

The Future of STAIRS

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Objectives

- **To understand what STAIRS is and why it developed**
- **To identify limitations in current animal models and how to interpret results**
- **Examples of failed translation and clinical trial design integration**

Same concept with a different name?

- STEPS
- RIGOR
- ARRIVE

Stroke therapy academic industry roundtable (STAIR) recommendations-1999

- Addressed rodent and primate studies
- Measure at least two outcomes
- Functional response and infarct volume in the acute (1–3 days) and in more chronic phases (7–30 days)
- Blinding
- Randomization
- Appropriate Statistical Methods
- Reproducibility
- Efficacy in multiple species
- Sex Differences
- Route of administration
- Clinically useful therapeutic window and response (still not good)

STAIRS 2.0 (2009)

- **Eliminating randomization and assessment bias**
- **Defining inclusion/exclusion criteria**
- **Conducting full power analysis and sample size calculations**
- **Disclosing potential conflicts of interest**
- **Sex (again)**
- **Aging and comorbidities (HTN, DM, high cholesterol)**

STEPS 1 and 2 (Cell based therapies)

- Test therapy in multiple strains
- Replication in a second species
- Consideration of age and gender (SEX!)
- Functional outcome (minimum of 1 month)
- Clinically useful therapeutic window and dose response
- Consideration of route of administration (intracerebral or systemic)
- Study ischemic and hemorrhagic stroke subgroups

STEPS II

- Reproducibility in multiple laboratories and in two species
- The possible addition of comorbidities (hypertensive, diabetic)
- “Positive, neutral, and negative” study outcomes should be reported

Wechsler L et al. Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. Stroke. 2009;40(2):510–5. Review.

Savitz SI et al L. Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) II. Stroke. 2011;42(3):825–829.

And still more: RIGOR

- Measured effect (improvement) was larger in studies without randomization
- Measured effect (improvement) was larger in studies without blinded assessment
- Only 36 % of published studies reported randomization
- Only 29 % of published studies were blinded
- Power analysis documented by 3 %
- No significant association between the quality of science and IF
- A significant amount of unpublished negative or neutral data cause an overestimation of efficacy because of published positive data

ARRIVE: What YOU need to KNOW for grants

Experimental design

- **Rationale for the selected models and endpoints (animal and/or cellular)**
- **Adequacy of the controls**
- **Route and timing of intervention delivery/dosing**
- **Justification of sample size, including power calculation**
- **Statistical methods used in analysis and interpretation of results (MUST HAVE POWER!!)**
- **Get some statistical help!**

ARRIVE

Minimizing Bias

- **Methods of blinding (allocation concealment and blinded assessment of outcome)**
- **Strategies for randomization and/or stratification**
- **Reporting of data missing due to attrition or exclusion**
- **Reporting of all results (negative and positive)**
- **<http://randomizer.org/>**

ARRIVE

Results

- Independent validation/replication, if available
- Robustness and reproducibility of the observed results
- Dose–response and therapeutic window results (and permeability)
- Alternative interpretations of the experimental data
- Discussion of effect size in relation to potential clinical impact
- Potential conflicts of interest

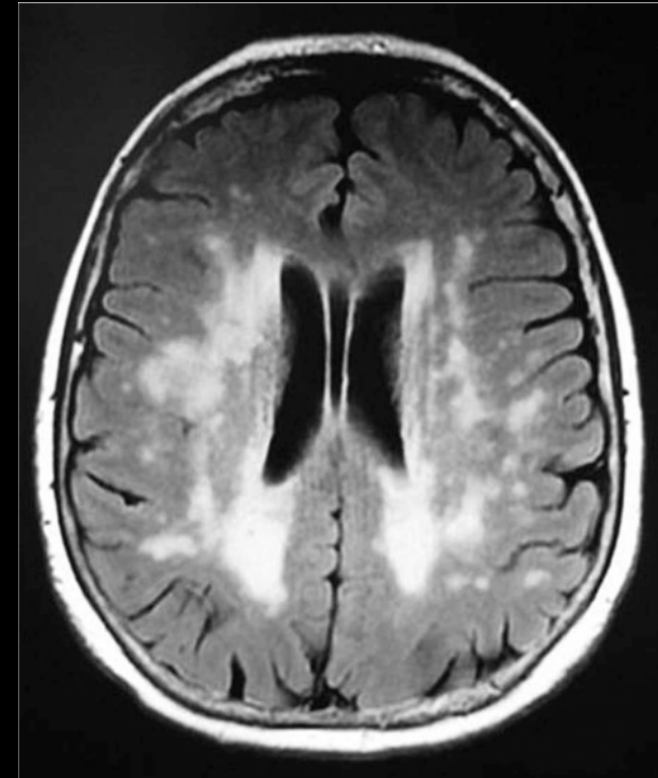
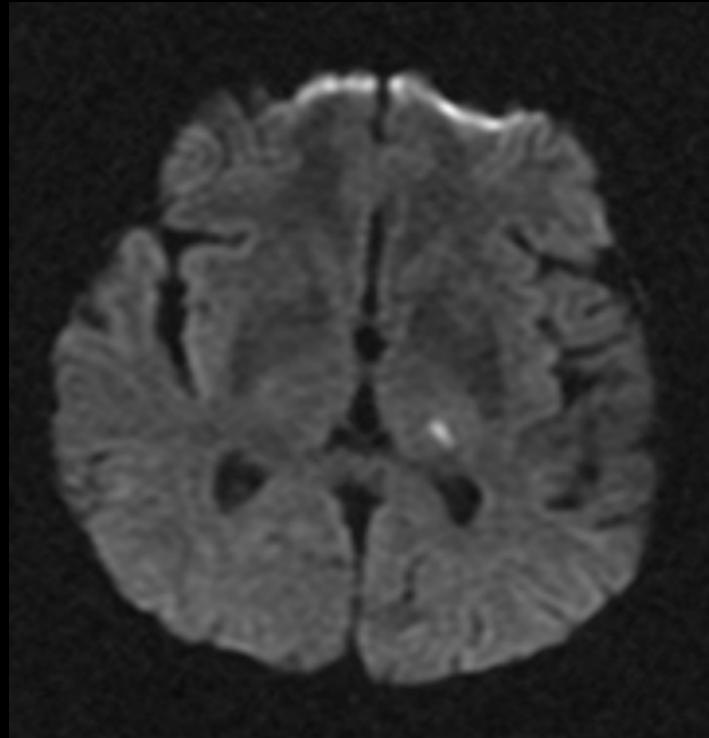
Translational studies....two labs-two species!

Well that was painful.....

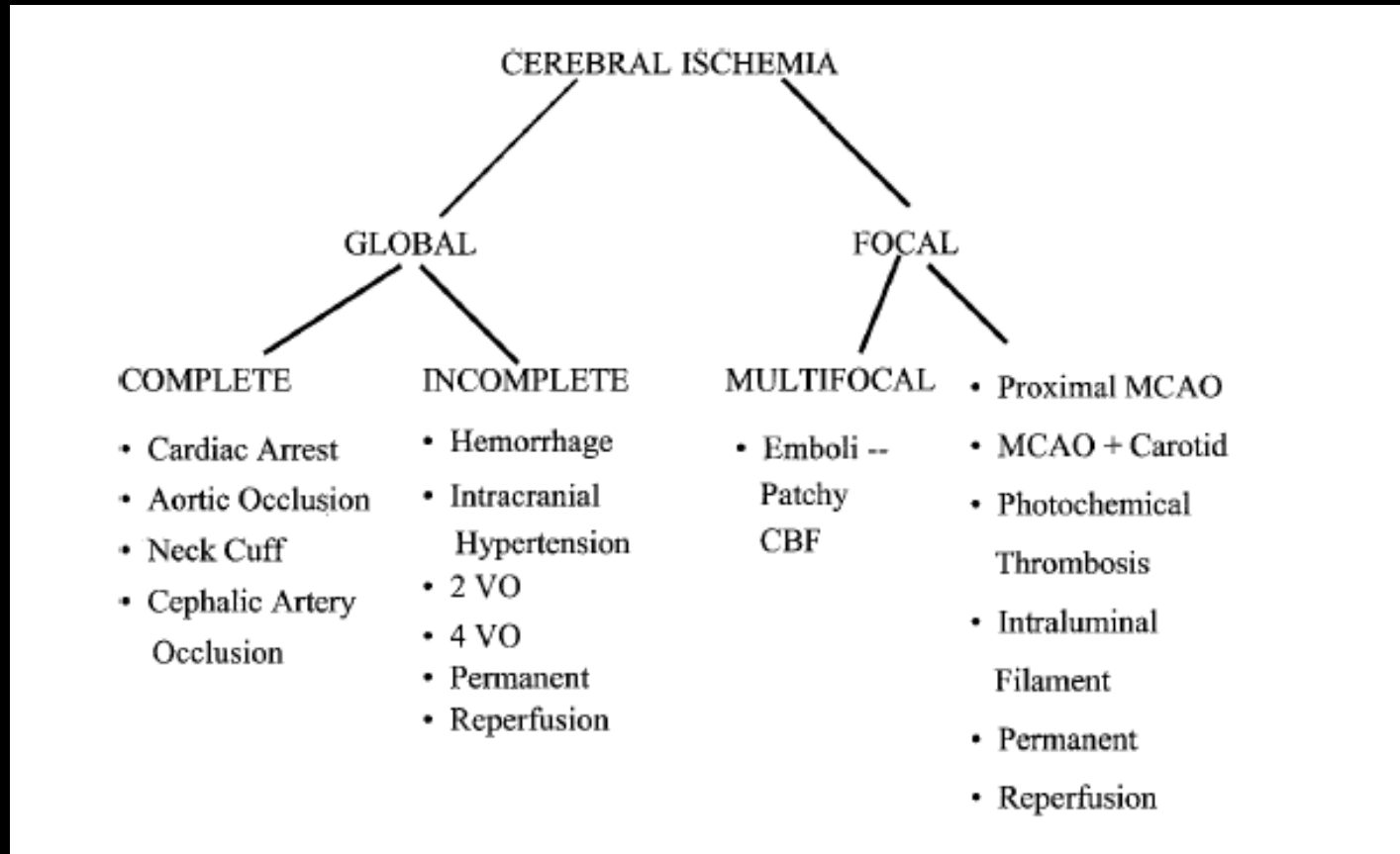
- Sex
- Stroke Subtype
- Animal Model
- Behavioral assays? What is relevant? Cognition/Mood issues often the major ones for patients.....
- Some examples

Models:

What types of stroke are we mimicking?

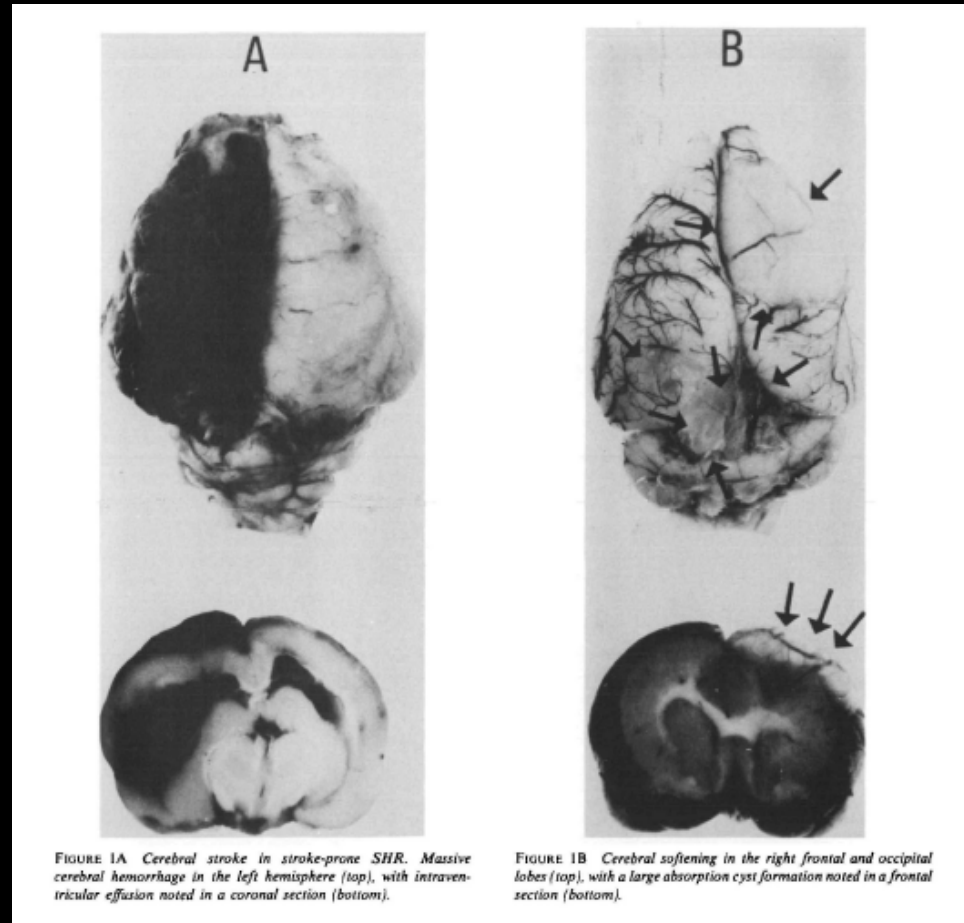


Animal Models of Stroke



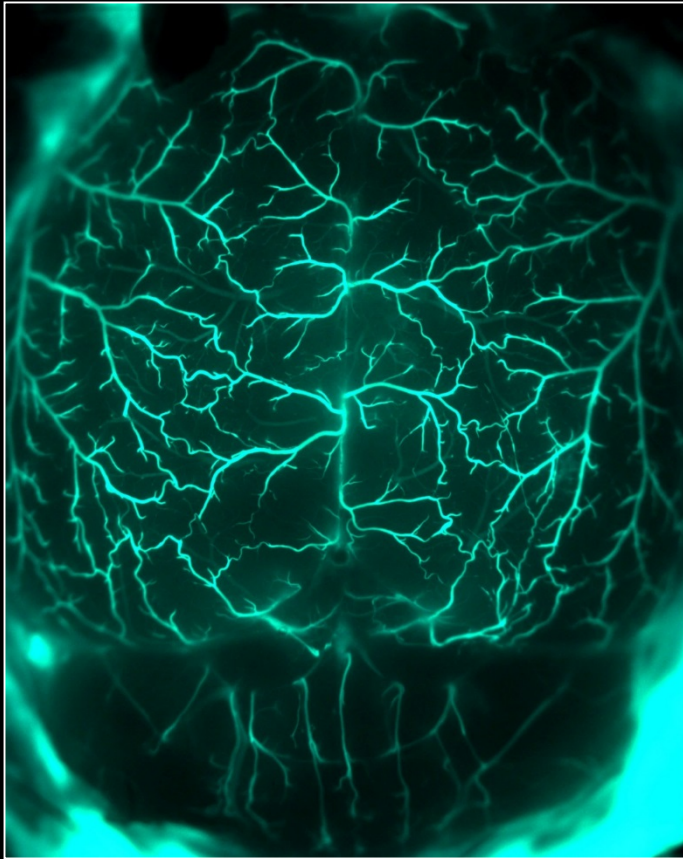
Traystman, RJ. ILAR Journal 2003 44:85-95

Stroke in Spontaneously Hypertensive Rats

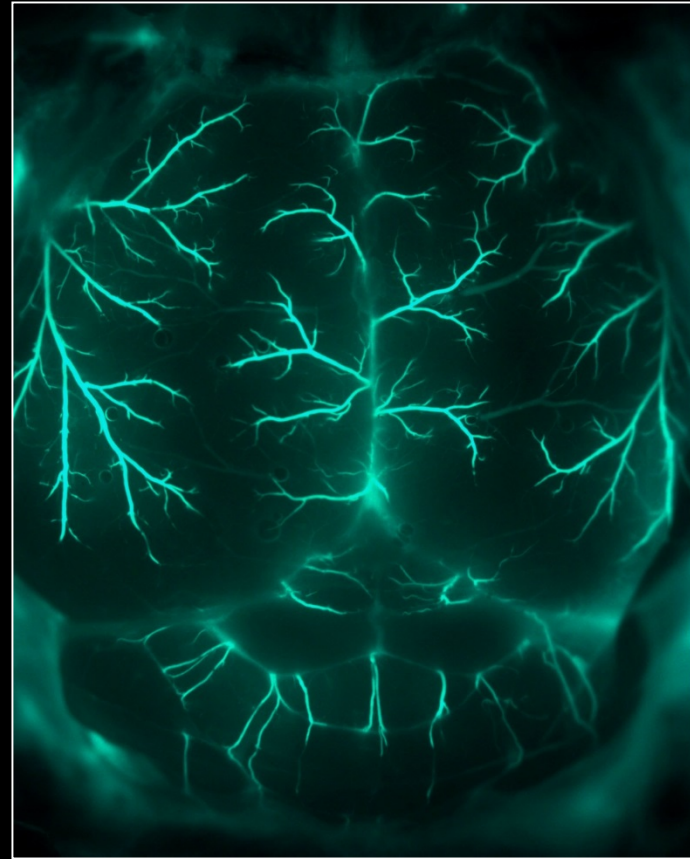


What animal/strain are you using?

