



# Physiology of nose-to-brain pathways and current approaches to intranasal treatment

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## Abstract

There are many factors that affect the passage of drugs through the blood-brain barrier and the blood-cerebrospinal fluid barrier. Generally, conventional treatments for many psychiatric disorders and central nervous system diseases are administered parenteral or per os to produce systemic effects. However, an increased dose or extended treatment time is required to achieve effective drug concentrations at the desired site. This significantly increases the risk of systemic toxicity. For this reason, strategies for the direct delivery of drugs to the central nervous system without the need of increasing their dose are being investigated intensively. Drug delivery methods to the central nervous system are divided into three groups non-invasive, invasive and alternative methods. Intranasal drug administration, which is one of the non-invasive methods that increase the solubility and permeability of the drug, prolongs the drug effects, minimizes enzymatic degradation, and increases the bioavailability of the drug, has become the focus of attention of researchers in recent years. The use of experimental animal models in studies to identify intranasal drug administration routes and potential brain targets limits the applicability of these results to humans. In this review, the routes of intranasal drugs to the brain, the properties of drugs that can reach the central nervous system, and drug examples used in clinical trials are discussed.



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## Introduction

The brain is one of the most complex vital organs in the skull bone system, located in the medulla spinalis and columna vertebralis (spine), receiving signals from the sense organs and regulating physical functions. It controls involuntary and voluntary movements, memory, hormone secretion, and the function of many other organs. Because of its critical role, the brain is protected both internally and externally. The brain is mainly protected by the blood-brain barrier (BBB), cerebrospinal fluid (CSF), and CSF-blood barrier. These barriers protect the central nervous system (CNS) from various toxins and pathogens in systemic circulation. Endothelial cells, carrier vesicles, and tight junctions (TJs) that restrict paracellular passage are the main components of BBB [1]. A very important challenge in the treatment of CNS diseases is the transport of active substances to the brain, especially to a certain area, and release at a controlled or constant rate. The most important factor in the emergence of this problem and in preventing the passage of most substances is the

BBB [2-4]. According to Lipinski's "rule of five", for a molecule to pass through the BBB: (i) the total number of hydrogen bond donors should not be greater than 5 (ii) the total hydrogen bond acceptor should not be greater than 10 (iii) molecular weight should be below 500 daltons (iv) the log P value should be less than 5. A molecule with Lipinski's properties can be accepted as a drug candidate, but not every molecule meeting these conditions can be considered an ideal drug candidate. It is not enough for the molecule to have these properties. The first two rules are about the atomic types of the molecule, the third rule is about the size of the molecule and the last rule is about the solubility of the molecule [5]. Thus, only a few low molecular weight and hydrophobic molecules can pass through the BBB, while others are prevented due to the properties of the BBB. This makes the development of drugs that target the brain-challenging. Conventional drugs used to treat central nervous system diseases typically reach the CNS through the systemic circulation. Achieving effective drug concentrations at the target disease sites in the CNS requires systemic drug levels to be raised through significantly increased doses or prolonged administration, although such practices often lead to a significantly in-

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creased risk of systemic toxicity. Therefore, studies have been carried out to identify alternative drug administration routes that facilitate the passage of therapeutic agents by removing barriers in the CNS, increasing the concentration in the target region, and contributing to the increase of drug efficacy.

