Dose-Response Effects of Low-Level Light Therapy on Brain and Muscle

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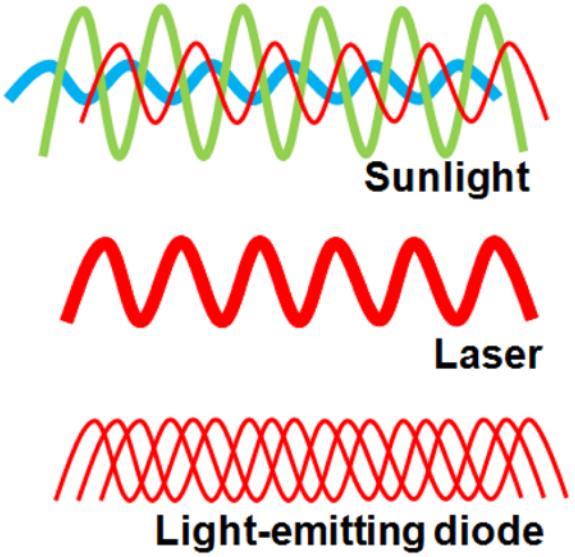
- Douglas Barrett, PhD, who coauthored the human experiments

Presentation Outline

1. Introduction to LLLT mechanisms
2. Oxygen consumption effects
3. Cytochrome oxidase effects
4. Superoxide dismutase effects
5. **Brain and muscle dose-responses**
6. Animal cognitive effects
7. **Human cognitive effects**
8. Conclusions

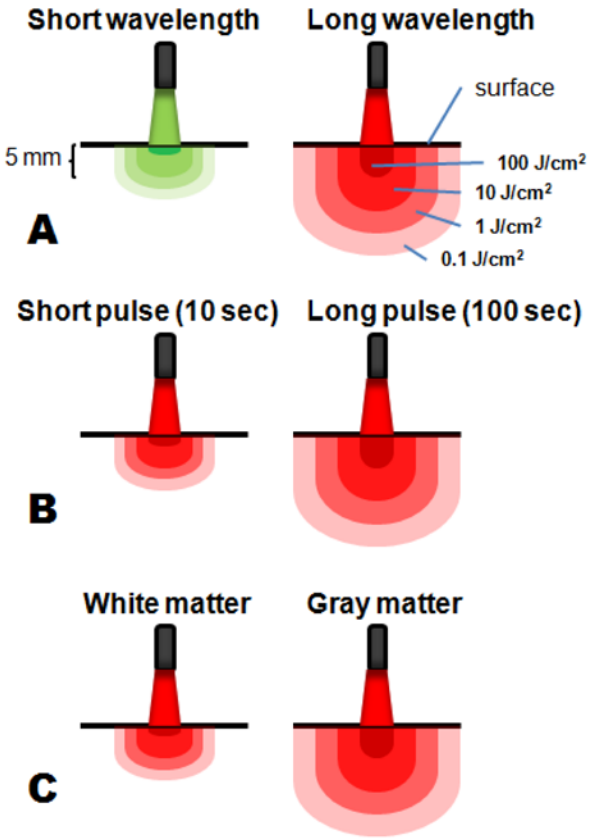
Properties of lasers and LEDs

- Sunlight has electromagnetic waves with different wavelengths
- Lasers emit waves of a single wavelength (monochromatic) that have spatial and temporal synchronization (coherence)
- Light emitting diodes (LEDs) produce light in a narrow wavelength range (quasimonochromatic)



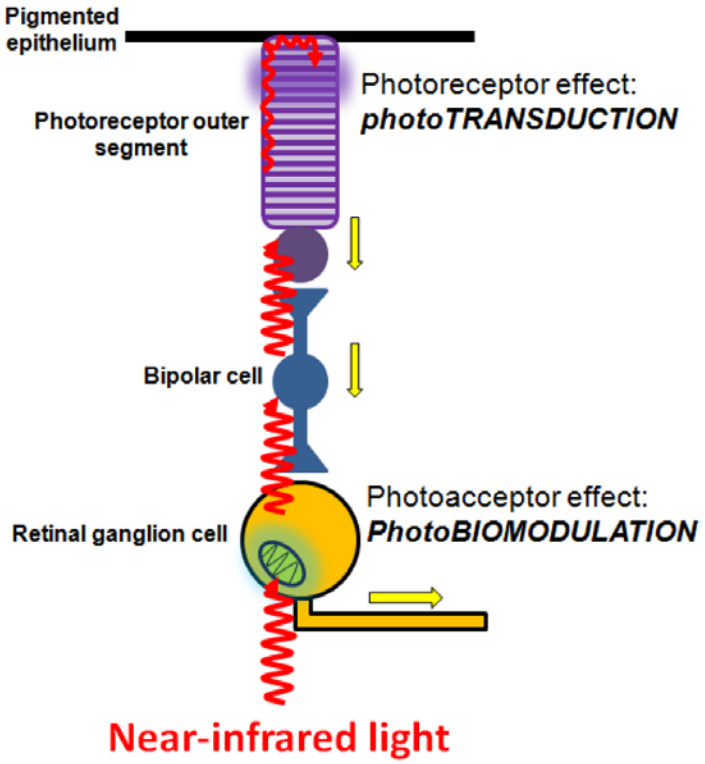
Principles of light-tissue interactions

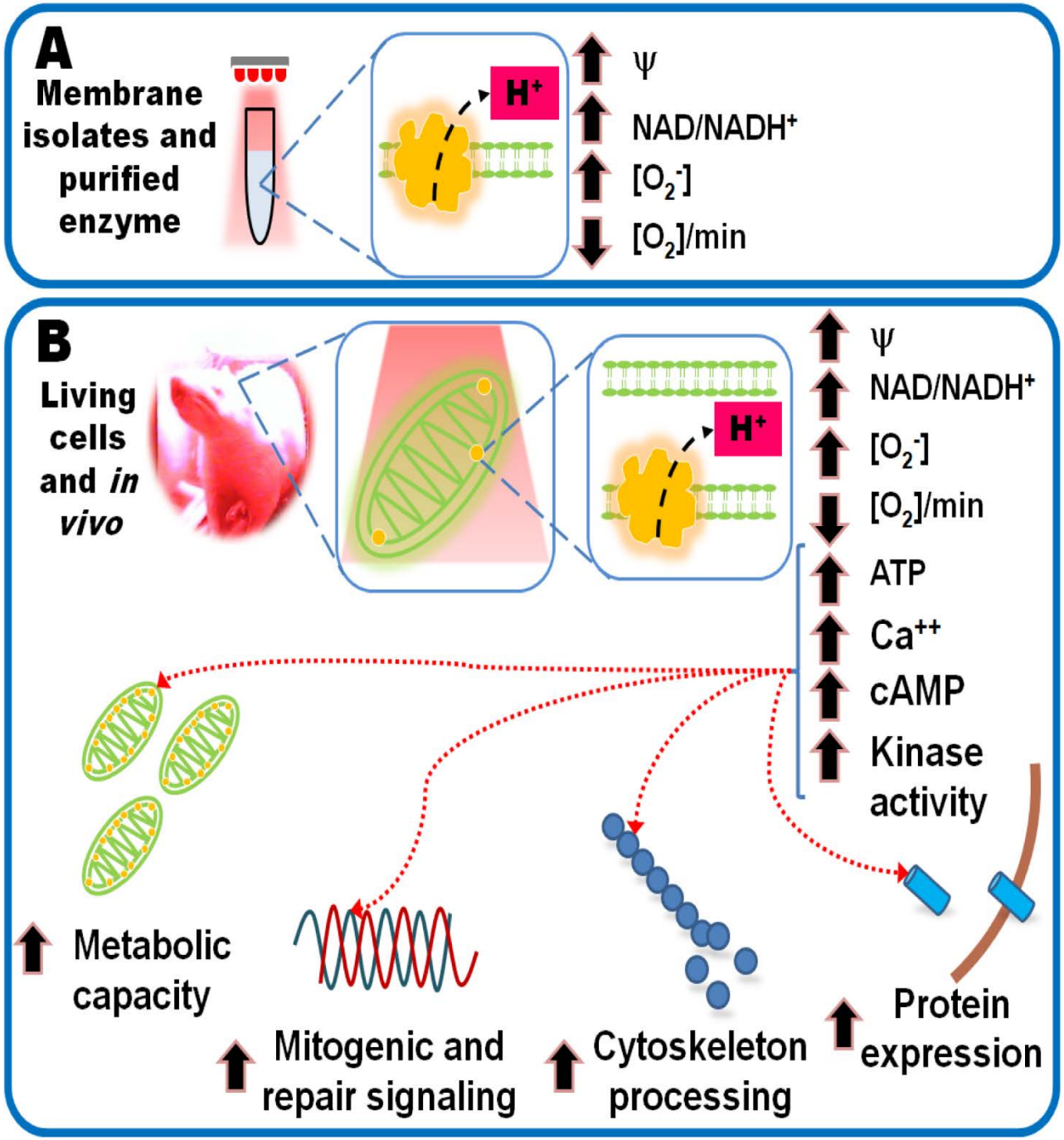
- Light at short wavelengths has low tissue penetration, but at high wavelengths displays high tissue penetration
- Tissues feature a relaxation time, which is the time needed to diffuse 50% of the absorbed energy
- Tissues vary in their transmittance and relaxation time

