



Medizinische Fakultät Heidelberg

Classical microglial activation is not neurotoxic *in situ* unless primed

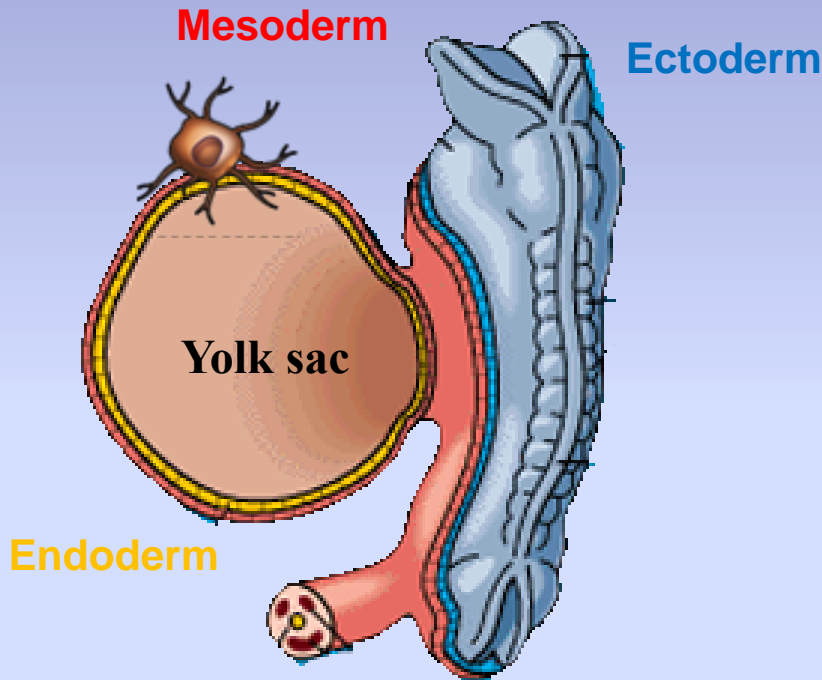
Dr. Ismini Papageorgiou, MD/PhD
Institut für Physiologie und Pathophysiologie
AG Kann

Lunchtime Seminar July 1st 2014, INF 306, R501, 13:00

Terminology

- Microglia
 - Microglia *in situ*
- Activation
 - Classical activation
- Lipopolysacchride (LPS) for classical activation
- Priming
 - Priming vs activation
- Interferon gamma (IFN γ) for priming

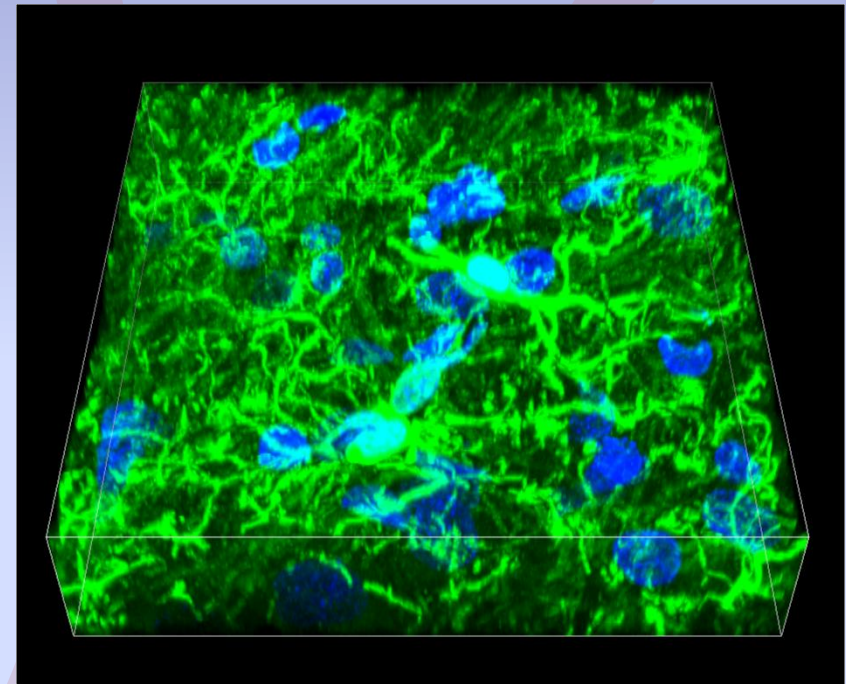
Microglia: macrophages behind the blood-brain barrier



Neural tube

E9-12 rat/E8-9 mouse

GW 6.5-8 human



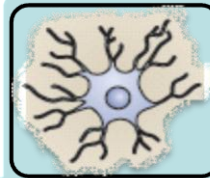
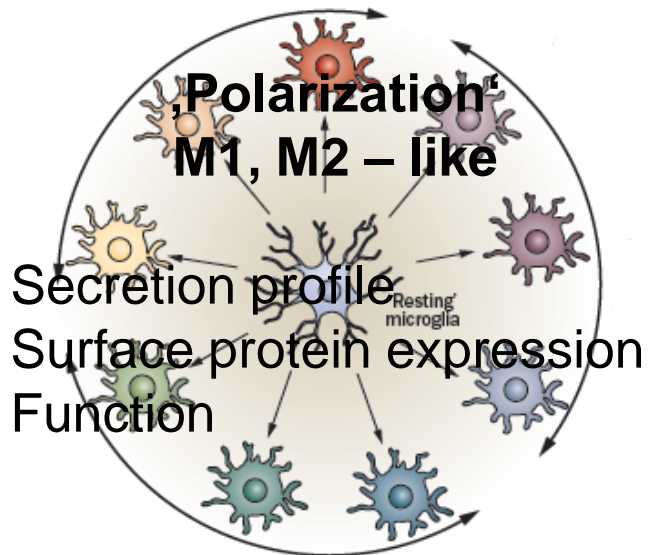
Adult Wistar rat

DAPI Iba1

Courtesy AG Kann

Activation spectrum

Neuroinflammation



Detection (**M0, surveying**)

- Pathogen (LPS)
- Damage



Recruitment & Toxicity (**M1, classical**)

- Adaptive immunity (Nph, Tcells, NK)
- Direct toxicity



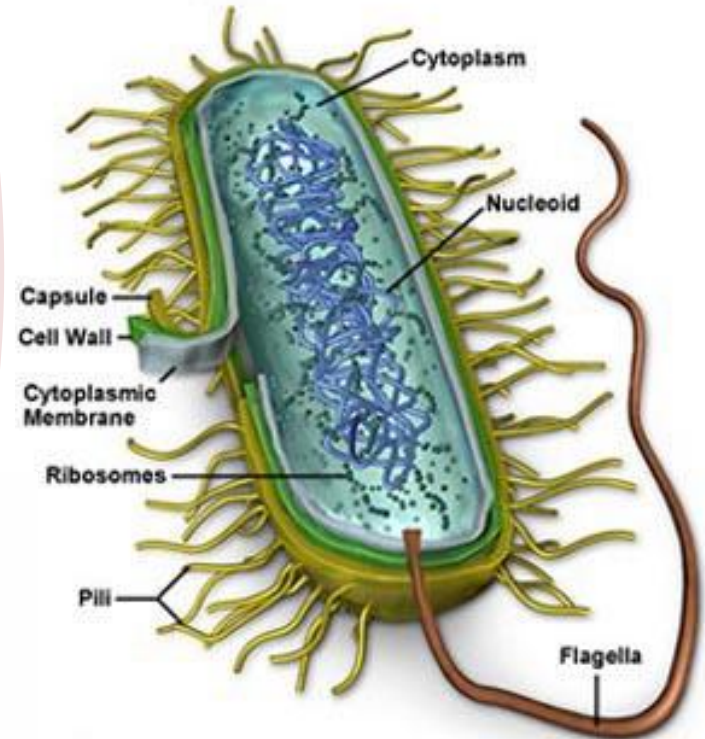
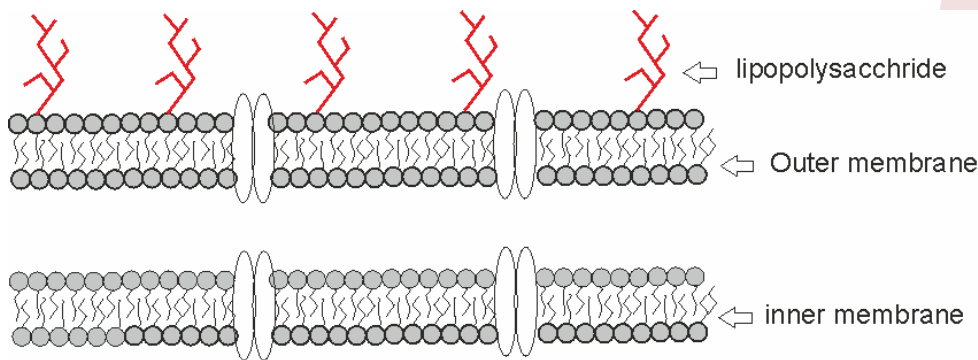
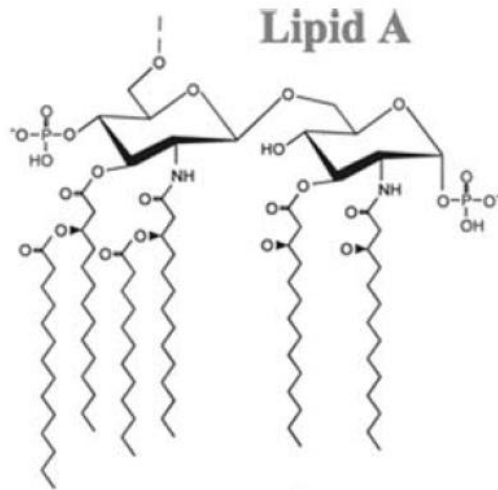
Resolution (**M2, alternative**)

