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“Interneurons” in the olfactory bulb revisited.

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Abstract

The main olfactory bulbs (MOBs) are now one of the most interesting parts of the brain in at least two points; the first station of the olfaction as an excellent model for understanding the neural mechanisms of sensory information processing and one of the most prominent sites whose interneurons are generated continuously in the postnatal and adult periods. Here we point out some new aspects of the MOB organization focusing on the following 4 issues; 1) there might be both axon-bearing and anaxonic periglomerular cells (PG cells), 2) most parvalbumin positive medium-sized neurons in the external plexiform layer as well as a few nitric oxide synthase positive PG cells and calretinin positive granule cells are anaxonic but display dendritic hot spots with characteristics of axon initial segments, 3) some of so-called “short-axon cells” project to the higher olfactory related regions and thus should be regarded as “nonprincipal projection neurons” and 4) tyrosine hydroxylase positive GABAergic (DA-GABAergic) juxtaglomerular neurons (JG neurons) are a particular type of JG neurons as a main source of the interglomerular connection, forming an intrabulbar association system.

Key words; projection neuron, local circuit neuron, association system, axon, dendrite, calcium binding protein, dopamine, GABA

Introduction

The main olfactory bulb (MOB) is the first relay station for transmission of olfactory information, which receives and processes the information from the olfactory sensory neurons in the nasal mucosa, and sends this information to different parts of the primary olfactory cortex in the forebrain. After the epoch-making study of Buck and Axel (1991), the MOB is considered as an excellent model for understanding the neural mechanisms of sensory information processing. The MOB is also attracting attention of many researchers for it is one of the most prominent sites whose interneurons are generated continuously in the postnatal and adult periods from the progenitor cells located in the subventricular zone (SVZ) of the lateral ventricle and migrate through the rostral migratory stream (Altman, 1969; Lledo et al., 2008). We reported a short summary of our data and our proposal on the glomerular organization and classification of periglomerular cells (PG cells) of the rodent MOB more than 10 years ago (Kosaka et al., 1998), in which we proposed the compartmental organization of the glomeruli and two types of PG cells based on the intraglomerular dendritic arborization correlating with their chemical properties (Kosaka et al. 1997). We also reported some reviews on the structural organization of the MOB focusing on the subtypes of PG cells and the intraglomerular neuronal interactions via synaptic and gap junctional connections (Kosaka and Kosaka, 2005, 2009a). However, several new findings of our own and other groups on the organization of the MOB are now changing the previous concept of the MOB organization. In the present article, showing some of our supplemental data unpublished previously, we point out the following 4 issues. 1) There might be both axon-bearing and anaxonic PG cells. 2) Most parvalbumin (PV) positive medium-sized neurons in the external plexiform layer (EPL) as well as a few nitric oxide synthase (NOS) positive PG cells and calretinin (CR) positive granule cells are anaxonic but

display dendritic hot spots with characteristics of axon initial segments (AISs). 3) Some of so-called “short-axon cells (SA cells)” project to the higher olfactory related regions and thus should be regarded as “nonprincipal projection neurons”. 4) Large tyrosine hydroxylase (TH) positive neurons, which had been regarded as external tufted cells but later revealed to be GABAergic (DA-GABAergic), are a particular type of juxtaglomerular neurons (JG neurons) as a main source of the interglomerular connection, forming an intrabulbar association system. Here we had better to pay attention to the term “juxtaglomerular neuron (JG neuron).” To our knowledge the term “JG neuron” appeared to be first used in relation with TH positive neurons around glomeruli without any clear definition; “TH staining in the juxtaglomerular neurons” (Baker et al. 1983). Later McLean and Shipley (1988) described as follows; “TOH (tyrosine hydroxylase) is most prevalent in periglomerular and external tufted neurons located around glomeruli in the main olfactory bulb. Collectively these cells are named juxtaglomerular cells.” Thus the initial proposal appears to indicate JG neurons are PG cells and external tufted cells. However, it is well known that there is another type of neurons around glomeruli, superficial short-axon cell (SSA cell). Then JG neurons are extended to those SSA cells. Thus the term “JG neuron” simply indicates neurons located around glomeruli irrespective of neuron types, including PG cells, external tufted cells and SSA cells. Thus the term “JG neurons” is usually used when the neuron types are not distinguished or un-identified or controversial.

