Translational Research: Inflammation and post-stroke cognitive decline

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Disclosures

- Research contract with Biogen
- Funding from NINDS, MJFF, Wu Tsai Neurosciences Institute, AHA, Allen Foundation
- Advisory Board, Omniox

Talk Outline

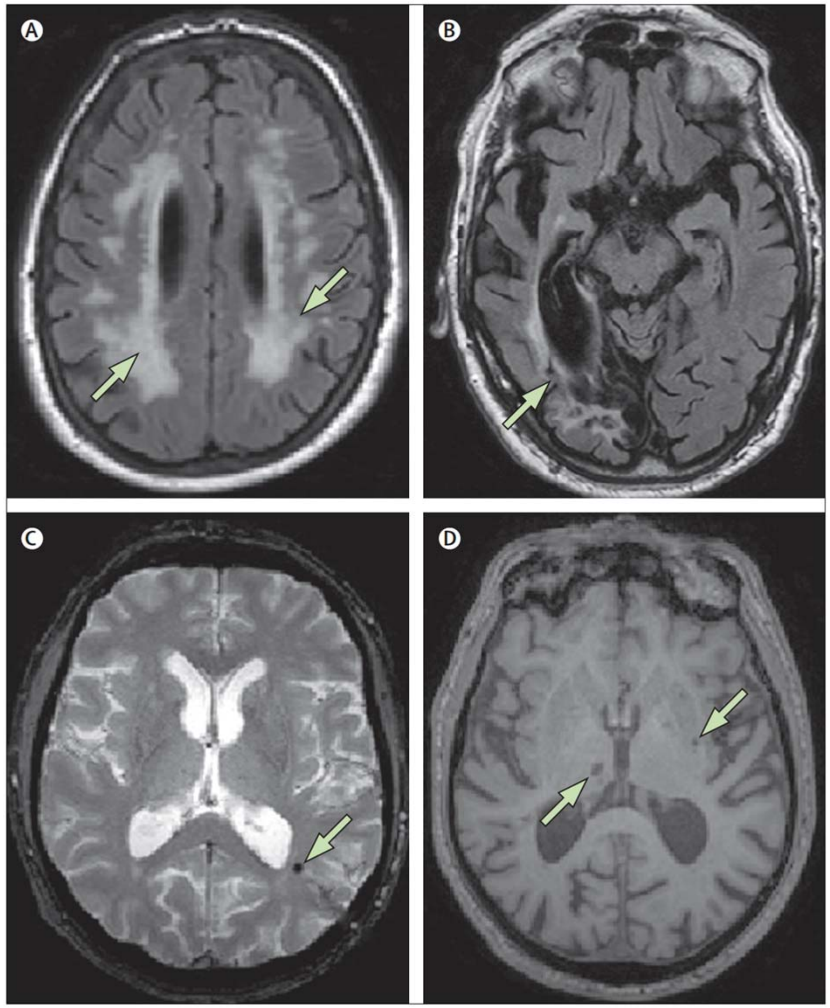
- Definitions: Post-stroke dementia vs. vascular dementia
- Hypothesis: Is post-stroke dementia an immune-mediated neurodegenerative disease?
- Mechanism: Mouse model of post-stroke dementia
- In progress: Testing the model in humans
- Relationship to other dementias

What is vascular dementia?

Ischemic /stroke (vascular) lesions

White matter disease (periventricular vs. subcortical)

Large Vessel



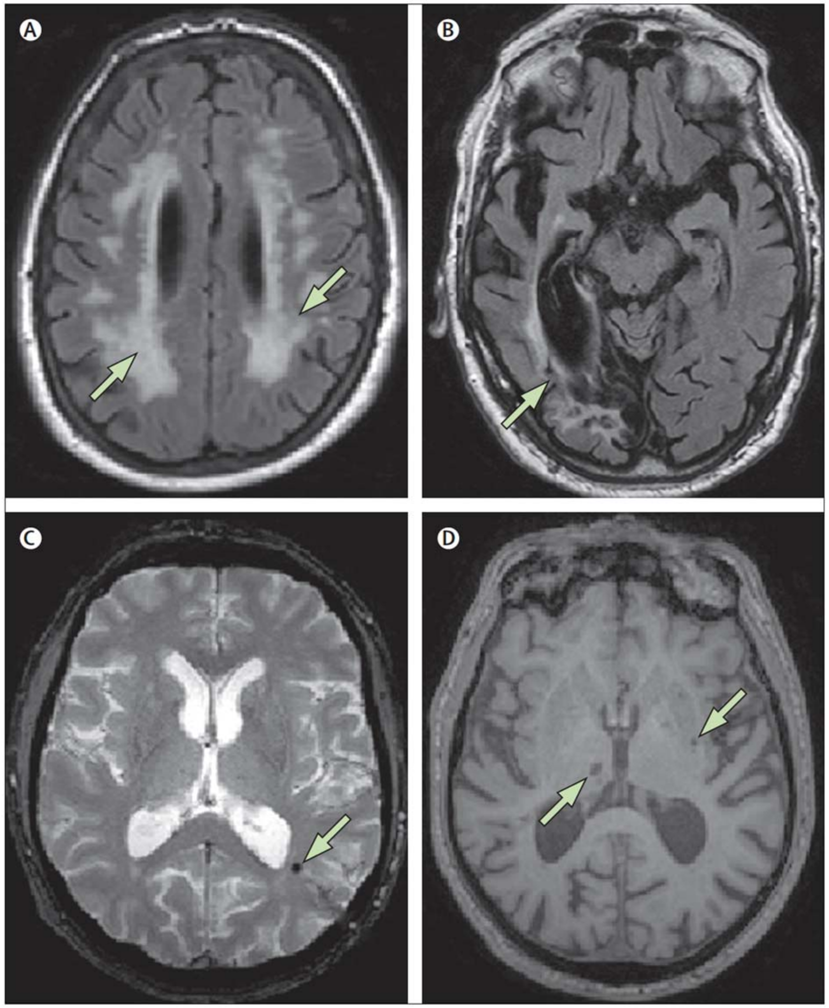
Microbleeds

Lacunar

O'Brien and Thomas, 2015. Lancet 386:1698-1706

Ischemic /stroke (vascular) lesions

White matter disease (periventricular vs. subcortical)



Large Vessel

Multi-infarct Dementia

Microbleeds (CAA) ICH

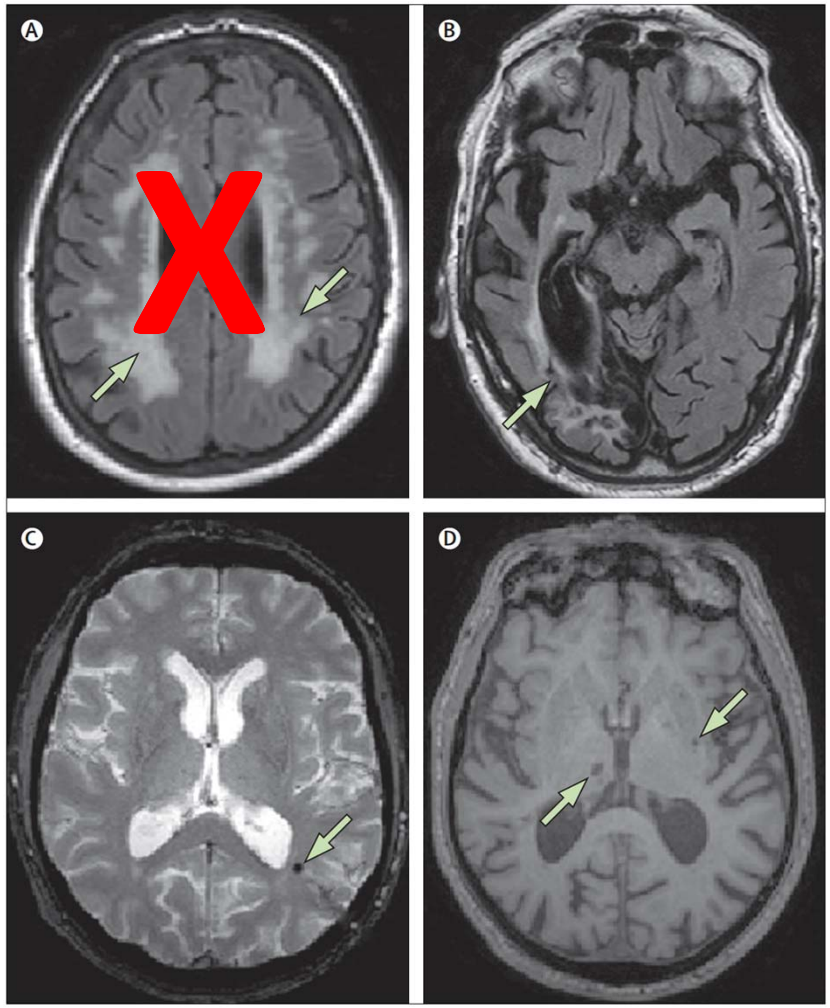
Lacunar

O'Brien and Thomas, 2015. Lancet 386:1698-1706

Post-stroke dementia lesions

White matter disease (periventricular vs. subcortical)

Large Vessel



Multi-infarct Dementia

Microbleeds
CAA
ICH

Lacunar

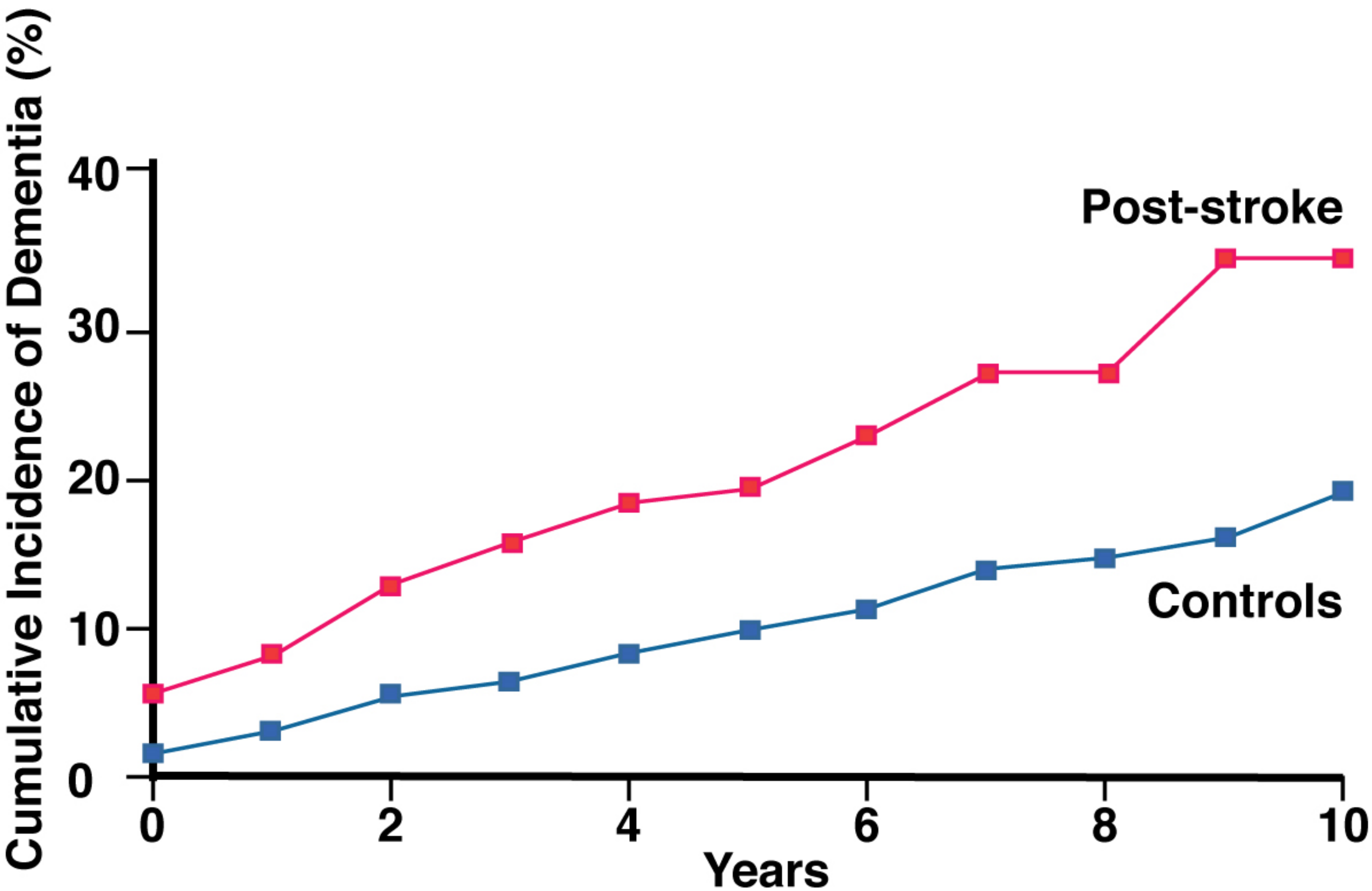
O'Brien and Thomas, 2015. Lancet 386:1698-1706

Post-Stroke Dementia

- Stroke doubles the risk of developing dementia in the decades after a stroke
- This is **incident** dementia after accounting for common / known risk factors of dementia (age, HTN, NIDDM, diet, exercise, tobacco)
- Absolute risk and relative risk depends on age
- Unclear mechanism with unknown risk factors

Savva, et al. 2010 *Stroke*, *41*(1), e41-e46.
Pendlebury, & Rothwell, 2009 *Lancet Neurology*, *8*(11), 1006-1018.
Corraini et al. Stroke. 2017;48:00-00. DOI: 10.1161/STROKEAHA.116.015242

Framingham sub-study



Ivan et al, 2004. Stroke 35:1264-1269

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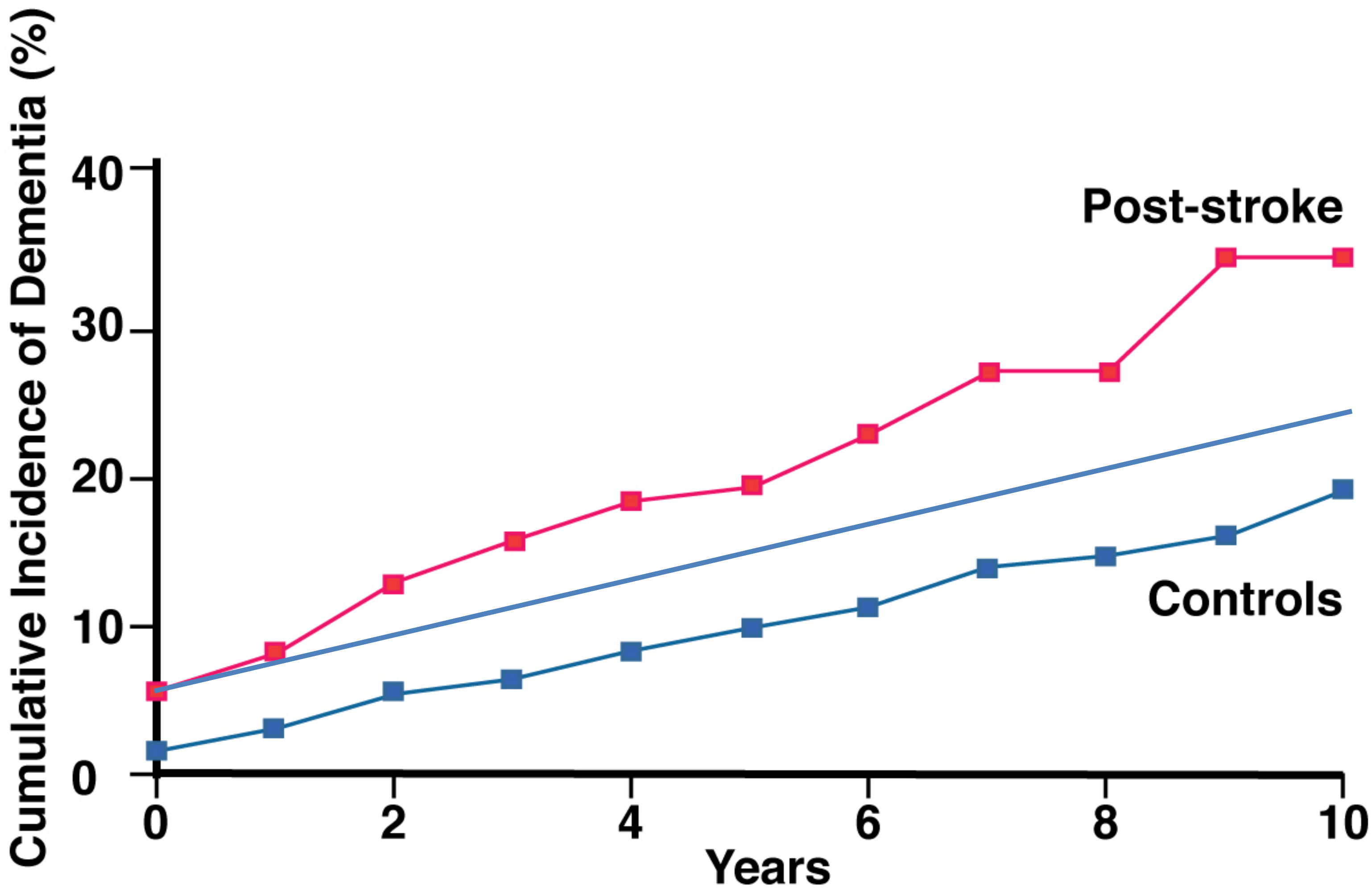

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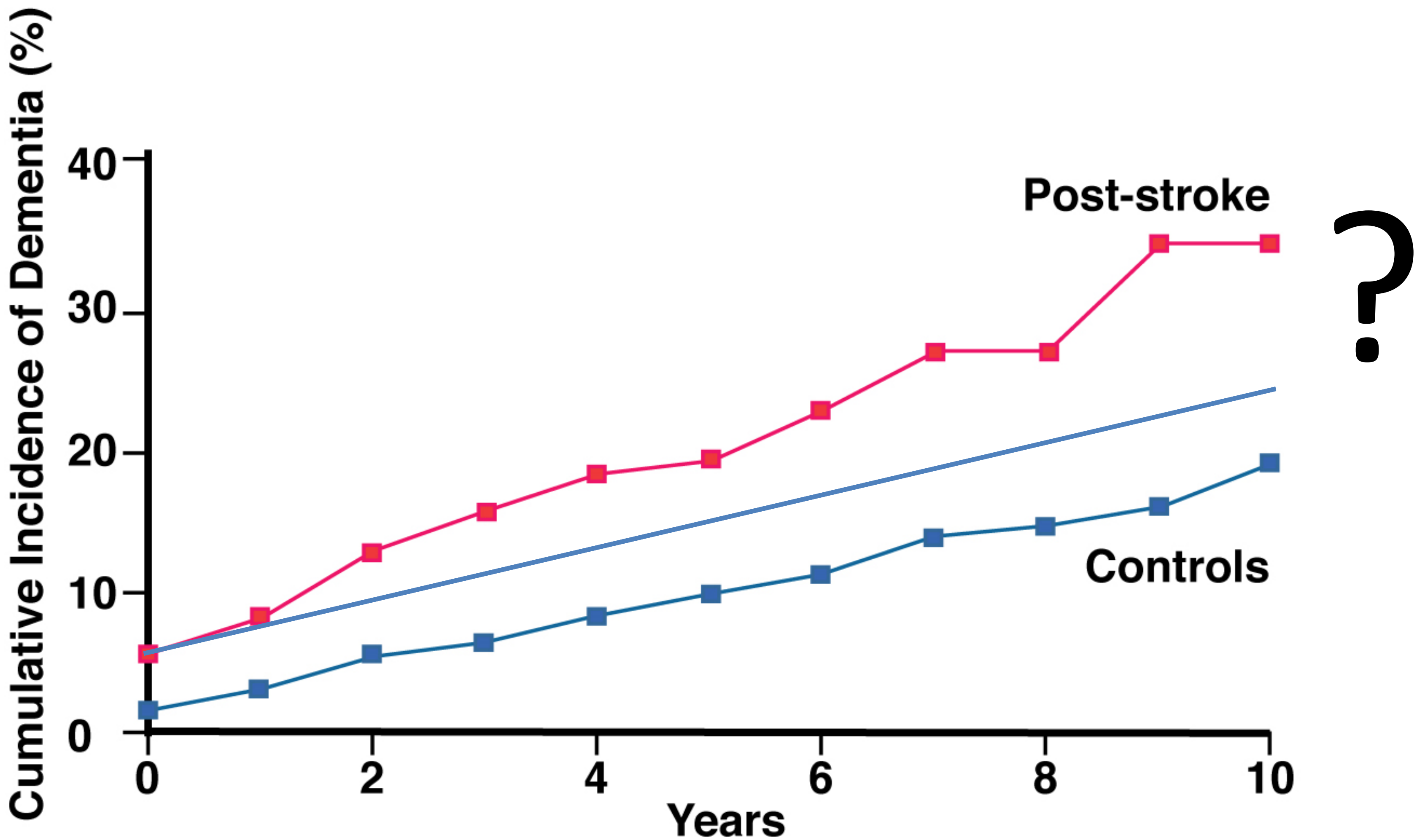
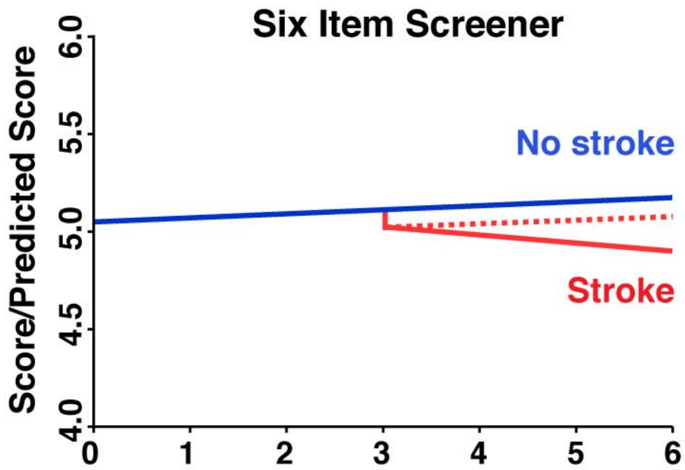


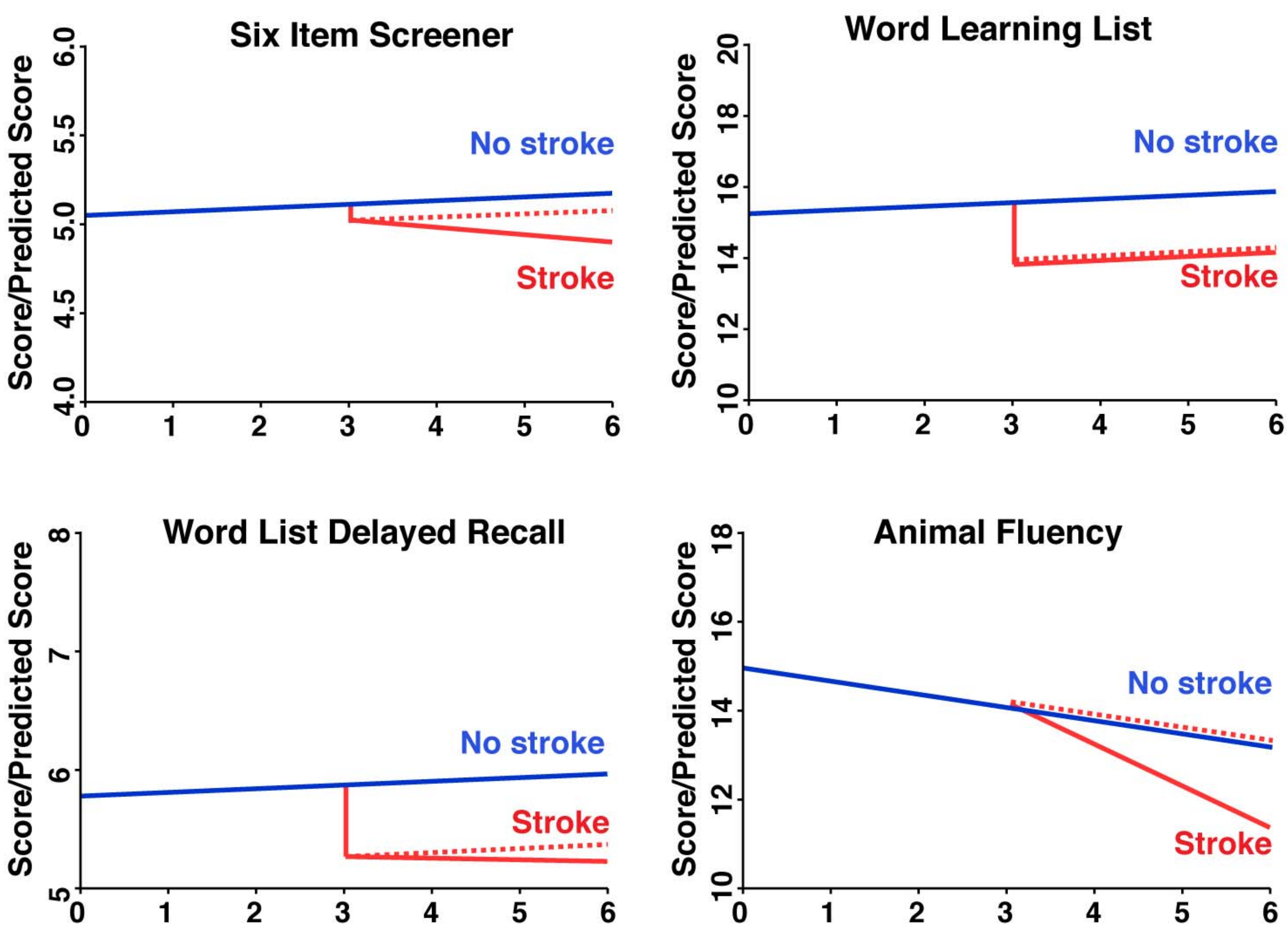
Ivan et al, 2004. Stroke 35:1264-1269

Cognitive trajectory after stroke-REGARDS cohort



Levine et al, 2015 JAMA 314:41-51.