- Termination of toxicity (anti-inflammation)
- Debris phagocytosis

Hao et al., 2012; Perry et al., 2010

Lipopolysachharide

Gram (-) bacterial endotoxin



Activation spectrum

Anti-inflammatory cytokines

IL-4
IL-10
IL13
Phagocytosis



IL-4



LPS

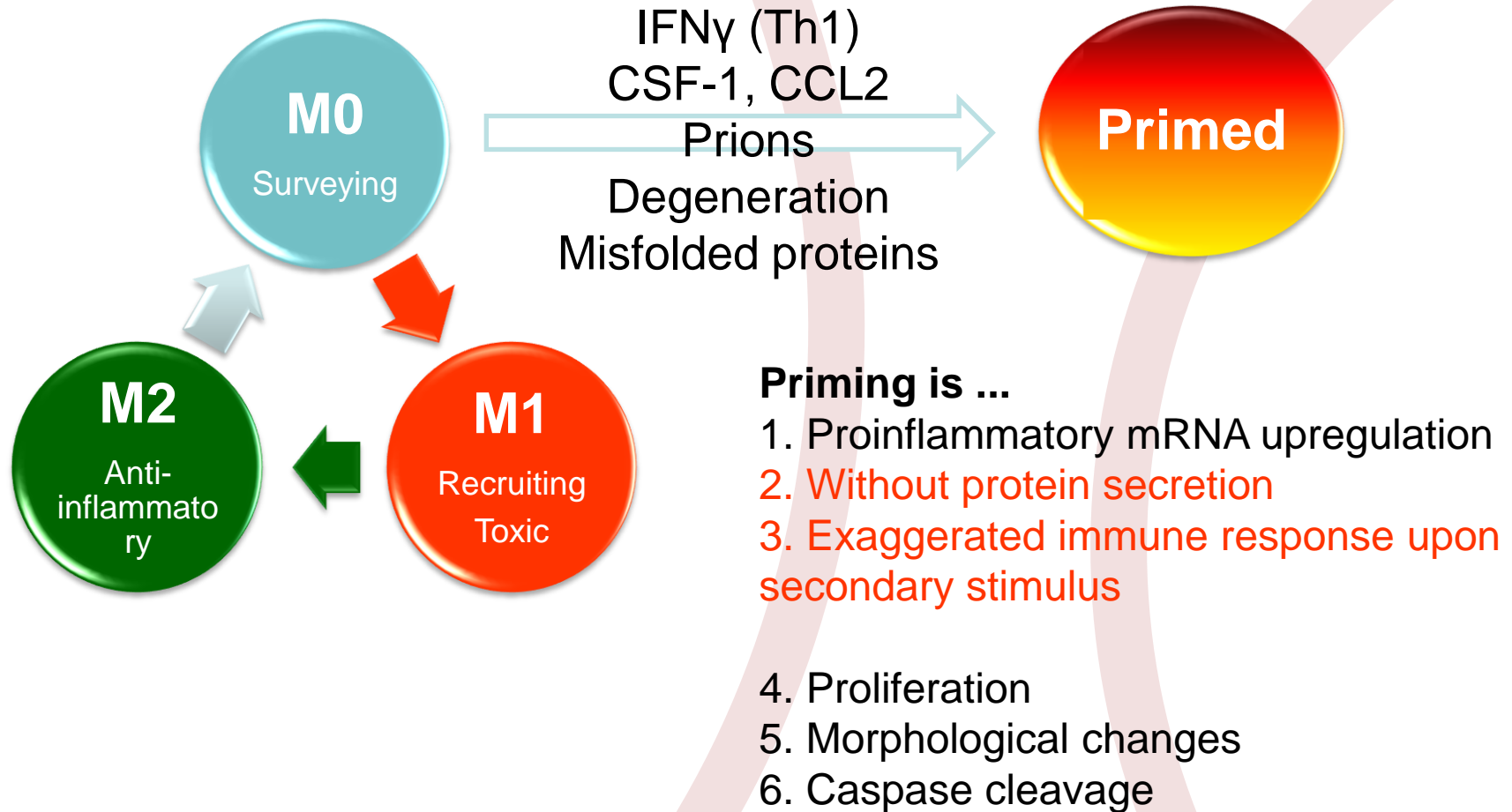
Proinflammatory cytokines

IL-1 β
TNF- α
IL-6
ROS, NOO-



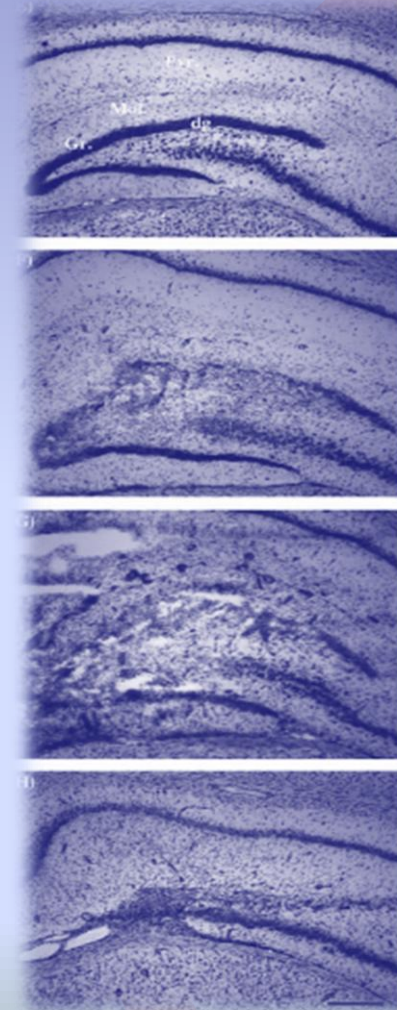
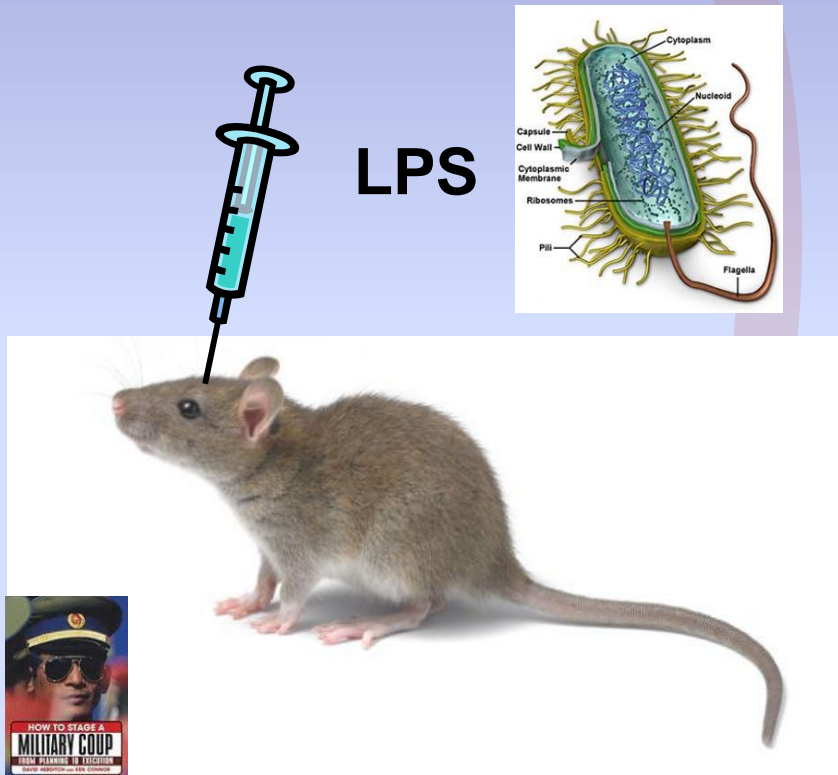
IL-4
IL13

Priming Vs activation



Microglia as neuronal executors

Classical microglial activation with LPS is an animal model for **degeneration**



Ambrosini et al, 2005



Microglial activation in neurodegenerative diseases (?)

Aguzzi et al., 2013 *Science*

Neurodegenerative	Alzheimer´s Disease Multiple sclerosis Parkinson´s Disease Epilepsy / Hippocampal sclerosis	Halle et al., 2008 Nat Neurosci Grathwohl et al., 2009 Nat Neurosci Helenka et al., 2012 Nature Sriram, 2011 J Neuroimmunology Amor et al., 2010 Immunology Pernot et al., 2011 Epilepsia Yeo et al., 2011 J Neuroimmunology
Neuropsychiatric	Bipolar disorder Schizophrenia Autism & Rett Syndrome	Derecki et al., 2012 Nature Blank & Prinz 2013 Glia Beumer et al., J Leukoc Biol Suzuki et al., 2013 JAMA Psychiatry Tetreault et al., 2012 J Autism Dev Dis
Chronic pain		Hains & Waxman, 2006 J Neurosci Hulsebosch, 2012 Exp Neurol Inoue & Tsuda, 2009 Glia

IFN γ priming (?) and neurodegenerative diseases

Mount et al. • IFN- γ and Parkinson's Disease

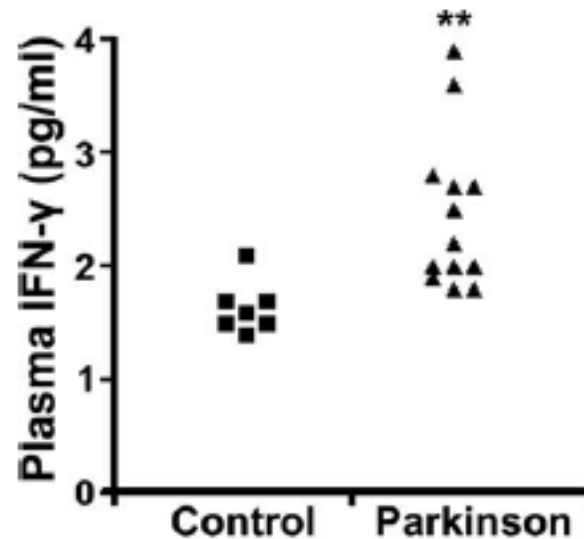
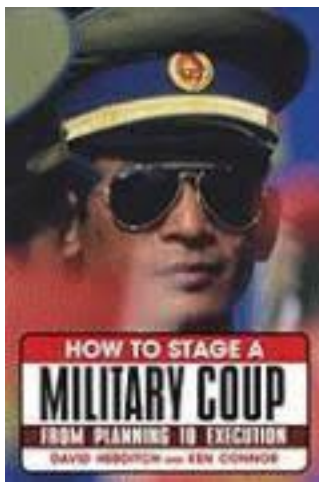
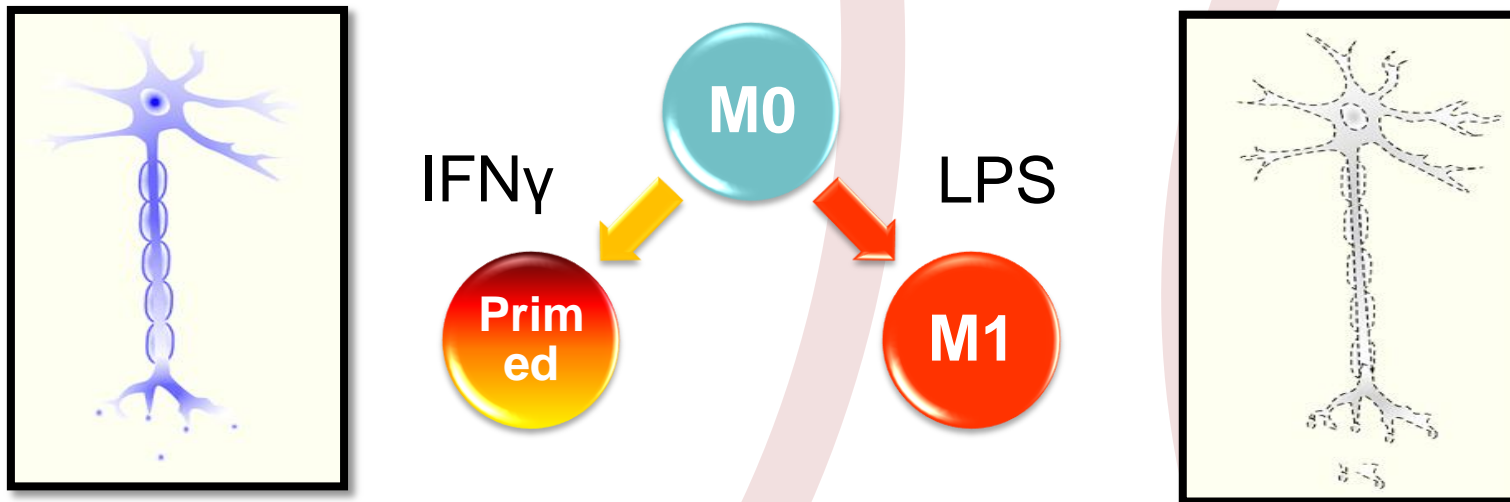


Figure 1. PD patients display elevated IFN- γ plasma levels relative to non-PD patients. Blood plasma from 13 PD and 7 control patients was analyzed by a multiplex bead assay system for several cytokines. Results for IFN- γ concentrations are provided here in dot plot representation (two-tailed *t* test, ***p* < 0.01).

Mount et al, 2007; Barcia et al., 2011; Perry and Holmes, 2014

Aim

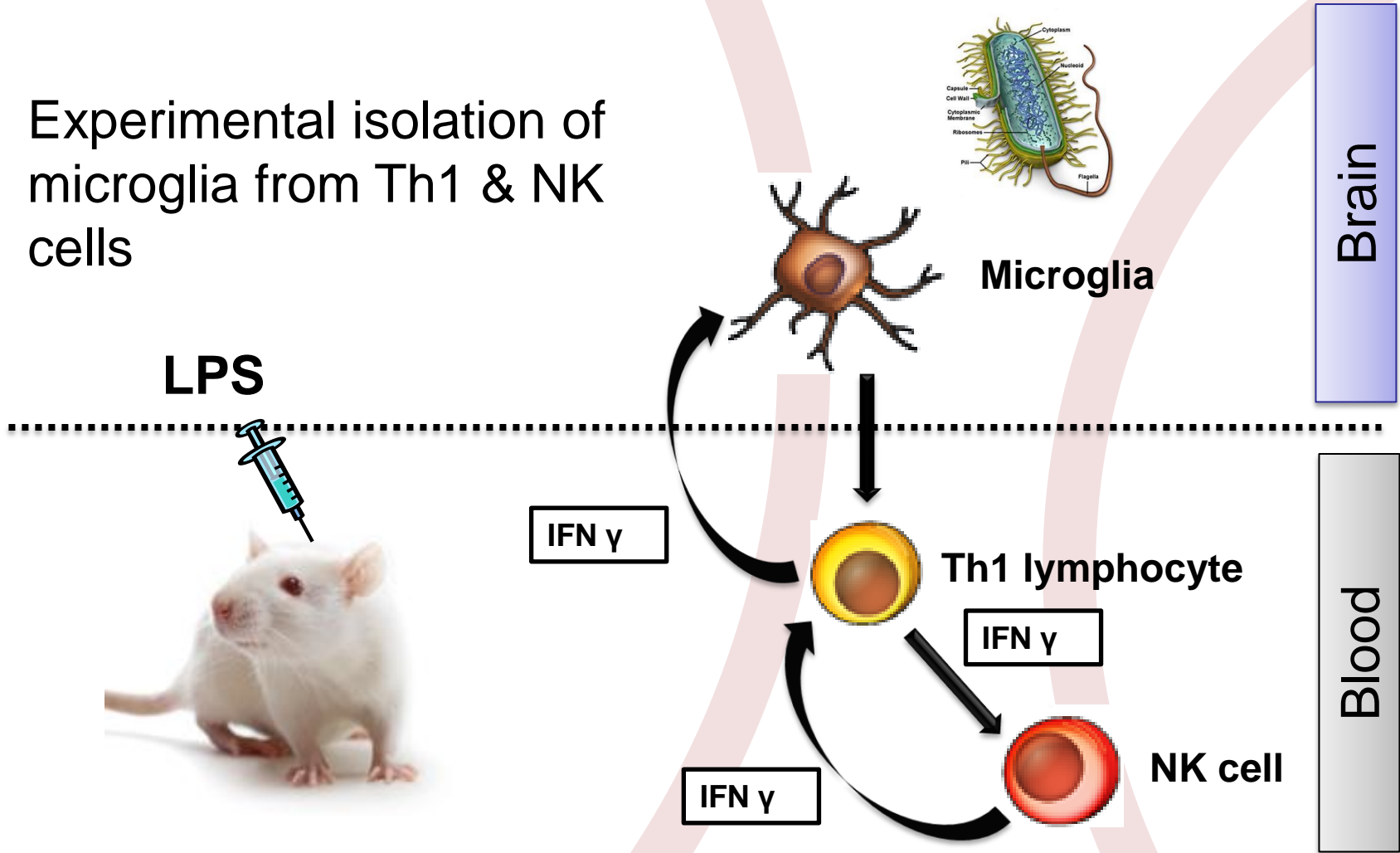
Investigate the effect of classically activated and primed microglia on neuronal **FUNCTION** and **SURVIVAL**



