
The Vertebrate Olfactory System

Chemical neuroanatomy, function and development

N. Halász



Akadémiai Kiadó, Budapest

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By

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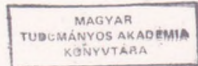
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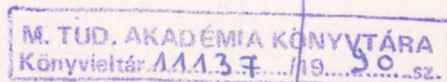


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FOREWORD

As neuroscience continues its explosive growth, it becomes increasingly important for pioneers in the field to synthesize recent developments in order to formulate new concepts of neural function. The present volume fulfills this need admirably.

Norbert Halász has been a pioneer in the analysis of the olfactory system. His work is an outstanding example of how one can use an interdisciplinary approach in the analysis of specific neuronal populations and neural circuits within defined regions. Among the regions suitable for such an approach, the olfactory system is particularly attractive, by virtue of its relatively simple and stereotyped structure. Halász has exploited these advantages brilliantly. His application of methods of immunocytochemistry, histology, neuropharmacology, electron microscopy and electrophysiology is a model that has helped to lay the foundations for our understanding of microcircuit organization in the olfactory pathway.

We are much in Halász' debt that he has found the time and effort amidst his experimental and administrative duties to summarize his work and place it in the context of a unifying overview of the olfactory system. Interest in the olfactory system has increased sharply in recent years, with wider recognition of the unique regenerative properties of olfactory receptor neurons, the accumulating evidence for the functions of microcircuits in the olfactory bulb, and the exploration of the network properties of olfactory cortex. The present volume is an excellent introduction to these and related issues. It also provides an exhaustive reference list, covering both classical and modern work, that is the most extensive I have seen. This is therefore a timely volume which makes a permanent contribution not only to the olfactory system but also to the principles of brain organization.

Gordon M. Shepherd
Yale University
September 4, 1989

PREFACE

In 1965 the Science Faculty at Szeged University got a new electron microscope, with sufficiently good resolution for studies on synaptic ultrastructure and I, with still a wet stamp on my diploma, was granted a position in the laboratory, established and operated by the Hungarian Academy of Sciences. While this laboratory became one of the roots of the later Biological Research Center, my interest was raised in fine structural characteristics of the rodent olfactory bulb, by my first teacher, Bert Csillik. This occurred around the early seventies, when the excellent studies of Tom Powell and his collaborators in Oxford, and contributions from several other laboratories brought about enormous progress in the knowledge of the structural details of this brain area.

Structural investigations and the basic achievements made through electrophysiological studies on the bulb prompted the necessity of a systematic exploration of the neurochemical basis of the bulbar machinery. An advance in this direction became available for me while spending a year at the Department of Histology of the Karolinska Institute in Stockholm, under the careful guidance of Tomas Hökfelt. Further widening of the scope of my interest, into functional and developmental aspects of the olfactory system was initiated and kept alive until now by Gordon Shepherd of Yale University. He made me aware, and also provided the way, of getting into contact with the admirably wide world of problems of the vertebrate olfactory system, and facilitated to build up and keep contact with leading scientists in these topics.

While pondering over and composing a review article with Gordon Shepherd, I, being a neurohistologist, encountered problems each day by looking for and finding (very frequently not finding) easy ways to correlate the view of a morphologist with biophysical, chemical, functional, developmental and behavioral findings on the same subject. Moreover, since the olfactory bulb is only a relay station between peripheral receptors and more central (and less specialized, for processing olfactory information) brain areas, I badly felt the need to familiarize myself more with relations, together with those of the other nasal chemosensory systems and their central representations.

These main reasons prompted me to attempt a summary of the fundamental morphological and neurochemical grounds of the nasochemosensory systems and their central connections, and complete the picture with basic recognitions, as well as with recent examples, of the beautifully rich world of the olfactory-related events in vertebrates. In this way, it is hoped to provide a complete account of the structural and histochemical aspects to specialists, working in this field, by drawing attention to the functional, developmental and behavioral significance of the olfactory system in all main groups of the vertebrates, including man. These latter aspects may give an overview for students and beginners in this field, and orientate them, by presenting the main features of the nasochemosensory systems and their significance, as a whole.

My special thanks are due to Prof. B. Csillik, Prof. J. Hámori, Prof. G.M. Shepherd and Dr. C.A. Greer for reading the entire book and giving numerous helpful suggestions. Although it is a single author book, it would not have been possible to write without the invaluable contributions of, first of all, my numerous collaborators, during original experiments and their discussions. These include my scientist colleagues whose names can be found in the reference list, and also the laboratory assistants, whom I want to express my deepest gratitude to here: Mr.B. Dusha, Miss Gabriella Gazdag, Mrs. Waldtraut Hiort, Mrs. Zsuzsanna Horváth, Miss Éva Mármárosi, Mrs. Etelka Mitrov, Mrs. Krisztina Mohácsi, Mrs. Anni Nygards-Tovi, Mr. J. Szeles and Miss Maria Szerletics. I am deeply indebted to Prof. O. Fehér, who read Sections, 2.4. and 4.4. and to Dr. L. Hernádi and Prof. E. Mugnaini who provided me with Figures 2.4a-c, 4.52. and 4.56. While processing the text with a computer, the kind help of Dr. Cs. Bagyinka was always available. The unwearing and efficient secretarial help of Mrs. Zsuzsa Gonda and Mrs. Mária Tartóczy, and correction of the English by Miss Renée P. Malone and Katalin Holland greatly contributed to the smooth preparation of the book. Editing of the text speaks highly for the careful and patient contribution by Ms Anikó Maleczky and Krisztina Takács.

My family and especially my wife shut their eyes to my lack of contribution to family cares over years of experimental work abroad and during the writing of this book; they were patient and even strong enough to keep continuous interest and inspire me.

The continuous financial support of the Hungarian Academy of Sciences, especially through the Institute of Biophysics and Laboratory for Molecular Neurobiology for experiments, accomplished in Hungary, and by granting time and fellowships for initiating and maintaining collaborations with foreign laboratories, was determinant in providing support for this work. I also greatly acknowledge additional support from the UNESCO/UNDP (funding a one-year fellowship) and from the following Swedish and American funds: Swedish Medical Research Council (04X-2887, 14P-3262 and 04X-04495), Knut och Alice Wallenberg Stiftelse, Magnus Bergvalls Stiftelse, Karolinska Institutets Forskningsfonder, U.S. National Institute of Health (NS-07609, NS-19174 and F32-06159), National Science Foundation (BNS 78-16545 and CB-8465).

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LIST OF ABBREVIATIONS

(additional abbreviations may appear at single figure legends or tables)

AC	anterior commissure	GCL	granule cell layer
ACh	acetylcholine	GFAP	glial fibrillary acidic protein
AChE	acetylcholinesterase	GL	glomerular layer
Ac-Asp-Glu	N-acetylasparyl glutamate	Glu	glutamic acid
AON	anterior olfactory nucleus	GR	granule cell
APB	2-amino-phosphonobutyric acid	³ H	tritium
AS	astrocyte	HRP	horseradish peroxidase
ATP	adenosine triphosphate	5-HT	5-hydroxytryptamine (serotonin)
BSA	bovine serum albumin	IPL	internal plexiform layer
CaBP	calcium-binding protein	28K-D-CaBP	28K-vitamin D-dependent calcium-binding protein
CCK	cholecystokinin	LC	locus coeruleus
CGRP	calcitonin gene related peptide	LEA	lateral entorhinal area
ChAT	choline acetyltransferase	Leu-Enk	leucine-enkephalin
CNS	central nervous system	LHT	lateral hypothalamus
DA	dopamine	LHRH	luteinizing hormone-releasing hormone
DADLE	D-alanine-D-leucine enkephalin	LOT	lateral olfactory tract
D-Asp	D-aspartic acid	MAB	monoclonal antibody
DBH	dopamine- β -hydroxylase	MCL	mitral cell layer
DDC	dopa decarboxylase	Met-Enk	methionine-enkephalin
2-DG	2-deoxyglucose	NA	noradrenaline
DNA	desoxyribonucleic acid	NFP	neurofilament protein
DOPAC	3,4-dihydroxy-phenylacetic acid	nHDB	nucleus of the horizontal limb of the diagonal band of Broca
E	embryonic day	nLOT	nucleus of the lateral olfactory tract
EEG	electroencephalogram	NMDA	N-methyl-D-aspartate
ENK-7	(Met)enkephalyl-Arg ⁶ -Phe ⁷ (an opioid heptapeptide)	NPY	neuropeptide Y
EOG	electro-olfactogram	NSE	neuron-specific enolase
EPL	external plexiform layer	OB	olfactory bulb
GABA	γ -aminobutyric acid	OG	oligodendroglial cell
GABA-T	GABA-transaminase	6-OHDA	6-hydroxydopamine
GAD	glutamic acid decarboxylase	OMP	olfactory marker protein
		ON	olfactory nerve

OT	olfactory tubercle	SP	substance P
P	postnatal day	TH	tyrosine hydroxylase
PC	piriform cortex	TN	terminal nerve
pcd	Purkinje cell degeneration	TRH	thyrotropin-releasing hormone
PG	periglomerular cell	VIP	vasoactive intestinal polypeptide
RIA	radioimmunoassay	VP	vasopressin
RNA	ribonucleic acid	WGA-HRP	wheat germ agglutinin-horseradish peroxidase
S-100	S-100 protein (a non-neural marker)		
SA	short-axon cell		
SOM	somatostatin		

CHAPTER 1: NASOCHEMOSENSORY SYSTEMS: MAIN OUTLINES (AN INTRODUCTION)

The chemical senses communicate an indispensable set of information for all living organisms, from bacteria to man. Without careful alignment of the metabolism of a single cell or multicellular organism to changing external and internal conditions, survival cannot be imagined. Such a control presumes immediate and continuous flow of information, its evaluation and implication in adaptive behavior.

Although there are obvious differences in the architecture of the chemosensory systems between unicellular, invertebrate and vertebrate organisms, similarities in signal detection, transduction processes, the association of chemoreception with immunorecognition, etc., all constitute undeniable evidence for common fundamental principles in organization. Phylogeny resulted in the highest olfactory sensitivity in insects (to sex pheromones), but certain vertebrates like fish and dogs also have an extreme acuity for the perception of certain odorants.

A major part of external stimuli are detected through chemosensation in less developed creatures, but taste and smell are essential also in vertebrate life during fundamental processes, such as feeding, reproduction and social relations. Among chemical senses those with their receptors localized in the nasal region play (in collaboration with gustatory reception) a particularly important role in the detection, finding and evaluation of food, mates, conspecifics and harmful external conditions. To serve the various aspects of these functions, a certain specialization of nasal chemoreceptive regions and their related central regions has developed in vertebrates as will be briefly summarized below, and discussed more extensively in the following chapters.

As this monograph is based on a structural perspective, morphological (see Section 1.1.) and neurochemical (see Section 1.2.) aspects will be primarily emphasized and correlated with selected physiological, developmental and behavioral aspects (see Sections 1.2., 1.3.).

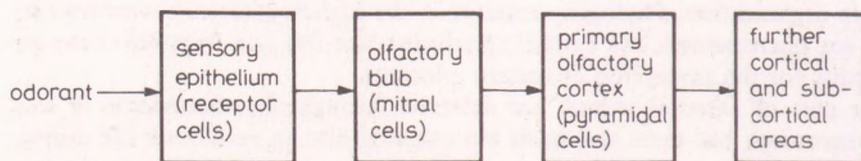
The specific external conditions (food availability, climate, etc.) of a given vertebrate species, and the degree of contribution of other senses to the adaptation of the organism, resulted in different complexities of these systems during phylogeny and created a rich world of variety. This variety, as reflected in the nasal chemosensory systems, is still far from clear as regards their structure and significance; a full presentation of the available data extends far beyond the potential and intentions of this book. A short outline on these aspects discussed in more detail in Chapters 2-9, will follow below.

1.1. Structural segregation of the nasal chemosensory systems and their central representations

The inner surface of the nasal region is lined by a mucous covered epithelium, parts of which are specialized primarily for the detection of chemical stimuli. These areas are rich in receptor cells, while the remaining regions (actually the major part) serve respiration. Nevertheless, the whole nasal mucosa may possess a certain degree of chemosensory capacity, especially for painful, irritant chemicals; these can be detected by the trigeminal nerve branches. The possible chemosensory role of the terminal nerve fibers is also under investigation (in this respect see Sections 7.3. and 7.4.).

Among the specialized receptorial fields of the nasal mucosa, the main olfactory epithelium is ubiquitously (although in certain species, only rudimentarily) present. Epithelial receptors project to the olfactory bulb and, after being relayed here, further on to the major part of the basal forebrain.

Transmission of olfactory information from the periphery to olfaction-related central brain areas may be depicted in a simplified form as follows:



Detailed characterization of this pathway constitute the central part of this book, from Chapters 2 to 6. The main olfactory bulb also seems to relay and process further information from another group of nasal (septal) chemosensory cells, those from the organ of Masera (where this special organ exists, see Section 7.2.).

Another primary nasal chemoreceptive pathway derives from the vomeronasal (Jacobson's) organ, which exists in most vertebrates. The primary fibers from this region are relayed in the accessory olfactory bulb and are directed to a restricted amygdaloid area of the forebrain (see Section 7.1.). Further limbic and neocortical connections of the main and accessory olfactory systems provide pathways towards other sensory, associational and motor areas of the brain.

1.2. Neurochemical, functional and developmental aspects

Most of the related studies were performed on the main olfactory system, or were not specified for a particular component of one single (i.e., main, vomeronasal or septal) nasal chemosensory system. The great importance of these neurochemical achievements will, however, be stressed throughout this book as they constitute the most obvious link between functional events and morphological findings. As we shall see, the olfactory organ and its representation in the brain (especially the olfactory bulb) provides an excellent model system for revealing this bridging role of neurochemistry.

Major questions of this field are: how the chemical stimuli are detected by the receptors, how are they transformed into neurochemically coded, odorant-

characteristic information, how are these signals transmitted to the primary and further olfactory-related brain areas and what is the basis of the sensory and perceptual discrimination of odors?

With respect to the odorant detection, many theories have been proposed (for a review, see Ottoson, 1963a). The most probable conceptions emphasize the significance of the stereochemical characteristics of odorants, trapped by specified molecular (proteinaceous) receptors, which—through a cascade of molecular events, involving cyclic nucleotides—may lead to changes in membrane potential (see Sections 2.3., 2.4.). An action potential is initiated, and the signal is transmitted to the first relay station, the bulbar complex and to further central brain areas by chemical neurotransmission (see Sections 2.3., 4.3., 5.2.); in turn, many central brain areas modulate this sensory processing (see Sections 4.2.3., 4.3.7., Chapter 6, Table 4.2.). A degree of spatio-temporal patterns in coding odor qualities was recognized at the periphery and in most of the central projections, providing anatomical and functional grounds for sensory discrimination. Field potential and intracellular recordings revealed odorant-elicited electrical activity of the respective neurons along the whole olfactory pathway and contributed significantly to our understanding of the processing of information, conveyed by this modality, to the brain (see Sections 2.4., 4.4.).

Both the olfactory epithelium and bulb develop from the olfactory placode and may become functionally active before birth or shortly thereafter when other senses are still immature. The epithelial region undergoes a continuous renewal throughout the life, because it preserves its regenerative capability (see Section 2.5.). A high capacity for plastic regenerative changes was also established for centers along the olfactory pathway (see Sections 4.5., 5.3., 6.1.7. and 7.1.4.).

1.3. Behavioral significance of nasal chemoreception and cooperation with other sensory modalities

For most vertebrates, nasal chemoreception constitutes the most effective sensory system for distant recognition of external factors determining or strongly influencing the conditions of their survival (see Chapter 8). The main olfactory system was implicated mainly in feeding-related behavior and self-preservation, the accessory olfactory system in reproductive processes, while a contribution of both systems may be involved in directing emotional, social and other adaptive processes. In this context, cooperation of the main olfactory system with the gustatory and visual systems, while cooperation of the accessory olfactory system with the endocrine system seems most plausible.

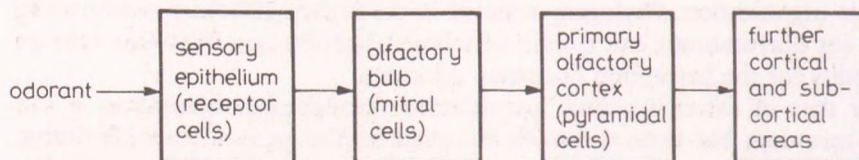
Finally, significance of the nasal chemosensory signals in a representative of the vertebrates (which, incidentally may not be the most interesting for many of us), man, will be also briefly surveyed (see Chapter 9). Although other sensory modalities may more significantly influence human life, we are still dependent on olfactory stimuli. This provides us with warnings about dangerous chemicals or air pollutants, but more pleasantly, the anticipation of the joy of some delicious food or drink, a freshly blooming rose, or just plain air, clear and refreshing—all being a joyful part of living nature on Earth.

1.1. Structural segregation of the nasal chemosensory systems and their central representations

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1.2. Neurochemical, functional and developmental aspects

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Both the olfactory epithelium and bulb develop from the olfactory placode and may become functionally active before birth or shortly thereafter when other senses are still immature. The epithelial region undergoes a continuous renewal throughout the life, because it preserves its regenerative capability (see Section 2.5.). A high capacity for plastic regenerative changes was also established for centers along the olfactory pathway (see Sections 4.5., 5.3., 6.1.7. and 7.1.4.).

1.3. Behavioral significance of nasal chemoreception and cooperation with other sensory modalities

For most vertebrates, nasal chemoreception constitutes the most effective sensory system for distant recognition of external factors determining or strongly influencing the conditions of their survival (see Chapter 8). The main olfactory system was implicated mainly in feeding-related behavior and self-preservation, the accessory olfactory system in reproductive processes, while a contribution of both systems may be involved in directing emotional, social and other adaptive processes. In this context, cooperation of the main olfactory system with the gustatory and visual systems, while cooperation of the accessory olfactory system with the endocrine system seems most plausible.

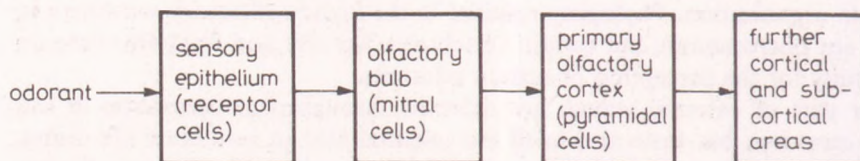
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1.1. Structural segregation of the nasal chemosensory systems and their central representations

The inner surface of the nasal region is lined by a mucous covered epithelium, parts of which are specialized primarily for the detection of chemical stimuli. These areas are rich in receptor cells, while the remaining regions (actually the major part) serve respiration. Nevertheless, the whole nasal mucosa may possess a certain degree of chemosensory capacity, especially for painful, irritant chemicals; these can be detected by the trigeminal nerve branches. The possible chemosensory role of the terminal nerve fibers is also under investigation (in this respect see Sections 7.3. and 7.4.).

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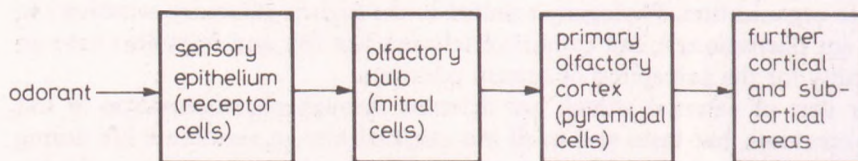
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CHAPTER 2: RECEPTOR LEVEL OF OLFACTION

Nasal chemoreception in vertebrates covers detection of volatile and non-volatile compounds of various physiological and behavioral significance. Diverse purposes and importance led to the development and territorial separation of different sensory areas: the main olfactory epithelium, the vomeronasal organ and the septal organ, while the trigeminal receptors may be more dispersed all over the inner lining of the nose. Significance of the vomeronasal, septal and trigeminal sensation is far from clearly understood; in many vertebrates, even anatomical proof of their presence is lacking (see also Chapter 7). In contrast, much interest has been shown in the olfactory receptors as reflected by the numerous original observations, communications and extensive reviews. In this Chapter these results will be summarized.

2.1. Anatomy

The olfactory receptor field can most clearly be recognized by the presence of the olfactory receptor neurons; such neurons are absent from the respiratory epithelial area. In *Amblystoma*, a simple depression serves as the olfactory receptor organ, by giving room to the chemoreceptive neurons, while in *Cyclostomata* paired nasal pits can already be found. In *Amphibians*, the receptor cells are grouped in a well circumscribed area of the paired nasal conchae, most characteristically in its dorsocaudal region (see Allison, 1953*b*). In most vertebrates and man, complex, convoluted, cartilaginous lamellae (turbinalia) and the septal wall support the enlarged area (from several up to more than 150 cm², see Adams, 1972) of the olfactory epithelium.

Macroscopically, the receptor area can be recognized by its yellowish color, different from the neighbouring (pinkish) respiratory mucosal area. The significance of this yellow pigment is unclear as yet (see Gerebtzoff and Schkapenko, 1952; Allison, 1953*b*; Moulton and Beidler, 1967; Moulton, 1971). Another difference is that the olfactory epithelium is regularly thicker than the respiratory epithelium. In aquatic animals, the mucosal area is bathed in water, while in the terrestrial animal glands provide fluids which spread on the surface of the mucosa. The characteristic gland of the olfactory receptor region was described first and named after Bowman, but other nasal (and lacrimal) glands also contribute to the moistening of the sensory epithelium. The Bowman gland is missing from the vomeronasal mucosa.

2.2.Histology

2.2.1. Light microscopy of the olfactory epithelium

Although the nose region of the vertebrates is highly variable according to the differences in habitats, feeding and other conditions, the olfactory mucosa shows a remarkable constancy in architecture and cellular components. It can be divided into two layers, the neuroepithelial layer and the lamina propria, while the surface of the epithelium is covered by a viscous mucus film.

In the lamina propria, blood and lymphatic vessels are distributed within a loose connective tissue; alveoli of the Bowman glands and Schwann cell bodies (providing the interfascicular insulation for the receptor cell axons) are also situated here. The thin basal membrane, which separates the lamina propria from the epithelial layer, is

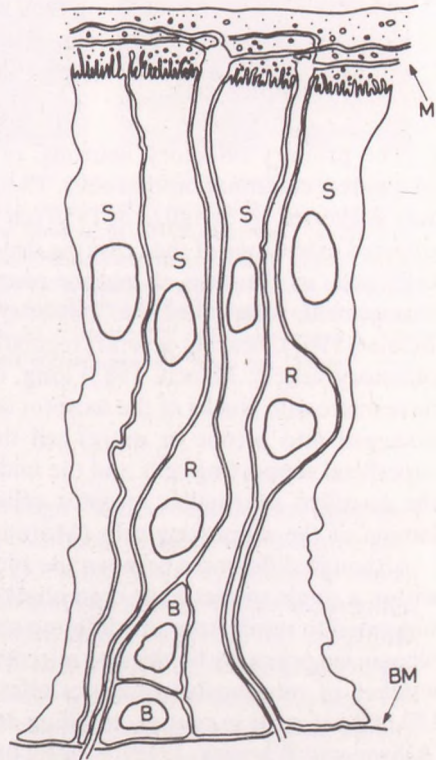


Fig. 2.1. Main cell types of the olfactory epithelium. Supporting cells (S) provide the uppermost nuclear sheet of the layer. Luminal surfaces of the cells are densely covered with microvilli (embedded into a mucus layer, M), while their lower extension lies on the basal membrane (BM). Basal cells (B) are sitting on the basement membrane, or gradually approach the level of the receptor cells (R), into which they are transformed. The most distal enlargements (knobs) of the mature receptor cell dendrites release several long cilia into the mucus, while the olfactory nerve axons, by penetrating the basal membrane, leave the epithelial layer in a central direction. For further details see text

penetrated by ducts of the Bowman glands (which finally open to the epithelial surface) and bundles of the receptor cell axons (see Fig. 3.1).

The three main cell types of the pseudostratified, columnar neuroepithelium were recognized in the middle of the nineteenth century (Kleerekoper, 1982) and were characterized by many authors (see for reviews, Cajal, 1911; Allison, 1953*b*; Baradi and Bourne, 1953; Moulton and Beidler, 1967; Graziadei, 1971; Andres, 1975). These cells are the primary receptor cells, the supporting (sustentacular) cells and the basal cells (Fig. 2.1). Occasionally mast cells may also be observed (see Saini and Breipohl, 1977), while rhinitis is accompanied by the appearance of polymorphonuclear leucocytes in the mucus and epithelium (Hinds et al., 1984).

Based on light- and electron microscopic observations, a columnar arrangement of the main cell types, including a limited number of sensory neurons, basal cells and the sustentacular cells, supporting them, has been recognized in mouse, and was suggested to constitute a unit in organization of the sensory epithelium (Graziadei and Monti Graziadei, 1979).

2.2.1.1. Receptor cells

The primary olfactory neurons, or receptor cells of the olfactory mucosa, are elongated, columnar bipolar cells. Their connection with the tiny, deep-directed axons was suggested by Schultze in 1856 (cit. Allison, 1953*b*). The other, rod-like, surface-directed extensions of the cells (the dendrites) follow the gaps between the supporting cells and, in the case of mature receptor neurons end with a knob-like terminal enlargement, were called the "olfactory vesicle" by Stricht in 1909 (cit. Moulton and Beidler, 1967), bearing several (regularly 3–15, but extending from 1 even to 150 per olfactory vesicle; Menco, 1983) long, immotile cilia. Also the olfactory vesicle may have microvilli. Nuclei of the receptor cells are discretely and tightly packed (Fig. 2.2), arranged into a (one or more) cell thick lamina, between the nuclei of the more superficial supporting cells and the underlying basal cells. The more bulky somata of the so-called microvillar receptor cells in humans lie more superficially above the lamina of the supporting cells (Moran et al., 1982*b*).

Although differences between the ciliated receptor cells of various species and even within a single species have been observed frequently, a large part of these were later suggested to result from the differing maturities of these neurons (see below), or were recognized primarily by electron microscopy, as discussed in the following section. The number of recognizable (mature) olfactory receptors was calculated to be around 120,000 per mm² in rabbits, totalling around 50×10^6 in each side of the nasal cavity (Allison and Warwick, 1949). In adult humans, this number might be around $6-7 \times 10^6$ as can be inferred from the studies of Moran et al. (1982*b*). Dendritic endings per cm² occurred in comparable density, as was found for the frog (cca. 4.5×10^6), rat (8.3×10^6), dog (6.0×10^6) or ox (7×10^6) (Menco, 1980).

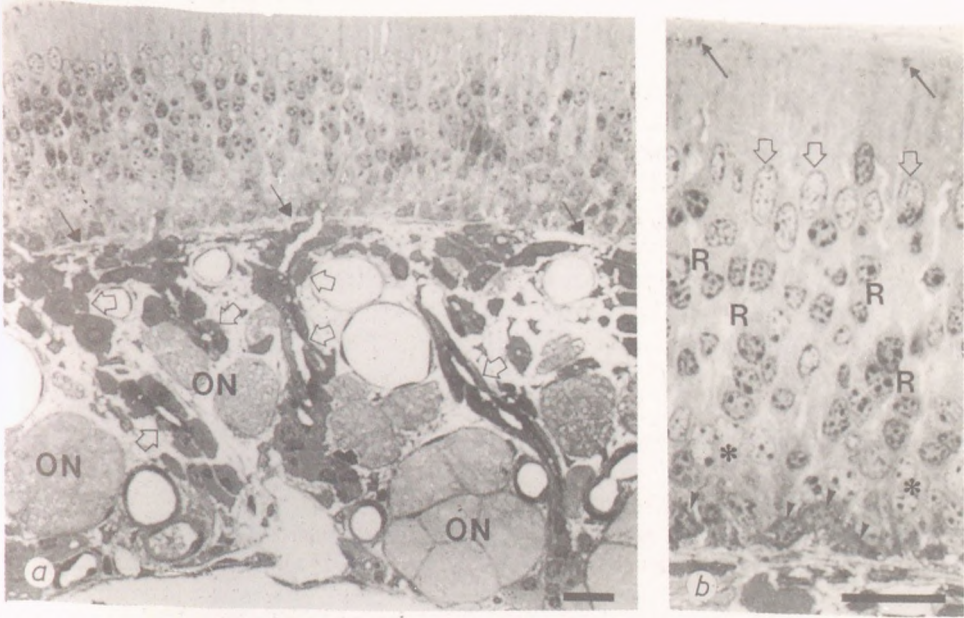


Fig. 2.2. Cross-sectional view of the olfactory mucosa of rat. Light micrograph, 2 μ m thick epoxy section, toluidine blue staining. *a*: At the deep aspect of the mucosa the loose connective tissue of the lamina propria includes fascicles of the olfactory nerve (ON), large vessels and Bowman glands (open arrows); the ducts of the latter cross the basal membrane (arrows), enter the epithelium and ascend to open on the surface. Scale bar, 20 μ m. *b*: high power light micrograph of the epithelium shows the main cell types: dark, deep basal cells (arrowheads), globose basal cells (asterisks) with lighter nuclei, followed above by the region of the mature receptor cells (R) and the layer of the elongated, larger nuclei of supporting cells (open arrows). The uppermost layers are free of nuclei; a few olfactory knobs are stained (arrows). Scale bar: 20 μ m

2.2.1.2. Supporting cells

Supporting (or sustentacular) cells traverse through the neuroepithelium from the basal lamina up to the mucus. The nuclei are positioned between the receptor cell nuclei and the mucosal surface (see Fig. 2.2), while their cytoplasmic mass predominate in the upper aspect of the layer. The mucosal surface of the supporting cells is also covered by cytoplasmic processes: microvilli are common, but in certain species (motile) cilia have also been observed (see Muller and Marc, 1984). Due to this, they may be mistaken for receptor cells, if one studies only the mucous surface. Of course, supporting cells do not have axons, as is evident from cross-sectional views.

Two types of supporting cells were identified recently by Golgi impregnation (Rafols and Getchell, 1983). Type I had an unbranched central stalk between the cell body and the basilar expansion, while the central stalk of the type II supporting cell had many rib-like processes, releasing numerous cytoplasmic veils to envelop receptor cell bodies.

Secretory granules within the supporting cells, regularly in the vicinity of the mucosal surface may also be recognized, as has been published in the frog (Bloom, 1954; Graziadei and Bannister, 1967).

2.2.1.3. Basal cells

These cells are the only cells of the neuroepithelium which are not elongated, and so, do not traverse through the layer; they are localized between the basal lamina and the nuclear region of the receptor cells. These stumpy cell bodies may appear in darker and lighter versions, as observed with toluidine blue staining (see Fig. 2.2): dark cells sit regularly at the bottom of the epithelial layer, contacting the basal lamina with their most expanded side, while the light ("globose") basal cells appear somewhat higher, close to the nuclei of the receptor neurons, into which they are thought to differentiate (see Nagahara, 1940; Andres, 1966; Graziadei and Metcalf, 1971; Moulton, 1974; Graziadei and Monti Graziadei, 1979; Hinds et al., 1984; Vollrath et al., 1985).

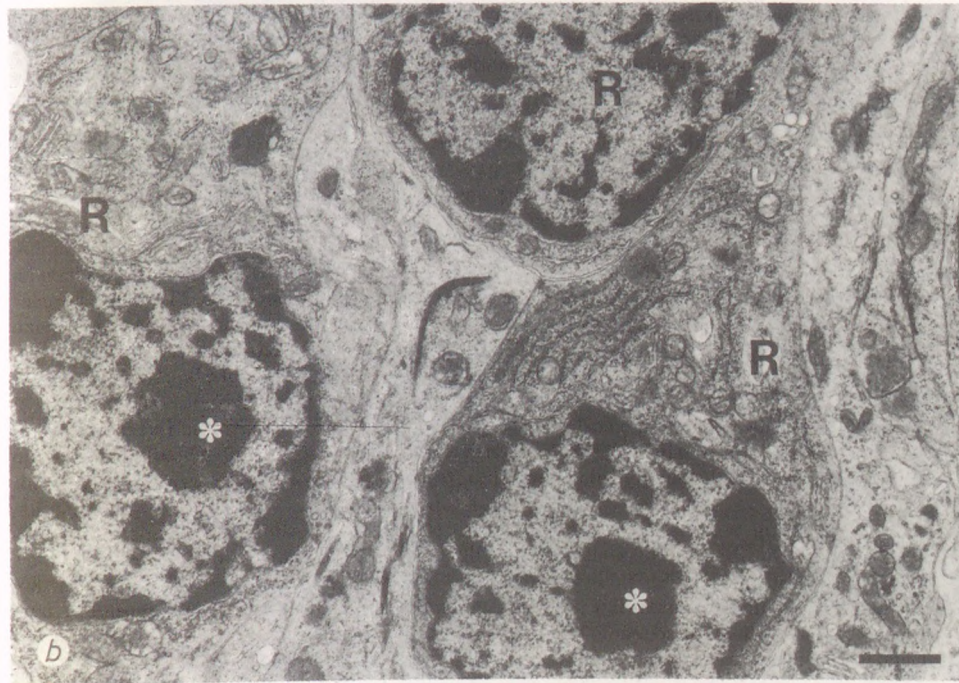
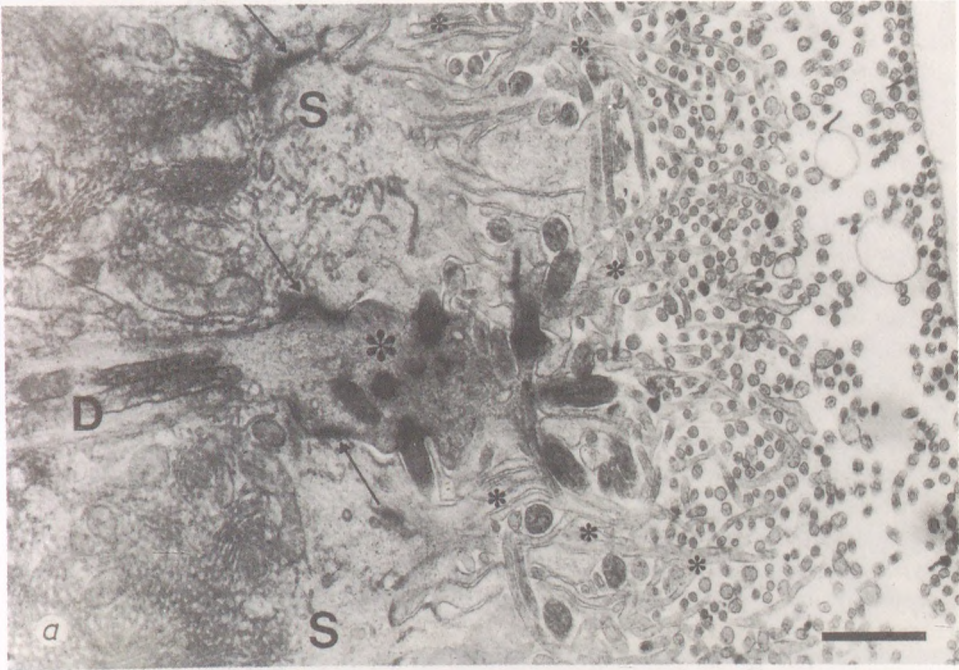
2.2.2. Electron microscopy of the olfactory epithelium

The relatively thin olfactory epithelium is a very suitable specimen for good fixation, required in electron microscopy and has been the subject of many ultrastructural studies, uncovering fine structural details of all main cell types of the layer in different vertebrate species from fish to man. Several subtypes of receptor cells have been established, as ciliar (in fish: type I and II) and microvillar cells. Sensory cells with rod-like peripheral processes have also been described ("rod cells"), but it seems that the rod is the result of fusion of the cilia of degenerating ciliar cells (Muller and Marc, 1984), thereby they will not be included in the following discussion. Because of the most frequent appearance throughout the vertebrate species and suggested primary importance in perception of olfactory signals, ciliated receptor cells will be most extensively discussed here, while other receptor cells, as well as supporting and basal cells will only be briefly mentioned.

2.2.2.1. Ciliar receptor cells

These receptor cells have been extensively studied and were found to show a remarkable similarity in most subcellular characteristics (see Bloom, 1954; DeLorenzo, 1957; Reese, 1965; Andres, 1966, 1975; Graziadei, 1966, 1971; Graziadei and Bannister, 1967; Thornhill, 1967; Cushieri and Bannister, 1975*b*; Loo, 1977;

Fig. 2.3. Details of the rat olfactory epithelial surface (*a*) and somata of receptor cells (*b*) on electron micrographs. Aldehyde and osmium fixation, lead citrate staining. *a*: the olfactory knob (large asterisk), the distal enlargement of the dendritic process (D) contains basal bodies and gives rise to several cilia. Distal parts of these cilia approach the surface of the mucus (see right side), while decreasing in thickness and number of filaments. Supporting cells (S) are extremely rich in organelles (especially microtubules) at the upper level of the receptor dendrite. Above this zone, tight junctions (arrows) provide close attachment between cellular components reaching the surface. A few microvilli of supporting cells are labeled with small asterisks. Scale bar, 1 μ m. *b*: Dense patches of the chromatin material and nucleoli (white asterisks) characterize the nuclei of the receptor cell somata (R). The majority of the cytoplasmic organelles are accumulated distal (upwards in this picture) from the nuclei. For more details see the text. Scale bar: 1 μ m



Graziadei and Monti Graziadei, 1979; Moran et al., 1982*b* and others). There may be, however, a continuous change in appearance and even content of organelles, according to the aging of the (continuously regenerating) receptor cells, as illustrated by Graziadei and Monti Graziadei (1979). During aging of the cell, a darkening of the ground plasma, swelling of the cisternal organelles, increase of lysosomal activity and on-going clumping of the nuclear chromatin material is evident. A "typical", mature olfactory receptor shows, however, several more stable ultrastructural characteristics.

The level at which the nucleus lies (Fig. 2.3) is too narrow to contain spacious systems of organelles, like Golgi apparatus and stacks of rough endoplasmic reticulum, ordered into Nissl-bodies. These organelles can be observed regularly distal to the nuclei. Mitochondria and free ribosomes are present throughout the elongated perikarya. Filaments are sparse, tubules are also rare, but always appear at the origin and within the two processes of the cell: the axons (see later in Chapter 3) and the dendrite.

The dendrites of the receptor cells are rich in mitochondria and tubules (and also smooth endoplasmic reticular vesicles), in the vicinity of their distal end. Numerous centrioles and basal bodies with similar ultrastructure (Reese, 1965) can be found, most characteristically, in the olfactory vesicle. Just below this knob-like enlargement, there is a constriction of the dendritic rod, where the dendrite is closely attached to supporting cell membranes by tight junctions (Graziadei, 1971; Kerjaschki and Hörandner, 1976; see also Fig. 2.3).

The olfactory vesicle provides the base for the cilia, the protoplasmic extension of most olfactory receptor cells, and has warranted much interest among investigators of the peripheral olfactory system. We have to note here, however, that microvilli may also be interspersed with cilia on the mucous surface of the ciliated receptor cells, as exemplified in the duck (Graziadei and Bannister, 1967) or in fish in the type II ciliar cell of Muller and Marc (1984) (for reviews, see also Graziadei, 1971; Yamamoto, 1982).

Both the internal structure and surface characteristics of cilia of the vertebrate olfactory organ have been extensively studied by transmission and scanning electron microscopy (Fig. 2.4), as well as by freeze-substitution and freeze-etching (Bloom, 1954; Reese, 1965; Andres, 1966, 1969, 1975; Moulton and Beidler, 1967; Thornhill, 1967; Graziadei, 1971; Breipohl et al., 1973, 1982; Kerjaschki and Hörandner, 1976; Menco et al., 1976; Moran et al., 1982*b*; Menco, 1983, 1984; Lidow and Menco, 1984; Muller and Marc, 1984).

The length of cilia varies from species to species, e.g. in lamprey they are just 5–6 μm long (Thornhill, 1967), while in frogs they may extend up to 200 μm (Reese, 1965). They exhibit a thicker proximal part (approx. 0.20–0.25 μm in diameter) and a thinner (0.08–0.15 μm) distal segment, resulting in a continuous decrease in diameter, as found in certain fish (Breipohl et al., 1973) and the tiger salamander (Breipohl et al., 1982), or a sudden constriction in other species (Reese, 1965; Andres, 1966; Moulton and Beidler, 1967; Thornhill, 1967).

At the level of the basal body in the olfactory vesicle, 7–9 triplets of subfibers (tubules) are stabilized by fine basal foot filaments. The fibers continue further in the proximal segment, which shows the typical ciliary arrangement of nine pairs of peripheral and two central tubules (Fig. 2.5). In the frog, the number of the distal

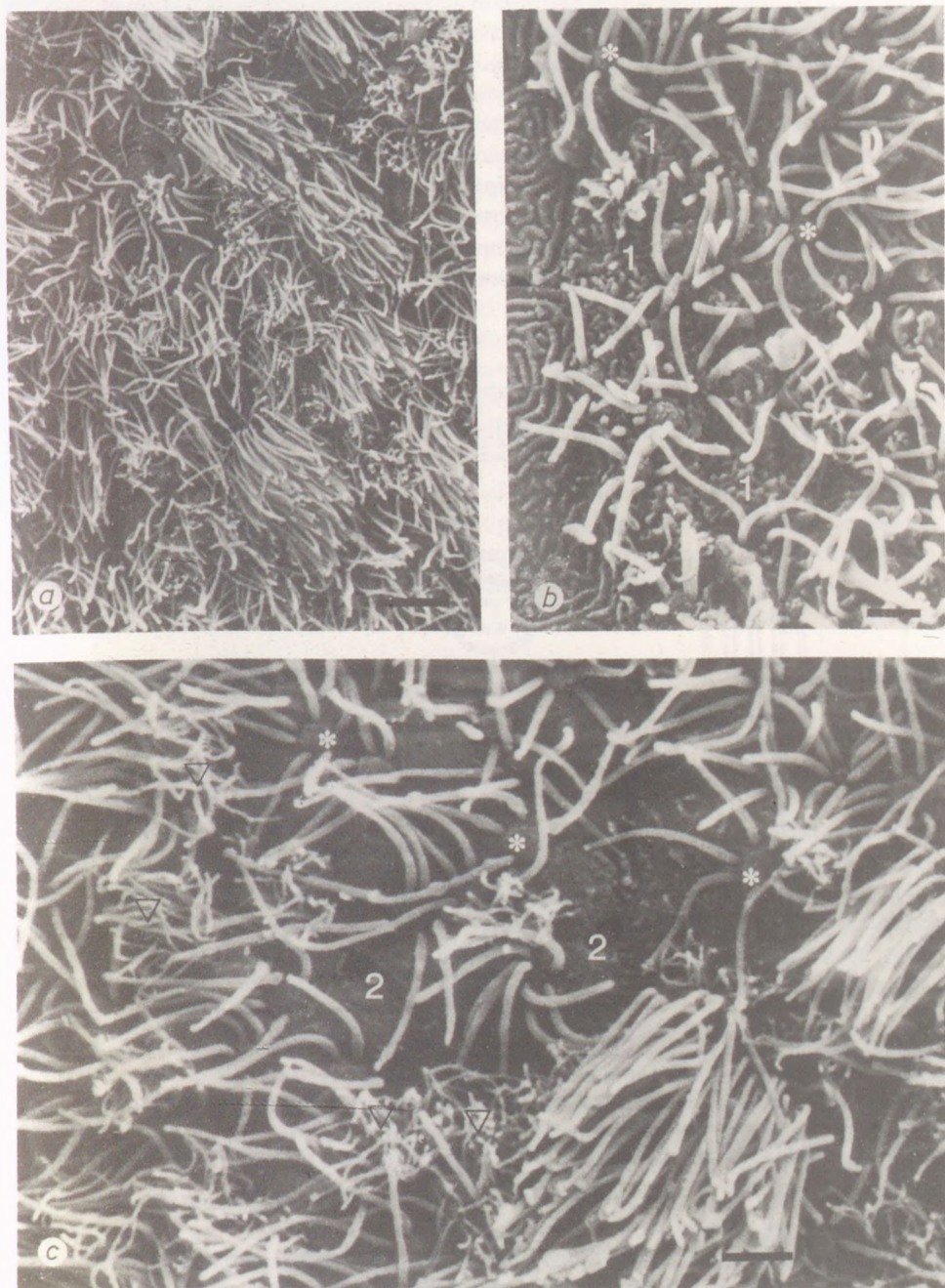


Fig. 2.4. Scanning electron microscopy of the olfactory receptive surface of the ablen (*Alburnus alburnus*), after removing the mucus. By courtesy of Dr.L. Hernádi. *a*: low power picture shows a dense covering of the apical surface of ciliated and microvillar receptor cells. Part of this picture is enlarged in *c*. Scale bar: 5 μ m. *b* and *c* show details, e.g., olfactory knobs (small white asterisks) and cilia of (ciliated) receptor cells, microvillar receptors (black triangles), supporting cell surface (1) and openings of glandular ducts (2). Scale bars: 2 μ m

fibers, may be similar (nine single peripheral and two single central tubules), or slightly less (in one third of the cases investigated, Reese, 1965), or may significantly be reduced (even to two at the most distal parts), as found, e.g., for rats (see Fig. 2.2), dogs and cats (Andres, 1966, 1969, 1975; Lidow and Menco, 1984).

During the course of the distal segment, cilia may become flattened and their axonemal structures (tubules) may be separated by double membrane folds, then unite again. Vesicular enlargements may also appear (Reese, 1965; Andres, 1969; Menco et al., 1976; Menco, 1983, 1984). The ultrastructure of the axonemes, number of tubules, lack or presence of arms and spokes, as connecting structures for the tubules does not seem essential for the olfactory transduction process. They are missing in the proximal

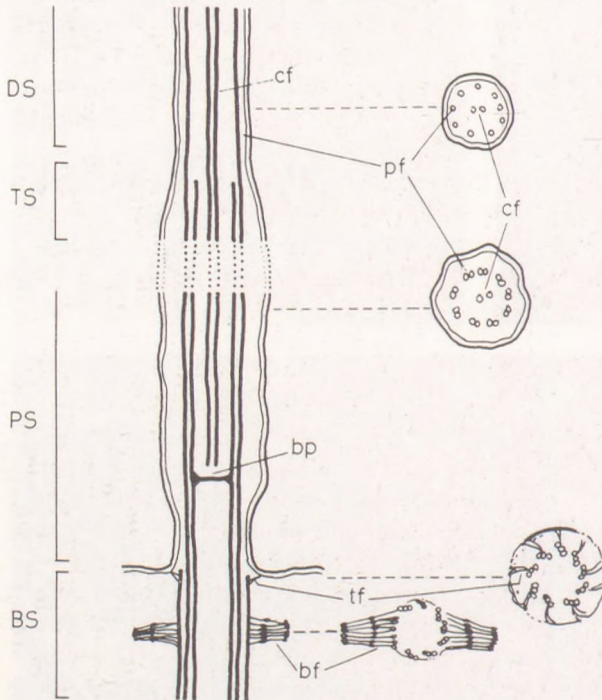


Fig. 2.5. Diagrammatic representation of olfactory ciliary substructure. The basal (BS), proximal (PS) and beginning of the distal (DS) segments are depicted in some detail (slightly modified after Thornhill, 1967). Left side: longitudinal section; right side: transectional view. At the base, in the olfactory knob, triplets of tubules are attached by basal foot (bf) filaments and transition fibers (tf). Above the basal plate (bp) a pair of central fibers (cf) and surrounding peripheral fibers (pf) can be seen; these latter, leaving the tapering segment (TS) continue their course as single tubules. For more details, see text. Reproduced with permission

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The olfactory ciliary membrane surface is rich in intramembrane particles, observed by freeze-etching first by Kerjaschki and Hörandner (1976) in mouse and Menco et al. (1976) in the frog epithelium. Their number varied from species to species, but was in

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Based mostly on studies investigating the mucosal surface of the fish olfactory epithelium, an axon-bearing, ciliated sensory cell type with an additional (not only receptor) function, similar to the nasal respiratory epithelial cells of tetrapods was also described (type I ciliar cell of Yamamoto and Ueda; see Yamamoto, 1982; or type II ciliar cell of Muller and Marc, 1984). They may occur single or grouped, interspersed with other sensory cells (goldfish), or segregated from the latter (as in catfish). They have 30–60 (motile), synchronously beating cilia (10–15 μm in length); their axons reach the olfactory bulb in *Carassius* and *Ictalurus*, like those of the microvillar and “regular” ciliated receptor cells.

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2.2.2.2. Microvillar receptor cells

Such receptors are much less numerous than the ciliar receptors, although not uncommon in fish (Bannister, 1965; Breipohl et al., 1973; Andres, 1975; Yamamoto, 1982; Erickson and Caprio, 1984; Muller and Marc, 1984) (see also Fig. 2.4) and in the human olfactory epithelium (Moran et al., 1982*a,b*), and have occasionally been observed in cats (Andres, 1969) and in the treeshrew as reported by Loo (1977). In man, the somata are flask-shaped and shorter than those of the ciliated receptor cells but most of the cytoplasm, including the Golgi apparatus, exhibits a supranuclear localization. The ground plasma is paler than that of the surrounding ciliated receptor or supporting cells, although rich in smooth endoplasmic cisterns and tubules; mitochondria and microfilaments are commonly found.

In human material it has been found that an axon-like, slender, centrally-directed process leaves the cell. The axonal nature of such processes and the identification of the microvillar cells as real sensory neurons in the fish olfactory epithelium, have been experimentally verified: goldfish microvillar cells degenerated following olfactory nerve transections. Also, both in the goldfish and catfish, such cells could be filled with horseradish peroxidase (HRP), injected into the olfactory bulb (Muller and Marc, 1984).

In fish, the apical part of the microvillar cells seemed to be more elongated and rich in mitochondria. These cells regularly gave rise to over 100, structurally uniform, 0.1 μm thick and 2–3 μm long, microvilli.

Graziadei and Monti Graziadei, 1979; Moran et al., 1982*b* and others). There may be, however, a continuous change in appearance and even content of organelles, according to the aging of the (continuously regenerating) receptor cells, as illustrated by Graziadei and Monti Graziadei (1979). During aging of the cell, a darkening of the ground plasma, swelling of the cisternal organelles, increase of lysosomal activity and on-going clumping of the nuclear chromatin material is evident. A "typical", mature olfactory receptor shows, however, several more stable ultrastructural characteristics.

The level at which the nucleus lies (Fig. 2.3) is too narrow to contain spacious systems of organelles, like Golgi apparatus and stacks of rough endoplasmic reticulum, ordered into Nissl-bodies. These organelles can be observed regularly distal to the nuclei. Mitochondria and free ribosomes are present throughout the elongated perikarya. Filaments are sparse, tubules are also rare, but always appear at the origin and within the two processes of the cell: the axons (see later in Chapter 3) and the dendrite.

The dendrites of the receptor cells are rich in mitochondria and tubules (and also smooth endoplasmic reticular vesicles), in the vicinity of their distal end. Numerous centrioles and basal bodies with similar ultrastructure (Reese, 1965) can be found, most characteristically, in the olfactory vesicle. Just below this knob-like enlargement, there is a constriction of the dendritic rod, where the dendrite is closely attached to supporting cell membranes by tight junctions (Graziadei, 1971; Kerjaschki and Hörandner, 1976; see also Fig. 2.3).

The olfactory vesicle provides the base for the cilia, the protoplasmic extension of most olfactory receptor cells, and has warranted much interest among investigators of the peripheral olfactory system. We have to note here, however, that microvilli may also be interspersed with cilia on the mucous surface of the ciliated receptor cells, as exemplified in the duck (Graziadei and Bannister, 1967) or in fish in the type II ciliar cell of Muller and Marc (1984) (for reviews, see also Graziadei, 1971; Yamamoto, 1982).

Both the internal structure and surface characteristics of cilia of the vertebrate olfactory organ have been extensively studied by transmission and scanning electron microscopy (Fig. 2.4), as well as by freeze-substitution and freeze-etching (Bloom, 1954; Reese, 1965; Andres, 1966, 1969, 1975; Moulton and Beidler, 1967; Thornhill, 1967; Graziadei, 1971; Breipohl et al., 1973, 1982; Kerjaschki and Hörandner, 1976; Menco et al., 1976; Moran et al., 1982*b*; Menco, 1983, 1984; Lidow and Menco, 1984; Muller and Marc, 1984).

The length of cilia varies from species to species, e.g. in lamprey they are just 5–6 μm long (Thornhill, 1967), while in frogs they may extend up to 200 μm (Reese, 1965). They exhibit a thicker proximal part (approx. 0.20–0.25 μm in diameter) and a thinner (0.08–0.15 μm) distal segment, resulting in a continuous decrease in diameter, as found in certain fish (Breipohl et al., 1973) and the tiger salamander (Breipohl et al., 1982), or a sudden constriction in other species (Reese, 1965; Andres, 1966; Moulton and Beidler, 1967; Thornhill, 1967).

At the level of the basal body in the olfactory vesicle, 7–9 triplets of subfibers (tubules) are stabilized by fine basal foot filaments. The fibers continue further in the proximal segment, which shows the typical ciliary arrangement of nine pairs of peripheral and two central tubules (Fig. 2.5). In the frog, the number of the distal

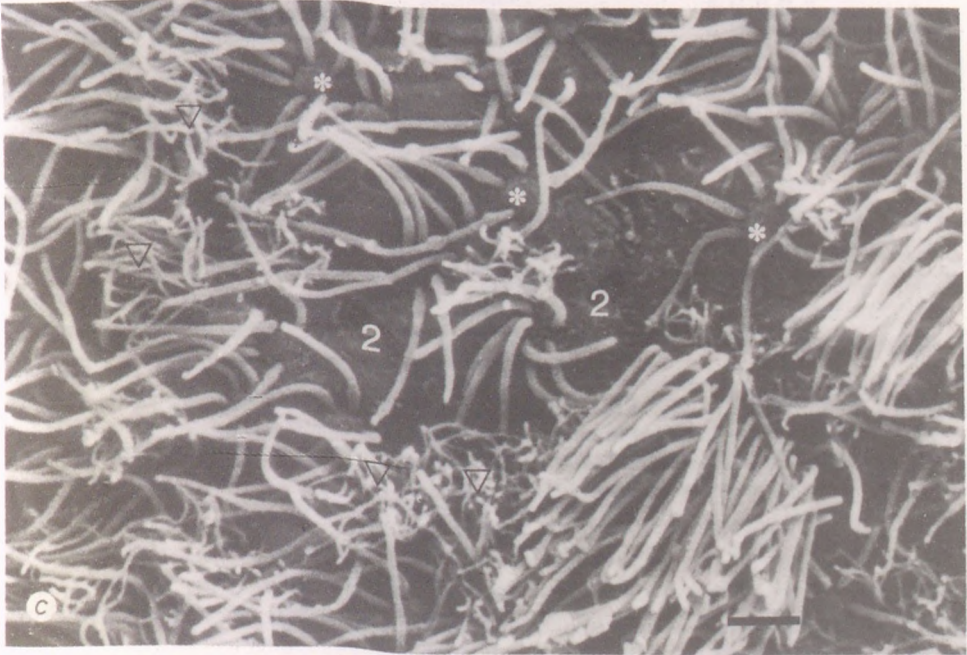


Fig. 2.4. Scanning electron microscopy of the olfactory receptive surface of the ablen (*Alburnus alburnus*), after removing the mucus. By courtesy of Dr. L. Hernádi. *a*: low power picture shows a dense covering of the apical surface of ciliated and microvillar receptor cells. Part of this picture is enlarged in *c*. Scale bar: 5 μ m. *b* and *c* show details, e.g., olfactory knobs (small white asterisks) and cilia of (ciliated) receptor cells, microvillar receptors (black triangles), supporting cell surface (1) and openings of glandular ducts (2). Scale bars: 2 μ m

fibers, may be similar (nine single peripheral and two single central tubules), or slightly less (in one third of the cases investigated, Reese, 1965), or may significantly be reduced (even to two at the most distal parts), as found, e.g., for rats (see Fig. 2.2), dogs and cats (Andres, 1966, 1969, 1975; Lidow and Menco, 1984).

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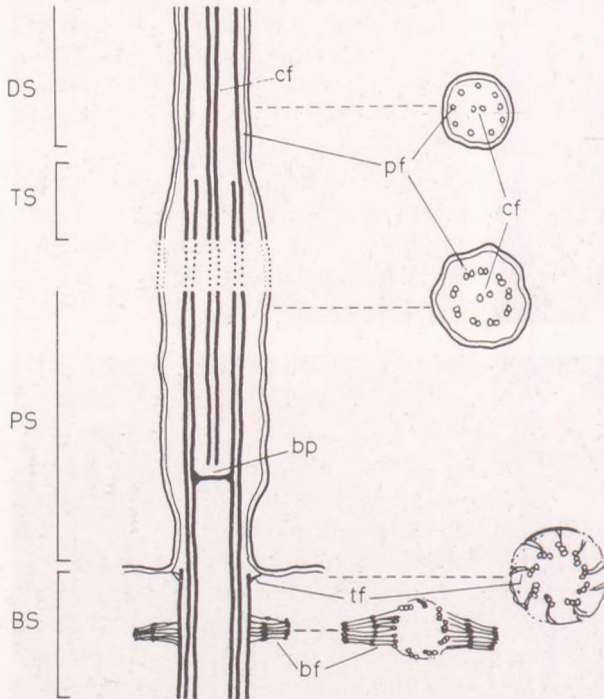


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2.2.2.3. Supporting cells

Electron microscopy has revealed that the region of the cell, distal to the nucleus, was very rich in organelles (Bloom, 1954; DeLorenzo, 1957; Reese, 1965; Andres, 1966, 1969; Graziadei, 1966, 1971; Graziadei and Bannister, 1967; Loo, 1977; Graziadei and Monti Graziadei, 1979; Moran et al., 1982*a,b*). The rough (and also the smooth) endoplasmic reticulum was arranged into circular complexes (Graziadei, 1971). The smooth endoplasmic reticular system was extremely rich, and filled almost the whole sectioned aspect of the peripheral part of the cell (see Fig. 2.3). Numerous mitochondria, free ribosomes, vesicles, dense bodies and secretory granules (these latter were characteristic in non-mammalian species; Graziadei and Monti Graziadei, 1979) were also observed here, contributing to the dense appearance of the sustentacular cells compared to the peripheral processes of the receptor neurons. The Golgi apparatus surrounded the nucleus.

The presence of tonofilaments is a very characteristic feature of this cell type. The filaments, running parallel with the surface membrane, establish a dense network (particularly at the basal pole of the cell); these filaments are absent in the receptor cells (Reese, 1965; Andres, 1966; Thornhill, 1967). In contrast, microtubuli are not found in supporting cells, they are characteristic of receptor cells.

Many finger-like protrusions emerge from the supporting cells, resulting in a strong attachment of these cells to each other and also between the supporting cells and the basal membrane (Thornhill, 1967). Further strengthening of the intracellular contacts is provided by junctional complexes, close to the epithelial surface, between supporting cells and also between these cells and the receptor cell dendrites.

The luminal surface of the supporting cells (which is broader than that of the receptor cells) is lined with microvilli (see Fig. 2.3) of differing number (in humans they may reach 1000; Bloom and Engström, 1952), length (from 0.2 to several μm) and thickness (70–100 nm) (see Bloom and Engström, 1952; Bloom, 1954; Andres, 1966; Graziadei, 1966; Moulton and Beidler, 1967; Loo, 1977 and others). Intramembranous particles and holes were found in largest numbers on the supporting cell microvilli (by freeze-etching) when compared to other processes on the mucosal surface (Menco, 1983, 1984).

Occasionally, 2–4 μm (wide) by 2–5 μm (long), finger-like protrusions were observed on the mucosal surface of the supporting cells (Graziadei, 1966); pinocytotic vesicles were commonly observed in close proximity to the mucus by many authors. In addition, ciliated supporting cells in lower vertebrates were also found, as documented for the isolated sustentacular cells of the catfish, which possessed 15–20 thin (100 nm in diameter), soft cilia (Cancalon, 1978).

2.2.2.4. Basal cells

Different types of basal cells (see Section 2.2.1.3.) have been distinguished with the aid of the electron microscope (Andres, 1966; Graziadei and Metcalf, 1971; Graziadei and Monti Graziadei, 1979; Graziadei et al., 1980). The smaller, deep-situated, dark-staining basal cells had the morphological characteristics typical for other,

undifferentiated epithelial cells, and were termed "basal cells proper" (Graziadei and Monti Graziadei, 1979). Their cytoplasmic matrix appeared to be electron-dense, contained numerous free ribosomes and tonofilaments (attached to desmosomes in places) and mitochondria. These cellular features were similar to the base of the supporting cells in the rabbit (DeLorenzo, 1957), cat, rat and dog (Graziadei, 1965; Andres, 1966), mole (Graziadei, 1966), duck (Graziadei and Bannister, 1967), primates (Loo, 1977) and man (Moran, 1982*b*). Thornhill (1967) found no filaments in the basal cells of the frog, instead, he observed a few microtubules and cilia, enclosed by invaginations of the cell surface.

Invaginations of the olfactory axons into the cell surface have been observed by several authors (Gasser, 1956; DeLorenzo, 1957; Graziadei, 1966). Basal cells may bear long, interdigitating processes (Graziadei and Bannister, 1967; Loo, 1977), like supporting cells, with which they make direct contact (Graziadei and Monti Graziadei, 1979).

As the "silent", dense basal cells are transformed into the other, lighter, "globose" (Graziadei and Monti Graziadei, 1979) basal cells ("blastema cells" of Andres, 1966) and later on, to receptor cells, density of the filaments decreases, while fine structural characteristics of the receptor neurons begin to appear (Andres, 1966; Graziadei, 1973; Graziadei and Monti Graziadei, 1979; Monti Graziadei and Graziadei, 1979; Graziadei et al., 1980; Moran et al., 1982*b*). Globose basal cells have an electron-lucent cytoplasmic matrix, rosettes of ribosomes, well-developed granulated endoplasmic reticulum and Golgi apparatus, distal to the nucleus. Microtubuli and a few basal bodies may also appear (Graziadei and Monti Graziadei, 1979; Graziadei et al., 1980). During transformation, a dendrite emerges from the distal part, and an axon-like, 0.25 μm thick process extends from the base towards olfactory nerve bundles (Moran et al., 1982*b*). Nuclei of the globose basal cells are also similar to those of the receptor cells: they are paler, have a distinct, clear chromatin pattern and may include one or two nucleoli.

By recognizing these intermediate forms of basal cells which represented morphologically a continuous line of transient cell types up to mature receptor cells, the formation and replacement of the sensory neurons could be proven convincingly (see also Section 2.5.). In accordance with these observations, mitotic figures were commonly seen in the basal cell layer (infrequently more superficially), reflecting the existence of cell divisions mainly in this level of the epithelium.

2.2.3. Summary

The sensory epithelium of the main olfactory system is a distinct region of the mucosal lining in the nasal cavity. It is different from the receptor areas of other nasal chemosensory systems (see Chapter 7) as well as from the respiratory epithelium in its localization and/or structure. Receptor, supporting and basal cells are the main cell types of the epithelium. Most receptor cells give rise to cilia, while the (apical) peripheral portion of supporting cells have dense, fine microvillar extensions. The surface of the epithelium is covered by a viscous mucus layer. Basal cells constitute the deepest layer of the epithelium. Electron microscopy reveals characteristic features of the receptor, supporting and basal cells.

A basal membrane separates the neuroepithelium from the lamina propria, in which axons of the receptor cells are collected into bundles, wrapped by Schwann cells and directed towards the olfactory bulb. The deep mucosal layer is rich in vessels and gives room also for the alveoli of the Bowman glands.

2.3. Molecular receptors and neurochemical markers

Since the detection of an odorant substance by the sense organ cannot be realized without interaction between the substance in question and the receptor cell surface, knowledge of the chemical-molecular structure of the exposed cell surface has an utmost importance in the understanding of the very early events of olfaction. Isolation techniques (Rhein and Cagan, 1980), *in vitro* experiments and molecular biochemistry appear to have contributed substantially in the last few years to the elucidation of a few basic steps in the odorant molecule detection, resulting in production of the receptor potential. Several specific markers were recognized exclusively for mature, functioning olfactory receptor cells (others only in subsets of topographically separated receptor cells and axons). Other neuroactive substances, distributed more widely in the peripheral or central nervous system (CNS) may also contribute to the final shaping of the signal, transduced through the olfactory nerve to the glomeruli of the bulb.

In the two following sections data for the molecular receptors (Section 2.3.1.) and those for specific marker substances, neurotransmitters and modulators (Section 2.3.2.) will be briefly summarized.

2.3.1. Molecular receptors

A method of discerning amongst the great variety of odors by the olfactory organ was proposed a long time ago, based on the presence of a chemically specified receptive surface (see Amoore, 1970). The numerous distinct smells, detected even by humans, suggested the existence of an equally high number of receptive structures. This idea generated numerous experiments, applying various pure odorants and searching for the specific receptor structure (cell or molecule) and physiological response in the epithelium, bulb and even higher centers. Detailed discussion and evaluation of these experiments extends far beyond the scope of this book. It turned out, however, that several basic receptor types and principal mechanisms might result in a sufficiently large amount of discernible information for the olfactory centers. These enable them to make clear distinctions between the various odors, by recognition of the pattern of activity (Beets, 1971; Getchell and Getchell, 1974; Price, 1981, 1984; see also Chapter 2.4.). No matter which olfactory theory will be preferred or predominate in the future, a few basic criteria of the molecules, detected as odors, must be fulfilled. Such as presence in a certain concentration, solubility in lipoproteins and/or water, fitting to the receptor surface (which includes size, shape, conformation, flexibility, etc. of the "odogen") (Moncrieff, 1954, 1967; Mozell, 1970; Hara, 1976; DeSimone, 1981; Hornung and Mozell, 1981; Wright, 1982).

Quite recently, several basic steps towards the understanding of the underlying mechanism of trapping the odorant molecule by molecular receptors and conditions leading to the generation of the receptor potential were clarified by the characterization of polypeptides involved in the detection of certain odorants (for review, see Lancet, 1986; Anholt, 1987; Lancet and Pace, 1987; see also Shepherd, 1985*b*; Shepherd et al., 1986).

The purification of proteins, sensitive to chemical signals, was first achieved from the taste buds of the cow (Dastoli and Price, 1966). Shortly thereafter Ash (1968) obtained an "olfactory preparation", a supernatant resulting from homogenization and centrifugation of the rabbit olfactory epithelium, the light absorbance of which changed when the odorants linalool (10^{-2} M) and/or linalyl isobutyrate (less than 10^{-5} M) were applied. Heating to 68°C inactivated this mechanism (i.e., the proteinaceous receptor molecule). Later other homogenates were also studied and characterized for their binding of specific odorant molecules, e.g., camphor (Fesenko et al., 1979, 1985), different amino acids (Rhein and Cagan, 1980) or a smell component of the bell pepper, 2-isobutyl-3-methoxypyrazine (Pelosi et al., 1982).

To date, odorant-binding proteins have been isolated (Bignetti et al., 1985; Pevsner et al., 1985; Chen et al., 1986) and localized to nasal glands and secretions of the cow (Pevsner et al., 1986), to the olfactory cilia of the frog (Chen et al., 1986*b*) and also to the rat olfactory epithelium (Fesenko et al., 1987). Such receptor protein candidates may interact with GTP-binding proteins (G proteins), such as those isolated and characterized by Chen and Lancet (1984), Pace and Lancet (1986) and Anholt et al. (1987) from frogs or Bruch and Kalinoski (1987) from fish. A novel, olfactory-specific G protein (G_{olf}) α subunit has also been characterized recently using the rat olfactory system (Jones and Reed, 1989). The signal-transducing cascade may continue in the modulation of the activity of adenylate cyclase (Pace et al., 1985; Chen et al., 1986*a*; Pace and Lancet, 1986; Shirley et al., 1986; Sklar et al., 1986; Nakamura and Gold,

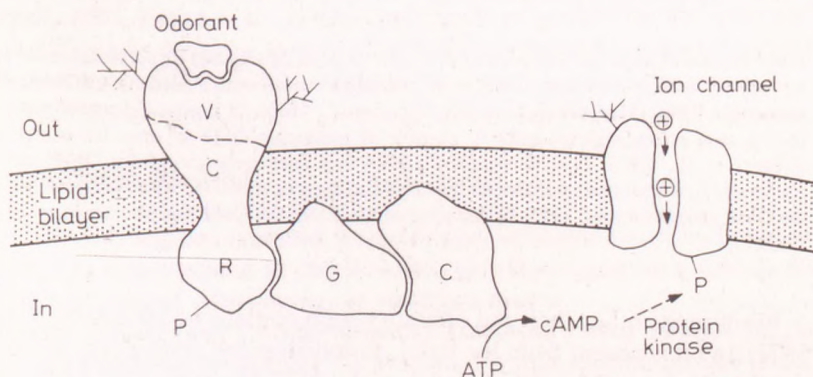


Fig. 2.6. Schematic drawing of the early events in olfaction: odorant reception and transduction (slightly modified after Lancet, 1986). The odorant is trapped by the variable region (v) of the receptor (R) molecule, while constant region (c) of this glycoprotein modulates the activity of adenylate cyclase (C), through a GTP-binding protein (G). This produces cyclic AMP (cAMP), which elicits membrane potential changes by activating the phosphorylation (p) of the channel polypeptides. Reproduced, with permission, from the Annual Review of Neuroscience, Vol. 9. © 1986 by Annual Reviews Inc.

1987; Vodyanoy and Vodyanoy, 1987), and finally, in protein phosphorylation (Heldman and Lancet, 1986), which may lead to opening of the ion channels and membrane potential change (Lancet, 1986; Anholt, 1987; Lancet and Pace, 1987; Fig. 2.6). Thus, basic molecular mechanisms of olfactory, visual, hormonal or neurotransmitter receptors appear similar in principle.

Other data also seem to support various steps of this proposal. Odorous chemicals perturbate the $\text{Na}^+ - \text{K}^+$ -dependent ATP-ase activity (Koch and Desiah, 1974; Koch and Gilliland, 1977; Dreesen and Koch, 1982). A selective, irreversible olfactory deficit can be elicited by chemical modification of the proteinaceous receptive components of the salamander olfactory epithelium (Mason et al., 1984). Deficiency in stimulatory G protein (G_s protein) parallels olfactory disfunction in humans (Weinstock et al., 1986).

2.3.2. Olfactory marker substances, neuroactive materials

2.3.2.1. Marker proteins

2.3.2.1.1. Olfactory marker protein, monoclonal antibodies to olfactory receptor cells

Margolis (1972), when investigating the olfactory system for a selective marker, discovered a protein in the primary olfactory pathway and called it olfactory marker protein (OMP). Extensive characterization of the distribution, biochemistry, synthesis and developmental significance has greatly increased our knowledge of this protein (for reviews see Margolis, 1975, 1977, 1980b, 1981, 1985; see also Halász and Shepherd, 1983). Here only a short summary of these results will be given.

OMP has a molecular mass of around 18.5–18.7 kDa (as determined by polyacrylamide gel electrophoresis and amino acid sequencing), consists of 162 amino acids, which predicts 486 bases for coding by the messenger RNA (Margolis et al., 1985; Sydor et al., 1986). In contrast, translational experiments have shown, that polyadenylated mRNA, capable of producing OMP *in vitro*, has about 2,500 nucleotides (Rogers et al., 1985). Double stranded cDNA has been generated from OMP mRNA, cloned into *Escherichia coli* and clones positive for the OMP coding region selected which will permit also the isolation of the OMP gene (Margolis, 1985). Sequencing of the cDNA for OMP has also been reported (Rogers et al., 1987).

Brain areas other than the olfactory bulb contain no detectable OMP (Margolis, 1972) and it is absent from the basal, supporting and respiratory mucosal cells of the nasal epithelium (Monti Graziadei et al., 1977; Farbman and Margolis, 1980). The OMP is widely distributed throughout vertebrate classes, including mammals and man (see Keller and Margolis, 1975, 1976; Nakashima et al., 1985) but is absent from immature neurons (Farbman and Margolis, 1980; Monti Graziadei et al., 1980). OMP appears first in intrauterine life, concomitantly with the appearance of mature olfactory synapses in the bulb (Farbman and Margolis, 1980; Monti Graziadei et al.,

1980). However, it was produced even when the regular target, the olfactory bulb was not available (Miragali and Monti Graziadei, 1982), but regularly depended on undamaged connections of the receptor cell axons with the glomerular counterparts in the bulb. Degeneration caused disappearance, while regeneration prompted reappearance of OMP (Margolis et al., 1974). The excised and *in vitro* cultured olfactory epithelium produced more (cca. $2 \times$) OMP (Chuah and Farbman, 1983) and grew more (cca. $1.6 \times$) ciliated receptor cell knobs at the membrane surface (Chuah et al., 1985) than those pieces having no direct contact with the co-cultured olfactory bulb.

In summary, OMP appears to be a sensitive and selective marker for the maturation, integrity and functioning of the primary olfactory pathway.

Further neuronal (and non-neuronal) markers were recently developed for the visualization of biochemical differences between olfactory cell types and their central projections, by the monoclonal antibody (MAB) technique. MABs specifically recognized certain cells and regions, e.g., neuronal, supporting and Bowman gland cells, basal cell layer and respiratory epithelial cells (Hempstead and Morgan, 1985a). The expression of some neuronal antigens was controlled by the bulb, as shown by unilateral bulbectomy (Hempstead and Morgan, 1985b). The existence of additional differences in antigenic determinants between the main olfactory and vomeronasal nerve, or subsets of these nerves, has also been suggested by this and other laboratories.

Immunofluorescence with a MAB, designated 2B8 was found only on receptor cells of the olfactory and vomeronasal organ (and their central projection) by Allen and Akeson (1985a). A subclass of 2B8 reactive cells did not react with OMP, also the development of the two markers differed in rat (Allen and Akeson, 1985b). The distribution pattern of the 2B8 immunopositive receptor cells resembled that of the MAB 2C5, used by Mori (1987) in the rabbit: a subset of the main olfactory (and most vomeronasal) receptor cells was labeled with this MAB. The same group developed another MAB (R2D5) which labeled all olfactory receptor cells and their axons in the rabbit, while their MAB R4B12 recognized only a subgroup of the olfactory nerve fibers (Mori et al., 1985, Fujita et al., 1985). These findings revealed biochemical and neurochemical differences between subsets of the olfactory receptors, and therefore suggest a functional specificity of their segregation.

2.3.2.1.2. Other marker proteins

A 20 kD protein was isolated from the frog olfactory epithelial tissue by Lee et al. (1987). This protein was confined to the Bowman glands, suggesting a role in the binding and transporting of odorants in the secretory mucus.

The continuous replacement of the olfactory receptors raised interest in the localization of neurofilament proteins in the mucosa. Neuron-specific enolase (NSE) was observed in the olfactory receptor cells of human fetuses; a population of nerve fascicles in the lamina propria had both NSE and neurofilament-protein (NFP) immunoreactivity (Takahashi et al., 1984). In cultured rat mucosal cells first glial fibrillary acidic protein (GFAP) was observed, which (when the cells became electrically excitable) disappeared, giving room to NSE (Schubert et al., 1985). In rats, antibodies used by Vollrath and co-workers (1985) to intermediate filament proteins

(NFP, GFAP and vimentin) revealed no immunopositivity in the mucosa, while Schwob et al. (1986), by using different sources of primary antisera observed reaction to GFAP antibodies; the immunopositivity was confined to certain, presumably glial, profiles, related to olfactory nerve fascicles in the submucosa. More interestingly, they revealed vimentin-like immunoreactivity in many (perhaps all) mature olfactory receptor cells, in contrast to the belief that mature neurons would not contain vimentin (Shaw et al., 1981). This might reflect again the special mechanisms in the life-time renewal and unique plastic capacity of the olfactory receptor neurons. In addition, rare, scattered olfactory receptor neurons, and also several axons, with NFP-like immunopositivity, were also observed in adult rats (Schwob et al., 1986).

Nerve growth factor-like immunoreactivity was established in the developing olfactory receptor neurons of the mouse (Williams and Rush, 1988). Another growth associated protein, B50/GAP43 was suggested to mark an intermediate state of maturation of the receptor cells (Verhaagen et al., 1989). B50/GAP43 and OMP was expressed "in a nearly reciprocal fashion".

2.3.2.2. Carnosine

Carnosine, a dipeptide (β -alanyl-L-histidine), which has long been known in the muscle, has been discovered in the primary olfactory pathway by Margolis (1974) and Neidle and Kander (1974) (for reviews see Margolis, 1980a, 1981). While most measurements were made on the target area of the olfactory nerves, the olfactory bulb, data are available also for the receptor (and nerve) region.

Synthetic and degrading enzymes, carnosine synthetase (E.C. 6.3.2.11) and carnosinase (E.C.3.4.13.3) were demonstrated in the epithelium and were found (together with carnosine) dependent on undamaged connections with the olfactory bulb (Harding et al., 1977; see also Section 2.5.2.). Irrigation of the mucosa with radiolabeled β -alanine or L-histidine resulted in synthesis of carnosine in the epithelium (Margolis and Grillo, 1977). 61–82% of radioactivity was related to authentic carnosine in the olfactory epithelium, 6–24 h following the application of (^3H) β -alanine into the hamster olfactory epithelium; autoradiography showed highest activity in the lamina propria, extending gradually to the olfactory nerve (Burd et al., 1982b). Since the normal epithelial concentration of alanine was well below the K_m value of carnosine synthetase for this substrate (but not for L-histidine), Margolis et al. (1985) successfully demonstrated that the availability of β -alanine might be at least one of the regulating factors of carnosine production.

Owing to its specific distribution, dependence on the presence of the functioning primary olfactory pathway and Ca^{++} -dependent release at the olfactory nerve terminals in the bulb, carnosine was suggested as the most likely transmitter candidate of the olfactory nerve (Margolis, 1980a). Further support from pharmacological experiments are required, however, before this idea can be accepted without reservations (see also Section 4.3.1.1.).

2.3.2.3. Other neuroactive substances

2.3.2.3.1. Acetylcholine (ACh)

No direct evidence is available for the demonstration of ACh itself in any of the cellular compartments of the olfactory mucosa. Certain data suggest, however, a possibility of involvement of ACh in peripheral olfactory events. The presence of muscarinic receptors (as determined by ^3H -quinuclidinyl benzilate, ^3H -QNB) has been reported in mouse (Hirsch and Margolis, 1981) and salamander (Hedlund and Shepherd, 1983) material. Unilateral bulbectomy resulted in a 30% decrease of muscarinic binding sites in the mouse olfactory bulb, while olfactory nerve transection almost completely abolished the specific ^3H -QNB binding. In addition, in salamander, acetylcholine esterase (AChE, E.C.3.1.1.7) and choline acetyltransferase (ChAT, E.C.2.3.1.6) were also measured and found to decrease slightly after nerve transection. These data suggested that the muscarinic cholinergic receptors were present on the receptor neurons, the responsiveness of which might be modulated by ACh (Hedlund and Shepherd, 1983). To date the origin of such ACh is unknown. Wirsig and Getchell (1986) observed AChE-positive nerve fascicles and also neuronal cell bodies, embedded into the trigeminal nerve in the ventral mucosa of the salamander nasal cavity.

Irrigation of the frog mucosa by 10^{-6} – 10^{-8} M ACh resulted in an electrical response, which could be strongly depressed by a prior application of substance P (10^{-8} M) in a similar manner (Bouvet et al., 1984). These effects seem, however, to be indirect for the receptor cell functioning, mediated possibly through influencing the blood supply or secretory activity of the Bowman glands.

2.3.2.3.2. Substance P (SP)

The short paper by Szabó et al. (1987) was the first note on SP-immunopositivity of the olfactory receptor cells and axons in certain gymnotid fish. Olfactory nerve transection, or destruction of the epithelium, abolished the immunoreaction in the bulb.

A dense plexus of SP-immunoreactive nerve fibers was reported first by Hökfelt et al. (1975b) from the rat olfactory mucosa. In this study, however, the level of the labeled fibers was not indicated. Papka and Matulionis (1983), who thoroughly examined the distribution of the nerve fibers with SP-like immunoreactivity in the nasal mucosa, found no immunoreaction in the olfactory epithelium proper or in the olfactory nerve. Instead, the labeled fibers accumulated at the border (basal membrane) between the epithelium and lamina propria, parallel to the layers, or were confined to vessels and Bowman glands in the connective tissue. A trigeminal origin for these supposedly sensory fibers was proposed (Papka and Matulionis, 1983), which seems to be supported also by another experiment. Desensitization with capsaicin of adult rats eliminated trigeminal responses to odorants and irritants, which left unaffected the behavioral responses directed by the sense of smell (and taste) (Silver et al., 1985).

Application of SP, however, modulated the electrical responses of the frog olfactory mucosa in experiments of Bouvet et al. (1984, 1987), similarly to that effect, elicited by electrical stimulation of the trigeminal nerve.

2.3.2.3.3. Luteinizing hormone-releasing hormone (LHRH)

LHRH-like immunopositive nerve fibers were observed intermingled with non-stained olfactory nerve fascicles (Jennes and Stumpf, 1980; Schwanzel-Fukuda and Silverman, 1980; Witkin and Silverman, 1983; Jennes, 1986; Wirsig and Getchell, 1986); sometimes immunoreactive cell bodies also appeared in the nasal mucosa (Wirsig and Getchell, 1986). None of these cells or fibers were, however, related directly to the olfactory receptor neurons; they were determined to be constituents of the terminal nerve (see also Section 7.).

Nevertheless, the nasal LHRH system may have a special importance: the developing olfactory pit (and vomeronasal organ) was suggested to be the source of all LHRH neurons of the (mouse) brain (Schwanzel-Fukuda and Pfaff, 1989).

2.3.2.3.4. Benzodiazepines

Although the olfactory bulb was the richest among brain areas in peripheral-type benzodiazepine receptors, as measured with a ligand (^3H)-Ro 5-4864 (4'-chlorodiazepam, Gallager et al., 1981) binding (879 fmol/mg protein; rest of the brain: 123 fmol/mg protein), the olfactory mucosa highly exceeded this (4,610 fmol/mg protein), constituting the most active tissue in this respect in the body of several mammals (Anholt et al., 1984). Irrigation of the nose with ZnSO_4 , causing degeneration of the receptor neurons, decreased the receptor density by 50%. The authors suggested a role in differentiation of receptor neurons from stem cells and axon growing for this type of molecular receptor.

2.3.3. Summary

Odorant detection occurs at the receptor surfaces of sensory cells. The molecular composition of the excitable membranes and other neurochemical conditions play a determinant role in signal detection and transduction. The cascade of molecular events, initiated by the trapping of the odorants, seems similar in principle to that found in the visual and neurotransmitter reception processes. Several specific odorant receptors have been isolated, and the contribution of nucleotide-binding proteins, adenylate cyclase and protein kinase were demonstrated.

A specific (olfactory marker) protein is characteristic of mature, functioning receptor cells and their projections. Monoclonal antibodies recognize subclasses of olfactory receptor cells. NSE, NFP and GFAP may also be present in these cells. Another neurochemical marker, carnosine also shows a function-dependent appearance within sensory neurons and in the primary olfactory pathway. LHRH and SP

appear in nerve fibers of the mucosa (but not in the receptor neurons or in their processes). The presence of cholinergic and benzodiazepine receptors suggests their involvement in peripheral olfactory events.

2.4. Functional aspects

The olfactory system of vertebrates is capable of detecting waterborn and/or airborne odorants. For fish water is the sole medium in which all chemical stimulants are dispersed. Sensory areas to subserve the gustatory and olfactory systems are both, however, well developed and separate from each other. The system which detects airborne odorants in terrestrial vertebrates achieves again a high level of development but there is a lack in peripheral olfactory apparatus of mammals returning to water habitats (like *Odontoceti*, see also Section 4.5.1.).

Recognition of the odorant, reaching and entering the mucus layer, transformation of this signal into an electrically coded message (production of a receptor potential) and generation of an action potential (and later, synaptic activation at the olfactory glomeruli) are principal aspects of the olfactory receptor cell activity. Although environmental odorants convey the basic, most important information to the organ of smell, concerning the external conditions of nutrition, reproduction and other vital needs, certain internal substances, like those supplied by the circulation, can also initiate receptor cell responses (Maruniak et al., 1983). In addition, external energy sources, far from being clearly understood in their relation to olfaction, like ionizing radiation, may also trigger odor-detecting mechanisms (Cooper, 1968; Garcia et al., 1969; Garcia and Koelling, 1971). In spite of these examples, olfactory cells of the epithelium detect primarily (physico-chemically characterizable) water- or airborne odorant molecules, which penetrate the mucus sheet, interact with the receptors and produce a generator potential in the receptor cells. Following this sensory transduction, an action potential is initiated through voltage-gated ion channels (Getchell et al., 1984; Getchell, 1986; see also Section 2.3.1.).

Physiological processes, resulting finally in the production of a propagated action potential in the olfactory nerve fibers have been extensively studied, characterized and reviewed in great detail. Here a short summarization and discussion of several particular aspects, related to the main (structural) line of the present monograph, will be presented. Receptor cell activity has been detected frequently not in the mucosa itself, but at a more central level of the olfactory pathway; these studies will be considered in the respective sections.

The following two short sections will pay attention to some aspects of the physiological studies directed to odorant receptive surfaces of the olfactory epithelium, and discuss studies on the electrical properties of the olfactory epithelial cells, generation of changes by stimulation and coding certain properties of odorants.

2.4.1. Generation of potential changes

Chemical stimuli generate potential change in the olfactory epithelium as observed first by Hosoya and Yoshida (1937). Electrical activity of the receptor cells was detected again later in small twigs of the olfactory nerve (Beidler and Tucker, 1955) or on the olfactory mucosal surface as a summated epithelial response to odorant stimulation; the latter has been called electro-olfactogram (EOG) (see Ottoson, 1956; Gesteland et al., 1965; Takagi, 1967; Getchell, 1974; Getchell and Getchell, 1977 and others). Thus, the EOG could be used to monitor neurochemical events. The term EOG, introduced by Ottoson (1956) was originally applied to the slow, monophasic, surface-negative voltage change he observed in the olfactory epithelium. Since, other components, including positive voltage transients, were later also recognized as parts of the summated voltage changes (Gesteland et al., 1965; Takagi and Yajima, 1964; Takagi, 1967), Getchell (1974) designated this characteristic surface-negative voltage change as $V_{\text{eog}(-)}$.

The source of this electronegative wave was considered to be localized at, or near, the epithelial surface as supported by histological, biochemical and physiological findings. As noted already in the discussion on the morphological characteristics of the receptor cells, the most probable site of the molecular receptors are the most peripheral portion of the dendritic rods, and especially cilia (Adamek et al., 1984) in the mucous sheet. There a high density of intramembranous particles was observed (see Section 2.2.2.1.) and from isolated cilia odorant-responsive *in vitro* preparations could be prepared (see Section 2.3.1.). Responses to odorants could also be elicited, however, in epithelia from which the vast majority of cilia were removed by detergents (Tucker,

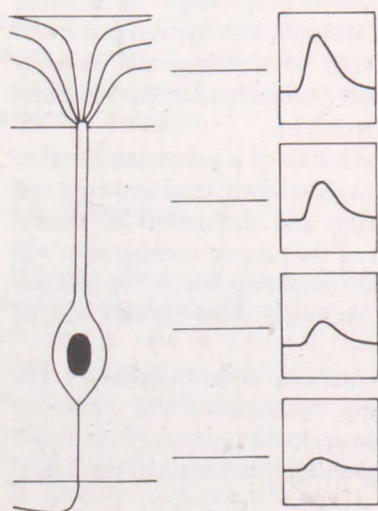


Fig. 2.7. Schematic drawing of the olfactory receptor neuron and waveforms of the odorant-evoked, slow, surface-negative potential change (electro-olfactogram, EOG), recorded at different depths of the olfactory mucosa of the frog (from Ottoson, 1956). Amplitude of the EOG decreased as the recording tip of the electrode was lowered. Reproduced with permission

1967). Microvilli might equally be able to detect odorants, as reported for microvillar receptor cells of the catfish olfactory epithelium (Erickson and Caprio, 1984). Microvillar receptor cells (of the salmonid fishes) have been, however, suggested to be specific to amino acids, while those of the ciliated receptors to bile salt-like substances (Thommesen, 1983). Physiologists detected a decreasing amplitude of the EOG to odorant stimulation as they lowered the tip of the recording electrodes (Ottoson, 1956; Gatchell, 1977) (Fig. 2.7).

