Extending Injury- and Disease-Tolerant Phenotypes by Repetitive Conditioning

Promoting Long-Lasting Protection in the CNS

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WHAT DOESN'T KILL YOU

Makes you stronger.
got hypoxia?
CNS Disease
Acute and Chronic Disease

Stroke

Retinal Ischemia and Glaucoma
Hypoxic Preconditioning
newborn rat stroke

Gidday et al., JCBFM, 1999
Hypoxic preconditioning
Adult mouse stroke

Miller et al., *NeuroReport*, 2000

Wacker et al., *JCBFM*, 2012
CNS Disease

Acute and Chronic Disease

Stroke

Retinal Ischemia

Glaucoma
Control

Ischemia

Hypoxic Preconditioning + Ischemia

Histology

Hypoxic Preconditioning-Induced Protection

Zhu et al., IOVS, 2002
Flash Electroretinogram
Quantifying the Retinal Response to Light

10-µsec flash

b-wave

a-wave
Hypoxic Preconditioning
functional protection against retinal ischemia

Zhu et al., IOVS, 2002
Ischemic Preconditioning

Protection Against Retinal Ischemic Injury

Roth et al., IOVS, 1998
Transient Protection – Rat Retina

ERG b-wave Amplitude (% of baseline)

Time after ischemia

- Ischemia
- 24 h PC + Ischemia
- 72 h PC + Ischemia
- 168 h PC + Ischemia
Transient Protection – Mouse Retina

Zhu et al., IOVS, 2002

Percent of Nonischemic Controls

layer thickness number of cells

OLM-ILM INL IPL INL GCL

Nonischemic controls
Ischemia
PC + ischemia 1 day later
PC + ischemia 3 days later
PC + ischemia 7 days later

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Retinal Phototoxicity Tolerance

**transient protection**

Hypoxic preconditioning in adult transient focal stroke

Reductions in leukocyte-endothelial adherence and infiltration
Home Sweet Home...?
**In vivo preclinical studies**

- Promotes hippocampal neurogenesis (Zhu et al., *J Neurosci* 2010)
- Enhances proliferation of neuroprogenitor cells (Zhu et al., *Brain Res* 2005)
- Upregulates egr-1 and other survival-promoting transcription factors (Rybnikova et al., *Behav Brain Res* 2005)
- Protects against iron-, MPTP-, and kainic acid-induced neuronal injury (Lin et al., *Exp Neurol* 2002)
- Prevents mitochondrial DNA deletion and inhibits opening of MPTPs in heart (Zhu et al., *J Mol Cell Cardiol* 2006)
- Prolongs survival in a leukemic mouse model via tumor arrest and differentiation induction (Liu et al., *Blood* 2010)
Intermittent Hypoxia

dose titration critical
Epaulette shark
*Hemiscyllium ocellatum*
Repetitive Hypoxic Preconditioning “RHP”

Experimental Design

RHP = Systemic hypoxia (11% oxygen, 6 treatments over 2 wks)
“Long-Term Tolerance” Against Stroke

Stowe et al., Ann Neurol. 2011
“Long-Term Tolerance” Against Retinal Ischemia

Zhu et al., IOVS. 2007
Prolonged Retinal Ischemic Tolerance Following Repetitive Hypoxic Preconditioning (RHP)

- Single Hypoxic Preconditioning (HP x 1)
- Repetitive Hypoxic Preconditioning over 2 wks (HP x 6)
Prolonged Retinal Ischemic Tolerance Following Repetitive Hypoxic Preconditioning (RHP)

Baseline

Post-Ischemia

Wave Amplitude (μV)

Stimulus Intensity (log cd.s/m²)

Ischemia

RHP + Ischemia (4 wks later)

Zhu et al., IOVS, 2007
Intermittent Hypoxia for SCI
sustained functional protection

Lovett-Barr et al., *J Neurosci.*, 2013
Mechanisms?
Hypoxia-Inducible Factor-1α (HIF-1α) and the Prolyl Hydroxylases
Hypoxia-Induced HIF-1α Protein Expression

Severity- and duration-dependent regulation

Stroka et al., FASEB J, 2001
HIF-1α Drives Many Cell Survival Responses

Sharp & Bernaudin, *Nat Rev Neurosci.*, 2004
Retinal HIF-1α protein expression following hypoxic preconditioning (SHP vs. RHP)

Zhu et al., IOVS, 2007
Retinal HO-1 protein expression following hypoxic preconditioning (SHP vs. RHP)

Zhu et al., IOVS, 2007
Cerebral HIF-1 dose-response
single or repetitive hypoxia

Shao G et al., Neurosignals, 2005
Daily Hypoxia... ...for Good Health ????
Rerpetitive Pre-Conditioning for Neurodegenerative Disease?
Repetitive Pre- and Post-Conditioning for Neurodegenerative Disease?
CNS Disease