C57BL/6 (B6)

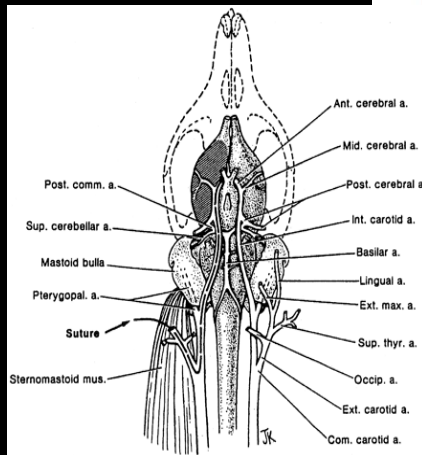


BALB/c (BC)

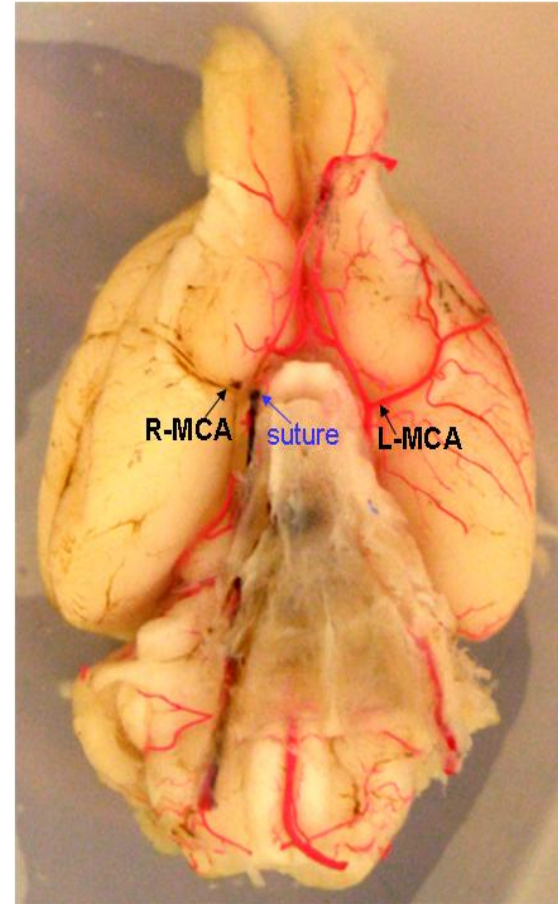
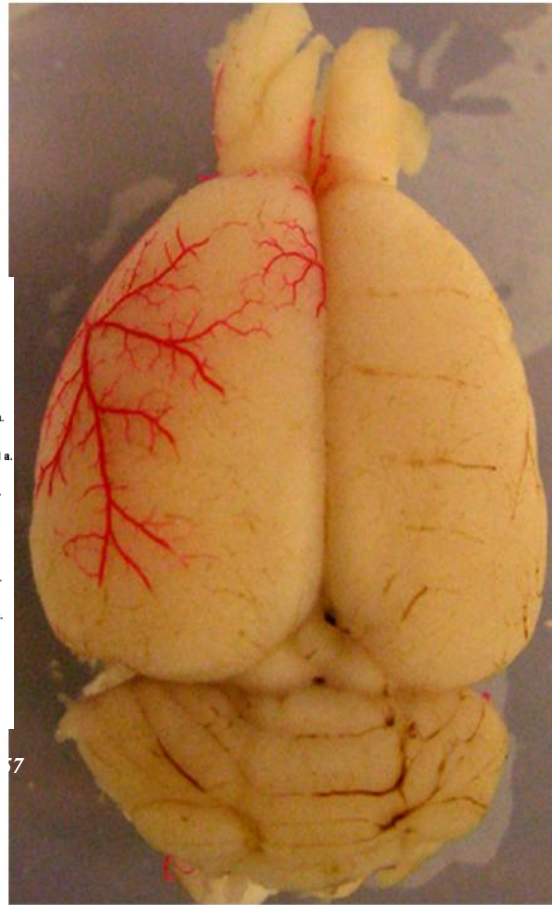


Suture model (intra-luminal occlusion)

MCAo in Rat



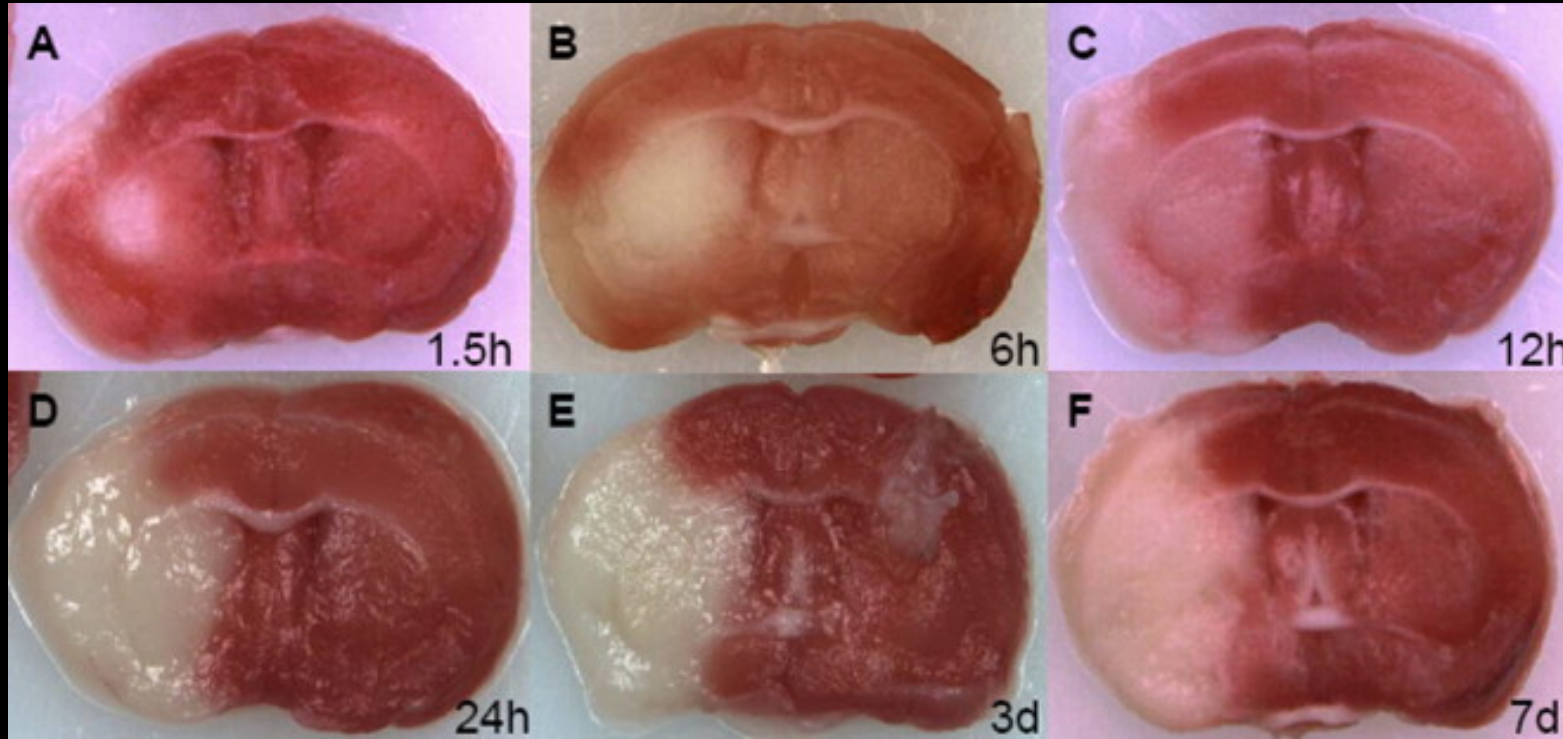
Dittmar, M. et al. Stroke 2003;34:225-237

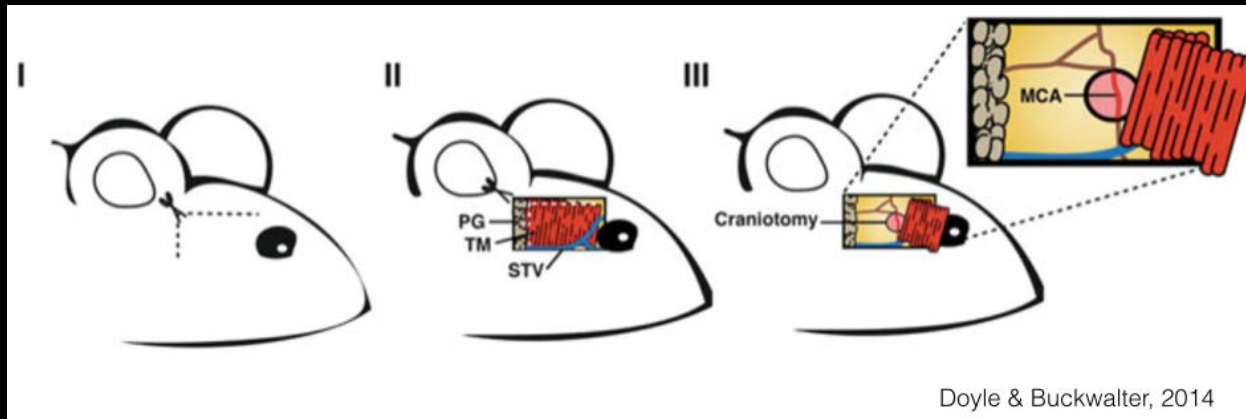


Triphenyl-tetrazolium chloride (TTC)

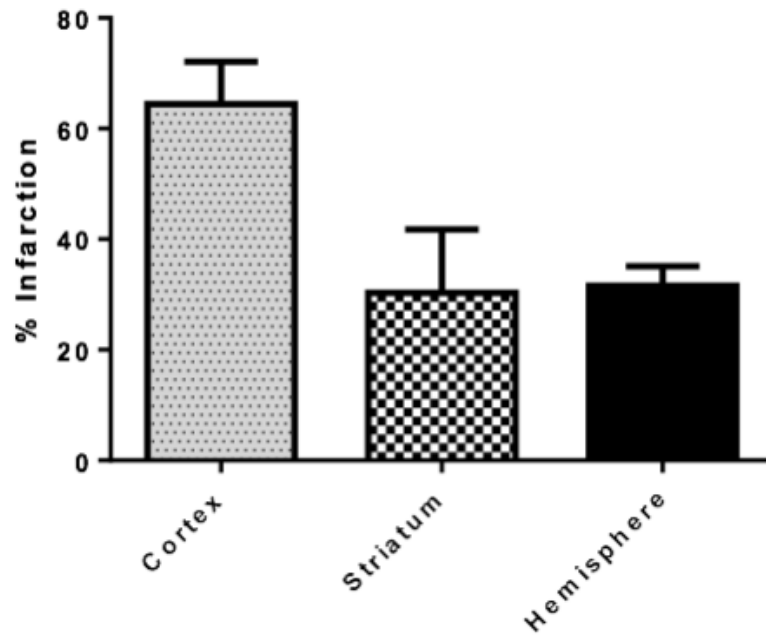


Chronology of Infarct



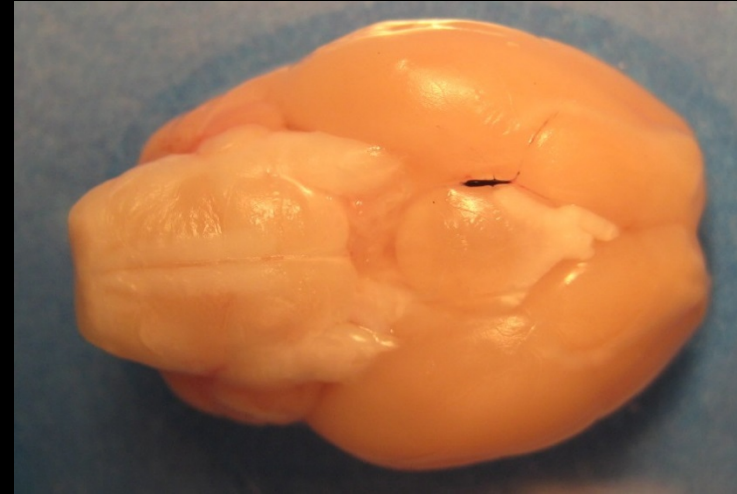
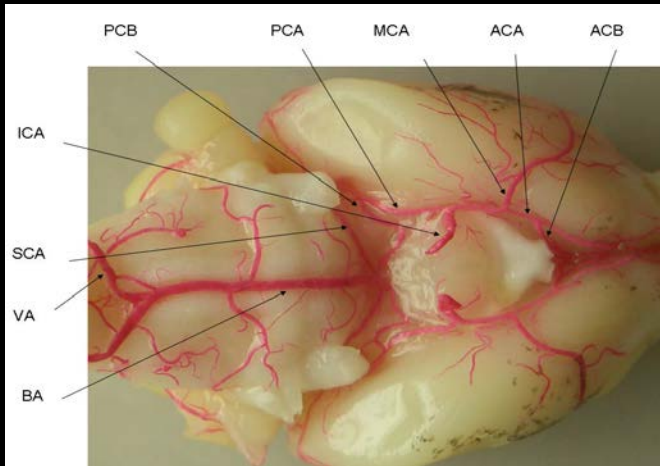


TTC Stain - Right Hemisphere

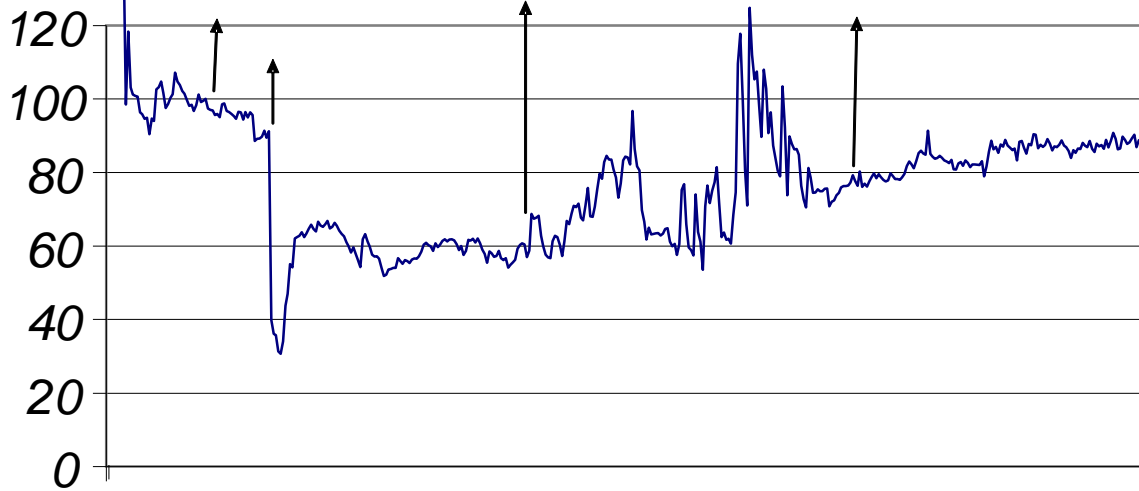


Example TTC from
72 hours post-stroke
(white = infarcted tissue).

