

Chapter 15

Intranasal Drug Delivery to the Brain



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Abstract The barriers that separate the blood from brain interstitial and cerebrospinal fluids present a significant challenge to efficient and practical drug delivery into the central nervous system (CNS). New strategies to circumvent the blood-brain barrier (BBB) have long been needed to utilize polar pharmaceuticals and large biotherapeutics for CNS disease treatment because the BBB is typically impermeable to such compounds. The increasing application of biologics as therapeutics over the past several decades has brought much new interest in routes of drug delivery that may be more easily utilized for chronic dosing of large molecules, e.g., oral, subcutaneous, transdermal, pulmonary, and intranasal administration. The intranasal route in particular offers a number of advantages for chronic dosing including its noninvasiveness, efficient uptake and absorption into a highly vascular submucosa, avoidance of hepatic first-pass elimination, rapid pharmacokinetic pro-

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files, and ease of administration. Importantly, the intranasal route has also been demonstrated to potentially allow a variety of drugs direct access to the brain and/or cerebrospinal fluid. Studies over the past few decades have shown that even large biotherapeutics may have access to the CNS along extracellular pathways associated with the olfactory and trigeminal nerves. This chapter provides an overview of the unique anatomic and physiologic attributes of the nasal mucosa and its associated cranial nerves that allow small but significant fractions of certain intranasally applied drugs to transfer across the nasal epithelia and subsequently be transported directly into the CNS. We also review some of the preclinical and clinical literature related to intranasal targeting of biologics to the CNS and comment on future directions for the further clinical translation of this route of administration.

Keywords Drug delivery · Nasal passage · Olfactory · Trigeminal · Proteins · Gene vectors · Stem cells

15.1 Introduction

The blood-brain barrier (BBB) and blood-cerebrospinal fluid barriers (BCSFB) are critical for the maintenance of central nervous system (CNS) homeostasis. Although these barriers restrict neurotoxic substances from entering the brain, they also restrict many potential therapeutics from reaching the CNS. The BBB, formed by brain endothelial cell lining microvessels, exhibits a low rate of pinocytosis and possesses tight junction (TJ) protein complexes on apposing cells that limit paracellular permeability (Reese and Karnovsky 1967). These TJ create a high transendothelial electrical resistance of 1500–2000 $\Omega \cdot \text{cm}^2$ compared to 3–30 $\Omega \cdot \text{cm}^2$ across most peripheral microvessels (Crone and Olesen 1982; Butt et al. 1990). This high resistance is associated with very low paracellular permeability, and typically, only small (<600 Da), lipophilic molecules appreciably cross the healthy BBB via transcellular passive diffusion, although some limited transport of certain peptides and peptide analogs has been reported (Banks 2009). Additionally, many potential therapeutics that would otherwise be predicted to cross the BBB based on their molecular weight (MW) and lipophilicity are restricted by the expression of drug transporters (e.g., P-glycoprotein) (Miller 2010; Ronaldson et al. 2007).

Nearly all CNS drugs in clinical use today can be categorized as small MW pharmaceuticals that either cross the BBB transcellularly (e.g., barbiturates) or utilize endogenous transporters expressed on endothelial cells (e.g., Parkinson's therapeutic levodopa). Just about all large MW substances are severely restricted from crossing the BBB under physiological conditions. Indeed, the only examples of large MW drugs approved for clinical use in treating neurological illnesses are those that act outside the CNS (e.g., type I interferons for treating multiple sclerosis), those with the chance to cross compromised endothelial barriers associated with some CNS tumors (e.g., the humanized monoclonal antibody bevacizumab for the treatment of recurrent glioblastoma); a peptide administered intrathecally to treat severe, chronic pain (the ~3 kDa cone snail toxin ziconotide); an antisense oligonucleotide

administered intrathecally to treat spinal muscular atrophy; an enzyme administered intraventricularly to treat Batten disease; and a recently approved AAV9-based gene therapy. Many other large MW peptides, proteins, oligonucleotides, and gene therapy vectors have been identified as potential CNS therapeutics based on studies utilizing *in vitro* systems and animal models; however, new drug delivery strategies are needed to allow these potential drugs to cross or bypass the BBB and BCSFB for these studies to translate to the clinic (Neuwelt et al. 2008). It is also likely that recent advances in cerebrovascular biology, e.g., single-cell transcriptomics analyses of the brain vasculature in mice (Vanlandewijck et al. 2018) and humans (Yang et al. 2021), coupled with advances in our understanding of the complex physiology of brain fluids (Abbott et al. 2018) may yield fresh, new ideas and previously unexplored novel approaches for CNS delivery.

The central input of substances through intraparenchymal, intracerebroventricular, or intrathecal injections/infusions represent one strategy, but these routes of administration are invasive and typically not ideal for chronic administration. Increasing evidence suggests the intranasal (IN) route of administration provides a noninvasive method to bypass the BBB and directly deliver therapeutics to the CNS along extracellular pathways associated with the olfactory and trigeminal nerves (Fig. 15.1). In addition to its noninvasiveness, the IN administration route has long

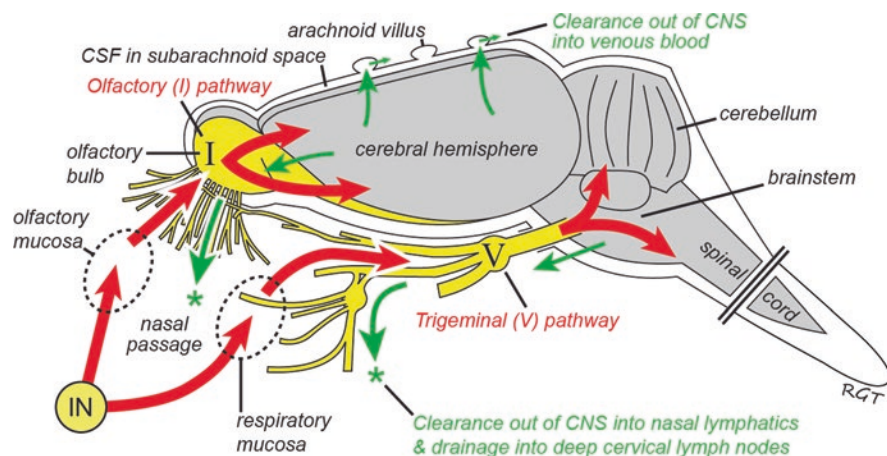


Fig. 15.1 Intranasal (IN) administration provides access to olfactory and trigeminal pathways (shown in red for the rat), potentially allowing certain peptides, proteins, and even cells to reach widespread CNS regions. Based on work utilizing radiolabeled proteins in rats and primates (Thorne et al. 2004a, b, 2008a, b), a small fraction of intranasally applied drug may be rapidly transported via components associated with the olfactory nerves to the olfactory bulbs and rostral brain regions or via components associated with the trigeminal nerves (the fifth cranial nerve) to the brain stem and caudal brain regions. Drug entry into the brain appears to occur rapidly following transport across the olfactory or respiratory epithelia. Other work has shown that a variety of substances may also be cleared out of the brain along possibly related pathways (shown in green) connecting CNS parenchymal tissue and cerebrospinal fluid (CSF) in the subarachnoid spaces with lymphatics in the nasal passages and, ultimately, the deep cervical lymph nodes of the neck (Bradbury and Cserr 1985; Kida et al. 1993). The principal clearance of CSF into the venous blood occurs through the arachnoid villi that extend from the subarachnoid space into the dural sinuses

been associated with a number of advantages (Lochhead and Thorne 2012), mostly based on the application of drugs with a systemic mode of action; these include typically rapid onset of effects, ease of administration by nasal drops or sprays, simple dose adjustment, avoidance of hepatic first-pass elimination, and a developing record of experience with clinically approved formulations (e.g., the nasal spray of the 3.5 kDa polypeptide hormone calcitonin has been used for many years to treat postmenopausal osteoporosis). The main disadvantages of the IN route comprise a limitation typically to potent drugs due to low nasal absorption (particularly for hydrophilic drugs, peptides, and proteins), limited solution volumes (typically, 25–200 μl in humans), active mucociliary clearance processes resulting in limited contact time with the absorptive epithelia, nasal enzymatic degradation for some drugs, interindividual variability, and low CNS delivery efficiencies ($<0.05\%$) for most proteins measured thus far (Lochhead and Thorne 2012; Costantino et al. 2007).

The IN administration route has a long, successful history of clinical application, where it has been used to deliver a number of drugs to the systemic circulation that cannot be given orally (Lansley and Martin 2001; Costantino et al. 2007). The possibility that IN administration may also deliver potentially therapeutic amounts of large MW drugs directly from the nasal passages to the CNS was first described relatively recently (Thorne et al. 1995; Frey 2nd et al. 1997). The delivery of small molecules, macromolecules, gene vectors, and even cells from the nasal passages to the brain has now been documented in numerous animal and clinical studies (Lochhead and Thorne 2012; Dhuria et al. 2010; Baker and Genter 2003; Illum 2004). This chapter provides an overview of relevant nasal anatomy and physiology as well as the potential pathways and transport mechanisms that are involved in the distribution of therapeutics from the nasal cavity to the CNS. We also summarize some of the most relevant preclinical and clinical studies that have presented evidence of brain entry and/or efficacy following intranasal targeting of biotherapeutics to the CNS and speculate on future directions.

15.2 Nasal Anatomy and Physiology

15.2.1 General Overview

The nasal chamber is divided into two separate passages by the nasal septum, with each nasal passage principally consisting of an olfactory region (containing the olfactory epithelium) and a respiratory region (containing the respiratory epithelium) extending from the nostrils (nares) to the nasopharynx. The general organization of the rat nasal passage is shown in Fig. 15.2. The olfactory region contains olfactory sensory neurons that are responsible for the detection of airborne odorants (i.e., mediating the sense of smell). Most of the nonolfactory epithelium in the nasal passages of laboratory animals and human beings consists of a respiratory epithelium specialized for warming and humidifying inspired air as well as the removal of

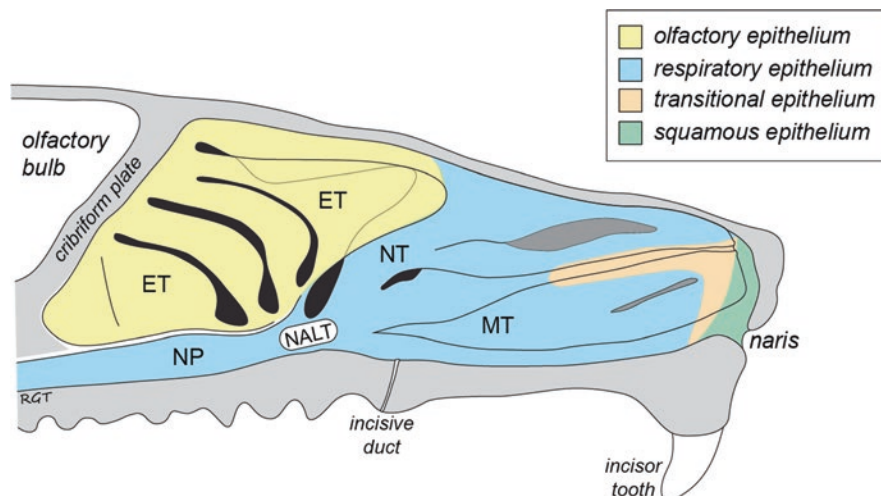


Fig. 15.2 Schematic diagram of the nasal passage showing the distribution of the surface epithelia on the lateral wall of the rat. Abbreviations: *ET* ethmoturbinates, *MT* maxilloturbinate, *NALT* nasal-associated lymphoid tissue, *NP* nasopharynx, *NT* nasoturbinate. (Figure partly based on Mery et al. 1994; Harkema et al. 2006)

allergens, microorganisms, and particulates (Harkema et al. 2006). The human nasal cavity has a large absorptive surface area of ~ 160 cm² due to three, comma-shaped bony structures called turbinates or conchae (inferior, middle, and superior) which filter, humidify, and warm inspired air (Harkema et al. 2006). The differences in nasal structure, organization, and physiology between primates and rodents may potentially be important in evaluating experimental data in support of nose-to-brain transport pathways (Lochhead and Thorne 2012), e.g., humans and monkeys are oronasal breathers, while rats are obligate nasal breathers with a turbinate architecture that is considerably more complex than that in primate species. Additionally, the olfactory region accounts for only about 10% of the total absorptive surface area in the human nasal cavity, whereas it comprises $\sim 50\%$ of the total nasal surface area in the rat, likely reflecting the greater importance of this sense for macrosmatic mammals such as rodents. By contrast, the absolute olfactory surface area does not differ too greatly between human beings (~ 12.5 cm²), rhesus monkeys (6–9 cm²), and rats (7 cm²) (Lochhead and Thorne 2012). While it is not yet clear if significant differences in nose-to-brain transport occur with different species, most investigations have utilized rodents simply because it has not been practical to conduct certain types of research in monkeys and human beings; further developments in noninvasive imaging may allow better comparisons between species in the future.