Olfactory nerve transection and subsequent retrograde degeneration of the receptor cells resulted in a parallel disappearance of EOG in bullfrog, as observed first by

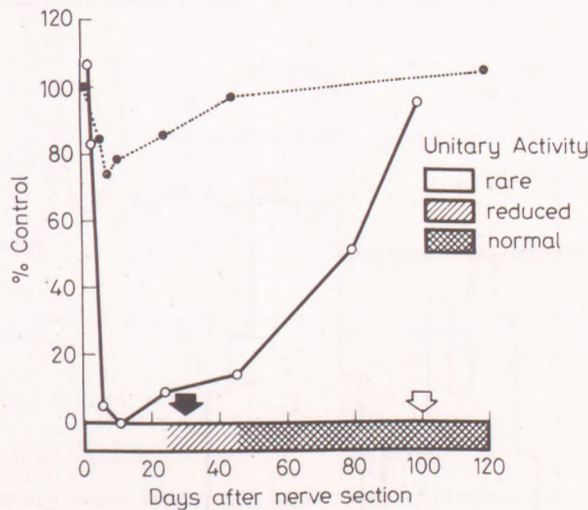


Fig. 2.8. Effect of salamander olfactory nerve section and subsequent regeneration of the odorant-evoked unitary activity (see coding of frequency at the abscissa) and slow surface-negative voltage transients (continuous line), parallel with reconstitution of the nasal epithelial thickness (dotted line), olfactory nerve connection in the bulb (closed arrow) and recovery of the glomerular layer (open arrow) (slightly modified after Simmons and Gatchell, 1981*b*). Reproduced with permission

Takagi and Yajima (1964). Regeneration of the nerve led to restoration of the EOG (Simmons and Gatchell, 1981*a,b*; Evans and Hara, 1985; Fig. 2.8.).

Amplitude of the EOG was a power function of the stimulus intensity and increased also with increasing flow rate of the odorized air, delivered to the mucosa; it depended highly on the quality of the odorant (see also below).

As for the temporal characteristics, the EOG showed a relatively long latency (which, however, shortened as the stimulus concentration was increased). The responses were certainly delayed significantly because of the time-consuming diffusion of odorants through the watery-mucous phase, before reaching the molecular receptor sites (Gatchell et al., 1980). Removal of the stimulating odorants may also be slowed down by this (diffusion) factor (Gatchell and Gatchell, 1977; Gatchell et al., 1984). Although the sense of a given smell weakens rapidly, this was suggested to be caused by mechanisms occurring at a more central level, and the olfactory receptors have been

regarded as slowly adapting ones (Ottoson, 1971; Getchell and Shepherd, 1978*b*; Getchell, 1986).

A better temporal resolution of the receptor phenomena was achieved by application of instruments delivering short step pulses of odorants (Kauer, 1974; Kauer and Moulton, 1974). This technique proved to be useful later in the activation of the olfactory receptor cells, and improved the conditions for their characterization (Getchell and Shepherd, 1978*a,b*).

As the EOG, recorded by large surface electrodes, was evidently a composite sign of activity going on at the cellular level, it was important to examine the intracellular electrical events and analyse responses to odorants (Getchell, 1977; Suzuki, 1977; Trotier and MacLeod, 1983, 1986; Masukawa et al., 1985*a,b*; Firestein and Werblin,

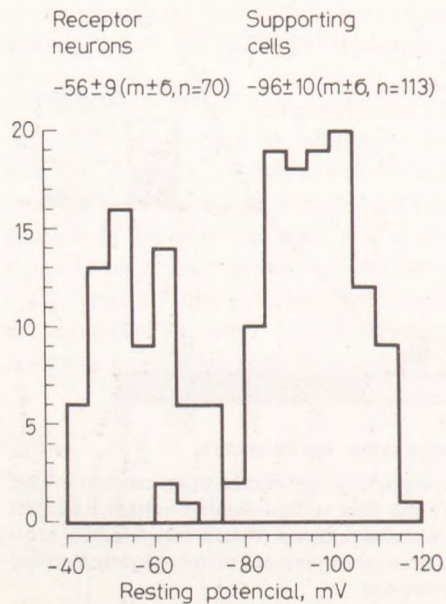


Fig. 2.9. Comparison of resting membrane potentials of receptor neurons and supporting cells in the salamander olfactory epithelium (from Trotier and MacLeod, 1986). Ordinate, number of intracellularly recorded cells; abscissa, potential values. Numbers above columns show mean $\pm$ S.D. Reproduced with permission

1987, 1989). Voltage-clamped olfactory receptor neurons in a slice of the salamander olfactory epithelium exhibited relatively high stimulus thresholds, long latencies and steep dose-response relations (Firestein and Werblin, 1989). In other preparations, supporting cells had a high (about -96 mV on average) resting potential and low input resistance, while receptor neurons had a lower (-50 to -56 mV on average) membrane potential (Fig. 2.9) and a high input resistance (Masukawa et al., 1985*a*; Trotier and MacLeod, 1986). Supposed immature receptor cells (globose basal cells?) showed intermediate values; their membrane properties were similar to those of the receptor cells, but they were not able to generate spikes (Masukawa et al., 1985*a,b*).

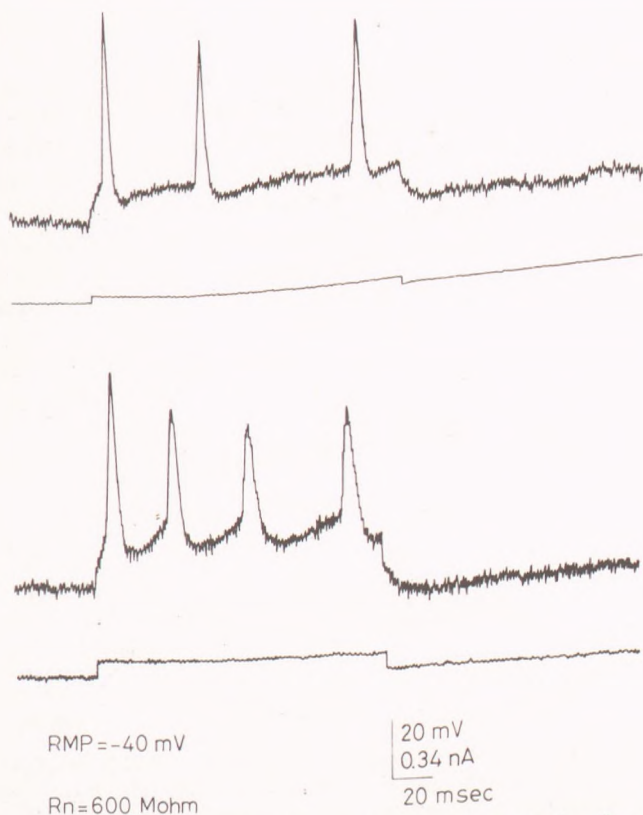


Fig. 2.10. Responses of salamander olfactory receptor neurons to a depolarizing current injection (from Masukawa et al., 1985a). Frequency of responses increased as current pulses increased (lower trace), but a reduction in amplitude was commonly observed. RPM: resting membrane potential; Rn: input resistance. Reproduced with permission

Mature receptor neurons showed spontaneous activity. Their firing frequency was enhanced by an increase of the odorant concentration (Getchell, 1977; Trotier and MacLeod, 1983) or of stimulating current (Masukawa et al., 1985a) (Figs 2.10 and 2.11). Supporting cells, however, depended also on the receptor cell activity: they responded with changes in membrane properties to olfactory nerve section (Masukawa et al., 1985b) or odorant stimulation (Trotier and MacLeod, 1986). In addition, certain odorants also stimulated the secretory activity of the Bowman glands and supporting cells. This may significantly contribute to the physico-chemical properties of the mucosa, by changing the conditions of the odorant binding and removal (Getchell et al., 1987).

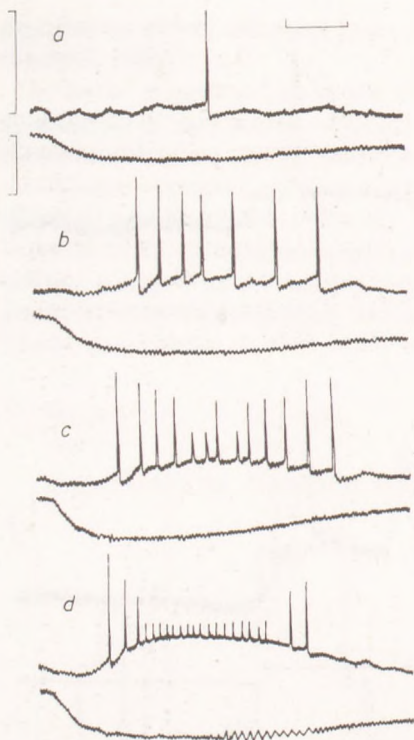


Fig. 2.11. Dependence of unit activity (upper traces at each record) and EOG (lower traces) on increased (from *a* to *d*) concentrations of stimulating isoamylacetate (from Trotier and MacLeod, 1983). Increasing concentration of the odorant evoked an increase in frequency (but decreased amplitude) of the response in the recorded unit, and increased amplitude of the summated receptor potential of the salamander mucosal surface. Calibration, horizontal scale bar: 500 ms; vertical bars: 60 mV (intracellular recordings) and 10 mV (EOG). Reproduced with permission

2.4.2. Coding of odorant properties

It is clear from the results summarized above that interaction of odorants with the receptors results in a potential change within the distal portion of the sensory cells which, by spreading down through the cell, may result in generating action potential of the olfactory nerve (see later in Section 3.4.). The intensity of stimulation was reflected, at least in part, by changing the temporal pattern of the firing, or the amplitude of EOG, but this information seemed to be insufficient for understanding the quality coding in olfaction. This initiated a number of extensive studies, based on chemical stimulation of the olfactory epithelium and subsequent recording at different levels of the olfactory system, or testing the effects of smell application on behavioral responses, and led finally to several theories of olfactory discrimination. Here I shall refer to only a few selected results and problems, regarding the classification of odors and physiological data related to the conception of spatio-temporal coding of odorant qualities.

Successful recordings of unit activity and their correlation to odorant stimulation were reported in the mid-sixties, first by Gesteland and co-workers (1965). Different odorants were used, which elicited excitation or inhibition at a given unit but failed to evoke similar responses in others. Responses to the same odorant had different time courses at different units, and discharge patterns varied even in the very same unit, depending on other factors like the delivery rate and/or stimulus intensity. In addition, most units responded to many or most of the odorants applied. It was necessary to find similarities between odorants, exerting similar physiological effects at the same receptor units. Such correlations had been recognized earlier between structurally closely related organic compound families and the pattern of the evoked EOG responses (Ottoson, 1958). At the level of the activity of olfactory units, multidimensional scaling of odorants, characterizing the degree of similarity in their evoked responses, grouping and categorization of stimulants are the subjects of many stimulating experiments (Leveteau and MacLeod, 1969; O'Connell and Mozell, 1969; Döving, 1970, 1974; Revial et al., 1978*a,b*; Duchamp et al., 1974; Sicard and Holley, 1984) (Fig. 2.12).

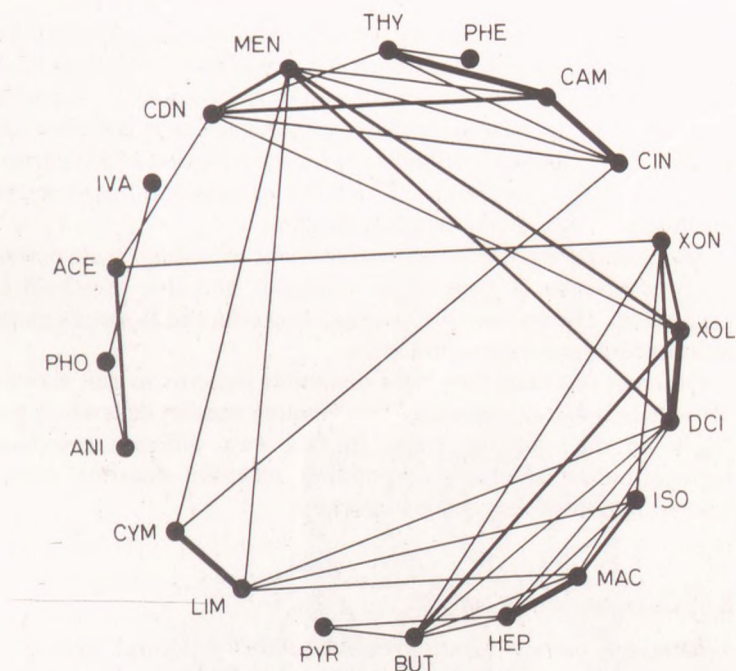


Fig. 2.12. Illustration of similarities between unitary responses to different pairs of odorants in the frog olfactory mucosa: representation of correlation coefficient values (r ; mean: 0,24) (from Sicard and Holley, 1984). Thick lines: $r > 0.60$; medium lines: $r > 0.50$; thin lines: $r > 0.38$. Dots represent odorants. ACE: acetophenone; ANI: anisole; BUT: n-butanol; CAM: DL-camphor; CDN: cyclodecanone; CIN: 1,8-cineole; CYM: p-cymene; DCI: D-citronellol; HEP: n-heptanol; ISO: isoamylacetate; IVA: isovaleric acid; LIM: D-limonene; MAC: methyl-amyketone; MEN: L-menthol; PHE: phenol; PHO: thiophenol; PYR: pyridine; THY: thymol; XOL: cyclohexanol; XON: cyclohexanone. Reproduced with permission

With the aid of unit recordings, only a low degree of specificity could be assigned to the receptors as represented by on- or off- responses to introduction of an odorant, inhibition or excitation and characteristic patterns of spiking (Getchell, 1986). Another important, structure-related finding was that receptor neurons, responding to a certain kind of odorant, were more or less restricted to an area and grouped in different (although widely overlapping) regions of the olfactory mucosal sheet. The olfactory sense organ seemed thus to provide separate epithelial areas for detection of different olfactory stimulants. Organization of the projection of the olfactory nerves preserved some degree of this spatial pattern as reflected by activation of separate branches of the nerve and of certain, topographically specified regions (glomeruli) in the bulb, by odorants (see also Sections 3.2.3. and 4.4. regarding this aspect). Thus, the spatio-temporal patterns of activity may provide the fundamental basis for smell recognition as suggested by Adrian (1951, 1953) and Clark (1951) (for reviews see Mozell, 1964, 1971; Moulton, 1967, 1976; Shepherd, 1985a).

2.4.3. Summary

Stimulation of the olfactory epithelium by odorants results in potential changes in a group of receptor cells. Therefore, a summated, slow voltage transient (EOG), with a large surface-negative component, can be recorded with macroelectrodes. The EOG is dependent on the odorant quality, stimulus intensity and flow rate; it has a relatively long latency. The most probable site for the initiation of this current is at the level of the olfactory receptor molecules. The EOG declines and reappears with the degeneration and regeneration of the receptor neurons.

Single unit extracellular and intracellular recordings from mature olfactory receptor neurons revealed a high input resistance and low threshold for action potential generation. The activity of the supporting cells and Bowman glands also seemed to be influenced by odorant stimulation.

Different odorants may elicit dissimilar patterns in unit activity. Specific odorants activate only certain subsets of the olfactory sensory cells which project into a specified region in the olfactory bulb. In this way, different response patterns and the segregation of similarly responding receptor neurons may contribute to the discrimination of the various odorants.

2.5. Developmental aspects

Although certain results, related to the structural, chemical and physiological development were touched upon in the appropriate previous sections, a more detailed presentation of several important aspects of the formation, maturation, aging, degeneration and regeneration of the olfactory receptors and their axons will be outlined here. On the other hand, problems related to the high regenerative capacity of the system have been extensively studied and the results reviewed quite recently (Breipohl et al., 1986; Brunjes and Frazier, 1986; Farbman, 1986; Graziadei and Monti Graziadei, 1986; Mair, 1986). In the following sections emphasis will be placed on questions regarding the development and regeneration of the receptor cells.

2.5.1. Formation, maturation and aging of the receptors
under normal circumstances

Primary receptor cells of the olfactory organ derive from the rostral thickening of the ectoderm, called the olfactory placode (see Graziadei and Monti Graziadei, 1978; Mendoza et al., 1982; Klein and Graziadei, 1983; Brunjes and Frazier, 1986). Differentiation of the receptor cells has been recognized as early as the 10th embryonic day (E10) in the mouse, followed by the main steps of maturation, i.e., outgrowth of the peripheral dendrite and the axon on the other side by E11–12 (Cushieri and Bannister, 1975*a,b*; Hinds, 1972*a,b*) and finally establishment of synapses with dendrites in the olfactory bulb (Hinds, 1976*a,b*). Dendritic endings gave rise one primary, followed by several, longer secondary cilia (Menco and Farbman, 1985*a,b*). By E17, mature receptors, supporting and basal cells, as well as Bowman glands were recognized in mouse (Cushieri and Bannister, 1975*b*). The number of mature receptors continued to increase during the rest of the prenatal life, in newborns and young animals, with a concomitant (numerical) increase of their synapses in the bulb until about the 15–20th postnatal days (P15–20), then slowly declined (Hinds and Hinds,



Fig. 2.13. Turnover of the olfactory receptor neurons (from Graziadei and Monti Graziadei, 1978). Mature neurons (n) have a limited life span, after which they degenerate (d), but are continuously replaced by immature receptor cells (in), produced by mitosis (m) of the deep basal (stem) cells (st). Reproduced with permission

1976a). Considering the limited life span of the receptors, and a fair constancy in the number of cells in the mucosa, degeneration and regeneration, i.e., a continuous turnover of the receptor cells (and other cells), axons and their terminals in the olfactory bulb was suggested and documented (Moulton et al., 1970; Thornhill, 1970; Graziadei and Metcalf, 1971) (Fig. 2.13).

Although a life span of about a month long had been proposed earlier (Graziadei and Monti Graziadei, 1979), protection of rats from rhinitis allowed the detection of receptor cell survival over a 12-month span (Hinds et al., 1984). From birth to the second year (in rats) the number of olfactory receptors (and their axon volume in the bulb) more than doubled, then declined markedly by the 33rd month, but young olfactory knobs were seen even at this age (Hinds and McNelly, 1981). In addition to the fixed life span, proliferation of the progenitor cells may also be influenced by external (environmental) factors and the hormonal state of the body (Breipohl et al., 1986).

Morphological maturation was followed by the appearance of the olfactory marker protein (mouse: E14; rat: E15–18) (Farbman and Margolis, 1980; Monti Graziadei et al., 1980; Allen and Akeson, 1985b), when synapses were established by the sensory axons in the bulb. In adults, OMP appeared in receptor cells seven days after ^3H -thymidine incorporation (Miragall and Monti Graziadei, 1982). Parallel with the occurrence of mature synapses and OMP, receptor cells seemed ready to respond to odorant stimulation. EOG responses were recorded as early as E14 in rats (Gesteland et al., 1982). Unit responses, although recorded already at E16 in rat, showed little selectivity to odorants; by E21 an increase in selectivity was observed: the units responded to about 50% of the chosen odorants (Gesteland et al., 1982). This was already comparable to the selectivity of units in the adult epithelium (see Section 2.4.2.). In the adults, the responses of the epithelium were suggested to derive from receptor cells at all stages of maturity, from young to senescent, according to the morphological composition of the mucosa. In spite of the continuous turnover of the receptor cells a perceptual constancy can, however, be achieved by the organisms (Mair, 1986).

2.5.2. Experimental degeneration and regeneration

Because of the relative ease of carrying out partial or total deafferentation of the epithelium, such degeneration studies were frequently used to characterize the developmental aspects of the epithelium, nerve or bulb (in the case of the latter, see also Section 4.5.). Two methods were commonly used: chemical deafferentation (irrigation of the mucosal surface by a destructive agent) or surgical interventions (ON cut or OB removal). The first treatment principally impaired the receptive surface and somata, followed by degeneration of the axon. Chemical deafferentation, depending on the severity of the destructive effect killed even the premature receptor and basal cells, while surgery affected the mature (functional, synapsing) sensory receptors only, sparing those having no processes yet within the area of injury (Takagi, 1971; Alberts, 1974; Margolis et al., 1974; Harding et al., 1978; Monti Graziadei and Graziadei, 1979; Simmons et al., 1981; Samanen and Forbes, 1984). Among the chemical agents, ZnSO_4

solution (introduced by Smith in 1938) proved to be the most effective and its consequences well defined (e.g. Alberts, 1974; Matulionis, 1976; Harding et al., 1977, 1978), but other agents, like certain salts (Cancalon, 1982), Triton (Margolis, 1981; Adamek et al., 1984), organic solvents (Ekblom et al., 1984), alkyl nitrosamines (Greenblatt and Rijshinghani, 1969) and the N-methyl-formimino-methylester (Rehn et al., 1981) were also reported to destroy the epithelium and/or impair olfaction. Ablation of the epithelium with ZnSO_4 and continuous treatment with hydroxyurea after a few days of regeneration was used successfully in producing a developmentally synchronized population of receptor cells in frogs (Lidow et al., 1987).

Both surgical and chemical denervation resulted, in parallel with the morphological alterations, in a specific decrease in carnosine, OMP and ornithine decarboxylase (for review see Margolis, 1981), loss of the transepithelial voltage transients (Simmons and Getchell, 1981a; Evans and Hara, 1985; Masukawa et al., 1985b) and of olfactory perception.

Following regeneration, the olfactory-guided behavior was normalized, although some physiological, biochemical and morphological losses persisted in the epithelium for more than several months, or were found to be totally irreversible (see e.g. Harding et al., 1978). In this respect, it is interesting to note that, according to the calculations of Harding et al. (1978), reconstitution of some 1–10% of functioning receptors enables the animal to perform normally in olfactory behavioral tests. This reflects a very high redundancy in receptorial supply in normal animals.

Functional regeneration, of course, involves growth of new axons and establishment of new synapses in the target area. Olfactory nerve growth was faster at higher temperatures and, after transection of the nerve in the garfish, included several, identifiable "waves" of new axons, according to the maturity of the cells they derived from (Cancalon, 1983a). The axolemmal membrane of the new, growing nerve showed signs of maturation, too, during elongation, as seen from the appearance and distribution of the intramembrane particles (Strichartz et al., 1984). Migration of Schwann cells were suggested to determine the speed of growth (Cancalon, 1986).

When new olfactory axons reached the brain, they re-innervated the olfactory glomeruli but were capable also of initiating formation of glomerular structures in other target areas as recognized first by Graziadei et al. (1978) and Shafa (1979). Mammalian cerebellum or occipital cortex, transplanted into the cavity of the removed olfactory bulb or the protruding forebrain were invaded by olfactory axons, which synapsed onto (unidentified) fibers in a "glomerular" structure (for review see Monti Graziadei and Graziadei, 1984). The mammalian nasal epithelium, transplanted into different brain areas, ventricle or anterior eye chamber, grew olfactory axons into the host tissue (even to the iris muscle), but did not result in glomerularization (Monti Graziadei and Graziadei, 1984). Transplantation of the embryonic (stages 23–24) olfactory placode in *Xenopus* (Magrassi and Graziadei, 1985), or the entire nasal pit of rats (age of the donor: E12) (Graziadei and Monti Graziadei, 1986) did, however, induce formation of glomerular structures in the eye chamber or in different (non-olfactory) brain areas. According to the recent view of these authors, "reciprocal recognition of complementary axons" might have triggered the glomerulus formation, so, it did not depend on the recipient area (Graziadei and Monti Graziadei, 1986).

Behavior of animals with ectopically formed glomerular structures was also tested. There is only one report of functional recovery after bulbectomy in adult mice (Wright and Harding, 1982); this finding was, however, not supported in later experiments on hamsters (Small and Leonard, 1983) or rats (Butler et al., 1984). On the other hand, as shown in a number of studies, reinnervation of the bulb resulted in reconstitution of the olfactory function. Events and principles of the development of the regular, functional target of the primary olfactory nerves, the olfactory bulb, will be discussed later in Section 4.2.5.

2.5.3. Summary

Olfactory receptor cells have an ectodermal origin. They emerge around the same time as the projection neurons in the olfactory bulb. Still during the late prenatal life the peripheral (dendritic) portion gives rise to, and subsequently increases the number of, cilia, while the centrally directed axon establishes synapses in the glomeruli of the bulb. The presence of carnosine and OMP in the primary olfactory pathway is accompanied by the functional maturity of this contact.

A life-long replacement of the sensory neurons from progenitor cells is the most remarkable feature of the olfactory mucosa. This process may be controlled, at least in part, by the limited life span of the receptor cells. Olfactory nerve transection or irrigation of the sensory epithelial surface by toxic chemicals initiates the degeneration of the receptor cells and sensory nerve, followed by a gradual structural, neurochemical and functional recovery. Olfactory nerve axons may also induce the formation of (ectopic) glomeruli in non-bulbar brain tissue, but without resulting in normal olfactory behavior. A continuous turnover of the sensory neurons within the epithelium and the re-establishment of their synapses in the regular olfactory glomeruli, however, ensures constancy in the perception of odorants.

CHAPTER 3: THE OLFACTORY NERVE

As we showed already in the preceding Chapter (Section 2.2.), the receptor cells in the olfactory mucosa give rise to axons in the deep aspect of the epithelium. These axons gather into small bundles, break through the basal membrane and leave the epithelium as discrete small fascicles of the olfactory nerve (ON). The fascicles approach the bulb, penetrate it and synapse within the glomerular layer onto different neuronal surfaces (see also Section 4.2.). This Chapter deals with some aspects of the structure, chemistry and physiology of the section of the olfactory pathway, extending from the nasal epithelium until the olfactory glomeruli.

3.1. Anatomy

Depending on the distance between the sensory epithelium of the nasal cavity and the olfactory bulb, the ON may vary in length from several hundreds of μ up to even 200 mm of the garfish olfactory bulb (OB) (Easton, 1971). Considering that the furthestmost points of the olfactory epithelium and latero-dorsal surface of the bulbs are well within the mm range in almost all vertebrates, we can estimate the average length of the individual fascicles as being regularly above one mm.

ON fascicles penetrate the cribriform plate, reach the bulbar tissue from the ventromedial aspect, then enclose the OB and provide its outermost layer (for OB layers see Figs 4.2–5). When lifting out the bulbs from their osseous cavity, their lower surface appears rough, uneven at the patch, lying opposite to the holes of the cribriform plate because of the torn nerve fascicles, the *fila olfactoria*.

3.2. Histology

The unique structure of the ON raised the interest of the first investigators of the olfactory system, because of its very delicate, unmyelinated axons (Owsiannikow, 1860; Golgi, 1875; Van Gehuchten and Martin, 1891; Blanes, 1898), which are central processes of the bipolar sensory cells of the nasal mucosa (Cajal, 1911). Morphological characteristics of the nerve, according to the course of the axons, will be described in three parts: the nasal region of the ON, the connecting nerve between the nose and the olfactory bulb and bulbar segment of the axons with special regard to the spatial distribution of the nerve fascicles and their endings in the bulb and synaptic terminals of the ON axons within the glomerular neuropil.

3.2.1. Nasal segment of the ON

In the lamina propria, bundles of the olfactory axons are embedded (already from their exit point at the basal membrane which separates the basal cells from the submucosal connective tissue) into Schwann cells (Fig. 3.1) in groups of several tens (DeLorenzo, 1957; Andres, 1966, 1975; Graziadei and Bannister, 1967; Graziadei et al., 1980; Moran et al., 1982*b*). The Schwann cell processes advance so close to the supporting cells at the basal membrane that Gasser (1956) thought they formed a syncytium with them. The Schwann cell envelope may be, however, incomplete at places as observed by Graziadei and Bannister (1967); at such places the basal membrane of the Schwann cell is the only covering over the outermost axons of a bundle. Within the fascicles, the axon diameter may vary between 0.1–1.25 μm (they may originate from morphologically distinct types of receptors; see Section 2.2.2.). The axons become more evenly thick at the segment between the nose and the bulb.

Fine structural features of ON axons are the same for the total length from the mucosa until entering the glomeruli. They may contain small, dense mitochondria, rarely small, smooth-surfaced cisternal elements and, as their most characteristic

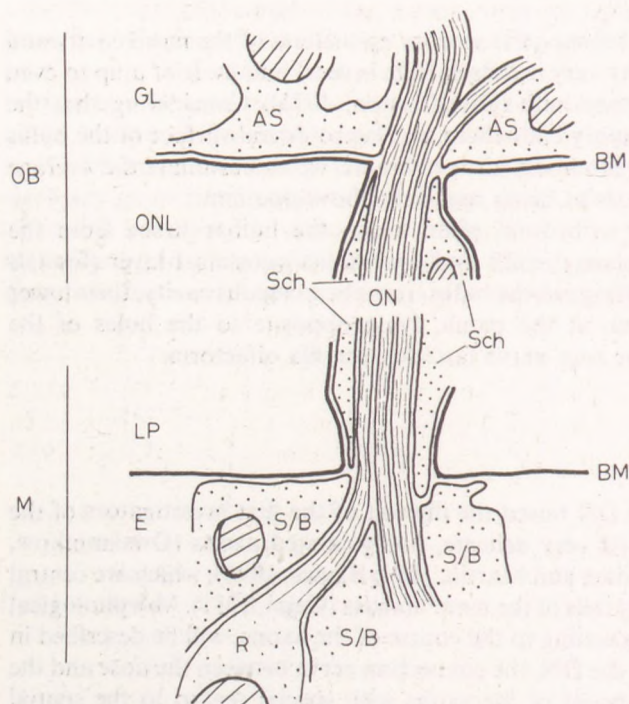


Fig. 3.1. Schematic drawing of the insulation of the olfactory nerve (ON) between the receptor (R) cells of the mucosa (M) and the glomerular layer (GL) of the olfactory bulb (OB). In the nasal epithelium (E) the ON axons run between supporting and basal cells (S/B) then, after penetrating the basal membrane (BM) between the epithelium and lamina propria (LP) they are covered by processes of Schwann cells (Sch). On the surface of the bulb, Schwann cells are still present in the olfactory nerve layer (ONL) while from the basal membrane of the OB, in the glomerular zone (GL), astrocytic (AS) cells insulate the bundles of the nerve

feature, microtubuli, the number of which is usually between three and eight per axon, but may reach even twenty (Andres, 1966). Occasionally neurofilaments are also observed (Andres, 1965; Capanna and Caravita, 1966). The Schwann cells of the ON resemble astrocytes of the OB because of their similar fine structure and glial fibrillary acidic protein content (Barber and Lindsay, 1982). Orthogonal arrays of particles between processes, what is typical for astrocytes (Landis and Reese, 1974, 1982; Massa and Mugnaini, 1982), are, however, absent (Mack and Wolburg, 1986).

3.2.2. The ON between the nasal epithelium and the OB

If the nasal epithelium and the bulb lie in close vicinity, the connective segment of the ON does not unite into a compact fascicle; smaller, individual branches, with a diameter between 80–300 μm in rat (Andres, 1965) slip through the cribriform plate as fila olfactoria and spread on the bulbar surface. The long, compact ONs, as seen in several fish with elongated noses (Easton, 1971) are especially good subjects to study the properties of this unmyelinated nerve bundle. In this section of the nerve, groups of the axons run in separate pockets of a Schwann cell, while domains of individual Schwann cells are separated further by connective tissue. The bundles regularly have also “empty” areas between the axons (in increasing number in older specimens) which may reflect degeneration of several receptors (Easton, 1971). Axons within the bundles are fairly similar but thinner (0.1–0.5 μm) than along the epithelial segment of the ON (DeLorenzo, 1957; Andres, 1966; Easton, 1971). On the basis of their size, they were categorized as C fibers by Gasser (1956).

The ultrastructural features of this segment are similar to those described above; the number of neurotubules may decrease somewhat in number, when compared to the initial segment in rats (Andres, 1965), while in frogs, they show the opposite tendency by breaking up in the vicinity of the terminal (Burton, 1987). The axons are very tightly packed within the bundles (the extracellular space between neighboring axons is between 5 and 20 nm) and are not supposed to branch before reaching the glomeruli. The extreme dense packing and close proximity, without any special insulation within an elementary bundle led even morphologists to suggest that a certain kind of synchronization, or electrotonic interaction may exist within a bundle (Clark, 1957; Thornhill, 1967).

Although the fascicles fall into smaller bundles in close vicinity of glomeruli, electron microscopy of this segment of the ON never revealed dividing axons. Based on light microscopical evidence, a 1:1 ratio was presumed to exist between receptor cells and individual olfactory axons (Allison and Warwick, 1949; Allison, 1953a; Clark, 1957).

3.2.3. The bulbar segment of the ON

ON fascicles are interwoven in a very complicated manner on the surface of the OB as noted by many observers. There exists, however, a kind of topographic organization between parts of the sensory epithelium and the surface of the bulb. Approval of a

strict topological organization in the projections would greatly support those theories of olfactory discrimination, based on spatial differences in coding of certain odors.

By placing fine surgical lesions in different parts of the rabbit OB, Clark (1951) was able to demonstrate degeneration in different, more or less circumscribed areas of the olfactory epithelium, although the projection, as a whole, seemed rather to be a diffuse one. Later, Shepherd and his co-workers made many efforts to find anatomical evidence for localized inputs from the nasal receptor field to certain areas of the bulb by small ON lesions (Land et al., 1970; Land, 1973), autoradiography of ^3H -leucine transport (Land and Shepherd, 1974) and wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) transport (Jastreboff et al., 1984), as summarized by Shepherd (1972, 1979, 1985a). These studies, together with autoradiography of 2-deoxy-glucose (2-DG) uptake (Stewart et al., 1979; Bell, 1985; see also later, Section 4.4.2.) and placing of horseradish peroxidase (HRP) (Stewart, 1985; Stewart et al., 1985) or ^3H -leucine (Mackay-Sim and Nathan, 1984) in restricted regions of the nasal mucosa, suggested that a particular region of the epithelium might be related to a restricted region of the bulb, although wide overlapping may exist between such representations. This was supported recently by Astic et al. (1987) who suggested similar principle in organization of the epithelial projections for any single glomerulus. The most restricted projection found as yet was revealed from a matrix of receptors within the nasal epithelium, extending rostro-caudally in the septal wall (ipsilaterally to the injection site) to the glomeruli of the so-called macroglomerular complex (Teicher et al., 1980; Greer et al., 1982a; Jastreboff et al., 1984; Pedersen et al., 1986). An even more delicate labeling was achieved by Kauer (1981) placing HRP injection to a single fascicle of the ON within the nasal sac of the salamander. This technique allowed the visualization of the corresponding receptor cells and their terminal endings within certain glomeruli. Further suggestions for the existence of topologically specified projections from the epithelium to the bulb, emerged from studies with monoclonal antibodies marking different subsets of the olfactory receptor cells (see Sections 2.3.2.1.1. and 3.3.1.).

Fascicles of the olfactory nerve have to pass the external limiting membrane of the outermost bulbar layer, provided by the processes of astrocytes (Andres, 1965; Willey, 1973; see also Section 4.2.5.). From this level, protoplasmic astrocytes insulate the ON bundles (see Fig. 3.1), instead of the peripheral Schwann cells, which are still present in high numbers, in the olfactory nerve layer (ONL) of the bulb (see Fig. 4.33). Astrocyte processes surround the glomeruli and also separate inner subcompartments, receiving axons from one bundle. According to calculations of Allison and Warwick (1949), some 26,000 ON axons (plus processes of 24 mitral and 68 tufted cells) may fall, on average, to each olfactory glomerulus in the rabbit OB. Upon entering, the bundles fall into finer fascicles in a brushlike manner (Valverde, 1965). Single axons may also branch as shown already by Van Gehuchten and Martin (1891) and Allison (1953b), based on Golgi-type impregnations and observed by electron microscopy later (Reese and Brightman, 1965) as well as by HRP filling of the nerve (Kauer, 1981). The observation of Van Gehuchten and Martin (1891) on olfactory axons, supplying two separate glomeruli after branching, however, has not been confirmed by modern techniques as yet.

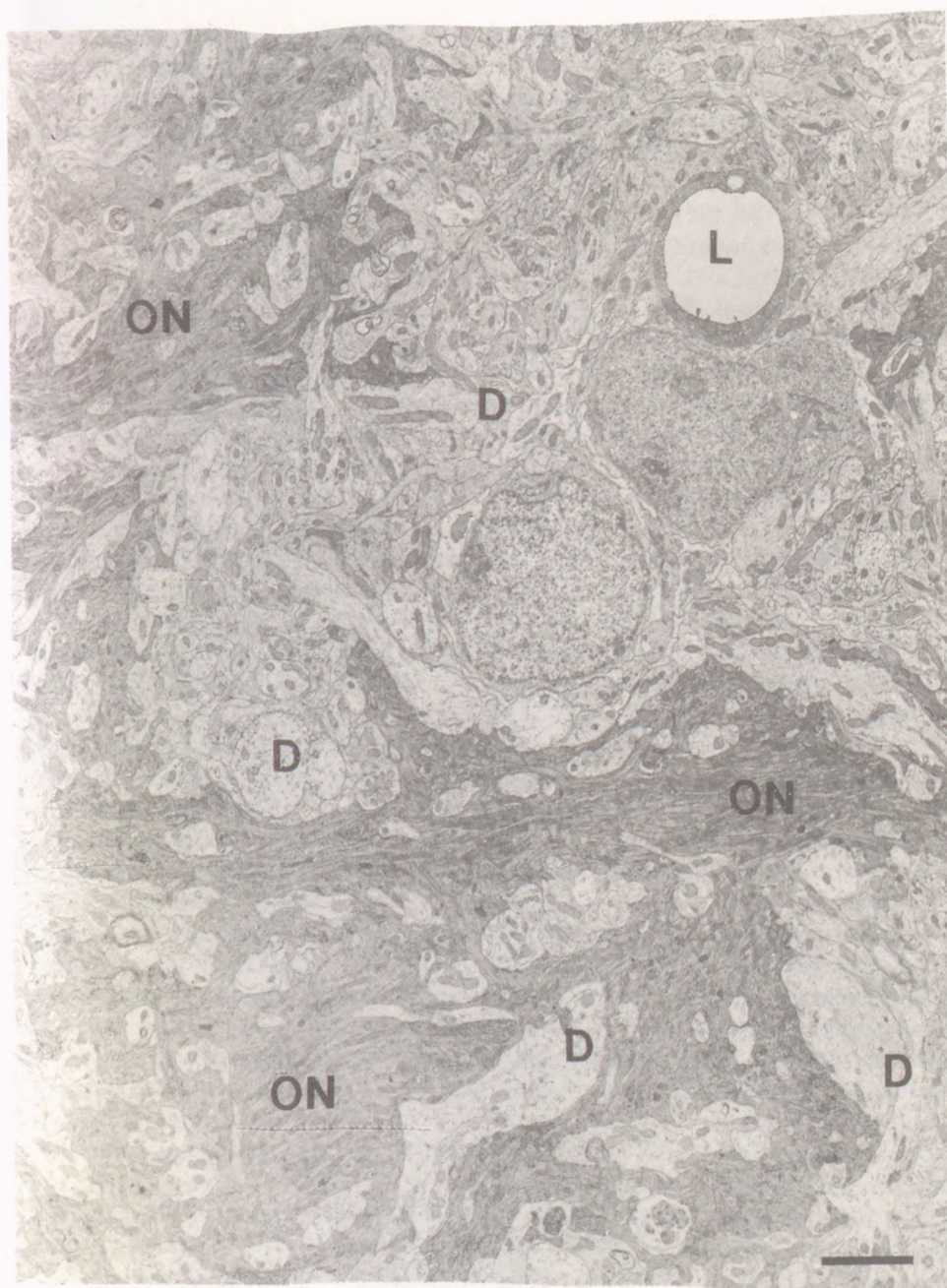


Fig. 3.2. Low-power electron micrograph of the glomerular neuropil. Dense fascicles of the preterminal and terminal segment of the olfactory nerve (ON) axons are considerably more electron dense than the various dendritic profiles (D) of mitral, tufted, or periglomerular cells, astrocyte processes or transected cell somata and nuclei. L: lumen of a capillary. Scale bar: 2 μ m

Under the electron microscope the olfactory axons and especially their terminal segments and vesiculated enlargements have a very characteristic, dense appearance (Figs 3.2 and see Fig. 4.10), offering an easy way for identification (Andres, 1965, 1970, 1975; Reese and Brightman, 1965, 1970; Pinching, 1969; Hinds, 1970; Pinching and Powell, 1971c; White, 1972, 1973; Willey, 1973). Their density increases as they enter the glomeruli and loose glial wrapping (Pinching and Powell, 1971c). Another change after entering is that the regular, parallel ON axons of the bundles become extremely irregular, beaded or varicose at places and tortuous in their course (Van Gehuchten and Martin, 1891; Cajal, 1911; Andres, 1965; Valverde, 1965) (see Figs 3.2 and 4.10). They give off several short branches and may terminate "en passant" (Reese and Brightman, 1965, 1970; Pinching, 1969; Pinching and Powell, 1971c; White, 1972; Willey, 1973). Groups of ON terminals and preterminal segments frequently form dark islands, surrounding the lighter dendritic elements and fill the space around the dendrites in a glia-like manner (see Figs 3.2 and 4.10). At their terminal segments, neurotubules are rarely seen, but a high density of round (35–75 nm) synaptic vesicles, a few small mitochondria, several coated vesicles and agranular reticular cisterns can also be found (Andres, 1965). The cytoplasmic matrix in the terminal zone, if any remained between the tightly packed synaptic vesicles and other organelles, has an osmiophilic, fine granular appearance. Synapses have asymmetric thickenings (see Fig. 4.10) and are directed predominantly towards fine branchings of the mitral, tufted and periglomerular cell dendrites, but dendritic trunks and cell somata may also occur as their postsynaptic targets (Pinching and Powell, 1971c; Willey, 1973) (for other details of glomerular synapses, see Sections 4.2.2. and 4.2.3.). ON axons or terminals have never been reported to be postsynaptic to any elements, although desmosome-like thickenings have been occasionally seen (Berger, 1969; Halász, 1974) between them.

3.3. Histochemical and neurochemical characterization

Since the olfactory nerve is the axonal extension of the receptor cells, synapsing in the glomeruli of the bulb, it should not differ sharply in neurochemical characteristics from the receptor cell region or the superficial layers of the OB. Both aforementioned areas are characterized in this book (see Sections 2.3. and 4.3.). Carnosine and the olfactory marker protein show a close and characteristic relationship to the functioning, undamaged olfactory nerve. Production and systematic use of monoclonal antibodies against determinants of (subsets or all) olfactory receptor cells (see Section 2.3.2.1.1.) may also be useful in uncovering the function-related chemical features of the olfactory nerve axons.

In this Section, neurochemical findings on the olfactory nerve will be discussed briefly, including studies on carnosine, olfactory markers and GABA. Although these substances may be synthesized in part in the synapsing terminal and alongside the nerve, presence of the synthesizing machinery in the nasal receptor area and a transport system has also been characterized in several aspects in rodents. Their olfactory nerve is short and not especially suitable for studies on axoplasmic transport. Certain fish, like the pike and garfish possess long nerves and were used in investigations of macromolecular transport (which will not be discussed extensively in

this book). Both fast and slow transport has been established in such preparates; transport velocities (and regeneration of the nerve) were highly dependent on temperature (Gross and Beidler, 1975; Cancalon, 1979, 1983*a,b*). In addition to slow macromolecular transport, smaller molecules, including those entering from the exposed nasal mucosal surface, might also be delivered to the bulb (and were suggested to be transported even transneuronally; Shipley, 1985).

3.3.1. Carnosine and other olfactory markers

Characteristic localization and the production of carnosine and OMP has been discussed more extensively along with other marker substances of the olfactory receptor region (see Section 2.3.2.) and, in the case of carnosine, will also be evaluated briefly as a suggested synaptic transmitter candidate of the primary olfactory nerve in the glomeruli (Section 4.3.1.1.). Here only data relating to the connective nerve between the receptor cell soma and the axon terminal, and to the transport of these markers from the epithelium to the bulb, will be considered.

Surgical or chemical deafferentation of the bulb led to the almost total disappearance of transported carnosine and other biochemical markers of carnosine metabolism; reappearance occurred in conjunction with morphological regeneration of the pathway in mice (Harding et al., 1977, 1978; Margolis, 1980*a*). Application of (^3H) β -alanine resulted in synthesis of radioactive carnosine and transport of the label to the olfactory bulb within several hours in hamsters (Burd et al., 1982*b*); the quantity of label increased further up to 24 h, but disappeared by the 18th day after application. Radioactive material, deriving from a similar application of (^3H) α -alanine, was transported much slower to the OB in these experiments; it was suggested to be incorporated, in a similar manner to other α -amino acids, into deposited or transported macromolecules (Burd et al., 1982*b*).

OMP was characteristic not only of the receptor cell somatic and axon terminal area, but for the interconnecting nerve, too (for reviews see Margolis, 1980*b*, 1985; see also Monti Graziadei et al., 1977, 1980; Monti Graziadei and Graziadei, 1981). OMP was detected in the olfactory nerve from embryonic day 15 in mice (Monti Graziadei et al., 1980). In the olfactory epithelium of adult mice, labeled ^3H -thymidine was shown to be incorporated into differentiating basal cells; the radioactive label appeared 7-10 days later in OMP-immunopositive olfactory nerve fascicles (Miragall and Monti Graziadei, 1982).

It is possible that the supply of either carnosine or the OMP or both by the receptor neuron to the bulb, through the olfactory nerve, is responsible for (or, at least, contributes to) the transsynaptic regulation of tyrosine hydroxylase and substance P expression in the glomerular region of the olfactory bulb (Kawano and Margolis, 1982; Baker et al., 1983; Kream et al., 1984).

Selective labeling of certain subpopulations of the ON fibers with monoclonal antibodies suggested a diverse neurochemical specificity of the ON fibers (see Fujita et al., 1985; Mori et al., 1985; Mori, 1987). This technique made it possible to follow the projection of subsets of the olfactory receptor cells to the bulbar glomeruli. MAB R4B12 labeled most ON fibers in the lateral, and a few in the medial olfactory

epithelium, projecting to the ventrolateral and caudal olfactory bulb (Fujita et al., 1985; Mori et al., 1985). The MAB 2C5-positive fibers were collected from receptor cells, distributed evenly in all areas of the olfactory epithelium, however, they tended to be concentrated in certain glomeruli, while absent from others (Mori, 1987). The recognition of such differences may help to understand the odorant-specific spatial activity patterns of the olfactory bulb.

3.3.2. γ -aminobutyric acid (GABA)

The long, dissected olfactory nerves of the pike and garfish were studied for GABA, the synthetic enzyme glutamic acid decarboxylase (GAD) and GABA-transaminase (GABA-T) in the experiments of Roskoski et al. (1974). They found about the same amount of GABA-T in the nerve, about 25% of GAD and 65-75% of GABA, as compared to brain slices (not including the olfactory bulb). A gradual, rostro-caudal increase in the concentration of these markers was also apparent. Among other proposals, a suggestion for (excitatory) transmitter role of GABA in the olfactory nerve appeared in the conclusion of the paper of Roskoski et al. (1974). This seems, however, very doubtful because denervation of the bulb did not change GABA and GAD levels or high affinity GABA uptake in the bulb (see for review Quinn and Cagan, 1981); ^3H -GABA did not label the olfactory nerve terminals (Halász et al., 1979) and these axons were not reported to be stained by immunohistochemical markers (see for review Mugnaini et al., 1984b). Instead, dendritic shafts and terminals in the glomerular layer in synaptic contact with the olfactory nerve were labeled either by radioactive GABA or, in other experiments, by antibodies against GAD (see more on GABA histochemistry in Section 4.3.).

3.4. Impulse conduction

Interaction of odorants generate a potential change in the distal segment of the receptor neuron, as discussed in Sections 2.3.1. and 2.4. This depolarization spreads through the cell and produces action potential in the axon hillock region of the olfactory nerve fibers (see Shepherd, 1983; Getchell, 1986). As noted in Section 3.2.2., these unmyelinated axons are very tightly packed, which led to the idea of a certain degree of synchronization in their activity (Adrian 1950; Clark, 1957; Tucker, 1963; Thornhill, 1967; Shepherd, 1972).

Electrical properties of the olfactory nerve were first described at the beginning of this century by Garten (for reviews, see Ottoson, 1963a; Moulton and Tucker, 1964), then extended by Gasser (1956). According to their caliber and slow conduction velocity, Gasser (1956) categorized them as C fibers. This velocity of spreading of an evoked wave was really one of the slowest, measured in vertebrates. For the olfactory nerve of frog, Gasser (1956) and Ottoson (1963a) reported 0.14 or 0.2 m/sec; this value was around 0.4 m/sec in the cat (Freeman, 1972), rabbit (Shepherd, 1972) and opossum (MacLean et al., 1957); on small, dissected twigs of the opossum olfactory nerve Beidler and Tucker (1955) measured somewhat higher velocities, ranging from 0.4 to

1.0 m/sec. Single fibers in these "*in vitro*" prepared nerves responded with a frequency of 8–15/sec to stimulation. The duration of the compound action potential, reported by Gasser (1956) was 30 msec (for comparison, duration of single action potentials was calculated to be 3–5 msec, Moulton and Tucker, 1964); such spikes were followed by long-lasting negative after-potentials in the pike olfactory nerve. In spite of these low velocities, due to the close vicinity of the mucosal sheet to the olfactory bulb in most vertebrates, signals, detected by the receptors, may reach the glomeruli within tens of milliseconds, through the olfactory nerve.

Although extracellular unit recordings as well as intracellular recordings from single receptor cells have already been successful (see Section 2.4.), most studies, testing the response of the receptors to odorant stimulation, were made by recording the evoked activity on the small olfactory nerve fascicles in the submucosa, on "*in vitro*" prepared nerve fibers or on the cut end of the nerve. This was supposed to give a real picture on the receptor cell activity, according to the recognition of a close correlation between the receptor potential and nerve discharge (Kimura, 1961). These recordings on nerve did, however, always detect a kind of a compound action, because single olfactory nerve axons were too small in caliber to be recorded separately. The next stage in olfactory information processing, recordable by the intracellular technique, would be the output neurons of the bulb (see Section 4.4.), but they are known to be influenced by a high number (a mode of 1000 axon per mitral cell in rabbit, Allison and Warwick, 1949) of olfactory nerve fibers, converging on the primary dendritic tree (see also Section 4.2.), and so, there is little, if any, chance to recognize the contribution of one single axon.

3.5. Summary

Fine, unmyelinated axons of the receptor cells are collected into bundles of the olfactory nerve. Carnosine and OMP may generally be present and transported centrally in all mature axons, while distinct neurochemical features, revealed by monoclonal antibodies, are characteristic only of subclasses of the ON fibers. Tract-tracing experiments, degeneration studies and immunochemistry suggest that glomerular foci of the olfactory bulb receive afferents from more or less restricted areas of the neuroepithelium. This suggests that organization of the ON fascicles may serve the transmission of discrete signals from topographically segregated receptor cells, and generation of odorant-specific spatial activity patterns across the superficial layers of the bulb.

Chapter 4: THE FIRST RELAY STATION: THE OLFACTORY BULB

4.1. Localization and anatomy of the bulb

As it has been shown in the previous chapters, the sensory organ for smell detection is located anteriorly to the brain, and the paired bulbs (which may be partly or totally fused in certain species, Nieuwenhuys, 1967), receiving the sensory input through the olfactory nerves, occupy usually the foremost space in the skull of most vertebrates. The bulbs can also vary in shape and in volumetric proportion to other brain areas (Fig. 4.1). They are absent in some cetaceans (Turner, 1891; Allison, 1953*b*; Edinger, 1955; Jacobs et al., 1971), but may also be duplicated, as in the rat-fish (Faucette, 1969). In Primates, and especially in humans, where neocortical areas have expanded in volume, and the significance of the olfactory sense has diminished, the bulbs are covered by other brain areas, and can be found rather on the base of the brain. In the latter case, the volume of the bulb, relative to other brain areas is also decreased. These volumetric changes may result in part from differences in the size or number of cells in different bulbs.

Numerical data for neurons counted in the bulbs is scarce in the literature, and can hardly be compared because of the differences in the age of the animals under study, sampling and evaluating techniques, individual variability, etc. For mammals, however, we have an estimate of 30,000–75,000 mitral cells in the adults (on average, 40–45,000), 130–160,000 tufted cells (Allison and Warwick, 1949; Meisami and Safari, 1981), and around one million internal granule cells (Holt, 1917; Allison and Warwick, 1949; Schönheit, 1970/71; Rosselli-Austin and Altman, 1979; Meisami and Safari, 1981). Smith (1928) found the highest cell counts of all, 3–6 million granule cells per bulb. In addition, he emphasized the strain differences which were obvious during his study, while comparing the cell counts from the wild Norwegian rat and different albino rats. Struble and Walters (1982) recently calculated around 3.1 million for the granule cells in 40 day old rats.

The number of cells is just one characteristic feature, which is responsible for the volumetric differences, there are also differences in the proportion of principal (larger) and (smaller) interneurons, in the patterns and frequency of dendritic and axonal arborizations, number of synaptic boutons, etc., which will also result in differences in thickness of layers. These questions clearly necessitate the morphological characterization of different neuronal (and, of course, glial) cell types of the bulb.

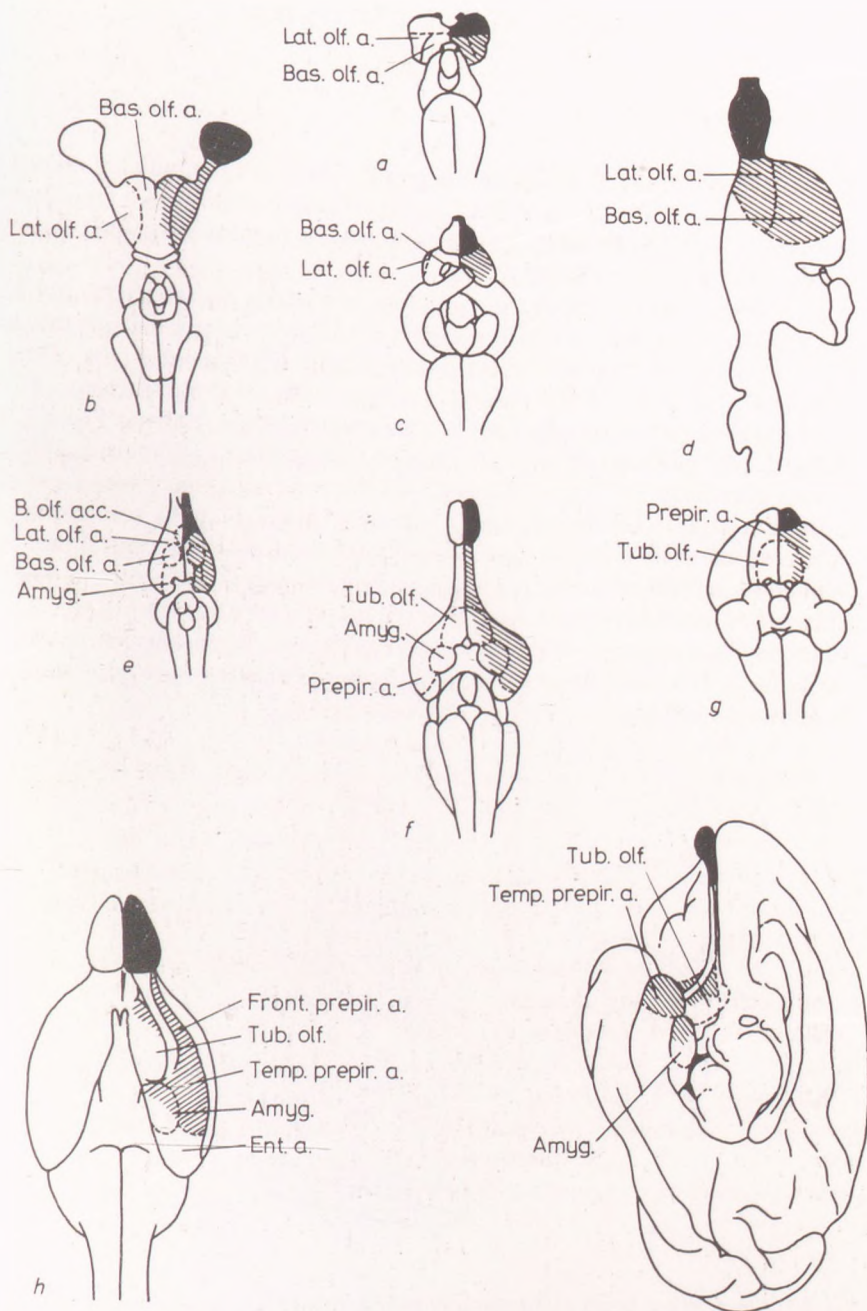


Fig. 4.1. Examples of sessile and pedunculated olfactory bulbs (solid black) and spreading of the secondary olfactory fibers (cross-hatched areas) in several vertebrate brains (from Allison, 1953a). a: *Lampetra*, b: *Acanthias*, c: *Salmo*, d: *Neoceratodus* (from the right side), e: *Rana*, f: *Alligator*, g: *Columba*, h: *Rattus*, i: *Macaca* (from the left side with the temporal lobe somewhat retracted). Amyg.: amygdala; B.olf.acc.: bulbus olfactorius accessorius; Bas.olf.a.: basal olfactory area; Ent.a.: entorhinal area; Front. prepir. a.: frontal prepiriform area; Lat.olf.a.: lateral olfactory area; Prepir.a.: prepiriform area; Temp. prepir.a.: temporal prepiriform area; Tub.olf.: tuberculum olfactorium. Reproduced with permission from the Publisher

4.2. Microscopic organization of the olfactory bulb

4.2.1. Layers of the bulb

Since classification and naming of several neurons of the bulb are related closely to their location, we have to deal briefly with the layers of this brain area. Lamination is less clear in lower vertebrates, and is more regular in more developed classes (Allison, 1953b; Nieuwenhuys, 1967).

The main olfactory bulb has a fairly symmetric (most frequently concentric) arrangement of layers, in which peripheral afferents occupy the surface, and central connections run deep, close to the center, where, in the caudal part of the bulb, the olfactory extension of the lateral ventricle may also be present. A schematic representation of this arrangement in higher vertebrates is shown in Fig. 4.2. The layers include, from surface to deep, the olfactory nerve (ONL), glomerular (GL), external (outer) plexiform (EPL), mitral cell (MCL), internal (inner) plexiform (IPL) and granule cell (GCL) layers. Small, densely packed cells, sometimes not fully differentiated even in adults, may also be observed in the most central part of the bulb (medullary layers), or just below the ependymal lining of the ventricle. The layers are shown on a frontal cryostat section of the rat olfactory bulb in Fig. 4.3. There is also an ependymal cell lining on the ventricular surface as deep as the ventricle extends into the bulb. In fish, lamination of the bulb is poor, with the only exception of *Myxine* (Cyclostomata) (Jansen, 1930; cit. Allison, 1953b).

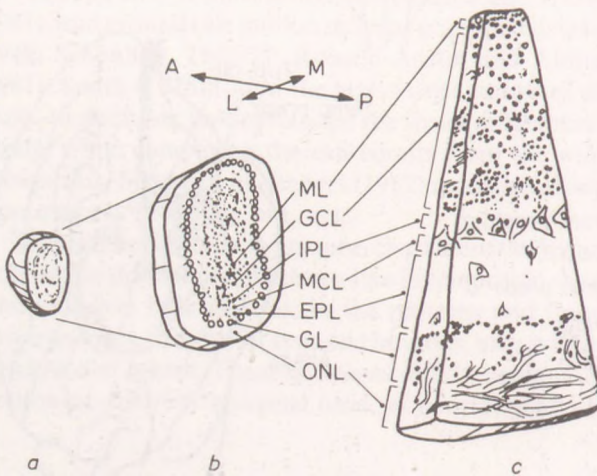


Fig. 4.2. Characteristic layers of the olfactory bulb in frontal view, as revealed by light microscopy of sections cut through the medial portion of the rodent olfactory bulb (a). The glomerular and the inner cellular strata are apparent already at low magnification (b), other details (c) can be resolved with regular staining and microscopical techniques. ML: medullary layer; GCL: granule cell layer; IPL: internal plexiform layer; MCL: mitral cell layer; EPL: external plexiform layer; GL: glomerular layer; ONL: olfactory nerve layer. A: anterior; L: lateral; M: medial; P: posterior

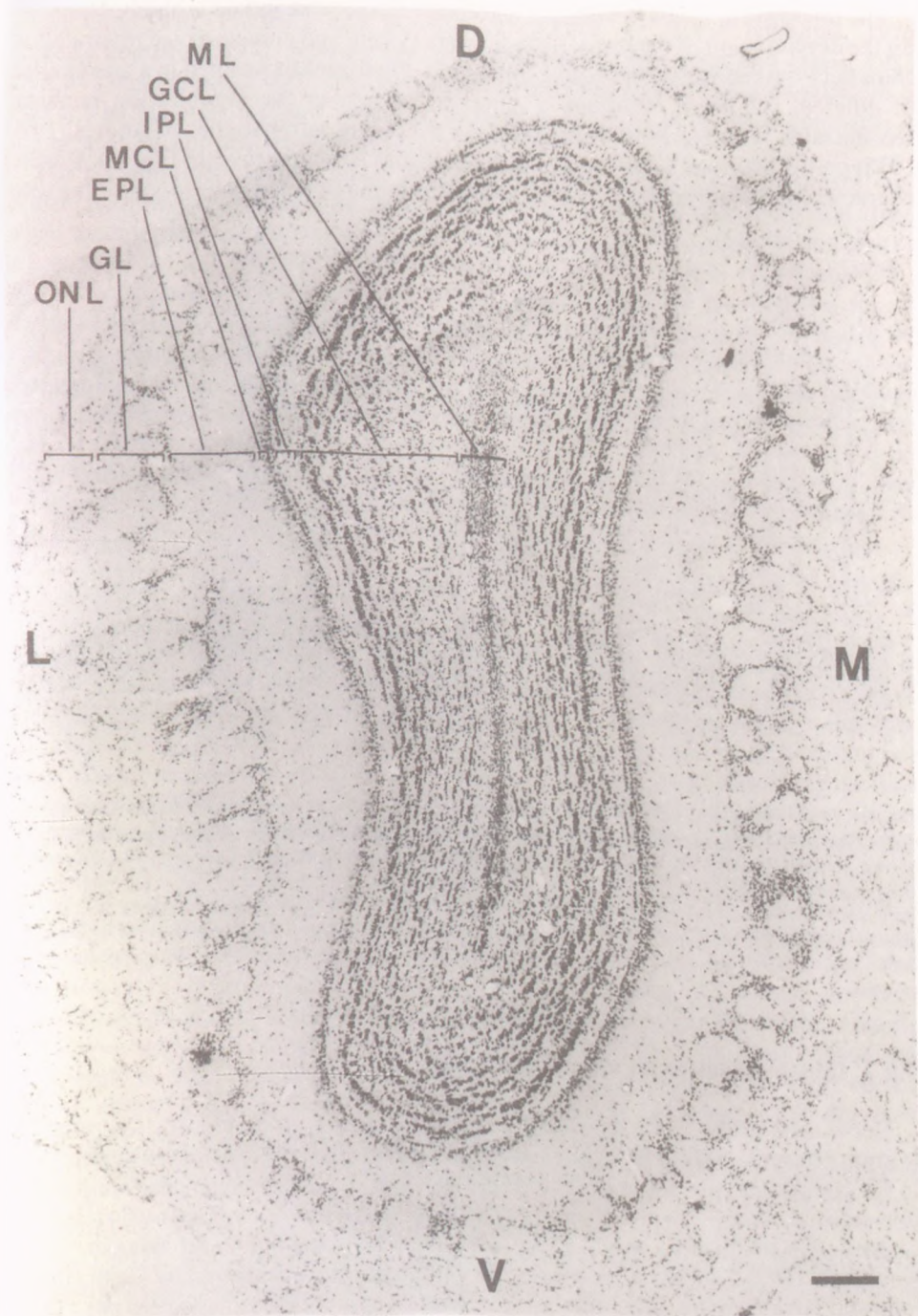


Fig. 4.3. Frontal section of the olfactory bulb, cut through the center of the bulbar complex. Adult rat, 10 μ m cryostat section, toluidine blue staining. Layers (outwards from the center): ML: medullary layer; GCL: granule cell layer; IPL: internal plexiform layer; MCL: mitral cell layer; EPL: external plexiform layer; GL: glomerular layer; ONL: olfactory nerve layer. D: dorsal; M: medial; V: ventral; L: lateral. Scale bar: 200 μ m

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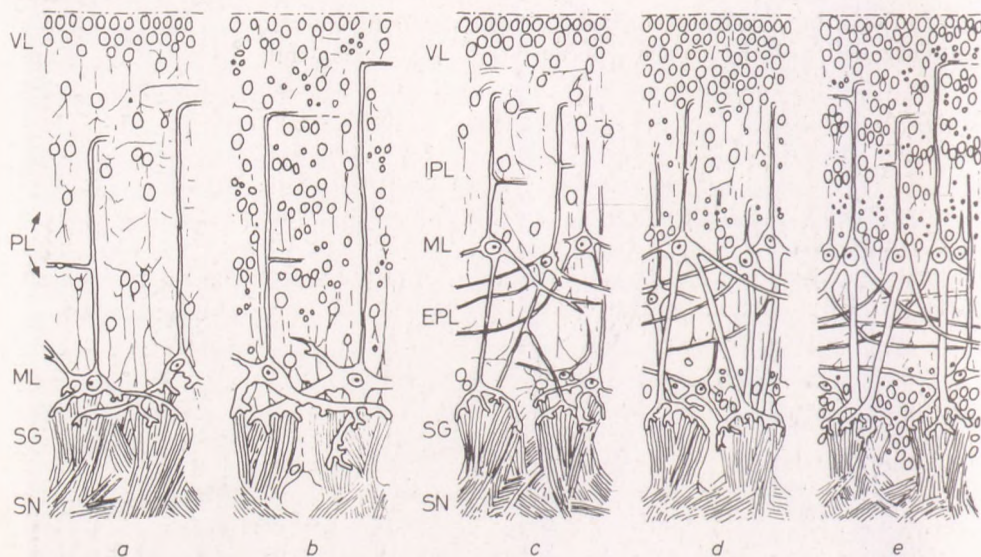


Fig. 4.4. Organization of the olfactory bulb: comparison of several representative vertebrates (from Andres, 1975). The largest, star- or miter-like cells represent mitral and tufted cells; their principal processes are also shown. Smaller, oval bodies represent (nuclei of) granule cells (and periglomerular cells, in *c-e*); some of their processes are drawn with thin lines. Small circles (thick line) show myelinated fibers. The surface of the bulb is at the bottom of the Fig., while deep lies at the top. VL: central zone (medullary layer); IPL: internal plexiform layer; PL: plexiform layer; ML: mitral cell layer; EPL: external plexiform layer; SG: glomerular layer; SN: olfactory nerve layer. *a*: lamprey; *b*: selachian; *c*: frog; *d*: crocodile; *e*: cat. Reproduced with permission.

4.2.2. Characterization of the neuron types

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The principal neurons are under direct and indirect influences of peripheral and central pathways and produce an output signal which is directed to more central areas, while the interneurons also contribute to the processing of the peripheral and central information. In well laminated bulbs, the principal output neurons are arranged in a more central position between superficial and deep layers, while the interneurons are divided regularly into two main layers, separated by the output neurons. Basically, the principal (output) neurons are morphologically (and also, functionally) similar throughout the vertebrate classes (as are the interneurons) (see Fig. 4.3), although minor differences exist in size, arborization, direction and frequency of contacts, etc., and, as a consequence, organization into more or less defined layers. However, there is increasing evidence, discovered recently, for the existence of subclasses of the different principal nerve cells and interneurons based on delicate morphological, transmitter histochemical or physiological dissimilarities between neurons of the same class.

4.2.2.1. Principal (output) neurons

These neurons can hardly be differentiated from other neurons in the olfactory bulb of lower fishes (Allison, 1953*b*), and are arranged in a single layer just from the Teleostei. At higher levels of organization in vertebrates, they can be divided into two main classes and several subclasses as shall be discussed later. According to their grouping, localization, and other (mostly morphological) criteria, the two classes consist of the mitral cells (ordered into a single layer, see, e.g., Fig. 4.11.) and tufted cells; the latter are scattered throughout neuropil, and lie more superficially, than the mitral cells. Some of the mitral cells may, however, be found more superficially than the mitral cell layer (referred to as "displaced" mitral cells), and were characterized extensively by the arborization pattern of their processes in rabbit by Mori et al. (1983) and Kishi et al. (1984). One study even suggests the existence of inwardly displaced members of this class of neurons. Haberly and Price (1977) noted that some neurons, resembling mitral cells in morphological characteristics, but lying in the granule cell layer could be filled with HRP injections into the rostral part of the olfactory cortex, and are therefore candidates of principal neurons. In the hedgehog, many displaced mitral cells were found (López-Mascaraque et al., 1986).

Early light microscopic descriptions of principal output neurons can be found in works of Golgi (1875) and Cajal (1911), while fine structural characterizations were published by Andres (1965), Price and Powell (1970*d*), Pinching and Powell (1971*b*), Willey (1973), Kosaka and Hama (1982), Oka (1983) and others. Several ultra-structural studies are based also in part on silver-impregnated material, and recently an increasing number of studies appear in the literature, which contribute to the light microscopic characterization of mitral (and other olfactory bulbar) cells with modern techniques (improved Golgi methods, HRP and fluorescent dye tracing experiments).

4.2.2.1.1. Mitral cells

The mitral cells are the biggest neurons in the bulb, although they are non-uniform in size and shape even within a single bulb. They have been measured by Price and Powell (1970*d*) in young rats, $20 \times 30 \mu\text{m}$, and found to be slightly elongated, parallel to the surface-center axis. The axon leaves the cell on the centrally extended side while several dendrites originate on the other (peripheral) part (Fig. 4.5 and see also Figs 4.4.



Fig. 4.5. Organization of the olfactory bulb: general scheme, based on Golgi-type silver impregnation (from Cajal, 1911). Layers (on the right side): *a*: glomerular layer; *b*: external plexiform layer; *c*: mitral cell layer; *d*: internal plexiform layer; *e*: granule cell layer and white matter. Cell- and fiber types: *a* and *b*: terminal arbor of the olfactory nerve within the glomeruli, *c*: arborization of the main (primary) dendrite of a mitral cell, *d*: tufted cells, *e*: mitral cell, *f* and *g*: axons and collaterals of mitral and tufted cells, *i* and *j*: granule cells

and 4.11–4.13). This gives the characteristic miter-shape to the cell, from which the name originated. Both the axon and the dendrites branch several times. The axon becomes myelinated in the vicinity of the cell body (at a distance of 15–20 μm ; Price and Powell, 1970*d*), but deeper stratum gives rise to collaterals which arborize in the GCL. In most cases, one or a few thick dendrites (termed, primary dendrites) are directed to the glomeruli, in which they ramify. The terminals of the final dendritic arbor contacted by the primary olfactory nerve fibers, and also by processes of local interneurons. In fish, amphibians and reptiles the mitral cells may send their primary dendrites into one or a few glomeruli, while in birds, this number is higher than eight (Allison, 1953). Kishi et al. (1982) found also several primary mitral dendrites, all of which arborized in one single glomerulus of the rabbit OB. The other (secondary) dendrites are found in most vertebrates, but not in teleosts (Nieuwenhuys, 1967; Ichikawa, 1976; Kosaka and Hama, 1982; Oka, 1983). These dendrites do not enter the glomeruli; they are somewhat thinner, but may surpass the primary dendrites in length, owing to their more oblique (tangential) direction. For example, Mori et al. (1981*a*) found secondary dendrites extending 1,500–1,800 μm from the perikaryon in turtle; Macrides and Schneider (1982) measured a 1,350 μm maximum length for a hamster mitral cell secondary dendrite, and an average of 792 μm (Table 4.1). Secondary dendrites terminate in the EPL, and make reciprocal contacts with the peripheral processes of the (internal) granule cells (Hirata, 1964; Andres, 1965). This was recognized later by Rall et al. (1966) as a true dendro-dendritic synapse (see also Price 1968*b*; Price and Powell, 1970*c*; Reese and Shepherd, 1972; Jackowski et al., 1978) (see also Section 4.2.2.2.3.). Secondary dendrites of mitral (and tufted) cells have been sometimes observed, however, to penetrate the GL and even the ONL, in the rabbit olfactory bulb (Mori et al., 1983). Synaptic connections of these dendrites were not studied.

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Mitral cells (48)	4.5 (2–9)	792 (330–1,350)	2.0 (1.6)

4.2. Microscopic organization of the olfactory bulb

4.2.1. Layers of the bulb

Since classification and naming of several neurons of the bulb are related closely to their location, we have to deal briefly with the layers of this brain area. Lamination is less clear in lower vertebrates, and is more regular in more developed classes (Allison, 1953*b*; Nieuwenhuys, 1967).

The main olfactory bulb has a fairly symmetric (most frequently concentric) arrangement of layers, in which peripheral afferents occupy the surface, and central connections run deep, close to the center, where, in the caudal part of the bulb, the olfactory extension of the lateral ventricle may also be present. A schematic representation of this arrangement in higher vertebrates is shown in Fig. 4.2. The layers include, from surface to deep, the olfactory nerve (ONL), glomerular (GL), external (outer) plexiform (EPL), mitral cell (MCL), internal (inner) plexiform (IPL) and granule cell (GCL) layers. Small, densely packed cells, sometimes not fully differentiated even in adults, may also be observed in the most central part of the bulb (medullary layers), or just below the ependymal lining of the ventricle. The layers are shown on a frontal cryostatate section of the rat olfactory bulb in Fig. 4.3. There is also an ependymal cell lining on the ventricular surface as deep as the ventricle extends into the bulb. In fish, lamination of the bulb is poor, with the only exception of *Myxine* (Cyclostomata) (Jansen, 1930; cit. Allison, 1953*b*).

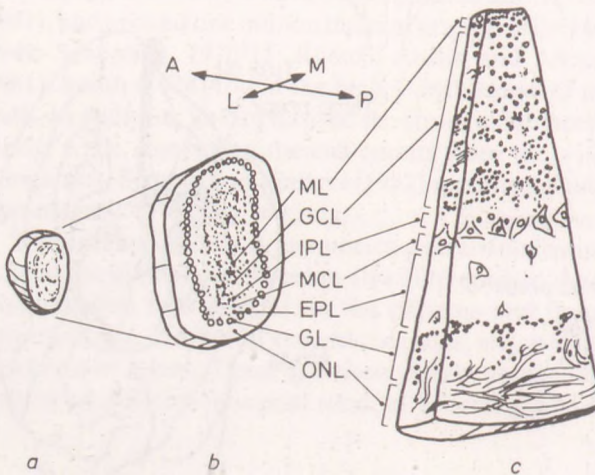


Fig. 4.2. Characteristic layers of the olfactory bulb in frontal view, as revealed by light microscopy of sections cut through the medial portion of the rodent olfactory bulb (a). The glomerular and the inner cellular strata are apparent already at low magnification (b), other details (c) can be resolved with regular staining and microscopical techniques. ML: medullary layer; GCL: granule cell layer; IPL: internal plexiform layer; MCL: mitral cell layer; EPL: external plexiform layer; GL: glomerular layer; ONL: olfactory nerve layer. A: anterior; L: lateral; M: medial; P: posterior

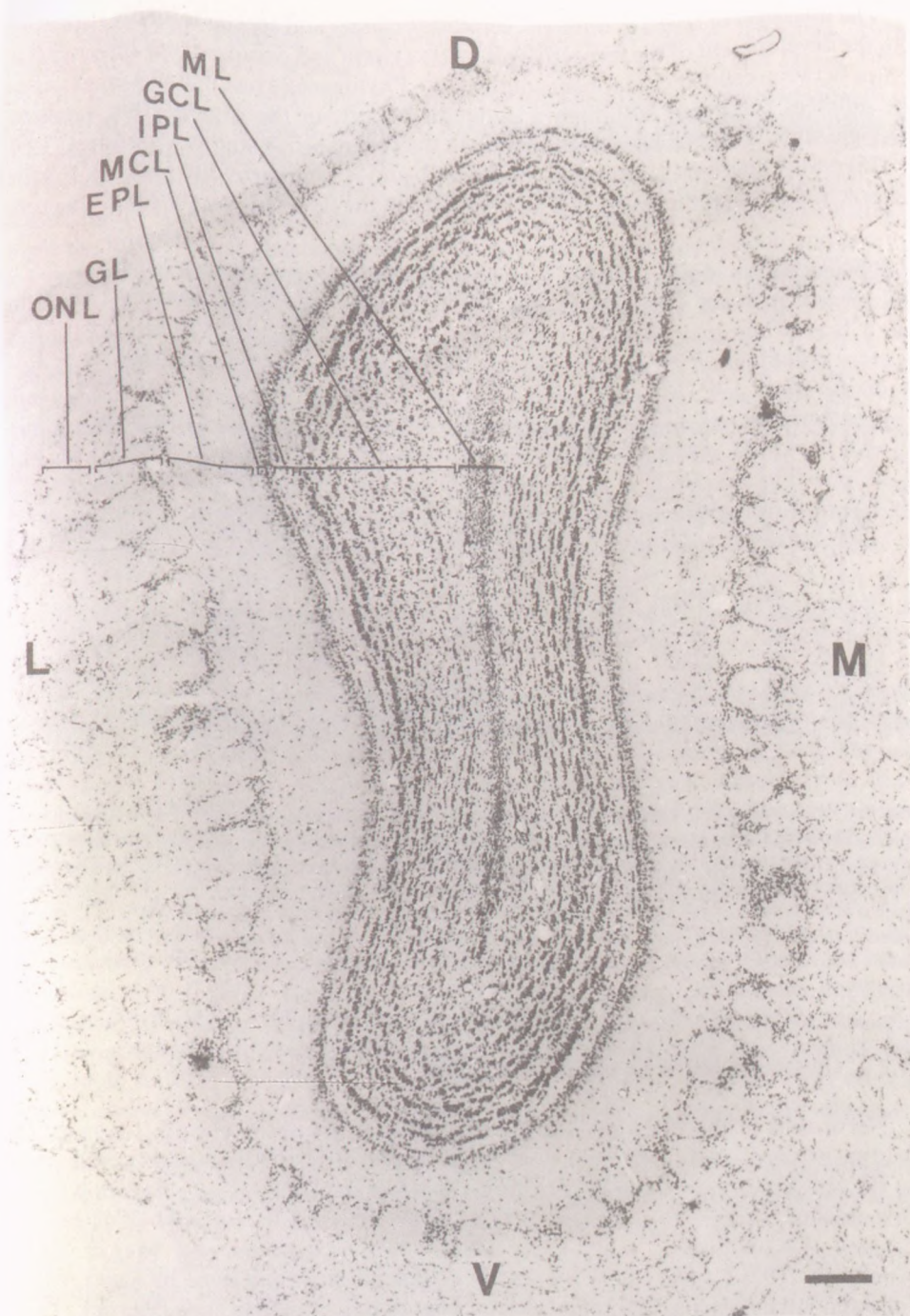


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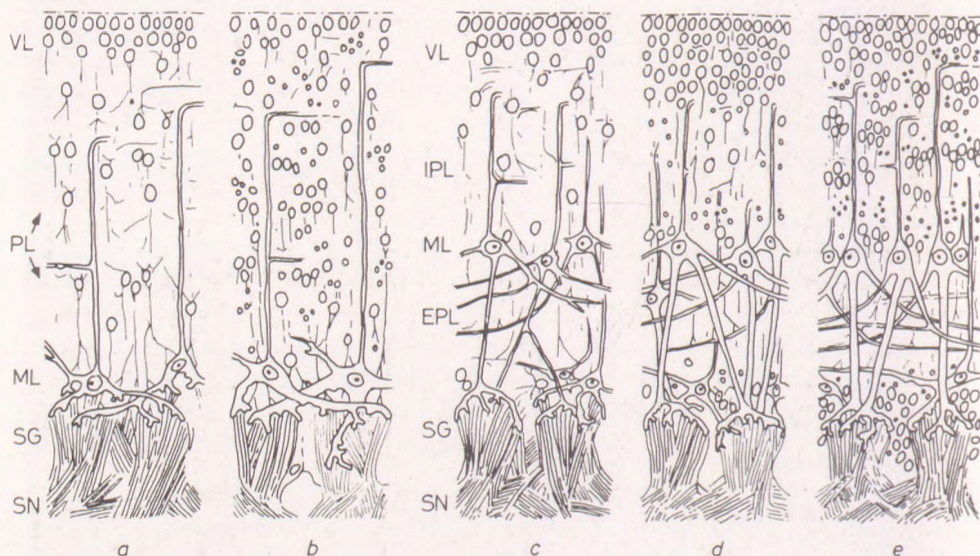


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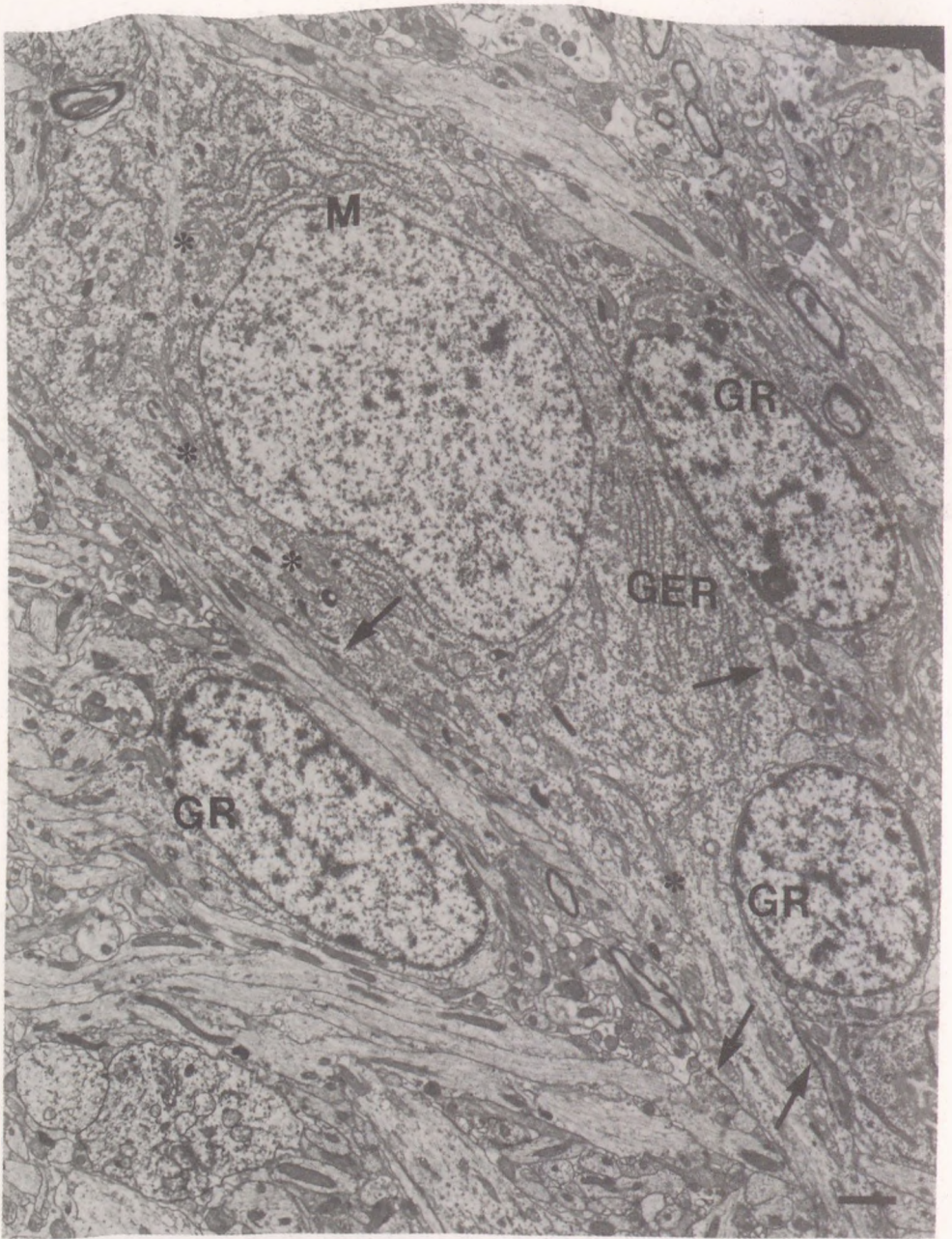


Fig. 4.6. Low-power electron micrograph of a mitral cell (M) and granule cells (GR) in a satellite position. Adult rat, aldehyde-osmium fixation. The large nucleus of the mitral cell is pale and is surrounded by a considerably thick cytoplasm, rich in organelles. The granulated endoplasmic reticulum (GER) is frequently seen as organized into large Nissl-bodies, but single cisternae are also frequent. Abundant Golgi-cisternae (asterisks) can be observed at both apices of this cell, extending also into a (dendritic) process at the lower right-hand corner. Dark lysosomes and mitochondria (blown up in part), ribosome rosettes and neurotubules (most characteristically at the origin of, and within the two processes) are organelles still recognizable at this magnification. Synapses (arrows) are commonly found on somata and dendrites. Scale bar: 1 μ m

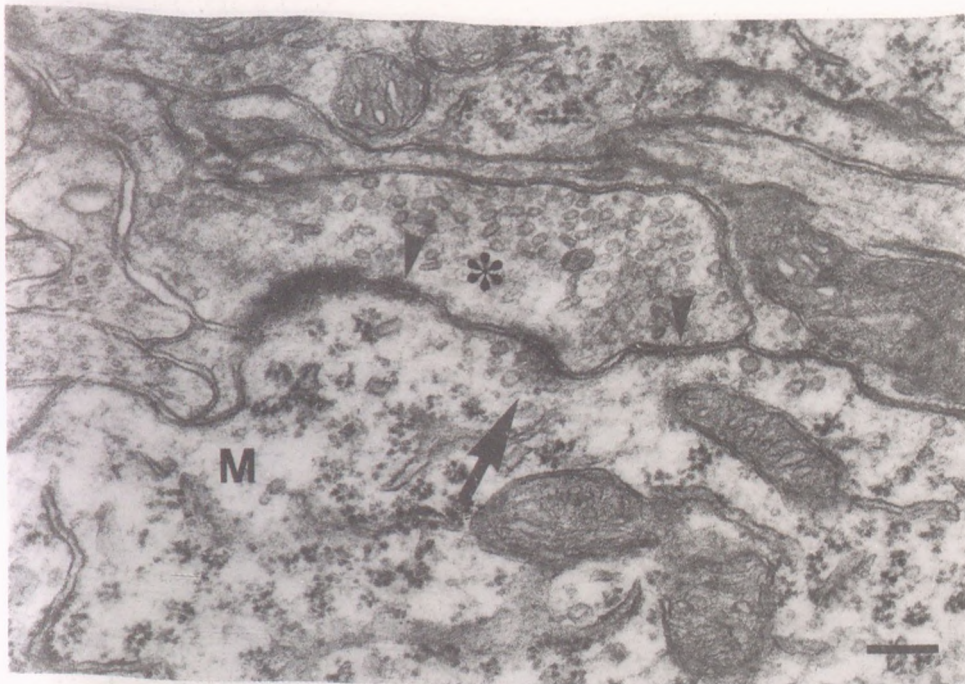


Fig. 4.7. Reciprocal synapse on a mitral cell perikaryon. Several round synaptic vesicles (arrow) are grouped in the mitral cell (M) at an asymmetric membrane thickening, suggesting the existence of an outwardly directed synapse. Flattened vesicles and symmetric thickenings (arrowheads) may represent the inwardly directed part of the synapse from a presumed granule dendritic ending (asterisk). Scale bar: 0.2 μ m

Under the electron microscope, perikarya of the mitral cells were found rich in organelles (Fig. 4.6), including an extensive rough endoplasmic reticular system, organized in parallel stacks, numerous mitochondria, free ribosomes, neurotubules, cisterns of smooth endoplasmic reticulum, a well-developed Golgi system, and many lysosomes and agranular ("clear") synaptic vesicles. These (spherical) vesicles were associated regularly with the outwardly directed part of reciprocal synapses between the perikaryon and (flattened) vesicle-containing processes, with a purported origin from the granule cells (Price and Powell, 1970*a,c*) (Fig. 4.7). A flattened subsurface sac, an extension of the rough endoplasmic reticulum of the mitral cell, is regularly also a component of this synapse (Hirata, 1964; Price and Powell, 1970*a*).

The dendrites of the mitral cells in the EPL (and GL) (Figs 4.8–4.10) exhibit regularly arranged microtubuli, (up to the final branchings), sacs of smooth endoplasmic reticulum, a few free ribosomes, mitochondria, and spherical (small) agranular synaptic vesicles, accumulated opposite to the synaptic specializations of Gray type I (see Fig. 4.9) (Gray, 1959, 1969). Although, both primary and secondary dendrites have reciprocal dendro-dendritic synapses with the flattened vesicle-containing granule dendritic "gemmules" (Price and Powell, 1970*a*), it is supposed that the secondary dendrites are under greater influence of the granule cells. Segments of mitral dendrites may be covered by several myelin lamellae.

