Impact of not including sex as a variable in your research

Farida Sohrabji
Texas A&M Health Science Center

ORWH Methods Workshop 2014

Texas A&M Health Science Center College of Medicine

Incorporating sex as a variable in neurologic disease: Stroke as a case study

Women's Health in Neuroscience
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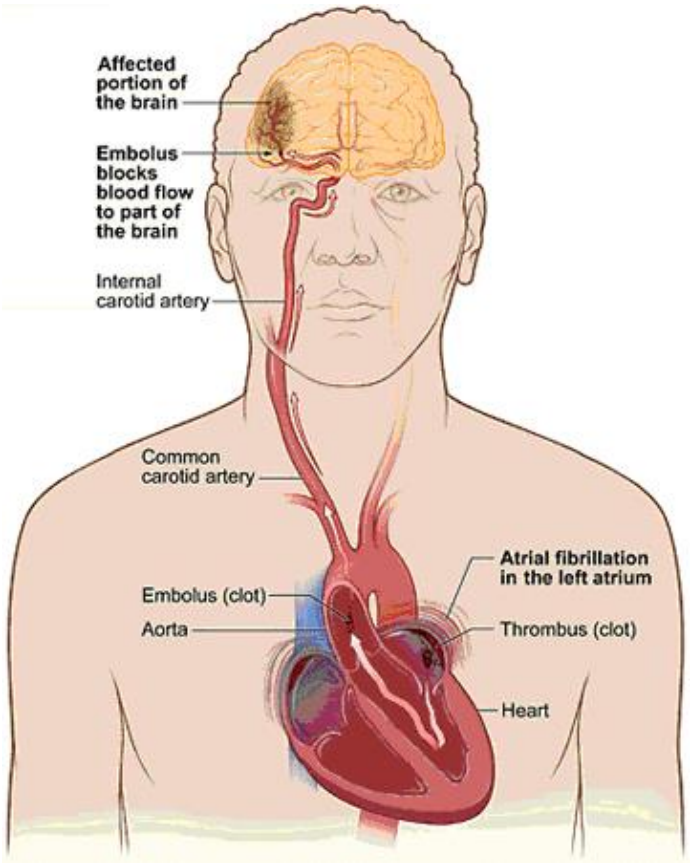
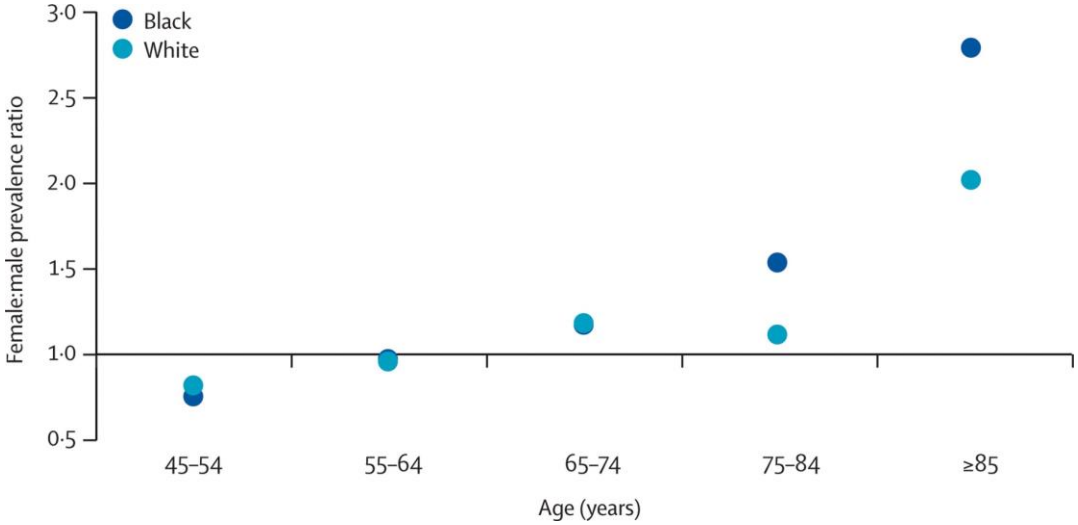
Why? Stroke incidence is influenced by age and sex





Incorporating sex as a variable in neurologic disease: Stroke as a case study

Sex differences in stroke prevalence

Table 1 Comparison of stroke prevalence in men vs women and across age groups 35 to 64 years in NHANES 1999 to 2004

Variable	Comparators		Odds ratio	95% CI	*p* Value
Age group	Women*	Men*			
35-44 years	1.2%	1.0%	1.2142	0.4715-3.1268	0.6876
45-54 years	2.5%	1.0%	2.3903	1.3205-4.3267	0.0040
55-64 years	3.4%	3.0%	1.1256	0.6218-2.0376	0.6961
Sex	Age group	Age group			
Female	45-54 years	35-44 years	2.1346	0.9491-4.8011	0.0667
Female	55-64 years	45-54 years	1.3969	0.6912-2.8229	0.3518
Female	55-64 years	35-44 years	2.9818	1.3329-6.6704	0.0078
Sex	Age group	Age group			
Male	45-54 years	35-44 years	1.0843	0.4303-2.7322	0.8637
Male	55-64 years	45-54 years	2.9665	1.5407-5.7115	0.0011
Male	55-64 years	35-44 years	3.2165	1.4735-7.0213	0.0034

* Mobile Exam Center weighted percentages.
NHANES = National Health and Nutrition Examination Survey.