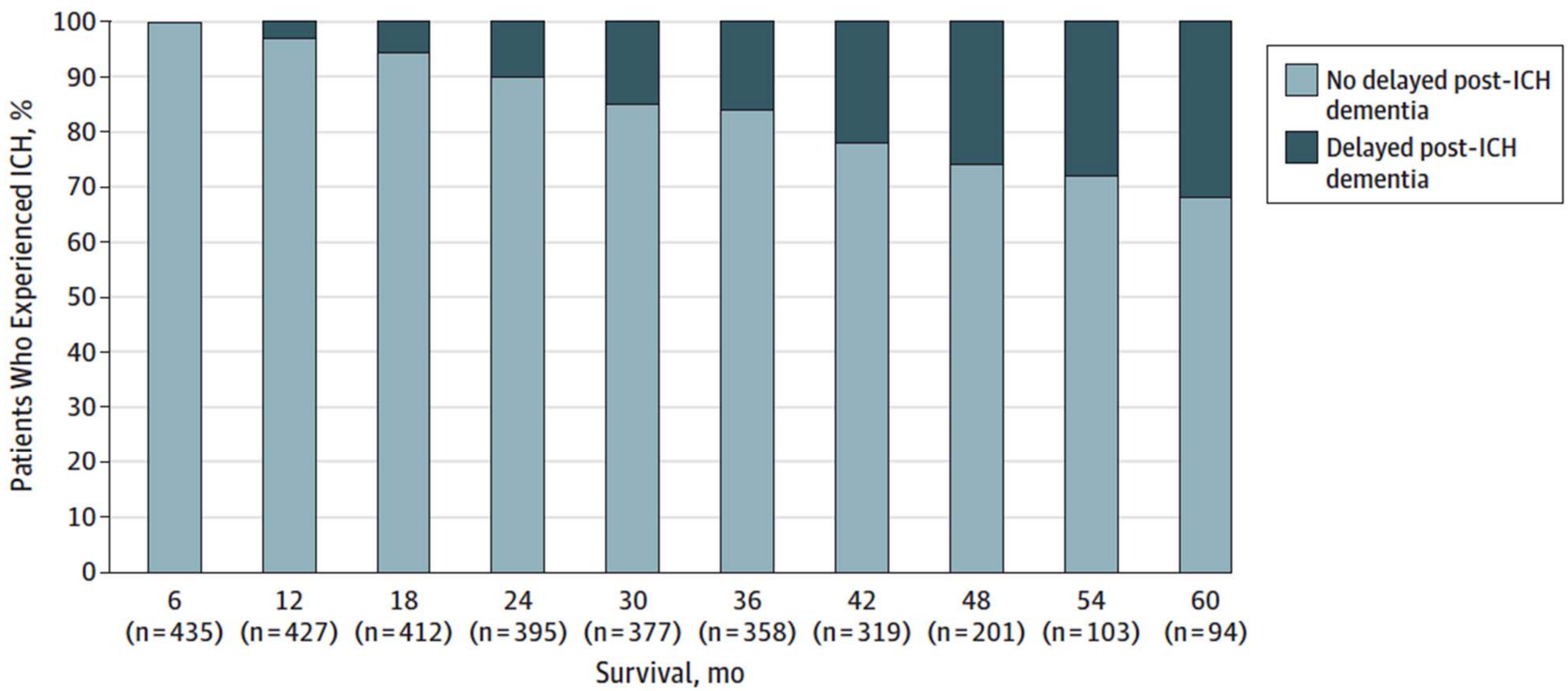
Cognitive trajectory after stroke-REGARDS cohort



Levine et al, 2015 JAMA 314:41-51.

Incident delayed cognitive decline is high after intracerebral hemorrhage

Figure 2. Incident Delayed Cognitive Decline Among Patients Experiencing Intracerebral Hemorrhage (ICH)



Biffi et al, 2016. JAMA 73(8):969-976

Delayed cognitive decline after ICH is not related to ICH volume or location

Table 3. Multivariable Analyses of Risk Factors for Early vs Delayed Dementia After ICH

Risk Factor	Post-ICH Dementia Risk, HR (95% CI) Early	P Value	Delayed	P Value	P Value for Heterogeneity
Model 1[a]					
No. of patients	619		435		
Age	1.02 (1.00-1.04)	.03	1.01 (1.00-1.01)	.05	.78
Educational level (≥10 y)	0.89 (0.61-1.30)	.55	0.60 (0.40-0.89)	.01	<.001
African American race	1.22 (0.96-1.55)	.11	1.48 (1.09-2.02)	.01	.55
Incident mood symptoms	0.66 (0.04-11.11)	.77	1.29 (1.02-1.63)	.04	.01
ICH volume (per 10-mL increase)	1.47 (1.09-1.97)	.01	1.10 (0.70-1.73)	.68	<.001
Lobar ICH location	2.04 (1.06-3.91)	.03	1.33 (0.25-7.03)	.74	.02
CT-WMD severity	1.34 (0.23-7.76)	.74	1.70 (1.07-2.71)	.03	.04

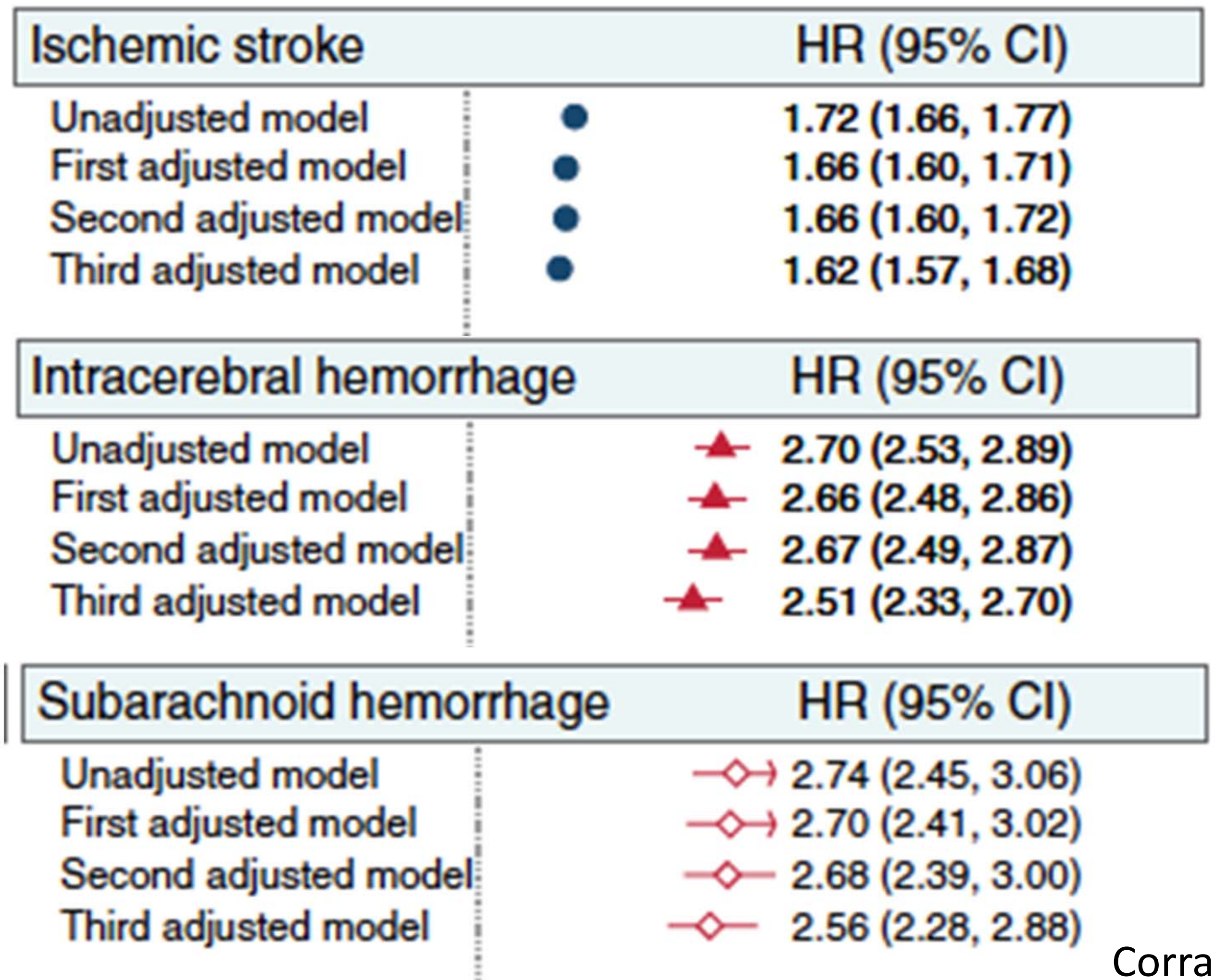
Biffi et al, 2016. JAMA 73(8):969-976

Risk of dementia after stroke in large Danish cohort

- Population-based cohort from national medical databases
- Included all Danish citizens with first time stroke between Jan 1989-Dec 2013
- Observed over 30 years, avg. 5 year f/u
- 279,349 patients with first ever stroke vs. 1,075,558 general population

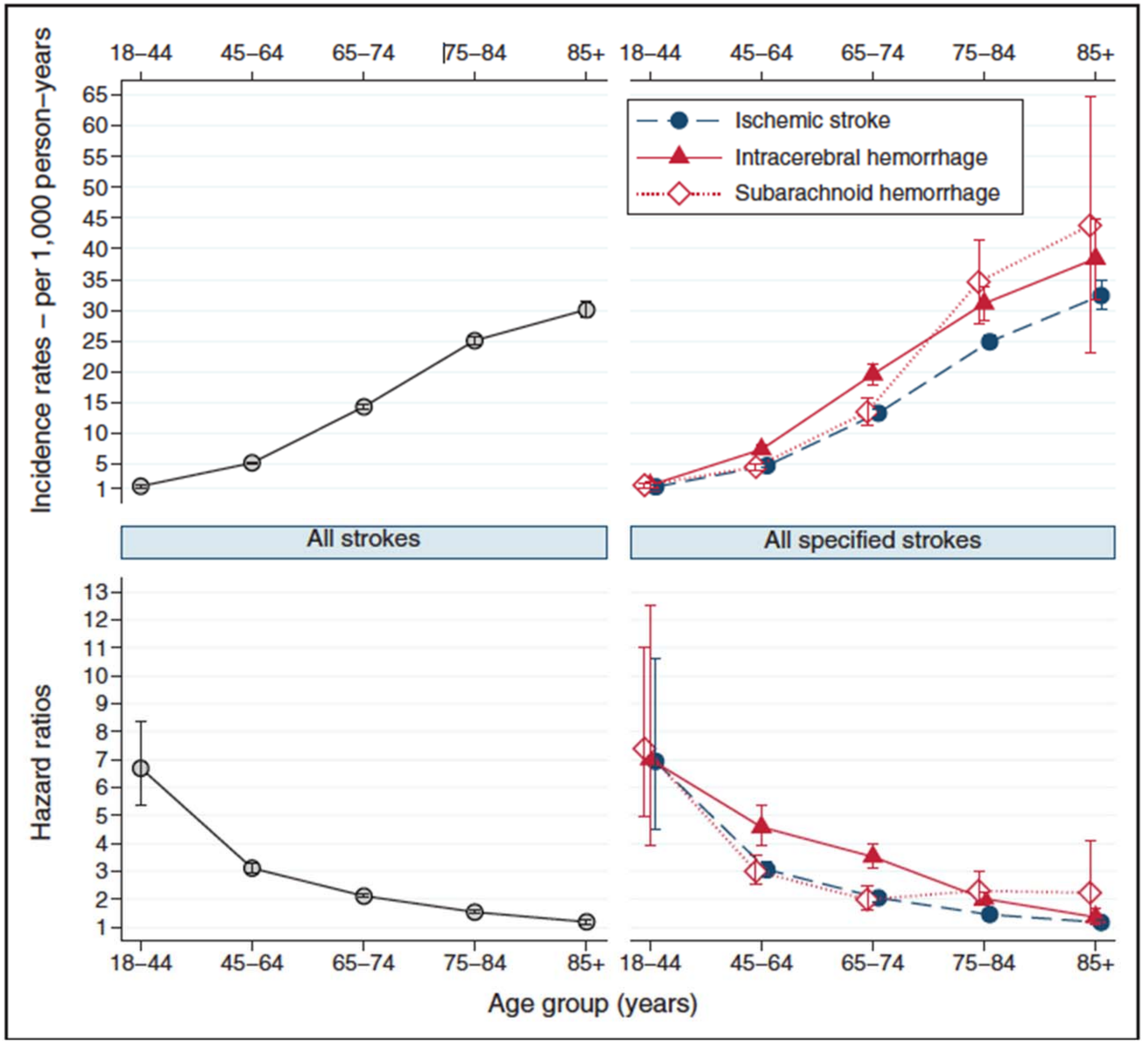
Corraini et al, Stroke 2017.
https://doi.org/10.1161/STROKEAHA.116.015242

Effect of stroke subtype on post-stroke dementia risk



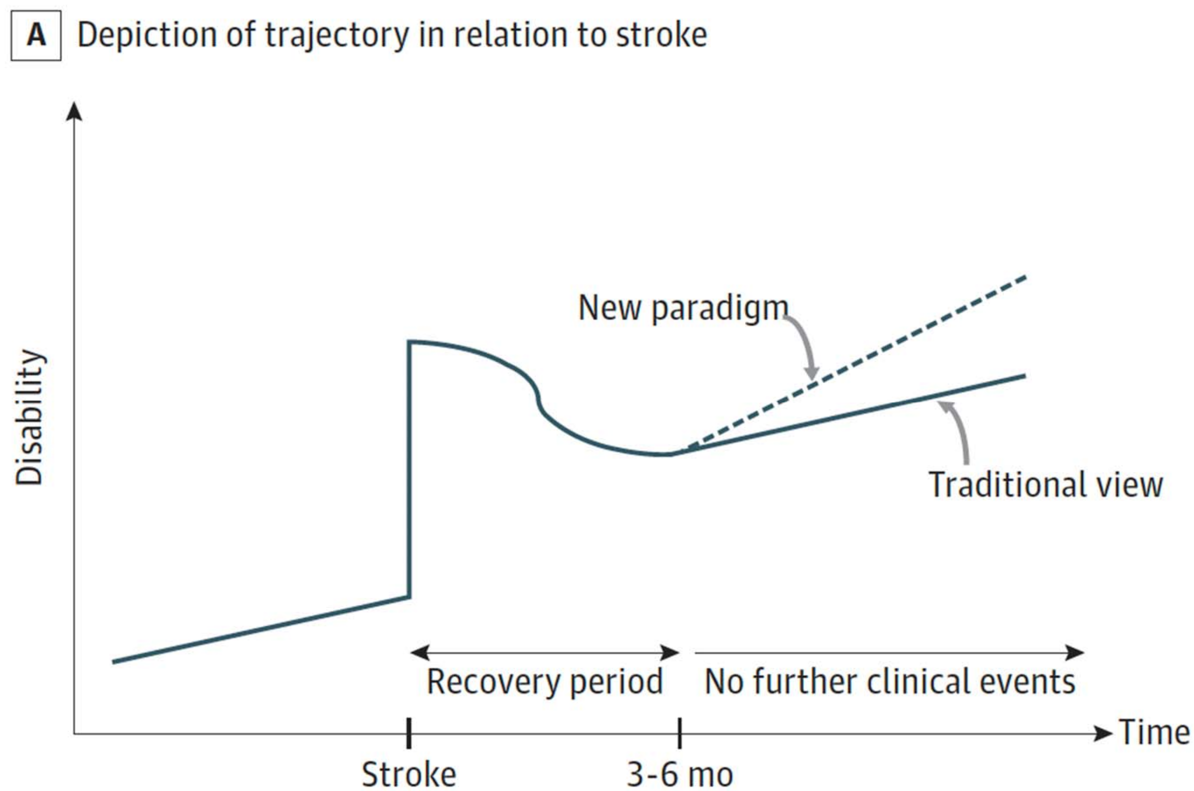
Corraini et al, Stroke 2017.
https://doi.org/10.1161/STROKEAHA.116.015242

Effect of age on post-stroke dementia risk



Corraini et al, Stroke 2017.
https://doi.org/10.1161/STROKEAHA.116.015242

Cardiovascular Health Study – disability trajectories after stroke



Dhamoon et al, 2017 JAMA Neurology (74):12, 1439-1445

What can we learn from prior trials about the causes of post-stroke cognitive decline & dementia?

- **Negative**
  - Blood pressure lowering – PRoFESS w telmisartan
  - Statins – simvastatin (HPS) or pravastatin (PROSPER)
  - IRIS - pioglitazone (JNNP 2018; *89*(1), 21-27.)
  - SPS3 – BP reduction+2 anti-plt (*Lancet Neuro*, 2014; *13*(12), 1177-1185.)
  - PODCAST – intensive BP and lipid lowering (PlosOne 2017, *12*(1), e0164608)
- **Positive**
  - ARTEMIDA – calf serum derivative, unclear mechanism. 250 treatment, 250 placebo, less ADAS-Cog decline (Stroke 2017;48:1262-1270)
  - PROGRESS trial BP lowering w perindopril

Brainin et al., 2015. Eur J Neurol 22:229-238.

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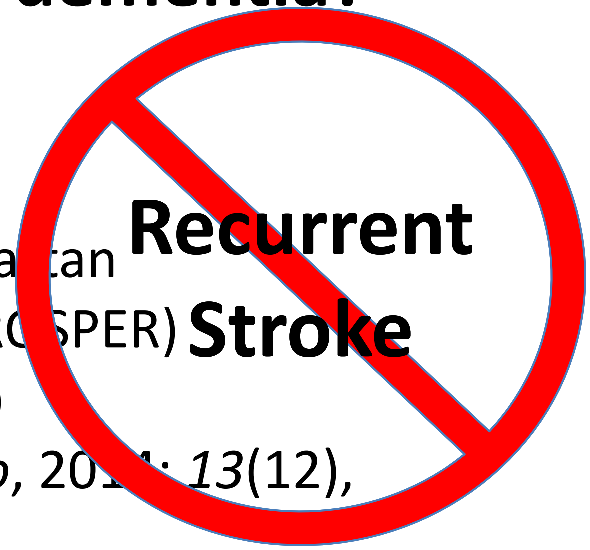

