Melatonin and N-acetyl-serotonin offer neuroprotection in experimental models of ischemic injury

Xin Wang, Ph.D.

Director, Neuroapoptosis Drug Discovery Laboratory
Assistant Professor,
Department of Neurosurgery
Brigham and Women's Hospital
Harvard Medical School

Background

Stroke is one of the leading causes of death worldwide. According to The World Stroke Organization, every six seconds someone somewhere will die from a stroke **and** one in six people will at some time have a stroke.

Central Nervous System Agent In Medicinal Chemistry. 2011. 11, 80
Current Molecular Medicine 2012. 12, 1282-1296

The long-term aim

No effective treatment has been found to prevent stroke except TPA (tissue plasminogen activator) with narrow therapeutic window. Developing novel drugs for neuroprotection from stroke is urgently needed.

The long-term aim of our Neuroapoptosis Drug Discovery Laboratory is to find new neuroprotective drugs for cure adult **ischemic stroke** and newborn hypoxic-ischemic (H-I) brain injury (H-I encephalopathy).

Types of Stroke

1) Ischemic Stroke

The most common type of stroke, accounting for almost 80 - 85% of all strokes, Is caused by a clot or other blockage within an artery leading to the brain.

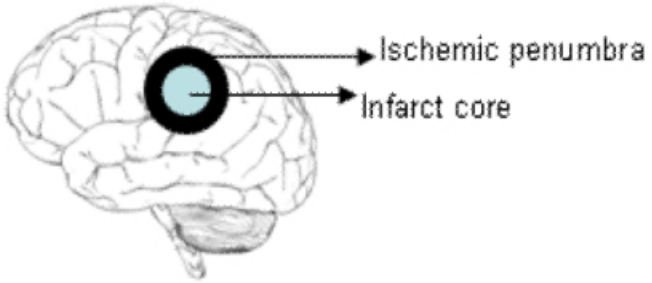
2) Intracerebral Hemorrhage (ICH)

A type of stroke caused by the sudden rupture of an artery within the brain. Blood is then released into the brain compressing brain structures.

3) Subarachnoid Hemorrhage (SAH)

Also a **type of stroke caused by the sudden rupture of an artery. A SAH differs from an ICH in that the location of the rupture leads to blood filling the space surrounding the brain rather than inside of it.**

CNS agents for ischemic stroke with neuroprotective mechanisms



ISCHEMIC STROKE

- **ANTI-OXIDANTS**
  - AM 36
  - Tempol
  - Ebselen
  - 4-HBA
  - GSRD
  - Carvedilol
  - IAC
  - PPBP
  - Bromocriptine
- **ANTI-INFLAMMATORY**
  - Melanocortins
  - IFN-β
  - 6-Mercaptopurine
  - BM derived MNC
- **ANTI-EXCITOTOXIC**
  - Suramin
  - YM-202074
  - Mefenamic acid
  - Nortryptyline
  - Memantine
  - Valdecoxib
  - Felbamate
  - Diazepam
  - Resveratrol
  - NBQX
- **MULTI-ACTION AGENTS**
  - PAN-811
  - Telmisartan
  - Minocycline
  - Melatonin
- **ANTI-APOPTOSIS**
  - Humanin
  - Nicotinamide
  - EPO
  - Acetaminophen
  - Baicalein
  - Neural stem cells
  - Aminoguanidine
- necrosis
- **HORMONAL THERAPY**
  - Estrogen
  - progesterone
- **COMBINED AGENTS**
  - Ethyl pyruvate & aspirin
  - Velcade & tPA
  - Urokinase & topiramate
  - Caspase inhibitors & MK 801
  - Eliprodil & rt-PA

Central Nervous System Agent In Medicinal Chemistry. 2011. 11, 81-97

Melatonin

A nutritional supplement Sleeping pill (*e.g.*, for jet lag) CVS, Walgreens. Oral

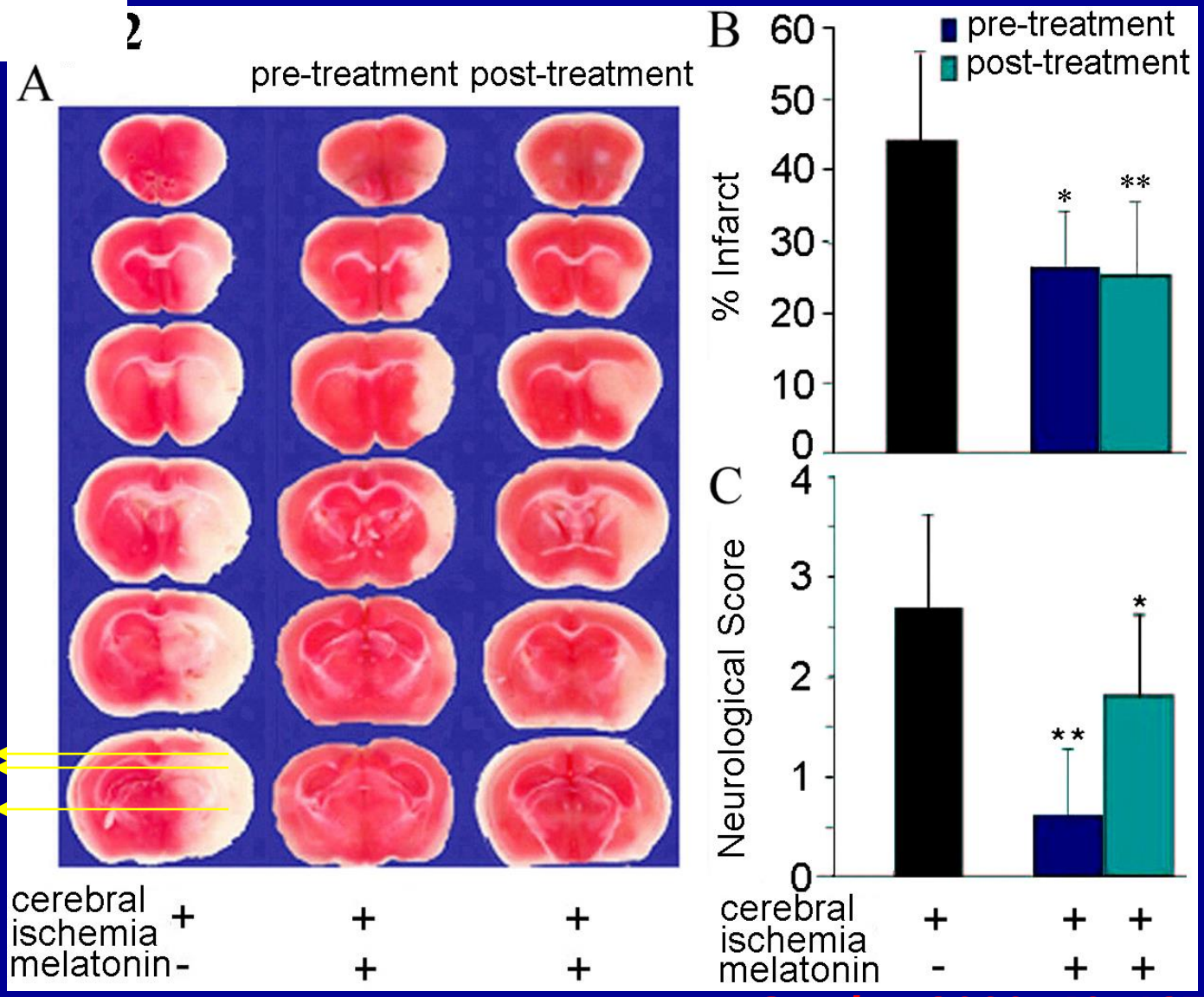
- A natural hormone secreted by the pineal gland; FDA approved drug with human safety and easily crosses Blood Brain Barrier (BBB); Beneficial in circadian rhythm/sleep wake cycle, cancer, aging.

Neuroprotection in neurological disorders

- **Stroke**
  - **adult ischemic stroke**
  (X Wang et al., Stroke 2009)
  - **newborn hypoxic-ischemic (H-I) brain injury**
- **Amyotrophic lateral sclerosis (ALS)**
  (Y Zhang et al., Neurobiol Dis 2013)
- **Huntington's disease (HD)**
  (X Wang et al., J Neurosci 2011)
- **Alzheimer's disease (AD)**
- **Parkinson's disease (PD)**

(X Wang, review, CNS Neurosci& Ther. 2009 and Taylor & Francis-CRC Press 2014)

Melatonin decreases damage in Middle Cerebral Artery Occlusion (MCAO) mice



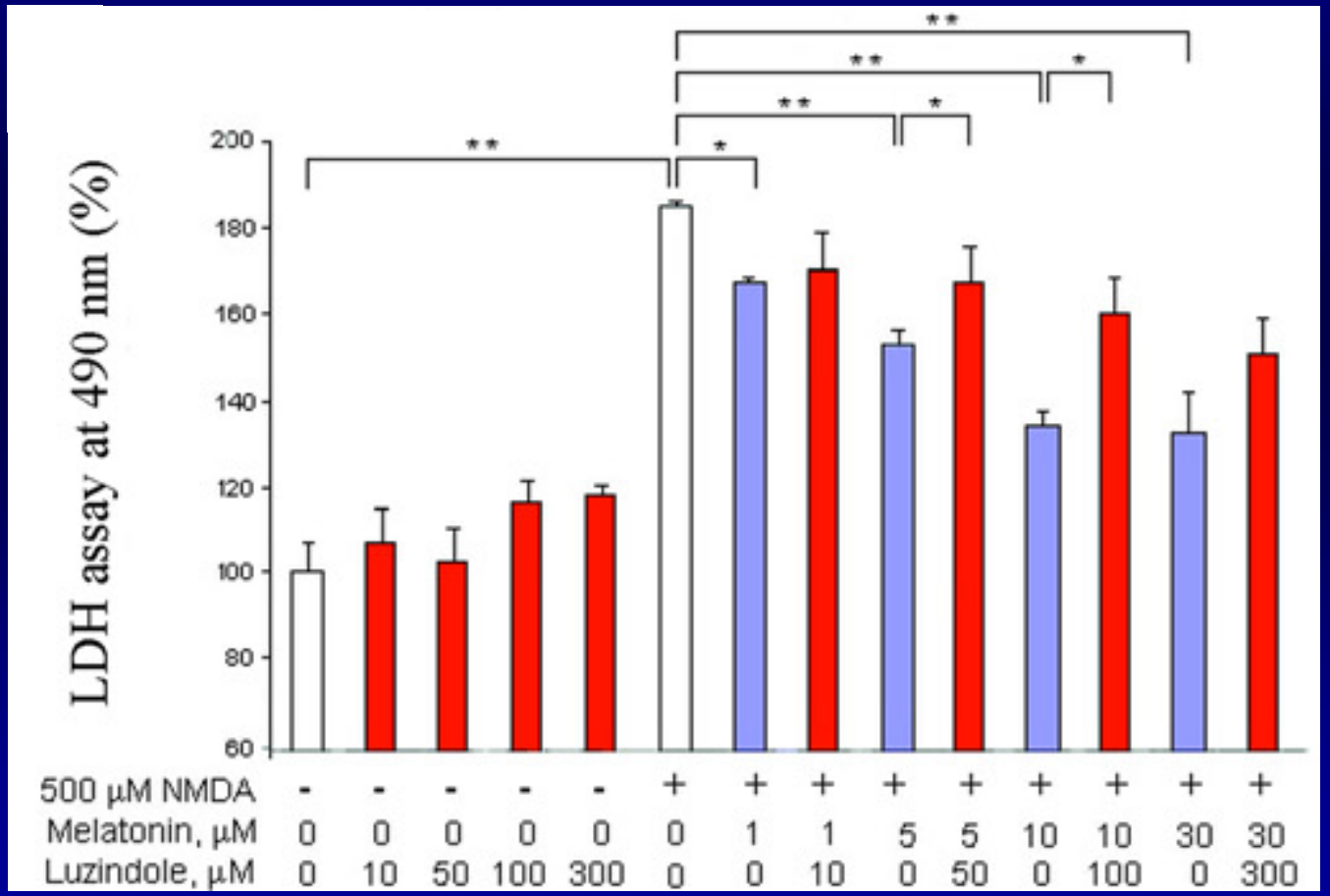
Cortex
Hippocampus
Striatum

Stroke. 2009. 40, 1877-1885

Central Nervous System Agent In Medicinal Chemistry. 2011. 11, 81-97

Melatonin inhibits NMDA-mediated cell death in PCNs while MT1 antagonist luzindole blocks this neuroprotection

