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Center for Radiopharmaceutical Sciences

Yale University PET Center

Radiosynthesis and evaluation of (*R*)- and (*S*)-^{18}F-OF-NB1 for imaging the GluN2B subunits of the NMDA receptor in non-human primates

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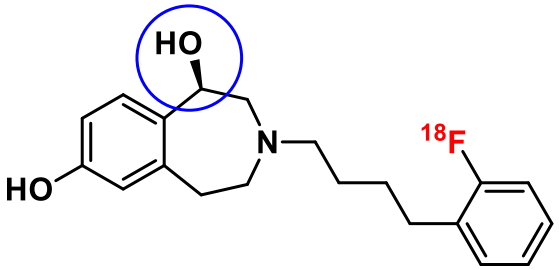
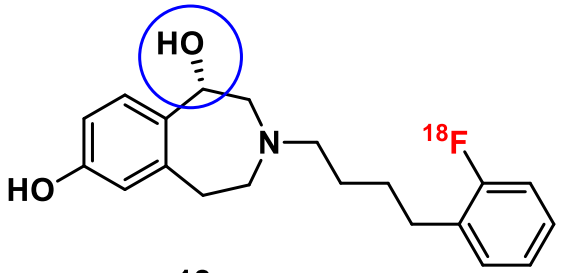


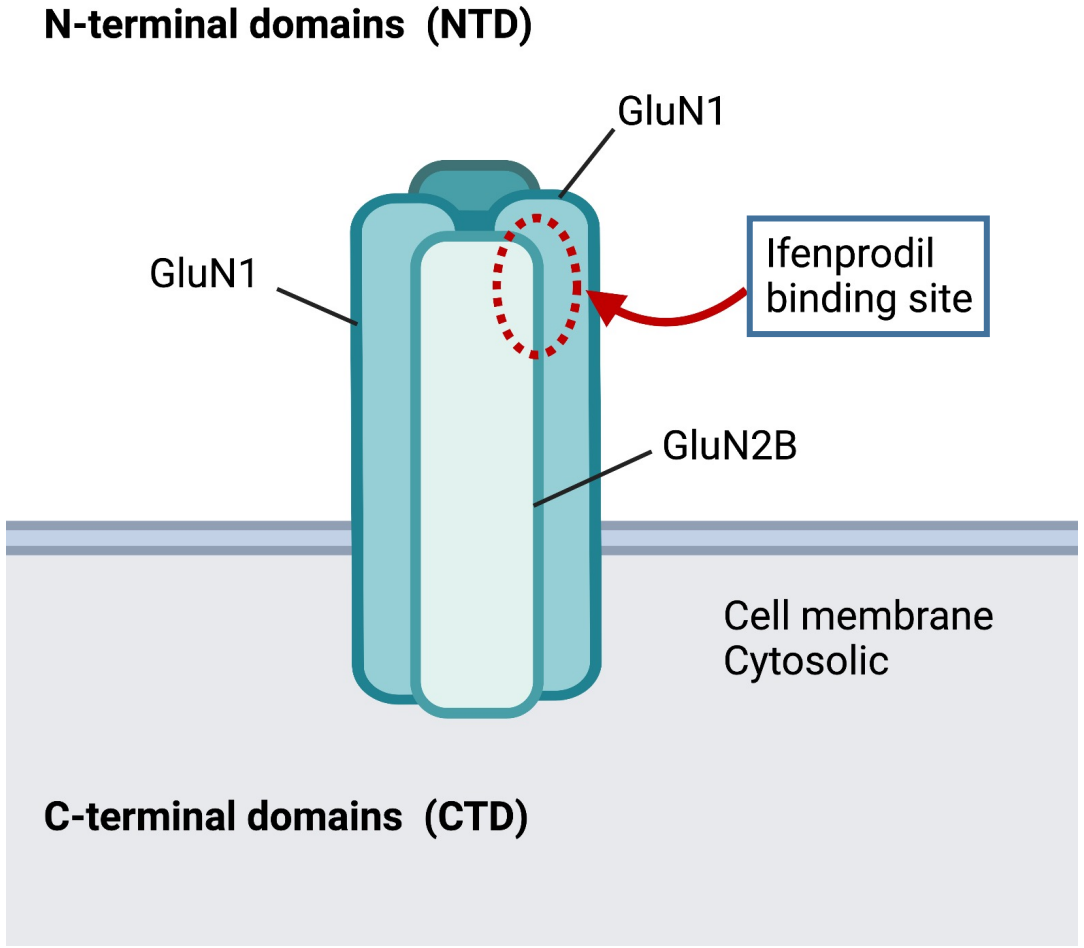
(*R*)-^{18}F-OF-NB1



(*S*)-^{18}F-OF-NB1

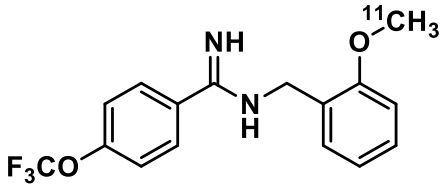
Target: The NMDA Receptor

- ✓ Ionotropic glutamate receptor
- ✓ Heterotetramer, consisting of three different subfamilies (GluN1a-h, GluN2A-D, GluN3A/B).
- ✓ GluN2 subunits exhibit heterogeneous expression and dictates the receptor function.
- ✓ Physiological: learning processes, memory function and synaptic plasticity.
- ✓ Pathological: neurological diseases comprising Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, schizophrenia and depression amongst others.

