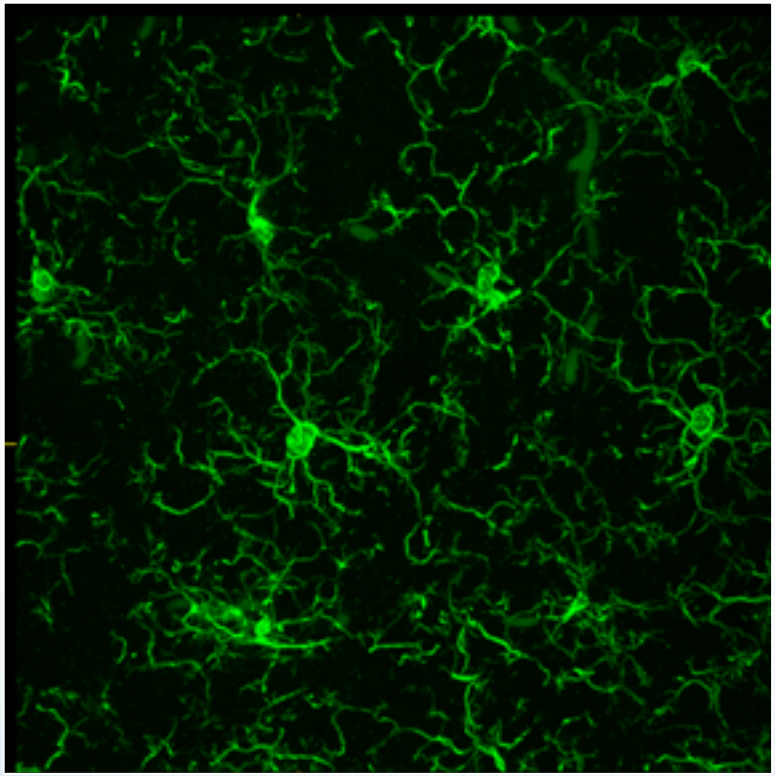
Microglia as a Therapeutic Target in Alzheimer's Disease

Kim Green, Ph.D.
Department of Neurobiology and Behavior
University of California, Irvine

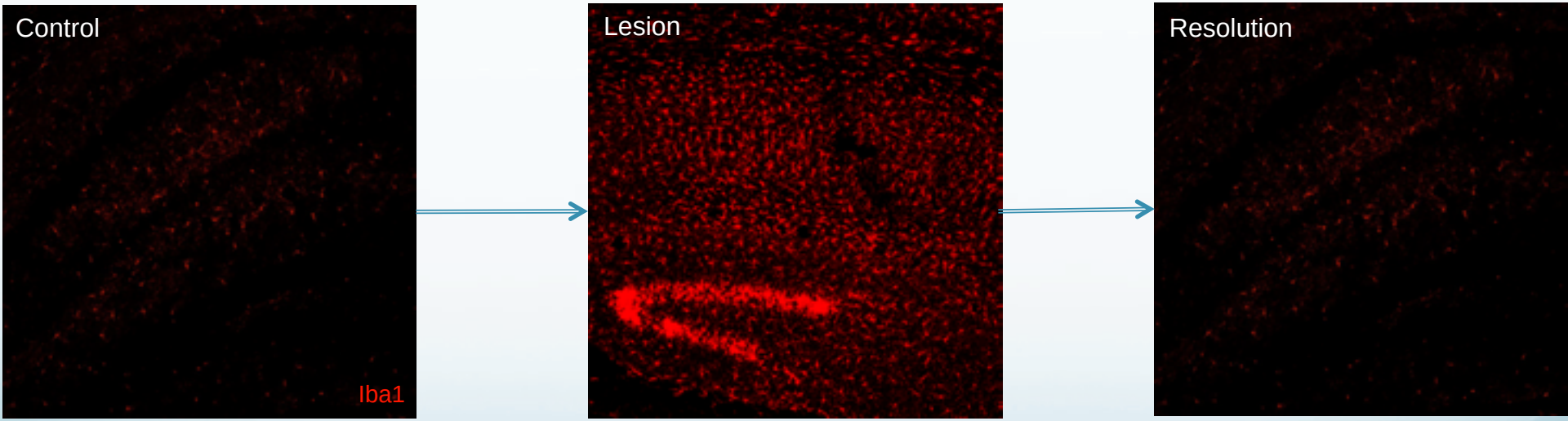
Microglia



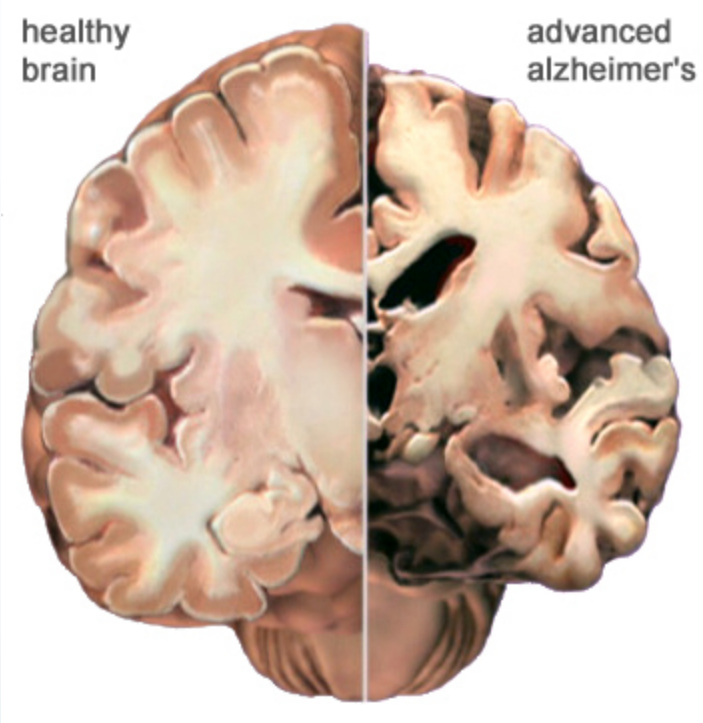
- Microglia are the immune cell of the brain.
- Comprise ~12% of all cells in the brain.
- Function to protect from infections, and to clean up debris following damage and injury.
- Microglial dysfunction implicated in traumatic brain injury, aging, and neurodegeneration

Activation of microglia

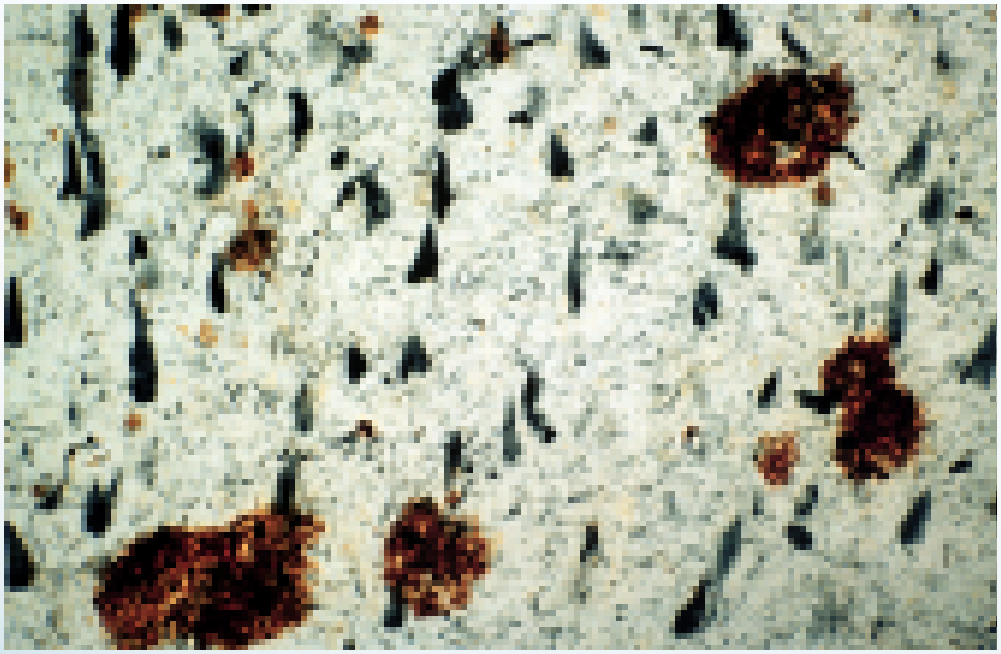
- Infection or damage causes microglia to become “activated”
- Primed to fight pathogens.
- After the infection/damage is contained microglia revert to resting state.



AD Neuropathology



AD Neuropathology

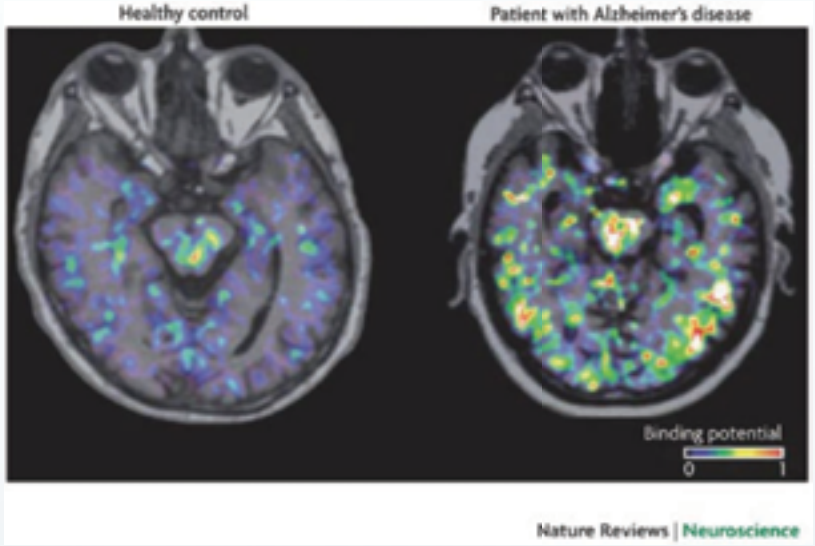


- Plaques composed of

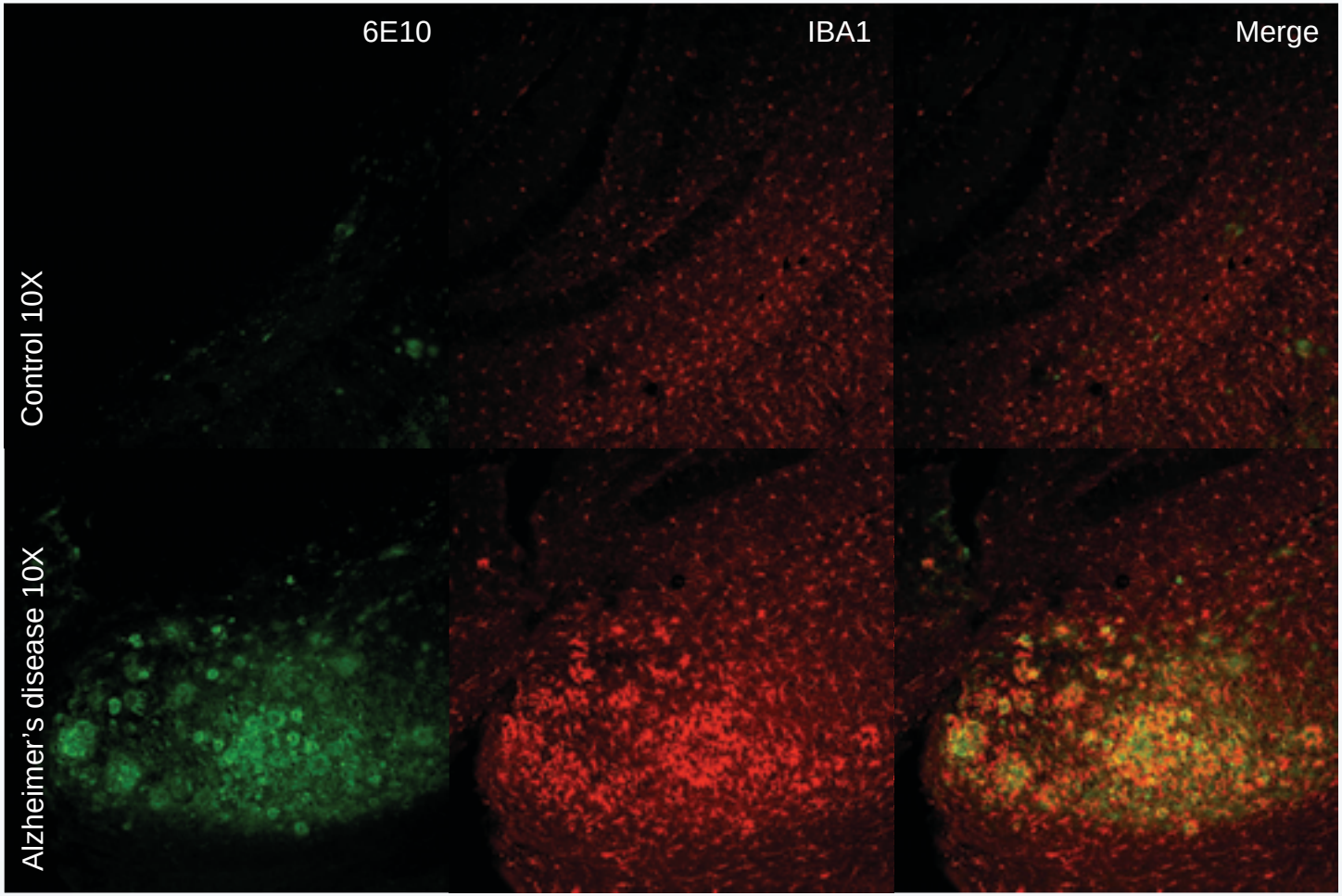
  β-amyloid (Aβ) peptide
  - Initiates in cortical regions
- Tangles composed of hyperphosphorylated tau
  - Initiates in hippocampus (CA1)
- Synaptic/Neuronal dysfunction and death

Inflammation and Alzheimer's disease

- The AD brain is characterised by the presence of plaques and tangles, extensive neuronal loss and an inflammatory response.



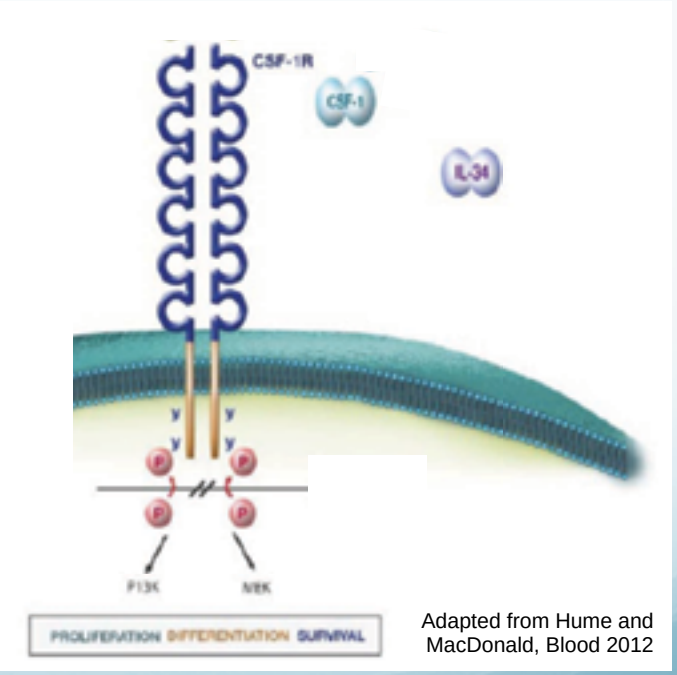
- Microglia surround plaques, in an attempt to phagocytose Aβ and remove it from the brain.



- They are unsuccessful, as the AD brain is still riddled with Aβ plaques.
- However, now there is also an increasing chronic inflammatory process...

CSF1R

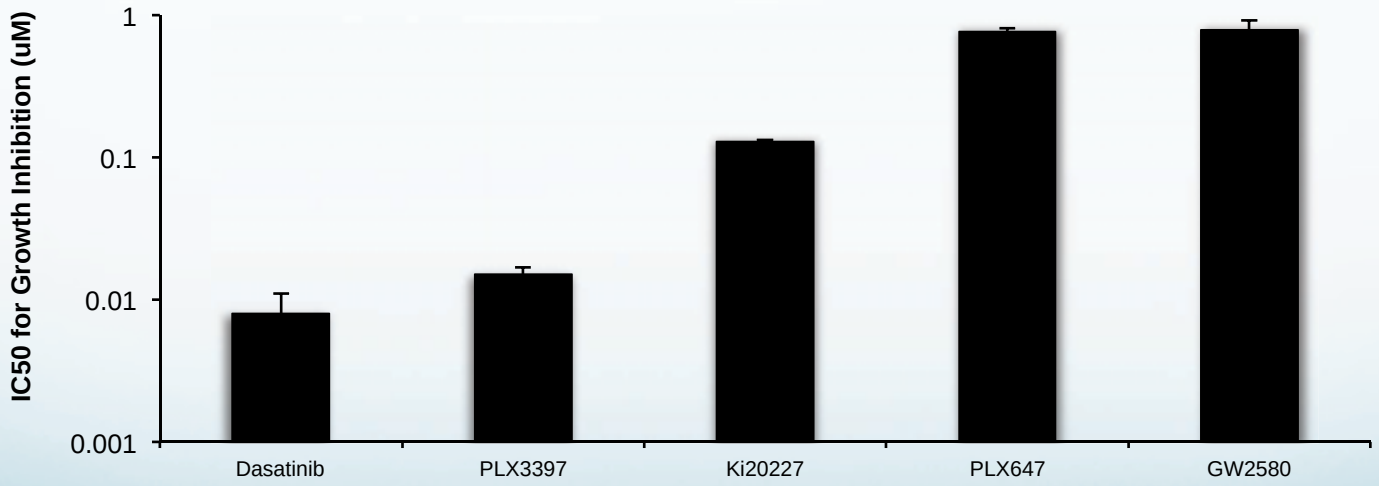
- In the brain, microglia express the majority of the colony stimulating factor 1 receptor
  - expressed on monocytes, macrophages, dendritic cells and osteoclasts
- Has 2 ligands CSF1 and IL34
  - Cause dimerization and autophosphorylation
- Involved in cell proliferation, survival and migration.



