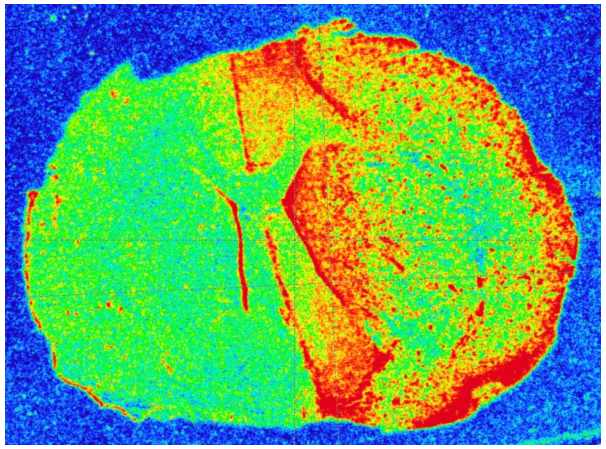
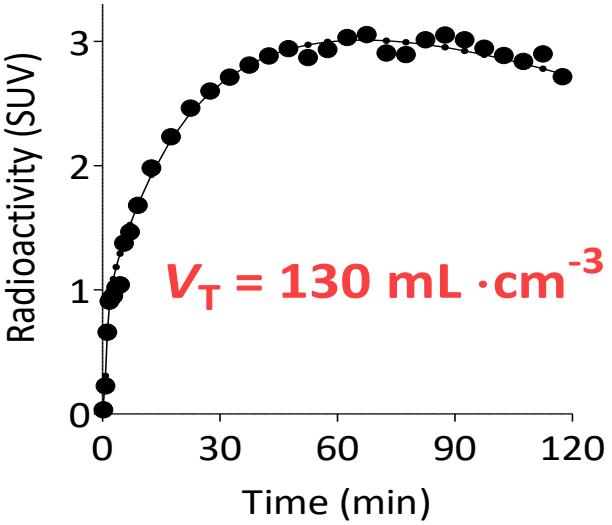
Translational Imaging of Inflammation with [^{11}C]PBR28

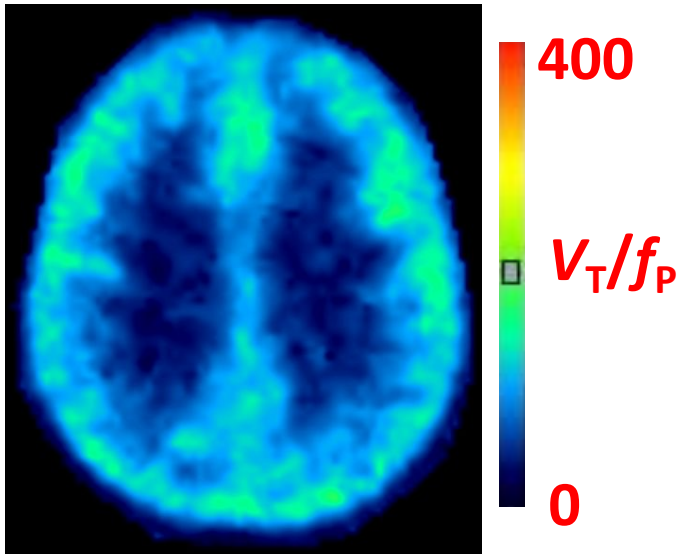
Rat Stroke



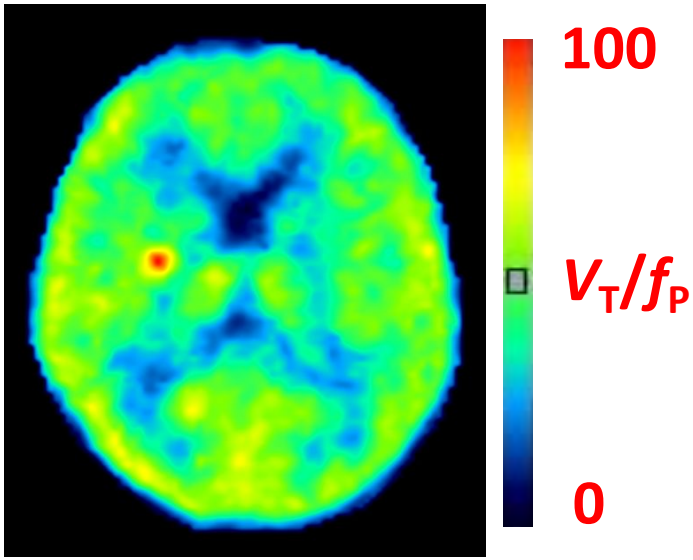
Monkey Quantitation



Healthy Human

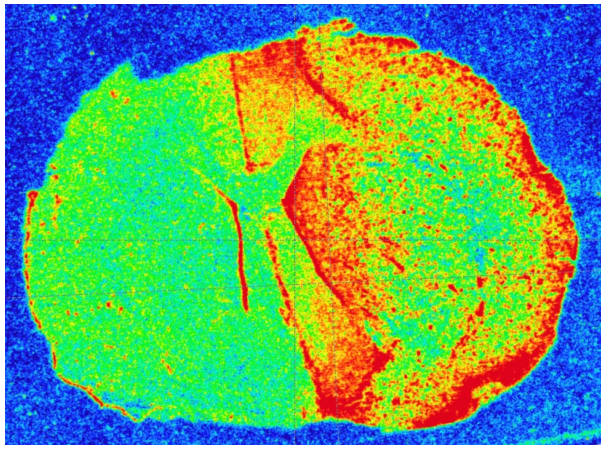


Human Stroke

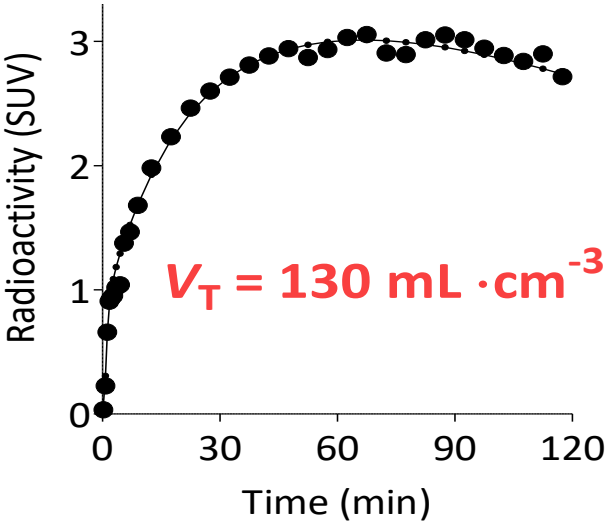


Translational Imaging of Inflammation with [^{11}C]PBR28

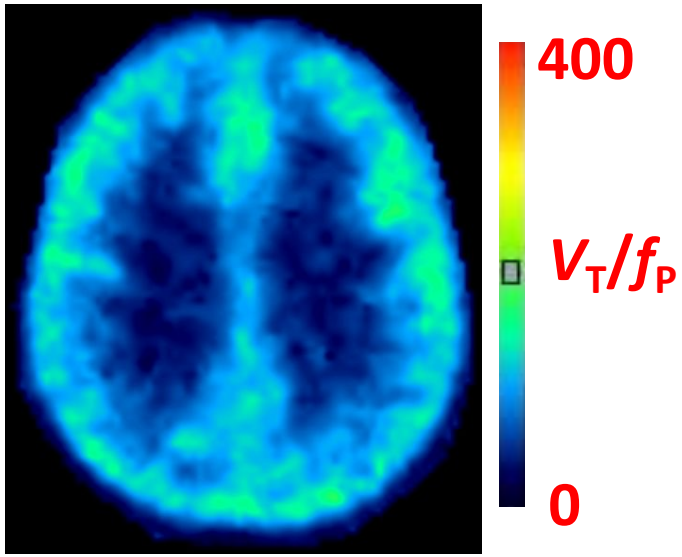
Rat Stroke



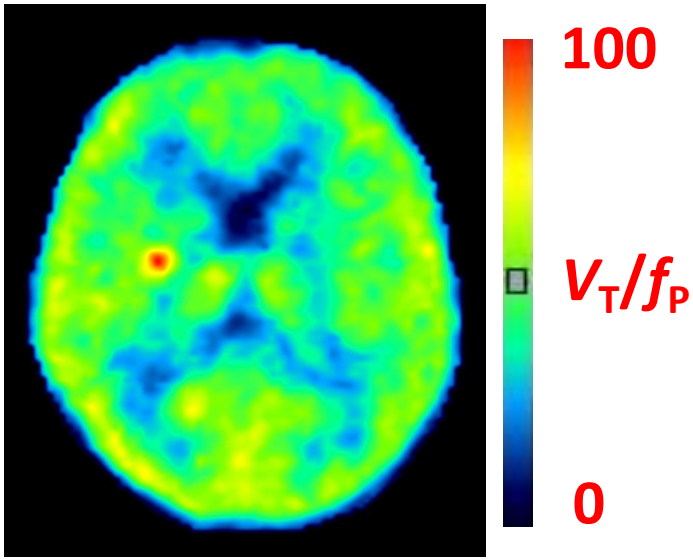
Monkey Quantitation



Healthy Human



Human Stroke



FUTURE: Linking PET to Treatment Trials

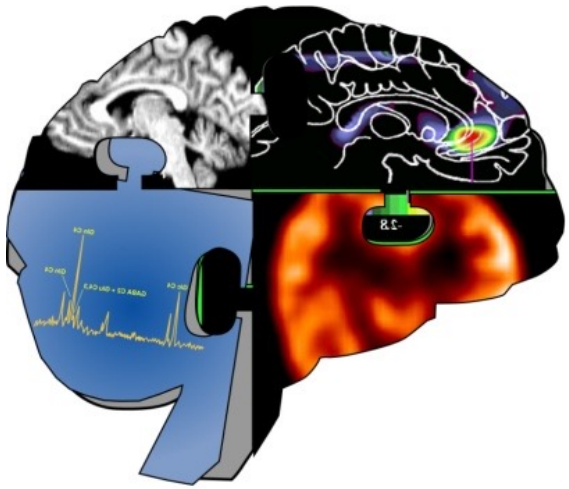
Pathophysiology

Do a subset of patients with depression have 'neuroinflammation' shown by PET imaging?

Treatment

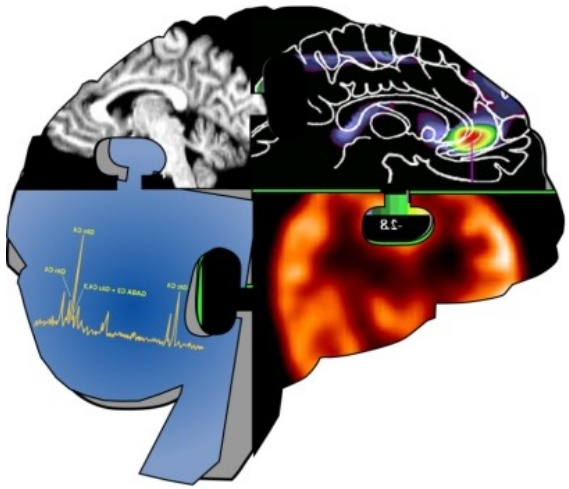
Can a drug that inhibits the activation of microglia decrease PET signal and treat depression?

Positron Emission Tomography of Human Brain can Monitor Neuroinflammation and cAMP Signaling: Applications to Alzheimer's Disease and Depression



Robert B. Innis, MD, PhD
Chief, Molecular Imaging Branch
NIMH

Linking Positron Emission Tomography to Therapeutic Trials in Dementia and in Depression



Robert B. Innis, MD, PhD
Chief, Molecular Imaging Branch
NIMH

PET vs. MRI

	PET	MRI
Spatial Resolution	2 – 6 mm	<< 1 mm
Sensitivity	10^{-12} M	10^{-4} M
Temporal Resolution	minutes	<1 sec

Radionuclide (^{11}C): high sensitivity
Ligand (raclopride): high selectivity
Radioligand [^{11}C]raclopride: high sensitivity & selectivity

Major Findings

Alzheimer's Disease

1) TSPO binding increased in Alzheimer's disease but not mild cognitive impairment
2) Increased TSPO binding binding correlates with disease severity (cross sectional study) and with disease progression (longitudinal study)

Major Depressive Episode

1) TSPO binding increased in unmedicated patients
2) TSPO binding not changed in medicated patients

Translocator Protein (TSPO)
aka Peripheral Benzodiazepine Receptor

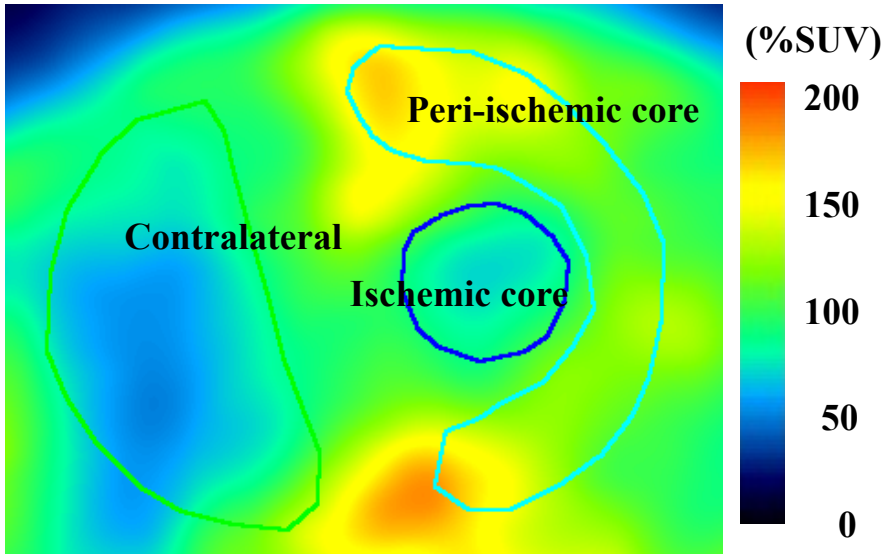
1. Mitochondrial protein transports cholesterol to enzyme that synthesizes pregnenolone plus other functions

2. Highly expressed in macrophages, activated microglia, and reactive astrocytes

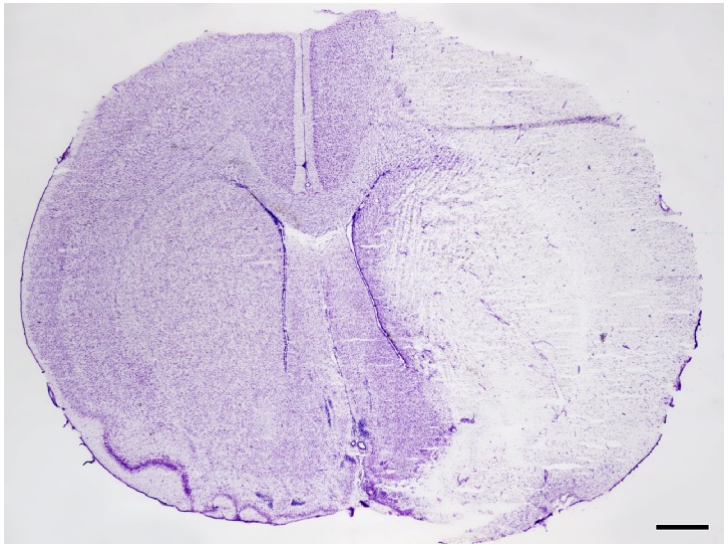
3. Putative biomarker for activation of the immune system in brain: ‘neuroinflammation’

