Pre-conditioning with low level light therapy: Light before the storm

Michael R Hamblin PhD
Mechanisms of Low Level Light Therapy.

Michael R Hamblin a,b,c,* and Tatiana N Demidova a,d

a Wellman Center for Photomedicine, Massachusetts General Hospital, b Department of Dermatology, Harvard Medical School, c Harvard-MIT Division of Health Sciences and Technology, d Graduate Program in Cell Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine


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The Nuts and Bolts of Low-level Laser (Light) Therapy

Hoon Chung,1,2 Tianhong Dai,1,2 Sulbha K. Sharma,1 Ying-Ying Huang,1,2,3 James D. Carroll,4 and Michael R. Hamblin1,2,5
Mitochondrial ATP

Respiratory chain

Complex I

Complex II

Complex III

Complex IV

$\text{H}^+$

$\text{H}^+$

$\text{H}^+$

$\text{H}^+$

$\text{e}^-$

$\text{e}^-$

$\text{e}^-$

$\text{e}^-$

$\text{NADH}$

$\text{NAD}^+$

$\text{H}^+$

$\text{succinate}$

$\text{fumarate}$

$\text{Cyt c}$

$4\text{H}^+$

$\text{O}_2$

$2\text{H}_2\text{O}$

$\Delta\text{P}$

$\text{ADP}$

$\text{ATP}$
Oxygen consumption ↑
Mitochondrial membrane potential ↑
ATP ↑ cAMP ↑
NO released
Brief burst of ROS
Calcium modulation
Main effects of LLLT on tissues

Vasodilation
Lymphatic drainage
Less pro-inflammatory cytokines
More anti-inflammatory cytokines
Less iNOS
More antioxidants
Less oxidative damage
Increased activation of stem/progenitor cells
Which tissues respond to light?

Tissues with high mitochondrial content respond well to light

Muscles, heart
**Neurons**, brain
Liver, kidney
Pain Pathways

Skin

Primary afferent axons

Viscera

Stimulus

Voltage

- 65 mV

Dorsal root ganglion

Pain

Primary afferent axon

Sympathetic ganglion of the ANS
Analgesics

TCA=tricyclic antidepressants
SSRI=selective serotonin reuptake inhibitors
SNRI=serotonin-norepinephrine reuptake inhibitors
LA=local anesthetics
NSAIDS=nonsteroidal anti-inflammatory drugs
ASA=aspirin
Mechanism of analgesic effect

Aδ and C

AP frequency

Disarray of cytoskeleton

Tubulin

Microtubule

Neurofilament

Microfilament
1\textsuperscript{st} Objective

Pain threshold alteration

DRG L4 / L5

LLLT
Laser irradiation and pain evaluation

$\lambda = 808$ nm

$P = 300$ mW; $A = 6$ cm$^2$ ; $PD = 50$ mW/cm$^2$

<table>
<thead>
<tr>
<th>Irradiation time (s)</th>
<th>0</th>
<th>24</th>
<th>120</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy density (J/cm$^2$)</td>
<td>0</td>
<td>1.2</td>
<td>6.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>
Pain evaluation (24 hours)

![Graph showing pain threshold over time for different energy levels: Sham, 1.2 J/cm², 6.0 J/cm², and 30.0 J/cm². The graph indicates a peak pain threshold at 6 hours for all conditions, with Sham showing a slightly lower peak compared to the energy levels.]

1 LLLT/day during 7 days

3h after LLLT

BASELINE

time of treatment (day)
What markers for analgesia?

- Prostatic acid phosphatase (PAP)
- Tubulin & cytoskeleton
- Glutamate receptor
PAP


Tubulin & cytoskeleton


Glutamate receptor


Immunofluorescence

Ganglion extracted 3h after LLLT

2 min LLLT

Sham
Searching for new points of LLLT
Transcranial LLLT for pain

![Graph showing pain threshold over time for different LLLT intensities.
- Sham: 14.4 J/cm²
- 14.4 J/cm²
- 72.0 J/cm²

Pain threshold (gf) vs. time (h)
LLLT on the right (R) paw

Pain threshold (gf) vs. time (h)

- Sham
- R paw
- L paw

Pain threshold (gf) vs. time (min)

- R paw
- L paw
Pain evaluation in several points

![Graph showing pain threshold over time for different groups.]

