

Erchonia National Laser Expo 2026

Dan Murphy, DC

5 Hours

Calcium

WHAT YOUR DOCTOR **STILL** DOESN'T KNOW

2013

NEW
UPDATED &
EXPANDED
EDITION

ROBERT THOMPSON, MD & KATHLEEN BARNES

THE Calcium LIE II

The Calcium Lie II **What Your Doctor Still Doesn't Know**

Robert Thompson, MD
2013

We were all led to believe that unless we get loads of calcium, our bones will break and crumble to powder. It's not true. It's never been true and basic science taught in every university in the world shows us the error of this belief system.

Calcium is only one of at least 12 minerals that build strong bones.

If you take calcium to strengthen your bones and you already have an excess of calcium in your body, you are signing your own death warrant.

Excess calcium can cause:

- Kidney and gallstones
 - Arterial plaque
 - Bone spurs
 - Calcium deposits in tissues other than bones
 - Brain cell dysfunction, brain shrinkage and dementia
- Calcium excess causes an increased chance of calcification, concretions, or gravel-like or concrete-like calcium deposits in our arteries (plaque), joints (spurs), eyes (cataracts), brain (shrinkage, dementia), kidneys and gallbladder (stones), and other health issues, including hardening of the arteries, heart disease, dementia, cancer, diabetes, hypothyroidism and hypertension (due to intracellular sodium depletion) to name a few.

The Calcium Cascade

Excess calcium in the human body begins a cascade of negative effects that have enormous adverse consequences to our health. This process cannot be diagnosed with standard blood tests. It requires a reliable, competent lab to conduct a hair tissue mineral analysis (HTMA).

If you have excess or relative calcium excess in your body

THAT LEADS TO

Calcium seeking and needing more magnesium to try to keep your body's calcium and magnesium in balance

THAT LEADS TO

A relative magnesium deficiency in proportion to calcium that leads to increased muscle tension, and nerve endings firing erratically and other 'electrical' malfunctions in your body

AND

In its need for more magnesium, your body has to suppress adrenal function in order to retain more magnesium to compensate for the high calcium

This adrenal suppression causes a continuous loss of sodium and potassium in your urine as well as immune compromise from the adrenal suppression

THIS LEADS TO

A continual depletion of the sodium and potassium that are stored inside the trillions of cells in your body

THAT LEADS TO

A loss of the sodium and chloride you need to produce the stomach acid you need to digest protein

AND

This increases the incidences of heartburn and other digestive disorders, and the use of prescription drugs that have further destructive effects and impede digestion

AND

Your body gradually loses its ability to digest protein and absorb the essential amino acids that are the building blocks of protein and neurotransmitters

ALSO

The sodium depletion leads to a failure of membrane electrical potential and ion exchanges necessary for cellular function, the mechanism by which our bodies get essential amino acids and glucose into all our cells, except fat cells which keep absorbing glucose without sodium while the rest of our bodies cells are starving

FURTHERMORE

Intracellular potassium levels decline dramatically and this leads to increasing degrees of thyroid hormone resistance (type 2 hypothyroidism), with all the symptoms of hypothyroidism and slowed metabolism with what are thought to be normal blood tests

SO

All cells (except fat cells) become starved for glucose and amino acids

RESULTING IN

Increased cravings for glucose and increased food intake

This loss of minerals also leads to more food cravings

AND

Intracellular deficiencies of sodium, potassium and essential amino acids, and more cravings

THE END RESULT IS

Multiple metabolic malfunctions, including, obesity, heart disease, type-2 hypothyroidism, type-2 diabetes, anxiety, migraines, depression, dementia, hypertension

Osteoporosis

Your bones are not made of calcium alone.

Osteoporosis, osteopenia and general brittle bones are not caused by a lack of calcium, and one cannot strengthen bones by taking calcium supplements, drinking lots of milk or eating loads of dairy products and calcium-rich and calcium-fortified foods, no matter what your doctor might tell you.

The Harvard and Cornell research on 122,000 women who participated in the Nurses' Health Study showed those who drank the most milk had the highest fracture rates.

Osteoporosis is caused by mineral deficiency because your body doesn't have adequate supplies of *all* the minerals it needs to build strong bones or it has too much of one of those minerals: calcium.

Bones are the storehouses of minerals for the entire body.

Bones are not made of calcium alone, but of at least 12 minerals, including calcium. Expecting to keep bones strong by giving someone calcium supplements is like expecting that you can make a loaf of bread from yeast alone. It simply won't work.

Calcium supplements can do great harm as crystallized excess calcium concretions make their way into arteries and joints; this forces the adrenals to compensate for the calcium excess to their own detriment. This problem leads to a continuous decline in sodium and potassium from the body, changes in cell membrane physiology and electrical potential, and causes the brain to shrink and become demented.

Our foods don't contain the minerals they once did because of mineral- depleted soil and lack of vine ripening.

Almost everyone needs trace minerals, not just calcium, because we simply cannot get all nutrients we need from food grown in our minerally depleted soils, picked before ripeness, especially in view of our society's propensity for nutritionally void foods.

Most importantly: Calcium hardens concrete, not bones. Excess calcium can do severe damage to the human organism, especially the brain, arteries and other soft tissue.

FEEL

GOOD

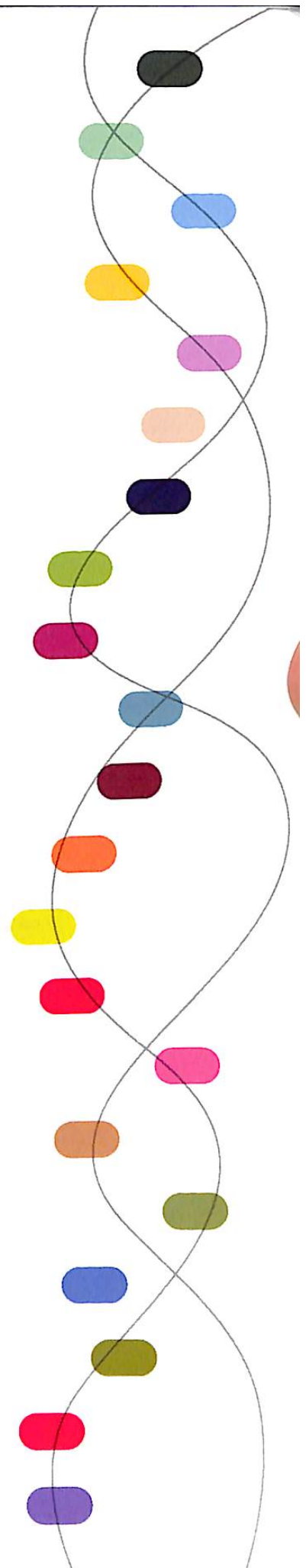
NUTRIGENOMICS

YOUR ROADMAP TO HEALTH

Dr. Amy Yasko

Ph.D., NHD, AMD, HHP, FAAIM

2014



Feel Good Nutrigenomics **Your Roadmap to Health**

Amy Yasko
2014

Chapter 7: "Speeding Through Life: the Gas Pedal is Stuck"

Balancing glutamate is the *first* step of the program.

Excess glutamate can overexcite your nerves.

"Glutamate works with calcium to stimulate your nervous system."

"The impact of excess glutamate in the body is like a car whose gas pedal is stuck, pressed to the floor."

Speeding through life virtually ensures that you will experience a major crash due to excessive speed.

"Too much glutamate will exhaust your nerves to the point of creating health issues. Glutamate is considered an 'excitotoxin'. Excitotoxins are compounds that have the ability to overexcite nerves to death."

"Too much calcium will work with glutamate to overexcite your nervous system."

"Glutamate is the gun and calcium is the bullet."

Glutamate opens a special channel that allows calcium to enter the neuron, "and it was calcium that triggered the cell to die."

Excitotoxins, including glutamate and aspartate work by opening calcium channels. "When those neurotransmitters are allowed to come into contact with the receptor in too high a concentration or for too long a period of time, the calcium channel gets stuck in the open position allowing calcium to pour into the cell in large amounts." This calcium *must* be balanced with magnesium.

"We are often unaware of the issue of excess glutamate until more than 80% of our neurons have been impaired." **"This is why prevention is so important."**

"Addressing imbalances in the glutamate/GABA ratio as well as the calcium to magnesium ratio is what I consider the starting point, or Step One of this [Methylation cycle] program."

"This is a critical ongoing way of life, to keep glutamate and calcium in balance as you move forward to work on the other aspects of multifactorial health conditions."

SCIENTIFIC AMERICAN

JULY 1991
\$3.95

Hope that strokes may soon be treatable.
Do "smart" airplanes have the Right Stuff?
When the local druggist sold heroin—legally.



Churning clouds of interstellar gas
veil the first glimmers of newborn stars.

© 1991 SCIENTIFIC AMERICAN, INC.

SCIENTIFIC AMERICAN MAGAZINE

July 1991

Volume 265, Issue 1

You are currently logged out. Please sign in to download the issue PDF.

[Sign In](#)

Features

Opium, Cocaine and Marijuana in American History

Over the past 200 years, Americans have twice accepted and then vehemently rejected drugs. Understanding these dramatic historical swings provides perspective on our current reaction to drug use

DAVID F. MUSTO

The Early Life of Stars

Violent collisions of gas dominate the childhood of stars. Eventually nuclear fusion begins, enabling mature stars to burn steadily for billions of years

STEVEN W. STAHLER

Stroke Therapy

Although no treatment to limit brain damage is yet available, many tantalizing possibilities are on the horizon. Some are already being evaluated in all-important human trials

JUSTIN A. ZIVIN, DENNIS W. CHOI

Stroke Therapy

**Scientific American
July 1991; pp. 56-63**

Justin A. Zivin and Dennis W. Choi: practicing physicians and research Scientists: Zivin is professor of neurosciences at the University of California, San Diego, School of Medicine and a staff neurologist at the San Diego Veterans Administration Medical Center; Choi is head of neurology at the Washington University School of Medicine in St. Louis.

BACKGROUND FROM DAN MURPHY:

Glutamate is both an amino acid (building block for proteins) and functions as an excitatory neurotransmitter in the brain.

Arachidonic Acid is an omega-6 fatty acid that becomes a structural component of the phospholipid neuronal (and other) membranes. It is derived from the oils of corn, soy, sunflower, safflower peanut canola and cottonseed.

Arachidonic Acid is enzymatically converted into inflammatory hormone-like molecules called eicosanoids.

As long as there is some intact blood supply (the "penumbra") low-level laser therapy may instantly increase energy (ATP) production helping to clear excess glutamate and downstream excitotoxic loss of neurons.

KEY POINTS FROM THIS ARTICLE:

- 1) "Researchers in many laboratories are developing ways to reduce brain injury associated with stroke."
 - 2) "In the U.S., according to the National Institutes of Health (NIH), the condition is the third leading cause of death and the most common cause of adult disability."
 - 3) Following a vascular clot, "cells at the center of the stroke, in the 'core' region that receives essentially no blood when a vessel is occluded, die."
 - 4) Tissue plasminogen activator (tPA), rapidly dissolves obstructions in the cerebral arteries and therefore limits tissue damage and diminished functional loss when used within an hour after a stroke.
- "Administration of tPA or similar drugs might have to be delayed until hemorrhage was ruled out with a brain scan." [to distinguish a clot stroke v. a bleed stroke]

- 5) "The brain depends on blood for a continuous supply of oxygen and glucose."
- "The affected cells die directly from a loss of energy." **[Key Point]**
 - Ischemic neurons are killed by a damaging cascade of chemicals, primarily glutamate; blocking this chemical cascade might spare many neurons.
- 6) "The glutamate cascade could well be a leading cause of damage in the surrounding, 'penumbral,' region, which receives some blood from non-occluded vessels." **[Key Point]**
- The glutamate cascade "is a major cause of ischemia-induced neuronal death." **[Important]**
 - The glutamate cascade "begins after terminals of ischemic neurons release excessive amounts of the neurotransmitter glutamate, an excitatory stimulus, into the spaces between cells."
 - "Many, perhaps a majority, of the neurons in the brain contain this [glutamate] transmitter."
 - Penumbral cells may be damaged by events that began when "ischemia induces neurons to oversecrete glutamate, an excitatory neurotransmitter."
- 7) When neurons release large amounts of glutamate, the sustained receptor binding "induces an abnormal movement of calcium ions (Ca^{2+}) into the recipient cells, producing a buildup of calcium, which apparently contributes to many of the events that destroy those cells." **[Key Point]**
- 8) The idea that glutamate neurotransmitter activity could be a critical link between ischemia and neuronal death, was advanced when it was shown that "magnesium impairs the ability of neurons to release" glutamate. **[Critical]**
- "The magnesium reduced the expected damage, implying that inhibition of signaling can protect neurons from hypoxia" during a stroke. **[Critical]**
 - "Work by John W. Olney and his collaborators at Washington University in the 1970s first established that excessive exposure to glutamate or related compounds can kill brain neurons, presumably by overstimulating them."
 - "Olney named the killing process excitotoxicity." **[Important]**
 - Glutamate excites neurons by stimulating glutamate receptors, especially the N-methyl-D-aspartate (NMDA) receptor.
 - "Activation of NMDA receptors may be required for glutamate-induced killing of cells."

- "NMDA receptors are the only glutamate receptors able to open channels that allow large amounts of calcium to pass into neurons across the cell membrane."

9) In cultures, death of neurons from the cerebral cortex could be reduced by removal of calcium from the extracellular fluid.

- "Calcium entry into the cell is a major mediator of glutamate's destructive effect." **[Very Important]**
- "Glutamate-bound NMDA receptors open calcium channels, which then usher a great deal of calcium into the cell."

10) "Calcium activates enzymes that attack several classes of molecules, including nucleic acids, proteins and lipids [phospholipids] (fats)." **[Important]**

11) "The breakdown of phospholipids in the outer cell membrane and in membranes of internal organelles may be particularly devastating."

- "When phospholipids are degraded, one by-product is arachidonic acid, the metabolism of which leads to formation of highly reactive compounds called oxygen free radicals."
 - "These compounds, in turn, can initiate a chain reaction, called lipid peroxidation, capable of destroying the cell membrane."
- "Arachidonic acid can harm cells in other ways."
 - "It participates in the formation of substances called eicosanoids, which increase the aggregation of blood cells and the constriction of blood vessels."
 - This creates a "vicious circle in which ischemic injury leads to further ischemia."

12) "Stroke-related brain damage can potentially be limited by blocking steps in the glutamate cascade." **[Important]**

- The easiest way to halt the glutamate cascade is to prevent the activation of NMDA receptors. **[Magnesium]**
- The glutamate cascade might be halted by "clearing the neurotransmitter from extracellular spaces." [This requires energy (ATP); and therefore it is of particular interest for those using low-level laser therapy].
- "In healthy tissue, neurons and glial cells would remove excess glutamate from extracellular spaces, but ischemic cells lack the energy they need for the job."

13) "For patients who have reached the expression stage, interference with the activity of oxygen free radicals holds special appeal, both because the compounds are highly destructive and because their suppression probably would not interfere with the functioning of healthy cells." [Particularly interesting for those who advocate taking antioxidant supplements]

14) The Stages of Stroke: "Excess release of glutamate may damage brain tissue by a three-stage process."

- STAGE 1: INDUCTION

"After a blood vessel becomes occluded neurons become starved for blood and, thus, for oxygen and glucose."

"The blood-deprived neurons then release excessive glutamate, which activates glutamate receptors on other neurons."

In response to glutamate, NMDA receptors open channels that allow passage of calcium (Ca^{2+}), resulting in its abnormal buildup.

- STAGE 2: AMPLIFICATION

The Ca^{2+} overload "activates enzymes that increase cellular sensitivity to glutamate."

Further buildup of Ca^{2+} triggers the release of more glutamate, "thus spreading the toxic cascade to other cells."

- STAGE 3: EXPRESSION

" Ca^{2+} activates enzyme that degrade DNA proteins and phospholipids."

"Phospholipid breakdown leads to the formation of arachidonic acid which, when metabolized, gives rise to oxygen free radicals that harm the cell membrane and to eicosanoid molecules."

Eicosanoids, a byproduct of phospholipid neuronal membrane breakdown, "promotes blockage of previously healthy vessels and the spread of ischemia."

The "continuing destruction of cellular constituents kills the neurons."

COMMENTS FROM DAN MURPHY

This fascinating study would add biological plausibility to the following:

- If an acute clot vascular event is suspected, delaying referral for imaging (to distinguish a clot v. bleed stroke) and the administration of anticoagulant (like tPA, only if a clot) could adversely affect the prognosis.
- Strategies to enhance mitochondria energy production will almost certainly reduced loss of neurons in the penumbra. Such strategies include low-level laser therapy, and supplements such as medium chain triglycerides (coconut oil), acetyl-L-carnitine, alpha-lipoic acid, PQQ, magnesium, etc.
- Glutamate is routinely added to processed foods to enhance taste and hence sales. It is disguised by many names on packaging labels. Increased dietary glutamate before or after brain injury/stroke is probably counterproductive to recovery.
- Consumption of the artificial sweetener, aspartame increases aspartate (another amino acid). This is also counter-productive because the "glutamate cascade" is enhanced not only by glutamate, but also by aspartate, as they both use the N-methyl-D-aspartate (NMDA) receptor.
- Taking Ca++ supplements after brain injury/stroke is probably counterproductive to recovery.
- Immediately taking a quality magnesium supplement about 600-900 mg daily, is probably critically important. [We prefer magnesium-malate with medium-chain triglycerides to enhance crossing of the blood-brain barrier: Nutri-West Complete Magnesium]
- Reduce the adverseness of arachidonic acid derived inflammatory eicosanoids (like prostaglandin E2) by immediately consuming high dose quality omega-3 fatty acids. We like higher levels of eicosapentaenoic acid (EPA) [Nutri-West Complete Omega-3 Essentials 2:1]. Eicosapentaenoic Acid is the biological nemesis to Arachidonic Acid.
- Taking antioxidant supplements to reduce dangerous and damaging oxygen free radicals is probably wise.

A supportive article is Article Review 45-13:

Post-concussion Syndrome: A Review of Pathophysiology and Potential Nonpharmacological Approaches to Treatment

This study advocates these supplements for improved recovery:

- | | |
|--------------------|--------------------|
| • Omega-3s EPA+DHA | • Vitamin D3 |
| • Curcumin | • Resveratrol |
| • Magnesium | • Green Tea (EGCG) |

PubMed

Display Settings: Abstract

NEJM FULL TEXT

N Engl J Med. 1994 Mar 3;330(9):613-22.

Excitatory amino acids as a final common pathway for neurologic disorders.Lipton SA, Rosenberg PA.

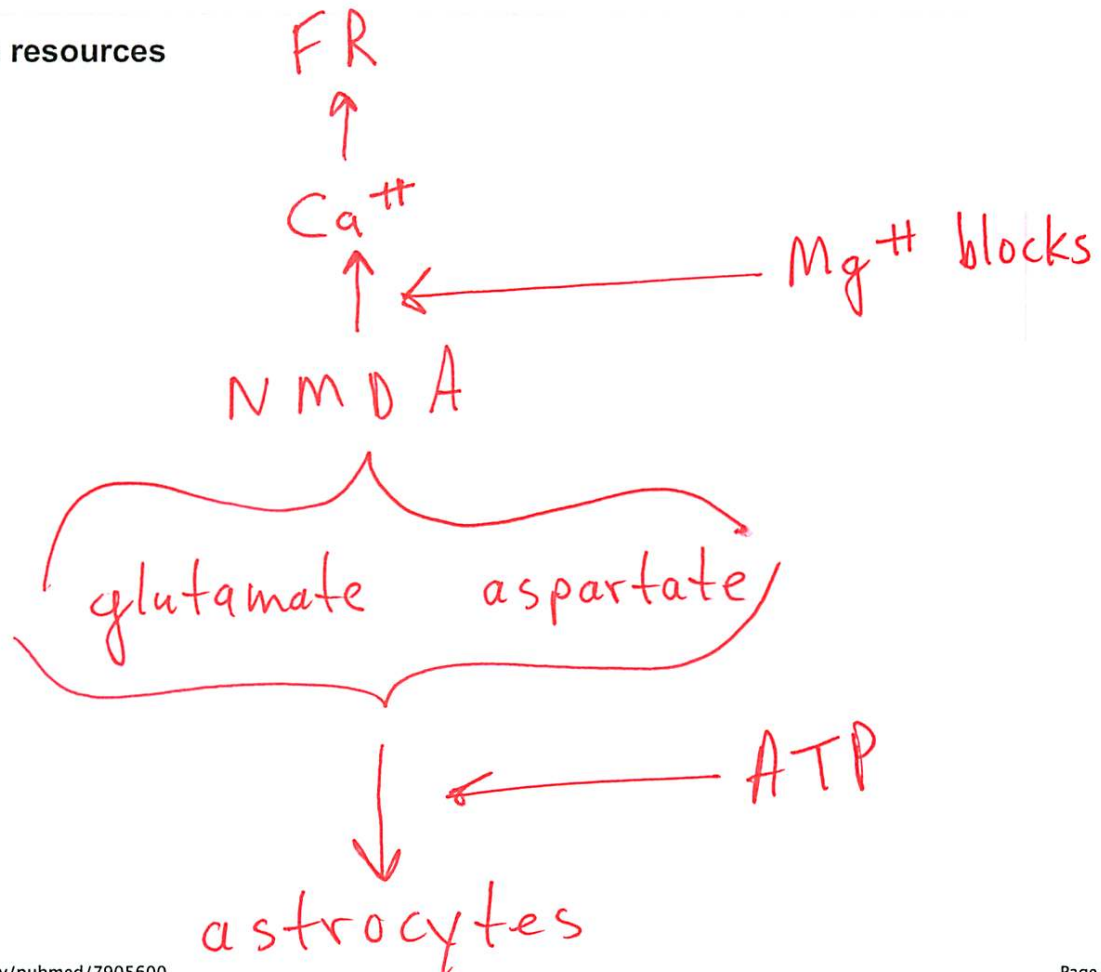
Harvard-Longwood Neurology Program, Boston, MA 02115.

Comment in

Excitatory amino acids in neurologic disorders. [N Engl J Med. 1994]

Excitatory amino acids in neurologic disorders. [N Engl J Med. 1994]

PMID: 7905600 [PubMed - indexed for MEDLINE]

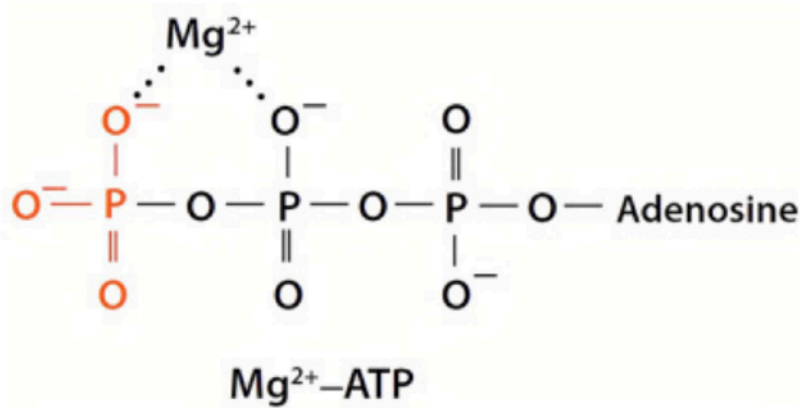
Publication Types, MeSH Terms, Substances**LinkOut - more resources**

Magnesium and the Hallmarks of Aging

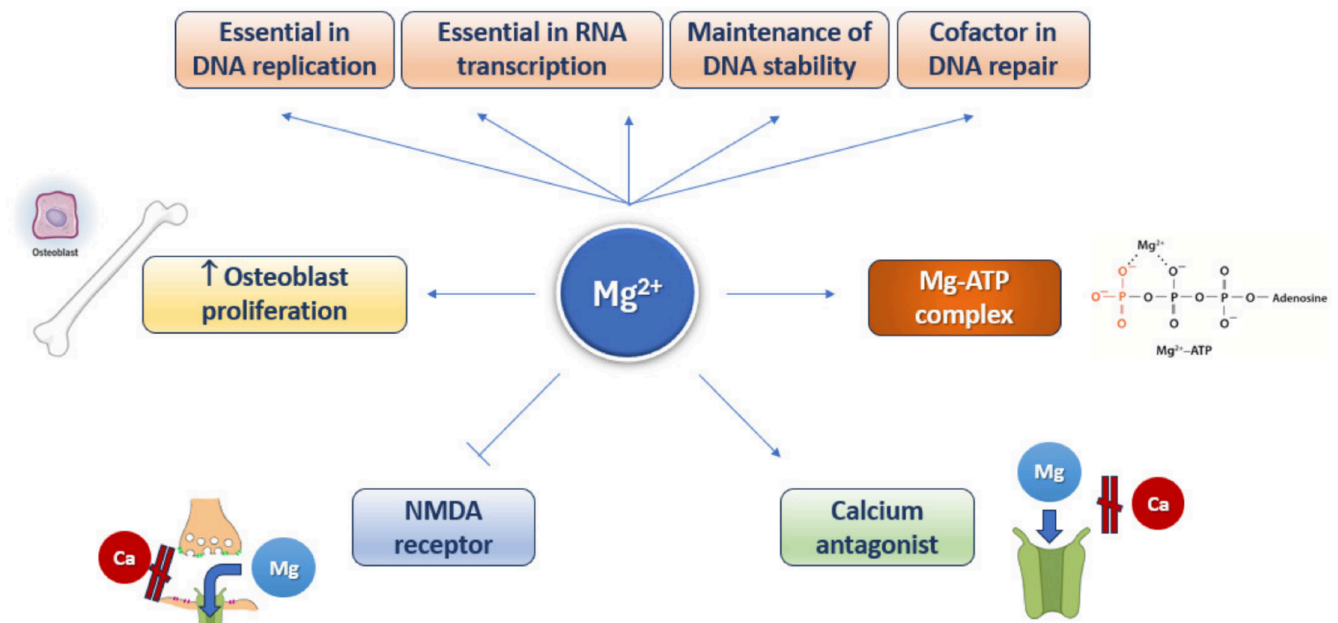
Nutrients

February 9, 2024

This Study Cites 254 References



.....



PubMed

Format: Abstract

Full text links



Neuron. 1995 Oct;15(4):961-73.

Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function.

Ankarcrona M¹, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P.

Author information

1 Division of Toxicology, Karolinska Institute, Stockholm, Sweden.

Abstract

During ischemic brain injury, glutamate accumulation leads to overstimulation of postsynaptic glutamate receptors with intracellular Ca²⁺ overload and neuronal cell death.

Here we show that glutamate can induce either early necrosis or delayed apoptosis in cultures of cerebellar granule cells. During and shortly after exposure to glutamate, a subpopulation of neurons died by necrosis. In these cells, mitochondrial membrane potential collapsed, nuclei swelled, and intracellular debris were scattered in the incubation medium. Neurons surviving the early necrotic phase recovered mitochondrial potential and energy levels. Later, they underwent apoptosis, as shown by the formation of apoptotic nuclei and by chromatin degradation into high and low molecular weight fragments. These results suggest that mitochondrial function is a critical factor that determines the mode of neuronal death in excitotoxicity.

PMID: 7576644

[Indexed for MEDLINE] [Free full text](#)



Publication types, MeSH terms, Substances, Grant support



LinkOut - more resources



PubMed



Format: Abstract

Full text links



Neurosci Lett. 2017 Aug 24; pii: S0304-3940(17)30695-X. doi: 10.1016/j.neulet.2017.08.048. [Epub ahead of print]

Beyond the critical point: An overview of excitotoxicity, calcium overload and the downstream consequences.

Bano D¹, Ankarcrona M².

Author information

- 1 German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. Electronic address: daniele.bano@dzne.de.
- 2 Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden.

Abstract

Over the past several decades, an overwhelming body of research has greatly expanded our understanding of the mechanisms underlying excitotoxicity in brain ischemia as well as in many chronic neurodegenerative diseases. The identification of an array of molecular targets has opened avenues for neuroprotective strategies and, consequently, has sparked considerable interest for their attractive therapeutic means as pharmacological options. The purpose of this work is to provide a general overview of neuronal excitotoxicity and the inevitable downstream consequences of Ca^{2+} overload. We also discuss the contribution of Ca^{2+} transporters in excitotoxicity. This article is part of a Special Issue entitled "Calcium Pumps and Exchangers in Neuronal Injury and Neurodegeneration".

Copyright © 2017 Elsevier B.V. All rights reserved.

KEYWORDS: Calcium; Calpains; Excitotoxicity; Mitochondria; $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX)

PMID: 28843346 DOI: [10.1016/j.neulet.2017.08.048](https://doi.org/10.1016/j.neulet.2017.08.048)



Publication type



2016

Mark F. BEAR
Barry W. CONNORS
Michael A. PARADISO

NEUROSCIENCE

Exploring the Brain

Enhanced Fourth Edition



ACCESS
CODE
INSIDE



NAVIGATE ²
PREMIER ACCESS

Neuroscience
Exploring the Brain

Second Edition, Lippincott Williams & Wilkins, 2001

Mark Bear, PhD
Professor of Neuroscience, Brown University

Barry Connors, PhD
Professor of Neuroscience, Brown University

Michael Paradiso, PhD
Associate Professor of Neuroscience, Brown University

●●●●●●●●●●

"The Brain's Exciting Poisons"

"Neurons of the brain rarely regenerate, so each dead neuron is one less we have for thinking."

"One of the fascinating ironies of neuronal life and death is that glutamate, the most essential neurotransmitter in the brain, is also one of the biggest killers of neurons."

The brain stores a large quantity of glutamate. **"An ominous observation is that when you apply [a small excess] amount of glutamate to the outside of isolated neurons, they die within minutes."**

"The voracious metabolic rate of the brain demands a constant supply of oxygen and glucose" to generate enough ATP to keep the neuron's glutamate and ion pumps working hard.

"If blood flow ceases, as in cardiac arrest [stroke, brain trauma, seizures, oxygen deficiency, and {insulin resistance}], neural activity will stop within seconds, and permanent damage will result within a few minutes."

"Excess glutamate depolarizes neurons, which raises intracellular Ca^{++} which causes still more glutamate to be released." [feedback loop]

"When glutamate reaches high concentrations, it kills neurons by overexciting them, a process called *excitotoxicity*."

"The NMDA type of glutamate channel is a critical player in excitotoxicity because it is the main route for Ca^{++} entry."

"Neuron damage or death occurs because of stimulation by Ca^{++} of intracellular enzymes that degrade proteins, lipids, and nucleic acids. Neurons literally digest themselves."

"Excitotoxicity has recently been implicated in several progressive neurodegenerative diseases, such as *amyotrophic lateral sclerosis* (ALS, also known as Lou Gehrig's disease, *Alzheimer's* disease, and *Parkinson's* disease."

"Glutamate receptor antagonists can obstruct these excitotoxic cascades and minimize neuronal suicide."

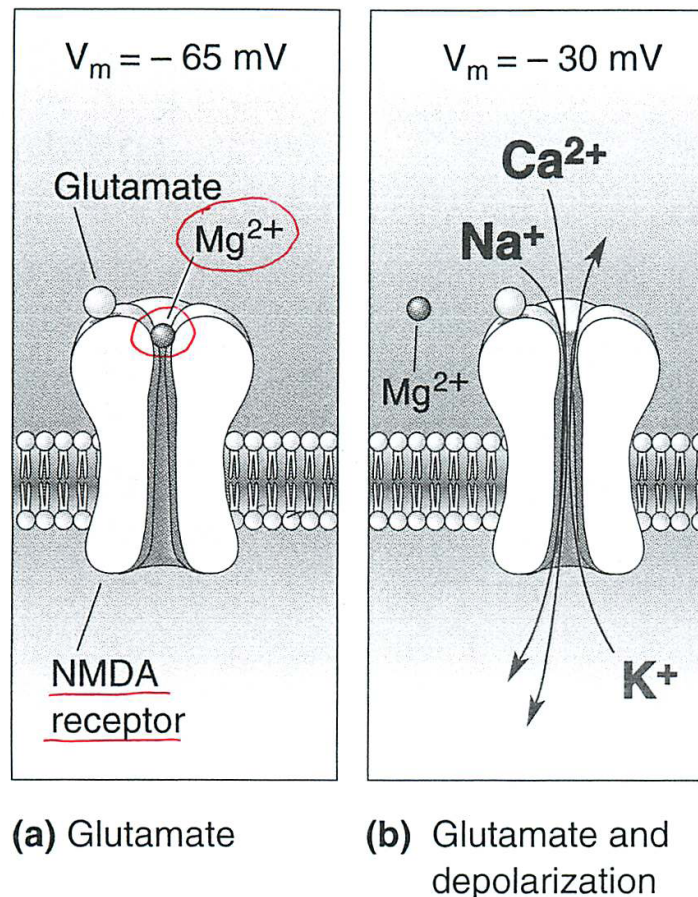


Figure 6.20

The flow of inward ionic current through the NMDA-gated channel. (a) Glutamate alone causes the channel to open, but at the resting membrane potential, the pore becomes blocked by Mg^{2+} ions. (b) Depolarization of the membrane

Amyotrophic Lateral Sclerosis

A small percentage of ALS cases are genetic and linked to a "mutation affecting the enzyme superoxide dismutase." "Superoxide radicals are extremely reactive and can inflict irreversible cellular damage."

"Another suspected cause of ALS is excitotoxicity," "overstimulation by the excitatory neurotransmitter glutamate and closely related amino acids can cause the death of otherwise-normal neurons."

"The slow accumulation of damage from toxic by-products of cellular metabolism probably occurs in all neurons and contributes to neuronal death over the lifespan of all people."

"This loss of neurons may not be inevitable. Vitamin C and E readily accept electrons from superoxide radicals and can be neuroprotective. Dietary supplement with these vitamins may help stem the tide of neuronal loss that occurs in the brain as we grow older."

Stress and the Brain

Chronic stress damages the brain.

"Stress leads to the release of the hormone cortisol from the adrenal cortex. Cortisol travels to the brain through the bloodstream and binds to receptors in the cytoplasm of many neurons."

"The direct effect of cortisol is damage to the hippocampus. These effects of cortisol and stress resemble the effects of aging on the brain." "Chronic stress causes premature aging of the brain."

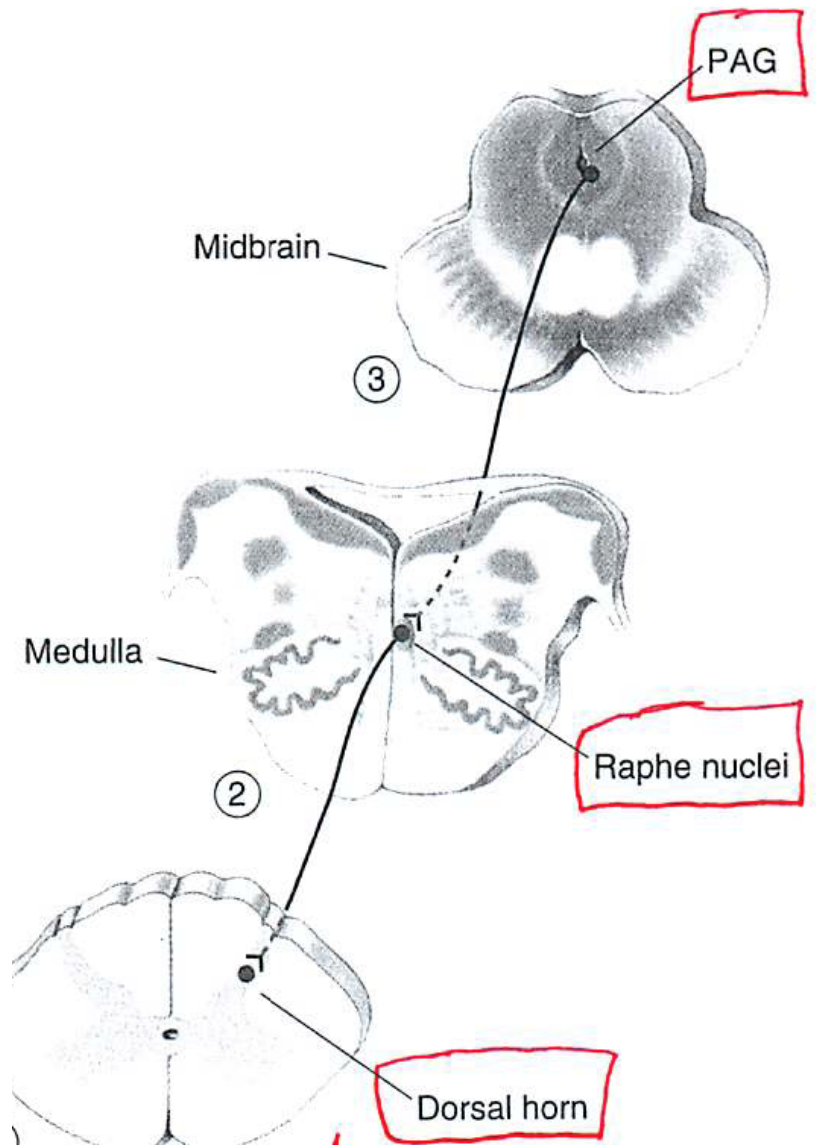
"One consequence of cortisol's action is that neurons admit more Ca^{++} through voltage-gated ion channels."

"Too much Ca^{++} can be a bad thing. If neurons become overloaded with Ca^{++} , they die (excitotoxicity)."

"Several brain regions are implicated in pain suppression, including the periaqueductal gray matter (PAG)."

"Electrical stimulation of the PAG can cause profound analgesia."

"PAG neurons send descending axons into various midline regions of the medulla, particularly to the *raphe nuclei*, which use the neurotransmitter serotonin. These medullary neurons project axons down to the dorsal horns of the spinal cord, where they can effectively depress the activity of nociceptive neurons."



"Opioids produce profound analgesia when taken systemically. They also produce mood changes, drowsiness, mental clouding, nausea, vomiting, and constipation."

**Exogenous opioids include opium, morphine, codeine, and heroin.
Endogenous opioids are called endorphins.**

Both exogenous and endogenous opioids use the opioid receptors that are concentrated in the PAG and raphe nucleus.

Opioids exert multiple effects, including "suppressing the release of glutamate from presynaptic [nociceptive] terminals and inhibiting pain."

Cell Cycle and Cell Death in Disease: Past, Present and Future

Journal of Internal Medicine

2010; 268: pp. 395–409

B. Zhivotovsky and S. Orrenius

From the Institute of Environmental Medicine, Division of Toxicology, Karolinska Institute, Stockholm, Sweden; This article has 75 references.

"Cell division and cell death are the two predominant physiological processes that regulate tissue homeostasis in the adult organism."

"Dysregulation of these processes in the pathogenesis of major diseases, such as cancer, myocardial infarction, stroke, atherosclerosis, infection, inflammation and neurodegenerative disorders, is becoming increasingly evident."

A FEW KEY POINTS FROM THIS ARTICLE

General Principles

"Two predominant physiological processes, cell division and cell death, are known to control tissue homeostasis in the adult organism."

"There is a relationship between inappropriate cell death and premature ageing."

Neurodegeneration and cancer may share a common mechanistic basis.

In the Alzheimer's brain there is an accumulation of amyloid B-peptides (AB). Accumulation of AB has also been detected in brain mitochondria from patients with Alzheimer's disease. AB causes mitochondrial dysfunction and a decrease in ATP levels.

The main mitochondrial target in Alzheimer's disease is *cytochrome c oxidase*.
[Very Important for those using low level laser therapy]

Calcium (Ca++) and Neurodegeneration

There is evidence to suggest that dysregulated Ca++ signaling plays a role in the pathogenesis of Alzheimer's disease.

Various Ca++ channels and pumps in the endoplasmic reticulum appear to be involved in the dysregulation of intracellular Ca++ signaling in Alzheimer's disease.

Some of the Ca++ signaling changes observed in Alzheimer's disease appear to be detrimental to neuronal function and represent a major neuropathological feature of Alzheimer's disease.

These abnormalities appear at the very early stages of the disease, prior to the formation of plaques. **[Important: “very early stages”]**

“Intracellular Ca^{++} overload owing to excessive stimulation of excitatory amino acid receptors and enhanced Ca^{++} influx through membrane channels appears to play an important role in various neurodegenerative disorders as well as in ischaemic brain damage.”

[The two primary excitatory amino acids are glutamate and aspartate]

[The receptor used by both glutamate and aspartate is the N-methyl-D-aspartic acid (NMDA) receptor]

[Ischemic brain damage occurs when ischemia decreases the production of ATP allowing for failure of the protective glutamate/aspartate pump]

“N-methyl-D-aspartic acid (NMDA) receptor/channel blockers prevented both necrosis and apoptosis in primary cultures of glutamate-treated neurons.”

[Mother Nature’s NMDA most effective blocker is one’s level of Mg^{++}]

“It is clear that intracellular Ca^{++} overload is an important factor indifferent in vitro models, and results from clinical studies appear to support the experimental data.”

“The outcome of Ca^{++} overload is in fact neuronal death is most probably determined by the magnitude of the cytoplasmic Ca^{++} rise.”

“The influx of extracellular Ca^{++} through plasma membrane channels results in necrosis.”

Necrosis is involved in NMDA-induced excitotoxicity in cortical neurons, “consistent with a role for excitotoxicity in the pathogenesis of neurodegenerative disease.”

Cancer

The Warburg Effect

“A characteristic feature of many tumour cells is the predominantly glycolytic production of ATP, including under aerobic conditions, known as the ‘Warburg effect.’”

“Cancer cell lines rely on glycolysis for ATP generation to different extents, typically the most glycolytic tumour cells were also found to be the most aggressive.”

“Thus, exploitation of the Warburg effect could represent a novel approach to overcome some of the limits of traditional radio- and chemotherapy, assuming that drugs that suppress glycolysis would have beneficial therapeutic properties.”

The Plant Paradox

The Hidden Dangers in “Healthy” Foods That Cause Disease and Weight Gain

Steven Gundry, MD

**Harper Wave
2017**

“The Cancer Connection”

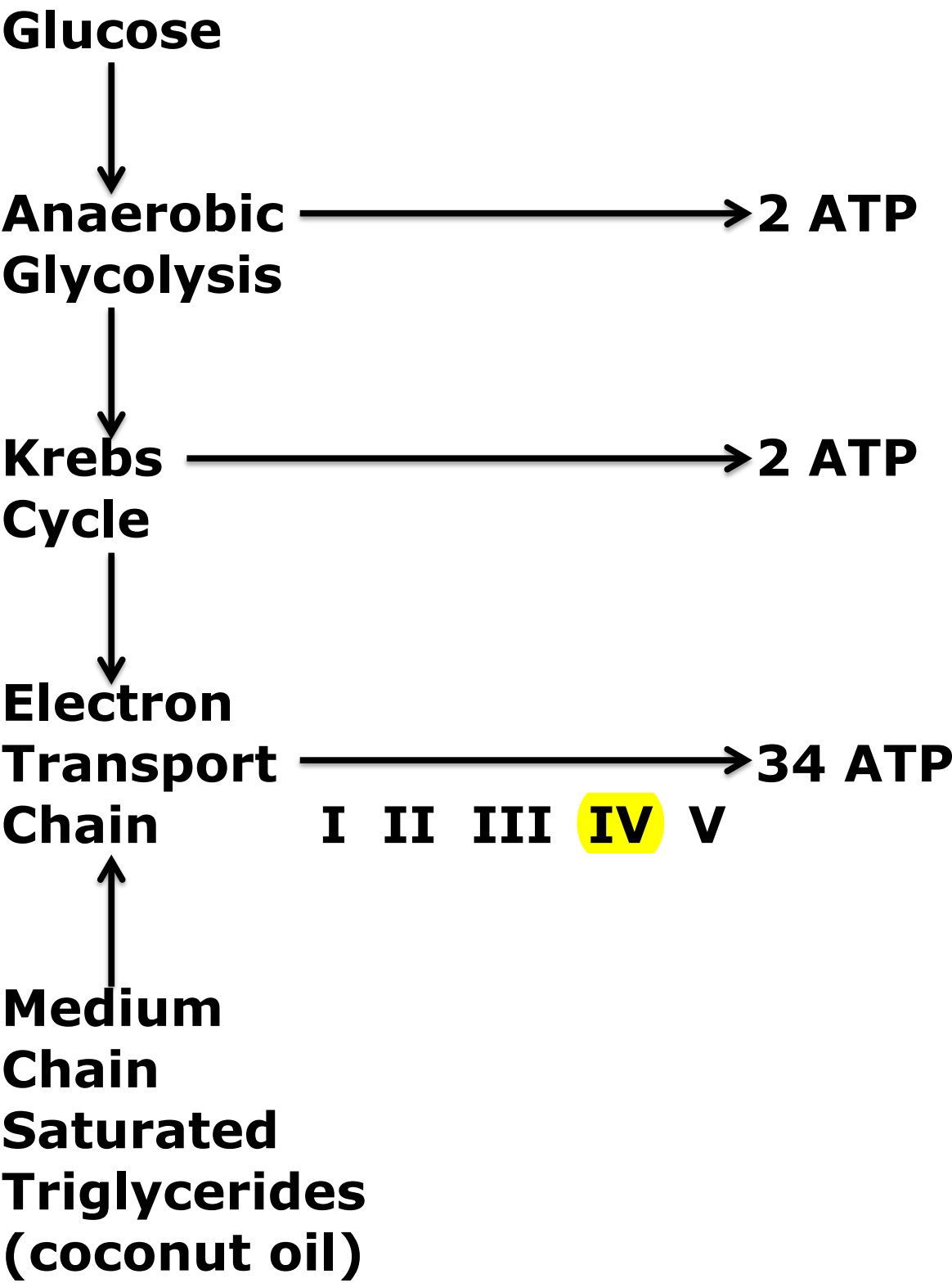
“Let's take a moment to thank Nobel Prize—winning German Physician Otto Warburg, who in the 1930s discovered the Achilles' heel of all cancer cell metabolism. Unlike in normal cells, the mitochondria in cancer cells are unable to use ketones to generate ATP.”

“Nor do they choose to combine sugar with oxygen to generate ATP, the way normal cells act. Instead, cancer cell mitochondria rely on the extremely inefficient system of sugar fermentation [anaerobic glycolysis] also used by yeasts and bacteria.”

“This means that the average cancer cell needs up to eighteen times more sugar to grow and divide than do normal cells.”

“Cancer cells prefer to ferment sugar in the form of fructose rather than glucose, so there's another reason to give fruit the boot.”

“If you or someone you love has cancer, we are going to starve those guys out of existence. While we are starving cancer cells, every other cell in your body, including your brain, can use ketones to power [the mitochondria].”



Neurology. 1990 Aug;40(8):1302-3.

Cytochrome oxidase deficiency in Alzheimer's disease.

Parker WD Jr¹, Filley CM, Parks JK.

Author information

Abstract

We assayed cytochrome oxidase and other electron transport chain activities in platelet mitochondria isolated from patients with Alzheimer's disease (AD). Five of 6 patients had striking reductions of platelet cytochrome oxidase activity (patient mean, 83.72 +/- 82.99 nmol/min/mg; control mean, 167.14 +/- 36.21 nmol/min/mg; n = 8). Other electron transport chain catalytic activities were not significantly different than control values. AD may be a systemic illness, a primary defect in cytochrome oxidase may be pathogenically important in its production, and the mitochondrial genes encoding cytochrome oxidase subunits may be important in producing the defect.

PMID: 2166249

[Indexed for MEDLINE]



Publication type, MeSH terms, Substance, Grant support ☐

LinkOut - more resources ☐

PubMed Commons

[PubMed Commons home](#)

0 comments

[How to join PubMed Commons](#)

PubMed

Format: Abstract**Neurology.** 1994 Jun;44(6):1086-90.

Reduced platelet cytochrome c oxidase activity in Alzheimer's disease.

Parker WD Jr¹, Mahr NJ, **Filley** CM, Parks JK, Hughes D, Young DA, Cullum CM.

Author information

Abstract

We evaluated a simplified method for preparation and analysis of platelet cytochrome c oxidase activity in Alzheimer's disease (AD) and control patients. Mean cytochrome c oxidase activity in controls (n = 17) was 0.233 sec-1/mg whereas mean cytochrome c oxidase activity in Alzheimer patients (n = 19) was 0.193 sec-1/mg, p = 0.033. Complex III (ubiquinol:cytochrome c oxidoreductase), complex II (succinic dehydrogenase), and citrate synthase were all assayed as internal controls and were not significantly different in controls and Alzheimer patients. There is a relatively specific loss of platelet cytochrome c oxidase activity in Alzheimer disease patients.

Comment in

Cytochrome c oxidase in Alzheimer's disease. [Neurology. 1995]

PMID: 8208406

[Indexed for MEDLINE]



Publication type, MeSH terms, Substance, Grant support

LinkOut - more resources

PubMed

Format: Abstract**Neurology.** 1994 Jun;44(6):1090-6.

Electron transport chain defects in Alzheimer's disease brain.

Parker WD Jr¹, Parks J, **Filley CM**, Kleinschmidt-DeMasters BK.

Author information

Abstract

Previous work suggested a deficiency in the terminal complex of the mitochondrial electron transport chain, cytochrome c oxidase (COX), in platelet mitochondria of Alzheimer's disease (AD) patients. The present study extends this observation to AD brain mitochondria through assay of electron transport chain activities in mitochondria isolated from autopsied brain samples from AD patients (n = 9) and from controls with and without known neurologic disease (n = 8). AD brain mitochondria demonstrated a generalized depression of activity of all electron transport chain complexes. This depression was most marked in COX activity ($p < 0.001$). Concentrations of cytochromes b, c1, and aa3 were similar in AD and controls. The electron transport chain is defective in AD brain, and the defect centers about COX.

Comment in

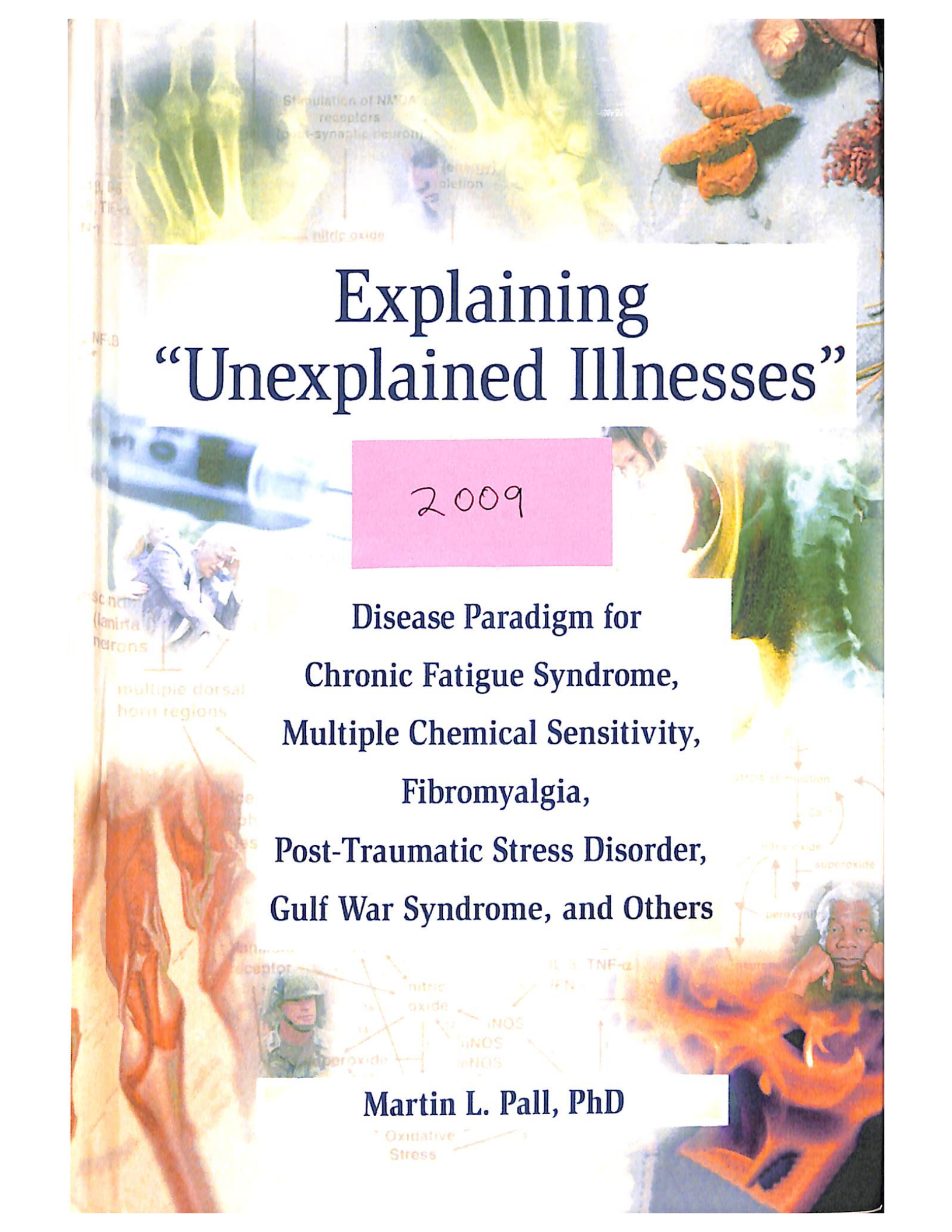
Electron transport chain defects in Alzheimer's disease. [Neurology. 1995]

Cytochrome c oxidase in Alzheimer's disease. [Neurology. 1995]

PMID: 8208407

[Indexed for MEDLINE]

**Publication types, MeSH terms, Substance, Grant support****LinkOut - more resources**



The background is a collage of various images and diagrams. At the top, there's a diagram of a neuron with labels like 'Stimulation of NMDA receptors (post-synaptic neuron)', 'nitric oxide', and 'apoptosis'. To the right, there's a microscopic image of orange, branching structures. Below the title, on the left, is a diagram of a brain section with labels like 'NF-κB', 'TNF-α', and 'IL-1β'. In the center, there's a pink rectangular box with the year '2009' written in it. Below the box, there's a diagram of a person's head and neck with labels like 'multiple dorsal horn regions' and 'pain receptors'. To the right of the box, there's a diagram of a person's head and neck with labels like 'nitric oxide', 'iNOS', 'eNOS', 'peroxide', 'IL-1', 'TNF-α', and 'IFN-γ'. At the bottom right, there's a diagram of a person's head and neck with labels like 'nitric oxide', 'superoxide', 'peroxynitrite', and 'Oxidative Stress'.

Explaining “Unexplained Illnesses”

2009

**Disease Paradigm for
Chronic Fatigue Syndrome,
Multiple Chemical Sensitivity,
Fibromyalgia,
Post-Traumatic Stress Disorder,
Gulf War Syndrome, and Others**

Martin L. Pall, PhD

PubMed



Abstract

Full text links

J Cell Mol Med. 2013 Aug;17(8):958-65. doi: 10.1111/jcmm.12088. Epub 2013 Jun 20.



Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects.

Pall ML¹.

Author information

¹Professor Emeritus of Biochemistry and Basic Medical Sciences, Washington State University, Portland, OR, USA. martin_pall@wsu.edu

Abstract

The direct targets of extremely low and microwave frequency range electromagnetic fields (EMFs) in producing non-thermal effects have not been clearly established. However, studies in the literature, reviewed here, provide substantial support for such direct targets. Twenty-three studies have shown that voltage-gated calcium channels (VGCCs) produce these and other EMF effects, such that the L-type or other VGCC blockers block or greatly lower diverse EMF effects. Furthermore, the voltage-gated properties of these channels may provide biophysically plausible mechanisms for EMF biological effects. Downstream responses of such EMF exposures may be mediated through Ca(2+) /calmodulin stimulation of nitric oxide synthesis. Potentially, physiological/therapeutic responses may be largely as a result of nitric oxide-cGMP-protein kinase G pathway stimulation. A well-studied example of such an apparent therapeutic response, EMF stimulation of bone growth, appears to work along this pathway. However, pathophysiological responses to EMFs may be as a result of nitric oxide-peroxynitrite-oxidative stress pathway of action. A single such well-documented example, EMF induction of DNA single-strand breaks in cells, as measured by alkaline comet assays, is reviewed here. Such single-strand breaks are known to be produced through the action of this pathway. Data on the mechanism of EMF induction of such breaks are limited; what data are available support this proposed mechanism. Other Ca(2+) -mediated regulatory changes, independent of nitric oxide, may also have roles. This article reviews, then, a substantially supported set of targets, VGCCs, whose stimulation produces non-thermal EMF responses by humans/higher animals with downstream effects involving Ca(2+) /calmodulin-dependent nitric oxide increases, which may explain therapeutic and pathophysiological effects.

© 2013 The Author. Journal of Cellular and Molecular Medicine Published by Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd.

PubMed



COVID-19 is an emerging, rapidly evolving situation.

Get the latest public health information from CDC: <https://www.coronavirus.gov>.Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

Full text links

**Format:** AbstractRev Environ Health. 2015;30(2):99-116. doi: 10.1515/reveh-2015-0001.

Scientific evidence contradicts findings and assumptions of Canadian Safety Panel 6: microwaves act through voltage-gated calcium channel activation to induce biological impacts at non-thermal levels, supporting a paradigm shift for microwave/lower frequency electromagnetic field action.

Pall ML.

Abstract

This review considers a paradigm shift on microwave electromagnetic field (EMF) action from only thermal effects to action via voltage-gated calcium channel (VGCC) activation. Microwave/lower frequency EMFs were shown in two dozen studies to act via VGCC activation because all effects studied were blocked by calcium channel blockers. This mode of action was further supported by hundreds of studies showing microwave changes in calcium fluxes and intracellular calcium $[Ca^{2+}]_i$ signaling. The biophysical properties of VGCCs/similar channels make them particularly sensitive to low intensity, non-thermal EMF exposures. Non-thermal studies have shown that in most cases pulsed fields are more active than are non-pulsed fields and that exposures within certain intensity windows have much large biological effects than do either lower or higher intensity exposures; these are both consistent with a VGCC role but inconsistent with only a heating/thermal role. Downstream effects of VGCC activation include calcium signaling, elevated nitric oxide (NO), NO signaling, peroxynitrite, free radical formation, and oxidative stress. Downstream effects explain repeatedly reported biological responses to non-thermal exposures: oxidative stress; single and double strand breaks in cellular DNA; cancer; male and female infertility; lowered melatonin/sleep disruption; cardiac changes including tachycardia, arrhythmia, and sudden cardiac death; diverse

neuropsychiatric effects including depression; and therapeutic effects. Non-VGCC non-thermal mechanisms may occur, but none have been shown to have effects in mammals. Biologically relevant safety standards can be developed through studies of cell lines/cell cultures with high levels of different VGCCs, measuring their responses to different EMF exposures. The 2014 Canadian Report by a panel of experts only recognizes thermal effects regarding safety standards for non-ionizing radiation exposures. Its position is therefore contradicted by each of the observations above. The Report is assessed here in several ways including through Karl Popper's assessment of strength of evidence. Popper argues that the strongest type of evidence is evidence that falsifies a theory; second strongest is a test of "risky prediction"; the weakest confirms a prediction that the theory could be correct but in no way rules out alternative theories. All of the evidence supporting the Report's conclusion that only thermal effects need be considered are of the weakest type, confirming prediction but not ruling out alternatives. In contrast, there are thousands of studies apparently falsifying their position. The Report argues that there are no biophysically viable mechanisms for non-thermal effects (shown to be false, see above). It claims that there are many "inconsistencies" in the literature causing them to throw out large numbers of studies; however, the one area where it apparently documents this claim, that of genotoxicity, shows no inconsistencies; rather it shows that various cell types, fields and end points produce different responses, as should be expected. The Report claims that cataract formation is produced by thermal effects but ignores studies falsifying this claim and also studies showing $[Ca^{2+}]_i$ and VGCC roles. It is time for a paradigm shift away from only thermal effects toward VGCC activation and consequent downstream effects.

PMID: 25879308 DOI: [10.1515/reveh-2015-0001](https://doi.org/10.1515/reveh-2015-0001)[Indexed for MEDLINE] [Free full text](#)

Publication type, MeSH terms, Substance☐

LinkOut - more resources☐

PubMed



COVID-19 is an emerging, rapidly evolving situation.

Get the latest public health information from CDC: <https://www.coronavirus.gov>.Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

Full text links

ELSEVIER
OPEN ACCESS**Format:** AbstractJ Chem Neuroanat. 2016 Sep;75(Pt B):43-51. doi: 10.1016/j.jchemneu.2015.08.001. Epub 2015 Aug 21.

Microwave frequency electromagnetic fields (EMFs) produce widespread neuropsychiatric effects including depression.

Pall ML¹.

Author information

- 1 Professor Emeritus of Biochemistry and Basic Medical Sciences, Washington State University, 638 NE 41st Avenue, Portland, OR 97232-3312, USA. Electronic address: martin_pall@wsu.edu.

Abstract

Non-thermal microwave/lower frequency electromagnetic fields (EMFs) act via voltage-gated calcium channel (VGCC) activation. Calcium channel blockers block EMF effects and several types of additional evidence confirm this mechanism. Low intensity microwave EMFs have been proposed to produce neuropsychiatric effects, sometimes called microwave syndrome, and the focus of this review is whether these are indeed well documented and consistent with the known mechanism(s) of action of such EMFs. VGCCs occur in very high densities throughout the nervous system and have near universal roles in release of neurotransmitters and neuroendocrine hormones. Soviet and Western literature shows that much of the impact of non-thermal microwave exposures in experimental animals occurs in the brain and peripheral nervous system, such that nervous system histology and function show diverse and substantial changes. These may be generated through roles of VGCC activation, producing excessive neurotransmitter/neuroendocrine release as well as oxidative/nitrosative stress and other responses. Excessive VGCC activity has been shown from genetic polymorphism studies to have roles in producing neuropsychiatric changes in humans. Two U.S. government reports from the 1970s to 1980s provide evidence for many neuropsychiatric

effects of non-thermal microwave EMFs, based on occupational exposure studies. 18 more recent epidemiological studies, provide substantial evidence that microwave EMFs from cell/mobile phone base stations, excessive cell/mobile phone usage and from wireless smart meters can each produce similar patterns of neuropsychiatric effects, with several of these studies showing clear dose-response relationships. Lesser evidence from 6 additional studies suggests that short wave, radio station, occupational and digital TV antenna exposures may produce similar neuropsychiatric effects. Among the more commonly reported changes are sleep disturbance/insomnia, headache, depression/depressive symptoms, fatigue/tiredness, dysesthesia, concentration/attention dysfunction, memory changes, dizziness, irritability, loss of appetite/body weight, restlessness/anxiety, nausea, skin burning/tingling/dermographism and EEG changes. In summary, then, the mechanism of action of microwave EMFs, the role of the VGCCs in the brain, the impact of non-thermal EMFs on the brain, extensive epidemiological studies performed over the past 50 years, and five criteria testing for causality, all collectively show that various non-thermal microwave EMF exposures produce diverse neuropsychiatric effects.

Copyright © 2015 The Author. Published by Elsevier B.V. All rights reserved.

KEYWORDS: Excessive calcium effects; Low-intensity microwave electromagnetic fields; Oxidative/nitrosative stress

PMID: 26300312 DOI: [10.1016/j.jchemneu.2015.08.001](https://doi.org/10.1016/j.jchemneu.2015.08.001)

[Indexed for MEDLINE] [Free full text](#)



Publication type, MeSH terms, Substance

☐

LinkOut - more resources

☐

Electrosmog and Autoimmune Disease

Immunology Research

February 2017; Vol. 65; No. 1; pp. 129–135

Trevor G. Marshall and Trudy J. Rumann Heil

Humans are constantly exposed to electromagnetic radiation, primarily from human-made sources such as televisions, cellular phones, and Wi-Fi, all of which use microwave frequency bands.

Recently, anti-collision vehicle radars are exposing humans to WiGig (gigahertz) radiations.

These new electromagnetic exposures have increased human exposure more than 1,000-fold. This increased exposure is termed "electrosmog." "Electrosmog" describes the electromagnetic waves surrounding us in our environment.

Human physiology operates in part via electromagnetic fields, and hence it is plausible that electrosmog would influence human biology.

KEY POINTS FROM THIS ARTICLE:

- 1) "Electromagnetic waves in electrosmog exert sufficient force to affect biological function."
- 3) Animal studies have shown that environmental electromagnetic waves tend to suppress the immune system with a potency similar to NSAIDs.
- 4) According to NASA:
"As you sit watching TV, not only are there visible light waves from the TV striking your eyes, but also radio waves, transmitting from a nearby station, and microwaves carrying cellphone calls and text messages, and waves from your neighbor's WiFi, and GPS units in the cars driving by. There is a chaos of waves from all across the spectrum passing through your room right now."
 - "Every year, the quantity and nature of radio and microwaves contained in this Electrosmog increases."
- 5) "Until the 1950s, electrosmog frequencies remained out of the microwave region, but by the 1960s television channels began microwave transmissions."
- 6) Cellular phone technologies emerged during the 1980s; WiFi during the 1990s. Both extensively use microwave frequency bands.

7) "The recent release of WiGig and anti-collision vehicle radars in the 60 GHz region embody a 1000-fold increase in frequency, and photon energy, over the exposures mankind experienced up until the 1950s."

8) There is evidence that low-level non-ionizing electromagnetic radiation will suppress the immune response in animals.

9) "Proteins are continually in motion, responsive to electromagnetic waves."

10) Lysozyme is an antimicrobial enzyme that is unstable and non-ionizing electromagnetic radiation alters its "proper biological function."

11) The human Vitamin-D receptor (VDR) and its ligand, 1,25-dihydroxyvitamin-D, are associated with many chronic inflammatory and autoimmune diseases. "The VDR pathway exhibits a susceptibility to electrosmog."

- The primary electromagnetic range that affects the VDR is in the microwave frequency range, which is currently populated by cellphone and WiFi communication signals.
- The action of electrosmog on human proteins includes an alteration in the atoms of the VDR.
- The hydrogen bond in the VDR exhibited structural resonances at frequencies typically found in modern electrosmog.
- The VDR molecule changes shape with characteristic frequencies found in today's electrosmog.
- The VDR is even more susceptible when bound with its natural ligand, 1,25 dihydroxyvitamin-D.

12) WiFi routers comprise a significant proportion of indoor electrosmog.

13) The reported bio-effects of electromagnetic radiation from cellular towers include:

- Headaches, concentration difficulties and behavioral problems in children and adolescents.
- Sleep disturbances, headaches and concentration problems in adults.

14) Microwave shielding sleeping caps and clothing are commercially available. "This clothing typically has silver-coated polyester threads interwoven with the supporting fabric so that the garment is capable of partially blocking microwave electrosmog."

Microwave Shielding Sleeping Cap



15) These authors used 64 patients with a variety of immune diagnoses including arthritis, lupus, multiple sclerosis, Sjogrens and celiac. They had them wear protective caps with the silver threads to block the electromagnetic fields and to protect the brain from microwave electrosmog, 4 hours at night and 4 hours during the day.

- 90% reported a *definite* or *strong* change in their symptoms.
- "Follow-up reports have indicated a durable response over many months."
- When the electrosmog in a patient's environment is reduced, it changes the function of the immune system.

16) "Autoimmune patients seem predisposed to electrosmog hypersensitivity at levels currently existing in typical home and work environments, and this factor may be affecting their therapeutic response." **[Key Point]**

17) Biological molecules are constantly moving and interact with electromagnetic fields, including electrosmog.

18) "We cannot ignore the increasing body of evidence showing electromagnetic effects on the immune system."

19) The high rate of electromagnetic hypersensitivity in a healthy population "suggests that effective control of environmental electrosmog immunomodulation may soon become necessary for successful therapy of autoimmune disease." **[Important]**

COMMENTS FROM DAN MURPHY

The improvement of autoimmune disease symptoms with shielding of electrosmog may be as a consequence of its effect on the vitamin D receptor. In this study, such shielding made significant symptom changes in 90% of autoimmune patients. See:

Article Review 42-17: **Vitamin D in Autoimmunity**

Format: Abstract**Full text links**

Sci Rep. 2017 Dec 13;7(1):17541. doi: 10.1038/s41598-017-16623-8.



Exposure to Magnetic Field Non-Ionizing Radiation and the Risk of Miscarriage: A Prospective Cohort Study.

Li DK¹, Chen H², Ferber JR², Odouli R², Quesenberry C².

Author information

- 1 Division of Research, Kaiser Foundation Research Institute, Kaiser Permanente, Oakland, CA, USA. dkl@dor.kaiser.org.
- 2 Division of Research, Kaiser Foundation Research Institute, Kaiser Permanente, Oakland, CA, USA.

Abstract

Magnetic field (MF) non-ionizing radiation is widespread and everyone is exposed to some degree. This prospective cohort study of 913 pregnant women examined the association between high MF exposure and miscarriage risk. Cox (proportional hazards) regression was used to examine the association. After controlling for multiple other factors, women who were exposed to higher MF levels had 2.72 times the risk of miscarriage (hazard ratio = 2.72, 95% CI: 1.42-5.19) than those with lower MF exposure. The increased risk of miscarriage associated with high MF was consistently observed regardless of the sources of high MF. The association was much stronger if MF was measured on a typical day of participants' pregnancies. The finding also demonstrated that accurate measurement of MF exposure is vital for examining MF health effects. This study provides fresh evidence, directly from a human population, that MF non-ionizing radiation could have adverse biological impacts on human health.

PMID: 29235463 PMCID: PMC5727515 DOI: 10.1038/s41598-017-16623-8

Free PMC Article

Grant support

PubMed



COVID-19 is an emerging, rapidly evolving situation.

Get the latest public health information from CDC: <https://www.coronavirus.gov>.Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

Full text links

ELSEVIER
OPEN ACCESS**Format:** AbstractEnviron Res. 2018 Jul;164:405-416. doi: 10.1016/j.envres.2018.01.035. Epub 2018 Mar 21.

Wi-Fi is an important threat to human health.

Pall ML¹.

Author information

- 1 Washington State University, 638 NE 41st Avenue, Portland, OR 97232-3312, USA.
Electronic address: martin_pall@wsu.edu.

Abstract

Repeated Wi-Fi studies show that Wi-Fi causes oxidative stress, sperm/testicular damage, neuropsychiatric effects including EEG changes, apoptosis, cellular DNA damage, endocrine changes, and calcium overload. Each of these effects are also caused by exposures to other microwave frequency EMFs, with each such effect being documented in from 10 to 16 reviews. Therefore, each of these seven EMF effects are established effects of Wi-Fi and of other microwave frequency EMFs. Each of these seven is also produced by downstream effects of the main action of such EMFs, voltage-gated calcium channel (VGCC) activation. While VGCC activation via EMF interaction with the VGCC voltage sensor seems to be the predominant mechanism of action of EMFs, other mechanisms appear to have minor roles. Minor roles include activation of other voltage-gated ion channels, calcium cyclotron resonance and the geomagnetic magnetoreception mechanism. Five properties of non-thermal EMF effects are discussed. These are that pulsed EMFs are, in most cases, more active than are non-pulsed EMFs; artificial EMFs are polarized and such polarized EMFs are much more active than non-polarized EMFs; dose-response curves are non-linear and non-monotone; EMF effects are often cumulative; and EMFs may impact young people more than adults. These general findings and data presented earlier on Wi-Fi effects were used to assess the Foster and Moulder (F&M) review of Wi-Fi. The F&M study claimed that there

PubMed



COVID-19 is an emerging, rapidly evolving situation.

Get the latest public health information from CDC: <https://www.cdc.gov/>Get the latest research from NIH: <https://www.nih.gov/>

Full text links

WILEY

Full Text
Article

PMC

FREE
Full text

Format: Abstract

Environ Mol Mutagen. 2020 Feb;61(2):276-290. doi: 10.1002/em.22343. Epub 2019 Nov 13.

Evaluation of the genotoxicity of cell phone radiofrequency radiation in male and female rats and mice following subchronic exposure.

Smith-Roe SL¹, Wyde ME¹, Stout MD¹, Winters JW², Hobbs CA², Shepard KG², Green AS², Kissling GE³, Shockley KR³, Tice RR¹, Bucher JR¹, Witt KL¹.

Author information

- 1 Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.
- 2 Integrated Laboratory Systems, Inc., Research Triangle Park, North Carolina.
- 3 Biostatistics and Computational Biology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Abstract

The National Toxicology Program tested two common radiofrequency radiation (RFR) modulations emitted by **cellular** telephones in a 2-year rodent cancer bioassay that included interim assessments of additional animals for genotoxicity endpoints. Male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice were exposed from Gestation day 5 or Postnatal day 35, respectively, to code division multiple access (CDMA) or global system for mobile modulations over 18 hr/day, at 10-min intervals, in reverberation chambers at specific absorption rates of 1.5, 3, or 6 W/kg (rats, 900 MHz) or 2.5, 5, or 10 W/kg (mice, 1,900 MHz). After 19 (rats) or 14 (mice) weeks of exposure, animals were examined for evidence of RFR-associated genotoxicity using two different measures. Using the alkaline (pH > 13) comet assay, DNA damage was assessed in **cells** from three brain regions, liver **cells**, and peripheral blood leukocytes; using the micronucleus assay, chromosomal damage was

assessed in immature and mature peripheral blood erythrocytes. Results of the comet assay showed significant increases in DNA damage in the frontal cortex of male mice (both modulations), leukocytes of female mice (CDMA only), and hippocampus of male rats (CDMA only). Increases in DNA damage judged to be equivocal were observed in several other tissues of rats and mice. No significant increases in micronucleated red blood cells were observed in rats or mice. In conclusion, these results suggest that exposure to RFR is associated with an increase in DNA damage. Environ. Mol. Mutagen. 61:276-290, 2020.

© 2019 Wiley Periodicals, Inc.

KEYWORDS: DNA damage; Sprague Dawley; brain; comet assay; glioma; micronucleus assay

PMID: 31633839 PMCID: [PMC7027901](#) DOI: [10.1002/em.22343](#)

Free PMC Article



Images from this publication. [See all images \(3\)](#) [Free text](#)

Grant support

☐

LinkOut - more resources

☐

Calcium Supplementation and Risk of Dementia in Women with Cerebrovascular Disease

Neurology

October 18, 2016; Vol. 87; pp. 1674–1680

Jurgen Kern, MD, PhD; Silke Kern, MD, PhD; Kaj Blennow, MD, PhD; Henrik Zetterberg, MD, PhD; Margda Waern, MD, PhD; Xinxin Guo, MD, PhD; Anne Börjesson-Hanson, MD, PhD; Ingmar Skoog, MD, PhD; Svante Östling, MD, PhD

This article has 40 references.

This study included 700 Swedish women aged 70–92 years, followed for 5 years. The average recommended dose of calcium supplements in Sweden is 1,000 mg daily

KEY POINTS FROM THIS ARTICLE:

- 1) “Dementia and osteoporosis are leading causes of disability in the elderly. Because calcium deficiency contributes to osteoporosis, daily dietary calcium intake of 1,000–1,200 mg is recommended. Such a large calcium intake through diet alone can be difficult; therefore, calcium supplements are widely used.”
- 2) The objective of this study was to determine whether calcium supplementation is associated with the development of dementia in women after a 5-year follow-up.
- 3) Calcium supplementation may increase the risk of developing dementia in elderly women.
- 4) “Women treated with calcium supplements had a higher risk of developing dementia (increased relative risk by 110%) and the subtype dementia with stroke (increased risk by 340%) than the women who did not take calcium supplementation.”
- 5) “We found a relationship between calcium supplementation and increased risk for dementia in elderly women in this 5-year follow-up study.”
- 6) “The frequency of fractures was 40.8% in the group that received calcium supplements and 20.8% in the group that did not.” **[Crazy, fewer fractures in women not taking calcium supplements, by half]**
- 7) “Calcium supplementation might have direct toxic effects on vulnerable neurons, because the increased calcium levels may amplify ischemic cell death and worsen the outcome after cerebrovascular events.” **[Key Point]**

- 8) There is a steep increase in serum calcium levels caused by calcium supplements. Neurons are especially vulnerable to the excitatory and excitotoxic effects of the calcium-level peaks caused by the supplementation.
- 9) "Calcium plays a central role in the mechanisms of cell death." **[Key Point]**
- 10) "Blocking calcium influx via NMDA receptors prevents both necrosis and apoptosis. This indicates the crucial role of calcium influx and intracellular calcium overload in the genesis of apoptosis and necrosis."
- 11) Calcium supplements may also potentiate changes in the vessel walls leading to atherosclerosis. "Calcium supplementation stimulates vascular calcification by abnormal extra-osseous deposition in atherosclerotic plaques."