Imaging Brain Infarction in Rats using [^{11}C]PBR28

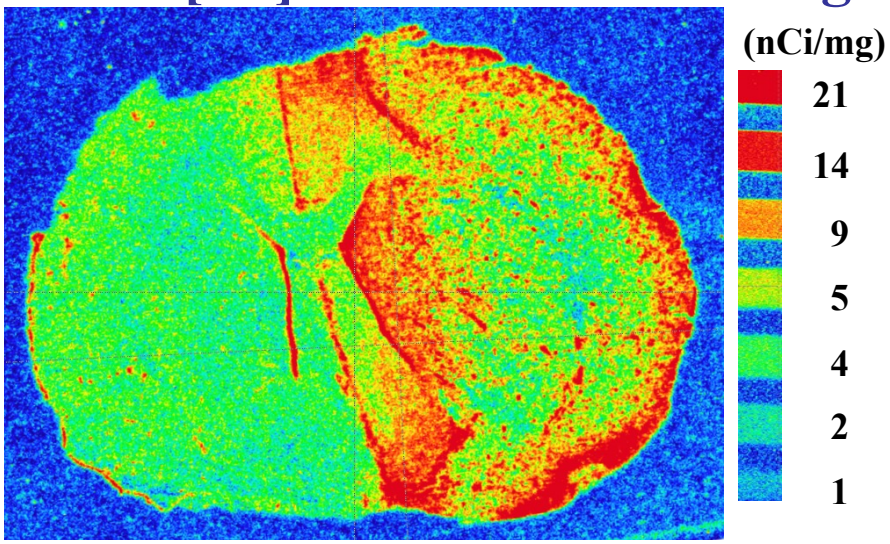
[^{11}C]PBR28 PET summation image



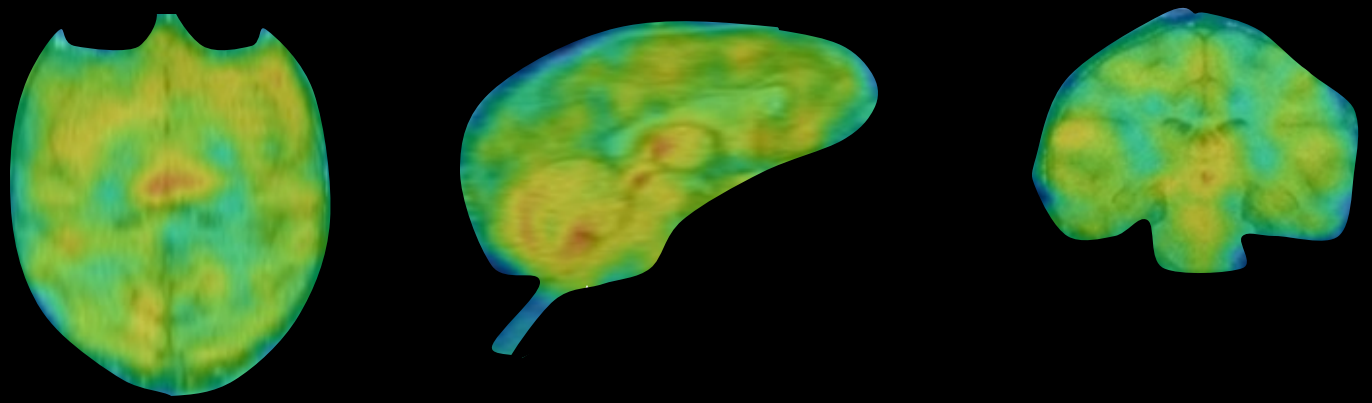
Cresyl violet staining



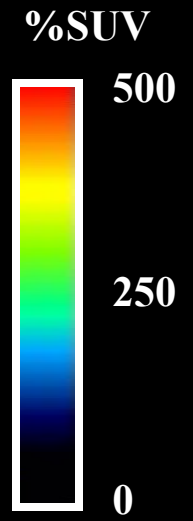
In vitro [^{3}H]PK11195 autoradiography

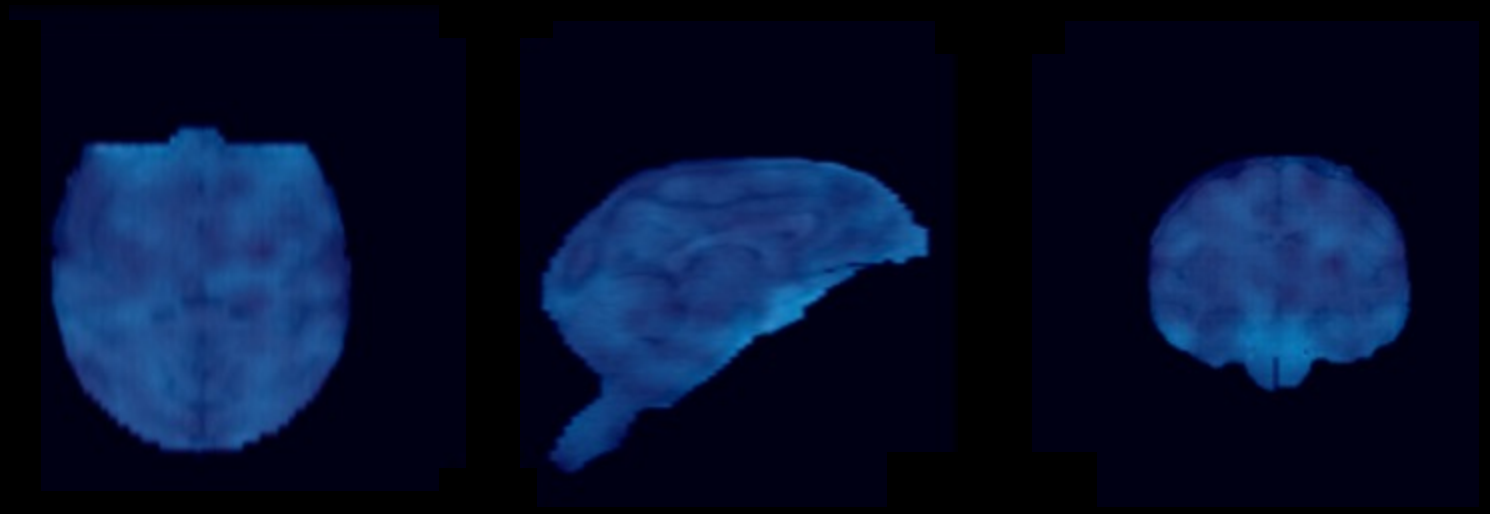


[^{11}C]PBR28 Monkey Brain: high total uptake & high specific binding



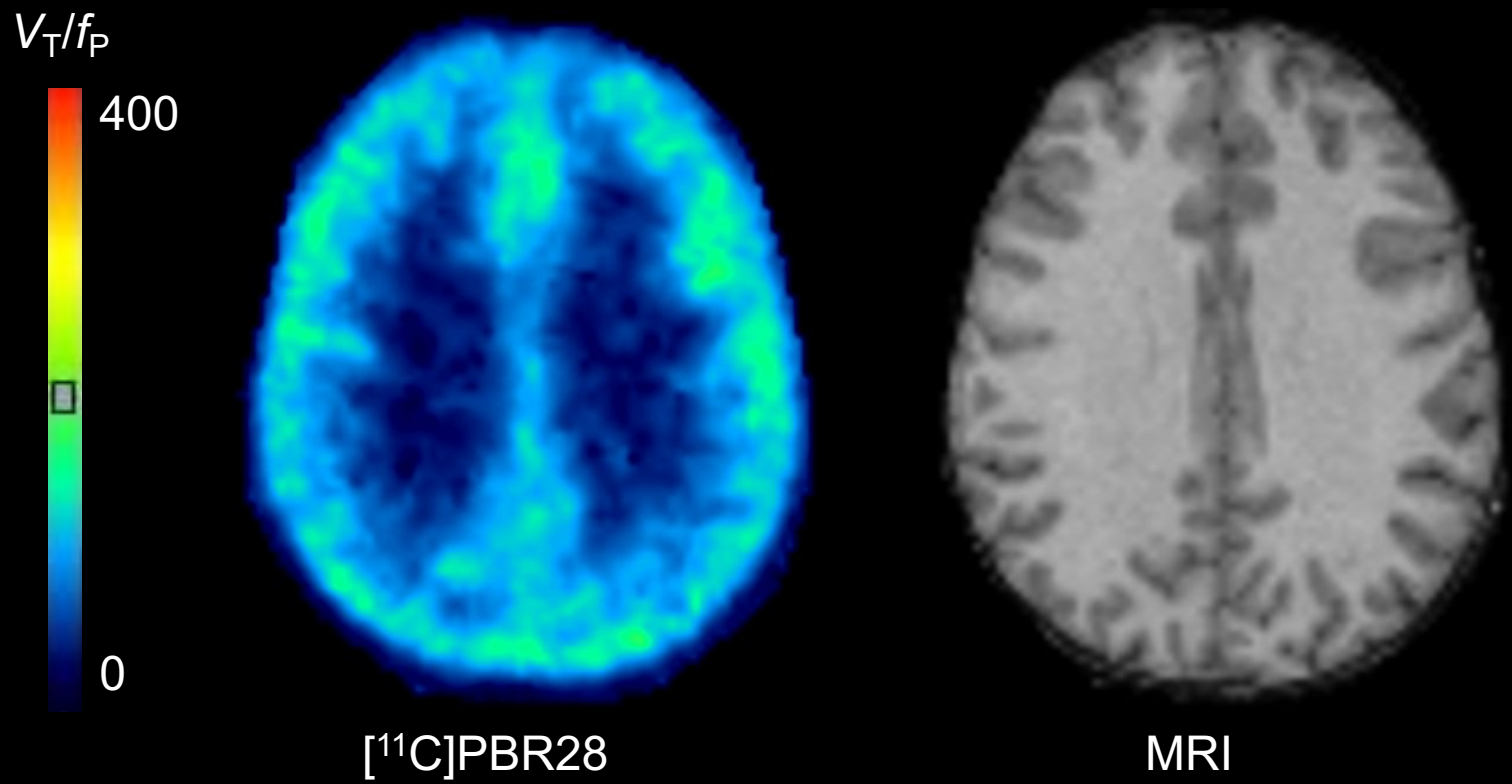
Total Uptake





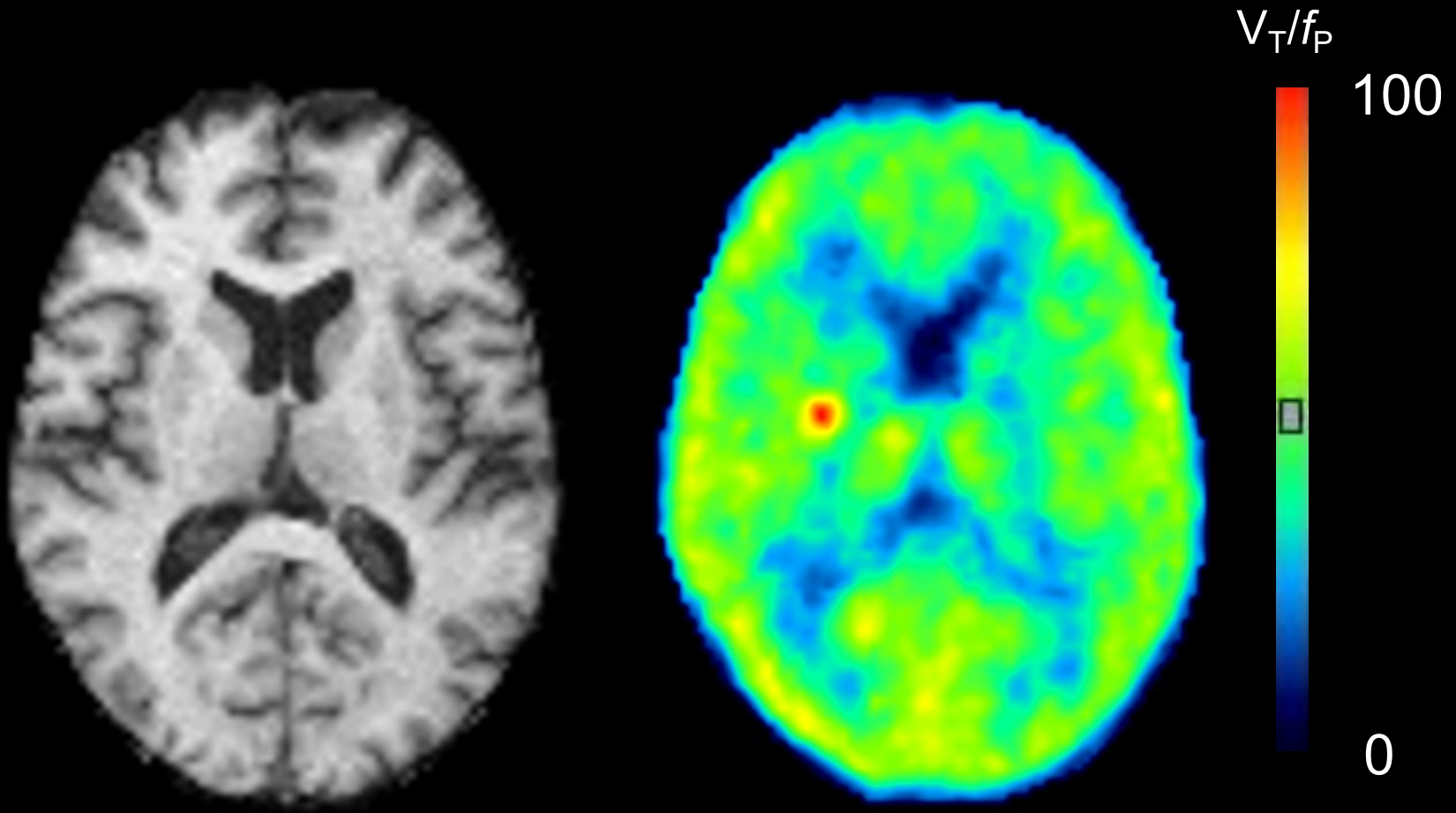
Nonspecific Uptake: Preblocked with PK11195

TSPO in Human Brain [^{11}C]PBR28: widespread; gray > white



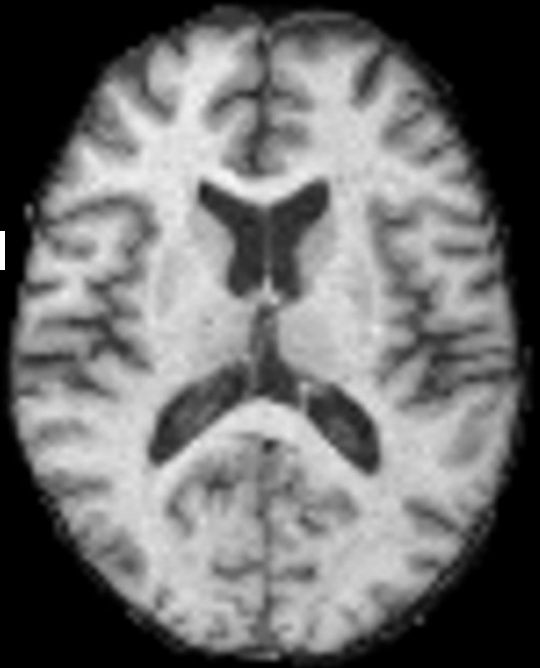
Incidental finding in subject from Hopkins

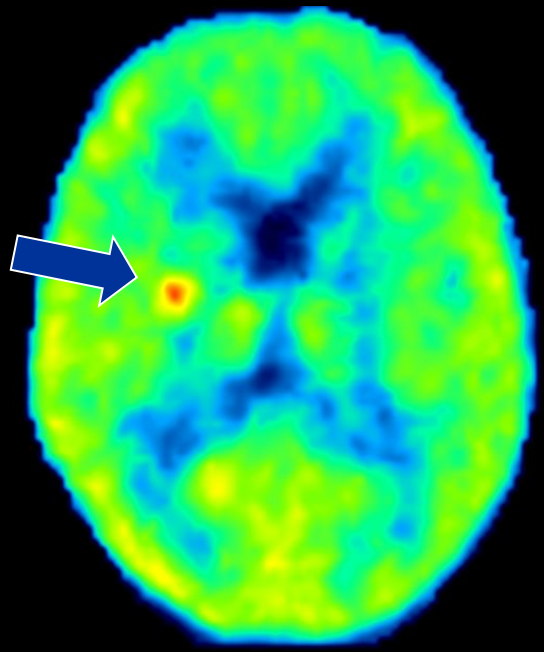
Stroke?



Incidental Stroke

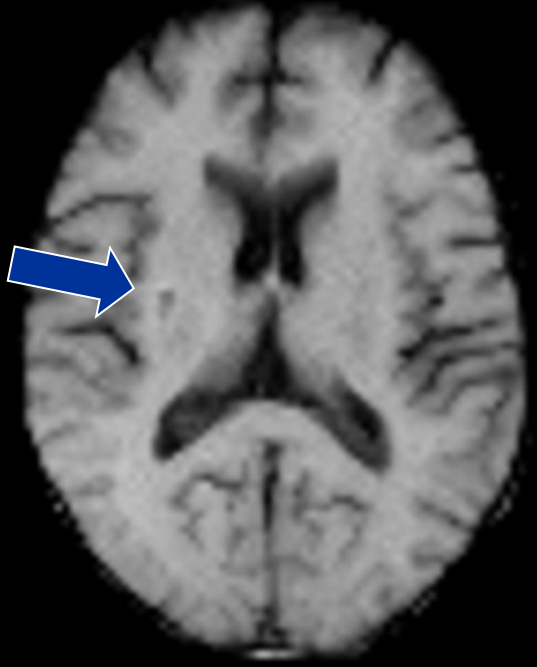
Original MRI (T1)

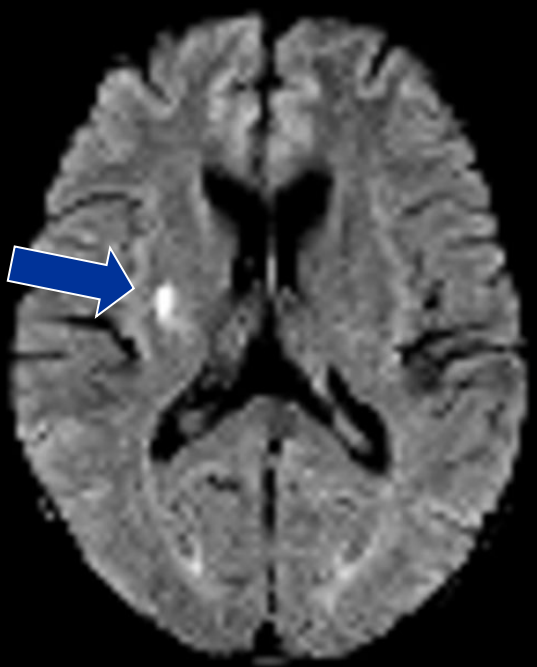




PET 6 weeks after MRI

Repeat MRI 8 weeks after PET





Repeat MRI (FLAIR , edema)

TSPO imaging in Alzheimer's disease

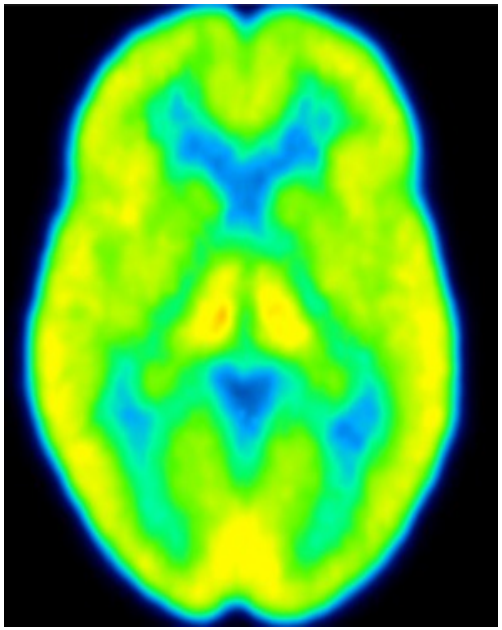
- Neuroinflammation a proposed contributor to Alzheimer's disease pathology
  - Unclear if early or late phenomenon
- Prior TSPO PET studies have shown conflicting results in AD and mild cognitive impairment
- PBR28 an improved TSPO radioligand
  - Genotype correction expected to detect differences in TSPO density in AD, MCI, and controls

TSPO imaging in Alzheimer's disease

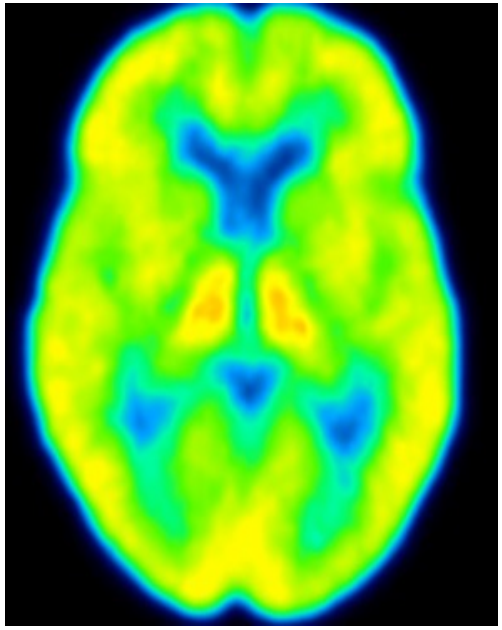
William Kreisl, MD (*Brain*, 2013)

	AD	**MCI**	**Healthy**
Number (n)	19	10	13
Age	63 ± 9	73 ± 10	63 ± 6
MMSE	20 ± 4	28 ± 2	30 ± 0.4
Amyloid	Positive	Positive	Negative

