Chem. Senses 30 (suppl 1): i107–i108, 2005 doi:10.1093/chemse/bjh137

Structural Organization of the Glomerulus in the Main Olfactory Bulb

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Key words: calbindin, compartment, GABA, gap junction, periglomerular cell, synapse, tyrosine hydroxylase

Introduction

According to the combinatorial receptor and glomerular codes for odors, the fine tuning of the output level from each glomerulus is assumed to be important to the information processing in the olfactory system, which might be regulated by numerous elements such as olfactory nerves (ONs), various interneurons and centrifugal nerves. Recently structural and physiological analyses at the cellular level has started to reveal that the neuronal organization of the main olfactory bulb (MOB) might be more complex than previously believed. In this article we describe on some aspects of the structural organization of the glomerulus we have revealed in our morphological studies.

The heterogeneity of periglomerular cells (PG cells) in their chemical and structural features and compartments of the glomerulus

Pinching and Powell (1971a,b) revealed, in a series of their classic studies, the structural and synaptic features of PG cells as well as other cells by Golgi impregnation method and electron microscopic examinations. In their studies they did not recognize the heterogeneity of PG cells clearly. On the other hand, many immunocytochemical studies have revealed that presumed PG cells contained various chemical substances and suggested that they were heterogeneous in chemical properties. So we first conducted the chemical classification of neurons in the periglomerular region of the rat MOB.

After colocalization analyses of various combinations of chemical markers we found that three substances, GABA, calretinin and calbindin, characterize three separate types of cells, which are ~20, 20 and 10% of all the cells in this region of the rat olfactory bulb, respectively. As our light and electron microscopic analyses of structural and synaptic features of these cells confirmed that most of these cells are PG cells, GABA-, calretinin- and calbindin-positive cells are considered as three nearly separate groups of PG cells. Among other chemical substances, tyrosine hydroxlase (TH) is contained almost exclusively in GABA-positive cells. About 50% of GABA-positive cells are also TH positive, and thus TH-positive cells are ~10% of cells in this region.

However, we must note two points. First, there are many as yet uncharacterized cells in this region. Secondly, the PG cell types described above does not necessarily hold true for others. For example, we have already confirmed that in some other species such as laboratory musk shrews and mice most of calbindin-positive cells in this region are also GABA-positive and thus the colocalization relationship is different from that of the rat MOB. At any rate, PG cells are chemically heterogeneous.

Then the next question is whether or not these chemically different PG cells are also different in their structural features. As described above, TH- and calbindin-positive PG cells are similar in number. However, our CLSM observations revealed that they are prominently different in the density, and thus in the number and extent, of their intraglomerular dendritic branches. In particular, we found that these two PG cells showed a prominent difference in the distribution of their intraglomerular dendrites in relation to ONs. The differences in their structural features of intraglomerular dendrites are far more clearly recognized when we consider the compartments of the glomerulus, as described below.

ONs do not spread diffusely within the glomerulus, but roughly speaking, they cluster and make many irregular tortuous strands or islands with many small holes. Thus the glomerulus is regarded to consist of two complementary compartments, the ON and non-ON zones, although we cannot define the border of these compartments strictly. Dendrites of TH and GABA-positive cells appear to penetrate into clusters of ONs and make many contacts with ONs, whereas dendrites of calbindin and calretinin-positive cells appear as if they avoid ONs. Then dendrites of TH- and GABA-positive cells are located both in the ON and the non-ON zones, whereas dendrites of calbindin and calretinin-positive cells are located in non-ON zone but rarely in the ON zone. So we can classify PG cells into two types, type 1 and type 2, on the basis of the distribution of their intraglomerular dendrites. The glomerular compartments can then be defined by the distributions of ONs and the distribution of intraglomerular dendrites of chemically different PG cells. Similar compartmental organization of the glomerulus has also been reported by Kasowski *et al.* (1999) and Chao *et al.* (1997). In the rat MOB type 1 PG cells include GABA- and TH-positive cells and type 2 PG cells include calbindin- and calretinin-positive cells. However, we must be careful not to oversimplify the classification of neurons. For example, even in the rat olfactory bulb we cannot regard all GABA-positive cells as type 1 PG cells a priori. We must consider the possibility that GABA-positive cells are heterogeneous and some are type 1 PG cells but others are type 2 PG cells. Furthermore, as there are many as yet uncharacterized cells in the periglomerular region, there must be GABA-negative type 1 PG cells and calbindin-/calretinin-negative type 2 PG cells.