A SIMPLE MODEL FROM DAN MURPHY

The brain's excitatory neurotransmitters, glutamate and aspartate, attach to the neuron's NMDA receptor.

Calcium enters the neuron enhancing free radical production and neuronal excitotoxicity, potentially causing neuronal sickness and death.

Higher levels of calcium enhance this process.

Magnesium blocks the calcium entry into the neuron, and as such is neuroprotective.

Higher levels of calcium through supplementation and lower levels of magnesium render neurons vulnerable to excitotoxic injury, death, and dementia.

Sugar- and Artificially Sweetened Beverages and the Risks of Incident Stroke and Dementia: A Prospective Cohort Study

Stroke
April 20, 2017; Vol. 48: pp. 1139-1146 [epub]

Matthew P. Pase, PhD; Jayandra J. Himali, PhD; Alexa S. Beiser, PhD; Hugo J. Aparicio, MD; Claudia L. Satizabal, PhD; Ramachandran S. Vasan, MD; Sudha Seshadri, MD; Paul F. Jacques, DS:

Primary authors are from **Boston University School of Medicine.**

This Article has 25 references.

This article used the community-based Framingham Heart Study Offspring cohort.

It used **2,888 participants** aged >45 years for stroke and 1,484 participants aged >60 years for dementia, and **the study extended for 10 years.**

The authors looked at these three combinations:

- Intake of total sugary beverages (combining sugar-sweetened soft drinks, fruit juice, and fruit drinks).
- Intake of sugar-sweetened soft drinks (high-sugar carbonated beverages, such as cola).
- Intake of artificially sweetened soft drinks (sugar-free carbonated beverages, such as diet cola).

KEY POINTS FROM THIS ARTICLE:

- 1) "Sugar- and artificially-sweetened beverage intake have been linked to cardiometabolic risk factors, which increase the risk of cerebrovascular disease and dementia."
- 2) Artificially sweetened beverages are typically sweetened with non-nutritive sweeteners, such as saccharin, acesulfame, aspartame, neotame, or sucralose.
- 3) "Greater recent consumption of artificially sweetened soft drinks was associated with an increased risk of stroke, with the strongest associations observed for ischemic stroke."
- 4) "Higher cumulative intake of artificially sweetened soft drinks was also associated with an increased risk of ischemic stroke."
- 5) **"In our community-based cohort, higher consumption of artificially sweetened soft drinks was associated with an increased risk of both stroke and dementia."**

6) "When comparing daily cumulative intake [of artificial sweeteners] to 0 per week (reference), the hazard ratios were 2.96 for ischemic stroke and 2.89 for Alzheimer's disease." **[This means that drinking intake of artificially sweetened soft drinks essentially tripled the risk of suffering both ischemic stroke and Alzheimer's].**

[The upper end of the risk range was an approximate 700% increased risk].

7) "When examining cumulative beverage consumption, daily intake of artificially sweetened soft drink was associated with an increased risk of both all-cause dementia and AD dementia."

8) "To our knowledge, our study is the first to report an association between daily intake of artificially sweetened soft drink and an increased risk of both all-cause dementia and dementia because of Alzheimer's disease."

9) Diabetes is an important risk factor for both stroke and dementia; importantly, both sugar intake (including sweetened drinks) intake and artificially sweetened drinks intake increase the risk of diabetes. "Diabetes mellitus status was identified as a potential mediator of the association between artificially sweetened beverage intake and the risk of both incident all-cause dementia and AD dementia."

10) Artificial sweeteners cause glucose intolerance by altering gut microbiota and are associated with dysbiosis and glucose intolerance.

11) "After adjustments for age, sex, education (for analysis of dementia), caloric intake, diet quality, physical activity, and smoking, higher recent and higher cumulative intake of artificially sweetened soft drinks were associated with an increased risk of ischemic stroke, all-cause dementia, and Alzheimer's disease dementia."

12) Conclusions: "Artificially sweetened soft drink consumption was associated with a higher risk of stroke and dementia." "The consumption of artificially sweetened soft drinks is increasing in the community, along with the prevalence of stroke and dementia." **[Important]**

13) "Sugar-sweetened beverages were not associated with stroke or dementia."

**THIS ARTICLE GENERATED THIS EDITORIAL:
Sugar-Sweetened and Artificially Sweetened Beverages in Relation to
Stroke and Dementia:
Are Soft Drinks Hard on the Brain?**

Editorial

Stroke

April 20, 2017; Vol. 48; pp. 1129-1131 [epub]

Heike Wersching, MD; Hannah Gardener, SCD; Ralph L. Sacco, MD

From the University of Munster, Institute for Epidemiology and Social Medicine, Germany, and the University of Miami Miller School of Medicine, Department of Neurology, FL.

This editorial has 26 references.

- 1) "Sugar-sweetened beverages are the leading sources of added sugars in the US diet and are increasing on a global level."
- 2) "A single can of sugar-sweetened soda contains about the upper limit of the recommended 25 to 50 g per day."
- 3) "The harmful effects of regular sugar-sweetened soda consumption, including weight gain, the metabolic syndrome, and type 2 diabetes mellitus, have been demonstrated in numerous large observational studies."
- 4) "A higher intake of sugar-sweetened soda has been repeatedly associated with increased risks of hypertension, coronary heart disease, and stroke, as well as with adverse changes in lipid levels and inflammatory markers."
- 5) "Artificially sweetened beverages are marketed as healthier alternatives to sugar-sweetened soda. Their consumption is rising in the United States, particularly among children."
- 6) Several large observational studies show a positive association between diet soda consumption and increased risks of the metabolic syndrome and type-2 diabetes mellitus.
- 7) Prior studies have shown diet soda consumption is associated with an increased risk of stroke, myocardial infarction, and vascular death.
- 8) In the present study, "higher recent and cumulative intake of artificially sweetened soft drinks was associated with an increased risk of ischemic stroke, all-cause dementia, and Alzheimer's dementia." "The effects persisted when analyses were adjusted for total caloric intake, diet quality, physical activity, and smoking status."
- 9) "Artificial sweeteners may increase cravings for high glycemic and high-calorie foods, induce glucose intolerance, or impair caloric compensation, thereby increasing calorie intake and body weight." **[Important]**
- 10) Artificially sweetened beverages may increase the risk of vascular disease and dementia by these mechanisms:
 - Advanced glycation end products, which are produced during the process of caramelization used in some artificially sweetened beverages and sugar-sweetened soda are pro-inflammatory and promote insulin resistance. **[Important]**

- They damage the microbiome, increasing insulin resistance.
- They contain phosphoric acid [as do sugar sweetened beverages] which damages the vascular system.

11) "The growing number of epidemiological studies showing strong associations between frequent consumption of artificially sweetened beverages and vascular outcomes suggests that it may not be reasonable to substitute or promote artificially sweetened beverages as healthier alternatives to sugar-sweetened beverages."

12) "Both sugar-sweetened and artificially sweetened soft drinks may be hard on the brain." **[Key Point]**

COMMENTS FROM DAN MURPHY

We have reviewed a number of studies pertaining to diet soda consumption. They show that regular consumption of artificially-sweetened beverages is not good for one's health:

Article Review 16-05:

Aspartame Induces Lymphomas and Leukaemias in Rats; Aspartame, a Leukaemogenic Compound; 2005

Article Review 14-08:

Life-Span Exposure to Low Doses of Aspartame Beginning During Prenatal Life Increases Cancer Effects in Rats; 2007

Article Review 04-13:

Diet Soft Drink Consumption is Associated with an Increased Risk of Vascular Events in the Northern Manhattan Study; 2012

Article Review 15-13:

Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain; 2008

Article Review 24-15:

Gain weight by "going diet?" Artificial Sweeteners and the Neurobiology of Sugar Cravings; 2010

Article Review 28-15

Consumption of Artificial Sweetener- and Sugar-containing Soda and Risk of Lymphoma and Leukemia in Men and Women; 2012



HHS Public Access

Author manuscript

Cell Calcium. Author manuscript; available in PMC 2019 March 01.

Published in final edited form as:

Cell Calcium. 2018 March ; 70: 47–55. doi:10.1016/j.ceca.2017.05.007.

The role of Ca^{2+} in cell death caused by oxidative glutamate toxicity and ferroptosis

Pamela Maher¹, Klaus van Leyen², Partha Narayan Dey³, Birgit Honrath⁴, Amalia Dolga⁴, and Axel Methner^{3,#}

¹Cellular Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, Ca, USA

²Harvard Medical School, Cambridge, Ma, USA ³University Medical Center and Focus Program Translational Neuroscience (FTN) of the Johannes Gutenberg University Mainz, Department of Neurology, Mainz, Germany ⁴Department of Molecular Pharmacology, University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, The Netherlands

Abstract

Ca^{2+} ions play a fundamental role in cell death mediated by oxidative glutamate toxicity or oxytosis, a form of programmed cell death similar and possibly identical to other forms of cell death like ferroptosis. Ca^{2+} influx from the extracellular space occurs late in a cascade characterized by depletion of the intracellular antioxidant glutathione, increases in cytosolic reactive oxygen species and mitochondrial dysfunction. Here, we aim to compare oxidative

Loss of glutathione and consequent oxidative stress is a feature of many diseases, especially those involving ischemia.

This is particularly relevant in brain pathologies, notably stroke. In neurons, glutathione represents the major antioxidant defense, so maintaining adequate amounts of reduced glutathione is critical.

Following a stroke, intracerebral glutathione levels drop within the first several hours and Ca^{2+} becomes dysregulated.

Supplementing glutathione from exogenous sources, which at first glance would appear to be the most straightforward way to counter glutathione loss, has to date not been very successful. Glutathione itself is highly water soluble, but does not readily cross the blood – brain barrier.

The increase in intracellular (Ca^{2+}) and excessive ROS production during oxytosis causes mitochondrial Ca^{2+} overload, extensive mitochondrial fragmentation and breakdown of the mitochondrial membrane potential.

Ca²⁺ overload, irrespective of the signaling pathway that induces it, is detrimental to the cell and induces cell death. Excessive Ca²⁺ influx into mitochondria leads to dissipation of the mitochondrial membrane potential, dysfunction of mitochondrial metabolism and opening of the mitochondrial permeability transition pore to induce cytochrome c-dependent cell death.

Preventing Ca²⁺ overload in conditions of oxidative stress overall restores mitochondrial respiration and blocks subsequent Ca²⁺ dysregulation.

In this review article, we described the signaling pathways that culminate in Ca²⁺ influx and cell death during oxidative glutamate toxicity or oxytosis, a form of programmed cell death caused by glutathione depletion.

Nobel Prize in Medicine Awarded for Work on Senses

BY DENISE ROLAND
AND BRIANNA ABBOTT

WJS

The Nobel Prize in physiology or medicine was jointly awarded to David Julius and Ardem Patapoutian for their work on how the human body senses temperature, touch and movement.

The Nobel Assembly said Monday that the scientists' discoveries had unlocked one of the secrets of nature by explaining the molecular basis for sensing heat, cold and mechanical force.

This basic understanding of how temperature and touch signal the nervous system is now being harnessed to research potential treatments for chronic pain and other medical conditions, the Nobel Assembly said.

"These sensations of heat and pain and touch, if those are all converted to electrical signals and sent to the brain, that opens up a whole new world of potential treatments," said Chad Bouton, a professor at the Feinstein Institutes for Medical Research in New York, whose work in-



David Julius and Ardem Patapoutian were honored for explaining the molecular basis for sensing heat, cold and mechanical force. Above right, Thomas Perlmann, secretary of the Nobel Assembly.

cludes the use of stimulation of the brain to restore the sense of touch to patients who have lost it as a result of a stroke or injury. "It's all made possible by these critical discoveries," he added.

Dr. Julius was born in 1955 in New York and is now a pro-

fessor and chairman of the department of physiology at the University of California, San Francisco. Dr. Patapoutian was born in 1967 in Beirut. He moved to the U.S. in his youth and is currently a professor at Scripps Research in La Jolla, Calif.

While at UCSF in the late 1990s, Dr. Julius used capsaicin, a compound in chili peppers that causes a burning sensation, to identify a chemical receptor in nerve endings of the skin that responds to heat. In separate research, Dr. Patapoutian used pressure-

sensitive cells to discover new receptors that respond to touch in the skin and internal organs.

Both the scientists' work involved identifying the specific genes involved in sensing either heat or touch and then figuring out what those genes did within cells.

Dr. Julius and his team assembled a library of millions of DNA fragments from sensory neurons and found a single gene that made cells sensitive to capsaicin. That gene directs nerve cells to make a so-called ion channel that opens up in response to capsaicin and high temperatures, allowing electrically charged particles called ions to flood into cells and send a pain message to the brain. That receptor was later named TRPV1.

"There is a time when you make a discovery where you are the only person on the planet, or at least you think you're the only person on the planet, who knows the answer to a particular question," Dr. Julius said in a media event. "And that's a really thrilling moment."

That discovery, published in a 1997 paper, sparked a wave of research by large drugmakers hopeful that blocking the TRPV1 receptor could treat chronic pain.

The work also paved the way for the discovery of other ion channels.

One of those channels was a cold-sensing receptor called TRPM8, which Drs. Julius and Patapoutian uncovered independently of one another, using menthol.

Dr. Patapoutian's work on touch uncovered two ion channels, named Piezo1 and Piezo2 after the Greek word for pressure, that open up in response to touch. He later showed that Piezo2 played a key role in how the body senses its position and movement. Subsequent work demonstrated how Piezo1 and Piezo2 help regulate bodily functions such as blood pressure and bladder control.

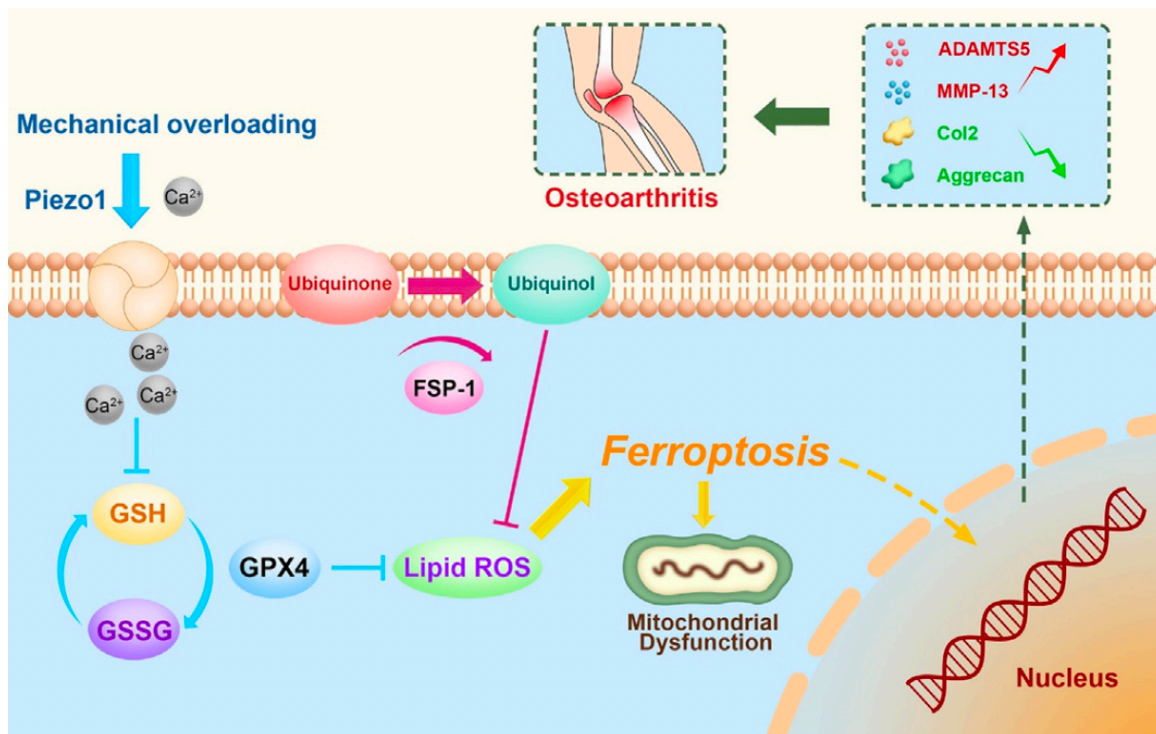
Dr. Patapoutian said that work was under way to find molecules that block the Piezo channels as these could form the basis of drugs for different types of pain.

JONATHAN NACKSTRAND/AGENCE FRANCE-PRESSE/GETTY IMAGES

10/5/2021

Mechanical Overloading Induces Glutathione-Regulated Chondrocyte Ferroptosis in Osteoarthritis via Piezo1 Channel Facilitated Calcium Influx

Journal of Advanced Research
November 2022



A Simplified Model from Dan Murphy

Piezo1 is a **mechanoreceptor** that was mentioned in the **2021 Nobel Prize** in physiology or medicine.

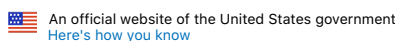
Piezo1 receptors are found in the membrane of chondrocytes.

Mechanical loading (overloading) opens the Piezo1 receptor which allows excessive influx of Ca⁺⁺ to enter into the cell. **[consider magnesium]**

The excessive intracellular Ca⁺⁺ inhibits glutathione which increases chondrocyte oxidative stress, leading to osteoarthritis. **[consider glutathione supplements]**

CoQ10 blocks the oxidative stress driven ferroptosis cartilage damage. **[consider CoQ10; consider donating blood]**

In an integrative narrative, this article has applications for the chiropractic subluxation and adjustment, Ca⁺⁺ excess, Mg⁺⁺ deficiency, glutathione status, and free radical damage. As such it would support not only the chiropractic adjustment, but also diet, nutrition, supplements, and laser therapy.



FULL TEXT LINKS



[Spine \(Phila Pa 1976\)](#). 2022 Mar 1;47(5):E203-E213. doi: 10.1097/BRS.0000000000004203.

Single Impact Injury of Vertebral Endplates Without Structural Disruption, Initiates Disc Degeneration Through Piezo1 Mediated Inflammation and Metabolism Dysfunction

Zhengang Sun ¹, Xinfeng Zheng ², Songbo Li ³, Baozhu Zeng ⁴, Jiaming Yang ⁴, Zemin Ling ⁵, Xizhe Liu ⁵, Fuxin Wei ⁴

Affiliations

PMID: 34431832 PMCID: [PMC8815838](#) DOI: [10.1097/BRS.0000000000004203](#)

[Free PMC article](#)

Abstract

Study design: In vitro experimental study.

Objective: To establish an axial impact injury model of intervertebral disc (IVD) and to investigate if a [single impact injury without endplate structural disruption](#) could initiate intervertebral disc degeneration (IDD), and what is the roles of Piezo1 in this process.

Summary of background data: Although IDD process has been confirmed to be associated with structural failures such as endplate fractures, whether a single impact injury of the endplates without structural disruption could initiate IDD remains controversial. Previous studies reported that Piezo1 mediated inflammation participated in the progression of IDD induced by mechanical stretch; however, the roles of Piezo1 in IVD impact injury remain unknown.

Methods: Rats spinal segments were randomly assigned into Control, Low, and High Impact groups, which were subjected to pure axial impact loading using a custom-made apparatus, and cultured for 14 days. [The degenerative process was investigated by using histomorphology, real-time Polymerase Chain Reaction\(PCR\), western-blot, immunofluorescence, and energy metabolism of IVD cell.](#) The effects of Piezo1 were investigated by using siRNA transfection, real-time PCR, western-blot, and immunofluorescence.

Results: [The discs in both of the impact groups presented degenerative changes after 14 days,](#) which showed significant up-regulation of Piezo1, NLRP3 inflammasome, the catabolic (MMP-9, MMP-13), and pro-inflammatory gene (IL-1 β) expression than that of the control group ($P < 0.05$), accompanied by significantly increased release of ATP, lactate, nitric oxide (NO), and glucose consumption of IVD cells at first 7 days. [Silencing Piezo1 reduced the activation of NLRP3 inflammasome and IL-1 \$\beta\$ expression in the nucleus pulposus induced by impact injury.](#)

Conclusion: [It demonstrated that not only fracture of the endplate but also a single impact injury without structural impairment could also initiate IDD, which might be mediated by activation of Piezo1 induced inflammation and abnormal energy metabolism of IVD cells.](#)Level of Evidence: N/A.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

Excessive Mechanical Stress-induced Intervertebral Disc Degeneration is Related to Piezo1 Overexpression Triggering the Imbalance of Autophagy/Apoptosis in Human Nucleus Pulposus

Arthritis Research & Therapy
May 23, 2022; Vol. 24; No. 1; Article 119

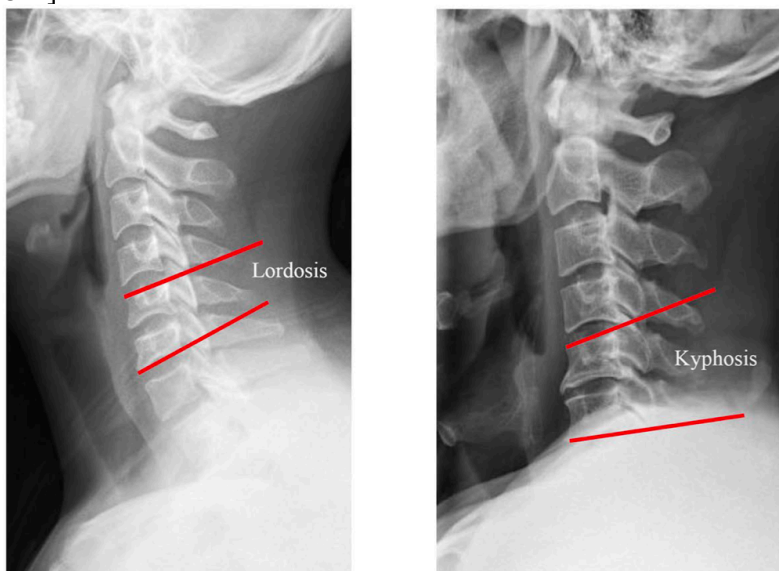
Sheng Shi, Xing-Jian Kang, Zhi Zhou, Zhi-Min He, Shuang Zheng, Shi-Sheng He

This study aimed to investigate the effect of the **Piezo1** mechanoreceptor on the mechanical stress-induced intervertebral disc degeneration (IVDD) and explore the possible mechanism. Immunohistochemistry staining antibodies against **Piezo1** were used in the analysis.

Cervical nucleus pulposus (NP) samples were collected via surgery from 26 patients (12 men and 14 women; age range: 22–65 years).

“In order to explore whether mechanical stress treatment can affect Piezo1 expression and cell function of NP cells, a mechanical compression with different intensity and/or duration was conducted on human NP cells.”

Cervical lordosis was assessed with x-rays using the protocol established by Deed Harrison, DC, and colleagues in the journal *Clinical Biomechanics* in 2001: [Harrison DE, Harrison DD, Janik TJ, William Jones E, Cailliet R, Normand M. Comparison of axial and flexural stresses in lordosis and three buckled configurations of the cervical spine; *Clinical Biomechanics*; May 2001; Vol.16; No. 4; pp. 276–784.]



The angle between the superior endplate of the rostral vertebra and the inferior endplate of the caudal vertebra on the lateral x-ray was measured.

The expression of ***Piezo1***, cervical curvature, and the stiffness of the nucleus pulposus (NP), was performed in normal and degenerated human intervertebral discs.

"Mechanical stress plays a crucial role in the pathogenesis of intervertebral disc degeneration (IVDD)."

"Intervertebral disc degeneration (IVDD) is the leading cause of degenerative spine diseases such as discogenic pain and disc herniation, which reduces the quality of life and increases the socioeconomical burden."

- "The onset and development of IVDD is a complex process involving numerous factors such as biomechanics, aging, genetic factors, nutrition, inflammation, and so on."
- "Mechanical stress as part of the biomechanical factors may contribute to playing a particularly prominent role in the pathogenesis of IVDD."

The nucleus pulposus (NP) "is considered to be the most crucial component for maintaining the pressure gradient and ensuring the infiltration and diffusion of metabolites and nutrients."

- "Abnormal mechanical load can promote cell death in the NP, cause matrix metabolism disorders, and hasten the process of IVDD." **[Important]**
 - "Compressive forces by bad postures or kyphosis may lead to intense stresses that act on the NP." **[Key Point]**
 - "Mechanical stress caused by kyphosis has been linked to the pathological process of IVDD." **[Key Point]**

"The mechanosensitive Piezo1 ion channel can sense the changes in mechanical stress and convert the mechanical signals into chemical signals." **[Key Point]**

- ***Piezo1*** is a new mechanosensitive ion channel, which can "sense the changes of mechanical stress and convert the mechanical signals into electrical or chemical signals."
- "[Piezo1] is widely distributed in a variety of different tissues including bone, articular cartilage and the intervertebral disc."

Findings:

- "Intradiscal pressure was shown to be definitely higher in forward leaning from a sitting position than in relaxed sitting, which indicates that intradiscal

pressure increases with application of compression and is dependent on the lifestyle."

- "The expression of Piezo1 had obviously increased in the NP tissues of the IVDD patients when compared to that of the control group."
- The expression of Type-II Collagen significantly declined in the IVDD group when compared to that in the control group.
- "The protein levels of Type-II Collagen and Aggrecan were markedly suppressed after mechanical stress."

[Aggrecan and Type-II Collagen form the major structural component of cartilage and the intervertebral disc].

- "Apoptosis was significantly increased in mechanical stress-treated NP cells and could be restricted when Piezo1 was inhibited."

Chiropractic Mechanical Connection:

- "The lateral radiograph revealed the local cervical curvature was significantly lower [kyphotic] in the high grade IVDD group."
- "Excessive mechanical stresses are found in the kyphotic areas of sagittal cervical spine when compared to the normal lordosis."
- "The increased mechanical stress induced by kyphosis was considered to be a risk factor for the development of IVDD or disc herniation."
- Cervical kyphosis resulted in the up-expression of **Piezo1** and the down-expression of Type II Collagen.
 - This increased nucleus pulposus stiffness and increased degeneration of human intervertebral discs.
- "The upregulated expression of Piezo1, poor curvature [kyphosis], and increased extracellular matrix (ECM) stiffness in the degenerated human NP."
- Increased **Piezo1** expression reduced **Type-II Collagen** in the degenerated human NP tissue, along with more kyphotic angle in the cervical spine, reveals that the "Piezo1 sensing the excessive stress partly attributed to poor curvature may affect the ECM homeostasis and further change the mechanical properties of NP." **[Key Point]**
- "Our data provide the evidence that Piezo1 upregulation under excessive compression can promote the apoptosis and senescence of NP cells, secrete

pro-inflammatory cytokines, and reduce the synthesis of ECM by mitochondrial dysfunction and the suppression of autophagy in NP."

- "The up-regulation of the mechanosensitive [calcium] ion channel Piezo1 by excessively external compressive stress can increase pro-inflammatory cytokines, and induce mitochondrial dysfunction, which may trigger the apoptosis and senescence of human NP cells via suppressing autophagy and resulting in the loss of ECM."
- This research confirms that "overactivated Piezo1 by excessive compression inhibits the synthesis of major parts in ECM involving the collagen II and aggrecan."
- The upregulation of Piezo 1 enhanced the apoptosis of NP cells and promotes the apoptosis and senescence of NP cells. **[Key Point]**
- "[Both] apoptosis and autophagy were induced by moderate mechanical compression in NP cells."

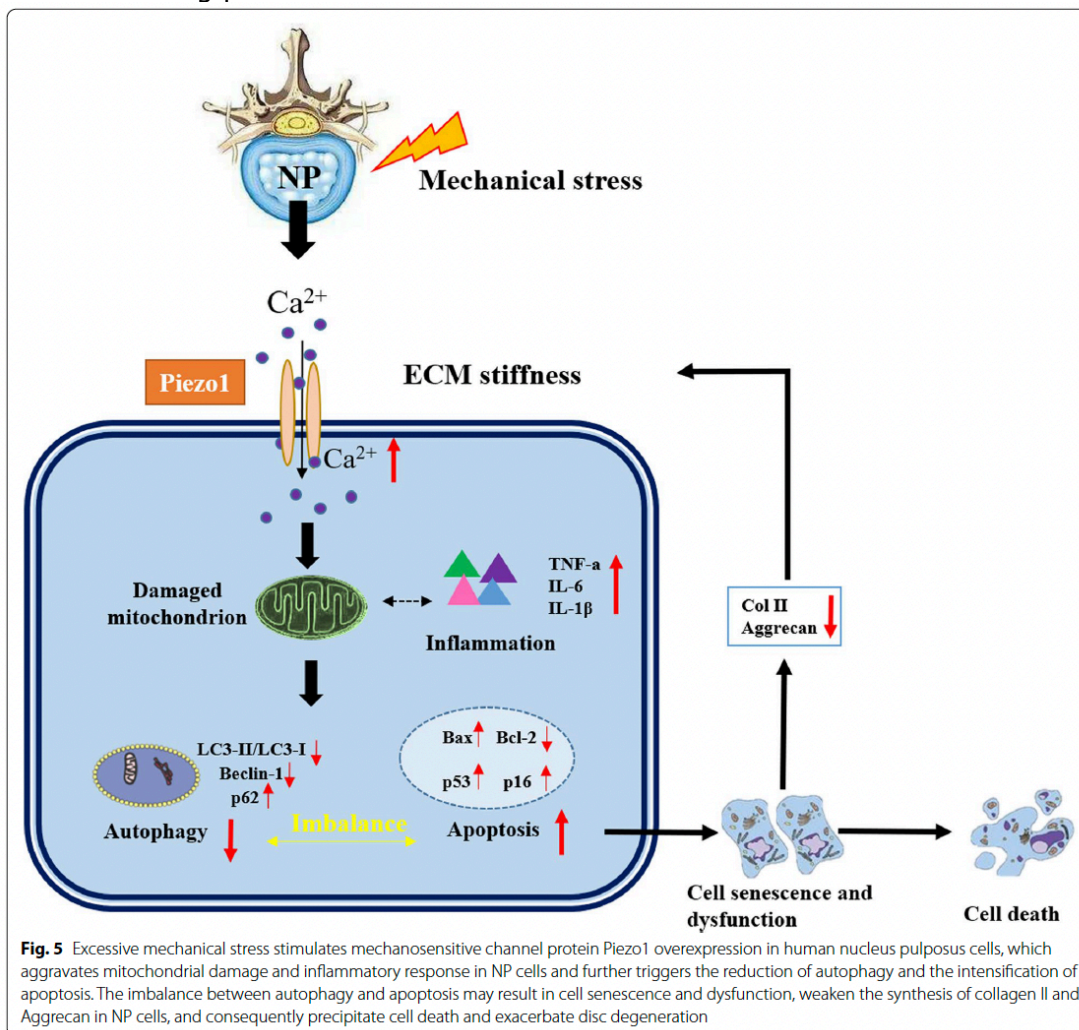
PUTTING IT ALL TOGETHER

Cervical kyphosis drives the following:

- Increases the load on the Piezo1 mechanoreceptor.
- Opens the Ca^{++} ion channel.
- The Ca^{++} influx damages the mitochondria.
- Damaged mitochondria increase inflammatory cytokine production.
- Damaged mitochondria reduce cell repair and the reusing of old and damaged cells or parts of cells. This is **autophagy**.
- Reduced autophagy increases **apoptosis** and **senescence**, leading to cell death.
- **Apoptosis** and **Senescence** reduce the production of **Type-II Collagen** and **Aggrecan**, which degrades the **extracellular matrix**.
- The results are acceleration of degenerative disc disease and spinal stiffness.

This supports correcting cervical kyphosis (we use Dennerolls from CBP), correct subluxations, take Mg^{++} (blocks the Ca^{++} ion channel), use low-level laser therapy (improves mitochondria health and physiology), take mitochondria health supplements (like CoQ10, PQQ, acetyl-L-carnitine, alpha-lipoic acid, etc.).

See the following picture:



Other Article Reviews on Piezo1 mechanoreceptors include:

Article Review 16-23:

Mechanical Loading Induces Piezo1 Calcium Influx and Osteoarthritis

Article Review 42-23:

Injury Initiates Disc Degeneration Through Piezo1 Mediated Inflammation

Other Article Reviews on cervical kyphosis include:

Article Review 24-11:

Is the Kyphosis Cause or Effect of Disc Degenerative Disease

Article Review 52-13:

Focal Kyphosis correction and Neurological Outcomes

Article Review 2-23:

Improvement in Cervical Curve in the Management of Lumbar Radiculopathy

The Emerging Role of Piezo1 Channels in Skeletal Muscle Physiology

Biophysics Review

September 29, 2023; Vol 15; 15; No. 5; pp. 1171-1184

Timur Mirzoev

Piezo1 channels are mechanically activated (MA) cation channels that are involved in sensing of various mechanical perturbations, such as membrane stretch and shear stress, and play a crucial role in cell mechanotransduction.

In response to mechanical stimuli, these channels open up and allow cations to travel into the cell and induce biochemical reactions that can change the cell's metabolism and function.

Skeletal muscle cells/fibers inherently depend upon mechanical cues in the form of fluid shear stress and contractions (physical exercise).

For example, an exposure of skeletal muscles to chronic mechanical loading leads to increased anabolism and fiber hypertrophy, while prolonged mechanical unloading results in muscle atrophy.

MA Piezo1 channels have recently emerged as key mechanosensors that are capable of linking mechanical signals and intramuscular signaling in skeletal muscle cells/fibers.

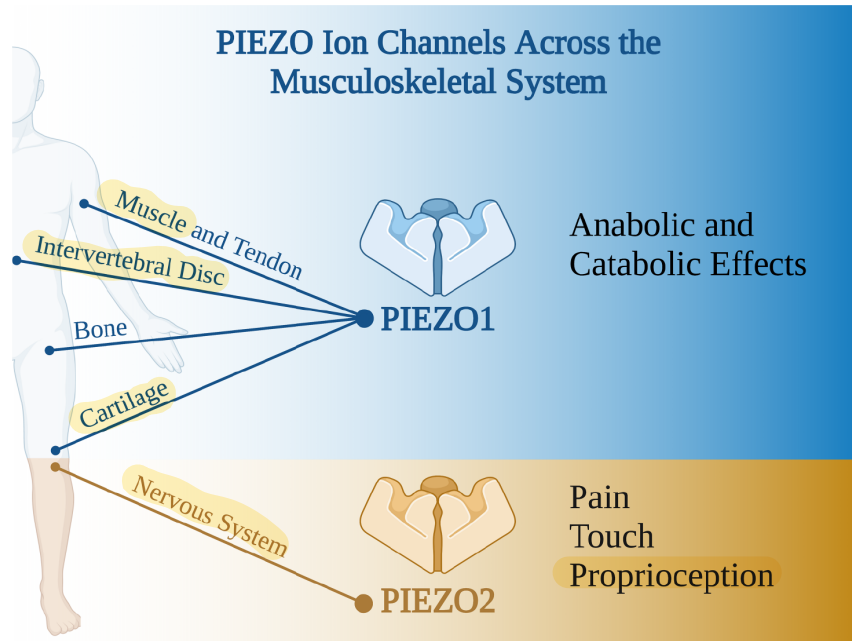
This review will summarize the emerging role of Piezo1 channels in the development and regeneration of skeletal muscle tissue as well as in the regulation of skeletal muscle atrophy.

In addition, an overview of potential Piezo1-related signaling pathways underlying anabolic and catabolic processes will be provided.

The Role of PIEZO Ion Channels in the Musculoskeletal System

March 14, 2023

Alireza Savadipour, Daniel Palmer, Erica Ely, Kelsey Collins, et. al.



AMERICAN JOURNAL OF PHYSIOLOGY

CELL PHYSIOLOGY. © 2023



PIEZO1 and PIEZO2 are mechanosensitive cation channels that are highly expressed in numerous tissues throughout the body and exhibit diverse, cell-specific functions in multiple organ systems.

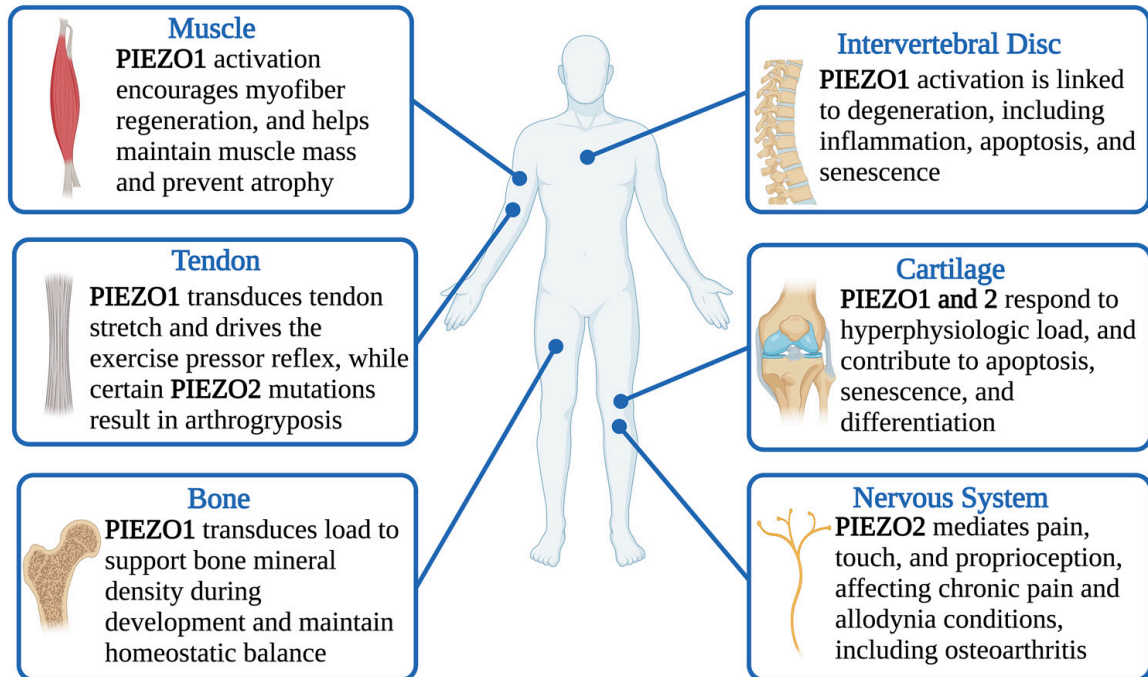
Within the musculoskeletal system, PIEZO1 functions to maintain muscle and bone mass, sense tendon stretch, and regulate senescence and apoptosis in response to mechanical stimuli within cartilage and the intervertebral disc.

PIEZO2 is essential for transducing pain and touch sensations as well as proprioception in the nervous system, which can affect musculoskeletal health.

PIEZO1 and PIEZO2 have been shown to act both independently as well as synergistically in different cell types.

Conditions that alter PIEZO channel mechanosensitivity, such as inflammation or genetic mutations, can have drastic effects on these functions.

PIEZO1 and PIEZO2 Regulate Essential Musculoskeletal Functions



Potential **PIEZO**-Related Therapies Include:

Targeted PIEZO Inhibition
Control **PIEZO1** and **2** sensitivity through regulated use of inhibitors such as GsMTx4

Inhibit VGCCs or TRPV4
Inhibit voltage gated cation channels (VGCCs) or TRPV4 to modulate **PIEZO** activity

Dietary Supplementation
Increase omega-3/omega-6 dietary fatty acid ratio to decrease **PIEZO1** and **2** sensitivity

Voltage-Gated Ion Channels in Neuropathic Pain Signaling

Ricardo Felix ^{1,*} , Alejandra Corzo-Lopez ² and Alejandro Sandoval ²

¹ Department of Cell Biology, Centre for Research and Advanced Studies (Cinvestav), Mexico City 07360, Mexico

² School of Medicine FES Iztacala, National Autonomous University of Mexico (UNAM), Tlalnepantla 54090, Mexico

* Correspondence: rfelix@cinvestav.mx; Tel.: +52-55-5747-3800

Abstract: Neuropathic pain is a chronic and debilitating disorder of the somatosensory system that affects a significant proportion of the population and is characterized by abnormal responses such as hyperalgesia and allodynia. Voltage-gated ion channels, including sodium (Na_V), calcium (Ca_V), and potassium (K_V) channels, play a pivotal role in modulating neuronal excitability and pain signal transmission following nerve injury. This review intends to provide a comprehensive analysis of the molecular and cellular mechanisms by which dysregulation in the expression, localization, and function of specific Na_V channel subtypes (mainly Na_V1.7 and Na_V1.8) and their auxiliary subunits contributes to aberrant neuronal activation, the generation of ectopic discharges, and sensitization in neuropathic pain. Likewise, special emphasis is placed on the crucial role of Ca_V channels, particularly Ca_V2.2 and the auxiliary subunit Ca_Vα₂δ, whose overexpression increases calcium influx, neurotransmitter release, and neuronal hyperexcitability, thus maintaining persistent pain states. Furthermore, K_V channels (particularly K_V7 channels) function as brakes on neuronal excitability, and their dysregulation facilitates the development and maintenance of neuropathic pain. Therefore, targeting specific K_V channel subtypes to restore their function is also a promising therapeutic strategy for alleviating neuropathic pain symptoms. On the other hand, recent advances in the development of small molecules as selective modulators or inhibitors targeting voltage-gated ion channels are also discussed. These agents have improved efficacy and safety profiles in preclinical and clinical studies by attenuating pathophysiological channel activity and restoring neuronal function. This review seeks to contribute to guiding future research and drug development toward more effective mechanism-based treatments by discussing the molecular mechanisms underlying neuropathic pain and highlighting translational therapeutic opportunities.

Keywords: voltage-gated ion channels; neuropathic pain; calcium channels; Ca_V channels; potassium channels; K_V channels; sodium channels; Na_V channels; PROTACs



Academic Editors: Carlo Musio and Marzia Martina

Received: 27 January 2025

Revised: 30 April 2025

Accepted: 8 May 2025

Published: 30 May 2025

Citation: Felix, R.; Corzo-Lopez, A.; Sandoval, A. Voltage-Gated Ion Channels in Neuropathic Pain Signaling. *Life* **2025**, *15*, 888. <https://doi.org/10.3390/life15060888>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The somatosensory system enables us to perceive and interact with our environment and body through highly specialized peripheral sensory neurons in the skin, muscles, joints, and internal organs that detect touch, pressure, pain, and temperature. These neurons detect environmental stimuli, convert them into action potentials (APs), and transmit them to the brain via the spinal cord (SC). These same neurons transmit pain signals through the peripheral nerves to the SC, where second-order neurons transfer them to the thalamus. The thalamus receives these signals and projects them to the primary somatosensory cortex, where the information is integrated [1] (Figure 1).

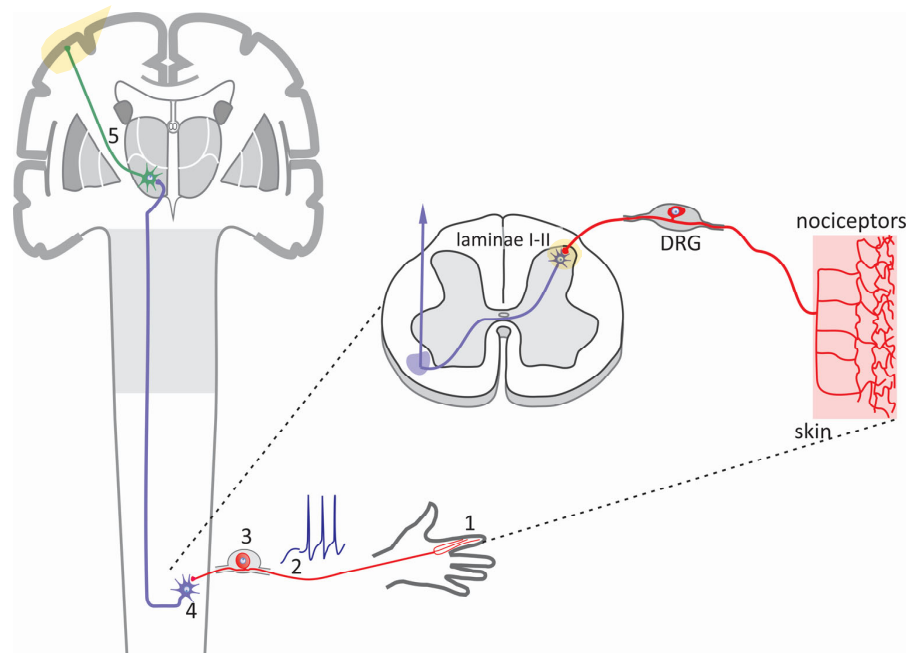


Figure 1. The sensory pathway. The somatosensory system comprises an intricate network of sensory receptors distributed throughout the skin, muscles, joints, and internal organs. These receptors include nociceptors activated in response to noxious stimuli and generate pain signals. The information generated in the periphery (1) is transmitted as action potentials (2), primarily via primary afferent fibers of the A δ and C type that have a peripheral axon innervating the distal regions, to the DRG (3), where the soma of the sensory neurons are located. The pain signals then travel to the second-order neurons in the laminae I-II of the spinal cord (4). Finally, these signals are transmitted to third-order neurons in the thalamus (5) and then to the primary somatosensory cortex to be integrated.

Neuropathic pain is a disorder of the somatosensory system that affects ~10% of the general population. It is more frequent in women and in individuals over 50 years of age and most frequently affects the lower back, upper and lower limbs, and the neck [2,3]. It is characterized by abnormal responses to stimuli, including hyperalgesia, an increased painful response to painful stimuli, and allodynia, the presence of pain associated with innocuous stimuli. Different conditions may cause neuropathic pain, with diabetes mellitus being one of the most significant [3]. However, neuropathic pain may also occur as a result of herpes virus infections, acquired immunodeficiency syndrome, or have a traumatic origin. Likewise, autoimmune disorders such as Guillain–Barré syndrome and multiple sclerosis, as well as some oncological treatments, may also cause peripheral neuropathy [1,3].

It is well-known that the changes responsible for neuropathic pain mechanisms lie in altered gene transcription or protein functional expression/localization in sensory neurons. Interestingly, after damage to peripheral sensory fibers, alterations in the different subunits that compose voltage-gated ion channels may contribute to the changes in pain transmission observed in allodynia and hyperalgesia, as we shall discuss next [1,4].

2. Voltage-Gated Sodium (Na_v) Channels and Neuropathic Pain

2.1. Structure and Function of Na_v Channels

Na_v channels play a relevant role in the development and maintenance of neuropathic pain. These proteins are essential components of the excitability machinery in excitable cells, including neurons, and alterations in their expression or function after nerve injury

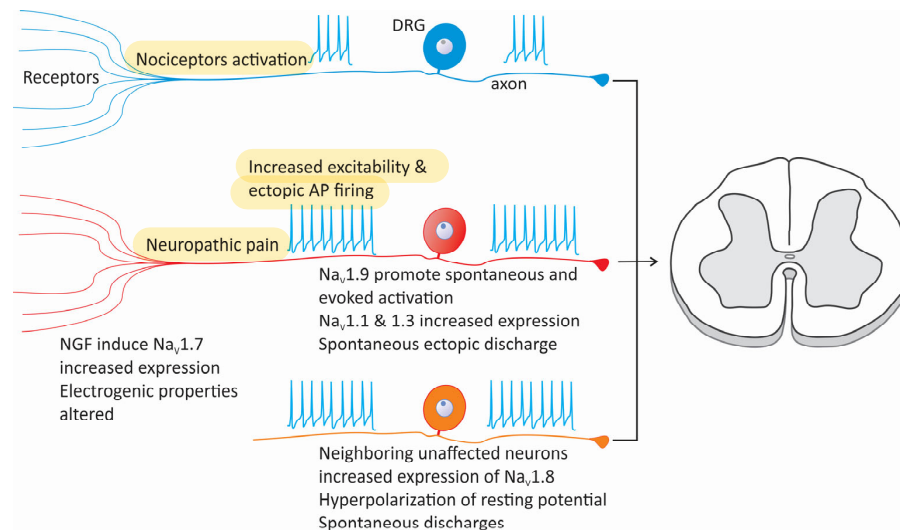


Figure 3. Changes in Na_V channel expression and cellular excitability in neuropathic pain. Alterations in the expression of various subtypes of sodium channels, such as $\text{Na}_V1.1$, $\text{Na}_V1.3$, $\text{Na}_V1.7$, $\text{Na}_V1.8$, and $\text{Na}_V1.9$, can increase cellular excitability, reducing the activation threshold of nociceptors. Similarly, after nerve injury, neurons adjacent to the damaged area may experience changes in the expression of Na_V channels, particularly $\text{Na}_V1.3$ and $\text{Na}_V1.8$, which causes the development of ectopic foci of neuronal activity. On the other hand, the expression of $\text{Na}_V1.8$ channels in neurons neighboring an injured nerve can also be compromised, contributing to the maintenance of neuropathic pain.

$\text{Na}_V1.9$ channels are expressed primarily in nociceptive neurons, where they play a relevant role in pain signaling, especially in neuropathic pain conditions [24,27]. In addition to those reviewed in the preceding sections, these channels generate APs and amplify pain signals, especially after nerve injury. While $\text{Na}_V1.9$ channels mediate TTX-R sodium currents that are crucial for maintaining neuronal excitability under physiological conditions [41,45], under pathological conditions, sodium currents through these channels can become dysregulated and contribute to chronic pain states. Numerous studies suggest that $\text{Na}_V1.9$ channels favor spontaneous and evoked activation in DRG neurons, which, in abnormal conditions, leads to exacerbated pain perception [24,27].

2.3. The Role of the Na_V Channel Auxiliary Subunits in Neuropathic Pain

Furthermore, as already mentioned, $\text{Na}_V\beta$ auxiliary subunits regulate the kinetic properties and voltage dependence of the ion-conducting subunits of Na_V channels [9,46,47]. Therefore, alterations in the functional expression of these proteins may influence the development of neuropathic pain due to their central role in the excitability of sensory neurons. On the other hand, it has also been observed that the expression of the auxiliary subunits $\text{Na}_V\beta_2$ and $\text{Na}_V\beta_3$ can be augmented after peripheral nerve injury, both in injured sensory neurons and neighboring uninjured nerve cells. This could be associated with neuronal hyperexcitability and the development of ectopic activity [48].

Previous work on $\text{Na}_V\beta_2$ subunit expression has revealed that this protein is upregulated following peripheral nerve injury, which may affect neuronal excitability [48]. This idea is supported by results obtained in $\text{Na}_V\beta_2$ null mice, showing decreased Na_V TTX-S channel expression in DRG neurons [10,49]. Remarkably, mechanical allodynia associated with peripheral nerve injury was attenuated in the knockout animals, consistent with the role of this protein in neuropathic pain [10,49].

The auxiliary subunit $\text{Na}_V\beta_3$ may also play a role in neuropathic pain. As discussed above, there is evidence that peripheral nerve injury induces an increase in currents passing

At the molecular level, VX-548 exhibits potent state-dependent inhibition of $\text{Na}_V1.8$ channels, characterized by a “reverse use dependence” mechanism. This means that it binds tightly to the channels in their resting (closed) state, but this inhibition can only be rapidly relieved by extensive and prolonged depolarizations. Consequently, VX-548 maintains tonic inhibition of these channels under physiological conditions. This unique mechanism distinguishes VX-548 from other Na_V channel inhibitors and supports its consistent and selective analgesic effect [75,76].

The clinical success of VX-548 validates $\text{Na}_V1.8$ channels as a viable pharmacological target for treating acute pain, confirming their central role in peripheral nociceptive signaling. Furthermore, initial data from the evaluation of VX-548 in managing pain in DPN and LSR suggest that $\text{Na}_V1.8$ channels also play a relevant signaling role during the development of neuropathic pain. However, further studies are required to validate this idea.

The treatment of neuropathic pain aimed at the selective inhibition of $\text{Na}_V1.8$ channels offers several advantages. These compounds provide analgesia and may improve tolerability compared to other therapies. By targeting peripherally located Na_V channels, these blockers may minimize the central side effects typically seen with more broad-spectrum Na_V channel inhibitors. Preclinical and clinical research will contribute to a better understanding of their role and effectiveness in broader pain management contexts.

On the other hand, therapy targeting the molecular mechanisms associated with neuropathic pain involving $\text{Na}_V1.9$ channels is still in development. Finding compounds that may alter $\text{Na}_V1.9$ currents has proven difficult [61]. This is because the expression of these channels in heterologous systems is complex and tends to run down quickly in sensory neurons [77]. An innovative strategy was developed in which individual voltage-sensor paddles from $\text{Na}_V1.9$ were transplanted into chimeric constructs of voltage-gated (K_V) channels to identify toxins that may interact with native $\text{Na}_V1.9$ channels [78]. Although this study showed that $\text{Na}_V1.9$ channels have a distinctive pharmacological profile and that the voltage-sensor paddles could be promising targets, it was unclear to what extent the chimeric channels reproduced the pharmacological properties of native channels.

3. Voltage-Gated Calcium (Ca_V) Channels in Neuropathic Pain

3.1. Structure and Function of Ca_V Channels

Ca_V channels are the preferential route for the entry of calcium ions into excitable cells. These channels are activated in response to the depolarization of the plasma membrane and, thus, allow for the selective entry of calcium. In this way, Ca_V channels contribute to determining cell excitability. Additionally, calcium entering cells acts as a second chemical messenger that initiates and regulates multiple physiological processes, including gene expression and neurotransmitter release, among many others. Therefore, Ca_V channels play a dual role by linking electrical signals at the cell surface with biochemical responses within the cell [79–82].

Based on their biophysical and pharmacological properties, voltage-gated calcium (Ca_V) channels have been classified into T, L, N, P, Q, and R subtypes. However, the most used classification is based on the voltage range at which they apparently activate, separating them into the following two categories: low- and high-threshold channels, LVA and HVA, respectively. The T-type channel is the only low-threshold channel described, while the L-, N-, P-, Q-, and R-type channels are considered to be high-voltage-activated channels [79–84] (Figure 4A).

Likewise, it is known that the $\text{Ca}_v\alpha_2\delta$ auxiliary subunits favor the membrane expression of Ca_v channels (Figure 4A). Four subtypes of these proteins have been described ($\text{Ca}_v\alpha_2\delta$ -1 to $\text{Ca}_v\alpha_2\delta$ -4), encoded by independent genes (*CACNA2D*). These genes are initially translated into precursor proteins that are proteolytically processed, giving rise to two peptides, $\text{Ca}_v\delta$ and $\text{Ca}_v\alpha_2$, with the first anchored to the plasma membrane through a GPI motif and the second being completely extracellular, which remain linked by a disulfide bond [88]. The $\text{Ca}_v\alpha_2$ peptide is highly glycosylated and contains diverse functional regions, including von Willebrand factor A (vWFA) motifs, a metal-ion-dependent adhesion site (MIDAS), and four cache regions. Similarly to the $\text{Ca}_v\beta$ subunit, the $\text{Ca}_v\alpha_2\delta$ subunits promote and stabilize the expression of Ca_v channels on the cell surface [79,85,89].

Finally, eight $\text{Ca}_v\gamma$ subunits have been identified, which, according to phylogenetic analyses, belong to a protein subfamily originating from a single gene. Biochemical and electrophysiological studies have shown the physical and functional interactions of these subunits with the Ca_v channel complex [90–93]. On the other hand, it is known that the $\text{Ca}_v\gamma_2$ subunit can also bind to proteins containing the PDZ domain and that it participates in the intracellular trafficking of the AMPA receptor [94].

In mammals, ten different $\text{Ca}_v\alpha_1$ subunits encoded by independent genes (*CACNA1*) are expressed, which, from a molecular point of view, group Ca_v channels into three subfamilies [81,82]. The first (Ca_v1) includes L-type channels with four members ($\text{Ca}_v1.1$ to $\text{Ca}_v1.4$). The Ca_v2 subfamily has three members ($\text{Ca}_v2.1$ to $\text{Ca}_v2.3$), which give rise to currents through P/Q-type, N-type, and R-type neuronal channels, respectively. P- and Q-type channels result from the alternative splicing of the *CACNA1A* gene encoding the $\text{Ca}_v2.1\alpha_1$ subunit [95,96].

Lastly, the Ca_v3 subfamily groups low-activation-threshold channels and consists of three members, $\text{Ca}_v3.1$ to $\text{Ca}_v3.3$ (Figure 4A). These channels allow for a basal calcium influx called a window current, which helps to maintain the resting membrane potential. Likewise, since they are activated at more negative potentials than the other Ca_v channels, they can significantly influence cell excitability, contributing to the generation of APs and rhythmic electrical activity [79–82].

It is widely accepted that Ca_v channels may play a key role in the fundamental mechanisms of neuropathic pain. The contribution of these proteins to cell excitability and neurotransmission, as well as their potential role in the treatment of the condition, stresses the need to understand neuropathic pain at the cell and molecular levels [82,85,97]. The association of Ca_v channels with the pathogenesis of the disease occurs predominantly through the HVA channels of the P/Q- ($\text{Ca}_v2.1$) and N-types ($\text{Ca}_v2.2$) and the ancillary $\text{Ca}_v\alpha_2\delta$ subunit. However, it has also been reported that the LVA channels of the $\text{Ca}_v3.2$ class may also contribute significantly to the pathophysiology of the condition [98–100].

As mentioned above, the entry of calcium ions in response to the activation of Ca_v2 channels determines the release of neurotransmitters. The calcium that enters the nerve terminal promotes the assembly of a subset of scaffolding proteins essential for anchoring synaptic vesicles containing neurotransmitters to the cell membrane and their eventual fusion [87,101,102] (Figure 4B). Therefore, alterations in the functional expression of Ca_v2 channels will alter synaptic transmission and consequently may affect pain signaling.

3.2. Role of Different Ca_v2 Channel Subunits in Nociceptive Pathways and Neuropathic Pain

Ca_v channels regulate neuronal excitability, synaptic transmission, and pain signaling. As we will see below, there are three subtypes of Ca_v2 channels. Among them, $\text{Ca}_v2.1$ (P/Q-type) and $\text{Ca}_v2.2$ (N-type) are particularly important in neurotransmission between primary afferent fibers and neurons of the SC's dorsal horn.

Ca_v2 channels contribute to the onset and maintenance of neuropathic pain through different cellular and molecular processes. Firstly, these channels are decisive in synaptic transmission, since their activation gives rise to transient increases in the concentration of intracellular calcium in the nerve terminals, which favors the release of neurotransmitters. In pathological conditions, however, synaptic transmission in sensory neurons may be altered, increasing the release of chemical pain mediators such as glutamate and substance P and inducing central sensitization, one of the main features of neuropathic pain. Second, the activity of Ca_v2 channels can be regulated by phosphorylation and other post-translational modifications, which may promote their functional expression during neuropathic pain. Finally, transient increases in intracellular calcium can cause, in pathological conditions, changes in gene expression patterns that promote the activation of transcription factors that target genes associated with chronic pain [103] (Figure 5A).

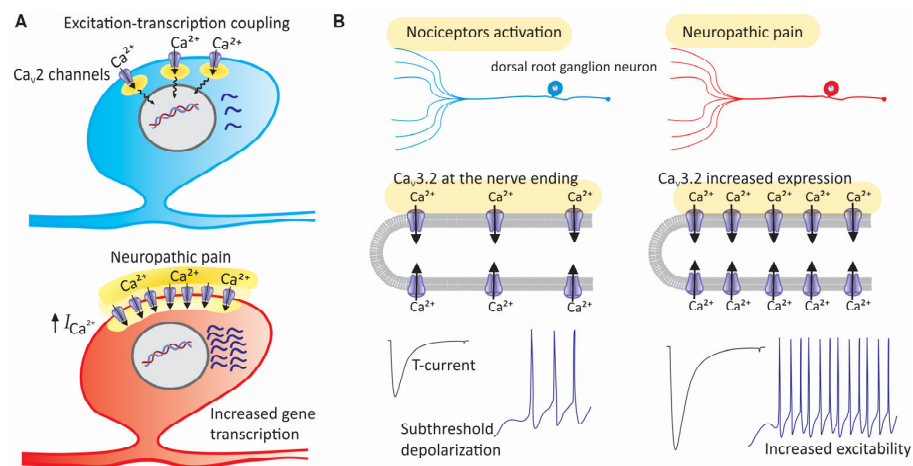


Figure 5. Contribution of Ca_v channels to the pathogenesis of neuropathic pain. **(A)** In addition to its effects on the release of neurotransmitters, alteration in the expression of Ca_v2 channels participates in the pathophysiology of neuropathic pain by affecting the excitation–transcription coupling. This fundamental cell process links electrical activity in excitable cells to gene transcription. This implies that the calcium, once inside the cells, can activate transcription factors, either directly or through protein kinases and second messengers that control the activity of these factors. **(B)** Overexpression of Ca_v3 channels in sensory neurons during neuropathic pain increases their excitability, decreases the firing threshold of afferent fibers, and favors repetitive firing.

The contribution of $\text{Ca}_v2.1$ (P/Q-type) channels to pain perception is being studied in detail and is beginning to be revealed. These channels are crucial for neurotransmitter release in the CNS, and pharmacological studies suggest their involvement in the pathophysiology of pain. While initial studies showed that intrathecal injections of specific blockers, such as AgaIVA, did not have an apparent effect on $\text{Ca}_v2.1$ channels in neuropathic pain [104–106], subsequent research has indicated that they may influence descending pathways that modulate pain transmission in some areas of the brain [107,108]. Likewise, studies conducted in $\text{Ca}_v2.1\alpha_1$ subunit knockout mice have revealed alterations in their response to nociceptive stimuli [109]. However, eliminating the pore-forming subunit of $\text{Ca}_v2.1$ channels does not significantly modify the response to thermal stimuli [109].

$\text{Ca}_v2.2$ channels are predominantly expressed in the presynaptic nerve terminals of central and peripheral neurons [110]. They are crucial for releasing neurotransmitters relevant to generating pain signals, such as glutamate and GABA. In addition, the activity of these channels is modulated by the activation of various G protein-coupled receptors (GPCRs) involved in nociception, including opioid, cannabinoid, neuropeptide Y, and

substance P receptors [111,112]. Research has shown a significant relationship between the knockout of $\text{Ca}_v2.2$ channels and neuropathic pain, consistent with their contribution to the molecular pathophysiology of this condition. Multiple studies suggest that the genetic ablation of these channels can result in reduced pain responses across various models of neuropathic pain [113,114]. The absence of $\text{Ca}_v2.2$ channels affects the release of substance P and CGRP, and this reduction in neurotransmitter release contributes to a decrease in overall pain signaling.

$\text{Ca}_v2.2$ channels are concentrated in nerve terminals located in laminae I and II of the dorsal horn of the spinal cord, where they transmit pain signals arriving on C and $\text{A}\delta$ fibers. Interestingly, these channels in primary afferent fibers contribute to developing allodynia and hyperalgesia after nerve injury [115], as discussed below.

Several studies have shown that mutually exclusive splicing patterns in the gene encoding the $\text{Ca}_v2.2\alpha_1$ subunit modulate the function of N-type channels in sensory neurons and can influence pain transmission. In particular, an exon 37a-containing isoform whose expression is restricted to DRGs correlates closely with significantly larger N-type currents in nociceptive neurons [116,117]. The preferential inclusion of exon 37a in sensory neurons generates a module in the C-terminus of the $\text{Ca}_v2.2\alpha_1$ subunit that mediates channel inhibition in a voltage-independent manner, which requires tyrosine kinase activation [118,119]. Furthermore, exon 37a enhances the μ -opioid-receptor-mediated inhibition of N-type channels [120], contributing to defining the molecular nature of the voltage-independent inhibition of N-type channels in the pain pathway.

Likewise, research in animal pain models has shown that the expression of the $\text{Ca}_v2.2\alpha_1$ subunit is significantly increased [100]. Mice subjected to partial sciatic nerve ligation showed an increased current amplitude through N-type channels and increased mRNA levels for the gene encoding the $\text{Ca}_v2.2\alpha_1$ protein in DRG neurons [121,122]. Likewise, in a chronic constrictive nerve injury model, the $\text{Ca}_v2.2\alpha_1$ protein was upregulated in lamina II of the SC dorsal horn [123]. Furthermore, in nerve ligation models, a significant increase in the expression of $\text{Ca}_v2.2$ channels with a subsequent increase in the amplitude of the current was reported, which further facilitated the excitatory synaptic transmission of $\text{A}\delta$ and C fibers in the SC dorsal horn [122].

Research on the cellular and molecular bases of neuropathic pain has shown that ubiquitination may contribute to its establishment and maintenance by regulating the turnover of synaptic proteins. Specifically, it has been described that the active zone protein RIM1 α participates in the development of the condition by binding and positively regulating the expression of $\text{Ca}_v2.2$ channels. It is also known that RIM1 α -associated spinal allodynia is mediated by Fbxo3, a protein that reduces the Fbxl2-dependent ubiquitination of RIM1 α . When deubiquitinated, RIM1 α can bind directly to these channels, increasing its expression in the nerve terminals of the dorsal horn of the SC [124].

The activation of nociceptin opioid peptide (NOP) receptors, also known as opioid-like receptor 1 (ORL-1), results in the G protein-dependent regulation of $\text{Ca}_v2.2$ channels [125–127]. This results in a decreased current amplitude, with consequent alterations in presynaptic calcium levels and impairment in neurotransmission [128]. Due to the widespread expression of both NOP and $\text{Ca}_v2.2$ channels in the brain, dorsal horn of the SC, and DRG, alterations in this system result in different neurological conditions, including neuropathic pain. As mentioned, $\text{Ca}_v2.2$ channels are crucial for pain processing by controlling the synaptic strength on C and $\text{A}\delta$ afferent fibers. Thus, reducing calcium influx by activating NOP receptors may decrease the release of CGRP and substance P, neurotransmitters involved in pain signaling. This is beneficial in neuropathic pain, where the expression of $\text{Ca}_v2.2$ channels is generally upregulated [126,129].

3.3. The Role of the $\text{Ca}_v\alpha_2\delta\text{-1}$ Auxiliary Subunit in Neuropathic Pain

The $\alpha_2\delta$ subunits of Ca_v2 channels have been shown to play crucial roles in nociceptive signaling [130–133]. As mentioned earlier, these proteins are essential in the function and regulation of these channels by contributing to the intracellular trafficking, voltage dependence, and kinetics of the currents [134–138]. In particular, the $\text{Ca}_v\alpha_2\delta\text{-1}$ subunit, which is expressed in excitable cells, including neurons, is essential for presynaptic functions such as synapse formation, the regulation of synaptic plasticity, and the control of the calcium concentration in the synaptic cleft [138–140]. The protein contains several functional regions that allow for interactions with the channel complex and other synaptic molecules. Research on the structure of $\text{Ca}_v\alpha_2\delta$ has identified a von Willebrand factor A (VWA) region along with four cache domains. The VWA region is critical for interaction with the $\text{Ca}_v\alpha_1$ subunit [138].

It has been reported that $\text{Ca}_v\alpha_2\delta$ expression may increase at both the mRNA and protein levels in sensory neurons after spinal nerve ligation and in animal models of diabetic neuropathy [130,141–145]. This change is accompanied by AP discharges in the injured neurons due to an increase in the functional expression of $\text{Ca}_v2.2$ channels mediated by the exacerbated expression of the $\text{Ca}_v\alpha_2\delta$ subunit. Consistent with this, genetic ablation of the $\text{Ca}_v\alpha_2\delta\text{-1}$ subunit significantly decreased the expression of $\text{Ca}_v2.2$ channels on the cell membrane of DRG neurons and in the dorsal horn of the SC [146]. This alteration in neuronal excitability can affect the release of neurotransmitters associated with neuropathic pain pathways [122]. Interestingly, mice overexpressing $\text{Ca}_v\alpha_2\delta\text{-1}$ exhibit neuropathic pain symptoms without nerve damage, whereas $\text{Ca}_v\alpha_2\delta\text{-1}$ -deficient mice show deficits in sensitivity after nerve injury [147–149].

Several studies support an important role of the interaction between the $\text{Ca}_v\alpha_2\delta\text{-1}$ subunit and thrombospondin-4 (TSP4), a glycoprotein found in the extracellular matrix, in nerve-injury-induced neuropathic pain, mediated through aberrant excitatory synapse formation and presynaptic neurotransmission in the SC [150–152]. Peripheral nerve injury induces the upregulation of both proteins in the SC that precedes the onset and correlates with the duration of neuropathic pain [130,141–143,153–155]. The inhibition of this regulation or the genetic ablation of $\text{Ca}_v\alpha_2\delta\text{-1}$ or TSP4 prevent the onset and development of the disease [152,155,156]. The mechanism by which an exaggerated expression of TSP4 alters the function of Ca_v channels remains to be established. In this regard, it has been reported that TSP4 can differently affect the distinct types of channels in sensory neurons, decreasing the currents passing through HVA channels and increasing those flowing through LVA channels [154], which is paradoxical given that, unlike what occurs with HVA channels, a clear role for auxiliary subunits, including $\text{Ca}_v\alpha_2\delta\text{-1}$, in the functional expression of LVA channels has not yet been established. Further research is required to better understand the origin of this discrepancy.

Similarly, research has demonstrated that the $\text{Ca}_v\alpha_2\delta\text{-1}$ subunit interacts with N-methyl-D-aspartate receptors (NMDARs) to create a complex that increases their activity by promoting their trafficking to synapses [157,158]. NMDARs are preferentially expressed in postsynaptic neurons, but are also present in presynaptic neurons, influencing neurotransmitter release and synaptic plasticity [159]. The activation of presynaptic NMDARs leads to increased calcium and the exocytosis of secretory vesicles, resulting in greater glutamate release. Under normal conditions, NMDARs are inactive; however, these receptors become tonically active in neuropathic pain [160]. Notably, models of neuropathic pain have shown an elevated expression of $\text{Ca}_v\alpha_2\delta\text{-1}$ /NMDAR complexes, indicating their potential involvement in pain mechanisms [149].

have shown that knocking down USP5 results in an increased ubiquitination of Cav3.2, decreased protein levels of the channel, and reduced whole-cell currents [170–172]. Conversely, increasing USP5 activity leads to greater activity of Cav3.2 channels in models of neuropathic pain.

3.5. Cav Channels as Potential Therapeutic Targets for Neuropathic Pain

Gaining insight into the function and potential of Cav channels as therapeutic targets has offered valuable information for managing chronic pain conditions. For instance, Cav2.2 channels are key in pain signaling because they help release neurotransmitters from sensory neurons. Blocking these channels can prevent the release of neuropeptides that transmit pain, making them a promising target for treating neuropathic pain.

Several studies stress significant advancements in studying Cav2.2 channels for treating neuropathic pain. Ziconotide, a synthetic version of the marine peptide ω -conotoxin MVIIA, has been established as an effective blocker of these channels for treating severe chronic pain [111,173]. It is administered intrathecally and has shown efficacy in several neuropathic pain models. The mechanism of ziconotide involves blocking calcium entry, which is crucial for releasing the neuropeptides substance P and CGRP in sensory neurons [174].

Furthermore, in animal models, treatment with ziconotide can prevent hyperalgesia and allodynia, confirming the role of Cav2.2 channels in establishing neuropathic pain. Ziconotide is about ten times more potent than intrathecally administered morphine [175]. However, its clinical use is limited by side effects [111]. Leconotide is a newer blocker of Cav2.2 channels, which has emerged as an alternative to ziconotide. This compound has been shown to have antihyperalgesic effects and offers a better side effect profile [176]. On the other hand, an alternative for pain relief has focused on developing small molecules that function as inhibitors of Cav2.2 channel activity. These molecules aim to provide similar benefits to ziconotide and leconotide without the disadvantages associated with peptide administration. Some of these peptides have been designed to disrupt the coupling of the main subunit Cav α_1 with other intrinsic or extrinsic proteins in the channel complex. In this context, various studies suggest that collapsin response mediator protein 2 (CRMP-2) is an important molecular interactor of Cav2.2, regulating its function and, consequently, the release of neurotransmitters in sensory neurons. The overexpression of CRMP-2 increases the current density through Cav2.2 channels and enhances the release of CGRP, which participates in pain transmission.

It has also been reported that disrupting the CRMP-2/Cav2.2 complex with specific peptides, such as TAT-CBD3, Ct-dis, and R9-CBD3-A6K, reduces the excessive neurotransmitter release associated with chronic pain, showing antinociceptive effects in neuropathic pain models [177–180]. Moreover, the potential of quinazolines and benzoylpyrazolines as agents that disrupt the coupling between Cav α_1 and Cav β subunits has been investigated. These compounds have shown the ability to decrease currents through Cav2.2 channels, alter their presynaptic localization, and inhibit the release of CGRP, exhibiting antinociceptive properties in various pain models, including neuropathic pain [181].

Likewise, Khanna and his colleagues also showed that the inhibition of the interaction between Cav α_1 and Cav β subunits reduces the excitability of DRG neurons, leading to a decrease in acute and neuropathic pain in several animal models [182]. Specifically, the authors developed a molecule identified as IPPQ that selectively binds to Cav β , inhibiting its coupling with the Cav α_1 subunit of Cav2.2 channels. This leads to the delocalization of presynaptic channels, a decrease in the amplitude of calcium currents in sensory neurons, and a reduction in the release of the nociceptive neurotransmitter CGRP in the SC. This same research group recently designed a small peptidomimetic molecule derived from

species production and potentiating the release of proinflammatory cytokines in the central nervous system.

Voltage-gated H⁺ channels allow for the flow of protons across the cell membrane in response to changes in electrical potential and play important roles in various cellular functions. When activated during membrane depolarization, they allow proton efflux, thus helping to maintain the acid–base balance within cells. Furthermore, Hv1 proton channels may directly influence neuronal excitability by conducting ions that depolarize the neuronal membrane. As mentioned above, they may also indirectly modulate excitability through their effects on ROS production, pH regulation, injury, and microglial activity.

The molecular structure of Hv1 channels differs from other voltage-gated ion channels due to their unique composition and function [262]. These channels consist solely of a voltage-sensing domain (VSD) composed of four transmembrane helices (S1–S4) and an additional amphipathic helix (S0) at the N-terminus. They are composed of two subunits, each with its own proton permeability pathway within the VSD [262,263]. The S4 helix in the VSD is crucial for sensing changes in transmembrane voltage. It undergoes outward displacement in response to depolarization, which changes the internal salt bridge network and reconfigures the proton permeability pathway [262]. The two subunits of Hv1 channels interact during the channel opening process, showing a positive cooperativity that modulates the voltage response of the two permeation pathways [264]. Furthermore, Hv1 channels exhibit unidirectional conductance, allowing protons to exit the cell but not enter it, which is essential for maintaining intracellular pH homeostasis.

The involvement of ROS in the development of neuropathic pain is reinforced by the fact that, following spinal cord injury, a large proportion of patients develop neuropathic pain [265]. Furthermore, NOX2-derived ROS in microglia have been associated with neuropathic pain induced by nerve injury [266]. It is also known that, in response to peripheral nerve injury, macrophages are recruited by DRGs and increase ROS production through a NOX2-dependent mechanism. Interestingly, NOX2^{−/−} mice display a reduced neuropathic pain phenotype [267]. Given the established link between Hv1–NOX and NOX2 and the development of neuropathic pain, it was initially suggested that microglial Hv1 channels could initiate and maintain neuropathic pain after spinal cord injury [268].

