LPS-induced neuroinflammation - *in vitro* and *in vivo* assays for development of new treatment strategies

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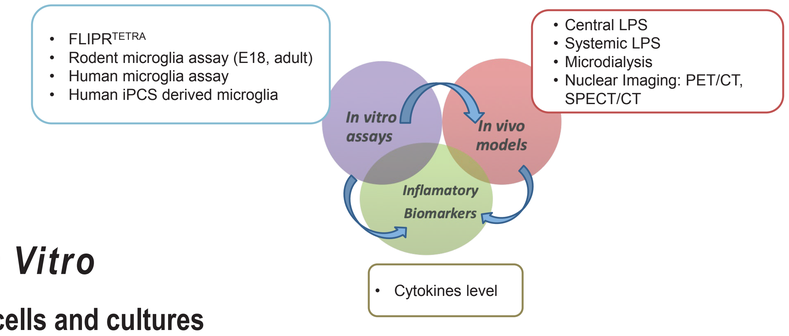
1 Introduction

Neuroinflammation is linked to the progression of neurodegenerative disorders. Activated inflammatory cells of the myeloid lineage, in particular microglia, play a key role in the pathogenesis of these chronic disorders. Activated microglial cells enhance the production of inflammatory cytokines and chemokines like interleukin-1β, interleukin-6, tumor necrosis factor α and make microglia more susceptible to secondary stimuli, promoting microglial activation.

In the described assays a common used bacterial endotoxin (lipopolysaccharide, LPS) is used as a proinflammatory stimulus for microglia cells. The combination of existing *in vitro* and *in vivo* assays gives us the ability to delineate the underlying molecular mechanisms of neuroinflammation, thus reinforcing the development of new treatment strategies and biomarkers for neurodegenerative disorders beyond the existing conventional approaches.

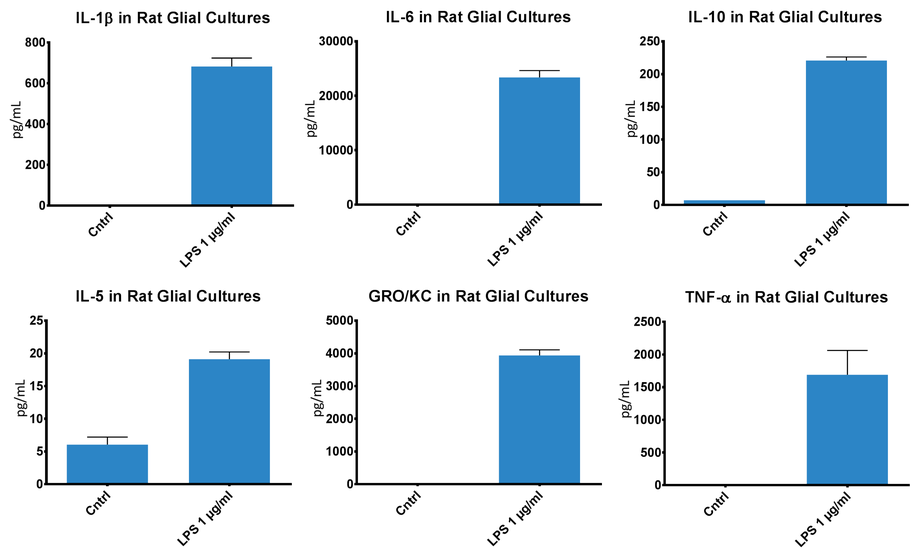


2 *In Vitro*

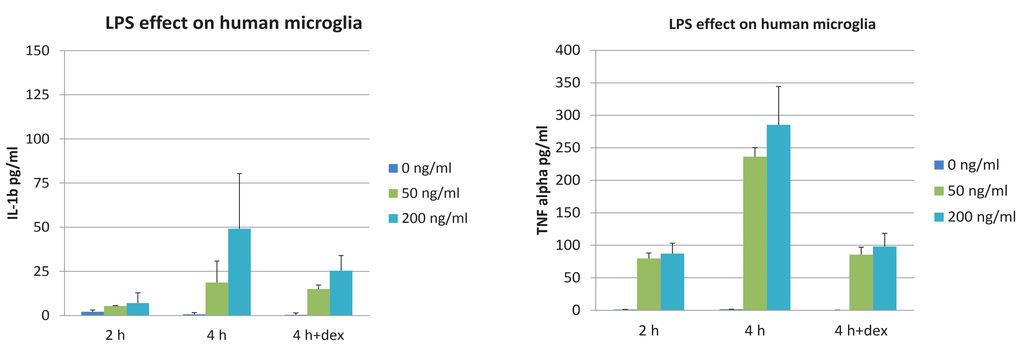
Microglia cells and cultures

Cortical glial or pure microglial cultures

- Bacterial lipopolysaccharide (LPS) induces inflammatory reactions in glial cells, 24 h exposure time
- Measurement of inflammatory cytokines from culture medium or cell lysate using ELISA or multiplexing with Magpix