#### *Nose-to-brain delivery pathways*

After intranasal administration of a drug formulation, drug-loaded particles or drug molecules are transported directly from the nose to the brain and pass into the systemic circulation [6]. All drugs that reach the trigeminal nerves in the respiratory and olfactory regions are transmitted to the brain in four main ways: 1. via the extra-neuronal pathway along olfactory neurons, 2. via the intra-neuronal pathway through endocytosis by olfactory neurons, 3. through endocytosis by supporting cells, or 4. via the intercellular gap by crossing tight junctions. The extra-neuronal pathway (pathway 1) is the main direct route, taking up to 30 minutes. In the second pathway, called the intraneuronal pathway, the drug undergoes endocytosis by olfactory neurons and is released into the olfactory bulb before it reaches different parts of the brain. Drug transit in the intraneuronal pathway may take several hours or days. A less important pathway is via the supporting cells (pathways 3 and 4) [7]. Transport pathways are represented in Figure 1. The intranasal drug administration results of animal studies cannot be generalized to humans due to physiological and anatomical differences. In humans, substances, when taken intranasally, enter the nasal vestibule. The surface of the nasal vestibule is covered with skin containing vibrissae or hair to filter the large particles. The substances reach the respiratory region, which consists of nasal cartilages and turbinates, and these nasal turbinates are alternately occluded and decongested every 3 to 7 hours due to autonomic arousal and is indicative of asymmetry in brain function [8]. The nasal valve region is the narrowest part of the airway and shows the highest resistance to airflow. The nasal valve limits the nasal airflow. Even small changes in the nasal valve reduce the amount of substance reaching the olfactory region [9]. In preclinical studies comparing drug efficacy in intranasal and arterial drug administration, higher concentrations of the drug were found in the cerebral perivascular spaces within 20 minutes after intranasal administration [10]. Drugs that do not reach the olfactory region are reabsorbed back into circulation through the respiratory mucosa [9]. The olfactory system is one of the systems that transmit the sense of smell. The olfactory epithelium consists of the olfactory nerve, olfactory bulb, olfactory pathway, and various cortical areas where primary olfactory fibers terminate. The trigeminal nerve (nervus trigeminus) is the fifth and largest of the twelve cranial nerves. It provides sensation on the face and movements such as biting and chewing [11]. The sensory nerves of the nose come from the ophthalmic and maxillary branches of the trigeminal nerve. The sensation of the nasal cavity is received mainly by the maxillary branch of the trigeminal nerve. The nerve passes through the sphenopalatine ganglion and innervates the nasal septum, lateral nasal wall, palate, and nasopharynx. The compounds diffuse through