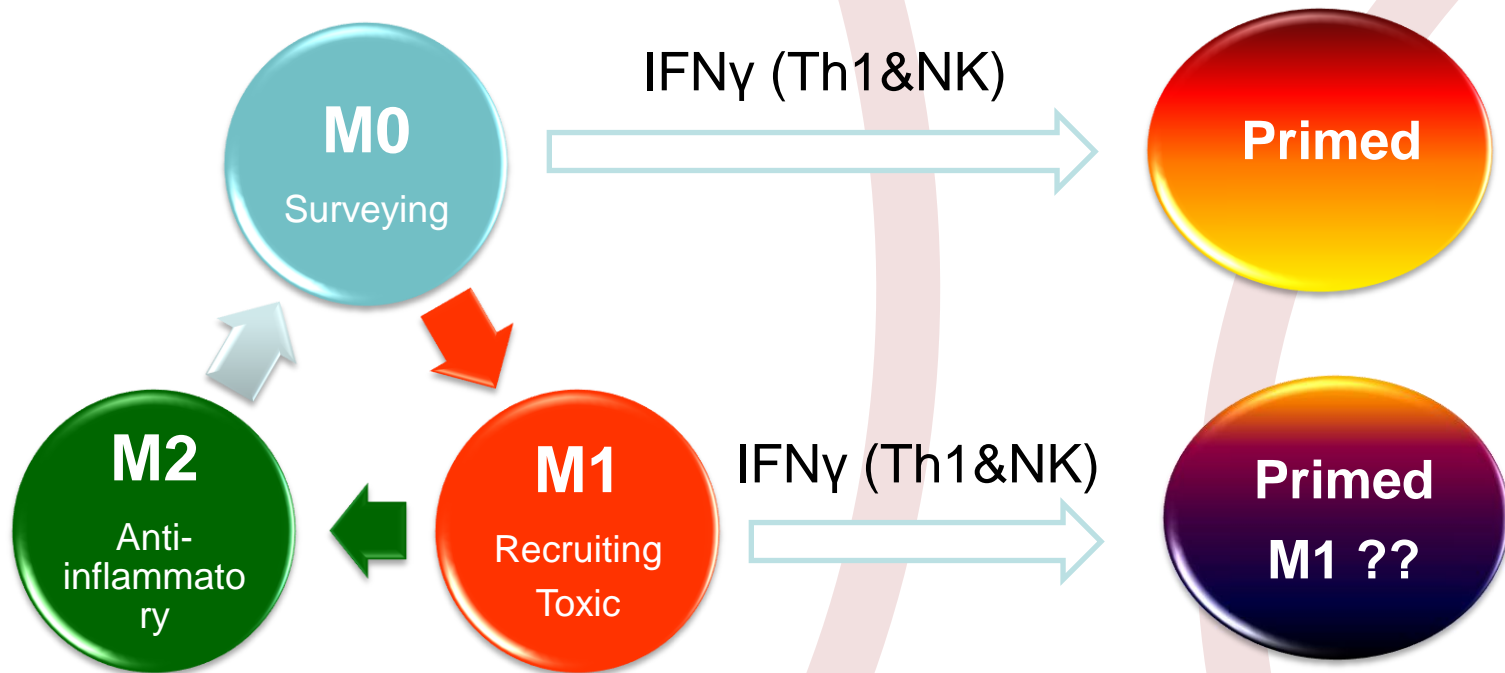
Block et al., 2007; Hanisch & Kettenmann, 2007

Objective

Experimental isolation of microglia from Th1 & NK cells

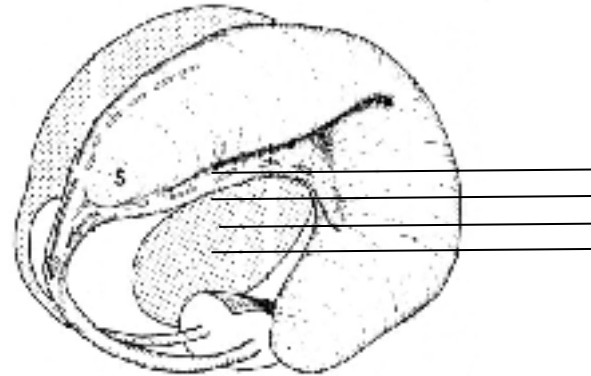


Priming Vs activation

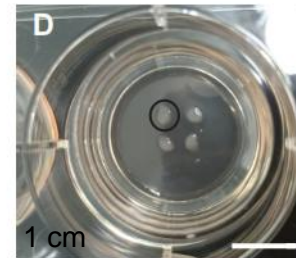
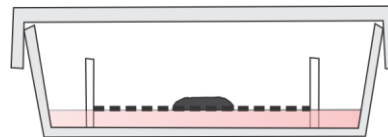


In situ model Organotypic hippocampal slice cultures

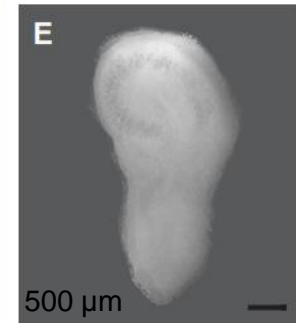
Transverse slices



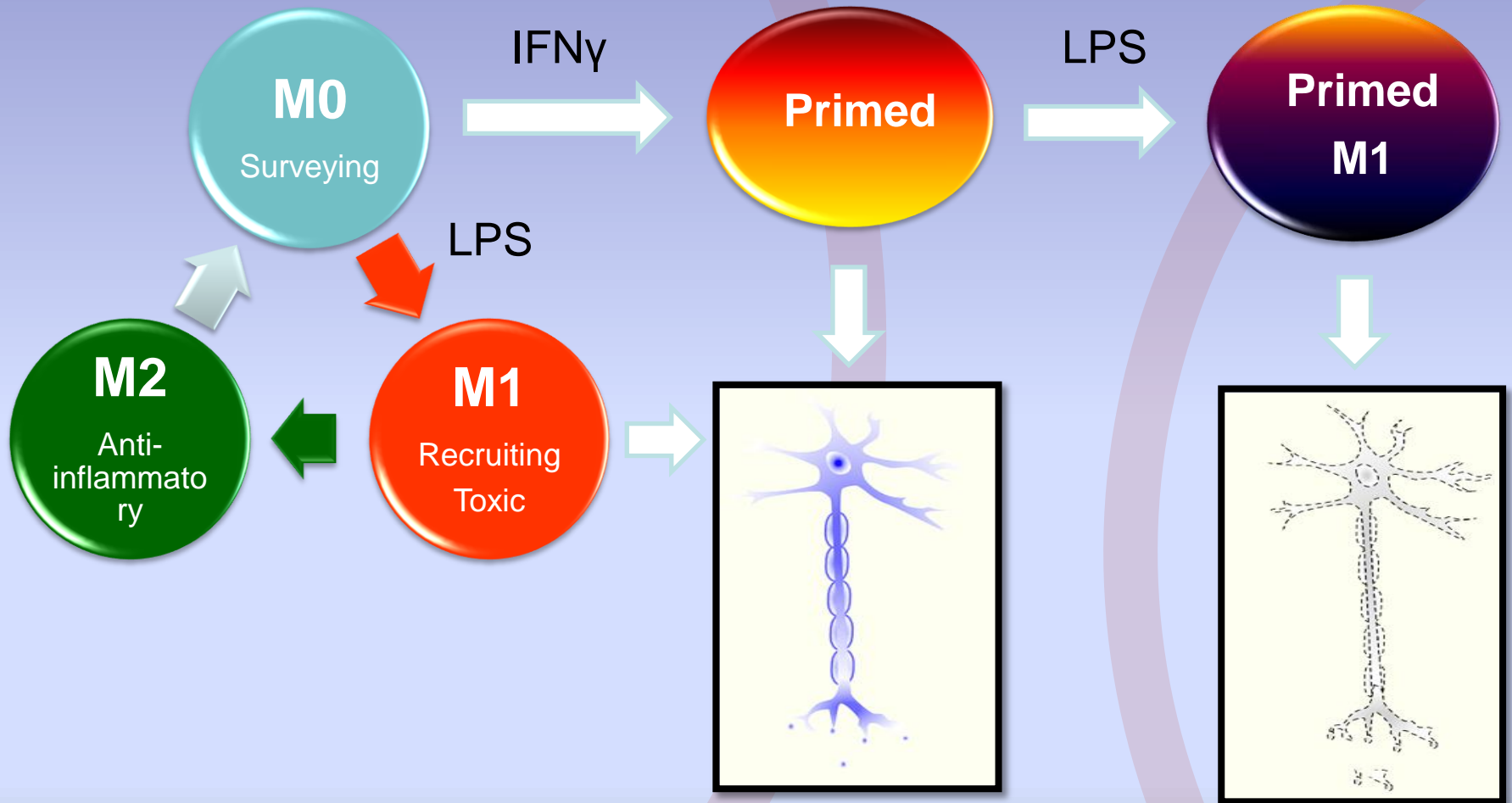
Stoppini protocol



DIV 11

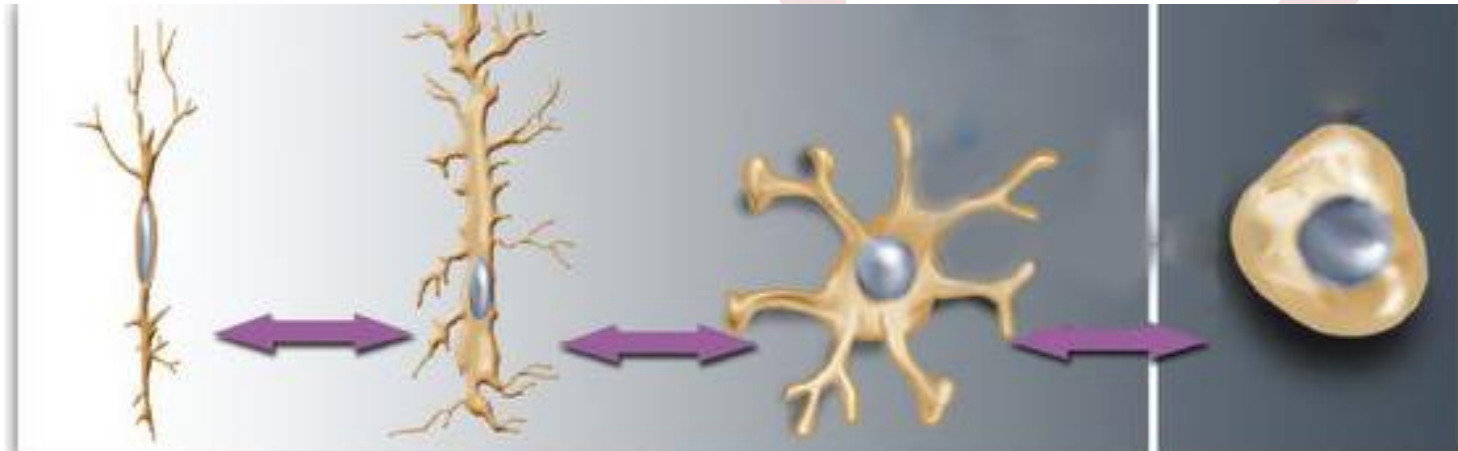


Results





Microglial morphology

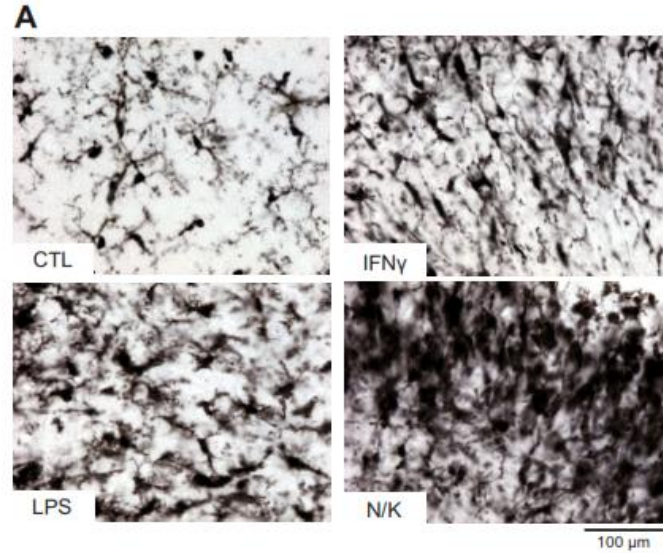
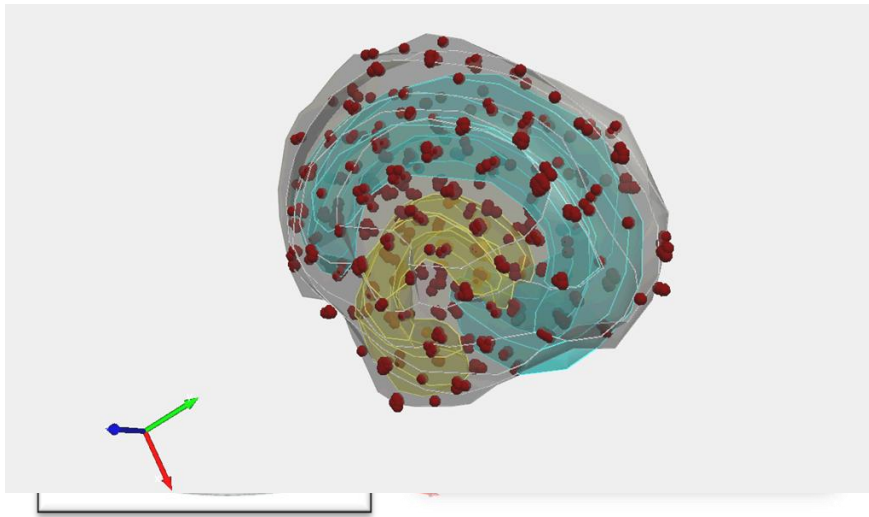


Ramified
Surveying ???

