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for
Cardiac and Thoracic
Diagnosis & Regeneration



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WIEN

Fingolimod Reduces Cerebral Lymphocyte Infiltration in Experimental Models of Rodent Intracerebral Hemorrhage

Rolland WB, Lekic T, Krafft PR, Hasegawa Y, Altay O,
Hartman R, Ostrowski R, Manaenko A, Tang J, Zhang JH

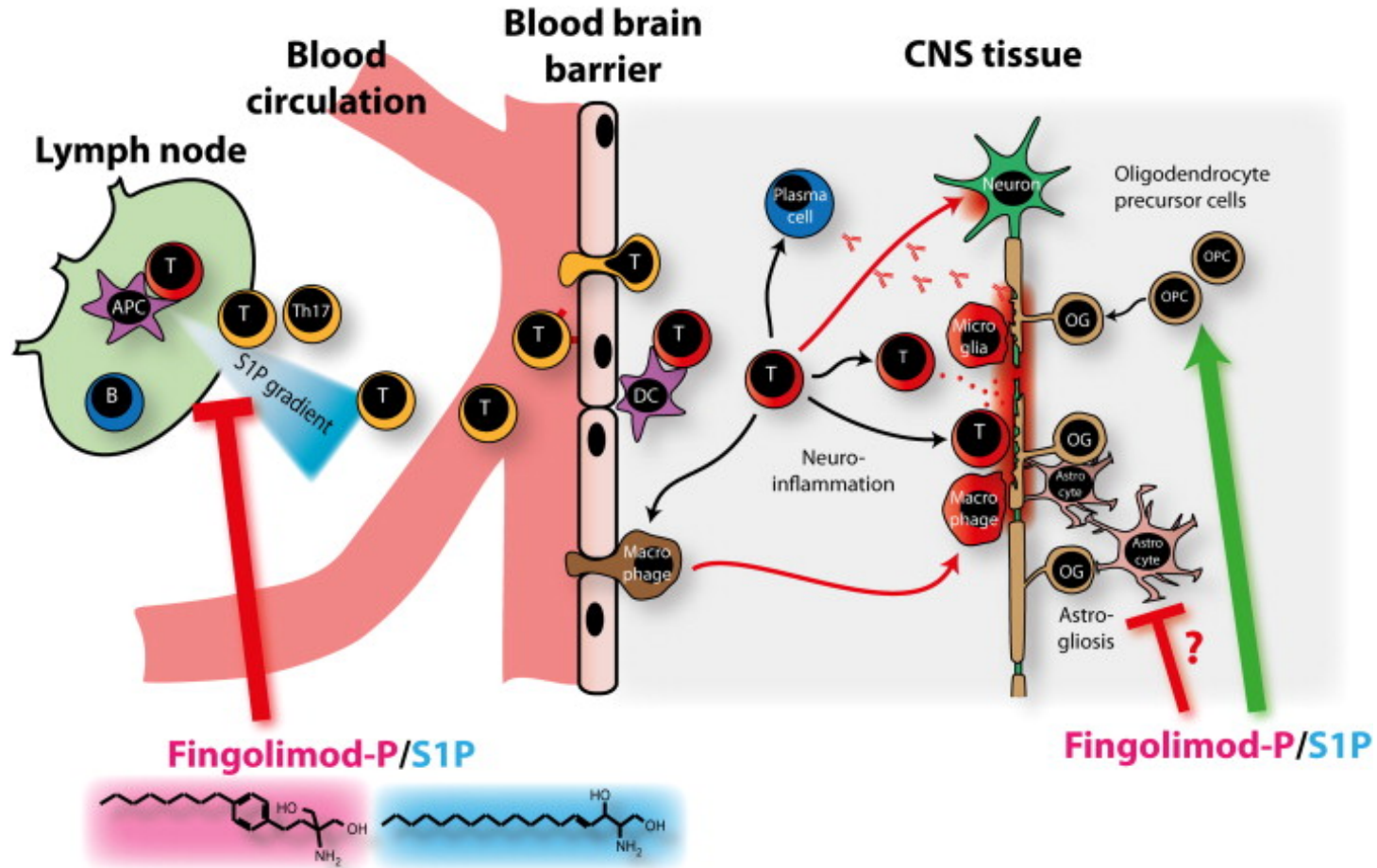
Exp Neurol 2013

*Patrick Altmann
April 2013*

- Background
- Methods/Results
- Discussion
- My Conclusion

- CONy 2013 in Istanbul

- Fingolimod (Gilenya®)
- Sphingosine 1-phosphate analog
- Reduces the expression of S1PR-1 on T-lymphocytes
 - preventing their egress from primary and secondary lymphoid organs
 - fewer immune cells are expected to migrate into the brain after experimental ICH



- Approved 2013 for RRMS
- Side effects:
 - First dose bradycardia
 - Lymphocytes ↓ by 30%
 - Vaccinate against VZV
 - Macular edema
 - Hepatic enzymes ↑
 - Stop, if lymphocytes $<200/\mu\text{L}$

➤ Male CD-1 mice (30-40g)

n=103

behavior, brain edema, peripheral leukocytes, brain-infiltrated T-Lymphocytes, cerebral pro-inflammatory proteins [24 and 72hrs postop]

➤ Male Sprague Dawley rats (280-350g)

n=28

short and long-term behavioral and neurocognitive performances, histopathological changes at 10 weeks after ICH

➤ Induction of ICH:

- *) cICH: intrastriatal infusion of bacterial collagenase
- *) bICH: intrastriatal infusion of autologous blood

➤ Experimental groups:

- *) sham
- *) vehicle
- *) single dose fingolimod
- *) daily fingolimod

➤ Behavioral testing:

in mice:

24 and 72 hrs after cICH

24 and 72 hrs after bICH

in rats:

24, 48, 72 hrs and 10 days after cICH

- Modified Garcia Neuroscore
 - 7 individual tests (motor, sensation...)
- Wire hang test
 - Distance moved along wire. 0=☹ 5=☺
- Beam balance test
 - Distance moved along rod. 0=☹ 5=☺
- Forelimb use asymmetry test
 - Monitor use of forelimbs.
- Corner test
 - Choice of turning sides.
- Paw placement test
 - Forward movement of paws after vibrissae-elicited excitation

