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NOSE-TO-BRAIN DRUG DELIVERY FOR NEURODEGENERATIVE DISEASE: CRITICAL APPRAISAL OF EVIDENCE, TRANSLATIONAL BARRIERS AND STANDARDS FOR PRECLINICAL REPORTING

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ABSTRACT

Intranasal administration is widely presented as a route that circumvents the blood-brain barrier and delivers therapeutics directly to the degenerating brain. Three decades of preclinical work support the existence of olfactory, trigeminal and perivascular pathways from the nasal mucosa to the central nervous system, yet no product is approved for a neurodegenerative indication on the strength of direct nose-to-brain transport, and the largest completed trial in the field returned a null result confounded by a mid-study change of delivery device. This review appraises that gap. The mechanistic case for the route is examined against the anatomical evidence, and the quantitative preclinical literature is evaluated on its own terms. Reported drug targeting efficiency values for broadly comparable rodent intranasal studies span roughly 158% to 2362%, a spread that is not explained by the physicochemical properties of the compounds involved, and a standardised rat screening programme found blood and tissue ratios to be highly variable and not always reproducible. Systematic quantitative analysis of the published record indicates that particulate and gel-based carriers confer limited advantage over simple drug solution. Against this, human data are sparse but instructive: intranasal glutathione raised brain glutathione measured by magnetic resonance spectroscopy yet failed to outperform placebo in a randomised phase IIb trial, and intranasal oxytocin raised cerebrospinal fluid concentrations on a time course dissociated from plasma. The argument advanced is that the binding constraint on translation is evidentiary rather than technological. The field reports a metric it cannot standardise, in species whose nasal anatomy does not scale, using devices that have no clinical counterpart. A route-specific reporting standard is proposed as an extension of ARRIVE 2.0, covering dose placement, administration volume, anaesthetic state, systemic controls, analytical validation, absolute delivered fraction and device disclosure.

Keywords: administration; intranasal; blood-brain barrier; neurodegenerative diseases; drug delivery systems; olfactory pathways; translational medical research; guidelines as topic.

1. Introduction

The pharmacology of neurodegeneration is, to an uncomfortable degree, a pharmacology of exclusion. Compounds that engage their targets convincingly in cell culture reach the human brain at concentrations too low to matter, and the barrier responsible is not a single obstacle but a coordinated physiological system. Passive transcellular diffusion across the blood-brain barrier is effectively restricted to small, lipophilic molecules below about 400 Da, which excludes most peptides, proteins, oligonucleotides and a large share of rationally designed small molecules [1]. Efflux transporters remove much of what does cross. For diseases in which the therapeutic window is measured in years of preserved function rather than hours of symptom relief, the cost of that exclusion compounds.

Intranasal administration has been offered as the way around it since the early 1990s, and the rationale is anatomically sound. The olfactory epithelium is the only site in the body where neurons sit in direct contact with the external environment, and their axons pass through the cribriform plate into the olfactory bulb. A drug deposited on that epithelium has, in principle, a route into the central nervous system that does not require it to survive the systemic circulation or negotiate the endothelial barrier. The trigeminal nerve provides a second entry, to the brainstem and more caudal structures [2,3].

The principle is not in dispute. What is in dispute, or ought to be, is how much of an administered dose actually takes that route in a human being, and whether the preclinical literature is capable of answering the question.

The numbers suggest it is not. Published values for drug targeting efficiency, the standard index of brain-selective delivery, range across more than an order of magnitude for studies using similar compounds in the same species under broadly similar conditions [4-8]. A systematic quantitative analysis of seventy-three publications reporting eighty-two compounds found that delivery efficiency does not correlate with the physicochemical properties of the drug, which is close to saying that the index is not measuring a property of the drug at all [9]. A pharmaceutical research group running a standardised screening model in rats, with every incentive to identify promising candidates, concluded that blood and tissue concentrations and ratios were highly variable and not always reproducible [10].

Set against that, the clinical record is thin. Four intranasal products acting on the central nervous system have been approved in the United States in recent years, and none of them on a nose-to-brain mechanism; the manufacturer of one states explicitly that direct nasal-to-brain delivery was not studied and is not considered relevant to the product [11]. The single largest randomised trial of an intranasal agent in Alzheimer disease, the Study of Nasal Insulin to Fight Forgetfulness, produced a primary outcome of 0.03 points on a cognitive scale with a confidence interval straddling zero, and did so in a population defined mid-trial by a change of delivery device [12].

This review argues that these observations describe a single problem rather than several. The field's binding constraint is not the biology of the olfactory pathway, and it is not the chemistry of the carriers. It is that the evidence base cannot distinguish a formulation that genuinely delivers drug to the brain from one that was measured in a way that produced a flattering ratio. Until that is fixed, further preclinical studies will add volume without adding confidence.

The scope is deliberately restricted to neurodegenerative disease, principally Alzheimer disease and Parkinson disease, because these indications carry the translational burdens that make the problem acute: chronic administration over years, a requirement for parenchymal rather than merely cerebrospinal exposure, and elderly patients whose nasal physiology and device handling differ from those of the young volunteers in whom deposition studies are usually performed. Acute intranasal indications such as seizure clusters and migraine are treated only where they illuminate what the route can and cannot be relied upon to do.

Sections 2 and 3 appraise the mechanistic and preclinical evidence. Section 4 examines the human data. Section 5 sets out the translational barriers in order of severity. Section 6 proposes the reporting standard that the preceding sections argue is necessary.

2. What is actually established about the route

Three transport processes have reasonable experimental support, and they operate on different time scales and carry different cargo. Distinguishing them matters, because much of the confusion in the applied literature comes from treating nose-to-brain delivery as one thing.