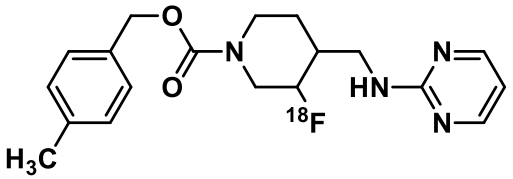


Challenges facing GluN2B PET Radioligands Development

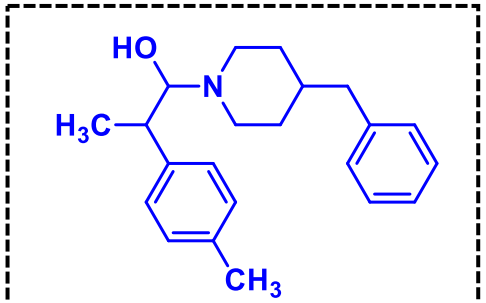
- No selectivity over other CNS receptors
- Low brain uptake
- Brain radiometabolites
- Brain uptake inconsistent with known GluN2B expression profile



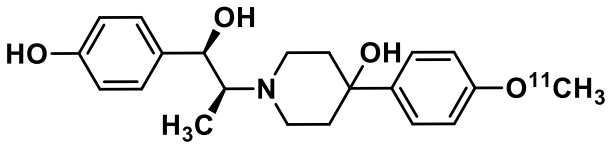
Astrad et. al, Bioorg. Med. Chem. 2006



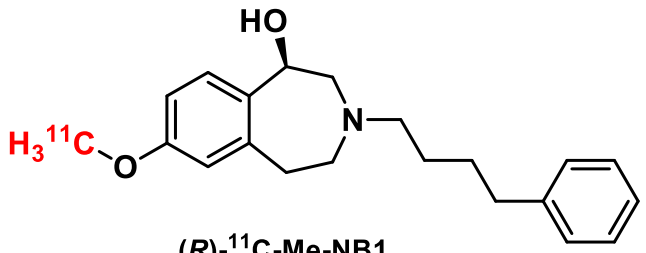
Koudih et. al, Org. Biomol. Chem. 2012



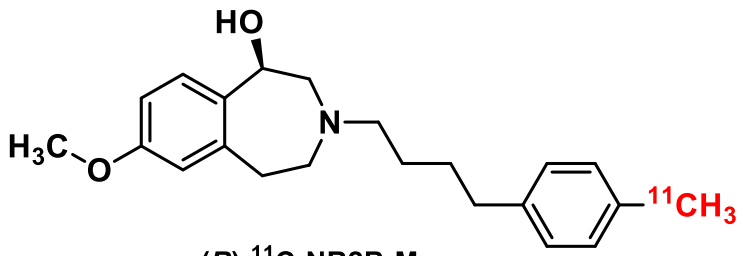
Ifenprodil (lead structure)



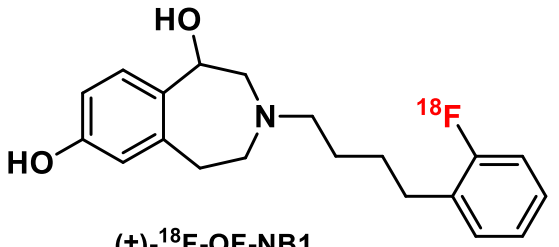
Haradahira et. al, Nucl. Med. Biol. 2011



(*R*)-^{11}C-Me-NB1
(Haider et al, JNM 2019)



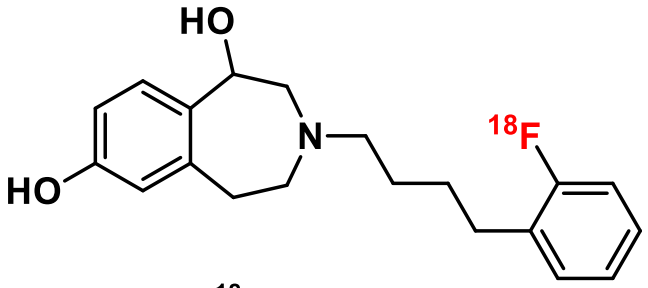
(*R*)-^{11}C-NR2B-Me
(Cai et al, JNM 2020)