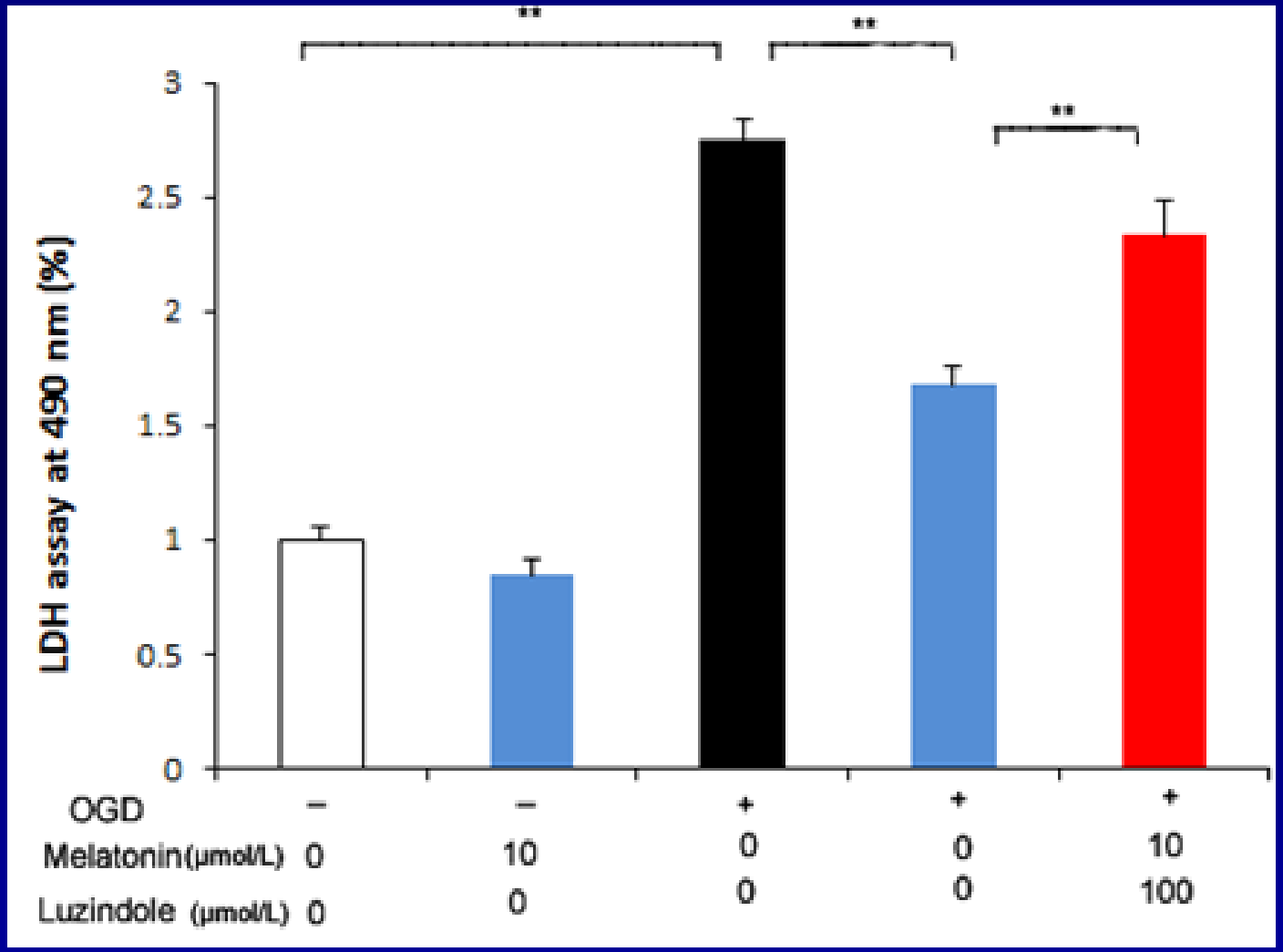
Primary cerebrocortical neurons (PCNs)



Journal of Neuroscience. 2011. 31, 14496-14507

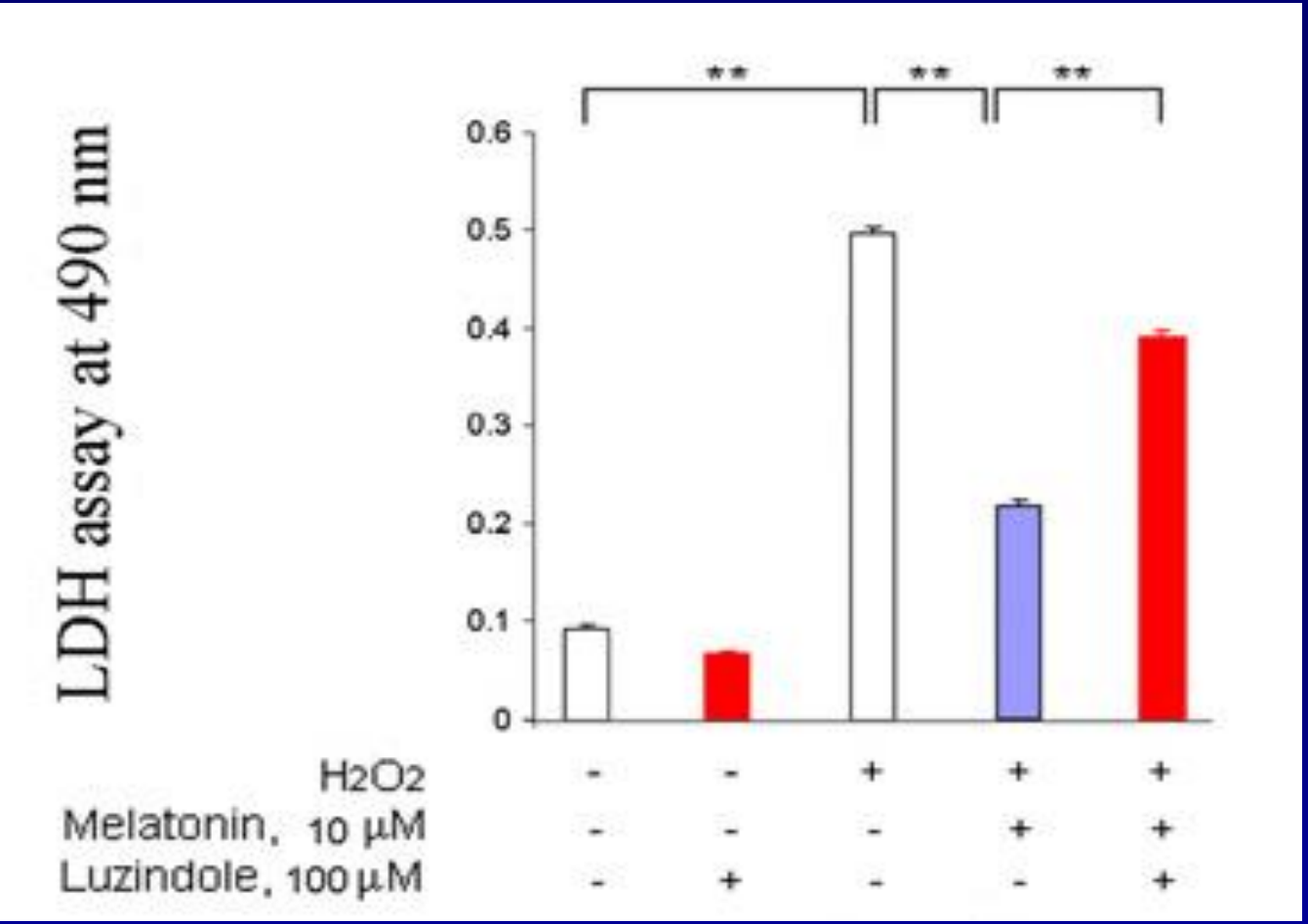
Melatonin inhibits Oxygen–Glucose Deprivation (OGD)-mediated cell death in PCNs, while MT1 antagonist luzindole blocks this neuroprotection

Primary cerebrocortical neurons (PCNs)



Melatonin inhibits H_2O_2-mediated cell death in PHNs while MT1 antagonist luzindole blocks this neuroprotection

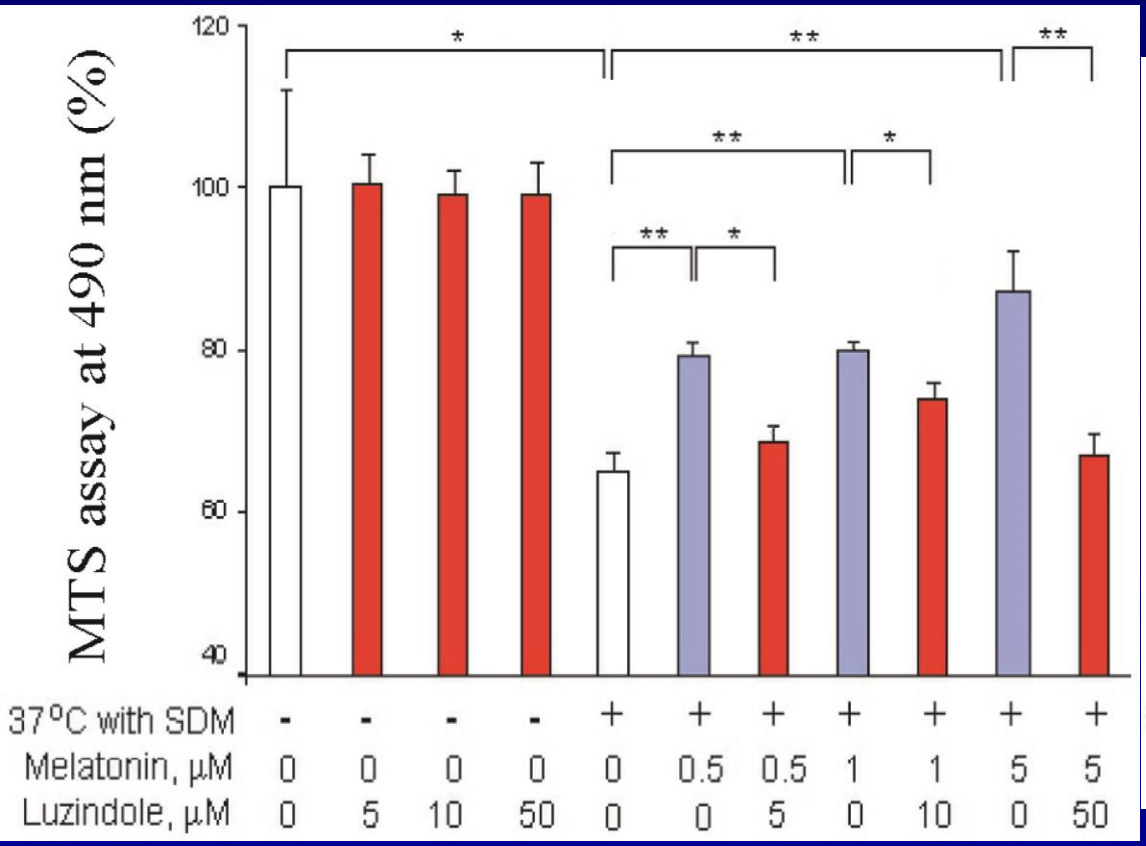
Primary hippocampal neurons (PHNs)

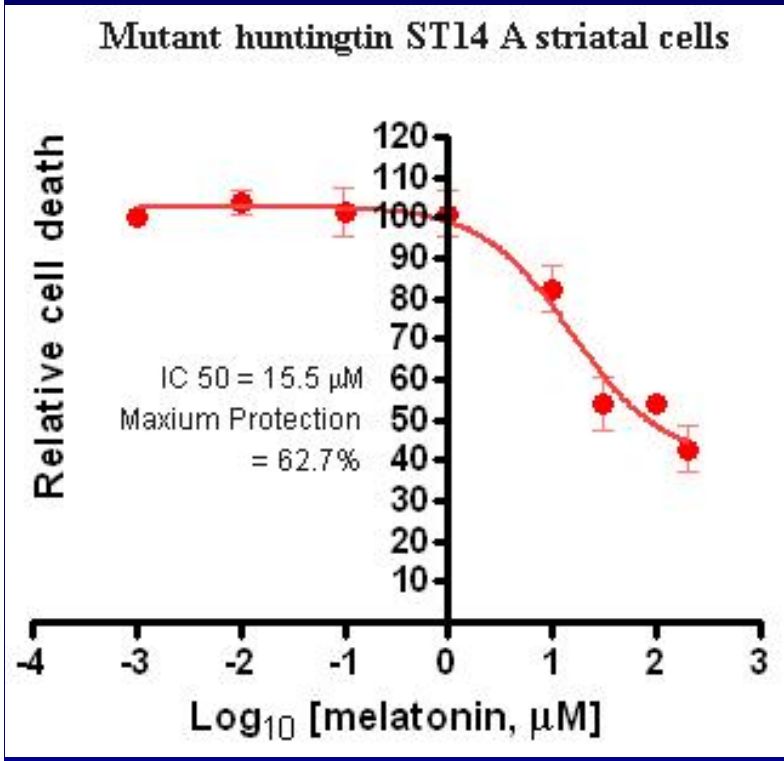


Journal of Neuroscience. 2011. 31, 14496-14507

Melatonin inhibits temperature shift with SDM-mediated cell death in ST14 striatal cell lines, while MT1 antagonist luzindole blocks this neuroprotection

