**Supporting Information:**

# Olfactory Drug Delivery in Rodents: Deposition and Pharmacokinetics

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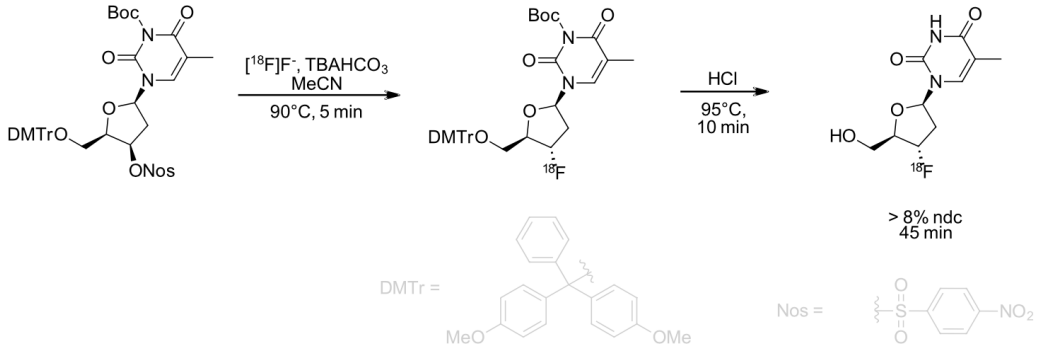
## Supplementary Methods

*Radiopharmaceutical Dose Preparation:*

[$^{18}$F]FDG was prepared as follows on a GE FASTLAB 2 instrument under GMP conditions at the Fedoruck Centre (Saskatoon, SK). The irradiated [$^{18}$O -water] is passed through an anion exchange cartridge where the $^{18}$F- fluoride ions are trapped. The $^{18}$F- ions are then eluted from the cartridge using a solution containing potassium carbonate, Kryptofix® K222, water and acetonitrile (eluent mixture). The solvents are evaporated and mannose triflate precursor is added to the dry residue. A nucleophilic substitution reaction occurs at 125°C, in which the trifluoromethane sulfonate group of the precursor is replaced by the $^{18}$F- ions, and results in 2-[$^{18}$F]- Fluoro-1,3,4,6-tetra-O-acetyl-Dglucose (also called FTAG). The mixture undergoes preliminary purification via a reverse phase cartridge. The acetylated compound (FTAG) is then converted into FDG by removing the 4 acetyl protecting groups. This de-protection is carried out through alkaline hydrolysis. The alkaline FDG solution then undergoes a neutralization step and final purification.

3-deoxy-2-[$^{18}$F]-fluorothymidine (FLT) was produced under good manufacturing practices (GMP) conditions for this study. [$^{18}$F]FLT was synthesized on a TRASIS All-inOne hot cell, according to the reaction scheme:




The expected yield was ~8%.

Cyclotron produced $^{18}$F water was passed over an ion exchange resin to capture the $^{18}$F and eluted with tetrabutylammonium bicarbonate (TBAHCO3) in water/ethanol and dried (120°C, 10 min) and reacted with the precursor (3-N-Boc-5'-O-dimetoxytrityl-3'-O-nosyl-thymidine) dissolved in acetonitrile at 90°C for 5 min. Protecting groups were removd with 2 M HCl at 95°C for 10 min and then neutralized with 1 M sodium hydroxide, diluted to 2.5% ethanol and passed through chromoafix PS-H+, Oasis WAX, Oasis HLB and alumin-N cartridges. The final formulation was 9% Ethanol diluted in citrate buffer.

According to the manual section 10.1 Radionuclide production (003150 version 2) "The radionuclide is produced in the form of fluoride ions by bombardment of $^{18}$O-water with accelerated protons. Usually the amount of irradiated enriched water ranges from 0.3 to 5 mL. Bombardment time is up to 2 hours. The energy of the protons ranges from 8 to 17 MeV. The amount of $^{18}$F-fluoride activity recovered is usually between 20 and 740 GBq (500 and 20000 mCi). This step is performed on the target."

The cyclotron at the University of Saskatchewan delivers ~2.5 mL of bombarded $^{18}$F water activity ranges of between 2 - 600 GBq were tested. Activities between 300 - 600 GBq were used

for experiments. All production runs were performed in a GMP clean-space using GMP materials and procedures.