(±)-^{18}F-OF-NB1
(Ahmed et al, JNM 2021)

^{18}F-OF-NB1 is a Promising GluN2B PET Ligand

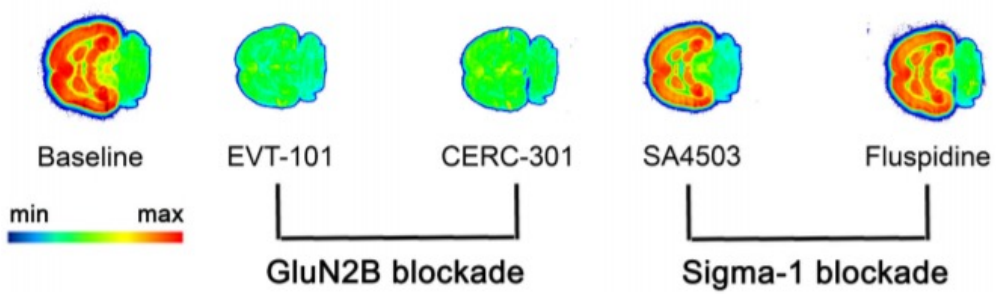
*Previous work (rodents)



(±)-^{18}F-OF-NB1

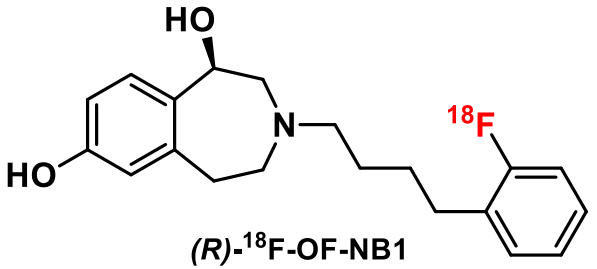
K_i (GluN1/2B): 10.4 ± 4.7 nM

K_i (sigma-1): 410 nM

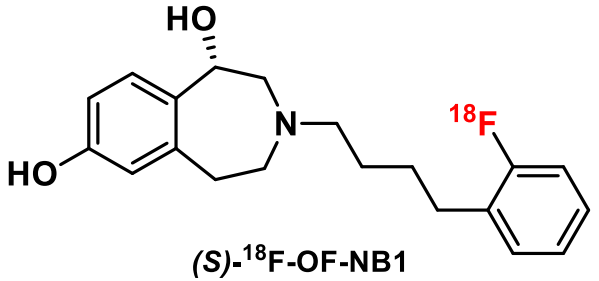


Current work (non-human primates)