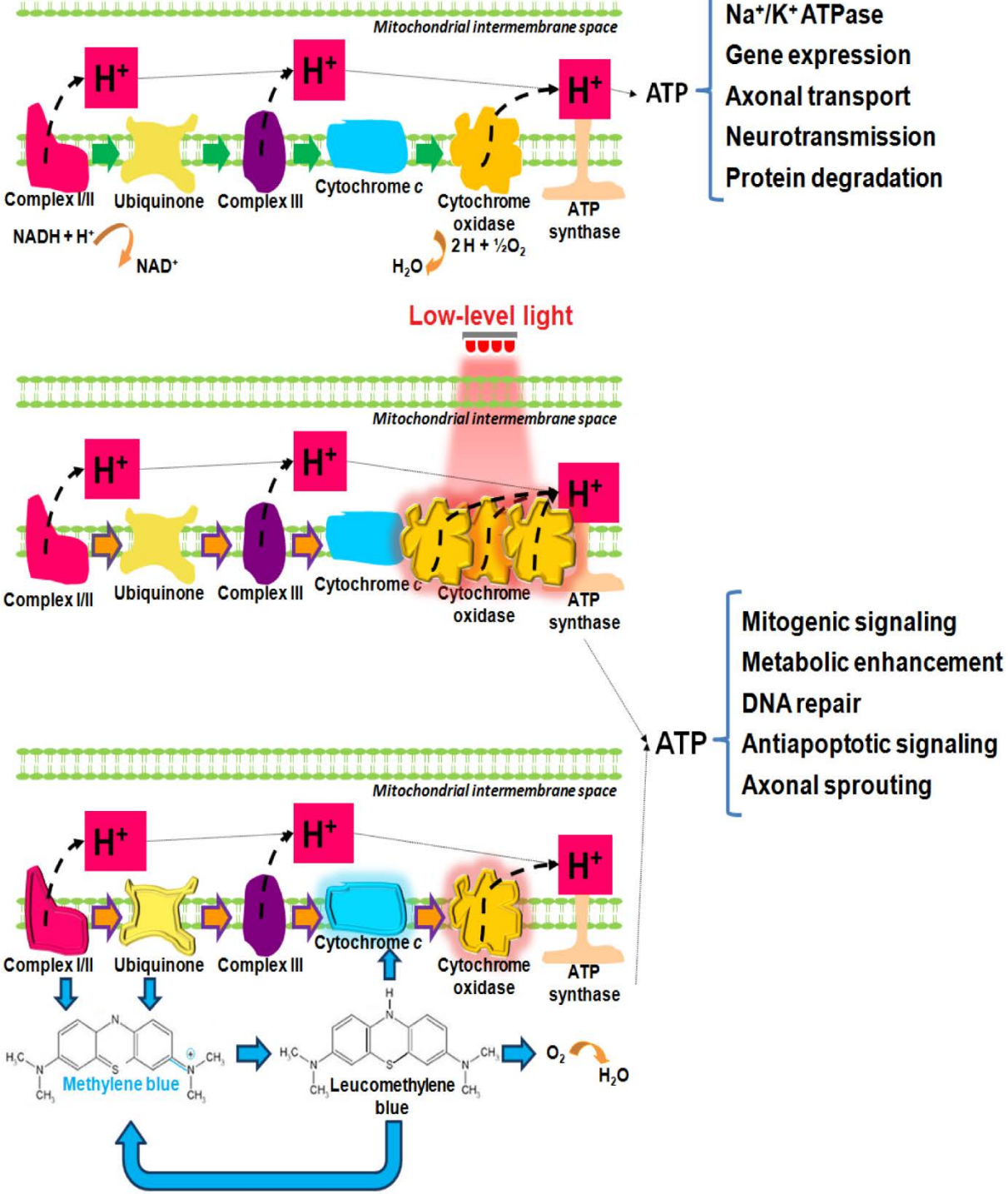


Different effects of light on photoreceptors and photoacceptors

- Light can excite *photoreceptors* in the retina and pineal gland
- Light can also directly excite *photoacceptors* in neurons
- The main photoacceptor in **red-to-near-infrared** spectrum (620-1150 nm) is the mitochondrial respiratory enzyme *cytochrome oxidase*

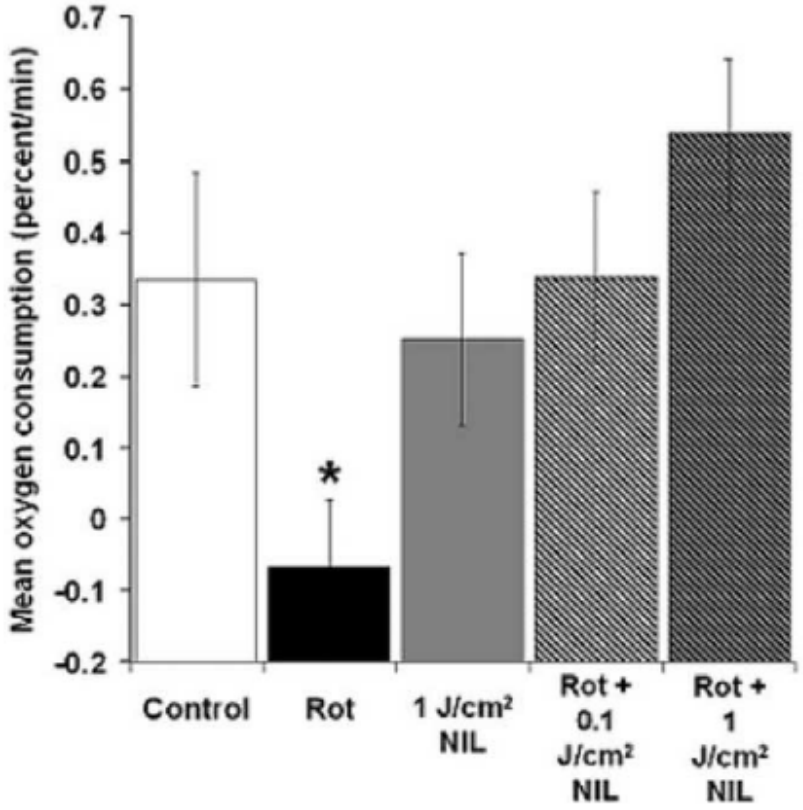



A
Membrane isolates and purified enzyme
H+
ψ
NAD/NADH+
[O2-]
[O2]/min
B
Living cells and in vivo
H+
ψ
NAD/NADH+
[O2-]
[O2]/min
ATP
Ca++
cAMP
Kinase activity
Metabolic capacity
Mitogenic and repair signaling
Cytoskeleton processing
Protein expression


