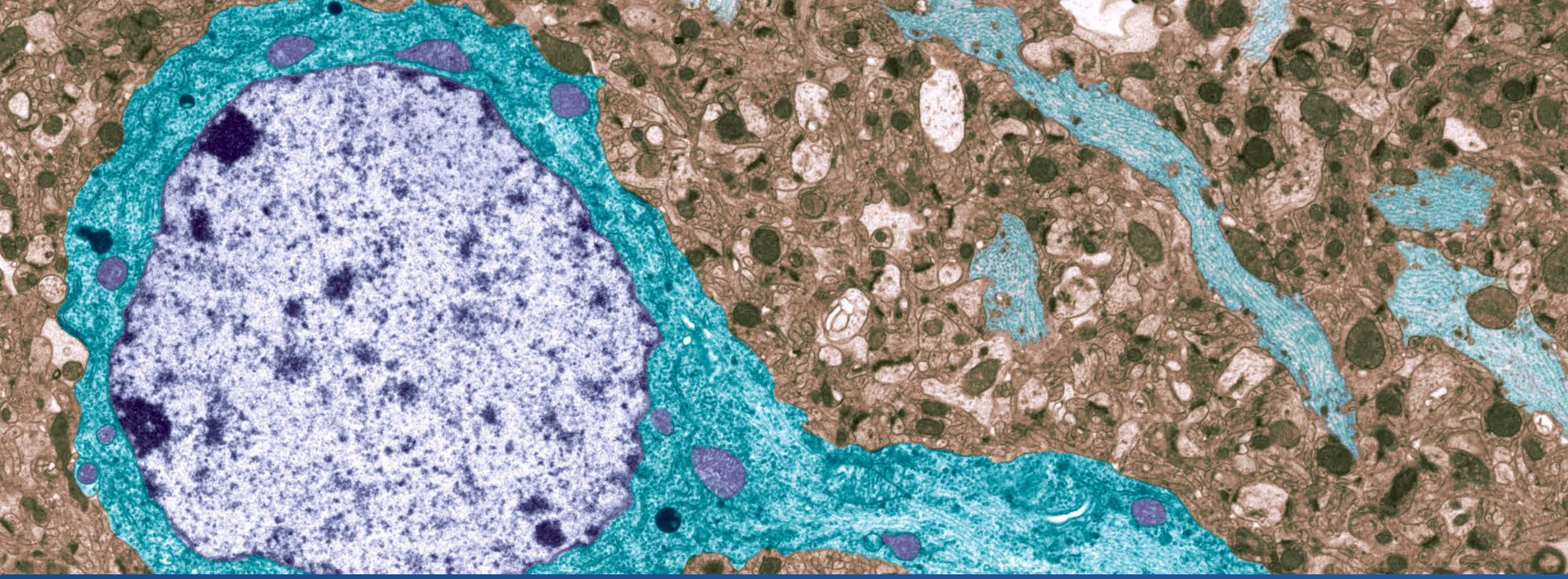


In Vivo Imaging of Neuroinflammation

Development of a P2X7R PET Tracer: [18F]JNJ-64413739

Hartmuth Kolb, Katrin Szardenings, Greg Chen, Wei Zhang, Anna Xia, Tamara Berdyyeva, Chaofeng Huang

Anindya Bhattacharya, Nicholas Carruthers, Michael Letavic,

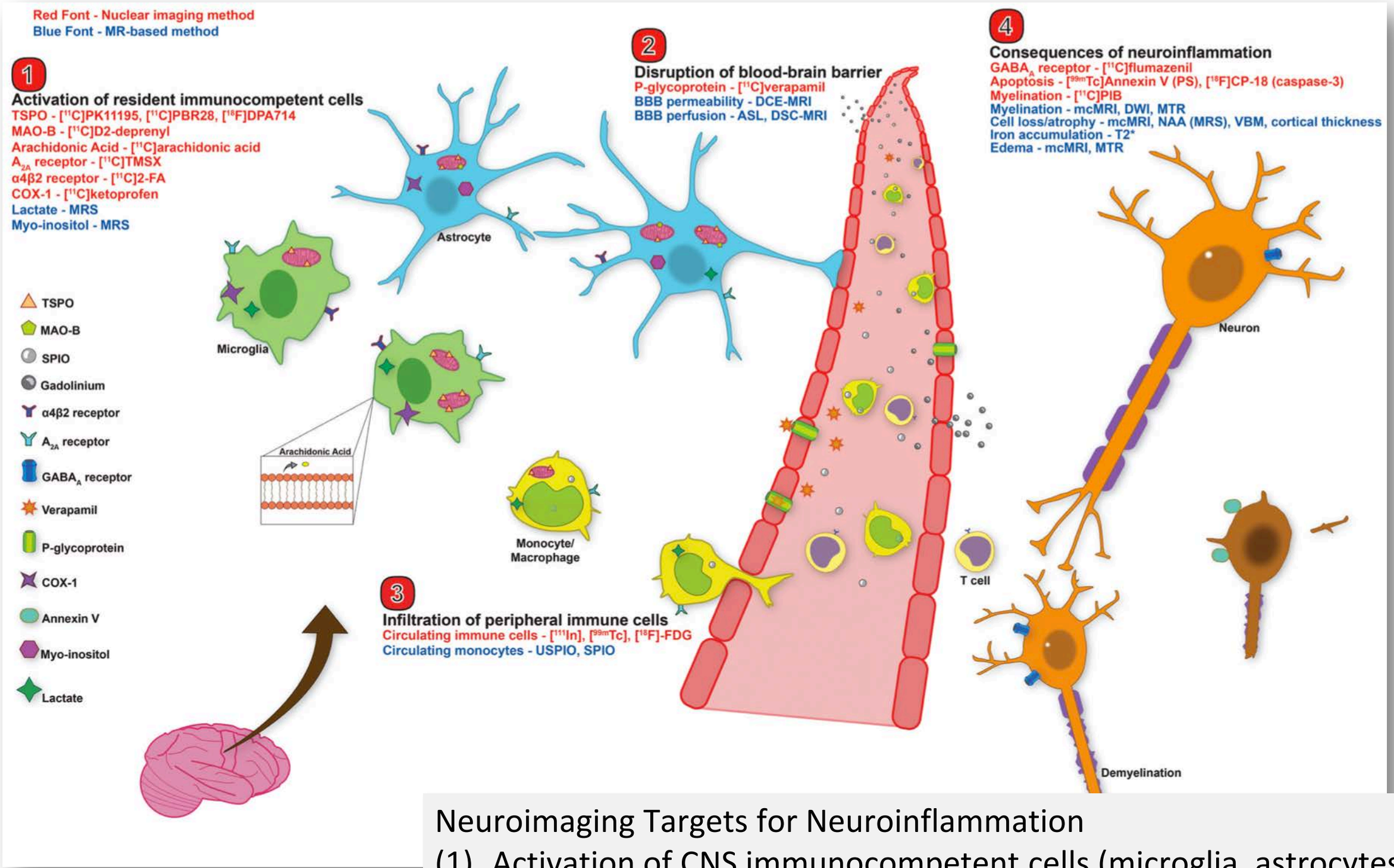
Gilles Tamagnan (MNI), Guy Bormans (KU Leuven)

Pictured above: Nerve Cell

janssen Neuroscience

PHARMACEUTICAL COMPANIES OF Johnson & Johnson

In Vivo Imaging of Human Neuroinflammation



Neuroimaging Targets for Neuroinflammation

(1) Activation of CNS immunocompetent cells (microglia, astrocytes),
(2) disruption of BBB,
(3) infiltration of peripheral immune cells, and
(4) consequences of neuroinflammation (e.g., demyelination, cell death)

D.S. Albrecht, C. Granziera, J.M. Hooker, M.L. Loggia ACS Chemical Neuroscience 2016

Session 3: Neuroimaging Biomarkers, Current Initiatives and Opportunities

- HARTMUTH KOLB, KATRIN SZARDENINGS
  Johnson and Johnson

- In vivo imaging of Neuroinflammation: P2X7R PET

- ROBERT INNIS, National Institute of Mental Health

- Positron Emission Tomography of Translocator Protein 18 kDa (TSPO) as a Biomarker of Neuroinflammation in Dementia and in Depression

- MARTINA ABSINTA, National Institute of Neurological Disorders and Stroke

- MR imaging of chronic inflammation in multiple sclerosis

- KATERINA AKASSOGLOU, Gladstone Institute of Neurological Disease

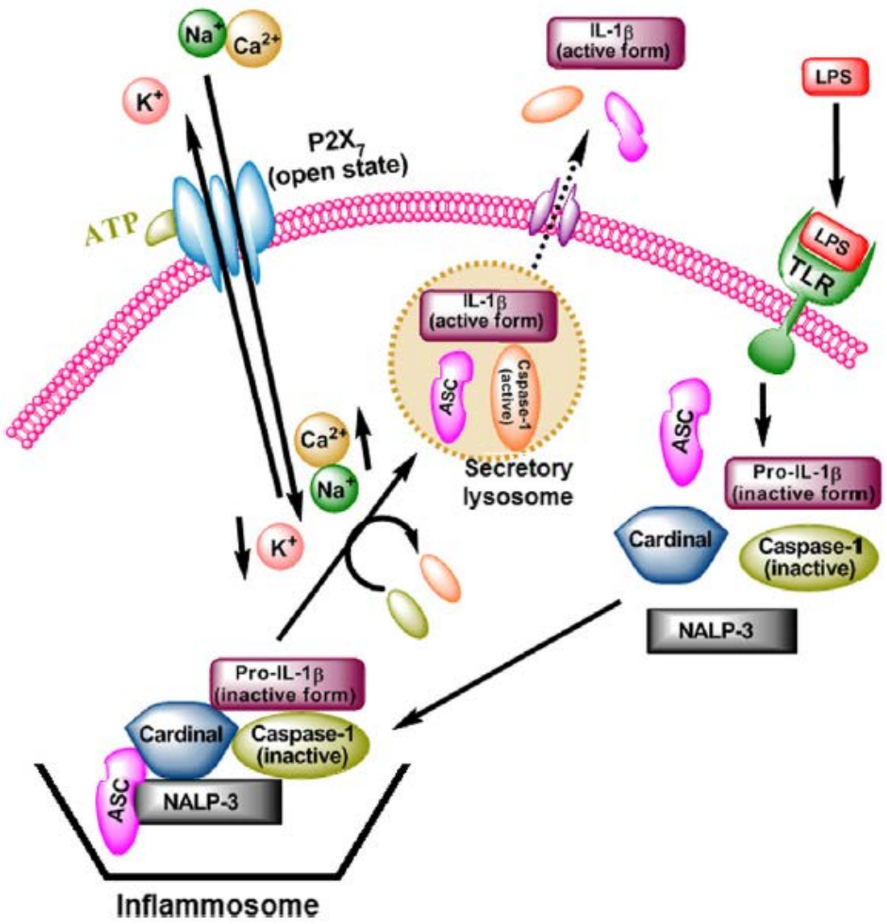
- Fibrin in Neurologic Diseases: Mechanisms, Imaging, Therapeutics

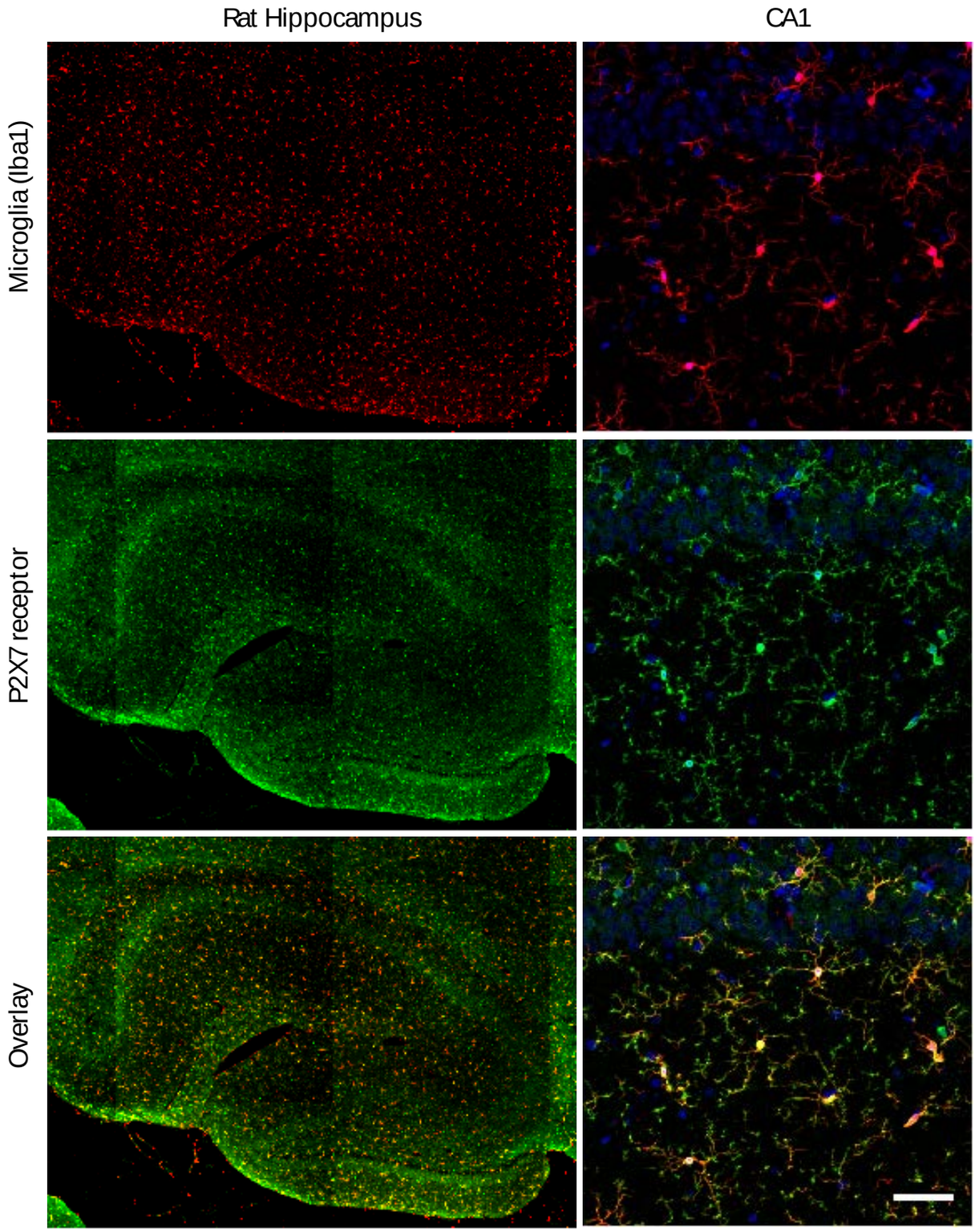
Potential Targets for Neuroimmune PET Imaging