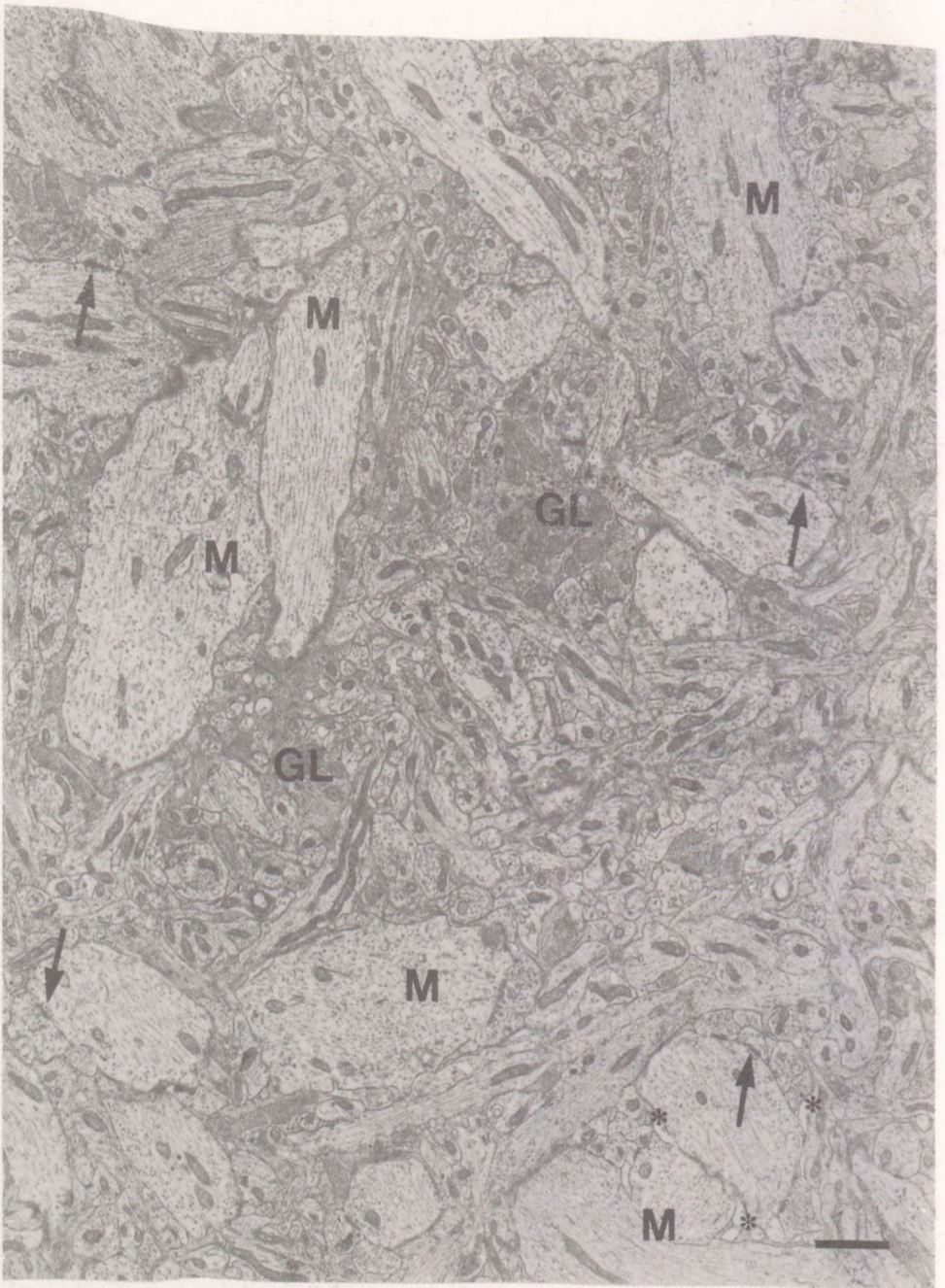


Fig. 4.8. Detail of the external plexiform layer of the rat olfactory bulb. Aldehyde-osmium fixation, electron micrograph. Several dendritic elements (M) of the output neurons and synapses (arrows) with granule cell dendritic gemmules are labeled. The mitral and tufted dendritic segments are considerably less electron dense than the rest of the neuropil layer, as are granule cell terminal gemmules, transient granule cell peripheral processes, other unmyelinated fibers and terminals, and dense glial (GL) compartments. A few "watery" astrocytic processes appear on the lower right-hand corner of this picture (asterisks). Scale bar: 1 μ m

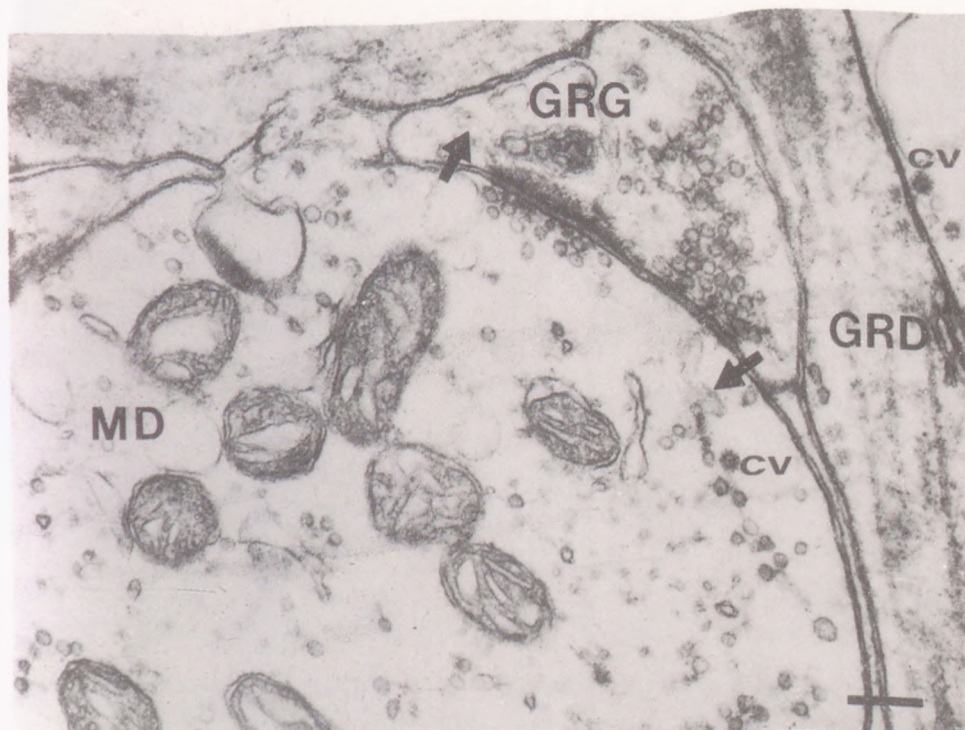


Fig. 4.9. Example of a reciprocal dendrodendritic synapse in the deep external plexiform layer of the rat olfactory bulb between a large mitral dendrite (MD) and a granule cell dendritic gemmule (GRG). Supposed directions are indicated by arrows. The mitral-to-granule synapse is an asymmetric (Gray type 1) synapse, while its counterpart is a symmetrical (Gray type 2) synapse. Small sacs and tubules of the smooth endoplasmic reticulum and mitochondria are common in both terminal segments. A higher density of small (regular) synaptic vesicles is seen in the granule dendritic terminal, while dispersed vesicles can be seen in all (also, non-synaptic) parts of the mitral dendrites. Coated vesicles (cv) may also be observed from time to time. GRD: granule cell dendrite. Scale bar: 0.2 μ m

Primary dendrites of the mitral (and tufted) cells enter the glomeruli where they ramify (Fig. 4.11 and see also Figs 4.12 and 4.13), and receive Gray type I synapses from the olfactory nerve endings (see Figs 3.2 and 4.10). Even these parts of fine terminal dendritic branches may contain agranular vesicles. The vesicles are more numerous at the contacts which are not rare between the mitral, and other, electron-lucent dendritic profiles (of periglomerular cell origin) (Hinds, 1970; Pinching and Powell, 1971c; White, 1973). At their primary branching, the mitral (and tufted) cell main dendrites are (unidirectionally) postsynaptic to axons of periglomerular cells from neighboring glomeruli (Pinching, 1970; Pinching and Powell, 1971d).

The axon initial segment of the mitral cells is to be pre- and postsynaptic for several terminals in the goldfish (Kosaka and Hama, 1979b) and cat (Willey, 1973), but not in the rat olfactory bulb (Price and Powell, 1970d). Axons of the mitral (and tufted) cells give rise to local collaterals, which arborize mainly in the GCL (rat: Kishi et al., 1984; rabbit: Orona et al., 1984). Under the electron microscope, the local collaterals were

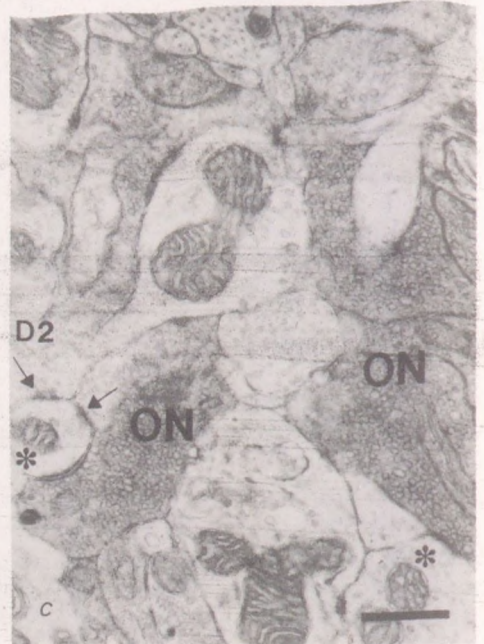
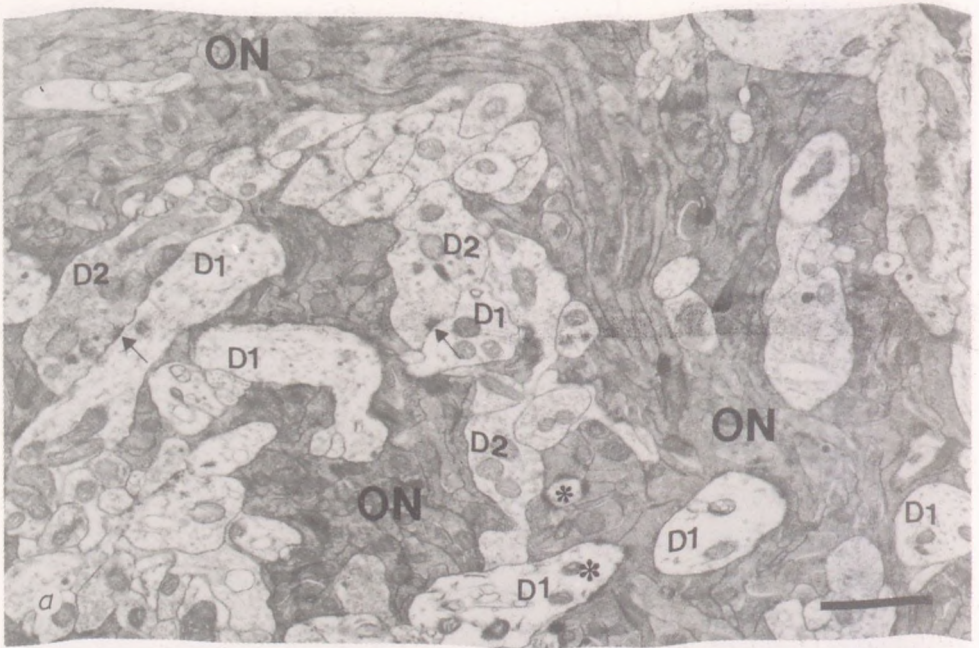


Fig. 4.10a-c: Glomerular synapses from the rat (a) and turtle (b,c) olfactory bulb. Aldehyde-osmium fixation. The dark olfactory axon terminals (ON) have the highest density of regular (small) synaptic vesicles and may synapse (asterisks) upon larger or smaller, but much paler dendritic shafts or terminal enlargements, having a few (D1) or a considerable amount (D2) of small synaptic vesicles. Such dendritic elements (of the output neurons and small periglomerular cells) may also establish dendrodendritic synapses (arrows). Scale bars: 9a: 1 μm , 9b: 0.5 μm , 9c: 0.5 μm

found to contain spherical agranular synaptic vesicles (Price and Powell, 1970c). The myelinated axons of the mitral cells leave the olfactory bulb through the olfactory tract(s). The axon terminals are distributed in the basal forebrain, and terminate in various cortical areas, as will be discussed below. In a few amphibians and reptiles, like the soft shell turtle (*Trionyx*) several mitral cell axons may terminate, however, in the contralateral olfactory bulb (Skeen and Rolon, 1982).

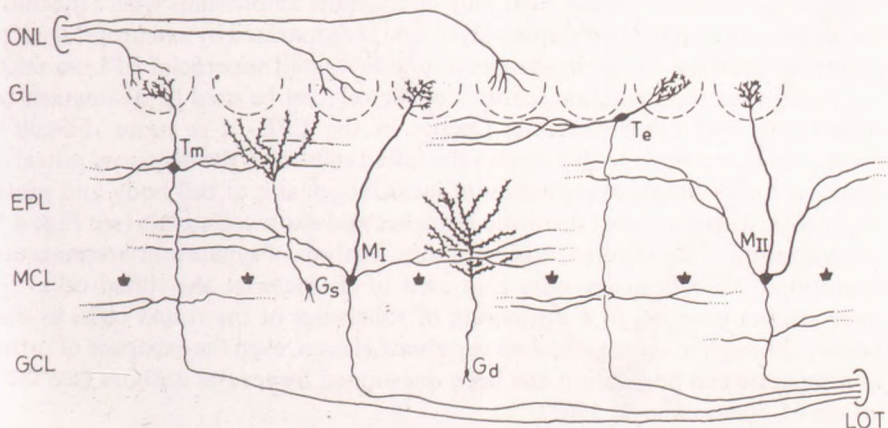


Fig. 4.11. Patterns of distribution of several mitral, tufted and granule cell somata and their processes (from Orona et al., 1984). Rat olfactory bulb, HRP tracing experiments. Primary dendritic trees of the output neurons arborize within the glomeruli, where they are postsynaptic to the olfactory nerve. Secondary dendrites of the external (T_e) and middle (T_m) tufted cells and those of the type II mitral (M_{II}) cells are distributed more superficially in the EPL, and may contact primarily the peripheral processes of the superficial granule cells (G_s), while the deeper lying tufted cells (not shown here) and type I mitral cells (M_I) may synapse principally with gemmules of the deep granule cells (G_d). ONL: olfactory nerve layer; GL: glomerular layer; EPL: external plexiform layer; MCL: mitral cell layer; GCL: granule cell layer; LOT: lateral olfactory tract. Reproduced with permission

Subclasses of mitral cells have also been distinguished in several species. Classification was based mostly on morphology differences. Differences in the length, position and complexity of secondary dendrites led Orona et al. (1984) to designate type I and type II mitral cells in rat (see Fig. 4.11). Secondary dendrites of the type II mitral cells arborized more superficially in the EPL than those of type I. Similarly, in rabbit, Mori et al. (1983) found a more superficial localization of secondary mitral dendrites for their "displaced" mitral cells (the cell body lay more superficially than the regular mitral cells). Kishi et al. (1984) found a difference in distribution of local axon collaterals of mitral cells in the rabbit GCL: the more superficial, displaced mitral cells tended to have their axon collaterals distributed more superficially. In the aforementioned experiments fine HRP injections were used to reveal these minor differences. Tonosaki (1982) completed his electrophysiological analysis with procion yellow filling of mitral cells in gecko, and assigned depolarization responses (to odor stimulation) to large ($25 \times 10 \mu\text{m}$), miter shaped mitral cells, and hyperpolarization to a smaller category ($18 \times 7 \mu\text{m}$) of oval mitral cells. Unfortunately, this paper lacks detailed morphological data, comparable to the HRP studies, to enable an easier structural comparison of the two sets of data.

4.2.2.1.2. Tufted cells

The definition of this cell type is not easy even in higher mammals, where the mitral cells form a single, shell-like layer, lying just deep to the EPL. Tufted cells are similar to mitral cells, but they are located superficially to the mitral cell body layer, i.e., in the EPL, and may extend also into the glomerular area. This implies that we can clearly recognize tufted cells on this basis only up from the amphibians where the mitral cells are arranged already into a distinct layer, and are separated by a neuropil area from the glomeruli (Andres, 1970). In the glomerular layer and superficial EPL no mitral cells are present, so the structural features of the cell can be used to distinguish between tufted cells and other neurons. Deeper in the EPL, it is more difficult to use morphological criteria to distinguish the tufted cells from the displaced mitral cells. In addition to the simple examination of localization, size of cell body and presence of organelles, distribution of dendritic branches and axon collaterals (see Figs 4.11–13), recognition of the terminal areas of axons, analysis of synaptic interconnections and transmitter histochemistry were also used to characterize the tufted cells. To date, these studies have led to a distinction of subclasses of the tufted cells, as discussed below, although in submammalian vertebrate classes, even the existence of tufted cells, as a separate cell population has been questioned by several authors (see the review article of Nieuwenhuys, 1967).

The general tendency is that the deep tufted cells have more similarities to the mitral cells in their appearance and distribution of dendrites than the superficial ones. On the other hand, axonal projections of the deeper tufted cells reach more caudal cortical regions than axons from the more superficial cells (Schneider and Scott, 1983). There is uncertainty in the literature as to whether axons of all external (superficial) tufted cells actually leave the bulb at all.

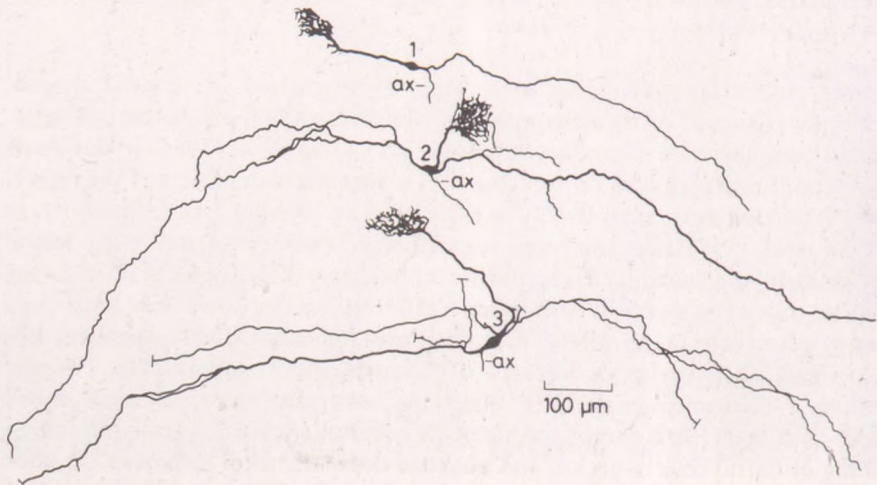


Fig. 4.12. Golgi-impregnated middle tufted cells of the hamster olfactory bulb. Camera lucida drawing (from Macrides and Schneider, 1982). These cells have regularly long, tangentially running secondary dendrites, with symmetric (neurons 2 and 3) or less frequently, asymmetric (neuron 1) secondary dendritic fields. ax: axon. Reproduced with permission

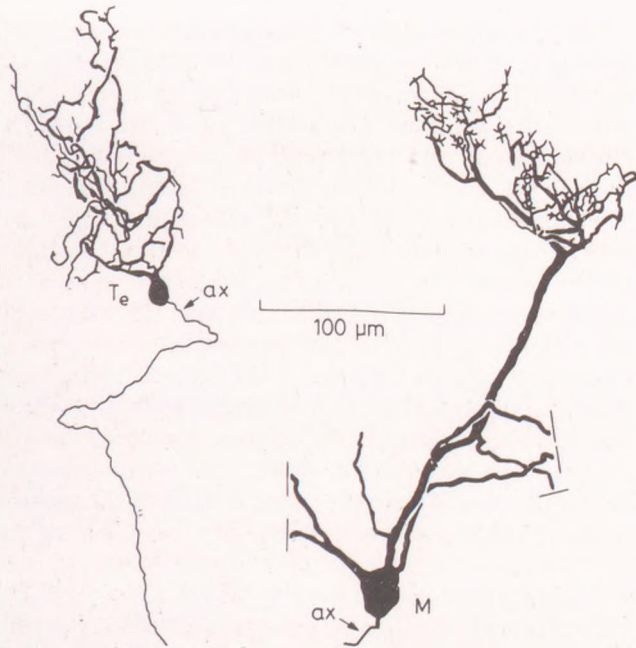


Fig. 4.13. External tufted cell (T_e), lacking secondary dendrites and a mitral cell (M) of the hamster olfactory bulb. Camera lucida drawing of a Golgi-impregnated sample (from Macrides and Schneider, 1982). Extension of the glomerular arbor of the two cells are similar. The mitral cell has several secondary dendrites, running out of the plane of the section. ax: axon. Reproduced with permission

According to Cajal (1911) we shall use the term “internal” (or deep) tufted cells to refer to those lying in close vicinity of mitral cells; “middle” tufted cells for those lying in the outer two-thirds of the EPL, and “external” (or superficial) tufted cells (Figs 4.12 and 4.13) for those located at the borderline of EPL and GL, or lying in the periglomerular area. The morphological characteristics of these cells will be described mainly on the basis of Golgi studies, performed by Van Gehuchten and Martin (1891), Blanes (1898), Cajal (1911), Pinching and Powell (1971*b*) and Macrides and Schneider (1982). Data from HRP studies of Kishi et al. (1984) and Orona et al. (1984), and from electron microscopic studies of Andres (1965), Pinching and Brooke (1973), Willey (1973) and our own (unpublished) experiments will also be incorporated in the description.

Tufted cells are somewhat smaller than the mitral cells. Internal tufted cells were measured in hamster, having their diameters within the range of $16 \times 27 \mu\text{m}$ (Macrides and Schneider, 1982), while external tufted cells were even smaller ($10 \times 14 \mu\text{m}$ in rat; Pinching and Powell, 1971*b*). The shape of the cells is largely dependent on the processes they emit; the internal tufted cells are more likely to be miter-shaped because of more numerous basal processes, while the more superficial ones have rather a fusiform appearance. As an example, numerical data for secondary dendrites of tufted cells of the hamster olfactory bulb are shown from Macrides and Schneider's (1982) quantitative analysis (see Table 4.1).

Our electron micrograph shows a fusiform middle tufted cell from the rat olfactory bulb (Fig. 4.14). The nucleus of tufted cells is regularly smooth in outline, with occasional (and not deep) indentations and a chromatin material with patchy appearance in places. The perikaryon is rich in rough endoplasmic reticulum, free ribosomes and mitochondria which may be found slightly blown up if one compares them to the mitochondria of the surrounding elements (Pinching and Powell, 1971*b*). The number of parallel stacks of the rough endoplasmic reticulum decreases parallel with the size of the cell and the more superficial localization. The Golgi system is well developed; smooth endoplasmic reticulum, neurotubules and lysosomes are also common components of the cytoplasm of the tufted cells. Although the surface of the cell is mostly wrapped by glial processes, and sometimes is partly ensheathed by a few thin myelin lamellae (Pinching, 1971; Burd, 1980; Tigges and Tigges, 1980; Remahl and Hildebrand, 1985), it is very typical to find reciprocal synapses between the soma and vesicle-containing nerve endings, similar to the mitral cells. These synapses are characterized by spherical vesicles and asymmetrical membrane thickening, when directed outwards from the tufted cell, while the opposite direction is a Gray type II synapse. The presynaptic component of the latter, in the EPL, might be the terminal gemmules of the granule cells, while in the glomerular layer, they are of periglomerular cell origin (Pinching and Powell, 1971*c*).

Dendrites of the tufted cells, as revealed by Golgi studies (see Figs 4.12 and 4.13) and HRP tracing (see Fig. 4.11) can also be similarly classified, as the mitral cell dendrites: some of them arborize in the glomeruli (primary dendrites), and others stay out of them (secondary dendrites) and synapse with the peripheral processes of the granule cells. Some tufted cells may completely lack secondary dendrites (Pinching and Brooke, 1973; Macrides and Schneider, 1982), as is shown also in Fig. 4.13. The number, length, complexity and distribution of the secondary dendrites is proportional to the size and localization of the cell body (hamster: Macrides and Schneider, 1982; rabbit: Mori et al., 1983; rat: Orona et al., 1984); there are differences also in the density of branching (see Figs 4.12 and 4.13 as well as Table 4.1).

Dendritic segments of the tufted cells in the EPL and GL were found sometimes to be ensheathed by a thin myelin wrapping (Pinching, 1971; Burd, 1980), but this myelin was different from that of regular axonal myelin, according to Remahl and Hildebrand's (1985) studies on the feline olfactory bulb. There, at the tufted cell dendrites, no correlation was found between the fiber diameter and the number of myelin lamellae; nodal differentiation was also absent.

Primary dendritic arborization of tufted cells within the glomeruli is similar to that of the mitral cells, and single fine sections of them are indistinguishable (Pinching and Powell, 1971*c*). Terminal portions of primary tufted cell dendrites are unidirectionally postsynaptic to the olfactory nerve terminals, and may form reciprocal synapses with the periglomerular cell dendrites, although at the point of primary branching, unidirectional synapses have also been recognized, with periglomerular dendritic endings as presynaptic components (Pinching and Powell, 1971*b*).

Secondary dendrites of the tufted cells form reciprocal dendrodendritic synapses in the EPL with the granule cell gemmules. Although these synapses cannot be distinguished under electron microscope from the mitral secondary dendrite-granule gemmule synapse, we have indirect evidence (Greer et al., 1982*a*) that the synapsing

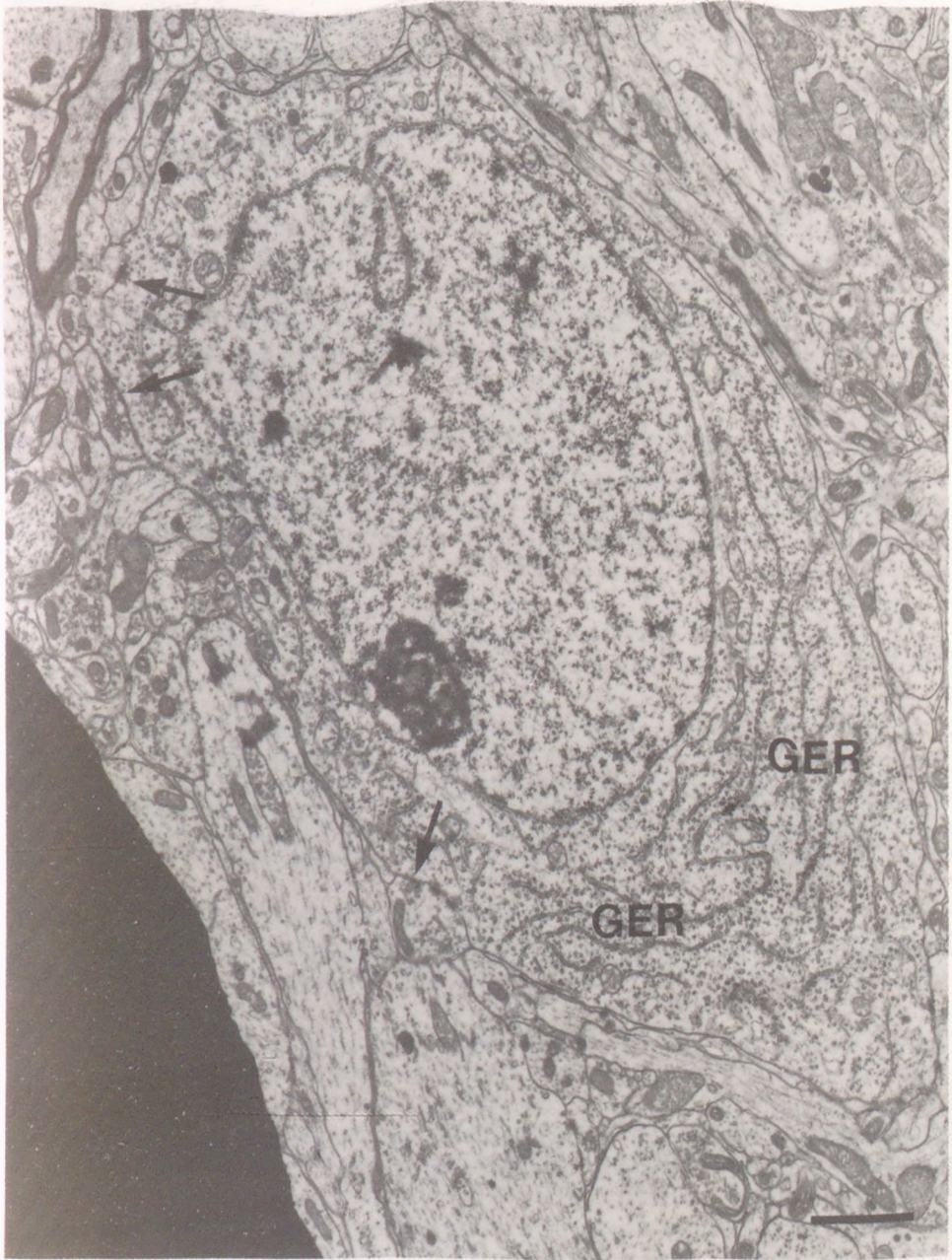


Fig. 4.14. Electron micrograph of a middle tufted cell soma. Adult rat, aldehyde-osmium fixation. Although the appearance of the cell is similar to the deep tufted cells and mitral cells, it is smaller, and has a relatively thinner cytoplasm, with a less well-organized granulated endoplasmic reticulum (GER). It is rich in ribosome rosettes and mitochondria; a few short stacks of Golgi- or smooth endoplasmic reticular cisternae, lysosomes, neurotubules, multivesicular bodies and various vesicles may also be found. Synapses (arrows), directed inwardly or outwardly from the cell are common. Scale bar: 1 μ m

secondary tufted cell dendrites are of smaller caliber than those of mitral cells. Axons of tufted cells regularly do not receive synapses before becoming myelinated. An exception was noted by Pinching and Powell (1971*d*) who observed rare symmetric synapses from gemmules onto the initial segment of the external tufted cells of rats, and noted reciprocal synapses in the same area of middle tufted cells. Pinching and Brooke (1973) described also a reciprocal synapse between an axon initial segment of a middle tufted cell and a characteristic granule dendritic gemmule.

Axon collaterals of tufted cells are distributed characteristically within the olfactory bulb. External tufted cells give rise to and distribute their local axon collaterals in the rat within the periglomerular area (Golgi-Kopsch technique: Pinching and Powell, 1971*b*), or deeper. Orona and collaborators (1984), with the aid of HRP injections, demonstrated the local axon collaterals of external and medium tufted cells exclusively in the MCL and IPL of the rat. It is possible, however, that in fact deeper tufted cells were studied in the latter investigation. In rabbits, local collaterals from middle tufted cells arise in the superficial GCL, or IPL, and branch within the same layers (silver impregnation: Cajal, 1911; Allison, 1953*a*; Valverde, 1965; HRP tracing: Kishi et al., 1984).

The termination area of tufted cell axons, outside of the ipsilateral olfactory bulb, is one of the most disputed questions. This problem will be discussed later in Section 5.1.

4.2.2.1.3. Other output neurons

Several neurons with a distinctly recognizable axon, leaving the olfactory bulb proper, could not be classified either into the mitral, or the tufted cell category. These cells were observed mainly in the olfactory bulb of fish and (with the only exception of the ruffed cell) were only sketchingly characterized. We discuss these cells in the sequence of phylogenetic order of the species, in which the respective olfactory bulb neurons were observed.

When characterizing the cyclostomes, Nieuwenhuys (1967) mentioned that the mitral cells were not the only cells which send their axons into the pathways leaving the bulb, but that some smaller elements do likewise. A distinct axon was described for *granule cells* of the selachian olfactory bulb (Sterzi, 1909, cit. Nieuwenhuys, 1967), and Johnston (1898) treats also the central processes of the sturgeon (*Acipenser rubicundus*) olfactory bulb granule cells as an axon, running similarly (centrally or backward) to all other axons. In addition, Johnston (1898) observed several other cells with centrally projecting axons: the *stellate cells* of the glomerular area and of the granule cell zone, the *spindle cells*, and a third type he designated *cells of Cajal*. These all are relatively large cells, with their greatest diameters between 8 and 40 μm and have a third feature in common: (at least some of) their dendrites contribute to the glomeruli, as the mitral cells do. Since their synaptology has not been studied yet by means of electron microscopy, we do not know whether further similarities exist.

Fortunately, much more is known about a proposed special output neuron of the goldfish, the *ruffed cell* (Fig. 4.15). The cell body, dendritic arborization and especially the axon initial segment was characterized in detail by Kosaka and Hama (1979*a,c*, 1980, 1981, 1982–83) and Kosaka (1980), although the course and final destination of

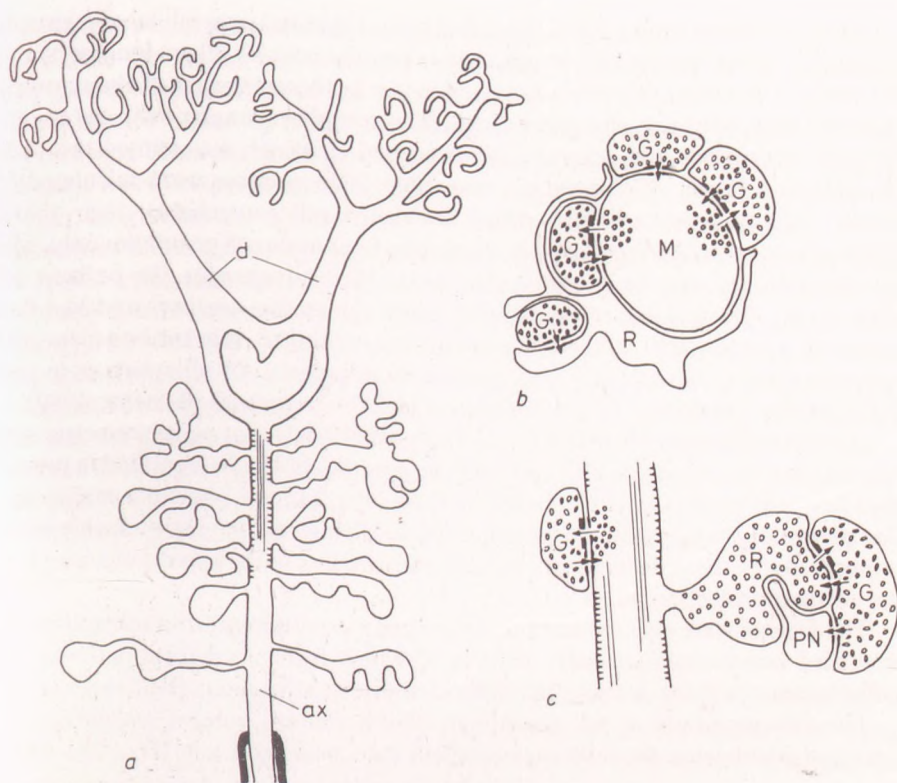


Fig. 4.15. Schematic drawing of the ruffed cell of the goldfish, based on Golgi-impregnation and electron microscopic studies (from Kosaka and Hama, 1982–83). *a*: dendritic arborization (d), cell body and initial unmyelinated segment of the axon (ax). *b*: synaptic relations between mitral dendrite (M), granule cell dendritic terminals (G) and the glial sheet-like dendritic processes (R) of the ruffed cell. *c*: granule dendritic gemmules (G) synapse onto protrusions of the ruffed cell axon initial segment (R) and dendrites of the perineuronal cell (PN). Single arrows: asymmetric synapses, double-headed arrows: symmetrical synapses. Reproduced with permission

the centrally directed axon has not been fully elucidated. The cell has a diameter between 10–20 μm , releases several dendrites extending about 100 μm from the cell body; the axon has an unmyelinated initial portion (70–250 μm long) with many protrusions (see Fig. 4.15a), then—because of lack of impregnation through the myelin sheath—could not be followed further. The nucleus is large and irregular, the cytoplasm is rich in organelles, especially in free ribosomes. Many unidirectional (somatofugal and somatopetal) as well as reciprocal synapses were observed on the cell body; between the contacting terminals, granule gemmules predominate.

The dendrites display a spherical receptive field, cca. 70–100 μm in diameter, distributed into many fine branches, which made a relatively small number of synapses. Only granule gemmules were identified as pre- or postsynaptic partners at these synapses (see Fig. 4.15b). No synaptic contacts were found between the olfactory nerve or mitral cell processes and the ruffed cells.

The most remarkable part of the ruffed cell is the initial unmyelinated segment of the axon. It is bare for about 20–40 μm , then (along the next 10–20 μm long part) gives rise to many collateral-like protrusions, while the furthestmost part of the unmyelinated portion does not have any protrusions. The complex synaptic field, involving these axonal protrusions and several other neuronal elements, was named the “nest” by Kosaka and Hama. Within such a nest, 1000–2000 synapses were calculated for one ruffed cell by Kosaka (1980). Here the ruffed cell protrusions, their shafts and appendages were found pre- and postsynaptic to granule cell gemmules only, while the granule endings were presynaptic also for another component, the perinest cell (see Fig. 4.15c). The granule cell gemmules made symmetric synapses and had flattened synaptic vesicles, the ruffed cell protrusions contained spherical vesicles and had asymmetrical synapses with their granule counterparts. Of all synapses in the nest, 63% were asymmetric, 12% symmetric and 25% reciprocal (Kosaka, 1980).

It was emphasized by Kosaka and Hama (1982–83) that no asymmetric synapses were found on the ruffed cells, and only the granule cells were identified in presynaptic position to this cell (Fig. 4.16). Based on these observations, it seems a challenging task for physiologists to study the contribution of such a cell, having probably inhibitory inputs only, to the processing of olfactory information in the teleost olfactory bulb and its cortical projection areas.

The Dipnoi were also reported to have a cell type, differing from the mitral cells, the so-called *interglomerular cells*, neurites of which form a separate pathway (tractus olfactorius externus), leaving the bulb for more central areas (Rüdebeck, 1945).

The olfactory bulb of the amphibian *Ablystoma* has certain “transitional cells”, axons of which leave the bulb together with the mitral cell axons (Herrick, 1924). The

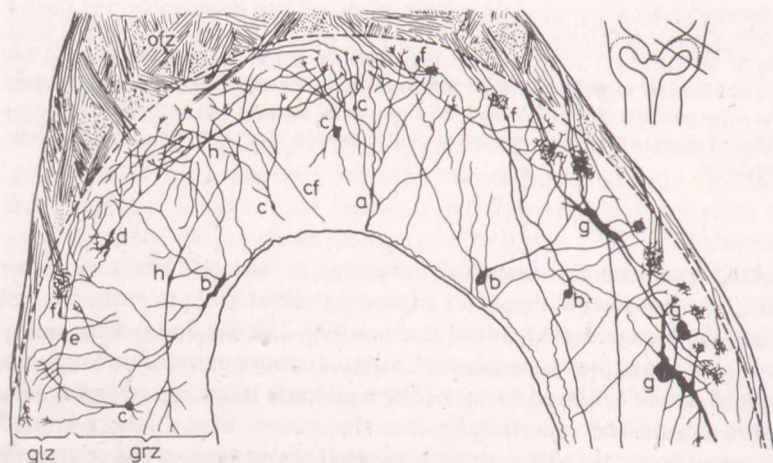


Fig. 4.16. Cell types of the olfactory lobe (bulb) of *Acipenser* (an actinopterygian fish), based on Golgi-type silver impregnation studies (redrawn from Johnston, 1898). *Inset* shows part of the forebrain with the area (between the strait lines) displayed on the scheme of a section in horizontal plane. ofz: olfactory fiber zone; glz: glomerular zone; grz: granule cell zone; a: spindle cell; b: stellate cells of the granule cell zone; c: granule cells; d: short-axon cell; e: stellate cells of the glomerular zone; f: small mitral cells; g: large mitral cells; h: Cajal cells

peripheral processes of these cells do not enter the glomeruli, but have their most distal endings below the glomerular layer. In this respect, this cell might be organized similarly to the teleostean ruffed cell (see above).

In the Alligator olfactory bulb, three peculiar (non-granule) cells (distinct from mitral cells) were observed in the granule layer. In the case of the “*intrinsic*” (type II) cell, no axon was distinguished, but axons of the two others, the *stellate cells* and the *goblet cells*, may enter the olfactory tract (Crosby, 1915, cit. Nieuwenhuys, 1967). Some dendrites of the goblet cells may contribute to the formation of the glomerulus. Allison (1953b) regarded the goblet cells as equivalents of the mammalian tufted cells.

All the above data were taken with Golgi-type silver impregnation techniques. Our last example for this cell category was identified, however, with retrograde HRP labeling, following injections of the tracer into the rat basal forebrain area (Haberly and Price, 1977). These labeled cells lay among the granule cells, some of them were smaller than the regular mitral cells, and they were stained less densely than the latter. The authors were uncertain in deciding, whether these labeled cells were “small mitral cells of Cajal”, displaced inwardly from the mitral cell layer, or whether they had discovered a third, yet unknown projection neuron in the olfactory bulb of the rat.

4.2.2.1.4. Summary

Sensory input from the neuroepithelium is relayed in the bulb and is conveyed to more central brain areas by axons of the output neurons. In the vertebrate bulb, mitral and tufted cells are the most frequent and best characterized output neurons, but certain other cells may also contribute to the olfactory tracts.

Perikarya of the output neurons are the largest in the bulb; they are rich in organelles. Primary dendrites of the mitral and tufted cells are contacted by the olfactory nerve axons in the glomeruli. In addition, both primary and secondary dendrites may synapse with local circuit neurons. Patterns of the distribution, branching and termination of the secondary dendrites and axon collaterals provide a morphological basis for the characterization of subclasses of the main output neurons.

4.2.2.2. Local circuit neurons (interneurons)

From lower vertebrates to man several types of interneurons can be distinguished in the bulb, which may be classified according to different aspects. These neurons are better known in mammals and especially in rodents and less distinctly characterized for other groups, like birds.

The projective neurons of the bulb receive peripheral (and central) information, process it and finally transmit their signals to more central brain areas. Interneurons are present in all areas where these functions are executed: they are in close proximity to the terminal dendritic field of the projective neurons, they may even be influenced directly by the peripheral receptor cell axon terminals. Local circuit neurons can also be found in the area of the thick dendritic shafts of output neurons and interact with them; they may receive central stimuli and; relay these signals for the mitral/tufted cells

even at their axon hillock and initial segment area. In addition to these interneurons, others may also be present in the same area, without having any direct extrinsic (peripheral or central) information, and establish connections just between the projective neurons and the former ("primary") interneurons (these latter can be called "secondary interneurons"). These secondary interneurons may also be found in the superficial and deep layers of the bulb.

In this Section we shall try to include all types of intrabulbar associational neurons, even if the information is valid for a limited group of vertebrates only, or includes a few or one single evidence for the existence of the respective neuron. As we shall see, a considerable variety of different neuron types were observed in adult vertebrates. It cannot be ruled out, however, that the continuous turnover of the receptor cells in the epithelium, and the production of new (deep) granule cells during adulthood, may induce more plastic changes in the bulb than in any other brain areas. This may suggest the appearance of temporary, transitional cell forms and connections, in addition to a few basic neuron types.

In most vertebrates, the interneurons of the olfactory bulb are grouped within a superficial, peripheral (glomerular) and deeper (granule) cell layer and, accordingly, interact with the "primary" dendritic tree, or with the secondary dendrites (where they exist) of the output neurons. In the following sections, the interneurons will be described in accordance with this anatomical organization. In lower vertebrates, however, in most cases, no strict lamination can be recognized for the interneurons. These scattered interneurons will be described first, followed by the superficially and deeply organized groups of interneurons of higher developed species.

4.2.2.2.1. Interneurons of the fish olfactory bulb

The lack of strict laminar organization of mitral cells into a single layer in olfactory bulbs of most fish (Allison, 1953*b*; Nieuwenhuys, 1963, 1967) implies that the interneurons, acting at different levels (dendritic appendages, dendritic shaft, cell body, axon initial segment) onto output neurons, may also be dispersed throughout the mitral cell area. Although there are a few exceptions of this arrangement, e.g., the olfactory bulb of the cyclostome *Myxine* (see Jansen, 1930; cit. Allison, 1953*b*, and Nieuwenhuys, 1967) and several bony fish and the Dipnoi (Nieuwenhuys, 1963, 1967), it is the class of amphibians, in which superficial and deep interneurons can easily be recognized as distinct groups (Herrick, 1924; Allison, 1953*b*; Nieuwenhuys, 1967; Andres, 1970, 1975). This is why interneurons of fish will be discussed in this separate Section. Numerous light microscopic observations contributed to the knowledge of this topic, as summarized by Nieuwenhuys (1963), but only a few electron microscopic studies were performed as yet; most of them on the goldfish olfactory bulb.

Although the lamination of these cells is not clear, there exists more than one single type of interneurons in the fish olfactory bulb, as can be judged from the cell size, location, arborization characteristics and synaptic relations. What most of the authors of the area agree on, is that granule cells do not have projections outside the olfactory bulb. In addition, some other cell types in different groups of fish may have similar connectivity patterns, as shall be discussed below.

Investigators of histology of the fish olfactory bulb regularly mention and characterize this type of cell, which is most abundant in the deeper aspect of the bulb, but may be interspersed in the neuropil area (plexiform layer) among the mitral cells, too. As a consequence, granule cell bodies have been observed even at the glomerular level (see Fig. 4.4a,b). Deep to the mitral cells, they are organized into the granule cell layer. The name "granule cells" or "granules" was used in the early literature for the Nissl-stained, small neurons of the bulb, which appeared as small granules under the light microscope. Several "granule cells" were shown (in the Acipenser) to have central projections (Johnston, 1898) or distinct axons (in the selachian olfactory bulb) (Sterzi, 1909; cit. Nieuwenhuys, 1967). Since these granule cells may have connections with an area outside of the olfactory bulb proper, we have mentioned them already in the section of various output neurons (other than the mitral or tufted cells).

Peripheral granule cell processes may have their endings at different depths extending from the glomeruli until the level of the deepest located mitral cells, i.e., the whole neuropil (plexiform) layer (Johnston, 1898; R debeck, 1945; Nieuwenhuys, 1967; Andres, 1970) (see Fig. 4.16). They have reciprocal synapses with the mitral cells—this is their main feature, as shall be discussed below. In addition, peripheral granule cell processes may establish the link between mitral cells and the ruffed cells (Kosaka and Hama, 1982–83). There is agreement between investigators that the granule cells in most vertebrates do not have true axons (Cajal, 1911; Allison, 1953b; Nieuwenhuys, 1967; Shepherd, 1979).

Electron microscopy revealed similar ultrastructure for this cell type in goldfish (Ichikawa, 1976) as found in mammals. The nuclei with a 5–6 μm diameter were surrounded by a tiny sheet of cytoplasm, which was generally poor in endoplasmic reticulum. The presumed granule cell of Kosaka and Hama, shown in their 1982–83 paper (see Fig. 4.18), which is presynaptic to a fiber, has its largest diameter around 13–14 μm (average for these cells: 10 μm) and resembles more closely the mitral cells of the goldfish (Ichikawa, 1976; Kosaka and Hama, 1982), particularly with regard to the richness of organelles, appearance of the nucleus and nucleus:cytoplasm volumetric proportion. It should be mentioned here that Kosaka and Hama (1979b, 1982–83) identified their granule cells as "the neuron which makes reciprocal synapses with the mitral cell", which does not exclude granule cells with centrally projecting true axons (if they exist) from the category (such granule cells shall not be included in our local circuit neuron category).

Synapses on the soma of the fish granule cells have been mentioned very rarely. In contrast, there is an abundance of contacts on the peripheral (and also central) processes of the granule cells, as characterized by electron microscopy for the goldfish (Ichikawa, 1976; Kosaka and Hama, 1979b, 1982, 1982–83, lamprey and shark (Andres, 1970). According to the majority of these observations, the peripheral granule cell dendrites arborize richly, and have many gemmule-like terminal enlargements. Oka (1983), nevertheless, observed reciprocal synapses exclusively between the dendritic *shaft* of certain (probable granule) cells and mitral cell dendrites. These presynaptic specializations are rich in synaptic vesicles (small, ovoid or mixed) and while they may contain mitochondria, they do not contain neurotubules. The

granule dendrites may establish reciprocal synapses with the mitral dendritic branches and shafts in the glomerular area and plexiform layer, as well as with the mitral cell bodies and axon initial segment (Kosaka and Hama 1982–83; Oka, 1983). At these contacts, presynaptic granule dendrites make symmetrical synapses onto the mitral component, and receive asymmetrical contacts from them. According to the serial sectioning studies by Kosaka and Hama (1982) 50% of the synaptic contacts between mitral and granule cell processes in the glomerular area of the goldfish were found to be reciprocal, 30% were mitral to the granule asymmetric synapse, and 20% were symmetrical, granule to mitral synapse. The peripheral granule cell processes also have reciprocal synapses with the peripheral processes of the ruffed cells (Kosaka and Hama, 1979a,c; 1980, 1981, 1982–83; Kosaka, 1980). In addition, granule cell dendritic endings may be presynaptic (symmetrical synapse) for another interneuron in the goldfish glomerular area, the perinest cell (Kosaka and Hama, 1982–83). Rare direct contacts between the olfactory nerve and peripheral granule cell processes have been observed by electron microscope (Kosaka and Hama, 1982–83).

The central granule cell processes have also several (unidirectional) inputs from nerve fibers of different origin, although no serial sectioning has been performed to

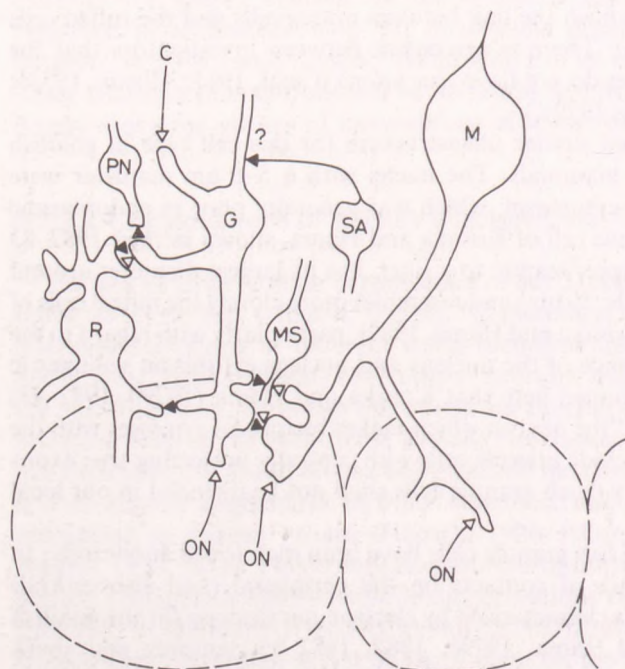


Fig. 4.17. Synaptic relations of the granule cells (G) of the goldfish olfactory bulb. This schematic drawing is based mainly on fine structural studies of Ichikawa (1976) and Kosaka and Hama (1982–83). Presence and direction of the symmetric synapses are represented by solid triangles, while empty triangles show asymmetric contacts. Outlines of glomeruli are marked by a broken line. Question marks denote uncertainty as to the origin of terminals, having symmetrical thickenings with central processes of granule cells. C: central fiber; G: granule cell; M: mitral cell; MS: mixed-synapse cell; ON: olfactory nerve axon; PN: perinest cell; R: ruffed cell; SA: short-axon cell

synapses from presumed central fibers or mitral axon collaterals in the plexiform layer of the shark and lamprey (Andres, 1970) and in the GLC of goldfish (Ichikawa, 1976; Ichikawa and Ueda, 1979) olfactory bulb and symmetrical synapses in the granule cell layer, originating supposedly from the short axon cells in the goldfish (Ichikawa, 1976). This complexity, including also the rare and ambiguous observations, is depicted in Fig. 4.17.

Short-axon cells

Cells grouped under this heading have been described from every level of the fish olfactory bulb. Johnston's (1898) "*cells with short axis cylinders*" were found in the glomerular area of the (Acipenser) olfactory bulb, or in the level of the mitral cells (according to his Fig. 1). Their dendritic branches are distributed in the glomeruli, similar to the dendrites of the "*cellules superficielles*" of Catois (1902). The latter cell body lies between the olfactory nerve bundles or glomeruli, and may be the predecessor of the mammalian periglomerular cells (Nieuwenhuys, 1967). Ichikawa (1967) described rare *short-axon cells* from the level of mitral or granule cells of the goldfish olfactory bulb, but did not study the dendritic arborization of these cells. Axons of his short-axon cells are presynaptic for the peripheral processes of the granule cells. We do not know about the course and extension of the axons of the "*cellules superficielles*" of Catois. Johnston's short-axon cells emitted an axon, which branched into finer fibers, which were lost in the glomerular zone.

Two other intrinsic associational cells were described by detailed Golgi-impregnation and electron microscope serial sectioning studies of the goldfish olfactory bulb (Kosaka and Hama, 1982-83). The one best characterized, is the so-called *perinest cell*, which lies around the "nest" of the ruffed cells (see Section 4.2.2.1.3.). The cell body is about 8 μm in diameter, has a relatively thin rim of cytoplasm around the nucleus, and is relatively poor in organelles. Processes originating from the cell body vary in shape, and may contain vacuoles, cisternae and mitochondria, in contrast to the vesicle-containing granule cell processes, which were found presynaptic to the perinest cell processes (Kosaka and Hama, 1982-83). Nothing is known about the presynaptic perinest cell processes. Even less data are available for another cell type of the goldfish olfactory bulb, the "*mixed-synapse cell*" (Kosaka and Hama, 1982-83). Their processes may receive asymmetrical synapses from the granule cell dendrites as is deduced from reconstructions of serial sections. One such mixed-synapse cell was shown in Fig. 10 of Kosaka and Hama (1982-83) after Golgi impregnation. It shows two major dendritic branches, with many thin cylindrical processes and spines. The tiny axon of this cell is divided into several branches at about 50 μm from the cell body; the branches were followed for another 50 μm . No postsynaptic structure for any of these processes were identified in this paper (Kosaka and Hama, 1982-83).

According to the available data, the "*interglomerular mitral cells*" of Holmgren, the *stellate cells*, described by Holmgren and Jansen from the Myxine olfactory bulb, several scattered "*interglomerular cells*" of R  debeck (1945) (in Dipnoi) and the follow the course of either of the synapsing components. These include asymmetrical

"*cellula axoramificata*" of Sterzi (from shark) (as cited by Nieuwenhuys, 1967) may also be different from the granule cells, although they may have short, intrinsic axonal processes, indicative of interneurons.

4.2.2.2.2. Superficial interneurons of higher vertebrates

This section describes the interneurons located at the glomerular level of the olfactory bulbs from amphibians to the mammals. These short-axon cells are in (direct or indirect) connection with the most peripheral dendritic arborization of the output neurons, immediately after the primary olfactory nerve has contacted the latter. This means that the cell bodies of the superficial interneurons are located in close proximity to the glomeruli and that their processes are distributed within the intraglomerular neuropil or in the periglomerular area. Although data point to the possibility that several superficial tufted cells may also lack long axons, projecting out from the olfactory bulb, and so serve only local functions, the vast majority of the superficial interneurons can be classified as periglomerular cells. This cell category, based on light microscope morphological observations, seemed to be a fairly homogeneous group (Golgi, 1875; van Gehuchten and Martin, 1891; Koelliker, 1892; Blanes, 1898; Cajal, 1911; Herrick, 1924; Allison, 1953*b*; Valverde, 1965; Nieuwenhuys, 1967). By careful analysis of serial thin sections, Pinching and Powell (1971*b*) supplied morphological proof for the presence of a rare, distinct type of neuron between common periglomerular neurons: the superficial short-axon cell. Thus, we will characterize first the (regular, common) small periglomerular cells, then the superficial short-axon cells and finally discuss data for the existence of local, interneuron-like (superficial) tufted cells.

Periglomerular neurons

Under the light microscope, small caliber neurons of the glomerular layer featured granule-like appearance in Golgi preparations, so, it is not surprising that several authors named these cells "outer granule cells". The size of the periglomerular (PG) cells, distribution of their cell bodies and processes were similar to those of the glial cells in the periglomerular area, which led Koelliker (1892) to question their neuronal nature. Shortly thereafter, Blanes' (1898) investigations on rabbits, cats and dogs supported again the original proposals of Golgi (1875), Cajal (1890, cit. Blanes, 1898) and van Gehuchten and Martin (1891) for PG cells, as true (local circuit) neurons.

The cell body has a round or oval shape, with maximal diameters from 5 to 10 μm . The cells can be found all around the glomeruli, but mostly between the GL and the EPL (Figs 4.18–4.19, and see also Fig. 4.28). As an exception, Herrick (1924) observed clumps of PG cells especially on the outer surface of the glomeruli. Dendrites of these neurons are distributed within one or two glomeruli (see Figs 4.18, 4.28), where they may have numerous varicosities and also spines.

The axons run to neighboring glomeruli, where they end in the periglomerular neuropil area, or within the superficial region of the EPL. These basic features might be

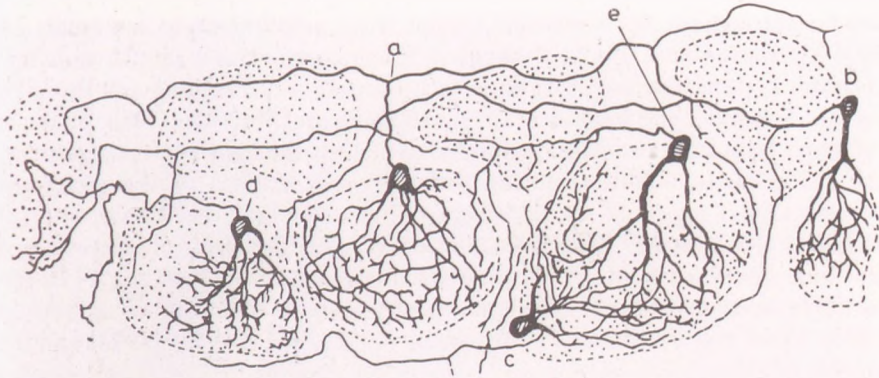


Fig. 4.18. Periglomerular cells: position of somata and distribution patterns of their processes in the glomerular layer, as seen by Golgi-type silver impregnation (redrawn from Blanes, 1898). Dendritic arbors may invade the whole neuropil area of a glomerulus (see cell "a"), or rather just a compartment of it (b,c,d). The axons may give rise to several collaterals, terminating in different glomeruli (see cell "e") but ending in the close vicinity of their parent cell bodies (see b,d)

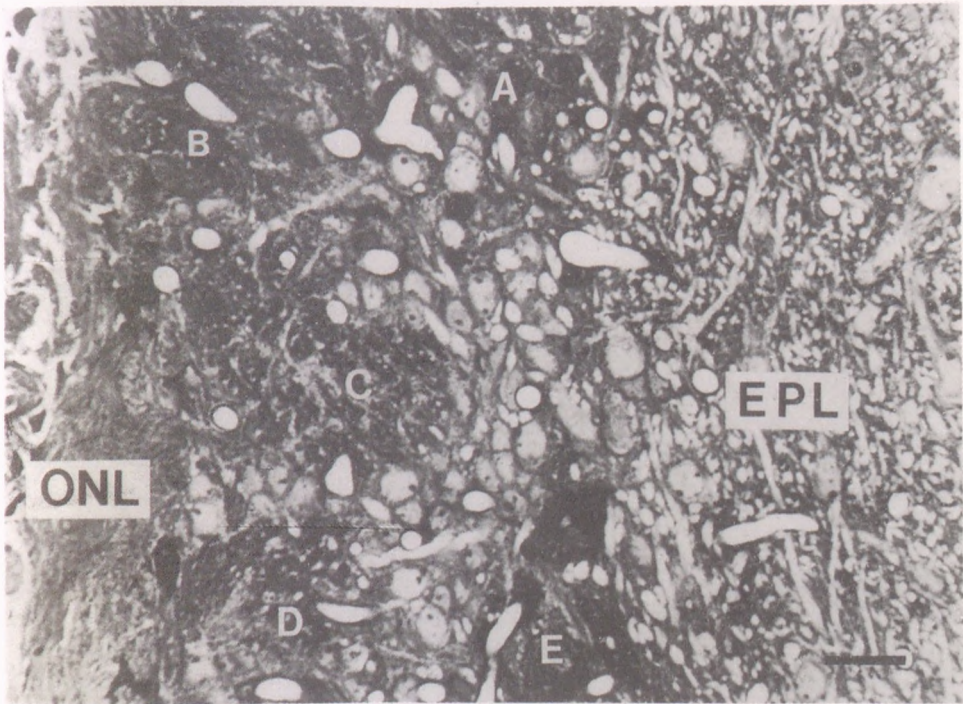


Fig. 4.19. High-resolution light micrograph of the glomerular layer of the rat olfactory bulb. Aldehyde-osmium fixation, Epon embedding, 2 μ m thick section, toluidine blue staining. Details of several (A-E) glomeruli, separated by cells (and their thick processes), are displayed. Periglomerular cells accumulate preferentially at the deeper part of the glomeruli, facing the external plexiform layer (EPL), opposite to the entrance of the axons of the olfactory nerve layer (ONL). Scale bar: 20 μ m

similar throughout the vertebrate classes from amphibians to mammals (Allison, 1953*b*; Nieuwenhuys, 1967), although in lower vertebrates a smaller number of PG cells was present. Recent Golgi impregnation studies of Pinching and Powell (1971*b*) on rats, Schneider and Macrides (1978) and Davis and Macrides (1983) on the hamster olfactory bulb led to similar conclusions as regards the light microscopic structure of the PG cells. In addition, during their extensive studies of the glomerular and periglomerular region of the rat olfactory bulb, Pinching and Powell (1971*a-d* and 1972*a-c*) characterized the PG cells in detail with the electron microscope. Other features, described here for the fine structural appearance of the PG cells, their processes and synaptic relations derive mostly from the observations of Andres (1965, 1970), Reese and Brightman (1970), White (1972, 1973), Willey (1973) and from our studies (Halász, 1974, 1976).

Very little cytoplasm surrounds the nuclei (Fig. 4.20 and see also Fig. 4.22). The nucleus follow the shape of the cell, so they are rarely a regular sphere, and may show several, sometimes deep, indentations. The nuclear plasma has a patchy appearance. The cytoplasm is rich in ribosome rosettes only; a few mitochondria and several cysternal elements are still common constituents. The well developed Golgi apparatus and the vast majority of the other cell organelles are regularly concentrated at the poles where the dendrites originate. Occasionally, somatic spines can also be recognized on PG cells. Very few (single) stacks of rough endoplasmic reticulum can be observed in this cell type. In spite of the low number of organelles, the appearance of the cytoplasm is darker than that of the other types in the PG area, and may remind the observer of densely stained glial cells. The glial appearance of the periglomerular neurons is further strengthened by the presence of thin, sheet-like processes, emerging from the cell body ("thin processes" of Reese and Brightman, 1970). These processes may wrap around other cellular elements, and fill the (sometimes) irregular space between the cells and fibers, in the same manner as the glial processes do. With increasing distance from the parent cell, the processes gradually loose their density of staining and number of organelles (see, e.g., Fig. 4.20, cells B and D).

Another type of ensheathing lamellae were also observed around several PG (and other bulbar) neurones (and dendritic segments) of the monkey olfactory bulb (Tigges and Tigges, 1980). This kind of myelin sheet is, however, different in ultrastructure from the (regular) axon myelin sheets: it lacks nodal structures, and is extremely thin and short (Remahl and Hildebrand, 1985). In addition, PG neurons and (regular) dendrites are surrounded frequently by non-compact glial sheets.

The common, large dendrites of the PG cells are pale and tortuous, have irregular neurotubules, a few mitochondria, and may bear spines. These spines often contain large, flattened vesicles, cysternae, ribosomes and sometimes mitochondria. The spines may receive asymmetrical synapses from primary dendritic branches of mitral and tufted cells, while the PG dendritic shafts and gemmules also have reciprocal synapses (with symmetrical thickenings, when directed away from the PG element) with dendrites from mitral and tufted cells (Pinching and Powell, 1971*b,c*; see also the section 4.2.2.1. and Fig. 4.10). Andres (1965) described the PG cell—tufted cell (reciprocal) connection in the glomerular layer, and emphasized its similarities to the (inner) granule cell—mitral secondary dendritic contacts, since Pinching and Powell (1971*b-d*) and later authors (including Andres himself, 1970) did not restrict this type

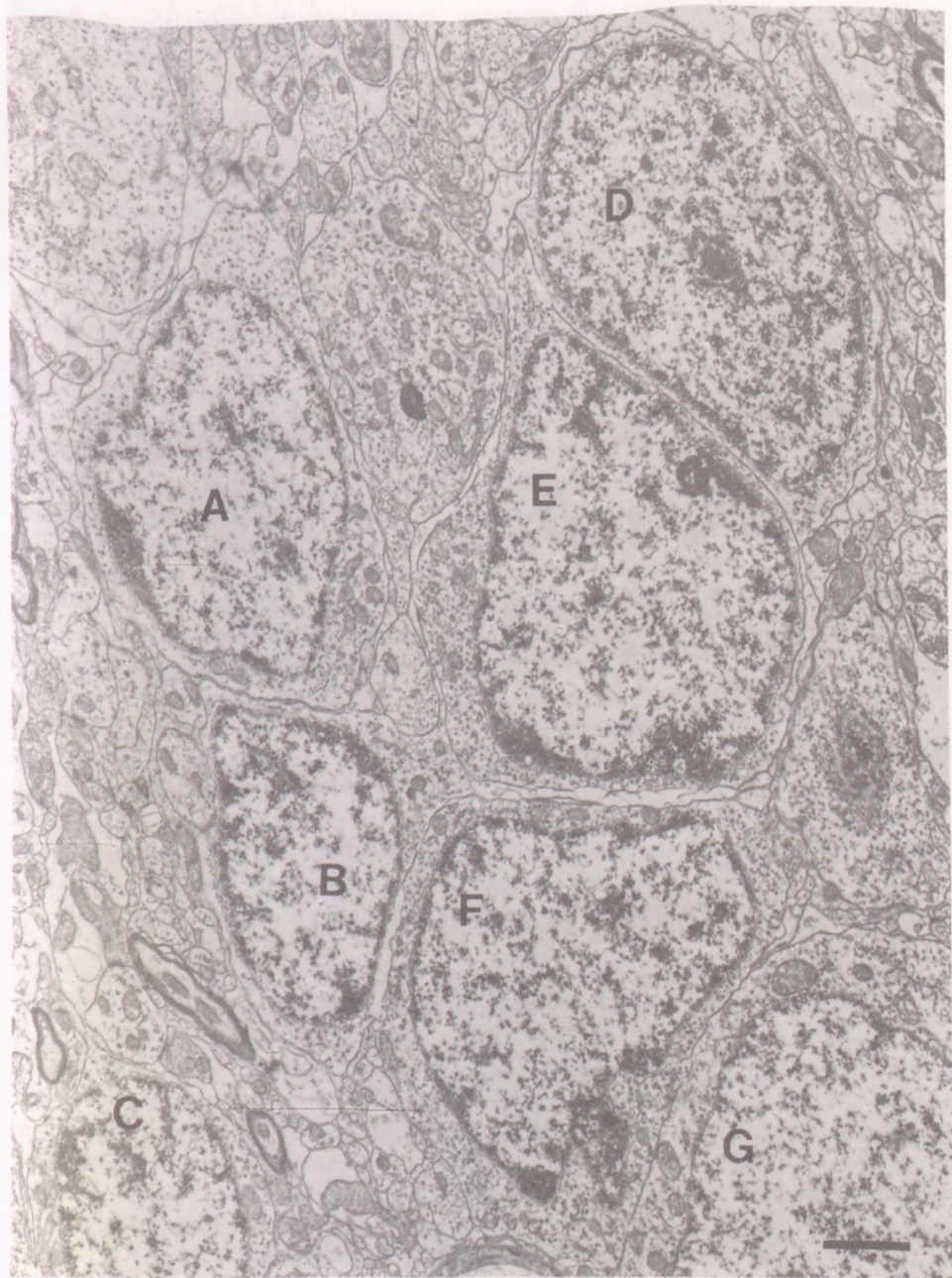


Fig. 4.20. Low power electron micrograph of periglomerular cells from the rat olfactory bulb. Adult rat, aldehyde-osmium fixation. All cells but one (G) appear to be small periglomerular neurons, while cell G resembles superficial tufted cells. Periglomerular cell nuclei have characteristic dense clumps of the chromatin material, so differ clearly from the protoplasmic astrocytes. Nuclei occupy the largest area of the periglomerular cells in this plane; the narrow cytoplasm surrounding them is rich in free ribosomes, but contains also small mitochondria, single short channels of the granulated endoplasmic reticulum and several small cisternae and vesicles. Narrow extensions of certain cells (see, e.g., cells B,D) ensheath parts of neighboring neurons; these processes gradually become very poor in organelles, resembling the "watery" processes of astrocytes. Scale bar: 1 μ m

of connection to the tufted cell dendrites, because of the difficulties in making distinctions between the mitral and tufted cell dendrites within the glomerular layer (see, e.g., the serial sectioning study of Pinching and Powell, 1971c).

Periglomerular cell bodies were also found postsynaptic to round or flattened vesicle containing processes (Pinching and Powell, 1971b) especially at places where somatic spines arose and a large number of vesicles accumulated inside the somatic cytoplasm. The thin, sheet-like dendritic processes of the PG cells have never been seen in a presynaptic position (Pinching and Powell, 1971b,d). Although mitral and tufted cell (primary) dendrites are regarded as the most important postsynaptic structures of the primary olfactory nerve, PG dendrites may also receive direct synaptic contacts from the olfactory receptor axons (Pinching and Powell, 1971c; White, 1973; Halász, unpublished data). It should be noted, however, that this contact might not be generally characteristic of all vertebrates. For example, in the Balb/c mouse, White (1972) was unable to detect any synaptic contacts of this type during his serial sectioning studies, although PG dendrites of the olfactory bulb of other closely related mammals, like other mice (*Mus musculus molossinus*) and the Sprague-Dawley rats, possessed this synapse (White, 1973).

Axons of PG cells regularly reach the nearest glomeruli of the periglomerular area (see also Figs 4.27 and 4.28). In the *Amblystoma*, these axons may terminate in the neighboring EPL, too (Herrick, 1924), and in anurans (Hoffman, 1963), fibers, originating in the glomerular layer, may contact mitral cell processes also deeper than the glomeruli. In rats, it was difficult to recognize the presence and follow the course of the Golgi-impregnated axon, but at the limited serial sectioning material of Pinching and Powell (1971b), the origin of an axon from the PG cells was always clearly observed. The axons were beaded during their course, occasionally branching, but "rarely reached beyond 3 or 4 glomeruli" (Pinching and Powell, 1971b). Studies, based on degeneration, caused by fine surgical lesions on the dorsal surface of the olfactory bulb (Pinching and Powell, 1971d), or retrograde transport of a poisonous lectin, ricin (Wiley, 1982), injected in small amounts into the glomerular layer (Gábel and Halász, unpublished data), revealed similar maximal extension for the periglomerular cell axons.

The axon initial segment shows a few microtubuli and many ribosomes and may receive symmetric synapses from supposed other periglomerular neurons (having large, flattened vesicles in their presynaptic terminals) or superficial short axon neurons (if small, flattened vesicles are present) (Pinching and Powell, 1971b). The terminal branch makes symmetrical synapses (with large flattened vesicles) and contacts all types of the neurons involved in the glomerular layer, but mostly the large mitral and tufted cell dendrites, preferentially in the region of their first branching (Pinching and Powell, 1971d). Other PG axon terminals have been described by the same authors on somata, axon initial segments and dendrites of other PG and short axon cells and on the base of the somatic spines of the PG cells.

Superficial short-axon cells

For lower vertebrates, no detailed histological study has yet been made to reveal the particular characteristics of this cell type. Our characterization is based mostly on the observations made on the olfactory bulbs of rats (Pinching and Powell, 1971*b,d*, 1972*c*; Halász, 1974, 1976 and unpublished results), cats (Willey, 1973) and hamsters (Schneider and Macrides, 1978; Macrides and Davis, 1983). While this cell of the rat olfactory bulb was recognized only in the GL, in cats and hamsters the superficial short-axon (SA) cells may extend into the superficial EPL, too (see Fig. 4.27). In rats and cats they were rarely seen, while in the hamster, their number was "comparable to that of periglomerular cells" (Schneider and Macrides, 1978). The size of this cell was also larger ($7-13 \times 12-21 \mu\text{m}$) in hamsters than in rats ($8 \times 12 \mu\text{m}$). The cell had a fusiform or oval shape and emitted several dendrites (Fig. 4.21).

The dendrites ramify mainly in the periglomerular area (and in the superficial EPL in hamsters), where they are distributed between the glomeruli, or surround them with characteristic, clasp-like branchings both in rats and hamsters. The dendrites may wear spines, which is a distinctive sign in contrast to the smooth tufted cell dendrites. In

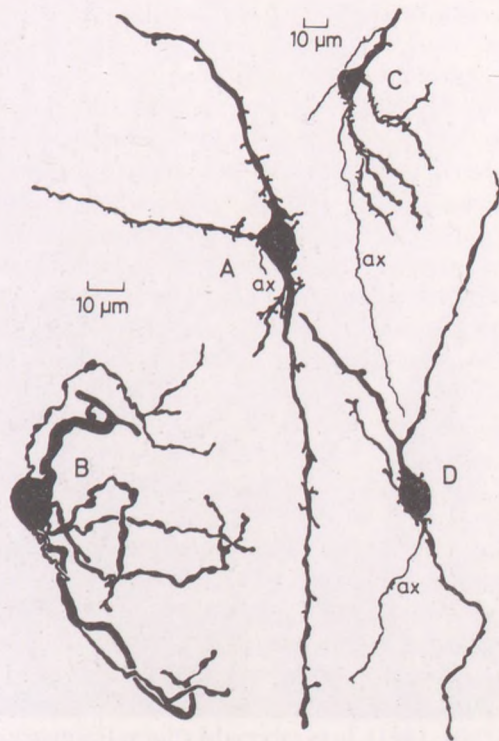


Fig. 4.21. Silver-impregnated examples of superficial short-axon cells. Camera lucida drawing (from Pinching and Powell, 1971*b*). Dendrites of cells C and B formed a "clasp" around a single glomerulus, while those of A and D were distributed over several adjoining glomeruli. Presence of varicosities and spines also differed. ax: axon (characteristic length is shown for cells C and D). Cell C is at lower magnification. Reproduced with permission

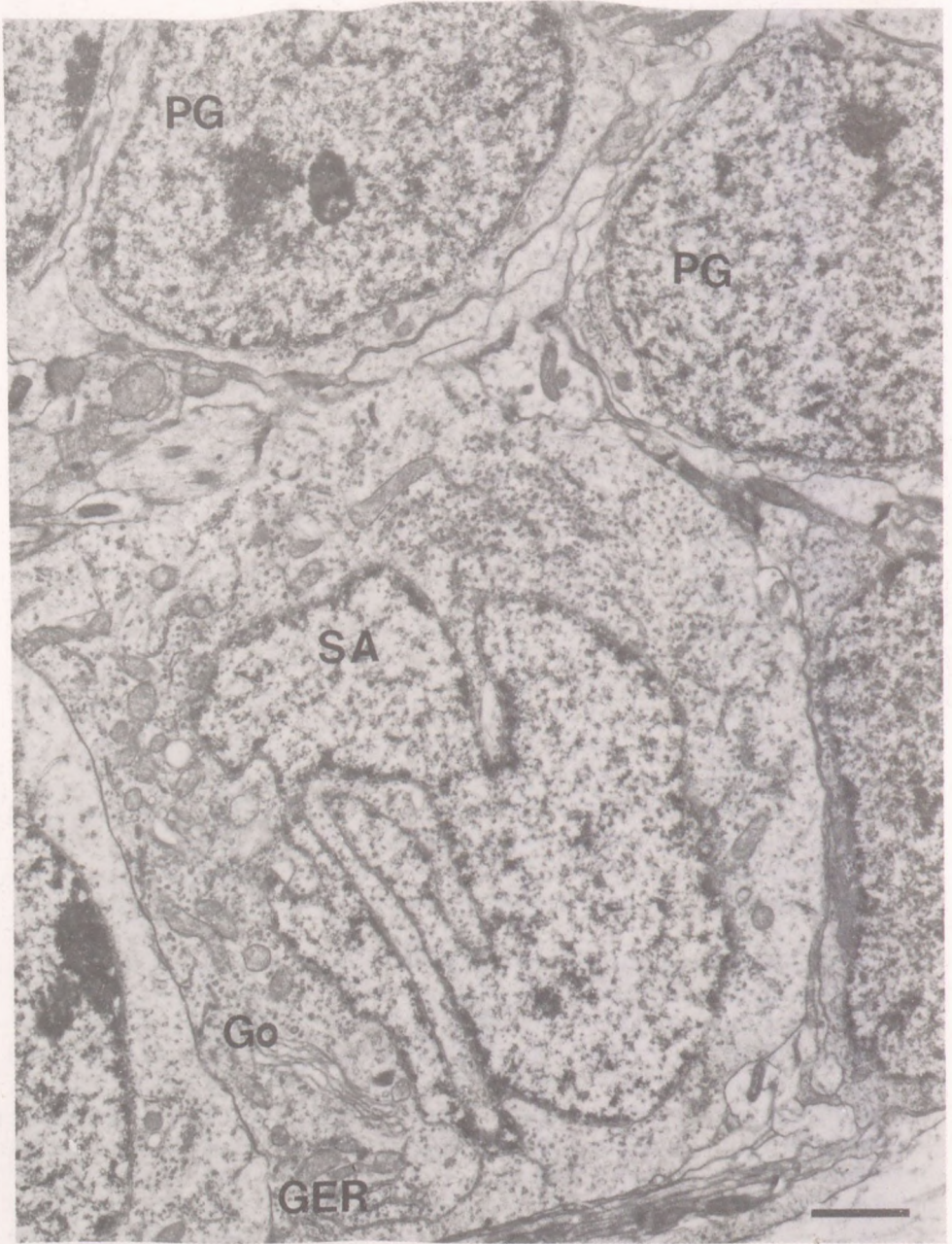


Fig. 4.22. Fine structural characteristics of a superficial short-axon cell (SA). The soma is larger than that of the periglomerular (PG) cells, but less electron-dense. The nucleus has less chromatin material and is regularly deeply indented. Numerous free ribosomes and mitochondria can be observed and a well-developed segment of the Golgi apparatus (Go) also appears. Several parallel stacks of the granulated endoplasmic reticulum (GER) may also be recognized at this magnification. Scale bar: 1 μ m

rats, the axons are shorter than those of the PG neurons (they and their collaterals do not traverse more than 1–3 glomeruli, Pinching and Powell, 1972c), while in hamsters, although rarely, superficial SA axons may ramify even in the internal plexiform and granule cell layers.

The ultrastructural appearance of the superficial SA cells resemble closely the deep SA cells, located in the granule cell layer (cf. Price and Powell, 1970d) similar to the way in which PG cells resemble granule cells. A relatively large part of the cross-sectional area of the cell is occupied by the nucleus, which is characteristically indented (Fig. 4.22). The nucleus (and the cytoplasm as well) appears paler than that of the periglomerular cells, the chromatin material forms a thin wedge just below the nuclear membrane. In the cytoplasm, there are several stacks of rough endoplasmic reticulum, although it is never so rich as that of the tufted cells in the same area. Density and distribution of mitochondria and free ribosomes is similar to the tufted cells, and is also commonly found at the base of the dendrites. The dendrites are thick and rich in organelles, but more irregular and varicose than those of the mitral or tufted cells. Spines may also be found on the soma and dendrites, but less in number than in PG cells.

A characteristic ultrastructural feature for the cell and dendrites is the lack of small (agranular) synaptic vesicles, and synapses, directed outwards from these structures. For the axons of the rat superficial SA cells, there is only one single synaptic target: the periglomerular cell (Pinching and Powell, 1971d). These contacts have symmetrical thickenings and small flattened vesicles. In contrast, synaptic inputs—although there is no direct, feed-forward information through the olfactory nerve—may reach this cell in many different ways. According to normal and experimental degenerative materials, synaptic endings of external tufted cell collaterals, centrifugal afferents as well as axon from local interneurons (frequently from PG cells) may all be found on the dendrites and cell body. The PG cells may also synapse on the axon initial segment (Pinching and Powell, 1971d). The most characteristic synapse type on the SA cell is made, however, by terminals containing round synaptic vesicles in the presence of asymmetric thickenings. Such synapses onto the PG and superficial tufted cell somata have very rarely been observed (Pinching and Powell, 1971b).

Superficial (external) tufted cells with intrabulbar projections

Experimental evidence for the existence of such a subgroup of the tufted cells in the mammalian olfactory bulb has been published very recently by Schoenfeld and co-workers (1985), although several earlier data pointed already to this possibility. Haberly and Price (1977) were unable to label a substantial portion of external tufted cells of the rat olfactory bulb (those, lying in the outer two-thirds or half of the glomerular layer), and questioned the existence of their bulbofugal projection. Based on similar tracing experiments and neurochemical results Macrides et al. (1981, 1985), Macrides and Schneider (1982), Davis and Macrides (1983) and Schoenfeld and Macrides (1984) reached the conclusion that the great majority of the external tufted cells, particularly those in the superficial two-thirds in the hamster olfactory bulb, do not project to the retrobulbar area. A recent paper of the same group (Schoenfeld et al.,

1985) classifies the external tufted cells of the hamster into three categories, only one of which is a true output neuron. The second group includes external tufted cells, located at the deeper aspect of the glomerular layer, establishing an associational pathway between opposing regions of the medial and lateral olfactory bulb while the third type is located in the outer two-thirds of the layer, and has only local connections. Therefore, the careful double-labeling experiments of Macrides and his co-workers furnish strong evidence for the existence of interneuron-like tufted cells in the hamster olfactory bulb. In other vertebrates, however, further observations should reveal clear distinctions between the supposed subclasses of the external tufted cells. Here we refer just to two other recent studies on rats, during which the output nature of axons of all external tufted cells studied, has been confirmed with orthodromic stimulation (Schneider and Scott, 1983) or HRP iontophoresis (Orona et al., 1984). It cannot be ruled out, however, that these very tufted cells would correspond to the first (mitral-like) category of the hamster olfactory bulb of Schoenfeld and colleagues (1985).

4.2.2.2.3. Deep interneurons of higher vertebrates

This Section deals with the local circuit neurons lying along the mitral cells or deeper, interspersed between the myelinated fibres of the olfactory tract. Peripheral processes of these cells, although they may extend until the most superficial part of the EPL, do not reach and enter the glomeruli. Consequently, they do not have direct inputs from the receptor cells, and have no connections with the primary branchings of the output neurons.

These cells form a distinct layer, extending from the mitral cells to the deepest portion of the bulb. Close to the mitral cells, however, a fiber layer (internal plexiform layer) of different thickness may separate (one or several rows of) the most superficial interneurons from the vast majority of the deeper lying ones. Most of these neurons are of small caliber, without a distinctive axon, and are designated granule cells, while several types of somewhat larger short-axon cells were also found in this depth, as is described below.

Granule cells

The granule (GR) cells are the most numerous neurons of the OB. Although their neuronal nature has been disputed (Koelliker, 1892), because of similarities to glial cells, and lack of a true axon, most of the early authors, using Golgi impregnation (Van Gehuchten and Martin, 1891; Blanes, 1898; Cajal, 1911; Herrick, 1924) were convinced that these cells are neurons, similar to the amacrine cells of the retina. Golgi (1875) described them as neurons, lying in the white matter of the deep aspect of the OB. He observed a pyramide-like shape of the cells, the base of which is lying centrally, while the superficially pointing apex releases a thick, thorny process, ramifying in the EPL. Other processes from the base (or other parts of the cell) were much thinner and shorter. In amphibians, characteristic "biramous" granule cells were observed (Herrick, 1924) with two similar processes, both directed obliquely to glomeruli,

branching in the EPL, and appearing thorny if compared to the smooth mitral dendrites. Based on these observations, Herrick supposed that the “amphibian granules are not structurally polarized”, i.e., both processes may serve as “emissive” or “receptive”. Some transient cells were also described between granule cells and cells of the anterior olfactory nucleus.

Recent studies with modified and refined Golgi impregnation of the rat (Price and Powell, 1970a), rabbit (Mori et al., 1983) and hamster (Schneider and Macrides, 1978) olfactory bulb essentially supported the early observations and revealed many finer details of the light microscopic appearance of these cells and their processes. Moreover, several subtypes of the granule cells of the rabbit and rat OB were also recognized, based mostly on data for localization of the cell bodies and distribution of the peripheral branchings in different depths of the bulb (Mori et al., 1983; Orona et al., 1983).

The GR cells are radially orientated; their elongated small ($6 \times 10 \mu\text{m}$ in rats, somewhat larger, $9 \times 13 \mu\text{m}$ in hamsters) cell bodies may lie close to each other, forming clumps, or 1–2 cell layer thick rows (Fig. 4.23). The peripheral processes are

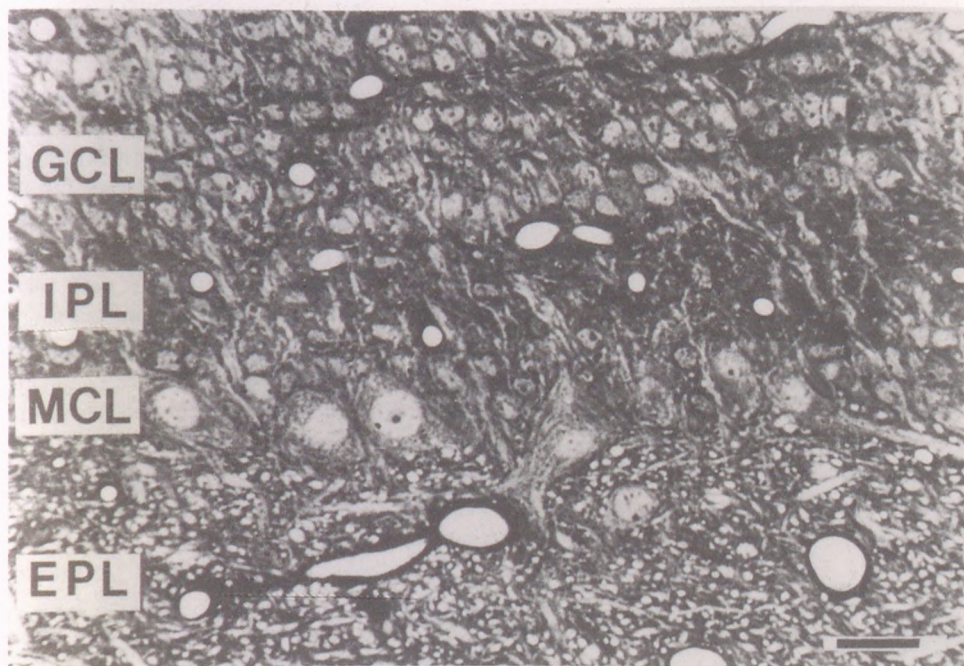


Fig. 4.23. Light microscopy of the superficial granule cell layer (GCL), internal plexiform layer (IPL) and mitral cell layer (MCL) in sagittal plane. Adult rat, aldehyde-osmium fixation, Epon embedding, toluidine blue staining of a $2 \mu\text{m}$ thick section. Granule cells in the GCL are tightly packed in groups, forming single- or double-layered sheets, separated by neuropil zones. Numerous granule cells may also be observed in the MCL; they accompany the mitral cells. Radial processes of the granule cells, appearing as light, wavy fibers, lying deep to or between the mitral cells, run perpendicularly to the layers. EPL external plexiform layer. Black scale bar: $20 \mu\text{m}$.

thick at their origin and are directed towards the mitral cell layer and EPL, where they then branch. Central processes of the cell are usually shorter (30–130 μm in hamsters) and thinner than the peripheral process, but have many swellings. All parts of the granule cells may bear spines, but these appendages differ in appearance, depending on their position (Fig. 4.24). In general, deeply localized spines are smaller and have thinner, slender pedicles, while the peripheral ones, especially those sitting on the branchings in the EPL are larger. These “gemmules” (Rall et al., 1966) contact the accessory (secondary) dendrites of the output neurons. The unbranched portion of the peripheral process has relatively few spines and varies in length according to the position (depth) of the cell body. This observation pointed to the possibility of the existence of GR cell subtypes (Shepherd, 1972).

As the deep output neurons (mitral and internal tufted cells) of the bulb distribute their secondary dendrites in the deep stratum of the EPL, and the more superficial tufted cells in the outer part of the same layer (see Section 4.2.2.1.), Mori and Kishi's (1982) observation by HRP filling (and electrical stimulation) showed, that deep spines may synapse mainly with mitral cell secondary dendrites, while the superficial spines of the GR cells interact rather with tufted cell dendrites. Shortly thereafter it was recognized simultaneously, by two groups that different subtypes of the GR cells exist, which contact either deep (preferentially mitral) or superficial (mostly tufted cell) secondary dendrites (Mori et al., 1983; Orona et al., 1983;). Orona et al. (1983) observed two classes of the rat olfactory bulb granule cells. The cells, lying in the deep stratum of the GCL, innervate exclusively the deep EPL, while the superficially located GR cells have a higher spine density (so, probably more numerous synaptic contacts) in the outer part of the EPL (see Fig. 4.11). In rabbit, Mori and colleagues (1983) reconstructed the distribution of branching and cell bodies of 31 GR cells of the OB and distinguished three different categories. Their type I granule cells distributed their gemmules in all depths of the EPL. Branchings (and spines of) the type II cells were confined to the deep half of the EPL; the cell bodies were found throughout the GCL (but deep GR cells tended to project deeper than the more superficial ones). Type III GR cell peripheral processes ran radially through the inner half of the EPL, giving branches to the superficial half of this layer (the cell bodies of these GR cells tended to be situated in the superficial part of the GCL or along the mitral cells). Golgi impregnation of the mouse OB revealed similar distribution patterns of dendritic branchings and spines of three granule cell types in recent studies of Greer (1987), performed on heterozygous (normal) littermates of the Purkinje cell degeneration (pcd) mice. Three types of granule cells were reported also from the *Podarcis* (a reptile) OB (Garcia-Verdugo et al., 1986). Granule cells of the hedgehog differed also in morphology and in position of cell somata but could terminate, regardless of their position, in any depth of the EPL (López-Mascaraque et al., 1986).