*Liquid Chromatography/Mass Spectrometry Instrument Information*

**Table S1a. Instrument Information for Cassette-Dosing Experiment**

| Equipment | ACQUITY UPLC System | | | |
|---|---|---|---|---|
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.1% FA in Water | | | |
| Mobile phase B | 0.1% FA in ACN | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.7 | 90 | 10 |
| | 0.3 | 0.7 | 90 | 10 |
| | 0.7 | 0.7 | 10 | 90 |
| | 1.2 | 0.7 | 10 | 90 |
| | 1.3 | 0.7 | 90 | 10 |
| | 1.5 | 0.7 | 90 | 10 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Labtalol | 329.2/162.1 | | | |
| Tolbutamide | 271.1/91.1 | | | |
| Ketamine | 238.2/125.0 | | | |
| Naloxone | 328.1/310.2 | | | |
| Diazepam | 285.0/193.1 | | | |
| Ondansetron | 294.0/170.1 | | | |

**Table S1b. Instrument Information for Diclofenac**

| Equipment | ACQUITY UPLC System | | | |
|---|---|---|---|---|
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.1% FA in Water | | | |
| Mobile phase B | 0.1% FA in ACN | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.6 | 80 | 20 |
| | 0.2 | 0.6 | 80 | 20 |
| | 1 | 0.6 | 5 | 95 |
| | 1.3 | 0.6 | 5 | 95 |
| | 1.31 | 0.6 | 80 | 20 |

| | | | | |
|---|---|---|---|---|
| | 1.5 | 0.6 | 80 | 20 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Diclofenac | 296.1/214.2 | | | |
| Verapamil (Brain) | 455.2/164.9 | | | |
| Celecoxib | 382.1/362.1 | | | |

**Table S1c. Instrument Information for Liraglutide**

| | | | | |
|---|---|---|---|---|
| Equipment | ACQUITY UPLC System | | | |
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.3% FA in Water | | | |
| Mobile phase B | 0.3% FA in ACN | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.65 | 77 | 23 |
| | 0.4 | 0.65 | 77 | 23 |
| | 2.2 | 0.65 | 2 | 98 |
| | 3.1 | 0.65 | 2 | 98 |
| | 3.11 | 0.65 | 77 | 23 |
| | 3.2 | 0.65 | 77 | 23 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Liraglutide | 938.7/1064.2 | | | |
| Verapamil | 455.2/164.9 | | | |

**Table S1d. Instrument Information for Lacosamide**

| | | | | |
|---|---|---|---|---|
| Equipment | ACQUITY UPLC System | | | |
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.1% FA in Water | | | |
| Mobile phase B | 0.1% FA in ACN | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.65 | 90 | 10 |
| | 0.2 | 0.65 | 90 | 10 |
| | 1.2 | 0.65 | 10 | 90 |
| | 1.5 | 0.65 | 10 | 90 |
| | 1.51 | 0.65 | 90 | 10 |

| | | | | |
|---|---|---|---|---|
| | 1.6 | 0.65 | 90 | 10 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Lacosamide | 251.2/144.1 | | | |
| Verapamil (Plasma) | 455.2/164.9 | | | |
| Labetalol (Tissue) | 329.2/161.9 | | | |

**Table S1e. Instrument Information for Tolcapone**

| | | | | |
|---|---|---|---|---|
| Equipment | ACQUITY UPLC System | | | |
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.3% FA in Water with 0.02% TFA | | | |
| Mobile phase B | 0.3% FA in ACN with 0.02% TFA | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.6 | 90 | 10 |
| | 0.4 | 0.6 | 90 | 10 |
| | 0.9 | 0.6 | 5 | 95 |
| | 1.2 | 0.6 | 5 | 95 |
| | 1.21 | 0.6 | 90 | 10 |
| | 1.5 | 0.6 | 90 | 10 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Lacosamide | 274.2/182.2 | | | |
| Verapamil | 455.2/164.9 | | | |