Mitochondrial intermembrane space
H+
H+
H+
Complex I/II
Ubiquinone
Complex III
Cytochrome c
Cytochrome oxidase
ATP synthase
NADH + H+
NAD+
H2O
2 H + ½O2
ATP
Na+/K+ ATPase
Gene expression
Axonal transport
Neurotransmission
Protein degradation
Low-level light
Mitochondrial intermembrane space
Complex I/II
Ubiquinone
Complex III
Cytochrome c
Cytochrome oxidase
ATP synthase
ATP
Mitogenic signaling
Metabolic enhancement
DNA repair
Antiapoptotic signaling
Axonal sprouting
Mitochondrial intermembrane space
Complex I/II
Ubiquinone
Complex III
Cytochrome c
Cytochrome oxidase
ATP synthase
Methylene blue
Leucomethylene blue
O2
H2O

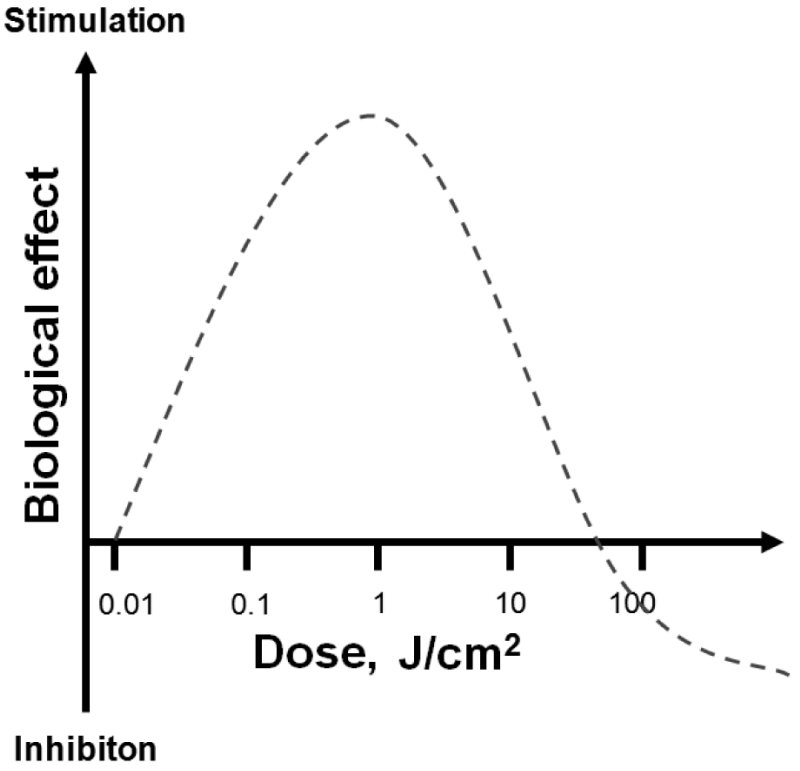
LLLT rescues inhibited brain oxygen consumption *in vitro*

In whole-brain homogenates, rotenone (Rot, 10 M) decreased the rate of oxygen consumption by 75% (*p <0.05). But 0.1 J/cm2 and 1 J/cm2 doses of NIL (633 nm LEDs, 2 mW/cm2) reversed the inhibitory effect of rotenone.



Hormesis of low-level light (LLLT)

- Hormetic dose-response (inverted U-shaped, biphasic or bell-shaped) by stimulation of bioenergetics at a low dose and inhibition at a high dose
- Photostimulatory or photoinhibitory *in vitro* effects are obtained with low (0.01 – 10 J/cm2) and high (> 10 J/cm2) energy densities, respectively
- Transcranial transmission, 5.8% rats (20x), 1.8% humans (60x)

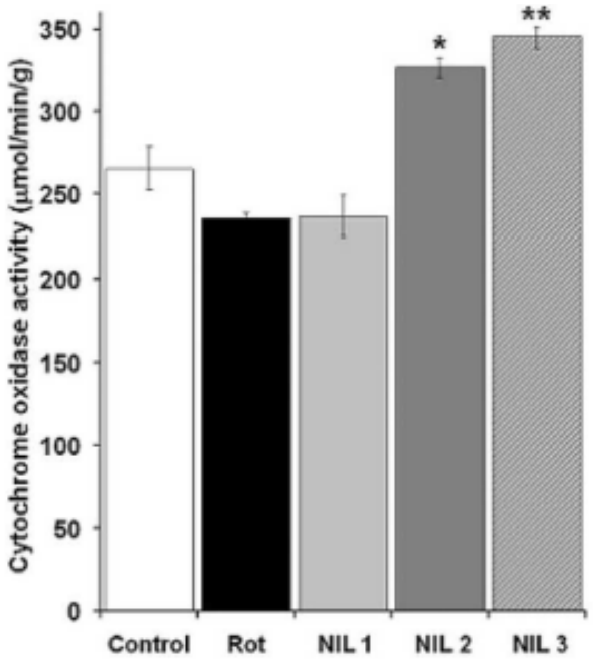


Transcranial effects on brain cytochrome oxidase (NIL: 633 nm, 2 mW/cm2, 30 min)

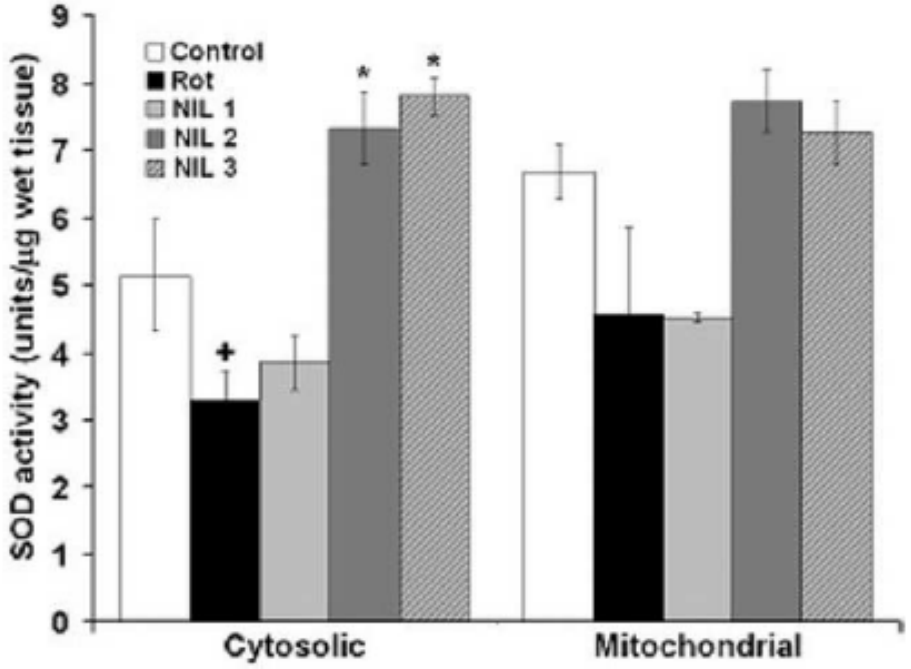
Table 2. Near-infrared doses and schedules