Indeed, Hv1 channels are functionally expressed in spinal cord microglia and show significant upregulation following peripheral nerve injury [269]. Furthermore, the activation of these channels contributes to the onset of neuropathic pain by favoring the production of reactive oxygen species (ROS). This production of ROS is associated with astrocyte activation, which worsens pain sensitivity [269]. Likewise, Hv1-null mice display decreased pain sensitivity after nerve injury compared to wild-type mice. Together these data suggest that Hv1 channels are relevant in mediating pain responses through microglial and astrocytic interactions.

6. Voltage-Gated Ion Channel Dysregulation in Supraspinal Pathways

Pain transmission involves complex neural circuits that run from the periphery to the brain. While spinal mechanisms are crucial for the initial processing of nociceptive signals, supraspinal pathways, predominantly cortical and brainstem circuits, are essential for the integration, modulation, and conscious perception of pain.

The cortex plays a central role in pain perception, integrating sensory, emotional, and cognitive aspects. Key regions include the somatosensory cortices (S1 and S2), which are responsible for the sensory-discriminative aspects of pain, such as location and intensity. S1 receives nociceptive inputs from the thalamus, while S2 integrates information from multiple body regions. The insula and anterior cingulate cortex (ACC) provide pain's affective and emotional dimensions. The insula contributes to the subjective experience, while

the ACC is related to emotional and motivational responses. The prefrontal cortex participates in cognitive pain evaluation, including attention and modulation. Its dysfunction is associated with chronic pain.

The brainstem bridges cortical centers and spinal circuits, modulating pain through several structures, including the periaqueductal gray matter that integrates descending pain control signals, producing analgesia by inhibiting nociceptive neurons. The rostral ventromedial nucleus of the medulla oblongata contains neurons that facilitate and impede pain, exerting bidirectional control. Finally, the parabrachial area involves pain's affective and motivational aspects and contributes to the transition from acute to chronic pain.

While alterations in peripheral voltage-gated ion channels predominate in initiating neuropathic pain, supraspinal mechanisms may maintain pain through central sensitization and altered descending control. However, direct evidence for the involvement of voltage-gated ion channels in supraspinal pathways is scarce and contrasts with their well-documented contribution to peripheral and spinal mechanisms. In this context, it is worth noting that neuropathic pain may involve important changes in neuronal excitability and synaptic transmission along the peripheral and supraspinal pathways. Indeed, a key event in the development of neuropathic pain is central sensitization, which involves alterations in neuronal excitability and synaptic transmission. Though the involvement of voltage-gated ion channels has not been directly documented in the supraspinal context, their contribution to neuronal excitability suggests that they may be indirectly involved in neuropathic pain, as discussed below.

It has been documented that $\text{Na}_V1.3$ channels, especially in the thalamus, can generate and maintain neuropathic pain due to their role in central hyperexcitability [14,201]. Particularly, after a spinal cord injury, $\text{Na}_V1.3$ channel expression increases significantly in thalamic neurons, which is associated with increased spontaneous neuronal activity contributing to pain generation. Furthermore, it has been observed that spinal cord injuries can trigger supraspinal changes in Na_V channel expression in thalamic neurons [14,37,270].

Specifically, four weeks after injury, immunostaining for $\text{Na}_V1.3$ channels was significantly increased in neurons of the ventral posterolateral nucleus of the thalamus. Electrophysiological recordings from neurons in this region in SCI animals showed a high rate of spontaneous activity, independent of ascending afferent input. Interestingly, antisense oligonucleotides targeting $\text{Na}_V1.3$ channel messengers reduced their expression in the thalamus and reversed the increase in spontaneous activity. Furthermore, the exacerbated spontaneous activity persisted even after complete spinal cord transection, indicating that afferent input is not essential for maintaining thalamic hyperexcitability, suggesting that this region may function as an intrinsic pain generator [37,270] and supporting the finding that $\text{Na}_V1.3$ overexpression in the post-spinal cord injury thalamus contributes to spontaneous neuronal activity and neuropathic pain. Although further research is needed to fully understand Na_V channels' contributions to neuropathic pain, their involvement in central sensitization and hyperexcitability suggests a potential role in the molecular mechanisms of the condition.

On the other hand, T-type calcium channels, especially those of the $\text{Ca}_V3.2$ class, are not only involved in neuropathic pain at the peripheral level, but also appear to play a role at the supraspinal level. Specifically, it has been found that $\text{Ca}_V3.2$ channels are expressed in GABAergic neurons and contribute to high-frequency firing activity in the reticular thalamic nucleus and anterior pretectum (APT), a region involved in pain perception [162,271]. This activity is increased in animal models of neuropathic pain, while the specific elimination of $\text{Ca}_V3.2$ channels in APT neurons reduces mechanical allodynia.

Likewise, the upregulation of T-type channels in the anterior cingulate cortex (ACC) has been shown to alleviate neuropathic pain [272]. After chronic nerve constriction injury,

redundancy and overlap among these channels complicate efforts to identify the most relevant candidates and design selective modulators without off-target effects.

There is also a significant translational gap between animal models and humans. Many of the findings on the involvement of voltage-gated ion channels in neuropathic pain come from animal models, which may not fully replicate the human disease. Differences in ion channel expression and regulation may limit the translation of preclinical findings into effective clinical treatments.

On the other hand, nonselective ion channel blockers can be effective in animal models, but they often have a limited clinical efficacy and cause adverse effects. Therefore, developing more selective compounds that target peripheral channels without central or cardiac impacts remains a significant challenge. Likewise, following nerve injury, the functional expression of voltage-gated ion channels can change dynamically, influenced by inflammatory mediators and neuronal activity. Understanding these temporal and spatial changes is crucial for producing effective therapeutic responses.

Lastly, the proteins that form voltage-gated ion channel complexes are targets of post-translational modifications such as phosphorylation, ubiquitination, SUMOylation, and glycosylation, which affect their activity, intracellular trafficking, and half-life. Studying how these processes are affected during the development of neuropathic pain would be a topic of interest for future studies, especially considering its therapeutic potential (see below). Furthermore, it would be of great interest to expand our knowledge of how the release of cytokines and chemokines during neuroinflammation can affect the function and expression of ion channels and how these processes can contribute to the molecular pathophysiology of neuropathic pain. Likewise, studying how DNA methylation or histone acetylation and other epigenetic modifications can affect the expression patterns of ion channels is another topic of great relevance, since these modifications can affect pain perception and promote the development of neuropathic pain.

9.2. Small Molecules Versus Biologics in the Future of Voltage-Gated Ion Channel Therapies in Neuropathic Pain

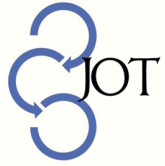
Small molecules remain the primary and most promising approach to targeting voltage-gated ion channels in neuropathic pain. Recent advances include highly selective inhibitors for Na_V and Ca_V channels, with successful clinical trials generating significant momentum for this approach. Small molecules offer advantages such as oral bioavailability, ease of synthesis, and the ability to modulate channel activity or trafficking in a reversible and tunable manner.

Innovative small-molecule strategies are emerging, such as targeted protein degradation (PROTACs), which can selectively degrade specific ion channel subtypes and potentially offer longer-lasting effects [206–208,297]. Furthermore, small molecules are being developed to disrupt protein–protein interactions critical to channel function and trafficking, expanding the scope of therapeutic mechanisms. PROTACs in the ion channel field represent a novel strategy to modulate ion channel function by targeting the channel proteins rather than through traditional inhibition, potentially offering increased specificity and overcoming drug resistance mechanisms. They are small, heterobifunctional molecules designed to selectively degrade target proteins by exploiting the cell's ubiquitin–proteasome system (UPS). They are composed of two ligands connected by a linker—one ligand binds to the protein of interest, and the other recruits an E3 ubiquitin ligase. This proximity induces the ubiquitination of the target protein, marking it for degradation by the 26S proteasome, effectively reducing the cellular levels of the protein rather than simply inhibiting its function [206–208].

$\text{Na}_V1.7$ and $\text{Na}_V1.8$ are also susceptible to PROTAC-mediated targeted protein degradation. Engineered PROTACs have been used to induce the rapid and near-

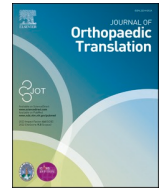
292. Ziegler, D.; Duan, W.R.; An, G.; Thomas, J.W.; Nothaft, W. A randomized double-blind, placebo-, and active-controlled study of T-type calcium channel blocker ABT-639 in patients with diabetic peripheral neuropathic pain. *Pain* **2015**, *156*, 2013–2020. [[CrossRef](#)]
293. Zhang, F.; Liu, Y.; Tang, F.; Liang, B.; Chen, H.; Zhang, H.; Wang, K. Electrophysiological and pharmacological characterization of a novel and potent neuronal Kv7 channel opener SCR2682 for antiepilepsy. *FASEB J.* **2019**, *33*, 9154–9166. [[CrossRef](#)] [[PubMed](#)]
294. Wang, J.; Liu, Y.; Hu, F.; Yang, J.; Guo, X.; Hou, X.; Ju, C.; Wang, K. Activation of neuronal voltage-gated potassium Kv7/KCNQ/M-current by a novel channel opener SCR2682 for alleviation of chronic pain. *J. Pharmacol. Exp. Ther.* **2021**, *377*, 20–28. [[CrossRef](#)] [[PubMed](#)]
295. Perucca, E.; Taglialatela, M. Targeting Kv7 Potassium channels for epilepsy. *CNS Drugs* **2025**, *39*, 263–288. [[CrossRef](#)] [[PubMed](#)]
296. Manville, R.W.; Abbott, G.W. Gabapentin is a potent activator of KCNQ3 and KCNQ5 potassium channels. *Mol. Pharmacol.* **2018**, *94*, 1155–1163. [[CrossRef](#)]
297. He, M.; Cao, C.; Ni, Z.; Liu, Y.; Song, P.; Hao, S.; He, Y.; Sun, X.; Rao, Y. PROTACs: Great opportunities for academia and industry (an update from 2020 to 2021). *Signal Transduct. Target. Ther.* **2022**, *7*, 181. [[CrossRef](#)]
298. Chamessian, A.; Payne, M.; Gordon, I.; Zhou, M.; Gereau, R. Small molecule-mediated targeted protein degradation of voltage-gated sodium channels involved in pain. *bioRxiv* **2025**. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.



Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Review Article

Electric currents in disc health: The role of ion channels in intervertebral disc pathophysiology

Hengxiang Shu^{a,1}, Yan Gao^{a,1}, Qin Zhang^{a,1}, Haobo Sun^{a,1}, Huazheng Wang^b,
Chengnan Jing^c, Peng Liu^d, Dechun Geng^{a,*}, Hao Shen^{a,**}, Minfeng Gan^{a,***}^a Department of Orthopaedic Surgery, The First Affiliated Hospital of Soochow University, Soochow University, Suzhou, 215000, Jiangsu, China^b Department of Orthopaedic Surgery, Soochow BenQ Medical Center, Suzhou, 215000, Jiangsu, China^c Department of Pain Clinic, Nanjing BenQ Medical Center, The Affiliated BenQ Hospital of Nanjing Medical University, Nanjing, 210000, Jiangsu, China^d Department of Orthopaedic Surgery, Longgang District People's Hospital of Shenzhen, Shenzhen, 518000, Guangdong, China

ARTICLE INFO

Keywords:

Low back pain
Ion channels
Intervertebral disc degeneration
Epigenetics
Targeted therapy
Tissue engineering

ABSTRACT

Intervertebral disc degeneration (IVDD), which is known as a common global health concern, has been a significant factor in neck and back pain. The intervertebral discs are avascular structures consisting of the nucleus pulposus, annulus fibrosus, and cartilage endplate, which are crucial for regulating the spinal motion, withstanding stress, and buffering vibration. Due to their special anatomical structure and functional role, they are highly susceptible to stimulation by external factors. Ion channels are transmembrane proteins which have attracted significant attention and great progress in cardiovascular diseases and neurological diseases, and the importance of them in the pathophysiology of IVDD is gaining recognition. They function as a receptor to stimulate the influx of calcium within cells, which acts as a second messenger to activate downstream pathways and upregulate the expression of transcriptional protein, thereby triggering IVDD. This review classified the ion channel families into three types based on their primary activation mechanisms, and then described the regulation of ion channels from transcription and translation to trafficking and expression. Subsequently, the function of ion channels in the pathophysiology of IVDD as well as their potential and practicality in treatment were the main topics of this review. We hope this review could help understand and develop new, specific therapies for IVDD.

The translational potential of this article: Targeted therapeutic strategies for ion channels are particularly critical in the treatment of IVDD. Ion channel-targeted drugs and tissue engineering strategies for ion channels have emerged as novel therapeutic targets for intervening in IVDD by modulating calcium homeostasis, inflammatory responses, and extracellular matrix metabolism in disc cells. In addition, as the development of nanotechnology, the integration of ion channel-targeted therapies with advanced drug delivery systems represents a promising frontier in the treatment of IVDD. Nanoparticle-based carriers and hydrogel-mediated sustained-release platforms have emerged as complementary strategies to enhance drug bioavailability and spatiotemporal control within the avascular, mechanically stressed intervertebral disc microenvironment. Furthermore, systematic exploration of combination therapies integrating ion channel-targeted drugs with complementary pharmacological agents like anti-inflammatory drugs and growth factors warrants rigorous investigation to enhance therapeutic efficacy in IVDD management.

1. Introduction

Low back pain (LBP) is an extremely common symptom affecting

individuals of all ages from youngsters to the elderly [1]. The Global Burden of Diseases, Injuries, and Risk Factors Study 2019 (GBD 2019) showed that LBP was the ninth leading cause of disability-adjusted

* Corresponding authors.

** Corresponding author.

*** Corresponding author.

E-mail addresses: szgengdc@suda.edu.cn (D. Geng), suda_shenhao@163.com (H. Shen), spineganmf@126.com (M. Gan).¹ These authors equally contributed to the work.

life-years (DALYs) in 2019, especially affects the 25–49-year age group [2]. The elements that make up the lumbar spine, such as soft tissues, vertebrae, sacroiliac joints, intervertebral discs, and neurovascular structures, are susceptible to numerous stressors, which can contribute to the LBP [3]. Among these, the intervertebral disc degeneration (IVDD) is considered to be the major culprit. Currently, most patients with IVDD have received conservative treatment such as bed rest, nonsteroidal anti-inflammatory drugs, and analgesics, or surgical interventions including lumbar discectomy and lumbar interbody fusion [4]. However, the application of oral NSAIDs is limited because of the gastrointestinal side effects they are linked to, and these drugs are primarily intended for the temporary relief of symptoms instead of targeting pathogenesis, thus remain unable to reverse the progression of IVDD [5]. In addition, despite the popularity and generally favorable outcomes of surgical interventions, the intervertebral disc's natural physiological structure cannot be fully restored, and there remains a probability of subsequent surgical interventions [6]. Given the high prevalence and negative effects of LBP, a novel therapeutic strategy that focuses on the mechanism of IVDD, aiming to delay or prevent its continued progression is desperately needed.

Intervertebral discs (IVDs) are fibrous cartilage structures located between two vertebrae in the spine, which are mainly composed of the translucent jelly-like nucleus pulposus (NP) tissue filled in the center, the outer annulus fibrosus (AF) tissue arranged in concentric circles, and the cartilaginous endplate (CEP) tissue which covers both top and bottom of the IVDs to separate the NP and the AF from adjacent vertebral bodies [7]. The pathogenesis of IVDD demonstrates multifactorial etiopathogenesis, characterized by a complex pathophysiological cascade involving dysregulated cellular metabolism, aberrant inflammatory mediator release, and disrupted extracellular matrix (ECM) homeostasis. Hypoxic/nutrient-deficient microenvironments disrupt cellular metabolism, triggering mitochondrial dysfunction and endoplasmic reticulum (ER) stress-induced apoptosis via caspase-3/9 activation [8]. Concurrently, NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) inflammasome activation by ECM fragments drives interleukin-1 β /interleukin-18 (IL-1 β /IL-18) maturation, exacerbating NF- κ B-mediated overexpression of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5), which degrade collagen and aggrecan [9]. ECM catabolism disrupts osmotic homeostasis and biomechanical integrity, further

promoting apoptosis and inflammation. These pathogenic elements engage in reciprocal molecular cross-talk through self-amplifying signaling pathways, ultimately accelerating the destruction of intervertebral disc structure including decreased intervertebral height, endplate sclerosis, and osteophyte formation [10] (Fig. 1).

While the precise pathogenesis of intervertebral disc degeneration (IVDD) remains incompletely elucidated due to its multifactorial nature, emerging evidence underscores the critical involvement of ionic homeostasis dysregulation, particularly calcium ion (Ca²⁺) imbalance, in driving disease progression. Notably, Ca²⁺ which has been recognized as the most extensively characterized secondary messenger system, exerts pivotal regulatory control over pathophysiological mechanisms underlying IVDD development [11]. Furthermore, emerging evidence substantiates that transmembrane channel-mediated disturbances in iron, copper, and potassium ionic gradients contribute to oxidative stress and disc matrix degradation in IVDD [12]. Since the pioneering application of the voltage-clamp technique in cardiac tissue research in 1964, the field of ion channel biology has witnessed remarkable advancements, particularly with the emergence and refinement of single-electrode patch-clamp methodologies [13,14]. As transmembrane protein complexes forming selective hydrophilic pores across lipid bilayers, ion channels exhibit precise ionic permeability critical for regulating cellular electrophysiological homeostasis [15]. Their fundamental role in orchestrating bioelectrical signaling has established ion channel dysfunction as a pathophysiological mechanism underlying various degenerative conditions, including cardiovascular disorders [16], neurological pathologies [17], oncogenesis [18], and musculoskeletal diseases [19]. In this review, we concentrated on the physiological and pathological functions of ion channels in IVDD, with the goal of elucidating novel therapeutic targets and providing novel insights for future investigative efforts.

2. Classification of ion channels

Ion channels are pore-forming proteins that provide the main pathways for ions to enter or leave the cells. Selectivity and gating are two common characteristics of ion channels. Selectivity allows specific ions to diffuse across membranes through their corresponding channels and prevents the passage of the others, while gating involves the mechanisms that regulate the closing and opening of channel gates [20]. In

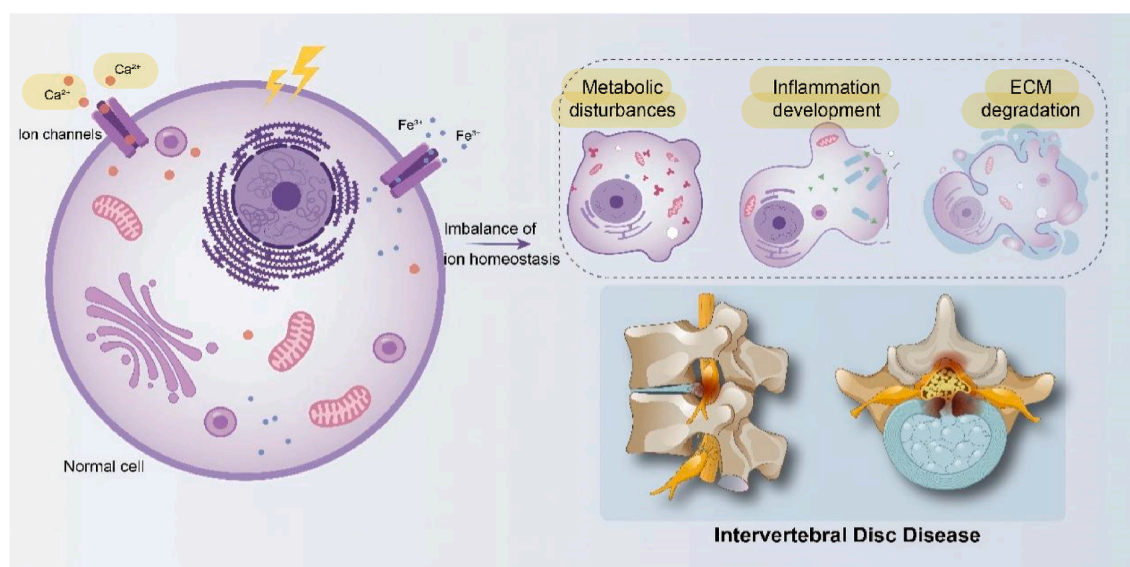


Fig. 1. An overview of ion channels mediating nucleus pulposus cell death as well as IVDD. Upon activation by diverse stimuli, ion channels located within the cellular membrane undergo activation-induced opening, facilitating transmembrane influx of divalent cations including Ca²⁺ and Fe³⁺, causing an imbalance of ion homeostasis and triggering a cascade of pathophysiological events, thus resulting in IVDD related performance.

most current studies, ion channels families are usually separated into sodium, potassium, calcium ion channels according to their selectivity. In view of the fact that the IVD cells are subjected to threats such as hypoxia, mechanical load, low pH, hypertonicity, and other factors in the progression of IVDD, we tend to classify the ion channel families into three types based on the primary activation mechanisms, which are ligand-gated channels, proton-gated channels, and physical stimuli-gated channels [21](Table 1).

2.1. Ligand-gated channels

Ligand-gated channels (LGCs) are transmembrane channels that, upon binding to ligands of extra acellular domains, namely neurotransmitters, undergo conformational changes and open transmembrane domains (TMD), allowing ions to flow through the membrane along their electrochemical concentration gradients. According to the difference of TMD, they are mainly divided into three superfamilies: the ATP-gated purine receptors are homotrimers, the ionotropic glutamate receptors family are tetramers, and the Cys-loop receptors superfamily are pentamers [30]. For instance, ATP is well known for its function as the main source of cellular energy, while it can also act as a signaling molecule by activating nucleotide receptors which are referred to as purinergic P2 receptors under certain conditions, such as inflammation, hypoxia, and ischemia [31]. P2X7 receptors (P2X7R) are ATP-gated non-selective cation channels, which have been widely concerned because of their important role in multiplying inflammatory and immune responses [32]. When the concentration of ATP exceed 100 μM, P2X7R are activated, resulting in a change in their molecular arrangement and the subsequent opening of ion channels, allowing potassium ions to exit and sodium and calcium ions to enter, which in turn induces alterations in the cell membrane potential, thereby affecting downstream Ca²⁺ signaling events [33]. Moreover, with the continuous action of ATP and its agonists, the TM1 and TM2 transmembrane segments of the P2X7R progressively open, forming larger membrane channels, which increased the cellular permeability to various ions and disrupted the equilibrium of intracellular small molecule metabolism, resulting in cell edema, cell necrosis, and cellular apoptosis [34].

2.2. Proton-gated channels

The functions of the proton-gated channels are primarily influenced by the protonation status of titratable groups distributed in discrete

functional domains or throughout the protein. Acid-sensitive ion channels (ASICs) are typical proton-gated cation channels which belong to the voltage-insensitive, amiloride-sensitive epithelial Na channel/degenerin family. In mammals, ASICs are composed of five subunits—ACCN1a, ACCN1ab, ACCN2a, ACCN2b, and ACCN3, which form five distinct subunits: ASIC1a, ASIC1b, ASIC2a, ASIC2b, and ASIC3 [35]. ASICs function as the detectors with the pH range of 7 to 4.0, and are highly sensitive to the decreases in extracellular pH [36]. The drop in extracellular pH, which is triggered by acidosis, lactate, and arachidonic acid, causes ASICs to open and facilitates the passage of calcium, potassium, and sodium ions—with sodium ions being particularly selective [37]. There are accumulating studies highlighted that ASICs modulate cell behavior via mediating Na⁺ and Ca²⁺ influx, leading to cell membrane depolarization and intracellular Ca²⁺ accumulation, which is crucial in several degenerative disorders, such as IVDD, arthritis, Parkinson's disease, Huntington's disease, and multiple sclerosis [38].

2.3. Physical stimuli-gated channels

Physical stimuli-gated channels are specialized membrane proteins that open or close in response to specific physical forces including mechanical stress, temperature, or voltage, enabling cells to detect and adapt to environmental changes. Voltage-gated ion channels (VGICs) serve as fundamental regulators of bioelectrical signaling. Their voltage-sensing domains detect transmembrane potential changes, triggering pore opening/closing to permit selective ion flux (e.g., Na⁺, K⁺, Ca²⁺) along electrochemical gradients. This mechanism underpins critical processes including action potential generation and synaptic transmission [39]. Temperature-gated ion channels operate through temperature-sensitive structural elements that modulate channel gating within physiological thermal ranges. A prominent family of temperature-gated channels is the Transient Receptor Potential (TRP) channel family. For instance, TRPV1 channels activate at noxious heat (>43 °C) and mediate thermal nociception, while TRPM8 responds to cooling (<25 °C) and mediates cold sensation [40]. These channels exhibit polymodal activation, often integrating thermal with chemical signals. Mechanosensitive ion channels (MSCs) are transmembrane proteins that convert mechanical stimuli, such as pressure, stretch, or shear stress, into electrochemical signals by allowing the flow of ions across cell membranes. These channels play critical roles in sensing touch, hearing, blood pressure regulation, and cellular responses to physical forces [41]. PIEZO, two-pore-domain potassium (K2P)

Table 1
Ion channels related to intervertebral disc degeneration.

Ion channels	Stimulating Factor	Selectivity	Cellular expression	Ref.
P2X7R	extracellular ATP	non-selective cation	immune cells, mast cells, glial cells, keratinocytes, retinal ganglion cells, intestinal epithelial cells, corneal cells, vascular endothelial cells, hepatocytes, fibroblasts, osteoclasts, skeletal muscle, vascular smooth muscle, and osteoblasts	[22]
ASICs	extracellular H ⁺ (acidosis, lactate, arachidonic acid)	non-selective cation	central and peripheral neurons, taste receptor cells, cochlear hair cells, photoreceptors and retinal cells, testis, lung epithelial cells, adipose cells, pituitary gland, vascular smooth muscle cells, immune cells and bone	[23]
Voltage-gated sodium channels	depolarization	sodium selective	myocardium, skeletal muscle, neuroendocrine cells, neurons, glial cells	[24]
Voltage-gated calcium channels	depolarization	calcium selective	myocardium, smooth muscle, skeletal muscle, endocrine cells, neurons, sensory, spinal cord, lymphoid tissue	[25]
PIEZO1	mechanical stress (sheer stress, membrane stretch)	non-selective cation	urinary tract epithelial cells, renal epithelial cells, lung epithelial cells, vascular endothelial, red blood cells, adipocytes, myeloid cells, bone marrow cells, osteoblasts and osteocytes	[26]
K2P channels (TREK1)	mechanical stress (membrane stretch), heat, depolarization	potassium selective	neurons and glia cells, cardiac muscle cells, lung epithelial cells, intestinal epithelial cells, bladder cells, uterine cells, adrenal cortical cells and vascular endothelium	[27]
TRPV1	noxious heat (>43 °C), depolarization	non-selective cation	DRGs, brain, kidney, pancreas, testis, uterus, spleen, stomach, small intestine, lung and liver	[28]
TRPV4	mechanical stress (osmotic stress), heat (>24 °C–32 °C), constitutively active	non-selective cation	smooth muscle cells, epidermal keratinocyte cells, lung epithelial cells, cornea epithelial cells, pancreatic cells, urinary tract epithelial cells, osteoblasts and osteocytes	[29]

channels, OSCA/TMEM63 channels, and mechano-electrical transduction channel complex are currently recognized as mainly mechano-sensitive ion channels. It is worth noting that this classification is not entirely accurate, as many channels may react to physical stimuli such as mechanical stress or voltage to some extent. For instance, ASICs are primarily activated by extracellular protonation states, while some studies have pointed out that they can also respond to mechanical stimulation of nerve endings [42].

3. The role of ion channels in IVDD

Emerging evidence indicates that ion channels may play a pathophysiological role in IVDD through their critical involvement in regulating transmembrane ion flux, thereby regulating the metabolism, inflammation, and structural integrity of intervertebral disc cells in response to various pathophysiological stimuli. The following summary summarized several ion channels linked to IVDD, categorized based on their major mechanisms of activation and discussed their specific mechanisms in IVDD (Table 2).

3.1. ATP-gated P2X7 receptor channels

A growing body of evidence has demonstrated the critical involvement of P2X7 receptors (P2X7R) in mediating cellular inflammatory responses. Under pathophysiological conditions, extracellular ATP accumulation triggers P2X7R activation through high-affinity binding, prompting its assembly into a macropore-forming cation channel. This structural reorganization enables coordinated K⁺ efflux and Ca²⁺

dysregulation, with the former acting as a key trigger for NLRP3 inflammasome assembly [61]. Subsequent caspase-1 activation within the inflammasome complex catalyzes proteolytic maturation of pro-IL-1 β and pro-IL-18, ultimately driving pyroptotic cell death and sustained release of bioactive cytokines that perpetuate sterile inflammation [62].

The association between P2X7R and IVDD has been well-established. Studies have revealed elevated expression of IL-1 β , P2X7R, and NLRP3 inflammasome components in severely degenerated IVD tissues. Intriguingly, spatial localization studies reveal distinct cellular distributions: NLRP3 primarily resides in the cytoplasm, while P2X7R exhibits nuclear localization under basal conditions. The stimulation of P2X7R agonists and lipopolysaccharide can induce receptor translocation to the cytoplasm, facilitating colocalization with NLRP3 [43]. Emerging evidence suggests nuclear P2X7R may function as a membrane-bound signaling protein capable of regulating specific gene transcription, potentially playing an understudied role in maintaining NP homeostasis [63]. Further investigation is required to elucidate the full functional significance of nuclear P2X7R in disc biology.

3.2. Aid-sensitive ion channels

The IVD, which represents the largest non-vascular tissue in the body, relying primarily on the vertebral body’s capillaries for nourishment, which diffuses to the NP cells through the CEP [64]. This special anatomical structure makes the IVD suffer from hypoxia for a long time, and glycolysis becomes the main energy source for the NPCs, coupled with the accumulation of lactate, which is the end product of glycolysis [65]. With the progression of calcification and degenerative changes in the cartilage endplate, the intervertebral disc’s ability to exchange nutrients and metabolites with surrounding tissues becomes increasingly compromised. This progressive impairment results in the accumulation of acidic metabolites such as lactate, subsequently creating an acidic microenvironment characterized by reduced pH levels. Studies have shown that the pH of healthy IVDs is kept between 7.1 and 7.4, while in highly deteriorated discs, it can fall as low as 6.5, thereby activating the ASICs on the membrane of IVD [66]. Notably, degenerated IVD tissue demonstrates distinct spatial expression patterns: AF regions show predominant upregulation of ASIC1 and ASIC4, while NP compartments exhibit increased expression of ASIC1, ASIC2, and ASIC3 [67].

Emerging evidence indicated that ASICs regulate IVDD through multifaceted mechanisms. Primarily, ASIC activation under acidic microenvironments triggers intracellular Ca²⁺ influx, subsequently disrupting cellular metabolic homeostasis. Mechanistic studies reveal that ASIC1 channel activation facilitates calcium entry, which functions as a secondary messenger to disrupt the mitochondrial membrane potential. This process is mediated through dual pathways: the ERK5 signaling cascade and mitochondrial dysfunction, ultimately leading to mitochondria-mediated apoptosis of nucleus pulposus cells [44–47]. Secondary to the degenerative process, the inflammatory cascade significantly contributes to the pathogenesis and progression of IVDD. ASIC activation facilitates NF- κ B and MAPK signaling pathway-mediated secretion of pro-inflammatory cytokines including IL-1 β , IL-6, and TNF- α , thereby establishing a self-perpetuating inflammatory feedback loop [68]. Furthermore, extracellular lactate modulates intracellular reactive oxygen species (ROS) homeostasis via ASIC1 and ASIC3 channels, which subsequently activates the NLRP3 inflammasome complex. This molecular mechanism potentiates localized inflammatory amplification and promotes pyroptosis within the degenerative disc microenvironment [48]. In addition, ASICs contribute to structural deterioration of intervertebral discs by modulating ECM degradation. Mechanistically, ASIC1a has been demonstrated to regulate metabolic homeostasis in endplate chondrocytes. Exposure to acidic extracellular microenvironments suppressed anabolic factor expression while upregulating catabolic factors, effects that are mitigated by ASIC1a siRNA through inhibition of NF- κ B transcriptional activity [49].

Table 2
The role of ion channels in IVDD.

Ion channel	Mechanism	Function	Cell	Ref.
P2X7R	P2X7R/NLRP3/IL-1 β pathway	Inflammatory response	IVD	[43]
ASIC1a	Ca ²⁺ /calcium-dependent proteases and signaling	Cellular apoptosis	CEP	[44]
ASIC1a	Ca ²⁺ /calpain and calcineurin	Cellular apoptosis	BMSC	[45]
ASIC1	ERK5 signaling pathway and mitochondrial dysfunction pathway	Cellular apoptosis	NP	[46]
ASIC1 and ASIC3	p53-p21/p27 and p16-Rb1 signaling	Cellular senescence	NP-MSCs	[47]
ASIC1 and ASIC3	ROS/NF- κ B/NLRP3/IL-1 β	Cellular inflammation and pyroptosis	NP	[48]
ASIC1a	NF- κ B/increase MMP expression and activity	ECM degradation	CEP	[49]
PIEZO1	Ca ²⁺ /ER stress and oxidative stress	Cellular senescence and apoptosis	NP	[50]
	Self-amplifying loop of NF- κ B and periostin	Cellular senescence	NP	[51]
	Calpain2/BAX/Caspase3 pathway	Cellular apoptosis	AF	[52]
	Piezo1-iron-ferroptosis axis	Cellular ferroptosis	NP	[53]
	increase pro-inflammatory cytokines, and induce mitochondrial dysfunction	Cellular apoptosis and autophagy	NP	[54]
	Ca ²⁺ /NF- κ B/NLRP3	Inflammatory response	NP	[55]
	Ca2+/F-actin/Yap signaling axis	ECM degradation	CEP	[56]
TRPV4	ERK MAPK pathway	Cellular apoptosis	NP	[57]
TRPV2	PI3K/AKT signaling pathway and Cyt c and caspase proteins	Cellular apoptosis	NP	[58]
TRPV4	COX2/PGE2 and activated p38 MAPK	Inflammatory response	AF	[59]
TRPV4	increased expression of proteoglycan and Prg4	ECM degradation	CEP	[60]

Concurrently, NP cells cultured under acidic conditions exhibit increased ASIC-3 expression at both transcriptional and translational levels. This upregulation promotes a catabolic phenotypic shift characterized by reduced aggrecan synthesis and elevated expression of matrix-degrading enzymes [69].

3.3. PIEZO1

The PIEZO channel family, comprising PIEZO1 and PIEZO2 subtypes, represents a class of mechanosensitive non-selective cation channels first identified as bona fide mechanical transducers in mammalian systems [70]. Compared to PIEZO2 which demonstrates more restricted tissue distribution, PIEZO1 exhibits broad expression patterns with particular enrichment in load-bearing musculoskeletal tissues [71]. This mechanotransduction channel can be activated by diverse physical stimuli including ultrasonic waves, tensile forces, and shear stress induced by fluid flow. Functionally, PIEZO1 demonstrates permeability to multiple divalent cations (Ca^{2+} , Mg^{2+} , Mn^{2+} , Ba^{2+}) and monovalent cations (Na^+ , K^+ , Cs^+), along with certain organic cationic molecules [72]. Of particular physiological significance is its role in mediating calcium influx, which modulates intracellular Ca^{2+} signaling cascades. This molecular mechanism enables PIEZO1 to serve as a critical mechanoelectrical transducer, converting extracellular mechanical stimuli into intracellular biochemical signals that regulate essential cellular processes including proliferation, differentiation, and tissue homeostasis [73,74].

In intervertebral disc cells, Piezo1 activation exacerbates disc degeneration by inducing NP and AF cell apoptosis and senescence, thereby disrupting cellular metabolic homeostasis. The stiffened ECM, a hallmark mechanical microenvironment in IVDD, triggers oxidative stress and endoplasmic reticulum (ER) stress through PIEZO1-mediated calcium influx. This pathway elevates intracellular Ca^{2+} and ROS levels, leading to mitochondrial Ca^{2+} overload and subsequent programmed necrosis via activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling cascade. In addition, mechano-stress activates the NF- κB p65 transcriptional pathway through PIEZO1- Ca^{2+} signaling, upregulating periostin expression [50,75,76]. Secreted periostin from senescent NPCs further amplifies NF- κB p65 activation, establishing a self-sustaining loop that accelerates cellular senescence and IVDD progression [51]. In AF cells, PIEZO1 activation promotes apoptosis by enhancing Ca^{2+} influx and dysregulating calcium-dependent signaling. This process initiates the Calpain2/Bax/Caspase3 proapoptotic pathway, which drives caspase-3 activation and irreversible cell death [52]. Emerging evidence implicates PIEZO1 in ferroptosis, an iron-dependent regulated cell death mechanism characterized by ROS accumulation. Under mechanical stress, PIEZO1 acts as a critical iron transporter, and its pharmacological inhibition significantly reduces iron overload, mitigates mitochondrial ROS production, and suppresses ferroptosis-associated changes, thereby alleviating mechanical injury-induced IVDD [53,77].

Moreover, Piezo1 channel activation mediates calcium ion influx that contributes to IVDD pathogenesis by exacerbating inflammatory responses and ECM degradation [54]. Mechanistically, the resultant intracellular Ca^{2+} surge triggers NF- κB pathway activation, which transcriptionally upregulates NLRP3 inflammasome components and IL-1 β expression. Simultaneously, elevated Ca^{2+} acts as a secondary messenger facilitating NLRP3 inflammasome oligomerization, thereby amplifying IL-1 β maturation and secretion [55]. Furthermore, Piezo1 orchestrates the Ca^{2+} /F-actin/YAP signaling axis that disrupts ECM homeostasis through downregulating collagen II and aggrecan synthesis while upregulating matrix-degrading enzymes (MMP-13 and ADAMTS-5) [56].

3.4. Transient receptor potential (TRP) channels

The TRP channel superfamily comprises seven distinct subfamilies:

TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV. These transmembrane proteins function as nonselective cation channels localized primarily in the plasma membrane [78]. TRP channels demonstrate ubiquitous tissue distribution and are expressed across nearly all mammalian cell types. These polymodal channels are activated by a diverse array of physical and chemical stimuli, including thermal gradients, mechanical forces, voltage fluctuations, nociceptive signals, photic stimuli, and chemosensory inputs [79]. Functioning as calcium-permeable nonselective cation channels, TRP channels serve as critical mediators of extracellular calcium influx, thereby modulating intracellular calcium homeostasis [80].

TRP ion channels are critically involved in the pathophysiology of IVDD, primarily mediated through their regulatory effects on calcium signaling dysregulation, secretion of pro-inflammatory cytokines, and activation of matrix metalloproteinases. These molecular mechanisms collectively contribute to the disruption of cellular metabolic equilibrium, amplification of inflammatory cascades, and progressive degradation of ECM homeostasis characteristic of IVDD progression. Firstly, pathophysiological mechanical stress mediated by TRP channels initiated a cascade of cellular injury mechanisms culminating in programmed cell death. The study demonstrated that TRPV4 modulated COX2/PGE2 signaling and mediated cellular injury induced by supra-physiological dynamic compression, potentially through the ERK pathway [57]. Furthermore, another research revealed that NPCs exhibited significant alterations in spreading morphology under ECM elastic modulus conditions. This mechanical stimulation concurrently activated TRPV2 channels and initiated the PI3K/AKT signaling cascade. Concomitantly, mitochondrial cytochrome c (Cyt c) release triggered caspase activation, ultimately promoting NP apoptosis [58]. Secondly, TRP channels play a key role in mediating the inflammatory response associated with IVDD. Upon TRPV4 activation, intervertebral disc cells exhibited significant upregulation of IL-6 family cytokines (IL-6, IL-11, IL-16, and leukemia inhibitory factor), concurrent with downregulation of T cell-recruiting chemokines and monocyte-associated chemokines. Notably, dynamic and static compressive loading elicited distinct chemokine correlation profiles. Pharmacological inhibition of TRPV4 during dynamic loading disrupted the coordinated expression between LIF and other cytokines, whereas TRPV4 blockade under static loading significantly altered the regulatory association of IL-16 with VEGFA [59,81,82]. These findings suggest mechanosensitive TRPV4 signaling differentially modulates cytokine-chemokine crosstalk in a loading modality-dependent manner. Finally, TRPV4 critically regulated ECM synthesis and modulated intervertebral disc tissue mechanotransduction in response to biomechanical stress. Activation of the TRPV4 channel in NP cells induced ECM-related transcriptional activity. Mechanistically, transient TRPV4 stimulation initiated a signaling cascade characterized by sequential Ca^{2+} flux, NF- κB pathway activation, and IL-6 upregulation. Chronic TRPV4 agonism potentiated degenerative progression via pathological glycosaminoglycan deposition and concomitant biomechanical stiffening of ECM. Notably, targeted TRPV4 inhibition significantly attenuated load-induced matrix catabolism and preserved tissue homeostasis under sustained compressive stress [60,83](Fig. 2).

4. Regulation of ion channels

The regulation of ion channels encompasses a multilevel regulatory cascade spanning transcriptional regulation, translational processes, intracellular trafficking, and membrane expression. This intricate modulation system is further influenced by the interactions with the lipid bilayer composition and exogenous pharmacological agents (Fig. 3). Such comprehensive regulatory mechanisms enable multifaceted modulation of ion channel function, establishing these transmembrane proteins as highly promising therapeutic targets for IVDD management.

The primary mechanisms of epigenetic regulation—DNA

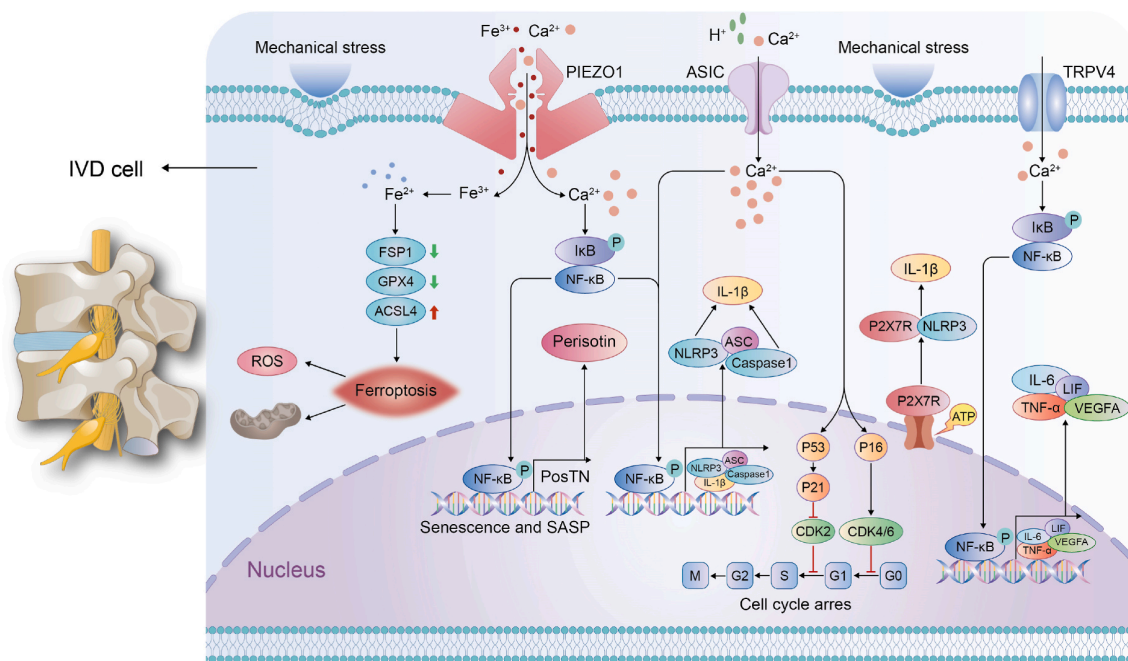


Fig. 2. The role of ion channels in IVD. When activated by mechanical stress and extracellular H^+ , ion channels distributed in IVD cell membrane including PIEZO1, ASIC and TRPV4 subsequently open and mediate the influx of Ca^{2+} and Fe^{3+} . Intracellular iron overload brought on by the Fe^{3+} influx ultimately results in ferroptosis as a stress response. The influx of Ca^{2+} activates the NF- κ B pathway to mediate senescence, apoptosis and pyroptosis of IVD cells via transcriptionally upregulating downstream proteins and pro-inflammatory cytokines. P2X7R distributed on the cell nuclear membrane can also bind to NLRP3 to promote the release of IL-1 β .

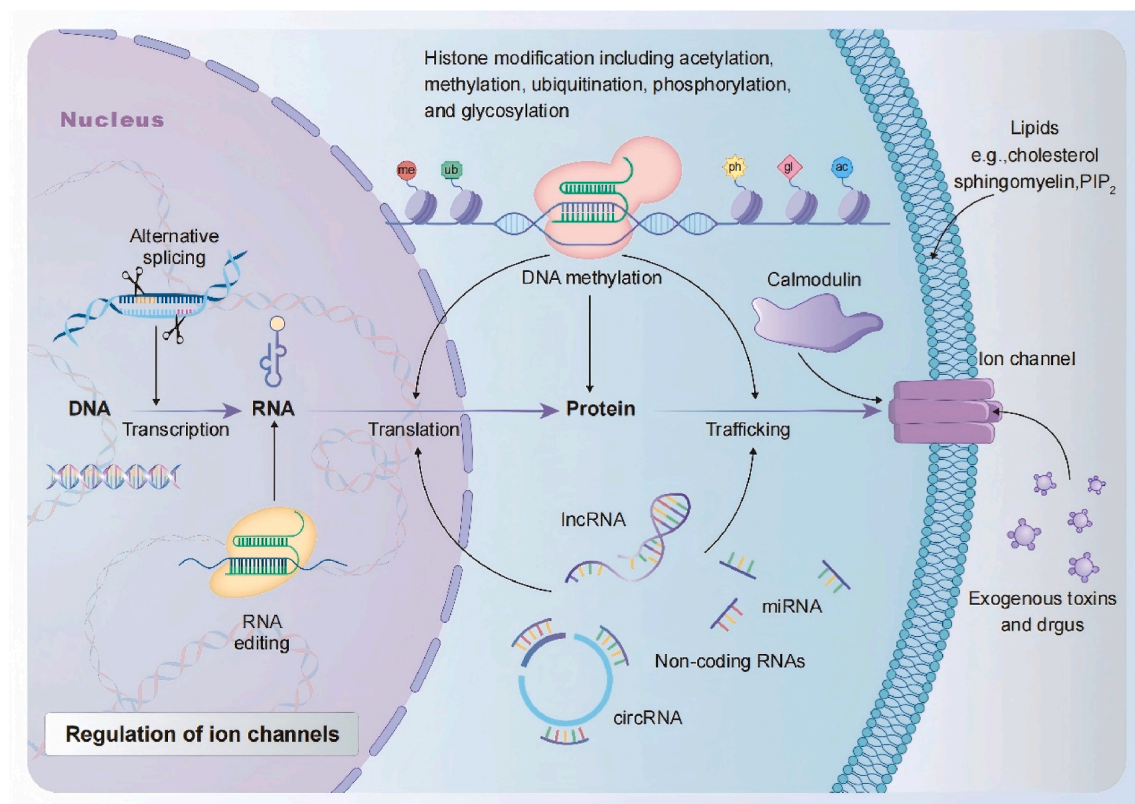


Fig. 3. Regulation of ion channels. Regulation of ion channels can occur at various steps from transcription and translation of the genetic code to trafficking of channel complexes and expression onto the plasma membrane, including alternative pre-mRNA splicing, RNA editing, epigenetic regulation, allosteric modulators, lipid environment, and exogenous drugs.

signaling pathways [103]. In the context of IVDD, circFOXO3 functioned as a ceRNA that sponged miR-185-3p and miR-939-5p, thereby relieving their inhibitory effects on ASIC1 expression. This regulatory axis culminates in ASIC1 upregulation and subsequent dysregulation of calcium ion homeostasis through enhanced Ca^{2+} influx, ultimately driving oxeiptosis in NPCs [104].

5. Possible therapeutic intervention

Ion channels are promising topics that may have important therapeutic implications for future research. Ion channels have been linked to the occurrence and prognosis of many diseases, especially neurodegenerative diseases and cardiovascular diseases (CVD). While a growing number of published studies now point to ion channels as a viable option for the treatment of IVDD.

5.1. Targeted drugs

Ion channel-related drugs are known for its applications in cardiovascular diseases and neurological diseases, such as hypertension and epilepsy. The European Society of Cardiology have stated that CVD medications like calcium channel blockers and antiarrhythmic drugs can also can affect cartilaginous tissues in addition to their own therapeutic effect [105]. Several studies have investigated how cardiovascular medications affect IVDD. Treatment with verapamil, a well-established blood pressure medication, effectively attenuated TBHP-triggered excessive generation of reactive oxygen species and suppressed NLRP3 inflammasome signaling. This therapeutic intervention consequently prevented cellular pyroptosis, apoptotic processes, and breakdown of extracellular matrix components [106]. Another calcium channel blocker nifedipine showed significant effect in the treatment of IVDD, resulting in altered cell calcium influx and downregulation of aggrecan and Col-II during dynamic compression [107]. In addition to CVD medications, the ion channels inhibitors have been shown to inhibit the progression of IVDD. For instance, GSMTx4, a selective PIEZO1 channel inhibitor, demonstrated significant anti-senescence effects by reducing senescence-associated β -galactosidase-positive cell populations. This compound effectively reversed the stiffness-dependent upregulation of MMP13 and POSTN expression in extracellular matrix environments [51]. Furthermore, Sa12b is a novel non-specific ASIC inhibitor that enhances the bioactivity of NP-MSCs (nucleus pulposus mesenchymal stem cells) in the severely acidic microenvironment of intervertebral discs by reducing Ca^{2+} influx through dual mechanisms of ASIC inhibition and Notch signaling pathway modulation [108] (Table 3).

5.2. Tissue engineering strategies

Current therapeutic approaches for IVDD, whether surgical or conservative, remain incapable of halting or reversing the underlying degenerative pathology. Mesenchymal stem cells (MSCs) have emerged as a promising regenerative strategy due to their potential to repopulate degenerated discs and restore ECM homeostasis. These multipotent adult stem cells, obtainable from bone marrow, adipose tissue, and muscular sources, exhibit self-renewal capacity and multilineage differentiation potential [109]. Notably, MSC differentiation into NP/AF-like phenotypes demonstrates enhanced synthesis of key ECM components, including collagen II and aggrecan, suggesting therapeutic potential for disc regeneration [110]. Preclinical and clinical studies have substantiated these regenerative properties [111]. However, the hostile microenvironment of degenerated discs—characterized by acidic pH, nutrient deprivation, and mechanical stress—compromises MSC viability, metabolic activity, and differentiation capacity. This necessitates tissue engineering approaches combining growth factors, biomaterial scaffolds, and cellular therapies [112].

Emerging evidence implicates ion channel modulation as a critical intervention point. The novel ASIC inhibitor Sa12b, isolated from *Sphex*

Table 3
Ion channel drugs associated with IVDD.

Drug	Target	Function	Cell	Ref.
Verapamil	VGCC	targeted the Nrf2/TXNIP/NLRP3 axis to suppress ROS overproduction and pyroptosis	NP	[106]
Nifedipine	VGCC	altered cell calcium influx and down-regulated col 1a, aggrecan and ADAMTS-4	NP	[107]
GsMTx4	PIEZO1	reduced the number of β -Gal ⁺ senescent cells and reversed the matrix stiffness-induced increase in expression of MMP13 and POSTN	NP	[51]
		alleviated AFCs apoptosis via inhibiting Calpain2/BAX/Caspase3 pathway	AF	[52]
GSK205	TRPV4	decreased NF- κ B activity, IL-6 and VEGFA production, and degenerative changes to the ECM	AF	[83]
GSK2193874	TRPV4	reduced compression-induced NP cell damage and inflammation via ERK MAPK pathway	NP	[57]
Sa12b	ASICs	improved the biological activity of NP-MSCs by reducing Ca^{2+} influx and the Notch signaling pathway	NP-MSCs	[108]
Amiloride	ASIC1a and ASIC3	suppressed NLRP3 inflammasome activation and pyroptosis through the ROS/NF- κ B signalling pathway	NP	[48]
APETx2	ASIC3	prevented the acidic pH induced proinflammatory and catabolic phenotype	NP	[69]
PcTx1	ASIC1 and ASIC3	alleviated premature senescence via inhibiting senescence programming pathways including p53-p21/p27 and p16-Rb1 signaling	NP-MSCs	[47]
	ASIC1a	decreased the activation of calpain and calcineurin and subsequent mitochondrial-mediated apoptosis	BMSC	[45]

argentatus venom, enhanced NP-MSC viability and bioactivity in acidic conditions by inhibiting ASICs and reducing calcium influx [108]. When integrated with RADA16-I self-assembling nanopeptides to form RAD/SA1 composites, this construct significantly outperformed conventional culture systems and RADA16-I alone in promoting NP-MSC proliferation and ECM synthesis—a process mediated through Ca^{2+} -dependent phosphorylation of ERK signaling pathways [113]. In addition, the mesenchymal stem cell differentiation can be controlled by mechanically stimulating ion channels. Innovative microgel platforms with upper critical solution temperature (UCST) properties demonstrate this principle: Adipose-derived MSCs (ADSCs) cultured on poly microgels exhibit omnidirectional stretching during thermoresponsive swelling, upregulating TRPV4 and Piezo1 channels. This mechanical priming promoted ADSC differentiation into NP-like cells with functional ECM remodeling capacity, offering a novel IVDD repair strategy [114] (Table 4).

6. Discussion and perspective

Intervertebral disc degeneration, as the predominant etiology of low back pain, substantially compromises patients' quality of life while imposing significant socioeconomic burdens. Contemporary therapeutic strategies, encompassing conservative management and surgical interventions, primarily provide transient symptomatic relief rather than addressing underlying pathological mechanisms. These approaches fail to halt disease progression or prevent IVDD development, underscoring the urgent need for pathogenesis-targeted therapies. Emerging insights from ion channel research in cardiovascular and neurological

Table 4
Summary of bioactive materials utilized to regulate ion channels in IVDD.

Bioactive materials	Target	Function	Cell	Ref.
RAD/SA1	ASICs	enhanced the Biological Activity of hNPMSCs via inhibiting the expression of p-ERK through Ca ²⁺ -dependent p-ERK signaling pathways	hNPMSCs	[110]
Injectable UCST Microgels	TRPV4 and Piezo1	upregulated TRPV4 and Piezo1 channel proteins and enhanced nucleus pulposus (NP)-like differentiation of ADSCs	adipose-derived mesenchymal stem cells	[114]
GM@CS-BP	ASIC3	maintained a balance between ECM synthesis and degradation by regulating the positive feedback between imbalanced oxygen metabolism in IVDs and inflammation	NP	[115]
Viscoelastic hydrogels	TRPV4	enhanced nucleus pulposus (NP)-like differentiation of ADSCs, coupled with an augmented secretion of aggrecan and type-II collagen	adipose-derived mesenchymal stem cells	[116]
HA hydrogel	TRPV1	altered glycosylation and modulated key inflammatory and signaling pathways, resulting in attenuation of inflammation and regulation of matrix components	NP and AF	[117]

pathologies have revealed promising therapeutic targets. Ion channels have been demonstrated to play a critical role in the pathogenesis of IVDD through their regulatory effects on cellular metabolic processes, modulation of inflammatory cascades, and maintenance the structural integrity of the intervertebral disc. In addition, the multifaceted regulatory capacity, combined with demonstrated druggability across multiple organ systems, positions ion channels as particularly viable therapeutic targets for IVDD intervention.

Ion channels exert stage-specific modulation of pathophysiological cascades during IVDD, with distinct regulatory patterns emerging in disease-specific microenvironments, where Ca²⁺ exerts pivotal regulatory control. The initial phase of IVDD is characterized by metabolic imbalances, compensatory adaptation to mechanical stress and acidic microenvironments, with calcium ions serving as crucial secondary messengers mediating cellular signaling pathways. ASIC1 channel activation facilitates calcium entry, leading to mitochondria-mediated apoptosis of nucleus pulposus cells via the ERK5 signaling cascade and mitochondrial dysfunction [46]. Meanwhile, the stiffened ECM triggered oxidative stress and ER stress through PIEZO1-mediated calcium influx, leading to mitochondrial Ca²⁺ overload and subsequent programmed necrosis [50]. When the degenerative process advances into an irreversible stage, the pathological progression becomes predominantly driven by the inflammatory cascade and catastrophic micro-architectural disintegration. Mechanistically, the pathological intracellular Ca²⁺ surge induces canonical NF-κB pathway activation through IκB kinase complex phosphorylation, leading to transcriptional upregulation of NLRP3 inflammasome core components and pro-IL-1β expression [55]. Furthermore, elevated cytosolic Ca²⁺ concurrently

promoted NLRP3 inflammasome oligomerization via P2X7 receptor and mitochondrial ROS generation, thereby potentiating caspase-1-dependent IL-1β maturation and subsequent pyroptotic secretion [43]. Finally, Piezo1 orchestrates the Ca²⁺/F-actin/YAP signaling axis that accelerate ECM catabolism through downregulating collagen II and aggrecan synthesis while upregulating matrix-degrading enzymes [56]. In summary, different ion channels play a role at different stages of IVDD, leading to corresponding pathological changes. It should be noted that the biomarkers for different stages of IVDD are still not very clear at present. Therefore, more research is needed to support the discovery of biomarkers for different stages of IVDD. Such research will help achieve stage-specific and sequential treatment of IVDD.

The regulation of ion channels occurs through multiple molecular mechanisms spanning from genomic expression to membrane localization. At the transcriptional level, alternative splicing of a single pre-mRNA transcript generates functionally distinct ion channel isoforms through precisely regulated mRNA processing [118]. RNA editing mechanisms further diversify protein products by introducing nucleotide modifications that alter coding sequences prior to translation. The research reported the use of CRISPR-Cas9 technology to successfully knock out TRPV4 in human AF cells, successfully reduced the stretch-induced upregulation of IL6, IL8 and COX2 gene expression, PGE2 release and p38 phosphorylation [59]. Another research developed multiplex CRISPR epigenome editing vectors that modulate endogenous expression of TRPA1, ASIC3, and Piezo2, abolishing degenerative IVD-induced mechanical nociception [119]. During protein synthesis and membrane trafficking, ion channels undergo essential co- and post-translational modifications including DNA methylation, histone modification, and non-coding RNA activity, which regulate gene expression through chromatin state modulation rather than altering DNA sequences. Common post-translational modifications include acetylation, methylation, ubiquitination, phosphorylation, and glycosylation. Allosteric regulation is achieved through calcium-dependent interactions with accessory proteins such as calmodulin, which induces conformational changes in channel gating properties [120]. In addition, the membrane lipid composition serves as a critical modulator of channel function, with specific lipid species exerting profound effects: PIP2 maintains voltage-gated channel activity through electrostatic interactions, cholesterol modulates mechanosensitive channels via membrane fluidity adjustments, and sphingomyelin-rich domains facilitate compartmentalized signaling [121]. Finally, ion channels are also regulated by exogenous toxins, and the development of related drugs has made them important targets for disease treatment. More mechanisms related to the regulation of ion channels in IVD need to be explored in the future, including alternative pre-mRNA splicing, RNA editing, histone modification, more non-coding RNAs, allosteric modulators, and lipid environment.

Emerging research highlights the therapeutic potential of ion channel-targeted pharmacological agents and bioactive materials for IVDD, though their clinical translation faces substantial limitations. Calcium channel blockers like nifedipine and verapamil exhibit established efficacy in hypertension management, while their application for IVDD requires rigorous preclinical and clinical validation to establish safety and efficacy profiles, particularly given systemic administration challenges posed by the disc's avascular architecture. The blood-disc barrier severely restricts therapeutic agent biodistribution, resulting in subtherapeutic concentrations at degenerative foci, while systemic verapamil use carries cardiovascular risks including bradycardia and conduction abnormalities, particularly in patients with preexisting cardiac comorbidities. Furthermore, monotherapeutic strategies targeting calcium homeostasis inadequately address IVDD's multifactorial pathophysiology involving inflammatory, oxidative, and biomechanical cascades. Parallel challenges exist for bioactive materials, where the disc's acidic, hypoxic, and hyperosmotic microenvironment compromises biomaterial integrity and homogeneous distribution, exacerbated by mechanical stresses during local injection [122]. Moreover, Current

- [78] Li H. TRP channel classification. *Adv Exp Med Biol* 2017;976:1–8. https://doi.org/10.1007/978-94-024-1088-4_1.
- [79] Zhang M, Ma Y, Ye X, Zhang N, Pan L, Wang B. TRP (transient receptor potential) ion channel family: structures, biological functions and therapeutic interventions for diseases. *Signal Transduct Target Ther* 2023;8:261. <https://doi.org/10.1038/s41392-023-01464-x>.
- [80] Ahern GP. Transient receptor potential channels and energy homeostasis. *Trends Endocrinol Metab* 2013;24:554–60. <https://doi.org/10.1016/j.tem.2013.06.005>.
- [81] Easson GWD, Savadipour A, Gonzalez C, Guilak F, Tang SY. TRPV4 differentially controls inflammatory cytokine networks during static and dynamic compression of the intervertebral disc. *JOR Spine* 2023;6:e1282. <https://doi.org/10.1002/jsp2.1282>.
- [82] Walter BA, Purmessur D, Moon A, Occhiogrosso J, Laudier DM, Hecht AC, et al. Reduced tissue osmolarity increases TRPV4 expression and pro-inflammatory cytokines in intervertebral disc cells. *Eur Cell Mater* 2016;32:123–36. <https://doi.org/10.22203/ecm.v032a08>.
- [83] Easson GWD, Savadipour A, Anandarajah A, Iannucci LE, Lake SP, Guilak F, et al. Modulation of TRPV4 protects against degeneration induced by sustained loading and promotes matrix synthesis in the intervertebral disc. *FASEB J* 2023;37:e22714. <https://doi.org/10.1096/fj.202201388R>.
- [84] Mattei AL, Bailly N, Meissner A. DNA methylation: a historical perspective. *Trends Genet* 2022;38:676–707. <https://doi.org/10.1016/j.tig.2022.03.010>.
- [85] Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005;6:597–610. <https://doi.org/10.1038/nrg1655>.
- [86] Ikuno A, Akeda K, Takebayashi S-I, Shimaoka M, Okumura K, Sudo A. Genome-wide analysis of DNA methylation profile identifies differentially methylated loci associated with human intervertebral disc degeneration. *PLoS One* 2019;14:e0222188. <https://doi.org/10.1371/journal.pone.0222188>.
- [87] Luo Z, Ma Y, Di T, Ma B, Li H, An J, et al. DNMT3B decreases extracellular matrix degradation and alleviates intervertebral disc degeneration through TRPA1 methylation to inhibit the COX2/YAP axis. *Aging (Albany NY)* 2021;13:20258–76. <https://doi.org/10.18632/aging.203410>.
- [88] Kouzarides T. Chromatin modifications and their function. *Cell* 2007;128:693–705. <https://doi.org/10.1016/j.cell.2007.02.005>.
- [89] Millán-Zambrano G, Burton A, Bannister AJ, Schneider R. Histone post-translational modifications - cause and consequence of genome function. *Nat Rev Genet* 2022;23:563–80. <https://doi.org/10.1038/s41576-022-00468-7>.
- [90] Kang L, Hu J, Weng Y, Jia J, Zhang Y. Sirtuin 6 prevents matrix degradation through inhibition of the NF- κ B pathway in intervertebral disc degeneration. *Exp Cell Res* 2017;352:322–32. <https://doi.org/10.1016/j.yexcr.2017.02.023>.
- [91] Xie T, Yuan J, Mei L, Li P, Pan R. Luteolin suppresses TNF- α -induced inflammatory injury and senescence of nucleus pulposus cells via the Sirt6/NF- κ B pathway. *Exp Ther Med* 2022;24:469. <https://doi.org/10.3892/etm.2022.11396>.
- [92] Zhou M, He S-J, Liu W, Yang M-J, Hou Z-Y, Meng Q, et al. EZH2 upregulates the expression of MAPK1 to promote intervertebral disc degeneration via suppression of miR-129-5p. *J Gene Med* 2022;24:e3395. <https://doi.org/10.1002/jgm.3395>.
- [93] Seal RL, Chen L-L, Griffiths-Jones S, Lowe TM, Mathews MB, O'Reilly D, et al. A guide to naming human non-coding RNA genes. *EMBO J* 2020;39:e103777. <https://doi.org/10.15252/embj.2019103777>.
- [94] Matsui M, Corey DR. Non-coding RNAs as drug targets. *Nat Rev Drug Discov* 2017;16:167–79. <https://doi.org/10.1038/nrd.2016.117>.
- [95] Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014;15:509–24. <https://doi.org/10.1038/nrm3838>.
- [96] Wang C, Cui L, Gu Q, Guo S, Zhu B, Liu X, et al. The mechanism and function of miRNA in intervertebral disc degeneration. *Orthop Surg* 2022;14:463–71. <https://doi.org/10.1111/os.13204>.
- [97] Zhang Y, Zhang Y-S, Li X-J, Huang C-R, Yu H-J, Yang X-X, et al. Overexpression of miR-150 inhibits the NF- κ B signal pathway in intervertebral disc degeneration through targeting P2X7. *Cells Tissues Organs* 2019;207:165–76. <https://doi.org/10.1159/000503281>.
- [98] Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem* 2012;81:145–66. <https://doi.org/10.1146/annurev-biochem-051410-092902>.
- [99] Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. Epigenetics of colorectal cancer: biomarker and therapeutic potential. *Nat Rev Gastroenterol Hepatol* 2020;17:111–30. <https://doi.org/10.1038/s41575-019-0230-y>.
- [100] Wang X, Li D, Wu H, Liu F, Liu F, Zhang Q, et al. LncRNA TRPC7-AS1 regulates nucleus pulposus cellular senescence and ECM synthesis via competing with HPN for miR-4769-5p binding. *Mech Ageing Dev* 2020;190:111293. <https://doi.org/10.1016/j.mad.2020.111293>.
- [101] Cao S, Liu H, Fan J, Yang K, Yang B, Wang J, et al. An oxidative stress-related gene pair (CCNB1/PKD1), competitive endogenous RNAs, and immune-infiltration patterns potentially regulate intervertebral disc degeneration development. *Front Immunol* 2021;12:765382. <https://doi.org/10.3389/fimmu.2021.765382>.
- [102] Liu C-X, Chen L-L. Circular RNAs: characterization, cellular roles, and applications. *Cell* 2022;185:2016–34. <https://doi.org/10.1016/j.cell.2022.04.021>.
- [103] Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet* 2019;20:675–91. <https://doi.org/10.1038/s41576-019-0158-7>.
- [104] Chen X, Song Y, Chen G, Zhang B, Bai Y, Sun C, et al. Circular RNA CircFOXO3 functions as a competitive endogenous RNA for acid-sensing ion channel subunit 1 mediating oxepitosis in nucleus pulposus. *Biomedicines* 2024;12:678. <https://doi.org/10.3390/biomedicines12030678>.
- [105] Abovans V. Introducing the 2019 ESC guidelines on diabetes, pre-diabetes, and CVD. *Eur Heart J* 2019;40:3217–9. <https://doi.org/10.1093/eurheartj/ehz688>.
- [106] Chen Y, Cao X, Pan B, Du H, Li B, Yang X, et al. Verapamil attenuates intervertebral disc degeneration by suppressing ROS overproduction and pyroptosis via targeting the Nrf2/TXNIP/NLRP3 axis in four-week puncture-induced rat models both in vivo and in vitro. *Int Immunopharmacol* 2023;123:110789. <https://doi.org/10.1016/j.intimp.2023.110789>.
- [107] Poillot P, Snuggs JW, Le Maitre CL, Huyghe JM. L-type Voltage-Gated calcium channels partly mediate Mechanotransduction in the intervertebral disc. *JOR Spine* 2022;5:e1213. <https://doi.org/10.1002/jsp2.1213>.
- [108] Wang Z, Han L, Chen H, Zhang S, Zhang S, Zhang H, et al. Sa12b improves biological activity of human degenerative nucleus pulposus mesenchymal stem cells in a severe acid environment by inhibiting acid-sensitive ion channels. *Front Bioeng Biotechnol* 2022;10:816362. <https://doi.org/10.3389/fbioe.2022.816362>.
- [109] Vadalà G, Russo F, Ambrosio L, Loppini M, Denaro V. Stem cells sources for intervertebral disc regeneration. *World J Stem Cells* 2016;8:185–201. <https://doi.org/10.4252/wjsc.v8.i5.185>.
- [110] Kasamkottil J, Gryadunova A, Martin I, Barbero A, Schären S, Krupkova O, et al. Spheroid-based tissue engineering strategies for regeneration of the intervertebral disc. *Int J Mol Sci* 2022;23:2530. <https://doi.org/10.3390/ijms23052530>.
- [111] Clouet J, Fusellier M, Camus A, Le Visage C, Guicheux J. Intervertebral disc regeneration: from cell therapy to the development of novel bioinspired endogenous repair strategies. *Adv Drug Deliv Rev* 2019;146:306–24. <https://doi.org/10.1016/j.addr.2018.04.017>.
- [112] Vadalà G, Ambrosio L, Russo F, Papalia R, Denaro V. Interaction between mesenchymal stem cells and intervertebral disc microenvironment: from cell therapy to tissue engineering. *Stem Cells Int* 2019;2019:2376172. <https://doi.org/10.1155/2019/2376172>.
- [113] Han L, Wang Z, Chen H, Li J, Zhang S, Zhang S, et al. Sa12b-Modified functional self-assembling peptide hydrogel enhances the biological activity of nucleus pulposus mesenchymal stem cells by inhibiting acid-sensing ion channels. *Front Cell Dev Biol* 2022;10:822501. <https://doi.org/10.3389/fcell.2022.822501>.
- [114] Huang X, Chen D, Liang C, Shi K, Zhou X, Zhang Y, et al. Swelling-mediated mechanical stimulation regulates differentiation of adipose-derived mesenchymal stem cells for intervertebral disc repair using injectable UCST microgels. *Adv Healthc Mater* 2023;12:e2201925. <https://doi.org/10.1002/adhm.202201925>.
- [115] Li Z, Cai F, Tang J, Xu Y, Guo K, Xu Z, et al. Oxygen metabolism-balanced engineered hydrogel microspheres promote the regeneration of the nucleus pulposus by inhibiting acid-sensitive complexes. *Bioact Mater* 2023;24:346–60. <https://doi.org/10.1016/j.bioactmat.2022.12.025>.
- [116] Liu Y, Li L, Li X, Cherif H, Jiang S, Ghezbash F, et al. Viscoelastic hydrogels regulate adipose-derived mesenchymal stem cells for nucleus pulposus regeneration. *Acta Biomater* 2024;180:244–61. <https://doi.org/10.1016/j.actbio.2024.04.017>.
- [117] Mohd Isa IL, Abbah SA, Kilcoyne M, Sakai D, Dockery P, Finn DP, et al. Implantation of hyaluronic acid hydrogel prevents the pain phenotype in a rat model of intervertebral disc injury. *Sci Adv* 2018;4:eaq0597. <https://doi.org/10.1126/sciadv.aag0597>.
- [118] Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet* 2008;40:1413–5. <https://doi.org/10.1038/ng.259>.
- [119] Stover JD, Trone MA, Lawrence B, Bowles RD. Multiplex epigenome editing of ion channel expression in nociceptive neurons abolished degenerative IVD-conditioned media-induced mechanical sensitivity. *JOR Spine* 2023;6:e1253. <https://doi.org/10.1002/jsp2.1253>.
- [120] Hoefflich KP, Ikura M. Calmodulin in action: diversity in target recognition and activation mechanisms. *Cell* 2002;108:739–42. [https://doi.org/10.1016/s0092-8674\(02\)00682-7](https://doi.org/10.1016/s0092-8674(02)00682-7).
- [121] Incontro S, Musella ML, Sammarini M, Di Scala C, Fantini J, Debanne D. Lipids shape brain function through ion channel and receptor modulations: physiological mechanisms and clinical perspectives. *Physiol Rev* 2025;105:137–207. <https://doi.org/10.1152/physrev.00004.2024>.
- [122] Koutsopoulos S, Zhang S. Long-term three-dimensional neural tissue cultures in functionalized self-assembling peptide hydrogels, matrigel and collagen I. *Acta Biomater* 2013;9:5162–9. <https://doi.org/10.1016/j.actbio.2012.09.010>.
- [123] Yilmaz I, Akalan H, Yasar Sirin D, Karaarslan N, Kaplan N, Ozbek H. Effects of an acetylcholinesterase inhibitor and an N-methyl-D-aspartate receptor antagonist on inflammation and degeneration of the nucleus pulposus. *Eur Rev Med Pharmacol Sci* 2022;26:4409–19. https://doi.org/10.26355/eurrev_202206_29080.



An official website of the United States government

[Here's how you know](#)

FULL TEXT LINKS



FASEB J. 2025 Jan 15;39(1):e70290. doi: 10.1096/fj.202400544RR.

Polyunsaturated fatty acids suppress PIEZO ion channel mechanotransduction in articular chondrocytes

Gabrielle K Marushack^{1 2 3 4}, Alireza Savadipour^{1 2 3 5}, Ruhang Tang^{1 2 3},
Jaquelin M Garcia-Castorena^{1 2 3 6}, Neda Rashidi^{1 2 3 5}, Robert J Nims^{1 2 3},
Natalia S Harasymowicz^{1 2 3}, Yu Seon Kim^{1 2 3}, Farshid Guilak^{1 2 3 5}

Affiliations

PMID: 39786170 DOI: [10.1096/fj.202400544RR](https://doi.org/10.1096/fj.202400544RR)

Abstract

Osteoarthritis (OA) is characterized by articular cartilage degeneration, leading to pain and loss of joint function. Recent studies have demonstrated that omega-3 (ω 3) polyunsaturated fatty acid (PUFA) supplementation can decrease injury-induced OA progression in mice fed a high-fat diet. Furthermore, PUFAs have been shown to influence the mechanical properties of chondrocyte membranes, suggesting that alterations in mechanosensitive ion channel signaling could contribute to the mechanism by which ω 3 PUFAs decreased OA pathogenesis. Here, we hypothesized that PUFAs may alter mechanical signaling through PIEZO1 (activated by changes in membrane tension) and TRPV4 (activated by physiologic mechano-osmotic signals), as these mechanosensitive cation channels have been shown to influence OA progression. Our results demonstrated that PUFAs reduced chondrocyte sensitivity to single-cell mechanical compression and to pharmacologic agonists of PIEZO1 and TRPV4, with ω 3 PUFAs having the most significant effects overall. We also found that supplementation with ω 6 PUFA linoleic acid (LA) altered the biophysical properties of chondrocytes, as evidenced by increased intracellular lipid droplet formation and more rapid membrane rupture in response to hypo-osmotic shock, suggesting that LA increases chondrocyte membrane susceptibility to damage. Our findings underscore the differential impacts of specific PUFAs on chondrocyte signaling and membrane properties and provide important considerations in the development of nutritional interventions to prevent or treat OA.

Keywords: PIEZO channels; TRPV4; articular cartilage; mechanosensitive ion channels; osteoarthritis; polyunsaturated fatty acids.

© 2025 Federation of American Societies for Experimental Biology.