In addition to the olfactory and respiratory regions, the nasal cavity also contains squamous and transitional regions, along with a small specialized area of the lymphoepithelium (Harkema et al. 2006). The squamous region extends from just inside the nares to the anterior portion of the inferior turbinates and is lined with stratified squamous epithelium containing coarse hairs in addition to sebaceous and sweat

glands. The transitional region is a non-ciliated cuboidal or columnar epithelium located between the squamous and respiratory epithelia. Nasal-associated lymphoid tissue (NALT) contains the lymphoepithelium, a region on both sides of the nasopharyngeal duct in rodents (Fig. 15.2) that appears to play a role in the induction of antigen-specific immune responses (Kiyono and Fukuyama 2004). The stimulation of protective systemic/mucosal immunity resulting from intranasal administration of specific antigens (usually requiring the co-administration of an enhancing adjuvant for adequate stimulation of NALT) provides the basis for nasal vaccine development. It is generally considered that the olfactory and respiratory epithelia are by far the most important sites for nasal absorption, so these regions will be covered individually in greater detail below.

15.2.2 Blood Supply and Lymphatic Drainage

The nasal mucosa is extremely vascular, a feature which allows efficient absorption into the systemic circulation for drugs possessing the right properties for this to occur (e.g., drugs that are sufficiently small to cross through the interendothelial clefts of nasal capillaries). Once in the systemic circulation, a substance would need to cross the BBB or BCSFB to enter the CNS. Although some nasal endothelial cells express TJ proteins such as zona occludens (ZO)-1, occludin, and claudin-5, capillaries in the nasal submucosa appear fenestrated with porous basement membranes, suggesting higher permeability than capillaries comprising the BBB (Cauna and Hinderer 1969; Wolburg et al. 2008). Nasal venules and arterioles are continuous and lack fenestrations. The vascular density and relative vascular permeability vary in different regions of the nasal mucosa and serve as important considerations when designing intranasal dosing strategies to maximize drug delivery to the CNS (Kumar et al. 2015). The caudal olfactory region has a ~ fivefold lower mean capillary density and lower vascular permeability to hydrophilic macromolecules than the anterior respiratory region of the nasal mucosa. Delivering drugs to the olfactory region may therefore minimize clearance into the systemic circulation and consequently favor more drug to access the extracellular cranial nerve-associated pathways to the CNS (Kumar et al. 2015). Indeed, intranasal devices have been designed to target the olfactory region with the goal of enhancing direct delivery to the CNS (Hoekman and Ho 2011a, b). Clearly, the utility of targeting different regions of the nasal passage with intranasally administered drugs for the purpose of enhancing brain targeting is an area that merits further study.

The blood supplying the nasal passages is chiefly provided by (i) branches of the ophthalmic artery, (ii) the sphenopalatine artery, and (iii) branches of the facial artery (Greene 1935; Standring 2021; Schuenke et al. 2010). The anterior and posterior ethmoidal arteries branch from the ophthalmic artery to supply the olfactory region, anterior septum, and anterior lateral wall. The sphenopalatine artery mostly supplies the posterior septum and posterior lateral wall with smaller branches extending to further areas. Branches of the facial artery supply the anteroinferior

septum and lateral wall. Species differences between rats and humans exist upstream of the ophthalmic and sphenopalatine arteries. The internal carotid artery gives rise to the ophthalmic artery in humans, while the ophthalmic artery branches from the pterygopalatine artery in rats. In humans, the sphenopalatine artery is a branch from the maxillary artery via the external carotid artery. The rat sphenopalatine artery, however, arises from the pterygopalatine artery via the internal carotid artery. It is also relevant that both olfactory and trigeminal arteries have been described in the rat and in other mammals, including human beings (Coyle 1975; Scremin 2004; Favre et al. 1995); these vessels travel at least some distance with their respective nerve bundles and likely provide complex anastomoses between nasal arteries in the nasal passages and cerebral arterial branches from the anterior and posterior brain circulations. Venous drainage in the posterior nasal passage occurs primarily through the sphenopalatine vein, while veins accompanying the ethmoidal arteries drain the anterior nasal passage. Some veins in the nasal passage connect with cerebral veins on the frontal lobe after passing through the cribriform plate.

Although there are no lymph nodes in the CNS, several studies have shown that extracellular and cerebrospinal fluids in the brain may drain either through the arachnoid villi to the venous blood or through the cribriform plate to the nasal lamina propria and then subsequently to the deep cervical lymph nodes in the neck (Fig. 15.1) (Bradbury and Cserr 1985). Intranasally administered substances that are absorbed to the nasal lamina propria but do not enter nasal capillaries (i.e., the systemic circulation) may therefore drain to the deep cervical lymph nodes. Lymphatic vessels have been found traversing the cribriform plate (Furukawa et al. 2008; Norwood et al. 2019). The potential involvement of these lymphatic vessels in the transport or clearance of intranasally applied substances to the CNS has not been established but warrants further examination. Radiolabeled protein tracers or dyes injected into the brain or CSF are cleared to the nasal lamina propria to reach the deep cervical lymph nodes at high concentrations (Bradbury and Cserr 1985; Kida et al. 1993). Sealing the cribriform plate with kaolin or acrylate glue significantly reduced the drainage of [¹²⁵I]-albumin following intraventricular infusion (Bradbury and Cserr 1985). Recent studies have demonstrated the presence of functional lymphatic vessels lining the dural sinuses (Louveau et al. 2015) and cranial nerves such as the olfactory nerve as it traverses the cribriform plate to innervate the olfactory mucosa (Aspelund et al. 2015). The drainage of CSF- or parenchymally administered tracers and macromolecules like antibodies has been shown to occur along olfactory perivascular/perineural spaces and/or lymphatics (Faber 1937; Kida et al. 1993; Pizzo et al. 2018; Aspelund et al. 2015). Relatively higher drainage to the olfactory region versus the respiratory region may have functional implications due to the former's lower vascularity/vascular permeability (Kumar et al. 2015), a circumstance which favors drainage to local cervical lymph nodes and potential induction of peripheral immune responses against CNS antigens (Cserr et al. 1992).

Dyes and macromolecules like antibodies can be found in the perineural sheaths of the fila olfactoria as well as the deep cervical lymph nodes following intranasal administration (Faber 1937; Yoffey and Drinker 1938; Kumar et al. 2018a). This localization of intranasally administered dyes and macromolecules is often similar

to what has been reported for dyes and macromolecules injected into the subarachnoid space CSF (Kida et al. 1993; Pizzo et al. 2018), suggesting that pseudolymphatic pathways leading out of the brain may be similar to pathways leading into the brain. These studies suggest the subarachnoid space, nasal lamina propria, and deep cervical lymph nodes are in communication. Importantly, the localization of microfil following injection into the CSF compartment of cadavers has confirmed that some of these connections also appear to be present in humans (Johnston et al. 2004).

15.2.3 *The Olfactory Region of the Nasal Passage*

The olfactory region consists of a pseudostratified columnar epithelium (Fig. 15.3a) located on the most superior aspect of the nasal cavity where the olfactory sensory neurons (OSN) reside. The OSN are the only first-order neurons possessing cell bodies located in a distal epithelium. The tips of their dendritic processes contain several nonmotile cilia which extend into the overlying mucus layer; odorant receptors are found in the plasma membrane of the olfactory cilia, where they are positioned to respond to olfactory stimuli in the external environment. The OSN are bipolar cells possessing unmyelinated axons which extend through the epithelial

Fig. 15.3 (continued) projecting to the olfactory bulb. Red arrows indicate potential pathways for drug delivery across the olfactory epithelium and into the brain following intranasal administration. Intranasally applied drugs may be transported by an *intracellular* pathway from the olfactory epithelium to the olfactory bulb within olfactory sensory neurons following adsorptive, receptor-mediated, or nonspecific fluid-phase endocytosis. Other drugs may cross the olfactory epithelial barrier by *paracellular* or *transcellular* transport to reach the lamina propria, where a number of different *extracellular* pathways for distribution are possible: (1) absorption into olfactory capillaries and entry into the general circulation; (2) absorption into olfactory lymphatics draining to the deep cervical lymph nodes of the neck; and (3) extracellular diffusion or convection in compartments associated with olfactory nerve bundles and entry into the cranial compartment. Transport within the perineural space bounded by olfactory nerve fibroblasts is shown, but other possibilities exist, e.g., transport within the fila olfactoria compartment contained by ensheathing cells, transport within the perivascular spaces of blood vessels traversing the cribriform plate with olfactory nerves (not shown), or transport within lymphatics traversing the cribriform plate with olfactory nerves (not shown). Possible pathways for distribution of substances from the perineural space into the olfactory subarachnoid space cerebrospinal fluid (CSF) or into the olfactory bulb are shown. (Figure adapted with permission from Lochhead and Thorne 2012). **(b)** The lymphatic drainage of the nasal mucosa is principally to the deep cervical lymph nodes. The deep cervical lymph nodes are present in the viscera of the neck deep into the superficial muscles and just lateral to the common carotid artery. **(c)** Rodent olfactory mucosa sections stained with hematoxylin and eosin or immunostained using an antibody to olfactory marker protein, a protein specific to mature olfactory sensory neurons (not sustentacular or basal cells). Sections show the pseudostratified layers of the olfactory epithelium with the relative positions of the cell bodies of sustentacular (S) cells and olfactory sensory (receptor, R) neurons indicated. Numerous blood vessels (BV) and Bowman's glands (BG) are also visible within the lamina propria. (Images of sections kindly provided by Professor Harriet Baker, Weill Medical College of Cornell University)

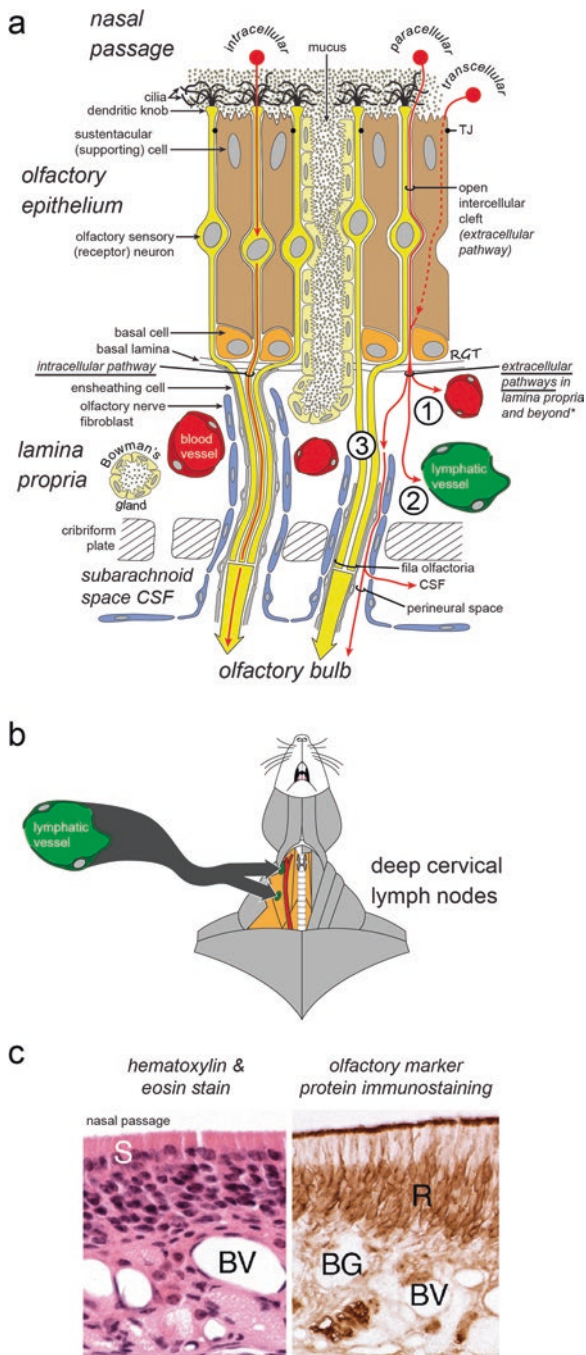


Fig. 15.3 The olfactory region: organization and histology. (a) The olfactory mucosa consists of the olfactory epithelium and the lamina propria. Axonal processes of olfactory sensory neurons converge into bundles (filia olfactoria), surrounded by ensheathing cells and fibroblasts, before

basal lamina and converge with axons from other OSN to form nerve bundles called fila olfactoria. Interlocking olfactory ensheathing cells (OEC) form continuous channels around the fila olfactoria from their origin to the olfactory bulb. Multicellular sheets of olfactory nerve fibroblasts enclose the OEC to form a perineural-like sheath around the fila olfactoria (Field et al. 2003). The olfactory nerve is comprised of the ensheathed fila olfactoria and travels through the cribriform plate of the ethmoid bone into the brain where its axons terminate on dendrites of mitral, periglomerular, and tufted cells in the glomeruli of the olfactory bulb. Axons of the mitral and tufted cells project to a number of areas including the anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala, and entorhinal cortex (Carmichael et al. 1994).