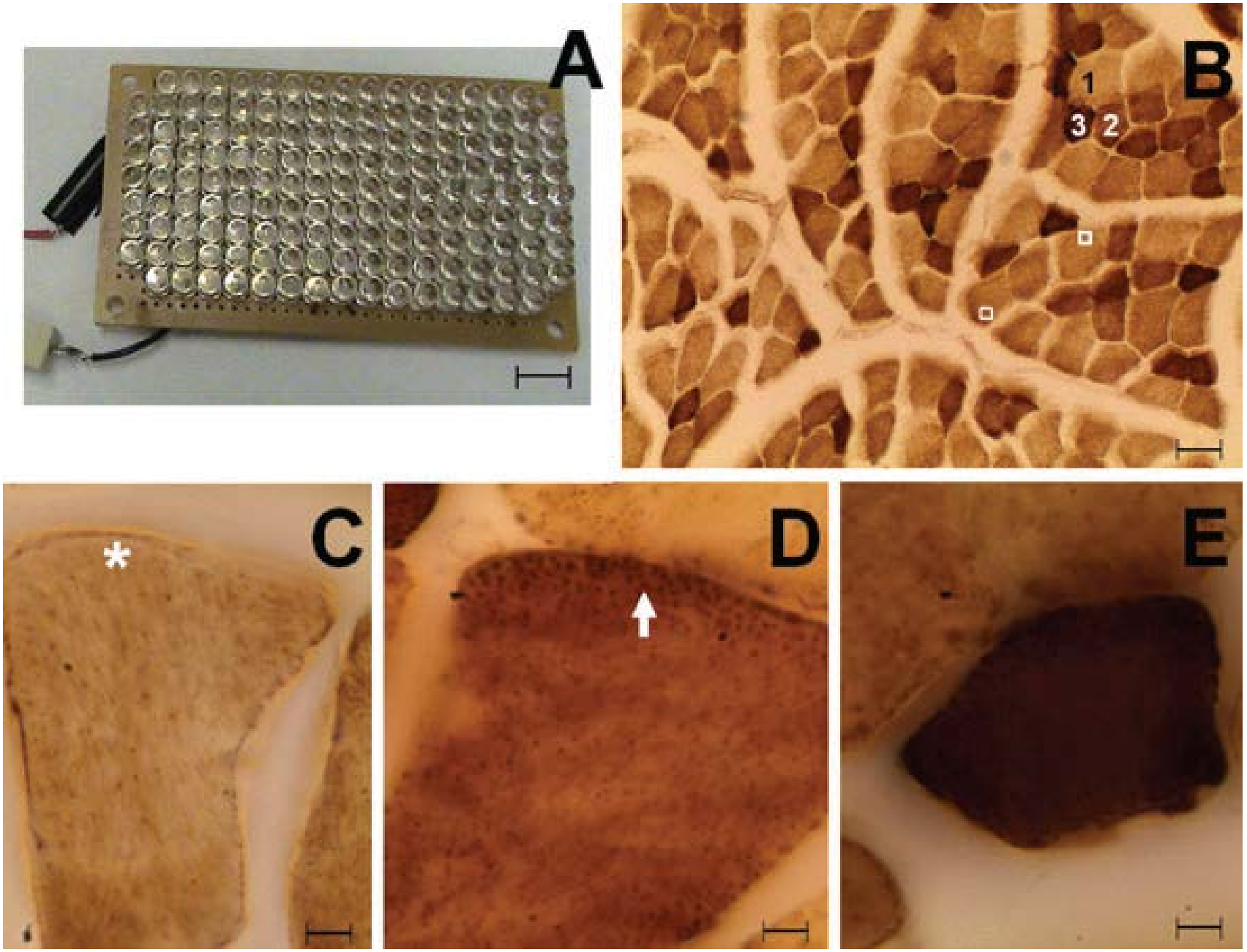
Protocol	Day								Dose (J/cm^2)	
	19	20	21[a]	22	23	24	25	26	Per day	Total
NIL 1			●	●	●				3.6	10.8
NIL 2			●	●	●	●	●	●	3.6	21.6
NIL 3	●	●	●	●	●	●			3.6	21.6

[a]Intravitreal injections of rotenone were done on this day.



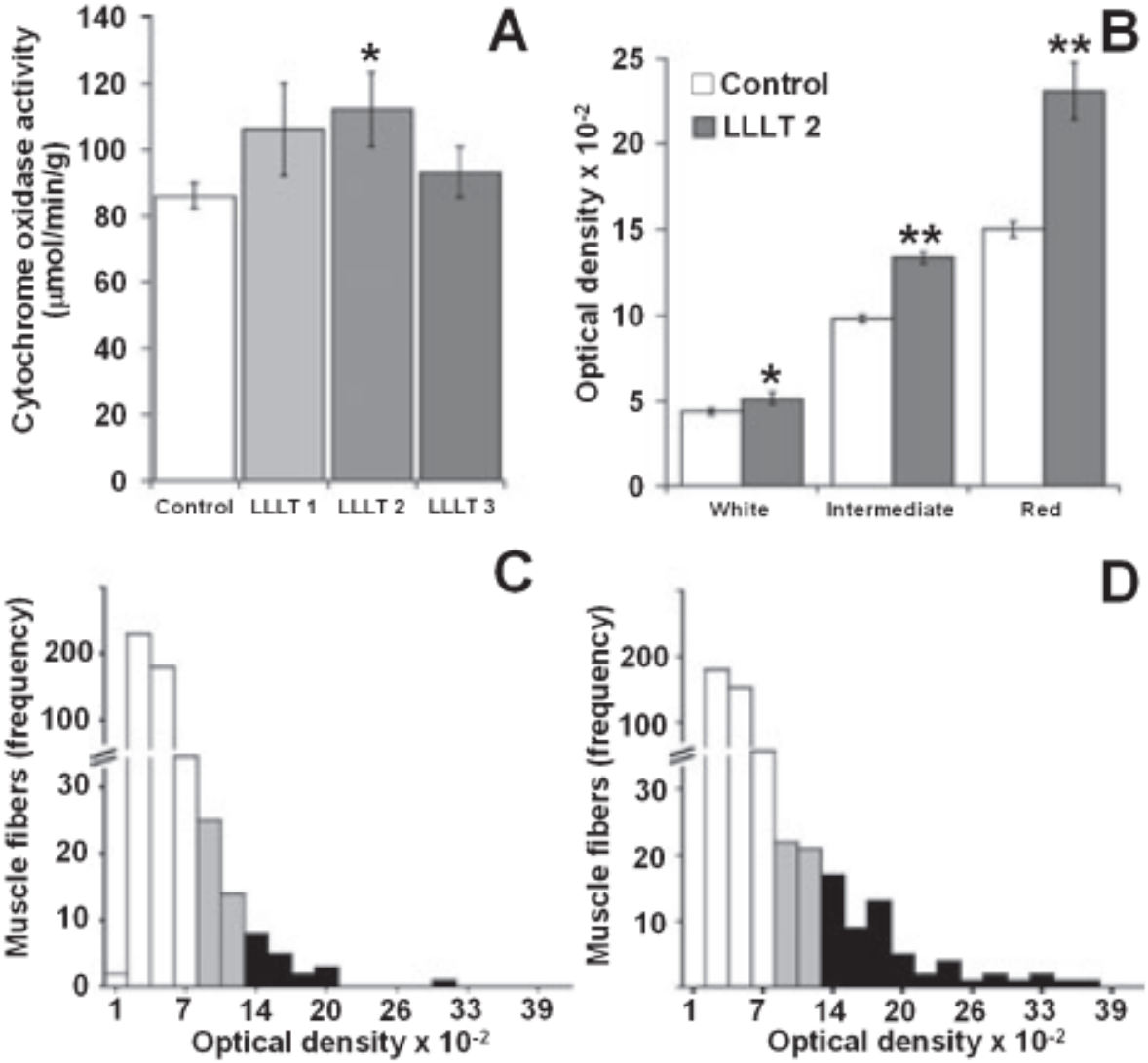
Transcranial effects on brain superoxide dismutase activity



