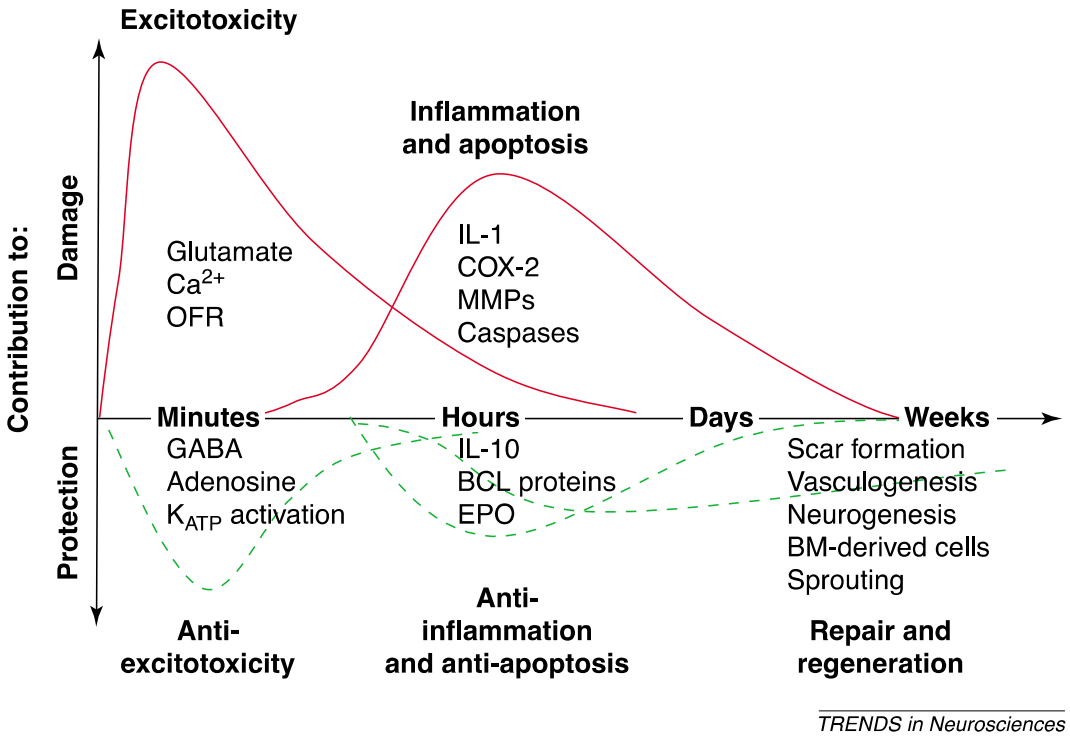
Preconditioning the Brain for Stroke Prevention

UNIVERSITY OF MIAMI

The Peritz Scheinberg Cerebral Vascular Disease Research Laboratories; Department of Neurology

Miguel A. Perez-Pinzon, Ph.D., FAHA

UNIVERSITY OF MIAMI
MILLER SCHOOL of MEDICINE


Excitotoxicity
Inflammation and apoptosis
Contribution to:
Damage
Protection
Glutamate
Ca^{2+}
OFR
IL-1
COX-2
MMPs
Caspases
Minutes
Hours
Days
Weeks
GABA
Adenosine
K_{ATP} activation
IL-10
BCL proteins
EPO
Scar formation
Vasculogenesis
Neurogenesis
BM-derived cells
Sprouting
Anti-excitotoxicity
Anti-inflammation and anti-apoptosis
Repair and regeneration
TRENDS in Neurosciences

The main goal in this field is to define the pathophysiological mechanisms of cerebral ischemia-induced cell death with the goal of finding therapies to ameliorate the consequences of the insult

Strategies for Neuroprotection

- Cocktail of drugs that target all these pathological mechanisms

- Or, the use of a drug that have pleiotropic properties that can protect against most of these pathological phases

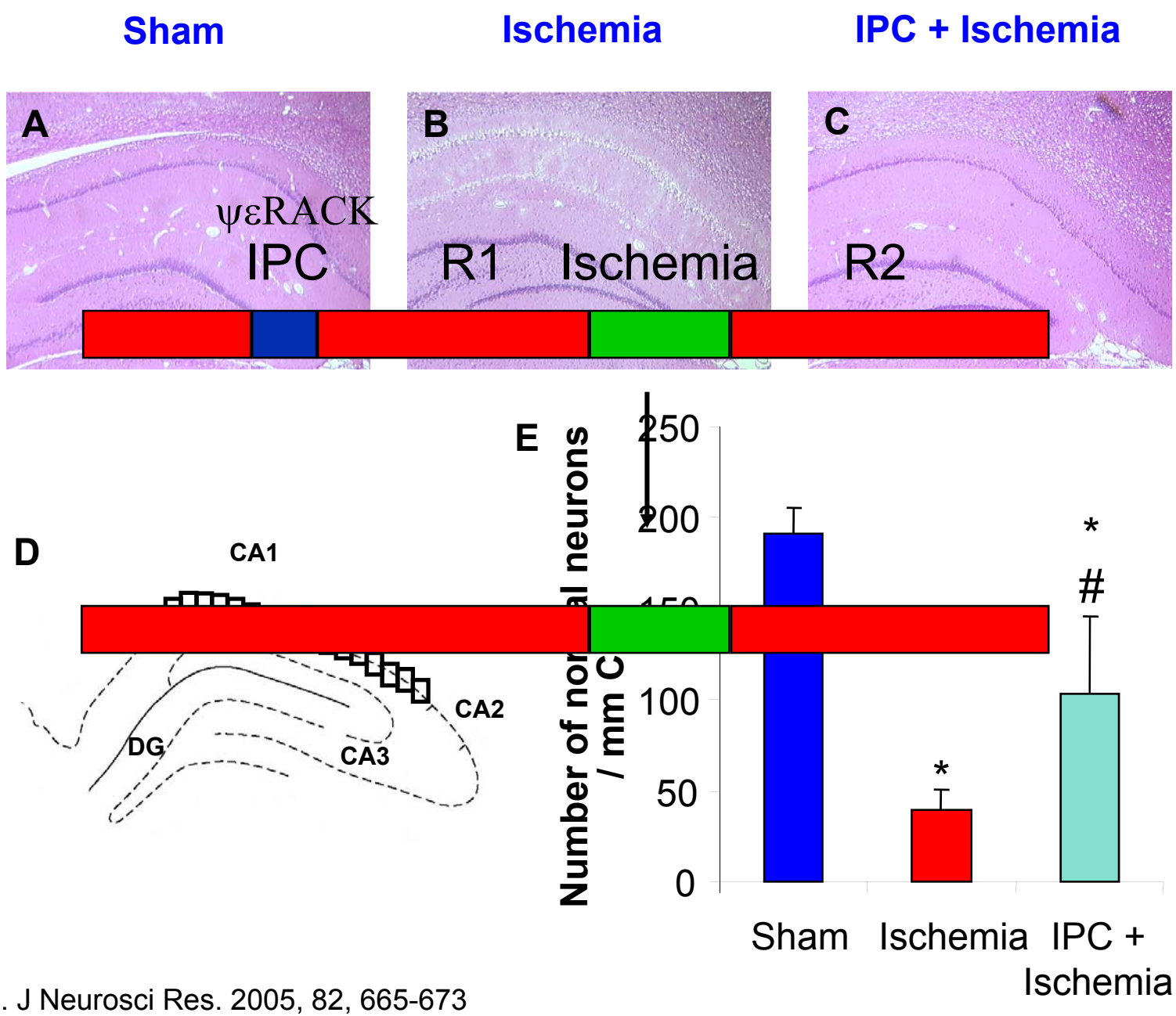
Strategies for Neuroprotection with Pleiotropic Properties

- Ischemic Conditioning

Ischemic Preconditioning

‘That which does not kill us makes us stronger’ Friedrich Nietzsche, Ecce Homo – (1908)

Ischemic preconditioning refers to the ability of a brief (“sublethal”) ischemic episode, or mild stress insult followed by a period of reperfusion, to increase an organ’s resistance to injury (ischemic tolerance)



Dave et al. J Neurosci Res. 2005, 82, 665-673

Other models of conditioning

Remote organ ischemic preconditioning protect brain from ischemic damage following asphyxial cardiac arrest

Kunjan R. Dave*, Isabel Saul, Ricardo Prado, Raul Busto, Miguel A. Perez-Pinzon

Cerebral Vascular Disease Research Center, Department of Neuroscience, University of Miami Miller School of Medicine, Miami, FL 33101, USA

Stroke

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Stroke Association℠

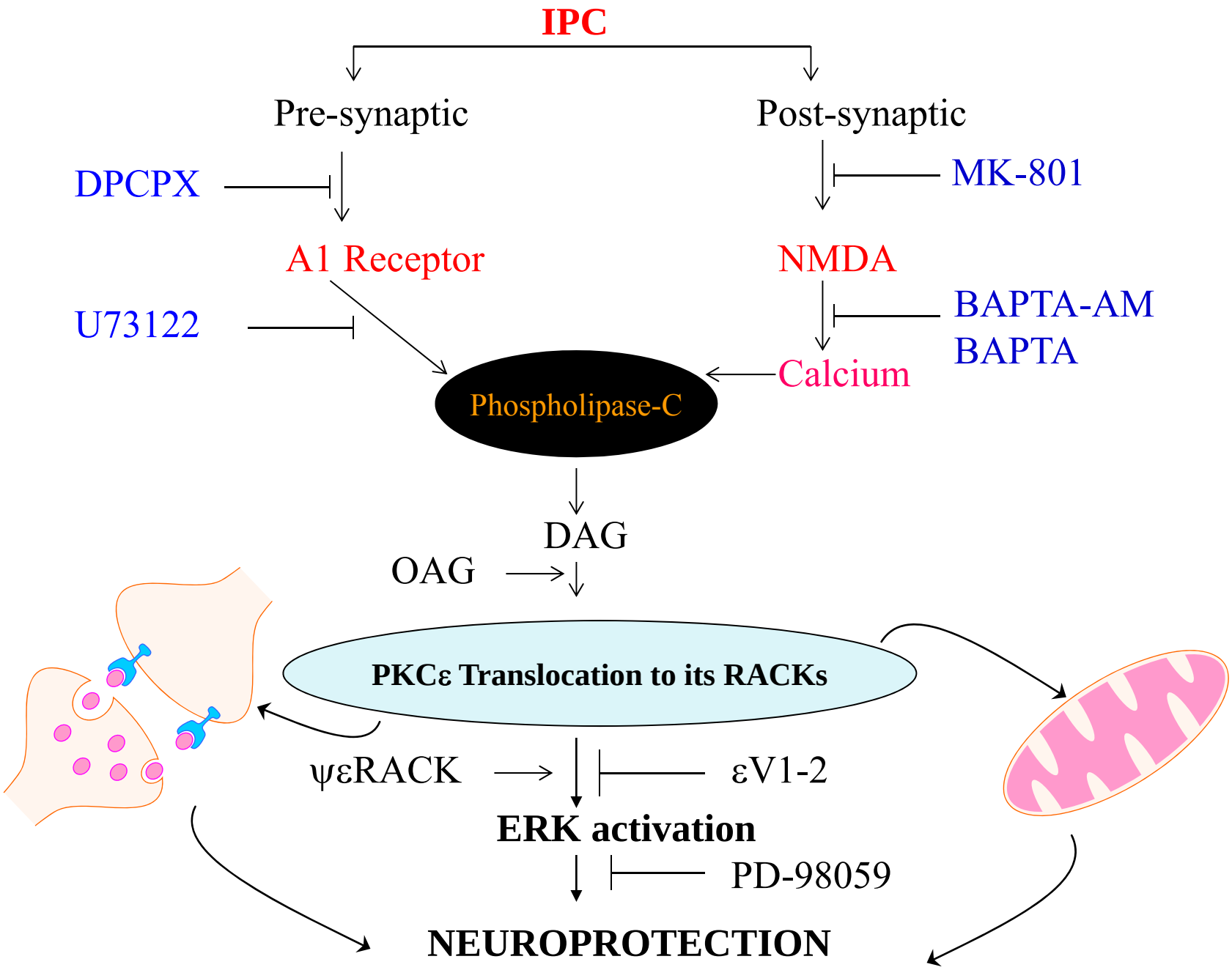
A Division of American Heart Association