Staining patterns of GR cells were also found to be dissimilar. Struble and Walters (1982) described darkly and lightly stained GR cells on 3 μm thick, methacrylate embedded and toluidine blue-stained rat olfactory bulb sections. Their “light granules” had the tendency to be located deeply, and comprised about 15% of the GR cells in the adult rat OB. These cells were thought to be formed mainly prenatally and, according to their position, might be correlated with the deeply situated, HRP-filled GR cells described by Orona and co-workers (1983). A difference in the staining

patterns of the granule cells was also observed in some anuran OBs (Hoffman, 1963). The cytoplasm of the lightly stained, larger cells was hardly visible, while the smaller cells had dark nuclei and possessed numerous cytoplasmic processes giving the cells a stellate appearance.

Structural characteristics of the GR cells and their processes, revealed by electron microscopy, have been published mainly in mammals (Andres, 1965, 1970, 1975;

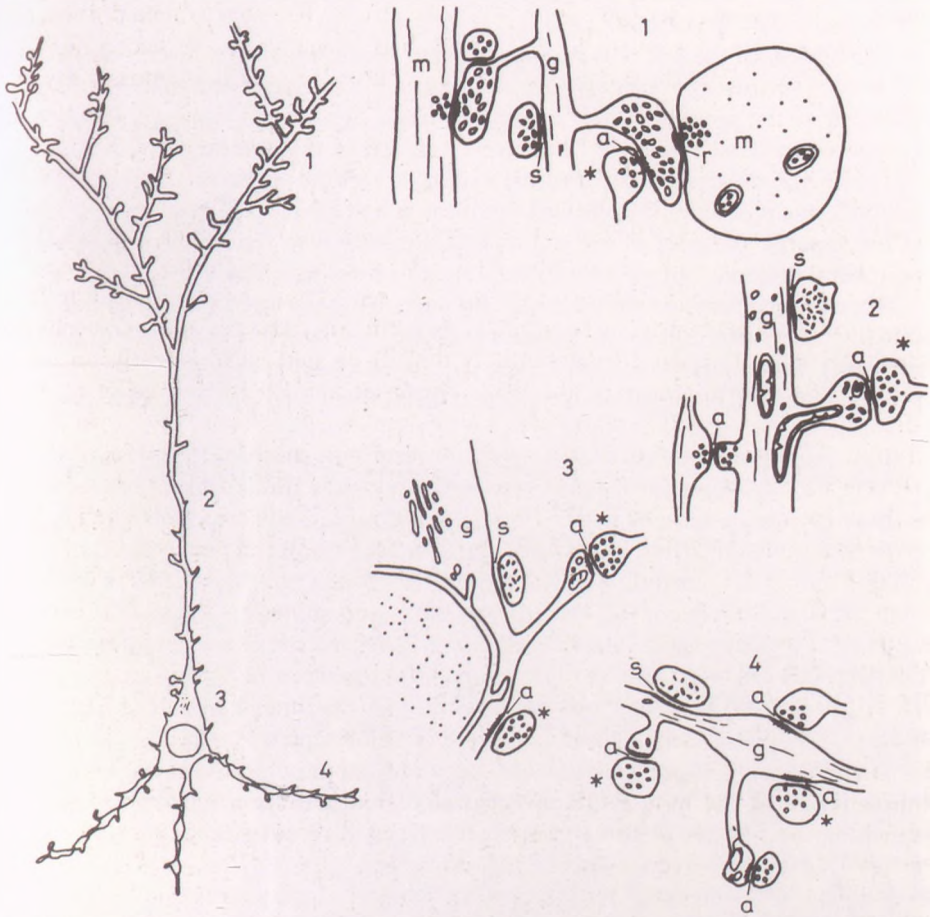


Fig. 4.24. Structural features and characteristic synaptic connections of the granule cells (based principally on and redrawn in part from Price and Powell, 1970b). On the left, a generalized granule cell is shown (periphery is up, central processes down) with characteristic branching, spines and varicosities. Synapses on the right show typical contacts found on the final branchings of the peripheral processes in the external plexiform layer (1), peripheral dendritic shaft (2), soma (3) and on central processes (4). Terminals of intrinsic axon collaterals (of the output neurons) and axon from the short-axon cells may synapse at all levels of the cell with asymmetrical or symmetrical membrane thickenings, respectively. Synaptic terminals of extrabulbar origin end at asymmetrical thickenings and may appear also along the total length of the granule cells. These were found to degenerate (asterisks) after damaging the ipsilateral anterior olfactory nucleus, commissura anterior or other retrobulbar connections. a: asymmetrical membrane thickenings; g: granule cell soma or dendrite; m: mitral cell soma or dendrite; r: reciprocal synapse; s: symmetrical synaptic membrane thickenings. Reproduced with permission

Hinds, 1969; Price and Powell, 1970a-c; Willey, 1973; Halász, 1974, 1976) and in finer detail for the rat by Price and Powell. The relatively large nucleus is surrounded by a shallow cytoplasmic rim (Fig. 4.25). Although the cell membranes of the GR cells may contact each other along a several μm long zone, desmosomes have never been observed in this region (Price and Powell, 1970a). At other places, glial lamellae may separate GR cells from their neighbors, or the surrounding fibers. Myelin lamellae have also been observed in a few cases to partially or completely surround GR cells of the human OB (Braak et al., 1977).

The nucleus of the GR cells may have indentations, which are never so deep as those of the short-axon cells or periglomerular neurons. The chromatin material tends to be clumped at the periphery. The cytoplasm has a moderately dark appearance when compared to other neurons of the layer; it is rich in ribosomes, and contains all the characteristic organelles, comparable in density to other neurons, but is very poor in endoplasmic reticulum. The Golgi apparatus is well developed. It is situated regularly at the superficial aspect of the cell and may extend into the initial part of the stout peripheral process, but never into the central (proximal) dendrites.

None of the processes has an initial segment, characteristic of the origin of an axon (fascicle of neurotubules, an increased density below the membrane of the initial segment), which renders inappropriate the use of the term "axon" for the peripheral process of the cell in the earlier ultrastructural studies (1965-70) of Andres. Both kinds

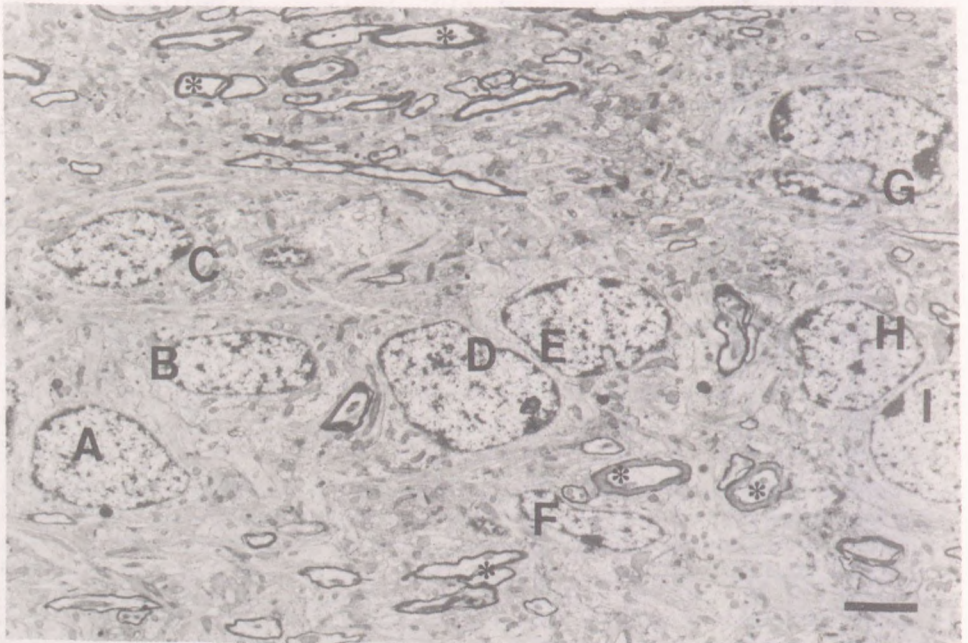


Fig. 4.25. Low-power electron micrograph of a group of granule cells (A-I) in the outer half of the layer. Adult rat, aldehyde-osmium fixation, sagittal section. The cells form a fairly compact sheet within the neuropil, composed of unmyelinated processes, synapsing terminals and numerous myelinated fibers (several labeled with asterisks). Three granule cells (C,E,G) show indented nuclei, two cells (D,F) give rise to a tortuous wavy process. Scale bar: 2 μm



Fig. 4.26. Axosomatic synapse on a granule cell perikaryon. One of the two granule cell (GR) somata is contacted by a terminal (asterisk). The synaptic membrane thickening (between triangles) and the presence of the small, flattened vesicles suggest that this may be an ending of a short-axon cell. Scale bar: 0.2 μ m

of processes, as well as the cell body may wear appendages, which frequently contain a spine apparatus, but differ widely in size and shape (Price and Powell, 1970a-c). They tend, however, to be smaller, and contain less vesicles in the deep portion of the cell than those lying more superficially. The biggest spines, the "gemmules" of the peripheral branchings possess the highest number of large, spheroidal synaptic vesicles and are the only output gates for the GR cells, forming reciprocal synapses with mitral and tufted dendritic or somatic counterparts (Hirata, 1964; Andres, 1965; Rall et al., 1966; Price, 1968b; Jackowski et al., 1978). At these synaptic sites (after aldehyde-osmium fixation) large, flattened vesicles accumulate (at symmetric membrane thickenings) within the granule-gemmules, while the opposite direction of the synapse is represented by spheroidal vesicles, opposed to asymmetric thickenings (see Fig. 4.9) (see also Section 4.2.2.2.1.).

Granule cell processes, soma, dendritic shafts and especially the various appendages have been found frequently postsynaptic to a variety of terminals of intrinsic and extrinsic fibers. According to Price and Powell (1970b,c), boutons filled with spherical and flattened vesicles terminate on any part of the GR cells. In addition to the mitral/tufted dendritic synapses, spherical vesicles and asymmetric thickenings are characteristic of terminals of the centrifugal fibers (terminating on spines of the peripheral process in the deep half of the EPL), endings from the fibers of the anterior commissure (observed on the soma and deep dendrites) and for the boutons of axon

collaterals from the anterior olfactory nucleus (synapsing perhaps on the entire GR cell, but certainly on the gemmules and spines of the peripheral process). Intrinsic axon collaterals and short-axon cell terminals may synapse at all levels of the cell, and are characterized by spherical vesicles and asymmetric thickenings, or small flattened vesicles and symmetrical thickenings, respectively (Fig. 4.26, and see also Fig. 4.24).

In the light of recent results, as regards segregation of different subtypes of GR cells, this complexity in wiring may be more simple in reality at the level of a single GR cell. Accordingly, deep or superficial GR cells may not necessarily receive all kinds of intrinsic and extrinsic afferents, and may participate in the integration of lateral and central influences just for a subtype of output neurons (see also later in Section 4.2.4.2.).

Short-axon cells

Short-axon (SA) cells comprise a non-homogeneous group, but they have some common characteristics. The size of the cell somata lies between that of the granule cells and the output neurons; most of the cell bodies lie deeper or along the mitral cells. All have a distinct axon, branchings of which are distributed to the depth of the perikarya or more superficially than the parent cell bodies. The most distal arborizations of the dendrites always wear spines. According to our recent knowledge, granule cells (or other deep SA cells) may be their only postsynaptic targets.

The various deep SA cell types, on the other hand, differ moderately in cell size and shape, richness and distribution of their dendritic and axonal arborization, orientation of the cells and their processes, density and distribution of spines (Fig. 4.27). The largest number of deep SA cells was observed and characterized in detail for the hamster OB by Schneider and Macrides (1978) and for the hedgehog by López-Mascaraque et al. (1986), but similar types have been described in other mammals and, rarely, from the olfactory bulbs of the less extensively studied submammalian vertebrate groups. For light microscopic reviewing we use the nomenclature applied to the hamster OB by Schneider and Macrides (1978).

Apart from the granule cells, two types of SA cells have been observed to extend from the deep GCL to the IPL or MCL, the Blanes and Golgi cells, and two other cell types located in the IPL and MCL: the horizontal cells and the vertical cells of Cajal (Fig. 4.28 and see also Fig. 4.27).

The *Blanes cells* have a relatively large cell body (13–19 x 16–23 μm in hamster; 15 μm on average in rat), and have been observed in other species. These cells in hamsters may correspond to the cells, categorized as “tipo grueso” (big type) of the Golgi cells by Blanes (1898), presumably in cat material, and also to the “most readily identified” deep SA cells of Price and Powell (1970*d*), from the rat olfactory bulb. This cell type was also observed by Van Gehuchten and Martin (1891) and Cajal (1911; see cell “D” in Fig. 4.26, adapted from Cajal’s work). Numerous dendrites emerge from all sides of the cell body and may extend 100–200 μm in a radial direction. Distal portions of the branches are rich in spines, while the proximal part of the dendrites and the somata are almost or completely spineless. The axons run parallel with the layers, and ramify most

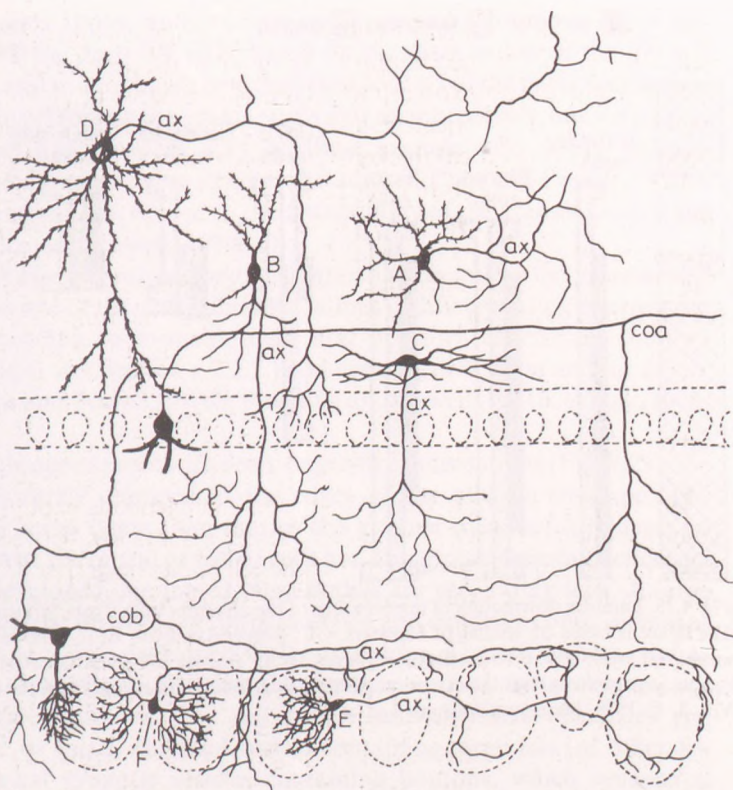


Fig. 4.27. Olfactory bulb neurons, possessing short axons. Based on Golgi-type impregnations, from Cajal (1911). A: short-axon cell; B: cell with peripherally directed short axon; C: fusiform horizontal cell of the internal plexiform layer; D: cell with a horizontal axon; E and F: periglomerular neurons. ax: axon; coa and cob: axon collaterals of a mitral and tufted cell, respectively

frequently in the GRL, but occasionally in the IPL (Blanes, 1898) or deep EPL (Schneider and Macrides, 1978), too.

Neurons with morphological features of the *Golgi cells* in the hamster OB tended to be located more peripherally than the Blanes cells, while Blanes (1898) found them ("tipo pequeno") deeper than the larger cells. Golgi (1875) observed these neurons only rarely in the dog OB, and noted their lack of orientation with respect to the direction of the histological layers. Such, relatively large SA cells were observed by Van Gehuchten and Martin (1891), Calleja (1893; cit. by Schneider and Macrides, 1978), Cajal (1911) and Price and Powell (1970*d*). Blanes (1898) characterized them to be smaller than the former type, although they were still larger than the "big" granule cells of his samples. In hamsters, they were measured to be $11\text{--}17 \times 12\text{--}22 \mu\text{m}$. The dendrites and axon were restricted regularly to the GRL (see, e.g., cell "A" in Fig. 4.27), but were observed infrequently also in the IPL and MCL (see Fig. 4.28). Axonal ramifications of such cells in rats were never found outside the GCL (Price and Powell, 1970*d*). The dendrites branched more frequently than those of the Blanes cells and

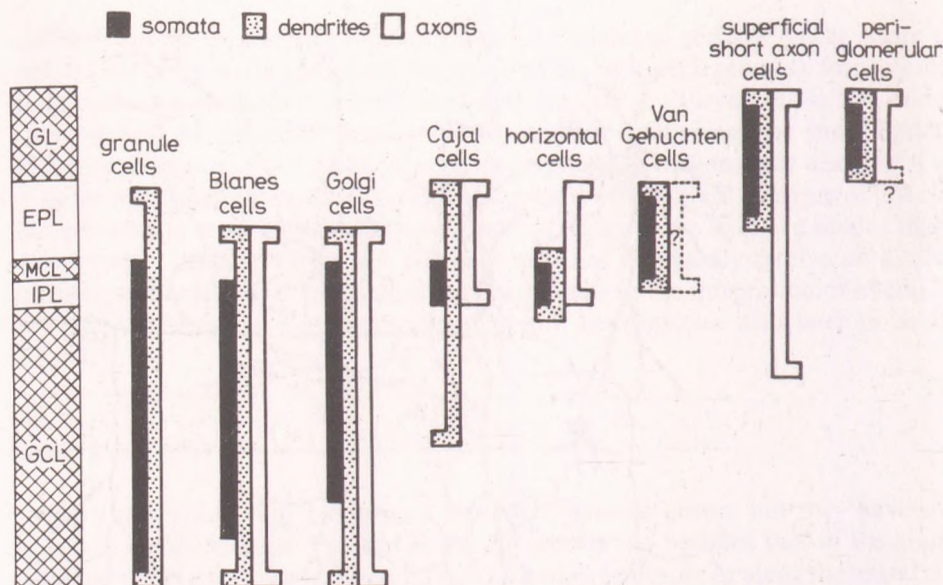


Fig. 4.28. Laminar distribution of interneurons of the olfactory bulb (from Schneider and Macrides, 1978). The figure is based on studies of hamsters. GL: glomerular layer; EPL: external plexiform layer; MCL: mitral cell layer; IPL: internal plexiform layer; GCL: granule cell layer. Question marks and dotted lines denote ambiguities about the extension of spreading. With permission, from the Brain Research Bulletin, Vol. 3, © 1978, Pergamon Journals Ltd

were relatively spineless. No clear orientation of the processes could be recognized in Golgi-impregnated material.

Two other, more superficially localized, SA cells were characterized predominantly for their distinct orientation. Cell bodies of the *vertical cells of Cajal* were found in the IPL and MBL of the hamster OB (Schneider and Macrides, 1978). Similar cells were observed in the superficial GRL by Van Gehuchten and Martin (1891), Blanes (1898), Cajal (1911, see cell "B" in Fig. 4.27) and Price and Powell (1970d; "perpendicular fusiform" cell). The somata of these cells were completely spineless in hamsters, and measured $8-12 \times 14-17 \mu\text{m}$ (Schneider and Macrides, 1978). Dendrites were arranged in a direction perpendicular to the mitral cell layer and were varicose, but poor in spines. The dendrites extended from the superficial GRL to the GL, without deep penetration of the latter in the hamster. The axons, after crossing the MBL, ramified within the EPL.

The *horizontal cells* (cell "C" in Fig. 4.27) were seen to be localized, as the vertical cells, in the IPL and MBL. Their dendrites were found almost exclusively within the same two layers and were orientated tangentially to the MBL (Cajal, 1911; Price and Powell, 1970d; Schneider and Macrides, 1978). The average somal diameter of the horizontal cells was $13 \times 16 \mu\text{m}$ in hamsters. The dendrites were varicosities and isolated spines only. The axons, penetrating the mitral cell body layer, branched in the EPL.

From the Golgi studies above, authors only could make assumptions about the synaptic connections of the deep SA cells, based on the distribution of the SA cell somata and processes, and other known neuronal components of the particular layers. Since all of these cells overlap with the granule cells and their processes, one very likely target for their axonal arborizations may be these granule cells. More positive knowledge can be gained from the fine structural studies of Price and Powell (1970*d*) and from their preceding studies, related to the synaptology of the granule cells of the rat olfactory bulb (Price and Powell, 1970*b,c*).

In single sections for *electron microscopy* it is impossible to follow the processes of the SA cells, so, we miss one of the characteristic features in differentiating between the various SA cells. According to available data and our own experience, the fine structure of the superficial (see Section 4.2.2.2.) and deep SA cells in rat and cat differs only very slightly (Price and Powell, 1970*d*; Pinching and Powell, 1971*b*; Willey, 1973; Halász, 1976).

The nucleus has a pale appearance with deep, finger-like indentations (Fig. 4.29), the chromatin material is rarely clumped at the edges of the nuclear envelope. The cytoplasm is definitely more dense than that of the granule cells, but it is never so abundant in organelles as the mitral or tufted cells are. Rough endoplasmic reticulum is not rare, but well-developed, laminated Nissl-bodies are very rarely seen. A large number of ribosomes can be observed in the perikaryon and dendritic cones, as well as many mitochondria, and a well-developed Golgi apparatus. The cell somata, just as those of the superficial SA cells, lacks agranular synaptic vesicles and presynaptic or reciprocal synaptic specializations. The cell bodies, and the basal dendrites are, however, postsynaptic to small, flattened vesicle containing terminals (of other SA cells?) and also spherical synaptic vesicles containing boutons, which synapse at symmetrical or asymmetrical membrane thickenings, respectively (Price and Powell, 1970*d*). Andres (1975) observed two different bouton types (of supposed central fibers) on the deep SA cells of the mammalian OB: a large, complex ending and a smaller (rare) one, with a dense-core and small vesicles in the second terminal, but did not define their origin.

Not much is known about the endings of the axon terminals of SA cells. Based on their studies with deafferentation and aldehyde-osmium fixation, Price and Powell (1970*d*) proposed that the intrinsic axon terminals with small flattened vesicles, synapsing at symmetrical thickenings, may belong to the SA cells. Such synaptic endings were observed in large numbers on cell somata, peripheral dendritic shafts and central processes of the granule cells (see Fig. 4.24) in addition to those synapsing onto another SA cells. Although the fine structural appearance of these axon terminals may be similar to different deep SA cells (cf. the similarity of these presynaptic boutons to those described by Pinching and Powell (1971*d*) for the superficial SA cells), indicate that they may relay different central or collateral influences onto (different) granule and SA cells, according to their localization and orientation.

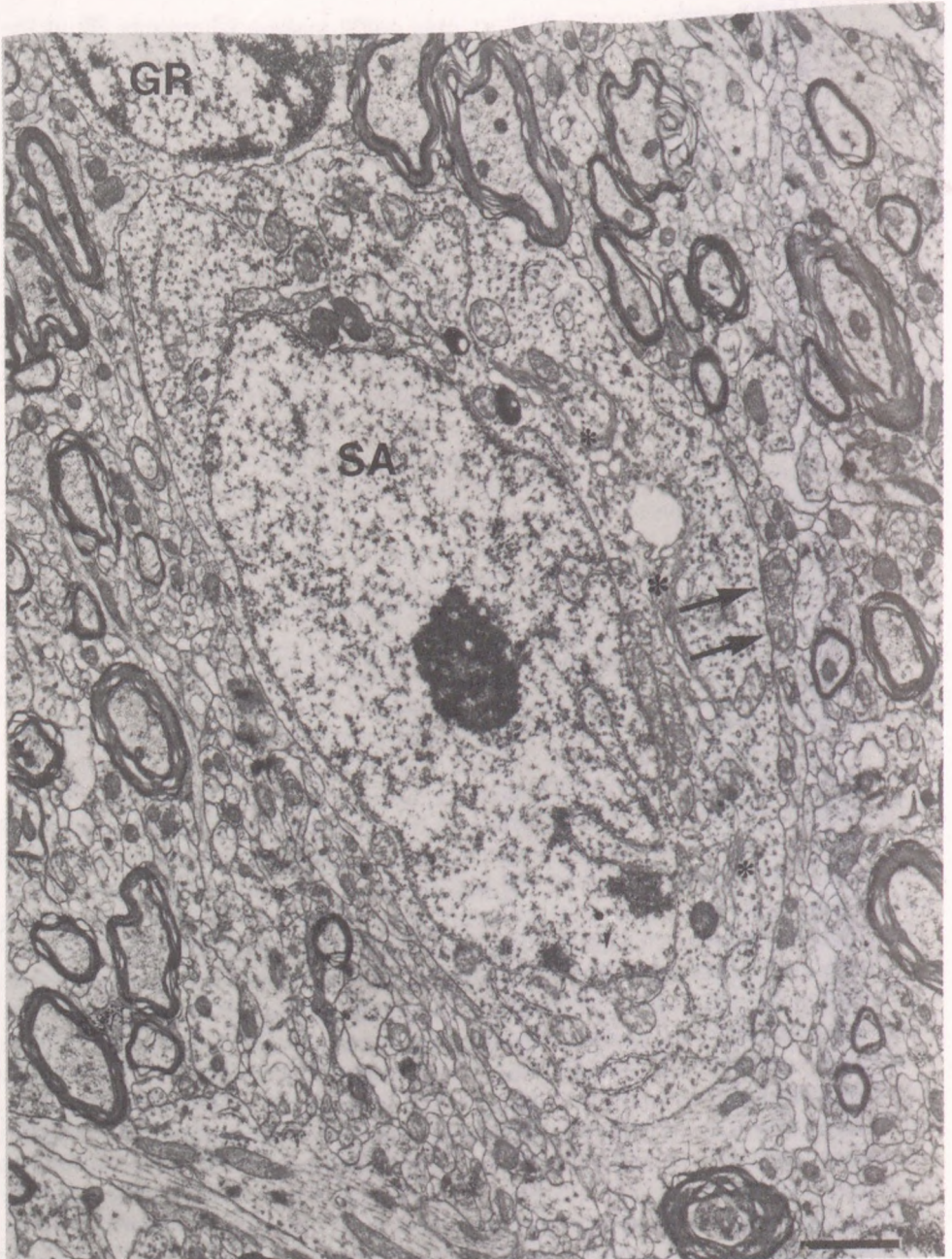


Fig. 4.29. Electron micrograph of a deep short-axon cell (SA). Adult rat, aldehyde-osmium fixation. An indented and elongated, pale nucleus and a fairly developed cytoplasmic sheet can be observed. The cytoplasm is rich in ribosome rosettes and mitochondria; a well-developed Golgi-apparatus (asterisks), a limited number of cisternae of granulated endoplasmic reticulum, several lysosomes and a multivesicular body is also apparent. A terminal is likely to contact the soma (see at arrows) and a granule cell (GR) is also apposed to the SA cell. Scale bar: 1 μ m

Axonless cells of the EPL

Although these neurons were localized more superficially than the GR cells and deep SA cells, and may overlap with dislocated superficial SA cells (cf. with Section 4.2.2.2.), according to the distribution of their dendrites, they may contribute to the processing of signals by the output neurons at the level of the secondary dendrites of the latter.

The *Van Gehuchten cells* have been observed and characterized only in the cat (Van Gehuchten and Martin, 1891) and the hamster (Schneider and Macrides, 1978) OB to date. Averages of minor and major diameters of the soma were measured as $10 \times 14 \mu\text{m}$ in the hamster. The spineless cell bodies were localized mostly in the EPL, and rarely in the MBL. No axon was clearly recognized for this cell type, while several thick, varicose dendrites (wearing isolated spines only) extended from the same (Schneider and Macrides, 1978). The dendrites branched elaborately, but were restricted practically to the EPL and MCL (see Fig. 4.28).

Finally, it should be mentioned that Gallego (1972) has also observed a small neuron with the Golgi technique in the EPL of the rabbit OB, which also lacked an axon. The cell body was fusiform or oval, and released several varicose dendrites, which ramified repeatedly, but was void of spines. This cell may be the equivalent of the Van Gehuchten cells in the rabbit OB.

Nothing is known as yet about the fine structure and functional significance of these cells. Combined Golgi-electron microscopic studies, HRP filling of the cells and/or serial sectioning may reveal further details.

4.2.2.2.4. Summary

The olfactory bulb of vertebrates is rich in local circuit neurons; they greatly outnumber the output neurons and may contact the latter anywhere from the dendritic appendages to the axon initial segment. Single types of interneurons synapse, however, onto a restricted segment of the relay neurons. The interneurons are regularly smaller than the output cells and have a less developed dendritic tree. Their axons extend for a limited distance only and terminate frequently within the histological layer of their somata of origin. At least two categories of local circuit neurons used to be distinguished in the vertebrate olfactory bulb: granule and short-axon cells. The fish granule cells and periglomerular cells of the higher vertebrates may have an input from the olfactory nerves, while other interneurons may not (e.g., granule cells of the higher vertebrates, short-axon cells). The morphological characterization of various types of these local circuit neurons except the rodent periglomerular and granule cells, is still very much incomplete. Electron microscopy of serial fine sections, or the combination of Golgi impregnation with ultrastructural studies would highly extend our knowledge in this field.

4.2.3. Termination of central afferents

In the former Section (4.2.2.), when characterizing the intrinsic neurons, we mentioned that practically all bulbar layers are invaded by extrinsic fibers. In the superficial region (ONL, GL) this is the olfactory nerve, while in the following deeper layers these extrinsic fibers are the various central afferents. As it turns out from studies designed to reveal the specific neurotransmitters of the neurons and their terminals (see Section 4.3.), the two—peripheral and central—afferent systems even mingle with each other in the glomerular layer, although the output neurons may be contacted directly only by the olfactory nerve.

It is clear from previous studies that the interneuronal systems of the OB are under the influence of central afferent fibers at all levels from the GL to the deep GCL. We might expect that fibers from the various higher brain centers terminate exclusively at a certain level or point of the intrabulbar network, but, as yet, there is little direct evidence to support this idea. Technical difficulties in labeling exclusively one pathway, without interfering with others, difficulties to reach the brain nuclei and pathways on the base of the brain, which supply the centrifugal fibers to the bulb, frustrated attempts to shed light on this problem. In addition, recent HRP tracing experiments led to the conclusion that perhaps the termination of (at least certain) central afferent pathways might, in fact, be quite diffuse and broadly overlapping as is summarized in Table 4.2.

4.2.3.1. Light microscopy

The existence and origin of centrifugal afferent fibers to the main OB was described already in early papers, based on Golgi-type silver impregnation techniques, and are still the subject of recent studies, applying fine surgical, electrolytic or chemical lesions, as well as using sophisticated micromethods to deliver and detect minute amounts of tracers. Among the most powerful techniques to elucidate such questions, perhaps the retrograde transport of HRP, and tritiated amino acids and histochemical detection of various neurotransmitters contributed most significantly to our present knowledge, as regards the origin, course and termination of the central afferents. In this section, emphasis will be given to the termination of the extrabulbar fibers, while their course and a more detailed characterization of the source area (origin) of these fibers will follow in the forthcoming Sections 5 and 6. A brief summary of these results seems, however, necessary at this point.

Extrabulbar central afferent fibers were supposed to originate and/or were traced from many retrobulbar areas, especially from the anterior olfactory nucleus (AON) (with the heaviest projection). According to their origin, the projections are categorized into three main groups here: (i) Projections from the contralateral OB, (ii) Projections from other contralateral brain areas (including those from the contralateral AON), and (iii) Projections from the ipsilateral retrobulbar brain (including the ipsilateral AON projections and those termed regularly as “centrifugal fibers” in previous literature).

Table 4.2. *Central afferent projections to the main OB from ipsilateral brain areas*
(as revealed by morphological techniques)

Species	Source brain area	Termination in the OB	Transmitter specificity	Technique	References
rat	PC, amygdala, preoptic area, hypothalamus	GCL, EPL, GL	—	silver degeneration	Powell and Cowan, 1963
rat	? OT	all layers except ONL	—	silver degeneration	Powell et al., 1965
rat	olfactory peduncle, PC and amygdaloid cortex, OT	all layers except ONL	—	silver degeneration	Heimer, 1968
rat	nHDB	GL (among PG cells)	—	silver degeneration	Price, 1969a
rat	nHDB	(mostly) GL and GCL	—	silver degeneration	Price and Powell, 1970e
rat	nHDB	—	—	HRP transport	Divac, 1975
rat	raphe nuclei	—	—	³ H-am.ac.transport	Halaris et al., 1976
rat	lateral preoptic area	molecular layers	—	degeneration,	Swanson, 1976
				³ H-am.ac.transport	
rat	transition area between lateral preoptic area and lateral hypothalamus	—	—	HRP transport	Shafa and Meisami, 1977
rat	AON, OT, PPC, nHDB, nLOT	—	—	HRP transport	Haberly and Price, 1978a
rat	PC, nLOT (weak)	—	—	HRP transport	Haberly and Price, 1978b
rat	AON, ventral TT	—	—	HRP transport	deOlmos, 1978
rat	AON (except pars externa), transition field (between AON and primary cortex), primary olfactory cortex, MFB area (deep to the OT), nHDB, part of nVDB, nLOT, ventral pre-commissural area (HC), sublenticular part of substantia innominata, anterior amygdaloid area, posterolateral cortical amygdaloid nuclei, transition area (between olfactory cortex and lateral entorhinal area), dorsal HT, prefrontal HT, lateral HT, LC, dorsal and medial raphe nuclei	—	—		
rat	raphe nuclei	—	5-HT	³ H-am.ac.transport	Bobillier et al., 1979
rat	raphe nuclei	—	—	biochemistry	Araneda et al., 1980a

Species	Source brain area	Termination in the OB	Transmitter specificity	Technique	References
rat	n. dorsalis and n. centralis raphe	—	—	^3H -5-HT retrograde transport	Araneda et al., 1980b
rat	supraoptic nucleus (HT)	—	VP	immunocytochemistry	Buijs, 1980
rat	diagonal band area	—	LHRH	immunocytochemistry	Bennet-Clarke and Joseph, 1982
rat	AON pars lateralis	(mainly) outer 2/3 of GCL; IPL	—	^3H -am.ac. transport	Luskin and Price, 1983a
	AON pars dorsalis	(mainly) dep 2/3 of GCL			
	AON pars medialis	deep GCL; EPL (weak)			
	AON pars ventroposterior	GL (mostly deep)			
	ventral TT (very weak)	GL (mostly deep)			
	dorsal peduncular cortex	(mostly) IPL and superficial GCL; EPL			
	anterior and posterior PC, nLOT, periamygdaloid cortex	(mostly) deep GCL, (faintly) outer GCL			
rat	AON, nHDB, anterior and posterior PC boundary between PC and AON	deep GCL (mostly)	—	^3H -am.ac. transport	Schwob and Price, 1984
rat	lateral preoptic area	IPL, deep GCL	ACh	histochemistry	Shute and Lewis, 1967
rat	LC	GL (in the glomeruli), MCL GRL	NA	histofluorescence	Lindwall and Björklund, 1974
rat	LC	GCL, GL	NA	histofluorescence, biochemistry and HRP transport	Fallon and Moore, 1978a
rat	VTA	EPL	DA	histofluorescence, ^3H -amino acid and HRP transport	Fallon and Moore, 1978b
rat	nHDB	—	ACh	HRP transport, histochemistry	Mesulam et al., 1983b
rat	AON, PC, nLOT	—	Asp or Glu	^3H -D-Aspartate retrograde transport	Watanabe and Kawana, 1984
rat	LC	GCL, MCL, GL (within glomeruli)	NA	WGA-HRP transport and histofluorescence	Shipley et al., 1985
rat	PC, medial frontal cortex	—	—	WGA-HRP transport	Neafsey et al., 1986
rat	nHDB, nVDB	—	ACh, GABA	fluorescent tracer transport, histochemistry	Záborszky et al., 1986

rat	subst. innominata	—	ACh (in part)	lectin and ChAT transport	Woolf et al., 1986
rat	dorsal and median raphe nuclei	—	5-HT	WGA-HRP and fluorescent tracer transport, lesion, immunocytochemistry	McLean and Shipley, 1987a
mouse	AON (except pars externa), nHDB, nVDB, PC, entorhinal cortex, HC, n. lateralis (HT), n. dorsomedialis (HT), n. ventromedialis (HT), LC, n. raphe dorsalis, n. centralis superior	—	—	WGA-HRP transport	Carson, 1984a
mouse	nHDB, nVDB, n. lateralis (HT), n. ventromedialis (HT), n. dorsomedialis, LC	—	ACh	histochemistry and WGA-HRP transport	Carson, 1984b
mouse	AON, TT, anterior continuation of HC, PC, lateral entorhinal area, transition area between lateral entorhinal area and PC, nHDB, nVDB, nLOT, posterolateral and medial cortical amygdaloid area, zona incerta, several HT nuclei, LC, dorsal and medial raphe nuclei, n. raphe pontis	—	—	WGA-HRP transport	Shipley and Adamek, 1984
hamster	entire AON, ventral HC, anterior PPC, nLOT, diagonal band, magnocellular preoptic area, raphe nuclei	—	—	HRP transport	Davis et al., 1978
hamster	AON pars lateralis, AON pars dorsalis	all layers, except ONL (mainly deep 1/3 of PG area and superficial 1/2 of GCL)	—	³ H-am.ac. transport	Davis and Macrides, 1981
	AON pars ventralis, AON pars posterior	superficial GCL and deep 1/3 of GL			
	AON pars medialis, ventral HC	(predominantly) deep 1/2 of GCL			
	PC	GCL (shifted progressively from superficial to deep, according to the origin from rostral to caudal PC)			

Table 4.2. (contd)

Species	Source brain area	Termination in the OB	Transmitter specificity	Technique	References
hamster	AON, ventral HC, dorsal peduncular cortex, PC, nLOT, medial septal area, nVDB, nHDB	— GL, GCL (in vicinity of IPL)	— ACh	HRP transport HRP and ^3H -am.ac. transport, ACh histochemistry, lesion	Macrides et al., 1981
	HT LC	— —	— ? NA	HRP transport	
hamster	raphe nuclei amygdala, LC	— GCL	— SP	HRP transport HRP transport immunocytochemistry	Macrides and Davis, 1983
rabbit	PPC	GCL, GL (PG area) GCL	— NA	silver degeneration histofluorescence	Cragg, 1962 Dahlström et al., 1965
rabbit and rat	lower brainstem	GL	5-HT	histofluorescence	Dahlström et al., 1965
rabbit and rat	AON	all layers (mostly deep GCL; IPL, GL)	—	HRP and ^3H -am.ac. transport	Broadwell, 1975b
rabbit	anterior PPC	—	—	HRP transport	Broadwell and Jacobowitz, 1976
	nHDB, lateral preoptic area, rostral lateral HC LC	— GCL (mostly) and GL (among PG cells) GL	? ACh NA 5-HT	HRP transport histofluorescence and HRP transport histofluorescence and HRP transport	
rhesus monkey	nHDB	—	ACh	HRP transport histochemistry	Mesulam et al., 1983a
goldfish	telencephalic areas: a. ventralis, p. dorsalis, a. dorsalis, p. lateralis	—	—	primuline transport	Oka, 1980
goldfish	a. ventralis, p. ventralis; a. ventralis, p. lateralis; a. dorsalis, p. lateralis; a. dorsalis, p. centralis; n. teniae	—	—	HRP transport	Bartheld et al., 1984
goldfish	dorsal n., anterolateral n., n. preglomerulosus (weak label), n. of the medial longitudinal fasciculus, raphe	—	—	WGA-HRP transport	Levine and Dethier, 1985

goldfish	posterior bulbopectal n., ventromedial n., LC	—	—	HRP and WGA-HRP transport	Hornby et al., 1987
piranha	a. ventralis	—	DA	immunocytochemistry	Ebbeson et al., 1981
	telencephalic nuclei (A1, A2, B1, B2, C, D, E)	—	—	HRP transport, silver degeneration	
<i>Sebasticus</i>	telencephalic a. dorsalis, p. posterior; a. dorsalis, p. centralis; a. ventralis, p. dorsalis;	—	—	HRP transport, silver degeneration	Murakami et al., 1983
<i>marmoratus</i>	a. ventralis, p. supra commissuralis	—	—		
(teleost)		—	—		
winter flounder	junctional area between the posterodorsal bulb and telencephalon, basal preoptic area, n. posterior tuber, LC, raphe nuclei	—	—	HRP transport, silver degeneration	Prasada Rao and Finger, 1984
bichir	a. ventralis	—	—	HRP, cobalt transport	Bartheld and Meyer, 1986a

ABBREVIATIONS

retrobulbar areas:

a	area
AON	anterior olfactory nucleus
HC	hippocampus
HT	hypothalamus
LC	locus coeruleus
MFB	medial forebrain bundle
n	nucleus
nHDB	nucleus of the horizontal limb of the diagonal band of Broca
nLOT	nucleus of the lateral olfactory tract
nVDB	nucleus of the vertical limb of the diagonal band of Broca
OT	olfactory tubercle
p	pars
PC	piriform cortex
PPC	prepiriform cortex
TT	tenia tecta
VTA	ventral tegmental area

main olfactory bulb:

EPL	external plexiform layer
GCL	granule cell layer

GL	glomerular layer
IPL	internal plexiform layer
MCL	mitral cell layer
ONL	olfactory nerve layer
PG	periglomerular area

transmitters:

ACh	acetylcholine
Asp	aspartate
DA	dopamine
Glu	glutamine
5-HT	serotonin
NA	noradrenaline
SP	substance P
VP	vasopressin
GABA	γ -aminobutyric acid

tracers:

HRP	horseradish peroxidase
WGA-HRP	wheat germ agglutinin-horseradish peroxidase conjugate
^3H -am.ac.	tritiated amino acid
ChAT	choline acetyltransferase

4.2.3.1.1. Direct interbulbar connections

This kind of connection is not common in higher vertebrates, and its existence was disputed widely after Cajal's statement on the tufted cell projections to the contralateral bulb (see Valverde, 1965). Interbulbar connections were described for cats (Fox and Schmitz, 1943), rabbits (Cajal, 1911; Clark and Meyer, 1947; Allison, 1953a; Cragg, 1962), caiman (Scalia et al., 1969), turtle (Skeen and Rolon, 1982) and fish (see e.g. Ichikawa and Ueda, 1979; Ebbeson et al., 1981; Bartheld et al., 1984; Prasada Rao and Finger, 1984). Such connections may be regular in fish and amphibians (Nieuwenhuys, 1967). In contrast, they are absent in the rat (Powell et al., 1965; Valverde, 1965; Heimer, 1968), guinea pig (Lohman, 1963), hamster (Davis et al., 1978; Schoenfeld et al., 1985) and lizard (Nieuwenhuys, 1967).

4.2.3.1.2. Projections from contralateral retrobulbar areas

The most significant projection to the contralateral OB was found to originate from the pars externa of the AON, which may project exclusively to the contralateral side, as was found in the two best characterized animals, the hamster (Ferrer, 1969; Davis et al., 1978; Davis and Macrides, 1981; Schoenfeld and Macrides, 1984; Schoenfeld et al., 1985) and the rat (Haberly and Price, 1978b; Luskin and Price, 1983a). In the hamster, the projection of the AON pars externa contributes to a "sector-to-sector topographical organization" between the olfactory bulbs (Schoenfeld and Macrides, 1984; Schoenfeld et al., 1985), but this seems to be the only topographically organized central projection also in the rat (Luskin and Price, 1983a). Laminar patterns of the termination of the AON afferents are prominent. The AON pars externa has exclusively contralateral projections to the OB also in the mouse (Carson, 1984a; Shipley and Adamek, 1984).

Other contralateral central afferents to the main OB, originating from several retrobulbar areas with bilateral projections, were also reported by several authors. Accordingly, the nucleus of the lateral olfactory tract (nLOT) in the rat (de Olmos et al., 1978; Haberly and Price, 1978a) and mouse (Carson, 1984a), the locus coeruleus (LC) of the rabbit (Broadwell and Jacobowitz, 1976), hamster (Macrides et al., 1981), mouse (Carson, 1984a) and of the rat (Shipley, 1985), raphe nuclei in the rabbit (Broadwell and Jacobowitz, 1976) and hamster (Macrides et al., 1981) certainly have a contralateral projection. In the rat OB, Shafa and Meisami (1977) revealed central afferents from the contralateral piriform cortex (PC), olfactory tubercle (OT) and nucleus of the horizontal limb of the diagonal band of Broca (nHDB). In contrast Davis and Macrides (1981) revealed an exclusively ipsilateral projection from the hamster PC, and de Olmos and co-workers (1978) were unable to demonstrate centrifugal collateral fibers, among other areas, from the OT and nHDB in the rat. These discrepancies may reflect, in part, species differences, but—before generalization—careful re-evaluation of the presented data seems to be important.

4.2.3.1.3. Projections from ipsilateral retrobulbar brain areas

Ipsilateral central afferent pathways to the main OB were demonstrated in a wide variety of species and by many authors, using different techniques, which consequently led to divergences in recognition of the pathways and their origin, as well as in lack of uniformity of their nomenclature. These questions fall far beyond the scope of this summarization, so here in the text we will just list the characteristic brain areas for the origin of ipsilateral central afferents to the main OB, while details of particular publications are summarized in Table 4.2.

The most significant contributions to morphological characterization of these pathways were made by careful degeneration experiments and/or tracing techniques and transmitter histochemistry. Accordingly, ipsilateral central afferent projections of mammals may arise from the substantial part of the basal retrobulbar area, including the olfactory peduncle (AON), OT, tenia tecta, anterior and posterior PC, nHDB (perhaps the most important source for the ipsilateral afferents to the OB), nLOT, rostral lateral hypothalamic area, ventral tegmental area, LC and raphe nuclei. Studies on the central connections of the fish OB concentrated on the goldfish brain. Based on these studies, central afferents may originate in both ventral and dorsal telencephalic areas as well as from the LC and raphe. Little is known about similar pathways in amphibians, reptiles or birds.

Although all layers (except ON) are supplied by centrifugal afferents, distribution of terminals from different central sources has a characteristic laminar pattern. For termination of the particular projections within the main OB, see again Table 4.2.

4.2.3.2. Electron microscopy

Our knowledge of the fine structure of the preterminal segment and terminal part of the central afferents to the bulb is incomplete. Direct data on this subject was collected only from degeneration experiments, when the terminal in question became recognizable by its altered staining density, distribution and content of organelles. This implies, that the "absolute" normal appearance of such terminals was never observed, although several features were recognized. Among these, central afferents were unequivocally determined to synapse at asymmetrical (Gray type 1) junctions, and to contain spherical vesicles, as found in the rat (Price 1968*a*, 1969*b*; Price and Powell, 1970*c*; Pinching and Powell, 1972*c*) and goldfish (Ichikawa and Ueda, 1979) olfactory bulb. In both species, granule cell dendrites and spines were determined as the most usual postsynaptic components of these synapses.

Although the termination of the central afferents exhibit distinct laminar patterns, terminals segregated according to the ipsilateral or contralateral origin of AON fibers, as suggested by Price (1969*b*) and Price and Powell (1970*c*) could not be supported by recent transport studies (Luskin and Price, 1983*a*). From different parts of the AON, however, projections to different segments of the granule cells could be demonstrated (see Table 4.2) while apparently there was no difference in laminar patterns of distribution of terminals from the anterior and posterior PC, nLOT and periamygdaloid cortex (Luskin and Price, 1983*a*).

Central afferent terminals formed synapses mostly on the spines of the granule dendrites and on somatic spines and swollen dendritic portions of the dendrites of the granule cells in the GCL, while in the EPL these terminals were found frequently on the gemmules (Price, 1968*a*, 1969*b*; Price and Powell, 1970*c*) (see Fig. 4.24). Similar terminals were observed by us in the EPL of the turtle OB. In the GCL, some degenerating terminals were quite large and synapsed with a complex spine (Price and Powell, 1970*c*). In the superficial layers, outside the EPL, central afferents were observed almost exclusively in the region surrounding the glomeruli (Pinching and Powell, 1971*d*). These boutons formed Gray type 1 synapses with the spines and gemmules of the periglomerular cells, as well as with their dendritic shafts (a few degenerating terminals were observed by the same authors to synapse on the dendrites of the short-axon cells, tufted and mitral cells, too). Similar degenerating terminals were seen to terminate upon dendrites in the GCL of the goldfish OB, after transection of the medial or lateral olfactory tract (Ichikawa and Ueda, 1979).

Synaptic endings, similar to those described in preceding paragraphs were described by Andres (1965, 1970, 1975) for the fish and mammalian OB. He emphasized the large size of such terminals (especially in the deeper level of the bulb), high number and relatively small size of synaptic vesicles (35–45 nm). Andres distinguished a unique type of central bouton, forming interdigitated synaptic complexes exclusively on short-axon cell dendrites. Unfortunately, no experimental evidence was presented in these studies for the central origin of these terminals. In this regard, it seems important to note that both symmetrical and asymmetrical synapses, surviving the surgical lesions, were reported for the fish and rat OB. The normal boutons (other than those from local interneurons) were supposed to be derived from collaterals of mitral and tufted cells. This means, that in the absence of specific lesions or serial sectioning it is difficult to recognize unequivocally the termination of the central afferents to the bulb. Non-degenerative labeling techniques, such as punctate injections of tritiated amino acids, or labeled neurotransmitters into the brain areas, purported to be the origin of central afferents, and demonstration of the transported marker within the undamaged terminals would supply the most convincing data in this matter.

4.2.3.3. Summary

Neuronal somata of the olfactory bulb are targets for sensory afferents and for fibers from various brain regions. These include most of the ipsi- and contralateral primary olfactory cortical areas (which receive bulbar afferents), in addition to hypothalamic, tegmental and brain stem nuclei, and even the contralateral olfactory bulb. A high degree of variety exists between the vertebrate classes and species in this regard, as is illustrated in the case of the ipsilateral centrifugal fibers. Central axons may be found in all bulbar layers; they prefer, however, the glomerular, external plexiform and granule cell layers and terminate at local circuit neurons. Asymmetrical synaptic junctions and the presence of spherical vesicles make it difficult to distinguish these synapses from those made by the local axon collaterals of the mitral or tufted cells.

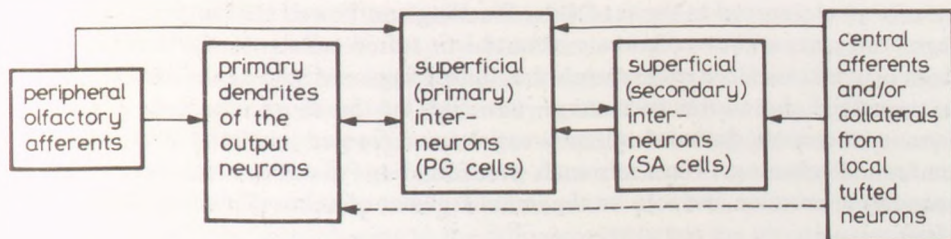
4.2.4. Synaptic organization

In the former Sections (4.2.1–3.) individual components of the neuronal assembly of the OB were discussed in detail. For a given neuron or fiber type, synaptic relations were also mentioned, but a comprehensive survey of the connections for the whole bulb was outlined only in the case of fish (see Section 4.2.2.2.1. and Fig. 4.17). Equivalent studies on mammals furnished a considerable amount of fine morphological details, even at the level of the particular layers and subcircuits, while less attention has been given to fine structural studies on the amphibian, reptile and bird OB. In these three classes observations were interpreted mainly as comparable to the equivalent mammalian neuron type or circuit. For the mammalian and especially the rodent olfactory bulb, intricate details of synaptic connectivity have been published. Although synaptic organization cannot be understood without taking into consideration findings in related disciplines (neurochemistry, physiology, developmental and behavioral studies), which will be discussed in detail in the forthcoming sections, it seems necessary to summarize briefly here the observations on the fine details of the connectivity.

The present survey is based mainly on findings obtained on the rat olfactory bulb from the comprehensive studies of Price and Powell (1970a–c) and Pinching and Powell (1971a–d, 1972a–c), but other studies on the rat, as well as other vertebrates will also be considered. Based on the lamination of the bulb, distribution of the primary dendritic branches of the output neurons within the glomeruli and those of the secondary dendrites of the EPL, as well as the preferential segregation of the interneurons into superficial and deep groups, two main levels of synaptic integration of the mammalian OB will be discussed here: (superficial) synaptic organization in the glomerular layer and (deep) synaptic assembly extending from the deep GCL to the superficial level of the external plexiform layer.

4.2.4.1. Organization in the glomerular layer

This is the layer, where main (primary) dendrites of the second-order neurons in the olfactory pathway, the output neurons of the OB, receive synapses from the primary olfactory nerve, and integrate the first central and local interneuronal modulatory effects. The general scheme of this organization may be represented as follows:



Block scheme of the principal synaptic connections in the superficial OB. Broken line denotes rare contacts. For further explanation see text

Fine structural details of the first synapse in this pathway, made by the *olfactory nerve*, were described first by Andres (1965) and Reese and Brightman (1965) for the rat OB and characterized by many others, for a wide range of species during the ensuing years. Most of the authors agree, that the ON is presynaptic not only for the final branchings of the output neurons, but also for superficial interneurons. One exception has been recorded through the serial sectioning studies of White (1972, 1973), when he was unable to find any ON—superficial interneuron synapse in the Balb/c mouse OB. The olfactory nerve was never found postsynaptic for any other neuronal element.

The final branchings of the *primary dendrites of the output neurons* were found to be the most common postsynaptic target for the olfactory nerve terminals in the OB of the lamprey (Andres, 1970), goldfish (Ichikawa, 1976; Oka 1983; Kosaka and Hama, 1982–83), turtle (Halász, unpublished data), duck (Rebiere and Dainat, 1981), rat (Pinching, 1969, 1970; Reese and Brightman, 1970, Pinching and Powell, 1971a,c; White, 1973; Halász, 1974, 1976), rabbit (Pinching and Powell, 1971a; White, 1973), mouse (White, 1972, 1973), cat (Willey, 1969, 1973) and dog (Andres, 1975). These dendrites are in reciprocal connection with dendrites of the primary interneurons, the granule cells of the lamprey (Andres, 1970) and goldfish (Ichikawa, 1976; Kosaka and Hama, 1982) or periglomerular cells of turtles (Halász, unpublished data), duck (Rebiere and Dainat, 1981), rat (Pinching, 1970; Reese and Brightman, 1970; Pinching and Powell, 1971c; White, 1972), cat (Willey, 1969), mouse and rabbit (White, 1973). Based on the observations of Pinching and Powell (1971d), occasionally a synapse can be observed on the primary dendritic shaft of the mitral or tufted cells, which may be derived from central afferent fibers and/or collaterals of other mitral/tufted cells. These and the following connections are depicted in some detail in Fig. 4.30.

The highest complexity of input and output synapses was observed in relation to the *periglomerular cells*, although all types of synapses were never recognized or identified on one single periglomerular cell. Since several subtypes of PG cells were categorized, with regard to the distribution and extension of their processes (see Sections 4.2.2.2.1. and 4.2.2.2.2.) and transmitter content (Section 4.3.), it is well worth to consider that this complexity might be of a lower degree in reality. Until this is fully elucidated, we can do no more than to simply list the synaptic types observed to date.

Two synaptic connections have been mentioned already in this section: input from the ON and reciprocal synapses of periglomerular neurons with the mitral or tufted cells. Another dendrodendritic (symmetric) synapse has been seen occasionally between two periglomerular cell dendrites (White, 1973). Additional inputs to PG neurons were observed in the rat OB by Pinching and Powell (1971d), established by central afferents and/or collaterals of mitral or tufted cells onto the dendrites and spines (asymmetrical synaptic membrane thickenings and spherical vesicles) and by the superficial short-axon cells. Axon terminals of the short-axon cells contained characteristic small, flattened synaptic vesicles and formed junctions at symmetrical membrane thickenings. These terminals were found on PG cell dendritic branches and appendages, on soma and also on the initial segment of axons (Pinching and Powell, 1971d).

Synaptic contacts of the ON axon terminals on the mitral and periglomerular dendrites, as well as the dendrodendritic synapses constitute the most important

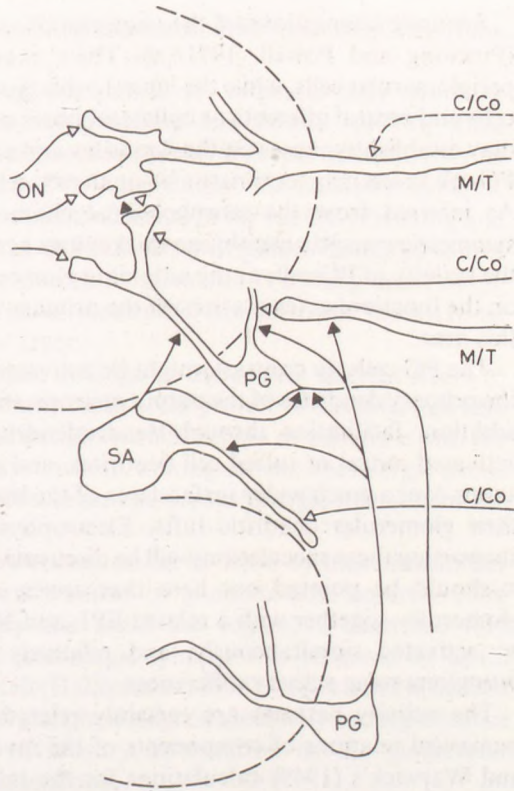


Fig. 4.30. Intraglomerular and periglomerular synaptic connections, as observed for the mammalian olfactory bulb. The drawing is based mostly on Pinching and Powell (1971*a-c*). For sake of clarity, synapses observed on dendritic shafts and/or somata, dendritic or somatic spines and gemmules were drawn only for *dendrites*. Those synapses, established by tufted cell somata onto periglomerular cells/dendrites (observed by Pinching and Powell, 1971*c*) are not included. Triangles show polarity of synapses, and denote morphological subtypes. Open triangles represent asymmetrical thickenings and spherical vesicles (presumed excitatory synaptic contacts), filled triangles show synapses with ovoid or (for synapses, made by short-axon cells) flattened vesicles and symmetrical thickenings (presumed inhibitory synapses). Dotted line at the upper right corner represents rare connections. C/Co: central afferents and/or collaterals of the local tufted neurons; M/T: principal dendrite of mitral or tufted cells; ON: olfactory nerve axons; PG: periglomerular neurons; SA: (superficial) short-axon cells

intraglomerular connections. The coordination of the function between individual (or groups of individual) glomeruli may be based to a large extent on *interglomerular* contacts. Activated periglomerular cells, as it can be inferred from their putative transmitter content (see Section 4.3.4.), may spread inhibition around these foci. Location of PG cells between peripheral and central afferents suggests that they are in a strategic position to influence more efficiently than all other neuronal components of the area, the excitability of the primary dendritic arbor of mitral/tufted cells. This may be a significant factor contributing to the differences between the physiological status and activity of particular glomeruli, as detected by electrophysiological techniques or 2-DG uptake experiments (see later).

Synaptic connections of the (superficial) *short-axon cells* were studied only in rat (Pinching and Powell, 1971*b,d*). Their axon terminals were observed solely on periglomerular cells, while the input to this type of cell (to SA cells) is derived from PG cells and central afferents or collateral fibers of the mitral or tufted cells. All afferents may establish synapses on the dendrites and soma of the SA cell and, in addition, the PG cell axons may terminate also on the axon initial segment of the superficial SA cell. As inferred from the morphological characteristics (small flattened vesicles and symmetric synaptic membrane thickenings at the SA terminals), SA cells may inhibit the activity of PG cells at the adjoining glomeruli, thus exerting a disinhibitory action on the functioning (excitation) of the primary dendritic tufts of the output neurons in this area.

The PG cells, in contrast, might be activated by the same fasciculi of ON fibers, as the primary dendrites of the output neurons, within a given glomerulus, and receive, in addition, facilitation through dendrodendritic synapses from the simultaneously activated mitral or tufted cell dendrites, and spread lateral inhibition through their axons over a much wider surface area of the bulb, as well as local interactions through their glomerular dendritic tufts. Electrophysiological and 2-DG uptake patterns, supporting these speculations will be discussed later in the corresponding sections, but it should be pointed out here that zones, corresponding to a single, or a few glomeruli—together with a related EPL and MCL column—were regularly found to be activated simultaneously, and relatively little data support the existence of *intraglomerular* activity differences.

The activity patterns are certainly related to the anatomical organization and numerical relations of components of the involved pathways. According to Allison and Warwick's (1949) calculations for the rabbit OB, some 26,000 olfactory nerve axons fall to one single glomerulus; 25–100 axons are grouped into fascicles. If the axons were distributed evenly for each glomerulus, 26,000 sensory fibers would contact there (in average) primary dendritic appendages of 24 mitral and 68 tufted cells (Allison and Warwick, 1949). Since a certain degree of topographical organization exists between the olfactory mucosa and the bulb (see Sections 2. and 3.), selective activation of a group of receptors and ON bundles may be maintained (or augmented) at the glomerular level (and also deeper, at the level of the output cell bodies and their secondary dendrites) by a contribution from the activity of the PG cells.

Conservation of topographical activity patterns, conveyed by ON fascicles to certain glomeruli, may supposedly be influenced by the *collaterals* of the *local tufted neurons* through self-inhibition (if synapsing onto the subset of PG cells, exerting feedback inhibition for the same glomerulus, or activating SA cells, which disinhibit neighboring glomeruli) or lateral inhibition (when activating SA cells, disinhibiting the respective glomerulus, or activating those PG cells, which inhibit glomeruli outside the activated focus). Another modulation of the glomerular activity may be supposed to result from activation of certain PG or SA cells by the *central afferents*.

Unfortunately, because of the lack of intracellular recording data and selective labeling of particular PG and SA neurons, it is as yet unknown, which PG cells are activated at a certain time by the action of the ON, output neuron dendrodendritic synapses, collaterals or central afferents. Some degree of specificity can, however, be

deduced from a few studies, related to the wiring and transmitter histochemistry of the area.

Axon collaterals, distributed in the glomerular layer, originate only from the superficial (external) tufted cells in the rat (Pinching and Powell, 1971*b*), while collaterals of all other subtypes of the output neurons arborize deeper in the rat (Orona et al., 1984) or rabbit (Kishi et al., 1984) OB. Accordingly, the collateral synaptic endings, which survive the lesions of the central afferents in the rat OB (Pinching and Powell, 1972*b*) and synapse mainly on the PG cells, may originate from this source only. Since these collaterals extend only over 6–12 glomeruli (Pinching and Powell, 1972*c*), they may integrate mostly local activity patterns, similar to the short axonal interneurons (PG, SA cells) of the same layer.

It is more difficult to assign the synaptic endings of the central afferent fibers to individual retrobulbar sources, because many brain areas project to this level (see also Table 4.2.) and may synapse either in the periglomerular region or penetrate into the intraglomerular neuropil. These pathways include, among others, cholinergic axons from the diagonal band area and nonadrenergic and serotonergic fibers from the midbrain.

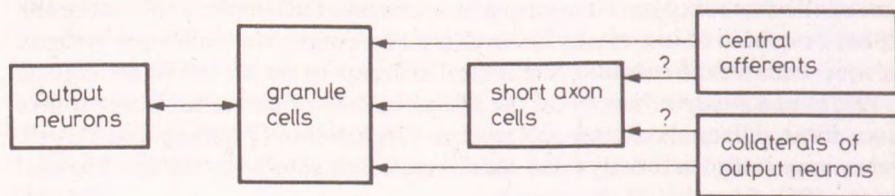
Certain central afferent pathways were characterized in detail by their transmitter content (see Section 4.3.7.). A significant number of glutamic acid decarboxylase (GAD) containing central afferents may also reach this region, as described very recently (Záborszky et al., 1986). A serotonergic synapse was observed by us on intraglomerular dendritic shaft of a PG cell (Halász et al., 1978). Much work is needed before the morphological, neurochemical and physiological specificity and significance of these pathways, in the processing of sensory information by the glomerular organization of the OB, will be fully understood.

4.2.4.2. Synaptic organization below the glomeruli

The spatial extension of the synaptic assembly passes far beyond the aforementioned (glomerular) system and its organization is none the less complex than that of the superficial system. Both are demarcated by the furthestmost extension of the primary interneuron of the assembly—the periglomerular cell in the superficial and the granule (GR) cell in the deep system. In the case of the granule cells, however, their wider distribution may certainly contribute in itself to some histological features. It may be such, that deeper or more superficial GR cells differ according to their preferential connections with deeper or more superficially located output neurons; they vary in the proportion of length of the central to the unbranched peripheral processes (according to the location of the GR cell somata) and distribution of spines (gemmules). These were certainly major factors in the categorization (and led to histological recognition) of subtypes of the GR cells, simultaneously with the appearance of the subtypes of the output neurons.

The picture is further complicated by the recognition of several subtypes of SA cells, according to their localization, orientation, and distribution of processes, segregation of the distribution of axon collaterals of the output neurons and characteristic laminar (but widely overlapping) pattern of central afferent fibers. These have been

characterized in detail in the preceding Sections 4.2.2. and 4.2.3. and may be dissimilar when comparing individual vertebrate species. The general scheme of connections, involving the granule cells and processes, may be summarized, however, as follows:



Block scheme of the main synaptic organization in the deeper layers of the olfactory bulb. Short axon cells may receive synapses from afferent central fibers and/or collaterals from the output cells. For further explanation see the text.

This general scheme based on numerous observations either by light- or electron microscopy is further supported by modern techniques (electron microscopy of serial sections, intracellular recording experiments, transport studies for intracellular tracers, multiple labeling, etc.) for several species, as in the rat (Price and Powell, 1970a-c; Orona et al., 1983, 1984) and rabbit (Mori et al., 1983; Kishi et al., 1984). Taken together, these results may furnish a more detailed account of the interrelations of the particular components.

At a level, below the glomeruli, both primary and secondary dendrites, soma and axon initial segments of the *output neurons* were found to be in synaptic contact with the deep primary interneuron, the GR cell. The reciprocal synapses between the secondary dendrites of mitral cells and GR cell peripheral processes are the most documented in this relation (Hirata, 1964; Andres, 1965; Rall et al., 1966; Price and Powell, 1970a-d; Willey, 1973; Halász, 1974, 1976; Ramón-Moliner, 1977; Jackowski et al., 1978). Reciprocal pairs of synapses, similar in morphological appearance to synapses in the EPL, have also been observed to lie between the soma of output neurons and a terminal, and were analysed through serial sectioning electron microscopy. Very probably, these synapses might also be granule-mitral cell interconnections (Price and Powell, 1970a,b; Willey, 1973; Halász, 1974), although earlier light microscope observations suggested the existence of tufted cell synaptic endings on the mitral cell soma (Cajal, 1911; Valverde, 1965). Willey (1973) described reciprocal contacts also on the axon hillock and initial segment of mitral cells in the IPL of the cat OB, but similar data for other types of the output neurons are not available.

Varying morphologies, with regard to granule cell contacts of different subtypes of tufted or mitral cells (Macrides and Schneider, 1982; Mori et al., 1983; Kishi et al., 1984) have not yet been shown, although an increasing amount of evidence appears to support the idea that different subpopulations of granule cells may synapse with output neurons in the deep part of the EPL or in the superficial half of this layer (Mori et al., 1983; Orona et al., 1983). Keeping in mind that local axon collaterals may terminate on the granule cells (Price and Powell, 1970b,c) and evidence for the segregation of the local *axon collaterals* of the output neurons (Kishi et al., 1984,

Orona et al., 1984), more than one feedback circuit may exist in the deep OB layers. If local collaterals of a subtype of the output neurons counteract with the same granule cells, which they contact (through reciprocal synapses) in the EPL, superficial (external) or medium tufted cell axon collaterals may preferentially terminate on superficial (in the rat OB), type III or type I (in the rabbit OB) granule cells in the IPL and superficial GCL. The displaced mitral cells in the rabbit OB have axon collaterals also in the medium (less frequently in the deep) layers of the rabbit OB (Kishi et al., 1984) and so may have the ability to contact also the deep GR cells, but distribution of their other local collaterals and secondary dendrites in the EPL are similar to the equivalent processes of the tufted cells, as described above.

A second subcircuit might be based on "regular" mitral cells of the rabbit (Kishi et al., 1984) or type I mitral cells of Orona et al. (1984) in the rat, which distribute their secondary dendrites in the deep EPL (where peripheral processes of the deep GR cells ramify and have most of their gemmules, both in rats and rabbits) (Kishi et al., 1984; Orona et al., 1984). Local collaterals of the rabbit (not displaced) mitral cells terminate in deep (and also in the superficial) GCL (Kishi et al., 1984) where they may contact the deep (but also the superficial) subgroup of granule cells. In the rat, no local axon collaterals for type I mitral cells were recognized by Orona and co-workers (1984), but their type II mitral cells had deep collaterals. Secondary dendrites of these type II mitral cells ascended until (and branched in) the medium level of the EPL, where both superficial and deep granule cells have gemmules.

According to physiological findings, direct influence of the mitral axon collaterals onto mitral dendrites may also be hypothesized (see e.g. Shepherd, 1972), but no mitral-to-mitral synapses have been described as yet.

Short-axon cells are another component of the local circuits, although it seems extremely difficult to assess, which type of SA cells (for cell types, see Section 4.2.2.2.3.) might serve more superficially and those serving the deeply localized subcircuits. It is known—mainly from Price and Powell's studies (1970b–d)—that they synapse onto the "bare" somata, peripheral or central dendritic shafts of the GR cells, as well as on another SA cell somata or basal dendrite. When presynaptic, all SA terminals contained small, flattened vesicles at symmetrical membrane thickenings and were thought to exert inhibition. No presynaptic granule cell terminals have been observed on SA cells to date; this is a divergency from the analogy which might exist between this and the superficial SA cell—periglomerular cell contact. The only afferent terminals on SA cell dendrites (spherical vesicles, asymmetric synaptic thickenings) were assigned to central afferents and/or local axon collaterals (Price and Powell, 1970d) (Fig. 4.31).

Central afferents were found to contain spherical vesicles and form synapses at asymmetrically thickened membranes not only onto SA cells but also over the entire GR cell surface, including the "smooth" surface of somata, peripheral and central processes as well as spines (gemmules and swellings) (Price and Powell, 1970c,d). Luskin and Price (1983a) found exclusive termination sites for central fibers from the anterior prepiriform and periamygdaloid cortices and nucleus of LOT in the deep half of the GCL, where deep GR cells lie, and their central processes may predominate. Pars lateralis and the dorsal wing of the pars externa of the contralateral AON and pars lateralis of the ipsilateral AON projected exclusively to the GCL. The posterior PC and dorsal peduncular cortex had almost all, and pathways from the remaining

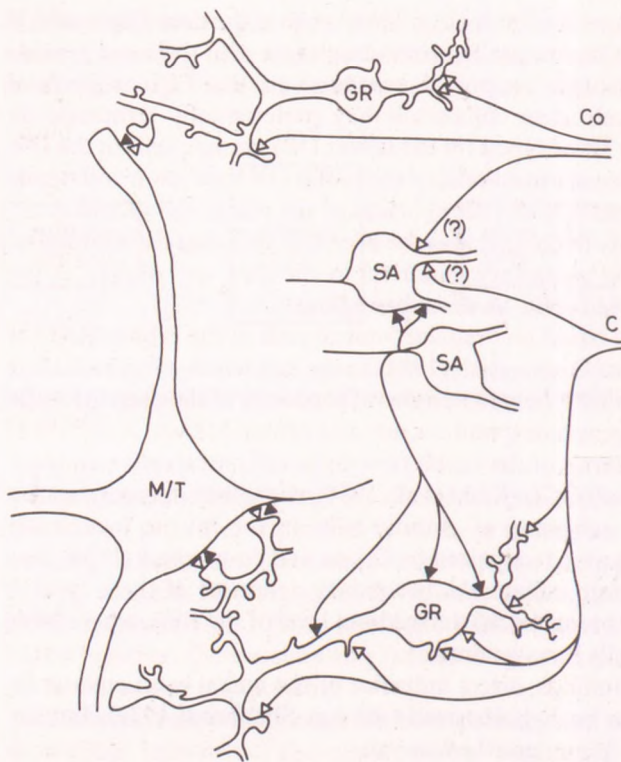


Fig. 4.31. Schematic drawing of synaptic connections in the granule cell, mitral cell and external plexiform layers of mammals. The figure demonstrates the specific targets of different terminals (on soma, dendritic shaft, spines/gemmules of individual cells) from certain cell (output neurons, granule and short-axon) and fiber (centrifugal afferents from different retrobulbar areas) types. The scheme is based principally on works of Price and Powell (1970a-c), and Willey (1973). For ease of demonstration, two granule (GR) and short-axon (SA) cells are included in the drawing, although pathways to and from both granule or short-axon cells may converge on one single respective example of that cell type. The drawing does not take into account the connectivity differences according to the segregated localization of subtypes of the mitral, tufted (M/T) and granule cells, or preferred layers of termination of the collaterals (Co) from mitral and tufted cells and central afferents (C). Triangles show polarity of synapses and morphological subtypes. Open triangles represent asymmetric thickening and spherical vesicles (presumed excitatory synapse), filled triangles designate synapses with ovoid or (for presynaptic endings of short-axon cells) flattened vesicles (both may be inhibitory) and symmetrical thickenings. Question marks reflect uncertainty as to the origin of asymmetrical synapses, formed on short-axon cell dendrites

aspects of the AON (ventral wing of the contralateral pars externa; pars dorsalis, lateralis and ventroposterior from both sides) and the posterior PC had most of their terminals in the GCL of the rat. These terminals are positioned to contact central processes, cell bodies and unbranched peripheral trunks, but not the gemmules, of GR cells. Although degenerating terminals of the central afferent fibers—other than those from the AON—were recognized exclusively in the inner half of the EPL by Price and Powell (1970c), in the latter study (Luskin and Price, 1983a) just a few terminals were recognized in the EPL (when compared to those in the GCL), in a position to synapse

on the peripheral dendritic branches and gemmules of the GR cells. No direct contacts between central afferents and output neurons of the bulb have been reported yet.

As illustrated in the above discussion, granule cells are the central elements of the synaptic integration in the deep OB. They may receive excitatory afferent impulses from central fibers and local axon collaterals of bulbar output neurons, and inhibitory influence from the SA cells (but not from another GR cell, unlike PG-PG cell contacts in the peripheral OB), from their deep until most superficial receptive surface. All these connections may serve the dendrodendritic connections, the only output gate of GR cells, established between the GR gemmules and the output neurons. According to physiological and neurochemical studies, the granule cells might be inhibitory interneurons. These (facilitated by central afferents or collaterals and inhibited by SA cells), by way of their dendrodendritic contacts, may contribute to local, self-inhibitory processes in the microenvironment (confined to the area of distribution of the dendrites and location of the output cell bodies) or spread lateral inhibition. Both effects may serve synchronization of the activity of subpopulations of the output neurons, initiated by volleys of the olfactory nerve fibers, shaped already at the glomerular level and conducted deeper into the system by primary dendrites of the mitral and tufted cells.

A further brief summary of the synaptic connections of the OB, after having discussed the detailed data furnished by neurochemistry, will appear at the end of the following Section (4.3.10.).

4.2.5. Glial system

Although the number of glial cells lies within the same range as that of the neurons in the olfactory bulb and they may have an indispensable role in maintaining favorable conditions for undisturbed stimulus processing and impulse conducting (for functions of glia, see the review by Kandel and Schwartz, 1985) in the OB, as in other parts of the brain, very little attention has been paid to their systematic study, as compared to neurons. Detailed light microscopic studies of the OB glial cells did not appear till the end of the last century (Van Gehuchten and Martin, 1891; Blanes, 1898) and have not been reinvestigated with recent techniques. A detailed ultrastructural characterization of these cells appears only in Andres (1965). Particular data for the morphology of several glial cells and their processes, or putative role, have been investigated by many authors of this field; these data will also be considered in this Section.

4.2.5.1. Light microscopy

All the main types of glial cells (for classification see Peters et al., 1974) have been recognized in the OB; their territory extended from the olfactory nerve layer until the ventricle (where it exists) (Fig. 4.32). The ON bundles are wrapped into separate bundles by Schwann cell processes, the elongated nuclei of the cells can be observed alongside the larger bundles (Fig. 4.33). Sheets of one single Schwann cell may enclose several hundreds of individual ON fibers in the rabbit (Clark, 1957). The ON

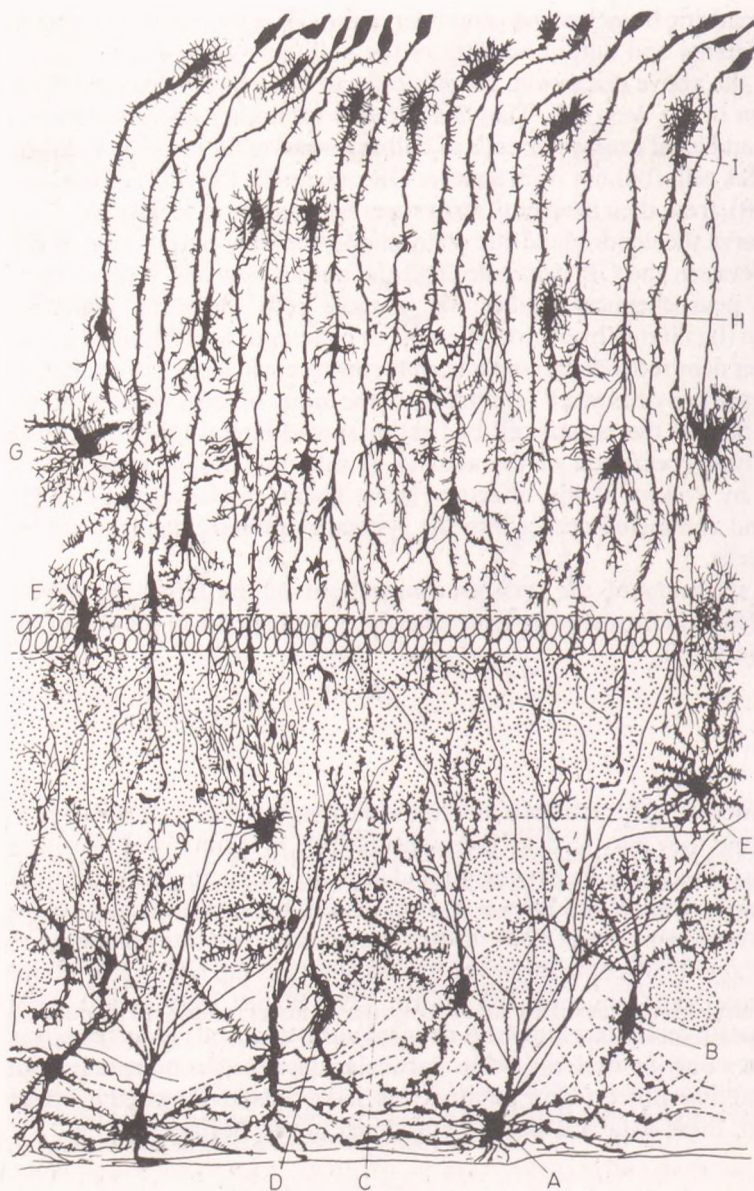


Fig. 4.32. Neuroglial cells of the bulbous olfactorius of a 2-month-old cat (redrawn from Blanes, 1898). A: neuroglial cell of the olfactory nerve layer; B and C: neuroglial cells with intraglomerular arborization; D: interglomerular neuroglial cell; E, F and G: neuroglial cells of the external plexiform, mitral cell and internal plexiform layers; H and I: dislocated epithelial cells; J: epithelium proper

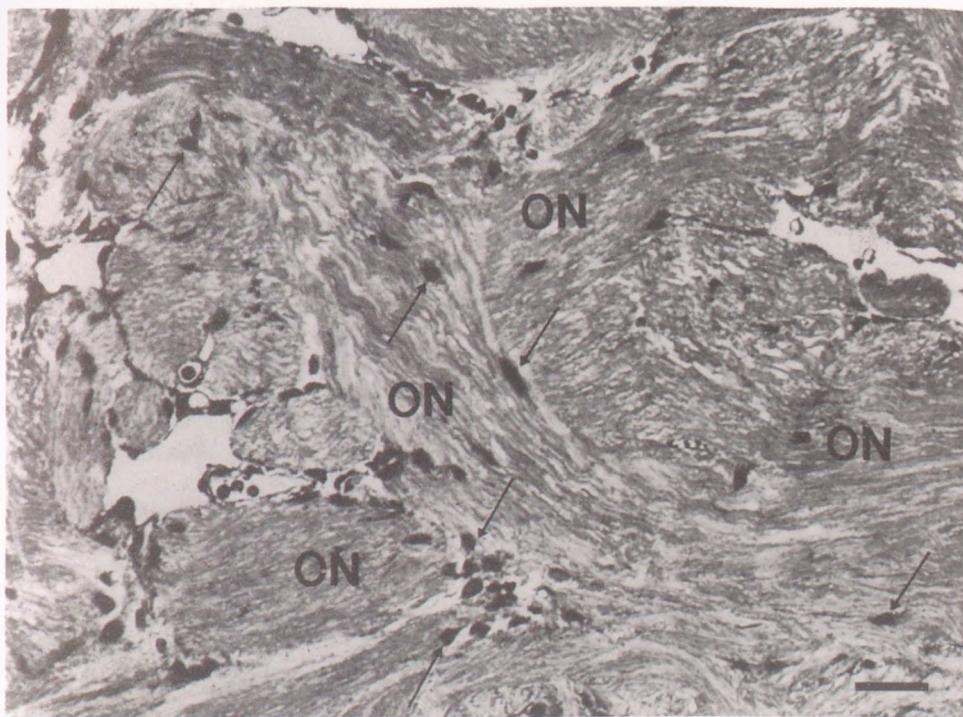


Fig. 4.33. Schwann cells (arrows) accumulate at the edges of the olfactory nerve (ON) fascicles. Rat olfactory bulb, aldehyde-osmium fixation, 4 μ m Epon section, toluidine blue staining. Scale bar: 20 μ m

penetrates the external limiting membrane, which is composed of processes/lamellae of the marginal astrocytes (Andres, 1965). Cells, contributing to this membrane may lie sometimes quite deep. In the amphibian OB, attachment of peripheral processes of the deep ependymal cells to the external limiting membrane have also been observed (Herrick, 1924).

Beneath this membrane fascicles of the olfactory nerve fibers, single neuronal cell bodies, compartments of the glomeruli and whole glomeruli may also be surrounded by glial processes provided most likely by astrocytes of the protoplasmic type. According to Blanes (1898), these contributing glial cells vary largely, according to the position, size and shape of the cell bodies as well as to morphology and distribution of their processes. He observed large, cylindrical or pyramid-like somata at the most external level of the glomeruli, having many branching collaterals parallel with the layers and long, straight central processes, extending even to the MCL in the cat OB. Somewhat smaller glial cells were situated around the glomeruli (still on the peripheral side). These distributed their richly branching processes within one or two glomeruli or in the superficial EPL. Small glial cells ("intraglomerular neuroglia") appeared within the glomeruli, their bushy arbors were restricted to the respective intraglomerular space.

The periglomerular glial cells (protoplasmic astrocytes) are quite similar to small periglomerular neurons either under the light or electron microscope (Koelliker, 1892;

Andres, 1965; Reese and Brightman, 1965; 1970; see also Section 4.2.2.2.2.). Deeper in the bulb, although protoplasmic astrocytes (and, also oligodendroglial cells) can be found all over the layers, they are more easily defined and there are more characteristic differences between the granule cells and glial cells of the GCL.

In the EPL and MCL, star-like glial cells were described by Blanes (1898), their processes were regularly restricted to the same layer (see Fig. 4.32), while the most peripheral extensions of the dislocated and regular epithelial (ependymal) cells reach this level also.

The extreme richness of the GCL in glial cells was emphasized already by Golgi (1875). Star-like neuroglial cells were observed in the zone between granule cells (Van Gehuchten and Martin, 1891; Blanes, 1898). These glial cells had an extremely rich, short arbor of processes which penetrated the space between the granule cells. Somata of the dislocated epithelial cells were found in the same layer. These cells also featured a very dense array of short processes, and released long, stout, rarely branching peripheral processes. In contrast, peripheral processes of the true ependymal cells were distributed into many terminal collaterals, arborizing regularly from the superficial GCL to the deep EPL (Blanes, 1898). Somata of these true ependymal cells were oval or cylindrical in shape and smooth-surfaced; they were found to have a stout central

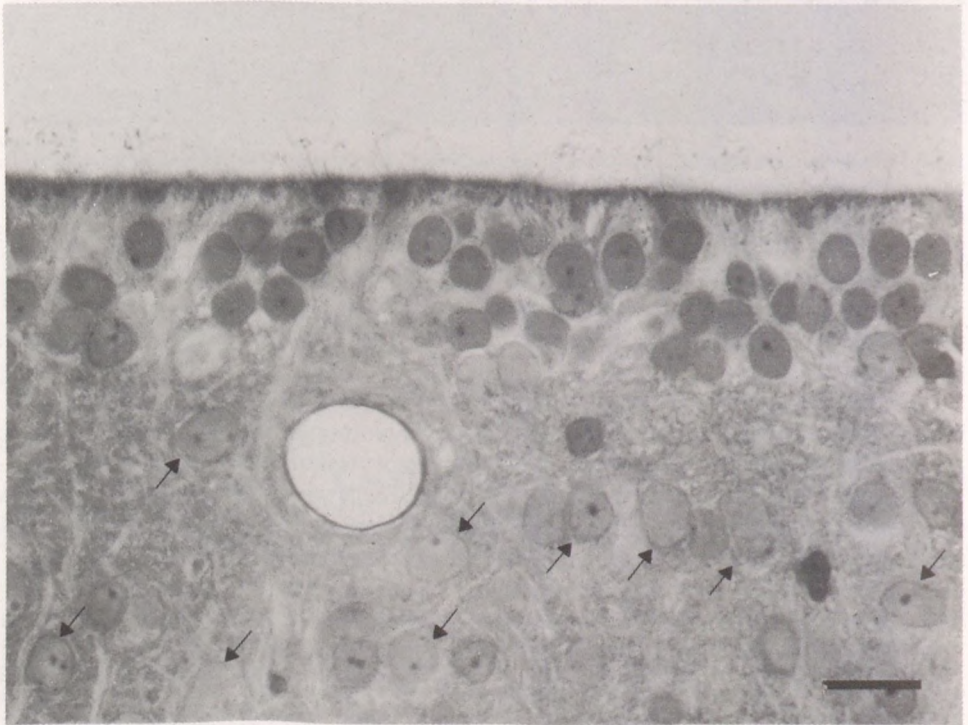


Fig. 4.34. Ventricular surface of the olfactory bulb of the turtle. The tightly packed, round or oval ependymal cell nuclei are somewhat smaller and more densely stained than the granule layer neurons (arrows). The central extension of the ependymal cells facing the ventricular surface are covered by microvilli and cilia. 2 μ m thick Araldite section, toluidine blue and thionin staining. Scale bar: 20 μ m

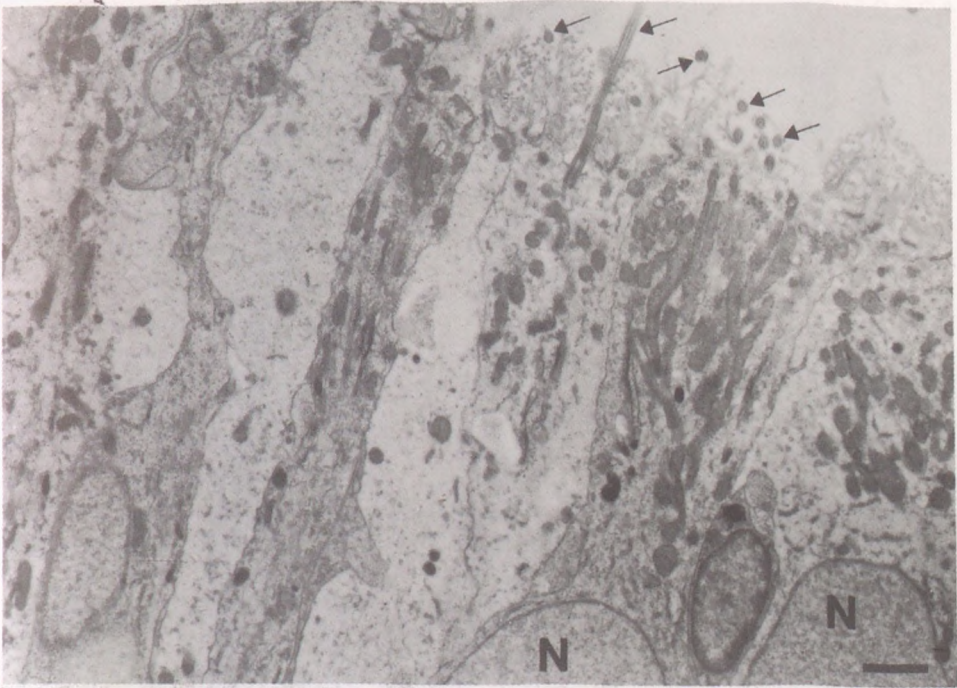


Fig. 4.35. Detail of the ventricular lining of the olfactory bulb of the turtle under electron microscope. Aldehyde-osmium fixation. Nuclei (N) compress the basal cytoplasm, while the ventricular extension of the cells is regularly rich in mitochondria, Golgi apparatus and other organelles. Cilia (arrows) and numerous microvilli arise at the luminal surface, where tight junctions are also frequently seen between neighboring ependymal cell processes. Scale bar: 2 μ m

process and constituted a tightly packed layer at the ventricular surface of the bulb (Fig. 4.34 and for electron microscopy see Fig. 4.35). At the amphibian ventricular surface, Herrick (1924) distinguished two types of ependymal cells, one having a stout, spiny peripheral process, while the other had a thinner and rarely spiny one. Both types arborized (very richly) predominantly in the layers exterior to the mitral cells and contributed to the structural link between the internal and external limiting membranes.

