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Olfactory Drug Delivery in Rodents: Deposition and Pharmacokinetics

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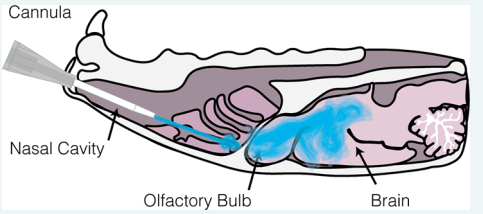
ABSTRACT: Olfactory drug delivery (ODD) is a route of administration that precisely targets the olfactory cleft, a region of the upper nasal cavity with privileged access to the brain. ODD offers better brain delivery compared to traditional intranasal (IN) delivery (e.g., nasal spray), which has imprecise, off-target drug deposition to the lower nasal cavities, with very little (if any) deposition at the olfactory region. ODD has immense potential for the noninvasive delivery of medication to the brain for the treatment of mental health and neurological disorders. Previously, we defined successful ODD as deposition of at least 50% of a dose to the olfactory cleft compared to the surrounding nasal cavities, assessed with a nasal endoscope. In this study, we investigate whether ODD is associated with increased brain uptake and compare the ODD, IN, and intravenous (IV) routes of administration across a range of compounds. First, using positron emission tomography (PET) imaging in rats, we demonstrate that ODD consistently targets the olfactory cleft, facilitating the brain uptake of fluorodeoxyglucose and fluorothymidine (FLT). These results successfully replicate previous work demonstrating direct nose-to-brain delivery of the poor blood−brain barrier penetrating molecule FLT. Additionally, we evaluated compounds that were selected for diversity of physiological properties and indications and demonstrated that for certain molecules, ODD offers superior brain delivery compared to IN and IV and demonstrates greater consistency than traditional IN administration. Remarkably, the volume of liquid required for ODD is approximately an order of magnitude smaller than that for the IN or IV methods, reducing variability in dosing, off-target deposition, and cost. These findings suggest that adopting the ODD method could enhance the accuracy, precision, and reproducibility of dosing across intranasal drug delivery applications. Importantly, ODD offers a consistent (less variable), accurate, and targeted method for improving the deliverability of medication to the brain.



INTRODUCTION

Mental health disorders are among the top 10 leading health conditions worldwide, and they have seen a dramatic increase in incidence since the onset of the COVID-19 pandemic.[1,2] Olfactory drug delivery (ODD) is a promising pain-free, needleless method for administering medication to the central nervous system (CNS) that many consider the next frontier of mental-health and neurological treatment options.[3−5] Previously, most intranasal (IN) devices (including spray bottles, pumps, nebulizers, droppers, etc.) have suffered from inconsistent dosage, varying widely in their clinical success.[6−9]

New research suggests that olfactory drug delivery (ODD) provides optimal brain uptake by targeting the olfactory cleft (OC): a region of the upper nasal passages where the olfactory nerve protrudes through the skull.[10−12] Medication deposited at the olfactory cleft may enter the brain through various pathways including axonal transport, bulk flow and perivascular pumping,[13] lymphatic drainage,[14] and endothelial transport through the olfactory nerve (for review, see refs 15 and 16). Previously, we defined successful ODD as deposition of at least 50% of a dose to the olfactory cleft, compared to the surrounding nasal cavities, assessed with a nasal endoscope.[17] In this study, we used a targeted method for olfactory drug delivery (ODD) (developed by Flamm et al.[18]) and compared it to standard intranasal delivery (IN) and intravenous delivery (IV) across an array of compounds with diverse targets.

Nasal sprays, which are the most common devices for IN drug delivery, suffer from several limitations. The volume of medication that can be deposited intranasally in humans is limited to about 1 mL.[5] When liquid is sprayed into the nose, only a small fraction of the already small dose reaches the olfactory cleft, with the remaining dose being swallowed, being inhaled, or dripping back out of the nose.[3] Sprays produce a turbulent plume of medication, and the precise deposition pattern is dependent on a variety of factors including the

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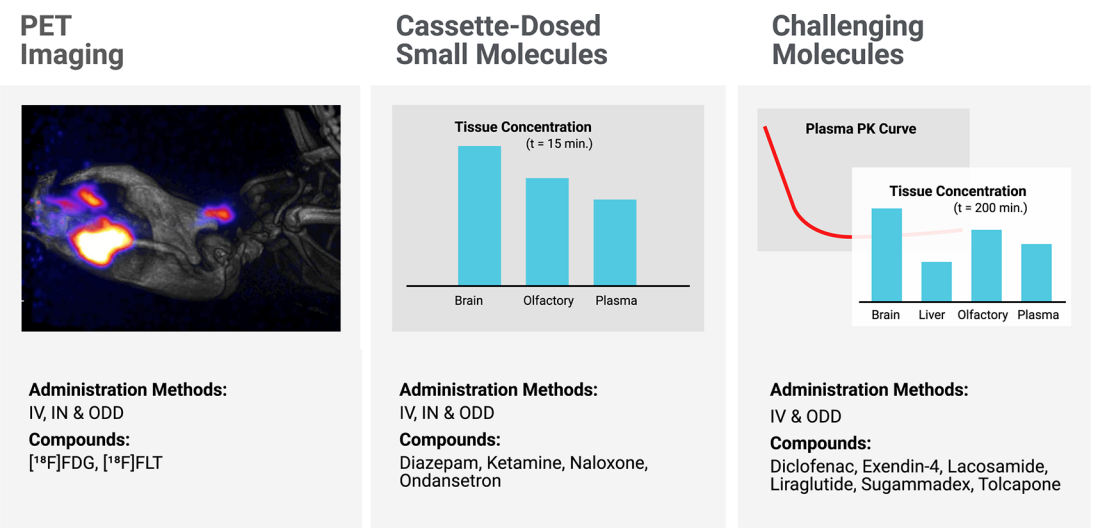

Figure 1. Outline of experimental procedures. Three primary experiments were conducted. First, PET imaging confirmed brain uptake via ODD using [^{18}F]FDG and [^{18}F]FLT. Second, the levels of ODD, IN, and IV administration were compared using four cassette-dosed small-molecule compounds. Finally, ODD and IV administration methods were compared using six challenging molecules separately administered. In the final experiment, detailed pharmacokinetics in plasma was also assessed.

Table 1. Compounds Investigated

experiment	compound	dose	crosses BBB?	description
deposition imaging and accumulation of [^{18}F]FDG	[^{18}F] fluorodeoxyglucose	~10 MBq	yes	radiolabeled glucose (marker of glucose metabolism)
	[^{18}F] fluorothymidine	~10 MBq	poorly	radiolabeled thymidine (marker of cellular proliferation)
PK analysis of cassette-dosed small molecules	ketamine	5.0 mg/kg	yes	dissociative anesthetic
	naloxone	0.5 mg/kg	yes	opioid antagonist
	diazepam	2.0 mg/kg	yes	anxiolytic benzodiazepine
	ondansetron	1.0 mg/kg	poorly	5HT$_3$ receptor antagonist; combats nausea induced by cancer chemotherapy
detailed analysis of ketamine PK	ketamine-HCl	5.0 mg/kg	yes	dissociative anesthetic
	ketamine-DMSO	5.0 mg/kg	yes	dissociative anesthetic in DMSO solvent
assessment of ODD for challenging molecules	diclofenac	5.0 mg/kg	yes	NSAID pain relief
	exendin-4	5.0 mg/kg	yes	GLP-1 receptor agonist; antidiabetic
	lacosamide	1.0 mg/kg	yes	enhances slow inactivation of voltage-gated sodium channels; treats partial-onset seizures
	liraglutide	1.0 mg/kg	yes	GLP-1 receptor agonist; antidiabetic
	sugammadex	5.0 mg/kg	poorly	neuromuscular reversal drug; reverses neuromuscular blockade elicited by muscle relaxants (rocuronium, vecuronium)
	tolcapone	0.25 mg/kg	yes	COMT inhibitor; symptom management in Parkinson's disease

angle/depth of insertion, plume geometry, and breathing patterns.[19] With the wrong setup, sprayed medication can be deposited in the lower nasal passages, failing to reach the brain. Our group has recently conducted proof-of-concept work for a laminar-fluid ejection method in humans, in which a device is inserted at a consistent angle and depth, depositing a steady stream of fluid at the olfactory cleft and optimizing nose-to-brain drug delivery.[17] The current work presents a set of studies to test focal olfactory drug delivery in rodents using a cannula method analogous to our human device across an array of compounds.

