Novel therapeutic strategies in maternal inflammation induced brain injury and cerebral palsy

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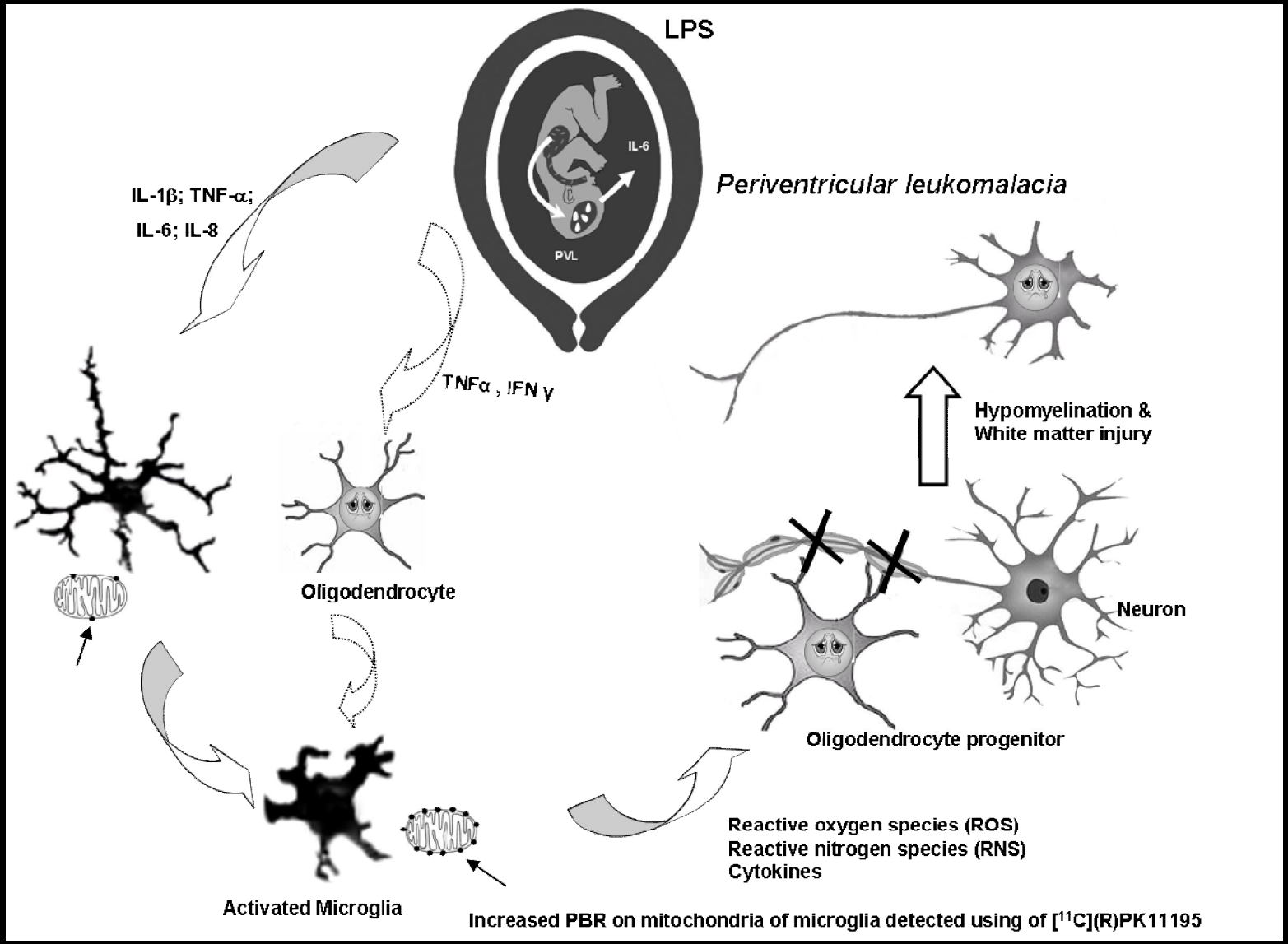
Introduction

- Injury to the developing brain is unique: different responses based on the timing of injury
- Maternal intrauterine infection implicated in brain injury resulting in cerebral palsy and neurodevelopmental disorders such as autism
- Significant correlation: chorioamnionitis and cerebral palsy; maternal infection and autism .
- Injury involves both grey matter and white matter.
- Motor, somatosensory and cognitive deficits noted in CP

Maternal Infection and FIRS

Mechanism of brain injury



S. Kannan, 2009, Journal of Child Neurology.

Animal Model

Pregnant New Zealand White rabbits (28 days)
Laparotomy and intrauterine injection

(Maternal serum for cytokines 0,2,6,24,48 hrs)

