Chapter 1

Anatomy and Physiology of Olfaction

Introduction

The evolution of life required organisms to sense chemicals suspended or dissolved in water. Some of these chemicals provided nourishment, whereas others were destructive and had to be avoided. Single-celled organisms, such as *Escherichia coli*, developed multiple chemical receptors critical for such survival. The rotatory direction of their flagellae – whip-like appendages used to propel them through their environment – is altered by the type of chemical encountered. Thus, chemicals important for sustenance induce a counterclockwise rotation of the flagella, facilitating a smooth and somewhat linear swimming path, whereas toxic chemicals provoke a clockwise flagellar rotation, resulting in tumbling and turning away from the offending stimulus (Larsen et al., 1974).

The sense of smell is one of nature's true wonders, being ubiquitous within the animal kingdom and capable of detecting and differentiating thousands of diverse odorants at very low concentrations. Humans possess far more odorant receptor types than any other sensory system, which explains, in part, their ability to perceive such a large number of stimuli. It is now well established, as described in subsequent chapters of this book, that the olfactory system provides a unique probe into the general health of the brain. Thus, smell loss is among the first signs of neurodegenerative diseases such as Alzheimer's or Parkinson's disease and provides insight into elements of brain development. Importantly, smell loss is one of the best predictors of future mortality in older populations, being a stronger predictor than cognitive deficits, cancer, stroke, lung disease, or hypertension even after controlling for the effects of age, sex, race, education, socioeconomic status, smoking behavior, alcohol use, cardiovascular disease, diabetes, and liver damage (Wilson et al., 2011; Gopinath et al., 2012; Pinto et al., 2014; Devanand et al., 2015). In the future, screening for a range of neurological disorders by olfactory biomarkers may be commonplace and may encourage the development of protective measures that delay or prevent central nervous system (CNS) degeneration.

We now describe the detailed anatomy, physiology, and pharmacology of the olfactory pathway, followed by factors that influence olfactory input and its interpretation.

Nasal Cavity

During normal inspiration, only 5–10 percent of inhaled air reaches the olfactory epithelium. This specialized pseudostratified neuroepithelium harbors the olfactory receptors. It is found high within the nasal vault, lining sectors of the upper nasal septum, cribriform plate, superior turbinates, and, to a lesser extent, the anterior aspect of the middle turbinates

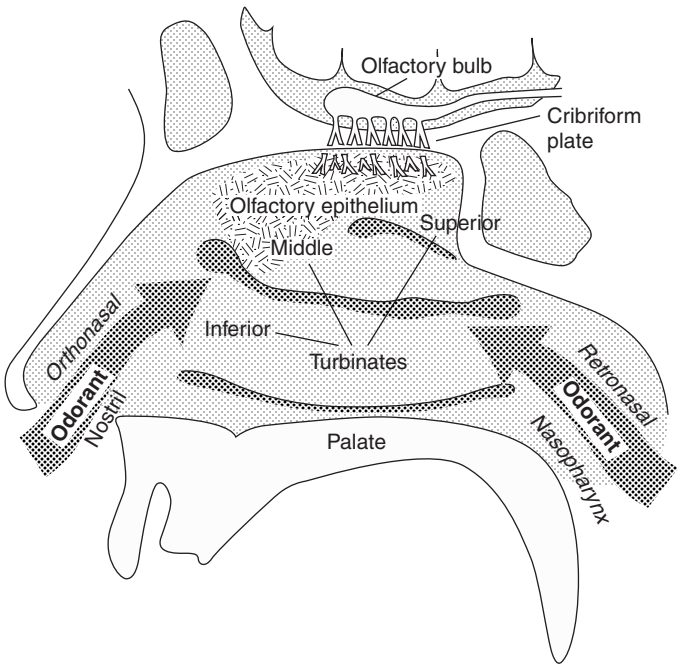

Figure 1.1 This figure shows the human nasal cavity and extent of the olfactory epithelium. Note the extension of the epithelium onto the anterior part of the middle turbinate. Odorants access the olfactory epithelium either directly through the orthonasal route (anterior arrow) or indirectly through the retronasal route as in chewing or swallowing (posterior arrow). Reproduced with permission from Rawson, N. (2000), Chapter 11, Human olfaction.