[PubMed Disclaimer](#)

Related information

[MedGen](#)[PubChem Compound \(MeSH Keyword\)](#)

LinkOut – more resources

Full Text Sources

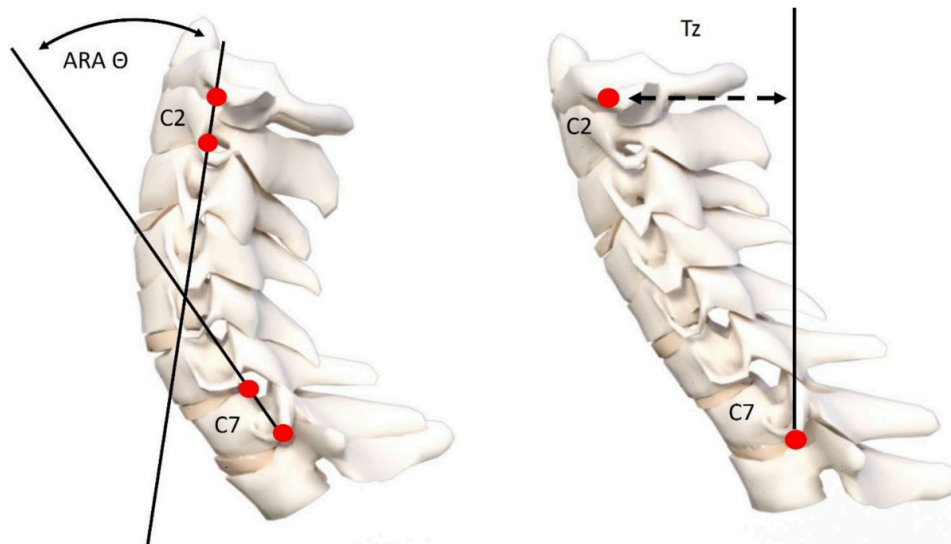
Utilizing machine learning to predict post-treatment outcomes in chronic non-specific neck pain patients undergoing cervical extension traction

Ibrahim M Moustafa^{1 2 3}, Dilber Uzun Ozsahin^{4 5 6}, Mubarak Taiwo Mustapha^{5 6 7},
Amal Ahbouch^{1 2}, Paul A Oakley^{8 9 10}, Deed E Harrison¹¹

Affiliations: [— collapse](#)

Affiliations

- 1 Department of Physiotherapy, College of Health Sciences, University of Sharjah, 27272, Sharjah, United Arab Emirates.
- 2 Neuromusculoskeletal Rehabilitation Research Group, RIMHS-Research Institute of Medical and Health Sciences, University of Sharjah, 27272, Sharjah, United Arab Emirates.
- 3 Faculty of Physical Therapy, Cairo University, Giza, 12613, Egypt.
- 4 Department of Medical Diagnostic Imaging, College of Health Science, University of Sharjah, Sharjah, United Arab Emirates.
- 5 Operational Research Centre in Healthcare, Near East University, TRNC Mersin 10, 99138, Nicosia, Turkey.
- 6 Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah, United Arab Emirates.
- 7 Department of Biomedical Engineering, Near East University, Nicosia, Mersin 10, Turkey.
- 8 CBP Nonprofit (a Spine Research Foundation), Eagle, ID, 83616, USA.
- 9 Private Practice, Newmarket, ON, L3Y 8Y8, Canada.
- 10 Kinesiology and Health Science, York University, Toronto, ON, M3J 1P3, Canada.
- 11 CBP Nonprofit (a Spine Research Foundation), Eagle, ID, 83616, USA.
drdeedharrison@gmail.com.



Mol Neurobiol. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Mol Neurobiol. 2018 August ; 55(8): 6601–6636. doi:10.1007/s12035-017-0852-4.

Brain Photobiomodulation Therapy: A Narrative Review

Farzad Salehpour^{a,b}, Javad Mahmoudi^a, Farzin Kamari^a, Saeed Sadigh-Eteghad^a, Seyed Hossein Rasta^{b,c,d}, and Michael R Hamblin^{e,f,g}

^aNeurosciences Research Center (NSRC), Tabriz University of Medical Sciences, Tabriz, Iran

^bDepartment of Medical Physics, Tabriz University of Medical Sciences, Tabriz, Iran

^cDepartment of Medical Bioengineering, Tabriz University of Medical Sciences, Tabriz, Iran

^dSchool of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

^eWellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts 02114, United States

^fDepartment of Dermatology, Harvard Medical School, Boston, Massachusetts 02115, United States

^gHarvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts 02139, United States

Abstract

Brain photobiomodulation (PBM) therapy using red to near-infrared (NIR) light is an innovative treatment for a wide range of neurological and psychological conditions. Red/NIR light is able to stimulate complex IV of the mitochondrial respiratory chain (cytochrome c oxidase) and increase ATP synthesis. Moreover, light absorption by ion channels results in release of Ca²⁺ and leads to activation of transcription factors and gene expression. Brain PBM therapy enhances the metabolic capacity of neurons and stimulates anti-inflammatory, anti-apoptotic, and antioxidant responses, as well as neurogenesis and synaptogenesis. Its therapeutic role in disorders such as dementia and Parkinson's disease, as well as to treat stroke, brain trauma, and depression has gained increasing interest. In the transcranial PBM approach, delivering a sufficient dose to achieve optimal stimulation is challenging due to exponential attenuation of light penetration in tissue. Alternative approaches such as intracranial and intranasal light delivery methods have been suggested to overcome this limitation. This article reviews the state-of-the-art preclinical and clinical evidence regarding the efficacy of brain PBM therapy.

Keywords

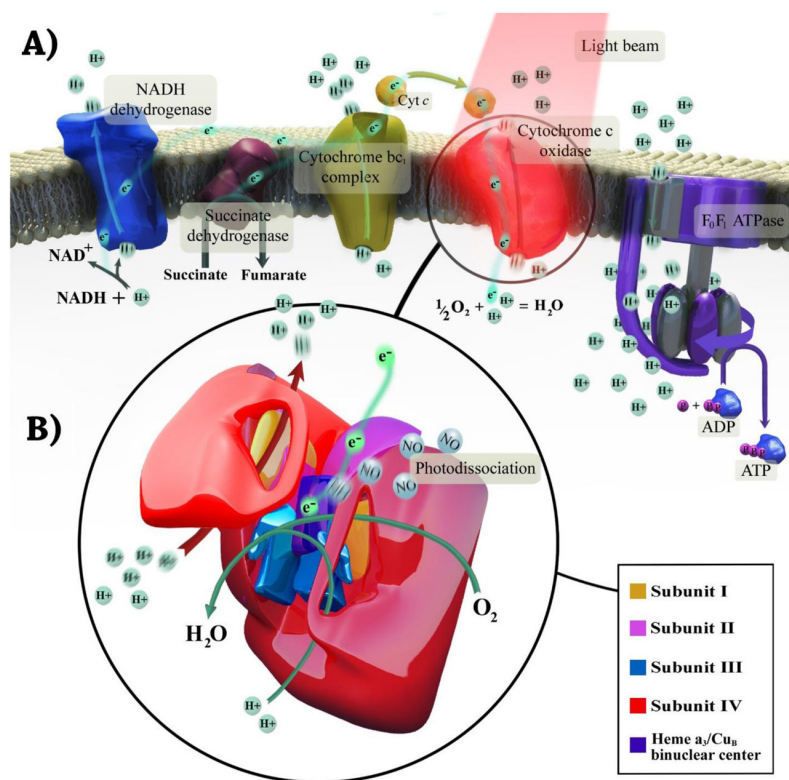


Fig. 1. Mechanism of photobiomodulation therapy in mitochondria

(A) The flow of electrons and protons through the mitochondrial respiratory chain.

Photobiomodulation stimulates cytochrome c oxidase, improves its catalytic activity, and elevates ATP synthesis. All these results in enhancement of neuronal respiration and metabolic capacity.

(B) The structure of cytochrome c oxidase and electrons path through its subunits. The complex contains two copper centers as well as two heme prosthetic groups.

Photobiomodulation may dissociate nitric oxide from binuclear center (a₃/Cu_B), allowing oxygen to return, and facilitates electron transfer and increases proton gradient.

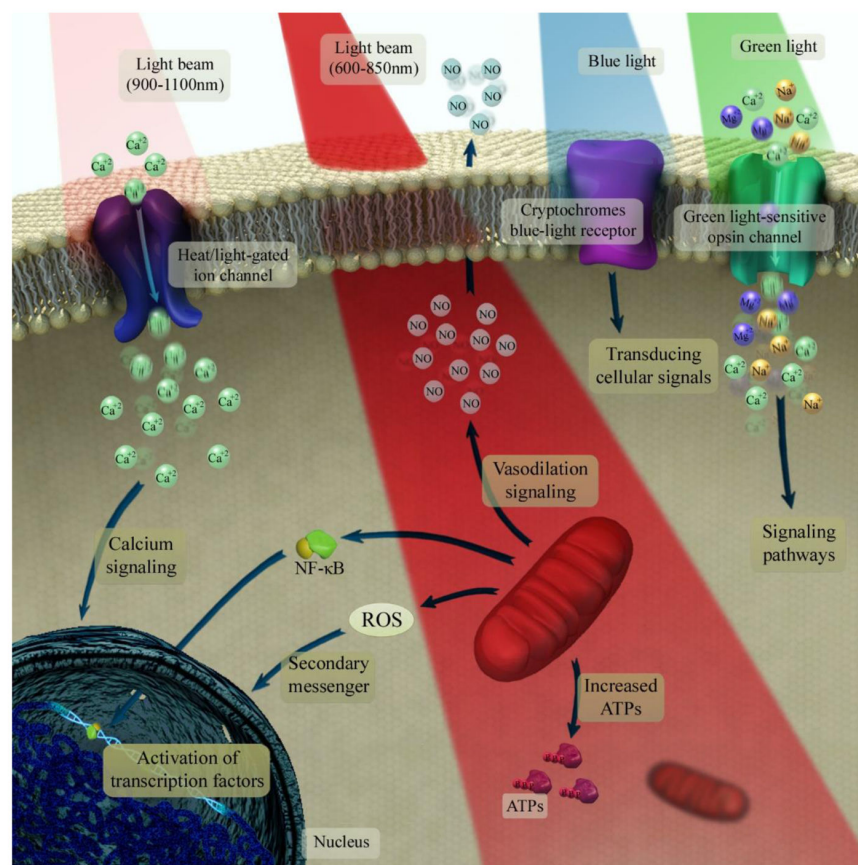


Fig. 2. Photobiomodulation underlying mechanisms at the cellular and molecular levels

Light at 600–850 nm is absorbed by the mitochondrial electron transfer chain and leads to upregulation of the neuronal respiratory capacity. The near-infrared light at range of 900–1100 nm is absorbed by structured water clusters formed in or on a heat/light-gated ion channels. An increase in vibrational energy of water cluster leads to perturb the protein structure and opening the channel which ultimately allows modulation of intracellular Ca^{2+} levels. The absorption of green light by neuronal opsin photoreceptors (OPN2–5) activates transient receptor potential channels which causes non-selective permeabilization to Ca^{2+} , Na^{+} and Mg^{2+} . The cryptochromes (a class of flavoprotein blue-light signaling receptors) absorb blue light and seems to activate the transducing cellular signals via part of the optic nerve to the suprachiasmatic nucleus in the brain, which is important in regulation of the circadian clock.

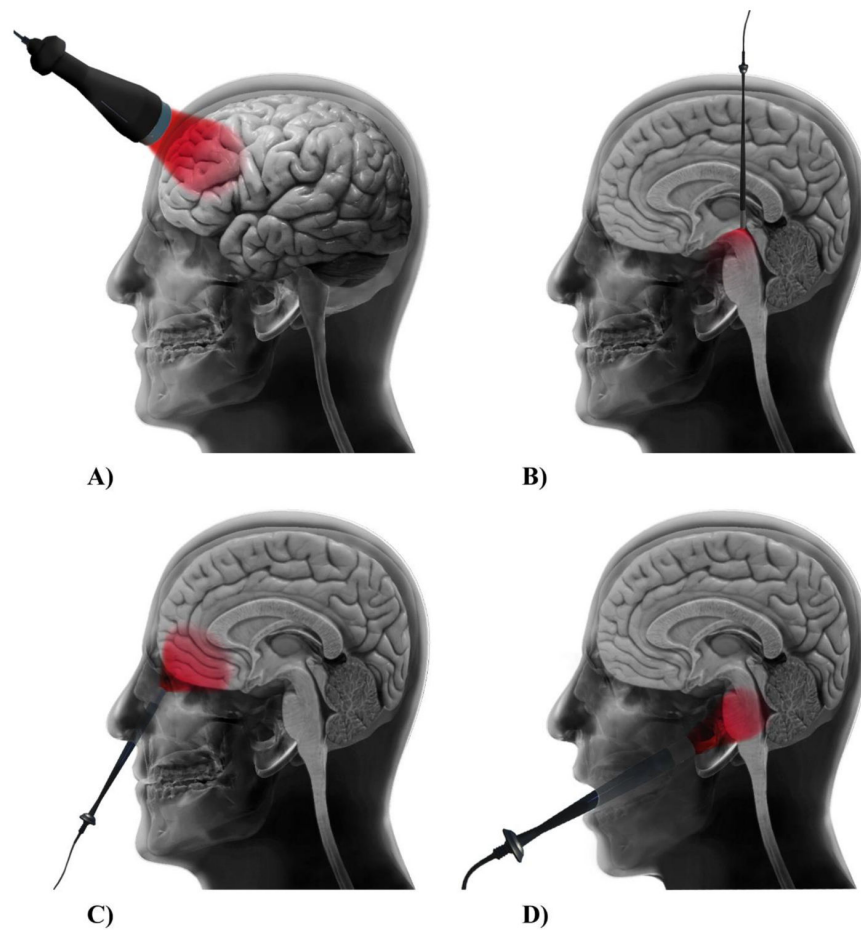


Fig. 3. Different approaches for light delivery to achieve brain photobiomodulation therapy (A) Transcranial, (B) intracranial, (C) intranasal photobiomodulation therapy, (D) brain photobiomodulation via oral cavity.

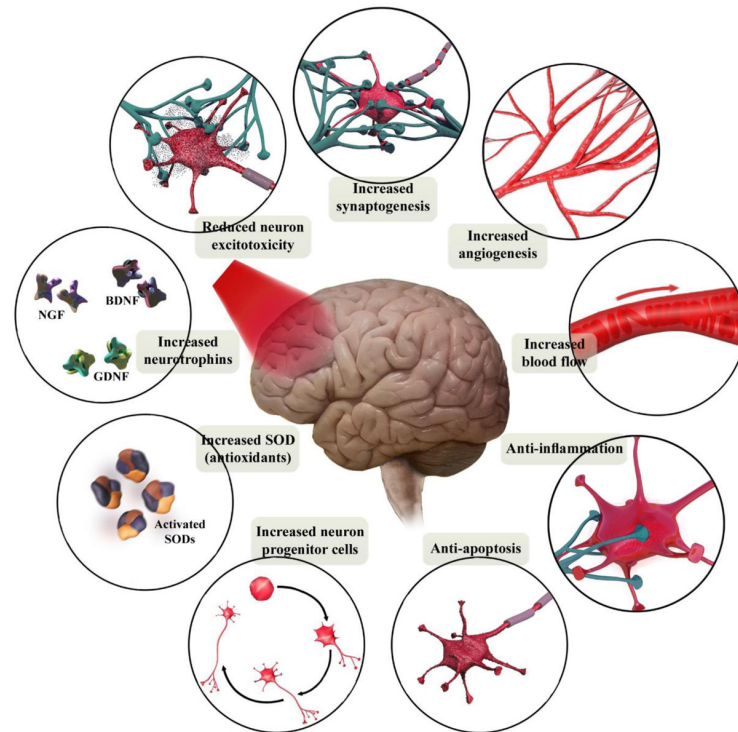


Fig. 4. Brain tissue-specific functional processes that occur after brain photobiomodulation therapy

On a cellular level, photobiomodulation can reduce apoptosis and excitotoxicity, increase antioxidants, neurotrophins and stimulate neuroprogenitor cells. On a tissue level, photobiomodulation therapy can increase blood flow and angiogenesis, reduce inflammation and help neurons form new connections. BDNF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; NGF, nerve growth factor; SOD, superoxide dismutase.

Review

Photobiomodulation Therapy on Brain: Pioneering an Innovative Approach to Revolutionize Cognitive Dynamics

Tahsin Nairuz , Sangwoo-Cho and Jong-Ha Lee *

Department of Biomedical Engineering, Keimyung University, Daegu 42601, Republic of Korea;

June 3, 2024; Vol. 13; No. 11; Article 966

Photobiomodulation (PBM) therapy on the brain employs red to near-infrared (NIR) light to treat various neurological and psychological disorders.

The mechanism involves the activation of cytochrome c oxidase in the mitochondrial respiratory chain, thereby enhancing ATP synthesis.

This cascade of events not only augments neuronal metabolic capacity but also orchestrates anti-oxidant, anti-inflammatory, and anti-apoptotic responses, fostering neurogenesis and synaptogenesis.

It shows promise for treating conditions like dementia, stroke, brain trauma, Parkinson's disease, and depression, even enhancing cognitive functions in healthy individuals.

However, delivering sufficient light to the brain through transcranial approaches poses a significant challenge due to its limited penetration into tissue.

Photobiomodulation (PBM) constitutes a sophisticated approach rooted in utilizing visible and near-infrared light at minimal power densities, and represents a captivating frontier in medical treatment.

This application necessitates precise control over the administered fluence to ensure the avoidance of thermal consequences.

Cytochrome c oxidase (CCO), an integral player in the electron transport chain (complex IV) of mitochondria, exhibits absorbance within the wavelength range of approximately 600 to 900 nm.

Beyond this range, longer wavelengths are theorized to engage with water molecules and light-gated ion channels.

PBM demonstrates the remarkable capability to mitigate oxidative stress through dislodging inhibitory nitric oxide (NO) from the CCO complex, thus orchestrating a cascade of intricate cellular responses conducive to therapeutic outcomes.

In 1967, PBM was serendipitously stumbled upon by Endre Mester of Hungary,

Despite Mester's ruby laser being significantly less powerful than the one used by McGuff, it yielded unforeseen results.

Instead of eradicating tumors, Mester observed remarkable outcomes: a stimulation of hair regrowth and accelerated wound healing at the tumor sites.

Mester termed "laser biostimulation", subsequently recognized as "low-level laser therapy" (LLLT).

PBM holds substantial promise as a therapeutic avenue for a diverse array of neurological disorders, spanning from stroke and traumatic brain injury (TBI) to Parkinson's disease (PD), Alzheimer's disease (AD), and depression, as well as potentially offering cognitive augmentation among healthy individuals.

... significant improvements in memory, executive function, and mood disorders, as well as promising therapeutic avenues for stroke, TBI, and neurodegenerative diseases.

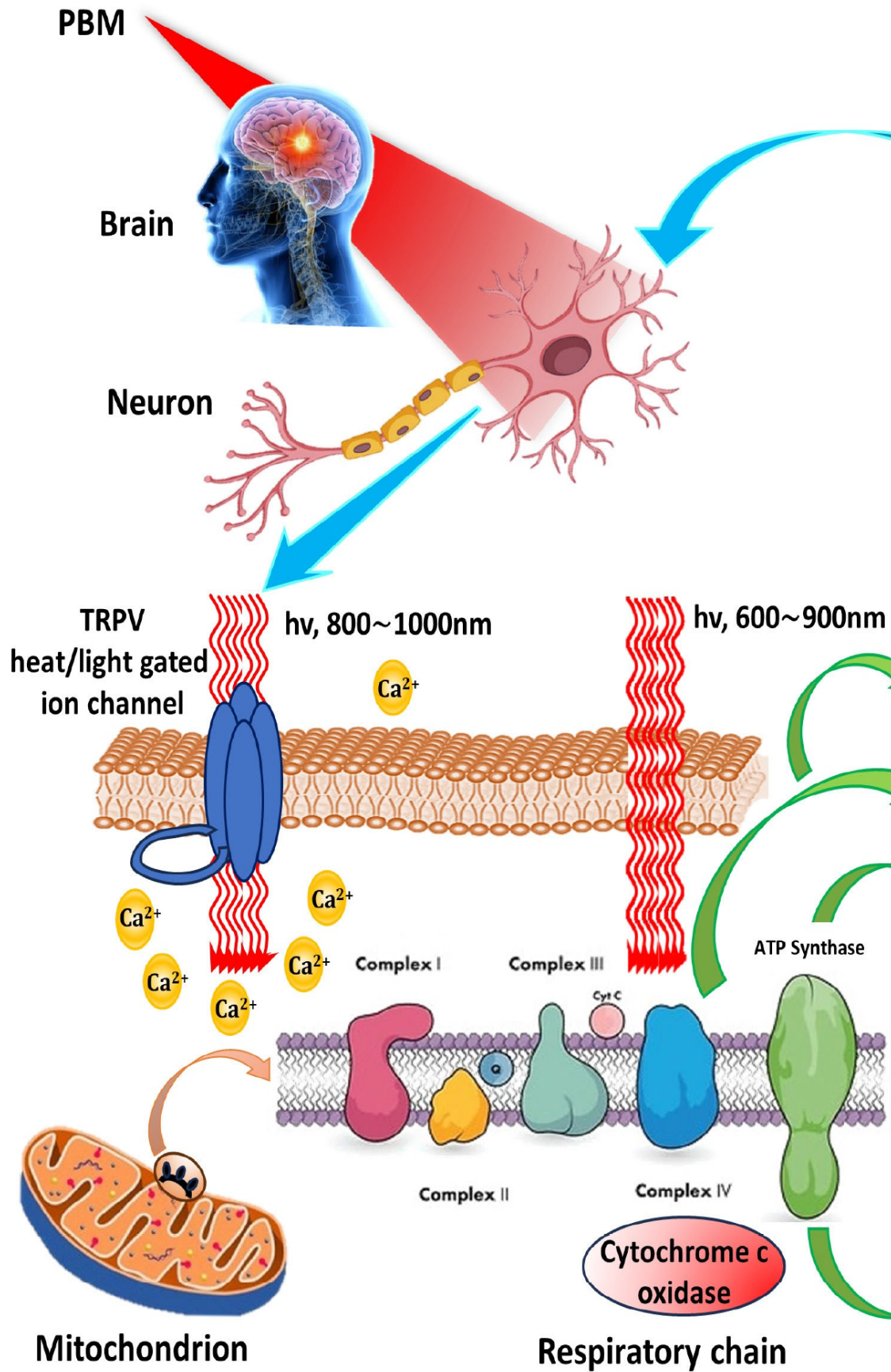
The predominant mechanism underlying PBM revolves around cytochrome c oxidase (CCO), an essential component in the respiratory chain of mitochondria.

... the final enzyme of complex IV of the mitochondrial electron transport chain.

In circumstances marked by hypoxia or cellular damage, nitric oxide (NO) can impede CCO enzyme activity.

Photons absorbed by CCO, especially within the red (600–700 nm) and near-infrared (760–940 nm) spectral ranges, can dissociate inhibitory NO.

This dissociation event triggers a cascade of effects: a surge in mitochondrial membrane potential, enhanced oxygen consumption, and intensified glucose metabolism, culminating in an amplification of ATP production within the mitochondria.



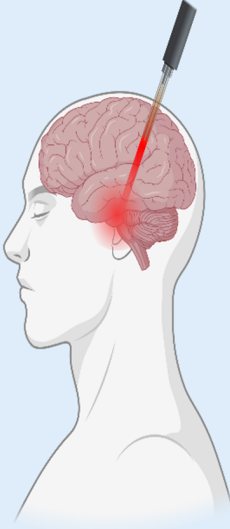
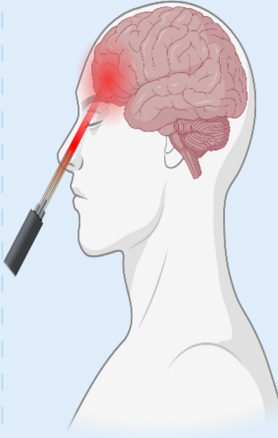
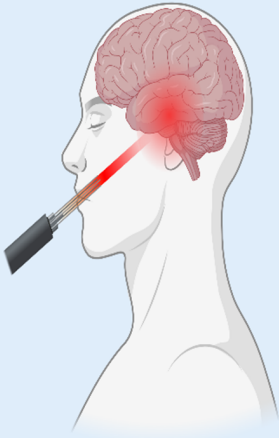
While green light has less practical clinical use due to its limited tissue penetration, infrared and near-infrared light are more promising.

As water predominantly absorbs infrared light ... [it] could conceivably induce alterations in protein conformation, potentially leading to the opening of ion channels.

PBM is thought to elicit retrograde mitochondrial signaling, an intriguing process where communication occurs between mitochondria and the cell nucleus, contrary to the typical flow of signals from the nucleus to other organelles.

Karu's insights suggest supplementary pathways, including directly increasing the expression of particular genes.

Transcranial photobiomodulation (tPBM) entails the non-invasive process of delivering light emitted by an external source, like a laser or LED, through several layers of the head, starting from the scalp, periosteum, skull bone, meninges, and dura, ultimately reaching the surface of the brain cortex.

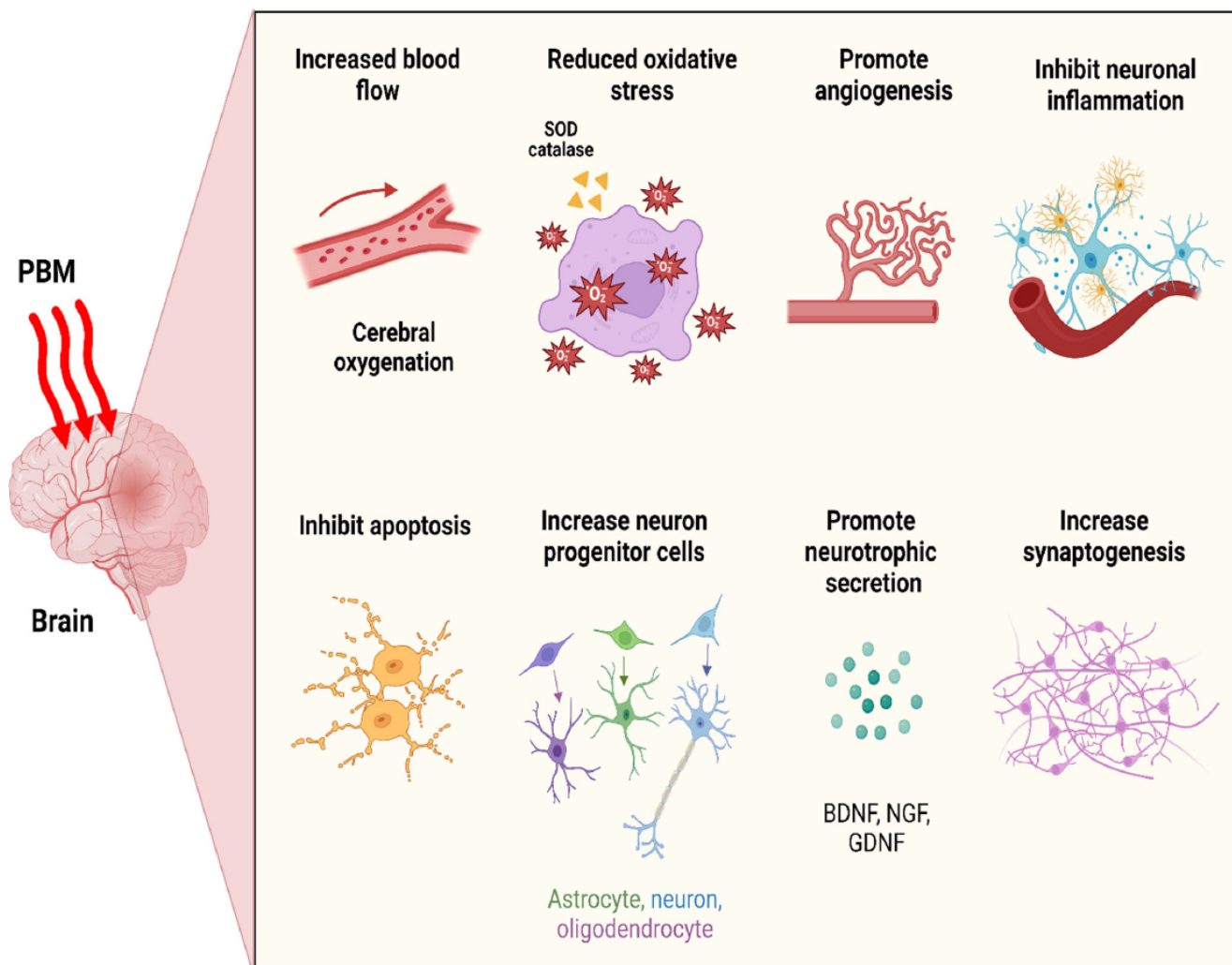
	Transcranial PBM (A)	Intracranial PBM (B)	Intranasal PBM (C)	PBM via Oral cavity (D)
Light delivery approaches				
Target(s)	• Scalp • Periosteum • Skull bone • Meninges • Dura • Cortical surface	• Midbrain • Substantia nigra pars compacta (SNc)	• Hypothalamus • Thalamus • Amygdala • Hippocampus • Orbitofrontal cortex	• Substantia nigra pars compacta (SNc)

There is also promising evidence that light radiation via the nasal and oral cavities can improve symptoms of dementia and PD.

Combining light exposure through the sphenoid sinus and mouth cavity ... demonstrated cognitive and functional improvements in dementia patients.

A contentious issue yet to be definitively resolved is whether or not coherent monochromatic lasers outperform non-coherent LEDs.

At the pinnacle of brain photobiomodulation therapy, pivotal outcomes emerge: optimizing cerebral metabolic activity, enhancing blood flow and angiogenesis, mitigating apoptosis while bolstering antioxidant levels and diminishing inflammatory responses, promoting neurotrophins secretion, fostering the activation of neuroprogenitor cells, and facilitating synaptogenesis, thereby enabling the formation of novel neuronal connections.



BDNF = brain-derived neurotrophic factor; GDNF = glial-derived neurotrophic factor; NGF = nerve growth factor; SOD = superoxide dismutase

Mitochondria undergo significant alterations under pathological circumstances, including diminished activity for respiratory chain complexes and attenuated ATP synthesis, alongside excessive ROS production, mitochondrial membrane potential (MMP) disruption, a transition of permeability in the inner mitochondrial membrane, and the subsequent discharge of cytochrome c in the cytosol.

Therefore, mitochondrial malfunction plays a critical role in the progression of numerous neurological and psychological disorders.

Neural tissue is notably abundant in mitochondria, and is thus highly responsive to light interactions, particularly with CCO, a key player in neuronal energy metabolism.

Neural tissues exhibit profound reliance on ATP generated by mitochondria for energy demands.

Diminished blood flow within the cerebral vasculature often represents one of the initial indicators of various brain disorders.

Nitric oxide (NO), a potent vasodilator, has the potential to be released from its binding sites during PBM via photodissociation in the respiratory chain.

PBM can widen blood vessel diameter, elevate NO levels in the brain, and enhance CBF.

PBM therapy on specific brain regions may influence regional CBF.

PBM has emerged as a potent modulator of angiogenesis, thereby facilitating improved blood flow.

Mitochondria are significant contributors to oxidative stress as they generate reactive oxygen species (ROS) that detrimentally impact neurons by impairing their mitochondrial functionality. A burgeoning body of literature has revealed links between oxidative stress and neurological conditions like AD, TBI, stroke, and major depressive disorder (MDD).

The beneficial or adverse impact of PBM is intricately tied to mitochondrial ROS production. While low PBM doses induce mild mitochondrial ROS, affecting cellular signaling, higher doses, can prompt excessive ROS generation, potentially activating cellular apoptosis.

The prolonged and excessive release of NO can exert neurotoxic effects, potentially leading to tissue damage.

Brain PBM emerges as a crucial initial intervention for ameliorating mitochondrial dysfunction induced by oxidative stress.

Neuroinflammation, a hallmark of brain disorders, is primarily facilitated by activated microglial cells.

Following various neuronal injuries, microglia undergo a cascade of morphological and proliferative changes, culminating in the secretion of pro-inflammatory mediators such as cytokines, chemokines, NO, and ROS.

PBM intervenes in this inflammatory cascade by impeding NF- κ B signaling pathways, thereby lowering the release of pro-inflammatory cytokines and mitigating inflammatory reactions.

PBM has been shown to reduce pro-inflammatory cytokines such as IL-1 β , IL-6, TGF β , and TNF α .

PBM exhibits a profound ability to downregulate the expression of ROS-generated inducible nitric oxide synthase (iNOS), further underscoring its anti-inflammatory potential.

There is abundant evidence indicating that PBM can be employed for neuroprotection, enhancing the survival and lifespan of neuronal cells by significantly ameliorating neuronal apoptosis through anti-apoptotic effects.

The potential of PBM to enhance both synaptogenesis and neurogenesis is one of its most remarkable and substantial effects on the brain.

PBM facilitates neuronal connectivity by intricately regulating the expression and function of different neurotrophic factors (neurotrophins) including brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), and nerve growth factor (NGF).

PBM can also stimulate the generation of neuroprogenitor cells, similar to neural stem cells, which could positively impact neurogenesis.

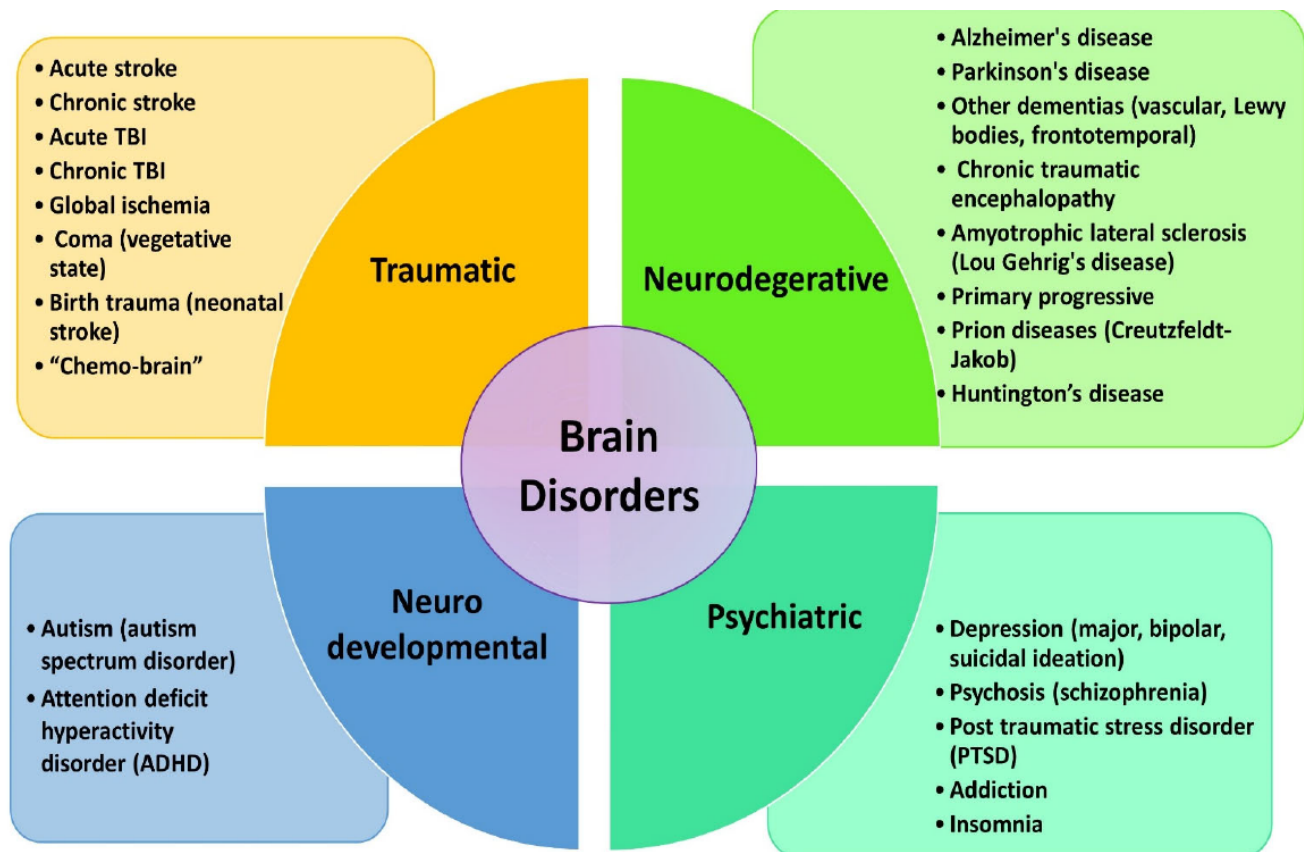
While direct brain PBM is considered the predominant therapeutic approach, there is a burgeoning understanding of neuroprotective effects stemming from irradiating specific non-brain regions. This implies that the light stimulation of certain organs may yield systemic benefits that indirectly influence the brain.

... enhanced immune cell activity, the modulation of cytokines, and increased mitochondrial ATP levels.

Mesenchymal stem cells may be mobilized via PBM of the bone marrow, allowing them to migrate to the brain and potentially restore cognitive function in AD patients as the disease progresses.

Particularly, noninvasive multisensory 40 Hz stimulation, comprising combined light and sound stimulation, reduces amyloid deposition across the cortex, notably the prefrontal cortex, thereby enhancing neural activity in an Alzheimer's disease.

A wide array of disorders afflicts the brain, and are broadly categorized into four groups: traumatic events, degenerative diseases, and neurodevelopmental and psychiatric disorders that could potentially benefit from various forms of transcranial photobiomodulation therapy.



As people age, there is often a decline in cognitive functions, making the enhancement or preservation of these abilities particularly important, especially for older adults who face increased susceptibility to dementia.

PBM therapy targeted towards the prefrontal region might enhance the brain's hemodynamics as well as oxygenation, which are critical to higher-level cognitive functions.

The future prospects of PBM in the field of neuroscience appear exceedingly promising, owing to its remarkably positive outcomes in animal models and the encouraging results from initial clinical trials.

Given the considerable challenges encountered in developing pharmaceutical interventions for conditions like stroke.

The favorable outcomes demonstrated by brain PBM therapy in mitigating symptoms of depression and anxiety may pave the way for novel trials targeting a broader range of psychiatric disorders including bipolar disorder, attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), as well as schizophrenia and autism.

Considering PBM's well-documented safety profile and its notable absence of adverse effects, alongside its relative cost-effectiveness, there is a strong argument for initiating comprehensive clinical trials for these indications. Furthermore, the disappointingly low efficacy rates and often distressing side effects associated with conventional psychiatric medications, such as antidepressants, highlight the need for novel therapeutic modalities like PBM.

This study cites 172 references.

THE NEW YORK TIMES BESTSELLER



2017

Why We Sleep

UNLOCKING THE
POWER OF SLEEP
AND DREAMS

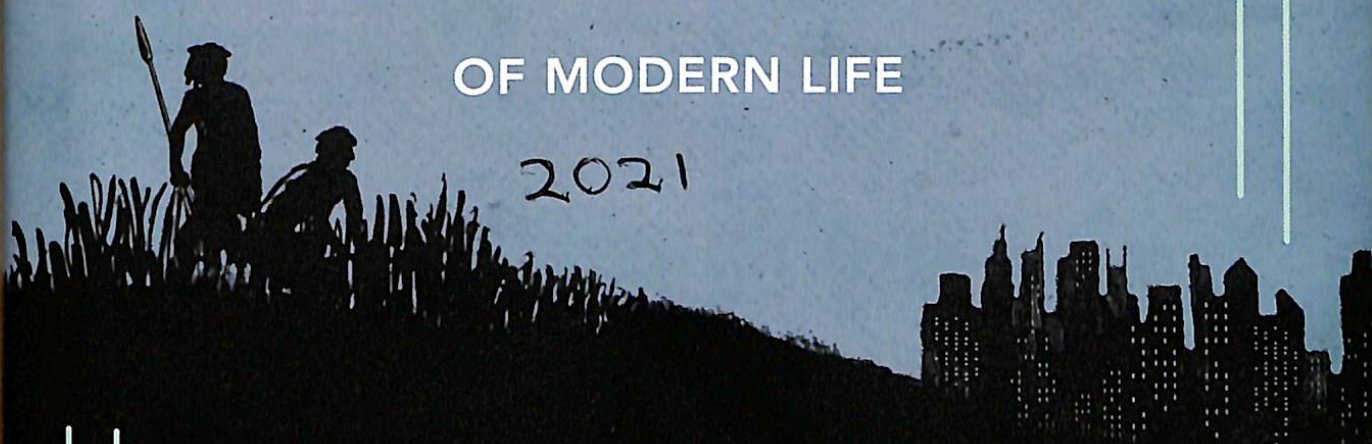
Matthew Walker, PhD

"A book on a mission... recommended for night-table reading in the most pragmatic sense." —*THE NEW YORK TIMES BOOK REVIEW*

A
HUNTER-GATHERER'S
GUIDE TO THE
21ST CENTURY

EVOLUTION AND THE CHALLENGES
OF MODERN LIFE

2021

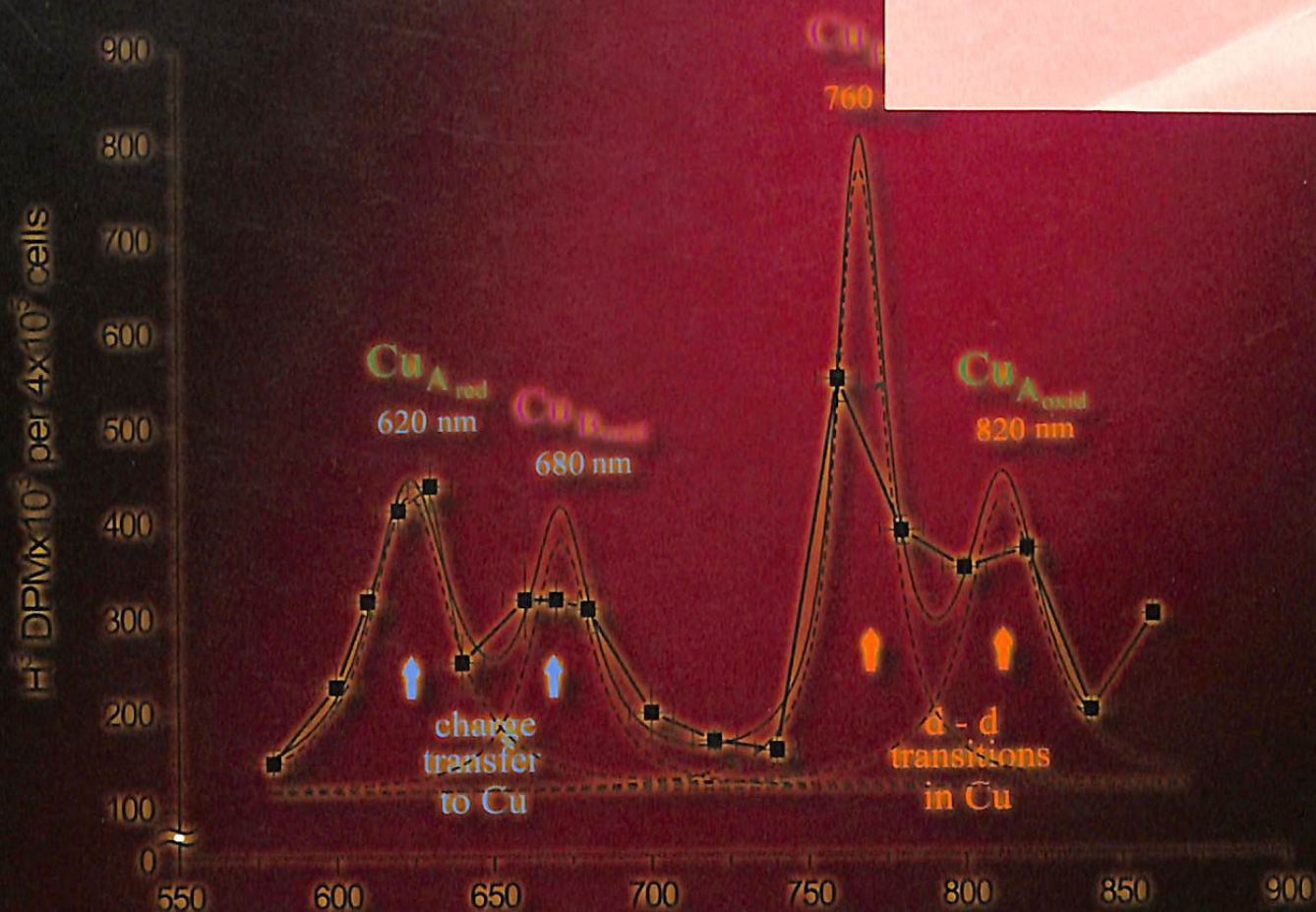
A silhouette illustration at the bottom of the cover. On the left, two figures are shown on a grassy hill; one is standing and holding a spear, while the other is crouching. To the right, a city skyline with several tall buildings is visible against the dark background.

Heather Heying
and Bret Weinstein

Ten Lectures on Basic Science of Laser Phototherapy

Tiina Karu

2007



Ten Lectures on Basic Science of Laser Phototherapy

Tiina Karu

Professor Tiina Karu is the Head of the Laboratory of Laser biology and Medicine at the Institute of Laser and Information Technologies of the Russian Academy of Sciences

Prima Books

2007

"One of the most important biological mechanisms based on activation/upgrading of the terminal enzyme of the mitochondrial respiratory chain, **cytochrome c oxidase**, is shown to be a universal mechanism controlling many aspects of the metabolism in different types of irradiated cells." Back Cover

"Monochromatic light in the visible-to-near region can be a subtle instrument to regulate cellular metabolism." (1)

Laser radiation can modulate cell metabolism through the mediation of a universal photoreceptor. **Cytochrome c oxidase** is an enzyme that catalyzes the final step in the mitochondrial respiratory chain. (2)

"**Cytochrome c oxidase** plays a central role in the bioenergetics of the cell." (120)

Laser irradiation may cause a shift in the cellular redox state. (4) Cells respond to the light stimulus in stronger or weaker manner depending on their redox potential at the moment of the irradiation. (113)

"Upgrading of **cytochrome c oxidase** may prove a protective influence against the detrimental effects of environmental pollutants like chemicals and ionizing radiation." (5)

Laser irradiation can reduce the formation of scar tissue. (6)

Laser phototherapy may be useful in reducing the severity of chemotherapy or radiation therapy. (9)

Laser irradiation can enhance bone repair about two-fold. (10)

632.8 nm laser irradiation can modulate immune responses. (12)

"Laser phototherapy is used by physiotherapists (to treat a wide variety of acute and chronic musculoskeletal aches and pains), by dentists (to treat inflamed oral tissues and to heal diverse ulceration), by dermatologists (to treat edema, indolent ulcers, burns, and dermatitis), by rheumatologists (to relieve pain and treat chronic inflammation and autoimmune diseases), and by other specialists, as well as by general practitioners. Laser phototherapy is also widely used in veterinary medicine (especially in racehorse-training centers) and in sports medicine and rehabilitation clinics (to reduce acute swelling and hematoma, relieve pain, improve mobility, and treat acute soft-tissue injuries." (21-22)

Clinical treatment laser dose intervals are generally administered on a daily or every other day basis. Multiple laser treatments enhanced the collagen growth in the repairing ligaments, superior to a single treatment with a similar dosage. (76-77)

Mitochondria are sensitive to irradiation with monochromatic visible and near IR light. (116)

Cytochrome c oxidase "plays a central role in the bioenergetics of the cell." (120)

Cytochrome c oxidase "is the photoacceptor responsible for various cellular responses connected with light therapy in the red-to-near IR region." (124)

"It is well known that it is only in the ideal case where the supply of electrons from **cytochrome c oxidase** is unlimited that electrons are always present to reduce oxygen," thus maximizing production of ATP and minimizing production of oxygen free radicals. (134)

Monochromatic visible and near IR radiation is initially absorbed by the mitochondria and eventually causes stimulation of DNA synthesis. (197)

"It is known that even small changes in the ATP level can significantly alter cellular metabolism. However, in many cases the regulative role of the redox homeostasis has proved to be more important than that of ATP." (198) This is because the redox-based regulation of gene expression appears to represent a fundamental mechanism in cell biology. (200)

In a laser irradiated muscle, the density of mitochondria increases. (205)

With laser irradiation, there is a "significant upregulation of gene expression involved in mitochondrial energy production and antioxidant cellular protection." (205)

Irradiation with monochromatic bands of visible light can change the redox properties of some respiratory chain components and therefore increase electron flow in the respiratory chain." (212)

Mitochondrial DNA plays a role in the processes of ageing and carcinogenesis. (222)

"Irradiation at 630-760 nm cannot cause mutations through direct action upon DNA. The energy of these photons is too low to cause ruptures of covalent bonds in a molecule." (248)

Laser irradiation can generate an electrical impulse in neurons, the action potential. "The action potential depended on the wavelength: the effect was strong at 540 and 632.8 nm, but radiation at 904, 660, 830, 880, and 950 nm had no effect." (277)

"The activation of cells via the mitochondria is suggested to be a universal photobiological mechanism." (305)

Format: Abstract**Full text links****J Invest Dermatol.** 2003 May;120(5):849-57.**ELSEVIER**
OPEN ACCESS

cDNA microarray analysis of gene expression profiles in human fibroblast cells irradiated with red light.

Zhang Y¹, **Song S**, **Fong CC**, **Tsang CH**, **Yang Z**, **Yang M**.

Author information

- 1 Applied Research Center for Genomic Technology, City University of Hong Kong, Kowloon, Hong Kong, China.

Abstract

In this paper, the cDNA microarray technique was used to investigate the gene expression profiles of human fibroblasts irradiated by low-intensity red light. Proliferation assays showed that the fibroblast HS27 cells responded with a curve effect to different doses of low-intensity red light irradiation at a wavelength of 628 nm. An optimal dose of 0.88 J per cm² was chosen for subsequent cDNA microarray experiments. The gene expression profiles revealed that 111 genes were regulated by the red light irradiation and can be grouped into 10 functional categories. Most of these genes directly or indirectly play roles in the enhancement of cell proliferation and the suppression of apoptosis. Two signaling pathways, the p38 mitogen-activated protein kinase signaling pathway and the platelet-derived growth factor signaling pathway, were found to be involved in cell growth induced by irradiation of low-intensity red light. Several genes related to antioxidation and mitochondria energy metabolism were also found to express differentially upon irradiation. This study provides insight into the molecular mechanisms associated with the beneficial effects of red light irradiation in accelerating wound healing.

PMID: 12713592 DOI: [10.1046/j.1523-1747.2003.12133.x](https://doi.org/10.1046/j.1523-1747.2003.12133.x)[Indexed for MEDLINE] **Free full text****MeSH terms, Substances**

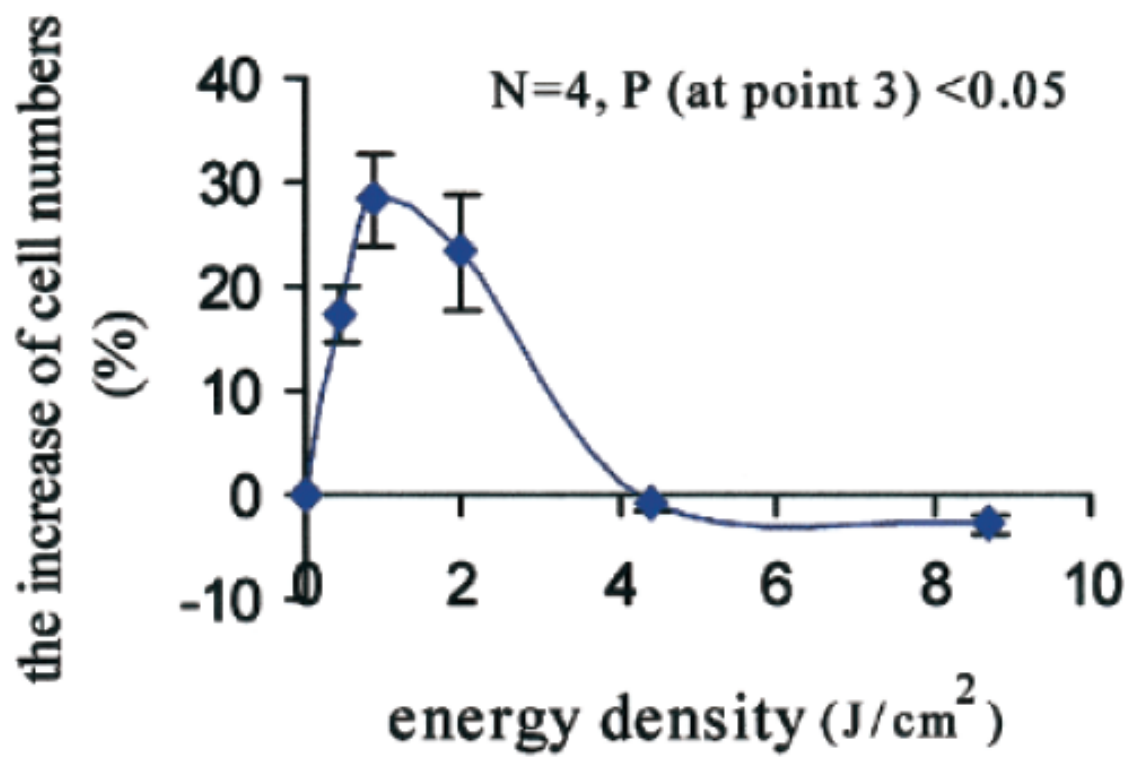
merous applications and controlled studies on the method have been reported. Low-intensity red laser light therapy has been applied to various medical conditions such as wound repair and dermatologic diseases, neurologic damage, blood disorders, musculoskeletal complications, and pain and inflammation (Abraham *et al*, 1994; Kawanishi *et al*, 1995; Tachibana *et al*, 1997; Doherty *et al*, 1998; Kawanishi *et al*, 1999; Kawanishi *et al*, 2000; Kawanishi *et al*, 2001).

Previous studies showed that low-intensity red laser light was capable of affecting cellular processes in the absence of significant thermal effects (Karu, 1989). Fibroblasts are an important cellular component of wound healing and it has been reported that low-intensity red laser light can stimulate the proliferation of fibroblasts (Kawanishi *et al*, 1994; Kawanishi *et al*, 1995; Kawanishi *et al*, 1997; Kawanishi *et al*, 1998; Kawanishi *et al*, 1999; Kawanishi *et al*, 2000). Red light irradiation has also been reported to enhance the activity of succinic dehydrogenase and promote the synthesis of procollagen and the release of basic fibroblast growth factor (Conlan *et al*, 1996; Yu *et al*, 1994a; Bolton *et al*, 1995; Kawanishi *et al*, 1996; Kawanishi *et al*, 1997; Kawanishi *et al*, 1998; Kawanishi *et al*, 1999; Kawanishi *et al*, 2000).

of the tissue culture plates at the irradiation distance of 0.75 cm. Samples were irradiated for various time periods to yield final energy doses of 0, 0.44, 0.88, 2.00, 4.40, and 8.68 J per cm². The temperature was monitored

and the changes in cell number were recorded. The HS27 fibroblasts responded to the irradiation dose as a nonlinear curve and the cells grew faster than the control at energy doses of 0.44, 0.88, and 2 J per cm², with an optimal proliferation effect at 0.88 J per cm² (Fig 1). When the energy dose was increased to over 4 J per cm², the cells grew at the same rate as or slightly slower rates than control cells. Previous studies have reported that irradiation using an He-Ne laser at 632.8 nm (660

proliferation of HS27 fibroblasts. In addition to its effects on cell proliferation, red light irradiation may also regulate the expression of genes in relevant cells that are related to microcirculation, antiapoptosis, antioxidation, and DNA repair.



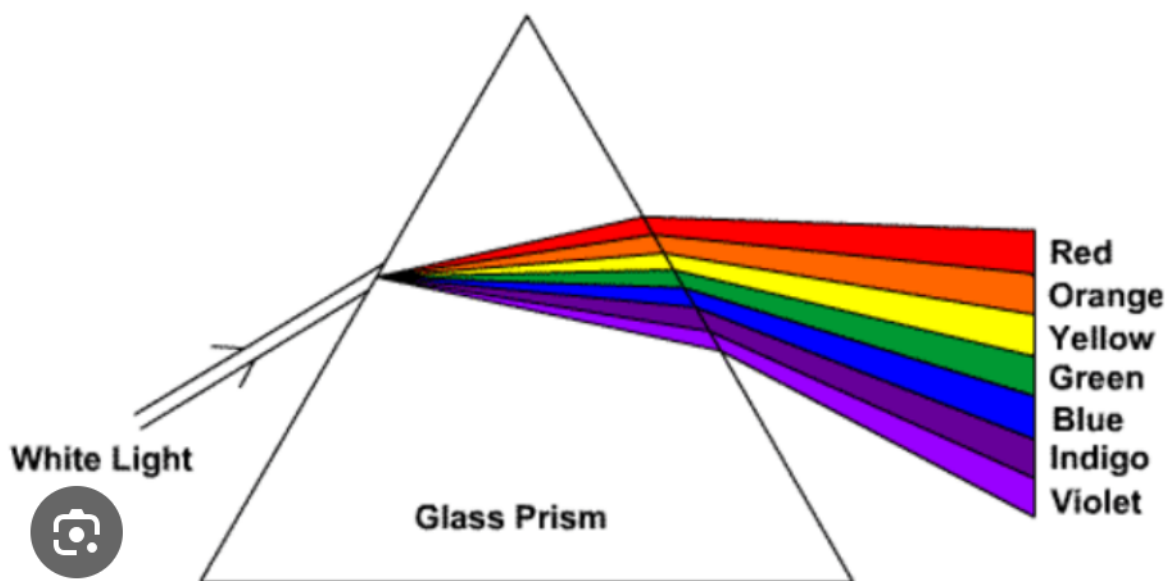
Sir Issac Newton: 1642-1727 (84 years)

AI Overview 8.20.26

In the 1660s, English physicist Isaac Newton used a glass prism to split a narrow beam of sunlight into a visible rainbow spectrum, proving that white light is a mixture of various colors rather than a single, pure entity.

The Experiment

- Newton darkened his room and let a small beam of sunlight through a hole in his window shutter.
- He placed a triangular glass prism in the path of the light beam.
- The light split into an oblong band of colors: red, orange, yellow, green, blue, indigo, and violet.
- Red light bent the least, while violet light bent the most.
- This proved the prism did not create color, but merely separated the different colors already present in white light due to their unique refractive indices.



AI Overview

Red light bends less than violet light through a prism because it has a longer wavelength, which causes it to travel faster inside the glass and experience a lower refractive index.

Why Wavelength Changes Bending

Wavelength and Speed

Red light has a long wavelength (around 700 nanometers), while violet light has a short wavelength (around 400 nanometers). When light enters a denser material like glass, these waves interact with the electrons inside.

Atomic Interaction

Shorter wavelengths carry higher energy and interact more strongly with the electrons in the glass, forcing them to oscillate more. This interaction slows violet light down more than red light.

A black and white photograph of a cluttered desk in front of a chalkboard. The desk is covered with papers, books, and a small container. The chalkboard contains mathematical equations and diagrams. Shelves filled with books and papers are visible in the background.

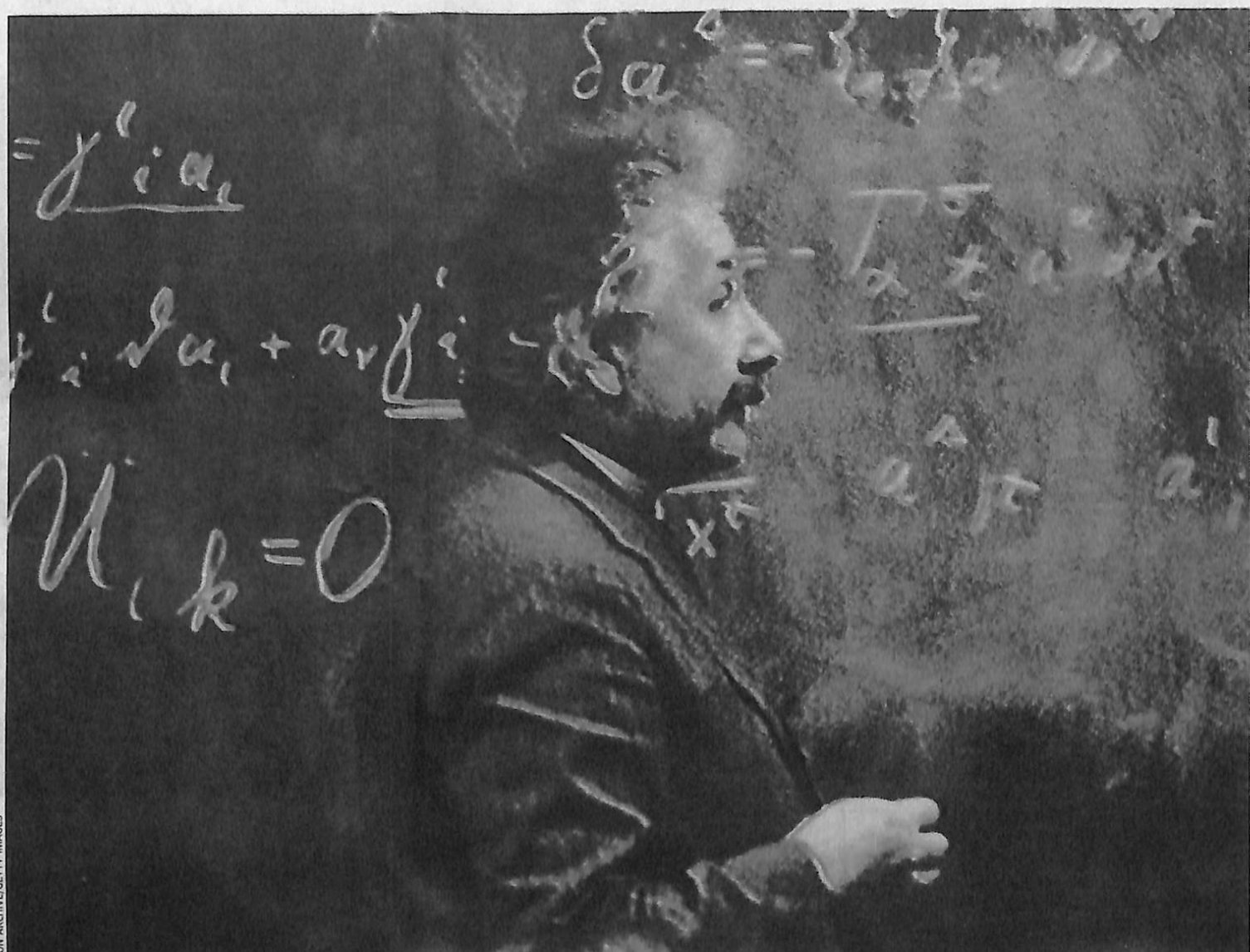




BOOKS

ing, but I do know that everything is interesting if you go into it deeply enough.' —RICHARD FEYNMAN

Genius and Awe



e

5 Einstein was a founder of quantum theory, realizing in 1905 that light could be regarded as consisting of particles. But the theory gradually developed into one based on probabilities, in

Einstein and the Photoelectric Effect, 1905

The Nobel Prize in Physics 1921 was awarded to Albert Einstein "for his services to Theoretical Physics, and especially for his discovery of the law of the photoelectric effect"

The Key Concept

Energy = (Planck's Constant) X (Frequency)
Therefore, as frequency increases, healing energy increases

(Wavelength) X (Frequency) = 186,282 miles/second

**Increase Wavelength Must Decrease Frequency
 and
 Therefore Decreases Energy**

**Decrease Wavelength Must Increase Frequency
 and
 Therefore Increases Energy**

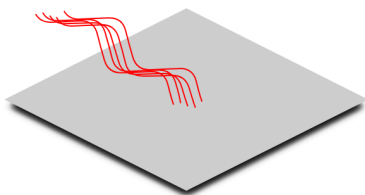
<u>Shorter Wavelengths:</u>	<u>Longer Wavelengths:</u>
Have <u>More</u> Energy	Have <u>Less</u> Energy
Have <u>Higher</u> Frequencies (Hertz)	Have <u>Lower</u> Frequencies (Hertz)
Do <u>Not</u> Penetrate as Deep	Penetrate Deeper
Do <u>Not</u> Generate Heat	Increase Molecular Motion and thus Generate Heat

The experimental results show that electrons are dislodged only when the light exceeds a certain frequency—regardless of the light's intensity or duration of exposure.

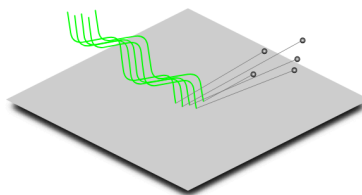
A low-frequency beam at a high intensity does not build up the energy required to produce photoelectrons.

Light with frequencies below a certain cutoff value, called the *threshold frequency*, would not eject photoelectrons from the metal surface no matter how bright the source was. These results were completely unexpected.

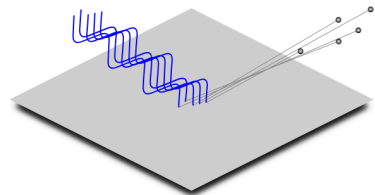
Given that it is possible to move electrons with light and given that the energy in a beam of light is related to its intensity, classical physics would predict that a more intense beam of light would eject electrons with greater energy than a less intense beam *no matter what the frequency*. This was not the case, however.



Red light does not eject photoelectrons (even if it is very bright).



Green light does eject photoelectrons (even if it is very dim).



Blue light ejects photoelectrons with more energy than green light (even if it is very dim).

The instant that light with an appropriate frequency of any intensity strikes a photoemissive surface, at least one electron will always pop out immediately.

The genius that figured out what was going on here was none other than the world's most famous physicist Albert Einstein.

In 1905, Einstein realized that light was behaving as if it was composed of tiny particles (initially called *quanta* and later called *photons*) and that the energy of each particle was proportional to the frequency of the electromagnetic radiation that it was a part of.

What we perceive as a continuous wave of electromagnetic radiation is actually a stream of discrete particles.

512

DOCS. 491, 492 MARCH 1918

491. To Otto H. Warburg

Berlin W., 5 Haberland St., 23 March 1918

Highly esteemed Colleague,

You are probably surprised at receiving a letter from me, because until now we have only walked past each other, without actually ever getting to know each other. I must even fear rousing some form of indignation in you with this letter; but it *has* to be.

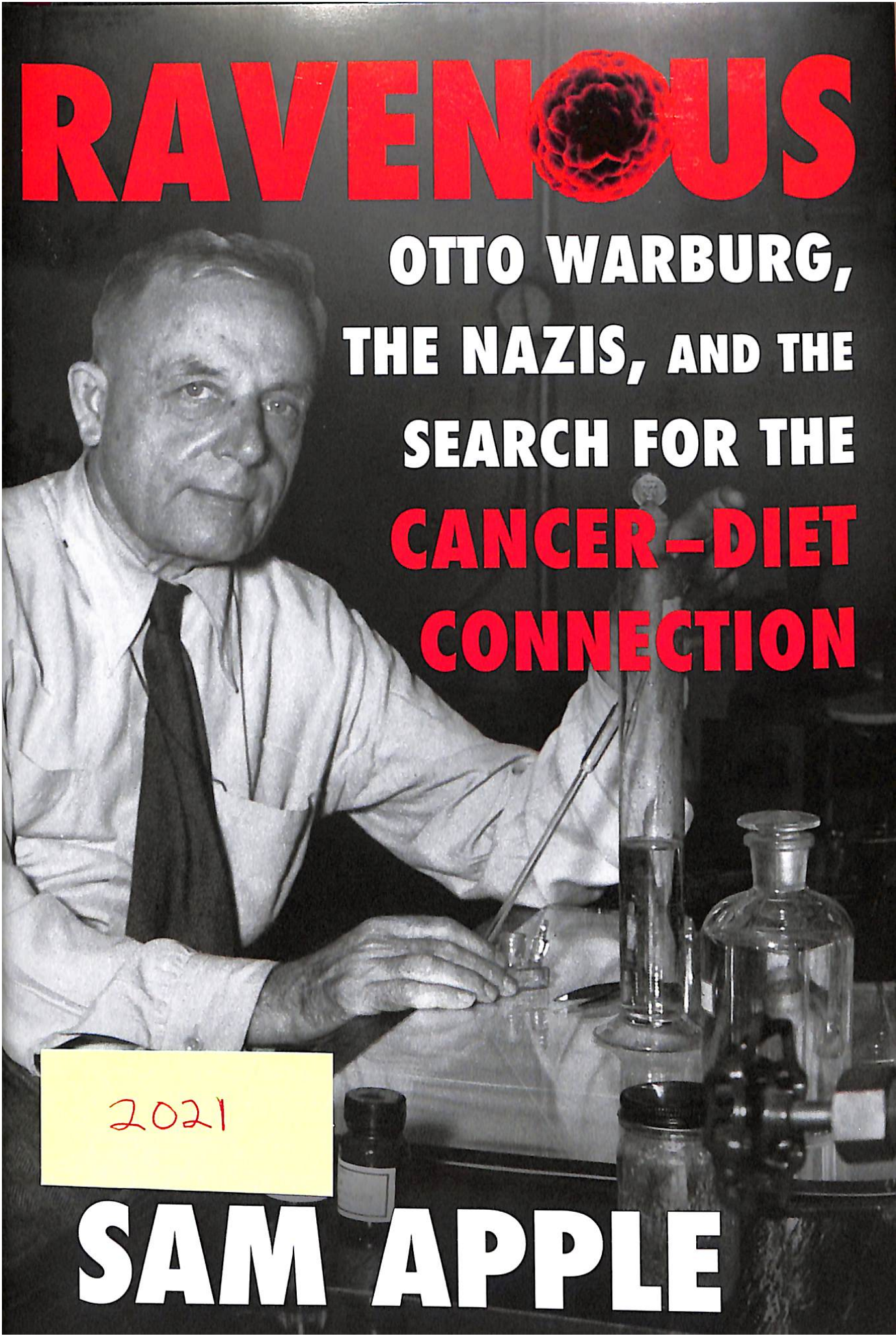
I hear that you are one of Germany's most talented younger biologists with great promise^[1] and that presently representatives in your particular field are quite mediocre here. I also hear, though, that you are stationed there at a very vulnerable outpost^[2] so your life is constantly hanging by a thread! Now, please step out of your shoes and into those of another discerning creature and ask yourself: Is this not madness? Can this post of yours out there not be filled by an unimaginative average person of the type that come 12 to the dozen? Is it not more important than all that big scuffle out there that valuable people stay alive? You know it yourself very well and must admit that I am right. Yesterday I spoke with Prof. Krauss, who is also entirely of my opinion and is also ready to arrange to have you recalled for another task.^[3]

My plea to you, which arises from what has been said above, is therefore that you support our efforts in securing your personal safety. Please, after a few hours of serious reflection, write me a couple of words so that we here know that our efforts will not founder on your attitude.

In the ardent hope that in this matter, for once, reason will exceptionally prevail, I am, with cordial regards, yours truly,

A. Einstein.

RAVENOUS



OTTO WARBURG,
THE NAZIS, AND THE
SEARCH FOR THE
**CANCER-DIET
CONNECTION**

2021

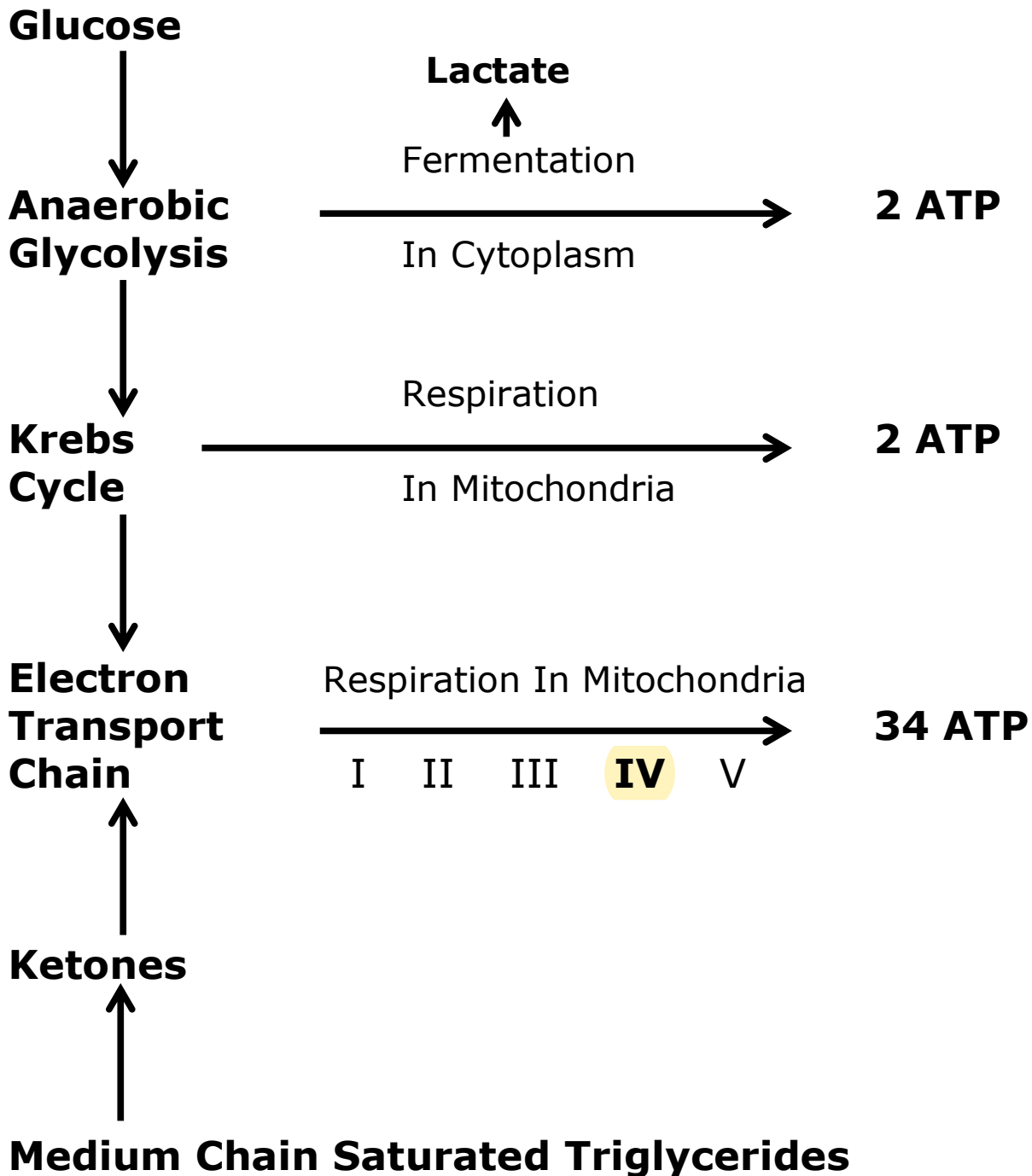
SAM APPLE

After the war (WWII), Warburg had political/scientific problems as many thought him to be a Nazi collaborator (he was not). Warburg could have easily escaped Germany if he had wanted to. Sam Apple portrays Warburg as making a conscious decision to stay to protect the lives of the Jewish researchers and their families that he employed; he assumed that if he fled, all would have been sent to concentration camps and probably not have survived.

Warburg believed that the initial genetic damage (mutations) that often-started cancer was attributed to exposure to chemical toxins.

Consequently, he insisted on consuming only organic food and he drank non-municipal water from a pristine well.

- 1883 Otto Warburg was born
- 1906 PhD in Chemistry
- 1911 MD degree (he never was in clinical practice)
- 1913 Discovers the Mitochondria
- 1914 WWI elite Calvary unit
Wounded
Mother writes to Einstein
- 1925 His **Respiratory Ferment was changed to cytochrome c oxidase** by David Keilin (a Russian at Cambridge)
- 1926 Runner-up for the Nobel Prize [the winner's work was later shown to be incorrect]
- 1931 Nobel Prize for respiratory enzymes
- 1944 Nominated for another Nobel Prize [Germany is still in WWII; Hitler forbade any German from receiving a Nobel Prize 1937-1945]
- 1970 Died just short of his 87th birthday



IV = Cytochrome c Oxidase (ATP)
III = Cytochrome c Reductase (O_2^- = Superoxide)

Fermentation may occur with or without oxygen
Respiration requires oxygen

Health

We may know how exercise fights cancer



CREATIVEPHOTO/ALAMY

EXERCISE can slow tumour growth in mice by shifting the body's metabolism so that muscle cells, rather than cancer cells, take the glucose and grow. A similar process may occur in people.

