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Abstract# 265
Radiosynthesis and Characterization in non-Human Primates of Three Enantiomerically Pure PET Radioligands for Imaging the GluN2B Subunit of the NMDA Receptor Complex

Ming-Qiang Zheng[1],Hazem Ahmed[2], Kelly Smart[1], Yuping Xu[1,3], Daniel Holden[1], Michael Kapinos[1], Zachary Felchner[1], Jim R. Ropchan[1], Gilles D. Tamagnan[1], Richard E. Carson[1], Yiyun Huang[1] , Simon M. Ametamey[2]

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[2]Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland
[3]Jiangsu Atomic Institute, Jiangsu, China.

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Background

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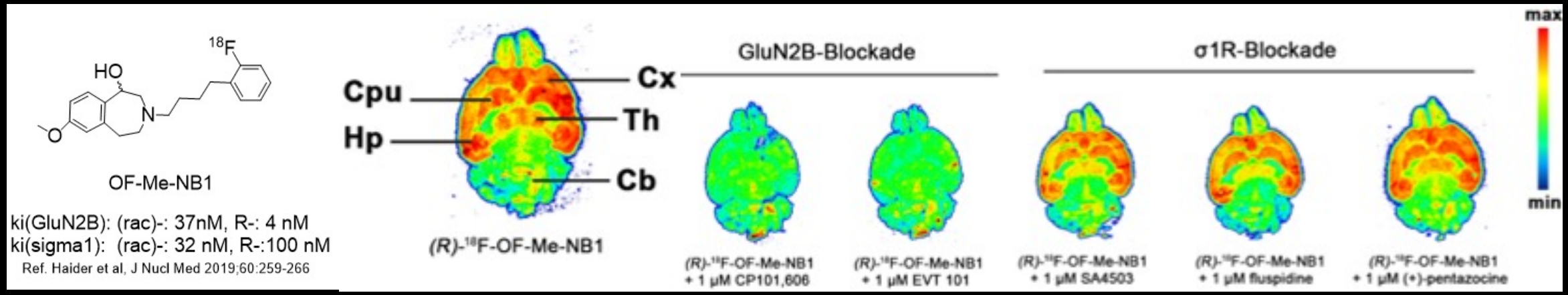

- ^{18}F-OF-Me-NB1 is a promising PET tracer for GluN2B subunit of NMDA receptors in rodents;
- Selected in MHPRD for monkey studies and potential human use. https://medicine.yale.edu/pet/mhprd/

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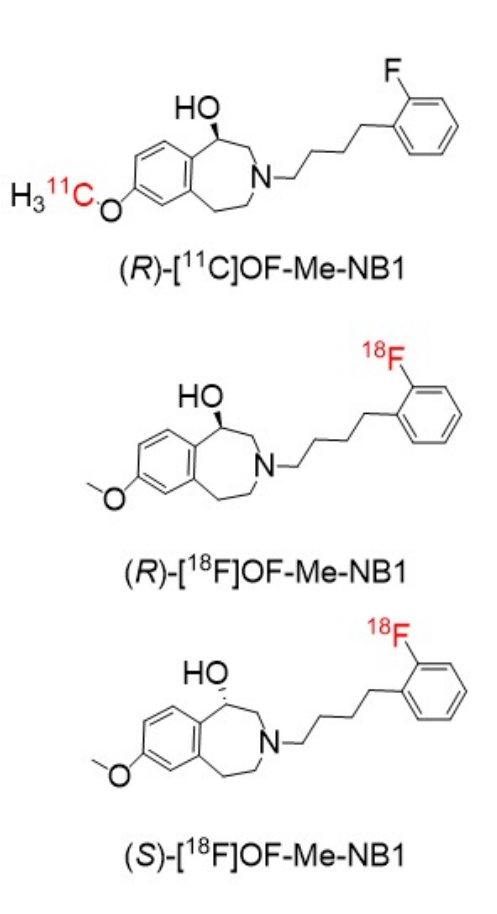
Objectives



- Preparation of three forms of ^{18}F-OF-Me-NB1
- Baseline and blocking scans in rhesus monkeys
- Metabolite analysis and input function measurement
- Modeling analysis

