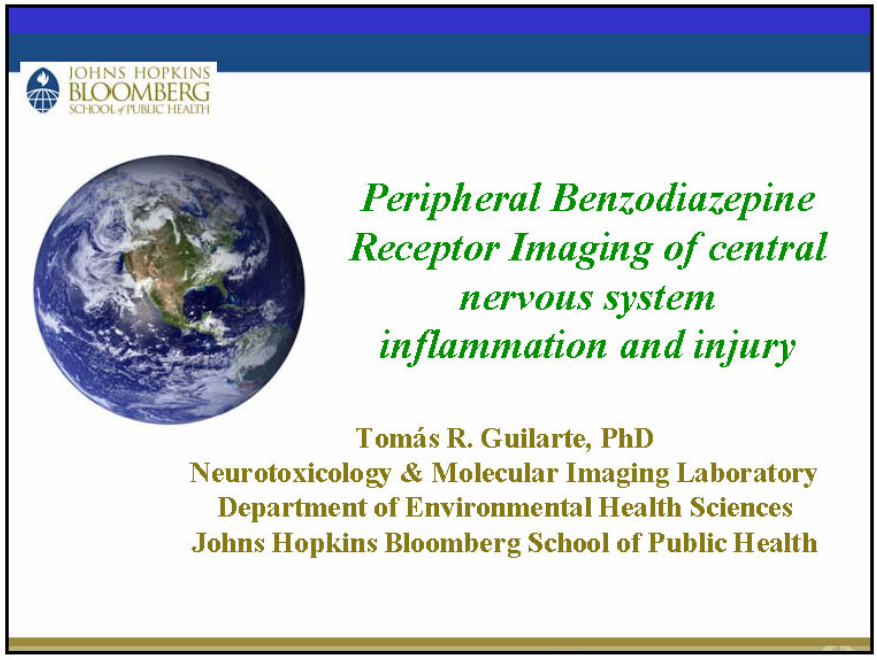
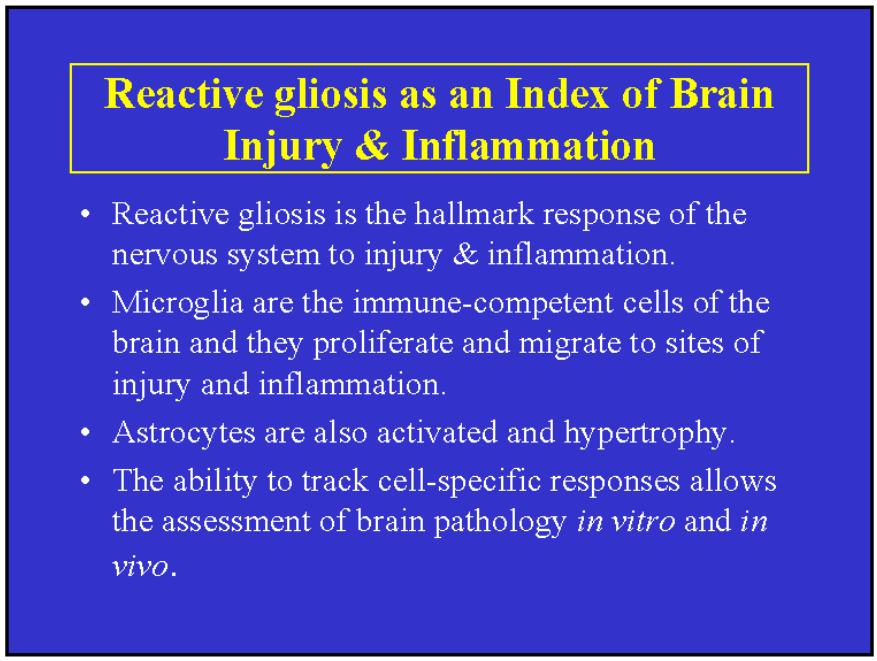
Presentation 8 – Tomás Guilarte

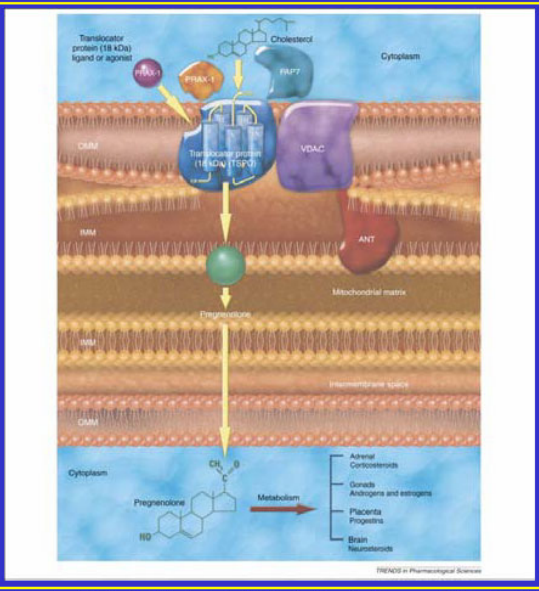




Peripheral Benzodiazepine Receptor

- Peripheral Benzodiazepine Receptor exclusively localized in glial cells.
- Different from the "central" type benzodiazepine receptors:
  - 1) Pharmacology
  - 2) Subcellular distribution (mitochondria)
  - 3) Function (***steroidogenesis***, cell growth & differentiation)

Schematic of the Peripheral Benzodiazepine Receptor



From: Papadopoulos V, Baraldi M, Guilarte TR et al., Trends Pharmacol. Sci. 27: 402-409, 2006

Peripheral Benzodiazepine Receptor

- Very low expression in the brain neuropil with high expression in the ependymal cells of the ventricles and in the choroid plexus.
- Availability of pharmacologically selective, high affinity (nM) radioligand (isoquinoline, PK11195. Now other ligands are available).
- PK11195 can be labeled with positron emission tomography (^{11}C and ^{18}F) and single photon emission computed tomography (^{123}I and ^{125}I) radioisotopes.

