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Microbial translocation is associated with neuroinflammation in HIV-infected subjects on cART

Jaime H Vera , Qi Guo, James Cole, Adriano Boasso, Louise Greathead , Peter Kelleher , Rabiner Ilan, Courtney Bishop, Paul Matthews, Roger Gunn, Alan Winston

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Risk factors associated with HIV-associated NCI

Pathogenesis	Likely / unlikely	Clinical risk factor
Inadequate exposure of cART	unlikely	• Antiretroviral therapy
Antiretroviral toxicity	maybe	• Antiretroviral therapy
Accelerated brain ageing	maybe	• Age
Co-morbidities	likely	• Cardiovascular • HCV • Lifestyle • Other infections: CMV ,STS • Yet unidentified
Immune restoration	maybe	• Nadir CD4 count
Persistent immune activation	likely	• Treating too late? • Nadir CD4 count

Winston A, Vera JH, 2013, ERF

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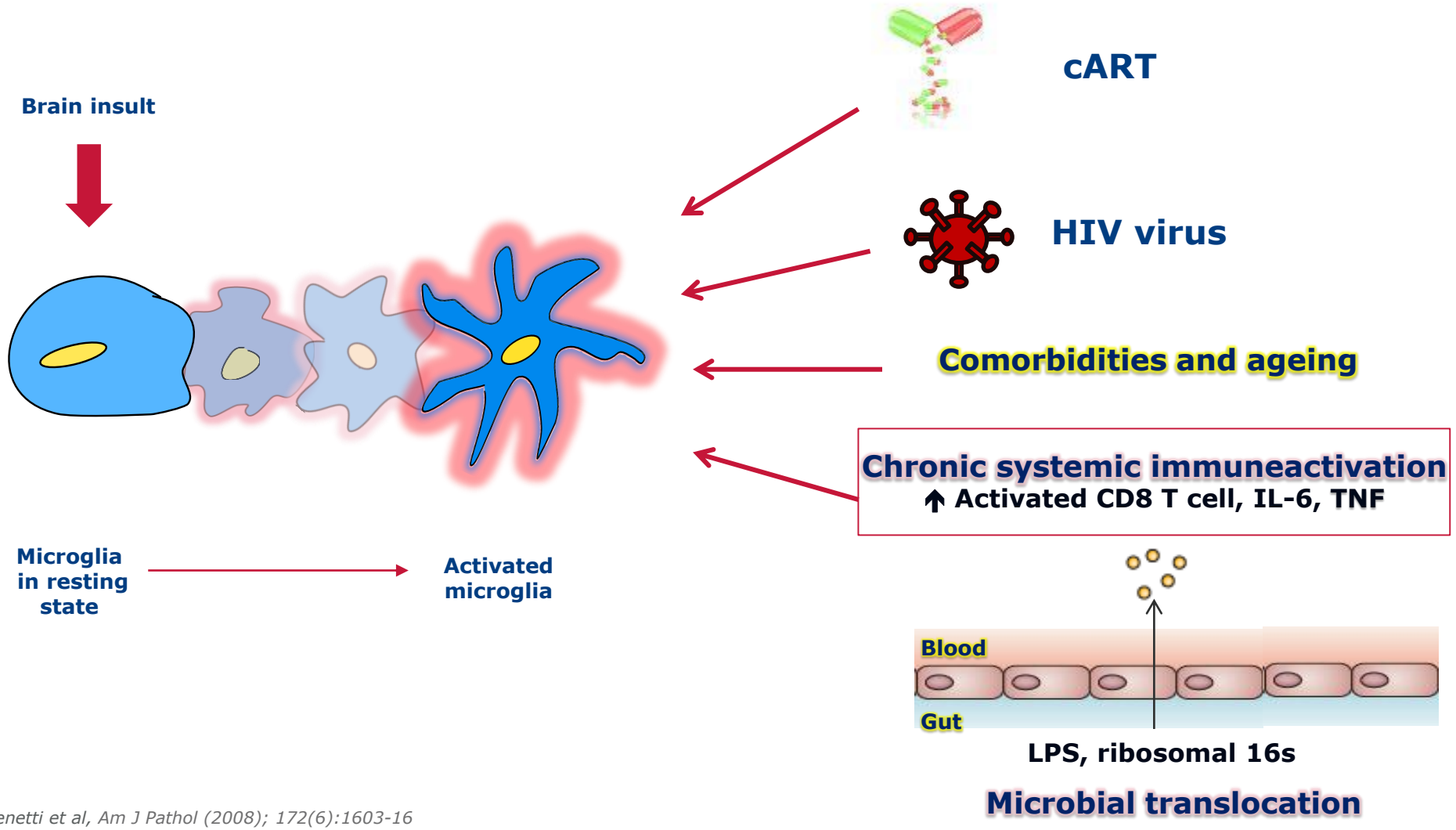
Microglial activation and immuneactivation