Chiral separation



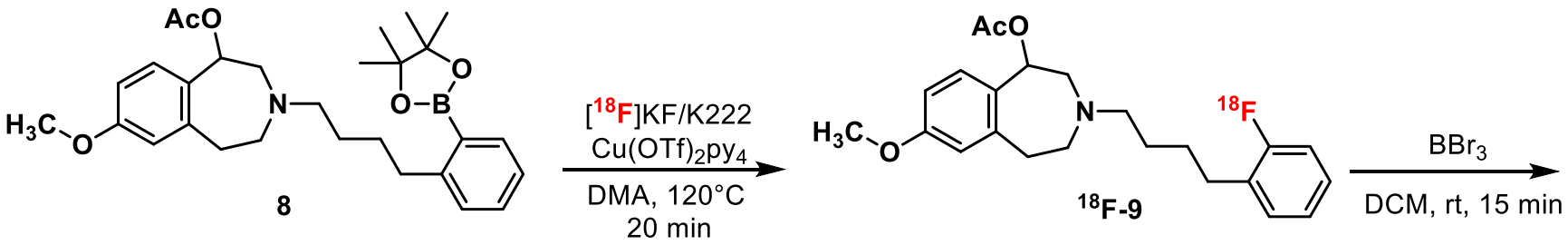
(*R*)-^{18}F-OF-NB1

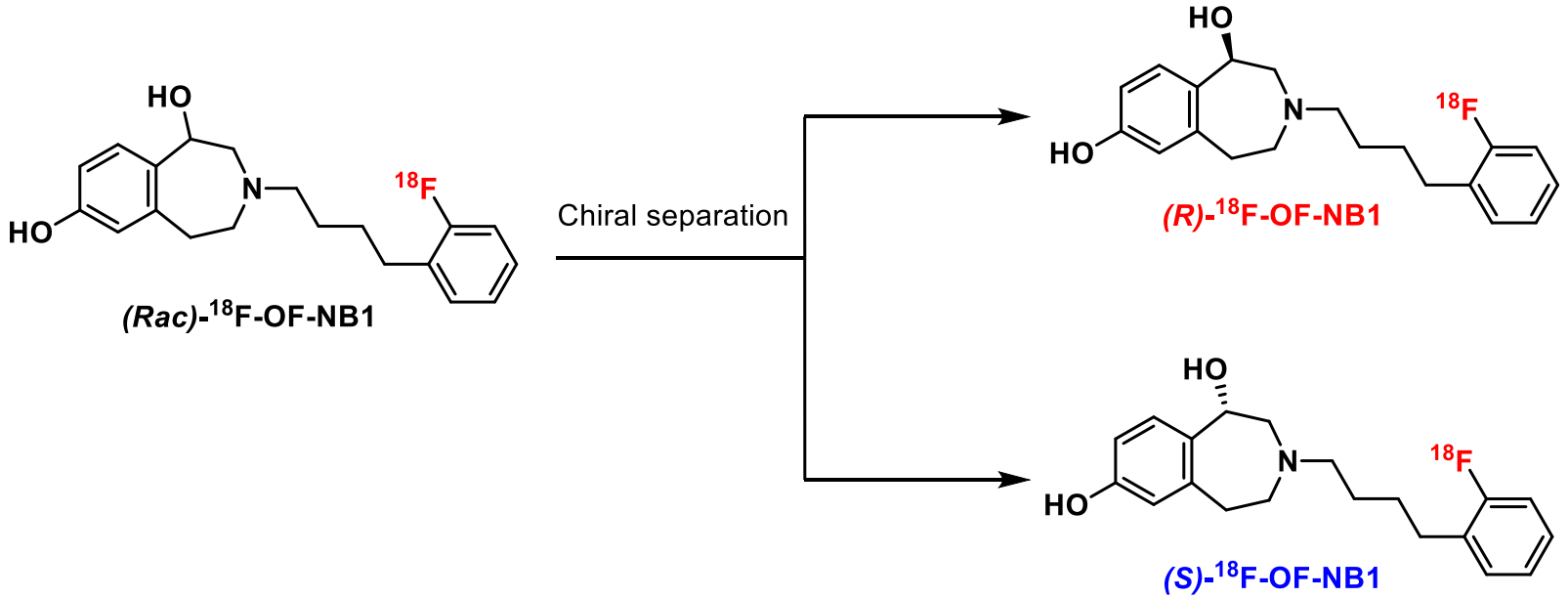


(*S*)-^{18}F-OF-NB1

* Ahmed et al, JNM 2021

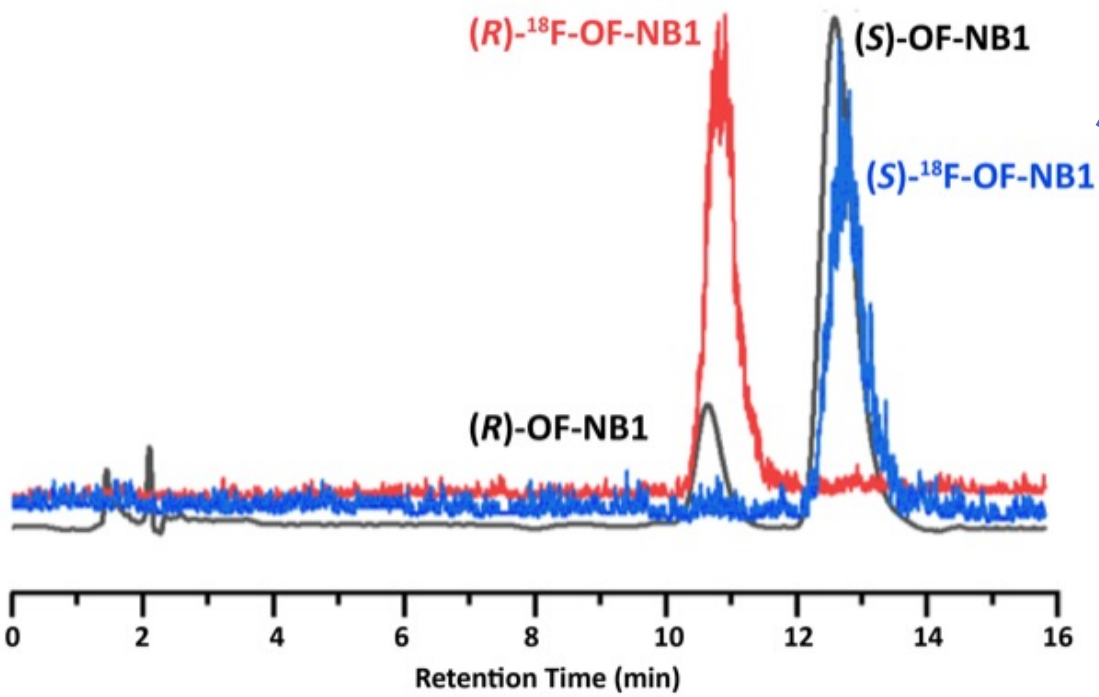
Radiosynthesis of ^{18}F-OF-NB1 & Chiral Purification





