Translational Neurodegeneration

REVIEW

Open Access

Nose-to-brain drug delivery: from bench to bedside

Isabell Drath[1,2], Franziska Richter[1,2*] and Malte Feja[1,2*]

Abstract

There is increasing interest in nose-to-brain delivery as an innovative drug delivery strategy for neurodegenerative disorders such as Parkinson's or Alzheimer's disease. The unique anatomy of the nose-brain interface facilitates direct drug transport via the olfactory and trigeminal pathways to the brain, bypassing the blood–brain barrier. Different administration techniques as well as advanced drug formulations like targeted nanoparticles and thermoresponsive systems have been explored to improve the delivery efficiency and the therapeutic efficacy. This review provides an up-to-date perspective on this fast-developing field, and discusses different studies on safety and pharmacokinetic properties. A thorough evaluation of preclinical and clinical studies reveals both promises and challenges of this delivery method, highlighting approved drugs for the treatment of epilepsy and migraine that successfully utilize intranasal routes. The current landscape of research on nose-to-brain delivery is critically discussed, and a rationale is provided for ongoing research to optimize therapeutic strategies.

Keywords Intranasal, N2B, Parkinson's, Alzheimer's, Neurodegenerative disease, Nanoparticle

Background

The blood–brain barrier poses a challenge for the treatment of central nervous system (CNS) disorders by preventing most therapeutic agents from reaching the brain after oral or parenteral administration. Recently, intranasal administration of drugs has gained increasing interest as it can bypass the blood–brain barrier. This route is suitable for daily application and allows therapeutic molecules to be transported directly into the brain, bypassing the blood–brain barrier and increasing drug concentrations in the CNS [1]. Although the exact mechanisms of transport from the nose to the brain are not fully understood, preclinical and clinical studies have shown that the nose-to-brain drug delivery is applicable in both animals and humans. Here we summarize the current state of knowledge regarding mechanisms of nose-to-brain drug delivery including clinical applications.

Anatomy and histology of the nose-brain interface

The nasal cavity of humans and other mammals including mice and rats is separated by the nasal septum into two parts. The two nostrils build the entrance to the nasal vestibule which merges into the nasal cavity. Within the nasal cavity there are three nasal conches, which form the nasal meatuses [2]. The whole nasal cavity except the nasal vestibule is coated with nasal mucosa which can be divided into the respiratory and the olfactory regions.

The respiratory region, which represents the major part of the nasal cavity, is coated with respiratory ciliated epithelium and serves primarily as a protective surface. It humidifies and warms the inhaled air and is able to remove particles and allergens [3]. Innervation of the respiratory mucosa is provided by branches of the trigeminal nerve [4].

*Correspondence:
Franziska Richter
franziska.richter@tiho-hannover.de
Malte Feja
malte.feja@tiho-hannover.de
[1] Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Bünteweg 17, 30559 Hannover, Germany
[2] Center for Systems Neuroscience (ZSN), Hannover, Germany

The significantly smaller olfactory region is located at the roof of the dorsal meatus and is characterized by olfactory mucosa. Fine nerve fibers, the fila olfactoria, innervate the olfactory mucosa. They originate from the olfactory nerve, which innervates from the olfactory bulb through the lamina cribrosa to the olfactory region (Fig. 1a). Thus, the olfactory nerve displays a direct connection between the brain and the nose, which could mediate the higher efficiency of nose-to-brain delivery via the olfactory mucosa compared to the respiratory mucosa [5]. In addition to the sensory innervation by the olfactory nerve, the nose is sensitively innervated by branches of the trigeminal nerve. The trigeminal nerve originates at the pons, swells to form the ganglion trigeminale and divides afterwards into three main branches: nervus ophthalmicus, nervus maxillaris and nervus mandibularis (Fig. 1b). The ophthalmic and the maxillary nerves are responsible for the innervation of the nasal mucosa [6]. Moreover, the perineural space surrounding the cranial nerves is connected to the subarachnoid space and thereby directly connected to the cerebrospinal fluid (CSF) [7–9]. One pathway of CSF drainage is along the cranial nerves into the nasal epithelium [7, 10]. Taken together, the nose is directly connected to the brain and the CSF via the trigeminal and the olfactory nerves.

Another distinct characteristic of the two regions is the capillary density, which is about 5-times higher in the respiratory region than in the olfactory region [11]. High vascular density efficiently eliminates drugs from the tissue; therefore, low vascular density of the olfactory region correlates with higher brain delivery efficiency [11]. Furthermore, it is important to note that the olfactory epithelium in rats and mice is about 40%–50% of the total nasal surface; however, in humans the percentage is less than 10% [12–14]. This anatomical difference has a significant impact on the translation of preclinical studies from laboratory animals to humans, particularly for dose finding and the investigation of nasal delivery devices.

The olfactory mucosa consists mainly of four different cell types: ciliated olfactory receptors, supporting cells, basal cells and microvillar cells [15]. Ciliated olfactory cells are bipolar neurons, with one process that terminates in the olfactory bulb and another superficial process ending in a ciliated apical extension called olfactory vesicle [16]. Supporting cells, also called sustentacular cells, are non-ciliated epithelial cells, which function as metabolic support and are able to introduce substances into the surface mucus as well as to remove substances from it [17]. Basal cells are stem cells which do not reach the surface and are able to differentiate into ciliated olfactory receptors or supporting cells to replace degenerated cells [17]. Microvillar cells serve as bipolar sensory neurons [18]. The olfactory mucosa lies above the lamina propria which consists of blood and lymph vessels, Bowman's glands, nerve bundles and connective tissue [15].

The nasal blood supply is mainly provided by the external and the internal carotid arteries. The nasal walls are supplied by the sphenopalatine artery which comes from the maxillary artery, a branch of the external carotid artery. The anterior part of the nose is supplied with blood by the anterior and posterior ethmoidal arteries. These are branches of the ophthalmic artery, which originates from the internal carotid artery. Additionally, branches from the facial artery supply the vestibule and the anterior portion of the nose [19].

The human nose has several functions regarding breathing, which include air conditioning, heating of the inhaled air as well as protective mechanisms like mucociliary activity to remove particles and pathogens. The human average mucociliary transport rate is about 6 mm per minute [20] and especially particles over 15 µm in size are removed [19]. This mucociliary clearance mechanism reduces the retention time of drugs in the nose, which needs to be taken into account when developing intranasal drugs. Moreover, the human nose serves as a resonating body for speaking and is critical for the olfactory sense.

Nose-to-brain transport routes

Nose-to-brain drug delivery is a promising avenue to circumvent the blood–brain barrier. Indications for a direct connection between the nose and the CNS were discovered at the beginning of the last century [8], as researchers were able to show that substances injected into the subarachnoid space can reach the nasal mucosa [21]. Numorous studies have substantiated this direct communication. However, the nose-to-brain pathway is still not completely understood and there appear to be different routes for drugs to reach the brain and distribute across its parenchyma (Fig. 1). Currently, two different routes of transport to the brain are proposed: the olfactory and the trigeminal routes. This concept is based on studies detecting high amounts of drugs in the lateral olfactory tract, olfactory bulb and trigeminal region after intranasal application [22–24].

First evidence for the olfactory route emerged almost 100 years ago when researchers suspected that viruses travel from the olfactory epithelium of the nose along the olfactory nerve to the brain [25]. This route of virus spread was brought back into focus of research by the COVID-19 pandemic [26]. Furthermore, latest research shows pathology in the olfactory bulbs after intranasal administration of alpha-synuclein preformed fibrils, which are thought to contribute to neurodegeneration in PD, underlining the connection between the nose and the olfactory part of the brain [27]. The olfactory route is

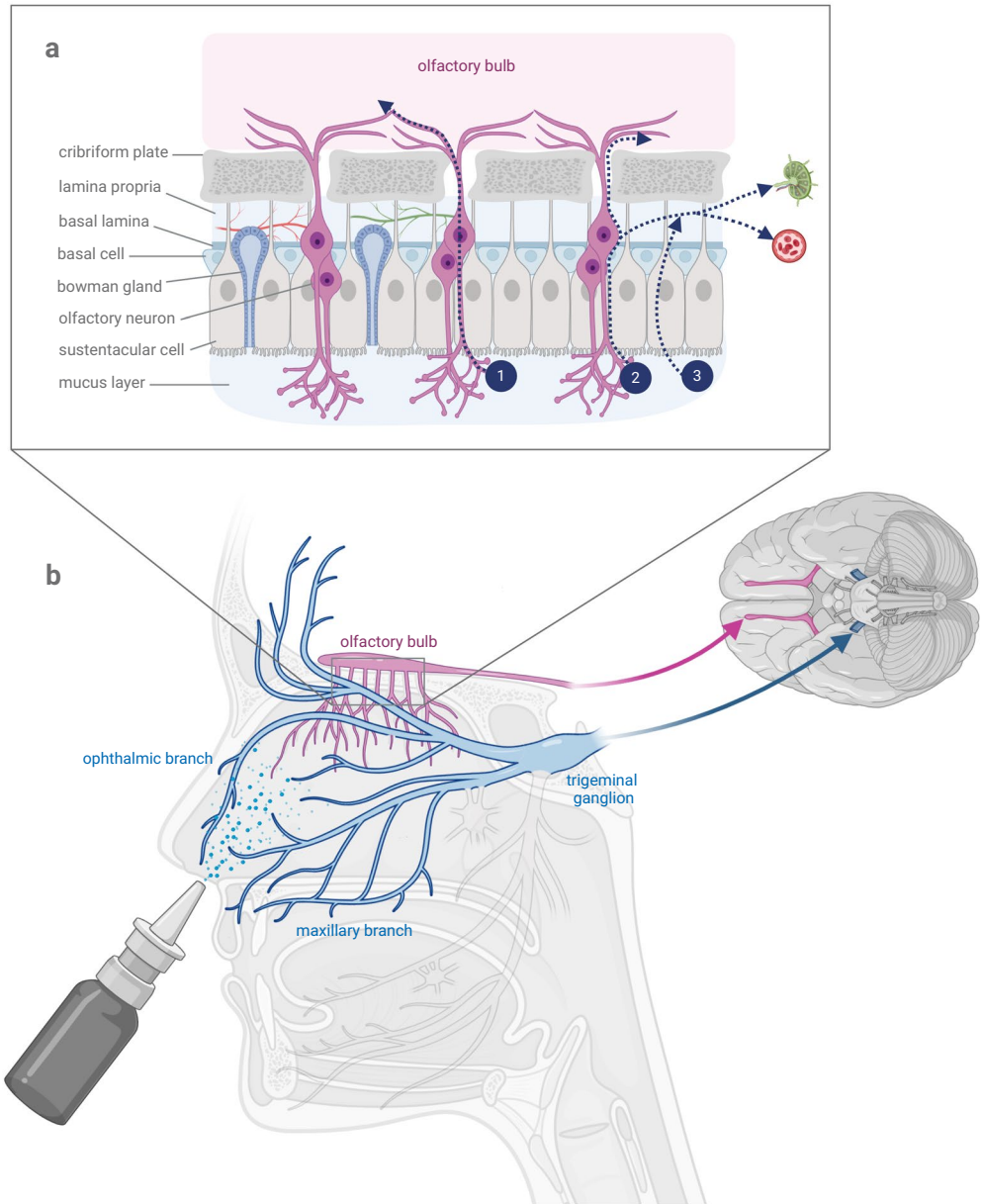

Fig. 1 **a** General anatomy of the nose-to-brain interface and potential transport pathways for nose-to-brain delivery. The olfactory epithelium is composed of bipolar olfactory neurons, sustentacular cells, Bowman's glands, basal cells and its underlying lamina propria, which contains blood and lymph vessels. Axonal processes of olfactory neurons are arranged in bundles known as the fila olfactoria, which traverse the cribriform plate and reach the olfactory bulb. Potential pathways for drug delivery from the olfactory mucosa to the brain are illustrated in dark blue. ① Intracellular pathway: drugs are transported within olfactory neurons via axonal transport and endocytosis. ② Extra/paracellular transport along cranial nerves: drugs are transported within the perineural space, either to the cerebrospinal fluid in the subarachnoid space, or to the lamina propria, and subsequently to the blood or lymph vessels. ③ Transcellular transport: drugs are transported through cells to the lamina proria with further transport to lymphatic vessels that are connected with the cervical lymph nodes or to blood vessels following entry to the systemic circulation. **b** Innervation of the nasal region by the olfactory nerve and branches of the trigeminal nerve. The olfactory nerve bundles, originating from the olfactory bulb, traverse the cribriform plate and provide innervation to the olfactory region of the nasal mucosa. The trigeminal nerve leaves the brainstem at the level of the pons and divides after the trigeminal ganglion into its three main divisions: V_1, ophthalmic nerve; V_2, maxillary nerve; V_3, mandibular nerve. Only V_1 and V_2 send branches to the nasal epithelium, thereby innervating the respiratory mucosa and thus participating in the process of nose-to-brain delivery

subdivided into two pathways: (1) via the olfactory nerve to the olfactory bulb with subsequent parenchymal distribution to different brain areas and (2) distribution via the CSF followed by entrance into the brain parenchyma [28].

Early studies administering wheat germ agglutinin-horseradish peroxidase intranasally found high concentrations in the olfactory nerve and the glomerular layer of the olfactory bulb [29, 30]. Intranasally administered labeled siRNA distributed along the olfactory pathway to the brain and was detected within the olfactory epithelium, the olfactory nerve as well as in the glomerular and mitral cell layer of the olfactory bulb [31]. In detail, substances enter olfactory dendrites and are transported via the intracellular pathway across olfactory neurons into the fila olfactoria [32], subsequently reaching the olfactory bulbs by endocytosis and axonal transport [29, 33]. The axonal transport velocity has been shown to be at maximum 130 mm per day, thus it would take a minimum of 45 min for substances to reach the brain of a mouse after intranasal application [34]. Given that several membrane barriers need to be crossed, transcellular transport is generally expected to require more time compared to extracellular transport.

By measuring drug concentrations in the CSF after intranasal application, previous studies confirmed the second branch of the olfactory route: a direct pathway from the nose to the CSF [35–37]. It was previously shown that substances injected into the CSF would drain not only through the arachnoid villi, but also along the cranial nerves [38, 39]. This pathway is bidirectional [8], supporting the assumption of a direct pathway from the nose to the CSF. Substances are most likely transported extracellularly via olfactory lymphatic, perivascular and perineural spaces to the CSF [40]. This route is assumed to be a direct transport, as model predictions of the time required for different transport pathways to reach the olfactory bulb suggest that only convective bulk flow processes are fast enough to account for experimentally observed data [14]. A pharmacokinetic study showed that the maximum concentration in the CSF can be reached only 5 min after intranasal phenytoin application; such rapid transport favours a direct route from the nose to the CSF [41].

The trigeminal route includes transport along different branches of the trigeminal nerve that innervate the nasal respiratory mucosa: the ethmoidal nerve originating from the ophthalmic nerve, as well as the posterior nasal branches and the nasopalatinal nerve originating from the maxillary nerve [42, 43]. They are projecting to the trigeminal ganglion and to trigeminal nuclei in the brain stem. Furthermore, there is evidence that some trigeminal ganglion cells with sensory endings in the nasal epithelium have direct branches reaching into the olfactory bulb [44, 45]. Studies have shown that substances can reach the trigeminal nerve after intranasal application [46]. More precisely, a GLP-2 derivative was detected in the trigeminal principal sensory nucleus (Pr5) in the pons three minutes after intranasal administration, accounting for a rapid intracellular axonal transport [47]. In another study, insulin was shown to reach the perineural space of the trigeminal nerve, providing evidence that extracellular transport processes are also involved in the trigeminal pathway [48].

As any transport of drugs across barriers, the nose-to-brain delivery route is dependent on particle size and formulation of the substance administered. However, small size does not necessarily improve neuronal transport. For instance, 520-nm Poly(lactic-co-glycolic acid) nanoparticles can be detected in neuronal bundles after intranasal administration, indicating their transcellular neuronal transport, whereas 80-nm and 175-nm particles were only detected in other cell types, albeit with a more rapid distribution [49]. Moreover, neuropeptides like GLP-1 and GLP-2 derivatives with functional sequences were shown to be rapidly transported through trigeminal axons after intranasal application in mice [47, 50]. Others reported that the trigeminal pathway serves as the dominant route for intact nanoparticles, whereas the olfactory pathway is more likely to deliver substances which are already released from nanoparticles [51].