(Figure 1.1). The existence of olfactory receptor neurons (ORN) on the middle turbinate is a useful aspect of applied anatomy for those wishing to biopsy olfactory receptor cells (ORC) for culture, histology, or patch clamp studies, as it is more accessible and less risky to sample than the main olfactory area.

Sniffing. Although sniffing assists smell recognition and identification, the first awareness of a new odor can be passive; sniffing then follows in an attempt to analyze the odor further and assess its behavioral significance. Sniffing redirects up to 15 percent of the inhaled air through the olfactory meatus, a ~1 mm-wide opening leading to the uppermost sector of the nose that contains most of the olfactory epithelium. Sniffing helps to increase the number of odorous molecules that ultimately reach this region. However, molecules must absorb into the mucus that forms across the nasal mucosa to make contact with the olfactory receptor cells. In some cases – particularly in the case of hydrophobic odorants – stimuli may be carried through this mucus by specialized "odorant carrier" proteins to the receptors (Pelosi et al., 1990). It should be emphasized that without a moist mucosal surface, detection of odors is largely impossible. As mentioned in Chapter 5, diseases with excessive nasal dryness such as Sjögren's syndrome are often accompanied by smell dysfunction.

Nasal Turbinates. The nasal turbinates are highly vascularized structures that extend into the nasal cavity from its lateral wall (Figure 1.2). They can rapidly expand or contract, depending upon autonomic nervous system tone and stimulation. Exercise, hypercapnia, and increased sympathetic tone constrict their engorgement, whereas cold air, irritants, hypocapnia, and

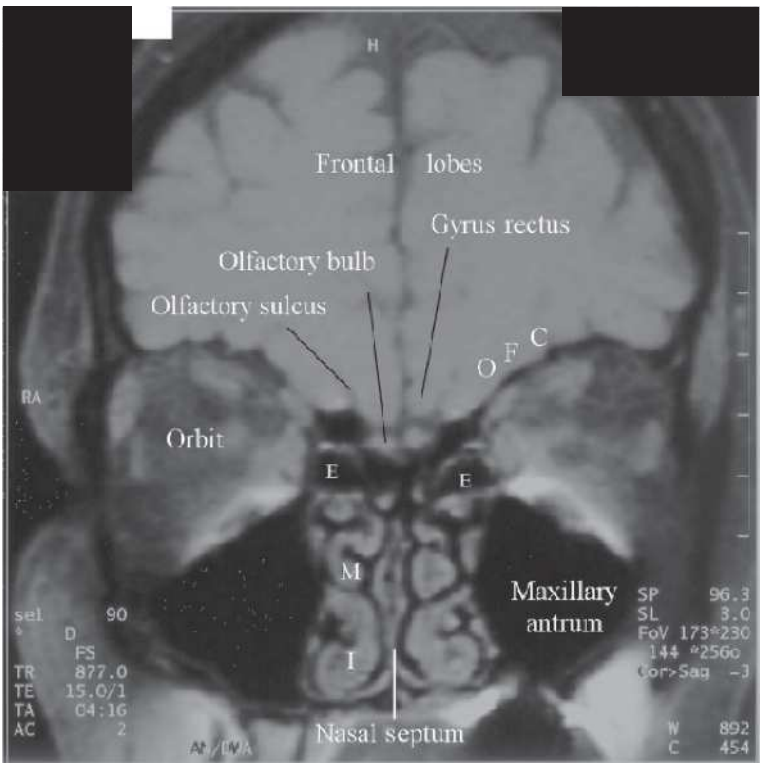

Figure 1.2 Coronal T1–weighted MRI scan to show the main structures around the nose.

increased parasympathetic tone can induce such engorgement. Turbinate engorgement can be influenced by pressure on sectors of the body, body position, or ambient temperature. Left-to-right fluctuations in relative engorgement, termed the nasal cycle, occur in many people over time, although these change with age and reciprocity is frequently the exception rather than the rule (Mirza et al., 1997). These fluctuations relate to changes in lateralized blood flow to various paired organs, including the two brain hemispheres, and belong to the basic rest-activity cycle, a continuation of the REM/non-REM sleep cycle that occurs during the daytime. Although the turbinates have never been thought relevant to the clinical neurologist, this concept may need to change given the recent suggestion that rhinorrhea, secondary to relative parasympathetic overactivity, may be a prodromal sign of Parkinson's disease (see Chapter 7 and Bower et al., 2006).