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Radiochemistry

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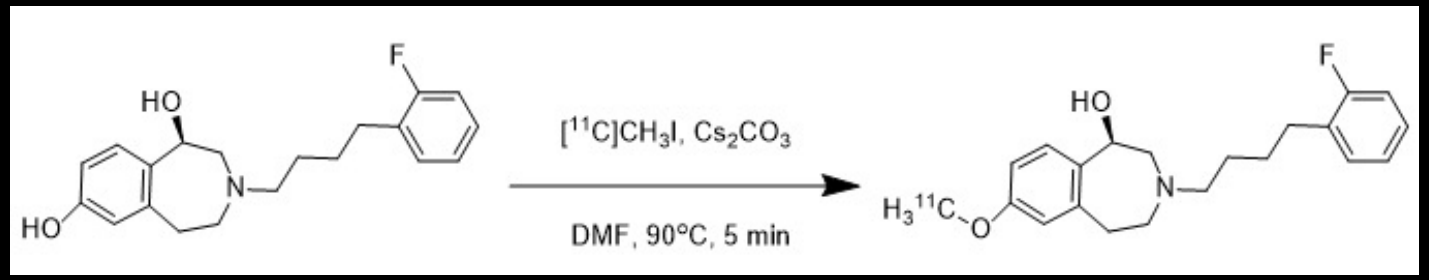
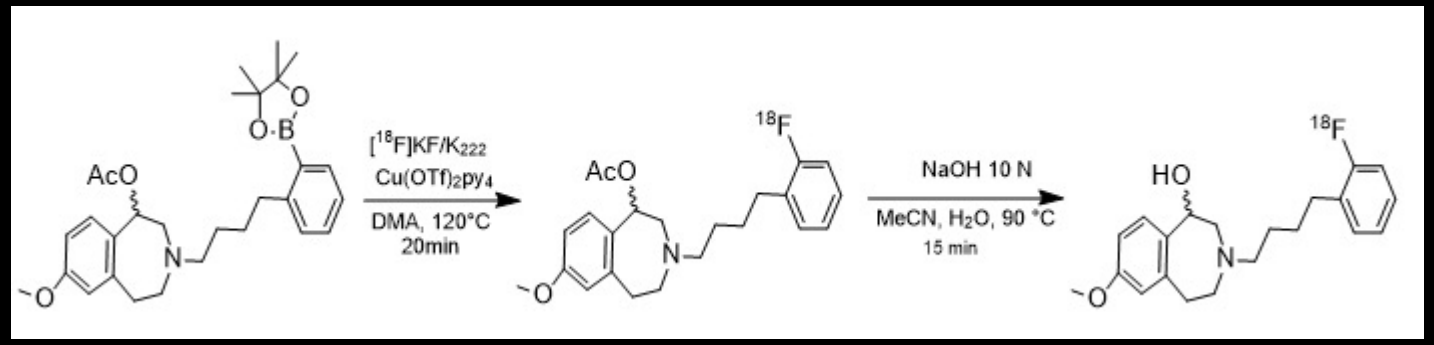


(*R*)-OF-NB1 (precursor)

(*R*)-^{11}C-OF-Me-NB1



(*R*)-BPin precursor

(S)-BPin precursor

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

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

Table 1. Summary of ^{11}C and ^{18}F tracers in QC analysis

	^{11}C (n=3)	^{18}F (n=5)
Molar activity (MBq/nmol)	518 ± 185	129.5 ± 44.4
RCP	>98%	>98%
ee	>95%	>95%

