Middle tufted cells drive the mitral cell spatiotemporal firing patterns through glomerular and granule cell microcircuits

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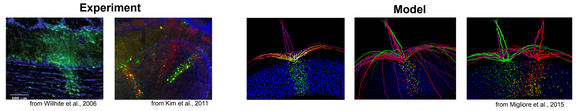
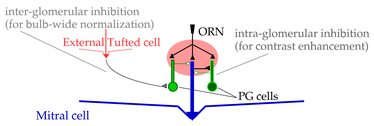
Our previous model has shown

3D model of the olfactory bulb

To investigate how the olfactory bulb (OB) represents natural odors, we have built the first 3D large scale model of it (Migliore et al., 2014; Migliore et al., 2015; Cavarretta et al., 2016).



The first version concerned the mitral-granule cell circuit; it reproduced the granule cell (GC) column observed experimentally in Willhite et al. (2006) and Kim et al. (2012) **(Left panels)**, by simulating odor learning **(Right panels)**. In Cavarretta et al., (2016), to simulate the learning of **natural odors, we have extended our model with the microcircuit of the glomerular layer (GL)** (Linster and Cleland, 2009; Cleland and Sethupathy, 2006).



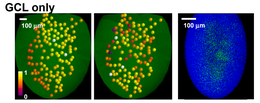
The GL microcircuit simulated the combined effects due to External Tufted Cells, Short Axon Cells, and Periglomerular Cells **(see the scheme on the left)**.

In particular, the GL performs a winner-take-all effect: in agreement with experiments, more glomeruli are recruited at higher concentrations.

The role of the GL in GC column formation with natural odors

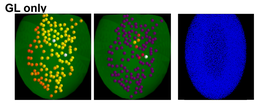
GL and granule cell layer (GCL) perform independent processing; they are both necessary to process natural odors (Cavarretta et al., 2016). Below is shown the glomerular input due to olfactory receptor neuron (ORN) activities **(Left)**, the resulting activity in mitral cell (MC) bodies in relation to a glomerulus **(Middle)**, and the GC column formed after learning **(Right).**

GCL only



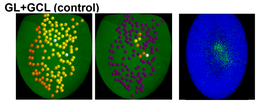
GCL does not perform contrast enhancement alone. Thus, training with natural odors results in a diffuse mitral-granule connectivity, without columnar organization.

GL only



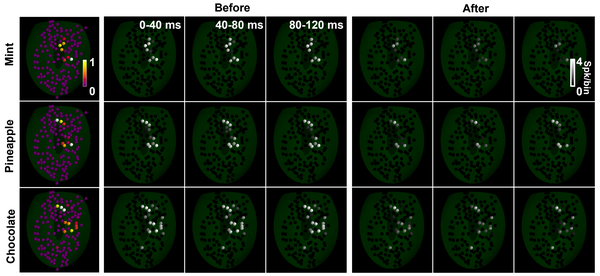
At the same time, GL transforms a dense odor representation into a sparse and contrast enhanced representation, without creating any GC columns.

GL+GCL (control)



Both GCL and GL lead to sparse, narrow, and segregated column formation, similar to experiments (Willhite et al., 2006; Kim et al., 2012).

Odor representation over time: before and after learning



Odors	Overlap			Overlap		
Mint, Pine.	0.49	0.70	0.51	0.59	0.63	0.33
Mint, Choc.	0.43	0.49	0.51	0.44	0.28	0.28
Pine., Choc.	0.77	0.73	0.74	0.76	0.63	0.59

Natural odor inputs are represented in the OB in terms of MC firing. Because of GL and GCL combined actions, odor learning decreases the relative spatial overlap among odors over time (Cavarretta et al., 2016).

Abstract

The olfactory bulb (OB) is a dual layer system, consisting of many glomerular units (GU) extending across the glomerular (GL) and the granule cell layers (GCL). The principal neurons are middle tufted (MT) and mitral cells (MC), whose activity encodes odor representation which is sent on the olfactory cortex (OC) through their axons. In turn, their activity is modulated by inter-glomerular lateral interactions which occur at both layers.

