Neuroinflammation in depression: microglia activation and dysfunction

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Strategic Program Areas:

- **Drug Discovery**
- Drug Design
- Drug Development
- Drug Usage

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9th International Symposium on Neuropsychiatry & HIV

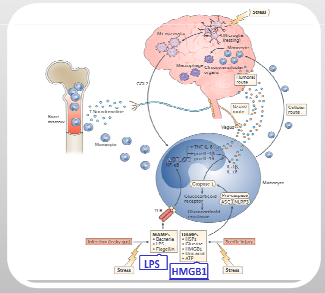
Barcelona, May 26 - 28, 2016

Conference Presentation Synopsis

People with HIV infection suffer from depression and inflammation spreading

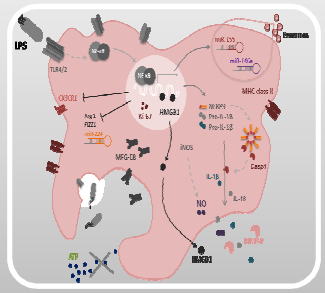
- Decline in attention, learning and executive function are associated to depression
- Elevated viral load in CNS - HIV crosses BBB (cell-free virus and within infected monocytes and T cells)
- Increased production of IL-1β and TNF-α and passage through BBB to activate microglia and astrocytes

http://slideplayer.com/slide/3023319/



Depression is a disorder of multifactorial origin, often associated with neuroinflammation

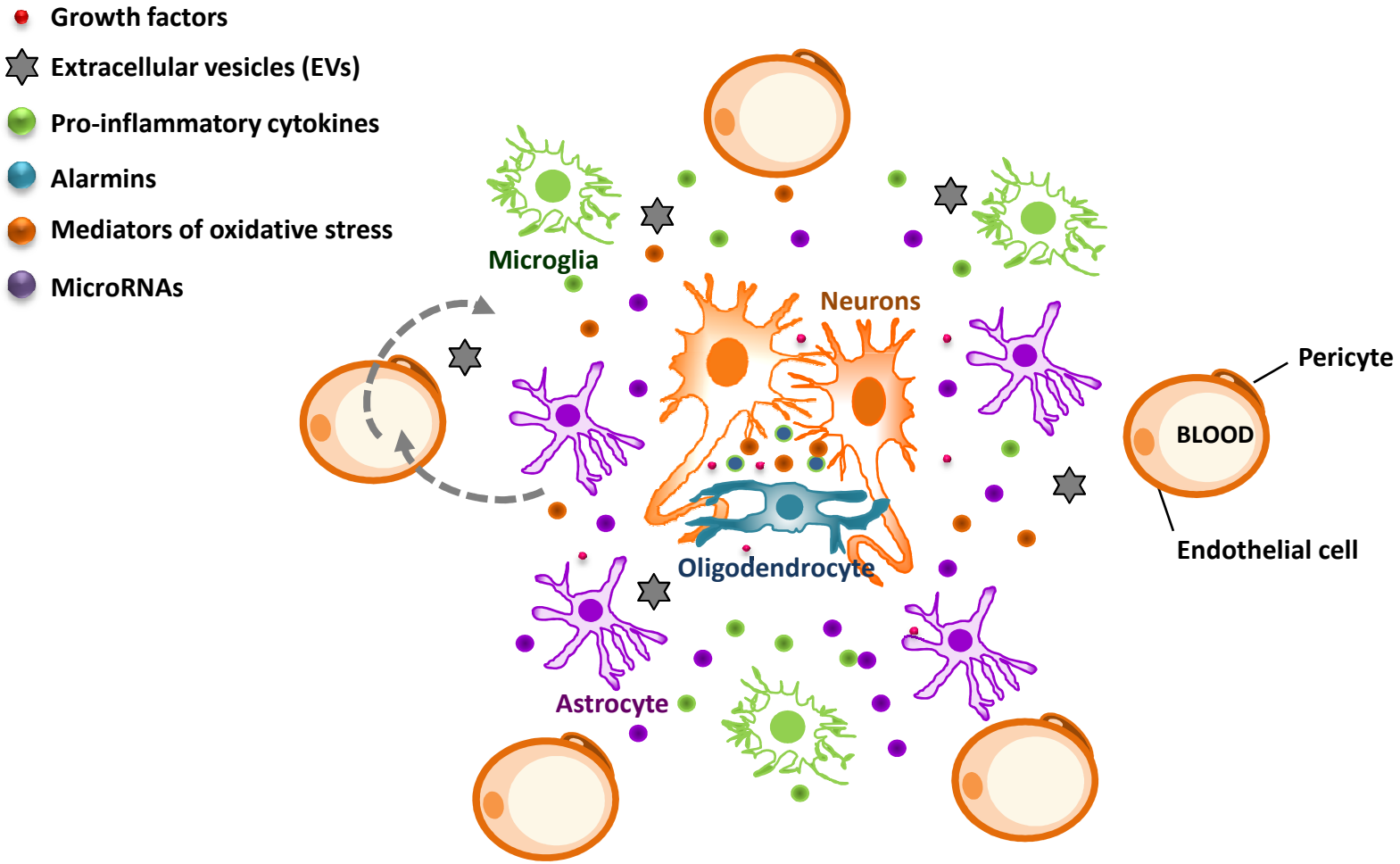
- **Stress**/Impaired neurogenesis/Defects in synaptic plasticity
- Loss of oligodendrocytes
- Astrocyte deficits (low density of GFAP+ cells)
- **Abnormal activation of microglia (excessive cell activation and increased cell number,microglia decline and senescence)**



Development of symptoms of depression results from chronic inflammation and impaired microglia function (microgliopathy)

- **Increased levels of proinflammatory cytokines**
- **NLRP3-inflammasome activation in microglial cells**
- **Induced nuclear factor kappaB (NF-kB) inflammatory pathway**
- **Pathogenic extracellular vesicles/exosomes (altered microRNA cargo? Not known)**

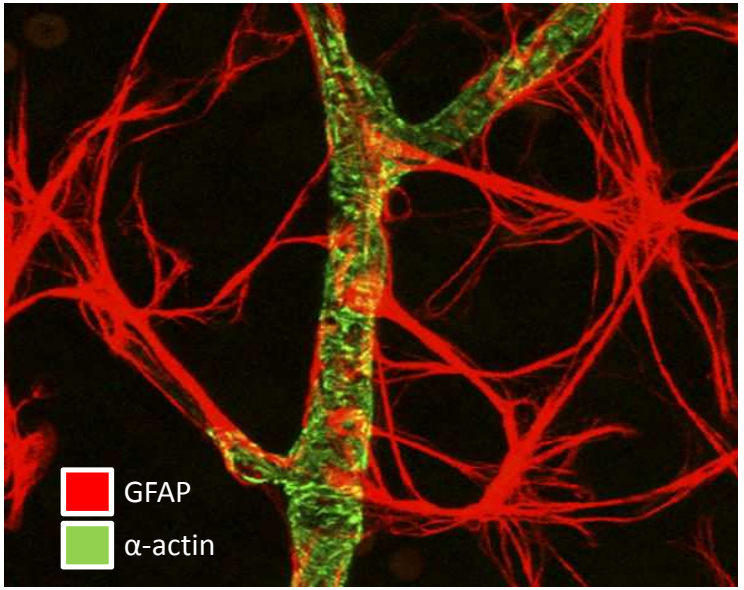
Neuron glia biology in health and disease



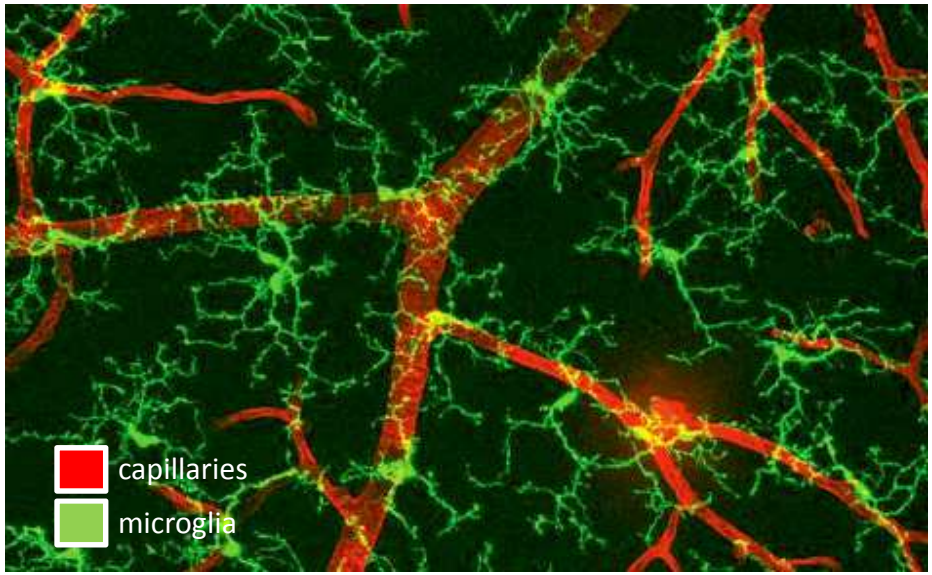
Adapted from: http://imed.ulisboa.pt/research/program-areas/drug-discovery/neuron-glia-biology-in-health-and-disease/

Role of astrocytes and microglia in homeostatic conditions

- **Astrocytes** are the most abundant cells in the central nervous system (CNS) that provide nutrients, recycle neurotransmitters and contribute to **intercellular network homeostasis**
- **Astrocytes** also regulate neuronal functions including the generation of new nerve cells and participate in functional **synapse remodeling**



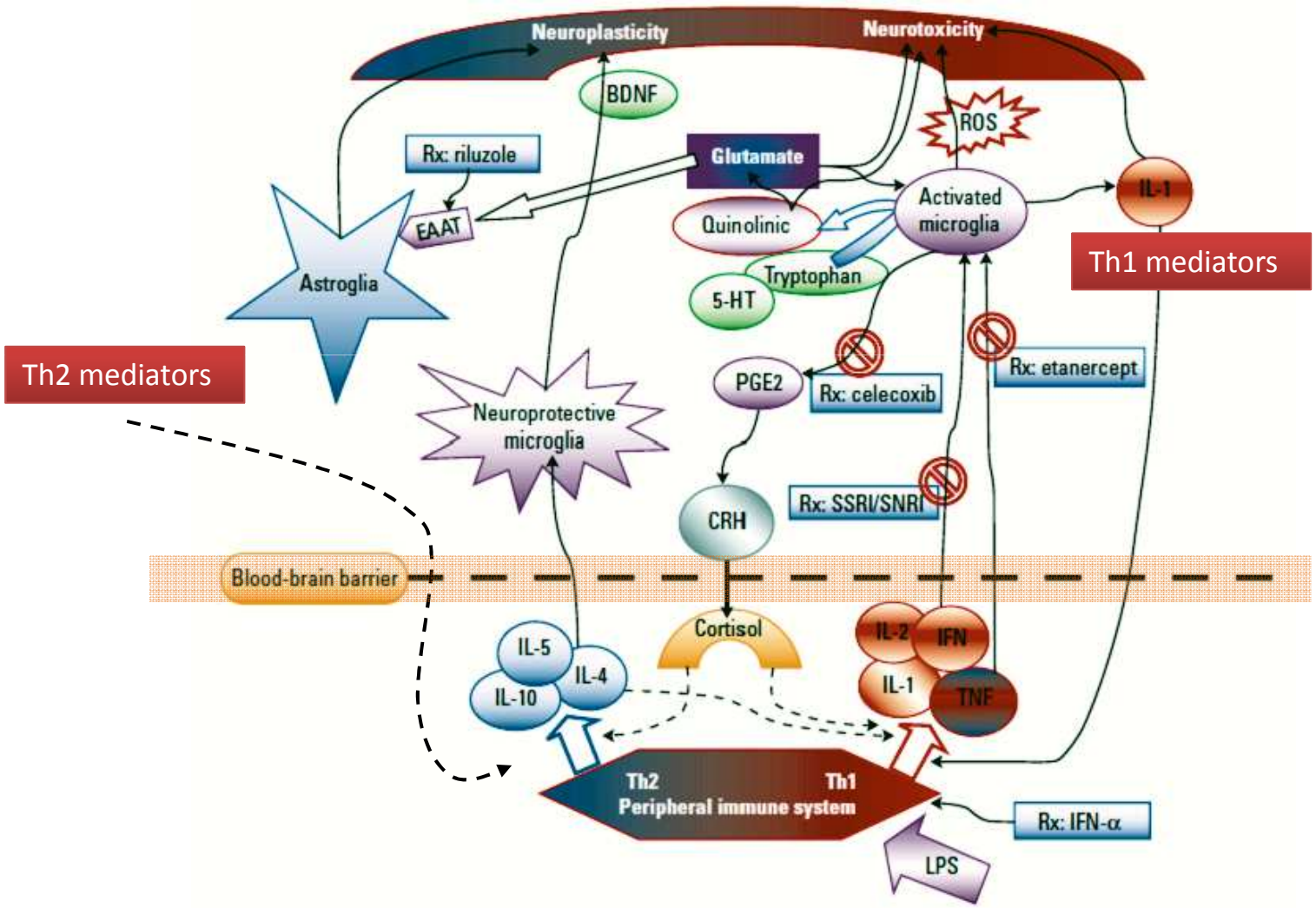
Pekny and Pekna , Physiol Rev 2014



http://usceye.org/vitreoretinal-correction/

- **Microglia** play a role in the innate immunity
- **Microglia** are involved in neural plasticity (**synapse remodeling**) and **homeostasis**
- **Microglia** have **highly motile processes** to monitor the microenvironment
- **Microglial** reactivity to stimuli can be **reparative** but if in excess is **detrimental**

Dysruption of neuroprotective/neurotoxic balance may lead to neuroinflammation and depression



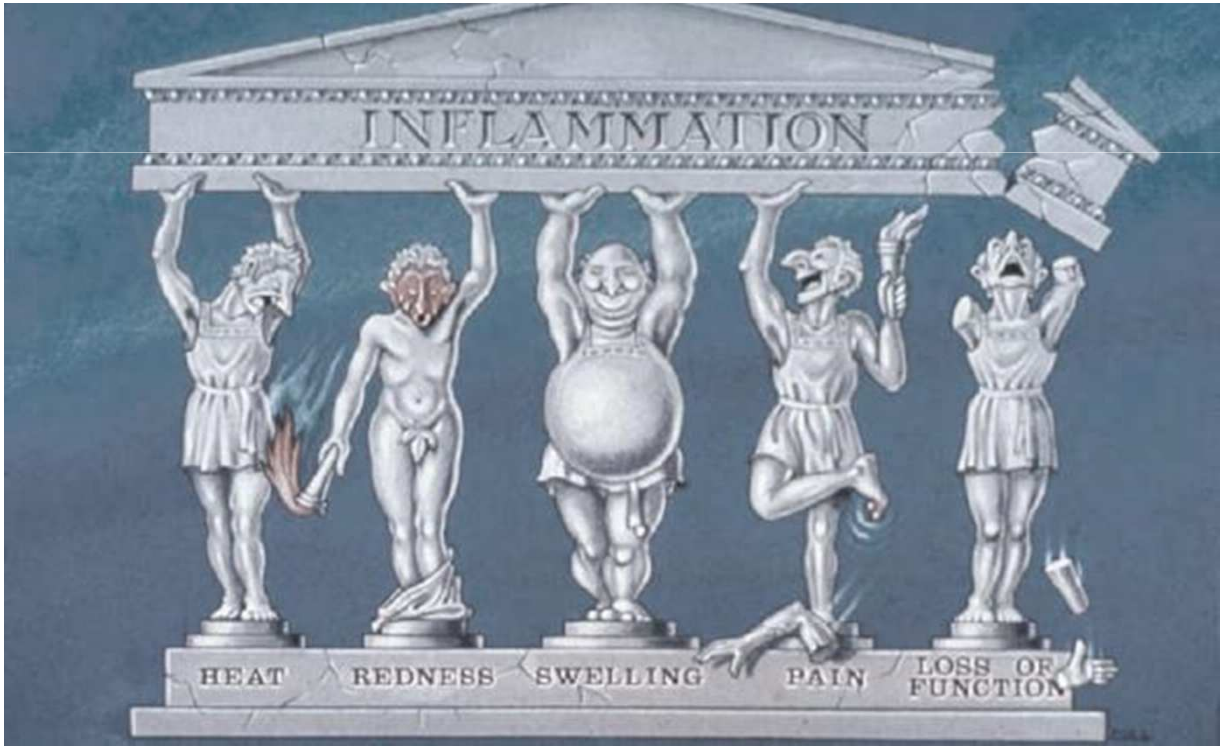
From Mcnally et al. CNS Spectr 2008, 13, 501-10

Inflammation in Chronic HIV Infection

"HIV-infected persons have persistent, **low-grade inflammation and immune activation** that are strongly associated with a heightened risk for **depression**"

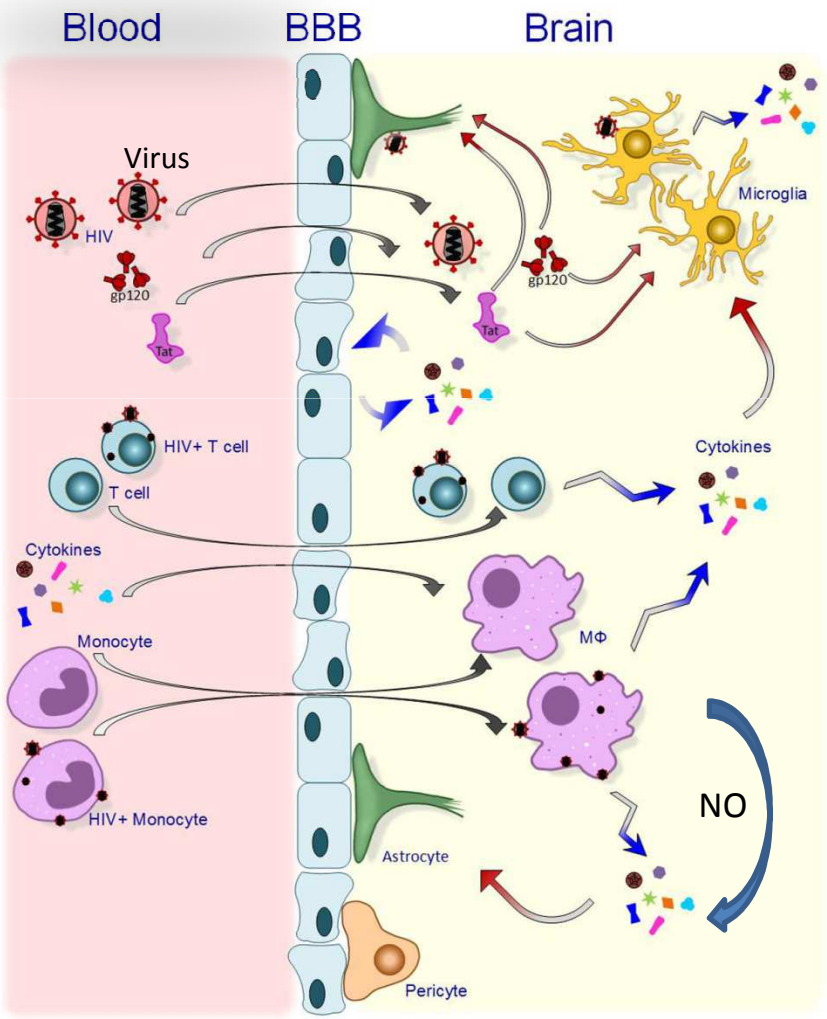
Erlandson and Campbell J Infect Dis. 2015

http://www.thebodypro.com /content/art58344.html



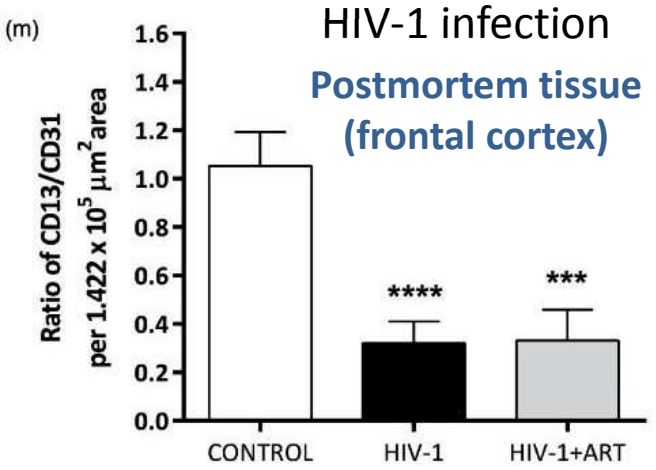
http://www.precisionnutrition.com/research-review-inflammation-exercise

Viral and cellular transmigration from peripheral blood to the brain in neuroinflammation by HIV



Adapted from Hong and Banks Brain Behav Immun 2015

HIV-infected monocytes and **T cells** not only infect brain resident cells upon migration into the CNS but also produce proinflammatory cytokines, such as **TNF-α and IL-1β** that further **activate microglia and astrocytes**, while causing a **diminished BBB coverage by pericytes**



ART - antiretroviral therapy
CD13 – pericyte marker
CD31 – endothelial cell marker

From Persisky et al J Cereb Blood Flow Metab. 2016

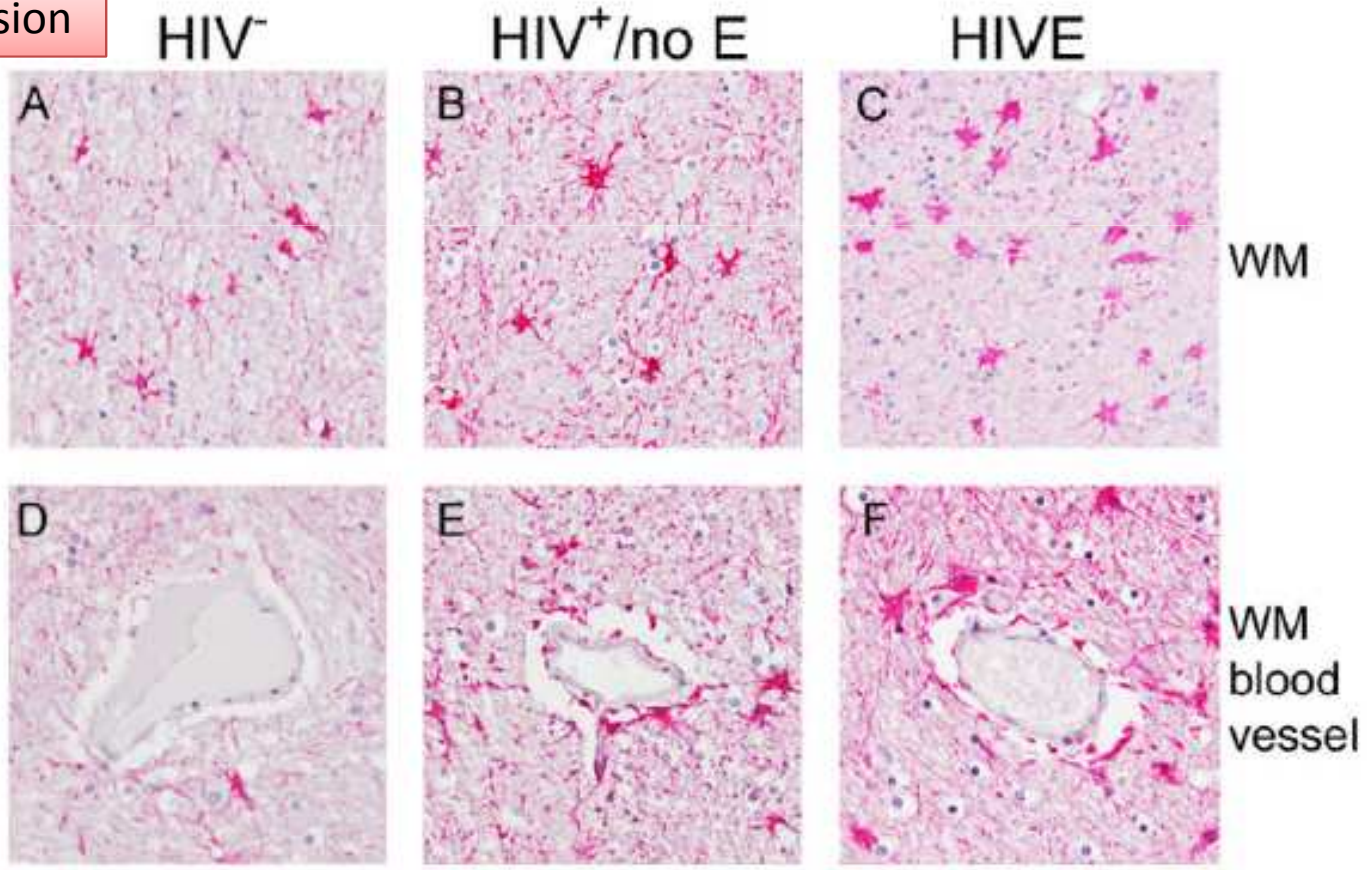
GFAP expression and astrocyte morphology in HIV infected patients with/without encephalitis