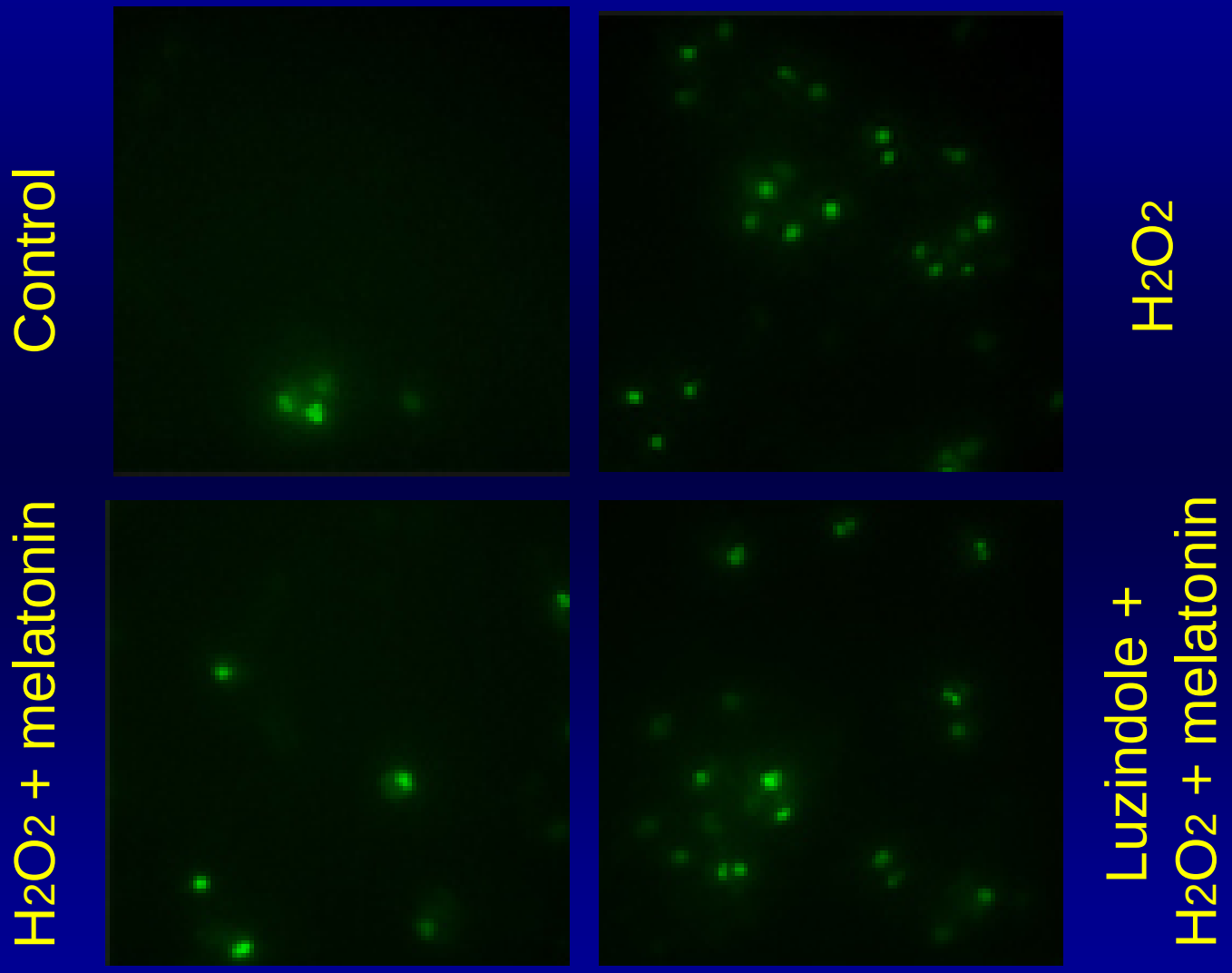
ST14A striatal cell lines





Journal of Neuroscience. 2008. 28, 9473-9485

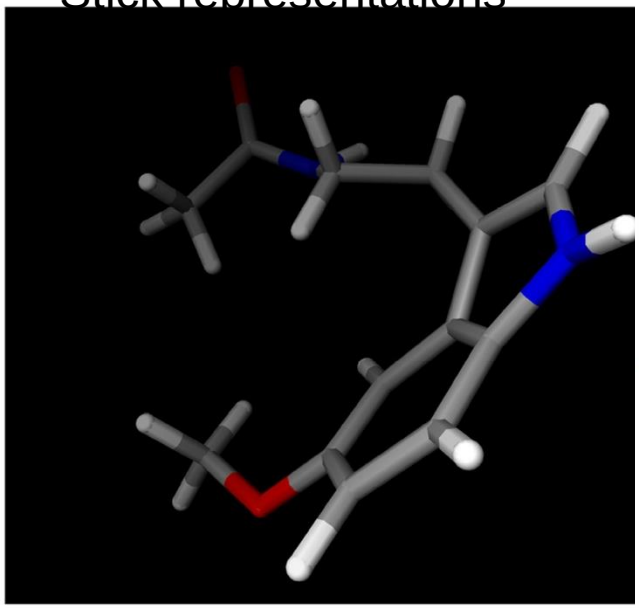
Melatonin inhibits H2O2-mediated PCN cell death, while MT1 antagonist luzindole blocks its role -- TUNEL staining



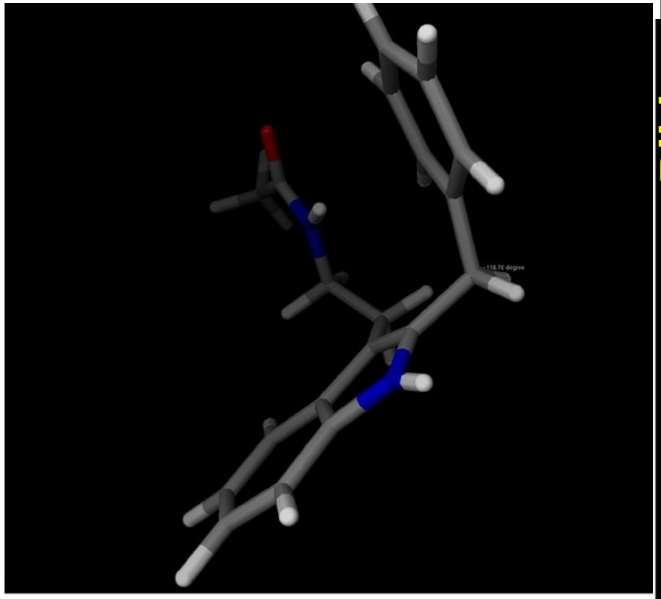
Stroke. 2009. 40, 1877-1885

CNS neuroscience & therapeutics 2009. 15, 345-357

Stick representations



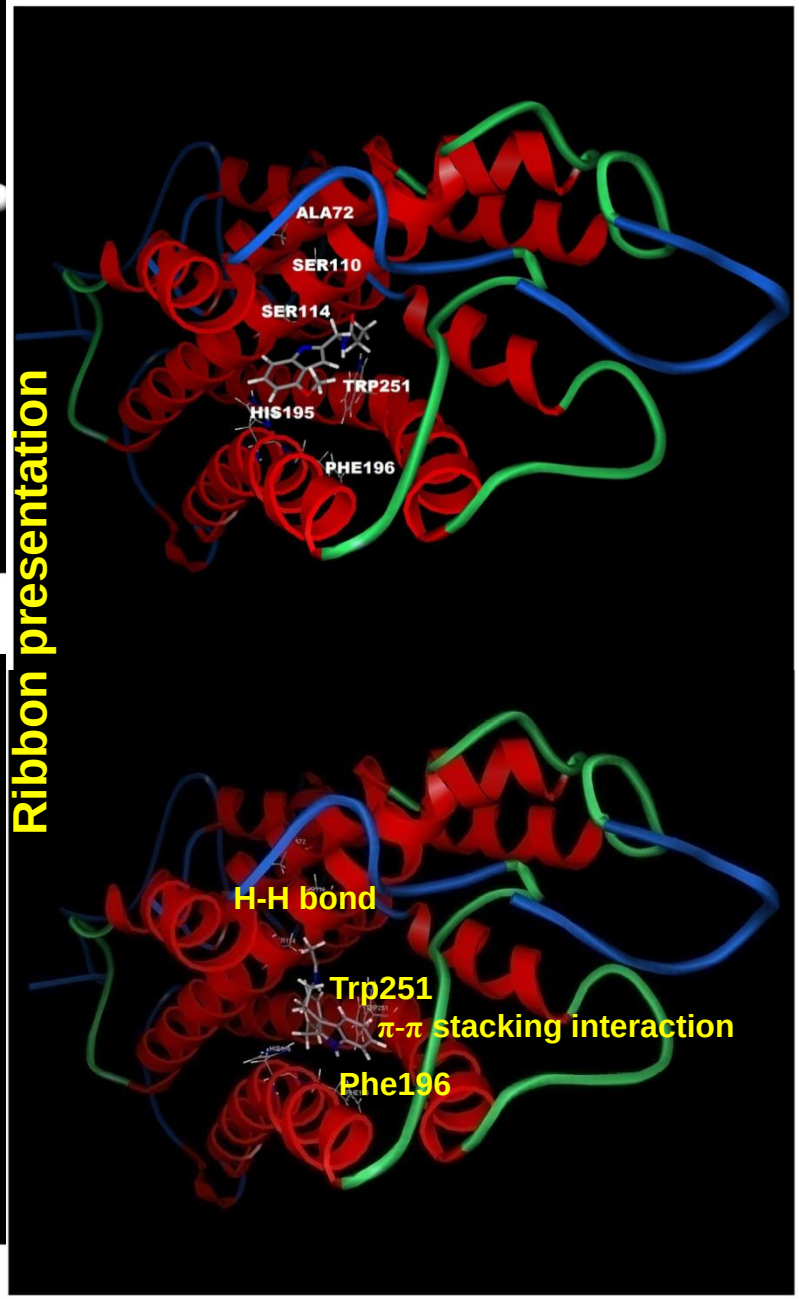
Melatonin



Luzindole

Melatonin -- MT1

Ribbon presentation



Luzindole -- MT1

Luzindole is an MT1 antagonist

Molecular docking analysis indicating a strong π-π stacking interaction and the bending of luzindole causes a shift of the hydrogen bond contribute to luzindole's effective antagonistic properties on the MT1 receptor

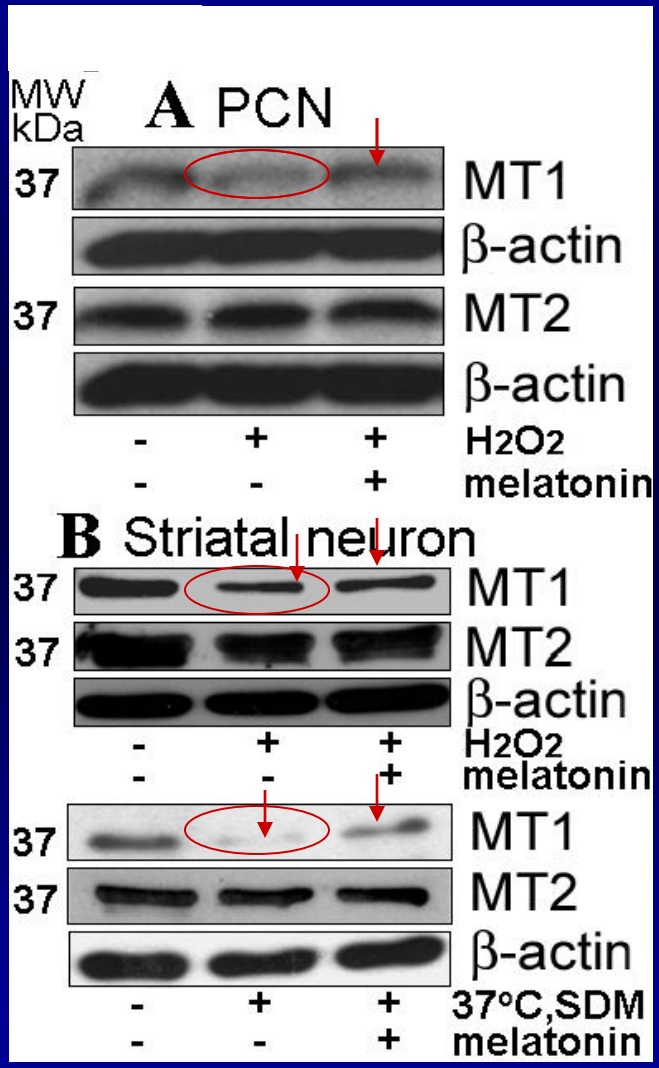
Molecular mechanisms of MT1 agonists

Is melatonin receptors involved in the neuroprotective effect of MT1 agonists in experimental models of stroke?

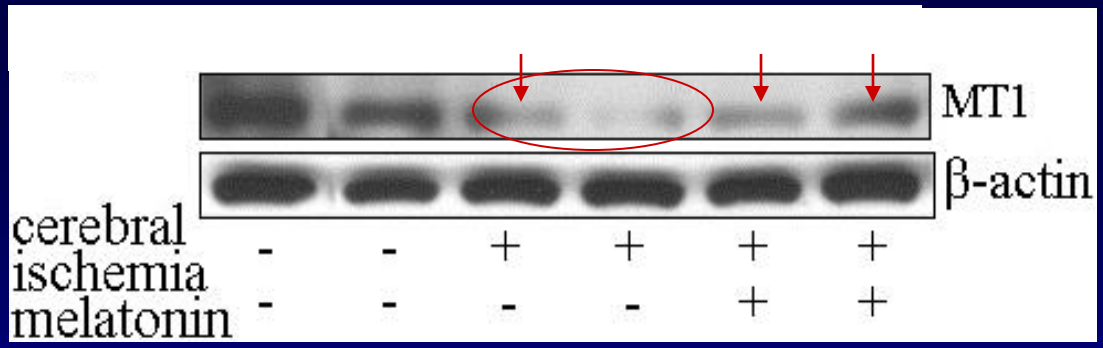
We focus on melatonin receptor 1A (MT1)

MT1 is lost/reduced in apoptotic cultured neurons and MCAO mice, while melatonin restores its deficiency