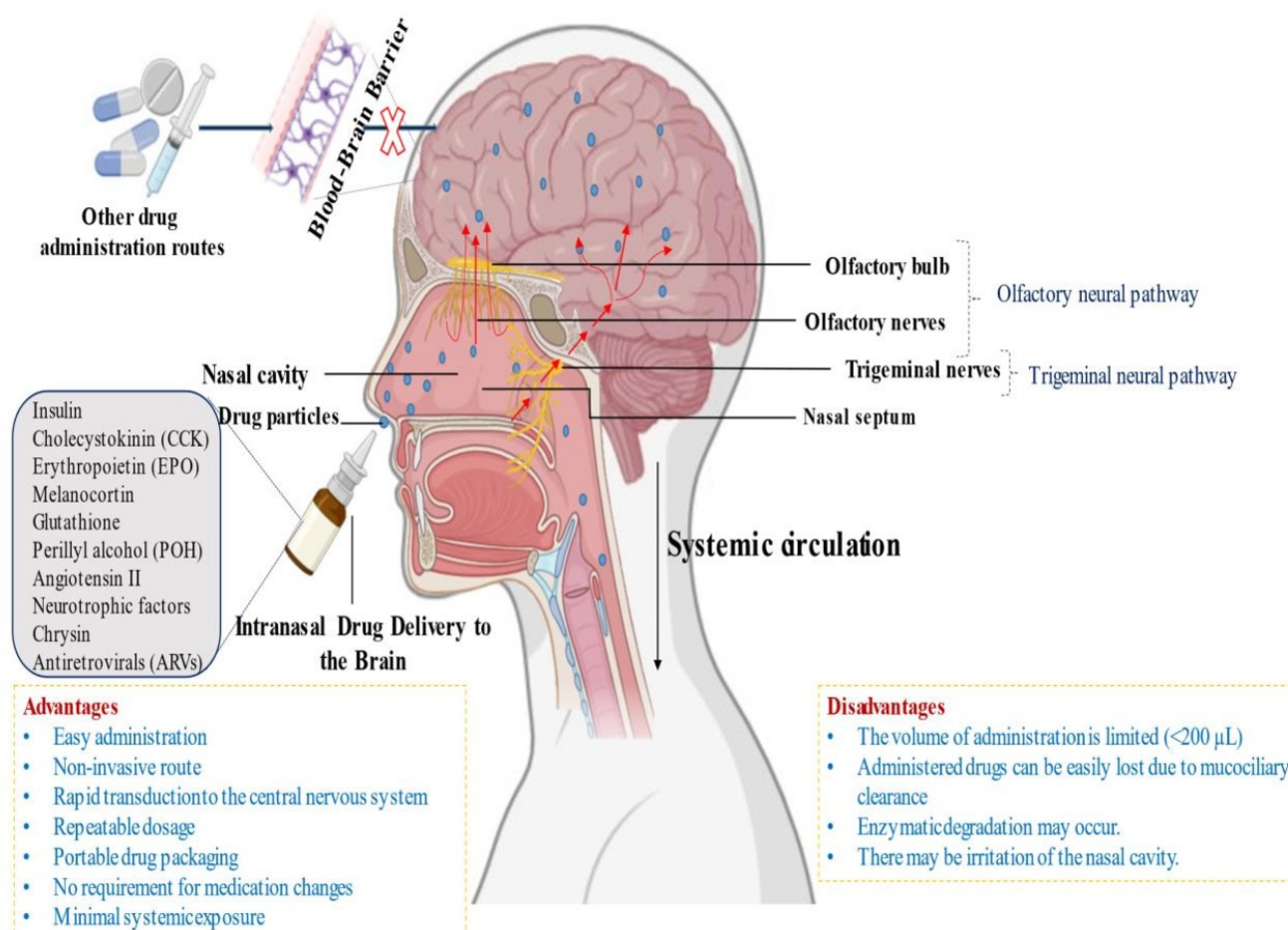
the nasal cavity mucosa and reach the branches of the trigeminal nerves. After reaching the trigeminal nerves, they are transported to the axonal pathway via the brain stem. This is the main part involved in the delivery of fractional therapeutics from the nasal cavity to the fore-brain [12]. Thorne et al. [13] reported in their study on the intranasal administration of insulin-like growth factor-I (IGF-I) that the growth factor has a rapid transition to the brain via the trigeminal neuronal pathway. After intranasal administration, drugs/nanoparticles are absorbed in the nasal cavity. After the molecules pass through the mucus, they are transported by different mechanisms such as transcellular, paracellular, transcytosis, carrier-mediated, and receptor-mediated transport [14]. Transcellular absorption refers to absorption through the epithelial cell membrane, while paracellular absorption refers to absorption through tight junctions between epithelial cells. Both absorption pathways take place by different mechanisms [15]. According to Kimura et al. [16], organic cation transporters, P-glycoprotein, and amino acid transporters are involved in carrier-mediated absorption, while the dopamine transporter acts as a molecule carrier in the nasal mucosa.

#### *Advantages of intranasal drug delivery to the brain*

Compared with parenteral administration, intranasal administration allows drug molecules to pass through the BBB more easily, providing faster delivery, and it can also improve drug bioavailability and targeting. Moreover, intranasal administration prevents the effects of drugs on hepatic and gastrointestinal metabolisms, reduces drug accumulation, and accordingly, reduces systemic side effects [17,18]. Nasal-to-brain drug administration can thus be considered an effective approach to the management of psychiatric disorders and CNS diseases and a practice that also reduces the risk of infection. The numerous side effects resulting from such invasive methods as the intracerebroventricular administration used for chemotherapeutics are eliminated, while other advantages include the increased bioavailability of the drug, the need for a reduced dosing, fewer side effects, and the avoidance of the hepatic first-pass metabolism [19]. The advantages of intranasal drug administration through nasal drops or nasal sprays include: 1) easy administration, requiring no significant medical training, 2) non-invasive route, 3) relatively rapid transduction to the central nervous system, 4) repeatable dosage, 5) portable drug packaging, 6) no requirement for medication changes, and 7) minimal systemic exposure [20].

