Imperial College London

Stroke: Clinical presentation, physiology and treatment strategies

Dr. Felicity N. E. Gavins

Wolfson Neuroscience Laboratories,
Imperial College,
Faculty of Medicine,
Hammersmith Hospital Campus,
Burlington Danes Building,
Du Cane Road,
London W12 0NN.

Tel: 020 7594 6586
Email: f.gavins@imperial.ac.uk

Imperial College London

Synopsis and Learning Objectives

Synopsis

1. Clinical features
2. Experimental models
3. Novel treatment strategies

Learning objectives

1. Describe the clinical differences between different types of stroke
2. Outline the therapies for intervention in stroke.
3. Describe different animal models of stroke and be able to discuss the cellular interactions that are involved.

Imperial College London

The Demands of the Brain

Brain uses:

10-20% of cardiac output

20% of body oxygen consumption

66% of liver glucose

Therefore, the brain is very vulnerable if the blood supply is impaired.

Imperial College London

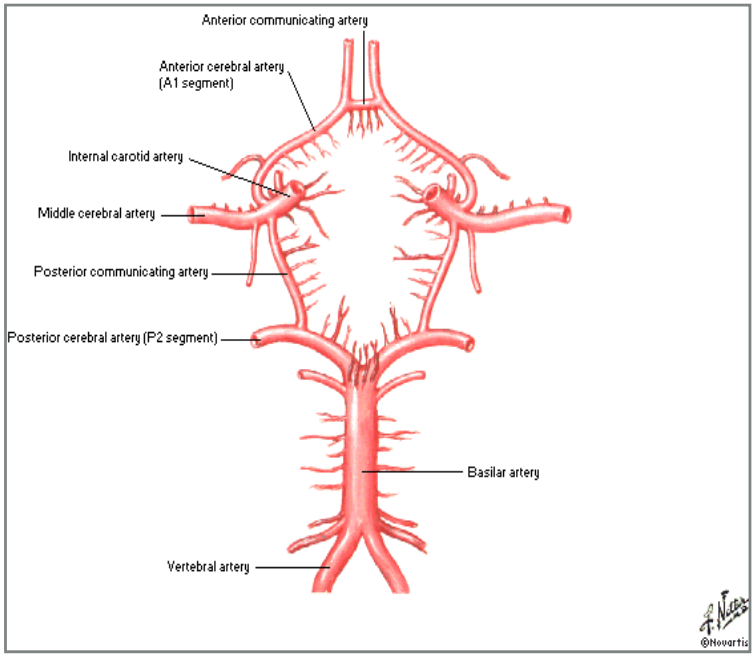
Blood Supply to the Brain

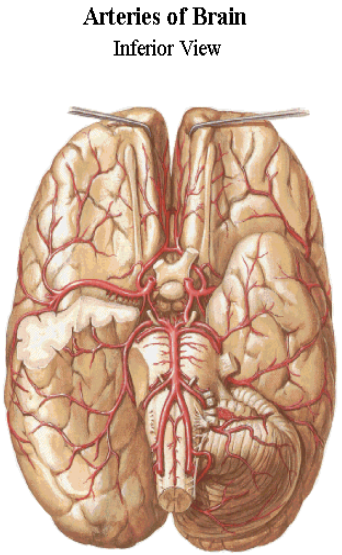
Two sources:

-Internal carotid arteries
-Vertebral arteries

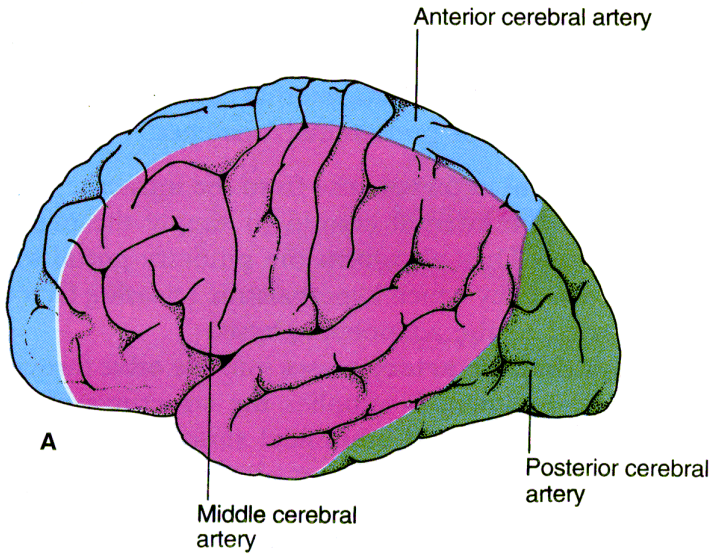
Circle of Willis

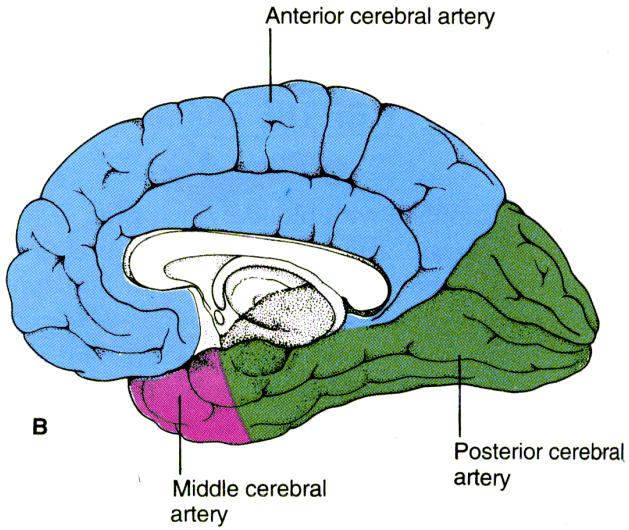
Cerebral Arteries





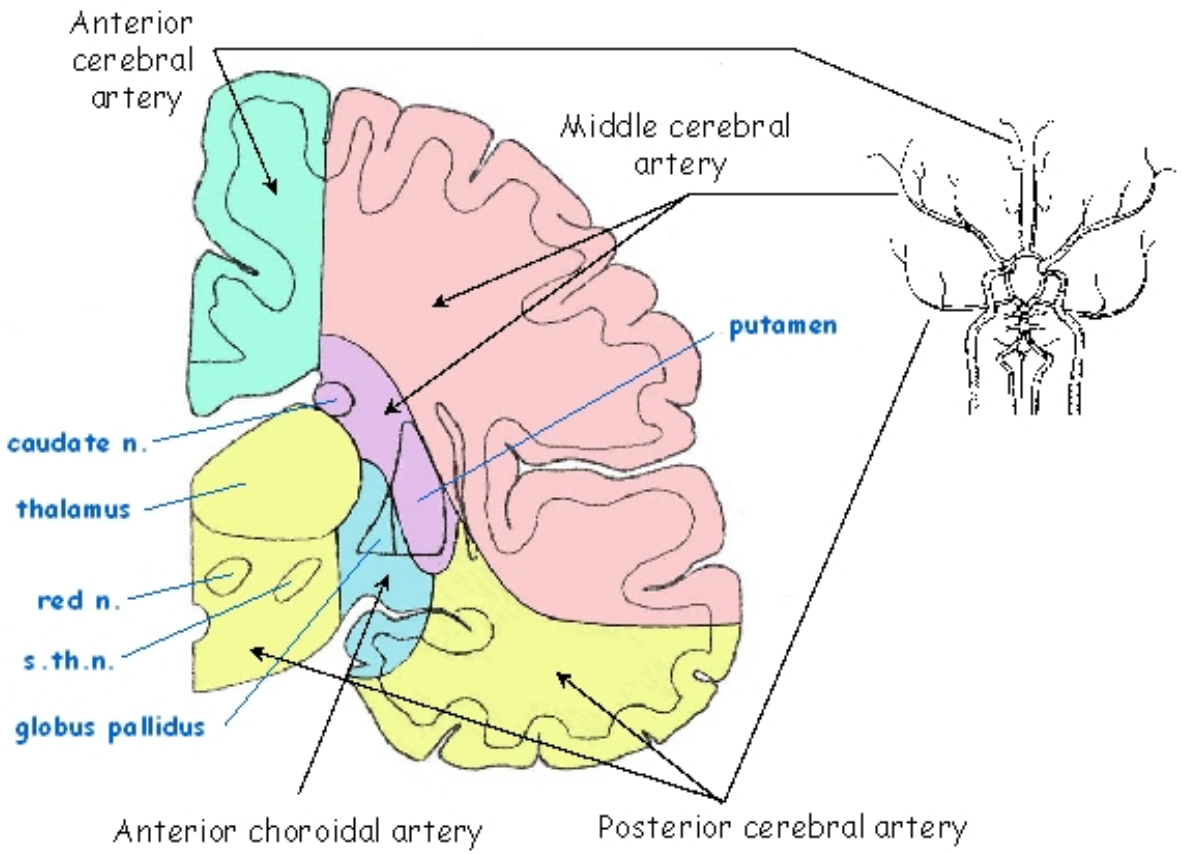
Anatomy


Anterior cerebral artery
A
Middle cerebral artery
Posterior cerebral artery


Anterior cerebral artery
B
Middle cerebral artery
Posterior cerebral artery

Imperial College
London

Anatomy

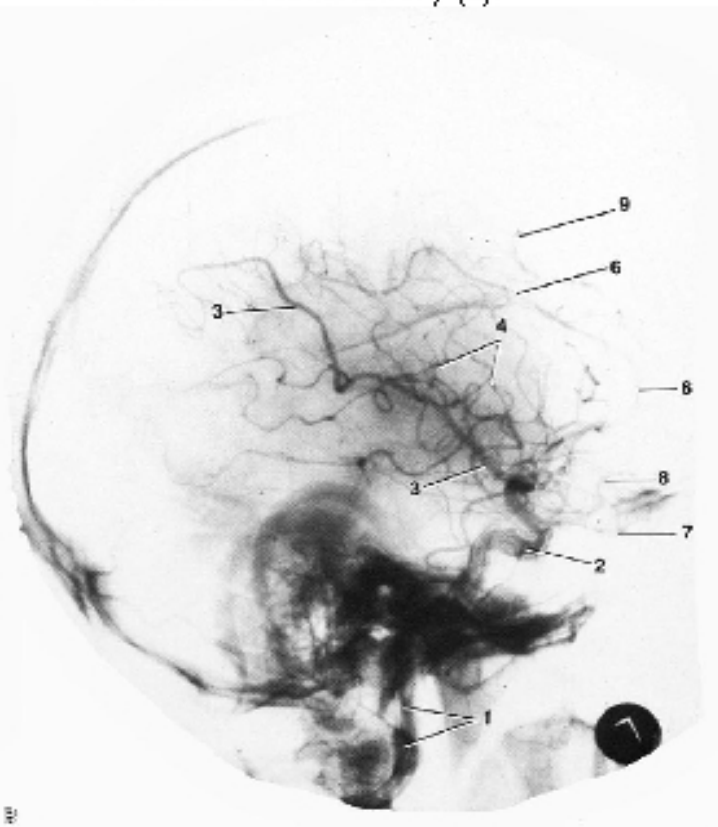


Imperial College
London

Cerebral Angiogram

1 Dye filling large arteries;
internal carotid (2)
and middle cerebral (3) arteries.

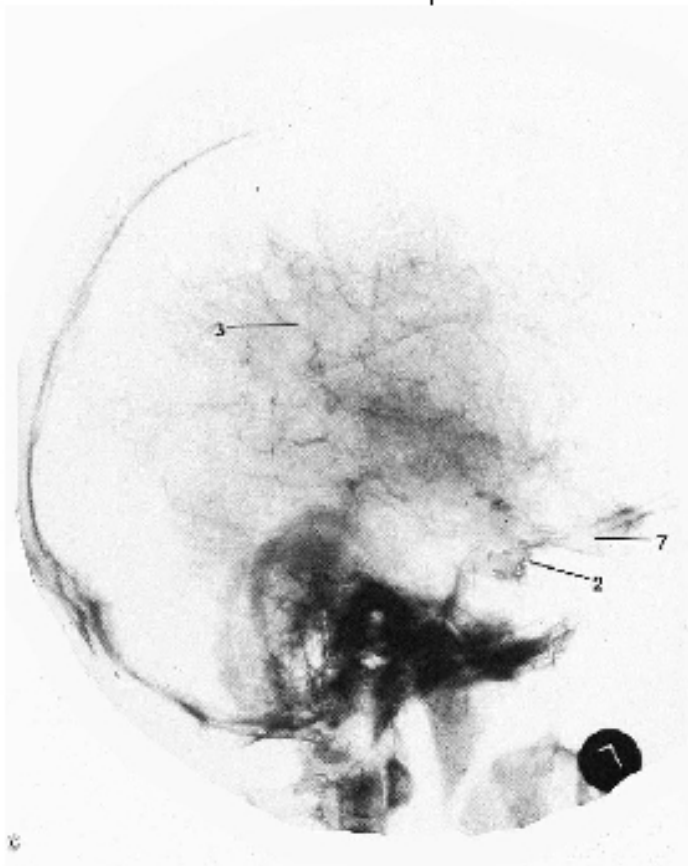
2 Dye spreading into smaller branches
of middle cerebral artery (3)