- LPS-induced cytokine release in enriched human microglia cultures

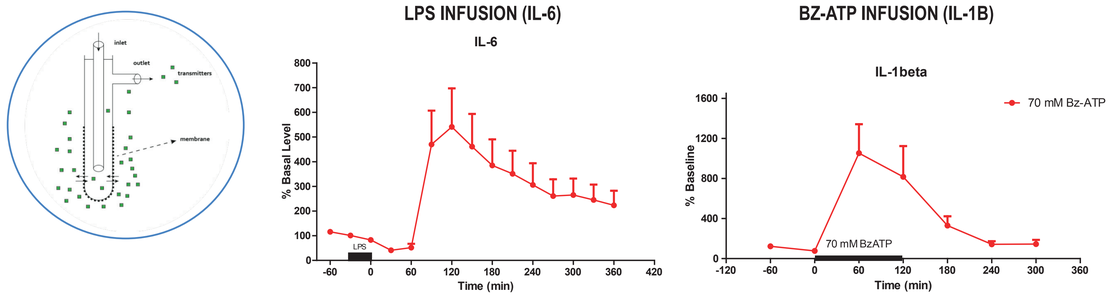


LPS-induced cytokine release and inhibition by dexametahsone

3 *In Vivo*

Microdialysis

- Microdialysis is a catheter-based technique to sample analytes from the interstitial fluid of 'any' target tissue.
- It provides the opportunity to measure endogenous compounds or the unbound concentration of exogenous compounds.



Nuclear Imaging

Dynamic PET imaging of radioligands without suitable reference region in brain requires metabolite corrected arterial input function (AIF) for quantification. For example, activation of TSPO is uniformly expressed in brain and hence reference tissue modelling is not applicable. Traditional blood sampling techniques for full AIF are not feasible due to limited blood volume of rodents.

LPS is a known potent immunostimulant, which was used to induce acute local neuroinflammation in rats. LPS was infused in the right striatum and rats were scanned with TSPO ligand ^{18}F-FEPPA. To obtain blood input function, the tail artery and vein were cannulated, connected to a coincidence counter and a peristaltic pump to maintain constant flow rate. Animals were scanned for 90 minutes and samples for plasma and parent fraction were collected during the scan (Figures 3 and 4).

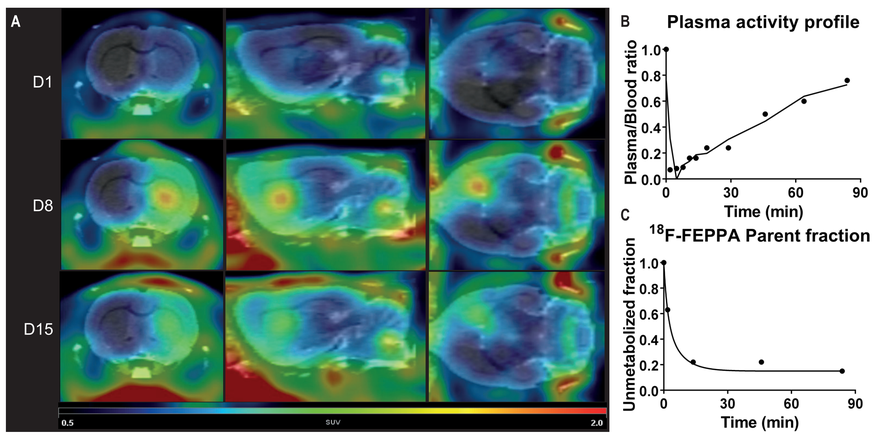


Figure 3. PET images 1, 8 and 15 days post LPS infusion with ^{18}F-FEPPA (A). Metabolite corrected AIF was generated for each scan. Higher tracer accumulation was seen to the injection site. Radioactivity distribution between whole blood and plasma during the PET scan (B). Fraction of unmetabolized ^{18}F-FEPPA in plasma during the PET scan (C). Plasma and parent fraction curves are required for quantitative PET analysis of tracer without reference region.

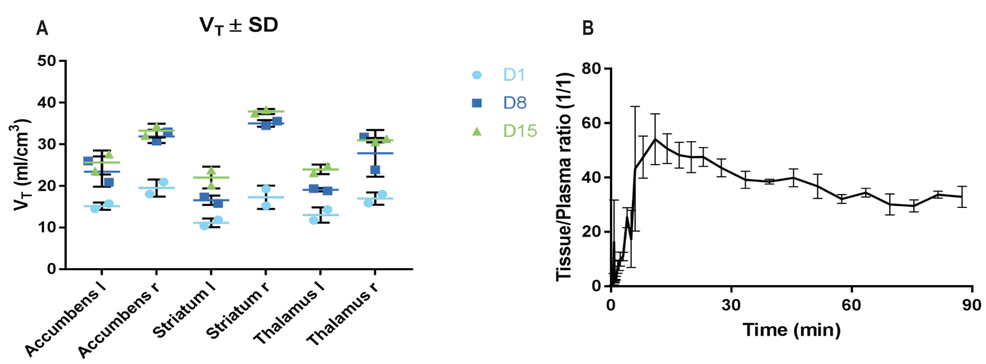


Figure 4. V_T-values of ^{18}F-FEPPA in nucleus accumbens, striatum and thalamus of both hemispheres. Highest values were seen in right striatum. V_T is low on D1, increased thereafter and remained elevated between D8 and D15. Individual data points are plotted in the graphs with mean ±SD (A). Tissue/Input curve ratio from right striatum shows equilibration of activity in tissue and plasma (B).