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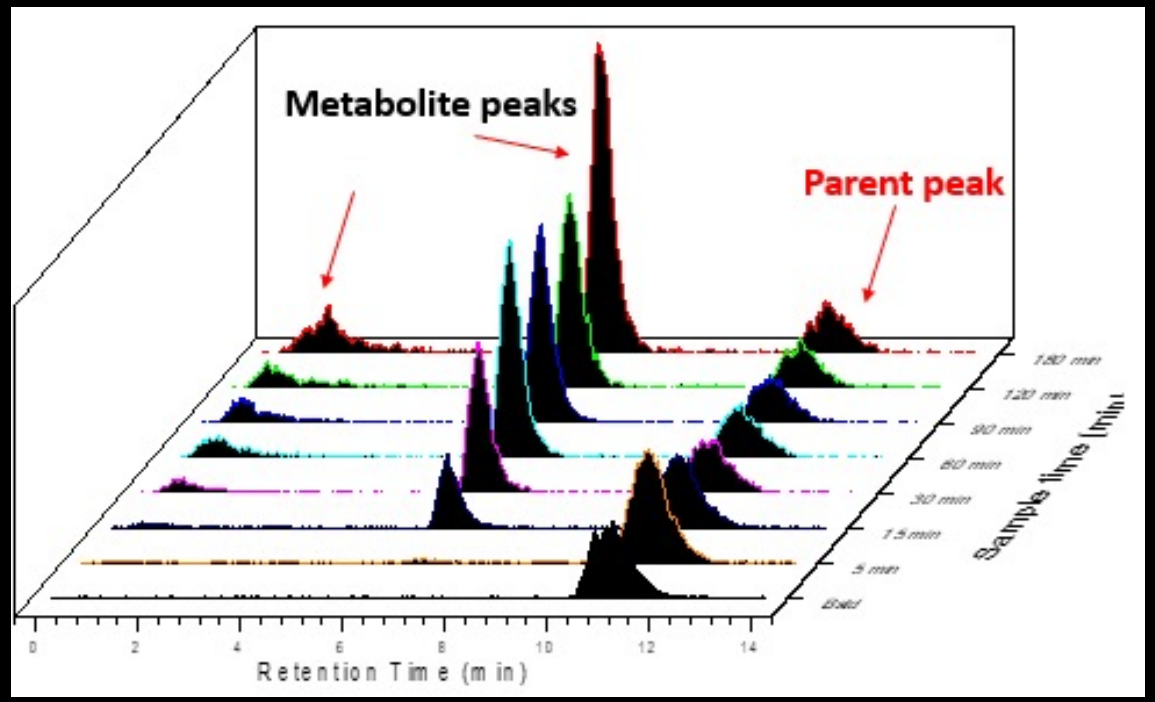
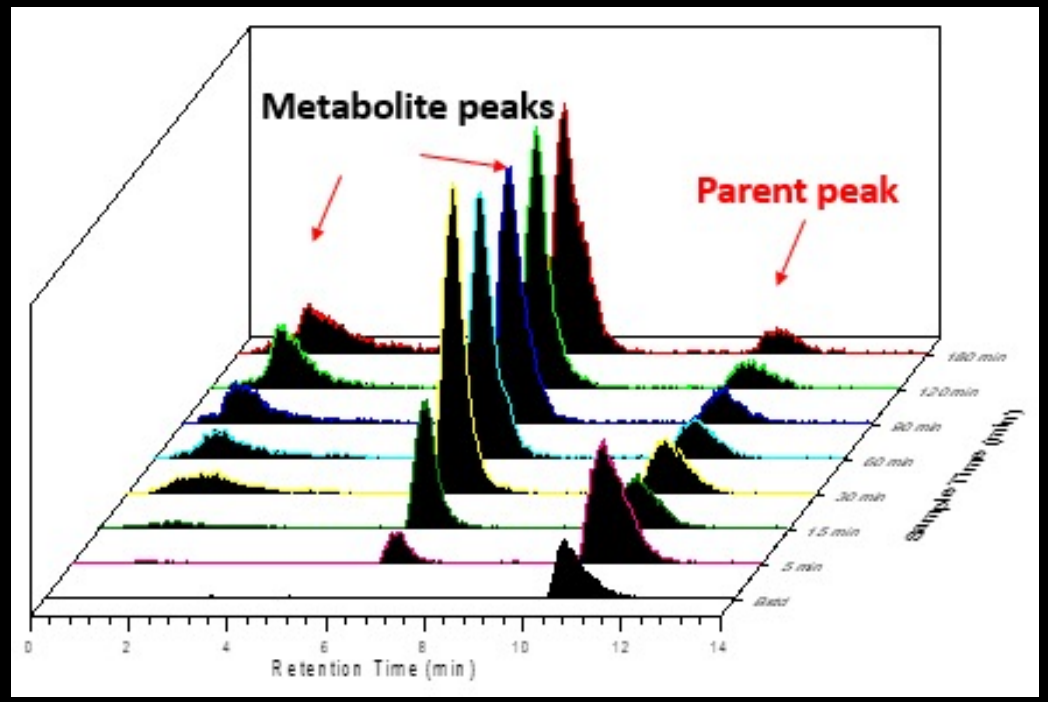
Metabolite Analysis in Monkey



***R*-^{18}F-OF-Me-NB1** 30% parent fraction @ 30 min p.i.

(Similar pattern for *R*-^{11}C-OF-Me-NB1)



***S*-^{18}F-OF-Me-NB1**

18% Parent fraction @ 30 min p.i.