* * p < 0.05 compared Sham group
Pain evaluation in several points

Pain threshold (gf) vs time (h)

- Sham
- L paw
- Tail
- Belly

p > 0.05 compared Sham group
Comparison with acupuncture

Neural mechanism underlying acupuncture analgesia

Zhi-Qi Zhao

Institute of Neurobiology, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China
## Therapeutic approaches for stroke/TBI

### Antioxidants
- Ebselen
- NXY-059 a nitrone spin-trap agent
- Tirilazad
- Edaravone
- Iron chelator
- Traditional Chinese medicine

### Anti-inflammatory
- Anti-neutrophil adhesion molecule
- Nitric oxide signal transduction down-regulator: lubeiluzole
- Corticosteroid
- Interleukin-1 receptor antagonist

### Circulation
- Volume expansion
- Flow enhancer
- Vasodilator
- Hemodilution
- Blood pressure-related strategy

### Neurochemical
- Serotonin antagonist
- Serotonin receptor agonist
- Serotonin uptake inhibitor

### Oxygen
- Hyperbaric oxygen
- Oxygenated fluorocarbon
- Oxygen supplementation

### Excitotoxicity
- Potassium channel opener
- Sodium channel blocker
- Calcium chelator
- Magnesium
- GABA agonist
- Glutamate/AMPA antagonist
- NMDA receptor/polyamine blocker

### Metabolism
- Ganglioside, Astrocyte modulator
- Beta blocker, CNS stimulant
- Phosphatidylcholine precursor
- Fibroblast growth factor
- Opioid antagonist
- Prostanoid, Statin

### Physical intervention
- Hypothermia (brain cooling)
- Hemicraniectomy
- Osmotic agent

### Clinical trials of pharmacological and physical therapies for stroke/TBI
Transcranial Laser may improve TBI

Neuron excitotoxicity

Neuron apoptosis

Survivin
Hsp70
Transcription Factors
SOD
Neuron prosurvival
ATP
ROS
 Mitochondrial function
Transcranial LLLT for TBI in mouse model (IACUC approved) closed head weight drop method based on Marmarou (1994)
Neurological performance testing

<table>
<thead>
<tr>
<th>Neurological Severity Score (NSS) for Brain-Injured Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of mono- or hemiparesis</td>
</tr>
<tr>
<td>Inability to walk on a 3-cm-wide beam</td>
</tr>
<tr>
<td>Inability to walk on a 2-cm-wide beam</td>
</tr>
<tr>
<td>Inability to walk on a 1-cm-wide beam</td>
</tr>
<tr>
<td>Inability to balance on a 1-cm-wide beam</td>
</tr>
<tr>
<td>Inability to balance on a round stick (0.5 cm wide)</td>
</tr>
<tr>
<td>Failure to exit a 30-cm-diameter circle (for 2 min)</td>
</tr>
<tr>
<td>Inability to walk straight</td>
</tr>
<tr>
<td>Loss of startle behavior</td>
</tr>
<tr>
<td>Loss of seeking behavior</td>
</tr>
<tr>
<td>Maximum total</td>
</tr>
</tbody>
</table>

One point is awarded for failure to perform a task.
Four different wavelength lasers

- 665-nm laser
- 732nm Laser
- 810-nm laser
- 980-nm laser
Low-Level Laser Therapy for Closed-Head Traumatic Brain Injury in Mice: Effect of Different Wavelengths