Peripheral Benzodiazepine Receptor

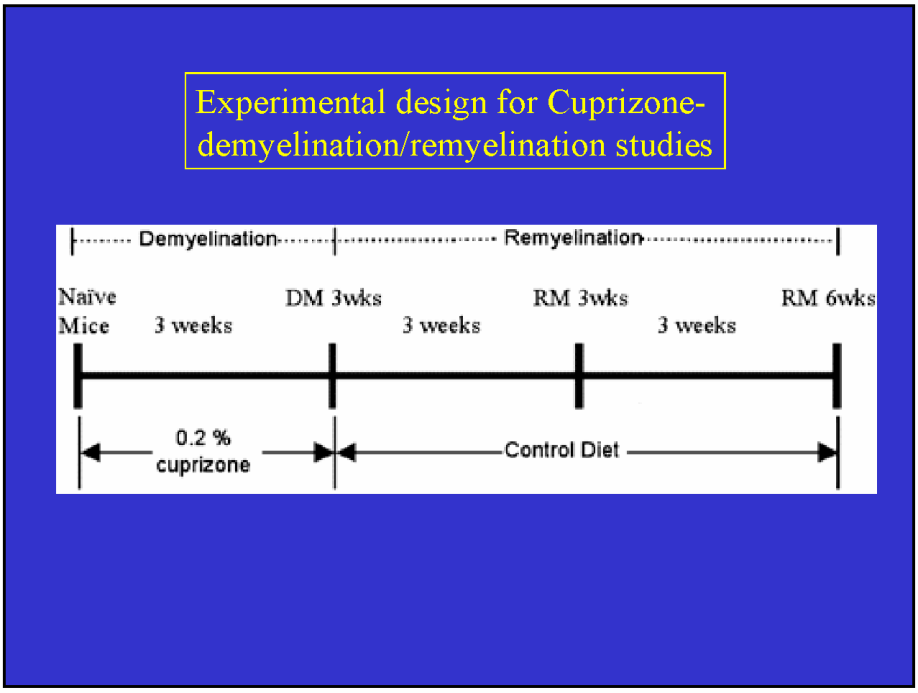
- PBR is the rate limiting step in neurosteroid synthesis. It controls the transport of cholesterol into mitochondria for the synthesis of pregnenolone. This has been demonstrated in glial cells.
- Emerging evidence suggests that activation of PBR may have neuroprotective effects [Ferzaz et al., JPET 301: 1067, 2002; Veiga et al., J. Neurosci. Res. 80: 129, 2005]
- The PBR has been validated as a marker of neuronal injury & inflammation, but its activation may provide an avenue for neuroprotection and recovery from brain injury.