Remote Limb Ischemic Postconditioning Protects Against Neonatal Hypoxic-Ischemic Brain Injury in Rat Pups by the Opioid Receptor/Akt Pathway

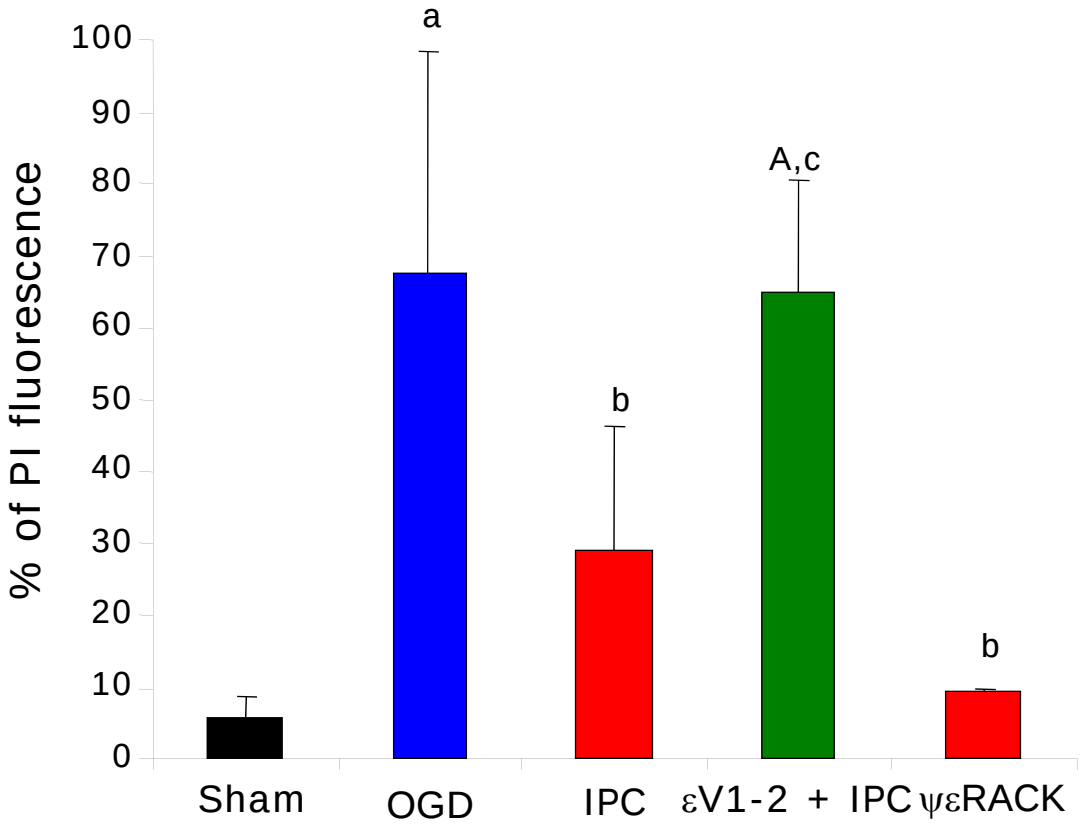
Yilin Zhou, Nancy Fathali, Tim Lekic, Robert P. Ostrowski, Chunhua Chen, Robert D. Martin, Jiping Tang and John H. Zhang

Stroke 2011;42;439-444; originally published online Dec 23, 2010;
DOI: 10.1161/STROKEAHA.110.592162

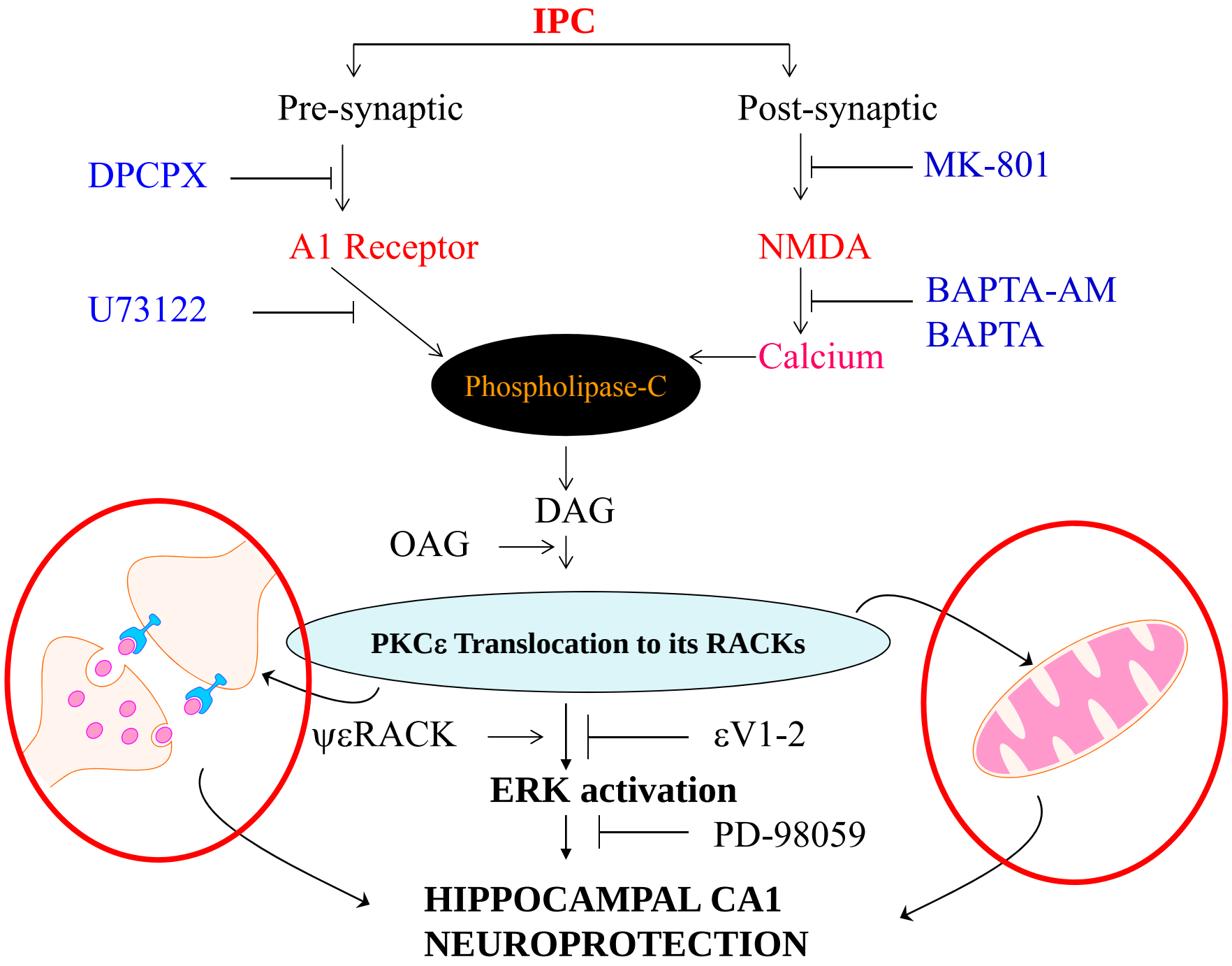
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214


IPC
Pre-synaptic
Post-synaptic
DPCPX
MK-801
A1 Receptor
NMDA
U73122
BAPTA-AM
BAPTA
Calcium
Phospholipase-C
DAG
OAG
PKCε Translocation to its RACKs
ψεRACK
εV1-2
ERK activation
PD-98059
NEUROPROTECTION

Protein Kinase C epsilon (PKCε) activation is necessary for IPC neuroprotection

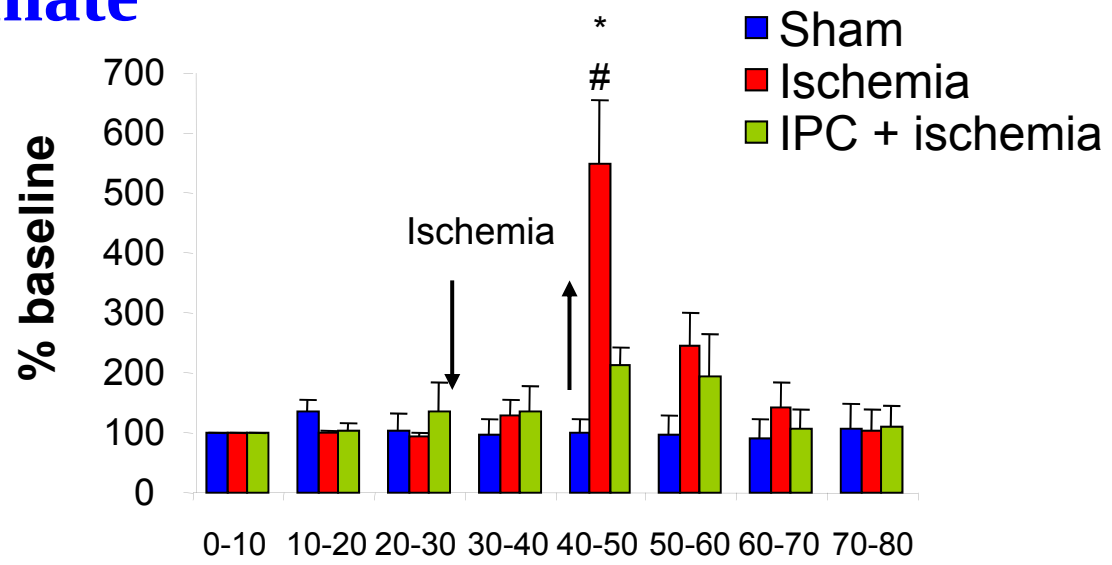


Raval et al., 2003, J Neurosci. 23(2):384-91.

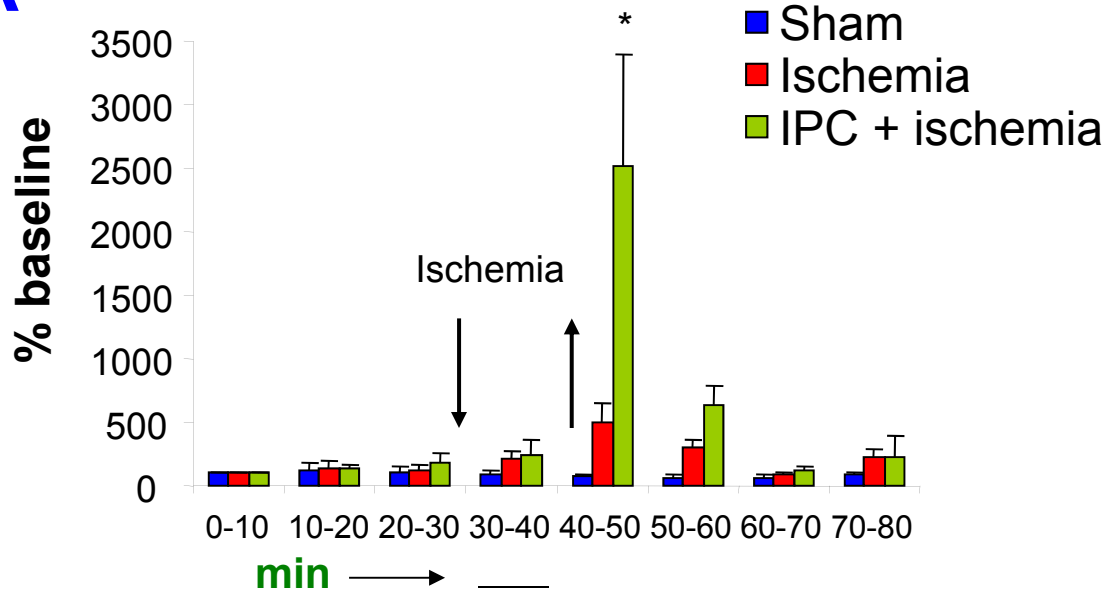

IPC
Pre-synaptic
Post-synaptic
DPCPX
MK-801
A1 Receptor
NMDA
U73122
BAPTA-AM
BAPTA
Calcium
Phospholipase-C
DAG
OAG
PKCε Translocation to its RACKs
ψεRACK
εV1-2
ERK activation
PD-98059
HIPPOCAMPAL CA1
NEUROPROTECTION