Innervation of the Nasal Cavity. In common with the nasal and oral mucosae, the olfactory epithelium also contains free nerve endings from the trigeminal nerve (CN V). Non-olfactory elements of nasal chemosensation, e.g., sharpness, coolness, warmth, and pungency, are mediated via free nerve endings of this nerve (Figure 1.3). These free nerve endings are supplied to the upper part of the nasal cavity by the anterior and posterior ethmoid nerves – branches of the nasociliary nerve which come from the ophthalmic (first division) of the trigeminal nerve. The nasopalatine nerve, a branch of the maxillary nerve (second division of the trigeminal nerve) is the source of the CN V fibers innervating the posterior nasal cavity. Most odorous compounds stimulate CN I and CN V, at least at higher

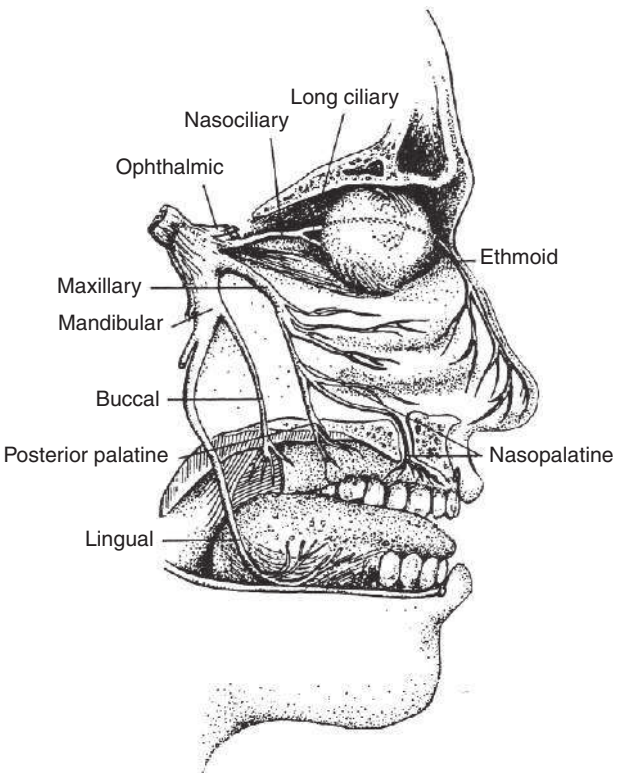

Figure 1.3 Schematic diagram of the branches of the trigeminal nerve that innervate the nasal, oral, and ocular epithelia. From Bryant & Silver (2000). Copyright © 2000, Wiley-Liss.

concentrations and/or volumes. Vanillin is one of few compounds that appear to have no perceptible CN V activity. This contrasts with ammonia, for example, which strongly stimulates the olfactory and trigeminal nerves. Thus, patients with anosmia can readily detect, via trigeminal sensation, several "impure" odors, such as menthol or camphor.

Vomeronasal Organ (VNO) and Nervus Terminalis. The VNO is a bilaterally symmetrical tubular structure located just above the palate at the base of the anterior nasal septum. This pouch-like structure is enclosed in a bony capsule and, depending upon the species, has a single opening into either the nasal or the oral cavity via the vomeronasal or nasopalatine duct, respectively. In the case of humans, the VNO is clearly vestigial and non-functional. In the developing human fetus there is a VNO which stains for gonadotropin-releasing hormone (GnRH), but its connections with the olfactory bulb disappear or become displaced and hard to locate at about 19 weeks of gestational age. In the adult human the vestigial VNO is present bilaterally on the anterior third of the nasal septum and opens through a pit about 1–2 cm behind the posterior margin of the nostril. However, VNO cells do not react to olfactory marker protein – a stain for functional olfactory neurons – and there is no neural connection to the CNS. Adult humans lack an accessory olfactory bulb, to which functional VNOs project. Moreover, most human VNO receptor genes are vestigial (pseudogenes) and the VNO-specific TRP2 cation channel critical for VNO function is lacking.