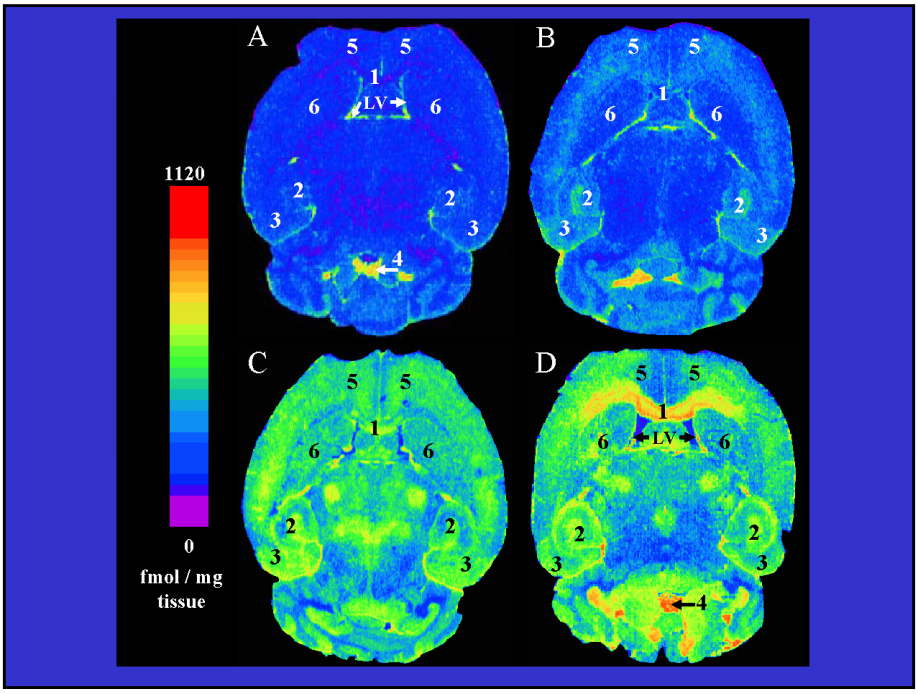
Animal models-PBR validation

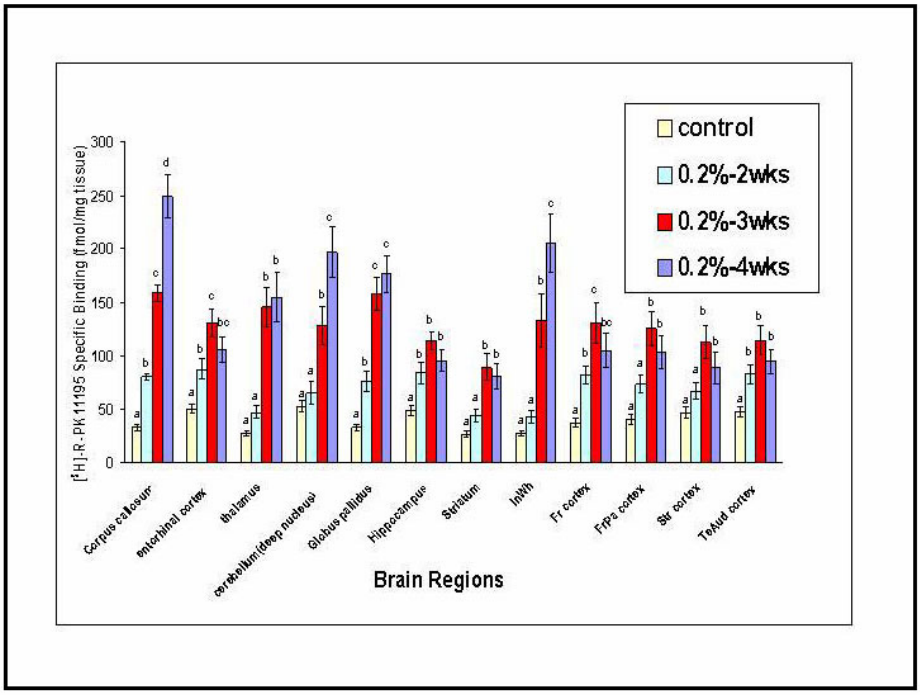
- Trimethyltin (TMT) - Hippocampus (Rat)
- Domoic Acid - Hippocampus (Rat)
- Kainic Acid - Hippocampus (Rat)
- MPTP - Basal ganglia (Mouse)
- Methamphetamine - Basal ganglia (Rat)
- Cuprizone - Demyelination (Mouse)
- Facial Nerve Axotomy (Rat)
- Transient Global Forebrain Ischemia (Rat)
- Traumatic Brain Injury (Rat)
- Simian Immunodeficiency Virus Encephalitis (Rhesus)