Towfighi et al., 2007

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Why? Sex differences in post stroke disability

Table 1

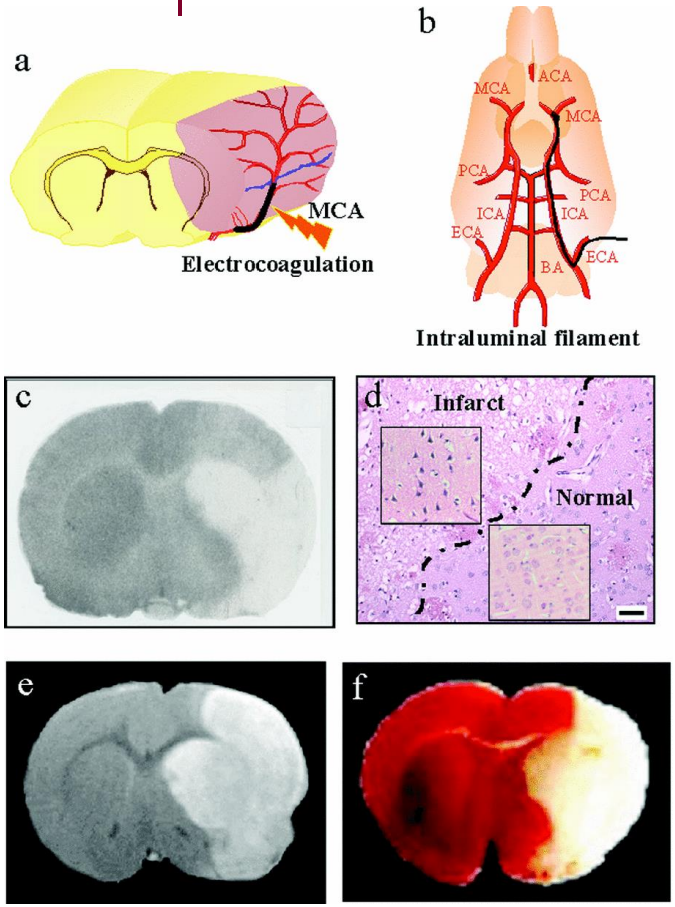
Differences in prevalence of stroke, incidence of stroke, disability, and mortality post-stroke betweei women in the United States

	Women	Men
Prevalence (as of 2005), million	3.9	2.6
Average age of first stroke, y	72.9	68.6
Incidence		
55–64 y	–	25% higher
65–74 y	–	50% higher
75–84 y	25% higher	
Disability: 3–6 mo post-stroke, unable to do independently, %		
Eating	15	9
Dressing	37	20
Grooming	32	17
Transfer from bed to chair	32	13
Walking	32	18
Mortality (as of 2005)	86,993	56,586

(*Data from* the American Heart Association [1•], Appelros et al. [4], and Petrea et al. [7].)

Persky et al., 2010

Experimental stroke models: measure severity not risk!



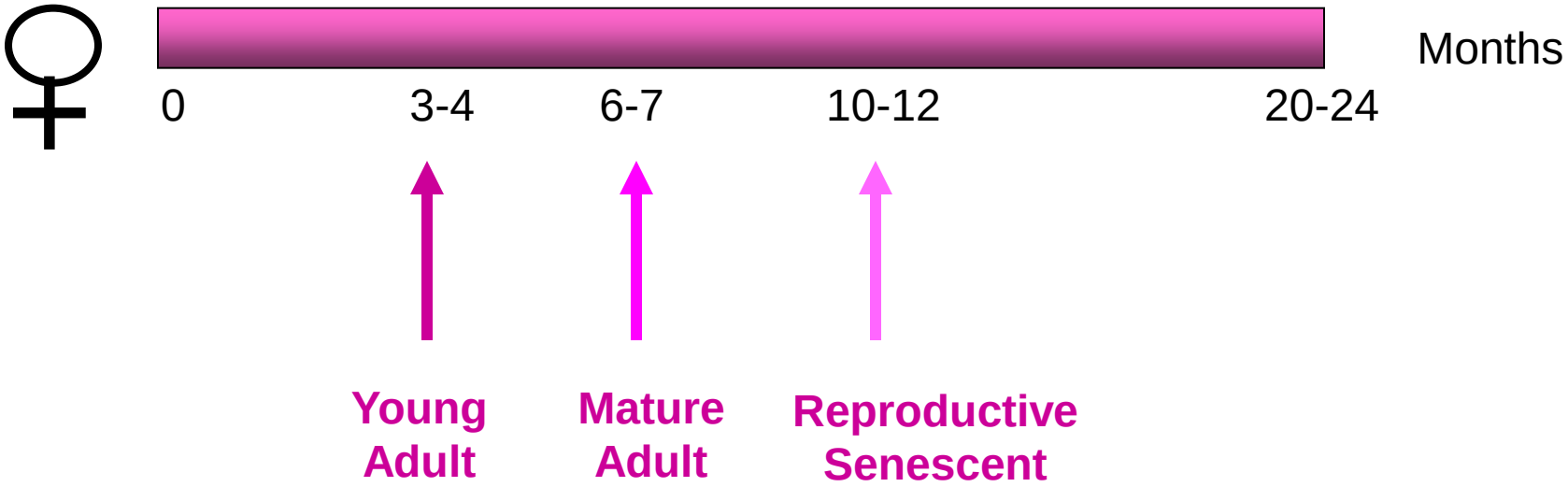
Nissl stained sections

TTC stained coronal slice.