#### *Disadvantages of intranasal drug delivery to the brain*

Despite these advantages, the delivery of intranasal drugs to the brain has several limitations that must be overcome when developing new formulations. First, the volume of administration per nostril in humans is limited (<200  $\mu$ L), preventing the intranasal administration of drugs that require high doses. Second, administered drugs can be easily lost due to mucociliary clearance, which prevents toxins, pathogens, and particles from entering the organism [21]. Third, many drugs are metabolized by enzymes in



**Figure 1.** Transport mechanism of therapeutics from nose to brain.

the nasal cavity and therefore these drugs must be protected from enzymatic degradation [22,23]. Various formulations have been developed recently, including emulsions, liposomes, inorganic and polymeric nanoparticles, nanostructured lipid carriers, and solid lipid nanoparticles [24]. In particular, formulations of nanostructured lipid carriers and solid lipid nanoparticles have been found to provide effective nose-to-brain transport and increase drug bioavailability by improving drug permeability, solubility, and stability, reducing enzymatic degradation, and prolonging drug effects [25].

#### Examples of Intranasal Administration

##### Insulin

Alzheimer's disease has recently been referred to as type 3 diabetes or diabetes of the brain. It has been suggested that anti-diabetic peptides, such as glucagon-like peptide-1 (GLP-1) receptor agonists and insulin enhance learning and memory through insulin receptor signaling and the consequent glucose uptake into hippocampal neuronal cells [26]. Thus, such anti-diabetic agents can be considered novel potential drug groups for the treatment of dementia. In general, drug delivery to the brain is significantly limited to the BBB, which is made up of astrocytes, microvascular endothelial cells, pericytes, and other

cells. Studies have also reported the intranasal administration of insulin in cases of early-stage dementia to enhance the therapeutic effect. Nasal administration is currently considered the ideal route for the delivery of peptides to the CNS, with many researchers reporting the efficient delivery of intranasally administered drugs to the brain through the BBB [27]. Intranasal administration of insulin with L-penetratin prevented the progression of mild cognitive dysfunction in a mouse model investigating dementia (senescence-accelerated prone mouse 8/SAMP8), although the same study found the treatment to be ineffective in preventing progressive cognitive dysfunction in aged SAMP8 [28]. Intranasal administration of insulin has been found to be effective in early-stage dementia. It is thought to be a promising strategy for the prevention of memory loss. Intranasal insulin delivery is the most commonly tested drug in randomized controlled drug trials for cognition, memory, and appetite control [29].

##### Cholecystikinin (CCK)

The intestinal and brain peptide cholecystikinin (CCK) was administered intranasally to test behavioral, cognitive, physiological, and motor effects in healthy adults [30].

In a study, the LPC (late positive complex) was observed to increase in women 30 min after CCK-8 administration, but not in men [31].

#### *Erythropoietin (EPO)*

Erythropoietin (EPO) is a glycoprotein hormone that is produced and released into the circulation mainly by the fetal liver and adult kidney, regulating erythropoiesis primarily in response to hypoxia. Erythropoietin has been tested as a neuroprotective factor in the prevention of amyloid accumulation in Alzheimer's disease and stroke. In a Phase I study, erythropoietin was found to be safe and well-tolerated in healthy subjects [32]. EPO can pass through the BBB through receptor-mediated transcytosis and can enhance its positive effects on the brain. Neuro-EPO is a 28 kD recombinant human glycoprotein. Since low sialic acid-containing EPO (Neuro EPO) is rapidly degraded by the liver, it's the preferred administration route in the nose as it does not induce EPO activity [33]. Recently, a phase I clinical trial reported that 0.5 or 1 mg of neuro-EPO administered intranasally every 8 h for 4 days was safe, causing no serious side effects [34]. Intranasally administered rhEPO can pass through the BBB and then into the brain parenchyma [35]. Compared to systemic administrations, intranasal administration avoids hepatic first-pass metabolism and reaches therapeutic drug levels of EPO in the CNS [20]. A previous study evaluating the therapeutic effects of rhEPO in mouse and rat cerebral ischemia models found intranasal delivery to be 10 times faster than the intravenous route [36]. A single intranasal administration of rhEPO (within 1 hour of injury) at a low dose produced histological neuro-repair in the hippocampal region [37]. Intranasal administration of rhEPO can thus be considered an alternative and promising approach to the treatment of ischemic stroke.