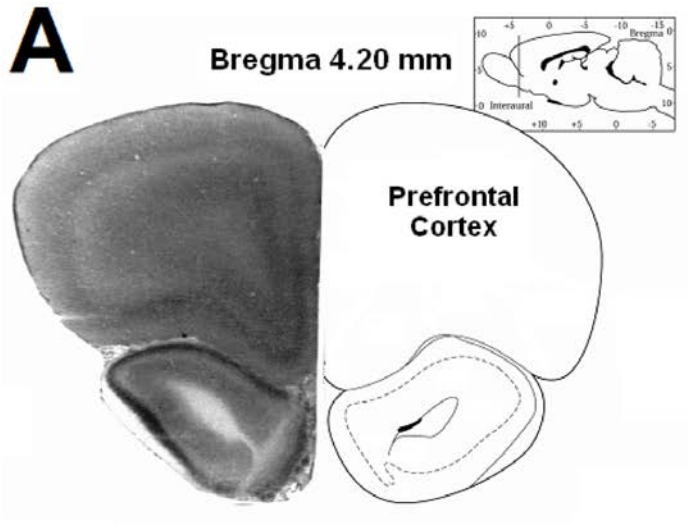

A
B
1
3
2
C
*
D
E

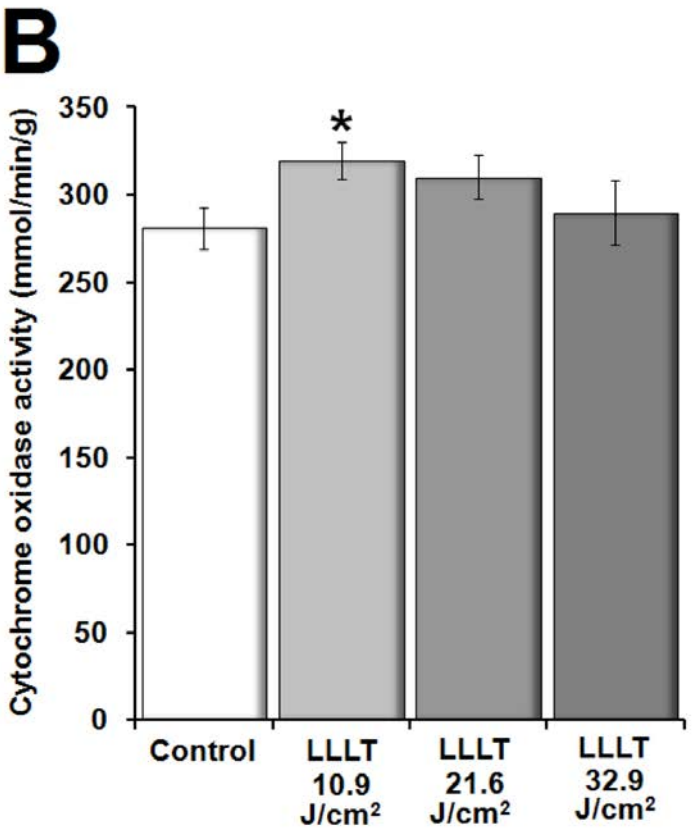
LLLT effects on skeletal muscle

(660 nm LEDs, 9 mW/cm2; LLLT 1=10.8, LLL2=21.6, LLL 3=32.4 J/cm2)