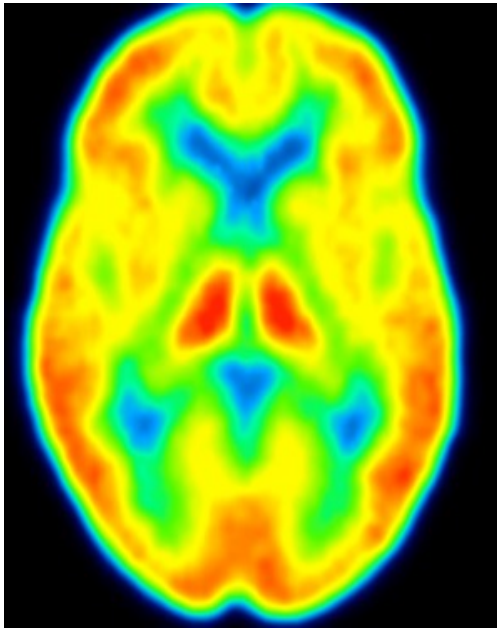
Increased TSPO in Alzheimer's Disease: Compared to Controls and MCI



Control



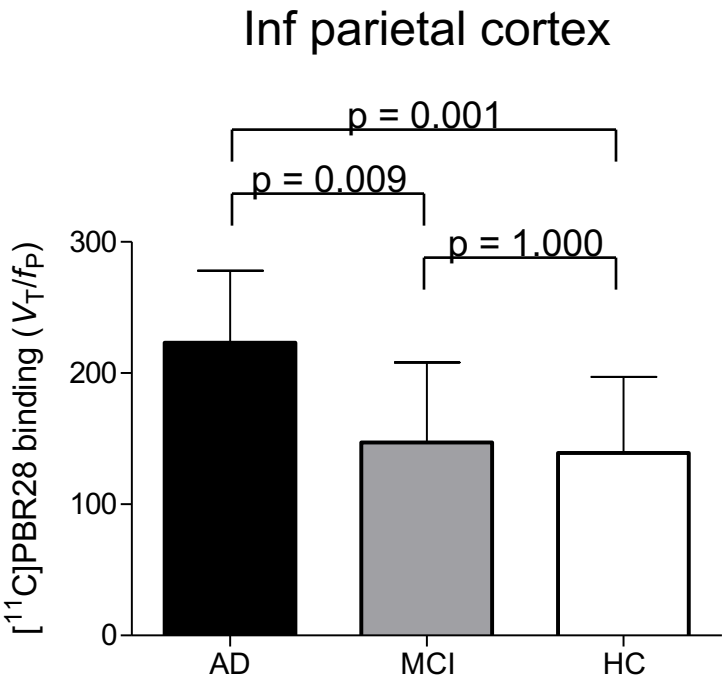
Mild Cognitive Impairment

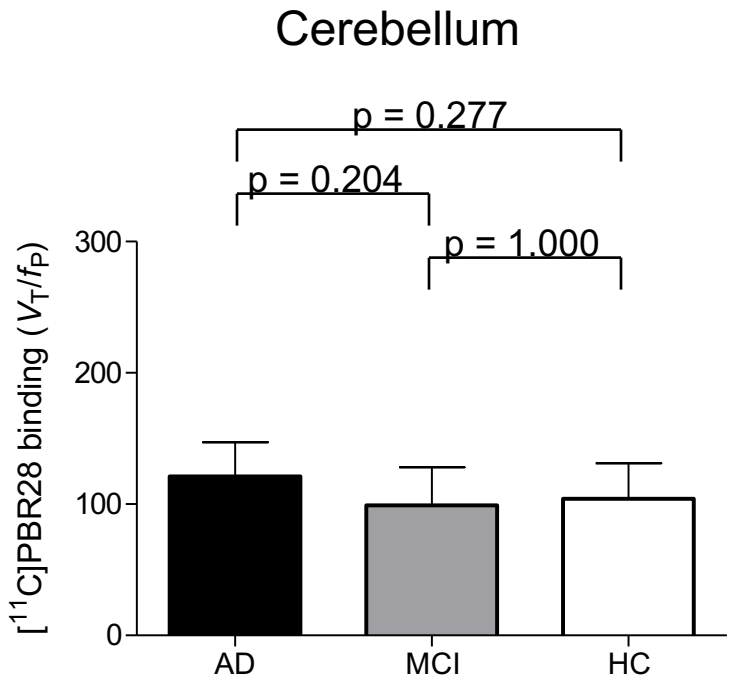


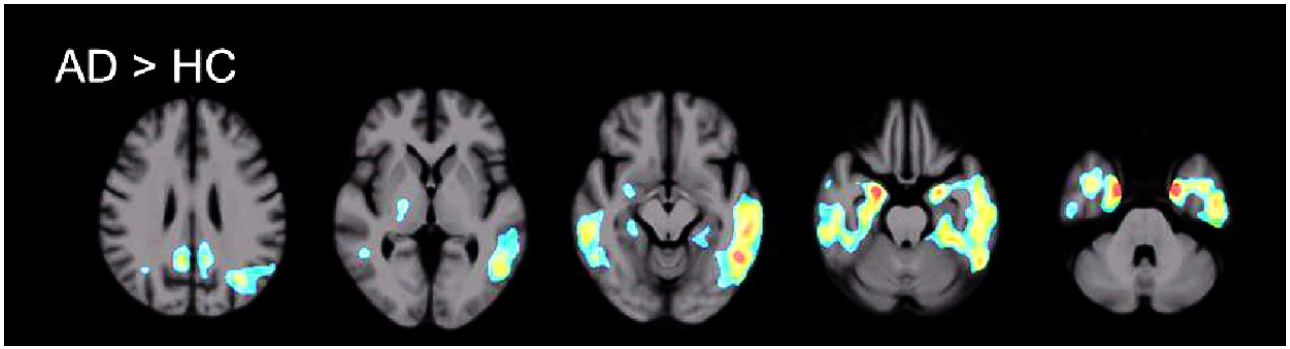
Alzheimer

Kreisl, Brain. 2013

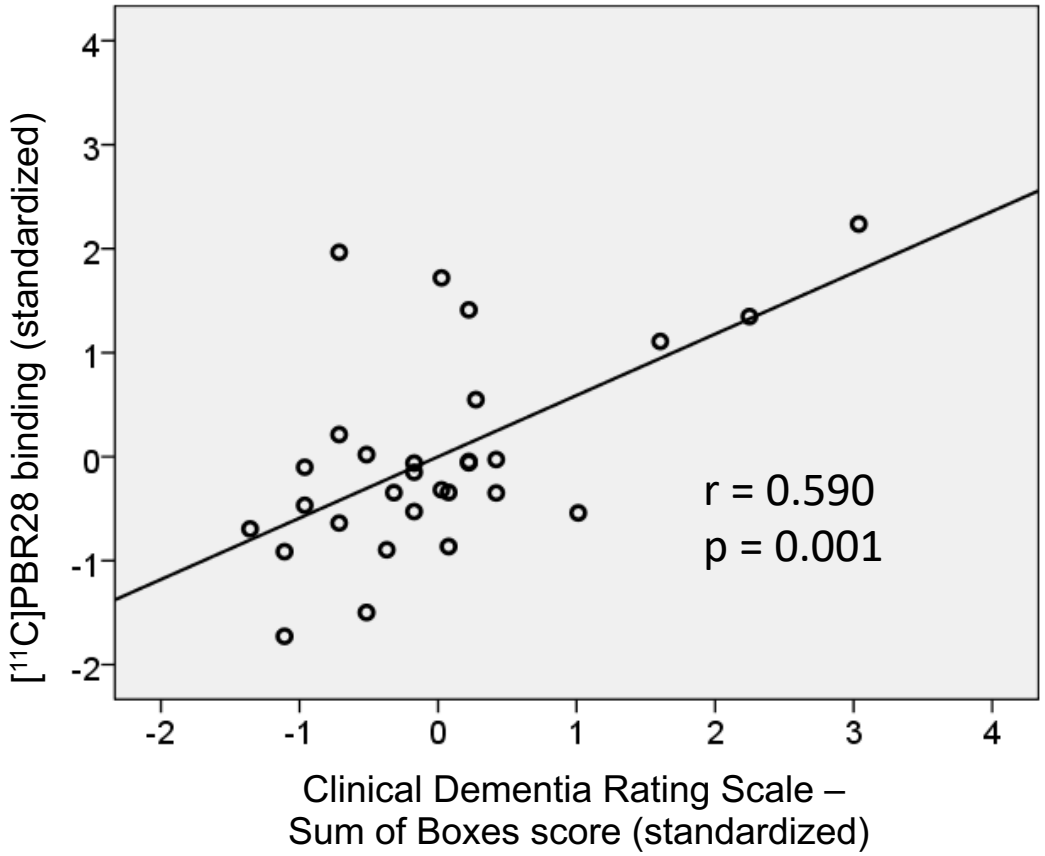
[^{11}C]PBR28 binding greater in Alzheimer's in target regions after correcting for TSPO genotype



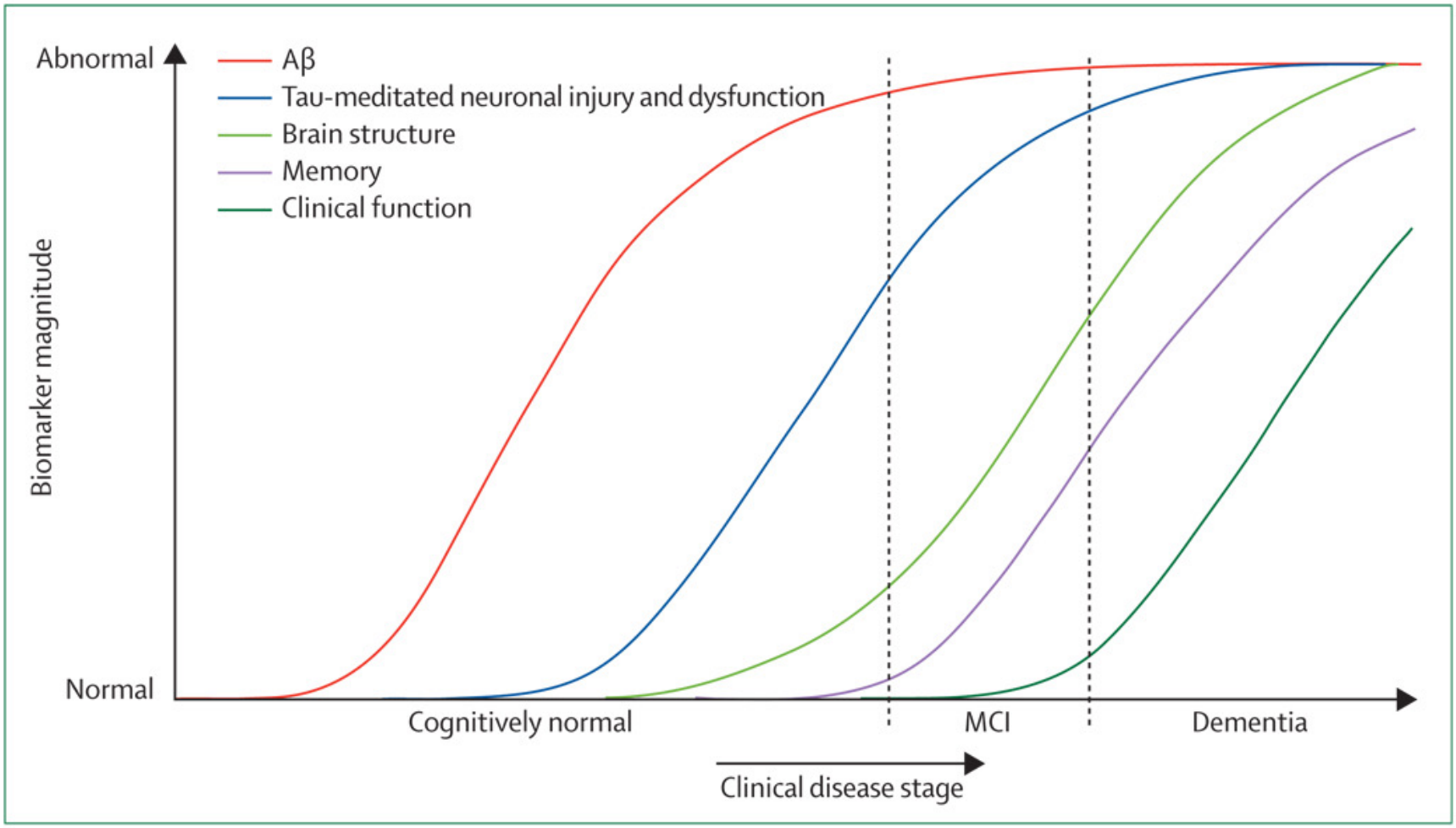




[^{11}C]PBR28 binding correlates with clinical severity across Alzheimer's disease spectrum



Alzheimer's disease as a continuum



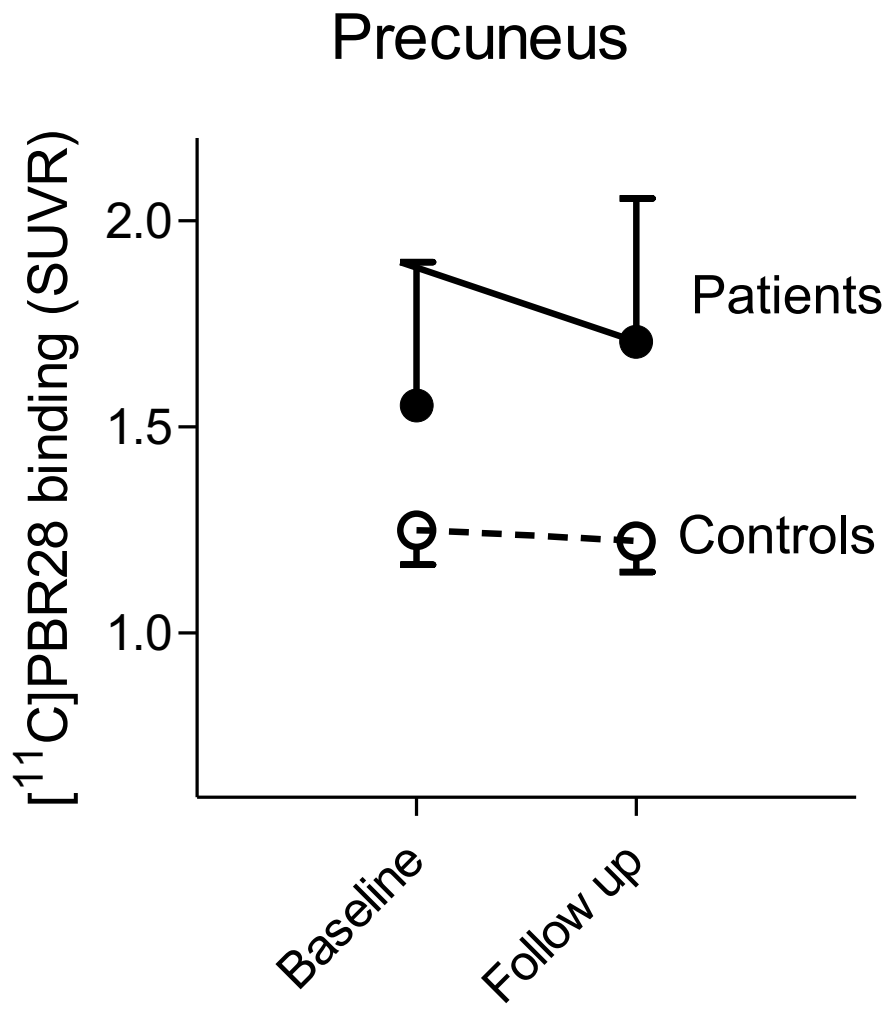
Jack CR Jr, Lancet Neurology 2010

Longitudinal [^{11}C]PBR28 study

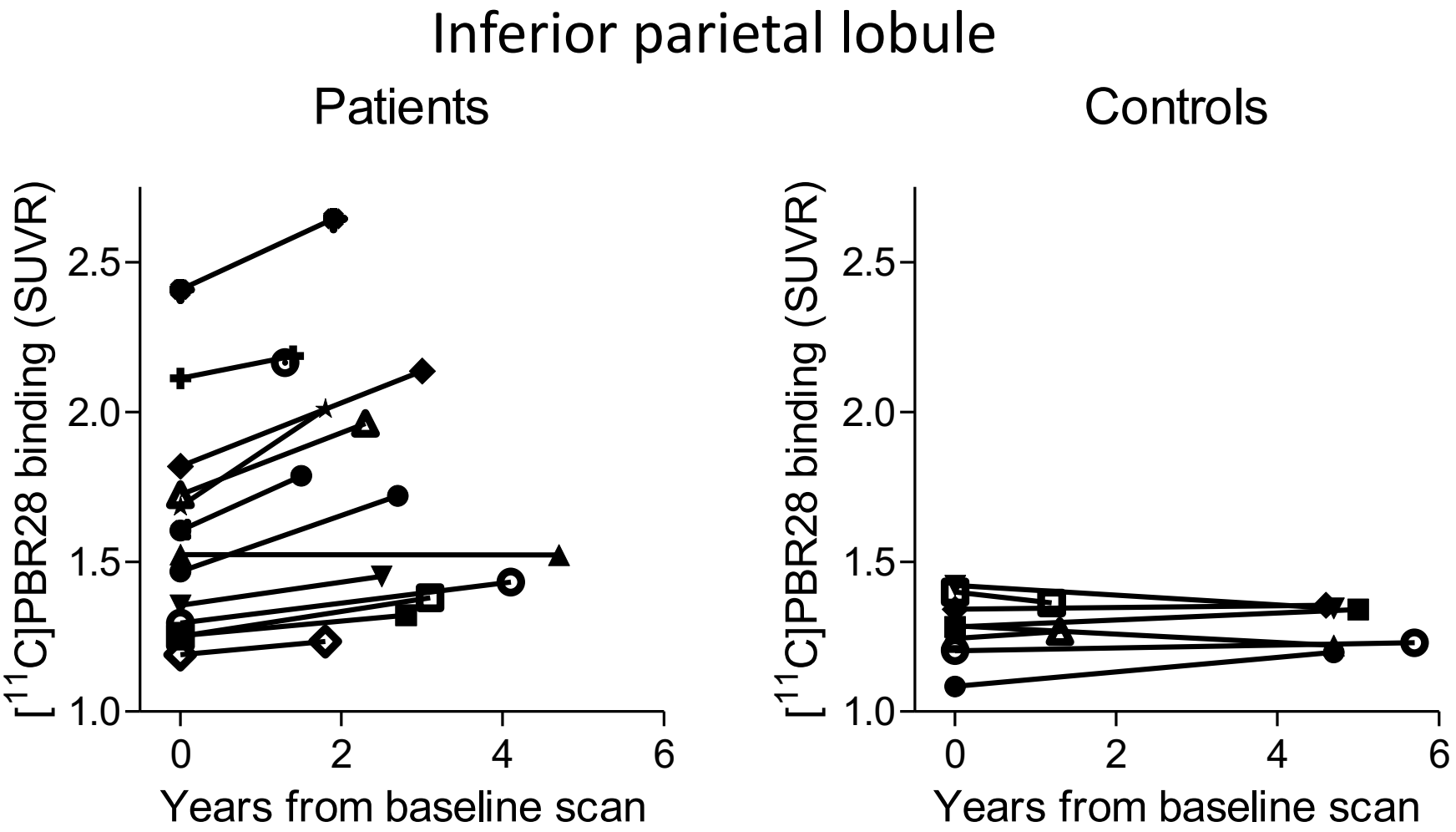
- Objective: Determine if TSPO binding increases during progression of AD and normal aging