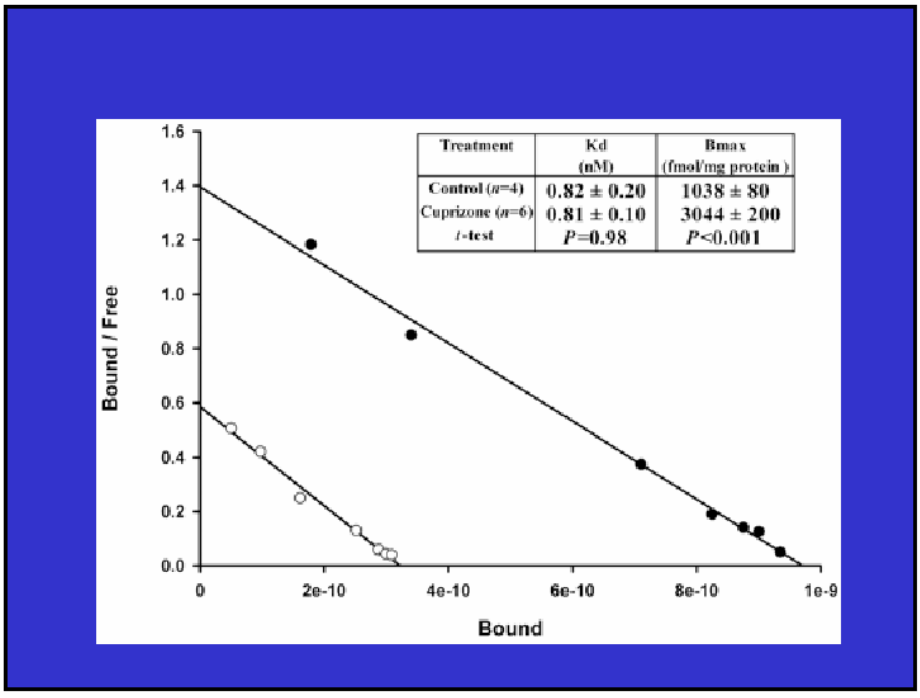
Animal Model of Demyelination
Cuprizone

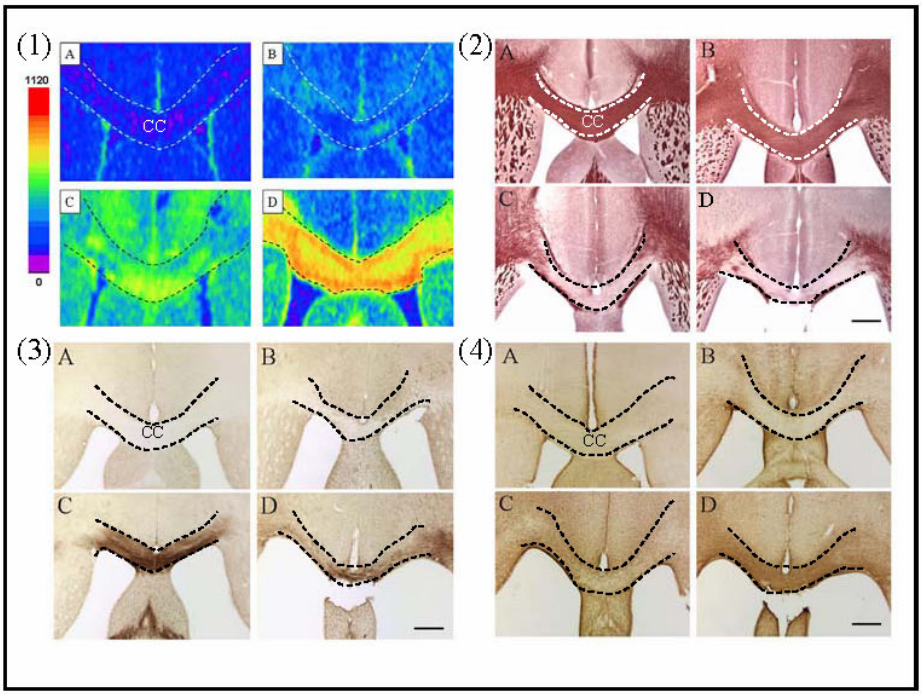
Chen et al., *Brain* 127: 1379-1392, 2004

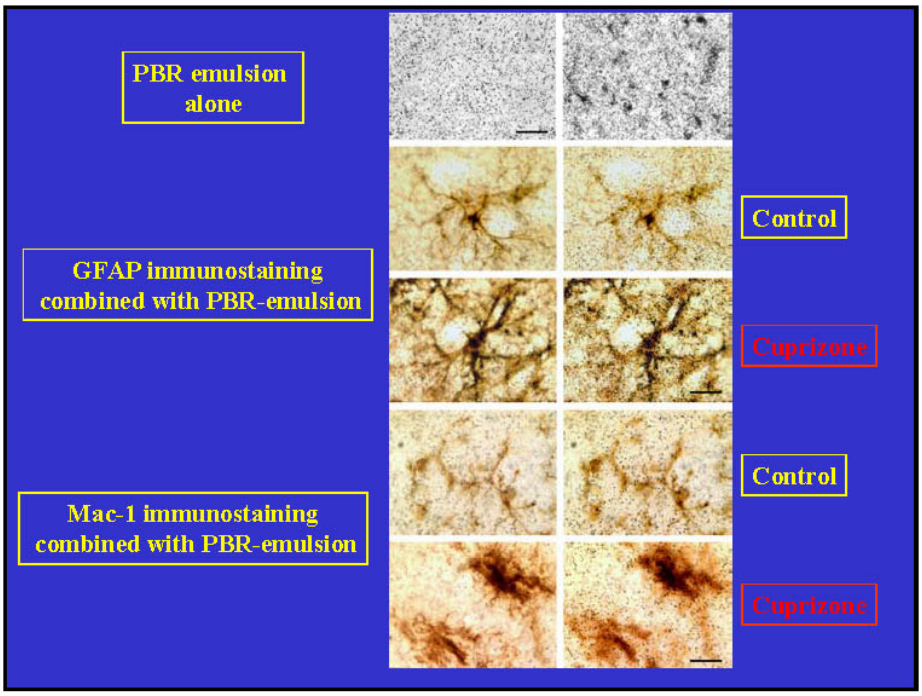

Experimental design for Cuprizone-demyelination/remyelination studies
Demyelination
Remyelination
Naïve Mice
3 weeks
DM 3wks
3 weeks
RM 3wks
3 weeks
RM 6wks
0.2 % cuprizone
Control Diet


A
B
C
D
1120
0
fmol / mg tissue
LV

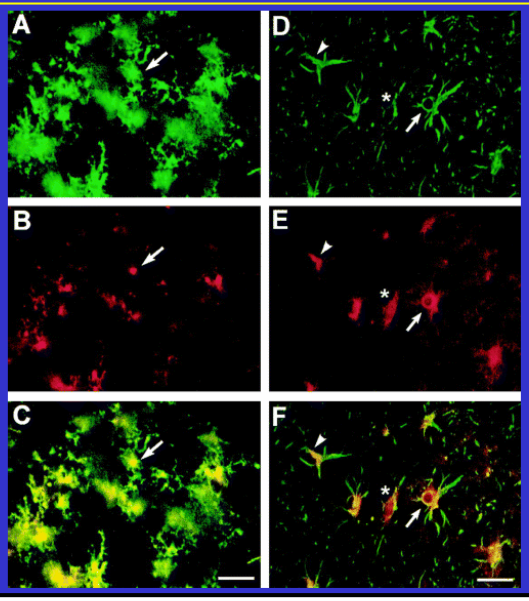

control
0.2%-2wks
0.2%-3wks
0.2%-4wks
[³H]-R-PK 11195 Specific Binding (fmol/mg tissue)
300
250
200
150
100
50
0
Corpus callosum
entorhinal cortex
thalamus
cerebellum(deep nucleus)
Globus pallidus
Hippocampus
Striatum
InWh
Fr cortex
Fr/Pa cortex
Str cortex
Tmd cortex
Brain Regions