Target	Distribution in healthy brain	Expression in Neuroinflammation model	PET Ligands	Key References
TSPO	Not in neurons; micro- and macroglial cells	Upregulated w/ microglial & astrocyte activation. High levels of non-specific binding, poor S/N, Ki influenced by polymorphism	**11C-PK11195**, 11C-PBR28, 18F-PBR06, 18F-GE-180, 18F-DPA-714 etc.	L. Vivash, T.J. O'Brien JNM 2016, 57, 165-168 (review); Hannestad, J. et al. NeuroImage 2012, 63 (1), 232–239 (LPS model); W.C. Kreisl, R.B. Innis et al. Brain 2013 (AD).
COX1	Neurons, endothelium, and microglia but not astrocytes	Upregulated primarily in microglia by neuroinflammaton	11C-ketoprofen methyl ester - Shukuri; **11C-PS13 & 18F-PS2**	Depboylu, Neurobio Disease 2011; Shukuri JNM 2011; Innis, Pike JNM 2015
COX2	Neurons	Markedly upregulated by inflammation in microglia and probably other cells	11C-Ibuprofen, Celecoxib; 11-C-GW406381x; **11C-MC1 & 18F-MC8**	Depboylu, Neurobio Disease 2011; Pulli, J Clin Cell Immunol 2014; Yiangou BMC Neurol. 2006, Gunn, J Cer Blood Flow Metab 2012; Innis and Pike JNM 2015
P2X7R	Widely expressed in microglia, astrocytes	2-5x increased expression in astrocytes and microglia in LPS rat model; 70% increased expression in AD microglia. Increased P2X7 expression drives microglial activation, rather than P2X7 overexpression being a consequence of microglial activation	**[18F]JNJ-64413739** [11C]GSK1482160; [11C]A-740003	H.B. Choi et al. J. Neuroscience 2007, 27, 4957; S.D. Skaper et al FASEB 2010, 24, 337-345; Y. Yiangou et al. BMC Neurol. 2006, 6. A.D. Michel et al. British J. Pharmacol. 2007, 151, 84; M. Gao et al. Bioorg. & Med. Chem Letters 2015, 25, 1965-1970
CB2R	Microglia, astrocytes, neurons - low expression	Up to 10x higher expression in activated microglia; seen in AD, ALS, AIDS dementia	11C-A-836339, **18F-29**, 18F-SAR343680; 11C-NE40	R-P Moldovan, M.G. Pomper, A.G. Horti et al. J.Med.Chem. 2016, 59, 7840; C. Benito et al. British J. Pharmacol. 2008, 153, 277-285; Y. Yiangou et al. BMC Neurol. 2006, 6.
CD11b/CD18 (Mac-1)	Microglia, (monocytes, macrophages)	Strong induction of CD11b expression in models of cortical impact and LPS.	Mostly mAbs; Leukadherins; 18F-VHH7 (mAB fragment)	D. Maiguel et al. Science Signalling 2013, 4(189); J.C. d'Avila et al. J. Neuroinfl. 2012, 9(31); R. Weissleder et al. PNAS 2015, 112(19)

P2X7 receptor expression in the brain

- P2X7: ATP gated ion channel





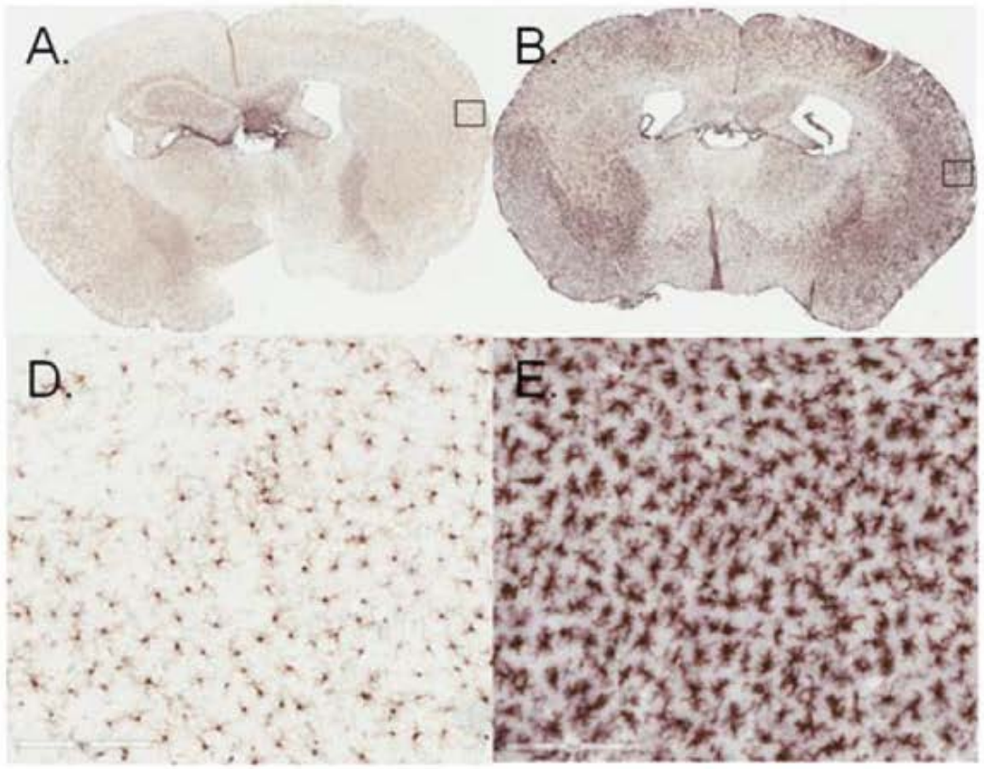
- Expressed throughout the brain & predominantly in microglia
- Similar expression observed with PET ligands in NHP
- **no reference region**

Neuroimmune Imaging - P2X7

LPS injection (IP with saline or 5 mg/kg LPS) causes increased Iba1 immunoreactivity and increased P2X7 expression

Iba1 IHC on coronal brain section of a mouse injected with Saline.

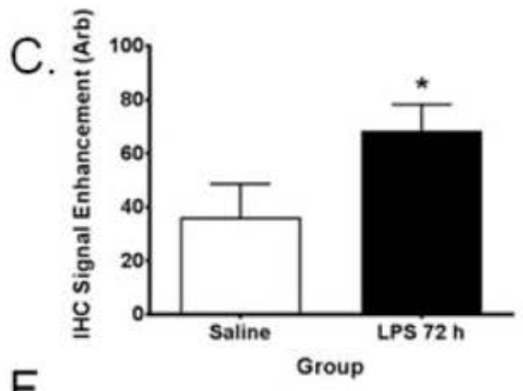
Iba1 IHC on brain section of a mouse injected with LPS @ 72h.



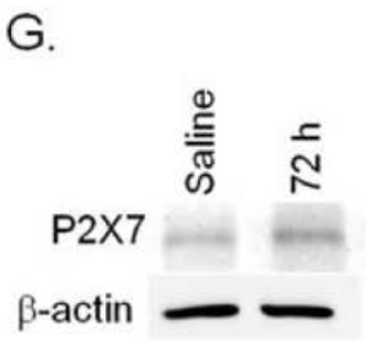
20X magnification

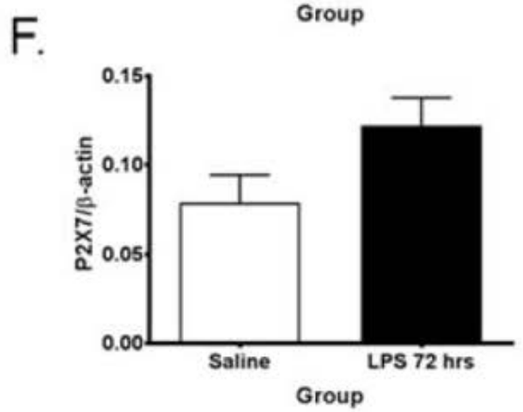
20X magnification

Iba1 IHC staining intensity in Saline (open) vs. LPS (filled) treated mice



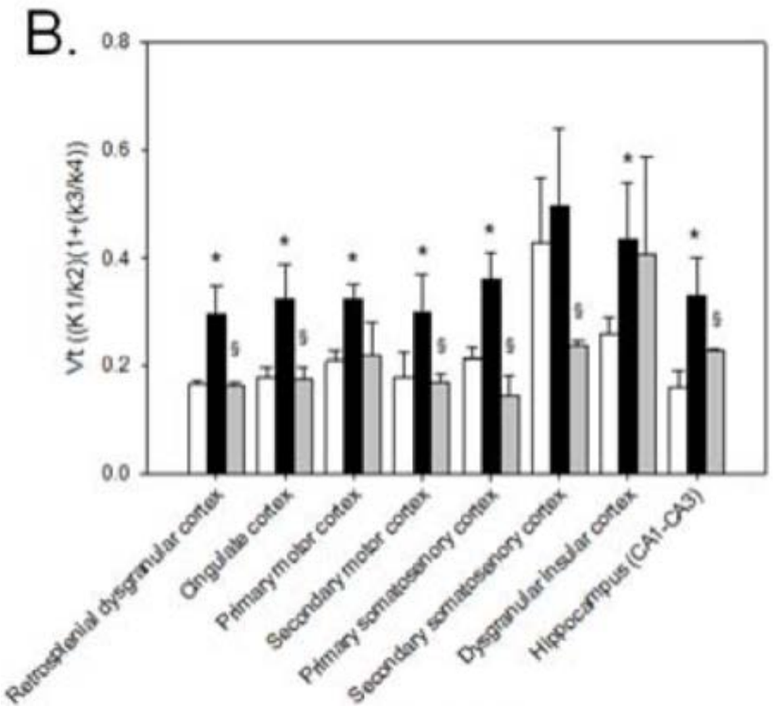
Western blot of P2X7 protein expression in the cortex of LPS treated mice after 72 hrs.





P2X7 expression levels ratioed to ß-Actin in Saline (open) vs. LPS (filled) treated mice.

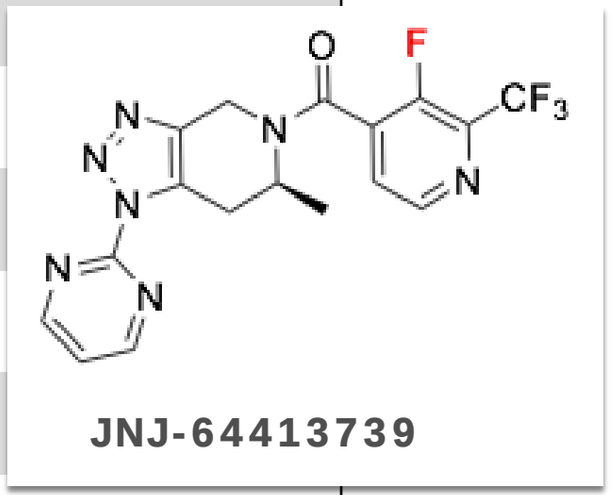
11C-GSK1482160 PET total volumes of distribution (VT) for saline (open), LPS (black), and LPS+Block (grey)