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PET Image & TACs-Baseline

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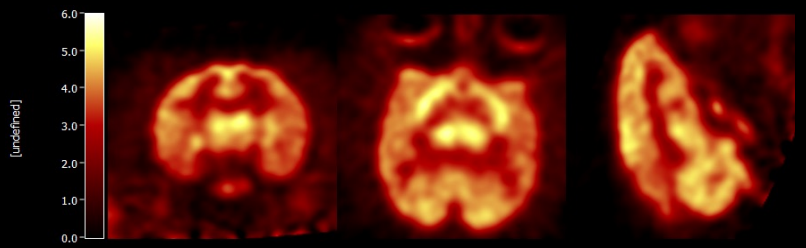
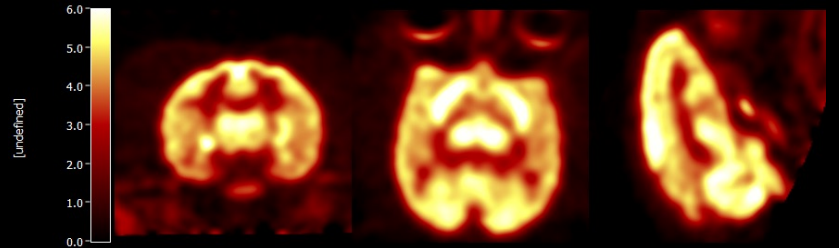
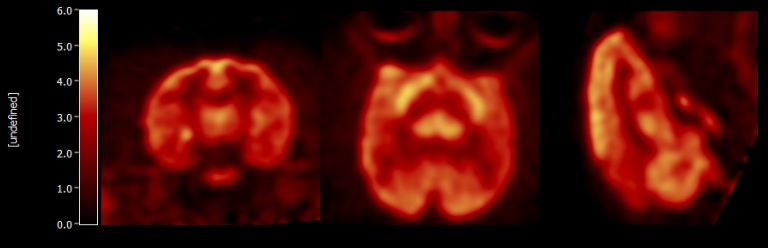
Summed SUV Images, 10-30 min