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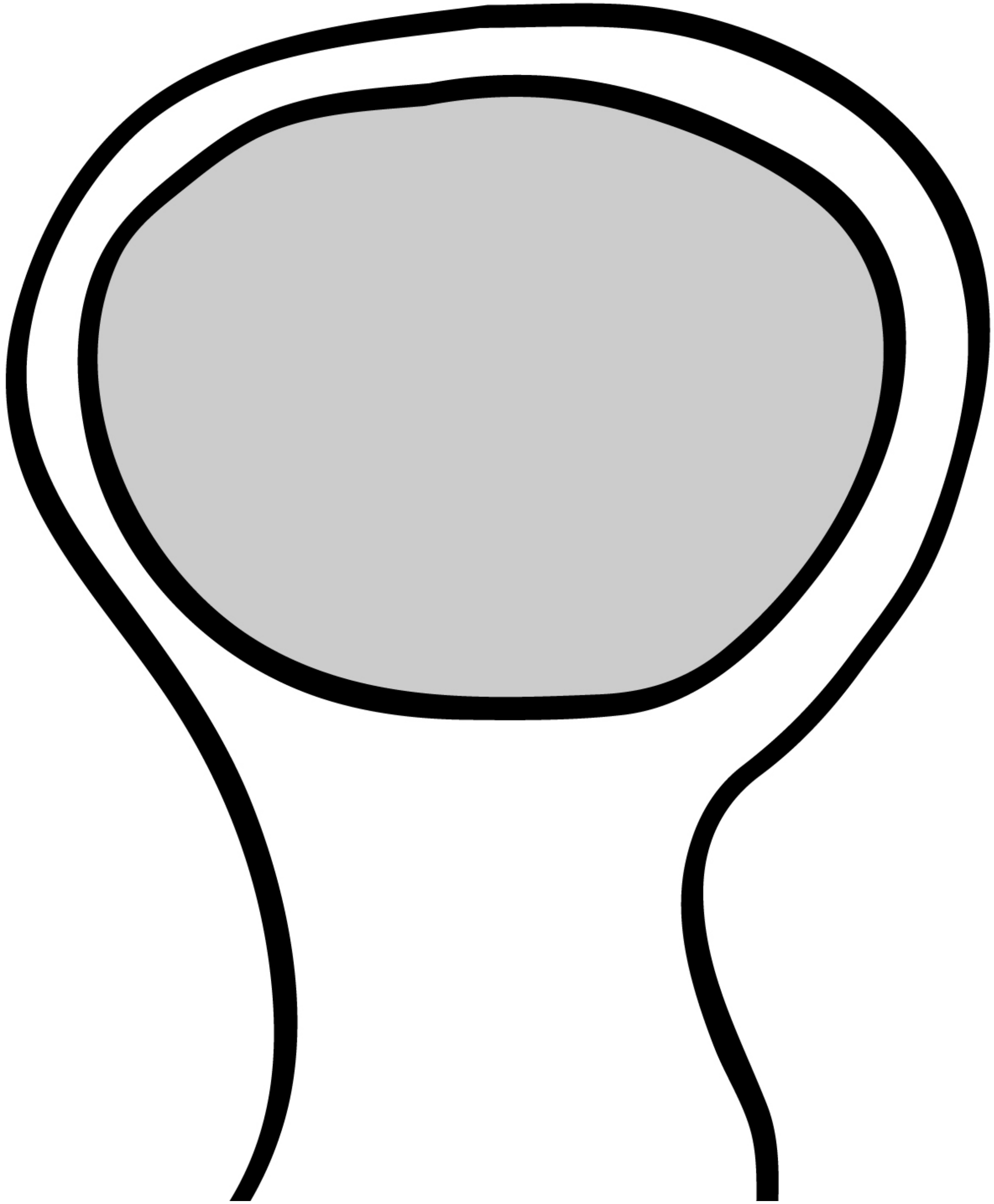
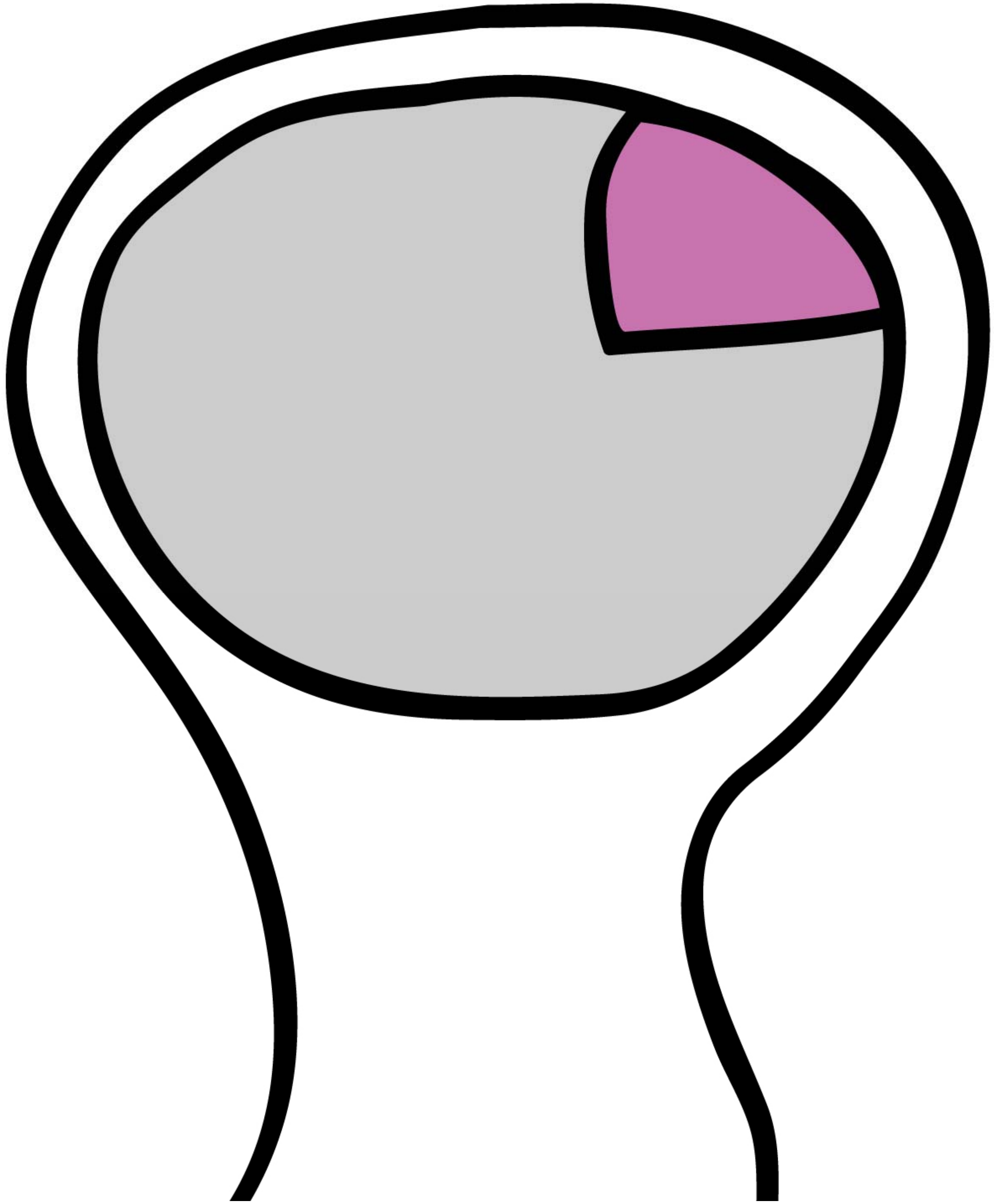
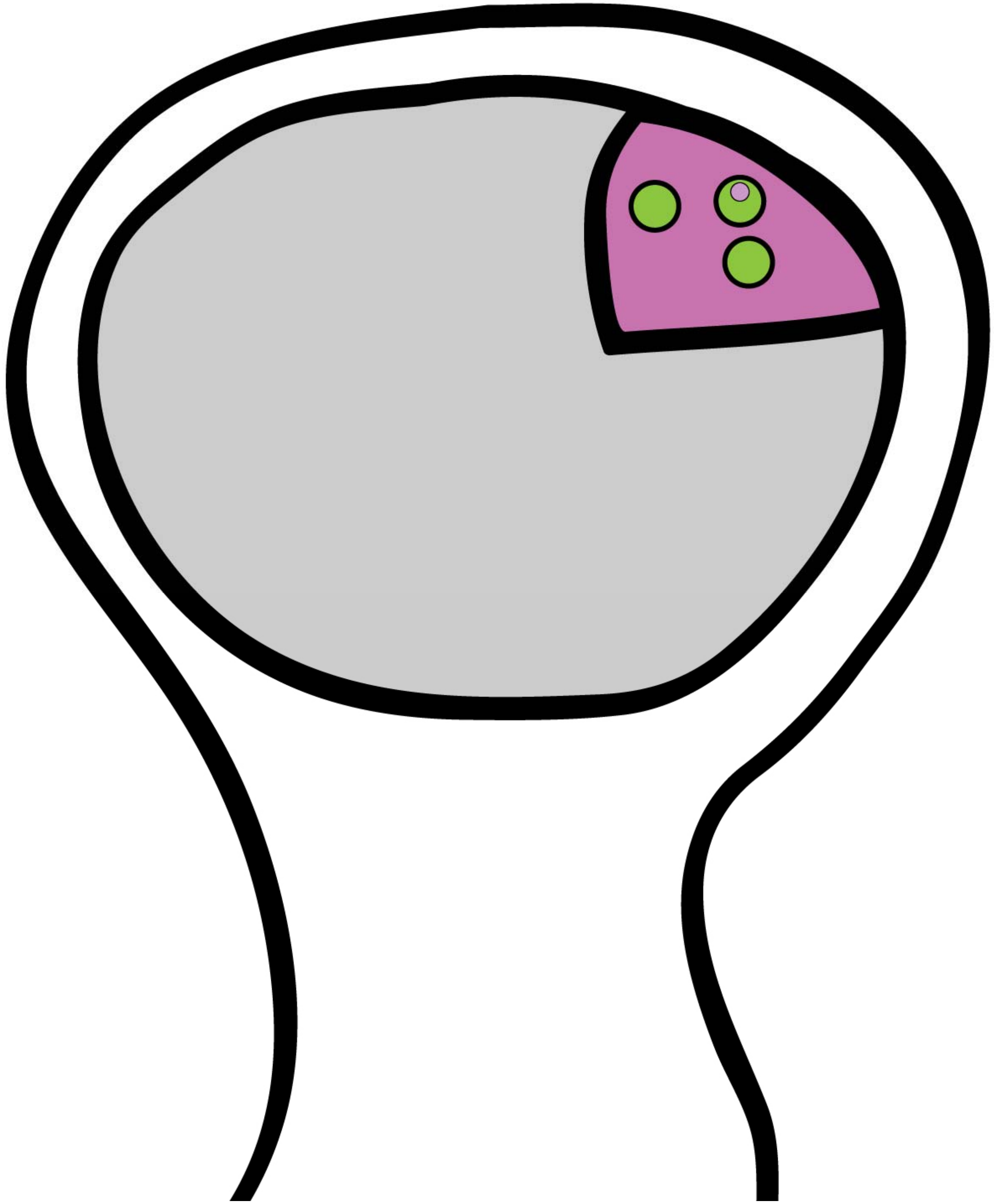
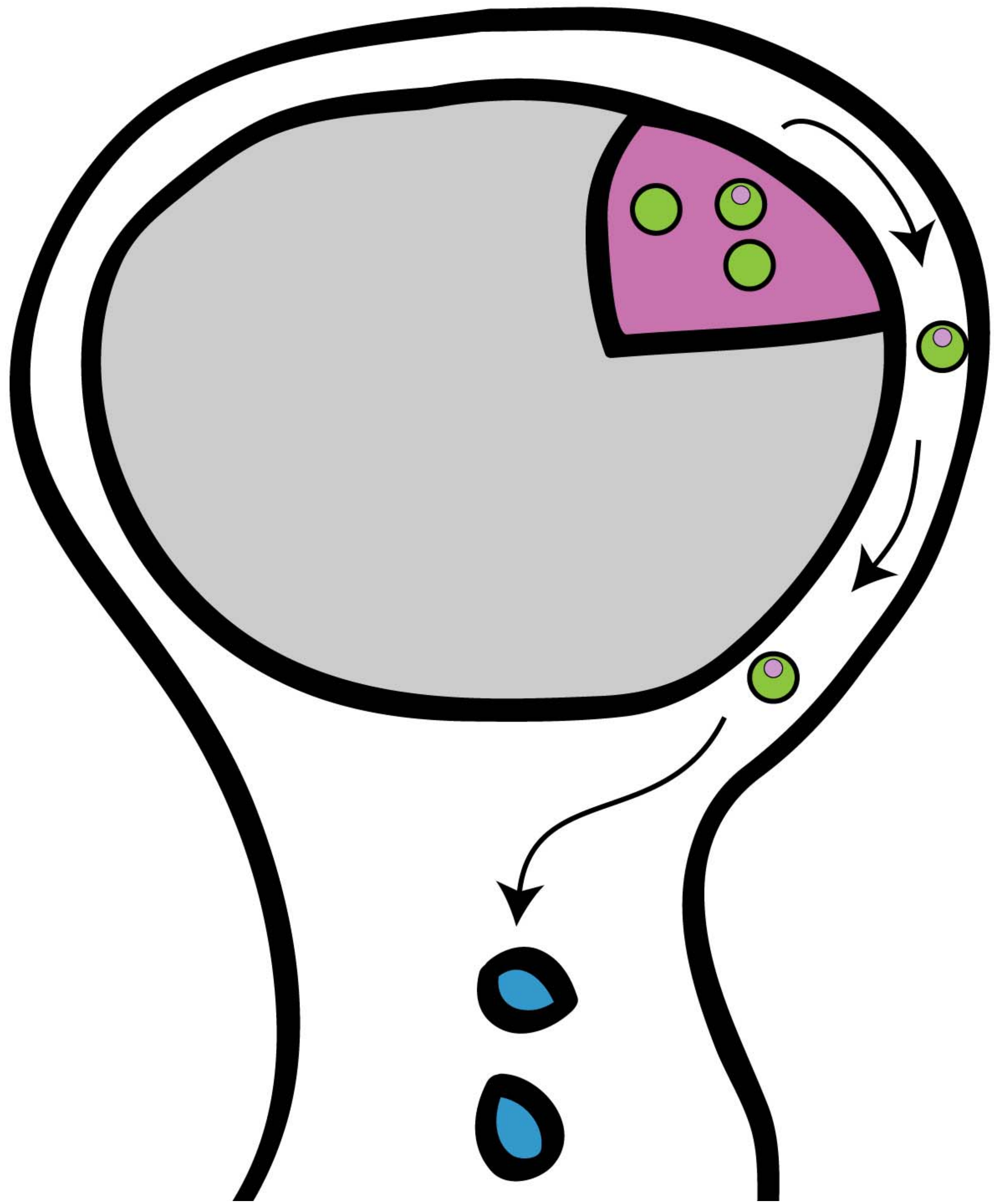
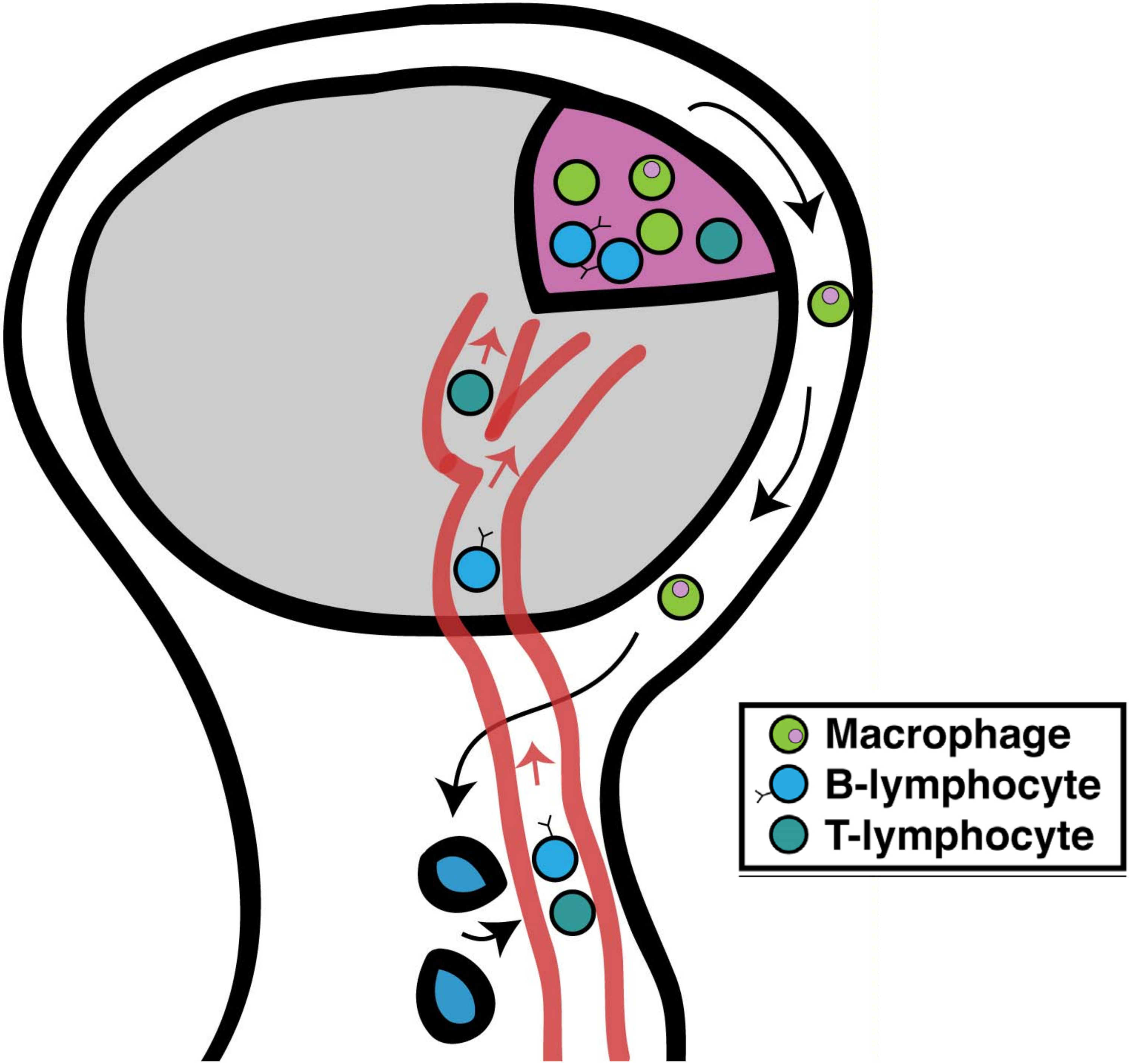
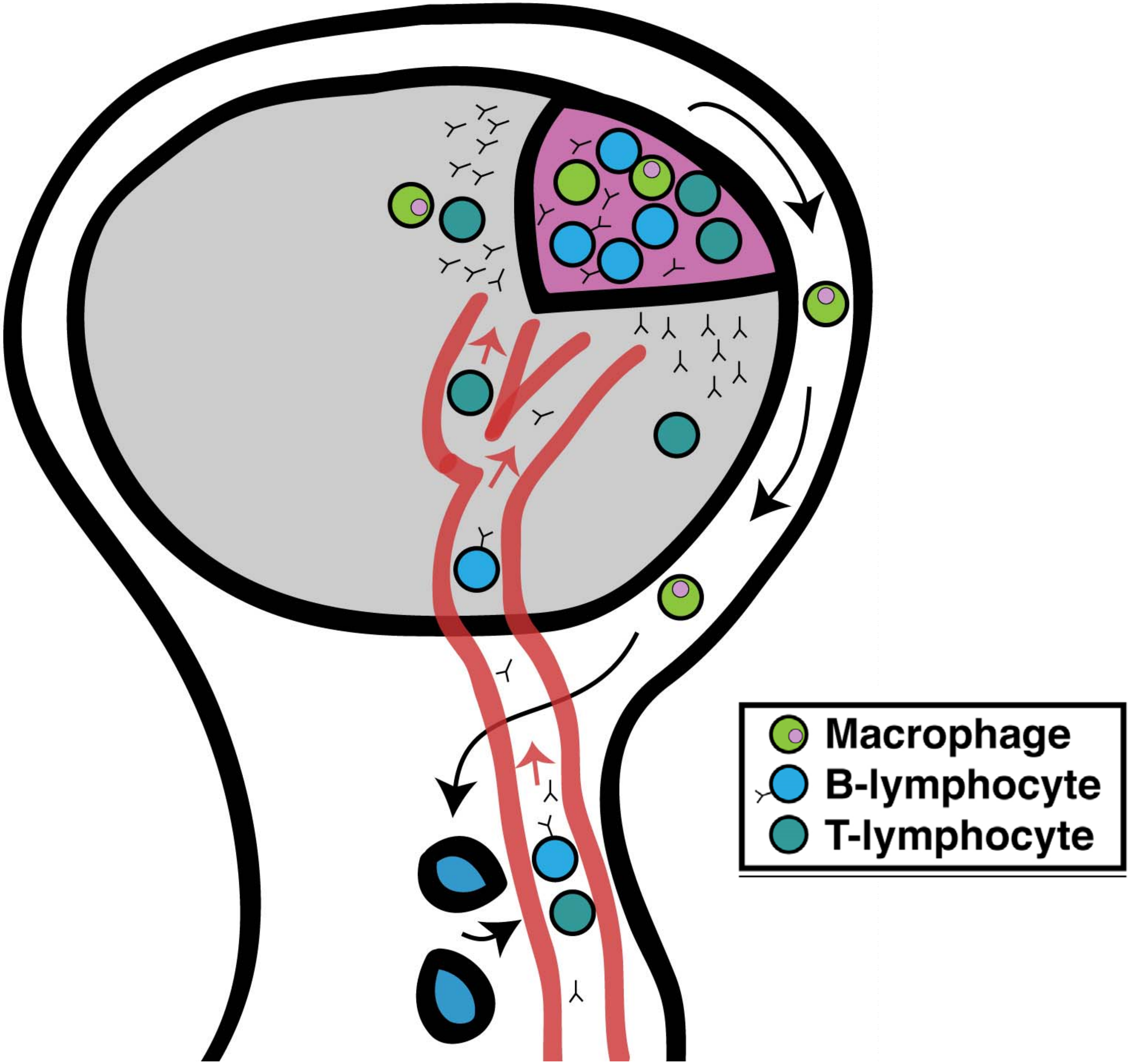









Macrophage
B-lymphocyte
T-lymphocyte


Macrophage
B-lymphocyte
T-lymphocyte

Antigens and autoimmunity after stroke

- Neuronal and glial proteins go up in the blood after stroke (NSE, GFAP, S100B)

- "Adjuvant" effects reported for non-cognitive outcomes
  - Rats given LPS at reperfusion
    - More proinflammatory Th1 responses to MBP, (66.7% versus 22.2)
    - More profound and persistent neurologic deficits than non-LPS-treated animals.
  - Humans with an infection within 15 days of stroke
    - more likely have a Th1 response to myelin basic protein and glial fibrillary acidic protein 90 days after stroke
    - More robust Th1 responses to myelin basic protein at 90 days were associated with a decreased likelihood of good outcome at 90 days (mRS), even after adjusting for baseline stroke severity and patient age (OR, 0.477; 95% CI, 0.244 to 0.935; P 0.031).

Becker, JCBFM 2005 & Becker, Stroke 2011

Histopathological changes after human acute ischemic stroke

Histopathological changes	n (%)
Astrogliosis	114 (83)
Neutrophils	31 (23)
Mononuclear inflammatory cells	61 (45)
Macrophages	103 (75)

n = 137

Mena et al, 2004. Acta Histopathologica 108:524-530.

Histopathological changes after human acute ischemic stroke

Histopathological changes	n (%)	Time
Astrogliosis	114 (83)	2 days-53 years
Neutrophils	31 (23)	1–37 days
Mononuclear inflammatory cells	61 (45)	3 days–53 years
Macrophages	103 (75)	3 days–53 years

n = 137

Mena et al, 2004. Acta Histopathologica 108:524-530.

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Modeling post-stroke dementia in a mouse

- Hypothesis: stroke ->

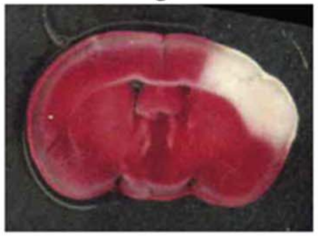
  chronic inflammation ->

  neurodegeneration

  / dementia

Kristian Doyle

- Needed a mouse model that has no immediate cognitive impairment



Doyle et al, 2012 J Neurosci methods.