In addition to OSN, several other cell types are located within the olfactory epithelium and the underlying lamina propria. Sustentacular (supporting) cells extend from the apical region of the epithelium to the basal lamina and possess long, irregular microvilli which intermingle with the cilia of the OSN (Hegg et al. 2009). In the lamina propria, the Bowman's gland forms tubular-type ducts which traverse the basal lamina to produce and secrete a serous fluid which serves as a solvent for inhaled odorants and intranasally applied drugs. Globose basal cells (GBC), located in the lamina propria, are neural progenitors which provide a source for the continuous replacement of the OSN throughout life (Caggiano et al. 1994). Horizontal basal cells are located superficial to the GBC and function as multipotent progenitors to the GBC, sustentacular cells, and cells of the Bowman's gland and ducts (Iwai et al. 2008). Microvillar cells also reside in the olfactory epithelium although their functions are not well defined (Elsaesser and Paysan 2007). Endothelial cells of blood and lymphatic vessels as well as inflammatory cells are also present in the lamina propria of the olfactory region (Fig. 15.3b and c).

15.2.4 *The Respiratory Region of the Nasal Passage*

The nasal respiratory region consists of a pseudostratified columnar secretory epithelium (Fig. 15.4a). Cell types of the human respiratory epithelium include goblet cells, ciliated cells, intermediate cells, and basal cells (Jafek 1983). Serous glands,

Fig. 15.4 (continued) of the trigeminal nerve shown together with the nasal blood supply. The cell bodies of the trigeminal nerve fibers are located in the semilunar ganglion; their axons project into the brain stem at the level of the pons and ultimately synapse with neurons in a number of areas including the principal sensory and spinal trigeminal nuclei. Of the three main trigeminal nerve divisions (V_1 , the ophthalmic nerve; V_2 , the maxillary nerve; and V_3 , the mandibular nerve), only V_1 and V_2 send branches to the nasal epithelium. Blood supply to the nasal passages is provided by ethmoidal branches of the ophthalmic artery, sphenopalatine branches of either the external carotid artery (ECA)/maxillary artery (in humans) or the internal carotid artery (ICA)/pterygopalatine artery (in rats), and nasal branches from the ECA/facial artery. Numerous anastomoses (*) are indicated; these specialized connections between arteries may experience directional change in blood flow depending on the relative pressures within parent arteries. (Figures adapted from Lochhead and Thorne 2012 with permission)

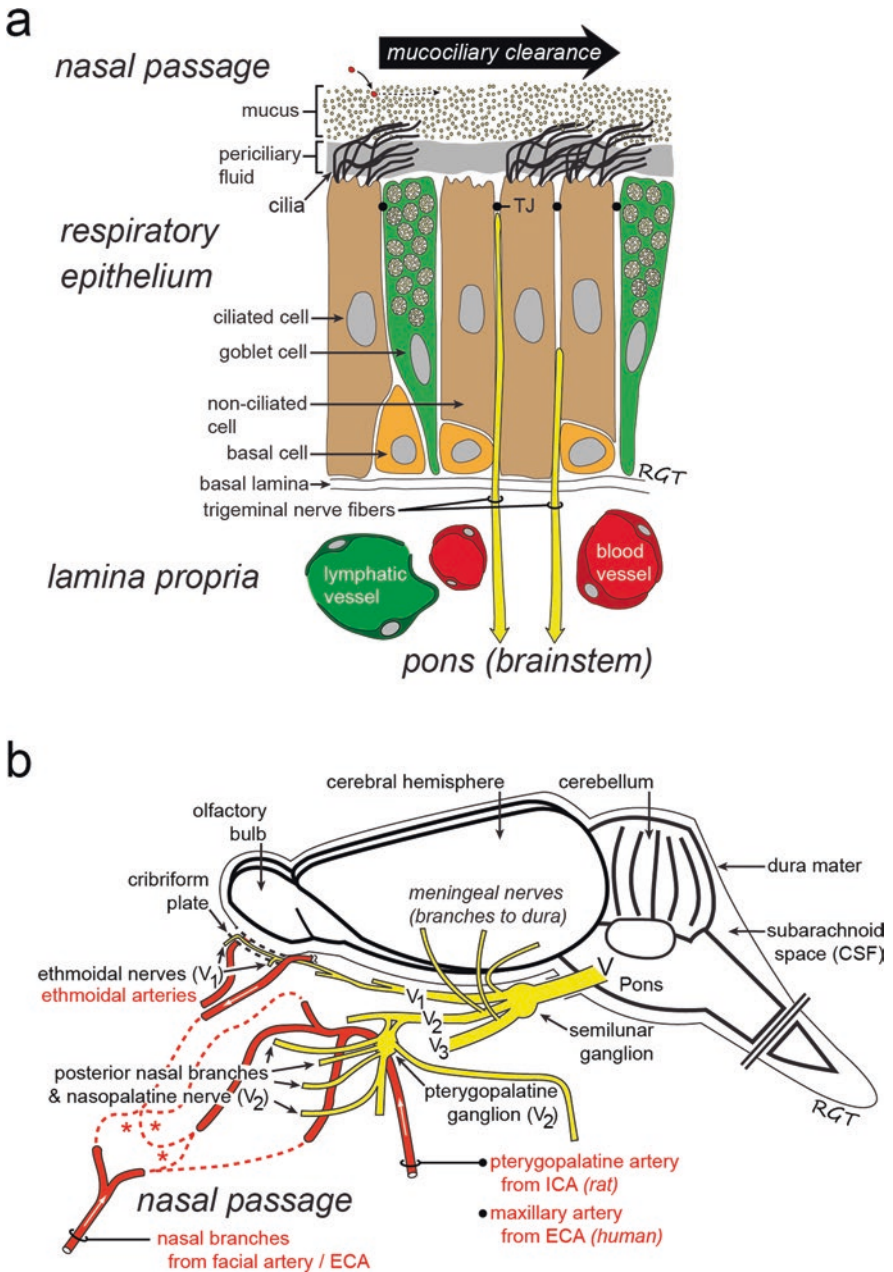


Fig. 15.4 The nasal respiratory region: general organization, trigeminal innervation, and blood supply. **(a)** The respiratory mucosa includes the respiratory epithelium and its underlying lamina propria. The trigeminal nerve, important for conveying chemosensory, nociceptive, touch, and temperature information, is found throughout the nasal epithelium; free nerve endings extend nearly to the epithelial surface, just beneath tight junctions (TJ). **(b)** Central projections

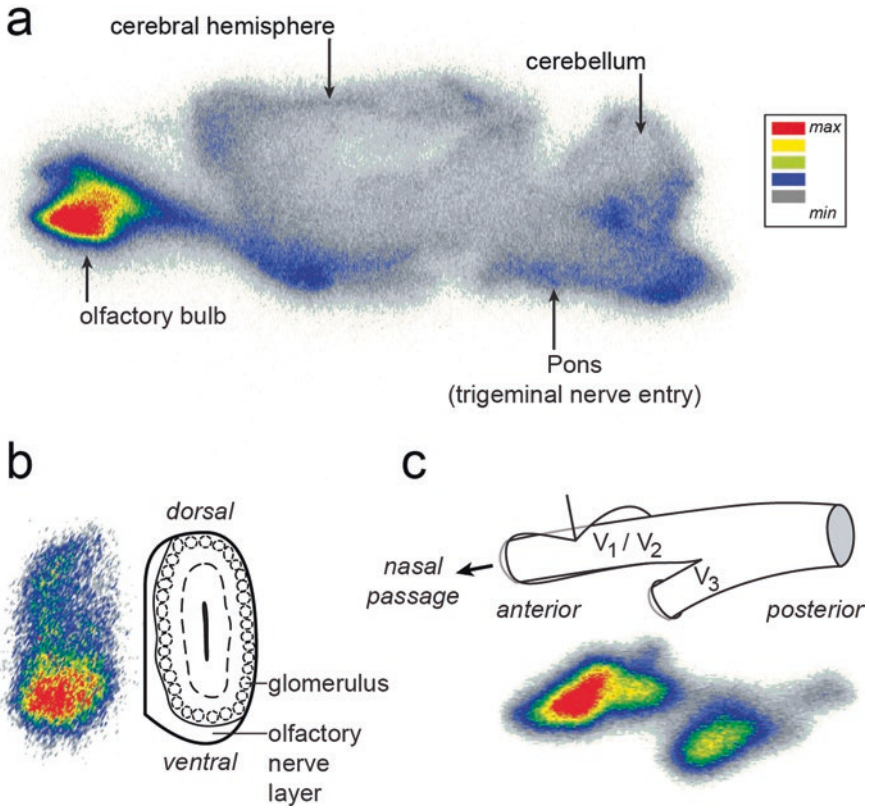


Fig. 15.5 The central distribution of $[^{125}\text{I}]$ -labeled IGF-I following intranasal application in anesthetized adult rats is characterized by high levels within the olfactory bulbs and trigeminal nerves. (a) Sagittal brain section from a rat approximately 30 min following intranasal administration of a low specific activity solution of $[^{125}\text{I}]$ -labeled IGF-I, allowing visualization of brain entry sites in the olfactory bulb (putative olfactory pathway) and pons (putative trigeminal pathway). (b) Coronal section through the olfactory bulb of a rat approximately 30 min following intranasal administration of a high specific activity solution of $[^{125}\text{I}]$ -labeled IGF-I. Signal intensity is highest in the ventral portion of the bulb in closer proximity to the olfactory nerve entry sites at the cribriform plate. (c) Transverse section through the trigeminal nerve of a rat approximately 30 min following intranasal administration of a high specific activity solution of $[^{125}\text{I}]$ -labeled IGF-I. Signal intensity is highest in portions of the ophthalmic (V_1) and maxillary (V_2) nerve divisions which innervate the nasal passage. (Figures adapted from Thorne et al. (2004a, b) with permission)

seromucous glands, and intraepithelial glands are also associated with the nasal respiratory epithelium. Most nasal secretions are produced by seromucous glands although goblet cells also secrete mucus. The primary role of the ciliated cells in primates is to propel mucus with their motile cilia toward the nasopharynx where it is either swallowed or expectorated. In rodents, mucus is propelled mostly in the anterior direction. Basal cells are relatively undifferentiated cells which give rise to other cell types in the nasal respiratory epithelium (Fig. 15.5).