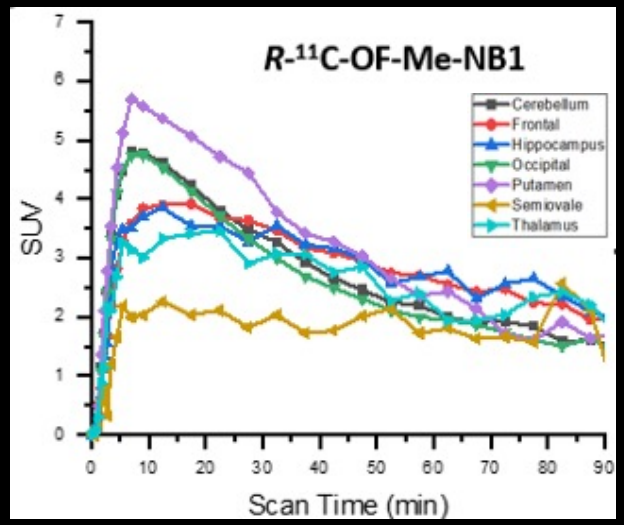
(*R*)-^{11}C-OF-Me-NB1 baseline

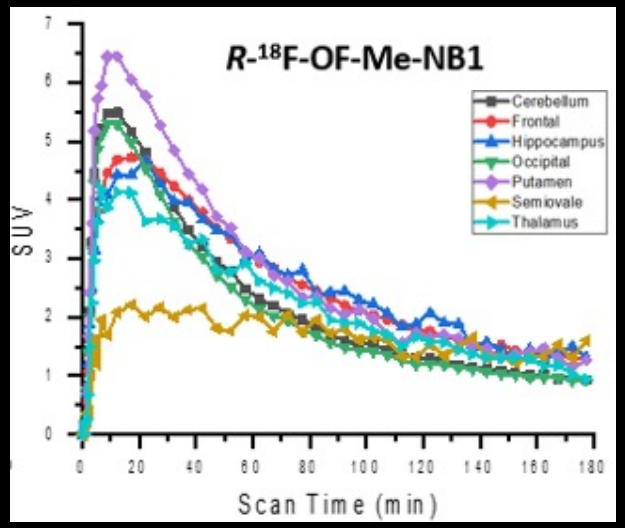


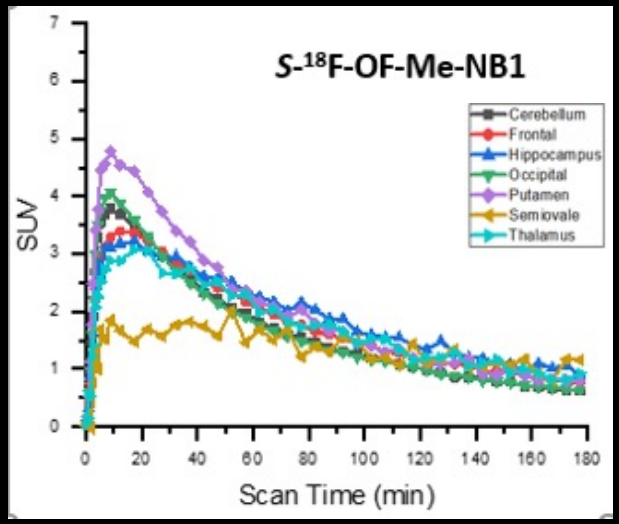
(*R*)-^{18}F-OF-Me-NB1 baseline



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







✓Higher brain uptake for (*R*)-enantiomer

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PET Images & TACs-Blocking

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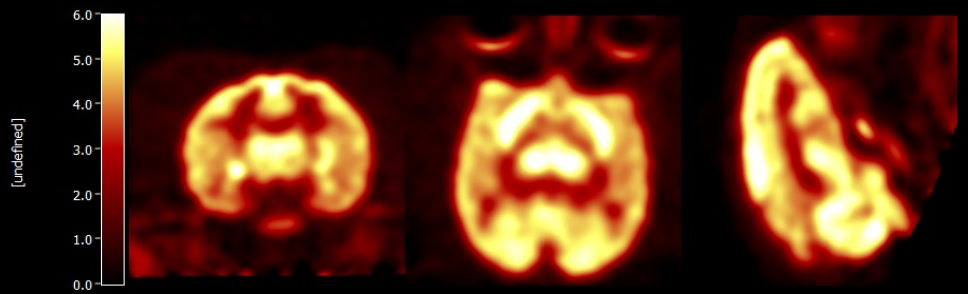
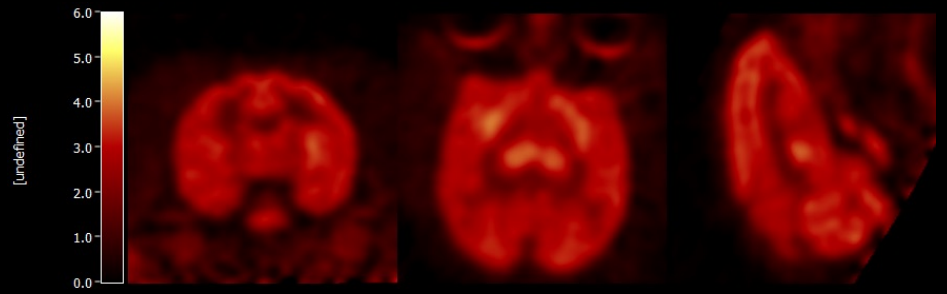
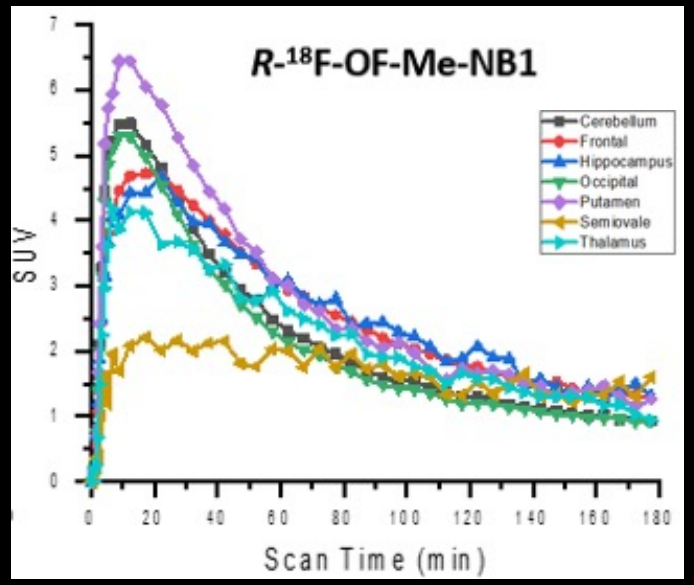
Summed SUV Images, 10-30 min