4.2.5.2. Electron microscopy

Electron microscopy did not reveal significant differences between glial cells according to layers, but distinguished clearly the (most numerous) astrocytes and oligodendroglial cells; rarely microglial cells were also observed (in normal, uninjured OB). A vast amount of neuroglial cells were identified as variations of the astroglial cells (Andres, 1965), so, these might be the correlates of most of the diverse glial cells (excluding the regular ependymal cells) reported by Blanes (1898), from the surface to the deep layers of the OB.

4.2.5.2.1. Astrocytes (AS)

The marginal and protoplasmic AS, described by Andres (1965) which constituted the external limiting membrane by their peripheral processes, were very similar in their ultrastructure; they had a cytoplasm poor in ribosomes, endoplasmic reticulum and mitochondria. The processes of these cells were very rich in tonofilaments, providing a firm support for the limiting membrane. The processes were in intimate contact with the pial vessels, although a continuous, poreless basal membrane separated them (Andres, 1965). According to Doucette (1984), such AS in the ONL had a relatively electron-lucent cytoplasm, they contained bundles of filaments, but never ensheathed olfactory nerve fibers. Another type of AS in the same layer had a more dense appearance than that of a "typical" AS, contained less filaments, contributed also to the external limiting membrane and was the only type providing processes to ensheath olfactory nerve axons.

In the glomerular zone protoplasmic AS provide their processes to encircle sets of 25–100 individual olfactory nerve axons (Reese and Brightman, 1970), and also intraglomerular packets of ON nerve fibers, terminals and dendritic processes (Andres, 1965; Reese and Brightman, 1965, 1970; Pinching and Powell, 1971c; Willey, 1973; Barber and Lindsay, 1982). They may also partially ensheath periglomerular neurons with tiny membranes. Periglomerular neurons have a tendency to overlap each other (see Section 4.2.2.2.2.) and because of the similar morphology and intermingling with protoplasmic AS, it is difficult to differentiate between the two cell types even at the electron microscopic level. In the GL, and also deeper, the fine structure of the AS is similar to that of surface-related protoplasmic AS, although they do not have tonofilaments. In general, AS of the OB were found to be poor in filaments (Andres, 1965). They may contain, however, small bundles of filaments, glycogen granules and dense bodies with a lamellar structure (Andres, 1965; Reese and Brightman, 1965, 1970; Willey, 1973). In addition, in the glomerular area, certain protoplasmic astrocytes may reach the density of organelles characteristic of neurons (Fig. 4.36).

Deeper in the bulb, more pale ("watery") processes and cellular cytoplasm is characteristic of the AS, but still shows a rare occurrence of filaments. The cell has an irregular shape; the soma and the processes conform to the shape of the surrounding neuronal processes. AS provide ensheathing lamellae to mitral and tufted cell dendrites and somata, the unmyelinated initial portion of the ruffed cells in goldfish (Kosaka and Hama, 1979c, 1982), synaptic complexes in the EPL, IPL and GCL, rarely also granule cells, and myelinated dendrites regularly all these wrappings are just partial or incomplete. Complete covering by processes of AS appear around blood vessels throughout the layers, as well as around synaptic complexes established by a certain axon and one or more dendritic spine-like processes in the GCL (Price and Powell, 1970b). The postsynaptic profile of these latter complexes (resembling usually granule cell dendritic spines in our material) frequently contain small, regular synaptic vesicles, various cisternal elements and 80–90 nm dense core vesicles (Fig. 4.37).

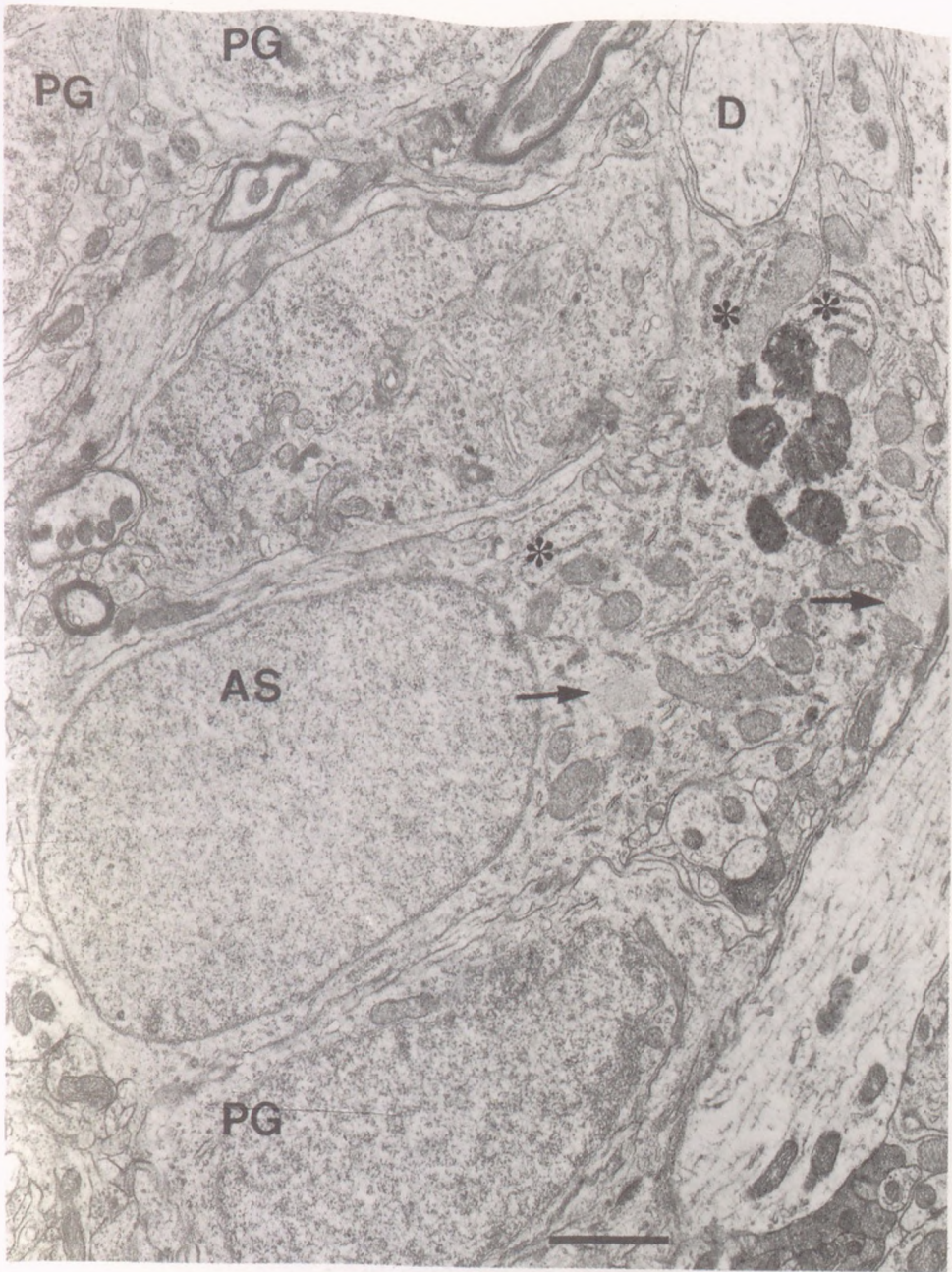


Fig. 4.36. Protoplasmic astrocyte (AS) from the periglomerular region of the rat olfactory bulb: electron micrograph. Adult rat, aldehyde-osmium fixation. Parts of periglomerular neurons (PG) are also included in the picture, their nuclei have a chromatin material, patchy in appearance. The astrocyte is rich in mitochondria, short, dispersed segments and parallel stacks of granulated endoplasmic reticulum (asterisks), free ribosomes and a Golgi-apparatus, just like small neurons in the same area. The presence of dark, laminated bodies and several bundles of fine filaments (arrows) and the fairly irregular outlines of the soma may, however, characterize this cell as a glial one. An extension of the cell encompasses part of the large dendrite (D) in the upper right of the picture. Scale bar: 1 μ m

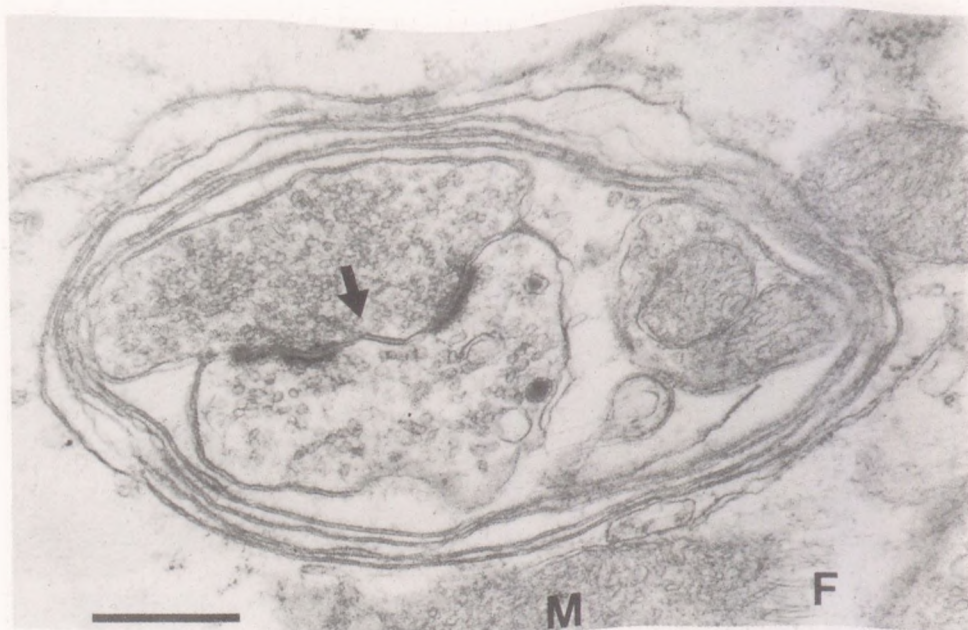


Fig. 4.37. A large synaptic complex from the granule cell layer of the rat olfactory bulb, engulfed completely by several layers of "watery" astrocyte processes. Aldehyde-osmium fixation. The pronounced asymmetry of the synapse clearly defines the polarization of this synapse, as indicated by the arrow. Even the postsynaptic fiber and a third one (not related to the former couple) contain small, regular synaptic vesicles. A bundle of filaments (F) appears to be attached to a mitochondrion (M) in the non-laminated compartment of the astrocyte. Scale bar: 0.5 μ m

4.2.5.2.2. Oligodendroglial (OG) cells

Such glial cells are much less frequent in the OB than the AS and more uniform in appearance. They can be found preferentially around the myelinated fibers in the GCL, MCL and IPL, more rarely in the GL or EPL, and are situated in both typical positions, as described by Peters and co-workers (1974) for "interfascicular" and "satellite" OG cells. Chromatin material in OG cell nuclei is aggregated into granules and the whole cell is characteristically dark because of the high electron density of the cytoplasmic matrix and richness of organelles (free and membrane-bound ribosomes, numerous mitochondria, fairly well-developed Golgi apparatus, microtubuli and vesicles).

Glial cells with ultrastructural features corresponding to the description above can be found occasionally alongside the capillaries of the bulb, too, where they may contribute to the wrapping of the wall of vessels, but they coexist much more typically with myelinated fibers (Fig. 4.38).

In this context, we may refer to the myelinated segments of the dendrites of output neurons in the bulb and the phenomenon of partly myelinated cell bodies, as discussed already (see Section 4.2.2.2.2. and also Fig. 4.39). OG cells were suggested (Diaz-

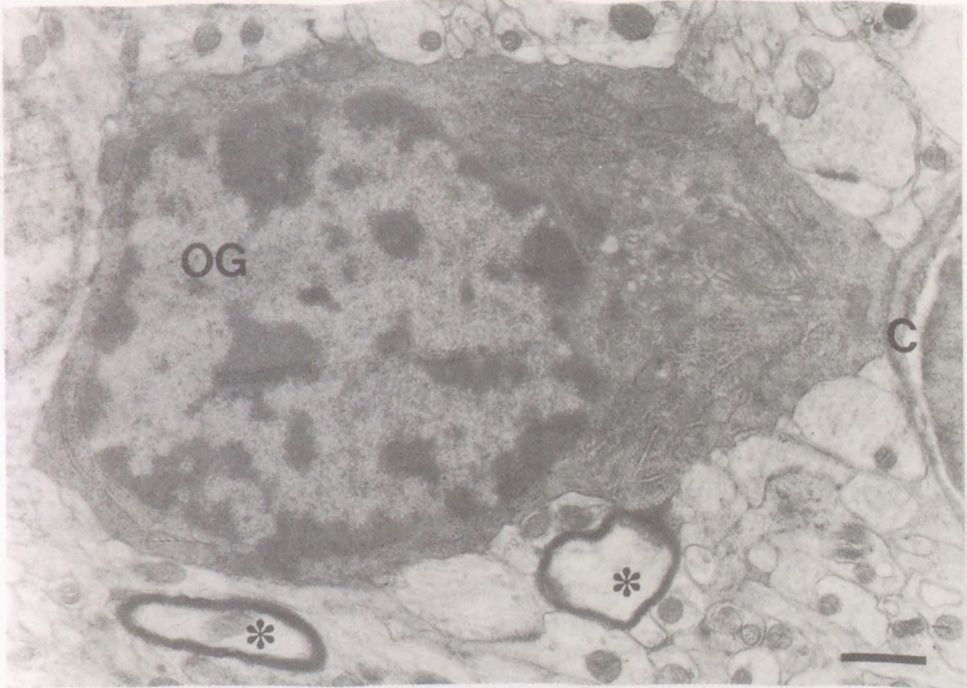


Fig. 4.38. Electron micrograph of an oligodendroglial cell (OG), apposed to a capillary (C). Adult rat, aldehyde-osmium fixation. Both the nucleus and the cytoplasm are more dense than those of any other neuronal or glial cell types in the olfactory bulb. The density is further increased in the nucleus by the presence of many chromatin aggregates and dark, grainy ground plasma containing numerous organelles: mitochondria, free ribosomes, dispersed granulated endoplasmic reticulum and an extensive Golgi-apparatus. The cell contacts one of the neighboring myelinated neuronal fibers (asterisks). Scale bar: 1 μ m

Flores et al., 1977), to provide these lamellae; close apposition of somata of satellite OG cells, or clearly recognized OG cell processes were observed by almost all investigators of this question (Braak et al., 1977; Burd, 1980; Tigges and Tigges, 1980; Remahl and Hildebrand, 1985). Regularly, these myelin lamellae are not exclusive components, constituting the wrappings around dendrites and cell bodies, because uncompacted sheets of other glial (AS) processes have also been found. Pinching (1971) suggested that the central processes of some glial cells could provide these myelin lamellae, peripheral processes of which ensheathed the ON fibers. In contrast, wrapping of the ON has no resembling features observed for typical OG cell processes, instead, they were clearly astrocytic in their appearance. Processes of OG cells in the OB (Andres, 1965) and elsewhere in the central nervous system (Peters et al., 1974) were characterized by the presence of large numbers of microtubuli, which are absent in the ensheathing lamellae between fascicles of the ON. So, if Pinching's (1971) proposal was correct, this myelin sheet would be produced by astrocyte-like glial cells.

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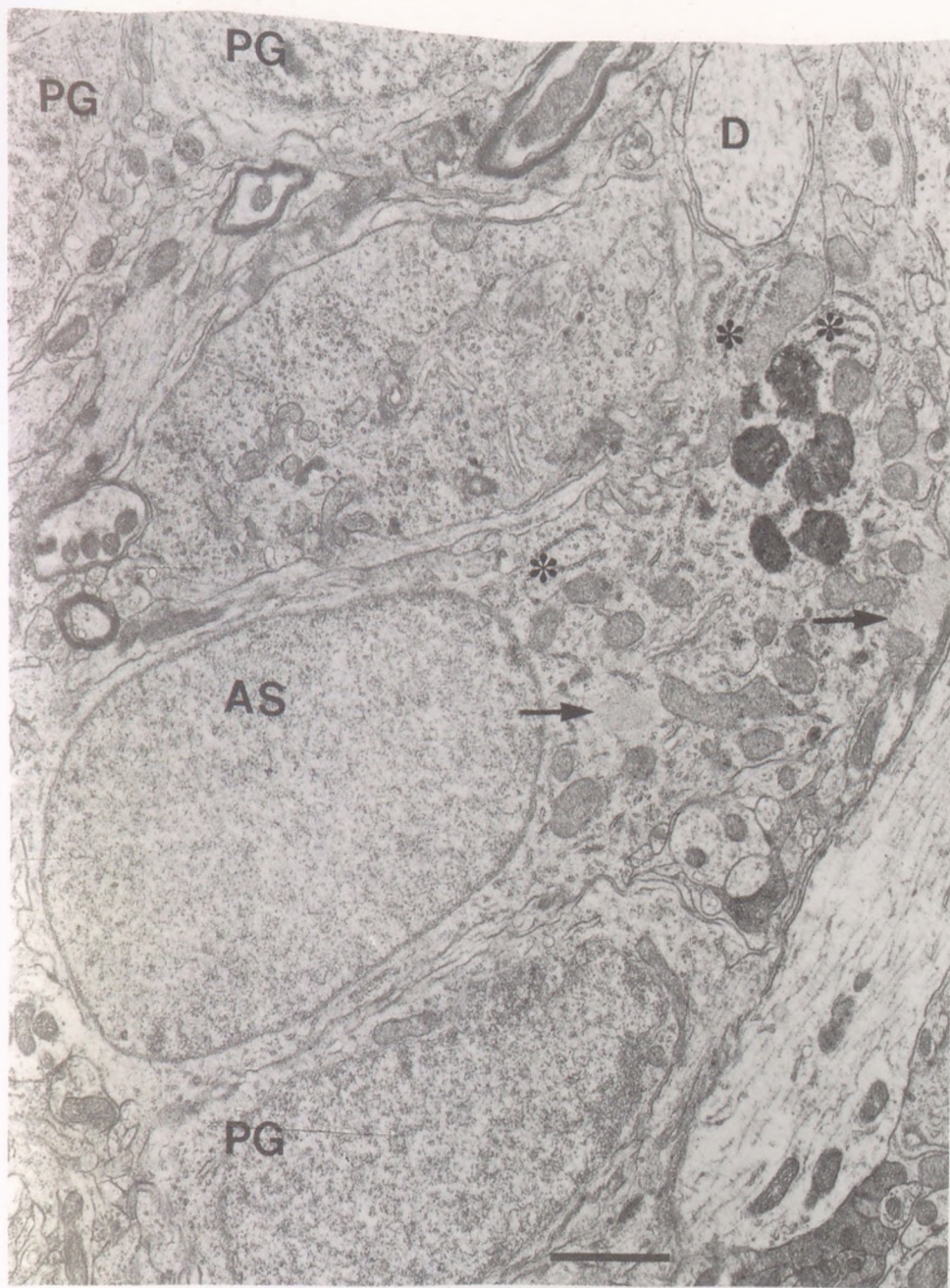


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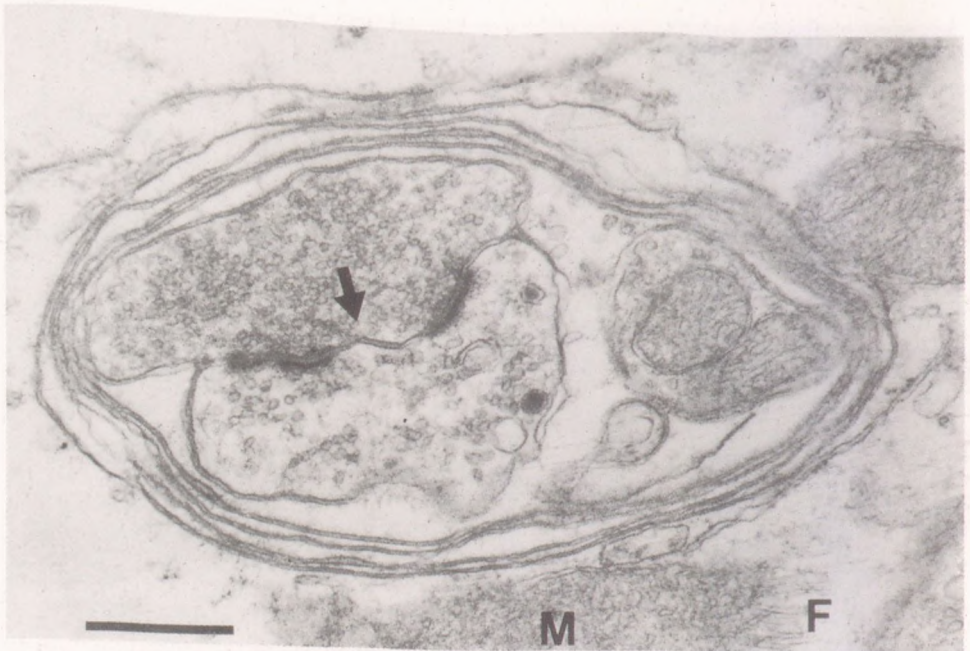


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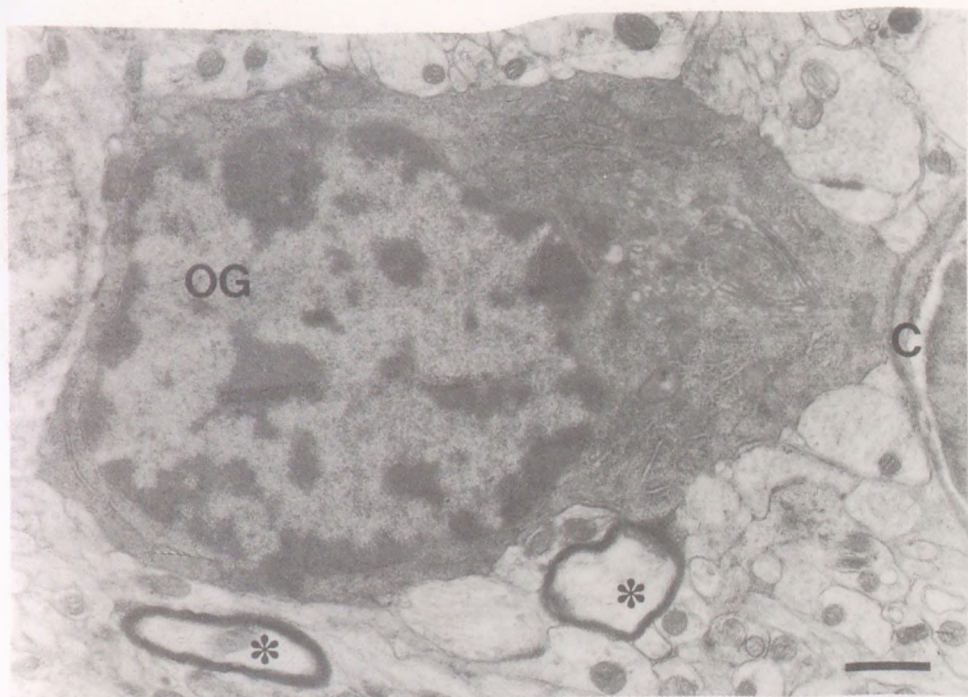


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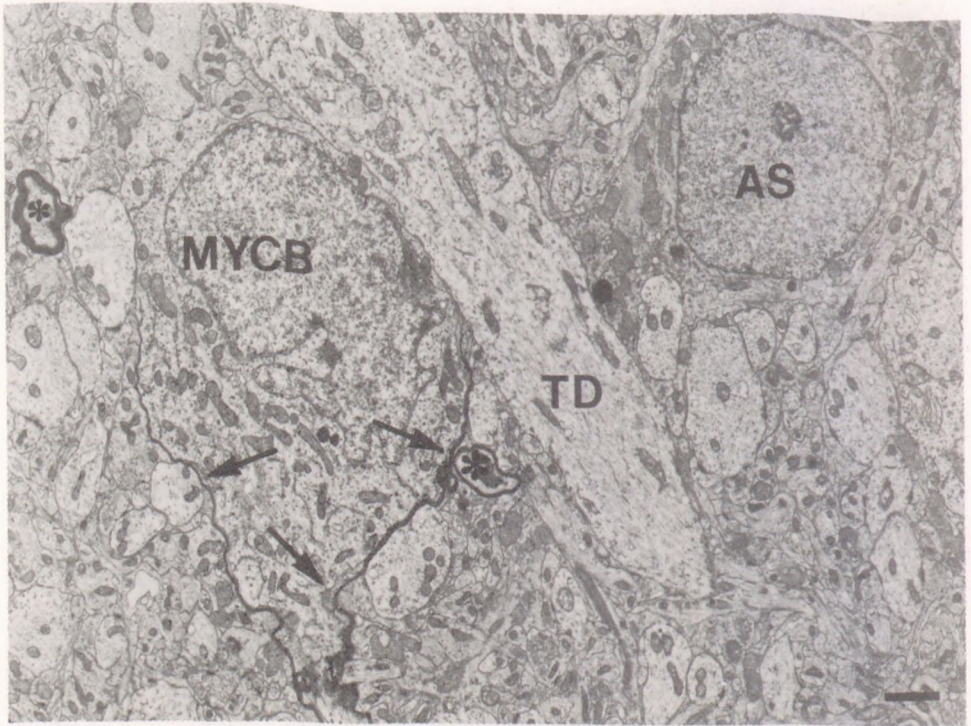


Fig. 4.39. Partly myelinated cell body (MYCB) from the superficial part of the external plexiform layer of the rat olfactory bulb. Aldehyde-osmium fixation. This cell (supposedly a short-axon cell) and its process is covered by several myelin lamellae (not resolved one by one at this magnification) and some other glial processes (see arrows) beneath the myelin. Two other myelinated fibers appear close by (asterisks) while, on the other side of a large tufted cell dendrite (TD), a protoplasmic astrocyte (AS) is situated at a position close enough to contribute to the ensheathing processes. Scale bar: 1 μ m

4.2.5.2.3. Ependymal cells

They have large, round or oval nuclei (lacking indentations) and relatively pale cytoplasm with irregularly dispersed, short cisternae of the granulated endoplasmic reticulum (see Fig. 4.35). Free ribosomes and mitochondria are also rare around the nucleus, while the central extension of the cell, which approaches (and reaches) the ventricular surface, is extremely rich in mitochondria, tubular and cisternal elements (the Golgi apparatus is located regularly here); lysosomes may also appear. The ventricular surface of the ependymal cells is completely covered by microvilli; cilia are also commonly found here. Tight junctions between neighboring ependymal cells and their processes are frequent, especially in the immediate vicinity of the ventriculus.

4.2.5.2.4. Microglial cells

It is widely accepted that these cells are not related specifically to the nervous system, they are a sort of phagocytic cells, distributed all over the brain and spinal cord and appear in high number in the case of injury.

Although they were described by light microscopy (Cajal, 1911; Andres, 1965) and characterized as small glial cells, having elongated or triangular nuclei and scanty cytoplasm, identification of the corresponding cells (in the OB) by electron microscopy is difficult because of the variability of their appearance and intermediate characteristics between AS and OG cells. Their large, contrasted phagosomes are, however, a peculiar feature and these cells may contain a considerably higher number of pinocytotic vesicles and microtubuli than the protoplasmic or marginal AS; also the granulated endoplasmic reticular system is somewhat less developed than that of the OG cells (Andres, 1965).

Resting microglial cells have rarely been observed. In the case of an injury, other glial cells, like the astrocytes appeared also to be activated, the territory of spreading extended, the number of cells increased and their "normal" appearance changed (in this state, they have been designated, "reactive glia"). In the early stage of

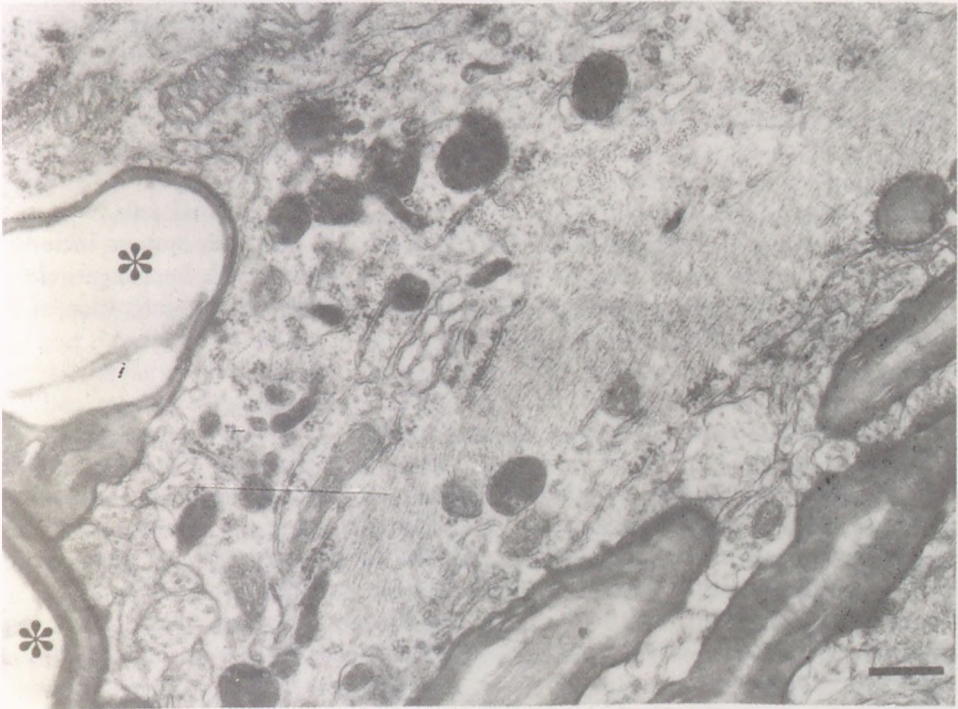


Fig. 4.40. Densely filamentous process of a reactive glial cell in the granule cell layer of the rat olfactory bulb. Adult rat, 6 days after transection of the olfactory peduncle; aldehyde-osmium fixation. A marked increase of phagocytic cells and processes, being very rich in filaments and dense lysosomes, were found predominantly around degenerating myelinated fibers (asterisks). Scale bar: 0.5 μ m

degeneration, Pinching and Powell (1971a) found an extension of pale, "watery" glial processes into the space, left behind by the degenerated neuronal elements. Such glial processes and cells were concentrated therefore at the site of the injury and could be characterized by high numbers of (dense) phagocytic vacuoles, extension of densely filamentous compartments and glycogen granules in their pale cytoplasm (Price and Powell, 1970c; Pinching and Powell, 1972a; Halász, 1976) (Fig. 4.40).

Although the origin of such "reactive" glial cells has been suggested to be astrocytic, microglia may also contribute to the same processes. Positive identification of the origin of such reactive glial cells, as well as of those cells, possessing intermediate characteristics between OG and AS, or subtypes of the main glial cell classes, even in the normal brain samples (Andres, 1965; Peters et al., 1974; Ford, 1975; Kandel and Schwartz, 1985) is awaiting the systematic application of antibodies to glial fibrillary acidic protein, glycerol phosphate dehydrogenase, 2',3'-c-nucleotide phosphohydrolase, myelin basic protein, galactocerebrosides, or other markers at the light- and electron microscopic level.

4.2.5.2.5. Summary

Between various types of glial cells, astrocytes can be most frequently observed. They are present within all layers, from the external limiting membrane to the deepest level of the granule cell layer. In addition to the usual appearance of their flattened processes around neuronal somata, axons and dendrites, they may tightly encircle large synaptic complexes in the granule cell layer or contribute to the wrapping of partly myelinated neuronal cell bodies and/or dendrites. Oligodendroglial cells are common, they occur most frequently in the GCL and GL; microglial cells can be observed only rarely. The ventricular surface is lined by ependymal cells.

With regard to the high degree of plasticity within the olfactory system, including also the adult bulb, the glial system may have an important role here in providing guidance for the ingrowing sensory axons as well as for the outward migration of the new granule cells.

4.3 Chemical architecture of the olfactory bulb

The olfactory bulb is one of the best known areas of the brain, as regards data for the presence of neuroactive substances (transmitters, neurohormones, regulatory peptides), precursors, metabolites and enzymes involved in their machinery, and mapping of these mediators or their receptors.* Drug applications may influence the functioning of the whole bulb, but little is known in detail about the role of a particular substance at a given synaptic contact. Unfortunately, histochemical localizations at

* Several reviews have summarized these achievements for the whole bulb in the last few years (Margolis, 1981; Macrides and Davis, 1983; Halász and Shepherd, 1983).

the fine structural level are scarce, although light microscopy has furnished much data to relate certain supposed transmitters to particular neuron or fiber types.

In the following sections we shall go through the various neuron types and bulbofugal fiber systems and summarize briefly the biochemical, neurochemical and histochemical evidence for their likely chemical mediators.

4.3.1. Olfactory nerve

According to the results summarized in the Chapter 3, two characteristic neurochemical markers of the ON have been discovered and characterized in detail to date. These include carnosine, as a suggested transmitter material, and the olfactory marker protein (OMP), the presence of which is an indication of functionally mature receptor cells, their axons and terminals (see more on OMP in Chapters 2 and 3). Selective labeling of the ON or its portions also became feasible by using the monoclonal antibody technique. In addition, a calcitonin gene-related peptide (CGRP) was also found in a small proportion of ON fibers (Rosenfeld et al., 1983), epithelial cells (Denis-Donini, 1989) and nerve fibers containing luteinizing hormone-releasing hormone (LHRH) were reported to join the fila olfactoria towards the nasal mucosa (see Section 2.3.). In amphibians, even neuronal cell bodies with immunoreactivity for LHRH antibodies were embedded into the olfactory nerve fascicles, entered the glomeruli and arborized there (Wirsig and Getchell, 1986). Several such embedded neurons showed also acetylcholinesterase positivity. These systems were interpreted as part of the terminal nerve (Wirsig and Getchell, 1986).

4.3.1.1. Carnosine

Although certain neurochemical data point to the specific occurrence of carnosine in the primary ON (Margolis, 1980a; see also Sections 2.3.2.2. and 3.3.1.), pharmacological and physiological evidence for its expected excitatory action upon postsynaptic targets, the periglomerular and output neurons of the OB has not been clearly shown.

When synaptosomes, prepared from the OB were lysed and synaptic vesicles released, carnosine was not found to be associated with the vesicles (Harding and O'Fallon, 1979). Ligand binding studies (Hirsch et al., 1978; Hirsch and Margolis, 1979), however, suggested the existence of a physiologically relevant receptor for carnosine. Different types of carnosine application (perfusion of the bulbar surface or iontophoretic delivery to the MCL, EPL or GL) and recording (extracellular: Tonosaki and Shibuya, 1979, 1985: gecko bulb; intracellular: Nicoll et al., 1980: frog and turtle OB; Frosch and Dichter, 1984: cultured OB cells) revealed weak or no excitation onto the spontaneous or odor-evoked activity of mitral cells. In contrast, Gonzales-Estrada and Freeman (1980), based on their EEG and slow potential recordings on rabbits, concluded that carnosine might excite the output neurons but this was obscured by granule cell inhibition. A similar explanation was offered by Tonosaki and Shibuya (1985): they proposed that PG cells, being affected by carnosine, suppressed, in turn, the activity of mitral cells. This assumption must,

however, still be supported further since PG cells were mostly inhibited by carnosine in the rat OB (MacLeod and Straughan, 1979).

In summary, acceptance of the suggestion for the transmitter role of carnosine in the primary olfactory pathway depends on further experiments.

4.3.2. Mitral and deep tufted cells

The neurotransmitter substance of these output neurons has not been determined. There are, however, strong suggestions for aspartic acid, (to a lesser extent) glutamic acid and most recently for a dipeptide of these amino acids, N-acetylaspartyl-glutamate (French-Mullen et al., 1985), as the likely transmitter candidates. Most of these data derive from experiments involving the LOT and its distribution area on the basal forebrain, the primary olfactory cortex.

4.3.2.1. Aspartate (Asp) and glutamate (Glu)

The whole bulb is rich in both Asp and Glu (Popov et al., 1967) but no sharp peak was shown as an indicator for specific accumulation of these amino acids at the levels of somata, primary or secondary dendritic processes and endings (Godfrey et al., 1980; Nadi et al., 1980).

Attempts to visualize markers of Glu or Asp at the cellular level in these cell categories were unsuccessful until very recently. Liu et al. (1989), however, revealed a glutamate-like immunoreactivity within mitral and tufted cell somata and mitral cell dendrites with a monoclonal antibody raised against Glu-BSA. Radiolabeled Asp or Glu, injected into the olfactory ventricle, bulbar tissue or piriform cortex, or added to the incubation medium of olfactory bulb slices of the rat or guinea pig, failed to label selectively any of the cellular compartments of these cells (Halász, unpublished data). Kainic acid treatment of the bulb did not damage nerve elements, being postsynaptic to the mitral and deep tufted cells (Halász, 1979; Krammer et al., 1980). This latter finding might be explained by assuming a lack of kainate receptors in the OB. In the rat olfactory cortex, quisqualate receptors were shown first to be involved in the LOT—pyramidal cell synapse (Collins, 1982), but later the contribution of N-methyl-D-aspartate (NMDA) and kainate were also postulated (Surtees and Collins, 1985).

The only positive result for microscopic visualization of a label for Asp in the projection neurons of the the OB was reported briefly by Saito and co-workers (1986). They were able to show, by using the indirect immunofluorescence technique, the presence of Asp-like immunoreactivity within mitral cells and their dendrites in the EPL (and also in thin fibers and several cells in the IPL and GCL).

All the rest of data, supporting the view of amino acid transmitters within these cells, are indirect and derive from pharmacological studies on the olfactory cortex and OB, or from analysis of the consequences of the LOT transection.

Iontophoretic application of either Glu or Asp within the rabbit OB resulted in suppression of mitral cell activity (Nicoll, 1971a). This was explained by suggesting granule cell excitation by these excitatory (Curtis and Watkins, 1960; Krnjevic and

Phillis, 1963; Curtis and Johnston, 1974) amino acids and subsequent inhibition of the output neurons through dendrodendritic synapses. Asp and/or Glu was released by electrical stimulation of the LOT from the olfactory cortex slice preparation of guinea pigs (Yamamoto and Matsui, 1974, 1976; Matsui and Yamamoto, 1975; Bradford and Richards, 1976) and rats (Collins, 1979a). Asp and Glu (and GABA) was present in, and could be released from synaptosomes, prepared from the rat olfactory bulb (Osborne et al., 1976). Transection of the LOT, or removal of the OB resulted in a substantial loss of both amino acids from the cortex (Harvey et al., 1975; Graham, 1977; Collins, 1979b, 1984; Godfrey et al., 1980; Scholfield et al., 1983) preferentially from layer IA, where the bulbofugal axons terminate (Godfrey et al., 1980; Scholfield et al., 1983). This reduction was suggested to be the result of the decrease in levels of glutaminase and malate dehydrogenase in the cortex (Sandberg et al., 1984). Upon electrical stimulation of the LOT, both Glu and Asp were released from the cortex slices by calcium-dependent processes (Bradford and Richards, 1976; Collins, 1979a).

Data are emerging, however, which question the proposed transmitter role of both Asp and Glu. Jacobson and co-workers (1986) by analysing the effect of receptor antagonists on the LOT-evoked antidromic responses on superfused slices of the rabbit olfactory bulb concluded that neither Asp nor Glu were the likely transmitters at the mitral-to-granule cell (dendrodendritic) synapse. Prepiriform cortex responses to LOT stimulation, Glu agonists or Glu itself, were not effected by L-glutamic acid diethyl ester (a Glu antagonist at the crustacean neuromuscular junction); similarly, stimulation of the cortex with iontophoresis of Glu was not disturbed by 2-amino-4-phosphonobutyric acid (APB) (Hori et al., 1981, 1982). Repetitive application of Glu or Asp desensitized receptors of the prepiriform cortex slices to these amino acids but not to the endogenous substance released by electric stimulation of the LOT (Braitman, 1986). The piriform area was one of the cortical regions containing the highest density of N-methyl-D-aspartate receptors (Cotman et al., 1987).

4.3.2.2. N-Acetylaspartylglutamate (Ac-Asp-Glu)

After questioning the transmitter role of Asp/Glu in the principal output neurons (Hori et al., 1981, 1982; Braitman, 1986), a dipeptide, Ac-Asp-Glu was reported to be endogenous in the brain, and was suggested to be the transmitter of these cells (French-Mullen et al., 1985). APB blocks the cortical response to Ac-Asp-Glu (similar to LOT stimulation) and bulbectomy causes a significant reduction of Ac-Asp-Glu in the piriform cortex. These data strongly support that at least part of the LOT fibers (mitral and deep tufted cell axons) may use Ac-Asp-Glu as their synaptic transmitter. This suggests also that their parent cell bodies (and dendrites) would contain the same peptide in the OB, too. Blakely et al. (1987) recently revealed the selective immunostaining of the mitral cells with a polyclonal antibody against Ac-Asp-Glu. It is also possible that the antibody of Saito et al. (1986), raised against bovine serum albumin (BSA)-linked aspartate, could recognize a determinant for aspartate on the endogenous Ac-Asp-Glu, and reveal, in this way, this very peptide in the mitral cells. The percentage for the occurrence of cross-reactivity of this aforementioned antibody with Ac-Asp-Glu was, unfortunately, not reported by Saito et al. (1986).

4.3.2.3. Cholecystokinin (CCK)

One paper documents the presence of CCK in the deep output neurons of the OB. Seroogy and co-workers (1985) found CCK-like immunoreactivity within large cell somata in the bulb, and also terminal-like structures within the layer IA of the AON, OT and most rostral PC, all being (the closest) projection area for the output neurons of the OB.

4.3.3. Medium and superficial (external) tufted cells

These subpopulations differ from the deep output neurons not only in position, projection field and morphology, but also in content of their neuroactive substances. Asp, Glu or their derivatives were not localized in their somata or processes. CCK was, however, observed in all kinds of tufted cells, including those of the deep ones. In the middle and external tufted cells, tyrosine hydroxylase and a specific Ca-binding protein was found. In superficial tufted cells only, substance P (hamster OB) and vasoactive intestinal polypeptide (rat OB) content was also demonstrated.

4.3.3.1. Dopamine (DA)

The existence of dopaminergic tufted cells was confirmed and they were identified in several ways and by many laboratories, regularly together with the dopaminergic periglomerular neurons. Presence of DA in the OB was recognized and measured by several authors in rats (Popov et al., 1966; Versteeg et al., 1976; Palkovits et al., 1979) and tortoise (Juorio, 1969). The expression and content of DA depended highly on the normal sensory input. Unilateral olfactory deprivation caused a significant reduction in the OB-DA content (without a comparable change in noradrenaline content) (Brunjes et al., 1985). Chemical or surgical deafferentation of rats and mice resulted in a significant reduction of tyrosine hydroxylase (TH), DA, 3,4-dihydroxyphenylacetic acid (DOPAC) in rat, mouse and hamster OB, and a dramatic loss of TH immunoreactivity in the juxtglomerular neurons (Nadi et al., 1981; Kawano and Margolis, 1982; Baker et al., 1983, 1984; Kream et al., 1984). Laminar distribution of DA and TH across bulbar layers showed a peak over the glomerular layer, indicating the presence of dopaminergic tufted and periglomerular neurons (Jaffé and Cuello, 1980a; Nadi et al., 1981). Presence of dopaminergic D-2 receptors has been proven by a new, specific photoaffinity labeling method, and also for the OB, although without information about the laminar distribution (Redouane et al., 1985).

Histochemical observations started with fluorescence microscopy of catecholamines after condensation with formaldehyde and revealed fluorescent cells predominantly at the deep aspect of the PG zone (Dahlström et al., 1965; Lichtensteiger, 1966; Lidbrink et al., 1974; Parent et al., 1978). Clear recognition of a cell category, differing from the periglomerular neurons, was achieved only by later studies, using labels for the enzymes TH (E.C. 1.14.16.2) and dopadecarboxylase (DDC, E.C.4.1.1.28) (also named as aromatic L-amino acid decarboxylase), during high resolution light

microscopy of the rat (Hökfelt et al., 1975a; Halász et al., 1977a,b, 1981a; Baker, 1986a), hamster (Davis and Macrides, 1983; Kream et al., 1984; Macrides et al., 1985; Baker, 1986a,b) guinea pig, mouse, cat and rabbit (Baker, 1986a), gecko (Smeets et al., 1986) and sheep (Tillet et al., 1987) olfactory bulb. Further details became evident when distribution of the same antibodies was studied by electron microscopy of single sections (Halász et al., 1977a,b) or by reconstruction of labeled cells from serial electron micrographs (Halász et al., 1981a).

From these studies, it is clear that not all tufted cells were immunoreactive, but particular morphological features of tufted cells with TH-like activity as opposed to those having no such marker were not described. Their size, distribution and intrabulbar arborization might be similar to other, non-reactive medium and superficial tufted cells (Fig. 4.41). The fine structure of dopaminergic tufted cells reveals also the features characteristic of a "regular" tufted cell (Halász et al., 1977b, 1978, 1981a) (Figs 4.43 and 4.45). Dendrites of the labeled cells, as determined by serial sectioning, or identified tentatively on single electron micrographs, based on their fine structural appearance, were recognized in the EPL and GL by the presence of the label

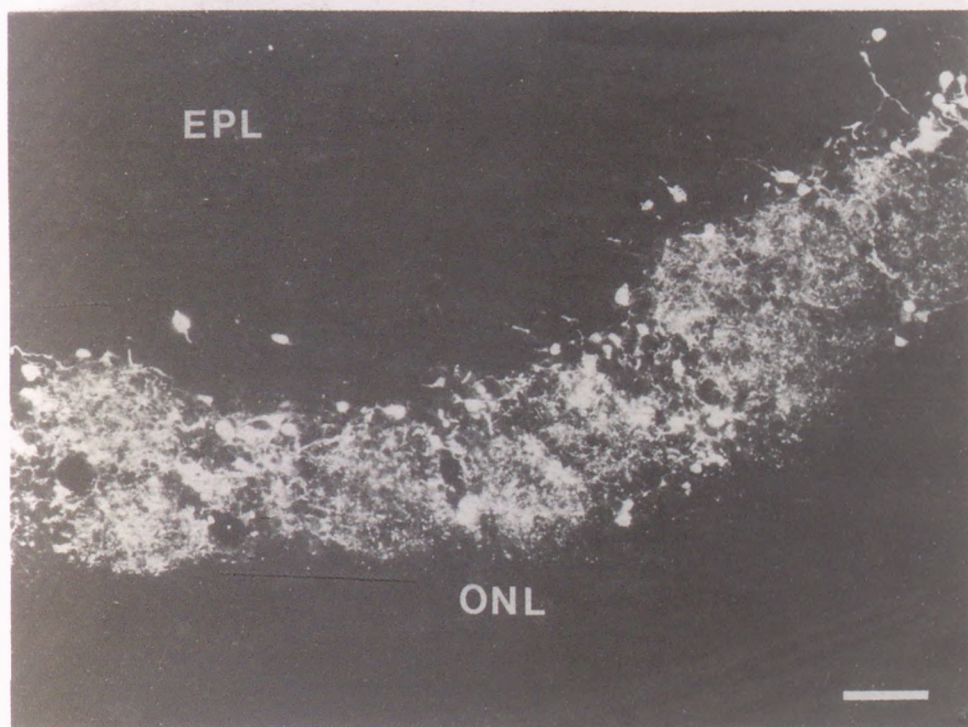


Fig. 4.41. Tyrosine hydroxylase immunoreactive structures in the superficial layers of the rat olfactory bulb. Indirect immunofluorescence technique. Most of the label is confined to the glomerular layer, while no specific label appears in the olfactory nerve layer (ONL). Numerous cell somata, surrounding the glomerular neuropil (like superficial tufted and periglomerular neurons) as well as their varicose processes show strong immunopositivity. In the external plexiform layer (EPL) several single cells and their processes fluoresce; they may represent a dopaminergic subpopulation of the medium tufted cells. Scale bar: 50 μ m

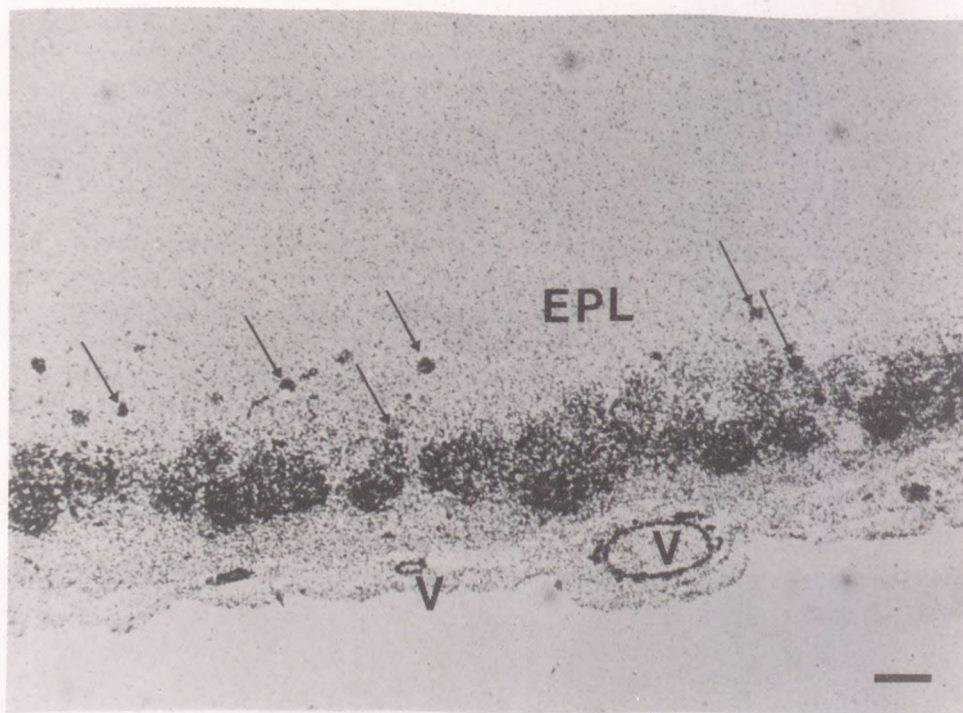


Fig. 4.42. Uptake of the radiolabeled dopamine by cells and processes in the superficial layers of the turtle olfactory bulb. Light microscopic autoradiogram of a 2 µm thick Epon section, Kodak NTB-2 emulsion, Kodak D19B developer, no counterstaining. The glomerular layer exhibits strong activity, as demonstrated by numerous labeled intraglomerular fibers and terminals. A few labeled cell somata (arrows) are obvious in the glomerular layers and in the superficial aspect of the external plexiform layer (EPL). Pial vessels (V) also show heavy accumulation of the label. Scale bar: 50 µm

within their cytoplasm (Figs 4.43–4.44, and see also Fig. 4.50). The reaction product was regularly aggregated around the organelles (see Figs 4.43 and 4.44).

Light- and electron microscopic autoradiography of the uptake of radiolabeled catecholamines contributed to the recognition of distribution of catecholaminergic cell somata and fibers in the rat (Halász et al., 1978; Priestley et al., 1979) and turtle (Halász et al., 1983) olfactory bulb (Fig. 4.42 and see also Figs 4.45, 4.49, 4.51 and 4.61). During such studies, ultrastructure of the labeled cells could be preserved better than through processing for immunohistochemistry (e.g. Figs 4.45, 4.49 and 4.51). It should be emphasized, however, that the specificity of the immunolabeling usually exceeds that obtained by radiolabeling.

TH activity has been shown to appear in several superficial tufted cells together with glutamate decarboxylase immunoreactivity. This points to the possibility of coexistence of DA and γ -aminobutyric acid (GABA) within the same tufted cell (Kosaka et al., 1985, 1987a). In contrast, although both superficial and middle tufted cells may contain a specific, vitamin D-dependent calcium binding protein (28K-D-

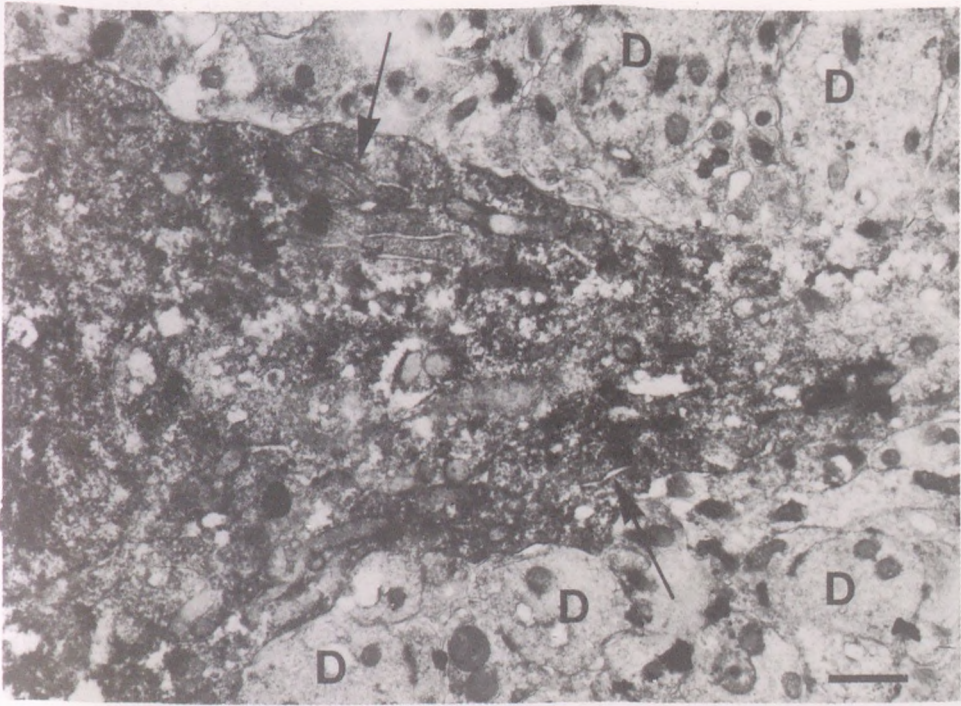


Fig. 4.43. Detail of a medium tufted cell of the rat olfactory bulb. Tyrosine hydroxylase immunocytochemistry, PAP technique at the electron microscopic level, lead citrate staining. The reaction product is distributed across the cell, but is concentrated around cytoplasmic organelles, like mitochondria and granular endoplasmic reticulum (arrows). Large, unlabeled (mitral and tufted cell) dendrites and smaller, vesiculated fibers of the external plexiform layer surround the labeled compartment. Scale bar: 1 μ m

CaBP), immunoreactivities for TH and 28K-D-CaBP were present in different juxtglomerular cells (Halász et al., 1985b; for 28K-D-CaBP immunoreactivity; Fig. 4.46).

As for the central projection of the dopaminergic tufted cells, they might terminate in layer IA of the same cortical basal forebrain areas, as the non-dopaminergic tufted cells do. Presence of receptors for DA (and noradrenaline, too) have been revealed in the olfactory cortex slice, but they were studied solely as targets of the neocortical dopaminergic system (originating in the ventral tegmentum and substantia nigra), without considering a possible afferent input from the OB (Collins et al., 1985).

4.3.3.2. Cholecystokinin (CCK)

CCK was assigned to the most superficial and medium (in part, also to the deep) tufted cells of the rat OB very recently (Seroogy et al., 1985). High levels of CCK (Rehfeld, 1978; Larsson and Rehfeld, 1979; Beinfeld et al., 1981) and CCK precursors (Beinfeld et al., 1981) were found in the OB. A significant amount of this CCK content,

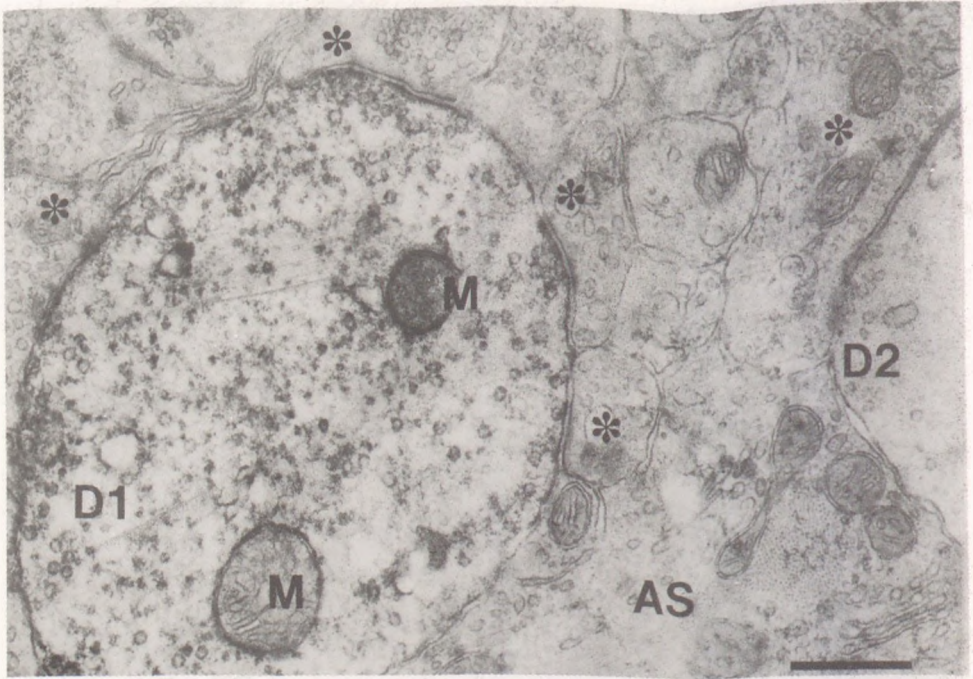


Fig. 4.44. Cross-sectional view of a large, labeled tufted cell dendrite of the rat olfactory bulb. Tyrosine hydroxylase immunohistochemistry, PAP technique, lead citrate staining. The large, immunopositive dendrite (D1) and another, non-labeled dendrite of the same type (D2) seems to be contacted by densely vesiculated profiles (asterisks). The PAP reaction product encircles mitochondria (M) and virtually enhances the density of synaptic vesicles and neurotubuli within labeled processes. Part of a fibrous astrocyte (AS) is also included in the photographed area. Scale bar: 0.5 μ m

according to the mapping study of Seroogy et al. (1985) at the light microscopic level, might be contained by middle (and external) tufted cells and their axon collaterals, distributed in the IPL, in addition to other, less frequently observed intrinsic neurons and central afferent CCK fibers (Cho et al., 1984). CCK-immunoreactivity in tufted cells was most apparent in somata and dendrites of the tufted cells, found in highest numbers in the superficial EPL, facing the glomeruli, but the axons have also been frequently visualized with this technique. In another structure-related experiment, performed on guinea pigs, there is no mention of the localization of immunoreactive CCK in the OB, although a high CCK content was revealed by radioimmunoassay (RIA) (Larsson and Rehfeld, 1979).

The significance of the CCK-content and CCK-containing nerve compartments in the OB is still under investigation. It might be related to feeding, since the CCK content of the OB jumps very significantly at the age of rat pups (between postnatal days 7–14) (Varró et al., 1983) when they start to search for complementary food over the mothers milk. Food deprivation causes wide changes in the CCK content of various brain areas, including the OB (Finkelstein et al., 1983).

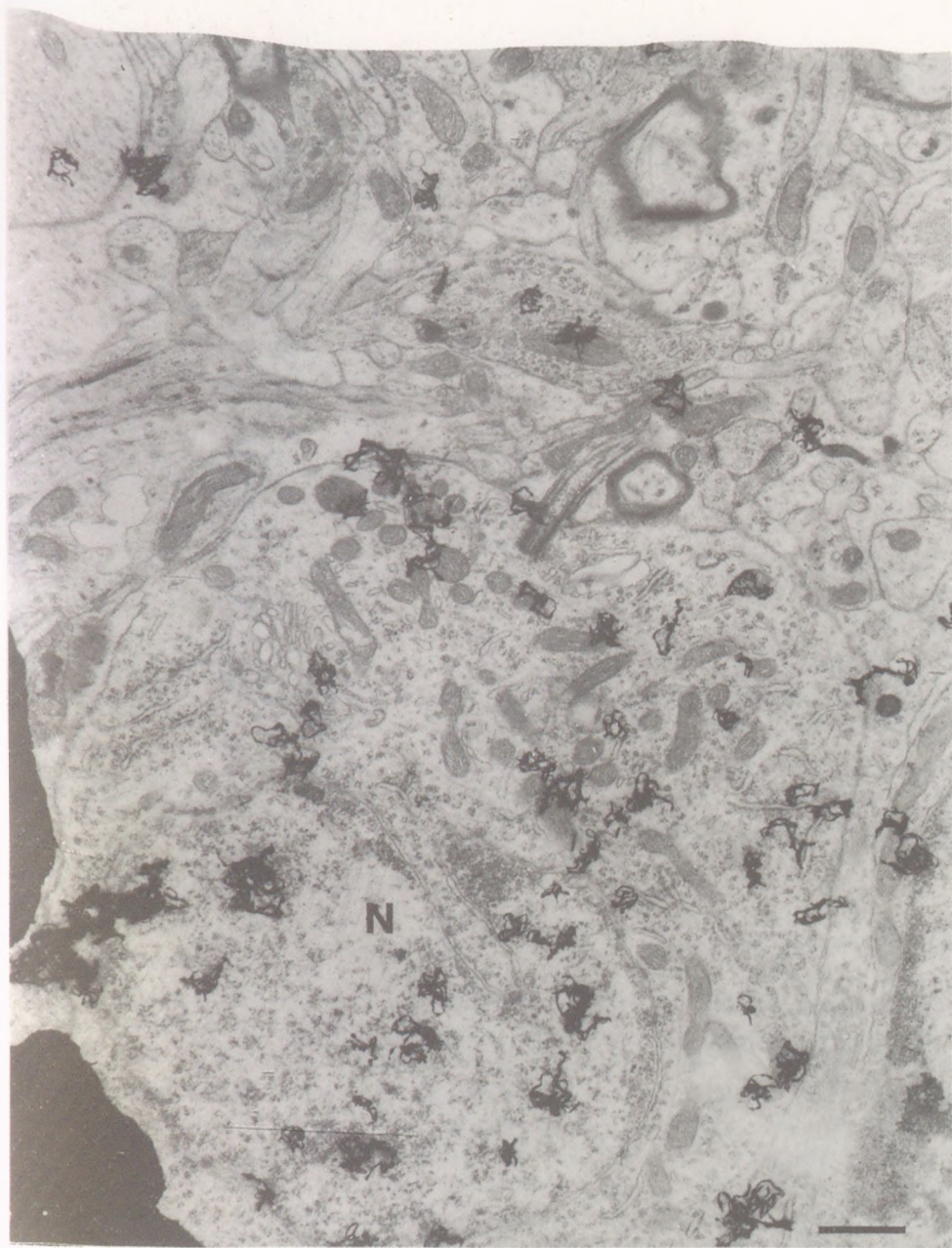


Fig. 4.45. A large (presumably tufted), ciliated neuronal soma, featuring dense accumulation of silver grains after ^3H -noradrenaline uptake into the rat olfactory bulb. Electron microscopic autoradioagram. Nialamide pretreatment (500 mg/kg, i.p.), aldehyde-osmium fixation, uranyl acetate and lead citrate staining, Ilford L4 emulsion, Amidol developer. Silver grains labeled both nuclear (N) and perikaryal plasm. Scattered grains, although much lower in density, are also visible over the surrounding glomerular neuropil. Scale bar: 1 μm

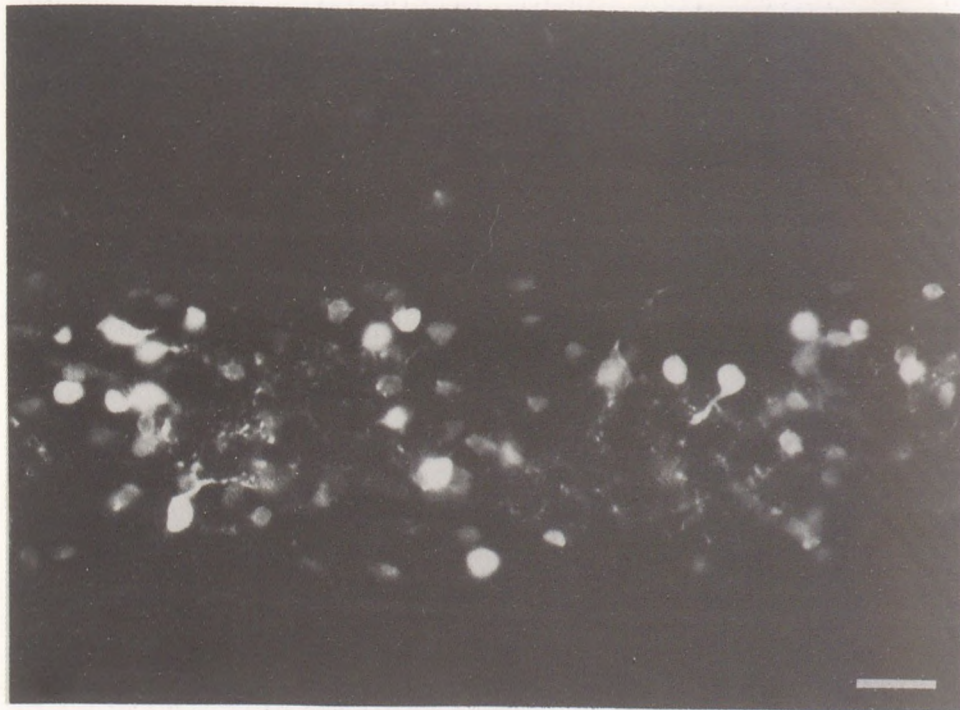


Fig. 4.46. Presence of a calcium-binding protein in juxtaglomerular cells of the rat olfactory bulb. Adult rat, immunofluorescence of fluorescein-labeled, 28K-vitamin D-dependent calcium-binding protein antibodies. Neuronal (supposed periglomerular and tufted) cell somata and several stout or thinner, varicose intraglomerular processes are labeled. Scale bar: 20 μ m

4.3.3.3. Substance P (SP)

SP immunoreactivity was also found in tufted cells, although only in members of the most superficial subclass of these neurons, as described for hamsters (Burd et al., 1982a; Davis et al., 1982; Davis and Macrides, 1983; Kream et al., 1984; Baker, 1986a,b). Baker (1986a) recognized, however, differences in the distribution pattern of the SP neurons in two (Syrian and Chinese) strains of hamsters and found no SP-immunopositive neurons in the main olfactory bulb of other mammalian (mouse, rat, guinea pig, rabbit, cat) main OB. Inagaki and co-workers (1981a) reported also SP immunoreactivity for neurons in the glomerular (and granule cell) area of the frog OB, but without making distinctions between tufted and other neurons.

A moderate SP content was measured in the OB, and SP receptors were found to be highly concentrated "in the external layers of the olfactory bulb" and other brain areas (Quirion et al., 1983).

Burd et al. (1982a) and Davis and Macrides (1983) found the SP-labeled cells exclusively between the EPL and GL of the hamster. These cells were definitely larger than the regular, small periglomerular neurons, and distributed their dendrites within

the intraglomerular neuropil, unlike the superficial SA cells. These observations, together with ultrastructural immunocytochemical studies (Burd et al., 1982a) excluded periglomerular and short-axon cells, and proved the SP-content of the superficial tufted cells, as the single neuron type being immunoreactive in the Syrian hamster OB. In the Chinese hamster, however, similarly labeled cells occurred also in the superficial EPL (Baker, 1986a). SP-like and TH-like immunoreactivity was earlier supposed to coexist in several external tufted cells (Davis and Macrides, 1983), but this could not be demonstrated in a following experiment (Baker, 1986b). According to the degeneration and regeneration experiments of Kream et al. (1984), expression of SP in these superficial neurons depended on the existence of an undamaged connection between the olfactory epithelium and nerve.

In the rat, no SP-containing cell bodies have been reported to date. Bulbofugal SP-containing fibers and terminals were described, however, by several authors and for more species (for review see Macrides and Davis, 1983; Halász and Shepherd, 1983; see also Section 4.3.7.10.).

4.3.3.4. Vasoactive intestinal polypeptide (VIP)

Very few large neurons ($10 \times 15 \mu\text{m}$ in diameter), lying at the border between the EPL and GL and showing immunoreactivity for VIP, arborized within the glomerular neuropil of the rat OB (Gall et al., 1986). Based on light microscopic observations, these cells were identified as superficial tufted cells. Observations on VIP-immunoreactive superficial neuronal (juxtaglomerular) somata of the hedgehog OB were also published (López-Mascaraque et al., 1989).

4.3.4. Periglomerular neurons

These neurons show in part similar neurochemical characteristics to superficially situated tufted cells: samples of both types are able to synthesize dopamine, or may contain the same Ca-binding protein or CCK; this is why careful parallel structural studies, along with histochemistry, are needed for clear recognition. On the other hand, several neuroactive substances, like GABA, methionine-enkephalin and somatostatin were recognized in periglomerular neurons, but not in tufted cells. Electron microscopic approvals for the clearly localized immunoreaction in periglomerular cells were published as yet only for dopamine, GABA and methionine-enkephalin.

4.3.4.1. Dopamine

Biochemical evidence for the presence of a dopaminergic mechanism was summarized in Section 4.3.3.1. (for reviews see Margolis, 1981; Macrides and Davis, 1983; Halász and Shepherd, 1983).

Histofluorescence (induced by formaldehyde or glyoxylic acid) revealed the presence of catecholamine-containing juxtaglomerular (mixed, periglomerular and tufted) neurons (Dahlström et al., 1965; Lichtensteiger, 1966; Ungerstedt, 1971; Lidbrink et al., 1974; Lindvall and Björklund, 1974; Fallon and Moore, 1978*a,b*; Halász et al., 1978). Identification of DA, as the representative catecholamine within these cells, was achieved through immunohistochemistry, by revealing the presence of DA itself (Smeets et al., 1987), or of TH (and DDC) (Hökfelt et al., 1975*a*; Halász et al., 1977*a,b*, 1983, 1985*a,b*; Ljungdahl et al., 1977; Specht et al., 1981; Baker et al., 1983, 1984; Davis and Macrides, 1983; Kream et al., 1984; Mugnaini et al., 1984*a*; Kosaka et al., 1985, 1987*b*; Baker, 1986*a,b*; Rosser et al., 1986; Smeets et al., 1986; Tillet et al., 1987), first on embryonic day 21 in rats (Specht et al., 1981). The DA concentration of the bulb increased further during early postnatal life, e.g., more than doubled between postnatal days 8–12 (Brunjes et al., 1985).

Antibodies to the enzyme TH strongly labeled the somata (Figs 4.47–4.48 and see also Figs 4.41 and 4.43) and processes (see Figs 4.43, 4.44 and 4.50) of juxtaglomerular



Fig. 4.47. Tyrosine hydroxylase immunopositive periglomerular cells (arrows) from the rat olfactory bulb. This picture was taken by photographing the trimmed surface of an Epon block containing an immunostained and osmicated, 50 μ m thick cryostat section. Adult rat, PAP technique. One glomerulus (white asterisk) shows a deeper density than others. Larger and smaller vessels (V) are apparent in all layers; several erythrocytes (remaining, in spite of perfusion, within capillaries) show endogenous peroxidase activity. ONL: olfactory nerve layer; EPL: external plexiform layer. Scale bar: 20 μ m

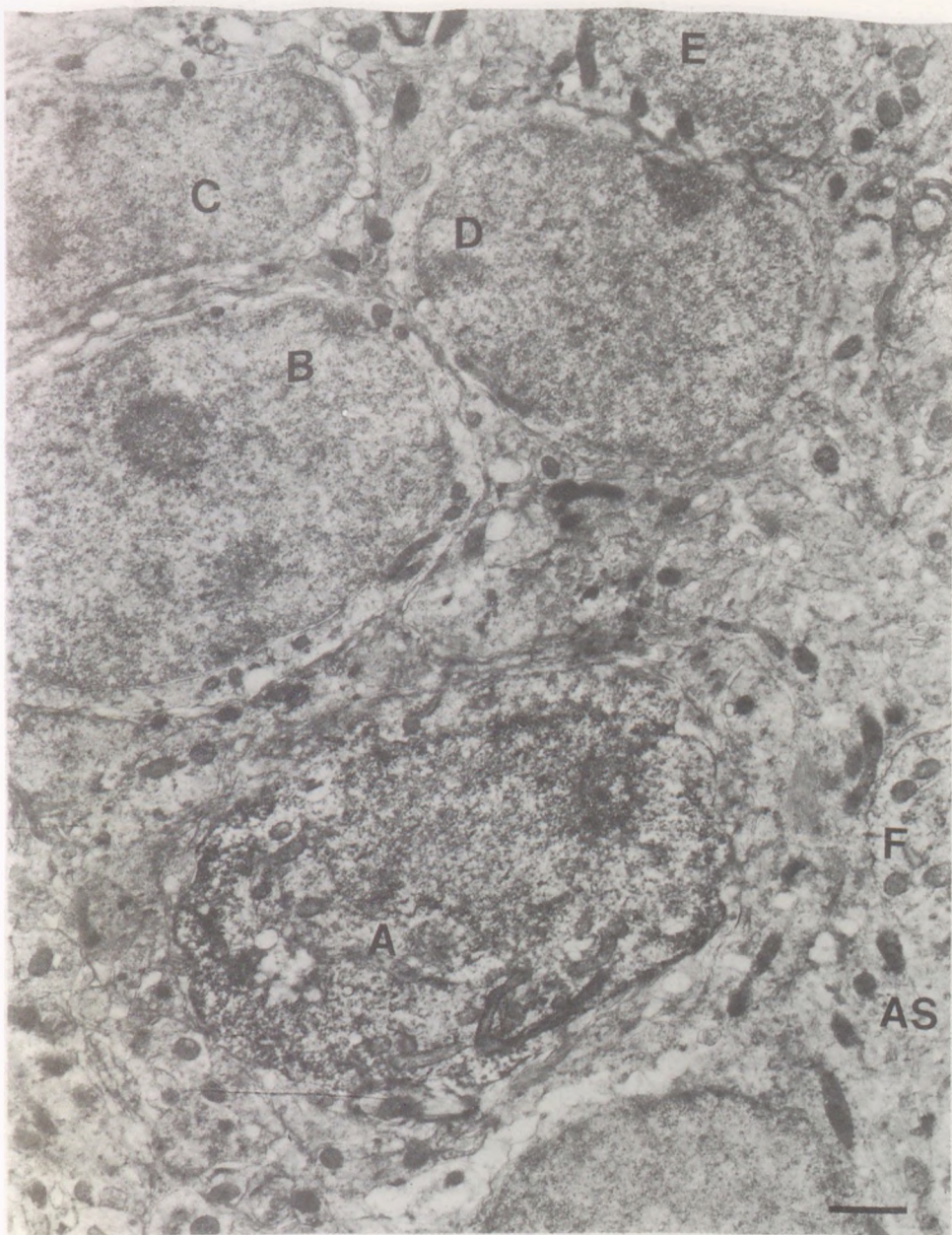


Fig. 4.48. Labeled and unlabeled juxtglomerular cells in the rat olfactory bulb. Tyrosine hydroxylase immunohistochemistry, PAP technique, lead citrate staining. One labeled (A) and details of four unlabeled (B,C,D,E) periglomerular neurons are seen. In addition, part of a second, weakly labeled periglomerular cell (F) and detail of an unlabeled astrocyte (AS) are also shown, while the surrounding neuropil area is composed mainly of unlabeled neural and glial constituents. Scale bar: 1 μ m

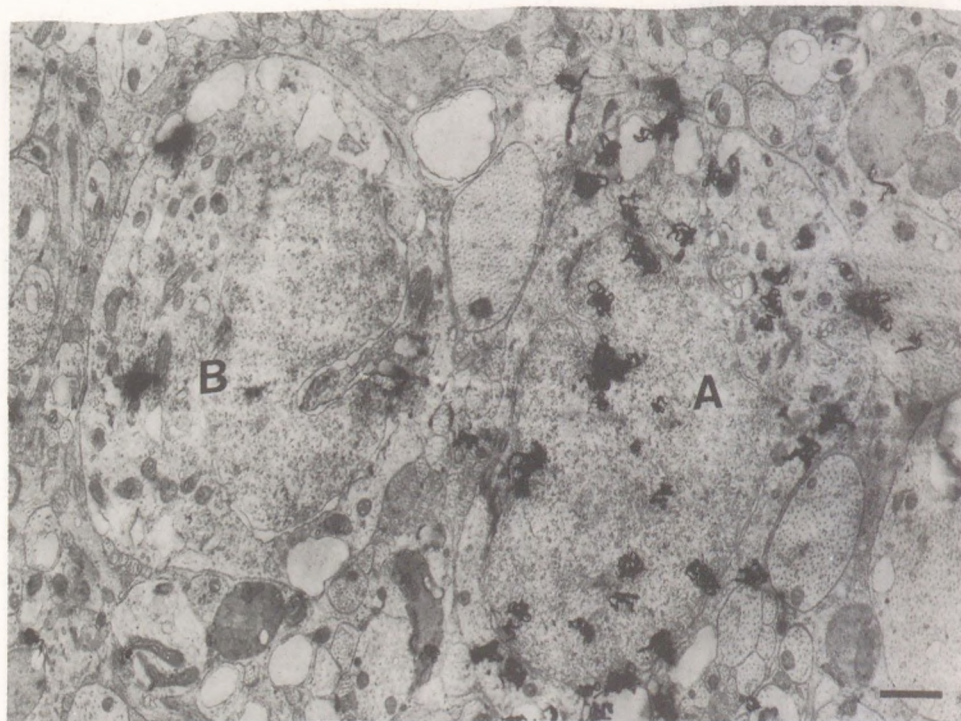


Fig. 4.49. Labeled (A) and unlabeled (B) periglomerular cells of the turtle olfactory bulb after ^3H -dopamine uptake. Electron microscopic autoradiogram. 500 μm thick hemislice, aldehyde-osmium fixation, lead citrate staining, Kodak NTB-2 emulsion, Amidol developer. Large dendrites, olfactory nerve and other axons and glial processes are unlabeled. Scale bar: 1 μm

cells. In the rat, a large contribution of the PG cells to all immunoreactive neurons was found, while in hamster, their ratio was low, when compared to TH-immunoreactive tufted cells. Electron microscopy of the immunolabeled cells in the rat OB also frequently revealed the particular characteristics of the periglomerular cells (see Fig. 4.48). Glomeruli were surrounded by unlabeled neurons and glia, with interspersed labeled periglomerular cells between them. Localization of the reaction product, when using the unlabeled peroxidase-antiperoxidase technique of Sternberger (Sternberger et al., 1970) was cytoplasmic, either in somata or within axon- or dendrite-like periglomerular and intraglomerular processes (Fig. 4.50 and see also Fig. 4.48), but invaded sometimes the nuclear plasma, too. Labeled and unlabeled finer and larger processes, as dendritic shafts, preterminal segments and also pre- and postsynaptic terminal parts of the supposed PG (and tufted) cells were observed (see Figs 4.44 and 4.50).

By electron microscopy of serial sections from the rat OB, both labeled periglomerular and superficial tufted cells were identified and reconstructed (Halász et al., 1981). Diameters of their cell bodies were measured as 6–9 and 10–15 μm , respectively; the more superficial tufted cells were generally smaller.

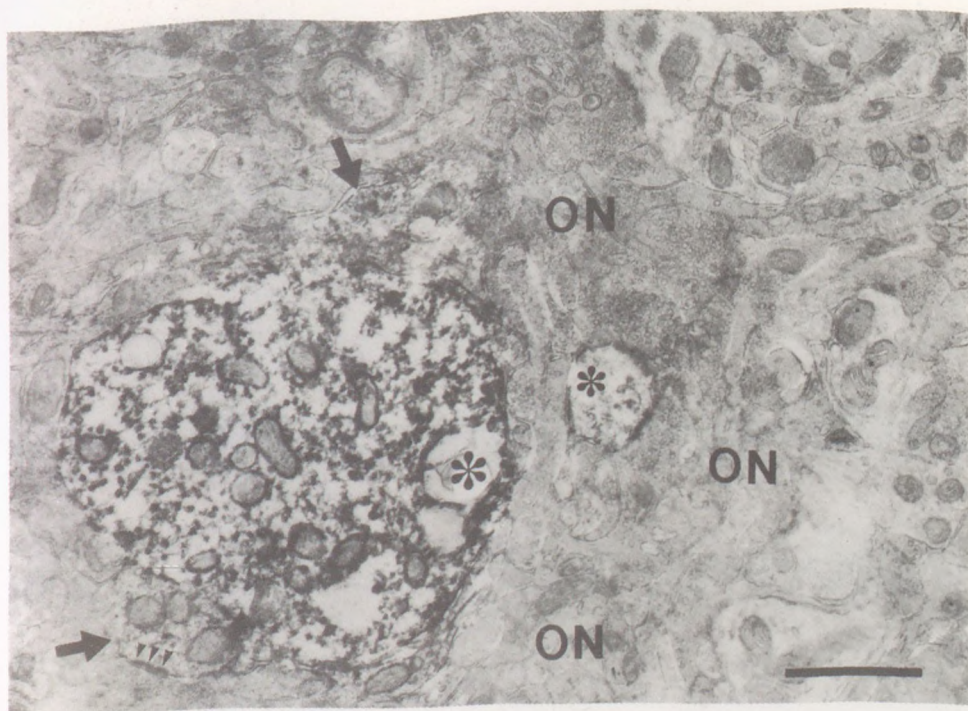


Fig. 4.50. Two labeled, dendrite-like processes (asterisks) in the glomerular neuropil of the rat olfactory bulb. Tyrosine hydroxylase immunohistochemistry, PAP technique, lead citrate counterstaining. A small dendrite (small asterisk) receives clearly recognizable synapses from olfactory nerve axons. Two other, more vesiculated profiles (arrows), apposed tightly to the large labeled dendrite (large asterisk), also show immunopositivity for antibodies to tyrosine hydroxylase; one of them is contacted (arrowheads) by an olfactory nerve axon. Other light dendritic, or glial processes as well as the olfactory nerve axons (ON) show no immunoreactivity. Scale bar: 1 μ m

Besides immunohistochemistry, autoradiography also contributed to the recognition of the dopaminergic PG cells, especially at the electron microscopic level (Halász et al., 1977a, 1978, 1983, 1985a; Priestley et al., 1979). Both labeled and unlabeled PG cells were distinguished after uptake of ^3H -labeled catecholamines by the turtle (Fig. 4.49) or rat (Fig. 4.51) OB. With autoradiography, it was more difficult to recognize the labeled processes than with immunohistochemistry, because of the higher background within the glomeruli and poor resolution. However, specific fiber- or terminal-like uptake was clearly recognized at both the light- (see e.g. Halász et al., 1982) and electron microscopic level.

Catecholamine-containing cells were recognized in the glomerular region of lower vertebrates, too, but without clear distinction as whether they were contained by superficially located output neurons or interneurons. Parent (1975) observed juxtglomerular fluorescent cells (Falck-Hillarp technique) in the frog bulb, but could not reveal catecholaminergic neurons in the sunfish OB (Parent et al., 1978). Yoshida and co-workers (1983b) were, in contrast, successful in visualization of supposed

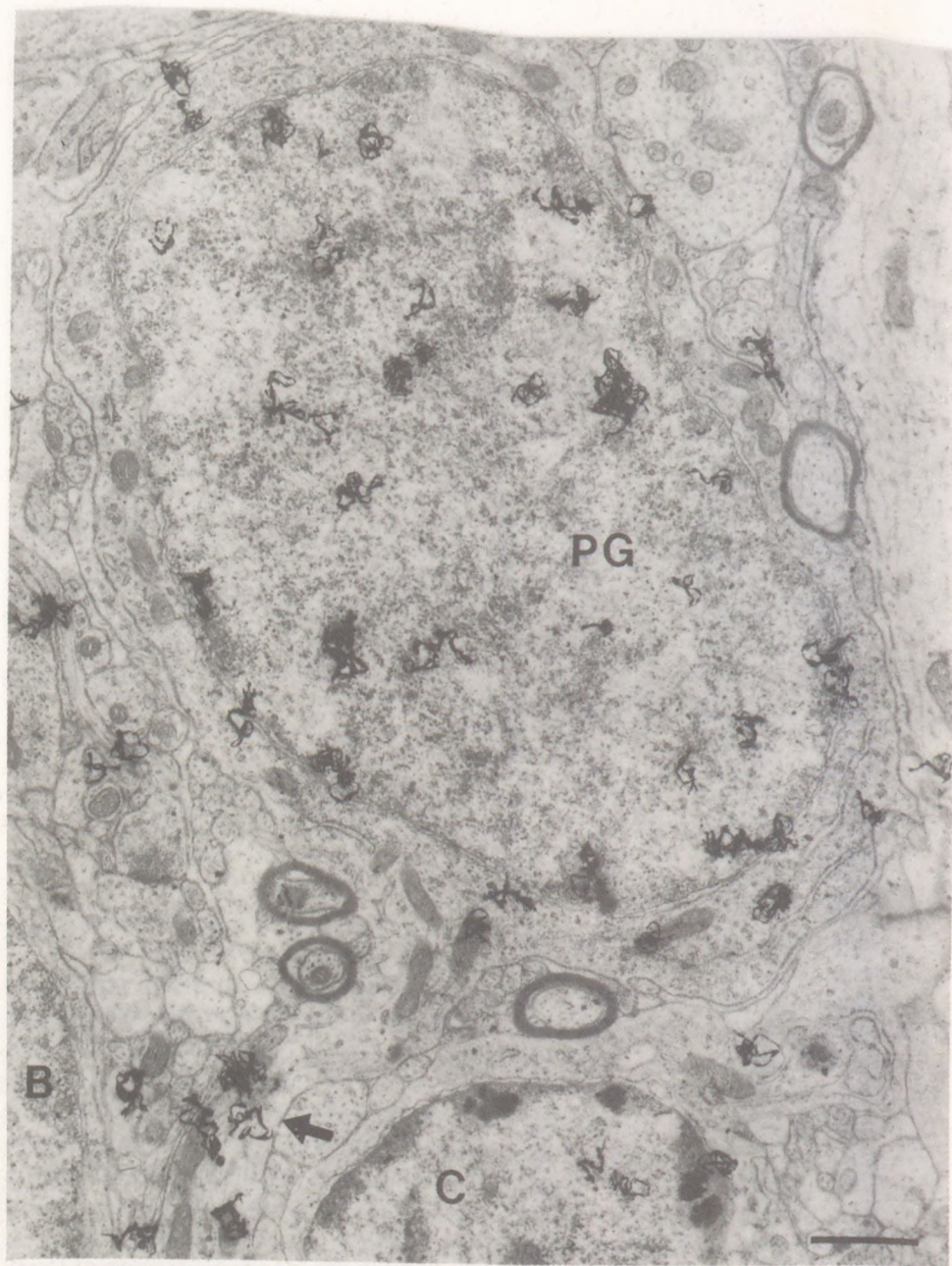


Fig. 4.51. Periglomerular cell (PG) of the rat olfactory bulb: uptake of ^3H -noradrenaline. Electron microscopic autoradiogram. Nialamide pretreatment (500 mg/kg, i.p.), ventricular injection of the label, aldehyde-osmium fixation, uranyl acetate and lead citrate staining, Ilford L4 emulsion, Amidol developer. Silver grains label the nucleus, the perikaryon and also the process emerging from the cell (arrow). Parts of other, unlabeled (B) or weakly labeled (C) periglomerular cells are also visible. Myelinated fibers and other neuronal and glial compartments are unlabeled. Scale bar: 1 μm

dopaminergic cells (TH immunochemistry at the light microscopic level) in the goldfish. In the frog OB, Inagaki and co-workers (1981*b*) also observed the formaldehyde-induced fluorescence of catecholamines in few neurons in the periglomerular region (and GCL), but the majority of fluorescent cells appeared in the mitral cell layer.