DENSITY OF GFAP-IMMUNOREACTIVE ASTROCYTES IS DECREASED IN LEFT HIPPOCAMPI IN MAJOR DEPRESSIVE DISORDER

J. A. COBB,[a†] K. O'NEILL,[a†] J. MILNER,[a] G. J. MAHAJAN,[a] T. J. LAWRENCE,[a] W. L. MAY,[b] J. MIGUEL-HIDALGO,[a] G. RAJKOWSKA[a] AND C. A. STOCKMEIER[a,c*]

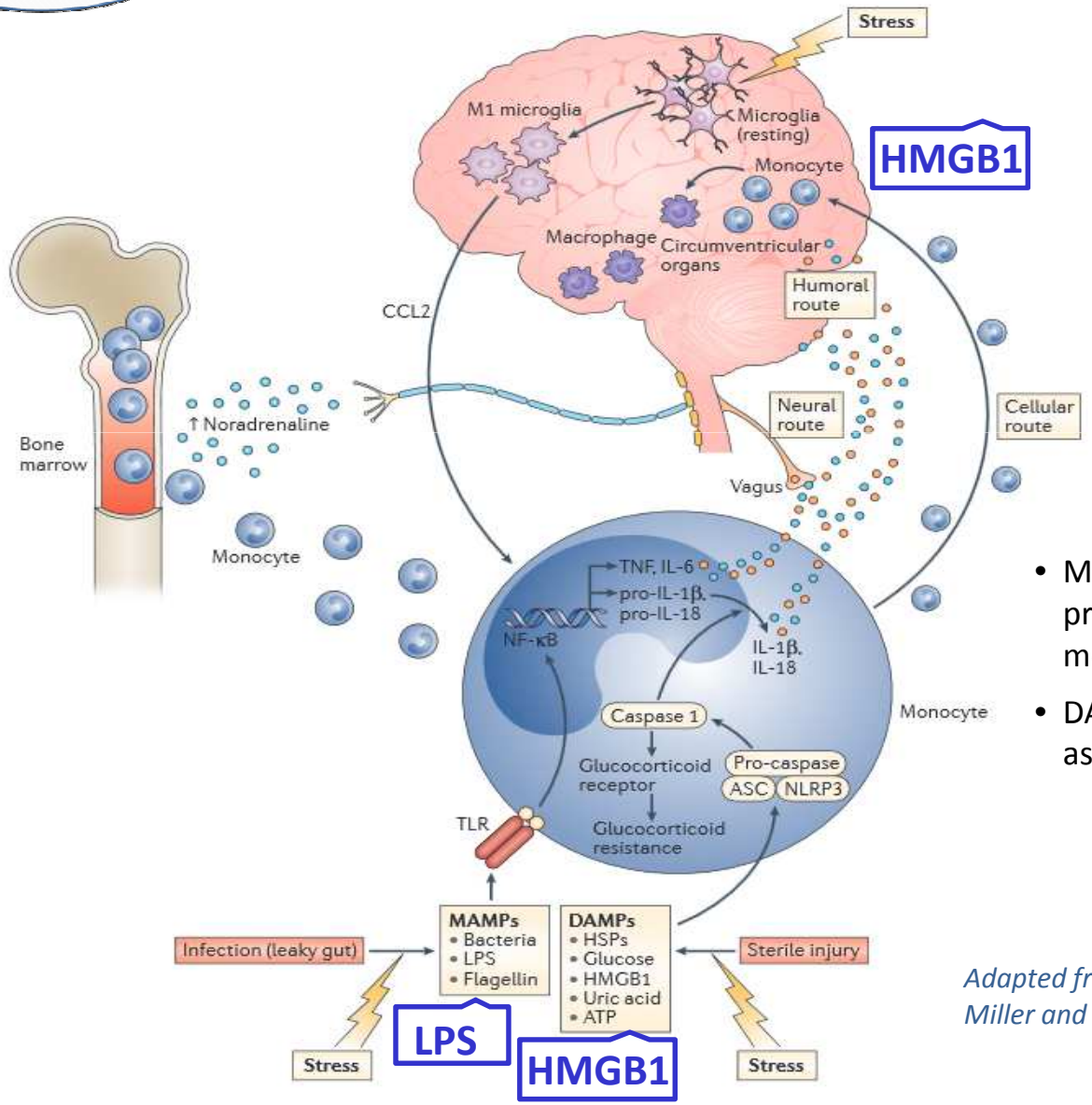
Neuroscience 316 (2016) 209–220

GFAP expression



Tavazzi et al Curr HIV Res 2014

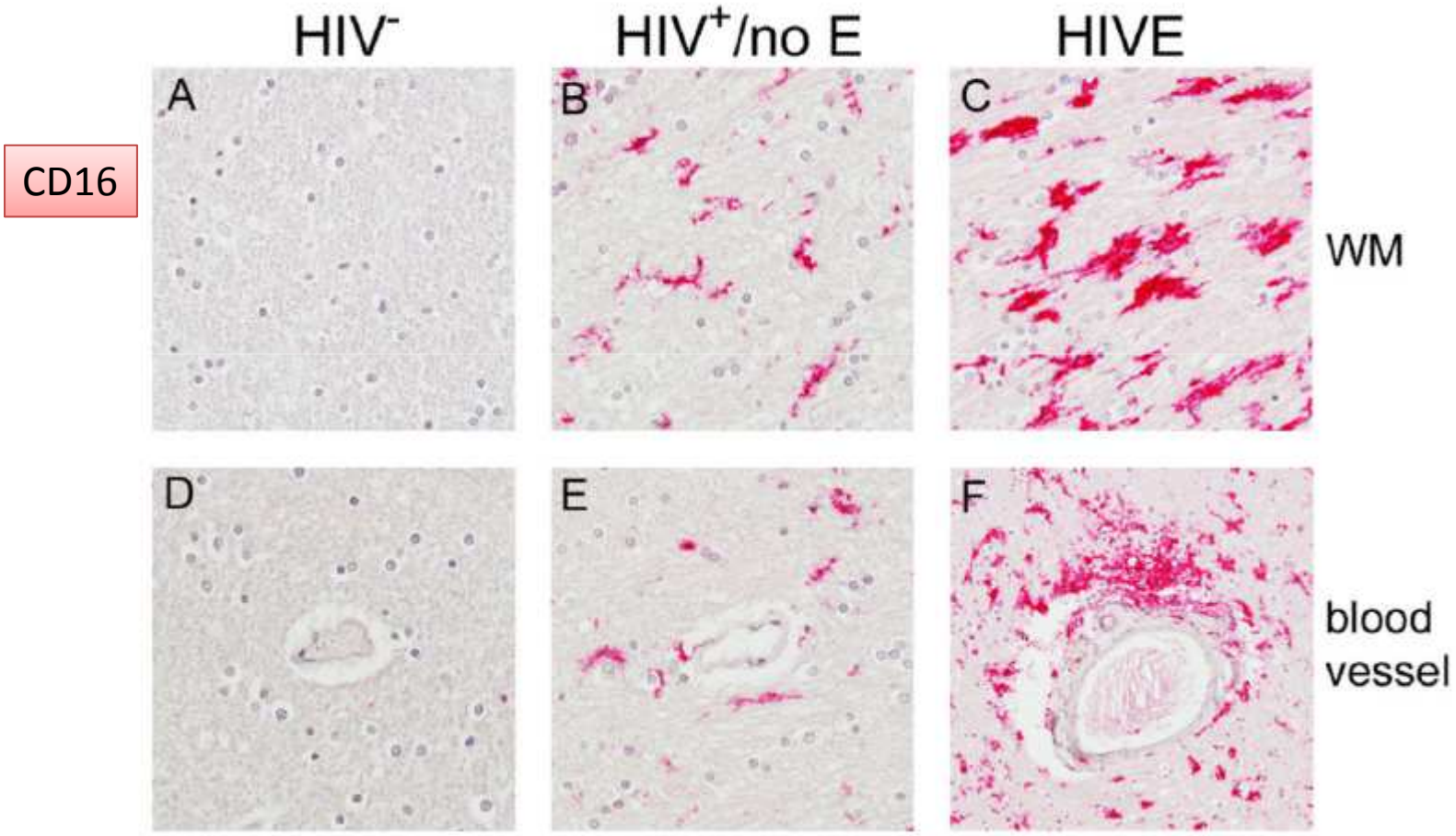
Transmission of stress-induced inflammatory signals from the periphery to brain



- MAMPs - Bacteria and bacterial products such as microbial-associated molecular patterns leaked from the gut
- DAMPs - Stress-induced damage-associated molecular patterns

Adapted from:
Miller and Raison Nature Reviews|Immunology 2016

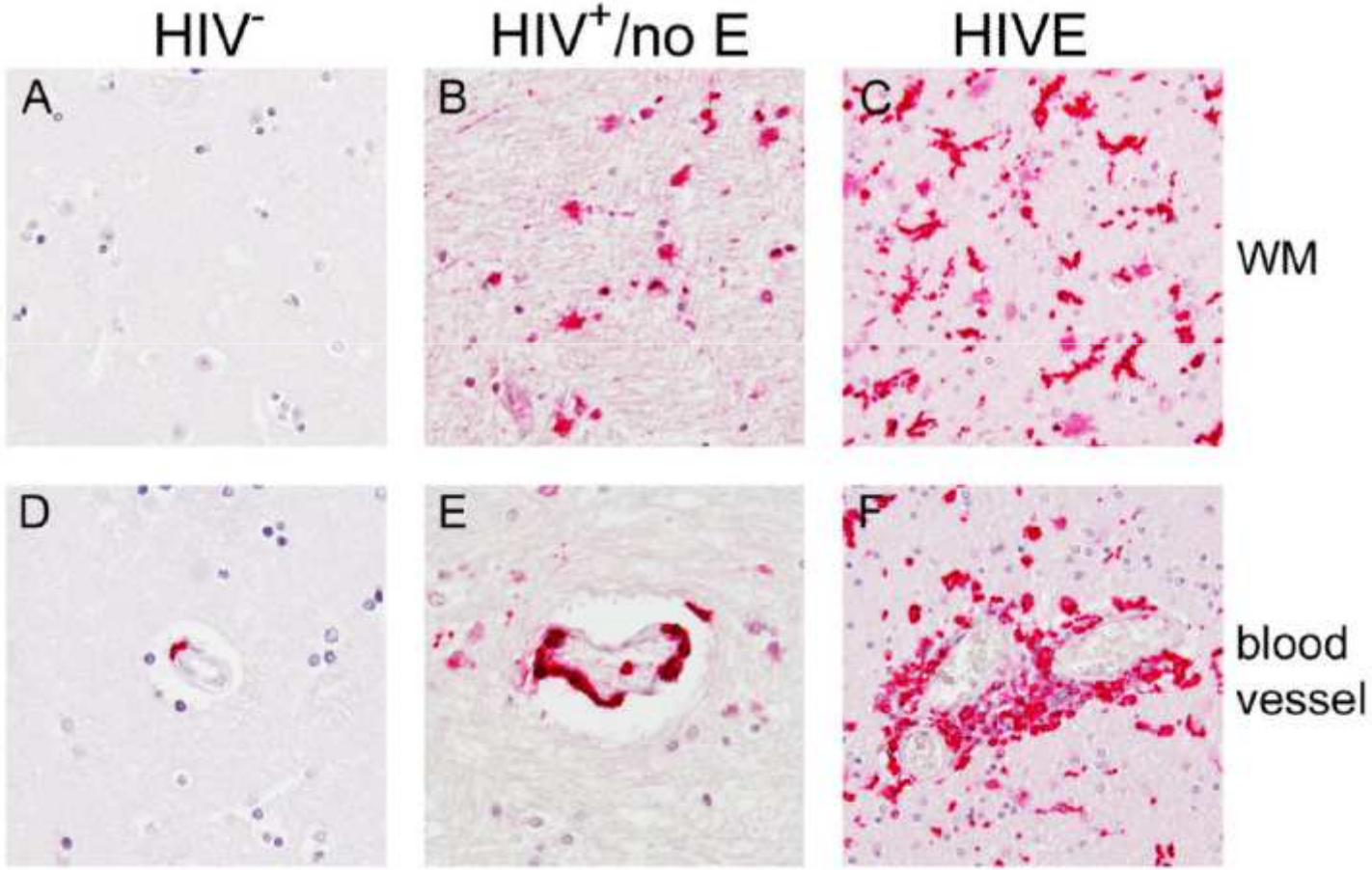
CD16^{+} MΦs/microglia are observed in the CNS of HIV+ patients with/without encephalitis



Tavazzi et al Curr HIV Res 2014

CD163^{+} MΦs/microglia are observed in the CNS of HIV+ patients with/without encephalitis

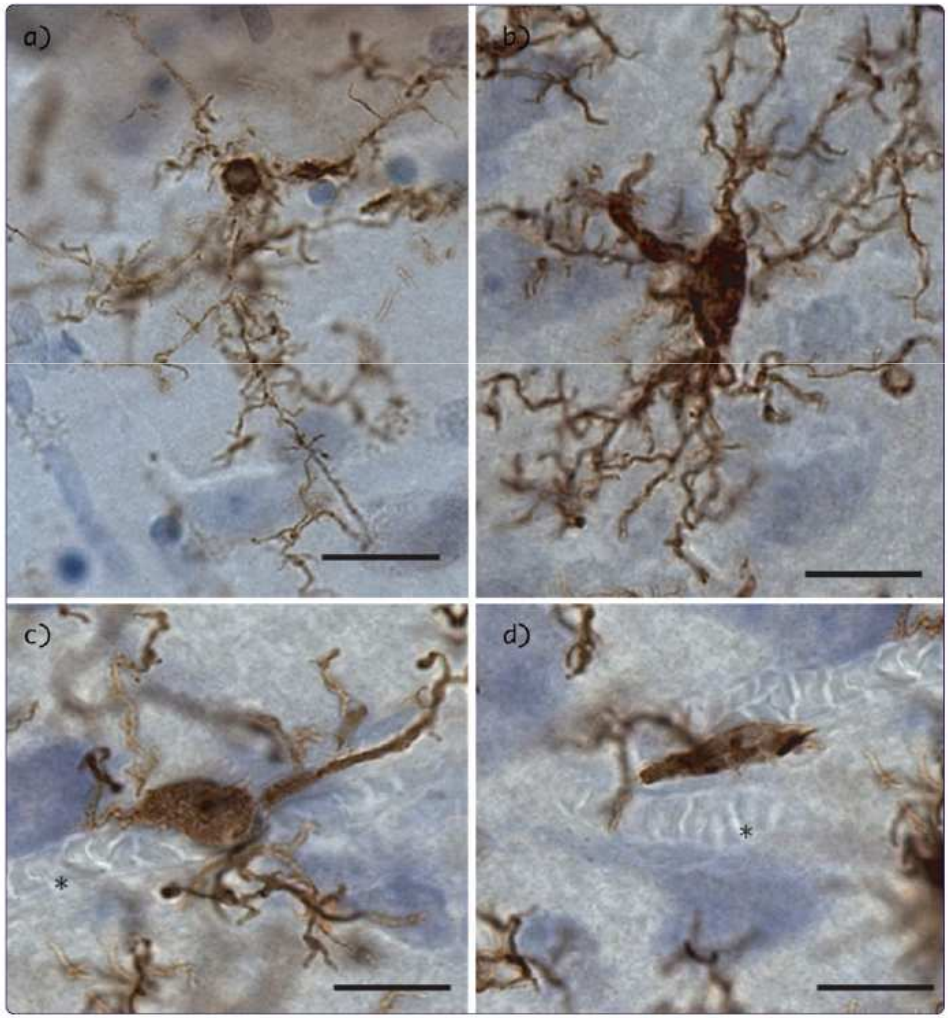
CD163



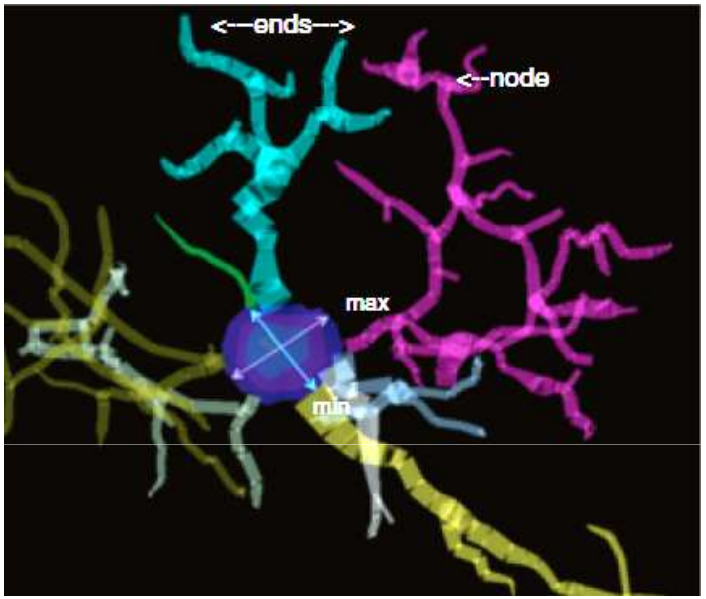
Tavazzi et al Curr HIV Res 2014

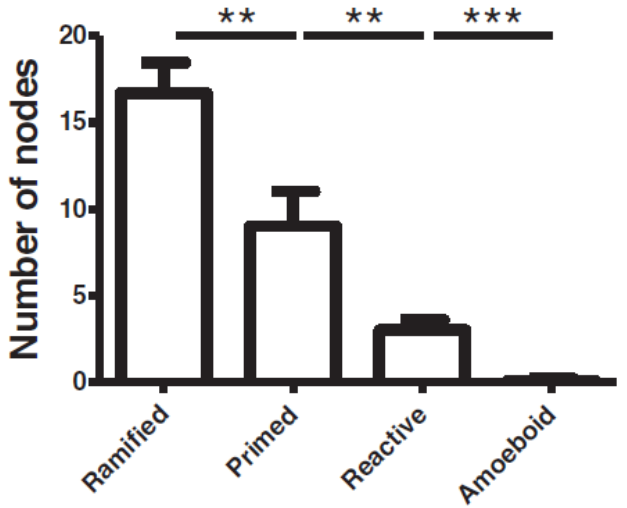
Microglia morphologies and morphometric characterization

Ramified microglia *Primed microglia*



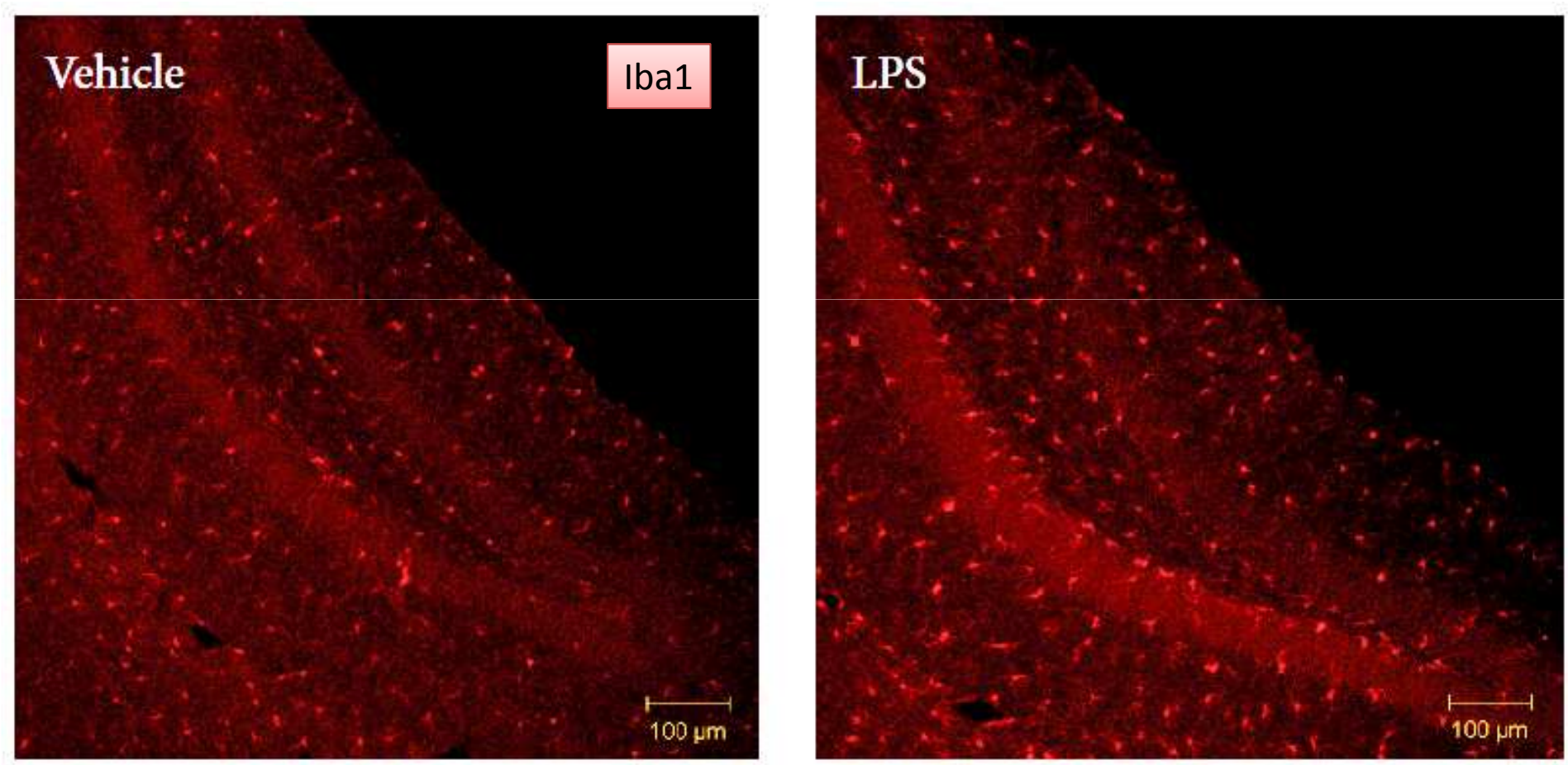
Reactive microglia *Amoeboid microglia*





From Torres-Platas et al. J Neuroinflammation 2014

Peripheral LPS increases IBA1 immunoreactivity in the hippocampal dentate gyrus



LPS injection (0.63 mg/kg, i.p.) induces microglial activation (increased IBA1 immunoreactivity) 24 h after administration

From Biesmans et al. Mediators Inflamm. 2013

Systemic LPS and glial reactivity

Cardoso *et al. Journal of Neuroinflammation* (2015) 12:82
DOI 10.1186/s12974-015-0299-3