Preconditioning enhances GABA release

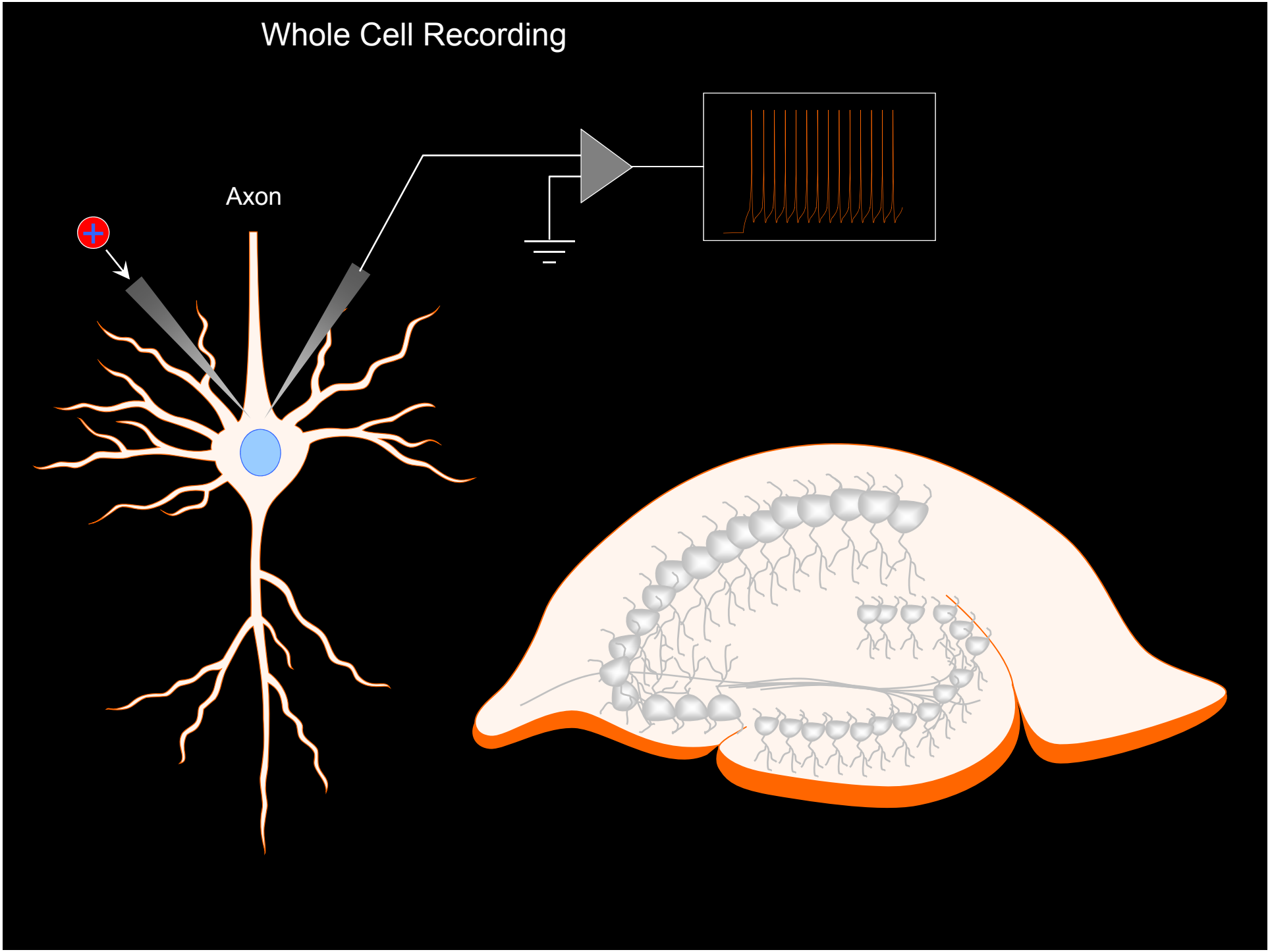
Glutamate



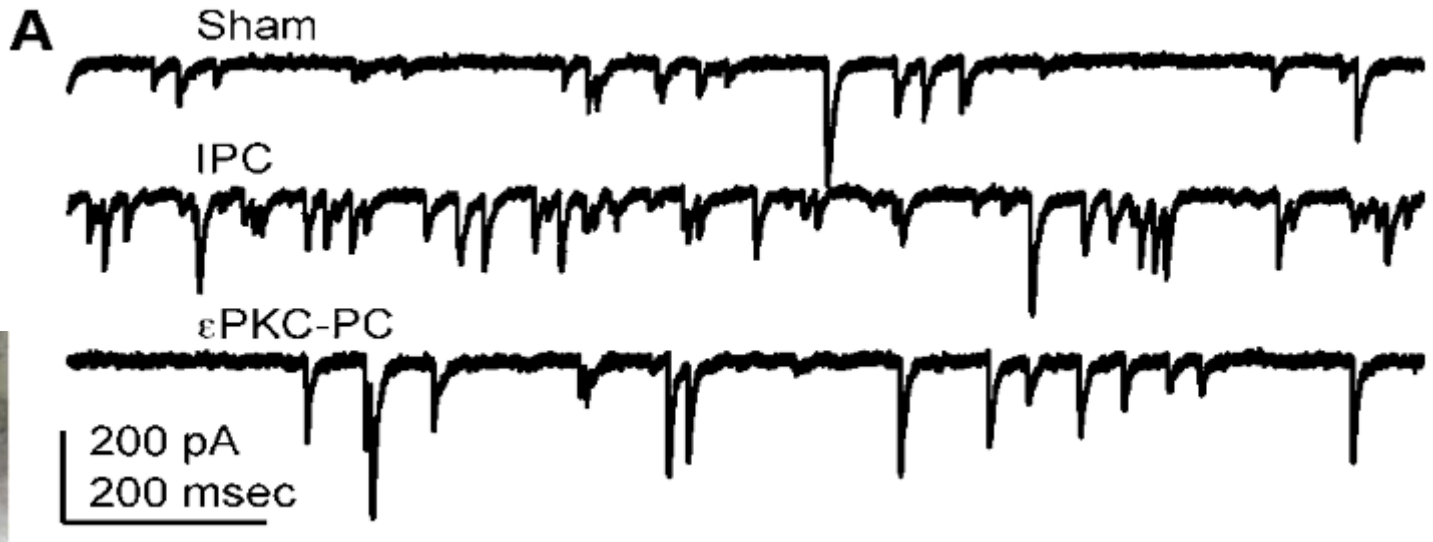
GABA



Dave et al. J Neurosci Res. 2005, 82, 665-673

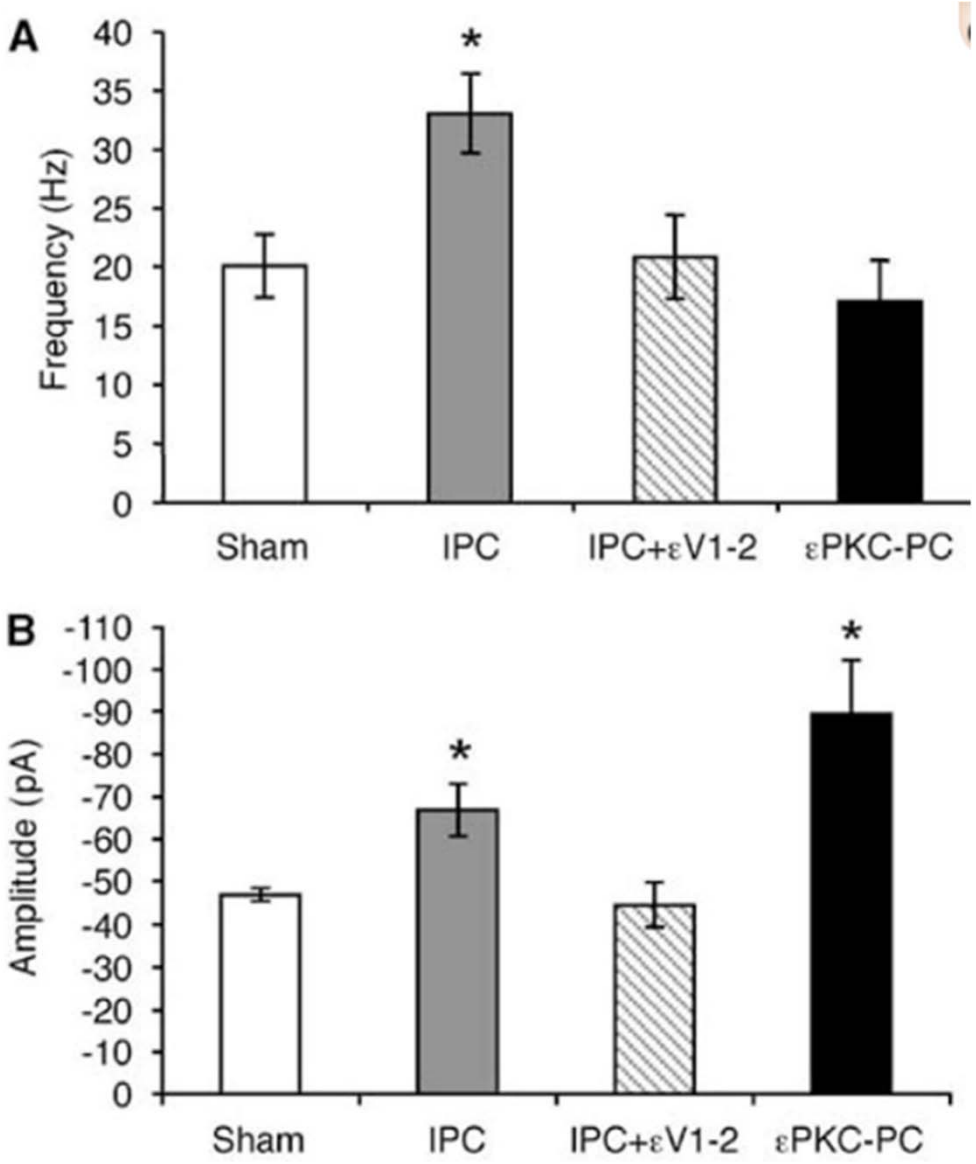

Whole Cell Recording
Axon

Preconditioning alters GABA mPSC



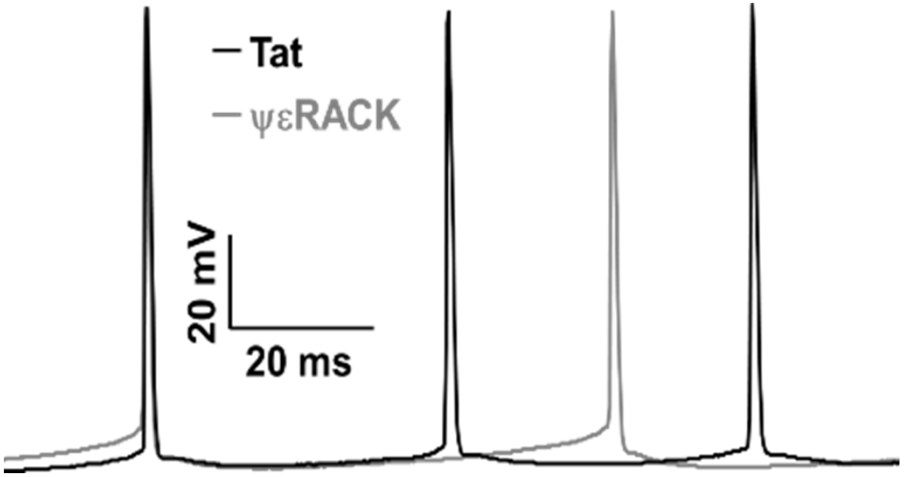
Defazio et al. JCBFM. 2009, 29, 375

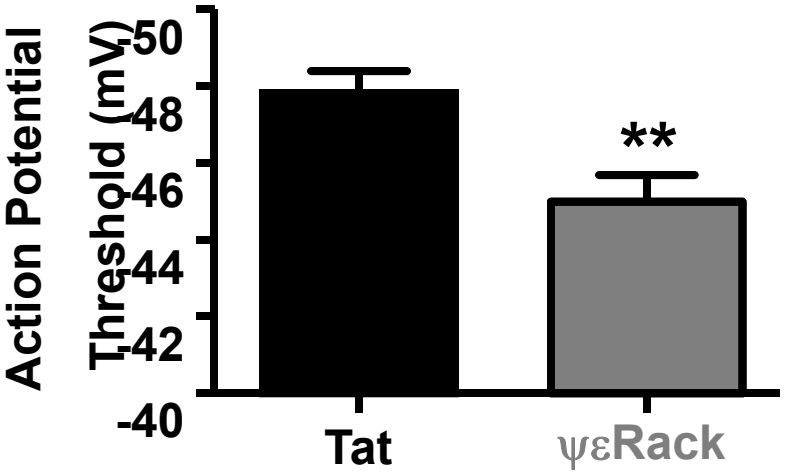
Preconditioning alters GABA mPSC