Qiuhe Wu, MD, PhD,1,2,3 Weijun Xuan, MD, PhD,1,2,4 Takahiro Ando, MS,1,5 Tao Xu, MD, PhD,1,2,6 Liyi Huang, MD, PhD,1,2,7 Ying-Ying Huang, MD,1,2,8 Tianghong Dai, PhD,1,2 Saphala Dhital, PhD,1,9 Sulbha K. Sharma, PhD,1 Michael J. Whalen, MD,10 and Michael R. Hamblin, PhD1,2,11
Comparison of Therapeutic Effects between Pulsed and Continuous Wave 810-nm Wavelength Laser Irradiation for Traumatic Brain Injury in Mice

Takahiro Ando¹,², Weijun Xuan¹,³,⁴, Tao Xu¹,³,⁵, Tianhong Dai¹,³, Sulbha K. Sharma¹, Gitika B. Kharkwal¹,³, Ying-Ying Huang¹,³,⁶, Qiuhe Wu¹,³,⁷, Michael J. Whalen⁸, Shunichi Sato⁹, Minoru Obara², Michael R. Hamblin¹,³,¹⁰∗
A single laser Tx of 36 J/cm² at 50 mW/cm² pulsed at 10 Hz is better than CW or 100 Hz.
Body weight (overall health)

Forced swim test (depression)

A

B

1 day

28 day

Forced swim test (depression)
Tail suspension test (depression and anxiety)

1 day

28 day
Treated mice   - 1X (4 h post TBI)
- 3X (1 per day for 3 days)
- 14X (1 per day for 2 weeks)
mice were sacrificed after 7 and 28 days
14X laser after 7 days = 7X laser
Different numbers of daily 810-nm laser Tx (18 J/cm² at 25 mW/cm²) in CCI TBI
Fluoro Jade C for neurodegeneration (lesion) 14 days
BrdU+ cells for neurogenesis (lesion) 28 days
Caspase 3 in lesion area measured at 4 days post-TBI
BDNF in hippocampus at days 7 & 28
BDNF in hippocampus (hipp) and subventricular zone (SVZ) at days 7 and 28.
Synapsin-1 in lesion at days 7 & 28
Synapsin-1 in lesion, hipp & SVZ at days 7 & 28
Injury-Induced Neurogenesis in the Adult Mammalian Brain

JACK M. PARENT

The persistence of neurogenesis in the adult mammalian forebrain suggests that endogenous precursors may be a potential source for neuronal replacement after injury or neurodegeneration. Limited knowledge exists, however, regarding the normal function of neurogenesis in the adult and its alteration by brain injury. Neural precursors generate neurons throughout life in the mammalian forebrain subventricular zone (SVZ)—olfactory bulb pathway and hippocampal dentate gyrus. Accumulating evidence indicates that various brain insults increase neurogenesis in these persistent germinative zones. Two brain injury models in particular, experimental epilepsy and stroke in the adult rodent, have provided significant insight into the consequences of injury-induced neurogenesis. Studies of dentate gyrus neurogenesis in adult rodent epilepsy models suggest that seizure-induced neurogenesis involves aberrant neuroblast migration and integration that may contribute to persistent hippocampal hyperexcitability. In contrast, adult rat forebrain SVZ neurogenesis induced by stroke may have reparative effects. SVZ neural precursors migrate to regions of focal or global ischemic injury and appear to form appropriate neuronal subtypes to replace damaged neurons. These findings underscore the need for a better understanding of injury-induced neurogenesis in the adult and suggest that the manipulation of endogenous neural precursors is a potential strategy for brain reparative therapies. NEUROSCIENTIST 9(4):261–272, 2003. DOI: 10.1177/1073858403252680

KEY WORDS Stem cell, Neural regeneration, Neurogenesis, Epilepsy, Stroke

Medline citations
Adult neurogenesis = 4,580
Synaptic plasticity = 17,841
BDNF = 10,522
Hippocampus = 104,022

Fig. 1. Schematic sagittal view of the adult rat brain showing the two regions of persistent neurogenesis. Neural precursor cells in the subventricular zone (SVZ; purple) undergo a long-distance, tangential migration in the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they differentiate into granule (green) and periglomerular (yellow) neurons. Precursors (purple) in the hippocampal dentate gyrus migrate a short distance into the dentate granule cell layer (outlined in red) and give rise to dentate granule neurons (green).
BrdU+ cells in lesion area