Of note, substances transported predominantly via the olfactory route rapidly appear in different brain areas, suggesting extracellular transport to reach the brain and the CSF [52]. In contrast, substances transported mainly via the trigeminal pathway take longer to reach the brain, indicating that they are more likely to be transported transcellularly [47]. Moreover, the trigeminal nerve is longer than the olfactory nerve, leading to longer transport duration.

Techniques for intranasal administration

An optimal technique for intranasal application is crucial for successful delivery of drugs to the brain. Several different techniques and devices have been developed for nose-to-brain delivery, including ultrasound-mediated methods [53], electric guidance of charged particles [54] and catheter-based administration [55]. In preclinical studies using small laboratory animals, micropipettes are frequently used to place small droplets at the entrance of the nostrils to be breathed in by the animal. In order to define factors that impact delivery efficacy, influences of animal position, body weight and age were investigated [56]. Interestingly, older animals with higher body weights require increased intranasal dosages to reach the same drug concentration in the brain as in young

animals. Furthermore, placement of animals in a supine or an upright position has no effect on the delivery efficiency [56]. To further enhance delivery efficiency and precision, catheters are applied to specifically target the olfactory region of the nasal cavity in mice and rats [55, 57]. The catheter reduces untargeted distribution to the periphery compared with the standard pipette-based method. However, the procedure is more invasive and requires anesthesia.

Another method to enhance delivery efficiency is the use of external magnetic fields to guide charged particles to the olfactory region. Electric guidance reduces particle loss in the anterior nose and increases particle deposition in the olfactory region [54]. With the use of a magnetic field and certain improvements in particle diameter and injection angle, the olfactory deposition in an in silico study of the human nose was 65-times higher compared to standard injections without magnetic fields [58]. This indicates that improvement of application methodology and the use of external magnetic fields can enhance nose-to-brain delivery.

One disadvantage of the above described techniques for nose-to-brain drug delivery is that drugs are delivered to the entire brain unspecifically, whereas most CNS diseases primarily affect certain brain regions or cell types. Thus, concentrated drug application to the target cells could enhance efficacy and reduce off-target effects. The combination of intranasal delivery and focused ultrasound with microbubbles has been suggested as a targeted strategy [53, 59]; however, this method can be quite disruptive for brain tissue and its use may be limited to severe conditions such as certain brain tumors. In addition to brain region targeting, it is also possible to use selective cell-targeting nanoparticles to deliver drugs to disease-related cells. For example, in glioblastoma therapy, it is of great importance to target cancer cells while reducing exposure of healthy cells. Co-layering nanoparticles with poly-l-glutamate and hyaluronate increases glioblastoma targeting in tumor-bearing mice after intracranial injection [60]. Targeting dopaminergic neurons could be promising in the field of Parkinson's disease (PD), whereas targeting amyloid plaques may be an interesting therapeutic approach in Alzheimer's disease (AD) [61]. Modified silica nanoparticles with dopamine ligands that bind to neuronal dopamine receptors showed superior delivery of glutathione to SH-SY5Y cells compared to unmodified nanoparticles, as well as improved cytoprotective and anti-apoptotic effects in vitro [62]. Furthermore, a study has demonstrated neuron-selective delivery of microRNA using a D3-peptide-conjugated nanopolymer injected into the tail vein in an AD mouse model [63]. Cationic siRNA complexes have also been shown to precisely target amyloid plaques in the brains of AD mice after intravenous injection [64]. Whether such selective cell-targeting can be combined with nose-to-brain delivery remains to be determined.

Another challenge for nose-to-brain drug delivery is the development of appropriate delivery devices, since standard devices are optimized for local effects and therefore deliver only a small amount of drug to the olfactory region [65]. For instance, traditional spray pumps deliver only around 5% of the drug to the upper nasal space where the olfactory mucosa is located [66]. The development of intranasal drugs with appropriate delivery devices is limited by the fact that their use in preclinical studies cannot be accomplished with the same delivery devices as in humans because of the different size and anatomy of laboratory animals. Mathematical models and human nasal replica casts are used to overcome this species barrier; however, successfull delivery in clinical studies remains challenging [67] (Fig. 2). For instance, four different nasal spray pumps and four nasal nebulizers failed to deliver a therapeutically significant amount of certain particles to the olfactory region [65]. Therefore, it is argued that nasal replica casts most times do not imitate a human nose sufficiently to draw valid conclusions for a regulatory drug deposition study [68]. Despite these challenges, there are several different nasal delivery devices on the market, some of which are specifically designed for nose-to-brain delivery. One such device uses a propellant-powered delivery technology which claims to reduce the amount of drugs that get trapped in the nasal vestibule, thereby delivering more medication to the upper part of the nose compared to traditional nasal sprays [69]. This device has been used in clinical studies to deliver dihydroergotamine mesylate for acute treatment of migraine attacks and has shown rapid pain relief as well as good tolerability [70, 71].

Further developments in the field are bidirectional breath-powered delivery devices [72], one of which has already been approved for migraine treatment with sumatriptan nasal powder. The device consists of an exhalation mouthpiece connected to the nosepiece by the device body. During exhalation the soft palate closes and seperates the nasal from the oral cavity, which prevents unintentional delivery to the oropharynx or lungs. The breath-powered delivery devices deliver more drugs to the target site with a better pharmacokinetic profile than traditional nasal sprays [73, 74].

Pharmacokinetics of nose-to-brain drug delivery

The goal of pharmacokinetic studies for nose-to-brain drug delivery is to develop the optimal drug formulation, determine dosing regimens, and gain understanding of drug interactions. Previous pharmacokinetic studies have shown that the peak concentrations of substances

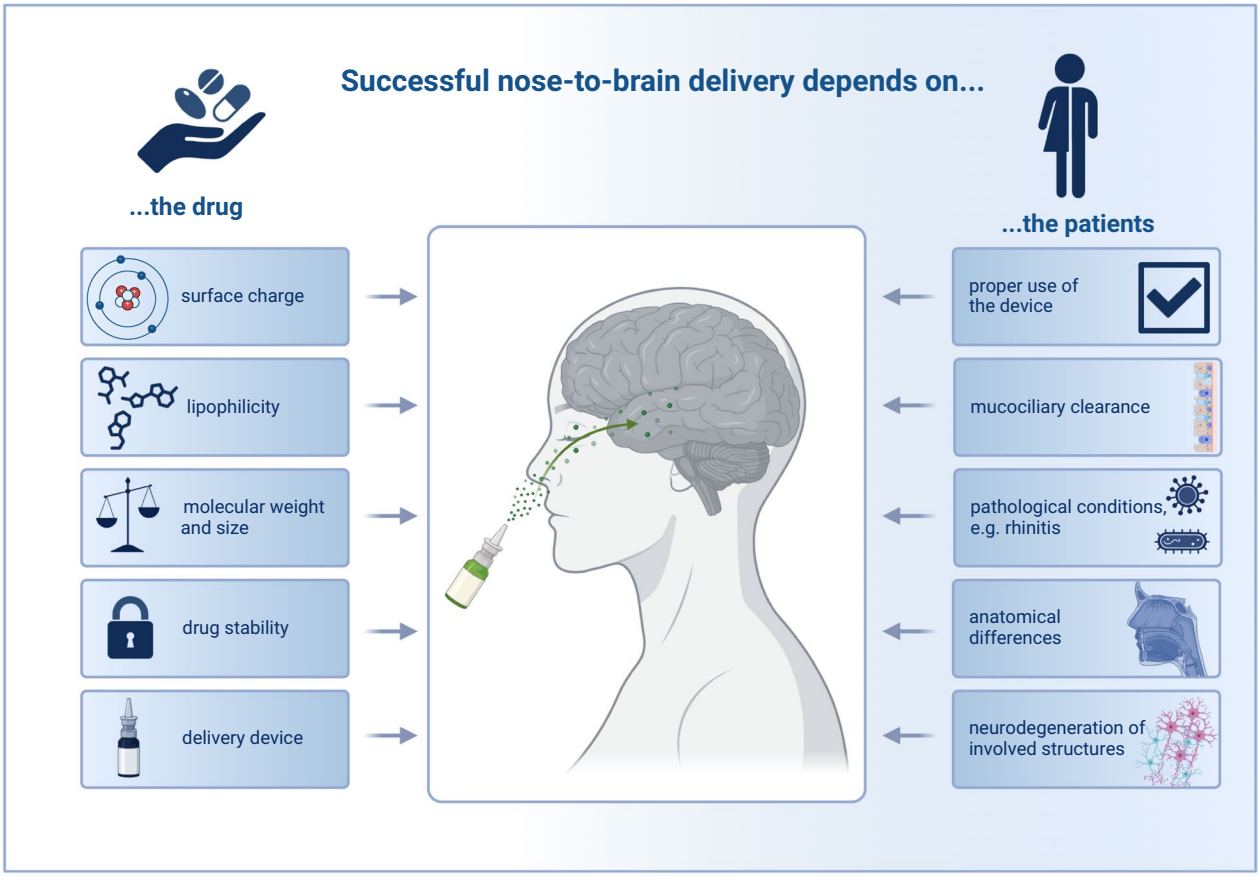

Fig. 2 Factors influencing nose-to-brain delivery

like clomipramine nanoparticles [75], dantrolene [76] or donepezil liposomes [77] in the brains of rodents can be reached as early as 20–30 min after intranasal application. In contrast, another study measured the highest concentration of intranasally administered nanoparticles containing fibroblast growth factor after 30 min only in the olfactory bulb, while the peak concentration in the rest of the rat brain was reached 4 h after application [78]. Further studies generated pharmacokinetic profiles of intranasal vascular endothelial growth factor in rats and intranasal allopregnanolone in mice, which measured the highest drug concentrations in the trigeminal nerve, optic nerve and olfactory bulb followed by the cerebrum and hippocampus [79, 80]. The lowest concentrations in the brain were measured in the cerebellum and even lower concentrations in the serum after intranasal application of fibroblast growth factor in rats [81]. Due to a good vascularization of the nasal mucosa, drugs are reaching the systemic circulation. In addition to different brain areas, drugs like methotrexate reach even the cervical lymph nodes of rats with peak concentrations one hour after intranasal administration [79, 82]. Different studies have revealed superiority of the nasal route compared to other administration routes. Intranasal administration of a hematopoietic growth factor, which has limited capacity to cross the blood–brain barrier, is 8–12 times more effective than subcutaneous application in brain and CSF delivery [83]. Furthermore, a recent study demonstrated that the brain uptake of human recombinant erythropoietin, curcumin, glucagon-like peptide 1 and anti-Aβ antibodies given to mice by intranasal administration is more than 5 times higher than that by intraperitoneal injection [84]. The superior efficiency of brain delivery by intranasal administration compared to intraperitoneal administration has also been observed for a peptide capable of Aβ hydrolysis [85]. Compared to oral administration, the nasal route achieved 25-fold higher bioavailability of harmine nanocrystals in the brain [86]. Moreover, intranasal applications achieved higher, longer-lasting brain drug concentrations compared to intravenous administration [81, 87]. For instance, the area under curve for carmustine concentration in the brain after intranasal administration was 14.7 times that of intravenous administration [88]. Furthermore,

oxytocin, as well as high-molecular-weight substances such as cobrotoxin, reach the brain in greater amounts when administered intranasally than when administered intravenously [89, 90].

Differences in pharmacokinetic profiles are apparent between experimental animals and humans. After intranasal administration of insulin lispro in humans as well as in dogs, the insulin lispro was detectable in the CSF of dogs, while its level in the CSF was below the limit of quantification in humans [91]. A computational model comparing intranasal delivery patterns between mice and humans has been developed for further pharmacokinetic studies [92]. The model predicted that nasally administered nanomaterials reach the mouse brain at an amount of two orders of magnitude compared to that reaching the human brain. This suggests that extrapolation of pharmacokinetic studies from laboratory animals to humans is of limited validity and should always be done with caution.

Drug formulations for nose-to-brain delivery

Another key aspect in drug discovery is finding an appropriate drug formulation that delivers the drug in a stable, safe and effective manner (Fig. 2). For nose-to-brain administration, drugs can be formulated as powders, solutions or gels. Furthermore, biochemical features like particle size, pH and charge can be adjusted. By optimizing the formulation, it is possible to enhance brain uptake by more than 10 folds [93].

As stated above, particle size is an important determinant of neuronal uptake. Furthermore, particles of different sizes also distribute differently inside the nasal cavity after application, which can be addressed by optimizing the formulation. In a 3D model of human nose, microparticles with a size of 10 µm reach the olfactory region at a higher amount than particles with a size of 2 µm [94]. The maximum olfactory deposition was observed with particles of 8–12 µm in size [95]. In line with these findings, another study reported that inertial 10-µm particles and diffusive 1-nm particles have higher olfactory deposition than particles in the size range of 10 nm–2 µm, probably due to the inertial force of 10-µm particles and the Brownian motion of 1-nm particles [96]. This is consistent with another study that found highest olfactory deposition for very small particles with a size of 1–2 nm [97].

To avoid nasal irritation and achieve efficient drug absorption from nasal mucosa, it is important to adjust the pH of the formulation [98]. While the pH level of the nasal mucosa is approximately 6.3 [99], studies have revealed a higher absorption of nasal formulations at pH below 4.79 [100]. Furthermore, to prevent irritation and maintain microbial defense, the pH of the nasal formulation should be adjusted slightly acidic, as lysozymes in nasal secretions effectively destroy bacteria at acidic pH but become inactivated under alkaline conditions, leaving tissue vulnerable to infection [101]. Considering the advantages of an acidic pH and the physiological environment of the nose, intranasal formulations should ideally have a pH level between 4.5 and 6.5, which is important to avoid adverse effects on the mucosa or the ciliary movement [102].

In addition, lipophilicity, molecular weight and surface charge of intranasal drugs also affect the delivery efficiency. Increasing lipophilicity is correlated with higher drug concentrations in the CSF after intranasal administration [103]. Furthermore, decreased molecular weight of drugs is associated with higher drug concentrations in the CSF [104]. Anionic drug carriers provide a 20% increase in drug targeting efficiency compared to cationic carriers [105].

Formulations in gel, solution or powder?

One obvious disadvantage of solutions is the short residence time in the nasal cavity. Gels with higher viscosity remain longer at the mucosa and deliver significantly higher brain concentrations of the drug compared to solutions [106, 107]. To prolong the residency time of gels further and overcome rapid clearance of the drug, gels that respond to temperature, ions or pH with higher viscosity are being developed [108, 109]. A thermo-responsive gel turning viscous at 32 °C not only increases drug concentrations in the brain compared to a non-thermo-responsive gel, but also sustains the concentrations for a longer period of time [110]. Besides, it is possible to add mucoadhesive agents to the formulation, which can improve biodistribution by increasing the retention time in the brain and the brain/blood ratio [111]. In most cases, either solutions or gels were used, but a small number of studies also investigated powder formulations. Some studies showed superiority of powders, while others achieved better delivery results using other formulations, depending on several factors, such as the drug administered. For *L*-3,4-dihydroxyphenylalanine (*L*-DOPA) administration in PD patients, powders appear to act more rapidly than solutions, which may be an advantage depending on the treatment goal [112]. Two different nasal powders applied with an active delivery device provide direct transport rates over 60% [113]. In contrast, other studies showed superiority of eutectic formulations compared to powders [114].

Nanoparticle-mediated nose-to-brain delivery

However, efficient delivery to the brain is challenging, and whether using powders, sprays, solutions or gels, vectors are commonly used in the treatment of CNS diseases.