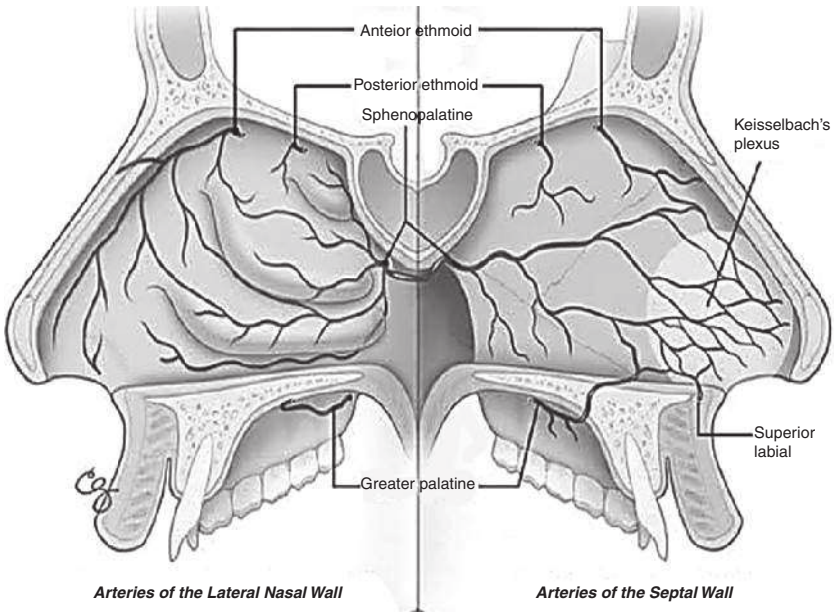

Figure 1.4 Arterial supply of the lateral and medial nasal walls. Reproduced with permission from Chris Gralapp in Medscape. (www.medscape.org/viewarticle/723327_2)

Another nerve in the noses of humans and many other vertebrates is the terminal nerve, also termed the nervus terminalis or Cranial Nerve zero. This unmyelinated and highly ganglionated nerve plexus is found within both the olfactory and non-olfactory sectors of the nasal cavity, and is the most anterior of the vertebrate cranial nerves. In some human specimens it can be seen intracranially at the base of the brain with the naked eye, lying along the inside surface of the dura mater medial to the main olfactory bulb near the cribriform plate. In the few species that have been examined, this understudied nerve releases GnRH into the region of the olfactory receptors, thereby modulating their function (Eisthen et al., 2000; Zhang & Delay, 2007). Damage to this nerve in hamsters alters copulatory behavior, presumably reflecting its close relation to the hypothalamus and its high gonadotropin-releasing hormone (GnRH) content (Schwanzel-Fukuda & Pfaff, 2003). At present, its function in humans is not known, although a sensory role, per se, is highly unlikely (Fuller & Burger, 1990).

Vascular Supply. The vascular supply of the nasal cavity comes from tributaries of the external and internal carotid arteries (Figure 1.4). The ophthalmic branch of the internal carotid artery gives rise to anterior and posterior ethmoid arteries that supply the upper part of the nasal cavity. The sphenopalatine artery, also derived from the internal carotid, feeds the posterior nasal cavity. The anterior sectors of the nasal chamber are fed by branches of the facial and internal maxillary arteries which derive from the external carotid artery. Impaired blood supply in the anterior and posterior ethmoidal arteries (branches of the ophthalmic arteries) may interfere with olfaction, as noted in Chapter 5. Thus, impairment is seen occasionally in vasculitic disorders such as systemic lupus erythematosis, giant cell arteritis, and Churg-Strauss syndrome. In