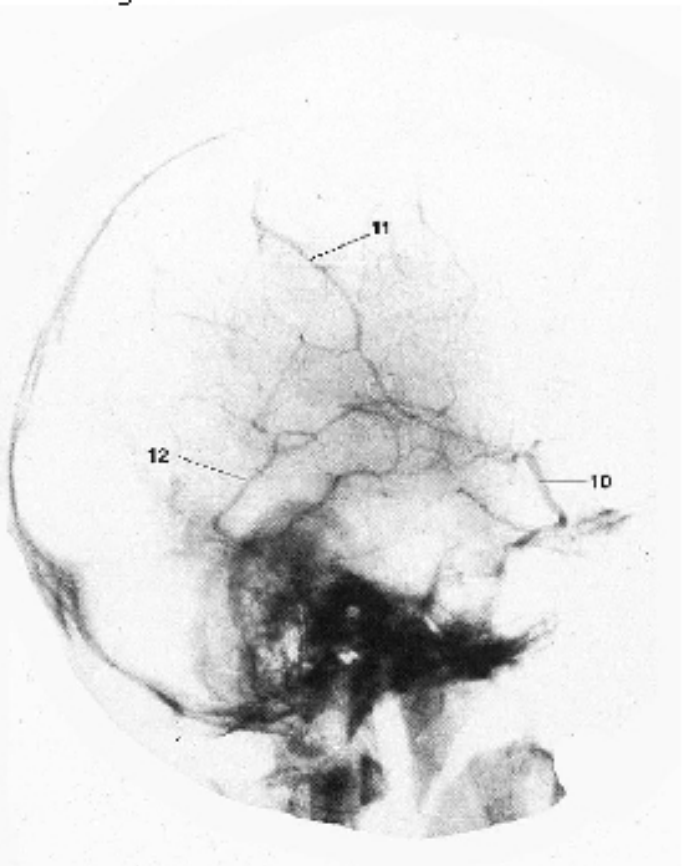
Imperial College
London

Cerebral Angiogram

3 Dye has now spread into the small microvessels and capillaries

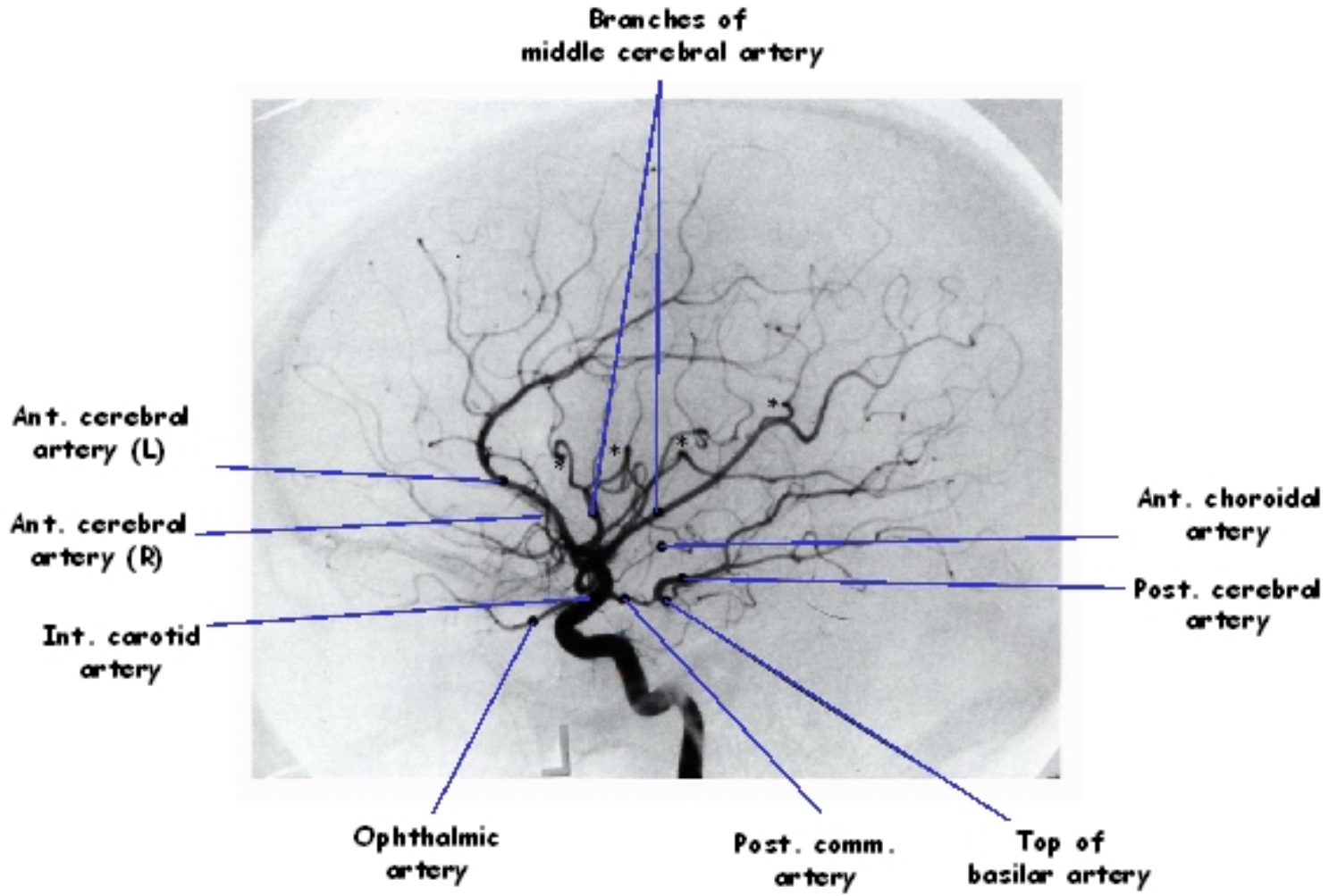


4 Dye moving from capillaries into larger veins



Imperial College
London

Cerebral Angiogram



Imperial College London

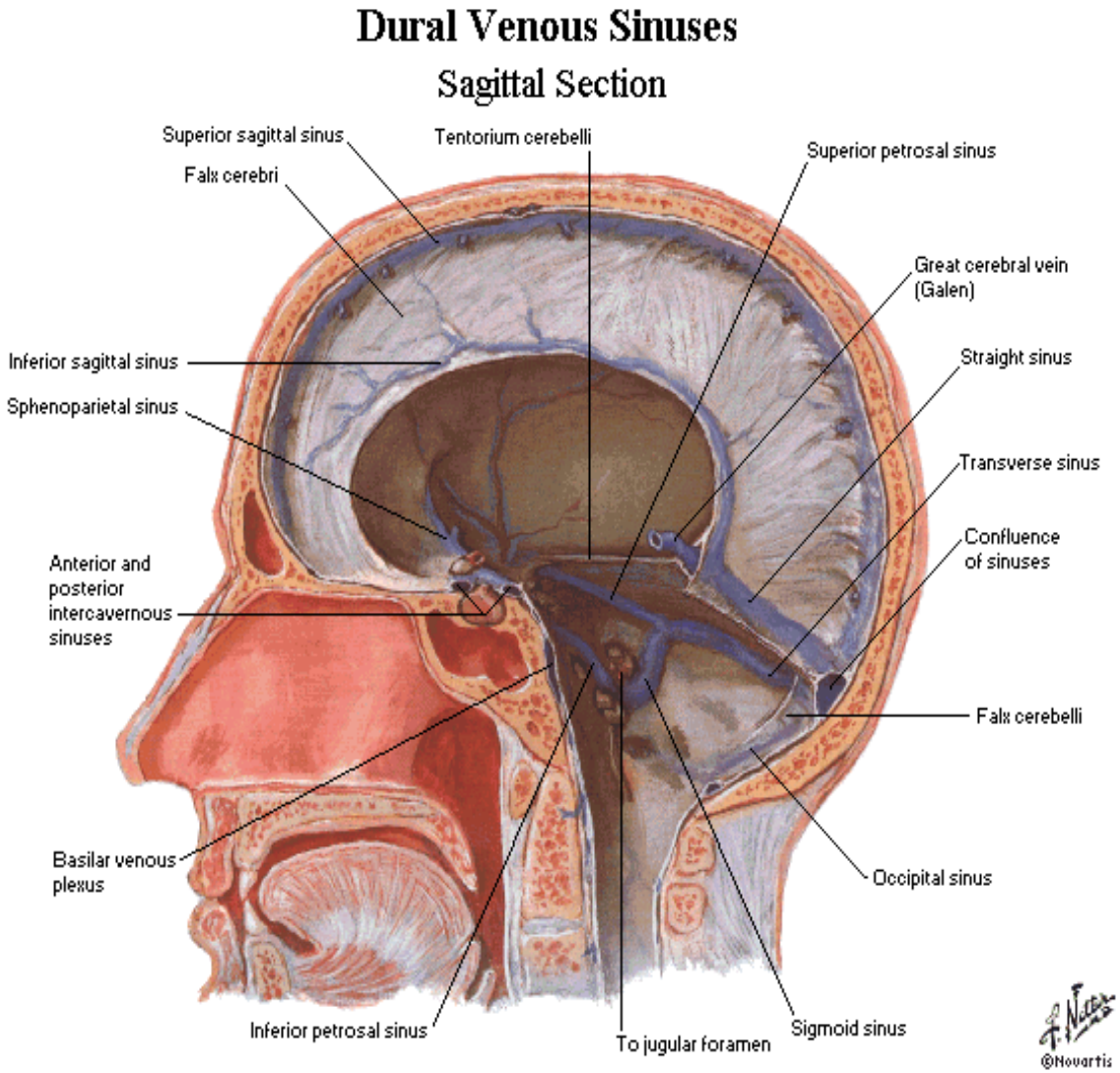
Venous Drainage of the Brain

Cerebral veins

Venous Sinuses

Dura Matter

Internal Jugular vein



Imperial College
London

Measuring Cerebral Blood Flow

Invasive :	radioactive microspheres
	autoradiography (^{14}C-antipyrine ^{14}C-2-deoxyglucose)
Non-invasive :	Doppler / electromagnetic
	N_2O method, Fick principle
	Isotope clearance (^{133}Xenon)
	PET, positron emmision topography (^{11}C, ^{13}N, ^{15}O, ^{18}F) no biological damage