Notably, in the field of neurodegenerative diseases, adeno-associated viruses are the most commonly used carriers. However, they have some disadvantages, such as limited loading capacity, difficult vector production and inflammatory reactions [115–117]. Non-viral vectors avoid these disadvantages and are safer for patients. For this reason, several studies have explored non-viral carriers, such as liposomes or nanoparticles to enhance brain bioavailability of various drugs. It has already been demonstrated that nanoscaled carriers support the transport of substances to the brain and at the same time increase the stability of active ingredients [118]. Lipid nanoparticles loaded with paclitaxel and miltefosine improved drug concentration in the mouse brain by 5 folds compared to the free drug [118]. A substantial number of studies have indicated superiority of nanoparticle-mediated delivery versus plain delivery in terms of pharmacokinetic parameters as well as treatment efficacy [119–121]. There is a wide range of materials that can be used to create nanoparticles. Materials should be carefully selected depending on the purpose. Nanoparticles should reach the brain and penetrate target cells while being non-toxic to the nasal mucosa or the brain. Meanwhile, they may have a direct effect on the efficacy of the drug. For instance, tyrosine modification on nanoparticles for siRNA therapeutics improves the siRNA-mediated knockdown efficacy [122]. Whether nanoparticles have a branched or a linear structure also makes a difference to biocompatibility [122, 123]. Moreover, a meta-analysis reported that lipid nanoparticles are significantly superior to polymeric nanoparticles in enhancing the brain bioavailability of drugs [124]. In addition, the coating of nanoparticles can also influence their properties: chitosan coating appears to improve mucoaffinity and diffusion efficiency in vitro [125]. In conclusion, there are several ways to modify nanoparticles and improve nose-to-brain delivery of a particular drug.

Safety considerations for nose-to-brain delivery

Safety is a fundamental aspect that needs to be addressed when developing drugs towards regulatory approval. In the development of intranasal drugs it is important that not only the drug itself but also the excipients of the drug formulation like mucoadhesives or nanoparticles are safe and do not cause any side effects. Regarding nose-to-brain delivery it is important to consider systemic side effects as well as local nasal mucosa and CNS toxicity.

A major advantage of intranasal compared to systemic delivery is the limited amount of drug reaching the systemic circulation and the liver [126], thereby reducing the risk of systemic adverse effects and rapid metabolization [127]. Different studies have confirmed general safety of intranasal drugs or have shown even higher safety compared to other administration routes. For instance, clinical studies testing intranasal recombinant erythropoietin recorded only mild adverse events without severe adverse events. Moreover, the number of adverse events in the treatment group was not increased compared to the placebo group [128, 129]. Furthermore, intranasal delivery of paliperidone palmitate, a drug which causes serious adverse events after oral administration, did not cause any alterations in blood parameters in rats, suggesting intranasal delivery as a promising tool to reduce systemic side effects [130].

Due to the direct contact of the drug with the nasal mucosa, it is very important to screen for local toxic effects and to consider different factors influencing nasal conditions such as temperature, humidity or conditions like rhinitis. Previous evaluation of cytotoxicity to the nasal mucosa was done in cell culture models of nasal mucosa, mainly including primary cells collected from the olfactory region of rats [131] and the immortalized cell line RPMI 2650 isolated from a squamous cell carcinoma of the nasal septum [132]. These models can be used to carry out cytotoxicity assays as well as permeation studies, e.g., measurement of transepithelial electrical resistance [133]. The nasal tissue has protective functions which, among others, include mucociliary activity to prevent exogenous particles reaching the upper airways. Thus, it is essential to ensure that the drug has no adverse effects on the ciliary movement. To this purpose, ciliotoxicity ex vivo studies on sheep nasal mucosa were conducted, monitoring for ciliary or epithelial damage, necrosis or hemorrhage in response to nanoparticle-mediated drug delivery [77]. Interestingly, nanoparticle formulation may decrease local nasal toxicity and improve safety at effective dosages. In rats, mucosa irritation was assessed after intranasal administration of free-diazepam solution compared with an aqua-triggered in-situ gelling microemulsion containing diazepam. While the free-diazepam led to mild to moderate histopathological mucosal lesions, the gelling microemulsion left the mucosa intact [107]. Thus, if a drug is known to cause local mucosal irritation, it may be possible to find carriers that can deliver the drug without local side effects [134].

Besides the adverse effects on mucosa and cilia, it is crucial to ensure that there are no effects on the olfactory nerve and the CNS. To predict effects on neuronal cells, in vitro assays using neuroblastoma cell lines such as SH-SY5Y cells or primary neuronal cells, can be utilized [135, 136]. Lactate dehydrogenase, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) or MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assays can be employed to assess cell

viability and cytotoxicity in response to different drugs. More advanced models would also include models of blood–brain barrier penetration, like transwell or microfluidic models [137]. In addition, computational models could be used to predict CNS side effects [138]. General health and potential adverse effects in the CNS can be preclinically assessed in laboratory animals using the modified Irwin screen or the functional observation battery [139]. Moreover, preclinical evaluation includes neurotoxicity studies in rodents using histopathological evaluation of brain and nerve tissue to check markers for neuroinflammation or cytotoxicity. Neuroinflammation and neurotoxicity could lead to deficits in cognition or olfaction [140, 141]. Nonclinical CNS safety assessments of chronic nose-to-brain drug delivery should therefore incorporate evaluations of cognitive and olfactory functions in laboratory animals, as the olfactory bulb and prefrontal cortex, which play key roles in olfaction and cognition, respectively, are anatomically proximal to the intranasal administration site. Relevant behavioral tests include the buried food or olfactory habituation/dishabituation tests for assessing olfactory function [142], as well as tasks probing executive functions such as decision-making and inhibitory control (delay discounting, five-choice serial reaction time task) [143, 144], working memory (delayed alternation task) [145], attention (attentional set-shifting task) [146], and behavioral flexibility (reversal learning task) [147], which are heavily dependent on prefrontal cortex activity.

Clinical relevance of nose-to-brain delivery and pre-clinical studies

Neurodegenerative diseases are one of the leading causes of death worldwide with an increasing prevalence in the aging society [148]. Although first slight improvements were shown for pathology-targeting antibody therapy, there is a lack of efficient disease-modifying treatment options due to incomplete knowledge of the pathophysiology and the challenges to overcome the blood–brain barrier. Similarly, treatment of debilitating neurological diseases such as epilepsy or migraine is hampered by insufficient drug levels in the brain and by side effects. Despite the success in preclinical studies, translation of nose-to-brain efficacy to the clinics remains challenging. Nevertheless, over the last twenty years there has been an increasing number of clinical studies evaluating nose-to-brain delivery as a novel option for the treatment of neurodegenerative and neurological diseases. The following sections will summarize current pre-clinical drug development and clinical trials, as well as successfull applications for nose-to-brain drug delivery.

Drug development for nose-to-brain delivery for PD

PD is the second most common neurodegenerative disease and the most common movement disorder worldwide, affecting more than 1% of the population over the age of 60 [149–151]. In addition to the classic motor symptoms such as rigor, tremor and postural instability, patients also show non-motor symptoms like cognitive deficits, anxiety and gastrointestinal dysfunction [152, 153]. Symptoms like nausea, dysphagia and delayed gastric emptying make alternatives to oral treatment even more necessary [154, 155]. The symptoms are based on a complex pathology that includes degeneration of dopaminergic neurons in the substantia nigra [156] and a striatal loss of dopamine [157], as well as accumulation and aggregation of alpha-synuclein [158], the main component of Lewy bodies [159], in various regions of the brain [160]. Current PD therapy is limited exclusively to improving hypokinetic, motor symptoms with dopamine substitutes, such as *L*-DOPA, and therefore improves the quality of life of patients for several years, but also leads to the development of uncontrolled, involuntary movements, known as dyskinesia [161, 162]. In addition, this therapy cannot address many motor (e.g., changes in speech) and non-motor disturbances (e.g., memory loss, anxiety disorders, gastrointestinal dysfunctions), or prevent or halt the progressive loss of dopaminergic neurons. Thus, there is no disease-modifying treatment for PD, even though the number of patients continues to increase [152, 163]. There are different treatment approaches in the pipeline of drug development, with some focusing on further improvement of dopamine replacement, while others targeting alpha-synuclein-related pathology [164]. In the following section, we point out some examples for preclinical (Fig. 3) and clinical studies (Table 1) with intranasal delivery for treatment of PD.

(See figure on next page.)

Fig. 3 Preclinical studies using nose-to-brain drug delivery in different rodent models of PD. The Thy1-aSyn mouse model overexpresses human alpha-synuclein under the Thy1 promoter, while the A53T mouse model overexpresses the A53T-mutant human alpha-synuclein under the mouse prion protein promoter. Models generated by injection of different toxins include 6-hydroxydopamine (6-OHDA), which is a synthetic monoaminergic neurotoxin. Haloperidol is an antipsychotic medication that has been observed to induce parkinsonism as an adverse effect. Rotenone is an isoflavonoid that is typically utilized as an insecticide and acaricide. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that selectively targets dopaminergic neurons

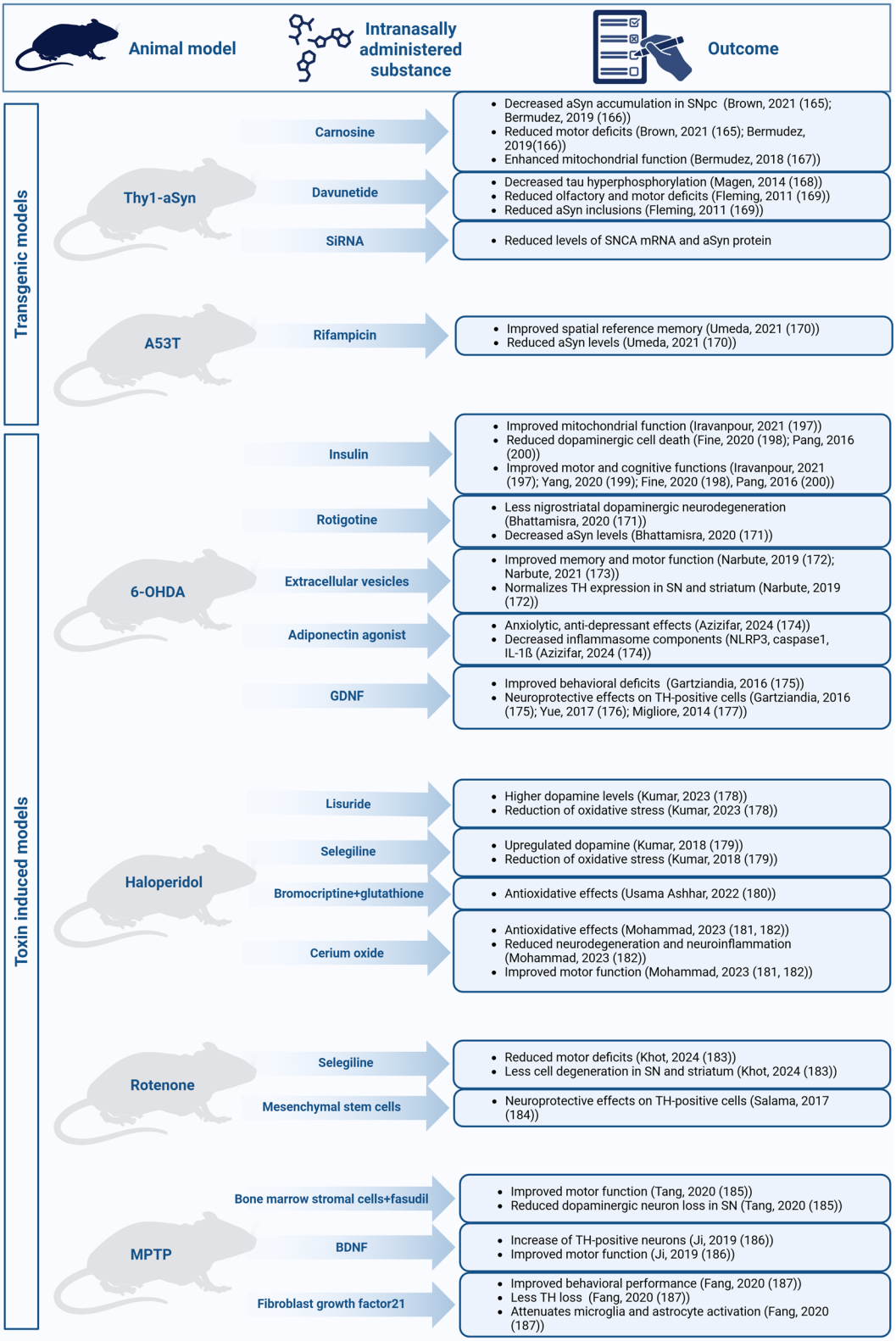

Fig. 3 (See legend on previous page.)

Table 1 Clinical studies using nose-to-brain drug delivery for the treatment of PD

Study title	Treatment	Study number (NCT)	Study Phase	Subject number	Status	Location	Related publication
Treatment of Parkinson Disease and Multiple System Atrophy Using Intranasal Insulin	Insulin vs. placebo	NCT02064166	2	15	Completed	Worcester, Massachusetts, United States, University of Massachusetts Medical School	[205]
The Effect of Intranasal Insulin on Motor and Non-motor Symptoms in Parkinson's Disease Patients	Insulin vs. placebo	NCT04687878	2	40	Recruiting	Tehran, Iran, Shahid Beheshti University of Medical Sciences, Shohada-e-Tajrish Hospital	n.a
Intranasal Insulin in Parkinson's Disease	Novolin R vs. placebo	NCT04251585	2	30	Recruiting	Saint Paul, Minnesota, United States, HealthPartners Neuroscience Center	n.a
CNS Uptake of Intranasal Glutathione	Reduced glutathione	NCT02324426	1	15	Completed	Seattle, Washington, United States, University of Washington	[210]
Intranasal Glutathione in Parkinson's Disease	Glutathione vs. placebo	NCT01398748	1	34	Completed	Kenmore, Washington, United States, Bastyr Clinical Research Center	[209]
Phase IIb Study of Intranasal Glutathione in Parkinson's Disease	Reduced glutathione 100 vs. 200 mg vs. placebo	NCT02424708	2	45	Completed	Kenmore, Washington, United States, Bastyr Clinical Research Center Seattle, Washington, United States, University of Washington	[211]
Intranasal Insulin and Glutathione as an Add-On Therapy in Parkinson's Disease	Novolin R + glutathion vs. matched placebo	NCT05266417	2	56	Recruiting	Davie, Florida, United States, Institute for Neuroimmune Medicine	n.a
Intranasal Human FGF-1 for Subjects With Parkinson's Disease	Human FGF-1 450 vs. 900 µg	NCT05493462	1	4	Not Yet Recruiting	Nassau, Bahamas, The Medical Pavilion Bahamas	n.a
Therapeutic Potential for Intranasal Levodopa in Parkinson's Disease -Off Reversal	*L*-dopa 35 mg vs. 70 mg vs. 140 mg vs. *L*-dopa 70 mg + carbidopa 70 mg vs. placebo	NCT03541356	2	32	Completed	Sydney, New South Wales, Australia, The Brain and Mind Centre/Scientia Clinical Research Brisbane; Queensland, Australia Q-Pharm Brisbane; Queensland, Australia The Mater Hospital Melbourne; Victoria, Australia, The Alfred Hospital Perth; Western Australia, Australia, Perron Institute	n.a
Tolerance to NeuroEPO in Parkinson Disease	NeuroEPO vs. placebo	NCT04110678	1/2	26	Completed	Havana, Cuba, Clinic of Movement Disorders, International Center for Neurological Restoration; La Habana, Cuba, Centro Immunologia Molecular CIM	[128, 215]
Parkinson's Disease Therapy Using Cell Technology	Autologous mesenchymal stem cells vs. placebo	NCT04146519	2 + 3	50	Recruiting	Minsk, Belarus, The Belarusian Medical Academy of Postgraduate Education	[216]

Rotigotine is a dopamine agonist, which has promising potential for the treatment of PD [188], but its use is challenging due to the low bioavailability and high first-pass effects after oral administration, as well as application-site reactions after transdermal treatment [189]. Nose-to-brain delivery could be an efficient tool to improve the bioavailability of rotigotine. A pharmacokinetic study revealed that intranasal delivery of rotigotine-loaded nanoparticles achieves higher brain levels than intravenous rotigotine application [87]. In line with these results, another study showed higher tyrosine-hydroxylase signal in nigrostriatal dopaminergic neurons in the 6-hydroxydopamine (6-OHDA) rat model of PD after intranasal administration of lactoferrin-modified rotigotine nanoparticles [190].

First-line therapy for PD includes monoamine oxidase B inhibitors, such as selegiline, as they reduce metabolic degradation of dopamine and its replacements. However, only 10% of the oral selegiline dose is bioavailable, leading to the need of high, daily doses causing a long list of adverse effects [191]. Preclinical studies have shown higher bioavailability as well as reduced motor deficits after intranasal administration of selegiline in a rotenone PD rat model [192, 193]. Further, intranasal application of selegiline nanoemulsion has led to increased dopamine in the brains of rats [179].

