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Review Article

Olfactory System of The Rat - Morphofunctional Organization

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Abstract

The olfactory system plays a key role in reproductive and maternal functions, neuroendocrine regulation, emotional responses, aggression, and recognition of congeners, predators, and prey. In addition, the sense of smell is critical in food selection because taste perception is formed through the integration of olfactory and gustatory cues. The influence of olfactory stimuli on animal memory and behavior has long been recognized, but the mechanisms of odor perception at the neural level are still poorly understood.

Kew Words: olfactory system; rat; neuron

Introduction

The olfactory system plays a key role in reproductive and maternal functions, neuroendocrine regulation, emotional responses, aggression, and recognition of congeners, predators, and prey. In addition, the sense of smell is critical in food selection because taste perception is formed through the integration of olfactory and gustatory cues. The influence of olfactory stimuli on animal memory and behavior has long been recognized, but the mechanisms of odor perception at the neural level are still poorly understood [1,5].

Odor molecules are transduced by olfactory receptor neurons (ORNs), primary neurons located in the olfactory epithelium within the nasal cavity. ORNs are both receptors that transform odors and primary neurons that transmit information to downstream neurons in the olfactory network. The axons of ORNs project into the olfactory nerve and synaptically terminate in the main olfactory bulb (GOL). The output neurons of the GOL, the mitral and bundle cells, transmit olfactory information to higher olfactory structures and other brain systems. Transmission from the nose to the mitral and bundle cells is strictly regulated by a local intraventricular circuit and centrifugal inputs to the GOL from other parts of the brain. Higher-order olfactory structures targeted by mitral and bundle cells include, from anterior to posterior, the olfactory trunk (anterior olfactory nucleus), piriform cortex, olfactory tubercle, entorhinal cortex, and some amygdala nuclei. From these primary olfactory cortical structures further form connections to brain regions that integrate olfactory information with other neural functions [2-4].

This sketch of the olfactory circuit may seem relatively simple, so it may give the impression that our understanding of the functional organization of the olfactory circuit is comparable to that of other sensory systems. However, this is not the case. Indeed, there are a number of critical gaps in our knowledge of olfaction that have prevented the kind of integrative analysis of structure and function that has led to advances in the visual, somatosensory, and auditory systems. Foremost among these gaps is our limited understanding of the nature of the "olfactory code," that is, the "dimensions" of olfactory stimuli that are extracted and processed by the olfactory system [5-7].

You raise important questions about the complexity of the olfactory system. At this point, scientists really haven't determined whether there are finite "classes" of odors, comparable to primary colors, that are recognized by different subfamilies of receptors. The selectivity of each of these receptors to different molecules varies, and the exact degree of this selectivity is still being actively studied [2].

Some olfactory receptor neurons (ORNs) may be involved in detecting the general properties of odors, similar to how the rods in the retina respond to the brightness of light. Other receptor neurons may be more specialized to classes of odors, similar to the way cones in the retina are sensitive to the wavelength of light.

Studies of olfactory codes and specificity have only been done for a limited number of molecules and receptors, leaving many questions open. This is one reason why the understanding of the olfactory system lags behind that of other sensory systems such as vision, touch, and hearing. Progress in this area requires further research and integration of data on various aspects of olfaction [10].

It is not known whether there are finite "classes" of odors, comparable to primary colors, that are recognized by different receptor subfamilies. How selective are each of these receptors to different molecules? Are some olfactory receptor neurons (ORNs) concerned with general properties of odors (analogous to luminance for sticks in the retina), while other receptor neurons are more specialized to classes of odors, just as cones are wavelength selective? In recent years, the issue of olfactory codes/specificity has only been addressed for a small number of molecules/receptors [2].

Another major gap in our understanding is how the brain analyzes the complex activity patterns triggered by a particular odor (e.g., the smell of coffee contains hundreds of different volatile and non-volatile components). At the cellular level, we are beginning to understand how neurons in the

olfactory bulb (MOB) respond to incoming signals and how some of the circuits at the bulb level function. It remains unclear how the bulb integrates combined odor mixtures and how bulb output is processed in higher cortical areas to elicit odor perception [11]. This chapter reviews the neurohistology, connections, and chemical anatomy of the central olfactory system. Despite significant progress in understanding olfactory receptor neurons (ORNs) and the olfactory epithelium, this topic has been the subject of numerous reviews. Therefore, a detailed discussion of these findings beyond the review presented below is not as important to a book that serves as a companion to a stereotaxic atlas as a review of central olfactory structures. Therefore, this chapter will focus on the current understanding of the organization of the main olfactory bulb (MOB) and higher olfactory centers [4].

Olfactory epithelium Organization

The sense of smell is accomplished by stimulation of olfactory receptor neurons (ORNs) by volatile chemicals. ORNs are contained in the neuroepithelial layer, which is located in the upper part of the nasal cavity along the upper part of the nasal septum, the region of the lattice plate and lining the endo- and ectoturbinate surfaces in rats, and the medial wall of the upper shell in humans [6].

In several species, a small area of olfactory neuroepithelium, the organ of Maser, is present on the ventral wall of the septum, although the function of this area of epithelium is unknown. Afferent information from ORNs is transmitted to the olfactory bulbs via the olfactory nerve, the first cranial nerve. To stimulate olfactory receptors, volatile molecules must enter the nasal cavity where they are exposed to relatively turbulent air currents. Duration, volume, and rate of inhalation are important determinants of the effectiveness of odor stimulation. Although these parameters vary considerably among individuals, they are fairly constant for each individual. Once volatile substances reach the olfactory epithelium, they must pass through the layer of mucus covering the olfactory epithelium. Thus, the relative distribution of odor between air and mucus also determines the stimulatory effectiveness of odor. Odor-binding proteins present in mucus may serve the function of binding odors and presenting them to receptors. Alternatively, odor-binding proteins may be required to remove odors from receptors and/or chemically inactivate them [12].

The olfactory neuroepithelium is a pseudostratified columnar epithelium that is thicker than the surrounding respiratory epithelium of the nasal cavity. This epithelium lies on a highly vascularized intrinsic lamina. Within the epithelium are bipolar olfactory receptor neurons (ORNs), supporting cells (sustentacular cells), microvillous cells, and basal cells. Bowman's glands are located in the underlying intrinsic lamina and extend into the nasal cavity. ORNs are true sensory neurons with a dendrite and axon. Their cell bodies are located in the basal two-thirds of the epithelium, with apical dendrites reaching the surface of the epithelium. The peripheral tip of this dendrite is slightly enlarged, forming an olfactory tubercle from which six to eight cilia extend into the mucosal layer [13].

Although human cilia do not appear to be mobile, in some vertebrate species, cilia length and mobility are related to age and receptor development. Cilia are the site of chemosensory transduction. An unmyelinated axon arises at the base of the ORN cell body and connects to a small bundle of other ORN axons. These axons pass through the basal lamina, where the bundles are enveloped by specialized Schwann cells, the sheath cells. These bundles subsequently combine to from 15 to 20 larger axon bundles (fila olfactoria) of the olfactory nerve, which pass through the lamina and synapse in the olfactory bulb [14].

The supporting cells of the olfactory epithelium separate and partially envelop the ORN. The apical surface of these cells in humans and some other vertebrates is covered with microvilli that protrude into the mucosal layer. These cells are postulated to regulate serum elements of mucus composition and express molecules of the P450 enzyme system, thereby suggesting a role in detoxification. A third cell type, microvillous cells, is present in humans, rodents, amphibians, and fish at approximately ten times the number of olfactory receptor neurons (ORN). These cells possess microvilli on the apical surface, which protrude into the mucus layer. The basal end of the cell tapers into a cytoplasmic extension that enters the lamina of the cell. In rats, retrograde tracing from the olfactory bulb indicates that this continuation can project to the olfactory bulb (Rowley et al., 1989). The ultrastructural appearance of microvillous neurons, in conjunction with their projection to the olfactory bulb, indicates that these cells represent a class of bipolar neurons. The supporting and microvillous cells are located deeper relative to the ORN. These cells are the globose and horizontal basal cells, which are located on the basal membrane just above the intrinsic lamina. These basal cells serve as stem cells to replace ORNs, which in mice have a life span of approximately 40 days. The intrinsic lamina contains Bowman's secretory glands, which provide the serum component of the mucous layer covering the olfactory epithelium [15].

Odor recognition and signal transduction.

Electrical signals are generated in olfactory receptor neurons (ORNs) when odorants penetrate the mucus layer and bind to specific membrane receptors located on ORN cilia. Traditionally, the existence of odorant receptors (ORs) as separate, specific molecules was largely a theoretical construct. However, in 1991, Buck and Axel discovered a large, multigene family of odorant receptor genes (ORGs). The ORG family consists of approximately 1000 genes that are expressed by ORNs. Our current understanding is that each mammalian ORN expresses only one unique ORG (a group of ORNs expressing the same ORG can be called an ORN-ORG cohort), although recent exciting data suggest that a subset of ORNs are capable of expressing two distinct ORGs. ORNs expressing the same odorant receptor gene are widely distributed in one of four broad zones of expression in the epithelium. However, there are also examples of cohorts of ORN-ORGs that are clustered in distinct regions rather than zones. These results indicate that there may be additional structuring for ORN-ORG subsets beyond the four major expression zones [17].