**Table S1f. Instrument Information for Exendin-4**

| | | | | |
|---|---|---|---|---|
| Equipment | ACQUITY UPLC System | | | |
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.1% FA in Water | | | |
| Mobile phase B | 0.1% FA in ACN | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.6 | 80 | 20 |
| | 0.3 | 0.6 | 80 | 20 |
| | 1 | 0.6 | 10 | 90 |
| | 1.4 | 0.6 | 10 | 90 |
| | 1.41 | 0.6 | 80 | 20 |

| | | | | |
|---|---|---|---|---|
| | 1.5 | 0.6 | 80 | 20 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Exendin-4 | 838.3/396.5 | | | |
| Labetalol | 329.2/161.9 | | | |

**Table S1g. Instrument Information for Sugammadex**

| | | | | |
|---|---|---|---|---|
| Equipment | ACQUITY UPLC System | | | |
| Analytical column | ACQUITY UPLC HSS T3 1.8 μm 2.1 × 50 mm | | | |
| Inject volume | 1 μL | | | |
| Mobile phase A | 0.1% FA in Water | | | |
| Mobile phase B | 0.1% FA in ACN | | | |
| Elution mode | Gradient | | | |
| Gradient | Time (min) | Flow Rate (mL/min) | A (%) | B (%) |
| | Initial | 0.6 | 85 | 15 |
| | 1 | 0.6 | 85 | 15 |
| | 4.5 | 0.6 | 70 | 30 |
| | 5.5 | 0.6 | 5 | 95 |
| | 6.7 | 0.6 | 5 | 95 |
| | 6.8 | 0.6 | 85 | 15 |
| Mass spectrometer | Triple Quad 6500 plus | | | |
| Ionization mode | ESI (+) | | | |
| Detective mode | MRM | | | |
| Ion Transition | | | | |
| Sugammadex | 501.0/251.1 | | | |
| Verapamil (Brian) | 455.2/164.9 | | | |
| Tolbutamide | 271.1/155.0 | | | |
| Labetalol (Plasma) | 329.2/161.9 | | | |

*Liquid Chromatography/Mass Spectrometry Calibration and Quality Control*

**Table S2a. Calibration and Quality Control (Cassette Dose Small Molecules)**

| Compound | Matrix | Calibration Standard Samples Conc. (ng/mL) | Quality Control Samples Conc. (ng/mL) | LLOQ (ng/mL plasma or ng/g tissue) |
|---|---|---|---|---|
| Ketamine | Male SD rat plasma | 1.00, 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 3.00, 800, 2400 | 1 ng/ml |
| Ketamine | Male SD rat Brain | 1.00, 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 3.00, 800, 2400 | 5 ng/g |
| Ketamine | Male SD rat Olfactory | 1.00, 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 3.00, 800, 2400 | 5 ng/g |
| Naloxone | Male SD rat plasma | 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 10.0, 800, 2400 | 3 ng/ml |
| Naloxone | Male SD rat Brain | 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 10.0, 800, 2400 | 15 ng/g |
| Naloxone | Male SD rat Olfactory | 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 10.0, 800, 2400 | 15 ng/g |
| Diazepam | Male SD rat plasma | 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 10.0, 800, 2400 | 3 ng/ml |
| Diazepam | Male SD rat Brain | 1.00, 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 3.00, 800, 2400 | 5 ng/g |
| Diazepam | Male SD rat Olfactory | 1.00, 3.00, 10.0, 30.0, 100, 300, 1000, 3000 | 3.00, 800, 2400 | 5 ng/g |
| Ondansetron | Male SD rat plasma | 1.00, 3.00, 10.0, 30.0, 100, 300 | 3.00, 80.0, 240 | 1 ng/ml |
| Ondansetron | Male SD rat Brain | 1.00, 3.00, 10.0, 30.0, 100, 300 | 3.00, 80.0, 240 | 5 ng/g |
| Ondansetron | Male SD rat Olfactory | 0.30, 1.00, 3.00, 10.0, 30.0, 100, 300 | 1.00, 80.0, 240 | 1.5 ng/g |

