Exercise is Beneficial in Models of Retinal Disease

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Conflicts statement

I have no commercial interests in the subject matter.
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Bummer.
Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.
When photoreceptors or retinal pigment epithelial (RPE) cells die,

- Age-related macular degeneration (AMD)
- Retinitis pigmentosa (RP)
- Other retinal dystrophies
Bruce, Keith, and Joe had just published a spinning training protocol that protected cognition in aged veterans...
Machelle’s idea:

Exercise will protect against retinal degeneration…

…just like it is beneficial to cognitive function as shown by our CVNR compadres…

Bruce Crosson, PhD  Keith McGregor, PhD  Joe Nocera, PhD
Background

• In rodents, aerobic exercise stimulates neurogenesis, and improves memory and learning.
  van Praag et al., 1999; Anderson et al., 2000; Wood et al., 2012

• Longer running distance is associated with better visual outcomes in human long-distance runners with ongoing age-related macular degeneration
  Williams, 2009

• Environmental enrichment, which includes access to voluntary running wheel, slows retinal function loss in the rd10 mice of retinitis pigmentosa
  Barone et al., 2012
Light-induced retinal degeneration (LIRD) mouse model

- Exposure of rodents to bright light is a classic model of induced photoreceptor degeneration and retinal function loss.
- “Over-drive visual transduction; produce lots of reactive oxygen species;”
- Light damage is accompanied by oxidative stress, apoptosis, and inflammation in photoreceptor cells and retinal pigment epithelial cells.
- Retinal cell death in LIRD somewhat models that seen in Age-Related Macular Degeneration (AMD).
- Several protocols with varying intensities and durations of light exposure.
ACME Mark I Lighterizer
High tech, temperature-controlled light damage chamber

No expense was spared!
Electroretinogram (ERG)
Measuring retina function in rats and mice (and humans!)

b-wave (bipolar cells)

a-wave (photoreceptors)
Mouse retina function
ERG waveforms

Photoreceptor response (a-wave)
Mouse retina function
ERG waveforms

- **Photoreceptor response** (a-wave amplitude)
- **Inner retina neuron response** (b-wave amplitude)
Example of how ERG data are presented
(Retinal function in \textit{rd}10 mice after TUDCA treatments at P30)
How will we exercise the mice?
Rodent treadmill
LIRD Treadmill Exercise

Start

Week 1

Begin Treadmill Running

Week 2

Light Exposure:
4 hours at bright (10,000 lux) or dim (50 lux) light

Week 3

ERG: 5-step scotopic

Week 4

ERG: 5-step scotopic. Sacrificed following ERG

Treadmill Exercise: 10 m/min; 60 minutes; 5 days/week*

Inactive Treadmill: Static treadmill

- Dim + Treadmill
- Dim + Inactive

- Bright + Treadmill
- Bright + Inactive

*Wood et al., 2012
LIRD Treadmill Exercise: Functional Preservation

![Graph showing a-wave and b-wave Amplitudes over time for different conditions.]
LIRD Treadmill Exercise: Structural Preservation

**Graph:***
- **# Photoreceptor Nuclei/2 mm**
- **Distance from ONH (mm)**
- **Treatment**
  - Bright + Inactive (n=3)
  - Bright + Treadmill (n=4)
- **p=0.029**

**Legend:**
- OS (Outer Segments)
- ONL (Outer Nuclear Layer)
- INL (Inner Nuclear Layer)
- RGC (Retinal Ganglion Cells)

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*Bright + Inactive (n=3)*
*Bright + Treadmill (n=4)***

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*0*
• Treadmill running protects against light-induced retinal degeneration
• Treadmill running protects against light-induced retinal degeneration
• How?
Brain-Derived Neurotrophic Factor (BDNF)

• Systemic and local brain BDNF levels increase following exercise
  Ploughman et al., 2007; Griffin et al., 2011

• BDNF TrkB signaling pathway is necessary for beneficial effects of exercise
  Real et al., 2013

• Hypotheses:
  – Aerobic exercise will increase BDNF protein levels in the retina.
  – BDNF TrkB signaling pathway must be intact for aerobic exercise to be protective in LIRD.
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Brain-Derived Neurotrophic Factor (BDNF)

- Naïve BALB/c mice exercised for 9 consecutive days at 10 meter/minute for 60 minutes/day
  - Mice sacrificed on 9\textsuperscript{th} day immediately following exercise
  - Brain, retina and serum collected for protein quantification
  - BDNF protein quantified with BDNF ELISA kit (Promega)
BDNF Protein Levels

**Retina**
- Inactive (n=6): p=0.024
- Treadmill (n=6): *

**Hippocampus**
- Inactive (n=6): p=0.004
- Treadmill (n=6): *

**Serum**
- Inactive (n=6): * p=0.003
- Treadmill (n=6): *
BDNF Protein Levels

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BDNF TrkB receptor antagonist:  