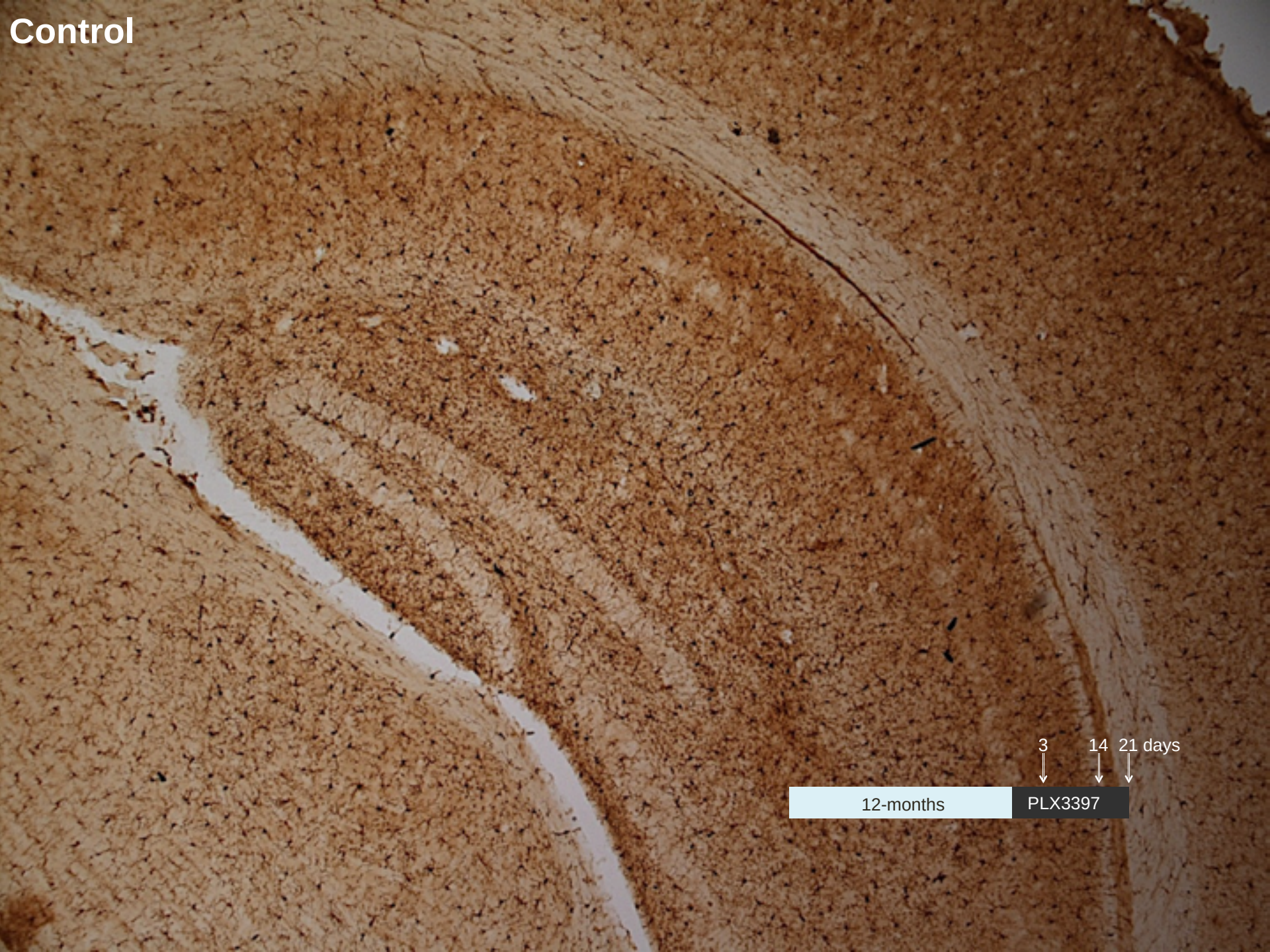
Adapted from Hume and MacDonald, Blood 2012

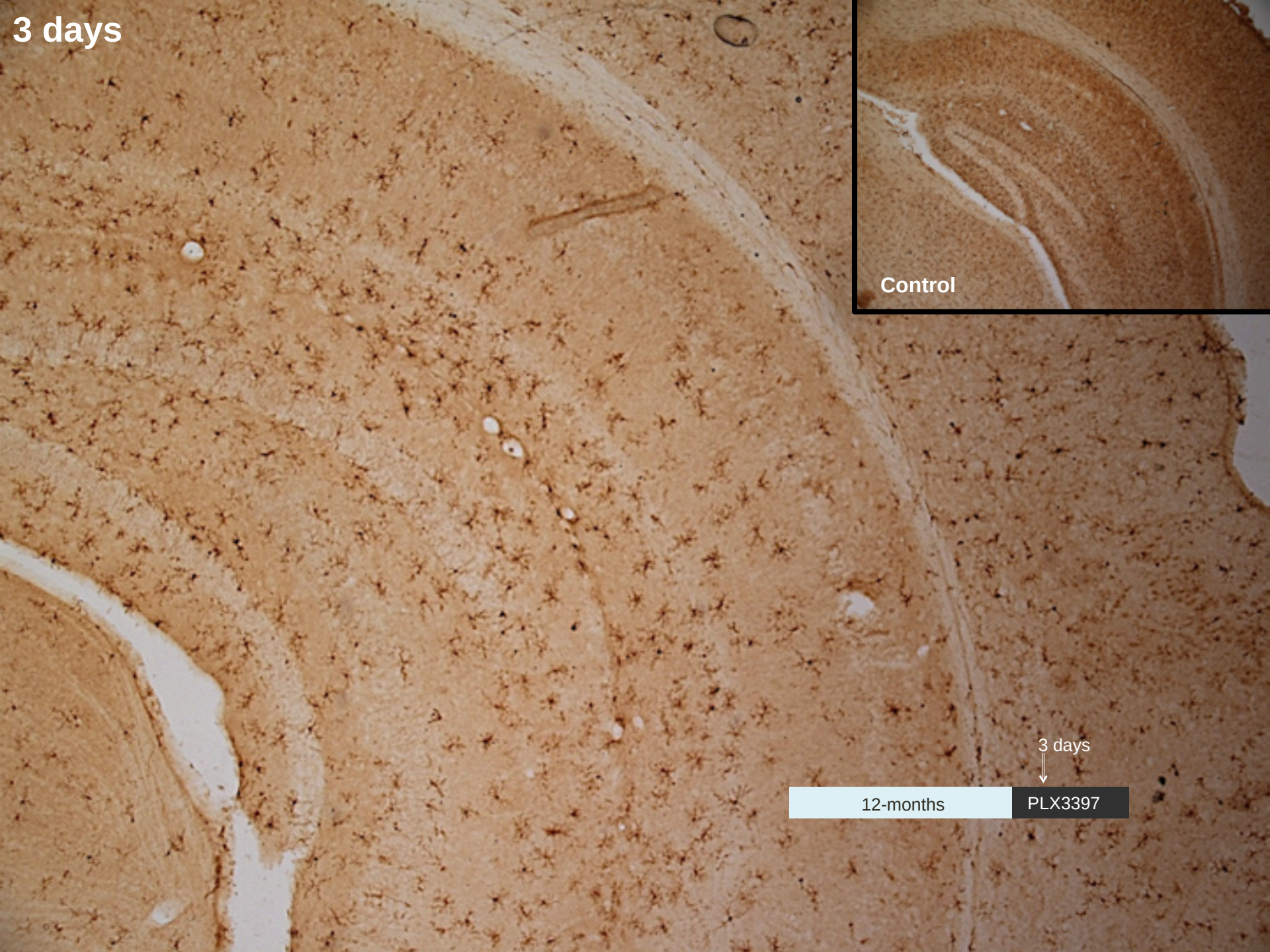
Evaluation of CSF1R antagonists

- We use PLX3397 (Plexxikon Inc.) – entering Phase 3 clinical trials for Pigmented villonodular synovitis (PVNS), and Phase 2 for oncology indications.
- Specific for CSF1R and also related receptor c-Kit.



Elmore and Najafi et al., *Neuron* 2014


Control
3
14
21 days
12-months
PLX3397


3 days
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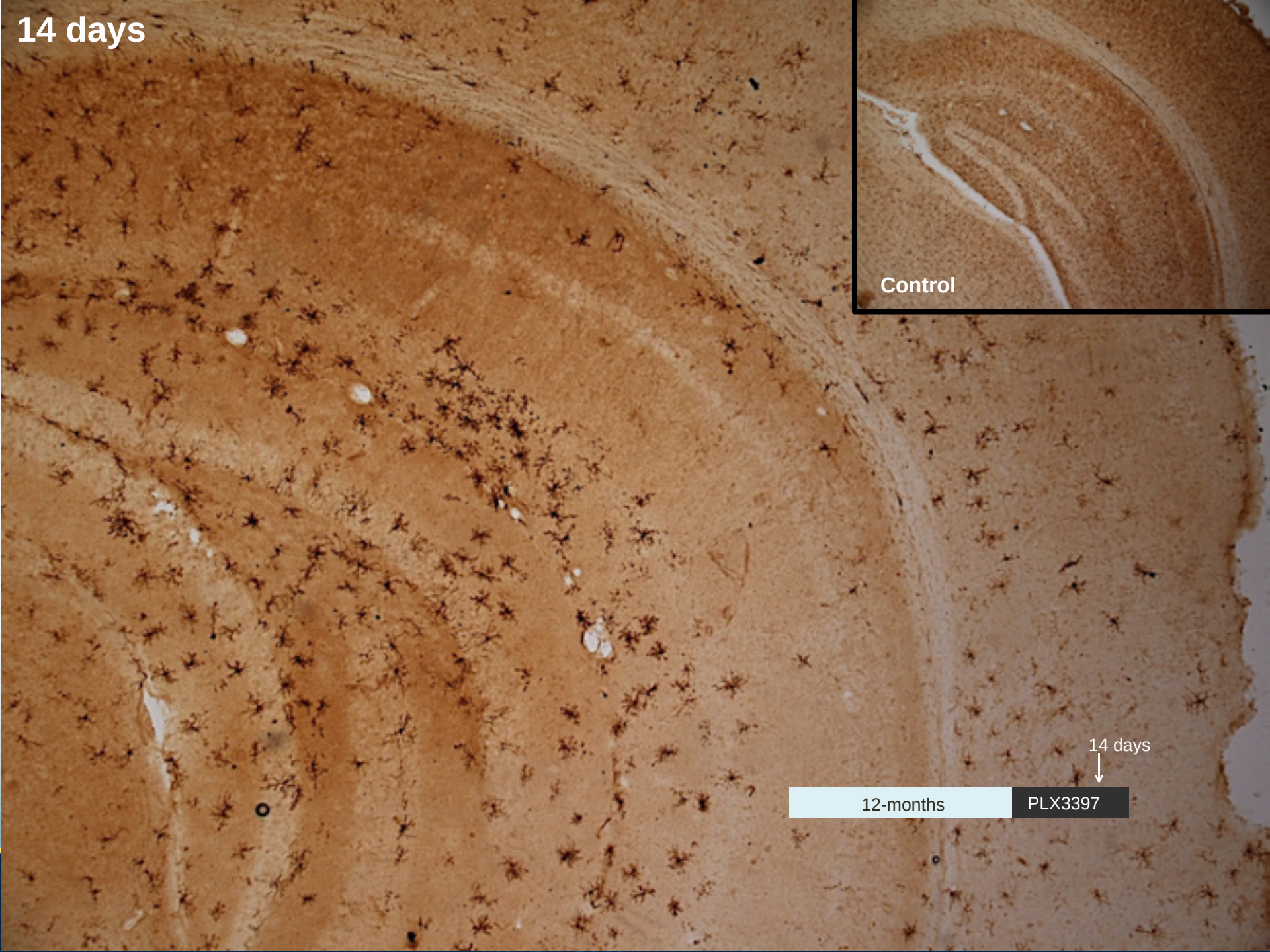
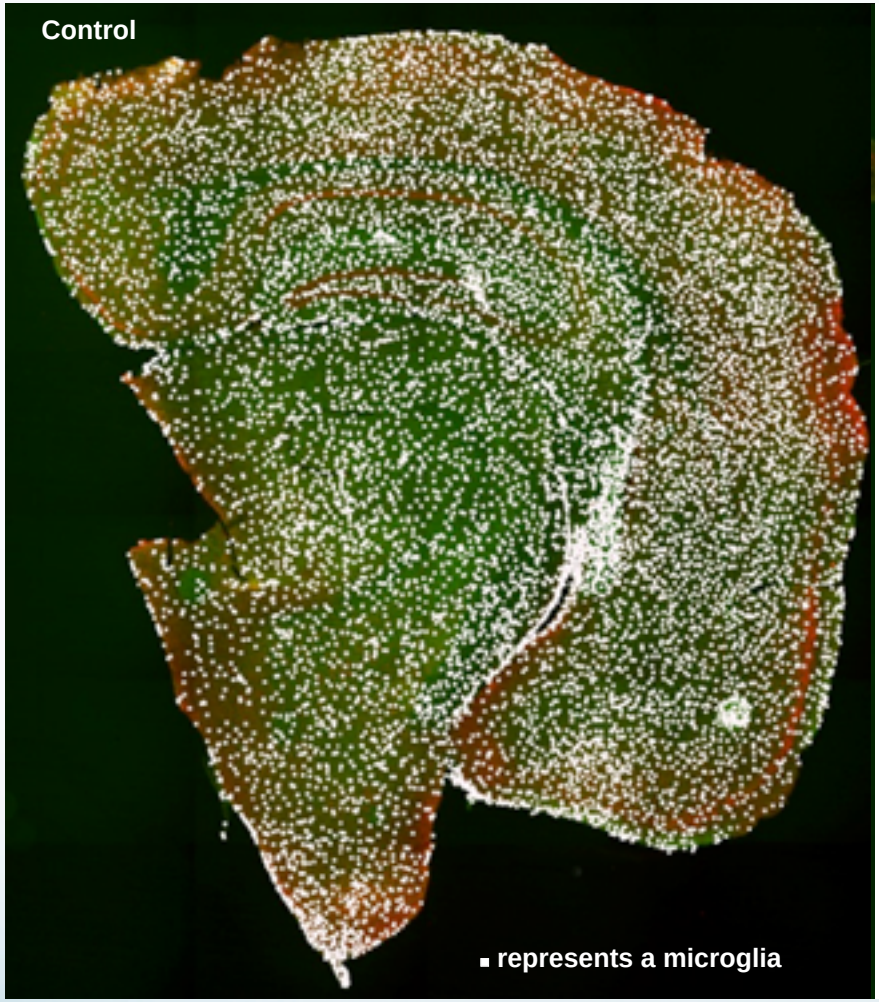
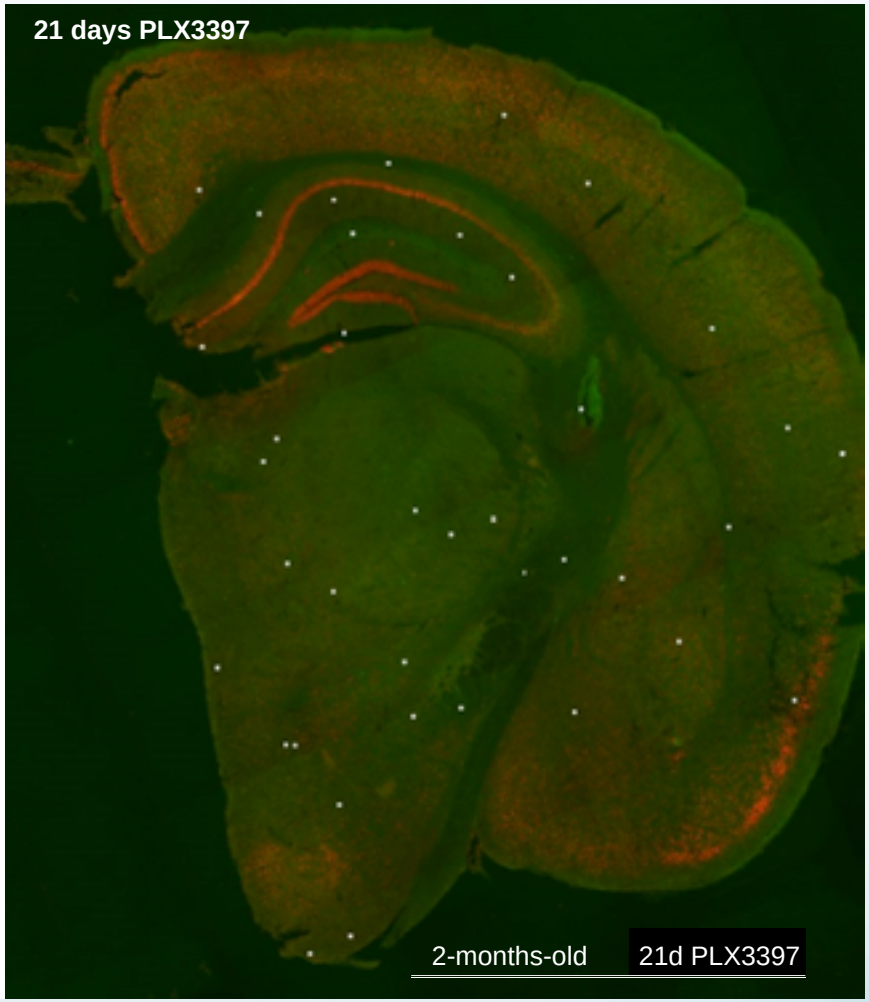