1) Axon-bearing and anaxonic PG cells

In the Cajal’s textbook (Ramón y Cajal, 1995) PG cells, or outer granule cells, are described as follows: “Their perikarya give rise to one or more thin dendrites that arborize within the depths or near the periphery of the glomeruli. In addition, they give

rise to a very thin axon that courses more or less tangentially between glomeruli and then ends by ramifying within one of them.” Pinching and Powell (1971) also confirmed the structural features of PG cells, but added some comments on the presence or absence of axons: “The axons of the periglomerular cells are not always evident in Golgi-stained material, even after repeated impregnations, a fact also noted by Blanes, who considered this to be impregnation defect. In view of the difficulty of staining these cells at all, and indeed of impregnating the axons of any of the cell types of the glomerular layer, such an explanation would seem fair; however, the absence of an axon in the granule cells, to which the periglomerular cells are in many ways analogous, suggests the possibility that *some* periglomerular cells may be entirely lacking in an axonal process.”

Logically it is very difficult to prove the absence of any structures; we cannot make an exception of axons of PG cells. However, several recent observations as well as classical Golgi examinations on the PG cells appear to support the Pinching and Powell’s notion described above, “*some* periglomerular cells may be entirely lacking in an axonal process.”

Generally the initial portion of axon arising from the soma or dendrite is called as “axon initial segment (AIS)”, which is considered as the spike initiation site and characterized by some particular molecular organizations as well as particular structural and physiological features (Rasband, 2010). The characteristic chemical markers of AISs include sodium channel clusters, ankyrinG, β IV-spectrin and phospho-IkBa.

Our immunocytochemical studies revealed that apparent projection neurons such as mitral cells (Fig. 1) and tufted cells (Fig. 2) and large so-called superficial and deep SA cells (SSA and DSA cells; PV positive SA cells shown in Fig. 9 of Kosaka and

Kosaka, 2008a, calbindin (CB) positive SA cells shown in Fig. 3 of this article and also in Figs. 2 and 12 of Kosaka and Kosaka, 2010, NOS positive SA cells shown in Fig. 5 of Kosaka et al., 2008) have apparent axons positive for these AIS markers. Among chemically defined JG neurons CB positive SSA cells (Fig. 3), PV positive SSA cells, large TH positive GABAergic (DA-GABAergic) JG neurons (Fig. 5; see also Fig. 5 of Kosaka et al., 2008), NOS positive SSA cells and NOS positive external tufted cells also displayed the AIS chemical markers on the proximal portion of their thin slender processes arising from the somata or dendrites, indicating that these neurons have apparent axons. We also examined the structural features of immunolabeled JG neurons by means of conventional light microscope equipped with a camera lucida apparatus and confocal laser scanning light microscope (CLSM). In our detailed examinations at high magnifications on numerous JG neurons immunolabeled for CB and NOS, we occasionally encountered PG cells with a thin axon-like process, as shown in some figures of our previous papers (Fig. 2 of Kosaka and Kosaka, 2007; Fig. 3 of Kosaka and Kosaka, 2010). However, so far examined none of those thin axon-like processes arising from PG cells showed the chemical markers for AISs (Fig. 5C of Kosaka et al., 2008), although NOS positive PG cells showed the AIS markers on their dendritic segments as described later. Thus so far we encountered no apparent AIS marker positive axon-like thin slender processes arising from small presumed PG cells of various chemical types characterized by immunoreactivity for CB (Fig. 3), CR (Fig. 3), or NOS (Fig. 5 of Kosaka et al. 2008). These observations suggest that some PG cells have no typical axons.

Recently JG neurons in slice preparations were labeled intracellularly with biocytin or fluorescent dyes (McQuiston and Katz, 2001; Zhou et al, 2006; Gire and Schoppa, 2009); some cells identified as PG cells displayed axons whereas others did not.

Particularly Zhou et al. (2006) mentioned that “Some PG cells showed no axons. It is unlikely that these were always cut during slicing because those cut axons usually displayed enlarged terminals ...” In addition, although not mentioned to the axons, some figures in those papers did not display apparent axons (Fig. 6 of Gire and Schoppa, 2009; Fig. 1 of Shao et al., 2009; Fig. 5 of Kiyokage et al., 2010). Structural features of individual PG cells were also revealed in transplantation studies of green fluorescent protein (GFP) labeled neurons or labeling the SVZ progenitor cells with virus vectors (De Marchis et al., 2007; Merkle et al. 2007; Mizrahi, 2007; Whitman and Greer, 2007). In most of these reports the authors mentioned to dendritic processes but not to axonal processes, presumably due to paying no attention to the differentiation between dendritic and axonal processes, but some of adult-generated PG cells shown in these reports appear to have no axons.