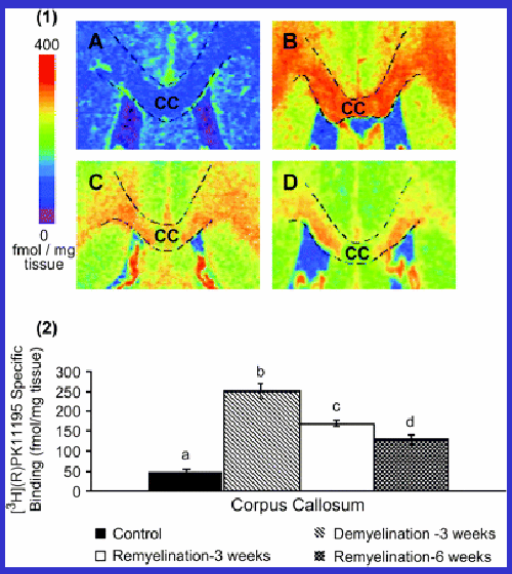

Treatment | Kd (nM) | Bmax (fmol/mg protein)
Control (n=4) | 0.82 ± 0.20 | 1038 ± 80
Cuprizone (n=6) | 0.81 ± 0.10 | 3044 ± 200
t-test | P=0.98 | P<0.001
Bound / Free
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2e-10
4e-10
6e-10
8e-10
1e-9
Bound

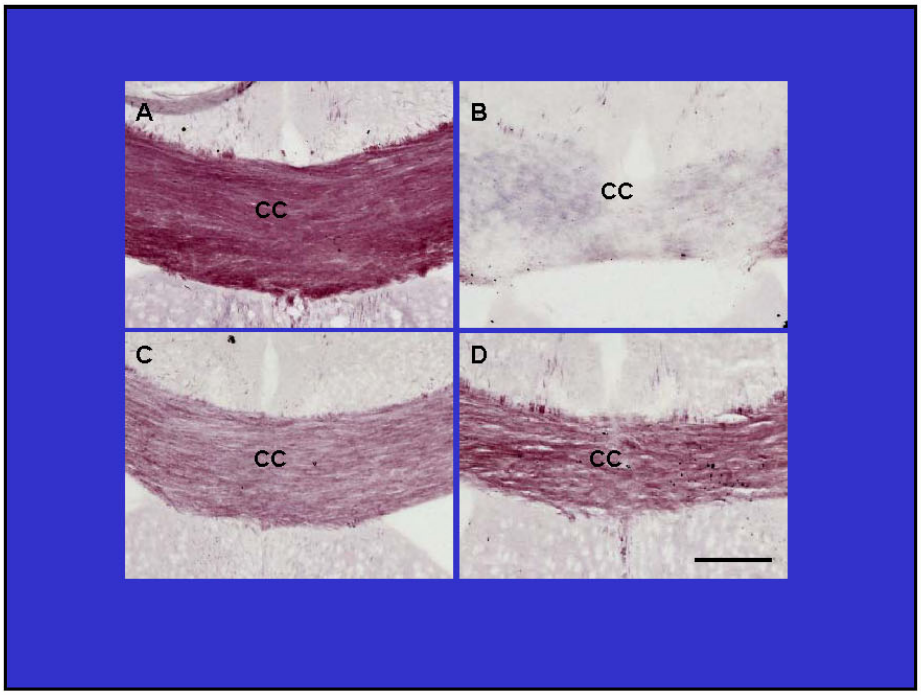

(1)
A
B
C
D
CC
1120
0
(2)
A
B
C
D
CC
(3)
A
B
C
D
CC
(4)
A
B
C
D
CC

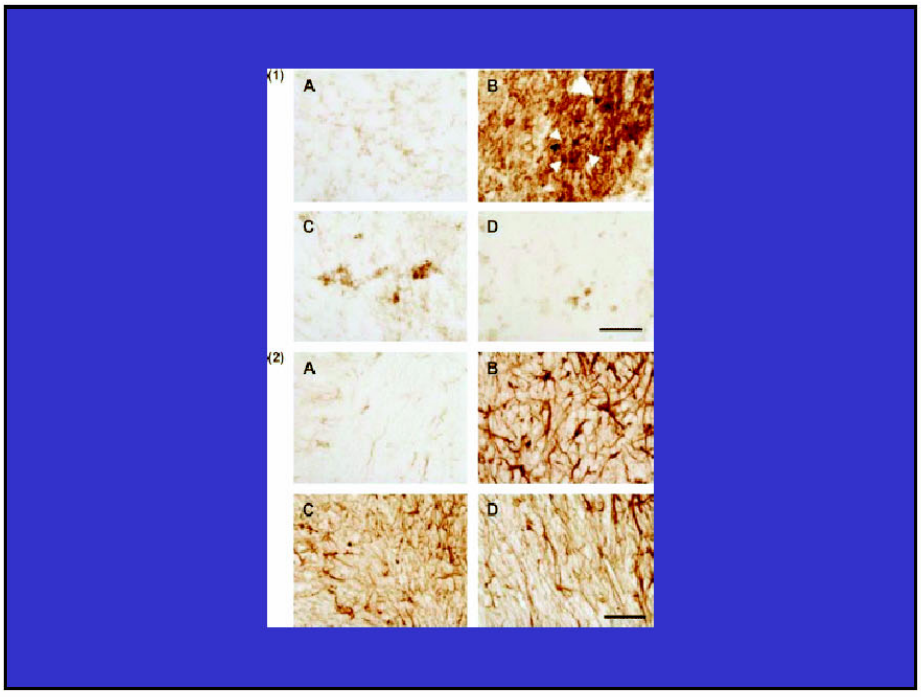

PBR emulsion alone
GFAP immunostaining combined with PBR-emulsion
Mac-1 immunostaining combined with PBR-emulsion
Control
Cuprizone
Control
Cuprizone