The spatial relationship of MT cell dendrites with ON terminals and PG cell dendrites

MT cell synapses are clearly segregated, that is excitatory ON inputs are located only on the tufts in the glomerulus, whereas inhibitory inputs are located on the secondary dendrites but also on the dendritic tufts. But are excitatory ON inputs and presumed inhibitory inputs from PG cells on the MT cell dendritic tufts segregated in a proximodistal pattern or distributed in a mosaic pattern? To address this question we labeled MT cells with biotinylated dextran amine (BDA) and conducted combined CLSM-EM analyses. Multiple immunofluorescent labeled samples were examined with a confocal microscope, then processed for the ABC-DAB method and analyzed with an electron microscope.

These analyses revealed that in contrast to type 2 PG cells MT cells extend their dendrites in the glomerulus without respecting the

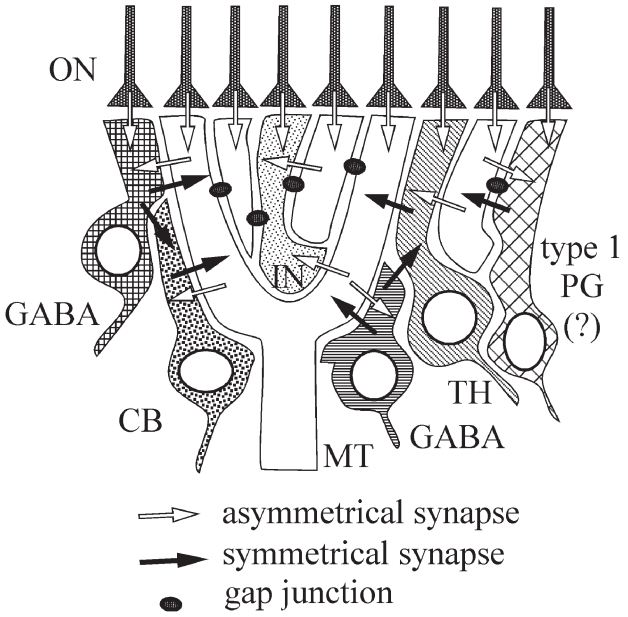

Figure 1 Cell types and interconnections among neuronal processes in the glomerulus.

border between the ON and non-ON zones. On the MT cell dendrites synapses from ON terminals and synapses from PG cells are not segregated proximodistally, but rather both are located on proximal as well as distal portions. Thus they are arranged in a mosaic pattern on the MT cell dendritic tufts.

Complex neuronal interactions via chemical synapses and gap junctions in the glomerulus

Our results on chemical synaptic connections among intraglomerular processes in the rat MOB are summarized as follows (Figure 1; calretinin-positive cells are not included in this description, which we are now analyzing). GABA- and TH-positive type 1 PG cells receive many synapses from ONs. GABA-positive dendrites occasionally make reciprocal synapses with MT cell dendrites. TH-positive cells also receive asymmetrical synapses from MT cells and make symmetrical synapses to MT cells. However, to date, we have encountered no TH-positive dendrites making reciprocal synapses. Calbindin-positive type 2 PG cells receive no or rare synapses from ONs, but make many synapses, including reciprocal pairs, with MT cell dendrites. On the other hand GABA positive, TH- and calbindin-positive PG cell dendrites receive synapses from other PG cell dendrites, at least some of which are GABA positive.

Among neuronal interactions, gap junctions (GJs) might be as important as chemical synapses, which have been proposed to play important roles in neuronal functions, i.e. synchronization of neuronal activities. In the glomerulus, we encountered numerous neuronal GJs. We revealed that MT cells made GJs with diverse types of neurons; some of them originated from PG cells but others did not show structural features characteristic to PG cells, and thus at least some were assumed to originate from hitherto uncharacterized neuron types (IN). GJ-forming PG cells occasionally received synapses from ONs, suggesting that at least some of them are type 1 PG cells. Type 1 PG cells include GABA-positive and TH-positive cells. So we examined whether or not GJ-forming processes are GABA- and TH-positive. So far we encountered no GJ -forming processes showing the intense GABA immunoreactivity or TH immunoreactivity in the rat MOB, although some were faintly GABA positive. Then when type 1 PG cells make GJs with MT cells, most of those GJ-forming PG cells might be different from GABA-positive or TH-positive type 1 PG cells we characterized before (type 1 PG (?) in Figure 1). So the majority of GJ-forming PG cells or other types of interneurons might be some as yet unidentified types of cells.

We confirmed GJs between MT cell dendrites and dendrites of PG cells and other presumed interneurons. But are there also GJs between MT cells, which are suggested by some physiological studies? In our recent study on the mouse MOB, we encountered several GJs between MT cells. Thus GJs are present between MT cells, as well as between MT cells and interneurons. Our EM serial sectioning studies revealed dendritic links consisting of several dendrites connected by GJs. Then we must consider both direct coupling between MT cells via GJs and indirect coupling between MT cells via intervening interneuron processes. Figure 1 summarizes the neuronal interactions in the glomerulus.

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