At present it might be most plausible that there are both axon-bearing PG cells and anaxonic PG cells. Structural features of axon-bearing PG cells such as shown in Figs. 414-416 of Cajal’s textbook led Cajal to think that “the small intra- and periglomerular cells (Kolliker’s outer granule cells) constitute association neurons between glomeruli”. Thus, PG cell axons have been proposed to be the main route of the interglomerular connections (Shepherd et al., 2004). However, recent physiological analyses indicated the importance of the intraglomerular self-inhibition rather than interglomerular lateral inhibition as a mechanism of odor signal filtering (Murphy et al., 2005; Gire and Schoppa, 2009). Taking these physiological analyses and proposals into consideration, there might be at least two functionally different groups of PG cells, one participating in the interglomerular lateral inhibition (see section 4 of this article) and the other in the intraglomerular self-inhibition or self-modulation. The former might be PG cells with axons extending to distant glomeruli and the latter might be PG cells

with no or, if present, short axons extending only near their somata. Although this notion might be oversimplified and there should be intermediate types of PG cells, the heterogeneity of PG cells in the structural, chemical and physiological properties must be important in future analyses of the microcircuit of the MOB.

2) Dendritic hot spots

As described above, the AIS, the spike initiation site, is characterized by particular morphological and chemical properties. However, our recent observations revealed that some neurons in the MOB were anaxonic but instead displayed some particular patch-like portions with AIS-characteristics, “hot spots”, on their dendritic processes (Kosaka and Kosaka, 2008b; Kosaka et al. 2008). Various types of interneurons were described in the EPL, such as Van Gehuchten cells, satellite cells, pyriform cells, multipolar type etc., but they might not be different group of neurons but rather comprise a single morphological continuum (Kosaka and Kosaka, 2008a); at present these neurons might be named tentatively “anaxonic multipolar EPL neurons”. Most PV positive medium-sized anaxonic multipolar EPL neurons expressed 2-7 hot spots, that is, patch-like β IV-spectrin and sodium channel cluster positive segments, on their dendritic branches (Fig. 4). Electron microscopic observations revealed that those dendritic hot spots displayed the membrane undercoating characteristic to the AISs (Kosaka et al. 2008). Importantly some other neurons in the MOB also displayed similar “hot spots” on their dendritic processes: these were some NOS positive PG cells (Fig. 5A-D of Kosaka et al. 2008) and a few CR positive granule cells (Fig. 5F of Kosaka et al. 2008). It is noteworthy that so far examined the vast majority of PV positive medium-sized anaxonic multipolar EPL neurons displayed such dendritic “hot spots”, but among NOS positive PG cells and CR positive granule cells, only a small proportion

of those neuron groups displayed dendritic hot spots. In addition, so far examined, we encountered neither CB positive PG cells nor CR positive PG cells displaying such dendritic hot spots, both of which appeared to be also anaxonic (Fig. 3). Our study suggested that these morphologically identified dendritic “hot spots” might correspond to dendritic spike generation sites of those neurons, which remains to be confirmed by physiological examinations.

3) Short axon (SA) cells

There are several types of so-called SA cells in the MOB, which are classified based on their position of somata (SSA or DSA cells), structural features revealed mainly with classical Golgi impregnation methods (Blanes cell, Golgi cell, Cajal cell etc.) and chemical properties (neuroactive substances and/or their synthesizing enzymes, calcium binding proteins, channels and receptors). Several recent physiological and morphological analyses revealed that those SA cells, particularly DSA cells, made synaptic contacts selectively on GABAergic local circuit neurons (Gracia-Llanes et al., 2003; Pressler and Strowbridge, 2006; Eyre et al., 2008), confirming and expanding the proposal of Schneider and Macrides (1978) that SA cells “could play a prominent role in the control of bulbar output activity, possibly by regulation of the inhibitory influences exerted upon output neurons.” However, recent studies started to reveal possible additional role of some so-called SA cells. In our previous study on the NOS positive neurons in the mouse MOB we revealed that some NOS positive neurons with interneuronal structural features projected to the higher olfactory areas (Fig. 6 of this article; Kosaka and Kosaka, 2007b). Projecting “SA cells or interneurons” in the MOB were also confirmed recently by Eyre et al. (2008), combining the retrograde tracer and intracellular labeling experiments. Furthermore we also revealed some CB positive “SA