- Methods:
  - 14 patients (5 AD + 9 MCI at baseline) and 8 controls returned for follow up
  - [^{11}C]PBR28 data analyzed using cerebellar ratio method (60 – 90 min scan data)
  - Image data analyzed with correction for partial volume effects

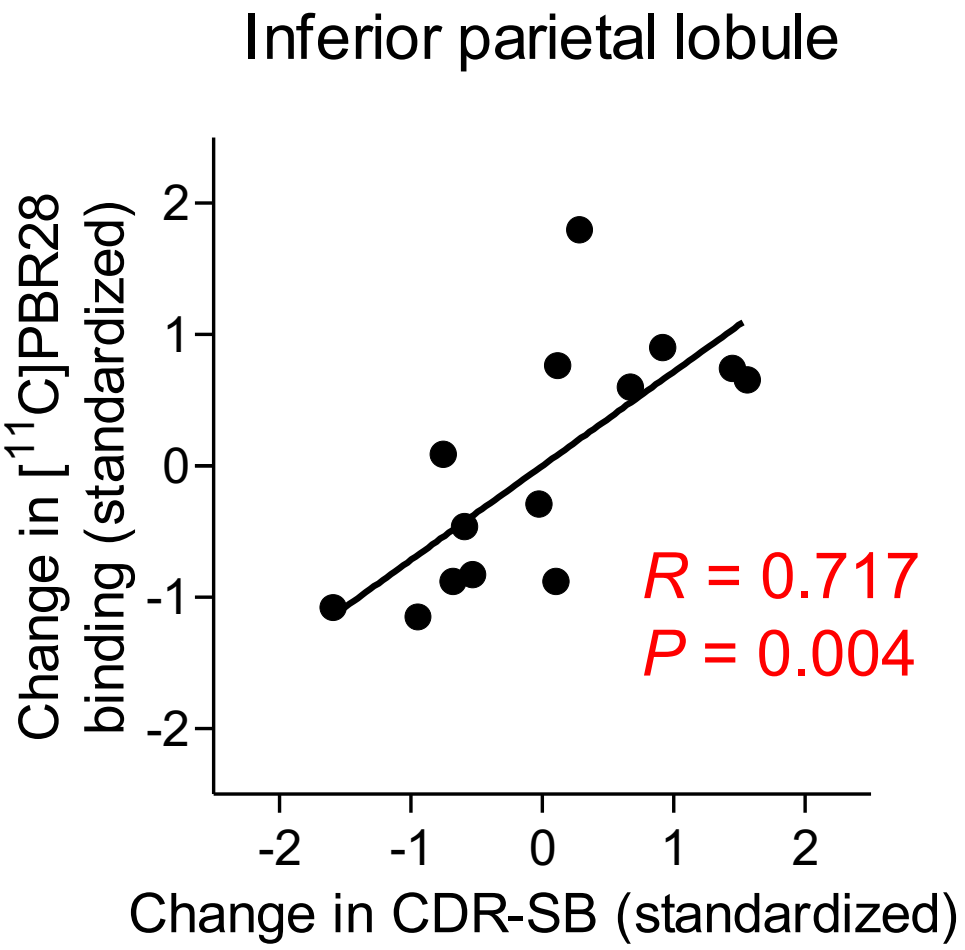
[^{11}C]PBR28 binding increased in patients but not in controls



Results: [^{11}C]PBR28 binding increased in patients but not controls



Increased [^{11}C]PBR28 binding correlates with increased clinical severity



Conclusions from Alzheimer's disease study

- Cross-sectional study: Neuroinflammation occurs after conversion of MCI to AD and worsens with disease progression.

  Biomarker of disease severity

- Longitudinal study: [^{11}C]PBR28 increases in AD but not in controls and correlates with disease progression.

  Biomarker of disease progression

TSPO binding in posterior cortical atrophy (PCA) is increased in posterior cortex

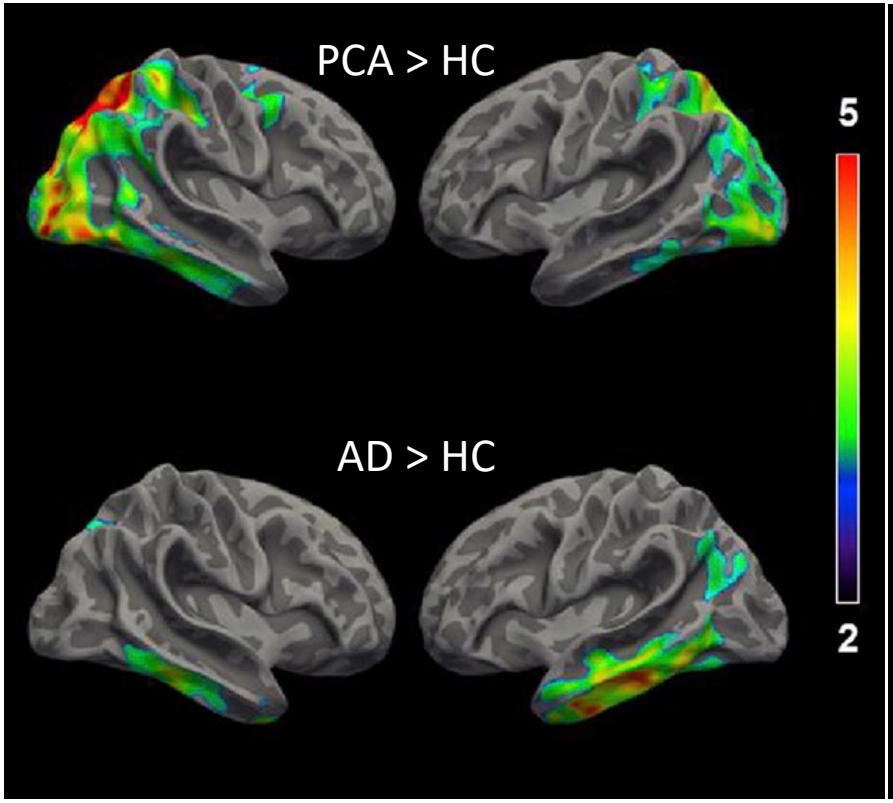
- PCA is rare variant of AD
  - Damage to dorsal “where” stream
  - Damage to ventral “what” stream
- Compared PCA (n=11), amnestic AD (n=11), and controls (n=15)
- [^{11}C]PBR28, [^{11}C]PIB, and [^{18}F]FDG

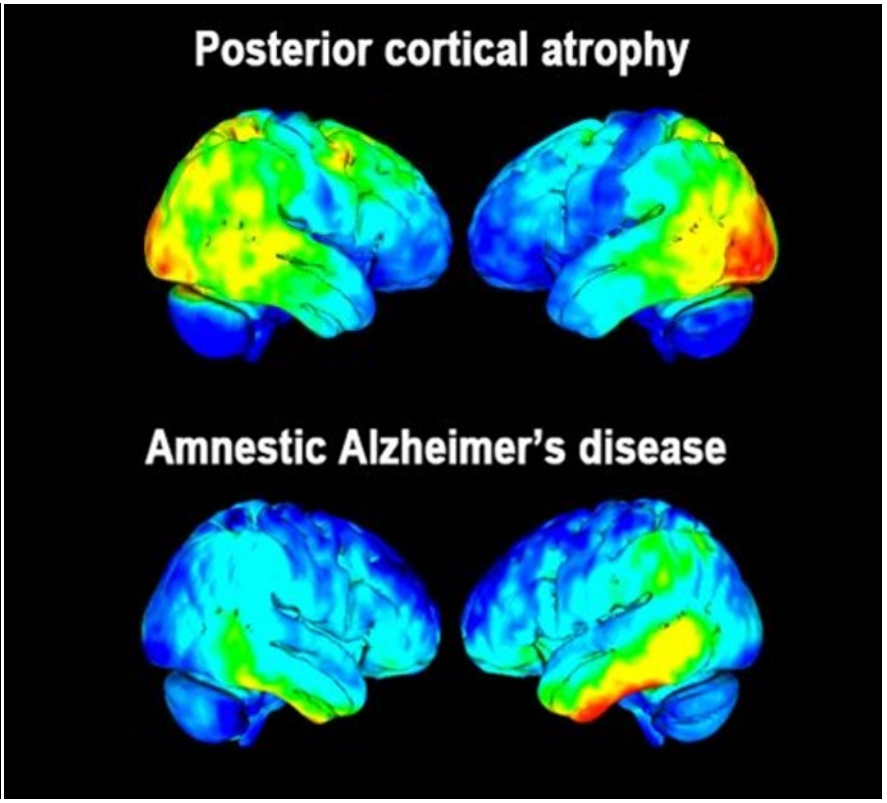
Kreisl, *Neurobio. Aging*, 2017

TSPO binding localizes with tau protein in AD and PCA

TSPO: ^{11}C-PBR28

Tau: ^{18}F-AV-1451





Kreisl, *Neurobio. Aging*, 2017

Ossenkoepele, *Brain*, 2016

TSPO Imaging in Major Depressive Episode

Erica Richards, MD, PhD
Paolo Zanotti Fregonara, MD, PhD*
Masahiro Fujita, MD, PhD
Wayne Drevets, MD†
Giacomo Salvadore, MD†
Robert Innis, MD, PhD
Carlos Zarate, Jr., MD

National Institute of Mental Health, Bethesda, MD, USA
*Houston Methodist Hospital
†Janssen Pharm R&D, Titusville, NJ, USA

Disclosure

- Supported by NIMH and Janssen / J&J

Study Aims

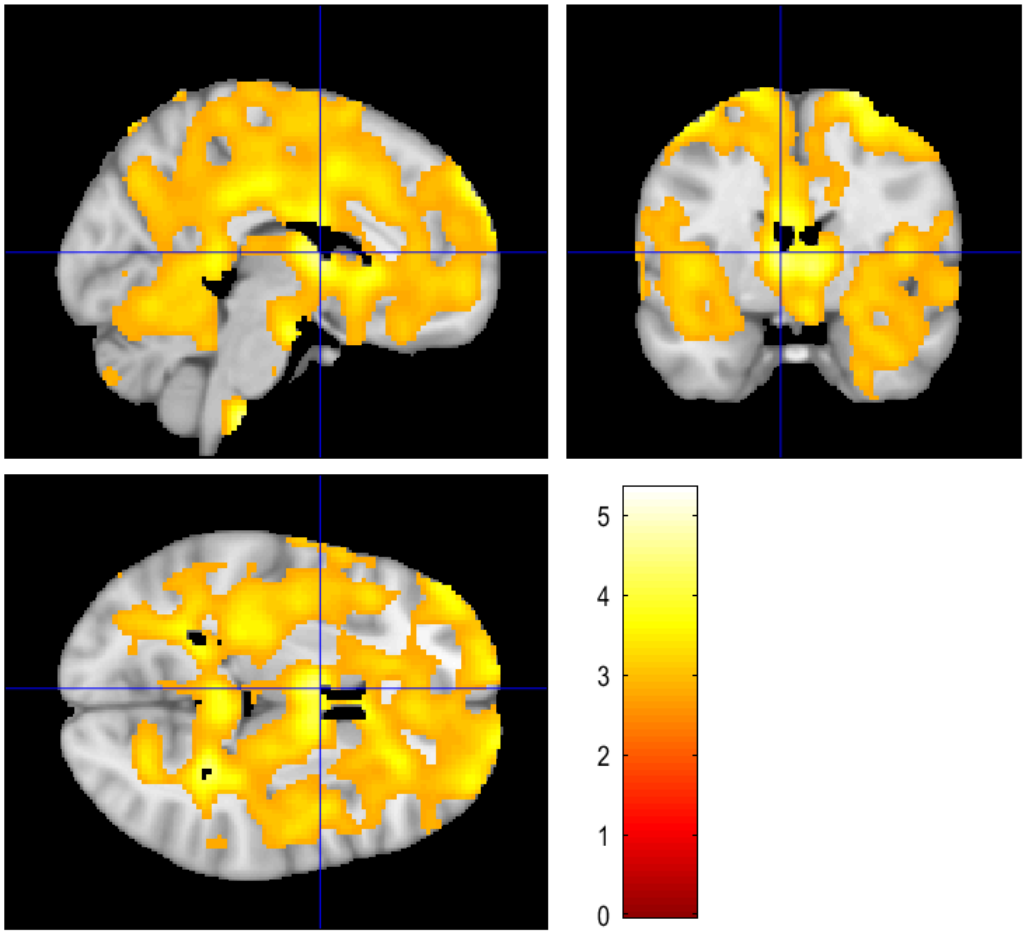
- To evaluate TSPO binding in MDE patients compared to healthy volunteers without a history of depression.

- To investigate any effects of medication on TSPO binding: half of MDE patients were on antidepressants.

Subject Demographics

	Healthy volunteers (N=20)	Medicated MDE (N=16)	Unmedicated MDE (N=11)
Age	32 ± 10	45 ± 10	34 ± 9
Sex	10M, 10F	10M, 6F	7M, 4F
MADRS	0.3 ± 0.57	31.0 ± 4.4	31.2 ± 3.7
HAMD	0.5 ± 0.89	18.9 ± 3.7	21.6 ± 3.3

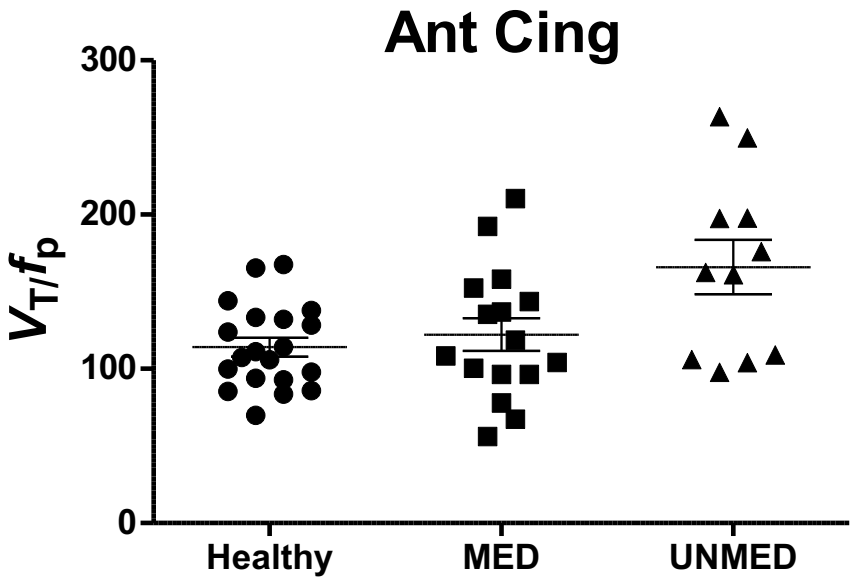
Widespread increase of TSPO in a large cluster over the whole brain: MDE > controls



p = 0.000 after family-wise correction for multiple comparisons; genotype and age as covariates

TSPO binding in anterior cingulate was increased in unmedicated MDE patients

	V_T/f_p
Healthy	114 ± 27
Medicated MDE	122 ± 42
Unmedicated MDE	166 ± 58



Healthy vs. Unmed MDE	p = 0.002
Med MDE vs. Unmed MDE	p = 0.033
Healthy vs. Med MDE	not significant

In unmedicated patients, TSPO binding was increased by 31% compared to healthy controls and by 27% compared to medicated patients.

Major Findings

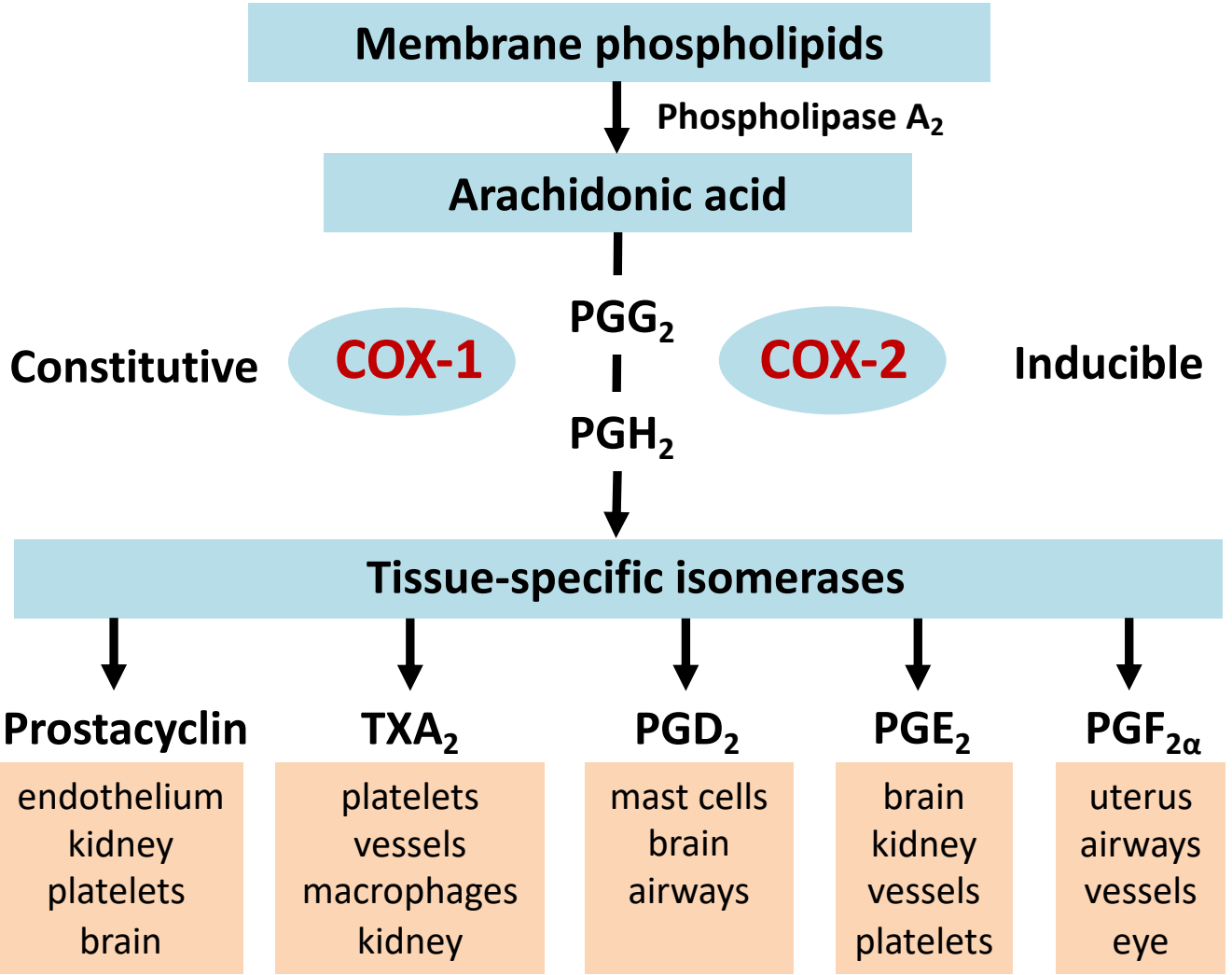
- TSPO binding showed widespread increase in unmedicated MDE patients compared to controls
  - Replicates findings of Meyer et al. (2015)
  - Four studies have now found increased TSPO in MDE

- But medicated MDE showed normal TSPO density
  - SSRI may modulate this PET inflammatory biomarker
  - Need a longitudinal study of patients before and after treatment

Summary

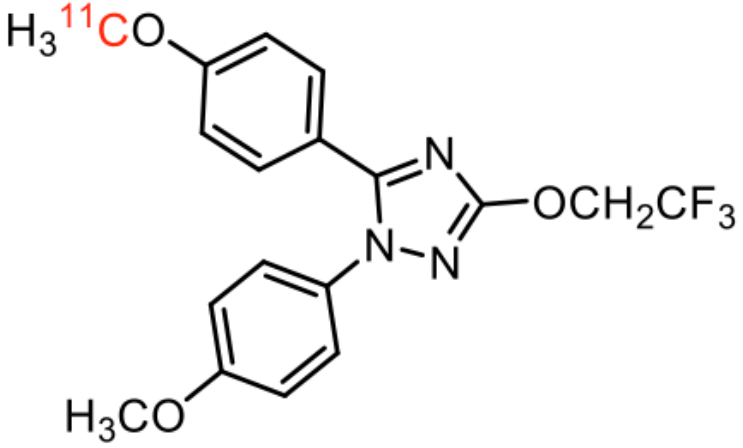
1. TSPO (translocator protein): marker of inflammation: activated microglia, reactive astrocytes, and macrophages
2. Alzheimer's disease: Increased TSPO binding correlates with disease severity (cross sectional) and with disease progression (longitudinal).
3. Major Depressive Episode: Four studies have now found increased TSPO in MDE
4. How can PET facilitate anti-inflammatory trials in dementia and depression?