Cellular model of stroke



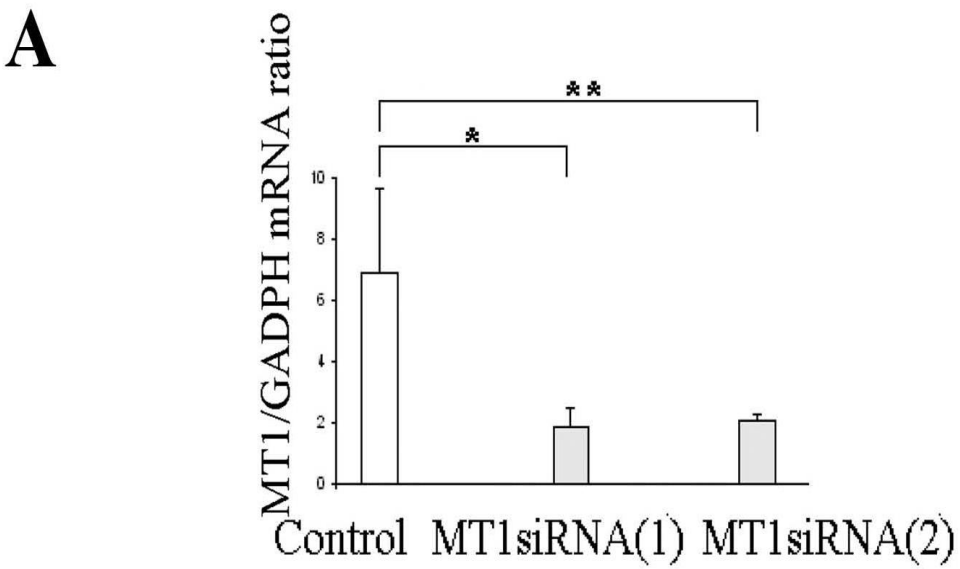
MCAO mouse model of ischemic stroke

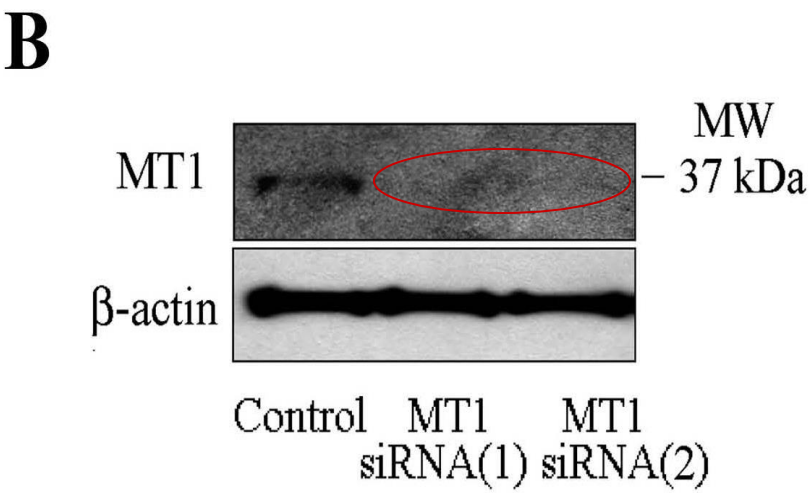


Journal of Neuroscience. 2011. 31, 14496-14507

Knockdown of MT1 in cultured neurons by siRNA assay

Cellular model of stroke





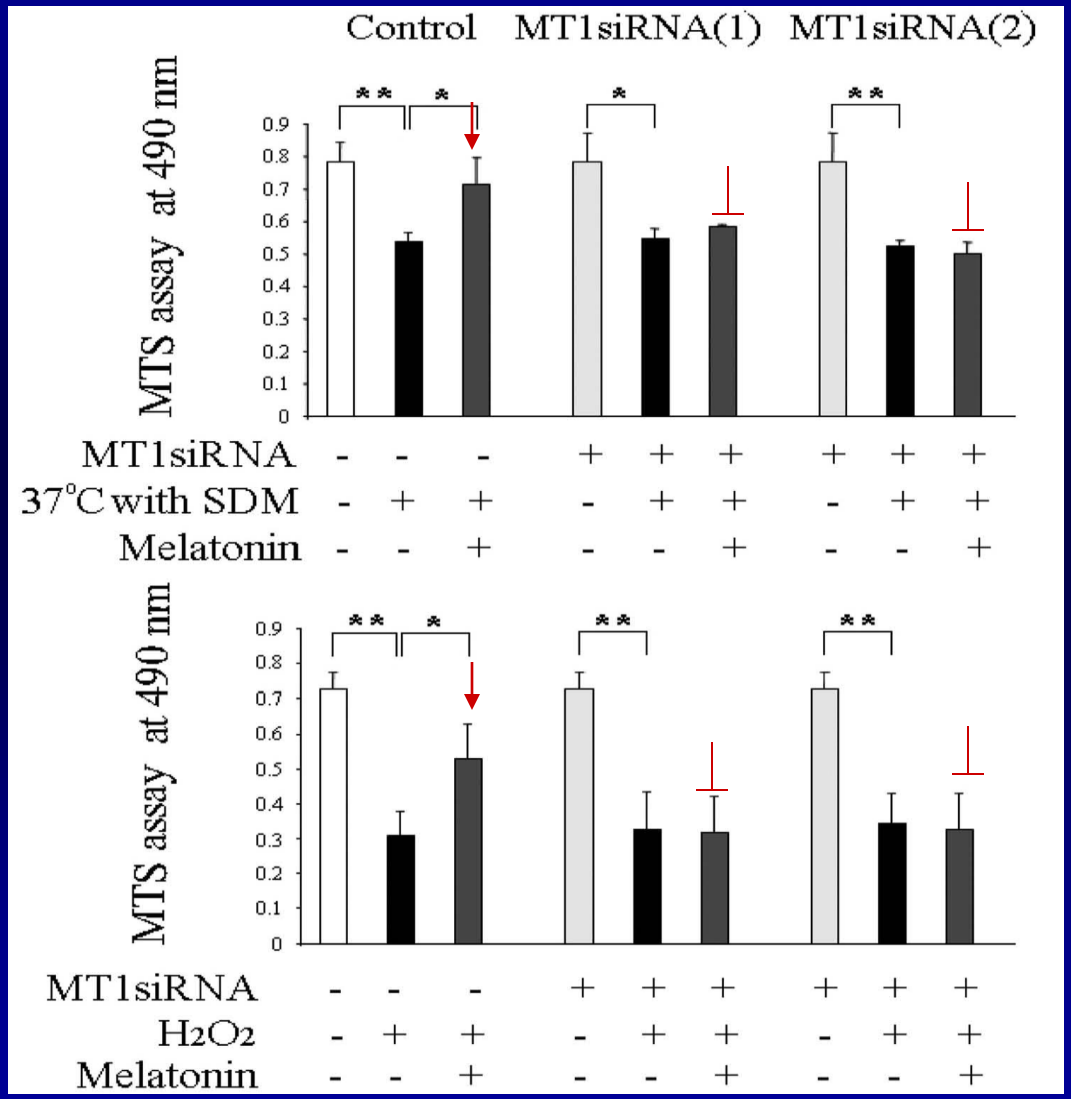
MT1siRNA(1) and MT1siRNA (2) targeting MT1 expression Cells -- transiently transfected with the siRNAs using HiPerFect Transfection Reagent (Qiagen)：

MT1 siRNA (1) targets sequence AACGCAATCATATACGGACTA and consists of 5’-CGCAAUCAUAUACGGACUAtt-3’ and 3’-ttGCGUUAGUAUAUGCCUGAU-5’

MT1 siRNA (2) targets sequence CGGGATCGCTATGAACCGCTA and consists of 5’-GGAUCGCUAUGAACCGCUAtt-3', and 3’-GCCCUAGC GAUACUUGGCGAU-5’

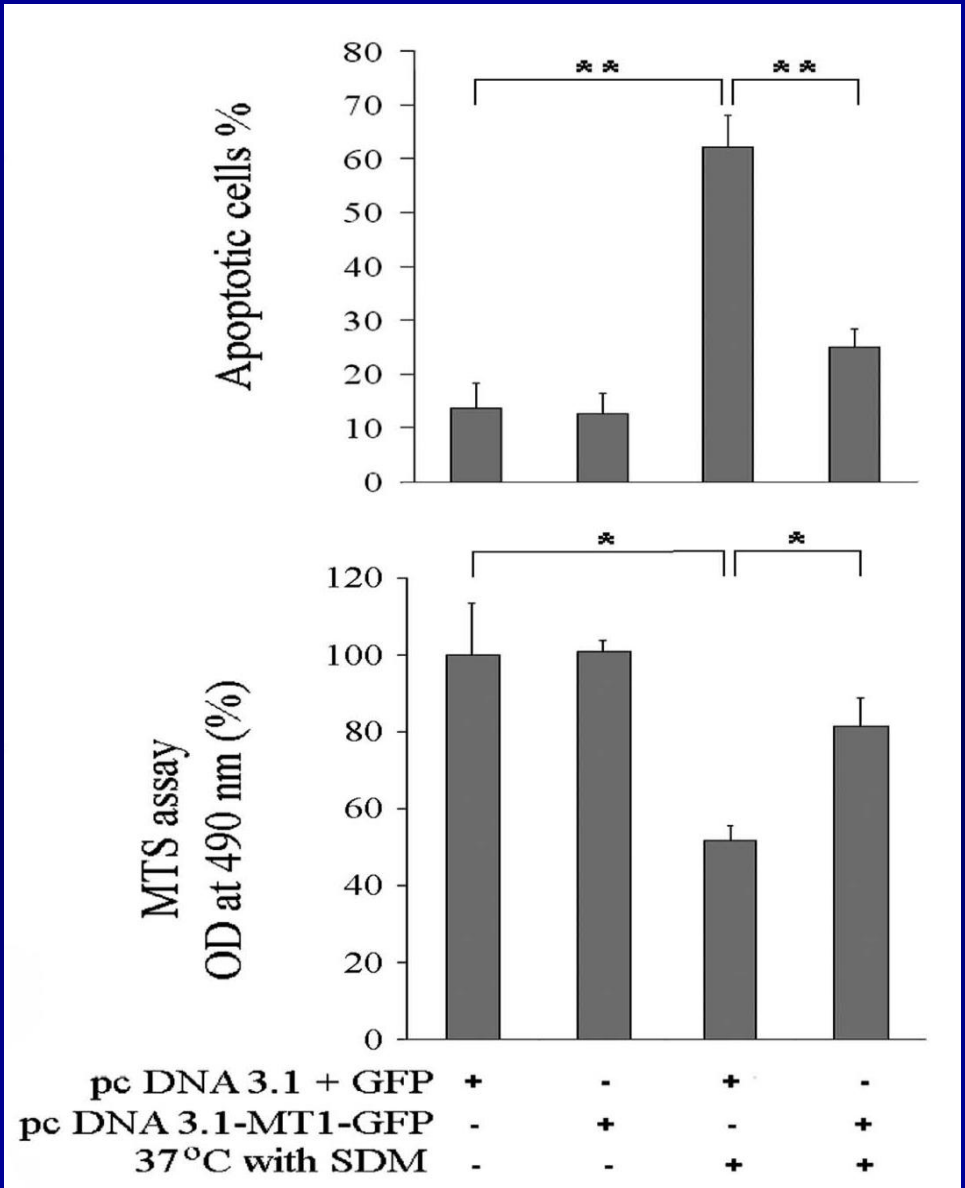
Neuroprotection by melatonin is eliminated/deleted by MT1siRNAs

Cellular model of stroke



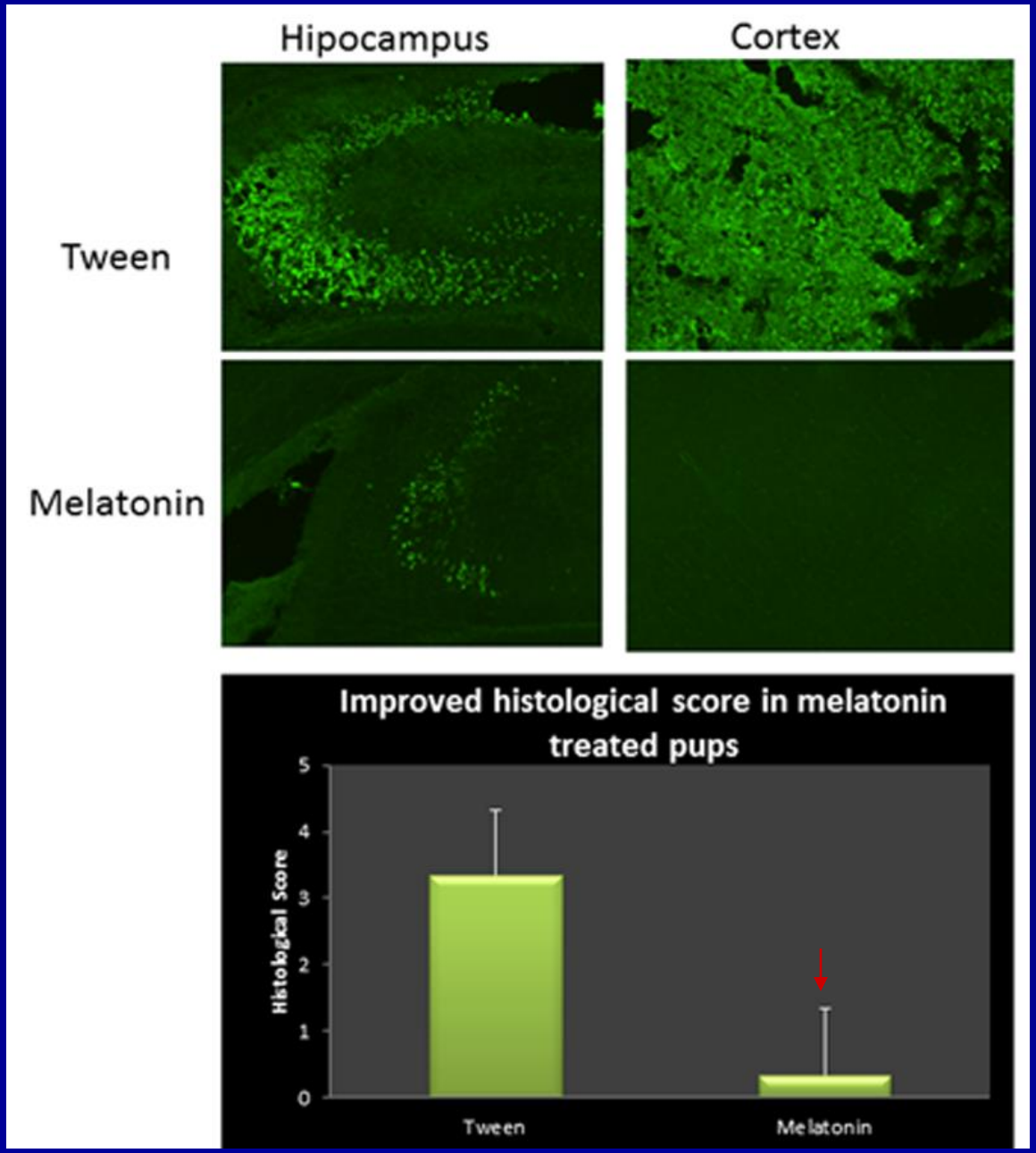
Journal of Neuroscience. 2011. 31, 14496-14507