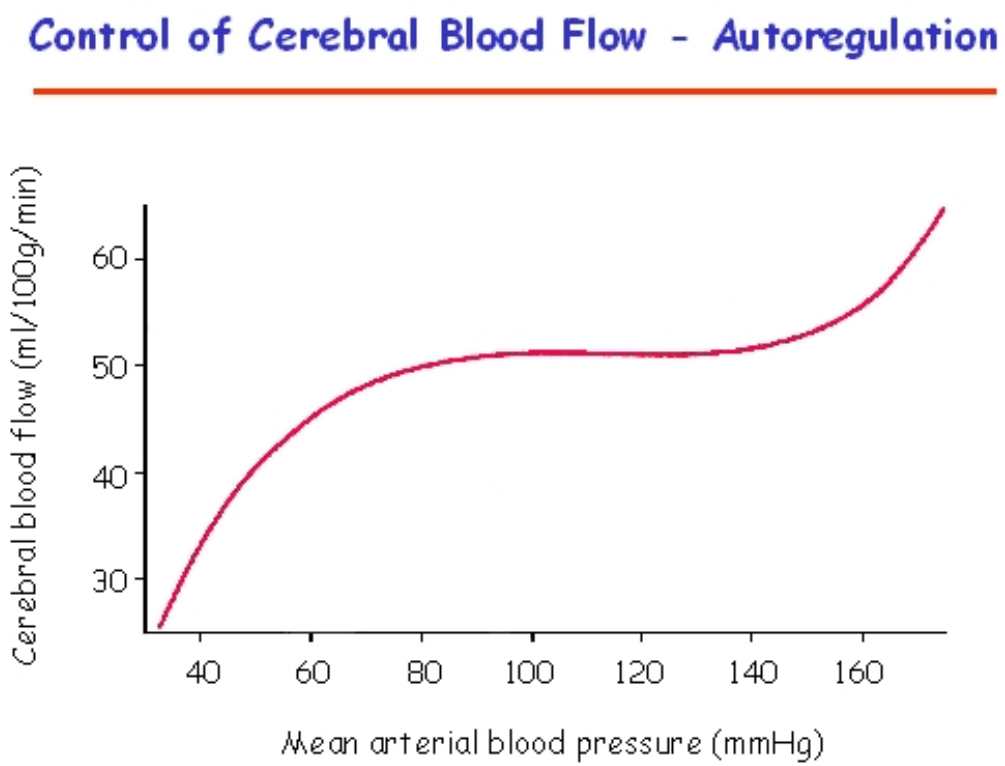
Total CBF in adult 750-1000 ml.min^{-1} (55 ml.min^{-1}.100g^{-1})

75% via carotid arteries, 25% via vertbro-basilar system

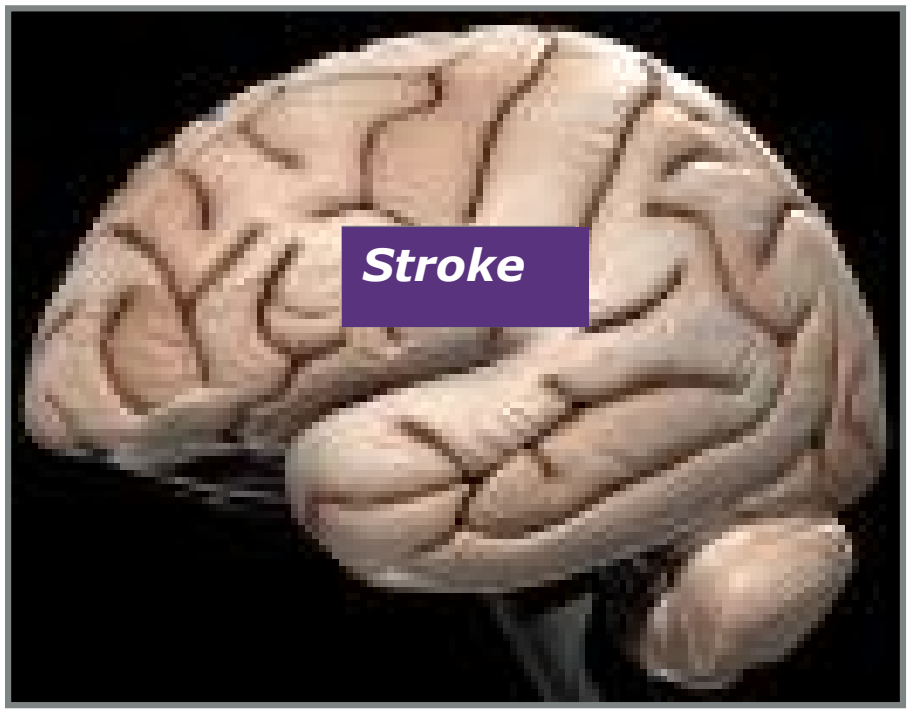
Flow to grey matter 4 times that to white matter

Imperial College
London

Control of Cerebral blood Flow - Autoregulation



Imperial College
London

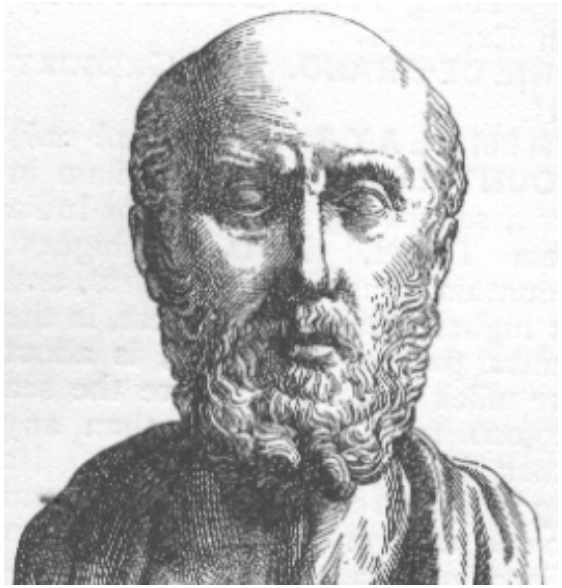


Imperial College London

History

Episodes of stroke and familial stroke have been reported from the 2nd millennium BC onward

Hippocrates (460 to 370 BC) was first to describe the phenomenon of sudden paralysis that is often associated with ischemia.



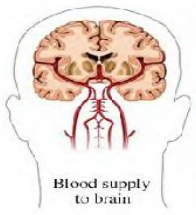
Imperial College London

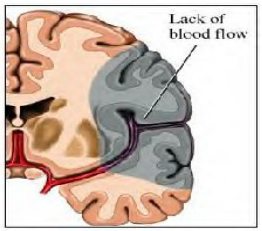
stroke

- *Continues to be a leading cause of death worldwide (9.5% of deaths).*
- *150,000 people in the UK have a stroke each year.*

What is a stroke?

- *A brain attack - part of the blood flow to the brain is restricted.*
- *Loss in blood flow can lead to hypoxia (deficiency of oxygen to tissues) and infarction (tissue death due to lack of oxygen-rich blood).*
- *Damage to brain can cause loss of speech, vision or movement in an arm or leg. - Depend upon the area of the brain affected.*





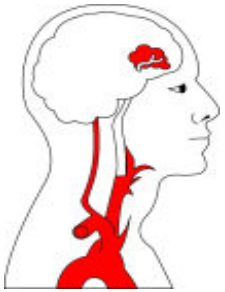
Imperial College London

Stroke: Causes

Ischaemic & Haemorrhagic

1. ***Ischaemic stroke*** *- clot blocks an artery carrying blood to the brain (85%)*

   - *Cerebral thrombosis*
   - *Cerebral embolism*
   - *Lacunar stroke*



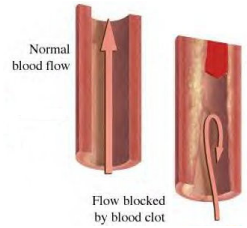
Imperial College London

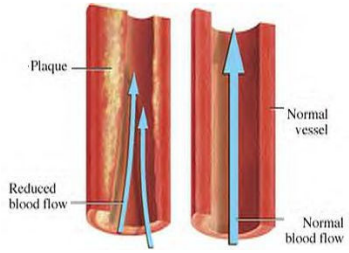
Ischaemic

(1)

Stroke caused by blocked blood flow - cerebral infarction or ischaemic stroke

85%





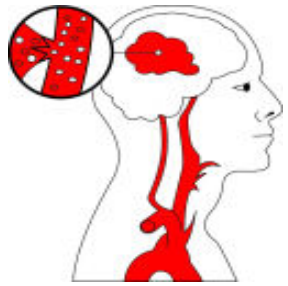
Usually in isachemic stroke 1 of 2 major arteries is involved:

- *carotid artery*
- *basilar artery*

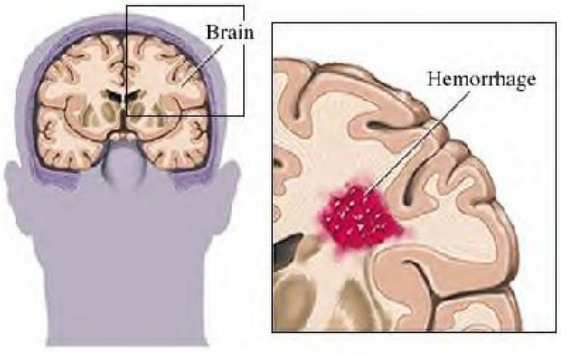
Imperial College London

Haemorrhagic stroke

2. Haemorrhagic stroke *- blood vessel bursts causing bleeding in the brain (15%)*



- *Intracerebral haemorrhage - Within the brain*
- *Subarachnoid haemorrhage - Between brain and skull*



Imperial College London

Global Ischaemia

- *Severe transient insult to the brain e.g. resusitation*
- *Produces ischaemia damage often associated with cytotoxic cerebral oedema*

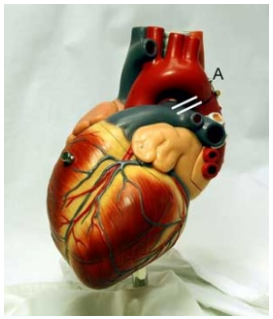
- *Pathologic changes depend upon*
  - *duration of ischaemia*
  - *Severity of ischaemia*
  - *Length of patient survival*

Imperial College London

Risk Factors for Stroke

What are they?

