Final publishable summary report

Executive summary

Sensory information is conveyed to specialized brain circuits and is translated into ensemble representations by various populations of projection neurons. Whether different channels of output neurons could form similar and stable representations in diverse behavioral contexts remains largely unknown. Here, we studied the olfactory bulb (OB), where two layers of output neurons, mitral and tufted cells (MCs and TCs respectively), jointly receive odorant information and in turn project to different target regions.

We chronically recorded the activity of MCs and TCs with two-photon Ca^{2+} imaging in awake head-fixed mice under different behavioral contexts. We investigated how the ensemble odor representation and discriminability by MCs and TCs may change during 1) repeated passive odor experience, where animals were simply exposed to odor stimulation over consecutive days and 2) odor discrimination learning, where animals were actively engaged in discrimination of two similar odors in a go/no-go task. We discovered state- and cell type-dependent ensemble plasticity in the OB: during passive sensory experience, both MCs and TCs displayed robust weakening of responses and constant remodeling of ensemble representation, yet with ensemble odor discriminability remaining stable. In contrast, after active sensory learning, MCs but not TCs showed significant improvement in ensemble odor discriminability, although both populations displayed constant reorganization of ensemble representation to a degree similar to that during passive odor experience. We thus uncovered a context-dependent long-term ensemble plasticity that is differentially implemented in distinct layers of output neurons within the same sensory circuit, allowing parallel transfer of non-redundant sensory information to distinct downstream centers.

Summary of project context and objectives

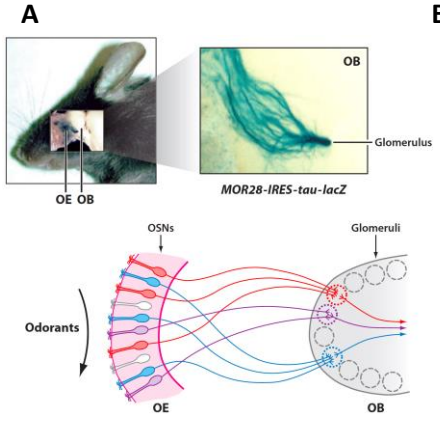
Collective activity of neuronal population, or neuronal ensemble representation, is proposed to be an important constituent of information processing in the brain. It has remained poorly understood, however, how the ensemble representation is maintained or modified over a long time scale (e.g. over days and beyond) by different types of output neurons. The recent advances in longitudinal and targeted large-scale recording with imaging offer the possibility to reliably track ensemble activity of identical neurons for a long time[3-5]. Although several studies have addressed the stability or the plasticity of ensemble representations in various brain regions in behaving [6-11], it still remains a hot topic of debate whether different populations of output neurons could display distinct forms of plasticity in diverse behavioral contexts[12-14]: when multiple groups of output neurons receive similar inputs yet project to different target regions, would they differently form and reorganize their ensemble representation depending on behavioral settings? Here we addressed this question in the mouse olfactory bulb, where pattern separation of complex odor information takes place [15-17,18].

The olfactory system processes information about food, predators, and mating partners, thereby providing animals with critically important information for survival as an individual and species. Odorant molecules activate olfactory sensory neurons (OSNs) on the olfactory epithelium located

inside the nasal cavity. It is believed that individual OSNs express only one type of olfactory receptors (ORs) among 1,000 types in mice[19]. In contrast to this simple organization principle at the periphery, the central olfactory system must integrate complex information about the combination of activated sensory neurons, since a single object contains dozens of odorant molecules, and a single odorant molecule activates multiple ORs and OSNs. The olfactory bulb (OB) is the first central relay in the olfactory pathway, which receives inputs from OSNs and sends outputs to other parts of the brain such as piriform cortex, amygdala, and entorhinal cortex (Figure 1). Besides its importance in olfactory information processing, the OB serves as an attractive model system for neural circuit analysis due to its highly organized layer structure with anatomically well-documented cell types. The axons of OSNs expressing the same OR converge onto one or two glomeruli among 1,800 in the OB, where odor information is transmitted to mitral and tufted cells (MCs and TCs respectively) via excitatory synaptic connections. A single M/T cell extends their apical dendrites into a single glomerulus, where it receives excitatory synaptic inputs from axons of OSNs expressing the same OR. M/T cells are the principal output neurons of the OB, and display selective responses to different odors and concentrations [20-24], with precise temporal pattern in the respiratory cycles [25-28]. The OB circuitry also contains several types of local inhibitory interneurons such as granule cells, periglomerular cells, and short-axon cells, which finely tune the activity of M/T cells [29, 30].