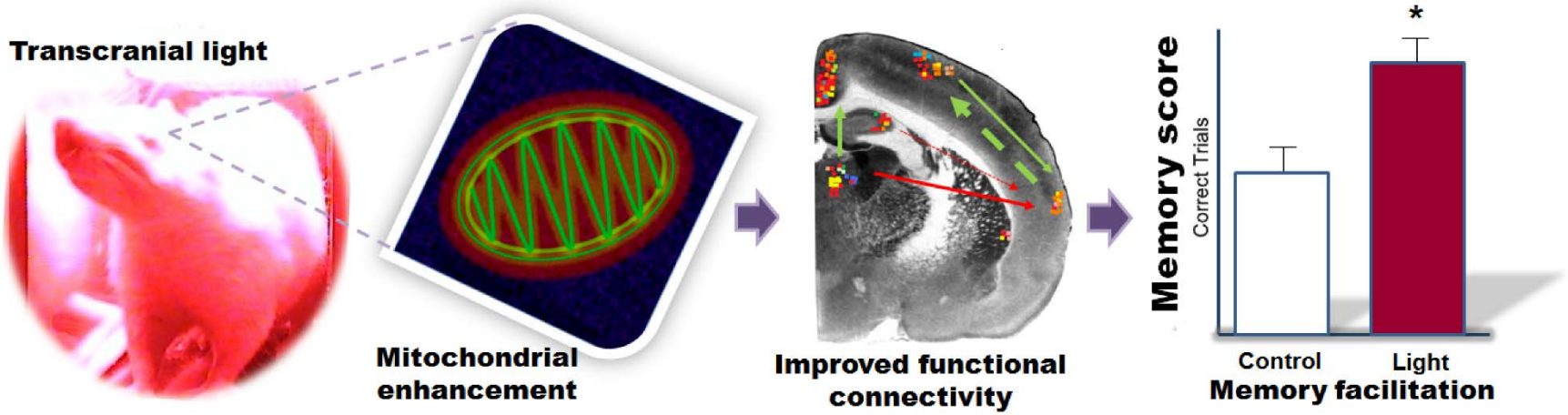
- 660 nm, 9 mW/cm2





Transcranial memory facilitation in rats

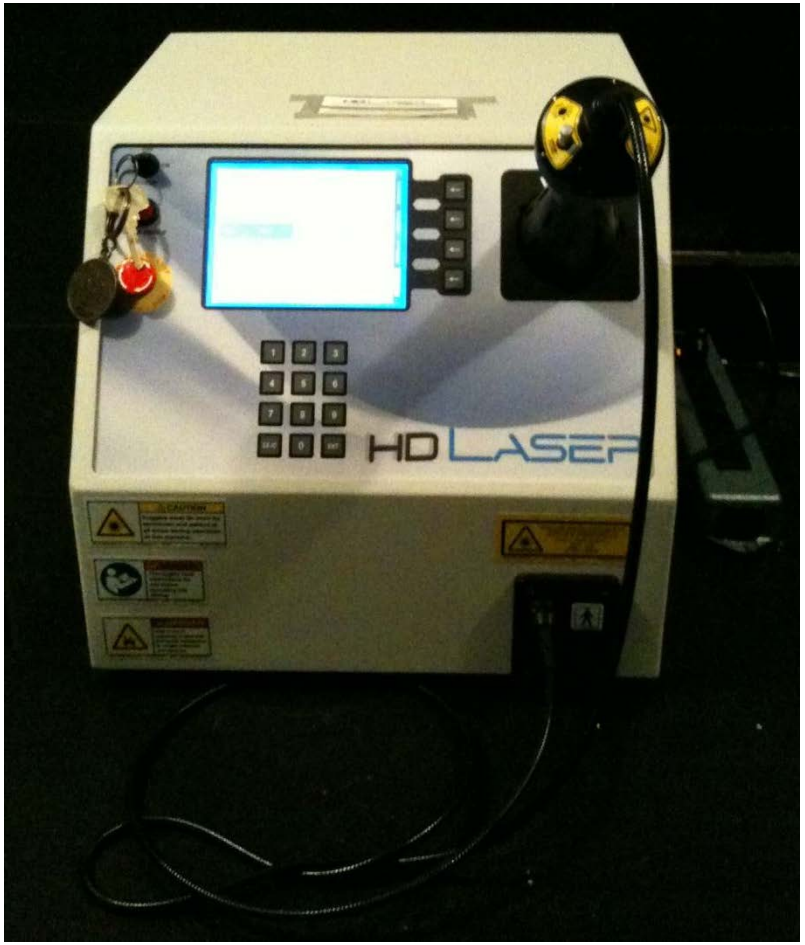
- Summary

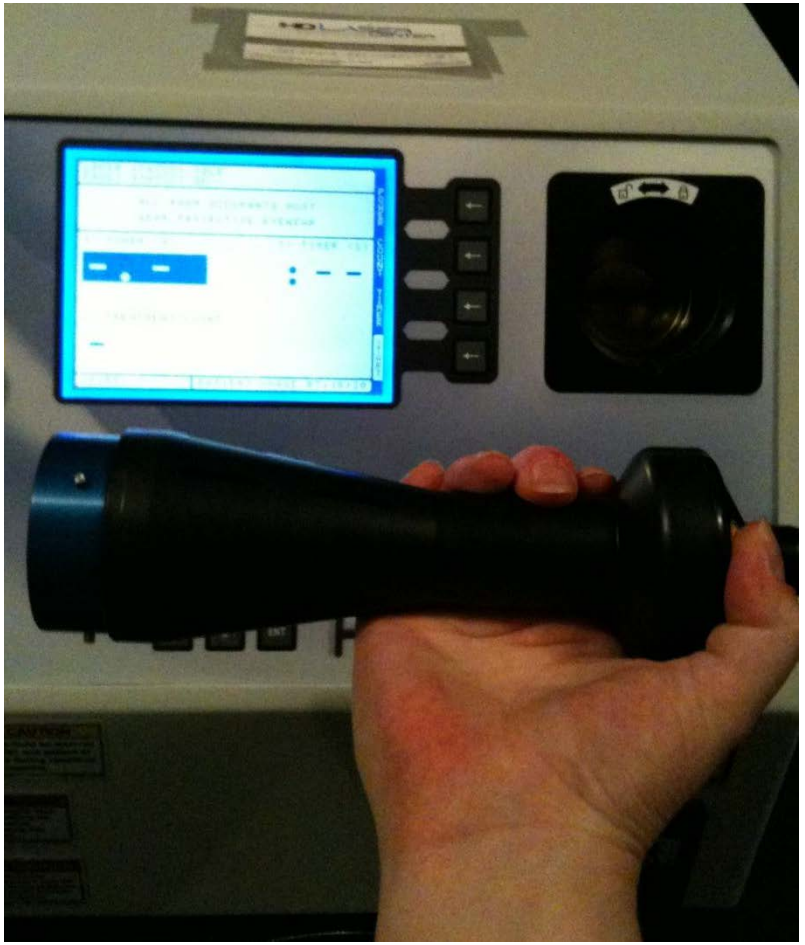


Transcranial photobiomodulation

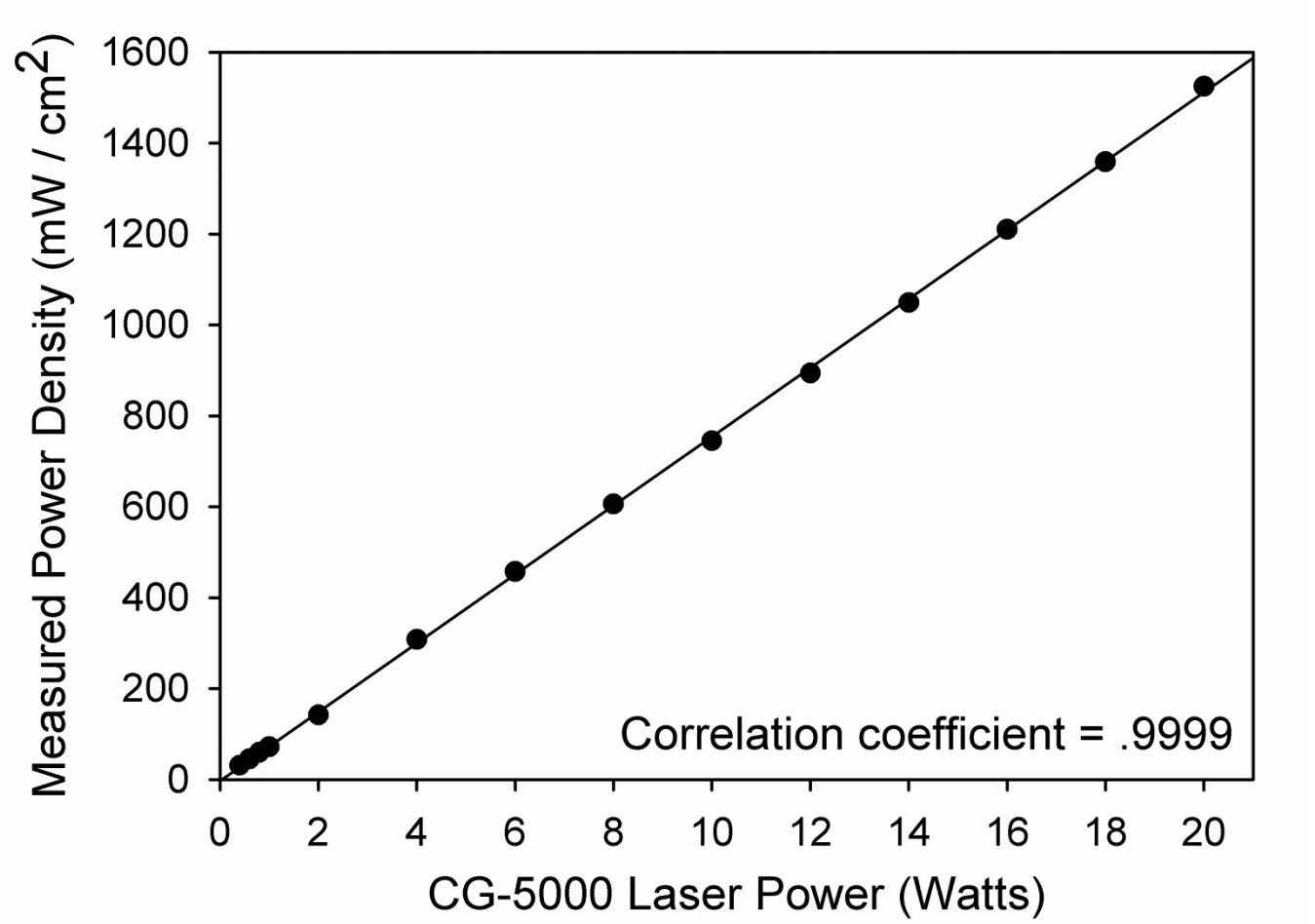
- Photobiomodulation with near-infrared light, also called low-level light therapy (LLLT), has been shown in both animals and humans. For example:
- In 2007, Lampl et al reported that infrared laser therapy to the head improved neurological outcome in controlled clinical trials of stroke
- In 2008, Rojas et al were the first to report that upon transcranial delivery *in vivo*, LLLT induces brain metabolic and antioxidant beneficial effects, as measured by increases in cytochrome oxidase and superoxide dismutase
- In 2009, Schiffer et al reported that LED treatment to the forehead may alleviate depression in an uncontrolled pilot study of 10 patients
- In 2011, Rojas and Gonzalez-Lima proposed LLLT as a novel paradigm to treat visual, neurological, and psychological conditions based on the stimulation of cytochrome oxidase activity in neurons
- In 2012, Rojas et al were the first to report that LLLT increased extinction memory retention and oxygen consumption in the rat prefrontal cortex *in vivo*
- In 2013, Barrett and Gonzalez-Lima reported the first controlled study of transcranial laser stimulation of psychological functions in humans