It is well known that exercise is linked to a lower risk of cancer, but the mechanisms behind this are only partly understood. Some of exercise's influence seems to come via impacts on the community of microbes in our guts and through its effects on the immune system.

To examine another possible route, Rachel Perry at Yale School of Medicine and her colleagues have injected breast cancer cells into 18 mice – 12 of which were fed a diet that caused obesity, which worsens the progression of several types of cancer – and let half the animals run as much or as little as they wanted on an exercise wheel.

They found that after four weeks, tumours in obese mice that chose to exercise were 60 per cent smaller than those in obese mice that didn't have access to a working wheel – and also slightly smaller than those of sedentary mice fed a normal diet (*PNAS*, doi.org/hbdbnz). A 30-minute bout of exercise was tied to increased uptake of oxygen and the key energy source glucose in skeletal and cardiac muscles, and decreased

Being active has long been linked to a lower risk of cancer

glucose uptake in tumours.

"This work reveals that aerobic fitness fundamentally reshapes metabolic competition between muscle and tumours," says Perry. "Importantly, the exercise intervention was voluntary. We're not talking about training for a marathon-type exercise, just what the mice wanted to do."

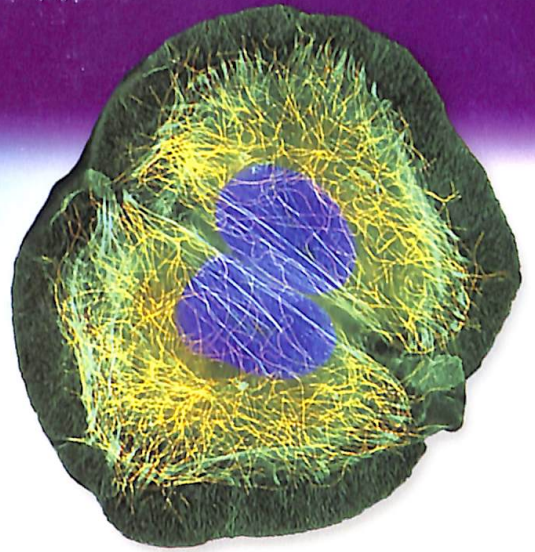
The researchers analysed gene activity and identified 417 genes in key metabolic pathways in mice that change as a result of exercise, essentially meaning the muscle tissues metabolise glucose more and the tumour tissues metabolise it less.

In particular, the researchers say down-regulation in cancer cells of mTOR, a protein involved in cell growth, may be restricting tumour growth.

Perry says these metabolic pathways are very similar across mammals, so she would expect the same process to happen in people.

"We need a clinical trial in people, but I really can't see any reason why you wouldn't have a similar effect in humans," says Rob Newton at Edith Cowan University in Perth, Australia. ■ CS

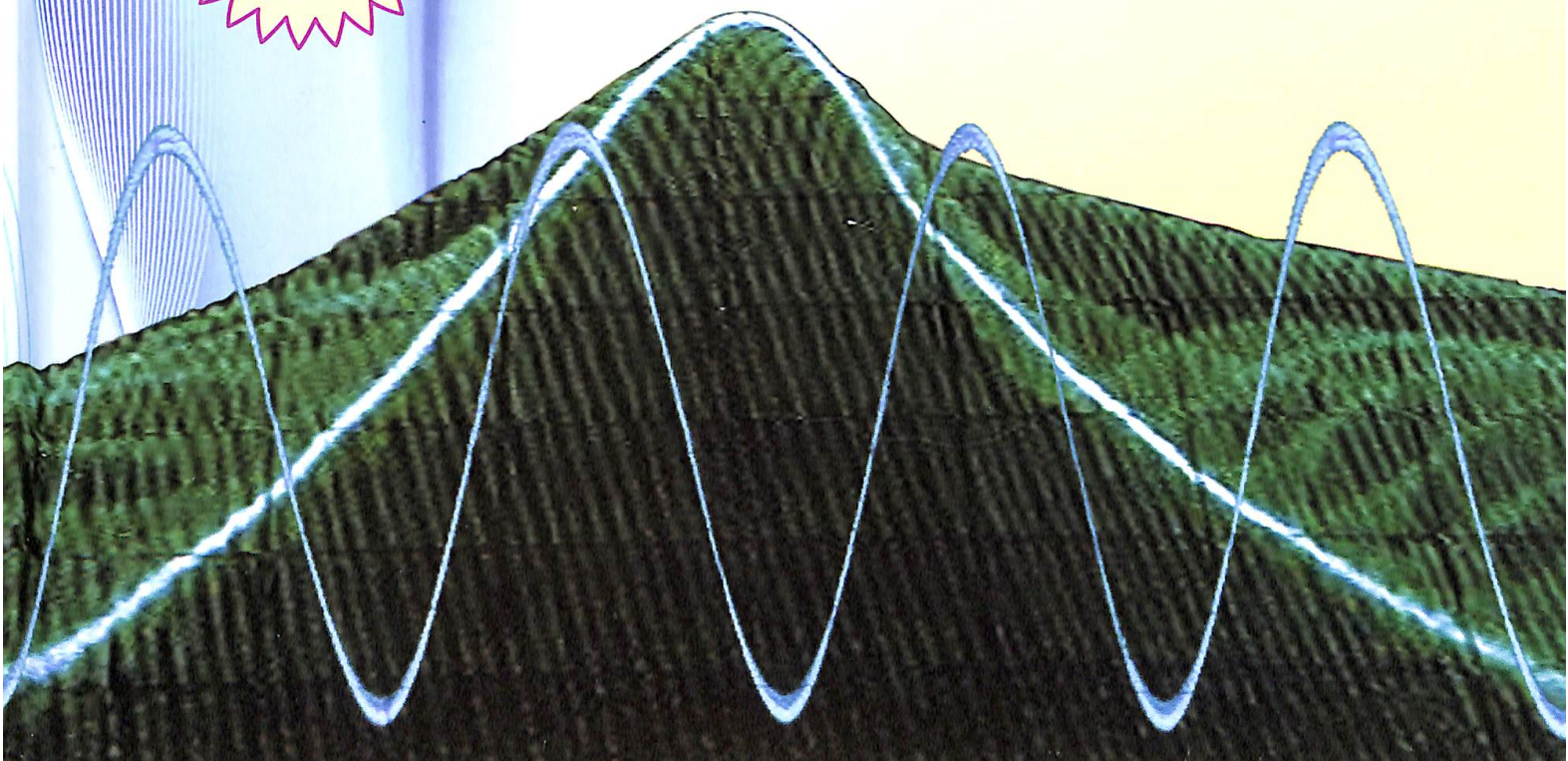
Nenah Sylver, PhD



The Rife Handbook

of Frequency Therapy and Holistic Health

REVISED
2011
EDITION



Nenah Sylver, PhD

The Rife Handbook of Frequency Therapy and Holistic Health

Royal Rife's Universal Microscope weighed 200 pounds, had 5,682 parts, and stood between 2 and 3 feet high. It was completed in 1933.

Rife used pulsed radio waves to find a pathogenic microbe's Mortal Oscillatory Rate.

"[It is] now known that selected [pulses] can regenerate tissues." 675

"Pulsing a wave means that the signal is 'on' for a brief period, then off, then on, then off, etc. Pulsing is independent of the frequency." 670 **[Key Point]**

[in light, frequency (wavelength) is a single color; in sound, frequency (wavelength) is a single note][pulsing is the rhythm]

"Any frequency can be pulsed." 670 **[Key Point]**

[In electromagnetic devices, pulsing will] "induce movement into the body. The movement of the EM radiation in the body translates into ion transport, increases in blood and lymph flow, and more." 670

"Pulsed electromagnetic radiation induces therapeutic results, which is why it is used." 671

"[Pulsing] means there is an 'off' period to the signal, it ensures that the human or animal receiving the signal does not become resistant to its effects. That is one of the secrets of electromedicine devices that are effective." 673 **[Key Point]**

"Correctly employed [pulsing] can increase cell energy, normalize membrane conductivity, lessen oxidative stress, reduce amounts of inflammatory chemicals in the blood, improve protein synthesis, boost feel-good endorphins, restore depleted adrenal function, and enhance immune function. The restoration of these metabolic processes leads to the regeneration of tissues as well as resistance to disease." 673

"Pulsing light stimulates healing." 680

"'Bigger is better' and 'More is better' figure prominently in the Western mindset."

"'Less is more'—reflects what the body usually needs. The exquisite sensitivity of cells to electromagnetic fields of all kinds explains why electromedical devices work—and why more gentle ones work the best. Low power energies might not be easily perceived subjectively, but they are the most compatible with living systems precisely because they are of lower power." 673

"Lasers and LEDs (short for *Light Emitting Diodes*) can be made to produce any color wavelength. The emission of the light (whether it's a red, green, blue, or other color) is due not to glass, paint, or pigment—it is solely the wavelength of the light itself that gives the beam its characteristic color. Since the wavelength is always a single frequency, the color is known as *monochromatic*." 679

"Laser and LED therapies differ in some important ways, but they also share similarities. Both light technologies are based on the energetic behavior of electrons. Normally, electrons occupy a fixed place in one or more orbital rings that sequentially surround the atom's nucleus. When they become excited, electrons move faster and jump to higher orbits. When they relax and return to their original position, electrons release energy in the form of light, or photon units. The wavelength of a photon—in other words, its color—is determined by the amount of energy released when the electron drops to a lower orbit. *It is this emitted light that is harnessed in visible light laser and LED technology.*" 679

"The frequency being used is almost always identified by the length of the wave, rather than the actual frequency itself as described in hertz. In the band of visible light, wavelengths are measured in nanometers (nm). One nanometer, the length of one complete wave, is one billionth of a meter and roughly about the size of a human cell."

"Research published in the *Journal of Clinical Laser Medicine Surgery* stating that a 630-nm wavelength appears "to be most commonly associated with bacterial inhibition. The findings of this study might be useful as a basis for selecting LLLT (low level laser therapy] for infected wounds." [ATTACH ABSTRACT Nussbaum] 680

"In this case, 'bacterial inhibition' consists of the retardation of the growth and functioning of pathogens. What is good for the body is usually bad for pathogens..."
 "635 nm is the worst wavelength for most pathogens but is beneficial for human tissue." 680

"Monochromatic red light stimulates blood circulation, increases lymphatic drainage, and promotes cell metabolism by stimulating photoreceptors in the mitochondria living within the cell. (Mitochondria are tiny living organelles with their own DNA and reproduction cycles, which live in symbiotic harmony with the cell, and control many important cellular processes including energy production.)" 680

"Except on the eyes in the case of a laser, the light can be applied to every part of the body: skin, soft tissue, muscle, bone, brain, organs, lymphatic fluid, glands, and blood. Used over an artery, the light can improve the condition of immune cells—leukocytes, T-cells, and B-cells within the bloodstream so they can more efficiently disable pathogens." 680

"There are photoreceptors at the molecular-cellular level which, when triggered, activate a number of biological reactions: DNA/RNA synthesis, increased cAMP levels [cyclic adenosine monophosphate, a molecule involved in many biological processes], protein and collagen synthesis, and cellular proliferation. The result is rapid regeneration, normalization, and healing of damaged cellular tissue. In essence, light is a trigger for the rearrangement of cellular metabolism." 680

"Single-wavelength light maintains its integrity while radiating. Its ability to travel along the meridians of the body without being dispersed into the surrounding tissues." 680

In low-level laser therapy, "the energy output is low enough so that the treated tissue does not rise above . . . normal body temperature." 681

"Pulse refers to the number of times the beam of light is turned on and off in one second." "Even though the eye cannot detect movement above 45 Hz or so...", "the body's tissue can clearly detect and recognize these pulse rates." 681

"Laser therapy works on all manner of conditions. These include injuries to ligaments, tendons, nerves, and other tissue; skin conditions; bone problems such as osteoarthritis; first, second, and third-degree burns; dental problems; infections including herpes; and, of course, chronic pain." 681

"The laser beam can be applied without risk to almost any part of the body (including trigger points and fascia). However, due to the precision of the beam, care must be taken to avoid shining the device directly into the eyes or even on the closed eyelid, because this can cause tissue damage and even blindness." 681

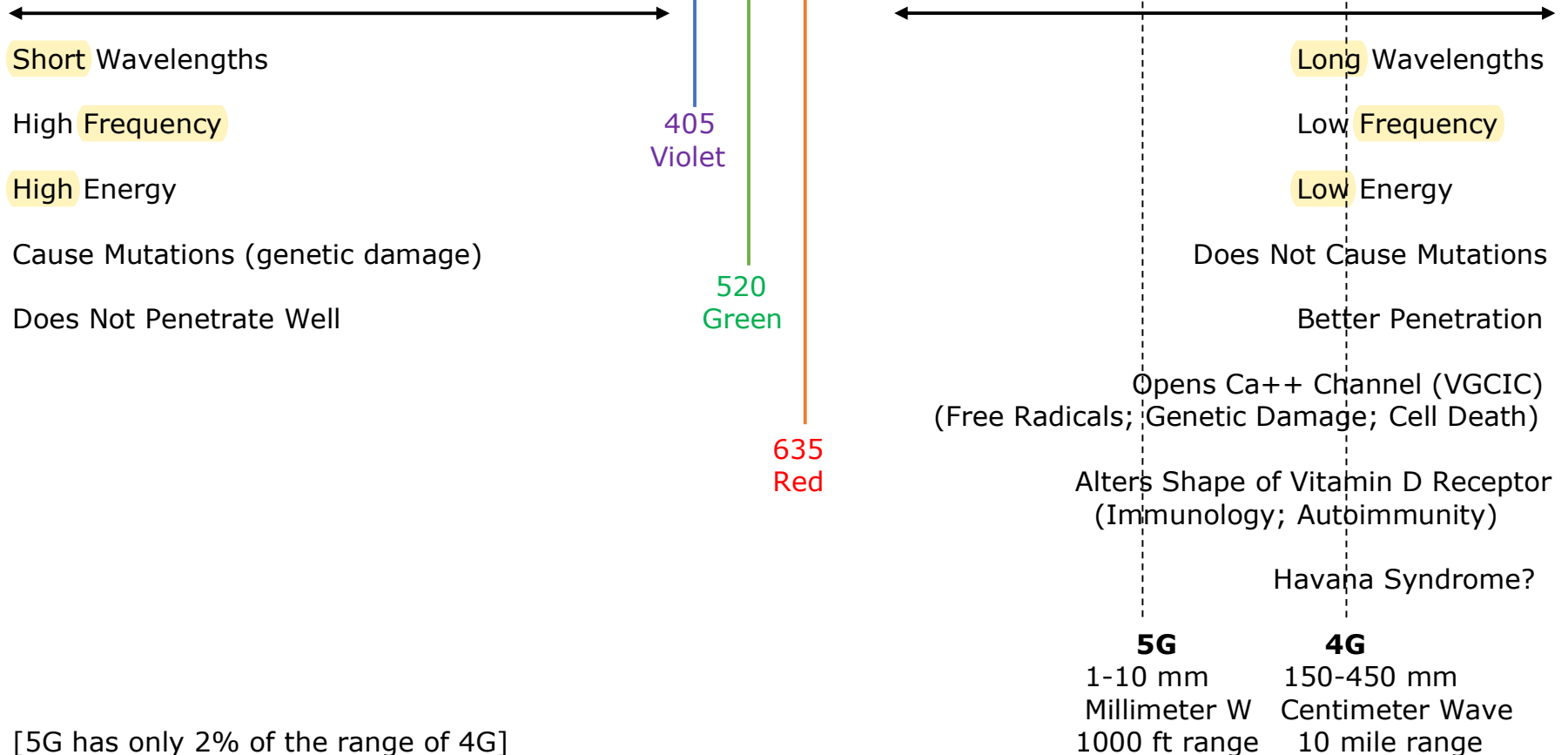
"LED is an acronym for *Light Emitting Diode*. It is sometimes erroneously called a soft laser or laser, but it is not—the monochromatic light emitted by LEDs is *incoherent*. This means, from a physics standpoint, that the waves are emitted at random intervals because the peaks and valleys of the waves do not line up. In practical terms, this means that the light is multi-directional and diffuse, *not* directional and focused (collimated)." 681

All light, visible and invisible, has a Wavelength and a Frequency

Wavelength X Frequency = 186,282 miles/second ALWAYS

The Electromagnetic Spectrum

Gamma	X-rays	Ultraviolet	Visible	Infrared	Microwave	Radio Waves
0.001- 0.1 nm	1-10 nm	10-400 nm	400-700 nm	700-1000 nm	1-100 mm	10 cm - 100,000 cm
				1000 nm = 1 mm	100 mm = 10 cm	100,000 cm = 100 km = 62 miles



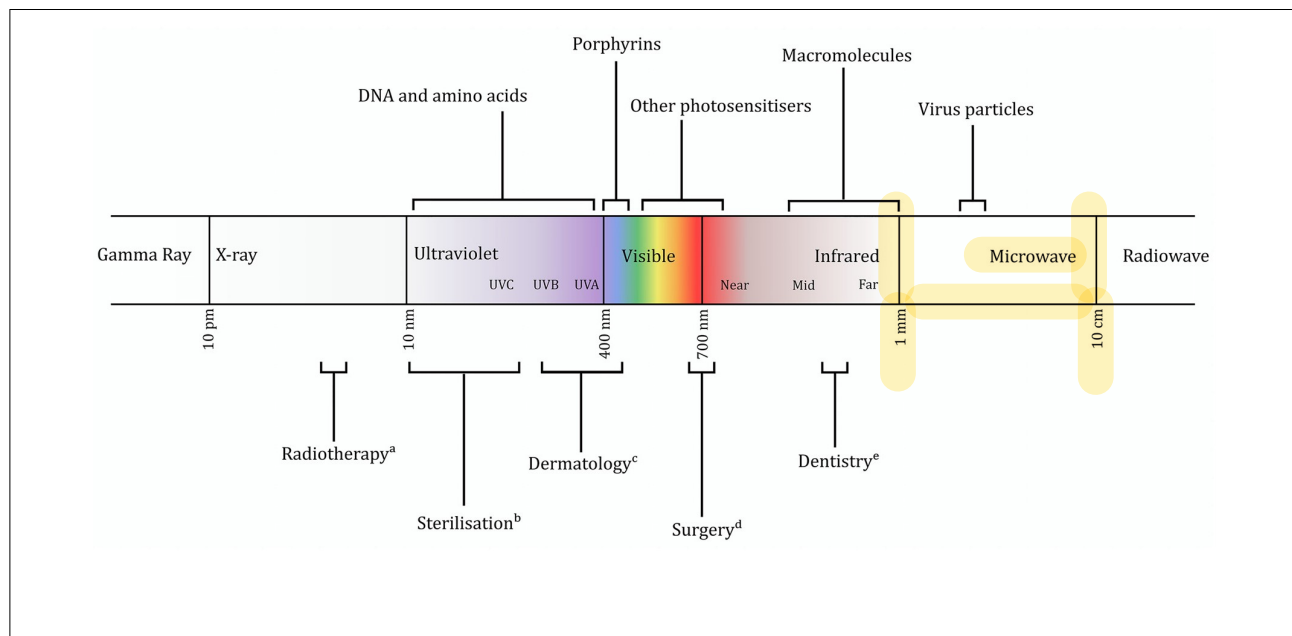


TABLE 1 | Optical properties of selected wavelengths in skin.

Wavelength (nm)	Absorption coefficient in skin (cm ⁻¹)	Scattering coefficient in skin (cm ⁻¹)	Approx penetration depth (μm)	Major interactions in tissue
300	45.0	260.0	6.0	Mutagenic
350	25.0	220.0	60.0	Mutagenic
400	13.5	34.3	90.0	Mutagenic, photochemical
500	6.2	25.1	230.0	Photochemical
600	3.8	18.6	550.0	Photochemical
700	2.4	14.8	750.0	Photochemical, thermal
800	1.9	12.4	1200.0	Photochemical, thermal
1000	1.6	9.2	1600.0	Thermal
1200	1.8	7.1	2200.0	Thermal

In general, shorter wavelengths scatter and are absorbed more in tissues, limiting penetration depth. Data from Anderson and Parrish (1981) and Bashkatov et al. (2011).



Published in final edited form as:

Support Care Cancer. 2016 June ; 24(6): 2781–2792. doi:10.1007/s00520-016-3152-z.

Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations

Judith A. E. M. Zecha¹, Judith E. Raber-Durlacher^{1,2}, Raj G. Nair⁴, Joel B. Epstein^{5,6}, Stephen T. Sonis⁷, Sharon Elad⁸, Michael R. Hamblin^{9,10,11}, Andrei Barasch¹², Cesar A. Migliorati¹³, Dan M. J. Milstein¹, Marie-Thérèse Genot¹⁴, Liset Lansaat¹⁵, Ron van der Brink¹⁶, Josep Arnabat-Dominguez¹⁷, Lisette van der Molen¹⁵, Irene Jacobi¹⁵, Judi van Diessen¹⁸, Jan de Lange¹, Ludi E. Smeele^{1,15}, Mark M. Schubert¹⁹, and René-Jean Bensadoun²⁰

Judith A. E. M. Zecha: j.zecha@amc.uva.nl; Judith E. Raber-Durlacher: j.e.raberdurlacher@amc.uva.nl; Raj G. Nair: r.nair@griffith.edu.au; Joel B. Epstein: joel.epstein@cshs.org; Stephen T. Sonis: ssonis@partners.org; Sharon Elad: SElad@URMC.Rochester.edu; Michael R. Hamblin: hamblin@helix.mgh.harvard.edu; Andrei Barasch: barasaff@cs.com; Cesar A. Migliorati: miglioni@uthsc.edu; Dan M. J. Milstein: D.M.Milstein@amc.uva.nl; Marie-Thérèse Genot: mtgenot@skynet.be; Liset Lansaat: l.lansaat@nki.nl; Ron van der Brink: RVanderbrink@coh.org; Josep Arnabat-Dominguez: josep.arnabat@ub.edu; Lisette van der Molen: l.vd.molen@nki.nl; Irene Jacobi: i.jacobi@nki.nl; Judi van Diessen: j.v.diessen@nki.nl; Jan de Lange: j.delange@amc.uva.nl; Ludi E. Smeele: l.smeele@nki.nl; Mark M. Schubert: mschuber@seattlecca.org

¹Department of Oral and Maxillofacial Surgery, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands ²Department of Medical Dental Interaction and Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands ³Oral Medicine Oral Pathology and Human Diseases, Menzies Health Institute Queensland, Gold Coast, QLD, Australia ⁴Department of Haematology and Oncology/Cancer Services, Gold Coast University Hospital, Queensland Health, Gold Coast, QLD, Australia ⁵Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA ⁶Division of Otolaryngology and Head and Neck Surgery, City of Hope, Duarte, CA 91010, USA ⁷Division of Oral Medicine, Brigham and Women's Hospital and the Dana-Farber Cancer Institute and Biomodels LLC, Boston, MA 02115, USA ⁸Division of Oral Medicine, Eastman Institute for Oral Health, and Wilmot Cancer Center, University of Rochester Medical Center, Rochester, NY 14620, USA ⁹Wellman Center for Photomedicine, Massachusetts General

Correspondence to: René-Jean Bensadoun.

Disclaimer

This article is based on a narrative review of existing data and the clinical observations of an international multidisciplinary panel of clinicians and researchers with expertise in the area of supportive care in cancer and/or PBM clinical application and dosimetry. This article is informational in nature. As with all clinical materials, this paper should be used with the clear understanding that continued research and practice could result in new insights and recommendations. The review reflects the collective opinion and as such does not necessarily represent the opinion of any individual author. In no event shall the authors be liable for any decision made or action taken in reliance on the proposed protocols.

Conflict of interest Judith E. Raber-Durlacher, Raj G. Nair, Joel B. Epstein, Ron van der Brink, Josep Arnabat Dominguez, and René-Jean Bensadoun have received travel expenses and hotel accommodation from THOR Photomedicine Ltd. UK. Raj Nair has received an honorarium from THOR, UK. Michael R Hamblin was supported by US NIH grant R01AI050875. All the other authors declare that they have no conflict of interest.

Keywords

Low level laser therapy; Low level light therapy; Photobiomodulation; Mucositis; Orofacial complications; Chemotherapy; Radiation therapy; Head and neck cancer; Safety; LLLT and PBM

Introduction

Nearly all patients with advanced head and neck cancer (HNC) suffer complications from treatment with radiation therapy (RT) or chemoradiotherapy (CRT) [1]. CRT is currently the standard of care with or without surgery in advanced HNC. An increased frequency and severity of side effects is seen, particularly when chemotherapy (CT) is combined with accelerated or hyperfractionated RT regimens. It is now recognized that organ preservation in HNC treatment is not synonymous with function preservation, and effects on quality of life (QoL) must be considered in cancer treatment planning and extending survival [2, 3].

RT to the head and neck, with or without CT, damages adjacent tissues within the radiation field despite continuing efforts to minimize these effects [1]. Furthermore, targeted therapies administered as single agents, and combined with RT or CRT, may generate additional symptoms [4–6]. Acute complications include oral mucositis (OM), pain, dysphagia, infections, salivary changes, dysgeusia, and dermatitis. Common chronic complications include hyposalivation and xerostomia, mucosal infections, mucosal atrophy, neuropathies including mucosal pain, dysgeusia, tooth demineralization and rampant caries, progression of periodontitis, soft tissue and/or bone necrosis, mucocutaneous and muscular fibrosis, dysphagia, trismus, lymphedema, dermatitis, and voice and speech alterations [7]. These complications are associated with morbidity and mortality, increased use of health care resources and costs, and may compromise patient adherence to cancer therapy protocols resulting in suboptimal outcomes.

Among the few supportive care measures available, low level laser or light therapy (LLLT) has shown significant promise. LLLT refers to light therapy that may stimulate tissue regeneration, reduce inflammation, and control pain. These treatments were originally referred to as “low level laser” because the light is of low intensity compared with other forms of medical laser treatment, which are used for ablation, cutting, and coagulation. At the 2014 joint North American Association for Laser Therapy (NAALT) and World Association for Laser Therapy (WALT) conference, photobiomodulation (PBM) was accepted as the preferred name with the following definition: “The therapeutic use of light [e.g. visible, near infrared (NIR), infrared (IR)] absorbed by endogenous chromophores, triggering non-thermal, non-cytotoxic, biological reactions through photochemical or photophysical events, leading to physiological changes” [8].

The potential utility of PBM in the management of side-effects of chemoradiation therapy in head and neck cancer

Although the biological mechanisms underlying the therapeutic effects of PBM have not been fully elucidated, studies suggest that PBM enhances wound healing [9], significantly

reduces inflammation, and prevents fibrosis [10–15]. Moreover, PBM reduces pain and improves function [16–19]. These photobiological reactions have been shown to occur in various tissues.

Systematic reviews have suggested efficacy of PBM for OM management in hematopoietic stem cell transplant (HSCT) recipients and in HNC patients [20–25]. Whereas in most studies, PBM is applied intra-orally on the oral mucosal tissues, studies indicate that it may also be administered extra-orally, with a resultant effect on structures at risk for OM transcutaneously, thereby enhancing the ease of delivery and possibly the efficacy of treatment [23, 26].

In addition, new generation PBM devices consisting of a cluster of laser or light-emitting diode (LED) beams, instead of a single laser point, provide exposure of larger fields. Monochromatic high-quality LED beams have the same properties as diode lasers with the same wavelength, but their light beam is less coherent. LED specifics need to be carefully matched to PBM using lasers when considering LED arrays.

When used with appropriate parameters, the light is able to penetrate into tissues sufficiently to activate cellular processes [27]. This finding suggests that extra-oral administration of PBM (with or without concurrent use of intra-orally administered PBM) enables the light to reach other anatomical structures of the head and neck at risk for RT- and CRT-induced complications. This may broaden the range of indications for PBM for the prevention and treatment of cancer treatment-induced complications.

Goals of this work

A task force consisting of an international multidisciplinary panel of clinicians and researchers with expertise in the area of supportive care in cancer and/or PBM clinical application and dosimetry was formed. The mission of this group is to aid in the design of PBM study protocols, identify validated outcome measures, and test the efficacy and safety of PBM for the management of complications related to cancer therapy.

In this paper, we review and discuss PBM mechanisms of action, dosimetry, and safety considerations. In a subsequent paper (part 2), we (i) identify selected oral, oropharyngeal, facial, and neck complications of treatment for HNC, in which PBM may have potential for prophylaxis and/or treatment; (ii) propose PBM parameters for prophylaxis and therapy to mitigate these complications based on current evidence and knowledge; and (iii) discuss directions of future research related to the use of PBM in HNC.

PBM mechanisms of action and therapeutic effects

The conclusion that PBM effectively modulates biological function is supported by a plethora of clinical and laboratory studies [17, 28]. Despite variations in instrumentation and dosing parameters, since its introduction in 1967, PBM has been shown to enhance wound repair and tissue regeneration by influencing different phases of injury resolution including the following: (i) the inflammatory phase, in which immune cells migrate to the site of tissue injury, ii) the proliferative phase, which includes stimulation of fibroblasts and macrophages

as well as other repair components, and iii) the remodeling phase, consisting of collagen deposition and rebuilding of the extracellular matrix at the wound site [29].

Although the complex biological mechanisms underlying the therapeutic effects of PBM have not been completely elucidated and may vary among different cell types and tissue states (healthy versus stressed or hypoxic), laboratory and clinical studies suggest that PBM significantly reduces inflammation and prevents fibrosis [10–15]. It has become increasingly clear that PBM's biological effects are closely dose-related. In fact, the historical lack of dosing consistency has been a confounder in the comprehensive interpretation of PBM activity. Nonetheless, confirmatory studies have contributed to a fundamental understanding of PBM biology.

Current data suggest that PBM acts predominantly on cytochrome c oxidase (CcO) in the mitochondrial respiratory chain by facilitating electron transport resulting in an increased transmembrane proton gradient that drives adenosine triphosphate (ATP) production [30]. ATP is the universal energy source in living cells essential for all biologic reactions, and even a small increase in ATP levels can enhance bioavailability to power the functions of cellular metabolism [31]. In addition, the absorption of red or NIR light may cause a short, transient burst of reactive oxygen species (ROS) that is followed by an adaptive reduction in oxidative stress. This action, impairment of ROS production, has been shown to favorably mitigate radiation-induced injury and mimics the activity of molecular agents that attenuate tissue damage (examples include amifostine, N-acetyl cysteine, and superoxide dismutase).

Low concentrations of ROS impact many cellular processes, including activation of key transcription factors such as nuclear factor kappa B (NF- κ B). This results in the expression of stimulatory and protective genes [32], which generate growth factors belonging to the fibroblast growth factor family, pro-inflammatory cytokines, and chemokines that are involved in tissue repair.

In hypoxic or otherwise stressed cells, mitochondria produce nitric oxide (mtNO), which binds to CcO and displaces oxygen [33]. This binding results in inhibition of cellular respiration, decreased ATP production, and increased oxidative stress (a state that develops when the levels of ROS exceed the defense mechanisms), leading to the activation of intracellular signaling pathways, including several transcription factors [34]. These include redox factor-1 (Ref-1), activator protein-1 (AP-1), NF- κ B, p53, activating transcription factor/cAMP-response element-binding protein (ATF/CREB), hypoxia-inducible factor (HIF)-1, and HIF-like factor [35]. These transcription factors induce downstream production of both inflammatory mediators, such as tumor necrosis factor-alpha (TNF- α), interleukin (IL)-1 and IL-6, cyclooxygenase (COX)-2, and prostaglandin E2 (PGE-2) [34, 36, 37] and anti-inflammatory mediators [transforming growth factor (TGF)-beta, IL-10]. There is evidence suggesting that when PBM is administered with appropriate parameters to stressed cells, NO is dissociated from its competitive binding to CcO, ATP production is increased, and the balance between prooxidant and antioxidant mediators is restored, resulting in a reduction of oxidative stress [38]. For example, PBM has been shown to attenuate the production of ROS by human neutrophils [39]. Silveira et al. [40] reported that PBM reduced ROS in an animal model of traumatic tissue injury, whereas a study in a model of

of 830 nm [85]. PBM (808 nm; 5.85 and 7.8 J/cm²) had an inhibitory effect on the proliferation of a human hepatoma cell line [86], and Sroka et al. [87] reported that glioblastoma/astrocytoma cells exhibited a slightly decreased mitotic rate after PBM at 805 nm and 5–20 J/cm². Similarly, 808-nm laser irradiation with an energy density of more than 5 J/cm² inhibited cell proliferation of glioblastoma cells in vitro [88]. Moreover, Al Watban et al. [89] observed growth inhibition of cancer cell lines at relatively high cumulative PBM doses. This prompted Crous and Abrahamse [90] to hypothesize that PBM may have a therapeutic potential in lung cancer.

Protective effects of PBM against cytotoxic therapy

There are no data to suggest that PBM may protect cancer cells against the cytotoxic effects of RT. On the contrary, Scharfetter et al. [91] observed a pro-apoptotic effect of PBM in head and neck SCC cells, whereas no anti-apoptotic effects occurred that might promote tumor cell resistance to cancer therapy. Increased apoptosis of human osteosarcoma cells was also induced by the administration of NIR (810 nm, continuous wave, 20 mW/cm², 1.5 J/cm²) prior to NPe6-mediated photodynamic therapy as a result of increased cellular ATP and a higher uptake of the photosensitizer [92]. Recently, it was reported that PBM administered to normal human lymphoblasts and leukemia cells prior to RT, resulted in a differential response of normal versus malignant cells suggesting that PBM does not confer protection and may even sensitize cancer cells to RT-induced killing [93]. Nevertheless, in vivo and clinical studies are warranted before firm conclusions can be drawn.

Carcinogenic effects of PBM on normal cells

It seems unlikely that PBM has carcinogenic effects on normal cells. The non-ionizing wavelengths of the red and NIR spectrum used in PBM are far longer than the safety limit of 320 nm for DNA damage [94]. No signs of malignant transformation in non-malignant epithelial cells and fibroblasts were observed following exposure to PBM with a wavelength of 660 nm, 350 mW for 15 min during 3 consecutive days [91]. In addition, no malignant transformation of normal breast epithelial cells was detected in an in vitro study comparing the effects of different doses and wavelengths of PBM during multiple exposures [84].

Data derived from in vivo and clinical studies

PBM (660 nm, 30 mW, 424 mW/cm², 56.4 J/cm², 133 s, 4 J), applied to chemically induced SCC in hamster cheek pouch tissue, increased tumor growth [95]. PBM at a dose of 150 J/cm² appeared safe, with only minor effects on B16F10 melanoma cell proliferation in vitro, and had no significant effect on tumor growth in vivo. Only a high power density (2.5 W/cm²) combined with a very high dose of 1050 J/cm² could induce melanoma tumor growth in vivo [96]. In a mouse model to study PBM effects on UV-induced skin tumors, the experimental mice received full body 670 nm PBM delivered twice a day at 5 J/cm² for 37 days, whereas controls received sham PBM [97]. No enhanced tumor growth was observed, whereas there was a small but significant reduction in tumor area in the PBM group, potentially related to a local photodynamic effect or PBM-induced antitumor immune activity.

Schaffer et al. [98] observed that PBM increased the locoregional blood flow that contributed to better local oxygenation and hypothesized that PBM applied shortly before cancer treatment might enhance the effect of ionizing RT and local delivery of chemotherapy. A recent randomized controlled trial in which PBM was administered for prevention of OM during CRT in HNC patients (diagnosed with SCC of the nasopharynx, oropharynx, or hypopharynx) reported that at a median follow-up of 18 months (range 10–48 months), patients treated with PBM had better locoregional disease control and improved progression-free or overall survival [99].

Current evidence suggests that PBM in the red or NIR spectrum, with an energy density of 1–6 J/cm² is safe and effective. However, as with drug-based therapies for comparable indications, disciplined follow-up studies in which subject cohorts large enough to represent the HNC tumor population with respect to gender, tobacco and alcohol use, human papilloma virus (HPV) status, primary stage, tumor therapy, and variations in PBM dose and fields are needed to definitely conclude that PBM fails to negatively impact survival and progression-free survival.

Discussion and concluding remarks

PBM mechanisms have not been fully elucidated, but based on its recognized therapeutic effects, PBM may have utility in a broad range of oral, oropharyngeal, facial, exocrine glands, and neck complications of HNC treatment.

Titration adequate doses and defining the other required PBM parameters according to evidence gathered in a systematic way for each indication is a prerequisite for a successful use of this technique. Without standardization in beam measurement, dose calculation, and the correct reporting of these parameters, studies will not be reproducible, and outcomes will not be consistent.

There are no known significant adverse side effects for PBM (administered with parameters discussed in more detail in part 2) in HNC patients. However, the potential effect on residual and new dysplastic and malignant cells has not been definitively resolved. Virtually, all studies have focused on cell-based assays rather than conventional xenograft or orthotopic animal models. And the results of in vitro investigations have been largely dependent on the experimental design and selection of target cells.

The lack of consistent findings and/or the latitude of interpretation of the clinical significance of molecular biology findings hamper meaningful conclusions. Continuing research addressing the molecular pathways affected by PBM is necessary.

It seems unlikely that PBM has carcinogenic effects on normal cells or protects to cytotoxic effects of RT; there is even some evidence suggesting that PBM may enhance treatment response.

Studies indicate that different tumor cells have distinct responses to specific PBM parameters and doses. In part, these differences may be also explained by variations in the cellular microenvironment, since these have been shown to affect cellular signal transduction



Published in final edited form as:

Support Care Cancer. 2016 June ; 24(6): 2793–2805. doi:10.1007/s00520-016-3153-y.

Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 2: proposed applications and treatment protocols

Judith A. E. M. Zecha¹, Judith E. Raber-Durlacher^{1,2}, Raj G. Nair³, Joel B. Epstein^{4,5}, Sharon Elad⁶, Michael R. Hamblin^{7,8,9}, Andrei Barasch¹⁰, Cesar A. Migliorati¹¹, Dan M. J. Milstein¹, Marie-Thérèse Genot¹², Liset Lansaat¹³, Ron van der Brink⁵, Josep Arnabat-Dominguez¹⁵, Lisette van der Molen¹³, Irene Jacobi¹³, Judi van Diessen¹⁴, Jan de Lange¹, Ludi E. Smeele^{1,13}, Mark M. Schubert¹⁶, and René-Jean Bensadoun¹⁷

Judith A. E. M. Zecha: j.zecha@amc.uva.nl; Judith E. Raber-Durlacher: j.e.raberdurlacher@amc.uva.nl; Raj G. Nair: r.nair@griffith.edu.au; Joel B. Epstein: joel.epstein@cshs.org; Sharon Elad: SElad@URMC.Rochester.edu; Michael R. Hamblin: hamblin@helix.mgh.harvard.edu; Andrei Barasch: barasaff@cs.com; Cesar A. Migliorati: miglioni@uthsc.edu; Dan M. J. Milstein: D.M.Milstein@amc.uva.nl; Marie-Thérèse Genot: mtgenot@skynet.be; Liset Lansaat: l.lansaat@nki.nl; Ron van der Brink: RVanderbrink@coh.org; Josep Arnabat-Dominguez: joseparnabat@ub.edu; Lisette van der Molen: l.vd.molen@nki.nl; Irene Jacobi: i.jacobi@nki.nl; Judi van Diessen: j.v.diessen@nki.nl; Jan de Lange: j.delange@amc.uva.nl; Ludi E. Smeele: l.smeele@nki.nl; Mark M. Schubert: mschuber@seattlecca.org; René-Jean Bensadoun: renejean.bensadoun@che-nice.com

¹Department of Oral and Maxillofacial Surgery, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105, AZ Amsterdam, The Netherlands ²Department of Medical Dental Interaction and Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University, P.O. Box 22660 1100 DD, Amsterdam, the Netherlands ³Oral Medicine Oral Pathology and Human Diseases, Menzies Health Institute Queensland and Oral Medicine Consultant, Department of Haematology and Oncology/Cancer Services, Gold Coast University Hospital, Queensland Health, Queensland, Australia ⁴Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA ⁵Division of Otolaryngology and Head and Neck Surgery, City of Hope, Duarte, CA 91010, USA ⁶Division of Oral Medicine, Eastman Institute for Oral Health, and Wilms Cancer Center, University of Rochester Medical Center, Rochester, NY 14620, USA ⁷Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA 02114, USA ⁸Department of Dermatology, Harvard Medical School, Boston, MA 02115, USA ⁹Harvard-MIT Division of Health Science and Technology, Cambridge, MA 02139, USA ¹⁰Division of Oncology, Weill Cornell

Correspondence to: René-Jean Bensadoun, renejean.bensadoun@che-nice.com.

Disclaimer This article is based on a narrative review of existing data and the clinical observations of an international multidisciplinary panel of clinicians and researchers with expertise in the area of supportive care in cancer and/or PBM clinical application and dosimetry. This article is informational in nature. As with all clinical materials, this paper should be used with the clear understanding that continued research and practice could result in new insights and recommendations. The review reflects the collective opinion and as such does not necessarily represent the opinion of any individual author. In no event shall the authors be liable for any decision made or action taken in reliance on the proposed protocols.

Disclosures Judith A.E.M. Zecha, Andrei Barasch, Sharon Elad, Steven Sonis, Cesar A. Migliorati, Marie-Thérèse Genot, Dan M.J. Milstein, Liset Lansaat, Irene Jacobi, Judi van Diessen, Jan. de Lange, Ludi E. Smeele and Mark M. Schubert have no disclosures relevant to this work to report.

Judith E. Raber-Durlacher, Raj G. Nair, Joel B. Epstein, Ron van der Brink, Josep Arnabat Dominguez, and Rene-Jean Bensadoun have received travel expenses and hotel accommodation for the founding meeting of iGLOB from THOR Photomedicine Ltd., UK. Raj Nair has received an honorarium from THOR, UK. Michael R Hamblin was supported by US NIH grant R01AI050875.

Medical Center, New York, NY, USA ¹¹Department of Diagnostic Sciences and Oral Medicine, Director of Oral Medicine, College of Dentistry, University of Tennessee Health Science Center, 875 Union Ave. Suite N231, Memphis, TN 38163, USA ¹²Laser Therapy Unit, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium ¹³Antoni van Leeuwenhoek Department of Head and Neck Oncology and Surgery, Netherlands Cancer Institute, Amsterdam, the Netherlands ¹⁴Antoni van Leeuwenhoek Department of Radiation Oncology, Amsterdam, Netherlands Cancer Institute, Amsterdam, the Netherlands ¹⁵Department of Oral Surgery, Faculty of Dentistry, University of Barcelona, Barcelona, Spain ¹⁶Seattle Cancer Care Alliance (SCCA), Oral Medicine, 825 Eastlake Ave E Ste G6900, Seattle, WA 98109, USA ¹⁷World Association for Laser Therapy (WALT) Scientific Secretary, Centre de Haute Energie (CHE), 10 Bd Pasteur, 06000 Nice, France

Abstract

Purpose—There is a large body of evidence supporting the efficacy of low-level laser therapy (LLLT), more recently termed photobiomodulation (PBM) for the management of oral mucositis (OM) in patients undergoing radiotherapy for head and neck cancer (HNC). Recent advances in PBM technology, together with a better understanding of mechanisms involved and dosimetric parameters may lead to the management of a broader range of complications associated with HNC treatment. This could enhance patient adherence to cancer therapy, and improve quality of life and treatment outcomes. The mechanisms of action, dosimetric, and safety considerations for PBM have been reviewed in part 1. Part 2 discusses the head and neck treatment side effects for which PBM may prove to be effective. In addition, PBM parameters for each of these complications are suggested and future research directions are discussed.

Methods—Narrative review and presentation of PBM parameters are based on current evidence and expert opinion.

Results—PBM may have potential applications in the management of a broad range of side effects of (chemo)radiation therapy (CRT) in patients being treated for HNC. For OM management, optimal PBM parameters identified were as follows: *wavelength*, typically between 633 and 685 nm or 780–830 nm; *energy density*, laser or light-emitting diode (LED) output between 10 and 150 mW; *dose*, 2–3 J (J/cm²), and no more than 6 J/cm² on the tissue surface treated; *treatment schedule*, two to three times a week up to daily; *emission type*, pulsed (<100 Hz); and *route of delivery*, intraorally and/or transcutaneously. To facilitate further studies, we propose potentially effective PBM parameters for prophylactic and therapeutic use in supportive care for dermatitis, dysphagia, dry mouth, dysgeusia, trismus, necrosis, lymphedema, and voice/speech alterations.

Conclusion—PBM may have a role in supportive care for a broad range of complications associated with the treatment of HNC with CRT. The suggested PBM irradiation and dosimetric parameters, which are potentially effective for these complications, are intended to provide guidance for well-designed future studies. It is imperative that such studies include elucidating the effects of PBM on oncology treatment outcomes.

chemotherapy, with or without total body irradiation, and to suggest a role for patients treated with RT for HNC [11, 18]. Evidence was derived from high-quality studies using specific PBM parameters, and the authors noted that there remains a need to identify optimal PBM parameters per cancer treatment modality.

Based on this evidence and on our experience, we propose the following regimen for the management of OM and mucositis affecting the oropharynx: wavelength of 633–685 or 780–830 nm; power output of between 10 and 150mW; *energy density* 2–3 J/cm², and no more than 6 J/cm² on the tissue surface treated; administered two to three times a week up to daily; and using successive intraoral applications on single spots on the mucosa, rather than a scanning motion over the entire mucosal surface. The upper safety limit was set as a precaution since no clinical data defining a safe upper limit are currently available. *Emission type*, continuous or pulsed (<100 Hz) as low-frequency **pulsed light may be superior to continuous wave light for wound healing or the prevention of injury**. Extraorally administered PBM may be effective for the management of OM of the buccal mucosa, vestibule, and inner epithelial surfaces of the lips which could be applied in combination with an intraoral device.

Dermatitis

Radiation dermatitis occurs in the majority of patients with locoregionally advanced HNC treated with RT.

The pathobiology of acute radiation dermatitis is complex and partially overlaps that of OM. Irradiation of the skin leads to direct tissue injury and inflammatory cell recruitment, involving damage to epidermal basal cells and connective tissue including endothelial cells and vascular components [19]. Radiation-induced generation of free radicals induces DNA injury and release of inflammatory cytokines [mainly interleukin (IL)-1 and IL-6] [20, 21]. This process leads to the development of erythema, edema, and possible ulceration. Late RT-induced changes involving skin are characterized by the loss of follicular structures, an increase in collagen and damage to elastic fibers in the dermis, and a fragile epidermal covering [22]. Transforming growth factor beta (TGF- β) is considered to play a central role in mediating RT-induced tissue fibrosis [23–25].

The severity of skin reactions is dependent on the total radiation dose, the dose per fraction, the overall treatment time, beam type and energy, the surface area of the skin exposed to radiation, the use of combined chemoradiotherapy with or without targeted therapies, and individual risk factors [20]. The severity of acute reactions has been shown to predict late effects. Radiation dermatitis impacts adversely on cosmesis and function and reduces QoL, especially in patients who develop secondarily infected dermatitis [19].

Patients with head and neck squamous cell carcinoma (HNSCC) treated with an epithelial growth factor receptor (EGFR) inhibitor may develop an acneiform skin rash in addition to radiation dermatitis [17, 22].

Based on the effects of PBM on the epidermis and dermis (reduced inflammation and improved wound healing), and on the shared similarities in pathobiology with OM, it seems

any alteration in muscle or tissue properties of these structures. Although voice and speech dysfunctions significantly affect QoL, these complications have received little attention and are likely underreported in efforts to preserve organ function after cancer therapy [117–119].

Currently, there is limited information on the prevalence of speech and voice dysfunction in advanced HNC patients treated with RT or CRT. Prospective studies are needed, including baseline measurements and standardized multidimensional assessment of functional aspects of voice and speech [118].

The etiology of voice and speech problems resembles that of dysphagia and may include neuromuscular weakness as a result of tumor invasion. Dependent on the dose tolerance of the critical organs involved, CRT-induced voice and/or speech dysfunction can result from mucositis of the soft palate, tongue and laryngeal soft tissues, edema, fibrosis, or atrophy of the vocal folds, pharyngeal and oral tissues, and altered saliva or hyposalivation [120–122].

New RT delivery techniques designed to spare these structures may prevent functional impairment.

A study using an animal model of reflux laryngitis (a condition including hoarseness, voice fatigue, globus, chronic cough, throat pain, and dysphagia) suggested that the anti-inflammatory effects of PBM may play in the management of this condition [123].

We are not aware of any studies on the effect of PBM on the quality of speech and voice in HNC patients. Since PBM may preserve function of the anatomical structures involved by its anti-inflammatory effects and may have indirect benefits by stimulating the salivary flow, future studies are warranted.

Conclusion

Acute and chronic complications induced by RT and CRT in patients with HNC represent a significant clinical challenge [1]. There are similarities with respect to pathophysiology across different complications, and patients may suffer from multiple concurrent and interrelated problems [13]. There is anecdotal evidence suggesting that the inflammation associated with acute complications is a harbinger for chronic complications. This observation suggests that preventive approaches starting before, and in the early phases of treatment with RT and CRT, may not only reduce the risk for developing acute problems but may also have an impact on the risk for late complications.

PBM has shown effectiveness in the management of OM and elicits several potentially beneficial effects, including reduction of inflammation and pain, promotion of tissue repair, reduction of fibrosis, and protection and regeneration of nerves. Therefore, there is a clear motivation for studies on the application of PBM for the prevention and treatment of a broad range of acute and chronic complications associated with RT or CRT in HNC patients.

The purpose of this article is to serve as a basis for establishing a platform for facilitating future collaborations among clinicians and researchers, in order to create firm scientific evidence for the use of PBM in patients with HNC. PBM protocols should be administered

What is Light Coherence?

Light coherence is important for deep-tissue photomedicine because it forms microscopic intensity patterns called laser speckles that increase the probability of photon absorption.

Coherence means order or synchronicity. It describes light waves that maintain a steady phase relationship with each other over distance and time. While standard light sources like LEDs emit chaotic, uncoordinated waves (incoherent light), lasers emit highly organized and coherent light.

Why Coherence Matters for Deep Tissues

Laser Speckles:

When coherent light enters tissue, it creates a pattern of tiny spots called speckles that have higher light intensity.

Overcoming Low Flux:

In deep tissue, most light gets scattered or absorbed before reaching deep targets. The high-intensity peaks inside individual speckles help the light cross the threshold needed to trigger a biological response.

Better Penetration:

Coherent laser light often penetrates deeper through structures like bone or thick tissue compared to non-coherent LEDs of similar power.

When Coherence Matters Less

Superficial Treatments:

For shallow wounds, skin surface issues, or hair growth stimulation, non-coherent polarized light and LEDs often perform just as well as coherent lasers.

Basic Absorption:

The primary chemical trigger in light therapy is photon absorption by the mitochondria, which depends on the wavelength and dose. Research reviewed by the National Institutes of Health shows that coherence acts as an optimizer for specific deep targets.

The importance of coherence in phototherapy

Tomas Hode*^a, Donald Duncan^b, Sean Kirkpatrick^b, Peter Jenkins^a, Lars Hode^c.

^aIrradia USA, 16275 Patrick Henry Hwy, Roseland, VA 22967, USA;

^bOregon Health & Science University, Div. Biomedical Engineering, Mail Code CH13B, 3303 SW Bond Ave., Portland, OR 97239, USA;

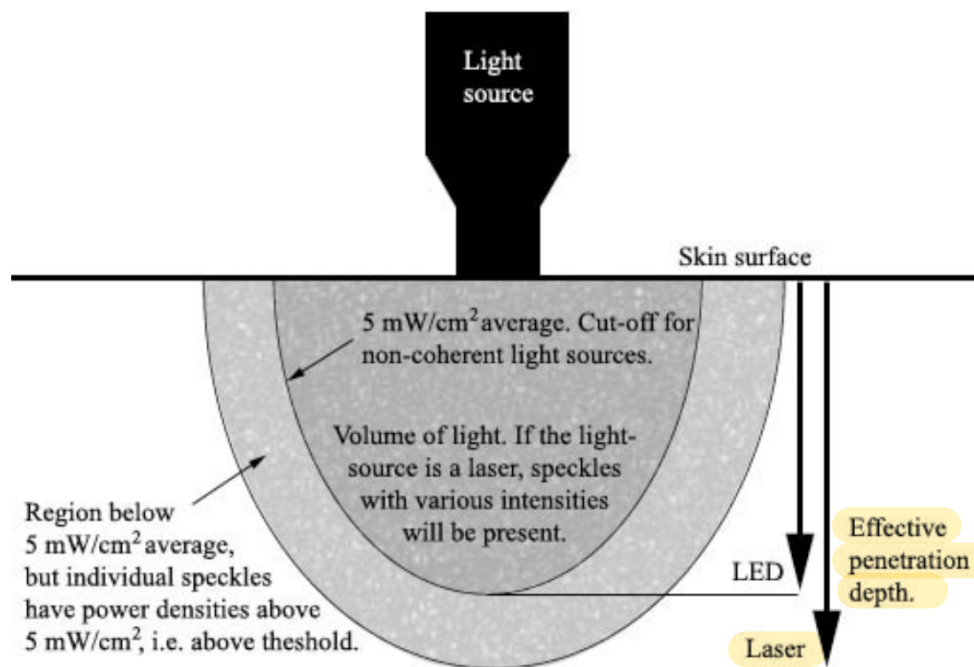
^cSwedish Laser-Medical Society, P.O. Box 1031, 181 21 Lidingö, Sweden.

... account for the effects of laser speckle in vivo. Laser speckles intensity is higher than the surrounding environment. This is important because the probability for a photon absorption event to occur largely depends on intensity and the photon absorption of the molecule.

In deep tissue where the photon flux is extremely low, the increased probability of photon absorption from individual laser speckles increases the probability of reaching the necessary power density thresholds.

Because of the non-coherent nature of radiation from light/IR emitting diodes speckle does not occur in the tissue with LED therapy, which may explain why head-to-head comparisons between lasers and LEDs in deep tissue seem to be in favor of lasers.

Several comparative studies have shown that laser phototherapy appears to be more effective than LED phototherapy in the treatment of deeper indications, as well as in in vitro and wound healing studies.



Before any speckle-effects in tissue can be discussed it is important to clarify that coherence is not lost in a scattering media (such as tissue), only reduced.

In the case of coherent light (where a speckle field is present), there will be individual speckles with intensities of up to 5X the average intensity, which means that tissue situated deeper than the average power density threshold cut-off will be exposed to power densities above the phototherapeutic threshold.

In other words, the effective penetration depth of coherent light-sources (i.e. lasers) is greater than for non-coherent light-sources (e.g. LED's).

The speckle intensity distribution shows intensities of up to five times the mean intensity will randomly occur in the speckle field.

The coherence is not lost when tissue is illuminated with a laser, which means that a 3-dimensional speckle pattern will appear in the tissue during laser phototherapy, but not when non-coherent light sources are used.

The speckle pattern has a significant impact on the effective penetration depth of in vivo laser phototherapy since it becomes easier to reach the required intensity thresholds.

In a clinical setting, this increased effective depth of penetration can make the difference between having to rely on systemic effects vs. getting direct effects in the target tissue.

Our simulations suggest that intensities of up to five times the mean intensity occur in a speckle field.

These observations could also explain why LED phototherapy in many cases appears to be comparable to laser phototherapy for superficial indications such as wound healing, but not as effective as laser phototherapy in cases of deeper-seated indications.

Low-Level Light/Laser Therapy Versus Photobiomodulation Therapy

Juanita J. Anders, PhD,¹ Raymond J. Lanzafame, MD, MBA,² and Praveen R. Arany, DDS, PhD³

Niels Ryberg Finsen pioneered the use of red and blue light to treat various human ailments, especially *Lupus vulgaris*.

He was awarded the Nobel Prize in medicine and physiology in 1903 for his pioneering demonstration that concentrated chemical rays from sunlight could be bactericidal as well as stimulating surrounding tissue.

Mester et al. noted that laser light at low doses demonstrated increased hair growth at an accelerated rate, and promoted excisional wound healing. This observation was termed “laser biostimulation.”

In addition to biostimulation, a few of the other names previously used for this therapy have included low-level laser (or light) therapy (LLLT), low-intensity laser therapy, low-power laser therapy, cold laser, soft laser, photobiostimulation, and photobiomodulation.

There is clearly a dismal lack of consistency and consensus on terminology. The most frequently used term is low-level laser therapy (LLLT).

LLLT, utilizes endogenous chromophores with low-dose illumination delivered at the target site.

... it distinguishes photobiomodulation therapy, which is nonthermal, from the popular use of light-based devices for simple heating of tissues as can be accomplished using near-infrared (NIR) lamps, or other applications of light energy that rely on thermal effects for all or part of their mechanism of action.

It is a nonthermal process involving endogenous chromophores eliciting photophysical and photochemical events at various biological scales.

This process results in beneficial therapeutic outcomes including but not limited to the alleviation of pain or inflammation, immunomodulation, and promotion of wound healing and tissue regeneration.



FULL TEXT LINKS



J Appl Biomater Funct Mater. 2019 Jan-Mar;17(1):2280800018816013.
doi: 10.1177/2280800018816013.

Laser versus LED polymerization: Comparison of in vitro preventive sealing procedures

Filiberto Mastrangelo ¹, Isabella Perraro ², Sabrina Mattia ², Giuseppe Troiano ¹,
Khrystyna Zhurakivska ¹, Elisabetta Polizzi ², Roberto Chiesa ³, Enrico Gherlone ²,
Lorenzo Lo Muzio ¹

Affiliations

Affiliations

- ¹ 1 Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy.
- ² 2 Department of Dentistry, Vita Salute University, IRCCS San Raffaele Hospital, Milan, Italy.
- ³ 3 Department of Chemistry, Materials and Chemical Engineering "G. Natta," Politecnico di Milano, Milan, Italy.

PMID: 30803291 DOI: 10.1177/2280800018816013

[Free article](#)

Abstract

Introduction:: The technique of sealing is a widely accepted procedure for prevention of caries. The aim of our in vitro study was to compare the effect of two different curing units (traditional LED source and innovative laser diode lamp) on the integrity of fissure sealant material and its interface with tooth enamel.

Materials and methods:: Sixty healthy third molars were randomly assigned to two groups. In group A were teeth intended for polymerization by LED B lamp, and group B comprised teeth to be polymerized by an innovative laser diode. Both groups were treated with the traditional sealing technique, subjected to a metallization process, and analyzed by scanning electron microscope.

Results:: Micro-gaps between the sealant and the enamel were found in specimens in both A (43%) and B (40%) groups ($p=0.793$), and sealant shrinkage was seen. Significant differences between the groups emerged in the percentage of perimetric micro-erosion sites (80% vs. 100%, $p=0.010$) and the presence of holes and micro-bubbles on the sealant surface (21% vs. 63%, $p=0.001$).

Conclusions:: Although macroscopic clinical polymerization occurred with both instruments, the microscopic evaluation showed significant differences between the studied groups in terms of perimetric micro-erosion sites and micro-bubbles, which were higher in laser-cured samples.

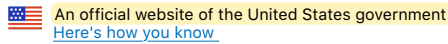
Keywords: LED; Preventive sealing; laser polymerization; polymerization.

[PubMed Disclaimer](#)

LinkOut – more resources

Full Text Sources

[Atypon](#)



Randomized Controlled Trial Lasers Surg Med. 2022 Mar;54(3):342-347.

doi: 10.1002/lsm.23480. Epub 2021 Sep 16.

HeaLED: Assessment of skin healing under light-emitting diode (LED) exposure-A randomized controlled study versus placebo

Florence Le Duff ¹, Eric Fontas ², Davide Guardoli ¹, Jean-Philippe Lacour ¹,
Thierry Passeron ^{1 3}

Affiliations

Affiliations

- ¹ Dermatology Department, Archet Hospital-CHU, University Côte d'Azur, Nice, France.
- ² Délégation à la Recherche Clinique et à l'innovation (DRCI), Université Côte D'Azur, CHU Nice, Nice, France.
- ³ INSERM U 1065, C3M, University Côte d'Azur, Nice, France.

PMID: 34529859 DOI: 10.1002/lsm.23480

Abstract

Background: Light-emitting diodes (LEDs) in the visible or near-infrared spectrum have been reported to promote wound healing. However, despite being frequently proposed in daily clinical practice, the efficacy of photobiomodulation treatment after a laser procedure relies on very limited clinical data.

Objective: To compare the relative efficacy of LED versus placebo treatment in decreasing erythema and transepidermal water loss (TEWL) after a fractional CO₂ session.

Methods: We conducted an open prospective intraindividual randomized controlled study with 10 healthy volunteers. An ablative fractional laser was performed on the seven forearm areas. Three consecutive daily sessions of LED (590, 630, and 850 nm [two tested irradiances each] and placebo) were applied after randomization. Physical measures (colorimetry, TEWL), photography, and clinical evaluation were performed on Days 1, 2, 3, 7, and 21. The main criterion of evaluation was the variation of parameter a* (erythema) at 72 hours for each LED parameter compared to placebo treatment.

Results: No significant differences in the variation of the parameter a* or any of the other studied parameters were found for the different LEDs compared to the placebo area.

Conclusion: Photobiomodulation failed to improve healing after laser ablation compared to placebo.

Keywords: CO₂ laser; ablative fractional laser; healing; light-emitting diode; photobiomodulation.

© 2021 Wiley Periodicals LLC.

[PubMed Disclaimer](#)



OPEN ACCESS

EDITED BY

Dwight E. Heron,
Bon Secours Health System,
United States

REVIEWED BY

Vinagolu K. Rajasekhar,
Memorial Sloan Kettering Cancer
Center, United States
Avraham Eisbruch,
University of Michigan, United States

*CORRESPONDENCE

René-Jean Bensadoun
renejean.bensadoun@che-nice.com

SPECIALTY SECTION

This article was submitted to
Radiation Oncology,
a section of the journal
Frontiers in Oncology

RECEIVED 24 April 2022

ACCEPTED 28 July 2022

PUBLISHED 30 August 2022

CITATION

Robijns J, Nair RG, Lodewijckx J,
Arany P, Barasch A, Bjordal JM,
Bossi P, Chilles A, Corby PM,
Epstein JB, Elad S, Fekrazad R,
Fregnani ER, Genot M-T, Ibarra AMC,
Hamblin MR, Heiskanen V, Hu K,
Klastersky J, Lalla R, Latifian S, Maiya A,
Mebis J, Migliorati CA, Milstein DMJ,
Murphy B, Raber-Durlacher JE,
Roseboom HJ, Sonis S, Treister N,
Zadik Y, Bensadoun R-J and “Cancer
Supportive Care” WALT Working
Group (2022) Photobiomodulation
therapy in management of cancer
therapy-induced side effects: WALT
position paper 2022.
Front. Oncol. 12:927685.
doi: 10.3389/fonc.2022.927685

Photobiomodulation therapy in management of cancer therapy-induced side effects: WALT position paper 2022

Jolien Robijns¹, Raj G. Nair², Joy Lodewijckx¹, Praveen Arany³,
Andrei Barasch⁴, Jan M. Bjordal⁵, Paolo Bossi⁶, Anne Chilles⁷,
Patricia M. Corby⁸, Joel B. Epstein⁹, Sharon Elad¹⁰,
Reza Fekrazad¹¹, Eduardo Rodrigues Fregnani¹²,
Marie-Thérèse Genot¹³, Ana M. C. Ibarra¹⁴,
Michael R. Hamblin¹⁵, Vladimir Heiskanen¹⁶, Ken Hu¹⁷,
Jean Klastersky¹⁸, Rajesh Lalla¹⁹, Sofia Latifian²⁰, Arun Maiya²¹,
Jeroen Mebis¹, Cesar A. Migliorati²², Dan M. J. Milstein²³,
Barbara Murphy²⁴, Judith E. Raber-Durlacher²⁵,
Hendrik J. Roseboom²⁵, Stephen Sonis²⁶, Nathaniel Treister²⁶,
Yehuda Zadik²⁷, René-Jean Bensadoun^{28*}
and “Cancer Supportive Care” WALT Working Group

¹UHasselt, Faculty of Medicine and Life Sciences, Diepenbeek, Belgium, ²Oral Medicine, Oral Pathology and Oral Oncology, Griffith University, Department of Haematology and Oncology, Gold Coast University Hospital, Gold Coast, QLD, Australia, ³School of Dental Medicine, Oral Biology and Biomedical Engineering, University at Buffalo, Buffalo, NY, United States, ⁴Harvard School of Dental Medicine, Division of Oral Medicine and Dentistry, Boston, MA, United States, ⁵Physiotherapy Research Group, IGS, University of Bergen, Bergen, Norway, ⁶Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy, ⁷Radiotherapy Department, Institut Curie, Paris, France, ⁸New York University College of Dentistry, Bluestone Center for Clinical Research, New York, NY, United States, ⁹City of Hope Duarte, CA and Cedars-Sinai Health System, Los Angeles, CA, United States, ¹⁰Eastman Institute for Oral Health, University of Rochester Medical Center, Rochester, NY, United States, ¹¹Department of Periodontology, Dental Faculty – Radiation Sciences Research Center, Laser Research Center in Medical Sciences, AJA University of Medical Sciences, Tehran, Iran, ¹²Oral Medicine Department, Hospital Sírio-Libanês, São Paulo, Brazil, ¹³Laser Therapy Unit, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium, ¹⁴Postgraduate Program on Biophotonics Applied to Health Sciences, Nove de Julho University, São Paulo, Brazil, ¹⁵Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, ¹⁶Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland, ¹⁷Department of Radiation Oncology, NYU Langone Health, New York, NY, United States, ¹⁸Medical Oncology, Institut Jules Bordet, Brussels, Belgium, ¹⁹Section of Oral Medicine, University of Connecticut School of Dental Medicine, Farmington, CT, United States, ²⁰Department of Medicine, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium, ²¹Manipal College of Health Professions, MAHE, Manipal, India, ²²Department of Oral and Maxillofacial Diagnostic Sciences, University of Florida College of Dentistry, Gainesville, Florida, United States, ²³Oral and Maxillofacial Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, ²⁴Department of Oncology, Vanderbilt University Medical Center, Nashville, TN, United States, ²⁵Department of Oral and Maxillofacial Surgery, Amsterdam UMC, University of Amsterdam, Department of Oral Medicine, Academic Centre for Dentistry Amsterdam, University of Amsterdam and VU University, Amsterdam, Netherlands, ²⁶Division of Oral Medicine and Dentistry, Brigham and Women's Hospital; Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine; Division of Oral Medicine and Dentistry, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Boston, MA, United States, ²⁷Department of Military Medicine, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel, and Department of Oral Medicine, Sedation and Maxillofacial Imaging, Hebrew University-Hadassah School of Dental Medicine, Jerusalem, Israel, ²⁸Department of Radiation Oncology, Centre de Haute Energie, Nice, France

Disclaimer: This article is based on recommendations from the 12th WALT Congress, Nice, October 3-6, 2018, and a follow-up review of the existing data and the clinical observations of an international multidisciplinary panel of clinicians and researchers with expertise in the area of supportive care in cancer and/or PBM clinical application and dosimetry. This article is informational in nature. As with all clinical materials, this paper should be used with a clear understanding that continued research and practice could result in new insights and recommendations. The review reflects the collective opinion and, as such, does not necessarily represent the opinion of any individual author. In no event shall the authors be liable for any decision made or action taken in reliance on the proposed protocols.

Objective: This position paper reviews the potential prophylactic and therapeutic effects of photobiomodulation (PBM) on side effects of cancer therapy, including chemotherapy (CT), radiation therapy (RT), and hematopoietic stem cell transplantation (HSCT).

Background: There is a considerable body of evidence supporting the efficacy of PBM for preventing oral mucositis (OM) in patients undergoing RT for head and neck cancer (HNC), CT, or HSCT. This could enhance patients' quality of life, adherence to the prescribed cancer therapy, and treatment outcomes while reducing the cost of cancer care.

Methods: A literature review on PBM effectiveness and dosimetry considerations for managing certain complications of cancer therapy were conducted. A systematic review was conducted when numerous randomized controlled trials were available. Results were presented and discussed at an international consensus meeting at the World Association of photobiomodulation Therapy (WALT) meeting in 2018 that included world expert oncologists, radiation oncologists, oral oncologists, and oral medicine professionals, physicists, engineers, and oncology researchers. The potential mechanism of action of PBM and evidence of PBM efficacy through reported outcomes for individual indications were assessed.

Results: There is a large body of evidence demonstrating the efficacy of PBM for preventing OM in certain cancer patient populations, as recently outlined by the Multinational Association for Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO). Building on these, the WALT group outlines evidence and prescribed PBM treatment parameters for prophylactic and therapeutic use in supportive care for radiodermatitis, dysphagia, xerostomia, dysgeusia, trismus, mucosal and bone necrosis, lymphedema, hand-foot syndrome, alopecia, oral and dermatologic chronic graft-versus-host disease, voice/speech alterations, peripheral neuropathy, and late fibrosis amongst cancer survivors.

Conclusions: There is robust evidence for using PBM to prevent and treat a broad range of complications in cancer care. Specific clinical practice guidelines or evidence-based expert consensus recommendations are provided. These recommendations are aimed at improving the clinical utilization of PBM therapy in supportive cancer care and promoting research in this field. It is anticipated these guidelines will be revised periodically.

KEYWORDS

photobiomodulation (PBM), cancer supportive care, guidelines, recommendations, mucositis, dermatitis, cancer-treatment side effects

1 Introduction

Despite the ongoing improvements in cancer therapy, it is still associated with severe life-impairing side effects. Both treatment- and patient-related risk factors determine the severity of the complications. Moreover, they negatively impact the patients' quality of life (QoL) and daily activities. Therefore, effective supportive care strategies are necessary (1). The biological effects of photobiomodulation (PBM) therapy were discovered by Endre Mester in 1965 (2). Currently, PBM is defined as: "the use of non-ionizing optical radiation in the visible and near-infrared spectral range is absorbed by endogenous chromophores to elicit photophysical and photochemical events at various biological scales without eliciting thermal damage, leading to physiological changes and therapeutic benefits" (3). There is a considerable body of evidence supporting the efficacy of PBM for the prevention of oral mucositis (OM) in patients undergoing radiotherapy (RT) for head and neck cancer (HNC), chemotherapy (CT), or hematopoietic stem cell transplantation (HSCT) (4). Recent advances in understanding the mechanisms of action of PBM and dosimetry parameters of PBM have resulted in examining other oncology-related conditions that may lead to effective management of a broader range of complications associated with cancer treatment. This could improve overall QoL, adherence to cancer treatment regimens, and their outcomes while reducing cost of care (5).

2 Methods

This position paper was developed based on reviewing existing literature on PBM in supportive cancer care. For oral mucositis, a systematic review was feasible. For all other indications, expert consensus opinions are based on a narrative review of the literature and the personal experiences of contributing experts. A level of evidence was assigned to these indications based on the Somerfield criteria based on the type and design of the study (6). The level of evidence for each intervention is then translated into a guideline recommendation where Level I or II evidence is achieved by at least one well-designed randomized controlled trial (RCT). A suggestion was possible for lower-level evidence, but only when consistent evidence from multiple studies and panel consensus on the interpretation of this evidence. Thus, to enable maximal, practical clinical use and promote future research for PBM in supportive cancer care, WALT makes recommendations in two categories as clinical practice guidelines and consensus expert opinion. We strongly recommend clinical judgment of the provider and individual patient scenario be carefully accounted for in interpreting and applying these WALT PBM recommendations.

3 Results

The reader is referred to comprehensive reviews in these WALT series for a detailed discussion of PBM parameters. A brief review of critical PBM parameters is presented below.

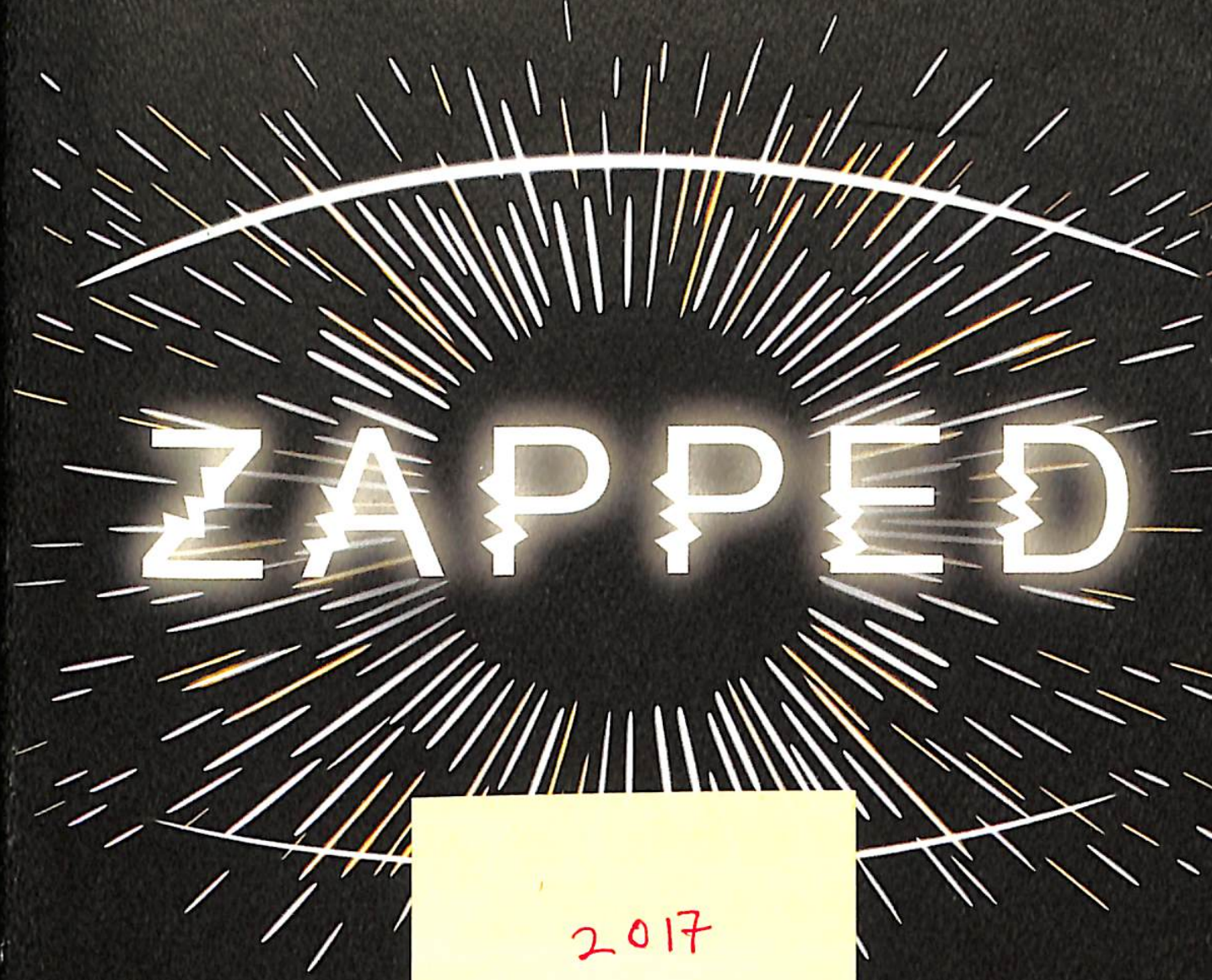
3.1 PBM parameters

PBM parameters using low-level lasers or light-emitting diodes (LEDs) in cancer supportive care (summarized in Table 1) are usually within the red and near-infrared (NIR) wavelength range between 600 nanometers (nm) and around 1,000 nm, with a power density from 5 (mW)/cm² to 150 mW/cm² (7). The duration of application varies according to the site, but it may well be within 30 - 60 seconds per point. While shorter efficacious treatment times have been used (2-10sec per point, multiple spots that are clinically laborious), this could be attributed to cumulative PBM dose effects. The therapeutic dosage is depicted as the energy density measured in Joules as J/cm² and varies between 0.1 to 12 J/cm² as per current literature. Low-level laser systems used include helium-neon (HeNe), neodymium-doped yttrium aluminum garnet (Nd : YAG), gallium aluminum arsenide (GaAlAs) diode lasers, indium gallium aluminum phosphorus (InGaAlP), and non-thermal, non-ablative carbon dioxide (CO₂) lasers (8). In recent years, LEDs with wavelengths in the red or NIR regions have become increasingly common due to their safety, low cost, and suitability for home use (8, 9).