14 days
Control
14 days
12-months
PLX3397

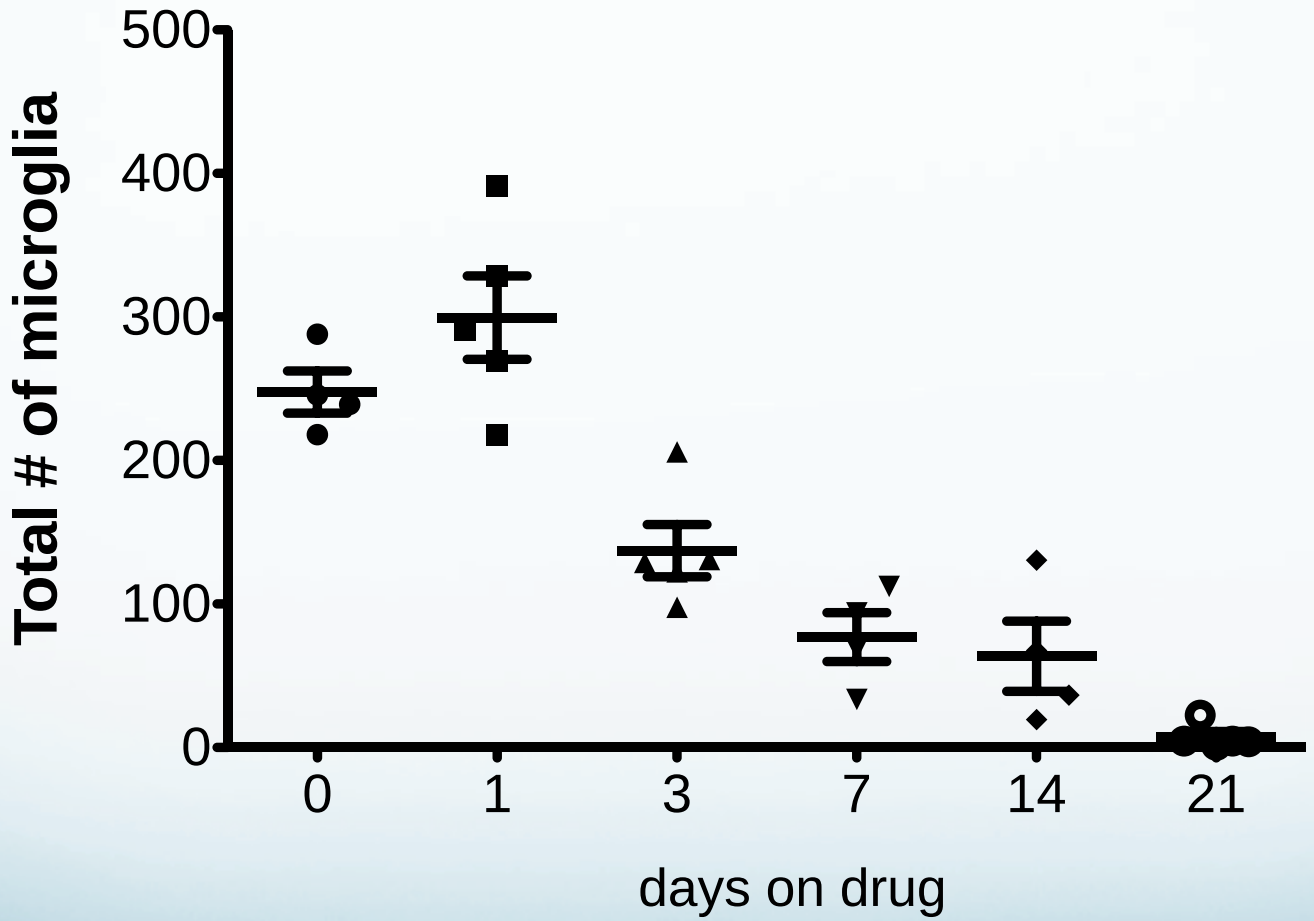

21 days
Control
21 days
12-months
PLX3397





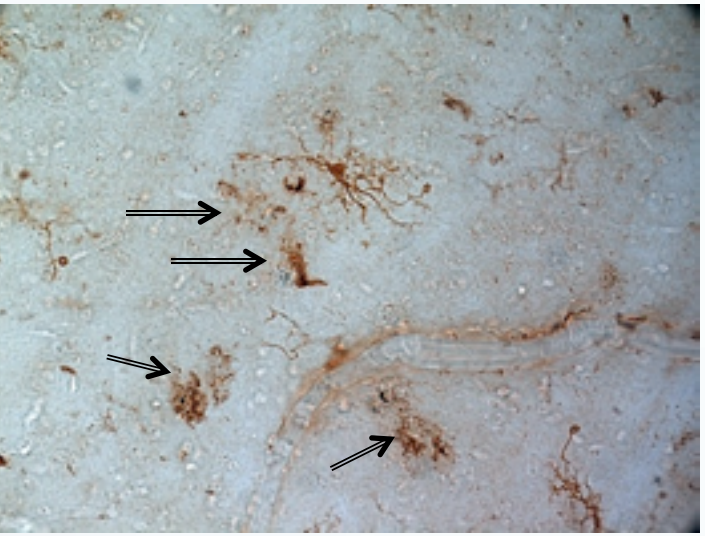
Elmore and Najafi et al., *Neuron* 2014

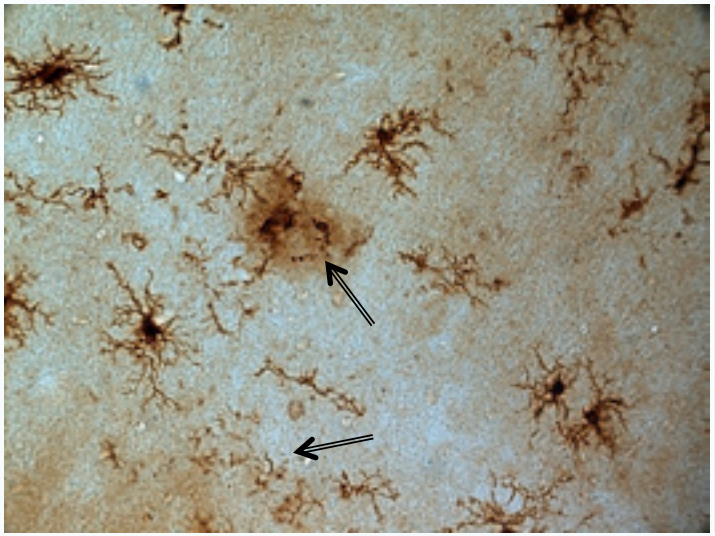
CSF1R inhibitors eliminate microglia

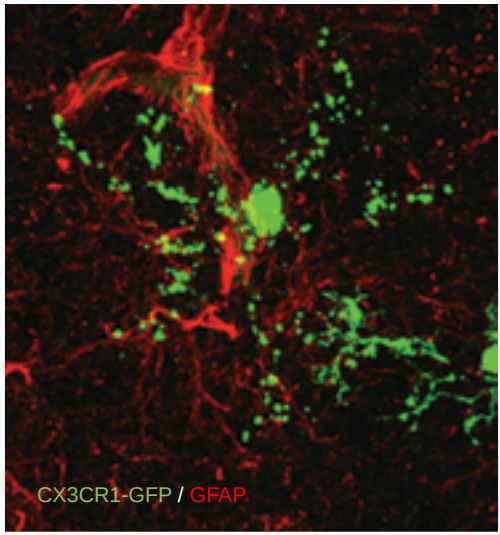


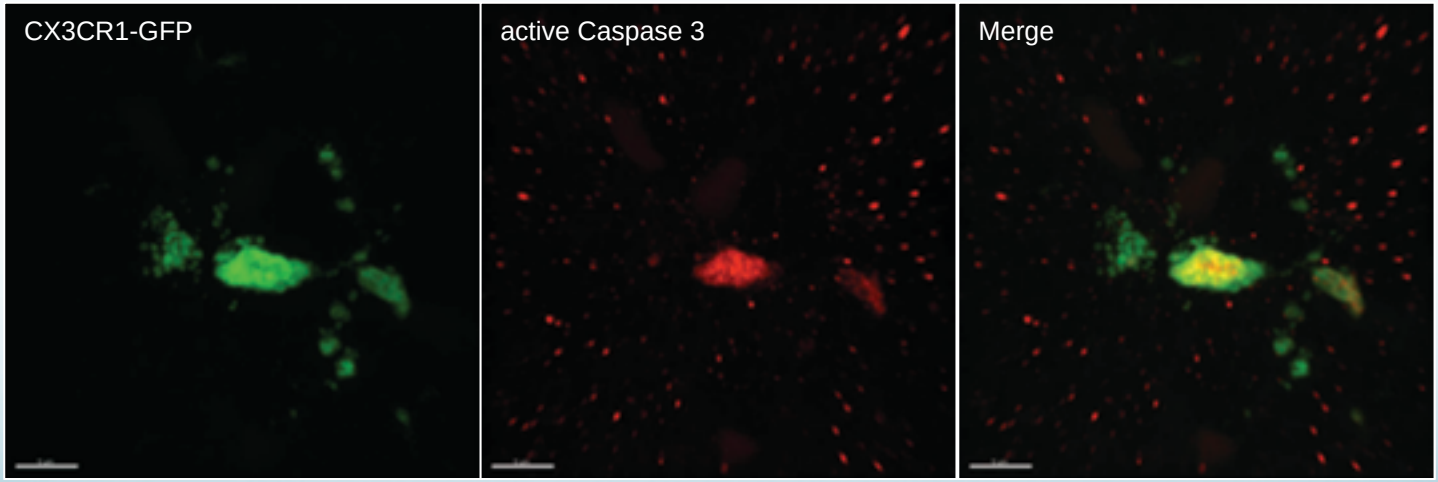
Elmore and Najafi et al., *Neuron* 2014

Microglial remnants seen throughout brain







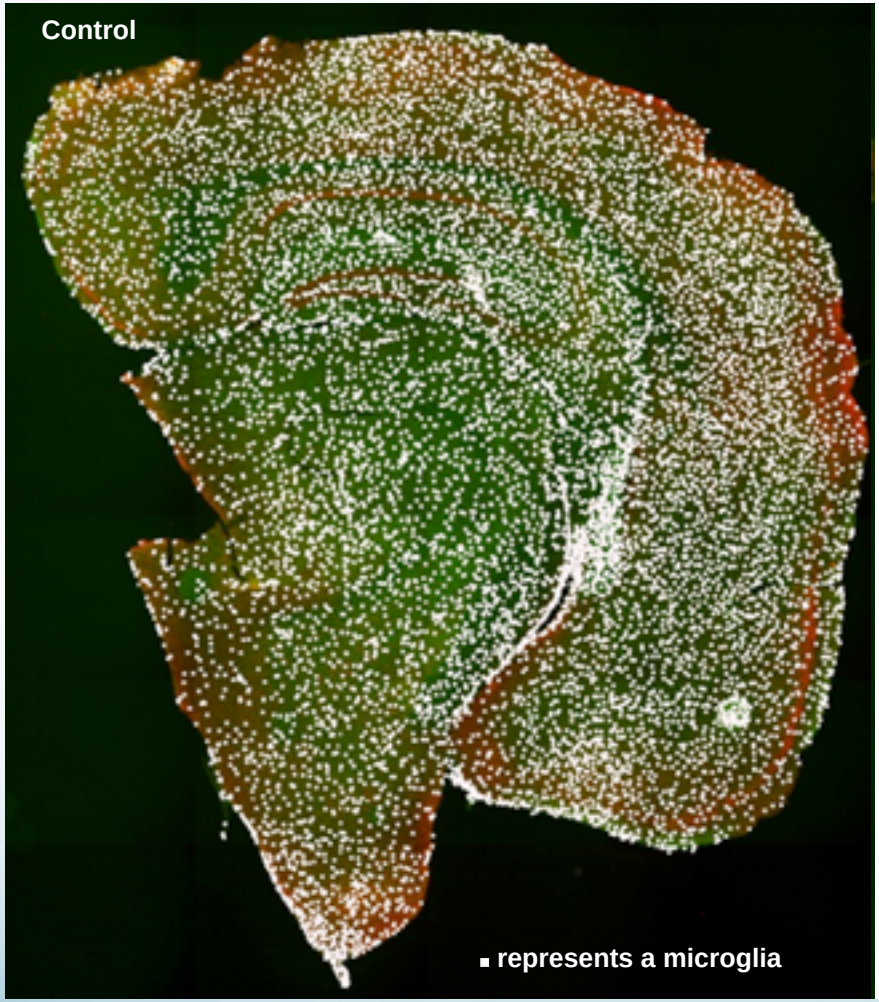


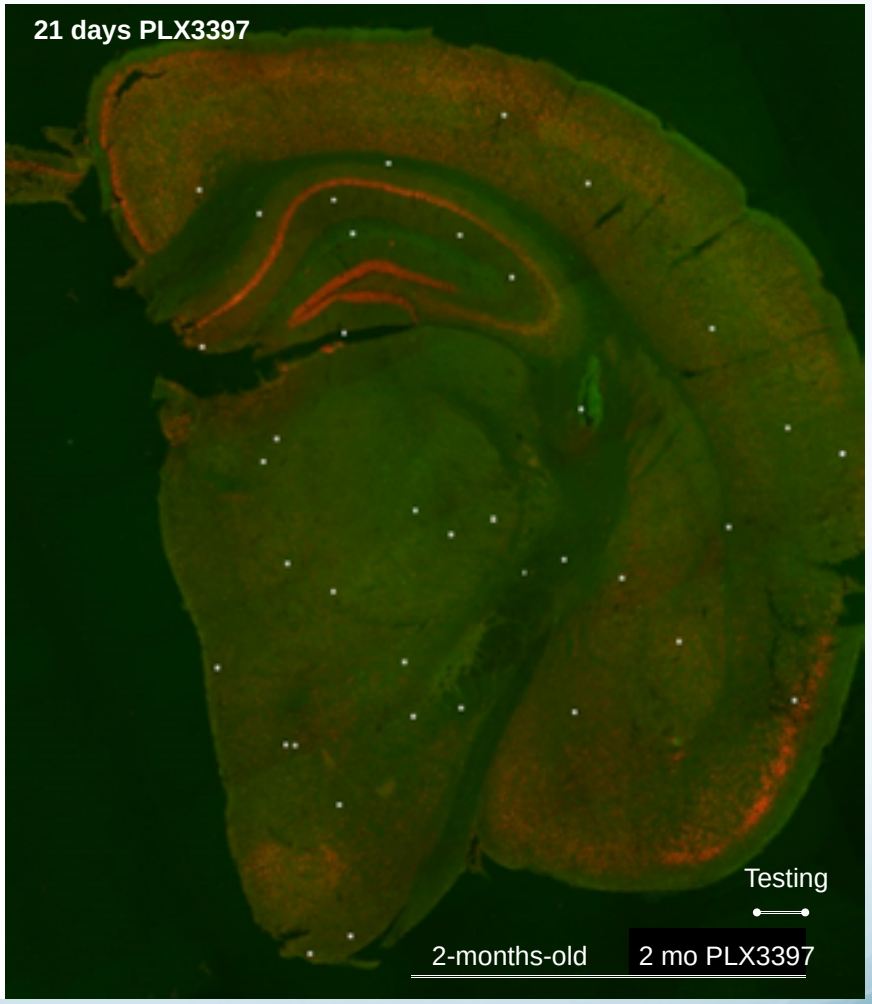
Elmore and Najafi et al., *Neuron* 2014

Microglia are dependent on CSF1R signaling

- Administration of CSF1R inhibitors that cross the blood brain barrier lead to the rapid elimination of microglia throughout the CNS.
- Microglia undergo cell death.
- Peripheral macrophage/monocyte populations are not depleted.
- As microglia are the only cell type in the CNS to express CSF1R it provides a useful tool to study microglial function, as well as a potential therapeutic target.

Phenotype of microglia-depleted mice:

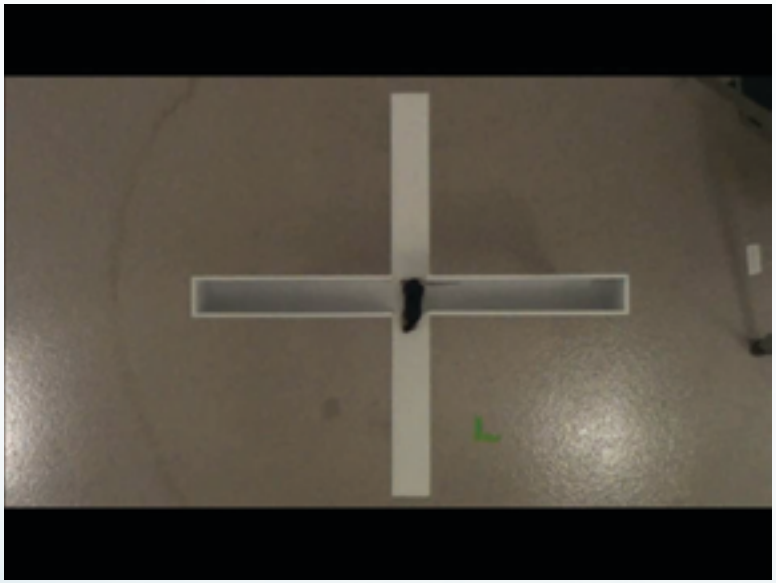




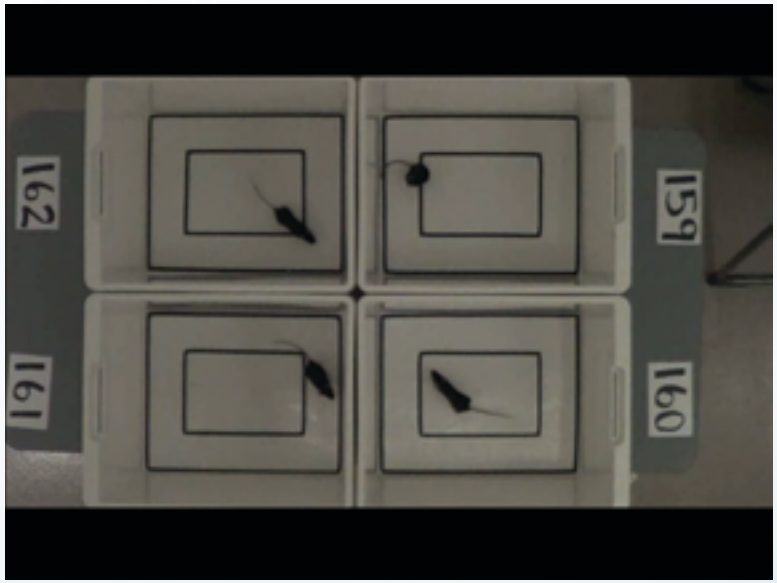
Elmore and Najafi et al., *Neuron* 2014

Tests of Anxiety

Elevated Plus Maze

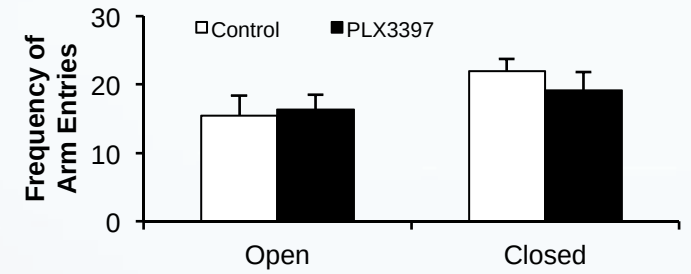


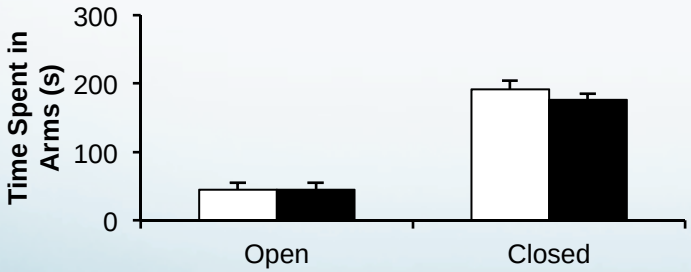
Open Field



No Effect of Microglial Elimination on Anxiety

Elevated Plus Maze





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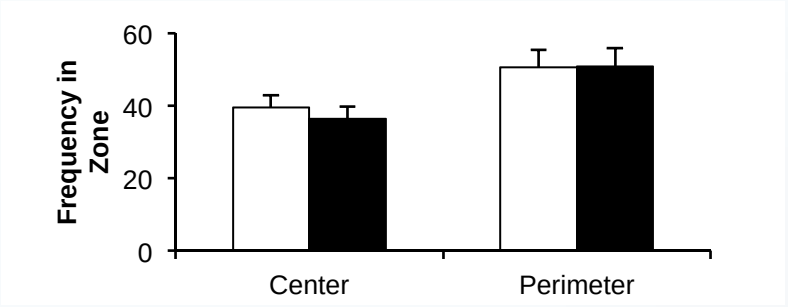
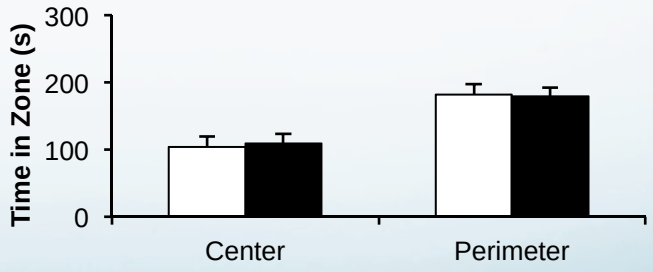
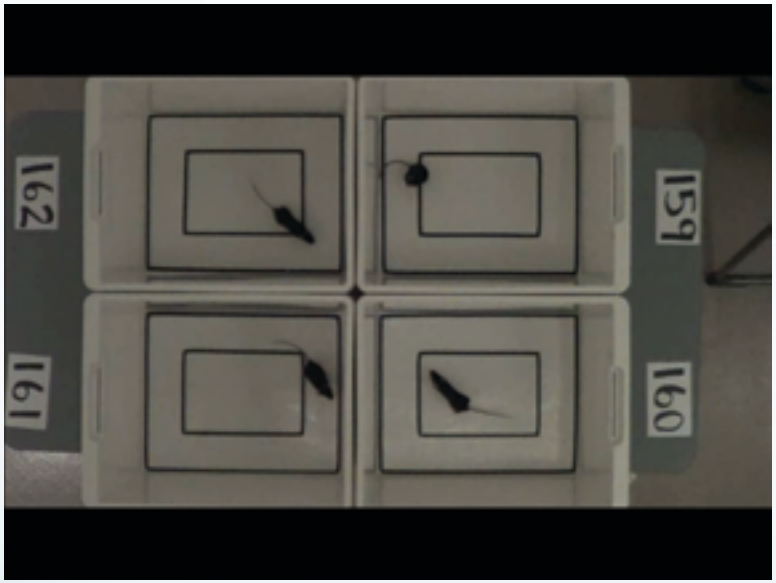