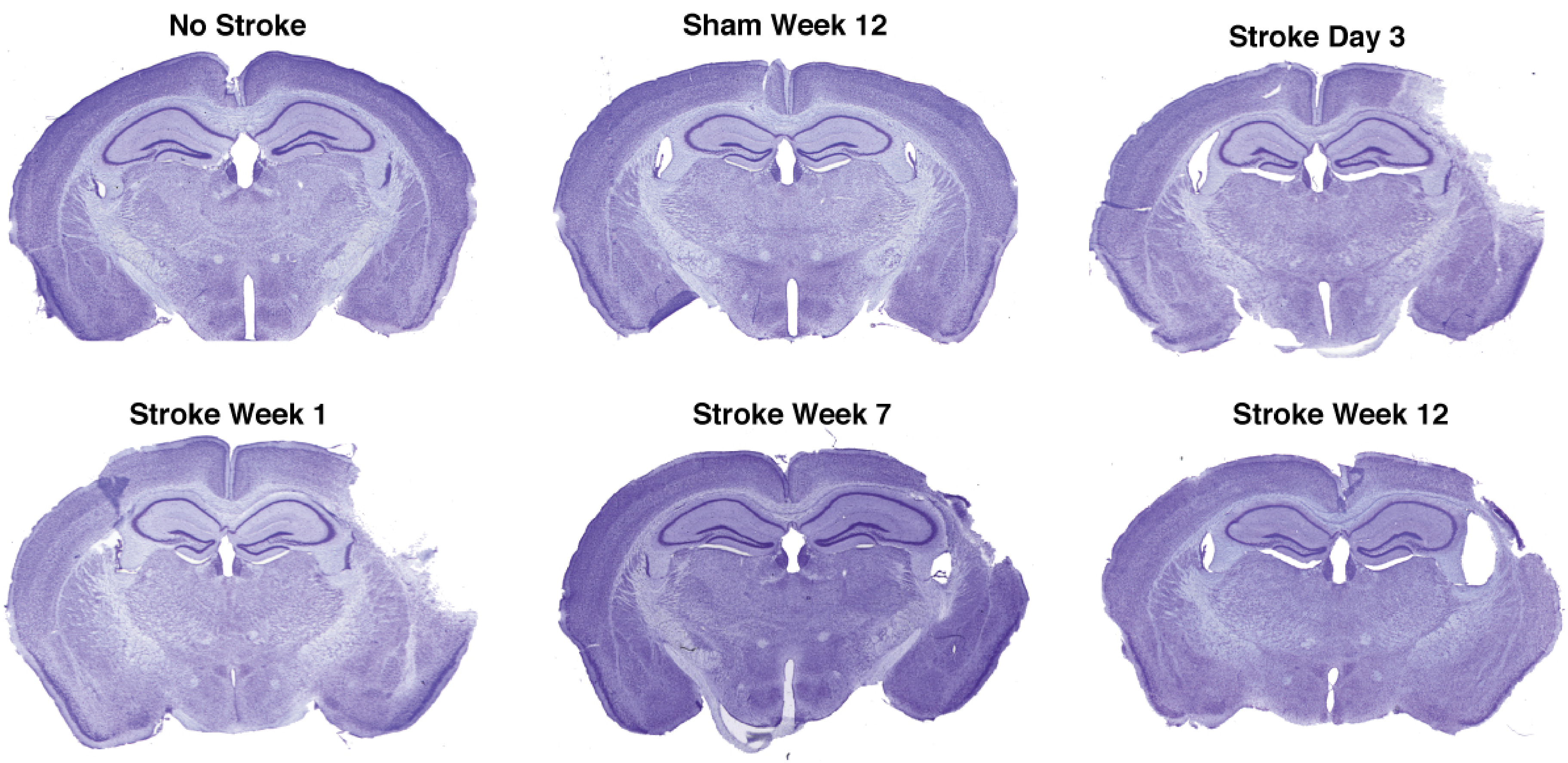
Hypothesis: stroke ->

chronic inflammation ->

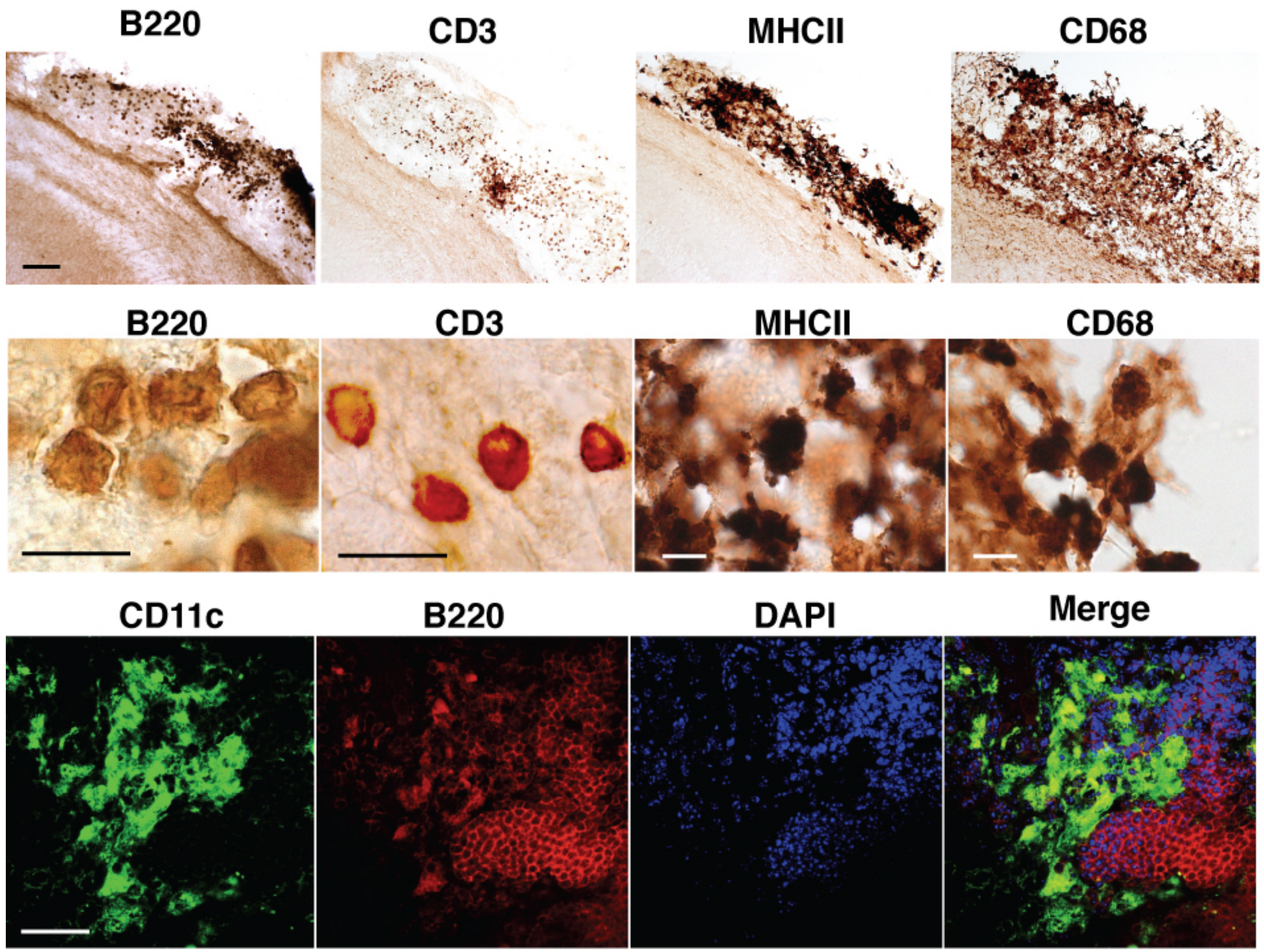
neurodegeneration

/ dementia

Cresyl violet shows no gross hippocampal cell loss



The stroke core contains immune cells 7 weeks after stroke



Doyle et al, J Neuroscience 2015

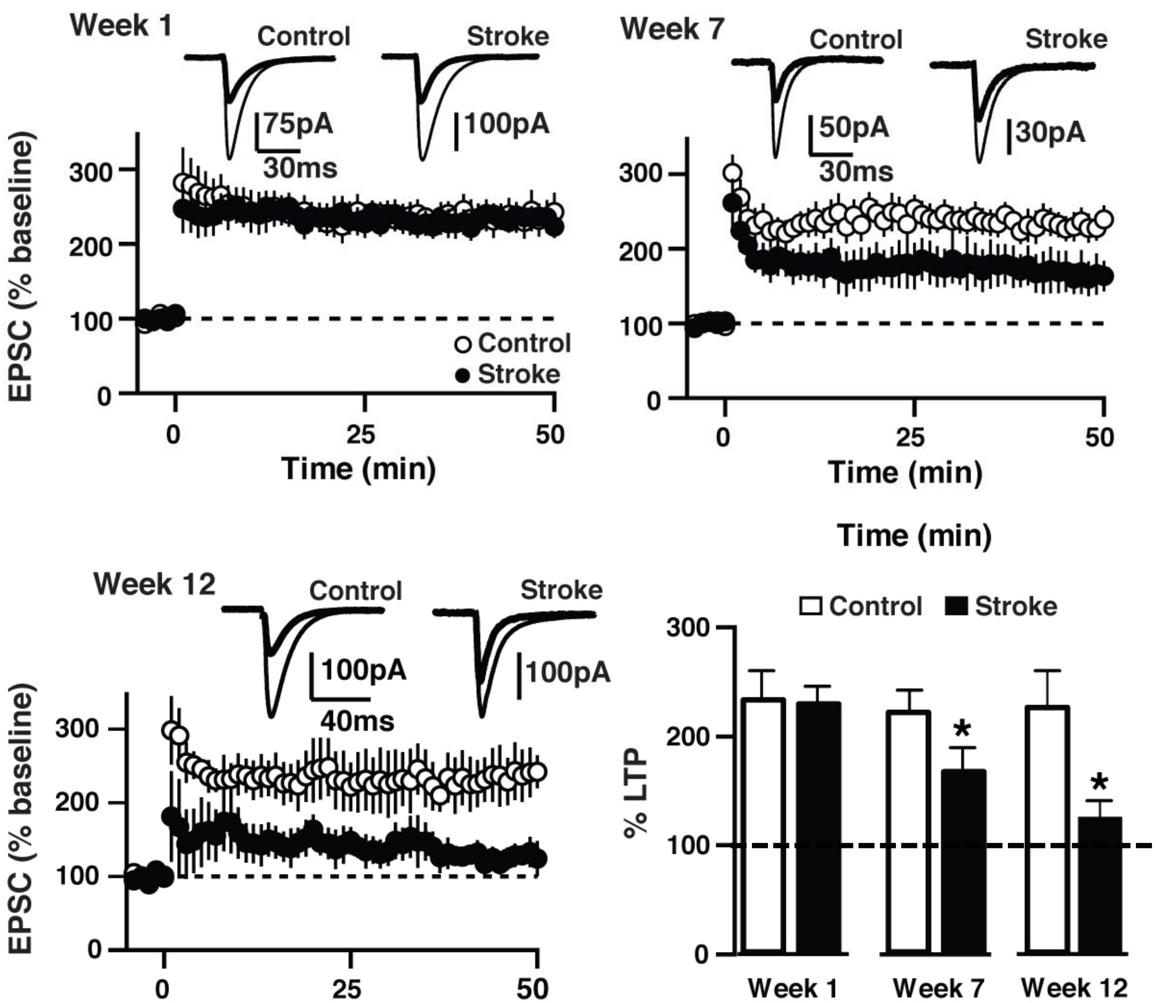
Hypothesis: stroke ->

chronic inflammation ->

neurodegeneration

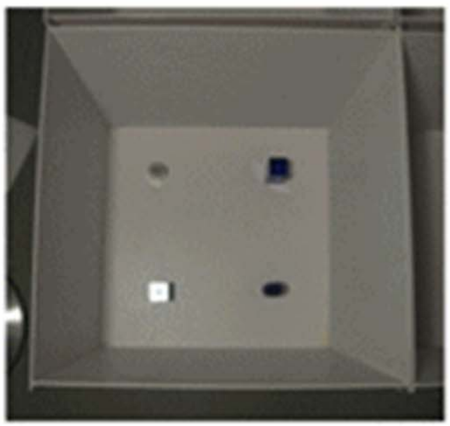
/ dementia

Hippocampal LTP is normal 1 week after stroke and then progressively worsens

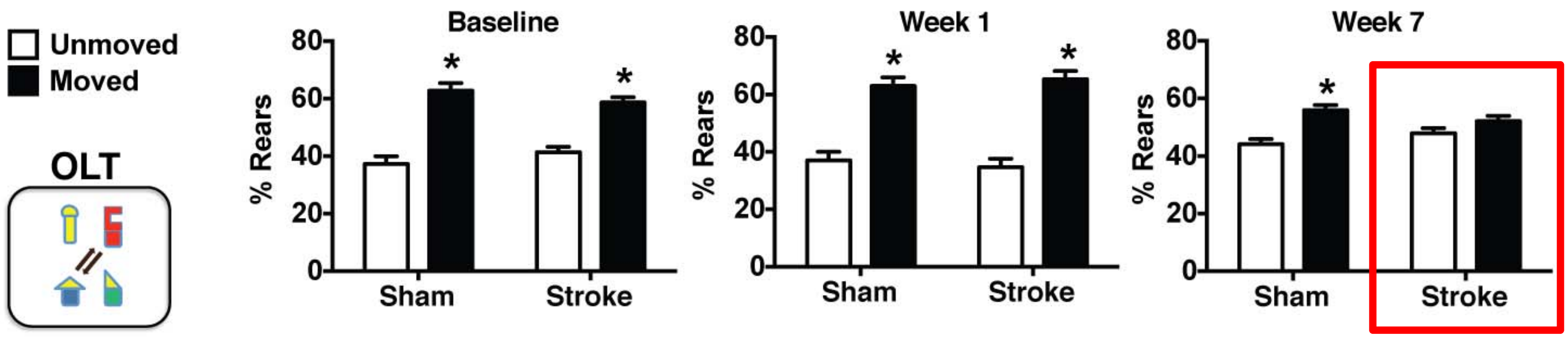


Doyle et al, J Neuroscience 2015

Object Location Task



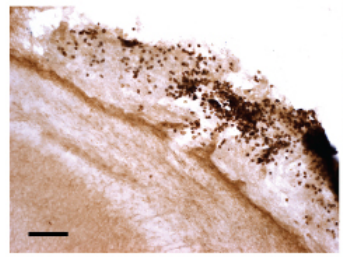
Deficits appear between weeks 1 and 7 in the Object Location Task



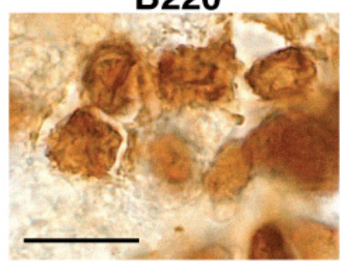
Doyle et al, J Neuroscience 2015

Are B lymphocytes necessary for the cognitive deficit?

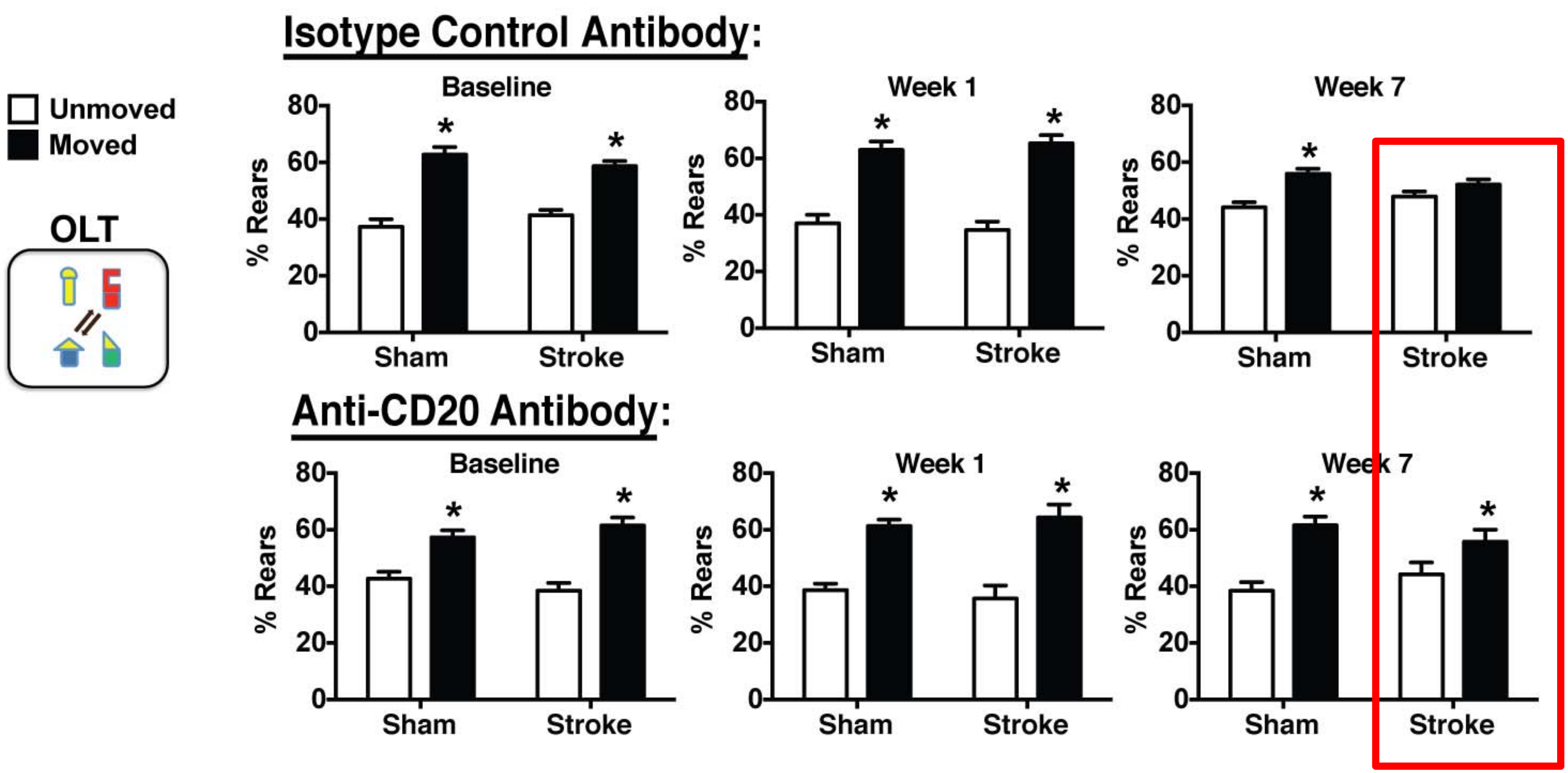
B220



B220



Anti-CD20-treated mice do not develop cognitive deficits



Doyle et al, J Neuroscience 2015

Post-Stroke Dementia Mouse Model

- Normal mice can develop delayed cognitive impairment after stroke
- This is associated with prolonged inflammation in the stroke core that includes B lymphocytes
- In the absence of B cells mice do not develop delayed cognitive impairment after stroke

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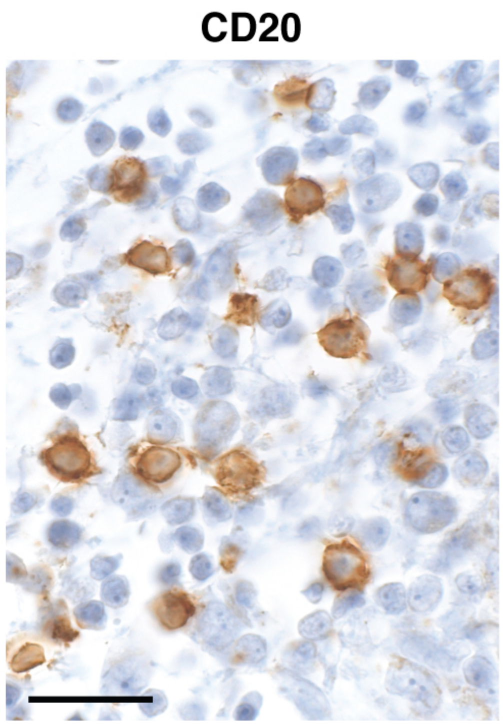
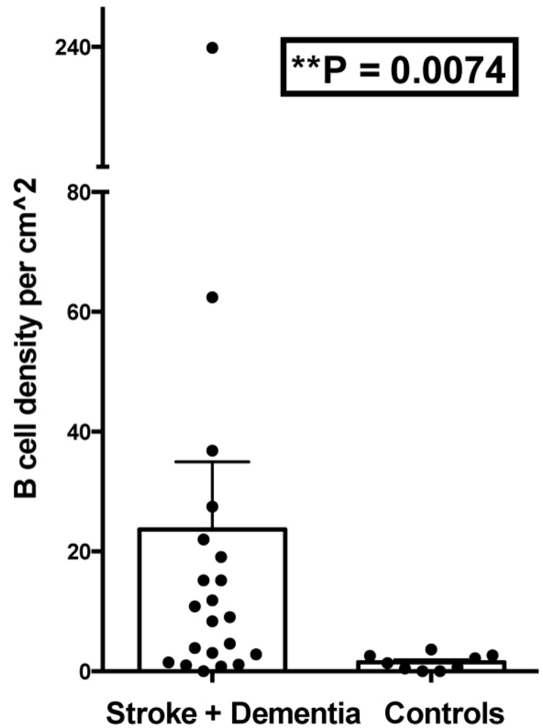
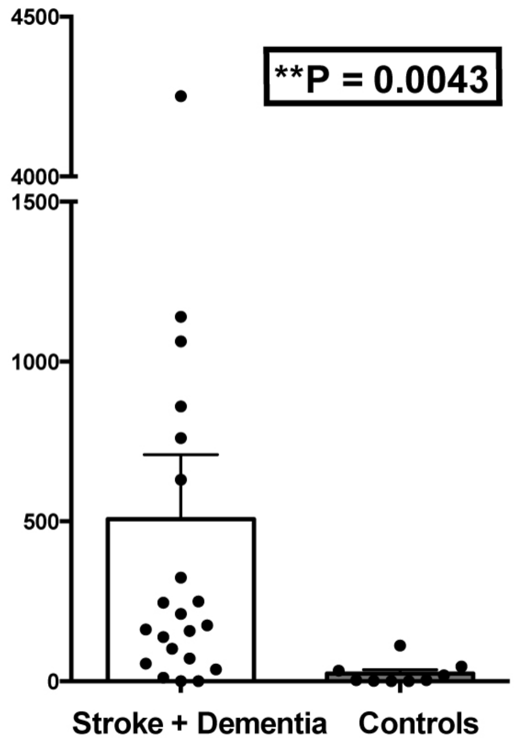
There are more B lymphocytes in the stroke core in people with stroke and dementia than in the controls





Doyle et al, J Neuroscience 2015
(collaboration with Julie Schneider at Rush)

There are also more T lymphocytes in the stroke core in people with stroke and dementia than in the controls



Doyle et al, J Neuroscience 2015
(collaboration with Julie Schneider at Rush)

What about the relationship between post-stroke cognitive trajectory and autoantibodies? (CASIS Cohort)

- 58 prospectively enrolled adults with ischemic stroke admitted to Harborview Medical Center from 2005-2009
- 40 subjects with no history of stroke as controls
- Serum autoantibody titers to MBP were determined by ELISA
- Antibody titers >95th percentile of the control group at any timepoint were considered significant.
- MMSE tested at 30, 90, 180 and 365 days after stroke

Shibata et al, 2012; Becker & Buckwalter2016

Serum autoantibodies: High anti-MBP antibody titer was associated with increased risk of MMSE decline

Characteristics (controlled for NIHSS)	OR (95% CI)	*P*
IV tPA use	3.88 (0.62, 24.14)	0.14
Age (per decade)	1.63 (0.59, 4.48)	NS
History of hypertension	4.34 (0.57, 32.95)	0.16
History of hyperlipidemia	1.03 (0.06, 24.14)	NS
Myelin basic protein (MBP) antibody titer >95% controls	9.02 (1.18, 68.90)	0.03

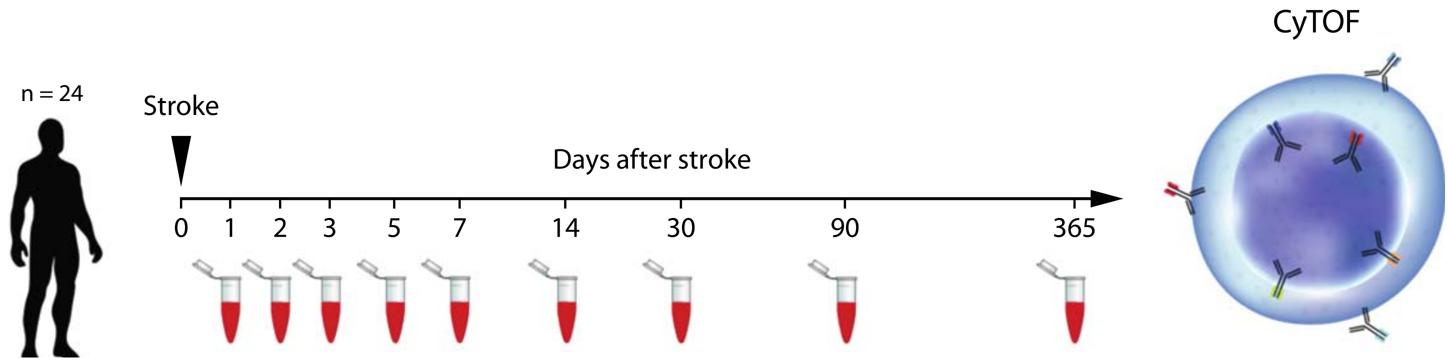
MMSE=mini-mental state exam, OR=odds ratio, CI=confidence interval, IV tPA=intravenous tissue plasminogen activator, NS=$P \geq 0.20$

Becker et al, J Neuroimmunol, 2016

StrokeCog

- A 5 year prospective clinical cohort study through the Stanford Stroke Recovery Program, first funded by the Wu Tsai Neurosciences Institute, now by an AHA/Allen Brain Health Initiative
- Goal 200 patients 6-12 months after ischemic stroke
- Yearly: level one cognitive battery, depression and fatigue scales, diet questionnaire, blood for CyTOF, plasma and serum
- Sister studies for additional immune biomarkers: StrokeCog LP (N=50) and StrokeCog PET (N=10)

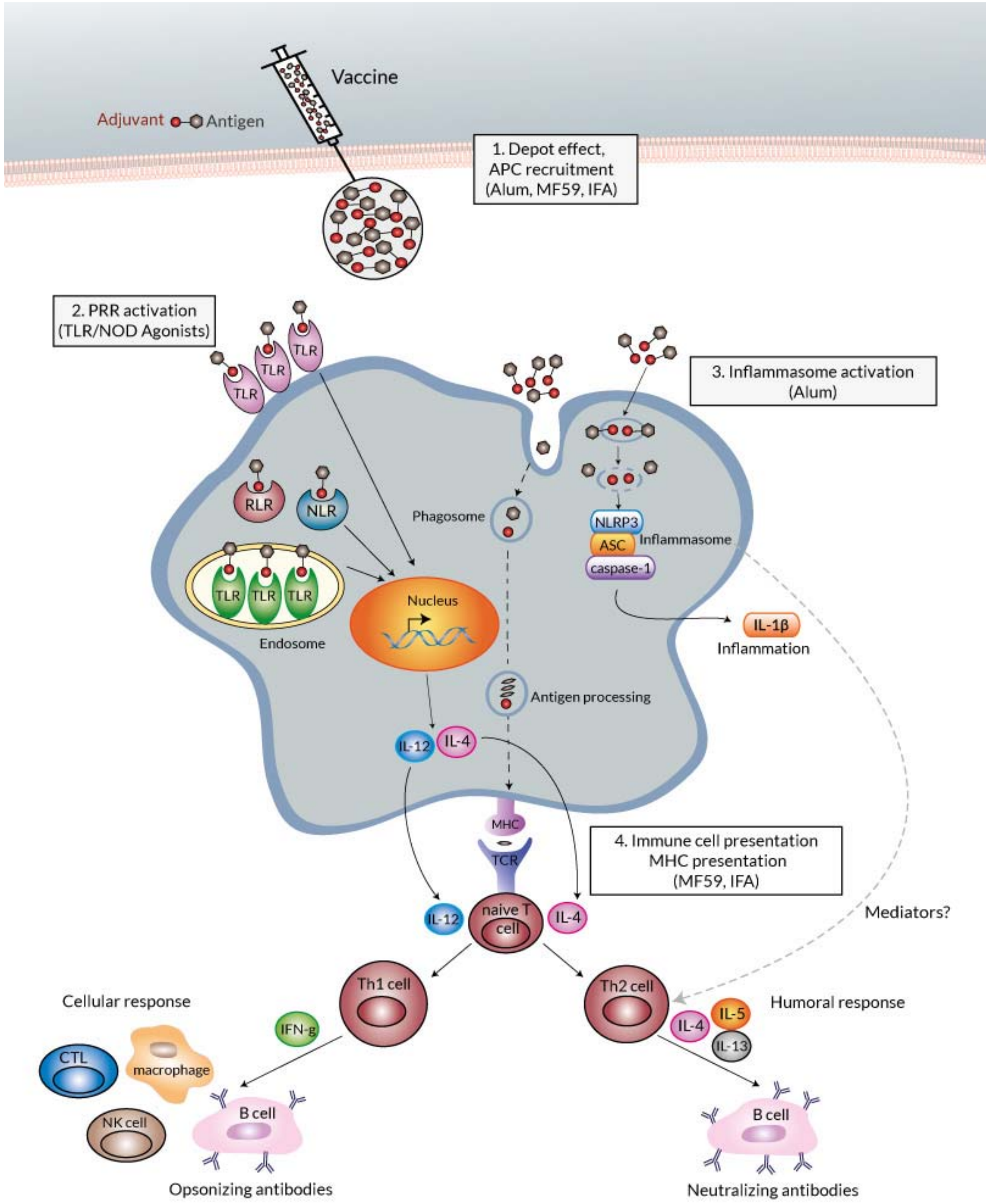
CyTOF: Can we detect peripheral immune signatures after stroke?