Saline

LPS (20µg/Kg) from *E. Coli*

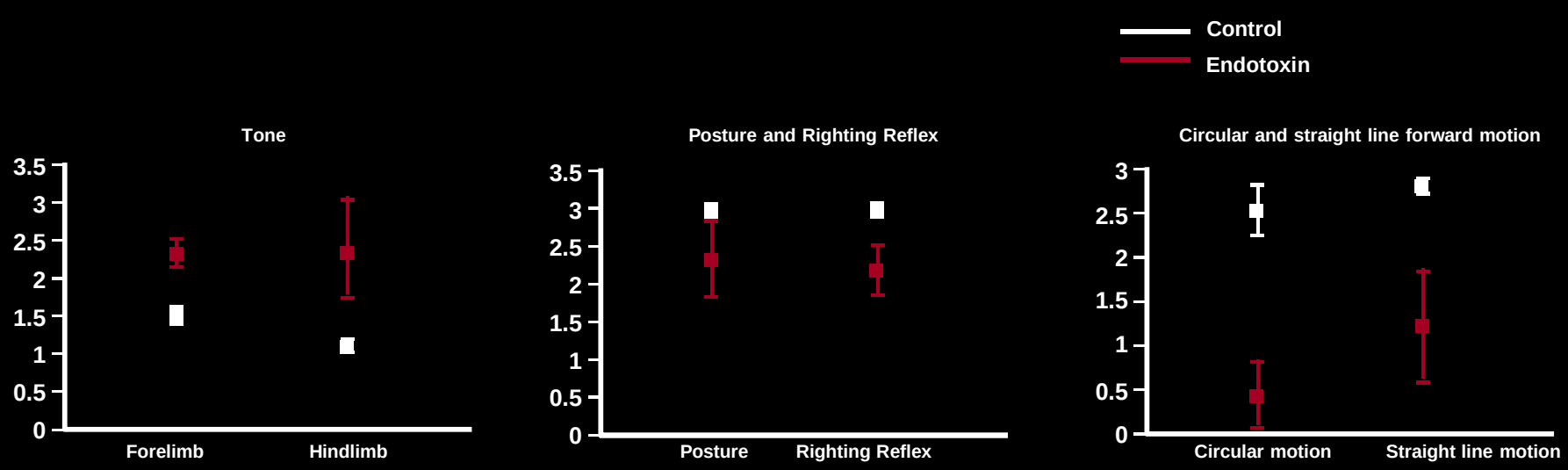
(Born spontaneously at term-31 days)

Control kits

Endotoxin kits

Neurobehavioral scoring, PET scan, MRI, and/or immunohistochemistry

Phenotype of Cerebral Palsy



Inability to maintain prone posture hypertonia and impaired locomotion noted in endotoxin kits; $p<0.01$ (Scoring for newborn rabbits based on Derrick et al. 2004)

S.Kannan group et.al. AJOG; 2008

Is this phenotype associated with presence of activated microglia in the neonatal brain?

PET scan

[^{11}C]PK11195= PET tracer (300-500 µci)