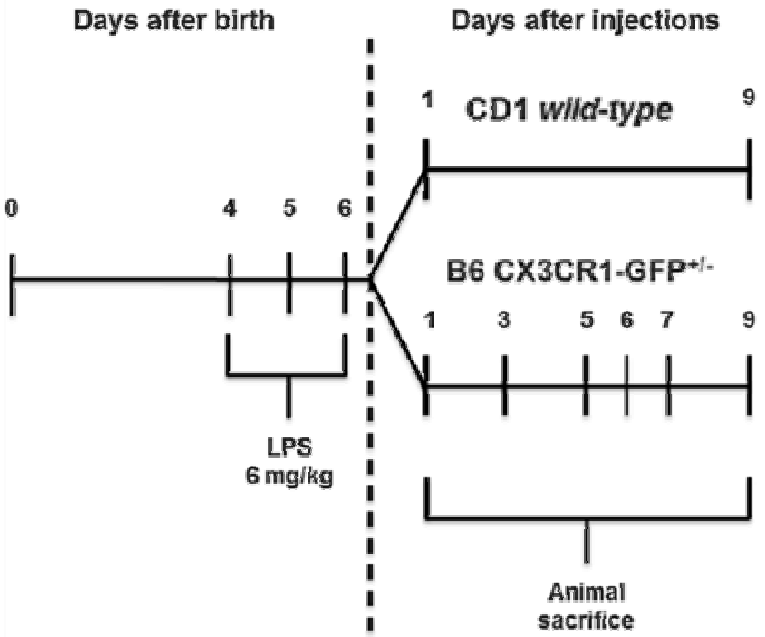
JOURNAL OF NEUROINFLAMMATION

RESEARCH Open Access

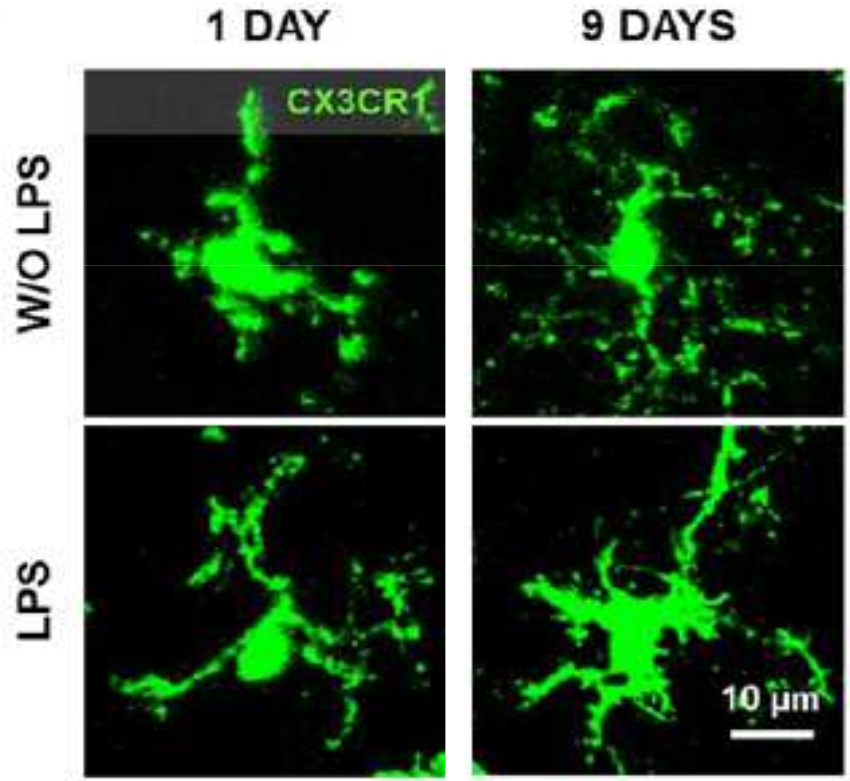
Systemic inflammation in early neonatal mice induces transient and lasting neurodegenerative effects

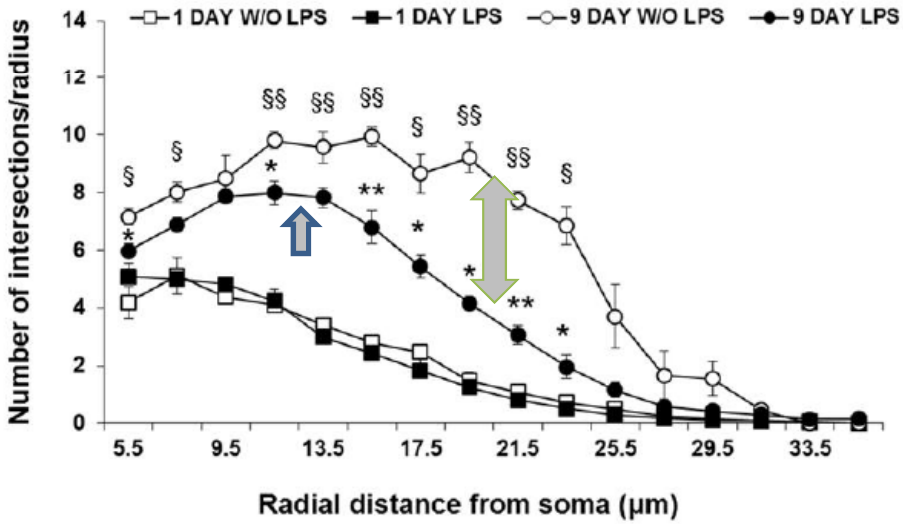
Filipa L Cardoso[1], Jasmin Herz[2], Adelaide Fernandes[1,3], João Rocha[1], Bruno Sepodes[1], Maria A Brito[1,3], Dorian B McGavern[2*] and Dora Brites[1,3*]

From: http://www.ahwla.org.uk/site/tutorials/BVA/BVA05-Mouse/Mouse.html



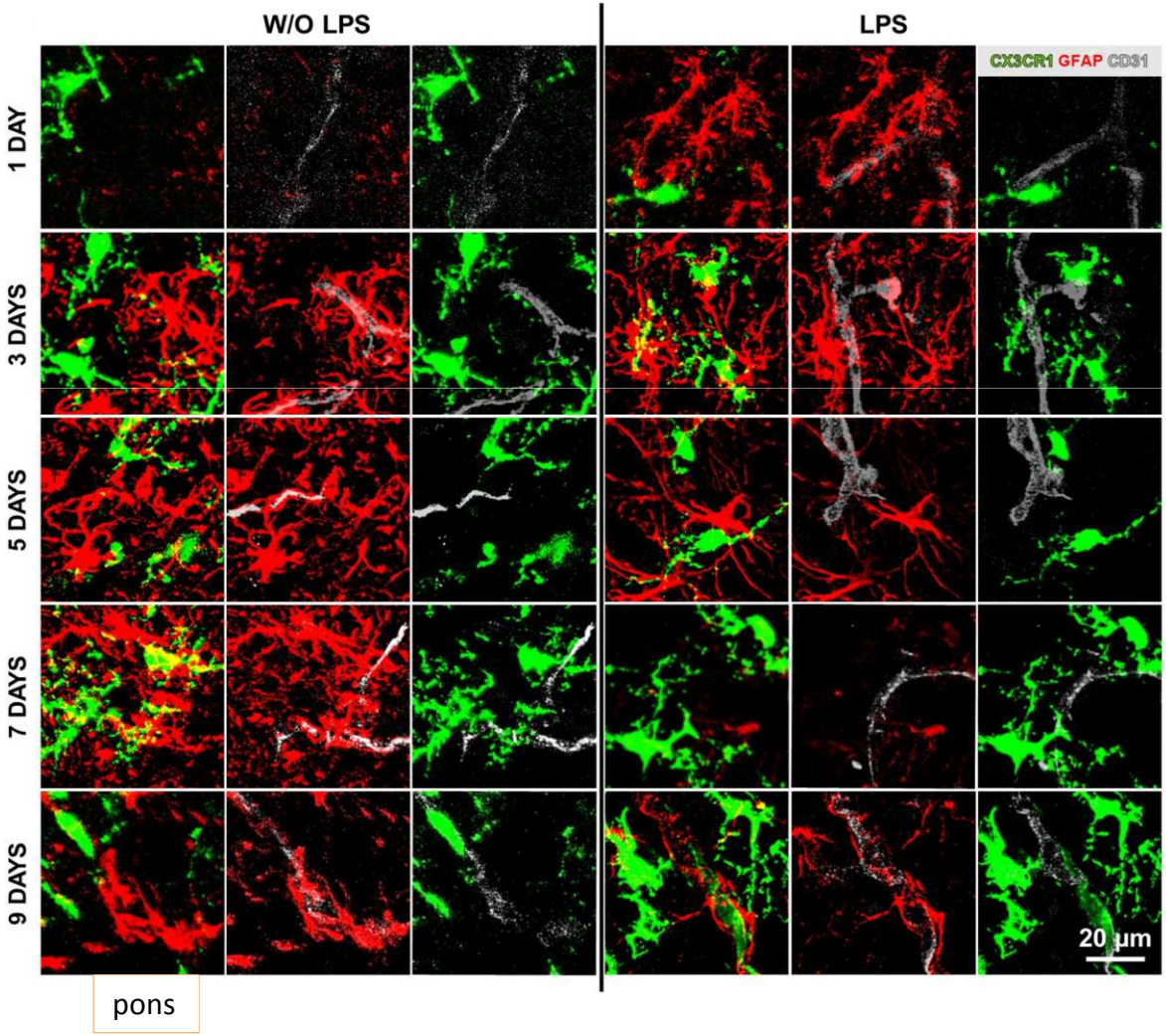
Systemic LPS triggers microglia amoeboid morphology



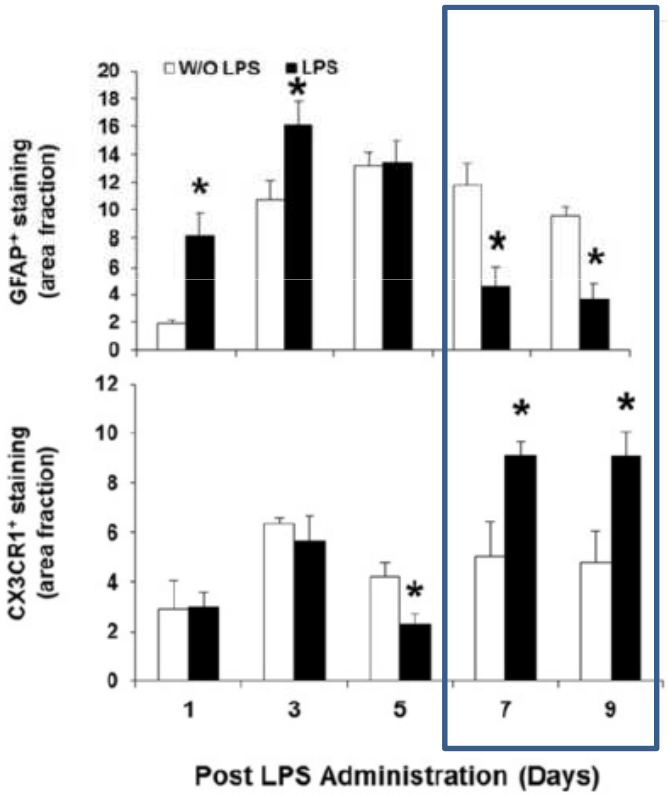


Cardoso et al. J Neuroinflammation 2015

Astrocyte reactivity precedes microglia activation

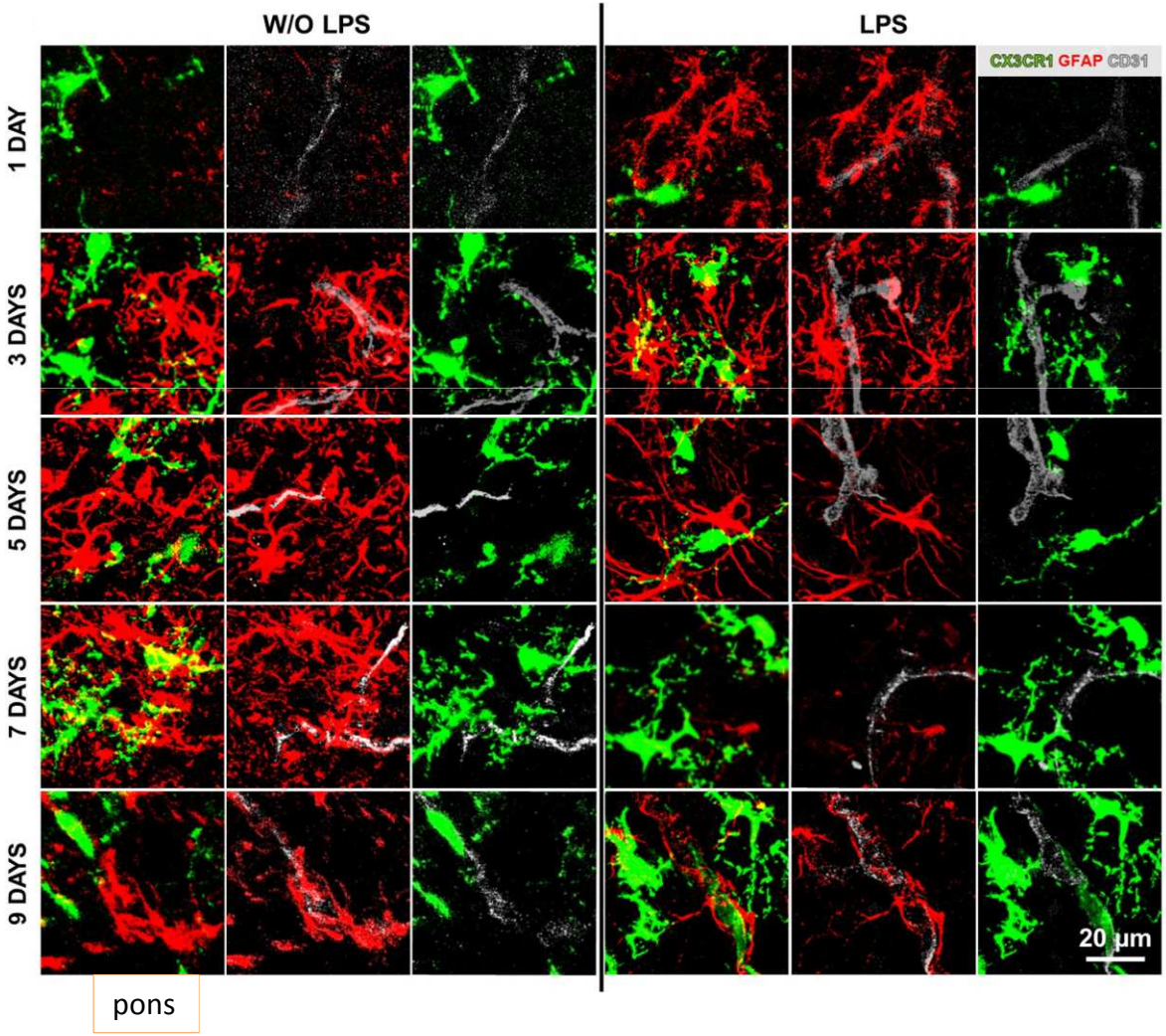


pons



Cardoso et al. J Neuroinflammation 2015

LPS triggers an early activation of astrocytes and microglia located near the blood vessels



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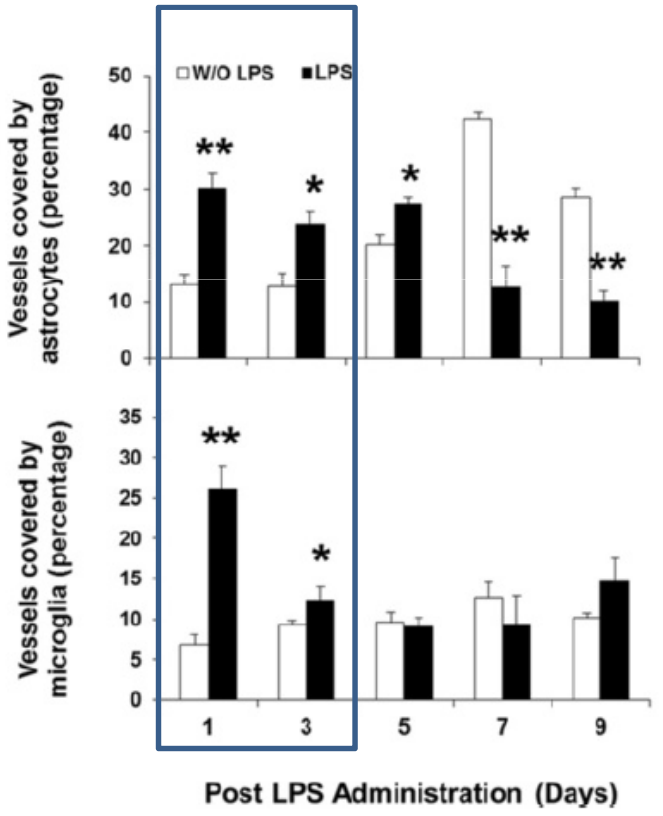
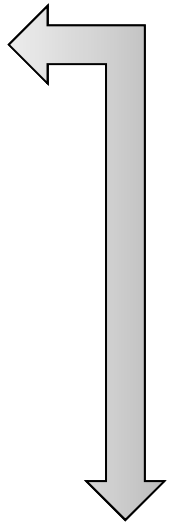


Cardoso et al. J Neuroinflammation 2015

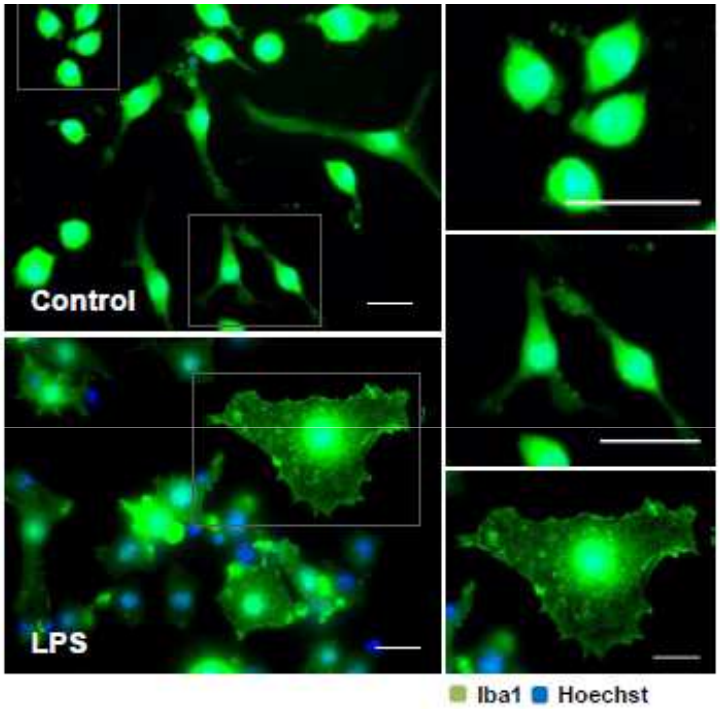
Conclusions

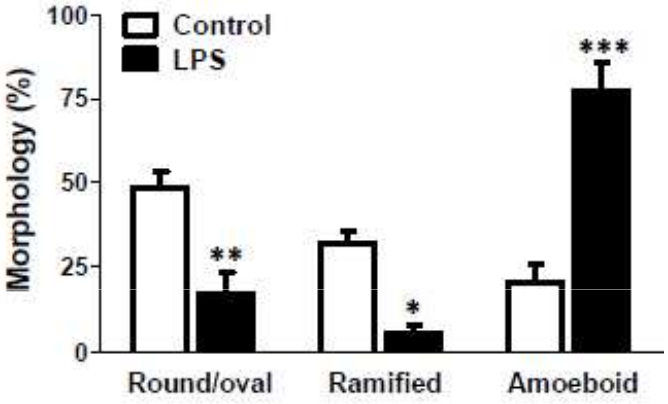
- Peripheral inflammation by LPS leads to ***neuroinflammation and changes in microglia cell shape***
- First changes are produced at the blood-brain barrier with increased ***astrogliosis*** and ***microgliosis***
- Delayed effects of LPS effects include ***decreased density of astrocytes*** and ***proliferation of microglia*** in the brain parenchyma

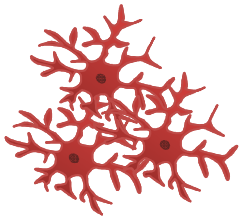


…In vitro studies

LPS switches microglia morphology towards an amoeboid shape and decreases cell migration



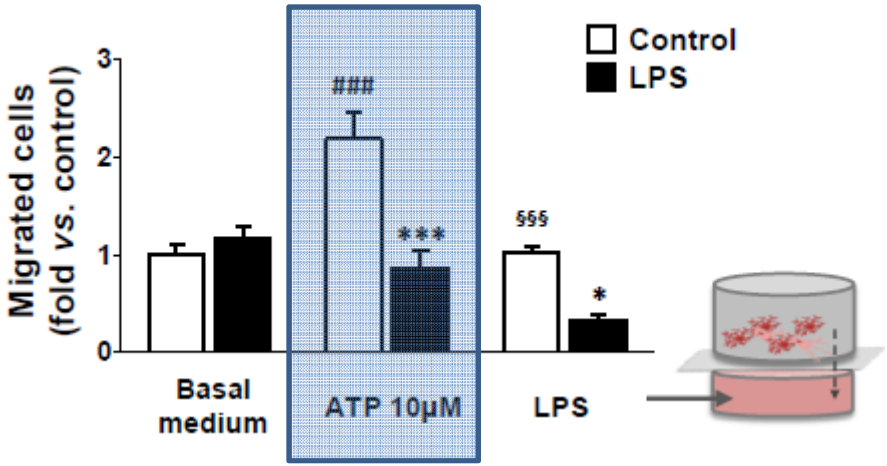




N9 microglia cell line

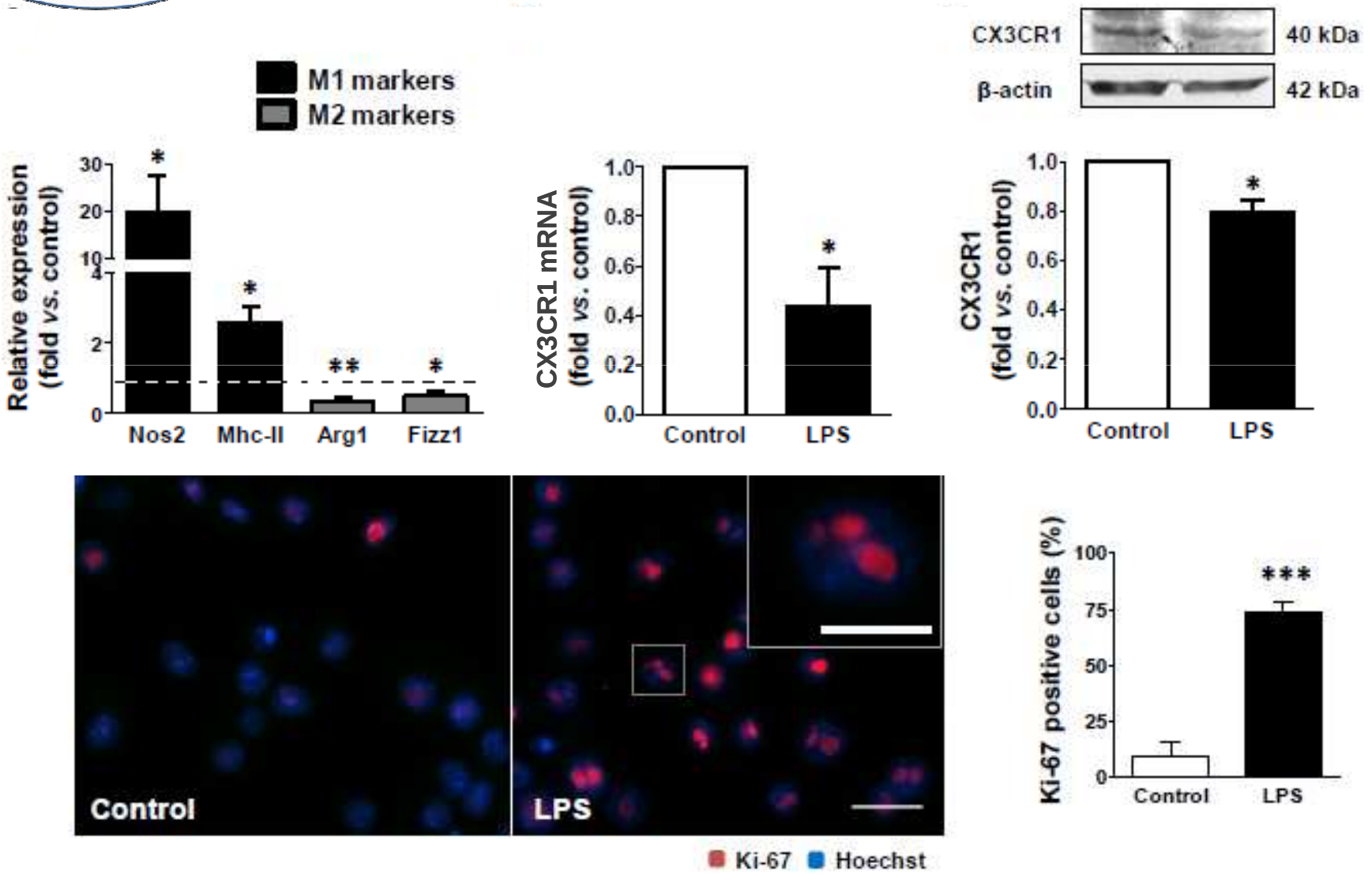
LPS 300 ng/ml

24 hours



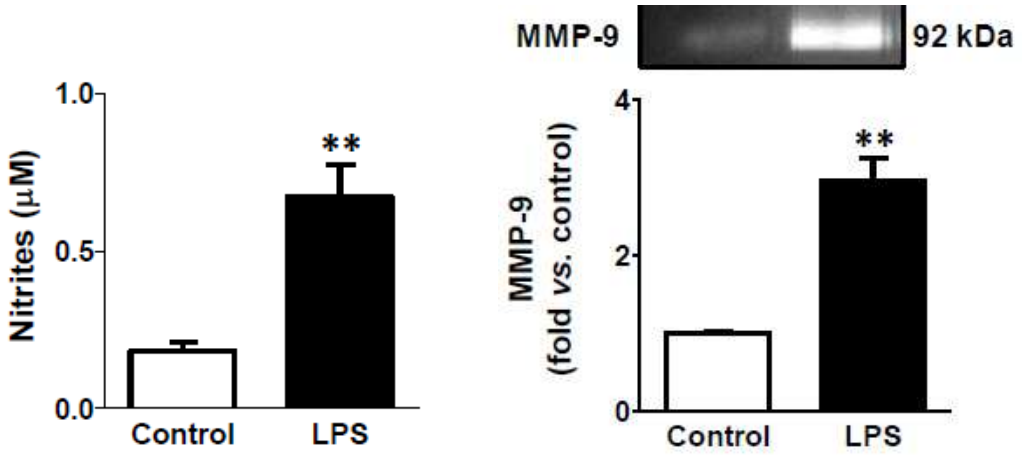
Adapted from from Cunha et al Mediators Inflamm (submitted by invitation)