Cyclooxygenase (COX)



Fitzgerald et al., *NEJM* 2001

COX-1: ^{11}C-PS13

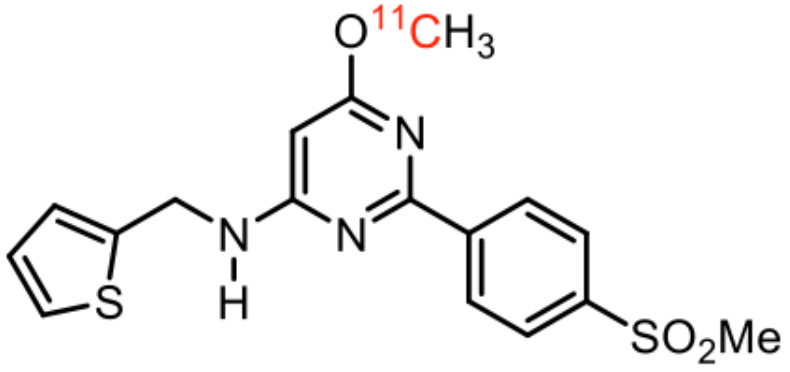


Human Enzyme	IC_{50} (nM)
COX-1	1
COX-2	>1,000

Constitutive

Microglia

COX-2: ^{11}C-MC1

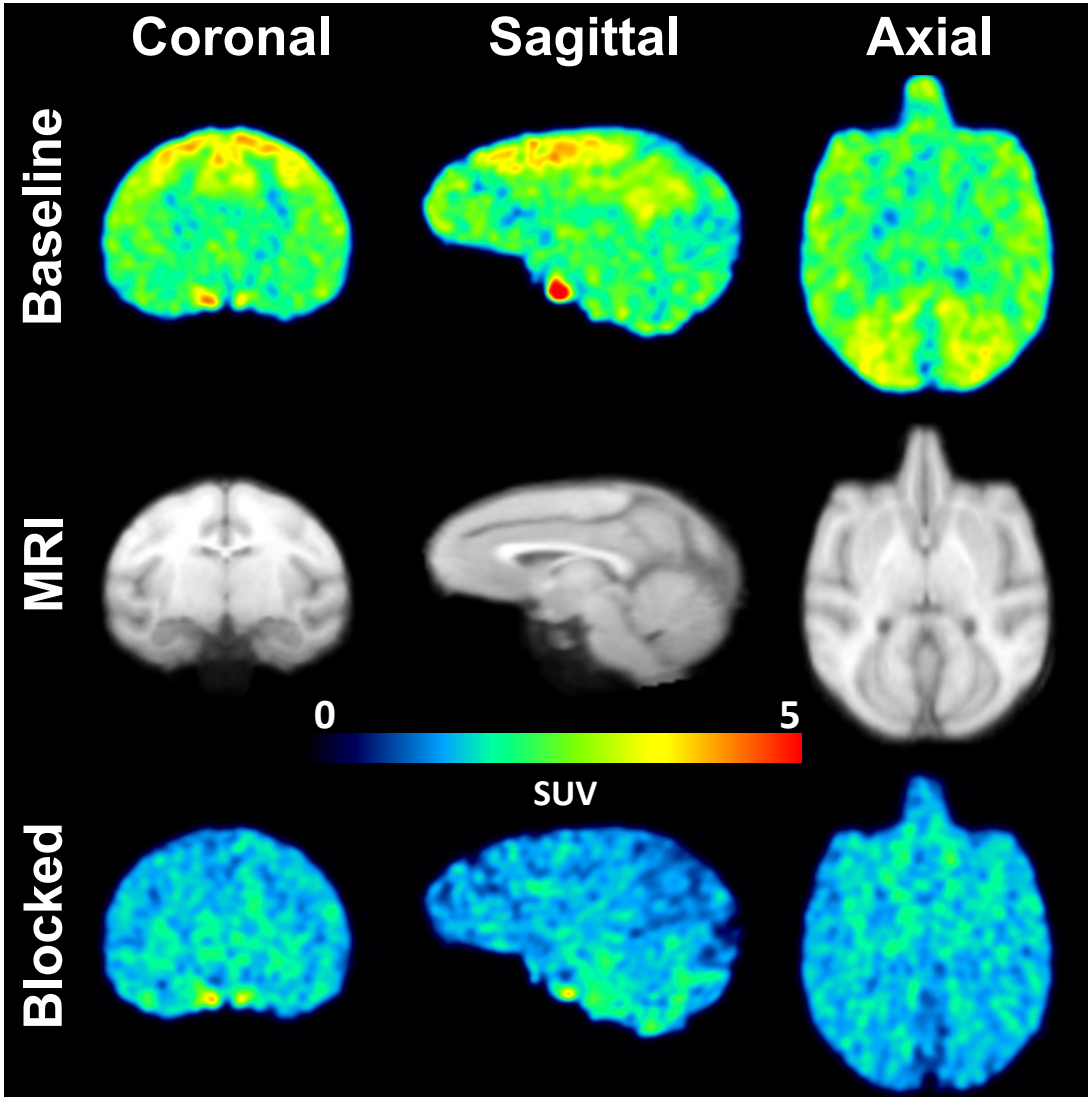


Human Enzyme	IC_{50} (nM)
COX-1	>1,000
COX-2	1

Inducible

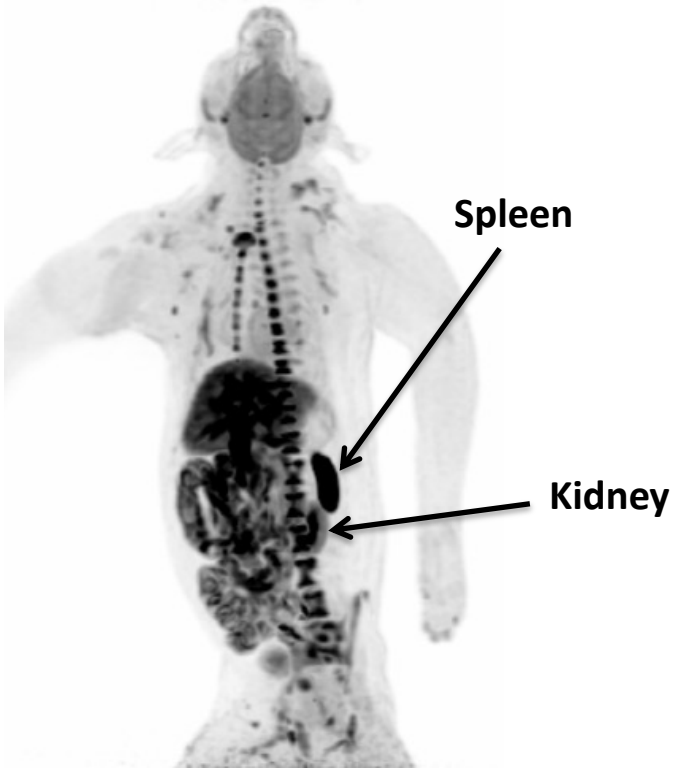
Neurons + Microglia

COX-1: Specific binding to [^{11}C]PS13 in monkey brain



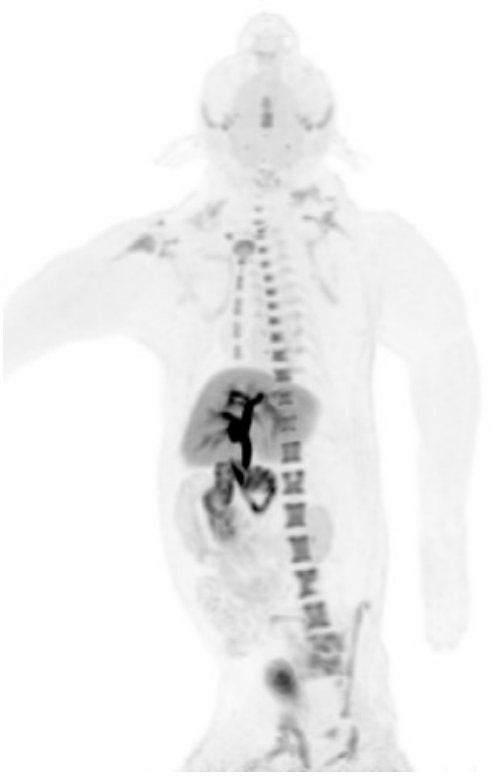
COX-1: specific binding of ^{11}C-PS13 in brain, spleen, GI tract, and kidney

Baseline

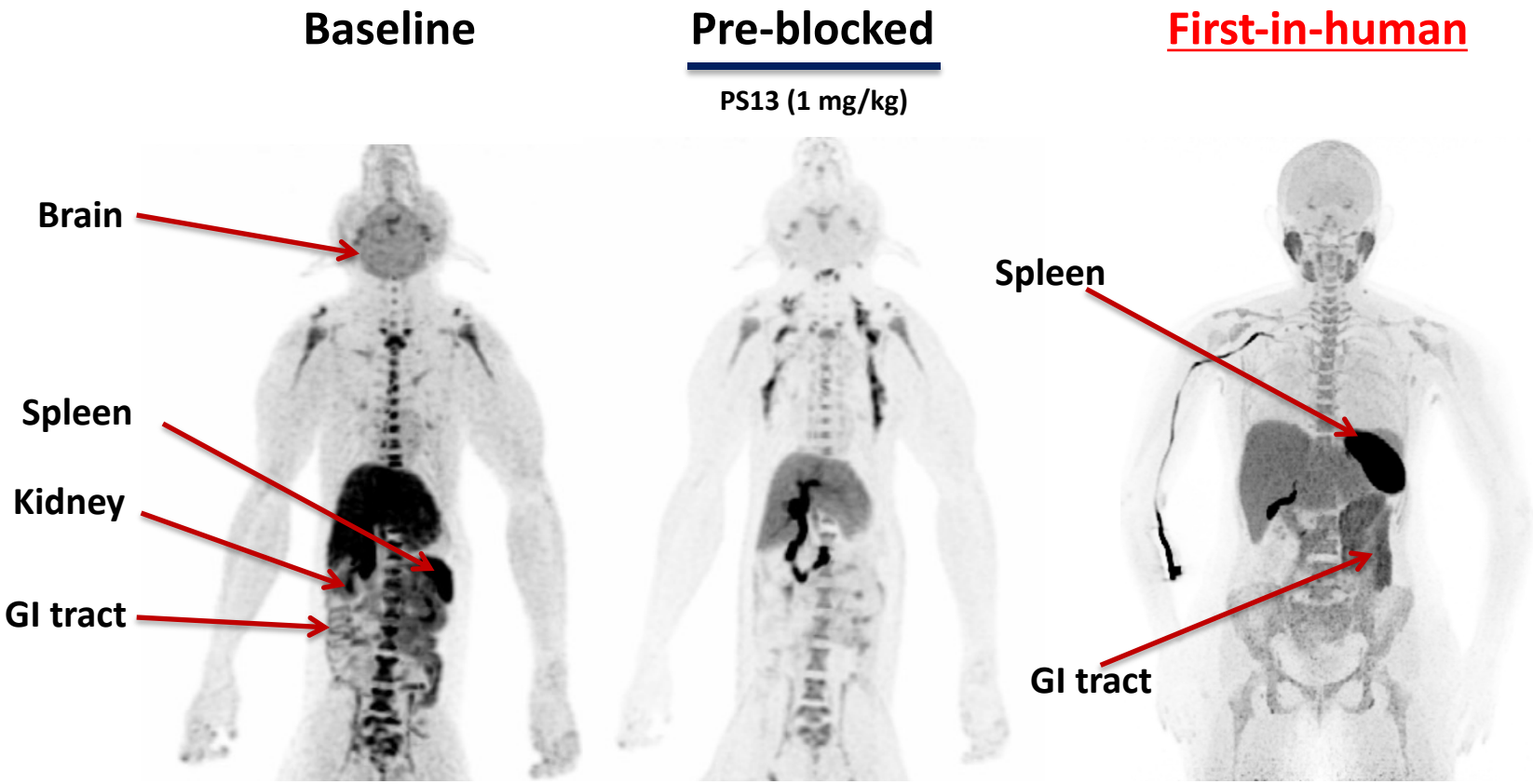


Blocked

PS13 (0.3 mg/kg)