**Table S2b. Calibration and Quality Control (Challenging Molecules)**

| Compound | Matrix | Calibration Standard Samples Conc. (ng/mL) | Quality Control Samples Conc. (ng/mL) | LLOQ (ng/mL plasma or ng/g tissue) |
|---|---|---|---|---|
| Diclofenac | Male SD rat plasma | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | 3.00, 40.0, 800, 2400, 4000 (DQC) | 1 ng/mL |
| Diclofenac | Male SD rat brain | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 5 ng/g |

| | | | | |
|---|---|---|---|---|
| Diclofenac | Male SD rat olfactory bulb | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 10 ng/g |
| Diclofenac | Male SD rat liver | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 10 ng/g |
| Liraglutide | Male SD rat plasma | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | 3.00, 40.0, 800, 2400, 4000 (DQC) | 1 ng/mL |
| Liraglutide | Male SD rat brain | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 5 ng/g |
| Liraglutide | Male SD rat olfactory bulb | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 10 ng/g |
| Liraglutide | Male SD rat liver | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 10 ng/g |
| Lacosamide | Male SD rat plasma | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | 3.00, 40.0, 800, 2400, 4000 (DQC) | 1 ng/mL |
| Lacosamide | Male SD rat brain | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 5 ng/g |
| Lacosamide | Male SD rat olfactory bulb | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 10 ng/g |
| Lacosamide | Male SD rat liver | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 10 ng/g |
| Tolcapone | Male SD rat plasma | 3.00, 5.00, 10.0, 48.0, 100, 500, 1000, 3000 | 9.00, 40.0, 800, 2400, 4000 (DQC) | 3 ng/mL |
| Tolcapone | Male SD rat brain | 3.00, 5.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 15 ng/g |
| Tolcapone | Male SD rat olfactory bulb | 3.00, 5.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 30 ng/g |
| Tolcapone | Male SD rat liver | 3.00, 5.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 30 ng/g |
| Exendin-4 | Male SD rat plasma | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | 3.00, 40.0, 800, 2400, 4000 (DQC) | 1 ng/mL |
| Exendin-4 | Male SD rat brain | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 5 ng/g |
| Exendin-4 | Male SD rat olfactory bulb | 1.00, 2.00, 10.0, 48.0, 100, 500, 1000, 3000 | NA | 20 ng/g |
| Sugammadex | Male SD rat | 5.00, 7.00, 10.0, | 25.0, 80.0, 800, | 5 ng/mL |

| | | | | |
|---|---|---|---|---|
| | plasma | 20.0, 50.0, 100, 500, 1000, 3000 | 2400, 4000 (DQC) | |
| Sugammadex | Male SD rat brain | 1.00, 2.00, 5.00, 10.0, 50.0, 100, 250, 500 | NA | 5 ng/g |
| Sugammadex | Male SD rat olfactory bulb | 1.00, 2.00, 5.00, 10.0, 50.0, 100, 250, 500 | NA | 10 ng/g |
| Sugammadex | Male SD rat liver | 1.00, 2.00, 5.00, 10.0, 50.0, 100, 250, 500 | NA | 10 ng/g |

**Table S2c: LCMS Detection Limits and Accuracy**

| Experiment | Compound | Detection limit | | | | Average Accuracy of QC |
|---|---|---|---|---|---|---|
| | | Plasma | Brain | Olfactory | Liver | |
| Cassette-Dosed Small Molecules in DMSO (Fig. 3) | Ketamine | 1 ng/mL | 5 ng/g | 5 ng/g | / | ±5.76% |
| | Naloxone | 3 ng/mL | 15 ng/g | 15 ng/g | / | ±7.91% |
| | Diazepam | 3 ng/mL | 15 ng/g | 15 ng/g | / | ±5.80% |
| | Ondansetron | 1 ng/mL | 5 ng/g | 5 ng/g | / | ±6.61% |
| Ketamine Detailed Pharmacokinetics (Fig. 4) | Ketamine-HCl | 1 ng/mL | 1.5 ng/g | 1.5 ng/g | / | ±4.55% |
| Challenging Compounds (Fig. 5) | Liraglutide | 1 ng/mL | 5 ng/g | 10 ng/g | 10 ng/g | ±7.37% |
| | Lacosamide | 1 ng/mL | 5 ng/g | 10 ng/g | 10 ng/g | ±5.13% |
| | Diclofenac | 1 ng/mL | 5 ng/g | 10 ng/g | 10 ng/g | ±5.01% |
| | Tolcapone | 3 ng/mL | 15 ng/g | 30 ng/g | 30 ng/g | ±9.95% |
| | Sugammadex | 1 ng/mL | 5 ng/g | 10 ng/g | 10 ng/g | ±14.65% |
| | Exendin-4 | 1 ng/mL | 5 ng/g | 20 ng/g | / | ±10.59% |
| Rocuronium Bromide with Sugammadex Challenge (Fig. S2) | Rocuronium Bromide | 0.2 ng/mL | 1 ng/g | 2 ng/g | / | ±3.91% |
| | Sugammadex | 5 ng/mL | 10 ng/g | 10 ng/g | / | ±8.68% |
| Naloxone Viscosity (Fig. S1) | Naloxone-DMSO | 3 ng/mL | 5 ng/g | 5 ng/g | / | ±4.27% |
| | Naloxone-mPEG | 1 ng/mL | 1.5 ng/g | 1.5 ng/g | / | ±6.24% |
| | Naloxone-HCl | 1 ng/mL | 1.5 ng/g | 1.5 ng/g | / | ±2.56% |