Elmore and Najafi et al. – Neuron (*2014*)

Tests of Motor Function

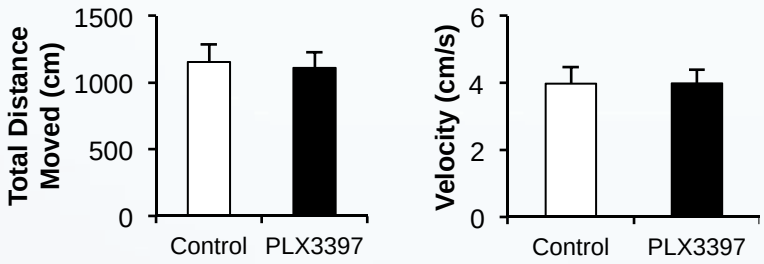
Open Field



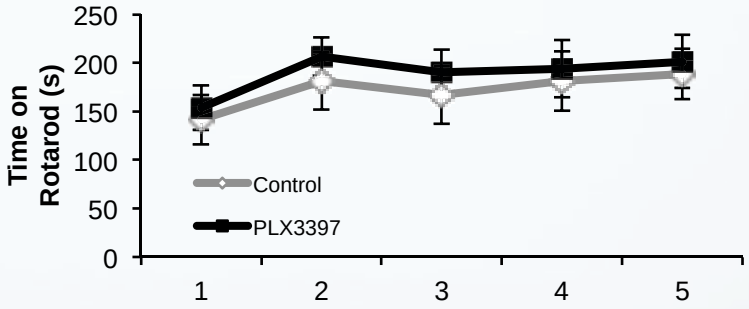
Rotarod

No Effect of Microglial Elimination on Motor Function

Open Field



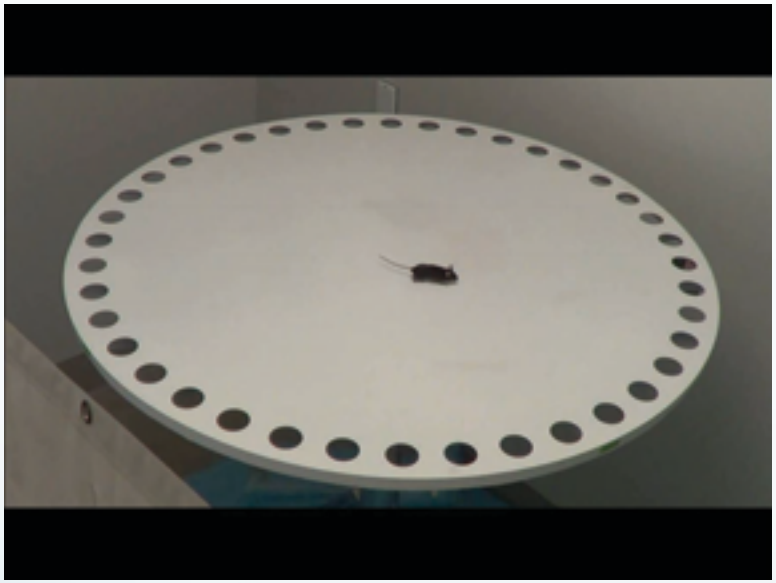
Rotarod



Elmore and Najafi et al. – Neuron (*2014*)

Tests of Cognitive Function

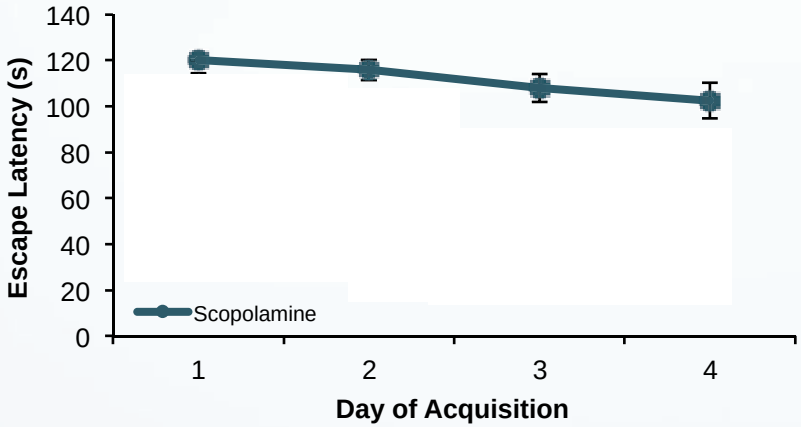
Barnes Maze

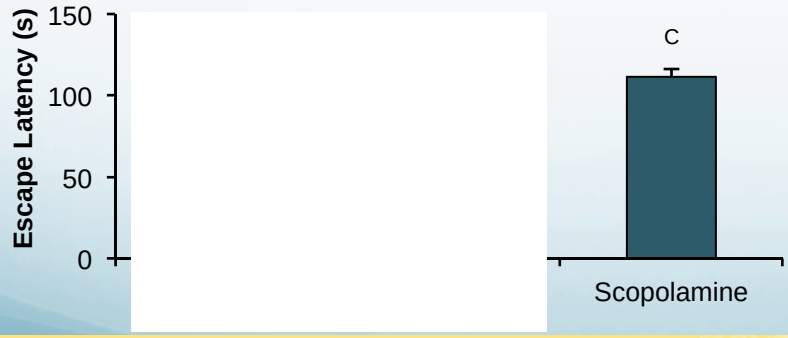


Fear Conditioning

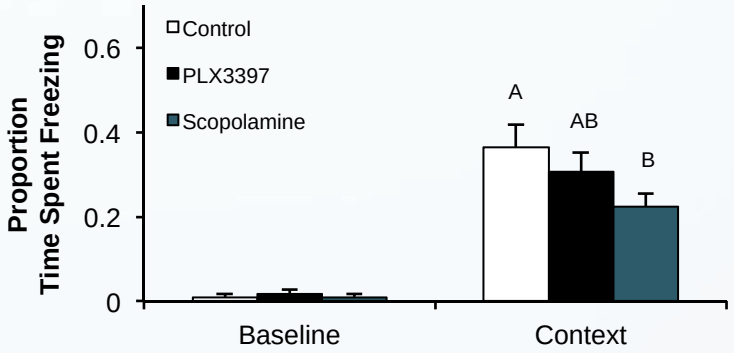
No Effect of Microglial Elimination on Cognitive Function

Barnes Maze





Fear Conditioning



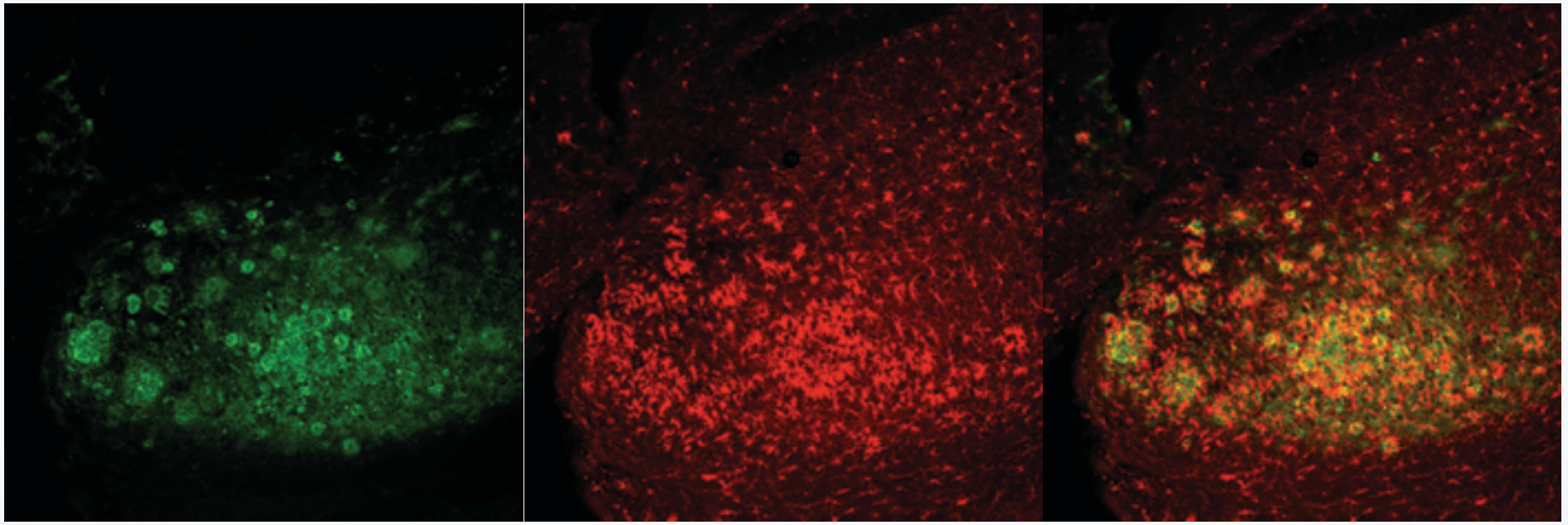
Elmore and Najafi et al. – Neuron (*2014*)

Interim Conclusions

- Pharmacological inhibition of the CSF1R results in rapid microglia elimination from the CNS in 7-21 days.
- Microglia are not overtly necessary for cognition or behavior.
- We can now directly study the roles of microglia in the healthy and diseased/injured/aged brain.
- Can microglial-elimination be a therapeutic for brain disorders?

Effects of microglial-elimination in Alzheimer's disease

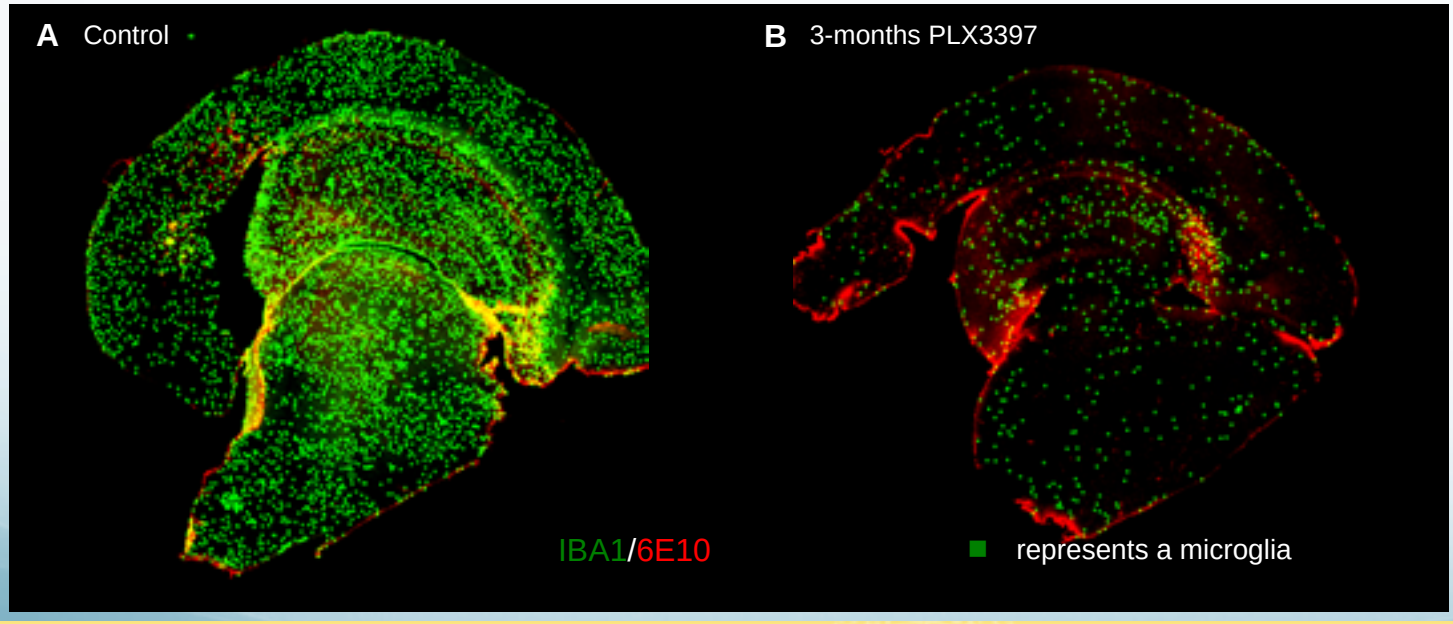
Alzheimer's disease:

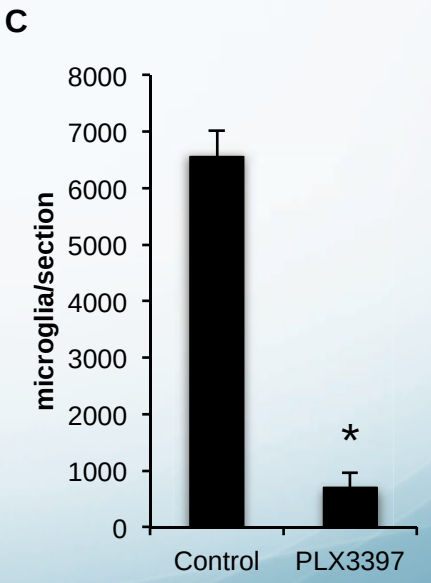


- Genetically modified mice develop Alzheimer's disease.
- They produce plaques in their brains, and become cognitively impaired.

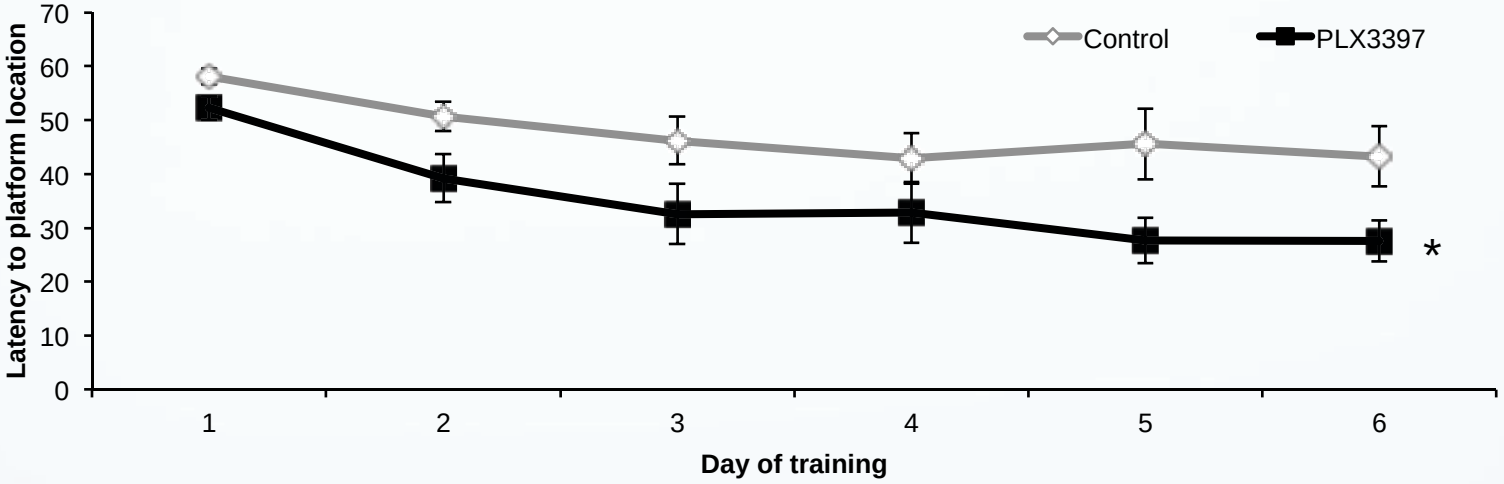
CSF1R inhibition eliminates microglia in AD mice

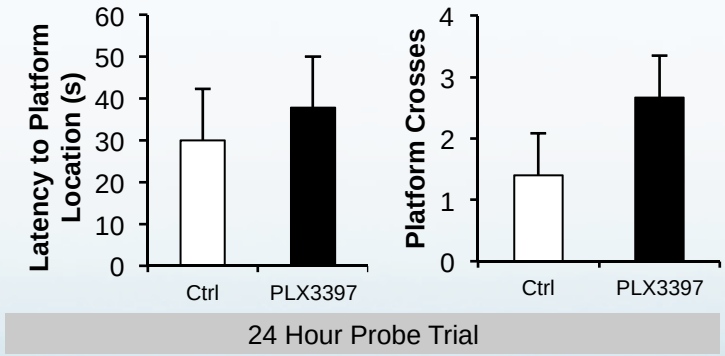
- 3 months treatment in 23 month old 3xTg-AD mice.
- Removes >95% of all microglia.
- Is this beneficial?