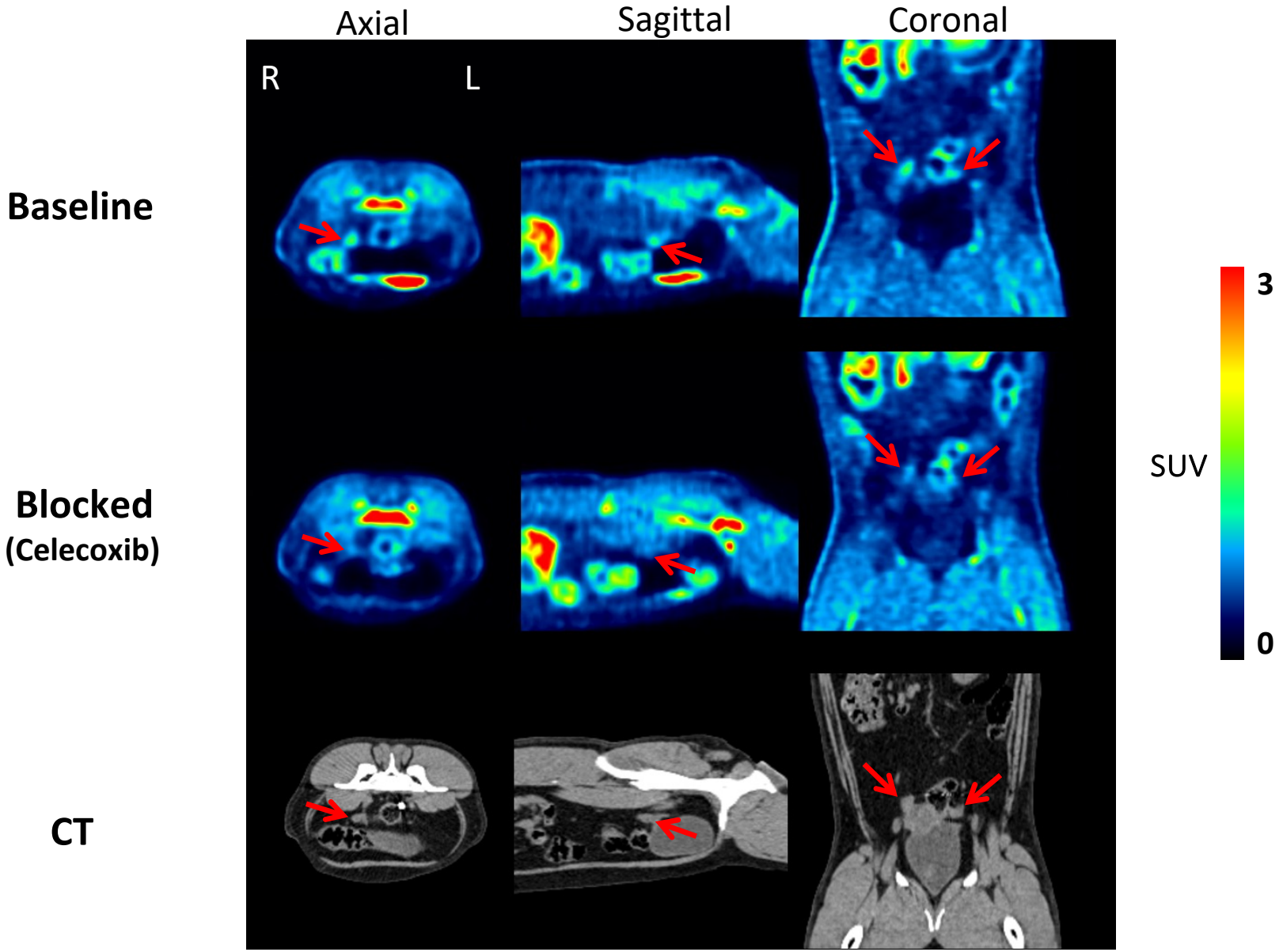


COX-1: extension from monkeys to humans

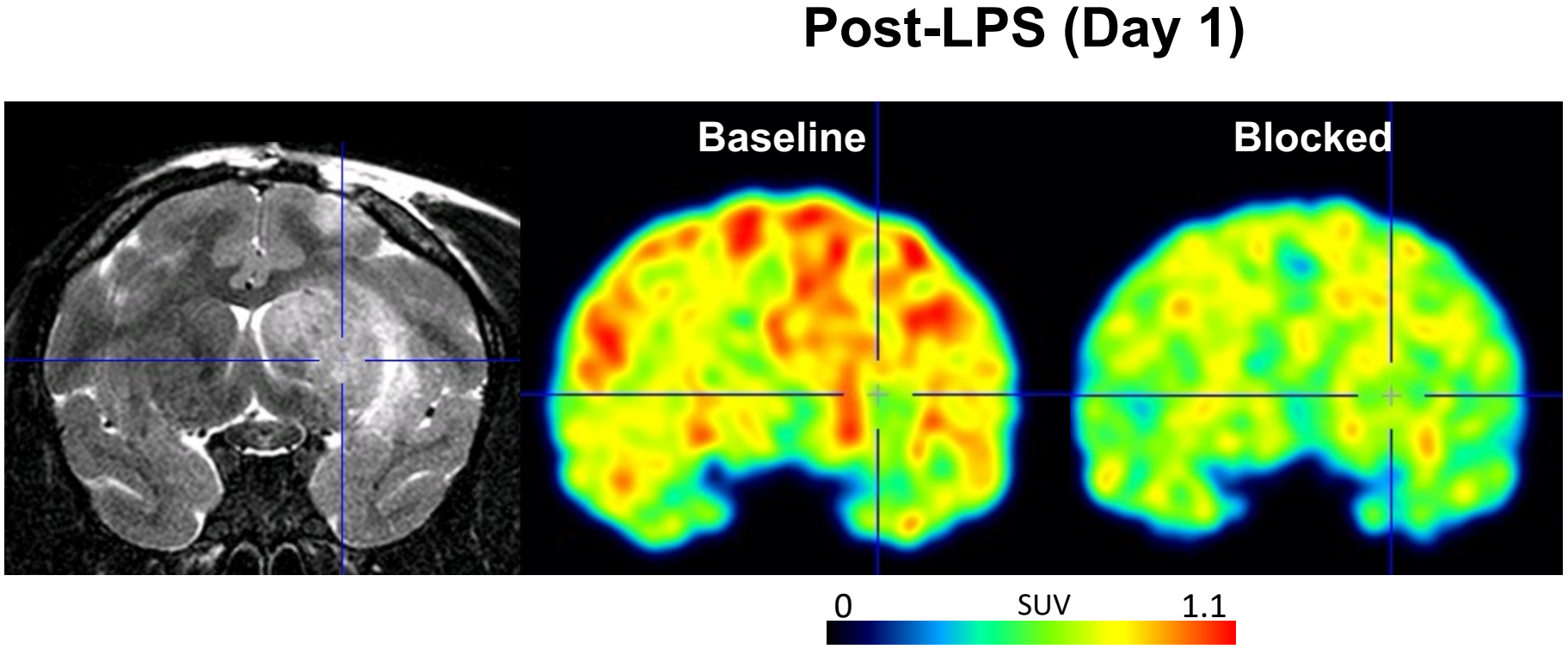


Min-Jeong Kim, MD, PhD
Blockade studies in progress

COX-2 specific binding undetectable except in ovary

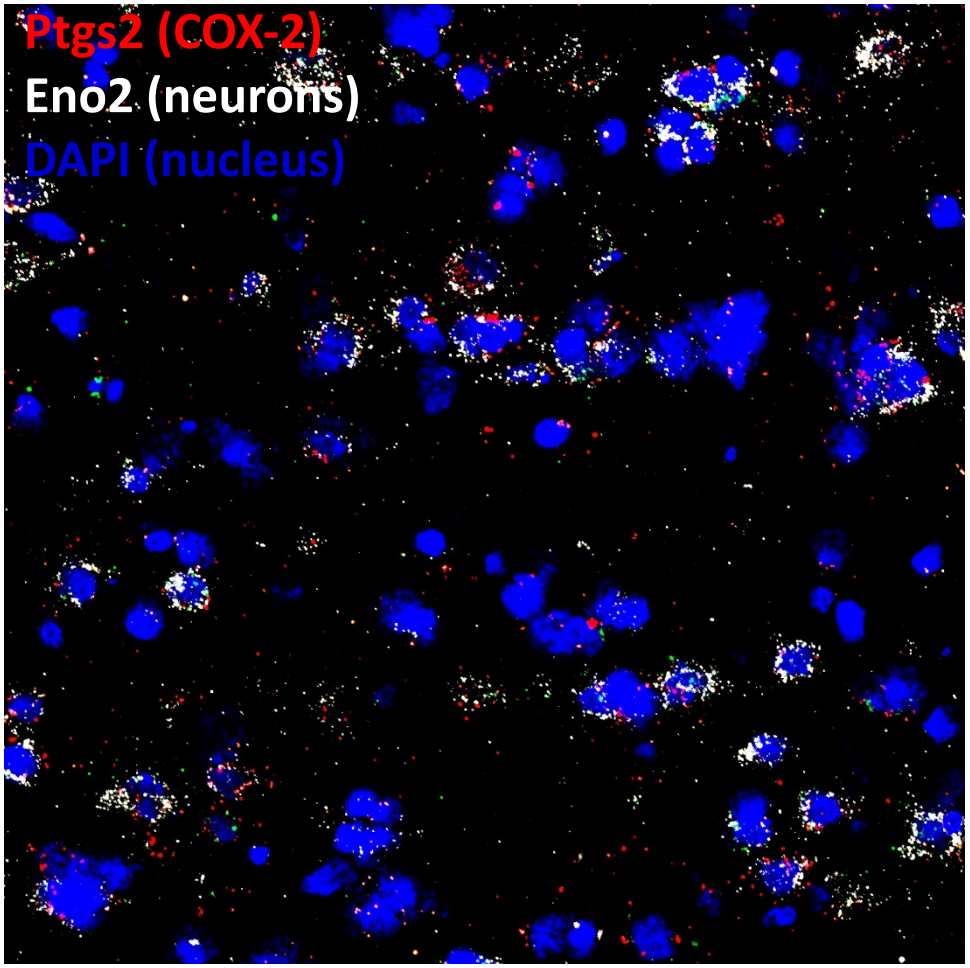


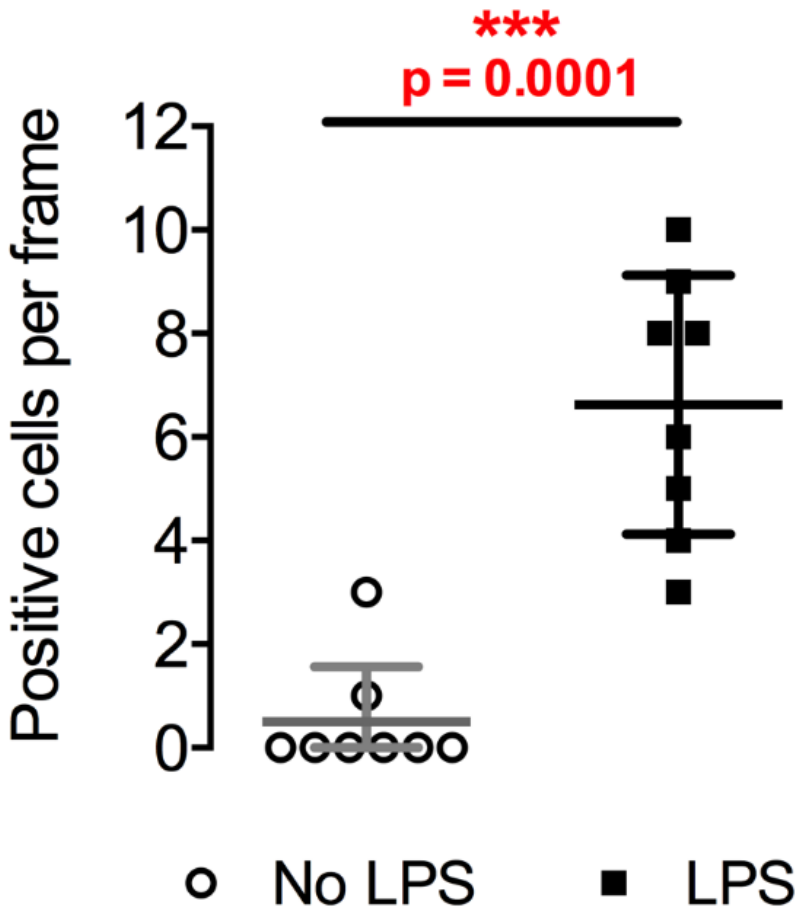
COX-2: LPS injection globally increased [^{11}C]MC1 binding about 50%



COX-1: LPS injection had no effect on [^{11}C]PS13 binding

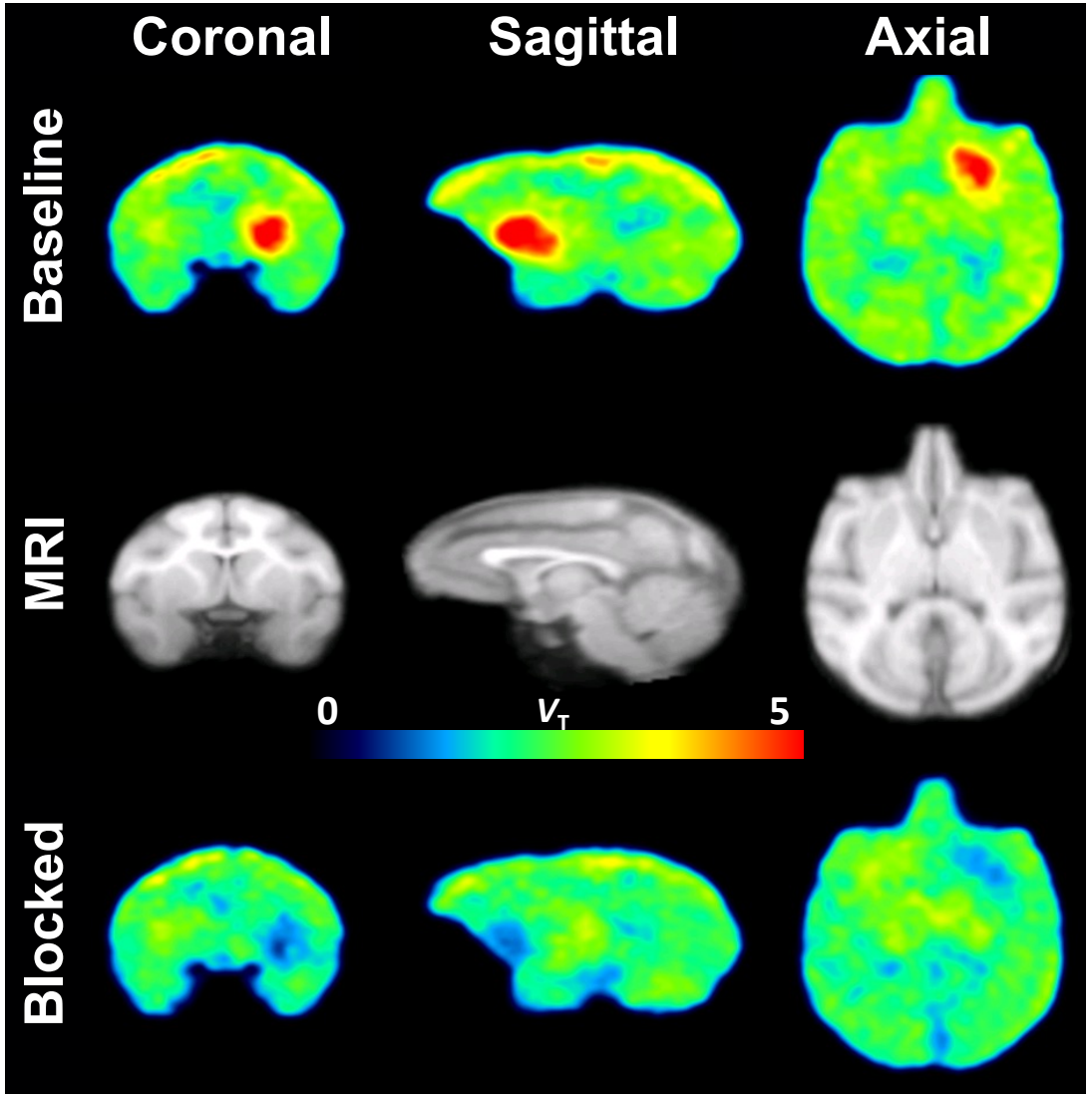
Inflammation increases COX-2 mRNA in neurons





^{11}C-MC1 is a novel, and specific inflammation marker for imaging COX-2

Post-LPS (Day 1)



Conclusion

1) ^{11}C-PS13 selectively binds COX-1, which is constitutively expressed in brain, spleen, GI tract, and kidney. Neuroinflammation does not increase its expression.

Whole body and brain imaging in healthy subjects (in progress)

2) ^{11}C-MC1 selectively labels COX-2, which is inducible by neuroinflammation;

- *Rheumatoid Arthritis and Myositis*
- *Developing new analogs with higher affinity*

3) COX-2 mRNA and protein are upregulated in inflamed brain and located primarily in neurons.

cAMP cascade in major depressive disorder: Downregulation in unmedicated patients and upregulation with treatment

Masahiro Fujita, MD, PhD
Erica Richards, MD, PhD
Victor W. Pike, PhD
Carlos Zarate, MD
Robert Innis, MD, PhD

National Institute of Mental Health, Bethesda, MD, USA

Outline

- In vivo binding of ^{11}C-(*R*)-rolipram to phosphodiesterase (PDE) 4 reflects the activity of cAMP cascade because of a feedback mechanism.

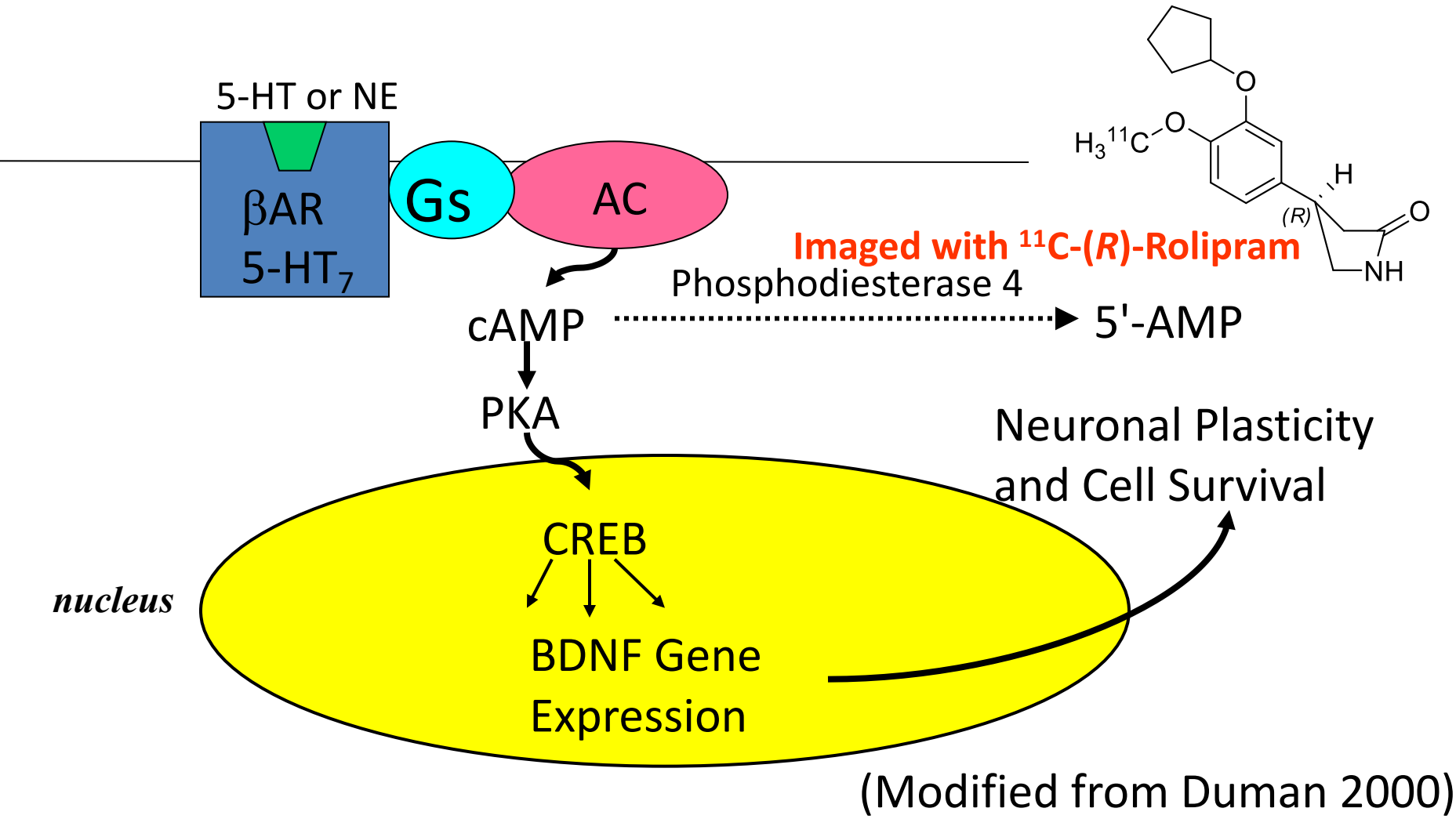
  ↑cAMP stimulates PKA, which phosphorylates PDE4, which

  ↑rolipram binding

- Rolipram binding was 18% lower ($p = 0.001$) in unmedicated patients with MDD ($n = 43$) than in controls ($n = 35$), indicating downregulation of cAMP cascade.

- SSRI treatment increased rolipram binding in patients by 13% ($p = 0.001$, $n = 21$), suggesting normalization of cAMP cascade.