Metabolic dysfunctions, including accumulation of lipids like polyunsaturated fatty acids and cholesterol, are involved in the misfolding and aggregation of alpha-synuclein [194]. In addition, abnormal binding of alpha-synuclein to oxidized lipid metabolites causes malfunction of mitochondria [195]. Accordingly, one approach to treating PD is targeting lipid metabolic abnormalities [196]. As antidiabetic drugs like insulin can regulate lipid metabolism, they have been explored in preclinical and clinical studies for PD. As mentioned before, insulin distributes along the trigeminal nerve and reaches the CNS after intranasal application in rats [14]. Several preclinical studies showed that intranasal insulin treatment leads to an improvement in mitochondrial functions as well as a reduction of dopaminergic cell death in a rat 6-OHDA model of PD [197, 198]. Intranasal insulin treatment also attenuates motor and cognitive deficits in the 6-OHDA rat model of PD [197–200].

With increasing attention paid to gene therapies, RNA interference has appeared as an interesting therapeutic approach to reducing alpha-synuclein and its downstream pathology. Small interfering RNAs (siRNAs) can be delivered to the brain more effectively via the nose than through the intravenous route [46]. In the alpha-synuclein-overexpressing Thy-1-aSyn mice [201, 202], intranasally administered siRNA-loaded polymeric nanoparticles are able to reach different brain regions including the substantia nigra, and significantly reduce *SNCA* mRNA expression as well as alpha-synuclein protein level in the brain [203]. Thus, intranasal administration using nanoparticles could provide a non-invasive route to chronically apply small nucleotides to the brain.

In PD, about half of the clinical trials using the intranasal drug delivery method focus on treatment with either insulin or glutathione, or a combination of both. Besides the beneficial effects of intranasal insulin in preclinical studies, a case study described a patient with manganese-induced parkinsonism whose motor and cognition symptoms improved after four weeks of intranasal treatment with insulin [204]. Furthermore, a clinical proof-of-concept study for intranasal insulin including 16 subjects with clinically diagnosed PD or multiple system atrophy [205] confirmed the safety and showed improvement of motor and cognitive symptoms. Other clinical trials in study phase II are running to test efficacy with an increased number of patients (Table 1).

Glutathione, which plays a protective role against oxidative stress, mitochondrial dysfunction and cell death [206, 207], is depleted in PD patients [208]. Clinical trials have been conducted to restore the glutathione levels in PD patients by delivering glutathione intranasally. Two different phase I studies have confirmed the safety and CNS uptake of glutathione after intranasal administration [209, 210]. The following phase IIb study showed symptomatic improvement after three months of intranasal administration, although after a wash-out period of four weeks, glutathione was not superior to placebo [211]. Of note, a survey revealed that over 86% of patients using glutathione nasal spray rated this route of administration as convenient and easy to use, supporting good compliance [212]. In the context of intranasal delivery of glutathione, the MAD Nasal™ mucosal atomization device (MAD; Teleflex, Morrisville, NC) has been used in a randomized, double-blind phase I/IIa study [209]. The MAD is a syringe equipped with a soft, conical nozzle that forms a seal with the nostril, thereby preventing expulsion of the drug. The liquid drug is atomized into particles ranging from 30 to 100 µm [213]. For clinical studies involving intranasal insulin, the ViaNase™ delivery device (Kurve Technology, Inc. Lynnwood, WA) has been used [205]. ViaNase™ is an electronic atomizer that nebulizes a metered dose of the drug into a chamber covering the patients nose. Patients then inhale the drug by breathing regularly for a predetermined time.

As shown in Table 1, there are further studies using various drugs for intranasal therapy in PD patients. For example, a study achieved positive effects on cognition by treating PD patients with intranasal recombinant human erythropoietin [214].

Drug development for nose-to-brain delivery for AD

AD is the most common cause of dementia worldwide with an increasing prevalence in the aging society. AD has two hallmark pathologies: extracellular beta-amyloid plaques [217] and neurofibrillary tangles composed of hyperphosphorylated tau [218]. The most prominent clinical symptoms are progressive memory deterioration, disordered behavior, and impairments in language, comprehension and visual-spatial skills [219]. There is no cure for AD, and current treatment options are mainly limited to symptomatic management. Recently, the U.S. Food & Drug Administration (FDA) has approved application of plaque-reducing antibodies, which is hampered by severe side effects in a subgroup of patients.

An extensively studied approach for the treatment of AD is intranasal insulin, which is already being tested in clinical trials. Intranasal insulin restores the cerebral glucose metabolism and mitigates astroglial activation and neuronal loss in a streptozotocin-induced AD rat model [220]. Further, intranasal insulin in rats restored levels of different AD-related, dysregulated microRNAs and decreased tau phosphorylation, amyloid-beta aggregation and neuroinflammation [221]. Moreover, intranasal insulin ameliorated memory and learning deficits in AD rat models in different preclinical studies [221–223].

Furthermore, researchers aim to slow the progression of neurodegeneration. Several AD mouse models (cholinotoxin-induced, amyloid-beta-induced [224] and transgenic APP/PS1 mice) showed improvement of memory function and spatial cognition after receiving intranasal application of colivelin [225, 226], a synthetic derivate of humanin that plays a role in suppressing neuronal death [227]. Furthermore, intranasal application of basic fibroblast growth factor leads to improved memory in a rat model of AD [81].

The use of stem cell transplantation for the treatment of neurodegenerative diseases is very promising, albeit limited by the blood–brain barrier. Intranasal delivery could make it more feasible in the future. Repetitive intranasal application of human neural stem cells decreases neuroinflammation, and enhances neurogenesis and expression of beta-amyloid-degrading enzymes in a APP/PS1 mouse model of AD [228]. Also, intranasal administration of the secretome of cortical neural stem cells to 5×FAD mice reduces amyloid-beta accumulation and ameliorates memory function [229]. Plaque reduction, alleviation of gliosis, and increased neuronal density in certain brain regions have been seen in an AD mouse model after intranasal delivery of mesenchymal stem cell secretome [230].

Intranasal siRNA application is also a promising approach for AD. Intranasal administration of siRNA targeting the β-site amyloid precursor protein cleaving enzyme 1 (*BACE1*) combined with rapamycin, an approved immunosuppressant, led to a reduction in amyloid-beta deposition and improvement of cognition in a transgenic AD mouse model [231]. Another preclinical study combined siRNA targeting *BACE1* and siRNA targeting caspase-3, to inhibit neuronal apoptosis in 3×Tg-AD mice. They constructed lesion-recognizing siRNA-nanoparticles for intranasal administration. Results showed positive treatment effects on memory deficits in the 3×Tg-AD mice [232].

Nose-to-brain delivery has been used in several clinical trials for AD over the last decades. A number of clinical studies have explored the potential of intranasal insulin application for AD, showing that insulin could reduce amyloid plaques and improve verbal memory [233, 234]. Past clinical trials employed various dosages, ranging from single-dose to long-term administration, and used different types of insulin, including fast-acting insulin aspart [235], fast-acting insulin glulisin [236], regular human insulin [237] and the long-acting analog insulin detemir [238]. In these studies, insulin aspart showed superiority to regular insulin in terms of treatment efficacy [235]. Several studies confirmed the safety of intranasal insulin in patients with mild cognitive impairment and AD [67, 239]. Recent evidence suggests that intranasal insulin treatment increases the volume of certain brain regions, which is associated with memory improvement [240]. Intranasal insulin also leads to changes in inflammatory markers in the CSF, suggesting that intranasal insulin may not only treat symptoms but also influence the progression of AD [241]. On the other hand, there are studies showing no significant treatment effects, which could be due to a small number of subjects [239] or due to inadequate delivery devices [67]. Pilot studies on intranasal insulin in AD patients and older adults at high dementia risk using the previously mentioned ViaNase™ for drug delivery, showed sufficient insulin penetration into the brain via CSF analyses [237, 242]. Nevertheless, the trial-specific modified device demonstrated insufficient reliability in a clinical study, necessitating its mid-trial replacement with the Precision Olfactory Delivery (POD®) device (Impel Pharmaceuticals, Seattle, WA) [67]. The POD® uses a liquid fluorocarbon propellant to inject a metered dose of liquids or powders to the olfactory epithelium without electronic assistance [69]. In addition, clinical trials are currently ongoing for intranasal stem cell treatment of AD. As mentioned above, preclinical studies in this field have shown promising results. The challenge is now to transfer these findings from preclinical to clinical feasibility. Two studies investigating intranasal administration of bone marrow-derived stem cells in 100 participants

(NCT03724136) and 500 participants (NCT02795052), respectively, are currently in progress and expected to be completed in July 2025.

Drug development for nose-to-brain delivery for epilepsy

Epilepsy is a chronic neurological disorder characterized by recurrence of seizures of central origin. Those seizures are the result of the interaction between pathological excitation and a lack of inhibition in the neuronal networks of the CNS. Current antiseizure medications do not cure epilepsy. They merely manage seizures and must be taken continuously, leading to lifelong exposure of the entire body to the drug, necessitating high safety standards. Although various antiseizure medications have been approved and most patients respond well to the therapy, about 30% of patients do not become seizure-free under currently available treatment [243, 244]. One possible cause is the insufficient drug levels in the brain. Therefore, strategies like acute and chronic intracerebral microinfusion via drug pumps were tested in a rat seizure model [244, 245]. Even though the studies showed beneficial effects in preclinical models, it is important to consider alternative, less invasive delivery routes. Non-invasive approaches such as nose-to-brain delivery may help achieve higher drug levels in the CNS while minimizing systemic exposure [126]. Another challenge in the treatment of epilepsy is to find an optimal application route to stop acute seizures. Emergency treatment in epilepsy patients is generally administered orally or intravenously, if the patient can swallow during the seizure or if a medically-trained person is available to inject intravenously. Therefore, intranasal emergency treatment could be an option to ease the administration especially in a non-medical setting at home and thereby accelerate treatment [246].

To maximize the effects of lamotrigine, a seizure-suppressing substance approved for oral application, researchers developed lamotrigine-containing nanocapsules or nanoparticles for intranasal use. By now, there are a few studies on the pharmacokinetics and bioavailability of intranasal lamotrigine in rats and mice, showing promising results with high brain-targeting efficacy [247, 248], but further research has to be done especially regarding efficacy. Another orally-administered seizure-suppressing drug carbamazepine has also been tested for intranasal delivery. Intranasal application of a mucoadhesive carbamazepine nanoemulgel to pentylenetetrazole-treated mice delayed the onset of convulsion and death compared to intravenously-injected animals [249]. The use of neuropeptides is another approach in the treatment of epilepsy. Intranasal treatment with nanoparticles containing thyrotropin-releasing hormone significantly reduced the seizure afterdischarge duration and increased the number of stimulations required to reach a generalized tonic–clonic seizure in a kindling model of temporal lobe epilepsy [250].

Benzodiazepines, including lorazepam, diazepam, and midazolam, are the most commonly used substances for acute seizure management. When administered intranasally in rats, a thermosensitive gel containing lorazepam-loaded nanostructured lipid carriers, reduced the prevalence of pentylenetetrazole-induced seizures by two-thirds and decreased the severity and duration of symptoms compared to the sham group [251]. Moreover, intranasal mucaoadhesive clobazam microemulsion showed enhanced brain uptake in a pentylenetetrazole-induced mouse model compared to intravenous clobazam, leading to a faster increase in seizure threshold [111].

Based on the promising preclinical studies, intranasal benzodiazepines were tested in human patients and approved for the treatment of acute seizures (Fig. 4). The first FDA-approved nasal treatments for acute seizures are midazolam (NAYZILAM) and diazepam (VALTOCO) nasal sprays [252]. In line with this, the European Medicines Agency (EMA) approved midazolam nasal spray (NASOLAM) in 2022 [253]. A retrospective study confirmed the efficacy and safety of midazolam nasal spray in humans [254], indicating it as a promising application route. For intranasal application in acute seizure management, it is important that the delivery device is user-friendly and does not require any active participation from the patient. Consequently, conventional unidose nasal sprays are commonly used in non-professional settings, whereas MADs are typically used by healthcare professionals for emergency treatment [255, 256].

Drug development for nose-to-brain delivery for migraine

Migraine is an episodic form of chronic headache that occurs in attacks, often accompanied by nausea and vomiting. There are different classifications of migraine by the International Headache Society Headache Classification Committee, such as migraine with or without aura [257]. An aura manifests itself, for example, in the form of very different neurological symptoms such as visual field defects, paresthesia and paresis [258, 259]. Migraine affects more than one billion people of all ages worldwide [260] and significantly reduces their quality of life with an impact on social life and work [261]. Women are three times more likely to be affected than men [262]. The pathophysiology is still not completely understood as it is a complex disorder of nervous system function including genetic causes and the influence of neuropeptides [263]. Therefore, treatment consists mainly of acute medication for pain relief, while preventive treatment is not very established. Oral application of triptans

like sumatriptan, zolmitriptan and rizatriptan is used most frequently. Despite the established migraine treatment with oral triptans, there is a need for optimization of the route of administration. After oral administration, triptans need to be absorbed from the gastrointestinal tract to reach the blood circulation and have to cross the blood–brain barrier to get to the CNS. On the way to the brain, a high amount of the drug gets lost, through first-pass metabolism of the liver, leading to the fact that high doses are required. Migraine patients often have gastro-intestinal complaints such as diarrhea, nausea or vomiting, which can occur as a symptom of the migraine or as an adverse side effect of treatment [264]. Nevertheless, low oral bioavailability of these compounds makes alternative delivery options for brain targeting necessary. In the following, we highlight preclinical studies investigating nose-to-brain delivery of triptans as well as clinical studies that showed efficiency for approved intranasal migraine treatment.

Solid lipid nanoparticles of sumatriptan succinate optimized for brain targeting showed fast permeation across nasal mucosa in an ex vivo study using goat nasal mucosa, without altering the integrity of the mucosa [265]. Furthermore, a pharmacokinetic study in rats showed higher brain levels after intranasal delivery of lipid sumatriptan nanoparticles compared to intravenous application [266]. A sumatriptan-loaded nano-ethosomal mucoadhesive gel showed beneficial effects on behavioral as well as biochemical parameters in a nitroglycerin-induced migraine rat model [267]. Different pharmacokinetic studies in rats and mice demonstrated that zolmitriptan reaches the brain faster and in greater amounts when administered intranasally compared to intravenously [114, 268].

For the treatment of migraine, there are already approved triptan nasal sprays on the market (Fig. 4). Sumatriptan nasal spray was approved by the EMA in 1996 under the trading name "Imigran nasal" and by the FDA in 2019 under the name "Tosymra". Sumatriptan nasal powder has a better outcome in the reduction of nausea compared to oral sumatriptan in human patients, indicating the advantages of nasal treatment [269]. Moreover, zolmitriptan was also approved by the EMA in 2002 and by the FDA in 2003 under the name "Zomig". Zolmitriptan nasal spray is superior to zolmitriptan tablets, with rapid onset of headache relief (only 15 min after dosing) [270]. Although zolmitriptan nasal spray has already been approved, efforts are still being made to further optimize the marketed nasal spray by using different kinds of nanocarriers like chitosan nanoparticles, nanoethosomes or novasomes [268, 271, 272]. Proper drug delivery devices play a crucial role in ensuring effective treatment. Most approved triptan nasal sprays use Advaspray®, a Unidose liquid nasal spray (Aptar, Crystal Lake, IL), which is user-friendly but does not explicitly target the upper nasal space [213, 273]. To improve drug delivery to the upper nasal cavity, particularly the olfactory epithelium, advanced bidirectional breath-powered delivery devices are used. One such approved delivery system is ONZETRA® Xsail® (Currax Pharmaceuticals, Brentwood, TN), which administers sumatriptan nasal powder [274]. Besides enhanced deposition to the upper nasal space, oral exhalation during application closes the soft palate, thereby preventing lung deposition [275]. However, one drawback of these advanced devices is that they require a certain level of patient compliance and are less convenient to use than traditional nasal spray.

