Neuroprotective Therapy Strategies after Stroke: Drug Delivery and VEGF

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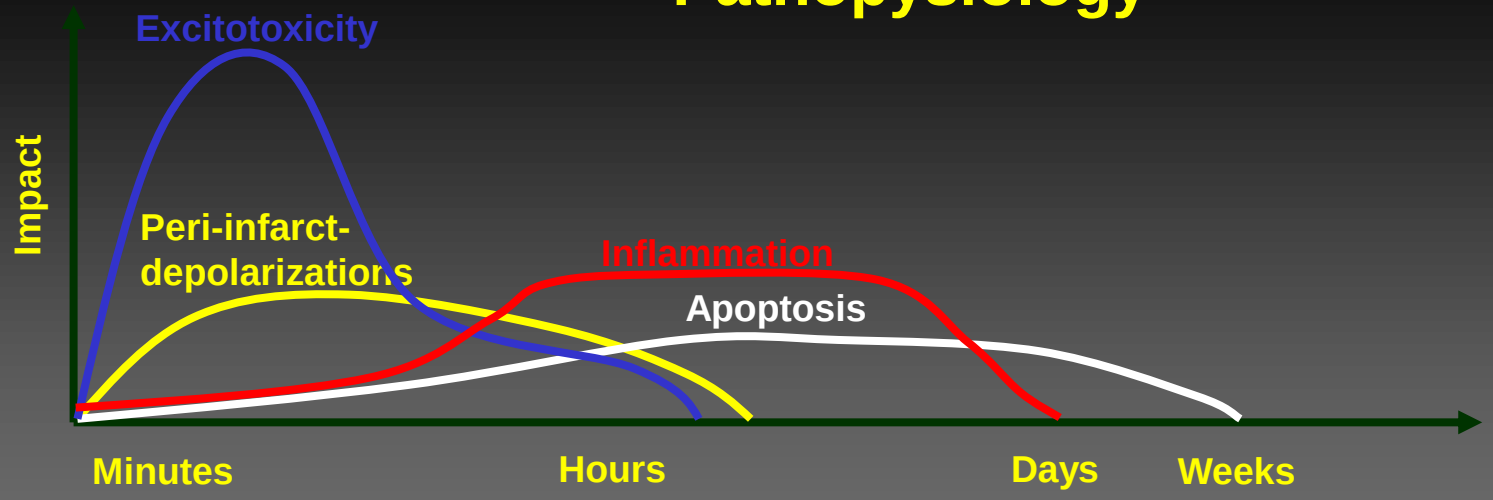
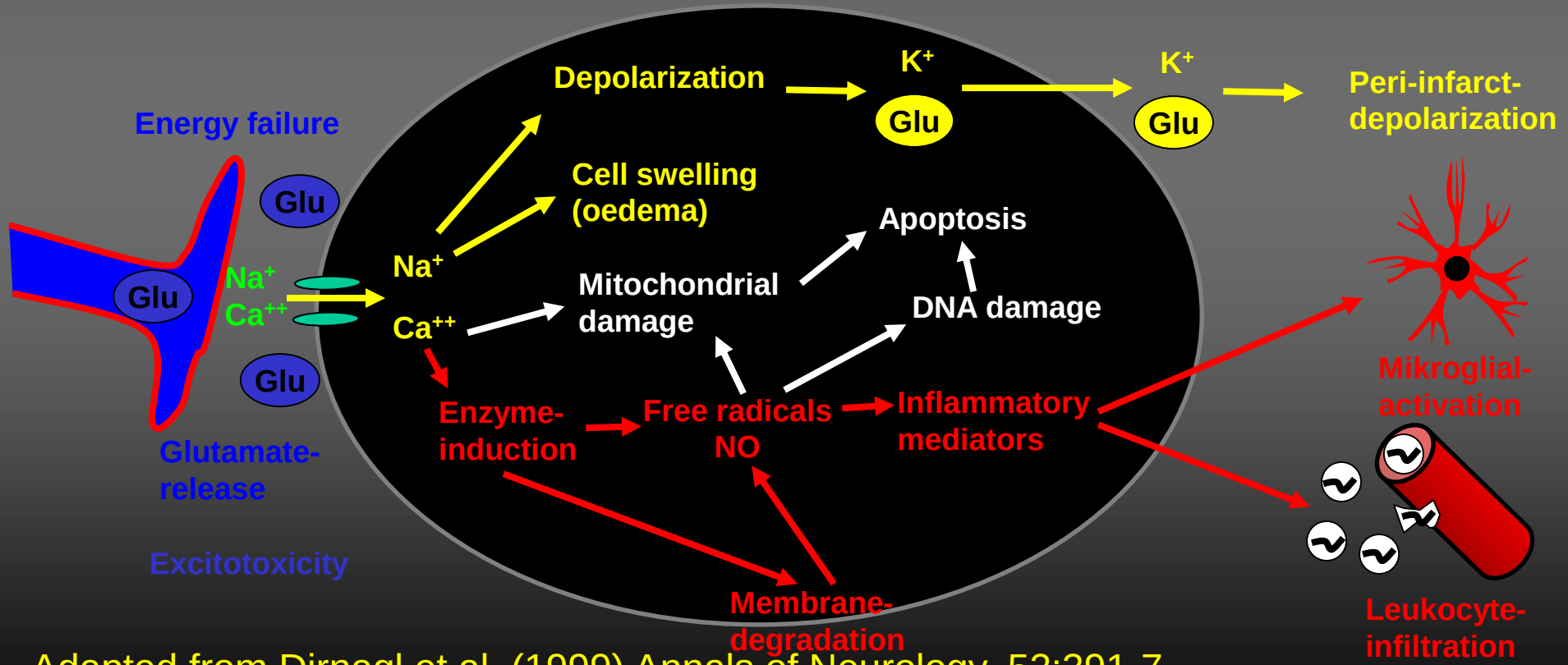
Ischemic Stroke

- caused by a deficiency in the blood supply to a part of the brain
- third most common cause of death worldwide
- cost of stroke and consecutive social services is nearly twice that of coronary heart disease

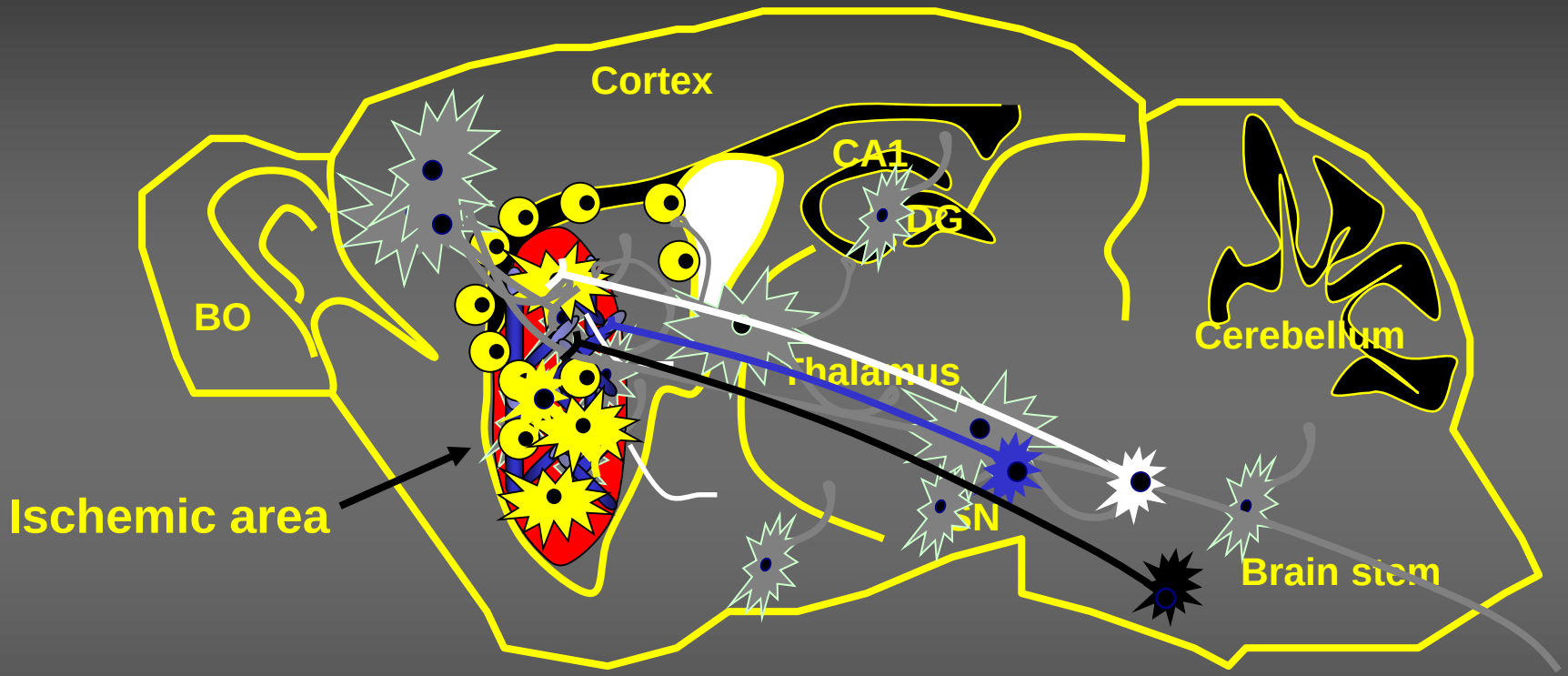
Pathopysiology





Adapted from Dirnagl et al. (1999) Annals of Neurology, 52:391-7

Pathophysiology



Retrograd axonal degeneration in ischemic brain regions

Cell death in Neuronal pathways

The sequelae of brain injury

Sensorymotor and neuropsychiatric (include a wide range of emotional and cognitive disturbances)

Clinical therapeutic approaches

Fibrinolytics:

- **t-PA**
- **Urokinase**
- **Desmoteplase**

REHABILITATION

NINDS (1995) N Engl J Med, 333:1581-87

Why have so many clinical stroke trials failed?

- Number of clinical studies
- Full characterization in models
- Timing
- Age and associated illnesses
- Evaluation of efficacy
- The expression of drug transporters (ABC) differs between animal species, strains and human.