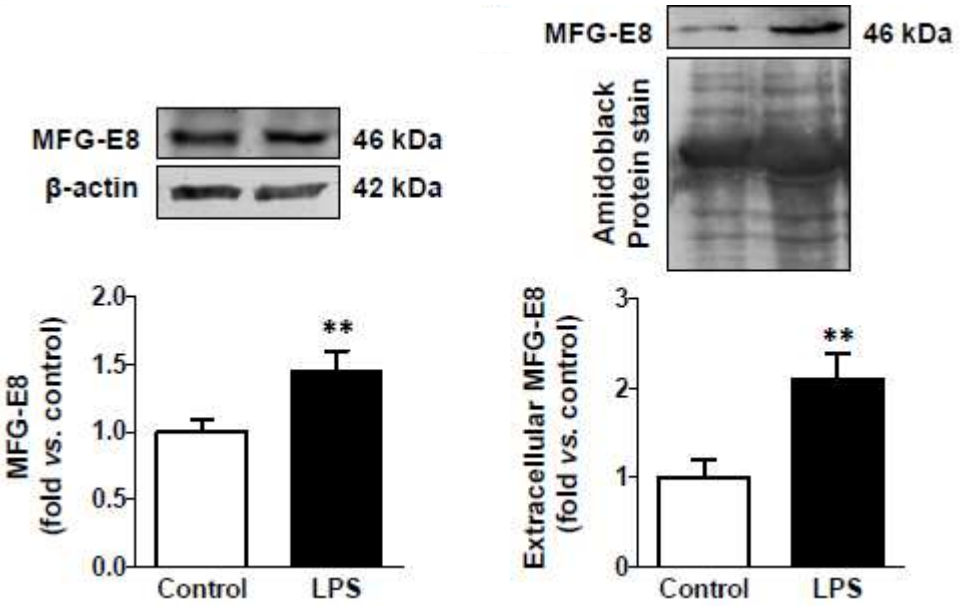
LPS switches microglia towards the M1 subtype and leads to increased cell proliferation

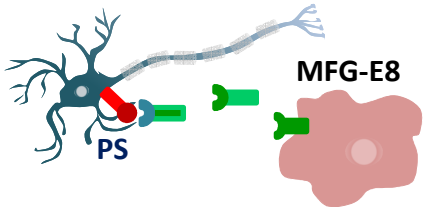


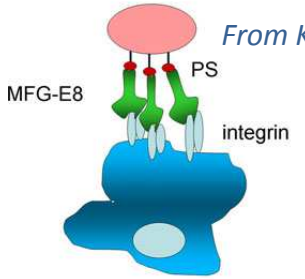
Adapted from from Cunha et al Mediators Inflamm (submitted by invitation)

M1 polarized microglia release NO, MMP-9 and MFG-E8





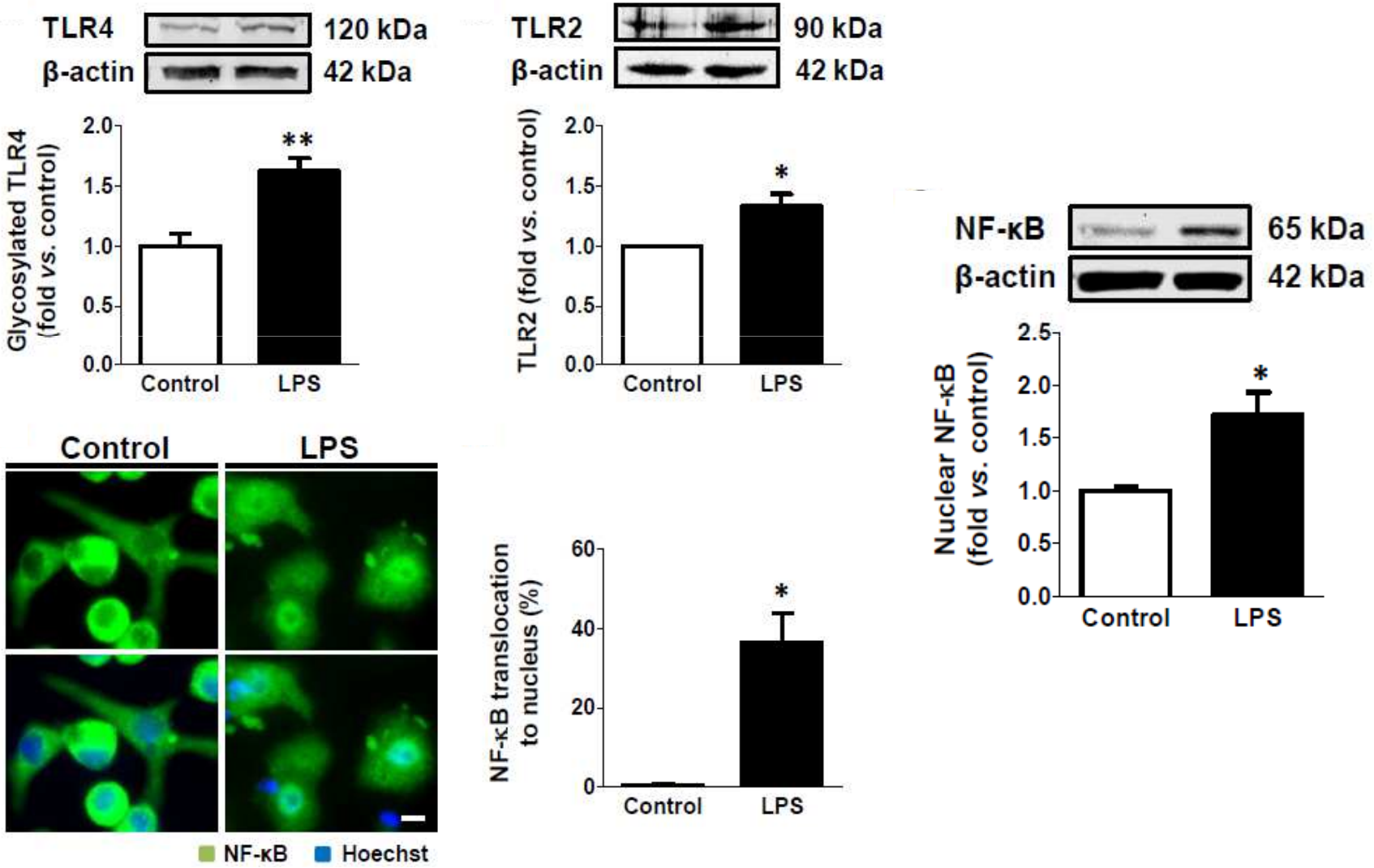




From Kruse Apoptosis 2010

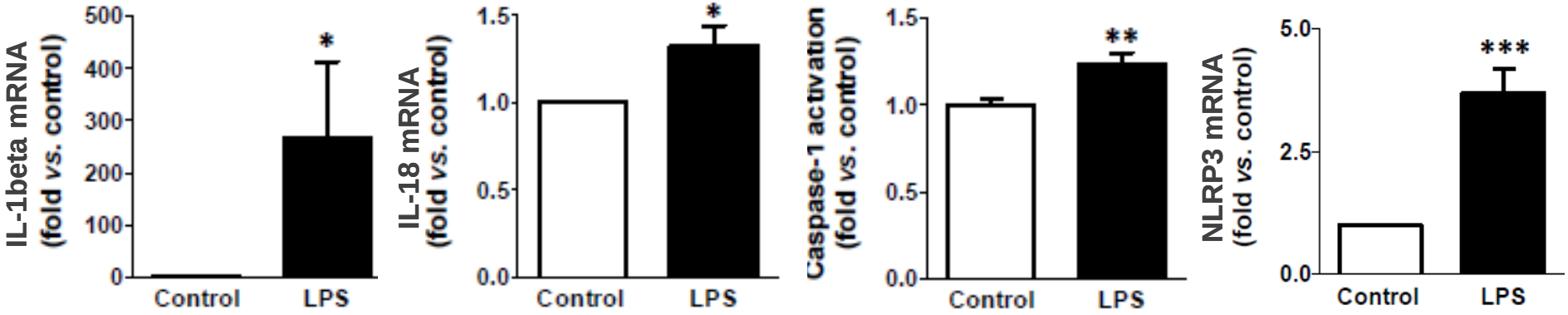
Adapted from from Cunha et al Mediators Inflamm (submitted by invitation)

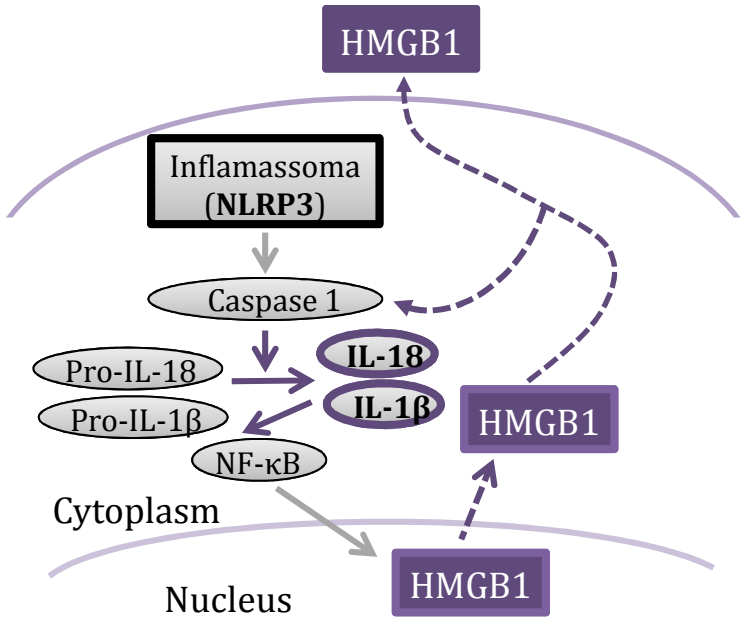
M1-polarized microglia evidence activation of TLR4/TLR2/NF-κB signaling pathway

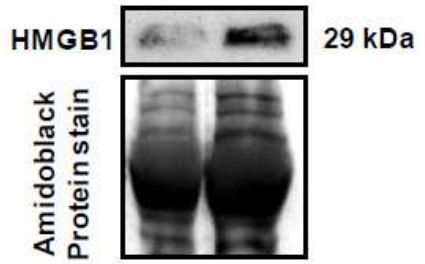


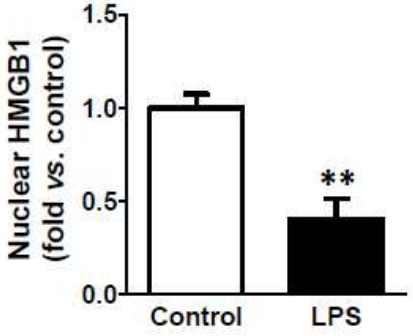
Adapted from Cunha et al Mediators Inflamm (submitted by invitation)

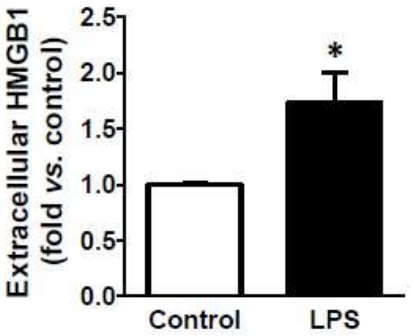
M1 microglia show inflammasome complex activation and HMGB1 release





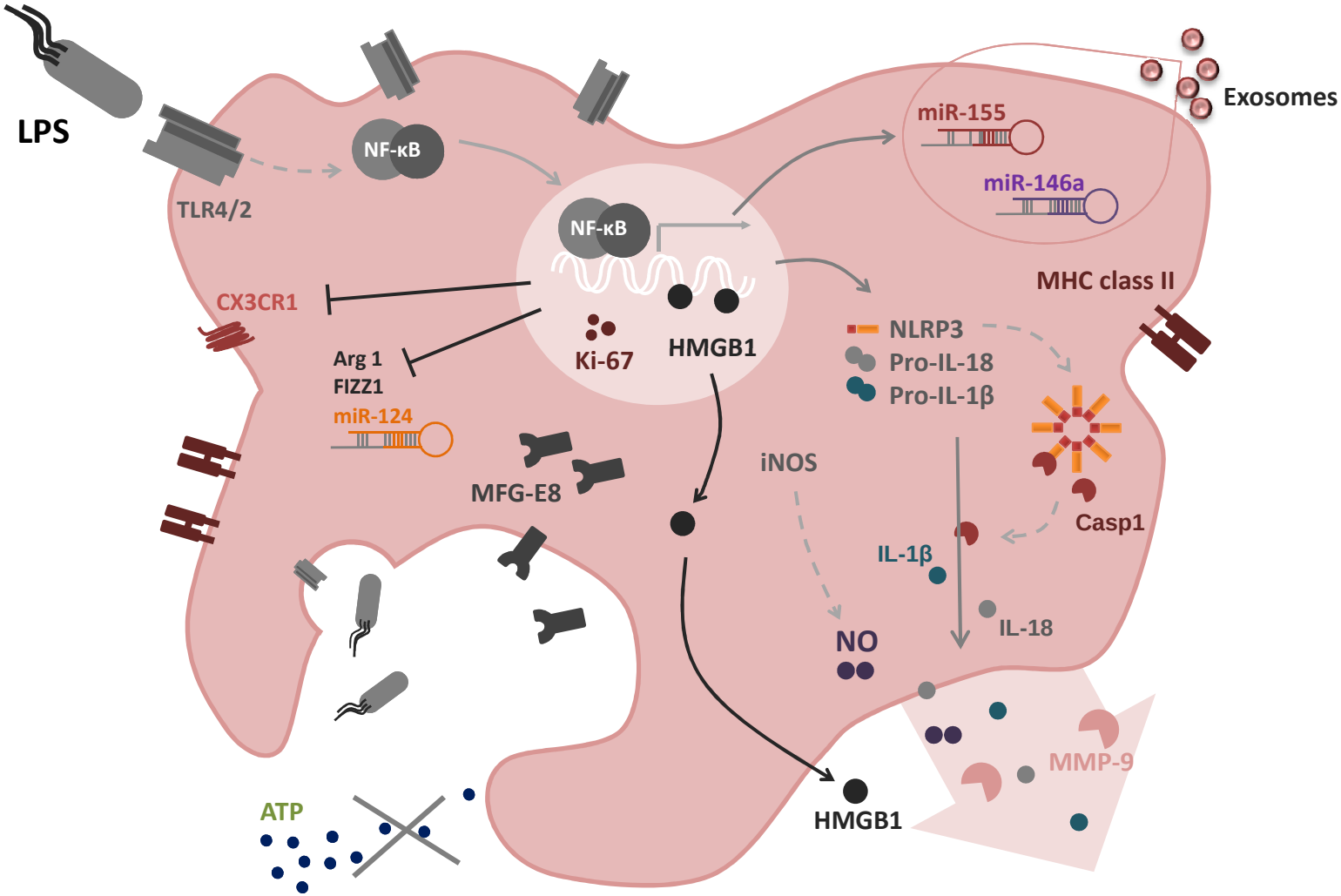






Adapted from from Cunha et al Mediators Inflamm (submitted by invitation)

Conclusions



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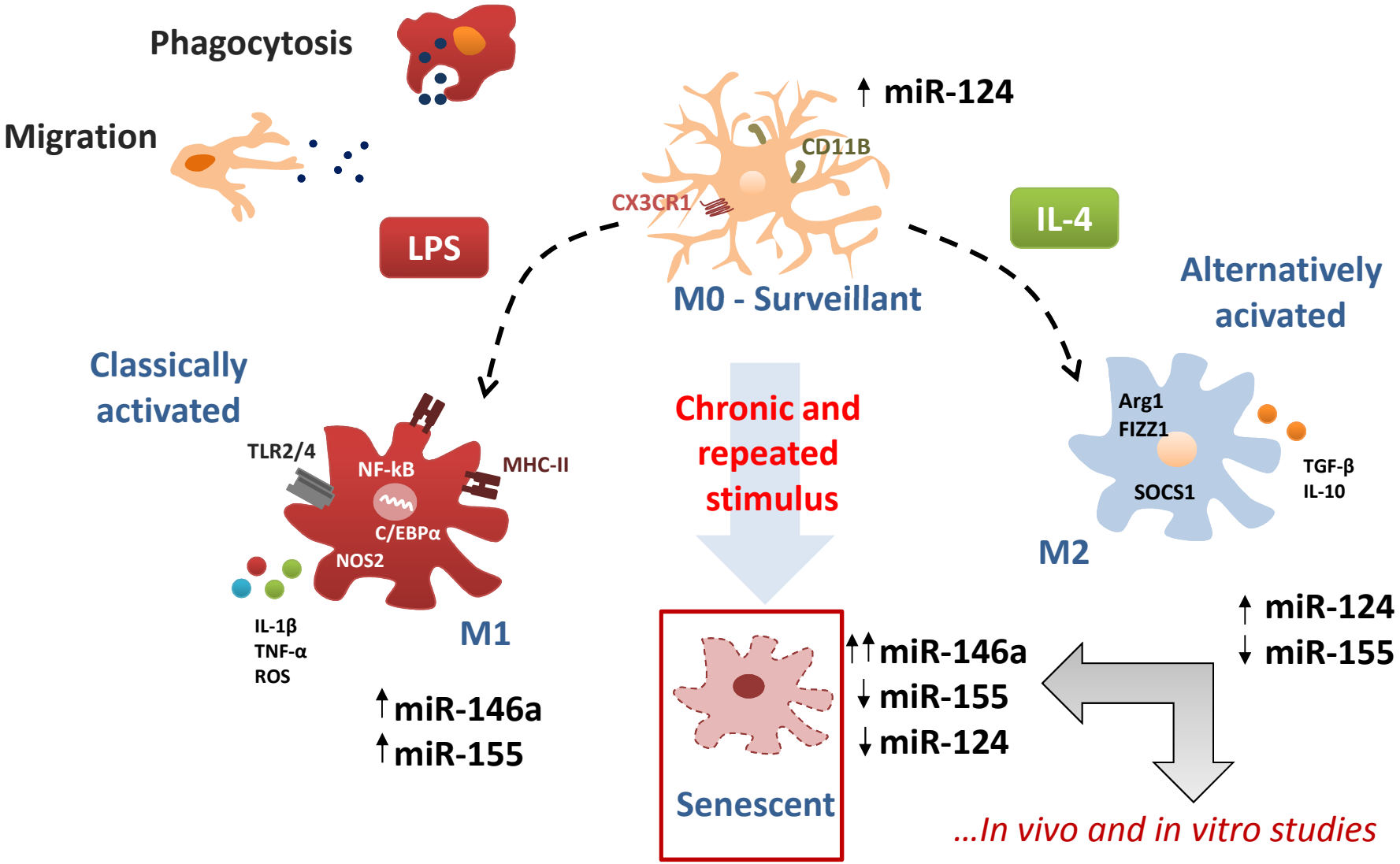
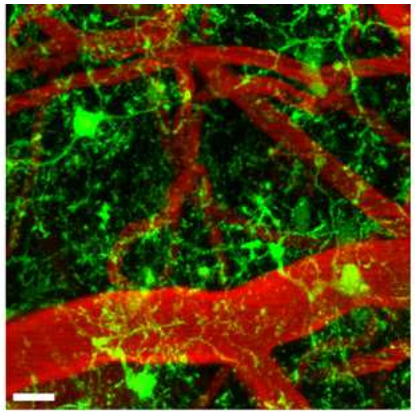
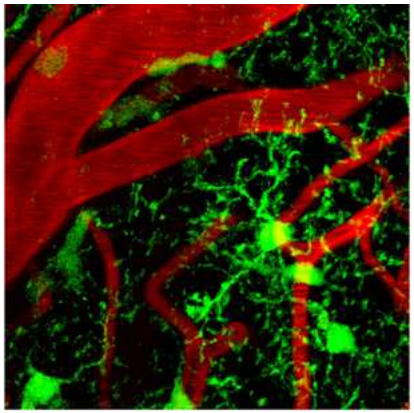
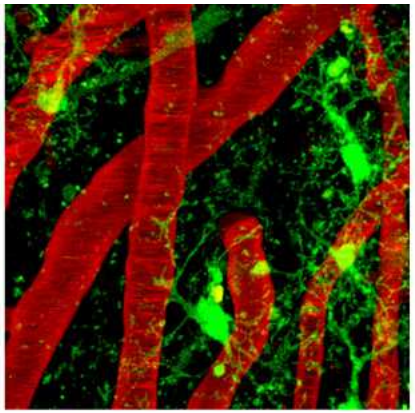
Diversity of microglia phenotypes