↓ IV

Control and Endotoxin kits

Customized head holders with markers for MRI and PET

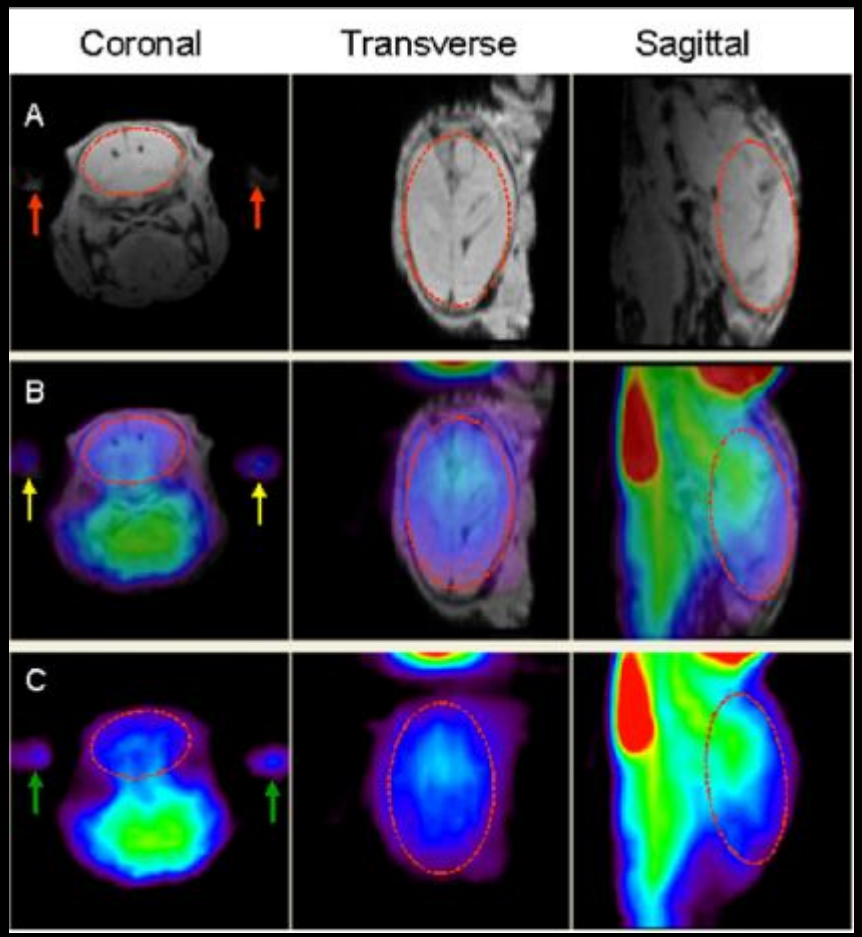
↓

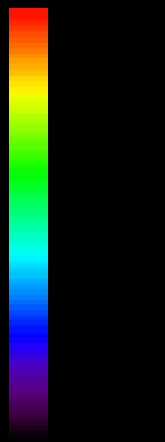
Scan for 60 min

↓

MRI for co-registration

MRI and PET Co-Registration





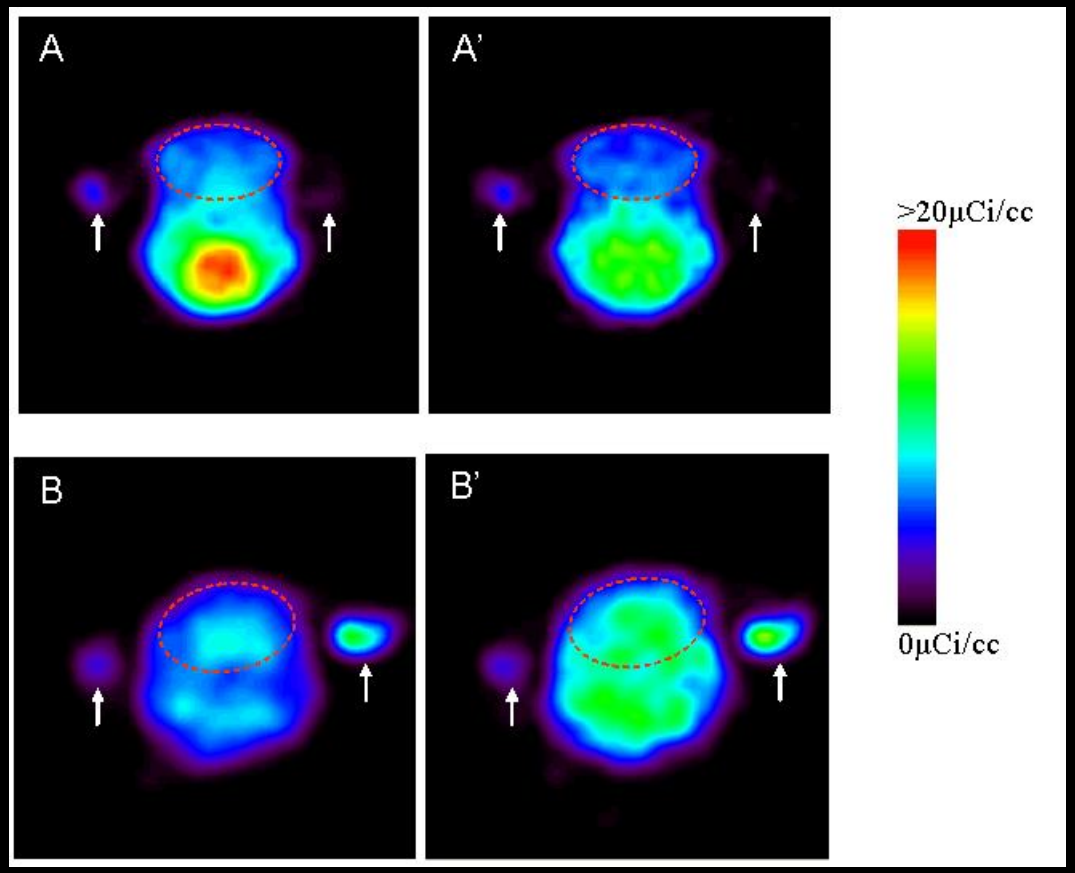
- Co-registration of PET and MRI images done with markers seen on both
- 3D Region of interest drawn for the cerebrum and midbrain
- SUV plotted over time and slopes and intercepts compared between groups

Kannan S et al, JNM 2007

$$SUV = \frac{\text{Activity in ROI } (\mu Ci/g) \times \text{Wt } (g)}{\text{Injected dose } (\mu Ci)}$$

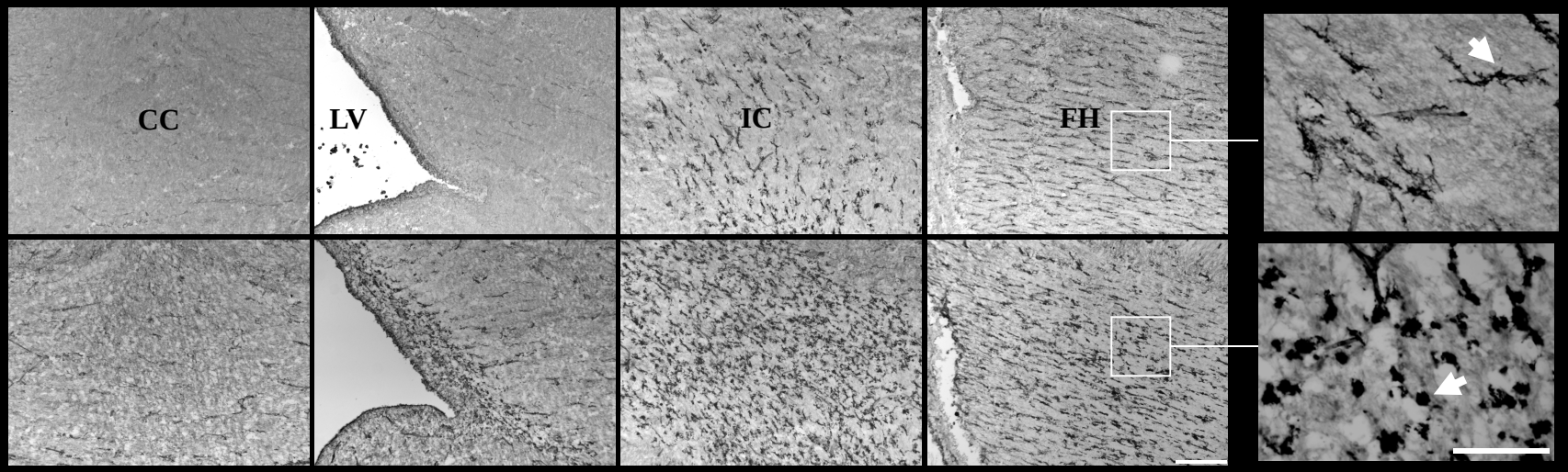
Detection of activated microglia by PET scan

^{11}C PK11195 uptake in the neonatal rabbit brain



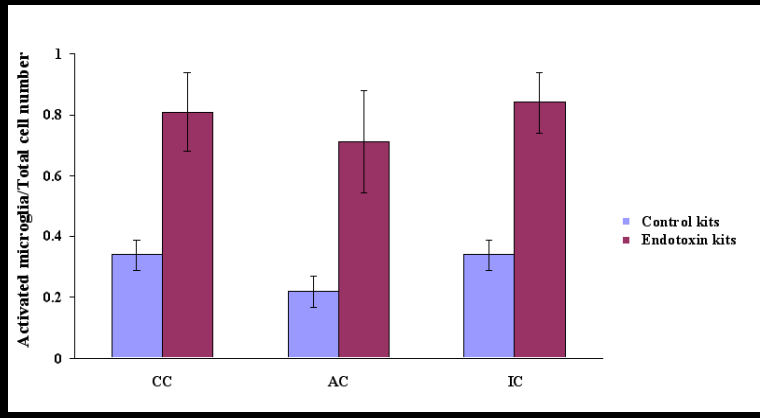
Increase in tracer retention seen over time in the endotoxin group indicating specific binding of the tracer to activated microglia