cells” in the internal plexiform layer (IPL) extended axons into the lateral olfactory tract (Kosaka and Kosaka, 2010). In addition we frequently encountered cells located in the IPL and granule cell layer (GCL) retrogradely labeled when tracers were injected into higher olfactory related regions (Fig. 6); they did not necessarily express any of chemical markers tested such as CB, NOS, PV or CR. Thus there might be various types of projecting cells other than principal projection neurons in the MOB, that is, mitral and tufted cells. It is well known that in addition to glutamatergic principal projection neurons such as pyramidal cells there are other nonprincipal presumed GABAergic projection neurons in various brain regions (e.g. hippocamposeptal GABAergic neurons). Those projecting “SA cells” in the MOB are another example and should be regarded as “nonprincipal projection (NPP) neurons” rather than traditional “SA cells.”

4) Large TH positive GABAergic (DA-GABAergic) JG neurons

Regarding the DA-GABAergic JG neurons we must discuss further to some extent here. It is well known that there are two types of TH-positive JG neurons with different soma sizes (Halász et al., 1981; Davis and Macrides, 1983; Kosaka and Kosaka, 2007a, 2008b). The larger type of TH-positive JG neurons had been regarded as dopaminergic external tufted cells, whereas the smaller type of TH-positive JG neurons as PG cells (Halász et al., 1981; Davis and Macrides, 1983). Davis and Macrides (1983) described as follows: “Based on their somal sizes and the association of their dendrites with glomeruli, most of the larger THLI (TH-like immunoreactive) neurons in the glomerular layer can be identified as external tufted cells, whereas the smaller and less common THLI neurons can be classified as periglomerular cells.....” However, the larger type of TH positive JG neurons as well as the smaller type of TH positive JG neurons were revealed to be also GABAergic (Kosaka et al., 1985, 1987; Kosaka and

Kosaka, 2007a), and thus both types of TH positive JG neurons have been regarded as “DA-GABAergic PG cells” rather than external tufted cells and PG cells.

We recently revealed that DA-GABAergic JG neurons are the main source of the interglomerular connections in the mouse MOB (Kosaka and Kosaka, 2008b). We showed that those DA-GABAergic JG neurons with the long interglomerular connection are large in soma size and extend at least some dendrites into glomeruli (Figs. 5, 7-9). Fig. 8 shows both TH positive somata and TH positive puncta labeled with cholera toxin B subunit (CTB), retro- and anterograde tracer, injected into the glomerular layer (GL) several 100 μ m distant from the region shown here. Retrogradely CTB-labeled TH positive somata extended their dendrites into glomeruli as well as around glomeruli, indicating that they are PG cells (see below). Anterogradely CTB-labeled TH positive puncta in glomeruli (mainly in the ON zone of two glomerular compartments) indicated that those large DA-GABAergic JG neurons near the injection site extended their axons into glomeruli located distantly.

On the other hand DA-GABAergic JG neurons are known to be one of the major groups of adult generated JG neurons (De Marchis et al., 2007; Merkle et al., 2007; Whitman and Greer, 2007). Then we examined the time of origin of DA-GABAergic JG neurons using thymidine analog 5-bromo-2'-deoxyuridine (BrdU), and showed that the time of origin of these large DA-GABAergic JG neurons is mainly pre- and perinatal but none of them are born in adult periods, whereas the small type of DA-GABAergic JG neurons are born even in adult periods (Kosaka and Kosaka, 2009b). Thus those large DA-GABAergic JG neurons participating in the interglomerular connection are different from the adult-generated DA-GABAergic JG neurons, which are the small type of DA-GABAergic JG neurons.