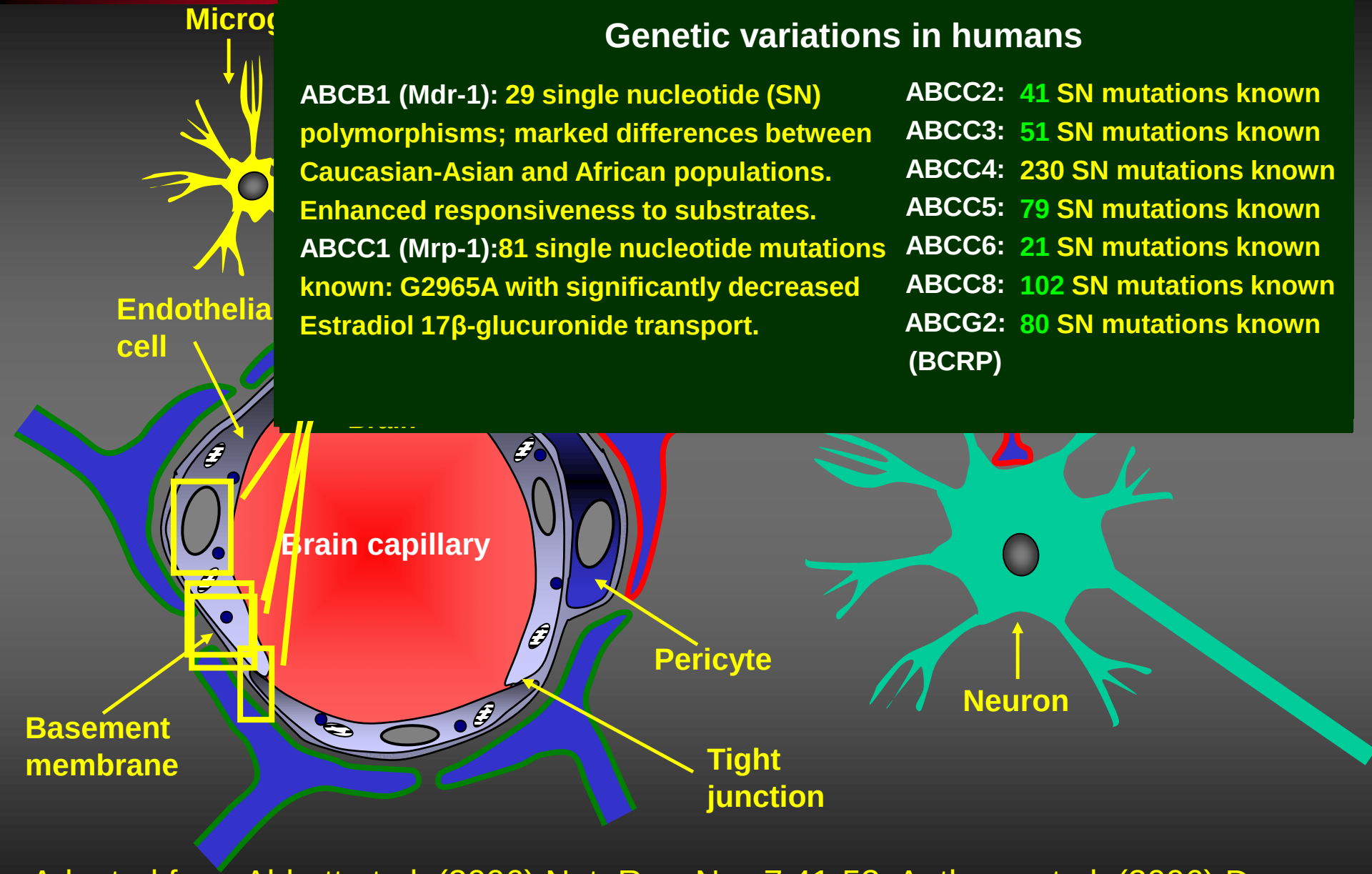
Blood Brain Barrier/ ABC (ATP-binding cassette) Transporters

Genetic variations in humans

ABCB1 (Mdr-1): 29 single nucleotide (SN) polymorphisms; marked differences between Caucasian-Asian and African populations. Enhanced responsiveness to substrates.

ABCC1 (Mrp-1):81 single nucleotide mutations known: G2965A with significantly decreased Estradiol 17β-glucuronide transport.

ABCC2: 41 SN mutations known
ABCC3: 51 SN mutations known
ABCC4: 230 SN mutations known
ABCC5: 79 SN mutations known
ABCC6: 21 SN mutations known
ABCC8: 102 SN mutations known
ABCG2: 80 SN mutations known (BCRP)



Adapted from Abbott et al. (2006) Nat. Rev. Nsc.7:41-53; Anthony et al. (2006) Drug Dis. Today, 11:17-8; Hermann et al. (2006) Ann. Neurology, 60:489-98

Drug Resistance in Brain Diseases and the Role of Drug Transporters

Drug resistance is an important hurdle in the therapy of brain disorders.

MDR-1 (ABCB1)

Substrates — LUMINAL-EFFLUX

Lipophilic compounds and Amphipathic organic anions: i.e.

- Antibiotics
- Anticancer
- Cardiacs
- Opioids
- Antipsychotic
- Anti-HIV
- Immunosupressants
- Lipid lowering agents
- Antiepileptics
- Antidepressants
- Ca channel bloc.
- Antirheumatic
- Benzodiazepine
- Antihelmintic
- H2 receptor antag
- Antihistaminic

Neuroprotective FK506

Mdr-1 inhibitor: Tariquidar

MRP-1 (ABCC1)

Substrates — ABLUMINAL-INFLUX

Glutathionized, glucuronidated and sulfated organic anions, lipophilic compounds, amphipathic organic anions: i.e.

- Anticancer
- Anti-HIV
- Immunosupressants
- Ca channel bloc.
- Metalloids
- Aflatoxin B1
- Bilirubine
- Colchicine
- Dehydroepiandrosteron (DHEAS)
- Fluorescein
- Folic acid
- Glutathione (GSH)
- S-nitrosoglutathione
- Leukotriene C4 / D4 / E4
- Rhodamine

Neuroprotective Estradiol-17β-D(+)-glucuronide (17βEG)

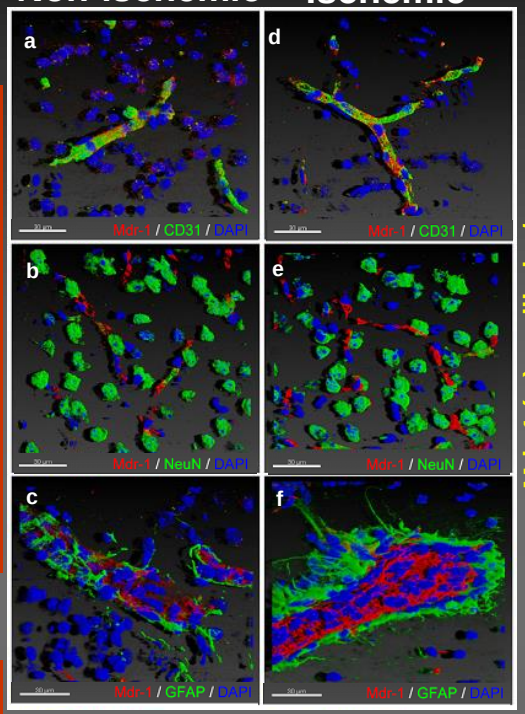
MRP-1 inhibitor: MK571

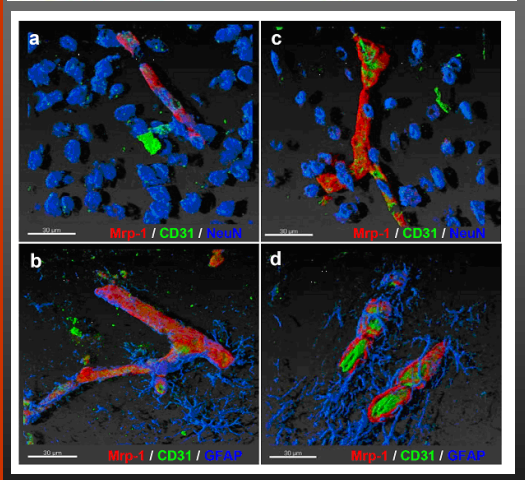
Mdr-1/ MRP-1 expressions 30 min after ischemia

Mdr-1: LUMINAL-EFFLUX

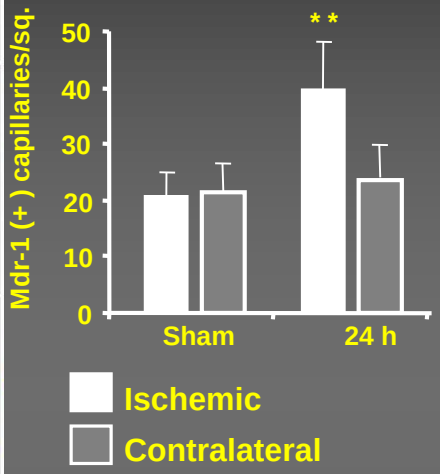
MRP-1: ABLUMINAL-INFLUX

Non-ischemic Ischemic

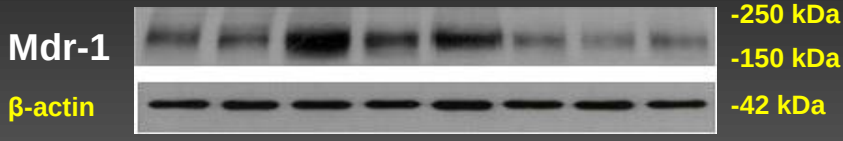


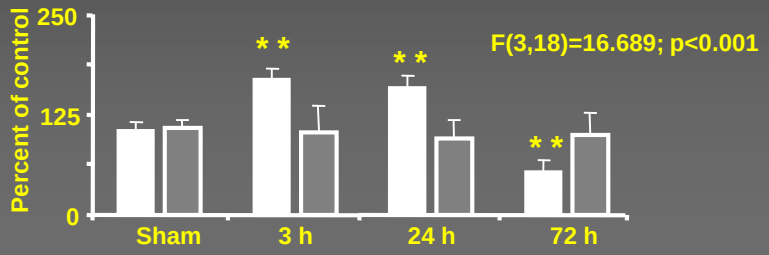


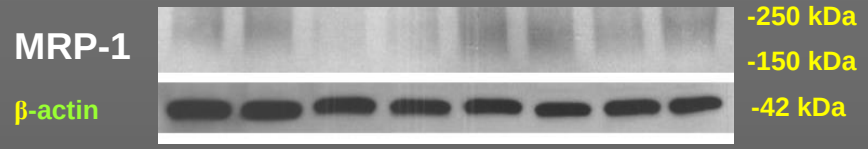
Mdr-1/ MRP-1 IHC on Capillary endothelium

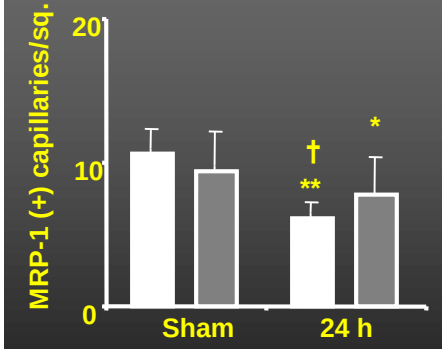


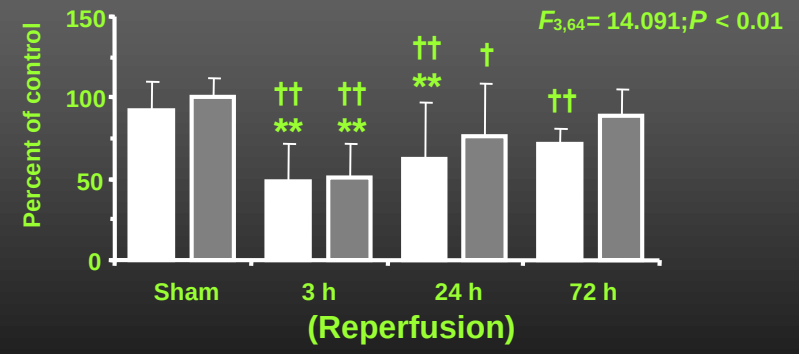
Mdr-1/ MRP-1 WB of enriched brain microvessel fraction









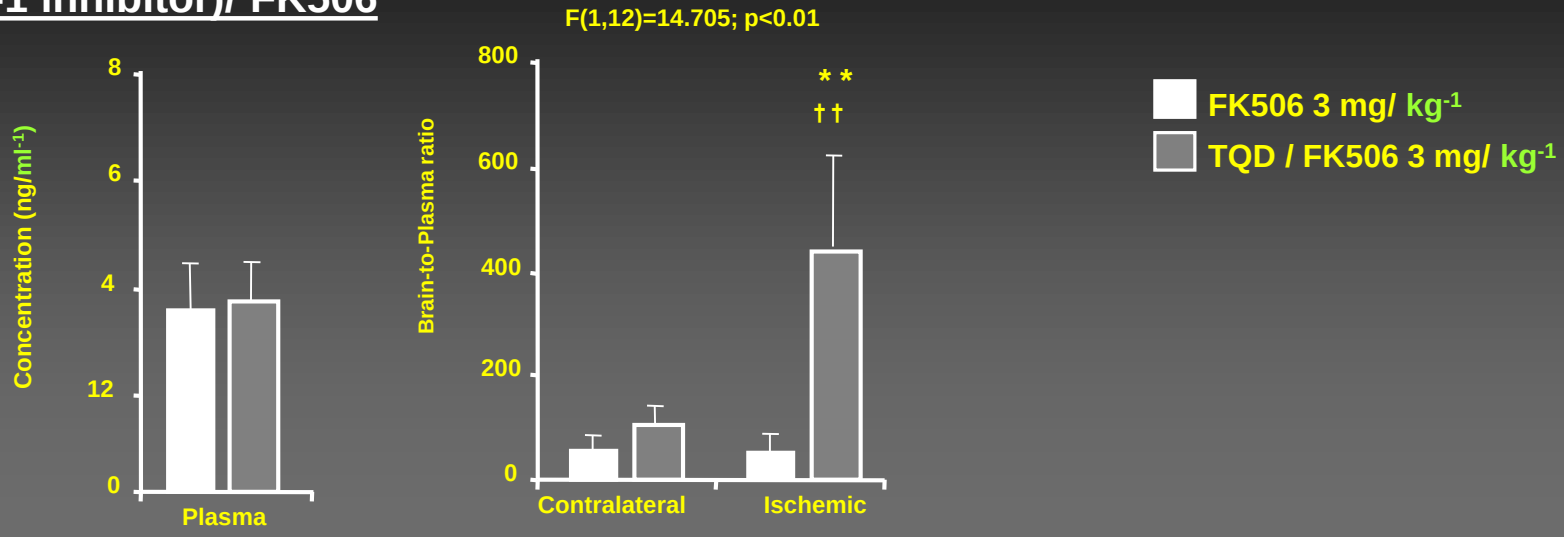


Spudich et al.,(2006) Nat. Neurosci.9:487-8; Kilic et al., (2008) Brain 131:2679-89

The Mdr-1 inhibitor tariquidar (TQD) increases concentration of FK506 in the ischemic brain.