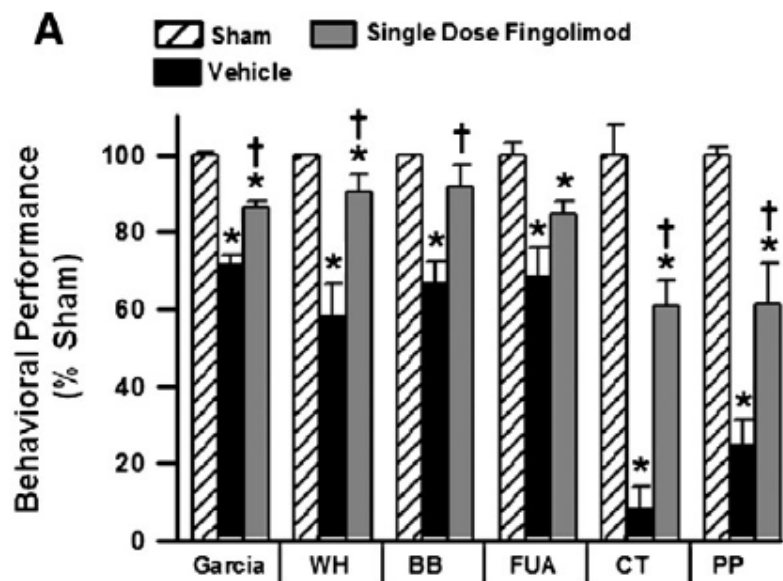


Brain edema

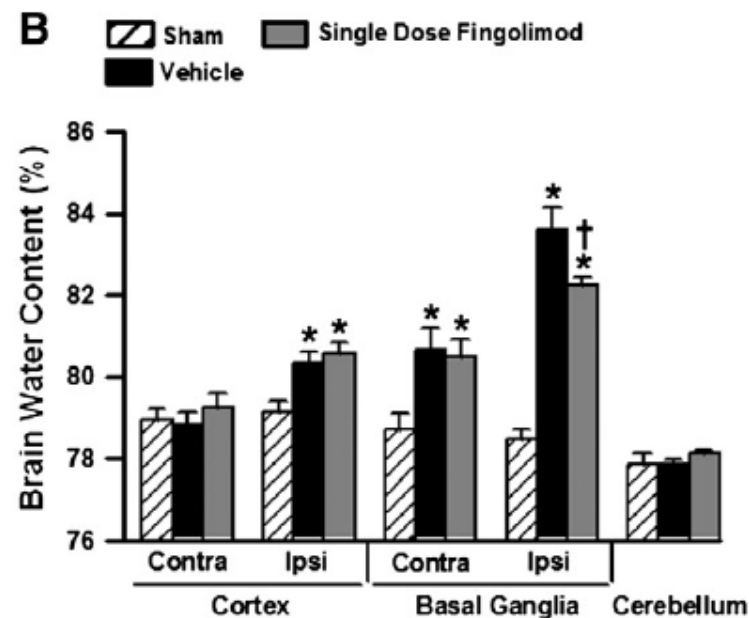


Mice were decapitated under deep isoflurane anesthesia, and brains were removed and immediately divided into five parts: ipsilateral and contralateral basal ganglia, ipsilateral and contralateral cortex, and cerebellum. All samples were weighed with an electronic analytical balance (AE100; Mettler Instrument Co, Columbus, OH) to obtain a wet weight (WW). Tissues were then dried at 100 °C for 24 h and the dry weights (DW) were determined. The brain water content (%) was calculated as $(WW - DW)/WW \times 100$.