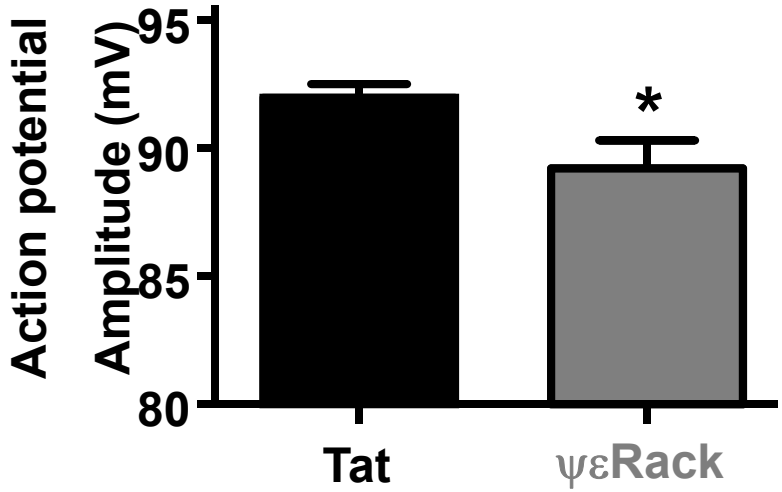


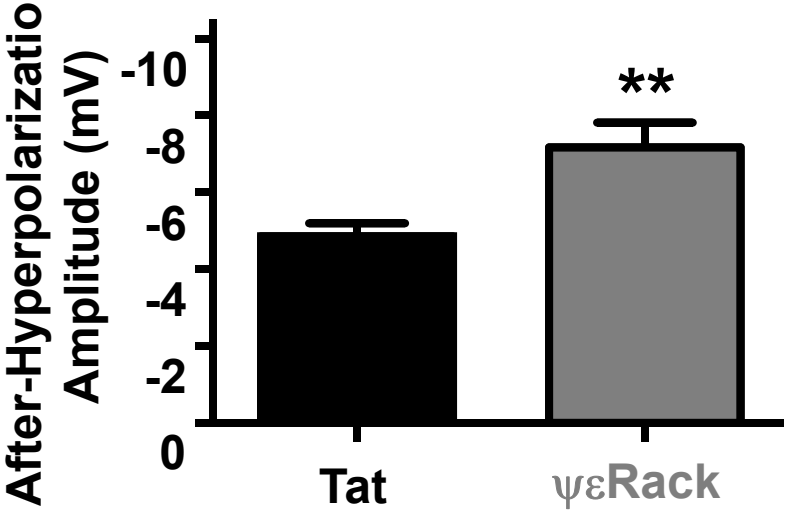
Defazio et al. JCBFM. 2009, 29, 375

PKCε preconditioning alters action potential properties in CA1 hippocampal neurons



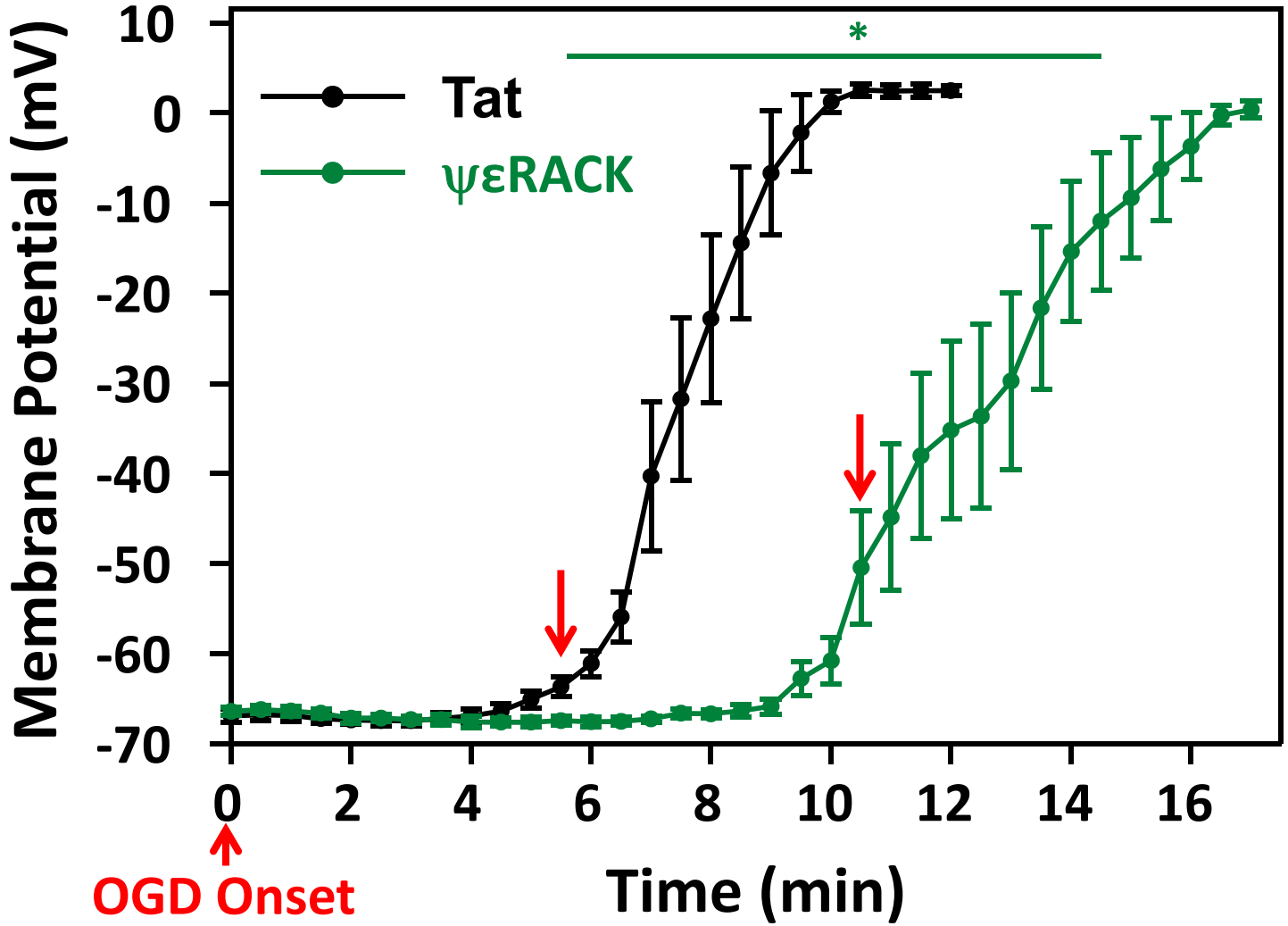






Neumann et al. JCBFM. 2015 Jan;35(1):121-30

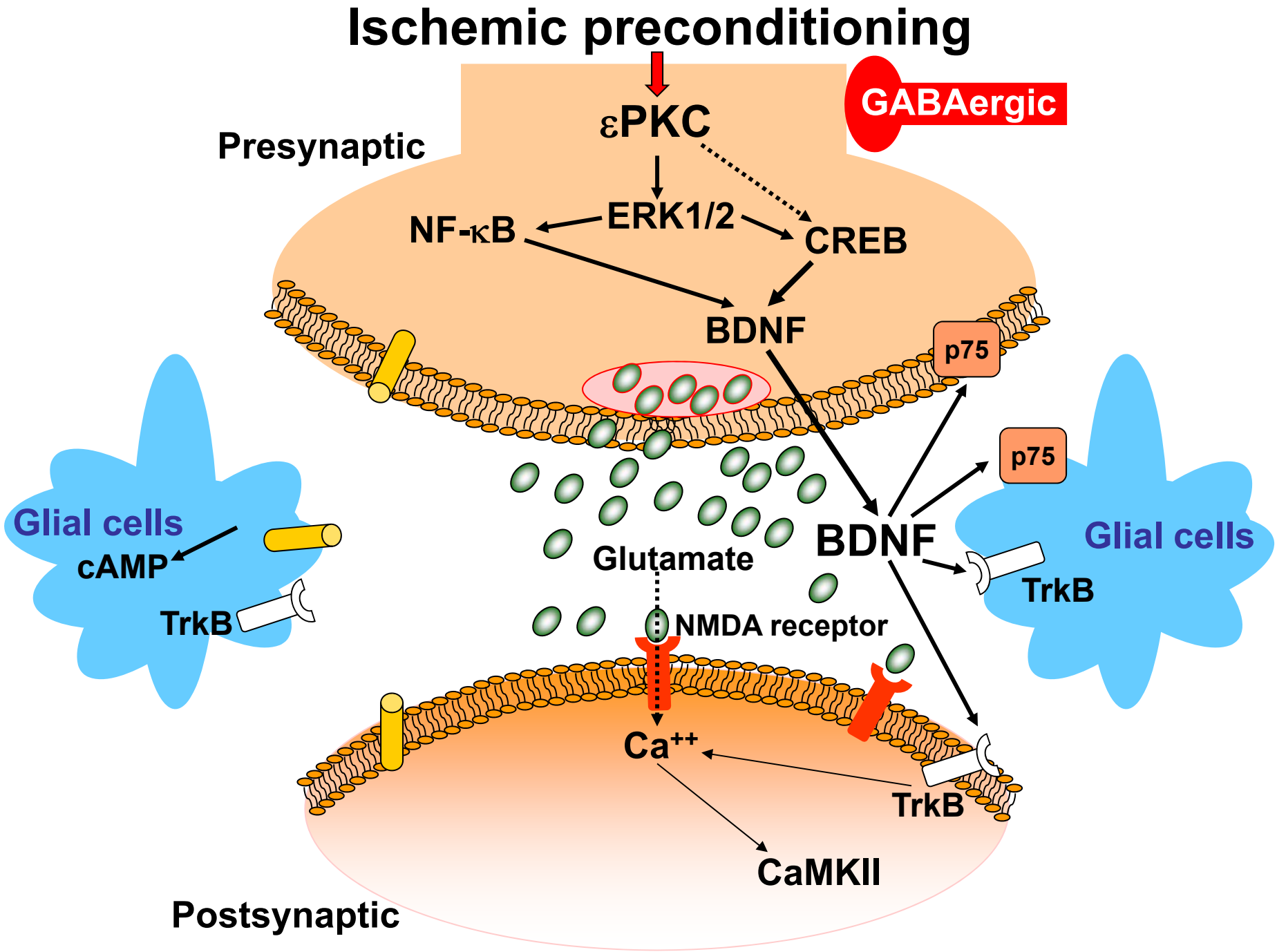
PKCε preconditioning induces a delay in membrane depolarization during OGD