The biological effects of PBM on the exposed tissues depend upon a number of variables, including the location of the cells in the field of exposure, cell type, molecular and redox state of the cell, the tissue microenvironment, PBM parameters such as wavelength, power density, type of delivery as in pulsing or continuous, beam or spot size, and duration of exposure (10, 11). It is well known that PBM therapy exhibits a biphasic dose-response that warrants optimal tissue-specific irradiation dose parameters. In other words, doses lower than the optimal value may have a diminished effect, while doses higher than optimal can have no beneficial or even adverse therapeutic outcomes (12, 13). The effect of such a phenomenon has been consistently evident in published data on the effectiveness and particular disparity of PBM therapy in cancer complications.

Titration adequate doses and defining the essential PBM parameters as per evidence gathered in a systematic way for each indication is a prerequisite for the successful use of this treatment modality. Without standardization in beam measurement, dose calculation, and the correct reporting of these parameters, studies will not be reproducible, and outcomes will not be consistent. A common misconception is that energy (in J) or energy density (J/cm²) is all that is necessary to replicate a successful treatment, irrespective of the original power, power

*From Infrared to X-rays,
the Curious History of Invisible Light*



ZAPPED

2017

BOB BERMAN

AUTHOR OF ZOOM

Zapped
From Infrared to X-rays, the Curious History of Invisible Light

Bob Berman
2017

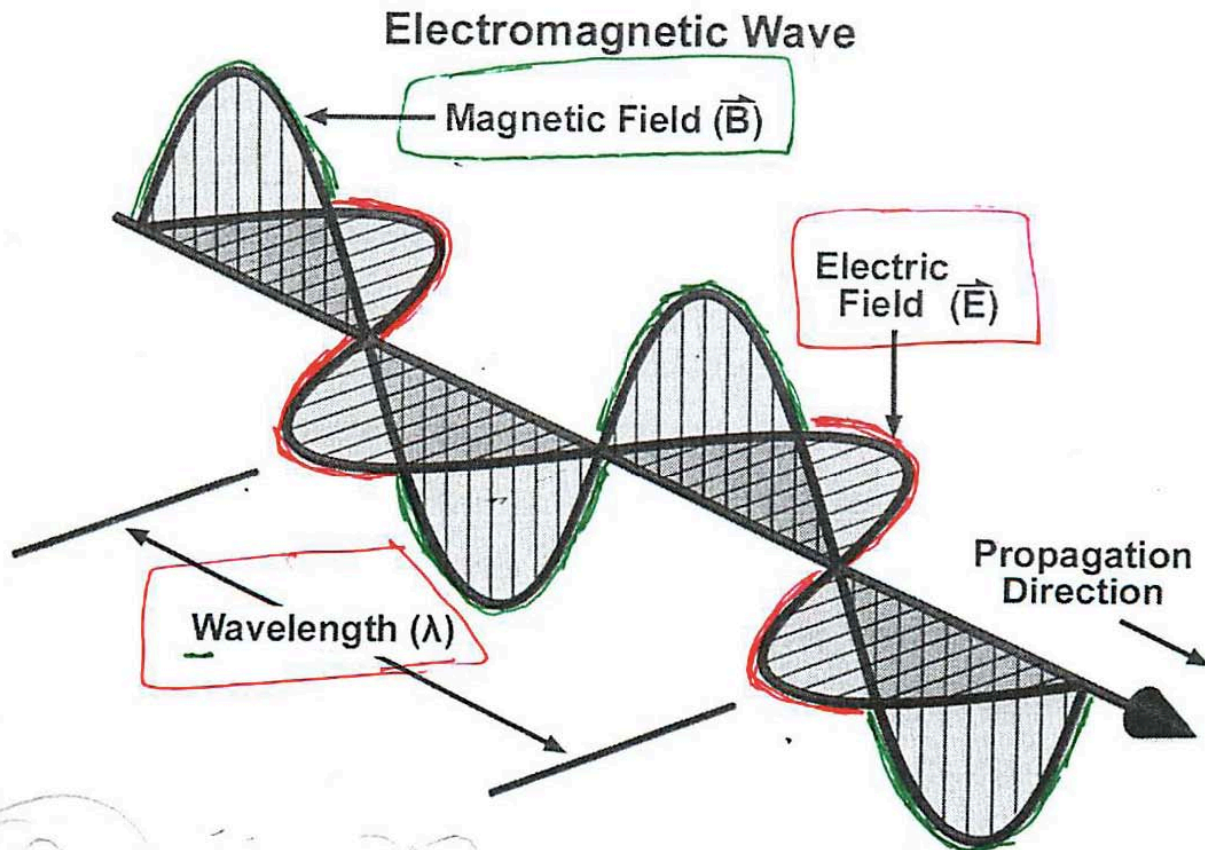
There is a "whole world of light outside our range of vision."

"Photons constitute 99.9999999 percent of everything."

"Light is made up of intertwined magnetic and electrical fields." Yet, "neither magnetism nor electricity is visible to our eyes."

All light consists of a dual wave:
 a magnetic pulse accompanied by an electric pulse positioned at a ninety-degree angle to it. Therefore, light is an electromagnetic phenomenon.

Light is a "manifestation of magnetism and electricity." Light is the unification of magnetic and electric fields.



All light consists of a dual wave. A magnetic pulse is accompanied by an electric pulse positioned at a ninety-degree angle to it. (*Molecular Expressions at*

Light Numbers

Healing is linked to photonic energy (E).

The higher the photonic energy the greater the healing.

All light has a wavelength, distance from crest to crest.
"Wavelength is the distance from one peak of a wave's electrical field to the next peak."

All light has a frequency, the period of time it takes the wave to pass you and to be replaced by the next wave. Frequency is the "number of wavelengths that pass you every second."

$$(\text{Wavelength}) \times (\text{Frequency}) = 186,282 \text{ miles/second}$$

$$(\text{WL}) \times (\text{F}) = 186,282 \text{ miles/second}$$

$$F = \frac{186,282}{\text{WL}}$$

Plank's Constant (Nobel Prize Physics 1918, Max Plank) = PC

$$E = \frac{(\text{PC}) \times (186,282)}{\text{WL}}$$

$$E = (\text{PC}) \times (\text{F})$$

Color Wavelength Frequencies

405 nm	535 nm	635 nm	800 nm	Micro	Radio
Violet	Green	Red	Infrared	In - Ft	Ft - Miles
Quadrillion	Trillions	Trillions	Billions	Millions	Thousands
				VGCIC	
				VDR	

Laser Basics

Laser
is

MONOCHROMATIC

This means it is of a single wavelength.

Background environmental light is a combination
of all wavelengths.

Laser eliminates all wavelengths but one.

Laser
is

COHERENT

This means that the light is not scattered.

All of the light is brought to a single point.

An Overview of Laser Principle, Laser-Tissue Interaction Mechanisms and Laser Safety Precautions for Medical Laser Users

International Journal of Pharmacology
2011; Vol. 7; No. 2; pp 149-160

Laser can now be regarded as practical and economical tool with unique properties that has been utilized effectively in several applications.

Medical, dental, biological and various chemical and physical investigations use lasers due to their advantages. The medical laser systems offer the physician and the dentist not only a window, but also a door into this high technology which facilitate a lot of clinical and therapeutic applications.

LASER is an acronym for Light Amplification by Stimulated Emission of Radiation.

The history of laser development should be traced as far back as 1917 when Albert Einstein showed that the process of stimulated emission must exist.

Coherence means the electromagnetic rays of light are in phase with each other. "Coherence is the most fundamental property of laser and distinguishes it from the light from other sources." "A laser may be defined as a source of coherent light."

Monochrome means that all the photons have the same wavelength.

- This contrasts greatly with a typical incandescent light bulb which emits wavelengths through the entire visible spectrum.

"Wavelength is the most important determinant in how light affects tissue."

- "Wavelength determines the quality of type of interaction between the laser and the tissue."

"In biological tissue, either water molecules or macromolecules such as proteins and pigments mainly cause absorption."

- "The absorption of infrared light can be attributed to water molecules."
- UV and visible light are absorbed by proteins and pigments.
- The absorbed portion of the laser radiation can produce ***photochemical*** (visible light) and/or ***photothermal*** (infrared wavelengths) effects.

Photothermal:

- "As the temperature is raised, the large, specially configured molecules necessary for life are shaken open."
- Most proteins, DNA, RNA, membranes and their integral structures start to unwind or melt at temperatures beginning at 40° C (104°F).
 - "The result is denaturation or loss of function."

Photochemical:

- "Photochemical reactions generally do not result in a significant rise in temperature."
- "Photochemical interaction mechanisms take place at very low power densities (typically, 1 W cm²)."
- "In most cases, wavelengths in the visible range are used because of their high optical penetration depths."
- **Biostimulation** using light is a photochemical effect.
 - The photochemical—biostimulation process uses "low energy, low light, soft, cold laser and low intensity, low power therapy with the application of monochromatic red-light energy close to infrared wavelengths to stimulate growth factor cells and improve wound/soft tissue healing."
 - "To many scientists and clinicians, the idea is that low intensity light energy can promote and upgrade metabolic processes that result in tissue repair and pain relief which is unbelievable."
 - "Reports from almost every region of the world indicate that low intensity lasers promote the repair process of skin, tendons, ligaments, bone and cartilage in experimental animals as well as wounds from various etiologies in humans."

BIPHASIC DOSE RESPONSE IN LOW LEVEL LIGHT THERAPY

Ying-Ying Huang □ Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, **Harvard** Medical School, Boston, MA; Aesthetic and Plastic Center of Guangxi Medical University, Nanning, P.R. China

Aaron C.-H. Chen □ Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; **Boston University** School of Medicine, Graduate Medical Sciences, Boston, MA

James D. Carroll □ THOR Photomedicine Ltd, 18A East Street, Chesham, HP5 1HQ, UK

Michael R. Hamblin □ Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, **Harvard** Medical School, Boston, MA; Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA

□ The use of low levels of visible or near infrared light for reducing pain, inflammation and edema, promoting healing of wounds, deeper tissues and nerves, and preventing cell death and tissue damage has been known for over forty years since the invention of lasers. Despite many reports of positive findings from experiments conducted in vitro, in animal models and in randomized controlled clinical trials, LLLT remains controversial in mainstream medicine. The biochemical mechanisms underlying the positive effects are incompletely understood, and the complexity of rationally choosing amongst a large number of illumination parameters such as wavelength, fluence, power density, pulse structure and treatment timing has led to the publication of a number of negative studies as well as many positive ones. A biphasic dose response has been frequently observed where low levels of light have a much better effect on stimulating and repairing tissues than higher levels of light. The so-called **Arndt-Schulz curve** is frequently used to describe this biphasic dose response. This review will cover the molecular and cellular mechanisms in LLLT, and describe some of our recent results in vitro and in vivo that provide scientific explanations for this biphasic dose response.

1. INTRODUCTION

1.1. Brief history

Low level laser therapy (LLLT) is the application of light (usually a low power laser or LED in the range of **1mW – 500mW**) to a pathology to **promote tissue regeneration, reduce inflammation and relieve pain**. The light is typically of narrow spectral width in the red or near infrared

Address correspondence to Professor Michael R. Hamblin, BAR 414, Wellman Center for Photomedicine, Massachusetts General Hospital, 40 Blossom Street, Boston, MA 02114; Phone: 617-726-6182, Fax: 617-726-8566, E-mail: hamblin@helix.mgh.harvard.edu

(NIR) spectrum (600nm – 1000nm), with a power density (irradiance) between 1mw-5W/cm². It is typically applied to the injury for a minute or so, a few times a week for several weeks. Unlike other medical laser procedures, LLLT is not an ablative or thermal mechanism, but rather a photochemical effect comparable to photosynthesis in plants whereby the light is absorbed and exerts a chemical change.

The phenomenon was first published by Endre Mester at Semmelweis University, Budapest, Hungary in 1967 a few years after the first working laser was invented (Mester *et al.* 1967). Mester conducted an experiment to test if laser radiation might cause cancer in mice. He shaved the hair off their backs, divided them into two groups and irradiated one group with a low powered ruby laser (694-nm). The treatment group did not get cancer and to his surprise, the hair grew back more quickly than the untreated group. He called this “Laser Biostimulation”.

1.2. Evidence for effectiveness of LLLT

Since 1967 over 100 phase III, randomized, double-blind, placebo-controlled, clinical trials (RCTs) have been published and supported by over 1,000 laboratory studies investigating the primary mechanisms and the cascade of secondary effects that contribute to a range of local tissue and systemic effects.

RCTs with positive outcomes have been published on pathologies as diverse as osteoarthritis (Bertolucci and Grey 1995; Ozdemir *et al.* 2001; Stelian *et al.* 1992), tendonopathies (Bjordal *et al.* 2006b; Stergioulas *et al.* 2008; Vasseljen *et al.* 1992), wounds (Caetano *et al.* 2009; Gupta *et al.* 1998; Ozelik *et al.* 2008; Schubert *et al.* 2007), back pain (Basford *et al.* 1999), neck pain (Chow *et al.* 2006; Gur *et al.* 2004), muscle fatigue (Leal Junior *et al.* 2008a; Leal Junior *et al.* 2008b), peripheral nerve injuries (Rochkind *et al.* 2007) and strokes (Lampl *et al.* 2007; Zivin *et al.* 2009); nevertheless results have not always been positive. This failure in certain circumstances can be attributed to several factors including dosimetry (inadequate or too much energy delivered, inadequate or too much irradiance, inappropriate pulse structure, irradiation of insufficient area of the pathology), inappropriate anatomical treatment location and concurrent patient medication (such as steroidal and non-steroidal anti-inflammatories which can inhibit healing) (Aimbire *et al.* 2006; Goncalves *et al.* 2007).

1.3. The medicine and the dose

As with other forms of medication, LLLT has its active ingredients or “medicine” (irradiation parameters) and a “dose” (the irradiation time). Table 1 lists the key parameters that define the medicine and Table 2 defines the dose. It is beyond the scope of this paper to exhaustively list and discuss every conceivable aspect of laser radiation or other light

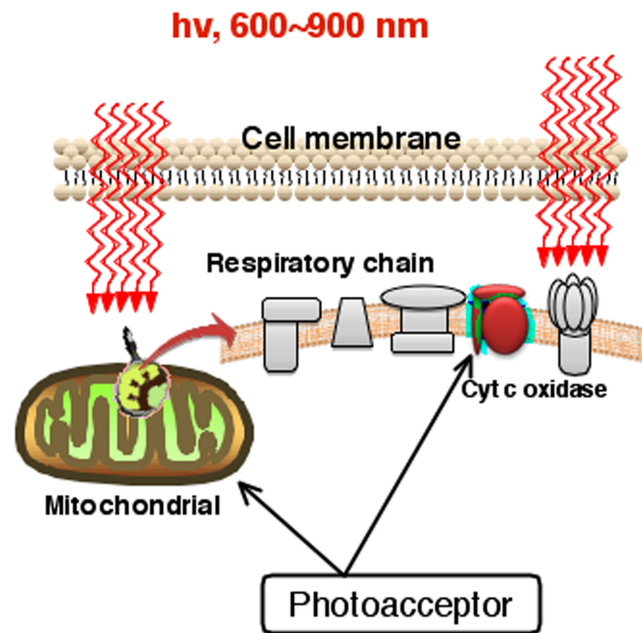


FIGURE 1. Schematic diagram showing the absorption of red and NIR light by specific cellular chromophores or photoacceptors localized in the mitochondrial respiratory chain

tissue penetration of light is maximized. This optical window runs approximately from 650 nm to 1200 nm. (Figure 2). The absorption and scattering of light in tissue are both much higher in the blue region of the spectrum than the red, because the principle tissue chromophores (hemoglobin and melanin) have high absorption bands at shorter wavelengths, tissue scattering of light is higher at shorter wavelengths, and furthermore water strongly absorbs infrared light at wavelengths greater than 1100-nm. Therefore the use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-1100-nm) (Karu and Afanas'eva 1995).

Phototherapy is characterized by its ability to induce photobiological processes in cells. Exact action spectra are needed for determination of photoacceptors as well as for further investigations into cellular mechanisms of phototherapy. The action spectrum shows which specific wavelength of light is most effectively used in a specific chemical reaction (Karu and Kolyakov 2005). The fact that defined action spectra can be constructed for various cellular responses confirms the first law of photobiology described above (light absorption by specific molecular chromophores).

2.3. Mitochondrial Respiration and ATP

Current research about the mechanism of LLLT effects inevitably involves mitochondria. Mitochondria play an important role in energy generation and metabolism. Mitochondria are sometimes described as

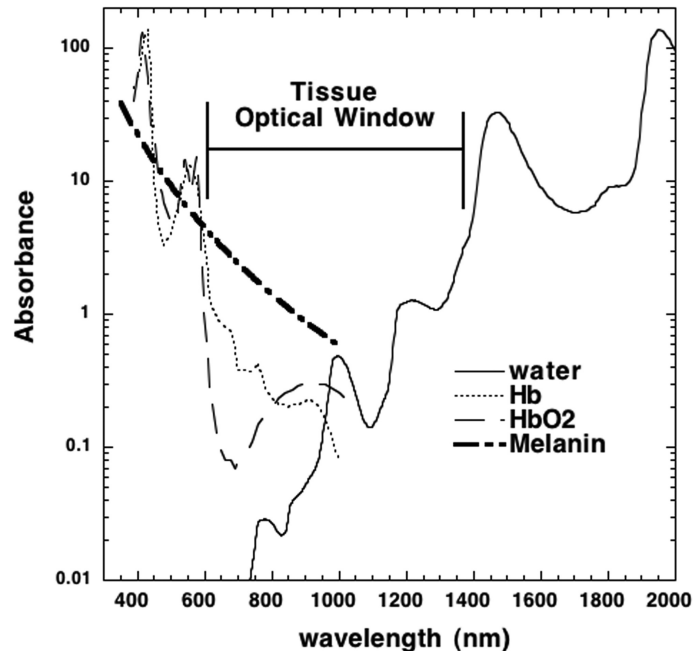


FIGURE 2. Absorption spectra of the main chromophores in living tissue on a log scale showing the optical window where visible and NIR light can penetrate deepest into tissue.

“cellular power plants”, because they convert food molecules into energy in the form of ATP via the process of oxidative phosphorylation (see Figure 3 for an illustration of the mitochondrial respiratory chain).

The mechanism of LLLT at the cellular level has been attributed to the absorption of monochromatic visible and NIR radiation by components of the cellular respiratory chain (Karu 1989). Several pieces of evidence suggest that mitochondria are responsible for the cellular response to red visible and NIR light. The effects of HeNe laser and other illumination on mitochondria isolated from rat liver, have included increased proton electrochemical potential, **more ATP synthesis** (Passarella *et al.* 1984), increased RNA and **protein synthesis** (Greco *et al.* 1989) and increases in oxygen consumption, membrane potential, and enhanced synthesis of NADH and ATP.

2.4. Cytochrome c oxidase and nitric oxide release

Absorption spectra obtained for **cytochrome c oxidase** (Cox) in different oxidation states were recorded and found to be very similar to the action spectra for biological responses to light (Karu and Kolyakov 2005). Therefore it was proposed that Cox **is the primary photoacceptor** for the red-NIR range in mammalian cells (Karu and Kolyakov 2005).

Nitric oxide produced in the mitochondria can inhibit respiration by binding to Cox and competitively displacing oxygen, especially in stressed

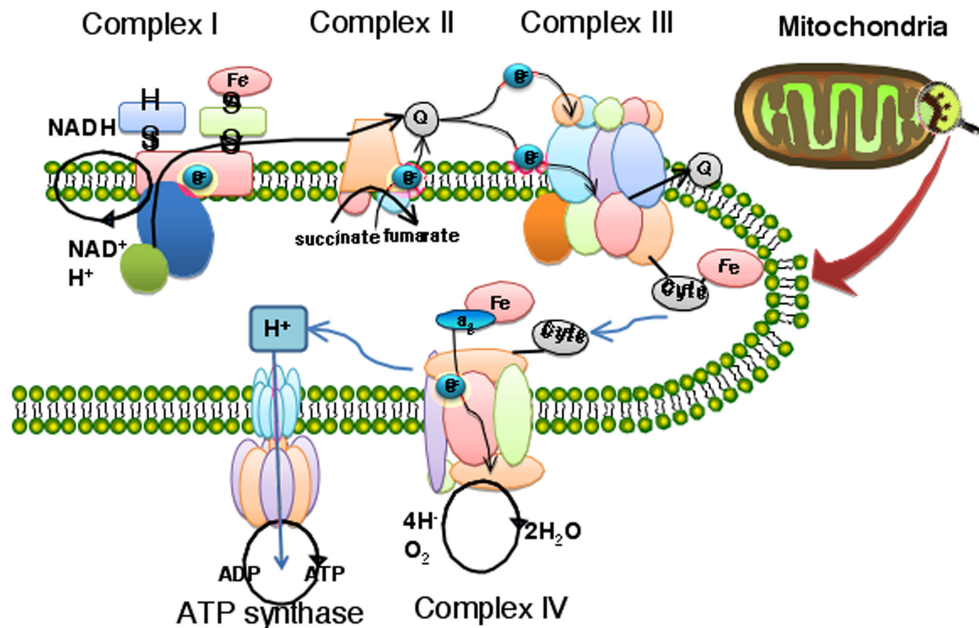


FIGURE 3. Mitochondrial respiratory chain consisting of contains five complexes of integral membrane proteins: NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), **cytochrome c oxidase (Complex IV)**, and ATP synthase (ComplexV).

or hypoxic cells (Brown 2001). Increased nitric oxide (NO) concentrations can sometimes be measured in cell culture or in animals after LLLT due to its photo release from the mitochondria and Cox. It has been proposed that **LLLT might work by photodissociating NO from Cox, thereby reversing the mitochondrial inhibition of respiration due to excessive NO binding** (Lane 2006). Figure 4 illustrates the photodissociation of NO from its binding sites on the heme iron and copper centers where it cometively inhibits oxygen binding and reduces necessary enzymic activity, thus allowing an immediate influx of oxygen and resumption of respiration and generation of reactive oxygen species.

2.5. NO signaling

In addition to NO being photodissociated from Cox as described, it may also be photo-released from other intracellular stores such as nitrosylated hemoglobin and nitrosylated myoglobin (Shiva and Gladwin 2009). Light mediated vasodilation was first described in 1968 by R F Furchgott, in his nitric oxide research that lead to his receipt of a Nobel Prize thirty years later in 1998 (Mitka 1998). Later studies conducted by other researchers confirmed and extended Furchgott's early work and demonstrated the ability of light to influence the localized production or release of NO and stimulate vasodilation through the effect NO on cyclic guanine

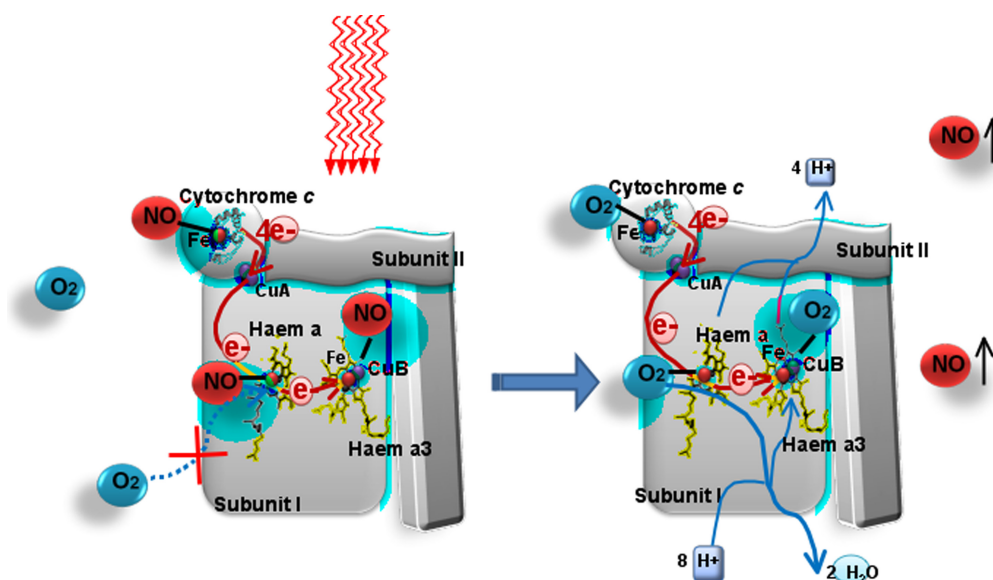


FIGURE 4. When NO is released from its binding to heme iron and copper centers in cytochrome c oxidase by the action of light, oxygen is allowed to rebind to these sites and respiration is restored to its former level leading to increased ATP synthesis.

monophosphate (cGMP). This finding suggested that properly designed illumination devices may be effective, noninvasive therapeutic agents for patients who would benefit from increased localized NO availability

2.6. Reactive oxygen species and gene transcription

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in the signaling pathways from mitochondria to nuclei. Reactive oxygen species (ROS) are very small molecules that include oxygen ions such as superoxide, free radicals such as hydroxyl radical, and hydrogen peroxide, and organic peroxides. They are highly with biological molecules such as proteins, nucleic acids and unsaturated lipids. ROS form as a natural by-product of the normal metabolism of oxygen and have important roles in cell signaling (Storz 2007), regulating nucleic acid synthesis, protein synthesis, enzyme activation and cell cycle progression (Brondon *et al.* 2005). LLLT was reported to produce a shift in overall cell redox potential in the direction of greater oxidation (Karu 1999) and increased ROS generation and cell redox activity have been demonstrated (Alexandratou *et al.* 2002; Chen *et al.* 2009b; Grossman *et al.* 1998; Lavi *et al.* 2003; Lubart *et al.* 2005; Pal *et al.* 2007; Zhang *et al.* 2008). These cytosolic responses may in turn induce transcriptional changes. Several transcription factors are regulated by changes in cellular redox state. But the most important one is nuclear factor B (NF- κ B). Figure 5 illustrates the effect of redox-sensitive transcription factor NF- κ B

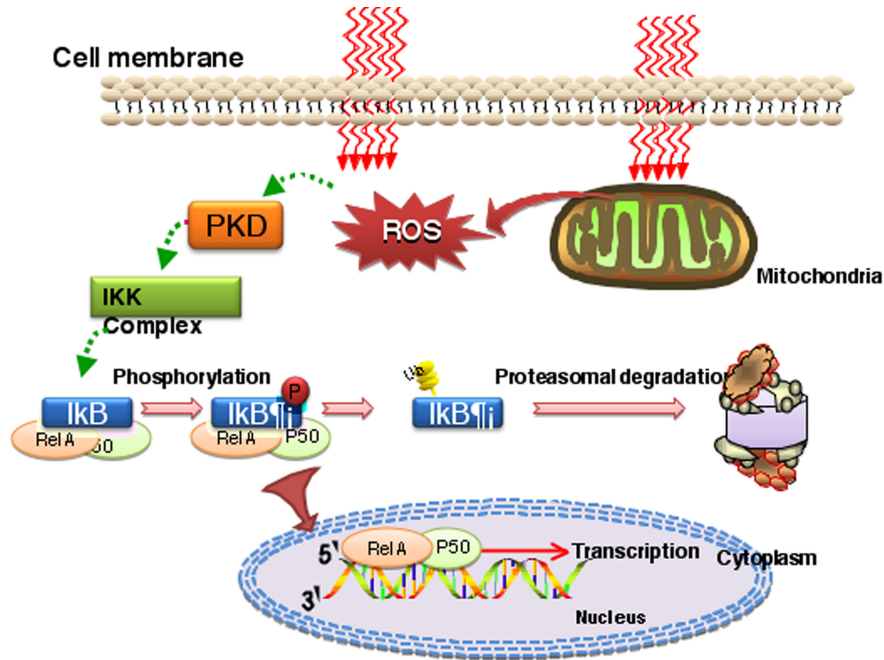


FIGURE 5. Reactive oxygen species (ROS) formed as a result of LLLT effects in mitochondria may activate the redox-sensitive transcription factor NF-κB (relA-p50) via protein kinase D (PKD).

activated after LLLT and is instrumental in causing transcription of protective and stimulatory gene products.

2.7. Downstream cellular response

Although the underlying mechanism of LLLT are still not completely understood, in vitro studies, animal experiments and clinical studies have all tended to indicate that LLLT delivered at low doses may produce a better result when compared to the same light delivered at high doses. LLLT can prevent cell apoptosis and improve cell proliferation, migration and adhesion at low levels of red/NIR light illumination (see Figure 6).

LLLT at low doses has been shown to enhance cell proliferation in vitro in several types of cells: fibroblasts (Lubart *et al.* 1992; Yu *et al.* 1994), keratinocytes (Grossman *et al.* 1998), endothelial cells (Moore *et al.* 2005), and lymphocytes (Agaiby *et al.* 2000; Stadler *et al.* 2000). The mechanism of proliferation was proposed to involve photostimulatory effects in mitochondria processes, which enhanced growth factor release, and ultimately led to cell proliferation (Bjordal *et al.* 2007). Kreisler *et al.* showed (Kreisler *et al.* 2003) that the attachment and proliferation of human gingival fibroblasts were enhanced by LLLT in a dose-dependent manner. LLLT modulated matrix metalloproteinase activity and gene expression in porcine aortic smooth muscle cells (Gavish *et al.* 2006). Shefer *et al.* showed (Shefer *et al.* 2002) that LLLT could activate skeletal

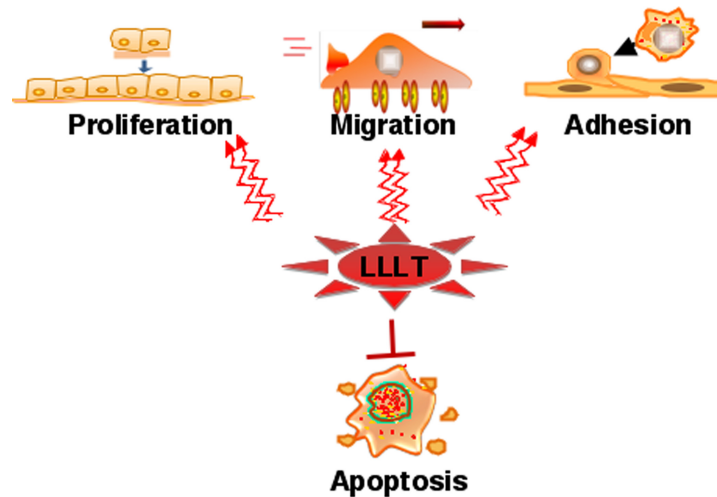


FIGURE 6. The downstream cellular effects of LLLT signaling include increases in cell proliferation, migration and adhesion molecules. Cell survival is increased and cell death reduced by expression of proteins that inhibit apoptosis.

muscle satellite cells, enhancing their proliferation, inhibiting differentiation and regulating protein synthesis.

2.8. Downstream tissue response

There have been a large number of both animal model and clinical studies that demonstrated highly beneficial LLLT effects on a variety of diseases, injuries, and has been widely used in both chronic and acute conditions (see Figure 7). LLLT may enhance neovascularisation, promote angiogenesis and increase collagen synthesis to promote healing of acute (Hopkins *et al.* 2004) and chronic wounds (Yu *et al.* 1997). LLLT provided acceleration of cutaneous wound healing in rats with a biphasic dose response favoring lower doses (Corazza *et al.* 2007). LLLT can also stimulate healing of deeper structures such as nerves (Gigo-Benato *et al.* 2004), tendons (Fillipin *et al.* 2005), cartilage (Morrone *et al.* 2000), bones (Weber *et al.* 2006) and even internal organs (Shao *et al.* 2005). LLLT can reduce pain (Bjordal *et al.* 2006a), inflammation (Bjordal *et al.* 2006b) and swelling (Carati *et al.* 2003) caused by injuries, degenerative diseases or autoimmune diseases. Oron reported beneficial effect of LLLT on repair processes after injury or ischemia in skeletal and heart muscles in multiple animal models in vivo (Ad and Oron 2001; Oron *et al.* 2001a; Oron *et al.* 2001b; Yaakobi *et al.* 2001). LLLT has been used to mitigate damage after strokes (in both animals (Lapchak *et al.* 2008) and humans (Lampl *et al.* 2007)), after traumatic brain injury (Oron *et al.* 2007) and after spinal cord injury (Wu *et al.* 2009).

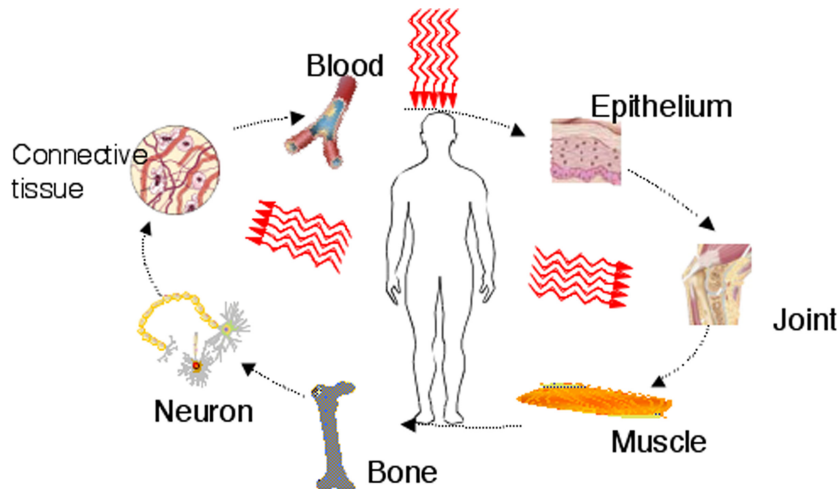


FIGURE 7. Beneficial tissue effects of LLLT can include almost all the tissues and organs of the body.

3. REVIEW OF BIPHASIC DOSE RESPONSES IN LLLT

3.1. Dose dependence and dose rate effects—the biphasic curve

A biphasic response has been demonstrated many times in LLLT research (Lanzafame *et al.* 2007; Oron *et al.* 2001a) and the “Arndt-Schulz Law” is frequently quoted as a suitable model to describe dose dependent effects of LLLT (Chow *et al.* 2006; Hawkins and Abrahamse 2006a; Hawkins and Abrahamse 2006b; Lubart *et al.* 2006; Sommer *et al.* 2001). The concept of the Arndt-Schulz Law dates from the years around the end of the nineteenth century, when H. Schulz published a series of papers that examined the activity of various kinds of poisons (iodine, bromine, mercuric chloride, arsenious acid, etc.) on yeast, showing that almost all these agents have a slightly stimulatory effect on the yeast metabolism when given in low doses (Schulz 1877; Schulz 1888). He then came into contact with the psychiatrist R. Arndt and together they developed a principle that later became known as the ‘Arndt-Schulz law’, stating that weak stimuli slightly accelerate vital activity, stronger stimuli raise it further, but a peak is reached and even stronger stimuli suppress it, until a negative response is finally achieved (Martius 1923). In 1960 Townsend and Luckey surveyed the field of classic medical pharmacology and published a list of 100 substances known to be capable of causing an inhibition at high concentrations and stimulation at low concentrations and termed the phenomenon “hormoligosis” (Townsend and Luckey 1960). The modern term “hormesis” was first used by Stebbing in 1982 (Stebbing 1982) and has been thoroughly reviewed by Calabrese (Calabrese 2001b; Calabrese 2002; Calabrese 2004a; Calabrese 2004b; Calabrese 2005).

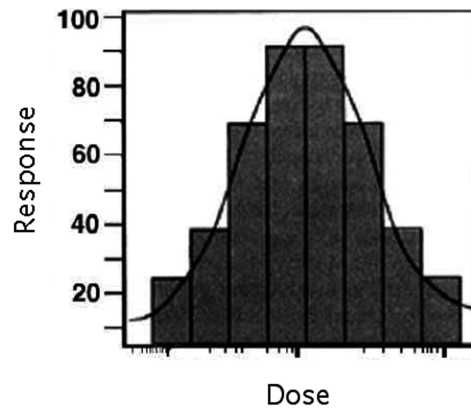


FIGURE 8. Idealized biphasic dose response curve (often termed Arndt-Schulz curve) typically reported in LLLT studies.

In the context of LLLT the increasing “stimulus” may be irradiation time or increased irradiance. This non-linear effect contradicts the Bunsen-Roscoe rule of reciprocity (which was originally formulated for visual detection of light by photoreceptors (Brindley 1952)), which predicts that if the products of exposure time in seconds and irradiance in mW/cm^2 are equal, i.e. the energy density is the same, then the changes in biological endpoint will be equal. This inverse linear relationship between irradiance and time has frequently failed in LLLT research (Karu and Kolyakov 2005; Lubart *et al.* 2006).

A “biphasic” curve can be used to illustrate the expected dose response to light at a subcellular, cellular, tissue or clinical level. Simply put, it suggests that if insufficient energy is applied there will be no response (because the minimum threshold has not been met), if more energy is applied then a threshold is crossed and biostimulation is achieved but when too much energy is applied then the stimulation disappears and is replaced by bioinhibition instead. An idealized illustration (Figure 8) similar to that suggested by Sommer (Sommer *et al.* 2001) helps understand the concept.

3.2. Biphasic Response—irradiance

As early as 1978 Endre Mester observed a “threshold phenomenon” after laser irradiation of lymphocytes in vitro (Mester *et al.* 1978). Peter Bolton in 1991 irradiating macrophages with two different irradiances (W/cm^2) but the same energy density (J/cm^2) recorded different results (Bolton *et al.* 1991). Karu (Karu and Kolyakov 2005) found a dependence of stimulation of DNA synthesis rate on light intensity at a constant energy density $0.1 \text{ J}/\text{cm}^2$ with a clear maximum at $0.8 \text{ mW}/\text{cm}^2$. In another study (Karu *et al.* 1997) the same group found no less than seven maxima in the dose vs. biological effect curves using a pulsed 810-nm diode laser.

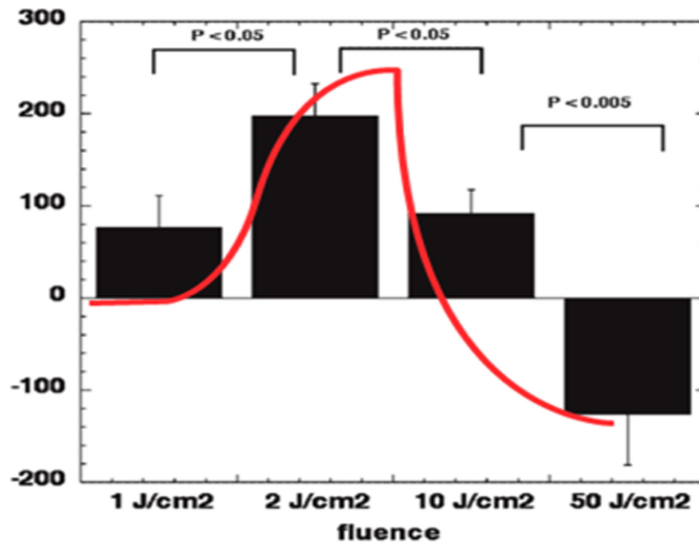


FIGURE 11. Biphasic dose response in measured difference in integrated area under the curve of time course of wound size compared to no treatment control, with a clear maximum seen at 2 J/cm², and the high dose of 50 J/cm² gave a worsening of the wound healing time curve.

sic dose response with positive effects (difference in integrated area under the curve of time course of wound size compared to no treatment control) seen in low doses with a clear maximum seen at 2 J/cm², and the high dose of 50 J/cm² actually gave a worsening of the wound healing time curve i.e. there was a greater expansion of the wound compared with non-treated controls.

4.3. Rat arthritis

In another in vivo study (Castano *et al.* 2007) we investigated whether LLLT using an 810-nm laser could have a therapeutic effect in a rat model of inflammatory arthritis caused by zymosan injected into their knee joints. In this model the severity of the arthritis is quantified by measuring the diameter of the swollen joint every day and plotting a time course for each joint. We compared illumination regimens consisting of a high and low fluence (3 and 30 J/cm²), delivered at high and low irradiance (5 and 50 mW/cm²) using 810-nm laser light daily for 5 days, with the positive control of conventional corticosteroid (dexamethasone) therapy.

As shown in Figure 12 three of the illumination regimens were effective in reducing the mean integrated knee swelling almost as much as the positive control of the powerful steroid, dexamethasone; these were 3 J/cm² delivered at 5 mW/cm² and 30 J/cm² delivered at 50 mW/cm² both of which took 10 minutes, and 30 J/cm² delivered at 5 mW/cm² which took 100 minutes. The only ineffective dose regimen was 3 J/cm²

ROS mediated activation of NF- κ B in MEF cells presented in 4.1 provides additional support for this hypothesis (Chen *et al.* 2009a). It is well-accepted that ROS can have both beneficial and harmful effects (Huang and Zheng 2006). Hydrogen peroxide is often used to kill cells in vitro (Imlay 2008). Other ROS such as singlet oxygen (Klotz *et al.* 2003) and hydroxyl radicals (Pryor *et al.* 2006) are thought to be harmful even at low concentrations. The concept of biphasic dose response in fact is well established in the field of oxidative stress (Day and Suzuki 2005). If the generation of ROS can be shown to be dose dependent on the delivered energy fluence this may provide an explanation for the stimulation and inhibition observed with low and high light fluences.

5.2. Excessive NO

The other mechanistic hypothesis that is put forward to explain the cellular effects of LLLT relates to the photolysis of nitrosylated proteins that releases free NO (see section 2.6). Again the literature has many papers that discuss the so-called two-faced or “Janus” molecule NO (Anggard 1994; Lane and Gross 1999). NO can be either protective or harmful depending on the dose and particularly on the cell or tissue type where it is generated (Calabrese 2001a).

5.3. Activation of a cytotoxic pathway

The third hypothesis to explain the biphasic dose response of LLLT is the idea that the protective and stimulatory effects of light occur at low doses, but there is an additional pathway that leads to damaging effects of light that only occurs at high doses, and effectively overwhelms the beneficial effects of low doses of light. Work from South China Normal University provides some support for this hypothesis. Low doses of LLLT were found to phosphorylate hepatocyte growth factor receptor (c-Met), and initiate signaling via cyclic AMP and Jun kinase and Src (Gao and Xing 2009). By contrast, high dose LLLT was found to induce apoptosis via a mitochondrial caspase-3 pathway and cytochrome c release was attributed to opening of the mitochondrial permeability transition pore caused by high-level intracellular reactive oxygen species (ROS) generation (Wu *et al.* 2009). A secondary signaling pathway through Bax activation was observed (Wu *et al.* 2009).

6. SUMMARY AND CONCLUSION

LLLT delivered at low doses tends to work better than the same wavelength delivered at high levels, which illustrates the basic concept of biphasic dose response or hormesis (Calabrese 2001b). In general, fluences of red or NIR as low as 3 or 5 J/cm² will be beneficial in vivo, but a large dose

like 50 or 100 J/cm² will lose the beneficial effect and may even become detrimental. The molecular and cellular mechanisms LLLT suggest that photons are absorbed by the mitochondria; they stimulate more ATP production and low levels of ROS, which then activates transcription factors, such as NF- κ B, to induce many gene transcript products responsible for the beneficial effects of LLLT. ROS are well known to stimulate cellular proliferation of low levels, but inhibit proliferation and kill cells at high levels. Nitric oxide is also involved in LLLT, and may be photo-released from its binding sites in the respiratory chain and elsewhere. It is possible that NO release in low amounts by low dose light may be beneficial, while high levels released by high dose LLLT may be damaging. The third possibility is that LLLT may activate transcription factors, upregulating protective proteins which are anti-apoptotic, and generally promote cell survival. In contrast, it is entirely possible that different transcription factors and cell-signaling pathways, that promote apoptosis, could be activated after higher light exposure. We believe that further advances in the mechanistic understanding of LLLT will continue to be made in the near future. These advances will lead to greater acceptance of LLLT in mainstream medicine and may lead to LLLT being used for serious diseases such as stroke, heart attack and degenerative brain diseases. Nevertheless the concept of biphasic dose response or LLLT hormesis (low levels of light are good for you, while high levels are bad for you) will remain.

ACKNOWLEDGMENTS

Research in the Hamblin laboratory is supported by the US NIH (grants R01CA/AI838801 and R01AI050875 to MRH).

REFERENCES

- Ad N and Oron U. 2001. Impact of low level laser irradiation on infarct size in the rat following myocardial infarction. *Int J Cardiol* 80:109-16.
- Agaiby AD, Ghali LR, Wilson R, and Dyson M. 2000. Laser modulation of angiogenic factor production by T-lymphocytes. *Lasers Surg Med* 26:357-63.
- Aimbire F, Albertini R, Pacheco MT, Castro-Faria-Neto HC, Leonardo PS, Iversen VV, Lopes-Martins RA, and Bjordal JM. 2006. Low-level laser therapy induces dose-dependent reduction of TNF α levels in acute inflammation. *Photomed Laser Surg* 24:33-7.
- al-Watban FA and Andres BL. 2001. The effect of He-Ne laser (632.8 nm) and Solcoseryl in vitro. *Lasers Med Sci* 16:267-75.
- Alexandratou E, Yova D, Handris P, Kletsas D, and Loukas S. 2002. Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy. *Photochem Photobiol Sci* 1:547-52.
- Anders J, Romanczyk T, Moges H, Ilev I, Waynant R, and Longo L. 2007. Light Interaction With Human Central Nervous System Progenitor Cells. NAALT conference proceedings. 2007
- Anggard E. 1994. Nitric oxide: mediator, murderer, and medicine. *Lancet* 343:1199-206.
- Basford JR, Sheffield CG, and Harmsen WS. 1999. Laser therapy: a randomized, controlled trial of the effects of low-intensity Nd:YAG laser irradiation on musculoskeletal back pain. *Arch Phys Med Rehabil* 80:647-52.
- Bertolucci LE and Grey T. 1995. Clinical analysis of mid-laser versus placebo treatment of arthralgic TMJ degenerative joints. *Cranio* 13:26-9.

Potential for Transcranial Laser or LED Therapy to Treat Stroke, Traumatic Brain Injury, and Neurodegenerative Disease

Margaret A. Naeser, Ph.D., L.Ac.^{1,2} and Michael R. Hamblin, Ph.D.^{3,4,5}

NEAR-INFRARED (NIR) LIGHT PASSES readily through the scalp and skull and a small percentage of incident power density can arrive at the cortical surface in humans.¹ The primary photoreceptors for red and NIR light are mitochondria, and cortical neurons are exceptionally rich in mitochondria. It is likely that brain cells are ideally set up to respond to light therapy. The basic biochemical pathways activated by NIR light, e.g., increased adenosine-5'-triphosphate (ATP) production, and signaling pathways activated by reactive oxygen species, nitric oxide release, and increased cyclic adenosine monophosphate (AMP) all work together to produce beneficial effects in brains whose function has been compromised by ischemia, traumatic injury, or neurodegeneration. One of the main mechanisms of action of transcranial light therapy (TLT) is to prevent neurons from dying, when they have been subjected to some sort of hypoxic, traumatic, or toxic insult. This is probably because of light-mediated upregulation of cytoprotective gene products such as antioxidant enzymes, heat shock proteins, and anti-apoptotic proteins. Light therapy *in vitro* has been shown to protect neurons from death caused by methanol,² cyanide or tetrodotoxin,³ and amyloid beta peptide.⁴

There is also probably a second mechanism operating in TLT; increased neurogenesis. Neurogenesis is the generation of neuronal precursors and birth of new neural cells.⁵ Two key sites for adult neurogenesis include the subventricular zone (SVZ) of the lateral ventricles, and the subgranular layer (SGL) of the dentate gyrus in the hippocampus.⁶ Neurogenesis can be stimulated by physiological factors, such as growth factors and environmental enrichment, and by pathological processes, including ischemia and neurodegeneration.⁷ Adult neurogenesis (in the hippocampus particularly) is now recognized as a major determinant of brain function both in experimental animals and in humans. Neural progenitor cells in their niche in the SGL of the dentate gyrus give birth to newly formed neurons that are thought to play a role in brain function, particularly in olfaction and in hippocampal-dependent learning and

memory.⁸ In small animal models neurogenesis can be readily detected by incorporation of bromodeoxyuridine (BrdU), injected before euthanasia, into proliferating brain cells. Increased neurogenesis after TLT, has been demonstrated in a rat model of stroke,⁹ and in the Hamblin laboratory after TLT for acute traumatic brain injury (TBI) in mice (W. Xuan, T. Ando, et al., unpublished data). These two mechanisms of action of TLT in ameliorating brain damage (prevention of neuronal death and increased neurogenesis) have motivated studies in both animals and humans for diverse brain disorders and diseases. TLT for acute stroke is the most developed,¹⁰ but acute TBI has also been shown to benefit from TLT.¹¹ These areas are reviewed further.

Stroke

In an early study with TLT to treat acute stroke in rats, significant beneficial results were obtained whether TLT was applied in a bilateral, ipsilesional or contralesional manner.¹² TLT (808 nm) significantly improved recovery ($p < 0.01$) at 3 weeks following ischemic stroke when treated once, at 24 h post-stroke (contralesional; power density, 7.5 mW/cm² to brain tissue).⁹ The number of newly formed neuronal cells, assessed by double immunoreactivity to BrdU and tubulin isotype III, as well as migrating cells (doublecortin immunoreactivity), was significantly elevated in the ipsilesional SVZ. There was no significant difference in the stroke lesion area between control and laser-irradiated rats. The authors suggested that an underlying mechanism for the functional benefit post-TLT was possible induction of neurogenesis. Other studies have also suggested that because improvement in neurologic outcome may not be evident for 2–4 weeks in the post-stroke rat model, delayed benefits may be caused, in part, by induction of neurogenesis and migration of neurons.^{13,14}

A recent study with embolized rabbits showed a direct relationship between level of cortical fluence (energy density, J/cm²) delivered, and cortical ATP content.¹⁵ Five minutes following embolization (right carotid), rabbits were exposed

¹VA Boston Healthcare System, West Roxbury, Massachusetts.

²Department of Neurology, Boston University School of Medicine, Boston, Massachusetts.

³Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts.

⁴Department of Dermatology, Harvard Medical School, Boston, Massachusetts.

⁵Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts.

Format: Abstract

Altern Ther Health Med. 2017 Oct 2. pii: AT5647. [Epub ahead of print]

Review of Literature on Low-level Laser Therapy Benefits for Nonpharmacological Pain Control in Chronic Pain and Osteoarthritis.

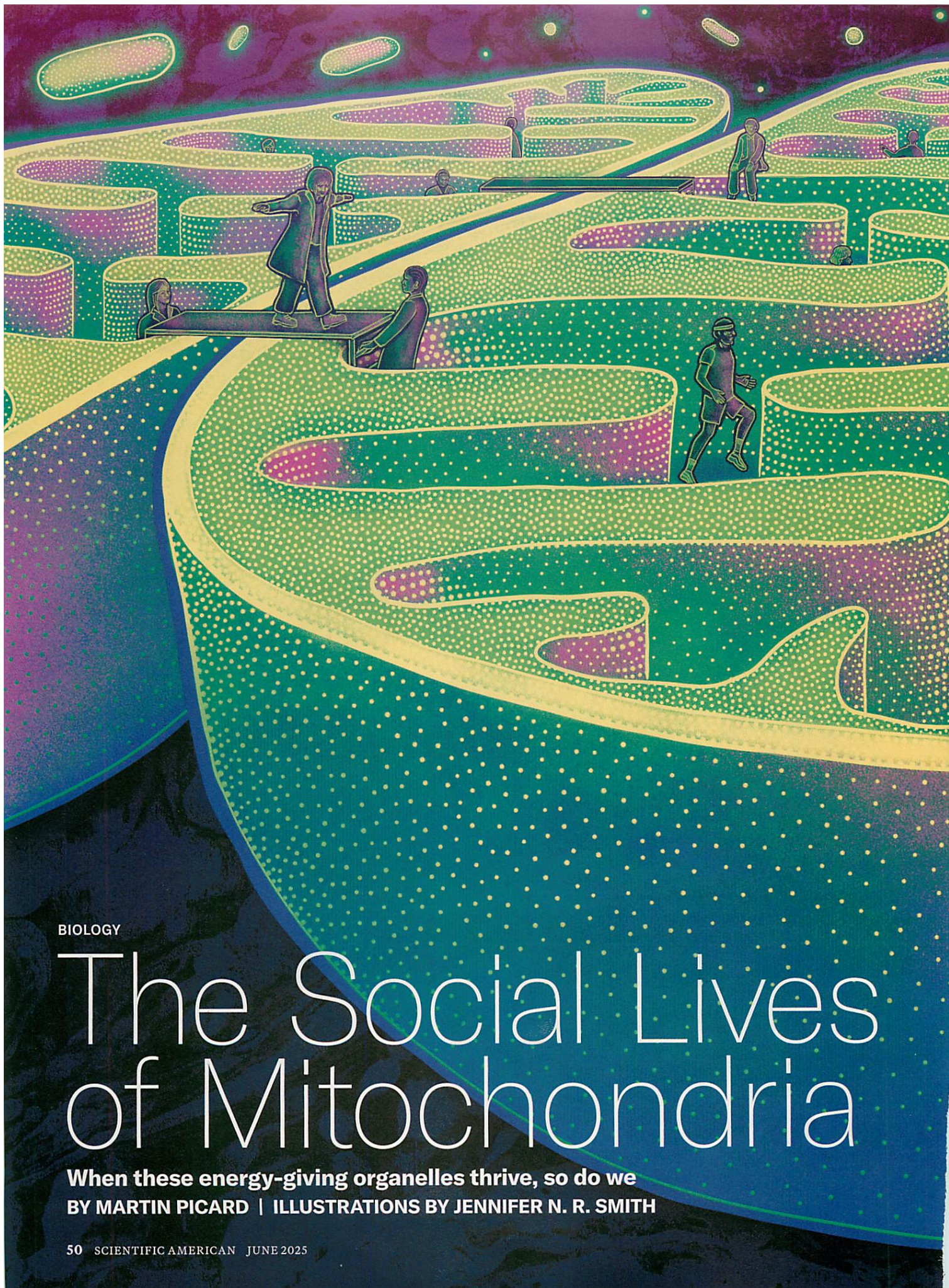
Dima R, Tieppo Francio V, Towery C, Davani S.

Abstract

Introduction • Low-level **laser** therapy (LLLT) is a form of light therapy that triggers biochemical changes within cells. Photons are absorbed by cellular photoreceptors, triggering chemical alterations and potential biochemical benefits to the human body. LLLT has been used in pain management for years and is also known as cold **laser** therapy, which uses low-frequency continuous **laser** of typically 600 to 1000 nm wavelength for pain reduction and healing stimulation. Many studies have demonstrated analgesic and anti-inflammatory effects provided by photobiomodulation in both experimental and clinical trials. **Objective** • The purpose of this research article was to present a summary of the possible pain management benefits of LLLT. **Results** • In cold **laser** therapy, coherent light of wavelength 600 to 1000 nm is applied to an area of concern with hope for photo-stimulating the tissues in a way that promotes and accelerates healing. This is evidenced by the similarity in absorption spectra between oxidized **cytochrome c oxidase** and action spectra from biological responses to light. LLLT, using the properties of coherent light, has been seen to produce pain relief and fibroblastic regeneration in clinical trials and laboratory experiments. LLLT has also been seen to significantly reduce pain in the acute setting; it is proposed that LLLT is able to reduce pain by lowering the level of biochemical markers and oxidative stress, and the formation of edema and hemorrhage. Many studies have demonstrated analgesic and anti-inflammatory effects provided by photobiomodulation in both experimental and clinical trials. **Conclusion** • Based on current research, the utilization of LLLT for pain management and osteoarthritic conditions may be a complementary strategy used in clinical practice to provide symptom management for patients suffering from osteoarthritis and chronic pain.

PMID: 28987080

Mitochondria



BIOLOGY

The Social Lives of Mitochondria

When these energy-giving organelles thrive, so do we
BY MARTIN PICARD | ILLUSTRATIONS BY JENNIFER N. R. SMITH



ALWAYS WANTED TO UNDERSTAND LIFE. What moves us? What allows us to heal and thrive? And what goes wrong when we get sick or when we eventually stop breathing and die? My search for answers to these stupendously ambitious questions led me, it now seems inexorably, to mitochondria.

In biology classes from high school through university, I learned that mitochondria are little objects that reside within each cell and serve as “powerhouses,” combining oxygen and food to yield energy for the body. This idea of mitochondria being little batteries with a built-in charger, about as interesting as the one in my phone, left me unprepared for the vital reality of these organelles when I first saw them under a microscope in 2011. They were luminous because of a glowing dye I had put in them, and they were dynamic—constantly moving, stretching, morphing, touching one another. They were beautiful. That night, a graduate student alone in a dark laboratory in Newcastle upon Tyne in England, I became a mitochondriac: hooked on mitochondria.

A profound insight by biologist Lynn Margulis helped me make some sense of what I was seeing. She postulated in 1967 that mitochondria descend from a bacterium that was engulfed by a larger ancestral cell about 1.5 billion years ago. Instead of consuming this tidbit, the larger cell let it continue living within. Margulis called this event endosymbiosis, which means, roughly, “living or working together from the inside.” The host cell had no energy source that used oxygen—which, thanks to plants, was already abundant in the atmosphere; mitochondria filled this gap. The unlikely union allowed cells to communicate and cooperate and let their awareness expand beyond their own boundaries, enabling a more complex future in the form of multicellular animals. Mitochondria made cells social, binding them in a contract whereby the survival of each cell depends on every other one, and thus made us possible.

Amazingly, my co-workers and I have discovered that mitochondria are themselves social beings. At

least, they foreshadow sociality. Like the bacterium they descended from, they have a life cycle: old ones die out, and new ones are born out of existing ones. Communities of these organelles live within each cell, usually clustered around the nucleus. Mitochondria communicate, both within their own cells and among other cells, reaching out to support one another in times of need and generally helping the community flourish. They produce the heat that keeps our bodies warm. They receive signals about aspects of the environment in which we live, such as air pollution levels and stress triggers, and then integrate this information and emit signals such as molecules that regulate processes within the cell and, indeed, throughout the body.

★ When our mitochondria thrive, so do we. When they malfunction—when, for instance, their ability to change energy into forms required for biochemical reactions is impaired—we may experience conditions as diverse as diabetes, cancer, autism and neurodegenerative disorders. And as mitochondria accumulate defects over a lifetime of stress and other insults, they contribute to aging and, ultimately, death. To understand these processes—to see how to sustain physical and mental health—it helps to understand how energy moves through our bodies and minds. That requires a deeper look into mitochondria and their social lives.

LONG BEFORE I GOT MY FIRST glimpse of mitochondria, I had boned up on the basics of their structure and biology. We inherit our mitochondria from our mother—from the egg cell, to be precise. Mitochondria have their own DNA, which consists of only 37 genes, compared with the thousands of genes in the spiraling chromosomes inside the cell nucleus. This ring of mi-

Martin Picard is an associate professor of behavioral medicine in Columbia University's departments of psychiatry and neurology. He directs the school's Mitochondrial Psychobiology Group, and he holds a chair in energy and health at the Robert N. Butler Columbia Aging Center.

tochondrial DNA, or mtDNA, is sheltered within two membranes. The outer shell, shaped like the skin of a sausage, encases the mitochondrion and selectively allows molecules to enter or exit. The inner membrane is made of densely packed proteins and has many folds, called cristae, which serve as a site for chemical reactions, much like the plates suspended inside a battery.

In the 1960s British biochemists Peter Mitchell and Jennifer Moyle discovered how electrons derived from carbon in food combine with oxygen in the cristae, releasing a spark of energy that is captured as a gradient in electrical voltage across the membrane. This voltage provides the driving force for all processes in the body and brain, from warming to manufacturing molecules to thinking. Mitochondria also produce a molecule called adenosine triphosphate, which serves as a portable unit of energy that powers hundreds of biochemical reactions within each cell.

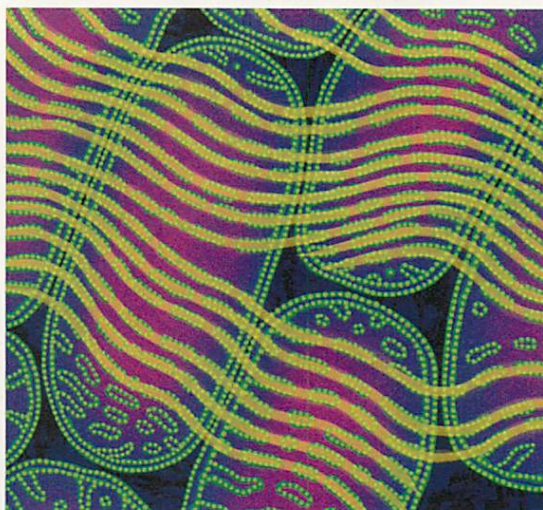
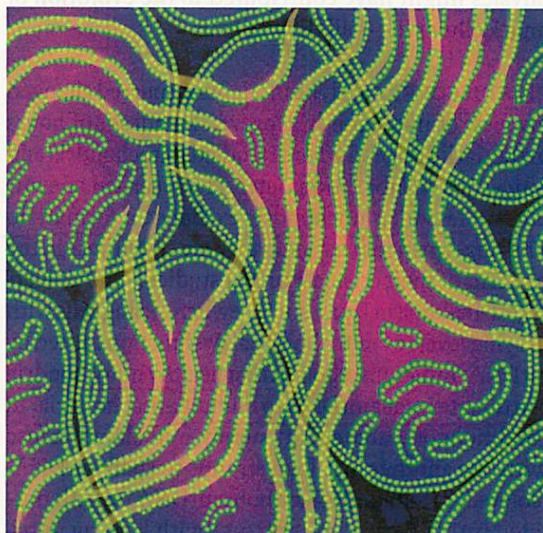
Returning from the U.K., I started a postdoctoral fellowship with geneticist and evolutionary biologist Douglas Wallace at the Center for Mitochondrial and Epigenomic Medicine at Children's Hospital of Philadelphia. In 1988 Wallace had discovered the first-ever link between a mutation in mtDNA and a human disease. He had gone on to map some of mitochondrial biology's fundamental connections to various diseases and the aging process, laying the foundation of the field of mitochondrial medicine. In Philadelphia, I began working with a fellow postdoc, Meagan McManus, who wanted to understand how defective mitochondria could cause cardiovascular and neurological diseases. McManus asked me to photograph with an electron microscope the mitochondria in the hearts of mice with a specific mtDNA mutation that led to heart failure.

Our team was also experimenting with three-dimensional imaging using electron tomography, the same technology that allows a radiologist to see a patient's internal organs in 3D. Weeks later, the director of this project, Dewight Williams of the University of Pennsylvania, brought me to a room where the million-dollar tomography microscope stood, as high as the ceiling, to show me reconstructed movies of mitochondria.

The tomography gave us a 3D view of the cristae. Some mitochondria in the hearts of the sick mice had jagged, highly irregular cristae—the unhealthy look I had been seeing in the 2D pictures. But one thing showed up in 3D that we had never seen in the flat images: even when mitochondria looked unhealthy, their cristae looked healthy at places where the mitochondria touched one another. They were interacting, helping one another's internal organization. These mitomito junctions also had more cristae than any other part of the same mitochondrion. “Meagan has to see this!” I thought, rushing to the lab across campus.

As I restarted the movie for McManus, I narrated what I had seen a few minutes earlier: “Mitochondria are influencing one another!” We watched the looping video a few times. Then McManus said, her voice pitched high with excitement, “And the cristae line up!

The cristae line up between mitochondria!” She drew a line with her extended finger across a junction between mitochondria.

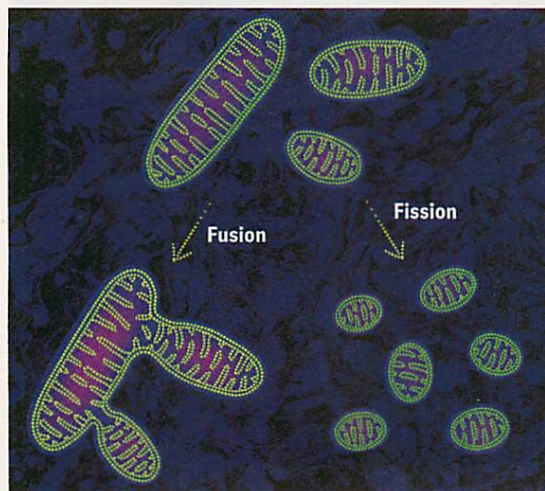


I had pored over thousands of electron microscopy images from the best microscopists. Never had I heard about cristae in one mitochondrion aligning with the cristae of another mitochondrion. While in Newcastle, I had seen a 1983 paper by Russian scientists Lora E. Bakeeva and Vladimir P. Skulachev describing “inter-mitochondrial contacts,” and I had demonstrated that these contacts increased after exercise—perhaps increasing energy efficiency. How had we all missed the alignment? Yet instead of lying there as parallel plates, like textbooks often portrayed them, the cristae formed parallel ribbons undulating across mitochondria. It almost looked like the cristae were helping their neighbors organize to achieve the typical, healthy, regular array.

At the next lab meeting, I suggested that these patterns looked like iron filings aligned around a magnet. Cristae are full of iron-sulfur clusters that may be paramagnetic. If they are, maybe there were electromagnetic fields induced by the flow of electrical charge across the cristae? Could they induce the cristae to line

up? So far this hypothesis appears to be the best one for how cristae align across mitochondria. For me, it also opened the door to thinking about how the forces of physics might have contributed to the evolution of multicellular life—all the way to us.

THIS DISCOVERY and the thoughts it spurred changed my view of mitochondria forever. Hundreds of hours in the dark dungeon where I studied mitochondria and numerous collaborations later, I had learned one important lesson: mitochondria exchange information. The fingerprint of that exchange lay right there in the patterns of their cristae. Further studies at the University of Tsukuba in Japan and elsewhere, using cells with varying levels of mitochondrial dysfunction caused by mtDNA mutations, showed that healthy mitochondria can donate intact mtDNA to mutant mitochondria. In conditions of scarce energy supply, mitochondria fuse with one another into long strands to share mtDNA. Isolated mitochondria without mtDNA or with mutated mtDNA can similarly fuse with healthy mitochondria, restoring their normal function.



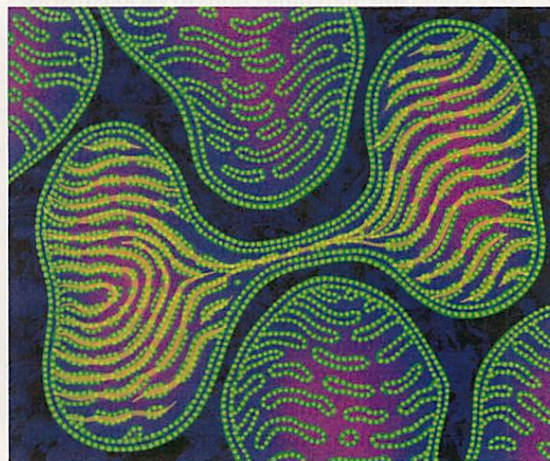
Fusion enhances the resilience not only of mitochondria but also of cells; interfering with these interactions leads to isolated mitochondria that accumulate mtDNA defects and ultimately die, along with the cells they live in. In people, decreased levels of mitofusin 2, a protein located in the outer mitochondrial membrane that helps with fusion, are correlated with neurodegeneration. And mice with mitochondria that have been engineered to impede fusion in the nucleus accumbens, a brain region involved in regulating reward, are more anxious.

Could there be yet other ways in which mitochondria communicate? Could they act like their bacterial ancestors, which build biofilms and use membrane protrusions, electrical fields and secreted molecules to cooperate and conquer the living world with their versatile collective behaviors? Could mitochondrial communication reveal a broader internal universe of energy and information exchange? Could mitochondrial junctions and aligned cristae operate like neu-

ronal synapses, with the resulting mitochondrial collective behaving essentially like an intracellular brain?

IN 2016, SHORTLY AFTER STARTING my own lab at Columbia University, I was back in Newcastle on a visit to Doug Turnbull's Wellcome Center for Mitochondrial Research. I was again sitting at the electron microscope, this time with a stellar British graduate student, Amy Vincent. We were imaging muscle from the calf of a woman with an mtDNA mutation that caused a rare mitochondrial disease. By coincidence, her mutation was similar to the one McManus's mice had had.

What Vincent and I found that afternoon opened another avenue of inquiry. In front of our eyes lay mitochondrial nanotunnels: thin membrane protrusions—the same kind that bacteria use to share their circular DNA! For the first time in humans, Vincent and I saw that mitochondria send thin tubular structures out toward one another, like feelers that some solitary cells use to search for a more hospitable environment or a healthy fellow cell. By imaging dozens of other muscle samples, we found that people whose mitochondria don't work well have more nanotunnels. It was as if unhealthy mitochondria with mtDNA mutations were reaching out for help.



Perhaps the most remarkable aspect of the mitochondrial collective, however, is that mitochondria from different parts of the body talk to one another, using hormones as their language. Mitochondria produce the steroid hormones we use for sustaining and reproducing life. Cortisol, the hormone that increases blood glucose levels to fuel the stress response, is made in the mitochondria of the adrenal glands, which sit on top of the kidneys. Testosterone, estrogen and progesterone are synthesized mainly by mitochondria in the reproductive organs. Interestingly, brain mitochondria have receptors to sense both stress and sex hormones. So we have a population of mitochondria in the adrenal glands that signal directly, via the blood, to mitochondria in the brain.

Further, mitochondria are not all created equal. In the same way that humans develop specialties in different social and economic roles and organs specialize

in executing complementary functions (the liver feeds other organs, the heart pumps, the brain integrates information and issues directives), mitochondria also specialize. Across organs and cell types, mitochondria look different. Their protein contents are different. They move differently. And their ability to sense, integrate and signal specific information varies according to the cell they inhabit. Mitochondrial specialization most likely affords gains in efficiency, allowing an organism to survive at a lower overall energy cost.

My co-workers and I recently built the first map of mitochondria in the human brain. Even within this single organ there are different types of mitochondria in different parts of the cortex and in deeper, subcortical brain regions. The brain uses 20 percent of the body's energy, despite constituting only 2 percent of the body's mass, so an efficient source of power is critical to its functioning. My colleagues, notably, Michel Thiebaut de Schotten of the French National Center for Scientific Research and Eugene V. Mosharov of Columbia, and I found that the more recently evolved brain areas, which have the highest energy expenditure, have mitochondria that are more strongly specialized for energy transformation.

Mitochondria within a cell may also look very different from one another. For example, in neurons, "dendritic" mitochondria are found in the fibers, or dendrites, through which neurons receive signals from other cells. These mitochondria are stable filaments that stretch over 10 to 30 microns—a stupendously long distance for this type of structure—and have several mtDNA copies. "Axonal" mitochondria move along the linear axons, which conduct signals to other neurons, as if they were cellular highways. They are generally short and stubby (up to a micron in length), and many lack mtDNA. "Cytoplasmic" mitochondria cluster around the nucleus and look like something between the dendritic and axonal types. Similar grouping and specialization of mitochondria exists in muscle and fat cells.

These findings, taken together, led behavioral neuroscientist Carmen Sandi of the Swiss Federal Institute of Technology and me to propose in 2021 that mitochondria are social organelles. If you are like me and your eyebrow rises when you hear the term "social" applied to a subcellular organelle, you are having a normal reflex. Nevertheless, Sandi and I argue that mitochondria show all the features of social beings—a shared environment inside the cell or body, communication, formation of groups or types, synchronization of behavior, interdependence, and specialization in the tasks they perform.

In a subsequent paper, which required a painfully long review of more than 400 studies, Orian S. Shirihai of the University of California, Los Angeles, and I established that the mitochondrial collective operates as a mitochondrial information-processing system, or MIPS. Like the animals they exist within and support, which must respond flexibly to the environ-

ment, the mitochondria sense signals, integrate this information in the membrane potential of their cristae, and produce signals that regulate the genes of the cell and shape cell behavior.

Your eyes transform light into electrical impulses that coalesce into an image in your visual field, and your ears transform air-pressure waves into electrical pulses that you eventually perceive as sounds. Likewise, mitochondria transform dozens of hormonal, metabolic, chemical, and other information streams into their electrical membrane potential. This "bioenergetic" state then leads to the production of secondary messenger molecules that are intelligible to the nucleus. So in the same way you read messages on your phone, which receives signals, transforms them and projects decipherable information onto its screen, the nucleus of your cells can "read" the environment through the MIPS that surrounds it.

Rather than having supplementary roles like those of battery chargers, mitochondria are more like the motherboard of the cell. Genes sit inert in the nucleus until energy and the right message come along to turn some of them on and some others off. Mitochondria provide these messages, speaking the language of the epigenome—the malleable layer of regulation that sits on top of the genome to regulate its expression.

My colleague Timothy Shutt of the University of Calgary likes to call mitochondria the "CEO of the cell": the chief executive organelle. This metaphor captures how mitochondria not only are involved in integrating information but also give orders. They dictate whether the cell divides, differentiates or dies. Indeed, mitochondria have a veto on cell life or death. If the MIPS deems it necessary, it triggers programmed cell death, or apoptosis—a form of self-sacrifice for the greater good of the organism.

So vital are mitochondria that in difficult times cells may donate entire mitochondria to other cells. "In cellular emergencies, newly arrived mitochondria might kick-start tissue repair, fire up the immune system or rescue distressed cells from death," journalist Gemma Conroy noted in a *Nature* news story last April. Inside tumors, cancer cells and immune cells appear to compete for mitochondria, using them as a kind of bio-weapon. An international effort I participated in, led by Jonathan R. Brestoff of the Washington University School of Medicine in St. Louis, recently created an entirely new lexicon to guide the emerging field of mitochondria transfer and transplantation.

ALL WELL AND GOOD, you may think. What does all this mean for my health or how long I'm going to live?

The short answer is that it may have everything to do with human health. Diabetes, neurodegenerative conditions, cancer and even mental health illnesses are all emerging as metabolic disorders involving malfunctioning mitochondria. And these findings are indicating new routes for intervention.

Mitochondria drive health—or disease—in several

epigenetics

ways. One route derives from their role as energy processors. In an electrical circuit, if we crank up the input voltage too much, we can blow it out. Similarly, if our cells are exposed to too much glucose or fat—or, worse, both together, causing what doctors refer to as glucolipotoxicity—the mitochondria undergo fission and fragment into little bits, accumulate mtDNA defects, and produce signals that end up prematurely aging or killing the cell. Experiments in cells and in mice have shown that pharmacologically or genetically preventing mitochondrial fission induced by excessive glucose and fats may protect against insulin resistance.

Cancer, too, may be a disorder of cellular metabolism. Cancer cells can burn glucose without oxygen, which suggests either that something is wrong with their mitochondria or that they prefer to reserve mitochondria for use in cell division—and proliferation.

A second pathway is through mitochondria's influence on gene expression. Mitochondrial signals alter the expression of more than 66 percent of genes in the nuclear chromosomes. By changing which genes are expressed and to what extent, mutations in mtDNA may completely alter the nature, behavior and stress resilience of cells and ultimately of the whole organism.

Mitochondria can look terribly odd when sick. In people with mtDNA defects that cause rare mitochondrial diseases, such as the person in whose mitochondria we first saw nanotunnels, the cristae in particular can look somewhat alien—like crop circles with regular angles, paracrystalline inclusions, and other weird shapes.



Notably, abnormal mitochondrial shape and function are emerging as biomarkers and potential causes of cognitive and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and others. Clinically, a neurobiological subtype of autism spectrum disorder involves defects in mitochondrial biology.