Previously, our 3D biophysically and morphologically accurate OB models have included only MCs, GL, and GCL. It has been predicted that the GL microcircuits transform a dense and disorganized spatial glomerular activation, such as that exhibited by natural odors, into a sparse, normalized and contrast-enhanced one. Together, the GCL, over time, decreases the spatial representation overlaps of different odors after learning (Cavarretta et al., 2016). However, many questions remain open, especially regarding the role of MTs.

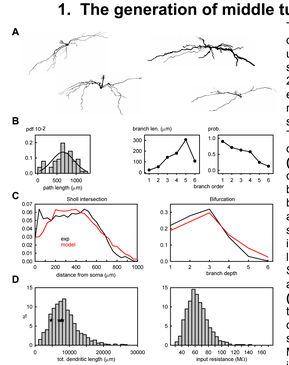
In this work, we have implemented MTs in our 3D model. We show that both MTs and MCs contribute to the columnar organization of the GCL, in particular demonstrating that they must be inhibited by the same GL neurons, in order to generate learning-dependent columns (Migliore et al., 2007) which conform to those observed in the experiments (Willhite et al., 2006). The inclusion of MTs in the model also allowed an estimation of the relative proportion of the 3 subtype of granule cells (Woolf et al., 1991), for which there are no quantitative data.

In addition, we found that MT membrane properties facilitate their precise inter-glomerular synchronization, which would be a key-feature to realize a fine temporal code relying on the inter-glomerular interactions in the GCL. Finally, assuming the MTs excite the deep short axon cells by their axon collaterals, we have inferred a plausible connection scheme in which the MTs could orchestrate the lateral inhibition produced by the GCL.

These results provide insights into the role of the MTs in spatial and temporal odor coding, which will eventually lead to also include the OC in the 3D OB model, starting from those regions that are targeted from MCs and MTs axons and could act as a gate connecting the OB and OC, such as the anterior olfactory nucleus.

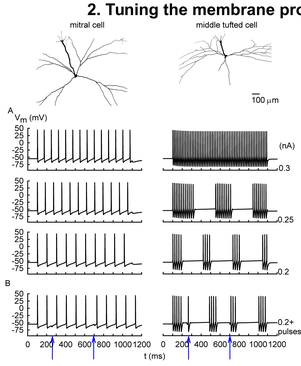
Adding middle tufted cells to the model

1. The generation of middle tufted cells



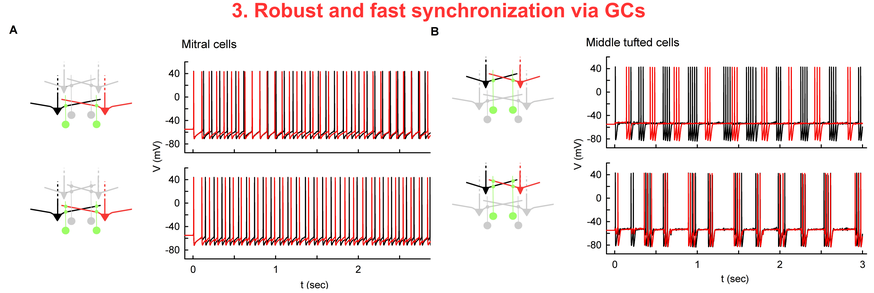
To generate the MT dendritic tree, we have used the same algorithm shown in Migliore et al., 2014. This requires the estimation from real MT reconstructions **(A)** of several parameters **(B)**. They are the distribution of overall dendritic extent **(Left)**, the branch length distribution **(Center)**, and bifurcation probability vs branch order **(Right)**. This algorithm generated MTs statistically indistinguishable from the labeled cells **(C)**, in terms of Sholl intersections **(Left)** and branch order proportion **(Right)**. In particular, **(D)** the distribution of overall dendritic length **(Left)** was similar to that of labeled MTs **(black stars)**, as was input resistance **(Right).**