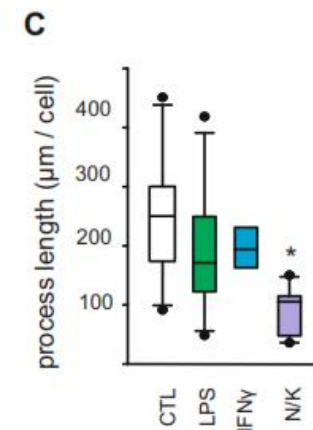
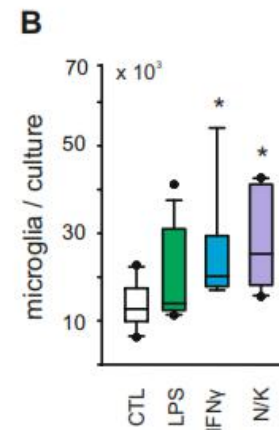
Amoeboid
Activated ???

Stence et al., 2001; Kettenmann et al., 2011

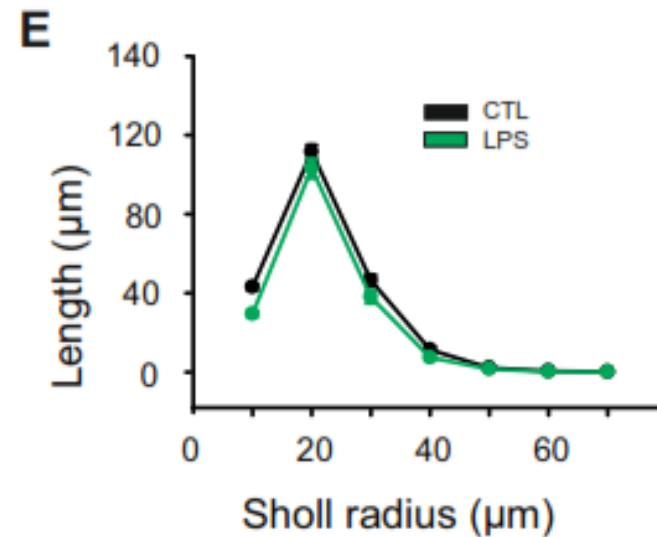
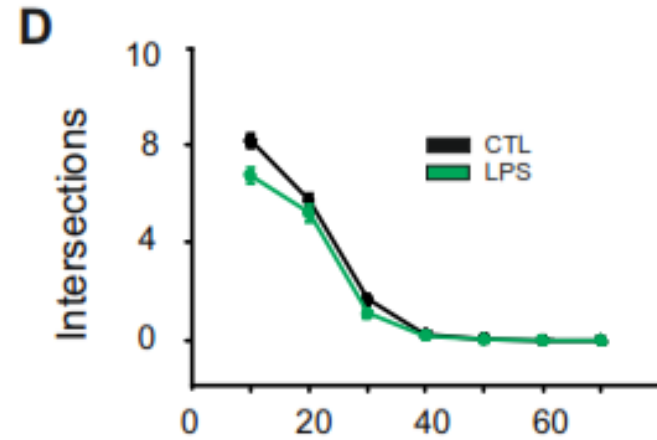
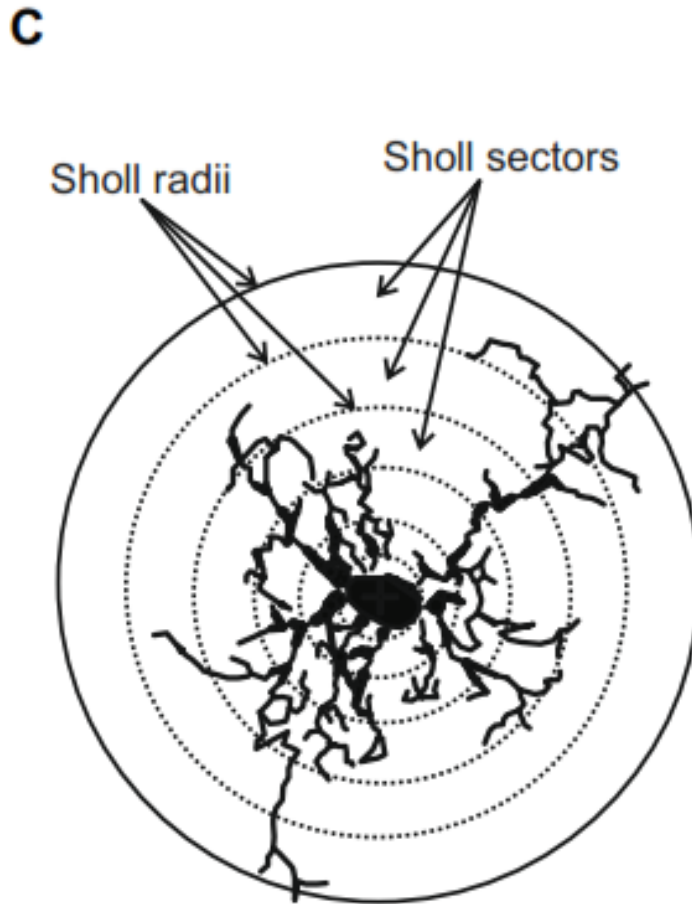
Population size and ramification



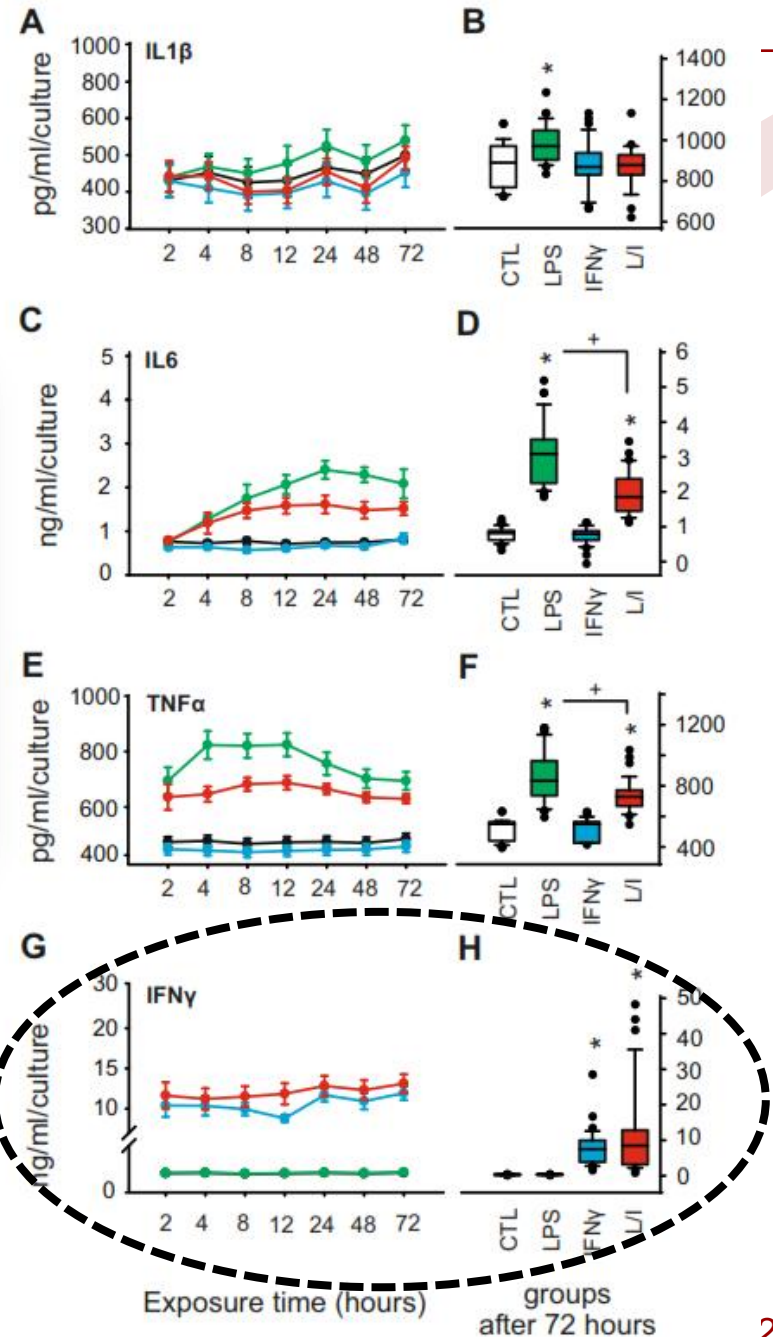
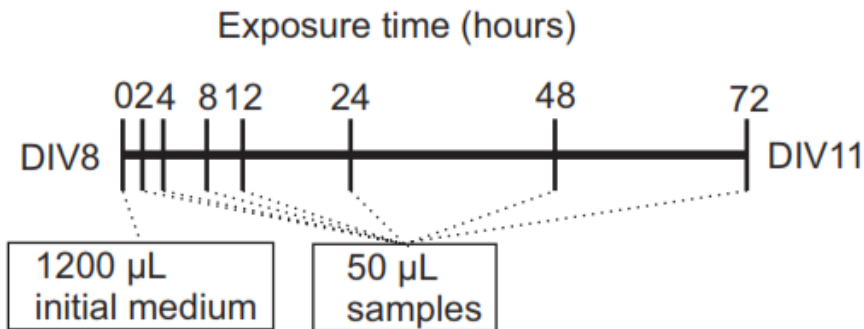
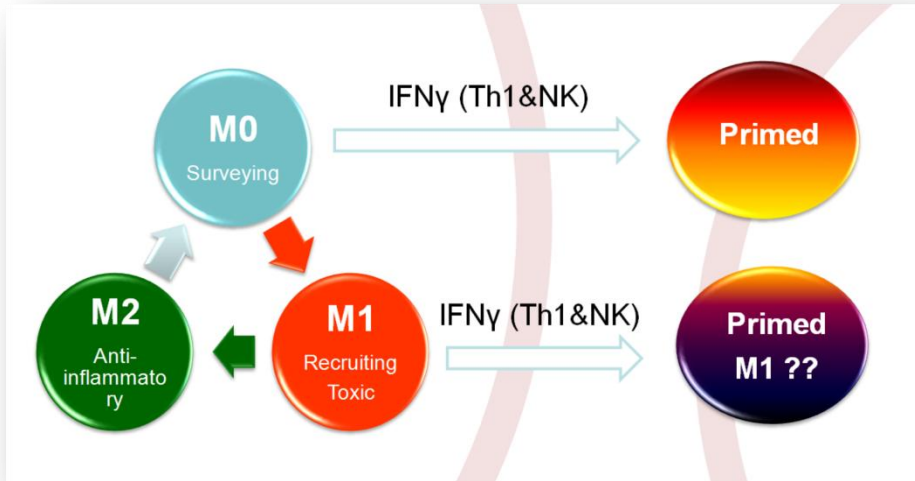
■ CTL	control, no medium exchange
■ LPS	lipopolysaccharide 10 μg/ml
■ IFN γ	Interferon gamma 100 ng/ml
■ N/K	NMDA 5μM + kainate 5 μM



