2RESEARCH ARTICLE

running head: Cortical Dynamics in Flavor Consumption

Retronasal olfaction coupled taste and olfactory cortex dynamics

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# ABSTRACT

N = 12 animals female rats

2 Sessions each

5 neurons per area minimum

IOC Hab 1: Retronasal odor battery: MV, EB, Carv, CisHex Triggered by Nosepoke

IOC Hab 2: Ortho odor battery: MV, EB Triggered by Nosepoke

Session 1: Retro v. Ortho: MV, EB Triggered by Nosepoke

Session 1: Retro v. Ortho: MV, EB Triggered by Nosepoke

When IR beam is ready turn off vacuum

1. IR triggered Ortho and water release
2. If in nosepoke for 0.5 reward with water 2 seconds later
3. 1 minute ITI
4. Turn on vacuum
5. Retro passive delivery
6. 1 minute ITI nosepoke on2
7. 15 Trials for each odor

Preference Task w/ 2 IOC

1 retro air and water

2 retro solution and water

3 ortho and water

4 retro air

6 animals per condition

The gustatory cortex (GC) and primary olfactory cortex (OC) share both poly and monosynaptic connections with each other and communicate during the consumption of food. Both regions have neurons which are responsive to taste and smell. Thus, it is no surprise that neural responses to taste in the posterior piriform cortex (a section of OC) are influenced by GC activity. It is reasonable to hypothesize that this influence occurs in the opposite direction as well and that odor information in OC changes the dynamics of GC taste responses. Furthermore, it is possible that aside from unisensory modulation of these cortico-cortical dynamics, that flavor consumption, a multisensory stimulus, has a dominating effect on this inter-region communication. Here we monitored changes in GC-OC ensemble dynamics in awake freely moving rats in response to a battery of unisensory and multisensory stimuli delivered intraorally. We found that individual GC neuron taste responses are amplified by the addition of odors and that OC neuronal ensembles become synchronous with GC ensemble dynamics in the palatability epoch. Using Hidden Markov Models (HMMs) to compare multisensory and unisensory conditions we discovered that taste responsive GC neuronal ensembles transition through states faster with multisensory stimuli and that this effect is independent of both the palatability of the flavor and the congruency of the taste palatability with the odor. These results support an enhancing effect of odor on the reliability and speed of GC dynamics.

## **NEW & NOTEWORTHY**

Communication between these two chemosensory regions is important for the percept of flavor and food choice. This study examines the dynamics of ensembles of neurons in two chemosensory cortical regions that are interconnected and important for flavor consumption. The speed of state transitions for taste cortex ensembles and the synchrony of both cortical ensembles increases with flavor stimuli.

**Keywords:** Chemosensation, Multisensory, Dynamics, Inter-Region, Flavor

# INTRODUCTION

Provide a brief overview of the scope and relevance of the study, especially regarding previous advancements in related fields.

## Subheading level 2

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### Subheading level 3

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#### Subheading level 4 (if needed)

Continuing text.

# MATERIALS AND METHODS

## Ethical approval

# Experimental procedures complied with the Wake Forest Baptist Medical Centre Institutional Animal Care and Use Committee guidelines, under the guidelines of the AVMA (protocol #A21-111) and conform to the principles and regulations as described in Grundy (2015). The investigators confirm that they understand these ethical guidelines and that they comply with the animal ethics checklist provided by The Journal of Physiology

## Animals

In total, 12 adult female Long–Evans rats (www.criver. com), weighing between 250 and 400 g at the time of surgery, served as subjects. All rats were individually housed and kept under a 12:12 h light/dark photocycle and received food and water ad libitum unless otherwise stated. Experiments were conducted during the light cycle. No animals were excluded from the analysis.