Recently Kiyokage et al. (2010) reported physiological and structural properties

of DA-GABAergic JG neurons and confirmed our conclusion on the interglomerular connections of DA-GABAergic JG neurons. Their confirmation also indicates, although not mentioned, that they corrected the mis-conclusion of the previous report of the same group on the “non-GABAergic excitatory SSA cells” as the main source of the intraglomerular connection (Aungst et al. 2003). However, Kiyokage et al. (2010) proposed that these DA-GABAergic JG neurons are “SA cells” rather than “PG cells”, for they extended their processes to several to many glomeruli. They described that “PG cells are generally depicted with dendrites ramifying within a single glomerulus, although the original descriptions of Kölliker (1896) and Blanes (1898) reported that dendrites can enter several glomeruli. TH neurons have traditionally been regarded as PG cells expressing dopamine and GABA. However, the present findings demonstrate that DAergic-GABAergic cells have a distinct morphotype, contacting multiple glomeruli. This does not fit the classical PG cell morphology, but rather, the morphology of SA cells (Pinching and Powell, 1971). our data indicate DAergic neurons are better considered short axon than periglomerular.” However, their DA-GABAergic “short-axon” cells extended presumed dendrites into several glomeruli. Thus the problem here might be the difference between the traditional definitions and those of Shipley’s group (Aungst et al. 2003; Kiyokage et al., 2010) of the PG cells and SA cells. In their classical paper, Pinching and Powell (1971) summarized as follows; “The superficial short-axon cells are characterized by the entirely periglomerular distribution of their dendrites, which are varicose and rarely branch.” Thus, in contrast to the notion described above by Kiyokage et al. (2010), JG neurons with intraglomerular dendrites (other than tufted cells) are traditionally considered as PG cells rather than SA cells irrespective of their mono- or oligoglomerular dendritic branchings, whereas SA cells are generally considered to have no (or few) intraglomerular dendritic branches.

Furthermore, in their paper the authors differentiated those DA-GABAergic “short-axon cells” into oligo- and polyglomerular types without differentiating processes into dendrites and axons. We showed at least large DA-GABAergic JG neurons have apparent axons with distinctive AISs (Fig. 5). Judging from the published figures of polyglomerular type neurons (Fig. 6 of Kiyokage et al., 2010), most parts of long projecting processes of those neurons appear to be axonal processes, whereas other possible dendrites appear to branch in several glomeruli near their somata of origin. Strangely, such differentiations of processes into axons and dendrites have been displayed in Fig. 2 of the previous paper of the same group (Aungst et al. 2003): two “excitatory SA cells” shown in Fig. 2 g, h of Aungst et al. (2003) might be included into “polyglomerular type neurons” of Kiyokage et al. (2010). Then there might be no distinctive differences between oligo- and polyglomerular types in their dendritic arborization. Although interesting, the proposed scheme shown in Fig.1 of Kiyokage et al. (2010) and the schemes shown in Fig. 6 of Aungst et al. (2003) are hopefully revised correctly.

Here we add some particular features of DA-GABAergic neurons. First, a few DA-GABAergic cells with relatively large somata scattered in the EPL and extended some smooth dendritic processes into glomeruli. They were also frequently labeled retrogradely when tracer was injected into the GL (Fig. 7). Thus these TH positive neurons in the EPL, although not located in the JG region, resemble large DA-GABAergic JG neurons, indicating that they could be regarded as “displaced DA-GABAergic JG neurons”. Second, we encountered frequently large DA-GABAergic JG neurons extending axons which descended in the EPL to some extent (Figs. 5, 9), then branched and extended laterally in the EPL; some collaterals ascended toward the GL (Fig. 9). Taken together, those large DA-GABAergic JG neurons participating in the

interglomerular connections are unique in their structural features.

At present we think that DA-GABAergic JG neurons are heterogeneous, consisting of small and large types. The small type of DA-GABAergic JG neurons (e.g. cell shown in Fig. 4D, G, L of De Marchis et al., 2007, which was erroneously labeled as CB positive neuron but corrected later as TH positive neuron) might be “conventional PG cells” (although it remains to be determined whether they have axons or not). The large type of DA-GABAergic JG neurons extend dendrites into up to several glomeruli nearby their somata and axons into numerous glomeruli located not only near their somata but also far from their somata (Figs. 10, 11). These structural features of the large type of DA-GABAergic JG neurons indicate that they are most plausibly considered to be a particular type of PG cells rather than SA cells. However, we might better reserve final judgment on the type of these particular neurons, that is, whether DA-GABAergic JG neurons are “PG cells” or another new category of JG neurons. We need more detailed characterization of these particular JG neurons before we reach the conclusion of their cell type. Classical PG cells described in Cajal’s textbook are considered to “constitute association neurons between glomeruli (Ramón y Cajal, 1995).” Interestingly those “association neurons” might correspond well to large DA-GABAergic JG neurons described above.