2.1 Intraneuronal transport

Endocytosis into olfactory sensory neurons followed by axonal transport to the olfactory bulb is the mechanism most often drawn in review figures, and it is the slowest and least quantitatively important of the three. Axonal transport over the relevant distance takes hours [1]. For a therapeutic intended to produce a measurable central effect within a dosing interval, a pathway with that latency cannot account for early brain concentrations, and studies reporting peak brain levels at twenty to thirty minutes after dosing are therefore not describing intraneuronal transport, whatever their schematic figures imply [2].

2.2 Extraneuronal transport along perineural spaces

The pathway that does account for rapid appearance is extracellular. Drug crossing the olfactory or respiratory epithelium paracellularly enters the lamina propria and moves along channels surrounding the olfactory nerve bundles through the cribriform plate, reaching the subarachnoid space and olfactory bulb without ever entering a neuron. Thorne and colleagues demonstrated this for insulin-like growth factor I, a 7.65 kDa protein, which reached the rat central

nervous system within thirty minutes of intranasal dosing, distributed along both olfactory and trigeminal routes, and achieved higher central concentrations than an equivalent intravenous dose while retaining receptor-activating bioactivity [3]. The paracellular component of this route favours molecules below about 1 kDa [1], a constraint that applied papers frequently pass over when proposing the route for antibodies and other large biologics [13].

2.3 Perivascular convection

The third process most changes how the route should be understood, and it is the least represented in formulation papers. Lochhead and colleagues tracked fluorescent dextrans after intranasal administration in anaesthetised rats and found labelled tracer in the perivascular spaces of leptomeningeal vessels on the ventral and lateral brain surfaces approximately twenty minutes after dosing, with perivascular fluorescence in over eighty per cent of animals for a 10 kDa tracer given with matrix metalloproteinase-9 pretreatment [14]. Two features of that study deserve more attention than they receive. Distribution was tracer-size dependent and could be modulated by altering nasal epithelial permeability, which means the pathway is accessible to formulation. More importantly, matched intravascular dosing in separate animals did not reproduce perivascular access. That comparison is the strongest available internal control against the objection that apparent brain levels after nasal dosing simply reflect systemic absorption followed by redistribution.

Perivascular convection places intranasal delivery within the broader framework of cerebrospinal fluid and interstitial fluid exchange along vascular sheaths described for solute clearance [15]. The practical implication is that a drug entering by this route distributes by bulk flow along vessels rather than by diffusion outward from the olfactory bulb, which explains why tracers appear in caudal structures far faster than axonal transport permits.

It also undermines a common experimental shortcut. Cerebrospinal fluid sampling is often taken as evidence of central delivery, but entry into cerebrospinal fluid does not invariably precede brain parenchymal delivery after intranasal dosing [16]. Cerebrospinal fluid concentration is therefore a proxy of uncertain validity, and a study reporting only cerebrospinal fluid levels has not demonstrated parenchymal exposure. The point returns in Section 4, where it bears directly on how the human oxytocin data should be read.

3. The preclinical evidence, critically appraised

3.1 What drug targeting efficiency actually measures

Two indices dominate the applied literature. Drug targeting efficiency (%DTE) compares the ratio of brain to plasma exposure after intranasal administration with the same ratio after intravenous administration. Direct transport percentage (%DTP) estimates the fraction of brain exposure attributable to the direct route rather than to systemic absorption and subsequent brain entry.

Both are ratios of ratios, and that is the source of the trouble. A %DTE of 300% can arise because intranasal dosing delivered substantially more drug to the brain. It can equally arise because intranasal dosing delivered substantially less drug to the plasma, because the intravenous comparator was dosed at a rate producing an unrepresentative plasma profile, or because the terminal phase of the brain concentration-time curve was sampled too sparsely to estimate area under the curve reliably. Nothing in a reported %DTE distinguishes these. The index is also unbounded above, which is why values in the thousands appear in the literature without attracting editorial scrutiny.

A fifteen-fold spread across studies using the same species, the same route and the same index is not a biological finding. It is a measurement problem.

3.2 The evidence that it is a measurement problem

Two independent lines of work support that reading.

The first is Kozlovskaya and colleagues' quantitative analysis of the published record. Seventy-three publications reporting eighty-two compounds, published between 1970 and 2014, were assessed for %DTE and %DTP against their experimental settings, formulation types and analytical methods [9]. Two findings from that analysis have not been adequately absorbed. Delivery efficiency differed widely between studies and did not correlate with the physicochemical properties of the drug, which is difficult to reconcile with the index measuring a real transport process governed by molecular size, lipophilicity and charge. And particle-based and gel-based delivery systems offered limited advantage for brain delivery compared with intranasal administration of drug in simple solution. The authors' own conclusion was that more detailed methodological characterisation and standardised reporting were required before brain targeting by this route could be reliably quantified.

That was published in 2014. The reporting practices it criticised have not materially changed.

The second line is experimental rather than bibliometric. Dhuyvetter and colleagues ran a standardised in vivo screening model in rats across a set of structurally diverse small molecules, under conditions designed to remove the between-laboratory variation that confounds cross-study comparison. Blood and tissue concentrations and ratios were found to be highly variable and not always reproducible, and the authors concluded that establishing a reliable screening model for direct brain transport remains problematic, with outcomes dependent on analytical methodology [10]. This is the strongest available evidence that the spread in Table 1 reflects the assay rather than the formulations, because the spread persists when the assay is deliberately held constant.

3.3 The carrier question

Formulation science has supplied an extensive catalogue of intranasal carriers: solid lipid nanoparticles, nanostructured lipid carriers,

polymeric and chitosan nanoparticles, liposomes, transferosomes, nanosuspensions, thermosensitive and ion-sensitive in situ gels, and extracellular vesicles. The mechanistic justifications offered for them are plausible. Mucoadhesion extends residence time against mucociliary clearance. Cationic surface charge promotes interaction with the anionic mucus layer. Lipid matrices facilitate transcellular partitioning. Particle size can be tuned toward the range that deposits preferentially in the olfactory region.