2. Tuning the membrane properties



Morphologies of mitral and tufted cells look very similar to each other, but **(A)** their firing patterns are different (Burton and Urban, 2014). In particular, the MC responds with tonic firing **(Left)** to depolarizing step current, whereas the MT switches from burst to tonic firing with increasing current intensity **(Right)**. Together, **(B)** a moderate and brief hyperpolarizing pulse does not affect MC firing **(Left),** whereas it can trigger a **rebound burst** in a MT cell **(Right)**, as experimentally confirmed by SD Burton (unpublished observations).

3. Robust and fast synchronization via GCs



The rebound burst may enable MTs to precisely synchronize with a synchronous inhibitory input. This is likely conveyed via GCs. To address this hypothesis, we have started by connecting a single GC to a single MC **(Left panels)** or MT **(Right panels)**. Because of the morphological differences, MCs or MTs fire asynchronously, even though they receive the same excitatory stimulus on their tuft dendrites **(Top panels)**. Single GCs do not affect significantly MC firing **(Left panels)**, while MTs burst synchronously when they share GCs **(Bottom, Right panels).**

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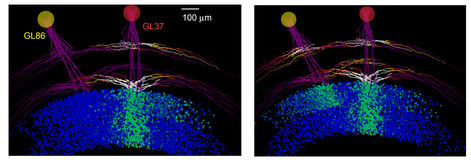
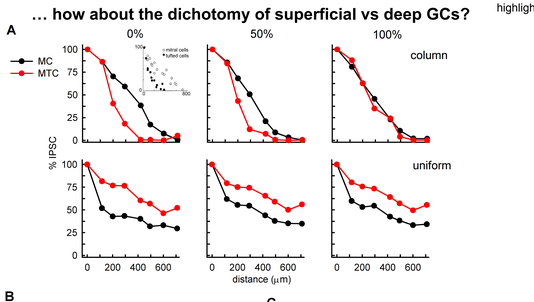
Finally the deep-short axon cell

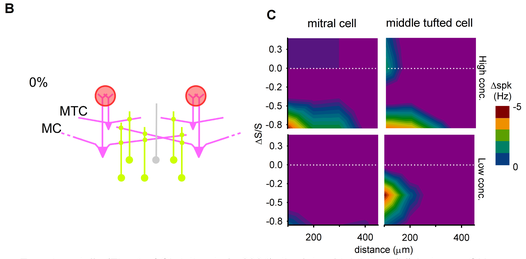
Are MTs controlled by the glomerular layer?



Column formation is driven by MC and MT activities, which connect to deep (dGC) and superficial GCs (sGC), respectively. Such subdivision can even be extended to the GC column. Experiments (Willhite et al., 2006) revealed that 95% of GC columns extended robustly through the full GCL depth **(Left)**. This gives insight into inhibition of sister MTs and MCs by the GL, because this makes them either active or silent together during learning. If MTs were not controlled by the GL, they might fire even if the MCs within the same GU are silent, forming GC columns only in the superficial half of the GCL **(Right).** This would contradict experimental evidence (Willhite et al., 2006), where no short columns were observed. Therefore, taken together, these results suggest that both MCs and MTs are controlled by the GL.

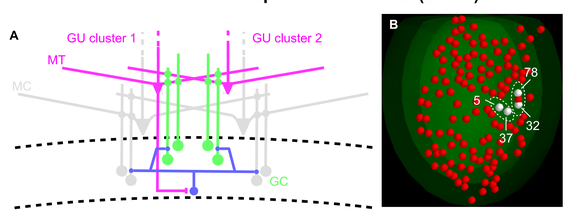
… how about the dichotomy of superficial vs deep GCs?