As is well known, there is a prominent intrabulbar association system linking reciprocally the mirror-symmetric isofunctional odor columns (Schoenfeld et al., 1985; Lodovichi et al., 2003), in which some subpopulation of excitatory external tufted cells are the main source of reciprocal connections making synapses on the granule cells on the opposite side (Fig. 10). The observations on the large type of DA-GABAergic JG neurons indicate that there might be another intrabulbar association system connecting glomeruli mainly on the ipsilateral side, in which the large type of DA-GABAergic JG

neurons are the main source of the interglomerular connections (Fig. 10). Then we would like to name this large type of DA-GABAergic JG neurons tentatively as “inhibitory juxtaglomerular association neuron (IJGA neuron)”, and those external tufted cells participating in reciprocal connections between two mirror-symmetric isofunctional odor columns on both sides as “excitatory juxtaglomerular association neuron (EJGA neuron)”. These two different intrabulbar association systems (Fig. 10) might be important in the organization of the MOB.

Figure 11 summarizes the neuronal organization of the MOB which incorporates the new findings described in the present article, although many details of the whole organization are omitted from this scheme.

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Figure legends

Figure 1. Pseudo-colored confocal laser-scanning light microscope (CLSM) partial projection image of biotinylated dextran amine (BDA)-labeled mitral cells (red) and axon initial segments (AISs) immunostained for β IV-spectrin (green) in the mouse MOB. BDA was injected iontophoretically into the external plexiform layer (EPL). Arrows indicate β IV-spectrin positive AISs of BDA-labeled mitral cells. Arrowhead indicates initial part of the glomerular dendritic tuft of the mitral cell primary dendrite. Layers: glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), granule cell layer (GCL). Scale bar is 10 μ m.

Figure 2. Pseudo-colored CLSM partial projection images of BDA-labeled external tufted cell (red), AISs immunostained for β IV-spectrin (green) and medium-sized neurons in the EPL immunostained for parvalbumin (PV ; blue) in the mouse MOB. Arrow indicates the β IV-spectrin positive AIS arising from descending dendritic stem of this BDA-labeled external tufted cell. Scale bar is 10 μ m.

Figure 3. Pseudo-colored CLSM partial projection image triple-immunostained for calbindin (CB; red), β IV-spectrin (green) and calretinin (CR; blue) in the mouse MOB. This frame was also shown in Fig. 2 of Kosaka and Kosaka (2010) as a stereo pair of double-immunostained images for CB (red) and β IV-spectrin (green). Arrow indicates the AIS arising from the CB positive superficial short axon (SSA) cell. CB or CR positive PG cells as well as CB or CR positive EPL neurons do not show β IV-spectrin positive processes. Scale bar is 10 μ m.

Figure 4. Pseudo-colored CLSM partial projection image double-immunostained for parvalbumin (PV; red) and β IV-spectrin (green) in the mouse MOB. This figure was also shown in Fig. 3 of Kosaka et al. (2008) as one of examples of Neurolucida analyses, although green and red colors are reversed. Arrowheads indicate the β IV-spectrin positive patch-like dendritic segments of PV positive medium-sized neurons in the EPL. Scale bar is 10 μ m.

Figure 5. Pseudo-colored CLSM partial projection image triple-immunostained for tyrosine hydroxylase (TH; red), β IV-spectrin (green) and CB (blue) of the mouse MOB. Two TH positive cells display β IV-spectrin positive AISs (arrows) descending in the EPL. Scale bar is 10 μ m.

Figures 6. Example of retrograde tracer fluorogold (FG) injection experiment. This frame is the same one shown in Fig. 1D of Kosaka and Kosaka (2007b). The injection site encroached the regions adjacent to the piriform cortex, that is, deeply located dorsal endopiriform nucleus and insular cortex, but the piriform cortex itself was not labeled intensely. CLSM projection image of the mouse MOB ipsilateral to the injection side triple-immunostained for FG (green), nitric oxide synthase (NOS; red) and PV (blue). Arrows indicate FG-labeled somata in the internal plexiform layer (IPL), one of which is NOS positive and shown in Fig. 9F of Kosaka and Kosaka (2007b) at a higher magnification. Arrowheads indicate FG-labeled mitral cell somata and FG-labeled NOS positive external tufted cell somata. Scale bar is 100 μ m.

Figure 7. Pseudo-colored CLSM projection image double-immunostained for FG (green) and TH (red). Mouse MOB. Arrows indicate retrogradely FG-labeled TH positive neurons in the GL and EPL. Arrowhead indicates a FG-labeled TH negative cell in the

IPL. FG was injected iontophoretically into the GL. Scale bar is 100 μm .