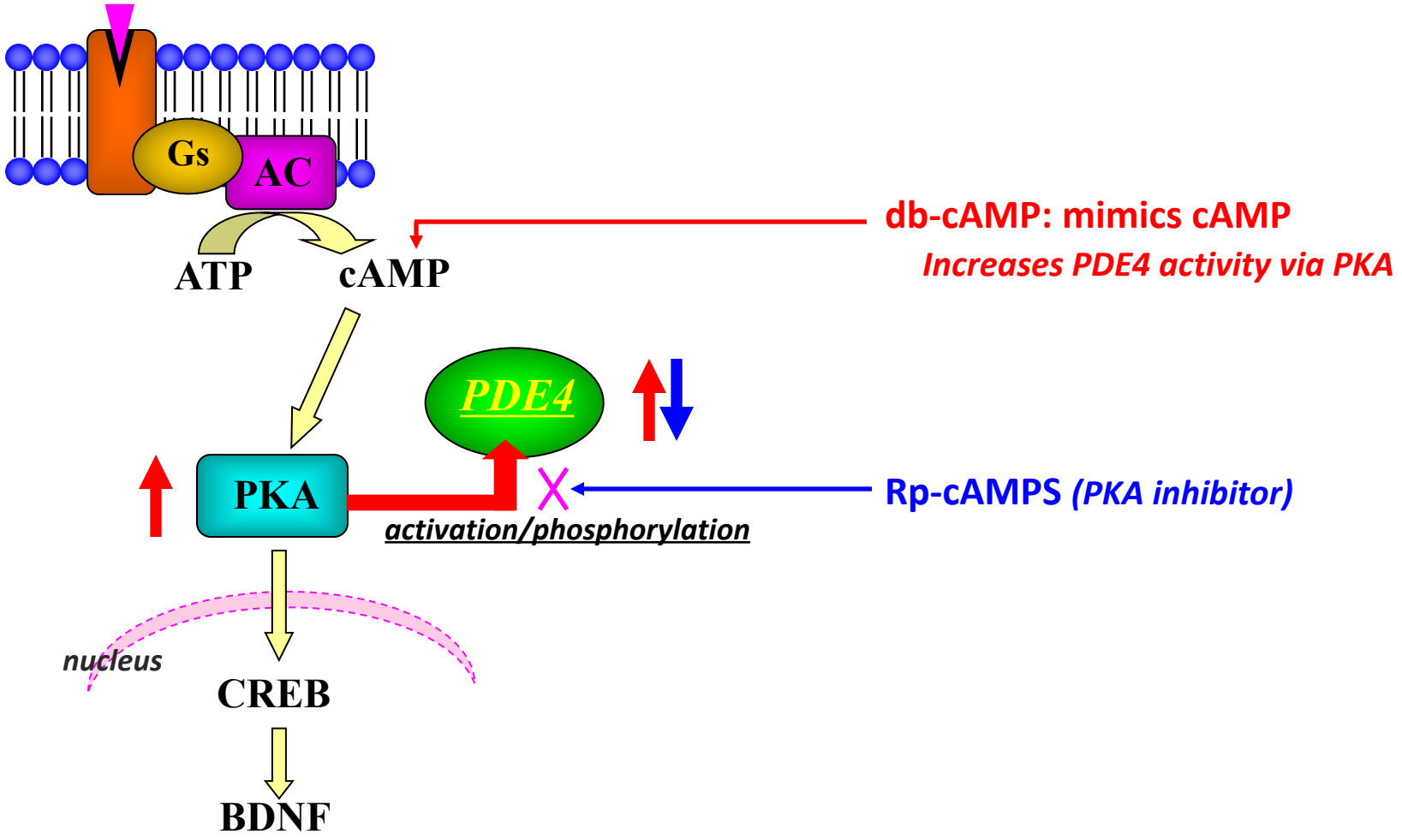
PET Imaging of cAMP Cascade using Phosphodiesterase Inhibitor ^{11}C-(*R*)-Rolipram



Enzyme activity and rolipram affinity are increased by phosphorylation of PDE4

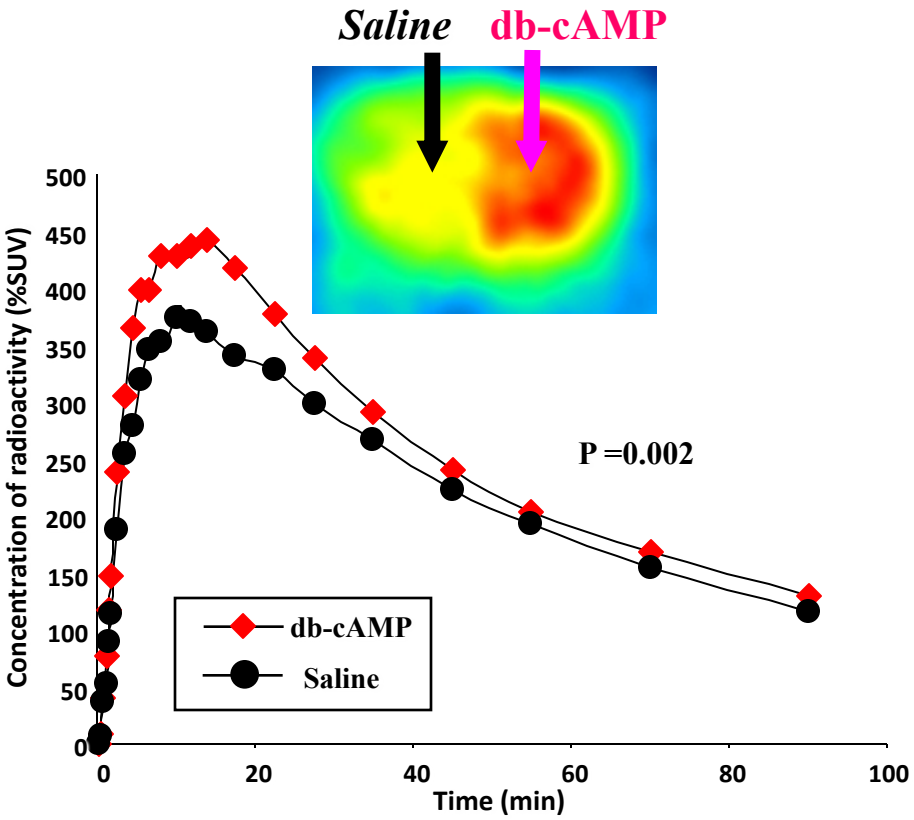
- Feedback mechanism: cAMP-stimulates PKA phosphorylation of PDE4 increases enzyme activity and affinity of rolipram binding.
- We measured density and affinity of PDE4 in rats: both in vivo and in vitro (postmortem).
  - Affinity is decreased five fold after death, consistent with rapid dephosphorylation of PDE4.
- Local injections to increase or decrease activity of PKA have expected effects on rolipram binding.
- ^{11}C-(*R*)-rolipram PET in humans provides unique in vivo measure of PDE4 density and affinity (enzyme activity), not possible in postmortem tissue.

PKA phosphorylates PDE4: increases enzyme activity and affinity of rolipram

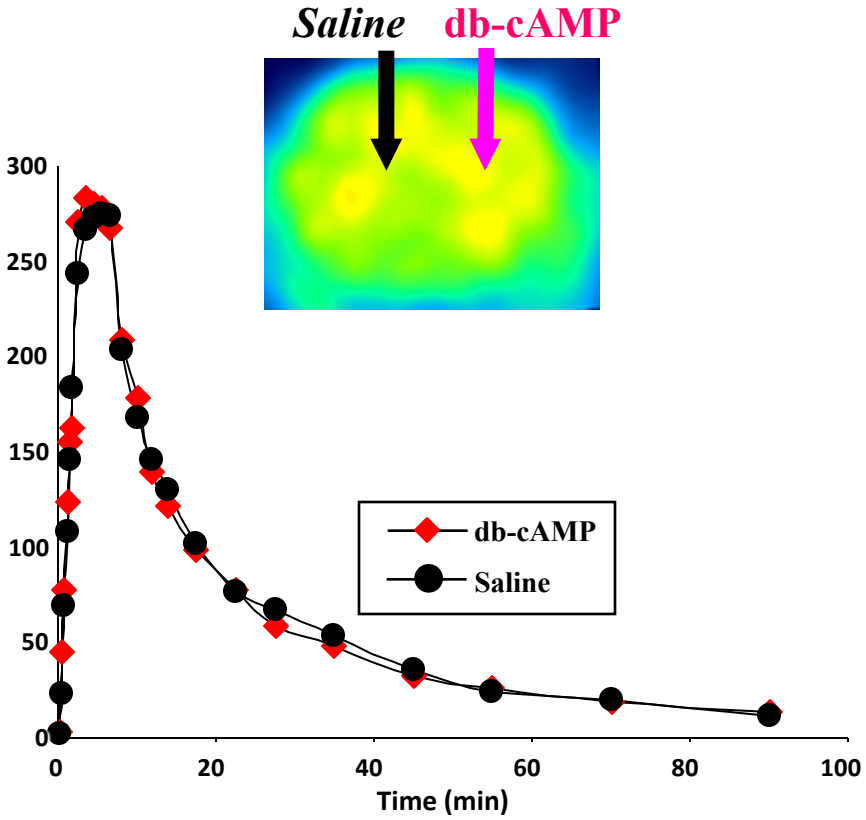


db-cAMP (PKA activator) increased ^{11}C-(*R*)-rolipram binding

^{11}C-(*R*)-rolipram: total activity



^{11}C-(*S*)-rolipram: nondisplaceable activity

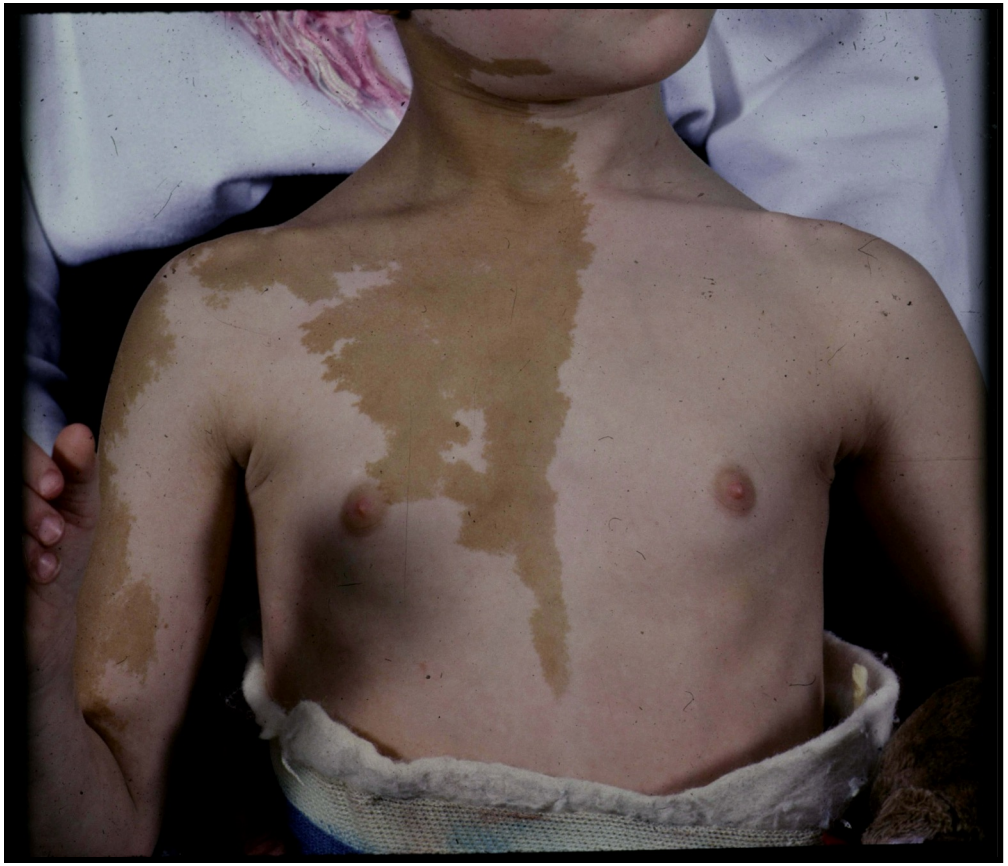


McCune-Albright Syndrome:
Rare Mosaic Genetic Disorder in $G_{s\alpha}$ leading to elevated cAMP

Café-au-lait

Precocious puberty

Fibrous dysplasia



Acromegaly

Hyperthyroid

Cushings

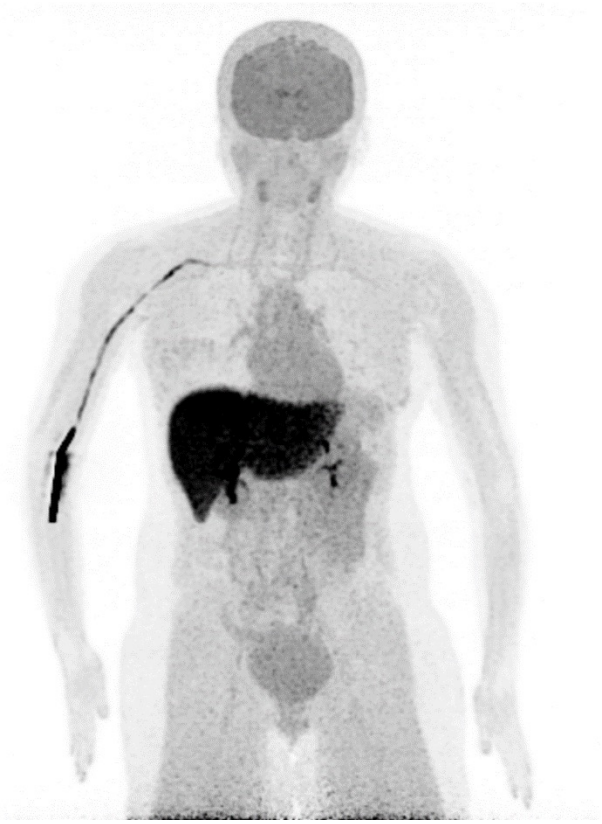
Rickets/ Osteomalacia

McCune-Albright Syndrome:

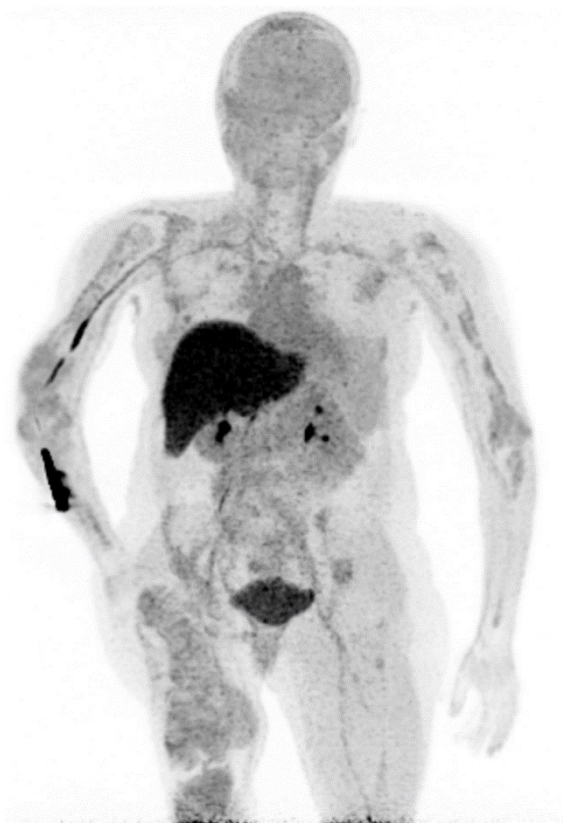
Organs respond by elevating / activating PDE4

PET ^{11}C-*R*-rolipram

Control



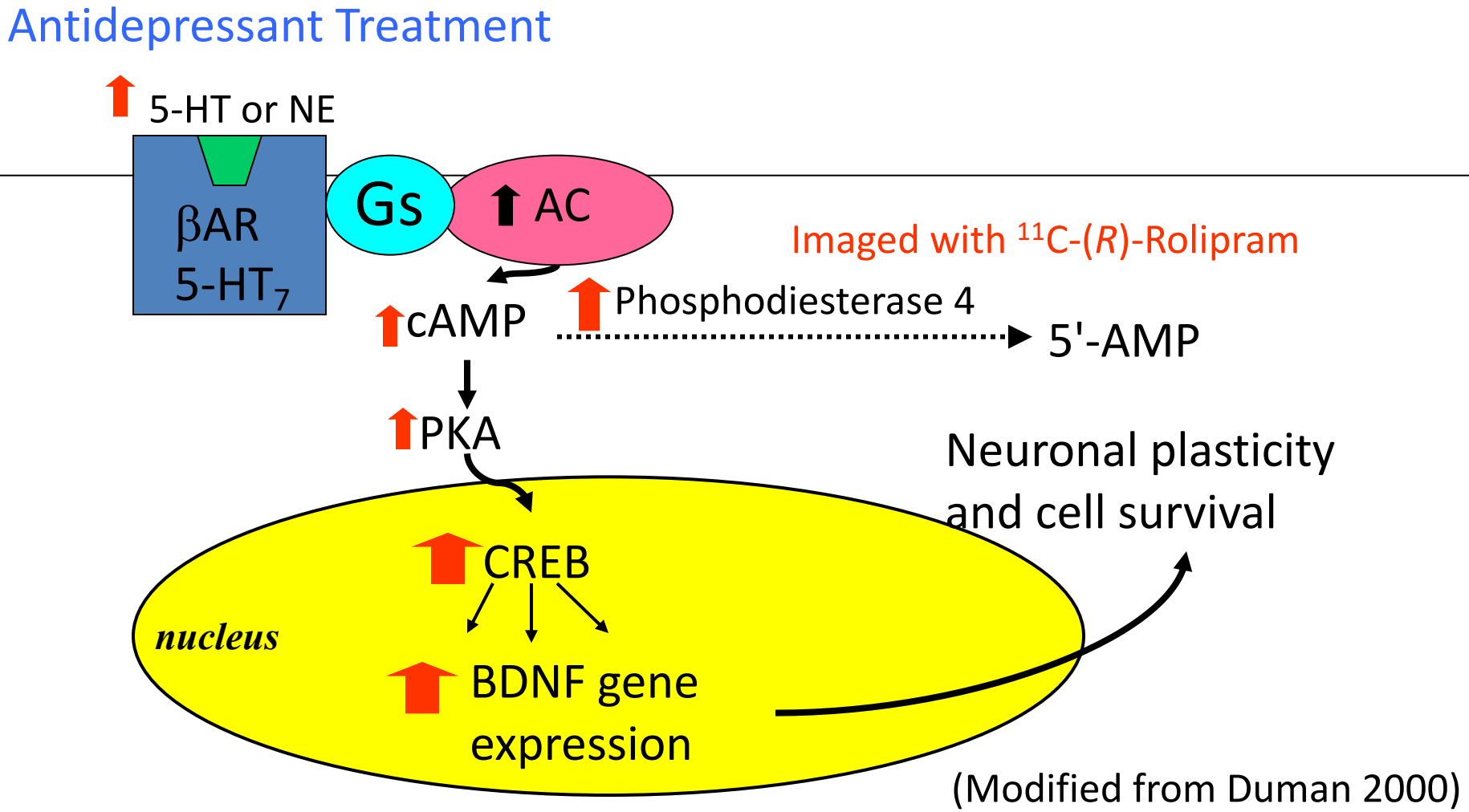
Patient



Weidner, Boyce, Collins et al.

Chronic (but not acute) antidepressant treatments upregulate the cAMP cascade

cAMP cascade – common action site of antidepressants?