Venetti et al, Am J Pathol (2008); 172(6):1603-16
Owen et al, J Cerbral blood and Meta; (2012);32(1):1-5.

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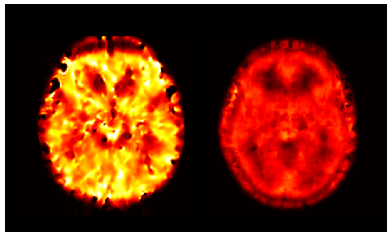
In vivo imaging of microglial activation: TSPO PET

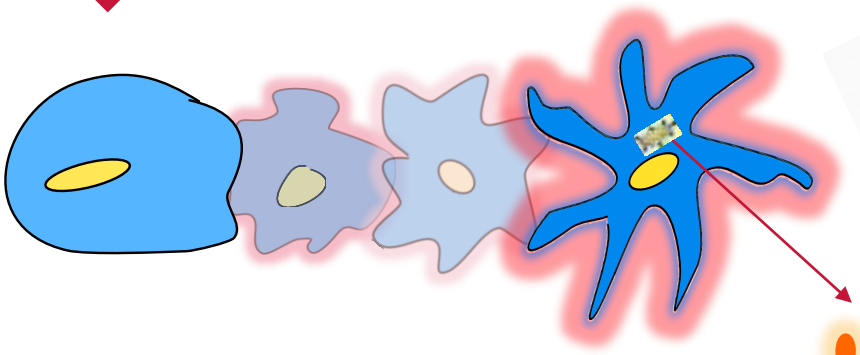
Brain insult

TSPO ligands
[^{11}C] PBR28

In vivo: PET-CT

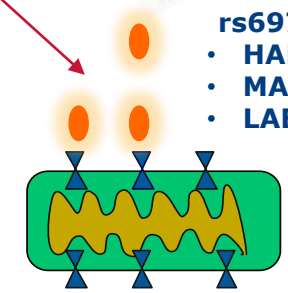


V_T



rs6971
- HAB
- MAB
- LAB

Microglia in resting state → Activated microglia =



TSPO

Increase expression of traslocator protein 18Kda

- MRI imaging biomarkers: MRS, DTI, fMRI
- Inflammatory biomarkers
- Neurocognitive parameters

Venetti et al, Am J Pathol (2008); 172(6):1603-16
Owen et al, J Cerebral blood and Meta; (2012);32(1):1-5.

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Aims of Study

- To determine the presence of *in vivo* microglial activation using [^{11}C] PBR28 PET CT in neuroasymptomatic HIV-infected individuals on effective cART

- To investigate the relationship between microglial activation and:
  - ✓HIV clinical parameters
  - ✓Cognitive performance
  - ✓MRI biomarkers: white matter integrity using diffusion tensor imaging (DTI)

- To examine the association between microglial activation and markers of CNS (cerebral chemokines) and peripheral immuneactivation (microbial translocation)

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Methods

Cross-sectional study conducted at Imperial College London between 2012 and 2014

Inclusion criteria
• Male adults between 20 to 50 years of age
• Chronic HIV infection
• On cART with a VL<50copies/mL
Exclusion criteria
• Any known or active neurological or psychiatric disease
• Current use of benzodiazepines or anti-inflammatories
• Use of recreational drugs or alcohol abuse (>6 units per week)
• Low affinity to TSPO on genotype testing

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Methods

Study procedures undertaken

1. Cerebral MRI scan
2. Diffusion tensor imaging MRI scan
3. PET scan with [^{11}C] PBR28
4. Plasma samples for analysis of ribosomal 16sDNA
5. Cerebrospinal fluid for analysis of chemokines
6. Computarised neurocognitive test (CogState^{TM})

Statistical analysis

- Differences in [^{11}C] PBR28 binding between HIV-infected patients and controls were determined on **parametric level** (whole brain) and **region of interest** (specific brain regions) using ANOVA adjusted for TSPO affinity status.