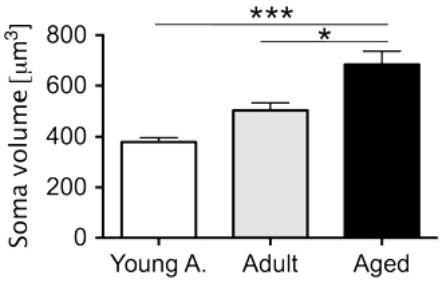


Age-related changes in microglia morphology assessed by in vivo imaging

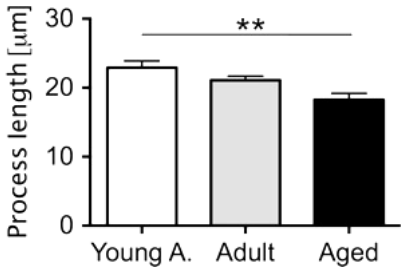

young adult


adult


aged animal



The processes show a significant reduction in volume coverage and **soma increase** with age



Hefendehl et al. Aging Cell 2014

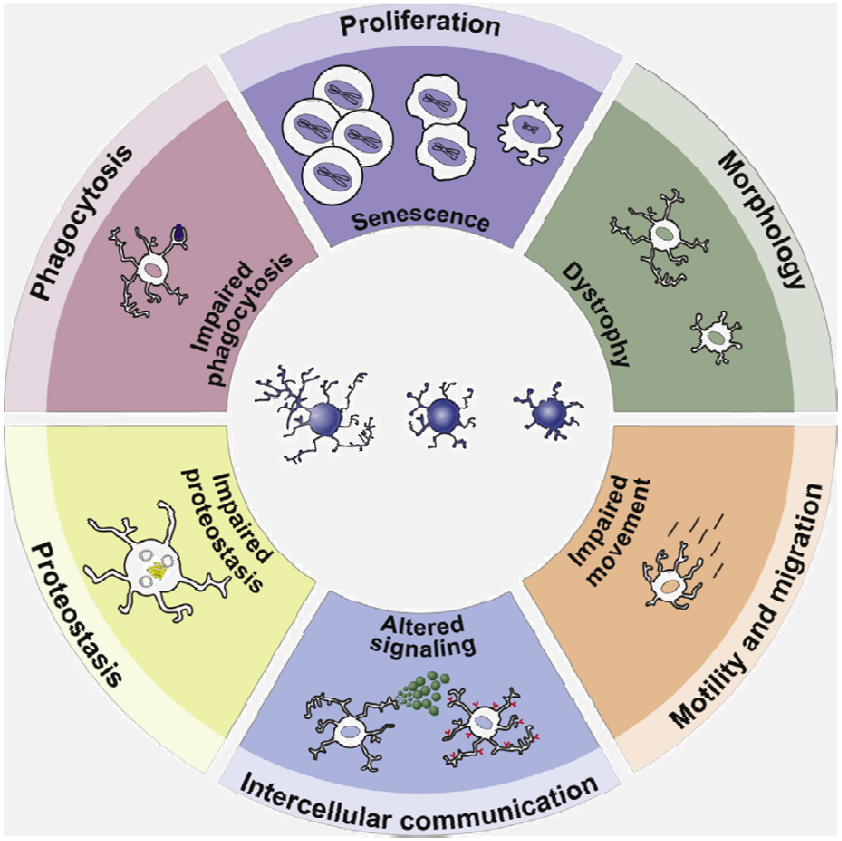
Microglia senescence

frontiers in
CELLULAR NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 02 June 2014
doi: 10.3389/fncel.2014.00152

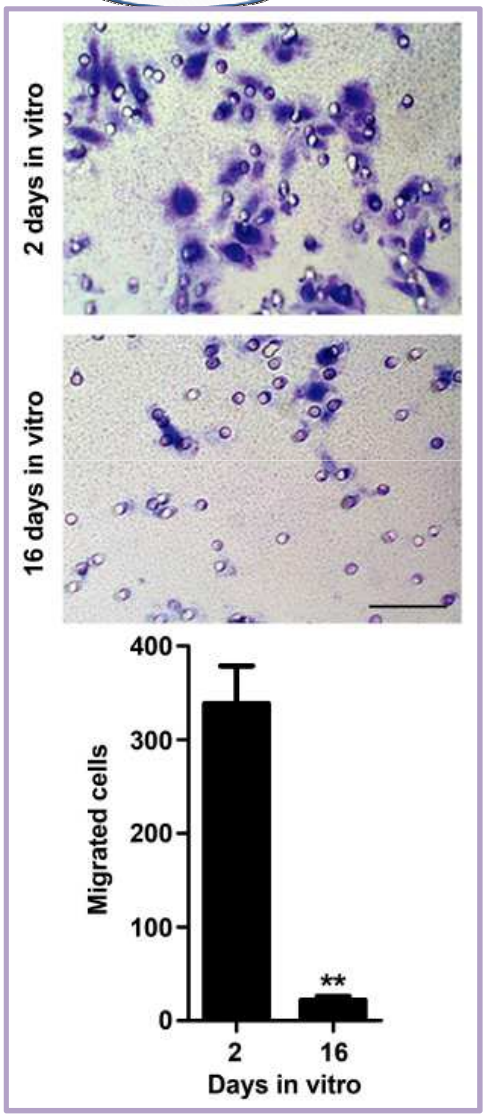
Microglia change from a reactive to an age-like phenotype with the time in culture

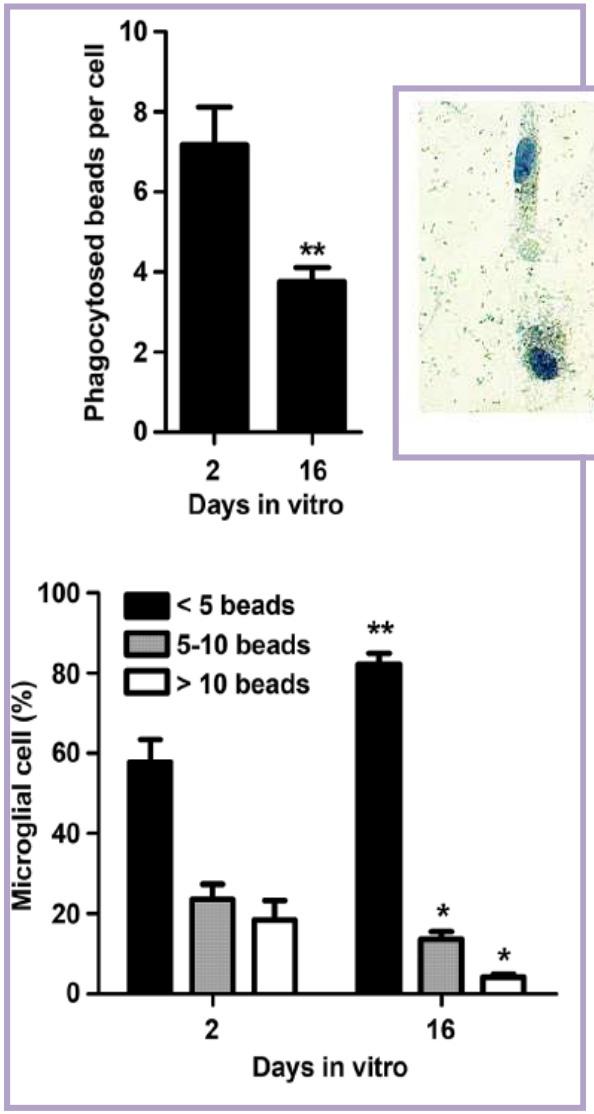
*Cláudia Caldeira[1,2], Ana F. Oliveira[1], Carolina Cunha[1], Ana R. Vaz[1,3], Ana S. Falcão[1,3], Adelaide Fernandes[1,3] * and Dora Brites[1,3] **

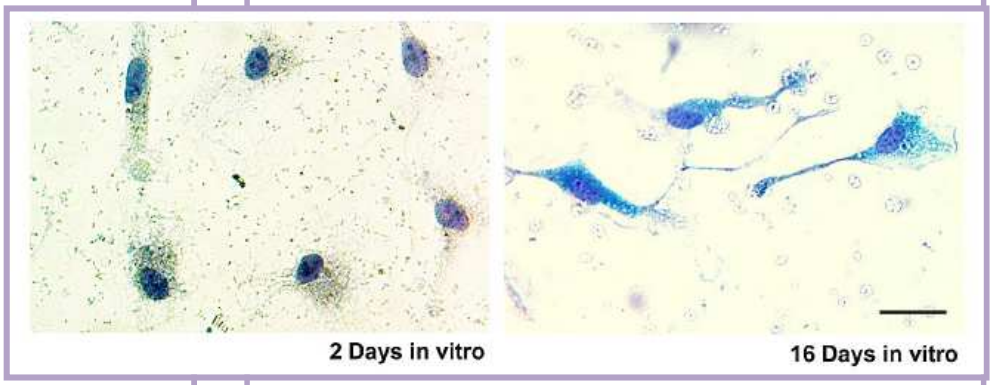


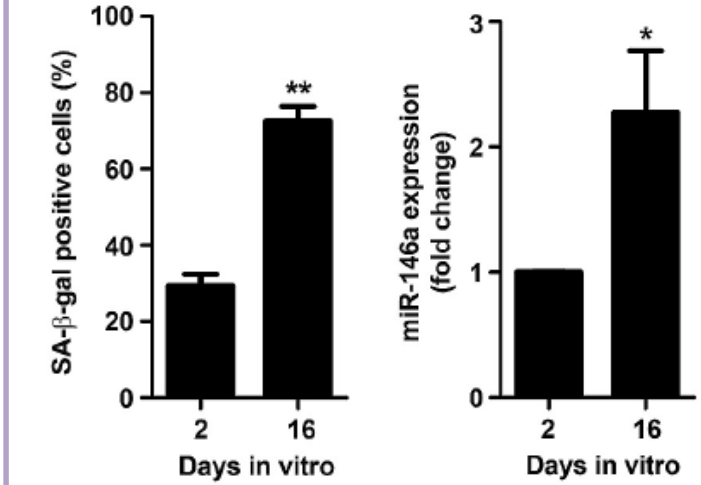
Mosher and Wyss-Coray, Biochem Pharmacol 2014

Aged microglia lose migration and phagocytic abilities, and show markers of cell senescence



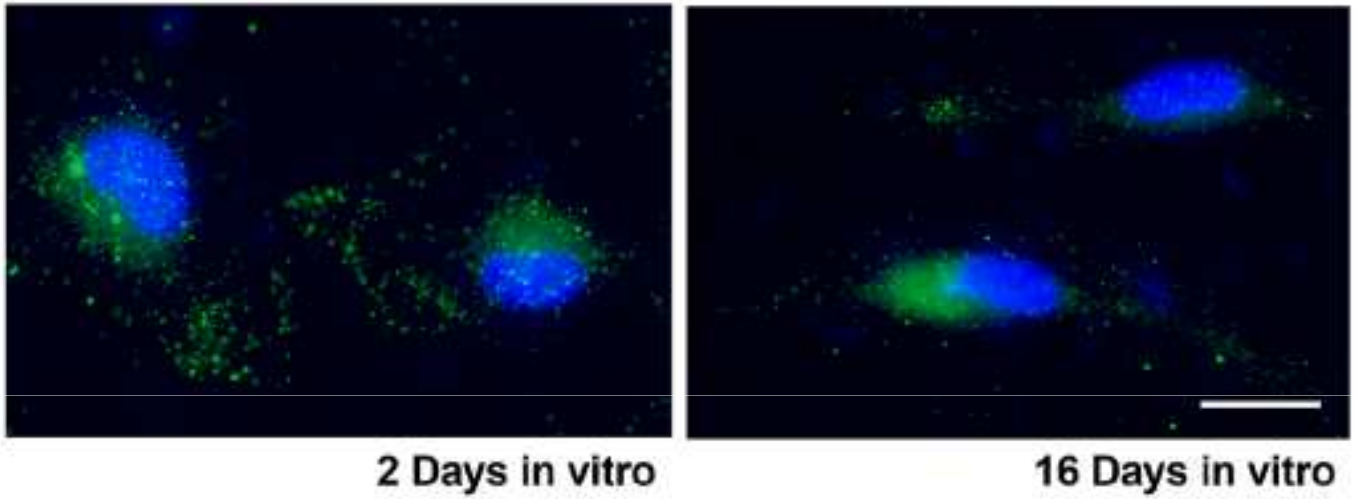


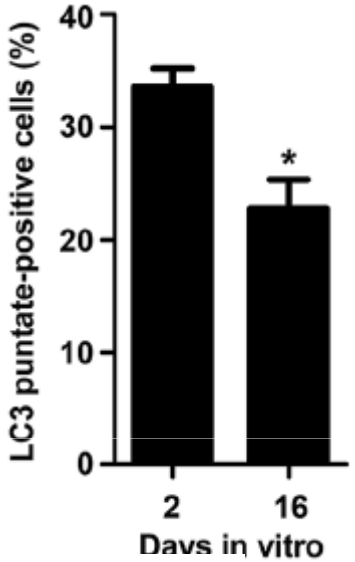


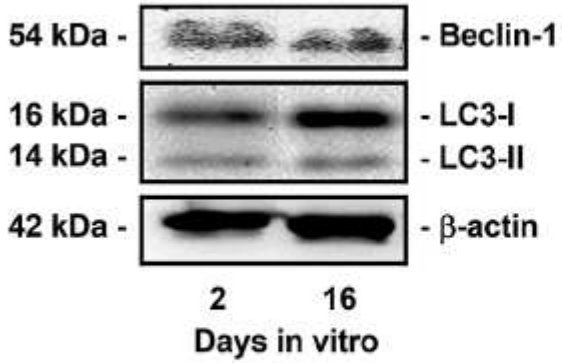


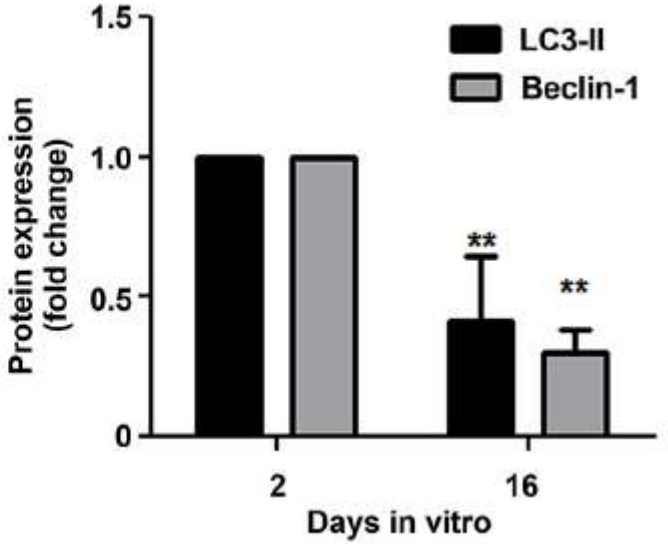
From Caldeira et al Front Cell Neurosci 2014

Senescent microglia show reduced autophagic capacity



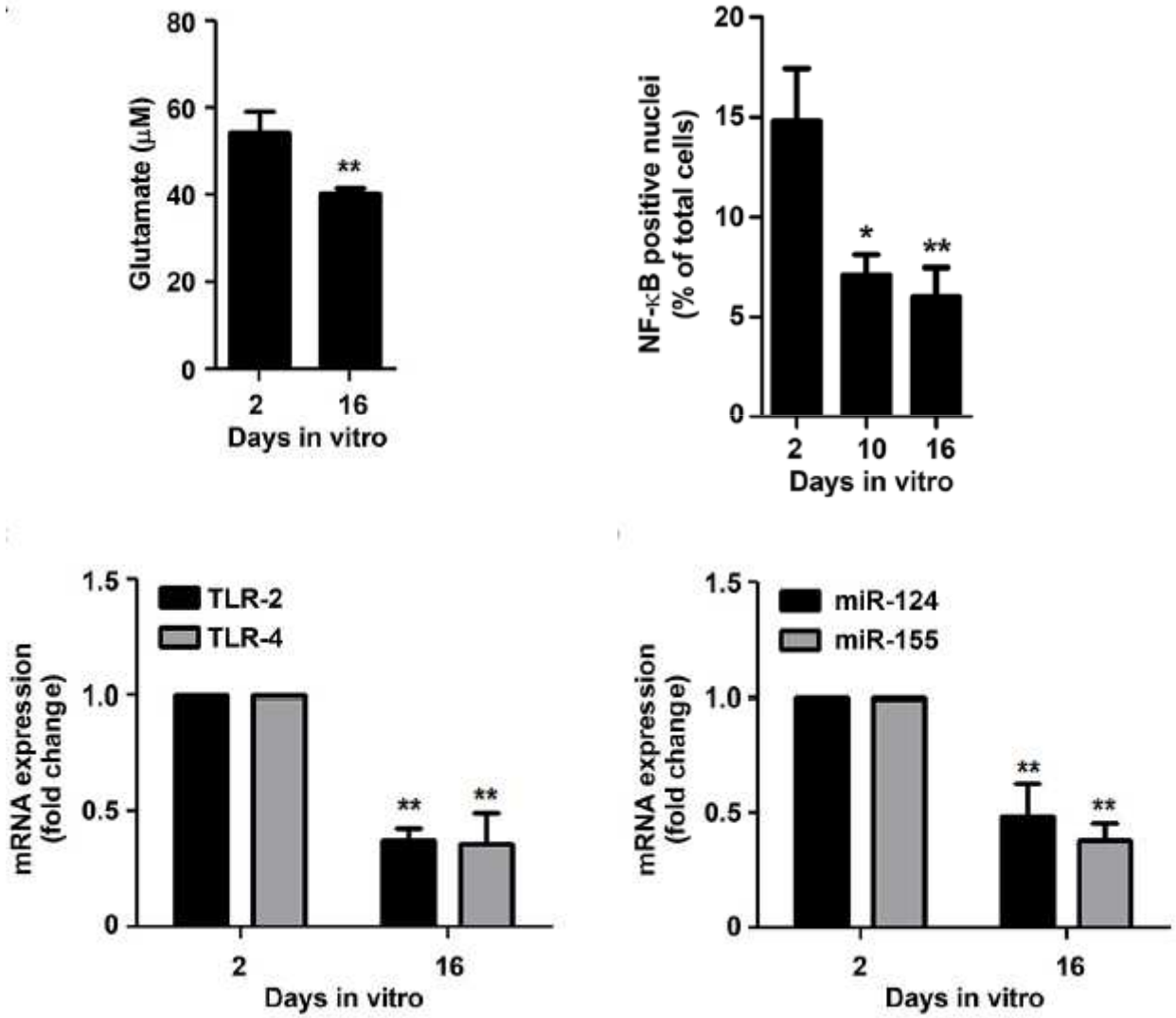






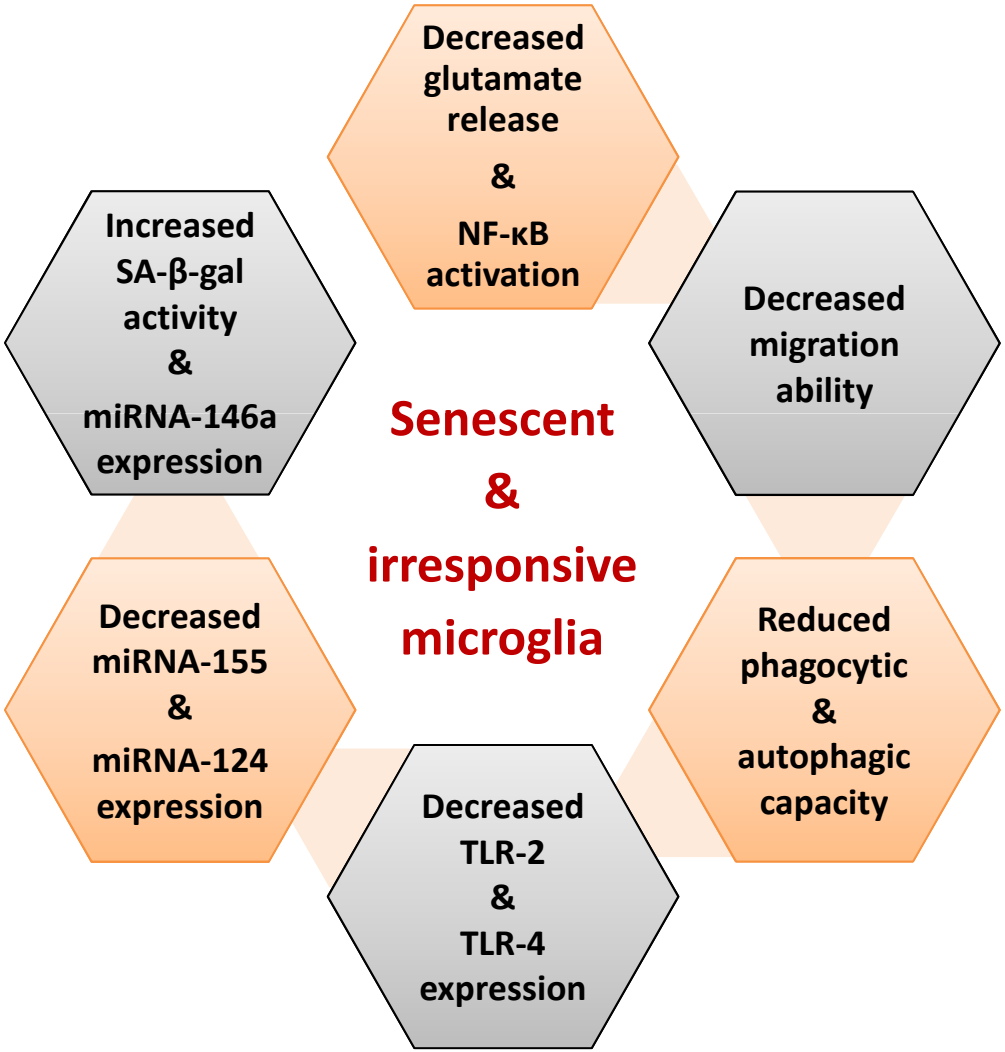
From Caldeira et al Front Cell Neurosci 2014

Senescent microglia show reduced glutamate release, NF-κB activation, and TLRs & inflamma-miRs expression