Excessive alcohol

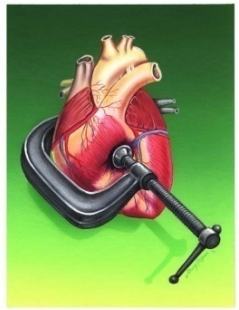


Cardiac Disease

Age

Diabetes Mellitus

Smoking



Hypertension

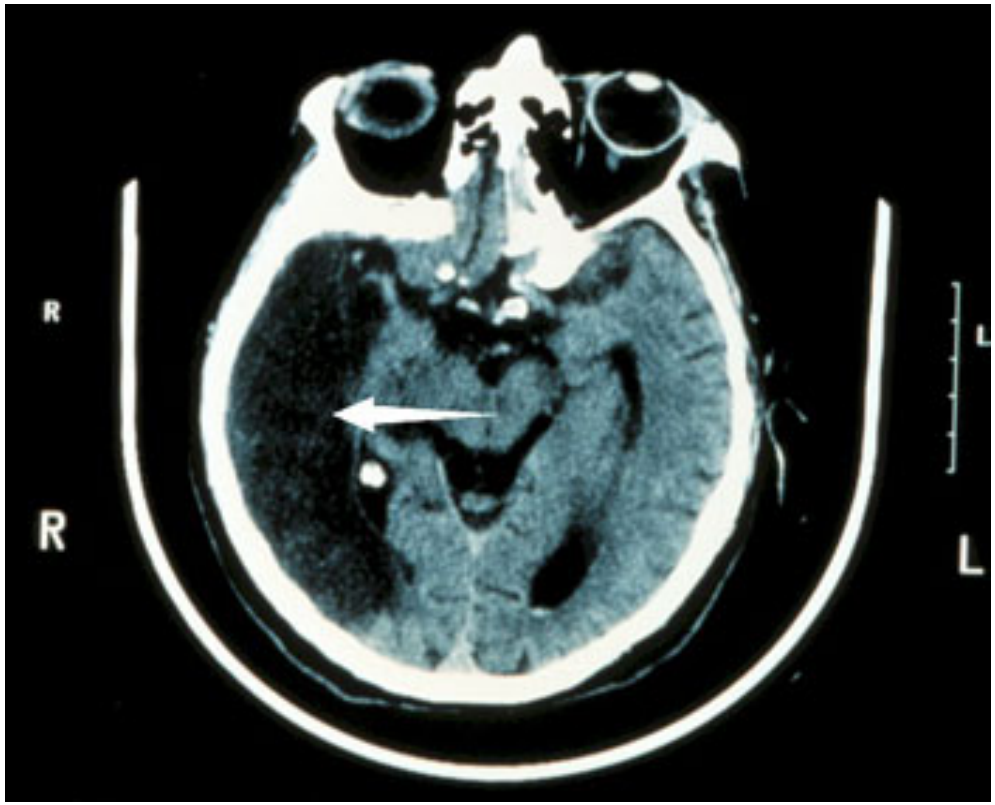
Obesity/high cholesterol

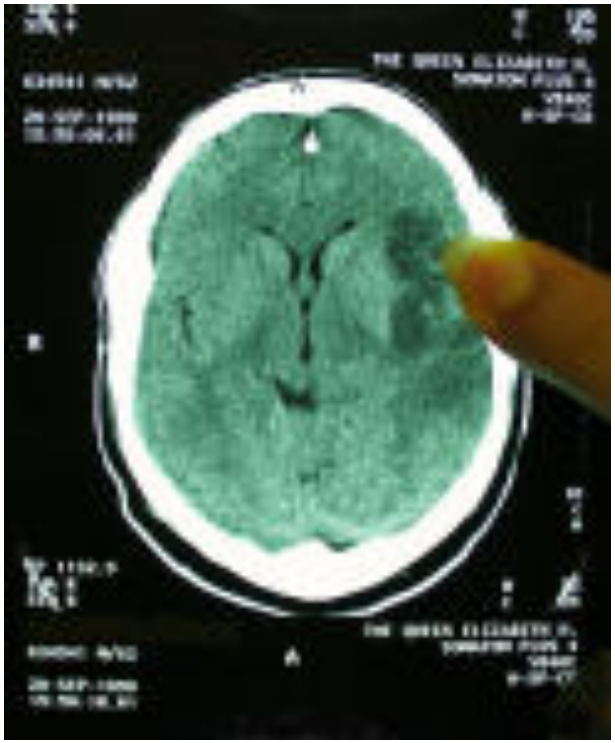
Imperial College
London

Imaging

Imperial College
London

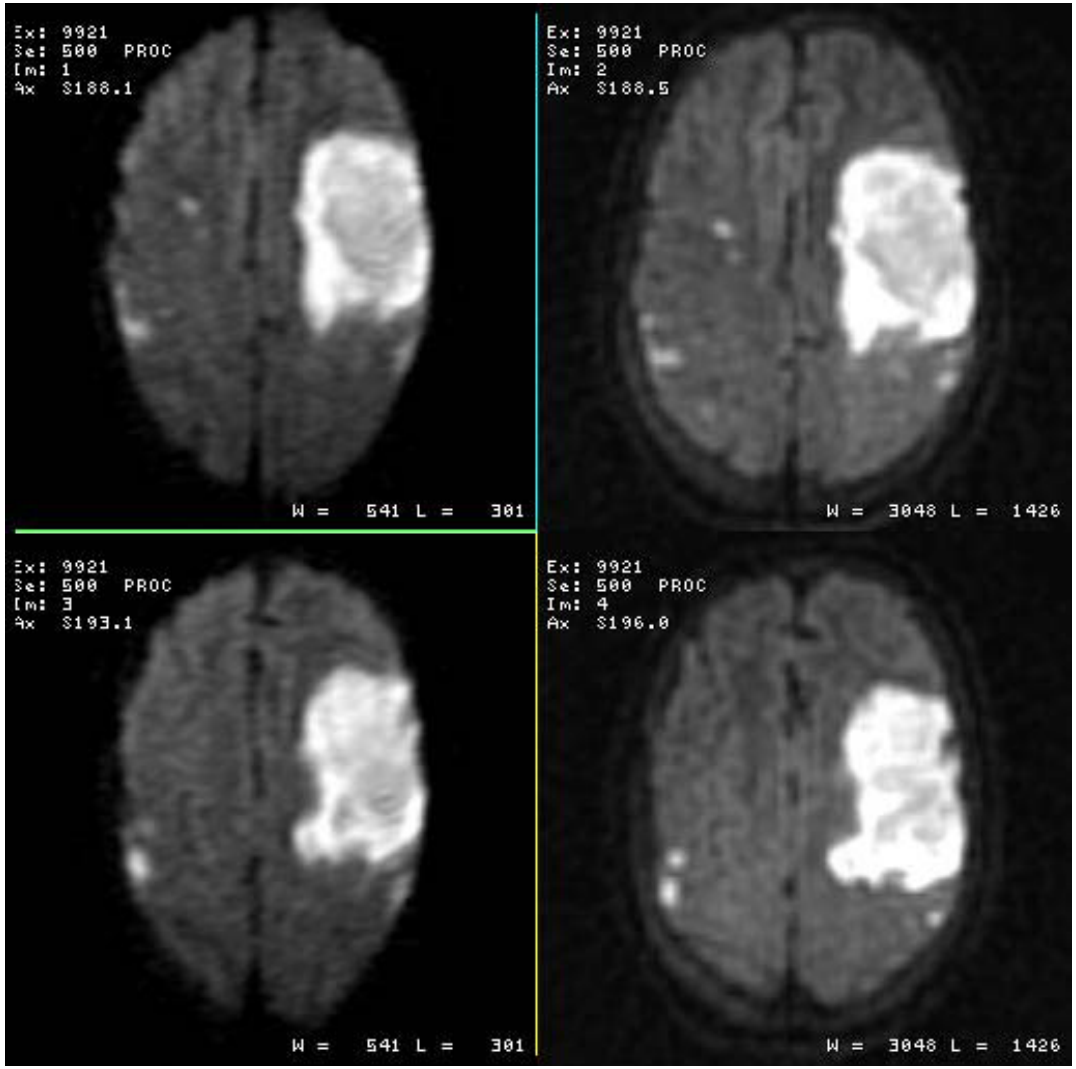
CT scan shows massive stroke





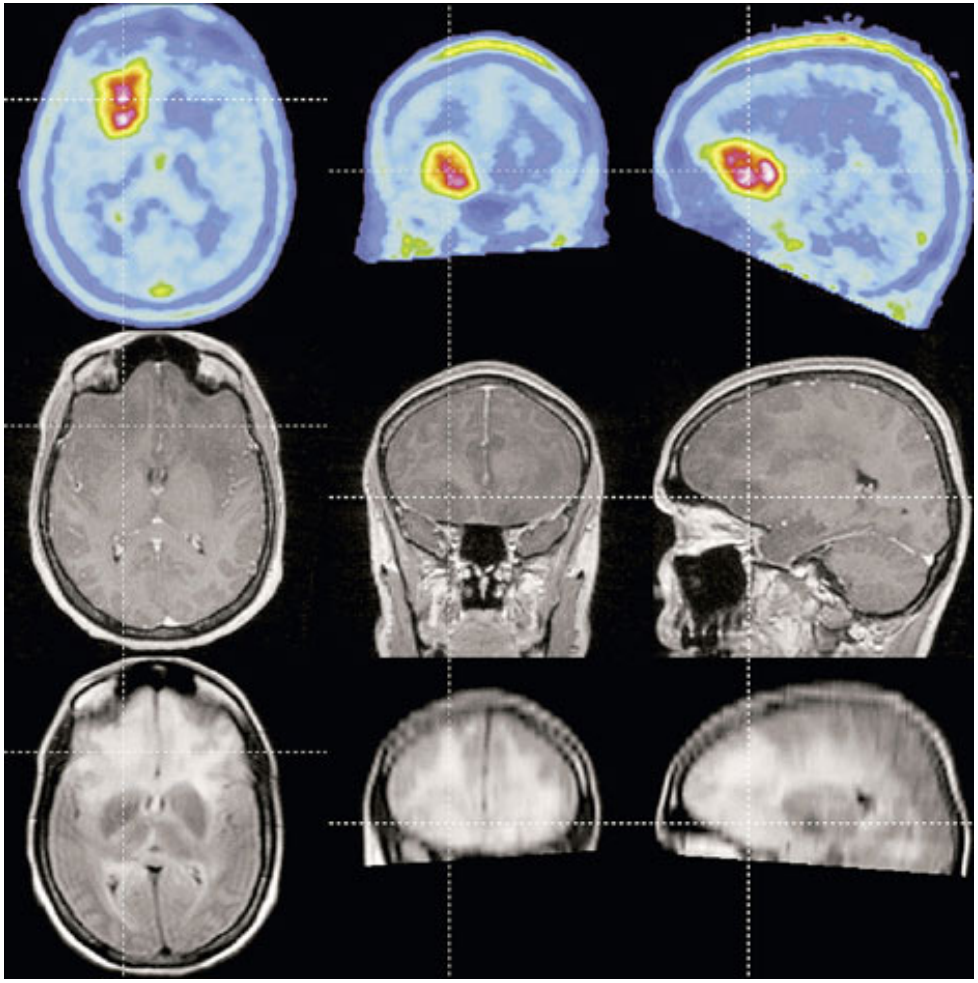
Imperial College
London

Diffusion Weighted Imaging



Imperial College
London

Combined MRI/PET for brain imaging



Imperial College London

Medical Treatment of Stroke

Primary Prevention *- no previous history of stroke*

- *Platelet anti-aggregants e.g. Aspirin*
- *HMG-CoA reductase inhibitors (Statins)*
- *Exercise*

Secondary Prevention *- have had a stroke*

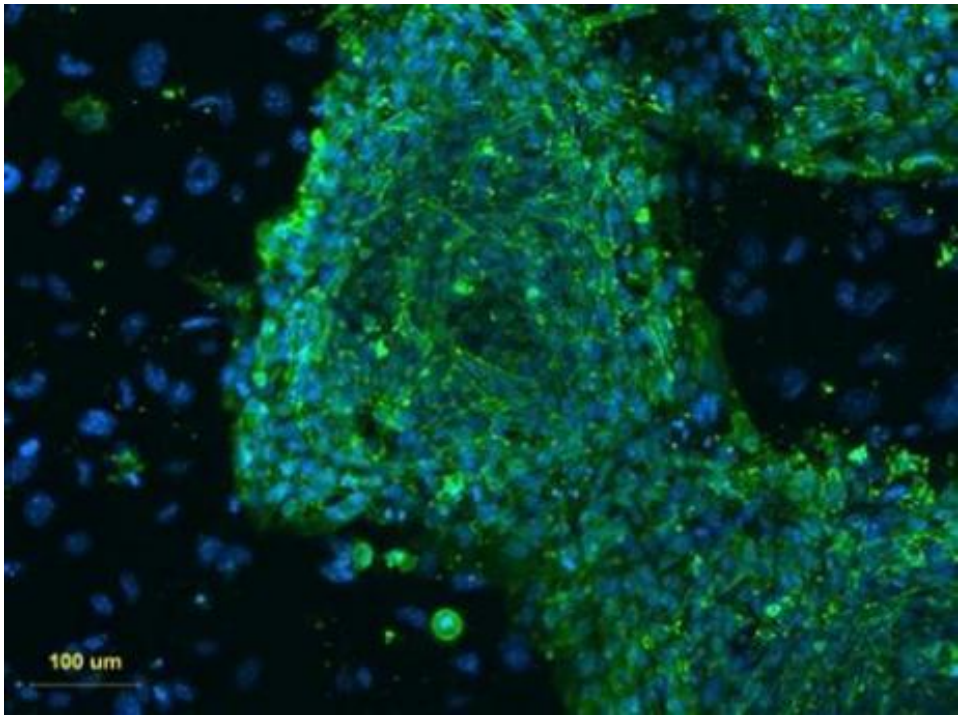
- *Platelet anti-aggregants e.g. Aspirin, Ticlopidine, Clopidogrel*
- *HMG-CoS reductase inhibitors (Statins)*
- *Anti-hypertensives*
- *Thrombin inhibitors e.g. Warfarin*
- *Life changes e.g. exercise, stopping smoking, diabetes control, weight loss*

Acute Treatment *- within 0-24 h of stroke onset*

- ***Thrombolytics e.g. tissue plasminogen activator, Prourokinase***
- *Anti-platelet agents e.g. Aspirin*
- *Anti-coagulants e.g. heparin*
- *Neuroprotectants*

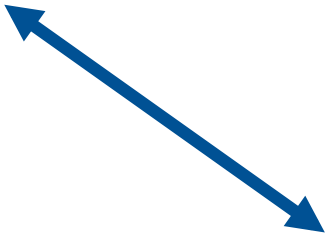
Imperial College
London

UK starts world's first stroke stem cell trial

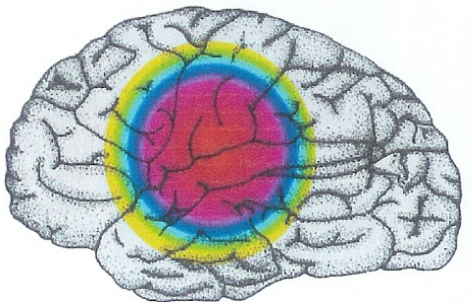


Doctors in Scotland working with British biotech company ReNeuron have injected stem cells into the brain of a man in a pioneering clinical trial to test the safety of a therapy for patients disabled by stroke.