Paul R Territo, Jill A Meyer et al. J Nucl Med. 2016

Profile of JNJ-64413739

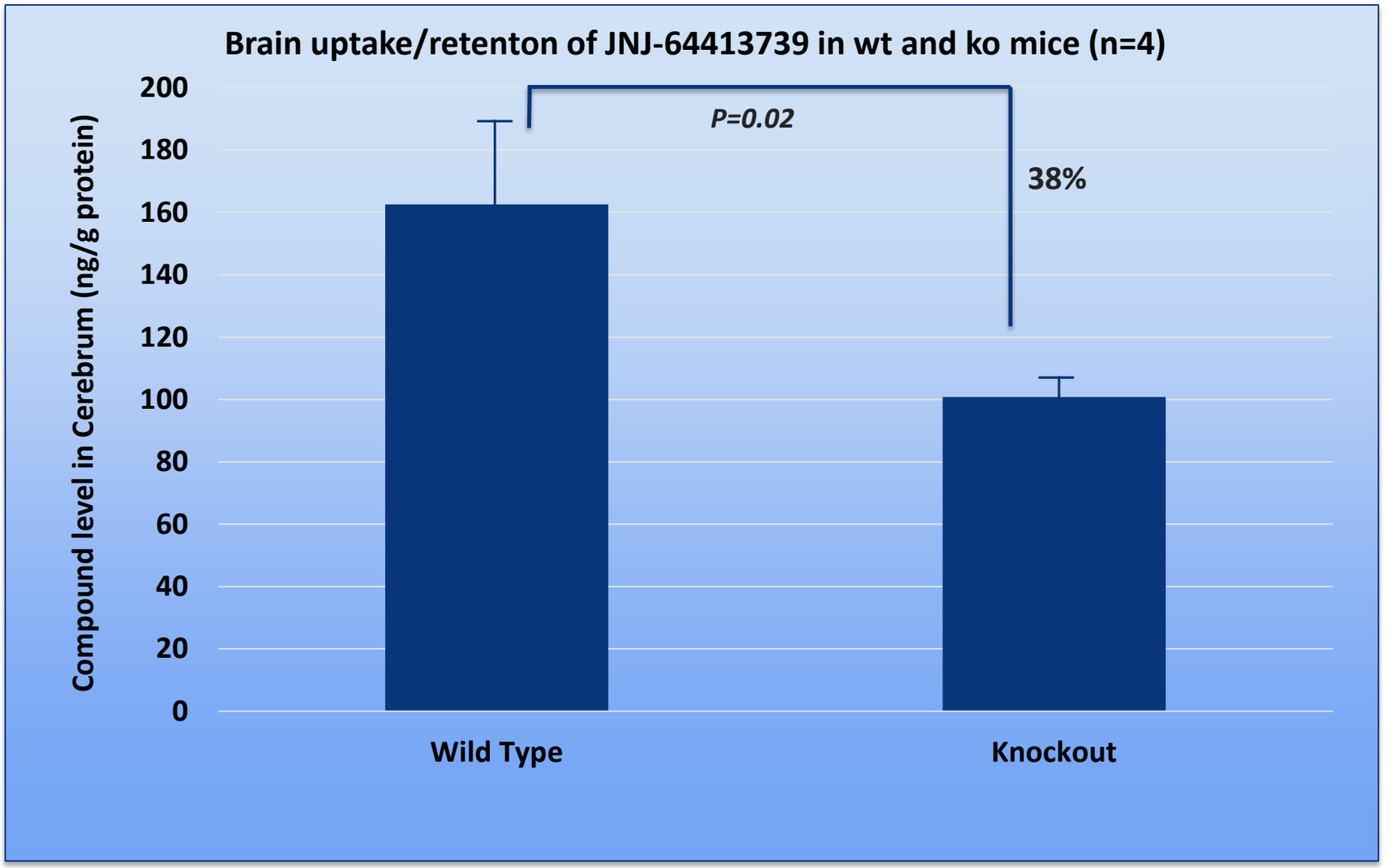
Feature / Test	JNJ-64413739
M.W./clogP/TPSA	407/1.3/85
IC_{50} P2X7 h/r (nM)	1.0/1.9
Ki P2X7 h/r (nM)	15/2.7
Plasma Protein Bdg (h/r, free-%)	63/67
Brain Tissue Bdg (rar, free-%)	18.7
MDCK ratio	13.8
ex-vivo ARG occupancy (rat, i.v., 1 mg/kg)	92% (@ 5min), 92% (@ 30min), 87% (@ 60min)
Brain/Plasma (rat, i.v.) by LC-MS/MS	0.81 (@ 5min), 0.91 (@ 30min)
IV PK rat	$T_{1/2}$ 0.5h; Cl 36.6 (mL/min/kg)
Liver microsome	r: $t_{1/2}$ 66min, ER 0.53; monkey: $t_{1/2}$ 27.5 min, ER 0.61; h: $t_{1/2}$ 180min, ER 0.16
Cerep off-target panel (@ 1 µM) 52 targets	clean
Microdosing blocking rats (30 min uptake)	42%
Microdosing ko versus wt mice (30min uptake; 100 µg/kg)	38% reduction ko vs wt
Monkey PET blocking with JNJ	57.5% receptor occupancy @ 2.5 mg/kg



JNJ-64413739

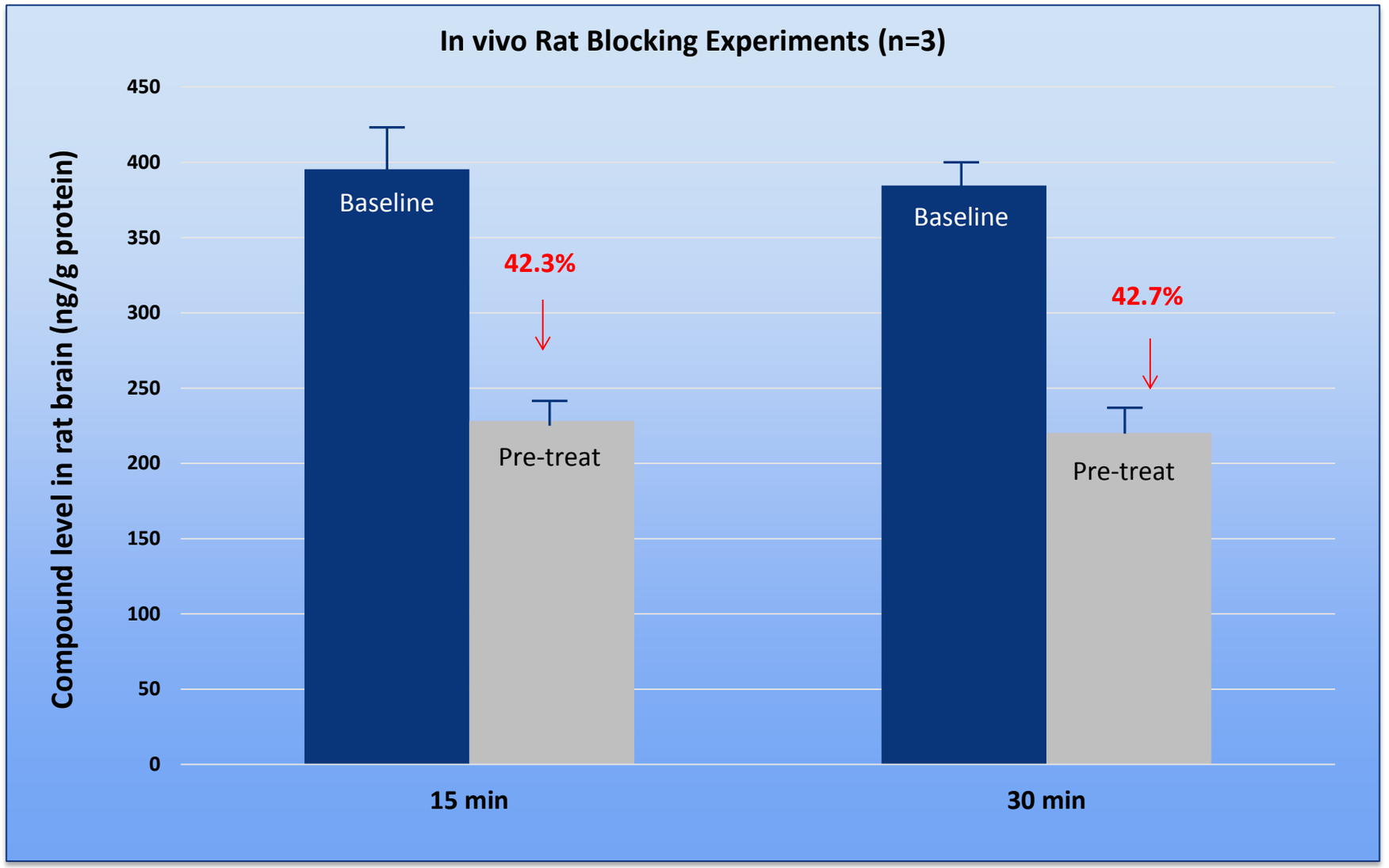
	Average	(± SD), n=6
Starting activity (mCi)	1227	(± 505)
[^{18}F]MNI-920 activity (mCi)	23.2	(± 8.9)
Decay-corrected yield (%)	3.3	(± 2.0)
Production time (min)	61	(± 11)
Radionuclidic identity	Pass	-
pH	6.0	(± 0.0)
Kryptofix content (≤ 50 µg/mL)	Pass	-
Radiochemical purity (%)	99.9	(± 0.2)
Identity	Pass	-
MNI-920 assay (µg/mL)	0.56	(± 0.36)
Total impurity assay (µg/mL)	2.96	(± 2.16)
Specific activity (mCi/µmol)	1371	(± 632)
Residual solvent content (< 410 ppm MeCN, < 5000 ppm DMSO)	Pass	-
Pyrogen content (<17.5 EU/mL)	Pass	-

Brain uptake/retention of JNJ-64413739 in wild type and knockout mice (n=4)



Compound levels in cerebrum are reduced by 38% for JNJ-64413739 in knockout mice compared with wild type (n=4, at 30 min after 100 μg/kg iv injection). Analyzed by LC-MS/MS.

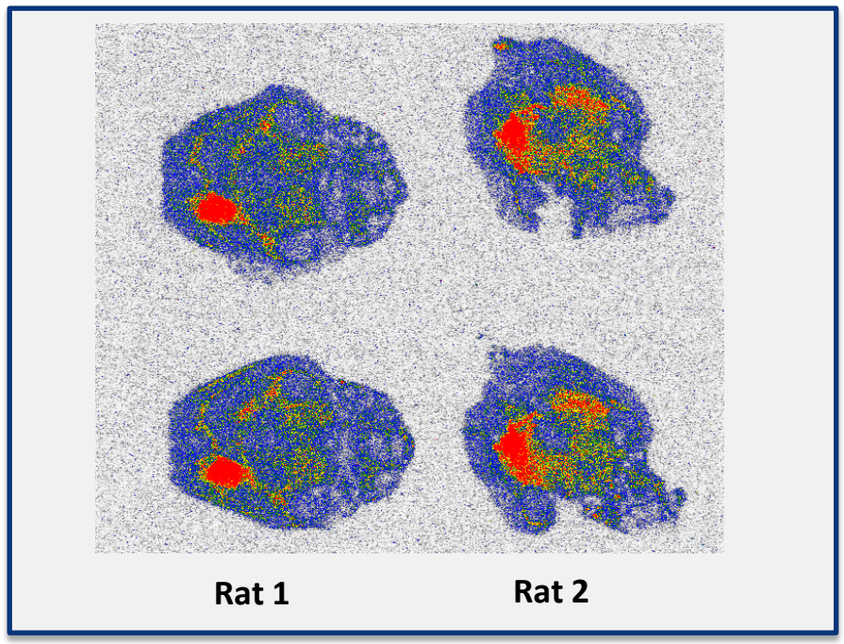
In vivo Rat Blocking Experiments with JNJ-64413739 (n=3)



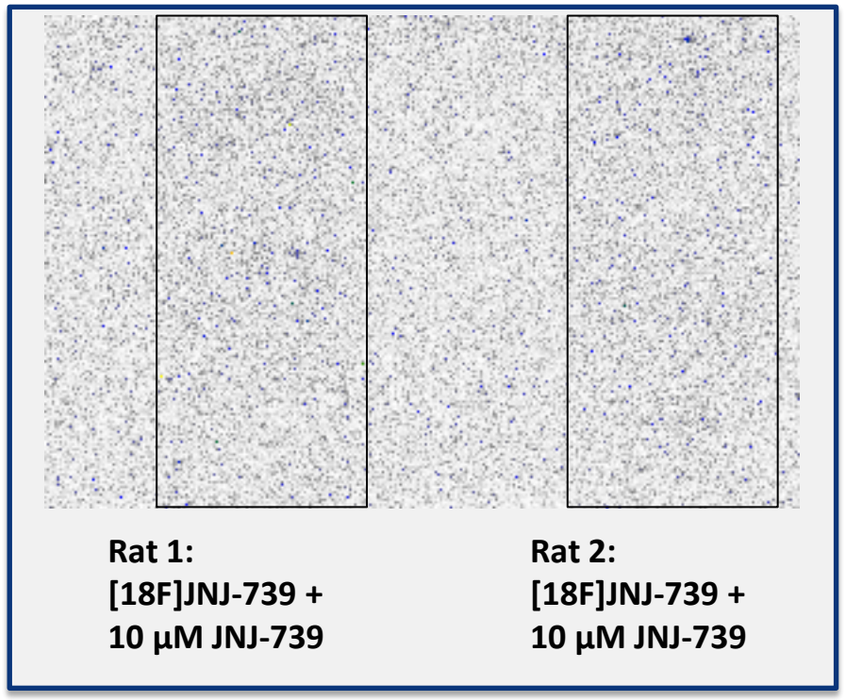
JNJ-55308942 (5 mg/kg, p.o. dose for 1hr) blocks JNJ-64413739 (30 µg/kg, iv) binding in rat brains (n= 3). The blocking effects are expressed as % reduction of baseline signal. Analysis by LC-MS/MS.

JNJ-64413739 shows high brain uptake in rats and exhibits a blocking effect with JNJ-55308942.

Humanized P2X7R Rat Model – in vitro ARG with [18F]JNJ-64413739



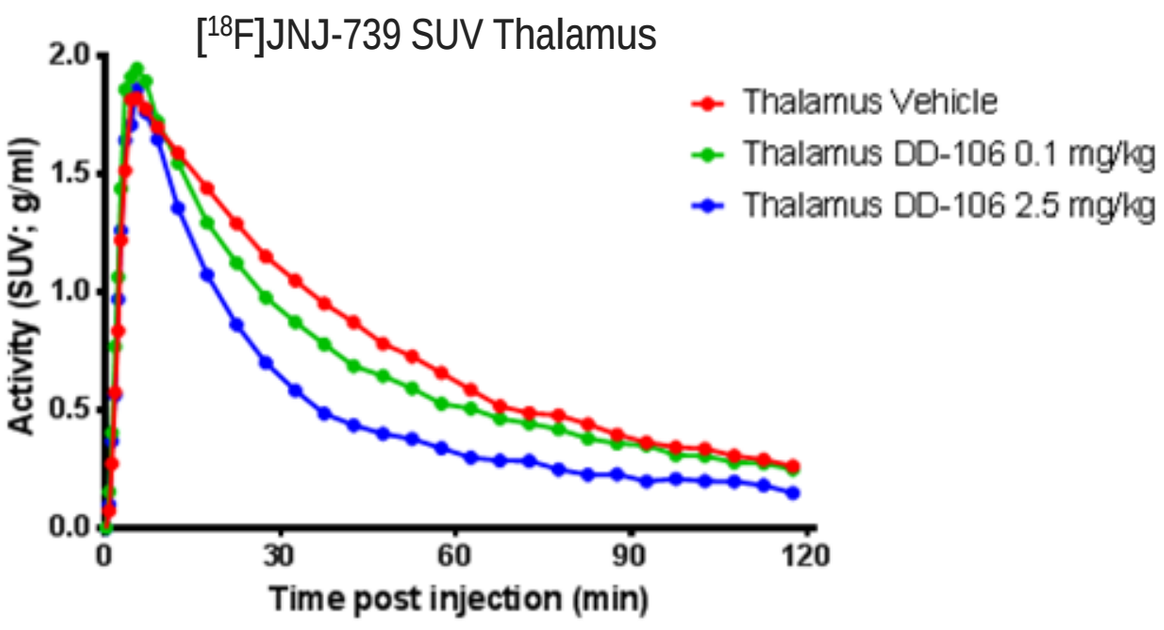
- Human P2X7R vector implanted in the rat right striatium
- hP2X7R expression monitored by Western Blot