Thromboembolic model

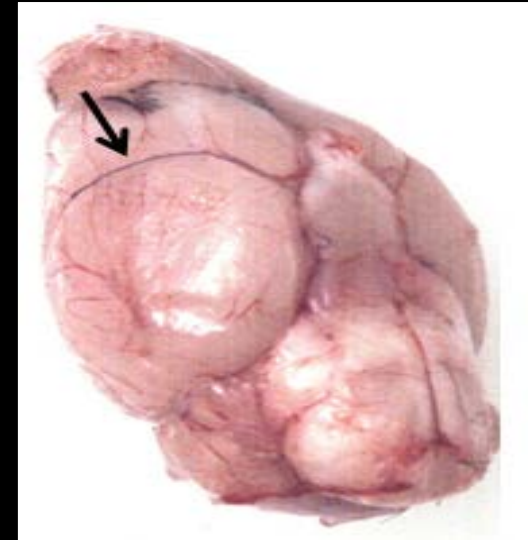


Cerebral Perfusion

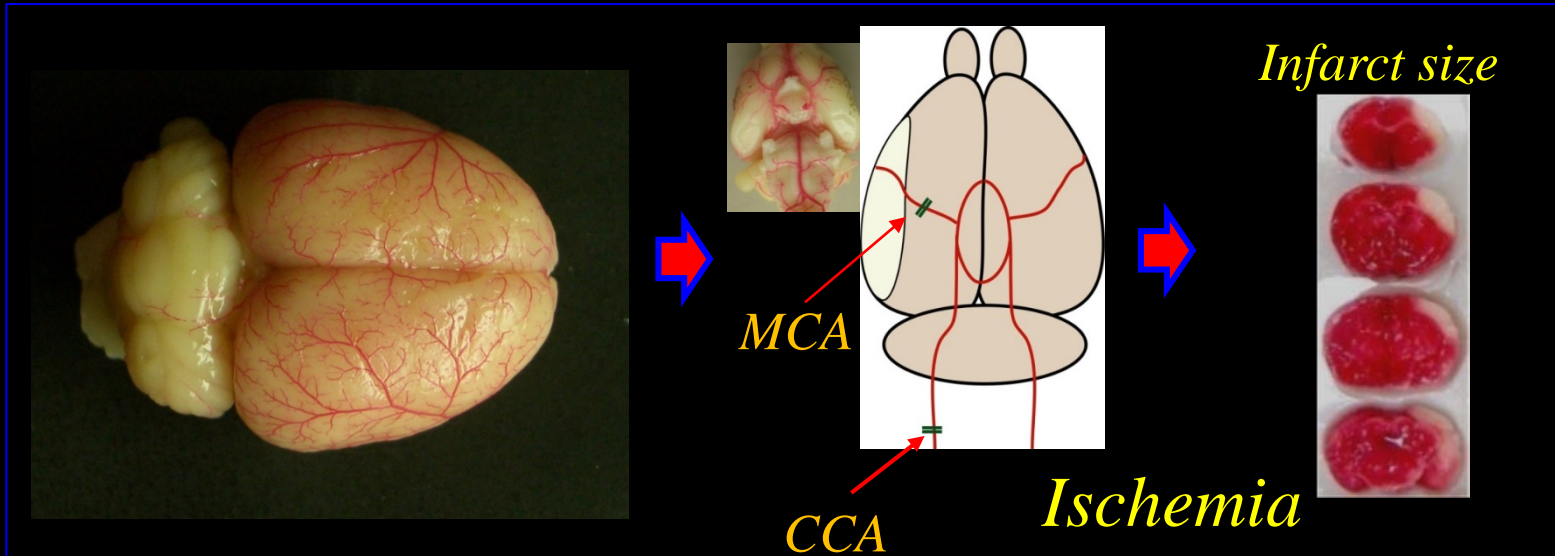




Hemorrhagic transformation (HT) after MCAO: blood marked by arrows-



Clot model MCA clot is stained with Evans Blue (arrows)

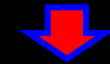


Infarct size

MCA

CCA

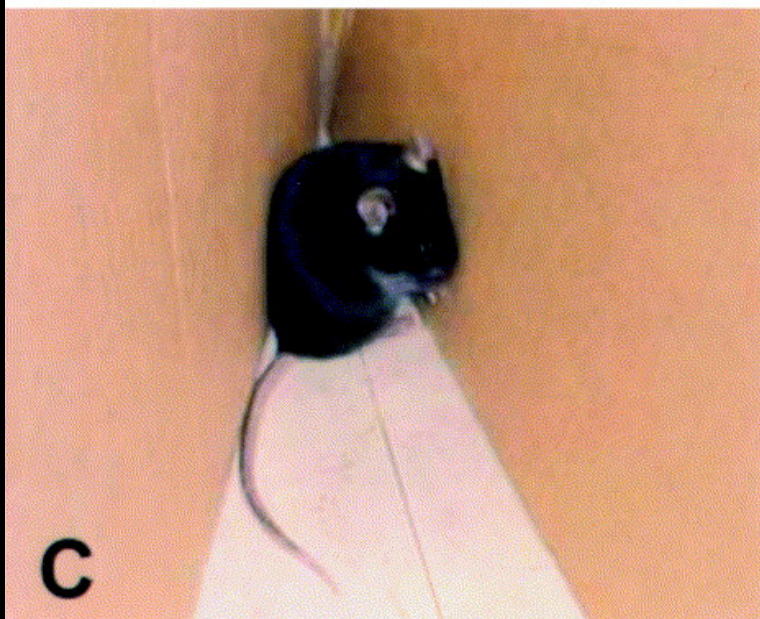
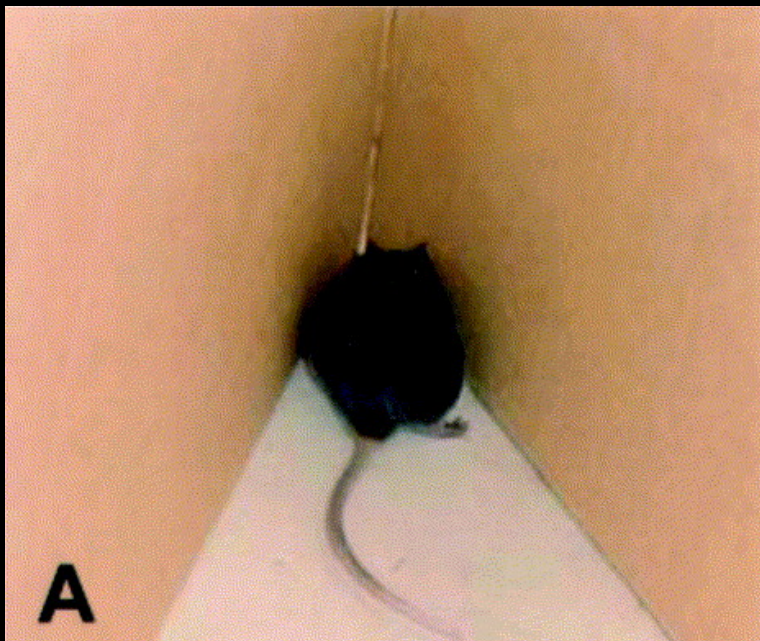
Ischemia



Behavioral dysfunction

Cylinder test







Epidemiology of stroke

- **1 in 5 women will have a stroke in her lifetime**
- **1 in 6 men will have a stroke in his lifetime**
- **In most age groups, stroke is more prevalent in men than women***
- **More women die after their strokes, are disabled after stroke, and require assistance (SNF placement in over 30%)**
- **Higher rates of secondary stroke, depression and post-stroke cognitive decline**

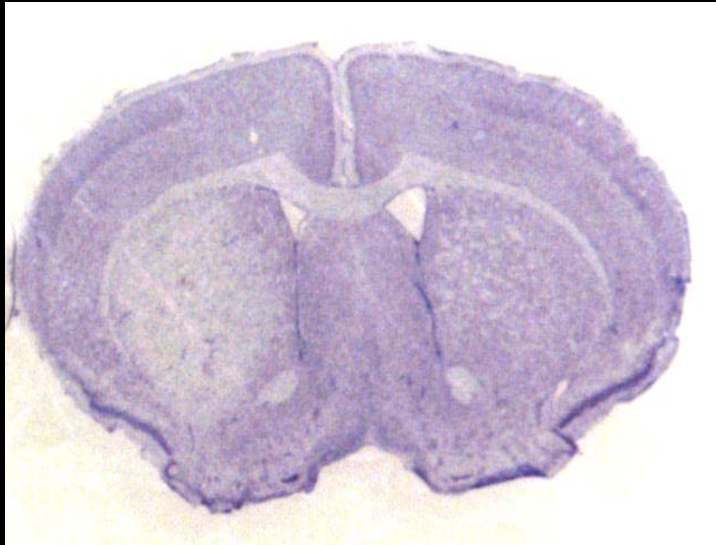
Table. Risk Factors for Stroke

Risk Factor	Sex-Specific Risk Factors	Risk Factors That Are Stronger or More Prevalent in Women	Risk Factors That Are Similar in Men and Women
Pregnancy, preeclampsia, or gestational diabetes	X		
Oral contraceptive or postmenopausal hormone use	X		
Migraine headache with aura		X	
Atrial fibrillation		X	
Diabetes		X	
Hypertension		X	
Physical inactivity, obesity, or unhealthy			X
Age			X
Prior cardiovascular disease			X
Smoking			X
The metabolic syndrome			X
Depression		X	
Psychosocial stress		X	

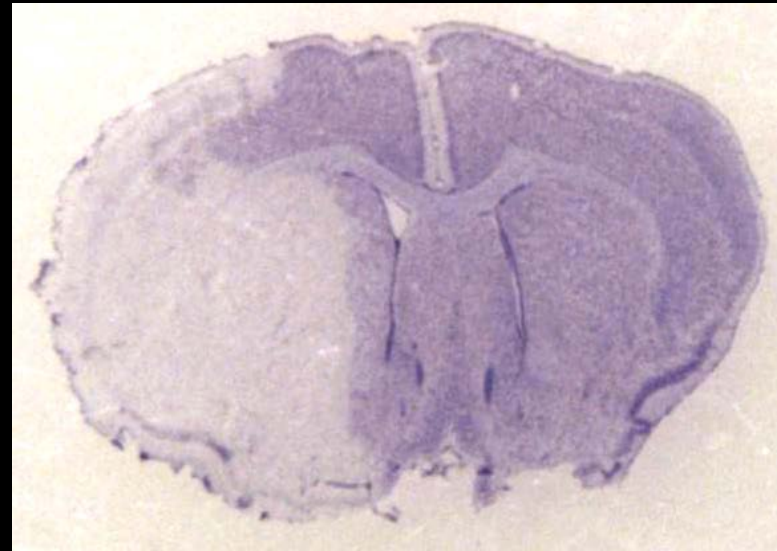
Hormone Therapy-An Example

- Epidemiology showing relative protection against vascular events in women until well after menopause
- Observational studies showing protection in HRT users from both stroke and MI/CAD
- Preclinical/Experimental evidence of robust neuroprotective effects in vivo and in vitro