The difficulty is that the evidence supporting these carriers is drawn almost entirely from the same %DTE and %DTP framework that Section 3.2 shows to be unreliable, and the one systematic attempt to evaluate carriers across the literature concluded that they add little over drug solution [9]. Where that analysis did identify a consistent benefit, it was for absorption enhancers, mucoadhesive components and targeting ligands rather than for particulate architecture as such.

Some recent work is considerably better. Lee and colleagues administered liposomal donepezil, memantine and BACE-1 small interfering RNA intranasally to APP/PS1 transgenic mice and reported substantial brain accumulation after intranasal dosing against only trace levels after intravenous dosing, with BACE-1 messenger RNA reduced ($p<0.01$), soluble and insoluble amyloid beta 40 and 42 reduced ($p<0.05$), and Y-maze spontaneous alternation restored from approximately 48% in untreated transgenic animals to approximately 78%, matching wild-type performance, where oral free drug reached only 64% and did not achieve significance [17]. That study is strong because it couples a delivery claim to a target engagement measure and a behavioural outcome, with an informative negative comparator. It is the shape the field should be aiming for.

Other frequently cited work is weaker than its citation count implies. Haney and colleagues' catalase-loaded macrophage exosomes for Parkinson disease report that a considerable amount of exosomes was detected in the brain following intranasal administration, alongside neuroprotection in vitro and in vivo, but the central delivery claim is qualitative and no targeting index, area under the curve ratio or quantified brain dose is given [18]. Ahmad and colleagues' levodopa chitosan nanoparticles report an approximately two-fold enhancement of absorption after intranasal dosing at 2.5 mg/kg in Wistar rats ($p<0.05$), but the measurement is of plasma concentration, with no brain sampling and no targeting index at all [19]. Neither observation is dishonest. Both are routinely cited as evidence of nose-to-brain delivery, which is not what either study measured.

3.4 The scaling problem, quantified

There is no equivalent for the intranasal central nervous system route of Wilhelm and colleagues' analysis of nanoparticle delivery to solid tumours, which found that a median of 0.7% of an administered nanoparticle dose reached the target across a decade of published work [20]. The absence of such an analysis is itself informative. The tumour figure reframed an entire field's

expectations by converting relative enhancement ratios into an absolute delivered fraction, and the intranasal literature has not undergone that conversion. Almost all reported outcomes remain ratios against a comparator rather than fractions of the administered dose, and a ratio can be large while the absolute quantity delivered is negligible.

One modelling estimate does attempt the cross-species conversion, and it is sobering. A computational model predicted that nasally administered nanomaterials reach the mouse brain in an amount roughly two orders of magnitude greater than that reaching the human brain [2]. If that estimate is approximately correct, a rodent study reporting a %DTE of 300% describes a delivery advantage that would largely disappear on translation, which is consistent with what the human trials in Section 4 actually show.

4. The clinical evidence

4.1 Intranasal insulin in Alzheimer disease

Insulin is the most thoroughly investigated intranasal agent in neurodegeneration, and its trial history is the most instructive failure in the field.

Early work was encouraging in a specific and qualified way. Acute intranasal insulin at 20 or 40 IU in twenty-six memory-impaired and thirty-five cognitively normal older adults facilitated verbal recall without altering plasma insulin or glucose, which is the pharmacokinetic signature a nose-to-brain advocate would predict [21]. The effect was confined to APOE ε4 non-carriers, with story recall improved at both 20 IU (p=0.0006) and 40 IU (p=0.0013), while ε4 carriers showed no improvement and performed worse on one memory measure. A subsequent twenty-one-day pilot in twenty-five participants receiving 20 IU twice daily reported improved delayed verbal recall (p=0.0374), attention (p=0.0108) and caregiver-rated function (p=0.0410), together with increased fasting plasma amyloid beta 40 (p=0.0471) and an increased amyloid beta 40/42 ratio (p=0.0207) [22].

The definitive test was the Study of Nasal Insulin to Fight Forgetfulness. Two hundred and eighty-nine participants with amnestic mild cognitive impairment or Alzheimer disease were randomised across twenty-seven sites to 40 IU insulin or placebo daily for twelve months, with a six-month open-label extension, and the primary outcome was change in the twelve-item Alzheimer's Disease Assessment Scale cognitive subscale [12].

What happened next is the part of the trial that matters here. The first forty-nine participants were dosed using an electronic nebuliser whose timer malfunctioned in some units, requiring repeated replacement, and whose reliability was ultimately judged unacceptable. The remaining two hundred and forty participants were dosed with a different device, a propellant-driven precision olfactory delivery system, and that group was designated the primary intention-to-treat population. In that population the twelve-month treatment difference was 0.0258 points (95% CI -1.771 to 1.822; P=.98). In the forty-nine participants dosed with the first device, the difference favoured insulin at twelve months (-

2.81 points; 95% CI -6.09 to 0.45; P=.09) and reached nominal significance at six months (-3.78 points; 95% CI -6.79 to -0.78; P=.01). No clinically important treatment-associated adverse events were observed.

The trial is usually cited as a negative result for intranasal insulin. It is more accurately a result that cannot be interpreted without knowing which device delivered drug to the brain, and that question was not answered. A primary analysis population redefined mid-trial around a device rather than around randomisation is a serious internal validity problem in its own right, and the small subgroup showing benefit is precisely the subgroup least able to support inference. The trial does not establish that intranasal insulin is ineffective in Alzheimer disease. It establishes that a trial of a delivery route cannot be interpreted when the delivery itself is not independently verified.