4.3.4.2. GABA

Much evidence for the presence of the classical inhibitory transmitter, GABA in the OB, has been reported to date. For reviews of the topic see Quinn and Cagan (1981) and Halász and Shepherd (1983). Also, more details of the biochemistry of bulbar GABA system appear here in Section 4.3.5.1., where granule cell transmitters are discussed.

Although the majority of the GABA in the bulb was assigned to the granule cells and their dendritic terminals in the EPL, the second richest cellular compartment for this

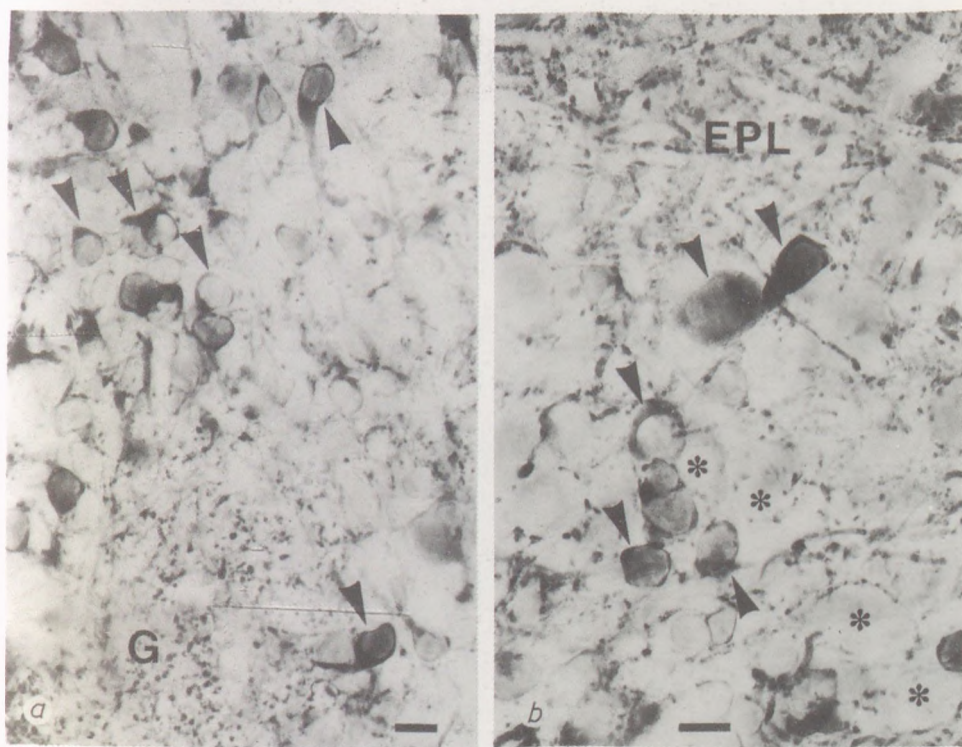


Fig. 4.52. GAD-immunoreactive structures in the superficial layers. PAP procedure performed on vibratome sections; light microscopy. By courtesy of Dr. E. Mugnaini. Many periglomerular neurons reveal stronger or weaker immunopositivity (arrowheads). In addition, numerous immunoreactive fibers and puncta are apparent within the neuropil of the glomerular (G) or external plexiform (EPL) layer. Immunoreactive structures seem to surround non-reactive smaller (periglomerular?) or larger (tufted?) cell somata (asterisks), as well as thick fibers (dendrites) in the EPL. These latter puncta may represent GABA-ergic granule dendritic gemmules. Scale bars: 10 μ m

transmitter might be the periglomerular cells. As for laminar distribution, the peak GABA, glutamic acid decarboxylase (GAD, E.C.4.1.1.15.), γ -aminobutyric acid transaminase (GABA-T, E.C.2.6.1.19.) and ^3H -muscimol binding was found mostly in the EPL, while the glomerular layer had roughly half of these. While the EPL was the richest in GABA_A receptors, the glomerular layer possessed the highest density of GABA_B binding sites in the rat OB (Bowery et al., 1987).

For structural localization at the cellular level, immunohistochemistry and autoradiography furnished most of the data. Nagai et al. (1983) reported intensive staining of the PG cell processes in the glomeruli with a specific histochemical technique, revealing GABA-T. Ribak and co-workers (1977, 1981) were the first to describe GAD activity for PG cells and their processes, as revealed by their antibody, raised in rabbits (Saito et al., 1974). The activity was strong around the glomeruli, and also within. Numerous GAD-positive puncta were observed, representing immunostained terminals. Under the electron microscope, the reaction product was localized within the cytosol. These authors did not find GAD-activity in short-axon, mitral or tufted cells (but did in granule cells; see Section 4.3.5.1.). As regards the

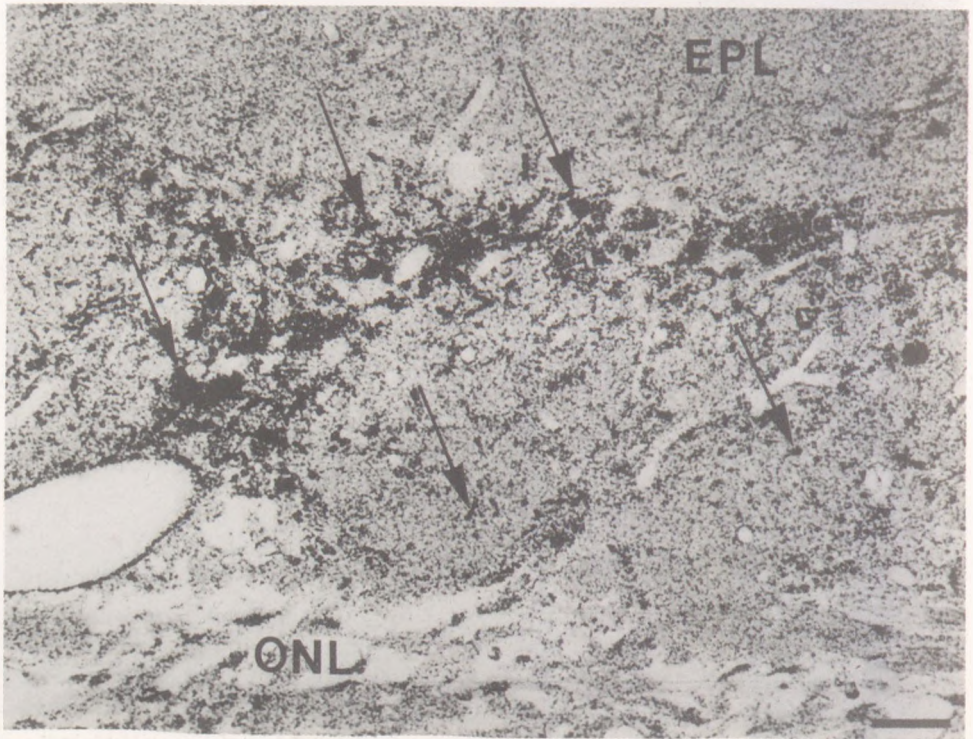


Fig. 4.53. Light microscopic autoradiogram of the uptake of ^3H -GABA by compartments in the glomerular zone of the rat. Aminooxyacetic acid pretreatment (40 mg/kg, i.p.), ventricular injection of the label, Epon embedding, 6 μm thick section, Ilford L4 emulsion, Kodak D19B developer, no counterstaining. Labeled juxtaglomerular cells, peri- and intraglomerular processes (arrows) appear. Schwann cells of the olfactory nerve layer (ONL) also accumulate a considerable amount of the label, while several labeled structures, not identified for this preparation, are also visible in the external plexiform layer (EPL). Scale bar: 20 μm

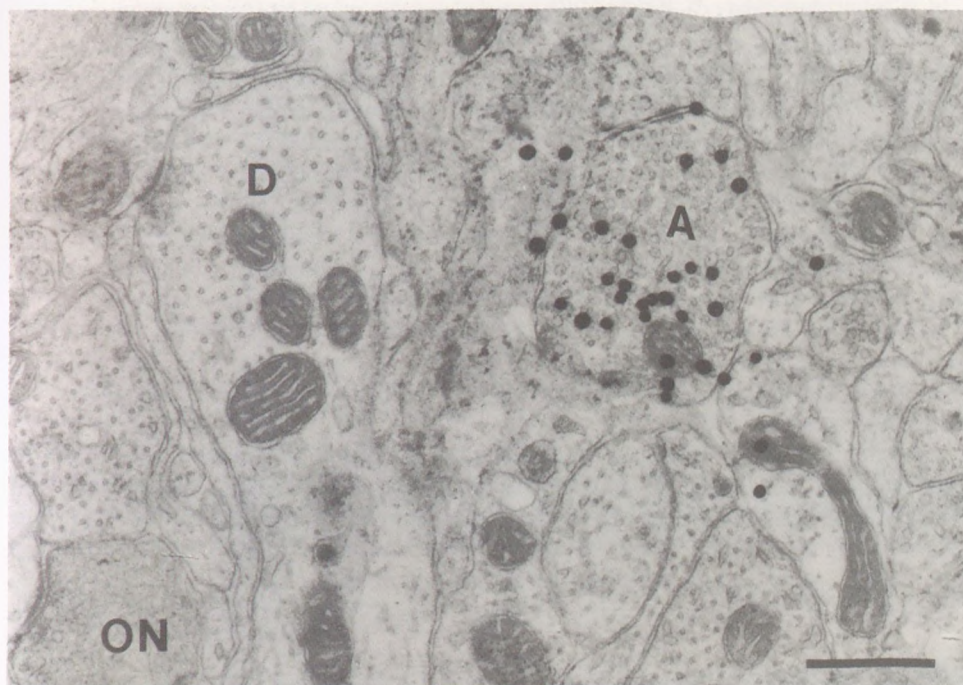


Fig. 4.54. Heavily labeled axon terminal (A) in the rat glomerular neuropil shows accumulation of ^3H -GABA. Electron microscopic autoradiogram. Aminooxyacetic acid (40 mg/kg, i.p.) pretreatment, ventricular injection of the label, aldehyde-osmium fixation, Epon embedding. Ultrathin section, uranyl acetate and lead citrate staining, Ilford L4 emulsion. Phenidone-ascorbic acid developer. Likely output neuron dendritic branches (D), olfactory nerve axons (ON) and other (glial) tissue components were not labeled in this preparation. Scale bar: 0.5 μm

localization of GAD in PG cells and their extensions, Ribak's results were supported by using another (sheep) antibody (Oertel et al., 1981) (Fig. 4.52*a,b*), which revealed GAD immunoreactivity in additional (SA) cells, too (Oertel et al., 1982*a*; Mugnaini et al., 1984*a,b*). These latter authors stated clearly that non-reactive PG cells also existed. They revealed GAD-immunoreactivity and TH-immunostaining in two separate subpopulations of PG cells (Mugnaini et al., 1984*a,b*) with their two-color immunohistochemical technique (Oertel et al., 1982*b*). In contrast, Kosaka and co-workers (1985), by incubating the facing surfaces of serial sections, one with an antibody to TH, and the other with antibodies against GAD, showed the coexistence of TH and GAD immunoreactivity in the same PG (and other) cells. Not all PG cells showed both immunoreactivities; different samples showed an uneven proportion of cells, featuring both antigens, or they had only one of the two; the intensity of staining also varied. A substantial proportion (9.6–55.6%) of labeled cells, however, possessed the synthetic capacity for both DA and GABA (Kosaka et al., 1985).

Immunoreactive juxtglomerular cells were revealed by a monoclonal antibody against GABA-BSA (Liu et al., 1989) supporting further the view of GABA-ergic periglomerular neurons.

Another, indirect line of evidence for GABA-ergic mechanisms in PG cells derived from our autoradiographic studies. Injection of the radiolabeled GABA in low concentration into the olfactory ventricle, the tissue itself, or incubation of slices with labeled GABA resulted in uptake of this amino acid, among others, into cells, processes and terminal-like puncta in the GL (Fig. 4.53) and GCL (see Fig. 4.55). The periglomerular area was strongly labeled, but intraglomerular varicosities were also clearly recognized. Under the electron microscope, the vast majority of labeled cells was found, however, to be glial in origin. Several labeled periglomerular (and superficial SA) cells were, however, recognized, and intraglomerular dendritic islands, surrounded by non-labeled olfactory nerve fibers and terminals, also retained the label (Halász 1976; Halász et al., 1979). Occasionally, strongly labeled, vesiculated terminals, more likely of axonal origin (Fig. 4.54) were also observed. They might be terminals of the GABA-ergic PG or SA cells, or GABA-containing bulbofugal central fibers (see later Section 4.3.7.5.).

All data discussed in this latter section suggest the role of GABA as a transmitter at least in several cells in the GL of the mammalian OB. Observations by Tonosaki and Shibuya on gecko (1979, 1985), however, argue against a similar role for GABA in this species. They were unable to show any effect of GABA, superfused or iontophoresized into the GL, upon odor-evoked excitation of mitral cells.

4.3.4.3. Opioid peptides

RIA measurements revealed a low enkephalin concentration for the OB (Hong et al., 1977), but receptor binding (Hirsch, 1980a; Hirsch and Margolis, 1980; Nadi et al., 1980) and high performance liquid chromatography (Margolis, 1981) studies supported their importance in this brain area. Laminar analysis of the distribution of ^3H -dihydromorphine binding in dog OB (Nadi et al., 1980) showed higher opiate receptor densities in the ON and in the mitral-granule cell layers, and about half of these in the glomerular area and white matter. In contrast, in adult rats, ^3H -naloxone receptors were most numerous in the GL and EPL (Herkenham and Pert, 1982). Density of δ -opiate receptors, as revealed by (^3H)D-Ala-D-Leu-enkephalin (DADLE) (McLean et al., 1986) or a tritiated bis-penicillinamine cyclic enkephalin analog (^3H -DPDPE) (Gulya et al., 1986) binding, was especially high in the EPL. There was, however, a shift in the distribution of opiate receptors from the EPL (as revealed by ^3H -dihydromorphine binding on postnatal day 2) to the GL and GCL (onwards from postnatal day 21) (Unnerstall et al., 1983).

Immunohistochemistry supported the existence of two main enkephalin systems in the OB: one represented by the periglomerular cells, and another (bulbofugal) fiber system localized predominantly in the GCL. In addition, several other scattered sources (granule and deep SA cells) were also described, and shall be characterized at the respective cell- or fiber type.

The majority of periglomerular cells in the hamster OB (Davis et al., 1982) and a significant proportion of the rat PG cells (Bogan et al., 1982; Merchenthaler et al., 1986), but only a few PG neurons in the guinea pig OB (Matsutani et al., 1989) showed immunoreaction with antibodies to leucine- or methionine- enkephalin (Leu-Enk and

Met-Enk, respectively) or to an opioid heptapeptide (ENK-7) (Merchenthaler et al., 1986), forming the carboxyl terminus of pre-proenkephalin. The high proportion of immunoreactive periglomerular cells suggested coexistence of an opioid peptide and GABA or DA (Macrides and Davis, 1983). By electron microscopic immunocytochemistry, the label was observed within somata, dendrites and intraglomerular processes of the periglomerular cells (Burd et al., 1981). This way, enkephalins may serve as a sort of direct effect of periglomerular cells onto the output neurons at their primary dendritic branching.

4.3.4.4. Somatostatin (SOM)

There is an indication in Johansson and co-workers' paper (1984) of the existence of SOM-containing periglomerular neurons. They found single, small (less than 10 μm in diameter) SOM-immunopositive cells in the rat OB, which sometimes released a varicose process into glomeruli. Moderate numbers of the periglomerular neurons in the frog olfactory bulb contained SOM (Inagaki et al., 1981b) and the high number of SOM-positive, varicose fibers in the same layer might originate from these cells.

4.3.4.5. Cholecystokinin

One paper reports the presence of CCK within the majority of small ($6.4 \times 10.8 \mu\text{m}$) cells around the glomeruli of the rat OB (Seroogy et al., 1985). These cells had also reactive processes, arborizing in a glomerulus nearby, or, very rarely, extending into two glomeruli. Although the ratio of these CCK-containing neurons was very low, it cannot be excluded that CCK is present in some GABA, DA or Met-Enk-containing PG cells, which taken together may account for a considerable proportion of the PG neurons.

4.3.4.6. Calcium-binding proteins

Periglomerular cells were reported by Jande et al. (1981) to be the only neurons of the OB which contained a vitamin D-dependent Ca-binding protein (D-CaBP). CaBP-immunostaining resulted in a high proportion of reactive juxtaglomerular cells, as illustrated in light micrographs by Baimbridge and Miller (1982) and Garcia-Segura and co-workers (1984), and especially rich intraglomerular arborization in Baimbridge and Miller's samples. These latter authors were, however, unable to demonstrate any additional cells as CaBP-immunoreactive. In contrast, Garcia-Segura et al. (1984) revealed, with their antibody to 28K-D-CaBP some reactive granule cells and radial immunopositive fibers (granule cell peripheral processes?) in the EPL, too. They found 26% of the PG cells reactive to this label.

By successive incubations of cryostat sections of the rat OB with the anti-28K-D-CaBP antibody and with another, directed against tyrosine hydroxylase (Markey et al., 1980) we reported recently that this CaBP-immunoreactivity and that of TH was

present in different subpopulations of the rat juxtaglomerular cells, including likely periglomerular, superficial and medium tufted cells (Halász et al., 1985b). The CaBP immunoreactive cells were somewhat smaller and more numerous, and had a less extensive intraglomerular arborization (see Fig. 4.46) than those of the TH-immunoreactive cells. This observation supported the proposal of Baimbridge and Miller (1982) for a coexistence of CaBP-immunoreactivity with a classical transmitter, GABA, in the periglomerular cells.

The first 28K-D-CaBP immunoreactive PG cells appeared on day E21 in rats; their number increased significantly during the first two postnatal weeks (Enderlin et al., 1987).

4.3.5. Granule cells

As the most numerous population of neurons in the OB, granule cells seem quite homogeneous in their neuroactive substances. While an overwhelming majority (or all) granule cells may use GABA as their chemical transmitter, small subpopulations in certain species were suggested to contain dopamine, enkephalins, calcium-binding protein and insulin.

4.3.5.1. GABA

Granule cells and their processes may contain the majority of GABA of the main OB, as suggested by numerous pharmacological, histochemical and biochemical studies (for reviews see Quinn and Cagan, 1981; Halász and Shepherd, 1983), although periglomerular cells (see Section 4.3.4.2.) and the central bulbofugal pathways (see Section 4.3.7.5.) also have GABA-ergic components.

GABA binding of the OB synaptosomes was the second highest (3.96 ± 0.36 pmole GABA per mg protein) among various brain areas, next to the thalamic synaptosomes (4.00 ± 0.32 pmole per mg protein) (Balfagón et al., 1975). Since dendrodendritic synapses between granule cells and the output neurons are the most characteristic contacts in the OB, there is a special interest in the synaptosomes, prepared from these endings (Kornguth et al., 1976; Osborne et al., 1976). 78–85% of the GAD activity of the rat bulb were assigned to such synaptosomes (Quinn and Cagan, 1980). Membranes, prepared from these synaptosomes, had the highest specific binding activity for ^3H -GABA (Quinn and Cagan, 1981, 1982) and ^3H -muscimol (Quinn and Cagan, 1982), accounting for 86% (with differential centrifugation) or 96% (by sucrose gradient centrifugation) of the total binding capacity of the homogenate.

Laminar analysis of the OB showed peaks for GABA level, GAD and GABA-T activity and ^3H -muscimol binding over layers, where the granule cell peripheral processes were distributed (i.e., in the EPL and MCL) (see for review Halász and Shepherd, 1983). High affinity, sodium-dependent uptake of the exogenous, labeled GABA was found in the EPL. Release of this amino acid at a high potassium concentration (Bergez et al., 1978; Jaffé and Cuello, 1980b, 1981) or veratridine (Jaffé and Cuello, 1980b, 1981) was largely calcium-dependent.

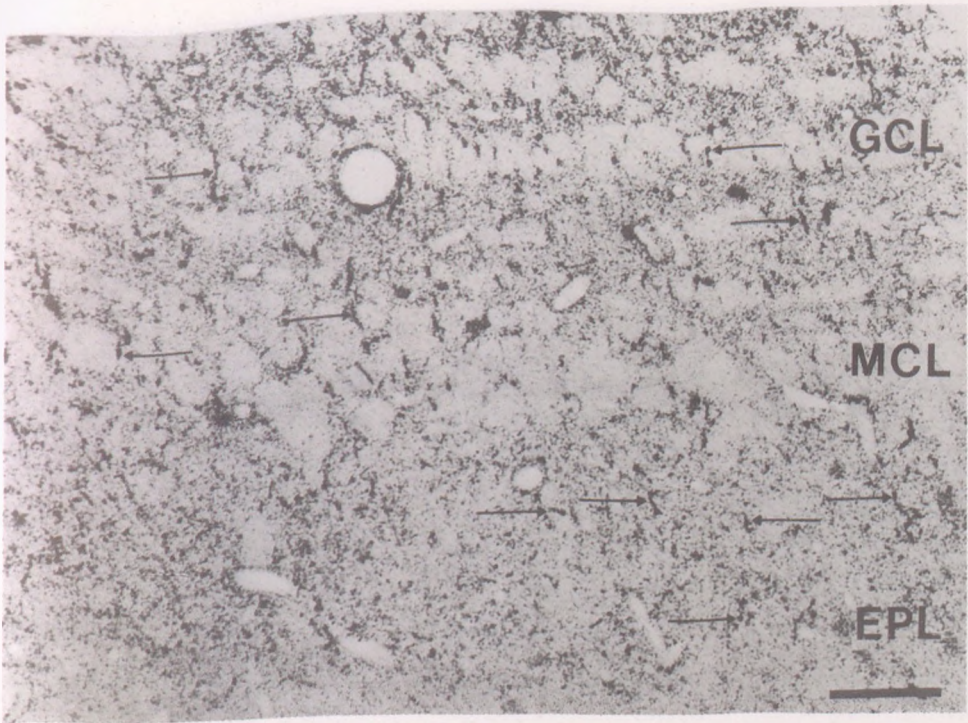


Fig. 4.55. Light microscopic autoradiogram of ^3H -GABA uptake into the rat olfactory bulb. Tissue and sample preparation as described in Fig. 4.53. Mitral and granule cell somata in their respective layers (MCL and GCL) exhibit no or very little activity above background, while fiber- or terminal-like grain accumulations (arrows) are obvious between granule and mitral cells as well as in the deeper aspect of the external plexiform layer (EPL). Scale bar: 50 μm .

Histochemical studies gave strong support for the localization of GABA within granule cells and their processes. GAD-immunohistochemistry at light (Ribak et al., 1977, 1981; Oertel et al., 1982*b*; Mugnaini et al., 1984*a,b*; Kosaka T. et al., 1985, 1987) and electron microscopic levels (Ribak et al., 1977, 1981) revealed the presence of the synthetic enzyme for GABA in very high proportion (nearly all) of the granule cells, their central and peripheral processes and gemmules (see Figs 4.52*b* and 4.56). A decreasing density of cellular immunostaining from superficial to deep GCL was noted by Mugnaini et al. (1984*b*). The reaction was especially strong within granule cell gemmules, synapsing with the dendrites of output neurons in the EPL (Ribak et al., 1977). Light microscopic localization of the GABA-T content revealed the presence of this (GABA-degrading) enzyme also in the granule cells (Nagai et al., 1983). GABA-like immunoreactivity was observed by a monoclonal antibody in many granule cells and radial processes (corresponding very likely to their peripheral processes) in the EPL (Liu et al., 1989).

Another, although less convincing line of evidence was derived from studies of the uptake of radiolabeled, exogenous GABA. This resulted in a high proportion of labeled neurons in a cell culture from 5-day-old rat olfactory bulbs (Currie and

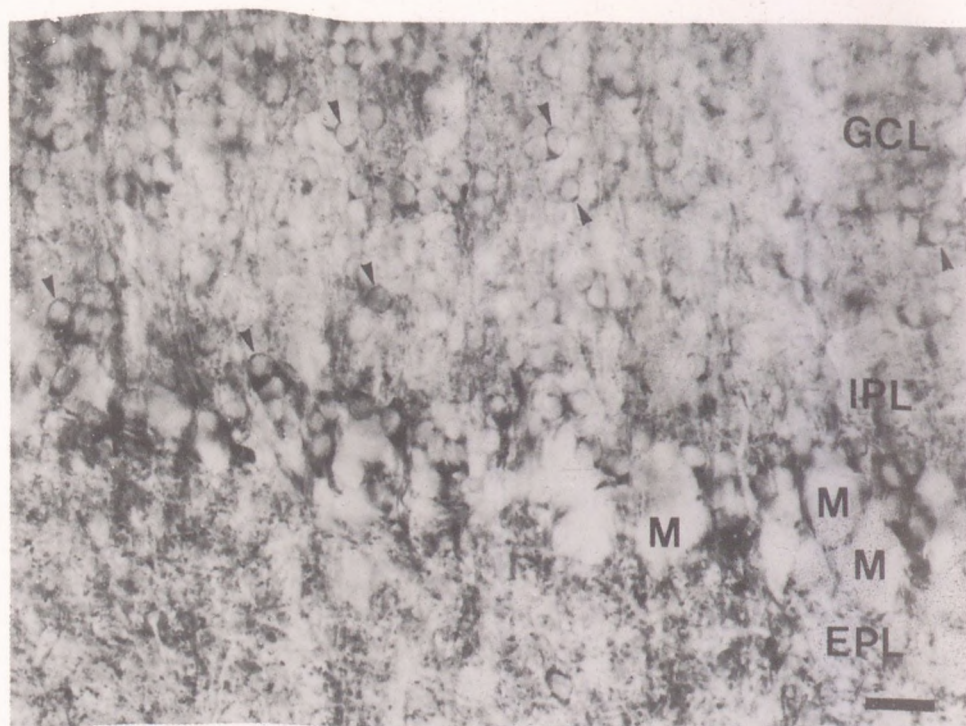


Fig. 4.56. GAD immunoreactivity around the mitral cell zone. PAP procedure on cryostat section. By courtesy of Dr. E. Mugnaini. Mitral cells (M) are void of immunoreaction, while numerous small (granule) cells are labeled (see e.g., at arrowheads) in the mitral, internal plexiform (IPL) and granule cell (GCL) layer. A high density of fine immunoreactive structures, corresponding to the granule dendritic gemmules, are apparent in the deep part of the external plexiform layer (EPL). Scale bar: 20 μ m

Dutton, 1980). Neurons comprised nearly 70% of all cells. Three-quarters of the neurons were labeled with ^3H -GABA; the overwhelming proportion of the labeled cells (96%) had somata with diameters of less than 10 μ m, i.e., they might have been mostly granule and periglomerular neurons. In our autoradiographic studies, the terminal segments of the granule cells, the gemmules, were labeled predominantly (Halász, 1976; Halász et al., 1979, 1981b) in the EPL of the rat main OB. Although we could visualize also labeled processes, most of the label appeared as puncta in the range of varicosity, on light microscopic autoradiographs (Fig. 4.55) referring to the immunolabeled varicosities in Fig. 4.56. A higher density of such label appeared in the inner half of the EPL. Similar fibers and (although less in number) labeled terminals were observed around mitral cells, in the IPL and CL.

Electron microscopic autoradiography revealed an accumulation of silver grains over certain vesicle-containing profiles, glial cells and their processes and close surroundings of capillaries and myelinated fibers. Glial extensions could correspond to the labeled, fiber-like processes, recognized at the light microscope level, while the labeled terminals were in part a subpopulation (about 1/3) of the granule cell gemmules

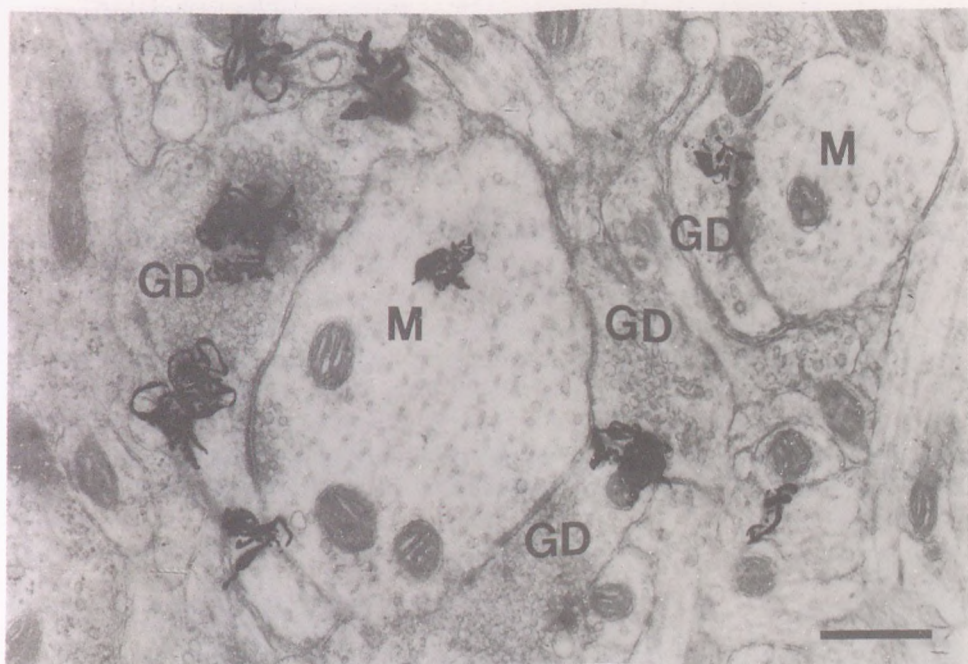


Fig. 4.57. Uptake of ^3H -GABA in the external plexiform layer of the rat olfactory bulb. Electron microscopic autoradiogram. Most silver grains are accumulated over granule cell dendritic gemmules (GD), while the mitral/tufted cell dendrites (M) show weak or no labeling. Scale bar: 0.5 μm

(Halász, 1976; Halász et al., 1979, 1981b) (Fig. 4.57) or were classified into a category of terminals, not identified during these studies. By counting the silver grains over labeled cut surfaces of different tissue components, and relating them to the respective domain, over which they appeared, a 4-fold increase of the exogenous label, as compared to the relative unlabeled mitral or tufted cell dendrites, was revealed (Halász et al., 1979).

By using a computer program for comparison of a hypothetical and the real grain distribution, a more significant accumulation of ^3H -GABA was observed (Halász et al., 1981b). Two-thirds of the likely granule cell gemmules, similar to those of labeled, synapsing or not synapsing, but filled with vesicles, remained unlabeled in these experiments. This uneven uptake might reflect differences in physiological status of GABA-ergic gemmules, or the existence of GABA-ergic and other granule cell terminals, containing a different neuroactive substance. We emphasize again that data obtained through such autoradiographic studies have only a limited degree of specificity, therefore they must be treated with caution.

Exogenous GABA was effective in depressing the mitral or (deep) tufted cell activity through the dendrodendritic synapses (Felix and McLennan, 1971; Nicoll, 1971a; Nowycky et al., 1981a; for review see also Halász and Shepherd, 1983). According to the original experiments, iontophoretically applied GABA depression could be

prevented or blocked by picrotoxin or bicuculline. Perfusion of the bulbar surface with GABA resulted also in a block of the mitral cells, activated by odor stimulation in gecko (Tonosaki and Shibuya, 1979).

4.3.5.2. Dopamine

Weak immunoreactivity for antibodies to TH in likely granule cells, as an indication of dopamine synthesis, was reported only for the turtle olfactory bulb by us (Halász et al., 1982, 1985a) so far. The labeled cells had regularly oval or round somata and one or two, peripherally directed, strongly fluorescent processes (Fig. 4.58). Most of the somata were distributed in the IPL and superficial one third of the GCL, while the labeled processes could be followed across the layer of mitral cell somata, then, after travelling a short distance (radially or obliquely), disappeared in the deep half of the EPL. Since only a fluorescent microscope study was performed, however, the possibility that the TH-immunopositive cells were, in fact, deep short-axon cells, could not be excluded.

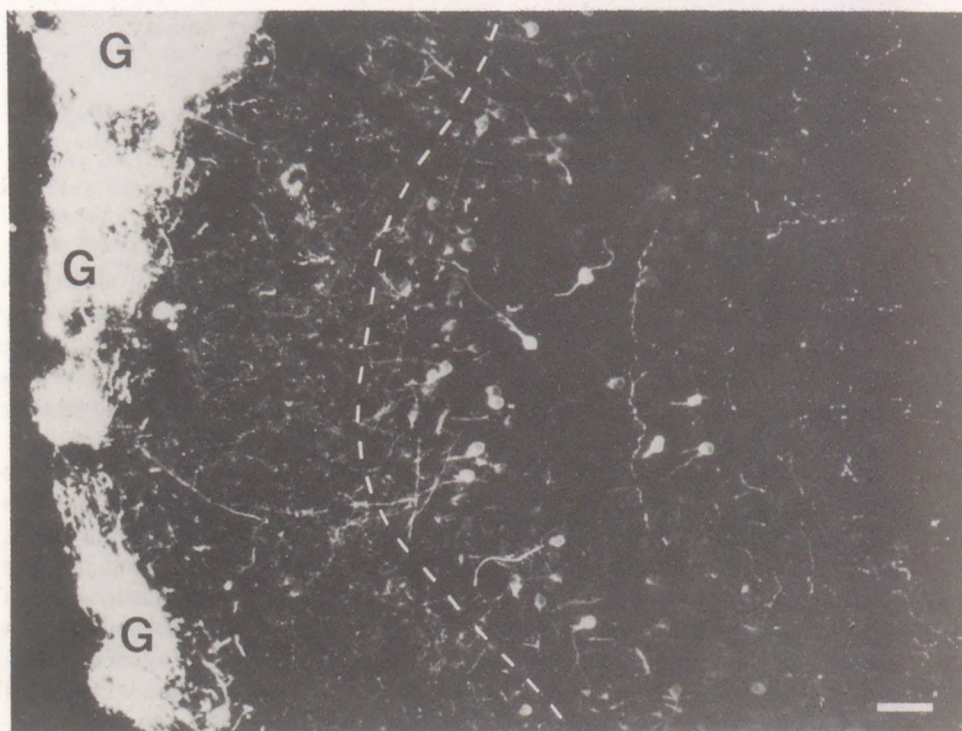


Fig. 4.58. Tyrosine hydroxylase immunoreactivity in the turtle olfactory bulb. Indirect immunofluorescence. Beside the very intense fluorescence of the glomeruli (G) weakly fluorescent cells and processes, especially deep to mitral cell layer (broken line), are apparent. Most of the weakly labeled cells send radial fluorescent processes towards the periphery. Scale bar: 50 μ m

4.3.5.3. Opioid peptides

Granule cells were visualized by antibodies to Met-Enk, Leu-Enk or to an enkephaline-heptapeptide, ENK-7 in the rat, hamster and guinea pig OB (Bogan et al., 1982; Davis et al., 1982; Merchenthaler et al., 1986; Matsutani et al., 1989). Although these labeled cells were numerous, they accounted for only a small proportion of the granule cells. In the studies of Bogan et al., granule cells were, however, the most frequently occurring neurons, reactive with the immunolabel. Processes of these labeled cells extended radially towards the MCL and EPL and arborized there.

4.3.5.4. Calcium-binding proteins

The antibody, raised against the chick 28K-vitamin D-dependent calcium binding protein (28K-D-CaBP) by Norman and co-workers (see Garcia-Segura et al., 1984) resulted in, besides staining of a considerable population of juxtaglomerular neurons, immunolabeling in several cells throughout the GCL and IPL (Garcia-Segura et al., 1984; Halász et al., 1985*b*). These cells, based on localization, size and distribution pattern of their labeled processes, as observed by light microscopy, were tentatively identified as granule cells. Varicosities of labeled, radial processes in the EPL (less in the GCL) were also visualized by Garcia-Segura et al. (1984).

4.3.5.5. Insulin

It is most likely that the small, tightly packed cells, which were slightly elongated and arranged into lamellae rather than circularly on a bent surface, and featuring immunoreactivity to insulin antibody (Havrankova et al., 1978*b*) were granule cells (the other, less likely possibility would be their identification as periglomerular cells). Although the highest insulin content and receptor density were found within the OB and hypothalamus of rats (Havrankova et al., 1978*a,b*; Sakamoto et al., 1980; Zahniser et al., 1984), the role of insulin in the olfactory system is not yet clear. Within the bulb, the highest density of insulin receptors was found in the EPL (Hill et al., 1986). Insulin may play a neuromodulatory role at the dendrodendritic synapses, since the dendrodendritic synaptosomes, prepared from the porcine OB, possessed 40–60% of the specific insulin binding sites, while the other (axonterminal-related) synaptosomes accounted only for 10–20% of binding (Rhoads, 1985). In addition, porcine insulin stimulated the uptake of GABA and Asp into dendrodendritic synaptosomes in these experiments.

4.3.6. Short-axon cells

SA cells are the most heterogeneous and still insufficiently characterized cell population of the vertebrate olfactory bulb. Their distribution extends from the glomerular level to the deep granule cell zone (see Section 4.2.2.2.). Although their

distribution is wide, they are relatively low in number and the somata are dispersed, so, occurrence of examples of one characteristic type on a usual microscopical section is low. Consequently, the recognition of a histochemical label for these cells is also more handicapped than, e.g., the regularly arranged mitral or granule cells. In spite of these difficulties, there is growing evidence for the existence of several neuroactive substances in certain well-recognized and defined subtypes of SA cells. In other cases, indirect evidence led investigators (by excluding all other neuron types in a given area) to point to SA cells as the characteristic cellular compartment for a neurotransmitter or modulator. The following neuroactive substances have been suggested to be contained by SA cells to date: dopamine, GABA, opioid peptides, somatostatin, vasoactive intestinal polypeptide, neuropeptide Y and cholecystokinin.

4.3.6.1. Dopamine

Superficial SA cells were never reported to be able to synthesize or take up catecholamines. Interneurons in the hamster EPL and deeper layers, as Van Gehuchten cells, Golgi and Cajal cells and horizontal cells (Baker et al., 1983; Davis and Macrides, 1983; Macrides and Davis, 1983; Kream et al., 1984) were, however, definitely recognized to express immunoreactivity for TH. Single, scattered TH-immunopositive cells, not resembling the granule or mitral cells, were reported also from the MCL, IPL and GCL of the sheep (Tillet et al., 1987) and rat (Halász et al., 1977b) OB; such cells were observed also in our turtle material (Halász et al., 1982; see also Fig. 4.58). Single cells, definitely larger than the granule cells were also labeled with ^3H -noradrenaline during our autoradiographic experiments on rats (Halász et al., 1978).

4.3.6.2. GABA

Suggestions for the existence of GABA-ergic mechanisms have been made for different types of SA cells, including superficial, medium (in the EPL) and deep SA neurons. GAD reaction product was not revealed in these cells by Ribak and collaborators (Ribak et al., 1977, 1981), but with the sheep-GAD antibody of Oertel et al. (1981) small and medium-sized neurons (other than the output neurons) were detected in the EPL, IPL and GCL (Oertel et al., 1982a; Mugnaini et al., 1984a,b). These authors suggested GABA-ergic functions for all deep SA cell types as well as for superficial SA cells (Mugnaini et al., 1984b). GABA-ergic immunoreactivity in SA cells was observed also by Gall et al. (1985) in the EPL of rat OB.

Light- and electron microscopic autoradiography of ^3H -GABA uptake in the rat OB revealed some large labeled neurons both in superficial and deep layers (Halász, 1976; Halász et al., 1979). Based on fine structural studies, as seen in single fine sections, larger (10–15 μm in diameter) SA cell somata took up and accumulated ^3H -GABA above background level (Fig. 4.59). In the GCL, several terminals, synapsing onto the granule cell somata or dendrites at symmetrical thickenings, and containing small vesicles, were selectively labeled (Fig. 4.60). With regard to the observation that

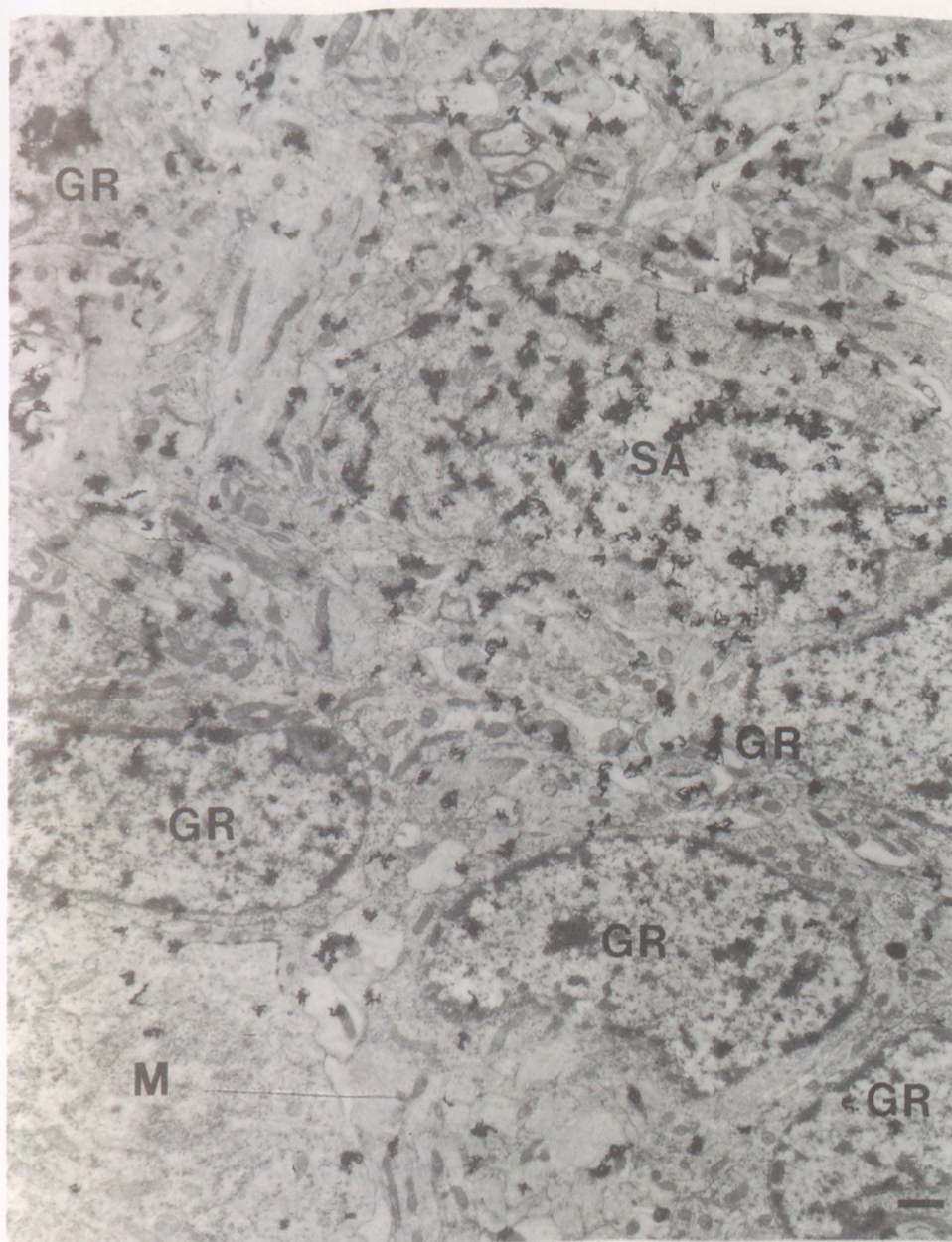


Fig. 4.59. Labeled deep short-axon cell (SA) after ^3H -GABA uptake. Electron microscopic autoradiogram. Tissue and sample preparation as described in Fig. 4.54. Amidol developer. Granule cells (GR) and especially the detail of a mitral cell (M), included in the picture, retained relatively little ^3H -GABA, while the surrounding neuropil, rich in "watery" astrocytic processes and vesiculated terminals, constitutes a somewhat higher "background" label around the labeled short-axon cell. Scale bar: 1 μm

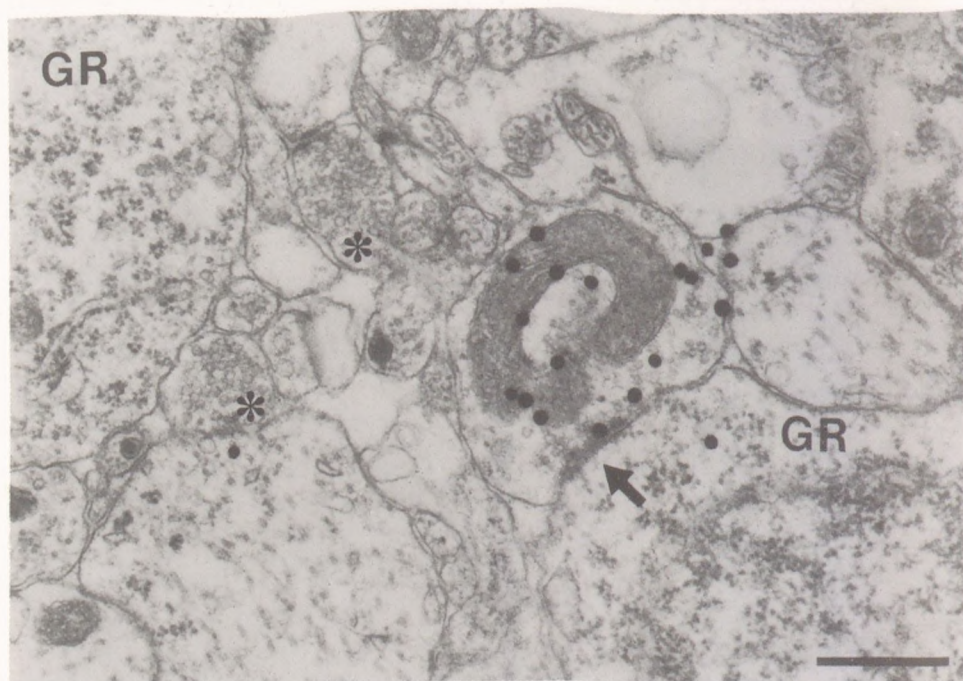


Fig. 4.60. ^3H -GABA uptake of an axon terminal (of probable short-axon cell origin) in the granule cell layer of the rat olfactory bulb. Electron microscopic autoradiogram. Tissue and sample preparation for exposure as described for Fig. 4.54. Phenidone-ascorbic acid developer. The labeled fiber synapses with a granule cell (GR) soma at symmetric membrane thickenings (arrow) and contains relatively small synaptic vesicles, when compared to the vesicles within two other, unlabeled profiles (asterisks). Detail of another unlabeled granule cell perikaryon is also included in the picture. Scale bar: 0.5 μm

centrifugal fibers and output neuron collaterals synapse at asymmetrical thickenings and contain large, spherical vesicles (processes of granule cells contain even larger, flattened synaptic vesicles), the aforementioned terminals were indirectly identified as belonging to a sort of deep SA cells.

4.3.6.3. Opioid peptides

Several types of SA cells in the GCL, MCL, IPL and EPL of the hamster and guinea pig OB were labeled by antibodies against enkephalins (Davis et al., 1982; Matsutani et al., 1989); their processes were also visualized under the light microscope and warrant more certain identification. The rat main OB contained also immunoreactive SA cells, labeled with antibodies to Leu-Enk and Met-Enk (Bogan et al., 1982). The processes of these cells travelled perpendicularly to the granule cell dendrites; the labeled cells were identified as horizontal cells and other SA cells. Fusiform cells of the GCL of the rat OB, continuing usually in a short, surface-directed process and being labeled for Met-Enk and enkephalin-7, were also mentioned (Merchenthaler et al., 1986), but were not characterized in detail.

4.3.6.4. Somatostatin

The hamster OB SOM-immunoreactive cells were found exclusively in the GCL (they were identified to be Blanes and/or large Golgi cells); they were more numerous in the deeper part of the layer, and had considerably larger cell somata than those of the enkephalin-containing neurons (Davis et al., 1982). In contrast, both superficial and deep SA cells of the rat OB displayed SOM-immunoreactivity (Scott et al., 1987). In the rat, the SOM-positive neurons appeared around the time of birth in the deep OB (Shiosaka et al., 1982). The frog olfactory bulb possessed also several deep SOM-positive neurons, although they were not claimed to be SA cells (Inagaki et al., 1981b).

4.3.6.5. Vasoactive intestinal polypeptide (VIP)

Although Lorén et al. (1979) were not able to show VIP-immunoreactive cells in the main OB at all, Gall and co-workers (1986) found a very remarkable array of small ($5-9 \times 8-11 \mu\text{m}$) labeled cells in the EPL of the rat OB. These cells had multiple branching dendritic arbors, confined to the same layer (preferentially in the deep two-thirds). Similarly, VIP-immunoreactive small neurons were observed by them in the GCL, while others were seen infrequently in the MCL. Larger ($10-15 \mu\text{m}$) VIP-immunoreactive neurons were also reported; they lay on the EPL/GL border and arborized in most cases in the periglomerular region. Based on these observations, the largest group of VIP-immunostained neurons were thought to be Van Gehuchten cells, while others would be deep or superficial SA cells, with a possible contribution from the superficial tufted cells (see Section 4.3.3.4.). Various VIP-immunoreactive short-axon cells were reported also from the hedgehog OB (López-Mascaraque et al., 1989).

4.3.6.6. Neuropeptide Y

NPY-containing large ($8-16 \times 14-28 \mu\text{m}$), multipolar neurons in the deep GCL (or periventricular area) of the rat OB were found by Gall et al. (1986) and characterized for the distribution of their processes under the light microscope. These neurons, together with very rare, $11 \times 17 \mu\text{m}$ cells in the EPL/GL border area were identified as SA cells. The latter neurons kept their processes entirely out of the glomeruli. These immunoreactive cells at the borderline of the EPL and GL, as well as the NPY immunoreactivity of the deep multipolar neurons were also characterized by Scott et al. (1985b, 1987), and by de Quidt and Emson (1986). A population of NPY-positive juxtglomerular cells, although not identified for the neuron type, was also reported by Everitt et al. (1984) and Woodhams et al. (1985). The "small cells in the periglomerular layer" (together with occasionally occurring cells in the GCL) expressed their NPY immunoreactivity very early, at embryonic days 15-16 (Woodhams et al., 1985); later, in the postnatal bulb, NPY staining was only occasionally seen in these samples, while de Quidt and Emson identified their deep NPY positive neurons as Blanes cells. In addition to the cellular immunoreaction, numerous reactive fiber- and terminal-like structures were also observed. Since all NPY-positive cells were identified tentatively

as a sort of short-axon cell, the low number of immunoreactive fibers in the LOT was suggested to represent a weak central supply to this system. A high density of NPY fibers was recognized in the bulbar component of the anterior commissure (de Quidt and Emson, 1986).

4.3.6.7. Cholecystokinin

CCK-containing SA cells were seen very rarely, but they were recognized definitely by Seroogy et al. (1985) in the rat main OB. The superficial immunostained SA cells they described arborized exclusively in the periglomerular area, while the deep SA cell examples (in the IPL) were identified as neurons, similar to the vertical cells of Cajal in the hamster OB (see Section 4.2.2.2.3.). Their immunoreactive, fine, varicose processes branched within the GCL.

4.3.7. Centrifugal afferents

An abundance of neuroactive substances are produced within intrinsic elements of the main OB (see Sections 4.3.2. through 4.3.6.). Supply of various neurohormones and transmitters from the retrobulbar regions (although topochemically not so well characterized as yet) is surprisingly rich. Histochemical and neurochemical characteristics of these pathways will be discussed here according to their suggested neuroactive substances in the following order: acetylcholine, noradrenaline, dopamine, serotonin, GABA, aspartate, luteinizing hormone-releasing hormone, opioid peptides, somatostatin, substance P, vasopressin and neuropeptide Y.

4.3.7.1. Acetylcholine

Till now, no direct evidence for an intrinsic cholinergic system of the OB has been reported; consequently, all cholinergic characteristics of the OB can be interpreted as depending on the extrabulbar input. Cholinergic bulbar afferents were reviewed by several authors (see Fibiger, 1982; Halász and Shepherd, 1983; Macrides and Davis, 1983; Wainer et al., 1984); here only a short summary of selected data will be given.

Cholinergic fibers are thought to terminate in all layers of the OB, except the ON and the intraglomerular neuropil (for the latter findings see Kan et al., 1979; Chao et al., 1982). Laminar analysis of the rat and mouse OB showed a relatively uniform distribution of choline acetyltransferase (ChAT, E.C.2.3.1.6.) (Godfrey et al., 1979; Jaffé and Cuello, 1980a). Binding studies for nicotinic (experiments with labeled α -bungarotoxin: Hunt and Schmidt, 1977, 1978; Arimatsu et al., 1981) and muscarinic (^3H -quinuclidinyl benzilate binding in dog OB: Nadi et al., 1980; ^3H -propylbenzilylcholine mustard binding: Rotter et al., 1979a) receptors revealed a relatively even distribution of receptor surfaces in different layers of the OB, with a somewhat higher number of receptors over the layers from the mitral to glomerular zone.

Light microscopical visualization of the acetylcholinesterase (AChE, E.C.3.1.1.7) revealed a high density of stained fibers in the GL and IPL. In addition, dense bundles of reactive fibers and patches of reaction were found on the dorsal and lateral surface of the posterior OB in the rat (Zheng et al., 1987). Stained fibers surrounded and entered certain glomeruli, having "atypical" architecture (Zheng and Jourdan, 1988) in the dorsomedial and ventrolateral OB. Acetylcholinesterase histochemistry at the electron microscopic level showed the highest concentration of label around fine fibers in the IPL and periglomerular region (Carson and Burd, 1980), or within dendritic structures (Zheng et al., 1987). Immunolabeling with antibodies against ChAT revealed a high density of fibers terminating in the GL (Kimura et al., 1981; cat experiments), in the periglomerular area and also in the IPL and GCL (of the dog OB) (Chao et al., 1982). In the rat OB, two cholinceptive groups of neuronal somata were revealed by Nickell and Shipley (1988): the deeper group was situated "just deep to the mitral cell layer", while those of the more superficial cells were localized in the superficial EPL and in the periglomerular region.

The specific AChE activity remained at the level of the newborn until postnatal day 10, then, with a five-fold increase during the following two weeks, reached the adult level (Meisami and Firoozi, 1985). The majority of muscarinic receptors was already available in the newborn rat (Rotter et al., 1979b). During postnatal development, a change in the OB muscarinic receptor forms was suggested by Large et al. (1986b), but a parallel, coordinated development of the (postsynaptic) receptor concentration and (presynaptic) ChAT activity was apparent between birth and the end of the first month. Based on ChAT and AChE activity measurements and autoradiography of muscarinic cholinergic receptors in different layers, a certain reorganization of the cholinergic synapses, during relatively early postnatal days (days 20–35 as opposed to days 10–20) in the rat OB was suggested (Rea and Nurnberger, 1986).

The origin of cholinergic fibers was traced first in AChE histochemical studies (Lewis and Shute, 1967; Shute and Lewis, 1967). The discovery of the reticular and limbic cholinergic systems was confirmed by Wenk et al. (1977). Until recently, many studies corroborated the view of the diagonal band area (especially the nucleus of the horizontal limb of the diagonal band of Broca) as the main source for the cholinergic afferent fibers to the rodent OB (Palkovits et al., 1974; Macrides et al., 1981; Macrides and Davis, 1983; Mesulam et al., 1983a,b; Carson, 1984b; Wainer et al., 1984; Brashear et al., 1986; Ichikawa and Hirata, 1986; Záborszky et al., 1986; see Table 4.2).

In spite of the high number of unequivocal data for the origin of the bulbar cholinergic systems outside the OB, as has been summarized above, several related, controversial findings could not be understood and interpreted on the basis of our present knowledge. Although a peripheral contribution of cholinergic fibers seems very unlikely (Margolis et al., 1974), a substantial muscarinic acetylcholine receptor binding was observed in the dog olfactory nerve layer (Nadi et al., 1980) and such receptors were observed also on the receptor cells of the salamander olfactory epithelium, with a concomitant presence of AChE and ChAT in the mucosa (Hedlund and Shepherd, 1983). There are still no explanations for the presence of nicotinic cholinergic receptors on the periglomerular dendritic terminals at the sites where they are postsynaptic to mitral dendrites (as shown with quantitative electron microscopic autoradiography by Hunt and Schmidt, 1977, 1978). Even the completely isolated,

developing rat OB was able to increase the specific activity of its AChE by more than one order of magnitude between birth and postnatal day 30 (intrinsic cholinergic sources?), while the centrally deafferented OBs did even better ($25\times$ increase; peripheral cholinergic input?) and reached one half of the increase rate of the normal bulb (Meisami and Firoozi, 1985).

In summary, there are still uncertainties in the interpretations of several experiments, related to the origin and role of the cholinergic fibers in the main OB, as might be inferred from the experiments above. Similarly, testing and uncovering of the physiological action of ACh onto different postsynaptic sites in various layers of the OB invites further studies.

4.3.7.2. Noradrenaline (NA)

Catecholamine content of central fibers to the main OB was recognized earlier than ACh. In fact, this was the first bulbofugal fiber system, in which the transmitter content was recognized (Dahlström et al., 1965).

NA content of the OB was shown to originate from central sources. Lesions placed upon the ascending NA pathways of the rat (Edwards et al., 1977) and hamster (Macrides et al., 1981) clarified that the OB does not contribute at all to the telencephalic NA content and, in contrast, all bulbar noradrenaline is dependent on the central supply. The source of these central fibers was found in the locus coeruleus (Dahlström et al., 1965; Lindvall and Björklund, 1974; Broadwell and Jacobowitz, 1976; Fallon and Moore, 1978a; Shipley et al., 1985). According to the WGA-HRP transport study of Shipley and co-workers (1985) at least 40% of the locus coeruleus cells projected onto the bulb.

The noradrenaline content of the OB was found to be comparably low, with only weak accumulation in the EPL, MCL, IPL and GCL (for review see Halász and Shepherd, 1983), and so, did not reflect clearly the distribution of NA containing fibers and terminals, as revealed by histochemistry. Similarly, the highest density of the α_1 adrenergic receptors in the EPL (Jones et al., 1985) could not be correlated to the histochemical findings. Potentiation of the adenylate cyclase activation by α -adrenergic receptors in the OB was the highest in the brain (Stone and Herrera, 1986).

Histofluorescence studies with the Falck-Hillarp technique or glyoxylic acid condensation method revealed the highest density of catecholaminergic fibers in the GCL (Dahlström et al., 1965; Lindvall and Björklund, 1974; Broadwell and Jacobowitz, 1976; Fallon and Moore, 1978a; Halász et al., 1978) of rodents, but such fibers were found (although in lesser density) also in the outer layers. In the frog and in fish, the catecholamine fibers preferred the more superficial layers (Parent, 1975; Parent et al., 1978). Immunohistochemical localization of the sites for dopamine- β -hydroxylase (DBH, E.C. 1.14.17.1.) furnished further support for these localizations, through experiments on rats (Swanson and Hartman, 1975; Halász et al., 1977b), turtles (Halász et al., 1982) and mice (Rosser et al., 1986). The highest concentration of DBH-immunoreactive fibers was observed in the IPL and most superficial GCL, while a moderate density of NA-fibers was also visualized in the whole extent of the GCL (Fig.4.61). Sparse labeled fluorescent fibers were also recognized in the EPL and, very

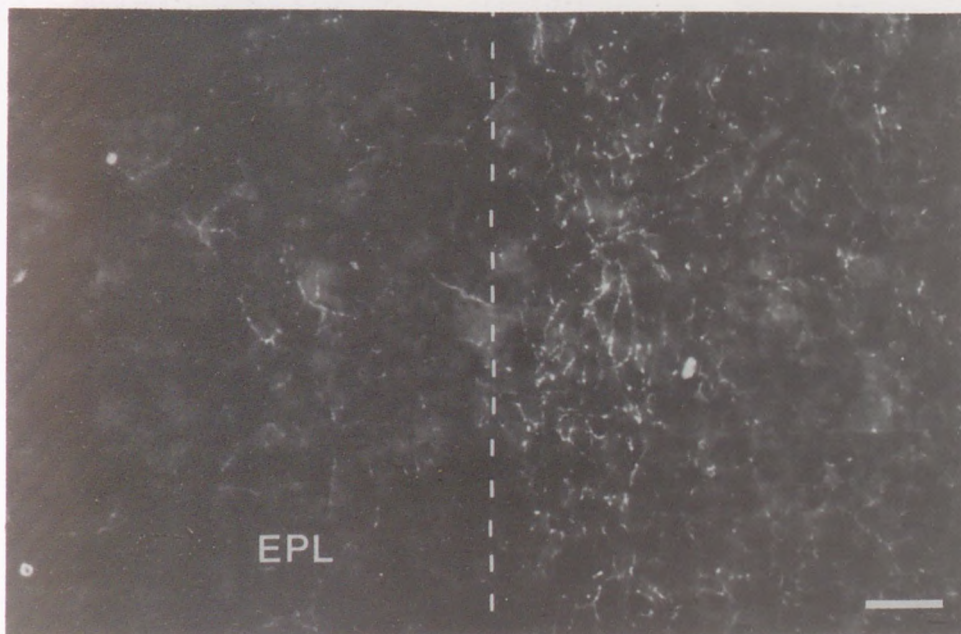


Fig. 4.61. Varicose fibers and terminals, possessing the enzyme dopamine- β -hydroxylase. Indirect immunofluorescence. Adult rat, 14 μ m thick cryostat section. Most of the labeled structures appear beneath the level of the mitral cells (broken line), in the internal plexiform and granule cell layers, while only sparse immunopositivity can be observed in the external plexiform layer (EPL). Scale bar: 20 μ m

occasionally, in the GL. In addition to these localizations, Shipley et al. (1985), however, revealed dense labeling confined to intraglomerular fibers and terminals, after injecting WGA-HRP into LC of the rats.

Predominance of labeled fibers within the deep layers was demonstrated by us after ^3H -NA uptake in rat (Halász et al., 1978), and ^3H -DA (Halász et al., 1983, 1985a) uptake in turtle OB. With autoradiography on an electron microscopic level, small labeled terminals were observed (Fig. 4.62). Such labeled terminals were never observed to synapse directly onto output neuronal surfaces. Instead, deeply located dendritic profiles (of granule and/or short-axon cells) might have been their postsynaptic targets.

Noradrenaline was shown to be released to peripheral (odor) (Chase and Kopin, 1968) or central (antidromic, electrical) (Brennells, 1974) stimulation. The released NA, according to its preferential localization in fibers and varicosities within the IPL and GCL, and distant termination from output neuronal postsynaptic surface, was suggested to act *via* excitation of granule cells, causing them to release GABA (Felix and McLennan, 1971; McLennan, 1971). This assumption was supported by revealing a suppression on mitral cell activity upon iontophoresed NA (Baumgarten et al., 1963; Bloom et al., 1964; Salmoiraghi et al., 1964; McLennan, 1971; Tonosaki and Shibuya, 1979, 1985). In contrast, Gervais (1987) found a (presynaptic) facilitatory effect of GABA application of K^+ -evoked NA-release in rat OB slices and suggested the

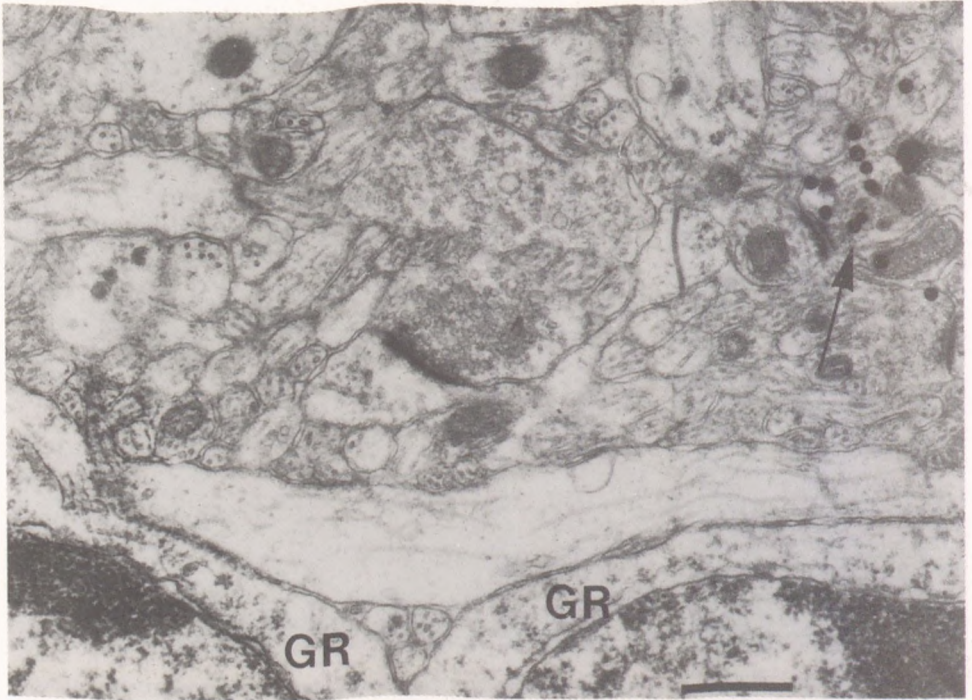


Fig. 4.62. Labeled axon terminal (arrow) from the granule cell layer of the rat olfactory bulb, after ^3H -noradrenaline uptake. Electron microscopic autoradiogram. Nialamide (500 mg/kg, i.p.) pretreatment, ventricular injection of the label, aldehyde-osmium fixation, Epon embedding, uranyl acetate and lead citrate staining, Ilford L4 emulsion, Phenidone-ascorbic acid developer. The labeled fiber makes a Gray type-I synapse onto a small caliber profile. Other terminals and fibers, astrocytic processes and granule cells (GR) are unlabeled. Scale bar: 0.5 μm

attenuation of GABA-ergic inhibition, through this excess NA, on output neurons. Jahr and Nicoll (1982a), by intracellular recordings, demonstrated also an indirect, disinhibitory action of NA on mitral cells to antidromic test stimuli, when they applied paired shocks to LOT. The amplitudes of the field potentials were, however, depressed also in these experiments.

Bulbar NA may be related in several ways to olfactory behavior. Lesion of the centrifugal NA system of the OB with 6-hydroxydopamine (6-OHDA) produced a reduction of neophobia to a novel food in rats (Royet et al., 1983), or by surgery, prevented the recognition of newborn lambs by the ewe (Pissonier et al., 1985). Intact noradrenaline afferents and NA supply to the bulb are also essential to the "olfactory memory" formation, resulting in interruption of pregnancy to strange male pheromones (Rosser and Keverne, 1985), or to normal recognition of conspecific odors (Cornwell-Jones and Marasco, 1980). NA might be related specifically to sexual and reproductive processes, since the NA content of the OB was significantly increased in male mice as a result of exposure to ovariectomized females (Dluzen and Ramirez, 1983) and in female vole (*Microtus orchrogaster*) when exposed to male vole urine (Dluzen et al., 1981).

4.3.7.3. Dopamine

Although most of the dopamine of the OB can be assigned to local, intrinsic sources (see Sections 4.3.3.1. and 4.3.4.1.), a minor projection from the ventral tegmentum and substantia nigra to the main OB of the rat has been reported (Lindvall and Björklund, 1974; Fallon and Moore, 1978*a,b*). In contrast, Macrides et al. (1981) were unable to detect any changes in the DA content of the hamster OB, after extensive medial forebrain bundle lesions, while (central) NA content of the bulb was abolished. Later, however, the same group (Kream et al., 1984) was able to demonstrate the presence of TH-containing (and so, likely dopaminergic) central fibers in the hamster olfactory bulb. Based on available data, it would appear that the contribution of central fibers to the OB DA content and mechanisms of action may be very limited.

4.3.7.4. Serotonin (5-hydroxytryptamine, 5-HT)

Unlike the other monoamine transmitter, DA, 5-HT has an exclusive extrabulbar supply, originating in the dorsal and median raphe nucleus (Dahlström and Fuxe, 1964; Broadwell and Jacobowitz, 1976; Halaris et al., 1976; DeOlmos et al., 1978; Moore et al., 1978; Bobillier et al., 1979; Araneda et al., 1980*a,b*; Andersen et al., 1983;



Fig. 4.63. ^3H -serotonin uptake by glomerular structures. Light microscopic autoradiogram. Adult rat, Nialamide (500 mg/kg, i.p.) pretreatment, intraventricular injection of the label, aldehyde-osmium fixation, Epon embedding. 6 μm thick section, Ilford L4 emulsion, Kodak D19B developer, no counterstaining. Silver grains are clustered mostly over structures within the glomerular neuropil, and may represent terminal accumulation of the label. In places, the necklace-like row of grain accumulations trace the course of likely serotonergic fibers with several varicosities. Much less such labeling is seen in the external plexiform layer (EPL), while no ^3H -serotonin accumulation appears in the olfactory nerve layer (ONL). Scale bar: 20 μm

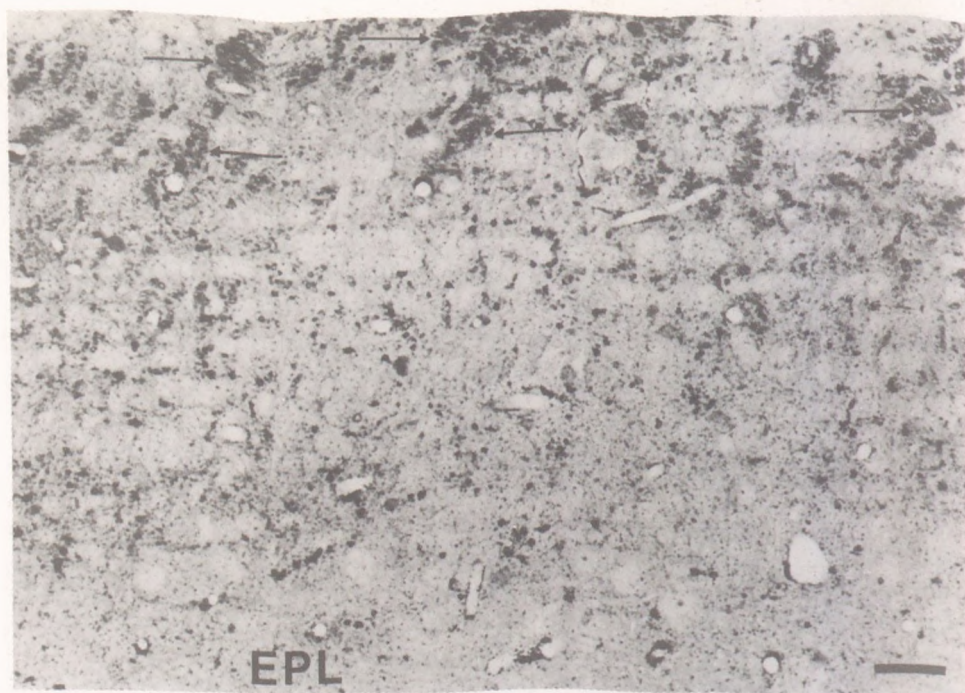


Fig. 4.64. Accumulation of ^3H -serotonin in deeper layers of the rat olfactory bulb. Light microscopic autoradiogram. Adult rat. Treatment and processing of the sample as described in Fig. 4.63. Some grain accumulation is present in the deep external plexiform layer (EPL), but more numerous labeled varicosities appear even deeper. Although all serotonergic fibers of the rat olfactory bulb were shown to have a central origin (see text), compartments occupied by bundles of myelinated fibers (arrows) are regularly devoid of silver grains. Scale bar: 20 μm

Kream et al., 1984; McLean and Shipley, 1987a). The serotonin content of cells in the raphe nuclei has been rendered by the Falck-Hillarp technique and immunohistochemistry of 5-hydroxytryptophan decarboxylase (E.C. 4.1.1.28.), tryptophan hydroxylase (E.C.1.14.16.4.) and antibodies to bovine serum albumin (BSA)-linked 5-HT. Serotonergic fibers and terminals of the rat OB have been recognized and quantitated by this last approach (Steinbusch et al., 1978; Steinbusch, 1981; McLean and Shipley, 1987a,b). The most dense supply of 5-HT containing structures was observed within the glomeruli (these fibers and varicosities had greater diameter than those found deeper; McLean and Shipley, 1987a), while the EPL contained only scattered fibers and the MCL, IPL and GCL a moderate plexus. Our autoradiography revealed a similar distribution for labeled terminals, after uptake of ^3H -5-HT (Halász et al., 1977a, 1978).

Figures 4.63 and 4.64 demonstrate the distribution of labeled terminals within the glomeruli and superficial GCL, respectively, after uptake of tritiated 5-HT. In places, labeled, thinner segments of fibers also became visible, but the vast amount of the label was confined to likely varicosities. Under the electron microscope, all of these label-accumulating fine structures were identified as vesicle-containing segments or terminal

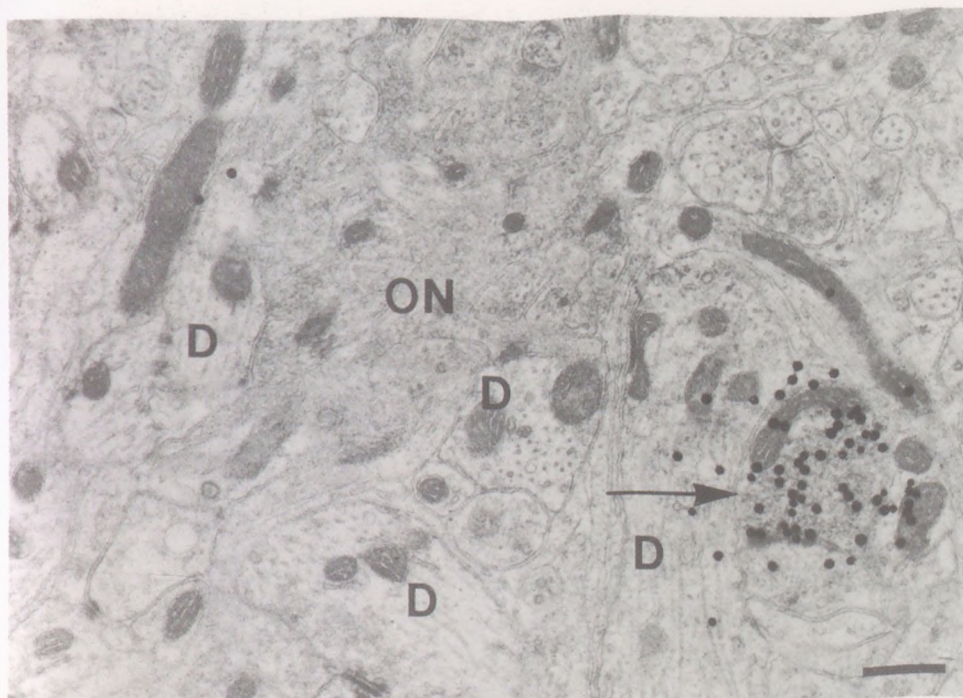


Fig. 4.65. Electron microscopic autoradiogram of ^3H -serotonin uptake in the rat olfactory glomerulus. Adult rat. Nialamide (500 mg/kg, i.p.) pretreatment, intraventricular injection of the label, aldehyde-osmium fixation, Epon embedding. The fine sections were contrasted with uranyl acetate and lead citrate. Ilford L4 emulsion, phenidone-ascorbic acid developer. Silver grains accumulate over a densely vesiculated profile (arrow), separated from the group of similarly dense, but unlabeled, olfactory nerve axons (ON). Other vesiculated neuropil constituents, axon-like small caliber fibers and dendrites (D) (these latter may belong to primary dendritic branchings of the output neurons and to periglomerular cells) are unlabeled. Scale bar: 0.5 μm

enlargements of fibers (Figs 4.65–4.67). In the GL, they entered the glomeruli, and were seen sometimes to form regular synapses. Olfactory nerve terminals and dendritic segments of the area were never labeled (see Fig. 4.65). In the EPL, output cell dendrites or granule cell terminals were not labeled; several medium-sized, vesiculated profiles appeared to be serotonergic (see Fig. 4.65). In the GCL likely granule cell dendrites, myelinated fibers and the large synaptic complexes, surrounded by astrocytic processes (see Section 4.2.5.2.1.) were devoid of silver grains (see Fig. 4.67).

As for the distribution of labeled fibers and terminals, an interspecific difference has been reported by Takeuchi et al. (1982). They revealed a low density of immunoreactive 5-HT fiber terminals in the monkey GL (in contrast to the high density of these structures in the cat and rat), while the rat had less numerous labeled fibers in the inner GCL than the two other species. The sheep OB had a 5-HT fiber network in the bulb similar to that observed in the rat (Tillet et al., 1987).

Immunoreactive serotonergic fibers were observed already in the newborn rat OB, but their number and arborization increased rapidly during the first two postnatal

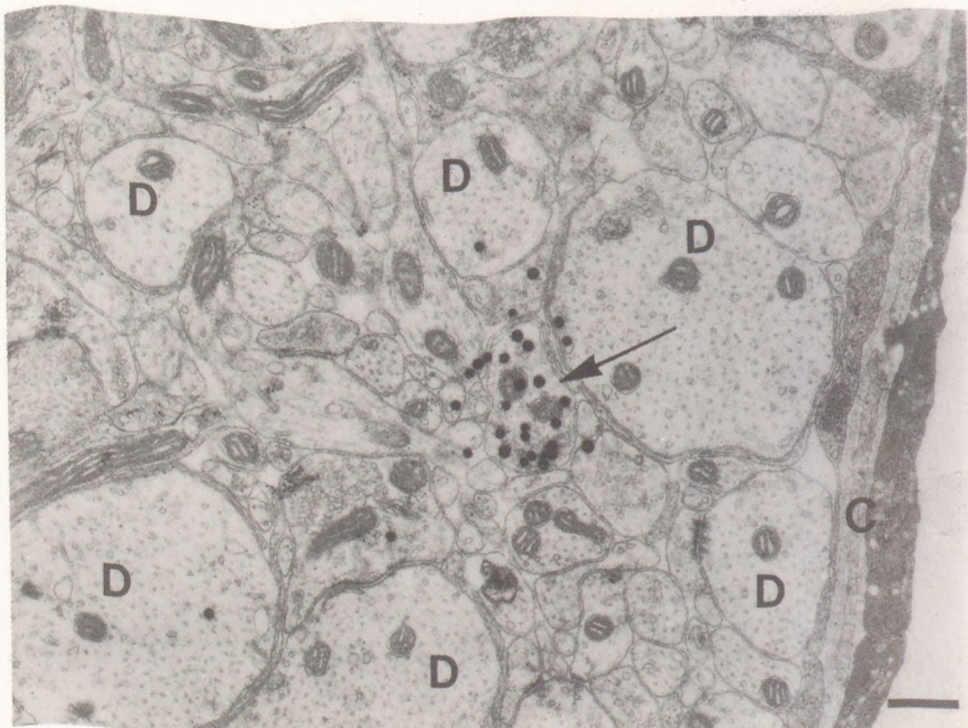


Fig. 4.66. Cross-sectional profile of a labeled fiber (arrow) in the external plexiform layer after ^3H -serotonin uptake. Electron microscopic autoradiogram. Adult rat; treatment of the animal and processing of the sample as described in Fig. 4.65. Large, light dendrites of the output (mitral and/or tufted) cell dendrites (D) and their synaptic counterparts, capillary wall (C), thin fibers and glial lamellae are unlabeled. Scale bar: 0.5 μm

weeks in all layers. In the GL, their density continued to further increase, resulting in a 2–3 times higher value in adults (per unit area of sections), when compared to the infraglomerular layers (McLean and Shipley, 1987b). Iontophoresis of 5-HT onto OB neurons resulted in suppression of mitral cell activity (Baumgarten et al., 1963; Bloom et al., 1964). The serotonin content of the bulb, and the inhibitory effect of serotonergic fibers could be reduced by direct injection of 5,6-dihydroxytryptamine (Gervais et al., 1984). It was concluded, however, from the behavioral experiments of the same group that the serotonergic fibers “could mediate some inhibitory influence on mitral cell activity during wakefulness only”, and 5-HT innervation of the OB would not be involved in the basic olfactory recognition mechanisms (Gervais et al., 1984). 5-HT might rather be responsible for involvement of the olfactory system in more general behavioral reactions (Cairncross, 1979).

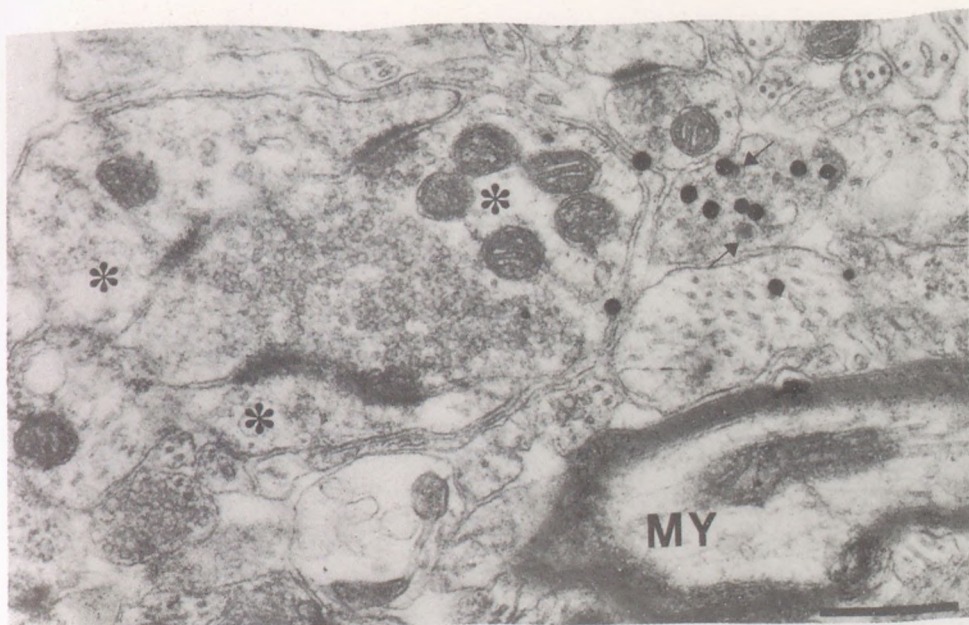


Fig. 4.67. Labeled small axon from the granule cell layer of the olfactory bulb after ^3H -serotonin uptake. Electron microscopic autoradiogram. Treatment of the animal and processing of the sample as described in Fig. 4.65. In this layer, small caliber axons and their terminals were labeled with silver grains, in a similar manner as for other layers. Dense-core vesicles (arrows) were regularly present in the labeled axons. Components of the large, complex synapses (their presynaptic terminals accumulated ^3H -GABA in another experiment) (asterisks) never accumulated ^3H -serotonin. MY: myelinated fiber. Scale bar: 0.5 μm

4.3.7.5. GABA

McLennan (1971) suggested the existence of a GABA-ergic inhibition on the (supposedly GABA-ergic) granule cells by central afferents. While granule cell terminal varicosities were labeled with ^3H -GABA only in the EPL (see Section 4.3.5.1.), in several experiments, a significant amount of dot-like labeled terminals appeared also in the GCL (see Fig. 4.54). During our studies with electron microscopic autoradiography of ^3H -GABA uptake, we observed some strongly labeled terminals in the GCL, which did not resemble either granule cell or SA cell terminals, so they were thought to have extrabulbar origin (Halász et al., 1979). In the deep GCL, central presynaptic components of large, complex synapses were labeled, but fine unmyelinated fibers also accumulated ^3H -GABA at their varicosities (Fig. 4.68).

The GABA-content of the OB decreased with increased extent of central lesions in the experiments of Godfrey et al. (1980), strongly suggesting the existence of a certain central GABA-supply to the rat OB. Recently, Záborszky et al. (1986) determined the source of such GABA-ergic bulbofugal fibers, by retrograde fluorescent tracer transport to, and simultaneous GAD immunocytochemistry of, the cells of the nucleus of HDB. The GAD-containing bulbofugal neurons were found predominantly in the caudal part of the nHDB; they appeared as large ($12 \times 20 \mu\text{m}$), multipolar neurons.



Fig. 4.68. Labeled, varicose enlargement of a tiny axon in the deep granule cell layer after ^3H -GABA uptake. Electron microscopic autoradiogram. Adult rat. Treatment of the animal and processing of the sample as described in Fig. 4.54. Phenidone-ascorbic acid developer. A higher density of silver grains appear only at the varicose, vesiculated segment of one axon (arrow). Other vesiculated profiles, dendrite-like compartments (D) and transient thin fibers are not labeled. Scale bar: 0.5 μm

About 33% of the GAD-immunoreactive HDB neurons projected to the OB, and some 30% of the nHDB neurons, labeled retrogradely from the OB, were GAD-positive (Záborszky et al., 1986). Degeneration of this pathway might have well been responsible for the significant loss of GABA in the deeper OB layers during the lesion experiments of Godfrey et al. (1980).

4.3.7.6. Aspartate, glutamate

Although doubts were raised concerning the suspected transmitter role of Asp and/or Glu in the output neurons in the OB (see Section 4.3.2.1.), several central afferents may utilize it, as would appear from immunohistochemical localization of glutamate-like reactivity in numerous terminals in the GCL (Liu et al., 1989) and from autoradiographic experiments with ^3H -D-aspartate (^3H -D-Asp). It was shown that this metabolically inert glutamate substitute is taken up and transported similarly to the endogenous, L-conformation (Davis and Johnston, 1976). ^3H -D-Asp was used successfully to reach a reasonably good selectivity in labeling of the presumed

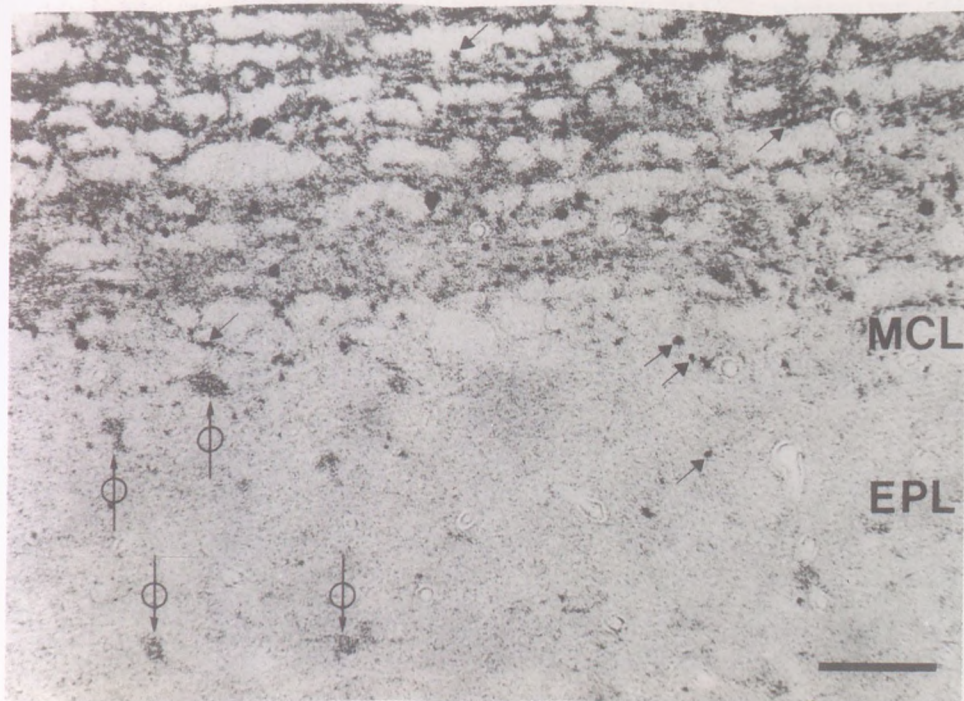


Fig. 4.69. ^3H -D-Aspartate uptake by terminal varicosities and processes in the rat olfactory bulb. Light microscopic autoradiogram. Adult rat, intraventricular injection of the label. Aldehyde-osmium fixation, Spurr resin embedding, $2\text{ }\mu\text{m}$ thick section. Ilford L4 emulsion, Kodak D19B developer, no counterstaining. Small arrows point to some heavy grain accumulations in the external plexiform layer (EPL) and deeper. Several weakly labeled cells also appear (arrows with a circle). MCL: mitral cell layer. Scale bar: $50\text{ }\mu\text{m}$

Asp/Glu-containing afferents in the rat OB (Watanabe and Kawana, 1984) and other mammalian brain areas (Matute and Streit, 1985). Although Watanabe and Kawana worked on a very limited number of rats, their retrograde labeling experiments of central afferents from the AON (both sides), ipsilateral piriform cortex and nucleus of the LOT, with ^3H -D-Asp, but lack of label in the taenia tecta, nucleus of HDB, locus coeruleus, dorsal raphe nuclei and amygdaloid nuclei (i.e., in areas which were proposed to deliver another transmitter to the main OB), are intriguing.