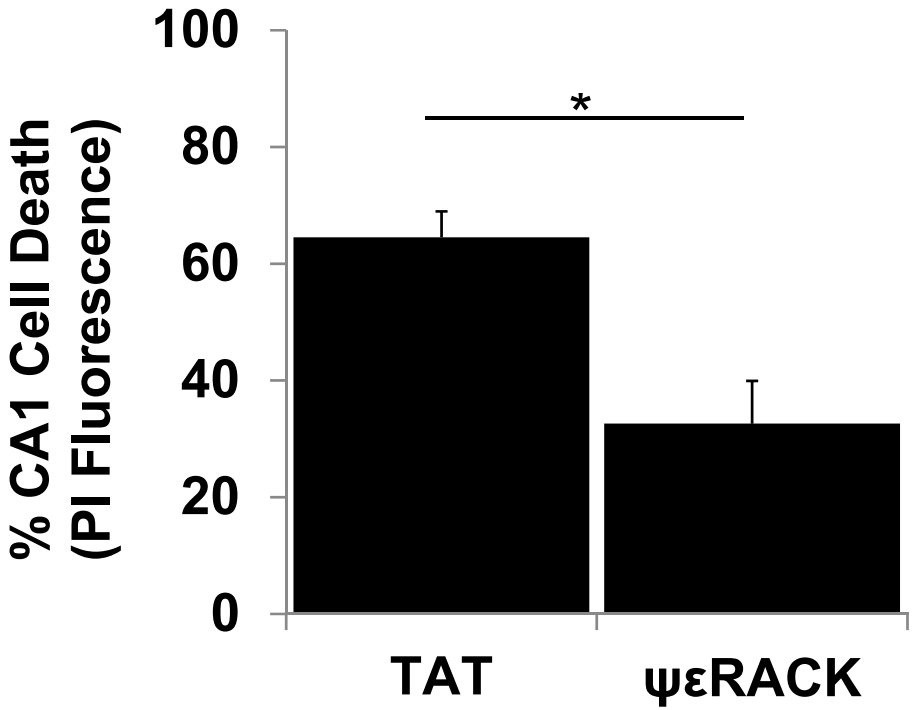
n=9

*p<0.05

Neumann et al. JCBFM. 2015 Jan;35(1):121-30


Ischemic preconditioning
GABAergic
Presynaptic
εPKC
ERK1/2
NF-κB
CREB
BDNF
p75
p75
Glial cells
cAMP
TrkB
Glutamate
BDNF
Glial cells
TrkB
NMDA receptor
Ca++
TrkB
CaMKII
Postsynaptic

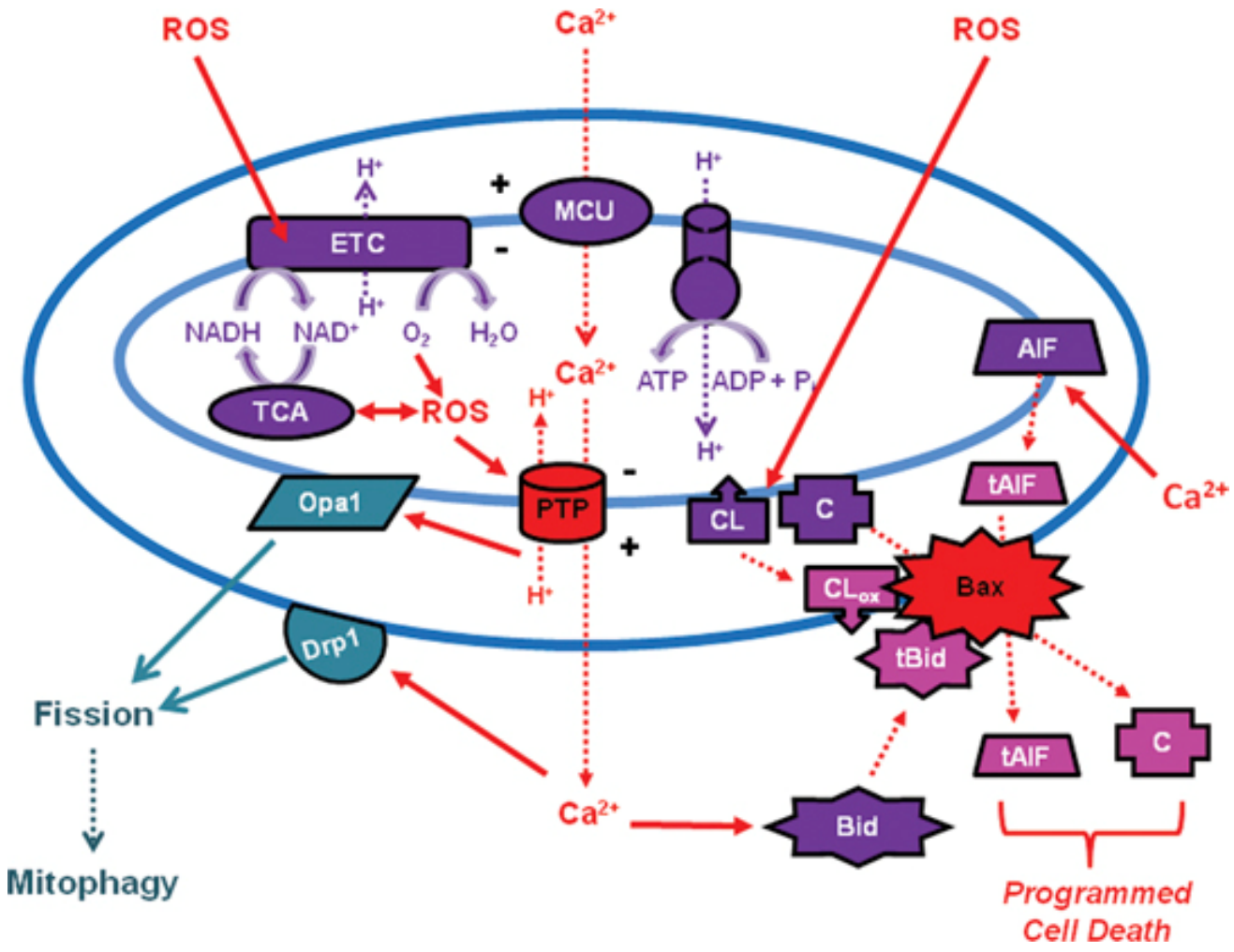
TrkB activation is necessary for ψεRACK mediated protection



SUMMARY

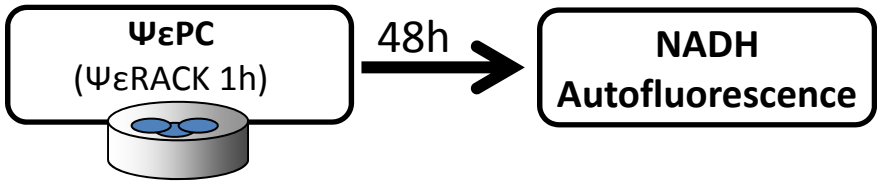
- ψεRACK increases BDNF expression and TrkB phosphorylation
- BDNF expression and TrkB phosphorylation alter action potential properties
- ψεRACK-mediated arc expression triggers a decrease in AMPAR mEPSCs
- ψεRACK delays the onset of anoxic depolarization
- TrkB activation and arc expression are necessary for ψεRACK-mediated neuroprotection

MITOCHONDRIAL DYSFUNCTION



Perez-Pinzon et al. Journal of Cerebral Blood Flow & Metabolism (2012), 1–15

PKCε Enhances Maximal NADH Fluorescence



Morris-Blanco et al. (2014) *J Cereb. Blood Flow & Metab*

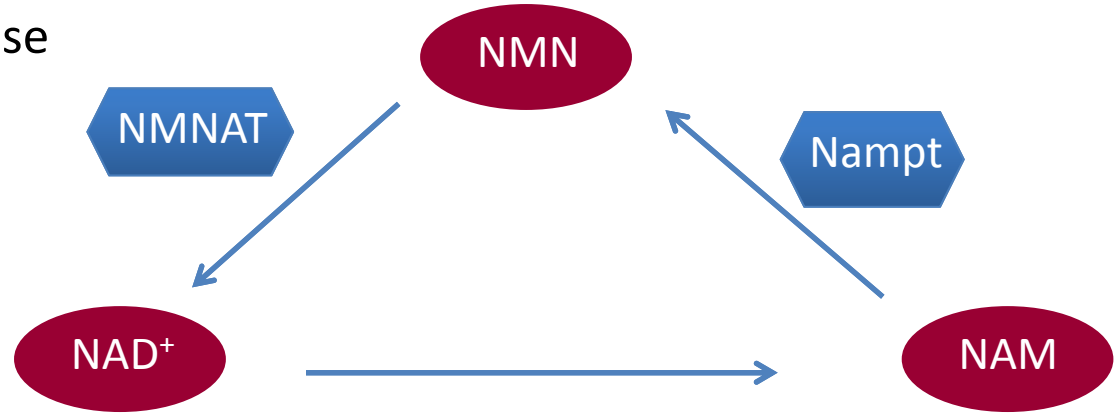
Major Biosynthetic Pathway for NAD^+ Production