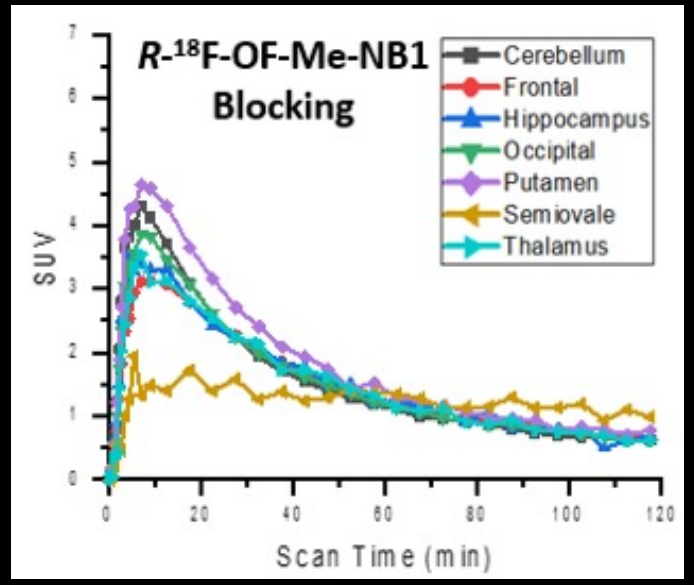


(*R*)-^{18}F-OF-Me-NB1 Baseline



(*R*)-^{18}F-OF-Me-NB1 Blocking with **0.25 mg/kg Co101244**





Occupancy: 77%

V_{ND}: 6.4

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Regional V_T and BP_{ND}

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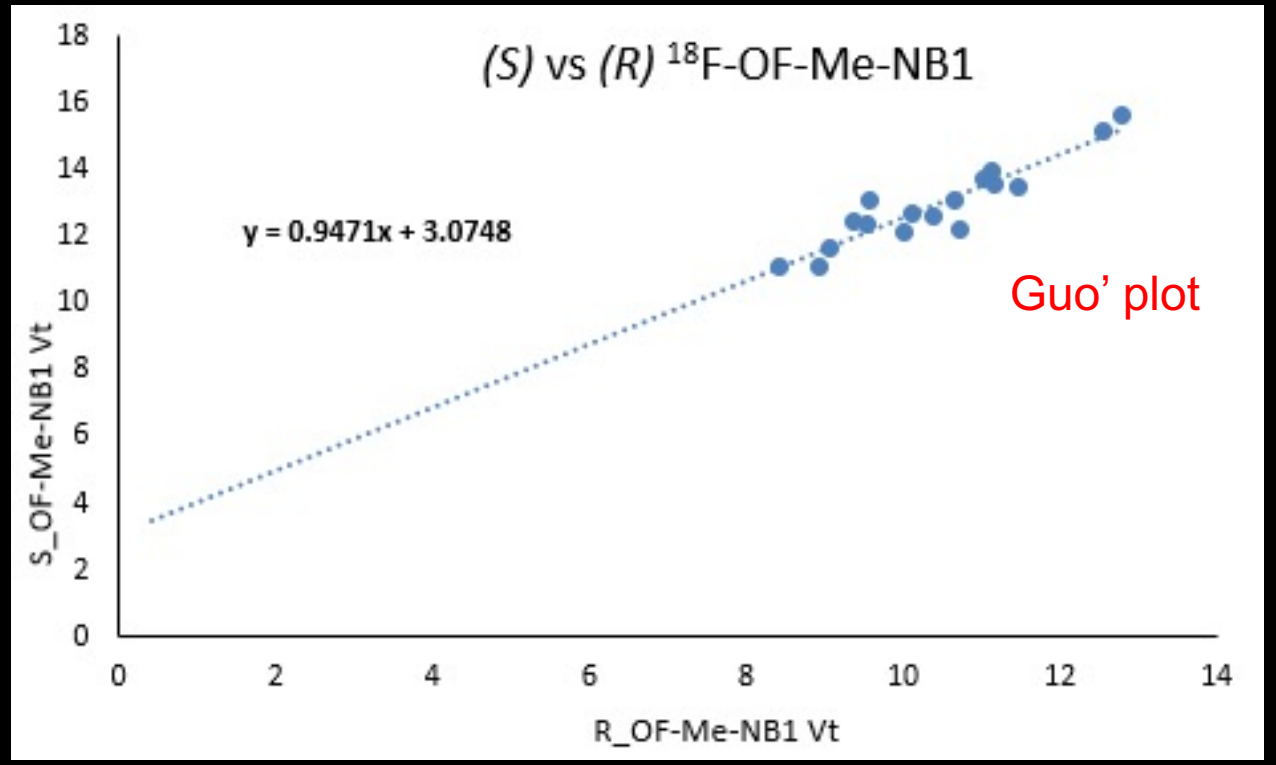
Table 2 1T Derived Regional V_T and BP_{ND} of The Three Radioligands in Different Monkey Brain Regions

Tracer/regions	Cerebellum	Frontal cortex	Hippocampus	Occipital cortex	Putamen	Cingulate cortex
R-^{11}C-OF-Me-NB1	9.6	11.8	11.8	9.0	11.4	13.9
R-^{18}F-OF-Me-NB1 (BP_{ND})	8.7 (0.37)	10.6 (0.67)	10.9 (0.71)	8.2 (0.29)	10.9 (0.72)	12.8 (1.00)
S-^{18}F-OF-Me-NB1	10.9	12.1	13.6	10.8	13.3	15.6
R-^{18}F-OF-Me-NB1 (Blocking)	6.7	6.7	6.8	6.7	8.0	8.0

$$BP_{ND} = (V_T - V_{ND})/ V_{ND}$$

Regional BP_{ND} for (*R*)-^{18}F-OF-Me-NB1: 0.29-1.00

(*R*)- vs. (*S*)-^{18}F-OF-Me-NB1



✓ Binding potential for (*R*)-enantiomer is slightly higher than the (*S*)-enantiomer.