Since the cloning and characterization of the ORG family, our understanding of the molecular biology of olfactory transduction has rapidly expanded. These odorant receptors are members of a superfamily of receptor genes with seven transmembrane domains that also includes a family of receptors highly homologous to ORGs isolated from testis, metabotropic glutamate receptors (mGluR), transforming growth factor receptors, and many other large gene families. In the mammalian genome, approximately 1000 individual genes encode the ORG family. Amazingly, of the 30-35,000 genes present in the mammalian genome, a full 1,000 genes (about 3% of the genome) encode odor receptors! In humans, about two-thirds of these ORGs have become pseudogenes; genes that have undergone evolutionary mutations that insert stop codons into the reading frame of the gene, rendering them inoperable. It has been suggested that heterogeneity in the human population and high levels of ORG pseudogenes may underlie some cases of specific anosmia [11-17].Despite recent successes in the genome project to identify ORG sequences, there are still many unanswered questions about the molecular specificity of odorant receptors. Several attempts have been made to create three-dimensional models to investigate how putative ligands can "fit" into putative binding pockets on ORGs. More conclusive evidence for receptor specificity has been provided by heterologous expression of ORGs combined with measurements of physiological responses. The most fully characterized ORG to date is the I7 receptor, which has a high affinity for octanol but responds weaker to bound molecules. The current model of OR specificity suggests that each OR is "tuned" to recognize a particular chemical group that may be present in a number of different odor molecules. The tuning of ORN responses appears to be quite broad. Receptors that respond strongly to one group may also respond to other groups when the concentration of the odorant is increased. It is clear from these initial studies that a Herculean effort may be required to determine receptor-ligand binding specificity for all 1000 ORGs [18].

Parallel advances have also been made in understanding the transduction events that occur between the binding of flavorants to ORs and the generation of action potentials in ORNs. Several lines of evidence suggest that the "cAMP" signal transduction pathway predominates in the

mammalian ORN. Binding of flavorant to the OR leads to activation of the G-protein Golf, which activates the adenylate cyclase type III (ACIII) molecule, resulting in an increase in cyclic nucleotides, opening of cyclic nucleotide-regulated olfactory channels (CNGA2/OCNC1), and calcium entry into cilia. Increased intracellular calcium triggers Na+ and Cl- conductance, leading to the generation of action potentials that propagate along the axon to the MOB. Genetic null mutations for Golf, CNGA2/OCNC1 and ACIII firmly establish the essential role of these molecules. Mouse null mutants for any of these three transduction elements are functionally anosmic [19]. In other species, especially invertebrates, a signal transduction pathway via IP3 is present. In this cascade reaction, binding of the flavoring agent to OR triggers interaction with G-proteins, which then activate phospholipase C (PLC), resulting in an increase in phosphatidyl inositol (IP3) levels. The secondary mediator IP3 also opens channels leading to calcium influx into cilia and initiation of action potentials in the ORN. However, in mammals, the necessity of ACIII for olfactory function suggests that the signal transduction pathway via IP3 is not a primary cascade reaction. It remains to be tested whether IP3 transduction in mammals is a modulator of the cAMP pathway or mediates the inhibitory responses to odorants reported in the ORN [20]. ORNs and axons also express high levels of olfactory marker protein (OMP), which is unique to ORNs. OMP is found in many mammalian species, including humans, and appears to be expressed in all ORNs, accounting for about 1% of the total protein content in these neurons. The role of OMP in ORN function is poorly understood; however, recent data from studies in mice with an OMP null mutation suggest that this protein may play a role in ORN adaptation to odors [21].Nevertheless, this exciting progress in understanding the fundamental molecular interactions at the level of the olfactory receptor will not lead to a complete understanding of olfaction, just as uncovering the mechanisms of retinal receptor transduction has not fully elucidated the neural mechanisms underlying vision perception. The ORN is only the first element in a complex neural network involving the MOB and higher cortical areas that leads to odor perception. The functioning of central olfactory networks and the anatomical organization of the olfactory system appear in some respects to be fundamentally different from the familiar topographically organized circuits of other major sensory systems. The remainder of this chapter focuses on the anatomical and neurochemical organization of these brain regions [23].

Basic olfactory projection

In general, each individual point on the surface of the MOB receives innervation from ORNs widely distributed in the olfactory neuroepithelium, and conversely, ORNs projecting to a point on the MOB are interspersed with other neurons projecting to another point. However, there is a general tendency for neurons located in the medial, lateral, dorsal, and ventral parts of the olfactory neuroepithelium to project to homologous bulb surfaces. Detailed analysis of the primary projection in hamsters shows that patterning in the olfactory neuroepithelium occurs as a series of mediolateral, dorsoventral zones, regardless of the presence of nasal shells. These epithelial "domains" broadly coincide with the four ORG expression domains. ORN subpopulations were also identified by expression of cytoplasmic and membrane antigens. Most of these subpopulations of ORN axons are "sorted" and terminate at broad regions of the MOB in templates conserved between animals [15].

In contrast to this diffuse topographic organization, there is a much more precise receptotopic organization between the MOE and the main olfactory bulb: ORNs expressing the same ORG appear to project to the same point on the surface of the MOB. In situ hybridization for odorant receptor mRNA has shown that axons containing mRNA for a particular receptor all terminate in one or more glomeruli. More recently, transgenic mice have been generated in which ORNs expressing a particular ORG coordinately express β-galactosidase or green fluorescent protein. In these mice, ORNs express the same ORG and project to one or two (or more) glomeruli. Thus, it is hypothesized that specific cohorts of ORN-ORGs innervate each glomerulus in the MOB, and each glomerulus receives homogeneous input from ORNs expressing the same ORG. Thus, glomeruli represent a spatial map of ORN activity in response to odor stimuli, a conclusion that has been suggested by various experimental approaches over several decades. These functional studies involving 2-deoxyglucose, c-fos, functional magnetic resonance imaging, calcium imaging, and internal imaging all suggest that glomeruli represent functional units. Thus, the neural computational challenge in odor identification becomes not how the brain recognizes odors, but how the brain recognizes patterns of glomerular activity elicited by different odors [10-17].

The central olfactory bulb

Because the brain's representation of odors is reduced to a neural computational problem, knowledge of the anatomy and physiology of central olfactory circuits is essential to understanding odor coding. The organization and development of olfactory circuits share common features with other neural systems, especially the cerebral cortex. An improved understanding of the neural operations performed by olfactory circuits and the properties of olfactory network function that are preserved in later evolutionarily evolved cortical structures may provide fundamental insights into the underlying computational features that contributed to the selective expansion of cortical structures during the evolution of the mammalian brain [4].

Laminar organization

The main olfactory bulb (MOB) is an allocortex that, like other cortical structures, has a characteristic laminar organization [1].

Olfactory nerve layer

The most superficial layer of the main olfactory bulb is the olfactory nerve layer (ONL). The ONL contains thin neomyelinated axons of olfactory receptor neurons (ORNs) and glial cells. These glial cells can originate from either the peripheral or central nervous system. A specialized glial cell called the "envelope cell" is present in the ONL, which provides an incomplete envelope that separates the ORN axons and glomeruli from the periglomerular region. The deepest third of the ONL contains astrocytes that express QPRT, a degrading enzyme for quinolinic acid, which is an agonist of the NMDA receptor. Quinolinic acid, like glutamate, can modulate glutamatergic transmission between ORN axon terminals and postsynaptic targets [3-10].

Glomerulus Layer (Gl)

Immediately beneath the olfactory nerve layer (ONL) is the glomerulus layer. The GL is one of the most distinctive structures in the brain. The glomeruli consist of neuropil-rich spheroidal structures surrounded by a characteristic sheath of small neurons and glial cells. Glomeruli are usually oval in shape and range in diameter from 80 to 160 micrometers in rats. Most estimates of the number of glomeruli are similar, about 2000-3000 glomeruli/bulb in rabbits and about 1800-2000 in mice. The number of glomeruli in rats is estimated at 3000. However, according to recent reassessments using stereological methods without distribution, it is suggested that rabbits may have up to 6300 glomeruli and rats up to 4200. It has also been estimated that a total of several million ORNs project to the olfactory glomeruli. Thus, several thousand ORN axons converge in each glomerulus, which is the initial site of synaptic integration in the olfactory system [4-6]. The glomerulus nucleus consists almost entirely of neuropil and is surrounded by a thin sheath of neuronal bodies and astrocytes. Astrocytes in the glomerulus sheath have a high degree of morphological specialization. The predominant type of astrocytes (called "wedge-shaped" astrocytes) has its body located in the glomerulus sheath and sends several thick, branched processes into the glomerular nucleus. Remarkably, the processes of these astrocytes are completely confined to a single glomerulus. The astrocytes seem to wall off neighboring glomeruli, reinforcing the long-standing idea that each glomerulus is a separate functional unit [19-21].