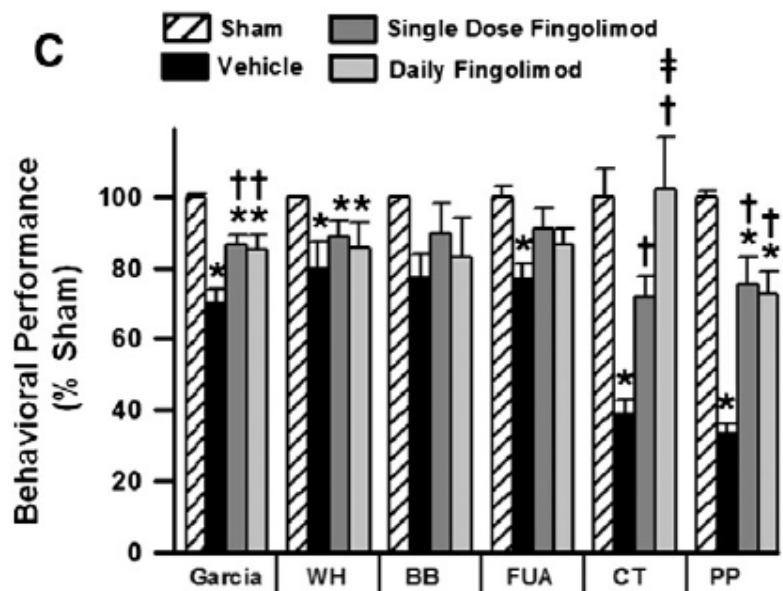
Behavior Outcome 24h Post-cICH



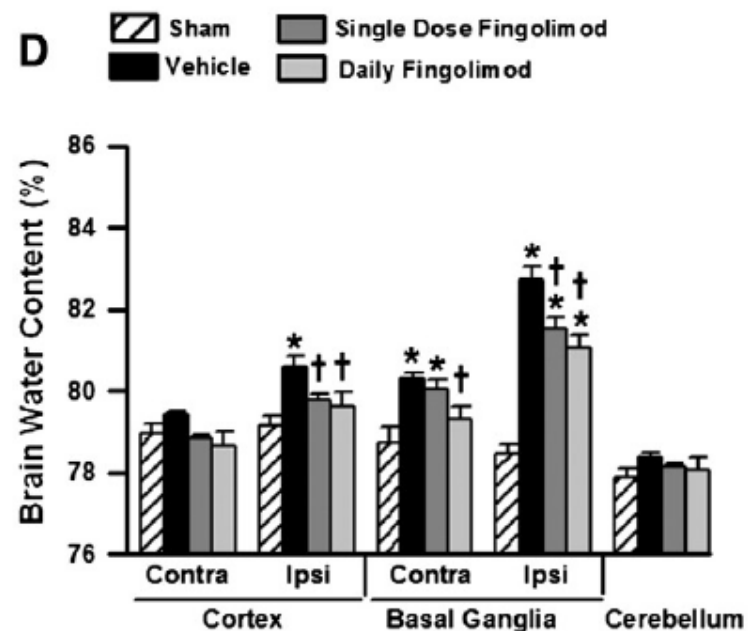
Brain Water Content 24h Post-cICH



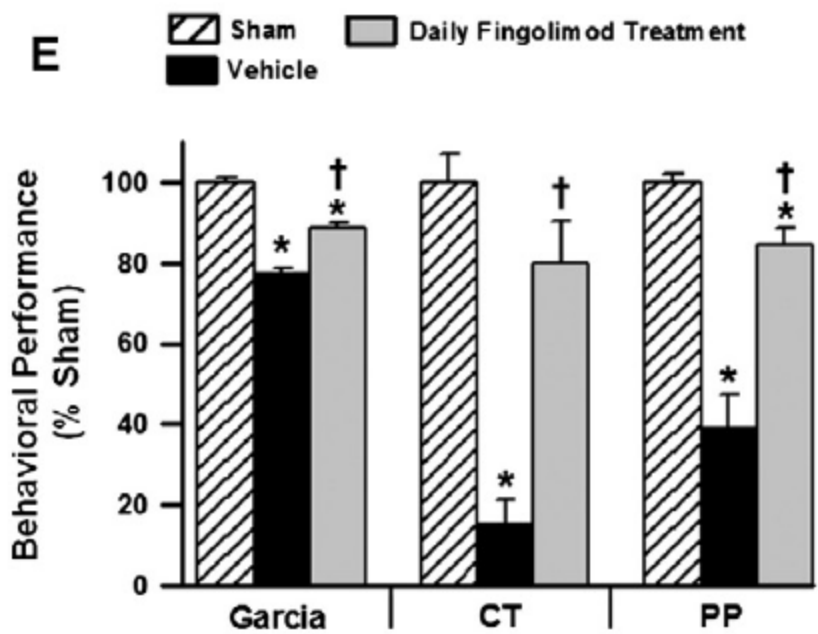
Behavior Outcome 72h Post-cICH



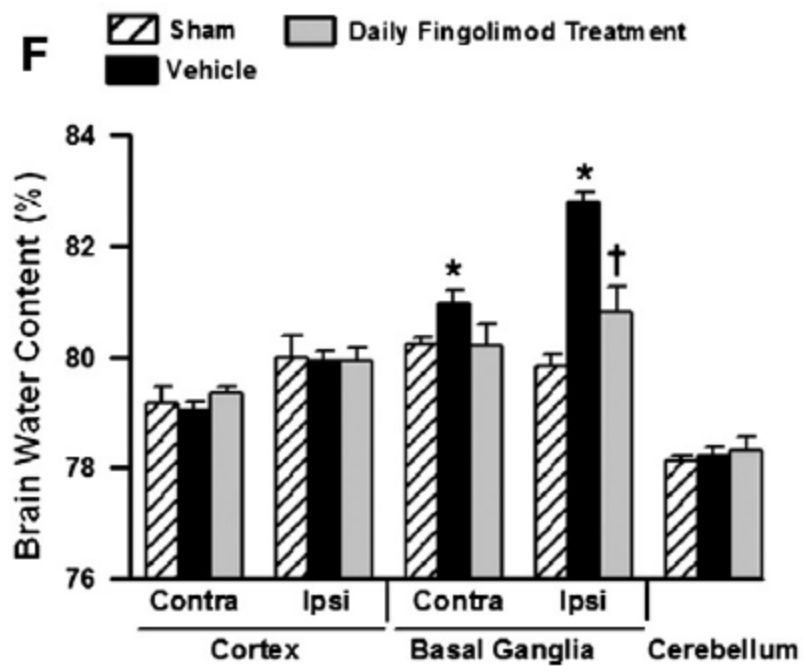
Brain Water Content 72h Post-cICH



Behavior Outcome 72h Post-bICH



Brain Water Content 72h Post-bICH

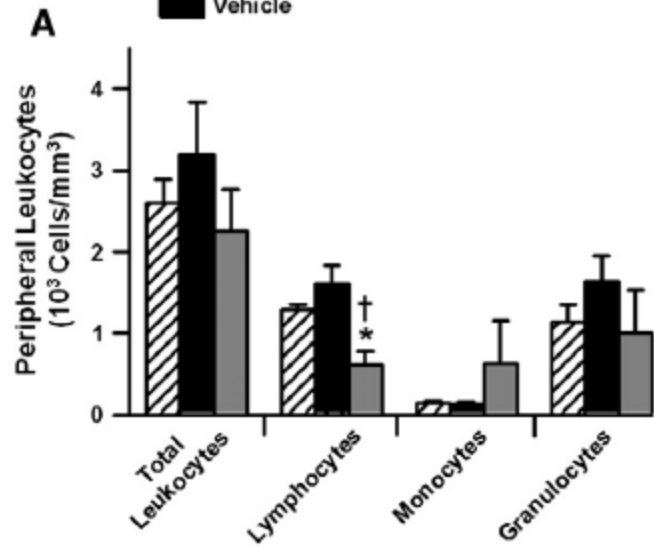


Peripheral Leukocyte Count

- In mice 24 hrs and 72 hrs post-clCH
- Retro ocular puncture
- Density centrifugation