Traditionally, IN administration of medication in rodents has been conducted using a standard pipet tip that is inserted just past the nostril opening. Like a traditional nasal spray in humans, the rodent pipet method is highly variable and deposits compound throughout the lower nasal cavities.[12] The traditional method also poses safety risks to rodents; they can only breath through their nose and may asphyxiate if the deposited compound blocks their airway. Previous work has proposed a new olfactory drug delivery method using a 0.699 mm diameter neonatal cannula to deposit medication directly to the olfactory cleft in the upper nasal cavities.[12,18] The primary goal of this study was to test whether the ODD of small molecules performed comparably to IV administration in delivering drugs to the brain. The experiments we conducted are summarized in Figure 1 and Table 1. First, in an experiment modeled after Ponto et al. (2017),[20] we performed in vivo PET/CT imaging to validate that the ODD method delivers the compound to the brain via the olfactory cleft. Next, we compared the ODD, IN, and IV methods using cassette dosing of four compounds: ketamine, naloxone, diazepam, and ondansetron. The four compounds were selected because they are common therapeutics with known targets in the brain that have been previously studied in the context of intranasal drug delivery in both humans and animals.[21−25] In a final series of experiments, we tested whether ODD exhibited noninferiority

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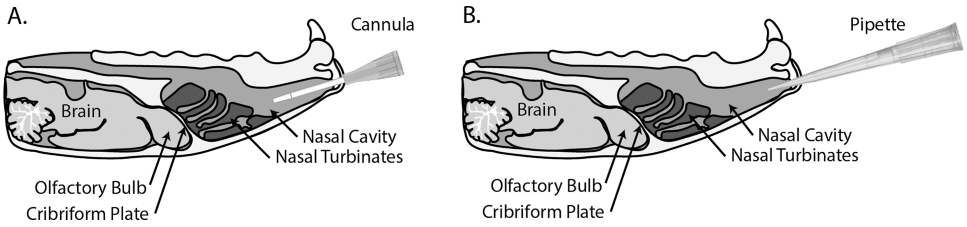

Figure 2. Routes of administration. (A) Direct deposition to the olfactory cleft (ODD) using a 0.699 mm diameter cannula. This simulates the laminar fluid ejection method, which has been prototyped in human subjects. (B) Intranasal delivery (IN) to the lower nasal cavity using a p200 pipet tip. This simulates traditional nasal sprays.

to IV delivery using six additional compounds with a range of targets (sugammadex, lacosamide, diclofenac, liraglutide, exendin-4, and tolcapone).[26−28] Results from these experiments suggested that ODD may offer comparable or even superior delivery when compared to IV delivery for a variety of compounds.

Altogether, our results suggest that the focal deposition of medication to the olfactory cleft optimally delivers compounds to the central nervous system. ODD provides a promising method for more consistent and efficient nose-to-brain drug delivery.

RESULTS AND DISCUSSION

In this study, we conducted a set of experiments to investigate whether the ODD provided superior brain uptake when compared to traditional IN and IV methods. First, using PET/CT imaging, we validated that the ODD method was capable of delivering a radiotracer to the olfactory cleft and into the brain. Next, we tested four cassette-dosed small molecules using the ODD, IV, and IN administration methods. Finally, we investigated the pharmacokinetics of more "challenging" molecules (e.g., larger molecular weight and failed brain delivery via oral route). Figure 2 displays a schematic of the IN and the ODD routes of administration. ODD administration was conducted by inserting a 0.699 mm diameter cannula into the upper nasal cavities and depositing the compound in a streamlined bolus. IN administration was conducted by inserting a p200 pipet tip into the lower nasal cavities and depositing the compound in a diffuse bolus. The IN method is meant to emulate standard nasal sprays used in humans, which are inserted to the lower nasal cavities and deposit medication via a dispersed plume or mist. IV administration was conducted via tail-vein injection.

Deposition Imaging and Accumulation of [^{18}F]FDG and [^{18}F]FLT. First, we conducted a PET/CT imaging study to investigate whether the onset of ODD was associated with region-specific brain uptake of a compound. Twenty-seven female Sprague−Dawley rats were used for this study. Each animal was assigned to one of six conditions determined by the route of administration (ODD, IN, and IV) and the compound that was administered ([^{18}F]fluorodeoxyglucose (FDG) and [^{18}F]fluorothymidine (FLT), see Methods for details). [^{18}F]FDG is radiolabeled glucose and readily crosses the blood−brain barrier. [^{18}F]FLT is radiolabeled thymidine and acts as a marker of cellular proliferation. [^{18}F]FLT is typically used to image tumor proliferation and minimally crosses the intact blood−brain barrier.[29,30] Even with access to the brain, [^{18}F]FLT is not expected to accumulate in healthy brain tissue, where there is little proliferation of cells.[31,32]

Time−activity curves for three regions of interest (frontal, occipital, and control) are displayed in Figure 3. Thin transparent lines and points represent time−activity curves from individual animals. Thick solid lines represent a logarithmic model ($y = a \log(bx)$) fit to the group data. The gray area around the curve represents the 95% confidence interval with respect to the fitted model. Lines and points are color-coded by the route of administration: green = intranasal/pipet (IN), orange = intravenous (IV), and blue = direct olfactory drug delivery via cannula (ODD). Panel A shows the results for [^{18}F]FDG imaging, and panel B shows the results for [^{18}F]FLT imaging.

Results from [^{18}F]FDG imaging demonstrated that all three administration methods (IV, IN, and ODD) resulted in significant delivery to frontal and occipital brain regions (confidence intervals for model curves did not overlap with zero). IV showed a significantly higher uptake in both regions compared to ODD and IN administration (confidence intervals for model curves did not overlap). ODD administration showed a significantly higher uptake compared to IN administration (confidence intervals for model curves did not overlap). In the control ROI, which was located outside of the brain, increased signal was observed in two animals during ODD administration. Notably, these two time−activity curves show greater noise, and the model fit for ODD shows a broader confidence interval than the other time−activity curves, leading us to interpret this as artifact. Biodistribution data measured after the scan demonstrated that IV administration provided the best delivery to the blood (IV vs IN: $T(4,5) = 3.86$, $p < 0.05$; IV vs ODD: $T(4,5) = 1.67$, $p < 0.05$), brain (IV vs IN: $T(4,5) = 13.8$, $p < 0.05$; IV vs ODD: $T(4,5) = 7.47$, $p < 0.05$), and cerebrospinal fluid (IV vs IN: $T(4,5) = 4.56$, $p < 0.05$; IV vs ODD: $T(4,5) = 2.91$, $p < 0.05$). Additionally, ODD provided better delivery compared to IN delivery in both the blood ($T(5,5) = 2.32$, $p < 0.05$) and brain tissue ($T(5.5) = 6.66$, $p < 0.05$). We note that because animals were not transcardially perfused prior to measuring biodistribution data, the measures of the radiotracer in the brain likely include signal from the blood. These results demonstrated that while IV administration remains superior for delivering [^{18}F]FDG to the brain, the ODD method performs better than the traditional IN method. These findings were consistent across dynamic PET imaging and biodistribution data acquired after the conclusion of the PET scan.