The neurons of the glomerular envelope are called juxtaglomerular neurons. Most juxtaglomerular neurons can be

classified as one of three types: small, periglomerular cells; slightly larger external bundle cells; and cells with a short axon. Dendrites of small (5-8 μm) periglomerular cells can enter more than one glomerulus, but usually favor a single glomerulus. These dendrites rarely fill the entire glomerular nucleus, instead branching in a distinct subregion of the glomerulus. The dendrites of periglomerular cells usually have many spike-like outgrowths, which are the main morphologic feature that distinguishes them from the small external bundle cells. Short axon cells are slightly larger than periglomerular cells (8-12 μm) and differ in Golgi material because their dendrites are mostly confined to the periglomerular envelope. Axons of periglomerular cells and cells with short axons usually run along the periphery of two to four glomeruli. The external bundle cells, measuring 10 to 15 μm in long axis, are located in the periglomerular region and usually have a single apical dendrite that branches within a single glomerulus, although some external bundle cells have two or three apical dendrites capable of branching into several different glomeruli [30].

The apical branching of the external bundle cell occupies a larger portion of the glomerulus than the apical bundles of other classes of bundle cells described below or mitral cells (unpublished observations). The apical dendrites of external bundle cells are varicose but rarely form spines. In addition to their thick apical dendrites, many external bundle cells also have thinner secondary or lateral dendrites that extend in the external plexiform layer, just beneath the glomerular layer, although these are less numerous than the lateral dendrites of mitral cells. Some external bundle cells have axons that project to nearby glomeruli; these axons do not appear to enter the glomeruli but terminate between the glomeruli, whereas other external bundle cells project an axon beyond the olfactory bulb. The presence of the apical bundle, lateral dendrites, and basal axon on external bundle cells is generally similar to the dendritic organization of superficial, middle, and deep bundle cells, as well as mitral cells. All of these types of bundle cells are often grouped with mitral cells when the output cells of the bulb are discussed; however, there are significant structural and physiological differences between these classes of cells that suggest different roles in processing olfactory information [30-35].

Other neural components are present in the glomeruli. These include dendrites from deeper fascicular and mitral cells, as well as axons from central centrifugal sources. Olfactory axons also synapse tightly on dendrites of mitral and bundle cells. Mitral/bunch cell dendrites and periglomerular cells also have other synaptic connections within the glomeruli. Reconstructions of electron microscope (EM) sections showed that mitral/beam cell dendrites make reciprocal synaptic contacts with dendrites and gemmules (spiking processes) of periglomerular cells. These specialized reciprocal synapses are often closely associated with each other [29-30].

Glomeruli have traditionally been viewed as relatively homogeneous structures composed of axons, dendrites, synapses, and glial processes. However, recent studies show that glomeruli exhibit a degree of subcompartmentalization that manifests itself as segregation of different types of synaptic contacts. ORN axons entering the glomerulus synapse onto target dendrites in subcompartments or islets within the glomerular neuropil, whereas dendrodendritic synapses occur in bundles of 4-100 dendrites that are separated from ORN axon islets by glia processes. In the last decade, several neurotransmitter/neuromodulators have been identified in different classes of juxtaglomerular neurons. "Synaptology" of dopaminergic neurons contrasts with juxtaglomerular neurons expressing calbindin D-28K, which receive almost no synapses from ORN axons but instead form dendrodendritic contacts with putative mitral/fascicular cell dendrites [10-21].

From these studies, it is clear that populations of juxtaglomerular neurons may differ substantially in their synaptic organization and role in processing olfactory input.

Neurotransmitters/peptides of neurons in the glomerular layer

The axons of olfactory receptor neurons (ORNs) are an important element of the glomerulus. They utilize glutamate as a major neurotransmitter that acts on glutamate receptors located on the dendrites of target MOB cells. Fast synaptic responses in mitral and bundle cells are mediated through AMPA/kainate receptors, whereas slow synaptic responses are mediated through NMDA receptors. AMPA/kainate and NMDA receptors also appear to mediate fast and slow synaptic responses in juxtaglomerular cells in response to ORN administration. Metabotropic glutamate receptors are also present in the glomerular layer, but their role(s) are currently unclear.

Many juxtaglomerular cells are dopaminergic or GABAergic. In hamsters, about 70% of dopamine (DA) neurons are reported to colocalize with GABA, whereas about 45% of GABAergic cells contain DA. In rats, almost all dopamine periglomerular neurons also contain GABA, whereas bundle-type dopamine cells do not express GABA [22-25].

Almost all juxtaglomerular neurons immunoreactive to substance P in the hamster MOB are reported to contain both GABA and DA based on the presence of immunocytochemical markers for these transmitters in the same cells, although juxtaglomerular neurons in rats do not appear to contain substance P. Thus, some juxtaglomerular cells may contain both a catecholamine (DA) and an amino acid inhibitory transmitter (GABA), and possibly also an excitatory neuropeptide (substance P), although there is considerable species variability. A few juxtaglomerular cells contain a vasoactive intestinal polypeptide. Some periglomerular cells with short axons that project into the deeper layer of granular cells contain NADPH diaphorase, neuropeptide Y (NPY), and somatostatin, which may provide a direct pathway for periglomerular cell effects on granular cells. NADPH in the glomerular layer is mainly contained in periglomerular cells. In addition to what has already been discussed, cholecystokinin, aspartate, thyrotropin-releasing hormone, enkephalin, and protein kinase C are also present in some juxtaglomerular cells. In addition, a subpopulation of small juxtaglomerular neurons is positive for acetylcholinesterase; hence, these cells may be cholinoreceptive [23-25].

At this point, several subclasses of juxtaglomerular neurons can be distinguished based on their expression of neurotransmitters/peptides. Although these neurochemical markers serve to identify potentally distinct cell types and were identified more than a decade ago, little is known about the functional significance of these transmitters/peptides for olfactory signal processing. Because most juxtaglomerular cells are very small (5-12 μm) and because most have widely varied local circuit connections and neurochemical content, their physiological characteristics have been difficult to study.

As noted above, the glomerular layer contains a large number of dopamine-containing neurons. In the MOB, the predominant DA receptor belongs to the D2 subtype. This receptor is localized in the glomerular layer and equally dense in the ONL. If $ZnSO_4$ is used to kill the olfactory epithelium, binding of the D2 receptor is eliminated in both the olfactory nerve and glomerular layers. D2 mRNA transcripts are also abundantly expressed by ORNs. Thus, although it has been assumed for two decades that olfactory nerve endings do not receive presynaptic contacts, it has been hypothesized that juxtaglomerular dendrites release DA that acts on olfactory endings but without classical synaptic specialization. Recently, electrophysiological studies and direct optical imaging of calcium transients in ORN axons have shown that DA, acting on the D2 receptor subtype, presynaptically inhibits ORN axons.

Interestingly, GABA, acting on GABAB subtype receptors, also exerts presynaptic inhibitory upregulation of ORN terminals in the MOB. GABAB receptors are present in high concentrations in the glomeruli. Recent electrophysiological results suggest that GABA released from juxtaglomerular neurons acts on GABAB receptors to presynaptically inhibit ORN terminals [25].

External plexiform layer

Immediately beneath the glomeruli is the External plexiform layer, which is characterized by a relatively low cell density but a very dense neuropil. The main neuronal elements of this layer are mitral/fascicular dendrites and

granular cells. The main types of neurons in the EPL are superficial, middle, and deep fascicular cells, named according to their relative depth in the EPL, the external fascicular cells at the EPL/GL boundary discussed above, and Van Geuchten cells, which appear to be local interneurons [26-34].

There is a gradual increase in the size of bundle cells from superficial to deep parts of the EPL. The dendritic morphology of bundle cells varies, but they usually have at least one apical dendrite that enters and branches into a single glomerulus, as in mitral cells. Some bundle cells may have two or three apical dendrites entering different glomeruli. Beam cells also have secondary dendrites that run tangentially into the EPL. These secondary dendrites are thought to form reciprocal synapses with the apical dendrites of granular cells, as do mitral cells. Glutamate released from secondary dendrites of bundle cells is thought to excite granular cells mainly through AMPA/kainate and NMDA receptors. Middle and deep bundle cells have similar but not identical axonal projections to mitral cells; thus, most bundle cells and all mitral cells can functionally be regarded as output cells of the olfactory bulb. The axons of many superficial bundle cells mainly project to other areas of the same (ipsilateral) olfactory bulb. Middle and deep bundle cells also have local collaterals in the ipsilateral bulb, but most appear to project from the MOB into the anterior olfactory nucleus and other rostral olfactory cortical structures; few bundle cell axons project into rostral cortical areas. Intratumoral collaterals of the surface bundle cell population form a highly organized intratumoral network called the intratumoral association system (IAS). These axons pass through the external plexiform and mitral layers into the inner plexiform layer (IPL), where they collect to form dense tracts that travel within this layer to the opposite side of the same bulb, where they terminate as a dense terminal field in the IPL. Thus, these bundle cells send a discrete, topographically organized projection to the opposite side of the same bulb, a point-to-point reciprocal projection between the lateral and medial bulb. The IAS has the highest degree of point-to-point topographic organization of any known circuit in the olfactory system.