MT1 receptor itself is neuroprotective



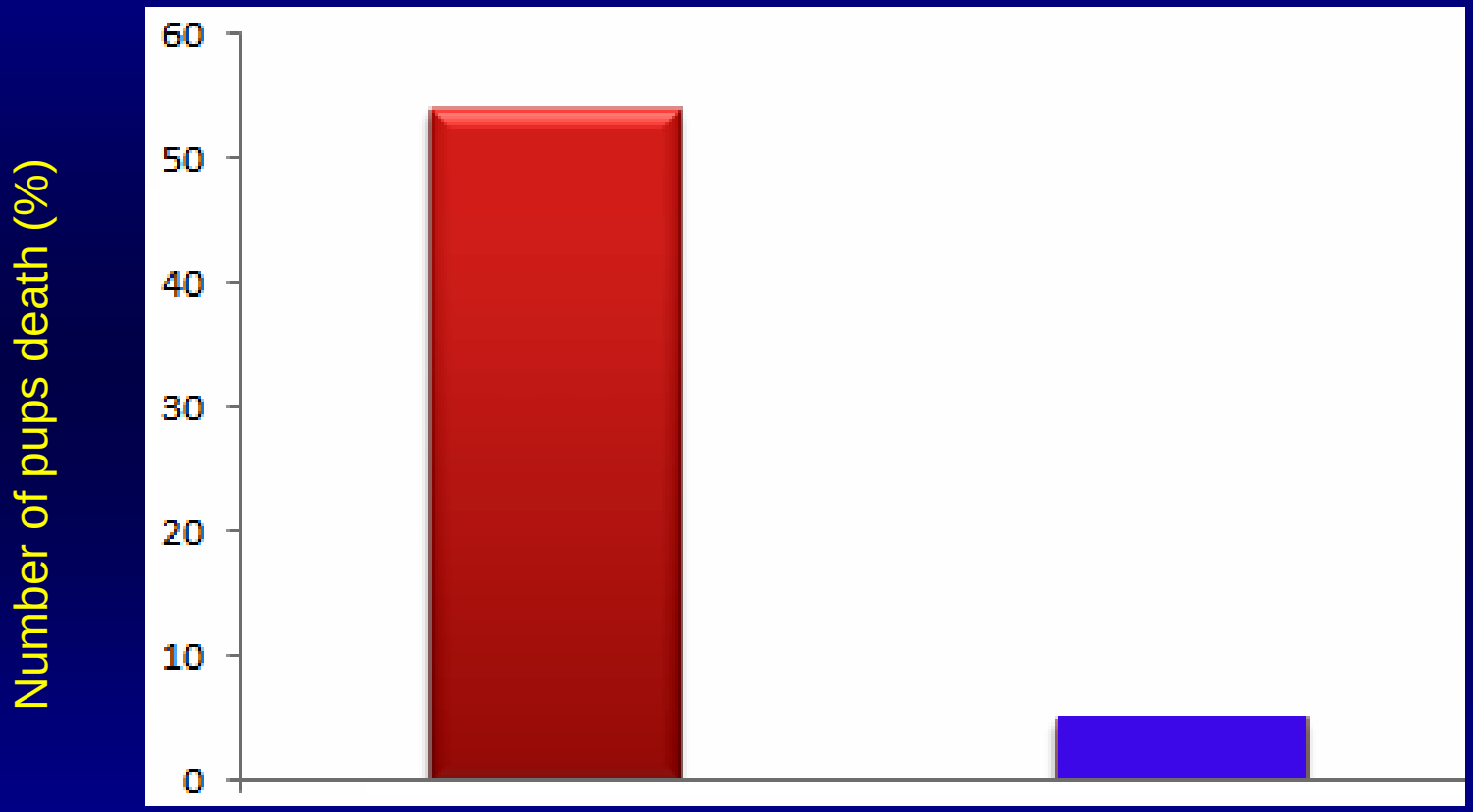
Journal of Neuroscience. 2011. 31, 14496-14507

Melatonin decreases damage from neonatal H-I brain injury



Fluorojade B Staining

Knockdown of MT1 sensitizes pups to death – Significantly increased mortality in MT1 KO mice



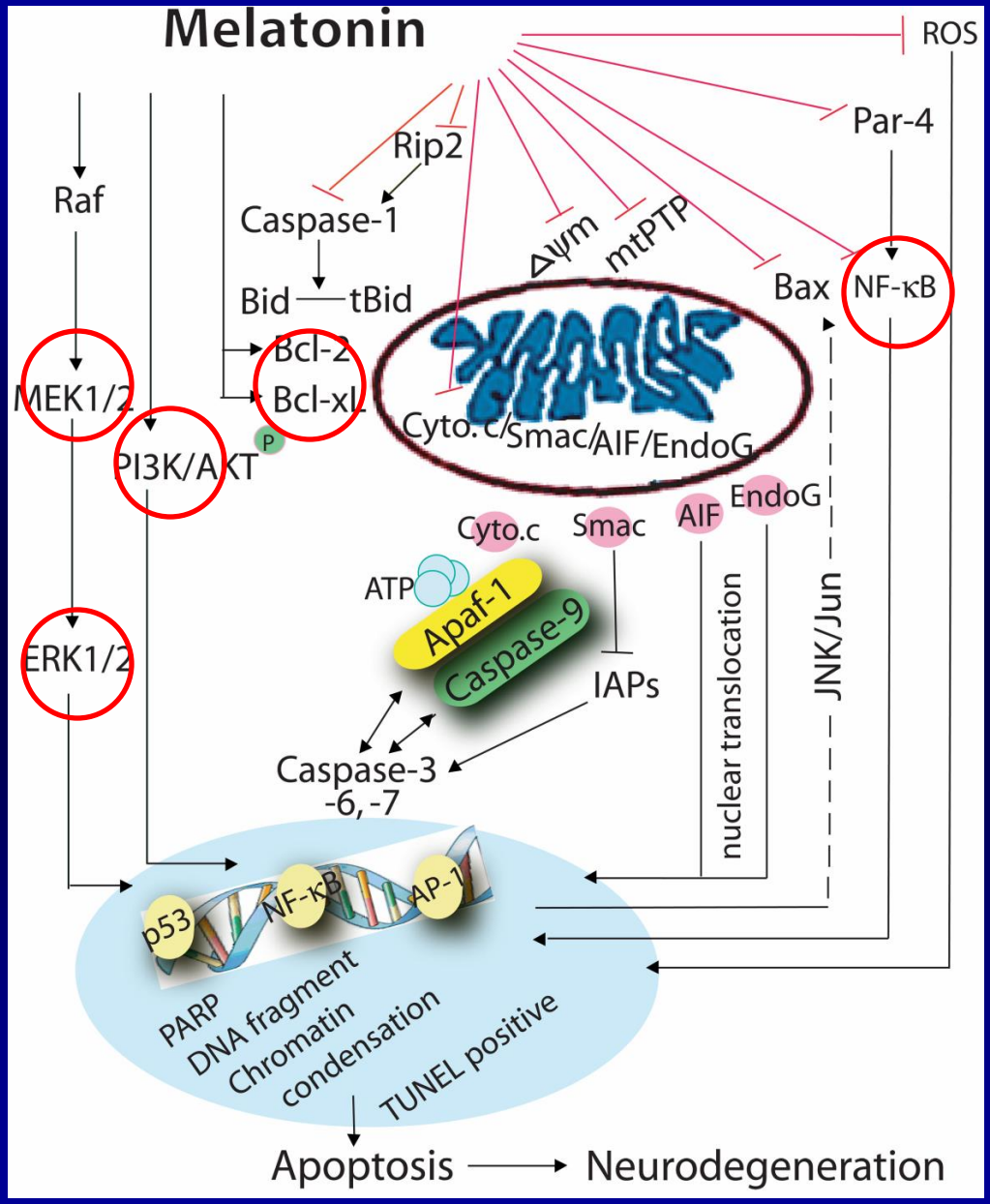
B. Sinha, et al., 2015. In the preparation

Molecular Mechanisms of melatonin

Melatonin offers neuroprotection through

1) inhibiting mitochondrial cell death pathway
2) activating anti-apoptotic survival signal pathway

Melatonin inhibits apoptotic death pathway and activates survival signal pathway



CNS neuroscience & therapeutics 2009. 15, 345-357

Inhibition of apoptotic cell death pathway by melatonin

Inhibits death pathway event	Diseases/ Models	Effects of melatonin	Species Cell line	Refs
Cyto. *c*	Neurodegeneration	Inhibits cyto. *c* release from purified mitochondria	Mouse	[76]
	Stroke/MCAO	Decreases cyto. *c* release	Rat; Mouse, PCN	[18,71]
	PD	Prevents cyto. *c* release	Astrocyte	[117]
Smac/Diablo	HD	Neuroprotective in HD models	Mu-htt ST14A	[unpublished data]
AIF	Stroke	Neuroprotective in PCN	PCN	[18]
$\Delta\Psi_m$	Stroke	Neuroprotective in PSN and PCN	PSN; PCN	[18,71]
	PD	Prevents $\Delta\Psi_m$ depolarization	Astrocyte	[117]
mtPTP	Stroke	Inhibits mtPTP in brain ischemia	PSN	[71]
	PD	Prevents mtPTP opening	Astrocyte	[117]
Bax	AD	Attenuates Aβ25-35-induced apoptosis	Microglial cell	[25]
Bad	Stroke/MCAO	Attenuates cerebral ischemic injury	Rat	[65,78]
ROS	PD	Prevents ROS formation	Astrocyte	[117]
	ALS	Reduces ROS in ALS model	NSC34 motoneuron	[1]
PARP	Stroke/MCAO	Attenuates cerebral ischemic injury	Rat	[65]
Caspase-3	Stroke/MCAO	Prevents caspase-3 activation	Rat; Mouse, PCN	[18,71,77]
	AD	Attenuates Aβ25-35-induced apoptosis	Microglial cell	[25]
	PD	Blocks caspase-3 activation	Astrocyte; Dopaminergic neuron; CGN	[116–118]
Caspase-9	HD	Neuroprotective in HD models	Mu-htt ST14A	[unpublished data]
Caspase-1	Stroke	Neuroprotective in PCN	PCN	[18]
IL-1β	Stroke	Neuroprotective in PCN	PCN	[18]
Rip2	HD	Neuroprotective in HD models	Mu-htt ST14A	[unpublished data]

CNS neuroscience & therapeutics 2009. 15, 345-357

Inhibition of the anti-apoptotic cell death pathway by melatonin

Inhibits death pathway event	Diseases/ Models	Effects of melatonin	Species Cell line	Refs
DNA Fragmentation	Stroke/MCAO	Displayes decreased DNA fragmentation; Neuroprotective in PCN	Rat; PCN	[18,71]
	AD	Attenuates Aβ25-35- or Aβ1-42-induced apoptosis	Astroglioma C6 cell	[102]
	PD	Prevents DNA fragmentation	SK-N-SH cell; Astrocyte; mesencephalic cell; striatal neuron; mouse; PC12 cells	[54,117,121,122]
TUNEL -positive	Neurodegeneration	Reduces number of DNA breaks	Rat	[80]
	Stroke/MCAO	Decreases TUNEL-positive cells	Rat	[65,78,79]
	Stroke/OGD	Neuroprotective in PCN	PCN	[18]
	AD/OVX	Improves spatial memory performance; Reduces apoptosis	Rat	[89]
	AD	Protects the wortmannin-induced tau hyperphosphorylation	N2a cells	[94]
JNK	PD	Inhibits cell death	SK-N-SH cell	[54,55]
Par-4	AD	Reducts Par-4 upregulation	Mouse	[92]
NF-κB	AD	Blocks Aβ25-35-induced apoptosis	Microglial cell; Mouse	[5,25]
	AD	Anti-inflammatory effect on Aβ vaccination in mice	Mouse	[26]

Abbreviations: OVX, ovariectomized.

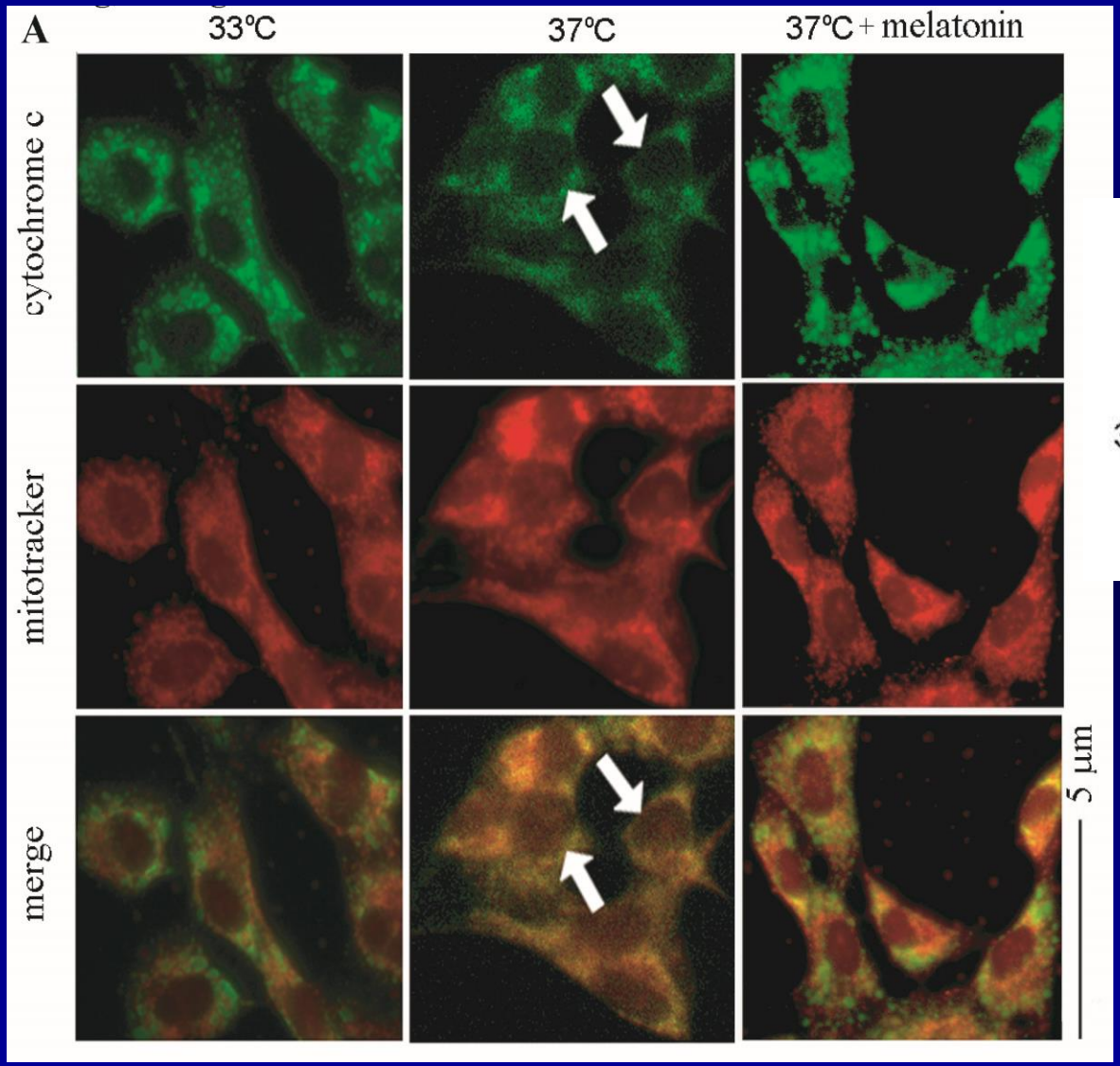
CNS neuroscience & therapeutics 2009. 15, 345-357

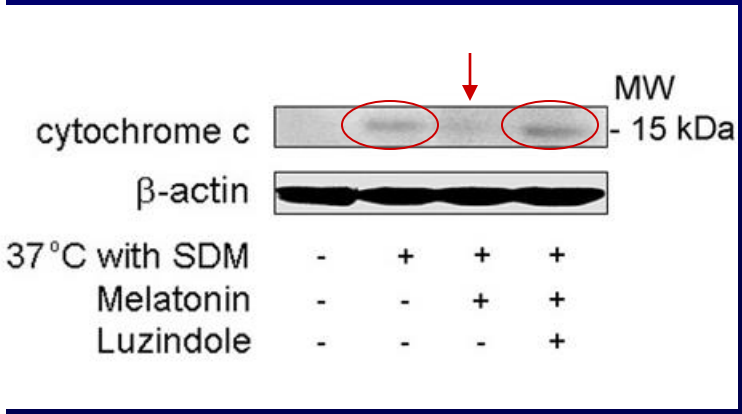
Activation of anti-apoptotic survival signal pathway by melatonin