Mdr-1: LUMINAL-EFFLUX

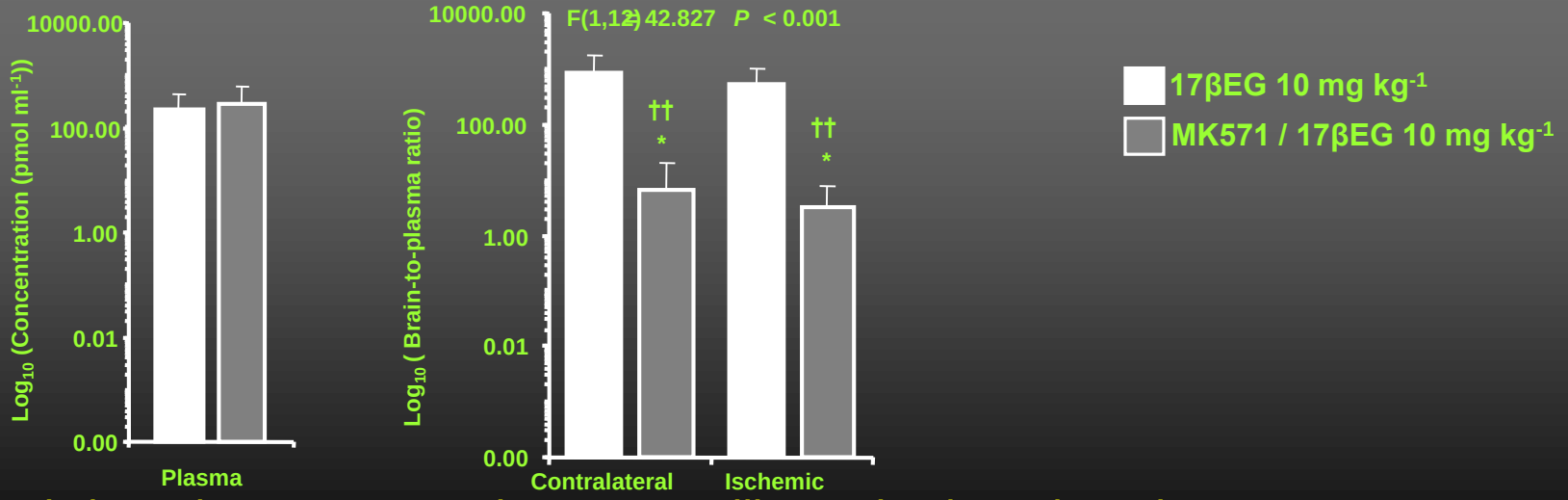
TQD (Mdr-1 inhibitor)/ FK506



The MRP-1 inhibitor MK571 decreases concentration of 17βEG in the ischemic brain.

MRP-1: ABLUMINAL-INFLUX

MK571 (MRP-1 inhibitor)/ estradiol-17β-D(+)-glucuronide (17βEG)



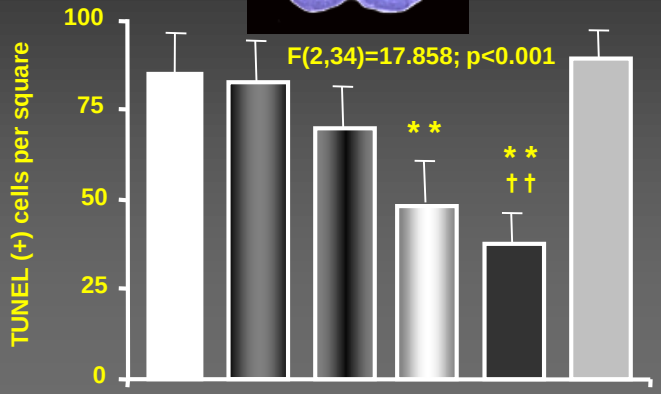
Spudich et al.,(2006) Nat. Neurosci.9:487-8; Kilic et al., (2008) Brain 131:2679-89

A

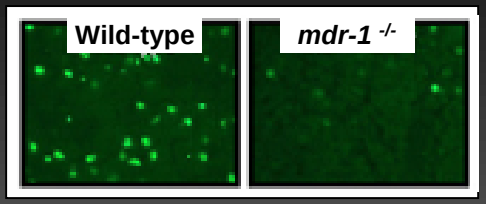
Effects of Mdr-1 inhibition by TQD on neuroprotective efficacy of FK506 after 30 min ischemia

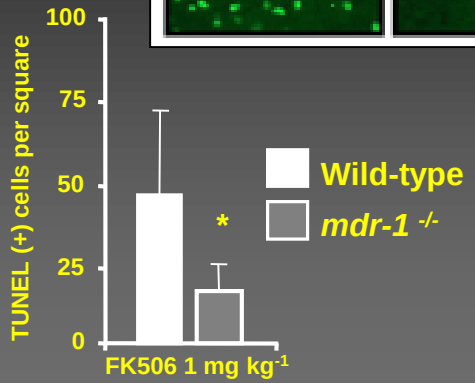
Mdr-1: LUMINAL-EFFLUX

TQD / FK506



Control
FK506 1 mg kg^{-1}
FK506 3 mg kg^{-1}
FK506 5 mg kg^{-1}
TQD / FK506 1 mg kg^{-1}
TQD

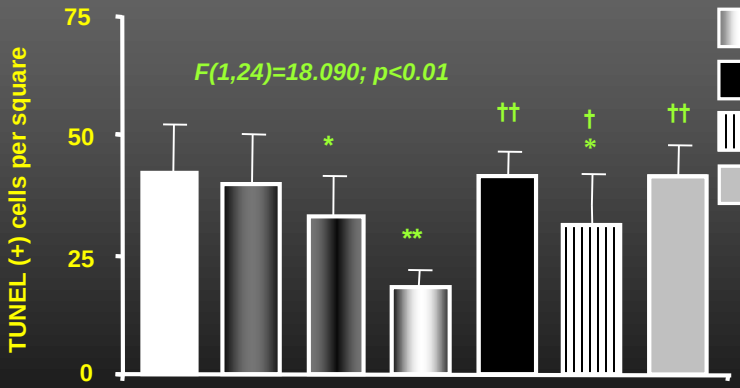




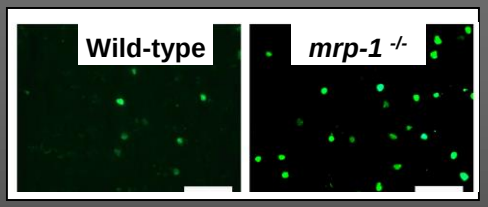
Effects of MRP-1 inhibition by MK571 on neuroprotective efficacy of 17βEG after 30 min ischemia

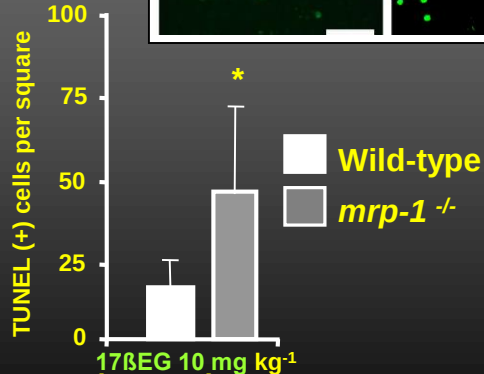
MRP-1: ABLUMINAL-INFLUX

MK571 / 17ßEG



Control
17ßEG 2 mg kg-1
17ßEG 5 mg kg-1
17ßEG 10 mg kg-1
MK571 / 17ßEG 10 mg kg-1
MK571 / 17ßEG 50 mg kg-1
MK571

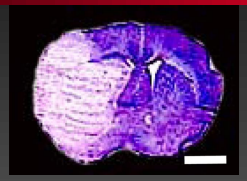


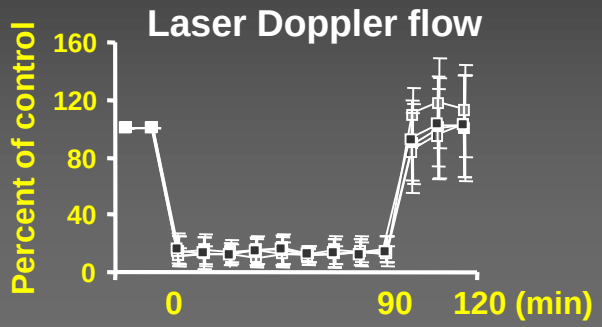


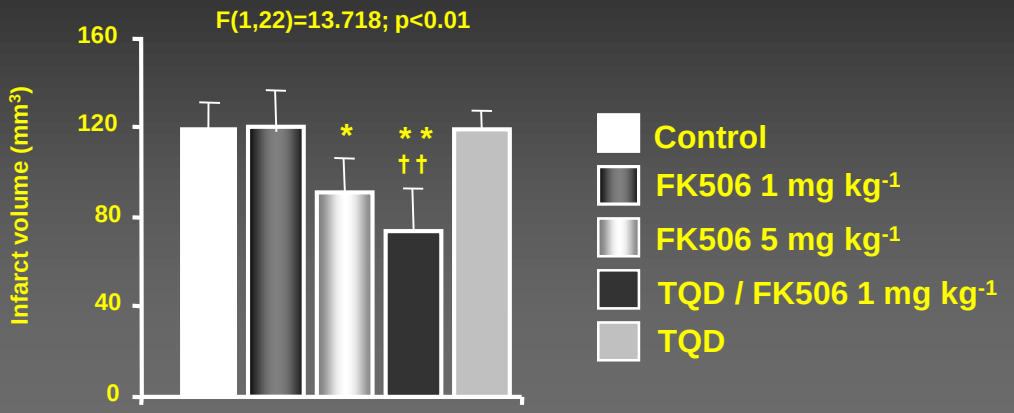
Spudich et al.,(2006) Nat. Neurosci.9:487-8; Kilic et al., (2008) Brain 131:2679-89

A Effects of Mdr-1 inhibition by TQD on neuroprotective efficacy of FK506 after 90 min ischemia