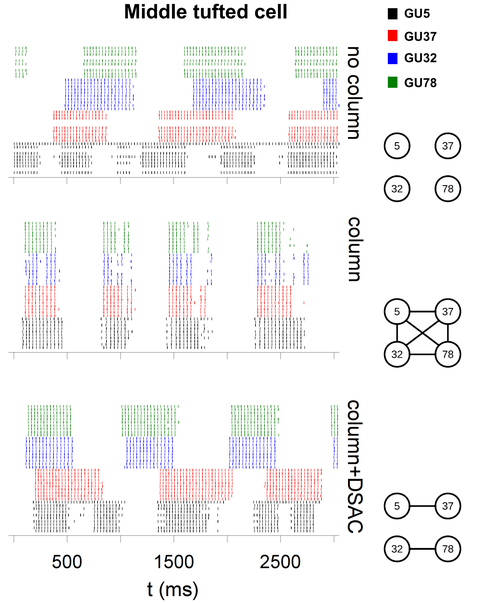
Experimentally (Fig. 4 of Christie et al., 2004), the lateral inhibition (LI) between GUs, induced via GCs, decreases as a function of inter-glomerular distance **(A)**; it is slower for MCs than MTs. Our model is able to reproduce this result **(top)** but only if there are GC columns **(down)**. Taken together, these results suggest MC and MT connect to separate subsets of GCs **(upper left panel with inset)**, forming two segregated and parallel signal pathways. This difference between MCs and MTs is due to the different extent of their lateral dendrites **(B)**. Moreover, LI decreases the firing rate in a distance-dependent fashion **(C)**. At higher conc., LI is effective on both MCs **(I)** and MTs **(II)**, especially when one GU is relatively more weakly activated than another **(DS/S, negative values)**. At low conc., LI effectiveness is reduced in MCs **(III)**, while it is strong in MTs **(IV)**. Thus, together with the M/T-GC connectivity, the odor conc. modulates the intensity of LI, and the spread of LI with inter-glomerular distance. In particular, LI seems to be more effective in MT at low conc.

Possible role of deep-short axon cells (DSAC)



DSACs may play an important role in modulating the MC and MT output from the OB. Experimental findings (Igarashi et al., 2012; Schoppa and Westbrook, 1998; Burton and Urban, 2015) suggested that MTs may drive DSAC activity, whereas no information is available on their connectivity. We have hypothesized a simple "fire together wire together rule", and assumed that DSACs axons target GCs shared between different GUs, as schematically represented in panel **(A)** using 4 GUs; we assumed that previous learning history resulted in the DSAC inhibiting the GCs shared between the GU pair (GU5,GU37) and (GU32,GU78). These were 4 of the 128 GUs implemented in our 3D model, and their spatial location in the dorsal part of the olfactory bulb is highlighted in panel **(B)**.

Middle tufted cell



To test their possible functional consequences, we ran simulations under different conditions of synaptic connectivity. The relative raster plots of the MTs belonging to each GU are shown under different conditions of: **1) no GC columns, 2) with GC columns, and 3) with columns and DSACs**. The raster plots highlight the different synchronization properties obtained in each case, and the underlying effective GU connectivity is schematically represented by the graph on the right. These results suggest that DSACs could have an important role in promoting the formation of GU clusters in such a way to synchronize MTs within the same cluster, but not between different clusters.

Conclusions

This is the first model of the olfactory bulb that includes a realistic implementation of both middle tufted and mitral cell parallel output pathways, which has suggested experimentally testable predictions on their interaction and computational functions.

1. The model predicts why mitral cells and middle tufted cells must share the same circuits in the glomerular layer.
2. In contrast, starting from experimental evidence, the model predicts that mitral cells and middle tufted cells must instead be connected to separate circuits at the granule cell layer
3. The model suggests that deep short axon cells may have an important role to play in shaping the spatial representation of odors by forming separate clusters of synchronized glomerular units.