Stroke



Inflammation



Imperial College London

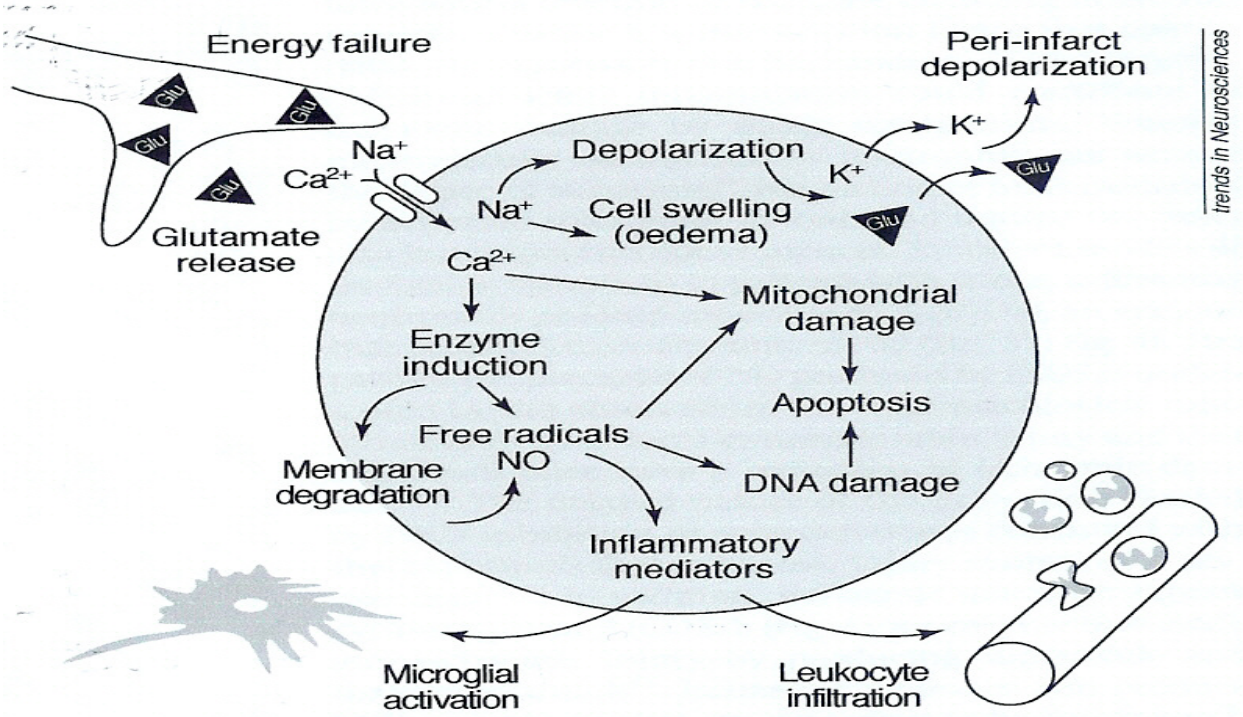
Inflammation in the Brain

Focal and global associated with inflammation

neutropenia, inhibition of inflammatory mediators or anti-

adhesion molecule therapy -----> protection

Pathophysiological Mechanisms in Focal Ischaemic Brain



Dirnagl et al., 1999, Trends Neurosci. 22, 391-397

What is the difference between the brain and other vascular beds?

Imperial College London

Blood Brain Barrier

- *refers to highly restrictive properties of cerebral capillaries*
- *interendothelial junctions fused - very tight*
- *glucose crosses by facilitated diffusion*
- *transport systems for Na^+, K^+, amino acids*

Imperial College London

Major Concepts: Cerebral Circulation

- *cerebral blood flow is*
  - *- tightly coupled to metabolic activity*
  - *- perfectly auto-regulated over a wide range of arterial pressures*
  - *- responds dramatically to changes in pCO_2*
  - *- unresponsive to autonomic nerve stimulation*
- *blood-brain barrier restricts extravasation of most solutes*

Imperial College
London

Research

Imperial College London

Animal Models of stroke

Type of model	Representative models	Notes
Global ischaemia	Bilateral carotid occlusion	Primarily in gerbils, rapid screening technique
	Two-vessel occlusion plus hypotension	Normally in rats
	Four-vessel occlusion	Normally in rats
Focal ischaemia	Middle cerebral artery occlusion:	Several species used:
	(1) transient	(1) uses clips, intraluminal thread and snare
	(2) permanent	(2) uses intraluminal thread, clips and coagulation
	(3) thrombotic	(3) injection of either microspheres or clots into cerebral vessels, including middle cerebral artery
Haemorrhagic	Infusion of collagenase into brain	See [41]

Green et al, Trends in Pharmacological Sciences Volume 24, Issue 8, August 2003402-408

Imperial College
London

Failure of drugs for acute ischaemic stroke

Compound	Mechanism of action[a]	Inclusion period (h)	Outcome (clinical phase)	Reason	Refs
Selfotel	NMDA receptor antagonist	6	Negative (III)	Adverse events	[59]
Cervene	Kappa opioid peptide receptor antagonist	6	Negative (III)	Lack of efficacy	[60]
Lubeluzole	NOS inhibitor and Na^+ channel blocker	8	Negative (III)	Lack of efficacy	[61]
Gavestinel	Antagonist at the glycine site of the NMDA receptor	6	Negative (III)	Lack of efficacy	[62]
Enlimomab	Anti-ICAM antibody	6	Negative (III)	Lack of efficacy and adverse events	[63]
Citicoline	Cell-membrane stabilizer	24	Negative (III)	Lack of efficacy	[64]
Ca^{2+} antagonists	Ca^{2+} channel antagonists	6–24	Negative (meta-analysis)	Lack of efficacy	[30]
Aptiganel	NMDA receptor antagonist	6	Negative (III)	Lack of efficacy	[65]
Clomethiazole	$GABA_A$ receptor modulator	12	Negative (III)	Lack of efficacy	[36]
BMS204352[b]	K^+ channel blocker	6	Negative (III)	Lack of efficacy	[66]

Imperial College London

Transferability of animal results to human stroke

Side effects: Many highly potent neuroprotective drugs display side effects which inhibit the application of effective doses in patients (e.g. MK-801)

Delay: Whereas in animal studies the time of incidence onset is known and therapy can be started early, patients often present with delay and unclear time of symptom onset

"Age and associated illnesses: Most experimental studies are conducted on healthy, young animals under rigorously controlled laboratory conditions. However, the typical stroke patient is elderly with numerous risk factors and complicating diseases (for example, diabetes, hypertension and heart diseases)" (Dirnagl 1999)

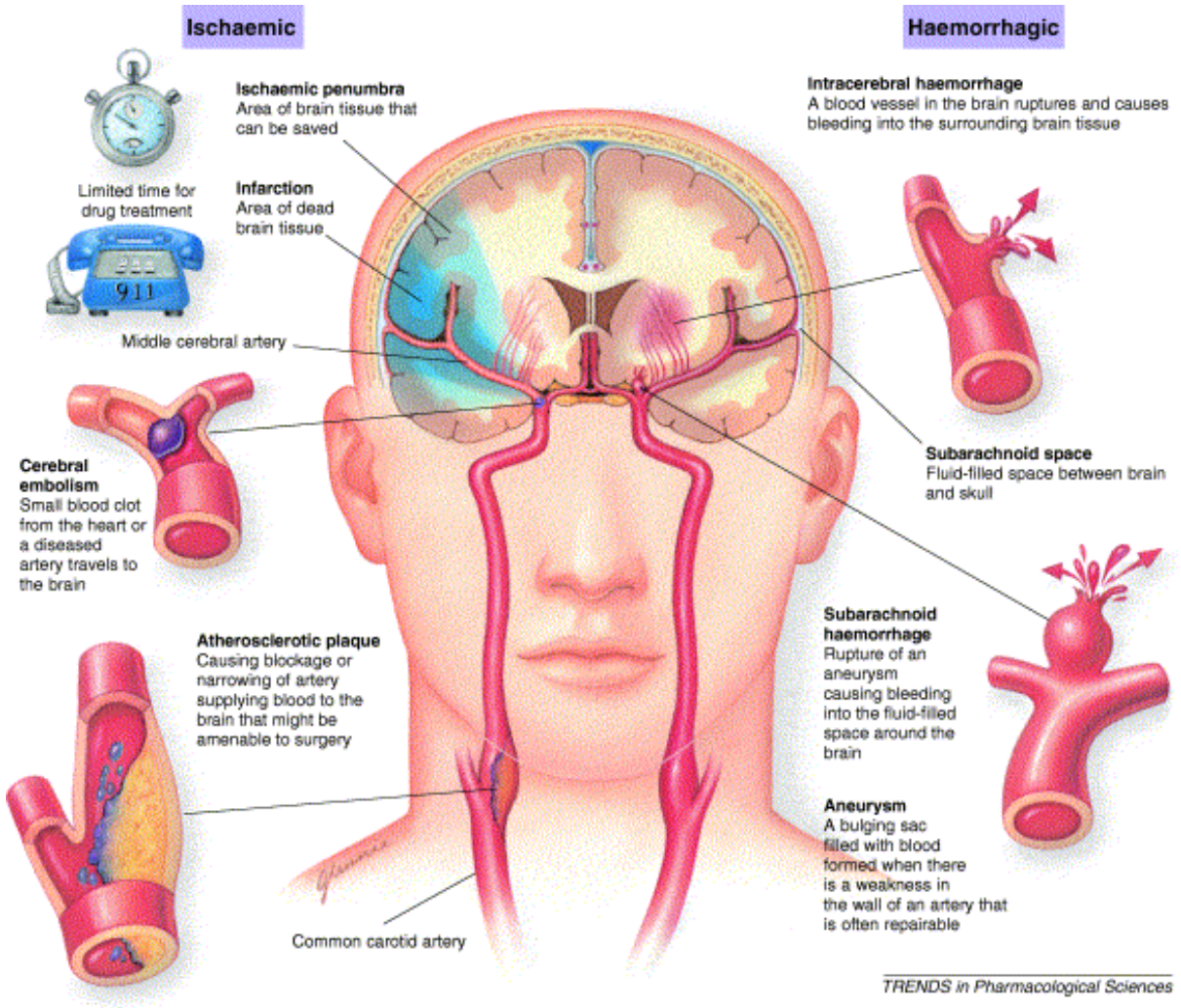
Morphological and functional differences between the brain of humans and animals: Although the basic mechanisms of stroke are identical between humans and other mammals, there are differences.

Evaluation of efficacy: In animals, treatment effects are mostly measured as a reduction of lesion volume, whereas in human studies functional evaluation (which reflects the severity of disabilities) is commonly used. Thus, therapies might reduce the size of the cerebral lesion

Summary

Imperial College
London

The major types of stroke and cerebral accident.



Imperial College
London

My Research

Imperial College London

Study of anti-inflammatory mediators

Mimic specific protective biochemical mechanism(s) in the host.