Estrogen



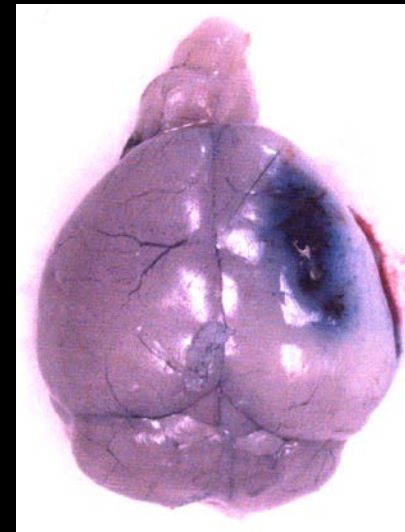
Intact



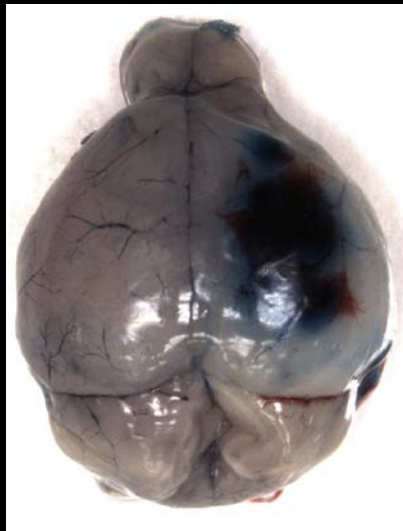
Ovx Female



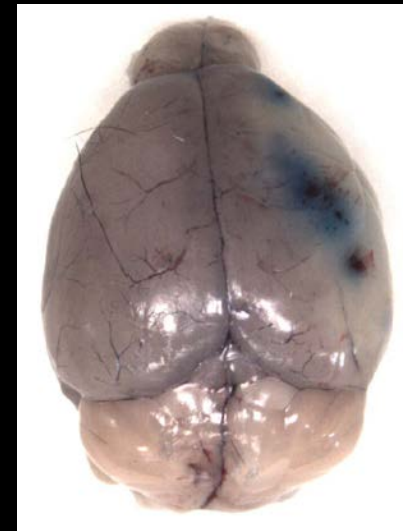
Aging male



Young male

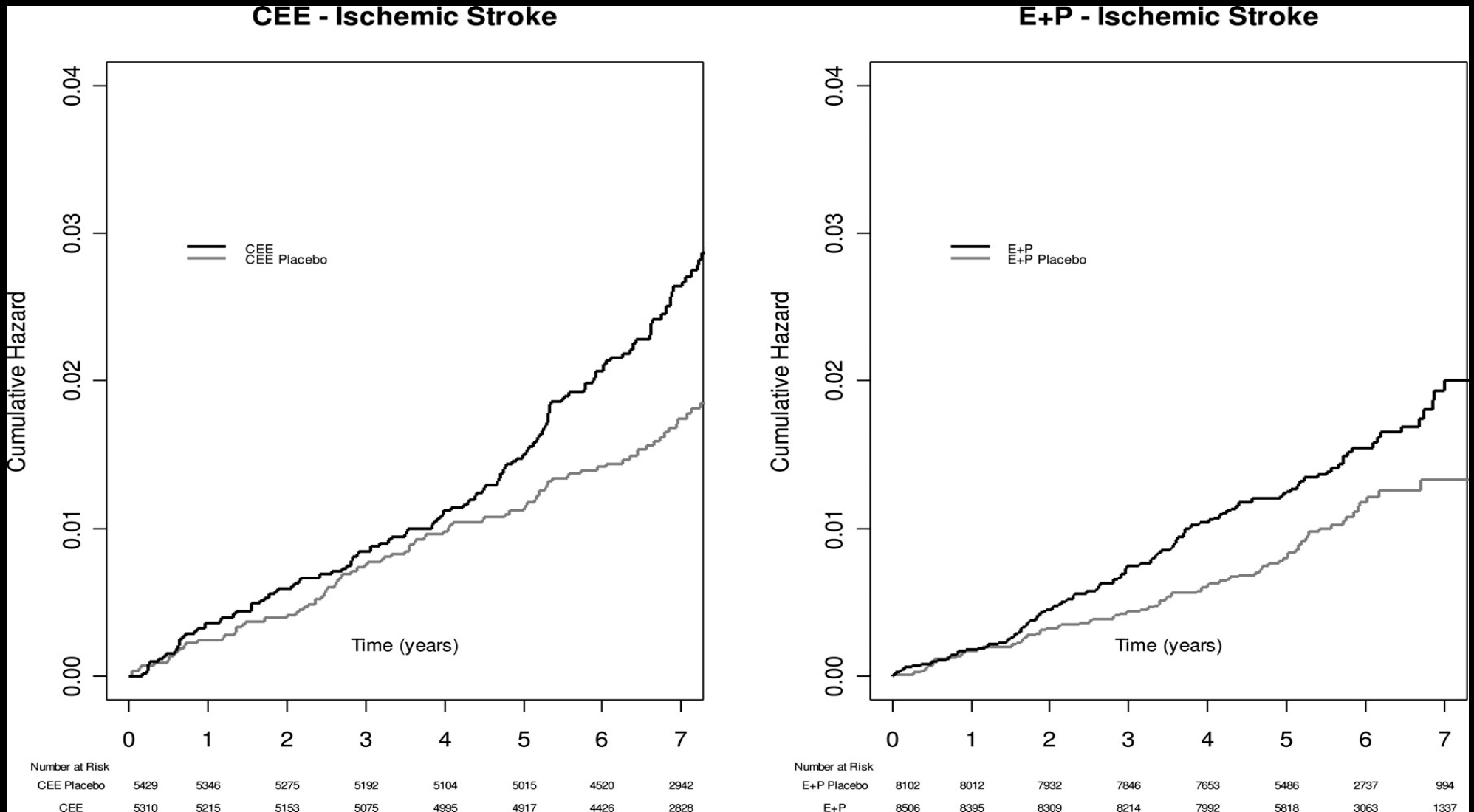


Aging female



Young female

Ischemic stroke risk- WHI hormone trial



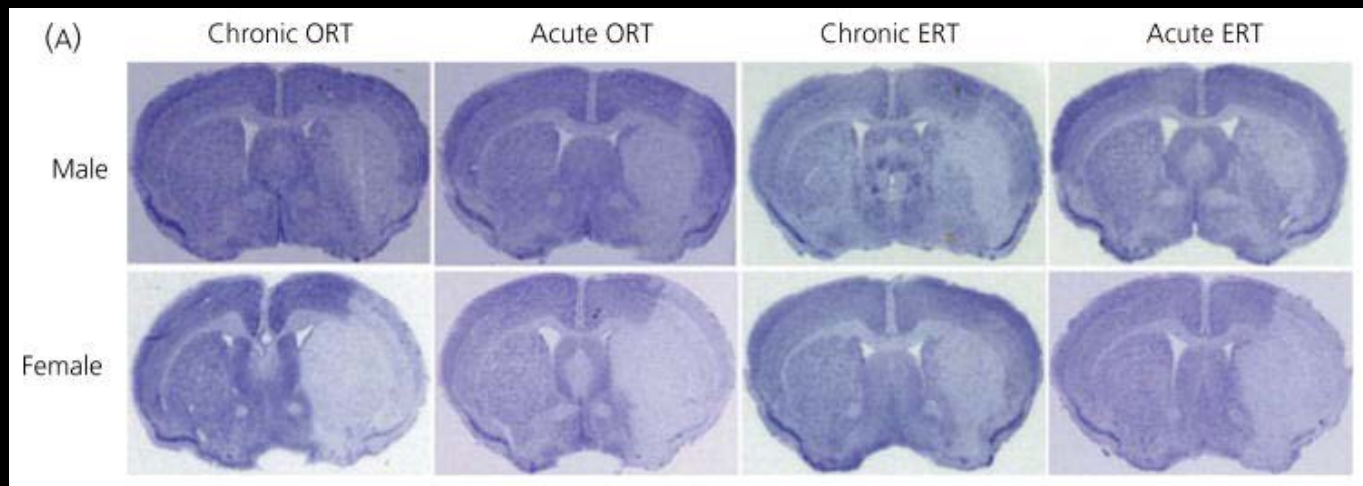
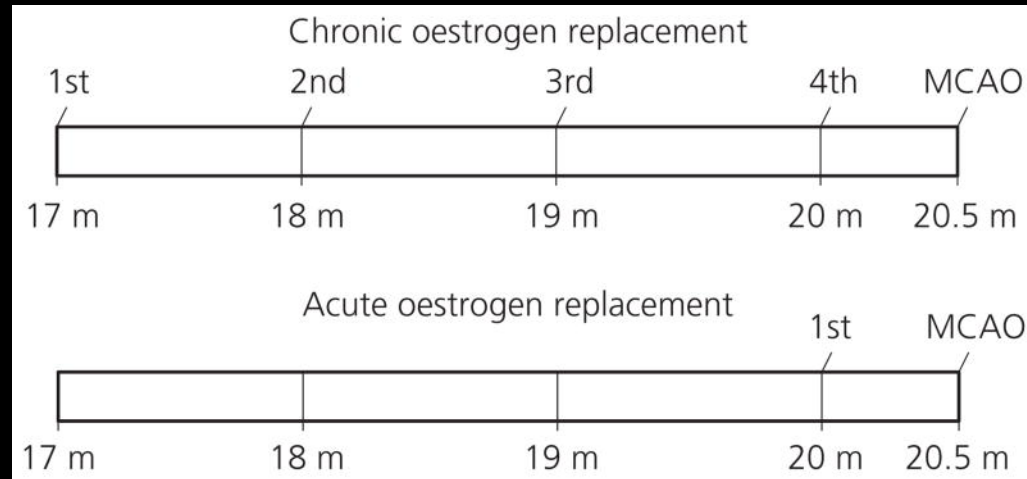
Postmenopausal HT Recommendations

- 1. For women with ischemic stroke or TIA, postmenopausal hormone therapy (estrogen with or without a progestin) is not recommended (Class III, Evidence A).*
- 2. Not recommended for primary prevention of CAD or CVD*

WHI: Possible Limitations?

- **Less than one-third of WHI were between the ages of 50 and 59, and women within a year of menopause were excluded (WHI)**
- **Older, more frequently diabetic, obese and more likely to smoke than women using HRT in prior observational studies**
- **HRT in observational studies: treatment early in menopause for control of symptoms (age of 51)**
- **In WHI HRT was started an average of 12 years after the menopause (mean ages for HRT; 63.3 and ERT; 63.6)**

What happens in animals?

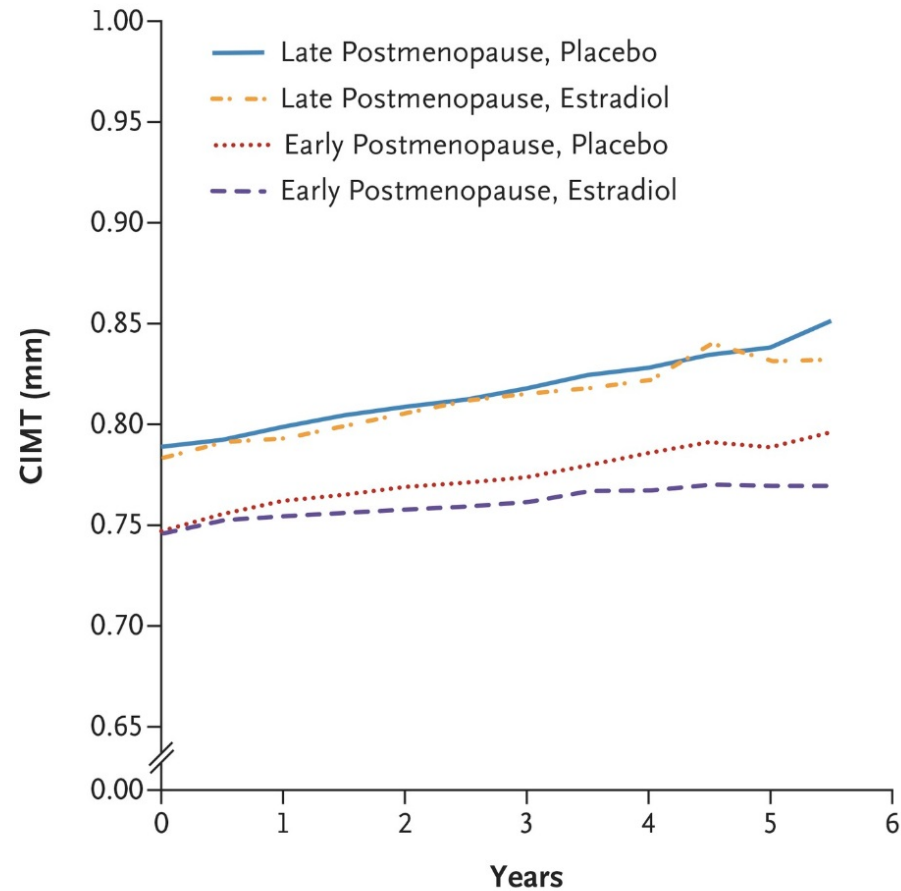


Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol

Howard N. Hodis, M.D., Wendy J. Mack, Ph.D., Victor W. Henderson, M.D., Donna Shoupe, M.D., Matthew J. Budoff, M.D., Juliana Hwang-Levine, Pharm.D., Yanjie Li, M.D., Mei Feng, M.D., Laurie Dustin, M.S., Naoko Kono, M.P.H., Frank Z. Stanczyk, Ph.D., Robert H. Selzer, M.S., Stanley P. Azen, Ph.D., for the ELITE Research Group

*N Engl J Med
Volume 374(13):1221-1231
March 31, 2016*

CIMT Progression According to Study Group and Postmenopause Stratum.



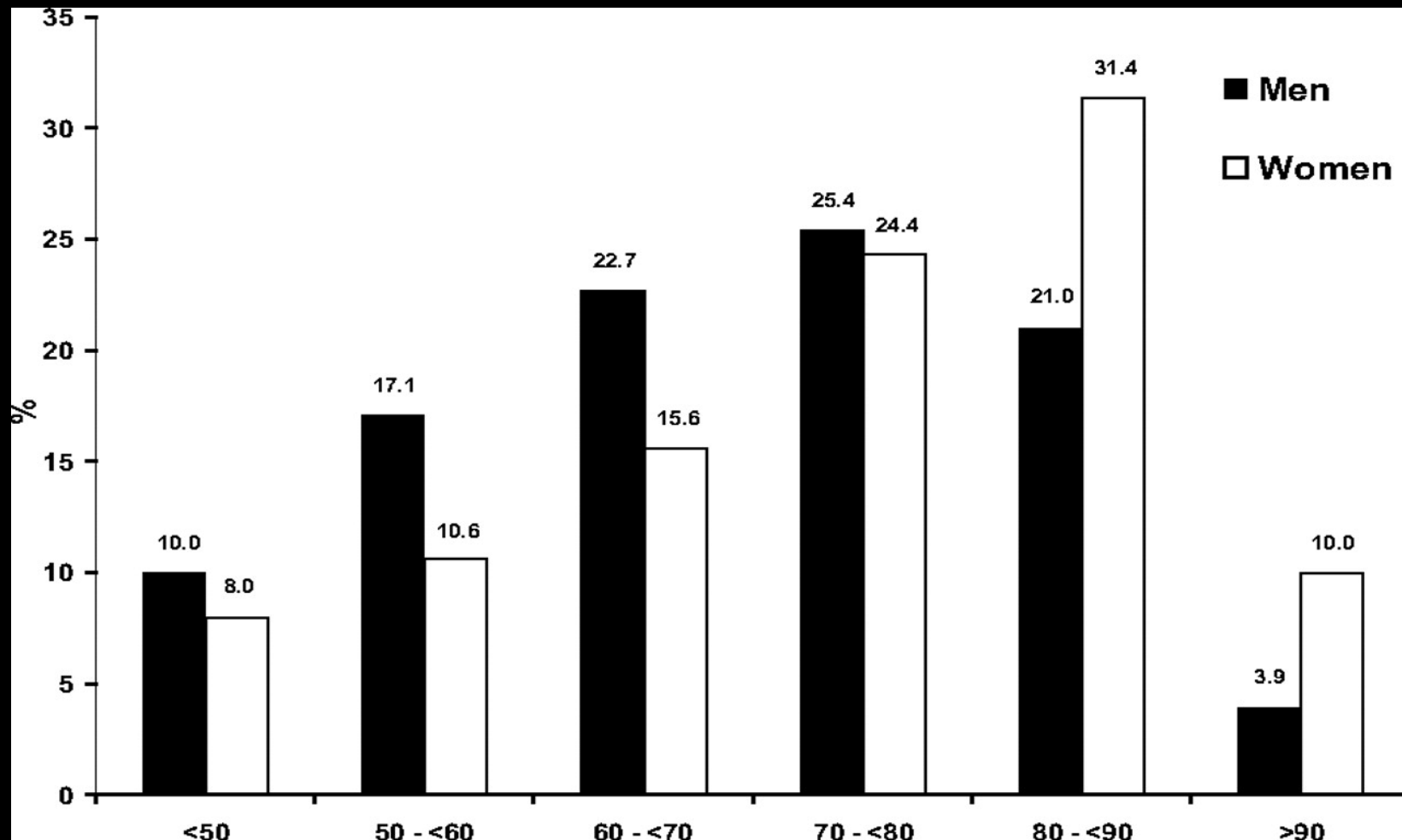
No. of Participants

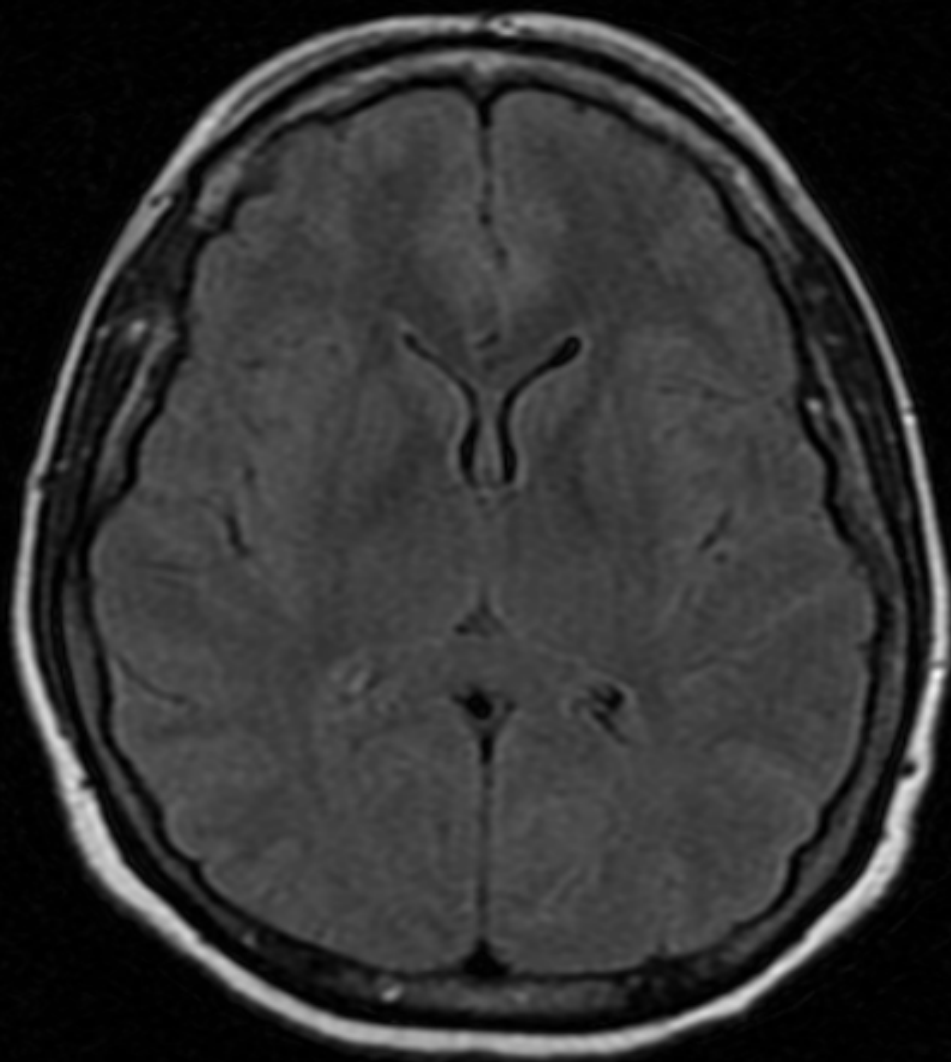
With CIMT data	643	533	522	515	424	295	56
Who completed or discontinued study	0	106	119	128	215	345	582
Without CIMT data	0	4	2	0	4	3	5