Co-localization of PBR protein with OX-42 (microglia) & GFAP (astrocytes)

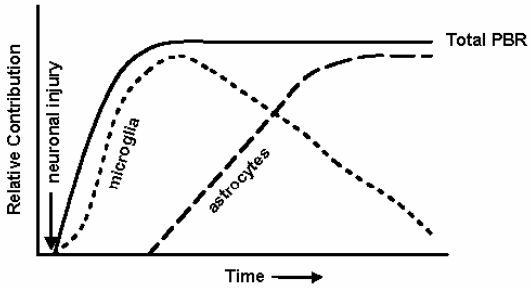




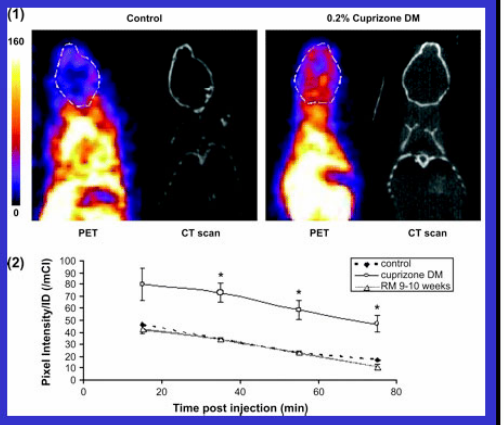

A
CC
B
CC
C
CC
D
CC


(1)
A
B
C
D
(2)
A
B
C
D

Cellular sources of PBR response in the brain following injury



Small animal PET



Experimental paradigm of chronic, low dose MPTP administration as a progressive model of Parkinson's disease in non-human primates