From MacRae and Carswell: Biochemical Society Transactions www.biochemsoctrans.org
Biochem. Soc. Trans. (2006) 34, 1362-1365

Clinically relevant model : Reproductive Aging

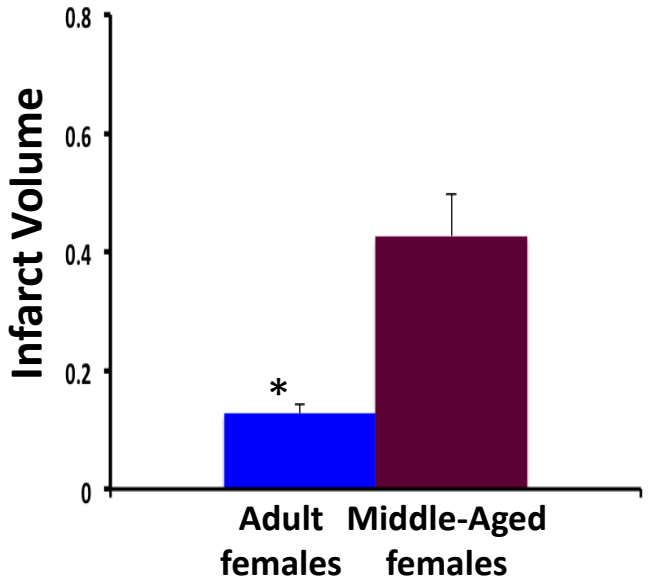
Sprague Dawley Rat

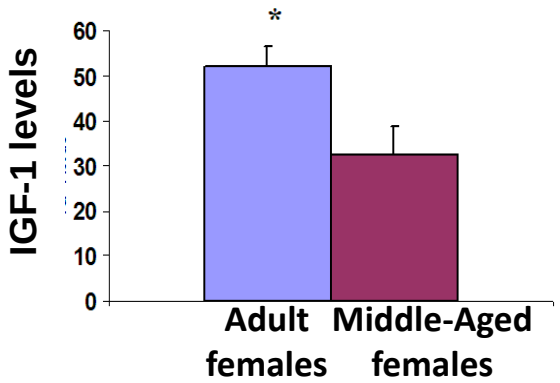


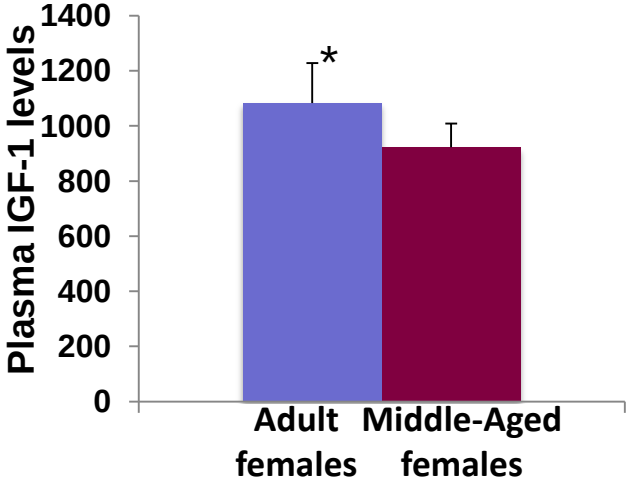
Estradiol	**YA**	**=**	**MA**	**>**	**RS**		
Progesterone			**MA**	**>**	**RS**		
FSH	**YA**	**<**	**MA**	**<**	**RS**		

Perils of not including sex as a biological variable: Incorrect interpretations

Hypothesis: Middle aged female rats have larger infarct volumes as compared to young females and this is due, in part, to low availability of IGF-1.







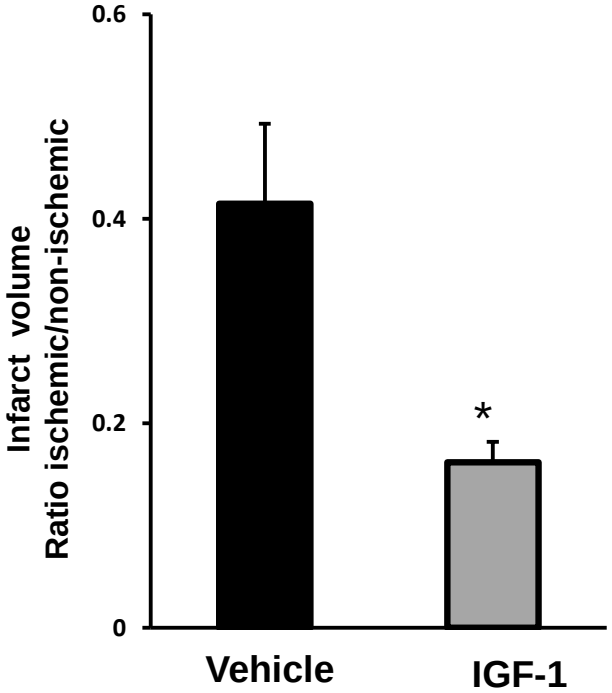
Selvamani and Sohrabji, J. Neuroscience 2010

Perils of not including sex as a biological variable: Incorrect interpretations

Hypothesis: IGF-1 treatment to middle aged female rats after ischemia will improve outcomes
Result: IGF-1 is neuroprotective
Conclusion: Age related loss of IGF-1 underlies more severe stroke outcomes.

Post-stroke 24h

NOT SO FAST….