Both the nasal respiratory and olfactory epithelia are innervated by branches of the trigeminal nerve (cranial nerve V), the largest of the 12 cranial nerves (Schuenke et al. 2010). Fibers from trigeminal ganglion cells ramify extensively within the nasal submucosa so that their free nerve endings stop at the TJ level near the epithelial surface (Finger et al. 1990). The trigeminal nerve exits the pons bilaterally and consists of a very large sensory root and a small motor root. Its motor fibers innervate the muscles of mastication, and the sensory fibers transmit information from the face, scalp, mouth, and nasal passages. The trigeminal nerve consists primarily of somatic afferent fibers which convey sensory information to nuclei located within the brain stem and spinal cord.

The trigeminal nerve is comprised of three major branches: the ophthalmic nerve (V_1), the maxillary nerve (V_2), and the mandibular nerve (V_3) (Fig. 15.4b). V_1 and V_2 are sensory nerves that also carry autonomic fibers, while V_3 contains the mixed portion of the trigeminal nerve. Importantly, ethmoidal (V_1), nasopalatine (V_2), and nasal (V_2) branches of the trigeminal nerve provide sensory innervation to the nasal passages (Tucker 1971; Bojsen-Moller 1975). A portion of trigeminal ganglion cells with sensory endings located in the nasal epithelium also send collaterals directly into the olfactory bulb in addition to the brain stem (Schaefer et al. 2002). Two other nerves, the nervus terminalis (terminal nerve; cranial nerve zero) and the vomeronasal nerve and organ (Jacobsen's organ), are also located in the nasal passages but have so far not been viewed as important for CNS delivery following intranasal administration, particularly in adult human beings where they may be vestigial or even absent.

15.3 Mechanisms and Pathways for Transport Into the CNS From the Nasal Passages

15.3.1 *Transport Across the Olfactory and Respiratory Epithelial Barriers*

The pathways and mechanisms governing the transport of substances from the nasal epithelium to various regions of the CNS are not fully understood. Substances which distribute throughout the CNS following intranasal administration must initially cross the nasal epithelial barrier through intracellular or extracellular (paracellular) routes. Proteins (e.g., albumin, horseradish peroxidase (HRP), wheat germ agglutinin-horseradish peroxidase (WGA-HRP)) and viruses (e.g., herpes, poliomyelitis, rhabdoviruses) endocytosed by OSN may reach the CNS (olfactory bulb) through intracellular axonal transport in the anterograde direction (Doty 2008; Kristensson and Olsson 1971; Broadwell and Balin 1985; Thorne et al. 1995; Baker and Spencer 1986; Kristensson 2011). HRP is taken up by OSN to a limited extent via pinocytosis, whereas WGA-HRP is internalized by OSN preferentially by adsorptive endocytosis (Broadwell and Balin 1985). Following intranasal

administration, WGA-HRP is also endocytosed and transported intracellularly through the trigeminal nerve to the brain stem (Anton and Peppel 1991; Deatly et al. 1990). Viruses and bacteria may also be transmitted to the CNS along trigeminal nerve components within the nasal passages (Deatly et al. 1990; Jin et al. 2001). Endocytosis by peripheral trigeminal nerve processes and subsequent intracellular transport to the brain stem could potentially occur at either the olfactory or respiratory regions of the nasal epithelium.

Substances may also cross the nasal epithelial barrier through transcytosis or paracellular diffusion to access the lamina propria. Electron micrographs of nasal epithelial cells have demonstrated the existence of TJ, but the paracellular permeability of the nasal epithelia remains poorly defined (Altner and Altner-Kolnberger 1974; Kerjaschki and Horander 1976); this is partly due to the difficulty in establishing and utilizing *in vitro* models to predict transport for epithelia having neurons as integral components. The TJ proteins ZO-1, ZO-2, and ZO-3; occludin; and claudin-1, claudin-3, claudin-4, claudin-5, and claudin-19 are expressed at the olfactory epithelium of rats (Wolburg et al. 2008; Steinke et al. 2008). Measurements across excised rabbit nasal epithelium have yielded electrical resistance values ranging from 40 $\Omega \cdot \text{cm}^2$ (Hosoya et al. 1993), suggesting a relatively permeable barrier, to 261 $\Omega \cdot \text{cm}^2$ (Rojanasakul et al. 1992), suggesting barrier properties comparable to the intestinal epithelium. The regular turnover of cells in the nasal epithelium may lead to continual rearrangement and loosening of the TJ as basal cells replace epithelial cells throughout life (Altner and Altner-Kolnberger 1974), resulting in a relatively high permeability compared to other epithelial sites. Electron microscopic studies in the intestinal epithelium have demonstrated colloidal gold nanoparticles cross the epithelial barrier and distribute to other tissues through spaces created by single, degrading enterocytes as they are extruded from the villus in a process known as persorption (Hillyer and Albrecht 2001). The replacement of cells throughout life at the nasal epithelial barrier may create similar potential spaces which may allow paracellular transport of substances to the lamina propria. Evidence for these spaces has recently been shown following intranasal administration of prions which could be found in holes approximately 5–20 μm near the surface of the nasal epithelium (Kincaid et al. 2015). Paracellular transport of molecules across nasal epithelia can be enhanced by modulating local tight junction complexes using MMP-9 at physiological concentrations (Lochhead et al. 2015; Kumar et al. 2018a). The expression of FcRn at the nasal epithelia and differences in pH between their apical and basal sides may facilitate directional transport of IgG from the epithelial surface to the lamina propria via an FcRn-dependent mechanism (Ye et al. 2011; Heidl et al. 2015). Substances that reach the lamina propria through transcellular or paracellular routes may be absorbed into the systemic circulation, drain to the deep cervical lymph nodes, or enter the CNS by direct pathways, utilizing components of the peripheral olfactory and/or trigeminal systems.

15.3.2 *Transport from the Nasal Lamina Propria to Sites of Brain Entry*

IN administration of [¹²⁵I]-insulin-like growth factor I (IGF-I, MW = 7.65 kDa) and [¹²⁵I]-immunoglobulin G (IgG, MW = 150 kDa) in rats and [¹²⁵I]-interferon-β1B (IFN-β1B, MW = 18.5 kDa) in monkeys all suggest that delivery to the CNS occurs along components associated with the olfactory and trigeminal nerves, followed by widespread distribution to other sites of the CNS within 30–60 min (Thorne et al. 2004a, 2008a; Kumar et al. 2018a, b). Substances may reach the brain from the nasal mucosa intracellularly following endocytosis by OSN or neurons of the trigeminal ganglion, as discussed above. There also appear to be extracellular pathways into the brain following transcytosis or paracellular diffusion across the nasal epithelium to the lamina propria; these pathways have been proposed based on much experimental evidence obtained by a large number of different groups (reviewed in several sources, including Thorne et al. 2004b; Illum 2004; Dhuria et al. 2010; Lochhead and Thorne 2012; Kumar et al. 2018a). The extracellular pathways potentially providing nose-to-brain transport routes include diffusion or convection within perineural, perivascular, or lymphatic channels associated with olfactory and trigeminal nerve bundles extending from the lamina propria to the olfactory bulb and brain stem, respectively.

The perineural distribution around olfactory nerve bundles extending from the lamina propria to the outermost layer of the olfactory bulb has been observed following the IN administration of potassium ferrocyanide and iron ammonium citrate solutions, 3 kDa and 10 kDa dextrans as well as IgG (Faber 1937; Jansson and Bjork 2002; Lochhead et al. 2015; Kumar et al. 2018a, b). This suggests that perineural spaces may act as pathways for molecules to distribute to the CNS from the nasal cavity. OEC maintain continuous open spaces in the nerve bundles to allow regrowth of olfactory nerve fibers (Li et al. 2005). These compartments provide a potential path that substances may take to reach the brain from the perineural space of entering olfactory nerve bundles. The perineural spaces of the olfactory and trigeminal nerves appear to also allow the distribution of certain substances to the CSF of the subarachnoid space, particularly smaller peptides and proteins, although the anatomical/physiological aspects of this perineural space-to-CSF distribution remain poorly understood. Indeed, the barrier between the perineural space and the CSF may be more permeable to some substances than others. Sakane and colleagues demonstrated a size-dependent entry of intranasally administered dextrans of varying sizes (4–20 kDa) into the CSF. Certain proteins, e.g., IGF-I, have not been detected in the CSF despite experimental evidence of brain entry following intranasal administration (Thorne et al. 2004b). Although a fairly large molecule, IgG has been found to enter the CSF in trace amounts within 30 min following intranasal administration (Kumar et al. 2018a). CSF IgG concentrations were ~ 2- to 30-fold lower than in the brain parenchyma, suggesting IgG access to the parenchyma occurred via pathways that do not require access to the CSF compartment first (Kumar et al. 2018a). Entry of intranasally administered IgG into the CSF, despite

its large size, may be due to the role it plays in immune surveillance and may be aided by FcRn-dependent transport mechanisms. For substances capable of accessing the CSF of the subarachnoid space following IN administration, further distribution to more distant sites of the CNS may occur along pathways of CSF flow.

The precise mechanisms underlying the rapid transport (30 min) of radiolabeled proteins from the rat nasal mucosa to widespread areas of the CNS along components of the olfactory and trigeminal nerves are at present unknown. Possibilities include intracellular (axonal) transport, extracellular diffusion, and extracellular convective (bulk) flow within perineural, perivascular, or lymphatic channels associated with olfactory and trigeminal nerve bundle. Recently, we have shown that fluorescently labeled insulin or IgG can be found within perineural and/or perivascular spaces of the trigeminal nerve within minutes after intranasal administration, suggesting bulk flow within these spaces are involved in the delivery of macromolecules to the CNS along the trigeminal route (Kumar et al. 2018a; Lochhead et al. 2019). We have previously estimated the time it would take for a molecule to reach the olfactory bulb and brain stem of rats by intracellular transport, diffusion, or convective flow (Lochhead and Thorne 2012). Intracellular (axonal) transport rates within olfactory or trigeminal nerves were estimated from experimental rates measured in fish olfactory nerves (Buchner et al. 1987). Rates of diffusion were based on experimental measurements and known correlations for protein-free diffusion coefficients (Thorne et al. 2004a). Convective flow rates were estimated from experimentally measured albumin transport within the perivascular spaces of pial arteries using an open cranial window preparation in rats (Ichimura et al. 1991).

In short, the intranasal delivery of macromolecular dextran tracers and proteins such as IGF-I and IgG, among others, resulting in transport to widespread areas of the CNS within 30 min of intranasal application, strongly indicates a convective (bulk) flow process along the olfactory and trigeminal nerve components that is likely the only plausible transport mechanism that can explain the experimental CNS distribution (Kumar et al. 2018a; Lochhead et al. 2015). This is an area clearly in need of further, careful study; more detailed discussion can be found elsewhere (Thorne et al. 2004b, 2008a, b; Lochhead and Thorne 2012; Kumar et al. 2018a; b).

15.3.3 Transport from Brain Entry Sites to Widespread Areas Within the CNS

The final distribution of substances to other CNS areas after they have reached the pial surface of the brain at the level of the olfactory bulb and brain stem has been shown to occur at least in part via bulk flow within perivascular spaces of cerebral blood vessels (Thorne and Frey 2001; Thorne et al. 2004b; Lochhead et al. 2015; Kumar et al. 2018a). It has been speculated that the normal expansion and contraction of cerebral blood vessels due to cardiac pulsatility could generate a pronounced fluid flow within the perivascular spaces. Different groups have attempted to

understand the direction and characteristics for such a flow by modeling the process, but thus far the results have produced conflicting ideas as to its directionality (Bilston et al. 2003; Schley et al. 2006; Wang and Olbricht 2011). It has been shown that increasing the blood pressure and heart rate results in a larger distribution of adeno-associated virus 2 capsids or fluorescent liposomes after injection into the striatum, suggesting the involvement of arterial pulsations in the intraparenchymal distribution of these large substances via the perivascular spaces (Hadaczek et al. 2006). Several groups have also observed rapid distribution along perivascular spaces following tracer application into the CSF (Rennels et al. 1985; Iliff et al. 2012); however, it must be noted that others have seen limited perivascular distribution following injection of tracers into the subarachnoid CSF (Kida et al. 1993; Szentistvanyi et al. 1984). Pizzo et al. showed full-length IgG (150 kDa) and smaller single-domain antibodies (sdAb; ~15 kDa) distributed via diffusion at brain-CSF interfaces and throughout the brain along perivascular spaces of cerebral blood vessels of all caliber in a size-dependent manner following intrathecal infusion into the cisterna magna (Pizzo et al. 2018). Intranasal administration of fluorophore-labeled 3 kDa dextran by itself or 10 kDa dextran and IgG following nasal pre-administration of MMP-9 (a physiologic nasal permeability enhancer) has been demonstrated to result in rapid access to the brain parenchyma. Such access to the brain has been suggested to occur first via transport along perivascular compartments of cerebral blood vessels followed by diffusion out of the perivascular space and into the brain parenchyma (Kumar et al. 2018a; Lochhead et al. 2015). Notably, the extent of macromolecule access to cerebral perivascular compartments following intranasal administration appears to be size dependent (Lochhead et al. 2015; Kumar et al. 2018a). The precise role that perivascular transport plays in dictating CNS distribution in health and disease following intranasal targeting of substances to the brain certainly deserves further study.