Issues in the use of animals in pre-clinical research

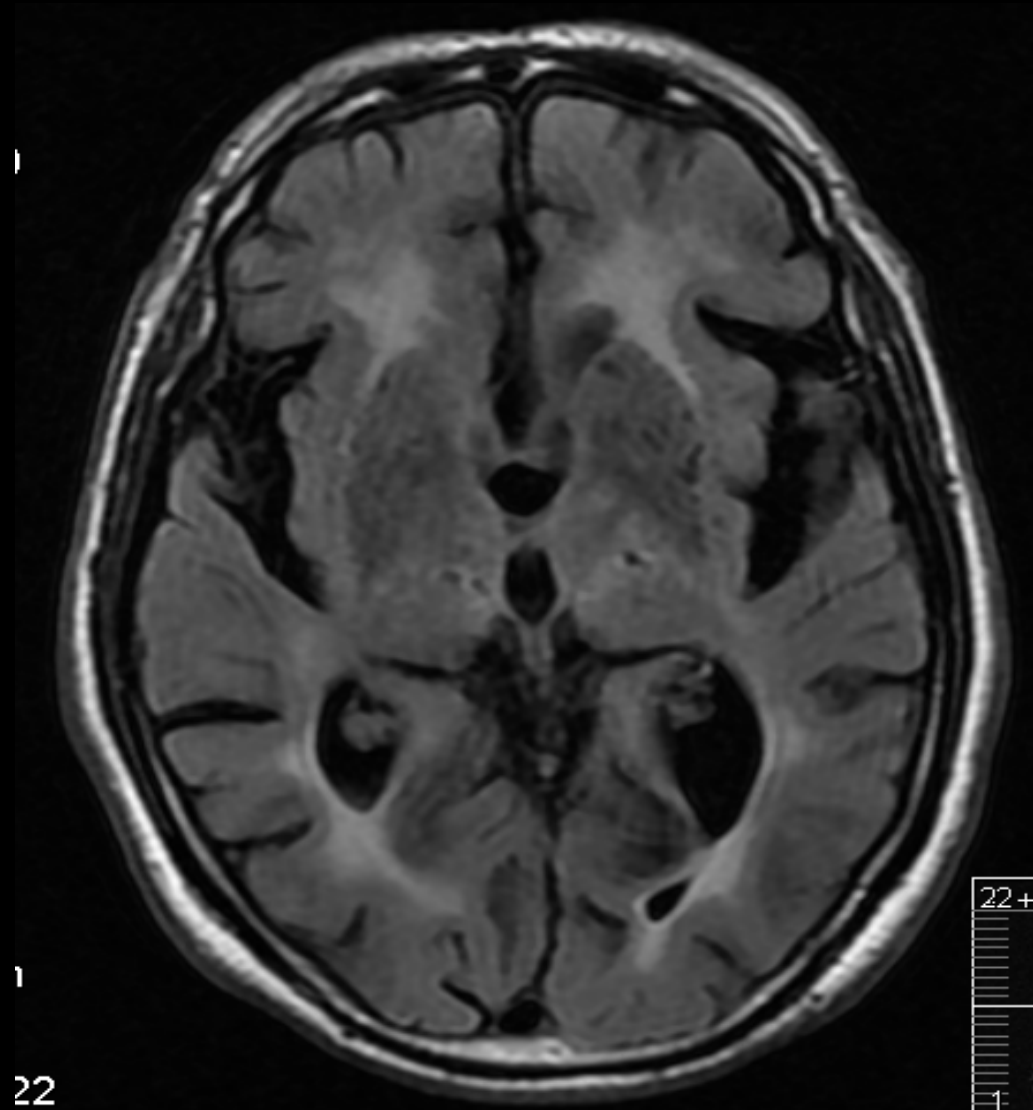
- Co-morbidities such as diabetes, obesity, high cholesterol, hypertension not modeled
- Lifestyle considerations (smoking, alcohol, pregnancy)
- Mimicking the actual “at risk populations”
- Interaction between risk factors and aging

Age distribution by sex of 502,036 ischemic stroke admissions in the GWTG-Stroke program





32 year old



78 year old

Policy: NIH to balance sex in cell and animal studies

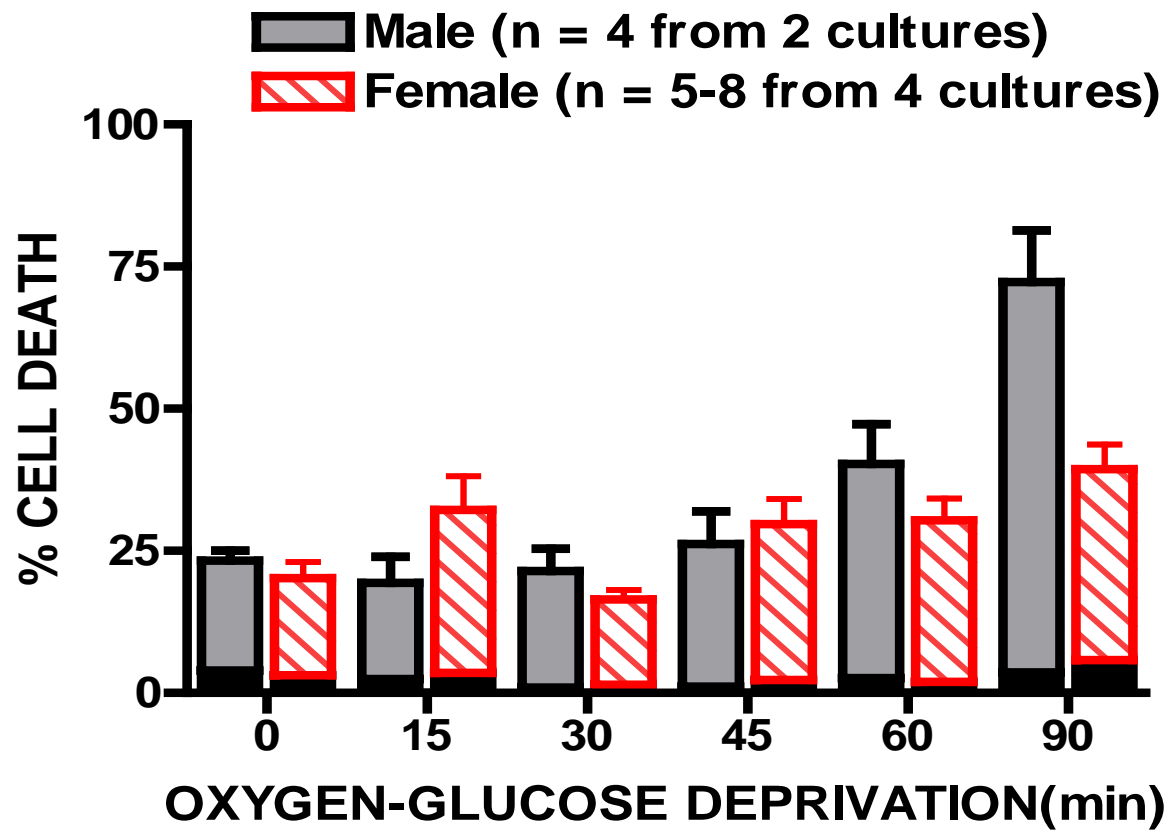


<http://orwh.od.nih.gov/research/index.asp>

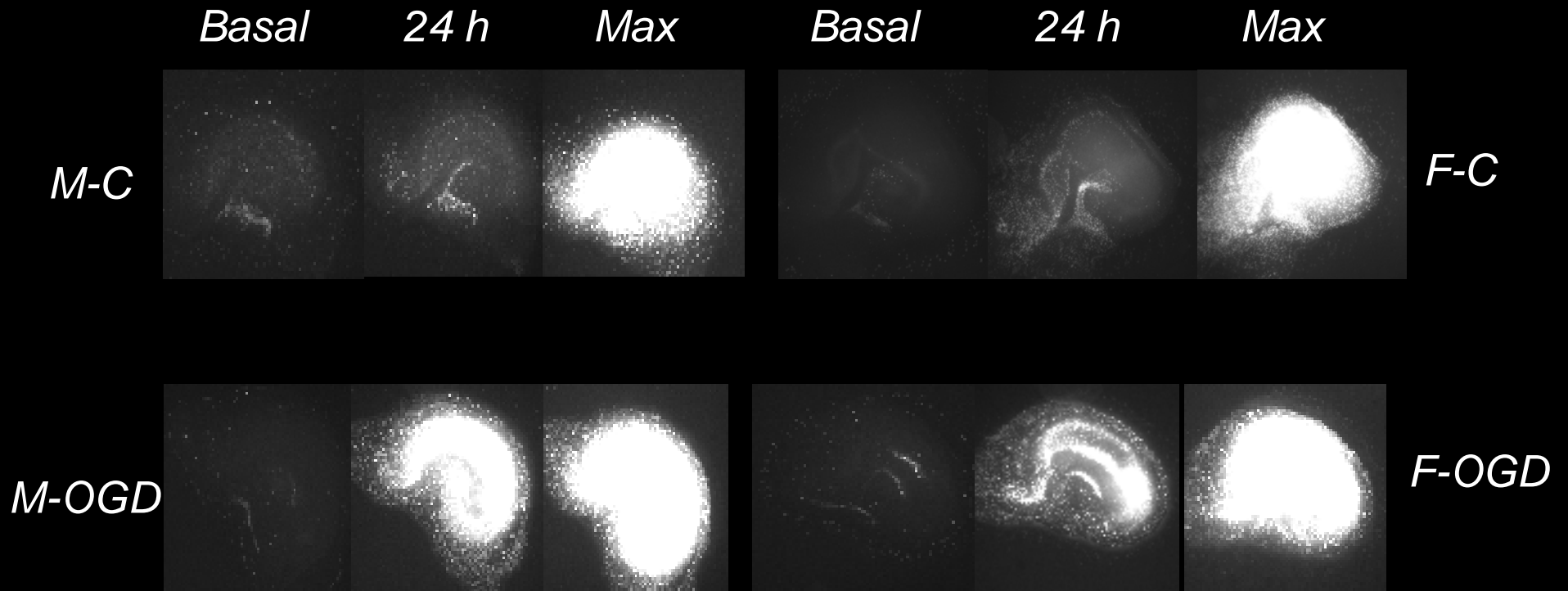
Translational Implications

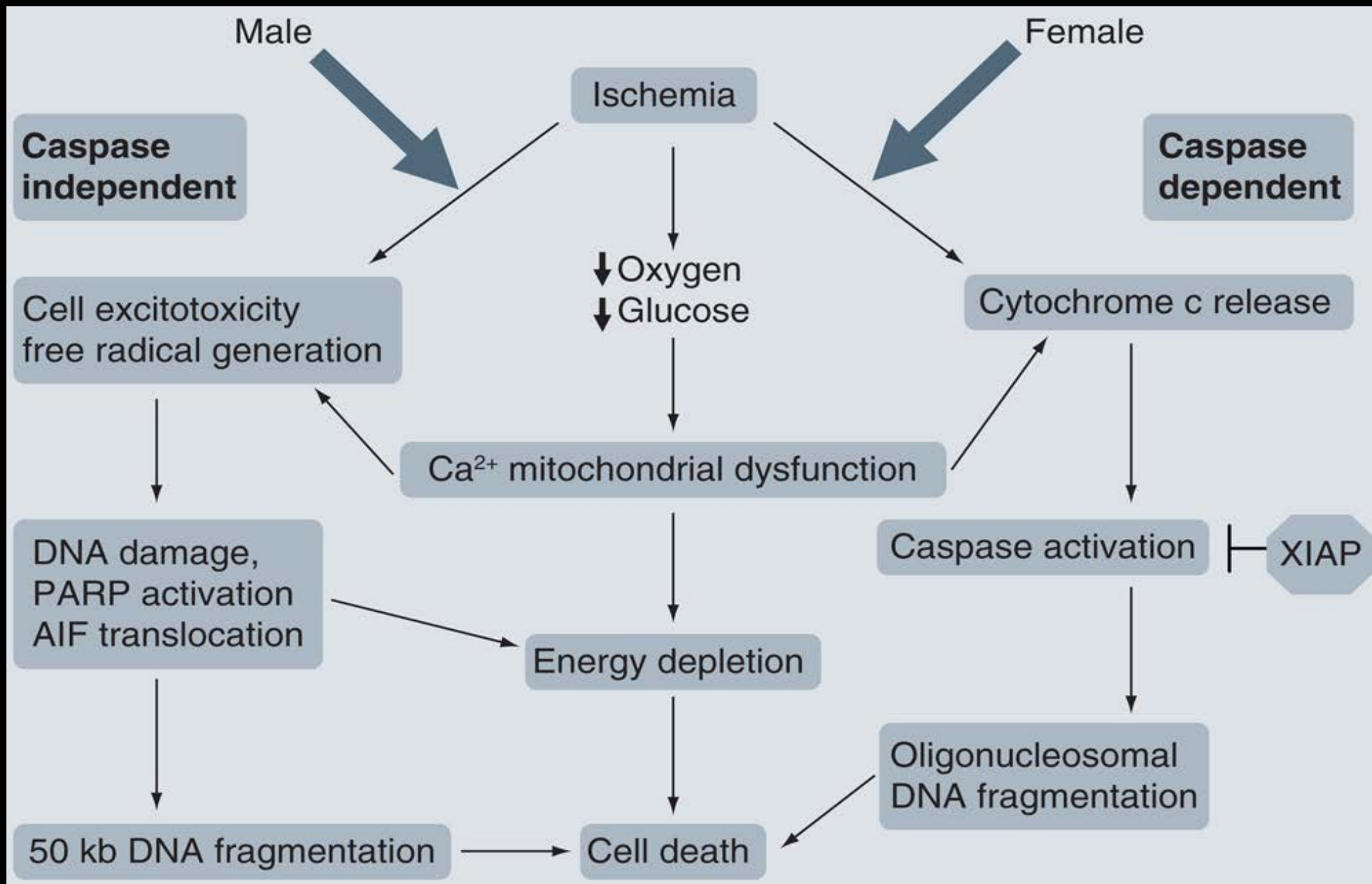
- In 1993, the NIH Revitalization Act required the inclusion of women in NIH-funded clinical research.
- However inclusion of women is not required at phases 1 and 2 of NIH-funded human subject trials when critical safety and dosage issues are addressed (Ambien)
- 80% of the drugs taken off the market between 1997 and 2000 “had disproportionately adverse effects on women”
- Call for Action for “Sex Balancing” in clinical and now pre-clinical studies

Sex differences *in vitro*



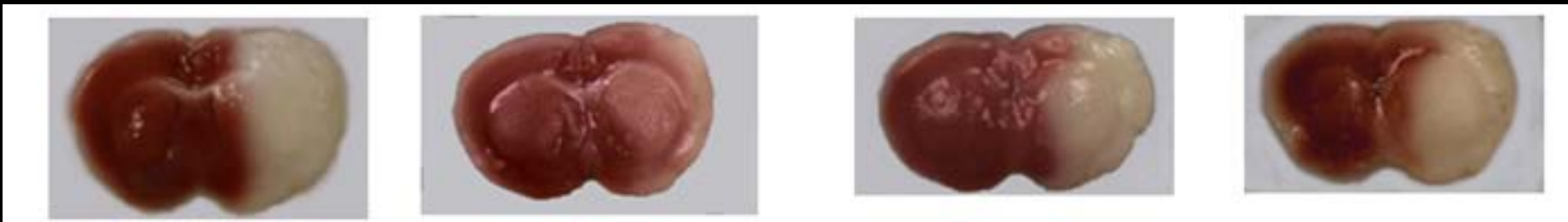
Sex Differences in OGD-induced Neuronal Injury





So who cares?

Does this actually mean anything to the treatment of stroke patients?

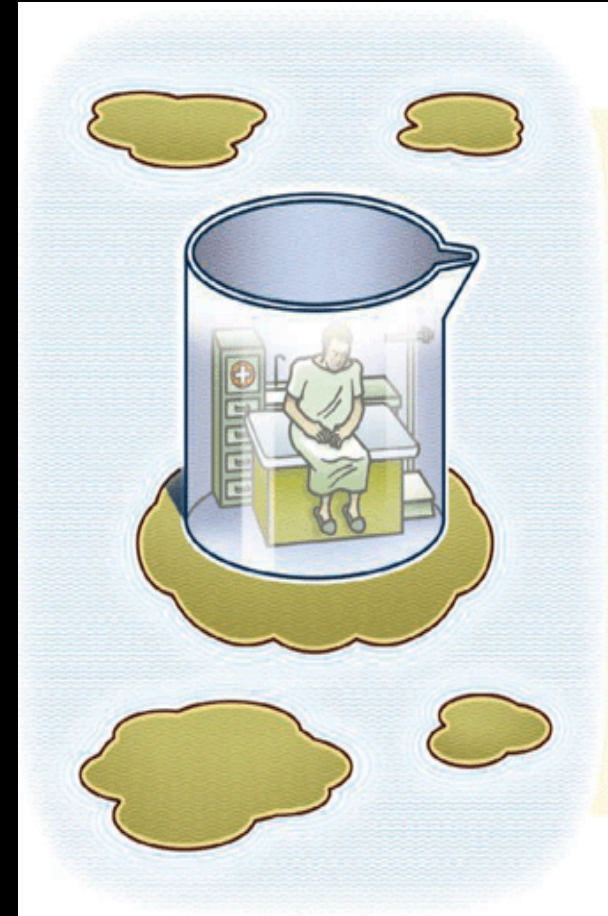


Amiri-Nikpour MR et al. An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect. Acta Neurol Scand 2015 Jan;131(1):45-50.