Activates element of survival pathway	Diseases/ Models	Effects of melatonin	Species Cell line	Refs
PI3-K/Akt	Stroke/MCAO	Restores phosphorylated Akt	Mouse; Rat	[56,77,78]
		Protects against brain injury	Rat	[81]
	AD	Impairs NADPH oxidase via PI3K/Akt signaling pathway	Microglia	[93]
Bcl-2	Stroke/MCAO	Enhances Bcl-2 upregulation	Rat	[79,82]
	AD/Aβ25-35	Attenuates Aβ25-35-induced apoptosis	Microglial cell	[25]
Bcl-xL	Stroke/MCAO	Elevates Bcl-xL in brain injury	Mouse	[77]
JNK1/2	Stroke/MCAO	Increases JNK1/2 phosphorylation	Mouse	[56]
ERK1/2	Stroke/MCAO	Increases ERK1/2 phosphorylation	Mouse; Rat	[56,65]
Raf-1	Stroke/MCAO	Attenuates cerebral ischemic injury	Rat	[65]
MEK1/2	Stroke/MCAO	Attenuates cerebral ischemic injury	Rat	[65]
NF-κB	Stroke	Relates with NF-kB-mediated protective signaling	Primary neuron	[27]

CNS neuroscience & therapeutics 2009. 15, 345-357

Melatonin, is an inhibitor of cytochrome c release (like minocycline does) – Luzindole blocks the role of melatonin

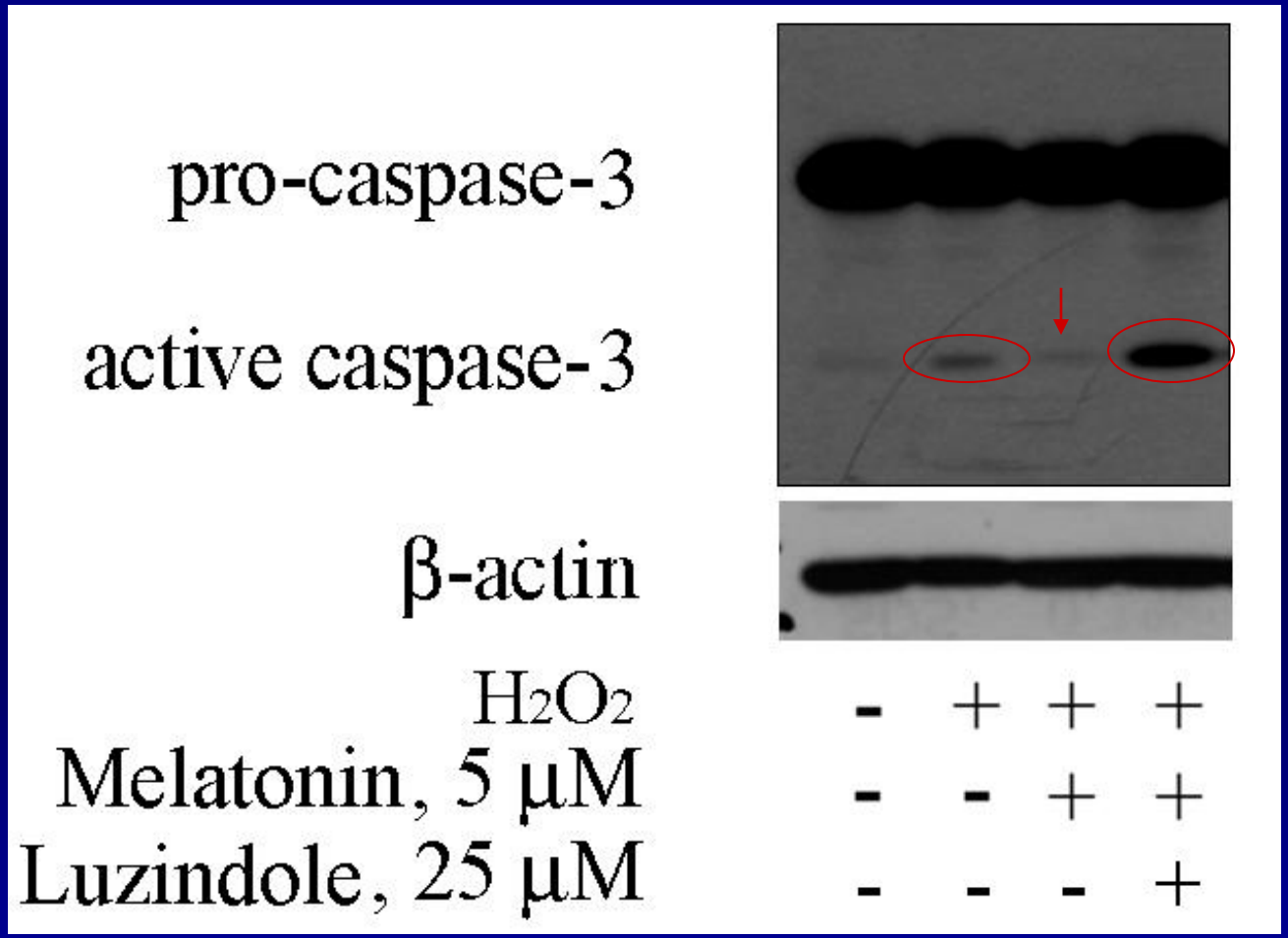




PNAS 2003. 100, 10483-10487

Journal of Neuroscience. 2011. 31, 14496-14507

Melatonin, reduces the activation of caspase-3, while luzindole blocks the role of melatonin



PNAS 2003. 100, 16012-16017

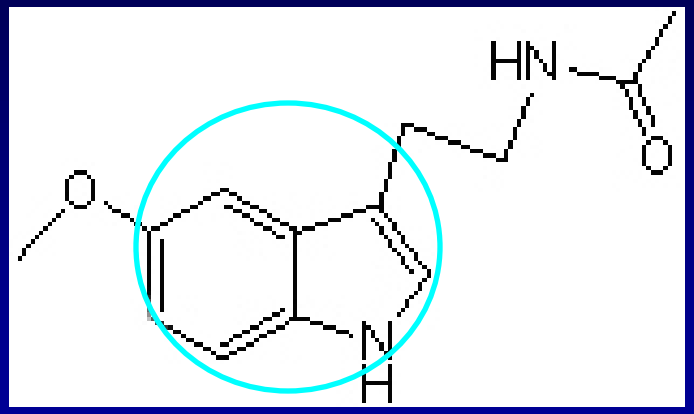
Conclusion

MT1 agonist melatonin-mediated neuroprotection through inhibiting of cell death pathway and activating survival signaling pathway in experimental models of stroke requires the MT1 receptor

The other agonists of MT1 May offer protection in stroke as well -- novel neuroprotective agents for stroke

146 MT1 agonists have been documented from the G-protein coupled receptor GPCR-Ligand Database. They are generally characterized by an indole core skeleton.

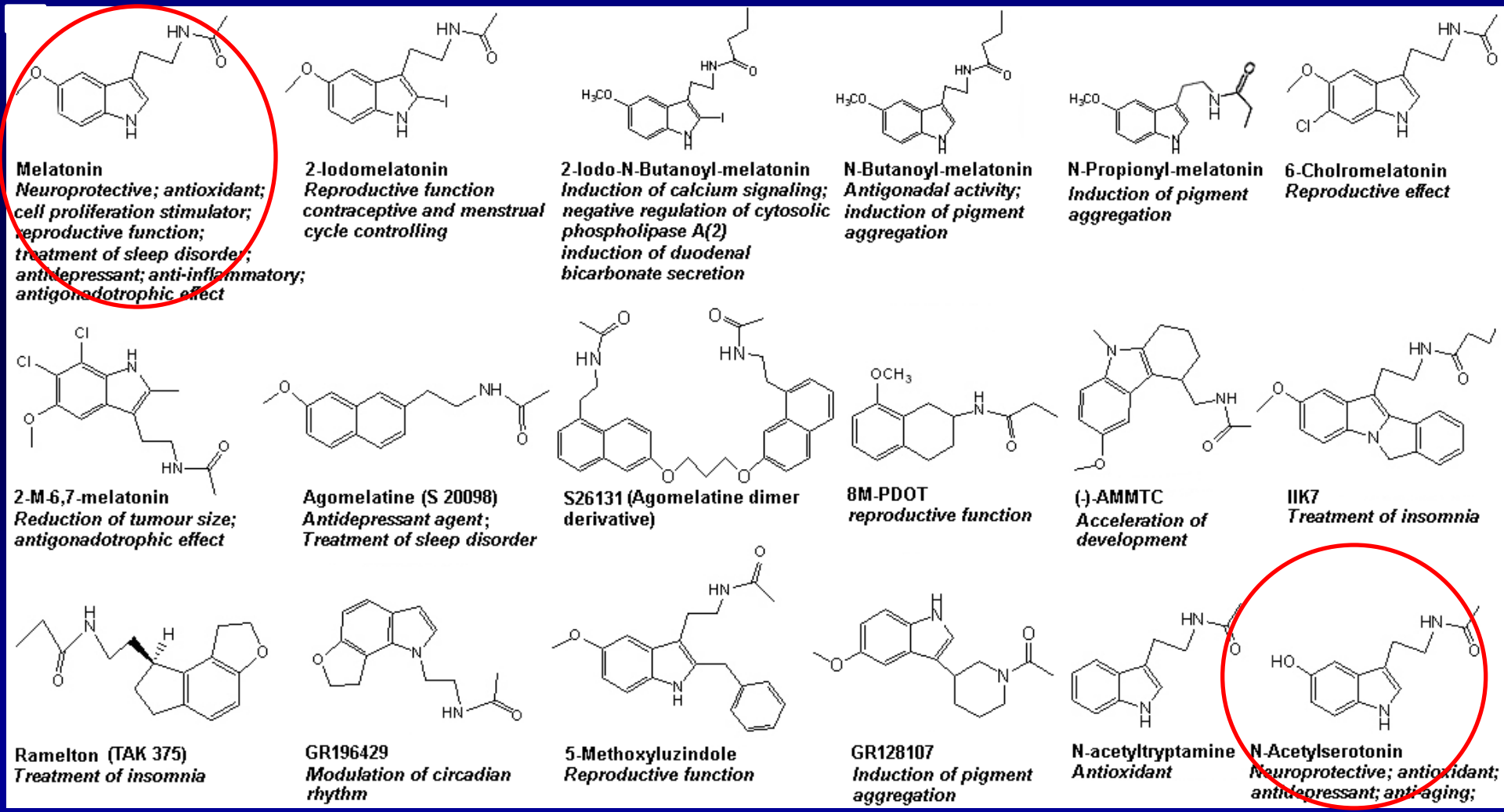
indole core skeleton



.

Eighteen relatively common MT1 agonists

There are 3 G-protein-coupled melatonin receptors: MT1, MT2, and MT3. They are generally characterized by an indole core skeleton. We aim to identify novel drugs from MT1 agonists for stroke



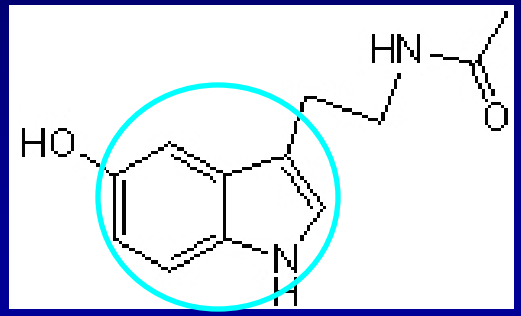
NAS (N-acetyl-serotonin, Normelatonin)

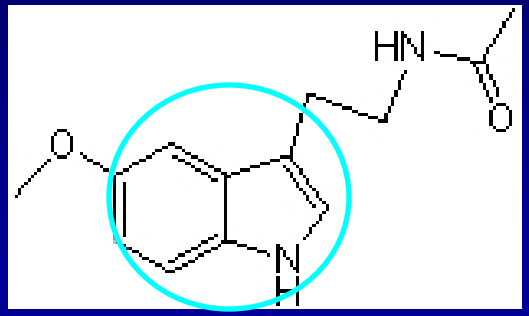
- NAS is the **immediate** precursor of melatonin.

NAS is a chemical intermediate, which produced from serotonin and is converted to melatonin.