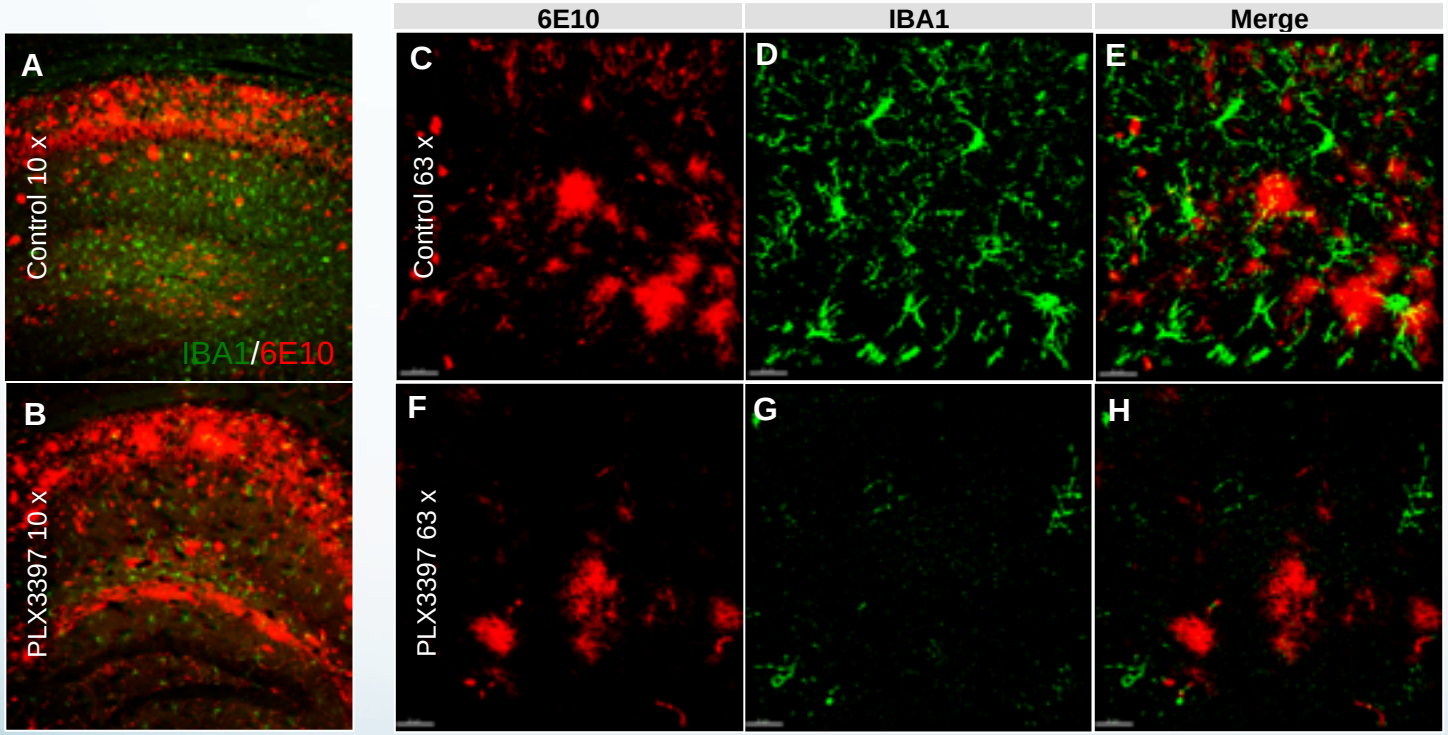


Elimination of microglia improves learning

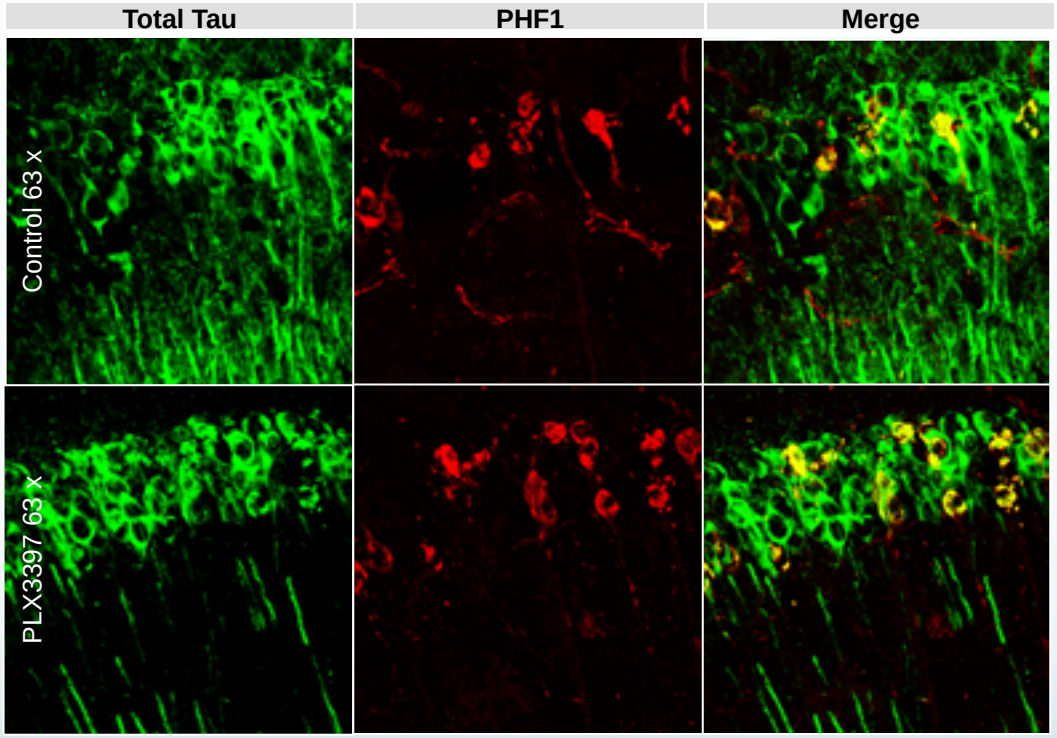




Elimination of microglia does not alter pathology

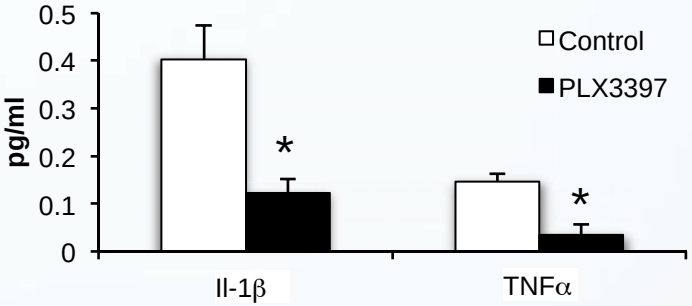


Elimination of microglia does not alter pathology



Elimination of microglia reduces inflammation

- Levels of Il-1β and TNFα are significantly reduced with microglial-elimination.



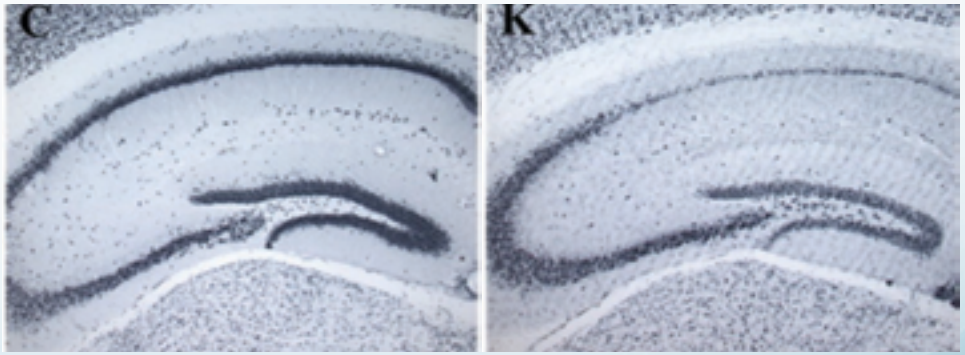
- Elevated levels of both of these are associated with memory impairments as well as synapto- and neurotoxicity.

Interim Summary

- Even microglia in the aged and diseased brain are fully dependent upon CSF1R signaling for their survival.
- We can achieve chronic microglial elimination in advanced AD mice.
- Elimination of microglia improves cognition, but has no impact on pathology.
- Treatment with CSF1R inhibitors may represent a useful therapy for AD and other disorders involving neuroinflammation.

Model of Neuronal Loss

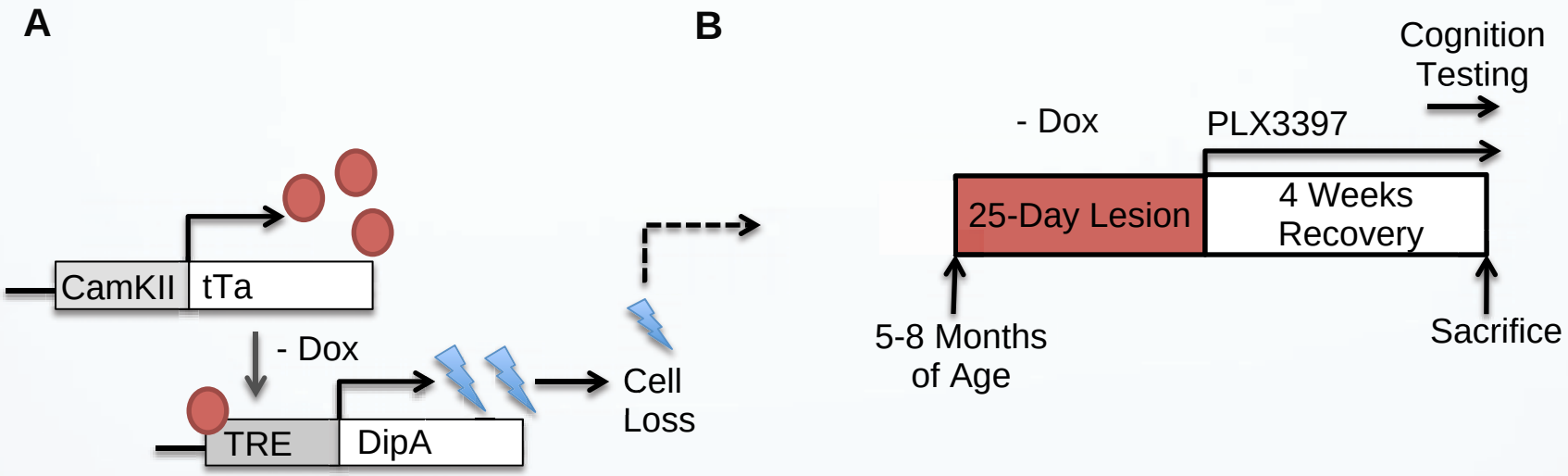
- AD models have plaques and tangles but not extensive neuronal loss.
- Many drugs have progressed into human clinical trials after testing in AD models, and have then failed!
- We also utilise a mouse model of extensive neuronal loss.



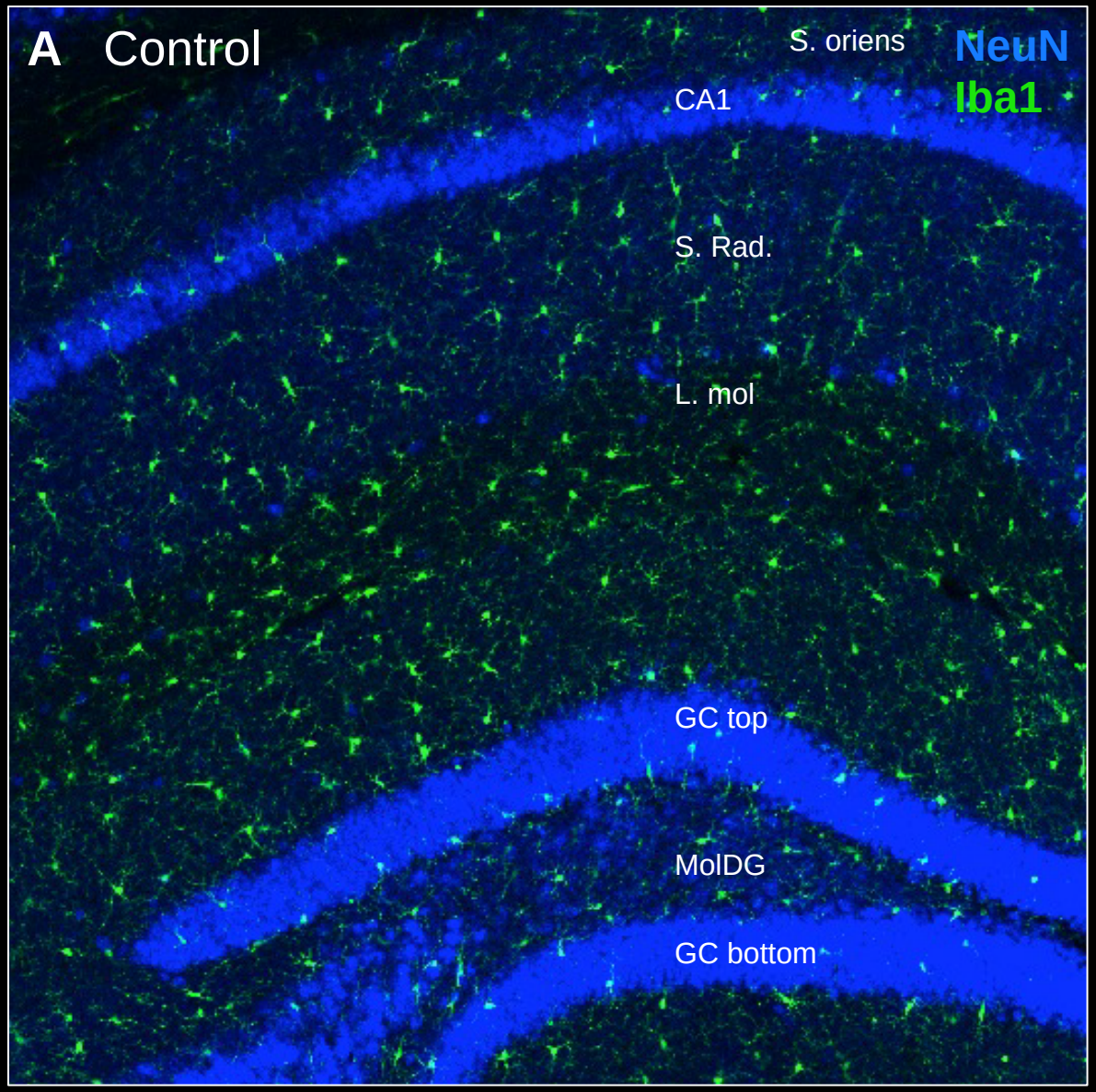
Control Lesion

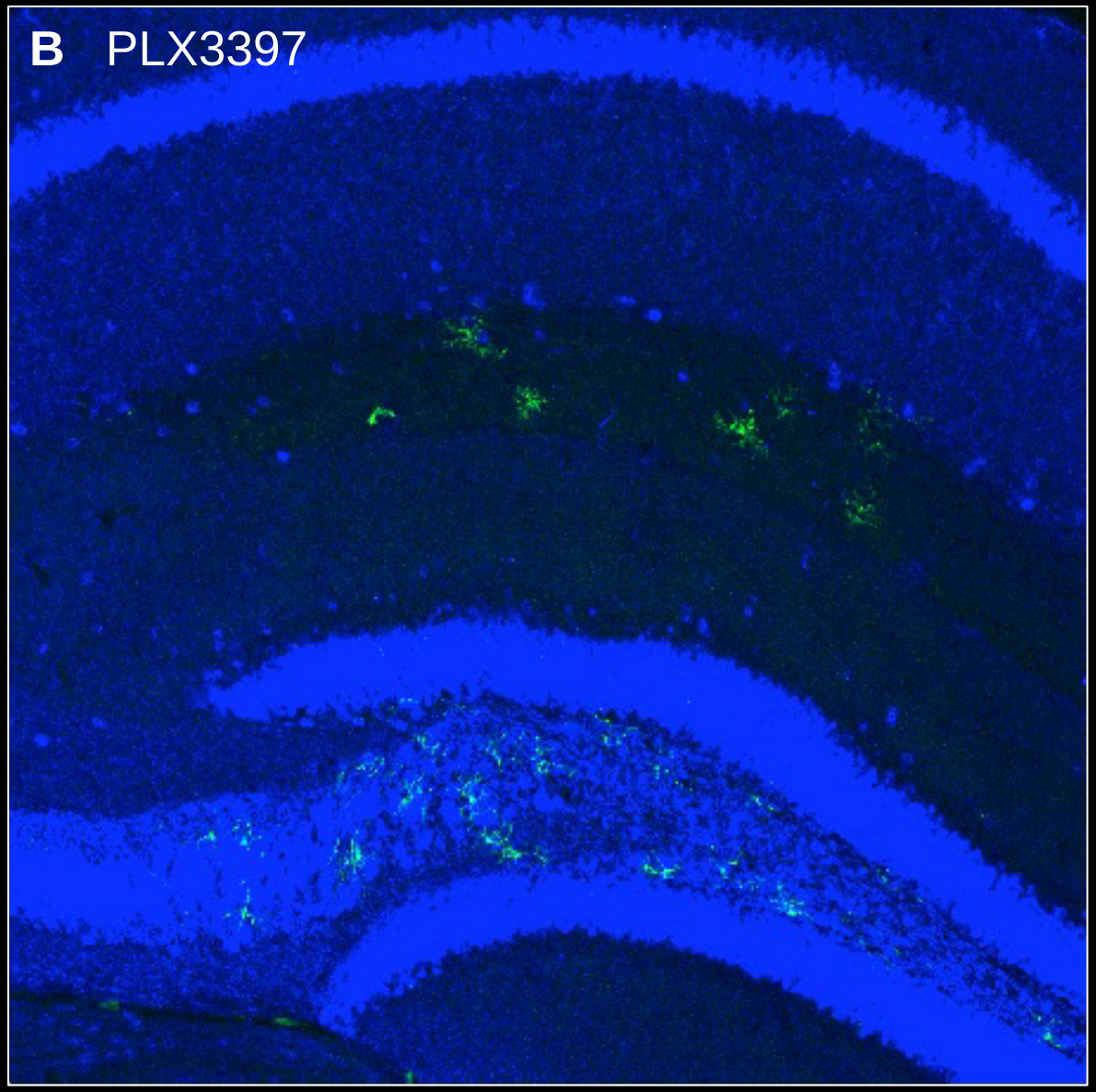
Yamasaki *et al*., *J. Neurosci*.

