Beyond Scent Detection: Inflammation Sculpts Neuronal Transcriptomes in the Mouse Nose

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Abstract

This study investigated how inflammation caused by viral infection affects the transcriptomes of olfactory sensory neurons (OSNs) in mice. We reanalyzed publicly available single-cell RNA sequencing data from mouse nasal tissues infected with influenza A virus at different time points (control, 2, 5, 8, and 14 days). After rigorous quality control, 115,761 high-quality cells were analyzed. We identified 19 distinct cell clusters, extracted 45,443 mature OSNs, and applied refined clustering analysis. We demonstrated the "one receptor per neuron"rule with 87.9% of cells expressing a single olfactory receptor. Temporal analysis revealed 4,341 genes significantly altered at day 2, 6,405 at day 5, 3,832 at day 8, and 6,387 at day 14. Genes with strongly increasing response patterns included Gm10076, mt-Atp8, and mt-Nd4l, while stress-related genes like Mt1 and Mt2 showed moderate increases. The results demonstrate that viral inflammation significantly modulates gene expression in OSNs with distinct temporal dynamics.

Keywords: ; olfactory sensory neurons; inflammation; transcriptome; influenza virus; single-cell sequencing

1 Introduction

The olfactory system plays a crucial role in detecting and processing odors, serving essential functions in animal survival through the identification of food, predators, and mates. Olfactory sensory neurons (OSNs) are located in the olfactory epithelium and express specific olfactory receptors that detect odorant molecules. Recent studies suggest that OSNs may encode signals that signify an animal's internal states and modulate sensory responses.

Inflammation caused by viral infections is known to affect the sense of smell, a phenomenon widely observed during the COVID-19 pandemic. However, the underlying molecular mechanisms of these alterations remain poorly understood. Influenza A virus represents a valuable experimental model for studying the effects of viral inflammation on the olfactory system.

The objective of this study was to test the model that olfactory sensory neurons encode signals that signify an animal's internal states and modulate sensory responses, specifically focusing on inflammation induced by viral infection. We reanalyzed publicly available single-cell RNA-sequencing data to examine the effects of inflammation on OSN transcriptomes and identify temporal dynamics of gene expression during influenza A virus infection.

2 Material and Methods

2.1 Dataset and Animal Model

We reanalyzed a publicly available single-cell RNA-sequencing dataset from Kazer et al. (2024) using nasal tissues from mice (6-8 weeks old) that underwent intranasal injection of influenza A virus (10 viral particles, 5l per nostril). Tissues were collected at various time points: naïve (control), 2, 5, 8, and 14 days post-infection. The original dataset contained 156,572 cells $\times$ 25,147 genes.

2.2 Single-Cell RNA Sequencing

Library preparation was performed using the 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1. The computational workflow began with unsupervised clustering to delineate discrete cellular subclasses, followed by the targeted extraction of OSNs using established marker genes.

2.3 Computational Pipeline

2.3.1 Quality Control

The following filters were applied:

a) cells with mitochondrial content 10% were removed;

b) cells with ribosomal content 20% were removed;

c) cells with $<$500 detected genes were removed;

d) doublet detection using Scrublet (28 doublets removed).

After quality control, 115,733 high-quality cells remained from the original dataset.

2.3.2 Data Processing

Data were normalized to 10,000 reads per cell followed by log1p transformation. We identified 2,500 highly variable genes and performed principal component analysis (PCA) with 50 components. A neighbors graph was constructed with 20 neighbors.

2.3.3 OSN Identification and Analysis

OSNs were identified using established marker genes:

- **OMP** (Olfactory Marker Protein) for mature neurons
- **GAP43** (Growth Associated Protein 43) for immature neurons

We identified 1,040 olfactory receptor genes, 13 TAAR genes, 110 V1R genes, and 53 V2R genes. The "one receptor per neuron"rule was implemented using UMI threshold analysis (5 UMIs).

2.4 Statistical Analysis

Statistical testing was performed using Mann-Whitney U test comparing Day 0 vs all other timepoints. Multiple testing correction was applied using False Discovery Rate (FDR < 0.05). Advanced gene response pattern categorization was performed using slope analysis and multiple criteria.

3 Results and Discussion

3.1 Cluster Identification and Cell Type Annotation

Leiden clustering (resolution 0.1) successfully identified 19 distinct cell types. Clusters 0, 1, and 15 were identified as mature olfactory sensory neurons based on high OMP expression (44.8% of cells expressing). Cluster 5 represented immature olfactory neurons with high GAP43 expression (9.6% of cells expressing).

We extracted 45,443 mature OSNs from the primary clusters and applied refined clustering, identifying 7 distinct OSN subclusters. The "one receptor per neuron"rule was successfully demonstrated with 87.9% single-receptor expression in mature OSNs (37,948 cells). After filtering for OSN subtypes present across all timepoints with 3 cells, 390 valid OSN subtypes comprising 26,002 cells were analyzed.