Results from the [^{18}F]FLT imaging demonstrated that both ODD and IN administration showed significantly greater signal in the frontal ROI compared to IV administration (model fits show nonoverlapping confidence intervals), with ODD administration showing the highest delivery, particularly at early time points (<20 min). In the occipital ROI, there were no significant differences in the signal observed across administration methods. Results from the control ROI demonstrated that the signal observed in the brain following

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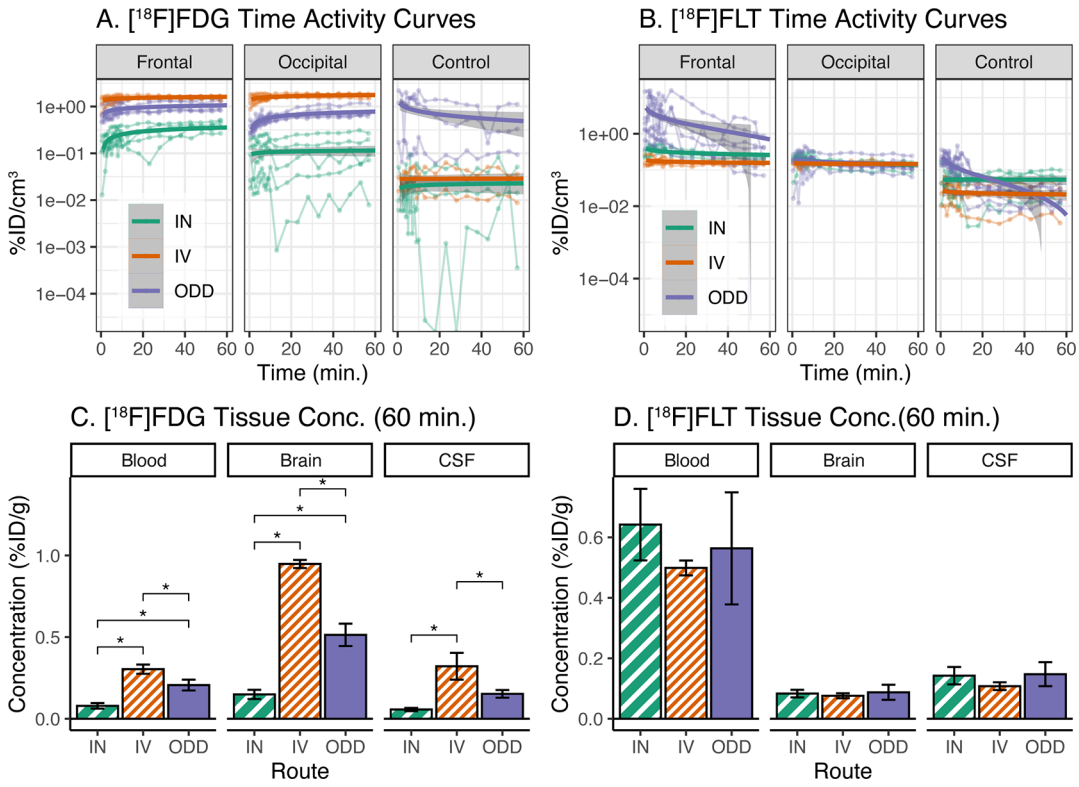

Figure 3. PET imaging results. (A, B) Time−activity curves show the percent injected dose per cm^3 of (A)[^{18}F]FDG and (B)[^{18}F]FLT. Transparent lines and points represent time−activity curves for individual animals. Group averages are plotted in thick solid and dashed lines, surrounded by the 95% confidence interval (gray). (C, D) Biodistribution data at t = 60 min are plotted. The height of each bar represents the group mean. Error bars represent standard error (* = $p < 0.05$, t test, Holm−Bonferroni adjusted for multiple comparisons). IN = intranasal delivery via pipet (striped green), IV = intravenous delivery (thin striped orange), ODD = olfactory drug delivery (solid blue), and CSF = cerebrospinal fluid.

ODD was likely not due to partial volume effects (note the different scales on the y axis of plots in Figure 3b). Biodistribution data measured after the scan demonstrated no significant difference in tissue uptake across the three routes of administration in the blood (IV vs IN: $T(4,5) = -1.33$, $p = 0.60$; IV vs ODD: $T(4,5) = -0.60$, $p = 0.95$; ODD vs IN: $T(5,5) = -0.73$, $p = 0.95$), brain (IV vs IN: $T(4,5) = -0.068$, $p = 1.0$; IV vs ODD: $T(4,5) = -0.107$, $p = 1.0$; ODD vs IN: $T(5,5) = 0.039$, $p = 1.0$), or CSF (IV vs IN: $T(4,5) = -0.32$, $p = 1.0$; IV vs ODD: $T(4,5) = -0.37$, $p = 1.0$; ODD vs IN: $T(5,5) = 0.04$, $p = 1.0$). These results demonstrated that no brain deposition was observed following the IV method. However, some frontal brain deposition may have occurred at early time points following ODD and IN administration (with the ODD method appearing to deliver the compound an order of magnitude higher than the IN method).

Ponto et al. (2017)[20] conducted a similar set of experiments comparing [^{18}F]FDG and [^{18}F]FLT administered via IV and IN routes. Those experiments found that both IV and IN administration of [^{18}F]FDG resulted in sustained brain uptake. For [^{18}F]FLT, however, brain uptake was observed only during early time points following IN administration. Interestingly, while both Ponto et al. and our study suggest a slight increase in brain uptake of [^{18}F]FLT following IN administration (compared to IV), our study showed superior frontal brain uptake of [^{18}F]FLT following ODD. Our results with ODD demonstrated increased [^{18}F]FLT signal in frontal brain regions at early time points and near-complete washout of the [^{18}F]FLT from the brain after the first 20 min of scanning (2017). The washout of [^{18}F]FLT was expected due to the low levels of cellular proliferation in healthy brain tissue. In our biodistribution data (measured at t = 60 min), we did not observe significant differences in brain tissue concentration across the three administration methods, which we attribute to the early washout of [^{18}F]FLT. Future experiments that measure biodistribution at earlier time points could confirm our PET imaging findings of increased [^{18}F]FLT in the brain at early time points. Additionally, because animals were not transcardially perfused prior to measuring biodistribution data, the measures of radiotracer in the brain likely include a signal from the blood. Overall, the results from Ponto et al.—including the timing and observed uptake (injected dose/mL)—are remarkably similar to the results from our study. It is possible that the ODD method outperforms the IN method both in our own study and compared to the previous work from Ponto et al. (2017).

Pharmacokinetic Analysis of Cassette-Dosed Small Molecules. In an initial experiment using cassette-dosed small molecules, we compared three routes of administration (ODD, IN, and IV) for the simultaneous delivery of four compounds (ketamine (5.0 mg/kg), naloxone (0.5 mg/kg), diazepam (2.0 mg/kg), and ondansetron (1.0 mg/kg)) to the brain, olfactory bulb, and plasma. The solution was dosed at 25 μL single nostril for the ODD and IN route or diluted in DMSO for IV dosing at 100 μL/animal injected to the tail vein. A follow-up experiment to test the effects of compound viscosity was also conducted (see the Supporting Information). Nine male Sprague−Dawley rats (weight 150−200 g) were used for this

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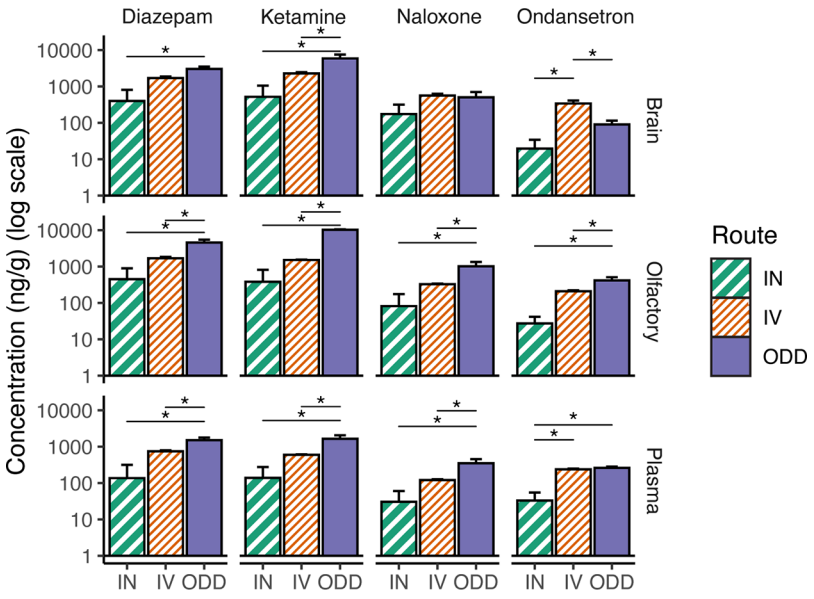

Figure 4. Comparing routes of administration. Concentration of diazepam, ketamine, naloxone, and ondansetron in the brain, olfactory bulb, and plasma 15 min after intranasal (striped green), intravenous (thin striped orange), or olfactory drug delivery (solid blue). The height of each bar represents the mean across animals, plotted on a log scale (n = 3 per route of administration). Error bars represent standard error. The asterisk indicates $p < 0.05$ for an unpaired t test, Holm−Bonferroni corrected for multiple comparisons.

experiment (three for each route of administration). The brain and olfactory bulb as well as blood plasma samples were extracted after the experiment and prepared for analysis via LC/MS. Results are presented in Figure 4. The height of each bar represents the mean concentration of the compound across all animals for a given route of administration, plotted on a log scale (n = 3 per route of administration). Error bars represent the standard error. Significant differences in concentration between routes of administration are indicated by an asterisk (unpaired t test, $p < 0.05$, Holm−Bonferroni corrected for multiple comparisons).