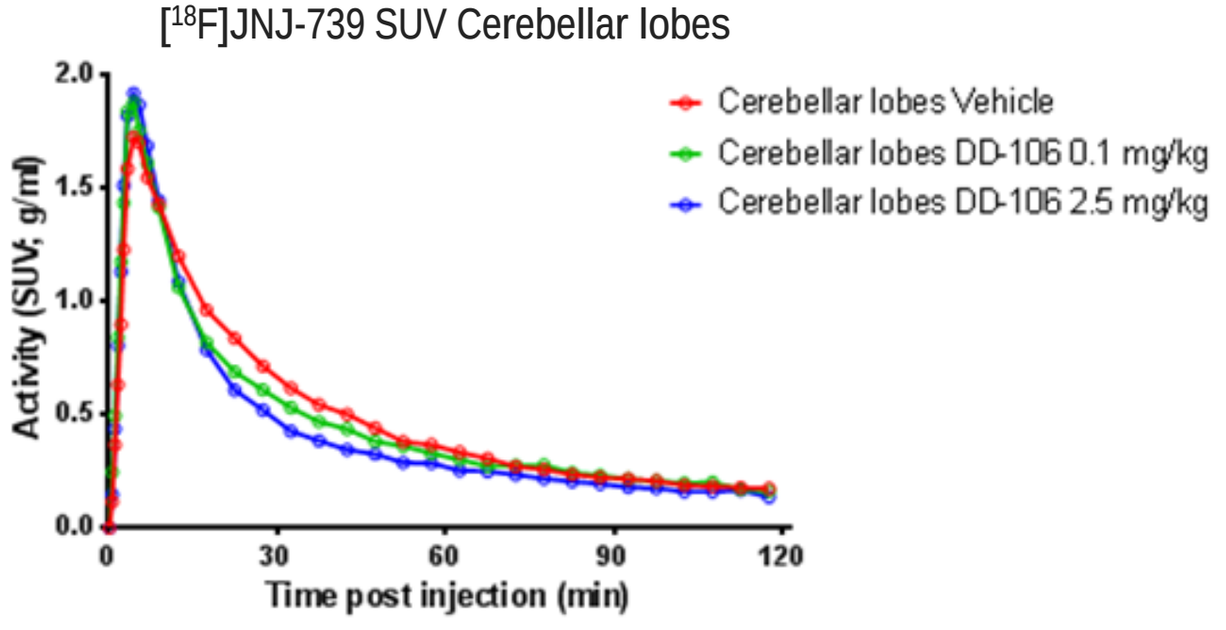


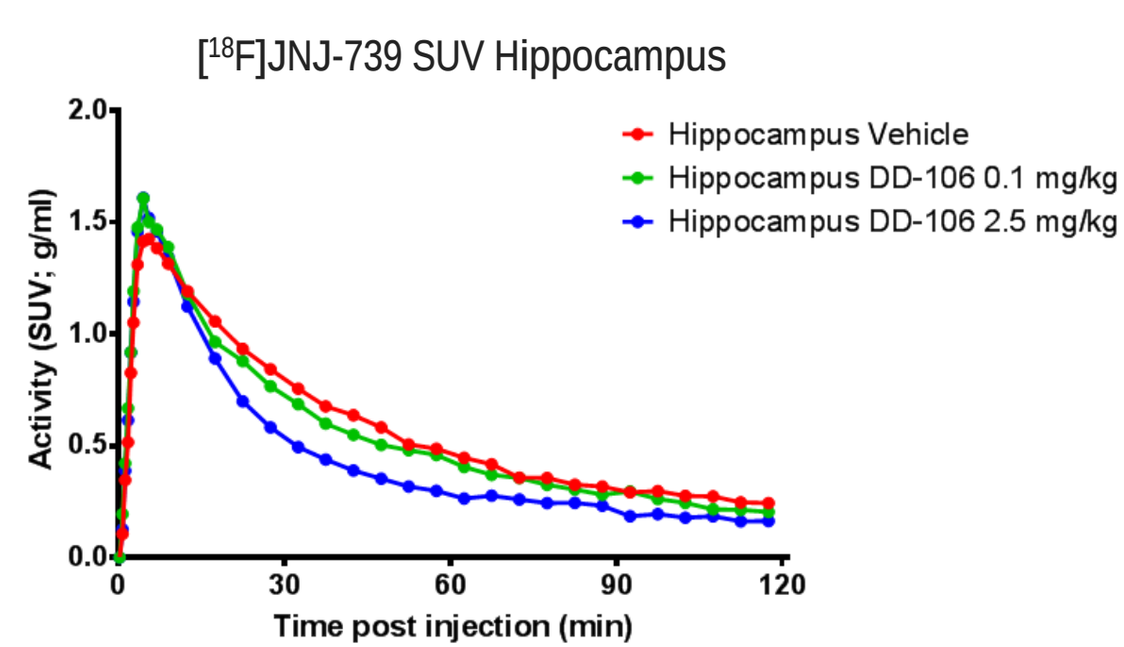
- 2 brain slices per slide
- Slices of two different rats
- Higher binding to injected striatum
- Complete blocking with 10 µM cold JNJ-64413739

Rat Model: D. Ory et al. J Nucl Med. 2016 Sep; 57(9):1436-41
KU Leuven

[18F]JNJ-64413739 Rhesus Monkey PET imaging: Blocking with "*JNJ (= DD-106)*"



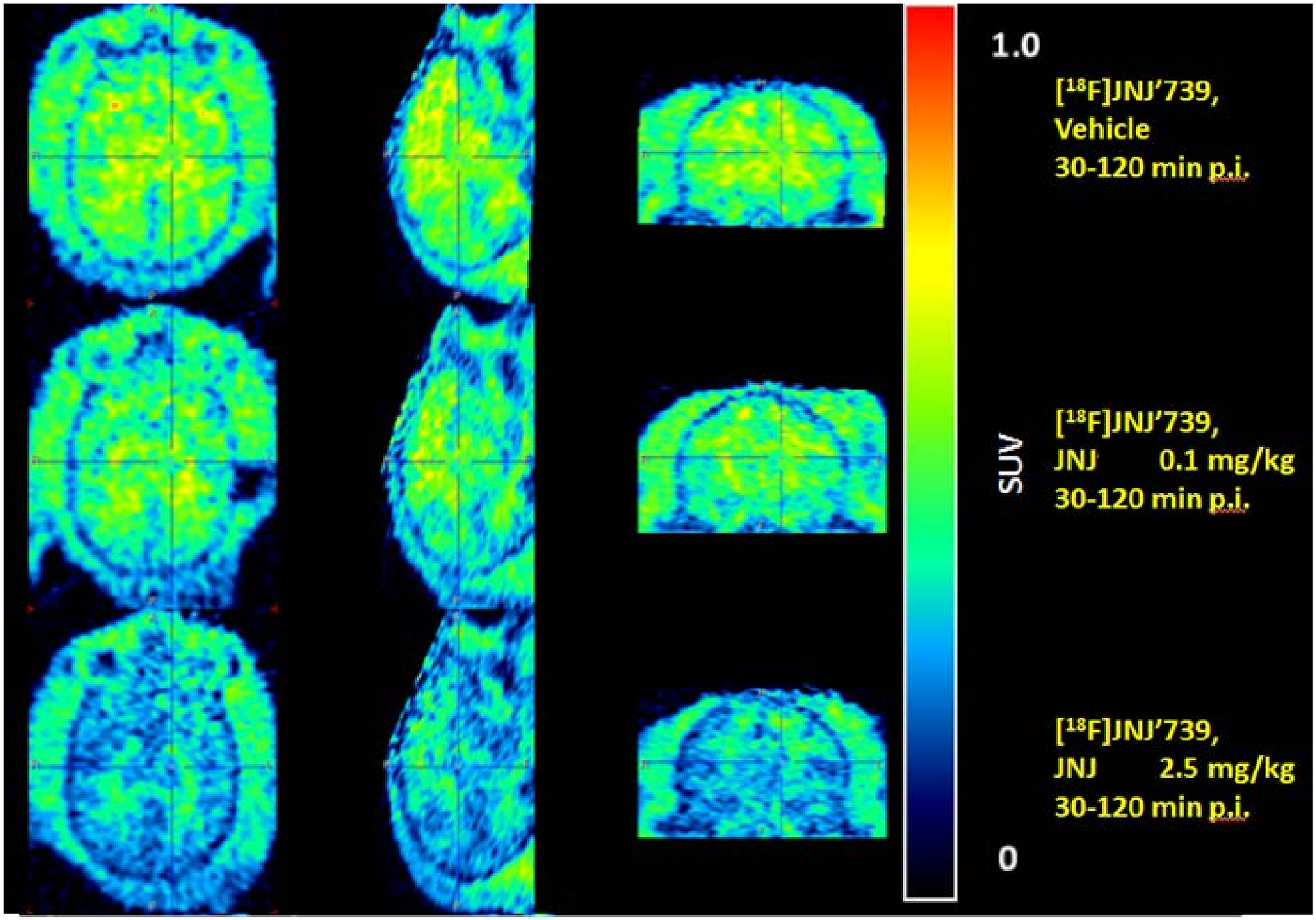




Wilma

[18F]JNJ-64413739 Monkey PET:

Blocking with a P2X7 inhibitor "JNJ": Summed images 30-120 min



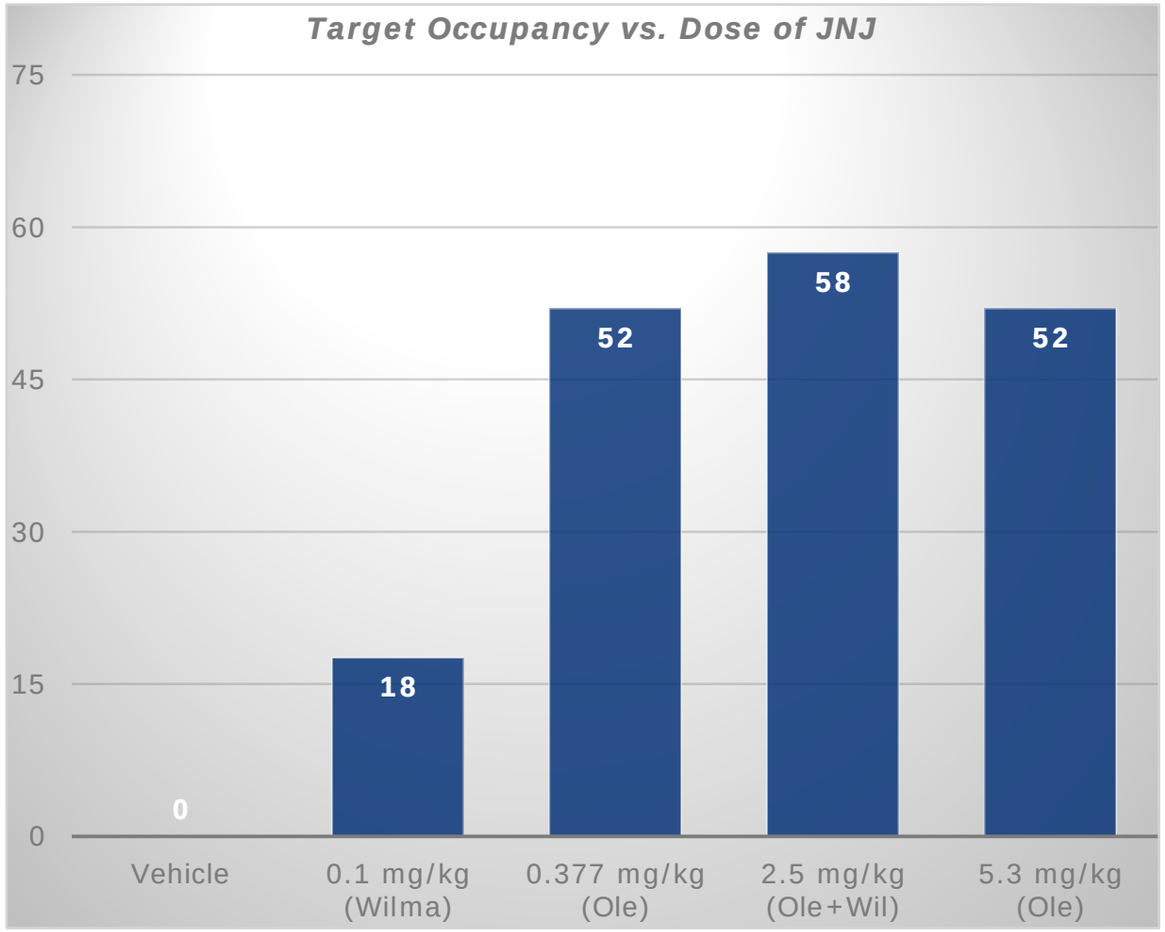
Baseline (vehicle)