Mdr-1: LUMINAL-EFFLUX

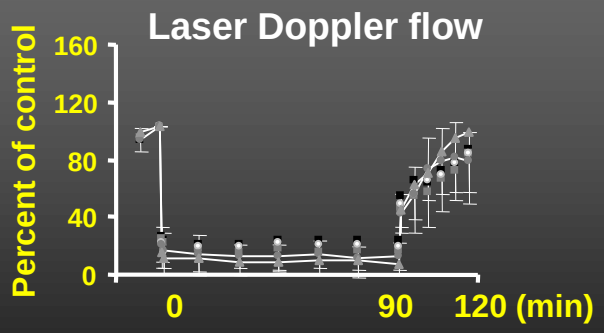


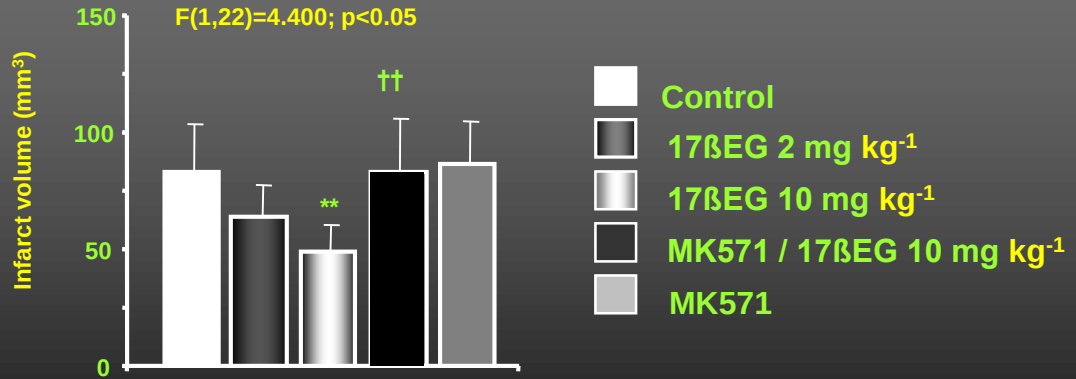




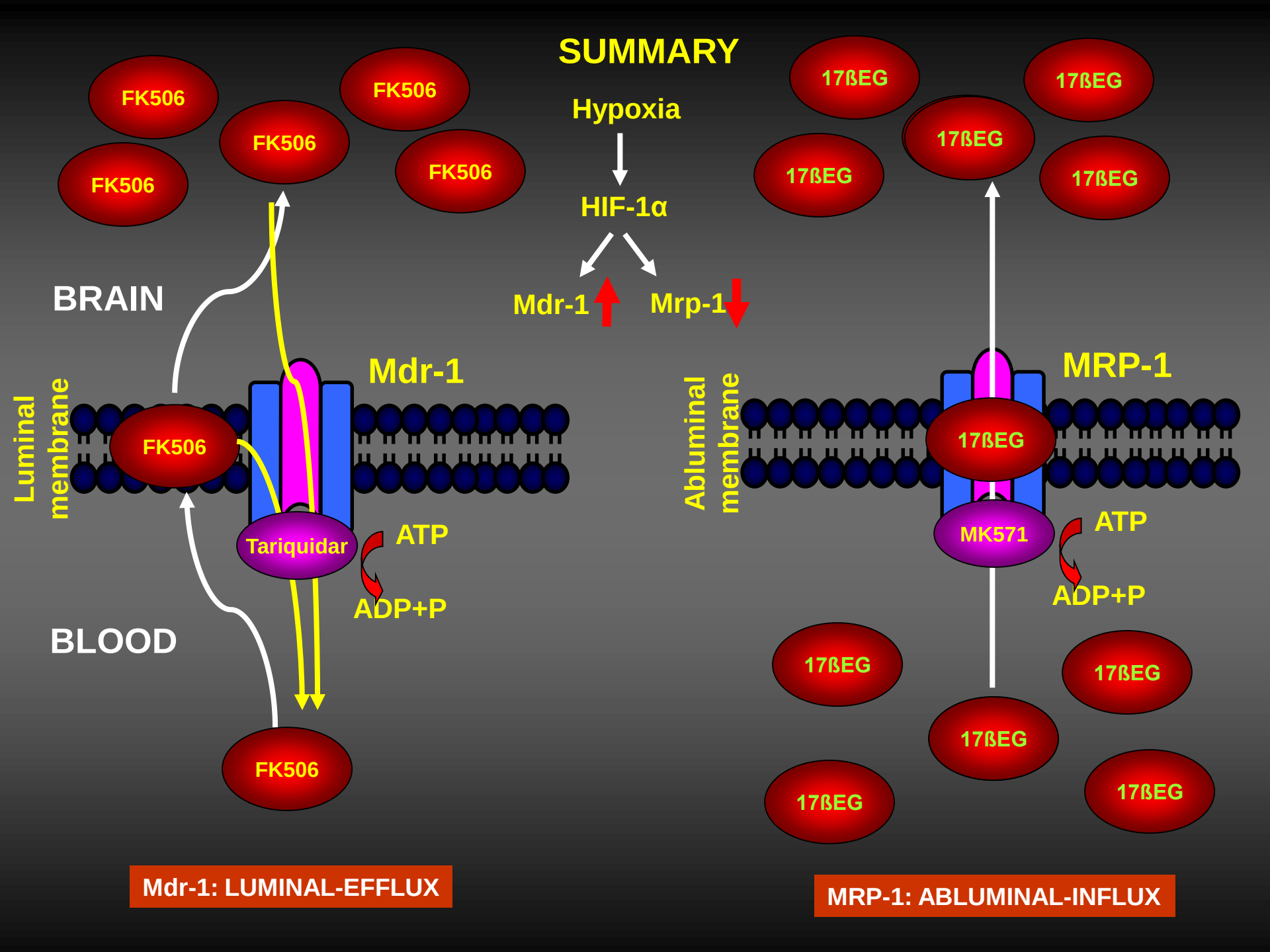
B Effects of MRP-1 inhibition by MK571 on neuroprotective efficacy of 17βEG after 90 min ischemia

MRP-1: ABLUMINAL-INFLUX





Spudich et al.,(2006) Nat. Neurosci.9:487-8; Kilic et al., (2008) Brain 131:2679-89


SUMMARY
Hypoxia
HIF-1α
Mdr-1
Mrp-1
BRAIN
BLOOD
Luminal membrane
Abluminal membrane
FK506
Mdr-1
Tariquidar
ATP
ADP+P
17ßEG
MRP-1
MK571
Mdr-1: LUMINAL-EFFLUX
MRP-1: ABLUMINAL-INFLUX

Conclusion and Outlook

- Multi drug transporter modulators might be used to promote the accumulation of drugs in various neurological disorders in future.

- Positron emission tomography (PET) enables measurements of pharmacological compounds in brain.

- The temporal analysis of the positron signal enables quantitative drug concentrations and precise information on the pharmacokinetics and pharmacodynamics of the compound.

- PET can be also used to evaluate the function of efflux proteins by using their inhibitors.

Conclusion and Outlook

The existence of ABC transporters has important implications for drug development that should be considered in the preparation of studies.

1- The expression of ABC transporters differ between animal species, strains and human. Thus biodistribution studies should be performed for clinical trials.

2-The expression and functionality of ABC transporters change in response to brain disease and drug exposure. As a consequence, tissue concentration should be assessed also in diseased brain.

3-The genetics and functionality of ABC transporters might vary between ethnic groups.

Vascular Endothelial Growth Factor (VEGF)

- stimulates angiogenesis (Angiogenesis is correlated with stroke recovery according to clinical studies), axonal outgrowth and neurogenesis
- protects neurons

- However, the cell death mechanism and signal transduction components underlying *in vivo* VEGF-mediated survival were largely unknown.

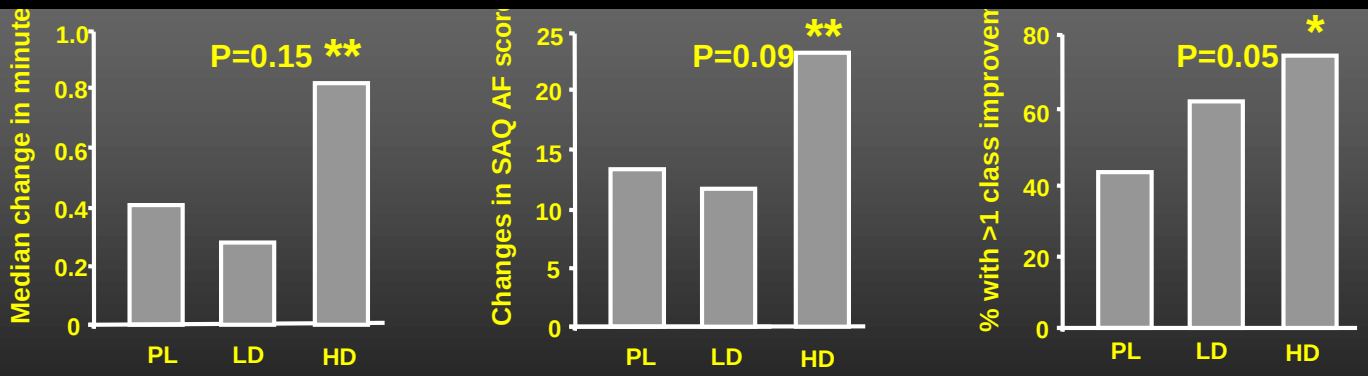
The VIVA (VEGF in Ischemia for Vascular Angiogenesis)-Trial

Patient Characteristics

	Placebo (n=63)	Low-Dose rhVEGF (n=56)	High-Dose rhVEGF (n=59)
Age, mean±SD	61±7	61±9	58±8
Male sex, %	87	89	92

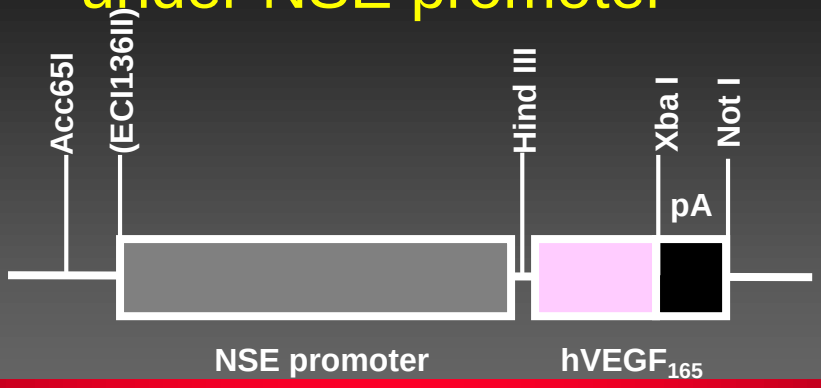
PL= Placebo,
LD= Low-Dose rhVEGF (17 ng·kg^{-1}·min^{-1})

According to this clinical study, rhVEGF is well tolerated, safe and beneficial in human.