A subsequent four-week phase 2A/B factorial study of intranasal insulin at 40 IU four times daily and oral empagliflozin, using a third delivery system, randomised forty-seven participants and reported a modest advantage on the modified Preclinical Alzheimer Cognitive Composite for insulin-treated participants (0.10; 95% CI -0.05 to 0.26) against non-insulin-treated participants (-0.08; 95% CI -0.25 to 0.09; p=0.0432), with changes in diffusion tensor fractional anisotropy in parietal grey matter (p=0.0207) and global white matter (p=0.0085), and reduced plasma glial fibrillary acidic protein (p=0.0402) [23]. Three devices across one research programme is a fair summary of where the field stands on delivery hardware.

4.2 Intranasal glutathione in Parkinson disease, and the lesson it teaches

The glutathione programme is the most methodologically complete demonstration available of what intranasal delivery can and cannot achieve, because it measured central delivery and clinical effect separately.

Delivery was demonstrated convincingly. Fifteen participants with mid-stage Parkinson disease self-administered 200 mg of glutathione intranasally inside a magnetic resonance scanner, with brain glutathione quantified by spectroscopy before and after. Brain glutathione rose significantly overall (P<0.001), with no significant increase at eight minutes but concentrations above baseline at all later time points (P<0.01), reaching a mean increase of 269% in the glutathione-to-creatine ratio and 240% in absolute glutathione by the forty-five minute scan and remaining elevated for at least sixty minutes [24]. This is one of very few human studies to show a central concentration rise after intranasal dosing by direct measurement rather than by inference from a peripheral compartment.

Efficacy was not demonstrated. A phase I/IIa study of thirty participants at 300 or 600 mg daily for three months established tolerability, with no substantial between-group differences in adverse events, compliance or withdrawals [25]. The subsequent randomised, double-blind, placebo-controlled phase IIb study in forty-five participants at Hoehn and Yahr stages 1 to 3,

comparing 100 mg and 200 mg three times daily against placebo over three months, found that all cohorts improved, including placebo. The high-dose group improved from baseline on total Unified Parkinson's Disease Rating Scale score (-4.6 [4.7]; P=0.0025) and on the motor subscore (-2.2 [3.8]; P=0.0485), but neither active group was superior to placebo [26].

The sequence is the important thing. Drug reached the brain, the concentration rise was large and sustained, and the clinical endpoint did not move. Delivery is necessary for efficacy and is not sufficient for it. A field that has spent three decades optimising a delivery index has implicitly assumed otherwise.

4.3 What the oxytocin data do and do not show

Intranasal oxytocin is frequently cited as human proof that the route works. Striepens and colleagues sampled blood and cerebrospinal fluid in participants receiving 24 IU of intranasal oxytocin (n=11) or placebo (n=4) and found concentrations rose in both compartments [27]. The kinetics are the informative part. Plasma concentrations peaked at fifteen minutes and declined after seventy-five minutes, whereas cerebrospinal fluid concentrations took up to seventy-five minutes to reach a significant level, and there was no correlation between the two compartments ($r<0.10$).

Dissociated kinetics and absent correlation are consistent with a route other than simple equilibration from blood, which is a genuine and useful finding. They do not identify that route as olfactory or trigeminal transport, and the sample sizes are small, with four participants in the placebo arm. More fundamentally, cerebrospinal fluid concentration is not brain parenchymal concentration, and cerebrospinal fluid entry does not reliably precede parenchymal delivery by this route [16]. The study is good evidence that something reaches a central compartment. It is not evidence of the quantity reaching the tissue where a drug would need to act.

4.4 The approved products, and what their labels do not claim

Four intranasal products acting on the central nervous system have been approved in the United States in recent years: esketamine for treatment-resistant depression in 2019, midazolam for seizure clusters in 2019, diazepam for seizure clusters in 2020, and dihydroergotamine mesylate delivered by a precision olfactory device for acute migraine in 2021. None is approved on a direct nose-to-brain mechanism. The manufacturer of the esketamine product states that nose-to-brain delivery was not studied and is not considered relevant to the product, and reports population pharmacokinetic analysis indicating that after a 28 mg dose approximately 54% is absorbed across nasal tissue and 46% is swallowed [11].

The dihydroergotamine product is the most relevant of the four, because it targets the upper nasal space where the olfactory epithelium lies. Its pivotal open-label safety study dosed 354 patients over twenty-four weeks at 1.45 mg, with drug-related treatment-emergent adverse events in 36.7% and discontinuation for adverse events in 6.8%, and exploratory two-hour pain freedom

of 38.0% [28]. The benefit is attributed to more consistent and rapid systemic absorption from the upper nasal mucosa rather than to demonstrated direct transport, and the study was powered for safety with exploratory efficacy and no comparator.

The commercial success of nasal central nervous system products therefore rests on rapid systemic absorption that avoids first-pass metabolism. That is a real pharmaceutical advantage. It is a different advantage from the one the nose-to-brain literature is pursuing, and conflating the two overstates the clinical validation of the direct route.

5. Translational barriers

5.1 The anatomy does not scale

This is the largest single barrier and the least remediable by formulation.

In the F344 rat, approximately 50% of the nasal cavity surface is lined by olfactory neuroepithelium. In humans, olfactory epithelium occupies an area of about 500 mm², roughly 3% of a total nasal cavity surface of approximately 150 to 200 cm² [29]. The formulation of this contrast most often quoted in the delivery literature, that olfactory epithelium constitutes around half the nasal surface in rodents and under 10% in primates, traces to the same comparative source [16].

Two consequences follow. The target tissue is proportionally more than an order of magnitude smaller in humans, and it is anatomically less accessible, sitting high in the nasal vault above the path taken by inspiratory airflow and by most sprayed droplets. A rodent's nasal cavity is a comparatively simple conduit lined predominantly with the target epithelium; a human's is a complex, turbinate-partitioned airway in which the target is a small patch at the roof. No formulation change alters this. It is the most likely explanation for the estimated two-order-of-magnitude difference in nanomaterial brain delivery between mouse and human [2], and for the conclusion drawn from an earlier review of roughly one hundred pharmacokinetic reports, in which only two of twelve methodologically sound studies supported direct nose-to-central-nervous-system transport, both of them in rats, with the authors finding no pharmacokinetic evidence that nasal administration in humans enhances delivery to brain target sites relative to intravenous dosing [30]. The human cerebrospinal fluid studies that posed the same question directly reached similarly cautious conclusions [31].