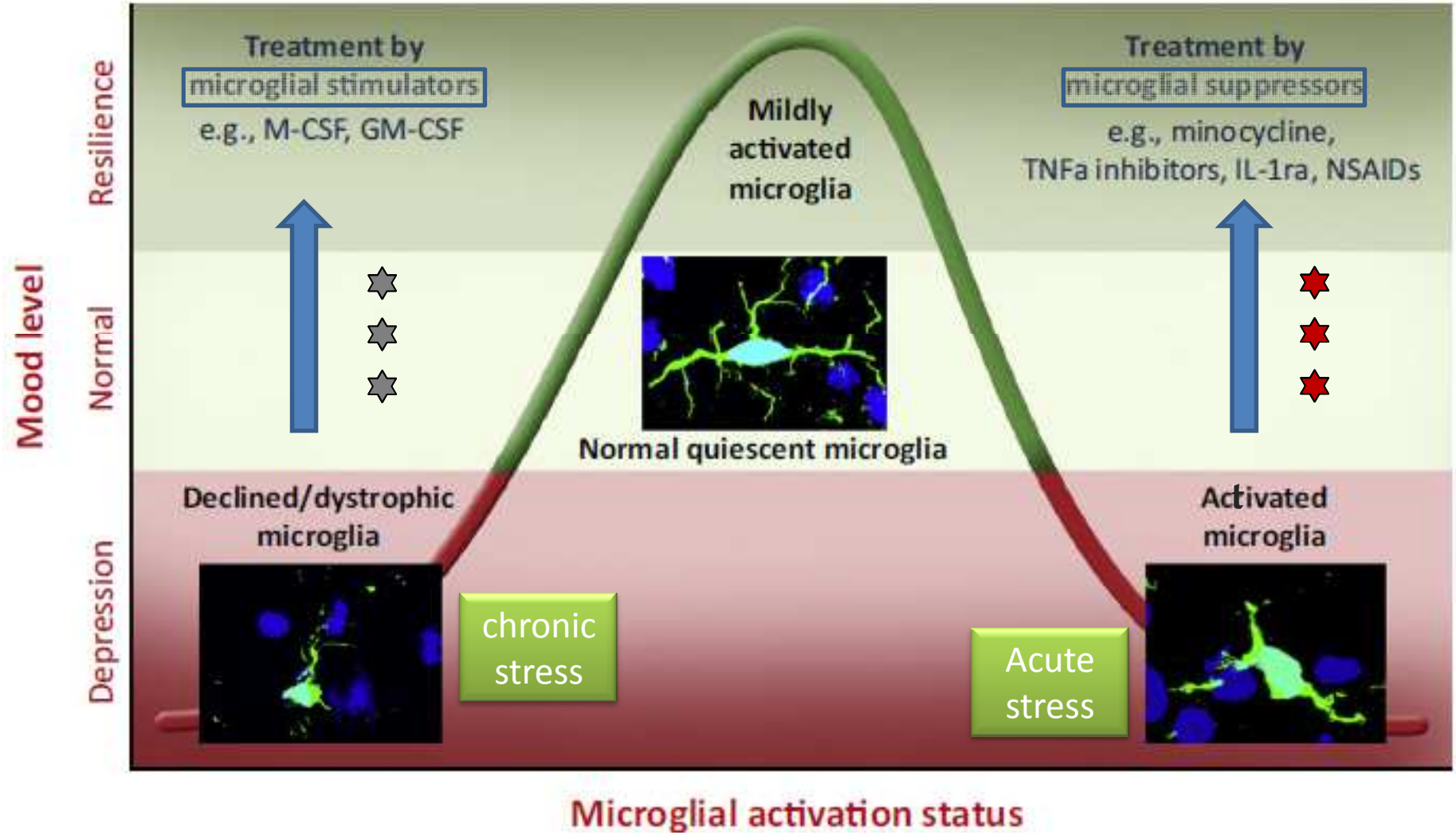
From Caldeira et al Front Cell Neurosci 2014

Conclusions



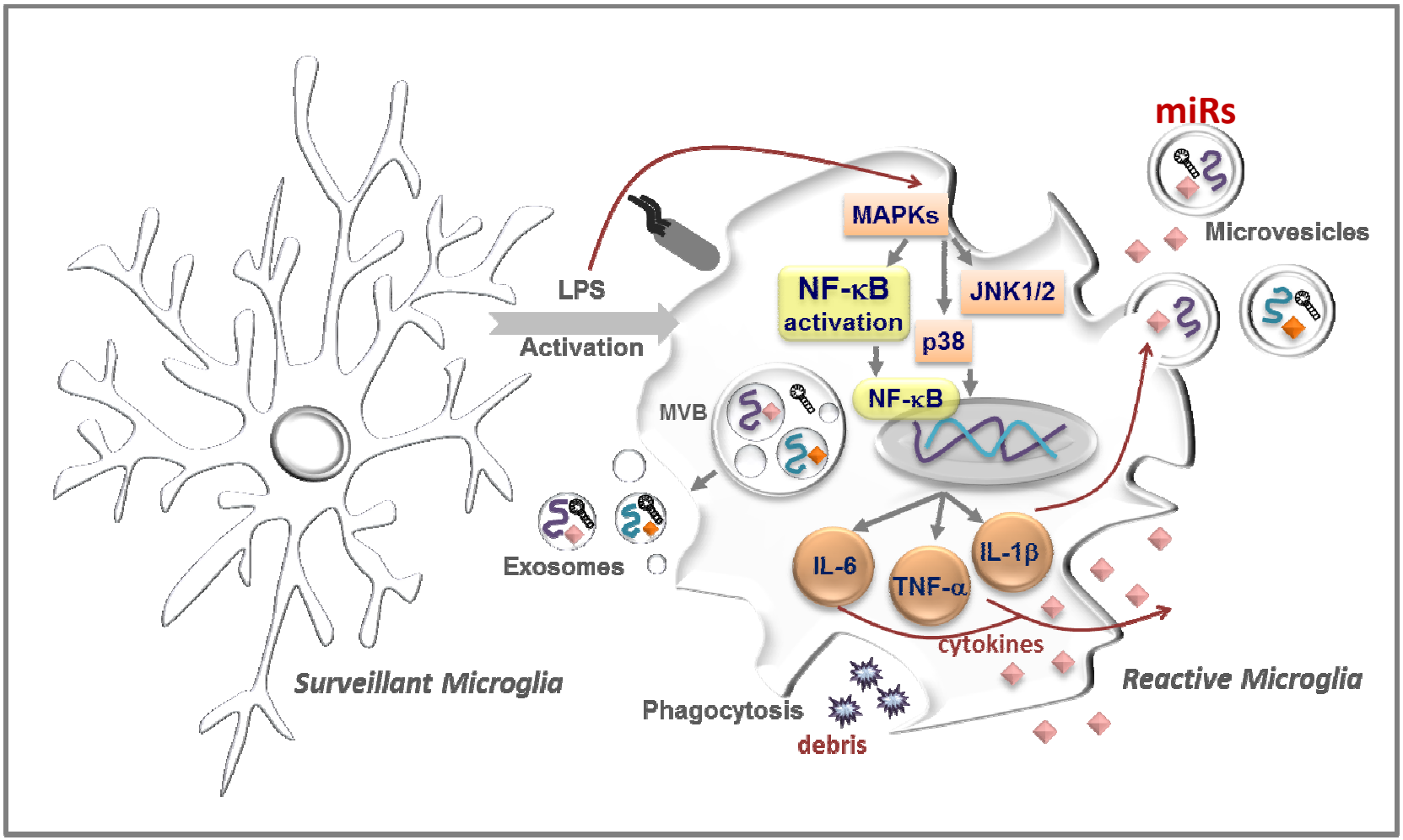
Adapted from Brites D AIMS 2015

Summary of microglia subtypes in mood disorders



Adapted from Yirmya et al Trends Neurosc 2015

Release of EVs by microglia can either potentiate activation or cause senescence



From Brites and Fernandes, Front Cell Neurosci 2015

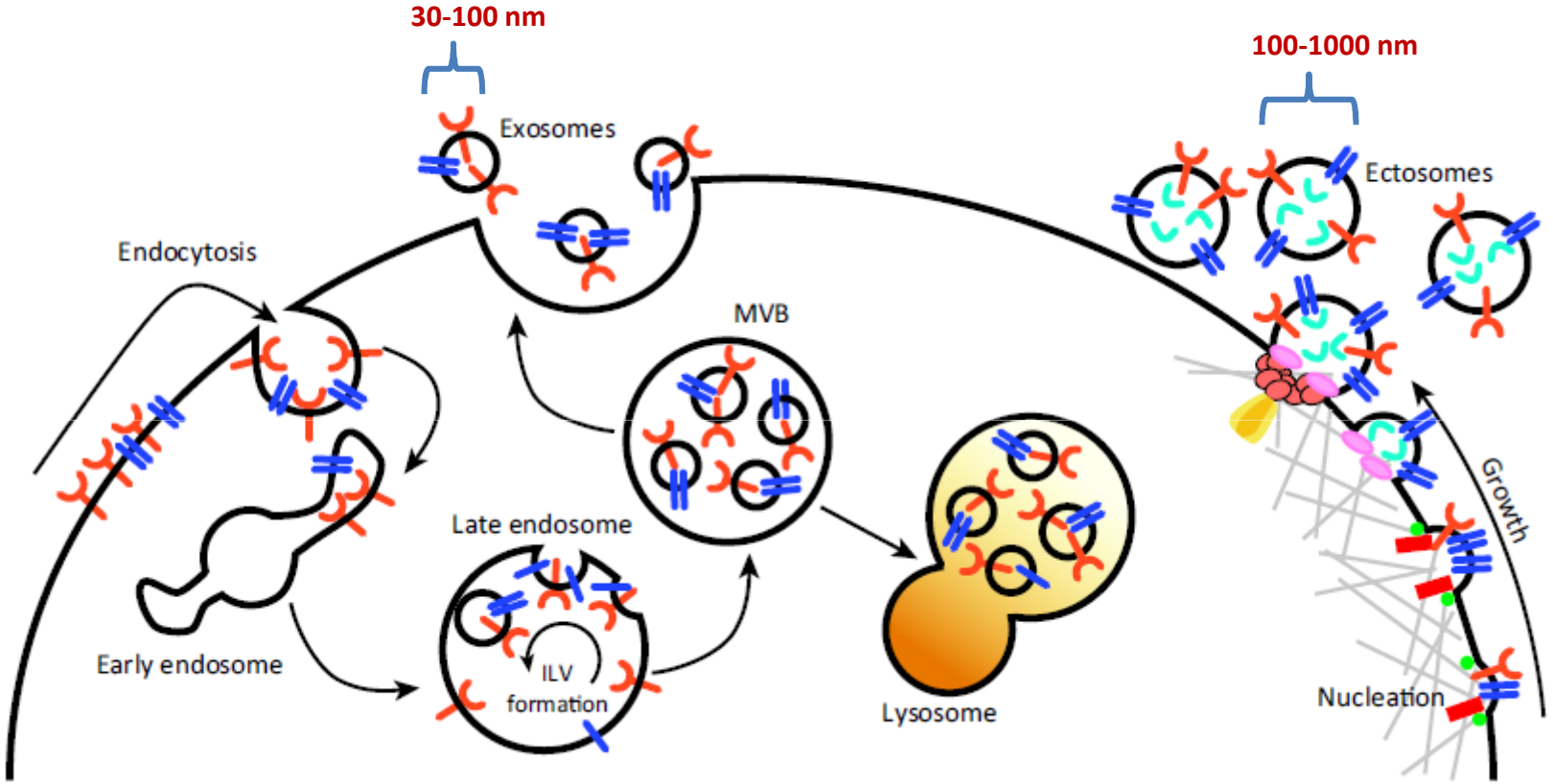
Long and short cell-to-cell communication

Extracellular vesicles (EVs):

- Exosomes
- Ectosomes

also… shedding vesicles, microvesicles, exosome-like vesicles, nanoparticles, microparticles and oncosomes

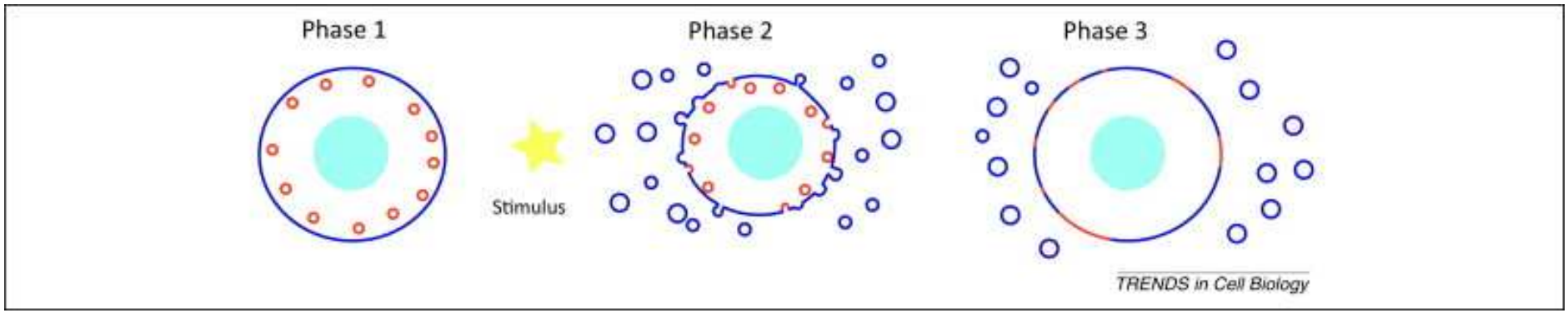
Exosome and ectosome biogenesis



ILVs – intraluminal vesicles
MVB – multivesicular bodies

Adapted from Cocucci and Meldolesi, Trends in Cell Biology 2015

Ectosome release is compensated by the exocytosis of intracellular vesicles



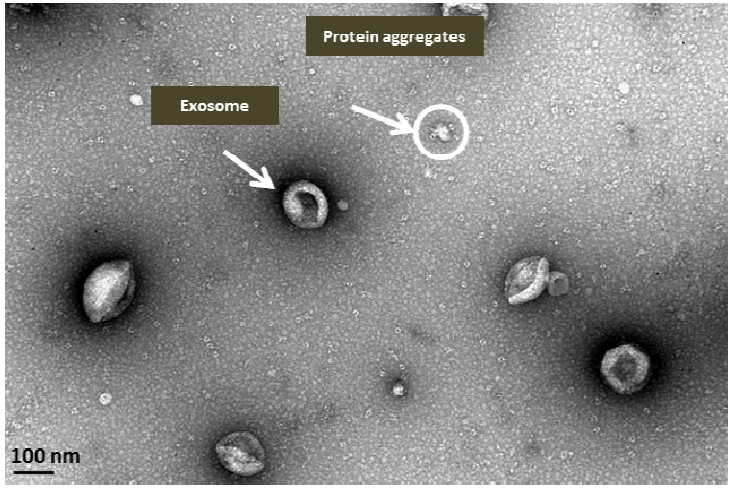
Phase 1 - Resting cell with ability to release ectosomes

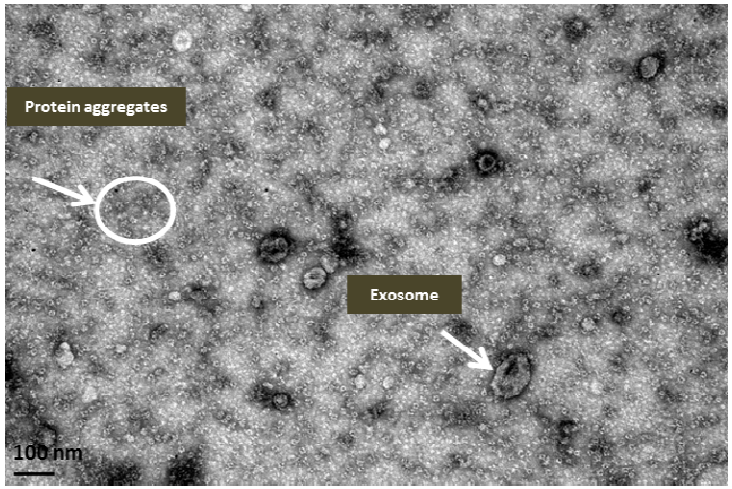
Phase 2 – Same cell a few tens of seconds after stimuli (e.g. **ATP**) with the *shrinkage* of the cell after releasing ectosomes (blue)

Phase 3 – Exocytosis of intracellular vesicles (red) and *compensation* of plasma membrane loss

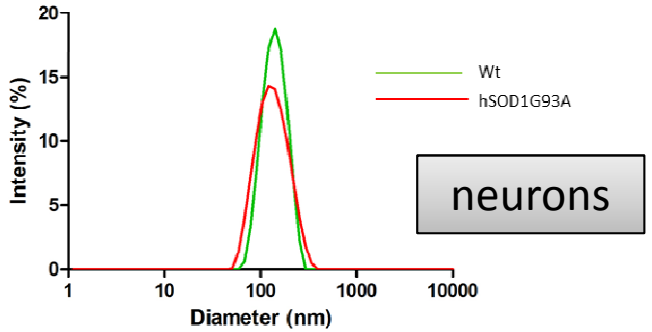
Adapted from Cocucci and Meldolesi, Trends in Cell Biology 2015

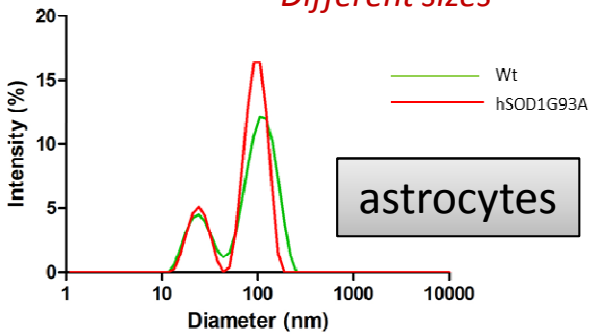
TEM and DLS of exosomes from motor neurons and astrocytes after differential centrifugation





Different sizes



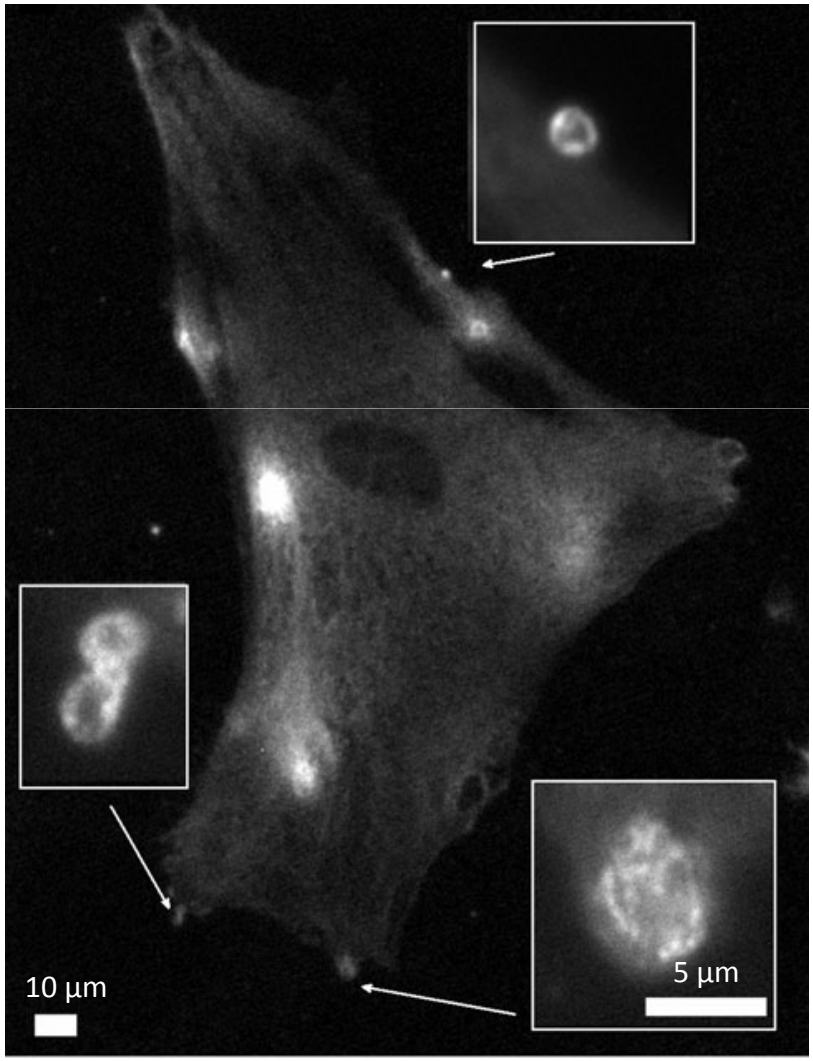


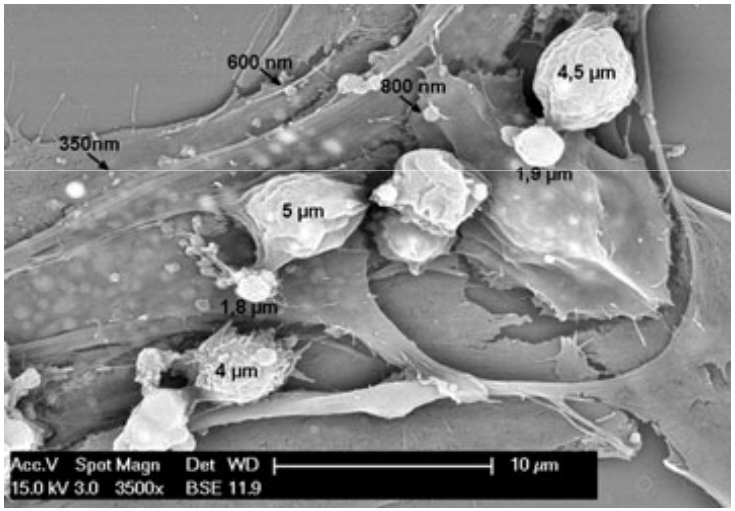
Sample	Size (diam. nm)	% Intensity
Wt	144.9	100
hSOD1G93A	141.4	100

Sample	Size (diam. nm)	% Intensity
Wt	113.3 25.5	76.1 23.9
hSOD1G93A	99.0 24.4	77.5 22.5

From Vaz et al New Developments in Astrocytes Research, Nova Science 2016 (in press)

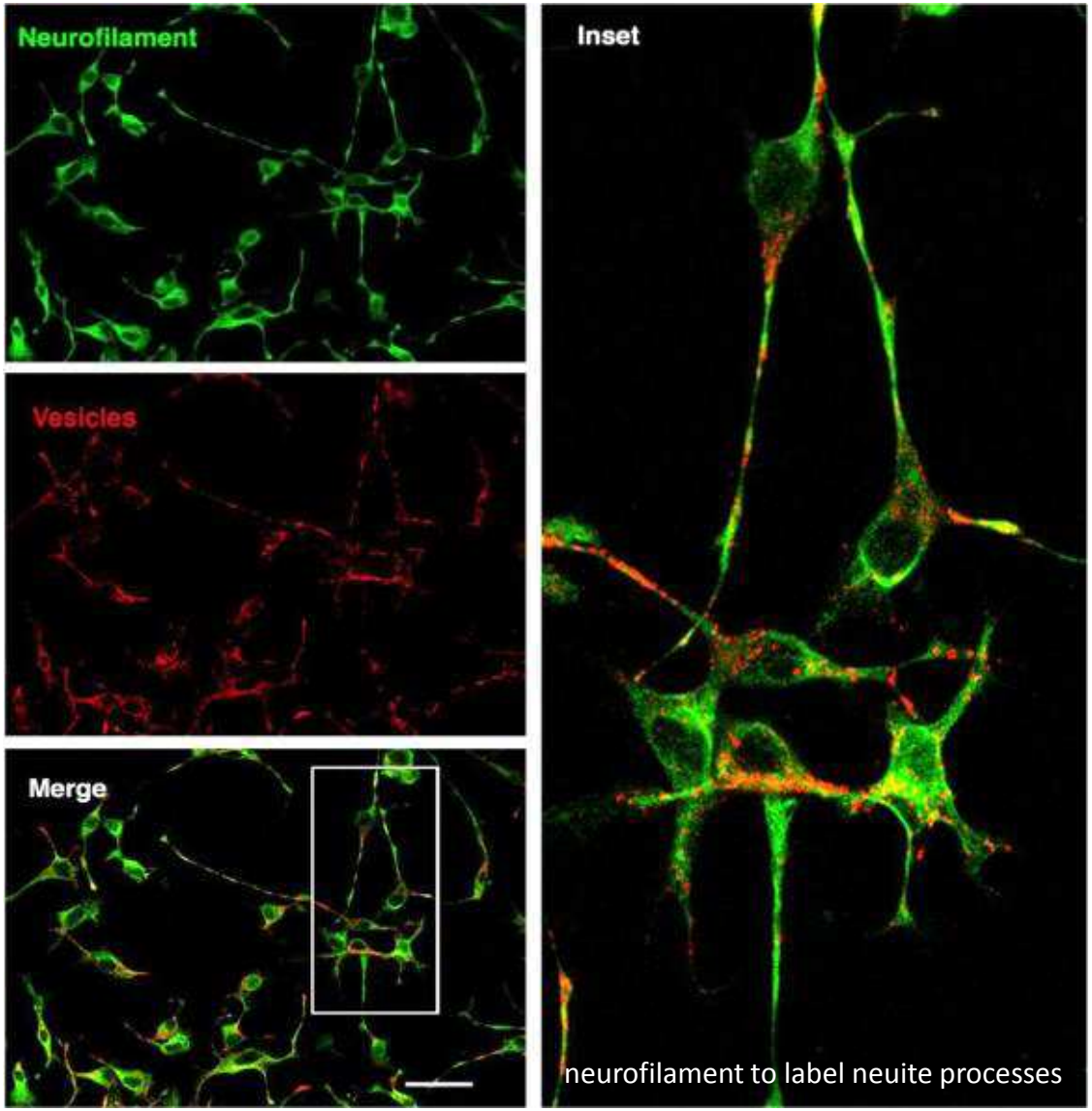
Human fetal astrocytes in culture shed membrane vesicles of several dimensions