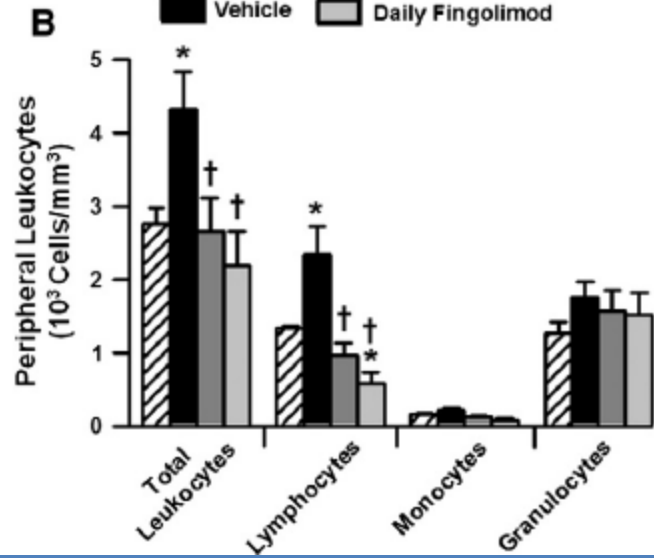
Leukocyte Count 24h Post-cICH

Sham Single Dose Fingolimod
Vehicle



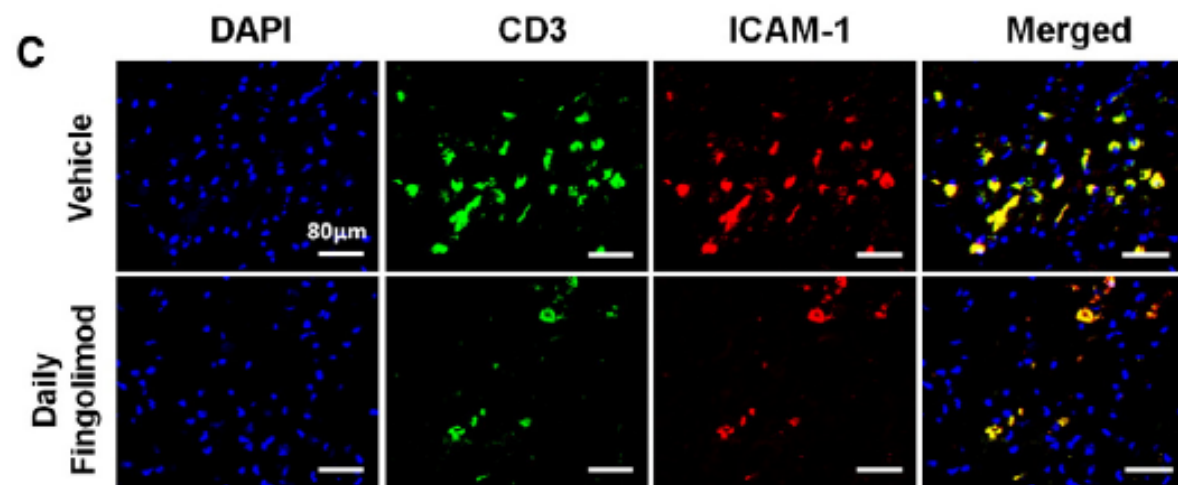
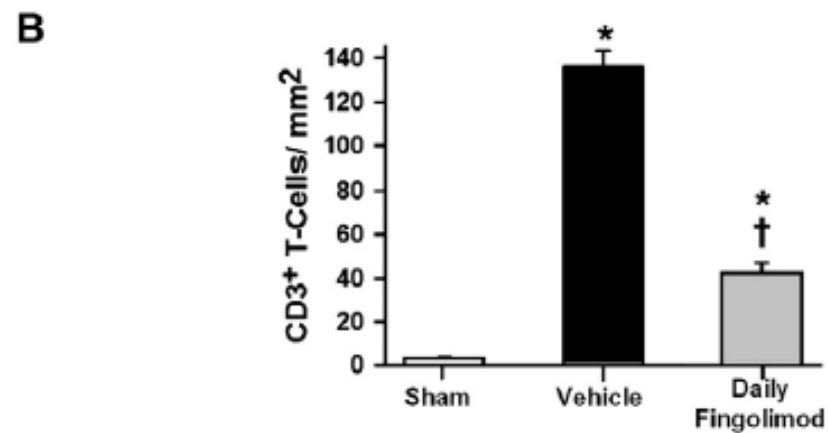
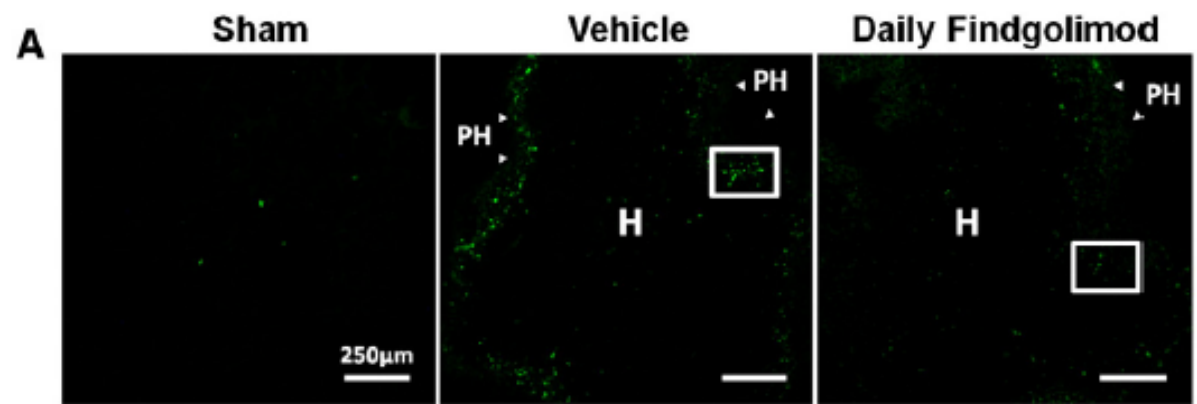
Leukocyte Count 72h Post-cICH