CG-5000 Class IV Laser 1064 nm, FDA-cleared for pain relief in humans





Laser Calibration Curve



Transcranial laser brain stimulation

- Light = type of electromagnetic radiation made of photons able to transfer energy (luminous energy)
- Energy (Joules) = Power (Watts) x Time (seconds)
- CG-5000 laser wavelength **1064 nm**
- Power **3.4 Watts**
- Irradiance (“intensity”) **250 mW/cm2**
- Exposure time 240 sec (**4 min**) per site
- Applied radiant exposure or fluence (“dose”) **60 J/cm2** (250 mW/cm2 x 240 sec divided by 1000 mW/W)
- 2% of 1064 nm laser light passed through frontal bone, so **1.2 J/cm2** reaches cortical surface

Human study design

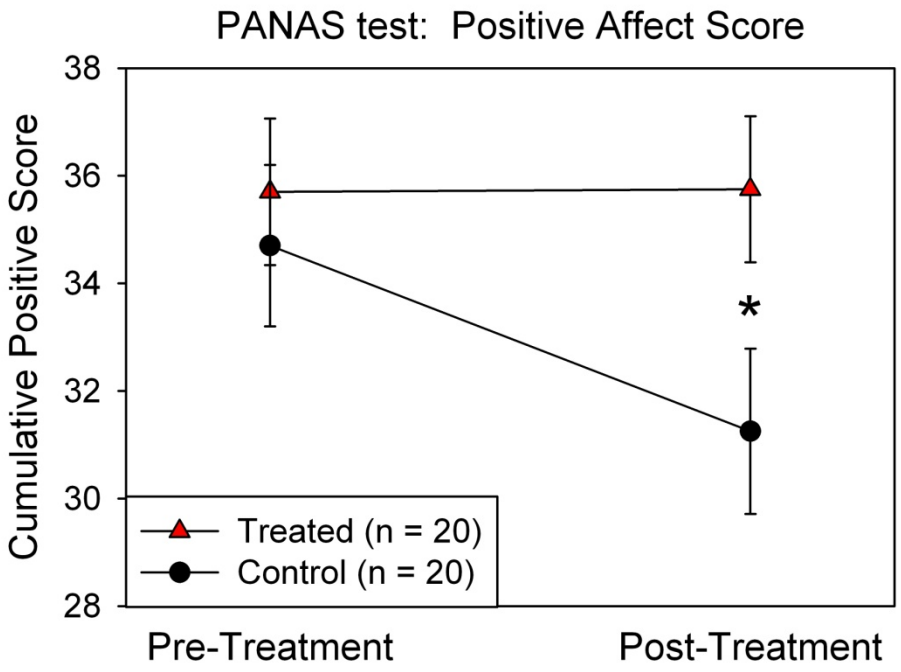
- **Hypothesis**: We tested whether low-level laser stimulation produces beneficial effects on frontal cortex measures of attention, memory and mood.
- **Groups**: Randomized, placebo-controlled blind trials of LLLT treated (n = 20) vs. active placebo control (n = 20) groups (10 males and 10 females per group, healthy UT students ages 18-35)
- **Analysis**: ANOVA using pre-post treatment measures as the within-subject variable, group assignment (treated vs. control) and sex (male vs. female) as independent variables
- **Tests**: transcranial LLLT protocol to right forehead, targeting frontal cortex-based cognitive tasks such as a **psychomotor vigilance task (PVT)** and a **delayed match-to-sample memory task (DMS)** immediately after LLLT, and also assessed emotional states before and two weeks after LLLT using the **Positive and Negative Affect Schedule (PANAS**, version X)

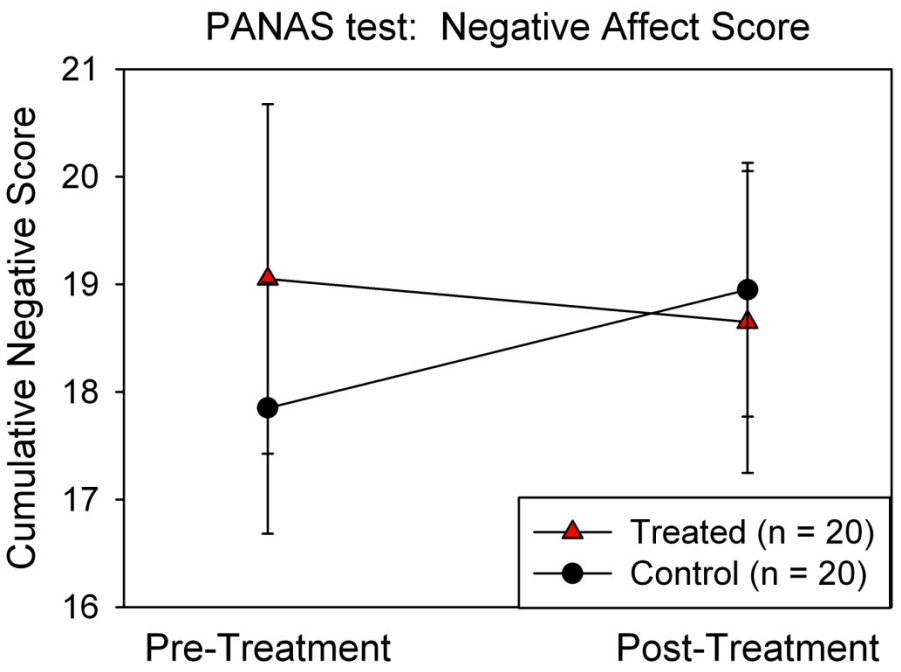
Experimental protocol

1. Verification of screening criteria.
2. Subject information collected.
3. Signing of informed consent form.
4. PANAS (pre-test).
5. TPQ (tri-dimensional personality questionnaire).
6. SSS (sensation-seeking scale, form V).
7. Medical history questionnaire.
8. One-minute practice of PVT.
9. Block 1 of PVT (pre-test).
10. One-minute practice of DMS.
11. Block 1 of DMS (pre-test).
12. LLLT or active placebo.
13. Block 2 of PVT (post-test).
14. Block 2 of DMS (post-test).
15. [Two weeks later] PANAS (post-test).

