Dense Odor Coding in the Mouse Olfactory Bulb

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Abstract

In this study, we explore odor-evoked activity representation in the olfactory bulb (OB) and how odor responses enable odor discrimination. Contrary to some previously cited theories that suggest a sparse representation, we hypothesize a more dense representation during odor presentation. A key question is how odors are reliably encoded in OB activity patterns, and how these patterns contribute to early odor processing. To address this problem, we recorded population level odor responses from the mouse OB with mesoscale two photon calcium imaging and applied machine learning techniques to suggest a model in which sparse coding is largely sufficient for olfaction, but redundant information may make odor coding more robust across different variables.

1. Introduction

A fundamental question in neuroscience is how features of the external world are converted to neuronal activity and represented in the brain. Odor encoding requires sampling a complex chemical sensory space and converting this information to neuronal activity. In mammals, odors are detected by a diverse array of olfactory receptors on sensory neurons which transmit odor information to the olfactory bulb (OB) via synapses in olfactory receptor-specific glomeruli (Buck & Axel, 1991; Vassar et al., 1994). Odor response patterns in the glomerular layer of the OB are spatially stereotyped due to regular patterns of receptor-specific projection input from sensory neurons (Ressler et al., 1994; Bozza et al., 2002; Mombaerts et al., 1996). This leads to spatially segregated representations of odors among relatively sparse populations of finely tuned OB projection neurons via their responses (Davison & Katz, 2007; Rinberg et al., 2006).

Sparse codes can be computationally advantageous because, by using only a small subset of neurons to represent each piece of information, they reduce the amount of neuronal activity needed to process and store information (Levy & Baxter, 1996). They can also provide greater selectivity and specificity in neuronal responses, allowing for more precise and reliable information processing (Zetsche, 1990). In olfaction, relatively small subsets of glomeruli and cortical neurons activated by specific odors, exhibiting population sparseness (Burton et al., 2022; Poo & Isaacson, 2009). Glomeruli, OB neurons, and cortical neurons are also sharply tuned to odors, exhibiting high lifetime sparseness (Poo & Isaacson, 2009). Additionally, activating sparse subsets of glomeruli in the OB is sufficient to drive odor-guided behavior (Chong et al., 2020), and the glomeruli responding with lowest latencies have the most influence driving downstream behaviors (Gill et al., 2020), suggesting that odor information is largely represented by sparse, low-latency glomerular activity.

Sparse codes, however, are susceptible to slight changes in the participating units. Accordingly, a sparser code for odors would be less robust to changes in input (i.e. odors encountered in different backgrounds), changes in gain (i.e. via habituation or state dependent neuromodulation) or changes in circuitry (i.e. learning related plasticity) than a denser, more redundant, and more distributed code (Spanne & Jörntell, 2015). In contrast to sparse codes, dense codes are advantageous in situations where robustness and redundancy are important. By involving a large number of neurons in the representation of each piece of information, dense coding increases the reliability of neural representations and reduces the risk of losing information due to noise, neuronal damage, or modulation from internal or environmental variables (Paiton et al., 2020). Dense codes can also improve generalizations and provide greater flexibility in neural responses, as different combinations of neurons can be activated to represent similar but distinct pieces of information (Foldiak, 2003). In the olfactory system, in addition to sparse, low latency odor evoked activity, dense, temporally complex, and state dependent activity is observed both during and after the odor exposure (Adefuin et al., 2022). In this study, we will train decoders in silico by applying linear and non-

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linear machine learning algorithms to decode odor identity from glomerular activity to determine what value – if any – does dense, distributed activity add to odor coding.

2. Results

Using meso-scale two-photon calcium imaging, we recorded odor evoked activity across a large population of glomeruli in the mouse OB with high spatial and temporal resolution. Fluorescence traces were extracted from each glomerular ROI, aligned to odor presentations and treadmill velocity, and deconvolved to isolate the timing of calcium signaling events.