Based on these experiments, we have attempted to localize the uptake sites of ^3H -D-Asp in the rat main OB, by means of light- and electron microscopic autoradiography. Labeled terminals appeared in highest numbers in the IPL and GCL (Fig. 4.69), with an occasional appearance in the MCL, EPL and GL, too. The terminal-like grain accumulations in the GCL were localized preferentially in the neuropil area of the layer, filling the space around granule cell islands, but they were present also in intimate proximity to unlabeled cellular compartments (Halász, 1986a).

Electron microscopy revealed the labeling of vesiculated, $1\text{--}2\text{ }\mu\text{m}$ thick terminal segments of fibers, differing from the granule cell processes, and synapsing sometimes onto such processes (Fig. 4.70), but myelinated fibers were also labeled frequently. In



Fig. 4.70. Labeled terminals (asterisks) after ^3H -D-aspartate uptake in the granule cell layer of the rat olfactory bulb. Electron microscopic autoradiogram. Adult rat; treatment and embedding as described in Fig. 4.69. Ultrathin section, Ilford L4 emulsion, uranyl acetate and lead citrate contrast, Amidol developer. Both labeled terminals synapse onto light, vesicle-poor, dendrite-like fibers. Scale bar: 0.5 μm

the EPL, an insignificant number of labeled small (10–12 μm in diameter) neurons and their stout dendrites were also labeled, but the overwhelming majority of labeled cells in this, and other layers, belonged to oligodendroglial and astroglial cells.

4.3.7.7. Luteinizing hormone-releasing hormone (LHRH)

The main OB has a limited source of intrinsic LHRH (the parent cell bodies were not identified according to the neuron type; several such cells lay around the course of the nervus terminalis and at the modified glomerular complex). The central LHRH fibers might be, however, the more significant component of the bulbar LHRH supply. The LHRH content of the posterior OB was reported to be higher than that of the anterior part in rats (Dluzen and Ramirez, 1981). Histochemical techniques revealed also a higher density of LHRH structures within the posterior bulb. While immunoreactive fibers of the hamster OB were segregated into a deep and superficial system (Hoffman et al., 1979; Jennes and Stumpf, 1980; Phillips et al., 1980), similar structures of the rat were found only in superficial layers (mainly in the EPL) (Witkin et al., 1982; Witkin and Silverman, 1983; Merchenthaler et al., 1984; Zheng et al., 1988) and also predominated in the superficial lamina of the posterior OB of mice (Rosser et al.,

1986). In *Macaca*, most of the LHRH fibers were seen in the GCL, while in two other primates (*Saimiri sciureus* and *Aotus trivirgatus*) they were again found mainly in the EPL (Witkin, 1985). Ultrastructural details for the termination of these systems in the main OB were not reported.

In mammals, the origin of the deep pathways with LHRH-like immunoreactivity was suggested to be the septal and diagonal band area and olfactory tubercle (Davis and Macrides, 1981; Bennet-Clarke and Joseph, 1982; Macrides and Davis, 1983; Merchenthaler et al., 1984), while the superficial fibers, supplying the GL and EPL might originate, at least in part, from the nervus terminalis (cf. Macrides and Davis, 1983; Witkin and Silverman, 1983; Merchenthaler et al., 1984; Zheng et al., 1988). These immunoreactive fibers ran alongside the terminal nerve, joined the ON and vomeronasal nerve and may have reached the olfactory epithelium, too (Jennes, 1986). Some LHRH fibers, originating from the n. olfaktoretinalis in platyfish (*Xiphophorus*), joined also the olfactory nerve, after having crossed the olfactory bulb, while several LHRH axons arborized in the OB itself (Münz et al., 1981).

Data have been published which support the role of LHRH fibers of the main (and accessory) OB in sexual and reproductive behavior, by receiving and mediating olfactory and/or pheromonal signals. The LHRH content in the posterior OB of the female vole changed to that of male vole urine (Dluzen et al., 1981). Exposure of male mice to intact males or ovariectomized females also resulted in a significant increase of LHRH in the same bulb region (Dluzen and Ramirez, 1983). Bulbectomy was shown to interfere with the daily changes of serum LHRH level in hamsters (Pieper and Mortiere, 1985).

It was not known which proportion of the LHRH-signals was closely related, or depended on collaboration with other mechanisms, the humoral LHRH-mediation or other hormonal or neuronal systems. In this respect, convergence of LHRH immunoreactive fibers and those having strong AChE activity should be mentioned (Zheng et al., 1988). It seems, that a particularly close link exists between the bulbar LHRH and NA system, since NA was also related to reproductive behavior (Keverne, 1983; see also Section 4.3.7.2.) and NA content of the bulb changed simultaneously with LHRH in behavioral experiments carried out by Dluzen and co-workers (Dluzen et al., 1981; Dluzen and Ramirez, 1983).

4.3.7.8. Opioid peptides

Since Bogan et al. (1982) did not find enkephalinergic fibers in the periventricular white matter of the rat, our only data for the presence of such central afferents derive from experiments on the hamster OB, as reported by Macrides and co-workers (Davis et al., 1982; Macrides and Davis, 1983). Sources of these fibers were determined to be the pars externa and medialis of the AON and ventral hippocampal rudiment, and their course was followed through the anterior and bulbar limbs of the commissura anterior (CA), but their preterminal or terminal segments could not be distinguished in the GCL from processes of intrinsic enkephalinergic structures.

4.3.7.9. Somatostatin

SOM-containing central afferents of the hamster main OB were traced by immunolabeling (Davis et al. 1982). These fibers may have originated in the olfactory peduncle or piriform cortex, and traveled through the AC, just like the enkephalinergic fibers. They could be followed into the GCL of the hamster; similar (sparse) immunoreactive fibers were noted in the rat OB by Johansson et al. (1984). Since intrinsic SOM-immunoreactive neurons were also described for the OB and a high density of SOM receptors was reported recently (Leroux et al., 1985), there seems to be increasing evidence to support the importance of this hormone in olfaction-related processes at the level of the main OB.

4.3.7.10. Substance P

Central SP-fibers were reported from the hamster OB (Davis et al., 1982; Macrides and Davis, 1983; Kream et al., 1984; Baker, 1986). They might originate from different central sources, including the amygdaloid area and locus coeruleus (Macrides and Davis, 1983), pass diffusely through the olfactory peduncle and enter the GCL. Such central SP-containing afferents were not affected by peripheral lesions (Kream et al., 1984). Receptors for these fibers in the GCL were not visualized in the autoradiographic experiments of Quirion et al. (1983), although they were able to show a high receptor density over the superficial layers of the rat olfactory bulb (which may on the other hand be the receptor domain for structures, which are postsynaptic to the SP-containing external tufted cells) (see also Section 4.3.3.3.). The existence of fibers with SP-like immunoreactivity was observed mainly also in the superficial layers of the main OB of the neonatal rat; their number slightly increased by postnatal day 20 (Inagaki et al., 1982).

4.3.7.11. Vasopressin (VP)

Vasopressin-containing fibers to the OB were followed from the hypothalamic supraoptic nucleus, until they entered the OB (Buijs, 1980). These fibers were later shown to reach the EPL of the rat OB (deVries et al., 1985), but no further details have been published, regarding their termination.

4.3.7.12. Neuropeptide Y (NPY)

Although several intrinsic NPY neuronal somata, and sometimes also their labeled axons, were visualized by light microscope immunohistochemistry, part of the labeled fibers in the IPL and GCL were suggested to have a central origin (Gall et al., 1985). This seems probable, because these fibers were observed to continue from the GCL into the anterior limb of the CA. On the other hand, they described NPY immunoreactivity for SA cells of the rat OB, which, in other studies, were never shown

to project outside the bulb. "Scattered fibers" with NPY immunopositivity were detected in the GCL of rats during the developmental studies of Woodhams et al. (1985) spanning from postnatal day 4 onwards.

4.3.8. Neuroactive substances of the main olfactory bulb with uncertain or unknown localizations

Indications of the presence and/or role of several more neuroactive substances of the OB of vertebrates also exist, but without satisfactory histological characterization. According to these, the following additional substances were suggested to be present: corticotropin releasing factor (in certain somata and fibers: Merchenthaler et al., 1982; Merchenthaler, 1984), neurotensin (in superficial cells of the OB: Hara et al., 1982; Matsutani et al., 1989; in addition to a very dense fiber network in the superficial GCL: Matsutani et al., 1989), oxytocin (Buijs, 1980; Dogterom and Buijs, 1980) and thyrotropin-releasing hormone (presumably in cell somata, also: Kreider et al., 1981, 1982). The recent study of Tsuruo et al. (1988) strongly supports that periglomerular cells are the most important sources for this peptide in the main OB. A small group of neurons (and fibers) with LHRH immunopositivity was described recently in the modified glomerular complex (Teicher et al., 1980; Greer et al., 1982b) of the main OB in mouse, but the neuron type of the cells could not yet be ascertained (Rosser et al., 1986). Parvalbumin immunoreactivity was observed in unidentified perikarya in the EPL of the rat OB (Kosaka et al., 1987b).

Very high density of atrial natriuretic factor binding sites was detected in the frog OB (Tong et al., 1989). In the rat OB, glycine immunoreactivity was apparent in a few cells in the glomerular layer; a diffuse staining was observed in neuropil areas (van den Pol and Görcs, 1988). Glycine receptors were visualized by the same authors, mostly in the EPL (postsynaptic mitral dendrites were stained). Evidences for the existence of glycinergic mechanisms in the olfactory bulb are, however, not available yet.

The high prostaglandin (PG) level was reported through RIA measurements: the concentration of PGD₂ in rat (Narumiya et al., 1982) and human (Ogorochi et al., 1984) OBs were amongst the richest brain areas; PGE₂ and PGF₂ levels of the human brain were also significant (Ogorochi et al., 1984). The synaptic membrane fraction of the OB showed the third highest level (after the pituitary and hypothalamus) PGD₂ binding capacity (Shimizu et al., 1982). Introduction of PGD₂ into the rabbit OB resulted in a modulation of the mitral cell responses to electrical or odor stimulation (Watanabe et al., 1986). This finding has been correlated with autoradiography: highest density of PGD₂ binding sites (in the rat OB) occurred in the mitral cell or internal plexiform layer (Yamashita et al., 1983). More details on cellular localization are not available yet. Presence of receptors for benzodiazepines (Anholt et al., 1984; Bolger et al., 1984; Richards and Möhler, 1984; Schoch et al., 1985; preferably in the superficial layers) and angiotensin-II (Chen and Printz, 1981; Chen et al., 1982; Speth et al., 1986) in the main OB were also reported. The ONL contained the highest concentration of peripheral-type benzodiazepine receptors, followed by the glomeruli (high density) and EPL (low concentration); the ligand binding of these receptors first appeared on day 16 of the embryonic life (Inagaki et al., 1982).

4.3.9. Single existence or coexistence of neuroactive substances in cellular compartments of the main olfactory bulb

As a closing word after the preceeding Sections of Chapter 4.3., we summarize the results of histochemical experiments in Table 4.3. As it is evident from this list, all types of somata, and afferent fibers contain more than one neuroactive substances (sometimes in a considerably large proportion of the given cell type), which would indicate their coexistence or cooperative action, too. Accordingly, several co-localization experiments have already been attempted (these will certainly increase rapidly in number in the near future), as reported in the previous sections, and shortly summarized here, as follows.

Table 4.3. Summarization of data for the presence of different neuroactive substances in various intrinsic and extrinsic neuronal compartments of the main olfactory bulb

Cell or fiber type	Mitral and deep tufted	Medium tufted	Superficial tufted	Periglomerular	Granule	Short axon	Retrobulbar afferent	Olfactory nerve
Neuroactive substance								
Acetylcholine							+	
Aspartate/glutamate	?+						+	
N-Acetyl-aspartyl-glutamate	?+							
CA-binding proteins		?	?	+	+	+		
Carnosine								+
Cholecystokinin	?+	?	?	+		+		
Calcitonin gene-related peptide								+
Dopamine		+	+	+	?+	+	+	
γ -aminobutyric acid				+	+	+	+	
Insulin					?+			
Luteinizing hormone-releasing hormone								+
Noradrenaline							+	
Neuropeptide Y						+	+	
Opioid peptides				+	+	+	+	
Olfactory marker protein								+
Serotonin							+	
Somatostatin				+		+	+	
Substance P			+				+	
Thyrotropin-releasing hormone				+		?+		
Vasoactive intestinal polypeptide			+	?+		+		
Vasopressin							+	

+ means demonstrated presence;

?+ means suggested presence.

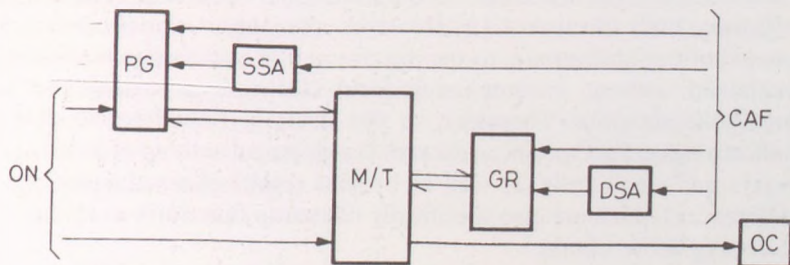
In periglomerular cells, there are strong suggestions for the coexistence of dopamine with several substances: Met-Enk (Macrides and Davis, 1983), CCK (Seroogy et al., 1985), GABA (Kosaka T. et al., 1985, 1987; Kosaka K. et al., 1987) (in contrast, Mugnaini et al. (1984a) reported different localizations for DA and GABA). Met-Enk and GABA may also coexist in periglomerular cells (Macrides and Davis, 1983). Kosaka T. et al. (1987) suggested a stratified organization of the GAD-immunoreactive neurons in the rat OB, according to their additional TH-like (in the GL), enkephalin-like (in the GCL and GL) or parvalbumin (in the EPL) immunopositivity.

Other experiments, investigating coexistence (in periglomerular cells again), were unable to demonstrate it, as happened in the case of DA plus NPY in rats (Everitt et al., 1984), DA with CaBP (Halász et al., 1985b) or DA with SP in the hamster OB (Baker, 1986b).

It would appear that evidence is still scarce for the coexistence of different transmitters and/or hormones, when compared to the high number of cell types with a multiple incidence of neuroactive substances. This reflects the difficulties of such experiments, but also raises the possibility for a reasonably strict segregation of neurons according to their topologically specified locations, different connectivity patterns and corresponding dissimilar mediator substances. These neurochemical differences might serve specialized physiological interactions in the processing of smell information at the level of the main olfactory bulb.

4.3.10. Summary and conclusions

Section 4.3 presents transmitter histochemical data and discusses their significance. These neurochemical findings offer a way for discerning subpopulations of morphologically indistinguishable neurons and fibers. In addition, they provide grounds, by which it is possible to correlate morphological data (Section 4.2.) with those characterizing the functions of neurons in the olfactory bulb (Section 4.4.). Accordingly, a tentative model of the bulbar machinery can be proposed. The following block diagram illustrates the main types of connections, in a much simplified and generalized form, between the various neurons of the olfactory bulb.



Olfactory nerve fibers (ON) activate the mitral/tufted (projection) neurons (M/T) which transmit the signal into central (olfactory cortical, OC) structures. The activity of the projection neurons may be modulated through the deep and superficial

interneurons, which are under the control of afferent systems. Neurochemistry suggests that GABA-containing granule cells (GR) and periglomerular cells (PG), as well as the dopaminergic PG cells may inhibit the projection neurons, while GABA-ergic deep (DSA) and superficial (SSA) short-axon cells may cause disinhibition, when activated. Projection neuron recurrent collaterals (RC) and central (mostly NA, 5-HT, ACh, DA, GABA or excitatory amino acid-containing) afferent fibers (CAF) may activate (or inhibit) segregated subgroups of local circuit neurons, situated in different lamina. This may result in either the amplification or suppression of the activity of a population of the projection neurons. Interactions between the projection neurons and (GABA and/or DA containing) periglomerular cells may also occur as a consequence of the activation of the latter by peripheral (ON) stimulation.

Thus, a transmitter-specific central and local circuit neuronal system serves the functioning of the bulb. This may provide the neurochemical basis for the formation of odorant-specific, focal activity patterns, to be characterized in more detail in the following section.

4.4. Functional aspects of the olfactory bulb

Odorant detection initiates a cascade of molecular events, resulting in generation of a receptor potential in the primary olfactory sensory neurons, which spreads down to the nerve and triggers an action potential (see Sections 2.4. and 3.4.). This liberates neuroactive substance(s) from the nerve in the bulb and produces potential changes in the primary output neurons of the bulb. This is followed (depending on wiring patterns of different vertebrate species; see Section 4.2.) by a direct or indirect activation of a whole range of interneurons, participating in the processing of the information. These activities may cause the release of a number of neuroactive substances from pools of local and extrinsic origin (see Section 4.3.), resulting again in potential changes in a number of postsynaptically localized neuronal elements, and shaping the output signal transmitted by the olfactory tracts from the bulb to more central brain areas.

The functioning of the neuronal systems of the OB has been partially characterized by histochemical and biochemical (to a lesser extent by certain related pharmacological) studies, in the discussion on the transmitter-specific structural connections of the olfactory bulb (Section 4.3.). However, the output signal-bearing local processes, contributing substantially to the discrimination and recognition of smells, cannot be explained without encompassing local electrophysiological and function-related metabolic activities. Therefore, in this Section, I shall try to gather observations concerning certain specific aspects of the electrical activity at levels of the whole bulb, layers and single cells, as well as present results characterizing regional metabolic differences (which are also specifically related to function), as revealed by mapping of 2-deoxyglucose uptake.

4.4.1. Electrical activity

Spontaneous, rhythmic activity of the bulbar neurons was observed first by Gerard and Young (1937). Introduction of odorants into the nasal cavity resulted in a disturbance of this rhythmicity, as reported first by Adrian (1950, 1953). Bulbar electrical phenomena were studied extensively during the following decades, by recording electroencephalographic (EEG) oscillations, which reflect the synchronous potential fluctuations of larger regions of the bulb. Even more studies were devoted to the analysis of the field- and unit potentials, characterizing the contribution of certain, anatomically localized subcompartments of this well-laminated brain area, by penetrating electrodes. These studies led to the detection of the complex wave forms from different depths, and also to suggestions for the explanation of physiological events, occurring at the cellular and synaptic level.

4.4.1.1. Spontaneous activity

Extensive studies on the electrical characteristics of the bulbar surface revealed a 15–100 Hz rhythmic oscillation of EEG waves. The frequency of these waves was decreased by application of anesthetics; their phase (and even frequency) differed according to the recording site on the bulbar surface; more importantly, they were modified according to respiration cycles and odorant application. During inspiration these EEG oscillations were substituted by waves, elicited by odorants. Such oscillations were observed in the bulb of a number of vertebrates, including man and have been characterized most extensively for cats and rabbits (see e.g. Adrian, 1950; Moulton and Tucker, 1964; Phillips and Mogenson, 1969; Sieck and Wenzel, 1969; Wenzel and Sieck, 1972; Freeman, 1972, 1978; Bressler and Freeman, 1980).

The source of this spontaneous activity was thought to be intrinsic, because it was not eliminated by peripheral (Ottoson, 1963a) or central (Gerard and Young, 1937) deafferentation, although a certain (15–25 Hz) frequency component disappeared in pigeons after bilateral olfactory nerve section (Sieck and Wenzel, 1969). Depth analysis indicated that granule cells were responsible for these rhythmic potential changes (Orrego, 1961). Granule cells of the rabbit OB were reported to have a spontaneous, rhythmic activity (Chaplain, 1976) which, through the dendrodendritic interactions with the output neurons, may initiate a regular oscillation of inhibition and excitation. Spontaneous activity was also observed in the output neurons (Green et al., 1962; Corrigan and Sherebrin, 1976). The spontaneous activity of the bulb was deeply influenced by extrinsic neuronal impulses, elicited by central (antidromic) or peripheral (orthodromic) stimulation.

4.4.1.2. Evoked activity

Stimulation of the olfactory bulb evoked several forms of electrical responses, including slow, sustained waves and changes in EEG oscillations, as observed on the bulbar surface, but the basic cellular and synaptic phenomena, underlying the surface-

recorded transient changes, were studied by way of recording of field potentials and unit activity in various layers. The following sections summarize briefly the related results in this sequence.

4.4.1.2.1. Slow potentials

Odorants evoked slow potential changes in the frog bulb (Ottoson, 1959*a*), which appeared parallel with the EOGs of the olfactory mucosa (Ottoson, 1959*b*); these potentials were characterized as their wave components by Beuerman (1975) in turtles. At higher resolution, it turned out that “induced waves” rode on the slow transients (Fig. 4.71); these “induced waves” were blocked by antidromic stimulation of the bulb,

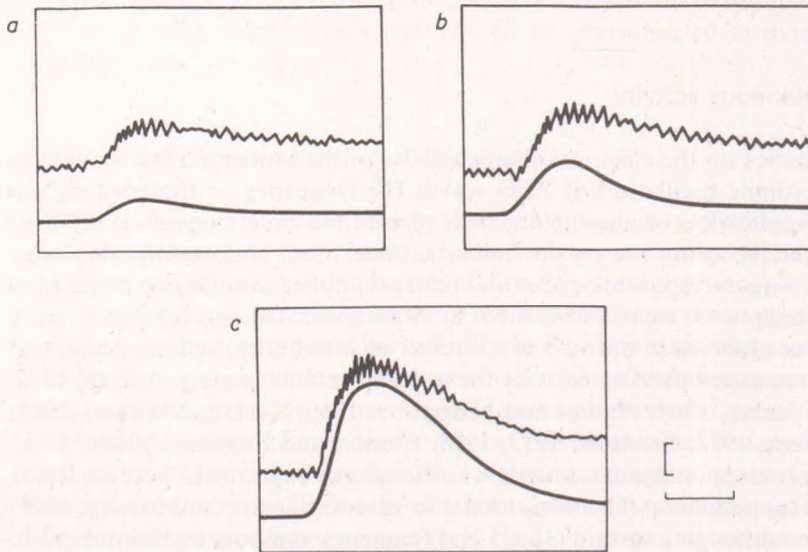


Fig. 4.71. Slow potentials, evoked by odorant stimulation of the olfactory mucosa (graphic reconstruction from Ottoson, 1963). *a, b* and *c* show responses to increasing concentrations of the odorant substance. Lower trace: response of the bulb; upper trace: simultaneously recorded electro-olfactogram (EOG) of the epithelium. Vertical bar: 1 mV; horizontal bar: 1 sec. Reproduced with permission

so, they were attributed to the activity of the output neurons of the bulb (Ottoson, 1963*b*).

These slow waves were supposed to be produced by the “dendritic network in the glomeruli” (Ottoson, 1963*b*). Indeed, in the case of odorant stimulation, similar slow potentials, following the EOG with a 40–50 msec delay, were recorded by Leveteau and MacLeod (1966) with the highest amplitude from single glomeruli. These latter authors used these potentials to examine the sensitivity of 42 glomeruli to 9 different odorants; different degrees of selectivity were reported. The potential change itself, however, was suggested to be rather of a glial origin, since it was resistant to the total blockade of the synaptic transmission by Mg^{2+} ions (Potapov, 1983).

4.4.1.2.2. EEG waves

These waves also represented a type of response to odorant application. According to the observation of Adrian (1950), in deep anesthesia, when the regular EEG oscillations were clearly apparent, introduction of an olfactory stimulus suppressed or substituted these oscillations. The substituting oscillations were termed "induced waves"; they had lower frequency, higher amplitude and regularity than the EEG waves, and were regarded as characteristic bulbar responses to chemical stimuli. Changes upon odorant stimulation were observed in various vertebrate species, as referred to briefly in many papers, or examined systematically (see e.g. Sieck and Wenzel, 1969; Wenzel and Sieck, 1972; Hara, 1973).

In contrast to the slow potentials, changes in EEG could be induced also by electric stimulation of the olfactory nerve. Foci of EEG activity were mapped with an array of surface electrodes, put onto the OB. In this way, bulbar projection fields of the primary olfactory nerve fascicles could be studied, as well as the temporal patterns of EEG oscillations monitored (Freeman, 1974) (Fig. 4.72).

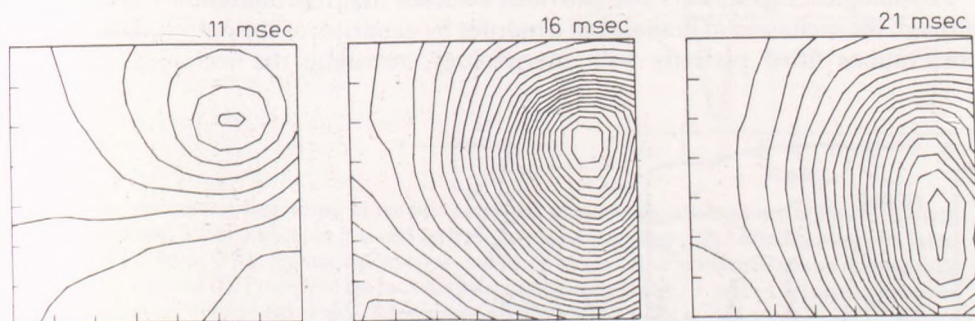


Fig. 4.72. Distribution and temporal changes of surface negativity (of the first negative wave, N1), as seen in the averaged evoked potentials (after Freeman, 1974). EEG waves were induced by electric stimulation of the olfactory mucosa and recorded by an array of 8×8 electrodes (0.5 mm spacing) on the lateral surface of the bulb, after 11 msec (rising phase), 16 msec (crest of negativity) and 21 msec (falling phase) of stimulation. Contour interval: $10 \mu\text{V}$. Reproduced with permission

4.4.1.2.3. Field potentials and unit recordings

In this Section, electrical properties of various neurons and their interconnections will be summarized, as revealed by studies using antidromic and orthodromic electric shocks, followed by others analysing the effects of odorants on unit activity.

Stimulation by electric shocks

Although the basic synaptic circuits of the olfactory bulb of vertebrates have been well known for a long time, electrophysiological examination of their functional interrelationships was not carried out until the early 1960s. Then, due to the

recognition of some characteristic principles of the system, first of all the main mechanisms of the peripheral and central stimulatory effects and those of the inhibitory processes elicited by antidromic activation of the output neurons, this field of physiological studies expanded rapidly.

As discussed previously, odorant stimulation activates the bulb; this activity proved to be sensitive to anesthetics and also to central (LOT) stimulation. Detailed analysis of antidromic stimulation suggested the existence of a direct (Green et al., 1962; Yamamoto and Iwama, 1962) or a synaptically activated inhibitory system (Ochi, 1963; Phillips et al., 1963; Shepherd, 1963*b*; Yamamoto et al., 1963). Some of the later observations also confirmed that part of this activation may be mediated by output cell axon collaterals (Westecker and Manns, 1983). The vast majority of experimental evidence showed, however, the involvement of the granule cells and their dendro-dendritic synapses in this inhibition (Rall et al., 1966; Rall and Shepherd, 1968; Nicoll, 1969; Westecker, 1970*b*; Mori and Shepherd, 1979; Shepherd and Brayton, 1979; Jahr and Nicoll, 1980; Mori et al., 1981*c*; see also related pharmacological studies cited in Section 4.3.).

Physiological experiments also provided evidence that this inhibition was induced through the excitation of granule cell dendrites by dendrites of the output cells. These observations fitted perfectly with morphology, revealing the reciprocal contact

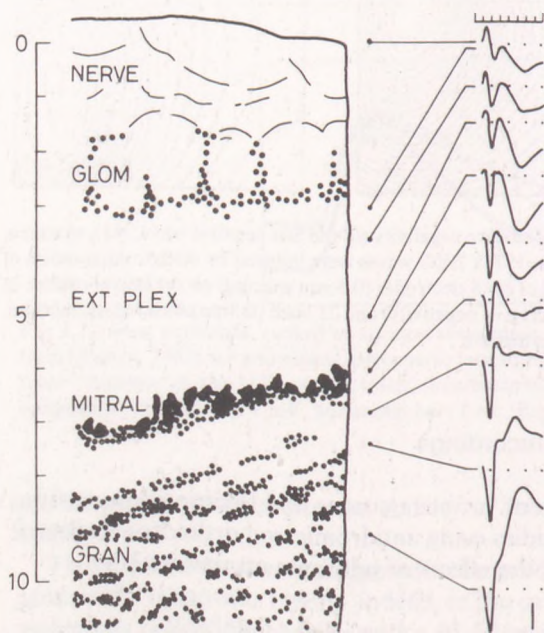


Fig. 4.73. Characteristic wave forms of the field potentials (right) elicited by LOT stimulation and recorded at different depths of the olfactory bulb (from Rall and Shepherd, 1968). The metric scale on the left has 100 μm intervals. Abbreviation of histological layers: NERVE: olfactory nerve layer; GLOM: glomerular layer; EXT PLEX: external plexiform layer; MITRAL: mitral cell layer; GRAN: granule cell layer. Dots connected to traces show exact location of each recording, in relation to the layers. Time scale on upper right has msec division, vertical bar at lower right represents 1 mV. Reproduced with permission

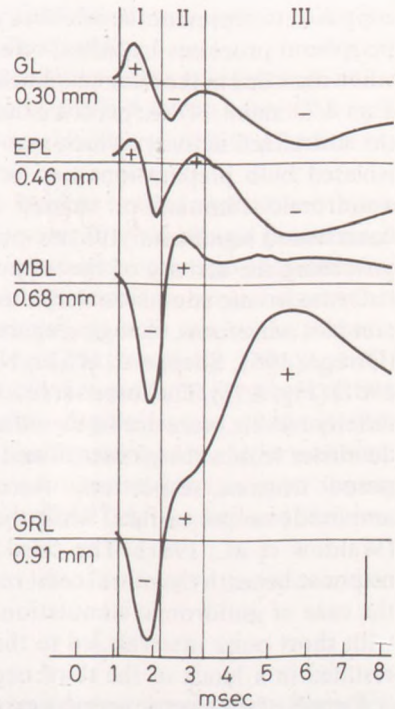


Fig. 4.74. Enlargement of wave forms of the field potentials from certain depths shown in Fig. 4.73. (from Rall and Shepherd, 1968). Depths in mm and layers (GL: glomerular layer; EPL: external plexiform layer; MBL: mitral cell layer; GRL: granule cell layer) are indicated on the left; characteristic phases of recordings are labeled by I, II and III. Polarity of the recording electrode is indicated by + and -, relative to reference electrode potential. Vertical bar, 1 mV. Reproduced with permission

between the two dendrites. Accompanying potential changes of this interaction, and contribution of different bulbar elements to this basic functional event could be studied in detail, when extracellular potentials were recorded with a fine recording electrode penetrating the various OB layers, and the waveforms were related to structurally identified cellular components (Figs 4.73 and 4.74). Such analyses were performed in a number of vertebrate species and there are many resulting publications (Orrego, 1961; Ochi, 1963; Rall and Shepherd, 1968; Nicoll, 1969; MacLeod, 1976, and others).

To a single antidromic electric shock, superficial to the mitral cell layer, early negative waves (as related to the reference electrode) dominated, the complex wave. These were suggested to reflect glomerular events, related to the invasion of mitral/tufted primary dendrites and/or to their interaction with the superficial set of interneurons. At the depth of the mitral cell layer, the polarity reversed and a large hyperpolarization appeared, as seen in the dominant positive component. Later, this reversal of the potential was regularly used in other studies to localize the level of the mitral cells. Here a large negativity, subsequent to the first negative waves, and reflecting the depolarization of the mitral cells, was clearly visible (see Fig. 4.74). Below this layer, the enlarged positivity, following the mitral cell depolarization, was

supposed to represent the inhibitory response of the granule cells to excitation of their peripheral processes by mitral cells. Response of the granule cells in this late phase, when recorded in the external plexiform layer or more superficially, did not appear (see Figs 4.73 and 4.74). Repetitive stimulation with short intervals led to suppression of the mitral cell activity, which was especially long lasting in reptiles, as observed in isolated bulb preparations (see below). In summary, analysis of the responses to antidromic stimulation, carried out with single, paired or repetitive stimuli, contributed significantly to our present understanding of the electrical phenomena governing the activity of the output neurons (Shepherd, 1972, 1979).

Orthodromic stimulation resulted also in an evoked potential, characterized by a complex waveform, changing again as the tip of the recording electrode was lowered (Orrego, 1961; Shepherd, 1963*a*; Nicoll, 1972; Jahr and Nicoll, 1981; Waldow et al., 1981) (Fig. 4.75). The three successive negative waves, representing depolarizations at different levels, were due to the olfactory nerve volley, their excitation of postsynaptic dendrites within the glomeruli and to the synaptic activation of granule cells by the output neurons, respectively. Accordingly, the first depolarization had the highest amplitude on the surface, while the second and third waves were higher in the EPL (Waldow et al., 1981). The third wave reversed into a positive (hyperpolarizing) response beneath the mitral cells, reflecting again the activity of granule cells, just as in the case of antidromic stimulation. Similarly, repetitive olfactory nerve stimulation with short pulse intervals led to the suppression of the output neuronal activity and resulted in a block of the third negative wave.

Details of the electric properties of single cellular constituents of the bulb with extra- and intracellular recording techniques and pharmacological tools have been extensively studied, but will be referred to only briefly here. A great advance in the understanding of the phenomena underlying mitral cell activity was achieved by the introduction of the isolated turtle olfactory bulb preparations (see Nowycky et al.,

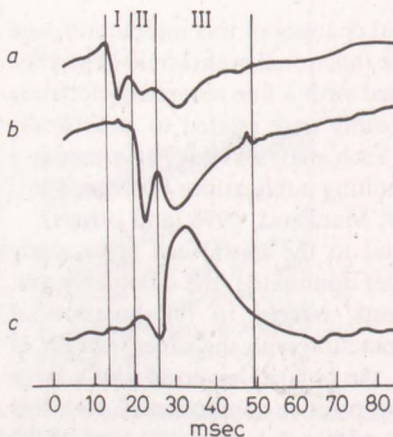


Fig. 4.75. Characteristic waveforms of field potentials, evoked by electric stimulation of the turtle olfactory nerve (after Waldow et al., 1981). *a* and *b* denote recordings from the external plexiform layer (*b*: shock intensity increased by $2.1\times$); *c* is a recording from the granule cell layer. I, II and III show characteristic phases in the responses. Reproduced with permission

1978; Mori and Shepherd, 1979; Jahr and Nicoll, 1980; Mori et al., 1981a-c; Waldow et al., 1981; Jahr and Nicoll, 1982b; Nicoll and Jahr, 1982).

Deep tufted cells may behave exactly in the same manner as the mitral cells (Getchell and Shepherd, 1975a); more superficial tufted cells in the EPL of the rabbit OB could also be separately recorded by intracellular electrodes (Nicoll, 1970; Getchell and Shepherd, 1975b).

Less information is available on neurons with smaller somata and more irregular geometrical characteristics. Mori and Kishi (1982) were able to drive and record single, morphologically identified granule cells by LOT or anterior commissure stimulation; the cells responded with one or a few spikes. This is of particular interest, because these cells have no axon (see Section 4.2.2.3.). Unit recordings from other cells, including those of the superficial interneurons, are also rare, but available (Nicoll, 1971b; Getchell and Shepherd, 1975b). A deeper insight into the particular features of these superficial neurons is of high importance, because depolarization of the primary dendritic branches of the output neurons by the olfactory nerve seems to be modulated already at the glomerular level by these interneurons and at least in part, through dendrodendritic interactions (Shepherd, 1963b; Nicoll, 1971b; Getchell and Shepherd, 1975b; Mori et al., 1984).

Stimulation by odorants

Changes in the slow potentials of the bulb to odorant stimulation have been observed, as mentioned earlier in this Section. At the level of the responding units, odorants regularly elicited more complex responses than electrical stimulation did. The "induced waves" (see above), produced by odorants, may be the consequence of modified activity of the bulbar units, i.e., facilitation or suppression of firing of the neurons, resulting in certain spatial and temporal activity patterns. These patterns were thought to be characteristic of the odorant quality and concentration.

This supposition initiated many studies, in which unit activity changes were investigated at the extra- and intracellular levels. Units recorded at different depths of the bulb, could clearly be identified only if they were output cells, which, in addition to their response to odorant application, could be driven by LOT stimulation, too.

Regularly, just a minority of the units failed to change the firing pattern to odorant introduction. Most of the cells responded with an increase or decrease, or, as observed by many investigators, with a more complex change of their firing. These included alternating excitations and suppressions and characteristic changes in firing patterns (e.g., rate of activity or latency of bursts) (Mancia et al., 1962; Cattarelli et al., 1972; Mathews, 1972; Shibuya et al., 1977; Tonosaki and Shibuya, 1979, 1985; Mair, 1982; Meredith, 1986). Even the same cell could respond differently to the same stimulant, applied under identical circumstances, within short intervals. This lack of consistency in responses made it difficult to correlate a certain stimulant with specific responses at the level of the OB (Scott, 1977).

In addition, certain factors, present in all such experiments may cause, by themselves, a response and so contribute to the final activity pattern. For example, the stimulating effect of the flow rate of odorant delivery must be taken into consideration,

as well as air and water, as solvents for odorants, since both were shown to elicit responses in bulbar units (Westecker, 1970a; Arito et al., 1978); although a mechanical component in the stimulations by the air flow could not be excluded (Ueki and Domino, 1961).

Coding of certain characteristics of the odorants was, however, evident in most of the similar experiments. A close (although not linear) correlation between the concentration of stimulants and the spike frequency was observed (Kauer, 1974; Kauer and Shepherd, 1975; Duchamp, 1982; Mair, 1982; Hamilton and Kauer, 1985; Harrison and Scott, 1986; Reinken and Schmidt, 1986; see also Section 4.4.4.), even fair quality coding was sometimes reported, as seen at the level of single unit responses (Döving and Hyvärinen, 1969; Mair, 1982; Chaput and Holley, 1985).

Effect of prolonged exposure to odorants

Repeated (food) odorant stimulation in the mitral cells of the goldfish OB caused well-reproducible responses over 40 repetitions (Schild and Zippel, 1986). In contrast, Potter and Chorover (1976) noted decrements in EEG and mitral cell responses of the hamster OB, to repetitive application of odorants for about the same (30 s) duration. In rabbits, more than 6 min (continuous) exposure to odor resulted in a reduced amplitude of response in mitral cells, although "gross changes in firing activity" did not occur (Chaput and Panhuber, 1982).

Continuous exposure of animals to odorants for a longer (several days to several months) duration produced more severe, odorant-dependent effects in some experiments, as seen in changes of mitral cell activity (Eckert and Schmidt, 1985; Wilson et al., 1985), and histology (Döving and Pinching, 1973; Pinching and Döving, 1974; Laing and Panhuber, 1978; Dalland and Döving, 1981; Harvey and Cowley, 1984; Apfelbach and Weiler, 1985b; Laing et al., 1985; Panhuber et al., 1987; Reasner, 1987), or on altered behavior (see Chapter 8). Continuous, lasting exposure of newborn rats to cyclohexanone or deodorized air resulted in a decrease of granule dendritic spine density on the lateral, or medial *and* lateral sides of the OB, respectively (Rehn et al., 1988). Prolonged rearing in deodorized air caused mitral cell shrinkage in restricted areas (Laing et al., 1985; Panhuber et al., 1987). The most severe histological consequences (degeneration of mitral cells) could not be reproduced, however, in the experiment of Cunzeman and Slotnick (1984).

4.4.1.3. Significance of the physical status

Responsiveness of neurons of the olfactory bulb was shown to be influenced by the physical status of the experimental animal, and especially highly dependent on breathing cycles. Although the synchronization of the recorded units was not always observed, most bulbar unit responses were enhanced during inspiration (Adrian, 1950; Macrides and Chorover, 1972; Kauer and Shepherd, 1977; Chaput and Holley, 1980; Onoda and Mori, 1980; Chaput, 1983, 1986; Pager, 1985). This synchronization was diminished by transection of the olfactory peduncle (Potter and Chorover, 1976;

Chaput, 1983). Similarly, central control of the bulb was evident in maintaining influence of the nutritional and motivational state upon unit activity. Increased responsiveness of the olfactory units to food odor during hunger was especially clearly documented (Giachetti et al., 1970; Pager et al., 1972; Pager, 1977); nutritional motivation-dependent enhancement of responsiveness disappeared with transections of the central fibers to the bulb (Pager, 1978). Contribution of the central noradrenergic supply to this special motivation was likely to be important (Gervais and Pager, 1983).

4.4.2. Metabolic activity

Mapping of the differences between various layers and smaller subcompartments of the OB, with the 2-deoxyglucose (2-DG) technique of Sokoloff and co-workers (Kennedy et al., 1975) has been performed for the resting (Sharp et al., 1975; Schwartz and Sharp, 1978; Hammer and Herkenham, 1984) and activated (see below) olfactory bulb. According to the first observations, the working OB had a high 2-DG uptake, and odorant stimulation led to increased activity in certain foci (Sharp et al., 1975). Further experiments in this system supported the view that the heavy 2-DG accumulation indicates an increased metabolic activity (see e.g. Sharp et al., 1977; Skeen, 1977; Stewart et al., 1979; Jourdan et al., 1980); moreover, these metabolic differences were suggested to reflect enhanced synaptic activity (Greer et al., 1981*a,b*). Different olfactory nerve subcompartments activated characteristically different regions of the bulb; various odorants resulted in increased uptake of 2-DG in different (but sometimes overlapping) areas; therefore, the existence of spatial patterns in projections and in quality coding at the level of the bulb were confirmed again (Fig. 4.76).

Stronger electric stimulation or higher odor concentrations threw larger zones into activity. Foci of active areas were found in most cases in the glomerular zone, although expanded into to EPL and ONL, too. With application of liquid emulsions and high-resolution autoradiography, activity increases could easily be assigned to certain glomeruli and even cells (Lancet et al., 1982; Benson et al., 1985*a*), but the correlation of these fine localizations with well-defined physiological events awaits further experiments. The modified glomerular complex, localized in the dorsomedial portion of the OB, next to the AOB showed the highest 2-DG uptake in suckling pups (Teicher et al., 1980; Greer et al., 1982*b*). Removal of foci, showing the highest metabolic activity upon certain odorant stimulations (like the modified glomerular complex) did not produce, however, an appreciable change in the related behavioral tests with the same odorants (Hudson and Distel, 1987; Slotnick et al., 1987). This suggests that the elevated 2-DG uptake marked those areas being the most heavily, but not exclusively, involved in processing of certain specific smell information.

Functional maturation of the system was monitored by use of this technique. Increased activity to odorants at specific glomeruli, as well as extension of the active, stimulated zones, corresponding to the adult pattern by the third postnatal week were observed in rats and guinea pigs (Greer et al., 1982*b*; Astic and Saucier, 1983). Specific (glomerular) 2-DG uptake was elicited in learning experiments, showing distinctive

distribution patterns after stimulation by familiar odors, in contrast to other patterns, appearing after conditioning with aversive stimuli (Coopersmith and Leon, 1984; Coopersmith et al., 1986; Sullivan and Leon, 1986).

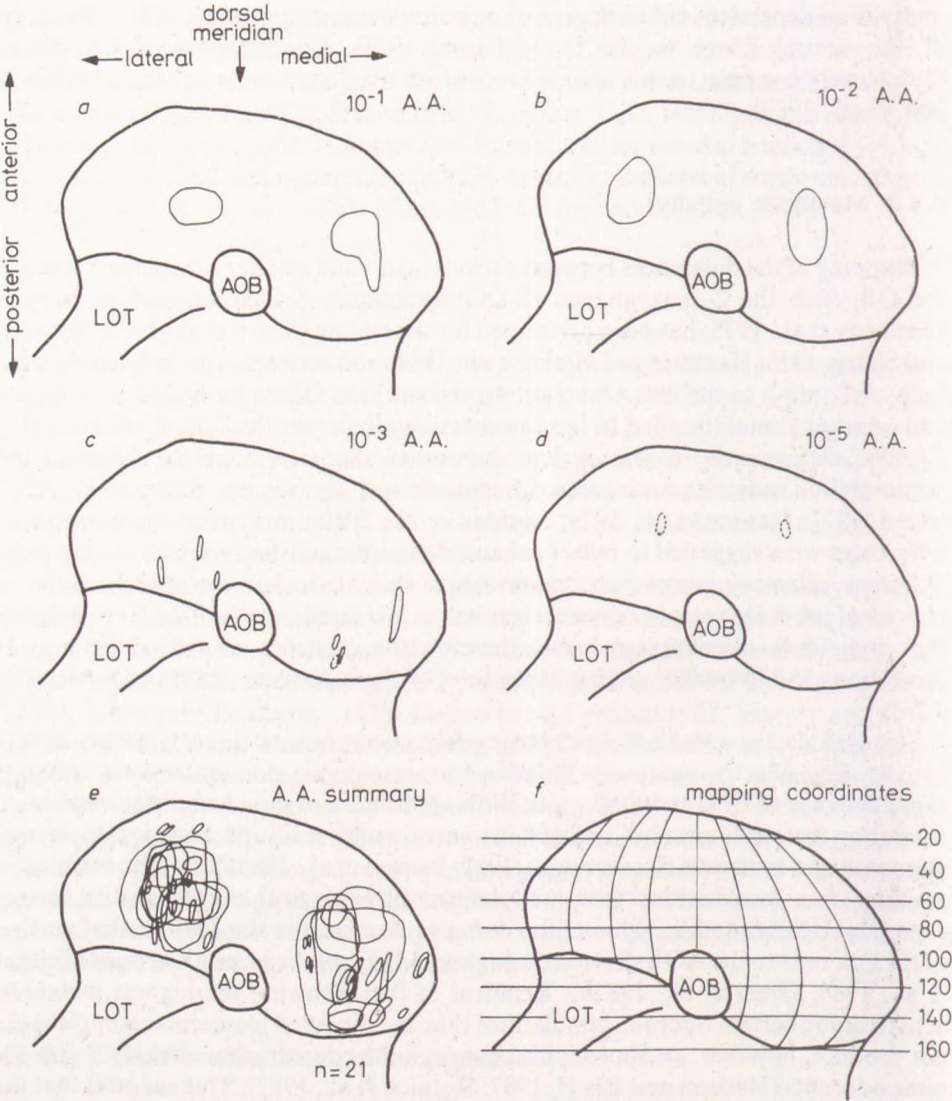


Fig. 4.76. Localization of areas with elevated 2-DG uptake to odorant stimulation, as seen on the unfolded glomerular sheet in rat (after Stewart et al., 1979). Adult rats were stimulated through a dilution olfactometer, by decreasing concentrations (from *a* to *d*, 10^{-1} to 10^{-5} M, as indicated) of amylacetate (A.A.). Characteristic spots of increased uptake are encircled by solid lines for the left and by dotted lines for the right olfactory bulbs. LOT: lateral olfactory tract; AOB: accessory olfactory bulb. Orientation of the maps is shown on upper left. Reproduced with permission from the Publisher

4.4.3. Coding of odor quality at the bulbar level

Mapping the foci of the characteristic, elevated 2-DG uptake (see Section 4.4.2.) and changes in the mitral cell morphology, upon prolonged odor exposure, provided morpho-functional evidence suggesting the view of the spatial coding of odorant quality at the bulbar level. When stimulated by a specific odorant, either shrinkage, or enlargement of the mitral cells occurred in specific regions of the OB, suggesting the lack or very high level of activity, respectively (Panhuber and Laing, 1987; Reasner, 1987). The elevated 2-DG uptake was associated with a significant increase in the width of the glomerular layer and the area of the underlying glomeruli in young rats (Woo et al., 1987).

Stereochemical and spatio-temporal patterns of odor quality coding at the epithelial level have been suggested and examined by many investigators. The same properties were studied at the level of the OB, by means of electrophysiology (see also Section 4.4.1.2.3.).

As noted in the former sections, some degree of spatial coding by the epithelium was preserved by the orderly projection of the olfactory nerves onto the bulbar surface. This resulted in a consequently higher electrical activity in certain large bulbar areas on stimulation with a specific odorant, and in lower or no activity in others (Mozell, 1958). Correlation of physiological activity of related mucosal and bulbar areas was studied later with better resolution: distinction at the level of glomeruli was recognized in frogs (Levetau and MacLeod, 1969), and intracellularly recorded mitral cells were also shown to receive inputs from a fairly restricted area of the epithelial sheet (with a diameter between 250 and 750 μm) in hamsters (Costanzo and O'Connell, 1980).

A novel approach, mapping the spatial distribution of odorant-elicited membrane potential changes, as observed by optical monitoring of voltage-sensitive dyes seems very promising. After electrical stimulation of the nerve, differences in the membrane potential of the *in vitro* and *in vivo* salamander olfactory bulb, could directly be visualized by this means (Orbach and Cohen, 1983; Kauer et al., 1987). This technique provided a resolution which is comparable to, or even better than mappings of EEG waves with an array of electrodes, or 2-DG uptake, in reflecting differences in local activity. The application of a video method for imaging the voltage sensitive fluorescence changes allowed the visualization of the temporal and spatial progression of the neuronal activity (Kauer, 1988).

Temporal patterns of electrical oscillations were also suggested to code odor intensity (see also Section 4.4.1.2.3.). Odorants of high molecular weight induced 37.5–75 Hz frequency waves, while for those of lower molecular weight, the frequency fell into a higher (75–125 Hz) range (Hughes and Hendrix, 1967). Freeman (1978), however, observed a smaller impact of odorants on the frequency changes of the induced waves; he assigned a decisive role to central effects, like motivation and expectance, or to interactions between the bulb and more central brain areas, in determining frequency distribution differences. The frequency of (35–85 Hz) oscillations of the OB were synchronously transmitted to more central brain areas, as seen by simultaneous EEG recordings from the bulb, nucleus olfactorius anterior (AON) or prepiriform cortex (Bressler and Freeman, 1980). At the level of activity of

the bulbar units, stereochemical differences between odorants were reflected by differences in bursting frequencies (Takeuchi, 1968).

Selectivity of epithelial units was regarded as relatively low (see Section 2.4.); however, it has been improved at the level of the bulbar output neurons (Tanabe et al., 1975b; Boulet et al., 1978; Duchamp and Sicard, 1984). Units of the rabbit AON were not able to discriminate between strong odor stimuli, whereas they had such ability, although poorer than the bulbar mitral cells, when stimulated weakly (Boulet et al., 1978). In monkeys, a gradual improvement of the capacity for odor discrimination was observed at the unit level, when recorded from the cells of the OB, the prepiriform cortex or medial portions of amygdala, and the latero-posterior region of the orbitofrontal cortex: cells responding to all (8) or more than one odorants decreased in number, while those activated with one odorant only, increased from 12.5% (OB) to 50% (cortex) (Tanabe et al., 1975a). These experiments clearly show that processing of signals by the higher olfactory centers substantially improve the quality of recognition of smell.

The participation of the OB may not be negligible, however, in the finer discrimination processes. Single unit analysis of the output cell responses to odorant stimulation, suggested that the OB may be the first level for coding the attractive value of an odor (Wilson et al., 1987).

4.4.4. Summary

Most data regarding the functioning of the olfactory bulb are derived from electrophysiological recordings and 2-DG uptake experiments. Rhythmic fluctuations of the EEG waves can be observed on the surface of the OB; this activity is synchronized by respiration and modified by the afferent inputs.

Recordings of the extracellular (field) potentials and unit activity, evoked by antidromic or orthodromic shocks or odorant stimulation shed light on the finer details of the electrical activity of the OB. Olfactory nerve stimulation activates the output neurons, this is followed by a strong hyperpolarization in deeper layers, due to the synaptic (dendrodendritic) excitation of the (inhibitory) granule cells. Antidromic stimulation of the bulb through the LOT results in a similar inhibition of the output cells.

Units in the OB usually change their firing patterns to odorant stimulation; their responses vary according to the quality, concentration and flow rate of the stimulant and depend on the nutritional and motivational state of the subject, as well as on previous experience. Mitral cells have a more acute selectivity in odorant discrimination than epithelial units, although the former is still worse than the units of the more central brain areas.

Various odorants result in characteristically localized foci of elevated 2-DG uptake. This may reflect the enhancement in synaptic activity in regions receiving specific input from a segregated group of receptor cells and being involved primarily in the recognition of a particular type of odorant.

4.5. Development of the bulb

In parallel with those developmental studies on the olfactory epithelium and nerve, the bulbus olfactorius, the central target of the developing olfactory nerves, was also scrutinized. Since the OB is a recipient of a number of centrifugal fibers (see Sections 4.2.3. and 4.3.7.), traveling through the easily accessible olfactory peduncle, the significance of the central or peripheral inputs, in maintaining the normal physiological activity and olfactory performance, can also easily be investigated by denervation studies. As in previous chapters, certain developmental aspects cannot be disregarded when discussing the structural, chemical or functional peculiarities of the OB, but a short account of several features of the ontogeny, which are of particular interest, will be surveyed here. This will be done in two sections, dealing with the normal development, and with impairments and reparative events, induced in various ways. Let me remind the reader of several comprehensive reviews in the field, like those written by Meisami (1979), Pedersen et al. (1985), Brunjes and Frazier (1986), Graziadei and Monti Graziadei (1986) and Mair (1986).

4.5.1. Normal ontogeny

Most data for the formation of the bulb are derived from experiments on rodents and reptiles (see Brunjes and Frazier, 1986). Researchers agree that the bulb, similar to the olfactory epithelium, develops from the olfactory placode of the cerebral ventricle, slightly after the nasal pit becomes recognizable, and from its thicker ventral portion. In toothed whales this peripheral olfactory apparatus (epithelial and bulbar anlage) undergoes rudimentation (Oelschläger and Buhl, 1985). In the rest of the vertebrates a primary proliferative zone (at the ventricular side) supplies the first neurons, migrating outwards and differentiating into mitral cells during the following days. In staged human embryos, the separated olfactory bulb was recognized at stage 19 (cca. 48 days), penetrated by olfactory nerve fibers between stages 21–23 (days 52–57), when mitral cells and bulbar layers were clearly recognized already (Bossy, 1980). Differentiation, outward migration, initial and final orientation and growth of processes by the mitral cells were described in most detail by Hinds (1972*a,b*) and Hinds and Ruffett (1973) for mice. Differentiation of mitral cells was followed by the formation of a second proliferative zone between the mitral cells and the ventricle, and sequential appearance of the other bulbar neurons, still during the prenatal life: the tufted, periglomerular and (in minor part) granule cells (mouse: Hinds, 1968*a,b*, 1972*a,b*; hamster: Grafe, 1981; Grafe and Leonard, 1982; rat: Bayer, 1983; duck: Rebiere et al., 1983) (Fig. 4.77).

Neuron formation continued in rodents after birth: periglomerular cells increased in number most extensively during the first postnatal week (but were proliferating even at the end of the third week), while the overwhelming majority of granule cells were differentiated only during postnatal life (Rosselli-Austin and Altman, 1979). At the level of the subependymal layer, around the lateral ventricle, the rostral migratory stream of proliferating cells (Altman, 1969) supplied new neurons to the granule cell layer also in the adult rat. Smoothly contoured bipolar cells made up the majority of the Golgi-impregnated subependymal cells during the first three postnatal weeks

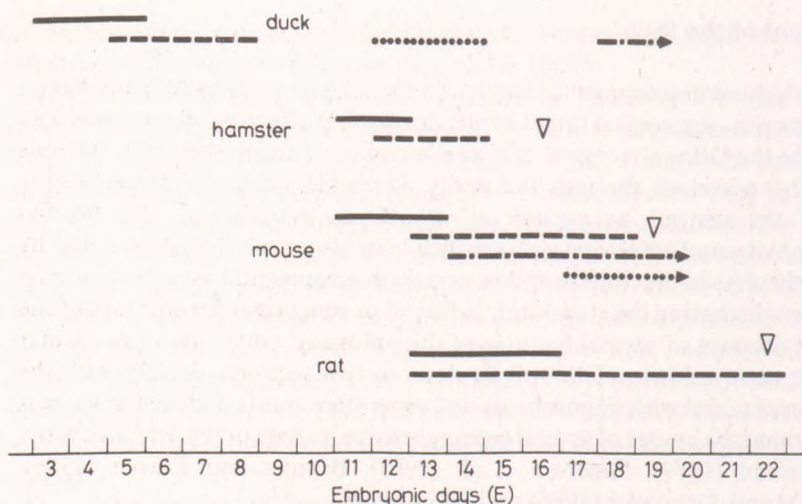


Fig. 4.77. Major times of prenatal formation of olfactory bulb neuron types, as found in duck (Rebiere and Dainat, 1983), hamster (Grafe, 1981), mouse (Hinds, 1968a) and rat (Bayer, 1983). In these rats, some 24% of periglomerular and 9% of granule cells were formed prenatally (not shown). Birth occurred approximately at the times indicated by triangles; ducklings hatched around E26–27. E: embryonic days of life (abscissa).

— : mitral cells; ---- : tufted cells; -.-.-.- : periglomerular cells; : granule cells

(Kishi, 1987). These cells migrated into the GCL and differentiated into the characteristic granule cells. ^3H -thymidine uptake (and turnover) of granule cells was reported even in senescent rats (Kaplan and Hinds, 1977; Kaplan et al., 1985). After appearance, certain changes in the arrangement of neurons, proportions of different neuron types and layer organization were also apparent during postnatal life; these changes could be correlated with the synaptic development and changes in odor-guided behavior (Grafe and Leonard, 1982; Mair et al., 1982; Derivot, 1984; Leon et al., 1984; Rehn et al., 1986).

The peak of final divisions of the neuroglial cells occurred between E17 and postnatal day 10 (P10) in mice (Hinds, 1968a). From P16 onwards, typical astrocytes replaced the radial glial system in rats (Hajos and Gallatz, 1987). The most significant development in vascularization took place between P10–P20 in rats (Singh and Nathaniel, 1975).

The first synapses in the glomerular layer of the OB were established soon after the arrival of the peripheral sensory axons, as seen in embryonic day 14 (E14) in mice (Hinds and Hinds, 1976a,b) and ducks (Rebiere and Dainat, 1981) and E22 in rabbit (Yaku and Kanda, 1982) (see also Section 2.5.1.). Although excitatory (as judged by morphological criteria) synapses appeared first, followed by the inhibitory synapses, the asymmetric type (Gray type 1) axodendritic synapses were preceded by transient symmetric thickenings by some 9–10 h, as found by Hinds and Hinds (1976b). Synapses occurred first in the glomerular layer, followed by the EPL and deeper lamina in mouse (Hinds and Hinds, 1976a), while in duck, the density of synapses in the EPL slightly exceeded the density of those in the glomerular layer at all ages (Rebiere and

Dainat, 1981). Peak density of synapses appeared around the second and third week of life, declining slightly during adulthood in mouse and duck, while the highest density of synapses (and granule cell spines) in the ferret EPL was reported between P60–P120, and interpreted as reflecting the time period for (prey) odor imprinting in these predators (Rehn et al., 1986).

Aging brought about changes not only in number of synapses, proportion of different fibers and neurons, but in intracellular organelles, too: in rats, the layers internal to mitral cells thickened, the size of mitral cells and their nuclei increased. Also, the number of somatodendritic synapses (at least until the age of 24–27 months), and the number of the granule cells increased; although significant strain differences were noted in these tendencies (Hinds and McNelly, 1977, 1979; Forbes, 1984; Kaplan et al., 1985).

Sporadical data for parameters, characterizing the chemical maturation of the bulb, are also available. Between P1–P25 the protein content, DNA and RNA concentration of the bulb was highly elevated (Meisami, 1979; Meisami and Mousavi, 1982); decrease in DNA concentration in adults was slower in this brain area than in others (Mareš, 1986). A high concentration of nerve growth factor was found even in the adult rat OB (Large et al., 1986a).

A few experiments referred to the appearance and development of various transmitter systems in the bulb, as mentioned in the respective preceding sections (Halász and Shepherd, 1983; Brunjes and Frazier, 1986; Margolis et al., 1986). Although, usually various neurotransmitters and their enzymes already appear on the last embryonic days and increase continuously during the first three postnatal weeks (for biochemical data see, e.g., Margolis et al., 1986), their proportion and significance may change in the developing bulb. Tyrosine hydroxylase is expressed in juxtglomerular cells only after they reached the glomerular region (McLean and Shipley, 1988). According to a recent suggestion, the inducing factor for this expression may be the CGRP (Denis-Donini, 1989). About 10% of the TH-immunoreactive juxtglomerular neurons may lose their GABA-like immunopositivity after the first postnatal week (Kosaka K. et al., 1987). A growing importance of the GABA-ergic systems in the deeper layers (during the following postnatal period) is suggested, however, by the significant increase of the number of granule cells. Similarly, the serotonergic system greatly expands (in the glomerular layer) during the early adulthood (McLean and Shipley, 1987b).

Glial markers increased postnatally: glial fibrillary acidic protein (GFAP) content of the OB in newborn rats was only 15% of the adult (Weir et al., 1984), while galactocerebrosides appeared first at P3, followed by the myelin basic protein from P7, in good accordance with the occurrence of the myelinated nerve fibers (Jacque et al., 1985).

Structural and neurochemical maturation provided a substrate for functional and behavioral development. High 2-DG uptake revealed intensive physiological activity first in modified glomerular complex in the dorsomedial aspect of the main OB of mice (Teicher et al., 1980; Greer et al., 1982b) as early as the first postnatal day, but other olfactory-governed activities were assigned already to fetal rats from E20 and young pups (Pedersen et al., 1985). As seen in 2-DG uptake, the adult-like metabolic activity was attained by the end of the second week in rat (Greer et al., 1982b) or third week in

guinea pig (Astic and Saucier, 1983). Unit activity in rats was recorded as early as the first postnatal day by Math and Davrainville (1980b), or during the following early days by others (Shafa et al., 1981; Mair and Gesteland, 1982), while in mice, Schäfer and Schmidt (1985) did not find spontaneously active (mitral) units before P10. Olfactory bulb explants from fetal (E18–E19) mice generated compound action potentials by day 1 *in vitro*, and extracellular potentials from presumptive mitral cells could also be recorded after the first day of explantation (Corrigall et al., 1976). EEG-like waves of the rat OB could be recognized first from P3–P4 by Salas et al. (1969) and Almli et al. (1985), but less regular activity at lower frequencies was present already in P2 in experiments of Schönfelder and Schwartz (1971). In contrast to the weak spontaneous electrical activity in the OB of newborn rats, responses could be evoked by a variety of odorants (Gregory, 1985). EEG waves from cat and guinea pig OBs were reported from P1 (Schwartz and Schönfelder, 1973). Adult characteristics of the EEG waves were attained gradually during the second to fourth postnatal weeks.

4.5.2. Degeneration and regeneration, plastic changes

4.5.2.1. Deafferentation

Injuries to olfactory nerve fibers led to peripheral deafferentation of the olfactory bulb (see Section 2.5.2.), resulting in structural, biochemical and functional impairment of the bulb. Surgical isolation of the OB was used frequently as a tool to recognize the origin of the extrabulbar fiber systems and also to localize the targets of their synaptic terminals (see Sections 2.2., 3.2., 4.2.). Central cuts resulted in degenerating terminals in all layers of the bulb, except ONL, which was, in contrary, affected seriously by peripheral denervation. Degeneration of the ON terminals went through swelling, then shrinkage, darkening, and finally they disappeared (Estable-Puig and de Estable, 1969; Pinching, 1969; Berger, 1971; Pinching and Powell, 1972a), while the postsynaptic thickenings persisted (Pinching, 1969). Olfactory nerve lesion, or removal of the olfactory epithelium were reported to elicit transneuronal degenerative changes in mitral, tufted and periglomerular cells in rabbits, rats (Pinching and Powell, 1971a) and C57/B16J strain of mice (Gozzo and Fülöp, 1984), but different time courses were observed in these species. Moreover, the SEC strain did not exhibit transneuronal degeneration (Gozzo and Fülöp, 1984), while in rabbits, degenerating dendrites were seen even in the piriform cortex at around 2 months following removal of the olfactory mucosa (Pinching and Powell, 1971a).

Morphological changes to peripheral deafferentation were accompanied by loss or impairment of certain biochemical markers of the OB. Carnosine and OMP disappeared from the sensory nerve fibers and terminals (Margolis, 1981); tyrosine hydroxylase, dopamine (DA) and substance P (SP) declined significantly (Nadi et al., 1981; Kawano and Margolis, 1982; Baker et al., 1983, 1984; Kream et al., 1984) and activity of the specific acetylcholinesterase (AChE) decreased, although much less in magnitude than compared to peduncle transection (Meisami and Firoozi, 1985).

Reinnervation brought about return of catecholamine markers to a normal level (Baker et al., 1984). DNA, total cell number (as inferred from the DNA content) and olfactory weight loss was about twice as large following central than after peripheral isolation, underlining the importance of the rostral migratory stream in cell accretion (Meisami and Hamed, 1986) although transection of axons of the projection (mitral and tufted) neurons certainly contributed to this effect. Interruption of the connection of the bulb with the epithelium inhibited the spontaneous and evoked activity of the mitral cells, while reconstitution of the structural link enabled these units to express normal electrical activity and discriminate between odor qualities and different concentrations, as seen in hamsters (Costanzo, 1985).

4.5.2.2. Genetic mutations

Modifications in architecture of the olfactory system of two types of neurological mutant mice were reported: the Purkinje cell degeneration (*pcd*) and the *reeler*.

In pcd mice, among other neurological defects, a slow, progressive degeneration, decrease of wet weight of the bulb and ongoing mitral cell loss were noted by Mullen et al. (1976). They observed certain degenerating fibers in the OB from the 13th week; this extended into a massive degeneration by weeks 38–45. Greer and Shepherd (1982) focussed their study on changes in the OB, and gave a detailed description. Smaller glomeruli, thinner EPL, but unchanged olfactory and granule cell layers, as well as “regular” 2-DG uptake were reported in a light microscopic study, in spite of the extensive loss of mitral cells (month 4: 80% loss; month 8: 96% loss). A decrease in the available postsynaptic sites initiated several reactive changes, as reported later. Peripheral processes of the granule cells followed a more tangential course than that of the normals. Peripheral truncation and decrease in number of gemmules, but not in dendritic bifurcations, was seen by Golgi impregnation of 4-month-old *pcd* mice (Greer, 1987). At around the same age, electron microscopy revealed reduction of the density of typical mitral cell dendrites in the EPL, but an increase in granule cell gemmules, contacting more peripheral (tufted cell) synaptic profiles (Greer and Halász, 1987). It seems likely that reorganization of the granule cell gemmules contributed to the survival and maintenance of normal-like functional activity, as observed earlier (Greer and Shepherd, 1982).

The reeler mutation affects the structure of the olfactory cortex (Caviness and Sidman, 1972; Devor et al., 1975) and also the olfactory bulb (Wyss et al., 1980). In the OB, all layers beneath the glomeruli were reduced in volume (by 26–46%), resulting in a decrease of total volume of the bulb. Mitral and granule cells were less orderly arranged, glomeruli were smaller; the deep periglomerular cells were less in number, while the average cell density in the EPL was 1.5–3 × greater than that of the heterozygous littermates (Wyss et al., 1980). In the case of the *reeler* mouse, however, a general rather than a specific loss of neuron or fiber constituents were apparent, when compared to the homozygous *pcd* mutations, gradually losing contributions from a single (mitral) cell type.

4.5.2.3. Deprivation of development

Several ways of deprivation, other than chemical destruction of the receptor field or surgical interruption of afferent neuronal connections of the OB, were reported and their consequences characterized. Such interventions, as sensory deprivation, low-dose X-irradiation, undernutrition and effects of certain other treatments will be briefly referred to here. Cauterization of one naris of *neonatal* rats and regeneration of the wound led to complete closure of the nose at the respective side, producing unilateral anosmia (Meisami, 1976). This treatment deprived the bulb on the affected side of normal sensory input during the early weeks, which is crucial for the development of the olfactory structures (Meisami, 1979; Brunjes and Borror, 1983). When compared to the control side, the deprived bulbs were significantly reduced in wet weight (by 25%), protein, RNA and DNA content, AChE and Na/K ATPase concentration and in the number of mitral (by 26%) and tufted (by 46%) cells in 25-day-old rats (Meisami, 1979; Meisami and Safari, 1981; Brunjes et al., 1985). Substantial reduction in the thickness of bulbar layers was reported by Benson et al. (1984) in mouse and Brunjes (1985) in the hooded rat. In the mouse, no reduction was observed in the number of mitral cells, but these cells were smaller and, more interestingly, the areal density of their somatic synapses was reduced to half that of the control. In addition, less than normal 2-DG uptake (i.e., reduced metabolic activity) occurred (Benson et al., 1984) (naris closure resulted in a decrease in the 2-DG uptake in the ipsilateral OB, even in adult rats, Sharp et al., 1975). The later developing, "dark" granule cells were also decreased by 33% in mice by P40 (Skeen et al., 1985). In hooded rats, a 33% decrease in the DA content accompanied morphological changes one week after occlusion (Brunjes et al., 1985).

A kind of (bilateral) sensory deprivation of normal composition of the odor environment was achieved by prolonged exposure to odors, or by maintaining odorless conditions. Both resulted in various, although less dramatic, effects (see Section 4.4.1.2.3.).

Undernutrition, protein deprivation during prenatal and the early postnatal period affected the development of the whole body, but the olfactory bulb showed a particular sensitivity in this respect (Morgane et al., 1978). Slowed cell migration in malnourished rats certainly contributed to this special vulnerability of the bulb (Debassio and Kemper, 1985). Volume of the bulb was reduced (Holt, 1917; Morgane et al., 1978). It is very probable (although not examined yet) that the main bulbar neurotransmitter systems were also affected by malnutrition, since parameters of cholinergic and aminergic neurotransmission and glutamic acid decarboxylase (GAD) level in certain brain areas were significantly, but differently, changed (Wiggins et al., 1984). Such a deprivation in the development of the GABA-containing granule cells could well account for the multiple enhancement of mitral cell unit activity in rats after undernutrition (Math and Davrainville, 1980a).

Low-dose X-irradiation impairs neurons most profoundly while they migrate and proliferate (Altman et al., 1968). Since several microneurons, like granule cells of the olfactory bulb, develop mostly after birth, this technique could be used to elicit selective loss of them (Bayer and Altman, 1975; Yanai and Rosselli-Austin, 1978; Struble and Walters, 1982). Although these treatments eliminated over 70% of the

granule cells, no appreciable behavioral changes could be revealed (Walters and Struble, 1978; Yanai and Rosselli-Austin, 1978; Schoonover and Slotnick, 1981). By application of a similar treatment during the prenatal life (*in utero*), vulnerable time periods (see Section 4.5.1. for sequential development of OB neurons) could be selected in order to interfere with the development of other neurons, too (Halász, 1986b, 1987a,b). In this way, a better understanding of the contribution from individual neuron types or transmitter machinery to the information processing in the olfactory system is expected.

Effect of certain chemical agents on development of the bulb or its components have been reported. Amitriptyline, an antidepressant, injected during the first 12 days into rats significantly reduced the thickness of the EPL (Chuah and Hui, 1986). Ethanol exposure (of mouse dams) from E13 and during early postnatal period of their offspring until P21 reduced the growth of the bulb, but did not specifically affect the maturation or synaptogenesis (Nyquist-Battie and Gochee, 1985). Interference with the thyroxine metabolism seemed promising: a precocious maturation in olfactory sensitivity was detected by Brunjes and Alberts (1980) in hyperthyroid rats. In spite of this, in animals treated similarly during the peak developmental period of granule cells, no morphological alteration could be revealed by Golgi analysis of these neurons (Brunjes et al., 1982). Hypothyroidism, however, decreased the wet weight of the OB, thickness of EPL and GCL, number of mitral cells, protein and nucleic acid content in 25-day-old rats, to a degree comparable to that found after unilateral sensory deprivation (Meisami, 1979).

4.5.2.4. Plasticity at the bulbar level

While the regenerative capacity of the nervous system is, in fact, very limited, the olfactory system seems to be its most exceptional region in this respect. The high adaptive capacity and the ability to preserve an appropriate level of activity is not restricted to the epithelium. A continuous renewal of the receptor cells and their terminals in the glomeruli (see Section 2.5.) may have crucial importance. An ongoing supply of the granule cells during adulthood (see Section 4.5.1.) is another important contribution to the "refreshment" of the bulbar circuit. These two renewing systems may provide some factors which enable the whole bulbar machinery to accommodate itself to the changing conditions.

Adaptive changes in the OB may be manifold, including modifications in the structure and neurochemical characteristics, in physiological and behavioral responses. The most intriguing morphological observations, involving plastic changes in various tissues, induced by the ingrowing olfactory nerve fibers were characterized in many detail by Graziadei and his group (Monti Graziadei and Graziadei, 1984; Graziadei and Monti Graziadei, 1985; see also Section 2.5). As regards the OB, reinnervation of the glomeruli in their regular layer (GL), after deafferentation, has also been extensively studied (see Sections 2.5.2, 4.5.2.1.). Partial bullectomy resulted in the formation of ectopic glomeruli, too, which deserve special attention. Such glomeruli were observed in the EPL, MCL and IPL, suggesting that ON fibers may establish contacts also with deep interneurons (Graziadei and Samanen, 1980). Certain

mitral dendrites were also described as reoriented towards these ectopically formed glomeruli. These experiments await further extensions by electron microscopy. Similarly, it is not known yet, whether the granule cell accretion in the adult bulb (Kaplan and Hinds, 1977; Kaplan et al., 1985) is accompanied by an increase in the available target sites on mitral cells, although indirect evidence points to this possibility (Kaplan et al., 1985). Early olfactory learning results in the enlargement of specific glomeruli (Woo et al., 1987), while olfactory deprivation accelerates the spine loss of granule cell dendrites (Apfelbach and Weiler, 1985b), or areal density of the synapses on mitral cell somata (Benson et al., 1984). A suggestion for the rearrangement of the granule dendritic endings, after the loss of their mitral dendritic counterparts was inferred from our electron microscopic studies in pcd mice (Greer and Halász, 1987).

The plastic morphological changes in the bulb are accompanied by adaptive changes in neurochemistry and function. Restoration of the olfactory function is synchronous with the reappearance of the OMP in the sensory afferents and tyrosine hydroxylase in juxtglomerular cells (Baker et al., 1984) (see Section 2.3.2.1.1.). Early olfactory learning increases the 2-DG activity at specific foci (see Sections 4.4.2, 4.4.3) and the response of the output neurons to learned odors is modified (Eckert and Schmidt, 1985; Wilson et al., 1987).

In conclusion, developmental and regenerative plasticity, as seen on the altered structure and function of the OB may be an important factor in the successful adaptation during a species life.

4.5.3. Summary

In the developing bulb (originating from the olfactory placode) mitral cells differentiate first, followed by tufted cells and various interneurons. A significant proportion of periglomerular cells and the majority of granule cells are acquired postnatally. The olfactory nerve axons and output neurons form the first synapses (in the GL), then contacts appear in increasing number in the EPL and deeper layers. From early adulthood onwards, a decrease in the density of synapses is apparent. Structural development is accompanied, or followed shortly by, neurochemical, functional and behavioral maturation.

Deprivation of the bulb of its normal sensory input, undernutrition, X-irradiation as well as certain types of genetic mutations may cause serious losses in the development of the normal architecture and function, while the recovery also reveals the unique regenerative and plastic capacity of this part of the brain. Consequently, the OB with its connections can be used as a suitable model system for studies of the principles of structural, neurochemical and functional restoration in the central nervous system.

CHAPTER 5: THE OLFACTORY TRACT

In the previous chapter, neuron types of the OB were classified into local circuit neurons (limited to intrinsic activity), and those, projecting out of the bulb. These latter neurons included several different nerve cells in lower vertebrates (see Section 4.2.2.1.), but the most prominent neurons of this kind were the mitral and tufted cells. Since bundles and terminals of the sensory nerve occupied the superficial aspect of the bulb, axons of the output neurons turned deep, and in most species, started to bundle already in the granule cell layer (see, e.g., Fig. 4.64). These fascicles constitute the olfactory tract. In the forthcoming sections a brief account of the morphology, distribution, neurochemistry and developmental questions of the tract will be given.

5.1. Morphology and distribution

As has been referred to already, axons of the output neurons become myelinated shortly after leaving the cell somata. Indeed, the olfactory tract was found to be composed mostly, although not exclusively, of myelinated fibers. As seen in the relatively compact lateral olfactory tract (LOT) of the rat, the myelinated fiber composition was not homogeneous: axons in the lateral part had $1.6 \pm 1.0 \mu\text{m}^2$ mean cross-sectional area, while those in the medial part measured only $0.7 \pm 0.6 \mu\text{m}^2$ (Price and Sprich, 1975). Presumed collaterals, leaving the tract laterally or medially had a $0.55 \pm 0.35 \mu\text{m}^2$ mean cross-sectional area in this study. Uneven fiber calibers were recognized through differences in conduction velocities: fast conducting (thick) fibers supplied the anterior, slow conducting (thinner) axons the more caudal regions of the basal forebrain in ferret (Dennis and Kerr, 1975).

Axonal outputs from the bulb varied widely in their organization, course and extension in distribution through the vertebrate phylum (Allison, 1953b; Nieuwenhuys, 1963, 1967; Scalia, 1968; MacLeod, 1971). While in mammals and many submammalian species a compact, lateral bundle was the most prominent pathway of the bulbofugal axons, a more or less separate medial tract was also observed frequently, especially in lower vertebrates, like fish (Nieuwenhuys, 1963, 1967), which may also have a special function (Döving and Selset, 1980; Ebbeson et al., 1981; Demski and Dulka, 1984; Levine and Dethier, 1985). The pigeon had a diffuse olfactory tract (Rieke and Wenzel, 1975); asymmetry of the whole (body and) brain of the winter flounder was reflected by different thickness (and extension of the projection field) of the olfactory tract (Prasada Rao and Finger, 1984). Toothed whales lack olfactory tracts (Turner, 1891; Jacobs et al., 1971; Oelschläger and Buhl, 1985).

As the motor, associational and non-olfactory sensory regions expanded during phylogenesis, the proportion of the brain, reached by the olfactory tract decreased. In addition, when compared to suggestions by the early investigators, the presence of a

more limited projection from the olfactory bulbar complex to defined anatomical regions could only be supported by later experiments. For example, no direct bulbar projection could be demonstrated in the hippocampus (Brodal, 1947). As a consequence, it seems justified that the term "rhinencephalon" (nose brain) (stressing the primary significance, for olfactory function, of certain brain areas, which are known now as limbic structures) is used less in more recent studies (Shepherd, 1979, 1983). Secondary and tertiary olfactory centers are involved, however, in the control of visceral motor functions and expression of emotions, through their limbic connections. This (limbic) system receives inputs from other sensory (like auditory, visual and somatic) modalities as well, in addition to those from the olfactory system.

Although there were variations in projection areas, olfactory tracts supplied a substantial part of the forebrain in almost all vertebrates, as reported by morphological studies of the normal brain, or after using the techniques of experimental degeneration, transport of tracers or evoked potentials. Accordingly, the pedunculus (anterior olfactory nucleus and tenia tecta), olfactory tubercle, nucleus of the LOT, piriform and periamygdaloid cortices and certain cortical amygdaloid nuclei and the ventrolateral entorhinal area were the most frequently reported brain regions to receive direct inputs from the main olfactory bulb (Allison, 1953*b*; Powell et al., 1965; White, 1965; Dennis and Kerr, 1968; Ferrer, 1969; Heimer, 1968; Scalia et al., 1969; Price and Powell, 1971; Northcutt and Royce, 1975; Skeen and Hall, 1977; Scott et al., 1980; Ojima et al., 1984; Room et al., 1984) (Figs 5.1 and 4.1). In pigeon, projection to the amygdala was restricted to a small portion of the dorsomedial part, while in turtle it extended to the entire mediolateral and rostrocaudal region (Reiner and Karten, 1985). In fish, the bulb projected even to the dorsal forebrain and preoptic areas (Ebbeson et al., 1981; Murakami et al., 1983; Bartheld et al., 1984; Levine and Dethier, 1985). A strict point-to-point projection from the bulb to these secondary centers could not be revealed (Lohman and Mentink, 1969; Price, 1973; Price and Sprich, 1975). Although individual bulbofugal afferents were seen to give collaterals to several cortical areas, they had dense arborization only at specific places, suggesting selectivity in termination (Ojima et al., 1984).

A major question related to this field was the contribution of tufted cell axons to the tracts. It seems likely that part of tufted cells do not project out of the bulb (see Section 4.2.2.2.2.), but the remaining category of these cells may convey information to more caudal brain areas through the olfactory tracts. Most tufted cell axons were suggested by earlier investigators to travel to the contralateral olfactory bulb through the anterior commissure (Cajal, 1911; Allison and Warwick, 1949), but the validity of this assumption could not be kept in light of more recent experiments (Powell et al., 1965; Heimer, 1968; Haberly and Price, 1977). Allison and Warwick (1949) calculated 45,000 mitral cells and 60,000 LOT fibers in each side of the rabbit brain, admitting that axons of some tufted cells might also be included in this pathway. Nicoll (1970) was able to drive tufted cells in the EPL of rabbits by LOT stimulation. The distribution of tufted cell axon terminals was different from those of mitral cells: most of them innervated the rostral part of the olfactory cortical areas only (Haberly and Price, 1977; Scott, 1981; Schneider and Scott, 1983). Deep tufted cells were suggested, however, to reach even the most posterior secondary olfactory centers (Schneider and Scott, 1983).

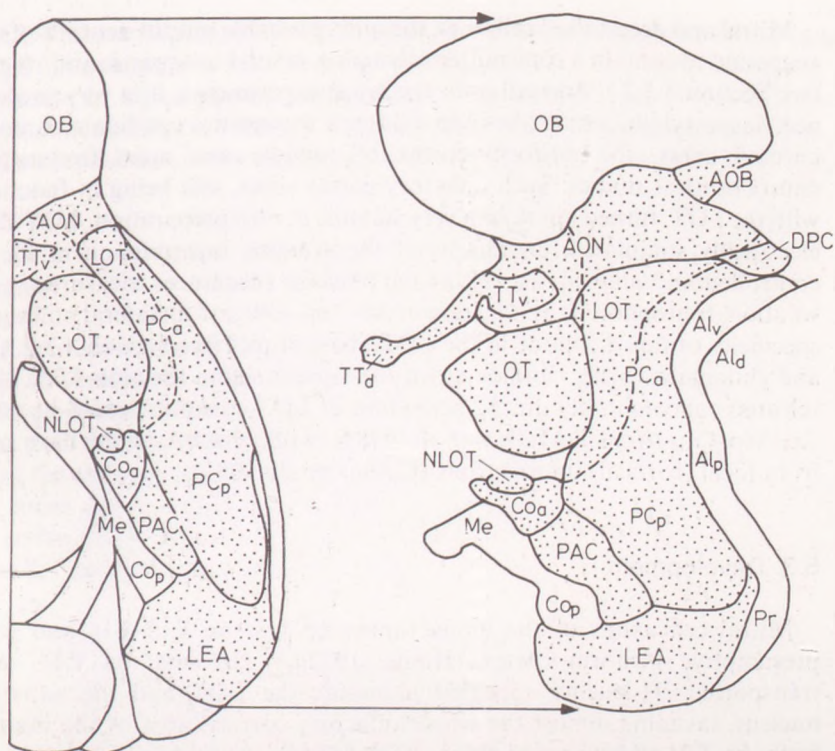


Fig. 5.1. Distribution of the centrifugal fibers from the main olfactory bulb on the basal forebrain of the rat (from Price, 1985). Dotted areas represent projection field of mitral and tufted cell axon terminals on the ventral forebrain. On the right, unfolded map shows more accurately, segmentation of the secondary olfactory centers, as seen on the surface. Al_d, Al_p and Al_v: dorsal, posterior and ventral agranular insular area, respectively; AOB: accessory olfactory bulb; AON: anterior olfactory nucleus; CO_a: anterior cortical nucleus of the amygdala; CO_p: posterior cortical nucleus of the amygdala; DPC: dorsal peduncular cortex; LEA: lateral entorhinal area; LOT: lateral olfactory tract; Me: medial nucleus of the amygdala; NLOT: nucleus of the lateral olfactory tract; OB: olfactory bulb; OT: olfactory tubercle; PAC: periamygdaloid cortex; PC_a: piriform cortex, pars anterior; PC_p: piriform cortex, pars posterior; Pr: perirhinal cortex; TT_d: tenia tecta, pars dorsalis; TT_v: tenia tecta, pars ventralis. Reproduced with permission

5.2. Neurochemistry

According to morphological studies, in addition to axons of (at least) two types of neurons from the OB, several centrifugal fibers from different sources of origin join, or travel close to, the LOT. Consequently, this must result in heterogeneity of the neurochemical and transmitter histochemical characteristics of this pathway. In the absence of studies, focussed exclusively on the tracts itself, we can gain some knowledge of its composition only from experiments performed at the site of their origin or termination. With regard to the bulbofugal axons, this means studies on mitral and tufted cells (in the bulb) and neurochemical results on their olfactory cortical projection areas (see below), while for those of centrifugal afferents, neurochemical data can be found in Table 4.2. and in the following chapter.

Mitral and deep tufted cells, i.e., the most probable output neurons of the OB, were suggested to contain a transmitter substance, related to aspartic and/or glutamic acid (see Section 4.3.2.). According to the latest experiments, it is very probable that N-acetylasparyl glutamate (Ac-Asp-Glu) is a transmitter candidate. Among olfactory cortical areas, the piriform cortex of rodents was most frequently used for neurochemical studies. Such olfactory cortex slices, still being in functional contact with the LOT, turned out to be a very suitable *in vitro* preparation. LOT fibers could be electrically stimulated, conditions of the synaptic transmission in the cortex slice controlled and aligned, as well as the released substances measured in the bathing solution. Ionophoretic application of Ac-Asp-Glu and unilateral bulbectomy proved specificity of this dipeptide in the LOT fibers of rats (see Section 4.3.2.2.). Aspartate and glutamate, earlier candidates for the same function (Section 4.3.2.1.) which were released concomitantly during activation of LOT synapses, could be metabolites of Ac-Asp-Glu (French-Mullen et al., 1985), while GABA might have been released from local (cortical) interneurons (Collins et al., 1981).

5.3. Development

Mitral cell axons of the mouse appeared first on E13-E14 and grew into the presumptive piriform cortex (Hinds, 1972*a,b*). In rats, on E17, anterogradely transported ³H-leucine appeared alongside the LOT and the anterior olfactory nucleus, invading almost the whole olfactory cortical area of the brain still before birth, by E21 (Schwob and Price, 1978, 1984*a*). As seen in 3-day-old hamsters, the medial quadrant of the bulb projected first to the cortex, and was equalized with the remaining quadrants by P8 (Grafe and Leonard, 1982). Degeneration studies at an early age in hamsters also revealed supply of the rostral olfactory tubercle and rostral amygdala first (Leonard, 1975).

By electron microscopy, presynaptic specializations within the LOT and underlying cortex were frequently seen in E18-E19 rats, and were suggested to initiate mature synapse formation (Newman-Gage and Westrum, 1984), which was seen to be completed by the end of the second postnatal week (Westrum, 1975*b*). Aging was accompanied by the regression of pyramidal neuron dendrites in layer Ia, presumably because of the reduction in peripheral input (Curcio et al., 1985). Extracellular cortical unit responses to orthodromic electrical stimulation of LOT fibers were evoked in newborn rats, differing in several aspects from responses of the more mature brain: thresholds were higher, conduction velocities slower and the inhibitory systems (as seen by stimulation with paired shocks) were still very weak (Schwob et al., 1984).

Bulbectomy or transection of the LOT resulted in degeneration, the severity and extension of which depended highly on age. LOT section in young hamsters brought about dense sprouting of axonal arbors proximal to cuts (Devor, 1976). LOT transection at P3 (but not at P9) was followed by reinnervation of the regions even caudal to the lesion (Sijbesma and Leonard, 1986). This was explained by the different glial response (less extensive astrocytic hypertrophy and survival of radial glial cells in hamsters, lesioned earlier). Neonatal olfactory bulb removal resulted in occupation of the available cortical postsynaptic sites by intracortical axons, in the absence of the

arrival of LOT fibers (Westrum, 1975a; Friedman and Price, 1986a), while the same experimental intervention in rats from the youngest pups to P21 animals caused a gradual increase in transneuronal degeneration of pyramidal cells of the olfactory cortex (Friedman and Price, 1986a,b). This was interpreted by the same authors as a consequence of reduction in the sprouting capacity of the intracortical axons in older animals.

5.4. Summary

Principal neurons of the olfactory bulb project onto a significant proportion of the forebrain in all vertebrates, although this projection is usually less extensive at higher levels of development. Axons of the deep output neurons may reach more caudal targets. The tract is already functional at birth, although further development is taking place during the early postnatal days. This is also the period when reinnervation of the projection areas can be observed following transection of the olfactory tract. An olfactory cortex slice with the connecting tract offers a very suitable *in vitro* preparation for neurochemical studies.