(38 antibodies, 27 cell surface and 11 intracellular markers)

Tsai et al., Brain 2019

Immunization & role of adjuvants

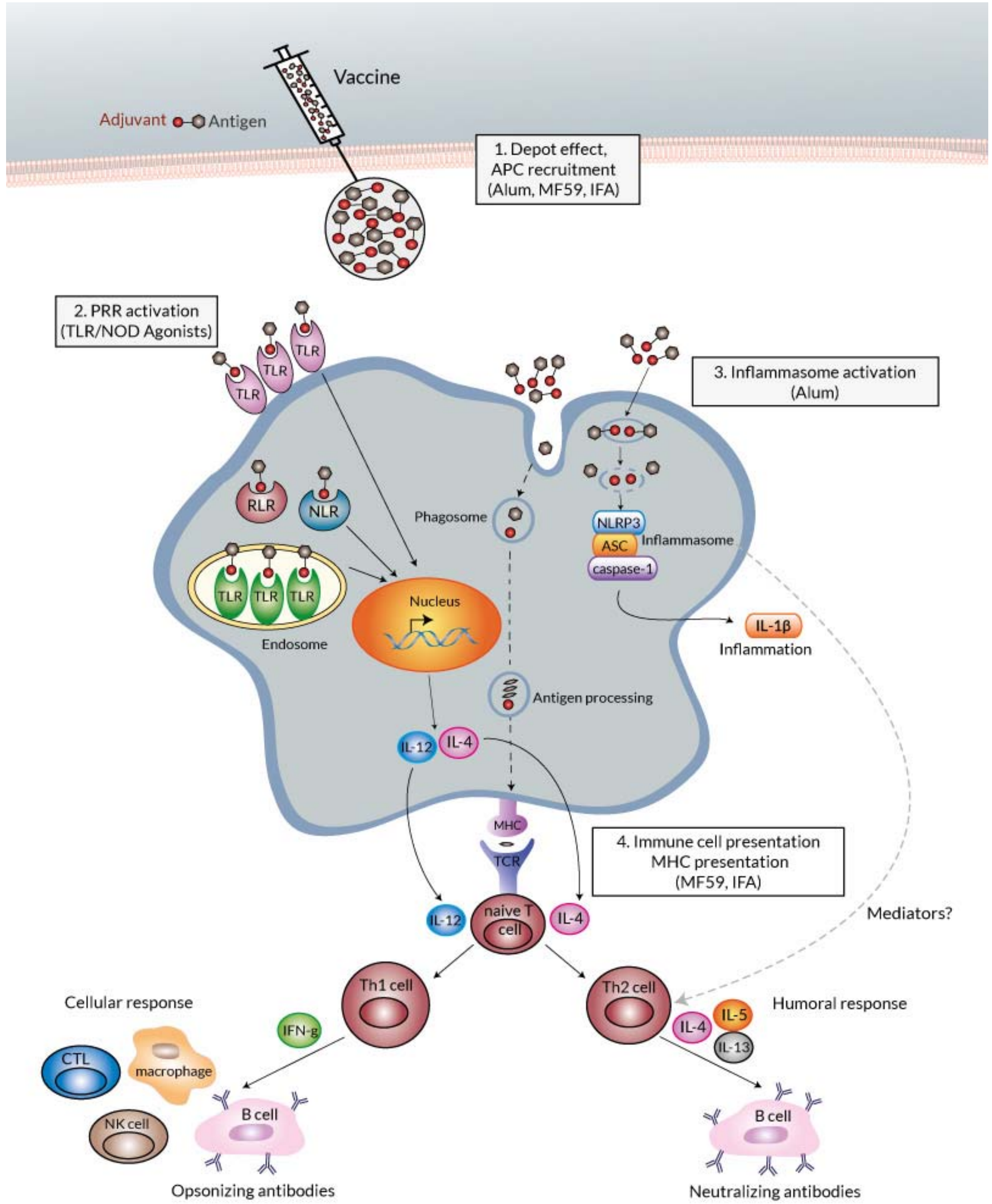


https://www.invivogen.com/review-vaccine-adjuvants

Immunization & role of adjuvants

Does the peripheral immune response act as an adjuvant after stroke?

And can we even detect an effect of stroke on the peripheral immune response?



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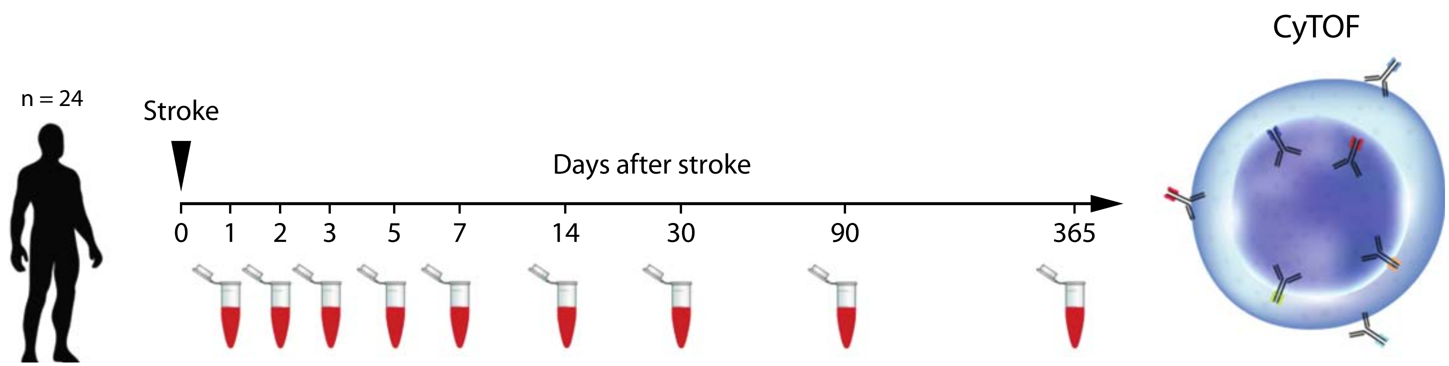

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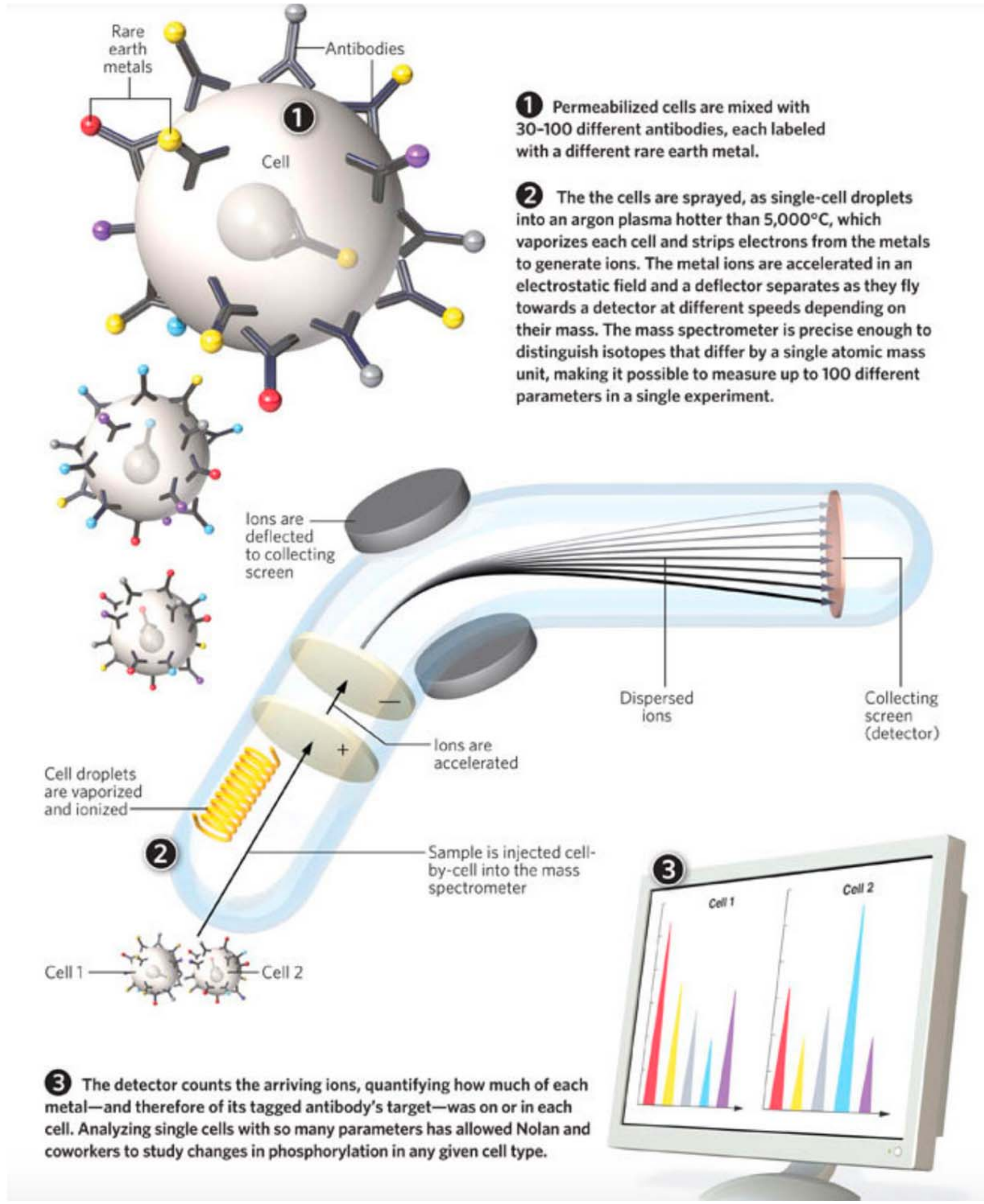
Tsai et al., Brain 2019

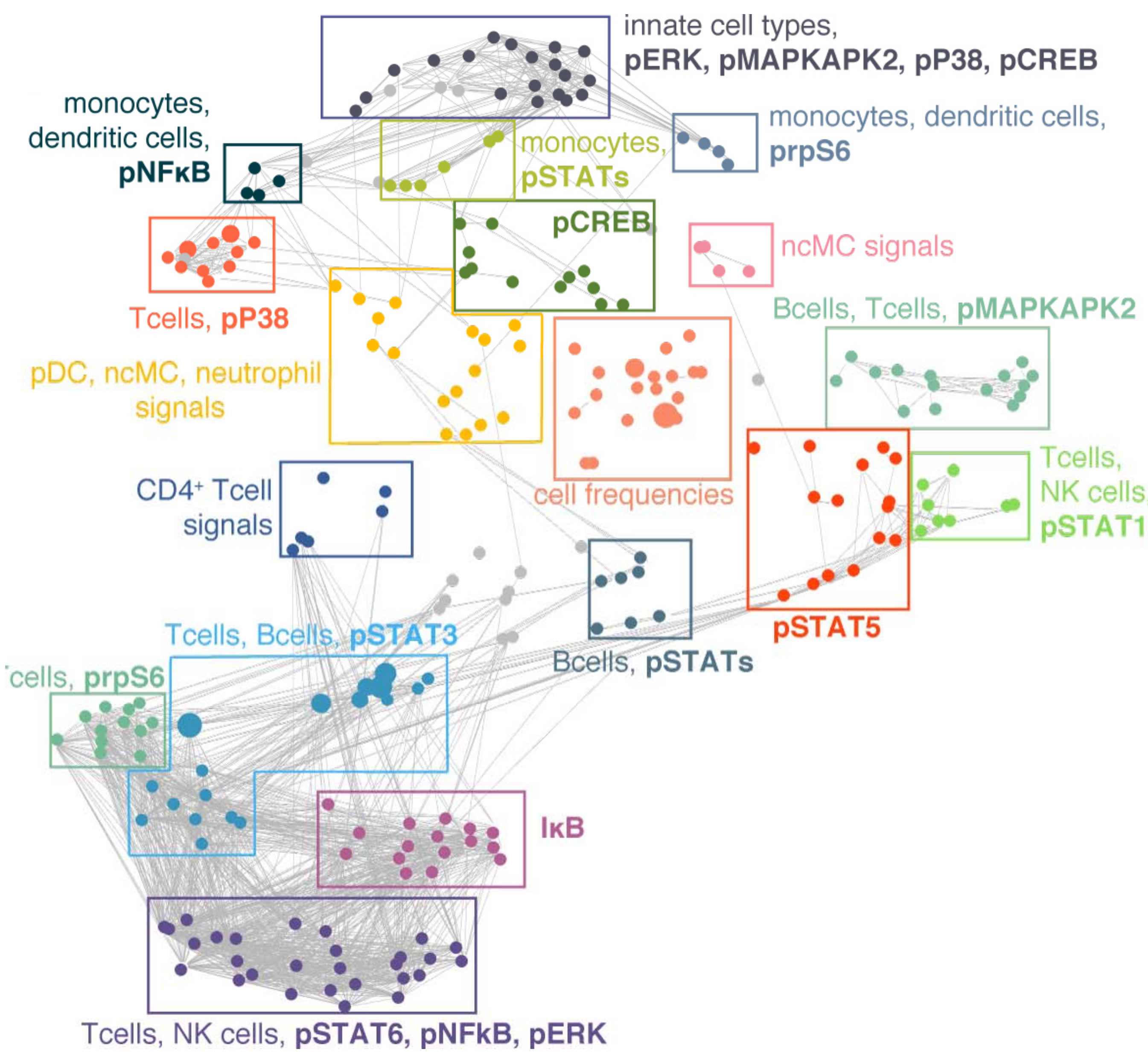
Mass Cytometry

Brice Gaudilliere

Nima Aghaeepour



Grant, 2011, The Scientist

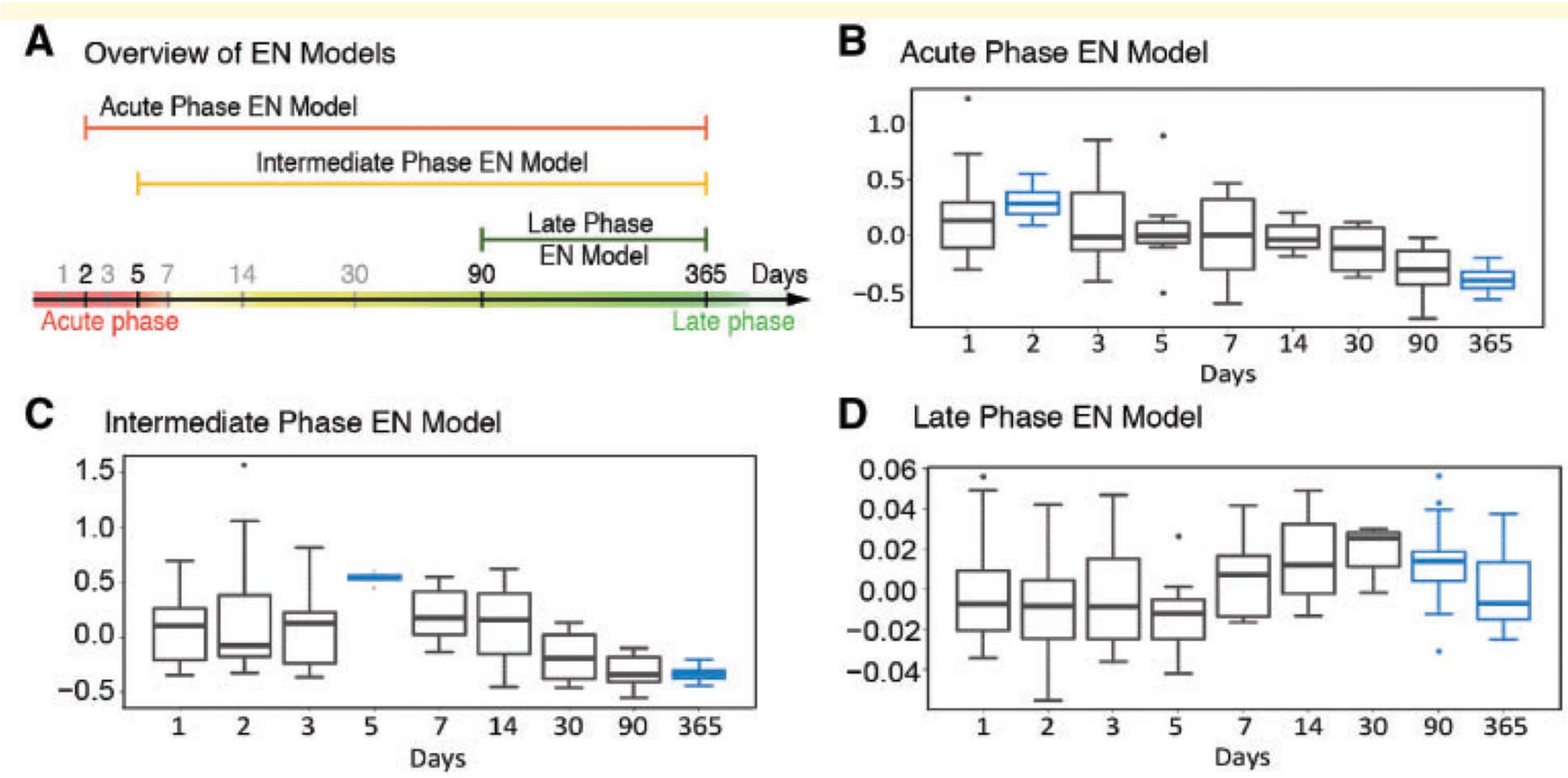


Tsai et al., Brain 2019

Subject	Days										Stroke Size (cc)
	0.5	1	2	3	5	7	14	30	90	365	
1	●	●	●	●	●		●	●	●	●	6.5
2		●	●	●	●	●	●			●	37.6
3		●	●	●	●	●	●	●	●	●	1
4		●	●	●	●	●					102
5		●	●	●		●					1
6		●	●	●	●					●	10
7		●	●						●	●	1
8		●	●	●	●	●			●	●	1
9	●	●	●					●	●	●	1
10	●	●	●								1.2
11		●	●	●	●	●					14.5
12		●	●					●		●	1
13		●	●			●			●	●	9
14		●	●	●							18.5
15	●										20
16		●	●	●					●	●	47.3
17		●	●	●					●	●	59.6
18		●	●	●					●	●	4.0
19		●	●	●					●	●	18.1
20		●	●	●					●	●	80.2
21		●	●	●					●	●	12.3
22		●		●						●	16.7
23		●	●	●						●	101.1
24		●	●	●					●	●	1
25		●	●	●					●	●	1