Neurological Assessment

Baseline Imaging/ Neurological Assessment

Chronic MPTP Exposure

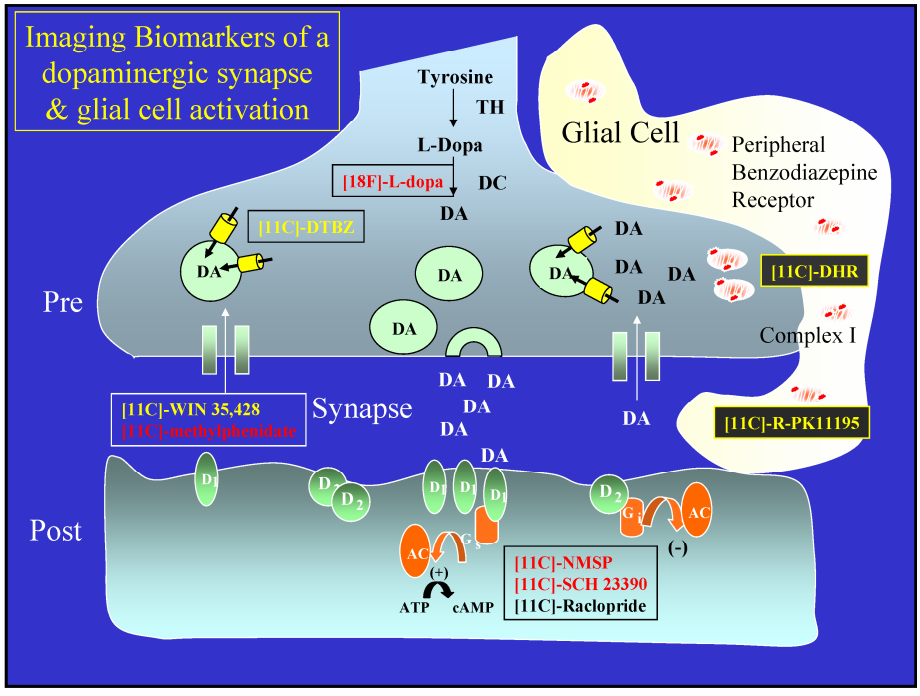
Sequential Imaging Sets

Molecular/ Neuropathology

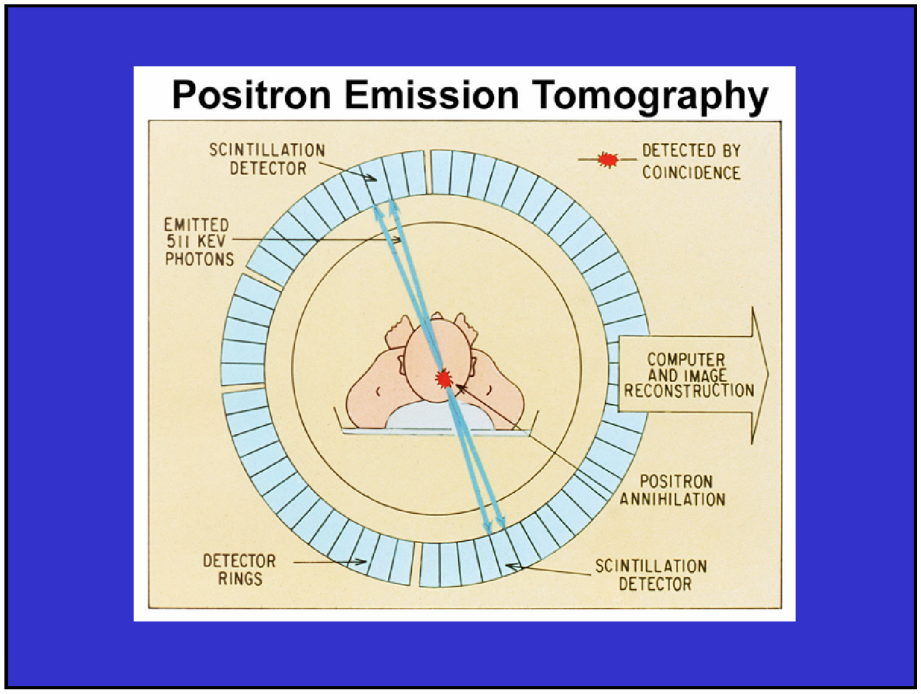
Baseline, 2, 6-7 and 9 months

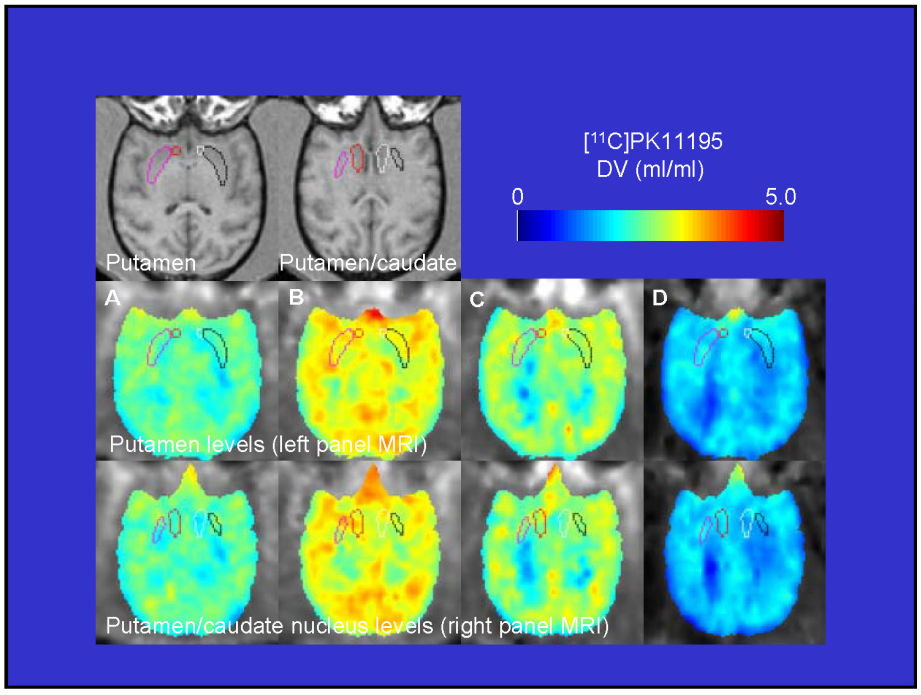
Imaging sets comprise of MRI for anatomical registration and PET studies for dopaminergic neuronal markers and glial cell activation

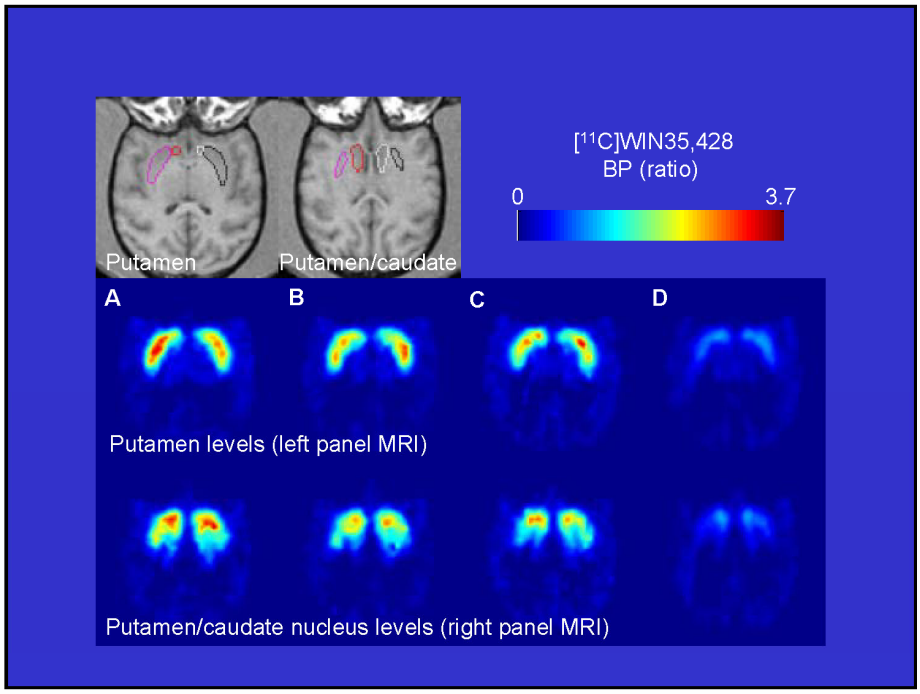
Imaging Biomarkers of a dopaminergic synapse & glial cell activation