15.4 Current Status of the Intranasal Route of Administration for CNS Targeting

IN administration has become an increasingly popular method to bypass the BBB and deliver therapeutics directly to the CNS. Numerous preclinical studies have indicated IN administration offers advantages over other routes of administration for delivery of some substances to the CNS. The published literature now includes a vast amount of animal work reporting positive effects following the intranasal administration of small molecules, peptides, proteins, oligonucleotides, gene vectors, or cell-based therapeutics using a number of different CNS disease models. Most importantly, several clinical trials involving IN administration for the treatment of CNS disorders have either been completed, are currently in progress, or are in the process of planning/recruiting. The sections below provide a brief summary of some notable preclinical and clinical work that has been conducted to date. This

review is by no means exhaustive; more comprehensive summaries may be found elsewhere (Lochhead and Thorne 2012; Dhuria et al. 2009).

15.4.1 Intranasal Delivery of Small Molecules to the CNS

Intranasal delivery has long been appreciated to offer unique advantages for small molecule administration across a variety of applications: (i) when local effects are desired (e.g., as with decongestants, antibiotics, and mucolytics); (ii) when noninvasive, needle-free access to the systemic circulation is needed for rapid drug onset (e.g., in the context of illicit drug overdose); and (iii) to avoid extensive hepatic first-pass elimination (e.g., as with the application of the opioid antagonist naloxone following opioid overdose). Indeed, multiple studies have demonstrated that intranasal delivery of both small molecules (e.g., zolmitriptan, sumatriptan, butorphanol tartrate, fentanyl, nicotine, and estradiol) and low-molecular-weight peptide drugs (e.g., calcitonin, desmopressin, busserelin, oxytocin) can yield drug absorption and disposition profiles capable of producing clinically meaningful responses in a safe, patient-friendly manner. The ability to achieve significant systemic exposure for intranasally applied small molecules and peptides (as compared to proteins and other large molecules) is likely due to their relatively high paracellular permeability across the nasal epithelia and efficient absorption into the blood stream through the extensive nasal vasculature present in the underlying lamina propria (Kumar et al. 2015; Nehra et al. 2021). Interestingly, it is often questioned whether intranasal delivery can truly yield improved CSF or brain exposures for small molecule therapeutics, due partly to a lack of careful studies capable of distinguishing direct delivery to the brain/CSF versus systemic absorption followed by brain/CSF entry across the BBB and/or blood-cerebrospinal fluid barriers (Nehra et al. 2021).

Small molecules may be able to directly access the CNS through the IN route of administration. The paracellular permeability of substances across the nasal epithelium is likely inversely proportional to their size. This would favor a higher percentage of small molecules than macromolecules reaching the lamina propria following IN administration. Small molecules, however, may also be more easily absorbed into the nasal capillaries due to their smaller size. Therefore, intranasally administered small molecules may be more likely to access the nasal lamina propria than large molecules, but their size may favor absorption into the systemic circulation. Small molecules which escape absorption into the nasal vasculature may directly access the CNS through olfactory or trigeminal nerve-associated pathways. Absorption into the CSF may favor small molecules over macromolecules. Small molecules distributed in the perineural space of the olfactory or trigeminal nerve may also more easily cross the perineural barrier than large molecules. Therefore, small molecules may have greater access than large molecules to the CSF within the subarachnoid space surrounding the olfactory and trigeminal nerves. Upon entry into the CSF, small molecules may also have access to more distant sites in the

CNS; conversely, small molecules may in some cases be cleared from the CNS compartment more quickly than larger molecules.

It has been questioned whether small molecules can directly access the brain following IN administration (Merkus et al. 2003). Merkus and coworkers measured the levels of melatonin (MW = 232 Da) in the CSF after IN or intravenous (IV) administration and concluded no direct delivery to the brain occurred. However, melatonin is able to cross the BBB, making it difficult to ascertain whether its detection in the CSF represents direct delivery from the nasal mucosa or delivery across the BBB or BCSFB from the systemic circulation. Furthermore, intranasally applied macromolecules such as IGF-I and vascular endothelial growth factor have been found in the brain but not the CSF following IN delivery, suggesting drug levels in the CSF may not always correlate with brain levels (Thorne et al. 2004a, b; Yang et al. 2009). In another study, the dopamine-D2 receptor antagonist remoxipride (MW = 371 Da) was measured in the brain extracellular fluid (ECF) using a microdialysis probe placed within the striatum; the brain ECF/plasma area under the curve (AUC) ratios was found to be significantly higher in rats administered remoxipride intranasally compared to intravenous application (Stevens et al. 2011). Elegant semi-physiologically based pharmacokinetic modeling by this group suggested 75% of remoxipride entering the brain following intranasal application did so using a direct nose-to-brain transport pathway. Similar results were obtained when levels of three glycine receptor antagonists and one angiotensin antagonist with varying degrees of BBB permeability (MW = 369–611 Da) were compared following IN or IV administration (Charlton et al. 2008). CNS/plasma AUC ratios were higher following IN versus IV administration for each compound. Autoradiographs further detected the angiotensin antagonist GR138950 in the olfactory nerves, CSF, and brain within minutes following IN administration. Finally, the local anesthetic lidocaine (MW = 234 Da) has also been shown to be transported to the brain along the trigeminal nerve pathway (Johnson et al. 2010).

Several disease models have been successfully treated with intranasally administered small molecule drugs. For example, the angiotensin type II receptor antagonist losartan (MW = 423 Da), which poorly penetrates the BBB, decreased amyloid β (A β) plaques and inflammation without inducing hypotension in an Alzheimer's disease (AD) transgenic mouse model (Danielyan et al. 2010). The iron chelator deferoxamine (MW = 561 Da) also exhibits neuroprotection in models of Parkinson's disease (PD), AD, and ischemic stroke (Febbraro et al. 2013; Guo et al. 2013; Hanson et al. 2009).

Finally, a number of studies, including clinical trials, have suggested that perillyl alcohol (POH; MW = 152 Da), a plant-derived monocyclic terpene and chemotherapeutic agent, may hold promise for the treatment of recurrent forms of primary brain cancers, particularly low-grade glioma (NCT02704858) following intranasal administration (see Nehra et al. 2021 for review).

15.4.2 *Intranasal Delivery of Peptides/Proteins to the CNS*

Peptides and proteins are the most widely used drugs which have been administered intranasally to treat disorders of the CNS in both animal models and humans. Most preclinical studies utilizing the intranasal route of administration have shown behavioral or pharmacodynamic effects but not presented pharmacokinetic data indicating direct delivery of the drug to the CNS. This makes it difficult to determine if the drug entered the brain through direct pathways from the nasal cavity, crossed the BBB or accessed circumventricular areas from the systemic circulation, or exerted its effects through direct action on the BBB itself. For some peptides and proteins, there is pharmacokinetic data to support their ability to directly enter the CNS from the nasal cavity.

A pioneering study by Born and colleagues was among the first studies to obtain CNS pharmacokinetic data following IN delivery of peptides in humans. The peptides melanocortin(4–10) (MW = 980 Da), arginine-vasopressin (MW = 1.1 kDa), and insulin (5.8 kDa) were all detected in the CSF within 30 min in healthy volunteers with a lumbar puncture (Born et al. 2002). Importantly, there was no increase in plasma concentration of melanocortin(4–10), insulin, or glucose with intranasal dosing of melanocortin or insulin in this study. CSF levels of the peptides remained elevated for at least 80 min following IN administration.

Insulin is one of the most widely studied biologics with regard to its effects on the CNS following intranasal administration. A number of studies have intranasally administered insulin to treat metabolic and cognitive disorders in animal models as well as in humans. IN administration of [¹²⁵I]-insulin to mice yields significantly higher CNS levels after 1 h when compared to subcutaneous administration (Francis et al. 2008). [¹²⁵I]-insulin distributed widely throughout the mouse brain following IN administration, with the highest levels detected in the trigeminal nerve and the olfactory bulbs (Francis et al. 2008). Electron microscopic studies have found insulin within olfactory nerve bundles minutes following IN administration in mice (Renner et al. 2012a, b). A recently completed clinical trial showed IN insulin improved memory and preserved general cognition in patients with mild cognitive impairment or AD (Craft et al. 2012). Changes in memory and cognitive function were associated with changes in A β ₄₂ levels and tau/A β ₄₂ ratio in CSF (Craft et al. 2012). IN insulin has also suppressed food intake and increased brain energy levels in humans, suggesting potential as a treatment for obesity (Jauch-Chara et al. 2012). As already discussed above, IN administration of [¹²⁵I]-IGF-I results in significantly higher CNS levels than comparable intravenous dosing, with widespread CNS distribution occurring via olfactory and trigeminal nerve pathways, and the activation of IGF-I signaling pathways in brain areas such as the olfactory bulb and brain stem trigeminal nuclei (Thorne et al. 2004b); IGF-I brain entry and effects following IN application may also be relevant for understanding how IN insulin exerts its central actions because the two proteins share significant structural homology. A large multicenter trial examining the effects of intranasal insulin in AD and mild cognitive impairment is now underway in the United States.

Oxytocin (MW = 1 kDa) is a neuropeptide which exhibits a wide range of effects on human behavior. Oxytocin receptors are expressed centrally in the accessory olfactory bulb, anterior olfactory nucleus, islands of Calleja, amygdala, CA1 of the hippocampus, ventral medial hypothalamus, nucleus accumbens, brain stem, and spinal cord (Stoop 2012). The BBB prevents the passage of peripheral oxytocin (Ermisch et al. 1985; Kang and Park 2000), and IN administration of oxytocin has increasingly become a popular method for assessing oxytocin's central effects. Oxytocin is currently being administered intranasally in clinical trials to treat autism spectrum disorders, schizophrenia, and alcohol withdrawal. Despite the widespread use of oxytocin in clinical settings, little is known in animals or humans about oxytocin distribution in the brain following IN administration, suggesting a need for further study in this area.