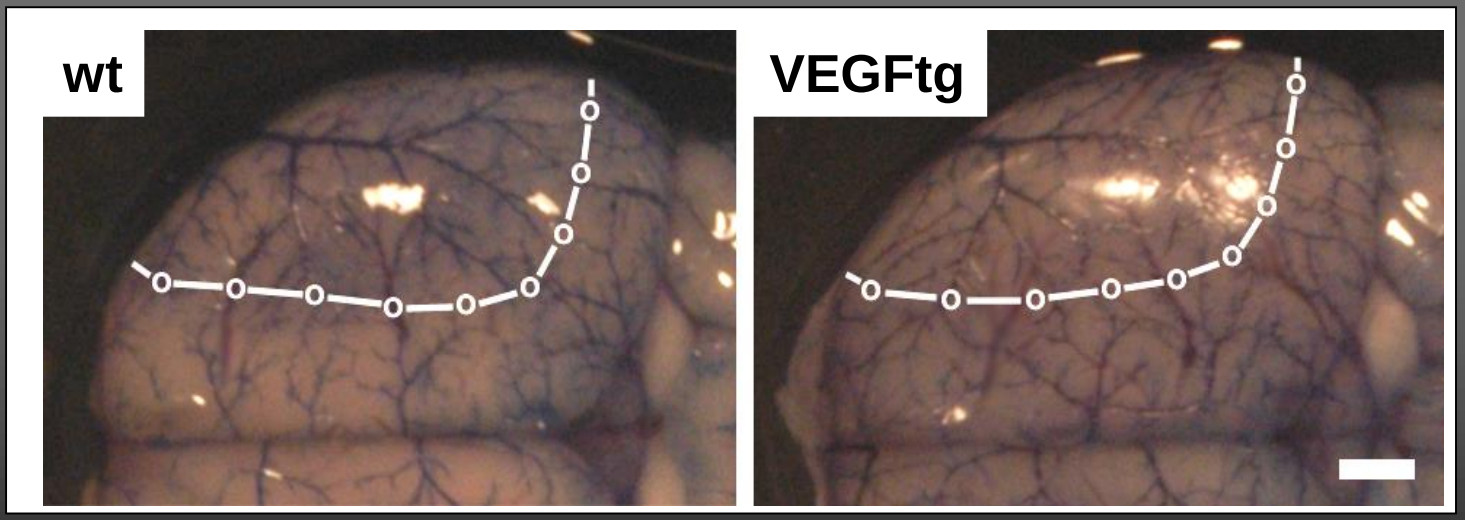


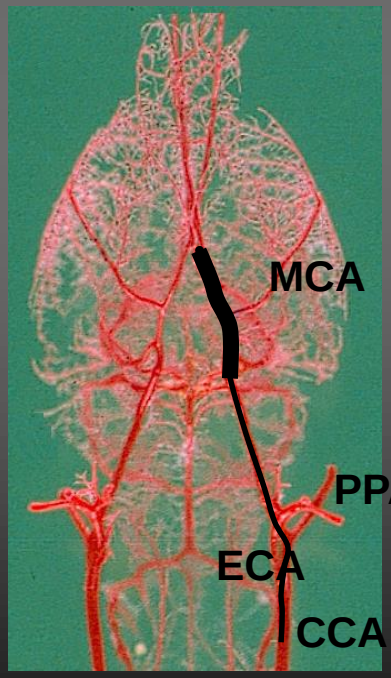
Henry et al., Circulation (2003) 107, 1359-1365

V1tg mouse: Brain-selective VEGF expression under NSE promoter



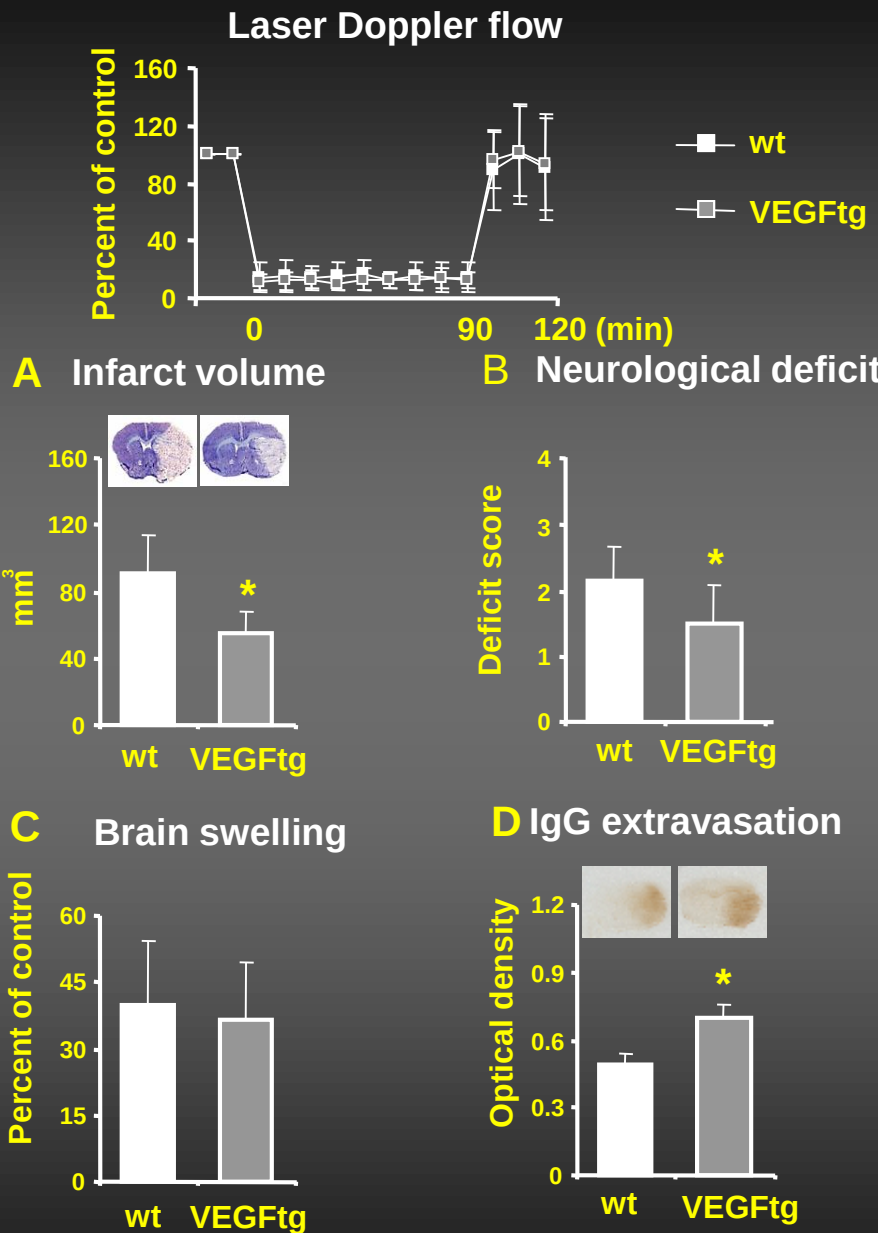
Macroscopic anatomy of the MCA territory