Serotonin ⟶ NAS ⇌ Melatonin

indole core skeleton





NAS (Normelatonin)

- **Common:**

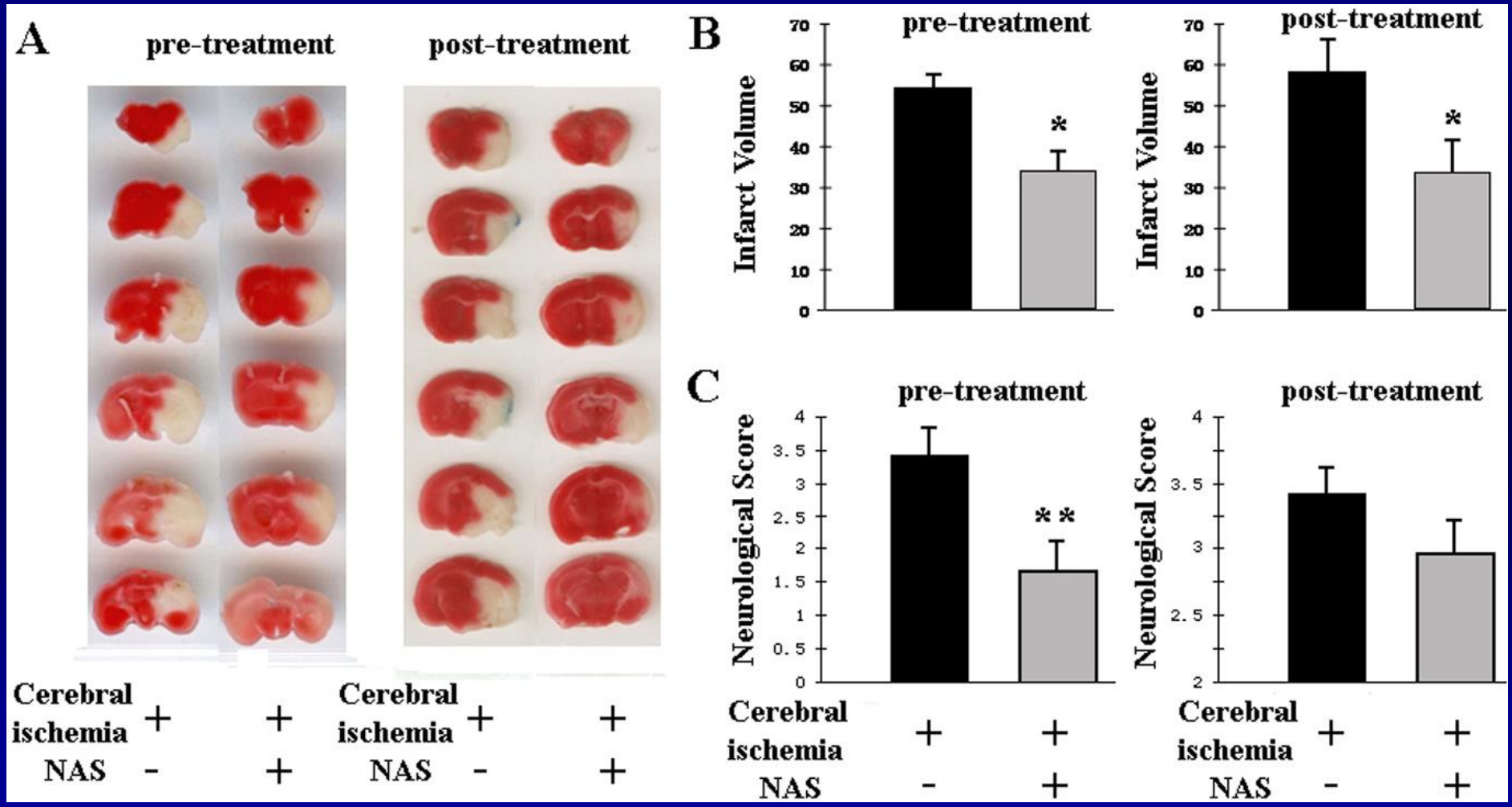
Both NAS and melatonin are 1) MT1 agonists; 2) NINDS 1040 compounds; 3) anti-oxidant; 4) anti-aging; 5) dietary supplements.

- **Different:**

NAS is a better antioxidant.

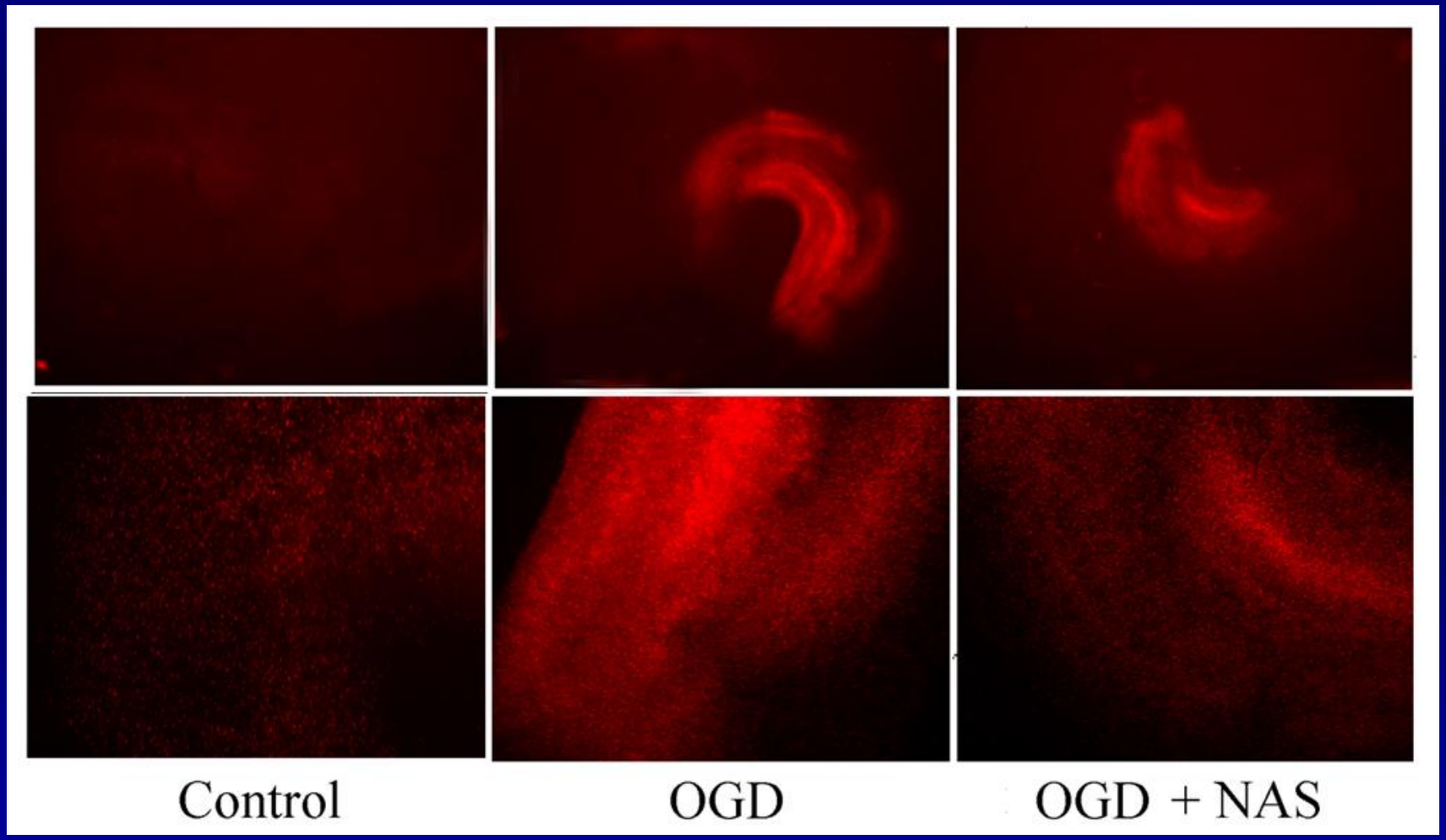
NAS has wider distribution (brainstem, cerebellum, hippocampus, motor nuclei). Melatonin is secreted mainly from the pineal gland, it distributes in brain tissue (neurons and glia), brain ventricles, and hippocampus.

NAS decreases damage in Middle Cerebral Artery Occlusion (MCAO) mice



Journal of Neuroscience. 2014. 34:2967-78

NAS inhibits cell death (PI staining) in organotypic hippocampal slice cultures



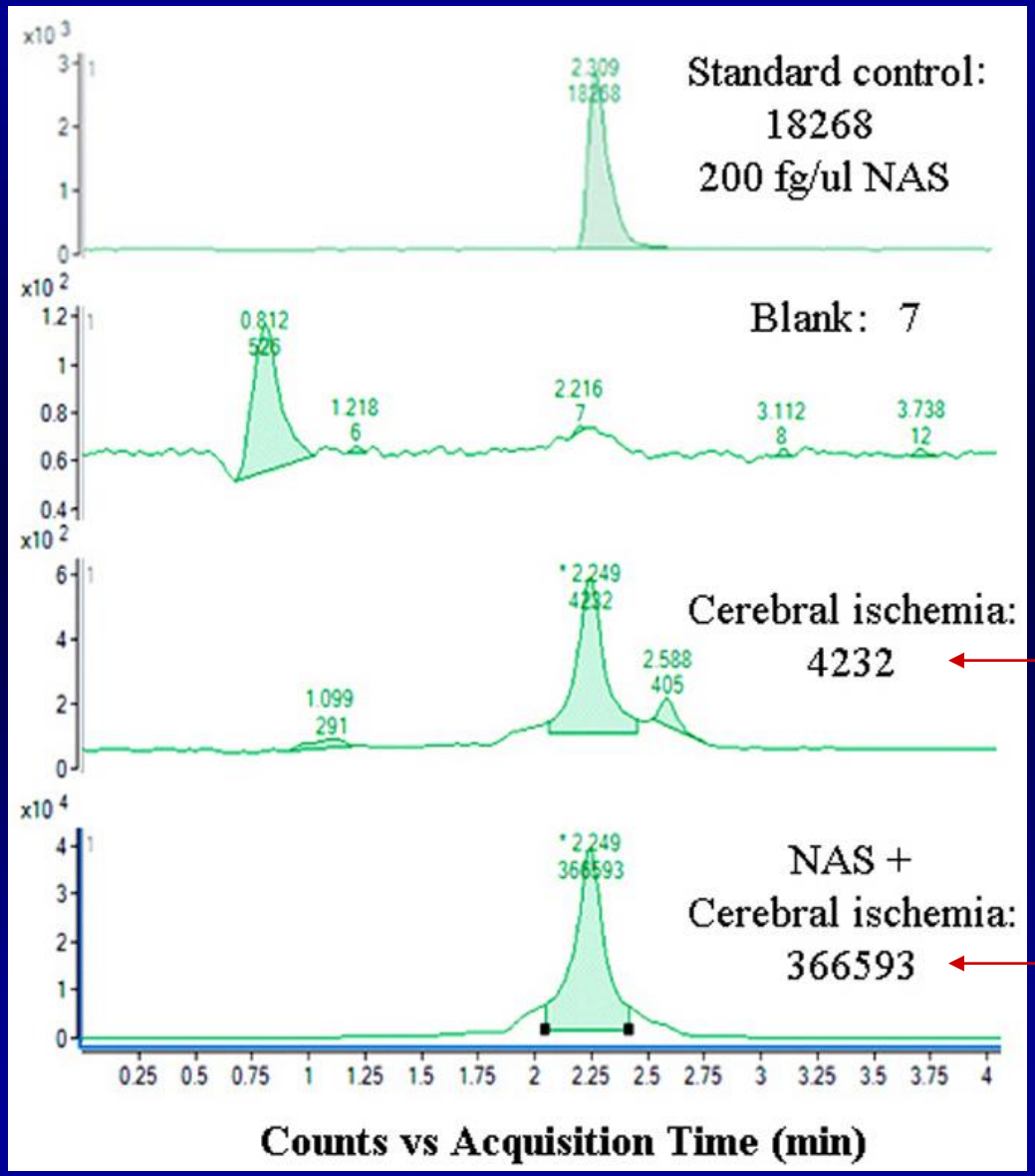
Journal of Neuroscience. 2014. 34:2967-78

Molecular Mechanisms of NAS

NAS offers neuroprotection through inhibiting

1) mitochondrial cell death pathways
2) autophagic cell death pathways

NAS determination in brains of mice with ischemic injury
Liquid chromatography/mass spectrometry (LC/MS)

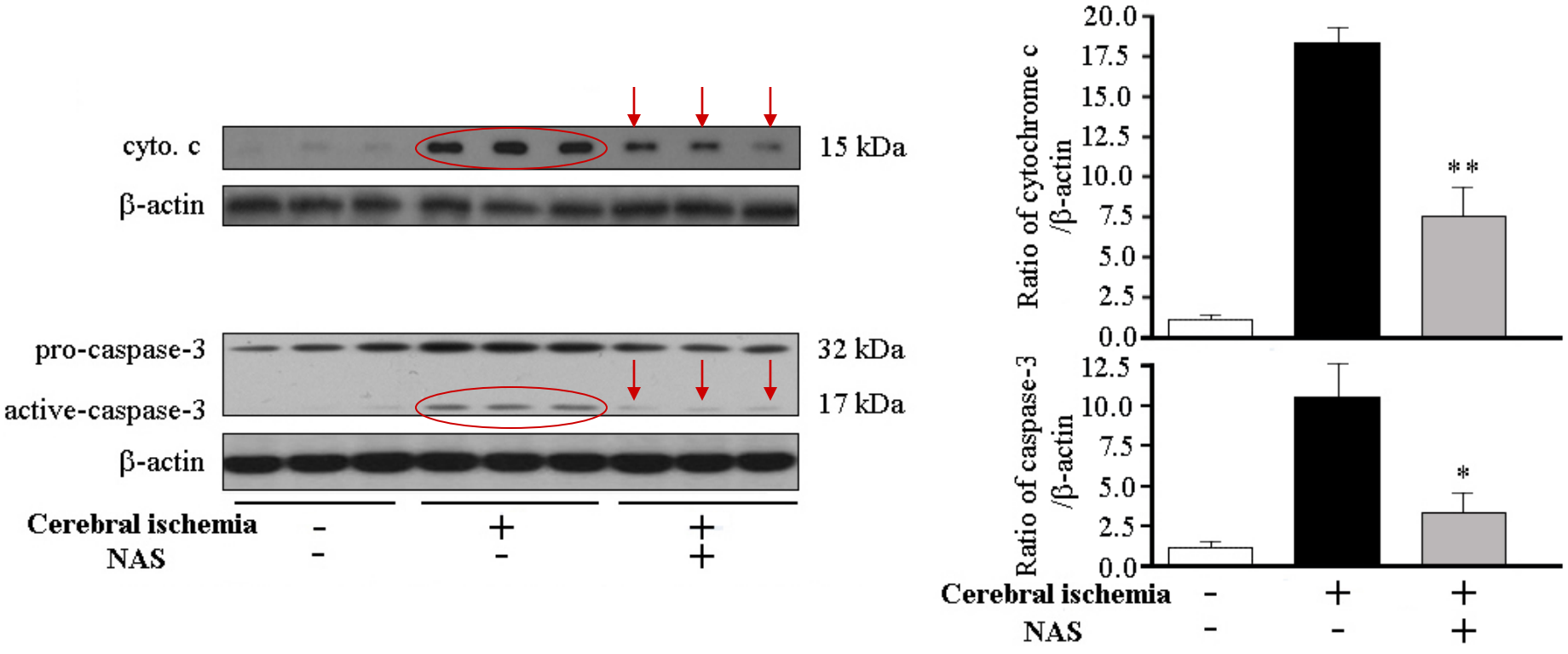


Endogenous NAS secretion in the brains of MCAO mice

NAS brain level in the brain of MCAO mice treated with NAS (~100 fold than non-NAS treated one)