*Assessing Influence of Viscosity on BBB Permeability:*

A follow-up experiment was conducted to assess whether viscosity of the solution affected the blood-brain-barrier permeability of naloxone. 18 male Sprague Dawley rats (weight 150-200g) were used in this follow-up experiment. Three formulations of naloxone (High Viscosity = naloxone-mPEG, Medium Viscosity = Naloxone-HCl, Low Viscosity = Naloxone-DMSO) were prepared according to the following procedure: For Naloxone 0.5mg/kg OD (40% mPEG): 5.31 mg of Naloxone was added to 0.412 ml of mPEG-350. After complete dissolution, 0.618 ml of ddH2O was slowly added multiple times. After mixing, the solution concentration was 5 mg/ml. For Naloxone-HCl 0.5mg/kg OD (Saline): 5.56 mg of Naloxone-HCl was added to 0.981 ml of saline, mixed well, and dissolved completely. The solution concentration was 5 mg/ml. For Naloxone 0.5mg/kg OD (100% DMSO): 4.76 mg of Naloxone was added to 0.923 ml of DMSO, mixed well and dissolved completely. The solution concentration was 5 mg/ml. Plasma time

courses for 200 minutes were generated according to procedures described in the main text. Concentration of compound in brain, olfactory bulb, and plasma at t = 200 minutes was quantified using LC/MS/MS as described in the main text. Results are plotted in **Figure S1**. T-tests were conducted to compare concentration of the various compounds within the brain, olfactory cleft, and plasma at t = 200 minutes. Associated p-values were Holm-Bonferroni corrected for multiple comparisons.

*Sugammadex Challenge to Rocuronium Bromide:*

A follow-up experiment was conducted to assess whether administration of sugammadex could remove rocuronium bromide from various tissues. Sugammadex is a neuromuscular reversal agent that reverses the neuromuscular blockade induced by muscle relaxants including rocuronium bromide. 35 Sprague Dawley rats (weight 150-200g) were used in this follow-up experiment. First, rats received 0.2mg/kg rocuronium bromide via IV injection into the tail vein. Next, rats received either IN, IO, or IV administration of 5mg/kg sugammadex. Plasma timecourses for 120 minutes were generated according to procedures described in the main texzt. Concentration of compound in the brain, olfactory bulb, and plasma at t = 120 minutes was quantified using LC/MS/MS as described in the main text. Results are plotted in **Figure S2**. T-tests were conducted to compare concentration of the various compounds within the brain, olfactory cleft, and plasma at t = 120 minutes. Associated p-values were Holm-Bonferroni corrected for multiple comparisons.

## Supplementary Results

*Assessing Influence of Viscosity on BBB Permeability:*

A follow-up experiment was conducted to assess how viscosity influenced BBB-permeability of naloxone. Comparison between high viscosity (naloxone_mPEG), normal viscosity (naloxone_HCl), and low viscosity (naloxone_DMSO) is presented in **Figure S1**. Results demonstrated that viscosity did not significantly affect delivery to the brain (Naloxone-DMSO vs. Naloxone-HCl: T = -1.90, p = 0.35; Naloxone-DMSO vs. Naloxone-mPEG: T = -0.71, p = 1.0; Naloxone-HCl vs. Naloxone-mPEG: T = -0.01, p = 1.0) or olfactory bulb (Naloxone-DMSO vs. Naloxone-HCl: T = -0.77, p = 0.47; Naloxone-DMSO vs. Naloxone-mPEG: T = -1.89, p = 0.35; Naloxone-HCl vs. Naloxone-mPEG: T = -1.66, p = 0.34) following OD administration. However, high viscosity naloxone showed increased delivery to plasma following ODD administration compared to low viscosity naloxone (Naloxone-DMSO vs. Naloxone-mPEG: T = -8.63, $p < 0.01$). There was no significant difference between naloxone-HCl the other two compounds (vs. naloxone-DMSO: T = -1.60, p = 0.34; vs. naloxone-mPEG: T = -0.50, p = 0.64).