Although MCs and TCs receive excitatory inputs from olfactory sensory neurons in common glomeruli [2, 31], they send output projection to distinct cortical and subcortical regions of the brain [32, 33]. Recent studies have started to shed light on the functional difference between MCs and TCs [32, 34-36], and yet little is known about the plasticity of the ensemble odor representations formed by the two populations in a behaviorally relevant context.

Neurons in the OB play critical roles in olfactory information processing as well as olfactory-dependent learning, and their activity can be modified in an experience-dependent manner [16, 37]. It has remained to be elucidated, however, how each cell type in the OB shows odor-evoked responses, and how these responses may change in the course of olfactory learning, where animals actively use odor information to guide their behavior.



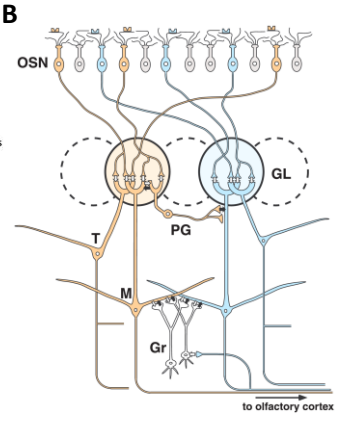


Figure 1. The anatomy of early olfactory system.

A. The projection from olfactory epithelium (OE) to olfactory bulb (OB) [1]. Staining of OSN axons (upper) and its schematic diagram (lower). B. Schematic cytoarchitecture of the OB [2]. M: mitral cell, T: tufted cell, Gr: granull cell, GL: glomerulus, PG: periglomerular cell

In this project, we employed *in vivo* 2-photon imaging using genetically encoded Ca^{2+} indicators (GECIs) to record the activity from each type of neurons in the OB. GECIs are chimera of fluorescent protein(s) and Ca^{2+}-binding protein, and show fluorescent changes in response to Ca^{2+} transients evoked by action potentials in neurons[38, 39]. Their expression can be chronically stable as well as targeted to specific cell types by use of appropriate promoters. We combined this imaging technology with a novel odor-dependent behavioral task developed in the host lab, where animals can learn to discriminate two different sets of odors under head-fixed condition[40]. We addressed the spatio-temporal pattern of population activity in the OB of awake mice and examine how the pattern may change in the course of olfactory learning.

Main results

We performed chronic two-photon imaging in awake head-fixed mice specifically expressing the genetically encoded Ca^{2+} indicator GCaMP6s[41] in MCs and TCs. Taking advantage of the highly organized layer structure of the OB, we separately recorded activity of MCs and TCs residing in different focal planes (Figure 2).

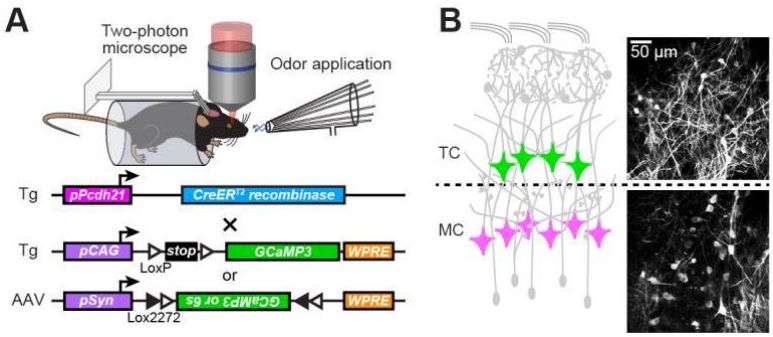


Figure 2. Imaging MCs and TCs in awake mice.

A. Schema of the experimental paradigm. B. Schema of the OB circuitry and example images of TCs (~120 μm deep; green) and MCs (~220 μm deep; magenta) expressing GCaMP6s.