- Molar activity:
  59 ± 16 GBq/µmol (n=6)
- RCP >98%
- Enantiopurity: >98%

Quality Control & PET Imaging in Rhesus Monkeys



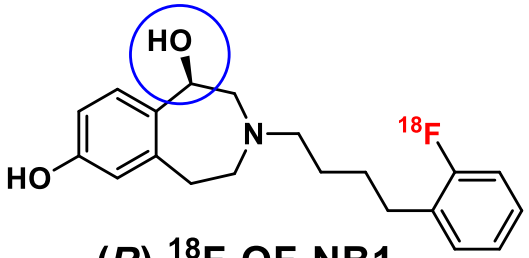
Semi-prep HPLC purification:

- Regis I-Amylose A 5 µm, 250 x 10 mm
- MeCN/0.05% aq. TEA (33/66), 5 mL/min

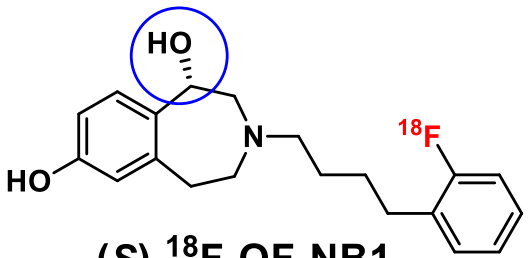
Dynamic PET Imaging (Rhesus Monkeys)

- 120 min scan time on a Focus 220 scanner
- Baseline and GluN2B blockade scans
- Plasma profile analysis & modeling
- Sigma-1 blockade scans

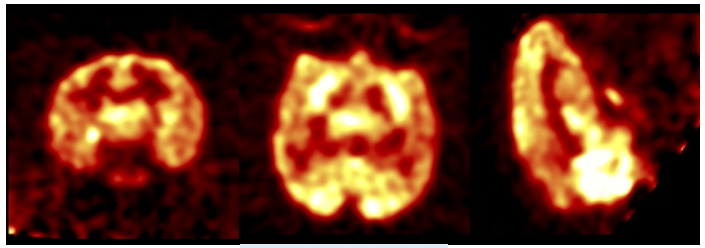
(R)- & *(S)*-^{18}F-OF-NB1: PET Imaging in Rhesus Monkeys