Social isolation and stroke

- **Clinical studies**

- SI increases risk of mortality
- SI independently increases stroke risk
- SI leads to poorer recovery (controlling for NIH etc)
- Stroke patients with low social support have increased risk of recurrent stroke

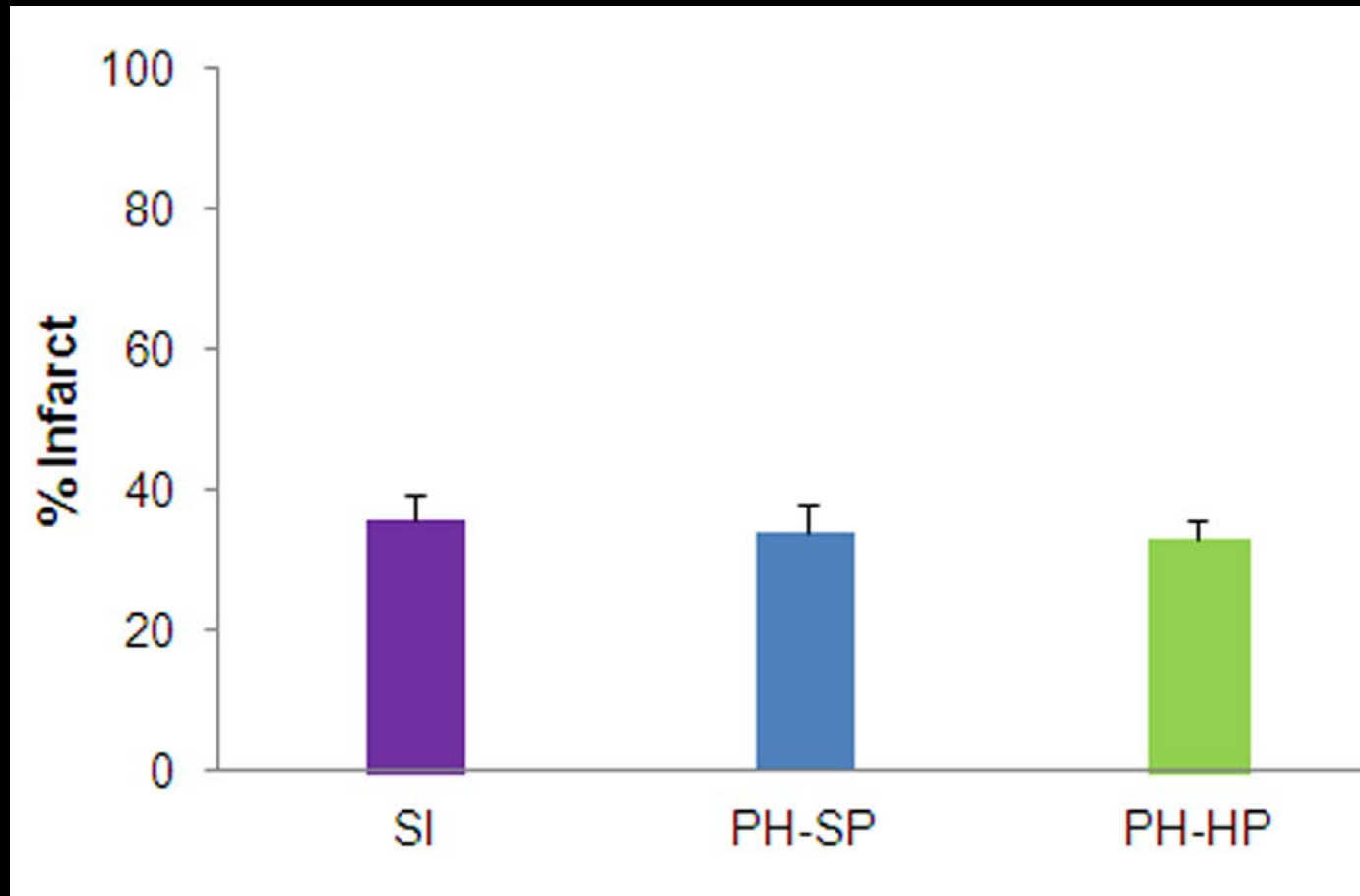


Epidemic of loneliness

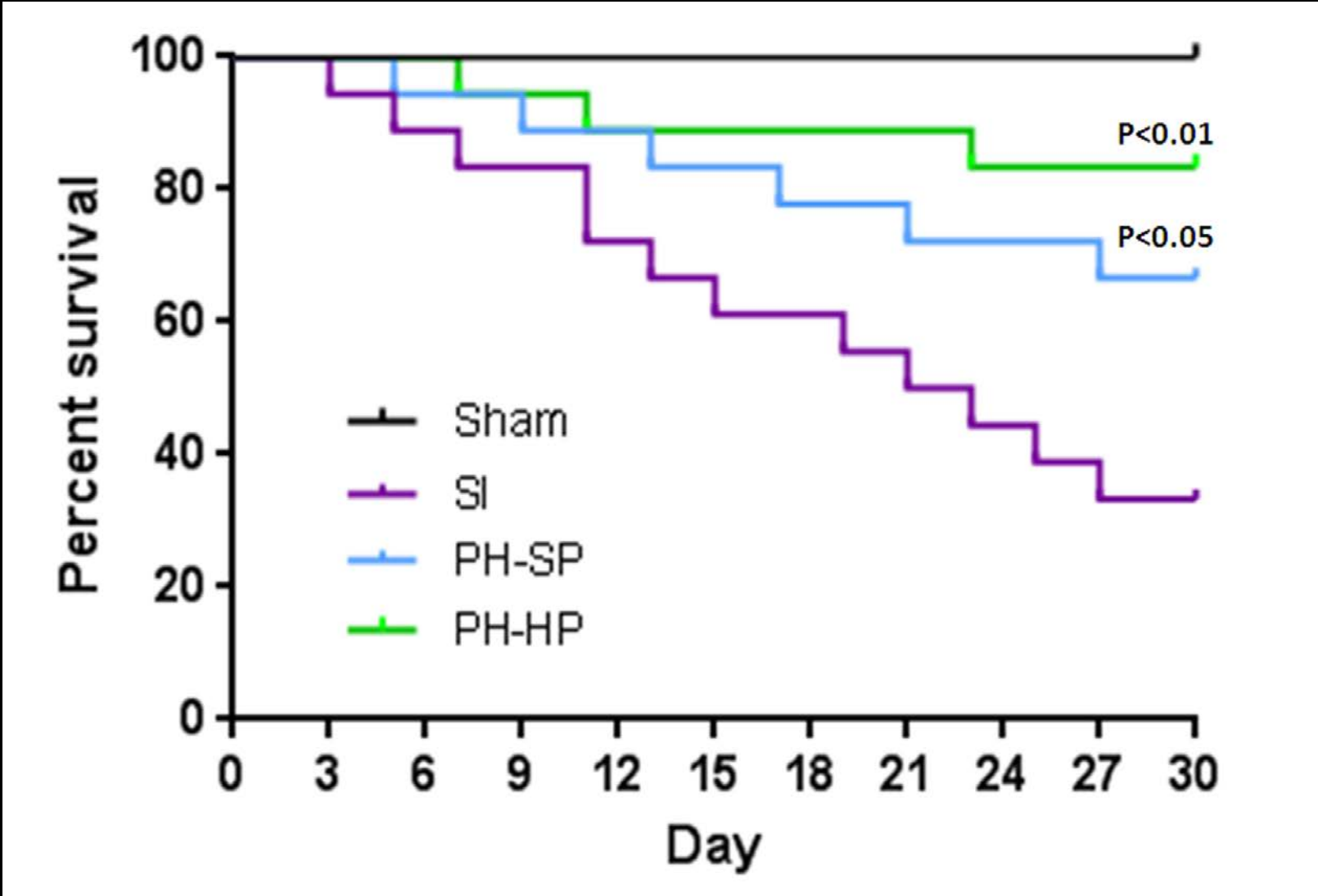
- **52% of women live alone compared to 29% for men after age of 80**
- **Many will have outlived not only spouses/partners but also their friends and children.**
- **Cardiovascular, nervous and immune targets**



Infarct Size was equivalent at 30 and 90 days when SI was delayed 72 hours after stroke



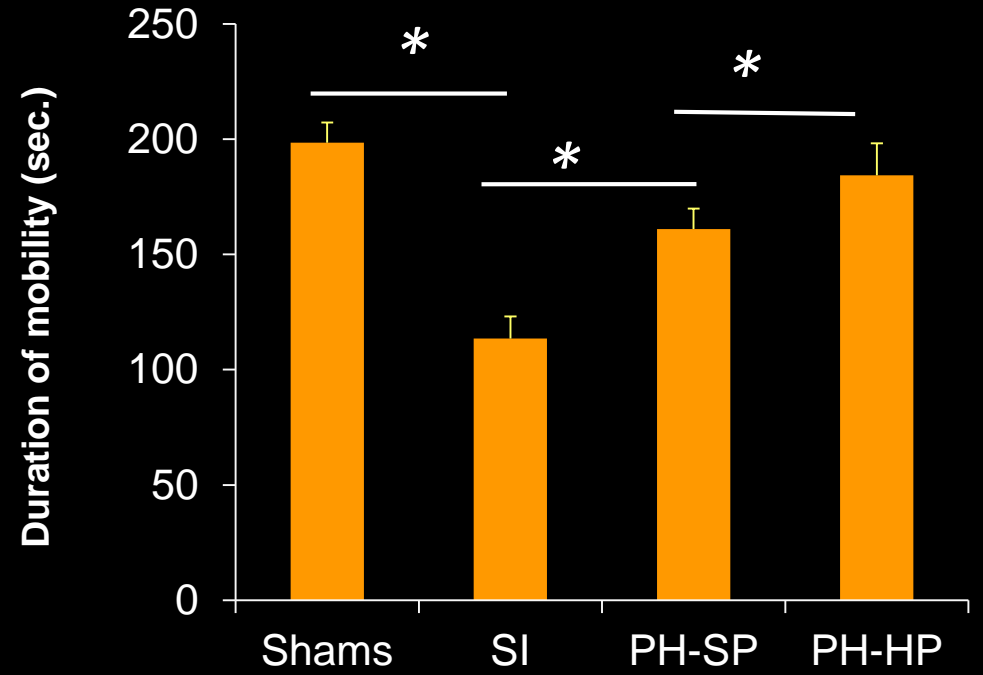
Mortality



Tail suspension Test at 90d



<http://lcn.brain.riken.jp>



Systemic Effects of Stroke

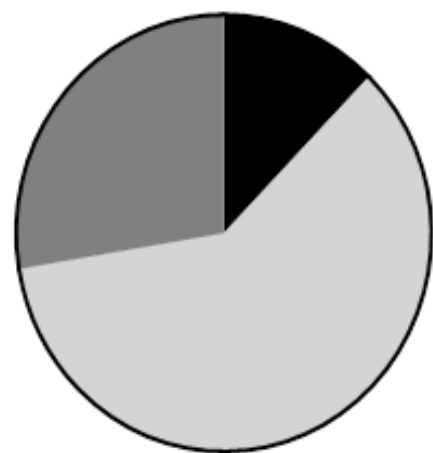
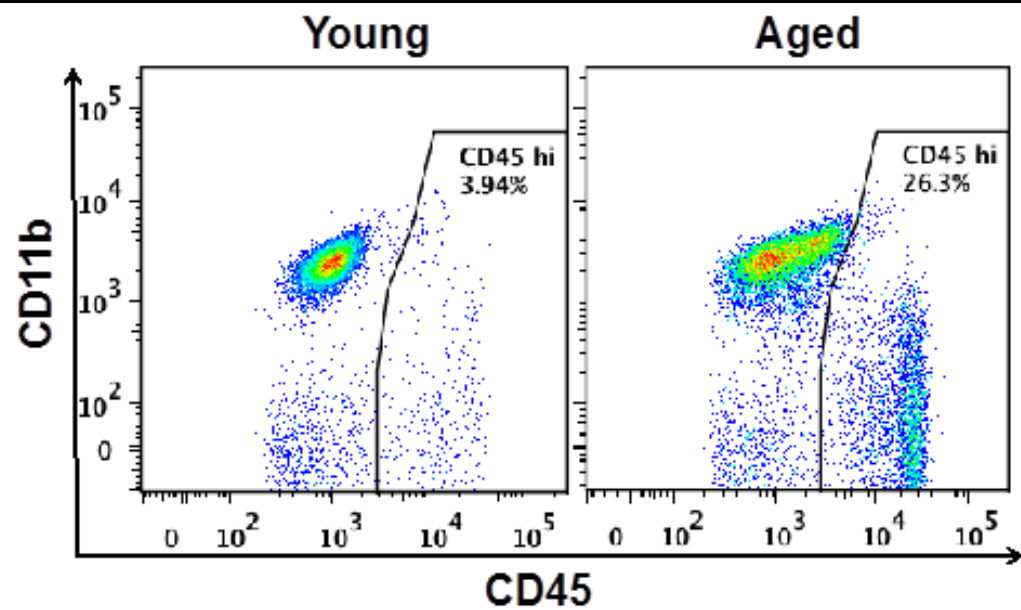
- **Peripheral Effects**



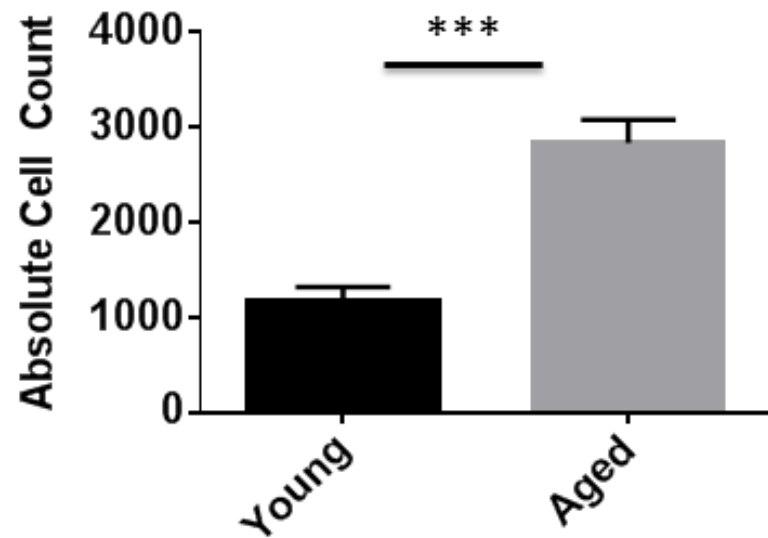
- **Role of Peripheral Leukocytes/Myeloid Cells**
- **Local Response...**
- **Ageing... T cell deficits? Infections?**