- Associations between clinical, imaging, cognitive and immunological parameters and PBR28 binding were calculated using correlation analysis adjusted for TSPO affinity status.

Demographic / clinical parameter Median range unless stated	HIV cases (n=12)	Controls (n=10)	CogState aged matched population data (n=873)
Male gender, n (%)	12 (100)	10	
Age, years	43 (26-49)	44 (28-52)	
White ethnicity, n (%)	10 (83.3)	10 (100)	
Education years	12 (2.5)		
Current CD4/CD8 ratio	0.7 (0.2-0.9)		
Current CD4 cell count (cells/uL)	645 (350-1240)		
Nadir CD4 cell count (cells/uL)	190 (70-350)		
CSF HIV RNA level<50 copies/mL, n(%)	9 (90)		
Pre-treatment HIV RNA (copies/mL)	5.0 (2.1- 6.2)		
Years since HIV diagnosis	15 (2-19)		
Antiretroviral therapy, n (%)	12 (100)		
PI based	4 (33)		
NNRTI based	8 (77)		
Cognitive assessment, mean (SD)			
Speed an visual attention	2.49(0.08)		2.49(0.09)
Executive function	52.2(13.02)		55.3(22.4)
Accuracy and visual learning	1.06(0.04)		1.01 (0.02)

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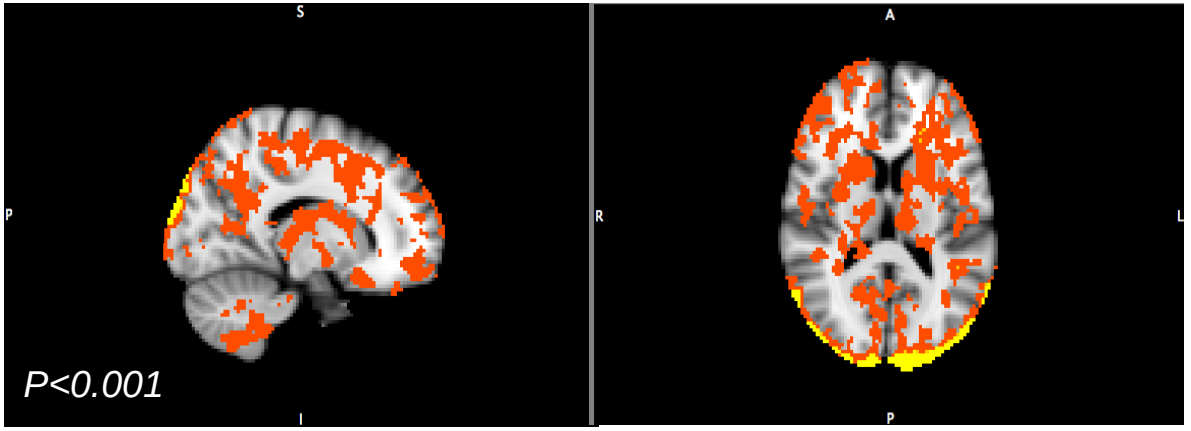
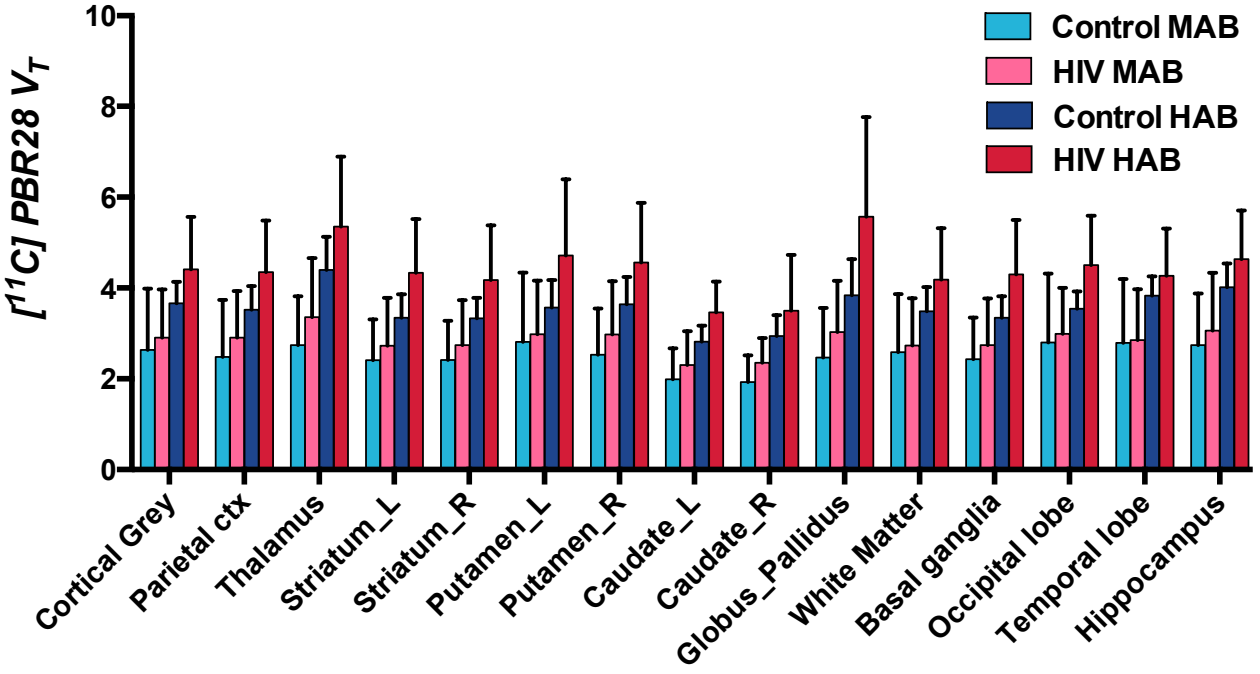
Results- Microglial activation HIV vs Controls