Sham Single Dose Fingolimod
Vehicle Daily Fingolimod



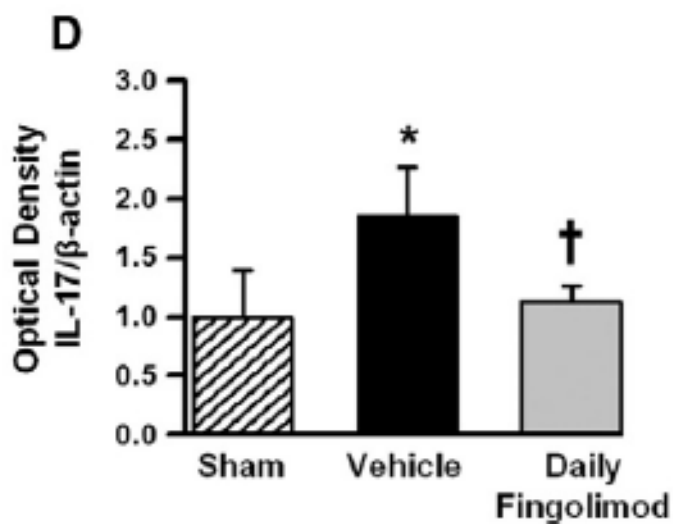
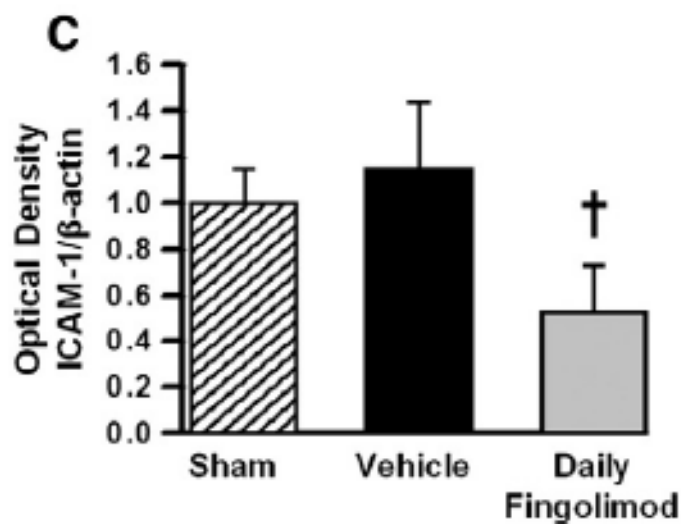
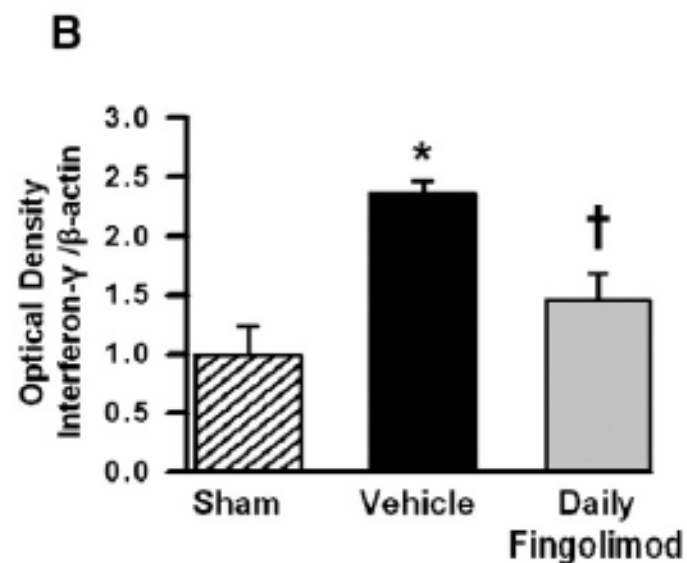
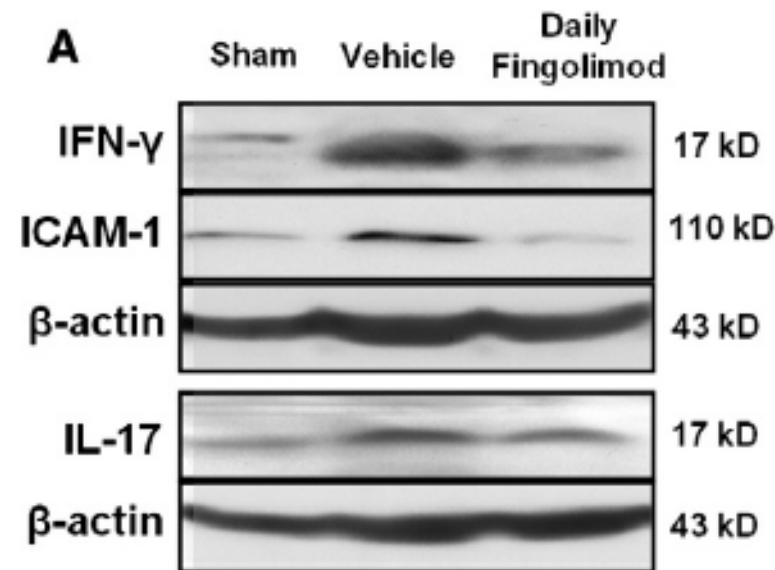
Immuno- fluorescence