Activated Microglial Cells



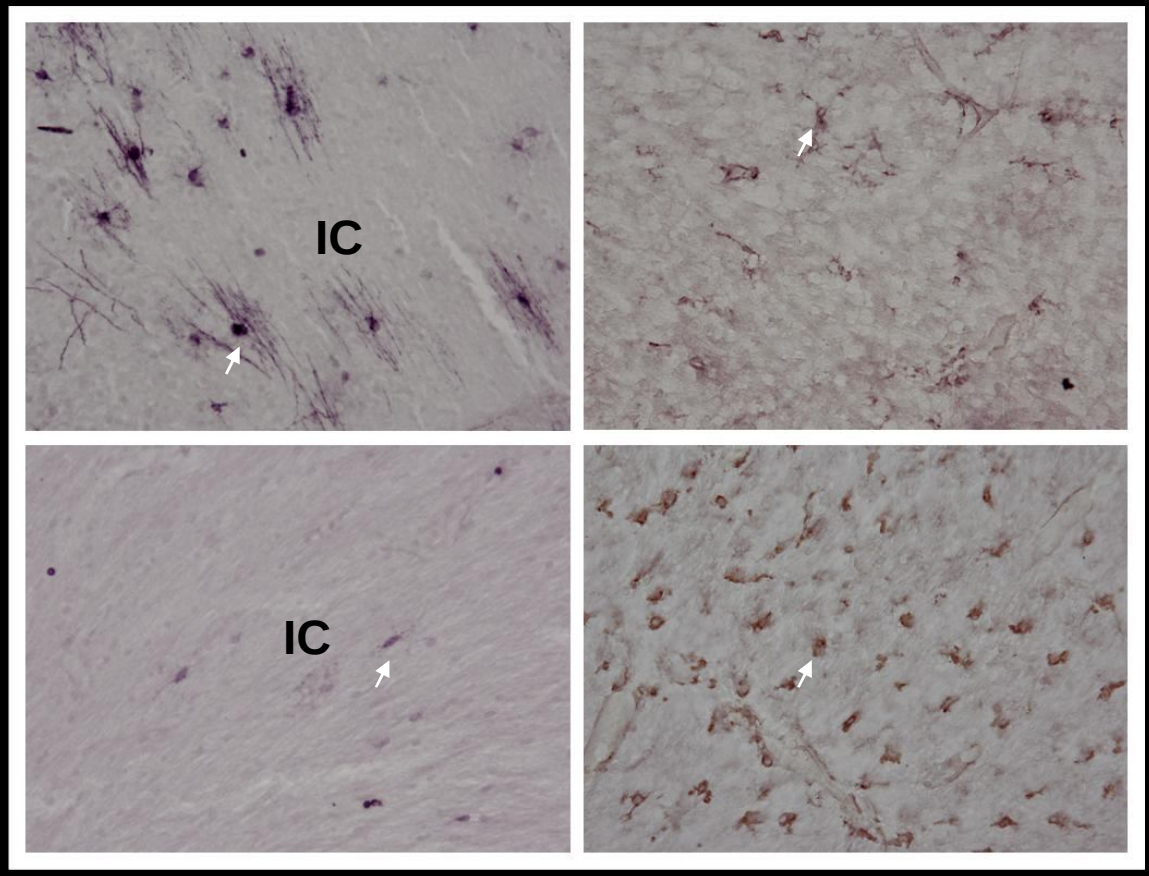
Change in microglial morphology from ramified to more amoeboid and rounded form with endotoxin exposure.

Increased activated microglia in white matter tracts in endotoxin kits.