We trained linear and non-linear classifiers to decode odors from population-level glomerular response patterns on individual trials. To define glomerular odor response patterns, we averaged the deconvolved fluorescence traces from individual glomeruli across the duration of individual odor presentations. To classify odors from glomerular response patterns, we employed supervised linear and non-linear machine learning methods. We tested five architectures on deconvolved calcium traces including one-vs-rest (OVR) logistic regression, support vector classification (SVC) (Gunn et al., 1998), convolutional neural network (CNN) (LeCun et al., 1995), random forest (RF) (Breiman, 2001), and XGBoost (Chen et al., 2015) with a train-test ratio of 80% training trials and 20% testing trials. We repeated each experiment five times. Then, we measured the decoding accuracy for testing trials across five mice. Among the linear methods, OVR logistic regression was the best performer with 87.94% accuracy (+/- 0.04 STD) on the testing data. Neural network methods were superior among non-linear models, but were outperformed by linear methods (Fig. 1A). OVR logistic regression was highly accurate across the whole panel of 11 odors and the odorless mineral oil control when trained and tested on full glomerular odor response patterns (Fig. 1B). Subsequent classifier analyses, therefore, apply the OVR logistic regression configuration described above. We

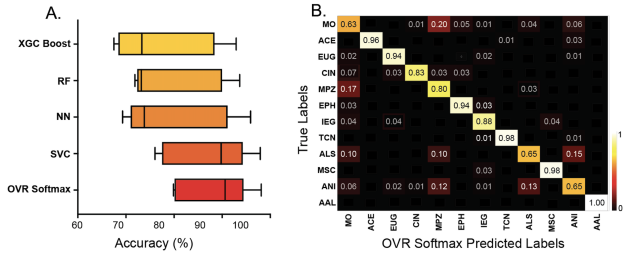

Figure 1. Odor discrimination using glomerular responses. A. Decoding accuracy calculated using linear and nonlinear methods with 5 repeats across 5 mice. B. Confusion matrix summarizing OVR softmax logisitic regression classifier predictions on the x axis vs true odor identities on the y axis.

used classifier testing accuracy to quantify odor information available in patterns of glomerular activity from individual trials. To determine how odor information is represented in subsets of glomeruli, we ranked glomeruli by various means and either added them into the classifier analysis one at a time to test sufficiency or removed them from the analysis one at a time to test necessity. First, we ranked glomeruli based on the magnitude of their largest odor response. This allowed us to target the highest responding glomeruli for addition or removal. We found that adding glomeruli based on response magnitude caused a sharp increase in classifier accuracy with a small subset of glomeruli added (Fig. 2A). This agrees with previous studies demonstrating that sparse glomerular activation is sufficient for odor decoding and olfactory discrimination.

Next, we wanted to test whether odor information was restricted to this small subset of sufficient glomeruli. To do this, we performed a similar analysis removing glomeruli one at a time based on response magnitude. If odor information is concentrated in a few glomeruli, then we would expect that the subset of glomeruli sufficient for odor decoding would also be necessary, and that removing them from the classifier analysis would cause testing accuracy to rapidly decrease to chance. However, when we removed glomeruli one at a time based on response magnitude, we found an unexpectedly gradual decrease in classifier test accuracy (Fig. 2B). This suggests that odor information is distributed across a broad population of glomeruli with a wide range of odor response magnitudes. Interestingly, accuracy declined uniformly across odors as glomeruli were removed from the analysis (Fig. 2C), further supporting the idea that odor information is densely represented across overlapping populations of glomeruli.

During odor presentations a subset of glomeruli are strongly activated across all trials, but a large population are activated at a lower magnitude with more variability across trials. To quantify the extent to which low magnitude responses are odor specific, we defined a metric of glomerular odor response reliability that was proportional to response magnitude and inversely proportional to response variability for a given odor. To determine whether reliability provided a more general metric of a glomerulus's contribution to odor decoding, we performed a similar classifier-based necessity and sufficiency analysis ranking glomeruli based on their reliability scores. Similar to ranking glomeruli by response magnitude, we found that ranking glomeruli by reliability led to a sharp increase in accuracy as glomeruli were added into the classifier analysis one at a time (Fig. 2D). Removing glomeruli based on reliability, we again found a slow, gradual decrease in accuracy (Fig. 2E). Together these data demonstrate that methods ranking glomeruli based on response magnitude and reliability are able to identify high-information glomeruli that are sufficient for odor classifi-