- 72 hrs after cIcH
- Antibodies:
 - Anti-CD3
 - Anti-ICAM-1



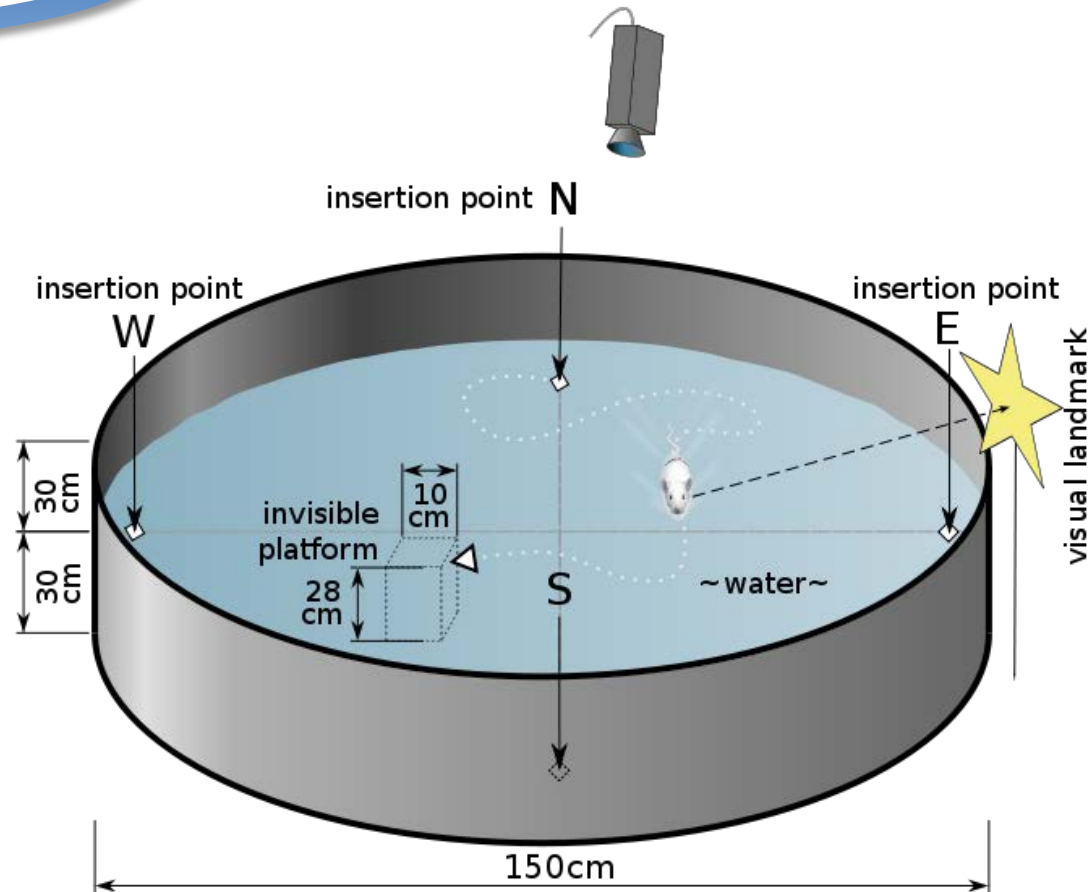
Pro-inflammatory Proteins

- 72 hrs after cIcH
- Antibodies:
 - Anti-ICAM-1
 - Anti-IFN- γ
 - Anti-IL-17
 - Anti- β -Actin

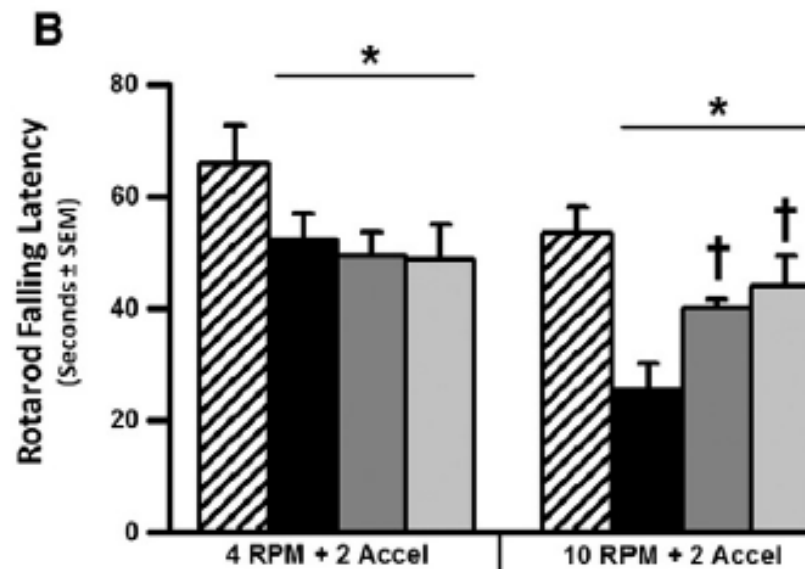
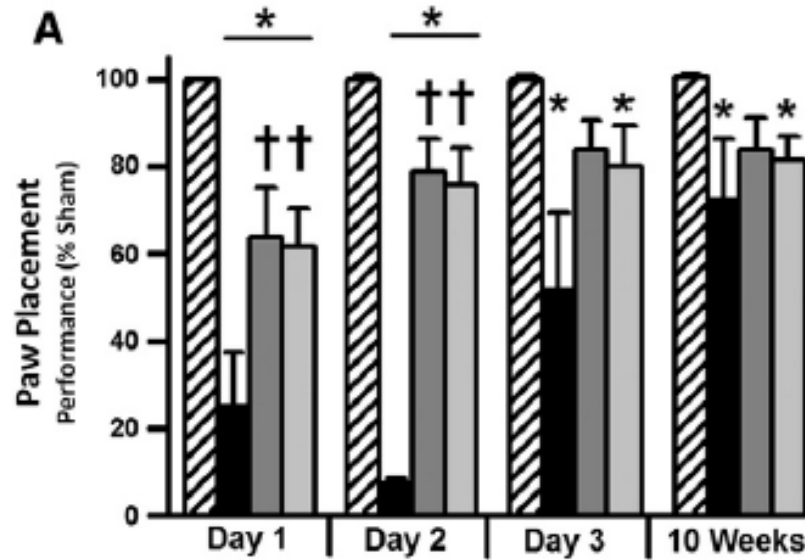


- Long-term behavioral testing:
 - Paw placement test (days 1,2,3 and at 10 weeks)
 - Rotarod (at 8 weeks)
 - Morris water maze test (at 8 weeks)
 - Distance between the rat and a submerged platform was recorded