When considering the compound concentration in the olfactory bulb, ODD showed greater delivery for all four compounds compared to both IV (diazepam: $T(3,3) = 4.12$, $p < 0.05$; ketamine: $T(3,3) = 20.70$, $p < 0.001$; naloxone: $T(3,3) = 3.35$, $p < 0.05$; ondansetron: $T(3,3) = 3.02$, $p < 0.05$) and IN delivery (diazepam: $T(3,3) = 5.47$, $p < 0.01$; ketamine: $T(3,3) = 22.78$, $p < 0.001$; naloxone: $T(3,3) = 4.18$, $p < 0.05$; ondansetron: $T(3,3) = 5.44$, $p < 0.01$). There was no significant difference in the olfactory bulb concentration detected between IN and IV delivery for any compound (diazepam: $T(3,3) = 1.35$, $p = 0.42$; ketamine: $T(3,3) = 2.09$, $p = 0.17$; naloxone: $T(3,3) = 0.83$, $p = 0.70$; ondansetron: $T(3,3) = 2.42$, $p = 0.11$). In the plasma, ODD showed greater delivery for diazepam, ketamine, and naloxone compared to IN (diazepam: $T(3,3) = 5.13$, $p < 0.01$; ketamine: $T(3,3) = 4.89$, $p < 0.01$; naloxone: $T(3,3) = 4.56$, $p < 0.01$) and IV delivery (diazepam: $T(3,3) = 3.17$, $p < 0.05$; ketamine: $T(3,3) = 3.65$, $p < 0.05$; naloxone: $T(3,3) = 3.53$, $p < 0.05$). For these three compounds, we did not detect a significant difference in plasma concentration for IN vs IV delivery (diazepam: $T(3,3) = 1.95$, $p = 0.21$; ketamine: $T(3,3) = 1.23$, $p = 0.48$; naloxone: $T(3,3) = 1.03$, $p = 0.59$). For ondansetron, ODD and IV administration exhibited greater plasma delivery compared to IN administration (ODD: $T(3,3) = 8.73$, $p < 0.001$, IV: $T(3,3) = 7.77$, $p < 0.001$). In the brain, results varied across the four compounds. For diazepam, ODD showed increased delivery compared to IN administration ($T(3,3) = 5.22$, $p < 0.01$). For ketamine, ODD showed increased delivery compared to both IN ($T(3,3) = 4.68$, $p < 0.01$) and IV administration ($T(3,3) = 3.39$, $p < 0.05$). For naloxone, no significant differences were detected across the three routes of administration (IN vs IV: $T(3,3) = 1.86$, $p = 0.23$; IN vs ODD: $T(3,3) = 1.82$, $p = 0.24$; IV vs ODD: $T(3,3) = 0.04$, $p = 0.99$). For ondansetron, IV administration showed increased delivery compared to ODD ($T(3,3) = 4.12$, $p < 0.05$) and IN administration ($T(3,3) = 5.28$, $p < 0.01$).

Together, these results demonstrated that ODD offered comparable delivery of compounds to the brain when compared to IV and IN. Perhaps most interestingly, ODD appears to offer a more consistent, reliable dose with a smaller standard deviation in tissue concentrations when compared to IN delivery. Notably, this study only measured the concentration of each compound at a single time point (15 min). It is possible that concentrations peak earlier or later depending on the administration method. Further work is needed to fully characterize how the pharmacodynamics of the ODD method compares to those of the IV and IN methods. Additionally, this study is limited by a small sample size (n = 3 per route of administration) and the fact that all four compounds were combined into a single solution. (e.g., it is possible that the compounds could interact with each other and/or the DMSO used to create the solution, increasing blood−brain barrier permeability). Generally, the results demonstrate that ODD effectively delivers medication to the olfactory bulb and plasma and may offer superior brain delivery of diazepam and ketamine when compared to IN and IV administration routes.

Detailed Analysis of IV vs ODD Ketamine Pharmacokinetics. A follow-up experiment was conducted to assess the pharmacokinetics of ketamine in greater detail. Twelve male Sprague−Dawley rats (weight 150−200 g) were used in this follow-up experiment. Animals were administered ketamine-HCl via IV or ODD administration (resulting in two

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experimental groups, each with $n = 6$). Blood samples were acquired at seven time points following compound administration: 5, 15, 30, 60, 90, 120, and 200 min. Pharmacokinetic parameters were calculated from the plasma curves of individual animals. Mean values for t_{max}, c_{max}, $t_{1/2}$, and AUC are summarized in Table 2. After 200 min, animals were euthanized via CO_2. The brain and olfactory bulb were extracted from each animal and prepared for analysis via LC/MS. t tests were conducted to compare the concentration of ketamine-HCl and ketamine-DMSO in plasma at $t = 15$ min across administration methods and to compare the concentration of ketamine-DMSO across administration methods in the brain, olfactory cleft, and plasma at $t = 200$ min. Associated p values were Holm−Bonferroni corrected for multiple comparisons.

Table 2. Ketamine-HCl Pharmacokinetic Parameters

route	T_{max} (min)	C_{max} (ng/mL)	terminal elimination half-life	AUC_{last}
IV	5.00	1713	23.70	3.05e4
ODD	11.67	9402	22.87	1.86e5

When comparing within route of administration, ketamine-HCl showed better delivery to plasma than cassette-dosed ketamine-DMSO at $t = 15$ min following ODD ($T = -4.21$, $p < 0.01$) but not IV administration ($T = -1.66$, $p = 0.15$). Across routes of administration, ketamine-HCl exhibited greater plasma concentration at 15 min following ODD compared to IV administration ($T = -5.10$, $p < 0.01$). Examining the tissue concentrations (at $t = 200$ min) of ketamine-HCl, we did not observe significant differences between IV and ODD in the brain ($T = -1.02$, $p = 0.37$), olfactory bulb ($T = -0.614$, $p = 0.58$), or plasma ($T = -1.49$, $p = 0.20$) concentrations. Results are plotted in Figure 5.

These results demonstrated that adding DMSO to ketamine did not markedly affect the amount of compound delivered to the brain. In fact, we observed that ketamine-HCl (without DMSO) showed significantly greater brain uptake following the ODD compared to IV administration. In additional follow-up experiments (Figure 5, Figures S1 and S2), we administered each compound independently in separate animals to guarantee that there were no pharmacokinetic interactions between compounds that could confound the results.