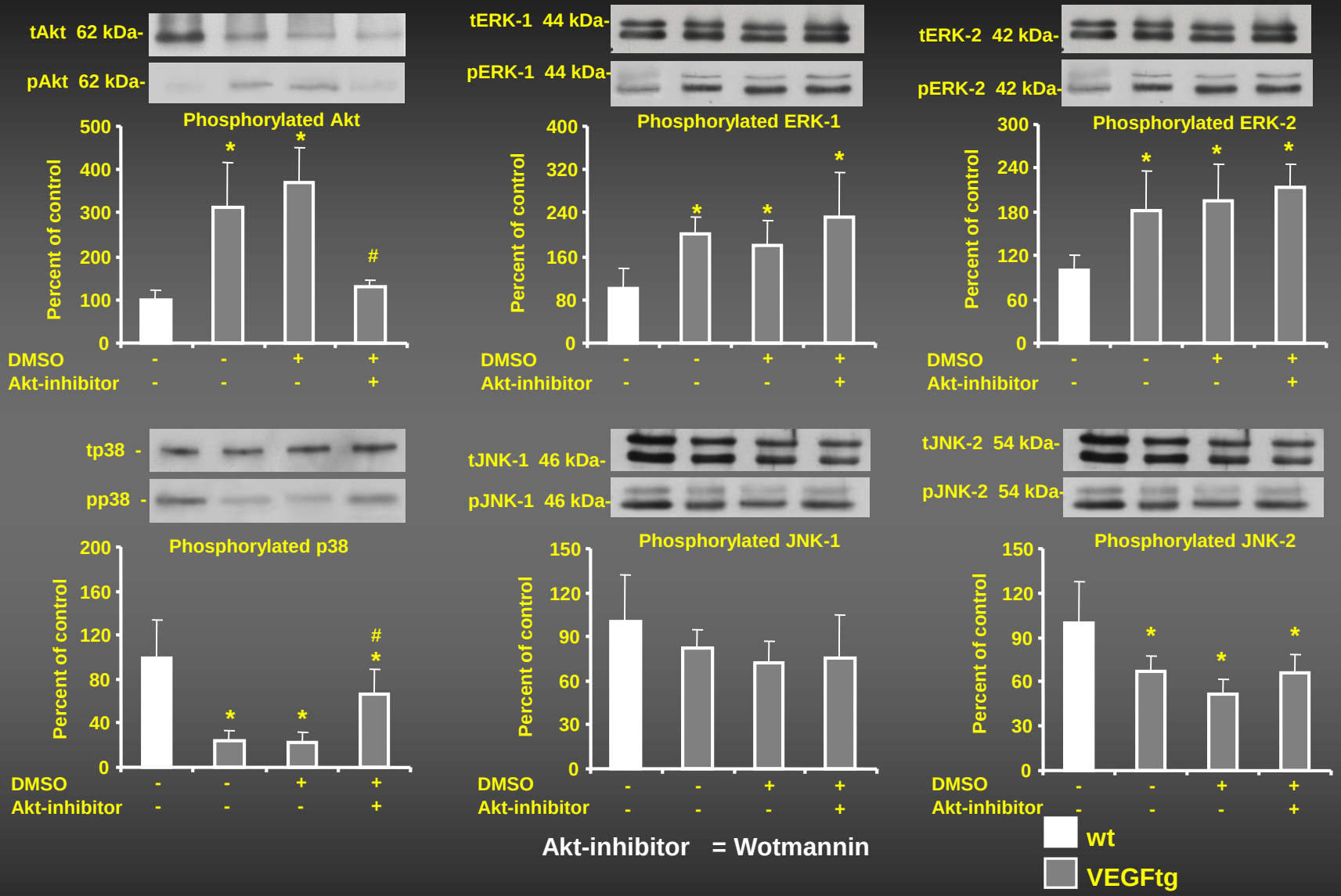
Kilic et al. (2006) FASEB J. 20:1185-7

Effects of human VEGF on ischemic injury



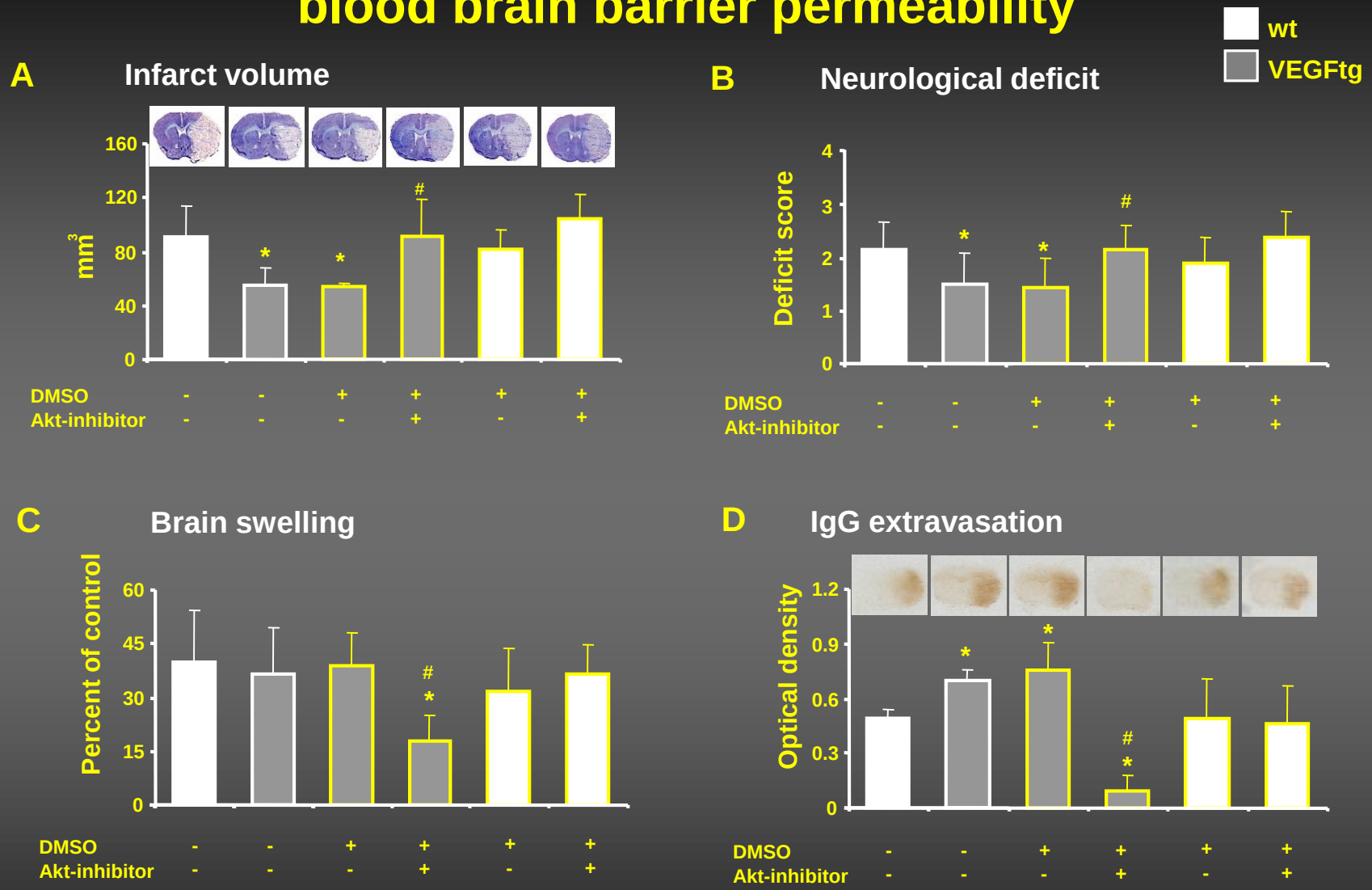
Kilic et al. (2006) FASEB J. 20:1185-7

Effect of VEGF on intracellular signal cascade: Western blots



Kilic et al. (2006) FASEB J. 20:1185-7

Role of PI3K/Akt pathway in ischemic neuroprotection and blood brain barrier permeability

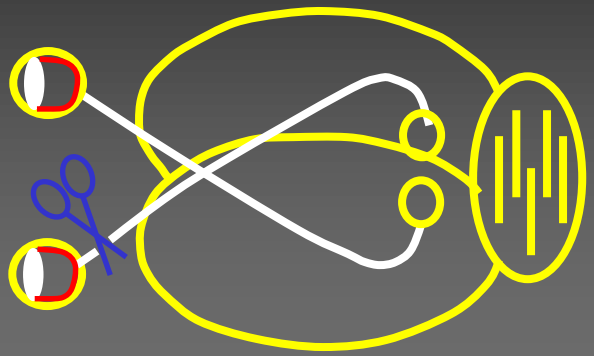


Akt-inhibitor = Wotmannin

Kilic et al. (2006) FASEB J. 20:1185-7

VEGF protects axotomized retinal ganglion cells

- **Optic nerve transection**

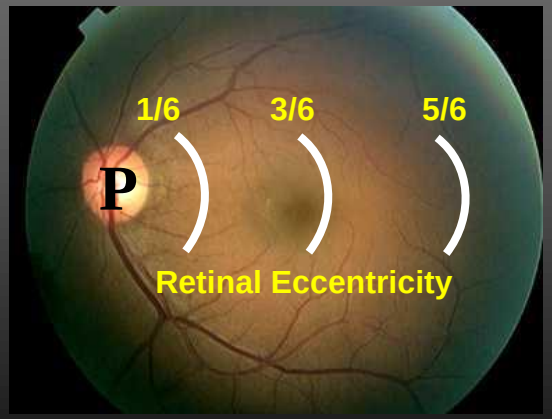


VEGFtg/ non-axotomized

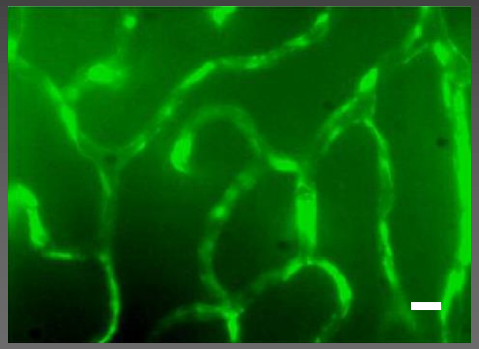
VEGFtg/ axotomized

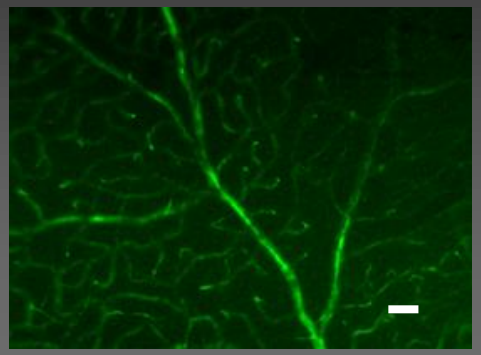
wt/ non-axotomized

wt/ axotomized

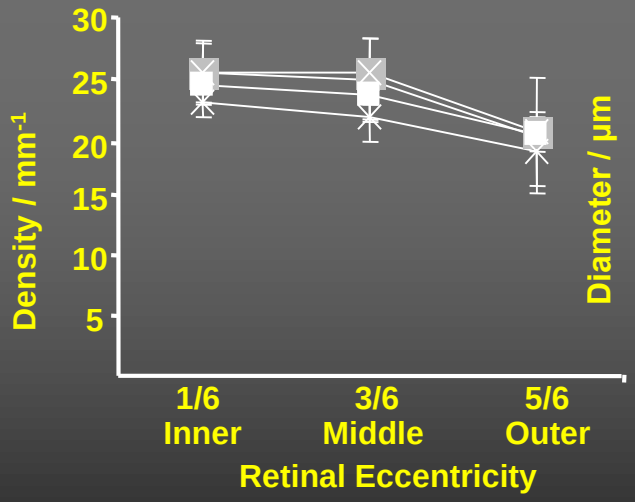


- **Retinal vascular networks are not influenced by human VEGF and ON transsection**

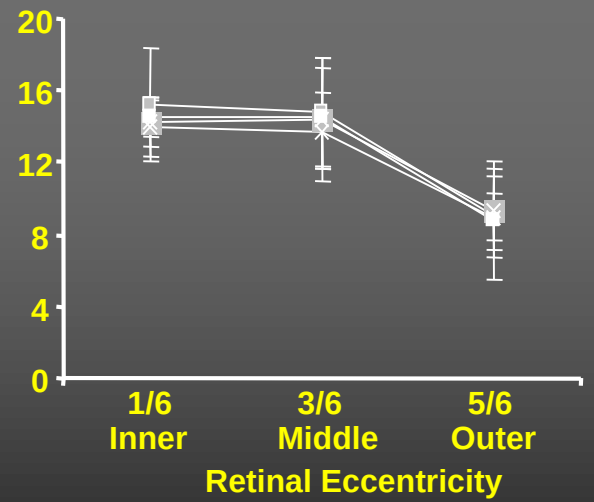




Density of retinal capillaries

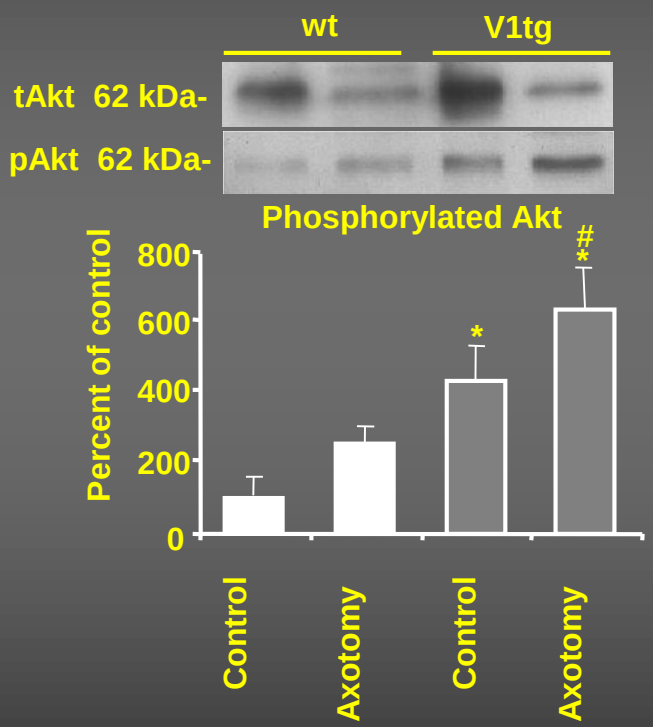


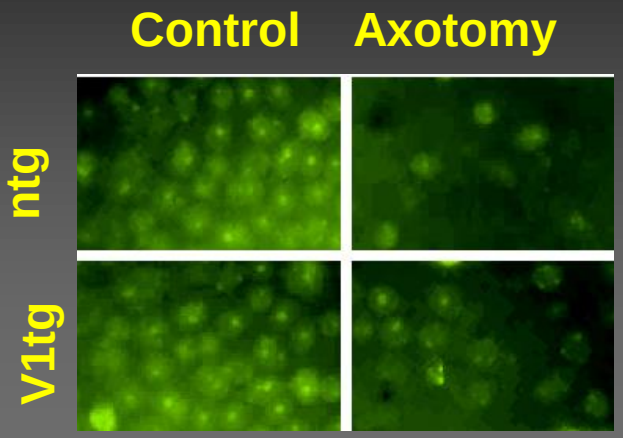
Diameter of main arterioles

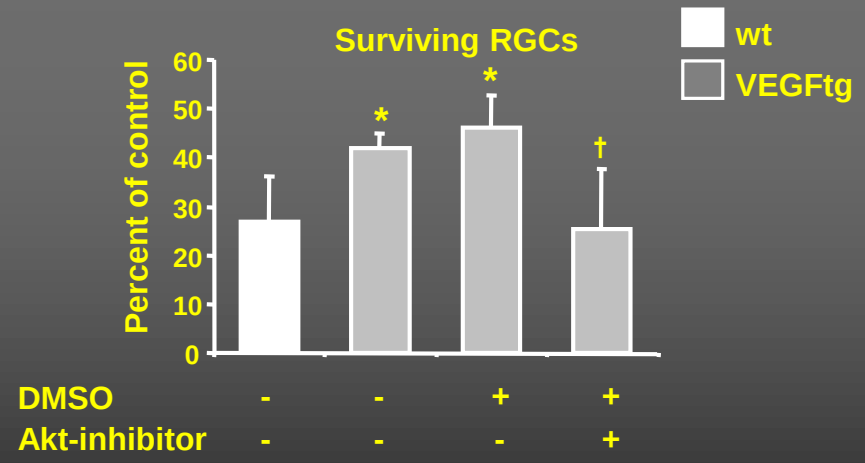


Kilic et al. (2006) J. Neurosci. 26:12439-46

VEGF protects degenerating RGCs by activating ERK-1/-2 and Akt pathways







Akt-inhibitor = LY294002

Kilic et al. (2006) J. Neurosci. 26:12439-46

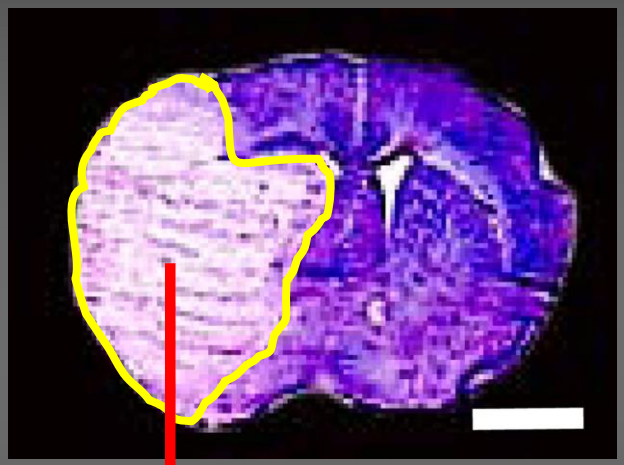
Conclusion

Based on our finding that the phosphorylated Akt pathway is responsible for VEGF`s neuroprotection and its BBB permeability, we predict that it may not easily be possible to make use of VEGF`s tissue survival without accepting its unfavourable consecuence, the increased BBB leakage.