Day 7

Day 28
BrdU+ cells in SVZ

Day 7

Sham control
TBI sham
TBI 1 laser
TBI 3 laser
TBI 7 or 14 laser

Day 28
BrdU in hipp at days 7 & 28
DCX and Tuj1 cells in DG (day28)

Double cortin (DCX) red
Tuj1 – green (Neuron-specific class III beta-tubulin is an excellent Neural Stem Cell Marker)

Sham control  TBI sham  TBI 1 laser  TBI 3 laser  TBI 14 laser

20X

60X
Mechanisms of Transcranial Light Therapy
<table>
<thead>
<tr>
<th>Traumatic</th>
<th>Degenerative</th>
<th>Psychiatric</th>
<th>Mitochondrial</th>
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</thead>
<tbody>
<tr>
<td>Acute stroke</td>
<td>Alzheimer’s</td>
<td>Depression</td>
<td>MELAS</td>
</tr>
<tr>
<td>Acute TBI</td>
<td>Parkinson’s</td>
<td>Anxiety</td>
<td>Fibromyalgia</td>
</tr>
<tr>
<td>Chronic stroke</td>
<td>Huntington’s</td>
<td>Schizophrenia</td>
<td>Gulf War Syndrome</td>
</tr>
<tr>
<td>Chronic TBI</td>
<td>Amyotrophic lateral sclerosis</td>
<td>Addiction</td>
<td>Tinnitus</td>
</tr>
<tr>
<td>Global ischemia</td>
<td>Primary Aphasia</td>
<td>Bipolar disorder</td>
<td>Attention deficit hyper activity disorder</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autism spectrum disorders</td>
</tr>
</tbody>
</table>
# Common pathways in neurodegenerative and psychiatric disease

<table>
<thead>
<tr>
<th>Neuroinflammation</th>
<th>Oxidative Stress</th>
<th>Excitotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low BDNF</td>
<td>Impaired Neurogenesis</td>
<td>Hippocampal Shrinkage</td>
</tr>
<tr>
<td>Alzheimer’s</td>
<td>Parkinson’s</td>
<td>Depression</td>
</tr>
<tr>
<td>Mitochondrial Dysfunction</td>
<td>Impaired Synaptogenesis</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Neuronal Apoptosis</td>
<td>Cortical Shrinkage</td>
<td>Bipolar Disorder</td>
</tr>
</tbody>
</table>
LLLT for Depression and PTSD

Behavioral and Brain Functions

Research

Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety

Fredric Schiffer*1, Andrea L Johnston3, Caitlin Ravichandran2, Ann Polcari1, Martin H Teicher1, Robert H Webb3,4 and Michael R Hamblin3,4,5

![Graph of Hamilton Depression Rating Scale score over time](image)

![Graph of Hamilton Anxiety Rating Scale score over time](image)
Transcranial LED therapy for cognitive dysfunction in chronic, mild traumatic brain injury: Two case reports

Margaret A. Naeser\textsuperscript{a,b}, Anita Saltmarche\textsuperscript{c}, Maxine H. Krengel\textsuperscript{a,b}, Michael R. Hamblin\textsuperscript{d,e,f}, Jeffrey A. Knight\textsuperscript{a,b,g}

\textsuperscript{a}VA Boston Healthcare System (12-A), 150 So. Huntington Ave., Boston, MA, USA 02130
\textsuperscript{b}Dept. of Neurology, Boston Univ. School of Medicine, 85 E. Concord St., Boston, MA, USA 02118
\textsuperscript{c}MedX Health Inc., 220 Superior Blvd., Mississauga, ON L5L 2L2, Canada
\textsuperscript{d}Wellman Center for Photomedicine, Massachusetts General Hospital, Boston MA 02114
\textsuperscript{e}Dept of Dermatology, Harvard Medical School, Boston MA 02115
\textsuperscript{f}Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA
\textsuperscript{g}National Center for PTSD - Behavioral Sciences Division, VA Boston Healthcare System

Case 1. 59 yo F, 7 yr. post-MVA after 8 weekly Tx.’s, ability to do computer work had improved 10-fold, obtained home unit and has used daily for 5 years.