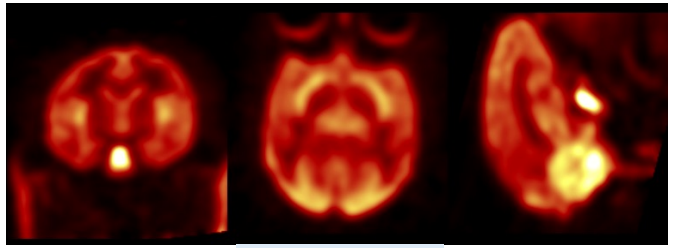
(R)-^{18}F-OF-NB1



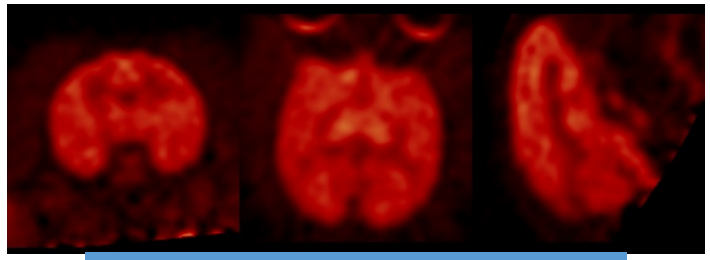
(S)-^{18}F-OF-NB1



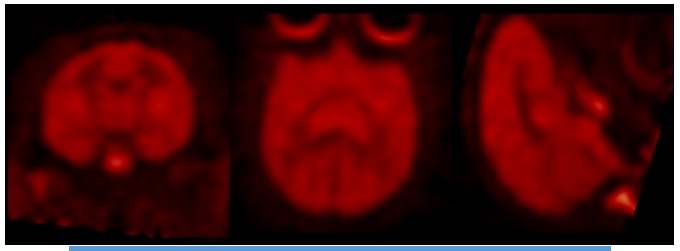
Baseline



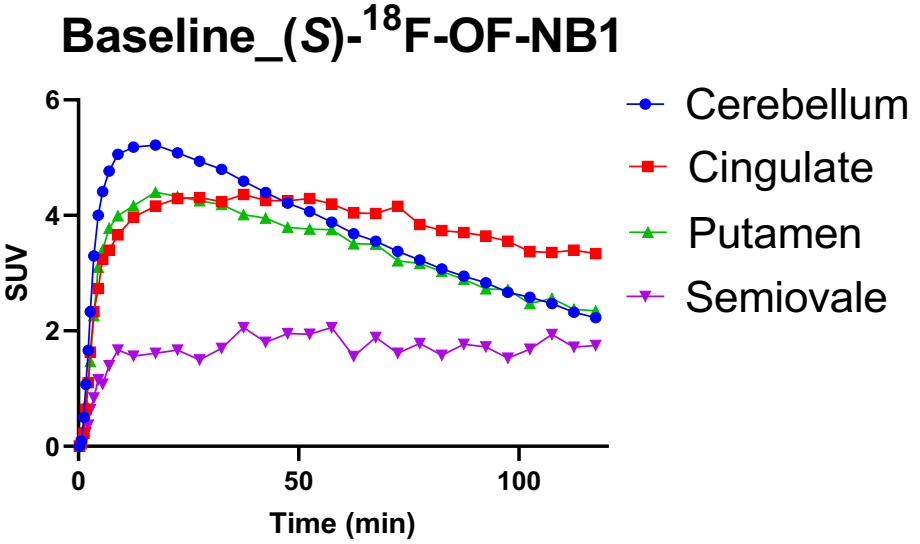
Baseline

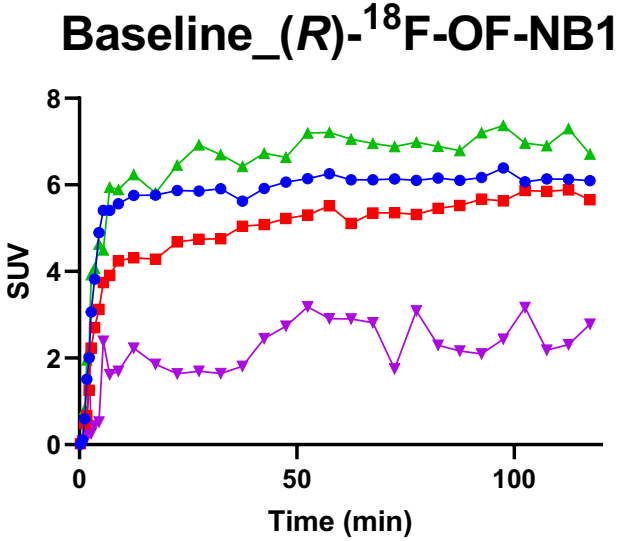


GluN2B Blockade
(CO-101244, 0.25 mg/kg)



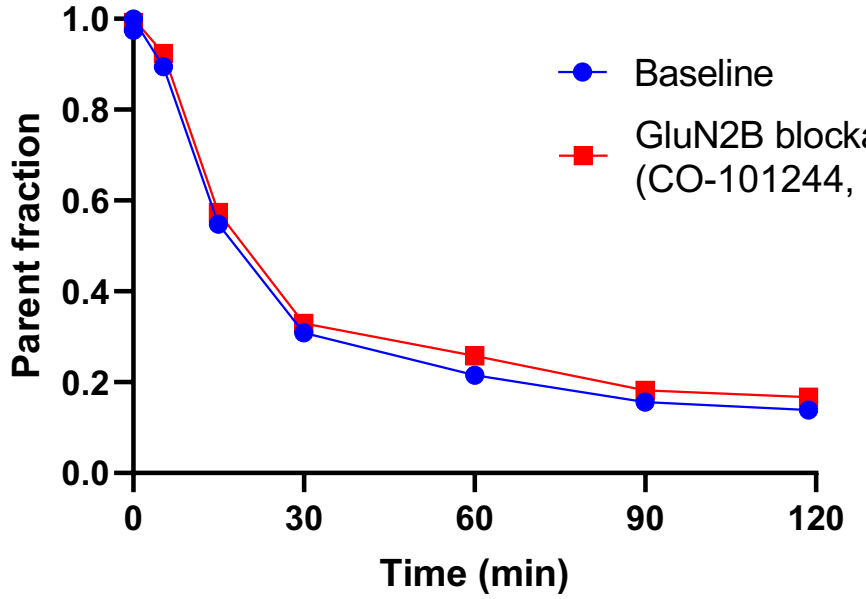
GluN2B Blockade
(CO-101244, 0.25 mg/kg)