Most ORN-ORG cohorts studied to date project to one glomerulus on the lateral bulb and a second on the medial bulb. There is speculation that the IAS may connect olfactory regions to the same ORN-ORG axons. The IAS is formed exclusively by bundle cells containing cholecystokinin (CCK), and the terminals of these CCKergic cells preferentially, if not exclusively, terminate on the apical dendrites of granule cells. Cholecystokinin (CCK) causes membrane depolarization in all neurons studied to date. Thus, it is likely that when CCKergic bundle cells are active, they cause depolarization of granular cells on the opposite side of the bulb. This may either increase or decrease GABA release, depending on whether the depolarization penetrates to dendritic release sites or acts as a shunt current that normally causes GABA release. Given the high topographical organization of the IAS, this could result in either high-focus inhibition or excitation on the opposite side of the bulb [1-12].

The second group of EPL neurons are the van Geuchten cells. These cells are characterized by the presence of two or more thick primary dendrites that remain in the EPL. The axons of these cells terminate around the mitral and bundle cells. Many of these Van Geuchten cells stain positive for vasoactive intestinal polypeptide in cats, and in rats for various calcium-binding proteins (calbindin D-28, calretinin, neurocalcin, and parvalbumin) and NADPH-diaphorase. Beam cells appear to utilize glutamate as their primary transmitter. As noted, large populations of bundle cells also contain the neuropeptide cholecystokinin. Using in situ hybridization, Substance P mRNA transcripts have been detected in some external bundle cells and in about half of the mitral MOB cells in rats, but to date, no study using immunocytochemistry has detected Substance P in mitral cells of any species. Even in hamsters, which have many juxtaglomerular-type substance P cells in the MOB, substance P is not present in mitral/fascicular cells, at least according to immunocytochemistry. Many of the medium bundle cells have been reported to contain vasoactive intestinal polypeptide (VIP) in rats, but in cats this peptide appears to be found in Van Geuchten cells rather than in bundle cells. In hamsters, several medium-sized bundle cells stain positively for NADPH-diaphorase [23-29].

Mitral cell layer (MCL)

Deeper than the external plexiform layer is the mitral cell layer. This is a thin layer containing mitral cell bodies (25-35 μm in diameter in rats) arranged almost in a single layer. These cells are the main output cells of the bulb and, with some small species variations, have a single apical dendrite that enters a single glomerulus where it branches extensively and synaptically contacts olfactory axons (Shepherd, 1972). The apical dendrites of approximately 25 mitral cells enter each glomerulus in rats. Passing through the EPL, the apical dendrite of mitral and bundle cells receives very few synapses, and the bulk of the apical dendrite is thought to be isolated by glial sheaths (unpublished observations). Secondary dendrites of mitral cells can extend up to 2 mm in the EPL and are oriented tangentially, that is, parallel to the bulb surface. These secondary dendrites participate in dendrodendritic synapses with granule cell dendrites. In addition, they can receive centrifugal and van Geuchten cell inputs. It should be noted that although rats have approximately 40,000 mitral cells, there are approximately 100,000 granular cells in the mitral cell layer. Thus, although it is called the mitral cell layer, mitral cells make up only about 35% of the cells in this layer [14-19].

Mitral cells are glutamatergic, but they were also thought to utilize N-acetyl-aspartate-glutamate (NAG), which was detected in mitral cells by immunocytochemistry. However, subsequent neurophysiologic studies have questioned the primary role of NAG as a transmitter in mitral cells. Also, several unusually small mitral cells are thought to contain aspartate and project into the piriform cortex [18].

A neuropeptide, corticotropin-releasing factor (CRF), has been demonstrated in mitral and some bundle cells using both immunocytochemistry and in situ hybridization in rats. CRF fibers were also observed in layer Ia of the piriform cortex. This finding is consistent with CRF being a released neuropeptide in mitral cells, as mitral cells synaptically terminate in layer Ia of the piriform cortex. Similar localization of CRF has been reported in squirrels, suggesting that this peptide may be a conserved transmitter/modulator in mitral/beam cells of many mammals. [17]

Inner Plexiform Layer (IPL)

Directly beneath the mitral cell layer is the internal plexiform layer (IPL), a thin layer with many axons and dendrites but low cell density. The IPL contains mitral/fascicular cell axons, granular cell dendrites, and axons from other centrilobular sources. Some axons in the IPL originate from the suture nuclei, coeruleus locus, and diagonal band nuclei. As noted earlier, the IPL also contains a very dense plexus of axons and terminals containing CCKs, which originate from surface bundle cells. The IPL also contains several multipolar neurons, larger than granular cells, that express AChE. Because these neurons do not express ChAT, the ACh synthesis enzyme, but lie in a layer richly targeted by cholinergic inputs from the diagonal band nucleus, they may be choline-sensitive [15-21].

Granular cell layer (GCL)

The granule cell layer is the deepest neuronal layer in the bulb. It contains many small (8-10 μm in long axis) granule cell neurons. Often three to five granular cells are arranged in rows, forming tightly packed aggregates of soma. Granular cells in these aggregates are connected by intercellular contacts that may serve to synchronize the functional activity of these neurons. Granular cells are also found mixed with mitral cells in the mitral cell layer. They have a limited basal dendritic arborization that branches into the GCL, and a thicker and longer apical dendrite that enters and branches extensively into the EPL. In general, the closer to the surface a granular cell body is located in the GCL, the closer to the surface its apical dendritic branching in the EPL, while granular cells located deeper in the GCL have dendrites branching in deeper parts of the EPL. However, not all granular cells follow this pattern. Exceptions include granular cells whose distal dendrites project deeper, toward the center of the bulb, and granular cells with dendrites that do not reach the external plexiform layer. In addition, the granular cell layer also contains non-granular cells with dendritic and axonal arborizations, including the Blanes cell and Golgi cell [26-35].

There is extensive synaptic communication between the dendrites of granular cells and the secondary dendrites of mitral/fascicular cells. Mitral/beam cell dendrites form excitatory glutamatergic synapses onto granule cell dendrites, and granule cell dendrites form inhibitory synapses onto mitral/beam cell dendrites. Excitation of mitral cells to granular cells, which causes the release of GABA from granular cells, appears to require the NMDA receptor. Most granule cells contain GABA, which inhibits mitral and bundle cells via GABAA receptors. Some peptides are also localized in other cells of the granular cell layer. For example, several cells containing NPY have been found in this layer in rats and humans. In rats, the axons of NPY cells appear to branch in more superficial layers such as the glomerular layer. These neurons are probably cells with short axons, in contrast to granular cells [32].

Subependymal layer

The deepest layer in the MOB is the subependymal zone, an area of low cellularity in adults. Cells in this layer line the ventricle (if present) and during development the precursors of many MOB cells originate from this zone, although in adults other MOB interneurons (mostly granular cells with a small number of juxtaglomerular cells) are generated in the more anterior subventricular regions of the forebrain and then migrate to the bulb via the so-called "anterior migratory stream [6].

Mediator regulation of the olfactory nerve

Input from the olfactory epithelium is important for normal transmitter expression in many types of MOB neurons. For example, the olfactory nerve influences DA phenotype expression in juxtaglomerular neurons. If the olfactory epithelium is destroyed by detergent or ZnSO4, or if functional activity in the pathway is disrupted by nostril closure, tyrosine hydroxylase (TH) immunoreactivity is lost in dopaminergic juxtaglomerular neurons. This phenomenon of transneuronal regulation of the transmitter phenotype has been demonstrated in adult rats, mice, dogs, hamsters, and developing rats. TH expression is also reduced in mice homozygous for a null mutation in CNGA2.OCNC1, rendering the mice functionally anosmic. Thus, these cells appear to require epithelial input to maintain TH and hence express their DA phenotype. Loss of TH immunoreactivity is not associated with cell death because neurons can be detected by antibodies to other DA enzymes. In addition, GABA continues to be expressed in juxtaglomerular neurons in which TH is reduced after deafferentation. The mechanism of TH regulation has been the subject of recent studies. In in vitro experiments, the induction of TH expression by depolarizing stimuli depends on calcium influx into bulb neurons. In a co-culture of olfactory bulb neurons and olfactory epithelium, the odorant-dependent transneuronal regulation of TH expression can be abolished by blocking glutamatergic signaling via the NMDA receptor. This suggests a mechanism whereby ORN glutamate release stimulates dopaminergic neurons, leading to calcium influx and regulation of TH expression. Epithelial input is also required to initiate initial developmental expression of TH. Thus, both the induction of development and maintenance of the DA phenotype depend on the presence and normal function of the olfactory nerve. Deafferentation does not affect GABA expression in periglomerular neurons, indicating that odorant-induced activity selectively regulates the expression of different transmitters.

DA is not the only transmitter affected by the olfactory nerve. In hamsters, many juxtaglomerular neurons express substance P, and this peptide is reduced after deafferentation. In addition, new results indicate that the influence of the olfactory nerve also extends to the expression of CCK in bundle cells and CRF in mitral cells.

The effects of the olfactory nerve on MOB cells have also been studied in other ways. Exposure of animals to clean air was found to reduce mitral cell size, whereas exposure to certain odors for prolonged periods of time caused a regional increase in mitral cell size. Light and dark granular cells have also been described, and these two subpopulations differ in their response to unilateral nostril closure: light granular cells, located in the deeper regions of the GCL, are the first to be affected by nostril closure. Light-colored cells are affected first because they are new arrivals to the GCL from an anterior migration stream. Nostril closure reduces the number of both light and dark granular cells, and it is hypothesized that the reduction is due to cell death rather than a reduction in cell division because granular cells continue to be born in the proliferative subependymal zone [21].