More selective in their action + less side effects

Imperial College London

Anti-inflammatory Mediators

Proteins -

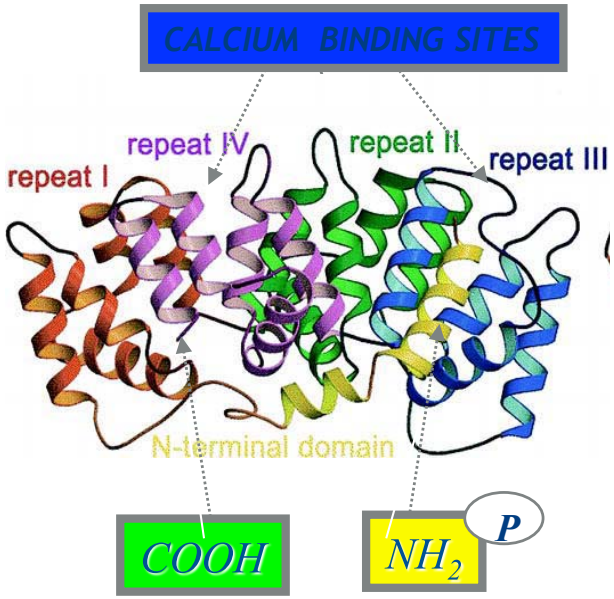
- *Annexin 1 (lipocortin)*
- *TSG-6*
- *Galectin-1*
- *ACTH and Melanocortins*
- *Heat Shock Proteins*
- *Interleukins*
- *Etc…*

Others -

- *Adenosine*
- *Cortisol*
- *Prostanoids*
- *Nitric Oxide*
- *Carbon Monoxide*
- *Lipoxin A_4*
- *Heparin*
- *Etc…*

Imperial College London

AnxA1



- *37kDa anti-inflammatory protein*
- *Consisting of: core (4 conserved repeats) and unique N-terminus*
- *Biological actions of full length protein are retained in first 25 amino acids of N-terminal region (peptide Ac2-26)*

Imperial College
London

Abundance in the cytosol

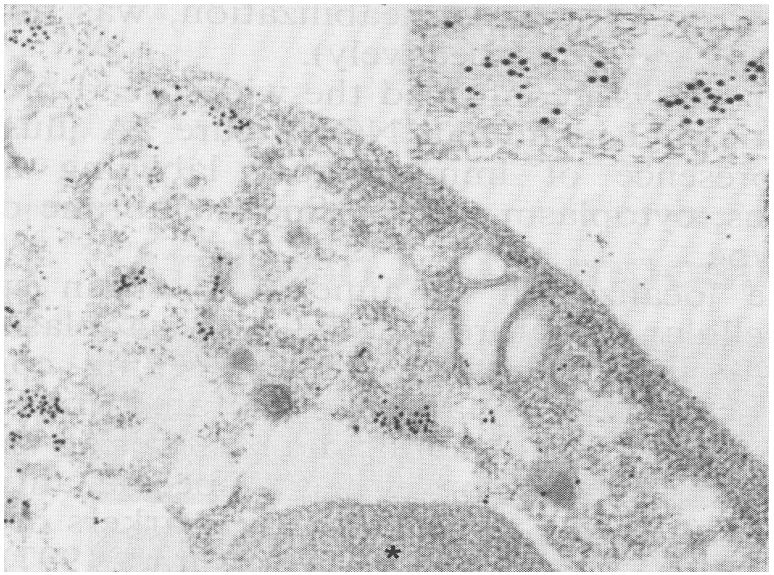
- ***AnxA1 - 3.60 %***
- ***AnxA2 - 0.02 %***
- ***AnxA3 - 0.75 %***
- ***AnxA4 - 0.05 %***
- ***AnxA5 - 0.19 %***
- ***AnxA6 - 0.80 %***
- ***AnxA7 - not detectable***

Perretti & Gavins, 2003

Imperial College London

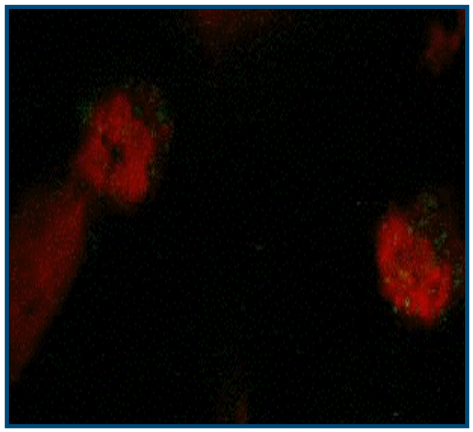
AnxA1

Storage

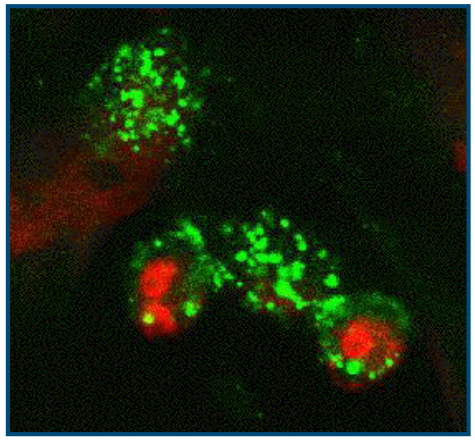


Oliani et al., Am J Pathol (2001)

Externalisation



Control IgG-FITC



AnxA1 IgG-FITC

Perretti et al., Cell Bio Int (2000)

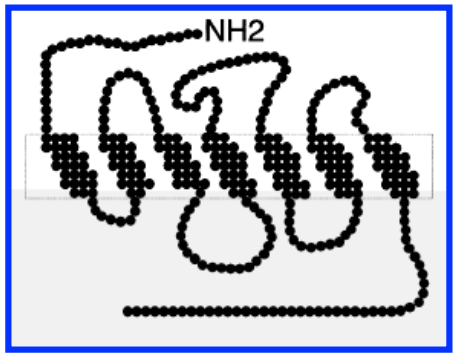
Imperial College London

AnxA1

FPR family

Multiple members in the mouse [chromosome 17 (analogous locus)]

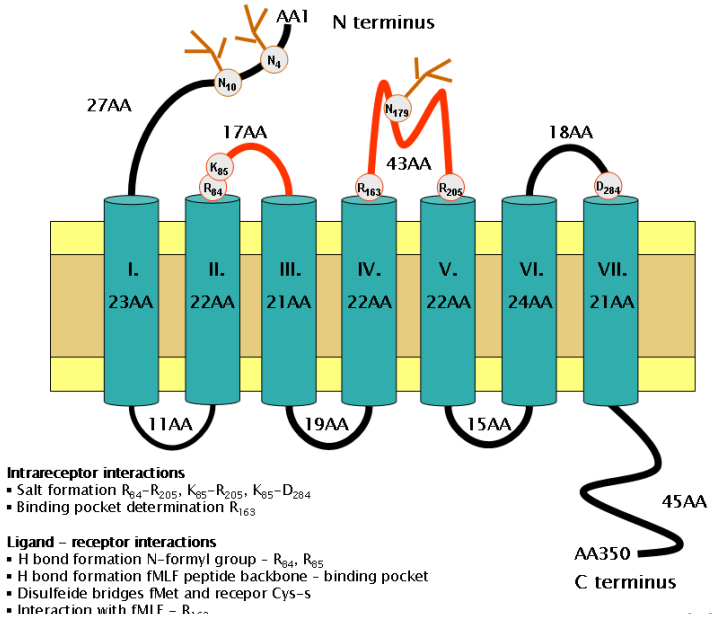
Human	Mouse	
FPR1	FPR1	Fpr1
FPR2	FPR2	Fpr-rs1
FPR3	FPR3	Fpr-rs2
		Fpr-rs3*
		Fpr-rs4*
		Fpr-rs5*
		Fpr-rs6*
		Fpr-rs7*

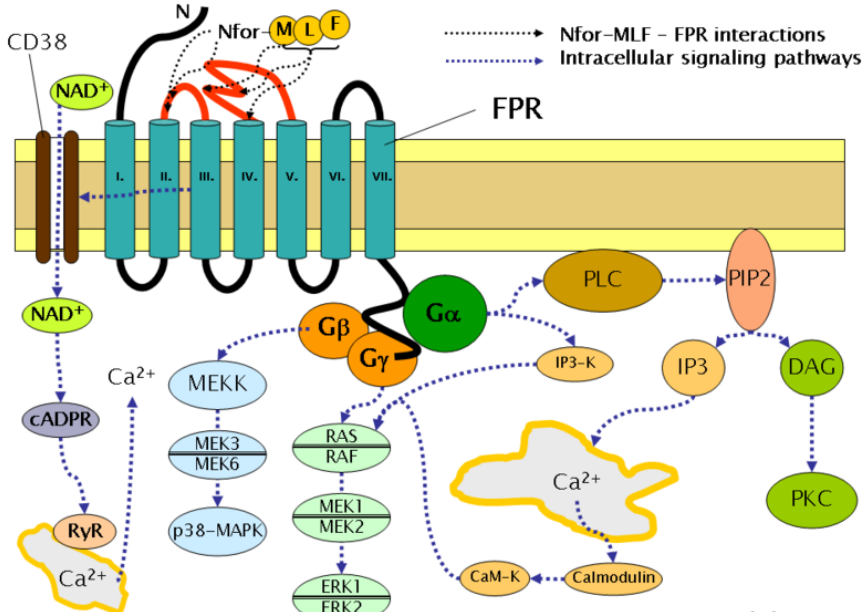


Imperial College London

Formyl Peptide Receptors (FPRs)

- *family of GPCRs*
- *Tissue/cellular distribution*
- *Ligands*

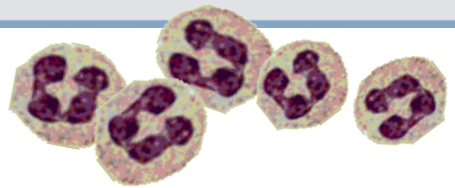




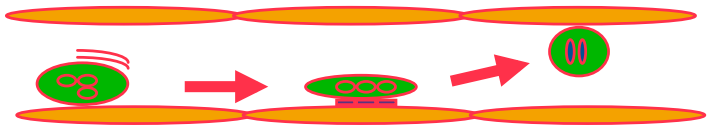
Signalling:

-G-protein dependent activation of PLC – PIP2, IP3 & DAG
-MAP kinase activation
-RAS/RAF activation
-CD38 activation induced – increase in cytoplasmin Ca2+ levels (required for directed migration of cells)

Imperial College London



Anti-migratory effect (peritonitis): partially FPR

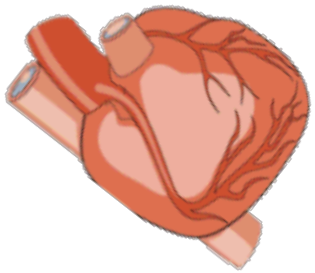


Anti-adhesive effect (mesentery): predominantly ALX

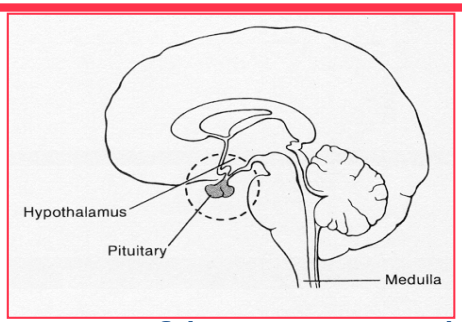
Receptor(s)

AnxA1

Pept



Acute cardio-protection (I/R injury): independent from FPR, (ALX?)



Inhibition of hormone release (Pituitary Gland):not FPR

Imperial College London

AnxA1 & Stroke: Evidence of involvement?

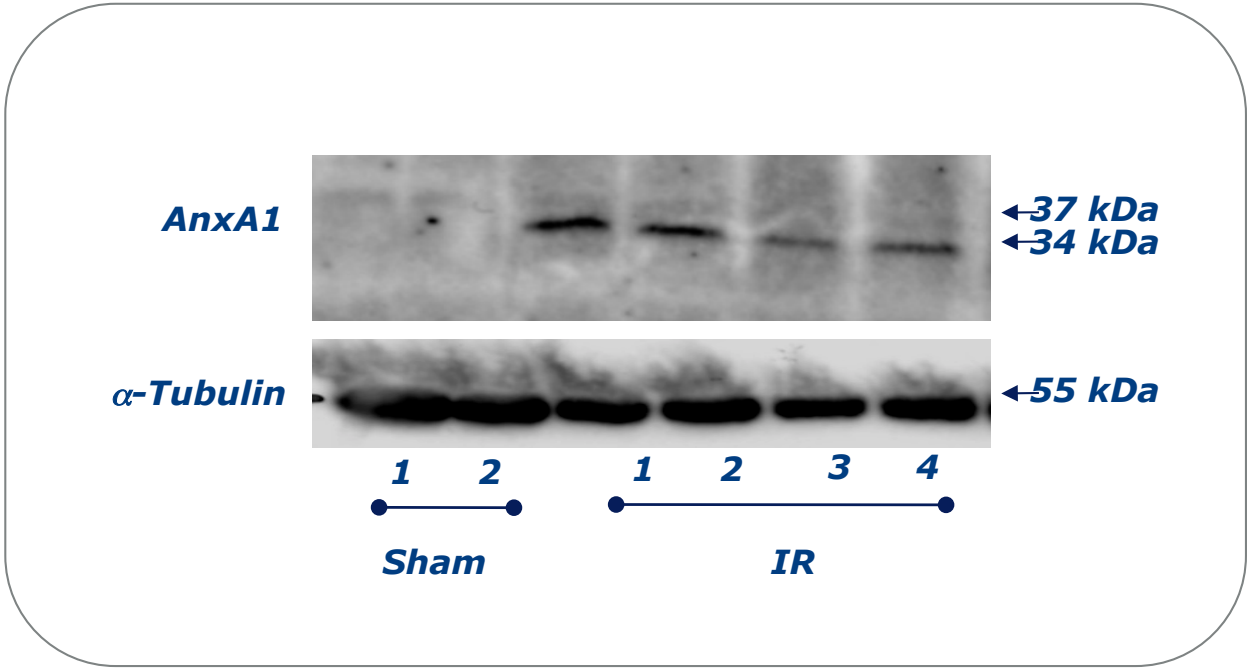
- *AnxA1 immunoreactivity in glial cells and neurons - detected in normal rat brain (Savchenko et al., Neuroscience, 2000, 96, 195-203).*