3.2 Temporal Gene Expression Analysis

Comprehensive temporal analysis revealed significant gene expression changes across different time points:

- **Day 0 vs Day 2**: 4,341 significant genes (572 upregulated, 3,769 downregulated)
- **Day 0 vs Day 5**: 6,405 significant genes (1,037 upregulated, 5,368 downregulated)
- **Day 0 vs Day 8**: 3,832 significant genes (460 upregulated, 3,372 downregulated)

- **Day 0 vs Day 14**: 6,387 significant genes (5,517 upregulated, 870 downregulated)

We selected the top 100 differentially expressed genes from each of the four time points (Day 2, 5, 8, and 14), then took the union of these gene sets, resulting in 218 unique genes for advanced gene response pattern categorization. This approach identified distinct temporal patterns across the combined gene set:

3.2.1 Strongly Increasing Genes

Seven genes (3.2%) showed consistent upregulation with strong positive trends:

1. **Gm10076** - Progressive increase (slope: 0.353)
2. **mt-Atp8** - Mitochondrial ATP synthase subunit (slope: 0.329)
3. **mt-Nd4l** - Mitochondrial NADH dehydrogenase (slope: 0.312)
4. **Gm42418** - Novel gene with strong Day 14 response (slope: 0.215)
5. **Lars2** - Leucyl-tRNA synthetase 2, mitochondrial (slope: 0.183)

3.2.2 Moderately Increasing Genes

Thirty genes (13.8%) showed moderately increasing patterns, with mean slope of 0.151 $\pm$ 0.033:

1. **Mt2** - Metallothionein 2 (slope: 0.250)
2. **Tomm7** - Translocase of outer mitochondrial membrane 7 (slope: 0.195)
3. **Mt1** - Metallothionein 1 (slope: 0.188)
4. **Lrrc3b** - Leucine rich repeat containing 3B (slope: 0.187)
5. **Gng13** - G protein subunit gamma 13 (slope: 0.186)

3.2.3 Strongly Decreasing Genes

Three genes (1.4%) showed strong decreasing patterns with mean slope of -0.395 $\pm$ 0.165:

1. **AY036118** - Novel gene with late-stage downregulation (slope: -0.629)
2. **Mbd2** - Methyl-CpG binding domain protein 2 (slope: -0.279)
3. **Brd1** - Bromodomain containing 1 (slope: -0.278)

3.2.4 Moderately Decreasing Genes

Sixteen genes (7.3%) showed moderately decreasing patterns with mean slope of -0.138 ± 0.038:

1. **Arl6ip5** - ADP ribosylation factor-like GTPase 6 interacting protein 5 (slope: -0.235)
2. **Hnrnpa0** - Heterogeneous nuclear ribonucleoprotein A0 (slope: -0.213)
3. **Nrn1l** - Neuritin 1-like (slope: -0.172)
4. **Cul3** - Cullin 3 (slope: -0.146)
5. **Gpr162** - G protein-coupled receptor 162 (slope: -0.144)

3.2.5 Mixed Pattern Genes

One hundred forty-five genes (66.5%) showed mixed patterns with no consistent temporal trend, including:

1. **Ubb** - Ubiquitin B with variable expression (R^2: 0.000)
2. **mt-Nd2** - Mitochondrial NADH dehydrogenase 2 (R^2: 0.000)
3. **mt-Co3** - Mitochondrial cytochrome c oxidase III (R^2: 0.000)
4. **Bsg** - Basigin with fluctuating expression (R^2: 0.001)
5. **Camk2d** - Calcium/calmodulin-dependent protein kinase II delta (R^2: 0.003)

3.3 Functional Implications

The results demonstrate that viral inflammation significantly modulates gene expression in OSNs with distinct temporal dynamics. The upregulation of mitochondrial genes (mt-Atp8, mt-Nd4l) suggests alterations in cellular energy metabolism. The increased expression of stress response genes indicates activation of cellular protection responses.

Notable Day 14 responders included Gm42418 with the strongest response (1.91 log2FC), mt-Atp8 (1.80 log2FC), and Lars2 (1.47 log2FC). Early response genes at Day 2 included Gpr162 (G-protein coupled receptor) and Arl6ip5 (ADP ribosylation factor-like GTPase).

4 Conclusions

This study successfully demonstrated that viral inflammation caused by influenza A virus significantly modulates the transcriptomes of olfactory sensory neurons in mice:

- Analysis of 115,761 high-quality cells revealed 19 distinct cell types in nasal tissue
- 87.9% of mature OSNs follow the "one receptor per neuron"rule
- Temporal gene expression showed distinct patterns throughout viral infection
- Mitochondrial and stress response genes were significantly upregulated
- 390 OSN subtypes were validated for future analyses

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Supplementary material

Single-cell RNA sequencing data are publicly available at the Single Cell Portal (SCP2216) from Kazer et al. (2024).

References

Kazer et al. (2024). Single-cell RNA sequencing dataset. Single Cell Portal (SCP2216).

Almushaddag, W.F. (2025). Beyond Scent Detection: Inflammation Sculpts Neuronal Transcriptomes in the Mouse Nose. GitHub repository. Available at: `https://github.com/WedyanFawaz/Beyond-Scent-Detection-Inflammation-Sculpts-Neuronal-Transcriptomes-in-the-Mouse-Nose`