Outlets of the Main Olfactory Bulb

Intrabulbar collaterals

Mitral axons give off collaterals within the bulb in the inner plexiform and granular layers. The main axons preferentially pass in the lateral olfactory tract, which forms at approximately the level of the AOB. Caudally directed axons give off collaterals in the anterior olfactory nuclear (AON) and other regions of the olfactory cortex. Beam cells form even more collaterals in the bulb than mitral cells. The association pathway within the bulb formed by SCergic bundle cells has been discussed previously [20].

Mitral/bundle cell axons

The main MOB output is via mitral cells and middle and deep bundle cells. Some MOB outputs have a moderate degree of topographic organization. For example, neurons in the dorsolateral quadrant of the MOB project into the dorsal part of the external subdivision of the AON, whereas output cells in the ventral half of the MOB project into the lateral subdivision. MOB output neurons projecting to more caudal regions (e.g., piriform cortex) are evenly distributed in the MOB. Intracellular injections of horseradish peroxidase (HRP) into mitral cells were used to demonstrate arborization of individual axons. Axons often have many collateral terminal arborizations in the AON and fewer in the more caudally located piriform cortex. Terminal arborizations have a patchy anteroposterior distribution in layer Ia of the AON and piriform cortex in the rabbit, resembling the patchy distribution of thalamic input to visual cortex. Some mitral cells branch to project to both olfactory cortex and olfactory tubercle. Mitral cells located close together are reported to have similar patterns of axonal projections to the olfactory cortex. Although there are some hints about the organization of MOB output projections, the preponderance of evidence from a large number of studies using a variety of tract tracing methods indicates that bulb outputs do not have a high degree of point-to-point topographic projection to their target structures, as is typical of other sensory systems [24].

Projections to the olfactory cortex

MOB mitral and bundle cells project to several structures in the ipsilateral hemisphere, including the superficial plexiform layer of the anterior olfactory nuclear, piriform, periamygdaloid, and lateral entorhinal cortices, taenia tecta, anterior hippocampal continuation, indusium griseum, and olfactory tubercle. Collectively, the regions directly innervated by the MOB output have been termed the primary olfactory cortex. Most of these projections have been described in several views. A direct projection of the MOB to the supraoptic nucleus has also been described. The organization of these projections is discussed below in the section on the primary olfactory cortex [22].

Centrifugal afferents to the MOB

Centrifugal afferent inputs to the MOB are very dense, arise from multiple sources, and play an important role in regulating neural processing in the MOB. These inputs exhibit some degree of anatomical organization and neurochemical diversity. Centrifugal afferents can be divided into two groups: (i) afferents from structures associated with olfaction and (ii) modulatory afferents from non-olfactory subcortical structures. These two groups are distinct because olfactory projections perform specific olfactory sensory and associative functions, whereas modulating afferents have broad projections that influence CNS functions in other neural systems. Olfactory centrifugal afferents arise from many sources, including the AON, piriform cortex, periamygdaloid cortex, entorhinal cortex, nucleus of the lateral olfactory tract, and amygdala. Subcortical modulatory afferents originate in the brainstem and basal forebrain. Olfactory centrifugal afferents to the MOB are discussed below on page 948. The organization of subcortical "modulating" inputs to the bulb is discussed below under "Non-ob olfactory modulating inputs to the olfactory system." [22]

Primary Olfactory Cortex

The MOB projects onto a set of structures that are collectively referred to as the primary olfactory cortex. These structures can be usefully divided into three groups: (A) the anterior olfactory nucleus; (B) the medial olfactory cortex, including the indusium griseum, anterior hippocampal extension, taenia tecta, infralimbic cortex, and olfactory tubercle; and (C) the lateral olfactory cortex, including, from rostral to caudal, the piriform, periamygdaloid, transitional, and entorhinal cortices [1-5].

Anterior Olfactory Nucleus (Aon)

AON architecture

Between the MOB and the piriform cortex lies a distinctive structure, the anterior olfactory nucleus. The AON is a laminated structure consisting of a plexiform layer and a fairly homogeneous layer of densely packed cells, except in the central region. Many output cells in the various subdivisions of the AON are pyramidal, with the exception of neurons in the external subdivision. Based on these architectonic features, the AON is considered a cortical structure. The anterior olfactory nucleus (AON) has been divided into several subdivisions based on cytoarchitecture and connections [15-23].

Inputs to the AON

As noted above, the AON intensively targets mitral and bundle cells. In addition, inputs to the AON come from other subdivisions of the ipsilateral AON and from the contralateral AON, from the piriform cortex and entorhinal cortex, from the anterior amygdaloid region, from the posterolateral cortical nucleus of the amygdala, from the olfactory tubercle, from the nucleus of the lateral olfactory tract, and from the temporal part of the CA1 division of the hippocampus. "Modulatory" inputs arise from the suture nuclei, the locus coeruleus, and the nucleus of the diagonal band [25].

AON outputs

All subdivisions of the ipsilateral AON project to both the ipsilateral and contralateral MOB, with the exception of the external subdivision (AON pars externa, AONe), which projects only to the contralateral MOB. The AON contains the largest number of neurons projecting to the bulb from any single source (Carson, 1984). Thus, there is an extensive, bilateral representation of olfactory information at the level of the AON [33].

There is some degree of laminar topography of terminal projections to the MOB from different subdivisions of the AON in rats and hamsters. In both species, ventral and posterior subdivisions of the AON project bilaterally to the GCL (mainly to the superficial half) and to the deep third of the GL. In hamsters, the lateral and dorsal subdivisions of the AON project mainly to the superficial GCL and GL (ipsilateral) with no projection to the glomeruli, whereas in rats, the dorsal subdivision terminates evenly in the GCL and weakly in the EPL but not in the GL. The external subdivision of the AON in both species terminates densely in a thin band deep relative to the IPL in the contralateral MOB. There are additional outlets of the AON, including projections to the ventral taenia tecta, piriform cortex and olfactory tubercle, endopyriform cortex, ventral agranular insular cortex, and nucleus accumbens (from the ventroposterior part of the AON) [34].

AON transmitters

Candidate transmitters in the AON and other olfactory cortical areas are summarized in Table 4, and neurotransmitter receptors are summarized in Table 5. Aspartate has been proposed as a transmitter for AON neurons (mainly dorsal and external AON subunits) based on selective retrograde transport of [3H] aspartate. Fewer neurons in other subdivisions of the AON contain aspartate, and no known afferents to the bulb contain aspartate. There are a few Met-enkephalin neurons and somatostatinergic neurons in the AON, and some of these appear to project to the MOB. All neurons in the AONe seem to contain the neuropeptide CRF [29].

Medial Olfactory Cortex

Several cortical regions located on the medial wall of the rostral hemisphere constitute part of the olfactory pathways that are often overlooked by olfactory researchers, probably because of confusion about the cytoarchitecture and connectivity of these regions. These regions include the indusium griseum (or dorsal extension of the hippocampus), the anterior extension of the hippocampus, and the taenia tecta. Anatomical studies have begun to reveal the cytoarchitecture and connections of these regions and the infralimbic cortex, which may have interesting functions integrating olfactory and visceral information [27].

Indusium Griseum

The indusium griseum (IG) or dorsal extension of the hippocampus receives input signals but does not project to the MOB. It is a thin cortical layer that runs parasagittally just above the corpus callosum. The IG has been the subject of debate as to whether it is more connected to the hippocampus or the MOB. It is now clear that the IG receives direct input to its tiny molecular layer from the MOB. This input is mainly directed to the rostral IG with fewer fibers running more caudally. The IG molecular layer also receives input from the lateral and medial entorhinal cortex. Because the entorhinal area receives direct inputs from the olfactory bulb (MOB) and, in turn, projects to the dentate gyrus of the hippocampus, it is suggested that the indusium griseum (IG) is the phylogenetically ancient part of the hippocampus that receives direct olfactory information, in contrast to most of the hippocampus, which receives only indirect olfactory information via the entorhinal area.

Continuation of the Anterior Hippocampus

The continuation of the anterior hippocampus (AHC) is located immediately ventral to the corpus callosum rostrum and dorsal to the taenia tecta. The inputs to the AHC are similar to those of the IG, as are their efferent connections, with the difference that the IG does not project to the MOB, whereas from the AHC there is a modest projection to the MOB. The other major efferent projections of IG and AHC are directed to the milky bodies and anterior thalamic nuclei [21].

Taenia Tecta

The taenia tecta proper projects strongly to the MOB. The pyramidal neurons of this cortical structure are relatively densely packed and are located dorsal to the anterior olfactory nuclei on the medial wall of the hemisphere anterior to the rostrum of the corpus callosum.

Infralimbic Cortex

This cortical area is located slightly rostrally but in the same general area as the AHC. Although most studies indicate that there is no direct relationship between the infralimbic cortex and the MOB, at least one study has reported a weak projection. The cells in the infralimbic cortex that are said to project to the bulb are in the same area as the projections to the visceral centers of the brain. The infralimbic cortex also has direct dense projections to the molecular and polymorphic layers of the rostral piriform cortex and possibly the endopyriform cortex. Because of its extensive connections with autonomous brain centers, the infralimbic cortex may be the link between olfaction and autonomous function [15-17].