#### *Melanocortin*

Melanocortin is used to reduce body fat and promote lipid metabolism in animals and humans, and it has been suggested to have direct effects on the central nervous system. In an experiment observing the changes in cerebrospinal fluid levels of melanocortin following intranasal administration, higher levels of melanocortin were determined [38]. It was shown that body fat, weight, and plasma levels of insulin and leptin were decreased after the intranasal administration of melanocortin in humans [39].

#### *Glutathione*

Glutathione (GSH) deficiency and/or imbalance in the brain has been reported in the pathogenesis of brain disorders, including epilepsy, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, autism, bipolar disorder, multiple sclerosis, Parkinson's disease, and schizophrenia. Glutathione is depleted early in the presence of Parkinson's disease and its deficiency triggers mitochondrial dysfunction, oxidative stress, cell death, and impaired autophagy. In a previous study, intranasal glutathione was administered to patients with moderate

Parkinson's, and the glutathione levels in CSF were monitored using magnetic resonance spectroscopy (MRS). The intranasal administration of GSH was found to increase brain GSH levels, with increases continuing for at least one hour in Parkinson's patients. Also, in a survey of intranasal glutathione therapy in Parkinson's patients, participants reported that the treatment was effective and had no significant side effects [40]. However, there are studies reporting different results. A Phase I study comparing the intranasal administration of glutathione and placebo in Parkinson's patients recorded no significant difference between the patient and placebo groups [41].

#### *Perillyl alcohol (POH)*

The potent anticancer activity of perillyl alcohol (POH), a natural compound, has been proven in preclinical studies, although POH failed in subsequent clinical trials due to intolerable gastrointestinal side effects when administered orally. Surprisingly, with intranasal administration, POH exhibited highly promising activity and was well tolerated in patients with recurrent glioma. As of 2018, POH is the only intranasally administered compound that has progressed to active clinical trials in the field of cancer therapy (other than for cancer pain) [42]. Intranasal perillyl alcohol administration in humans was first used to induce tumor shrinkage in the treatment of anaplastic oligodendroglioma. Numerous clinical trials have been conducted in the treatment of oligodendrogliomas, multiple gliomas, recurrent glioblastomas, and astrocytomas with intranasal perillyl alcohol, the majority of which have been successful [43]. The ineffectiveness of orally administered perillyl alcohol has been observed. In vitro studies have shown that POH and its most stable metabolite, perilic acid (PA), can inhibit cell proliferation and inflammation by inducing apoptosis. POH is actively being researched in clinical trials, especially for the treatment of brain cancers. Due to the dose-limiting gastrointestinal toxicity of POH, alternatively it was administered intranasally.

A previous study compared the plasma and CSF concentrations of POH and PA following the intranasal and intravascular administration of POH. Samples were collected for 70 minutes, and levels were measured using high-pressure liquid chromatography (HPLC). The intranasal administration route was found to produce a 10-times greater CSF-to-plasma ratio for POH, and a 10-times greater CSF level for PA when compared to equal-dose intravascular administration. Preclinical results show that POH, when used as a nasal chemotherapeutic agent for brain cancer, is directly transported from the nasal mucosa to the CSF [44].