Positive and Negative Affect

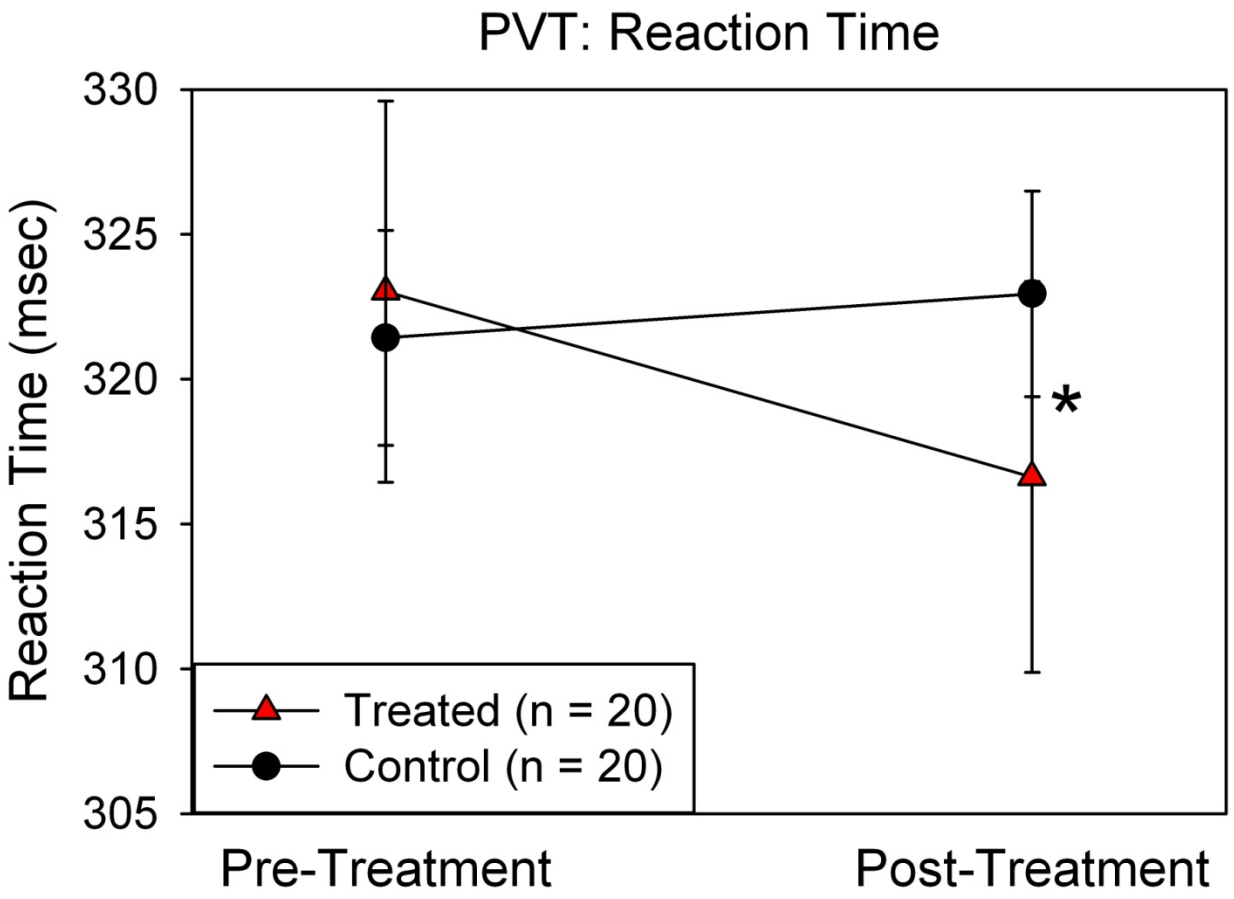
Laser effects led to two weeks of sustained positive emotional states





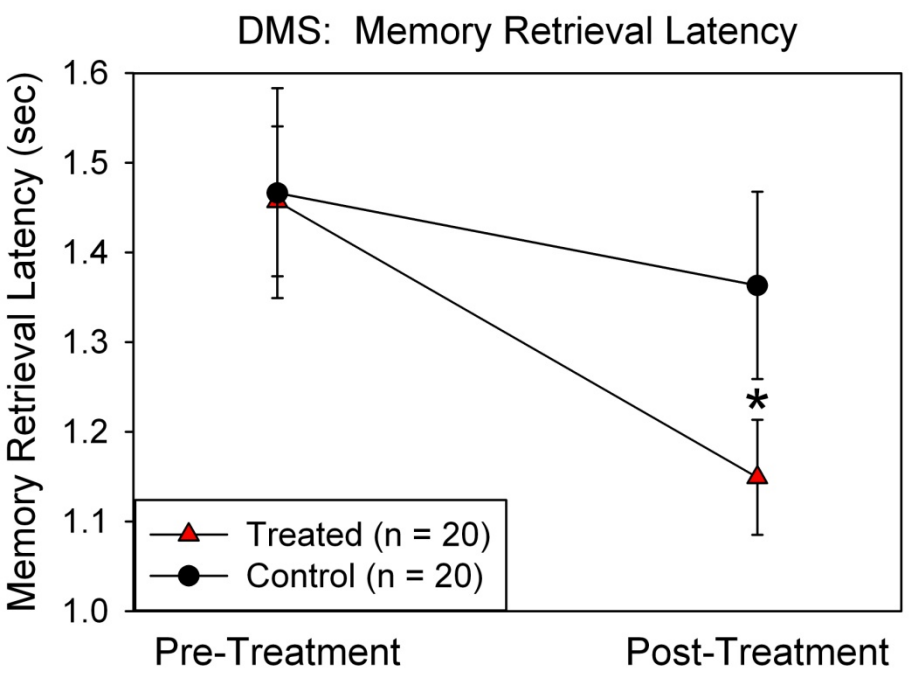
Psychomotor Vigilance Task

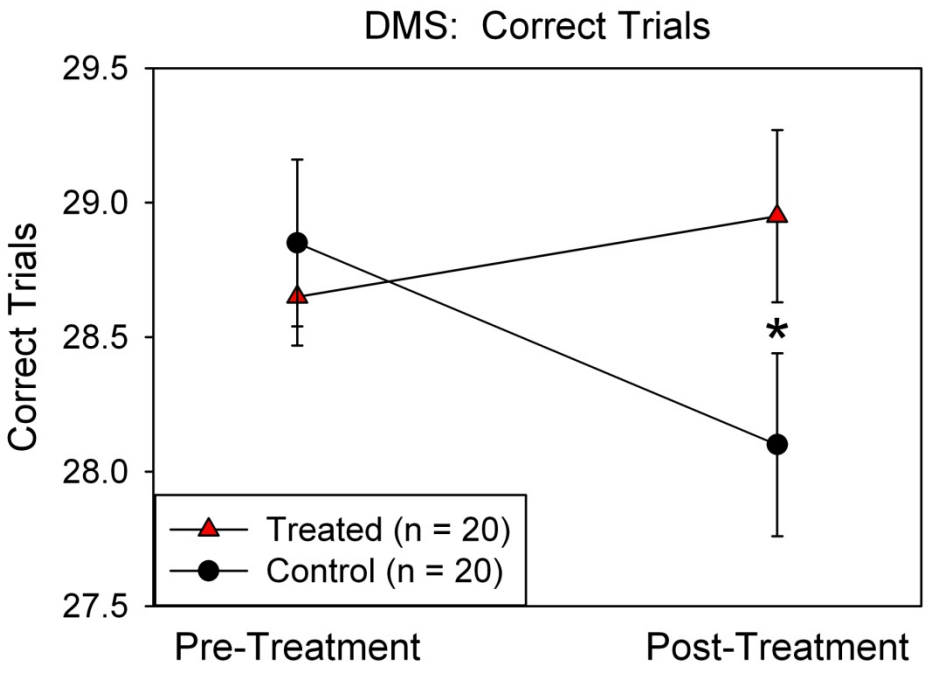
Reaction time in a psychomotor vigilance task was significantly improved



Delayed Match-to-Sample Task

Retrieval latency and correct match-to-sample trials improved significantly





Summary

- Transcranial laser stimulation improved cognitive and emotional functions in humans
- Randomized, placebo-controlled blind trials using attention, memory and mood tests
- Reaction time in a psychomotor vigilance task was significantly improved
- Memory retrieval latency and correct match-to-sample trials improved significantly
- Laser effects also led to two weeks of sustained positive emotional states

Conclusions

- These data imply that transcranial laser stimulation could be used as a non-invasive and efficacious approach to increase brain functions such as those related to cognitive and emotional dimensions.
- Transcranial infrared laser stimulation has also been proven to be safe and successful at improving neurological outcome in humans in controlled clinical trials of stroke.
- This innovative approach could lead to the development of non-invasive, performance-enhancing interventions in healthy humans and in those in need of neuropsychological rehabilitation.

References

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