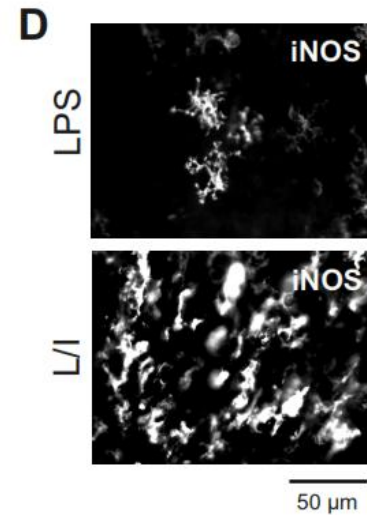
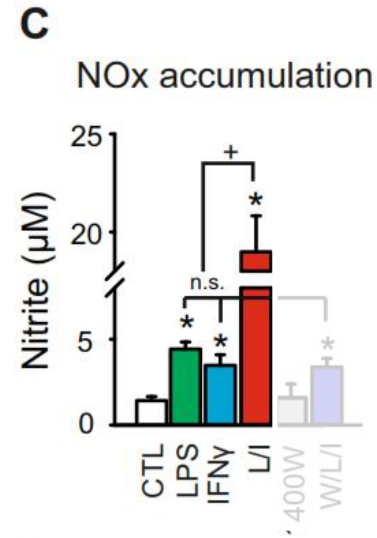
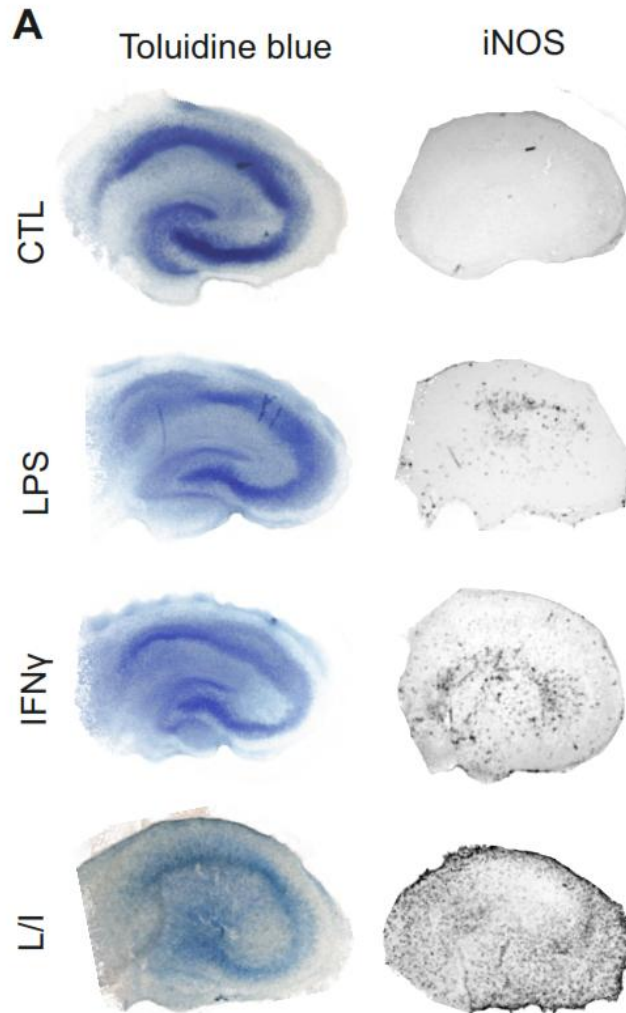
Classical activation with LPS: Microglial ramification, size and shape



Proinflammatory cytokine secretion



Inducible Nitric Oxide Synthase (iNOS)



Microglial activation profile

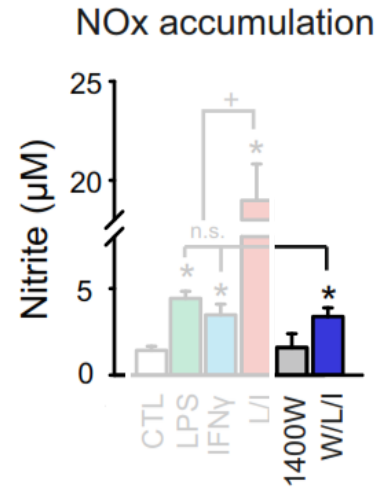
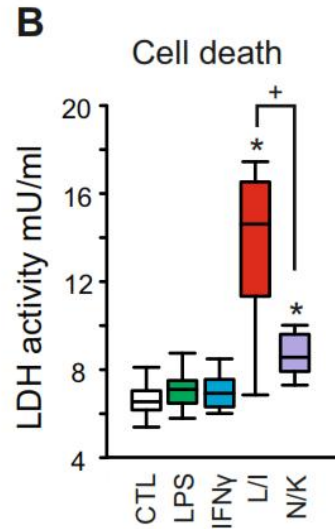
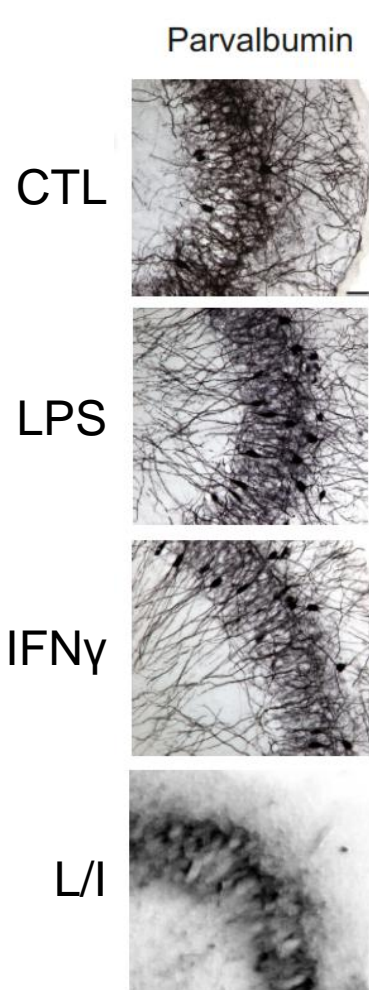


Activation trait

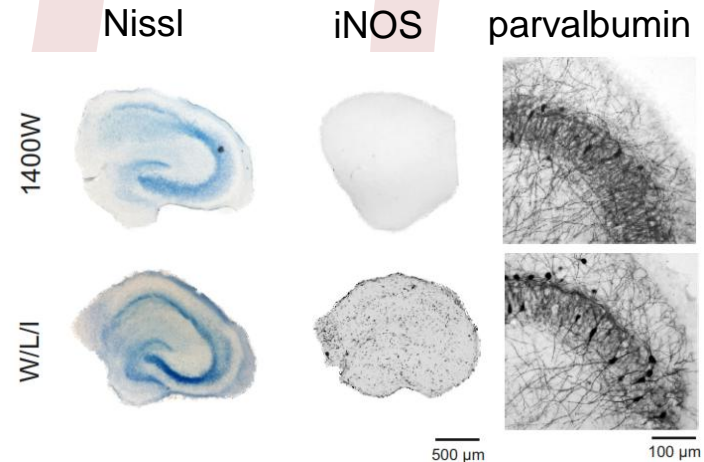
	LPS	IFN γ	LPS + IFN γ	NMDA + KA
De-ramification	-	-	+++	+++
Proliferation	-	+	+++	+++
Somatic shape / size	+	<i>n.a.</i>	<i>n.a.</i>	<i>n.a.</i>
Proinflammatory cytokine secretion	++	-	+	-
iNOS upregulation	+	+	+++	<i>n.a.</i>

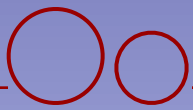
n.a. = not analyzed

iNOS and neurodegeneration



**iNOS
blockade
(1400W)
rescues
neurons**

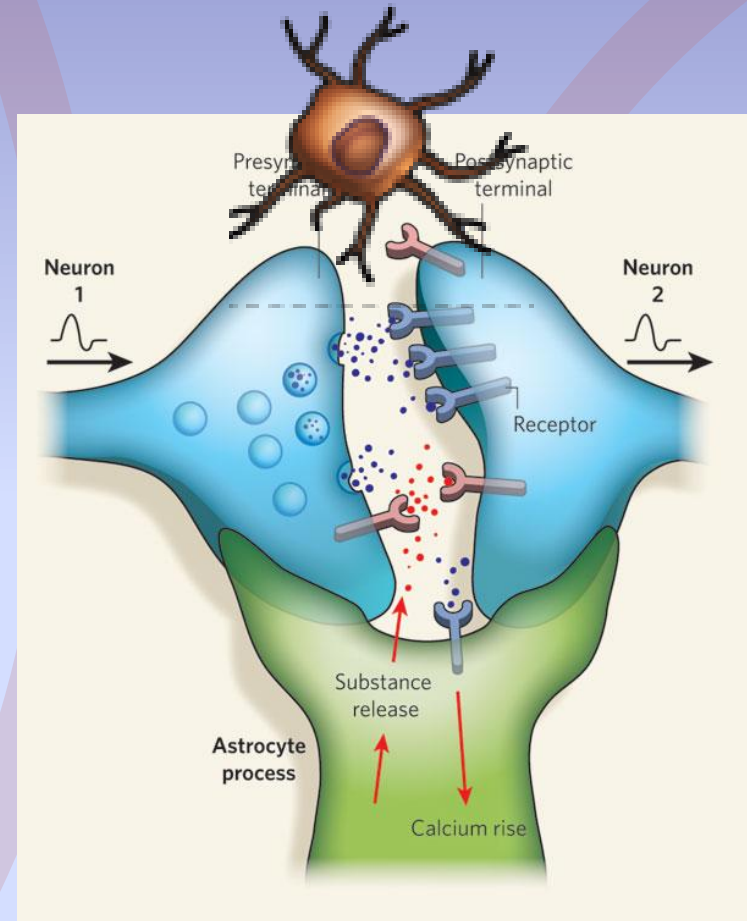
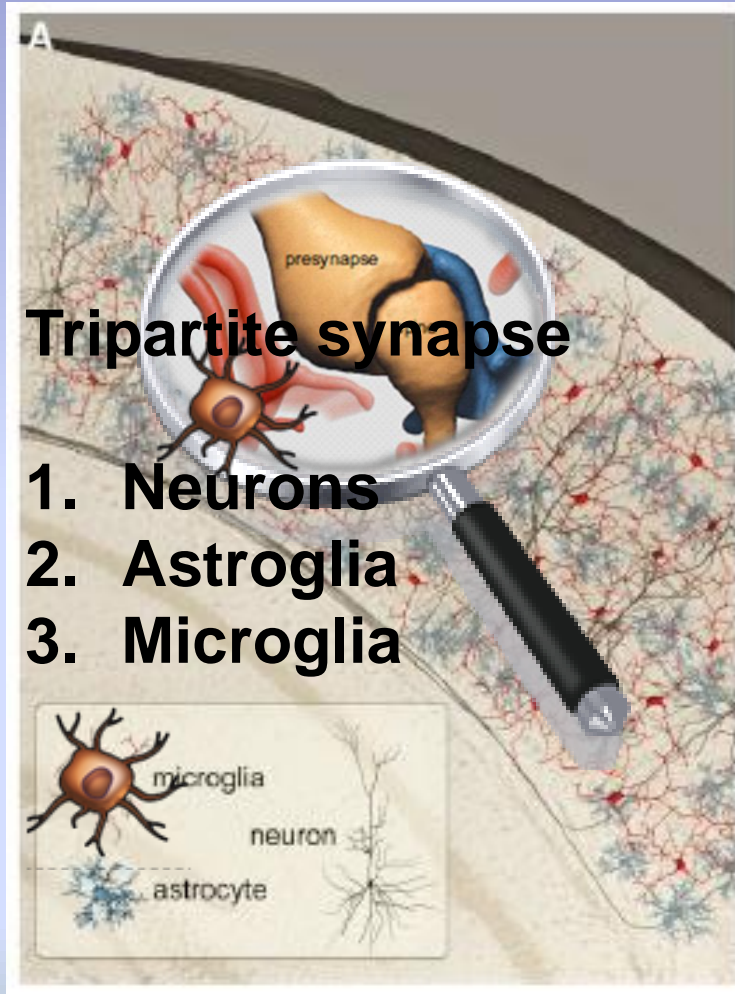