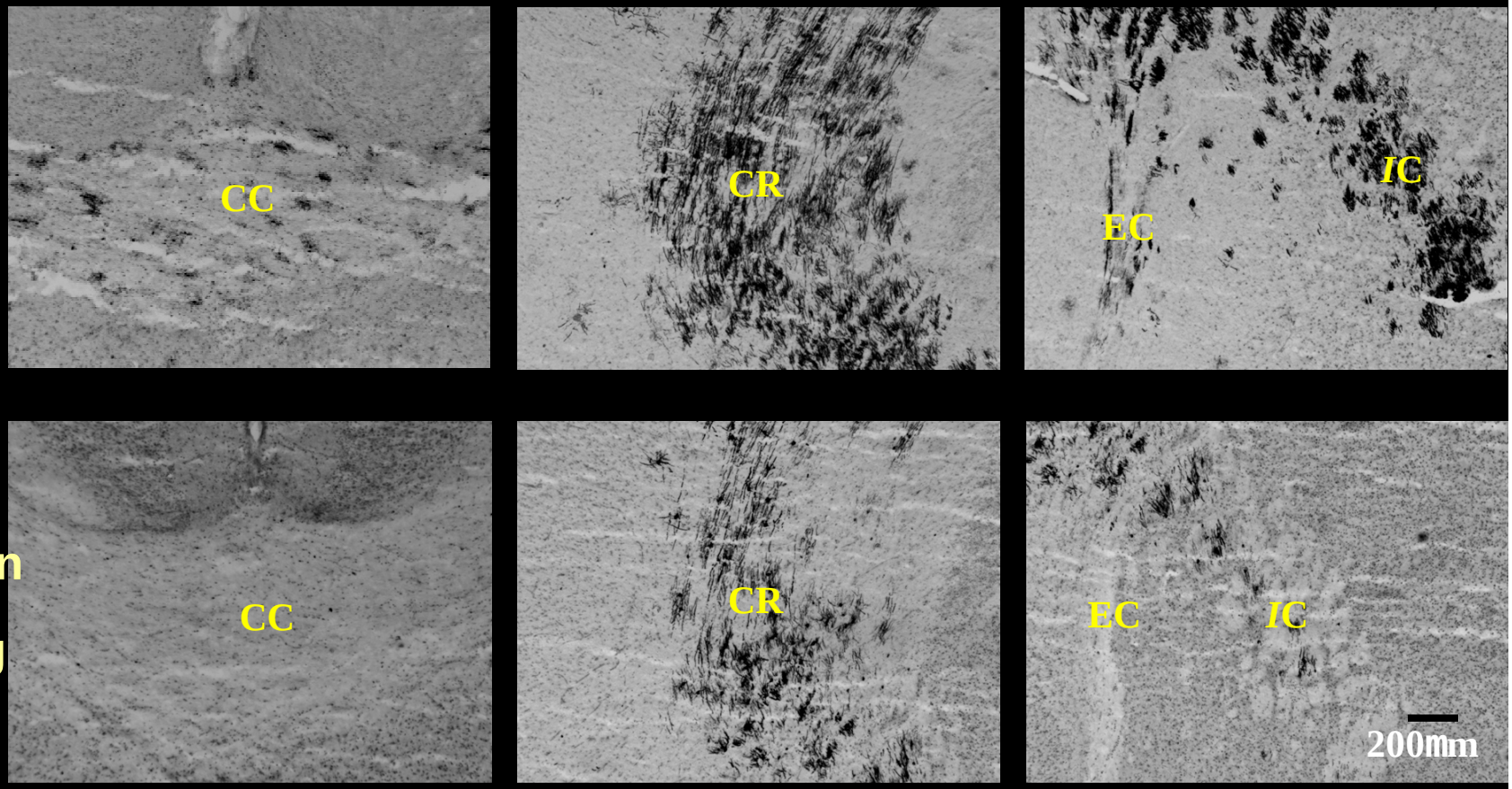
(Kannan S group et al JNM, 2007; Journal of Child Neurology, 2009)

Activated microglia and oligodendrocytes



A decrease in the number of mature oligodendrocytes (MBP staining) is noted with an increase in the presence of activated microglia in endotoxin kits ; IC=Internal Capsule

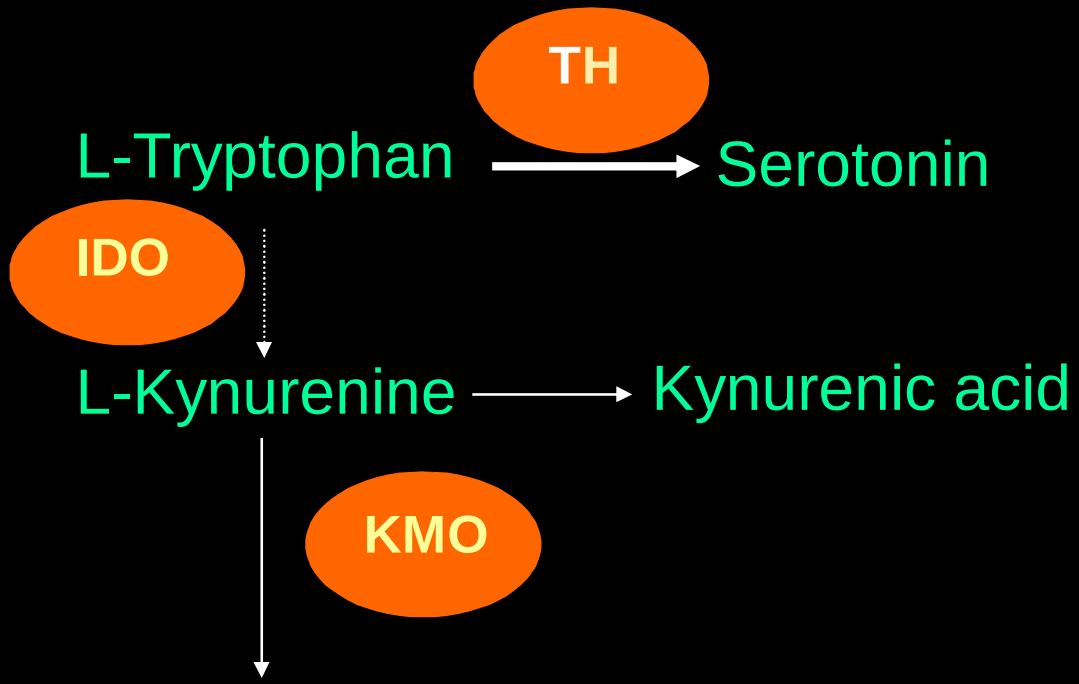
Myelination on Postnatal Day 5



Decrease in Myelin basic protein staining noticed on postnatal day 5 in the corpus callosum, corona radiata and internal capsule