Assessment of ODD for Challenging Molecules. We tested five additional compounds in a larger cohort of animals to investigate whether ODD offered noninferior delivery to the brain, olfactory bulb, plasma, and liver when compared to IV administration. The compounds were selected because of their larger molecular weight or previous failure to reach the brain via oral administration. Sixty male Sprague−Dawley rats (weight 150−250 g) were used for this experiment. Six compounds (diclofenac, exendin-4, lacosamide, liraglutide, sugammadex, and tolcapone) were tested for each of two routes of administration (ODD and IV), resulting in 12 experimental conditions. Blood samples were acquired at seven time points following compound administration: 5, 15, 30, 60, 90, 120, and 200 min. Pharmacokinetic parameters were calculated from the plasma curves of individual animals. Mean values for t_{max}, c_{max}, $t_{1/2}$, and AUC are summarized in Table 3. After 200 min, animals were euthanized via CO_2. The brain,

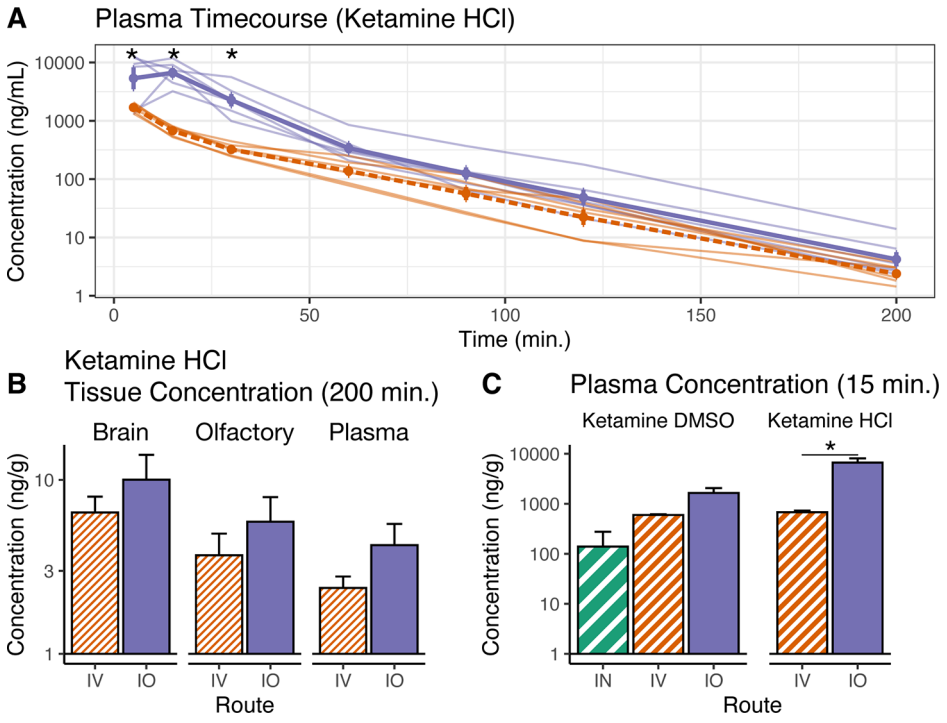

Figure 5. No significant influence of DMSO on BBB permeability. (A) Plasma timecourse of ketamine-HCl plasma concentration following IV (thin striped orange) or ODD (solid blue) administration. (B) Comparison of ketamine-HCl concentrations in the brain, olfactory bulb, and plasma at $t = 200$ min following IV (thin striped orange) or ODD (solid blue) administration. (C) Comparison of ketamine-HCl and ketamine-DMSO plasma concentrations at $t = 15$ min following IN (striped green), IV (thin striped orange), or ODD (solid blue) administration. Values are plotted on a log scale. The asterisk indicates statistically significant difference according to the t test, Holm−Bonferroni corrected for multiple comparisons.

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olfactory bulb, and liver were extracted from each animal and prepared for analysis via LC/MS.

Table 3. Pharmacokinetic Parameters for Challenging Molecules

compound	route	T_{max} (min)	C_{max} (ng/mL)	terminal elimination half-life	AUC_{last}
diclofenac	IV	5.00	42,040	34.63	1.69e6
	ODD	5.00	27,650	38.02	1.06e6
exendin-4	IV	5.00	12,220	32.99	2.02e5
	ODD	102.00	92.89	n/a	11,480
lacosamide	IV	5.00	2045	130.33	2.34e5
	ODD	5.00	4661	216.28	1.97e5
liraglutide	IV	7.00	25,640	109.62	2.76e6
	ODD	96.00	226.4	n/a	34,220
sugammadex	IV	5.00	14,010	36.02	2.56e5
	ODD	37.00	1857	97.54	1.79e5
tolcapone	IV	5.00	2661	58.69	7.58e4
	ODD	5.00	4655	62.19	9.68e4

Results are listed in Figure 6. Panel A displays plasma timecourses for each of the six compounds following ODD (blue) and IV (orange) administration. Solid points and lines mark the group mean, plotted on a log scale (n = 5 per route per compound). Error bars on each point show the standard error. Transparent thin lines show the timecourses for each individual animal. Panel B shows the concentration of each compound in the brain, liver, olfactory bulb, and plasma at time t = 200 min following compound administration. The height of each bar represents the group mean concentration, plotted on a log scale (n = 5 per route per compound). Error bars represent the standard error. Significant differences in concentration between routes of administration are indicated by an asterisk (unpaired t test, $p < 0.05$, Holm−Bonferroni corrected for multiple comparisons).

Diclofenac (5 mg/kg) demonstrated overall noninferior blood-plasma uptake (no significant difference) when comparing ODD and IV administration ($T(5,5) = 1.82$, $p = 0.11$). At early time points (t < 60 min), IV administration exhibited greater plasma concentration compared to ODD. Additionally, diclofenac showed similar brain ($T(5,5) = 1.32$, $p = 0.22$), olfactory ($T(5,5) = 1.30$, $p = 0.23$), and liver ($T(5,5) = 1.37$, $p = 0.21$) uptake across the two routes of administration. Lacosamide (1 mg/kg) demonstrated noninferior blood-plasma uptake when comparing ODD and IV administration ($T(5,5) = 2.16$, $p = 0.063$). Additionally, lacosamide showed similar brain ($T(5,5) = 1.50$, $p = 0.17$) and liver ($T(5,5) = 0.12$, $p = 0.91$) uptake across the two routes of administration. A greater concentration of lacosamide was observed in the olfactory bulb following IV administration compared to ODD ($T(5,5) = 3.93$, $p < 0.01$). Liraglutide (1 mg/kg) demonstrated superior blood-plasma uptake when administered via IV compared to ODD ($T(5,5) = 4.03$, $p < 0.01$). ODD was also associated with significantly reduced quantities in the liver compared to IV ($T(5,5) = 4.55$, $p < 0.01$) and undetectable quantities in the brain and olfactory bulb. Interestingly, increasing the dose from 1 to 20 mg/kg did not markedly increase blood-plasma uptake and failed to increase uptake in the brain or olfactory bulb (data not shown). Exendin-4 (5 mg/kg), a similar molecule to liraglutide, demonstrated noninferior uptake in plasma ($T(5,5) = 0.63$, $p = 0.55$). In two animals, exendin-4 showed increased olfactory uptake, and in three animals, exendin-4 showed increased brain uptake following ODD compared to IV administration. However, exendin-4 concentrations were below the quantifiable limits in the other animals in the ODD condition and across all animals in the IV condition, not allowing for a proper statistical comparison. Sugammadex (5 mg/kg) demonstrated superior blood-plasma uptake when comparing ODD and IV administration ($T(5,5) = -7.87$, $p < 0.0001$). At early time points (t < 30 min), IV administration exhibited greater plasma concentration compared to ODD. Ex vivo data demonstrated that ODD of sugammadex resulted in significantly reduced delivery to the liver ($T(5,5) = 3.25$, $p < 0.05$), a trending effect of increased delivery to the olfactory cleft ($T(5,5) = 2.41$, $p = 0.072$) and noninferior brain uptake ($T(5,5) = 2.63$, $p = 0.095$). Tolcapone (0.25 mg/kg) demonstrated noninferior blood-plasma uptake when comparing ODD and IV administration ($T(5,5) = -0.52$, $p = 0.62$). Tolcapone was detected at increased levels in the liver when comparing ODD to IV administration ($T(5,5) = -1.43$, $p = 0.19$), and both routes of administration failed to deliver the compound to the olfactory bulb and the brain (below the quantifiable limit).

This final set of experiments with more challenging compounds demonstrated that many compounds showed noninferiority for plasma uptake when comparing ODD to IV administration. Lacosamide, diclofenac, sugammadex, and tolcapone showed the most convincing noninferiority when considering plasma uptake. These same compounds (with the exclusion of tolcapone) also showed noninferior brain and olfactory uptake when comparing ODD and IV administration. Interestingly, tolcapone did not show any detectable brain or olfactory uptake following either the ODD or IV condition. This is surprising because tolcapone is prescribed as a catechol-*O*-methyltransferase (COMT) inhibitor in Parkinson's disease and has been shown to cross the blood−brain barrier and act on the central nervous system. It is worth noting that most of the tested compounds showed different plasma uptake levels at early time points when comparing ODD and IV administration. This may be worthy of further investigation given studies that suggest that the shape of a pharmacokinetic (PK) curve can influence where (and how effectively) a drug acts on the brain (e.g., Manza et al. (2023)[33]). Intriguingly, liraglutide showed brain uptake following IV but not after ODD administration. However, exendin-4, a closely related molecule, did show brain uptake following ODD in two of the five animals tested.