From Falchi et al Histochem Cell Biol 2013

Uptake of labelled vesicles (red) by the motor neuron cell line NSC-34



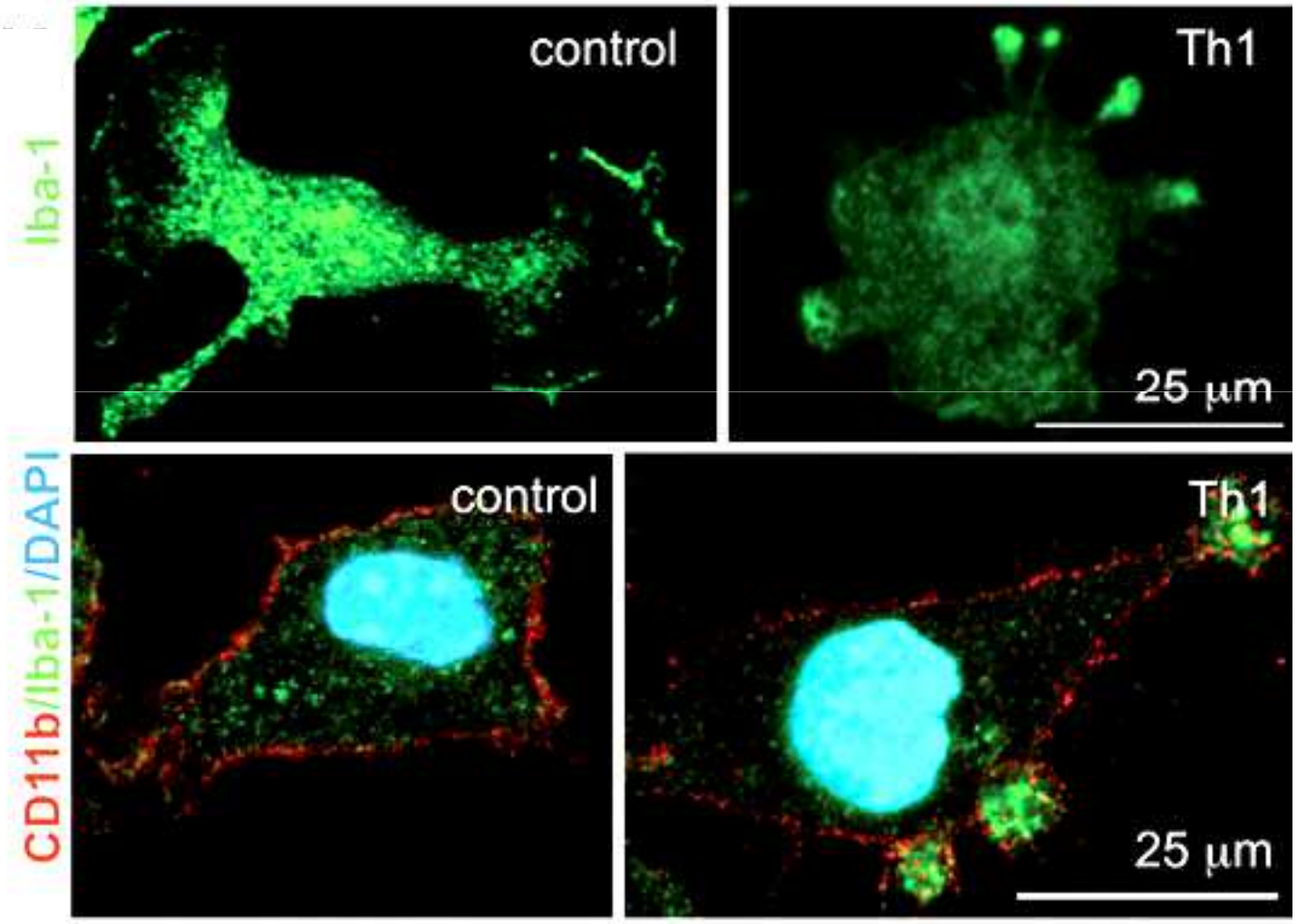
Motor neuron cell line NSC-34 exposed to fluorescently labelled vesicles for 3 hours, and stained for neurofilament (neurite processes)

It is observed extensive uptake of labelled vesicles by the neurite processes

Size bar=50 microns.

From Maddison et al J Extracellular Vesicles 2014

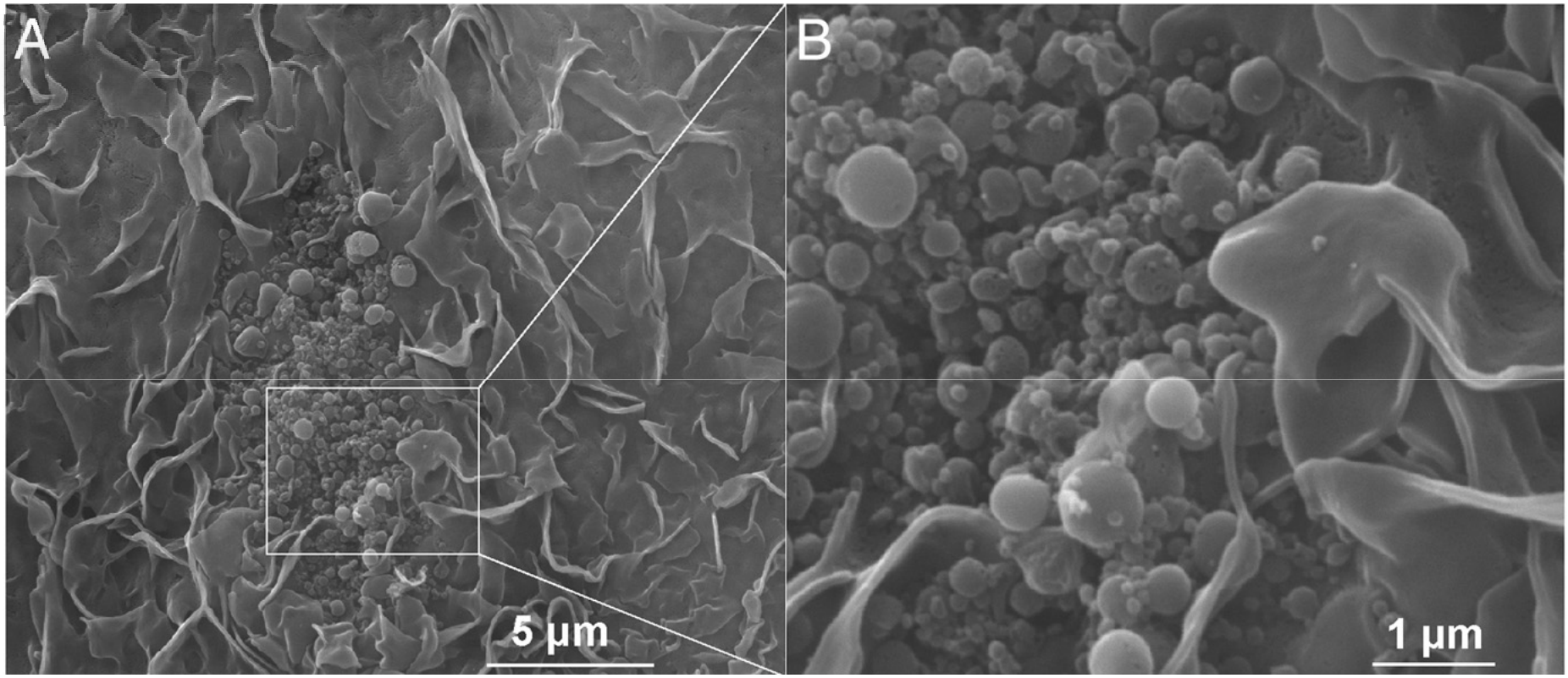
Microglial ectosomes after **Th1 cytokine** stimulation

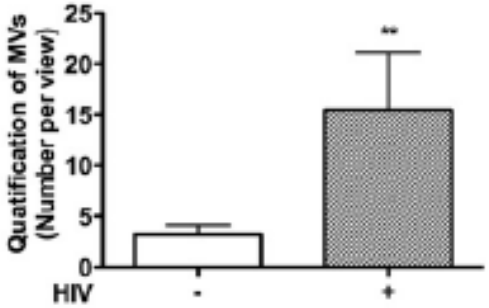


Cd11b – surface staining
Iba-1 – Intracellular staining

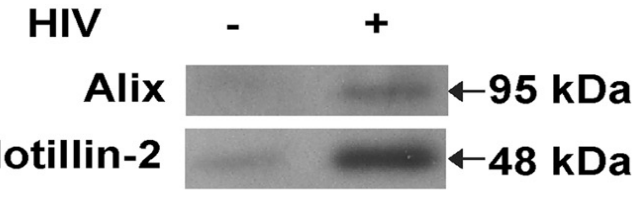
From: Verderio et al. Annals Neurol 2012

HIV-1 infection leads to an increased release of monocyte-derived MVs into the cell medium





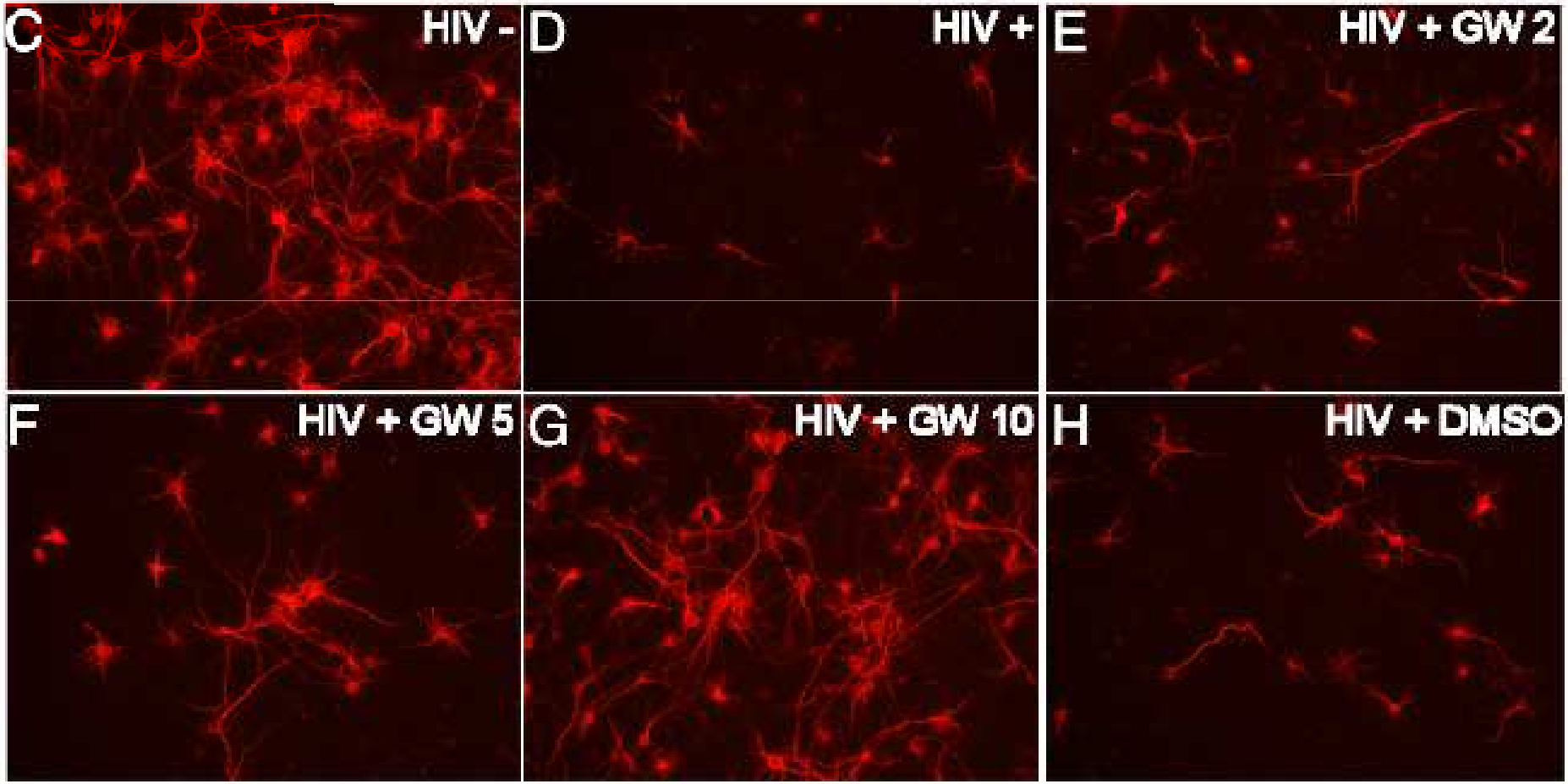
MVs biomarkers (Western blot)



Wu et al. Mol Neurodegener 2015

Exosomes collected from HIV-1 infected macrophages induce neurotoxicity

Rat cortical neurons

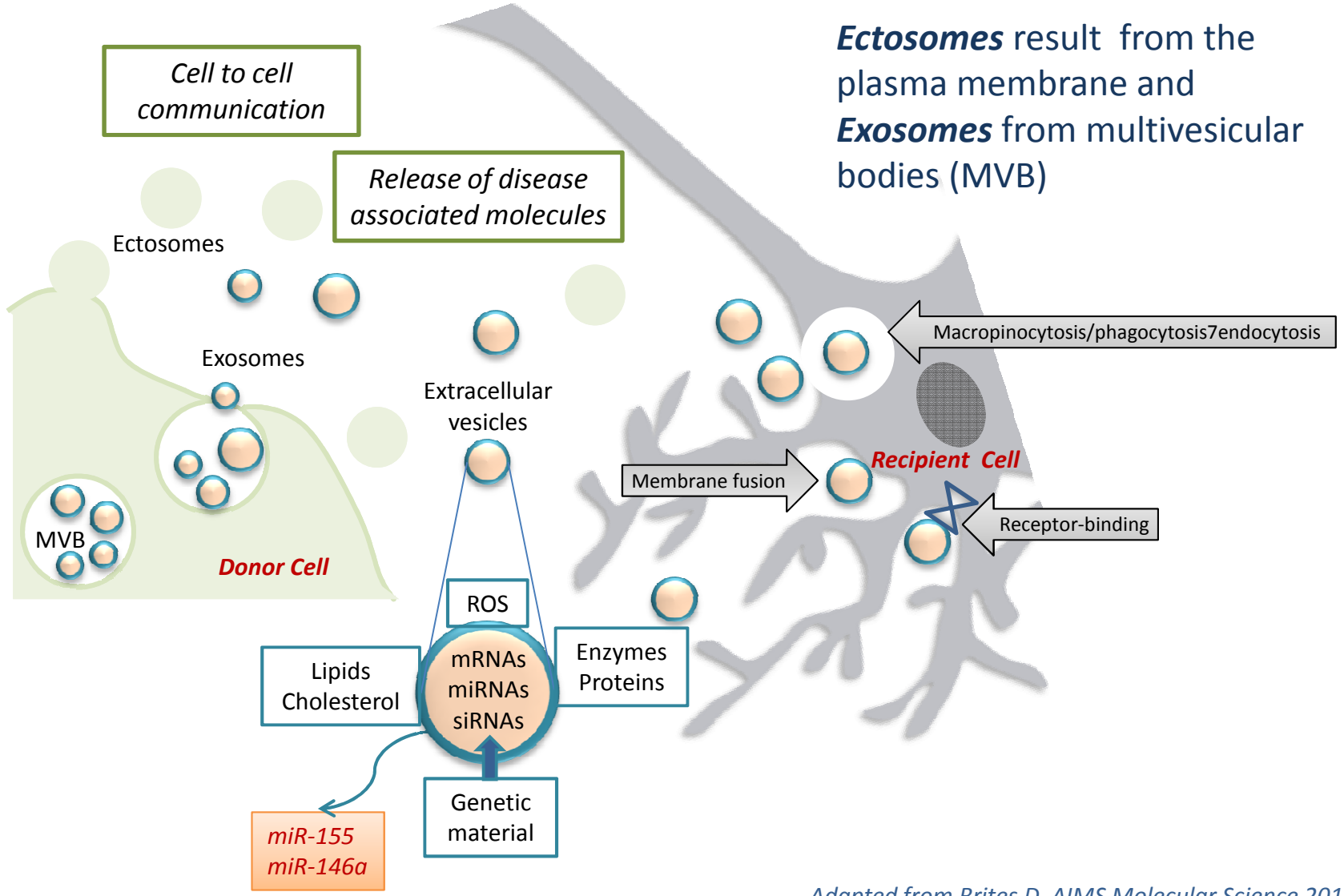


MAP2; GW4869, MV inhibitor; DMSO , GW solvent

Wu et al. Mol Neurodegener 2015

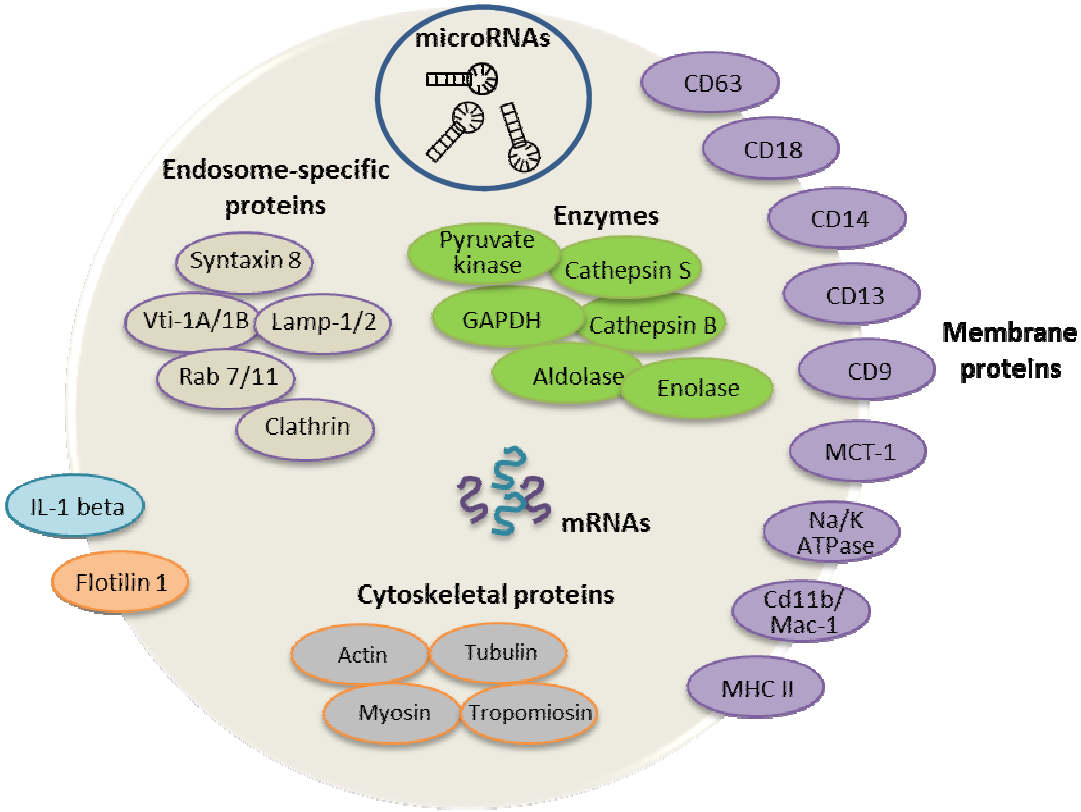
EVs cargo and disease spread

Ectosomes result from the plasma membrane and ***Exosomes*** from multivesicular bodies (MVB)



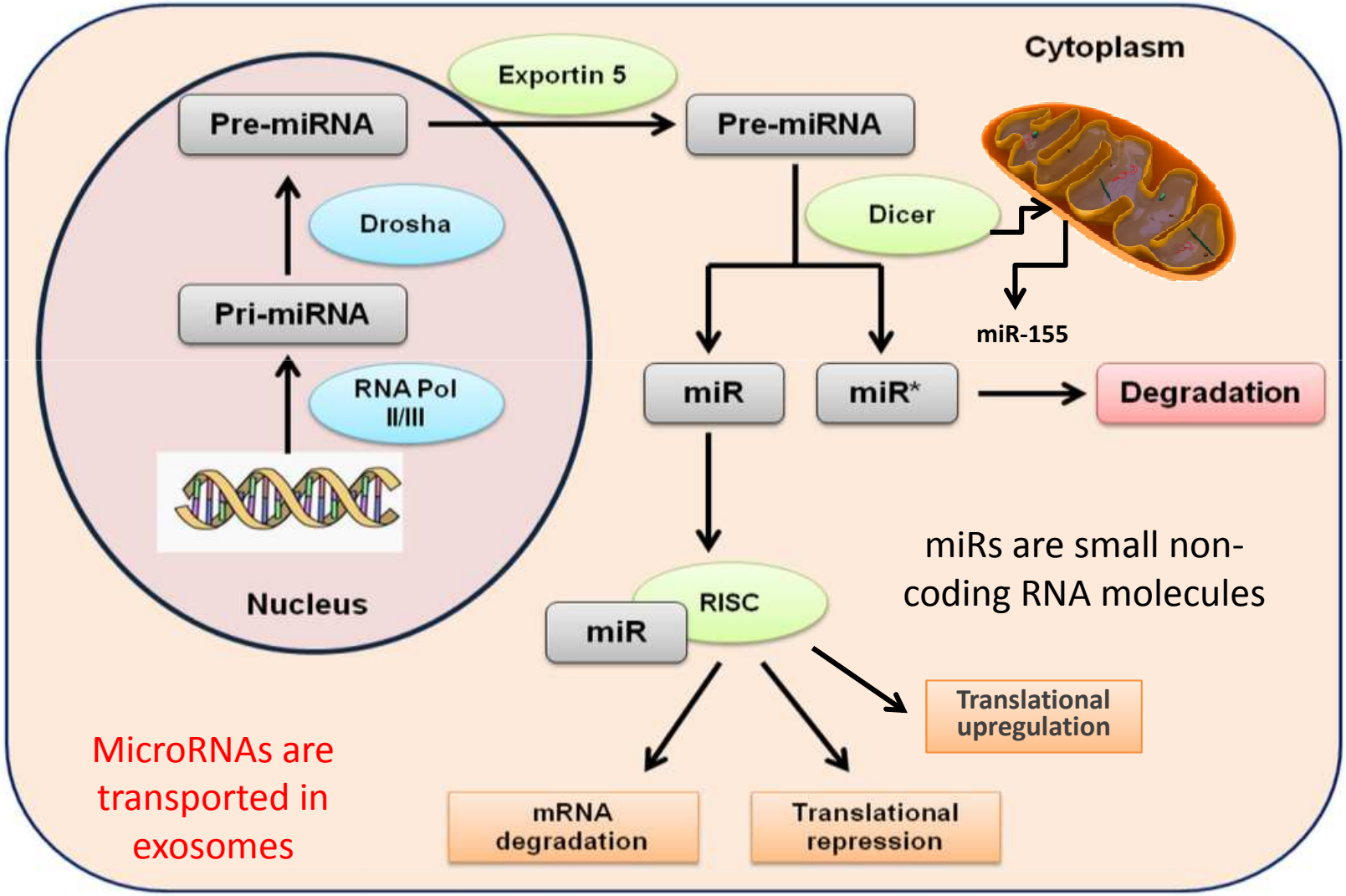
Adapted from Brites D. AIMS Molecular Science 2015