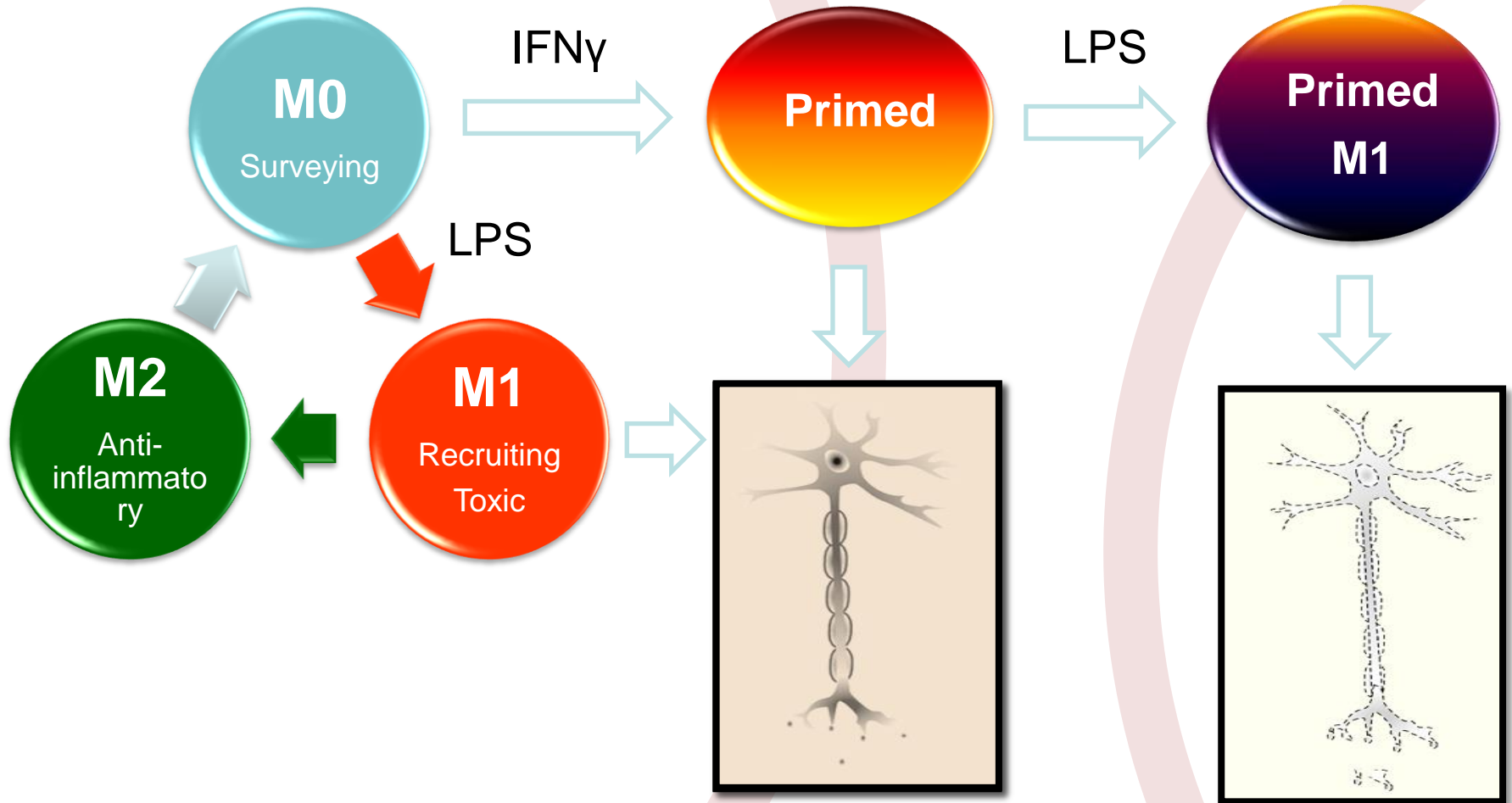
Results I

- Classical microglial activation with LPS or priming with IFN γ is **not neurotoxic** *in situ*
- Interferon γ **priming triggers** LPS-mediated neurotoxicity
- LPS/IFN γ neurotoxicity
 - **Without T-cell** invasion
 - With upregulation of iNOS
 - **Without upregulation** of proinflammatory cytokines

Microglia as neuronal sensors and effectors

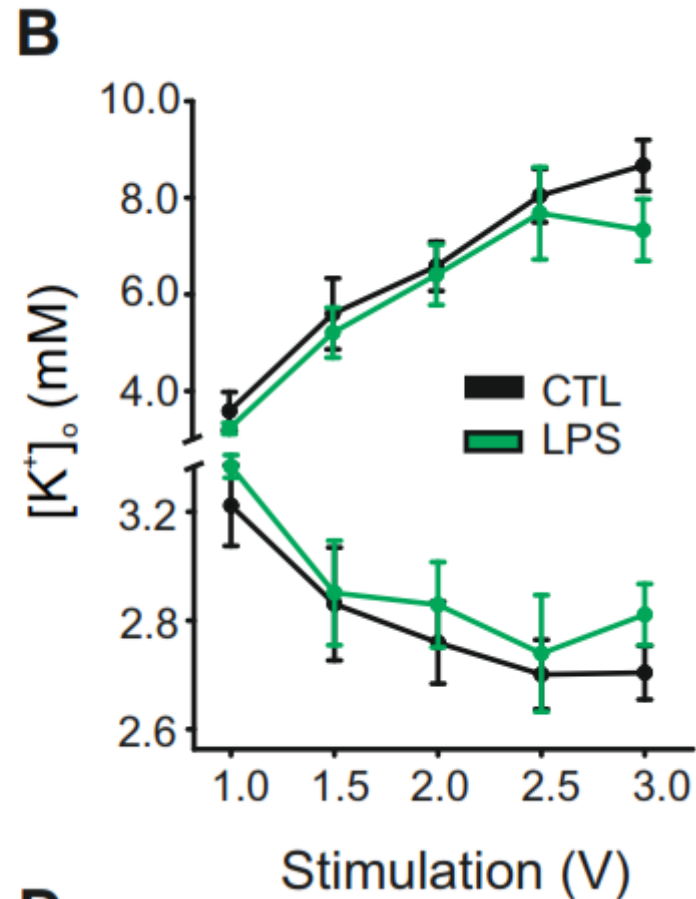
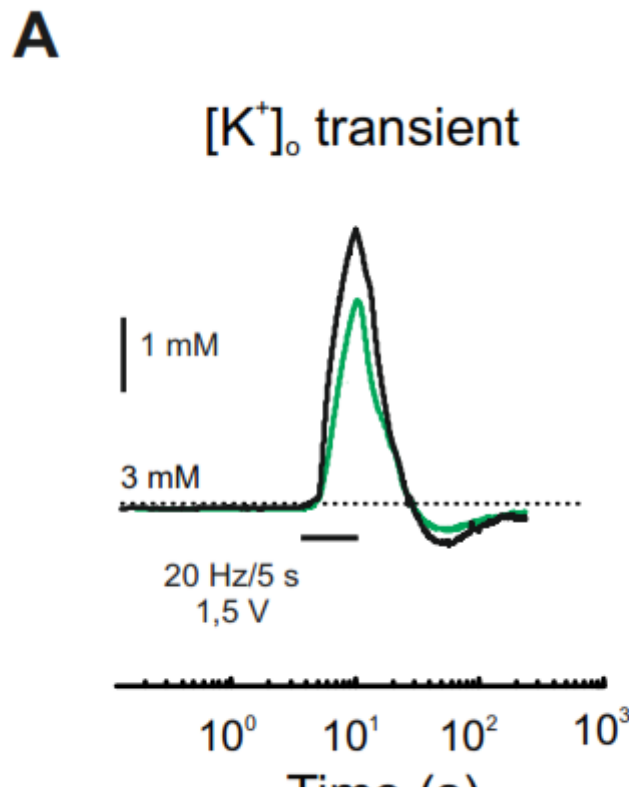


Neuronal functional status ?



Subramanian 2014; **Perry and Holmes, 2014**; Venero et al., 2011; Baylock and Maroon, 2011

Classical activation effect on potassium homeostasis

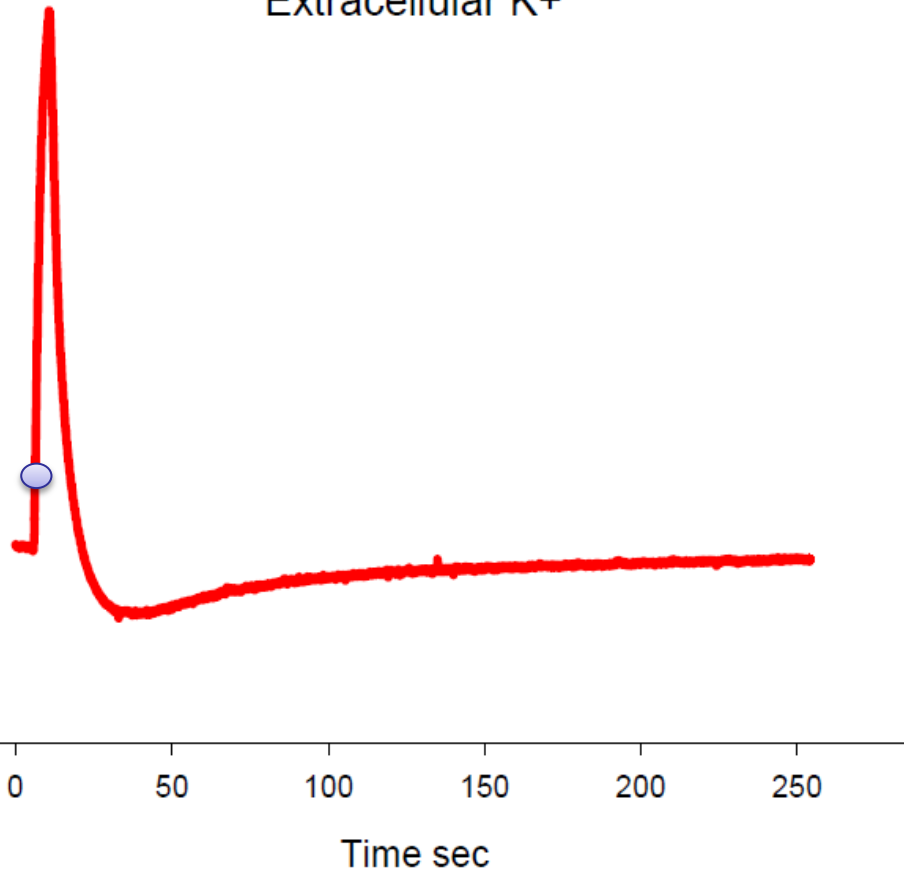


C

D

Why caring about potassium homeostasis ?

Extracellular K⁺



Rising Peak

Neuronal membrane repolarization
Astroglial-mediated K⁺ uptake

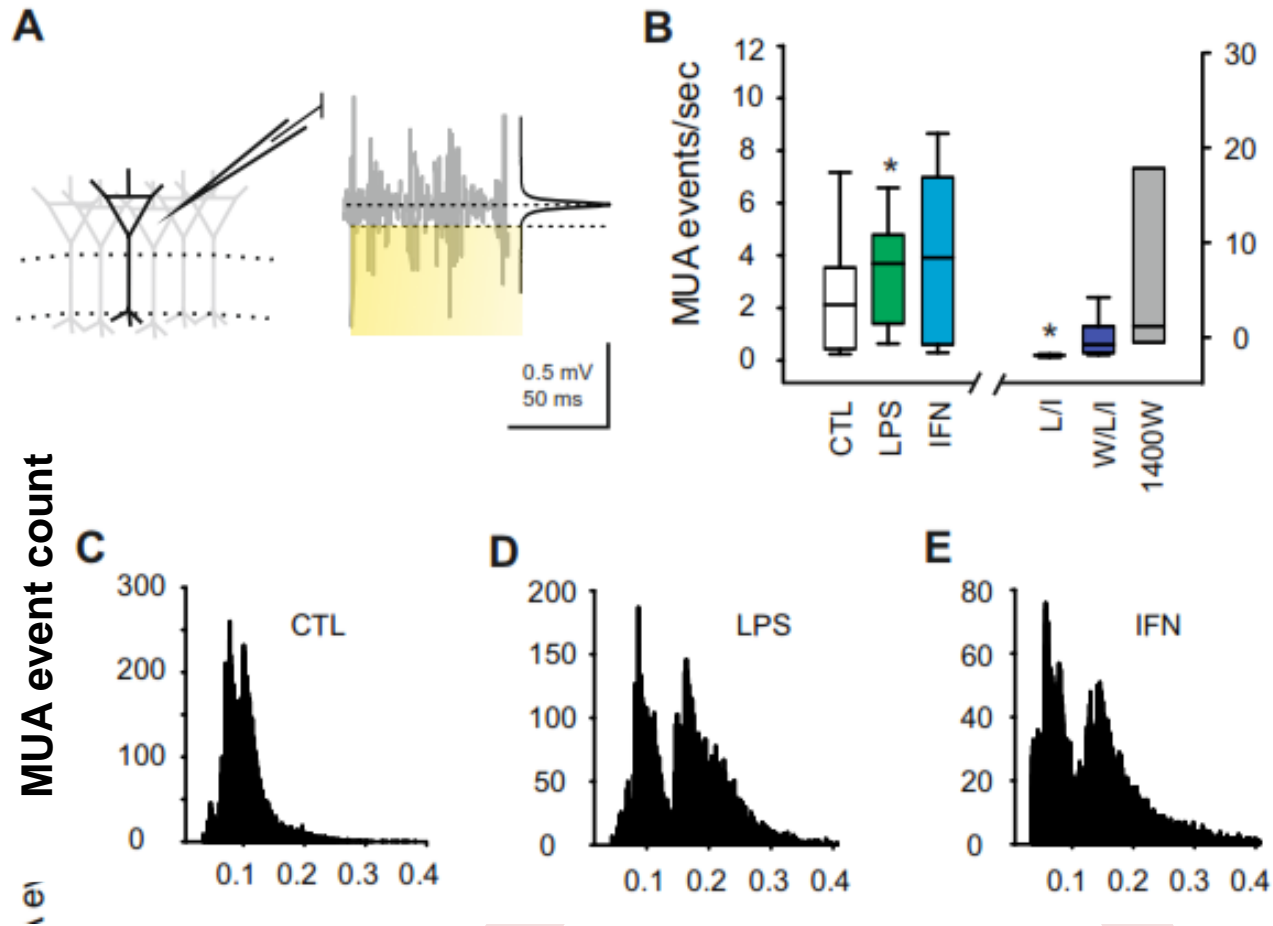
Decaying

K⁺ re-uptake by neurons and astroglia, Na-K-ATPase > metabolically sensitive!