A limitation of the current work is that timecourses are presented for plasma but not brain tissue. This means that brain tissue concentrations are presented at only a single time point, limiting the conclusions/comparisons we can make about brain penetrance across compounds. For example, it is possible that some compounds exhibit high brain uptake at early time points with quick washout resulting in the appearance of low brain uptake in our later-acquired brain tissue samples. Future studies employing ex vivo biodistribution analyses in larger cohorts of animals or additional in vivo PET imaging studies could examine the brain-tissue timecourses for each of the compounds we presented. While these studies are outside the scope of the current work, we present the current work as a screening/preliminary analysis of several compounds and encourage others to reproduce the work we present in greater temporal detail.

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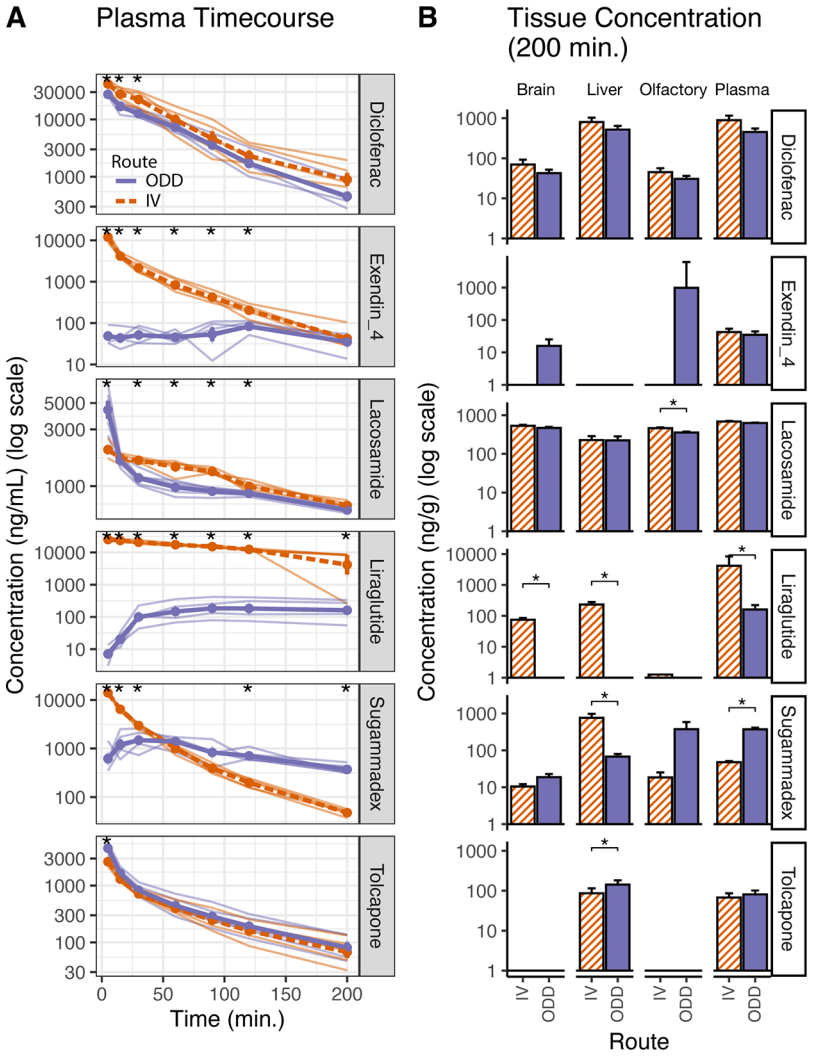

Figure 6. Testing for noninferiority of olfactory drug delivery compared to intravenous delivery.(A) Plasma timecourses showing the concentration of five compounds for 200 min following intravenous (thin striped orange) or olfactory drug delivery (solid blue). Points and thick lines represent the group means. Error bars on points represent the standard error. Transparent lines represent timecourses for individual animals (n = 5 per route per drug). (B) Concentration of five compounds at t = 200 min following intravenous (thin striped orange) or olfactory drug delivery (solid blue). Bar height represents the mean concentration across all animals, plotted on a log scale (n = 5 per route per drug). Error bars represent the standard error. The asterisk indicates $p < 0.05$ for an unpaired t test, Holm−Bonferroni corrected for multiple comparisons.

Considerations for Translation from Rodents to Humans. The compounds screened in this set of studies were selected due to their current or potential future use in humans. However, important differences between the rodent models used here must be considered before the method is translated to human use. In the current study, rats were positioned supine (on their back), which may have affected the distribution of the compound as it was deposited in the nasal cavities. Typically, humans are standing or sitting during intranasal administration, and their head is positioned upright, presenting a physical challenge. Moreover, the rat olfactory mucosa comprises a much larger surface area of the nasal cavities (∼50%) compared to the human olfactory mucosa (only 10%).[34] Given this smaller relative surface area of the human olfactory mucosa, the extent of the ODD must be even more precise in humans. Even slight anatomical variations in human nasal anatomy have the potential to impact whether the target tissue is reached. Ideal devices for human use will be designed for administration with an upright head position and may include flexible components to adapt to natural variations in human anatomy.

Focal Deposition to the Olfactory Cleft Is a Promising Target for Brain Delivery. Targeting the olfactory mucosa offers a variety of mechanisms by which a compound can be transferred to the brain, including axonal transport, bulk flow and perivascular pumping,[13] lymphatic drainage,[14] and endothelial transport through the olfactory nerve (for review, see Pardeshi and Belgamwar (2013)[15] and Trevino et al. (2020)[16]). Focal deposition at the olfactory cleft has been proposed as the primary route for intranasal delivery to the brain.[11] Direct transport from the olfactory mucosa into the brain bypasses first-pass metabolism of the compound in the liver, preventing side effects that could arise from compound metabolism or off-target deposition (e.g., in the digestive system).[3,4] While our experiments cannot determine the precise mechanisms of transfer to the brain, future work in rodent models could selectively lesion portions of the nasal anatomy (e.g., disconnect the olfactory nerve) to test methods of brain transfer. These types of experiments could help to

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Table 4. Potential Factors Influencing BBB Permeability

compound	description	molecular weight(g/mol)	H bond donors	topological polar surface area (Å^2)	superior brain penetrance with ODD?
[^{18}F] fluorodeoxyglucose	radiolabeled glucose	181.15	4	90.2	yes
[^{18}F] fluorothymidine	radiolabeled thymidine (marker of cellular proliferation)	243.22	2	78.9	potentially at early time points in frontal brain regions
ketamine	general anesthesia	237.72	1	29.1	yes
naloxone	opioid antagonist	327.4	2	70	yes
diazepam	benzodiazepine	284.74	0	32.7	yes
ondansetron	5HT-3 receptor antagonist	293	0	39.8	no
ketamine-HCl	general anesthesia	274.18	2	29.1	yes
diclofenac	NSAID; cyclooxygenase inhibitor	296.1	2	49.3	noninferior to IV
exendin-4	GLP-1 receptor agonist	4187	58	1780	possibly
lacosamide	decreased central nervous system; disorganized electrical activity	250.29	2	67.4	noninferior to IV
liraglutide	GLP-1 receptor agonist	3751	54	1510	no
sugammadex	gamma cyclodextrins	2002.2	24	972	noninferior to IV
tolcapone	COMT inhibitor	273.24	2	103	no
rocuronium bromide	neuromuscular nondepolarizing blockade	609.7	1	65	noninferior to IV

differentiate between endothelial transfer through the nerve and bulk flow and perivascular pumping through the vasculature.