Composition of typical microglial exosomes



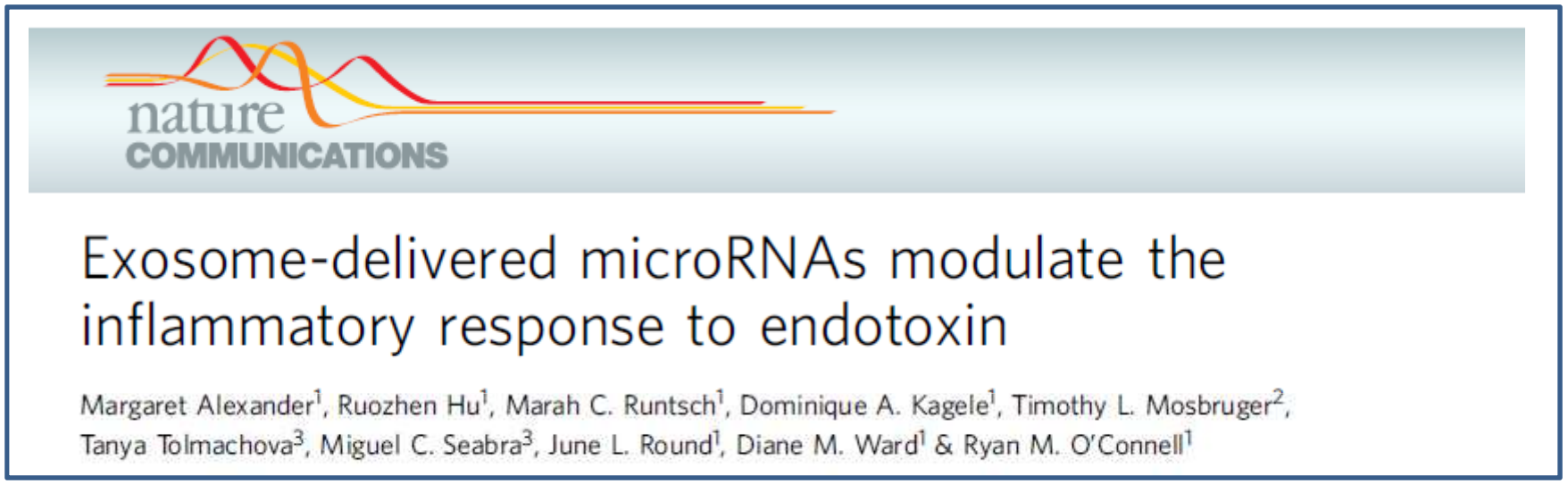
From Brites and Fernandes, Front Cell Neurosci 2015

miRNAs regulate gene expression



Fenoglio et al. Int. J. Mol. Sci. 2012
Wang et al Exp Neurol 2015

MicroRNAs are transported in exosomes

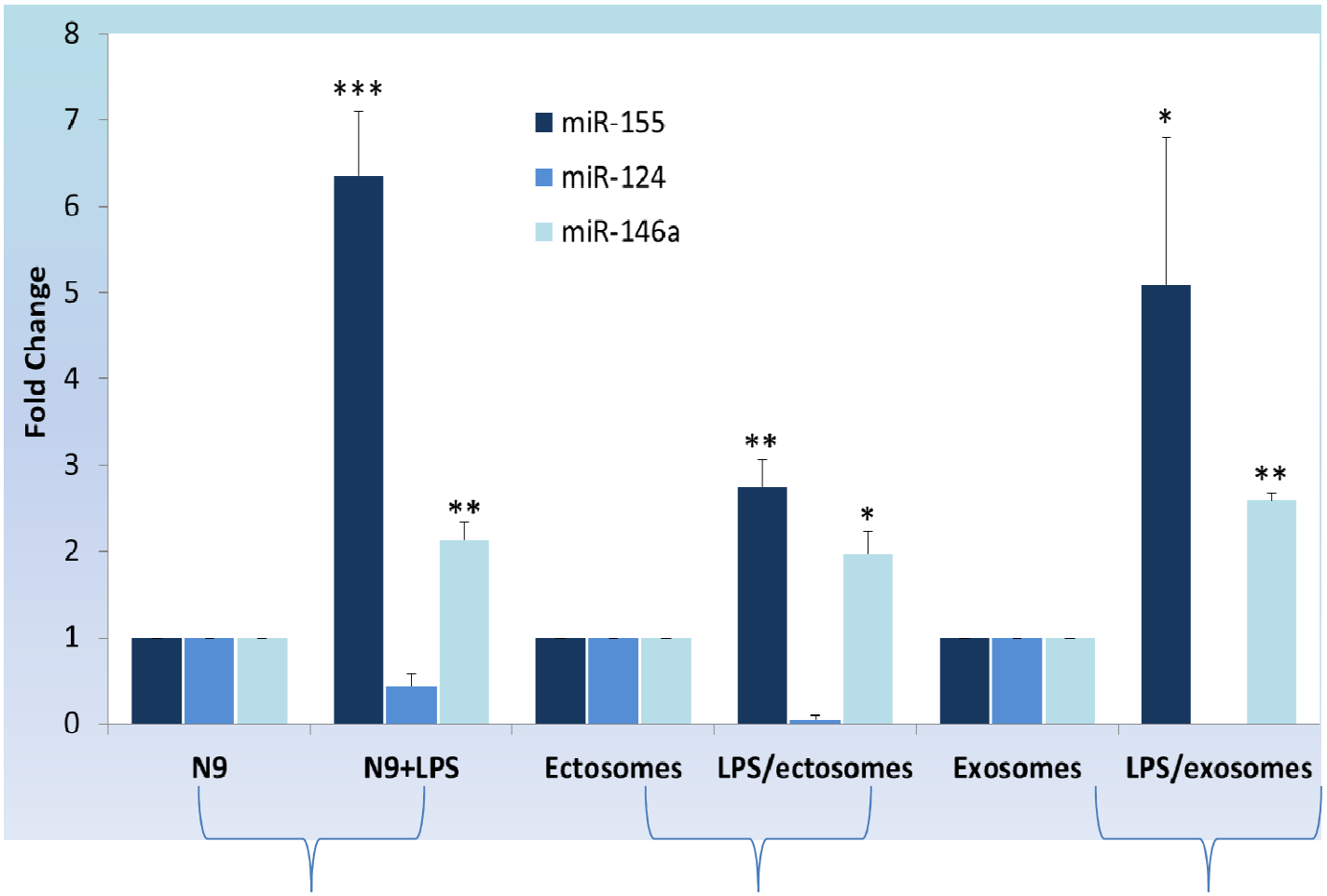


As exosomes appear to be a natural way that cells transfer miRNAs they may be used as:

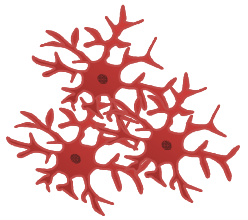
- Transportation of **specific miRNAs or their inhibitors**
- **Disease biomarkers** by their levels and presence in **serum**
- Ideal vehicle for **autologous therapies**
- **Repressors or promoters of target genes** and altered inflammatory responses
- **miR-155** promotes and **miR-146a** represses inflammation (when induced by endotoxin)

…However a great deal of future work is required to understand their role in EVs!

MicroRNA profile in exosomes and ectosomes mimics the cell of origin



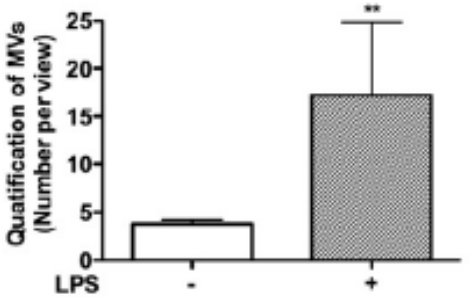
Adapted from Cunha et al Mediat Inflamm (submitted by invitation)



N9 microglia cell line

LPS 300 ng/ml

24 hours



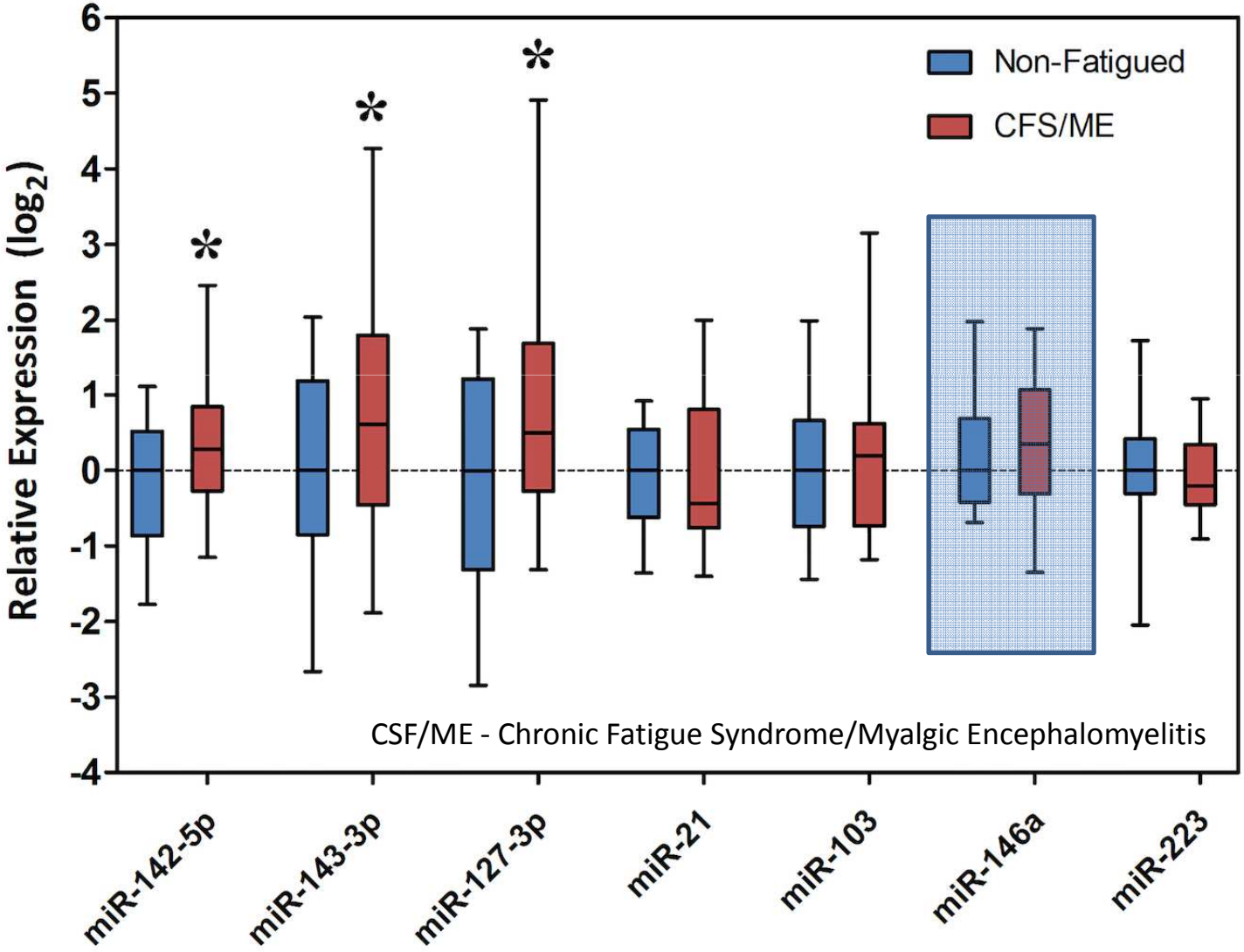
Wu et al. Mol Neurodegener 2015

MicroRNAs implicated in stress and depression

Restraint stress	Frontal cortex: miR-9, miR-9*, miR-26b, miR-29b, miR-30b, miR-30c, miR-30e, miR-125a, miR-126-3p, miR-129-3p, miR-207, miR-212, miR-351, miR-423, miR-487b, miR-494, miR-690, miR-691, miR-709, miR-711, and Let-7 a-e let-7a, miR-9, miR-26a/b, miR-30b/c, miR-125a
Immobilization stress	Hippocampus CA1, amygdala: miR-134, miR-183, miR-132, Let-7a-1, miR-9-1, miR-124a-1
Unpredictable chronic mild stress	Hippocampus: miR298, miR-130b, miR-135a, miR-323, miR-503, miR-15b, miR-532, and miR-125a and up-regulating miRNAs miR7a, miR-212, miR-124, miR-139, miR-182
Early life stress	Medial prefrontal cortex: pre-miRs 132, 124-1, 9-1, 9-3, 212, 29a
Animal model of depression	Learned helpless vs control frontal cortex: mmu-miR-184, mmu-miR-197, mmu-miR-107, mmu-miR-329, mmu-miR-125a-5p, mmu-miR-872, mmu-miR-181c, mmu-miR-18a*, mmu-miR-29b*, mmu-let-7a*, rno-let-7e*, rno-miR-20a*
Postmortem brain studies	Prefrontal cortex: hsa-miR-142-5p, hsa-miR-33a, hsa-miR-137, hsa-miR-489, hsa-miR-148b, hsa-miR-101, hsa-miR-324-5p, hsa-miR-301a, hsa-miR-146a, hsa-miR-335, hsa-miR-494, hsa-miR-20b, hsa-miR-376a*, hsa-miR-190, hsa-miR-155, hsa-miR-660, hsa-miR-552, hsa-miR-453, hsa-miR-130a, hsa-miR-27a, hsa-miR-497, hsa-miR-10a, hsa-miR-20a, hsa-miR-142-3p
Peripheral mononuclear cells	has-miR-107, miR-133a, miR-148a, miR-200c, miR-381, miR-425-3p, miR-494, miR-517b, miR-579, miR-589, miR-636, miR-652, miR-941, miR-1243
Whole blood cells (12 weeks of treatment with escitalopram)	hsa-miR-130b , hsa-miR-505 , hsa-miR-29b-2 , hsa-miR-26b, hsa-miR-22, hsa-miR-26a, hsa-miR-664, hsa-miR-494, hsa-let-7d, hsa-let-7g, hsa-let-7e, hsa-miR-34c-5p, hsa-let-7f, hsa-miR-629, hsa-miR-106b, hsa-miR-103, hsa-miR-191, hsa-miR-128, hsa-miR-502-3p, hsa-miR-374b, hsa-miR-132, hsa-miR-30d, hsa-miR-500, hsa-miR-770-5p, has-miR-589, hsa-miR-183, hsa-miR-574-3p, hsa-miR-140-3p, hsa-miR-335, hsa-miR-361-5p

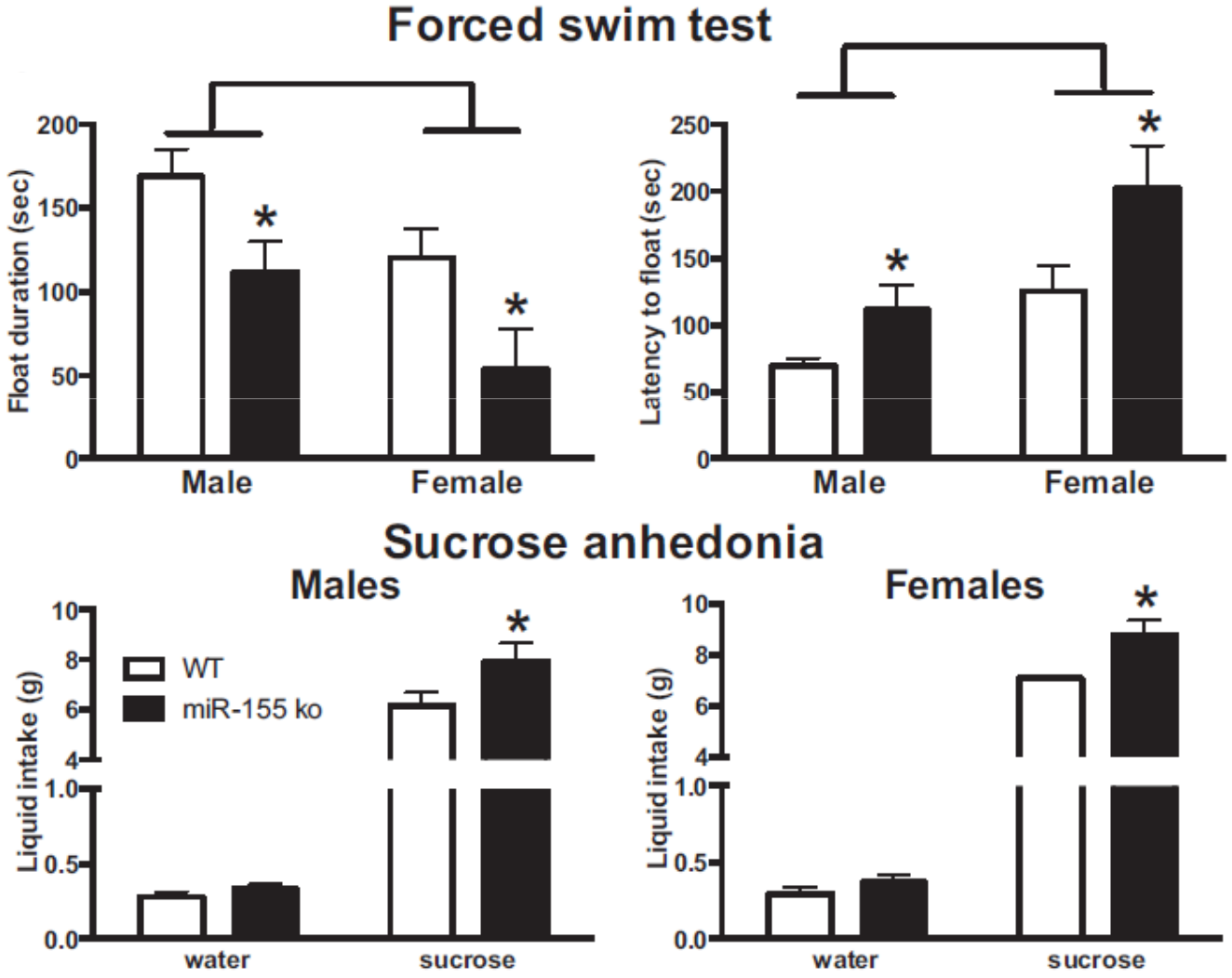
Dwivedi Y Dialogues in Clinical Neuroscience 2014

Plasma microRNA profile in CFS/ME



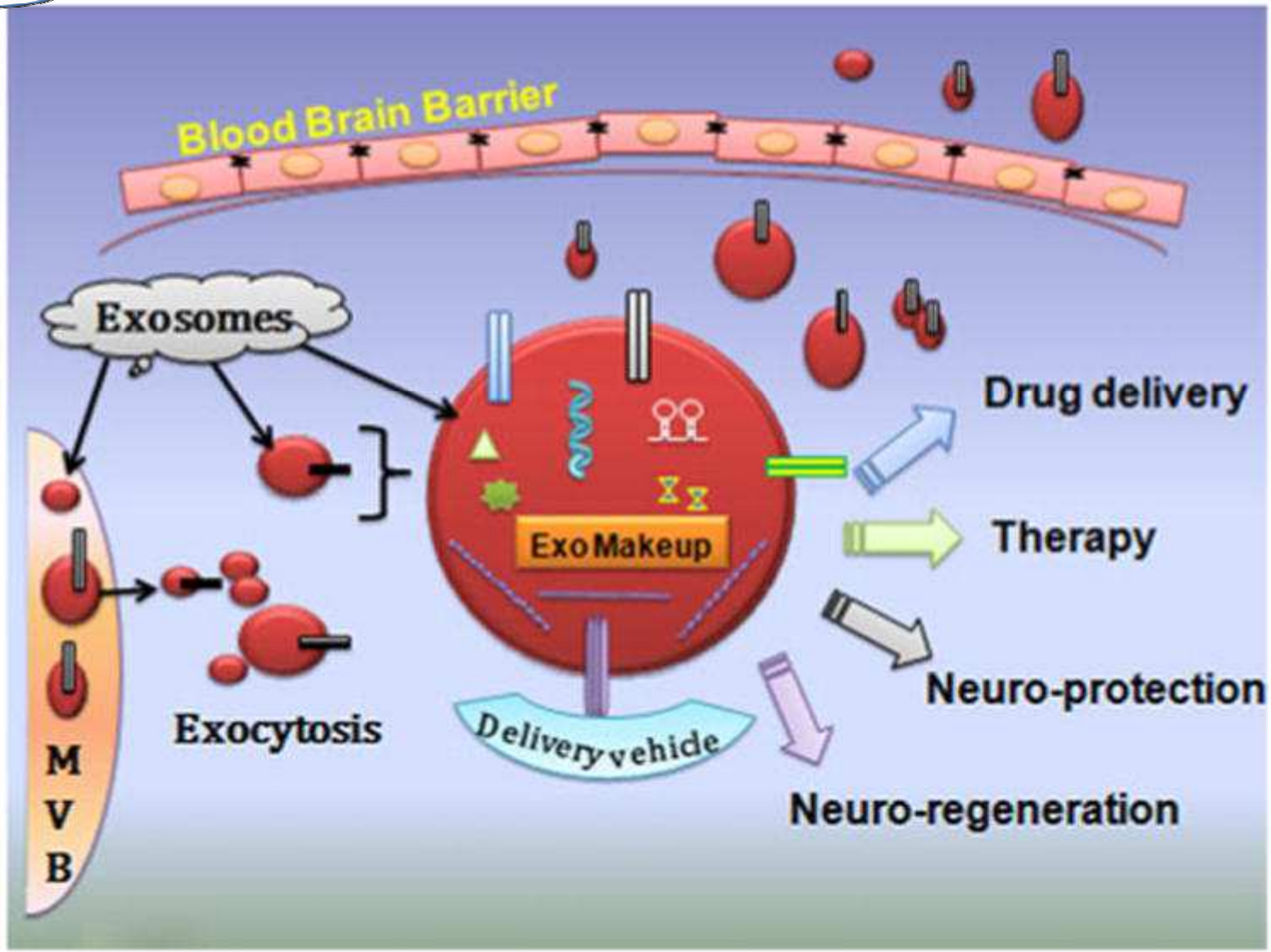
From Brenu et al. PLoS One 2014

MicroRNA-155 knockout mice show decreased depressive-like responses



From Fonken et al Psychoneuroendocrinology 2016

Exosome makeup and their use as delivery vehicles



From Kalani et al Mol Neurobiol 2014

Conclusions

Future studies should establish whether:

- deleting ***miR-155*** is protective against the development of depressive-like responses in animal models of depression (e.g., following chronic stress)
- Evaluation of exosome cargo in ***miR-155, miR-124 and miR-146a*** should be determined to assess whether depression propagation is mediated by ***exosomes/ectosomes*** containing inflammatory microRNAs.

*Results to be obtained may lead to the development of **novel therapies for depression** and other mood disorders.*

Take-home message

Mechanisms of astrocyte deficits in depression are not clarified

Microglia subsets may determine the prevalence of responsive/activated cells or irresponsive/senescent subtypes in a clinical condition, including HIV

Clarification of microglia dysfunction may help in personalized medicine – microglial inhibitors or stimulators?

Release of extracellular vesicles (EVs) are important features in cell-to-cell communication and disease propagation, and **are increased by inflammation**

MicroRNAs are transported in EVs that mimic parenteral cells and may act as biomarkers, as well as repressors or promotors of inflammation

EVs may be helpful in regenerative medicine, tissue engineering and patient directed medicine

Team, Partners and Funding

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Ana Luísa Cardoso, PhD
Ricardo Neves, PhD

- *PTDC/SAU-FAR/118787/2010 to DB*
- *UID/DTP/04138/2013 to iMed.ULisboa*

Advanced Experimental Models

Transnational project in the area of EU drugs policy – JUST/2014/JDRU/AG/DRUG

ご清聴ありがとうございました