- *AnxA1 fragments inhibit central & peripheral actions of cytokines on fever & thermogenesis in rat (Strijbos et al., Am J Phys 1992, 263, E632-6).*

- *Rat 2h post-MCA occlusion & administration of $AnxA1_{1-188}$ inhibited infarct size (60%) and cerebral oedema (46%; Relton et al., J Exp Med 1991, 174, 305-10)*

Imperial College
London

AnxA1 expression in infarcted brains



Gavins et al., FASEB, 2007

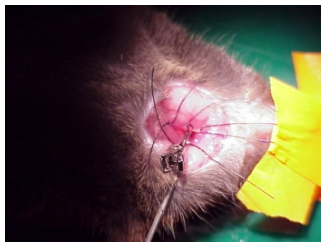
Imperial College London

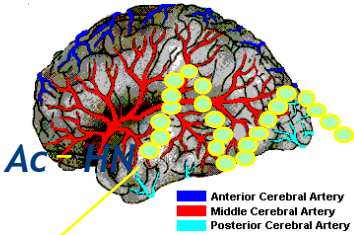
Method:MCAO

-60’ — Occlusion MCCA

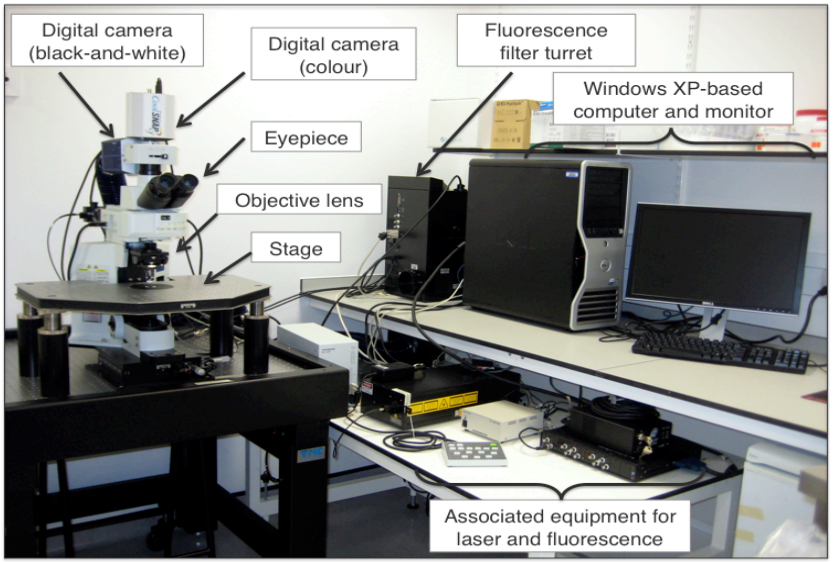
0 — • Begin Reperfusion • Drug treatment

+24h — Analysis





CH_3CO—AMVSEFLKQAWFIENEEQEYVQTV



Imperial College London

Analysis

- ***NEUROLOGICAL SCORE (Soriano et al., Stroke, 1999):***

0 = no deficit
1 = failure to extend right paw
2 = circling to the right
3 = falling to right
4 = unable to walk spontaneously

- ***INTRAVITAL MICROSCOPY*** *(Rhodamine 6G):*

1/ Leukocyte Rolling: $\frac{\text{No. rolling cells/time}}{\text{Diameter/1000}} = x \text{ cells/sec/mm}^2$

2/ Leukocyte Adhesion:

$$\frac{\text{No. cells/vessel diameter x 3.14 x vessel length}}{1{,}000{,}000} = x \text{ cells/mm}^2$$

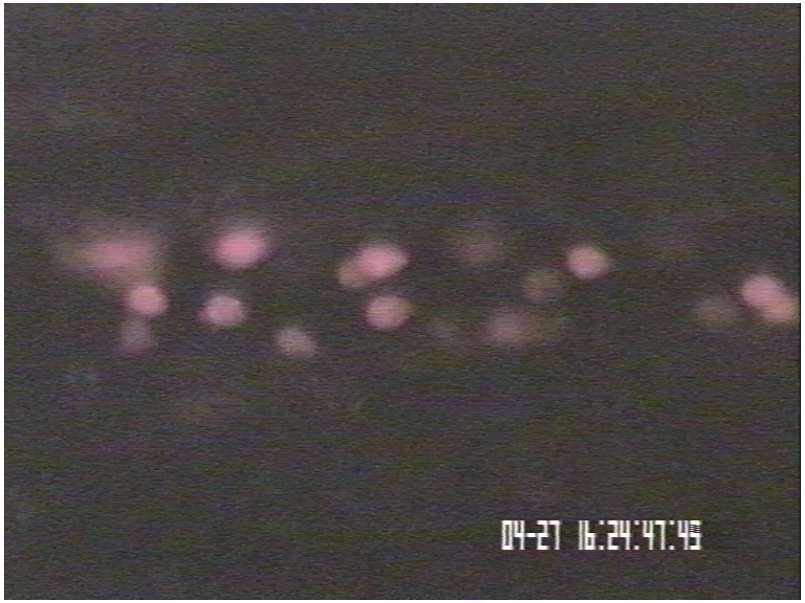
- ***INFARCT VOLUME:***

3/ Blood pressures, blood gases, leukocyte counts, serum samples
Brains stained with 2,3,5-triphenyltetrazolium chloride (TTC)
Sections photographed & quantified using NIH image.