The chemical and physical properties of drug compounds can affect how well they cross the blood–brain barrier. Previous work has identified the molecular weight, number of hydrogen bond donors, and topological polar surface area as several of the many factors that influence whether a molecule penetrates the blood–brain barrier.[35] In Table 4, we summarize these properties (sourced from PubChem) for each of the compounds we tested.[36] Our results demonstrate that ODD was most successful at delivering low-molecular-weight compounds into the brain. No obvious patterns emerged concerning hydrogen bond donors or topological polar surface areas, but we acknowledge that our experiments are limited to only a handful of compounds.

A large body of work examines how nasal spray medications can be modified to enhance their permeability through the blood–brain barrier (e.g., altering the spray pattern, plume geometry, droplet size, velocity, viscosity, thixotropicity, and surface tension) (for review, see Gänger and Schindowski (2018)[37] and Gao et al. (2020)[19]). Moreover, there is great interest in chemically shuttling medication through the blood–brain barrier (e.g., using peptides, lipid nanoparticles, or receptor-mediated routes).[38−42] Additional work has used thermoreversible gels to enhance brain uptake and mitigate mucociliary clearance of molecules administered via IN or ODD.[43,44] While a direct comparison of these factors is beyond the scope of this work, we did demonstrate that the ODD was effective across a range of viscosities/solubilities for naloxone and ketamine, drugs that are known to pass through the blood–brain barrier quite easily. It is possible that previous studies using traditional IN ("spray") delivery may have underperformed because they did not deposit the medication precisely at the olfactory mucosa. Future work should consider not only the compound formulation but also the deposition method/device together as a therapeutic platform.

Perhaps most intriguingly from a neuroscientific perspective, olfactory drug delivery presents an opportunity to target not only the central nervous system but also specific regions of the brain. Our PET imaging results complement prior work demonstrating that ODD was associated with increased uptake of the compound in the front of the brain compared to the back of the brain.[17,45] Delivery to the olfactory mucosa is thought to bring drugs to the brain via the olfactory nerve. The olfactory nerve is unique among the cranial nerves because it is the only nerve that projects directly from frontal cortical regions rather than the brain stem or thalamus.[46] The spatial topography of olfactory nerve projections may offer a unique access to prefrontal and medial temporal brain regions that are implicated in a range of neurological and psychiatric disorders (e.g., ADHD and Alzheimer's disease).

CONCLUSIONS

Our findings suggest that adopting the ODD method not only enables CNS delivery of certain non-blood–brain barrier permeable drug molecules but could also enhance the accuracy, precision, and reproducibility of dosing to the CNS versus IN and IV drug delivery applications. Importantly, ODD offers a consistent, accurate, and targeted method for improving CNS-targeted medication.

METHODS

Deposition Imaging and Accumulation of [^{18}F]FDG and [^{18}F]FLT. *Animal Preparation and Imaging.* All experimental procedures involving animals in this experiment were approved by the University of Saskatchewan Institutional Animal Care and Use Committee (Ethics Approval #2022097). Twenty-seven female Sprague–Dawley rats were used for this study. Each animal was assigned to one of six conditions based on the route of administration (IV, IN, or ODD) and compound administered ([^{18}F]FDG and [^{18}F]FLT) (see Table 5). Compound synthesis is described in the Supplementary Methods.

The animals were anesthetized with 3% isoflurane during ODD/IN administration and maintained with 2% isoflurane for the duration of the PET/CT scan. For biodistribution, the animals were anesthetized with 3% isoflurane and euthanized by cardiac puncture to collect blood. Approximately 5 min after the compound was administered, the rats were imaged using positron emission tomography/computed tomography (PET/CT) (Sofie, GNEXT scanner) to quantify the uptake of each compound in the brain, olfactory cleft, and other areas of the head/neck. Following the 60 min PET scan, the rats were

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Table 5. Animals Used in PET/CT Imaging

route	radiotracer	N	age (days) (mean ± SD)	weight (g) (mean ± SD)
intravenous (IV)	[^{18}F]FDG	4	62.8 ± 2.36	231 ± 18/1
	[^{18}F]FLT	3	58.0 ± 2.65	178 ± 14.7
pipette (IN)	[^{18}F]FDG	5	63.6 ± 2.79	207 ± 11.3
	[^{18}F]FLT	3	58.0 ± 2.65	159 ± 31.2
cannula (ODD)	[^{18}F]FDG	5	63.6 ± 2.79	196 ± 15.0
	[^{18}F]FLT	7[a]	56.7 ± 1.98	214 ± 32.1

[a]n = 7 animals completed PET imaging with ODD of [^{18}F]FLT, but biodistribution data were only collected from n = 3 of these animals.

anesthetized with 3% isoflurane and euthanized by cardiac puncture to collect blood. Ex vivo biodistribution of the radiotracer was quantified in the brain, blood, and cerebrospinal fluid (CSF) using a gamma counter. The brains were carefully collected from each animal without perfusion. The samples measured on the gamma counter were read as whole organs and were not homogenized. Biodistribution values were expressed in units of the injected dose per gram of tissue (ID/g).

PET/CT Image Acquisition and Data Analysis. PET images were acquired in list mode and reconstructed according to the default parameters on the GNEXT SOFIE (ordered subset expectation maximization (OSEM) 3D, matrix size: 240 × 240 × 191, data type: float 32). PET data were preprocessed and analyzed using the PMOD software (version 3.9). Reconstructed PET images were registered to the animal's CT images, and spherical regions of interest (ROIs) were generated in the frontal lobe and occipital lobe and in a control area outside of the brain. The control area and the frontal ROIs were located approximately the same distance away from the olfactory cleft but in a region outside the animal, where there could be no radiotracer accumulation. The purpose of the control ROI was to assess the potential partial volume effects that might have contributed to the observed signal in the frontal ROI following ODD. Time−activity curves were extracted from each ROI to compare regional uptake across the experimental conditions. Activity was expressed in units of percent injected dose per cm^3 (%ID/cm^3). Time−activity curves are presented in Figure 3.

Pharmacokinetic Analysis of Cassette-Dosed Small Molecules. *Animals.* All procedures involving animals in this experiment were approved by the HD Biosciences Institutional Animal Care and Use Committee (AUF-107). Nine male Sprague−Dawley rats (weight 150−200 g) were used. All animals were housed under standard ethical conditions with temperature/humidity control and a 12 h light/dark cycle. Animals had free access to chow and water. Animals were fasted overnight (free access to water) for a minimum of 6 h prior to beginning study procedures.

Compound Formulation. In this initial experiment, we utilized cassette-dosing, whereby all four tested compounds were administered in the same solution. Ketamine (Yuansi, Shanghai), diazepam (Yuansi, Shanghai), ondansetron (Aladdin, Shanghai), and naloxone (DC chem, Shanghai) were dissolved in DMSO at 50, 20, 10, and 5 mg/mL, respectively. The solution was dosed at 25 μL single nostril for the ODD and IN route or diluted in DMSO for IV dosing at 100 μL/animal injected to the tail vein. A follow-up experiment to test the effects of compound viscosity was also conducted (see the Supporting Information).

Drug Administration and Sample Collection. Throughout the experiment, animals were anesthetized with Zoletil 50 solution (Virbac, dissolved at 10 mg/mL in saline) mixed with xylazine (Shengxin, diluted at 8 mg/mL in slaine) in a 2:1 volume ratio. The anesthetic mixture was administered via intraperitoneal injection at a dosing of 4 mL/kg. Rats were administered the compound in one of three ways: (1) intranasally via standard pipet (IN) (25 μL single nostril), (2) directly to the olfactory cleft via cannula (ODD) (25 μL single nostril), or (3) intravenously (IV) (100 μL tail vein injection). IN and ODD methods were replicated from Maigler et al. (2021).[12] IN administration was conducted by placing a p200 pipet tip into the nostril just up to the nasal valve and gently depositing 25 μL of the solution to the lower nasal cavity. ODD was conducted by carefully inserting a 0.699 mm diameter cannula with lipophilic ointment spread on the catheter into the right nostril with a minimal angle of 20° to the target of the correct meatus. During insertion, the catheter was rotated gently to advance it through the tight nasal cavity. Once the catheter reached the ethmoid turbinate, it could not be advanced gently anymore (∼15 mm). Once in position, 25 μL was slowly instilled into the olfactory region.