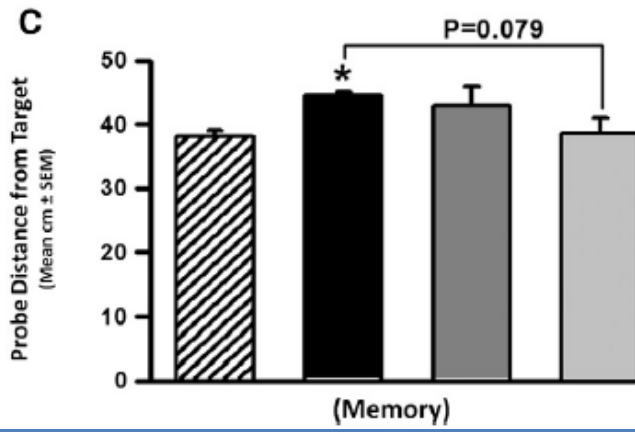
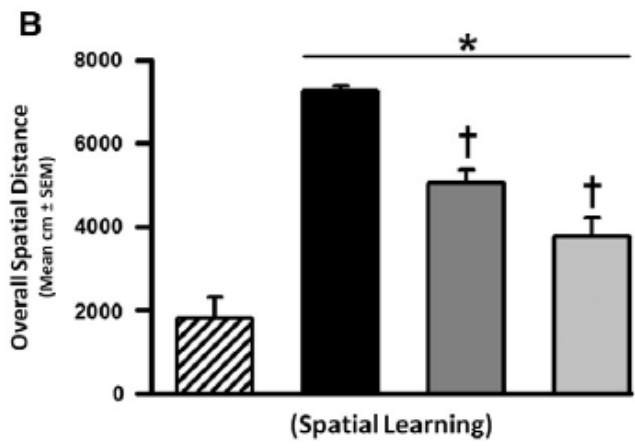
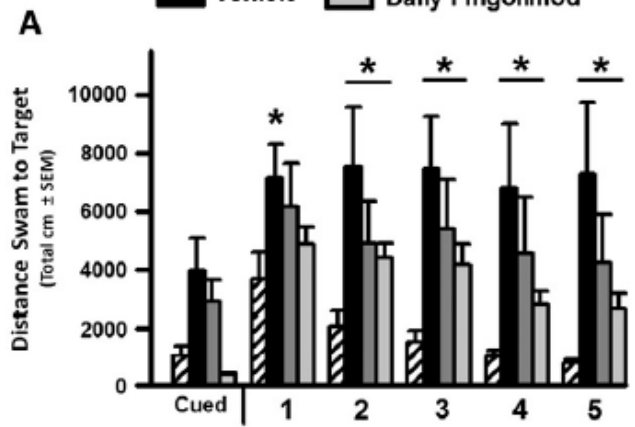
The Morris water maze test was additionally performed at 8 weeks post-cICH to assess learning and memory abilities in rats (Hartman et al., 2008; Lekic et al., 2010). This test required the finding of a slightly submerged platform in a pool of water (diameter: 110 cm). An overhead camera recorded the swim path of each rat, which allowed for quantification of swim distance as well as spatial distance from the platform, by a computerized tracking system (Noldus Ethovision, Tacoma, WA). Water maze testing was done over a 4 day period. Following the cued trials (visible platform) on day 1, the platform was submerged for all subsequent testing (days 2–4). The location of the platform was changed at the beginning of every testing day and the rats were released into the water on the several sites around the platform for 5 blocks of testing. At the end of days 2–4, each animal was subjected to a “probe” trial, in which the platform was removed from the water maze. The distance between the rat and the probe (where the platform used to be) was recorded for 60 s.



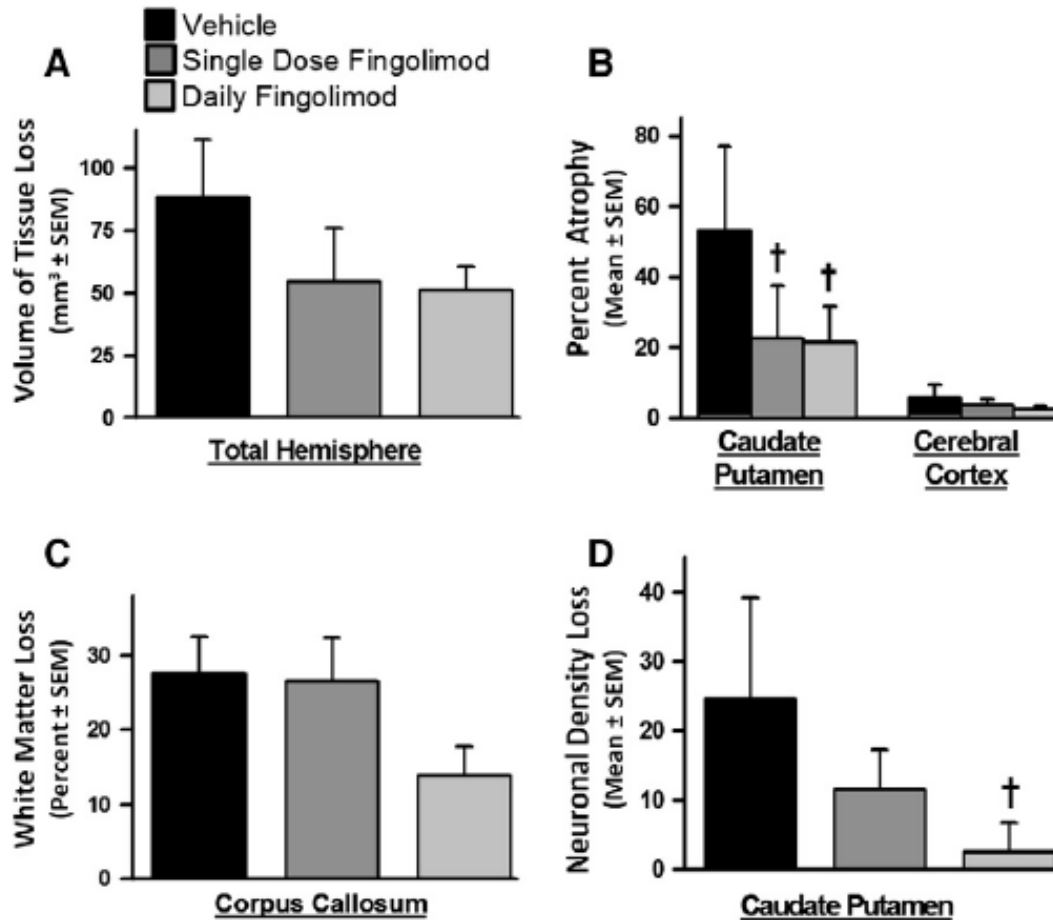
Sham Single Dose Fingolimod
 Vehicle Daily Fingolimod