(Modified from Duman 2000)

Hypotheses in the study of major depressive disorder

1. Unmedicated patients with major depressive disorder show lower ^{11}C-(*R*)-rolipram binding than healthy subjects.

2. Antidepressant treatment increases ^{11}C-(*R*)-rolipram binding in patients.

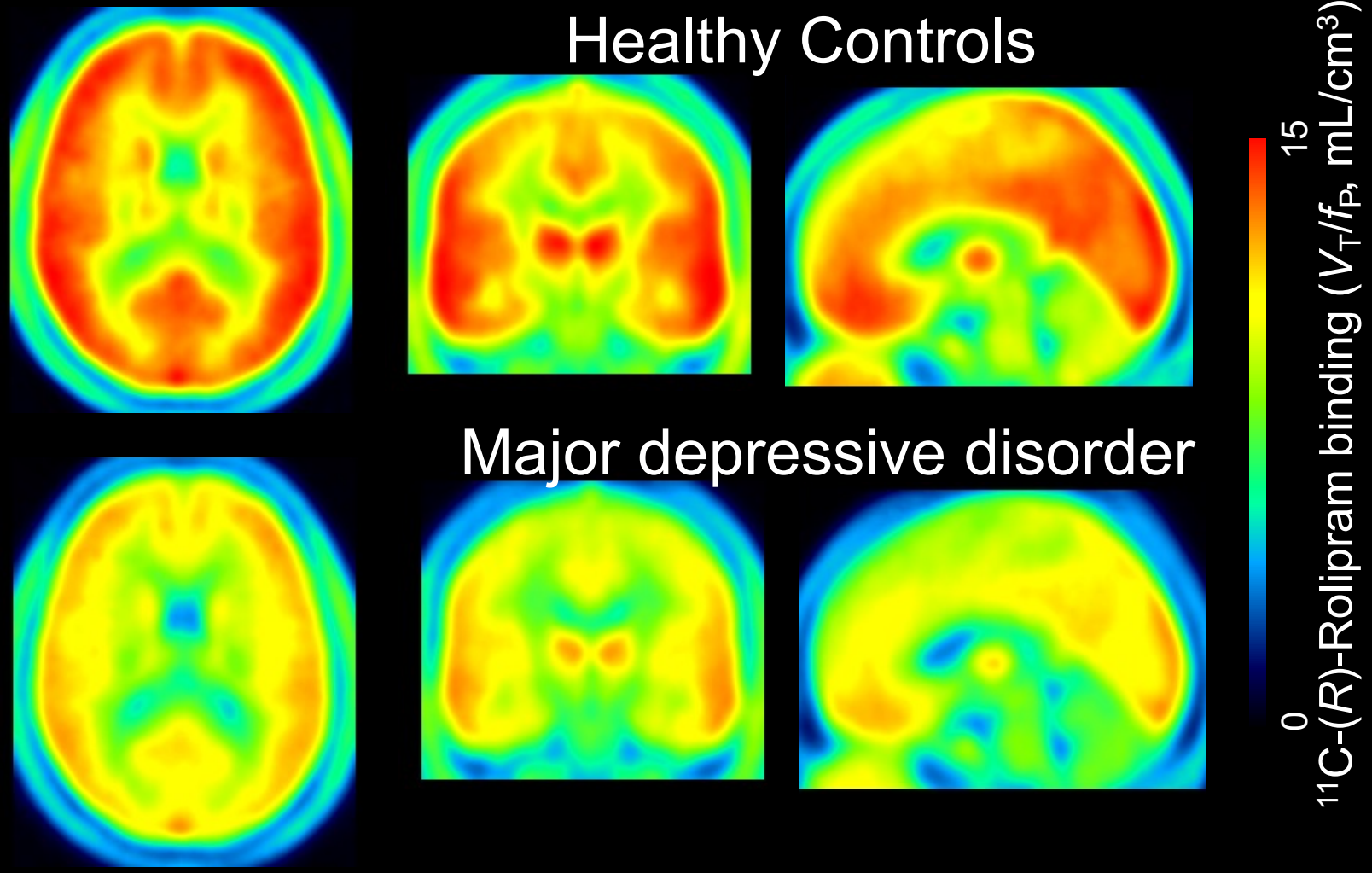
3. Increase in ^{11}C-(*R*)-rolipram binding correlates with symptom improvement.

Clinical Characteristics

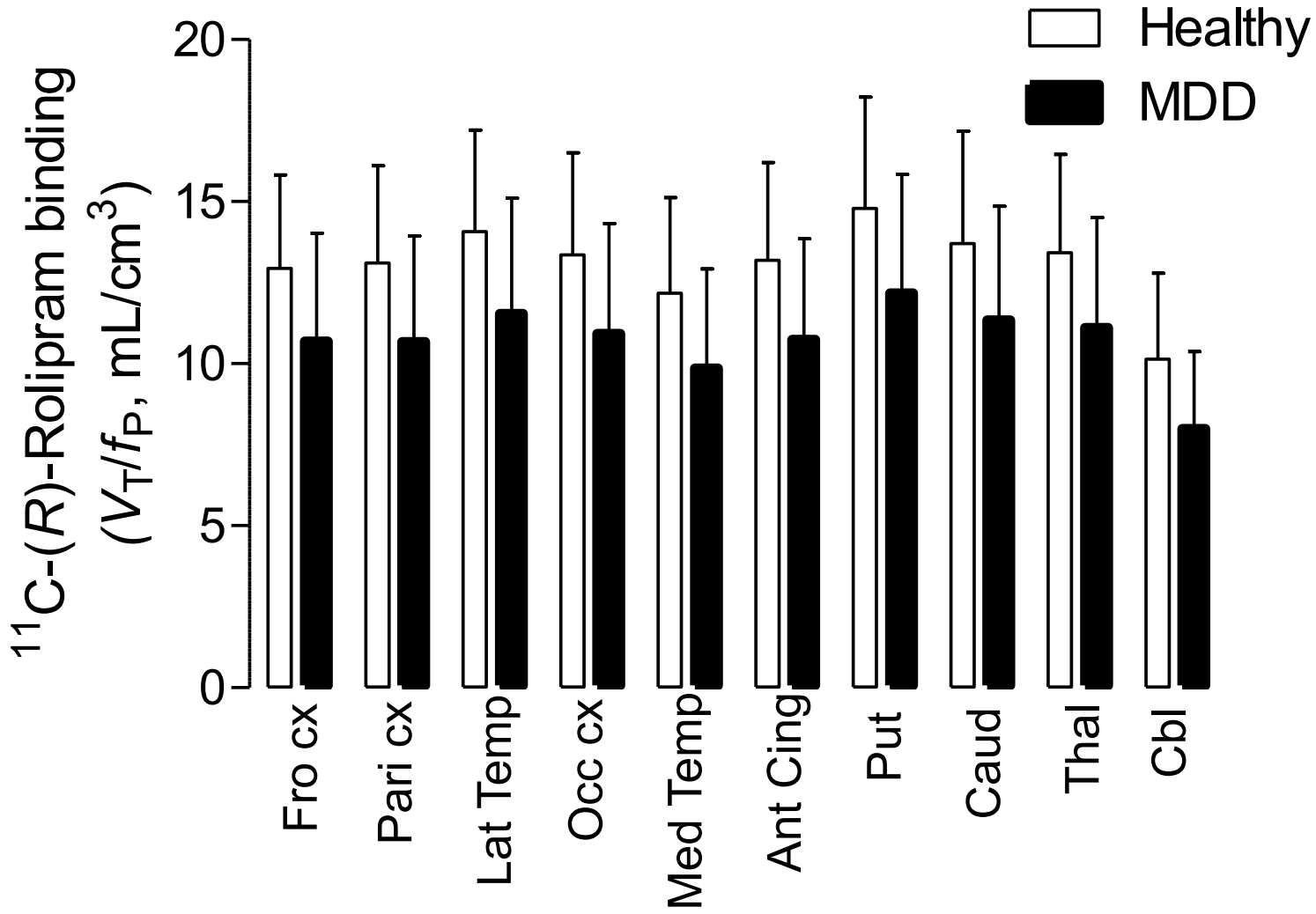
	Control (n = 35)	MDD (n = 44)
Gender (F/M)	11 / 24	12 / 32
Age	36 ± 11	38 ± 11
Baseline depression & anxiety ratings		
Montgomery-Asberg	0.7 ± 1.5	30 ± 6
Hamilton Dep. 17-item	0.7 ± 0.9	20 ± 6
Hamilton Anxiety	0.7 ± 0.9	18 ± 7
Treatment naïve (n)	NA	22 (50%)
Duration of med free (months)	NA	28 ± 37
Current comorbid anxiety disorders	NA	20 (45%)
Cigarette smokers	8 (24%)	10 (22%)

23 patients had two ^{11}C(*R*)-rolipram PET scans, before and after SSRI

Unmedicated patients with major depressive disorder showed global decrease of ^{11}C-(*R*)-rolipram binding



Unmedicated MDD patients had global decrease of ^{11}C-(*R*)-rolipram binding (-18%, p =0.001)



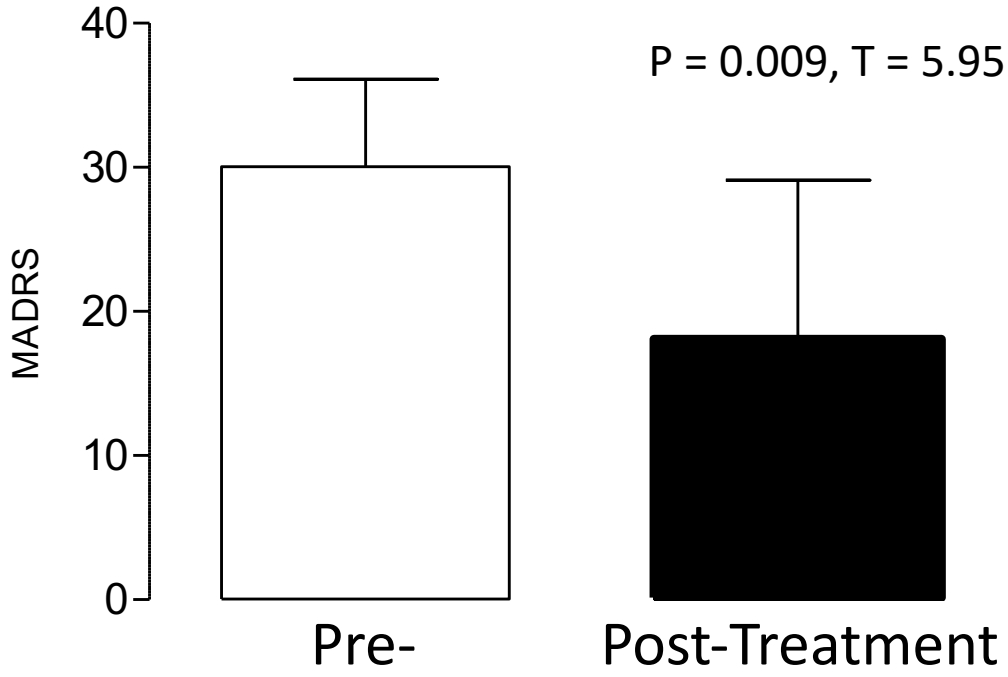
Global effect means decreased cAMP may predispose to, but not be the sole cause of, depression.

- The decrease of ^{11}C-(*R*)-rolipram was global and not region-selective based on both regional and voxel-level analyses.

- 'Two-hit Model': Global decrease may predispose to depression – but must combine with other parameters (e.g., ↓ 5-HT transmission) to affect specific circuits and functions.

Typical SSRI response at two months

- 43% (10/23) responded (>50% decline MADRS)
- 13% (3/23) remitted (MADRS < 10)



SSRI normalized ^{11}C-(*R*)- rolipram binding

Change in ^{11}C-(*R*)-rolipram after ~ 8 weeks SSRI

$$\frac{\text{*Patients (n = 23)} \quad +12 \pm 36\%}{\text{(2nd scan – 1st scan)/(1st scan)}}$$

*p < 0.001 using age as covariate

But no correlation of increased binding with symptom improvement

Summary

- ^{11}C-(*R*)-Rolipram binding in vivo reflects the activity of cAMP cascade in rat and in genetic human disorder..
- Rolipram binding was 18% lower ($p = 0.001$) in unmedicated patients with major depressive disorder ($n = 44$) than in controls ($n = 35$).
- SSRI treatment increased rolipram binding in patients ($n = 23$) by 12% ($p < 0.001$), exceeding retest variability in healthy controls.
  - Increased binding not correlated with symptom improvement.
- Implications:
  - This study goes beyond receptor to second-messenger system, modulated by PKA phosphorylation
  - Confirms cAMP theories of depression and of antidepressant action
  - Suggests MDD can be treated with PDE4 inhibitors

Future Directions: Linking PET to Clinical Trials

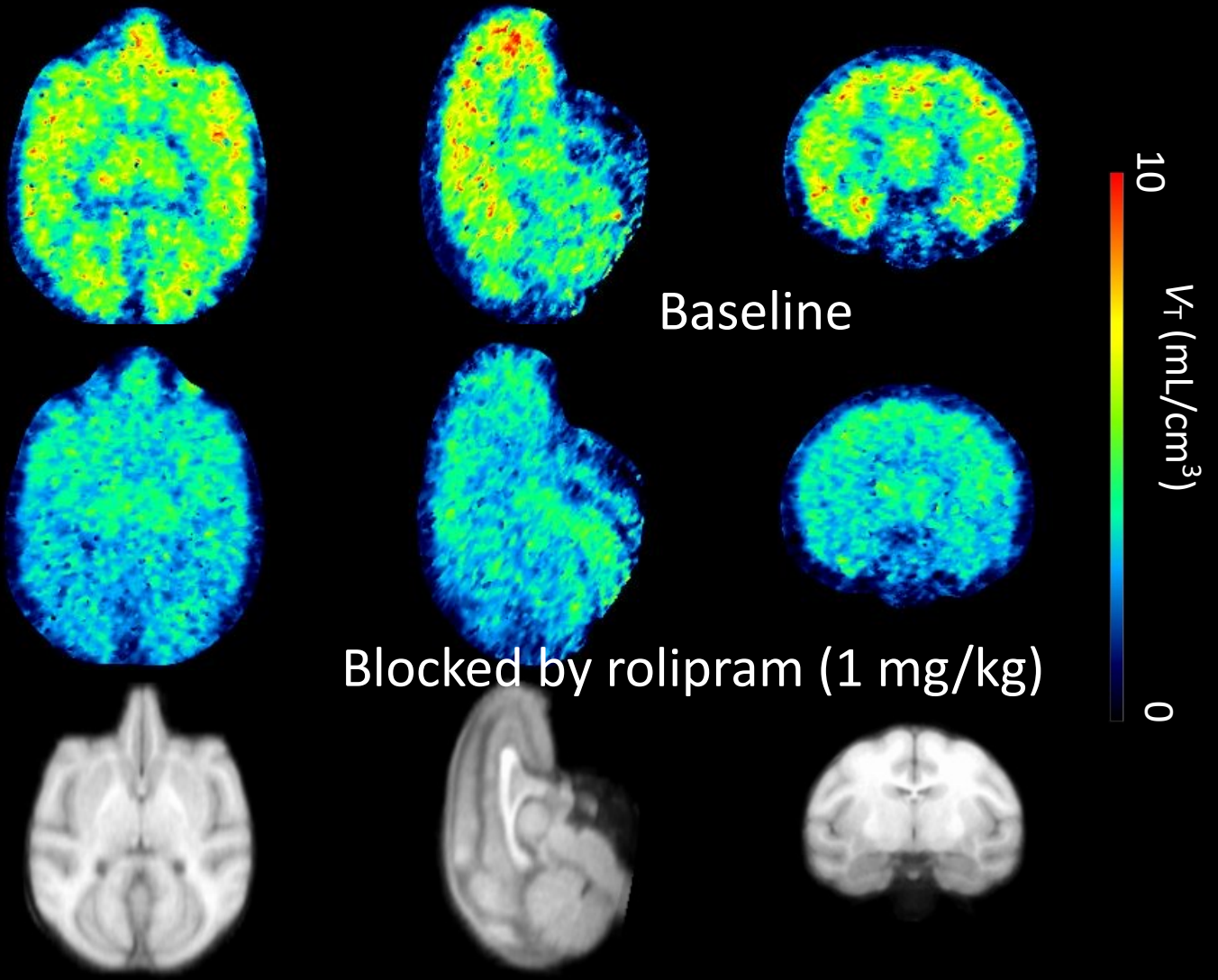
- *Rolipram.* Suggestive results as antidepressant but stopped because of nausea and vomiting. Rolipram inhibits all four subtypes: 4A, 4B, 4C, and 4D

- *Mark Gurney et al.* reported first subtype selective inhibitor: PDE4D in 2010 and PDE4B in 2014

- *Selective PET ligands.* Now developing PET radioligands selective for PDE4B and PDE4D

- *'Personalized / Precision Medicine'.* Determine which patients have low PDE4B activity and then treat with PDE4B inhibitor.

^{11}C-PDE4D selective radioligand in monkey brain



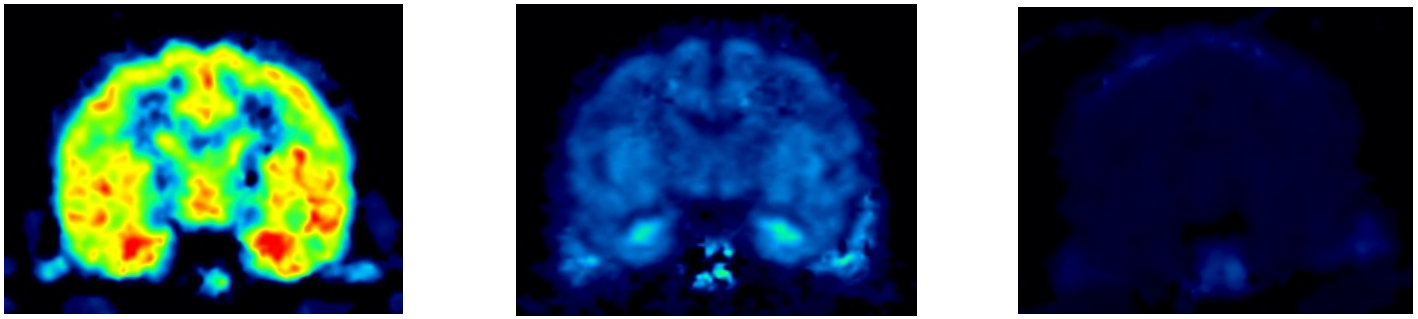
Using PET to Guide Treatment Trials

Patient Stratification: Precision Medicine

PET → High inflammation — High cAMP

PET → Low inflammation — Low cAMP

Drug Delivery to Brain: Target Engagement



No blockade — partial blockade — complete blockade

ACKNOWLEDGEMENTS

- **Victor Pike**: Director of Radiochemistry
- **Drs. Fujita, Zoghbi, and Liow:** Staff Scientists
- **William Kreisl**: Alzheimer's
- **Erica Richards & Carlos Zarate**: MDD
- **Masamichi Ikawa, Masato Kobayashi**: TSPO radioligands
- **Stal Shrestha, Min-Jeong Kim, Mark Eldridge:** COX

Radiochemistry and clinical staff in labs of Pike and Innis