Functional Recovery

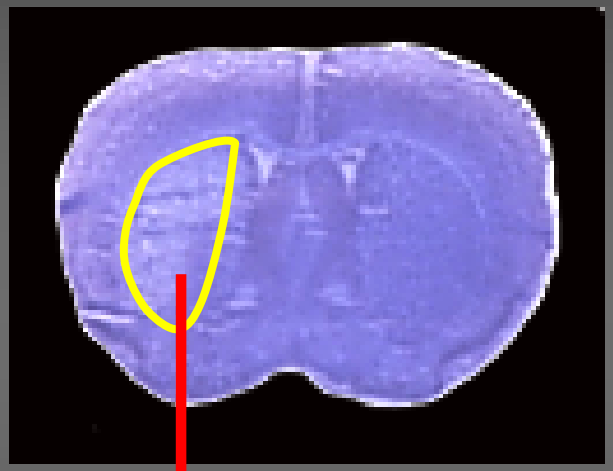
- Logically, infarct volume or the number of surviving neurons should be correlated with behavioral outcome.
- Behavioral outcome may not be correlated with infarct volume or surviving neurons due to long-term synaptic plasticity or diffuse morphological changes.

Infarct

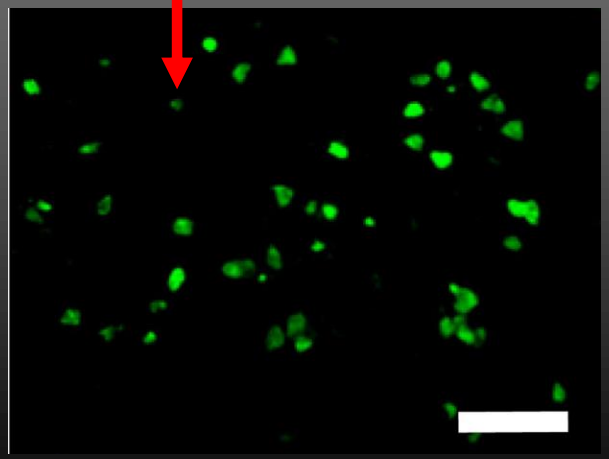


Necrosis

Disseminated cell death

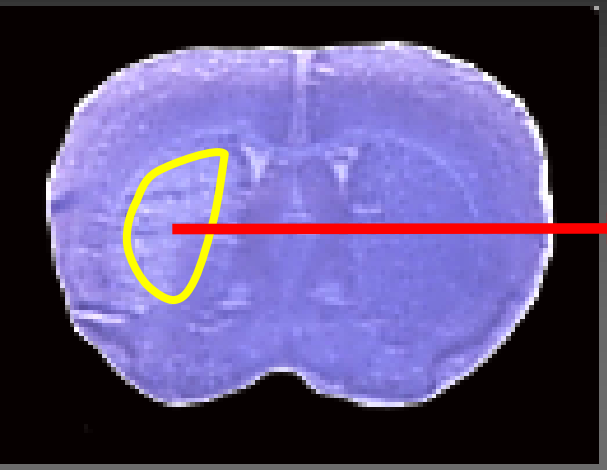


Apoptotic cells

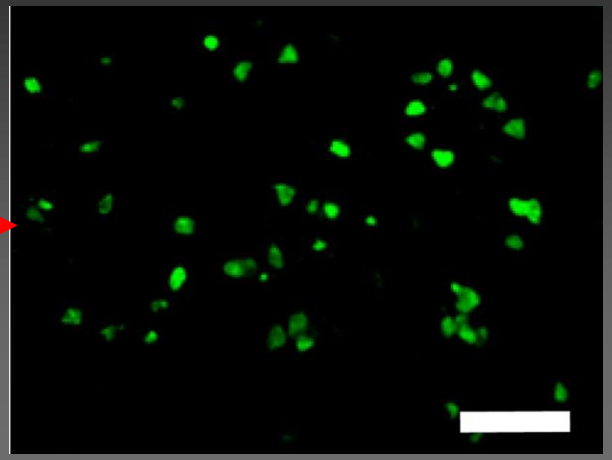


Restorative effects of exogenous VEGF

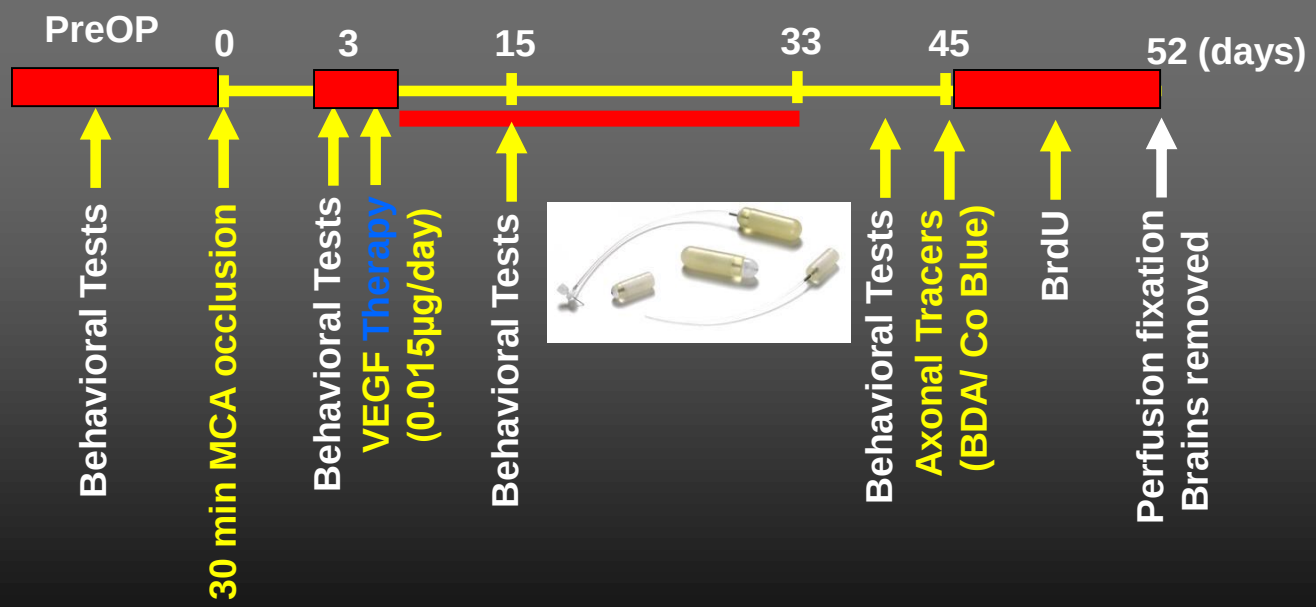
Disseminated cell death



Apoptotic cells

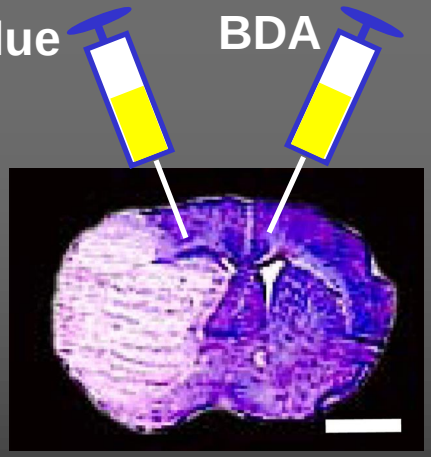


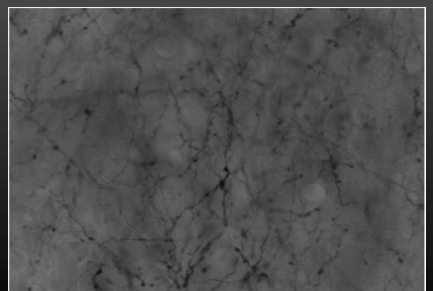
No apparent deficit



Co Blue

BDA

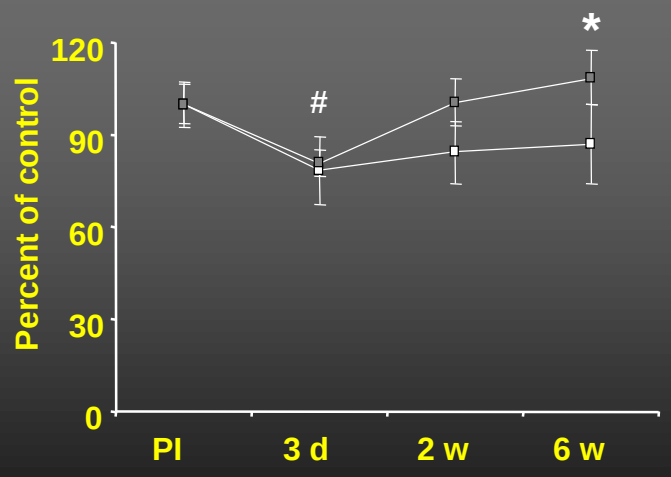




Behavioral Tests

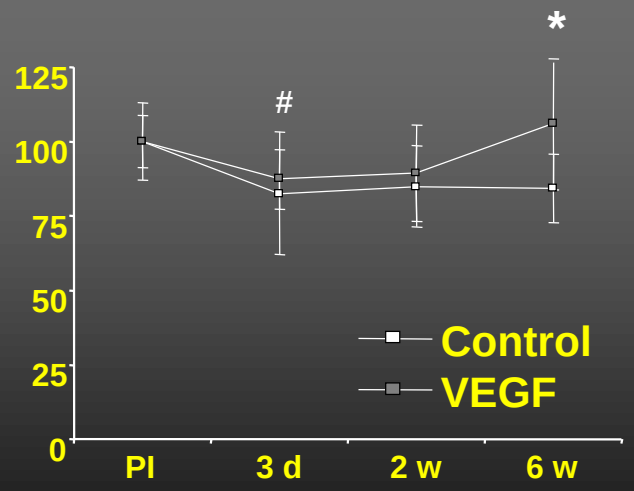
Grip Strength Tests

Paretic right forepaw



RotaRod Test

Motor coordination

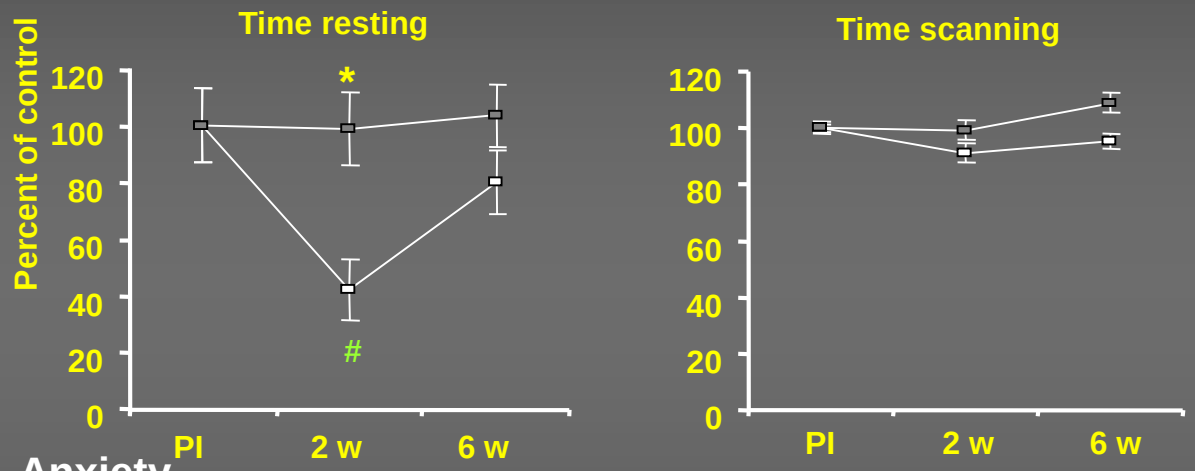


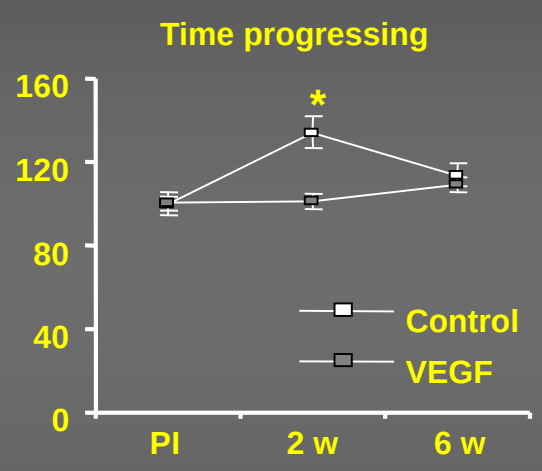
Kilic et al. (2009) in preparation.