Parametric analysis



Region of interest



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Results- Microglial activation and HIV clinical parameters

Brain region	CD4/CD8 ratio	Pre-treatment HIV RNA	Visual learning and memory (higher scores=better performance)
	Correlation coefficient (r)		
Occipital lobe	0.175	**0.684***	-0.315
Hippocampus	**-0.596***	-0.003	-0.221
Parietal lobe	0.100	**0.662***	**-0.580***
Amygdala_L	**-0.759***	0.259	-0.284
Thalamus_L	**-0.525***	-0.214	-0.259
Basal ganglia	**-0.555***	-0.080	**-0.660***
Striatum	0.333	**0.604***	-0.333
Midbrain	**-0.732***	-0.062	-0.059
Medulla	**-0.740***	0.263	0.115

**P<0.05, after adjusting for TSPO status*

No correlations between PBR28 binding in any brain region and age, nadir CD4 count, time since HIV diagnosis or type of ART (P=>0.1, all observations)

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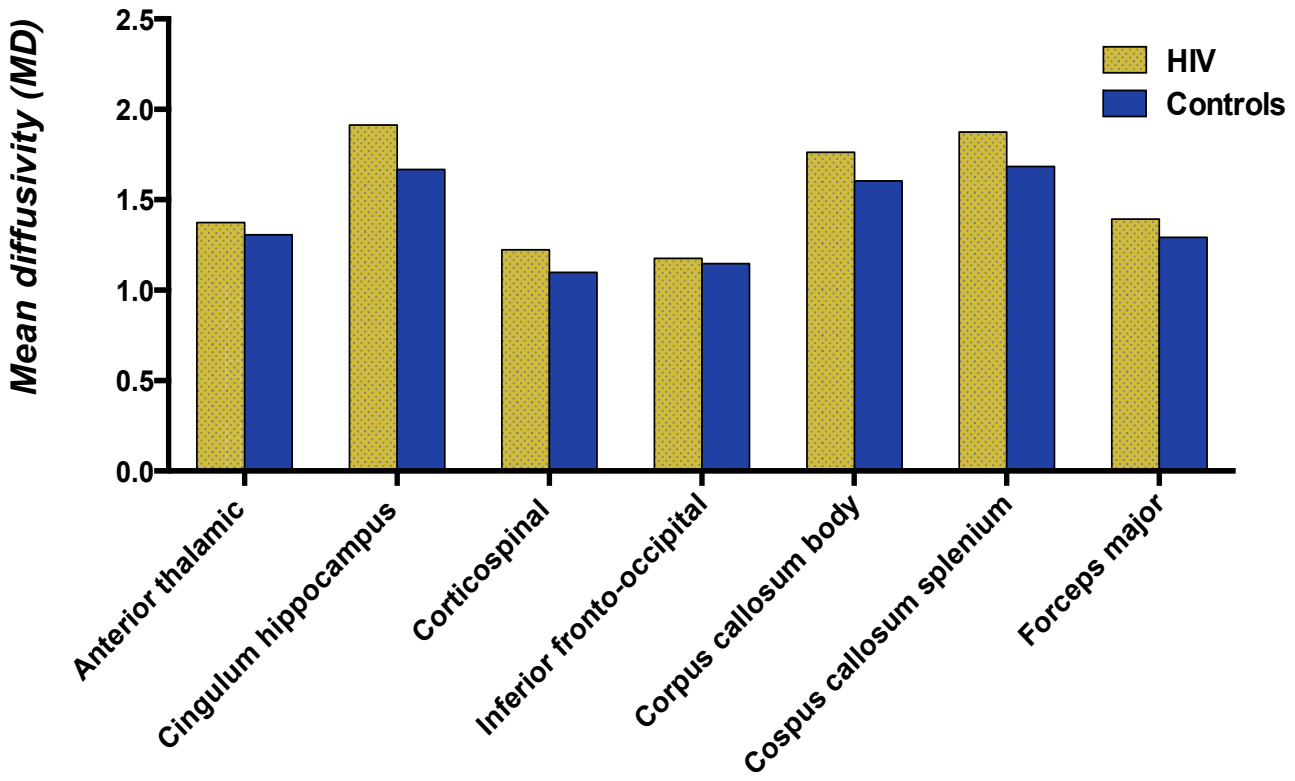
Results- White matter integrity HIV vs Controls



Significant alterations in white matter integrity (greater MD) in HIV-infected cases vs controls, (P=<0.05, all observations)

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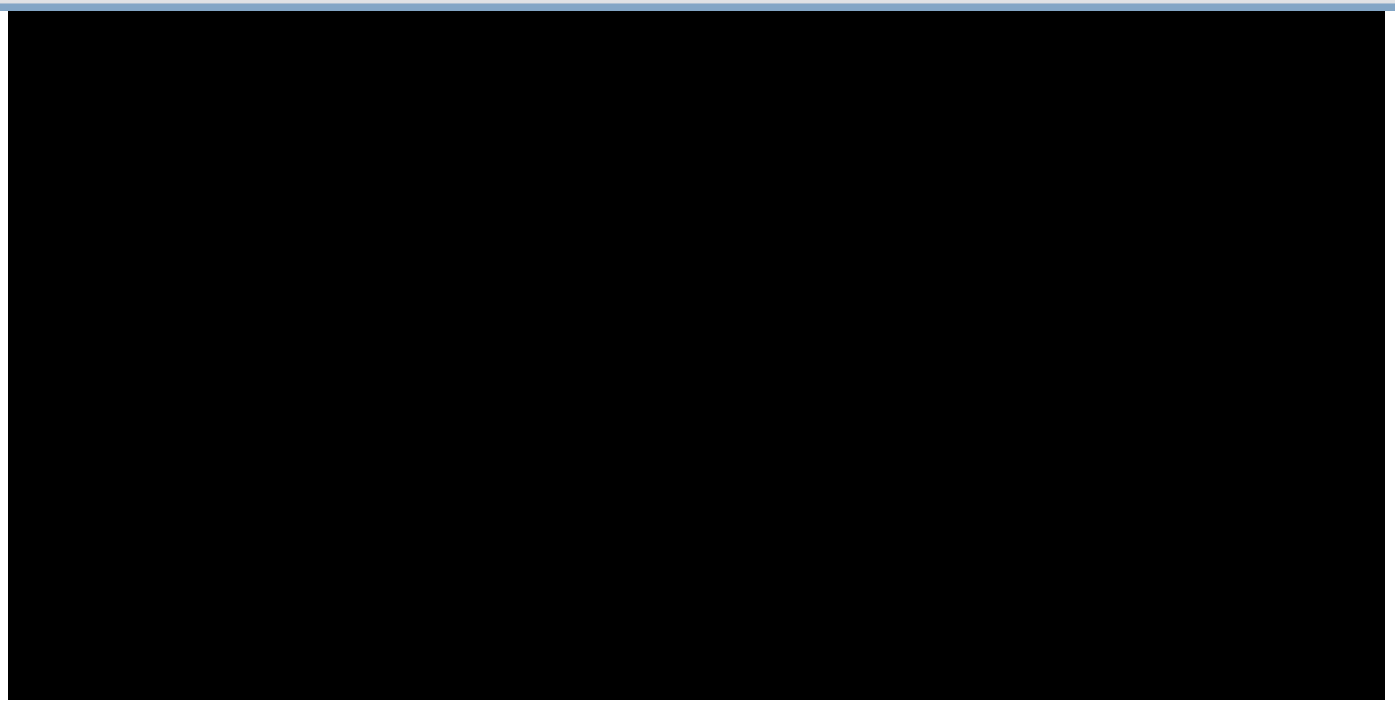
Results- microglial activation and white matter integrity