Bake et al., PLoS, 2014

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Perils of not including sex as a biological variable: Incorrect interpretations

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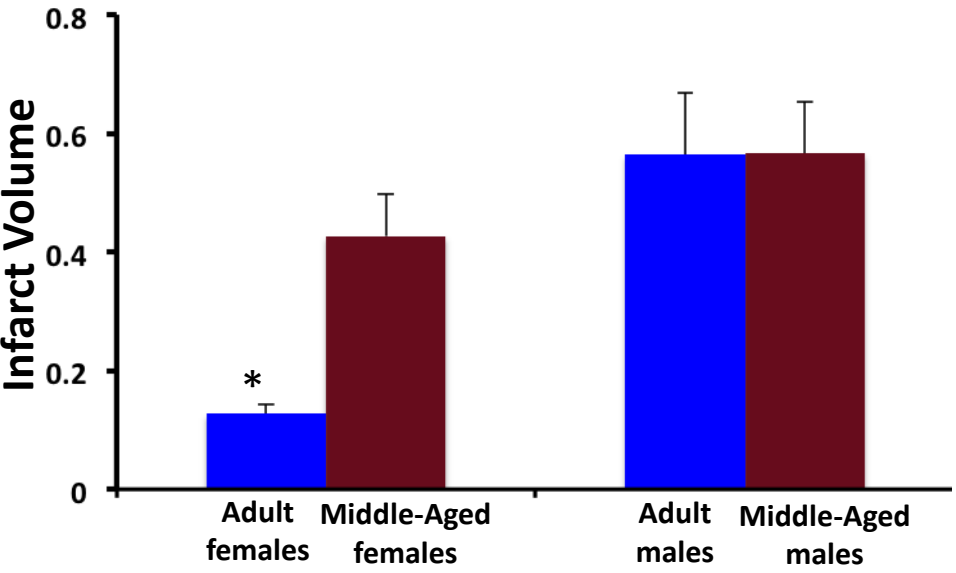
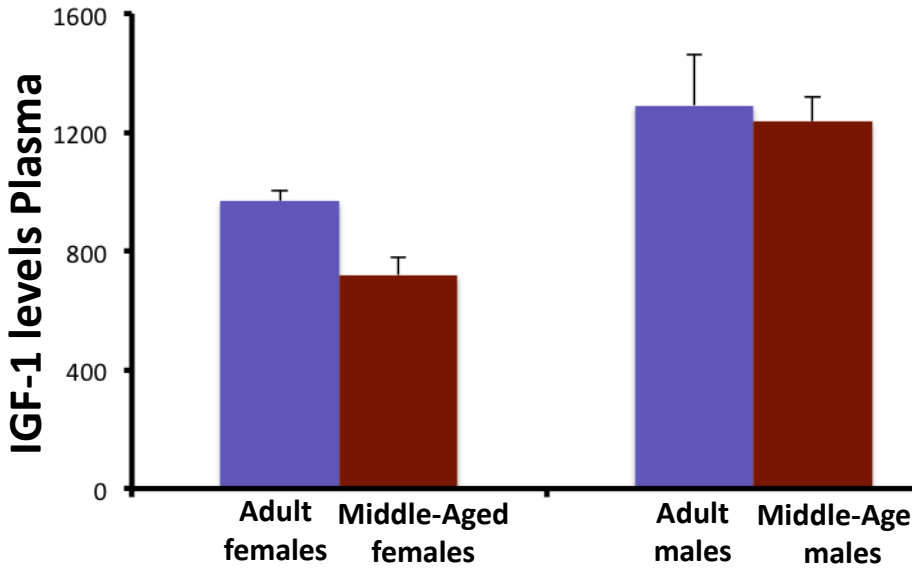
Age and Sex differences in infarct severity