There is an urgent need for better treatments for glioblastoma patients, especially in cases of recurrence. Clinical trials in Brazil have shown that the intranasal administration of POH may be effective in patients. NEO100, a highly pure, pharmaceutical-grade version of POH, is a compound that has been assessed for safety and efficacy in Phase I/IIa clinical trial in the U.S. in which it was reported that intranasal delivery of NEO100 has been shown to be very well tolerated at all dose levels, and no severe adverse events were observed. The intranasal administration

of NEO100 was noted to be well-tolerated in the treatment of glioma and to increase survival [45].

### *Angiotensin II*

Intranasal administration of angiotensin II occurs following the blockade of peripheral receptors. In a study, plasma levels of angiotensin II were found to be increased following intranasal administration, while plasma levels of norepinephrine and vasopressin were unaffected, and there was an acute decrease in blood pressure. The significant neuroprotective effects of the angiotensin II receptor agonists on the treatment of ischemic stroke have been shown in many studies to date, however, the administration routes of agonists used in these preclinical studies – being direct intracerebroventricular and systemic administrations – are not suitable for translation into humans [46].

### *Neurotrophic factors*

The intranasal administration of neurotrophic factors has produced successful results in animal models, however, human trials of neurotrophic factors were not sufficient and these studies are limited to case studies. In a case study with traumatic brain injury, a four-year-old boy with unresponsive wakefulness syndrome was administered intranasal NGF for 10 days, after which the CSF levels of nerve growth factor were found to be increased. Clinically, improvements were observed in nutrition, urinary and intestinal functions, voluntary movements, crying and cough reflex, and oral motility [47]. Significant neuronal death and functional losses occur as a result of damage to the CNS. In *in vitro* experiments, it has been observed that neurotrophic factors such as ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), and neurotrophin-4/5 (NT-4/5) contribute to neuronal survival, although these large protein molecules cannot adequately pass through the blood-brain barrier, and therefore reaching the damaged CNS is difficult. The intranasal administration of 70 µg of [125I]-radiolabeled BDNF, CNTF, NT-4, and erythropoietin resulted in neurotrophin concentrations of 0.1–1.0 nM in the brain parenchyma over 25 minutes. There is currently no effective treatment for traumatic, ischemic, or compressive injuries of the CNS [20].

### *Chrysin*

A study was conducted to explore the potential therapeutic effects of chrysin on doxorubicin-induced cognitive impairment and the possible underlying mechanisms in rats. Doxorubicin was found to reduce memory acquisition, short-term memory, and spatial memory in mice. The formulation of chrysin as transfersomes and chitosan composite vesicles supports the considerable improvement of its therapeutic performance against doxorubicin-induced cognitive impairment in rats. Chrysin has low oral bioavailability due to its poor solubility and first-pass elimination in the gut and liver. The intranasal administration of chrysin-loaded vesicles in doses of 0.5 mg/kg exhibited the same or superior effects than orally administered chrysin at a dose of 30 mg/kg. It has been observed to improve cognitive abilities by correcting AchE

activity in the prefrontal cortices and hippocampi in rats. Chrysin vesicles also corrected doxorubicin-induced histological changes and neurodegeneration and were found to increase cholinergic transmission by affecting acetylcholinesterase and preventing oxidative stress and apoptosis [48].

### *Antiretrovirals (ARVs)*

The neuronal abnormalities associated with HIV (Human Immunodeficiency Virus) or NeuroAIDS (Neuro Acquired Immunodeficiency Syndrome) remain a major health problem among AIDS patients. NeuroAIDS occurs when HIV enters the CNS by damaging neurons, directly crossing the BBB, or via a peripherally infected macrophage. Few studies to date have explored the efficacy of intranasal antiretrovirals in the treatment of neuroAIDS. Due to the low bioavailability of the drugs used in antiretroviral therapy in the brain, managing neuroAIDS becomes very difficult, resulting in a lack of any decline in neuroAIDS cases globally [49]. Despite the use of highly effective antiretroviral (ARV) agents, the virus causes significant damage to the brain, as brain cells act as a shelter for the virus and promote its replication. The virus has the ability to cross the BBB in various ways, and also to accumulate in the brain by infecting its resident cells. The intranasal administration of ARVs to bypass the BBB has produced positive results. The intranasal route is patient compatible due to its non-invasiveness, and helps target drugs to the brain, resulting in greater concentrations of the drug in the brain than in the blood. Formulations can be modified to allow the drug targeting of specific receptors, and the increased contact time of the formulation with the nasal mucosa also helps to open tight junctions, thus facilitating paracellular passage [20].