## Surgery

Animals were injected i.p. with the analgesic meloxicam (10 mg kg–1) prior to surgery. Stereotaxic surgery was performed under intranasally administered isoflurane anaesthesia (2–5%). First, the scalp was treated with lidocaine ointment, an incision was made and the skin was retracted from the skull. Using a dental drill, a craniotomy was made overlying the posterior piriform olfactory cortex (uni- or bilateral): 1.4 mm posterior to bregma, 5.2–5.6 mm lateral to the midline and 6.4–7.4 mm ventral from the surface of the brain (Paxinos & Watson, 1986). Five additional burr holes were made, evenly spread across the skull, for the insertion of skull screws that provide stability to the implant. Electrodes were then lowered to the piriform cortex over the course of 30 min using a manually-driven stereotaxic arm. Once in place, the craniotomy was filled with Kwik-Cast (www. wpiinc.com) and the electrode connector and skull screws were covered in dental acrylic, fixing the electrodes in place. Electrode assemblies consisted of microwire arrays with six or eight electrodes in circular arrangement, or 16 electrodes in square arrangement. Electrodes were 25 μm diameter stainless steel wires, spaced 100–200 μm apart (www.microprobes.com) or multi-electrode silicon probes (two shanks of 16 electrode contacts each, shanks spaced 500 μm apart; electrode contacts spaced 50 μm apart; model A2×16-10 mm-50-500-177-CM32; www.neuronexus.com). Intra-oral cannulae (IOC) were implanted to provide access to the oral cavity as described in detail previously (Maier & Katz, 2013; Phillips & Norgren, 1970). In short, flanged microbore polyethylene (PE) tubing (inner diameter 1.143 mm, outer diameter 1.574 mm) was inserted behind the second molar using a 20 G needle and guided under the skin overlying the zygomatic arch to exit at the edge of the cranial implant. Tubing was then attached to a coupler body (#SMF01; www.cpcworldwide.com) that could interface with a counterpart holding a manifold of fluid delivery tubes. Once in place, the coupler was secured to the rest of the implant with dental acrylic. Animals recovered in their home cage with ad libitum access to water and mashed rat chow for 4-5 days after surgery before the start of the experiment.

## Stimuli

Unisensory odour stimuli were exemplars of monomolecular odorants (www.sigmaaldrich.com), >98% purity): methyl valerate, amyl acetate, propyl acetate, methyl benzoate, 2-hexanone, citral, citronellal, octanal, nonanal and hexanoic acid in aqueous solution (0.025% v/v in distilled water). Unisensory taste stimuli (www.fishersci.com) were exemplars of basic taste qualities: saccharin (10 mm), sucrose (100 mm), sodium chloride (100 mm), citric acid (20 mm) and quinine-HCl (1 mm) in distilled water (comparable to taste stimuli use by other labs (Chen et al., 2021; Levitan et al., 2019; Roussin et al., 2012). Multisensory stimuli consisted of mixtures of a single odorant and a single tastant. All stimuli were presented intra-orally to allow, as much as possible, natural sensory stimulation dynamics associated with intra-oral evaluation of flavour.

## Stimulus presentation & recording procedures

The recording arena consisted of a 29 × 23 × 33 cm Plexiglas chamber encased in metal that served as a Faraday cage. Stimuli were delivered via syringe pumps directly onto the dorsal surface of the tongue when animals were moving freely around the arena. Syringes containing stimulus solution were connected to blunted needles fitted with strands of PE tubing (inner diameter 1.143 mm, outer diameter 1.574 mm) that fed into the recording arena via an opening in the roof. At the distal end of each strand of PE tubing, a 5 cm strand of polyimide (PI) microbore tubing (inner diameter 0.0254 mm, outer diameter 0.0270 mm) was glued, and all tubes were inserted into the through hole of a coupler body (#SMF02; www.cpcworldwide.com) and held together with glue. Before experimental sessions, the collection of PI tubes was fed into the IOC and secured in place by mating the coupler bodies on the tube manifold and IOC. Once connected, the tips of the PI tubes extended 0.5 mm below the tip of the IOC into the oral cavity. Animals were habituated to the recording setup and stimulus delivery apparatus for 1 or 2 days prior to recording by presenting drops of water through the IOC in the recording arena. During recording sessions, airborne odorants were cleared by a continuously running fan mounted in the ceiling of the recording arena. To encourage consumption of stimuli, animals were deprived of water for 6 h before recording sessions. Depending on the experimental context (see Results), all animals underwent one to five recording sessions (n = 42 sessions in total), during which two or three odorants, one or two tastants, and all binary combinations of taste and odour stimuli were presented (10 repetitions of each stimulus). Unisensory stimuli and plain water were presented via dedicated fluid lines; mixtures were presented as two simultaneous drops of unisensory taste and odour solution. To match concentration and total volume between conditions, unisensory stimuli were presented simultaneously with a drop of plain water. Concentrations listed above represent final concentration in the oral cavity. Stimuli were always presented in random order. Intra-oral stimuli were delivered in 30–50 μl aliquots (total duration of delivery <100 ms), with a random inter-trial interval in the range 30–45 s, allowing sufficient time for animals to swallow the fluid and clear their mouth. Animals did not have access to any of the stimuli prior to recording sessions and were therefore considered naïve. Each session (total duration <1 h) yielded between 1 and 26 (mean = 8.6) single neurons (0.3 neurons per electrode on average).