Olfactory tubercle

The olfactory tubercle in rodents, rabbits, and other macrosomatic mammals is a prominent bulge on the base of the hemisphere located immediately caudal to the olfactory trunk. In such species, mitral and bundle cell axons terminate in the superficial layer of the tubercle, as in the AON and primary olfactory cortex. The tubercle has a superficial plexiform layer similar to the AON and primary olfactory cortex, but the cellular architecture of the tubercle is intermediate between cortical and striatal structure. Immediately beneath the plexiform layer is a layer of neurons with apical dendrites that extend into the plexiform layer. However, the neurons deeper than this so-called cortical layer do not resemble the layer III pyramids of the primary olfactory cortex, but rather are polymorphic, and their dendrites do not

appear to preferentially penetrate the plexiform layer as do the dendrites of layer III pyramidal cells of the olfactory cortex. These polymorphic neurons more closely resemble striatum neurons, and indeed, extensive neuroanatomical analysis of the tuberosity and adjacent basal telencephalic gray matter has led to the concept of a "ventral striatum." [18]

Lateral Olfactory Cortex

Architecture of the Lateral Olfactory Cortex

The caudolateral portion of the AON passes continuously through transitional zones into the piriform cortex, which in turn passes caudally into the periamygdaloid and transitional cortex and then into the lateral entorhinal cortex. Collectively, these cortical structures constitute the entire temporal cortical mantle ventral to the rhinal sulcus. The piriform cortex is divided into three layers, which are further subdivided on the basis of cytoarchitecture and afferent connections. Layer I, the superficial plexiform layer, is divided into Ia and Ib, which receive different afferents: layer Ia receives afferents from the ipsilateral MOB, whereas layer Ib receives associative fibers from the AON and other parts of the primary olfactory cortex. Layer II, the superficial compact cell layer, is also divided into two sublayers; the more superficial zone has a lower cell density and the deeper division has a higher cell density. Layer III is the broadest cell layer. The endopyriform nucleus, although similar in many respects to the piriform cortex proper, was found to have unique internal and external connections that differ from the piriform cortex. In the piriform cortex, the pyramidal cells of layers IIb and III possess extensive basal dendrites, whereas some smaller cells of layer IIa lack basal dendrites and resemble the granular cells of the dentate gyrus of the hippocampus. Reconstructions of individually intracellularly filled pyramidal cells in the piriform cortex show that pyramidal cells can have extensive axonal projections covering almost the entire cerebral hemisphere, including local connections to the anterior and posterior piriform cortex, as well as branching in the orbitofrontal cortex, insular cortex, olfactory tubercle, perirrhinal cortex, entorhinal cortex, and amygdaloid cortex. Many of these cortical areas are involved in behavior, cognition, emotion, and memory [26].

The piriform cortex also contains numerous interneurons that are distributed throughout the layers and regions of this structure in the rat. Some interneurons are located in layer I, where they may function in direct inhibition systems. Many of these GABAergic interneurons, described as "basket cells", colocalize with calcium binding proteins (parvalbumin, calbindin), VIP or CCK . These basket interneurons are mainly distributed in layers II/III and exhibit a variety of molecular markers and morphologic characteristics. These cells are thought to preferentially form axosomal or close axodendritic synapses with layer II and III neurons and participate in both inverse and direct inhibitory circuits. Several information processing models for the functioning of the piriform cortex have been proposed, but it is beyond the scope of this chapter to present all the models and evidence.

There are several transitional regions (including the periamygdaloid cortex) between the piriform cortex and the entorhinal cortex, and between the olfactory cortices and the neocortex. These transitional regions have been described in detail previously, and readers are encouraged to refer to that article for further information. Caudal to the piriform and periamygdaloid cortex is the entorhinal cortex. This cortex is divided into medial, lateral, and intermediate sections and has six layers [16].

Olfactory Cortex Connections

Feedback to the olfactory bulb

The piriform cortex, lateral entorhinal cortex, and transitional cortical areas between them all project back to the MOB (olfactory bulb). Projections are more intense from rostral parts of the primary olfactory cortex than from caudal parts in rats and mice. A few cells in posterolateral and medial cortical amygdaloid regions can also project to the MOB. These back projections to the MOB arise mainly from layer II and to a lesser extent layer III pyramidal neurons in the primary olfactory cortex [20].

The transmitters of these olfactory cortical projections to the bulb are unknown, although glutamate is thought to be the transmitter. These back projections from the olfactory cortex to the MOB are thought to primarily excite GABAergic granule cells in the MOB, which in turn inhibit mitral cell activity via dendrodendritic synapses between dendrites of granule and mitral cells.

Intrinsic and associative connections

In addition to back projections to the MOB, the olfactory cortex has other extensive connections that can be discussed as four classes: intrinsic or local - short connections between neurons in different layers of the POC; associative - connections to different parts of the POC; extrinsic - connections to other structures; and modulatory inputs - afferents that terminate in the POC as part of the broader innervation of other cortical and subcortical neural systems [19-23].

Internal or local connections

The POC has two major layers of pyramidal neurons, layers II and III, which include several morphologic classes as well as several classes of non-pyramidal neurons. There are extensive translaminar or local connections between POC neurons. Layer II neurons give axonal collaterals to deeper layer III pyramidal cells, and there are local inhibitory interneurons in layers I and II that contact MOB terminals and with local pyramidal cell collaterals. Deeper pyramidal cells also give rise to extensive local collaterals that may synapse with local interneurons or with more superficial pyramidal cells. Thus, there are extensive translaminar connections both from superficial to deeper layers and vice versa [20].

Associative connections

Corticocortical projections within the POC are extensive and have a laminar and regional organization. Axons of layer IIb pyramidal cells are mainly directed to more caudal portions of the POC; layer III cells project predominantly to rostral portions of the POC. Commissural fibers to the contralateral POC arise from layer IIb anterior portions of the POC. The ipsilateral and commissural associative projections of the POC terminate in a highly laminar fashion in layer Ib, immediately beneath the area containing afferent input from the MOB; a lighter projection terminates in layer III. POC projections back to the AON also terminate in layer Ib, below the MOB reception zone in layer Ia. Neurons in layers IIb and III send a dense projection back to the MOB; as noted earlier, this return pathway mainly terminates in the GCL [10-30].

External outputs of the olfactory cortex

Two classes of POC outputs have been discussed above, the back projection back to the MOB and the associative connections between rostral and caudal olfactory cortex. A third class of outputs is discussed separately because it represents POC projections to brain regions that are not normally included in the olfactory system per se, although their receipt of inputs from the POC obviously involves these POC targets in olfactory function. External POC outputs are directed to both cortical and subcortical structures. MOB projections to the POC extend dorsally beyond the cytoarchitectonic boundaries of the POC into ventral portions of the granular insular and perirhinal cortex. There are also direct projections from the POC into the insular and orbitofrontal cortices. The insular and orbitofrontal cortices are also the primary cortical targets for ascending pathways originating in the nucleus of the solitary tract (Sol) in the brainstem, and appear to contain primary cortical representations for both gustatory and visceral sensations. Thus, olfactory projections to the insular and orbitofrontal cortices may be part of the circuitry that integrates olfactory and gustatory signals to produce integrated taste perception. These same cortical areas also have descending projections to the hypothalamus and back to the Sol, which may influence visceral-autonomic and possibly gustatory functions. Neurons in these cortical areas in primates respond to odors with a higher degree of selectivity than neurons in the MOB or POC. Thus, these neocortical areas may play a role in discriminating different odors [33].

Odors and cognition

Physiological studies in monkeys suggest that some degree of odor discrimination may occur in the lateral and posterior orbitofrontal cortex. This olfactory information is transmitted either through the mediodorsal thalamus or through corticocortical pathways. There are several studies showing a potential olfactory-neocortical circuit through the thalamus. For example, the olfactory tubercle, insular cortex, and piriform cortex receive input signals from mitral cell axons. The olfactory tubercle and piriform cortex project to the dorsomedial thalamic nucleus and the submedial thalamic nucleus, although projection from the piriform cortex (but not the insular cortex) has been challenged. The dorsomedial thalamic nucleus projects to the posterior orbitofrontal cortex, so this pathway may be involved in some aspects of odor discrimination. However, physiological evidence suggests that the projection of the dorsomedial thalamus is stronger to the centroposterior part of the orbitofrontal cortex, which is more involved in the integration of odor sensations than in odor discrimination, because individual cells in this region respond to multiple odors; individual cells in the lateral posterior orbitofrontal cortex are more likely to respond to a single odor. Thus, corticocortical pathways may be involved in higher olfactory functions.

The transmitters involved in the pathways discussed above are unknown, although it is conceivable that the excitatory amino acid neurotransmitter glutamate is involved, since it is found in all thalamic nuclei and projection cells of the piriform cortex [30-34].