Nampt= nicotinamide phosphoriboslytransferase

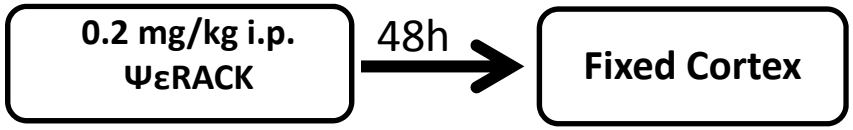
NAM= nicotinamide

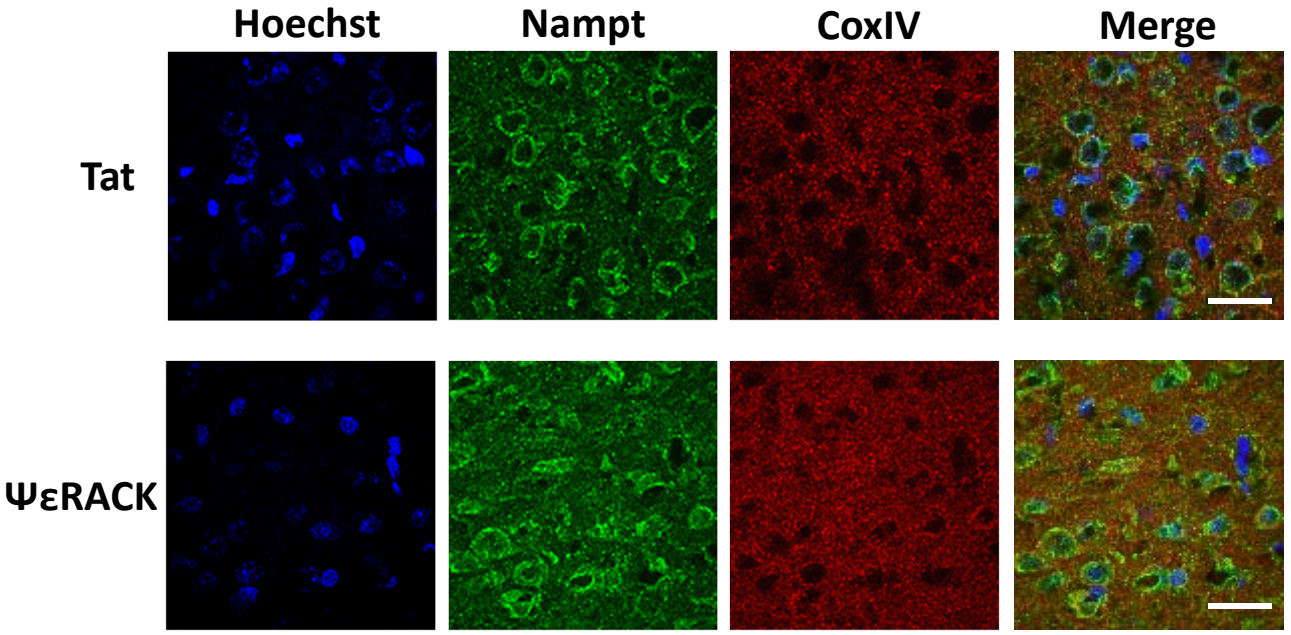
NMN=Nicotinamide mononucleotide

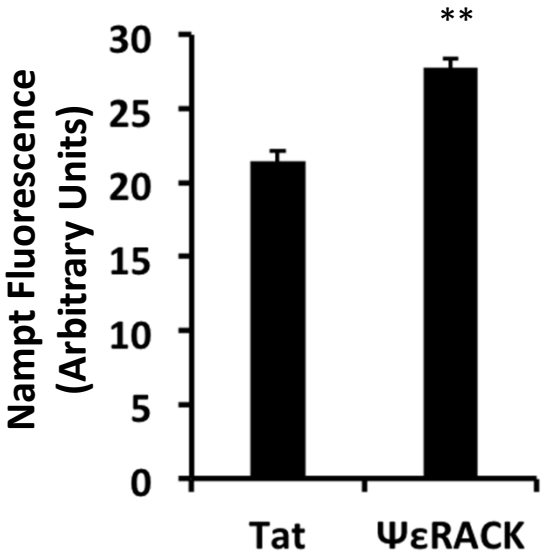
Nmnat=NMN adenyl transferase



PKCε Enhances Nampt Fluorescence in the Rat Cortex







Morris-Blanco et al. (2014) *J Cereb. Blood Flow & Metab*

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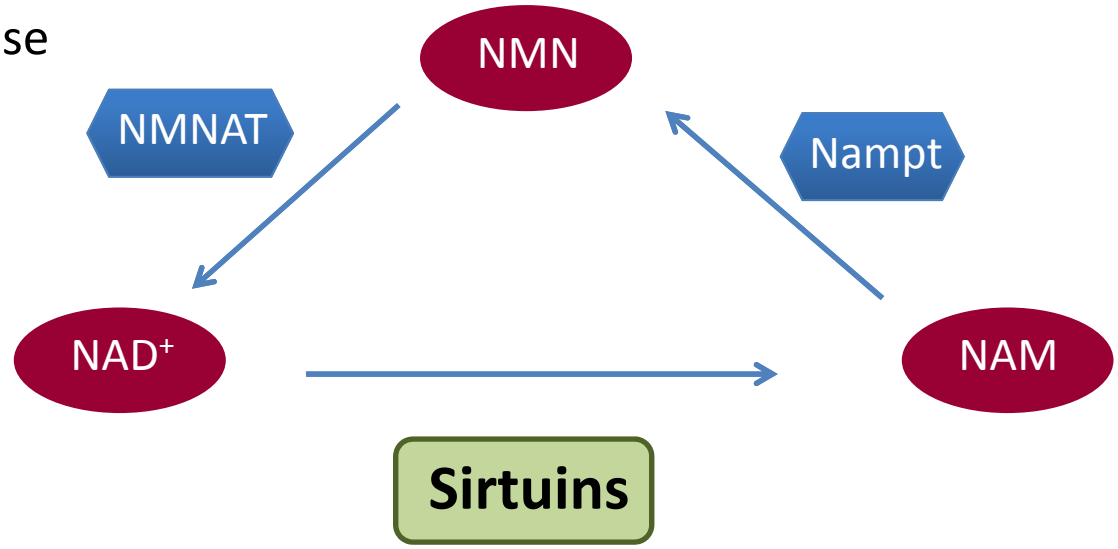
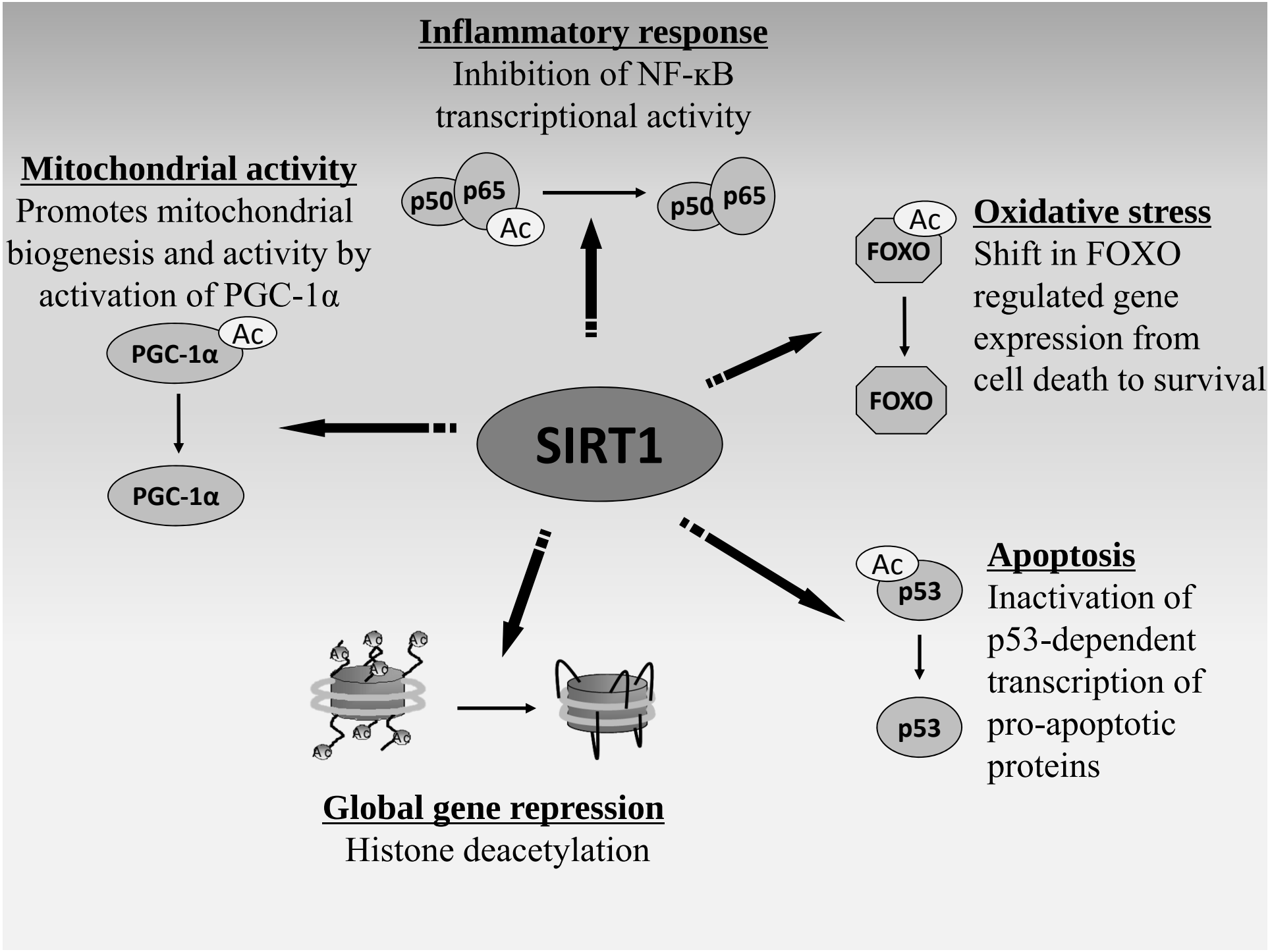
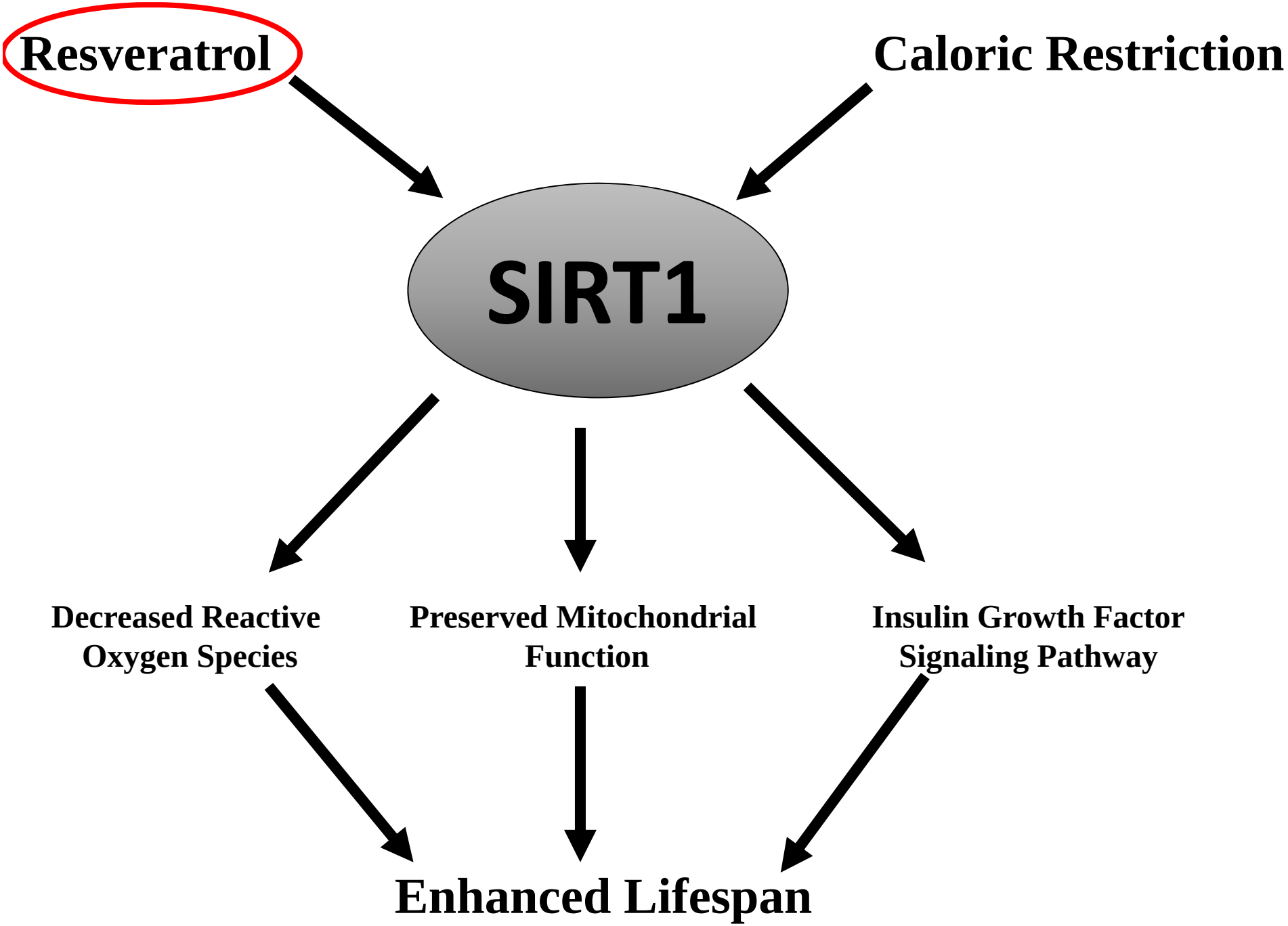
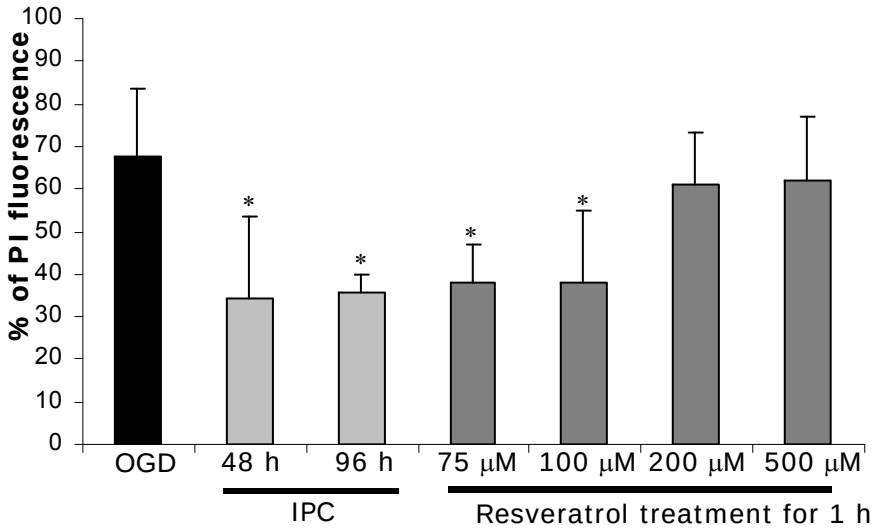
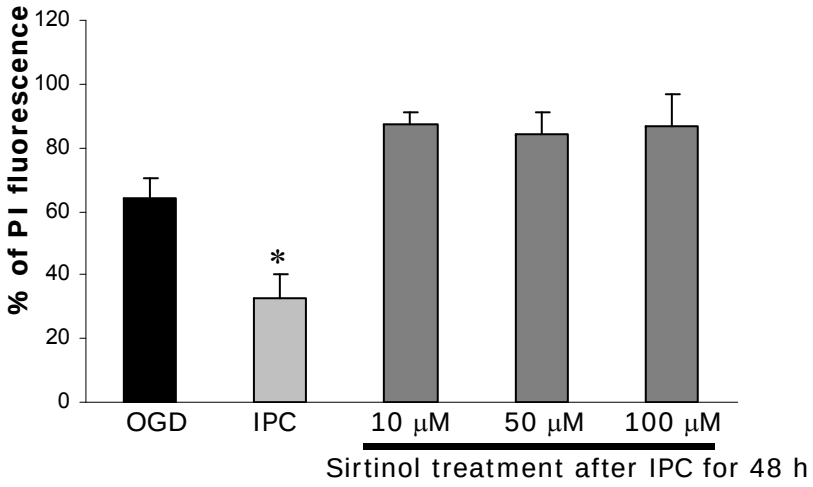