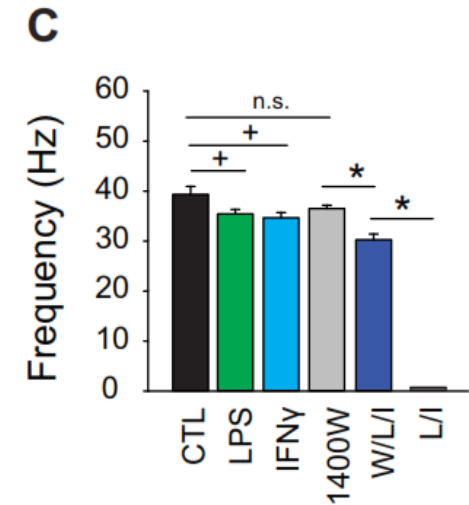
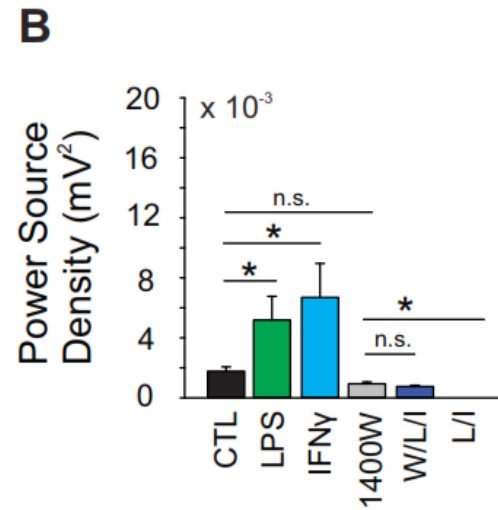
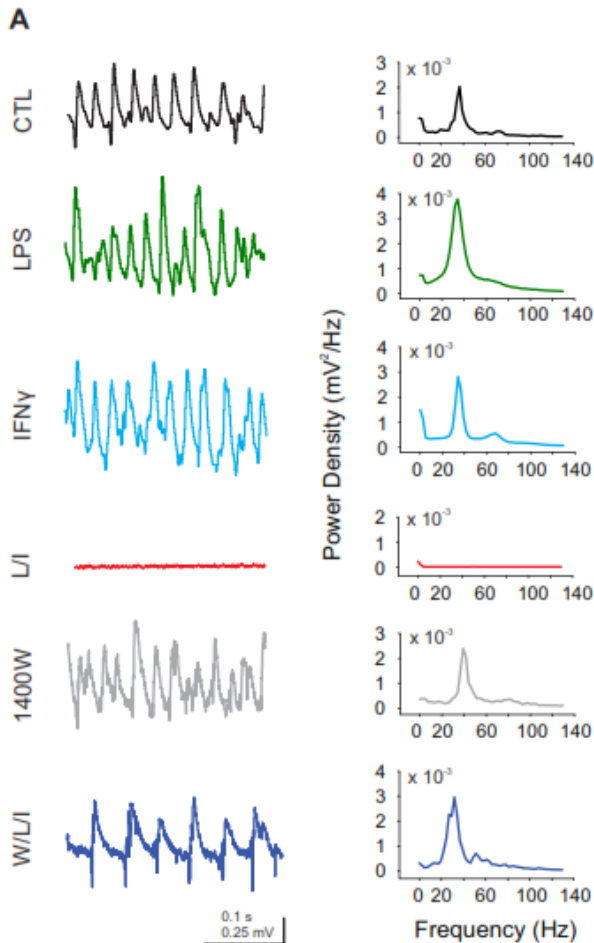
Undershoot

K⁺ spatial "buffering" in gap junction coupled astroglial syncytia

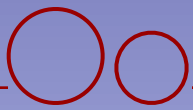
Enhanced spontaneous neuronal activity upon classical activation and priming



Modulation of γ -oscillation's amplitude and frequency



Acetylcholine/Physostigmine induced oscillations in CA3 str. pyramidale



Results II

Classical activation or IFN γ priming

Slightly INCREASE neuronal activity

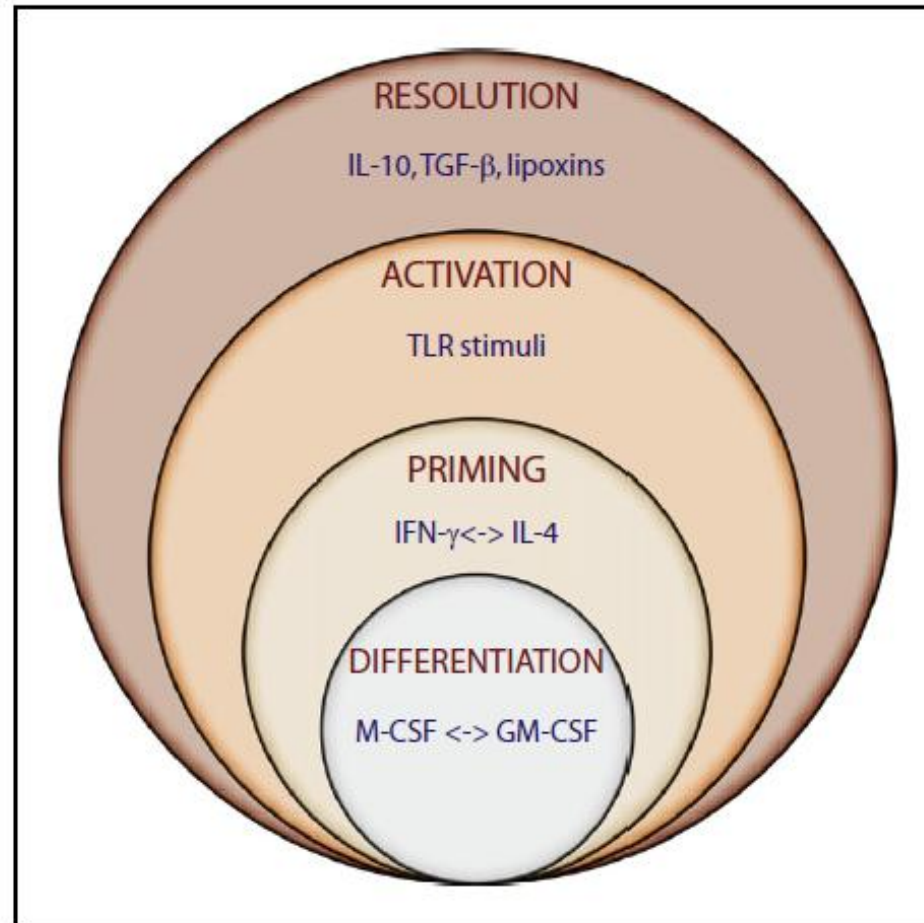
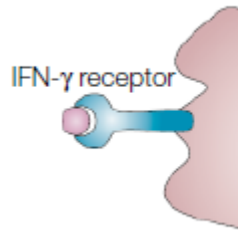
EXCITATION/INHIBITION (E/I) RATIO

Shown by:

1. Spontaneous activity
2. Oscillations
3. Electrically evoked postsynaptic responses

Literature evidence

c Classical activation



Microbicidal
Tissue damage
Cellular immunity
DTH

Literature evidence



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Available online at www.sciencedirect.com



Immunobiology 211 (2006) 511–524

Immunobiology

www.elsevier.de/imbio

REVIEW

Signal integration between IFN γ and TLR signalling pathways in macrophages

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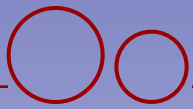
^c*School of Molecular and Microbial Sciences, University of Queensland, Brisbane, QLD 4072, Australia*

^d*Special Research Centre for Functional and Applied Genomics, Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD 4072, Australia*

Received 2 May 2006; accepted 23 May 2006

Norberg et al., 2005

Hellstrom et al., 2005



Contribution to the literature

- Introduction of OHSCs as an ***in situ* model** **which dissects** microglia from blood-born immune cells
- Microglia *in situ* are not IFN γ -primed
- Enhance the evidence that classical activation of non-primed microglia is **not neurotoxic**
- **Electrophysiological** consequences of classical activation and IFN γ -priming on neurons



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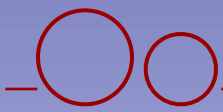
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Dr. Richard Kovacs
Dr. Siegrun Gabriel
Andriani Fetani & Kristin Lehmann
Katrin Schulze

Dr. Nikolaus Maier
Dr. Benedikt Salmen

Charité



Thank you for attending !!!





EXTRA SLIDES

■	CTL	control, no medium exchange
■	LPS	lipopolysaccharide 10 µg/ml
■	IFN γ	Interferon gamma 100 ng/ml
■	N/K	NMDA 5µM + kainate 5 µM
■	L/I	lipopolysaccharide 10 µg/ml + Interferon gamma 100 ng/ml
■	W/L/I	1400W 100 µg/ml + lipopolysaccharide 10 µg/ml + Interferon gamma 100 ng/ml
■	1400W	1400W 100 µg/ml

Some discussion points

- Is LPS activating or priming ?
 - Definitely activating , secretion of proinflammatory cytokines is against priming definition
- Activation & inhibition of iNOS in vivo ? Known results and perspectives? Is it a pharmacological target? (check literature)
- The secretion of proinflammatory cytokines under 1400w blockade has not been investigated but is not expected to change, unless a positive feedback loop (check literature).
- What is the ramification state of microglia under 1400W blockade??? Do they derammify or not at all??? If not at all, most likely deramification comes as result to CELL DEATH (secondary), and not primary to LPS-IFN γ activation (**Additional slide !!!**)
- IFN γ production after LPS stimulation:
 - Untriggered microglia do not produce IFN-gamma (primary culture)
 - Kawanoguchi et al., **2006** *Multiple sclerosis*
 - IFN gamma mRNA is not detected after LPS stimulation (OHSCs)
 - Fu et al **2010** *J Neuroinflammation*
- Is IFN γ priming specific for microglia?
 - See Häusler et al., 2002