Conclusions

- ✓ All three tracers were successfully prepared and evaluated in a rhesus monkey;
- ✓ Regional TACs were fitted well with the one-tissue compartment model (1TC) to obtain regional V_T values using the metabolite-corrected arterial input function.
- ✓ In vivo binding of (*R*)-^{18}F-OF-Me-NB1 is specific to the GluN2B subunit of NMDA receptors, as demonstrated by blocking study with the GluN2B specific antagonist Co-101244.
- ✓ Comparison of regional V_T values for (*R*)-^{18}F-OF-Me-NB1 and (*S*)-^{18}F-OF-Me-NB1 indicates that (*R*)-^{18}F-OF-Me-NB1 has slightly higher BP_{ND} values
- ✓ (*R*)-^{18}F-OF-Me-NB1 provides good specific binding signals (BP_{ND} = 0.29 – 1.00) and is a promising radiotracer for PET imaging of the GluN2B subunit.

Acknowledgement

Abstract #263 (Ahmed et al.)

Research supports

- 1. Swiss National Science Foundation;
- 2. NIH grant U01MH107803

- Yale PET Center Zoom Style Group Photo

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ePoster at the end of slides

Radiosynthesis and characterization in non-human primates of three enantiomerically pure PET radioligands for imaging the GluN2B subunit of the NMDA receptor complex

MingQiang Zheng[1], Hazem Ahmed[2], Kelly Smart[1], Yuping Xu[1,3], Daniel Holden[1], Michael Kapinos[1], Zachary Felchner[1], Jim R. Ropchan[1], Gilles Tamagnan[1], Richard E. Carson[1], Yiyun Huang[1], Simon M. Ametamey[2].

1 Yale University, New Haven, CT, USA, 2 ETH Zurich, Radiopharmacy, Zurich, Switzerland, 3 Jiangsu Institute of Nuclear Medicine, Wuxi, China.

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The NMDA receptors are involved in diseases of the central nervous system including Alzheimer's disease, ischemic brain injury, and schizophrenia. To date, there is no suitable PET radioligands for the GluN2B binding sites of brain NMDA receptors in humans. We have previously reported the evaluation of the novel radioligands ^{11}C- and ^{18}F-OF-Me-NB1 and demonstrated their in vivo binding specificity to GluN2B-containing NMDA receptor in rodents [1]. The data from in vitro autoradiography and in vivo baseline and blocking studies indicate this tracer is specifically and selectively binding to the NMDA GluN2B in rodent brain.

1. Radiosynthesis of three enantiomer pure PET tracers *R*-[^{11}C]-OF-Me-NB1, *R*-[^{18}F]-OF-Me-NB1, *S*-[^{18}F]-OF-Me-NB1;
2. Evaluate each tracer in rhesus monkey with arterial blood sampling and metabolite analysis;
3. Blocking study with *R*-[^{18}F]-OF-Me-NB1 and C_O101244 to test the binding specificity;
4. 1T and MA1 modeling, and comparison of *R* and *S*-[^{18}F]-OF-Me-NB1.

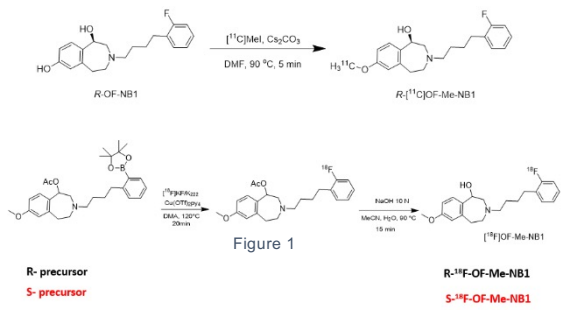

Figure 1

Enantiopure (*R*)-^{11}C-OF-Me-NB1 was synthesized by O-methylation with ^{11}C-MeI. (*R*)- and (*S*)-^{18}F-OF-Me-NB1 were prepared by ^{18}F-fluorination of the enantiomerically pure boronic ester precursors followed by cleavage of the ester protecting group with NaOH. PET scans of up to 180 min each in rhesus monkeys were conducted on the Focus 220 scanner. Plasma metabolite analysis was performed by HPLC and the arterial input function was calculated. Regional brain time-activity curves (TACs) were generated and analyzed with one-tissue (1TC) and two-tissue (2TC) compartment models and multilinear analysis-1 (MA1) method to obtain regional volumes of distribution (V_T, mL/cm^3).