Inflammatory response
Inhibition of NF-κB transcriptional activity
p50
p65
Ac
p50
p65
Mitochondrial activity
Promotes mitochondrial biogenesis and activity by activation of PGC-1α
PGC-1α
Ac
PGC-1α
SIRT1
Ac
FOXO
FOXO
Oxidative stress
Shift in FOXO regulated gene expression from cell death to survival
Ac
p53
p53
Apoptosis
Inactivation of p53-dependent transcription of pro-apoptotic proteins
Global gene repression
Histone deacetylation


Resveratrol
Caloric Restriction
SIRT1
Decreased Reactive Oxygen Species
Preserved Mitochondrial Function
Insulin Growth Factor Signaling Pathway
Enhanced Lifespan

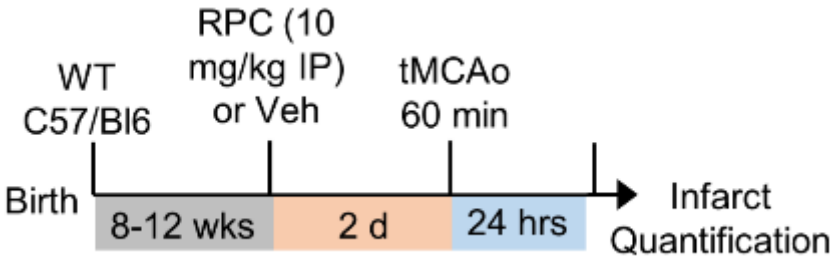
Resveratrol preconditioning (RPC) decreases CA1 cell death in organotypic slices after OGD

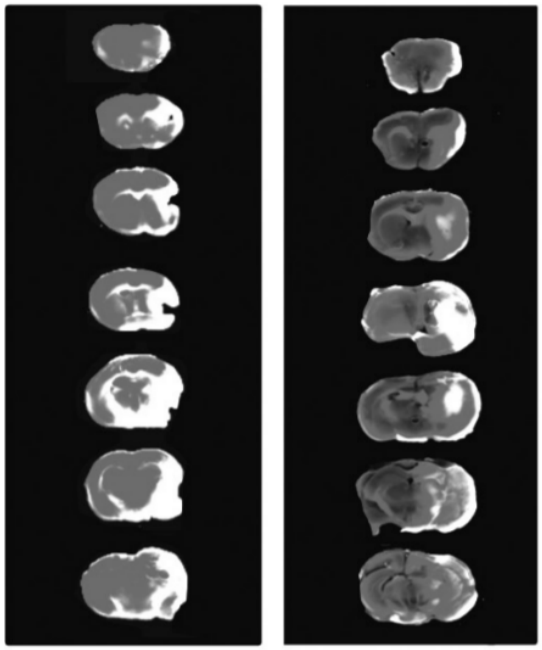




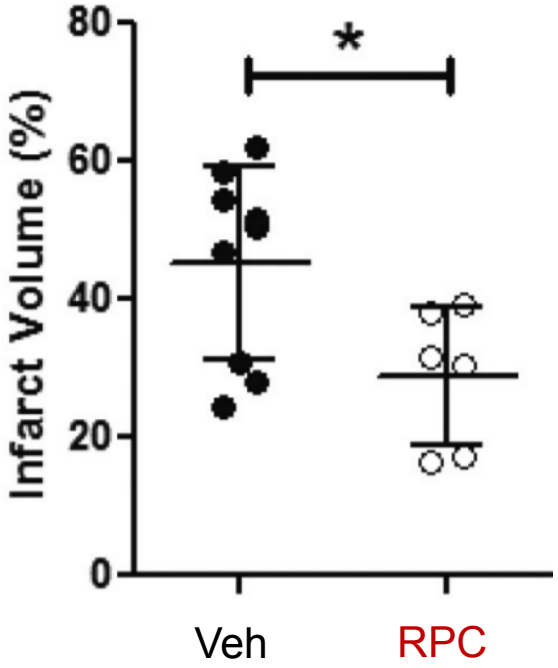
Raval et al. JCBFM. 2006 Sep;26(9):1141-7

Resveratrol preconditioning (RPC) reduces infarct after MCAo in mice





Veh RPC



Narayanan et al. Stroke 2015 46(6):1626-32

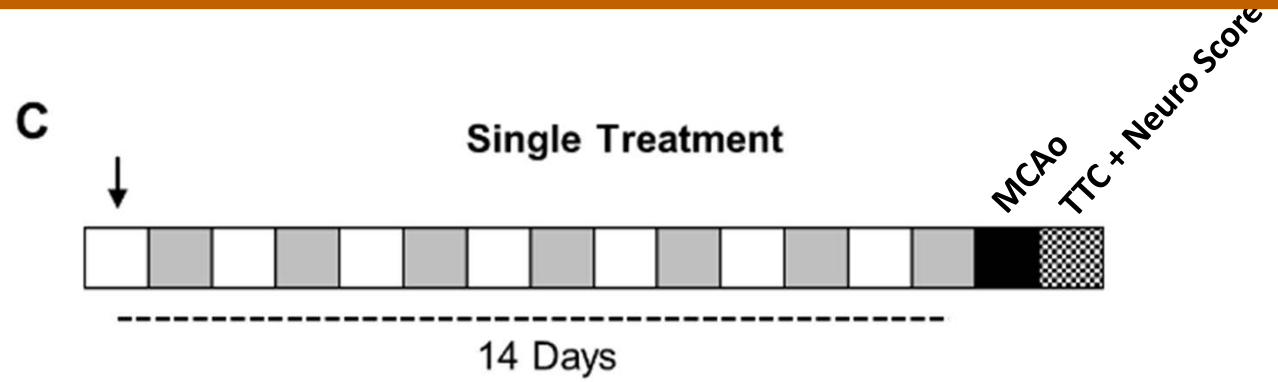
RPC decreases CA1 cell death after cardiac arrest in rats

Della-Morte et al. Neuroscience. 2009

CAN YOU DRINK YOUR WAY TO PRECONDITIONING???

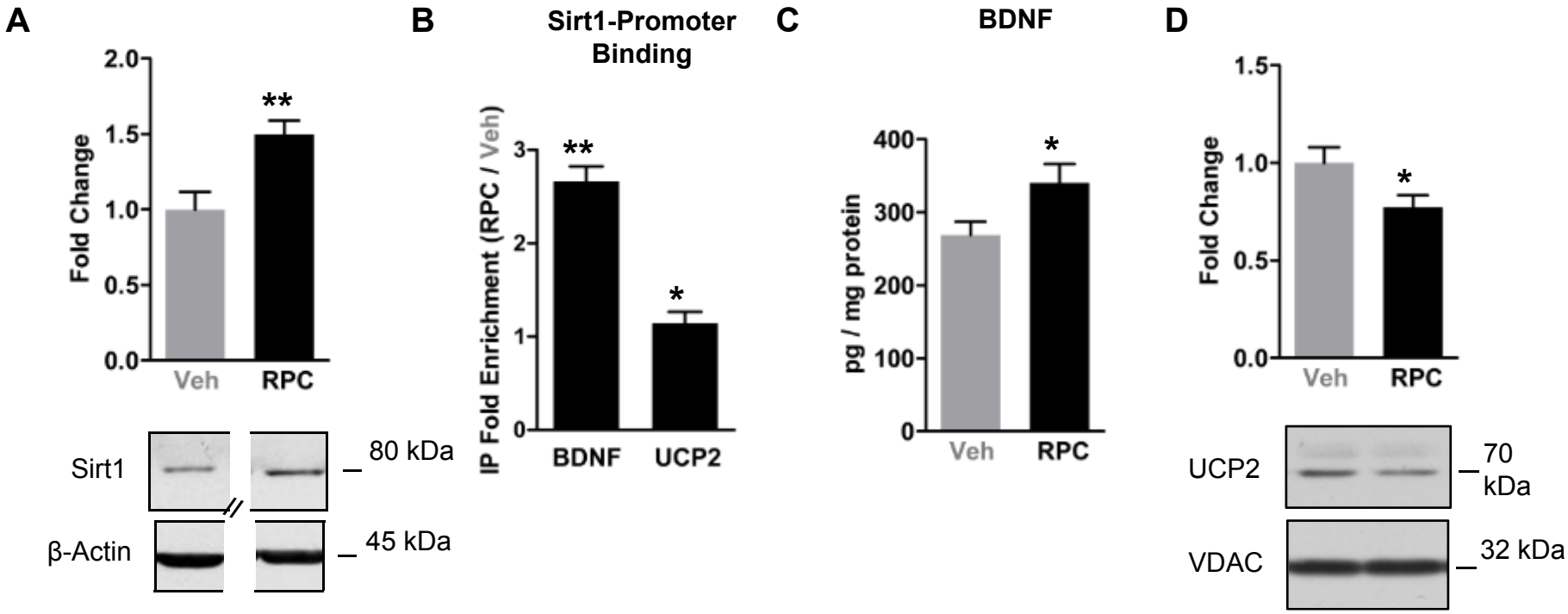
Images courtesy of Google Images

A SINGLE APPLICATION OF RPC INDUCES ISCHEMIC TOLERANCE AGAINST FOCAL ISCHEMIA FOR TWO WEEKS



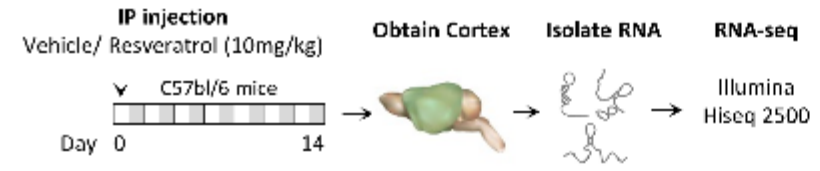
↓ **RPC (10 mg/kg) or Veh (DMSO-Saline)**

Koronowski et al. 2015. Stroke.