5.2 The device discontinuity

Rodent intranasal dosing is typically performed by micropipette, in an anaesthetised, supine animal, with the dose placed directly on or near the olfactory region and with anaesthesia suppressing swallowing and mucociliary clearance. Humans receive drug from a spray pump while upright and conscious.

These are not versions of the same procedure. Conventional spray pumps deliver only around 5% of the emitted dose to the upper nasal space where the olfactory mucosa lies [2]. Deposition data compiled by Maaz and colleagues indicate

that a breath-powered bidirectional powder device achieved upper posterior nasal deposition of 18.3% (SD 11.5) against 2.4% (SD 1.8) for a conventional spray [32,33], while computational and in vitro estimates for olfactory region deposition cluster between approximately 4.6% and 9% for optimised conventional and nebulised delivery [32]. Even the better figures describe most of the dose landing somewhere other than the target. Particle size is one of the few levers that reliably moves deposition, with maximum olfactory deposition reported for particles in the 8 to 12 μm range [2].

The discontinuity is structural rather than incidental. Preclinical studies cannot use the devices used in humans, because of the size and anatomy of laboratory animals [2]. A preclinical result therefore encodes an administration method that will never be reproduced clinically, and the efficiency of that administration is silently embedded in every reported targeting index.

5.3 Dose ceiling and residence time

The administrable nasal volume in humans is restricted to roughly 25 to 200 μL before run-off and swallowing dominate [1]. For a potent small molecule this is tolerable. For a protein, a nucleic acid, or any drug requiring milligram-level central exposure, it is a hard ceiling that formulation can raise only by increasing concentration, which is limited in turn by solubility and by local tolerability.

Residence time compounds the constraint. Saccharin transit time in healthy adults has a mean of approximately 17 minutes (SD 8.4), with a median of 16 minutes and an interquartile range of 12 to 20 minutes [34], and non-mucoadhesive nasal solutions are cleared within roughly 15 to 30 minutes [35]. Mucoadhesive and in situ gelling systems are a rational response to this, and prolonging nasal residence is the component of formulation strategy with the clearest supporting rationale [9,36]. The gain is nonetheless measured against a short clock, and a two-fold or three-fold extension of a fifteen-minute window is a smaller therapeutic lever than the enthusiasm around gelling systems suggests.

5.4 Potency, and what the route can realistically carry

Taking the constraints above together, the route suits agents that are potent at low central concentrations, act on targets accessible to bulk perivascular distribution, and are otherwise excluded from the brain. Neuropeptides, growth factors and oligonucleotides fit that description. Conventional small molecules with adequate oral bioavailability generally do not, and a substantial fraction of the published intranasal literature concerns compounds for which the route offers no obvious advantage over a systemic one.

5.5 Chronic tolerability

Neurodegenerative indications require daily administration for years, and the nasal safety literature is overwhelmingly acute.

The distinction is not academic. In three-dimensional human nasal epithelial models dosed daily over two weeks, an undiluted mometasone nasal spray reduced ciliary beat

frequency in a dose-dependent and cumulative manner, becoming significant by day seven and progressing to ciliostasis and cell shedding, while a ten-fold dilution produced no significant impairment [37]. Cumulative effects differed substantially from what acute assessment predicted. That study examined a marketed corticosteroid spray rather than a permeation enhancer, and equivalent chronic data for the cationic polymers and absorption enhancers on which many intranasal formulations depend are not available. Since these excipients act by loosening epithelial tight junctions, the property that produces their delivery benefit is precisely the property that warrants chronic-exposure assessment. A formulation that damages the olfactory epithelium also degrades its own delivery route over time, which makes this a question of efficacy as well as of safety.

5.6 Regulatory position

The applicable guidance is not framed for this route. The relevant United States guidance on bioavailability and bioequivalence studies for nasal aerosols and nasal sprays addresses products intended for local action and has remained in draft since 2003 [38]. The European guideline on the pharmaceutical quality of inhalation and nasal medicinal products was revised in July 2025 and addresses pharmaceutical quality rather than central delivery [39]. Neither document establishes how a sponsor should demonstrate direct nose-to-brain transport in humans, which means there is at present no agreed evidentiary standard for the claim on which the field is built.

6. Standards for preclinical reporting

The preceding sections make a specific diagnosis, and it admits a specific remedy. The problem is not that intranasal studies are poorly conducted. It is that they are reported in a way that makes the central quantity unverifiable and cross-study comparison meaningless.

ARRIVE 2.0 provides the general framework for reporting animal research, with an Essential 10 covering study design, sample size, inclusion and exclusion criteria, randomisation, blinding, outcome measures, statistical methods, experimental animals, experimental procedures and results, and a further eleven recommended items [40]. Compliance with the Essential 10 is necessary for an intranasal study and is not sufficient, because none of those items captures what varies most between intranasal laboratories.

Dose placement and administration geometry. Whether drug was placed on the olfactory region or in the anterior nares, the number and volume of instillations, the interval between them, and the animal's head position together determine what fraction of the dose contacts the target epithelium. Two laboratories using the same formulation and different placement will obtain different targeting indices, and at present a reader cannot tell which was done.

Anaesthetic state. Anaesthesia suppresses swallowing and slows mucociliary clearance, extending residence time well beyond what a conscious animal permits. An intranasal study in an anaesthetised rodent measures a best case with no clinical counterpart, and the anaesthetic

agent and depth belong in the methods as experimental variables rather than as husbandry detail.