Dopamine neuron-stimulating peptide-11 (DNSP-11; MW = 1.18 kDa) is a synthetic, amidated 11-amino acid peptide derived from the pro-domain of human glial cell line-derived neurotrophic factor (GDNF) that possesses broad neuroprotective and neurorestorative properties on dopaminergic neurons both in vitro and in vivo (Bradley et al. 2010; Kelps et al. 2011; Fuqua et al. 2014; Stenslik et al. 2015, 2018). In the first of a series of studies to examine the efficacy of repeated IN administration of DNSP-11 on the dopaminergic system, Stenslik et al. reported changes in d-amphetamine-induced rotation, recovery of dopamine turnover, and tyrosine hydroxylase (TH) neuronal sparing in a severe, unilateral 6-hydroxydopamine (6-OHDA) Fisher 344 (F344) rat model of parkinsonism (Stenslik et al. 2015). In the same report, a single, IN [¹²⁵I]-DNSP-11 dose in naïve F344 rats resulted in rapid, widespread distribution throughout the CNS, including the nigrostriatal system, and uptake in the CSF within 30 min. Highest levels of radiolabel were observed in the olfactory bulbs at 60 min (Fig. 15.6; Stenslik et al. 2015). In a subsequent report, Stenslik et al. developed a methodology to evaluate repeated IN administration of DNSP-11 in nonhuman primates (rhesus macaques) without the need for sedation (Stenslik et al. 2018). Stenslik et al. demonstrated that DNSP-11 administered IN to awake, chair-trained 1-methyl-4-phenyl-1,2,3,6-tetrahydroxyridine (MPTP)-treated rhesus macaques in a dose-escalating manner over the course of several weeks resulted in bilateral, neurochemical changes in the striatal system without observable, adverse behavioral effects or weight loss (Stenslik et al. 2018). In addition, a single, intranasal [¹²⁵I]-DNSP-11 dose revealed rapid, widespread distribution throughout the CNS and uptake in the CSF within 60 min, with the highest levels of radiolabel observed in the olfactory bulbs and trigeminal nerves (Fig. 15.7; Stenslik et al. 2015, 2018). These findings are consistent with other foundational nonhuman primate IN peptide/protein radiolabeled tracer studies discussed below (Thorne et al. 2008a, b). Collectively, these studies support the idea that DNSP-11 can safely and effectively deliver IN to target the dopaminergic system in both rodents and nonhuman primates.

Orexin-A (hypocretin-1, MW = 3.6 kDa) is a sleep-related peptide produced in the hypothalamus which has shown effects in monkeys and humans following IN administration. Intranasally administered orexin-A improved task performance and induced changes in the brain metabolic activity in sleep-deprived rhesus monkeys

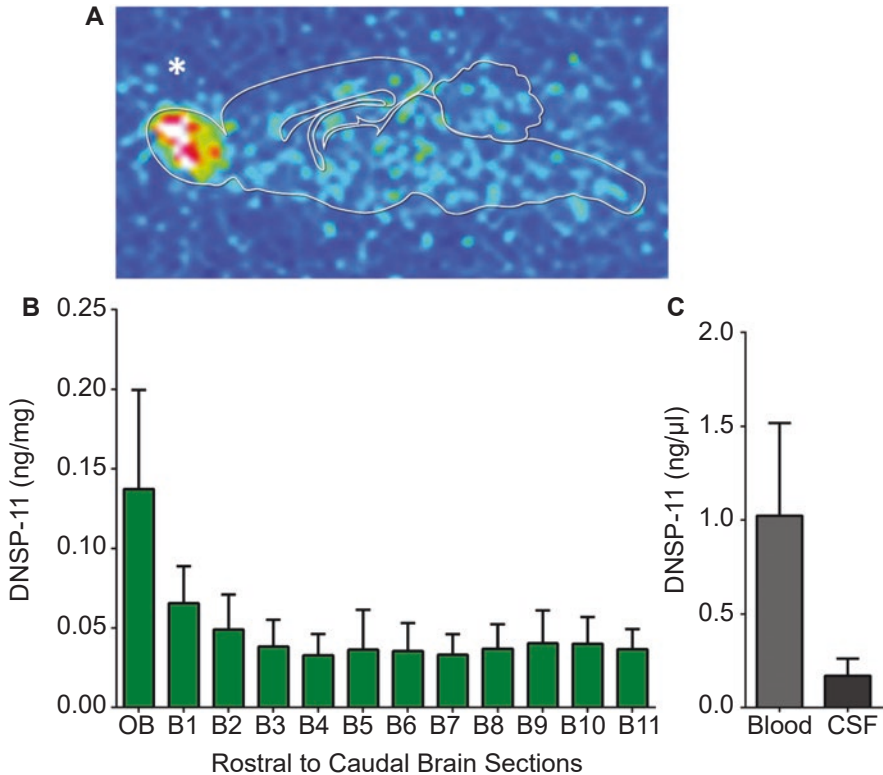


Fig. 15.6 Intranasal administration of DNSP-11 results in the delivery to the brains of Fischer 344 rats. Normal F344 rats were given a one-time intranasal dose of [^{125}I]-labeled DNSP-11 to determine the distribution in the brain. At 60 min the blood (500 μl), cerebrospinal fluid (100–120 μl), and brain tissue were collected from individual rats and processed by gamma counting ($n = 3$) and autoradiography ($n = 1$). (a) The distribution in a representative sagittal brain section (0.5 mm) supports a qualitative increase in radioactive signal found in the olfactory bulbs (OB) and diffuses signal throughout the brain. (b) Normalized DNSP-11 concentrations (ng/mg wet tissue weight) as analyzed by gamma counting were consistent with the autoradiography analysis. (c) Normalized DNSP-11 concentrations (ng/ μl) as analyzed by gamma counting indicate the presence of radioactive signal in the blood and cerebrospinal fluid samples at the single timepoint examined. * Denotes the olfactory bulb (OB) in a representative sagittal section of the midbrain following autoradiography analysis. B1–11 denote rostral to caudal serial brain sections taken for gamma counting analysis. (Figures adapted from Stenslik et al. 2015 with permission)

(Deadwyler et al. 2007). In humans suffering from narcolepsy with cataplexy, IN administration of orexin-A attenuates olfactory dysfunction and induces and stabilizes REM sleep (Baier et al. 2008, 2011). In rats, intranasally administered orexin-A distributed to the brain within 30 min, yielding tissue-to-blood concentration ratios that were 5–8 times higher in the posterior trigeminal nerve, olfactory bulbs, hypothalamus, and cerebellum compared to rats given IV orexin-A (Dhuria et al. 2009). High levels of orexin-A were found in the cerebral blood vessel walls, and

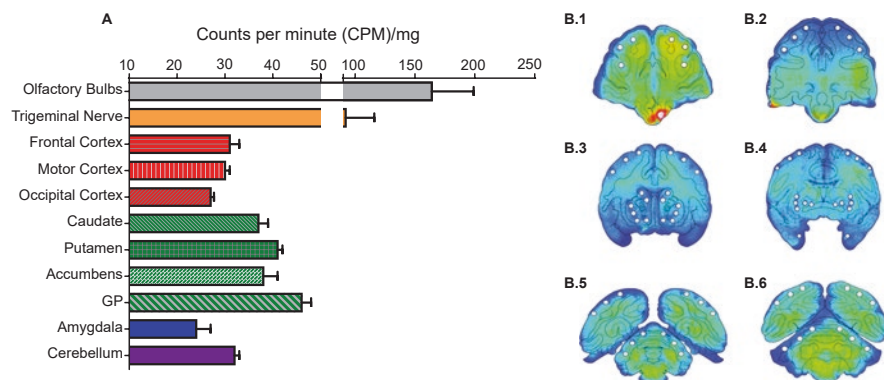


Fig. 15.7 The intranasal administration of DNSP-11 results in the delivery to the brain of a rhesus monkey (*Macaca mulatta*). To determine the distribution in the nonhuman primate brain, a single [^{125}I]-labeled DNSP-11 dose (5 mCi/10 mg DNSP-11; 2.5 mCi/5 mg/0.5 mL per naris) was administered to a rhesus macaque. (a) Whole olfactory bulbs and sections of trigeminal nerve were harvested, along with multiple 2-mm-diameter tissue punches from coronal sections of the frontal cortex ($n = 12$), motor cortex ($n = 12$), occipital cortex ($n = 12$), caudate nucleus ($n = 8$), putamen ($n = 12$), nucleus accumbens ($n = 2$), globus pallidus ($n = 4$), amygdala ($n = 2$), and cerebellum ($n = 12$) for gamma counting analysis. In the brain samples examined, normalized radioactive signal (CPM/mg) demonstrated highest signal in the olfactory bulbs and trigeminal nerves, with diffuse lower signal levels throughout the other brain regions sampled. (B1–6) The same 2-mm-thick coronal sections that were used for tissue biopsy mapping were subsequently processed for autoradiography. Qualitative visual assessment supports the highest radioactive signal in the olfactory tracts, with high levels also observed in the white matter regions. Circles represent tissue punches taken for gamma counting analysis. (Figures adapted from Stenslik et al. 2018 with permission)

low levels were found in the CSF of these rats, suggesting transport pathways may have involved distribution within the perivascular spaces.

NAP (davunetide) is an eight-amino acid neuroprotective peptide (MW = 825 Da) derived from activity-dependent neurotrophic factor. Intact levels of [^3H]-labeled NAP are found in the cortex and cerebellum of rats within 30 min following IN administration (Gozes et al. 2000). IN administration of NAP reduced levels of A β and hyperphosphorylated tau in an AD mouse model (Matsuoka et al. 2007) and decreased neurofibrillary tangles in a model of tauopathy (Shiryaev et al. 2009). IN NAP decreased hyperactivity and protected visual memory in a mouse model of schizophrenia (Powell et al. 2007). Unfortunately, IN NAP failed to show efficacy in a recent clinical trial to treat progressive supranuclear palsy. Clinical trials evaluating whether IN NAP is beneficial in the treatment of schizophrenia and tauopathies are currently in progress.

The 18.5-kDa protein interferon- β 1B (IFN- β 1B) is a cytokine therapeutic approved to treat the relapsing-remitting form of multiple sclerosis. Studies in rats have shown that IN application of [^{125}I]-labeled IFN- β 1B results in significantly higher CNS levels than intravenous dosing (Ross et al. 2004). High IFN- β 1B levels were measured in the olfactory bulbs and trigeminal nerves, with significant but

lower levels in other brain regions and the spinal cord, approximately 30 min after the start of administration. A subsequent study evaluating CNS delivery following IN application of [125 I]-labeled IFN- β 1B in cynomolgus monkeys (*Macaca fascicularis*) also demonstrated widespread distribution within the brain, with highest levels again in the olfactory bulbs and trigeminal nerves (Thorne et al. 2008a, b). Importantly, this study also showed an anatomically unique and significant central localization of [125 I]-labeled IFN- β 1B to regions of the basal ganglia that was remarkably consistent between different animals (Fig. 15.8). This study was among the first to describe the precise distribution and concentrations achievable in the CNS of a primate species following IN administration; [125 I]-IFN- β 1B concentrations in the olfactory bulbs, trigeminal nerves, and many other brain areas were found to be above the levels required for the antiviral, antiproliferative, and immunomodulatory actions of IFN- β 1B.

Antibodies are immunoglobulin proteins which are able to bind peptides and proteins with high affinity. This property makes them attractive drug candidates to treat diseases of the CNS, but antibodies have shown limited BBB penetration when administered systemically (Banks 2004; St-Amour et al. 2013; Kumar et al. 2018a). The efficiency of IgG transport from the systemic circulation into the brain parenchyma via sites such as the circumventricular organs, across the blood-CSF barrier, or the BBB has remained largely unknown, and it is very likely that BBB transport of IgG has been overestimated due to systemically derived exogenous IgG remaining sequestered within the brain endothelial compartment with limited entry into the parenchyma itself (St-Amour et al. 2013). Anti-A β immunoglobulin G (IgG) has been administered intravenously in several clinical trials to treat or prevent AD (Kumar et al. 2018b). A few studies have intranasally administered antibodies or antibody fragments in mouse models of AD. Full-length IgG (MW = 150 kDa) as well as a single-chain variable fragment antibody (scFv) (MW = 26 kDa) directed against the C terminus of A β (1–42) reduced amyloid plaque levels following intranasal administration to APP^{swe}/PS1^{dE9} transgenic mice (Cattepoel et al. 2011). The scFv was detected in the brain immunohistochemically, while the full-length antibody was not, suggesting greater delivery of the smaller fragment. Another study in 5XFAD mice showed improved spatial learning and lower levels of A β following IN administration of an anti-A β oligomer antibody (Xiao et al. 2013). Limited levels of HRP-labeled antibody have been reported in the brain following the development with diaminobenzidine (Xiao et al. 2013). Kumar et al. (2018a, b) performed a quantitative evaluation of [125 I]-labeled IgG delivery to the brain and CSF 30 min following intranasal delivery in rats. The highest concentrations of