We investigated how the ensemble odor representation and discriminability by MCs and TCs may change over time during either repeated passive odor experience, where animals were simply exposed to odor stimulation over consecutive days and or odor discrimination learning, where animals were actively engaged in discrimination of two similar odors in a go/no-go task. We discovered, for the first time to our knowledge, state- and cell type-dependent ensemble plasticity in the OB, which we detail in the following.

1) We characterized fluorescence changes in response to passive odor exposure in individual neurons, and we report both increase (excitatory) and decrease (inhibitory) in fluorescence during (ON response) and after (OFF response) odor application in both cell types (Figure 3).

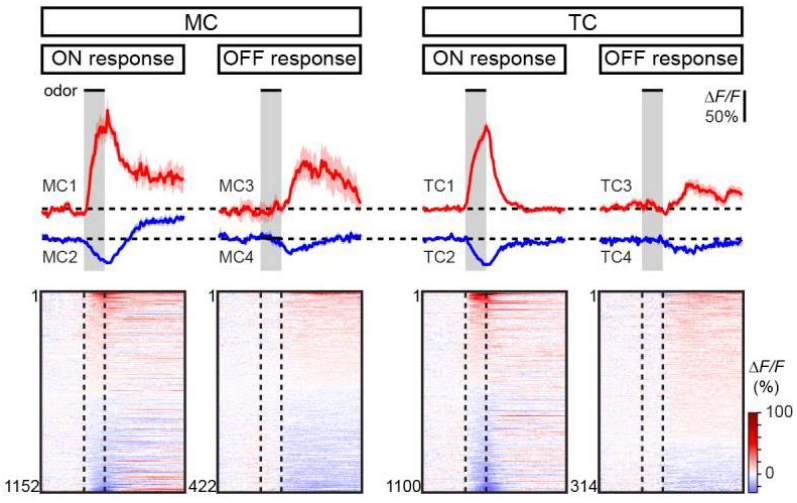


Figure 3. Odor-evoked responses of MCs and TCs in awake mice.

Example traces (upper panels; mean ± SEM across trials) and heat maps (lower panels; mean across trials) of responses evoked by 2-s odor application (black bars and dotted lines). Cell-odor pairs were sorted by their peak amplitude and grouped according to their responses observed either during (ON) or after (OFF, 7.3 s post-odor) odor application. Excitatory ($\Delta F/F > 0$) and inhibitory ($\Delta F/F < 0$) responses are shown in red and blue, respectively.

Although the results of this unbiased sampling are comparable to what can be observed in electrophysiological recordings, it was surprisingly overlooked in other studies using 2-photon calcium imaging in the OB. The proportion of cell-odor pairs for each response type were similar in MCs and TCs, suggesting that the overall inputs they receive in the local circuit might be indeed comparable. In summary, both populations of output neurons exhibited very similar behavior upon odor presentation.

2) We next asked how repeated passive sensory experience might alter the ensemble odor representation by MCs and TCs across days. We repeatedly applied daily the same set of odorants and imaged the response of the same cell assemblies. We found a general weakening in amplitude of both excitatory and inhibitory responses (Figure 4).