Acute and Chronic Disease

Stroke

Retinal Ischemia

Glaucoma
Glaucoma

Progressive retinal ganglion cell (RGC) death
Repetitive Preconditioning for Glaucoma

Experimental Design

- Chronic Elevation of Intraocular Pressure
  - 2 weeks Repetitive Hypoxic Preconditioning (RHP)
  - 3 or 10 weeks

- Surgical induction of glaucoma

- RGC cell survival and function

= Hypoxia (11% oxygen for 2 h)
Glaucoma

Retinal ganglion cell injury/protection

Morphology

- Cell bodies (in retina)
- Axons (in retina and optic nerve)
RGC Cell Body Survival

Glaucoma eye

Fellow eye

Untreated

RHP
RGC Axon Survival
Glaucoma

Retinal ganglion cell injury/protection

Morphology
- Cell bodies (in retina)
- Axons (in retina and optic nerve)

Function
- Visual acuity
- Visual performance
  visual evoked potentials (VEPs)
Visual Acuity

Optokinetic Response

Prusky et al., IOVS, 2004
Flash Visual Evoked Potentials

- P1
- N1
- P2

Scotopic, White, 0 dB Flicker 1.9 Hz
Cell Body Survival


3 weeks

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<thead>
<tr>
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<th>NT</th>
<th>RHP</th>
<th>Sham</th>
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<tbody>
<tr>
<td>Brn-3 Positive RGCs (per mm²)</td>
<td>650</td>
<td>700</td>
<td>600</td>
</tr>
<tr>
<td>21% loss</td>
<td></td>
<td>2%</td>
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<tr>
<td>91% protection</td>
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10 weeks

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<th>NT</th>
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<tbody>
<tr>
<td>Brn-3 Positive RGCs (per mm²)</td>
<td>600</td>
<td>700</td>
<td>600</td>
</tr>
<tr>
<td>30% loss</td>
<td></td>
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<tr>
<td>3% loss</td>
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<tr>
<td>87% protection</td>
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Axon Survival


35% loss

87% protection

5% loss
Repetitive Preconditioning

GLAUCOMA

months → years
Repetitive Postconditioning

GLAUCOMA

months    years
Intraocular Pressure

Control
20.4 ± 0.6 mmHg
19.2 ± 0.6 mmHg

RHP

Intraocular Pressure (mm Hg)

Time After Initial Episceral Vein Ligation (wks)

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* Control
* # RHP
Visual Performance

Flash VEP Amplitude (% of fellow eye)

F  0-N1  P1-N1
GL  RHP
Control

22-23% loss

62-73% protection

6-9% loss
Visual Acuity

![Graph showing visual acuity comparisons between control and post-treatment conditions with specific percentages indicated.]

- **Control:**
  - F: Visual Acuity 30% loss
  - GL: Visual Acuity 10% loss

- **HX-Post:**
  - F: Visual Acuity 100%
  - GL: Visual Acuity 67% protection

Legend:
- *: Significant difference
- #: Significant difference compared to control
Retinal Ganglion Cells

Cell Body Survival

16% loss

85% protection

2% loss
Retinal Ganglion Cells

Axon Survival

ON SMI32 Fluorescence Intensity (% of fellow eye)

- F Control
- GL Control
- F RHP
- GL RHP

19% loss
5% loss
74% protection
FROM STRESS TO SUCCESS
Natural Selection vs. Lamarckism

Taller neck

- Reaches more food than short neck

- Reproduces more than short neck

- Long necks more common in population

Cannot reach taller branches

- Neck lengthens

- Offspring born with longer neck
Inheritance of Acquired Characteristics

Jean-Baptiste Lamarck, 1809
Transgenerational Epigenetics

Experimental Design

- Breeding
- 4 months of Repetitive Hypoxic Preconditioning (RHP)
  + Normoxic Controls
- Gestation
- 3 wks
- Births
- Growth/Maturation
- 10 wks
- Retinal Ischemia
- ERGs
Transgenerational Epigenetics
children inherit injury resilience of parents

![Graph showing f-ERG wave amplitude comparison between control and RHP groups for a-wave and b-wave.]

- a-wave:
  - Control: 21% loss
  - RHP: 61% protection
- b-wave:
  - Control: 12% loss
  - RHP: 78% protection

**Note:**
- * indicates significant difference compared to control.
- # indicates significant difference compared to RHP.

*Graphs depict f-ERG wave amplitude (% of fellow eye) with error bars representing standard deviation.*
WHY YOUR DNA ISN’T YOUR DESTINY

The new science of epigenetics reveals how the choices you make can change your genes—and those of your kids

BY JOHN CLOUD
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