Association between PBR28 binding in the basal ganglia, globus pallidus, temporal and occipital lobe and greater mean diffusivity (MD):

- ***Forceps minor***
- ***Right inferior longitudinal fasciculus***
- ***Right inferior fronto-occipital fasciculus***

(P=<0.001 all observations)

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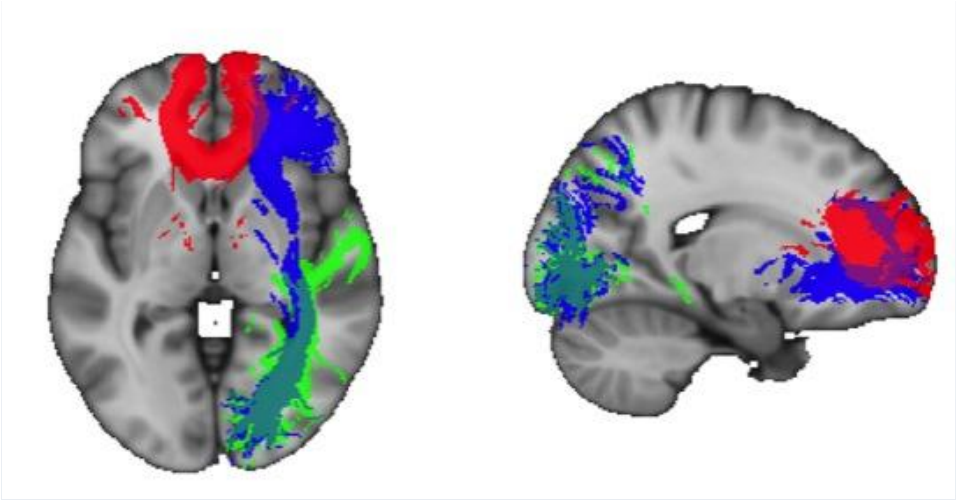
Results-microglial activation and microbial translocation

- **Association between PBR28 binding and plasma ribosomal 16s**

Brain regions with increased PBR28 binding	Correlation coefficient (r)
Basal ganglia	0.881
Globus pallidus	0.928
Temporal lobe	0.818
Parietal lobe	0.832
Occipital lobe	0.861
Caudate	0.877
Striatum	0.871

(P=<0.001 all observations)

- **Plasma ribosomal 16s is associated with increase CSF IL-8 concentration (r=0.599, p=0.024)**



- **Plasma ribosomal 16s and CSF IL-8 are associated with increased MD**
  (P=<0.005 all observations):
  - **Forceps minor**
  - **Right inferior longitudinal fasciculus**
  - **Right inferior fronto-occipital fasciculus**

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Conclusions

- Neuroinflammation was present neuroasymptomatic HIV-infected individuals on effective cART
- Neuroinflammation was associated with poor performance in cognitive tests evaluating verbal and visual memory and abnormalities of white matter integrity
- Neuroinflammation was associated with markers of systemic immune activation
  - Lower CD4/CD8 ratio
  - High-pre ART HIV RNA
  - Increase ribosomal 16S DNA (microbial translocation marker)
- We postulate that microbial translocation might be one of the factors associated with neuroinflammation in treated HIV-infected individuals

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