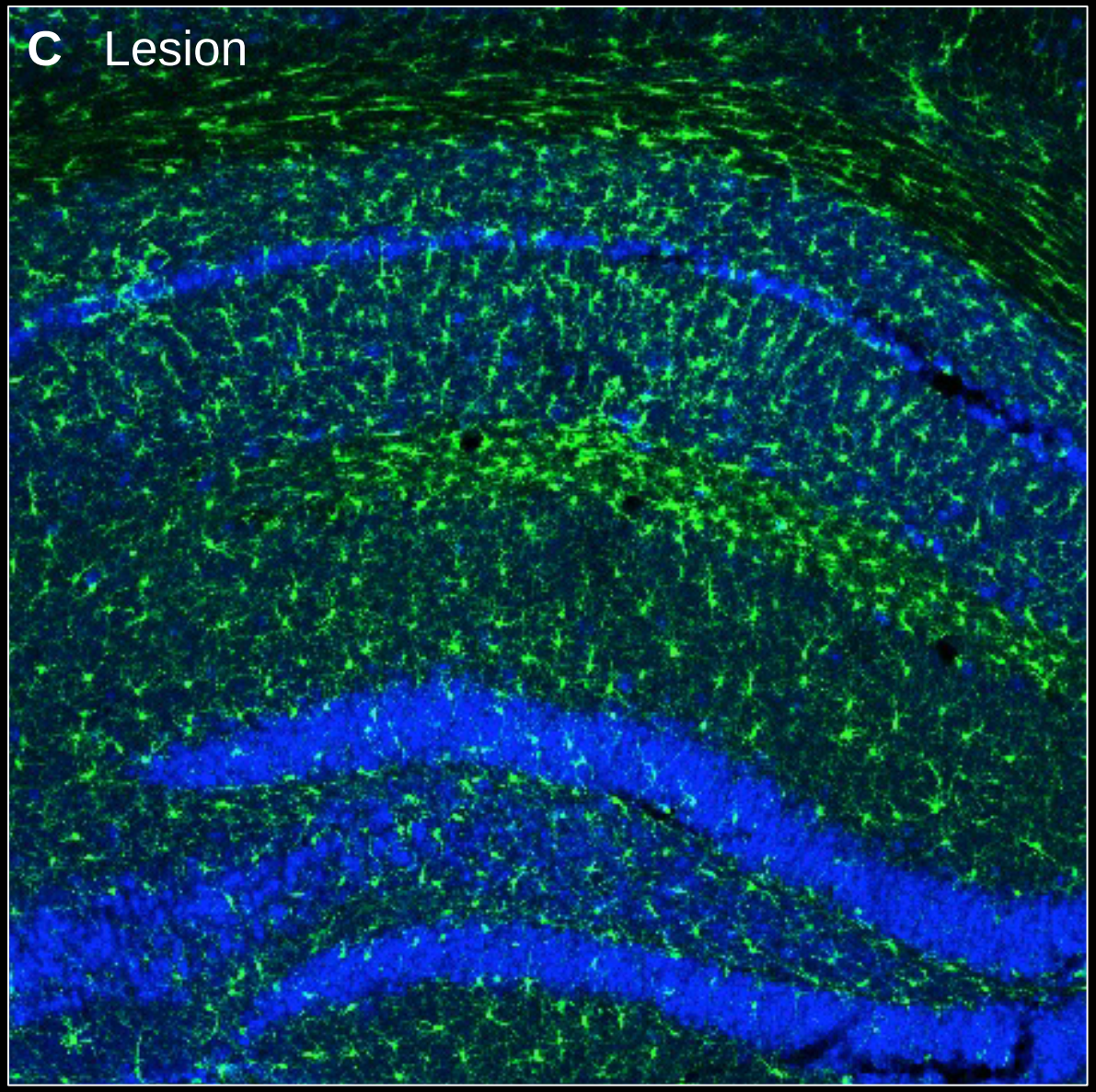
Experimental Design

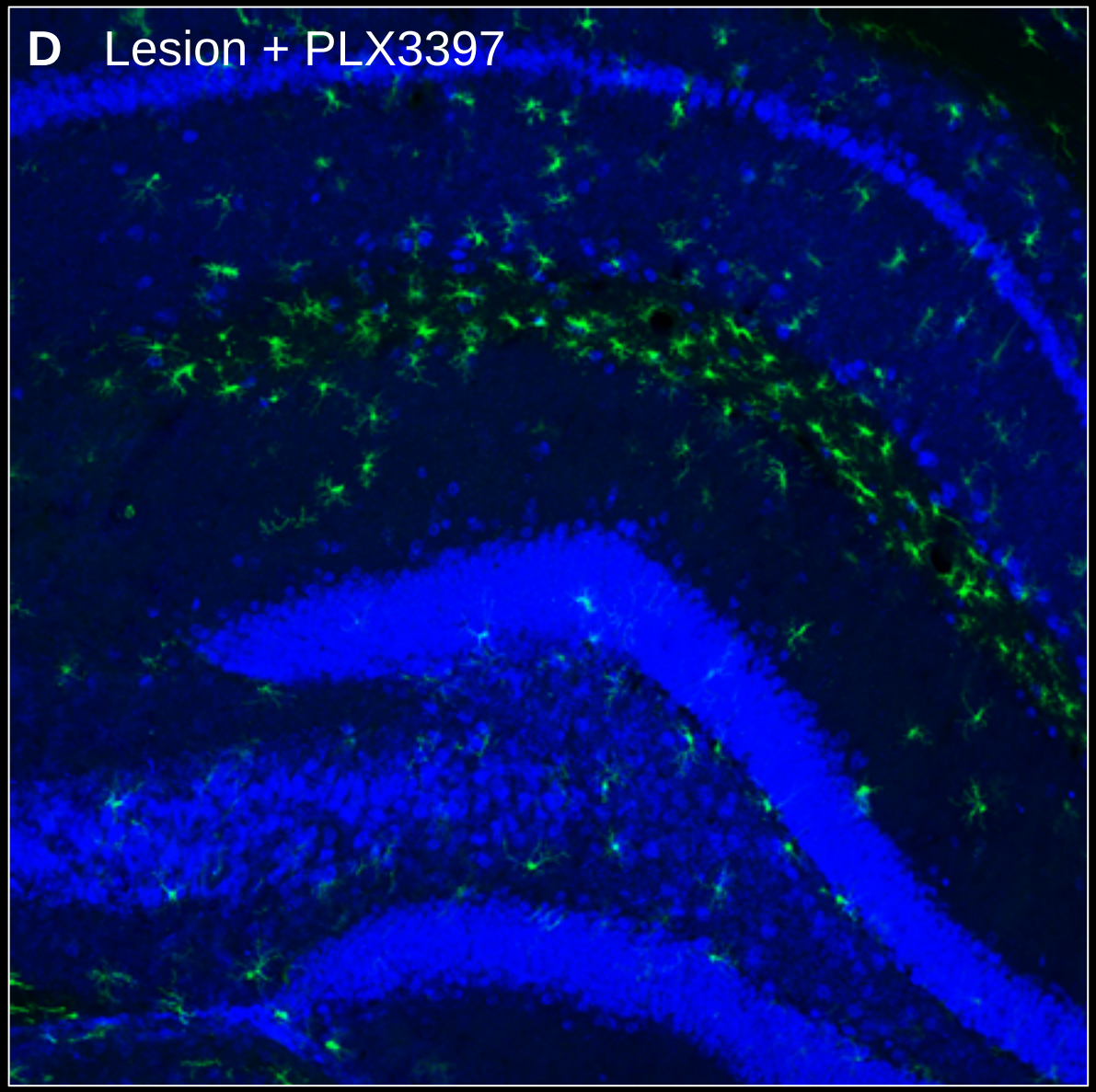


Control	PLX3397	Lesion	Lesion + PLX3397

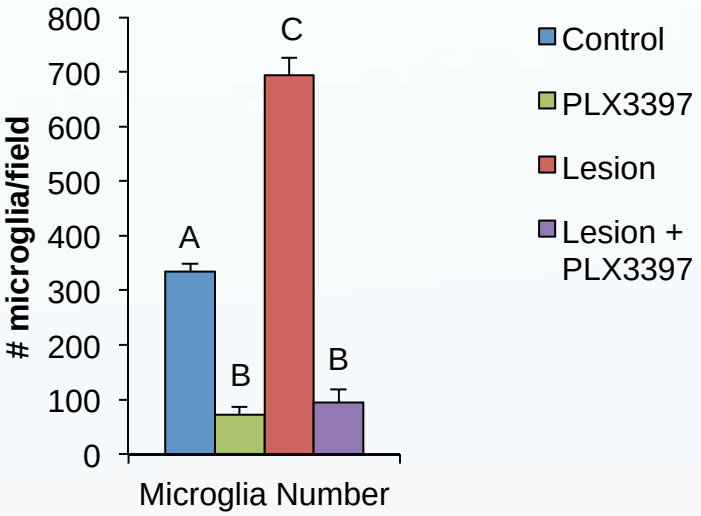

A Control
S. oriens
NeuN
Iba1
CA1
S. Rad.
L. mol
GC top
MolDG
GC bottom


B PLX3397

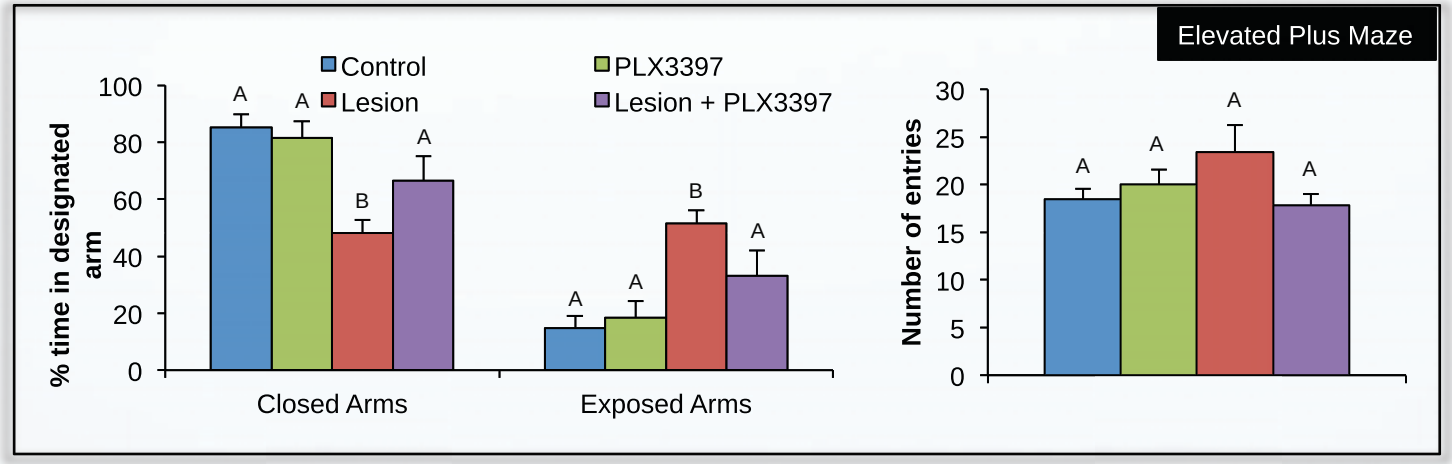

C Lesion


D Lesion + PLX3397

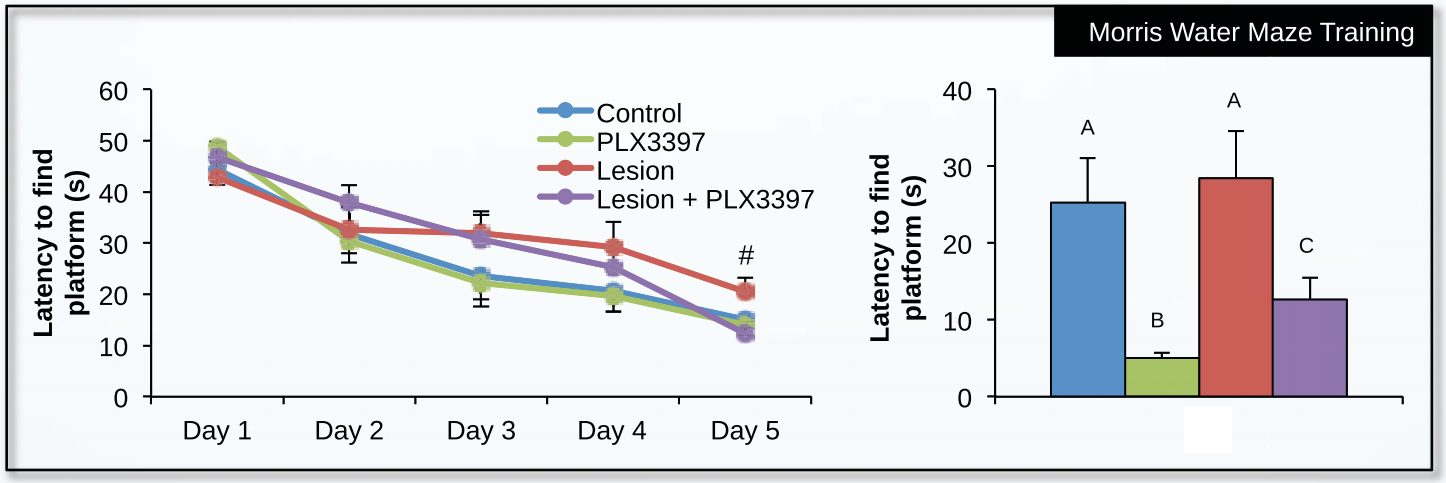
Activated microglia are dependent upon CSF1R signaling for survival



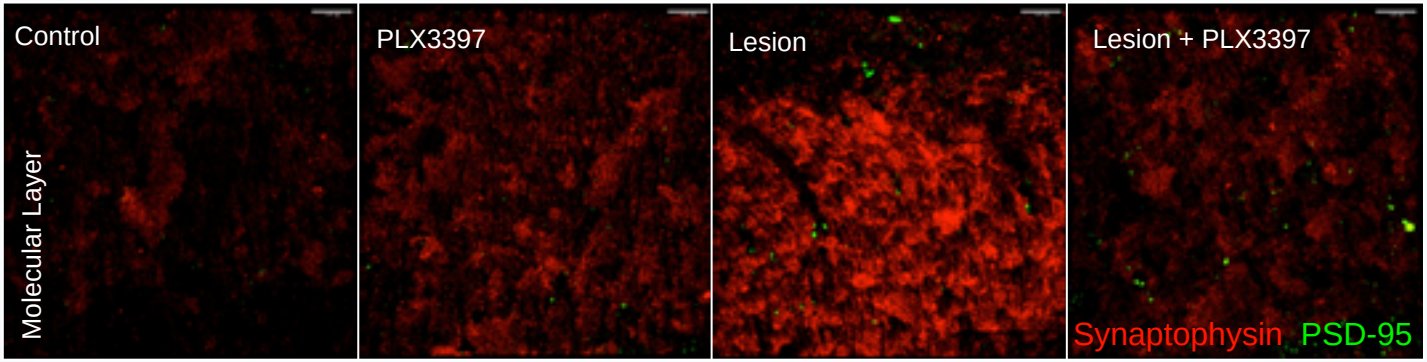
Microglia elimination improves lesion-associated deficits on elevated plus maze

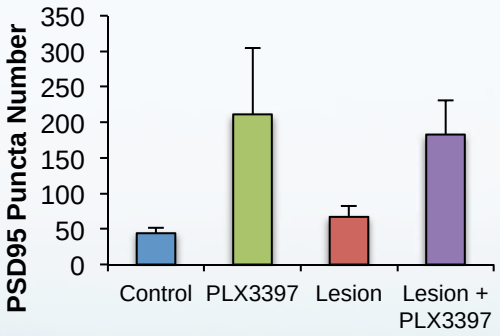


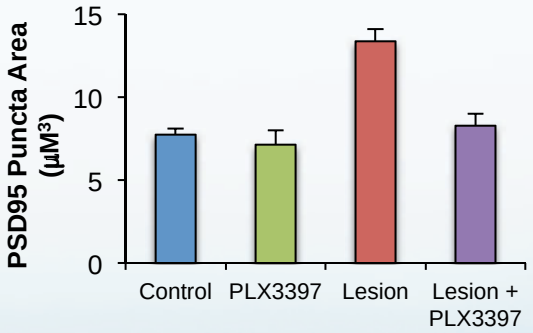
Microglia elimination improves performance on Morris water maze



Microglia elimination restores lesion-induced synaptic alterations







Conclusions

- Activated microglia are dependent upon signaling through CSF1R for survival
- Elimination of microglia following neuronal lesion facilitates:
  - Functional recovery
  - Synaptic alterations
- CSF1R inhibitors improve cognition in a model of AD and a model of robust neuronal loss. Therefore good rationale for developing further for neuroinflammatory disorders.