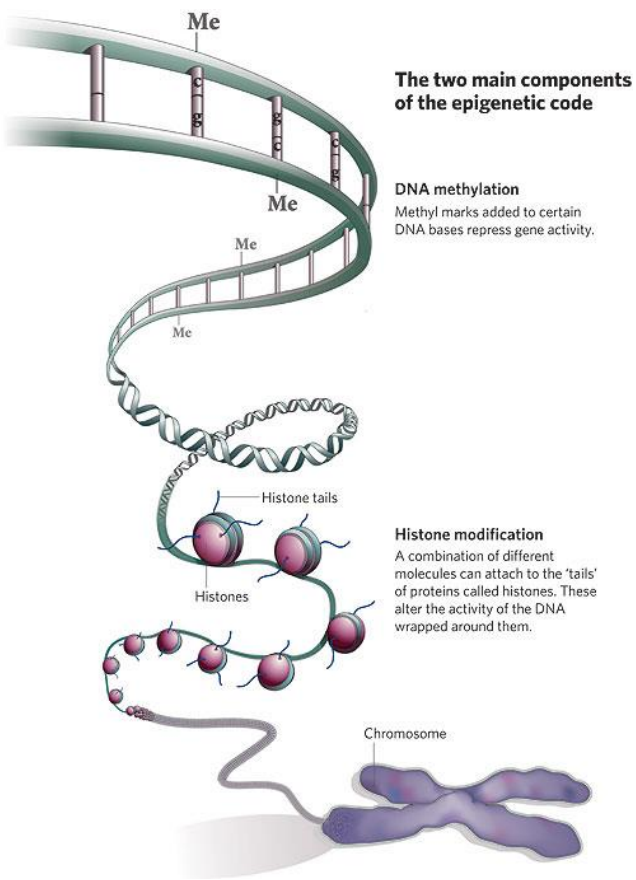
Age and Sex differences in IGF-1

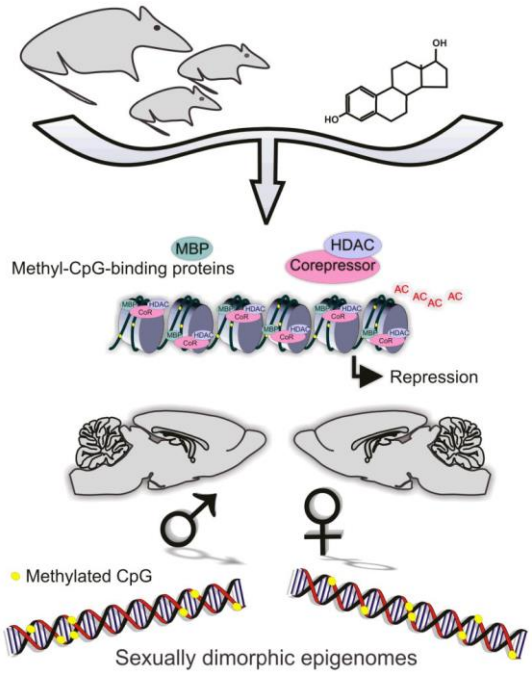


Male rats have much higher levels of IGF-1, but worse infarct volumes.
Interpretation: It's not just IGF-1 availability!

Selvamani et al., Clinical Science, 2014

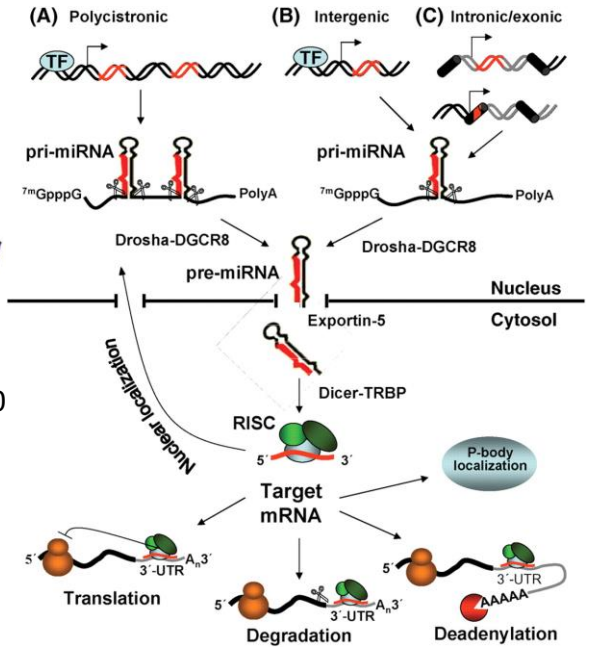
Perils of not including sex as a biological variable:
Missed opportunities





McCarthy et al., J. Neurosci, 2010

microRNA



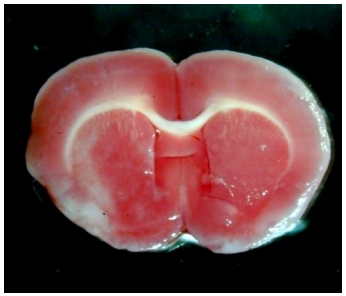
Perils of not including sex as a biological variable: Missed opportunities