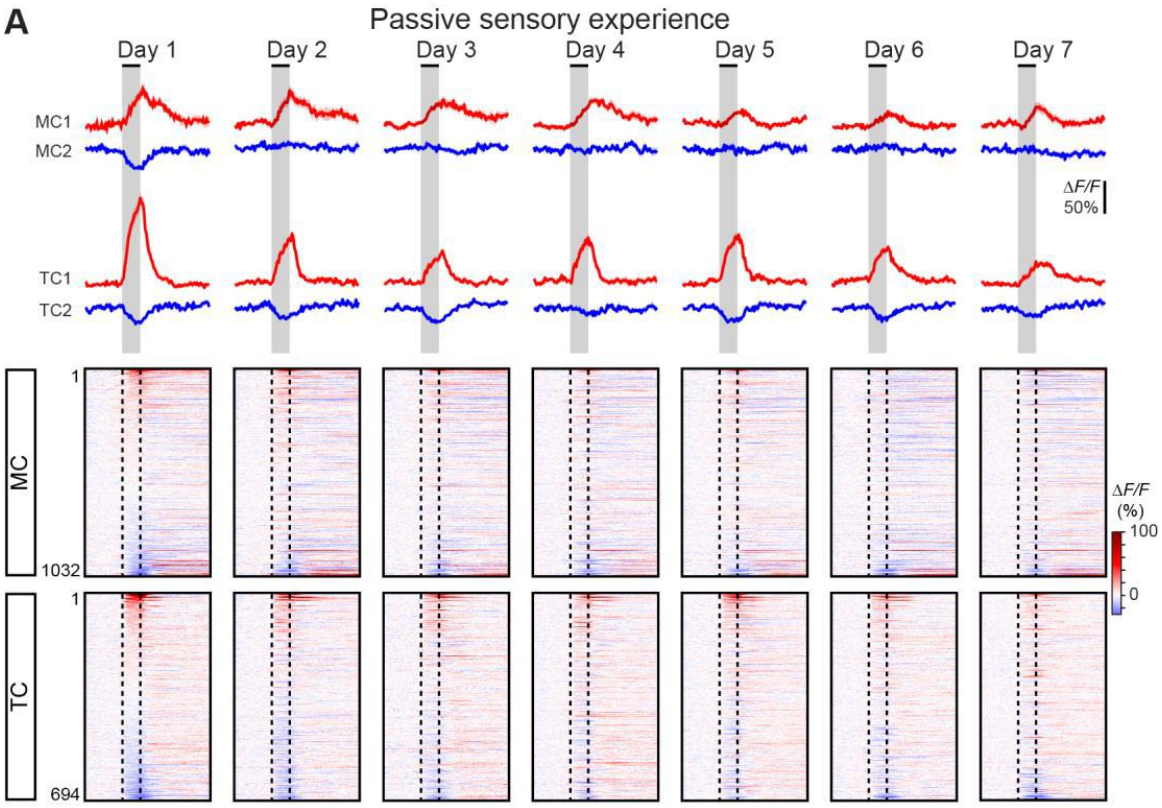


Figure 4. Reorganization of odor representations in MCs and TCs after repetitive passive odor experience.

Example traces (upper panels) and pseudo-color raster plots (lower panels) of all the responses evoked by 2-s odor application over 7 days of passive sensory experience. Data were acquired from 1032 cell-odor pairs, 125 cells and 6 mice for MC; 694 cellodor pairs, 105 cells and 5 mice for TC, respectively. Cell-odor pairs were sorted by peak amplitudes during ON period on day 1.

However, new cells were also becoming active over time. As a consequence, we found that the ensemble odor representation was constantly reorganized on a daily basis, but that the ensemble odor discriminability remained stable over time. Interestingly, this form of ensemble plasticity was similar in both populations of output neurons.

3) Finally, we examined the effect of active learning on ensemble odor representation and discriminability in MC and TC assemblies. Mice were trained to discriminate a pair of similar odors under head fixation, while population responses of MCs and TCs were imaged (Figure 5).

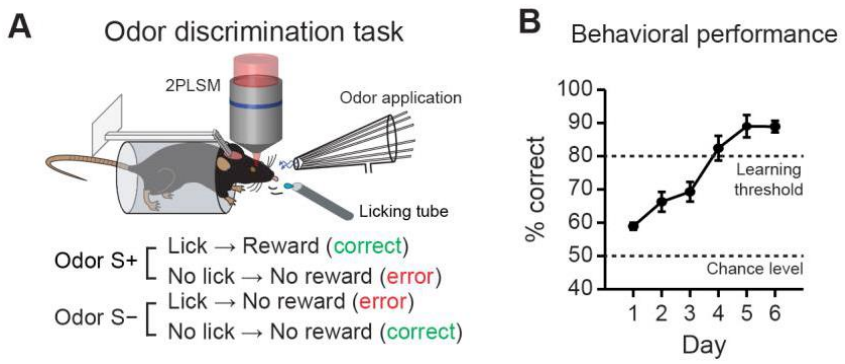


Figure 5. Odor-evoked responses of MCs and TCs in awake mice.

A. Schema of odor discrimination task. B. The odor discrimination performance.

Although a plasticity of ensemble representation was again observed, it differed from the passive sensory experience state. This new form of plasticity improved odor discriminability. Surprisingly, this form of long-term ensemble plasticity was observed exclusively in the MC population but not in the TC population (Figure 6). Thus MCs might serve as a specialized output channel that can be trained to disambiguate similar odors depending on behavioral context.