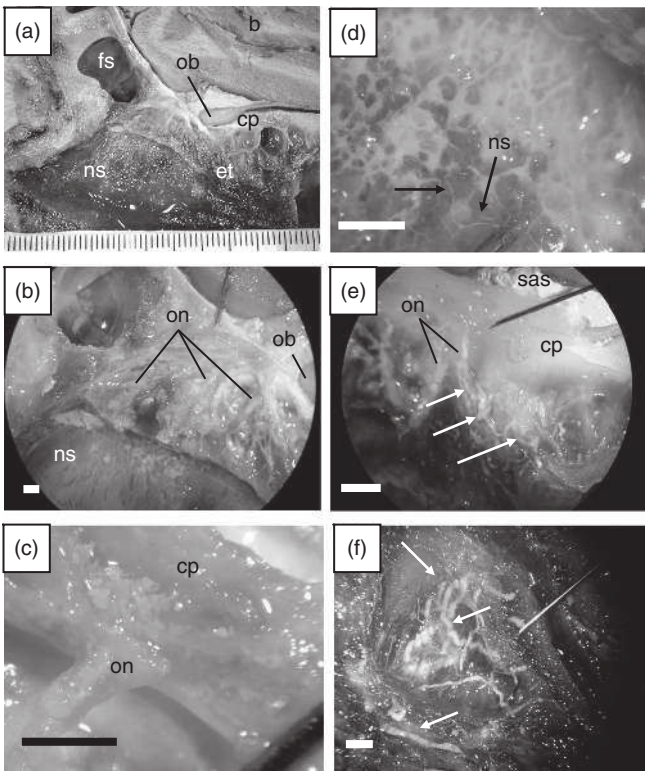

Figure 1.5 Lymphatic drainage through the nasal cavity. Silicone (Microfil) injection distribution patterns in the head of a human (a-f). All images are presented in sagittal plane with gradual magnification of the olfactory area adjacent to the cribriform plate. Reference scales are provided either as a ruler in the image (mm) or as a longitudinal bar (1 mm). Microfil introduced into the subarachnoid space was observed around the olfactory bulb (a), in the perineurial spaces of the olfactory nerves (b, c), and in the lymphatics of the nasal septum (d), ethmoid labyrinth (e), and superior turbinate (f). Some lymphatic vessels ruptured and Microfil was noted in the interstitium of the submucosa of the nasal septum (d). In (e), Microfil is observed in the subarachnoid space and the perineurial space of olfactory nerves. The perineurial Microfil is continuous with that in lymphatic vessels (arrows). Intact lymphatic vessels containing Microfil are outlined with arrows (d-f). Key to abbreviations: b – brain; fs – frontal sinus; cp – cribriform plate; et – ethmoid turbinates; ob – olfactory bulbs; on – olfactory nerves; ns – nasal septum; sas – subarachnoid space. Reproduced with permission from Johnston, Zakharov et al. (2005). (A black and white version of this figure will appear in some formats. For the color version, please refer to the plate section.)

general, there is a high rate of blood flow within the human nasal *respiratory* epithelium (~42 ml/100 g/minute), as measured by Laser-Doppler flowmetry, but the blood flow rate in the human *olfactory* epithelium is unknown.

Lymphatic Drainage. It is not widely appreciated that the nasal cavity is a major route for lymphatic drainage of cerebrospinal fluid (CSF). It is clear from observations in mice (Weller et al., 2009) and humans (Johnston et al., 2005) that CSF drains along olfactory perineural lymphatics from the subarachnoid space through the cribriform plate into the nasal cavity, terminating in cervical lymph nodes (Figure 1.5). As explained in Chapter 7, pathogens could use this drainage pathway in reverse direction to access the CSF and brain.

Figure 1.6 Electron photomicrograph of transition zone between the human olfactory epithelium (bottom) and the respiratory epithelium (top). Note the long olfactory receptor cell cilia compared to the cilia in the respiratory epithelium. Arrows signify two examples of olfactory receptor cell dendrites with cilia that have been cut off. Bar = 5 µm. From Menco & Morrison (2003), with permission. Copyright © 2003, Richard L. Doty.