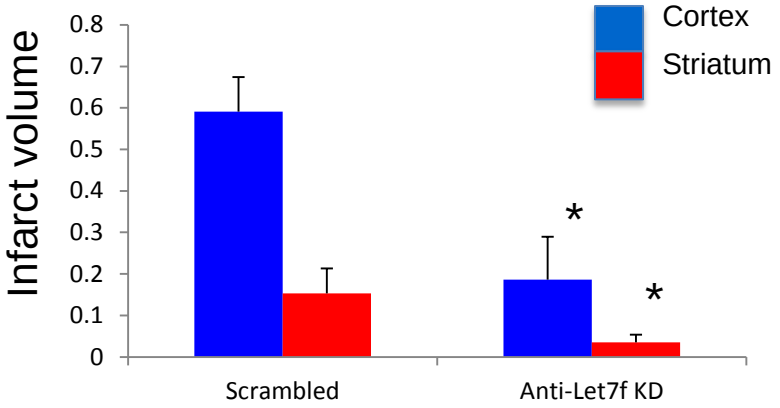
MicroRNA as stroke therapy

Adult females

Control

Anti-Let 7f

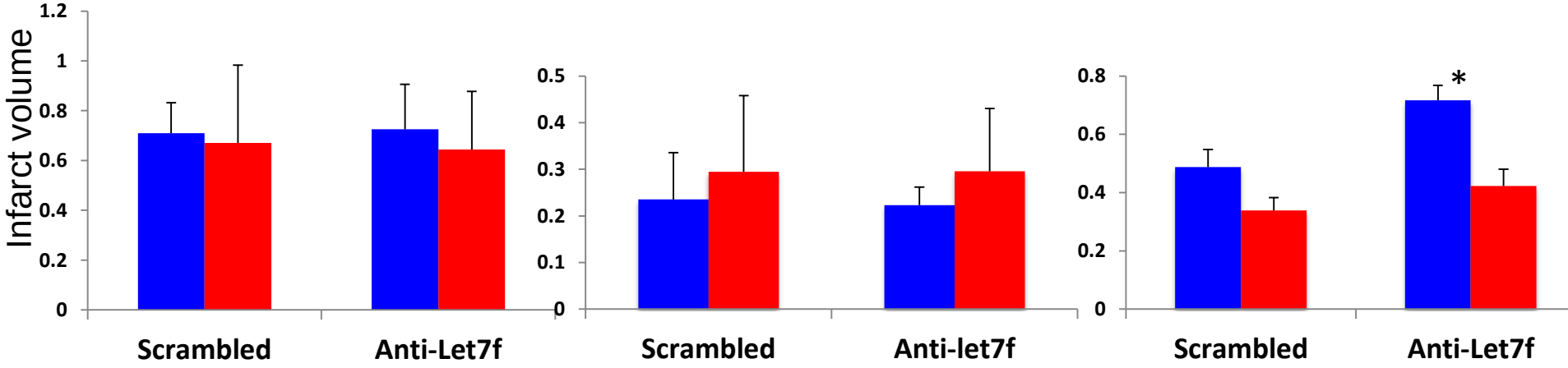




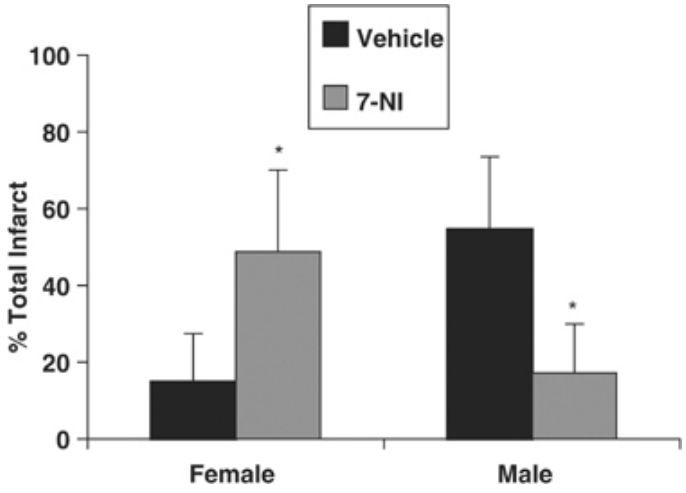
No effect in Males

No effect in OVX Females

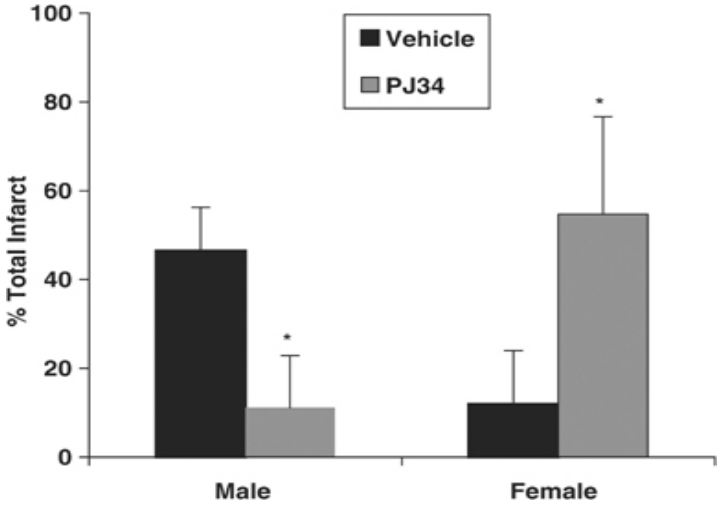
Toxic effect in middle aged females



Cell death pathways that are effective in males but not females



nNOS inhibitor

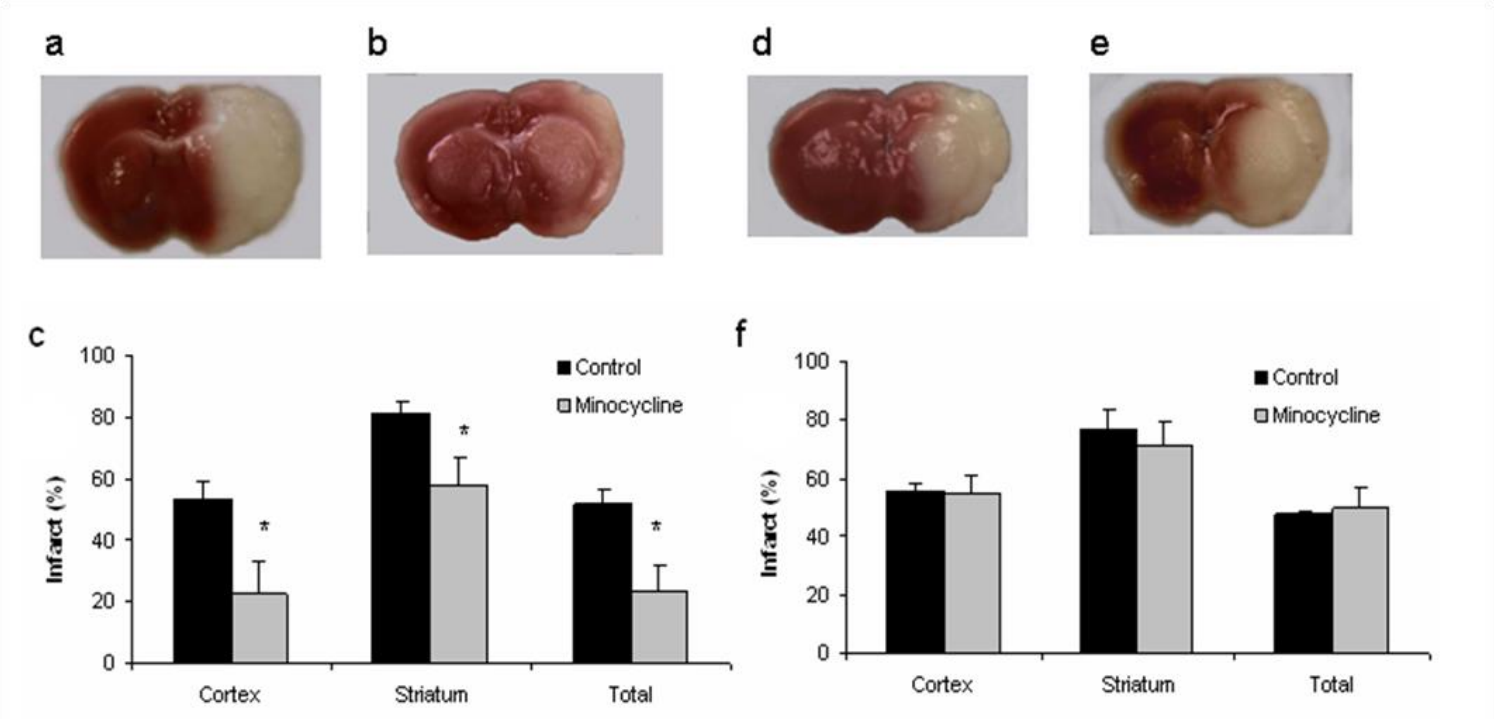


PARP inhibitor

McCullough et al, JCBFM, 2005

Perils of not including sex as a biological variable:
Sex differences in therapies

Minocycline treatment is ineffective for stroke in females



Li and McCullough 2009