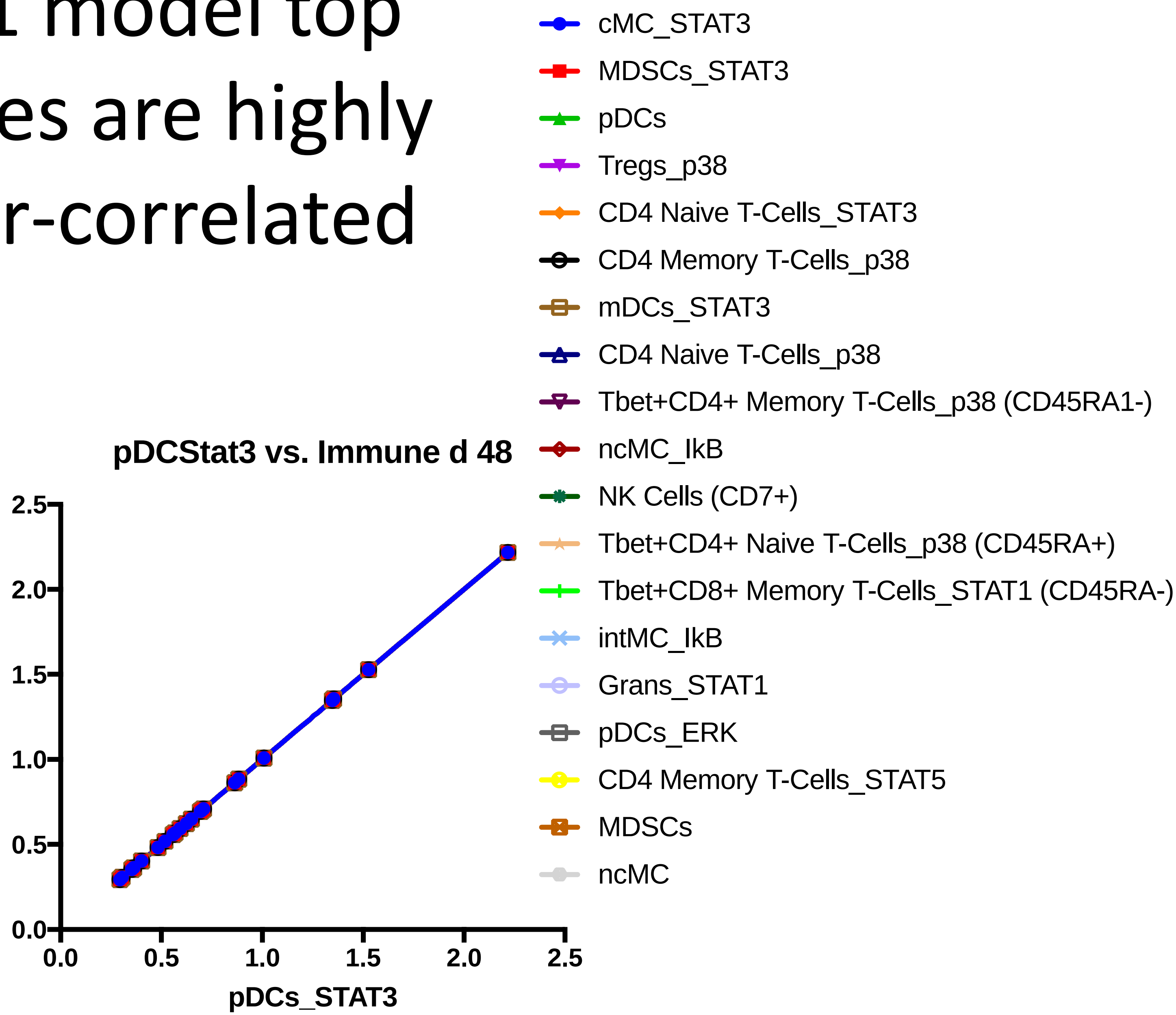
Tsai et al., Brain 2019

Elastic Net Models

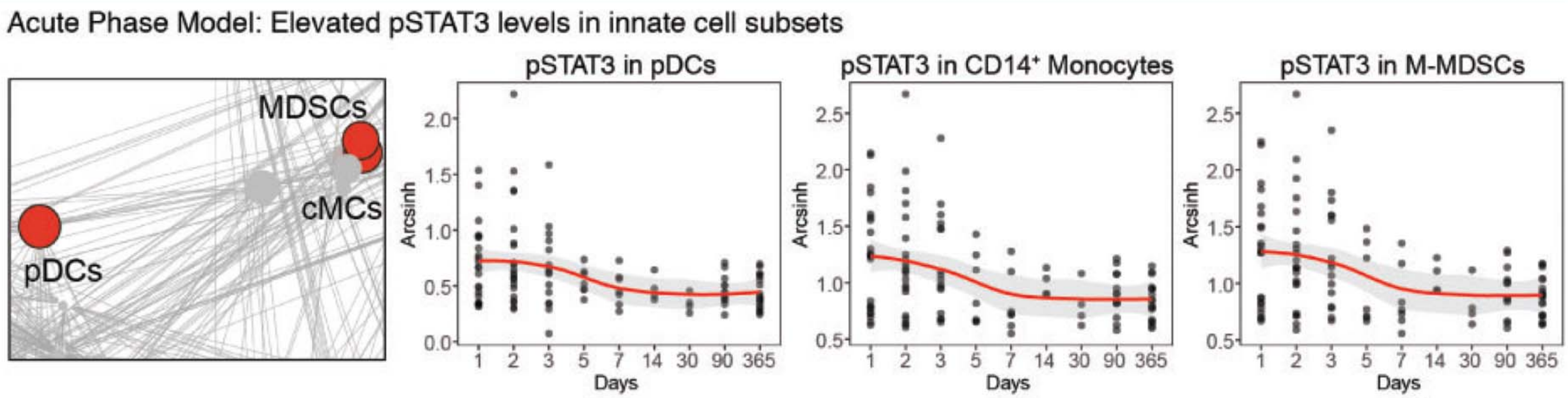


Tsai et al., Brain 2019

EN1 model top values are highly inter-correlated

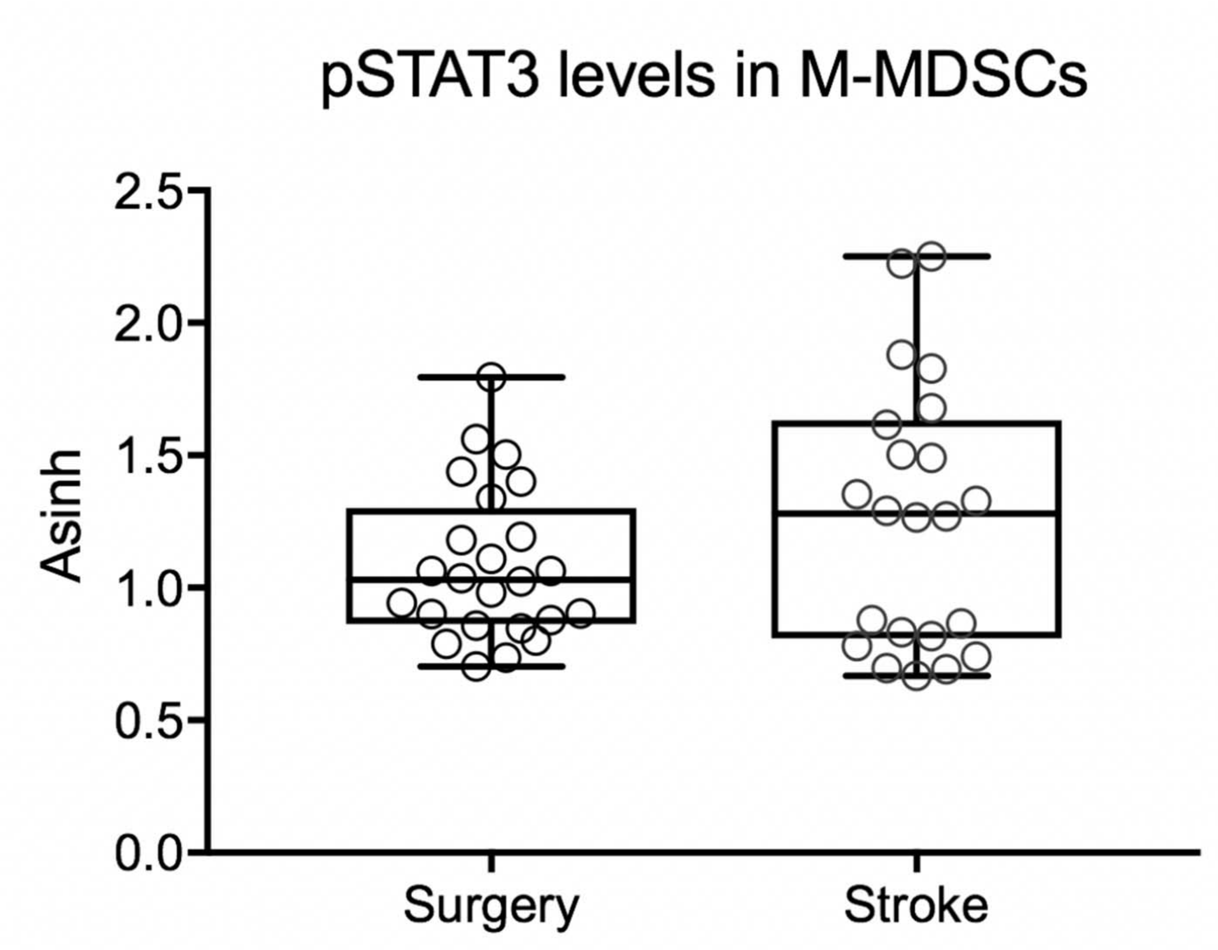


Subject-to-subject variability



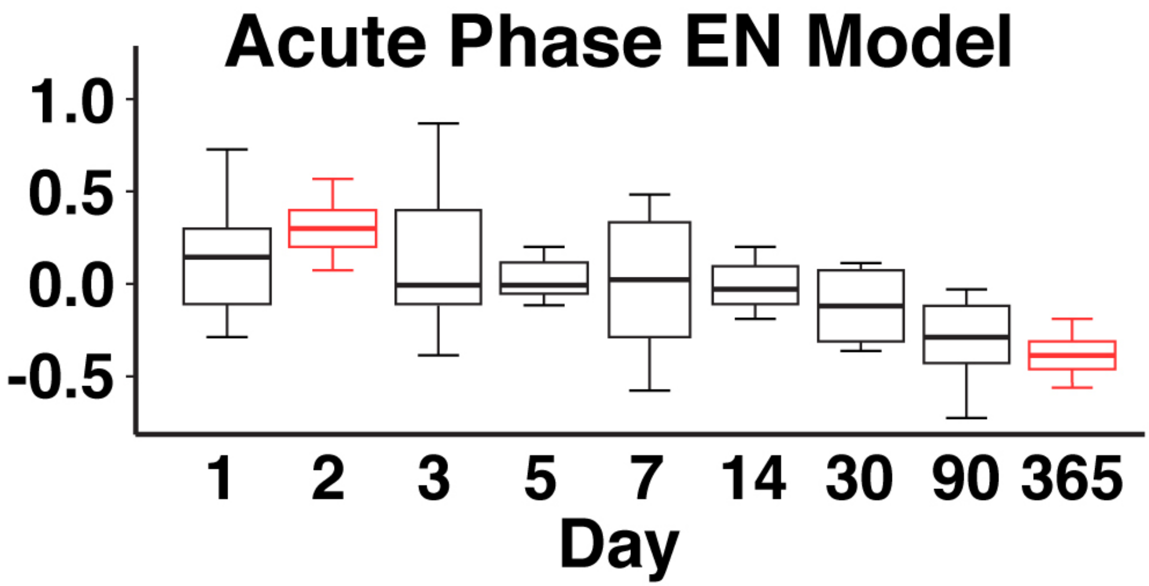
Tsai et al., Brain 2019

How does the d2 inflammatory response compare to the post-surgical immune response?



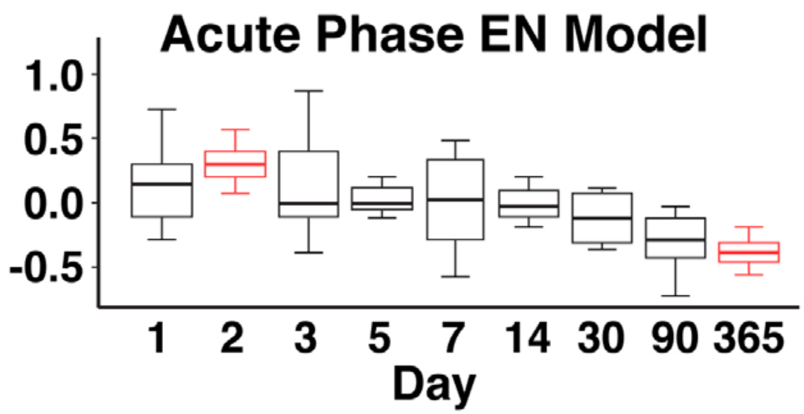
Tsai et al., Brain 2019

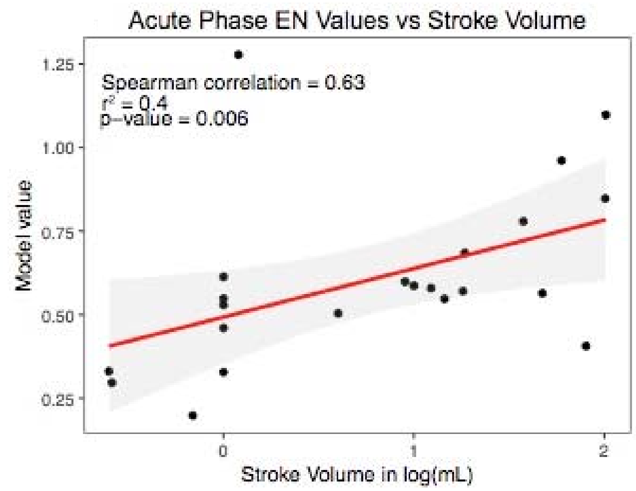
Acute phase EN model



Tsai et al., Brain 2019

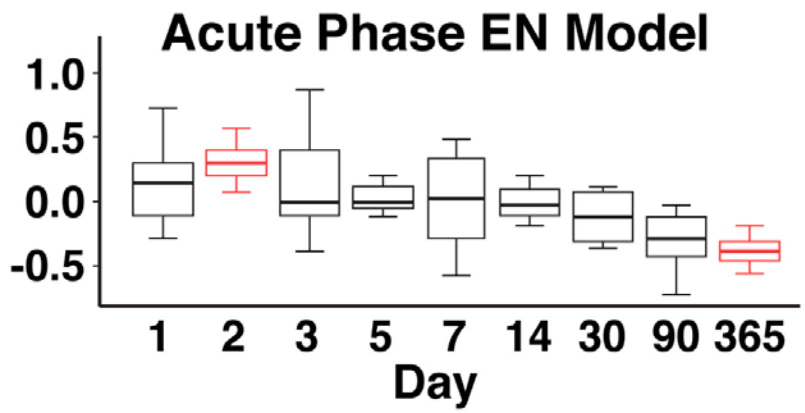
Acute phase EN model predicts stroke size

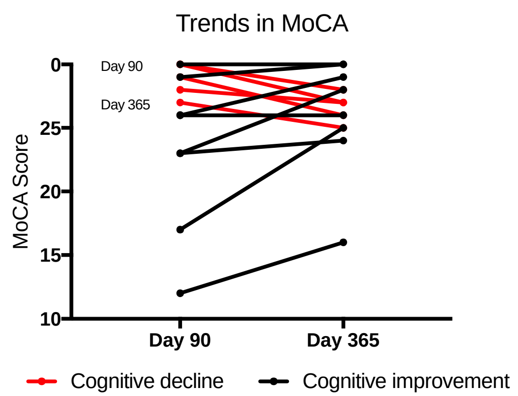




Tsai et al., Brain 2019

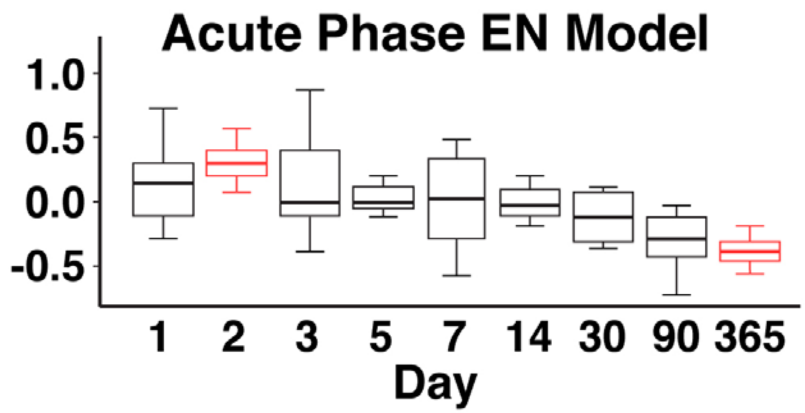
Acute phase EN model predicts change in cognition

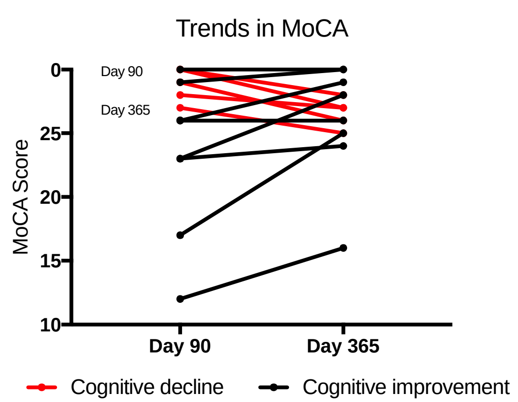


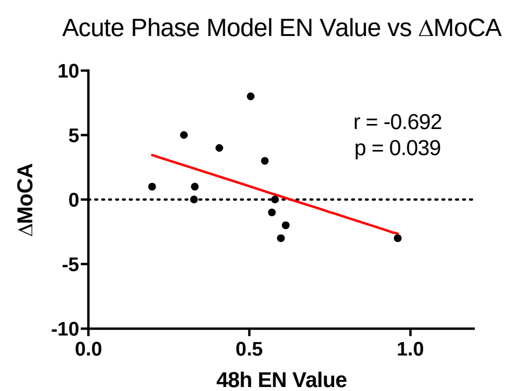


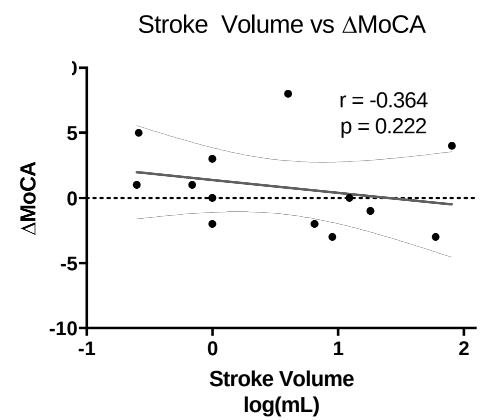
Tsai et al., Brain 2019

Acute phase EN model predicts change in cognition





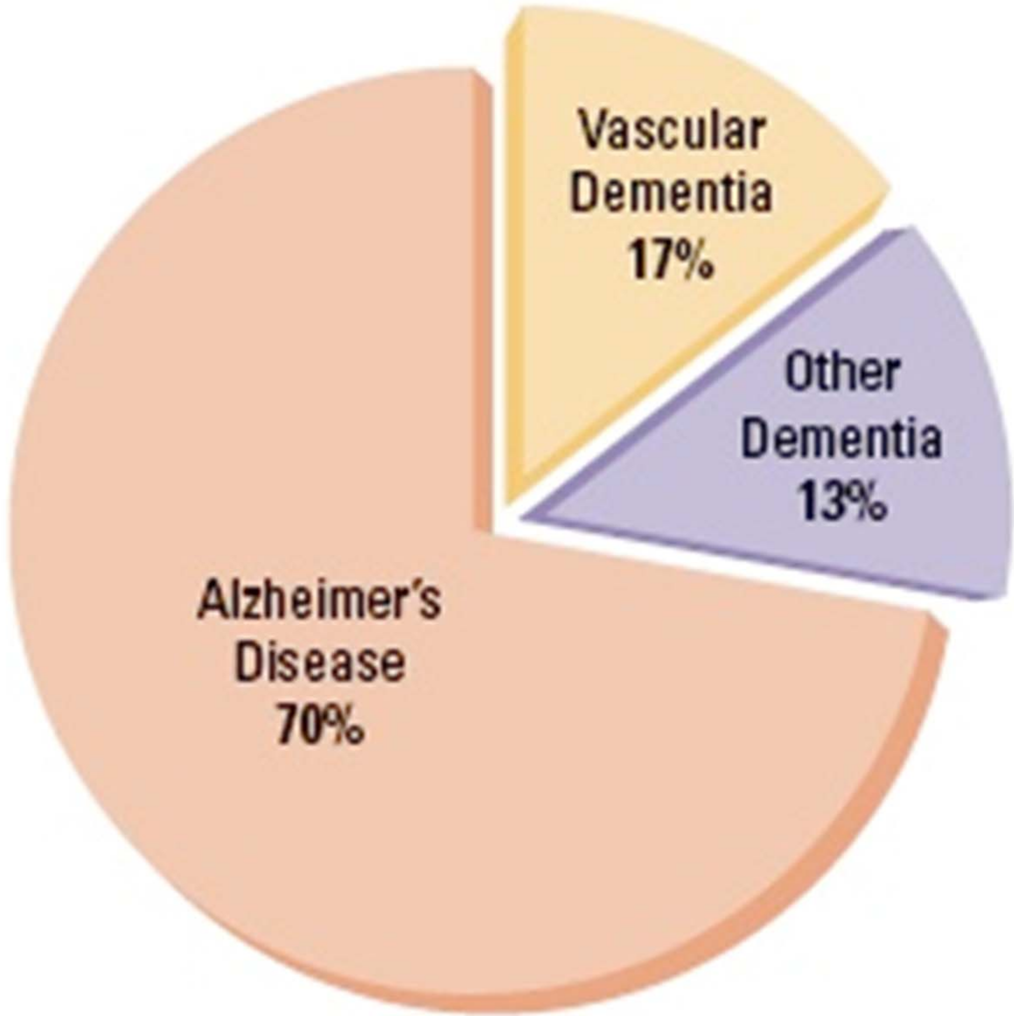




Tsai et al., Brain 2019

Talk Outline

- Definitions: Post-stroke dementia vs. vascular dementia
- Hypothesis: Is post-stroke dementia an immune-mediated neurodegenerative disease?
- Mechanism: Mouse model of post-stroke dementia
- In progress: Testing the model in humans
- Relationship to other dementias

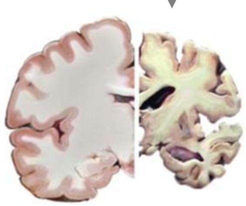


Source: Plassman, BL; Langa, KM; Fisher, GG; Heeringa, SG; Weir, DR; Ofstedal, MB, et al. "Prevalence of Dementia in the United States: The Aging Demographics, and Memory Study. *Neuroepidemiology* 2007; 29:125-132.[31]

Infarcts and vascular pathology in old brains

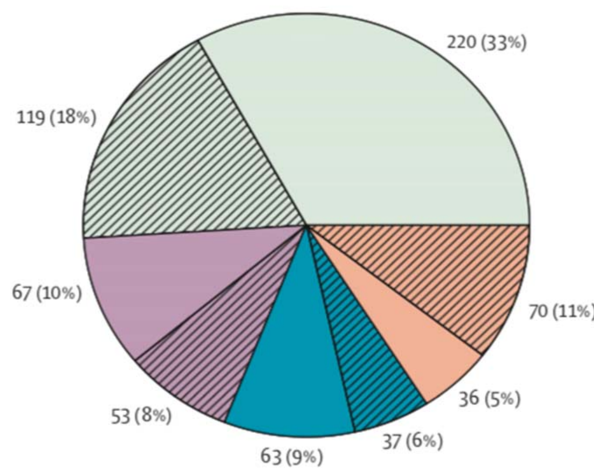
Religious Orders Study
Rush Memory and Aging Project
(>65 age longitudinal study)

1143 brains
age 84-93



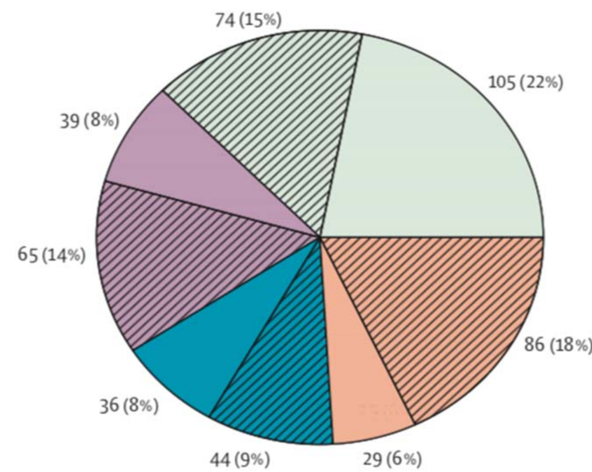
Pathological analysis

No dementia



43% with infarcts
67% with vascular pathology

Alzheimer's disease



56% with infarcts
78% with vascular pathology

- No significant cerebral vessel pathology
- Atherosclerosis only
- Arteriolosclerosis only
- Atherosclerosis and arteriolosclerosis
- Hatched lines indicate those with infarcts

Schneider, Bennett 2016

Buckwalter lab:
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Kristy Zera
Judy Zhu
Lisa Quach
Kendra Lechtenberg
Tawaun Lucas
Evan Brahms

Stanford Stroke Recovery Program:
Maarten Lansberg
Elizabeth Osborn
Michael Sharp
Emily Huang
Alay Parikh
Kara Flavin

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NIA/Stanford ADRC
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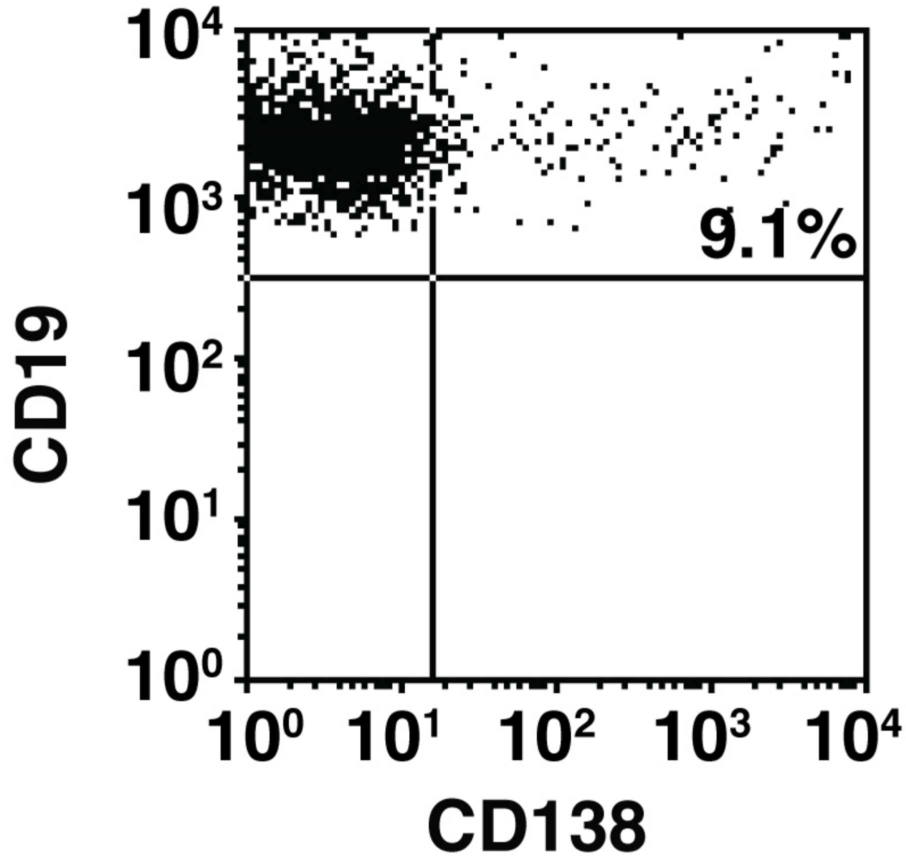
Greg Zaharchuk
Audrey Fan
Tony Wyss-Coray
Hanadie Yousef
Frank Longo
Vivian Nguyen