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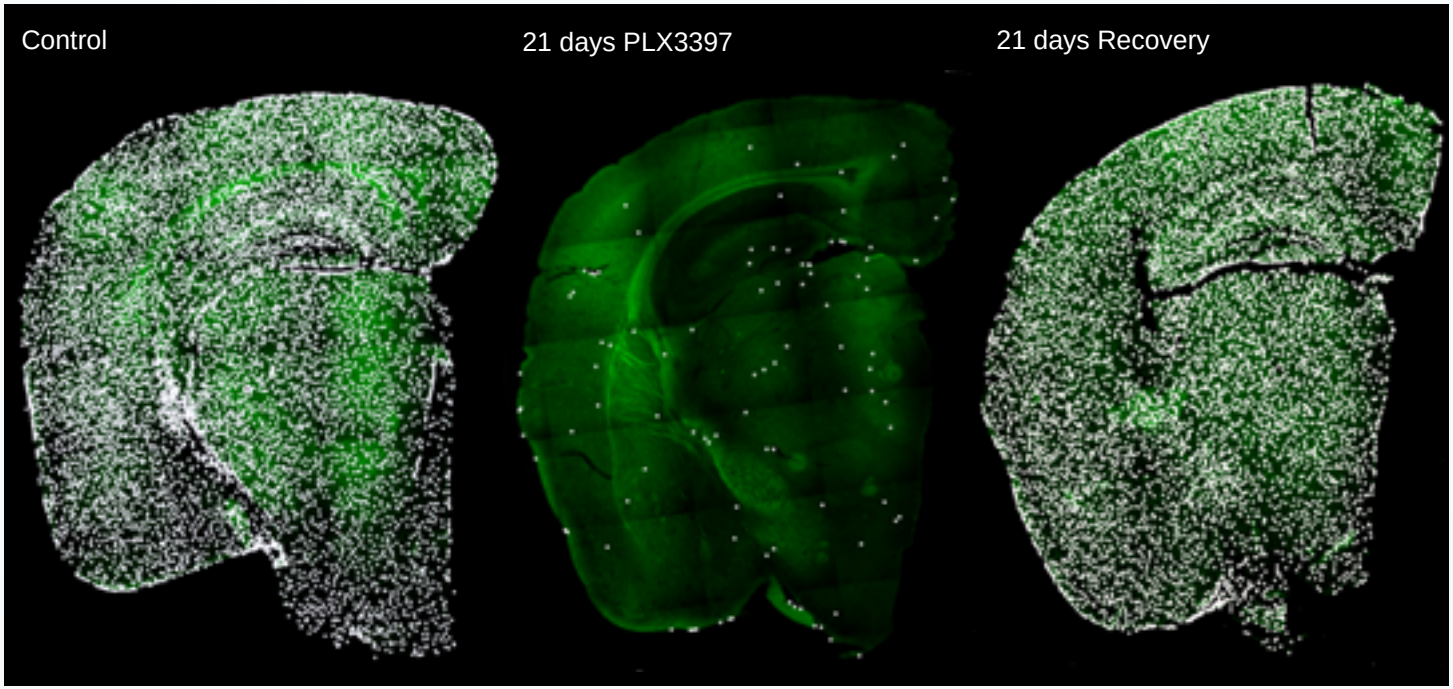
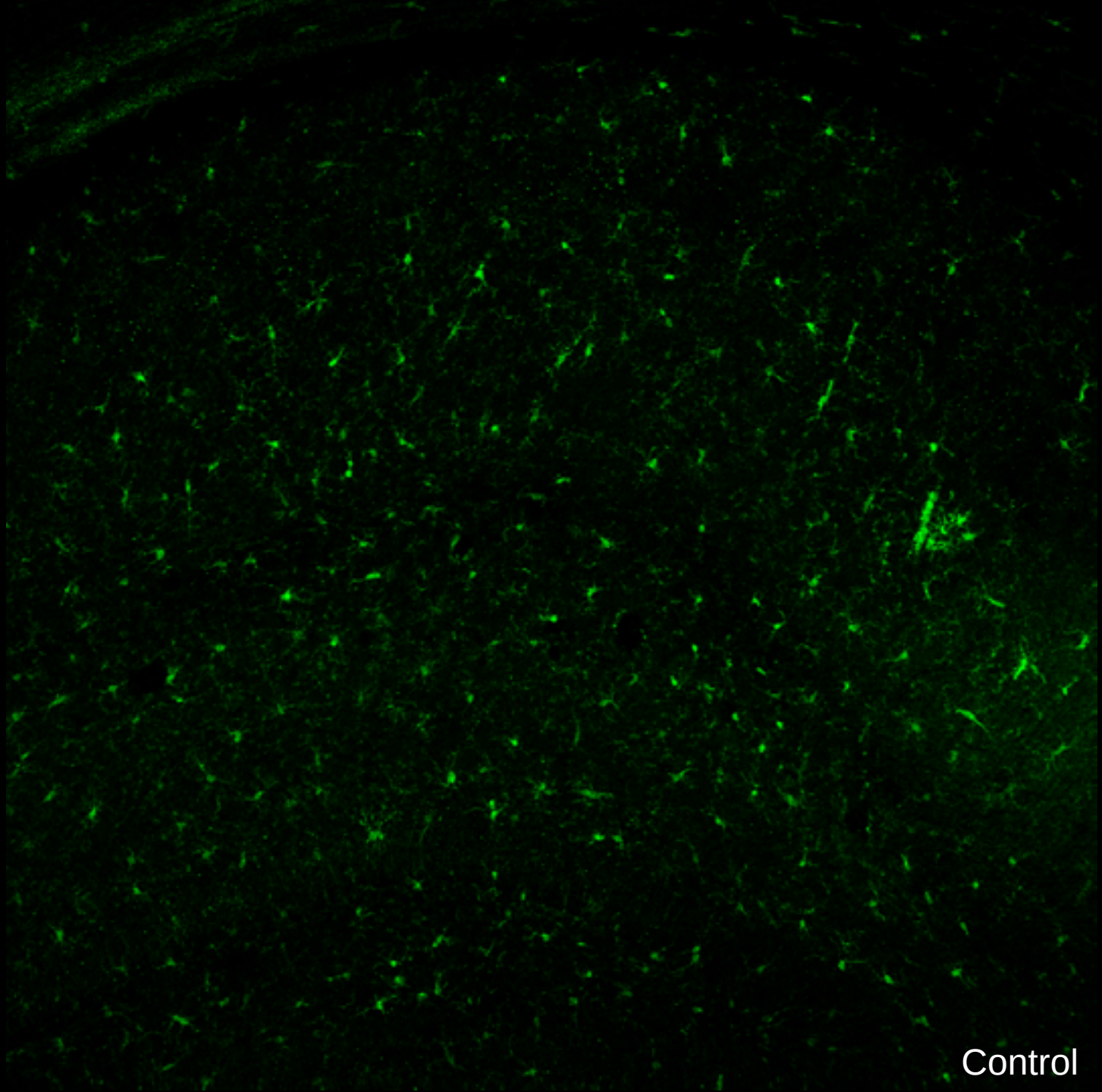
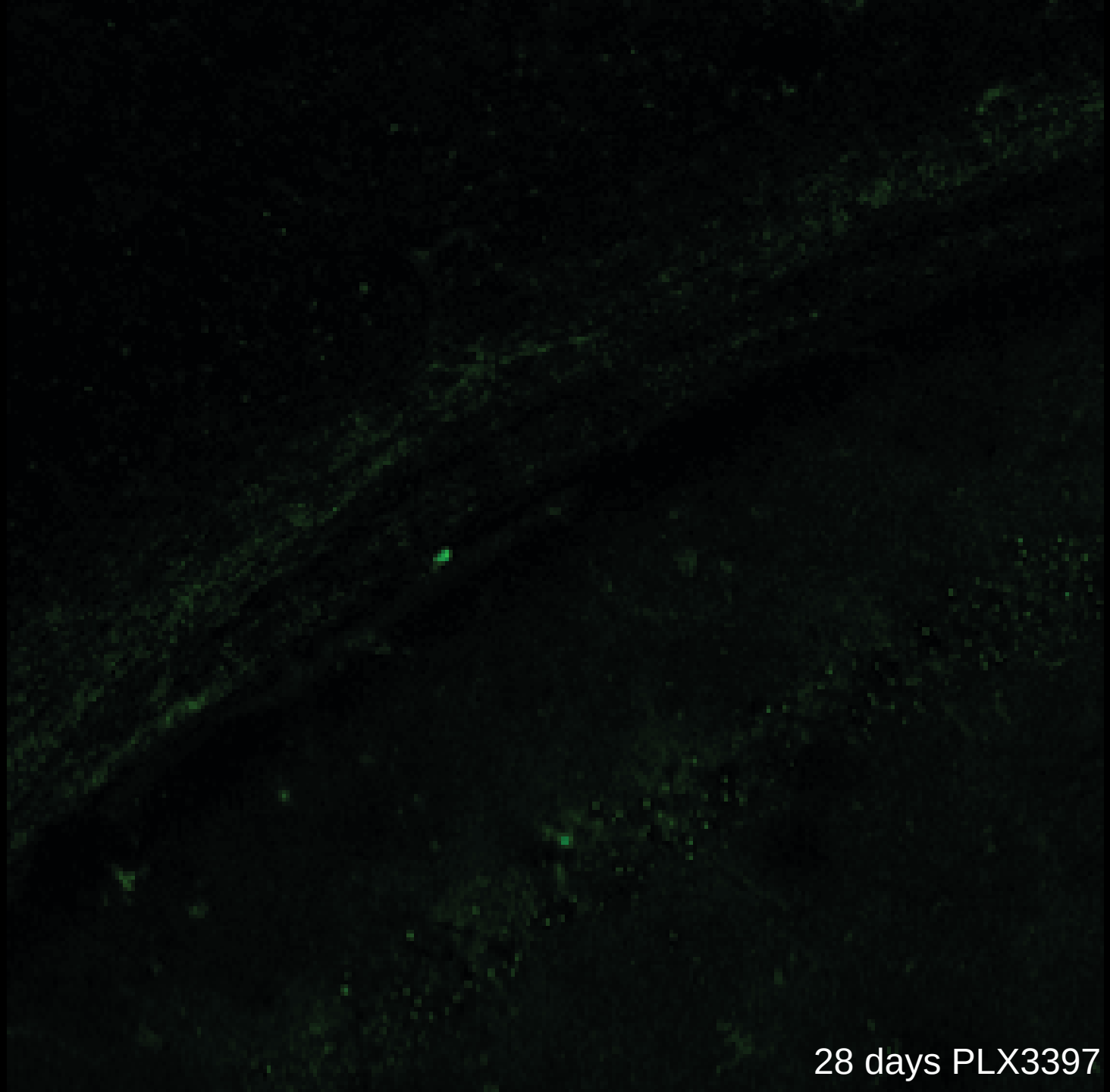
Microglial-elimination is fully reversible

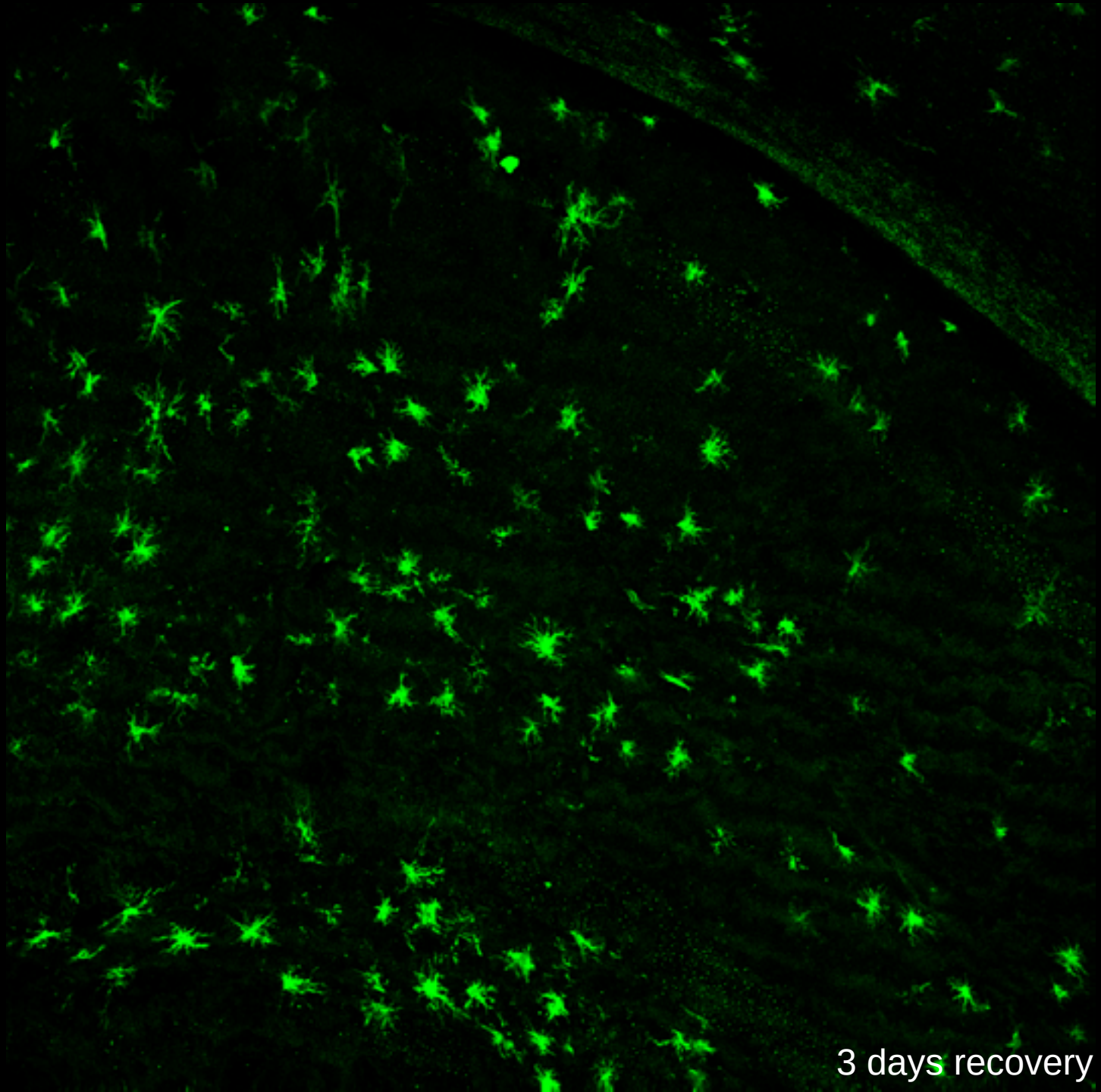


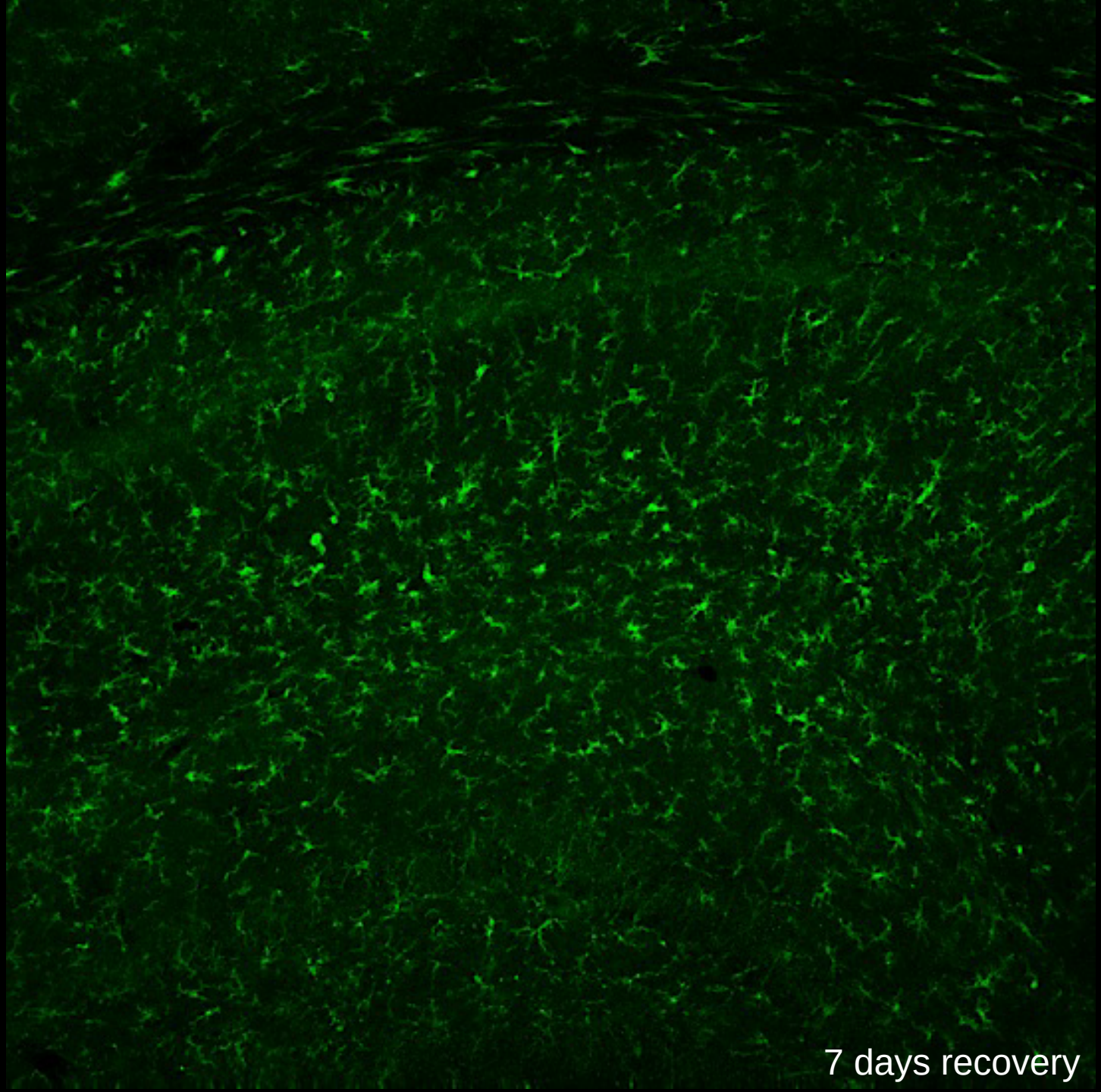
- Once microglia are eliminated with CSF1R inhibitors then withdrawal of CSF1R inhibitors stimulates rapid repopulation with new microglia.
- New microglia arise from stem cells found throughout the CNS that divide and then differentiate into new microglia.