Involvement of somatosensory cortex and neuronal injury

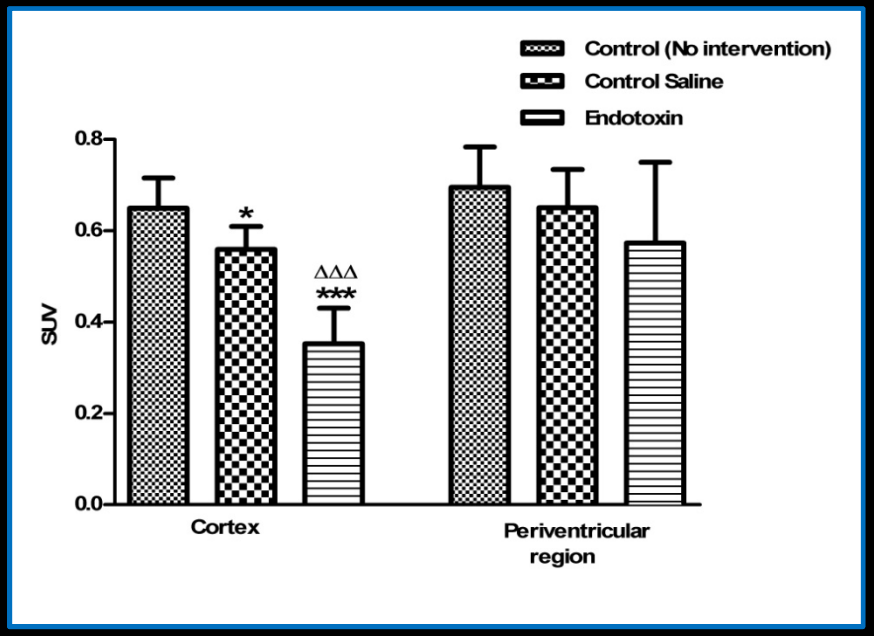
Tryptophan metabolism in the brain



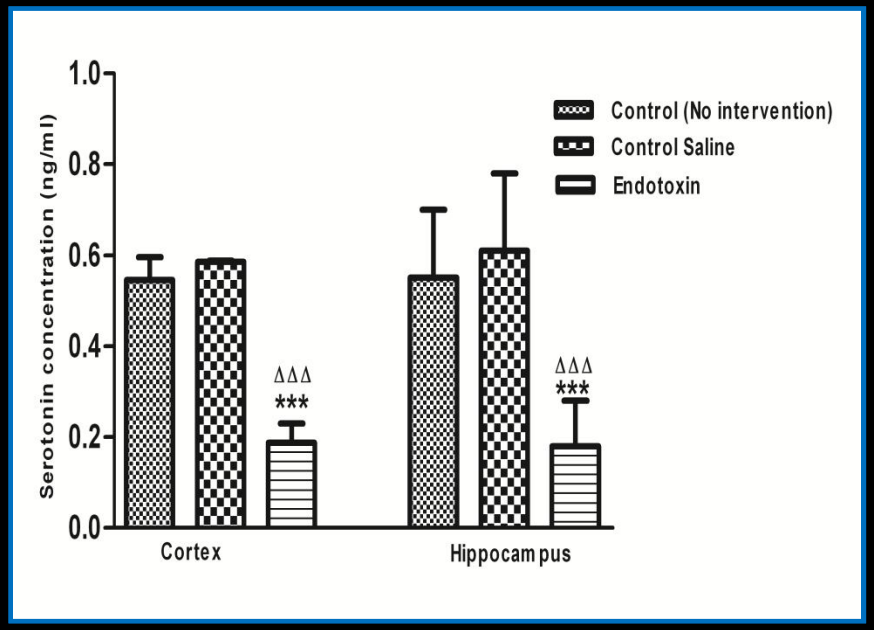
Tryptophan metabolism can be evaluated by PET using 11C AMT as a tracer

Serotonin in the newborn rabbit brain

11C AMT metabolism



Serotonin concentration

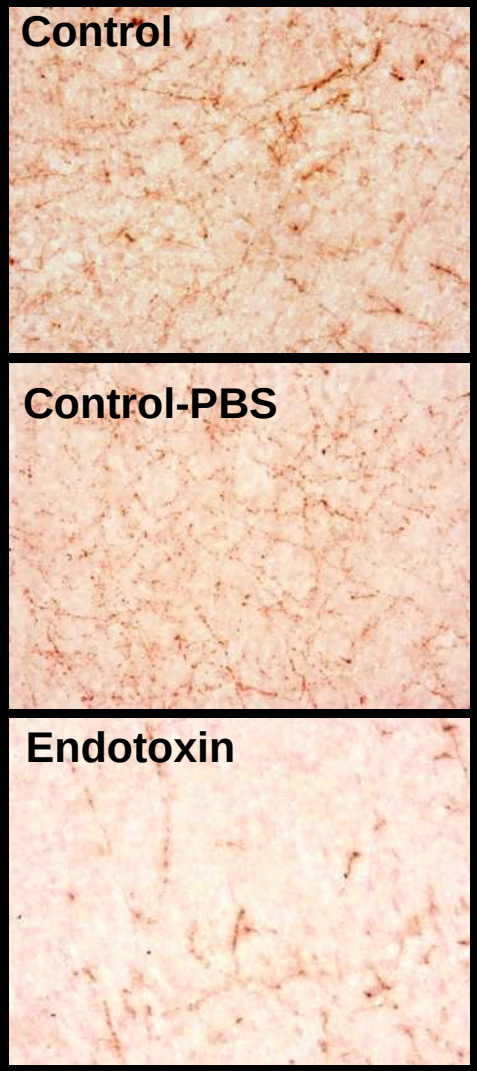


- Decreased [11C] AMT uptake in cortex suggestive of decreased serotonin synthesis in the endotoxin kits.
- Decreased serotonin concentration in the cortex and hippocampus
- Tryptophan metabolism along non-serotonin pathway in PVR (Kynurenine pathway)
- Decreased serotonin synthesis noted in the cortex of autistic children (Chugani D, 1997,1999, 2004)

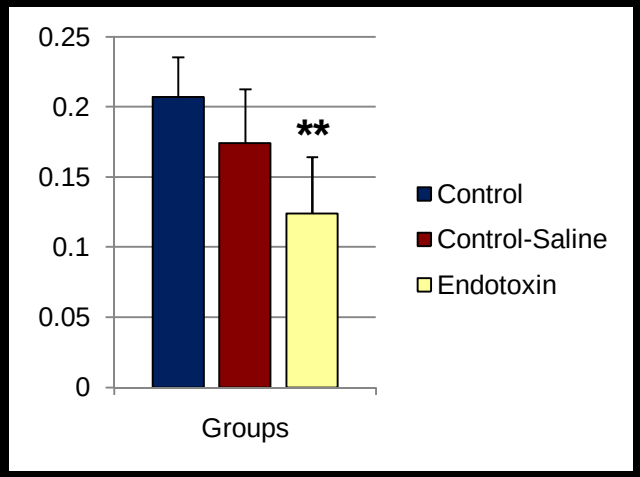
Kannan S et al., In press , JCBFM 2010.