Conclusion

Nose-to-brain delivery provides a promising option to circumvent the blood–brain barrier in a non-invasive route suitable for a long-term, repetitive and easy-to-apply regimen. Nasal application is proven to be safe or even enhance safety of drugs compared to systemic application in preclinical and clinical studies. Moreover, many studies have shown superior delivery efficacy of nasal applications compared to other application types. Nevertheless, the development of specific nasal delivery devices that target primarily the olfactory region for high delivery efficacy is challenging, currently limiting the application to potent drugs. Furthermore, a better understanding of the precise delivery routes and uptake mechanisms

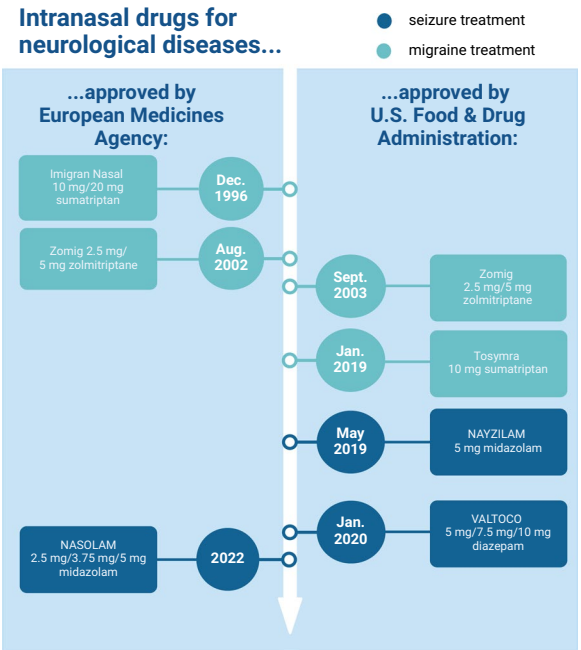

Fig. 4 Approved drugs using nose-to-brain delivery for the treatment of neurological diseases

will enable improvement of strategies, such as optimizing carrier systems and targeting specific brain regions or cells. In conclusion, the nose-to-brain delivery is a promising, innovative form of application with enormous implications for treating CNS diseases.

Abbreviations

6-OHDA	6-Hydroxydopamine
AD	Alzheimer's disease
BACE1	β-Site amyloid precursor protein cleaving enzyme 1
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
CSF	Cerebrospinal fluid
EMA	European medicines agency
FGF-1	Fibroblast growth factor 1
MAD	Mucosal atomization device
PD	Parkinson's disease
siRNA	Small interfering RNA

Acknowledgements
We acknowledge financial support by the Open Access Publication Fund of the University of Veterinary Medicine Hannover, Foundation. Figures 1, 2, 3 und 4 were created with BioRender.

Author contributions
ID wrote the original draft and prepared the figures. MF and FR reviewed, edited and approved the final manuscript.

Funding
Open Access funding enabled and organized by Projekt DEAL. This review was funded by intramural funds with no role in writing of the manuscript.

Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 12 December 2024 Accepted: 18 March 2025
Published online: 19 May 2025

References

1. Kiran P, Debnath SK, Neekhra S, Pawar V, Khan A, Dias F, et al. Designing nanoformulation for the nose-to-brain delivery in Parkinson's disease: advancements and barrier. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2022;14(1):e1768.
2. Dahl R, Mygind N. Anatomy, physiology and function of the nasal cavities in health and disease. Adv Drug Deliv Rev. 1998;29(1–2):3–12.
3. Pires A, Fortuna A, Alves G, Falcão A. Intranasal drug delivery: how, why and what for? J Pharm Pharm Sci. 2009;12(3):288–311.
4. Schuenke M, Schulte E, Schumacher U, Ross LM, Lamperti ED, Taub E, editors. Thieme Atlas of Anatomy: Head and Neuroanatomy. Stuttgart, Germany: Thieme; 2010.
5. Sasaki K, Fukakusa S, Torikai Y, Suzuki C, Sonohata I, Kawahata T, et al. Effective nose-to-brain drug delivery using a combination system targeting the olfactory region in monkeys. J Control Release. 2023;359:384–99.
6. Gizurarson S. Anatomical and histological factors affecting intranasal drug and vaccine delivery. Curr Drug Deliv. 2012;9(6):566–82.
7. Spera I, Cousin N, Ries M, Kedracka A, Castillo A, Aleandri S, et al. Open pathways for cerebrospinal fluid outflow at the cribriform plate along the olfactory nerves. EBioMedicine. 2023;91:104558.
8. Faber WM. The nasal mucosa and the subarachnoid space. Am J Anat. 1937;62(1):121–48.
9. Gopinath P, Gopinath G, TC AK. Target site of intranasally sprayed substances and their transport across the nasal mucosa: a new insight into the intranasal route of drug-delivery. Curr Therapeut Res. 1978;23(5):596–607.
10. Norwood JN, Zhang Q, Card D, Craine A, Ryan TM, Drew PJ. Anatomical basis and physiological role of cerebrospinal fluid transport through the murine cribriform plate. Elife. 2019;8.
11. Kumar NN, Gautam M, Lochhead JJ, Wolak DJ, Ithapu V, Singh V, et al. Relative vascular permeability and vascularity across different regions of the rat nasal mucosa: implications for nasal physiology and drug delivery. Sci Rep. 2016;6:31732.
12. Schroeter JD, Kimbell JS, Gross EA, Willson GA, Dorman DC, Tan YM, et al. Application of physiological computational fluid dynamics models to predict interspecies nasal dosimetry of inhaled acrolein. Inhal Toxicol. 2008;20(3):227–43.
13. Gross EA, Swenberg JA, Fields S, Popp JA. Comparative morphometry of the nasal cavity in rats and mice. J Anat. 1982;135(Pt 1):83–8.
14. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Adv Drug Deliv Rev. 2012;64(7):614–28.
15. Moran DT, Rowley JC 3rd, Jafek BW, Lovell MA. The fine structure of the olfactory mucosa in man. J Neurocytol. 1982;11(5):721–46.
16. Frisch D. Ultrastructure of mouse olfactory mucosa. Am J Anat. 1967;121(1):87–120.
17. Graziadei PP. Cell dynamics in the olfactory mucosa. Tissue Cell. 1973;5(1):113–31.
18. Moran DT, Rowley JC 3rd, Jafek BW. Electron microscopy of human olfactory epithelium reveals a new cell type: the microvillar cell. Brain Res. 1982;253(1–2):39–46.
19. Geurkink N. Nasal anatomy, physiology, and function. J Allergy Clin Immunol. 1983;72(2):123–8.
20. Proctor DF, Andersen IHP. The nose: upper airway physiology and the atmospheric environment. Amsterdam: Elsevier Biomedical Press; 1982.
21. Tchudnosovetof VA. The influence of types of breathing on the injection of the mucosa of the nose after subarachnoidal iniroduction of china-ink. Acta Otolaryngol. 1934;21:199–218.
22. Dahlin M, Bergman U, Jansson B, Björk E, Brittebo E. Transfer of dopamine in the olfactory pathway following nasal administration in mice. Pharm Res. 2000;17(6):737–42.
23. Correa D, Scheuber MI, Shan H, Weinmann OW, Baumgartner YA, Harten A, et al. Intranasal delivery of full-length anti-Nogo-A antibody: a potential alternative route for therapeutic antibodies to central nervous system targets. Proc Natl Acad Sci U S A. 2023;120(4):e2200057120.
24. Dahlin M, Jansson B, Björk E. Levels of dopamine in blood and brain following nasal administration to rats. Eur J Pharm Sci. 2001;14(1):75–80.
25. Brodie M, Elvidge AR. The portal of entry and transmission of the virus of poliomyelitis. Science. 1934;79(2045):235–6.
26. Ueha R, Ito T, Furukawa R, Kitabatake M, Ouji-Sageshima N, Ueha S, et al. Oral SARS-CoV-2 inoculation causes nasal viral infection leading to olfactory bulb infection: an experimental study. Front Cell Infect Microbiol. 2022;12:924725.
27. Sawamura M, Ohira J, Hikawa R, Ishimoto T, Nakanishi E, Yamakado H, et al. Single-dose intranasal administration of α-syn PFFs induce lewy neurite-like pathology in olfactory bulbs. Parkinsonism Relat Disord. 2023;112:105440.
28. Danielyan L, Schäfer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, et al. Intranasal delivery of cells to the brain. Eur J Cell Biol. 2009;88(6):315–24.
29. Thorne RG, Emory CR, Ala TA, Frey WH 2nd. Quantitative analysis of the olfactory pathway for drug delivery to the brain. Brain Res. 1995;692(1–2):278–82.
30. Broadwell RD, Balin BJ. Endocytic and exocytic pathways of the neuronal secretory process and trans-synaptic transfer of wheat

germ agglutinin-horseradish peroxidase in vivo. J Comp Neurol. 1985;242(4):632–50.
31. Renner DB, Frey WH 2nd, Hanson LR. Intranasal delivery of siRNA to the olfactory bulbs of mice via the olfactory nerve pathway. Neurosci Lett. 2012;513(2):193–7.
32. Baker H, Spencer R. Transneuronal transport of peroxidase-conjugated wheat germ agglutinin (WGA-HRP) from the olfactory epithelium to the brain of the adult rat. Exp Brain Res. 1986;63:461–73.
33. Kristensson K, Olsson Y. Uptake of exogenous proteins in mouse olfactory cells. Acta Neuropathol. 1971;19(2):145–54.
34. Buchner K, Seitz-Tutter D, Schönitzer K, Weiss DG. A quantitative study of anterograde and retrograde axonal transport of exogenous proteins in olfactory nerve C-fibers. Neuroscience. 1987;22(2):697–707.
35. Sakane T, Akizuki M, Yoshida M, Yamashita S, Nadai T, Hashida M, et al. Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. J Pharm Pharmacol. 1991;43(6):449–51.
36. Zhang QZ, Zha LS, Zhang Y, Jiang WM, Lu W, Shi ZQ, et al. The brain targeting efficiency following nasally applied MPEG-PLA nanoparticles in rats. J Drug Target. 2006;14(5):281–90.
37. Sakane T, Yamashita S, Yata N, Sezaki H. Transnasal delivery of 5-fluorouracil to the brain in the rat. J Drug Target. 1999;7(3):233–40.
38. Erlich SS, McComb JG, Hyman S, Weiss MH. Ultrastructural morphology of the olfactory pathway for cerebrospinal fluid drainage in the rabbit. J Neurosurg. 1986;64(3):466–73.
39. Walter BA, Valera VA, Takahashi S, Ushiki T. The olfactory route for cerebrospinal fluid drainage into the peripheral lymphatic system. Neuropathol Appl Neurobiol. 2006;32(4):388–96.
40. Kumar NN, Lochhead JJ, Pizzo ME, Nehra G, Boroumand S, Greene G, et al. Delivery of immunoglobulin G antibodies to the rat nervous system following intranasal administration: Distribution, dose-response, and mechanisms of delivery. J Control Release. 2018;286:467–84.
41. Nair SC, Vinayan KP, Mangalathillam S. Nose to brain delivery of phenytoin sodium loaded nano lipid carriers: formulation, drug release, permeation and in vivo pharmacokinetic studies. Pharmaceutics. 2021;13(10):1640.
42. Schuenke M, Schulte E, Schumacher U, Stefan C. Thieme Atlas of Anatomy: Head, Neck, and Neuroanatomy, 3rd ed. New York: Thieme; 2020.
43. Bojsen-Møller F. Demonstration of terminalis, olfactory, trigeminal and perivascular nerves in the rat nasal septum. J Compar Neurol. 1975;159(2):245–56.
44. Schaefer ML, Böttger B, Silver WL, Finger TE. Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. J Comp Neurol. 2002;444(3):221–6.
45. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience. 2004;127(2):481–96.
46. Kanazawa T, Akiyama F, Kakizaki S, Takashima Y, Seta Y. Delivery of siRNA to the brain using a combination of nose-to-brain delivery and cell-penetrating peptide-modified nano-micelles. Biomaterials. 2013;34(36):9220–6.
47. Akita T, Oda Y, Kimura R, Nagai M, Tezuka A, Shimamura M, et al. Involvement of trigeminal axons in nose-to-brain delivery of glucagon-like peptide-2 derivative. J Control Release. 2022;351:573–80.
48. Lochhead JJ, Kellohen KL, Ronaldson PT, Davis TP. Distribution of insulin in trigeminal nerve and brain after intranasal administration. Sci Rep. 2019;9(1):2621.
49. Spindler LM, Feuerhake A, Ladel S, Günday C, Flamm J, Günday-Türeli N, et al. Nano-in-micro-particles consisting of PLGA nanoparticles embedded in chitosan microparticles via spray-drying enhances their uptake in the olfactory mucosa. Front Pharmacol. 2021;12:732954.
50. Akita T, Shimamura M, Tezuka A, Takagi M, Yamashita C. GLP-1 derivatives with functional sequences transit and migrate through trigeminal neurons. Eur J Pharm Biopharm. 2024;195:114176.
51. Li Y, Wang C, Zong S, Qi J, Dong X, Zhao W, et al. The trigeminal pathway dominates the nose-to-brain transportation of intact polymeric nanoparticles: evidence from aggregation-caused quenching probes. J Biomed Nanotechnol. 2019;15(4):686–702.
52. Frey WH, Liu J, Chen X, Thorne RG, Fawcett JR, Ala TA, et al. Delivery of 125I-NGF to the brain via the olfactory route. Drug Delivery. 1997;4(2):87–92.
53. Ye D, Chen H. Focused ultrasound-mediated intranasal brain drug delivery technique (FUSIN). Methods Mol Biol. 2022;2394:501–13.
54. Xi J, Yuan JE, Alshaiba M, Cheng D, Firlit Z, Johnson A, et al. Design and testing of electric-guided delivery of charged particles to the olfactory region: experimental and numerical studies. Curr Drug Deliv. 2016;13(2):265–74.
55. Flamm J, Hartung S, Gänger S, Maigler F, Pitzer C, Schindowski K. Establishment of an olfactory region-specific intranasal delivery technique in mice to target the central nervous system. Front Pharmacol. 2021;12:789780.
56. Krishnan JKS, Arun P, Chembukave B, Appu AP, Vijayakumar N, Moffett JR, et al. Effect of administration method, animal weight and age on the intranasal delivery of drugs to the brain. J Neurosci Methods. 2017;286:16–21.
57. Maigler F, Ladel S, Flamm J, Gänger S, Kurpiers B, Kiderlen S, et al. Selective CNS targeting and distribution with a refined region-specific intranasal delivery technique via the olfactory mucosa. Pharmaceutics. 2021;13(11):1904.
58. Li B, Feng Y. In silico study to enhance delivery efficiency of charged nanoscale nasal spray aerosols to the olfactory region using external magnetic fields. Bioengineering. 2022;9(1):40.
59. Chen H, Chen CC, Acosta C, Wu SY, Sun T, Konofagou EE. A new brain drug delivery strategy: focused ultrasound-enhanced intranasal drug delivery. PLoS One. 2014;9(10):e108880.
60. Pickering AJ, Lamson NG, Marand MH, Hwang W, Straehla JP, Hammond PT. Layer-by-layer polymer functionalization improves nanoparticle penetration and glioblastoma targeting in the brain. ACS Nano. 2023;17(23):24154–69.
61. Guo S, Yi CX. Cell type-targeting nanoparticles in treating central nervous system diseases: challenges and hopes. Nanotechnol Rev. 2023;12(1):20230158.
62. Zhang H, Jiang Y, Zhao SG, Jiang LQ, Meng Y, Liu P, et al. Selective neuronal targeting, protection and signaling network analysis via dopamine-mediated mesoporous silica nanoparticles. MedChemComm. 2015;6(6):1117–29.
63. Israel LL, Sun T, Braubach O, Cox A, Shatalova ES, Rashid HM, et al. β-Amyloid targeting nanodrug for neuron-specific delivery of nucleic acids in Alzheimer's disease mouse models. J Control Release. 2023;361:636–58.
64. Zheng X, Pang X, Yang P, Wan X, Wei Y, Guo Q, et al. A hybrid siRNA delivery complex for enhanced brain penetration and precise amyloid plaque targeting in Alzheimer's disease mice. Acta Biomater. 2017;49:388–401.
65. Xi J, Yuan JE, Zhang Y, Nevorski D, Wang Z, Zhou Y. Visualization and quantification of nasal and olfactory deposition in a sectional adult nasal airway cast. Pharm Res. 2016;33(6):1527–41.
66. Silberstein SD, Shrewsbury SB, Hoekman J. Dihydroergotamine (DHE) - then and now: a narrative review. Headache. 2020;60(1):40–57.
67. Craft S, Raman R, Chow TW, Rafii MS, Sun CK, Rissman RA, et al. Safety, efficacy, and feasibility of intranasal insulin for the treatment of mild cognitive impairment and Alzheimer disease dementia: a randomized clinical trial. JAMA Neurol. 2020;77(9):1099–109.
68. Djupesland PG, Messina JC, Mahmoud RA. Role of nasal casts for in vitro evaluation of nasal drug delivery and quantitative evaluation of various nasal casts. Ther Deliv. 2020;11(8):485–95.
69. Cooper W, Ray S, Aurora SK, Shrewsbury SB, Fuller C, Davies G, et al. Delivery of dihydroergotamine mesylate to the upper nasal space for the acute treatment of migraine: technology in action. J Aerosol Med Pulm Drug Deliv. 2022;35(6):321–32.
70. Shrewsbury SB, Jeleva M, Satterly KH, Lickliter J, Hoekman J. STOP 101: a phase 1, randomized, open-label, comparative bioavailability study of INP104, dihydroergotamine mesylate (DHE) administered intranasally by a I123 precision olfactory delivery (POD(®)) device, in healthy adult subjects. Headache. 2019;59(3):394–409.
71. Smith TR, Winner P, Aurora SK, Jeleva M, Hocevar-Trnka J, Shrewsbury SB. STOP 301: A Phase 3, open-label study of safety, tolerability, and exploratory efficacy of INP104, Precision olfactory delivery (POD(®)))