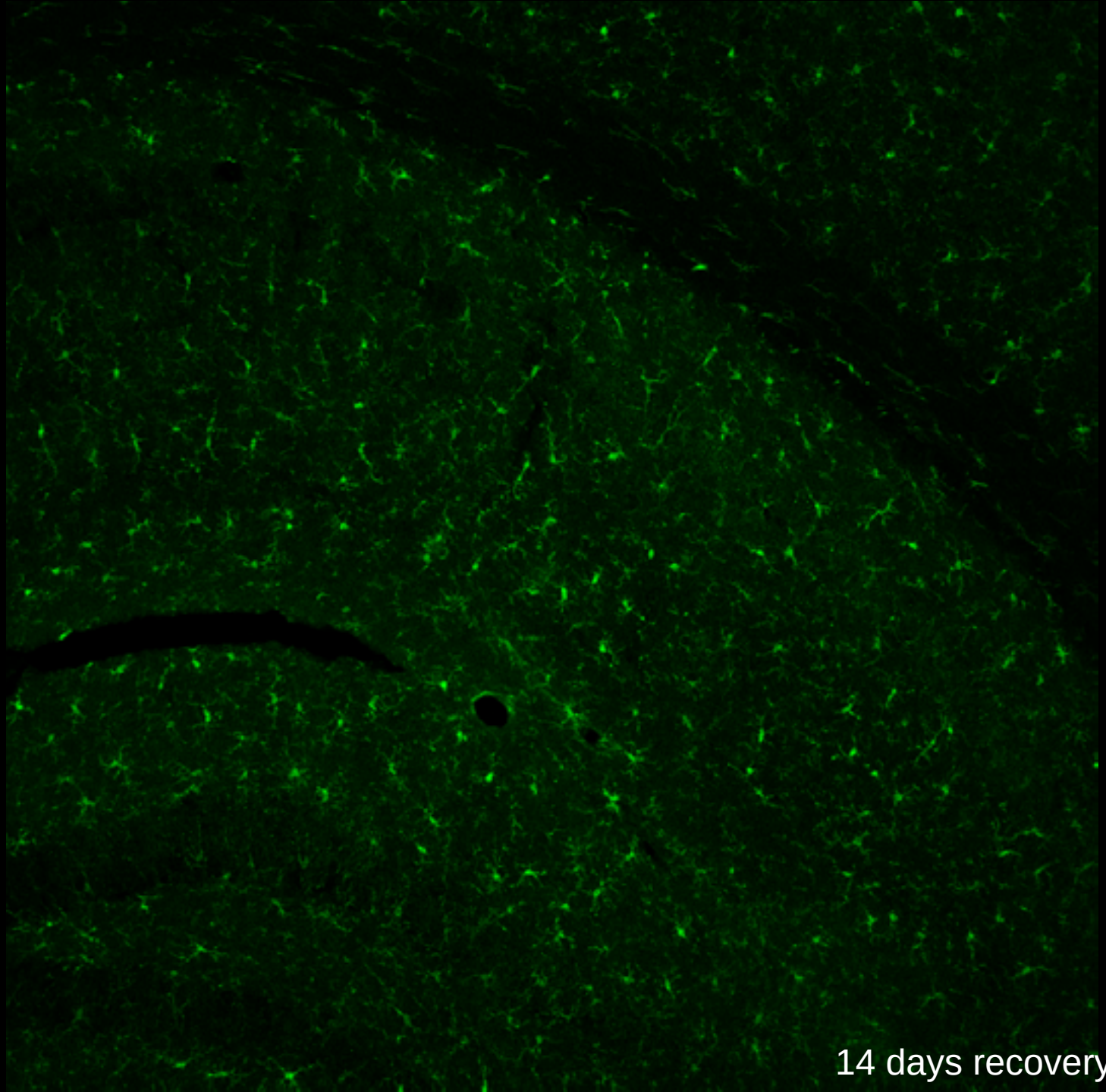
Elmore and Najafi et al., *Neuron* 2014


Control

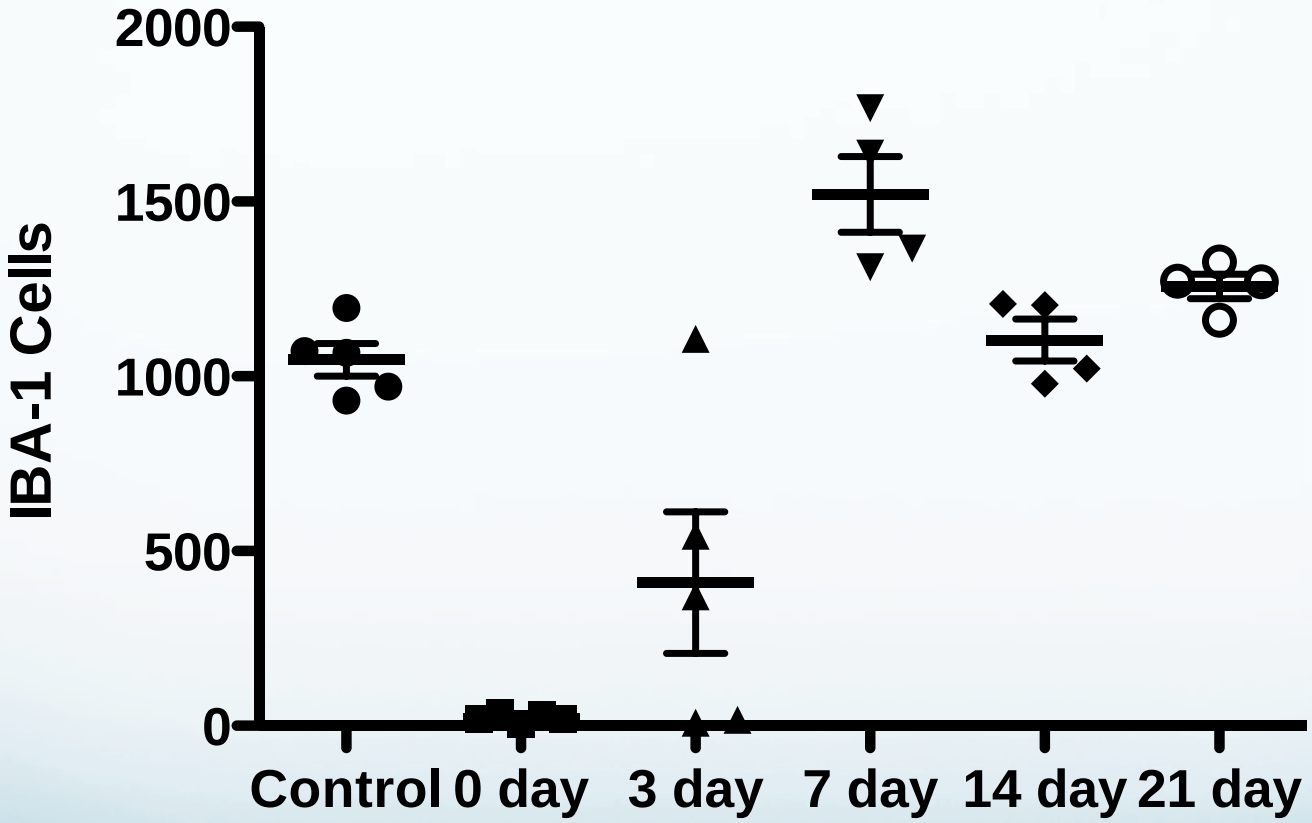

28 days PLX3397


3 days recovery

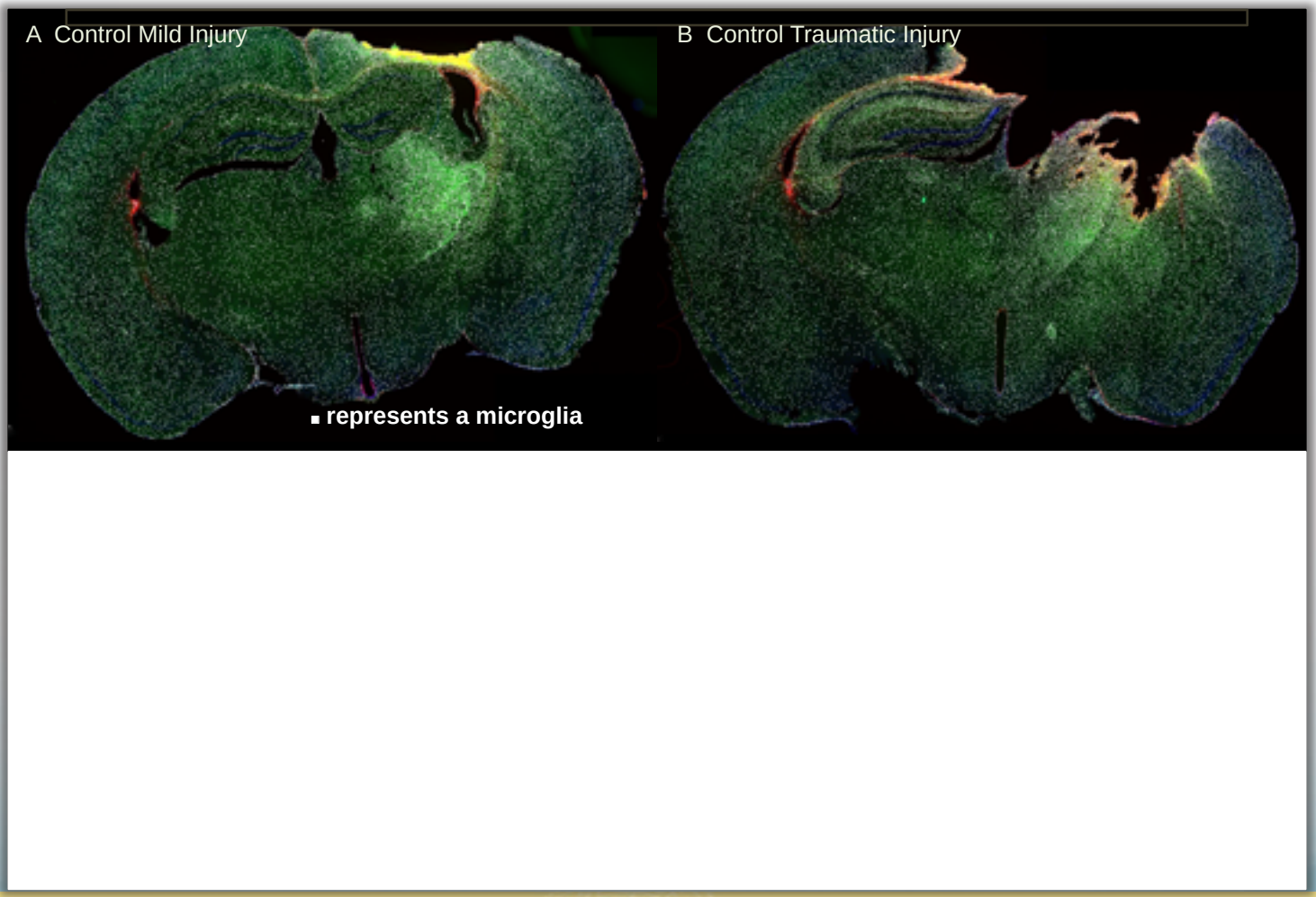

7 days recovery


14 days recovery

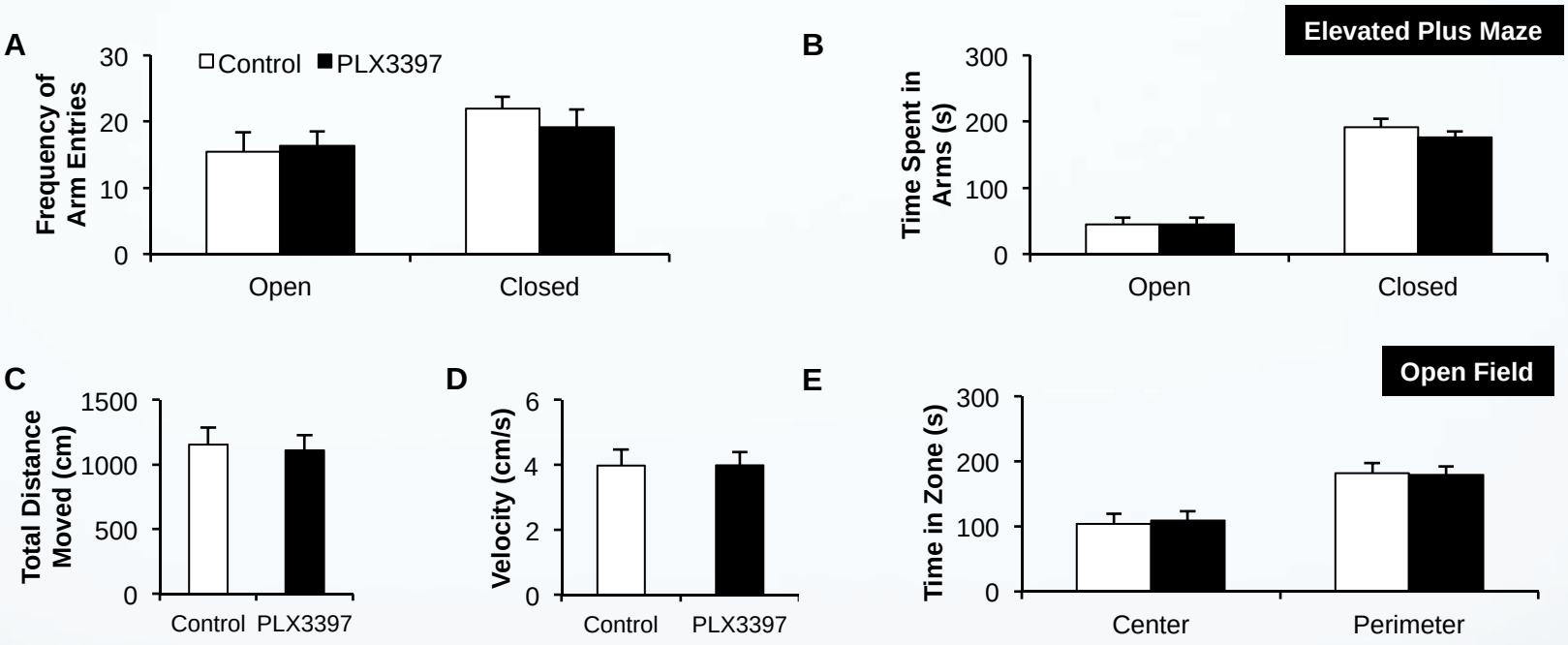
Rapid repopulation of the microglia-depleted brain



CSF1R inhibitors eliminate microglia during brain injury



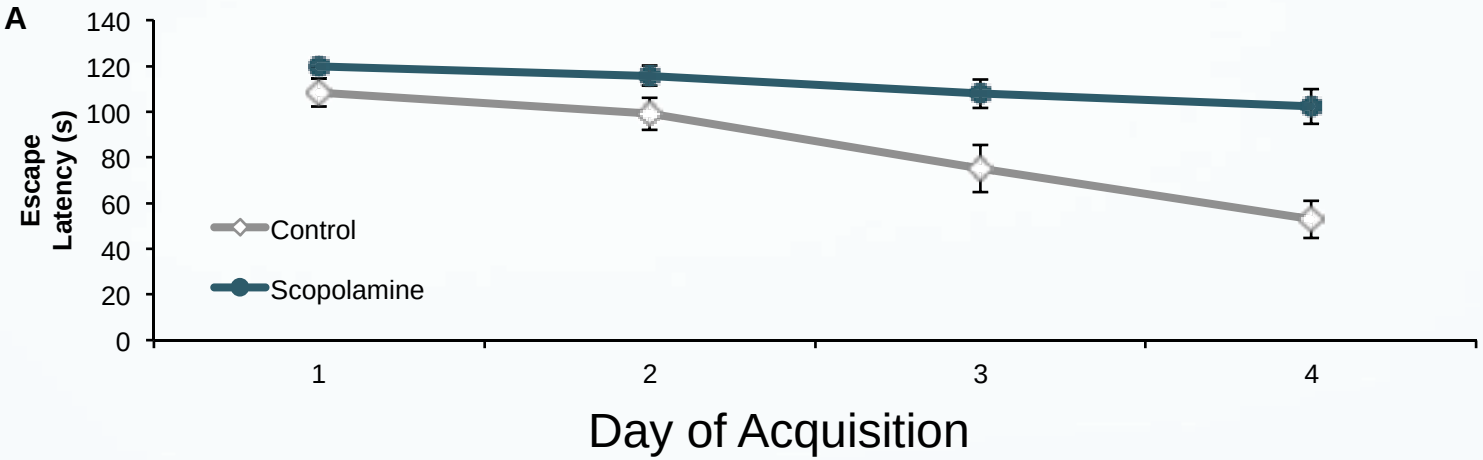
Microglia-depleted mice have no behavioral deficits

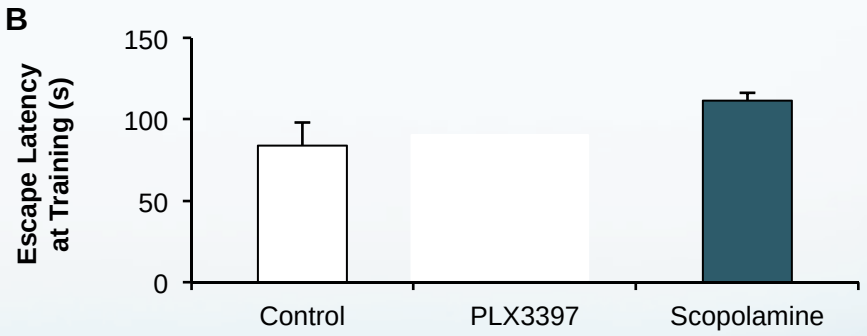


- Depletion of microglia for 2 months does not effect Elevated plus maze, or Open field analyses.

Elmore and Najafi et al., *Neuron* 2014

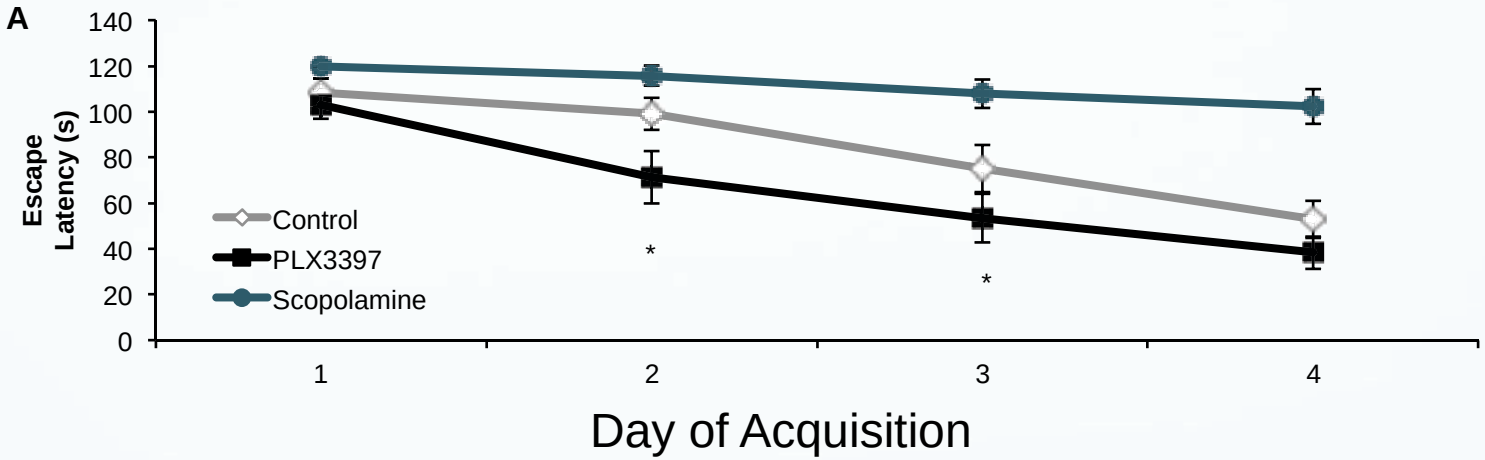
Microglia-depleted mice have no learning deficits

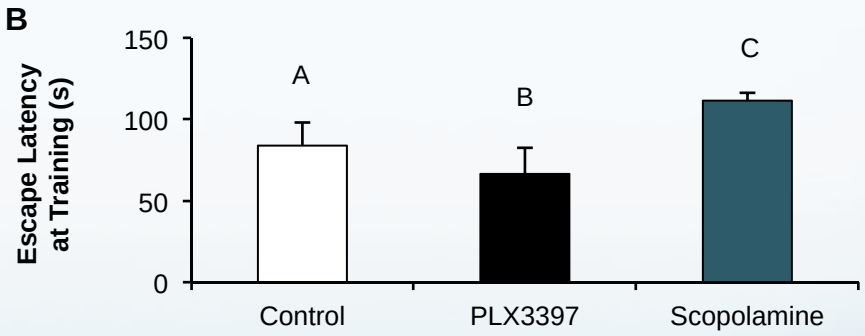




- Mice depleted of microglia have significantly improved learning compared to intact animals.

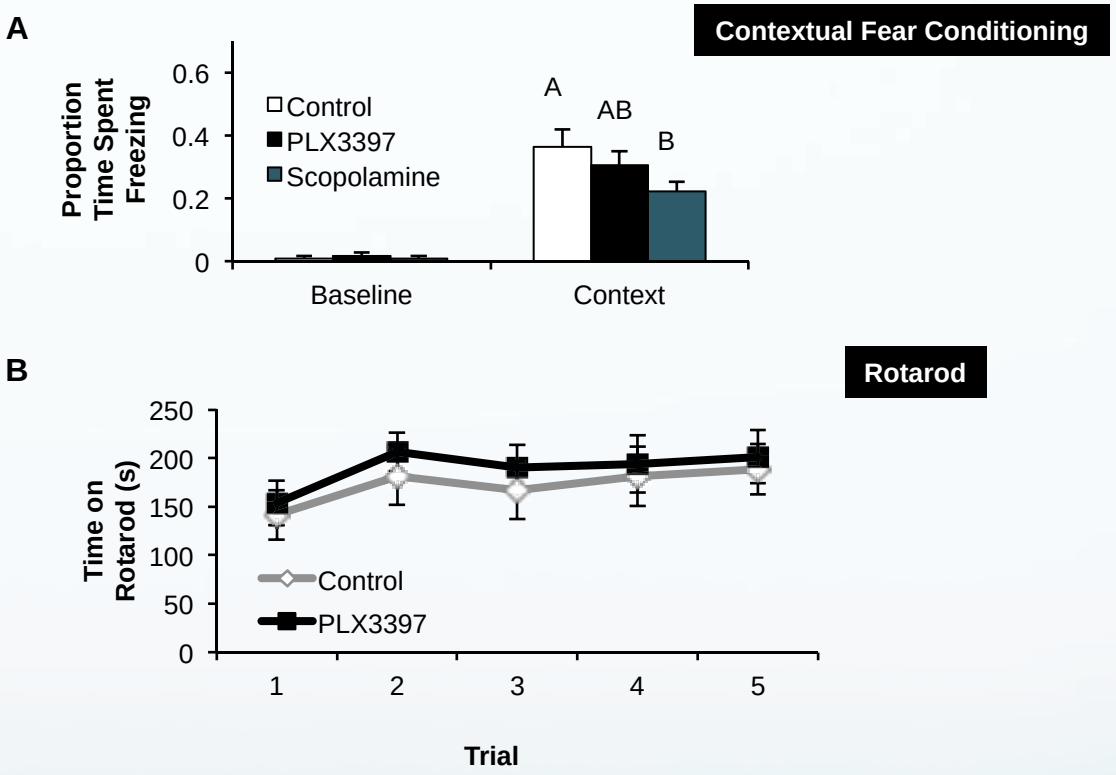
Microglia-depleted mice have no learning deficits





- Mice depleted of microglia have significantly improved learning compared to intact animals.

Microglia-depleted mice have no motor deficits



- No deficits in Contextual Fear Conditioning
- No deficits in motor function in healthy mice

Elmore and Najafi et al., *Neuron* 2014