A third pathway is inflammation. When cells are injured or stressed, they may leak mtDNA into the cell's interior, the cytoplasm, or even into the blood. Along with Caroline Trumpff of Columbia, Anna

Marsland and Brett Kaufman of the University of Pittsburgh, and other co-workers, I found that mental stress induced by having to speak in public for five minutes increased the amount of free-floating mtDNA in the blood. People in intensive care units who are grievously ill tend to have very high levels of mtDNA in their blood. Because mtDNA rings resemble bacterial DNA, immune cells see them as pathogens and mount an attack that can develop into inflammation. And inflammation, clinicians know well, is linked to the onset and progression of a host of chronic health conditions.

Just how defective mitochondria lead to illness in the body and mind is a question that has yet to be answered. But there are simple ways to ensure our mitochondria stay healthy. One is exercise. When you move vigorously, your cells consume energy rapidly, powering up the membrane potential of your mitochondria. If your exercise leaves you feeling out of breath, it is a sign that your mitochondria are working hard. Because the brain-body entity is expert at anticipating and preparing for the future, if you move in a way that activates your mitochondria, your body thinks, "Next time this happens, I'll be ready!" To get ready, it makes more mitochondria and keeps them working at their best.

Surprisingly, social connections, too, may promote the health of our brain mitochondria. In a major study led by David A. Bennett of Rush Medical College in Chicago, researchers asked hundreds of individuals aged 65 and older in the Chicago area to fill out surveys, take cognitive tests and give blood every year until they died. After death their brains were collected to enable analysis of their mitochondria. My colleague Trumpff used those data to ask whether positive mental states such as feeling purpose in life, optimism and a sense of connectedness—or, in contrast, negative mental states such as perceived stress, depression and social isolation—could be related to the mitochondria's ability to transform energy.

What Trumpff learned was remarkable: the amount of energy-transforming proteins in mitochondria in the prefrontal cortex was significantly correlated with how many positive and negative experiences people reported in the year before they died. This finding aligned with previous studies relating early-life adversity or daily mood markers such as feelings of love, closeness or trust to mitochondria in blood immune cells. Our states of mind might affect the biology of our mitochondria, modulating how well they transform energy.

Another intervention that can be remarkably effective is diet. Medical ketogenic therapy or "nutritional ketosis," which involves cutting out all refined sugars, limiting intake of carbohydrates, and making up the calorie difference with more proteins and fats, has been shown to sustainably reverse insulin resistance and type 2 diabetes. The ketogenic diet has been used for decades to ward off seizures and thereby "stabilize" the brain in children and adults with intractable and otherwise incurable epilepsy. A ketogenic diet can even ameliorate the mental state and cognitive function of people with Alzheimer's. It enhances brain-network

stability, a marker of brain aging—and this function may explain why some people on the diet sleep better.

The ketogenic diet can have astonishing effects in other diseases as well, as evinced by the story of Lauren Kennedy West, a Canadian woman diagnosed with schizophrenia and bipolar disease at the age of 25. Her life progressively felt too difficult to navigate, “like there was no space for me in the world,” she explained in a moving account of her journey posted on YouTube last year. In December 2023 West began nutritional ketogenic therapy. A couple of weeks later, she noticed she had more energy. Many of her symptoms lessened. After about nine months she was symptom-free, had tapered her medication in collaboration with her care team, and continued to feel better. Late in 2024 she took her last dose of antipsychotic medication.

West’s experience parallels initial positive results from a pilot trial of 21 people with bipolar disease and schizophrenia. Numerous other clinical trials of the ketogenic diet for people with severe mental illnesses such as schizophrenia, depression, anxiety and obsessive compulsive disorder are currently in progress around the world. (Many of these trials are by funded by the Baszucki Group, a philanthropic foundation formed after Matt Baszucki, the son of the group’s founders, successfully treated his bipolar disease with the ketogenic diet. In 2024 I received the Baszucki Prize in Science, which helps to fund my lab at Columbia.)

A new study of 28,995 people in the U.S., 4,484 of whom had significant depression symptoms, also supports the protective effects of low-sugar diets on mental health. People whose diet was “more ketogenic”—low in carbohydrates and sugars relative to lipids and proteins—were less than half as likely to develop depression compared with people whose diet was fairly rich in sugars.

How does it work? From a mitocentric perspective, the ketogenic diet does three things. First, it leads to the supply of an efficient fuel source by the liver, which feeds other organs in the body. If you fast or eat a ketogenic diet, your liver takes the fats from your love handles or your food and breaks them down into smaller bits called ketone bodies. This process happens inside the liver’s mitochondria. Second, after entering the blood, ketone bodies reach the organs, some of which, including the brain, prefer ketone bodies over other fuels such as glucose, proteins and fats. So in the presence of various fuel sources, the brain will preferentially burn ketones.

The third thing may have to do with efficiency—and might explain why ketone bodies are the preferred fuel for the brain. Glucose has to traverse a number of hurdles before getting to neuron mitochondria—it detours through astrocytes, crosses several membranes and goes through several enzymatic reactions. In contrast, ketone bodies are taken up directly by the mitochondria in neurons, where they are burned. It’s a far less convoluted path.

So ketosis, or the burning of ketone bodies, may exert its effects on the brain by enabling energy to di-

Rather than being like battery chargers, mitochondria are more like the motherboard of the cell.

rectly flow between mitochondria. Ketones in your blood open a stream of communication between producer and consumer mitochondria, fostering their sociality throughout your body.

ONCE WE REGARD MITOCHONDRIA as dynamic energy and information processors, an entirely new perspective of life emerges. Think of yourself as a waterfall. The waterfall exists only insofar as the water molecules keep flowing down. You learn as much about the waterfall when you scoop up a few inert H_2O molecules as you learn about how healthy a person is by sequencing their genome: close to nothing.

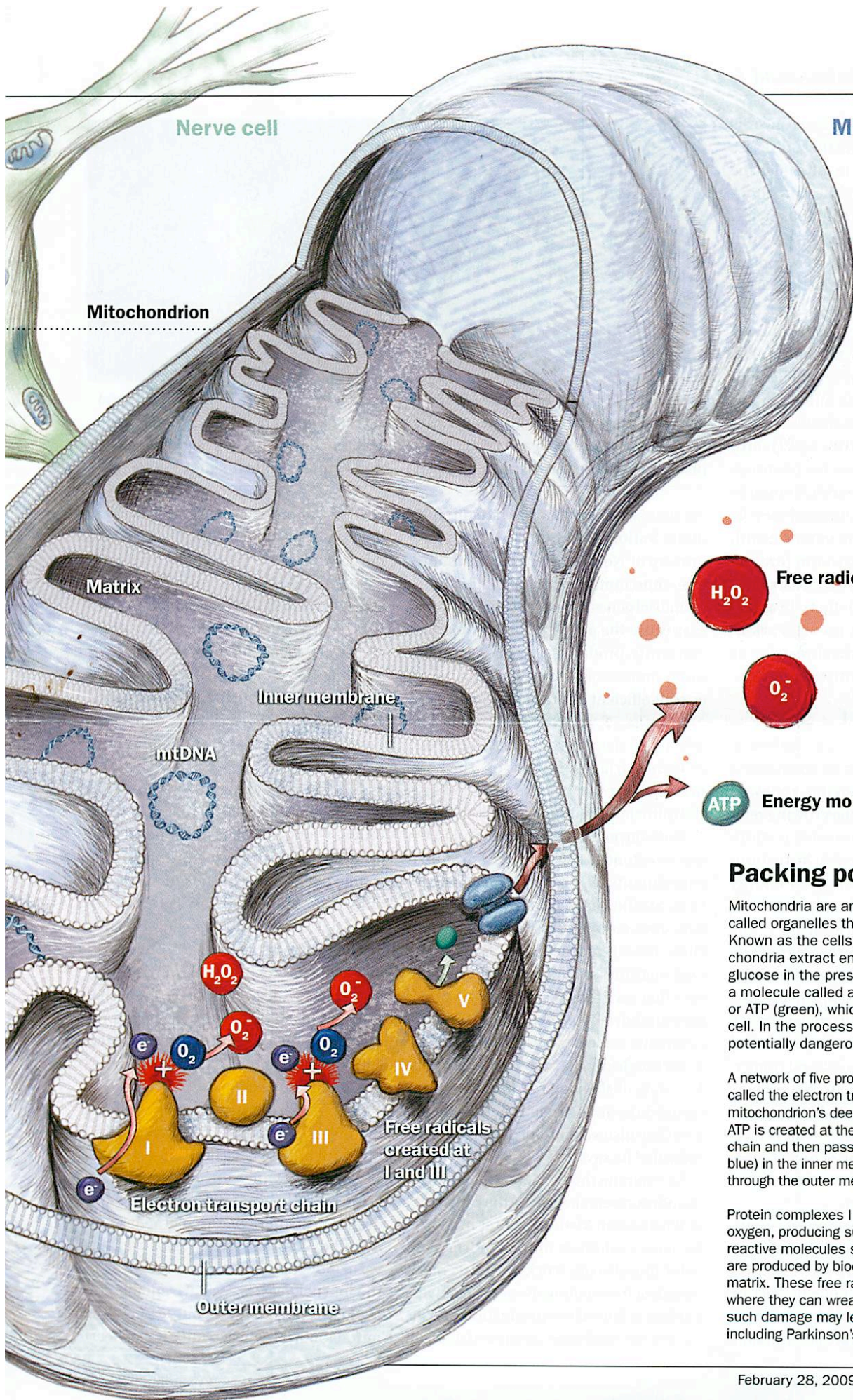
The waterfall cannot be understood from its parts, only from its movement. And once the flow stops, there is no more waterfall. The waterfall is not a thing that appears and disappears. It is a *process*—a process that flows and stops flowing. Like a waterfall, you are not a thing. You are a process—an energetic process, to be precise.

Your fundamentally energetic nature has two main implications. The first is that as a dynamic process, you are bound to change. Your body continually sheds, kills and makes cells. Your mind also changes. Some parts of your mind, such as your personality, are relatively stable. But then again, that can change, too—when you are “hangry,” for instance, and become less than your best self. That’s an energy deficit changing your mind.

Some drugs can dramatically change your mind. Psychedelics, for example, act on the serotonin system to desynchronize the human brain. They also dissolve the sense of self, or “ego.” Change brain-energy patterns, change the mind. The mind, then, may essentially be an energy pattern. Further, energy flowing through your brain somehow *feels* like something. As Nirosha Murugan of Wilfrid Laurier University in Ontario and I recently postulated in a paper, humans may be wired to experience excessive resistance to energy flow as aversive. In contrast, smoothly flowing energy, as occurs after an enjoyable exercise session or when you are working on a stimulating project, feels good. When energy ceases flowing to your brain—if your heart stops, for example—your conscious awareness quickly fades, and you no longer are.

Does all this say anything useful about my original questions? I suspect we now have answers. The key to life and health may lie in how easily energy flows through your mitochondria with each breath you take. So next time you skip that appealing sugary treat, go outside for a stroll, hit the gym or decide to spend time with someone you care about, know that you are supporting your mitochondria. Keeping energy flowing through your mitochondrial collective may be the key to good health and a meaningful life. ● ✖ ✖ ✖

FROM OUR ARCHIVES
A New Understanding
of the Cell. Philip Ball;
February 2025.
ScientificAmerican.
com/archive



Packing power

Mitochondria are among the small structures called organelles that reside within a cell. Known as the cells' powerhouses, mitochondria extract energy from fuels such as glucose in the presence of oxygen to produce a molecule called adenosine triphosphate, or ATP (green), which provides energy for the cell. In the process, mitochondria generate potentially dangerous free radicals (red).

A network of five protein complexes (yellow, I–V) called the electron transport chain sits in the mitochondrion's deeply folded inner membrane. ATP is created at the last complex (V) within the chain and then passes through a channel (light blue) in the inner membrane before diffusing through the outer membrane into the cell.

Protein complexes I and III leak electrons to oxygen, producing superoxide radicals. Highly reactive molecules such as hydrogen peroxide are produced by biochemical reactions in the matrix. These free radicals move into the cell, where they can wreak havoc. Accumulation of such damage may lead to human diseases, including Parkinson's and Alzheimer's.

Mitochondria Gone Bad
Problems in the cell's energy
factories power new ideas on disease
and aging

Laura Bell
Science News
February 28, 2009

"Mitochondria might even be the biological epicenter of aging itself."

"Mitochondrial metabolism is the key to aging."

"People are only as young as their mitochondria."

Anna Gvozdjaková
Editor

Mitochondrial Medicine

*Mitochondrial Metabolism,
Diseases, Diagnosis and Therapy*



Springer

LEE KNOW, ND

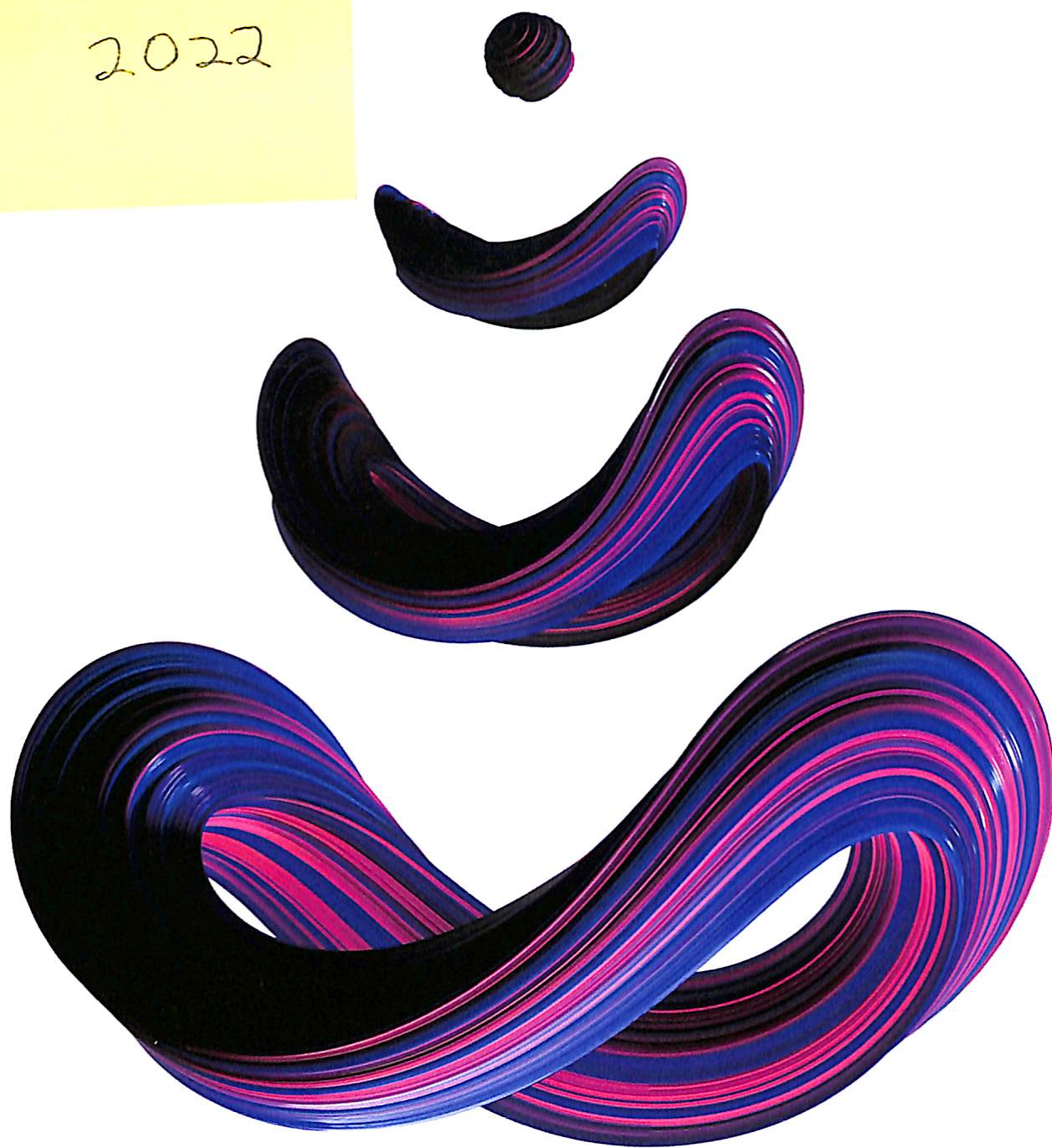
MITOCHONDRIA AND THE FUTURE OF MEDICINE

The Key to Understanding Disease,
Chronic Illness, Aging, and Life Itself

A microscopic image showing several mitochondria, which are bean-shaped organelles with internal folds called cristae. They are stained in a way that makes them appear as glowing, translucent structures against a dark background.

2018

2022



TRANSFORMER

The Deep Chemistry of Life and Death

NICK LANE

The experiment for which Warburg won the Nobel Prize in 1931 was just so beautiful I have to tell you about it.

Warburg knew he could block respiration by using the gas carbon monoxide (CO), which in cells binds to metal atoms in the catalyst that converts oxygen to water.

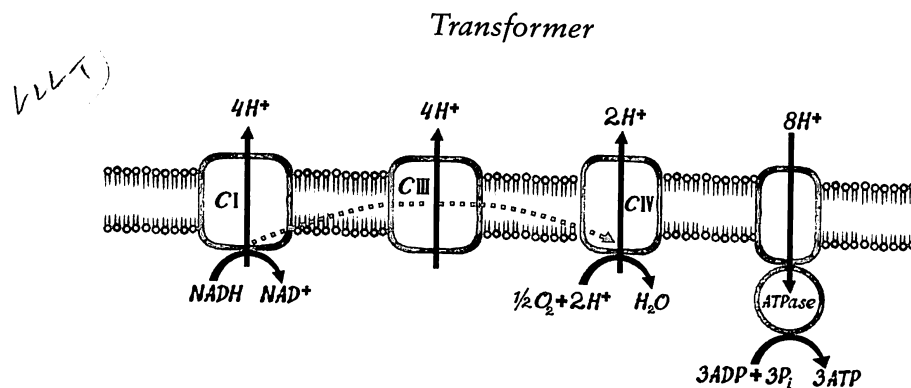
The binding of CO therefore halts the final step of respiration.

Here's the clever bit. CO can be detached from the catalyst through the absorption of light. That kick-starts respiration and oxygen consumption over again.

But not all wavelengths of light worked equally; some were absorbed by the catalyst, which then releases its bound CO, while other wavelengths just bounce off, leaving the CO bound.

Warburg used light sources that emitted photons with specific wavelengths, ... By measuring the rate of respiration at different wavelengths of light, he could reconstitute the absorption spectrum for the catalyst, showing that it was a heme pigment, closely related to hemoglobin, the pigment that transports oxygen in our own red blood cells..."

"The heme protein that Warburg discovered is now called "cytochrome oxidase."



A grossly simplified schematic of the respiratory chain. The transfer of electrons from NADH to oxygen is shown by the dotted line. These electrons pass through three great protein complexes embedded in the membrane (complex I, III and IV; we'll meet complex II in Chapter 4). This current of electrons powers the extrusion of ten protons across the membrane, generating a combined electrical charge and concentration difference (the proton-motive force) that drives ATP synthesis from ADP (adenosine diphosphate) and inorganic phosphate (P_i). In humans, eight protons pass through the ATP synthase for one turn of the cycle.

Otto Warburg
The Oxygen-transferring Ferment of Respiration
Nobel Lecture, December 10, 1931

The Effect of Light on Inhibition of Cellular Respiration by Carbon Monoxide

Not only cellular respiration but also simpler iron catalyses are reversibly inhibited by carbon monoxide and prussic acid.

•••

But it would never have been possible to reach a definite conclusion if Nature had not endowed the iron compounds of carbon monoxide with the remarkable property of becoming dissociated - with splitting off from the carbon monoxide - under the action of light.

•••

By using monochromatic light and comparing the amount of light energy absorbed with the amount of carbon monoxide set free, it is found that Einstein's law of photochemical equivalence is very exactly fulfilled.

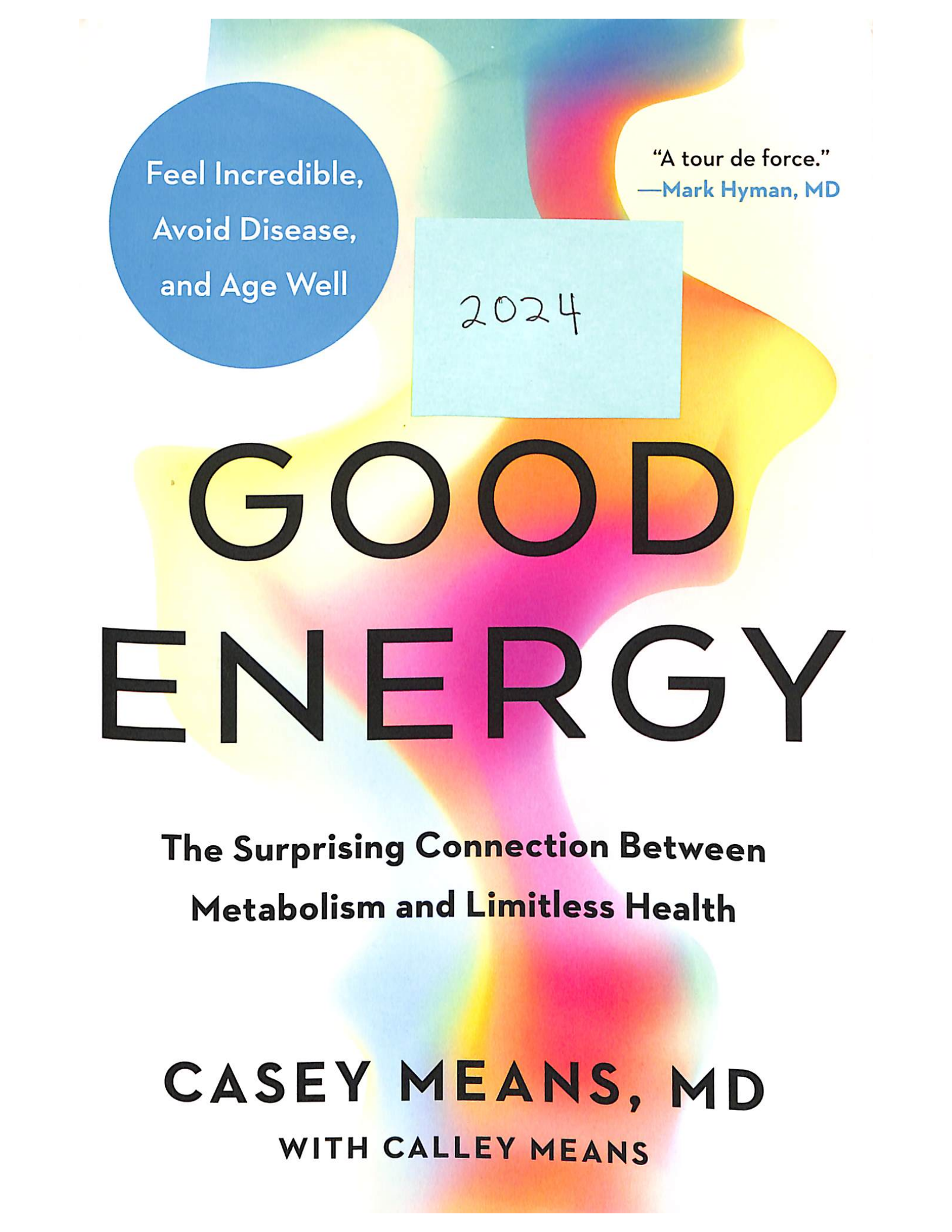
•••

If then this is exposed to monochromatic light of various wavelengths and of measured quantum intensity, and the effect of light W measured the increase in the rate of catalysis - it is found that the effects of the light are proportional to the quanta absorbed.

•••

The respiration of living cells was inhibited by carbon monoxide.

We then irradiated with monochromatic light of various wavelengths and of measured quantum intensity, and the increase of respiration measured together with the relative absorption spectrum.



Feel Incredible,
Avoid Disease,
and Age Well

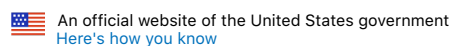
"A tour de force."
—Mark Hyman, MD

2024

GOOD ENERGY

**The Surprising Connection Between
Metabolism and Limitless Health**

CASEY MEANS, MD
WITH CALLEY MEANS



FULL TEXT LINKS



[Review](#) > [Nat Rev Rheumatol](#). 2011 Mar;7(3):161-9. doi: 10.1038/nrrheum.2010.213.

Epub 2011 Jan 4.

The role of mitochondria in osteoarthritis

Francisco J Blanco ¹, Ignacio Rego, Cristina Ruiz-Romero

Affiliations

PMID: 21200395 DOI: [10.1038/nrrheum.2010.213](#)

Abstract

Mitochondria are important regulators of cellular function and survival that may have a key role in aging-related diseases. Mitochondrial DNA (mtDNA) mutations and oxidative stresses are known to contribute to aging-related changes. Osteoarthritis (OA) is an aging-associated rheumatic disease characterized by articular cartilage degradation and elevated chondrocyte mortality. Articular cartilage chondrocytes survive and maintain tissue integrity in an avascular, low-oxygen environment. Recent ex vivo studies have reported mitochondrial dysfunction in human OA chondrocytes, and analyses of mitochondrial electron transport chain activity in these cells show decreased activity of Complexes I, II and III compared to normal chondrocytes. This mitochondrial dysfunction may affect several pathways that have been implicated in cartilage degradation, including oxidative stress, defective chondrocyte biosynthesis and growth responses, increased cytokine-induced chondrocyte inflammation and matrix catabolism, cartilage matrix calcification, and increased chondrocyte apoptosis. Mitochondrial dysfunction in OA chondrocytes may derive from somatic mutations in the mtDNA or from the direct effects of proinflammatory mediators such as cytokines, prostaglandins, reactive oxygen species and nitric oxide. Polymorphisms in mtDNA may become useful as biomarkers for the diagnosis and prognosis of OA, and modulation of serum biomarkers by mtDNA haplogroups supports the concept that mtDNA haplogroups may define specific OA phenotypes in the complex OA process.

Related information

[MedGen](#)

LinkOut – more resources

Full Text Sources

[Nature Publishing Group](#)

[Ovid Technologies, Inc.](#)

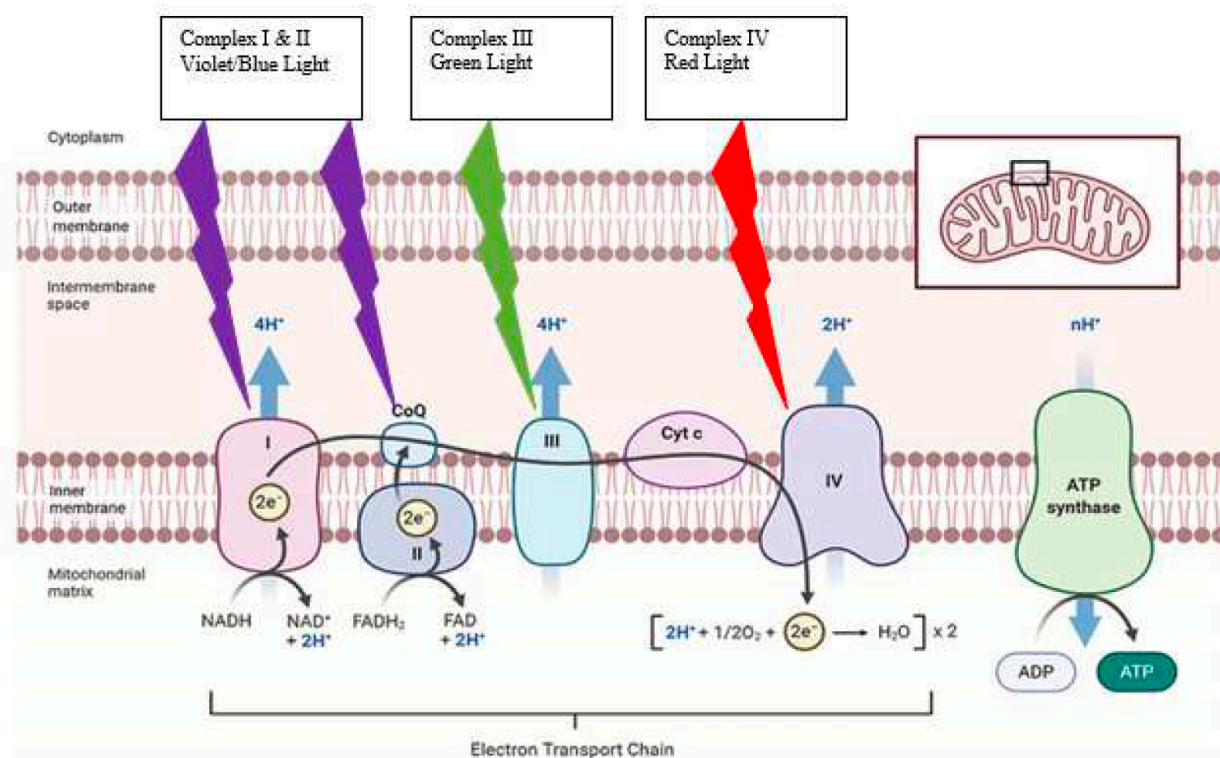
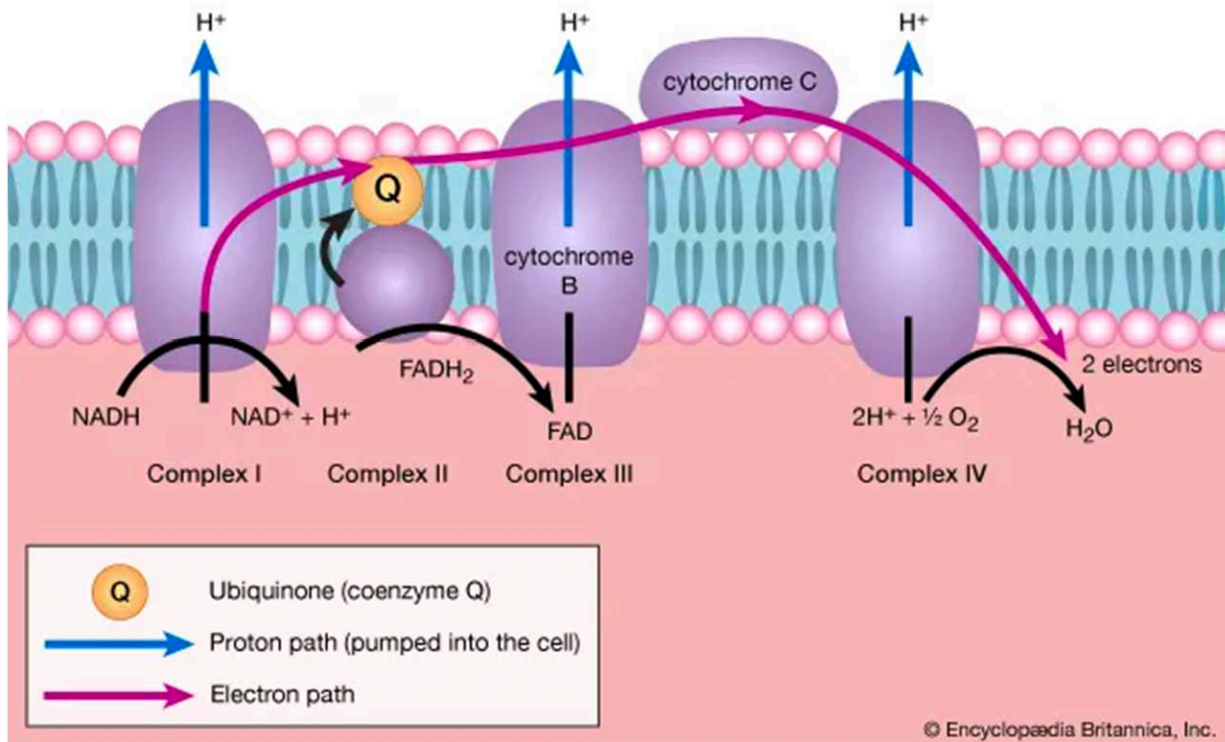
Other Literature Sources

[The Lens - Patent Citations](#)

Medical

[MedlinePlus Health Information](#)

Electron transport chain



Pain

Myofascial Trigger Points Show Spontaneous Needle EMG Activity

SPINE

October 1, 1993; Volume 18, Number 13, pp. 1803-1807

David R. Hubbard, MD and Gregory M. Berkoff, DC

From the Department of Neurosciences, University of California, San Diego

KEY POINTS FROM THIS ARTICLE:

- 1) Trigger points are a common cause of chronic muscle pain.
- 2) Janet Travell, MD, first described the **trigger point** in 1942, in the Journal of the American Medical Association.
- 3) This article attempts to determine the underlying pathophysiology of the trigger point.
- 4) The diagnosis of **myofascial pain syndrome** has these 3 specific criteria:
 - A palpable firm area of muscle, referred to as the taut band.
 - Within the taut band there is a localized spot of exquisite tenderness to manual pressure, the trigger point (TrP).
 - A characteristic pattern of pain, tingling, or numbness occurs in response to sustained pressure on the trigger point (TrP).
 - A local twitch of the taut band occurs when the TrP is distorted transversely.
- 5) The trigger point itself is only a few millimeters in size.
- 6) There are two types of **trigger points**:
 - **Active trigger points** (TrPs) that cause clinical pain syndromes.
 - **Latent trigger points** (TrPs) which are painless and not associated with clinical pain syndromes; 50% of normal asymptomatic people have **latent** TrPs of the shoulder-girdle musculature.
- 7) **Tender points** (TePs) are areas of tenderness that may or may not be in muscle tissue, do not have palpable taut bands, and do not refer pain to adjacent areas.
- 8) **Myofascial Pain Syndrome** has TrPs localized to one or two muscles.
- 9) **Fibromyalgia** has multiple or generalized TrPs or TePs.

10) These authors recorded trapezius needle EMG simultaneously from the 1-2 mm nidus of trigger points and from adjacent nontender muscle fibers of the same muscle. They used:

- 29 patients that had chronic tension headaches and active trigger points.
- 25 patients with fibromyalgia.
- 8 normal controls that had **latent** triggerpoints.

11) Spontaneous EMG activity was recorded from the trigger points of all normal subjects and patients. "Spontaneous EMG activity was found in the 1-2-mm nidus of myofascial trigger points (TrPs)."

12) The EMG activity disappeared when the needle was moved by as little as 1 mm. "The activity disappeared if the recording needle was advanced or withdrawn as little as 1 mm."

13) There was no spontaneous EMG activity in the non-trigger point normal muscle tissue in the subjects or patients.

14) When the needle was inserted into the trigger point in all normal subjects and patients, there was an immediate appearance of spontaneous EMG activity, and the subject/patient reported pain, described as a deep aching or squeezing sensation with a referral into the cervical, occipital, or temporal areas, and "often associated with autonomic symptoms such as light-headedness, diaphoresis, or nausea."

15) The spontaneous EMG activity was present for as long as the needle remained in the trigger point, up to 50 minutes.

16) The mean trigger point EMG amplitudes were significantly greater (more than 6X greater) in the patients than in the normal control group.

17) The sympathetic nervous system innervates the intrafusal fibers of the muscle spindle. The trigger point EMG activity is generated from sympathetically stimulated intrafusal muscle fiber contractions.

18) Multiple studies have shown that stimulation of the cervical sympathetic nerves increased the tension in the jaw muscles. In contrast, cutting the cervical sympathetic nerves immediately blocks jaw muscle tension. "Sympathetic nerve stimulation produced an immediate increase in jaw tension and cutting it reduced the tonic jaw tension." This response is totally blocked by the sympathetic nerve block. Sympathetic nerve block significantly reduces pain in patients with fibromyalgia.

19) Increased sympathetic tone increases output muscle tone (muscle hypertonia), and is associated with clinical syndromes and pain symptomatology. "It is now recognized that there is direct sympathetic innervation to the intrafusal fibers of muscle spindles, and that sympathetic stimulation causes muscle tension."

20) Since the density of muscle spindles is greatest in the neck muscles, increased sympathetic tone would have the greatest influence on neck muscle tone and therefore on neck muscle pain syndromes.

21) These authors believe that sympathetically stimulated muscle spindle intrafusal fiber contraction "causes an involuntary low-grade but symptomatic muscle tension."

22) "Sympathetic activity explains the autonomic symptoms associated with trigger points and provides a mechanism by which local injury and nociception causes local tension, and by which emotional factors cause widespread tension and pain."

CHIROPRACTIC MODEL FROM DAN MURPHY:

The Trigger Point is the Sympathetically Contracted Intrafusal Muscle Fibers of the Muscle Spindle.

Chiropractic adjusting improves the function of mechanoreceptors that are located in the disc, facets, ligaments, muscles, fascia, etc.

Improving mechanical function inhibits pain (closes the pain gate).

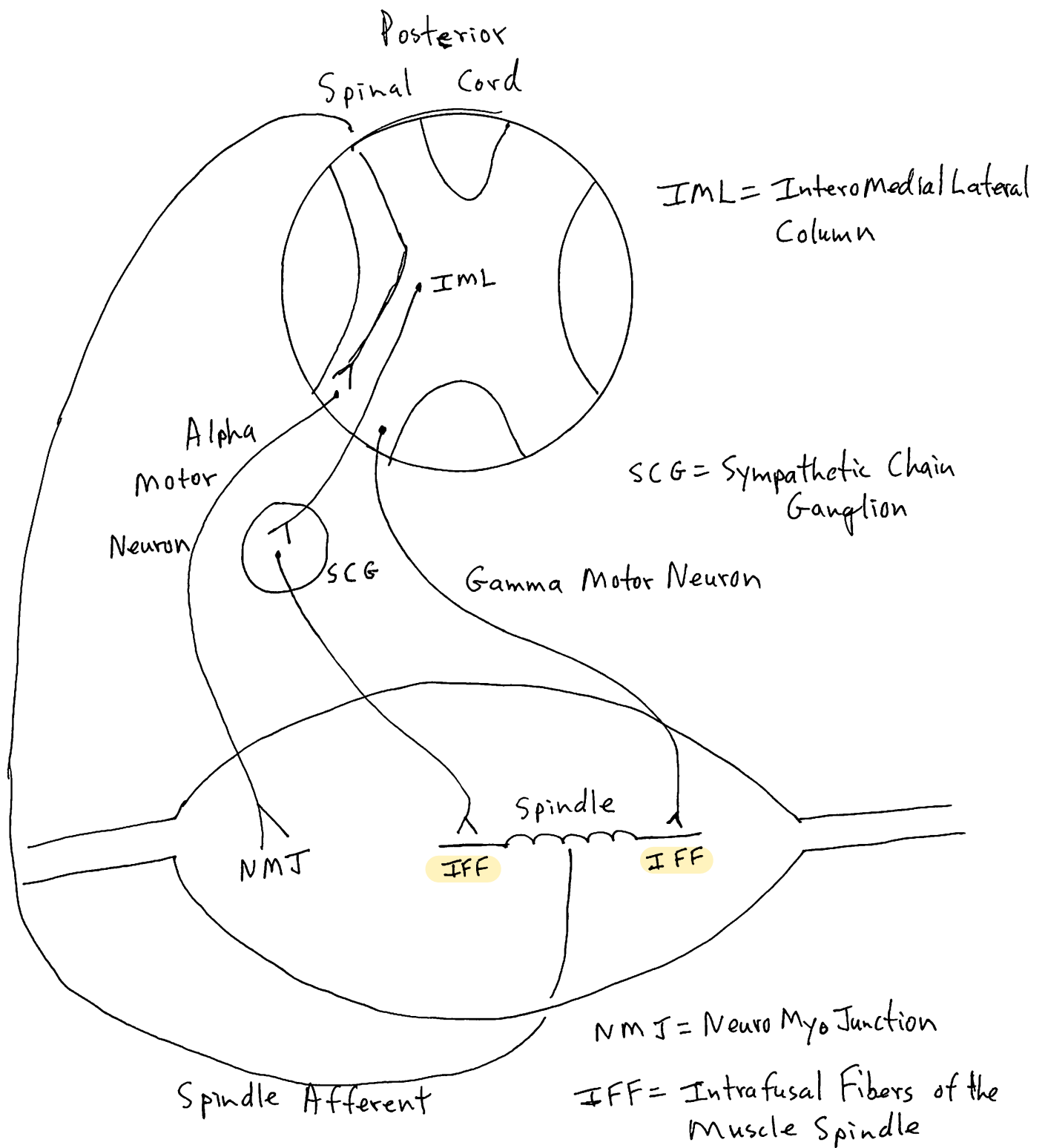
Improving mechanical function inhibits sympathetic tone.

The sympathetic nervous system innervates the intrafusal muscle fibers of the muscle spindle.

Increased sympathetic tone causes contraction of the intrafusal muscle fibers of the muscle spindle, which increases the firing of the muscle spindle afferents.

Increased muscle spindle afferents into the spinal cord communicate and excite the alpha motor neuron, causing the muscle to contract. This contraction increases muscle tone, reduces range of motion, and if prolonged, makes the muscle sore and painful.

Chiropractic adjusting, in part, improves mechanical afferentation, inhibits sympathetic tone, relaxes the intrafusal muscle fibers of the muscle spindle, reduces spindle afferent discharge, reduces muscle tone, improves the range of motion, reducing inflammation and pain.



SECOND EDITION

The Gunn Approach to the **TREATMENT OF CHRONIC PAIN**

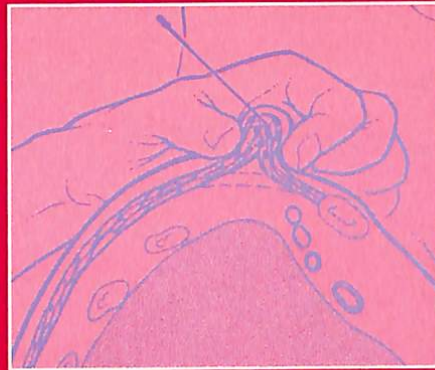
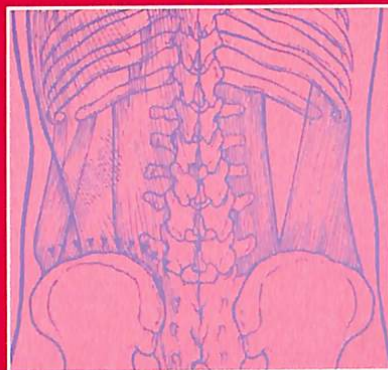
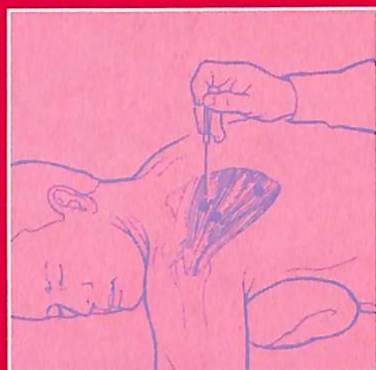
*Intramuscular Stimulation for
Myofascial Pain of Radiculopathic Origin*

C. CHAN GUNN

Foreword by

Patrick D. Wall

1996



CHURCHILL LIVINGSTONE

The Gunn Approach to the **TREATMENT OF CHRONIC PAIN**

*Intramuscular Stimulation for
Myofascial Pain of Radiculopathic Origin*

C. Chan Gunn MD

Clinical Professor, Multidisciplinary Pain Center,
University of Washington Medical School, Seattle, USA

Foreword by

Patrick D. Wall FRS DM FRCP

Professor Emeritus
United Medical and Dental Schools, London, UK

1996



CHURCHILL
LIVINGSTONE

NEW YORK EDINBURGH LONDON MADRID MELBOURNE SAN FRANCISCO AND TOKYO 1996

G. Stux · R. Hammerschlag (Eds.)

Clinical Acupuncture

Scientific Basis

With Contributions by

B. M. Berman

S. Birch

C. M. Cassidy

Z. H. Cho

J. Ezzo

R. Hammerschlag

J. S. Han

L. Lao

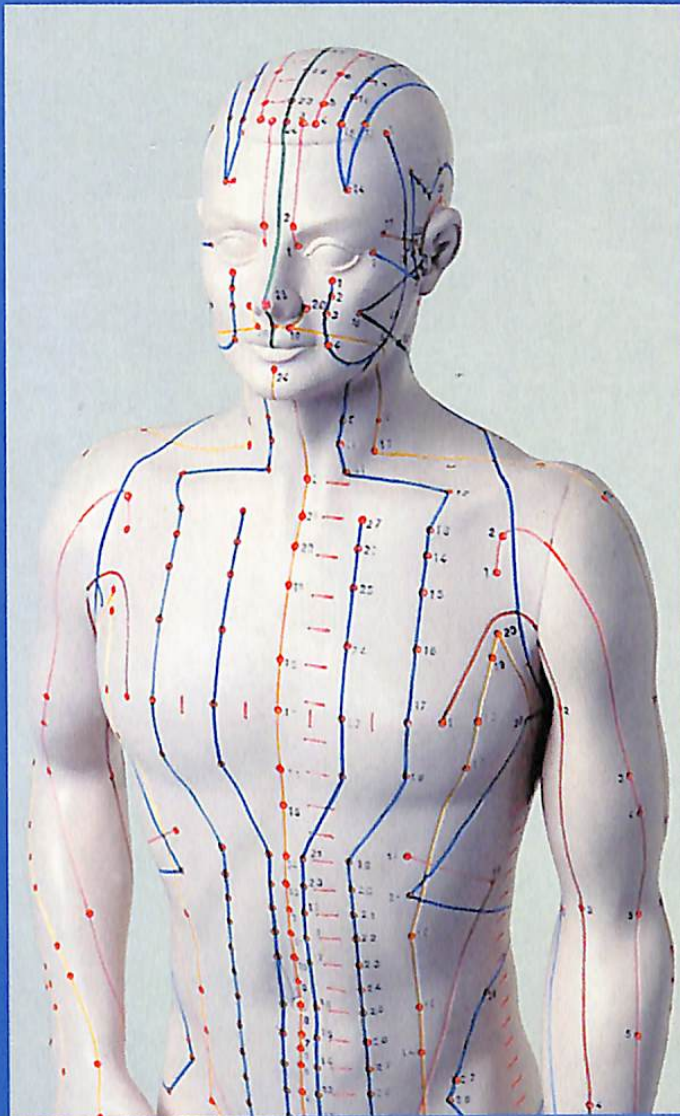
T. Oleson

B. Pomeranz

C. Shang

G. Stux

C. Takeshige



鍼灸

2001



Springer

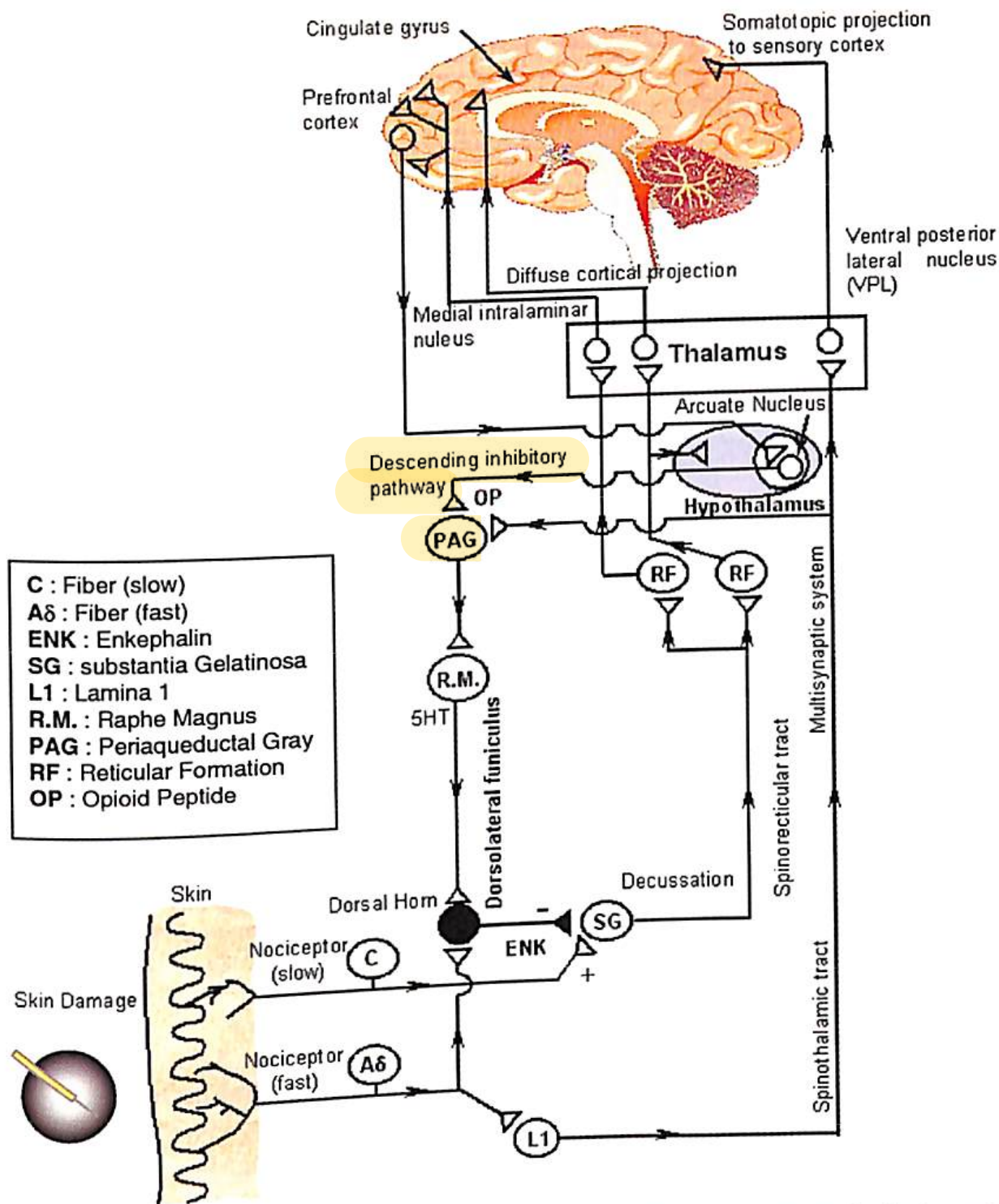


Fig. 6. A pain relief hypothesis involving higher cortical areas. In this example, the hypothalamus and other higher cortical areas are involved in secretion of beta-endorphin as well as other opioids and neurotransmitters. This hypothesis suggests involvement of higher cortical areas such as the sensory cortex as well as the frontal cortex, especially the prefrontal cortex and the limbic areas [11]

Inflammatory Chemicals Brief Summary

Eicosanoids	Cytokines
Prostaglandin E2 PGE2 Hormone-Like Molecules Derived from Omega-6 Fatty Acid <i>Arachidonic Acid</i> Linoleic Acid (Corn/ Soy) ↓ Arachidonic Acid ↓ PGE2 Insulin-D5D → COX → High Omega-6/Omega-3 ratio >3/1 <u>Ratio Improved By</u> •Limiting omega-6 and grained meat/egg consumption •Increasing omega-3 and wild fish consumption COX Enzymes are Inhibited by Red LLLT Red LLLT Reduces PGE2 Accumulation Motion Disperses Accumulation Chiropractic, etc. Inflammatory Eicosanoids Increase Inflammatory Cytokines	Interleukin-6 IL-6 Proteins Derived from Immune Cell Activation Infection Excessive Immunological Responses Autoimmune Diseases Leaky Gut Low Vitamin D Altered Vitamin D Receptor Low Levels of IL-10 <u>Improved/Reduced By</u> Immune Enhancing Supplements •Vitamin D •Vitamin C •Magnesium •Zinc •Omega-3s Heal the Leaky Gut Avoid Harmful Wavelengths >850 nm Violet LLLT Are Antimicrobial Violet LLLT Reduce/Inhibit IL-6 Red LLLT Reduce/Inhibit IL-6 Red LLLT Increases IL-10 Vigorous Exercise Increases IL-10 Chiropractic Increased IL-10 Chiropractic May Be Immunoenhancing Motion Disperses Accumulation Chiropractic, etc. Inflammatory Eicosanoids Increase Inflammatory Cytokines

PubMed

Display Settings: AbstractInt J Clin Pharmacol Ther. 1995 Apr;33(4):208-11.**Successful management of female office workers with "repetitive stress injury" or "carpal tunnel syndrome" by a new treatment modality--application of low level laser.**Wong E, Lee G, Zucherman J, Mason DT.**Author information****Abstract**

Female office workers with desk jobs who are incapacitated by pain and tingling in the hands and fingers are often diagnosed by physicians as "repetitive stress injury" (RSI) or "carpal tunnel syndrome" (CTS). These patients usually have poor posture with their head and neck stooped forward and shoulders rounded; upon palpation, they have pain and tenderness at the spinous processes C5-T1 and the medial angle of the scapula. In 35 such patients we focused the treatment primarily at the posterior neck area and not the wrists and hands. A low level laser (100 mW) was used and directed at the tips of the spinous processes C5-T1. The laser rapidly alleviated the pain and tingling in the arms, hands and fingers, and diminished tenderness at the involved spinous processes. Thereby, it has become apparent that many patients labelled as having RSI or CTS have predominantly cervical radicular dysfunction resulting in pain to the upper extremities which can be managed by low level laser. Successful long-term management involves treating the soft tissue lesions in the neck combined with correcting the abnormal head, neck and shoulder posture by taping, cervical collars, and clavicle harnesses as well as improved work ergonomics.

PMID: 7620690 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms**LinkOut - more resources****PubMed Commons**[PubMed Commons home](#)

0 comments

"All laser treatment [should be] proceeded by a stimulating irradiating of the vertebra that innervates the damaged area." Page 146

The biostimulatory action of laser therapy is countered by the presence of NSAIDs and steroids that are non-acceptors of laser therapy, as the latter block the membrane channels and antennae pigment receptors that laser therapy relies upon." Page 91

Laser Therapy

Clinical Practice and Scientific Background

A guide for research scientists, doctors, dentists, veterinarians and other interested parties within the medical field.

By
Jan Tunér and Lars Hode

Prima Books AB
2002

570 pages

1281 references

Laser Phototherapy Clinical Practice and Scientific Background

Lars Hode (physicist) and Jan Tuner (dentist)

Prima Books 2014

936 Pages; 2534 References

Systemic or indirect irradiation include the irradiation of (p. 117):

- Acupuncture Points
- Trigger Points
- Spinous Processes
- The Dermatome
- The Blood
- Lymph Nodes

"In these instances we irradiate areas outside or far from the area where the patient would otherwise locate the problem."

"Direct and indirect treatment can be combined. In fact, even direct irradiation is a mode of indirect irradiation."

"The beam will always find blood, lymph and nerves, influencing the circulating metabolites."

"So every local treatment is also a systemic treatment."

Spinal Processes (p. 131)

"Irradiation of the spinal processes corresponding to the area where the pain is located will reduce the pain."

It may be a nerve entrapment in the nerve passage leading into the spinal cord and laser treatment may reduce edema around the nerve and hence pain relief.

Format: Abstract**Full text links**Science. 1973 Mar 9;179(4077):1011-4.

Opiate receptor: demonstration in nervous tissue.

Pert CB, Snyder SH.

Abstract

Tritiated naloxone, a powerful opiate antagonist, specifically binds to an opiate receptor of mammalian brain and guinea pig intestine. Competition for the opiate receptor by various opiates and their antagonists closely parallels their pharmacological potency. The opiate receptor is confined to nervous tissue.

PMID: 4687585

[Indexed for MEDLINE]

MeSH terms, Substances☐**LinkOut - more resources**☐**PubMed Commons**[PubMed Commons home](#)

0 comments

[How to join PubMed Commons](#)

Candace B. Pert, Ph.D.

Foreword by Deepak Chopra, M.D.



1997

Molecules of Emotion

THE SCIENCE
BEHIND MIND-BODY
MEDICINE

"Candace B. Pert . . . has managed to take the study of the emotional connection to the body . . . and present this information in not only an understandable manner, but an enjoyable one."

—Caroline Myss, Ph.D., author of *Why People Don't Heal and How They Can*

"An important book."

DAVID S. LUDWIG, MD, PhD, PROFESSOR, HARVARD MEDICAL SCHOOL;
NEW YORK TIMES BESTSELLING AUTHOR

2017

MEMORY

MEMORY

RESCUE

*SUPERCARGE YOUR BRAIN,
REVERSE MEMORY LOSS, AND
REMEMBER WHAT MATTERS MOST*

THE OFFICIAL PROGRAM OF THE AMEN CLINICS

DANIEL G. AMEN, MD

“The prefrontal cortex (which is involved with focus, forethought, judgment, and impulse control) is the area of the brain most densely populated with mitochondria.”

THE BRAIN AND PAIN

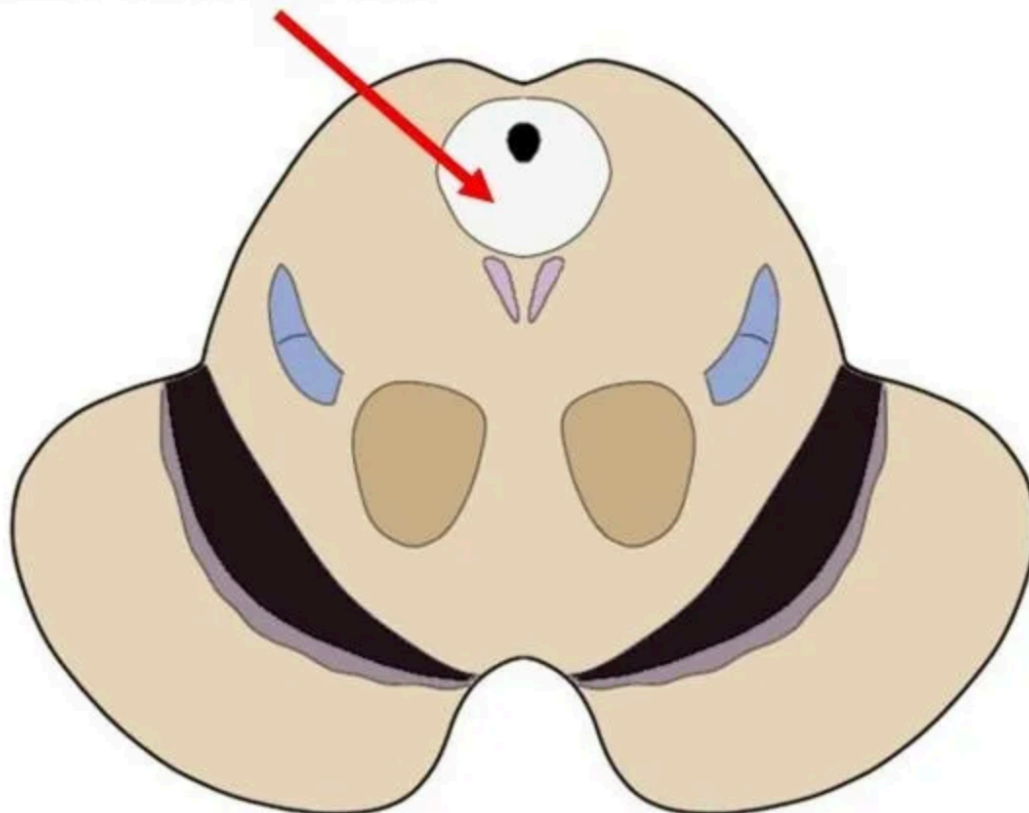
Breakthroughs in Neuroscience

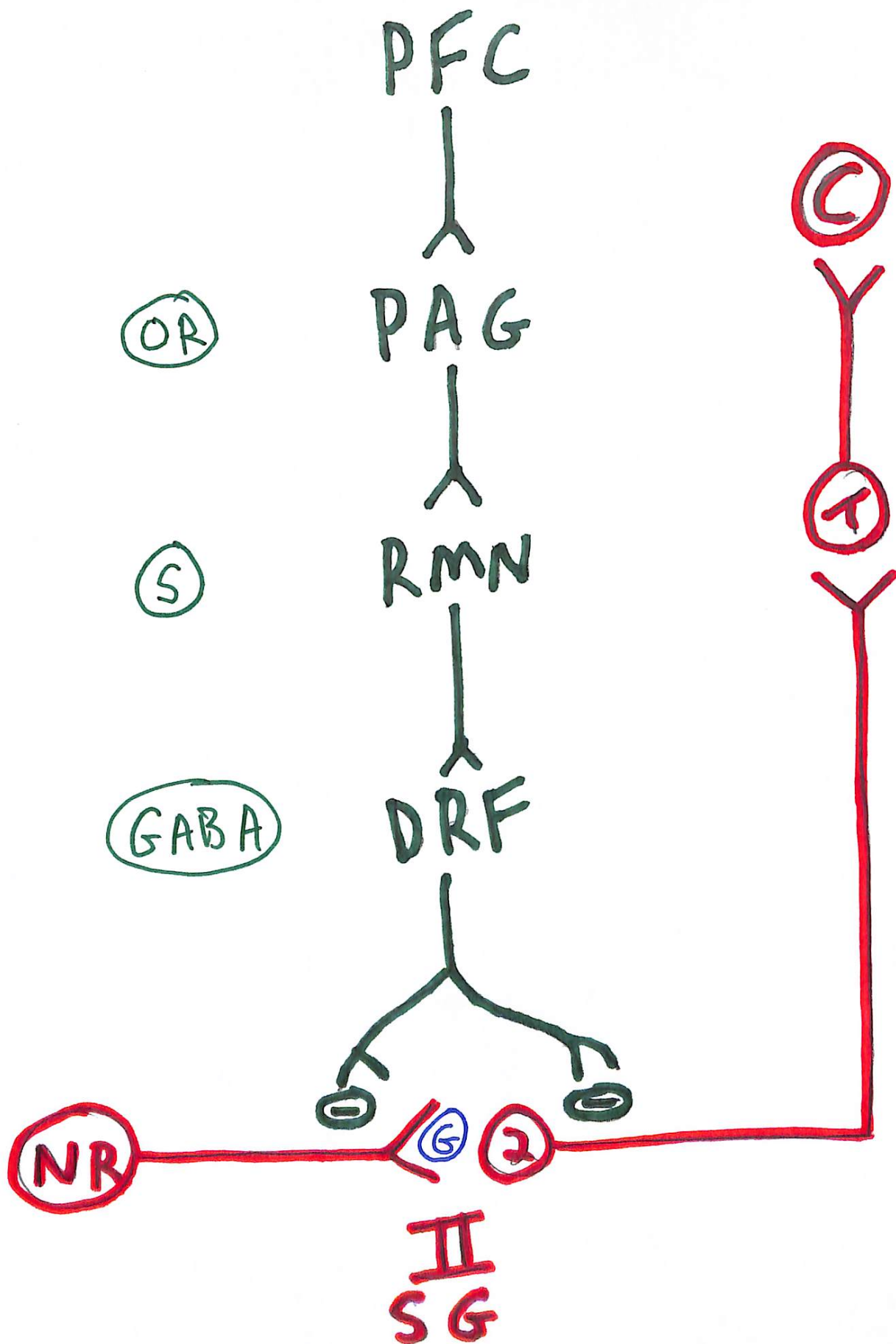


RICHARD AMBRON

Illustrated by Ahmet Sinav

PERIAQUEDUCTAL GRAY





Richard Ambron

emeritus professor of pathology, anatomy, and cell biology at Columbia University

PAG = periaqueductal gray

"Studies show that electrical stimulation of the PAG could diminish pain without interfering with touch, pressure, or temperature sensations."

The PAG is "placed" "as an essential center for the perception of pain."

The PAG blocks "synaptic transmission between the first and second-order neurons."

"Activation of neurons in the PAG results in the release of the endogenous opiates in the spinal cord that shuts down the nociceptive pathway to the thalamus."

"The suppression of injury-induced pain is most likely due to the activation of the opiodergic neurons in the PAG."

PFC = prefrontal cortex

"The prefrontal cortex (PFC) comprises the cortical neurons found in the frontal region of the frontal lobe. Its functions are probably the most important for what separates us from other primates."

An increase in the activity of circuits in the PFC reduce responses to noxious stimulus.

Activation if circuits within the "PFC will have a significant impact on the experience of pain."

"The modulation of painfulness is associated with increased activity in the PFC."

"We know that there are connections between the PFC and the PAG."

"The activation of the PAG is controlled by circuits in the PFC."

"Inputs from the PFC to the PAG result in the activation of the opiodergic neurons whose axions descend to the spinal cord, where the release of opioids prevents synaptic transmission at the synapse between the first- and second-order neurons in the nociceptive pathway."

.....

"Only the cell body contains the genome and machinery for synthesis of proteins and other macromolecules."

Format: Abstract**Full text links**Neurochem Res. 2015 Dec;40(12):2583-99. doi: 10.1007/s11064-015-1581-6. Epub

The Glymphatic System: A Beginner's Guide.

Jessen NA¹, **Munk AS**², **Lundgaard I**², **Nedergaard M**².

Author information

- 1 School of Medicine and Dentistry, University of Rochester Medical Center, 601 Elmwood Ave, Box 645, Rochester, NY, 14642, USA. Nadia_Aalling@urmc.rochester.edu.
- 2 School of Medicine and Dentistry, University of Rochester Medical Center, 601 Elmwood Ave, Box 645, Rochester, NY, 14642, USA.

Abstract

The glymphatic system is a recently discovered macroscopic waste clearance system that utilizes a unique system of perivascular tunnels, formed by astroglial cells, to promote efficient elimination of soluble proteins and metabolites from the central nervous system. Besides waste elimination, the glymphatic system also facilitates brain-wide distribution of several compounds, including glucose, lipids, amino acids, growth factors, and neuromodulators. Intriguingly, the glymphatic system function mainly during sleep and is largely disengaged during wakefulness. The biological need for sleep across all species may therefore reflect that the brain must enter a state of activity that enables elimination of potentially neurotoxic waste products, including β -amyloid. Since the concept of the glymphatic system is relatively new, we will here review its basic structural elements, organization, regulation, and functions. We will also discuss recent studies indicating that glymphatic function is suppressed in various diseases and that failure of glymphatic function in turn might contribute to pathology in neurodegenerative disorders, traumatic brain injury and stroke.

KEYWORDS: Aging; Astrocytes; Cerebrospinal fluid secretion; Neurodegenerative diseases; Perivascular spaces; Sleep; The glymphatic system; Traumatic brain injury; Virchow–Robin spaces

PMID: 25947369 PMCID: [PMC4636982](#) DOI: [10.1007/s11064-015-1581-6](#)

[Indexed for MEDLINE] **Free PMC Article**

Images from this publication. [See all images \(6\)](#) [Free text](#)

The Longevity Paradox

How to Die Young at a Ripe Old Age

Steven Gundry, MD
2019

Sleep and the Glymphatic System

"The glymphatic system is most active during the specific stage of deep sleep that happens very early in the sleep cycle."

"The glymphatic system, just like your digestive system, requires a great deal of blood flow. This means that if you eat too soon before going to bed, your blood will all flow to your gut to aid in digestion and will not be able to reach your brain to complete the all-important brain wash."

"If your glymphatic system cannot wash out your brain effectively, you end up with a building up of amyloid and other toxins, including lectins and LPSs, in the brain."

"This wash cycle is probably the single most overlooked and misunderstood aspect of neurodegenerative diseases."

The "simple solution: leaving as big a gap as possible between your last meal of the day and your bedtime."

"The minimum amount of time between finishing your last meal and going to sleep should be four hours."

The Brain's Waste-Disposal System May Be Enlisted to Treat Alzheimer's and Other Brain Illnesses

An Internal Plumbing System Rids the Brain of Toxic Wastes

Sleep is When this Cleanup Ritual Occurs

Scientific American

March 1, 2016

[modified]

Maiken Nedegaard and Steven Goldman: from the University of Rochester Medical Center and the University of Copenhagen

The human brain weighs about 3 pounds, or roughly 2% of the average adult body mass. Yet its cells consume 20-25% of the body's total energy. In the process, inordinate amounts of potentially toxic protein wastes and biological debris are generated.

Each day, the adult brain eliminates 7 grams of worn-out proteins that must be replaced with newly made ones, a figure that translates into the replacement of about half a pound of detritus a month and nearly 6 pounds, twice the brain's own weight, over the course of a year.

To survive, the brain must have some way of flushing out debris. It is inconceivable that an organ so finely tuned to producing thoughts and actions would lack an efficient waste disposal system.

Disturbances in waste clearance could contribute to cognitive problems encountered in neurodegenerative disease because the disruption would lead to the accumulation of protein debris in and around brain cells. The aggregates could impede the transmission of electrical and chemical signals in the brain and cause irreparable harm.

These authors found an undiscovered system for clearing proteins and other wastes from the brain—and this system is *most active during sleep*. The need to remove potentially toxic wastes from the brain may help explain the mystery of why we sleep.

The Glymphatic System

In most regions of the body, a network of intricate fluid-carrying vessels, known as the *lymphatic system*, eliminates protein waste from tissues. Waste-carrying fluid moves throughout this network between cells. The fluid collects into small ducts that then lead to larger ones and eventually into blood vessels. This duct structure also provides a path for immune defense, because lymph nodes, a repository of infection-fighting white blood cells, populate ducts at key points throughout the network. Yet,

neuroscientists had believed that the lymphatic system did not exist in the brain or spinal cord.

Astrocytes are support cells that perform a multitude of functions for the interconnected network of neurons that relay signals throughout the brain. The astrocytes' extensions—astrocytic end feet—completely surround the arteries, capillaries and veins in the brain and spinal cord. The hollow, tube-like cavity that forms between the feet and the vessels remains largely free of obstructions, creating a spillway that allows for the rapid transport of fluid through the brain.

Arterial pulsations might drive the CSF through the perivascular space. From there, some of it could enter astrocytes through their end feet. Using astrocytes as conduits, the CSF then moved through the brain tissue, where it left the astrocytes and picked up discarded proteins.

The fluids exited the brain through the perivascular space that surrounded small veins draining the brain, and these veins in turn merged into larger ones that continued into the neck. The waste liquids went on to enter the lymph system, from which they flowed back into the general blood circulation.

The astrocytes play a critical role in the brain's counterpart of a lymphatic system.

The authors named this discovery the glymphatic system. They combined the words “glia”—a type of brain cell of which the astrocyte is one example—and “lymphatic,” thus referencing this newly discovered function of the brain's glial cells.

In a healthy brain, Alzheimer's beta-amyloid is cleared by the glymphatic system. Other proteins implicated in neurodegenerative diseases, such as the synuclein proteins that turn up in Parkinson's, Lewy body disease and multisystem atrophy, might also be carried away and could build up abnormally if the glymphatic system were to malfunction.

Many patients with Alzheimer's experience sleep disturbances long before their dementia becomes apparent. In older individuals, sleep becomes more fragmented and shallow and lasts a shorter time. Epidemiological studies have shown that patients who reported poor sleep in middle age were at greater risk for cognitive decline than control subjects when tested 25 years later.

Healthy individuals who are forced to stay awake exhibit symptoms more typical of neurological disease and mental illness—poor concentration, memory lapses, fatigue, irritability, and emotional ups and downs. The sleep difficulties of dementia might not just be a side effect of the disorder but might contribute to the disease process itself. If the glymphatic system cleared beta-amyloid during sleep at a higher rate than when awake, perhaps the poor sleeping patterns of patients with neurodegenerative disorders might contribute to a worsening of the disease.

CSF in the glymphatic system falls dramatically while awake. Within *minutes after the onset of sleep* influxes of the fluid increased significantly. The brain's interstitial space—the area between cells through which glymphatic fluid flows on its way to perivascular spaces around veins—rose by more than 60% with sleep. The flow of glymphatic fluid increases during sleep because the space between the cells expands, which helps to push fluid through the brain tissue.

Norepinephrine appears to regulate the volume of the interstitial area and consequently the pace of glymphatic flow. Levels of norepinephrine rise when awake and are scarce during sleep, implying that sleep-related dips in norepinephrine led to enhanced glymphatic flow.

The Power of Sleep

The expansion and contraction of the interstitial space during sleep is important to both brain function and protein-waste clearance. The *waste clearance rate more than doubled with sleep*. The sleeping brain exports protein waste, including beta-amyloid, through the glymphatic transport system.

Normal-pressure hydrocephalus, an illness typically seen in the elderly, is a form of dementia in which excessive CSF accumulates in the hollow central brain cavities, the cerebral ventricles. When a procedure called lumbar puncture removes the fluid by draining it out, patients often exhibit remarkable improvements in their cognitive abilities.

Fluids moving through the glymphatic system may do more than remove wastes; they may deliver various nutrients and other cargo to brain tissue, including delivery of glucose to neurons to provide energy. The glymphatic system may deliver both nutrients and materials needed for maintaining the cells' structural integrity.

MORE TO EXPLORE

The Glymphatic System: A Beginner's Guide. Nadia Aalling Jessen et al.; *Neurochemical Research*; December 2015; Vol. 40; No. 12; pp. 2583–2599.



Review

The Glymphatic System (En)during Inflammation

Frida Lind-Holm Mogensen ^{1,†}, Christine Delle ^{1,†}  and Maiken Nedergaard ^{1,2,*}

¹ Center for Translational Neuromedicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark; Frida.lind-holm@lih.lu (F.L.-H.M.); christine.delle@sund.ku.dk (C.D.)

² Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY 14642, USA

* Correspondence: Nedergaard@sund.ku.dk

† Contributed equally.

Abstract: The glymphatic system is a fluid-transport system that accesses all regions of the brain. It facilitates the exchange of cerebrospinal fluid and interstitial fluid and clears waste from the metabolically active brain. Astrocytic endfeet and their dense expression of the aquaporin-4 water channels promote fluid exchange between the perivascular spaces and the neuropil. Cerebrospinal and interstitial fluids are together transported back to the vascular compartment by meningeal and cervical lymphatic vessels. Multiple lines of work show that neurological diseases in general impair glymphatic fluid transport. Insofar as the glymphatic system plays a pseudo-lymphatic role in the central nervous system, it is poised to play a role in neuroinflammation. In this review, we discuss how the association of the glymphatic system with the meningeal lymphatic vessel calls for a renewal of established concepts on the CNS as an immune-privileged site. We also discuss potential approaches to target the glymphatic system to combat neuroinflammation.

Keywords: glymphatic system; astrocytes; glia limitans; AQP4; ocular glymphatic system; inflammation; immune privilege; CSF; ISF; immune surveillance



Citation: Mogensen, F.L.-H.; Delle, C.; Nedergaard, M. The Glymphatic System (En)during Inflammation. *Int. J. Mol. Sci.* **2021**, *22*, 7491. <https://doi.org/10.3390/ijms22147491>

Academic Editors: Graça Baltazar and Cláudio Roque

Received: 9 June 2021

Accepted: 8 July 2021

Published: 13 July 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The blood–brain barrier (BBB) is a critical component of the central nervous system (CNS) that dampens environmental perturbations in interstitial ionic and chemical concentrations, and thereby supports the stability of synaptic transmission. Long-distance neural networks require a protected environment with limited fluctuations of the concentrations of interstitial ions, neurotransmitters, essential metabolic intermediates, and toxic xenobiotics. The BBB also restricts the entry to the brain of peripheral inflammatory mediators and pathogens that might negatively impact neuronal activity. In the eye, the blood–retina barrier (BRB) similarly protects the environment of the neuroretina. The CNS lacks a parenchymal lymphatic vessel system that supports fluid homeostasis and provides a path for immune surveillance in the peripheral organs. The lack of lymphatic vessels in the retina and brain presented a conceptual challenge in understanding how fluid homeostasis and the export of waste products are obtained within the confines of the CNS [1]. With the discovery of the glymphatic system initially in brains of rodents [2–5], and later in human brain by magnetic resonance imaging (MRI) studies [6–10], a glial-associated functional homologue of the lymphatic system was established. More recently, the existence of an ocular glymphatic clearance system was demonstrated [11]. Astrocytes play a key role in the glymphatic system. Astrocytes create with their vascular endfeet the perivascular spaces that surround the cerebral vasculature. The perivascular spaces are utilized as “highways” for fast transport of cerebrospinal fluid (CSF) into deep brain regions.