### *Clinical Trials*

Many preclinical and clinical studies have been conducted investigating intranasal drug administration in neurological disorders. The efficacy and safety of compounds that have recorded positive results in preclinical studies have been clinically tested on volunteers. An analysis of international clinical studies revealed that 51% of the studies are to be completed, 28% are recruiting, and 9% have been terminated. In addition, 8% were unknown, and 4% were withdrawn. The withdrawn drugs include sphenopalatine, midazolam, chloral hydrate, oxytocin, and E-selectin. The drugs included in the studies were oxytocin (16%), insulin (11%), ketamine (4%), diazepam (4%), glutathione (3%), midazolam (3%), nerve growth factor (2%), dexmedetomidine (2%), vasopressin (2%), and other agents (53%).

Regarding the distribution of the studies by age groups, 11% were conducted with children aged 0–17, 50% with adults aged 18–64, and 39% with participants over the age of 65. Of these studies, 7% were supported by the National Institutes of Health (NIH), 2% by other U.S. federal agencies, 32% by industry, and 59% by others (individuals, universities, and organizations) [50].

All clinical studies conducted in Turkey were supported financially by universities and hospitals.

**Table 1.** Studies from Turkey on intranasal drugs for neurological complications [51].

Status	Study characteristics	Intervention/treatment	Purpose	Locations	Phase
Completed	60 participants Randomized	Dexmedetomidine Ketamine	Premedication and sedation of children	Erciyes University Hospital	Phase 4
Completed	40 participants Cohort	Dexmedetomidine	Reduction of preoperative anxiety in preschool children	Karaman Training and Research Hospital	-
Completed	78 participants Randomized	Midazolam Alfentanil Ketamine	Prevention of agitation in 1–8-year-old children	Yeditepe University Hospital	Phase 4
Completed	41 participants Randomized	Emthexate Prednol Mometasone furoate	Patients with treatment-resistant chronic rhinosinusitis with nasal polyposis	Dokuz Eylul University School of Medicine	Phase 2
Completed	140 participants Cohort	Dexmedetomidine	Delirium Pediatric disorderanesthesia	University of Health Sciences Turkey, Bursa Yuksek Ihtisas Training and Research Hospital	-
Completed	90 participants Randomized	Lidocaine Ketamine	Decrease in postoperative pain scores in infiltration anesthesia during rhinoplasty	Turgut Özal Medical Center	Phase 4

## Conclusion

As a result of many preclinical and clinical studies in which intranasal drug administrations have been tried, this drug administration approach has been shown to be safe and effective. Physiological barriers in the central nervous system and physicochemical drug properties such as drug molecule size, lipophilicity, and drug delivery apparatus can alter the efficiency of drug delivery to the brain. Newly developed intranasal formulations will help to expand and exploit the therapeutic potential of the intranasal pathway and will particularly contribute to the treatment of neurodegenerative disorders. Studies focused on clinical trials are studies on drugs used in central nervous system diseases and neurodegenerative diseases, which are frequently used by the elderly group. Another group of drugs is sedation and anxiety-relieving drugs used in pediatric patients before and after the operation. Since intranasal drug administration is non-invasive, it will be a good treatment approach for pediatric and geriatric patients who are sensitive populations. Therefore, the development of ideal nasal-to-brain intranasal drug administration strategies, especially for these two age groups, will provide ease of application in sensitive patient groups as well as increase therapeutic effectiveness.

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