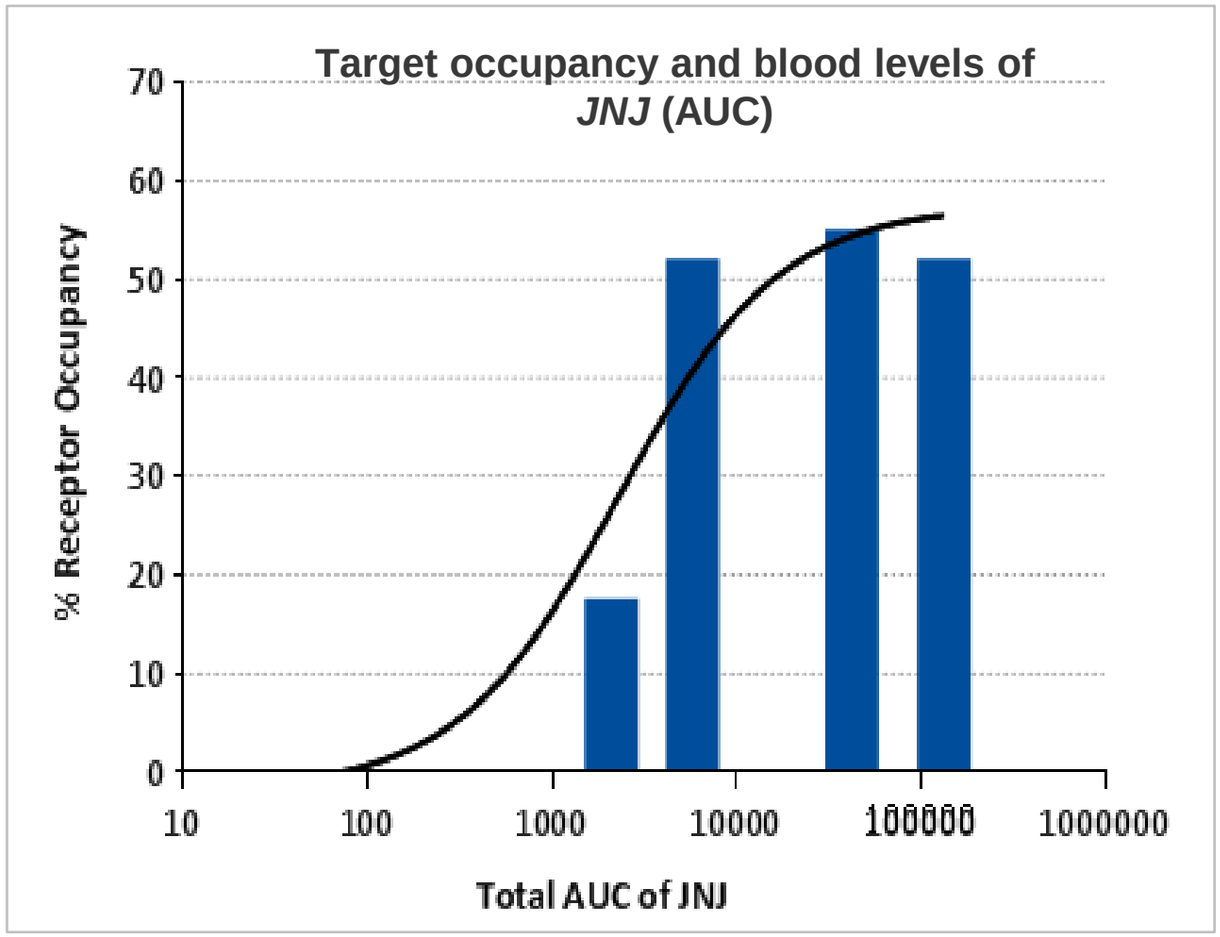
0.1 mg/kg *JNJ*

2.5 mg/kg *JNJ*

Wilma

[18F]JNJ-64413739 Rhesus Monkey PET:
Dose dependent target occupancy





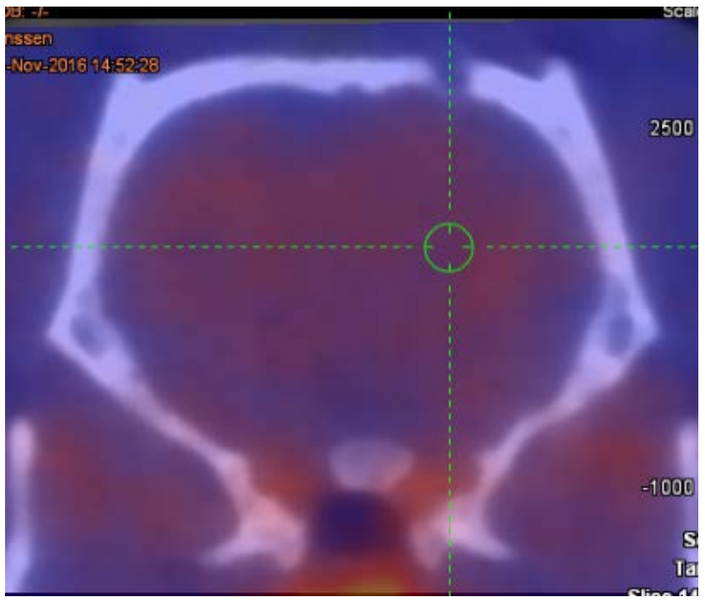
Dose(mg/kg)	AUC	%RO	monkey
5.3	132470	52	#1
2.5	42315*	57.5	#1 + #2
0.377	5750	52	#1
0.1	2094	17.6	#2
0	0	0	#1 + #2

PET images from day 2 with [18F]JNJ-64413739

Animals were PET imaged on day 2 and brains harvested/frozen after scan. Images are summed 10-60 min.

P2X7 (PET, VOI 6x4.5x4.5 mm)

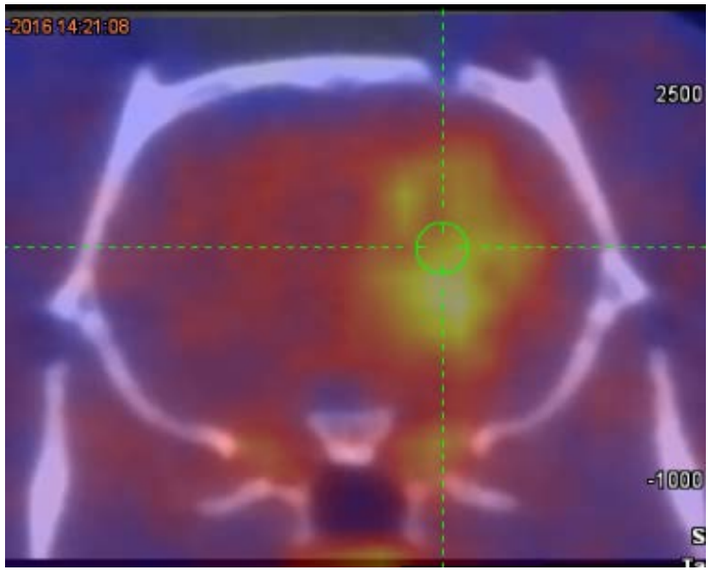
PBS (control, rat #11)



SUV 2

0

LPS (rat #6)

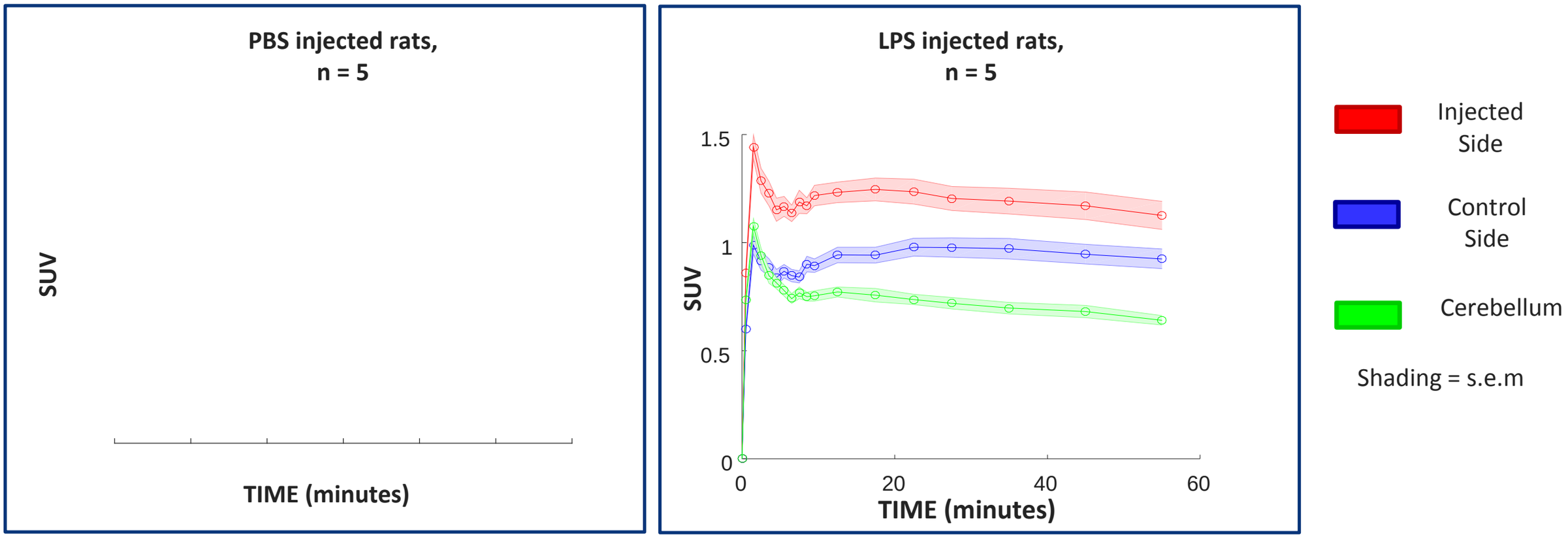


SUV 2

0

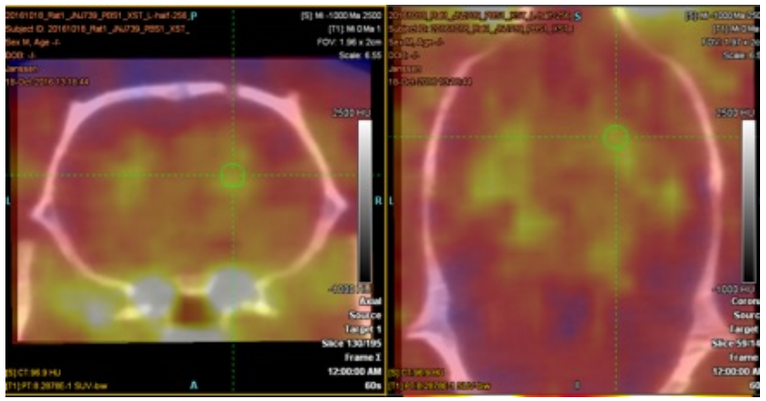
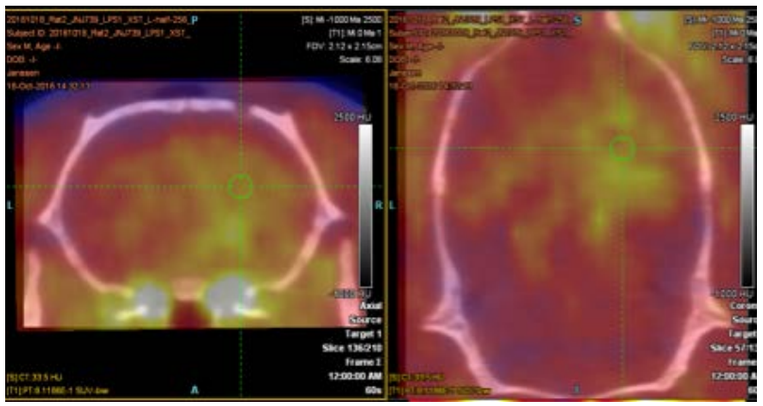
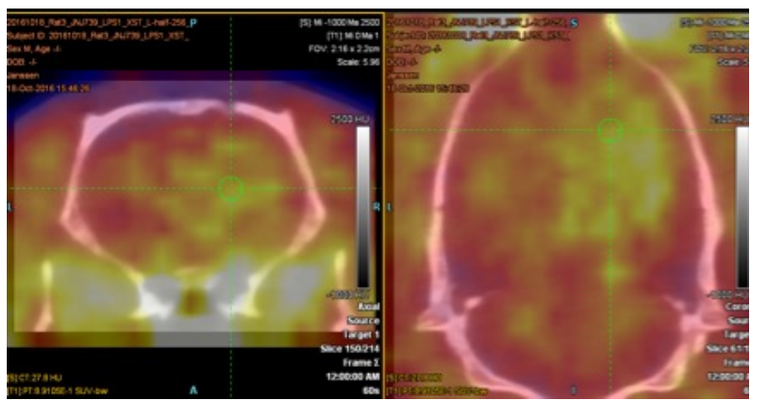
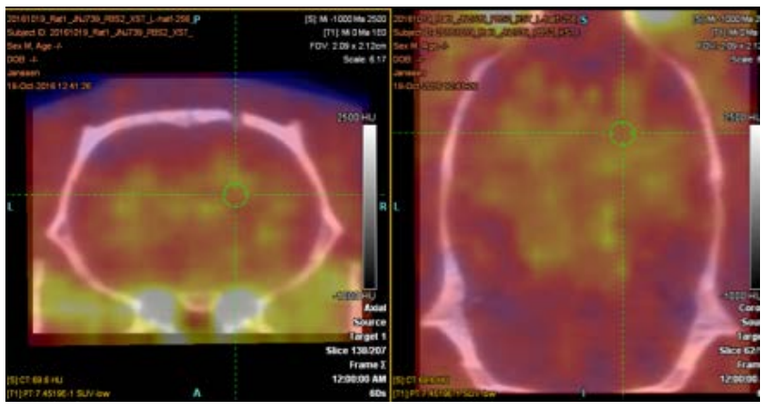
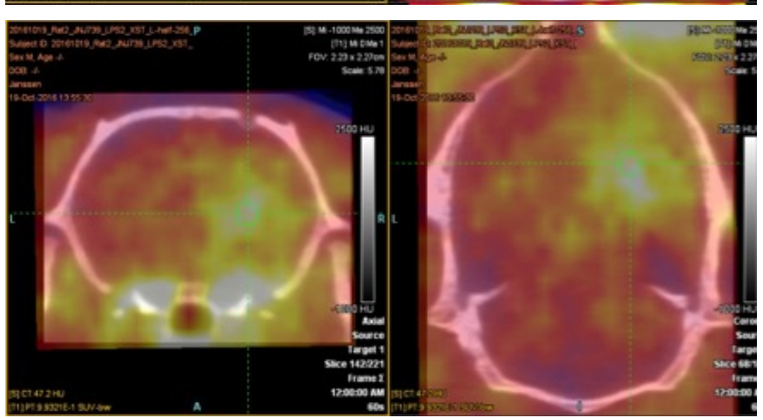
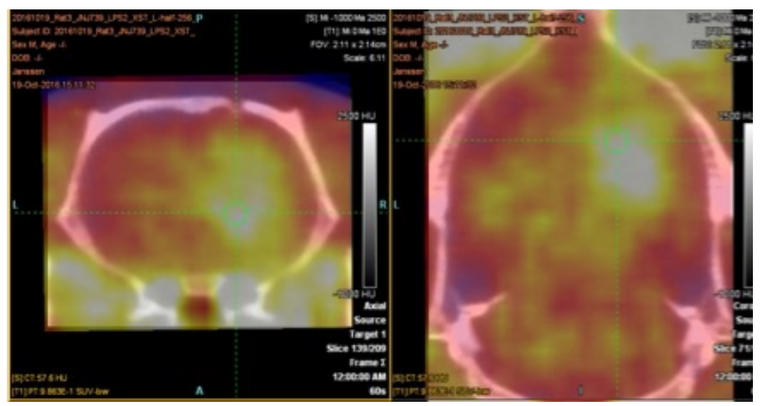
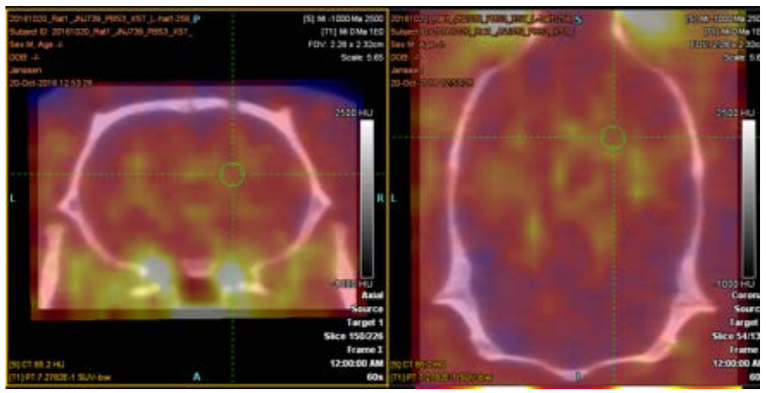
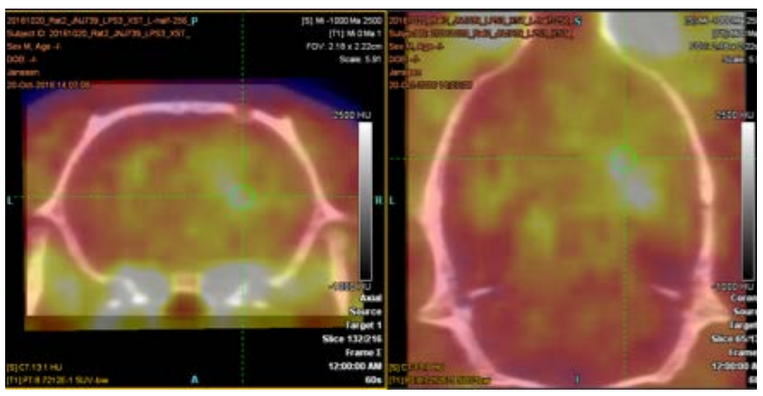
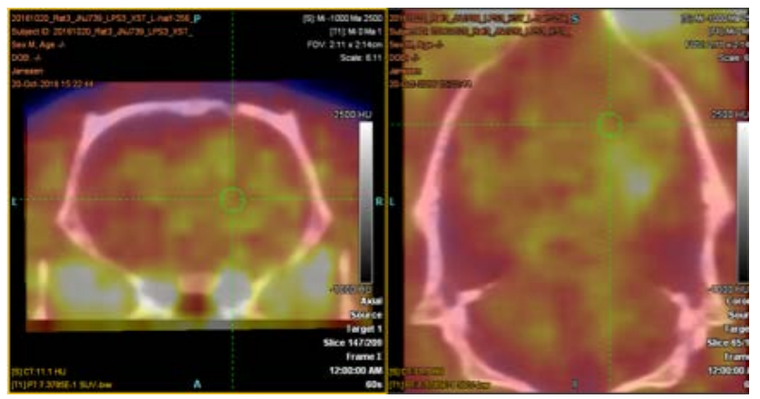
[18F]JNJ-64413739 P2X7 PET of LPS-Induced Neuroinflammation in Rats:

Local Injection of LPS (20 µg, 5µL, right striatum, 2 days post-injection) vs Local Injection of PBS (5µL, right striatum, 2 days post-injection)



- Hemispheres of the rats injected with LPS had significantly increased uptake of [18F]JNJ-739 relative to the hemispheres of control animals injected with PBS (34% increase in SUV measured over entire scanning period, p = 0.036, t-test).
- The uptake in the contralateral hemispheres and cerebellums were not significantly different (p = 0.80 and p = 0.96 correspondingly) between the groups of animals.
- Blocking studies were performed to confirm P2X7-specific PET signal

P2X7R PET imaging with [18F]JNJ-64413739 after PBS (control, 5 µL) versus 20 µg LPS (in 5 µL PBS) local injection into striatum

	PBS Rat 1, control	LPS Rat 2	LPS Rat 3
Day 1			
Day 2			
Day 3			

P2X7R PET imaging with [18F]JNJ-64413739 after PBS (control, 5 µL) versus 20 µg LPS (in 5 µL PBS) local injection into striatum

PBS Rat 1, control

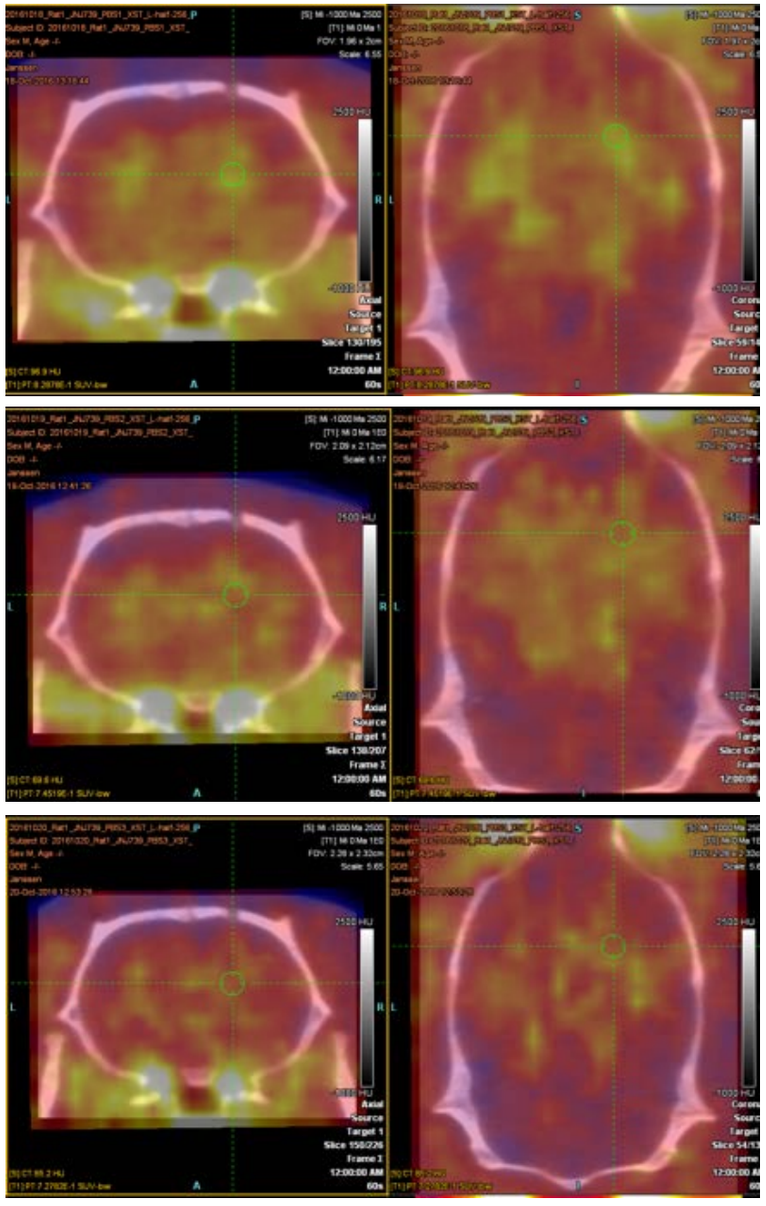
LPS Rat 2

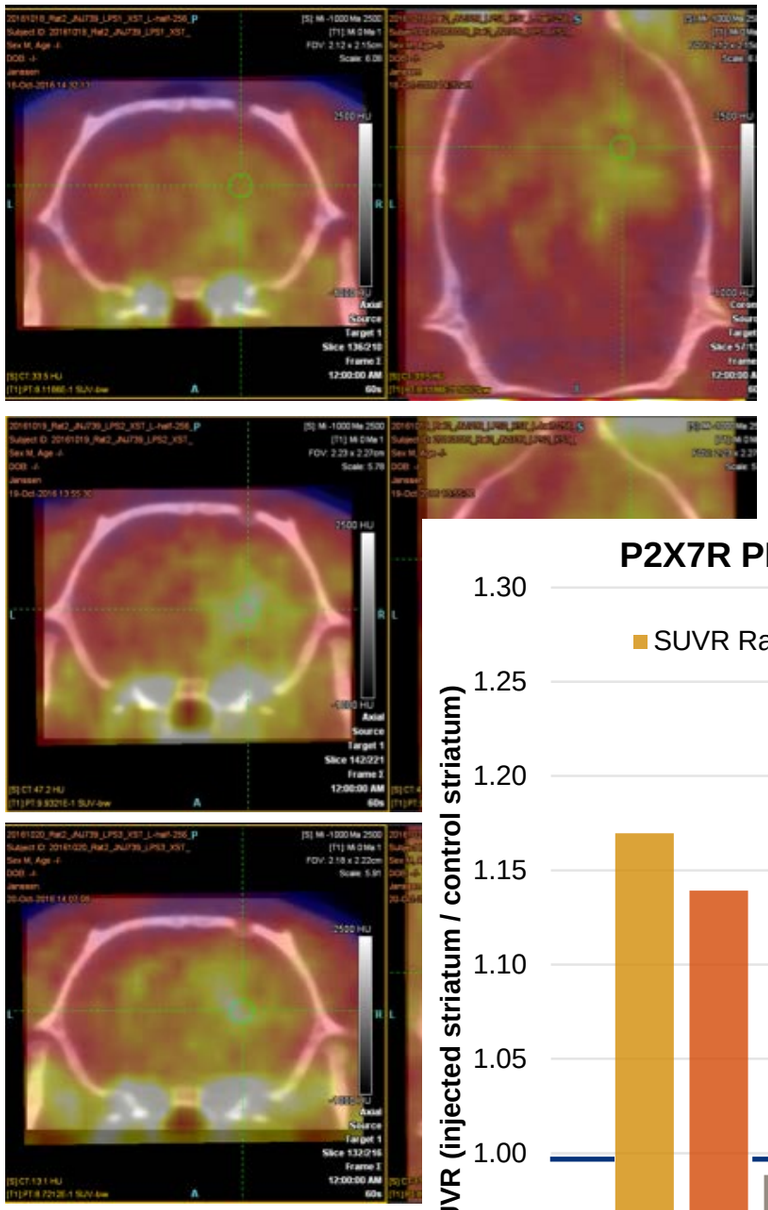
LPS Rat 3

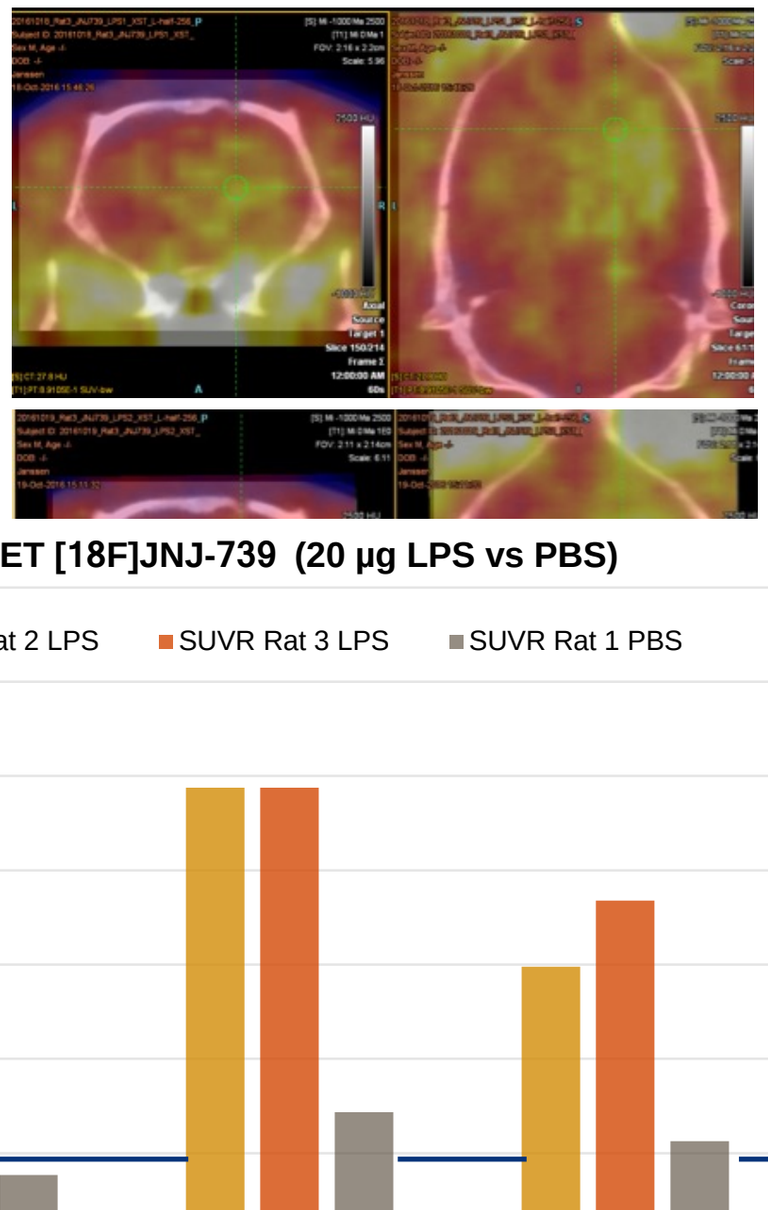
Day 1

Day 2

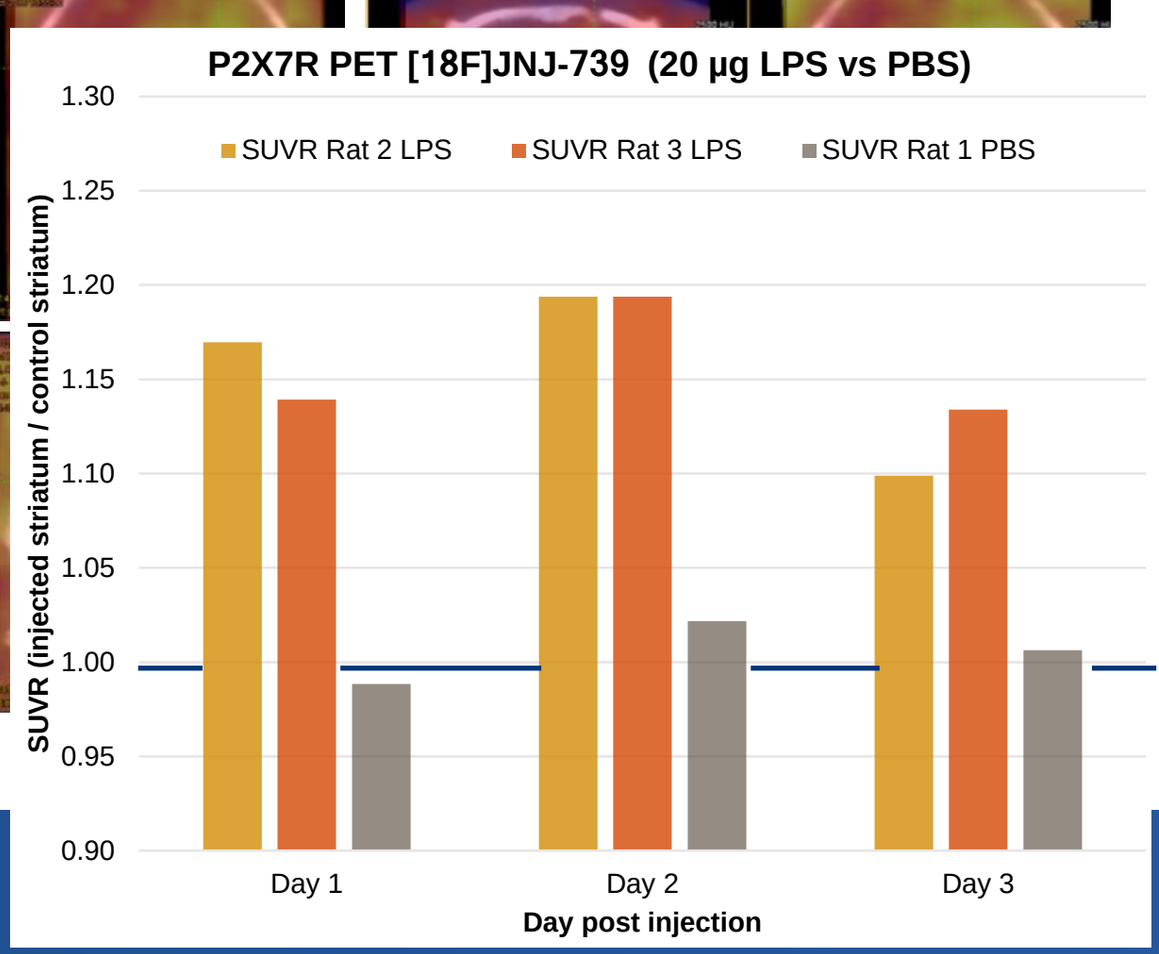
Day 3







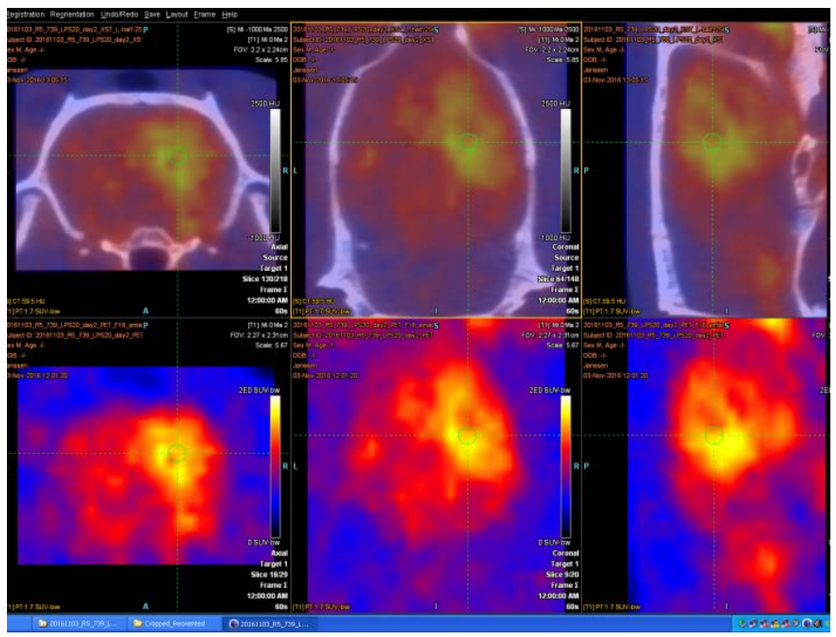
0 1.0 SUV



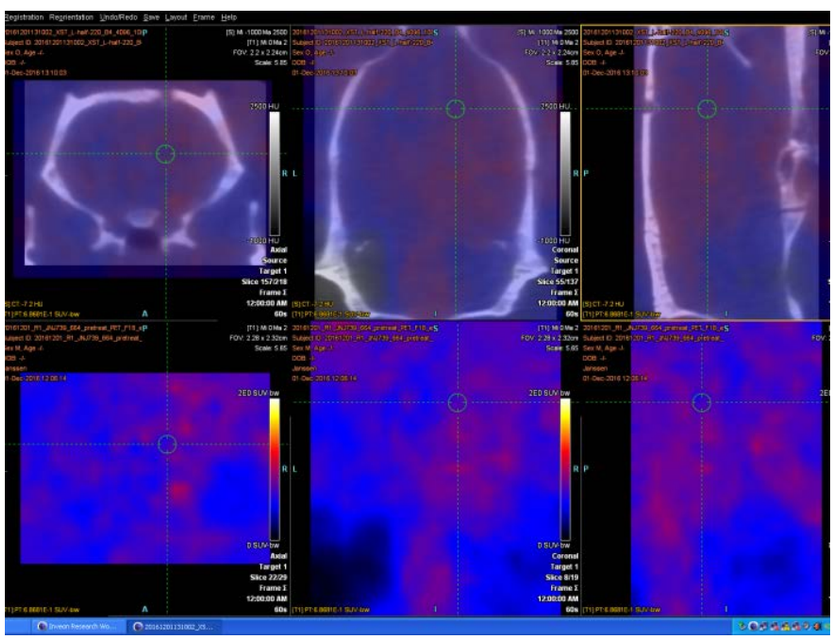
[18F]JNJ-64413739 P2X7R PET Blocking Experiments:

LPS Rat Model (20 µg, 2 days post-injection), baseline (left) vs. blocking (right) with JNJ (2.25 mg/kg, 10 min pre-treat)

LPS Rat5, Day 2 (baseline)



LPS Rat1, Day 2 (*JNJ*, 2.25 mg/kg, 10 min pretreatment)



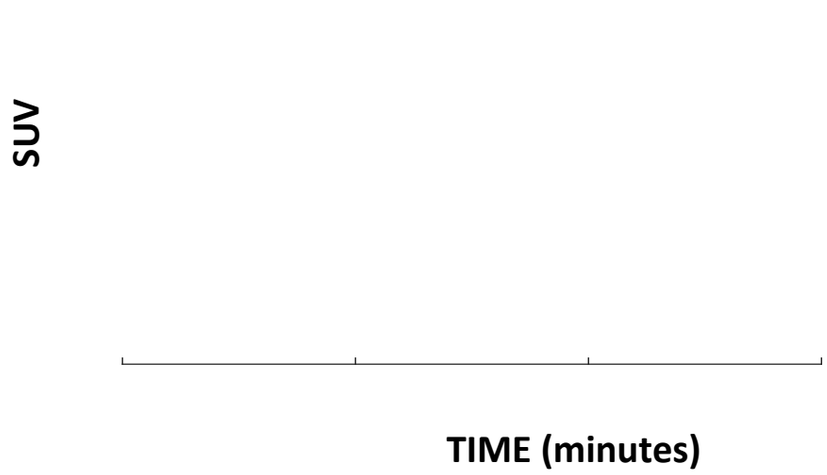
2.0 SUV

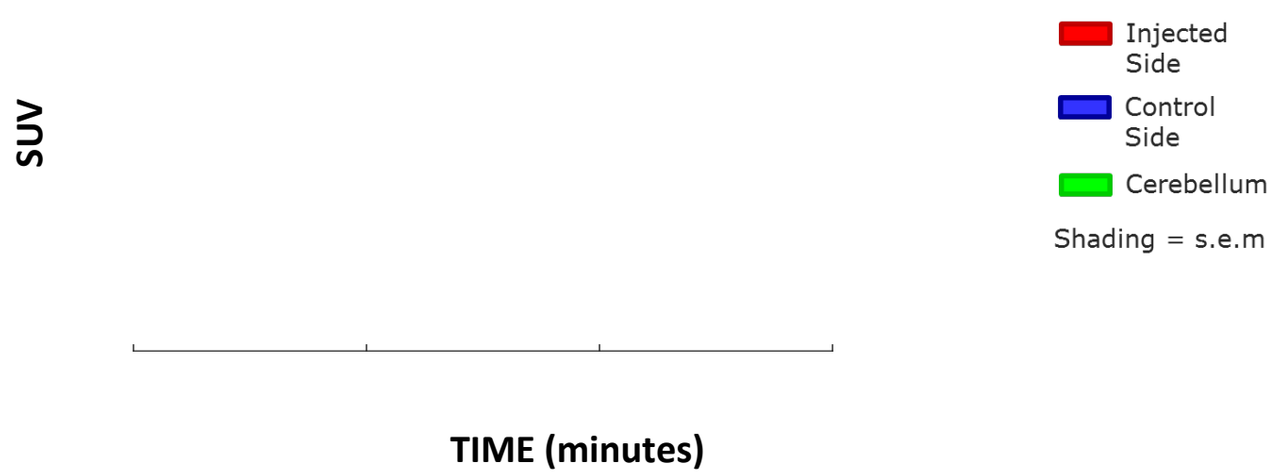
Images: Sum from 10 to 60 min

0

Vehicle; N=5; 2 days post-LPS (20 µg) injection pre-treated 10 min w/ vehicle

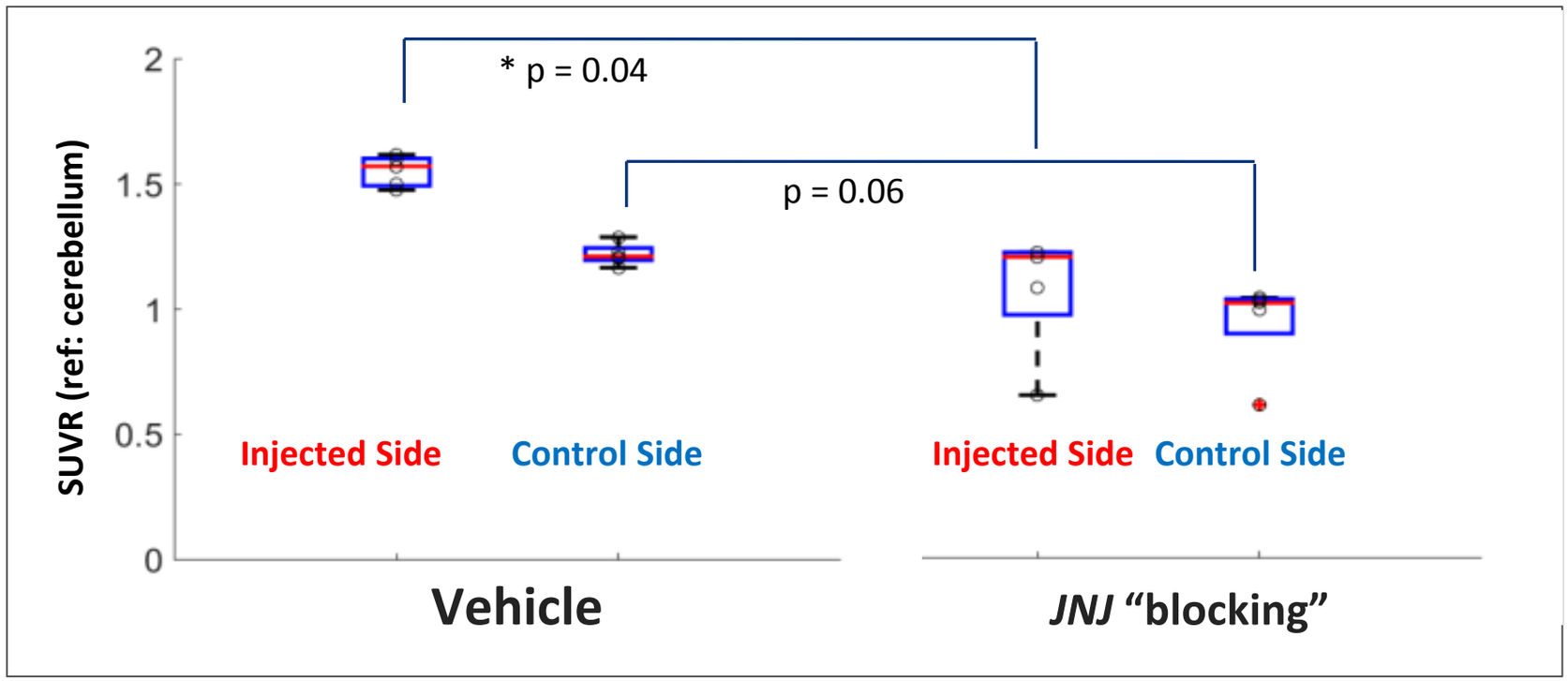
Blocking; N=5; 2 days post-LPS (20 µg) injection pre-treated 10 min w/ *JNJ* (2.25 mg/kg)