**Microglial
deramification :**
Toxic phenotype
OR
secondary to
degeneration ???

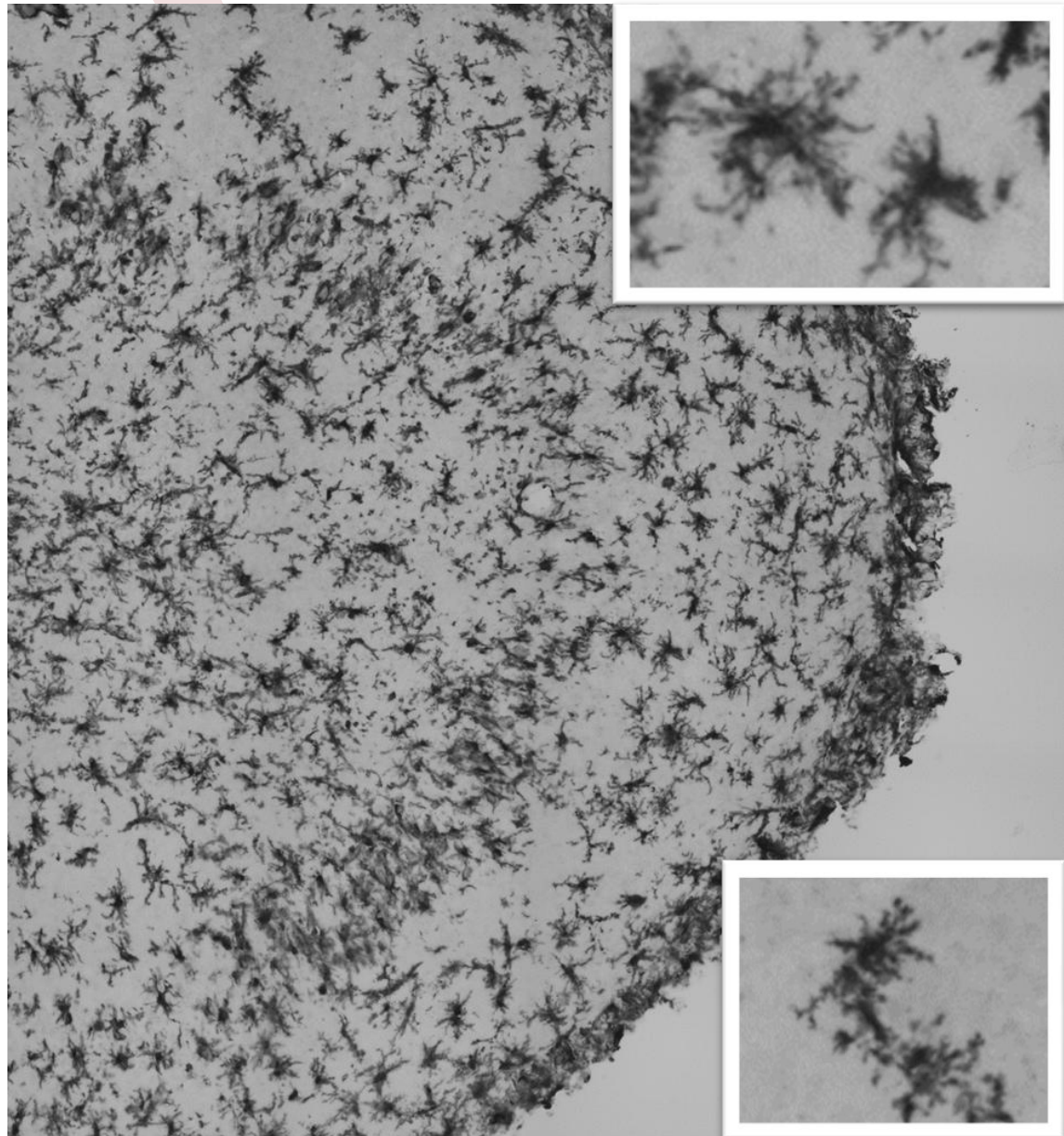
Primed, classically activated
microglia

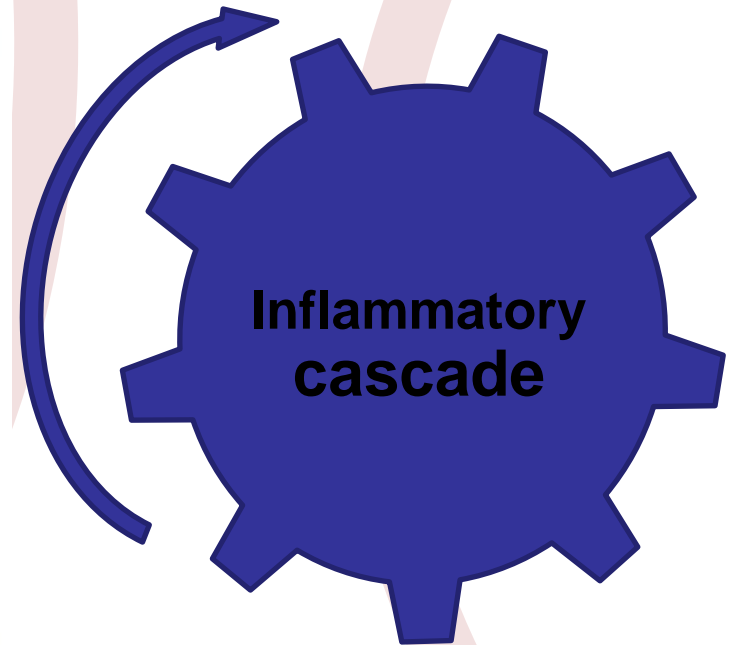
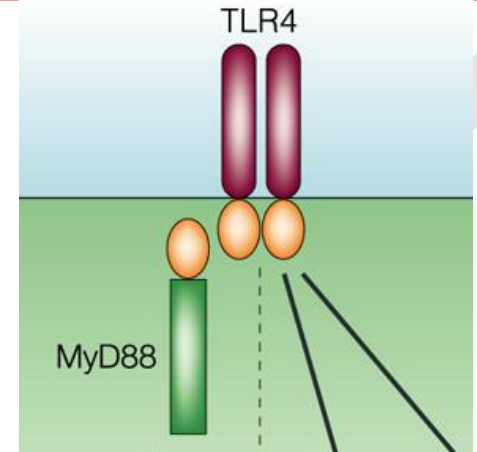
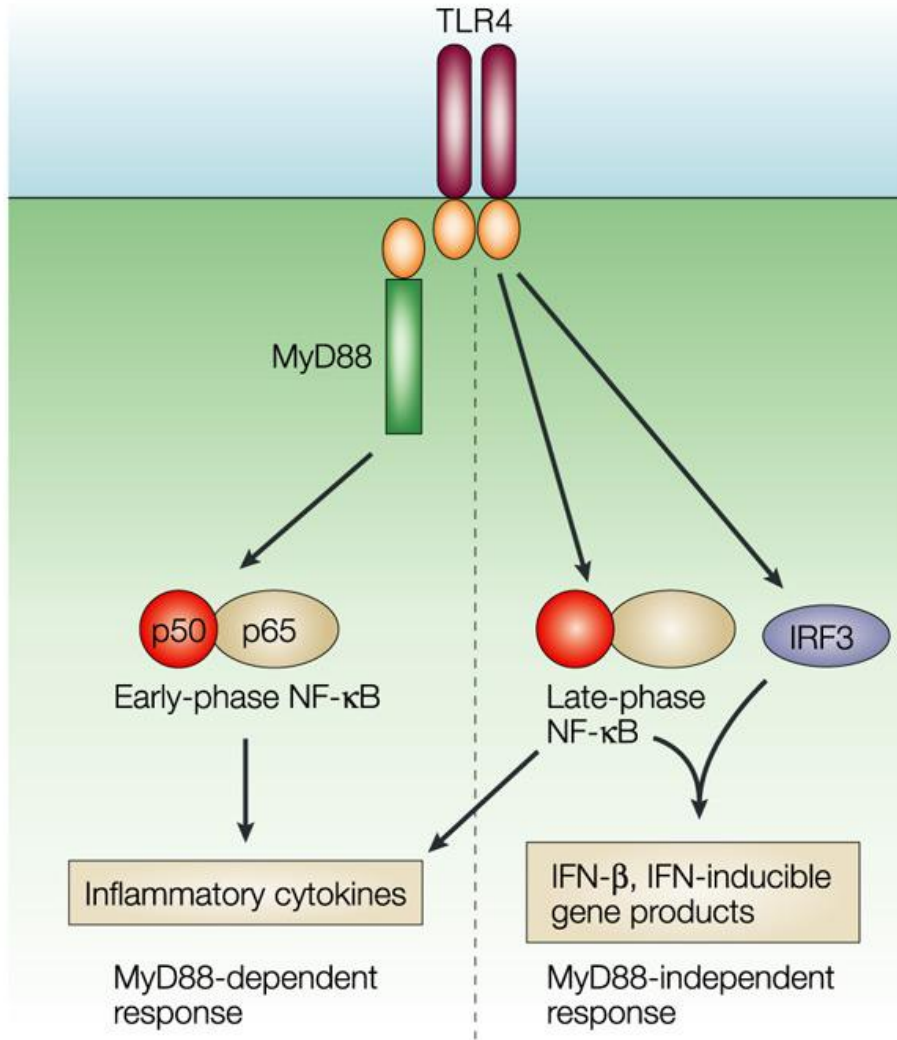
DO NOT DERAMIFY

Upon iNOS blockade

Likely due to lack of
degeneration

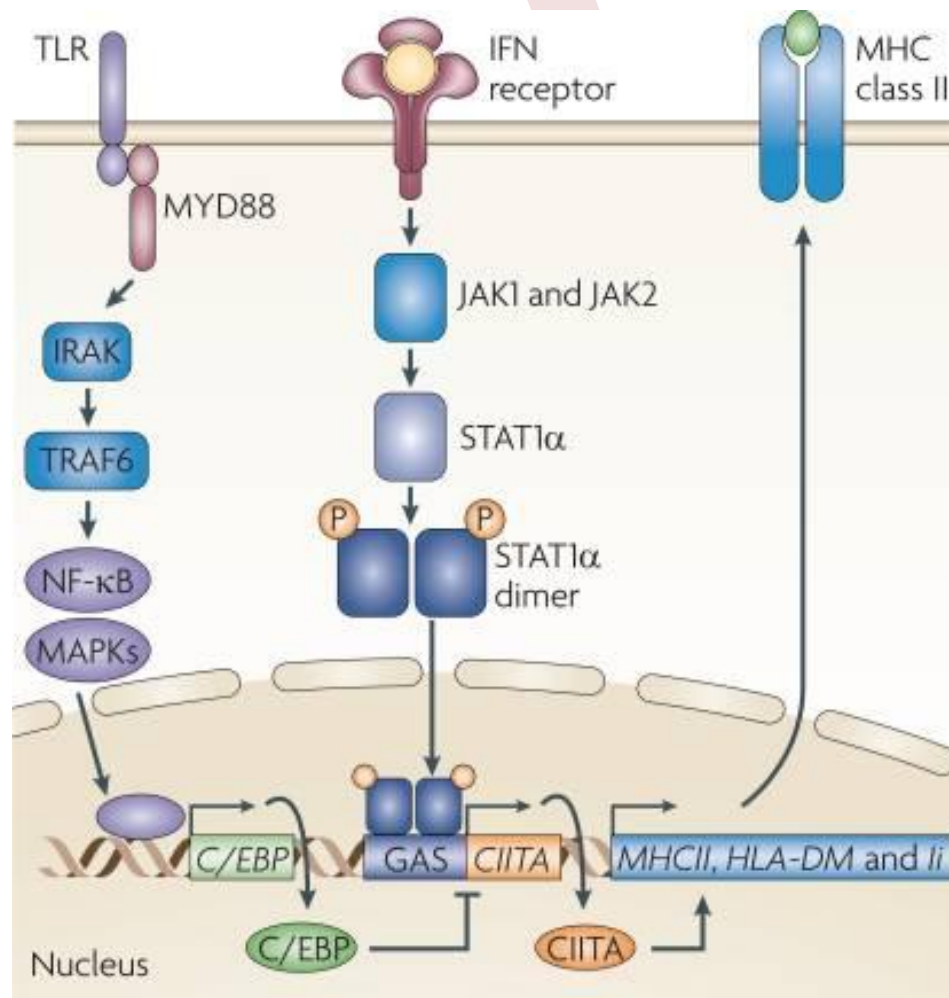
1400W+LPS+IFN γ
Iba-1 immunohistochemistry







Nature Reviews | Immunology

IFN γ signaling is necessary for antigen presentation



Harting, Nat Rev Microbiol. 2010 April; 8(4): 296–307.

IFN-gamma

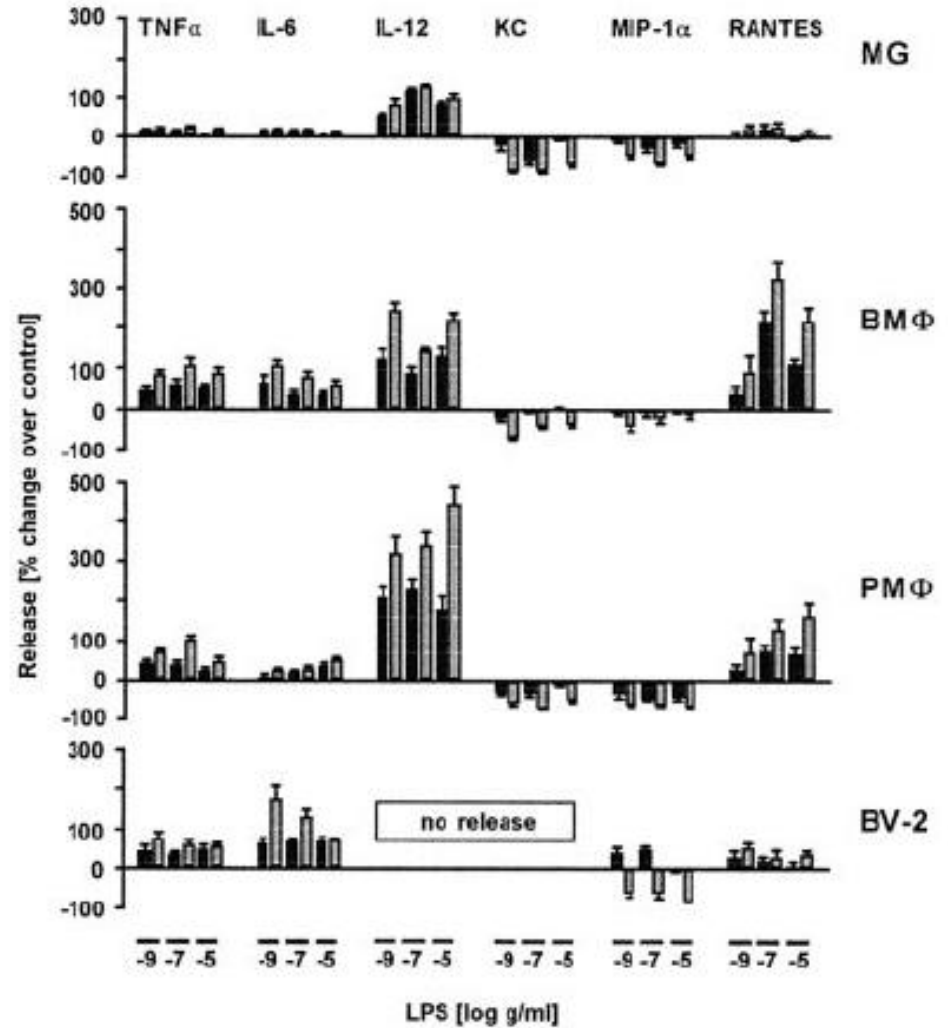
0.1 ng/ml 
 10 ng/ml 

MG = microglia

BMφ = bone marrow macrophages

PMφ = peritoneal macrophages

BV2 cells = immortalized mouse primary microglial cell line



Astroglial activation