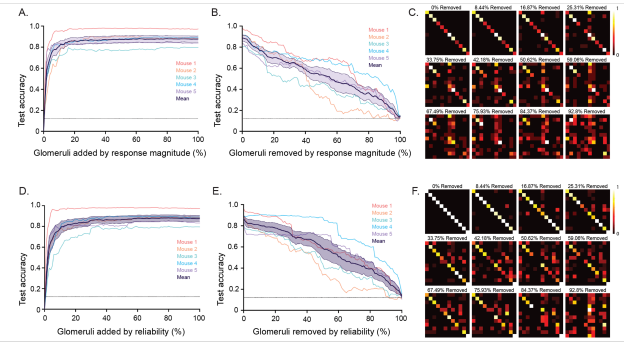

Figure 2. High response magnitude, reliable glomeruli are sufficient but not necessary for odor decoding. A. Classifier testing accuracy when glomeruli are added to OVR logistic regression training and testing one at time based on odor response magnitude. Thin lines are averages of 10 training and testing runs for one mouse. Thick line is average across mice. Shaded area is 95% confidence interval across mice. Dotted line is chance. B. Classifier testing accuracy when glomeruli are removed from OVR logistic regression training and testing one at time based on odor response magnitude. C. Confusion matrices showing classifier predictions (x axis) vs true odor presentations (y axis) at different percentages of glomeruli removed based on response magnitude. D. Classifier testing accuracy when glomeruli are added to OVR logistic regression based on their reliability scores. E. Classifier testing accuracy when glomeruli are removed from OVR logistic regression based on their reliability scores. F. Confusion matrices showing classifier predictions (x axis) vs true odor presentations (y axis) after different percentages of glomeruli were removed from classifier training and testing based on reliability score.

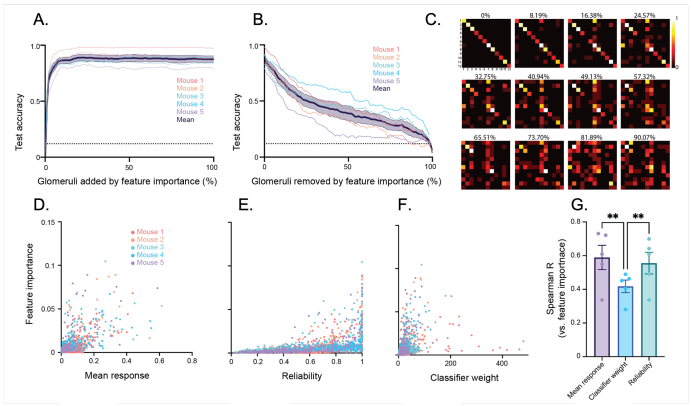

Figure 3. Employing RFGI for feature selection. A. Value-added analysis of glomerular responses based upon the importance scores averaged over animals (n=5). B. Value-added analysis of glomerular responses based upon the importance scores averaged over animals (n=5). C. Classifier confusion by odor after removing different percentages of features. D. Correlations of feature importance score with mean odor response. E. Correlations of feature importance score with reliability. F. Correlations of feature importance score with classifier weights. G. Spearman correlations calculated for data in G for individual mice (filled circles). Importance scores correlate more strongly with mean response and reliability compared to Softmax weights.

cation, but they fail to show that these same populations of glomeruli are necessary for odor classification. This means that either (1) odor information is broadly and redundantly distributed across a large population of glomeruli or (2) response magnitude and reliability are not effectively identifying the glomeruli that are most necessary for odor decoding (even if they are effectively identifying sufficient glomeruli). In either case, the discrepancy between the analyses adding glomeruli and removing glomeruli suggest that odor information is denser and more redundant across glomeruli than has been previously appreciated.

We then sought to measure the contribution of individual glomeruli to odor decoding without predefining specific measures of importance (i.e. response magnitude or reliability). To accomplish this, we applied information theoretic measures to determine the importance of individual glomeruli for odor decoding as a whole. There are several methods to measure the feature importance, see (Altmann et al., 2010) and (Toghani & Allen, 2021). Here we will focus on the RF feature selection which has the advantage of being highly applicable to high dimensional data. RF is an ensemble classifier that uses a variety of decision trees to subdivide data according to specific criteria (Breiman, 2001). At each split in a set of decision trees, a selected criteria is used to divide the data into two sets. The splits are then used to calculate the importance of individual features in the data for correct classification. In the current study we used a measure of inequality - Gini impurity - as the criteria to determine splits in the RF decision trees.

Using random forest Gini importance (RFGI) scores, we then performed a similar classifier-based necessity and sufficiency analysis. We added or removed glomeruli one at time based on their importance score. We recorded classifier performance and recalculated feature importance for remaining glomeruli at each iteration. Similar to analyses ranking glomeruli by response magnitude and reliability, we found that adding glomeruli based on feature importance sharply increased classifier accuracy (Fig. 3A). This indicates that RFGI scores are accurately identifying a small subset of glomeruli sufficient for odor classification. Intriguingly, the subset of glomeruli identified by RF feature detection as being sufficient for our odor classification task is smaller than the subsets of glomeruli identified by response magnitude or reliability suggesting that RF feature detection more accurately identifies the smallest subset of glomeruli sufficient for odor classification. Removing glomeruli by feature importance again revealed a gradual decline in classifier accuracy (Fig. 3B). However, examining the rate of decay in classifier accuracy, we found the decline is sharper during removal of the top 20% of glomeruli, indicating that they

contribute proportionally more to odor decoding. Accuracy reduced more gradually upon removal of the middle 60% of glomeruli (from 20% - 80%) though overall accuracy remained well above chance. Accuracy again declined steeply upon removing the last 20% of glomeruli.