Olfaction and the integration of taste/visceral sensations

Olfactory stimuli can activate visceral responses and autonomous adjustments such as gastric secretions, salivation, and heart rate changes. The circuits mediating these functions are poorly understood. The lateral olfactory cortex projects mainly to the lateral hypothalamus, which is known for its involvement in visceroautonomic functions. Another possibility is that connections of the MOB and piriform cortex with the insular cortex may be involved in these. Part of the granular insular cortex is the site of considerable overlap between olfactory and visceral information in mice and rats. In addition, the medial olfactory cortex may be an area of efferent control of visceral activity. There are direct projections from the MOB to the ventral part of the medial frontal cortex, as well as reciprocal connections between the insular and medial frontal cortices. Olfactory inputs to the insular cortex and medial frontal cortex may influence autonomic and visceral functions via direct cortical projections to the cardiovascular regions of the ventral medulla and solitary nucleus or by less direct pathways. For example, the central nucleus of the amygdala, which receives a dense projection from the insular cortex, projects to stem autonomous centers such as the gray matter around the conduit and the dorsal vagal complex. Parts of the gray matter around the water pipe project to the ventral-lateral medulla and may be involved in pressor and depressor responses of the cardiovascular system.

Another area that may be involved in the integration of different sensations is the posterolateral orbitofrontal cortex. This cortex receives inputs from the insular cortex, and many neurons in the primate orbitofrontal cortex respond to both taste and odor as well as visual inputs. Thus, the orbitofrontal region may be the site of higher-level integration of multiple sensory modes (taste, odor, vision) [22].

Olfaction and Motor Activity

As noted earlier, several pathways link structures related to olfaction to what has been termed the ventral striatum. These connections are hypothesized to be the means by which limbic (and possibly olfactory) information is integrated with motor control regions of the striatum, so that visceral and somatic effectors may be controlled by these pathways. Olfactory connections to the ventral striatum are made by parallel projections from the MOB, AON, and piriform cortex to the olfactory tubercle. Neurons in the olfactory tubercle and some in the piriform cortex project into the nucleus accumbens (part of the ventral striatum), which in turn projects into the ventral pallidum and substantia nigra pars reticulate [29].

Olfaction and Memory

The entorhinal cortex receives significant input from the MOB. In turn, the medial and lateral entorhinal cortices project into the dentate gyri and CA fields of the hippocampus. MOB projections to the entorhinal cortex directly contact stellate cells located in layer II, which in turn project through the perforant pathway into the hippocampus. In addition, the piriform cortex has direct connections with the entorhinal cortex. Because the hippocampus is important for memory function, these olfactory-entorhinal-hippocampal circuits may be important for the formation or recall of olfactory memories created or associated with other events [13-16].

Accessory Olfactory System

In macrosmatic mammals such as the rat, two components of the olfactory system are recognized: the primary and accessory olfactory systems. These two components are parallel but largely anatomically and functionally separate.

Vomeronasal organ (VNO)

ORNs in the olfactory epithelium convert mainly volatile odors and transmit this information to the MOB. In contrast, ORNs located in the vomeronasal organ are exposed to non-volatile odors by activating a physiologically regulated pump mechanism. The olfactory epithelium can be divided into an apical zone and a basal zone. VRNs in the apical zone express the odorant receptor genes V1R and V3R and the G-protein Gα. VRNs in the basal zone express the V2R and G-protein Gαo receptor genes [21].

In the basic olfactory system, our current understanding is that ORNs respond to chemical epitopes that may be present on different odor molecules and that the tuning of basic olfactory neurons is broad; that is, a single ORN responds to related chemical epitopes with different affinity. However, VRNs show a narrower tuning, as individual VRNs respond specifically to only one putative pheromone molecule. This response occurs at very low concentrations (e.g., 10-11M), which is several orders of magnitude lower than observed in the main olfactory epithelium. The response profile of vomeronasal neurons (VRNs) to a single putative pheromone molecule does not broaden with increasing concentration, indicating their very narrow specialization for putative pheromones. VRN populations also show strong selectivity for male or female urine, although the molecules responsible for this response are unknown. Thus, assuming that olfactory receptor neurons (ORNs) in the main olfactory epithelium are sensors of epitopes, VRNs may be detectors for individual putative pheromone molecules.

Axons of vomeronasal neurons project exclusively into the accessory olfactory bulb (AOB), located in the dorsocaudal part of the main olfactory bulb (MOB). VRNs from the apical zone project into the anterior half of the AOB, whereas VRNs from the basal zone project into the posterior half of the AOB. In contrast to macrosmatic mammals, in microsmatic mammals such as humans, the VNO-AOB is either absent or present only during fetal development. Some mammals, such as harbor porpoises, are anosmic and lack an olfactory bulb. Indeed, the size of structures associated with olfaction reflects the importance of olfaction to the animal. For example, the olfactory bulb in humans is relatively small compared to the rest of the brain, whereas it is relatively large in the rat, which relies heavily on olfaction for reproduction and survival [23-25].

Accessory olfactory bulb (AOB)

The AOB is located at the caudodorsal end of the MOB. The AOB has some cytoarchitectural features similar to the MOB but is much smaller in size. The vomeronasal nerve transmits information from the VNO to the glomeruli of the AOB. The glomerular layer in the AOB is less pronounced than in the MOB because the glomeruli in the AOB are smaller and fewer in number. In addition, there are far fewer periglomerular cells than in MOB, resulting in the glomeruli not being as clearly labeled by the cell envelope. The term

"periglomerular" is thus less appropriate in AOB than in MOB because the few periglomerular cells tend to be located superficially or deeply relative to the glomeruli rather than between them. The external plexiform layer of the AOB and the mitral cell layer are also less pronounced than the corresponding layers of the MOB. The inner plexiform layer of the AOB is inconspicuous and is located between the mitral cell layer and the lateral olfactory tract. The granular layer of the AOB, located deep from the lateral olfactory tract, contains the same type of small cells as the granular layer of the MOB. Although many authors refer to it as the mitral cell layer, the output cells in the AOB are much more polymorphic than their counterparts in the MOB. Indeed, there are five major categories of mitral cells based on dendritic cell arrangements in the Golgi-impregnated material. These mitral cells may have one to five apical dendrites that branch into different glomeruli. Vomeronasal receptor neurons (VRNs) expressing the same ORG also project to multiple glomeruli in the AOB (6 to 20 glomeruli per gene). Interestingly, the dendrites of at least some mitral cells in the AOB specifically project to different glomeruli innervated by VRNs expressing the same ORG [30].

Neurotransmitters

Electrophysiological studies suggest that glutamate is a transmitter of vomeronasal receptor cells. Based on retrograde transport of labeled amino acids, aspartate is hypothesized to be a transmitter of output neurons of the AOB. It appears that mitral AOB cells are more aspartatergic than mitral MOB cells. Electrophysiologic studies also suggest that dendrodendritic signal transduction from mitral cell to granule cell is mediated by glutamate or aspartate, as in MOB. Many mitral cells in guinea pig AOB contain neurotensin, whereas in rat mitral cells transiently express substance P, but expression in these output cells gradually decreases after day 10 after birth. Interestingly, the number of granular cells immunoreactive for substance P increases while mitral cell expression decreases.

Few "periglomerular cells" in AOB are neurochemically different from those in MOB. The most obvious difference is the absence of dopaminergic periglomerular cells in the AOB. Also absent are the external bundle cells containing substance P, which are abundant in the MOB of some species. GABA-ergic periglomerular and granular cells are present in the AOB. Cells containing substance P are most prominent in rat GCLA. In contrast, there are fewer cells immunoreactive to substance P in the GCLA of rabbits, guinea pigs, cats, and hamsters, and these cells appear to be absent in mice [17-32].

Outputs of the accessory olfactory bulb (AOB)

The central connections of the accessory olfactory bulb (AOB) and the main olfactory bulb (MOB) to higher olfactory structures have little overlap. The AOB has direct projections to the amygdala, particularly to the medial and posterior cortical nuclei, the nucleus of the stria terminalis, and the nucleus of the accessory olfactory tract. These pathways may be involved in the processing of pheromonal information. Neurons in AOB target structures express gonadal steroid receptors and thus can be modulated directly by circulating hormones.

Sexual dimorphism of the AOB and its target structures

The growth of AOB is dependent on gonadal steroids. The AOB in male rats is significantly larger than in females, but if the male is castrated early in development, the size of the AOB will be similar to that in females. These findings correlate with sexual dimorphism in other structures (e.g., preoptic area and hypothalamic nuclei) known to influence sexual behavior and receive direct or indirect projections from the AOB.

Centrifugal afferents to the AOB

There are significant differences between centrifugal inputs to the MOB and the AOB. First, centrifugal inputs to the AOB originate from a much smaller number of brain regions than inputs to the MOB. The main afferents to the AOB come from the nucleus of the stria terminalis, the nucleus of the accessory olfactory tract, the medial nucleus of the amygdala, and the posterior medial cortical nucleus of the amygdala. A limited portion of the medial AON sends a dense projection to the granular layer of the AOB, but all other sections of the AON have no connections to the AOB [32-35].