Start  
Begin Treadmill Exercise and injections  

Week 1  
Treadmill Exercise: 10 m/min; 60 minutes; 5 days/week  
Inactive Treadmill: Static treadmill  
I.P. Injections: ANA-12 or Vehicle; 2.5 hours prior to exercise*  

Week 2  
Light Exposure: 4 hours at bright (10,000 lux) or dim (50 lux)  

Week 3  
ERG: 5-step scotopic. Sacrificed following ERG  

*Cazorla et al., 2011
BDNF TrkB receptor antagonist: Functional results

*a-wave*

*b-wave*

*p=0.002*

#p=0.009
BDNF TrkB receptor antagonist: Structural Results
• Treadmill running protects against light-induced retinal degeneration
• How? Apparently by increasing BDNF TrkB receptor activation
Wheel running

- Voluntary; less stressful
- Allows exercising of young pups whose degeneration starts too early to treadmill train (e.g., genetic models like rd10)
- Negative control is simply a locked wheel that mouse can explore and play on, but it does not spin so doesn’t allow running
- Some adult mice run over 20 km per night!

1. Locked or unlocked wheels placed in cage with one mouse
2. Two weeks later, mice exposed to toxic light to induce LIRD
3. One week later, ERGs taken
Effect of wheel running on LIRD mouse retinal function (ERG)
Will exercise protect in a genetic disease model?
**rd10 mouse model of retinal degeneration**

- Missense point mutation in exon 13 in β subunit of rod cGMP PDE gene
- Considered a model of autosomal recessive retinitis pigmentosa
- Postnatal day (P) 16: photoreceptor degeneration onset (significant by P18)
- P30: Less than one cell layer in the outer nuclear layer (ONL)
- P50: No rods, sparse-to-no cones
Wheel running of rd10 pups

1. Wheels placed in cages prior to birth (moms run, too), locked (control) or spinning.
2. Weanlings individually housed with wheel in same state as with mom.
3. Visual acuity assessed at various ages.
Effect of wheel running on rd10 mouse retinal function (ERG)

Scotopic b-waves for p27 rd10 Running Wheel Experiment

Photopic b-waves for p27 rd10 Running Wheel Experiment
Visual Acuity
Mouse tracks the movement of the lines with a reflex movement of its head. The fundamental measure is “yes/no” – does the mouse’s head move? Width of lines varied to assess visual acuity. Darkness of lines varied to assess contrast sensitivity.
Wheel running delays loss of acuity in rd10 mice
Running wheel exercise preserves retinal morphology in *rd10* mice
ANA-12 treatment (i.e., blocking TrkB receptors) prevents protective effect of running wheel exercise in *rd10* mice
Conclusions

Treadmill training and wheel running:

• Protect against function and structure loss in LIRD and rd10 models

• Protection is mediated by and requires BDNF TrkB activation
Discussion

Will exercise be prescribed specifically as a retina treatment?

- Recall, some people scoffed at “prescribing vitamins and vegetables” prior to AREDS!
- But everyone knows to exercise – and we never do!
- THE most compliant patient population is the RD population.
- They “see” their loss everyday.
Discussion

Will we identify new mechanisms of health and disease? New therapeutic targets?
• Is this a way to provide BDNF-mediated retinal neuroprotection?
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• Is muscle acting as an endocrine organ – sending out myokines?
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• Is this a way to provide BDNF-mediated retinal neuroprotection?
• Is muscle acting as an endocrine organ – sending out myokines?
• Is this protective preconditioning (Local hypoxia? Remote ischemia?)
Discussion

Will we identify new mechanisms of health and disease? New therapeutic targets?

- Is this a way to provide BDNF-mediated retinal neuroprotection?
- Is muscle acting as an endocrine organ – sending out myokines?
- Is this protective preconditioning (local hypoxia or energy stress?)

Or have we simply discovered something weird about mice?
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• Wheel running protects against the retinal degeneration of the rd10 mouse model of retinitis pigmentosa

• Effect mediated by BDNF TrkB activation
Daily elevated lighting preconditions against LIRD: Retinal function (ERG)

N.B.: Effect is similar to retinal ischemic preconditioning. Light or ischemic preconditioning protect against LIRD or retinal ischemia.
Light preconditioning and treadmill exercise have similar effects on LIF expression.

[Graphs showing relative quantification of LIF for different conditions]
Treadmill training increases LIF and Hmox1 expression
P18 rd10 photoreceptors are apoptotic

Wildtype  
**rd10**

TUNEL

Anti-active Caspase-3
Will exercise protect in a genetic disease model?

- rd10 mouse degeneration starts at P16
- Pups don’t want to run on a treadmill when they’re that young
Effect of N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC; a BDNF TrkB agonist) on LIRD mouse
Effect of N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC; a BDNF TrkB agonist) on LIRD mouse
What does all of this mean for us today, here in beautiful Amherst?

Enjoy a sunny day at the big rock...
Of course, wear proper eye protection…
Of course, wear proper eye protection…
... and it’s OK to drink a beer or three...
...as long as it is brewed with lots of hops and other proper extracts...
...and as long as you’re willing to exercise away the side effects...
Rodent treadmill