Fat in aged versus young mice

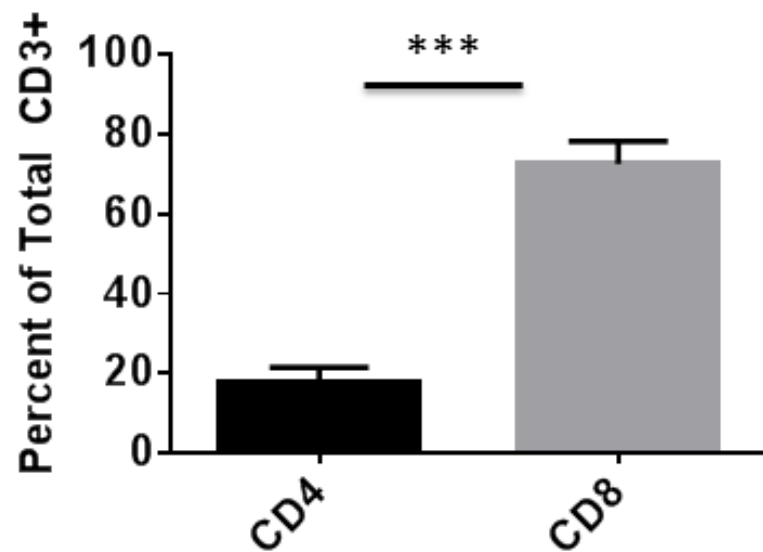




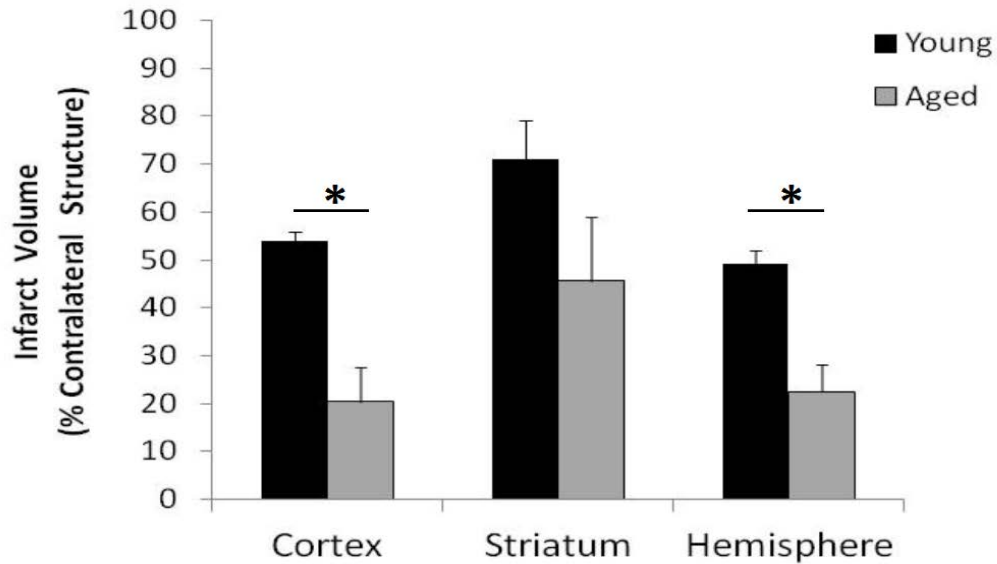
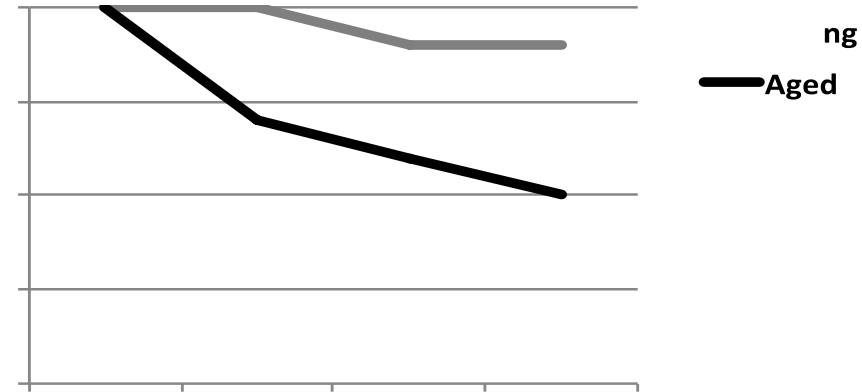
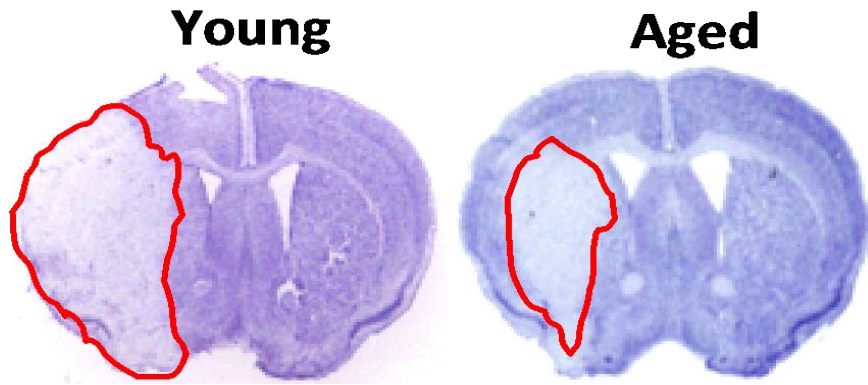
■ 12% Myeloid
 ■ 60% T cells
 ■ 28% Other



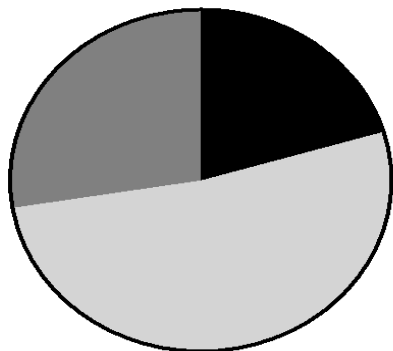
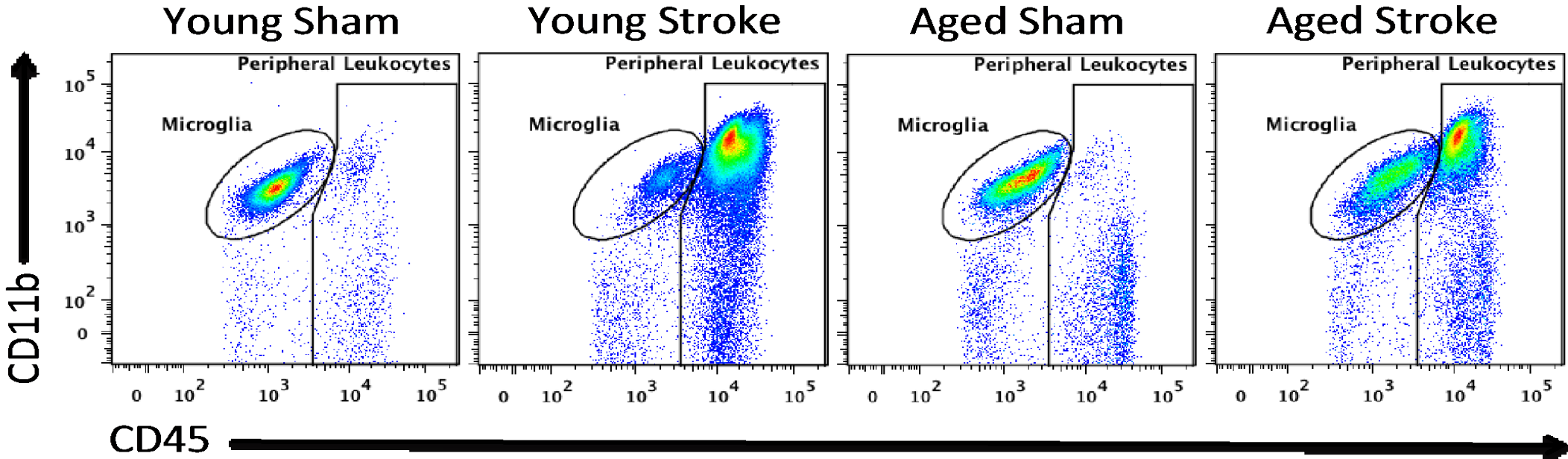
D



Aged mice have smaller infarcts but MUCH worse recovery

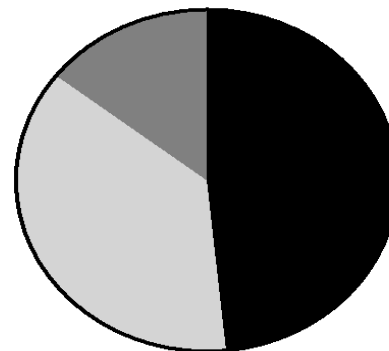


The Composition of Infiltrating Leukocytes After Stroke Differs with Age



20% Ly6G⁺ Neutrophils
52% Ly6C⁺ Monocytes
28% Ly6G⁻/Ly6C⁻ Other

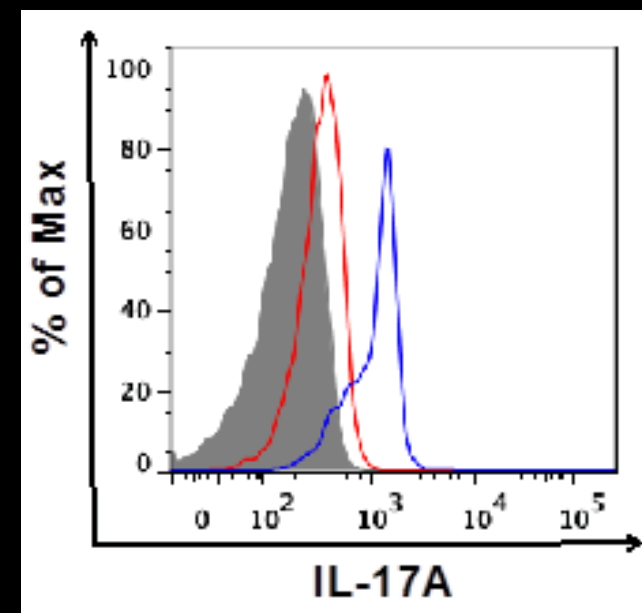
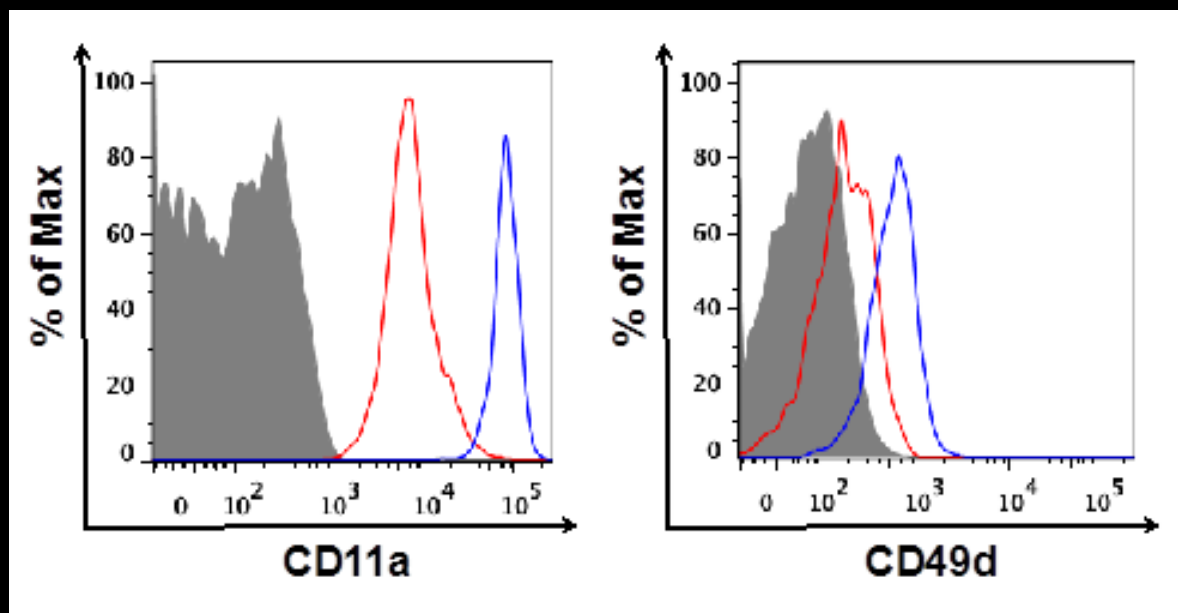
Monocytic



48% Ly6G⁺ Neutrophils
37% Ly6C⁺ Monocytes
15% Ly6G⁻/Ly6C⁻ Other

Neutrophilic

High expression of T cell adhesion markers on CD8 T cells in aged in blood and brain compared to young mice

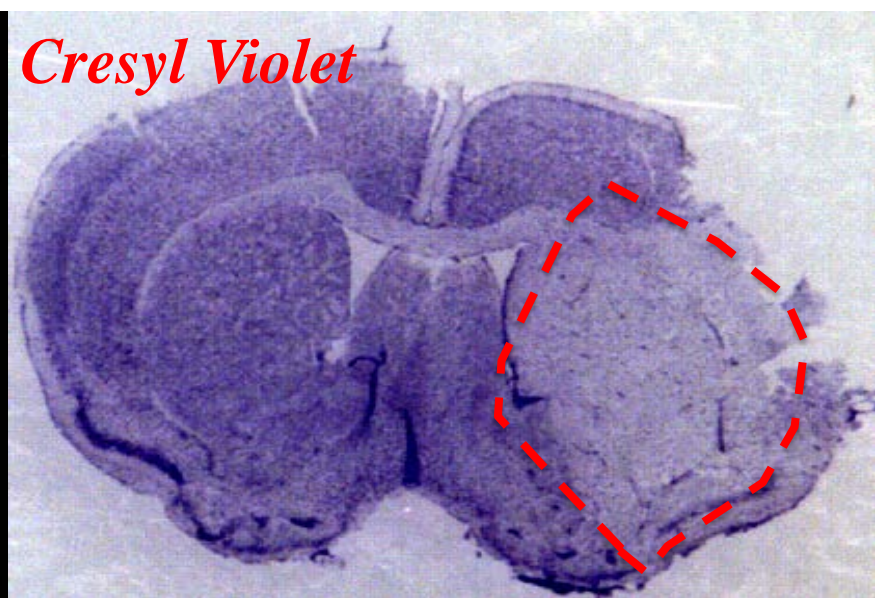
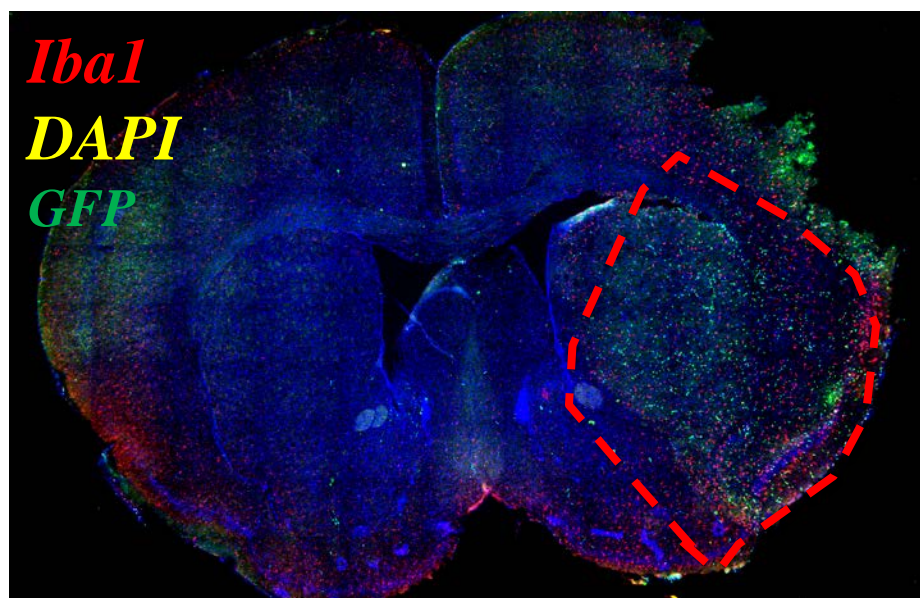
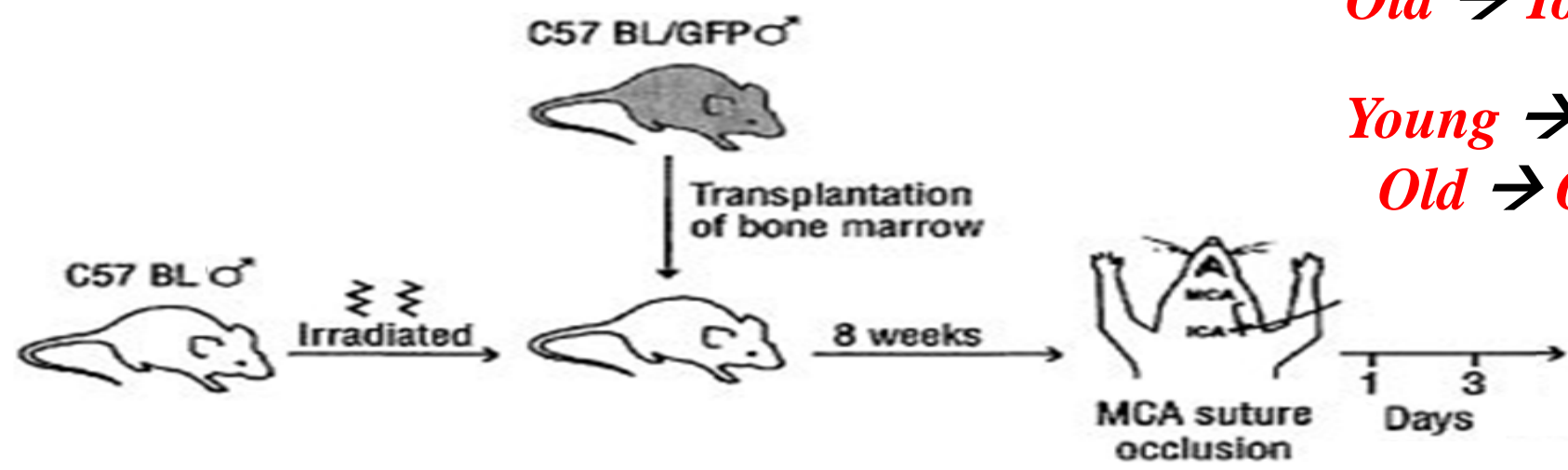