Rush University ADRC
Julie Schneider

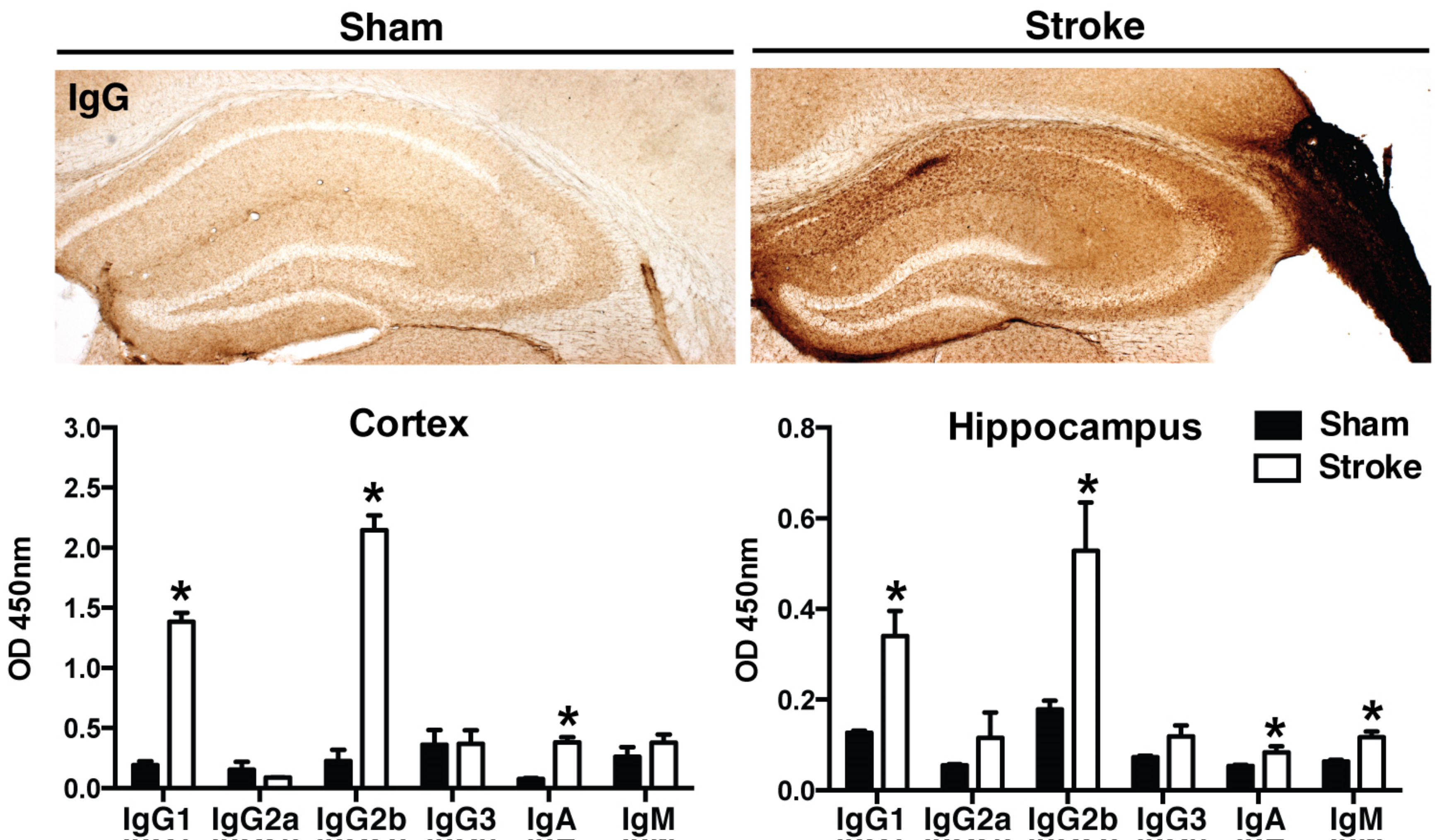
University of Washington
Kyra Becker

Plasma cells are also present in the stroke core



Doyle et al, J Neuroscience 2015

At 7 weeks after stroke, IgG is present in the tissue surrounding the stroke lesion



Doyle et al, J Neuroscience 2015

Is it valid to use d365 as baseline?

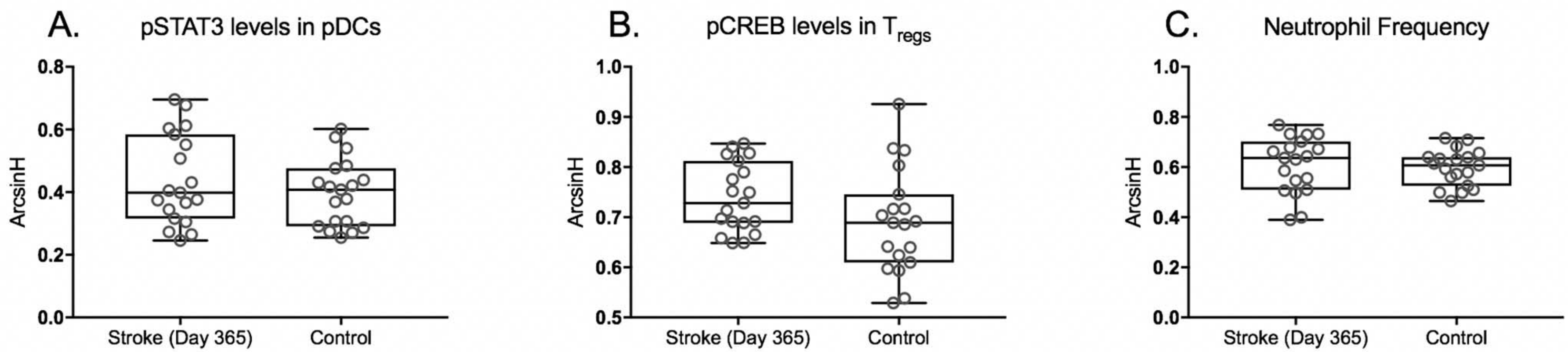


Figure S2. EN components in patients one year after stroke compared to a control cohort.

Tsai et al., Brain 2019

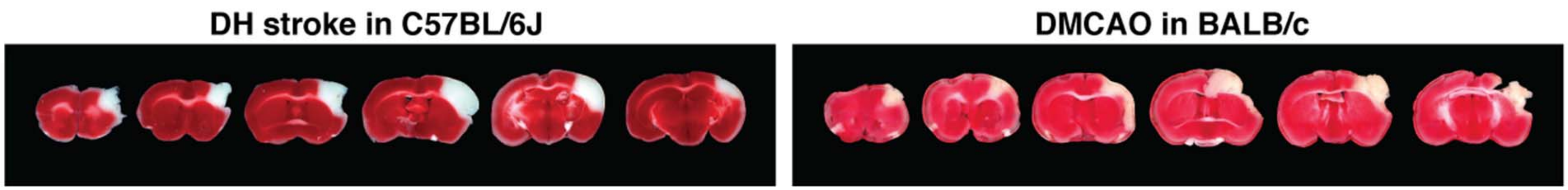
	Entire Cohort N=58	MMSE decrease by ≥2		
		No N=48	Yes N=10	*P*
Patient Characteristics				
Age (years)	52 (42, 63)	49 (41, 62)	64 (54, 70)	0.02
Sex (female)	21/58 (36%)	18/48 (38%)	3/10 (30%)	NS
Past medical history				
Hypertension	25/58 (43%)	18/48 (38%)	7/10 (70%)	0.06
Hyperlipidemia	39/58 (67%)	30/48 (62%)	9/10 (90%)	0.09
Coronary heart disease	12/48 (25%)	9/48 (19%)	3/10 (30%)	NS
Atrial fibrillation	8/56 (14%)	7/48 (15%)	1/10 (10%)	NS
Diabetes	13/58 (22%)	10/48 (21%)	3/10 (30%)	NS
Prior stroke	17/58 (29%)	16/48 (33%)	1/10 (10%)	0.14
Stroke Characteristics				
NIHSS score	8 (3, 14)	7 (3, 14)	10 (6, 14)	NS
Infarct volume (cc)	4.6 (0.5, 46.9)	4.1 (0.4, 43.5)	11.1 (1.4, 50.1)	NS
Treatment with IV tPA	14/48 (29%)	9/48 (19%)	5/10 (50%)	0.04

Becker et al, J Neuroimmunol, 2016

	Entire Cohort N=58	MMSE decrease by ≥2		
		No N=48	Yes N=10	*P*
Serum Antibodies				
Brain				
Myelin basic protein >95% control	13/58 (22%)	7/48 (15%)	6/10 (60%)	0.002
Proteolipid protein >95% control	10/58 (17%)	8/48 (17%)	2/10 (20%)	NS
Anti-phospholipid				
Anticardiolipin IgM positive	26/58 (44%)	21/48 (44%)	5/10 (50%)	NS
Anticardiolipin IgG positive	10/58 (17%)	7/48 (15%)	3/10 (30%)	NS
β-2-glycoprotein IgG positive	6/58 (10%)	6/48 (12%)	0/10	NS
Tetanus toxin (TT) >95% control	9/58 (16%)	9/48 (19%)	0/10	0.14

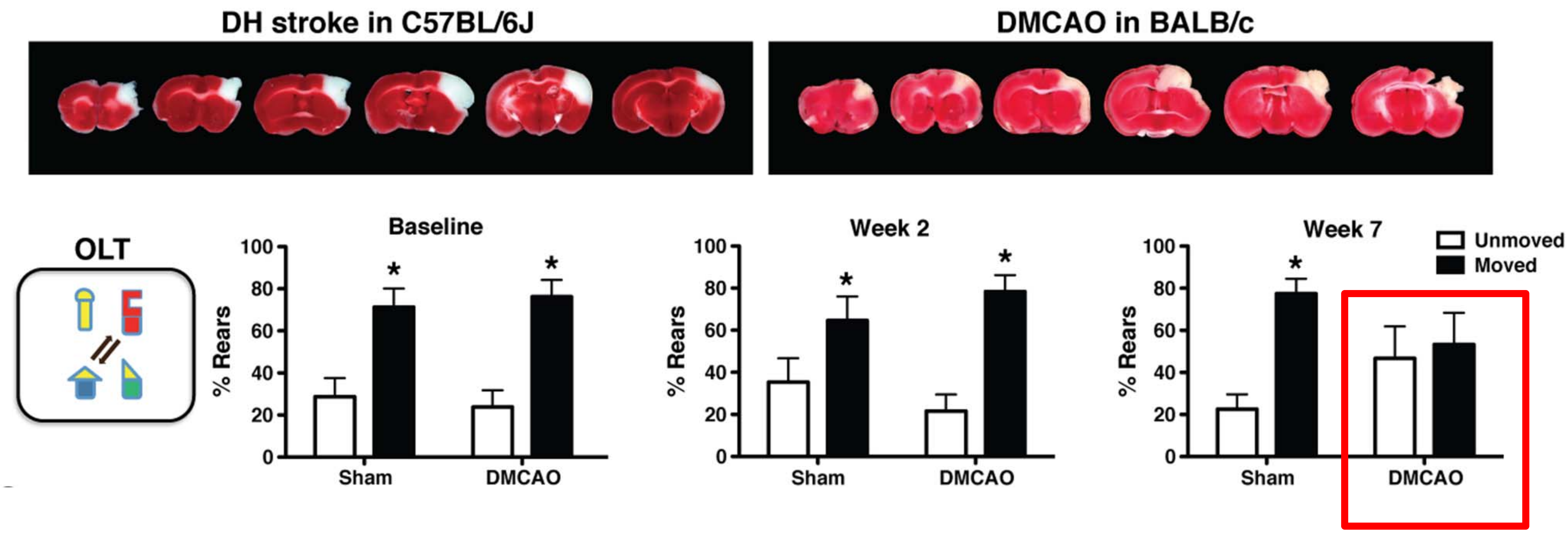
Becker et al, J Neuroimmunol, 2016

Is the delayed cognitive dysfunction due to hypoxia?



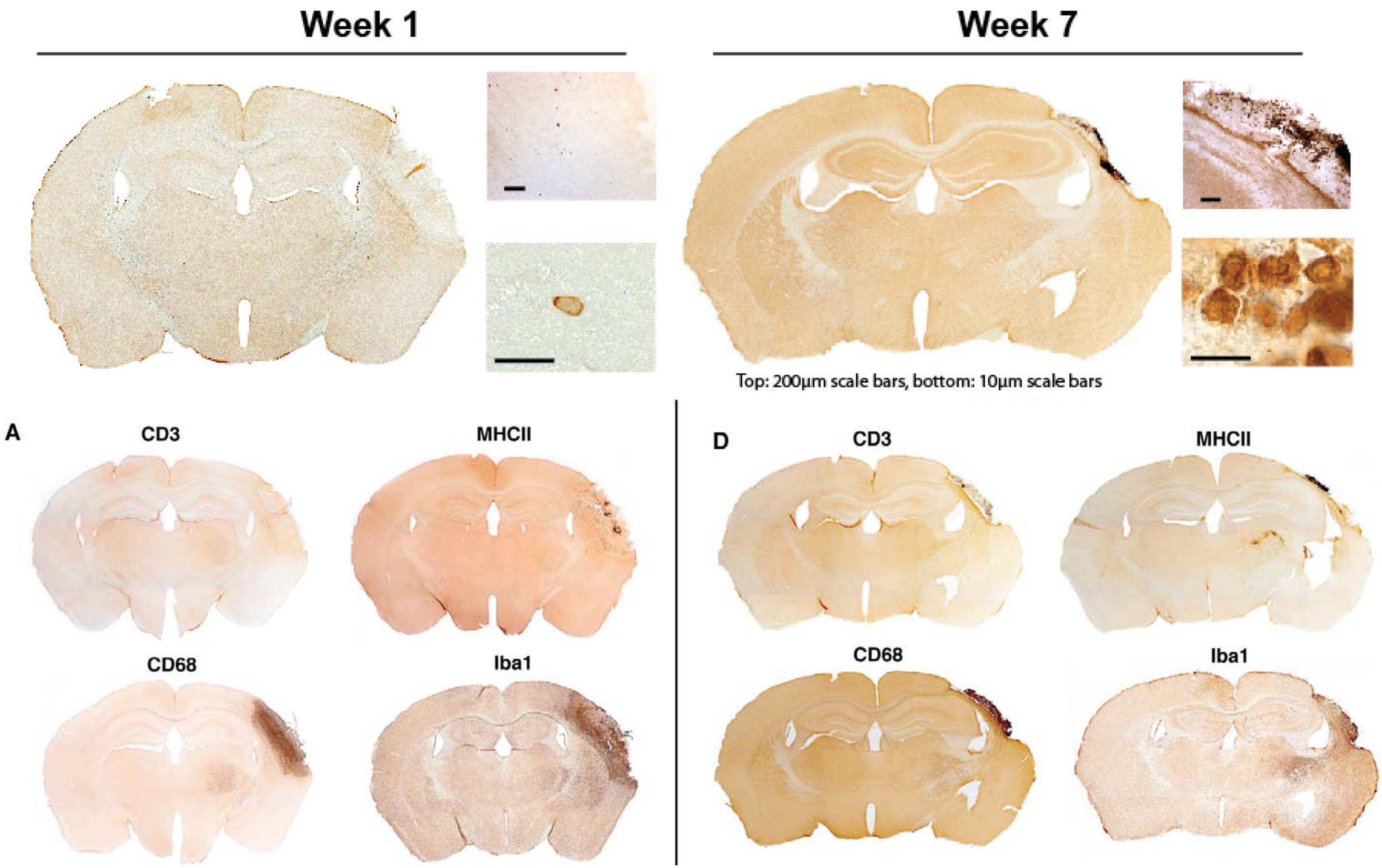
Doyle et al, J Neuroscience 2015

Delayed cognitive dysfunction is not due to hypoxia.



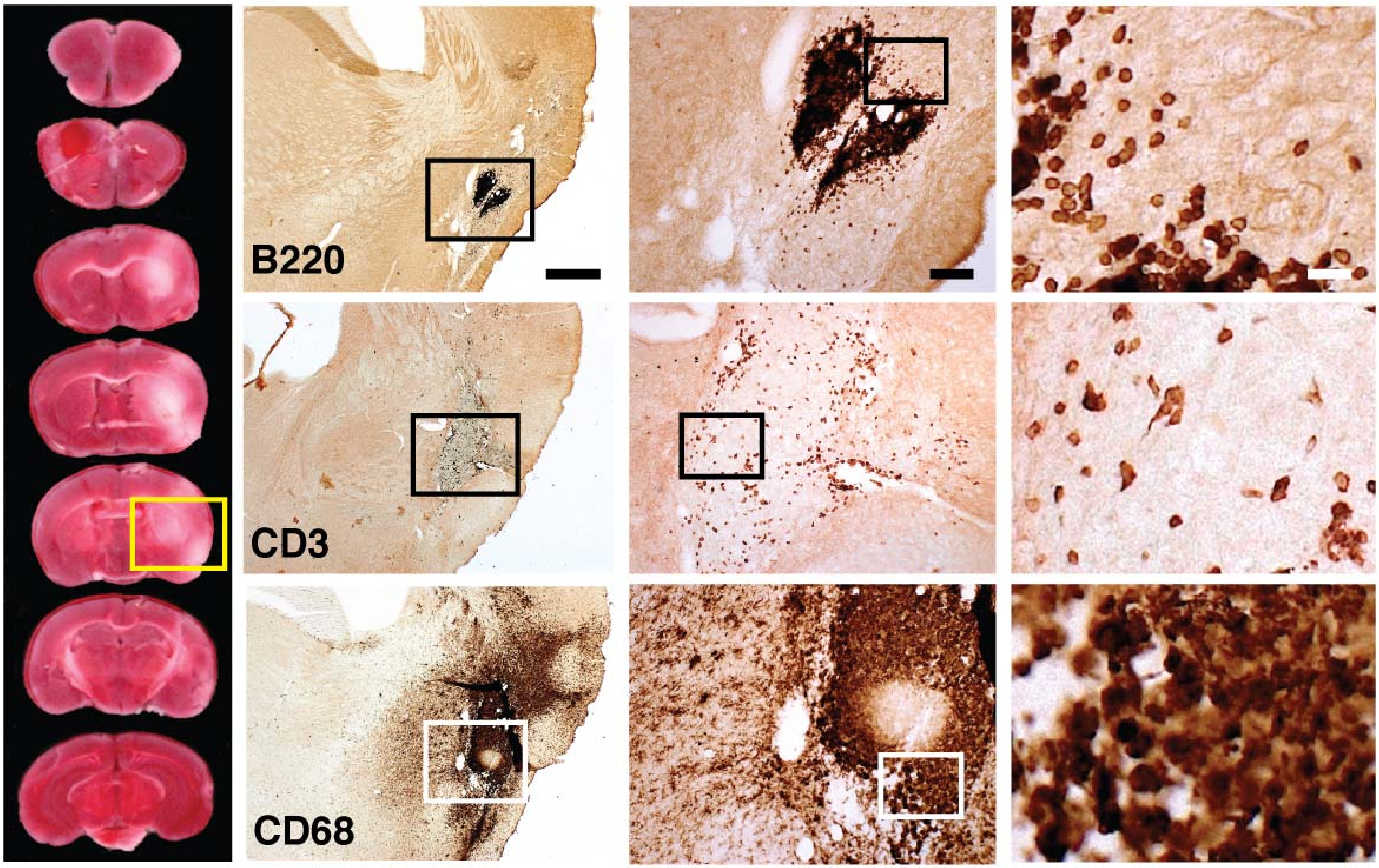
Doyle et al, J Neuroscience 2015

Inflammation after stroke



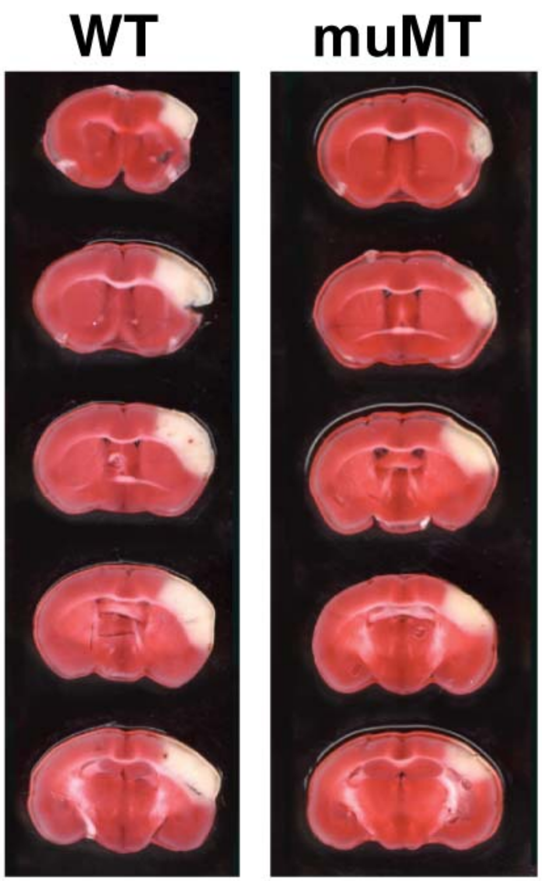
Doyle & Buckwalter, unpublished

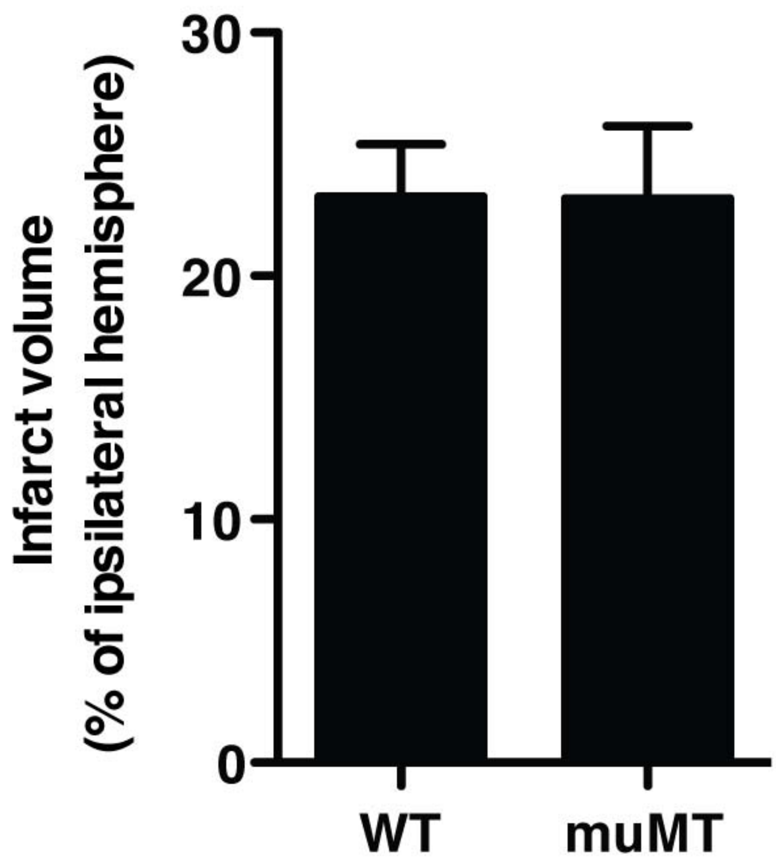
MCAO (Suture Model)



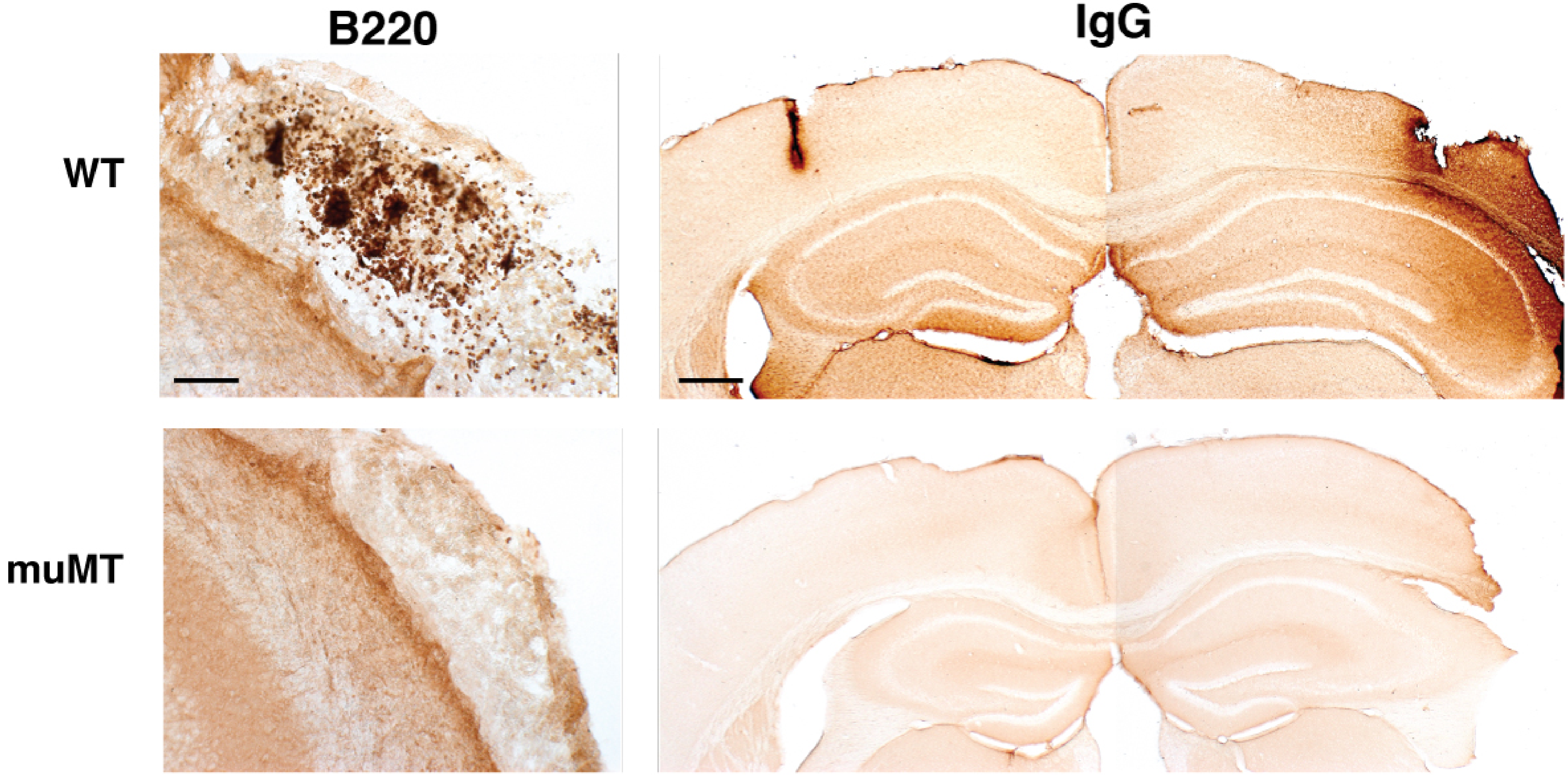
Doyle et al, J Neuroscience 2015

Are B lymphocytes responsible for the cognitive deficit?