Activity: Open Field Test

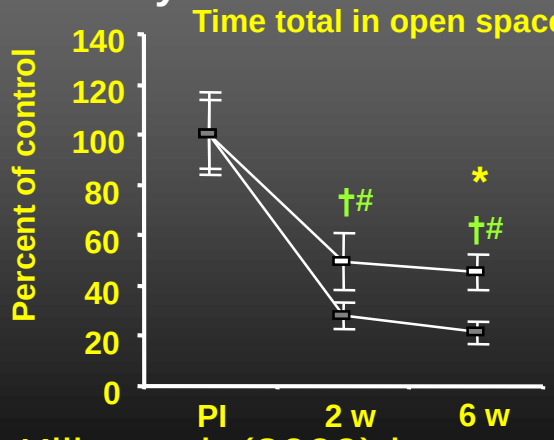
Anxiety: Elevated 0 Maze Test

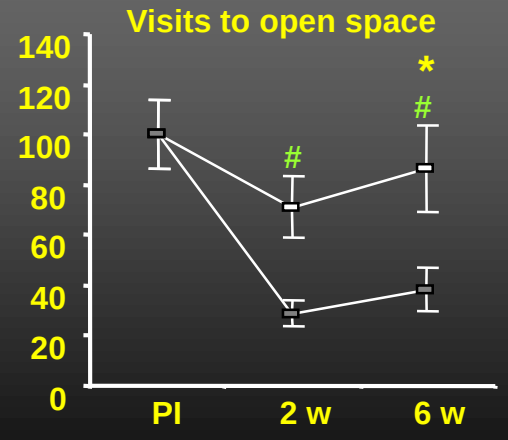
A- Hyperactivity

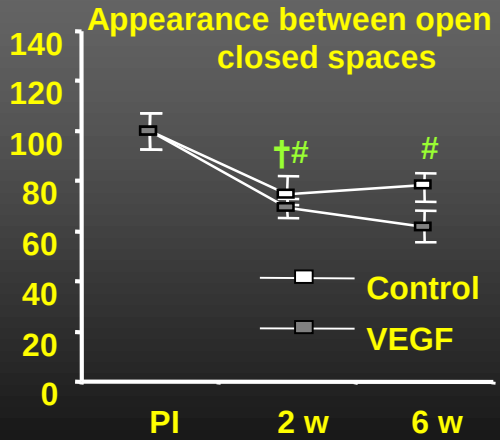




B- Anxiety

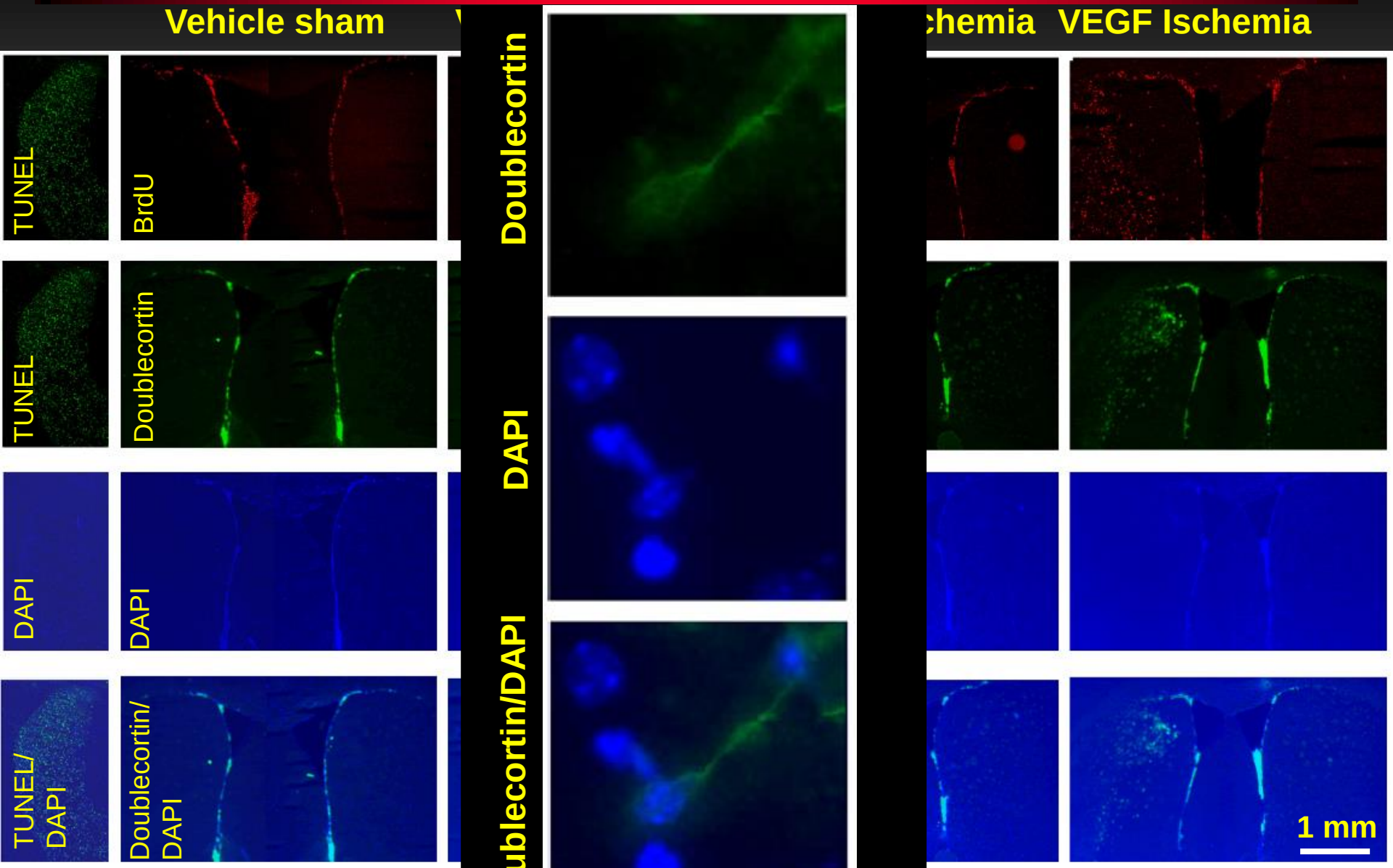




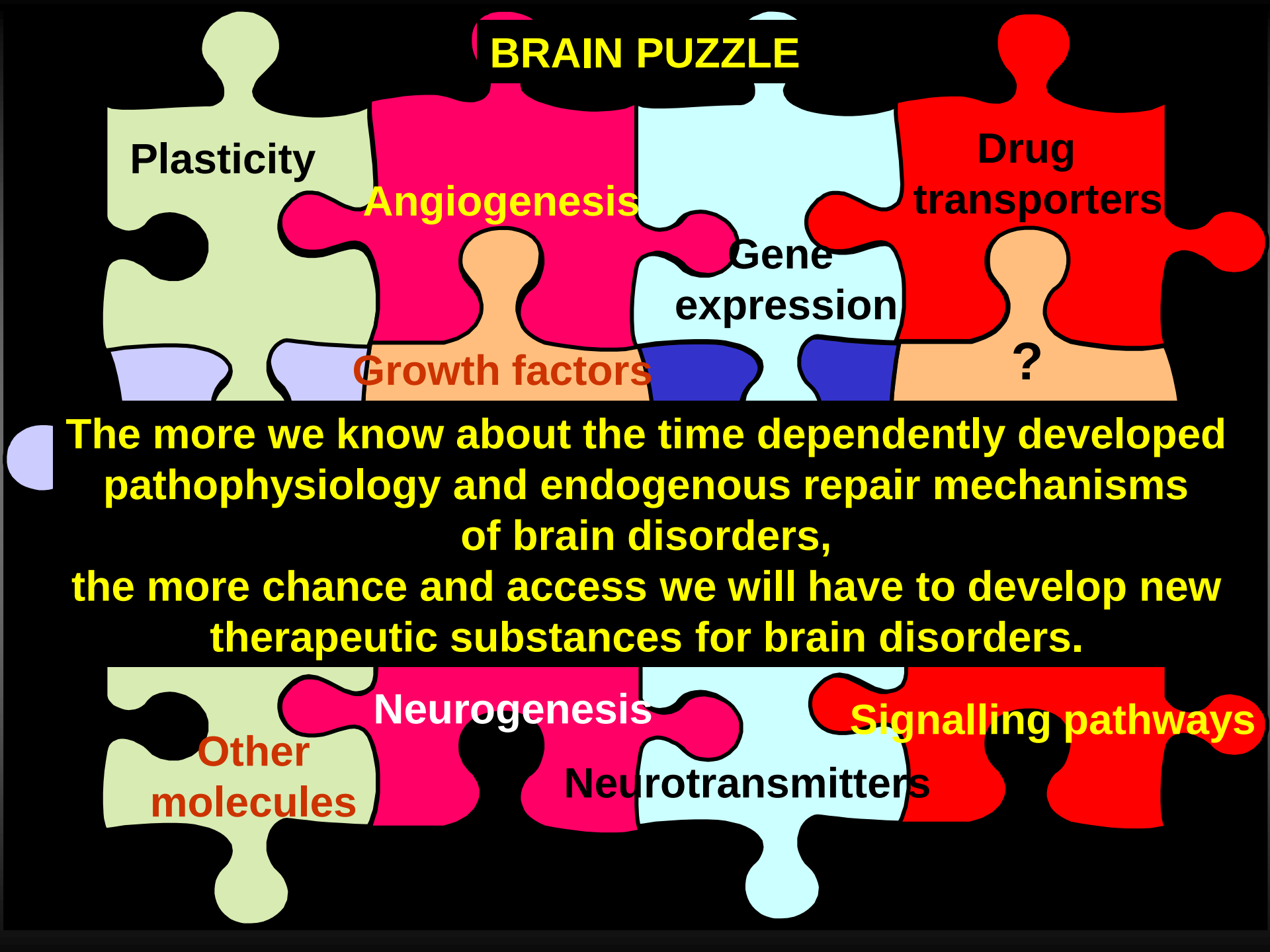


Kilic et al. (2009) in preparation.

Cell Proliferation



Kilic et al. (2009) in preparation.


BRAIN PUZZLE
Plasticity
Angiogenesis
Gene expression
Drug transporters
Growth factors
?
The more we know about the time dependently developed pathophysiology and endogenous repair mechanisms of brain disorders,
the more chance and access we will have to develop new therapeutic substances for brain disorders.
Neurogenesis
Signalling pathways
Other molecules
Neurotransmitters