Serotonin fibers in the somatosensory cortex

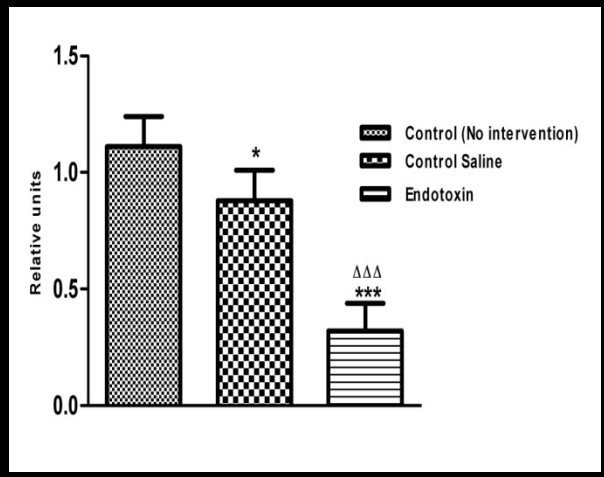
5-HT staining



Fiber length in μm/ μm²

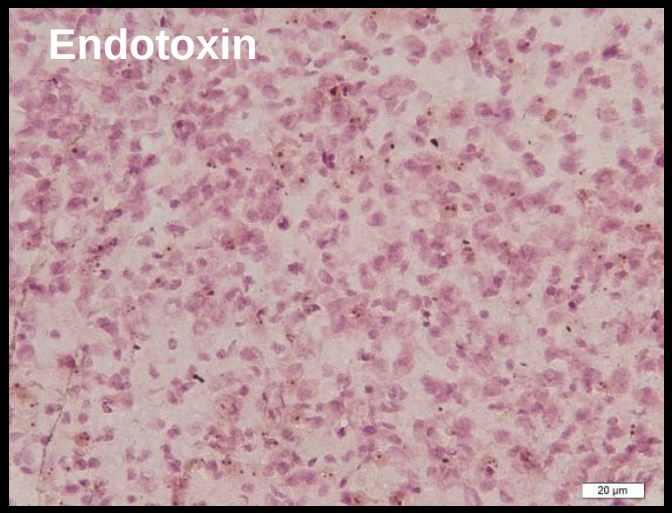


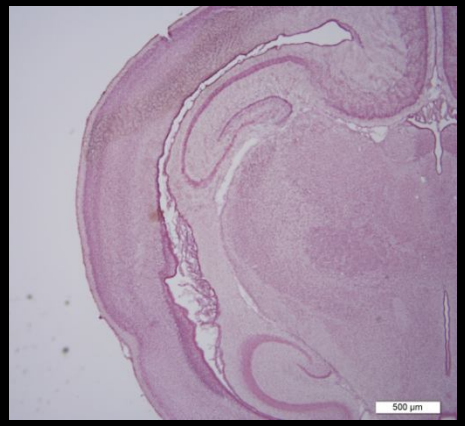
5HTT mRNA expression

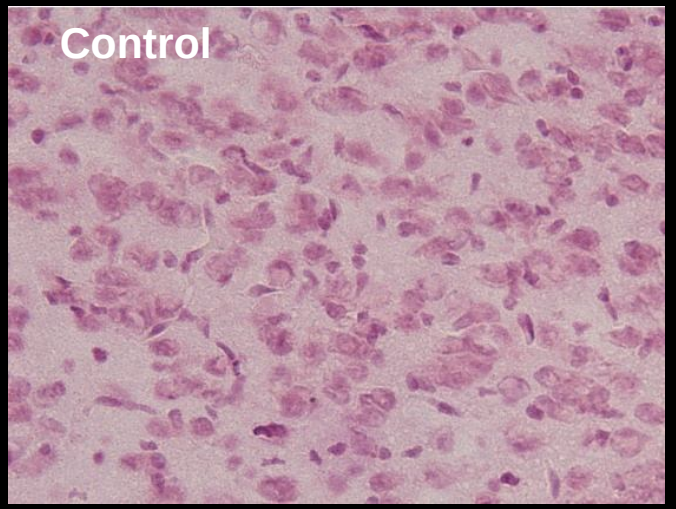


- A decrease in 5HT staining fibers noted in layer IV of the somatosensory cortex.
- 5HT fibers project from serotonergic neurons in raphe nucleus and from thalamus
- Thalamocortical fibers transiently express 5HT transporter (5HTT) during development
- Decrease in 5HTT expression indicates loss of thalamocortical fibers