In contrast to ranking glomeruli based on response magnitude or reliability, the sharper initial decline in accuracy when removing glomeruli based on feature importance suggests that this method better identifies necessary and important glomeruli. This implies that the RF feature selection method relies on more complex aspects of glomerular population activity to define which glomeruli are contributing to odor decoding. Accordingly, this method outperforms methods relying on response magnitude and reliability. However, further investigating the relationship between importance scores and other methods for ranking glomeruli, we found that importance scores were strongly correlated with both response magnitude and reliability (Fig. 3D, E, F). Surprisingly, we found a weaker correlation between importance scores and weights attributed to glomeruli by the classifier when trained on the full set of glomeruli (Fig. 3F, G). Together, these results suggest that (1) the overall importance of particular glomeruli to odor decoding is related to the magnitude and reliability of their odor responses and (2) the RF approach identifies a more efficient subset of important glomeruli.

3. Discussion

Previous studies using various imaging or electrophysiological methods to record neuronal activity in the OB have largely relied on recording from anesthetized animals, quantifying trial-averaged responses from a small subset of reliably time-locked glomeruli with high magnitude responses, and/or recording from small populations of active neurons (Vassar et al., 1994; Burton et al., 2022). These approaches, however, can mask state-dependent variability, trial-to-trial variability, and lower-magnitude glomerular activity in population-level odor responses, and therefore, they will tend to emphasize a sparse subset of active units that are sufficient for odor decoding, but does not reveal which patterns are necessary or which are employed under physiological circumstances. In this work we directly examine the information available in individual odor responses by using in silico classifiers to predict odor category. Our current study adds to the idea that odors are encoded by sparse subsets of glomeruli by providing evidence that relatively dense, low-magnitude, and variable-latency responses also contain extensive odor information.

A limitation of the current study is that it does not include behavioral testing or circuit manipulations to examine these possibilities directly. In the future it will be important and interesting to address how redundant odor information is utilized in these contexts to impact olfactory perception and odor guided behavior. While sparse, low-latency glomerular activity is directly triggered by olfactory sensory neuron (OSN) input, the density and temporal characteristics of the redundant glomerular activity that we observe make it unlikely that this activity is directly downstream of activated OSNs. Odor specific activity may be centrally maintained in the OB by either changes in cell intrinsic properties, persistent local circuit activity, or recruitment of long-range feedback. In insect models, it has been shown that persistent odor-specific activity relies on a combination of cellular adaptation and local inhibitory feedback circuits (Saha et al., 2017).

In the mouse OB, local circuits include both excitatory and inhibitory neurons (Nagayama et al., 2014) and recruitment of these circuits, particularly feedforward excitation via external tufted cells, is an intriguing candidate mechanism for driving secondary, dense, odor specific activity in the OB. At the same time, the mouse OB receives extensive top-down input from olfactory areas including the piriform cortex and anterior olfactory nucleus (Rothermel & Wachowiak, 2014), as well as from neuromodulatory centers like the locus coeruleus and basal forebrain (Gielow & Zaborszky, 2017). Feedback projections from olfactory cortical areas exhibit odor specific activity and may therefore be capable of directly driving secondary odor specific activity in the OB. Neuromodulatory feedback, while not odor specific, may serve to amplify or alter the properties of odor-specific cortical feedback or local circuit activity – providing a mechanism for modulating secondary odor-specific activity separately from activity directly downstream of OSNs.

4. Conclusion

Here we have described how odor information is redundantly and persistently represented in the activity of a large population of glomeruli in the mouse OB. In contrast to previous work framing odor encoding as exclusively sparse, we suggest a model in which sparse coding is largely sufficient for olfaction, but redundant information may make odor coding more robust across different internal and environmental variables. Future work will be needed to determine the extent to which dense, redundant, odor coding is the result of local circuit or feedback activity, how dense activity is transformed between glomerular input and mitral and tufted cell (MTC) output, how this activity contributes to odor coding across different states and contexts, and to how it is influenced by neuromodulation. Ultimately, uncovering mechanisms that drive and modulate the redundancy, distribution, and persistence of odor information in early olfactory circuits will help reveal how these circuits optimize odor encoding for perception under widely variable real-world conditions and changing internal states.

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