of dihydroergotamine mesylate, over 24/52 weeks in acute treatment of migraine attacks in adult patients. Headache. 2021;61(8):1214–26.
72. Silberstein S. AVP-825: a novel intranasal delivery system for low-dose sumatriptan powder in the treatment of acute migraine. Expert Rev Clin Pharmacol. 2017;10(8):821–32.
73. Djupesland PG, Skretting A, Winderen M, Holand T. Breath actuated device improves delivery to target sites beyond the nasal valve. Laryngoscope. 2006;116(3):466–72.
74. Obaidi M, Offman E, Messina J, Carothers J, Djupesland PG, Mahmoud RA. Improved pharmacokinetics of sumatriptan with Breath Powered™ nasal delivery of sumatriptan powder. Headache. 2013;53(8):1323–33.
75. Menshutina N, Majouga A, Uvarova A, Lovskaya D, Tsygankov P, Mochalova M, et al. Chitosan aerogel particles as nasal drug delivery systems. Gels. 2022;8(12):796.
76. Wang J, Shi Y, Yu S, Wang Y, Meng Q, Liang G, et al. Intranasal administration of dantrolene increased brain concentration and duration. PLoS ONE. 2020;15(3):e0229156.
77. Rajput A, Butani S. Donepezil HCl liposomes: development, characterization, cytotoxicity, and pharmacokinetic study. AAPS PharmSciTech. 2022;23(2):74.
78. Zhang C, Chen J, Feng C, Shao X, Liu Q, Zhang Q, et al. Intranasal nanoparticles of basic fibroblast growth factor for brain delivery to treat Alzheimer's disease. Int J Pharm. 2014;461(1–2):192–202.
79. Yang JP, Liu HJ, Cheng SM, Wang ZL, Cheng X, Yu HX, et al. Direct transport of VEGF from the nasal cavity to brain. Neurosci Lett. 2009;449(2):108–11.
80. Zolkowska D, Wu CY, Rogawski MA. Intranasal allopregnanolone confers rapid seizure protection: evidence for direct nose-to-brain delivery. Neurotherapeutics. 2021;18(1):544–55.
81. Feng C, Zhang C, Shao X, Liu Q, Qian Y, Feng L, et al. Enhancement of nose-to-brain delivery of basic fibroblast growth factor for improving rat memory impairments induced by co-injection of β-amyloid and ibotenic acid into the bilateral hippocampus. Int J Pharm. 2012;423(2):226–34.
82. Furubayashi T, Inoue D, Kimura S, Tanaka A, Sakane T. Evaluation of the pharmacokinetics of intranasal drug delivery for targeting cervical lymph nodes in rats. Pharmaceutics. 2021;13(9):1363.
83. Sun BL, He MQ, Han XY, Sun JY, Yang MF, Yuan H, et al. Intranasal delivery of granulocyte colony-stimulating factor enhances its neuroprotective effects against ischemic brain injury in rats. Mol Neurobiol. 2016;53(1):320–30.
84. Chauhan MB, Chauhan NB. Brain uptake of neurotherapeutics after intranasal versus intraperitoneal delivery in mice. J Neurol Neurosurg. 2015;2(1):009.
85. Hatakawa Y, Tanaka A, Furubayashi T, Nakamura R, Konishi M, Akizawa T, et al. Direct delivery of ANA-TA9, a peptide capable of Aβ hydrolysis, to the brain by intranasal administration. Pharmaceutics. 2021;13(10):16734.
86. Huang G, Xie J, Shuai S, Wei S, Chen Y, Guan Z, et al. Nose-to-brain delivery of drug nanocrystals by using Ca(2+) responsive deacetylated gellan gum based in situ-nanogel. Int J Pharm. 2021;594:120182.
87. Wang F, Yang Z, Liu M, Tao Y, Li Z, Wu Z, et al. Facile nose-to-brain delivery of rotigotine-loaded polymer micelles thermosensitive hydrogels: in vitro characterization and in vivo behavior study. Int J Pharm. 2020;577:119046.
88. Ahmad S, Khan I, Pandit J, Emad NA, Bano S, Dar KI, et al. Brain targeted delivery of carmustine using chitosan coated nanoparticles via nasal route for glioblastoma treatment. Int J Biol Macromol. 2022;221:435–45.
89. Tanaka A, Furubayashi T, Arai M, Inoue D, Kimura S, Kiriyama A, et al. Delivery of oxytocin to the brain for the treatment of autism spectrum disorder by nasal application. Mol Pharm. 2018;15(3):1105–11.
90. Li F, Feng J, Cheng Q, Zhu W, Jin Y. Delivery of 125I-cobrotoxin after intranasal administration to the brain: a microdialysis study in freely moving rats. Int J Pharm. 2007;328(2):161–7.
91. Lowe S, Sher E, Wishart G, Jackson K, Yuen E, Brittain C, et al. An assessment of the central disposition of intranasally administered insulin lispro in the cerebrospinal fluid of healthy volunteers and beagle dogs. Drug Deliv Transl Res. 2017;7(1):11–5.
92. Kolanjiyil AV, Kleinstreuer C, Kleinstreuer NC, Pham W, Sadikot RT. Mice-to-men comparison of inhaled drug-aerosol deposition and clearance. Respir Physiol Neurobiol. 2019;260:82–94.
93. See GL, Arce F Jr, Dahlizar S, Okada A, Fadli M, Hijikuro I, et al. Enhanced nose-to-brain delivery of tranilast using liquid crystal formulations. J Control Release. 2020;325:1–9.
94. Yarragudi SB, Richter R, Lee H, Walker GF, Clarkson AN, Kumar H, et al. Formulation of olfactory-targeted microparticles with tamarind seed polysaccharide to improve nose-to-brain transport of drugs. Carbohydr Polym. 2017;163:216–26.
95. Yarragudi SB, Kumar H, Jain R, Tawhai M, Rizwan S. Olfactory targeting of microparticles through inhalation and bi-directional airflow: effect of particle size and nasal anatomy. J Aerosol Med Pulm Drug Deliv. 2020;33(5):258–70.
96. Dong J, Shang Y, Inthavong K, Chan HK, Tu J. Numerical comparison of nasal aerosol administration systems for efficient nose-to-brain drug delivery. Pharm Res. 2017;35(1):5.
97. Garcia GJ, Schroeter JD, Kimbell JS. Olfactory deposition of inhaled nanoparticles in humans. Inhal Toxicol. 2015;27(8):394–403.
98. Loftus LT, Li HF, Gray AJ, Hirata-Fukae C, Stoica BA, Futami J, et al. In vivo protein transduction to the CNS. Neuroscience. 2006;139(3):1061–7.
99. Washington N, Steele RJ, Jackson SJ, Bush D, Mason J, Gill DA, et al. Determination of baseline human nasal pH and the effect of intranasally administered buffers. Int J Pharm. 2000;198(2):139–46.
100. Ohwaki T, Ando H, Kakimoto F, Uesugi K, Watanabe S, Miyake Y, et al. Effects of dose, pH, and osmolarity on nasal absorption of secretin in rats II: histological aspects of the nasal mucosa in relation to the absorption variation due to the effects of pH and osmolarity. J Pharm Sci. 1987;76(9):695–8.
101. Yenigun VB, Yenigun A, Sagiroglu AA, Kocyigit A, Ozturan O. Formulation of nasal analgesic sprays with diclofenac sodium, ibuprofen, paracetamol, and evaluation of in vitro toxicity. Sci Prog. 2024;107(4):368504241304200.
102. Romeo VD, deMeireles J, Sileno AP, Pimplaskar HK, Behl CR. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv Drug Deliv Rev. 1998;29(1–2):89–116.
103. Sakane T, Akizuki M, Yamashita S, Nadai T, Hashida M, Sezaki H. The transport of a drug to the cerebrospinal fluid directly from the nasal cavity: the relation to the lipophilicity of the drug. Chem Pharm Bull (Tokyo). 1991;39(9):2456–8.
104. Sakane T, Akizuki M, Taki Y, Yamashita S, Sezaki H, Nadai T. Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the molecular weight of drugs. J Pharm Pharmacol. 1995;47(5):379–81.
105. Gabal YM, Kamel AO, Sammour OA, Elshafeey AH. Effect of surface charge on the brain delivery of nanostructured lipid carriers in situ gels via the nasal route. Int J Pharm. 2014;473(1–2):442–57.
106. Wang JT, Rodrigo AC, Patterson AK, Hawkins K, Aly MMS, Sun J, et al. Enhanced delivery of neuroactive drugs via nasal delivery with a self-healing supramolecular gel. Adv Sci (Weinh). 2021;8(14):e2101058.
107. Bachhav SS, Dighe V, Mali N, Gogtay NJ, Thatte UM, Devarajan PV. Nose-to-brain delivery of diazepam from an intranasal aqua-triggered in-situ (ATIS) gelling microemulsion: monitoring brain uptake by microdialysis. Eur J Drug Metab Pharmacokinet. 2020;45(6):785–99.
108. Uppuluri CT, Ravi PR, Dalvi AV, Shaikh SS, Kale SR. Piribedil loaded thermo-responsive nasal in situ gelling system for enhanced delivery to the brain: formulation optimization, physical characterization, and in vitro and in vivo evaluation. Drug Deliv Transl Res. 2021;11(3):909–26.
109. Hao J, Zhao J, Zhang S, Tong T, Zhuang Q, Jin K, et al. Fabrication of an ionic-sensitive in situ gel loaded with resveratrol nanosuspensions intended for direct nose-to-brain delivery. Colloids Surf B Biointerf. 2016;147:376–86.
110. Dalvi A, Ravi PR, Uppuluri CT. Design and evaluation of rufinamide nanocrystals loaded thermoresponsive nasal in situ gelling system for improved drug distribution to brain. Front Pharmacol. 2022;13:943772.
111. Florence K, Manisha L, Kumar BA, Ankur K, Kumar MA, Ambikanandan M. Intranasal clobazam delivery in the treatment of status epilepticus. J Pharm Sci. 2011;100(2):692–703.
112. Chun IK, Lee YH, Lee KE, Gwak HS. Design and evaluation of levodopa methyl ester intranasal delivery systems. J Parkinsons Dis. 2011;1(1):101–7.