Higher orders of connectivity of the additive olfactory system and reproductive functions

Olfaction plays an important role in the sexual behavior of many animals. The ability to use the sense of smell to identify sexual partners, enemies, and food is highly developed in macromammalian animals; that is, these animals use the sense of smell for survival and continuation of the species. The link between reproductive behavior and olfaction is not as strong in humans, but we may still possess a neural apparatus that links odors to sexual arousal, and certainly the profit and loss reports of the perfume industry attest to the key role of olfaction in human sexual drives. In macrosmotic animals, the AOB is involved in the processing of pheromones, which are initially transduced by vomeronasal neurons projecting to the AOB. The AOB projects to the anteromedial (Me) and posterior cortical (PCo) nuclei of the amygdala, the nucleus of the stria terminalis, and the nucleus of the accessory olfactory tract. Me and PCo have projections to other amygdala nuclei, particularly the posterior amygdala nucleus, as well as to the preoptic area and hypothalamus. The posterior amygdala nucleus (Po) appears to receive convergent input from Me and PCo and projects intensely to some of the same structures to which Me and PCo specifically project, namely the medial preoptic area and the ventromedial nucleus of the hypothalamus. Some of these secondary olfactory connections strongly influence sexual drive, and neurons involved in these connections contain steroid receptors and secrete peptides that mediate sexual responses. For example, the posterior dorsal Me part of the Me (MePD) contains neurons that project to four groups of cells known for their sexual dimorphism and differences in roles in reproduction. The medial preoptic nucleus (MPO) is one of the sexually dimorphic targets of the MePD, and damage to the MPO reduces male copulatory behavior. Estrogen regulates CCK expression at the mRNA level in MePD cells; many cells containing CCK in the MePD project to the MPO. In female rats, injection of CCK into the medial preoptic region enhances luteinizing hormone secretion, although its effects on male sexual responses are unknown [32].

"Non-olfactory" MODULATORY INPUTS TO THE OLFACTORY SYSTEM

The olfactory system is intensely targeted by inputs from the neobontic subcortical modulatory systems. These inputs come from three main sources: the diagonal band nucleus, the dorsal and median suture nuclei, and the locus coeruleus. The diagonal band (DB) nucleus is a component of the basal anterior magnocellular system, including the DB, basal nucleus, and medial septum. These basal forebrain neurons innervate most regions of the neocortex, hippocampus, and many other forebrain regions including the amygdala and thalamus. The locus coeruleus nucleus and the dorsal and median suture nuclei innervate cortical and subcortical structures throughout the CNS [26-31].

Diagonal striatum (DB) nucleus

In mice, about 3.5% of all neurons projecting to the olfactory bulb originate from the horizontal branch of the DB; far fewer originate from the vertical branch of the DB. At least two distinct populations of transmitter-specific DB neurons project to the MOB. About 20% of the DB neurons projecting to the bulb are cholinergic; most of these cells are concentrated in the rostromedial portion of the horizontal branch of the DB. At least as many of the DB neurons projecting to the bulb are GABAergic and are predominantly localized primarily in the lateral-caudal regions of the horizontal branch of the DB. Acetylcholinesterase (AChE) is one of the markers of cholinergic axons. AChE staining in the bulb is predominantly concentrated in the IPL, GCL, inner third of the EPL and GL. Some glomeruli are more densely stained for AChE and correspond to regions of LHRH innervation. The source of LHRH in these specialized glomeruli is unknown. Glomeruli densely labeled for AChE and LHRH may include a modified glomerular

complex. It is hypothesized that these glomeruli may be areas of specialized olfactory processing during development.

There are also neurons in the bulb that are positive for AChE. A more specific marker of cholinergic axons is choline acetyltransferase (ChAT), an essential enzyme for acetylcholine synthesis. ChAT-stained axons are arranged in layers similar to those described for AChE and are very thin in diameter. The glomeruli of the AOB do not contain ChAT and AChE staining. There are no neurons positive for ChAT in the bulb. The GABAergic projection from DB is more difficult to characterize than the cholinergic projection because the intrinsic GABAergic periglomerular and granular cells in the bulb provide such massive intrinsic GABAergic innervation of the bulb [12-19].

Suture nuclei

The medial dorsal and median raphe nuclei of the brain provide strong inputs to the MOB. In rats, approximately 1000 dorsal and 300 median suture neurons project to the bulb. These neurons are serotoninergic and do not contain tyrosine hydroxylase or substance P. Thick serotoninergic fibers preferentially innervate the glomeruli of the MOB, whereas thinner serotoninergic axons preferentially innervate the inframitral layers. In the neocortex, thick axons arise from the median suture and thin axons from the dorsal suture, and the same separation occurs in the MOB. Serotoninergic axons do not innervate the glomeruli of the AOB, just as cholinergic axons avoid this layer. Because there are far fewer PG cells in the AOB than in the MOB, the lack of innervation of the 5HT and ACh glomerular layer of the AOB suggests that serotoninergic and cholinergic inputs target PG cells in the MOB. The relative absence of PG neurons in the AOB may thus explain the lack of serotonergic/cholinergic innervation of this layer in the AOB [10-23].

Locus Coeruleus (LC)

The locus coeruleus (LC) plays a significant modulatory role in the olfactory bulb, originating from the pontine nucleus. All rat LC neurons contain the neurotransmitter noradrenaline (NE), and the LC contains the largest population of NE neurons in the brain. Estimates suggest that up to 40% of LC neurons (400-600 of the total 1600 LC neurons) project to the bulb. LC axons mainly target the infraglomerular layers of the bulb, particularly the inner plexiform and granular layers. The external plexiform and mitral layers are moderately innervated, whereas the glomerular layer is almost devoid of NE input. This highly specific laminated innervation pattern, unusual for terminal LC fields, is observed in both the MOB and AOB. In AOB, the inner plexiform layer is in fact clearly marked by dense NE fibers running through it just beneath the multilayered output cell layer (mitral layer). Based on these light microscopic studies, it is hypothesized that the primary target of NE entry is granular cells.

The physiologic action of NE in the bulb has been a matter of debate. NE has been shown not to reduce mitral cell firing rate when bicuculline, a specific antagonist of the GABAA receptor, is co-administered with NE. This has been interpreted as an increase in GABA release by granular cells under the action of NE, thereby inhibiting mitral cells. However, using whole turtle bulb in vitro, NE application was found to cause an increase in mitral cell firing rate. Other studies show that LC activation, and hence synaptic release of NE, causes a twofold increase in mitral cell responsiveness to weak, but not to strong shocks applied to the olfactory nerve. Application of NE in vitro also resulted in a similar enhancement of mitral cell responses to weak olfactory nerve input. This is consistent with the idea that NE preferentially enhances responses to weak stimuli. The functional significance of this action may be to increase the sensitivity of mitral cells to weak olfactory input. Thus, when the LC is activated by novel or unanticipated events, there may be a temporary increase in the sensitivity of mitral cells to weak odors. This may allow the animal to detect weak but potentially important odor cues, such as a predator or a pup that has strayed from its nest.

NE input to the AOB appears to have a very interesting function in mice. Newly mated female mice abort if presented with odors from a strange male who is not a partner, a phenomenon known as the Bruce effect in honor of the discoverer. This effect is blocked if NE input to the female's AOB is removed immediately after mating, presumably before olfactory memories of the partner are formed. Thus, NE appears to be important for reinforcing memory of the "husband" odor. The mechanism of AOB memory formation is important in the context of pregnancy blocking. Kevern and colleagues suggest that the dendrodendritic synapse between granule cells and mitral cells in the accessory olfactory bulb (AOB) may be critical for memory formation, and that noradrenaline (NE), by enhancing inhibition of a subset of mitral cells for several hours after mating, may contribute to the formation of selective odor memory. As a result of this neural activity, presentation of a partner's odor to a pregnant female elicits mitral cell activity consistent with that during mating, whereas odors from unfamiliar males elicit different patterns of mitral cell activity, leading to neuroendocrine responses that terminate the pregnancy [23-24].

NE has also been shown to be required for other olfactory memories such as maternal recognition in sheep and odor preference in young rats. Noradrenergic fibers appear in the bulb before birth and increase in density while MOB circuits are still forming in the bulb. The timing of the arrival of noradrenergic axons in the MOB correlates with pharmacologic evidence for the effect of noradrenaline on mitral cell excitability in the immature bulb [22-29].

Differential innervation of the MOB and AOB

Modulating centrifugal afferents from the diagonal band, the dorsal and median suture nuclei, and the locus coeruleus are common to the MOB and AOB. The terminal distribution of these shared inputs differs in AOB and MOB, especially with respect to cholinergic and serotoninergic inputs. In the AOB, both cholinergic and serotoninergic inputs avoid the glomeruli, whereas they intensely innervate the glomeruli of the MOB. Cholinergic and serotoninergic inputs to the AOB are mainly directed to the granule cell layer and the inner plexiform layer. NE input appears to have similar laminar termination patterns in the MOB and AOB [22].

Olfactory cortex

Modulatory inputs to the olfactory cortex have not been studied in as much detail as those in the MOB. However, a recent preliminary report indicated that ACh, 5HT, and NE all intensely innervate the olfactory cortex and, in addition, there is an intense extragenic DA-ergic input. Similarly, the functions of these modulating inputs to the olfactory cortex are poorly understood. There is evidence that ACh may be involved in associative learning and that excessive release of ACh, as during anti-AChE intoxication, causes the generation of epileptic seizures. The functions of 5HT, NE, and DA are unknown [23-25].

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