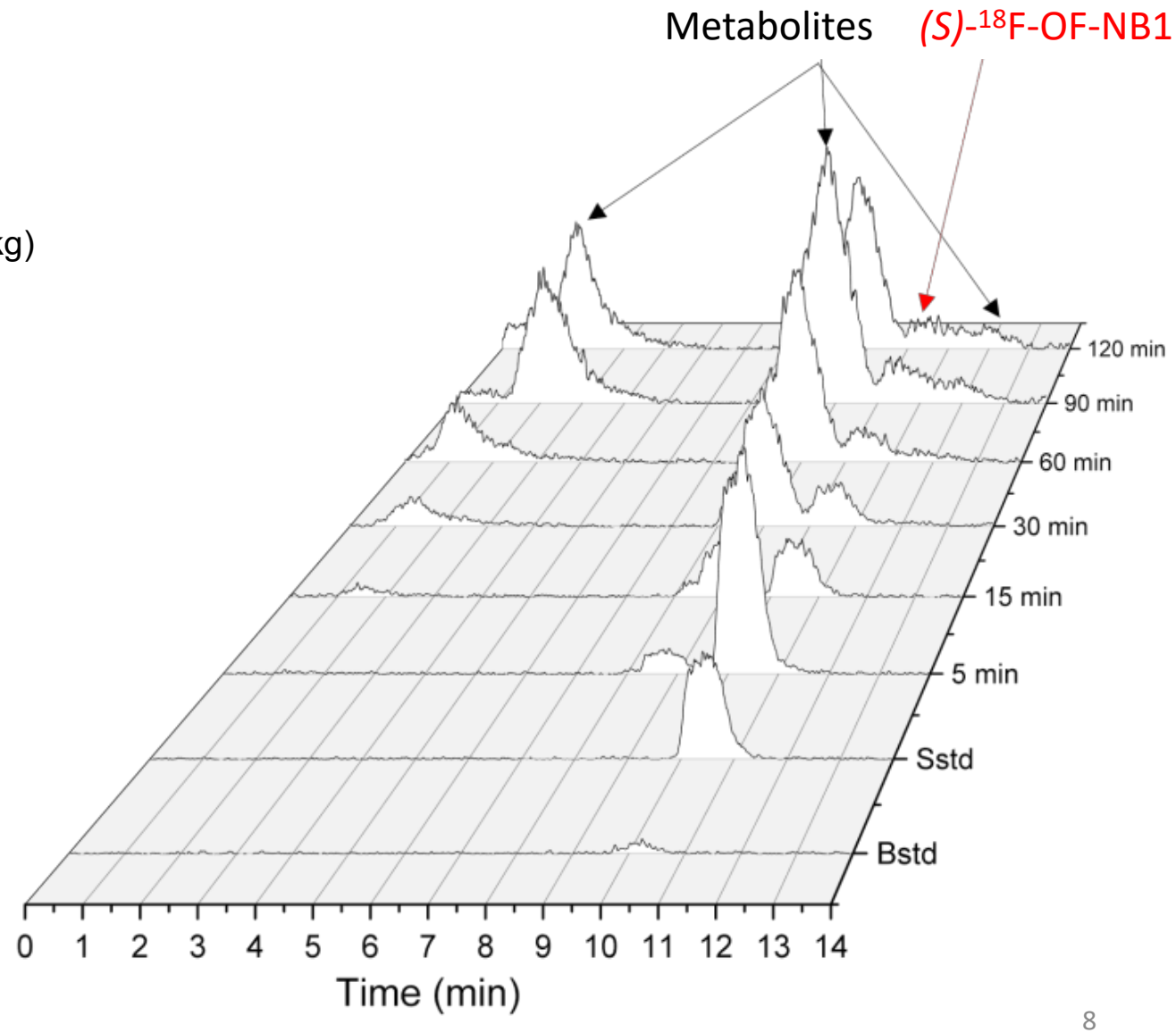


(S)-^{18}F-OF-NB1: Plasma Profile

(*S*)-^{18}F-OF-NB1_Plasma Analysis

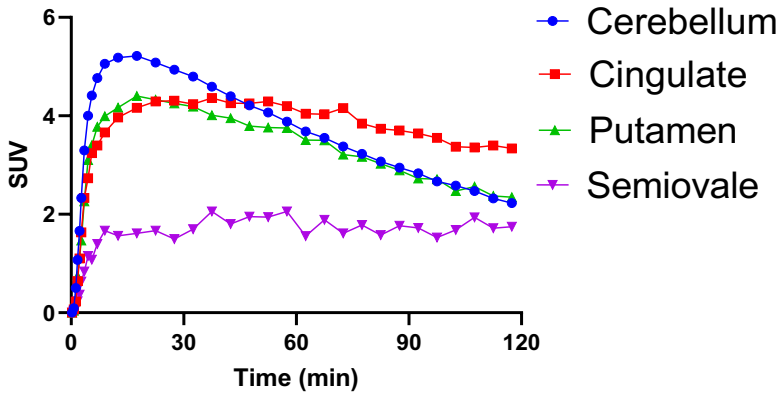


- Free fraction: 0.15
- Parent fraction @30 min: 0.31

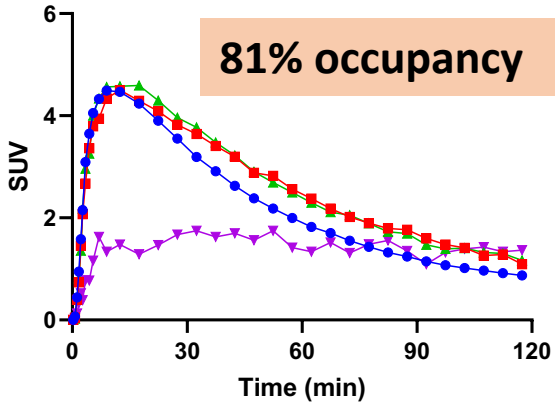


(S)-^{18}F-OF-NB1: High receptor occupancy & Selectivity

Baseline_(*S*)-^{18}F-OF-NB1

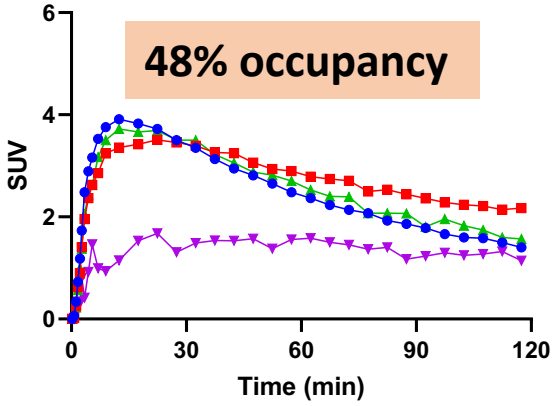


GluN2B Blockade_(*S*)-^{18}F-OF-NB1

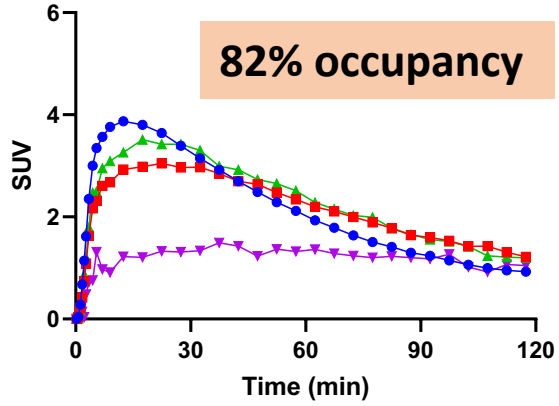


- **GluN2B blocker:**
  - Co-101244 (0.25 mg/kg)
- **Sigma-1 blocker:**
  - FTC-146 (0.125 mg/kg, high dose)
- **GluN2B + Sigma-1 blockers:**
  - (0.25 mg/kg + 0.125 mg/kg)

Sigma-1 Blockade_(*S*)-^{18}F-OF-NB1



GluN2B + Sigma-1 Blockade_(*S*)-^{18}F-OF-NB1



No additional binding reduction

(S)-^{18}F-OF-NB1: Kinetic Modeling Results

	V_T (mL/cm^3)		BP_{ND} (V_T/V_{ND} -1)
Region of interest	Baseline	GluN2B blockade (0.25 mg/kg Co101,244)	
	Monkey #1 (#2)	Monkey #1 (#2)	Monkey #1 (#2)
Thalamus	24.6 (23.3)	10.4 (10.2)	1.64 (2.20)
Cerebellum	27.3 (25.5)	10.8 (10.2)	1.93 (2.50)
Cingulate cortex	38.8 (32.3)	12.6 (12.1)	3.16 (3.44)
Frontal cortex	31.1 (28.9)	11.0 (10.7)	2.34 (2.97)