Neurons in the Ventro-posterior thalamus



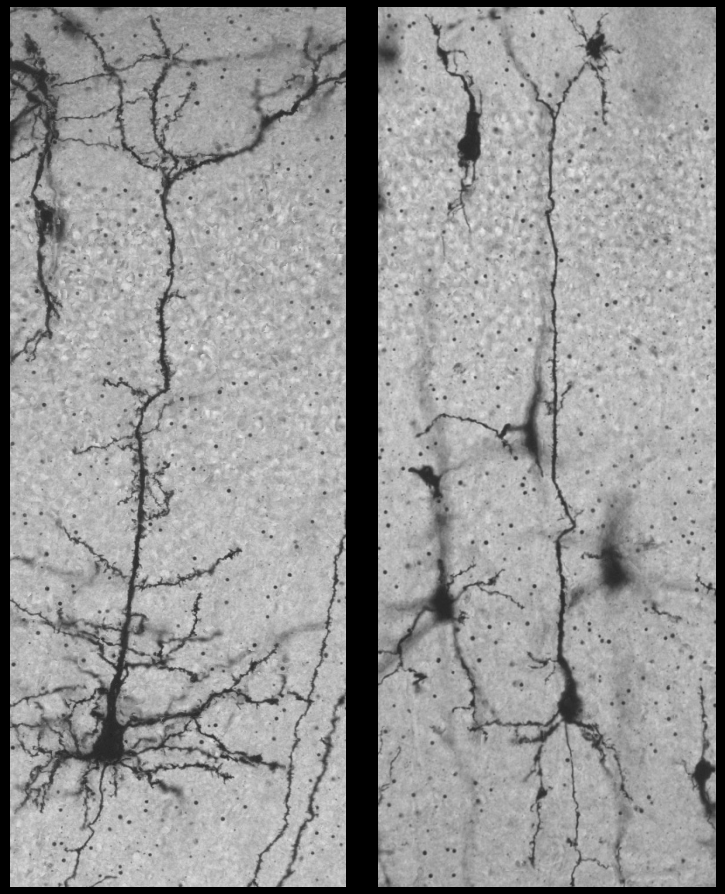




- Increased apoptotic cells seen in VP thalamus of endotoxin kits when compared to controls on day 1 of life.
- This may result in the loss of thalamocortical afferent fibers that transiently express 5HTT.
- Decrease in fibers may be a result of direct injury to the fibers and/or loss of thalamic neurons that project to the sensory cortex.

Injury to dendrites

CONTROL ENDOTOXIN

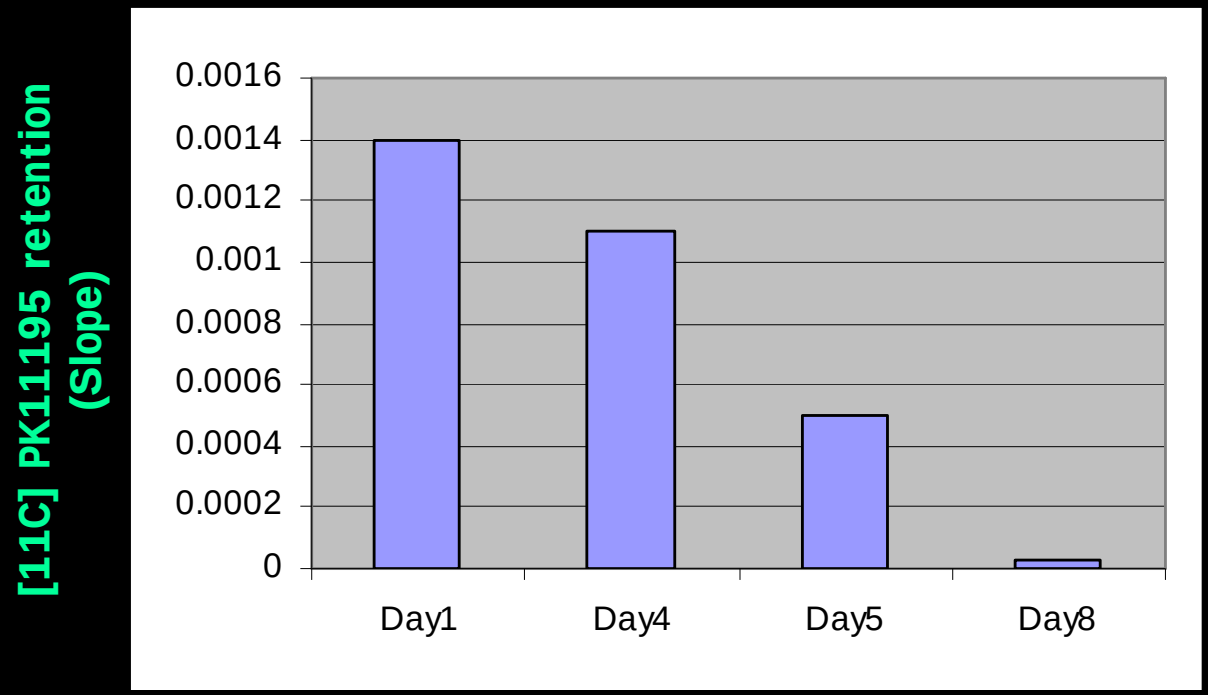


- Impairment in dendritic branching, organization and decreased spines seen in endotoxin kits upon Golgi staining.
- Associated with learning deficits and memory impairment
- Seen in brains of patients with mental retardation

Determine if there is impairment in learning associated with this injury

Molecular markers responsible for synaptogenesis, dendrite formation and axon guidance

Longitudinal PET Measure of Microglial Activation



Endotoxin kits scanned longitudinally

The slope decreases over time with the greatest drop (decrease in slope) after day4 indicating a decrease in the number of activated microglial cells

Conclusions

- Maternal endotoxin exposure induced microglial activation is associated with a phenotype of cerebral palsy in the rabbit model
- PET imaging of microglial cells may be used as a diagnostic tool to determine the presence of neuroinflammation in the neonate.
- Maternal inflammation is associated with decreased serotonin in the cortex with injury to thalamocortical afferents. May explain somatosensory impairment in CP.
- Targeting activated microglia with anti-inflammatory agents may help in attenuating injury and improving deficits.

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