Case 2. 52 yo F, multiple concussions and PTSD, Tx.’d daily with home unit, memory and “executive function” tests improved >2 SD, after 9 months. Off “Medical Disability” status after 4 months of home treatments; returned to full-time work.
P2, Pre- and Post-LED Tx., *Neuropsychological Test Results*  
Post-LED Testing, Post-9 months, nightly, transcranial LED Tx.

2 Stories, *Immediate Recall*  
Wechsler Memory Scale-R

* Significant Improvement, +1 SD: Memory

2 Stories, *Delayed Recall (30 minutes)*  
Wechsler Memory Scale-R

* Significant Improvement, +2 SD: Memory
Forthcoming Clinical Trial, at VA Boston Healthcare System (VABHS), Jamaica Plain

“Transcranial, Light-emitting Diode (LED) Therapy to Improve Cognition in Gulf War Veterans’ Illnesses”

PI: Margaret Naeser, PhD
VABHS and Department of Neurology, Boston University School of Medicine (BUSM)
Co-Investigators and Consultants:
Maxine Krengel, PhD, Neuropsychologist, VABHS, and Neurology, BUSM
Jeffrey Knight, PhD, Neuropsychologist, National Center for PTSD, VABHS
Michael R. Hamblin, PhD, Wellman Center for Photomedicine, Massachusetts General Hospital,
Beatrice Golomb, MD, PhD, Univ. of Calif., San Diego; and VA San Diego Healthcare System
Marc S. Goldstein, MD, Primary Care, Worcester VA Outpatient Clinic
Manisha Thakore-James, MD, Neurology VABHS and Neurology, BUSM
Carlos Tun, MD, Rehabilitation Medicine VABHS and Harvard Medical School

VA-funded, 4-Yr. - blinded, randomized, sham-controlled, cross-over study.

◆ 160 GWI Veterans with cognitive problems; recruited through Ft. Devens, MA Cohort Previously participated in GWI surveys, under direction of Maxine Krengel, PhD

2 Groups: A) Sham LED helmet 1st series; Real, 2nd series. B) Real LED helmet 1st series; Sham 2nd.
15 Tx.’s per series; 2x/Wk for 7.5 Wks. Tested at 1 Week and 2 Mo. post-Tx.
Each red/NIR LED pod inside helmet: 692.5mW; 36.5mW/cm²; CW, 13 J/cm², 10 min per LED pod locus.

Primary Outcome Measures: Attention/Executive Function; Learning & Memory; Psychomotor/Visuospatial
Secondary Measures: Pain, Fatigue, Mood, Blood Tests: mitochondrial function; inflammation; coagulation; general health
Forthcoming Clinical Trial at MGH

Acute Low-Level Laser Therapy for the Treatment of Moderate Traumatic Brain Injury
PI: Benjamin Vakoc, PhD

Double-blinded, randomized, and placebo-controlled. 82 moderate TBI patients admitted to Massachusetts General Hospital will be randomly assigned into equal treatment and control (sham therapy) groups. LLLT or sham treatment will be given daily for 1 week after injury.

Endpoints: Neuroimaging data (MRI-BOLD, ASL, DTI); clinical evaluation, neurofunctional tests at 3 and 6-month follow ups.
Previous transcranial helmet devices
“Brain Cap”
PhotoTherapeutics

PhotoMedex
Dermatology • Surgical
Conclusions

Mechanisms of LLLT are becoming understood

Pre-conditioning nerves with LLLT reduces pain

Transcranial LLLT improves acute TBI

Transcranial LLLT may be applicable for large range of brain disorders
Acknowledgments

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Tao Xu PhD
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Fatma Vatansever MD, PhD

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