The systemic control. A %DTP value is interpretable only against a comparator establishing what brain exposure the same systemic profile would produce without nasal administration. The intravenous comparator's dose, infusion rate and sampling schedule should be reported in full, and where feasible the nasal cavity should be excluded from the systemic route so that the direct component is isolated rather than inferred. The matched intravascular control used by Lochhead and colleagues is the design to emulate [14].

Absolute delivered quantity. Every study reporting a targeting ratio should also report brain concentration as a percentage of administered dose per gram of tissue, or an equivalent absolute measure. This single requirement would do more than any other to correct the field's expectations, for the reason the tumour nanomedicine literature discovered when it made the same conversion [20]. A %DTE of 2362% and a delivered fraction of a hundredth of a per cent of dose are compatible statements, and only the second tells a reader whether the formulation is worth developing.

Four further recommendations follow from Sections 3 and 4 and concern study design rather than reporting.

Delivery claims should be coupled to target engagement. A study reporting brain concentration without evidence that the delivered drug did something to its target has demonstrated distribution, not therapy, and the glutathione programme shows how far apart those two can be [24,26]. Analytical methods should be validated in brain tissue specifically, with the vascular contribution to measured brain concentration addressed by perfusion or by correction, since analytical methodology was identified as a source of the variability observed under standardised conditions [10]. Sample sizes should be justified against the observed variability of the intranasal measurement rather than against convention, because that variability is large. And the simple drug solution, not an untreated control, should be the comparator for any novel carrier, since that is the comparison many carriers were found to fail [9].

None of this requires new technology. It requires the field to report what it did.

7. Discussion and future directions

The evidence supports a narrower and more useful conclusion than either enthusiasts or sceptics usually draw.

Direct nose-to-brain transport is real. The perivascular and perineural pathways are experimentally demonstrated with appropriate internal controls, human brain concentrations of an intranasally administered compound have been shown to rise by direct spectroscopic measurement, and cerebrospinal fluid kinetics after intranasal peptide dosing are dissociated from plasma kinetics in a way that systemic absorption alone does not explain [14,24,27]. Whether the route exists is not the question.

The question is capacity, and on capacity the evidence is discouraging in humans and unreliable in animals. Human olfactory epithelium is roughly 3% of the nasal surface against about half in the rat [29]. Conventional devices place around 5% of the dose in the upper nasal space [2]. The best available modelling estimate puts human brain delivery two orders of magnitude below mouse [2]. Meanwhile the preclinical literature reports targeting indices spanning fifteen-fold for comparable studies, does not correlate those indices with drug properties, and cannot reproduce them within a standardised model [9,10].

Four things would change the picture, and they are worth naming precisely.

An absolute-fraction analysis of the published intranasal record. The single most valuable contribution now available is a systematic recomputation of the literature in terms of percentage of administered dose delivered to brain tissue, in place of the ratio metrics. It requires no new experiments, only access to published concentration-time data, and it would establish whether the route is capable of clinically meaningful delivery at all. The tumour nanomedicine field was reoriented by precisely this exercise [20].

Human deposition imaging as a routine prerequisite. Gamma scintigraphic or equivalent imaging of the deposition pattern of the actual clinical device, in patients of the age and disease stage to be treated, should precede any trial premised on the direct route. Had this been done in the insulin programme, the device question that renders its central result uninterpretable would not have arisen [12].

A non-rodent stage before clinical commitment. Rodent studies are appropriate for mechanism and are poor predictors of human delivery efficiency, for anatomical reasons that are fully understood and will not change. A species with more human-like nasal architecture, interrogated with a device resembling the clinical one, would test the single variable that rodent work systematically flatters.

Payload selection matched to the route's real capacity. The route should be reserved for agents potent at low central concentrations that are otherwise excluded from the brain, which is where its theoretical advantage is largest and where no alternative exists. Neurotrophic factors, therapeutic peptides and oligonucleotides meet that description. Reformulating orally bioavailable small molecules for intranasal administration is a use of the route that its capacity does not justify.

The limitations of this review should be stated. It is a critical narrative synthesis rather than a systematic review, so the selection of studies reflects argumentative relevance rather than an exhaustive protocol-driven search, and the quantitative values in Table 1 are drawn from studies that the review itself argues are not reliably comparable. Several sources central to the argument were accessible in abstract and summary form rather than in full text, and a small number of deposition figures are cited as compiled in a secondary review rather than from the primary reports; these are identified at the

relevant points in the text and in the footnote to Table 1.

8. Conclusion

Intranasal administration reaches the central nervous system by pathways that are anatomically genuine and experimentally demonstrated. It does so in quantities that the preclinical literature has not credibly measured and that human anatomy and device performance suggest are small.

The field's central weakness is therefore evidentiary rather than technological. Drug targeting efficiency and direct transport percentage are ratios that can be large while the delivered quantity is negligible. They vary fifteen-fold across comparable studies, they do not track the physicochemical properties of the compounds they describe, and they are not reproducible within a standardised model. Three decades of formulation optimisation have been conducted against an index with these properties, which is why accumulated preclinical enthusiasm has not converted into a single approved product for a neurodegenerative indication.

Two decisions would put the field on firmer ground. Report absolute delivered dose alongside every ratio, and couple every delivery claim to a measure of target engagement. Both are available immediately, neither requires new technology, and together they would make the next three decades of work interpretable in a way the last three were not.

Declarations

Conflicts of interest: None declared.

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Abbreviations

ADAS-cog-12, twelve-item Alzheimer's Disease Assessment Scale cognitive subscale; ARRIVE, Animal Research: Reporting of In Vivo Experiments; AUC, area under the concentration-time curve; BACE-1, beta-site amyloid precursor protein cleaving enzyme 1; BBB, blood-brain barrier; CI, confidence interval; CSF, cerebrospinal fluid; %DTE, drug targeting efficiency; %DTP, direct transport percentage; IGF-I, insulin-like growth factor I; IU, international units; MRS, magnetic resonance spectroscopy; NLC, nanostructured lipid carrier; PLGA, poly(lactic-co-glycolic acid); POD, precision olfactory delivery; SLN, solid lipid nanoparticle; UPDRS, Unified Parkinson's Disease Rating Scale.