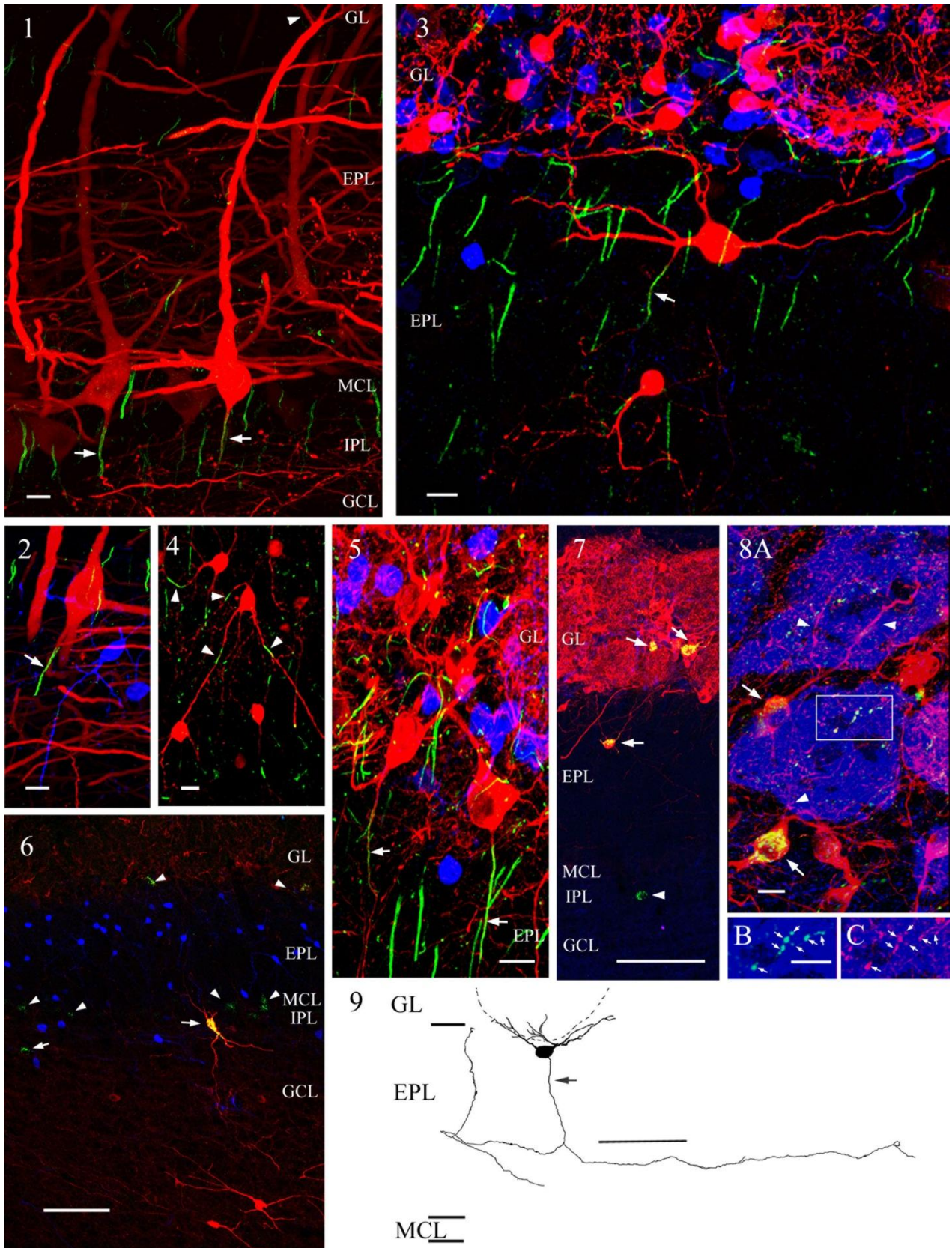
Figure 8. A: Pseudo-colored CLSM partial projection image triple-immunostained for TH (red), cholera toxin B subunit (CTB; green) and vesicular glutamate transporter 2 (VGluT2; blue), a marker for olfactory nerves and terminals. Mouse MOB. CTB is injected iontophoretically into the GL several 100 μm distant from the area shown here. Arrows indicate two CTB-labeled TH positive somata extending their dendrites into glomeruli (arrowheads). The area outlined by a rectangle is shown in **B** and **C** at a higher magnification displaying two channels. **B** and **C**: small arrows indicate intraglomerular CTB-labeled TH positive puncta. In **B** CTB (green) and VGluT2 (blue), and in **C** TH (red) and VGluT2 (blue) channels are shown, respectively. CTB-labeled TH positive puncta are mainly located in the VGluT2 positive ON zone. Scale bars are 10 μm .