Olfactory Receptor Cells (ORCs). In humans, the 6–10 million ORCs, collectively termed the first cranial nerve (CN I), are found at different stages of maturity in the olfactory epithelium. These bipolar cells are the first-order neurons of the system, and their central axons project directly from the nasal cavity to the olfactory bulb without an intervening synapse, making them a major conduit for CNS pathogens (Doty, 2008). When mature, each ORC projects up to 30 cilia into the mucus, which form knob-like dendritric extensions (Figure 1.6). These cells are unique in several ways. For example, they serve as sensory transducers, whereas in most other sensory systems the transducing cell is not a neuron, but it is synaptically connected to an effector sensory neuron. When damaged, ORCs, like the other cell types within the olfactory neuroepithelium, can be replenished from neuronal progenitor cells and basal stem cells located near the basement membrane (Loo et al., 1996).

Many other types of cells are located within this unique epithelium. Glial-like supporting (sustentacular) cells separate the ORCs from one another and may play some role in paracrine modulation of olfactory receptor cell activity. Another cell linked to paracrine activity is the ubiquitous microvillar cell, which is involved in modulation of ORCs via secretion of acetylcholine into the mucosa (Schiffman & Gatlin, 1993). A small number of such cells appear to project to the olfactory bulbs (Rowley et al., 1989). Most of the mucus that bathes the olfactory epithelium is derived from Bowman glands, whose ducts permeate the olfactory epithelium (Figure 1.7).

It is important to recognize that many odorants and chemicals are actively metabolized by the olfactory mucosa, being deactivated, detoxified, or, in rare cases, transformed into

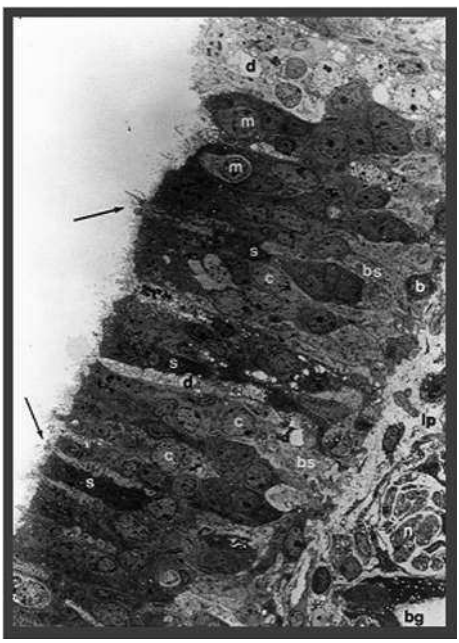

Figure 1.7 Low-power cross-section of the human olfactory neuroepithelium depicting the four major types of cells: bipolar receptor cells (arrows point to cilia at dendritic knob; c, cell body), microvillar cells (m), sustentacular cells (s), and basal cells (b). Key to abbreviations: bg – Bowman's gland; lp – lamina propria; n – collection of axons within an ensheathing cell; d – duct of Bowman's gland; bs – basal cell undergoing mitosis. Electron photomicrograph courtesy of Dr. David Moran, Longmont, Colorado.

toxic metabolites. The sustentacular cells, acinar and duct cells of Bowman glands, are enriched with xenobiotic-metabolizing enzymes. More than ten different P450 enzymes have been identified in the olfactory mucosa of mammals, including members of the CYP1A, 2A, 2B, 2C, 2E, 2G, 2J, 3A, and 4B subfamilies. Many P450s are preferentially expressed in the olfactory region, such as CYP2G1.

The ORN are among the few cells of ectodermal origin capable of regeneration. Others include cells within the subgranular zone of the hippocampal dentate gyrus, the sub-ventricular zone (SVZ) in the wall of the lateral ventricle, the organ of Corti, and the granule and periglomerular cells of the olfactory bulb (Maier et al., 2014). The latter cells arise from neuroblasts formed within the SVZ that migrate to the bulb along the rostral migratory stream. Approximately 95 percent of these neuroblasts become granule cells, whereas the remainder become GABAergic and dopaminergic periglomerular cells.

The olfactory receptor proteins are found on the cilia that, in many cases, lie along the surface of the mucus covering the epithelium. Unlike the cilia of the respiratory epithelium, these long cilia lack contractile (dynein) arms and, thus, intrinsic motility.

Olfactory Transduction

The primary second messenger for mammalian odor transduction is cAMP, whose essential function is to amplify the incoming signal from odorant receptors and ultimately facilitate release of glutamate, the primary neurotransmitter of the olfactory receptor cells. Opening