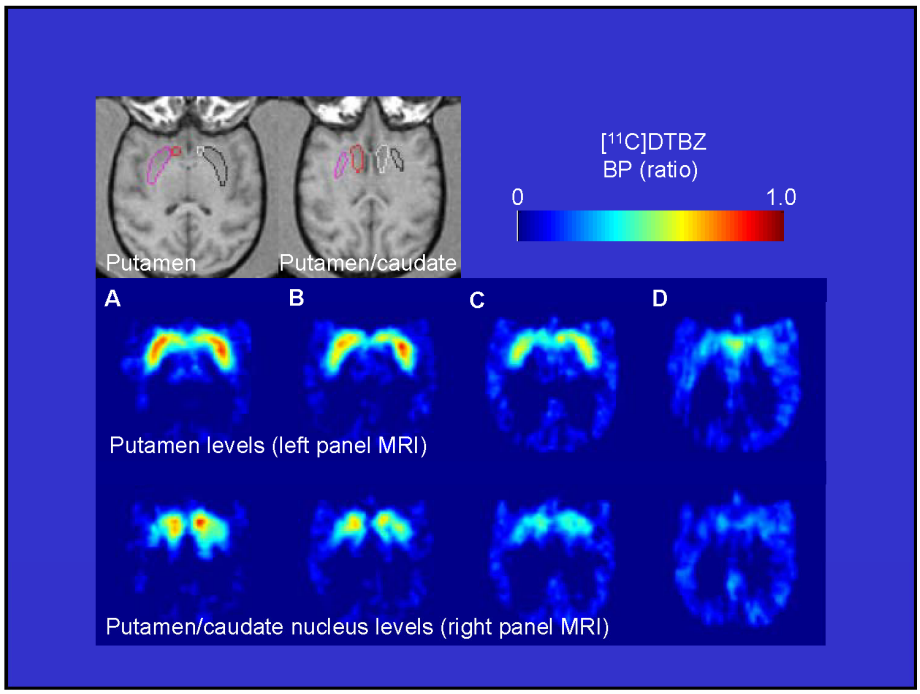



PET Advance


Positron Emission Tomography
SCINTILLATION DETECTOR
DETECTED BY COINCIDENCE
EMITTED 511 KEV PHOTONS
COMPUTER AND IMAGE RECONSTRUCTION
POSITRON ANNIHILATION
DETECTOR RINGS
SCINTILLATION DETECTOR


[11C]PK11195
DV (ml/ml)
0
5.0
Putamen
Putamen/caudate
A
B
C
D
Putamen levels (left panel MRI)
Putamen/caudate nucleus levels (right panel MRI)

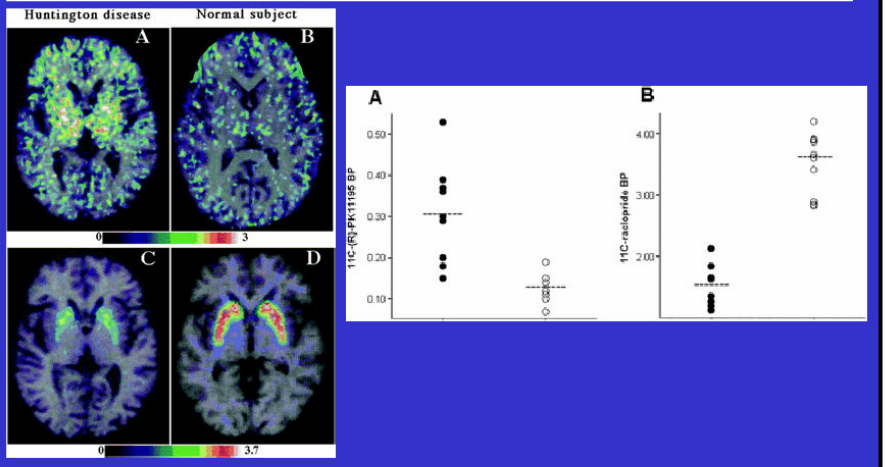

[11C]WIN35,428
BP (ratio)
0
3.7
Putamen
Putamen/caudate
A
B
C
D
Putamen levels (left panel MRI)
Putamen/caudate nucleus levels (right panel MRI)


[11C]DTBZ
BP (ratio)
0
1.0
Putamen
Putamen/caudate
A
B
C
D
Putamen levels (left panel MRI)
Putamen/caudate nucleus levels (right panel MRI)

Microglial activation correlates with severity in Huntington disease: A clinical and PET study

N. Pavese, A. Gerhard, Y. F. Tai, A. K. Ho, F. Turkheimer, R. A. Barker, D. J. Brooks and P. Piccini

Neurology 2006;66;1638-1643
DOI: 10.1212/01.wnl.0000222734.56412.17



Advantages of the Peripheral Benzodiazepine Receptor as a Biomarker of Neuronal Injury & Inflammation

1) Able to detect primary and secondary sites of brain injury with no *a priori* knowledge.

2) Highly sensitive to brain injury from frank neuronal loss to subtle damage to nerve terminals. It is able to detect areas of brain damage prior to commonly used histological methods.

3) Because it uses the principle of receptor autoradiography, it is quantitative.

Advantages of the Peripheral Benzodiazepine Receptor as a Biomarker of Neuronal Injury

4) Availability of selective and high affinity iodinated radioligands allows a high throughput for *in vitro* screening. Further, because of recent advances in small animal imaging instrumentation it can be used in rodent models of CNS disease.

Ongoing Studies using PBR-PET

- Application of PBR imaging and quantification in rodent and non-human primate brain in models of neurodegeneration.
- Application of PBR imaging in human neurodegenerative & inflammatory disease.

Concluding Remarks

- The PBR is a sensitive and early indicator of brain injury and inflammation.
- It can be used to detect primary and secondary sites of injury from physical, chemical, viral or other types of brain insults.
- *In vivo* application to animal and/or human studies is now feasible and it may be useful in the early detection of brain disease, help in our understand of disease progression and to monitor the effectiveness of therapeutic interventions.

Acknowledgements

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