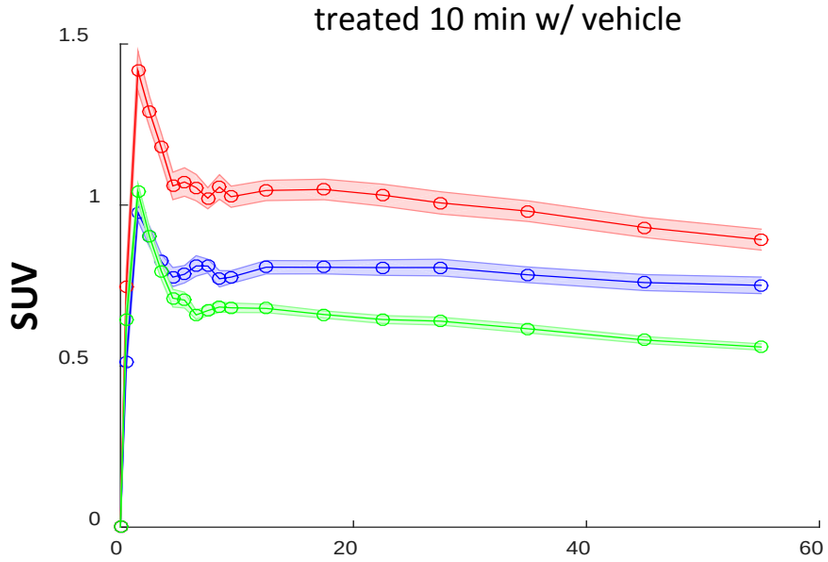


[18F]JNJ-64413739 P2X7R PET Blocking Experiments:

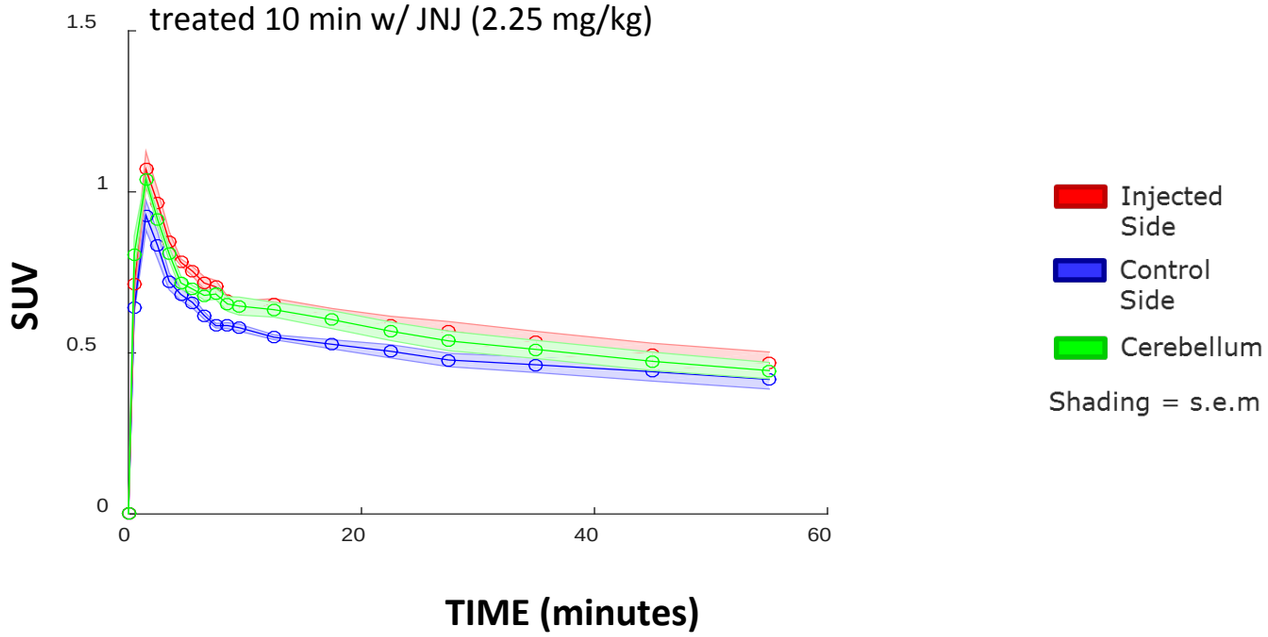
LPS Rat Model (20 µg, 2 days post-injection), baseline (left) vs. blocking (right) with JNJ (2.25 mg/kg, 10 min pre-treat)



Vehicle; N=5; 2 days post-LPS (20 µg) injection pre-treated 10 min w/ vehicle

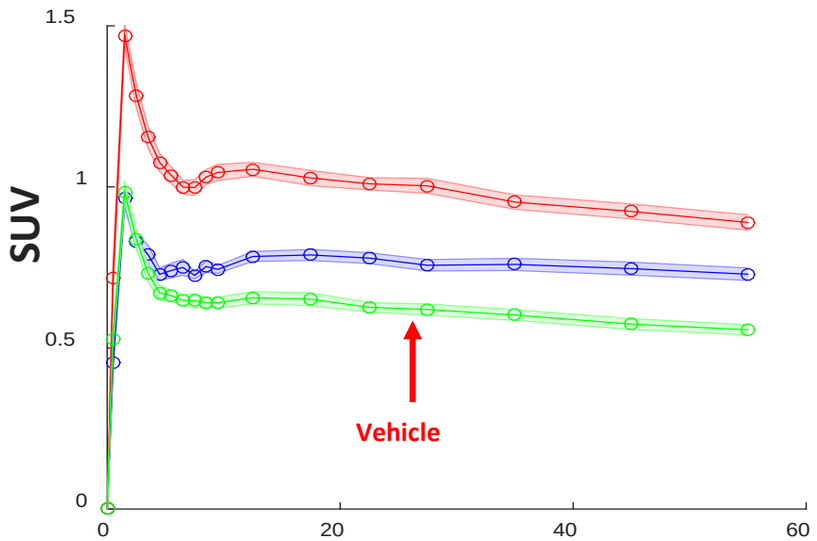


Blocking; N=5; 2 days post-LPS (20 µg) injection pre-treated 10 min w/ JNJ (2.25 mg/kg)

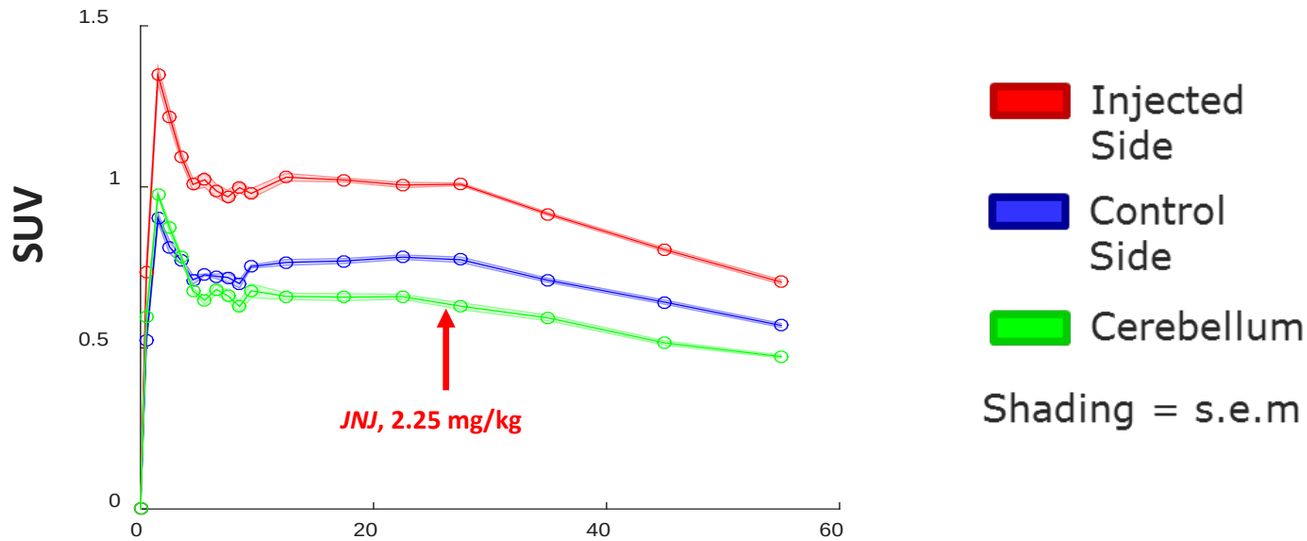


[18F]JNJ-64413739 P2X7R PET Blocking Experiments:
Chasing with JNJ (2.25 mg/kg); 2 days post LPS injection (20 µg)

Chasing w/ Vehicle; N=5; LPS (20 µg), 2 days post-injection



Chasing w/ *JNJ* (2.25 mg/kg); N=5; LPS (20 µg), 2 days post-injection



Chasing (25 min p.i.) with JNJ (2.25 mg/kg):

- Reduced tracer retention relative to vehicle chase.
- "Relative SUV drop" = SUV (25min) – SUV (60min), normalized to SUV (25min); **Significantly higher drop in "chase" vs vehicle** (injected side: 30% vs 10%, p = 0.002, t-test; control side: 26% vs 4%, p = 0.003).

Summary: [18F]JNJ-64413739 for P2X7 PET Imaging

- **Non-Human Primate Results:**
  - No “true” reference region (devoid of P2X7) in the brain could be identified, but a blood input function can be used to quantify the signal.
  - Target occupancy is reproducible in 2 different NHPs: Monkeys showed very similar tracer uptake at baseline and the 2.5 mg/kg dose level.
  - Non-specific binding in healthy NHP is approx. 40-45% (as estimated by the receptor occupancy plateau of 55-60%); this matches rodent data.
- **Neuroinflammation Rat Imaging Results:**
  - Time dependent [18F]JNJ-64413739 PET signal: strongest on day 2 post injection, 20% higher in LPS versus PBS control.
  - Statistically significant blocking of [18F]JNJ-64413739 PET signal by cold *JNJ* on the LPS side, but not the contralateral side.
  - Agreement with IBA1 immunostaining.
- **Summary P2X7R PET:**
  - [18F]JNJ-64413739 PET shows promise as an imaging agent for measuring target engagement of P2X7R therapeutics.
  - [18F]JNJ-64413739 may be useful to image neuroinflammation, but the lack of a reference region hampers image analysis (similar to TSPO).

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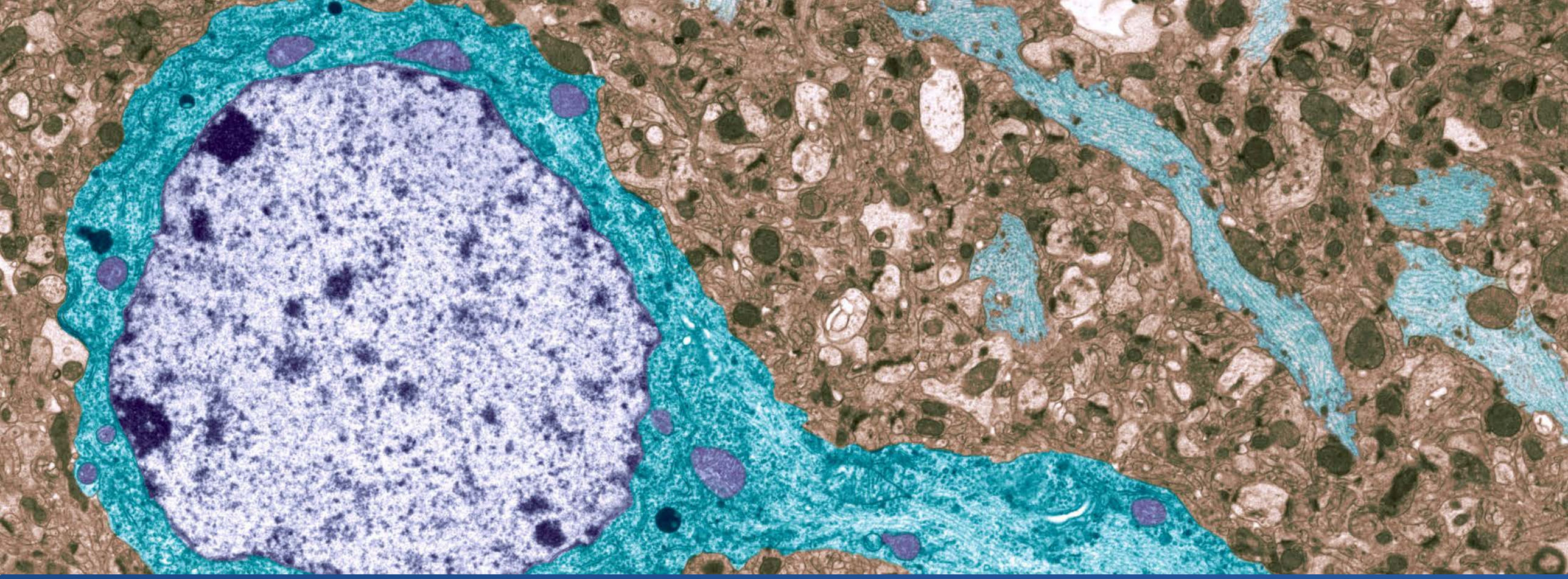

Thank you

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