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Perils of not including sex as a biological variable: Missed opportunities

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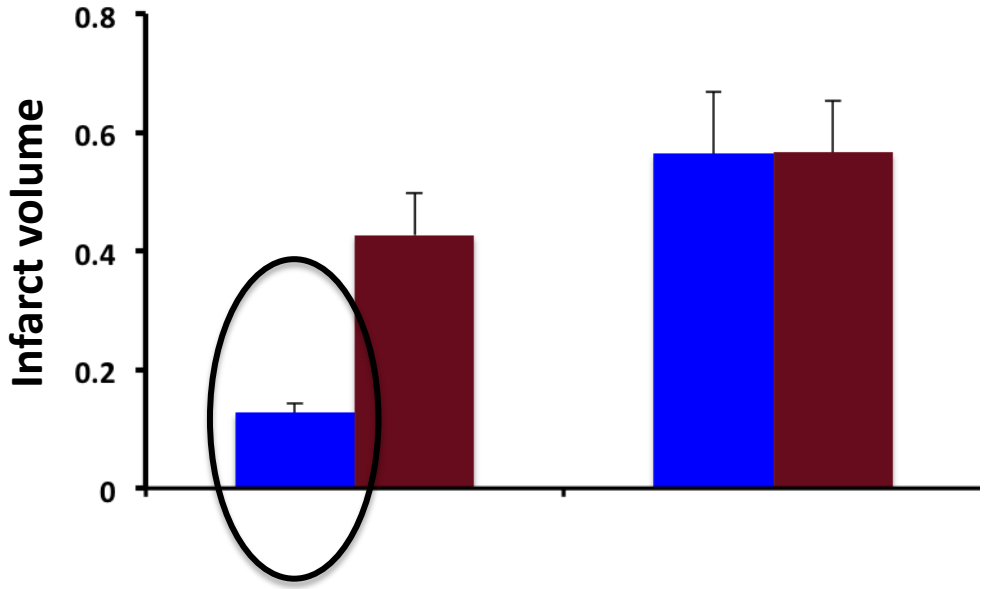
Developing a therapeutic strategy for stroke in aging populations

Exploit age and sex differences in stroke severity



Selvamani et al., Clinical Science, 2014

Differences in estrogen levels?
Has been tested, but has anomalous effects in older population

What else would have comprehensive effects on the transcriptome?
Active and repressive epigenetic marks
Alterations in microRNA

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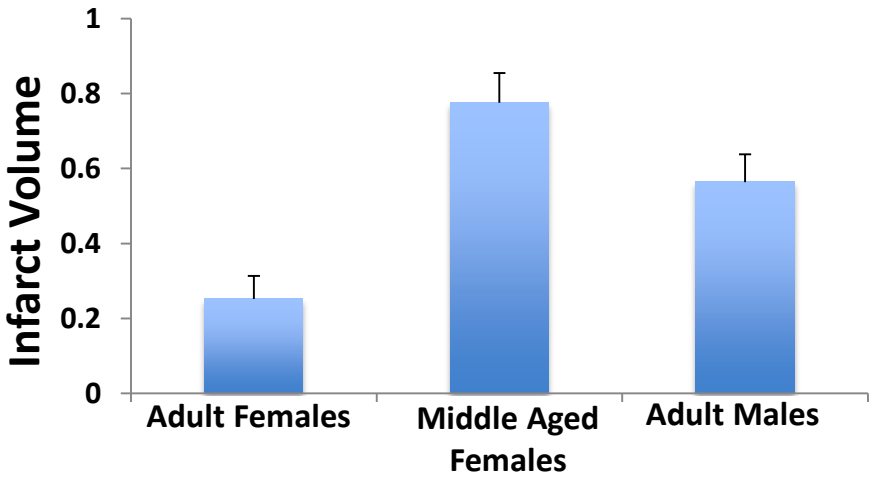
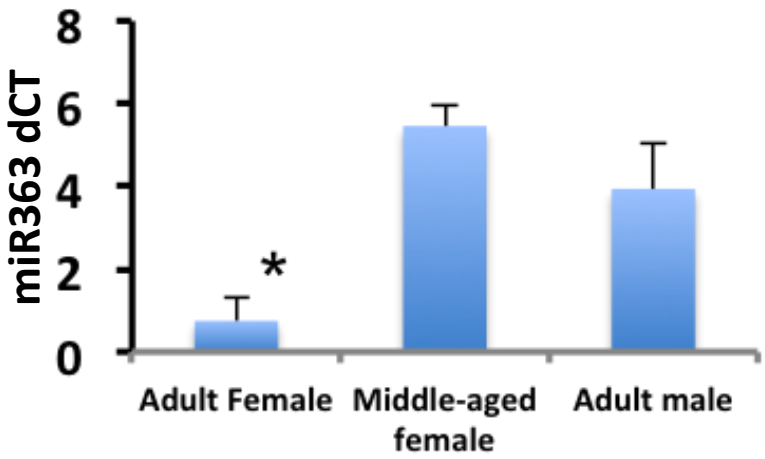