Sham Single Dose Fingolimod
 Vehicle Daily Fingolimod



Brain Atrophy



➤ 10 weeks after cICH-induction

- Met the STAIR criteria
 - 2 different experimental models
 - 2 species
 - 2 treatment regimes
 - Multiple behavioral and histopathological evaluations

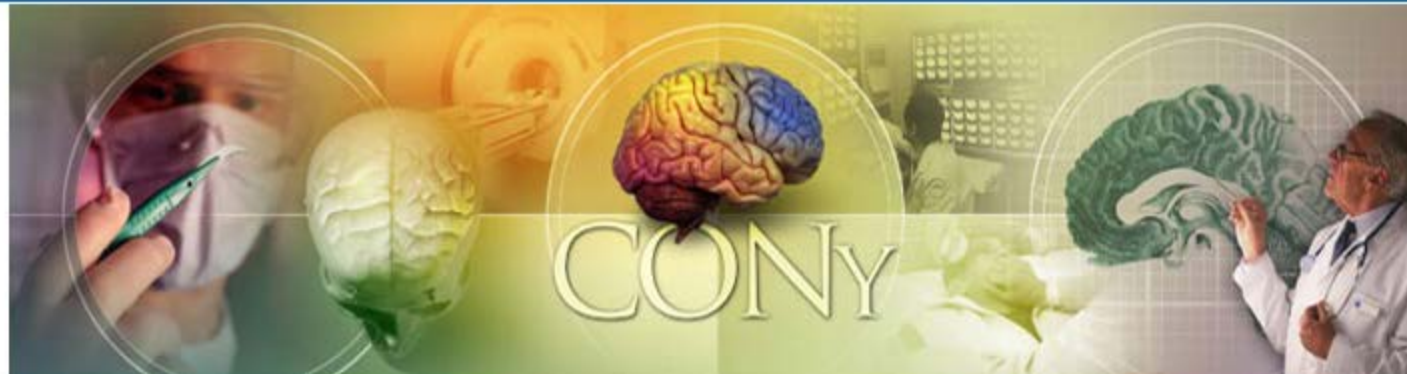
- Primary ICH most commonly affects the basal ganglia
 - Hemorrhagic lesions were induced within the basal ganglia through intrastriatal infusions
- cICH mimics a spontaneous bleed
 - This happens in 14-20% of all ICH patients
- bICH mimics a rapidly developing hemorrhage
- There is a close relationship between the degree of perihematomal brain edema and poor outcome in ICH patients

- Fingolimod reduces brain edema in experimental ischemic stroke as well
- A positive correlation between the amount of brain-infiltrated immune cells and neuronal apoptosis following ICH has been established
- BUT: inflammatory cells are also needed for phagocytosis of cellular debris and for induction of diverse repair mechanisms

- Fingolimod furthermore...
 - Reduces neuronal apoptosis
 - Enhances BBB integrity

- Keeping out Lymphocytes ameliorates damage...
- ... Surprise?
- A lot of testing
- How many animals were actually involved?
- Good figures, well structured paper

Highlights CONy 2013



➤ *Debate: RCTs for post-stroke neurorehabilitation: Are we on the right path?*

***) Yes: D. Muresanu (Romania)**

Neuroprotection+neurorecovery is the future

Use multimodal drugs

***) No: H. Binder (Austria)**

Impairment makes you focus in the environment rather the individual

***) Comment: V. Homberg (Germany)**

Sapere aude. Squeeze the biostatisticians

➤ *Debate: In AUS evaluation, is multimodal CT superior to MRI?*

***) Yes: K. Butcher (Canada)**

MRI supposedly delays treatment

***) No: L. Ranganathan (India)**

***) Comment: D. Heiss (Germany)**

Define DWI lesions, oligaemia, mismatch, healthy tissue

➤ *Debate: Intervention vs. BAMT in ACAS*

***) BAMT: D. Spence (Canada)**

only patients with high risk stenosis (very low CBF) need CEA
CEA only prevents emboli, it does not augment CBF

***) Intervention: O. Ozdemir (Turkey)**

***) Comment: J. Norris (UK)**

complications: stent → stroke

CEA → heart attack

➤ *Debate: AF-related stroke should be treated only with new anticoagulants*

***) Yes: A. Shuaib (Canada)**

decrease in stroke + (!!)-bleeding

no biweekly visits

***) No: K. Kutluk (Turkey)**

AEs: dyspepsie, GI bleed

no way to monitor them

***) Comment: M. Brainin (Austria)**

We don't know enough. When to start? How to monitor? How to treat bleeds?

➤ Debate: Should patients with cortical strokes be treated prophylactically for seizures?

Yes: N. Dericioglu (Turkey)

Stroke is one of the frequent underlying causes of symptomatic focal epilepsies

No: W. Theodore (USA)

AEDs might impair stroke recovery

Antiglutamatergic effects might be counterproductive (repair processes)

➤ Other debates:

- *) PFO – to close or not to close?
- *) Are neuroprotective agents still an option for AIS?
- *) Expensive mistakes in stroke thrombolysis
- *) Biological molecules: Progesterone Vs. Trophic factors in TBI
- *) MS – environmental or genetic?
- *) Are MS and NMO two polarized diseases? Does this have treatment implications?

➤ Other debates:

- *) RIS in patients are at high risk of developing MS and warrant treatment with disease modifying drugs
- *) Proposition: DBS should only be used after VNS has failed
- *) New Players in MS

Thank you for your attention