*Sugammadex Challenge to Rocuronium Bromide:*

A follow-up experiment was conducted to assess whether administration of sugammadex could remove rocuronium bromide from various tissues. Sugammadex is a neuromuscular reversal agent that reverses the neuromuscular blockade induced by muscle relaxants including rocuronium bromide. **Figure S2A** presents plasma timecourses for both sugammadex and rocuronium bromide following IN (green), IV (orange), or ODD (purple) administration of sugammadex. **Figure S2B** shows plasma timecourses for rocuronium bromide following IV administration of sugammadex

(red) or vehicle (blue). **Figure S2C** presents concentration of sugammadex in the brain, olfactory bulb, and plasma at t = 120 min. Results demonstrate that sugammadex concentration is statistically greatest in the brain following IV administration (vs. IO: $T(5,5) = 2.86$, $p < 0.05$; vs. IN $T(5,5) = 9.84$, $p < 0.05$). Moreover, ODD administration also showed significantly greater brain concentration of sugammadex compared to IN administration ($T(5,5) = 3.57$, $p < 0.05$). No significant differences in plasma or olfactory bulb concentration were observed for sugammadex at t = 120 min. Figure 2D presents concentration of rocuronium bromide in the brain, olfactory bulb, and plasma at t = 120 min. No significant differences in rocuronium bromide concentration were observed across the three administration methods. Additionally, rocuronium bromide concentration following administration of sugammadex did not significantly differ from rocuronium bromide concentration following administration of a vehicle via IV.