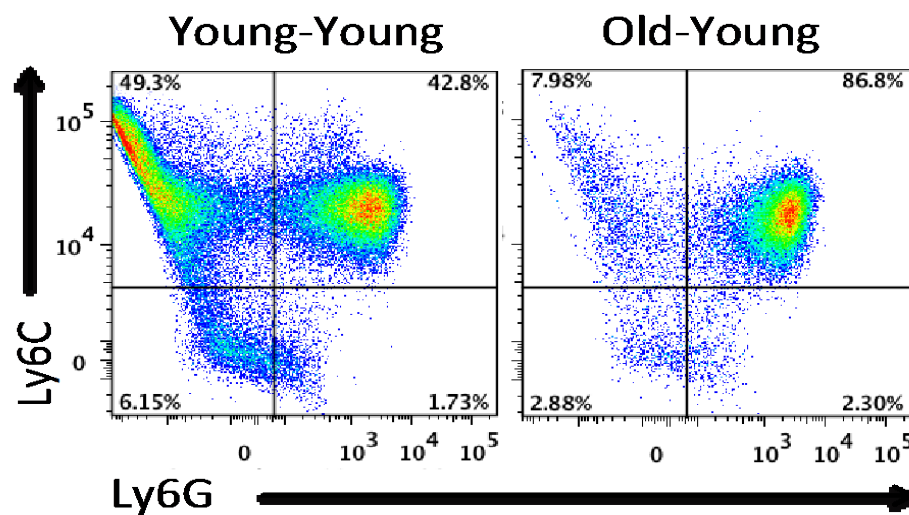
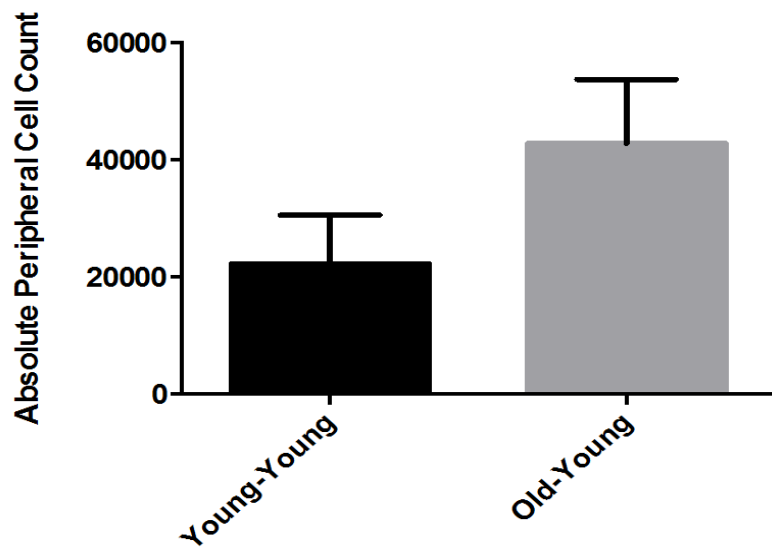
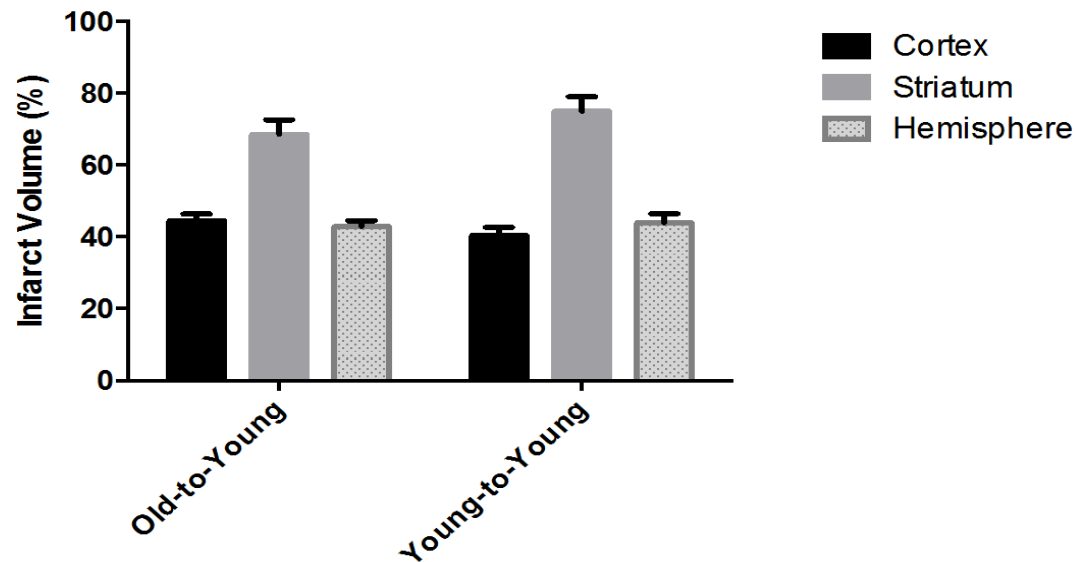
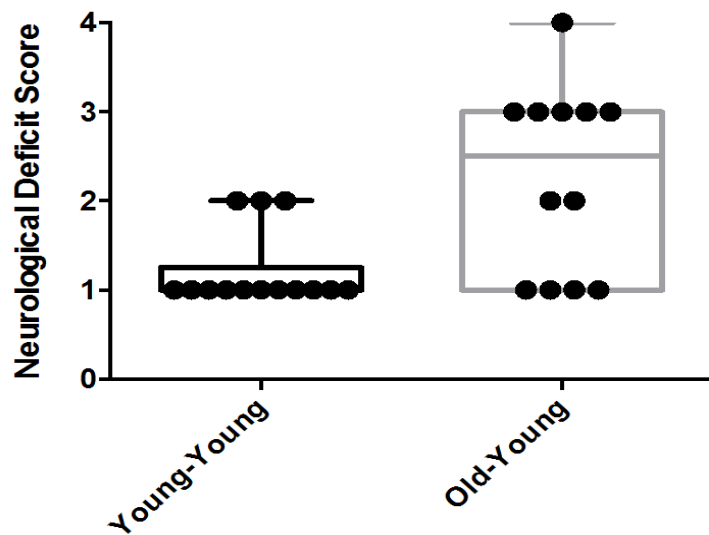
Generating Heterochronic Bone Marrow Chimeras

Young → Young
Old → Young

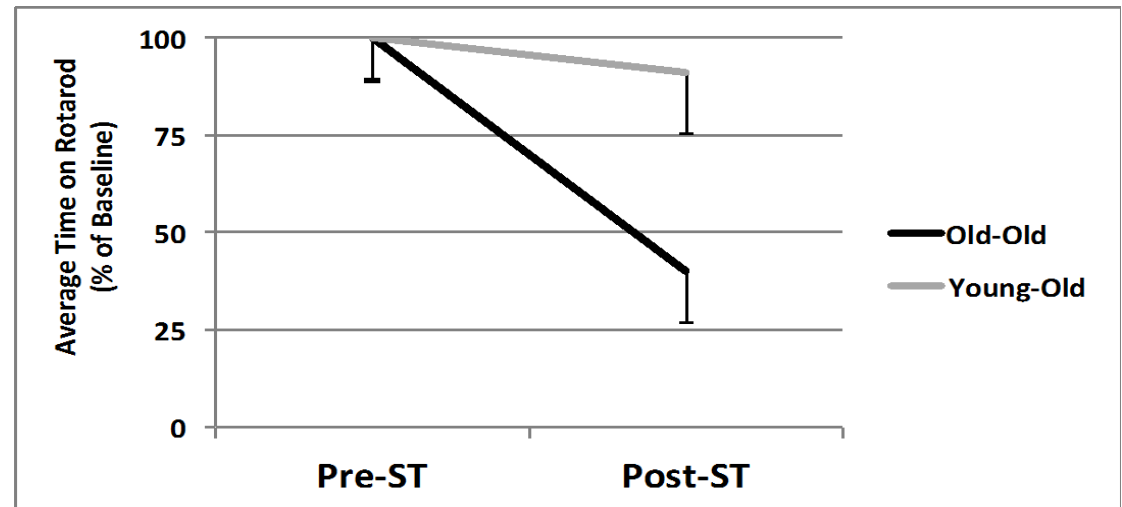
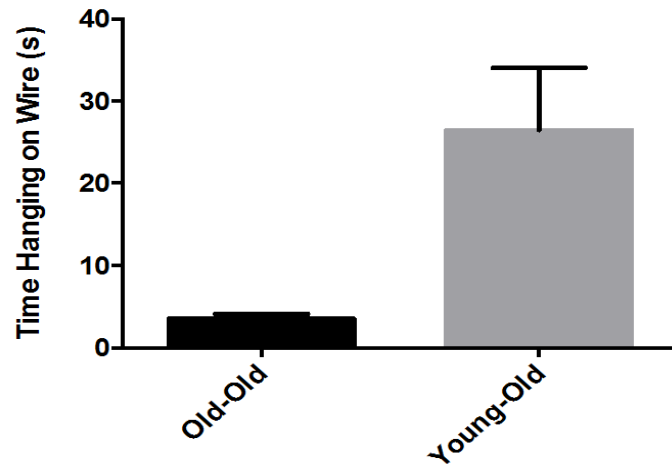
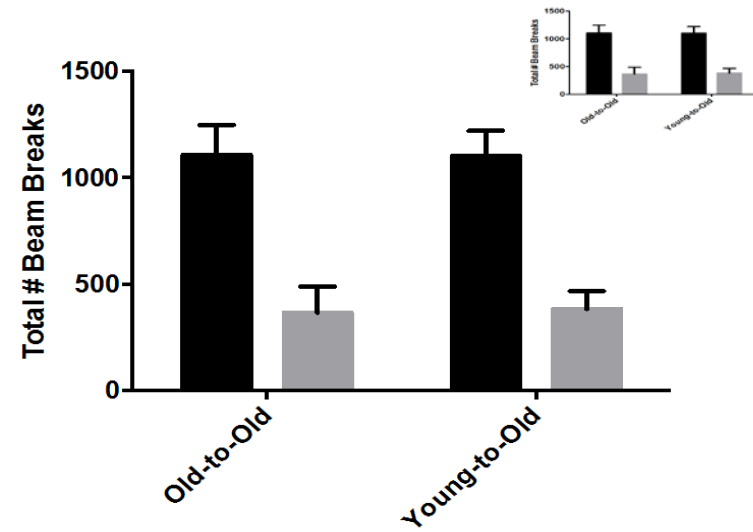
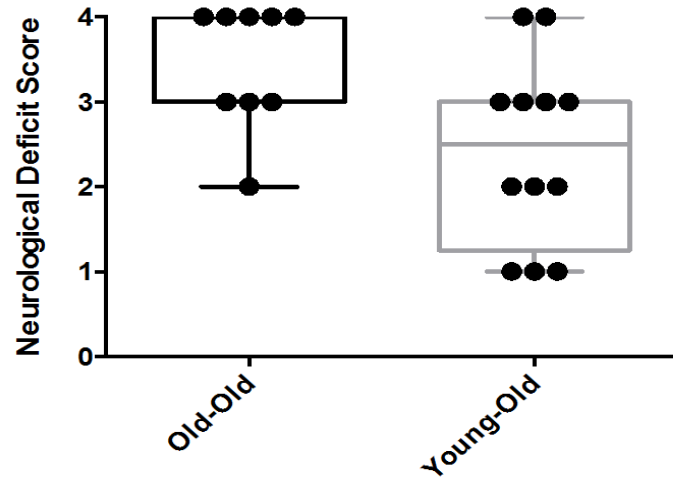
Young → Old
Old → Old



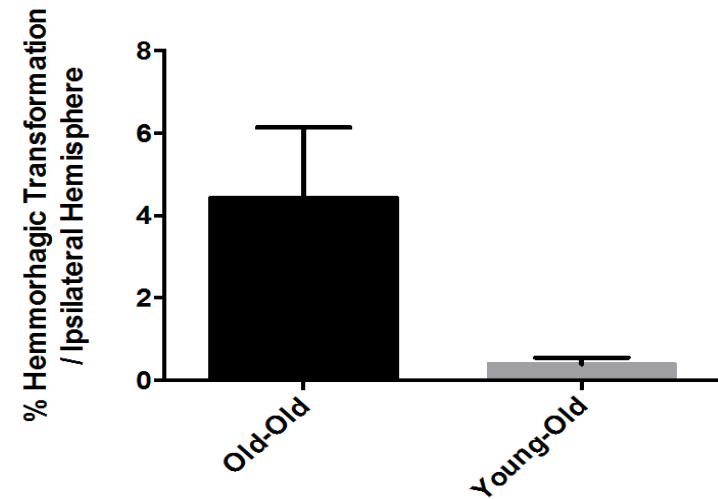
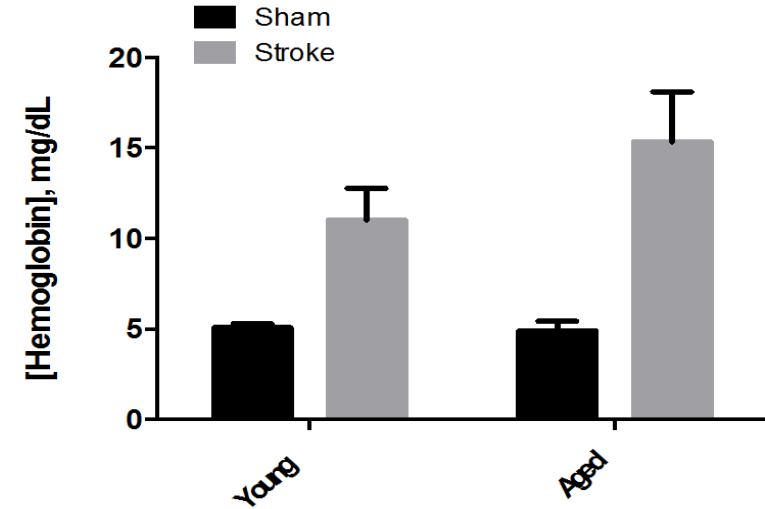
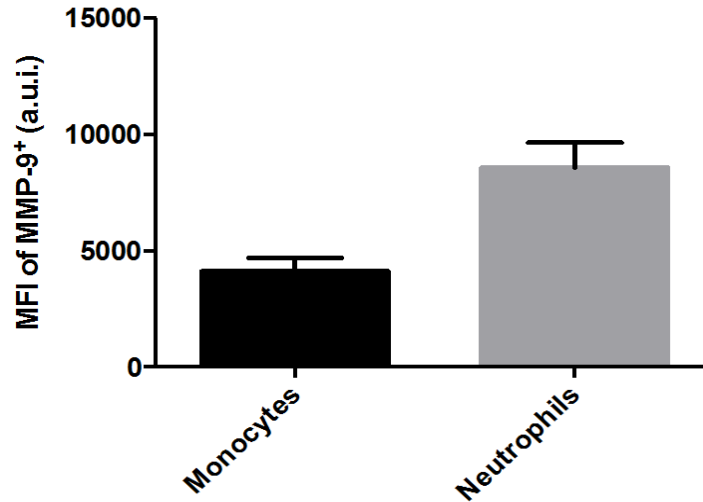
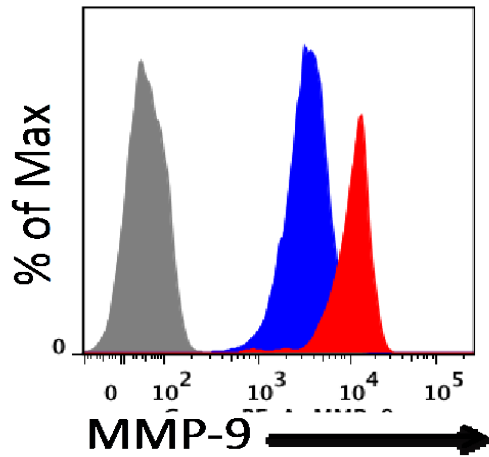
Aged bone marrow contributes to worse recovery in young mice



Young bone marrow contributes to enhanced recovery in Aged mice



Aged Bone Marrow Contributes to Increased Hemorrhagic Transformation



Translational Research

- Targeting mechanisms and developing therapies based on preclinical information from young animals may be futile
- We need better animal models-but these are expensive
- Validate pre-clinical targets in human populations (IP-10)
- Translation to CLINICAL TRIALS----Better clinical trial design!
- Really helps to have people that know both involved EARLY on in clinical trial design