Three animals were used for each route of administration. During IV and ODD, the rats were in the supine position. Fifteen minutes following administration, animals were euthanized by CO_2. The brain with an olfactory bulb was carefully collected from each animal without perfusion. The surface was gently rinsed with saline and dried. Each rat's olfactory bulb and brain were collected and snap-frozen in liquid nitrogen. The tissue was stored at −80 °C until analysis. Plasma: Full blood was collected into an EDTA-2K tube and centrifuged at 4 °C and 1700g. The plasma was stored at −80 °C until analysis.

Sample Preparation. An aliquot of 30 μL plasma was spiked into a 1.5 mL tube, and 120 μL of acetonitrile containing the internal standard was added for protein precipitation. The mixture was vortexed and centrifuged at 18,000g for 10 min. The 50 μL supernatant was resolved with 150 μL H_2O and then injected for LC/MS/MS analysis. The brain and olfactory samples were homogenized with ice-cold phosphate buffer saline (pH 7.4) at a ratio of 4 (buffer): (tissue) (v/w). An aliquot of 30 μL of homogenate was spiked into a 1.5 mL tube, and 120 μL of acetonitrile containing the internal standard was added for protein precipitation. The mixture was vortexed and centrifuged at 18,000g for 10 min. Fifty microliters of the supernatant was then mixed with 150 μL of water, and the final solution was injected for LC/MS/MS analysis.

Liquid Chromatography/Mass Spectrometry. An ultra-performance liquid chromatography (UPLC) chromatographic system (Waters), equipped with an AB Sciex QTRAP 6500 mass spectrometer, was used to analyze the samples. Chromatographic separation was achieved on a Waters HSS T3 column (50 × 2.1 mm ID, 1.8 μm) with a gradient mobile phase changing from 90% H_20 (0.1% formic acid) (A) to 90% acetonitrile (0.1% formic acid) (B). The flow rate was maintained at 0.7 mL/min. Analyst 1.6 software packages (Applied Biosystems) were used to control the LC/MS/MS system as well as for data acquisition and processing. A calibration curve was constructed in a blank olfactory bulb homogenate. Calibration and quality control curves are presented in the Supporting Information.

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Statistics. *t* tests were conducted to compare tissue concentrations following the three routes of administration. Corresponding *p* values were corrected for multiple comparisons using the Holm−Bonferroni method.

Detailed Analysis of IV vs ODD Ketamine Pharmacokinetics. Twelve male Sprague−Dawley rats (weight 150−200 g) were used in this follow-up experiment. Animals were administered ketamine-HCl via IV or ODD administration (resulting in two experimental groups, each with $n = 6$). Blood samples were acquired at seven time points following compound administration: 5, 15, 30, 60, 90, 120, and 200 min. After 200 min, animals were euthanized via CO_2. The concentration of the compound in the brain, olfactory bulb, and plasma at $t = 200$ min was quantified using LC/MS/MS as described above. Results from the follow-up experiment are presented in Figure 5. To directly compare ketamine-HCl with ketamine-DMSO data from the cassette-dosed experiment, plasma concentrations at $t = 15$ min are plotted in Figure 5a. Pharmacokinetic parameters were calculated from the plasma timecourses in R (v4.2.2) using the "ncappc" package.

Assessment of ODD for Challenging Molecules. *Procedure.* All experimental procedures involving animals were approved by the HD Biosciences Institutional Animal Care and Use Committee. Anesthesia procedures were identical to the conditions outlined for the cassette-dosed small-molecule experiment. Sixty male Sprague−Dawley rats (weight 150−250 g) were used for this experiment. Six compounds (diclofenac, exendin-4, lacosamide, liraglutide, sugammadex, and tolcapone) were tested for each of two routes of administration (ODD and IV), resulting in 10 experimental conditions. Blood samples were acquired at seven time points following compound administration: 5, 15, 30, 60, 90, 120, and 200 min. After 200 min, animals were euthanized via CO_2. The brain, olfactory bulb, and liver were extracted from each animal and prepared for analysis via LC/MS.

Compound Formulation. Diclofenac (Targetmol, Shanghai) was prepared at 50 mg/mL in 5% DMSO + 20% mPEG-350; exendin-4 (Targetmol, Shanghai) was prepared in 1× PBS at 50 mg/mL; lacosamide (DC Chem, Shanghai) was prepared in 5% DMSO at 10 mg/mL; liraglutide (Targetmol, Shanghai) was prepared in water at 10 mg/mL; sugammadex (Targetmol, Shanghai) was prepared in water at 50 mg/mL; and tolcapone (Targetmol, Shanghai) was prepared in 30% mPEG-350 at 2.5 mg/mL. All solutions were dosed at 25 μL single nostril in the rat for the ODD dosing route and were diluted five times in the corresponding formulation and dosed at 100 μL/rat for the IV dosing route.

Sample Preparation. The following procedure was conducted for processing plasma using a 96-well plate: An aliquot of 20 μL of unknown sample, calibration standard, quality control and dilution quality control, single blank, and double blank samples were added to the 96-well plate. Each sample (except the double blank) was quenched with 400 μL of IS1 (the double blank sample was quenched with 400 μL of ACN), and then the mixture was vortex-mixed for 10 min and centrifuged for 15 min at 3220*g* and 4 °C. A 60 μL aliquot of the supernatant was transferred to another clean 96-well plate and centrifuged for 5 min at 3220*g* and 4 °C, and then the supernatant was directly injected for LC/MS/MS analysis.

Brain homogenate was prepared by homogenizing tissue with 4 vol (w:v) of homogenizing solution (MeOH/15 mM PBS (1:2, v:v)), and the dilution factor was 10. One ball was added, and samples were shaken in a high-throughput cryogenic grinder (SCIENTZ-48LD) (temp: −20 °C, 45HX, 200 s, six times) to ensure homogeneity. The process was completed on wet ice. No protein precipitation was observed. The olfactory bulb homogenate and liver homogenate were prepared by homogenizing tissue with 9 vol (w:v) of homogenizing solution (MeOH/15 mM PBS (1:2, v:v)), and the dilution factor was 5. One ball was added, and samples were shaken in a high-throughput cryogenic grinder (SCIENTZ-48LD) (temp: −20 °C, 45HX, 200 s, six times) to ensure homogeneity. The process was completed on wet ice. No protein precipitation was observed. Protein precipitation for the brain, olfactory bulb, and liver homogenate was performed using the following procedure: An aliquot of 40 μL of unknown sample, calibration standard, single blank, and double blank samples were added to the 96-well plate. Each sample (except the double blank) was quenched with 800 μL of IS1 (the double blank sample was quenched with 800 μL of ACN), and then the mixture was vortex-mixed for 10 min and centrifuged for 15 min at 3220*g* and 4 °C. A 60 μL aliquot of the supernatant was transferred to another clean 96-well plate and centrifuged for 5 min at 3220*g* and 4 °C, and then the supernatant was directly injected for LC/MS/MS analysis.

Liquid Chromatography/Mass Spectrometry. The LC/MS/MS equipment used for experiment 2 was identical to that used in experiment 1. The mobile phase conditions and flow rate differed slightly for each compound and are described in the Supporting Information (Tables S1a−g). Calibration and quality control information is presented in the Supporting Information(Tables S2a−c).

Pharmacokinetics and Statistics. Pharmacokinetic parameters were calculated from the plasma timecourses in R (v4.2.2) using the "ncappc" package. *t* tests were conducted to compare tissue and plasma concentrations following each of the three routes of administration. Corresponding *p* values were corrected for multiple comparisons using the Holm−Bonferroni method.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.5c00206.

Additional experimental details, materials, methods, and results from follow-up experiments (PDF)

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Notes

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■ ABBREVIATIONS

CNS: central nervous system
COMT: catechol-*O*-methyltransferase
CSF: cerebral spinal fluid
CT: computed tomography
FDG: fluorodeoxyglucose
FLT: fluorothymidine
ID/g: injected dose per gram
IN: intranasal
IV: intravenous
OC: olfactory cleft
ODD: olfactory drug delivery
OSEM: ordered subset expectation maximization
PET: positron emission tomography
PK: pharmacokinetic
ROI: region of interest
UPLC: ultra-performance liquid chromatography

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