CHAPTER 6: HIGHER OLFACTORY CENTERS

Signal output from the olfactory bulb, the brain area specialized mainly for processing the olfactory information, is conveyed through the olfactory tract to the basal forebrain regions, as discussed in Chapters 4 and 5. The first part of this Chapter gives an account of these primary olfactory cortical areas (secondary centers), while the second part discusses the tertiary olfactory centers.

A significant portion of the primary olfactory cortical areas has reciprocal connections with the main OB. In addition, the secondary centers are interconnected with each other (Luskin and Price, 1983*a,b*), receiving olfactory signals processed already by another secondary center. These connections are depicted for rats in Fig. 6.1. Further pathways from the secondary centers are sent to tertiary centers, which may also be connected reciprocally to the primary olfactory cortical areas. Finally, the secondary centers may be recipients of projections from those brain areas, which send "centrifugal" fibers to the main olfactory bulb (but have no direct bulbar input), like the monoamine systems of the brainstem, cholinergic nuclei of the diagonal band and preoptic areas, hypothalamic peptidergic systems and others. Mapping of these systems, with regard to their structural, neurochemical and functional specificities is one of the most challenging tasks of recent neurobiology, because they are still far from a detailed description and clear understanding. An increasing knowledge has been gained, however, about the sources of the bulbofugal fibers. In this respect, the reader is referred to Sections 4.2.3.1.2. and 4.2.3.1.3. and especially to Table 4.2.; for a diagram summarizing this, see Fig. 6.2.

Although an increasing number of studies deals with the neurochemical characteristics of the olfactory cortical areas (see experiments on olfactory cortex slice preparations, designed to reveal LOT transmitters, Section 4.3.2.1.) and with centrifugal afferents of the bulb, traveling through the basal forebrain, little is known on the endogenous transmission. However, GABA has been suggested as an inhibitory transmitter of the local interneurons (Collins et al., 1981) and DA receptors have also been revealed (Collins et al., 1985) in the cortical slice. Due to the lack of detailed neurochemical results, the brief survey of the secondary and tertiary olfactory centers in the following two sections, will be based mostly on morphological findings.

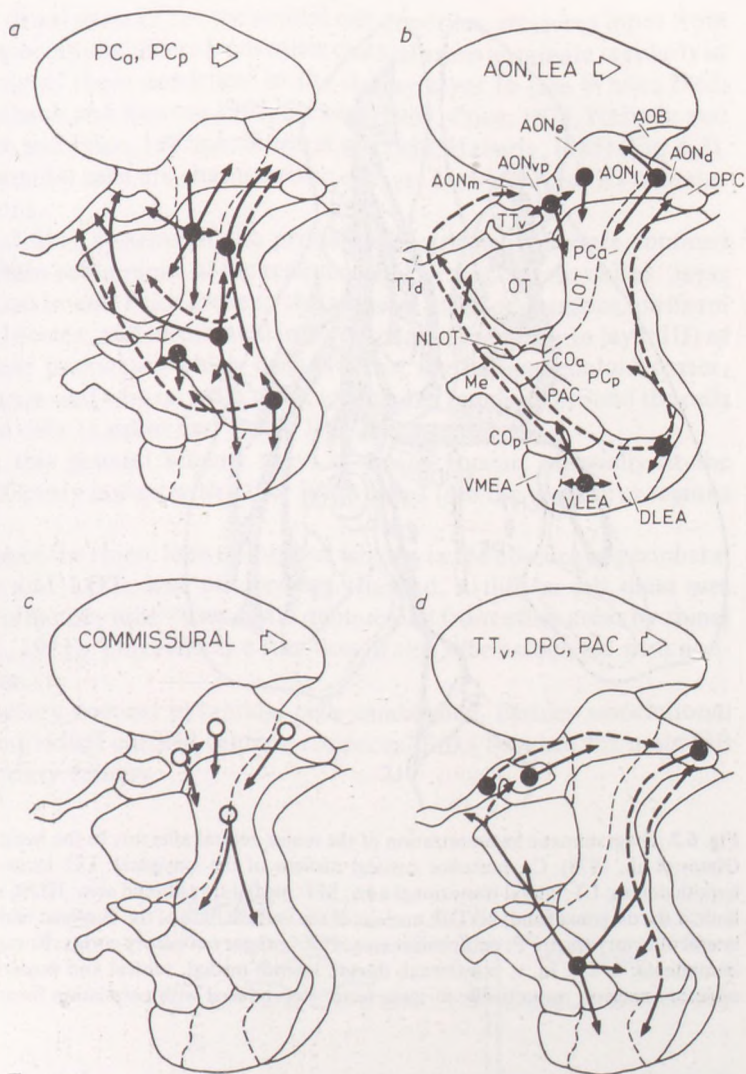


Fig. 6.1. Associational pathways of the secondary olfactory centers in the rat (from Luskin and Price, 1983a). Filled circles represent origin of ipsilateral connections, while open circles, those from the contralateral brain areas. Arrows show heaviest cortical projections. Dashed lines mark the course of pathways towards a target, but without projecting heavily into the area they pass through. *a* and *b* illustrate ipsilateral, *c* contralateral projections of the layer Ib fiber system, while *d* shows projections of the layer II—deep Ib fiber system of Luskin and Price (1983b). AOB: accessory olfactory bulb; AON: anterior olfactory nucleus; AON_d, AON_e, AON_m, AON_i and AON_{vp}: pars dorsalis, external, medialis, lateralis and ventroposterior of the anterior olfactory nucleus, respectively; CO_a: anterior cortical nucleus of the amygdala; CO_p: posterior cortical nucleus of the amygdala; DLEA: dorsal part of the lateral entorhinal area; DPC: dorsal peduncular cortex; LEA: lateral entorhinal area; LOT: lateral olfactory tract; Me: medial nucleus of the amygdala; NLOT: nucleus of the lateral olfactory tract; OT: olfactory tubercle; PAC: periamygdaloid cortex; PC_a: piriform cortex, pars anterior; PC_p: piriform cortex, pars posterior; TT_d: tenia tecta, pars dorsalis; TT_v: tenia tecta, pars ventralis; VLEA: ventral part of the lateral entorhinal area; VMEA: ventromedial part of the lateral entorhinal area. Reproduced with permission

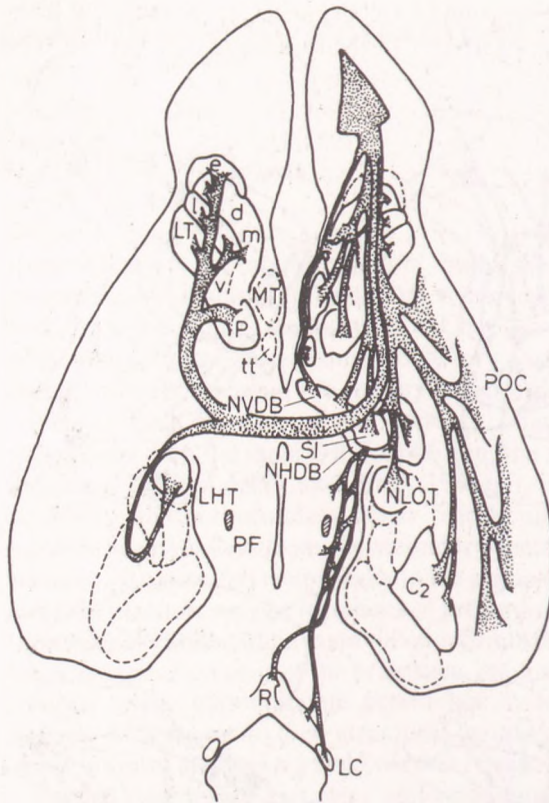


Fig. 6.2. Diagrammatic summarization of the major central afferents to the main olfactory bulb (from de Olmos et al., 1978). C₂: posterior cortical nucleus of the amygdala; LC: locus coeruleus; LHT: lateral hypothalamus; LT: lateral transitional area; MT: medial transitional area; HDB: nucleus of the horizontal limb of the diagonal band; NVDB: nucleus of the vertical limb of the diagonal band; NLOT: nucleus of the lateral olfactory tract; PF: perifornical area; POC: primary olfactory cortex; R: raphe nuclei; SI: substantia innominata; e, d, l, m, v, p: external, dorsal, lateral, medial, ventral and posterior parts of the anterior olfactory nucleus, respectively; tt: tenia tecta. Reproduced with permission from the Publisher

6.1.Secondary centers

6.1.1. Lamination patterns

The cortical areas, receiving peripheral afferents from the main OB follow a general plan of construction which shows, at first glance a superficial fiber layer and deeply located cell somata. The most remarkable area of the primary olfactory cortex (piriform and peririform area) and its closest surroundings show trilaminar structure. The most superficial layer I can be divided into two sublaminae: layer Ia lies on the

surface, and harbors distal parts of the pyramidal cell dendrites, receiving input from the main OB, while associational fibers from other cortical areas terminate regularly at the proximal segments of these dendrites, in the deeper layer Ib (see White, 1965; Heimer, 1968; Biedenbach and Stevens 1969; Stevens, 1969; Price, 1973; Haberly and Price, 1978*a,b*; Luskin and Price, 1983*a,b*; Skeen et al., 1984; Haberly, 1985) (Fig. 6.3). Closely apposed pyramidal cells are characteristic of layer II, while layer III contains more scattered neurons.

Many associational fiber systems of the primary olfactory cortex were confined predominantly to certain sublaminae of the recipient area in rats. The so-called "layer Ib fiber system" of Luskin and Price arose in the anterior olfactory nucleus, piriform cortex and entorhinal cortex, and terminated in layer Ib (and frequently in layer III) of the target, while those pathways, which originated in the periamygdaloid cortex, dorsal peduncular cortex and ventral tenia tecta, terminated regularly around the cells of layer II pyramidal cells (Luskin and Price, 1983*a,b*).

Divergences from this general scheme may, of course, occur, especially at the boundaries of the olfactory cortex with other brain areas (see the respective regions below).

Distinct lamination of the rhinic lobe of toothed whales, in the absence of peripheral olfactory apparatus and LOT, was not present. Instead, a diffuse cell mass was apparent filling the "olfactory lobe" tissue, still demarcated from other areas by rhinal sulci (Jacobson et al., 1971). This reflects a function of this lobe associated with non-olfactory activity, too.

Axons of the olfactory cortical pyramidal cells established, besides associational pathways between individual cortical centers, reciprocal links between the main OB and almost all secondary centers.

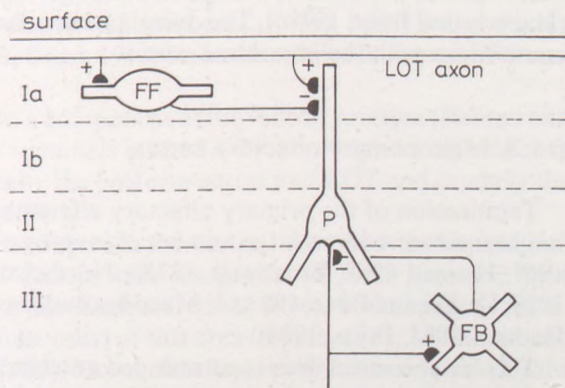


Fig. 6.3. Asymmetric (presumed excitatory) (+) and symmetric (presumed inhibitory) (-) synaptic inputs to pyramidal neurons of the piriform cortex in rats (from Haberly, 1985). Symmetric synaptic connections in the plexiform layer (Ia and Ib) are supposed to originate from superficial interneurons, which may provide a feed-forward (FF) inhibition, while those on the axon initial segment and deep dendrites (in layers II and III) could be endings of deep interneurons, exerting a feed-back (FB) inhibition on pyramidal cell activity. P: pyramidal cell; LOT: lateral olfactory tract. Reproduced with permission

6.1.2. Peduncular structures

The anterior olfactory nucleus (AON), which is the most rostral bulbar projection field in the cortex, lies adjacent to the main and accessory OB; cell somata of the AON may even intrude in between bulbar laminae. Cytoarchitectonically the AON seems to be a transitional area towards the main portion of the olfactory cortex. The rostral AON has two main layers, a superficial plexiform and a deep cellular layer, which differentiates gradually into a trilaminar structure more caudally (in part posterior), where it is apposed to and connected by the anterior commissure (AC).

The most significant contribution to the contralateral OB, through the AC, was reported to come from the pars externa of the AON (heaviest OB afferents were traced to the same subdivision of the AON) (see Section 4.2.3.1.2. and Table 4.2.). Other subdivisions (pars posterior, lateralis, ventralis, medialis and dorsalis) projected primarily onto the ipsilateral main OB and other olfactory cortical areas (Broadwell, 1975*b*; Shafa and Meisami, 1977; Davis et al., 1978; Haberly and Price, 1978*b*; Luskin and Price, 1983*a*; Shipley and Adamek, 1984; Macrides et al., 1985). A number of AON neurons, projecting to the contralateral main OB may send thin collaterals also to the ipsilateral bulb, as seen in rabbits (Mori et al., 1979). Projections between the OB and pars externa of the AON were topographically organized (Luskin and Price, 1983*a*; Schoenfeld and Macrides, 1984; Schoenfeld et al., 1985; Scott et al., 1985*a*).

Several smaller cell groups, forming a transitional zone towards the main region of the primary olfactory cortex, have also been described (de Olmos et al., 1978; Luskin and Price, 1983*a*). In the rat olfactory peduncle, in continuation with the AON, Price and co-workers (Haberly and Price, 1978*b*; Luskin and Price, 1983*a*; Price, 1985) described such areas as the ventral and dorsal parts of tenia tecta (anterior hippocampal rudiment) and the dorsal peduncular cortex. The dorsal tenia tecta had no bulbar afferents, since the two other areas had reciprocal connections with the main OB, in addition to their associational pathways with other secondary olfactory centers (Haberly and Price, 1978*b*). The dorsal peduncular neurons had prominent reciprocal connections with the entorhinal cortex.

6.1.3. Main primary olfactory cortex

Termination of the primary olfactory afferents in the central basal forebrain area has been observed by a large number of investigators (Valverde, 1965; Nieuwenhuys, 1967; Heimer, 1968; Broadwell, 1975*a*; Northcutt and Royce, 1975; de Olmos et al., 1978; Luskin and Price, 1983*a,b*; Macrides and Davis, 1983; Haberly, 1985; Levine and Dethier, 1985; Price, 1985).

This basal cortical area is surrounded anteriorly by the peduncular cortical areas, laterally by the rhinal sulcus (and so, by neocortical areas), caudally by the entorhinal cortex and medially by the amygdaloid cortex and the olfactory tubercle. This area may be divided into more rostral (anterior, prepiriform) and caudal (posterior, piriform) parts, both having a trilaminar structure and a similar basic arrangement of synaptic inputs onto pyramidal cells (Haberly, 1985; see also Fig. 6.2).

Cell types of the opossum piriform cortex were characterized recently in detail by light- and electron microscopic studies (Haberly, 1983; Haberly and Feig, 1983). Both LOT and associational terminals had asymmetric synaptic contacts and contained round synaptic vesicles (Haberly and Behan, 1983).

Associational fibers arising in the piriform cortex terminated in layer *Ib* (and were classified accordingly as a component of the "layer *Ib* fiber system", Luskin and Price, 1983*a,b*) in all of their projection fields. Pyramidal cells in the rostral piriform cortex projected chiefly to the ipsilateral AON, tenia tecta and olfactory tubercle, while those in the medial and posterior part, mainly to the neighboring caudal piriform and entorhinal area, but also to the peduncular region (see Fig. 6.1). The pyramidal cells projected back heavily to the ipsilateral main olfactory bulb (Davis and Macrides, 1981; Luskin and Price, 1983*a*).

Beyond the experiments on the olfactory cortex slice—LOT preparation (see Section 4.3.2.1.), electrical stimulation of the primary olfactory cortex helped to elucidate the organization of mitral and tufted cell projections, while orthodromic stimulation of the LOT and other inputs contributed to the understanding of functional aspects of this cortical field (for review see Haberly, 1985). Unit responses to odorant stimulation were recorded in the piriform cortex (Haberly, 1969; Tanabe et al., 1975*a*; Nemitz and Goldberg, 1983) and reflected increasing selectivity of the cortical neurons in discrimination of odorants, when compared to the more peripherically located (bulbar or epithelial) olfactory units.

6.1.4. Olfactory tubercle (OT)

This region is clearly delineated from the surrounding basal brain areas, as seen in amphibians to mammals. In cyclostomes, selachians and dipnoans the rostral part of the basal forebrain may be equivalent to the OT in higher vertebrates (Allison, 1953*b*). In lower mammals, it is highly developed (Kappers et al., 1936), but it is also readily recognized in rodents (Calleja, 1893; Cajal, 1911; Valverde, 1965; Heimer and Wilson, 1975).

The OT was considered recently as a component of the ventral striatum (Heimer and Wilson, 1975), but it is supplied by terminals of the LOT, too. Neighboring olfactory areas are anteriorly the AON, laterally the piriform cortex and LOT and caudally the amygdaloid cortex (see Figs 5.1 and 6.1).

Two main parts of the OT were observed: the cortical part and the cap region. Dorsal to the latter, the islands of Calleja were recognized (in adult humans, these islands could not be demonstrated; see Kappers et al., 1936), containing the most homogeneous cell population (granule cells) of this area (White, 1965; Millhouse and Heimer, 1984; Meyer and Wahle, 1986). The surface layers were characterized by the presence of a number of neurons, which differed widely in Golgi preparations of the rat (Millhouse and Heimer, 1984) and cat (Meyer and Wahle, 1986).

Medially the OT rather resembled the striatal organization, while the lateral part, especially in the vicinity of the LOT, revealed a typical, trilaminar olfactory cortical lamination (see Section 6.1.1.). At least three types of synaptic terminals, two of Gray type 1 and one of Gray type 2, were present in the cortical region of the rat OT

(Anderson and Westrum, 1972). Although the OT received associational pathways from various other secondary olfactory centers, like the piriform cortex, lateral entorhinal area and the amygdaloid complex (see Fig. 6.1), no reciprocal connections were sent, in turn, to any of the olfactory cortical areas or main OB (Haberly and Price, 1978a; Luskin and Price, 1983a).

While the role of OT in olfaction is obscure as yet, a number of various neurotransmitter systems, characteristic of both the striatal and pallidal systems, have been traced here. The cortical part, similarly to the striatum, displayed SP-like, NPY and opioid immunoreactivity (Wahle and Meyer, 1986). The cap region contained cholinergic and peptidergic somata (but no labeled cells in the islands of Calleja) as well as extrinsic fibers with opioid and SP-like immunoreactivities, as reported in this study. A strong dopaminergic projection terminates in the OT, originating in the ventral tegmentum (Ungerstedt, 1971). Pyramidal cells and their dendrites may be the direct target for this pathway, as seen on the distribution of the dopamine-sensitive adenylate cyclase (Krieger et al., 1977). In addition, Fallon et al. (1978) observed a topographically organized dopaminergic projection from the substantia nigra—ventral tegmental area system to the islands of Calleja. Markers for the presence of AChE, ChAT, GAD and TH were also reported (Gilad and Reis, 1979; Wahle and Meyer, 1986).

6.1.5. Amygdaloid complex

Development of the amygdaloid complex differs in various vertebrates; its clear distinction can be recognized in reptiles and higher levels (Allison, 1953b). It is a brain region which is shared by projections from the accessory olfactory system (projecting to the mediocortical amygdaloid region, posterior cortical nucleus and bed nucleus of the accessory olfactory tract; see also Chapter 7) and the main olfactory bulb. Accordingly, in rats, the nucleus of the lateral olfactory tract (nLOT), anterior cortical amygdaloid nucleus and the periamygdaloid cortex were regarded as centers of the main olfactory system (Haberly and Price, 1978a; Krettek and Price, 1978; Luskin and Price, 1983a), similar to homologous amygdaloid subdivisions in other mammals (Broadwell, 1975a; Scalia and Winans, 1975). These structures, related to the main OB, were bound laterally by the piriform cortex, posteriorly by the lateral entorhinal area and medially by the medial amygdaloid nucleus in rat.

Cytoarchitectonics and connections of all amygdaloid nuclei were described in much detail by Krettek and Price (1977a,b, 1978) through studies of ³H-amino acid transport and the nLOT by Millhouse and Uemura-Sumi (1985) with the rapid Golgi method. The periamygdaloid cortex and nLOT had a characteristic trilaminar structure, although layers II and III were less developed in the periamygdaloid cortex than in the main part of the primary olfactory cortical area (Krettek and Price, 1978). The biggest neurons of the nLOT were situated deep, in layer III; they were categorized into several subclasses (Millhouse and Uemura-Sumi, 1985).

The periamygdaloid cortex projected to several other olfactory cortical areas (entorhinal and piriform cortices, OT). Projections from nLOT were traced bilaterally in the OT and medial edge of the piriform cortex; the anterior cortical nucleus of

amygdala terminated also in primary olfactory and neocortical areas. In the recipient regions, these latter projections terminated in both superficial and deep layers; the nLOT fibers in and around the islands of Calleja (in the OT), while those from the anterior cortical nucleus could be traced to both layers Ia and Ib in rat (Luskin and Price, 1983a,b). Out of these three areas, only the anterior olfactory nucleus lacked reciprocal connections with the OB.

Lesions in the amygdala produced only minor changes in discrimination of odorants and olfactory-related behavior, as seen in monkeys (Schuckman et al., 1969) or rats (Slotnick, 1985). This would suggest that olfactory inputs into these, relatively posterior areas bear only a minor significance in direct processing of smell information. They could, however, contribute to regulation of feeding, because of their extensive connections with the hypothalamic centers. In support of this view, alterations in responses to odorants were recorded from amygdala (and OB) units, when rats were injected with insulin (Cain, 1975).

6.1.6. Lateral entorhinal area (LEA)

The lateral part of the entorhinal cortex was reported to be the most caudal part of the basal forebrain (see Fig. 5.1) which received afferent olfactory tract fibers from the main OB (White, 1965; Heimer, 1968; Price, 1973; Scalia and Winans, 1975; Davis et al., 1978), although earlier there was an uncertainty about its close relation to the primary olfactory cortex (Price and Powell, 1971). This was because of its heterogenous cytoarchitectonics. In rats, at the antero-lateral boundary region, no stratification of layer III could be recognized, while the more caudal region of LEA had a typical trilaminar arrangement. LOT fibers terminated in layer Ia, but the LEA did not project back to the bulb (Luskin and Price, 1983a). Terminals of the associational projections from the LEA to other secondary olfactory centers were found in layer Ib of the respective regions (Luskin and Price, 1983a,b), but an even heavier projection was observed from LEA to the hippocampal formation. In turn, fibers from the piriform cortex, lateral amygdaloid nucleus and periamygdaloid cortex terminated in restricted subdivisions of the LEA (Krettek and Price, 1977b).

The LOT fibers synapsed on GAD-containing local circuit neurons in layer Ia (in addition to dendrites of pyramidal cells), which were suggested to provide the neurochemical basis for feed-forward inhibition in this layer (Haberly, 1985; Wouterlood et al., 1985). Lesions in LEA of rats, i.e., denervation of the hippocampus, shed some light on the importance of this system in olfaction: formation of the olfactory memory was impaired (retention disappeared 3 h after acquisition) (Staubli et al., 1986).

6.1.7. Development

Neurogenesis in the rat primary olfactory cortex was followed by ^3H -thymidine autoradiography (Bayer, 1980, 1983, 1986a,b). In general, a caudal to rostral neurogenetic gradient was recognized. Two other gradients were also reported by

Bayer: primary olfactory cortical areas had an "inside-out" pattern during proliferation (deep to the superficial neurogenetic gradient), while regions with "ganglionic cytoarchitectonic" organization (amygdaloid nuclei and peduncular structures, like the ventral tenia tecta) showed the opposite direction. The "lateral transitional area" in the peduncle featured no neurogenetic gradient.

The majority of neurons originated between E15–E21 in AON, E14–E19 in the transitional peduncular areas, E14–E20 in the dorsal peduncular cortex, E14–E18 in the piriform area and E1–E17 in the periamygdaloid cortex. The appearance and increase of neurons in the respective areas was correlated with the arrival of peripheral projections through the olfactory tracts (Bayer, 1983).

Structural development of the projections was studied by many authors and was followed by studies using transport of tritiated amino acids (Schwob and Price, 1978) or by combination of this technique with HRP transport (Schwob and Price, 1984*a,b*), degeneration argyrophylia (Leonard, 1975) and with Timm staining (Friedman and Price, 1984); the latter was suggested to signal the ingrowth of axons and establishment of synapses (see also Section 5.3 for development of LOT). Through the use of these methods, completion of the adult-like morphological characteristics were recognized during the third postnatal week.

During these early postnatal days, neurochemical and physiological maturation also occurred. In the OT, Gilad and Reis (1979) observed adult-like levels of GAD and ChAT around the end of the fourth postnatal week and that of TH by P40. In the piriform cortex, inhibition of the extracellular responses, elicited by paired electrical shocks of the LOT, possessed adult-like intershock-intervals (200–300 msec) not before the third week, but the inhibition was still weaker than in adults (Schwob et al., 1984). Earlier, during the first two postnatal weeks, facilitation of the summated responses was found within this range, in the same experiment, reflecting slow maturation of the inhibitory systems in this olfactory cortical area.

6.2. Tertiary olfactory centers and connections with the limbic system

In this Section, brain areas receiving projections from the secondary olfactory centers will be surveyed.

Whether or not the central olfactory system is an integrative, perhaps fundamental part of the limbic system, has long been the subject of debate. Suggestions in early textbooks about the primary importance of olfaction in the visceral functions of the brain were based on neuroanatomical studies, which described numerous connections between the marginal (limbic) cortical areas and undisputed olfactory centers (Cajal, 1911). Later it appeared that certain "rhinencephalic" regions had little or no direct olfactory projections, although they were intimately implicated in several types of behavior (e.g., feeding, sexual activity, expression of emotions). Papez (1937), in view of the available data in his time, suggested a more circumscribed idea for the role of such structures in the perception of, and reaction to, emotionally significant external and internal signals. This was suggested to be realized by a mechanism, integrated by the gyrus cinguli, anterior thalamic nuclei, hypothalamus and hippocampus. The term "limbic system", as it is used now, was proposed by Paul McLean in 1952 to designate

those parts of the brain involved in visceral and emotional functions (see Shepherd, 1983). This functionally related system of connections included the so-called Papez-circuit (see above) and other related regions.

Comparison of the evolution of the limbic system and of olfactory centers in lower and higher vertebrates favored the view that the olfactory system is one among a group of contributors to the facilitation or suppression of the limbic activity (Riss et al., 1969). Since then, connections of several higher brain centers with the formerly discussed secondary olfactory areas have been experimentally supported or newly discovered, as summarized recently by Price (1985).

Importance of the olfactory inputs in limbic function was also supported by neurochemical and behavioral consequences of unilateral or bilateral ablation of the bulb or transection of the main olfactory pathways (for reviews, see Hirsch, 1980*b*; Leonard and Tuite, 1981; Jesberger and Richardson, 1986). Bulbectomy affected complex behavioral responses, including aggression, irritability, sexual and locomotor activity. The effects of lesioning of the various neurotransmitter systems were dealt with in the respective sections of this book, when the neurochemical aspects were discussed, or shall be referred to in the forthcoming parts. Behavioral aspects of olfaction, to be summarized in Chapter 8, are also relevant in the further characterization of connections between higher olfactory (including those of limbic) centers. In the following sections, relationships of the secondary and tertiary olfactory centers will be briefly surveyed.

6.2.1. Deep amygdaloid nuclei

Anterogradely labeled axons from the deep primary olfactory cortical region (anterior cortical amygdaloid nucleus) resulted in labeled axons in the deep amygdaloid nuclei (Luskin and Price, 1983*a*); the same connection could be revealed also by retrograde HRP labeling, in addition to other, retrogradely labeled primary olfactory cortical areas (Ottersen, 1982).

6.2.2. Hypothalamus

In the hypothalamus, there are sources of various peptidergic fibers, directed towards the primary olfactory cortical area and the OB. As another olfactory relation in this region, hypothalamic areas are recipients of projections from secondary olfactory centers.

Degeneration (Scott and Leonard, 1971) as well as tritiated amino acid transport (Price, 1985) studies traced pathways from the primary olfactory cortical areas to the lateral hypothalamus (LHT). WGA-HRP retrograde transport from the same area to many secondary olfactory centers further supported the existence of these pathways (Price, 1985). Electrical stimulation of the OB or LOT produced activity in LHT, especially close to the medial forebrain bundle (Scott and Pfaffman, 1967; Scott and Leonard, 1971; Tanabe et al., 1975*b*). Stimulation by odorants elicited unit responses (Scott and Pfaffmann, 1967; Scott and Leonard, 1971; Kogure and Onoda, 1983), but

without selectivity of units to different odorant qualities or concentration. This suggested that the hypothalamus would not contribute significantly to discrimination processes. It was shown, however, that olfactory, gustatory and somatosensory (i.e., external) and internal (as shown by stimulation with hypertonic glucose and salt solutions) information converge onto lateral (and also, ventromedial) hypothalamic neurons. The main trend of their development during the early postnatal days is a fine tuning for different modalities (Fisher and Almli, 1984; Almli and Fisher, 1985).

6.2.3. Thalamus

The mediodorsal thalamic nucleus of the rat, but not of the cat, was reported to receive afferents originating deep to the primary olfactory cortical areas, like the polymorph cell zone below the OT, endopiriform nucleus (deep to the piriform cortex) and deep primary amygdaloid olfactory structures (Krettek and Price, 1977a; Price and Slotnick, 1983; Price, 1985). Electrical stimulation of the OB evoked responses in the mediodorsal and submedial thalamic nuclei in rats (Price and Slotnick, 1983; Price, 1985). While small lesions in the amygdala did not affect the acquisition of an olfactory task, severe deficits were caused by lesions in the mediodorsal thalamic nucleus, underlying the importance of the olfactory thalamocortical connections in learning (Slotnick and Kaneko, 1981).

6.2.4. Hippocampus

Strong anterogradely transported label in the dentate gyrus, and a lighter one in the CA1 and CA3 region of the hippocampus proper was observed after injection of tritiated leucine into the lateral entorhinal cortex; somata of these projecting cells could be located in layer II (Price, 1985). The limbic θ rhythm was monitored on slow wave activity of the hippocampus, and was reported as being best correlated with temporal patterns of sniffing activity, when rats evaluated the behavioral relevance of a given stimulus (Macrides et al., 1982).

6.2.5. Neocortex

Sources for neocortical projections were retrogradely traced back to the piriform, periamygdaloid and entorhinal cortices in rat (Price, 1985); these areas projected most heavily (as seen by anterograde transport studies) to the orbital and insular cortices (Krettek and Price, 1977a; Luskin and Price, 1983a; Price, 1985) in rats and cats. Responses to OB stimulation were recorded in these areas in rats (Price, 1985), but also in the monkey orbitofrontal cortex (Tanabe et al., 1975a).

The importance of neocortical areas in detection and discrimination of odorants, an increase in selectivity of units, was observed during experiments on monkeys (Tanabe et al., 1975a,b), rabbits (Onoda et al., 1984) and also humans with temporal lobe lesions (Rausch and Serafetinides, 1975). In the mouse insular cortex, convergence of

more sensory modalities (olfactory, gustatory and visceral) was also evident, as suggested on anatomical grounds by Shipley and Geinisman (1984), supporting the view of the importance of olfactory information in feeding mechanisms.

6.3. Summary

Primary olfactory cortical areas are situated in most vertebrates in the base of the forebrain. In these regions, olfactory tract fibers terminate typically on the apical dendrites of pyramidal cells, while interneurons as well as the associational fibers may synapse onto the whole receptive surface of these cells. As seen in mammals, the first efferent fibers reach their cortical targets during the late prenatal period, while inhibitory mechanisms are completed relatively late.

Most primary olfactory cortical areas (the olfactory tubercle may be an exclusion) have reciprocal connections with each other and with the olfactory bulb. In addition, these olfactory cortical areas may project heavily onto the other, primarily non-olfactory brain regions. These central brain areas may be important in increasing the selectivity of olfactory discrimination, by tuning the activity of the bulb (according to the actual hormonal, behavioral, etc. state of the animal) or by themselves (as seen by unit recordings). On the other hand, the higher olfactory centers (especially the olfactory tubercle, the amygdala, as well as the hypothalamic, thalamic and hippocampal connections) are implicated in limbic functions, therefore they may communicate (and integrate) olfactory information for the control of hormonal, visceral and emotional state of the subject.

CHAPTER 7: ACCESSORY NASAL CHEMOSENSORY SYSTEMS

The olfactory epithelial receptors, the main olfactory bulb and its central projections play the most significant role in detecting smells and, by processing them, provide an important information to the animal about their behavioral relevance. There are, however, several other nasal chemoreceptive regions, working in parallel with the main olfactory system. Among these, the vomeronasal sensory epithelium (Jacobson's organ) and its central representation may be the most significant (and also most extensively studied), but chemical senses are also detected by the septal organ (of Masera) and perhaps by the trigeminal nerve. Moreover, there are suggestions for a chemosensory role of the terminal nerve system. In the following sections, these four systems will be briefly characterized and their physiological and behavioral importance assessed. During these descriptions, only the most significant aspects will be referred to and a few examples will also be given for illustrative purposes.

7.1. The accessory olfactory system

This system shows many similarities to the main olfactory system, with regard to its development from the olfactory placode, structure of the sensory (vomeronasal) epithelium, course of the vomeronasal nerve, situation of the primary center (the accessory olfactory bulb) and central projections, although information conveyed by the two systems merge first only at the basal cortical areas (for reviews see Allison, 1953*b*; Moulton and Beidler, 1967; Tucker, 1971; Graziadei, 1977; Pedersen et al., 1985; Brunjes and Frazier, 1986).

In fish, the separation of the nasal sac into the "main" and "accessory" olfactory organ can hardly be recognized. A separate diverticulum, representing the vomeronasal organ, is clearly apparent in anurans and is especially well developed in snakes and lizards (Allison, 1953*b*). This may be correlated with the importance of non-volatile olfactory stimuli in these species' behavior (Halpern and Kubie, 1984). In birds and the majority of mammals the main olfactory system seems to play the primary role in detecting and processing olfactory signals, while the accessory system remains vestigial.

Three main aspects of this system will be surveyed in this Section: the receptor level, the accessory olfactory bulb and its central connections; the proposed role of the accessory olfactory system will also be briefly summarized.

7.1.1. The vomeronasal receptor field (Jacobson's organ)

Separately grouped vomeronasal receptors may line the wall of a separate pit or a tubular duct (Fig. 7.1), and so the peripheral part constitutes a well-recognizable, separate anatomical unit, the vomeronasal organ (VNO, organ of Jacobson), but it may also be an almost indistinguishable, plain mucosal region at the basal aspect of the nasal conchae in others, or absent, as in adult humans, old-world monkeys, birds, toothed whales and most fish (Allison, 1953*b*; Tucker, 1971). The well-formed VNO opened into the nasal cavity or, more commonly, into the nasopalatine canal (or both) and was regularly filled with secretory fluid (Allison, 1953*b*; Negus, 1956; Moulton and Beidler, 1967; Tucker, 1971).

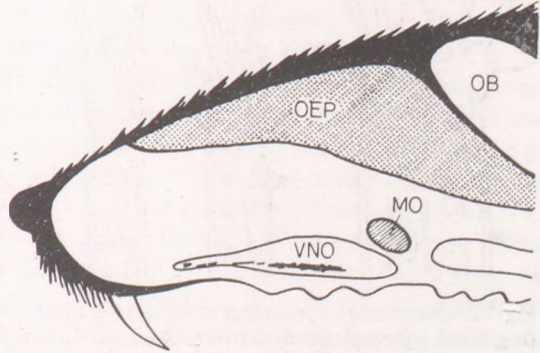


Fig. 7.1. Localization of the receptive fields on the nasal septum, as seen from the lateral side (from Miragall et al., 1984). OB: olfactory bulb; OEP: (main) olfactory epithelium; MO: septal organ of Masera; VNO: vomeronasal organ. Reproduced with permission

The structure of the receptorial region was similar to the olfactory mucosa; the vomeronasal epithelium contained receptor, basal and supporting cells and was underlined by a submucosal layer, which was rich in glands (no Bowman's glands were seen, however) and vessels (Negus, 1956; Vaccarezza et al., 1981; Adams and Wiekamp, 1984). Several differences were also recognized between the two regions. Most authors of the field argued that distal segments of the vomeronasal receptors did not wear cilia (Graziadei and Tucker, 1968; Kolnberger, 1971; Altner and Kolnberger, 1975; Ciges et al., 1977; Vaccarezza et al., 1981) (Fig. 7.2). Both VNO receptor cells and supporting cells wore, however, microvilli in all species studied, and were rich in organelles. Supporting cells showed only weak or no signs of secretory activity.

Electrical responses from the VNO were elicited with odorant stimulation (Tucker, 1963; Müller, 1971). EOG signals in the VNO were recognized similarly as in the olfactory epithelium, but different vertebrate classes (amphibians, reptilians and mammals) were suggested to differ in their sensitivity (Müller, 1971).

Unmyelinated axons of vomeronasal receptors were gathered into bundles, which coursed alongside the medial septum, and after penetrating the cribriform plate, approached the ipsilateral accessory olfactory bulb (AOB), to terminate there.

The most significant neurochemical labels of the olfactory nerve, carnosine and the olfactory marker protein were revealed in the vomeronasal system, too, although at

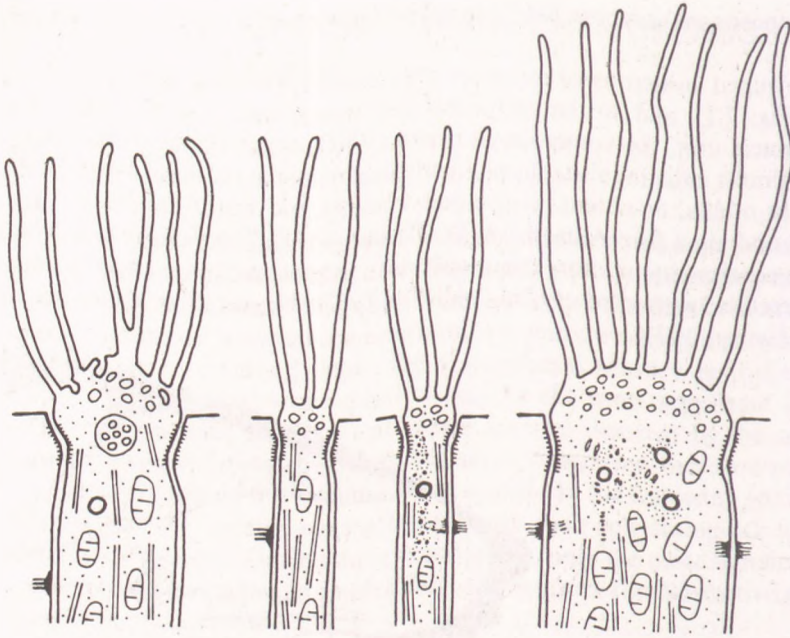


Fig. 7.2. Characteristic appearance of the distal endings of vomeronasal receptors of the (from left to right) frog, lizard, viper and tree shrew (from Altner and Kolnberger, 1975). Microvilli are confined to the terminal knobs, while cilia are absent. Reproduced with permission

lower concentrations (Monti Graziadei et al., 1980; Burd et al., 1982b). The distribution of the label for carnosine was similar in the VNO-accessory olfactory bulb system to that of the main olfactory system (Burd et al., 1982b); ontogenetically, OMP appeared somewhat later in the accessory olfactory system (Monti Graziadei et al., 1980).

Recently, with the advent of monoclonal antibodies, subclasses of the vomeronasal nerve have been recognized with the MAB R4B12, which project exclusively to the rostralateral part of the accessory olfactory bulb, while the MAB R5A10 labeled a complementary set of the VNO terminals in the mediocaudal part of the AOB (Imamura et al., 1985; Mori et al., 1987). The localization of other antibodies against lactoseries carbohydrates (MAB 4C9) was similar to that of the MAB R4B12 (Mori, 1987).

7.1.2. The accessory olfactory bulb (AOB)

According to the developmental differences in appearance and extension of the VNO, the AOB also differs widely between various vertebrates; it is fairly well developed (and studied most frequently) in rodents, where it is a wedge-shaped structure, positioned between the dorsocaudal OB and olfactory peduncular structures. No correlation between the degree of development of the main OB and the

AOB was, however, revealed; similarly, differences between the female and male, left or right AOBs were not apparent in insectivores and certain primates (Stephan et al., 1982).

Microscopical structure of the AOB is reminiscent to that of the main OB, but with several alterations. VNO fibers enter relatively poorly defined glomeruli, to contact mitral cell dendrites; mitral cell somata are not arranged in a single layer, but rather scattered within the EPL (Cajal, 1911; Herrick, 1924; Allison, 1953b; Hoffman, 1963). Periglomerular and granule cells were relatively few in number, while no tufted cells have been described for the AOB. Mitral cells of the rabbit AOB showed, however, similarities in the distribution of their dendrites and axon collaterals with those of the main OB (Mori, 1983). Their axons entered the dorsomedial aspect of the lateral olfactory tract in their course towards the amygdaloid complex.

The AOB also showed many similarities to the main OB with regard to its neurochemical properties. Among the major neurotransmitter systems, cholinergic fibers to the deep layers of the AOB were reported from the mouse AOB (Carson and Burd, 1980); TH-immunoreactive (supposedly dopaminergic) somata were seen, e.g., in the hamster (Davis and Macrides, 1983; Baker 1986b) and mouse (Rosser et al., 1986), noradrenergic fibers were reported from the mouse AOB (mostly in the IPL) (Broadwell and Jacobowitz, 1976; Halász et al., 1977b; Fallon and Moore, 1978a; Rosser et al., 1986); scattered GAD-immunopositive cells (including those from the granule and periglomerular cell population, Mugnaini et al., 1984a) and peptidergic structures were also apparent (Matsutani et al., 1989). With regard to the latter, the LHRH systems might have the greatest significance (Phillips et al., 1982; Witkin and Silverman, 1983; Witkin, 1985; Rosser et al., 1986); in addition, thyrotropin-releasing hormone (TRH) supply of the AOB was also prominent (Manaker et al., 1985).

Field potentials were evoked by VNO (or antidromically, by amygdala) stimulation and recorded with penetrating electrodes in different depths of the AOB: similar to the main OB, a reversal, of the polarity was observed at the level of the mitral cells (MacLeod and Reinhardt, 1983; Reinhardt et al., 1983; van Groen et al., 1986). Unit responses of the mitral cells in the AOB were also similar to those evoked in mitral cells in the main OB (Mori, 1983). In contrast, ON or LOT stimulation elicited only very weak or no responses in the AOB, reflecting a strict separation of these two systems (MacLeod and Reinhardt, 1983).

7.1.3. Central projection

Like the peripheral and bulbar segment of the accessory olfactory system, central projections were also segregated from those of the main olfactory system. The accessory olfactory tract terminated at a basal cortical area, where main olfactory afferent terminals were absent (Price, 1973; Broadwell, 1975a; Leonard, 1975; Northcutt and Royce, 1975; Scalia and Winans, 1975; Devor, 1976; Skeen and Hall, 1977; Davis et al., 1978; de Olmos et al., 1978; Kevetter and Winans, 1981a; Luskin and Price, 1983a). According to these studies the accessory olfactory tract terminated in the medial and posteromedial (posterior) cortical nuclei of the amygdala, nucleus of the accessory olfactory tract and bed nucleus of the stria terminalis (most of these areas

projected back to the AOB). Further projections of these areas include those of hypothalamic and preoptic areas, containing nuclei, which were closely related to hormonal regulations of the reproductive system and sexual behavior. Behavioral studies further supported the importance of the VNO/AOB system in reproduction (for a review see Wysocki, 1979; see also Section 7.1.4.).

7.1.4. Development and functional significance of the accessory olfactory system

The vomeronasal organ first became apparent on E12 in mouse (Brunjes and Frazier, 1986), while the majority of mitral cells of the AOB were reported to emerge on E10–E11 and the granule cells between E14 and E18 (Hinds, 1968*a*). In rats, neurogenesis of most mitral cells was complete by E15–E16; granule cells of the ventrolateral area arose before birth in about 90% (in contrast to the much later development of granule cells in the main olfactory bulb; see Section 4.5.). Central projection from the AOB reached their targets also slightly earlier than the LOT fibers and were suggested to “prepare” the amygdaloid cortex to receive main olfactory afferents (Bayer, 1983).

Metabolic activity was first seen in the AOB, as reflected by 2-DG uptake, and later in other olfactory-related areas: elevated 2-DG activity was detected already before birth (Pedersen et al., 1983, 1985). This might support recognition of the earliest nasal chemical stimuli (conveyed by fluid-to-fluid, from the amniotic to VNO secretion) before birth, and shortly thereafter, when the accessory olfactory system may contribute to maternal recognition (Teicher et al., 1984).

A direct contact between the stimulus-bearing secretion and VNO seems important at a later age, too: licking, tongue flicking and contraction of muscles at the nasopalatine area may help suction of the stimulus (pheromone?) containing fluids and so, constitute necessary prerequisites of the vomeronasal sensation. Another significant contribution of this system to successful reproduction seems to occur when animals reach sexual maturity, as investigated by numerous laboratories (for references see e.g. Wysocki, 1979; Keverne, 1983; Clancy et al., 1984*b*). Recognition of female pheromones, guiding of sexual activity and regulation of some related behavioral and limbic functions, like male aggression and also prey tracking had also been implicated in those functions assigned to the accessory olfactory system (Wysocki, 1979; Keverne, 1983; Clancy et al., 1984*a,b*; Halpern and Kubie, 1984).

7.2. The septal organ (of Masera)

Rodolfo-Masera (1943) described a separate chemosensory epithelial spot, surrounded by respiratory epithelium in the nose of a marsupial (*Didelphys*), rabbit and certain rodents (including rat and mouse). It was a paired organ, situated on the nasal septum, in close vicinity to the nasopharyngeal canal (see Fig. 7.1).

Microscopical structure of the septal organ closely resembled that of the (main) olfactory epithelium; Bowman's glands were present in the submucosa, main cell types

(receptor, supporting and basal cells) of the epithelium were revealed and receptor cells were cilia (Rodolfo-Masera, 1943; Adams and McFarland, 1971; Bojsen-Møller, 1975; Graziadei, 1977; Miragall et al., 1984). The centrally projecting nerves of the septal organ are collected on both sides and join the olfactory nerves towards the main OB, through the cribriform plate. These nerves are confined to the ipsilateral dorso-caudo-medial bulb and feature a degree of topographic organization in their projection (Benson et al., 1985b; Pedersen and Benson, 1986).

EOG potentials were recorded from the septal organ of rats. The responses to short pulses of odorants were comparable to, or even exceeded, those elicited in the surrounding main olfactory epithelial receptors (Marshall and Maruniak, 1986).

According to the situation of this sensory area within the main passage of the respired air, its special role was suggested to provide the main olfactory system with information during quiet respiration, when no voluntary exploratory activity (sniffing) occurred.

7.3. The trigeminal nerve

Branches of the trigeminal nerve innervate both respiratory and sensory epithelial regions of the nasal cavity (Bojsen-Møller, 1975) and apparently have the capability of detecting, beside pain sensation, chemical stimuli (Allen, 1928; Beidler and Tucker, 1955; Tucker, 1971).

The tortoise trigeminal nerve responded to some odorants at lower thresholds than the olfactory nerves did (Tucker, 1971). Increasing sensitivity to n-aliphatic alcohols with smaller carbon chain length was reported for the rat trigeminal nerve and so, lipid solubility was emphasized as the main determinant in effectiveness of stimulation (Silver et al. 1986). According to other experiments, the trigeminal nerve was involved rather in the regulation of respiration and pain reception (both of which are, however, very closely related to the chemosensory function of the nose).

Electrical stimulation of the nerve slowed the frequency of inhalation and increased expiration in rats (Ulrich et al., 1972). Capsaicin did not affect olfactory recognition and only slightly the gustatory sensation, while trigeminal responses were eliminated by this treatment (Silver et al., 1985). In light of these experiments, the trigeminal nerve may influence indirectly smell detection, rather than being involved in direct odorant reception. Fine structural characterization of the most peripheral segment of the trigeminal nerve branches in the nasal mucosa would greatly facilitate the elucidation of its exact role in nasal chemoreception.

7.4. The terminal nerve (TN)

If the role of the trigeminal nerve in olfactory recognition was questioned, so much more was the case with TN. Accordingly, in spite of the numerous documentation on TN in the literature, it will be discussed only briefly here.

The terminal nerve was defined as "a cranial nerve closely associated with the olfactory nerve and tract" (Bartheld, 1987). Clustering of neuronal somata into several

ganglia of the nerve was obvious on the surface of the medial forebrain, especially at the bulbar level, but scattered cells of the system were seen even along the olfactory and vomeronasal nerves. Both sensory regions of these latter nerves were supplied by TN fibers. The TN system may be present in all classes of vertebrates (see Bartheld et al., 1987).

The most striking neurochemical feature of the TN was the LHRH content (Münz et al., 1981; Halpern-Sebold and Schreibman, 1983; Stell et al., 1984; Zheng et al., 1988; see also Section 2.3.2.3.3.). Less data referred to an AChE (Wirsig and Leonard, 1986*b*) or molluscan cardioexcitatory tetrapeptide (FMRF amide, Stell et al., 1984) containing component, in rat and goldfish, respectively.

If the lack of physiological experiments, designed to study the TN exclusively, is viewed, it is hard to decide whether it has a motor or rather a sensory function. Lesion studies performed on different segments of the hamster TN revealed an accumulation of the LHRH-immunoreactive material at the peripheral segment of the system (Wirsig and Leonard, 1986*a*). This supports the view of the terminal nerve as being a centripetally projecting, separate chemosensory system which might be involved primarily in the regulation of sexual behavior (Demski and Northcutt, 1983).

In contrast, this system has been suggested to serve rather a non-olfactory (vasomotor?) function by Buhl and Oelschläger (1986), since toothed whales have well developed terminal nerves, but are anosmic. Also, termination of TN fibers in the periventricular nuclei (in goldfish) suggests that this cranial nerve may have a function different from olfaction (Bartheld and Meyer, 1986*b*).

7.5. Summary

Besides the receptor cells of the main olfactory epithelium, the involvement of the vomeronasal (accessory olfactory) and septal organs, as well as of branches of the trigeminal and terminal nerves has also been proven or suggested. These systems have, however, different significance in various vertebrates.

The chemosensory function of the vomeronasal and septal organs seems unquestionable; their sensitivity to certain odorants may be equal to, or even higher than that of the receptors of the main olfactory epithelium. Signals from the VNO are transmitted to the accessory olfactory bulb, while the septal organ projects to the main olfactory bulb.

Vomeronasal organs, where well developed, are narrow tubules or sacs, filled by secretory fluid and accessible to (non-volatile) stimulants by direct contact only. The accessory olfactory bulb shares many structural and neurochemical features with the main olfactory bulb. The accessory olfactory system may have a special importance in intraspecific communication, including the mother—offspring relationship and sexual behavior.

The trigeminal nerve fibers may have little significance in the recognition and discrimination of chemical cues. They seem to be restricted to the detection of the irritant or painful aspect of a chemical stimulant. Even less data support, although there are certain suggestions for, a limited role of the terminal nerve in nasal chemoreception.

CHAPTER 8: BEHAVIORAL SIGNIFICANCE OF OLFACTION IN VERTEBRATES

According to the main scope of this book, morphological and neurochemical grounds of nasal chemoreception has been surveyed in the previous Chapters 2–7 and completed with functional and developmental aspects, although reference to behavioral relations was unavoidable in certain places. This was inevitable because of the strong relevance of certain behavioral data in proving structural, chemical, etc. findings. The extent and emphasis on these scattered, complementary data, however, were far from reflecting even the main lines of significance of nasal chemoreception in the vertebrate's life. Such disciplines include a wide range of fundamental aspects related to the survival of the individual and the species, as summarized in a number of reviews (see e.g. Moncrieff, 1967; Cain, 1974; Wysocki, 1979; Little, 1982; Halpern and Kubie, 1984; Doty, 1986) and characterized in most detail in the monograph of Stoddart (1980). Among these aspects, two main groups can be recognized according to the function they serve: those contributing the feeding and others related to events of the reproductive life. Emotional and social manifestations of odorant-dependent behavior may support both groups while, of course, neither of these main aspects could provide exclusive grounds in maintaining the life of any vertebrate species. Doty (1986) lists 24 types of information, constituting different aspects of olfaction-mediated messages; accordingly, many aspects were characterized through olfaction-related studies.

Evaluation of these experiments extends far beyond the scope of the present book. For our purposes, a selection of findings, with a more or less close relation to structural and neurochemical characteristics of the vertebrate olfactory system will be, however, attempted here, by calling the attention of the reader to some basic aspects of olfactory learning and memory, reproductive life and feeding behavior. Many aspects of the olfactory-related behavioral manifestations of the vertebrate life (like those of making distinctions between biologically meaningful and "less important" chemical signals, prey–predator relations, social behavior, parent–offspring relations, recognition of conspecifics and individuals by smell, aggression, trail marking, territoriality, migration, alarm and defense through chemical signals, indication of the reproductive stage, etc.) will, however, be omitted in this Chapter or shall only be briefly mentioned.

8.1. Some general aspects

In this Section, experiments based on peripheral (mostly odorant) stimulation and the resulting behavior of the individual will be summarized. This implies that the whole olfactory system, from periphery to connections of the tertiary centers may contribute to the resulting reaction.

As another aspect, recognition and evaluation of the relevance of an olfactory stimulus has resulted often, from more than one, perhaps all, nasal chemoreceptor systems, where an individual component could not be separated from others in most experiments.

8.1.1. Detection and discrimination of odorants

Certain previous parts of this book have dealt already with the problems of olfactory sensitivity, recognition and identification of the stimulus (see especially Sections 2.3., 2.4., 4.4. and 7.1.4.); some additional findings may further complete this topic.

Thresholds for olfactory sensitivity were found to vary within a very wide range, from 10^{-1} to even 10^{-17} M, depending mainly on chemical characteristics, biological relevance and the experimental subject (Passe and Walker, 1985). Champions in detecting odorants at the lowest (10^{-15} – 10^{-17} M) concentrations were the eel and the dog; most of the odorants tested on other species were detected at concentrations lying between 10^{-6} – 10^{-9} M. Many factors, in addition to those mentioned above certainly also influenced sensitivity of the given experimental animal (as was clear from the related studies), like various conditioning signals, introduced by the experimenter, age, developmental stage, maturity, gender, hunger, arousal state, etc., even biological rhythms (through circadian and seasonal changes of hormone levels) influenced thresholds.

Evidently, structural and neurochemical integrity of the system also strongly influenced the detection and recognition of an odor. The effect of interventions leading to impairment of the receptor and/or more central levels of the system were discussed in Sections 2.5., 4.5. and also briefly in Chapters 5–7, when discussing developmental aspects. While complete peripheral deafferentation totally eliminated the olfactory-guided behavior, more central lesions impaired only certain aspects of the olfactory recognition. For example, complete transection of the LOT did not produce anosmia in rats; medial projections could effectively substitute for the loss (Slotnick and Berman, 1980). Small lesions in the anterior amygdala (Slotnick, 1985) or mediodorsal thalamic nucleus (Eichenbaum et al., 1980; Slotnick and Kaneko, 1981) only slightly affected the thresholds of smell detection, while more extensive lesions, which interrupted the connections between the olfactory cortical and neocortical areas seriously impaired the discrimination abilities of rats (Eichenbaum et al., 1980). Also, habenular lesions elevated thresholds for olfactory stimuli and resulted in a reduced locomotor activity (Rausch and Long, 1974).

Special attention was paid to the study of olfactory performance after prolonged exposure to an odor (as regards the structural and functional consequences see Section 4.4.1.2.3.). Long exposure to an odorant apparently did not eliminate the reactivity of the animal to the same stimulus (Porter and Treadway, 1974; Laing and Panhuber, 1980; Cunzeman and Slotnick, 1984).

8.1.2. Learning and memory

Although many experiments on discrimination, cited in the previous section also involved learning, e.g., adaptive changes in behavior to repeated application of olfactory stimuli, a few further examples will be given in this Section, which stress the importance of the acquisition and recovery of the olfactory experience in guiding the appropriate behavior of animals. Significance of the connections between "olfactory" and "non-olfactory" brain centers has been evidenced by various lesion experiments, as cited above, and also by olfactory bulbectomy (Hirsch, 1980b; Leonard and Tuite, 1981; Jancsár and Leonard, 1984; Leonard, 1984; Jesberger and Richardson, 1986).

Formation of the memory could be impaired by lesions in the hippocampus or lateral entorhinal area (Stäubli et al., 1984, 1986) or by inhibition of calcium-sensitive proteinases by intraventricularly applied leupeptin (Stäubli et al., 1985). Deficits in discrimination learning were observed after lesions of the mediodorsal thalamus (Slotnick and Kaneko, 1981). In light of their similar experiments, Stäubli et al. (1987) suggested that the poor acquisition of olfactory discrimination sets were due to a strong, temporary procedural impairment and to a lasting encoding deficit. In the same paper they reported on the reduced ability of rats to acquire olfactory discriminations after lesions of the piriform cortex.

Interruption of connections with the central monoaminergic (mostly noradrenergic) systems led to the assumptions that these systems were mainly responsible for the losses, induced by bulbectomy (see the papers cited above, and also Gervais and Pager, 1983; Royet et al., 1983). Aversive olfactory learning, when conditioned with LiCl₂ revealed reciprocal interrelationships between the olfactory and the digestive systems: beside poisoning, as detected by symptoms affecting the digestive tract, specific glomeruli of the olfactory bulb showed an increased metabolic activity, differing from those activated by a familiar odor only (Coopersmith et al., 1986; see also Section 4.4.2.).

The formation of memory for olfactory preference may start as early as the last gestational days (*in utero*) or in the early postnatal period in rats and rabbits (Pedersen and Blass, 1982; Pedersen et al., 1985; Hudson and Distel, 1987; Wilson et al., 1987). Conditioning at an early postnatal age (6-day-old rats), when commissural connections were still immature, revealed that odor learning can unilaterally be achieved (Kucharski et al., 1986).

It was also in the first postnatal-week, when neural and behavioral responses could most successfully be associated with learned odors (Woo and Leon, 1987). In mice, exposure to an odorant during the first two postnatal weeks nearly doubled the number of neurons, responding to the introduction of the same stimulant during the third month of life (Reinken and Schmidt, 1987). The association of tactile stimuli with an attractive odor resulted in an enhanced 2-DG uptake in specific olfactory bulb glomeruli (Sullivan and Leon, 1986) and a behavioral preference for that odorant in young rats (Wilson et al., 1987). Recovery of memory by an odor was especially effective, when lithium poisoning was associated with the same odor, but combined simultaneously with a taste stimulus (Palmerino et al., 1980). Olfactory signals were found to be superior over other modalities, like tones or light, in recovery of learned

behavioral performance in rats, proving the more salient nature of olfactory stimuli, in this animal (Slotnick, 1984).

Prolonged olfactory exposure of rats (see above and also Section 4.4.1.2.3.) and chicks (Jones and Gentle, 1985) presumed a successful formation of memory for odors. Such treatment in newborn mice was suggested to result in establishment of new pathways for processing a specific olfactory signal (Harvey and Cowley, 1984).

8.2. Feeding

Many experiments, investigating characteristics of discrimination, learning and memory formation in olfactory-related behavioral tasks were based on stimulations with food odor because of the superior biological relevance of this type of stimulus in survival. Olfaction was involved first of all in initiation of awareness of the presence and approach to the food. In addition, olfaction contributed to the evaluation of food palatability, marking and re-recognizing the trail to it (or hiding those parts not eaten immediately), the protection of the remainder against competitors and so, in social relations, aggression, territoriality, migration and navigational behavior, which were linked in this way to the olfactory-controlled feeding behavior. With regard to internal conditions, the strongest correlation and cooperation of olfaction was recognized, of course, with gustatory system, the other feeding-related chemosensory system.

In the process of detection and selection of food, odor information was proven to be essential and sufficient in all experiments when the olfactory system was structurally and functionally unimpaired, and in all groups of animals, as noted in the previous sections, when functional and developmental aspects were discussed (in this respect, see also the reviews by LeMagnen, 1971; Ottoson, 1963a; Stoddart, 1980; Little, 1982; Doty, 1986). Smelling was used even by birds in the detection of food (Wenzel, 1968; Wenzel and Sieck, 1972; Harriman and Berger, 1986), although because of the relatively low level of development of their olfactory system they have been rarely cited in this book. [Let me remind the reader that the bird olfactory system could also be activated, of course, with "artificial" odors or electrical stimulation, as was shown by Tucker (1965); Henton et al. (1966); Sieck and Wenzel (1969) and Wenzel and Sieck (1972)].

Experience with a species-specific, palatable food resulted in imprinting, leading even to formation of a characteristic morphological substrate, as seen by electron microscopic studies in the polecat bulb (Apfelbach and Weiler, 1985a,b; Apfelbach, 1986).

Motivation of olfactory behavior was most deeply influenced by the nutritional state (food deprivation or satiety) of the experimental animals (as discussed already in Section 4.4.1.3.; Pager, 1977). During sleep, the importance of the central serotonergic pathways in the inhibition of the olfactory system in recognition of food odors has been reported (Gervais, 1979; Gervais and Pager, 1979; Gervais et al., 1984).

Structural integrity was necessary for correct processing of food odors, too, as has been summarized already in the previous Section 8.1.1. It is interesting to note here that a different subcompartment of the olfactory tract, conveying bulbar information on food, and another for signaling significance in reproduction (sexual behavior) was

revealed in male goldfish (Stacey and Kyle, 1983). This implies that processing of signals, determining feeding and reproductive behavior, involves specialization of telencephalic areas and pathways projecting to them.

8.3. Reproduction

In different aspects of reproduction, even the peripheral (receptor) apparatus and their bulbar (and further central) segregation has been documented. The accessory olfactory system and the macroglomerular complex of the OB (with peripheral connections from receptors, separated from the main olfactory epithelial region) were suggested to be implicated more frequently in reproduction-related behavior than that of the main olfactory system and its projections, but regularly a cooperation of the nasal chemoreceptive systems was evidently necessary for correct and successful fulfilment of the behavioral needs at all critical periods of the reproductive life. In newborn rodents (and also in embryonic rats, shortly before birth) (Pedersen et al., 1983), elevated metabolic activity of the accessory olfactory system was already detected, followed by activation of the macroglomerular complex of the OB and, during the early postnatal days, by the main olfactory system (for a review see Pedersen et al., 1985). The accessory olfactory system and the macroglomerular complex projections were suggested to be inevitable in early feeding, i.e., suckling behavior (Teicher et al., 1980; Greer et al., 1982*b*; Pedersen et al., 1983, 1986; see also Section 7.1.), but in maternal recognition, necessity of the main olfactory system was also established (Teicher et al., 1984). Moreover, integration of information from tactile stimuli was also necessary during orientation, localization of the nipple and successful attachment to it (Larson and Stein, 1984; Distel and Hudson 1985).

Citral-scented, washed nipples were selected by newborn pups, if they were exposed to citral (injected into amniotic fluid) during the last gestational days and stroked in its presence for 1 h post-parturition (Pedersen and Blass, 1982). Before weanling, rats were attracted rather by nest odors, while afterwards, they preferred odors of the alternate sex (Brown, 1982). Interestingly, (citral) conditioning of male rat pups during suckling resulted, after their sexual maturation, in a significantly shorter latency of ejaculation when they encountered similarly scented females (Fillion and Blass, 1986).

Involvement of the nasal chemosensory systems in sexual behavior has also been studied in much detail because of their suggested importance in male attraction (receptive female recognition, courtship, mating, pregnancy and related behavior) (for reviews see Stoddart, 1980; Little, 1982; Meisel et al., 1984; Doty, 1986). It was not clear, however, to what extent the individual nasal chemosensory systems were involved during different aspects and phases of sexual behavior. At least in hamsters, as one of the species studied in most detail in this respect, it seemed plausible that both the main and the accessory olfactory systems were indispensable for the recognition of receptive females by the males and successful mating. In the detection of females, volatile stimuli, processed by the main olfactory system may play a determinant role (Johnston and Rasmussen, 1984; Kelche and Aron, 1984; O'Connell and Meredith, 1984), while for the undisturbed copulatory behavior, direct contact and vomeronasal detection of a high molecular weight (and non-volatile) vaginal secretion was necessary

(Clancy et al., 1984a,b; Macrides et al., 1984; Singer et al., 1984a,b). Nasal chemoreception was important also in females: scenting of the bedding with male urine before mating significantly enhanced ovulation and/or luteinization in rats, while olfactory bulbectomy prevented this increase (Antz-Vaxman and Aron, 1986). Also, olfaction was implicated in eliciting the pregnancy block by the introduction of a strange male shortly after the reception (Bruce, 1960; Keverne, 1983).

Interruption of connections of the bulbar complex with more central brain regions clearly proved the involvement of the limbic structures in integration of the nasal chemosensory signals and sexual behavior (see e.g. Cain, 1974; Sapolsky and Eichenbaum, 1980). Steroid-receptive neurons of the limbic system, neuropeptide supply and ascending noradrenergic pathways to the olfactory region may play a unique role in this control (Everitt et al., 1983). The noradrenaline and LHRH supply to the bulbar complex was especially strongly implicated in reproduction processes (Keverne and de la Riva, 1982; Dluzen and Ramirez, 1983; Keverne, 1983; Rosser and Keverne, 1985; Rosser et al., 1986; see also Sections 4.3.7.2. and 4.3.7.7.), as being necessary at a critical period for successful reception of females.

8.4. Summary

Olfactory afferents in vertebrates are intimately associated with the basal forebrain and that of the limbic system. Consequently, olfaction is implicated within a wide range of behavioral responses, directed by these structures.

The detection and recognition of smells are prerequisites for an olfactory influence on behavior. Thresholds of different odorants vary across the various vertebrates and may be proportional to the biological significance of an odorant. The integrity of the primary olfactory cortical regions and their central connections is necessary for the learning of olfactory tasks and the formation of an olfactory memory. Simultaneous stimulations by other modalities greatly enhance this memory function. The early postnatal period is crucial in the development of olfactory preference.

Olfaction, together with taste, has a determinant importance in the finding and selection of food. The olfactory-guided feeding behavior is also influenced, however, by other systems, primarily by those controlling the nutritional and emotional state of the subject. Similarly, the complexity of olfactory and non-olfactory influences are apparent in sexual and reproductive behavior. Olfaction may have a special importance in finding and selecting a mate, sexual behavior, stabilization of pregnancy as well as in the formation and maintenance of animal communities.

CHAPTER 9: THE SIGNIFICANCE OF OLFACTION IN HUMAN LIFE

Acuity of the odor sensation and importance of olfaction in human life, as is generally agreed, is far less important than in most other vertebrates. In "civilized", "modern" human life, sight and hearing are the most important senses and odors may be important only as a component, in determining one aspect of the flavor of food and drink (as surveyed in many reviews and monographs). In addition, olfaction is regarded only as a complementary contribution to our comfort feelings, when spending more and more hours of the day, while working or resting, within human-built and human-equipped rooms. Odors motivate us also only slightly, when we try to recreate ourselves as part of nature, outdoors. For most vertebrates, in contrast, chemical senses convey information on vital needs, as those providing grounds for the preservation of the self and the species, as has been discussed in Chapter 8.

Nevertheless, human nasal chemosensation is based on existing and functioning morphological substrates, it is able to influence our emotions and produce delight or disgust resulting in attraction or refusal of nutritional, reproductional and social conditions and so, interacts significantly with our chances of survival as individuals and species.

Aspects of human relations to nature, to the productions of modern civilization, to human societies, everyday nutrition and disposition are all influenced by perception of nasochemosensory stimuli (Moncrieff, 1967; Le-Magnen, 1971; Doty, 1976, 1981; Cain, 1979; Stoddart, 1980; Engen, 1982). These topics constitute the central interest of discussions at scientific societies, investigating problems of human nutrition, environment, air pollution, household chemicals, perfumes, cosmetics, etc. According to the main scope of this book, certain general aspects of human olfaction, which are related to the morphology, normal and abnormal functioning and olfactory communication will be surveyed in this Chapter.

9.1. Structural and developmental aspects of the human olfactory system

The human olfactory system does not differ substantially from that of other mammals (see Section 2.). The olfactory sensory epithelium covers about 1 cm² of the superior nasal cavity in each side. The sensory epithelium is about 70 μ m thick, pseudostratified columnar layer, containing basal (which implies regenerative capacity see Sections 2.2.2.4. and 2.5.), supporting and sensory cells (Andres, 1975; Moran et al., 1982a,b; Nakashima et al., 1984).

The fetal receptorial region is compact, while in adults, a large area in the same region is substituted by respiratory epithelium (Nakashima et al., 1984). Distal portions of the receptor cells, the olfactory knobs (olfactory vesicles) bear cilia (Andres, 1975; Moran et al., 1982b; Nakashima et al., 1984; see also references in the

review article of Menco, 1983); in addition, existence of a microvillar receptor cell type has also been suggested (Moran et al., 1982a). Supporting cells are lined by microvilli (Bloom and Engström, 1952; Moran et al., 1982b; Nakashima et al., 1984). In adults, zonal distribution of supporting and sensory cell nuclei gradually disappears (Naessen, 1971), and many degenerated receptor cells are apparent, which may be one reason for the weakening of the sense of smell in elderly persons (Nakashima et al., 1984; Moran et al., 1985).

As is the case for other vertebrates, human olfactory receptor cells, axons and their terminals contain olfactory marker protein. OMP immunopositivity appears in the epithelium first around the 28th week, and within the nerve and glomerular layer of the bulb at about the 32nd week of gestation (Chuah and Zheng, 1987).

The olfactory bulbs in humans are sagittally elongated structures, measuring cca. $11 \times 5 \times 2$ mm (Smith, 1941). The olfactory nerves, after penetrating the cribriform plate, terminate in glomeruli on the ventral, medial and lateral aspects of the bulb. Consequently, the dorsal aspect of the bulb does possess ON and glomerular layers; the distribution of mitral cells also follows this asymmetry (Smith, 1941; Andres, 1975; Fig. 9.1).

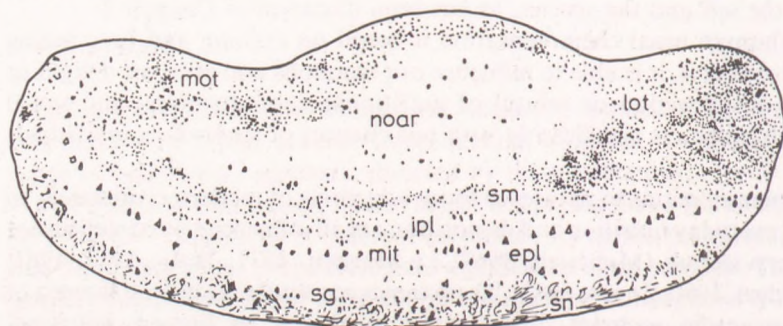


Fig. 9.1. Drawing of the transectional view of the human olfactory bulb (redrawn after Andres, 1975). epl: external plexiform layer; ipl: internal plexiform layer; lot: lateral olfactory tract; mit: mitral cell layer; mot: medial olfactory tract; noar: nucleus olfactorius anterior; sg: glomerular layer; sm: layer of myelinated nerve fibers; sn: olfactory nerve layer. Reproduced with permission

Mitral and granule cell densities are lower than, e.g., in rodents; the mitral cells are more scattered, and the internal plexiform layer is relatively wide (Andres, 1975; Braak et al., 1977). The number of glomeruli were estimated cca. 10,000 in each bulb; there was, however, a high incidence of atrophy and a substantial decrease in this number in many cases, as reported by Smith (1941), who studied a large population of human bulbs ($n = 163$) at the light microscopic level.

The accessory olfactory bulb in humans was also readily observed during the embryonic age (Humphrey, 1940; Chuah and Zheng, 1987), but regressive changes were already apparent from the 11th week of embryonic age (Humphrey, 1940). The presence of the vomeronasal nerve was, however, reported from the more mature fetuses and VNO (Jacobson's organ) even from adult humans. Although the vomeronasal reception of odorants in humans is very unlikely, vascularization of the VNO remains, constituting the most frequent cause of epistaxis.

A medial and lateral portion (stria) of the olfactory tract was recognized, travelling towards the basal forebrain areas; neurons of the rostral part of the nucleus olfactorius anterior were present already where all bulbar layers were still visible (Andres, 1975). The two tracts were then separated; they lined the medial and lateral side of the olfactory trigone.

Olfactory tract fibers terminate in the central area of the basal aspect of the brain. The medial stria (tract) distributes its terminals mainly in the olfactory tubercle and adjacent cortex of the olfactory trigone, but also in the paraolfactory areas, supracallosal gyri and septum pellucidum. The main portion of the bulbar projection fibers, the lateral stria (tract) terminate in the piriform area (in the rostral part of the hippocampal gyrus, the uncus and the lateral olfactory gyrus) and, to a lesser extent, in the amygdaloid area (Kuntz, 1950). Further connections of these structures include those with the limbic brain areas (and so, provided anatomical grounds for interactions with the supposed centers of the emotional life; see also Sections 5.1 and 6.2. in this respect) and also with motor neurons of the lower brain stem (see the same reference).

9.2. Normal functioning and dysfunction

9.2.1. General aspects

To collect data on the functioning of the human olfactory system at any level, or evaluating the operation of this system in humans as a whole poses several difficulties, when compared to similar experiments on other vertebrates. The nasal olfactory mucosa is almost inaccessible for recording (not to mention the unpleasantness and pain which would accompany such an intervention); living human brain tissue is not available for experiments. What remains is the reaction of the person, as seen in behavior or understood through verbal communication. This implies that a higher degree of dependence on the subject's earlier (largely unknown) experiences, emotions and communication abilities must be taken into consideration more so than in similar experiments on other vertebrates, which certainly decreases the confidence in obtaining exact results. In other words, a much greater interaction of subjective with objective factors may be expected. An improving factor may be, however, that the human subject can be prepared for the situation and convinced about the harmless nature of, e.g., sniffing probes. As a consequence, the influence of stress would be less significant in human experiments, while eliciting a certain amount of stress in animals simply could not be avoided.

In summary, planning and evaluation of experiments on human olfaction needed special care in application of the techniques of olfactometry and interpretation of the data obtained in this way. The history and recent status of these methods (and evaluation theories) has been reviewed recently by Berglund et al. (1986). As it turned out, development of portable olfactometers provided valuable new facilities for field experiments (in contrast to the former, exclusively stationary laboratory equipment); this is indispensable in monitoring, e.g., the air pollution. On the other hand, Doty and

co-workers developed a very simple test system, based on microencapsulation of standard odorants which allowed simple scaling of olfactory abilities in very large populations (UPSIT, University of Pennsylvania Smell Identification Test, Doty et al., 1984a). This seems to provide a great tool for the more general recognition of diseases with manifestations, implicating losses in olfactory performance.

Psychophysical experiments revealed regularly lower acuity in recognition and discrimination of odor qualities and concentration in humans than in most animals, but these processes were influenced by factors similar to those in other (terrestrial) vertebrates (flow rate, temperature and humidity of the inhaled air, dependence on olfactory stimuli preceding the test stimulus, other conditioning factors, nutritional and vigilance state, emotions, sex, etc.). Indications of the contribution by the trigeminal chemoreception (especially, when stimuli were irritant) were also noted (see e.g. Allen, 1929; Doty, 1975; Doty et al., 1978a; Benignus and Prah, 1980; Laing, 1985).

The olfactory memory of humans, like in other vertebrates, is extremely good (Lawless and Cain, 1975), although olfactory abilities decrease with age (Doty et al., 1984a,b; Stevens et al., 1984). Olfactory performance is also decreased in patients with temporal lobe lesions, showing the importance of this neocortical area in odor identification (Rausch and Serafetinides, 1975; Levin et al., 1985).

During the extensive studies of Doty et al. (1984a) on a high population of subjects, significant correlations between the olfactory performance of subjects and their age, gender, ethnic background, smoking behavior (as mentioned earlier) and known olfactory disorders (see below Section 9.2.3.) were revealed. Women performed better at all ages (Doty et al., 1984a; Fig. 9.2) and with most odorants tested (Comfort, 1971; Doty, 1981; Doty et al., 1984a).

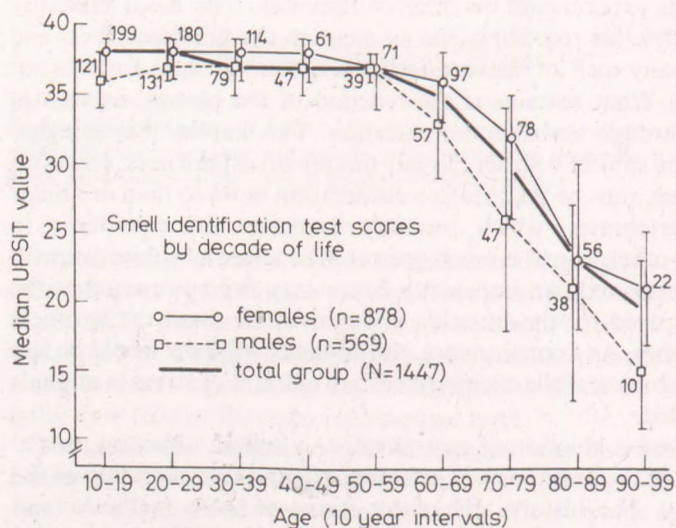


Fig. 9.2. Relationship between the olfactory performance (as evaluated by the University of Pennsylvania Smell Identification Test, UPSIT), age and gender (from Doty et al., 1984a). The test was based on recognition of 50 (natural, complex) encapsulated odor samples. Reprinted, with permission, from the *Physiology and Behavior*, Vol. 32, © 1984, Pergamon Journals Ltd.

9.2.2. Human communication by odors

Influence of external factors, relating to social behavior and reproductive life of vertebrates, and detected through nasal chemoreception has been widely accepted for a long time. Progression of studies on pheromones and corticosteroids saw a growing interest over the last two decades into similar questions, relating to human communication, and resulted in several interesting recognitions (Doty, 1981).

Awareness of the presence of human pheromones arose from observations on the likely close coincidence of the onset of the menstrual cycle in women living together for at least several months. The experiments of McClintock (1971) on college room mates provided significant data for the existence of such synchronization. This observation initiated suggestions for the production of pheromones by glands from various regions of the human skin (Comfort, 1971). Brooksbank and co-workers (1974) were able to reveal a steroid from men's axillary secretion; a very high sensitivity of humans to this musky odor was also noted.

Significance of the human "body odor" was discussed and the main sources of their production were characterized by Doty (1981). These were the sweat and sebaceous glands of the skin (including the hands), the axillary and vaginal glands. In general, human body odor was rated unpleasant by most observers; increasing intensity enhanced this judgement. Gender identification was possible through examination of body, axillary, hand and breath odors (Russell, 1976; Hold and Schleidt, 1977; Doty et al., 1978b, 1982; Doty, 1981), but the correct identification of men, as the source, was suggested to be dependent in part on (expected and anticipated) higher smell intensity only. Kin recognition was reported through studies of families and siblings (Porter and Moore, 1981); partners could be recognized (Hold and Schleidt, 1977) and even formerly unknown persons could be discriminated by human observers, through body odors (Wallace, 1977). Babies were able to discriminate between the odors of breast pads worn by their own or a strange mother (MacFarlane, 1975; Russell, 1976), not impossibly by investigating their own odors. A change in the acuity of odor recognition, as related to the phases of the menstrual cycle was also established (Doty, 1981; Doty et al., 1981).

In summary, these experiments clearly demonstrated that the (healthy) human body communicates with other humans through volatile chemicals and (nasal) chemoreception. In addition, characteristic odors of the human body, mostly breath and urine, helped physicians for long time in the recognition of certain diseases (with respect to children, for a summary see Table 1 in Doty, 1981).

9.2.3. Dysfunctions of the human olfactory system

Since impairments in the olfactory system accompany several disorders of the central nervous system, an examination of the olfactory abilities at the sensory and perceptual level may give an indication of serious diseases and so, may provide important information for neurologists. As the olfactory recognition and discrimination tests are harmless, their wide-range application in making a presumptive diagnosis would be desirable.

The best human olfactory performance was reported for non-smoking women around the 30–50 age group (Doty et al., 1984*a,b*). At earlier ages, although the receptor region may be more intact (less exposed to damaging environmental effects; see in this respect also Sections 2.5. and 9.2.3.1.), the experience is still limited. Later, accumulating effects of the environmental factors, accidents, medical interventions and certain diseases may seriously impair the olfactory acuity and perception. Although maldevelopment may also result in absence of smell sensation, limited olfactory capabilities (hyposmia) or complete lack of this sense (anosmia) seems to be caused mainly by impairments acquired during postnatal life. Specifically elevated thresholds, to certain odorants only, were also reported for people, having otherwise normal olfactory sensitivity; this was termed “specific anosmia” or “smell blindness” (Amoore et al., 1968). In special cases, heightened (when compared to normal) olfactory acuity may also occur. In the following two sections, a brief summarization of these aspects will follow. For factors affecting the optimal olfactory performance, see also the last paragraph of Section 9.2.1.

9.2.3.1. Disorders resulting primarily from impairments in the olfactory system

As one of the airways of the breathing system in direct contact with the environment, the nose is exposed to all, (volatile or non-volatile) airborne material of the given surroundings. Consequently, the olfactory receptors are accessible to destructive chemicals, allergenes, dramatic changes in temperature and humidity of the flowing air, virusoidal infections, accidents, medical interventions, etc. These invading factors may cause obstruction of the nasal airways, vasomotor, allergic or infectious rhinitis and subsequent degeneration of the receptor cells, resulting in a decrease or complete disappearance of olfactory abilities (Douek, 1974; Doty, 1979; Wright, 1987).

Thus, impairment of odorant detection at the receptor level may be an everyday hazard for a large proportion of the population. Capsaicin (at regular chili pepper-consumers) causes decrements in flavor identification and masks the olfactory sensation (Lawless et al., 1985). Decreased olfactory sensitivity is apparent in industrial workers, inhaling volatile air pollutants and chemicals (Doty, 1979), ozone (Prah and Benignus, 1979) and in smokers (Doty et al., 1984*a,b*). A simultaneous exposure to carbon monoxide and ethanol may also reduce the olfactory sensitivity (Engen, 1986).

Due to the easy access of carcinogens to the nasal mucosa it is not surprising that olfactory tumors, involving the peripheral olfactory apparatus, have also been reported, although in relatively low instances. These tumors were characterized morphologically and also histochemically. The presence of (cca. 100 nm) dense-core vesicles in processes (McGavran, 1970) was a characteristic symptom of olfactory neuroblastomas. Presence of neuron-specific enolase (NSE), S-100 protein and keratin (rarely neurofilament proteins) was also reported from olfactory neuroblastoma tissues (Tamada et al. 1984; Choi and Aderson, 1985, 1986; Taxy et al., 1986).

More central olfactory areas were also included in certain tumors (Bakay and Cares, 1972); however, they were relatively rare. Combination of X-ray therapy with surgical

interventions was suggested to provide the best result in the treatment of olfactory system-related tumors (Bailey and Barton, 1975; Mills and Frierson, 1985). The (chemically induced) rat olfactory epithelial tumor model, as introduced by Vollrath et al. (1986) may significantly improve the chance to increase our knowledge of this kind of human tumor.

9.2.3.2. Other diseases, involving the olfactory system

Addison's disease (an adrenal cortical failure) was the only syndrome, which was reported to dramatically enhance (by a factor of 100,000) the olfactory acuity; corticosteroid treatment normalized thresholds of these patients (Henkin and Barter, 1966). All other diseases resulted in a decrease or complete elimination of the perception of olfactory signals, as illustrated in the following examples.

Hypogonadotropic hypogonadism, *Kallmann's syndrome* (Kallmann et al., 1944; De Morsier, 1962) was associated with complete anosmia (absence of the olfactory bulb) (see also Males et al., 1973; Doty, 1979; Doty et al., 1984a). Investigation of the sense of smell may help to recognize Kallmann's syndrome in hypogonadal patients (Males et al., 1973). Another endocrine-related olfactory deficit was also discussed by Doty (1979), like *Turner's syndrome* (chromatin negative gonadal dysgenesis). These syndromes, together with Addison's disease seemed to provide further support for close interrelationships between the human olfactory and reproductive systems.

Reduced capability in olfactory (and gustatory) discrimination was recognized in alcoholic *Korsakoff patients* (Jones et al., 1975, 1978). In this organic brain disease, the mediodorsal thalamic nucleus and its neocortical projections were also damaged; this observation was correlated with impairments in perception, but not in sensory recognition, of odorants (Mair et al., 1980).

In *Parkinson's disease* a decrease in dopamine content in olfactory-related brain areas (Ansari and Johnson, 1975) as well as decrements in olfactory recognition (Ansari and Johnson, 1975; Ward et al., 1983; Corwin et al., 1985) was observed; it was related again to perceptual rather, than to sensory, impairments.

Much attention has been paid to the involvement of the olfactory system in the *Alzheimer's disease* during the last few years. Cell loss and the appearance of senile plaques were reported in the accessory olfactory bulb (Averback, 1983; Esiri and Wilcock, 1984) and anterior olfactory nucleus (Ohm and Braak, 1987), neurofibrillary tangles and threads occurred in the latter and also in the main OB (Ohm and Braak, 1987). Presence of neuritic masses and specific staining with antibodies against Alzheimer tissue or phosphorylated neurofilaments was also apparent in the nasal epithelium (as removed in autopsy) of Alzheimer patients (Talamo et al., 1989). In this disease, choline acetyltransferase (ChAT) content decreased in rhinencephalic areas (Simpson et al., 1984). In addition to these morphological and neurochemical symptoms, sensory and perceptual losses in Alzheimer patients, as regards the olfaction, were also shown (Corwin et al., 1985; Peabody and Tinklenberg, 1985; Warner et al., 1986; Doty et al., 1987; Rezek, 1987). It is interesting to note that the peripheral part of the olfactory system, the nose was hypothesized as a door for

aluminosilicates, which may be transported transsynaptically into the brain and may cause Alzheimer's disease (Roberts, 1986).

A decrease in the ChAT content of the olfactory tubercle in patients, featuring symptoms of *Down's syndrome* and *Huntington's disease* (Simpson et al., 1984) draws attention to a possible involvement of the olfactory system/function in these diseases, too. *Schizophrenic* patients performed significantly worse in olfactory discrimination tests than other psychiatric patients, who were on similar neuroleptic medication, or than the normal controls (Hurwitz et al., 1988). *Cystic fibrosis* was accompanied by elevation of the olfactory threshold in experiments of Weiffenbach and McCarthy (1984). Similarly, detection and recognition thresholds were significantly elevated in patients with *Sjögren's syndrome* (accompanied by rhinitis sicca) (Henkin et al., 1972).

A lower proportion (17%) of *hypothyroid* patients were found to be hyposmic (39% had distortions of taste) in McConnell and co-workers' (1975) experiments; thyroxine treatment largely improved their sensation. Antithyroid drugs may result in a contrary effect, i.e., loss of taste and smell (Schneeberg, 1952; Grossman, 1953). Finally, let me remind the reader of the *type 1a pseudohypoparathyroid* patients who are deficient in a guanine nucleotide binding protein, which results in olfactory dysfunction (Weinstock et al., 1986), most likely through a deficiency in the sensory (receptor) level, as discussed in Section 2.3.1.

9.3. Summary

Human nasochemosensory systems are similar in many respects to those found in other vertebrates. With respect to the number of epithelial receptors, size of the bulb (proportional to the rest of the brain) and extension of its central projection, the human olfactory system can be regarded as a moderately developed one. Both the main olfactory and the trigeminal systems are capable of detecting odorants. This enables healthy humans to detect and discriminate a high number of smells at low thresholds, and even to gain information on other humans. Several impairments in the central nervous system also involve the olfactory system. Consequently, a rating of human olfactory capabilities by standardized smell identification tests may have a special importance in making presumptive diagnoses in neurology.

Although the sense of smell in humans has a more limited importance than in many other vertebrates, it provides the brain with information influencing behavior from birth to the declining years. The contribution of this sense may be most relevant to warnings about environmental hazards, nutrition and emotional life. Odorants may provide the newborn baby with a clue to maternal care and safety; they may help the young and adult human to recognize and select optimal conditions. Moreover, they bring about a delight, when such conditions are found and experienced, through the activation of the oldest cortical area of our brain, belonging to the olfactory system.

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Olfaction (as a kind of chemical senses) is one of the most ancient sensory modalities. It may have priority over other senses or may be overshadowed by others, all vertebrates develop—most of them also use and simply cannot survive without—the nasal chemosensory systems and their central connections. This book is an attempt to summarize a selection of the knowledge gained about the vertebrate olfactory system from fish to man and from macroscopic observations to molecular events. The main emphasis is on the neurochemical studies, as they constitute the most obvious link between morphological findings and functional events. The structure and neurochemistry of the olfactory bulb constitute the most voluminous and richly illustrated part of this monograph. In addition, an account is given about the accessory nasal chemosensory systems; developmental aspects are correlated with morphology, and the significance of olfaction in the vertebrate—including human—behavior is also illustrated.