All target compounds were obtained in >95% radiochemical and enantiomeric purity, with molar activity of 14±5 mCi/nmol for ^{11}C-OF-Me-NB1 (n=3) and 3.5±1.2 mCi/nmol for ^{18}F-OF-Me-NB1 (n=5) at the end of the synthesis (Fig.1). Metabolism was fast with ~30% parent compound for (*R*)-^{11}C-OF-Me-NB1 and (*R*)-^{18}F-OF-Me-NB1 at 30 min after injection, and ~18% for (*S*)-^{18}F-OF-Me-NB1(Fig.2). Plasma free fraction for all three forms of the radiotracer was ~2%. In the monkey brain both (*R*)-^{11}C-OF-Me-NB1 and (R)-^{18}F-OF-Me-NB1 displayed very similar pattern of fast uptake and clearance, while (*S*)-^{18}F-OF-Me-NB1 showed lower brain uptake and faster clearance in all brain regions (Fig. 3). Radioactivity uptake was high in the putamen, hippocampus and thalamus, medium in the occipital cortex and cerebellum, and low in the white matter (centrum semiovale)(Fig.4). Both the 1TC model and MA1 method fitted the TACs well and provided reliable VT estimates, ranging from 8.2 in the Occipital cortex to 13.9 in the cingulate cortex for (*R*)-^{11}C- and (*R*)-^{18}F-OF-Me-NB1, compared to 10.8 in the occipital cortex to 15.6 in the cingulate cortex for (*S*)-^{18}F-OF-Me-NB1 (Table 1). The binding potential for the (*R*)-^{18}F-OF-Me-NB1 ranges from 0.29 to 1.00. Blocking study with 0.25 mg/kg of Co1012444 for (*R*)-^{18}F-OF-Me-NB1 results in 77% of occupancy (Fig.5). The binding potential of R is slightly higher than the S- ^{18}F-OF-Me-NB1 (Fig. 6, Guo's plot).

NA

Fig 2

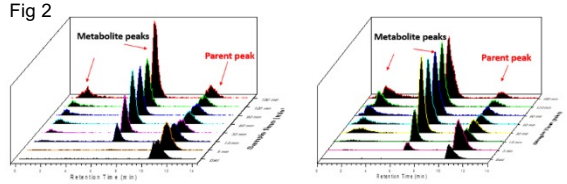

Figure 2

Fig 3

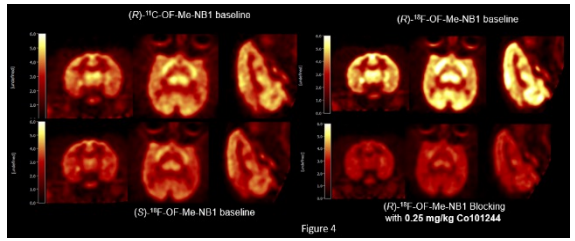

Figure 4

Fig 4

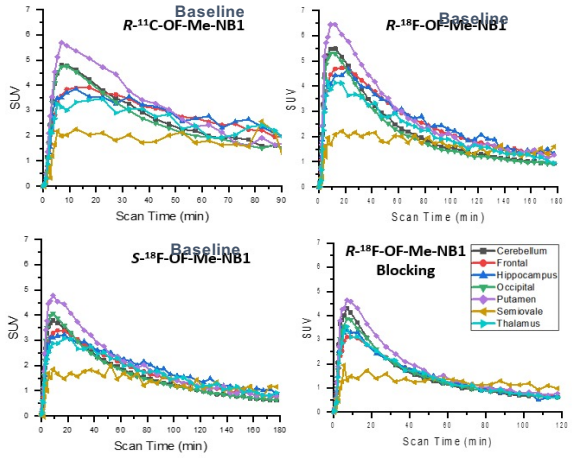

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Fig 5

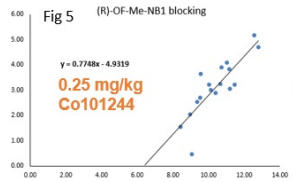
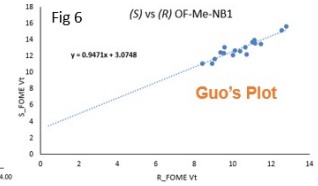


Fig 6



We have successfully synthesized and evaluated three enantiomerically pure radioligands for targeting the GluN2B subunit of the NMDA receptor complex. The (*R*)-^{11}C-OF-Me-NB1 and the (*R*)-^{18}F-OF-Me-NB1 have similar in vivo behavior in metabolism, brain uptake and V_T, but F-18 tracer gives better brain signals in the PET images due to its longer half-life. The binding signals for (*S*)-^{18}F-OF-Me-NB1 are lower than the R tracer. Blocking studies with Co101244 for (*R*)-^{18}F-OF-Me-NB1 results in 77% of occupancy indicates the in vivo binding is specific. (*R*)-^{18}F-OF-Me-NB1 provides good specific binding signals (BP_{ND} = 0.29 – 1.00) and is a promising radiotracer for PET imaging of the GluN2B subunit of the NMDA receptors.

1. Haider A et al. J Nucl med 2019; 60:1167

1. Swiss National Science Foundation; 2. NIH grant U01MH107803.