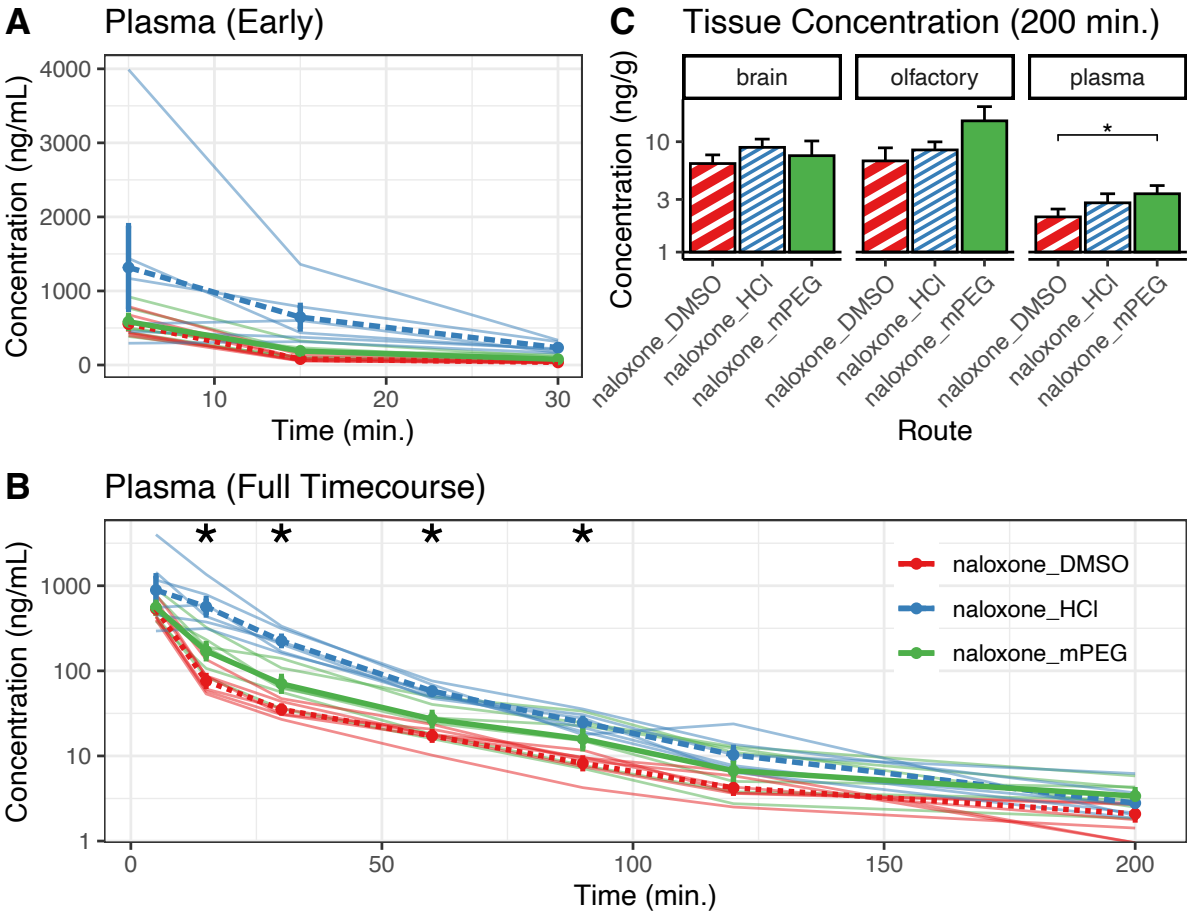



**Figure S1. No Significant Influence of viscosity on brain transfer. A.** Early time points of naloxone_DMSO (dotted red), naloxone_HCl (dashed blue), and naloxone_mPEG (solid green) plasma concentration following OD administration. **B.** Full time course of naloxone DMSO (dotted red), HCl (dashed blue), and mPEG (solid green) plasma concentration following OD administration. **C.** Comparison of naloxone DMSO (striped red), HCl (thin striped blue), and mPEG (solid green) concentration in the brain, olfactory bulb, and plasma at t = 200 min. following OD administration. * indicates statistically significant difference according to T-test, Holm-Bonferroni corrected for multiple comparisons. * = $p < 0.05$, Holm-Bonferroni corrected for multiple comparisons.

**Table S3: Pharmacokinetic Parameters for Naloxone at various viscosities**

| Compound | Route | $T_{max}$ (min.) | $C_{max}$ (ng/mL) | Terminal Elimination Half Life | $AUC_{last}$ |
|---|---|---|---|---|---|
| **Naloxone-DMSO** | **ODD** | 5.00 | 554.2 | 44.37 | 4845 |
| **Naloxone-HCl** | **ODD** | 8.33 | 1328.5 | 29.50 | 21261 |
| **Naloxone-mPEG** | **ODD** | 5.00 | 583.7 | 39.09 | 8320 |