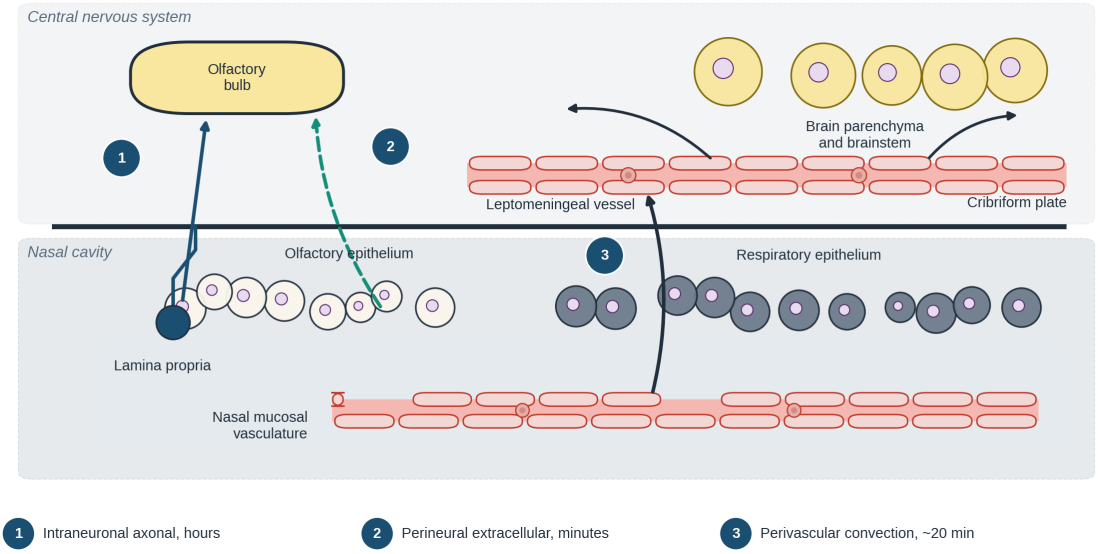

Figure 1. Three transport processes carry drug from the nasal mucosa to the central nervous system, and they differ by an order of magnitude in speed. Schematic of the nasal cavity (lower compartment) and central nervous system (upper compartment), separated by the cribriform plate. Route 1, solid navy: intraneuronal uptake into an olfactory sensory neuron followed by axonal transport to the olfactory bulb, which takes hours and therefore cannot account for brain concentrations measured at twenty to thirty minutes [1,2]. Route 2, dashed teal: extraneuronal movement through the olfactory epithelium into the lamina propria and along perineural channels traversing the cribriform plate to the subarachnoid space and olfactory bulb, occurring within minutes and favouring molecules below approximately 1 kDa through its paracellular component [1,3]. Route 3, solid dark: convection along perivascular spaces surrounding mucosal and then leptomeningeal vessels, distributing tracer to the brain surface and caudal structures by roughly twenty minutes, and not reproduced by matched intravascular dosing [14]. The olfactory epithelium is drawn as a discrete patch and the respiratory epithelium as the larger surface, reflecting the human proportions quantified in Figure 2a rather than the rodent proportions in which most of these routes were characterised [29]. The schematic is not to anatomical scale and the two vascular beds are drawn in a single colour because they represent the same structure rather than a colour-coded contrast. Time courses are those reported in the cited studies.

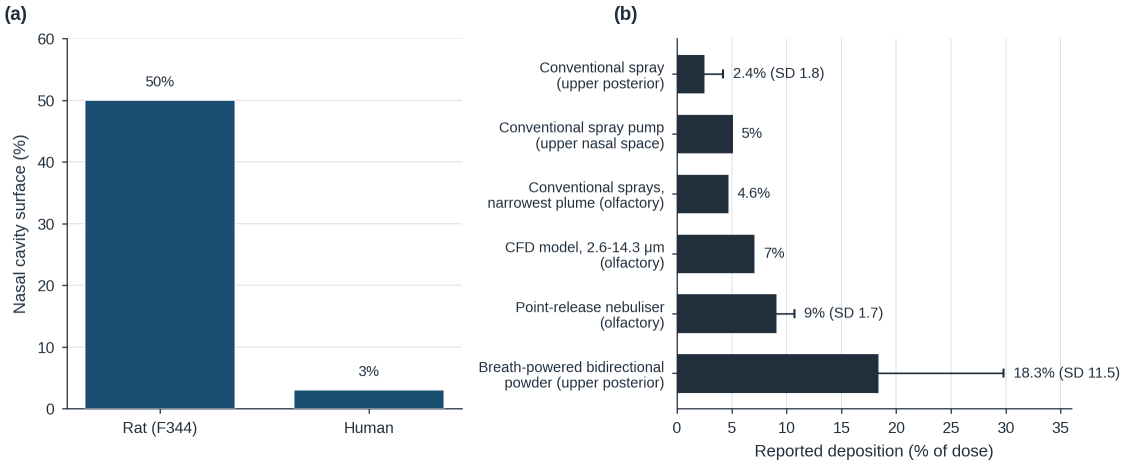

Figure 2. The two quantities that most limit translation, olfactory epithelial area and device deposition, are both small in humans. (a) Olfactory neuroepithelium as a percentage of total nasal cavity surface area in the F344 rat and in humans; the human value corresponds to an absolute area of approximately 500 mm² against a total nasal cavity surface of approximately 150 to 200 cm² [29]. (b) Reported deposition to the upper nasal

space or to the olfactory region specifically, by delivery method, expressed as a percentage of the administered or emitted dose. Bars show the reported point estimate; horizontal lines are standard deviations where the source reported dispersion, and their absence indicates a single reported value or a modelled estimate rather than a measurement without variability. The parenthetical text in each label states which region the figure refers to, because upper nasal space and olfactory region are not interchangeable. Values are from a published compilation of in vitro and computational deposition studies [32], from the primary breath-powered device comparison reported therein [33], and from a review reporting conventional spray pump performance [2]. Sources differ in method, particle size range and anatomical model and are presented together to show the range rather than to support direct statistical comparison between them.