Hippocampus	32.1 (26.3)	11.5 (10.6)	2.44 (2.61)
Semiovale	20.3 (16.9)	10.6 (9.0)	1.18 (1.32)

-Monkey#1 V_{ND} = 9.32 mL/cm^3

-Monkey#2 V_{ND} = 7.28 mL/cm^3

Summary & Future Perspectives

- Tissue kinetics is slow for (*R*)-^{18}F-OF-NB1 and fast for (*S*)-^{18}F-OF-NB1.
- (*S*)-^{18}F-OF-NB1 has high plasma free fraction (~15%); parent fraction was 31% at 30 min.
- Receptor occupancy of 81-90% using a GluN2B antagonist; 48-49% with a sigma-1 antagonist at two different doses.
- No additional blocking achieved with a sigma-1 antagonist that was sequentially administered after a GluN2B antagonist.
  - Possible effects of sigma-1 drugs on GluN2B binding site?
- BP_{ND} ranges from 1.18 (semiovale) to 3.44 (cingulate cortex) for *(S)*-^{18}F-OF-NB1, indicating high levels of specific binding.
- (*S*)-^{18}F-OF-NB1 appears to be a specific PET radioligand for the GluN2B subunit of NMDA receptor with appropriate tissue kinetics in rhesus monkey and warrants further investigation.

Acknowledgements

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CRS

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