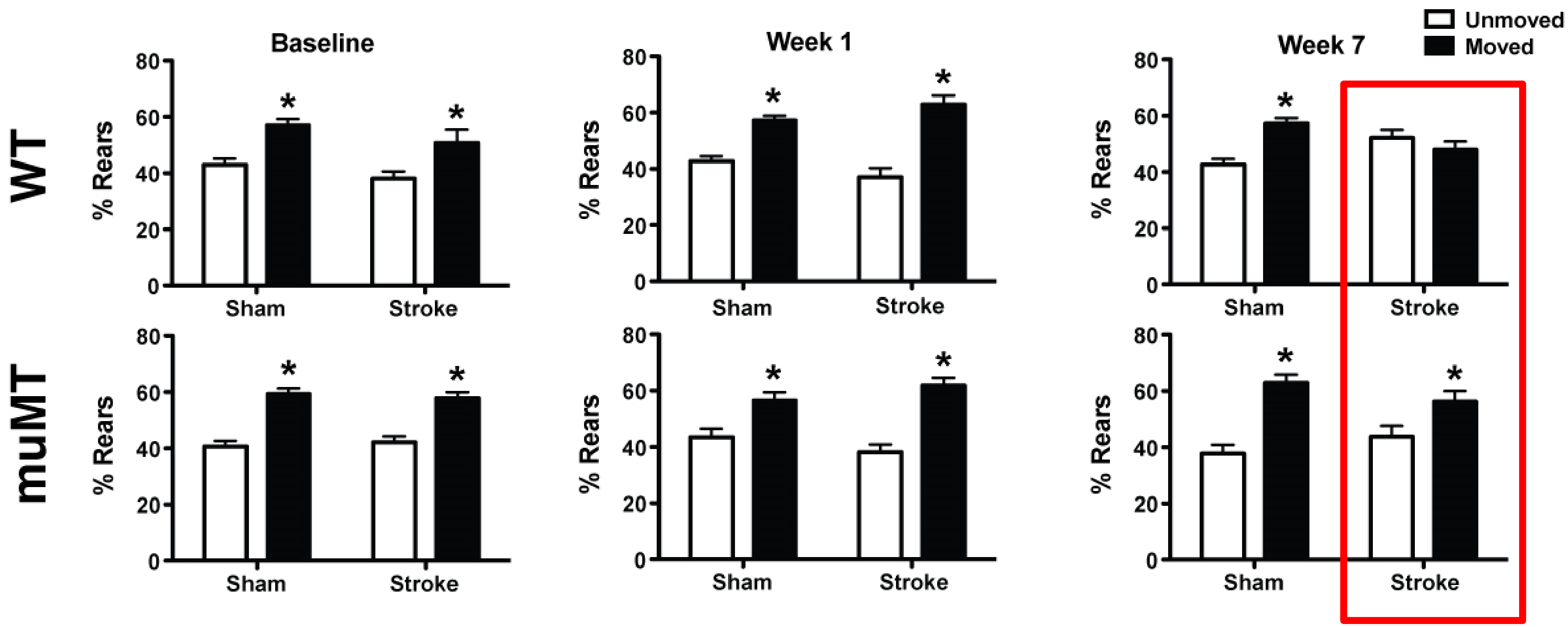




Validation of muMT mice

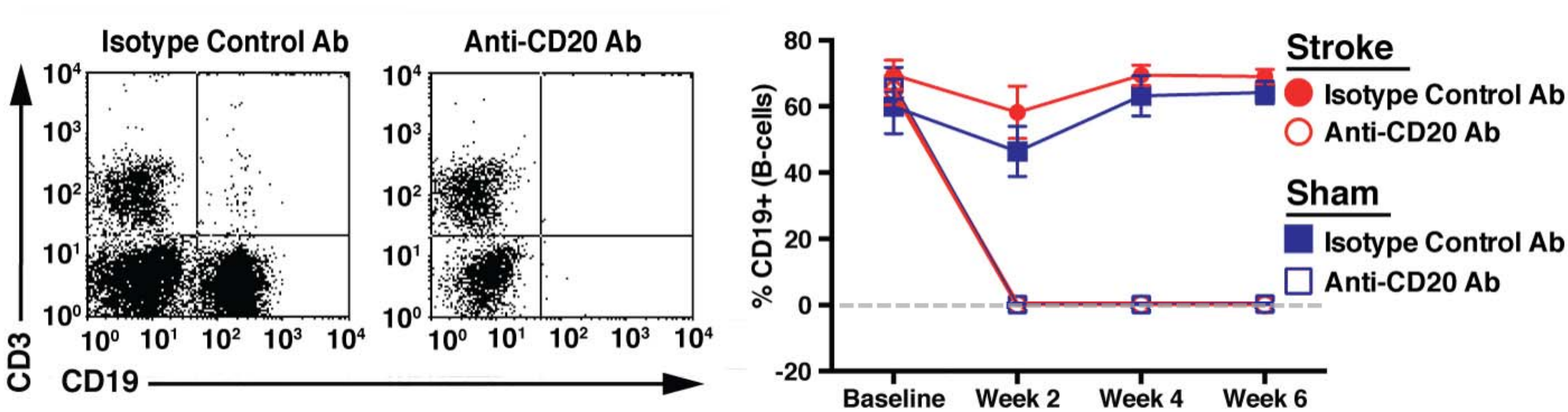


MuMT mice do not get delayed OLT impairment



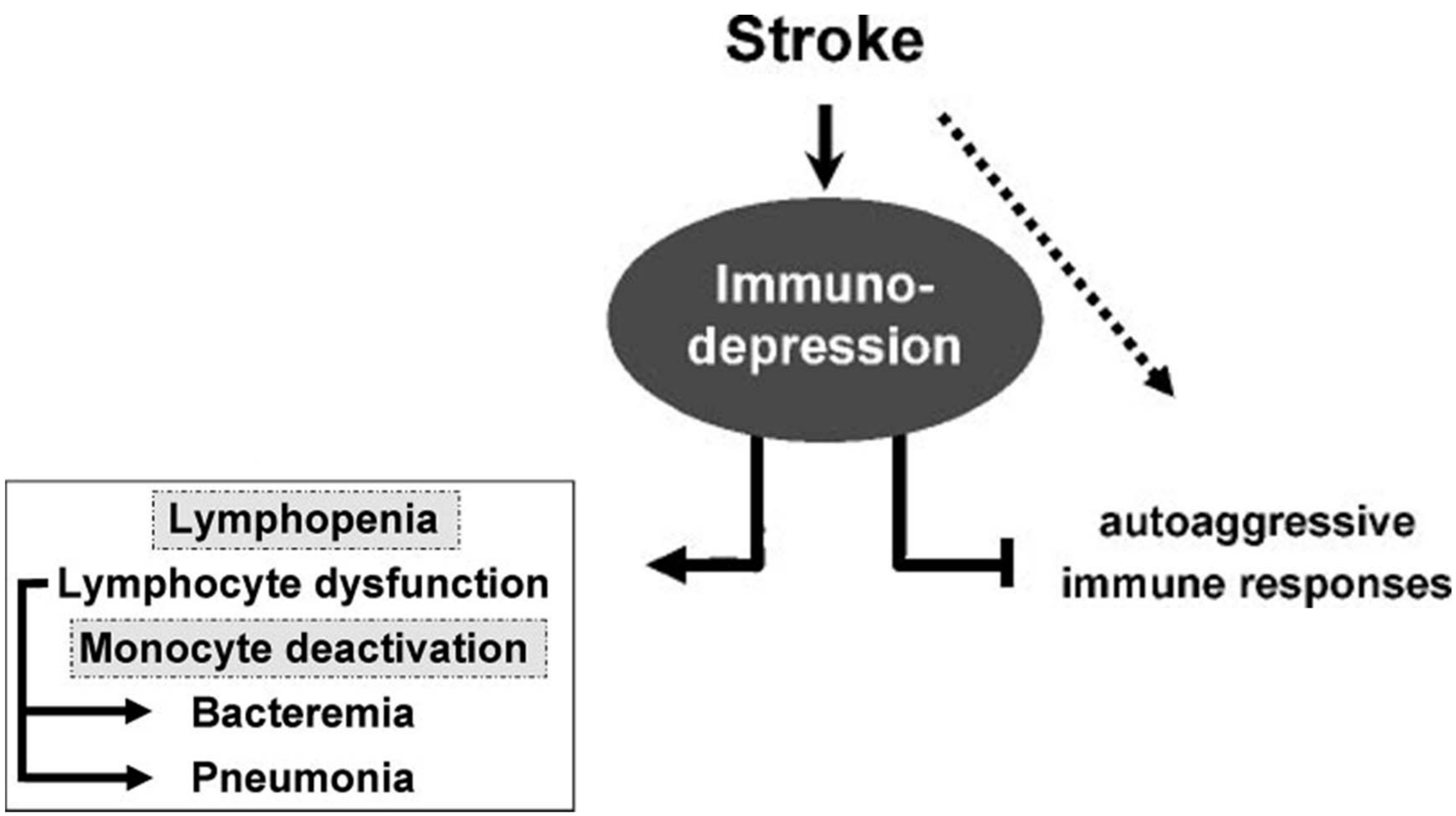
Doyle et al, J Neuroscience 2015

Anti-CD20 Antibody treatment 5 days after stroke ablates B cells



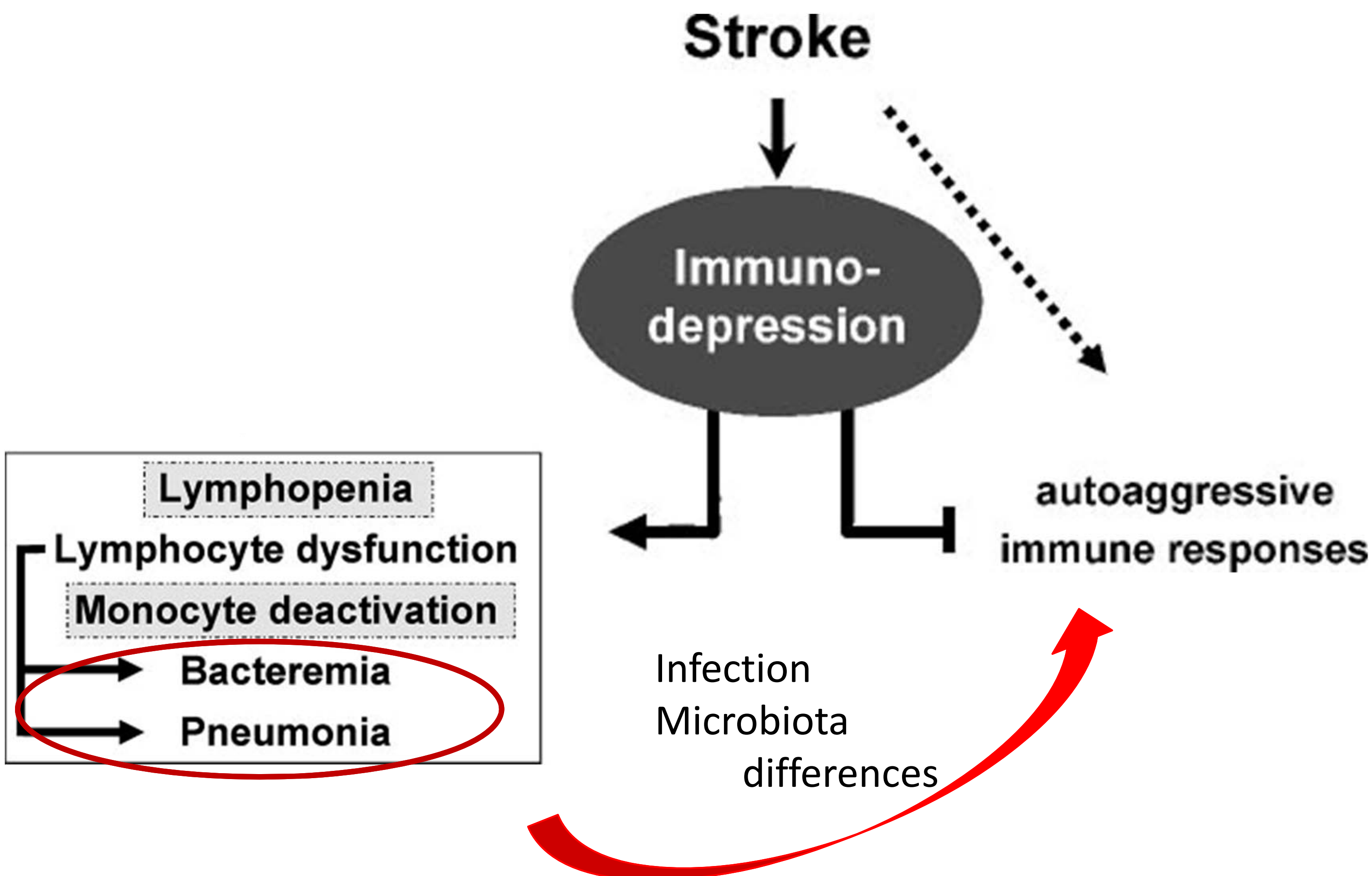
Doyle et al, J Neuroscience 2015

Stroke-induced Immunodepression



Dirnagl et al, Stroke. 2007;38[part 2]:770- 773

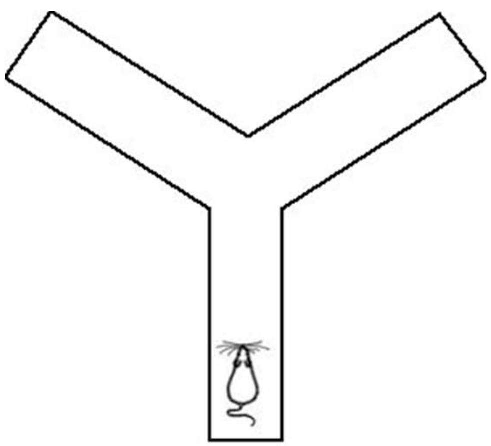
Stroke-induced Immunodepression

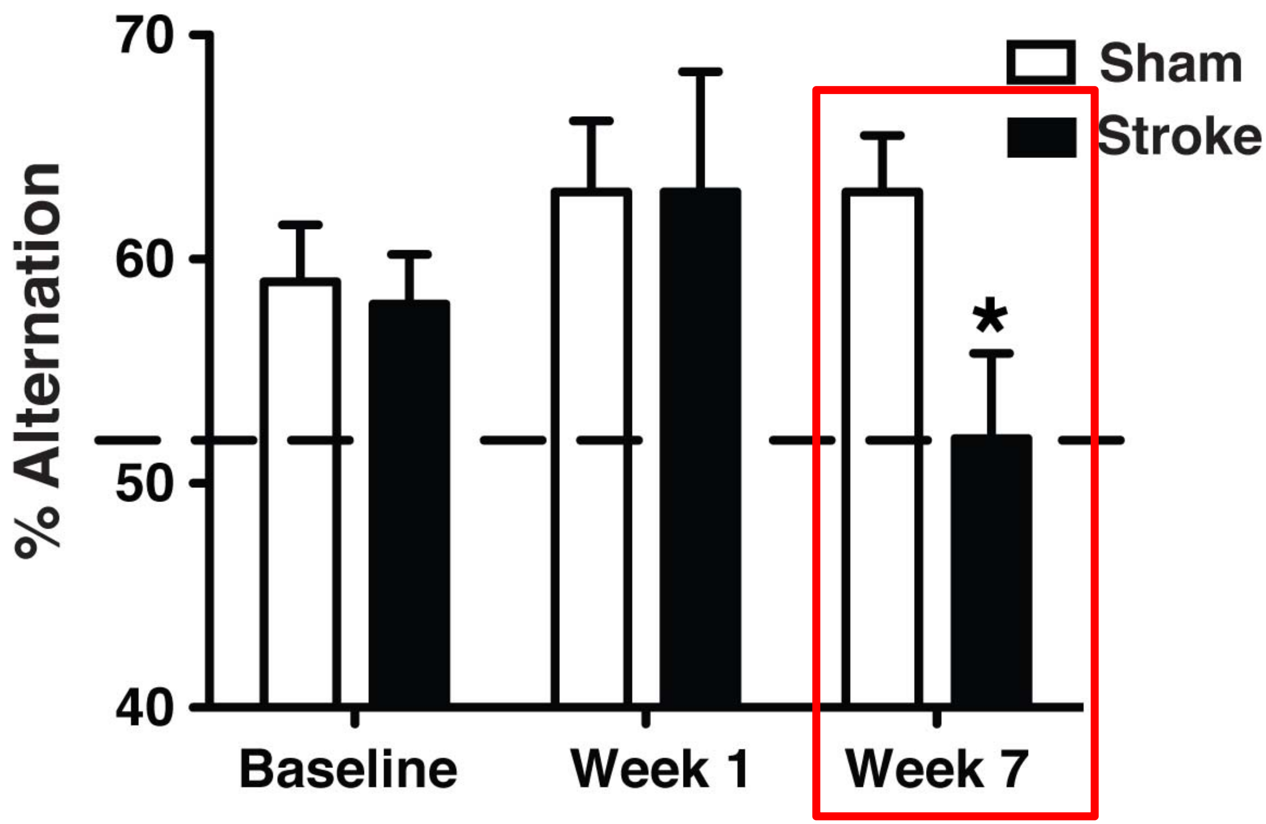


Becker Stroke 2011
Sadler Brain Behav Imm 2017

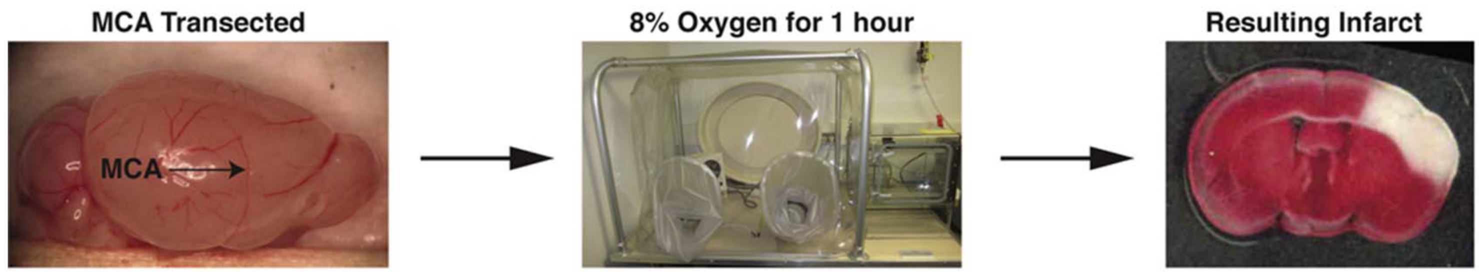
Dirnagl et al, Stroke. 2007

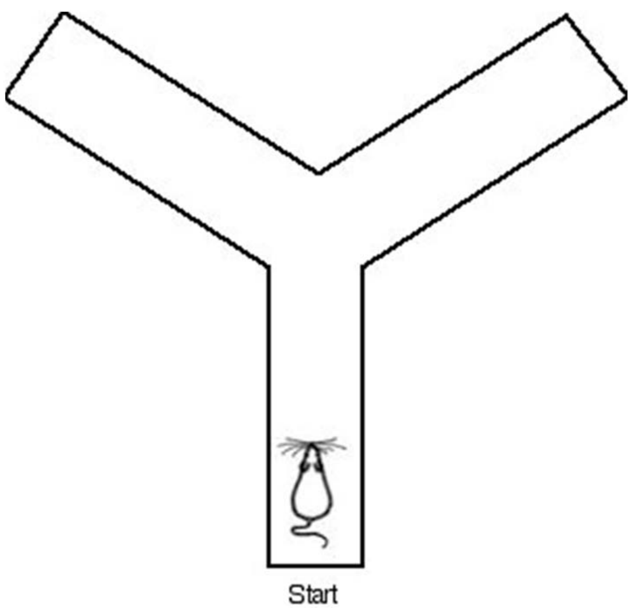
Y-maze performance deteriorates between weeks 1 and 7 after stroke

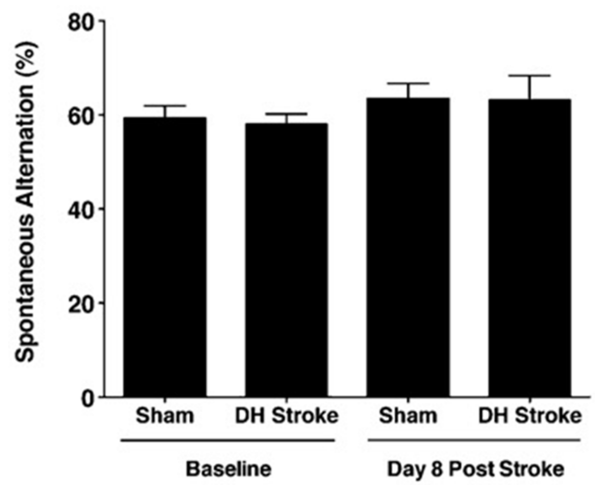




DH stroke







Doyle et al, J Neurosci Methods 207 (2012) 31– 40

Inflammation 7 weeks after stroke