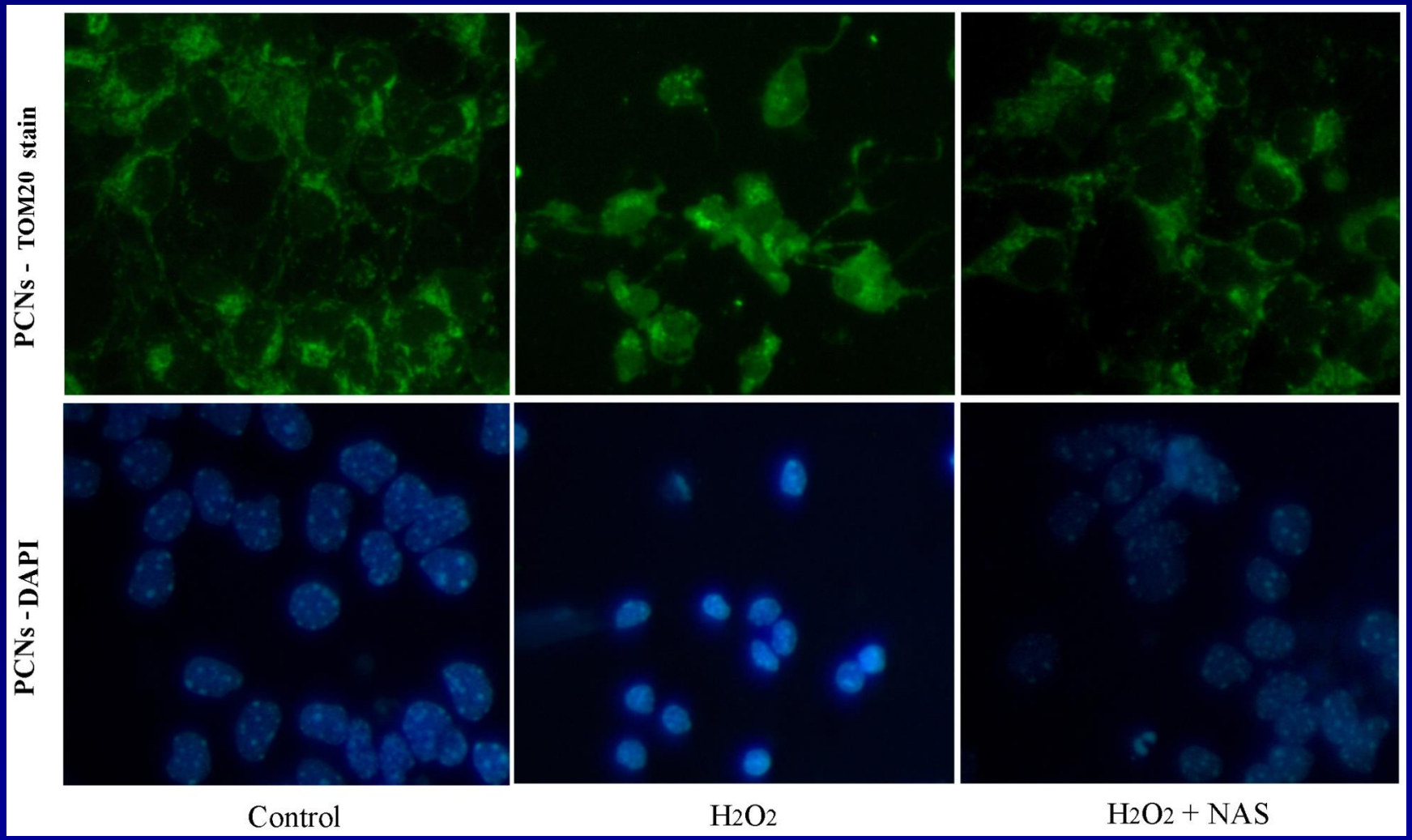
Journal of Neuroscience. 2014. 34:2967-78

NAS reduces the release of cytochrome c and activation of caspase-3 in MCAO mice



Journal of Neuroscience. 2014. 34:2967-78

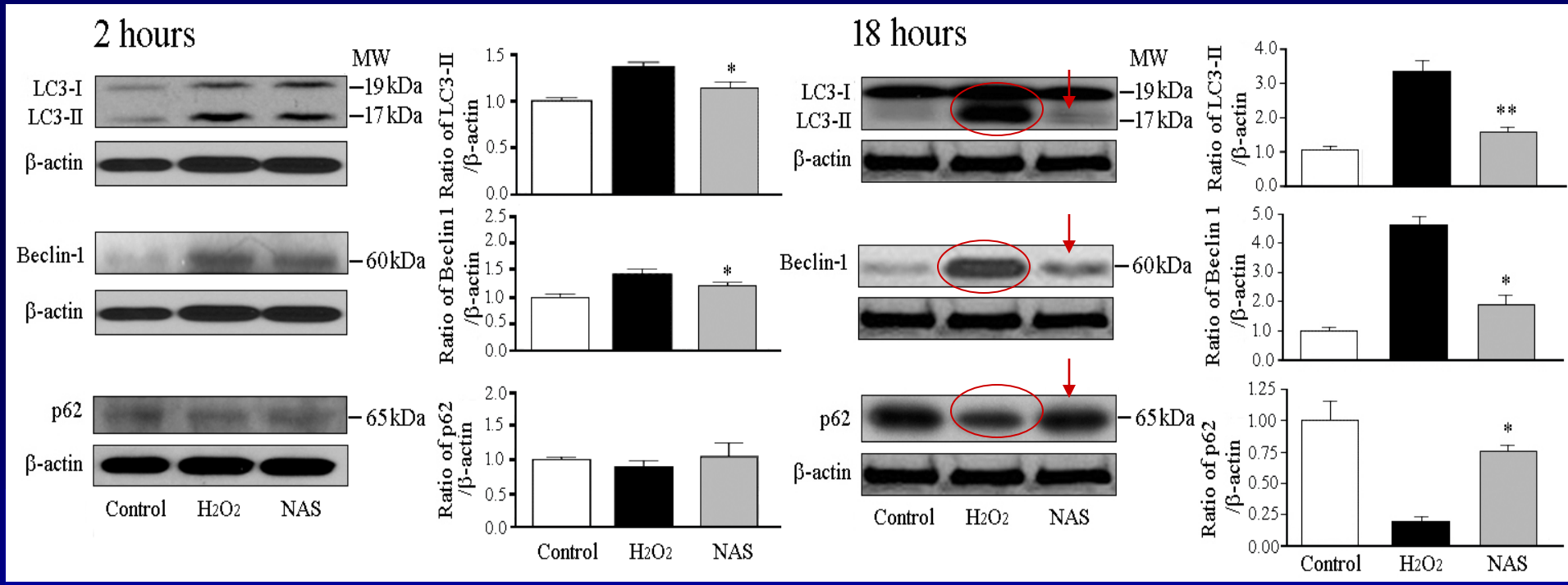
NAS offers neuroprotection through inhibiting mitochondrial fragmentation in PCNs



Journal of Neuroscience. 2014. 34:2967-78

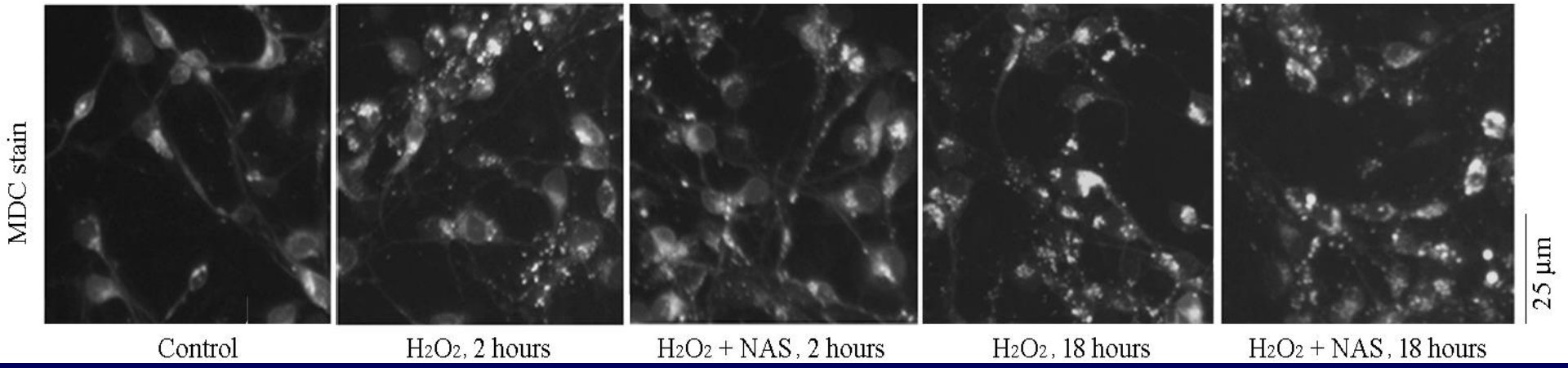
NAS offers neuroprotection through inhibiting autophagic cell death in cell model of ischemic stroke

Primary cerebrocortical neurons (PCNs)



Journal of Neuroscience. 2014. 34:2967-78

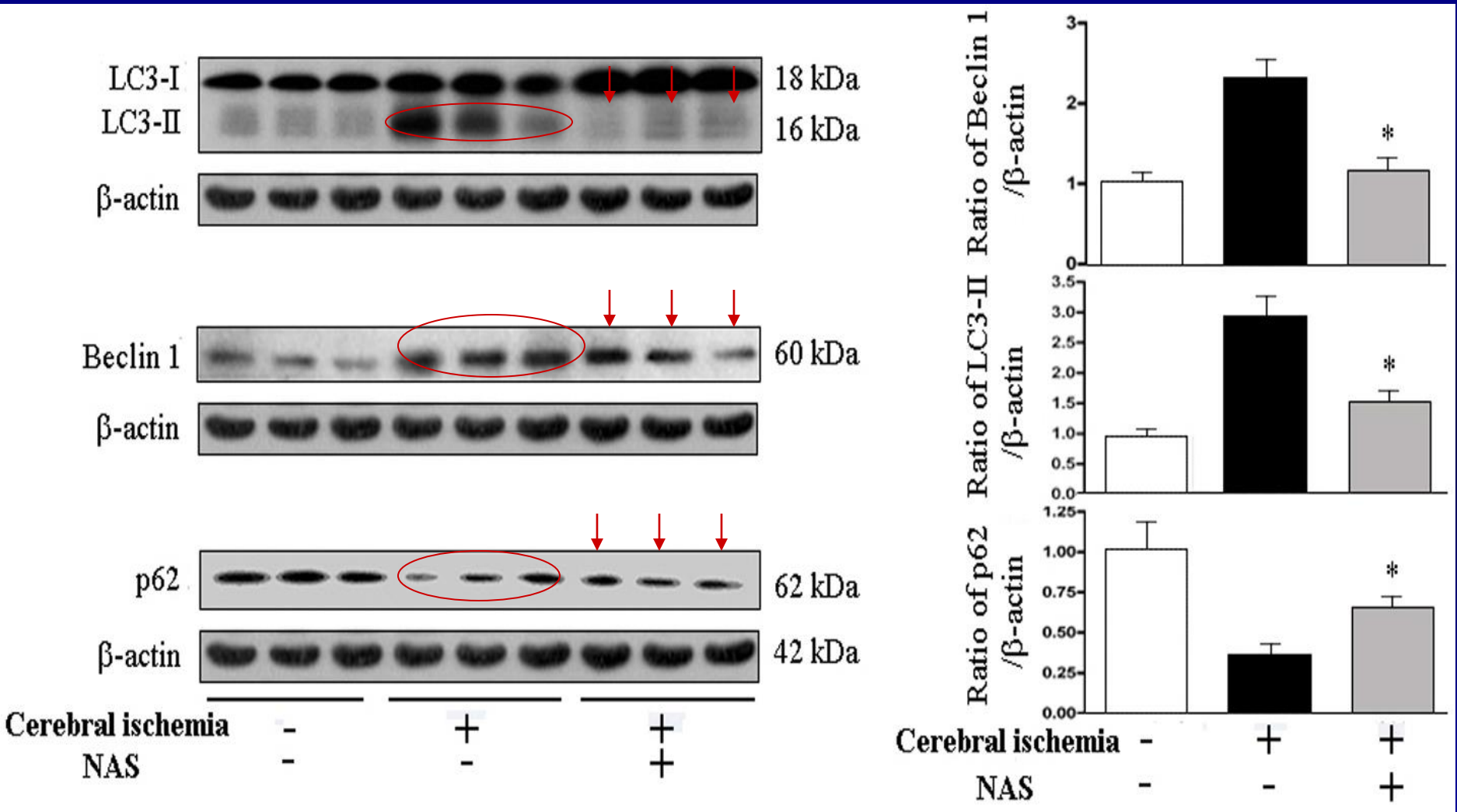
NAS inhibits H_2O_2-induced autophagic vacuoles/autophagosomes



Journal of Neuroscience. 2014. 34:2967-78

Monodansylcadaverine (MDC) is a specific marker for autophagic vacuoles/autophagosomes. H_2O_2 caused accumulation of autophagic vacuoles in PCNs.

NAS offers neuroprotection through inhibiting autophagic activation in MCAO mouse model of ischemic injury



Journal of Neuroscience. 2014. 34:2967-78

NIH/NINDS
Bill & Melinda Gates Foundation
Muscular Dystrophy Association
ALS Therapy Alliance
Prize4 Life Company
Hereditary Disease Foundation

Acknowledgments

Wang Lab:

Hannah Zhou
Bharati Sinha
Jian Wang
Xinmu Zhang
David Li
Wu Chen
Qiaofeng Wu
Ana C. Sirianni
Jiying Jiang
Rachna Pandya
Robert Luo
Anastasios Fotinos
Yanchun Chen
Daphne A. Kroessler
Yanyang Tu

Collaborators (HMS)

Bruce S. Kristal (BWH)
Irina G. Stavrovskaya (BWH)
Merit Cudkowicz (MGH)
Shuanhu Zhou (BWH)
Rose Du (BWH)

Other Collaborators:

Robert M. Friedlander (Univ. of Pitt)
Lilly L. Mao (Aimcan Pharma Inc Canada)
Yi Zhang (FDA)
Jiang Zeng (Univ. of Toronto, Canada)
Mingchang Li (Wuhan Univ., China)

Happy Global Summit on Stroke-2015!