Imperial College London

Intravital Microscopy: Pial Vessels

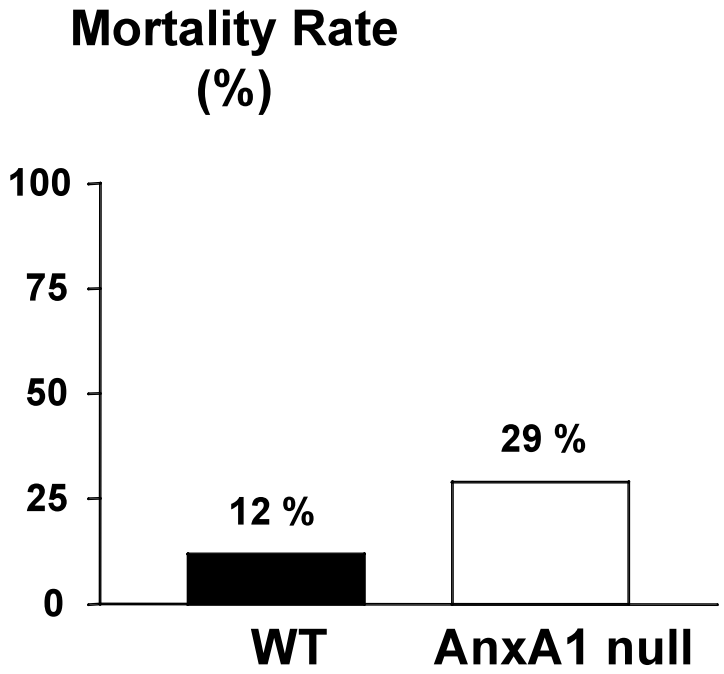
Sham

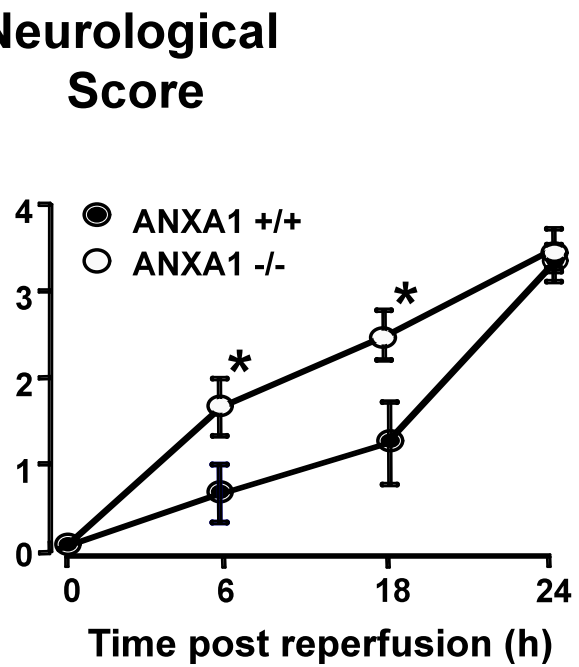


IR

Imperial College London

Endogenous AnxA1 & stroke



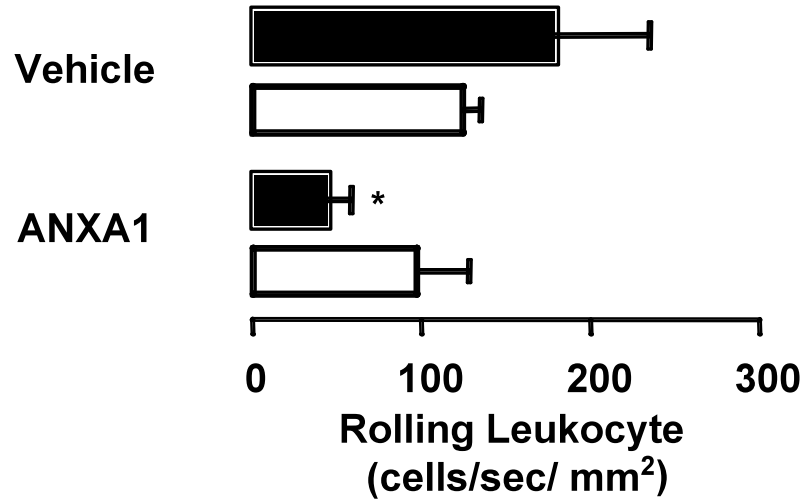


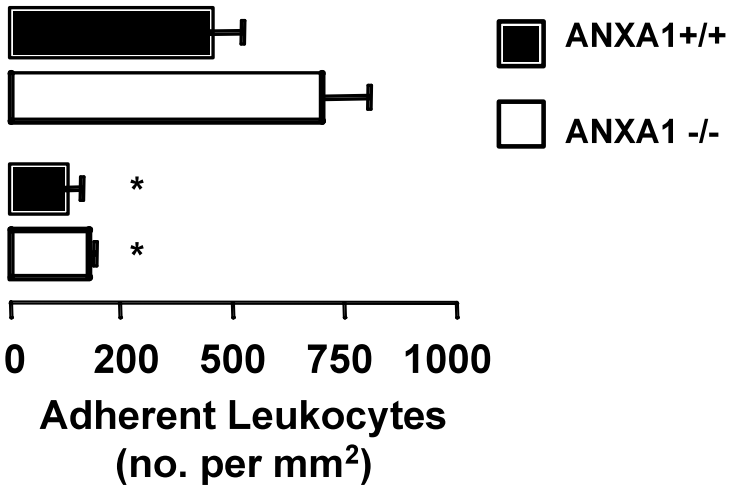
Gavins et al., FASEB, 2007

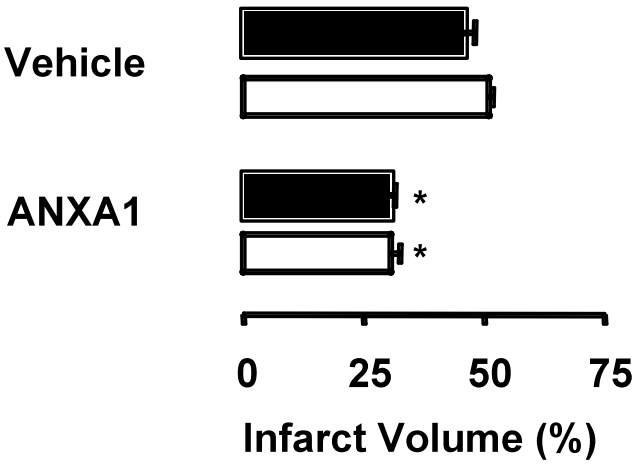
Imperial College
London

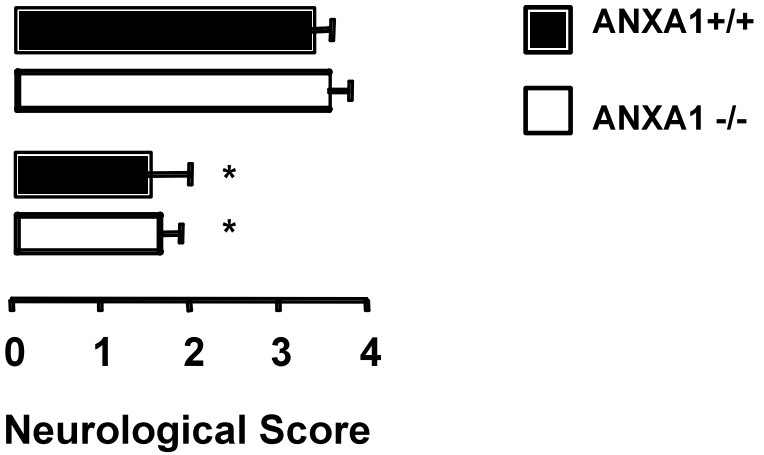
Imperial College London

AnxA1 rescues phenotype









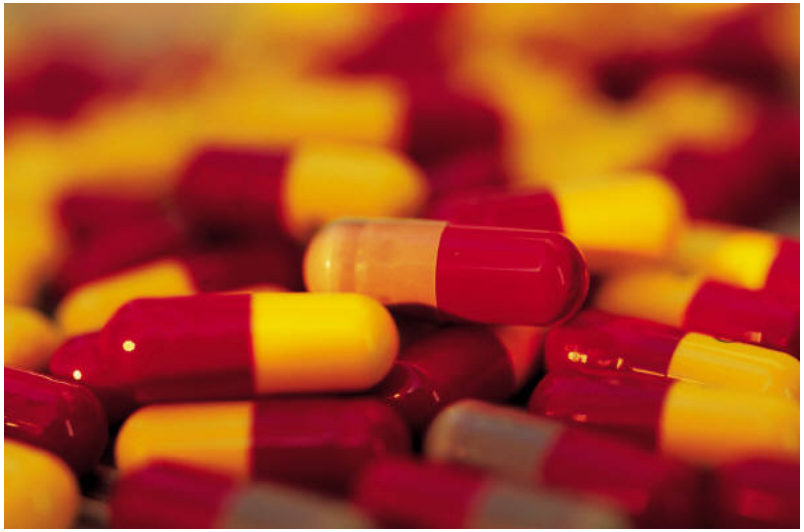
Gavins et al., FASEB, 2007

…tissue protective effect for exogenous AnxA1 in either genotype. - Highlighting the pharmacological potential.

Imperial College
London

❖*AnxA1 null mice more affected by stroke*

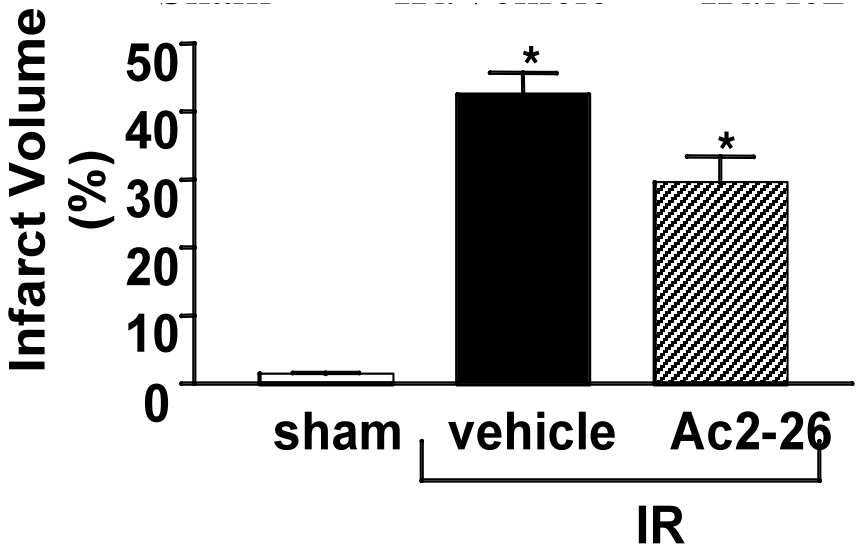
❖*Endogenous AnxA1 plays a role in stroke*



•*Effect of Ac2-26*

Imperial College London

Efficacy of Peptide Ac2-26

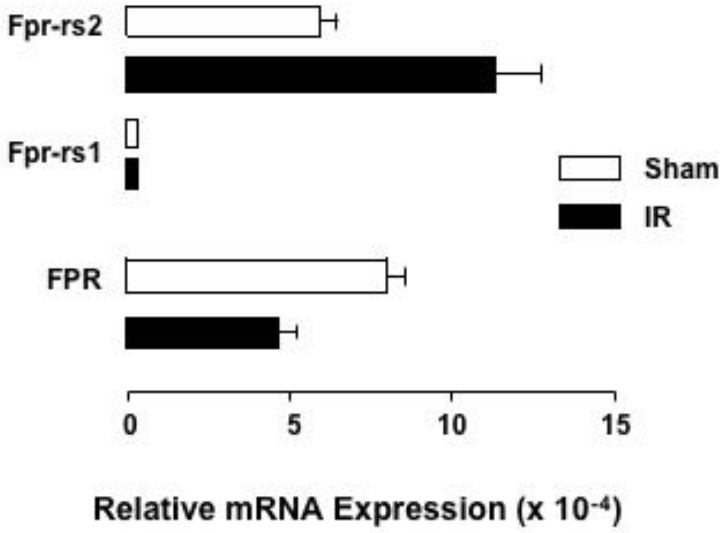


Gavins et al., FASEB, 2007

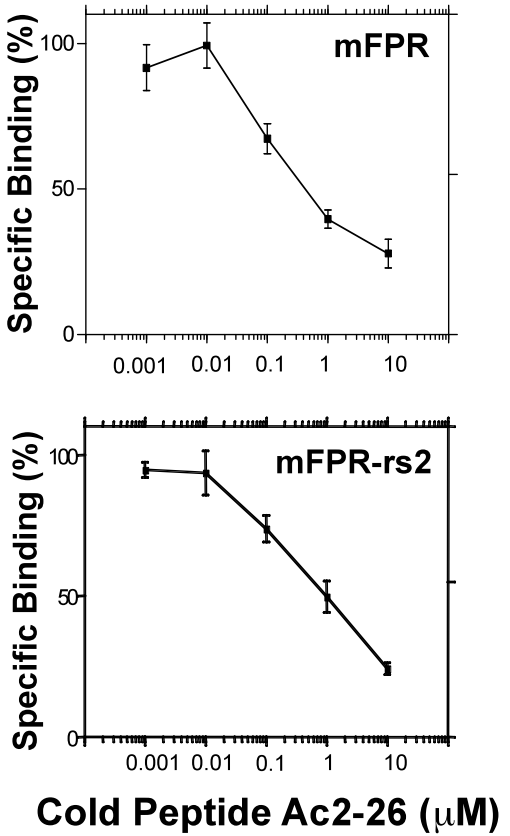
Imperial College London

Protein expression and binding

Real-Time PCR

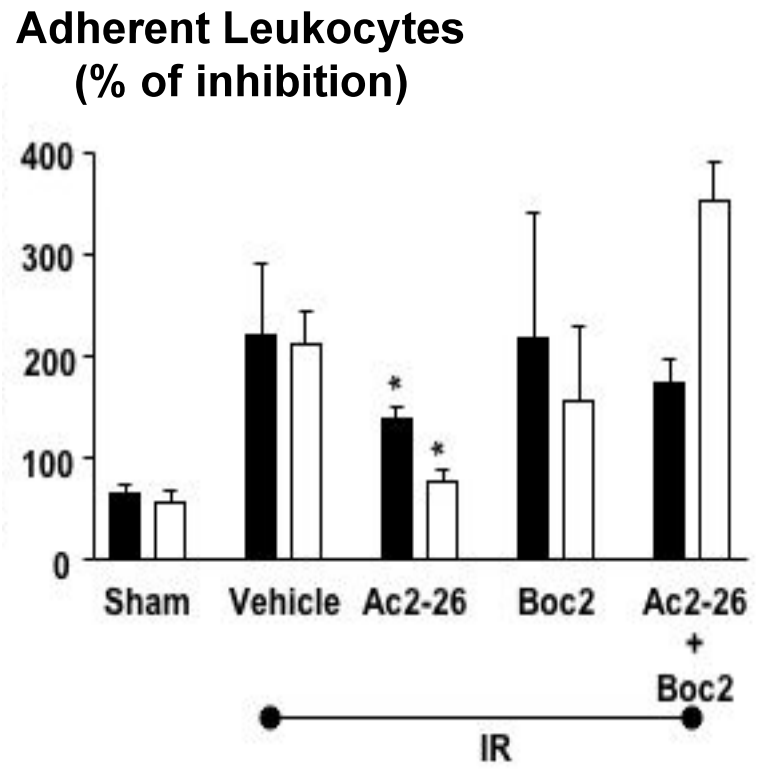


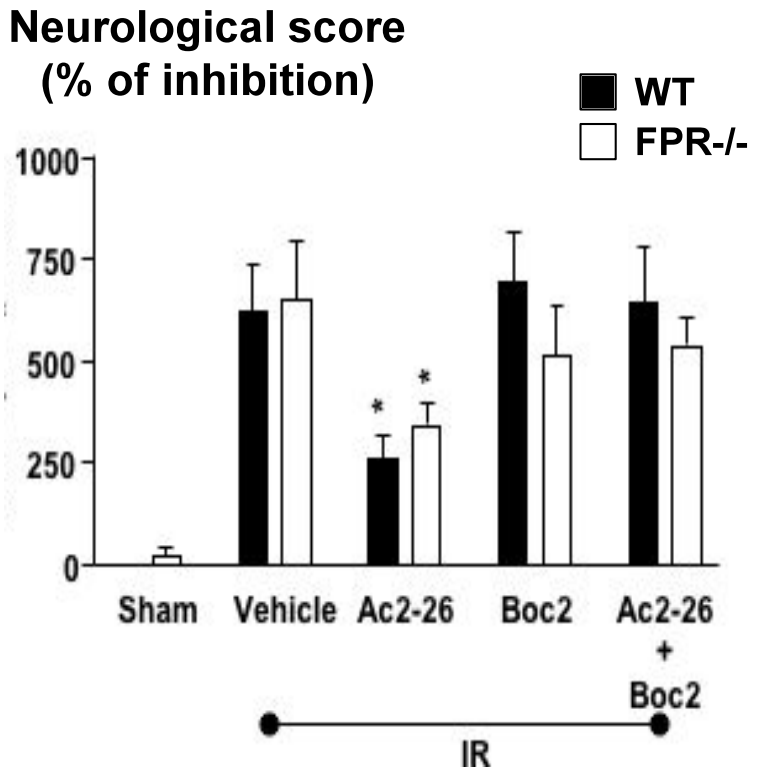
Specific Binding



Imperial College London

Peptide Ac2-26 Protective Actions in FPR Null Mice





Imperial College London

What have we learned from our research.....

- *Administration of both whole length AnxA1 and its peptide mimetic Ac2-26 are protective*

- *contribution blood borne cells vs. resident (microglia, astrocytes and neurons)*

- *FPR-rs2 (blood cells and microglia) may mediate the effects of AnxA1*

Goal.....

The endogenous AnxA1 pathway may provide a key for the development of novel anti-stroke therapies.

Imperial College London

Reading list

•Pharmacology, Rang, Dale and Ritter. Churchill Livingstone Press. 2007.

•John F. Cryan & Andrew Holmes. The ascent of mouse: advances in modelling human depression and anxiety. Nature Reviews Drug Discovery 2005:4;775-790.

•Smith MW, Gumbleton M. Endocytosis at the blood-brain barrier: from basic understanding to drug delivery strategies. J Drug Target. 2006 May; 14:191-214.

•Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007 Sep;7(9):678-89.

•Havekes R, Abel T. Genetic dissection of neural circuits and behavior in Mus musculus. Adv Genet. 2009;65:1-38.

•D. Mor, A.L. Bembrick, P.J. Austin, P.M. Wyllie, N.J. Creber, G.S. Denyer, K.A. Keay. Anatomically specific patterns of glial activation in the periaqueductal gray of the sub-population of rats showing pain and disability following chronic constriction injury of the sciatic nerve. Neuroscience 2010: 166:1167-1184