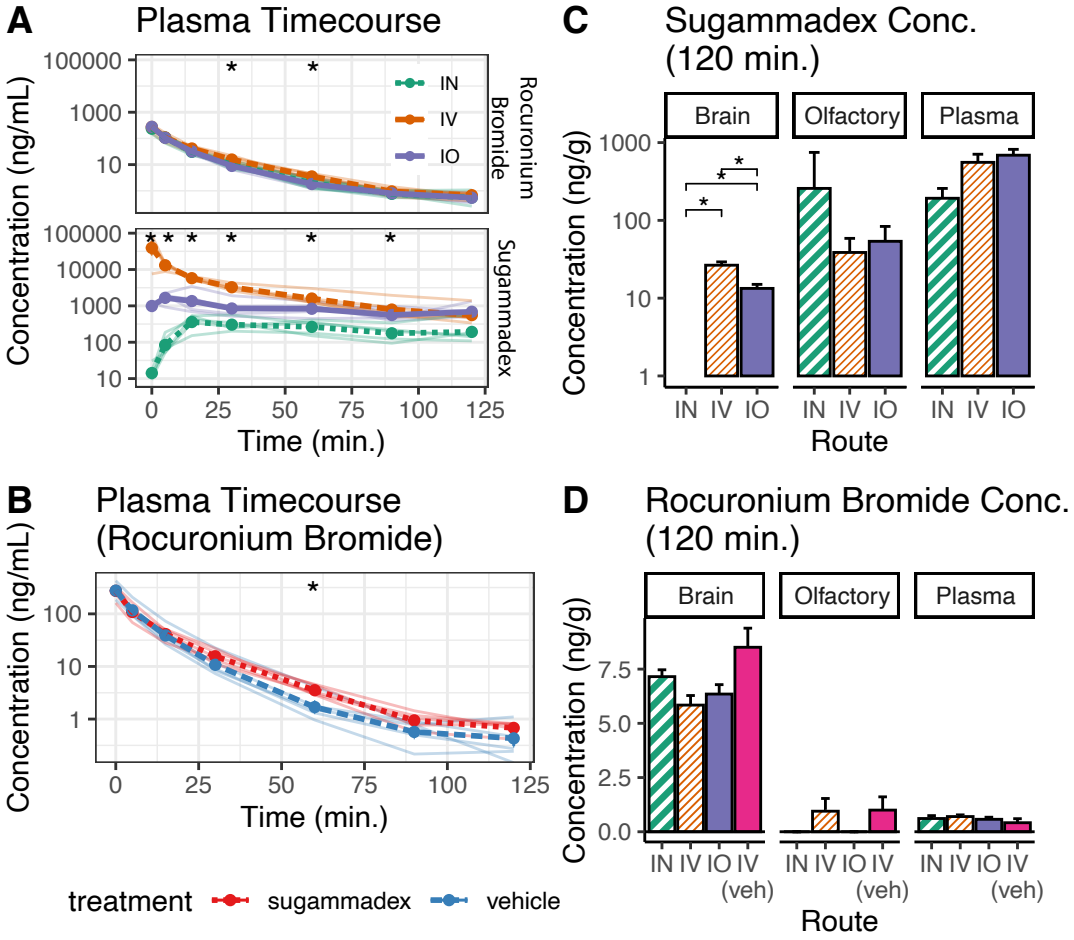



**Figure S2. Sugammadex Challenge to Rocuronium Bromide.** 5mpk sugammadex was administered via intravenous (IV), intranasal pipette (IN), or olfactory drug delivery via cannula (ODD) following 0.3mpk IV administration of rocuronium bromide. **(A)** Plasma timecourses for rocuronium bromide (top) and sugammadex (bottom) are plotted following IN (dotted green), IV (dashed orange), or ODD (solid blue) administration of 5mpk sugammadex. **(B)** Plasma timecourses for rocuronium bromide are presented following IV administration of either 5mpk sugammadex (dotted red) or IV administration of vehicle (dashed blue). **(C)** Tissue concentrations of sugammadex are shown for the brain, olfactory bulb, and plasma at t=120 min. following IN (striped green), IV (thin striped orange), or ODD (solid blue) administration of 5mpk sugammadex. **(D)** Tissue concentrations of rocuronium bromide are shown for the brain, olfactory bulb, and plasma at t = 120min. following IN sugammadex administration (striped green), IV sugammadex administration (thin striped orange), ODD sugammadex administration (solid blue),

or IV administration of vehicle (solid pink). * = $p < 0.05$, Holm-Bonferroni corrected for multiple comparisons.

**Table S4: Pharmacokinetic Parameters for Rocuronium Bromide by Treatment**

| Treatment | Route | $T_{max}$ (min.) | $C_{max}$ (ng/mL) | Terminal Elimination Half Life | $AUC_{last}$ |
|---|---|---|---|---|---|
| **Sugammadex** | **IV** | 0 | 285.4 | 17.84 | 2380 |
| **Vehicle** | **IV** | 0 | 287.2 | 19.53 | 2281 |

**Table S5: Pharmacokinetic Parameters for Rocuronium Bromide and Sugammadex by Route**

| Compound | Route | $T_{max}$ (min.) | $C_{max}$ (ng/mL) | Terminal Elimination Half Life | $AUC_{last}$ |
|---|---|---|---|---|---|
| | **ODD** | 0 | 278.4 | 24.49 | 1976 |
| **Rocuronium Bromide** | **IV** | 0 | 285.4 | 17.84 | 2380 |
| | **IN** | 0 | 242 | 18.31 | 1972 |
| | **ODD** | 6 | 2033 | 2072 | 1.110e5 |
| **Sugammadex** | **IV** | 1 | 50590 | 35.08 | 4.260e5 |
| | **IN** | 42 | 458.4 | 68.5 | 30640 |