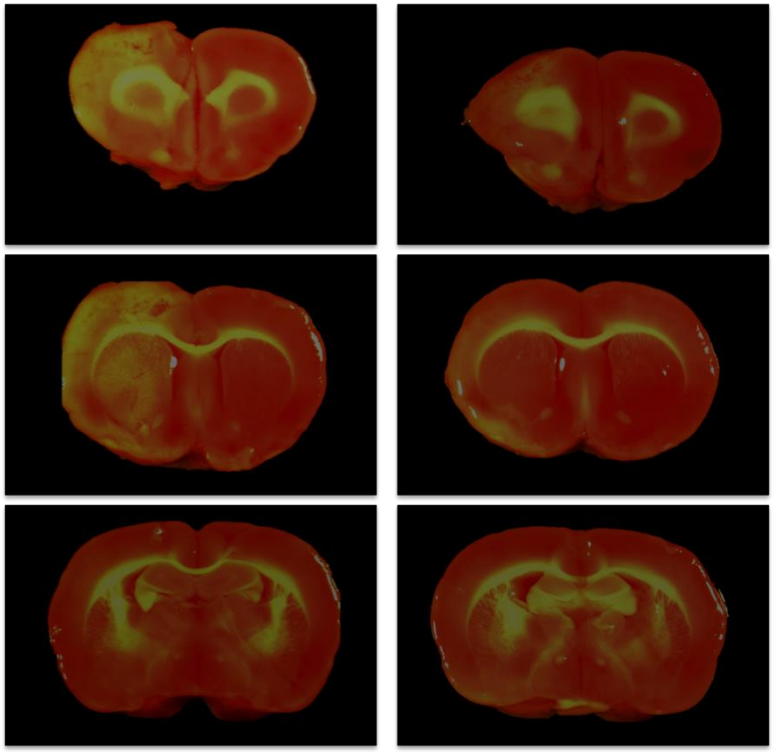


Selvamani et al., Clinical Science, 2014, and Unpublished observations

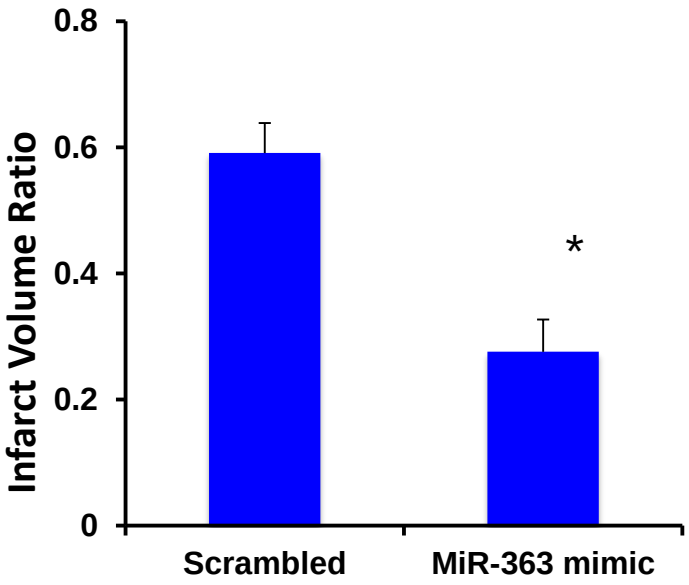
Identifying a therapeutic target by exploiting age and sex differences.

Middle Aged Females

Scrambled **miR-363 mimic**



Infarct volume



Selvamani and Sohrabji, Unpublished observations

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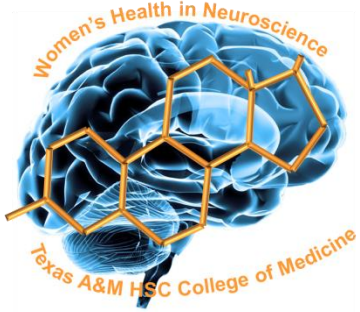
Random thoughts and lessons learned the hard way



- Including sex as a biological variable is expensive and time consuming; excluding sex as a biological variable is bad science

- Females are not "more difficult" to work with than males. Special considerations are needed for the estrous cycle in young animals, and for midlife events.
  - These events won't affect all outcomes
  - They can be controlled for, like any other variable

- Besides the obvious need to study both sexes in biomedical research for generalizability and applicability, sex differences can serve as a means to understand mechanism, develop therapeutic targets, exclude competing hypotheses.
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Thanks and Questions

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