Figure 9. Camera lucida drawing of a large TH positive juxtaglomerular neuron in the mouse MOB. Dendritic branches extending into a glomerulus (hatched line) as well as an axonal collateral extending to the GL could not be traced well due to intermingling with other TH positive processes. Arrow indicates the initial portion of the axon arising from the soma. Scale bar is 100 μm .

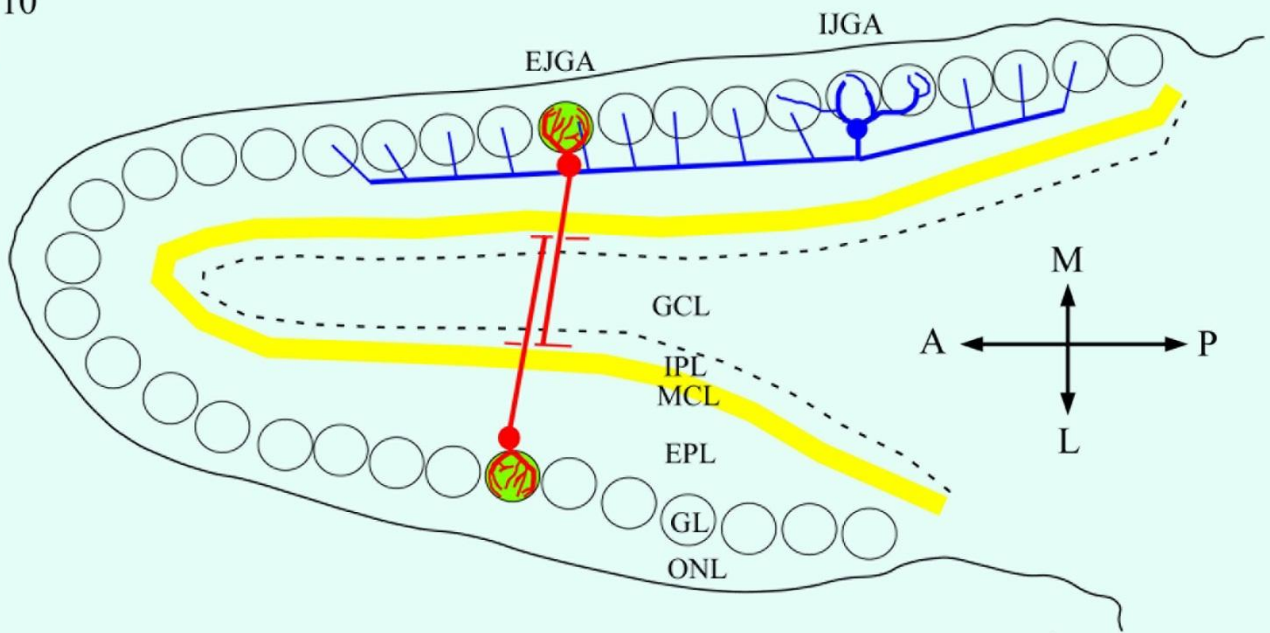
Figure 10. Scheme of the neuronal organization of rodent MOB showing two intrabulbar association systems. One is the intrabulbar association system linking reciprocally the mirror-symmetric isofunctional odor columns, excitatory juxtaglomerular association (EJGA) system, in which excitatory external tufted cells are the main source of reciprocal connections. Another is the intrabulbar association system connecting

glomeruli mainly on the ipsilateral side, inhibitory juxtaglomerular association (IJGA) system, in which the large type of DA-GABAergic JG neurons are the main source of the interglomerular connections. Layers: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), granule cell layer (GCL). Anterior (A)- posterior (P) and medial (M)- lateral (L) orientations are indicated.

Figure 11. Scheme of the neuronal organization of rodent MOB showing neuron types and their interaction. Red segments indicate AISs and dendritic hot spots. DSA; deep short axon cell. ET; external tufted cell. G; granule cell. IJGA; inhibitory juxtaglomerular association neuron (large DA-GABAergic juxtaglomerular neuron). LOT, lateral olfactory tract. M; mitral cell. NPP; nonprincipal projection neuron. PG; periglomerular cell, which includes both axon-bearing and anaxonic PG cells as well as type 1 and type 2 PG cells. PVAM; parvalbumin positive anaxonic multipolar EPL neuron. SSA; superficial short axon cell. T; tufted cell. Layers: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), granule cell layer (GCL).



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