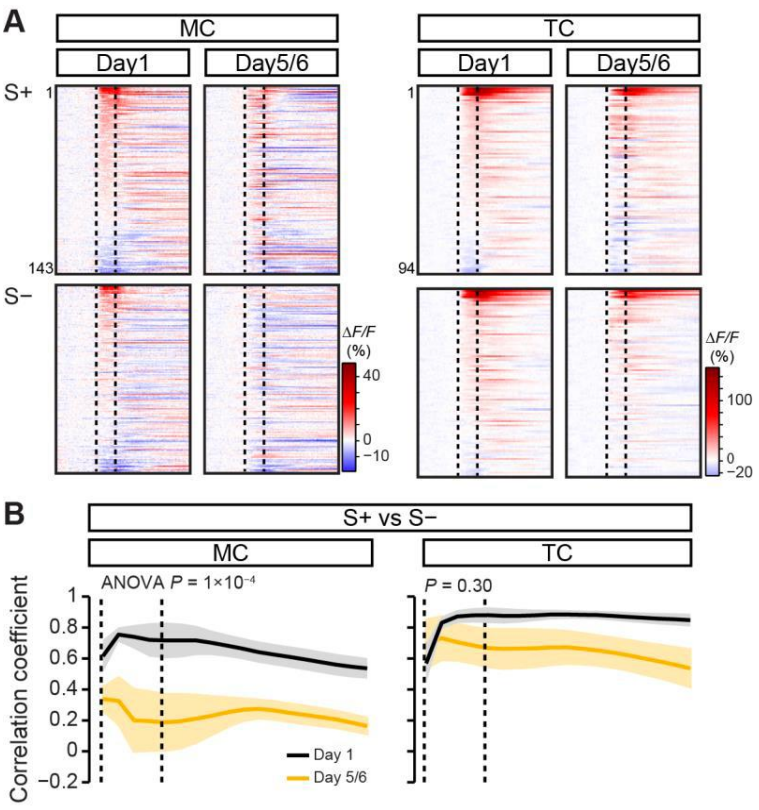


Figure 6. Long-term plasticity of ensemble representations after active sensory learning.

A. Heat maps of odor responses from all cells. Cell-odor pairs were sorted by peak amplitudes during S+ presentation on day 1.

B. Ensemble correlation between responses to S+ and S−. For each mouse, population vectors of ΔF/F values from all cells were calculated for S+ and S− from binned traces (4 points/bin). Correlation coefficient was calculated between a pair of population vectors constructed from responses to S+ and S−, and further averaged across mice (n = 5 mice for both MCs and TCs, mean ± SEM). The CC during odor application period (dotted lines, 4 bins) was significantly smaller on day 5/6 than that on day 1 in MCs but not in TCs (repeated measures ANOVA).

In conclusion, we uncovered two forms of plasticity in output neurons of the mammalian OB: 1) experience-dependent and cell type-independent constant reorganization of ensemble odor representation as well as 2) active learning-dependent and MC-specific long-term improvement in

ensemble odor discrimination. We propose that these plastic changes might be useful to optimize information coding in the OB.

Potential impact

In olfaction, whether and how MCs and TCs perform the division of labor has been a long-standing question. It has been postulated that TCs might contribute to odor detection, while MCs might facilitate odor discrimination, based on their spontaneous and odor-evoked activity timing (TCs early vs MCs late). To the best of our knowledge, our results provide the first direct evidence to this hypothesis by showing that TCs and MCs could display remarkable differences during active learning but not during passive experience. In the future, it would be interesting to assess the behavioral consequences of separate manipulation of TCs and MCs, although there are no genetic tools currently available to target these two populations independently. It would be also important to examine the functional difference of the cortical areas that are differentially innervated by TCs and MCs.

In other brain regions, the primary somatosensory cortex for instance, layer 2/3 pyramidal cells with different projection targets form non-overlapping subpopulations, namely those projecting to the primary motor cortex (M1P) and those to the secondary somatosensory cortex (S2P). Recently, it was shown that M1P and S2P display distinct plasticity following active tactile learning[12, 42]. Thus, our finding may point to a general and fundamental feature of early sensory information processing in the brain, where segregated output channels are implemented with differential plasticity to expand their capacity to code and transfer non-redundant sensory information to distinct downstream regions.

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