Representative intranasal studies relevant to neurodegenerative indications, ordered by reported drug targeting efficiency. %DTE, drug targeting efficiency, the ratio of brain to plasma exposure after intranasal dosing divided by the same ratio after intravenous dosing; %DTP, direct transport percentage, the estimated fraction of brain exposure attributable to direct nose-to-brain transport; AUC, area under the concentration-time curve; NLC, nanostructured lipid carrier; PLGA, poly(lactic-co-glycolic acid); SLN, solid lipid nanoparticle; IN, intranasal; IV, intravenous. The final column identifies what each study does not report, which is the basis of the argument in Section 3. No study listed reports brain concentration as a fraction of the administered dose. Values are as stated by the cited authors and have not been recalculated; the studies are not directly comparable, and Section 3.2 argues that this is the point rather than a limitation of the table.

Table 1: Reported brain-targeting outcomes vary by more than an order of magnitude across intranasal studies that share species, route and metric.

Payload	Carrier	Model	Reported outcome	Not reported
Haloperidol [8]	SLN	Wistar rat	%DTE 2362.43; %DTP 95.77; brain/blood ratio at 0.5 h 1.61 (IN-SLN) vs 0.031 (IV)	Absolute delivered fraction; target engagement
Resveratrol [7]	Ion-sensitive in situ gel, nanosuspension	Mouse	%DTE 458.2; %DTP 78.18; 2.88-fold bioavailability increase	Absolute delivered fraction; behavioural outcome
Rasagiline mesylate [6]	Transferosomal in situ gel	Wistar rat (n=66)	%DTE 304.53; %DTP 67.16; brain bioavailability 131.17%; nasal mucosa histologically unaffected	Absolute delivered fraction; efficacy endpoint
Tarenflurbil [5]	PLGA nanoparticle	Sprague-Dawley rat	%DTE 287.24; %DTP 65.18	Absolute delivered fraction; target engagement
Tarenflurbil [5]	SLN	Sprague-Dawley rat	%DTE 183.15; %DTP 45.41	As above
Charge-series comparison, payload not extracted here [4]	Anionic NLC in situ gel	Wistar rat	%DTE 158.5, the highest of the surface-charge series; cationic bioavailability 77.3%	Absolute delivered fraction; disease model

Donepezil, memantine, BACE-1 siRNA [17]	Liposome	APP/PS1 mouse (n=5-6 per group)	Substantial brain accumulation after IN vs trace after IV; BACE-1 mRNA reduced ($p<0.01$); amyloid beta 40 and 42 reduced ($p<0.05$); Y-maze alternation restored from ~48% to ~78% ($p<0.05$) vs 64% for oral free drug (not significant)	%DTE and %DTP; absolute delivered fraction
Catalase [18]	Macrophage-derived exosome	Parkinson disease mouse model	Qualitative statement of considerable brain exosome detection; neuroprotection in vitro and in vivo	Any quantitative delivery metric
Levodopa [19]	Chitosan nanoparticle (553 ± 52 nm, zeta +46.2 mV)	Wistar rat, 2.5 mg/kg	Approximately two-fold enhancement of absorption ($p<0.05$)	Brain concentration; %DTE and %DTP; the measurement is of plasma only

Each item names a variable that differs materially between laboratories, is rarely reported, and plausibly contributes to the between-study spread documented in Table 1. Items 1 to 6 concern administration, items 7 to 10 concern controls and analysis, items 11 to 14 concern interpretation and disclosure. The rationale for items 1, 3, 7 and 9 is developed in Section 6. ARRIVE, Animal Research: Reporting of In Vivo Experiments [40].

Table 2: A route-specific reporting extension for intranasal central nervous system delivery studies, to be applied in addition to the ARRIVE 2.0 Essential 10.

No.	Item	What must be stated
1	Dose placement	Anatomical site of deposition, whether anterior nares or olfactory region, and how placement was confirmed
2	Administration volume and schedule	Volume per instillation, number of instillations per nostril, interval between them, and total volume as a proportion of nasal cavity capacity for the species
3	Anaesthetic state	Whether animals were conscious or anaesthetised, the agent and depth used, and the interval between induction and dosing
4	Body and head position	Posture during and after dosing, and duration held
5	Delivery method	Instrument used, including pipette tip dimensions or device specification, and emitted dose accuracy
6	Formulation properties at administration	pH, osmolality, viscosity and, for gelling systems, the gelation condition and time under nasal conditions
7	Systemic comparator	Intravenous or other systemic route dose, infusion rate and sampling schedule, and whether the nasal cavity was excluded from that route
8	Tissue handling	Whether animals were perfused before brain collection, and how residual vascular content was corrected for
9	Absolute delivered quantity	Brain concentration as percentage of administered dose per gram of tissue, or an equivalent absolute measure, reported alongside any ratio metric
10	Analytical validation in matrix	Validation of the assay in brain tissue specifically, with lower limit of quantification and recovery

11	Sampling adequacy	Number and spacing of time points, and the proportion of AUC extrapolated beyond the last measured concentration
12	Carrier comparator	Comparison against the drug in simple solution by the same route, not against an untreated control alone
13	Target engagement	A pharmacodynamic or molecular measure demonstrating that delivered drug acted on its target
14	Translational disclosure	Explicit statement of the olfactory epithelium proportion in the species used, and of the expected direction and magnitude of change on translation to human nasal anatomy

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