Fig. 15.8 (continued) solution. The highest concentrations were evident in regions of the basal ganglia (putamen, caudate, and globus pallidus) with slightly lower signal in other subcortical structures (e.g., hippocampus and amygdala). (c) Coronal brain autoradiographs and labeled templates from two different monkeys at the same level as in (b) demonstrating remarkably low variability in central distribution across different subjects. The distribution for each animal is shown approximately 60 min following intranasal administration of [125 I]- IFN- β 1b (Portions of (a), (b), and (c) adapted from Thorne et al. (Thorne et al. 2008a) with permission)

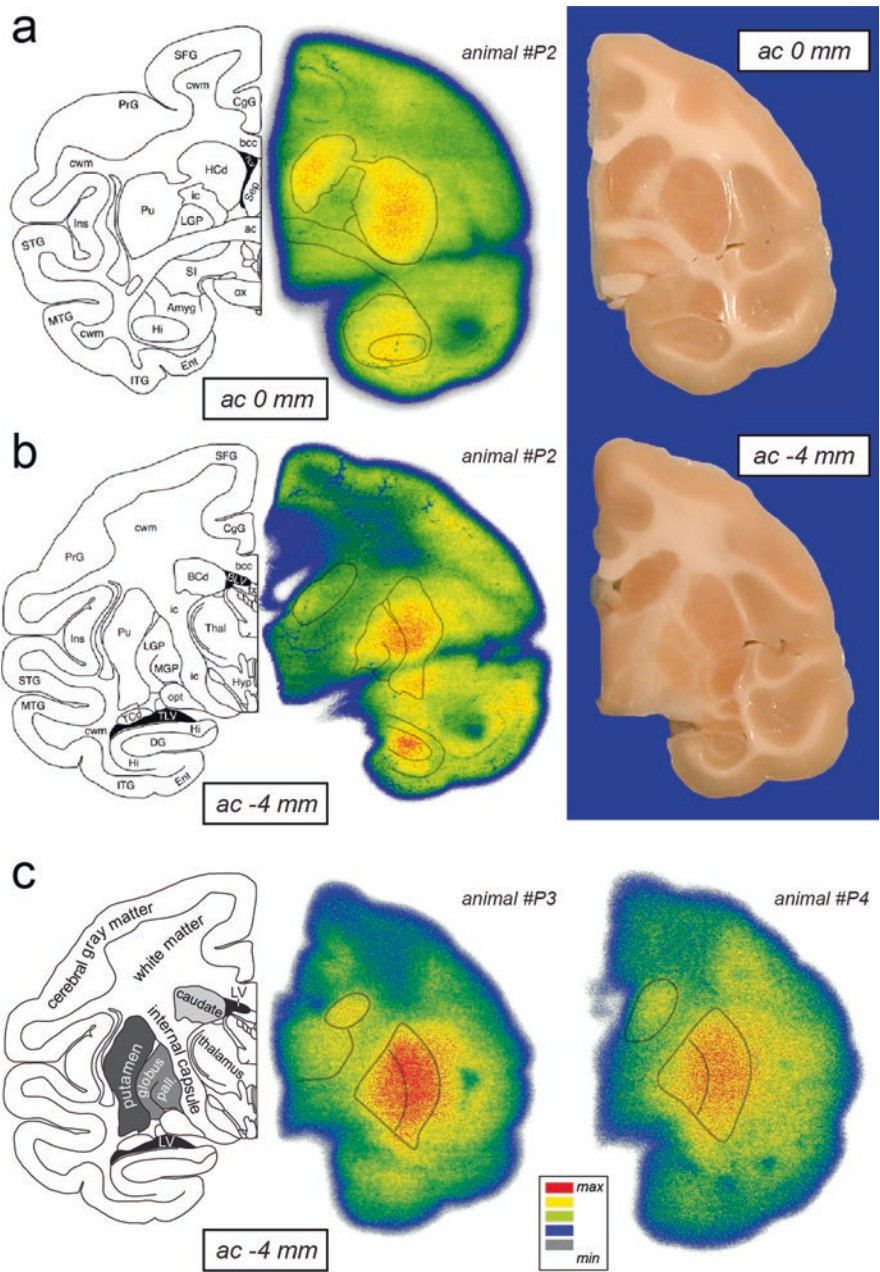


Fig. 15.8 Central distribution of [¹²⁵I]-labeled IFN-β1b following intranasal application in anesthetized cynomolgus monkeys (*Macaca fascicularis*). Coronal brain autoradiographs and labeled templates at (a) the level of the anterior commissure (ac 0 mm) or (b) 4 mm posterior to the anterior commissure (ac, 4 mm) with corresponding brain sections provided to illustrate the highly anatomical distribution in a single monkey receiving a very high specific activity [¹²⁵I]-IFN-β1b

radiolabeled IgG were observed in the olfactory bulbs, trigeminal nerves, and the walls of leptomeningeal blood vessels, supporting IgG access to perineural and perivascular pathways during nose-to-brain transport (Kumar et al. 2018a). The CNS delivery of radiolabeled IgG was significantly higher following intranasal administration versus systemic (intra-arterial) administration at doses resulting in similar endpoint blood levels (Kumar et al. 2018a). A positive dose-response was observed following intranasal radiolabeled IgG delivery with increasing doses resulting in higher CNS targeting, but, importantly, such a dose-response was not observed following intra-arterial delivery (Kumar et al. 2018a). A single intranasal radiolabeled IgG tracer dose (50 μg) in rats resulted in low-to mid-picomolar brain concentrations, while higher intranasal radiolabeled IgG doses (1 mg and 2.5 mg) resulted in low nanomolar levels in the CNS. This suggests that therapeutic levels of IgG may be achieved in the CNS following intranasal dosing, particularly at higher doses. The pre-administration of MMP-9 was used to enhance the transport of fluorophore-labeled IgG across the nasal epithelial barrier in rats and achieved a sufficiently high signal-to-noise ratio, allowing microscopic examination of IgG transport pathways to the CNS. Fluorescence microscopy images showed distribution across the nasal epithelium into the lamina propria, along fila olfactoria into the olfactory nerve layer, and along perivascular spaces surrounding perineural and cerebral blood vessels (Kumar et al. 2018a, b). Follow-up studies in C57BL6 mice and in the APP^{swe}/PS1^{dE9} transgenic AD mouse model have demonstrated that the intranasal administration of [¹²⁵I]-IgG either alone (Fig. 15.9) or following intranasal MMP-9 pretreatment (Fig. 15.10) results in widespread brain delivery compared to systemic administration using autoradiography (*Unpublished*; Nehra et al. 2021).

15.4.3 Intranasal Delivery of Gene Vectors and Oligonucleotides to the CNS

Long-term induction or suppression of gene products in the CNS has great potential to treat many neurological disorders. Many viral vectors, plasmids, and oligonucleotides exhibit low BBB permeability, making IN administration a potentially attractive alternative route. Several studies have investigated the IN route of administration to induce or repress gene products in the CNS.

Viral vectors, such as the recombinant adenoviral vector ADRSV β gal (Draghia et al. 1995), the growth compromised herpes simplex virus type 2 mutant Δ RR (Laing and Aurelian 2008), and herpes simplex virus type 1 (Broberg et al. 2004), have all been reported to induce gene expression in widespread areas of the brain following IN administration. Δ RR encoded the anti-apoptotic gene ICP10PK and prevented kainic acid-induced seizures, neuronal loss, and inflammation in rats. IN administration was more efficient at delivering herpes simplex virus type 1 DNA in the brain than corneal or intralabial infection. A filamentous bacteriophage (1000 nm long, 6 nm wide) expressing anti-A β scFv has also been shown to bind amyloid

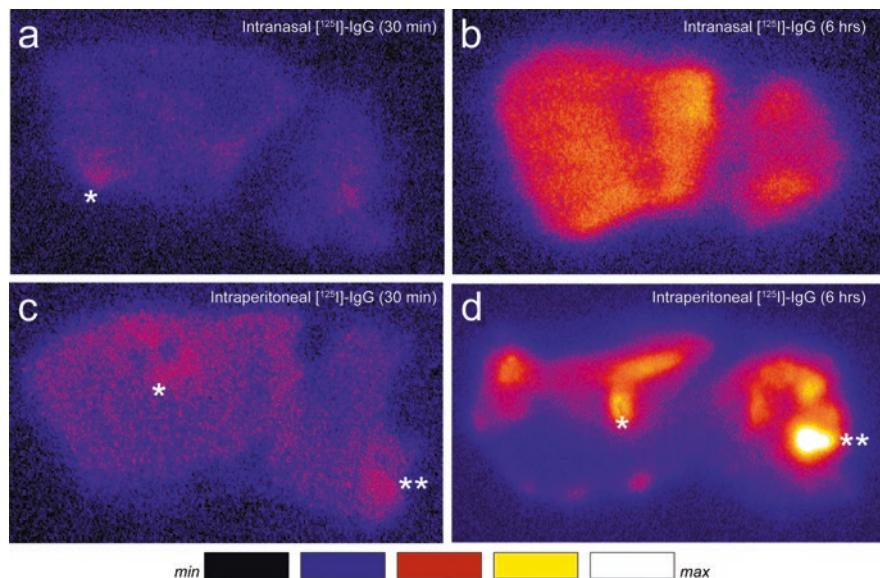


Fig. 15.9 Autoradiography images of $[^{125}\text{I}]\text{-IgG}$ distribution in sagittal brain sections of C57BL6 mouse after intranasal administration demonstrate brain delivery. (a) $[^{125}\text{I}]\text{-IgG}$ exposure was observed at ventral cortical regions 30 min following intranasal administration of $20\ \mu\text{g}$ $[^{125}\text{I}]\text{-IgG}$ (*). (b) $[^{125}\text{I}]\text{-IgG}$ distribution and intensity increased across widespread regions in the brain 6 h following intranasal $[^{125}\text{I}]\text{-IgG}$ delivery. (c) Equal-dose intraperitoneal administration of $20\ \mu\text{g}$ $[^{125}\text{I}]\text{-IgG}$ resulted in $[^{125}\text{I}]\text{-IgG}$ profiles at the ventricular region and brain stem, indicating putative access at the level of the choroid plexus (CP) in the lateral ventricle (*) and possibly also brain entry at circumventricular organs (CVOs) such as the area postrema (***) at the early time point of 30 min. (d) The $[^{125}\text{I}]\text{-IgG}$ signal showed a similar distribution, albeit with higher intensity, at 6 h post intraperitoneal $[^{125}\text{I}]\text{-IgG}$ administration, suggesting IgG entry across the blood-CSF barrier at the level of the CP in the lateral ventricle (*) and a region near the area postrema (***) dominated the biodistribution profile

plaques in an AD mouse model following IN administration (Frenkel and Solomon 2002). While the filamentous phage was detected in the brain, a spheroid phage was not, suggesting the shape of the phage may be important for IN delivery to the brain.

Plasmid DNA has also been delivered to the brain through the IN route. A 7.2-kb pCMV β and a 14.2-kb pN2/CMV β (encoding the gene for β -galactosidase) have been detected in the brain within 15 min of IN administration (Han et al. 2007). β -galactosidase activity was significantly higher in brain homogenates 48 h later and the brain-to-serum AUC ratio of pCMV β levels was ~ 2600 -fold higher 10 min after IN administration when compared to IV administration. Higher levels of plasmid DNA were detected in brain endothelial cells than microglia, while it was unclear if neurons were transfected.