## Electrophysiological recording and data processing

The continuous extracellular signal recorded from each electrode was amplified, digitized and stored for offline analysis at a sampling rate of 25 kHz using INTAN RHD2000 hardware and acquisition software (www.intantech.com). Action potentials were extracted, clustered and sorted using the klusta/phy toolbox to obtain single neuron spike time stamps (Rossant et al., 2016). For silicon probe recordings, spikes were clustered taking into account the possibility that the same action potentials could be recorded by up to three neighbouring channels. Spike time stamps were then binned at 1 ms resolution and aligned to stimulus delivery before further analysis. Only action potentials that exceeded 3.5 standard deviation units of the high-pass filtered (400 Hz) voltage signal were included in the clustering analysis, and only clusters that contained less than 2% of action potentials occurring at an inter-spike interval of 2 ms or less were included in the dataset (Gadziola et al., 2015).

## Data analysis

## Histology

Electrodes were labelled with a drop of Vybrant® DiI cell-labelling solution (www.thermofisher.com), applied with a needle tip before implantation, allowing post mortem reconstruction of the implant location. Accordingly, rats were perfused trans-cardially with saline and 10% formalin, their brains extracted and placed in 30% sucrose for 3–5 days. Brains were then frozen; coronal sections were cut around the implant location using a sliding microtome, mounted on glass slides in DAPI Fluoromount-G medium (www.southernbiotech.com) and a cover slip was applied. Epifluorescence microscopy was used to visualize 1,1 -dioctadecyl-3,3,3 ,3 -tetramethylindocarbocyanine perchlorate (DiI) and 4 ,6-diamidino-2-phenylindole (DAPI). Figure 1 shows histological reconstruction of electrode placement in pPC for two representative animals.

## Statistical analysis

# RESULTS

Present the experimental data and their statistical significance.

# DISCUSSION

Explain your interpretation of the data, especially compared with previously published material cited in the References. Significance and limitations may also be present.

Perspectives and Significance

This section is required at the end of all *AJP-Regulatory, Integrative and Comparative Physiology* DISCUSSION sections.

# APPENDIX

This section may be included in mathematical modeling or computational papers, e.g., to provide details of a solution strategy.

# GLOSSARY

This section is only included for equation-laden articles with many different symbols (such as mathematical modeling or computational papers). See [this article](https://doi.org/10.1152/advan.00171.2021) for an example.

Abbr. definition

# DATA AVAILABILITY

Provide a statement declaring where the source data can be found, including DOI / URL (accession number).

# SUPPLEMENTAL MATERIAL

List all cited Supplemental Figures, Tables, Audio, Videos, etc. and where they can be found, including DOI / URL (accession number).

# ACKNOWLEDGMENTS

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# AUTHOR CONTRIBUTIONS

Identify which authors participated in the research: Conceived and designed research, performed experiments, analyzed data, interpreted results of experiments, prepared figures, drafted manuscript, edited and revised manuscript, approved final version of manuscript. The information must be the same as in the online submission site.

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# FIGURE LEGENDS

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# TABLES

Footnotes should appear below table with all symbols and abbreviations defined. Example:

**Table 1.** Effect of ANG II infusion on biomechanical properties of parenchymal arterioles

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Male** | | ***P* Value** | **Female** | | ***P* Value** | **Female** | ***P* Value** |
|  | **Sham** | **ANG II** |  | **Sham** | **800 ng/kg/min ANG II** |  | **1,200 ng/kg/min ANG II** |  |
| Wall stress, dyn/cm2 | 229.6 ± 21.4 | 274.2 ± 24.1 | 0.20 | 260.6 ± 12.9 | 267.4 ± 30.2 | >0.99 | 273.0 ± 16.9 | >0.99 |
| Wall strain | 0.195 ± 0.02 | 0.174 ± 0.02 | 0.47 | 0.202 ± 0.01 | 0.210 ± 0.02 | >0.99 | 0.178 ± 0.02 | 0.50 |
| Distensibility | 19.5 ± 1.5 | 17.4 ± 2.2 | 0.47 | 20.21 ± 1.2 | 21.0 ± 1.7 | >0.99 | 17.8 ± 1.5 | 0.50 |
| β-Coefficient | 12.2 ± 0.9 | 13.8 ± 1.1 | 0.31 | 13.0 ± 0.75 | 11.9 ± 0.88 | 0.49 | 12.6 ± 0.50 | 0.92 |

Values are means ± SE; n = 7–9. ANG II, angiotensin II. Comparisons made vs. same-sex sham controls at 40 mmHg by two-tailed Student’s *t* test (males) or one-way ANOVA with Bonferroni post hoc correction (females).