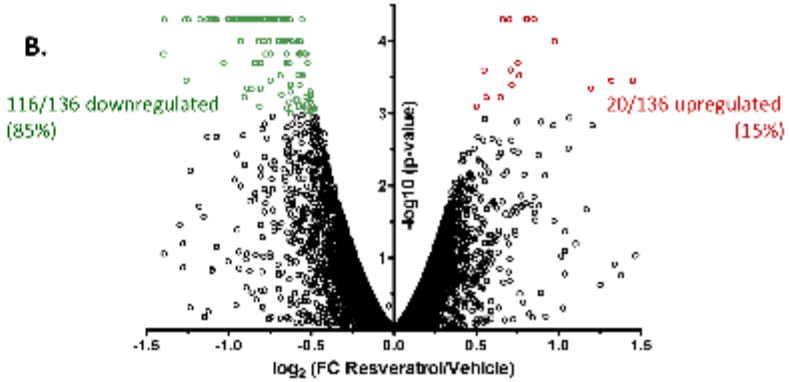

A
Fold Change
2.0
1.5
1.0
0.5
0.0
**
Veh
RPC
Sirt1
80 kDa
β-Actin
45 kDa
B
Sirt1-Promoter Binding
IP Fold Enrichment (RPC / Veh)
3
2
1
0
**
*
BDNF
UCP2
C
BDNF
pg / mg protein
400
300
200
100
0
*
Veh
RPC
D
Fold Change
1.5
1.0
0.5
0.0
*
Veh
RPC
UCP2
70 kDa
VDAC
32 kDa

Transcriptomic changes induced by RPC within the long-term window of IPC

A.



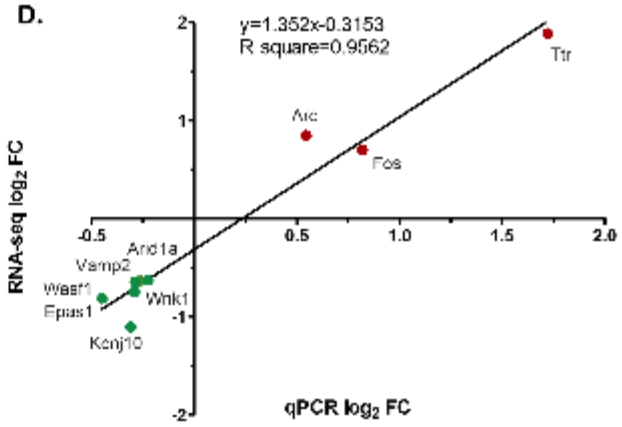
B.

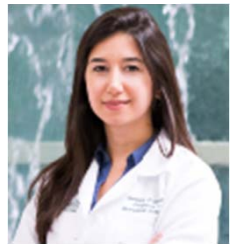


C.

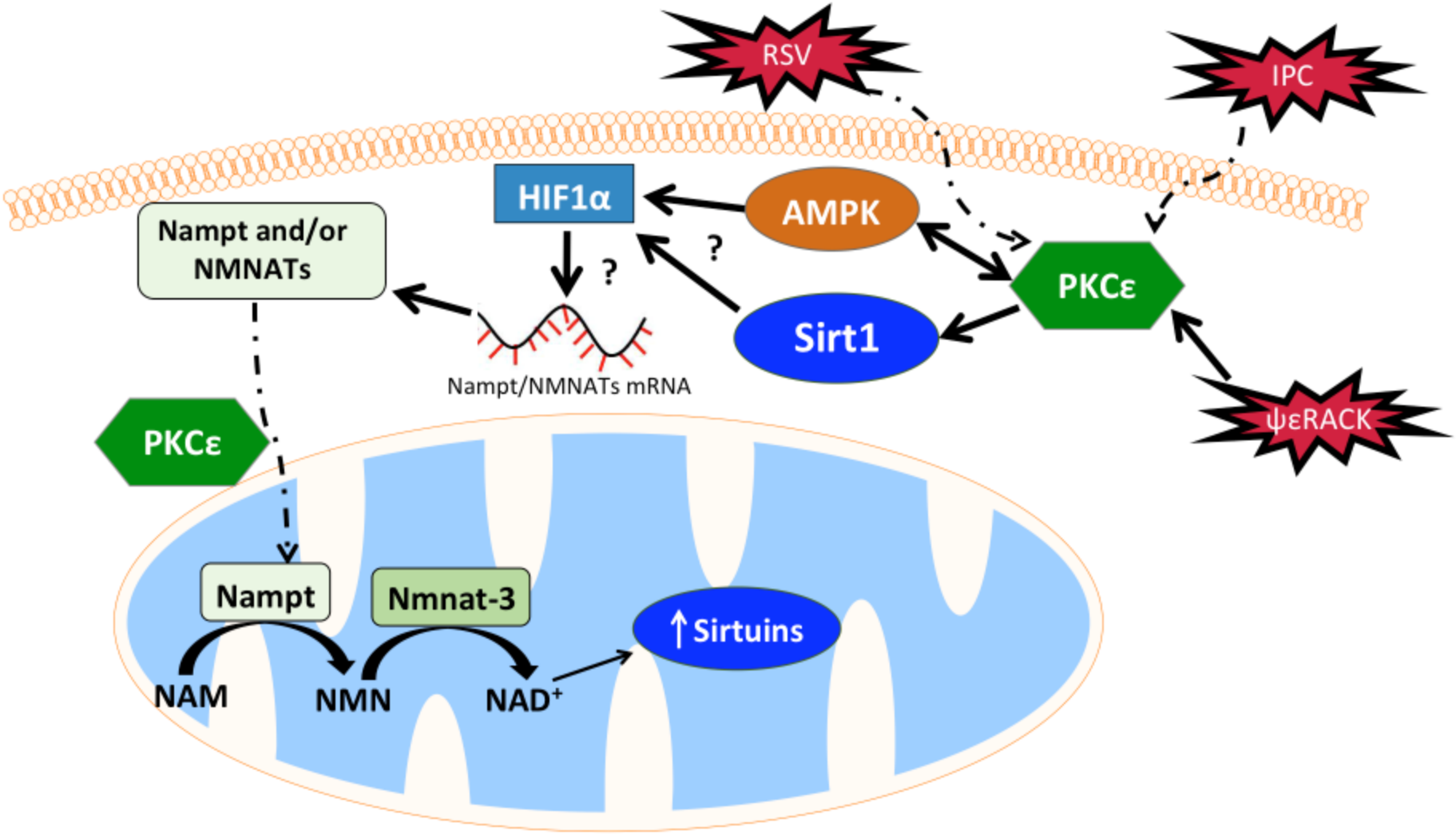
[Biological Processes(GO)]	p-value	Genes
Transcription from RNA polymerase II promoter	2.18E-08	Auts2, Npas4, Ep300, Fos, Junb, Nfia, Six3, Sall1, Lef1, Foxo1, Kmt2a, Foxk1, Zmiz1, Nr4a1, Tead1, Polr2a, Notch1, Ago2, Brd4, Nos1, Hivep3, Hipk2, Gli3, Sox9, Kmt2d, Zfhx3, Epas1, Rnf165, Arid1a, Atn1
Regulation of signaling	2.26E-08	Unc13c, Npas4, Ep300, Kcnj10, Six3, Sall1, Lef1, Foxo1, Kmt2a, Zmiz1, Doc2b, Folr1, Kl, Peg10, Shank1, Tacr1, Vamp2, Shisa6, Col1a1, Notch1, Ksr2, Shisa7, Sipa1l3, Ubqln2, Nos1, Trim59, Hipk2, Gli3, Sox9, Kmt2d, Cacng4, Dusp1, Kcne2, Arc, Flt1, Rnf165, Adcy5, Adgrg1, Wasf
Synaptic signaling	2.26E-05	Gpr88, Unc13c, Npas4, Kcnj10, Slc5a7, Kmt2a, Doc2b, Shank1, Tacr1, Vamp2, Shisa6, Shisa7, Nos1, Arc
Regulation of ion transmembrane transport	6.17E-05	Kcnj10, Atp1b2, Scn9a, Shank1, Vamp2, Nos1, Cacng4, Kcne2, Arc, Actn2
Positive regulation of neurotransmitter levels	3.77E-04	Unc13c, Kcnj10, Slc5a7, Doc2b, Vamp2, Nos1
Histone H3 acetylation	3.03E-04	Ep300, Lef1, Kmt2a, Brd4
Histone H4 acetylation	2.04E-05	Auts2, Ep300, Lef1, Kmt2a, Brd4
Histone acetylation	8.06E-05	Auts2, Ep300, Lef1, Kmt2a, Brd4, Nos1

D.





Nathalie Khoury et al. Mol Neurobiol. 2018 Oct 20


RSV
IPC
HIF1α
AMPK
Nampt and/or
NMNATs
?
?
PKCε
Sirt1
Nampt/NMNATs mRNA
PKCε
ψεRACK
Nampt
Nmnat-3
↑Sirtuins
NAM
NMN
NAD+

CONCLUSIONS

1. Signaling pathways activated by Ischemic Preconditioning exhibit pleiotropic properties that lead to neuroprotection
2. PKCε and Resveratrol are two pharmacological agents that emulate IPC and have pleiotropic properties
3. Pharmacological agents that emulate IPC could be used in a prophylactic manner to enhance ischemic tolerance in patients prone to cerebral ischemia

Clinical Scenarios for IPC Implementation

a) Carotid endarectomy and coronary artery bypass graft (CABG) which both result in high incidence of strokes and cognitive deficits
b) Transient ischemic attacks (TIA) patients, from which 10% will have a stroke within a month
c) Sub-arachnoid hemorrhage (SAH) patients from which 20-30% undergo delayed cerebral ischemia (DCI)
d) Neurosurgical procedures
e) All stroke patients have a higher incidence of subsequent strokes

“Precondition-able” population

Stroke Survivors
~ 660,000
Carotid Endarterectomy
~ 132,000
Coronary Artery Bypass Graft
~ 500,000
TIA
~ 240,000
Sub-arachnoid Hemorrhage
~ 55,650
Neurosurgical Procedures

Acknowledgements

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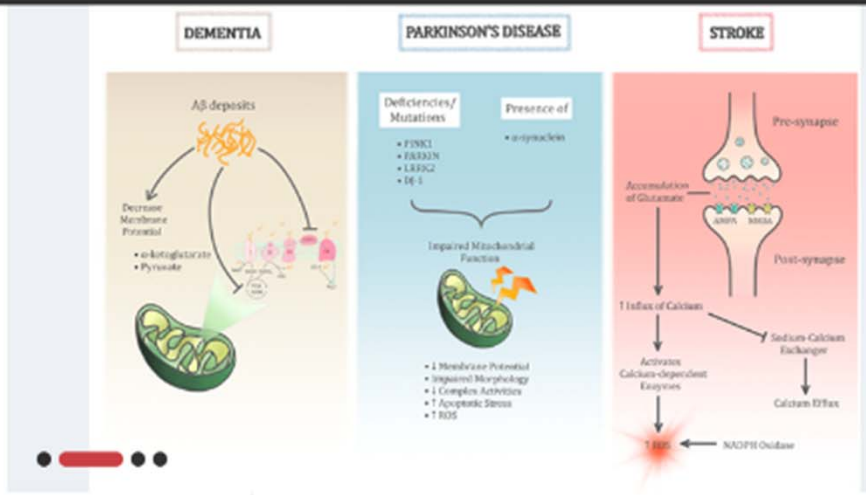

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The role of mitochondrial dysfunction in age-related neurodegenerative diseases is significant.

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Editorial

- **Negative Conditioning: Confounding the benefits of preconditioning and postconditioning**

  Giuseppe Pignataro, Eng H. Lo, Derek J. Hausenloy
  Conditioning Medicine, 2019, 2(1):1

  Full Text (HTML) | Full Text (PDF)

Review Articles

- **Diabetes mellitus worsens outcomes in tPA thrombolytic stroke therapy**

  Yinghua Jiang, Ning Liu, Zeyuan Cao, Lena Huang, Zhanyang Yu, Qiuchen Zhao, Fang Zhang, Ming-Ming Ning, Klaus van Leyen, Eng H. Lo, Xiaoying Wang
  Conditioning Medicine, 2019, 2(1):2-9

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- **Cardioprotection with Ischemic Conditioning: The Diabetes Dilemma**

  Joseph M. Wider, Karin Przyklenk
  Conditioning Medicine, 2019, 2(1):10-17