Finally, IN delivery of oligonucleotides to the CNS has also been reported. In one study, IN delivery of αB -crystallin small interfering RNA (siRNA) complexed with DharmaFECT 3 resulted in reduced expression of αB -crystallin in neurons and astrocytes in the olfactory bulb, amygdala, entorhinal cortex, and hypothalamus

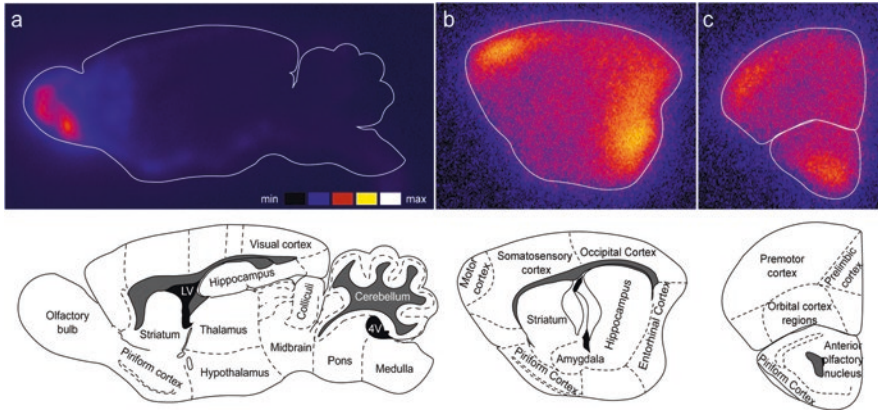


Fig. 15.10 Autoradiography images of [^{125}I]-IgG distribution in APPsw/PS1dE9 mouse brain following intranasal administration of $20\ \mu\text{g}$ [^{125}I]-IgG following intranasal MMP-9 pretreatment ($100\ \text{nM}$) to enhance absorption. (a) Sagittal brain section autoradiograph (approximately $1.5\ \text{mm}$ lateral to the midline; Paxinos and Franklin 2019) demonstrating high signal associated with the olfactory bulb following MMP-9 pretreatment at $30\ \text{min}$ post-administration. The lower schematic corresponds to the approximate position of the sagittal section. Settings optimized for high olfactory bulb signal intensity. (b) Sagittal brain section autoradiograph (approximately $3\ \text{mm}$ lateral to the midline; Paxinos and Franklin 2019) demonstrating relatively high signal intensity associated with cortical regions. The lower schematic corresponds to the approximate position of the sagittal section. (c) Coronal brain section autoradiograph (approximately $2.5\ \text{mm}$ anterior to the bregma; Paxinos and Franklin 2019) demonstrating relatively high signal intensity in both the rostral cortex and olfactory tract (anterior olfactory nucleus). The lower schematic corresponds to the approximate position of the coronal brain section. LV lateral ventral, 4 V fourth ventricle

both 3 and $12\ \text{h}$ following administration (Kim et al. 2009). In another study, a 21 base pair fluorescently labeled siRNA was delivered to the olfactory bulb following IN administration (Renner et al. 2012a). CNS delivery of the 22 base pair antagomir AM206 has also been reported following IN application in an AD transgenic mouse model (Lee et al. 2012). The authors concluded that AM206 increased brain-derived neurotrophic factor levels and memory function in mice by neutralizing microRNA-206.

15.4.4 Intranasal Delivery of Cell-Based Therapies to the CNS

Several recent studies have reported that the IN application of stem cells results in brain delivery and therapeutic effects in disease models. It is not known whether cells utilize the same pathways that molecular therapeutics use to reach the CNS after IN administration, but mesenchymal stem cells (MSC) have been found crossing the cribriform plate adjacent to the olfactory nerve bundles and in the olfactory nerve layer of the olfactory bulb $1\ \text{h}$ after IN administration (Galeano et al. 2018). These observations suggest both cells and molecules may access the CNS from the

nasal passages through the olfactory route. Fluorescently labeled rat mesenchymal stem cells (MSC) have been detected in the olfactory bulb, hippocampus, thalamus, cortex, and subarachnoid space of mice 1 h after IN delivery (Danielyan et al. 2009). Intranasally administered MSC have shown therapeutic potential in models of PD (Danielyan et al. 2011) and several models of stroke (Wei et al. 2012; Donega et al. 2013; van Velthoven et al. 2013). 1.5 h following IN administration, Hoechst labeled MSC could be found lining the blood vessels as well as in the parenchyma after ischemic stroke in mice (Wei et al. 2012). A study in which neural stem/progenitor cells (NSPC) were administered by the IN route found that the cells were targeted to the site of an intracerebral glioma within 6 h (Reitz et al. 2012). The enhanced green fluorescent protein expressing NSPC were located in the olfactory bulb within 6 h and the olfactory tract at 24 h. Few cells were observed in the trigeminal nerve at 24 h suggesting NSPC migrated into the brain within the first 24 h by the olfactory pathway as well as via the systemic circulation. Finally, IN administration of T cells engineered to express a chimeric antigen receptor targeting myelin oligodendrocyte glycoprotein have been reported to result in brain delivery and to suppress inflammation in a mouse model of multiple sclerosis (Fransson et al. 2012).

15.5 Future Challenges and Directions for Intranasal Drug Delivery to the Brain

15.5.1 *Methods to Enhance CNS Delivery Following Intranasal Administration*

A number of absorption enhancers have been used in experimental and clinical settings to enhance intranasal drug delivery to the systemic circulation. In theory, absorption enhancers may also increase the delivery of intranasally administered substances to the brain by increasing access to transport pathways from the lamina propria to the CNS. The mechanisms by which most absorption enhancers typically work are by enhancing the permeability of compounds across the nasal epithelial barrier and/or decreasing mucociliary clearance (Deli 2009; Illum 2012). Materials that have been used as intranasal absorption enhancers include surfactants, bile salts, bile salt derivatives, phospholipids, cyclodextrins, cationic polymers, proteases, and lipids (Davis and Illum 2003). Enhancing the permeability of mucosal membranes is often associated with irritation or damage (Sezaki 1995). Most absorption enhancers have not been well tolerated when administered intranasally in humans; one exception may be chitosan, which is produced by the deacetylation of chitin, a polysaccharide found in crustacean cells. Chitosan transiently opens TJ in mucosal membranes, has bioadhesive properties, and has been shown to be nonirritating with low local and systemic toxicity (Illum 2012). The development of non-toxic, physiological absorption enhancers is needed and may increase the delivery

of substances to the brain following IN administration, particularly for larger substances that have difficulty crossing the nasal epithelial barriers.

15.5.2 Unresolved Questions

Despite the increasing use of IN administration as a means to bypass the BBB and deliver substances directly to the CNS, a number of basic questions remain with regard to the mechanisms governing transport from the nasal mucosa to the brain. It is unknown if there is a size limit governing what can be delivered to the brain via the intranasal route. Studies with dextrans suggest there is an inverse relationship between MW and the CSF concentration following IN administration (Sakane et al. 1995). The permeability of the nasal epithelial barrier is not well characterized and may differ between the olfactory and respiratory regions. As the trigeminal nerve innervates both the respiratory and olfactory regions, it is unclear whether preferentially targeting one of these regions favors the delivery of substances to the brain stem along trigeminal nerve-associated pathways. What are the sites and rates of bulk flow that govern the extracellular transport of substances from the lamina propria to the CNS? Is the bulk flow associated with perineural, perivascular, and perilymphatic channels or some combination of these pathways? A summary of the hypothetical pathways into the brain from the nasal passages given our current state of knowledge is depicted in Fig. 15.11.

Further questions revolve around the effect of brain diseases on the pathways and mechanisms underlying brain uptake following intranasal administration. For example, it is poorly appreciated how or, if disease states, might affect brain delivery and/or the distribution of substances in the CNS following IN administration. It will be important to establish whether the capacity for nose-to-brain transport is compromised, unaffected, or otherwise altered by specific pathology, disease, or other factors. Finally, it is not yet clear how cells reach the brain by the IN route of administration; whether cells use the same or different pathways/mechanisms to gain entry to the brain from the nasal passage that have been identified for small molecules, peptides, and proteins remains an open question. There is a clear need for further research to address these questions and advance knowledge so that clinical applications utilizing intranasal targeting of drugs to the CNS can be evaluated with the best possible opportunities for success. Recent years have witnessed new studies that have expanded our understanding of nasal physiology (e.g., Kumar et al. 2015), factors governing intranasal delivery to the brain and CSF of smaller-molecular-weight substances (e.g., Stenslik et al. 2015, 2018; Nehra et al. 2021), and factors governing the intranasal delivery to the brain and CSF of large molecules (e.g., Lochhead et al. 2015; Kumar et al. 2018a, b). Despite this recent progress, more work is clearly warranted.

15.6 Conclusions

Drug delivery to the CNS remains a challenge due to the restrictive nature of the BBB and BCSFB. A number of studies suggest the IN route of administration may allow rapid, noninvasive delivery of substances directly to the CNS along pathways associated with the olfactory or trigeminal nerves. These pathways are not yet fully characterized, presenting opportunities for further investigation. Methods to enhance the delivery of substances from the nasal cavity to the CNS are also needed due to the typically low delivery efficiencies that have thus far been measured (<0.05% for proteins). A better understanding of the mechanisms governing the transport of substances into the CNS from the nasal mucosae may lead to improvements in the efficiency of IN administration. While IN drug delivery to the brain has shown great promise in animals and humans, it is clearly an area where more research is needed to fully exploit its potential.

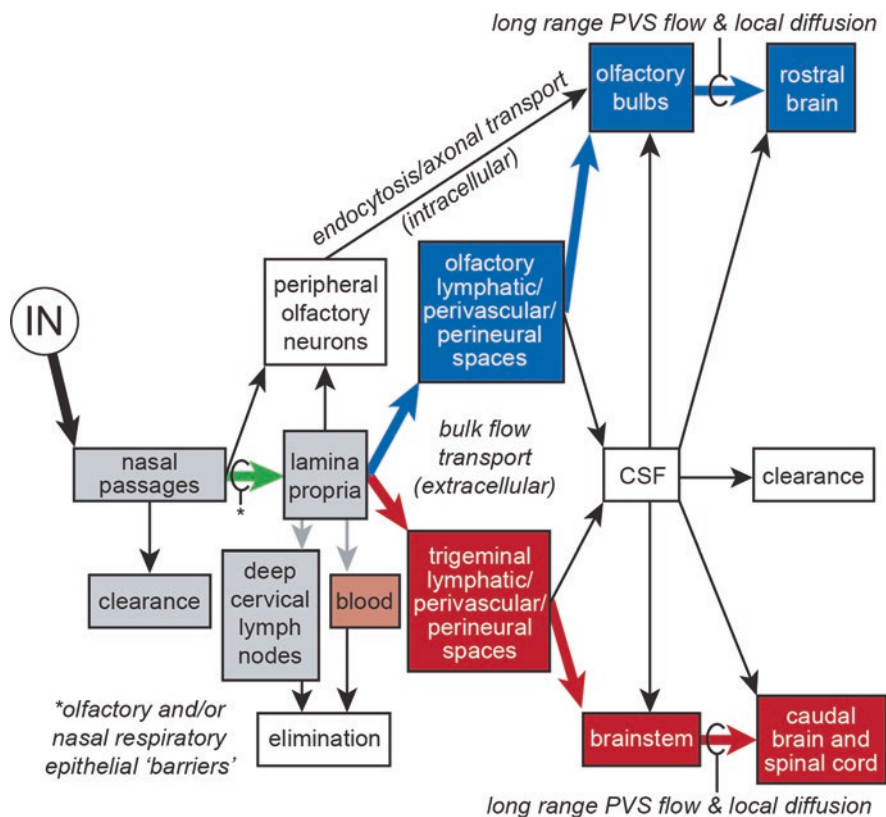


Fig. 15.11 Proposed pathways and mechanisms responsible for drug transport into the CNS following intranasal administration, based primarily upon studies utilizing radiolabeled proteins

15.7 Points for Discussion

- What advantages does the intranasal route of administration offer for chronic administration? Compare and contrast intranasal application requirements with those of other strategies designed to target biotherapeutics to the brain (e.g., are trained health professionals required for administration? Can administration be performed in an outpatient setting or is hospitalization necessary? What economic resources will likely be required to accomplish administration? Can the methods be easily applied in both developed and developing countries?).
- Why might knowledge of nasal epithelial organization and physiology be important for implementing, optimizing, and adjusting brain targeting following intranasal drug administration?
- What are the likely pathways that underlie nose-to-brain transport? What are the likely mechanisms that underlie nose-to-brain transport? What additional information is needed to more fully understand the pathways/mechanisms responsible for intranasal targeting of drugs to the CNS?
- Discuss each of the steps that may be required for an intranasally applied drug to reach CNS target sites?
- How might the transport of small molecules, macromolecules, and cell-based therapies into the brain following intranasal application be similar? How might their transport be different? What areas require further study?
- What key questions remain regarding the clinical translation of intranasal approaches targeting the CNS? How and why are rodent and monkey studies of intranasal administration different with respect to translational relevance for human studies?

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