113. Tiozzo Fasiolo L, Manniello MD, Banella S, Napoli L, Bortolotti F, Quarta E, et al. Flurbiprofen sodium microparticles and soft pellets for nose-to-brain delivery: serum and brain levels in rats after nasal insufflation. Int J Pharm. 2021;605:120827.
114. Khan T, Ranjan R, Dogra Y, Pandya SM, Shafi H, Singh SK, et al. Intranasal eutectic powder of zolmitriptan with enhanced bioavailability in the rat brain. Mol Pharm. 2016;13(9):3234–40.
115. Merten OW. State-of-the-art of the production of retroviral vectors. J Gene Med. 2004;6(Suppl 1):S105–24.
116. Bouard D, Alazard-Dany N, Cosset FL. Viral vectors: from virology to transgene expression. Br J Pharmacol. 2009;157(2):153–65.
117. Khodr CE, Sapru MK, Pedapati J, Han Y, West NC, Kells AP, et al. An α-synuclein AAV gene silencing vector ameliorates a behavioral deficit in a rat model of Parkinson's disease, but displays toxicity in dopamine neurons. Brain Res. 2011;1395:94–107.
118. Sandbhor P, Goda J, Mohanty B, Gera P, Yadav S, Chekuri G, et al. Targeted nano-delivery of chemotherapy via intranasal route suppresses in vivo glioblastoma growth and prolongs survival in the intracranial mouse model. Drug Deliv Transl Res. 2023;13(2):608–26.
119. Musumeci T, Di Benedetto G, Carbone C, Bonaccorso A, Amato G, Lo Faro MJ, et al. Intranasal administration of a TRAIL neutralizing monoclonal antibody adsorbed in PLGA nanoparticles and NLC nanosystems: an in vivo study on a mouse model of Alzheimer's disease. Biomedicines. 2022;10(5):985.
120. Dimiou S, Lopes RM, Kubajewska I, Mellor RD, Schlosser CS, Shet MS, et al. Particulate levodopa nose-to-brain delivery targets dopamine to the brain with no plasma exposure. Int J Pharm. 2022;618:121658.
121. Sandbhor P, Goda J, Mohanty B, Chaudhari P, Dutt S, Banerjee R. Non-invasive transferrin targeted nanovesicles sensitize resistant glioblastoma multiforme tumors and improve survival in orthotopic mouse models. Nanoscale. 2021;14(1):108–26.
122. Noske S, Karimov M, Aigner A, Ewe A. Tyrosine-modification of polypropylenimine (PPI) and polyethylenimine (PEI) strongly improves efficacy of siRNA-mediated gene knockdown. Nanomaterials. 2020;10(9):1809.
123. Karimov M, Schulz M, Kahl T, Noske S, Kubczak M, Gockel I, et al. Tyrosine-modified linear PEIs for highly efficacious and biocompatible siRNA delivery in vitro and in vivo. Nanomedicine. 2021;36:102403.
124. Hathout RM, El-Marakby EM. Meta-analysis: a convenient tool for the choice of nose-to-brain nanocarriers. Bioengineering. 2022;9(11):647.
125. Deruyver L, Rigaut C, Gomez-Perez A, Lambert P, Haut B, Goole J. In vitro evaluation of paliperidone palmitate loaded cubosomes effective for nasal-to-brain delivery. Int J Nanomed. 2023;18:1085–106.
126. Pusic KM, Kraig RP, Pusic AD. IFNγ-stimulated dendritic cell extracellular vesicles can be nasally administered to the brain and enter oligodendrocytes. PLoS ONE. 2021;16(8):e0255778.
127. Kataria I, Shende P. Nose-to-brain lipid nanocarriers: An active transportation across BBB in migraine management. Chem Phys Lipids. 2022;243:105177.
128. García-Llano M, Pedroso-Ibáñez I, Morales-Chacón L, Rodríguez-Obaya T, Pérez-Ruiz L, Sosa-Testé I, et al. Short-term tolerance of nasally-administered NeuroEPO in Patients with Parkinson disease. MEDICC Rev. 2021;23(1):49–54.
129. Santos-Morales O, Díaz-Machado A, Jiménez-Rodríguez D, Pomares-Iturralde Y, Festary-Casanovas T, González-Delgado CA, et al. Nasal administration of the neuroprotective candidate NeuroEPO to healthy volunteers: a randomized, parallel, open-label safety study. BMC Neurol. 2017;17(1):129.
130. Gadhave D, Gupta A, Khot S, Tagalpallewar A, Kokare C. Nose-to-brain delivery of paliperidone palmitate poloxamer-guar gum nanogel: Formulation, optimization and pharmacological studies in rats. Ann Pharm Fr. 2022.
131. Gartziandia O, Egusquiaguirre SP, Bianco J, Pedraz JL, Igartua M, Hernandez RM, et al. Nanoparticle transport across in vitro olfactory cell monolayers. Int J Pharm. 2016;499(1):81–9.
132. Wengst A, Reichl S. RPMI 2650 epithelial model and three-dimensional reconstructed human nasal mucosa as in vitro models for nasal permeation studies. EurJ Pharm Biopharm. 2010;74(2):290–7.
133. Boyuklieva R, Zagorchev P, Pilicheva B. Computational, in vitro, and in vivo models for nose-to-brain drug delivery studies. Biomedicines. 2023;11(8):2198.
134. Truzzi E, Rustichelli C, de Oliveira Junior ER, Ferraro L, Maretti E, Graziani D, et al. Nasal biocompatible powder of Geraniol oil complexed with cyclodextrins for neurodegenerative diseases: physicochemical characterization and in vivo evidences of nose to brain delivery. J Control Release. 2021;335:191–202.
135. Xicoy H, Wieringa B, Martens GJ. The SH-SY5Y cell line in Parkinson's disease research: a systematic review. Mol Neurodegener. 2017;12(1):10.
136. Goshi N, Morgan RK, Lein PJ, Seker E. A primary neural cell culture model to study neuron, astrocyte, and microglia interactions in neuroinflammation. J Neuroinflammation. 2020;17(1):155.
137. Gastfriend BD, Palecek SP, Shusta EV. Modeling the blood–brain barrier: Beyond the endothelial cells. Curr Opin Biomed Eng. 2018;5:6–12.
138. Fan J, Yang J, Jiang Z. Prediction of central nervous system side effects through drug permeability to blood–brain barrier and recommendation algorithm. J Comput Biol. 2018;25(4):435–43.
139. Gauvin DV, Zimmermann ZJ. FOB vs modified Irwin: What are we doing? J Pharmacol Toxicol Methods. 2019;97:24–8.
140. Liu K, Gao YZ, Wu XM, Hu XY, Shi CN, He QL, et al. Microglia phagocytosis of PNNs mediates PV-positive interneuron dysfunction and associated gamma oscillations in neuroinflammation-induced cognitive impairment in mice. Neuropharmacology. 2025;262:110205.
141. Fleming SM, Tetreault NA, Mulligan CK, Hutson CB, Masliah E, Chesselet MF. Olfactory deficits in mice overexpressing human wildtype alpha-synuclein. Eur J Neurosci. 2008;28(2):247–56.
142. Yang M, Crawley JN. Simple behavioral assessment of mouse olfaction. Curr Protoc Neurosci. 2009;Chapter 8:Unit 8.24.
143. Floresco SB, Magyar O. Mesocortical dopamine modulation of executive functions: beyond working memory. Psychopharmacology. 2006;188(4):567–85.
144. Bari A, Dalley JW, Robbins TW. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat Protoc. 2008;3(5):759–67.
145. Horst NK, Laubach M. Working with memory: evidence for a role for the medial prefrontal cortex in performance monitoring during spatial delayed alternation. J Neurophysiol. 2012;108(12):3276–88.
146. Bissonette GB, Powell EM, Roesch MR. Neural structures underlying set-shifting: roles of medial prefrontal cortex and anterior cingulate cortex. Behav Brain Res. 2013;250:91–101.
147. Rudebeck PH, Saunders RC, Prescott AT, Chau LS, Murray EA. Prefrontal mechanisms of behavioral flexibility, emotion regulation and value updating. Nat Neurosci. 2013;16(8):1140–5.
148. Ding C, Wu Y, Chen X, Chen Y, Wu Z, Lin Z, et al. Global, regional, and national burden and attributable risk factors of neurological disorders: The Global Burden of Disease study 1990–2019. Frontiers in Public Health. 2022;10.
149. Tysnes OB, Storstein A. Epidemiology of Parkinson's disease. J Neural Transm (Vienna). 2017;124(8):901–5.
150. Dorsey ER, Sherer T, Okun MS, Bloem BR. The emerging evidence of the Parkinson pandemic. J Parkinsons Dis. 2018;8(s1):S3-8.
151. Dorsey ER, Elbaz A, Nichols E, Abbasi N, Abd-Allah F, Abdelalim A, et al. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2018;17(11):939–53.
152. Balestrino R, Schapira AHV. Parkinson disease. Eur J Neurol. 2020;27(1):27–42.
153. Lai TT, Gericke B, Feja M, Conoscenti M, Zelikowsky M, Richter F. Anxiety in synucleinopathies: neuronal circuitry, underlying pathomechanisms and current therapeutic strategies. NPJ Parkinsons Dis. 2023;9(1):97.
154. Ray Chaudhuri K, Qamar MA, Rajah T, Loehrer P, Sauerbier A, Odin P, et al. Non-oral dopaminergic therapies for Parkinson's disease: current treatments and the future. NPJ Parkinson's Dis. 2016;2(1):1–7.
155. Suttrup I, Warnecke T. Dysphagia in Parkinson's disease. Dysphagia. 2016;31(1):24–32.
156. Damier P, Hirsch EC, Agid Y, Graybiel A. The substantia nigra of the human brain: II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain. 1999;122(8):1437–48.
157. Ehringer H, Hornykiewicz O. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Parkinsonism Relat Disord. 1998;4(2):53–7.

158. Galvin JE, Lee VM, Trojanowski JQ. Synucleinopathies: clinical and pathological implications. Arch Neurol. 2001;58(2):186–90.
159. Pollanen MS, Dickson DW, Bergeron C. Pathology and biology of the lewy body. J Neuropathol Exp Neurol. 1993;52(3):183–91.
160. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197–211.
161. Heumann R, Moratalla R, Herrero MT, Chakrabarty K, Drucker-Colín R, Garcia-Montes JR, et al. Dyskinesia in Parkinson's disease: mechanisms and current non-pharmacological interventions. J Neurochem. 2014;130(4):472–89.
162. Lane EL. L-DOPA for Parkinson's disease-a bittersweet pill. Eur J Neurosci. 2019;49(3):384–98.
163. Bloem BR, Okun MS, Klein C. Parkinson's disease. Lancet. 2021;397(10291):2284–303.
164. Vijiaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. Lancet Neurol. 2021;20(7):559–72.
165. Brown JM, Baker LS, Seroogy KB, Genter MB. Intranasal carnosine mitigates α-synuclein pathology and motor dysfunction in the Thy1-aSyn mouse model of Parkinson's disease. ACS Chem Neurosci. 2021;12(13):2347–59.
166. Bermúdez ML, Seroogy KB, Genter MB. Evaluation of carnosine intervention in the Thy1-aSyn mouse model of Parkinson's disease. Neuroscience. 2019;411:270–8.
167. Bermúdez ML, Skelton MR, Genter MB. Intranasal carnosine attenuates transcriptomic alterations and improves mitochondrial function in the Thy1-aSyn mouse model of Parkinson's disease. Mol Genet Metab. 2018;125(3):305–13.
168. Magen I, Ostritsky R, Richter F, Zhu C, Fleming SM, Lemesre V, et al. Intranasal NAP (davunetide) decreases tau hyperphosphorylation and moderately improves behavioral deficits in mice overexpressing α-synuclein. Pharmacol Res Perspect. 2014;2(5):e00065.
169. Fleming SM, Mulligan CK, Richter F, Mortazavi F, Lemesre V, Frias C, et al. A pilot trial of the microtubule-interacting peptide (NAP) in mice overexpressing alpha-synuclein shows improvement in motor function and reduction of alpha-synuclein inclusions. Mol Cell Neurosci. 2011;46(3):597–606.
170. Umeda T, Hatanaka Y, Sakai A, Tomiyama T. Nasal rifampicin improves cognition in a mouse model of dementia with Lewy bodies by reducing α-synuclein oligomers. Int J Mol Sci. 2021;22(16):8453.
171. Bhattamisra SK, Shak AT, Xi LW, Safian NH, Choudhury H, Lim WM, et al. Nose to brain delivery of rotigotine loaded chitosan nanoparticles in human SH-SY5Y neuroblastoma cells and animal model of Parkinson's disease. Int J Pharm. 2020;579:119148.
172. Narbute K, Piļipenko V, Pupure J, Dzirkale Z, Jonavičė U, Tunaitis V, et al. Intranasal administration of extracellular vesicles derived from human teeth stem cells improves motor symptoms and normalizes tyrosine hydroxylase expression in the substantia nigra and striatum of the 6-hydroxydopamine-treated rats. Stem Cells Transl Med. 2019;8(5):490–9.
173. Narbute K, Pilipenko V, Pupure J, Klinovičs T, Auders J, Jonavičė U, et al. Time-dependent memory and gait improvement by intranasally-administered extracellular vesicles in Parkinson's disease model rats. Cell Mol Neurobiol. 2021;41(3):605–13.
174. Azizifar N, Mohaddes G, Keyhanmanesh R, Athari SZ, Alimohammadi S, Farajdokht F. Intranasal AdipoRon mitigated anxiety and depression-like behaviors in 6-OHDA-induced Parkinson 's disease rat model: going beyond motor symptoms. Neurochem Res. 2024;49(11):3030–42.
175. Gartziandia O, Herrán E, Ruiz-Ortega JA, Miguelez C, Igartua M, Lafuente JV, et al. Intranasal administration of chitosan-coated nanostructured lipid carriers loaded with GDNF improves behavioral and histological recovery in a partial lesion model of Parkinson's disease. J Biomed Nanotechnol. 2016;12(12):2220–30.
176. Yue P, Gao L, Wang X, Ding X, Teng J. Intranasal administration of GDNF protects against neural apoptosis in a rat model of Parkinson's disease through PI3K/Akt/GSK3β pathway. Neurochem Res. 2017;42(5):1366–74.
177. Migliore MM, Ortiz R, Dye S, Campbell RB, Amiji MM, Waszczak BL. Neurotrophic and neuroprotective efficacy of intranasal GDNF in a rat model of Parkinson's disease. Neuroscience. 2014;274:11–23.
178. Kumar S, Gupta SK, Pahwa R. Designing lisuride intranasal nanocarrier system for reduction of oxidative damage with enhanced dopamine level in brain for Parkinsonism. J Psychiatr Res. 2023;165:205–18.
179. Kumar S, Dang S, Nigam K, Ali J, Baboota S. Selegiline nanoformulation in attenuation of oxidative stress and upregulation of dopamine in the brain for the treatment of Parkinson's disease. Rejuvenation Res. 2018;21(5):464–76.
180. Usama Ashhar M, Vyas P, Vohora D, Kumar Sahoo P, Nigam K, Dang S, et al. Amelioration of oxidative stress utilizing nanoemulsion loaded with bromocriptine and glutathione for the management of Parkinson's disease. Int J Pharm. 2022;618:121683.
181. Mohammad, Khan UA, Saifi Z, Bora J, Warsi MH, Abourehab MAS, et al. Intranasal inorganic cerium oxide nanoparticles ameliorate oxidative stress induced motor manifestations in haloperidol-induced parkinsonism. Inflammopharmacology. 2023;31(5):2571–85.
182. Mohammad, Khan UA, Warsi MH, Alkreathy HM, Karim S, Jain GK, et al. Intranasal cerium oxide nanoparticles improves locomotor activity and reduces oxidative stress and neuroinflammation in haloperidol-induced parkinsonism in rats. Front Pharmacol. 2023;14:1188470.
183. Khot KB, DS S, Gopan G, Deshpande NS, Shastry P, Bandiwadekar A, et al. Enhancing selegiline hydrochloride efficacy: Box Behnken-optimized liposomal delivery via intranasal route for Parkinson's disease intervention. J Liposome Res. 2024;34(4):575–92.
184. Salama M, Sobh M, Emam M, Abdalla A, Sabry D, El-Gamal M, et al. Effect of intranasal stem cell administration on the nigrostriatal system in a mouse model of Parkinson's disease. Exp Ther Med. 2017;13(3):976–82.
185. Tang Y, Han L, Bai X, Liang X, Zhao J, Huang F, et al. Intranasal delivery of bone marrow stromal cells preconditioned with fasudil to treat a mouse model of Parkinson's disease. Neuropsychiatr Dis Treat. 2020;16:249–62.
186. Ji R, Smith M, Niimi Y, Karakatsani ME, Murillo MF, Jackson-Lewis V, et al. Focused ultrasound enhanced intranasal delivery of brain derived neurotrophic factor produces neurorestorative effects in a Parkinson's disease mouse model. Sci Rep. 2019;9(1):19402.
187. Fang X, Ma J, Mu D, Li B, Lian B, Sun C. FGF21 protects dopaminergic neurons in Parkinson's disease models via repression of neuroinflammation. Neurotox Res. 2020;37(3):616–27.
188. Chen F, Jin L, Nie Z. Safety and efficacy of rotigotine for treating Parkinson's disease: a meta-analysis of randomised controlled trials. J Pharm Pharm Sci. 2017;20:285–94.
189. Zhou CQ, Li SS, Chen ZM, Li FQ, Lei P, Peng GG. Rotigotine transdermal patch in Parkinson's disease: a systematic review and meta-analysis. PLoS ONE. 2013;8(7):e69738.
190. Yan X, Xu L, Bi C, Duan D, Chu L, Yu X, et al. Lactoferrin-modified rotigotine nanoparticles for enhanced nose-to-brain delivery: LESA-MS/MS-based drug biodistribution, pharmacodynamics, and neuroprotective effects. Int J Nanomedicine. 2018;13:273–81.
191. Mahmood I. Clinical pharmacokinetics and pharmacodynamics of selegiline. Clin Pharmacokinet. 1997;33(2):91–102.
192. Sridhar V, Gaud R, Bajaj A, Wairkar S. Pharmacokinetics and pharmacodynamics of intranasally administered selegiline nanoparticles with improved brain delivery in Parkinson's disease. Nanomedicine. 2018;14(8):2609–18.
193. Sridhar V, Wairkar S, Gaud R, Bajaj A, Meshram P. Brain targeted delivery of mucoadhesive thermosensitive nasal gel of selegiline hydrochloride for treatment of Parkinson's disease. J Drug Target. 2018;26(2):150–61.
194. Sarchione A, Marchand A, Taymans JM, Chartier-Harlin MC. Alpha-synuclein and lipids: the elephant in the room? Cells. 2021;10(9):2452.
195. Ruipérez V, Darios F, Davletov B. Alpha-synuclein, lipids and Parkinson's disease. Prog Lipid Res. 2010;49(4):420–8.
196. Tong B, Ba Y, Li Z, Yang C, Su K, Qi H, et al. Targeting dysregulated lipid metabolism for the treatment of Alzheimer's disease and Parkinson's disease: current advancements and future prospects. Neurobiol Dis. 2024;196:106505.
197. Iravanpour F, Dargahi L, Rezaei M, Haghani M, Heidari R, Valian N, et al. Intranasal insulin improves mitochondrial function and attenuates motor deficits in a rat 6-OHDA model of Parkinson's disease. CNS Neurosci Ther. 2021;27(3):308–19.
198. Fine JM, Stroebel BM, Faltesek KA, Terai K, Haase L, Knutzen KE, et al. Intranasal delivery of low-dose insulin ameliorates motor dysfunction

and dopaminergic cell death in a 6-OHDA rat model of Parkinson's disease. Neurosci Lett. 2020;714:134567.
199. Yang L, Zhang X, Li S, Wang H, Zhang X, Liu L, et al. Intranasal insulin ameliorates cognitive impairment in a rat model of Parkinson's disease through Akt/GSK3β signaling pathway. Life Sci. 2020;259:118159.
200. Pang Y, Lin S, Wright C, Shen J, Carter K, Bhatt A, et al. Intranasal insulin protects against substantia nigra dopaminergic neuronal loss and alleviates motor deficits induced by 6-OHDA in rats. Neuroscience. 2016;318:157–65.
201. Chesselet MF, Richter F, Zhu C, Magen I, Watson MB, Subramaniam SR. A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. Neurotherapeutics. 2012;9(2):297–314.
202. Richter F, Stanojlovic M, Käufer C, Gericke B, Feja M. A mouse model to test novel therapeutics for Parkinson's disease: an update on the Thy1-aSyn ("line 61") mice. Neurotherapeutics. 2023;20(1):97–116.
203. ESGCT Abstract Author Index. Human Gene Therapy. 2024;35(3-4):A337–A65.
204. Salari M, Etemadifar M, Dargahi L, Valian N, Rezaee M. Manganese-induced parkinsonism responsive to intranasal insulin: A case report. Clin Case Rep. 2022;10(3):e05562.
205. Novak P, Pimentel Maldonado DA, Novak V. Safety and preliminary efficacy of intranasal insulin for cognitive impairment in Parkinson disease and multiple system atrophy: a double-blinded placebo-controlled pilot study. PLoS One. 2019;14(4):e0214364.
206. Liu RM, Gaston Pravia KA. Oxidative stress and glutathione in TGF-beta-mediated fibrogenesis. Free Radic Biol Med. 2010;48(1):1–15.
207. Sreekumar PG, Ferrington DA, Kannan R. Glutathione metabolism and the novel role of mitochondrial GSH in retinal degeneration. Antioxidants. 2021;10(5):661.
208. Korczowska-Łącka I, Hurła M, Banaszek N, Kobylarek D, Szymanowicz O, Kozubski W, et al. Selected biomarkers of oxidative stress and energy metabolism disorders in neurological diseases. Mol Neurobiol. 2023;60(7):4132–49.
209. Mischley LK, Leverenz JB, Lau RC, Polissar NL, Neradilek MB, Samii A, et al. A randomized, double-blind phase I/IIa study of intranasal glutathione in Parkinson's disease. Mov Disord. 2015;30(12):1696–701.
210. Mischley LK, Conley KE, Shankland EG, Kavanagh TJ, Rosenfeld ME, Duda JE, et al. Central nervous system uptake of intranasal glutathione in Parkinson's disease. NPJ Parkinsons Dis. 2016;2:16002.
211. Mischley LK, Lau RC, Shankland EG, Wilbur TK, Padowski JM. Phase IIb study of intranasal glutathione in Parkinson's disease. J Parkinsons Dis. 2017;7(2):289–99.
212. Mischley LK, Vespignani MF, Finnell JS. Safety survey of intranasal glutathione. J Altern Complement Med. 2013;19(5):459–63.
213. Shrewsbury SB. The upper nasal space: Option for systemic drug delivery, mucosal vaccines and "Nose-to-Brain." Pharmaceutics. 2023;15(6):1720.
214. Pedroso I, Garcia M, Casabona E, Morales L, Bringas ML, Pérez L, et al. Protective activity of erythropoyetine in the cognition of patients with Parkinson's disease. Behav Sci (Basel). 2018;8(5):51.
215. Bringas Vega ML, Pedroso Ibáñez I, Razzaq FA, Zhang M, Morales Chacón L, Ren P, et al. The effect of neuroepo on cognition in Parkinson's disease patients is mediated by electroencephalogram source activity. Front Neurosci. 2022;16:841428.
216. Boika A, Aleinikava N, Chyzhyk V, Zafranskaya M, Nizheharodava D, Ponomarev V. Mesenchymal stem cells in Parkinson's disease: Motor and nonmotor symptoms in the early posttransplant period. Surg Neurol Int. 2020;11:380.
217. Barage SH, Sonawane KD. Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides. 2015;52:1–18.
218. Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. Hum Mol Genet. 2010;19(R1):R12-20.
219. Atri A. The Alzheimer's disease clinical spectrum: diagnosis and management. Med Clin North Am. 2019;103(2):263–93.
220. Chen Y, Guo Z, Mao YF, Zheng T, Zhang B. Intranasal insulin ameliorates cerebral hypometabolism, neuronal loss, and astrogliosis in streptozotocin-induced Alzheimer's rat model. Neurotox Res. 2018;33(4):716–24.
221. Bazrgar M, Khodabakhsh P, Dargahi L, Mohagheghi F, Ahmadiani A. MicroRNA modulation is a potential molecular mechanism for neuroprotective effects of intranasal insulin administration in amyloid βeta oligomer induced Alzheimer's like rat model. Exp Gerontol. 2022;164:111812.
222. Farzampour S, Majdi A, Sadigh-Eteghad S. Intranasal insulin treatment improves memory and learning in a rat amyloid-beta model of Alzheimer's disease. Physiol Int. 2016;103(3):344–53.
223. Rajasekar N, Nath C, Hanif K, Shukla R. Intranasal insulin administration ameliorates streptozotocin (ICV)-induced insulin receptor dysfunction, neuroinflammation, amyloidogenesis, and memory impairment in rats. Mol Neurobiol. 2017;54(8):6507–22.
224. Yamada M, Chiba T, Sasabe J, Nawa M, Tajima H, Niikura T, et al. Implanted cannula-mediated repetitive administration of Aβ25–35 into the mouse cerebral ventricle effectively impairs spatial working memory. Behavi Brain Res. 2005;164(2):139–46.
225. Yamada M, Chiba T, Sasabe J, Terashita K, Aiso S, Matsuoka M. Nasal Colivelin treatment ameliorates memory impairment related to Alzheimer's disease. Neuropsychopharmacology. 2008;33(8):2020–32.
226. Wu M, Shi H, He Y, Yuan L, Qu X, Zhang J, et al. Colivelin ameliorates impairments in cognitive behaviors and synaptic plasticity in APP/PS1 transgenic mice. J Alzheimers Dis. 2017;59(3):1067–78.
227. Matsuoka M, Hashimoto Y, Aiso S, Nishimoto I. Humanin and colivelin: neuronal-death-suppressing peptides for Alzheimer's disease and amyotrophic lateral sclerosis. CNS Drug Rev. 2006;12(2):113–22.
228. Lu MH, Ji WL, Chen H, Sun YY, Zhao XY, Wang F, et al. Intranasal transplantation of human neural stem cells ameliorates Alzheimer's disease-like pathology in a mouse model. Front Aging Neurosci. 2021;13:650103.
229. Mo H, Kim J, Kim JY, Kim JW, Han H, Choi SH, et al. Intranasal administration of induced pluripotent stem cell-derived cortical neural stem cell-secretome as a treatment option for Alzheimer's disease. Transl Neurodegener. 2023;12(1):50.
230. Santamaria G, Brandi E, Vitola P, Grandi F, Ferrara G, Pischiutta F, et al. Intranasal delivery of mesenchymal stem cell secretome repairs the brain of Alzheimer's mice. Cell Death Differ. 2021;28(1):203–18.
231. Yang X, Yang W, Xia X, Lei T, Yang Z, Jia W, et al. Intranasal delivery of BACE1 siRNA and rapamycin by dual targets modified nanoparticles for Alzheimer's disease therapy. Small. 2022;18(30):e2203182.
232. Li J, Peng H, Zhang W, Li M, Wang N, Peng C, et al. Enhanced nose-to-brain delivery of combined small interfering rnas using lesion-recognizing nanoparticles for the synergistic therapy of Alzheimer's disease. ACS Appl Mater Interf. 2023;15(46):53177–88.
233. Craft S, Asthana S, Cook DG, Baker LD, Cherrier M, Purganan K, et al. Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interactions with apolipoprotein E genotype. Psychoneuroendocrinology. 2003;28(6):809–22.
234. Reger MA, Watson GS, Green PS, Wilkinson CW, Baker LD, Cholerton B, et al. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology. 2008;70(6):440–8.
235. Benedict C, Hallschmid M, Schmitz K, Schultes B, Ratter F, Fehm HL, et al. Intranasal insulin improves memory in humans: superiority of insulin aspart. Neuropsychopharmacology. 2007;32(1):239–43.
236. Rosenbloom MH, Barclay TR, Pyle M, Owens BL, Cagan AB, Anderson CP, et al. A single-dose pilot trial of intranasal rapid-acting insulin in apolipoprotein E4 carriers with mild-moderate Alzheimer's disease. CNS Drugs. 2014;28(12):1185–9.
237. Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol. 2012;69(1):29–38.
238. Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, et al. Long-acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. J Alzheimers Dis. 2015;44(3):897–906.
239. Rosenbloom M, Barclay TR, Kashyap B, Hage L, O'Keefe LR, Svitak A, et al. A phase II, single-center, randomized, double-blind, placebo-controlled study of the safety and therapeutic efficacy of intranasal glulisine in amnestic mild cognitive impairment and probable mild Alzheimer's disease. Drugs Aging. 2021;38(5):407–15.
240. Craft S, Claxton A, Baker LD, Hanson AJ, Cholerton B, Trittschuh EH, et al. Effects of regular and long-acting insulin on cognition and Alzheimer's disease biomarkers: a pilot clinical trial. J Alzheimers Dis. 2017;57(4):1325–34.

241. Kellar D, Register T, Lockhart SN, Aisen P, Raman R, Rissman RA, et al. Intranasal insulin modulates cerebrospinal fluid markers of neuroinflammation in mild cognitive impairment and Alzheimer's disease: a randomized trial. Sci Rep. 2022;12(1):1346.
242. Davidy T, Yore I, Cukierman-Yaffe T, Ravona-Springer R, Livny A, Lesman-Segev OH, et al. A feasibility study of the combination of intranasal insulin with oral semaglutide for cognition in older adults with metabolic syndrome at high dementia risk- Study rationale and design. Mech Ageing Dev. 2024;218:111898.
243. Kalilani L, Sun X, Pelgrims B, Noack-Rink M, Villanueva V. The epidemiology of drug-resistant epilepsy: a systematic review and meta-analysis. Epilepsia. 2018;59(12):2179–93.
244. Gernert M, MacKeigan D, Deking L, Kaczmarek E, Feja M. Acute and chronic convection-enhanced muscimol delivery into the rat subthalamic nucleus induces antiseizure effects associated with high responder rates. Epilepsy Res. 2023;190:107097.
245. MacKeigan D, Feja M, Meller S, Deking L, Javadova A, Veenhuis A, et al. Long-lasting antiseizure effects of chronic intrasubthalamic convection-enhanced delivery of valproate. Neurobiol Dis. 2023;187:106321.
246. Holsti M, Dudley N, Schunk J, Adelgais K, Greenberg R, Olsen C, et al. Intranasal midazolam vs rectal diazepam for the home treatment of acute seizures in pediatric patients with epilepsy. Arch Pediatr Adolesc Med. 2010;164(8):747–53.
247. Shah P, Dubey P, Vyas B, Kaul A, Mishra AK, Chopra D, et al. Lamotrigine loaded PLGA nanoparticles intended for direct nose to brain delivery in epilepsy: pharmacokinetic, pharmacodynamic and scintigraphy study. Artif Cells Nanomed Biotechnol. 2021;49(1):511–22.
248. Abdelmonem R, El-Enin HAA, Abdelkader G, Abdel-Hakeem M. Formulation and characterization of lamotrigine nasal insert targeted brain for enhanced epilepsy treatment. Drug Deliv. 2023;30(1):2163321.
249. Samia O, Hanan R, El Kamal T. Carbamazepine mucoadhesive nanoemulgel (MNEG) as brain targeting delivery system via the olfactory mucosa. Drug Deliv. 2012;19(1):58–67.
250. Veronesi MC, Aldouby Y, Domb AJ, Kubek MJ. Thyrotropin-releasing hormone d, l polylactide nanoparticles (TRH-NPs) protect against glutamate toxicity in vitro and kindling development in vivo. Brain Res. 2009;1303:151–60.
251. Taymouri S, Minaiyan M, Ebrahimi F, Tavakoli N. In-vitro and in-vivo evaluation of chitosan-based thermosensitive gel containing lorazepam NLCs for the treatment of status epilepticus. IET Nanobiotechnol. 2020;14(2):148–54.
252. Samanta D. Rescue therapies for seizure emergencies: current and future landscape. Neurol Sci. 2021;42(10):4017–27.
253. Chmielewska N, Szyndler J. Intranasal administration of antiseizure medications in chronic and emergency treatment: hopes and challenges. Seizure. 2024;115:62–7.
254. von Blomberg A, Kay L, Knake S, Fuest S, Zöllner JP, Reif PS, et al. Efficacy, tolerability, and safety of concentrated intranasal midazolam spray as emergency medication in epilepsy patients during video-EEG monitoring. CNS Drugs. 2020;34(5):545–53.
255. Bancke LL, Dworak HA, Rodvold KA, Halvorsen MB, Gidal BE. Pharmacokinetics, pharmacodynamics, and safety of USL261, a midazolam formulation optimized for intranasal delivery, in a randomized study with healthy volunteers. Epilepsia. 2015;56(11):1723–31.
256. Holsti M, Sill BL, Firth SD, Filloux FM, Joyce SM, Furnival RA. Prehospital intranasal midazolam for the treatment of pediatric seizures. Pediatr Emerg Care. 2007;23(3):148–53.
257. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018;38(1):1–211.
258. Kelman L. The premonitory symptoms (prodrome): a tertiary care study of 893 migraineurs. Headache. 2004;44(9):865–72.
259. Villar-Martinez MD, Moreno-Ajona D, Goadsby PJ. Familial hemiplegic migraine. Handb Clin Neurol. 2024;203:135–44.
260. Ashina M, Katsarava Z, Do TP, Buse DC, Pozo-Rosich P, Özge A, et al. Migraine: epidemiology and systems of care. Lancet. 2021;397(10283):1485–95.
261. Tam ACT, Naik H, Trenaman L, Lynd L, Zhang W. Health-related quality of life among women and men living with migraine: a Canada-wide cross-sectional study. J Headache Pain. 2024;25(1):170.
262. Peters GL. Migraine overview and summary of current and emerging treatment options. Am J Manag Care. 2019;25(2 Suppl):S23-34.
263. Charles A. The pathophysiology of migraine: implications for clinical management. Lancet Neurol. 2018;17(2):174–82.
264. Salonen R, Ashford E, Dahlöf C, Dawson R, Gilhus NE, Lüben V, et al. Intranasal sumatriptan for the acute treatment of migraine. J Neurol. 1994;241:463–9.
265. Yadav RK, Shah K, Dewangan HK. Intranasal drug delivery of sumatriptan succinate-loaded polymeric solid lipid nanoparticles for brain targeting. Drug Dev Ind Pharm. 2022;48(1):21–8.
266. Masjedi M, Azadi A, Heidari R, Mohammadi-Samani S. Nose-to-brain delivery of sumatriptan-loaded nanostructured lipid carriers: preparation, optimization, characterization and pharmacokinetic evaluation. J Pharm Pharmacol. 2020;72(10):1341–51.
267. Shafique U, Din FU, Sohail S, Batool S, Almari AH, Lahiq AA, et al. Quality by design for sumatriptan loaded nano-ethosomal mucoadhesive gel for the therapeutic management of nitroglycerin induced migraine. Int J Pharm. 2023;646:123480.
268. Abd-Elal RM, Shamma RN, Rashed HM, Bendas ER. Trans-nasal zolmitriptan novasomes: in-vitro preparation, optimization and in-vivo evaluation of brain targeting efficiency. Drug Deliv. 2016;23(9):3374–86.
269. Lipton RB, McGinley JS, Shulman KJ, Silberstein SD, Wirth RJ, Buse DC. AVP-825 (sumatriptan nasal powder) reduces nausea compared to sumatriptan tablets: results of the COMPASS randomized clinical trial. Headache. 2018;58(2):229–42.
270. Diener HC, Evers S. Effectiveness and satisfaction with zolmitriptan 5 mg nasal spray for treatment of migraine in real-life practice: results of a postmarketing surveillance study. Clin Drug Investig. 2007;27(1):59–66.
271. Shelke S, Shahi S, Jalalpure S, Dhamecha D. Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: formulation, optimization, evaluation and permeation studies. J Liposome Res. 2016;26(4):313–23.
272. Khezri F, Lakshmi CSR, Bukka R, Nidhi M, Nargund SL. Pharmacokinetic study and brain tissue analysis of Zolmitriptan loaded chitosan nanoparticles in rats by LC-MS method. Int J Biol Macromol. 2020;142:52–62.
273. Gawel M, Aschoff J, May A, Charlesworth BR. Zolmitriptan 5 mg nasal spray: efficacy and onset of action in the acute treatment of migraine–results from phase 1 of the REALIZE Study. Headache. 2005;45(1):7–16.
274. Tepper SJ, Johnstone MR. Breath-powered sumatriptan dry nasal powder: an intranasal medication delivery system for acute treatment of migraine. Med Devices (Auckl). 2018;11:147–56.
275. Djupesland PG, Skretting A, Winderen M, Holand T. Bi-directional nasal delivery of aerosols can prevent lung deposition. J Aerosol Med. 2004;17(3):249–59.