The heterogenic nature and function of astrocytes in neurological diseases has been studied excessively at molecular and cellular levels. Many excellent reviews exist on this topic [12–14]. The existence of the glymphatic system provides, however, a novel perspective on the role of astrocytes in pathological processes: Astrocytes are not only

note that fluid transport in the posterior segment of the optic nerve close to the brain is supported by CSF influx along the central retinal artery [11,60]. However, the periarterial influx of CSF does not reach the eye, but is drained along the dural lymphatic vessels, similarly to aqueous humor efflux from the eye [11]. Thus, CSF tracers do not enter the eye, even in glaucoma models, and (like the aqueous humor) leave the optic nerve via dural lymphatic vessels. In anatomic terms, the brain and optic nerves are ensheathed in the same meningeal–dural membrane, and thus share the same CSF pool. The main difference between the brain and the ocular glymphatic system is the source of fluid entering the neuropil; i.e., CSF in the brain and aqueous humor fluid in the retina. In other respects, the polarized fluid transport is organized in a similar manner.

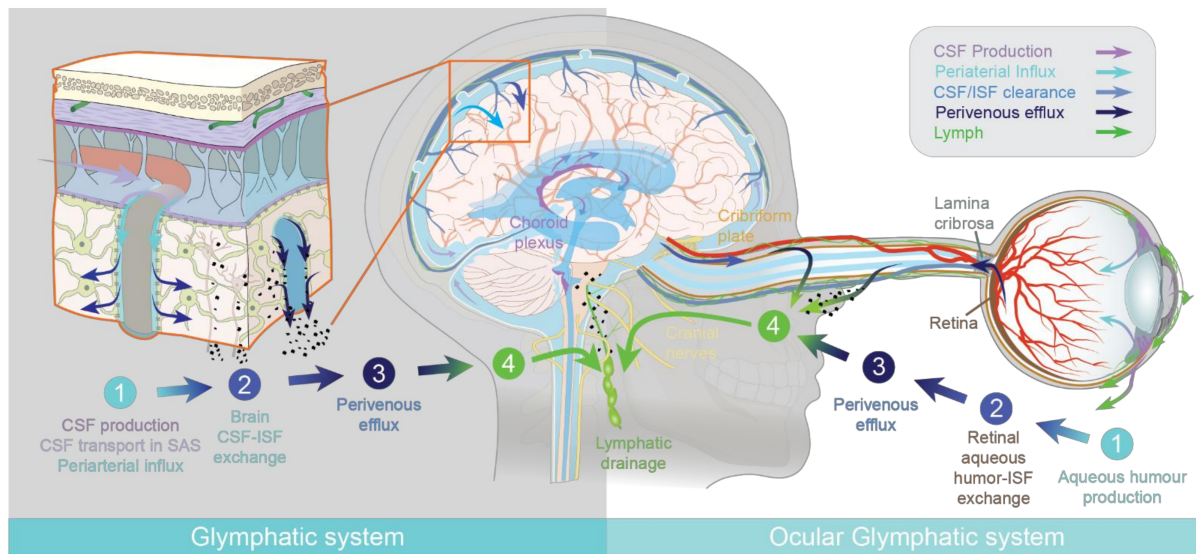


Figure 1. The glymphatic systems in the brain and the eye export fluid and solutes from metabolically active neural tissue. The organization of brain and ocular fluid flow can be divided into four distinct segments that share similarities, but differ in specific respects. (1) The first segment of the brain glymphatic system includes CSF production (purple arrows) and circulation in the subarachnoid space (SAS, light purple arrow), followed by periarterial influx of CSF into the brain tissue (light blue arrows). Two influx paths exist in the eye: the first path is the ciliary body, which produces the aqueous humor fluid. Most of the aqueous humor fluid leaves the eye via the anterior chamber. However, aqueous humor also moves posteriorly, passing the vitreal body before entering the neuroretina to support removal of waste products from that metabolically active tissue. The second inflow path is limited to periarterial influx of CSF along the posterior segment of the optic nerve. CSF does not pass into the eye, but drains into lymphatic vessels located in the dural sheet surrounding the optic nerve prior to the lamina cribrosa barrier [11,60]. (2) The second segment of the brain glymphatics is CSF–ISF exchange supported by AQP4 channels in the vascular endfeet plastered along the arterioles (blue arrows). In the eye, the aqueous humor mixes with interstitial fluid and is transported along axons across the lamina cribrosa barrier. From here the fluid leaves the axons and moves towards the perivenous space in a path supported by astrocytes. Astrocytic AQP4 water channels facilitate this segment as deletion of AQP4 suppresses ocular glymphatic activity [11]. (3) The third segment of the glymphatic system, which is common to the brain and eye, consists of perivenous efflux of interstitial fluid (ISF, dark blue arrows), which drains to the dural lymphatic vessels surrounding the brain and the optic nerve (green). (4) Ultimately, the drainage of perivenous waste from the eye ends up in the cervical lymph nodes (green), which constitute the fourth segment of the fluid-transport system. Fluids from both the brain and the eye thus drain via the cervical lymphatic vessels, which empty into the venous system at the level of the subclavian veins.

2. How Does Neuroinflammation Affect the Glymphatic System?

Among the various pathological events that can lead to neuroinflammation are traumatic brain injury (TBI) [61], meningitis triggered by either viral or bacterial invasion [62], autoimmune diseases such as multiple sclerosis [63], and neurodegeneration [64–69]. An

New Scientist

WEEKLY October 25 - 31, 2025 No3566 US \$7.99 Canada CAN\$9.99

WHAT HAPPENS WHEN
THE AI BUBBLE BURSTS?

BRAIN IMPLANT GIVES
TELEPATHIC CONTROL OF
ANOTHER PERSON'S BODY

HOW A GENETIC MISMATCH
CURSED NEANDERTHAL-
HUMAN HYBRIDS

THE BODY'S SECRET NETWORK

We're finally revealing
the lymphatic system's
extraordinary powers

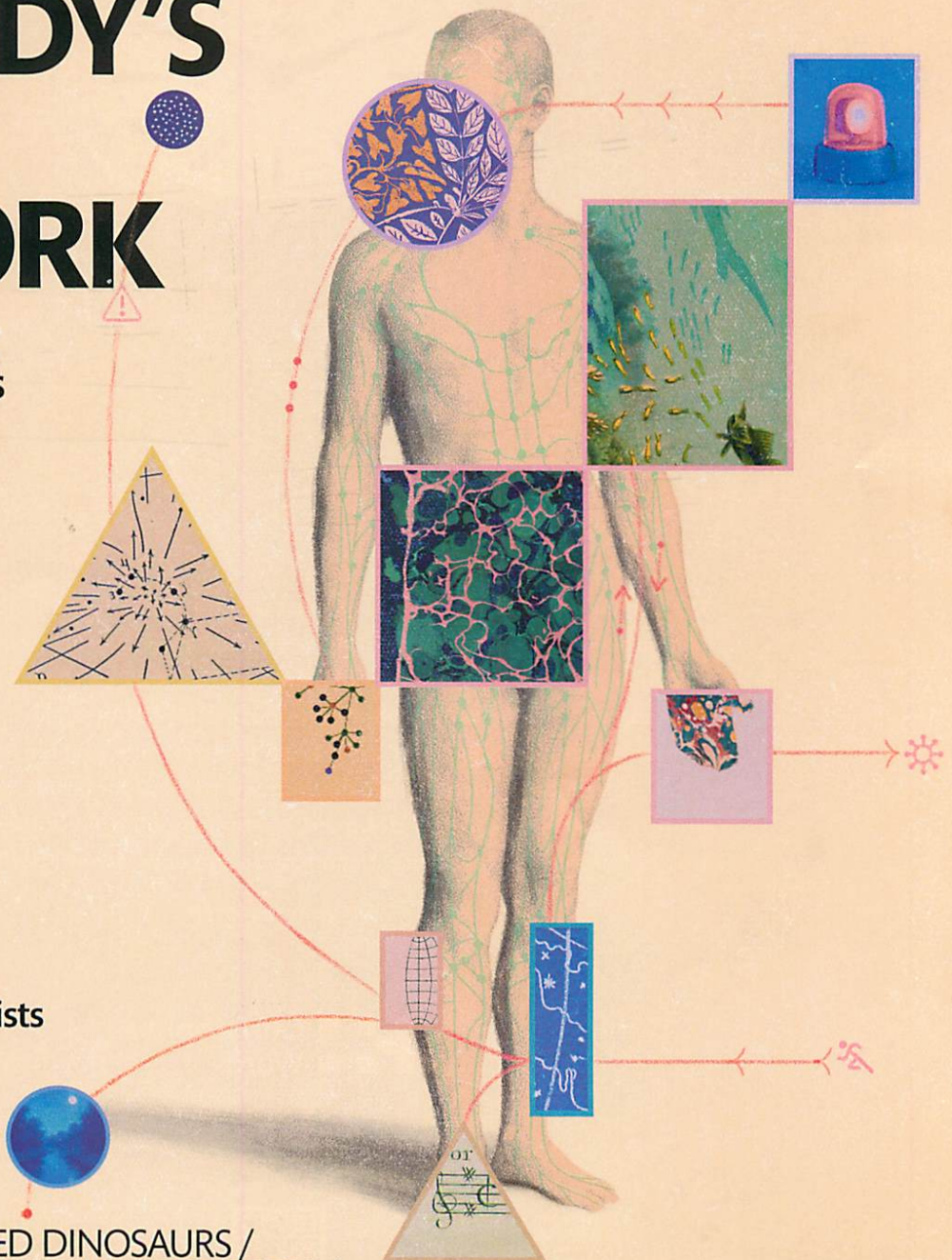
EXCLUSIVE SPECIAL REPORT

Majority of climate scientists
expect geoengineering
this century

PLUS

ORIGINS OF LONG-NECKED DINOSAURS /
THE DEAD SATELLITE POLLUTION PROBLEM /
WHAT 350 THEORIES OF CONSCIOUSNESS REVEAL ABOUT REALITY

Science and technology news www.newscientist.com



Your hidden helper

Our lymphatic system has long been overlooked, but it may hold the key to treating everything from dementia to cancer, finds **Carissa Wong**

I PLACED the cold, jade rolling pin under my cheekbone and gently glided it to the edge of my face. To be honest, I felt a bit like a piece of pastry. But my grandma assured me that I was engaging in a serious traditional Chinese practice that would make my skin glow and my face less puffy. It would be more than a decade before face rolling became the next big beauty fad on social media – and several years more before I realised its supposed benefits may hinge on the lymphatic system, a network spanning the entire body, consisting largely of thread-like vessels and bean-shaped nodes.

Despite having been described in the first known medical scientific paper, the lymphatic system remained a mystery for millennia afterwards. Being extremely elusive and incredibly delicate, it is hard to study – the ruby-red blood system, in contrast, got far more attention. “Lymphatic vessels permeate nearly every organ of our body and yet they sit in our tissues practically invisible,” says Kathleen Caron at the University of North Carolina at Chapel Hill. Only in recent decades have researchers begun to appreciate the profound role the system plays in health and illness, thanks to advances in imaging techniques and molecular beacons that shine a light on its inner workings.

This growing interest has fuelled discoveries that suggest our lymphatic network could be a secret ingredient in treating some major health conditions, including Alzheimer's disease and cancer. “In the past 20 years, we have experienced a renaissance in lymphatic biology,” says Caron. “It would be transformative to harness the powerful roles of lymphatics in keeping us healthy.”

The earliest surviving mention of this ghostly web comes from Egyptian hieroglyphs in a treatise from around 1600 BC that refers to swollen masses in the neck and armpits – structures we now recognise as lymph nodes. A millennium later, the Greek physician and

philosopher Hippocrates described the vessels running between these structures, noting that the nodes often swell during infections.

It wasn't until the 1700s, however, that we started to understand the lymphatic network as a sort of waste-disposal system. Blood vessels supply your tissues with a clear, yellowy fluid containing oxygen, nutrients and immune cells. As well as nourishing cells, it sweeps up their metabolic waste products along with fragments of damaged cells and pathogens, before draining back into the vascular system. However, about 10 per cent of the fluid remains in the tissues, and this is where lymphatic vessels come in; they collect the leftovers.

Once inside lymphatic tubes, the fluid is known as lymph – derived from the ancient Roman deity of fresh water, Lympha, and ancient Greece's nymphs, creatures associated with clear streams. Lymph travels to a nearby node, of which there are about 500 to 600 distributed throughout our bodies. There, immune cells called phagocytes gobble up much of the detritus and, in turn, activate nearby T-cells to recognise any pathogens or cancer cells that are present. Lymph, along with these activated T-cells, then travels through the lymphatic system, re-entering the blood via major ducts near the heart. Any remaining waste products are eventually filtered out by the kidneys and excreted. The T-cells, however,

continue to circulate until they reach sites where they fight off intruders or mutant cells.

Given the lymphatic system's roles in immunity and waste disposal, it seems surprising that scientists long thought it was disconnected from our most vital organ, the brain. But there's a reason. A healthy immune response includes inflammation, which, as well as fighting pathogens, can damage our cells. The brain was thought to have evolved to avoid this collateral damage. It has a structure called the blood-brain barrier to keep pathogens and toxins out, and was seen as “immune privileged”, meaning it lacks immune cells. So, it was assumed not to require the immune services of the lymphatic system – and circulating brain fluid was thought to drain out via non-lymphatic routes, including spaces along nerves that run to lymphatic vessels in the nose.

Such thinking meant that, in the late 1700s, when anatomist Paolo Mascagni injected corpses with mercury and discovered silvery branches, which he claimed were lymphatic vessels bordering the brain, he wasn't taken seriously. It would be more than two centuries before two groundbreaking studies upended the scientific consensus. In 2015, Kari Alitalo at the University of Helsinki in Finland and his team made a surprising discovery while exploring how T-cells move around the brain, which we now know has many immune cells. They noticed that the cells, which they had labelled with a fluorescent green marker, lined up in a vessel-shaped arrangement in the dura mater, the outermost of three membranes – collectively known as the meninges – that are wedged between the brain's surface and the skull.

Taking a closer look, the team found that the structures had all the features of lymphatic vessels, and drained fluid into so-called cervical lymph nodes in the neck. Just two weeks later, Jonathan Kipnis at Washington University in St. Louis, Missouri, and his

“CAN WE CURE DEMENTIA BY IMPROVING THE LYMPHATIC DRAINAGE FROM THE BRAIN?”

colleagues reported finding the same network, this time using mice genetically engineered to have fluorescent lymphatic vessels. "It was this eureka moment for the entire field," says Sandro Da Mesquita at the Mayo Clinic in Jacksonville, Florida, who wasn't involved in either study.

In 2017, Kipnis's team confirmed that humans also have this meningeal lymphatic network. Soon, a wave of rodent studies showed that the vessels become thinner, shorter and less numerous with age. Such degeneration slows the rate of fluid drainage from the brain and worsens cognitive decline. "Ageing leads to regression of these vessels," says Da Mesquita. "In a very old mouse, the lymphatic system that drains the brain is very impaired, which is even more interesting because in most peripheral tissues you don't see that [so much]."

Crucially, reversing such decline brings cognitive benefits. For instance, Da Mesquita and Kipnis injected the brains of old mice with the genetic code for a protein called VEGFC, which can dilate lymphatic vessels. This boosted the drainage of brain fluid through

nodes with age. Irani says this suggests that age-related deterioration of the meningeal lymphatic vessels may reduce their ability to drain and filter out problematic proteins, so they accumulate in the brain, where they could contribute to neurodegenerative conditions like dementia.

"Some of these diseases, at least in some cases, might even be starting or being partly triggered by degeneration of the lymphatics," says Irani. Da Mesquita cautions that much larger studies involving thousands of people are needed to verify such potential links. Nevertheless, these early findings hint at a tantalising possibility. "There's this big therapeutic angle," says Irani. "Can we cure dementia by trying to think of how you might improve the lymphatic drainage from the brain?"

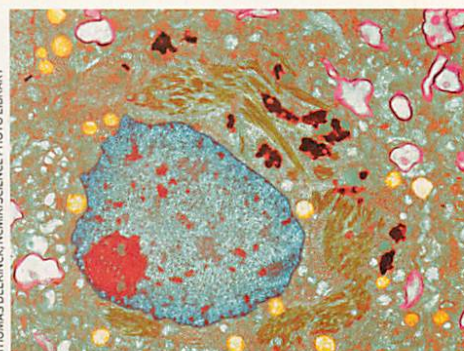
Indeed, some researchers are already pursuing this idea. In a study in May, Rong Hu at the Army Medical University in China and colleagues recruited 26 people with Alzheimer's disease to undergo surgery to their cervical lymphatic vessels that boosted the drainage of fluid from the brain. One month later, the participants performed slightly better in memory, attention and language tests, with about 60 per cent of their caregivers noticing such improvements. But the study was small and lacked a control group of participants with Alzheimer's disease who didn't undergo the procedure, plus the surgery is highly invasive.

In search of a simpler method, Gou Young Koh at the Korea Advanced Institute of Science and Technology in South Korea and his colleagues recently found that gently massaging lymphatic vessels around the face and neck in a very specific pattern could boost brain fluid drainage in old mice to the levels seen in young mice. The researchers didn't assess how this affected the animals' cognitive abilities, but they plan to explore whether the technique can improve such abilities in mice with features of Alzheimer's disease.

Cancer is another key area where tapping into our meningeal lymphatic system could help. After learning about the 2015 discoveries, Eric Song at Yale University and his colleagues wondered whether the VEGFC approach might boost immune responses to glioblastoma, the deadliest form of brain cancer. To put this idea to the test, they injected the genetic code for VEGFC into the brains of mice with this tumour, along with an immunotherapy called anti-PD-1. A control group got just the anti-PD-1 injection and the genetic code for a fluorescent protein that



SUSUMU NISHINAGA/SCIENCE PHOTO LIBRARY



THOMAS DEERINCK, NCMI/SCIENCE PHOTO LIBRARY

Above: Lymph nodes remove tau proteins from the brain, which can clump together in neurons in Alzheimer's disease

Below: People with Alzheimer's disease could benefit from improved lymphatic drainage



ELISABETH SCHNEIDER CHARPENTIER/SCIENCE PHOTO LIBRARY

"RESEARCHERS ARE HARNESSING LYMPH NODES TO GROW ORGAN TRANSPLANTS"

the meningeal lymphatic vessels and enhanced the animals' performance in memory and learning tests. The same approach partly reversed cognitive decline in mice with features of Alzheimer's disease, the most common form of dementia. It seemed to work by boosting clearance of a toxic form of beta-amyloid, a protein implicated in the condition.

Boosting drainage

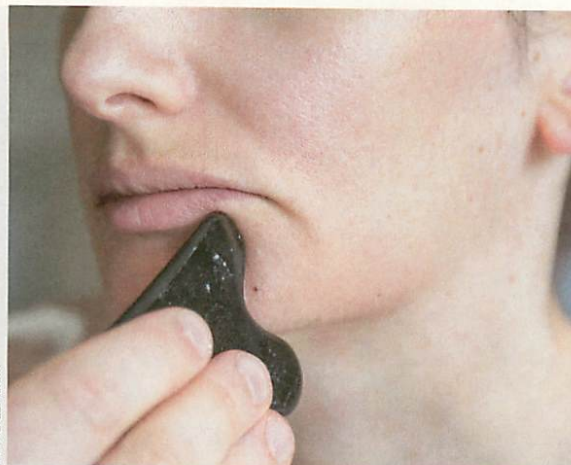
Such findings have prompted scientists to wonder whether lymphatic ageing also impairs cognition in people. There are very early hints that it does. In a study out earlier this year, Sarosh Irani at the Mayo Clinic, Da Mesquita and their colleagues analysed lymph extracted from the cervical lymph nodes of 25 people aged between 22 and 84 who didn't have neurodegenerative disease. They discovered that levels of tau, a protein linked to Alzheimer's, seem to decrease in lymph

doesn't affect the lymphatic system. Three months later, about 20 per cent of mice from the latter group had survived. In the former, the figure was 80 per cent – in some cases, their tumours had completely disappeared. "We were over the moon," says Song.

Further analysis revealed that the treatment probably worked by enhancing the drainage of cancer proteins through the meningeal lymphatic vessels into the cervical lymph nodes, enhancing the activation of cancer-killing T-cells that then migrated into the brain, says Song. That's not all. Mice that survived their initial tumours were also able to resist a subsequent injection of brain cancer cells, suggesting the treatment generated lasting immunity.

But there is one problem with the VEGFC approach. It can also enhance the growth of blood vessels, which is known to help tumours spread to distant sites. To address this, Song and his colleagues at the biotechnology start-up Rho Bio have developed an altered version of VEGFC that only acts on lymphatic vessels. They are now testing its safety in non-human primates and hope to trial it for a range of conditions, including brain cancer and dementia in humans.

Lymphatic therapies could treat cancers beyond the brain, too. Natalie Livingston at Massachusetts General Hospital and her colleagues have developed injectable artificial lymph nodes that boost immune responses against tumours in mice. The approach involves extracting T-cells from a mouse and



The traditional Chinese technique of gua sha supposedly stimulates the lymphatic system

mixing them with a gel containing a cocktail of proteins that activate T-cells to destroy the cancer. This mixture is then injected back into the mouse to create a factory of cancer-killing T-cells – the artificial lymph node. "It's about half a centimetre in diameter, and forms just like a little mosquito bite under the skin," says Livingston. This procedure nearly halved the size of colon tumours in mice, and improved survival rates compared with a placebo implant. And it worked just as well against melanoma, the deadliest form of skin cancer.

Internal bioreactor

Lymphatic therapy doesn't stop there, though. Other researchers are harnessing lymph nodes to grow organ transplants. The approach involves implanting cells from a donor organ into a lymph node near the failing organ. Nourished by the lymphatic system's rich supply of blood, the cells grow into a mini organ that eventually replaces the lymph node and supplements the damaged tissue. "It turns out that the lymphatic system and lymph nodes are just incredibly effective bioreactors for a variety of tissue types," says Michael Hufford, CEO at Lygenesis.

In experiments with mice, his team has shown that such mini organs can reduce symptoms of liver disease and kidney disease, as well as type 1 diabetes where there is damage to the pancreas. The body can afford to lose one lymph node and the rest of the lymphatic system is unaffected, so the technique doesn't seem to cause any side effects, says Hufford. His team is now trialling the approach in 12 people with end-stage liver disease, four of whom have now received their implants. The results should be available within the next two years.

It is already clear that lymphatic biology has huge and exciting potential in treating disease. But there is still much we don't understand. One key question is the degree to which

lymphatic vessels differ across the body. In an ongoing study that has yet to be published, Caron is exploring how gene activity varies between lymphatic vessels associated with eight organs, including the lungs, liver and heart. It is the first comparative study. "Prior studies looked at gene expression profiles in just one organ," says Caron. "We found a lot of very fascinating differences that really underscore just how different lymphatic vessels are throughout the body."

In another ongoing study, Caron is generating a list of protein receptors on lymphatic vessels that are potential targets for drugs. This could reveal new ways of manipulating our lymphatic system – it may even uncover previously unknown ways that existing treatments work. "Lots of drugs might be acting on lymphatics and we just didn't know it," says Caron. Supporting this idea, her team has recently shown that many migraine drugs boost the drainage of brain fluid through meningeal lymphatic vessels. "It's awe-inspiring when you consider just how much we need to learn about this understudied vascular system that is so critical for keeping us healthy," she says.

All this may leave you wondering whether popular "lymphatic drainage" practices might actually work. Here the news is less rosy. Admittedly, face rolling can temporarily sharpen your jawline by increasing fluid drainage, but there is little good-quality evidence to back up health claims for things like reduced inflammation or cellulite removal. So, although I have come to marvel at my lymphatic system, I won't be including a jade rolling pin in my morning routine. Sorry, Grandma! ■



Carissa Wong is a health reporter at New Scientist



BODY & BRAIN

Sound, light may fight Alzheimer's

Plaques shrank in mice's brains after clicks and flashes

BY LAURA SANDERS

Fast clicking sounds can boost brain-power in mice with signs of Alzheimer's disease. Like flickering lights, these external sounds spur a type of brain wave that seemed to sweep disease-related plaques from mice's brains, researchers report in the March 14 Cell.

It's too early to say whether flickers and clicks could help people with Alzheimer's. If so, the treatment would represent a fundamentally new way to target the neurodegenerative disease.

An earlier study by MIT neuroscientist Li-Huei Tsai and colleagues focused on the eyes. Lights that flickered exactly 40 times a second kicked off gamma waves, a brain wave thought to happen during concentration. In mice, the brain waves seemed to somehow

reduce amyloid-beta, a protein that piles up in the brains of Alzheimer's patients (*SN*: 1/21/17, p. 13). But A-beta was reduced only in the part of the brain that handles vision — an area not thought to be key to Alzheimer's progression.

Sounds that hum at a rate of 40 clicks per second, or 40 hertz, also spur gamma waves that appeared to clear mice's A-beta, Tsai and colleagues now find. But that happens in a more relevant brain area: the hippocampus, which is involved in memory and affected by Alzheimer's.

A daily hour of fast clicks for a week also improved the memories of mice genetically engineered to have signs of Alzheimer's. Compared with mice that heard randomly spaced clicks, mice that listened to 40-hertz clicks found a hidden platform in a water maze faster and better recognized a previously seen object.

"That's a very exciting finding, that it can actually affect cognition," says neuroscientist Tara Tracy of the Buck Institute for Research on Aging in Novato, Calif.

Sound-triggered gamma waves appear to kick off other beneficial changes, too. Brain levels of a harmful form of tau,

another protein implicated in Alzheimer's, dropped, and blood vessels in the brain expanded, perhaps easing A-beta's disposal. Immune cells called microglia also grew more active, attacking A-beta.

When mice were treated with both flickering lights and clicks, the effect was even stronger, Tsai says. There were fewer A-beta plaques across a big stretch of the brain, including the hippocampus and the prefrontal cortex, an area important for complex thinking. And microglia swarmed into a feeding frenzy. "Microglia pile up on each other, all congregated around the amyloid plaques," Tsai says.

It's not yet clear how increasing gamma waves led to these effects. "How is this particular stimulation affecting all of these properties?" Tracy asks.

Also unknown is whether results from mice that mimic a rare and aggressive form of Alzheimer's apply to people. Tsai has cofounded a company, Cognito Therapeutics, that is testing the light-and-sound approach in older people with mild to moderate cognitive impairment. So far, Tsai says, "we haven't seen any undesirable effects." ■

"THE RAREST OF BOOKS: A COMBINATION OF PAGE-TURNING DISCOVERY AND
REMARKABLY READABLE SCIENCE JOURNALISM." —MARK HYMAN, M.D.

THE ANGEL AND THE ASSASSIN

THE TINY
BRAIN CELL
THAT CHANGED
THE COURSE OF
MEDICINE

DONNA JACKSON
NAKAZAWA

2020

Multisensory gamma stimulation promotes glymphatic clearance of amyloid

<https://doi.org/10.1038/s41586-024-07132-6>

Received: 21 February 2022

Accepted: 25 January 2024

Published online: 28 February 2024

Open access

 Check for updates

Mitchell H. Murdock¹, Cheng-Yi Yang¹, Na Sun^{2,3}, Ping-Chieh Pao¹, Cristina Blanco-Duque¹, Martin C. Kahn¹, TaeHyun Kim¹, Nicolas S. Lavoie¹, Matheus B. Victor¹, Md Rezaul Islam¹, Fabiola Galiana¹, Noelle Leary¹, Sidney Wang¹, Adele Bubnys¹, Emily Ma¹, Leyla A. Akay¹, Madison Sneve^{4,5,6}, Yong Qian^{4,5,6}, Cuixin Lai⁷, Michelle M. McCarthy⁸, Nancy Kopell⁸, Manolis Kellis^{2,3}, Kiryl D. Piatkevich^{4,5,6,7}, Edward S. Boyden^{4,5,6} & Li-Huei Tsai^{1,3}✉

The glymphatic movement of fluid through the brain removes metabolic waste^{1–4}. Noninvasive 40 Hz stimulation promotes 40 Hz neural activity in multiple brain regions and attenuates pathology in mouse models of Alzheimer's disease^{5–8}. Here we show that multisensory gamma stimulation promotes the influx of cerebrospinal fluid and the efflux of interstitial fluid in the cortex of the 5XFAD mouse model of Alzheimer's disease. Influx of cerebrospinal fluid was associated with increased aquaporin-4 polarization along astrocytic endfeet and dilated meningeal lymphatic vessels. Inhibiting glymphatic clearance abolished the removal of amyloid by multisensory 40 Hz stimulation. Using chemogenetic manipulation and a genetically encoded sensor for neuropeptide signalling, we found that vasoactive intestinal peptide interneurons facilitate glymphatic clearance by regulating arterial pulsatility. Our findings establish novel mechanisms that recruit the glymphatic system to remove brain amyloid.

The glymphatic system is thought to have a critical role in clearing metabolic waste from the brain¹. According to the glymphatic model, arterial pulsation drives cerebrospinal fluid (CSF) along an interconnected network of perivascular spaces surrounding blood vessels in the brain^{2,9}, and the water channel aquaporin-4 (AQP4) localized on astrocytic endfeet facilitates exchange between CSF and interstitial fluid (ISF)^{2,10}. Glymphatic transport has been shown to clear parenchymal metabolites, including pathogenic proteins such as amyloid^{3,11}. Ageing and Alzheimer's disease impair the fitness of glymphatic clearance by attenuating arterial pulsation¹², reducing CSF–ISF exchange^{11,13}, and diminishing lymphatic drainage, leading to the pathogenic accumulation of amyloid and tau. Therapeutic interventions to promote glymphatic clearance have been hypothesized to alleviate Alzheimer's disease pathology¹⁴.

In previous studies, we found that 1 h of optogenetic-driven 40 Hz stimulation attenuated pathogenic amyloid burden in a mouse model of Alzheimer's disease compared with non-gamma control stimulations⁵. To extend the clinical translatability of this intervention, we explored noninvasive approaches to induce 40 Hz neural activity. In humans and mice, sensory stimuli at specific frequencies can be used to promote neural activity corresponding to the sensory stimulation¹⁵. Noninvasive 40 Hz stimulation promotes 40 Hz neural activity in multiple brain regions, including the prefrontal cortex^{6,7,16}, and we and others found that noninvasive 40 Hz sensory stimulation attenuates amyloid burden in Alzheimer's disease model mice⁸. In particular, multisensory 40 Hz stimulation (that is, combined light and sound stimulation)

attenuates amyloid burden throughout the cortex, including the prefrontal cortex⁶.

Brain metabolites are removed via glial-mediated phagocytosis and vascular-mediated clearance¹⁷. It has been unclear whether 40 Hz stimulation attenuates amyloid burden through glymphatic routes. Previous reports suggest that CSF influx to the brain is increased during anaesthesia and sleeping^{1,18}, indicating that distinct brain rhythms influence glymphatic transport. Arteriolar vasomotion, which drives CSF influx^{2,9} and paravascular clearance¹⁹, is coupled to gamma oscillations²⁰. We hypothesized that noninvasive gamma stimulation may clear amyloid in part via glymphatic transport.

Gamma stimulation promotes glymphatic clearance

We found that 40 Hz multisensory audio-visual stimulation increased 40 Hz local field potential power in frontal cortex areas of 6-month-old 5XFAD mice (Extended Data Fig. 1a–c), as expected on the basis of prior reports in mice⁶ and humans^{8,16}. In separate cohorts of 6-month-old 5XFAD mice, 40 Hz stimulation attenuated amyloid burden compared with no stimulation, 8 Hz stimulation and 80 Hz stimulation controls (Fig. 1a,b). Reductions in amyloid by 40 Hz multisensory stimulation were not associated with alterations in plasma corticosterone (Extended Data Fig. 1d), time spent moving (Extended Data Fig. 1e–f) or sleep architecture (Extended Data Fig. 1g,h), including neither rapid eye movement (REM) nor non-REM (NREM) sleep states (Extended Data Fig. 1i–k).

¹Department of Brain and Cognitive Sciences and the Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA. ²MIT Computer Science and Artificial Intelligence Laboratory, Cambridge, MA, USA. ³Broad Institute of MIT and Harvard, Cambridge, MA, USA. ⁴Departments of Biological Engineering and Brain and Cognitive Sciences, McGovern Institute, Cambridge, MA, USA. ⁵Koch Institute, Massachusetts Institute of Technology, Cambridge, MA, USA. ⁶Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA, USA. ⁷School of Life Sciences, Westlake University, Westlake Laboratory of Life Sciences and Biomedicine, and Westlake Institute for Advanced Study, Hangzhou, China. ⁸Department of Mathematics and Statistics, Boston University, Boston, MA, USA. ✉e-mail: lhtsai@mit.edu

Health

Light flashes may slow Alzheimer's by making the brain remove toxins

Clare Wilson

A NEW explanation has emerged for why an experimental treatment for Alzheimer's disease involving sounds and flickering lights may help slow cognitive decline. The frequencies involved seem to ramp up the brain's waste disposal networks, which boosts the clearance of beta-amyloid and other toxic proteins that contribute to memory and concentration problems.

"Once we understand the mechanism, we can probably figure out how to further optimise this whole concept and improve the efficacy," says Li-Huei Tsai at the Massachusetts Institute of Technology.

The treatment involves exposing people to lights flickering at a frequency of 40 times a second, or 40 hertz, and a low-pitched sound, also at 40 Hz. Typically, the stimulation is given for an hour a day.

Key to the novel approach is that large networks of brain cells naturally fire in sync with each other at different

Highlighted neurons in a mouse brain that seem to boost toxin clearance

frequencies – known as brainwaves. Brainwaves of around 40 Hz are often seen when people are concentrating and when they are forming or accessing memories.

As it was known that visual or auditory stimulation at a certain frequency can boost brainwaves at that same frequency, in 2016, Tsai's team investigated if stimulation at 40 Hz might boost cognitive abilities in people with Alzheimer's.

40 Hz

Frequency of bursts of light and sound used to treat Alzheimer's

Their group and others showed that this lowered amyloid build-up in mice with a version of Alzheimer's and had cognitive benefits in small trials in people with the condition. But it wasn't clear how the treatment works, with one idea being that it boosts the function of the brain's immune cells.

Now, it seems the lights and sound work by enhancing the function of the brain's drainage network, also known as the glymphatic system.

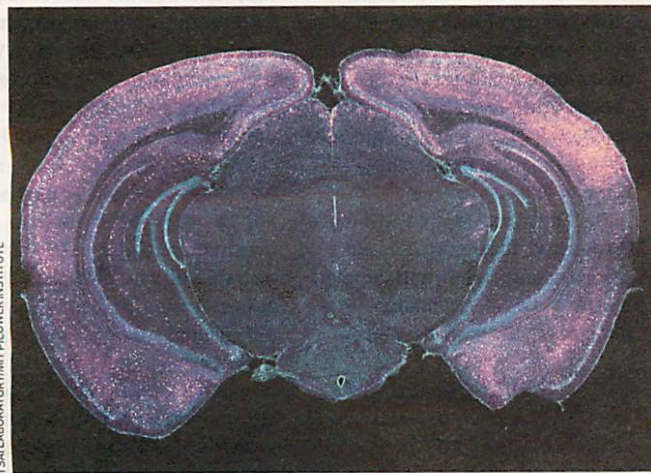
In the latest work, Tsai's team did experiments to study the treatment's mechanism in mice that had been genetically altered so that they usually develop a build-up of amyloid as they get older and have worse memories than typical mice.

As expected, when the animals were exposed to the light and sounds, they had less amyloid. The new finding was that during the treatment, they had a greater amount of cerebrospinal fluid entering the brain and more waste fluid leaving it through the glymphatic vessels (*Nature*, doi.org/mjjs).

This seemed to be happening because nearby blood vessels were pulsating more, which could help push the glymphatic fluid through its vessels, and because of more water flowing into the glymphatic system.

The team also found that the activity of brain cells known as interneurons seemed to be triggering the increased glymphatic flow by releasing a molecule called vasoactive intestinal peptide. When the team chemically blocked this molecule's production, the treatment no longer boosted amyloid clearance.

Maiken Nedergaard at the University of Rochester in New York, who helped discover the glymphatic system, says the finding fits with what we know about the system. "The brain, blood and cerebrospinal fluid are all contained within the skull. If the blood volume expands, the volume of the cerebrospinal fluid has to move since brain tissue is not compressible." A better understanding of toxin clearance in the brain could help us unlock its therapeutic potential, she says. ■



News

Cancer

Light and sound therapy may prevent 'chemo brain'

Clare Wilson

AN EXPERIMENTAL treatment for Alzheimer's disease that involves flickering lights and low-pitched sound might also help prevent cognitive problems after cancer treatment, a study in mice suggests.

For Alzheimer's disease, the method has been shown to ease people's memory and concentration problems in small trials, but it is still being investigated in larger studies.

The lights flicker 40 times a second, or 40 hertz, with the sound also having a frequency of 40 Hz. This frequency was selected because people with Alzheimer's have a lower intensity of 40 Hz brainwaves, which are linked with memory processing. Recent research suggests such waves bring brain benefits, including increasing the activity of immune cells and boosting its drainage system.

Curious about whether the approach might also help people who get memory and concentration problems after chemotherapy, sometimes called chemo brain, Li-Huei Tsai at the Massachusetts Institute of Technology and her colleagues turned to mice.

They found that cancer-free mice exposed to the lights and sounds for 1 hour a day while being dosed with the chemotherapy drug cisplatin showed less of a decline in mental sharpness – assessed by seeing how much interest they showed in novel or familiar objects – than those that just had chemotherapy.

The therapy also lowered brain inflammation, reduced DNA damage and lessened the loss of myelin, the insulation around nerve cell fibres (*Science Translational Medicine*, doi.org/mkkw).

Nazanin Derakhshan at the University of Reading, UK, says the method needs to be tested in people. If the treatment reduces cell death in the brain, it could promote the survival of cancer cells there, she says. ■

The Potential Mechanisms of High-Velocity, Low-Amplitude, Controlled Vertebral Thrusts on Neuroimmune Function: A Narrative Review

Medicina

May 27, 2021; Vol. 57; No. 6; pp. 536

Heidi Haavik, Imran Khan Niazi, Nitika Kumari, Imran Amjad, Jenna Duehr, Kelly Holt: This study cites 301 references. HVLA = High-Velocity Low Amplitude

"This review aims to elucidate the underlying mechanisms that can explain the HVLA controlled vertebral thrust-neuroimmune link and discuss what this link implies for clinical practice and future research needs."

KEY POINTS FROM THIS ARTICLE:

- 1) "The current COVID-19 pandemic has necessitated the need to find healthcare solutions that boost or support immunity."
- 2) "There is some evidence that high-velocity, low-amplitude (HVLA) controlled vertebral thrusts have the potential to modulate immune mediators."
 - 21 published papers were found that explored the impact of HVLA controlled vertebral thrusts on neuroimmune markers; 18 found a significant effect.
[Important] 18/21 = 86%
 - "HVLA controlled vertebral thrusts influence the levels of immune mediators in the body, including neuropeptides, inflammatory markers, and endocrine markers."
 - The mechanisms most for how HVLA manipulation could impact immune markers "are most likely due to the known changes in proprioceptive processing that occur within the central nervous system (CNS), in particular within the prefrontal cortex." **[Key Point]**
 - "The prefrontal cortex is involved in the regulation of the autonomic nervous system, the hypothalamic-pituitary-adrenal axis and the immune system."
- 3) "There are biologically plausible direct and indirect mechanisms that link HVLA controlled vertebral thrusts to the immune system, suggesting HVLA controlled vertebral thrusts have the potential to modulate immune function." **[Key Point]**
- 4) Pain-related stress alters neuro-immune interactions.
 - Stress-induced sympathetic nervous system activity alters vertebral motor control.

12) "Adjusting [vertebral subluxation or joint dysfunction] problems in the spine alters the afferent input from the small paraspinal muscles around the spine and skull to the brain." **[Key Point]**

- "This affects how the CNS processes and integrates all subsequent sensory input, such that the brain senses more accurately what is happening in and around the body, so there is improved brain–body awareness, adaptability, and coordination and therefore, better function."
- "One of the keys to this proposed model is the proprioceptive role played by the small intervertebral muscles closest to the spine and skull."
- "Maladaptive changes in neural function affect the way we respond to our internal and external environment, potentially leading to a lack of adaptability and resilience and the development of symptoms, diseases, and disorders."
- "By adjusting [vertebral subluxation or joint dysfunction] problems, the associated rapid stretch of the deep paraspinal muscles enhances proprioceptive afferent input from these muscles; this is thought to improve brain–body awareness, adaptability, and resilience."
- "It is quite clear that HVLA controlled vertebral thrusts can impact vital parts of the brain involved in creating internal and external schemas or maps of the internal and external environment."

13) "There is evidence suggesting that chiropractic HVLA controlled vertebral thrusts have a significant effect on the prefrontal cortex." **[Key Point]**

- "The prefrontal cortex is a high-level processing centre of the CNS and is described as the executive controller of the CNS."
- "The prefrontal cortex is also known to be vital for proper immune system function."
- "The prefrontal cortex is critically involved in regulating the autonomic nervous system, the endocrine system and therefore the immune system."
- "The prefrontal cortex regulates the ANS, directly activates the vagal cholinergic anti-inflammatory system, and impacts the hormonal system, in particular the hypothalamus–pituitary–adrenal (HPA) axis." **[Key Point]**
- "HVLA controlled vertebral thrusts influence the CNS, in particular the prefrontal cortex, which in turn has an impact on the ANS, HPA axis, and immune system, this provides a biologically plausible mechanism for how HVLA controlled vertebral thrusts may impact immune function." **[Key Point]**

Neuroplastic Responses to Chiropractic Care: Broad Impacts on Pain, Mood, Sleep, and Quality of Life

Brain Science

November 7, 2024; Vol. 14; No. 11; Article 1124

Heidi Haavik, Imran Khan Niazi, Imran Amjad, Nitika Kumari, Usman Ghani, Moez Ashfaq, Usman Rashid, Muhammad Samran Navid, Ernest Nlandu Kamavuako, Amit N. Pujari, Kelly Holt; the authors are from the New Zealand College of Chiropractic, Auckland University, Aalborg University (Denmark), Riphah International University (Pakistan), University of Hertfordshire (UK), Radboud University Medical Center (The Netherlands), King's College (UK), and the University of Aberdeen (UK). This study cites 169 references.

"This study evaluated the underlying neurophysiological mechanisms of chiropractic care using a multifaceted approach, recording behavioral, subjective, and neurophysiological data."

The objectives of this study were to "elucidate the mechanisms of chiropractic care using resting electroencephalography (EEG), somatosensory evoked potentials (SEPs), clinical health assessments (Fitbit), and Patient-Reported Outcomes Measurement Information System (PROMIS-29 [attached at end of review])."

- EEG and SEPs were collected pre and post the first intervention and post 4 weeks of intervention.
- PROMIS-29 (Patient-Reported Outcomes Measurement Information System) was measured pre and post 4 weeks to measure health-related quality of life (QOL).

PROMIS-29 assesses pain intensity using a single 0–10 numeric rating item and seven health domains (physical function, fatigue, pain interference, depressive symptoms, anxiety, ability to participate in social roles and activities, and sleep disturbance), using four items per domain.

The PROMIS-29 is a reliable and valid instrument to assess the impacts of health care and track changes in health over time. [attached at end of review]

- Fitbit data was recorded continuously for four weeks.

76 people with chronic low back pain (CLBP) were randomized to a chiropractic group (n=38) and a control group (n=38).

The chiropractic care included 4 weeks (approximately 3 times per week) of manual high-velocity low-amplitude (HVLA) adjustments to the spine or pelvis identified as being subluxated.

asymmetric intervertebral muscle tension, and altered joint play and end feel.”

3) “Vertebral subluxation involves altered afferent input from muscle spindles in the paraspinal muscles at the subluxated levels.”

- “This disruption in sensory input can cause maladaptive central neural plastic changes, resulting in impaired sensorimotor integration and control.”
- “These maladaptive central plastic changes in the central nervous system (CNS) can worsen over time, which is thought to contribute to dysfunction, pain, and other symptoms by disrupting normal sensorimotor control.”
 - “These maladaptive central plastic changes are thought to be reversed or improved through chiropractic care.”

4) “Over the past decade, multiple studies have shown that chiropractic adjustments alter central neural function and improve the ability of peripheral muscles to produce force.”

5) “Chiropractic adjustments have been shown to influence various aspects of neurophysiology, including somatosensory processing, sensorimotor integration, and motor control, all crucial for executing motor tasks accurately and recovering from central nervous system injuries.”

- Chiropractic care drives neuroplastic brain changes in “structures such as the primary somatosensory cortex, primary motor cortex, prefrontal cortex [PFC], and cerebellum.” **[Important]**
- “Improvements in either prefrontal cortex or cerebellar function could explain many of the clinically relevant changes that have been documented following chiropractic care, such as improved joint position sense error, cortical processing, reflex excitability, reaction time, cortical sensorimotor integration, motor control, upper and lower limb muscle functions, and pain changes.”
- Both the PFC and cerebellum influence emotional control and mental health, as well as neuroendocrine responses, autonomic nervous system function, and immune function. **[Very Important]**

6) Findings:

- “Resting EEG showed a significant increase in Theta, Alpha and Beta, and a significant decrease in Delta in the chiropractic group post intervention.”
- “Significant improvements in light sleep stage were observed in the chiropractic group along with enhanced overall quality of life post 4 weeks, including significant reductions in anxiety, depression, fatigue, and pain.”

- Fitbit data showed “significant differences between the control and chiropractic groups, with the chiropractic group showing significantly increased light sleep stage duration.”

“Fitbit data revealed that the light sleep stage significantly increased in duration in the chiropractic group over the four weeks of this study. Improved sleep length is consistent with existing research indicating that chiropractic adjustments help to alleviate tension from within the body, encouraging blood flow, enabling the muscles to relax and making it easier to also mentally relax and fall asleep.”

“As the participants in this study reported improvements in CLBP over the four weeks of the study, the chiropractic adjustments appear to have positively influenced pain processing, another factor known to improve sleep quality.”

“The current study supports this finding and suggests that chiropractic care does have an impact on sleep quality.”

- The PROMIS-29 showed an overall improvement in quality of life in the chiropractic care group compared to the control group.

“Chiropractic care had a significant effect on the PROMIS-29 domains of anxiety, depression, fatigue, pain intensity, and pain interference.”

7) Alpha rhythms are lower in individuals with chronic pain, compared with healthy controls.

- “In the current study, there were clear increases in Alpha power connectivity involving the frontal lobes.”
- “The Alpha power frequency changes found in this study likely reflect changes in pain perception in the chiropractic patients.”

8) The prefrontal cortex (PFC) plays a crucial role in executive functions.

- “Any alteration in prefrontal activity after chiropractic care could potentially elucidate or establish connections with the diverse enhancements in neural function.” **[Very Important]**
- Chiropractic adjustments altered proprioceptive input from the paraspinal tissues which may be powerful enough to alter processing within the PFC.
- Chiropractic adjustments may activate the descending pain inhibitory pathway.

- "It is well known that the descending modulatory pain pathway is malfunctional in chronic pain patients."

9) "[This study supports] the notion that part of the mechanisms of chiropractic care is that it might improve the descending modulatory pain pathway in CLBP patients, and this may be why it has been found to be clinically effective for both acute and CLBP problems and is a recommended option in relevant clinical guidelines."

- The HVLA adjustments of vertebral subluxations over the four weeks has likely altered the sensory stimuli coming from the spine.
- "Their internal narrative sense of self appears to have been altered over the four weeks of care, with changes in the emotional processing associated with internal and external stimuli which are influencing how they perceive themselves and the world around them, reflected by the reported decrease in fatigue, depression, anxiety, and pain."

10) "Numerous previous studies have also reported enhanced quality of life and well-being following chiropractic care, possibly linked to reduced pain and improved mobility."

- "Several studies have found quality of life improvements beyond just improvements in pain or pain related disability, similar to what has been documented in the current study." **[Important]**

11) "Pain and pain-related diseases are considered to be the leading causes of disability and the greatest disease burden worldwide."

- There is an association between depression and anxiety, and chronic pain.
- "Chiropractic care not only improved symptoms of pain and unpleasantness, but also resulted in improvements in fatigue, anxiety, and depression."
- "Chiropractic care may potentially prevent pain from becoming chronic and the current study provides some insight into the mechanisms of this." **[Important]**
- "Having ongoing maintenance chiropractic care (i.e., after the initial care period improves symptoms) results in less days of pain and improved QOL on an ongoing basis."
- Long-term chiropractic care for CLBP may "significantly lower rates of hospitalisations, emergency department visits, advanced diagnostic imaging, specialist visits, lumbosacral surgery, interventional pain medicine techniques, i.e., a reduced escalation of care."

- “Ongoing chiropractic care, even after the initial pain symptoms improve, will likely continue to impact somatosensory networks, thus maintaining positive neuroplastic changes that prevent such escalations of care, worsening of symptoms, and decreases in QOL.” **[Very Important]**

12) Possible Mechanisms:

- The contemporary model of the vertebral subluxation suggest that it can lead to abnormal multisensory processing and lead to poor motor control of the vertebral column and other muscles in the body.
- “The current findings provide compelling evidence for why chiropractic care, that includes the HVLA adjustments directed towards subluxated segments, can impact not only chronic pain symptoms, but also influence anxiety and depression symptoms.”
- There is evidence that proprioceptive input from the deep paraspinal muscles is essential for intervertebral motor control.
 - “[A] change in proprioceptive input to the CNS may ultimately be responsible for the development and maintenance of musculoskeletal pain syndromes.” **[Important]**
 - Over time, abnormal or reduced proprioceptive input from dysfunctional spinal regions (i.e., vertebral subluxations) would be capable of causing ongoing central maladaptive changes. **[Very Important]**
 - “With ongoing poor motor control, as the CNS is less accurately aware of what is occurring at the vertebral column level (due to the reduced/abnormal proprioceptive inputs from that paraspinal region); this would likely lead to repeated microtraumas at the subluxated spinal levels as well as other areas of the body that require accurate sensory input from the spine, such as accurate upper and lower limb motor control.”
 - “Over time, it makes sense that this could explain the development of chronic musculoskeletal pain problems.”
 - “This contemporary model also explains how spinal adjustments, i.e., HVLA thrusts delivered to subluxated spinal segments, can improve vertebral column motor control by bombarding the CNS with mechanoreceptive input from the segments that are dysfunctional.” **[Very Important]**
 - “Once the CNS is more accurately aware of the vertebral column, and it changes the body and world schemas, and therefore controls spinal

movement better this will also naturally improve whole body perception and functions as well."

"This research contributes significantly to the scientific discourse on the mechanisms of chiropractic care, paving the way for further investigations into its holistic impacts on health and well-being." **[Key Point]**

13) Conclusions:

- "Many health benefits of chiropractic care are due to altered brain activity." **[Key Point]**
- "These results further our understanding of the multifaceted impacts of chiropractic care on various health parameters, as well as improving our understanding about the mechanisms for these changes."
- "These results provide support for the contemporary model of the vertebral subluxation and help to explain how chiropractic HVLA adjustments impact the way the brain integrates both interoceptive and exteroceptive sensory information in a multimodal fashion, including the consideration of past memories and future expectations." **[Key Point]**
- This study suggests that "chiropractic care alters a person's narrative sense of self, that can enable that person to think, feel and function better." **[Key]**

Comments From Dan Murphy:

Heidi Haavik and her team of researchers are publishing the most important neurophysiological research for the chiropractic profession, and I believe that all chiropractors should financially support their efforts:

<https://chiropractic.ac.nz/research/support-our-research/>

We have reviewed these other studies by Dr. Haavik:

Article Review 25-07:

Cervical Spine Manipulation Alters Sensorimotor Integration

Article Review 26-22:

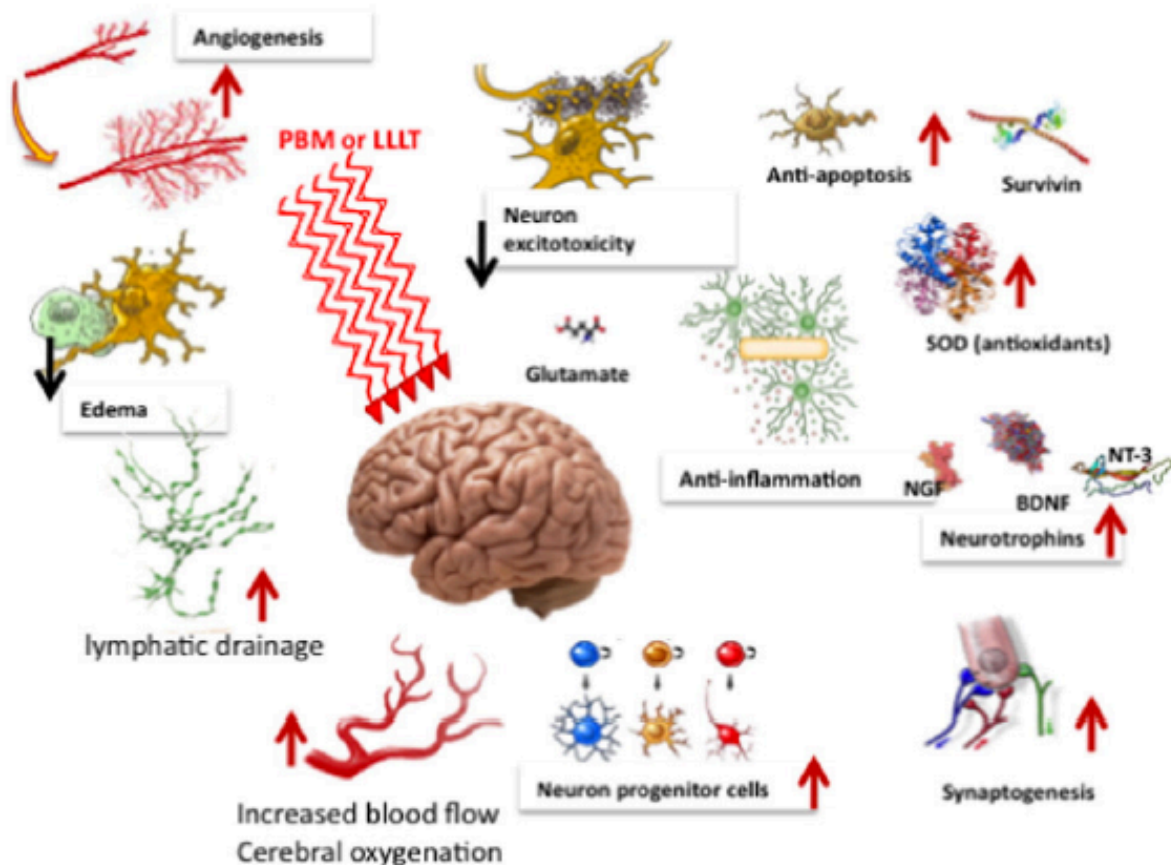
The Potential Mechanisms of High-Velocity, Low-Amplitude, Controlled Vertebral Thrusts on Neuroimmune Function

Heidi's book, The Reality Check: A Quest to Understand Chiropractic from the Inside Out, is also a must read for chiropractors. My signed copy cover page is attached below:

Shining Light on the Head: Photobiomodulation for Brain Disorders
BBA Clinical (Biochimica et Biophysica Acta Journal)
2016; No. 6; pp. 113-124

Michael R. Hamblin

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston;
Department of Dermatology, Harvard Medical School, Boston;
Harvard-MIT Division of Health Sciences and Technology



Photobiomodulation (PBM) describes the use of red or near-infrared light to stimulate, heal, regenerate, and protect tissue that has either been injured, is degenerating, or else is at risk of dying.

One of the organ systems of the human body that is most necessary to life, and whose optimum functioning is most worried about by humankind in general, is the **brain**.

The brain suffers from many different disorders that can be classified into three broad groupings: traumatic events (**stroke**, **traumatic brain injury**, and global ischemia), degenerative diseases (**dementia**, **Alzheimer's** and **Parkinson's**), and psychiatric disorders (**depression**, **anxiety**, **post traumatic stress disorder**). There is some evidence that all these seemingly diverse conditions can be beneficially affected by applying light to the head.

There is even the possibility that PBM could be used for **cognitive enhancement** in normal healthy people.

Published in final edited form as:

PM R. 2010 December ; 2(12 Suppl 2): S292–S305. doi:10.1016/j.pmrj.2010.10.013.

Role of Low-Level Laser Therapy in Neurorehabilitation

Javad T. Hashmi, MD,

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, Harvard Medical School, Boston, MA Disclosure: nothing to disclose

Ying-Ying Huang, MD,

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, Harvard Medical School, Boston, MA; Aesthetic and Plastic Center, Guangxi Medical University, Nanning, PR China Disclosure: nothing to disclose

Bushra Z. Osmani, MD,

Aga Khan Medical College, Karachi, Pakistan Disclosure: nothing to disclose

Sulbha K. Sharma, PhD,

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA Disclosure: nothing to disclose

Margaret A. Naeser, PhD, LAc, and

VA Boston Healthcare System, Boston, MA; Department of Neurology, Boston University School of Medicine, Boston, MA Disclosure: nothing to disclose

Michael R. Hamblin, PhD

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, Harvard Medical School, 40 Blossom St BAR414, Boston, MA 02114 ; Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA. Address correspondence to: M.R.H.; hamblin@helix.mgh.harvard.edu

Abstract

This year marks the 50th anniversary of the discovery of the laser. The development of lasers for medical use, which became known as low-level laser therapy (LLLT) or photobiomodulation, followed in 1967. In recent years, LLLT has become an increasingly mainstream modality, especially in the areas of physical medicine and rehabilitation. At first used mainly for wound healing and pain relief, the medical applications of LLLT have broadened to include diseases such as stroke, myocardial infarction, and degenerative or traumatic brain disorders. This review will cover the mechanisms of LLLT that operate both on a cellular and a tissue level. Mitochondria are thought to be the principal photoreceptors, and increased adenosine triphosphate, reactive oxygen species, intracellular calcium, and release of nitric oxide are the initial events. Activation of transcription factors then leads to expression of many protective, anti-apoptotic, anti-oxidant, and pro-proliferation gene products. Animal studies and human clinical trials of LLLT for indications with relevance to neurology, such as stroke, traumatic brain injury, degenerative brain disease, spinal cord injury, and peripheral nerve regeneration, will be covered.

also reported that, in patients with long-term peripheral nerve injury, 780-nm laser therapy (250 mW) can progressively improve nerve motor function, which leads to significant functional recovery [84].

Barbosa et al [85] observed that, compared with the 830-nm laser group and the sham group, rats in the 660-nm laser group had the best sciatic functional index scores on average, which indicates that the use of these parameters was more efficient. Differences in sciatic functional index were found among the 660-nm laser group and the other ones at the 14th day [85]. However, Gigo-Benato et al [86] found that pulsed (905 nm) continuous (808 nm) combined laser biostimulation showed the best effectiveness in promoting peripheral nerve regeneration.

CONCLUSION

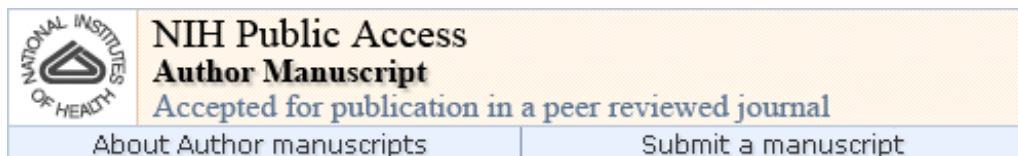
LLLT is steadily moving into mainstream medical practice. As the Western populations continue to age, the incidence of the degenerative diseases of old age will only continue to increase and produce an evermore severe financial and societal burden. Moreover, despite the best efforts of “big pharma,” distrust of pharmaceuticals is growing in general because of uncertain efficacy and troublesome adverse effects. LLLT has no reported adverse effects, and no reports of adverse events can be directly attributed to laser or light therapy. We believe that the high benefit:risk ratio of LLLT should be better appreciated by medical professionals in the rehabilitation and physical medicine specialties. The introduction of affordable LED devices powered by rechargeable batteries will lead to many home-use applications of LLLT. The concept of “wearable” light sources is not far off. Moreover, the particular benefits of LLLT to both the central and peripheral nervous systems suggest that much wider use of LLLT could or should be made in cases of both brain diseases and injuries.

Acknowledgments

Disclosure: research in the Hamblin laboratory supported by NIH grant R01AI050875, Center for Integration of Medicine and Innovative Technology (DAMD17-02-2-0006), CDMRP Program in TBI (W81XWH-09-1-0514), and Air Force Office of Scientific Research (FA9950-04-1-0079)

REFERENCES

1. Mester E, Szende B, Tota JG. Effect of laser on hair growth of mice. *Kiserl Orvostud.* 1967; 19:628–631.
2. Mester E, Spiry T, Szende B, et al. Effect of laser rays on wound healing. *Am J Surg.* 1971; 122:532–535. [PubMed: 5098661]
3. Mester E, Szende B, Spiry T, et al. Stimulation of wound healing by laser rays. *Acta Chir Acad Sci Hung.* 1972; 13:315–324. [PubMed: 4659882]
4. Huang YY, Chen AC, Carroll JD, et al. Biphasic dose response in low level light therapy. *Dose Response.* 2009; 7:358–383. [PubMed: 20011653]
5. Aimbire F, Albertini R, Pacheco MT, et al. Low-level laser therapy induces dose-dependent reduction of TNF α levels in acute inflammation. *Photomed Laser Surg.* 2006; 24:33–37. [PubMed: 16503786]
6. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J Photochem Photobiol B.* 1999; 49:1–17. [PubMed: 10365442]
7. Passarella S, Casamassima E, Molinari S, et al. Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser. *FEBS Lett.* 1984; 175:95–99. [PubMed: 6479342]



From: [PM R. Author manuscript; available in PMC 2011 December 1.](#)

<< Prev Figure 1 Next >>

Published in final edited form as:

PM R. 2010 December; 2(12 Suppl 2): S292-S305.

doi: 10.1016/j.pmrj.2010.10.013

[Copyright/License](#)

[Request permission to reuse](#)

Figure 1

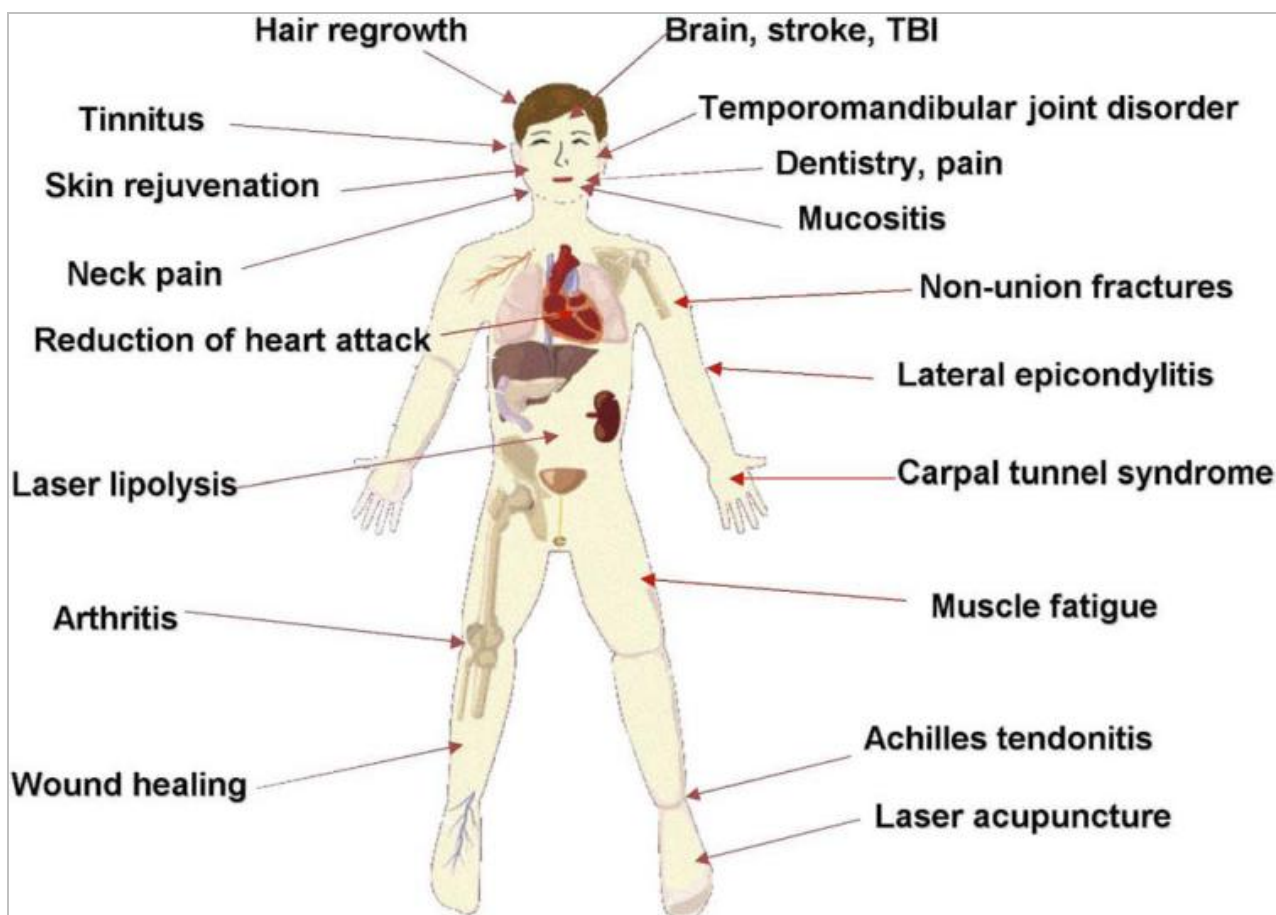
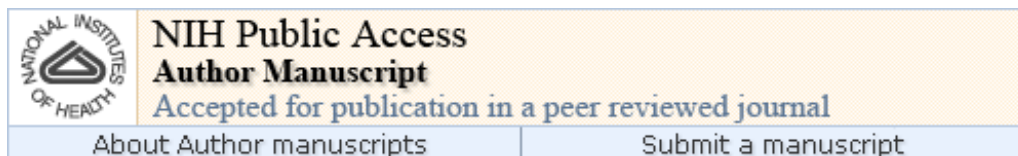


Diagram of the various medical applications of low-level light therapy.

Images in this article



From: [PM R. Author manuscript; available in PMC 2011 December 1.](#)

<< Prev Figure 4 Next >>

Published in final edited form as:

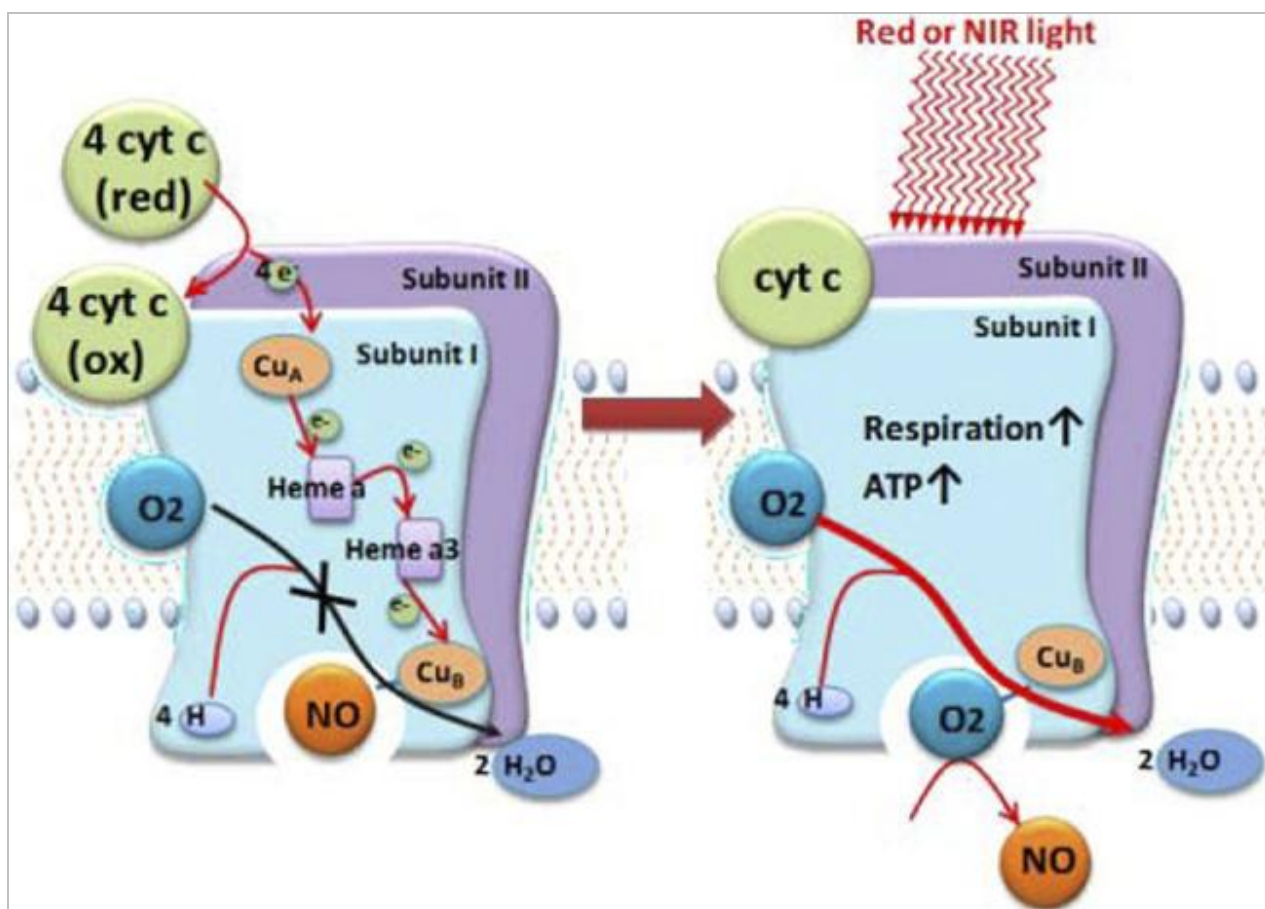
PM R. 2010 December; 2(12 Suppl 2): S292–S305.

doi: 10.1016/j.pmrj.2010.10.013

[Copyright/License](#)

[Request permission to reuse](#)

Figure 4



Nitric oxide can bind to copper (or heme) centers in cytochrome c oxidase and inhibit respiration. The nitric oxide may be photodissociated by absorption of red or near infrared light, allowing oxygen to return and sharply increasing respiration and adenosine triphosphate formation.

Lasers in Surgery and Medicine

Supplement 22, 2010

#185

THE APPLICATION OF LOW-LEVEL LASER THERAPY FOR THE SYMPTOMATIC CARE OF LATE STAGE PARKINSON'S DISEASE: A NON-CONTROLLED, NON-RANDOMIZED STUDY

Ryan Maloney, Steven Shanks, Jillian Maloney

Erchonia Medical, McKinney, TX; University of Arizona, Tucson, AZ

Background: Parkinson's Disease (PD) is the degeneration of dopaminergic neurons of the substantial nigra resulting in a reduction of striatal dopamine content. The exact mechanism is not well understood; however, several pathophysiologic mechanisms such as oxidative stress, mitochondrial dysfunction, and degradation of major intracellular proteins have been postulated to explain the progressive neuron loss. Numerous studies have revealed that LLLT can modulate cell bioenergetics influencing the intracellular redox state of a cell. With histological evidence suggesting that laser therapy can repair oxidatively damaged cells and increase neuronal axonal transport, the objective of this non-controlled, non-randomized study was to evaluate the improvement of symptoms associated with late stage PD.

Study: Eight volunteers between 18 to 80 years with late stage PD participated in a non-controlled, non-randomized study. Participants received LLLT treatments (PL5000, manufactured by Erchonia Medical Inc.) daily for two weeks. Each participant received treatment of the brain stem, bilateral occipital, parietal, temporal and frontal lobes, and treatment along the sagittal suture. Patients were asked, using the Visual Analog Scale (VAS), to record the severity of their symptoms of balance, gait, freezing, cognitive function, rolling in bed, and difficulties with speech pre-procedure and at study endpoint with 10 being most severe and 0 as no symptom.

Results: Compared with baseline, all participants demonstrated a numerical improvement in the VAS from baseline to study endpoint. A statistically significant reduction in VAS rating for gait and cognitive function was observed with average mean change of -1.87 ($p < 0.05$) for gait and a mean reduction of -2.22 ($p < 0.05$) for cognitive function. Further, freezing and difficulty with speech ratings were significantly lower at study endpoint with a mean reduction of -1.28 ($p < 0.05$) for freezing and -2.22 ($p < 0.05$) for difficult with speech.

Conclusion: These data suggest that laser therapy may serve as a non-invasive instrument for symptom reduction of PD.

Author of the *New York Times* bestseller

THE BRAIN THAT CHANGES ITSELF

The Brain's Way of Healing

REMARKABLE DISCOVERIES
and RECOVERIES from the FRONTIERS
of NEUROPLASTICITY

NORMAN
DOIDGE MD

Neurodegenerative Prevention Protocol; Cognitive Enhancement Brain Trauma Rehabilitation Protocol

Stop consuming refined/glycating carbohydrates and ultra-processed foods

Balance omega-6/omega-3 ratio (1.5 – 3 /1)

Get vitamin D levels above 50 ng/ml

Take vitamin K2-4 and K2-7

Be mindful of cholesterol lowering drugs (optimum total cholesterol for brain physiology 200-240 mg/dl)

Supplement with magnesium, about 500 mg/day

No copper water or copper in supplements

Take antioxidant vitamin/mineral supplements (watch the iron and copper)

Take curcumin/resveratrol

Elevate glutathione (Undenatured Whey Protein / NAC supplementation / Sublingual)

Detoxify (passive sweating / oil pull)

Treat/Protect the gut/microbiome (especially prebiotics/probiotics)

Low Level Laser Therapy (XLR8 two 7.5mW diodes: 1-10-40-60 or 1-10-40-528) [while doing your goals, gratitude, and forgiveness lists] [laser works best with mitochondrial supplements] [Trevor, Michelle]

- 2 minutes prefrontal cortex (most important)
- 2 minutes cerebellum/atlas junction (for CSF)
- 2 minutes at frontal/parietal junction (vertex of skull)
- 2 minutes for each cymba conchae (ear): use 10-40-10-40, Trevor
- 2 minutes carotid sheath medial to SCM low neck: use 10-10-10-10, Trevor
- 2 minutes GI Vagus (above umbilicus): use 1-10-1-10, Trevor

Avoid brain trauma (especially repetitive sub-concussive blows)

Exercise

Do not smoke

Minimize television and other mindless activities

Do crossword puzzles and other challenging brain games/activities

Consume Medium Chain Saturated Triglycerides (coconut oil, 1 Tbs./meal)

Do not retire from work (increases risk 3% per year of retirement)

Minimize consumption of Anti-Cholinergic Drugs

Take Cholinergic supplements

Blood contains circulating cell-free respiratory competent mitochondria

Zahra Al Amir Dache¹ | Amaëlle Otandault¹ | Rita Tanos¹ | Brice Pastor¹ |
Romain Meddeb¹ | Cynthia Sanchez¹ | Giuseppe Arena² | Laurence Lasorsa¹ |
Andrew Bennett³ | Thierry Grange³ | Safia El Messaoudi¹ | Thibault Mazard¹ |
Corinne Prevostel¹ | Alain R. Thierry¹

Blood Contains Circulating Cell-free Respiratory Competent Mitochondria

The FASEB Journal

[Federation of American Societies for Experimental Biology]

March 2020; Vol. 34; No. 3; pp. 3616-3630

We believe that circulating cell-free intact mitochondria have crucial biological and physiological roles because mitochondria are already known as systemic messengers in cell-cell communication by transferring hereditary and nonhereditary constituents.

Mitochondria were recently discovered to translocate from one cell to the other.

Indeed, intercellular mitochondria trafficking was observed in vitro and in vivo, both in physiological and pathophysiological conditions including tissue injury and cancer.

We speculate that mitochondrial clearance and degradation occurs principally by phagocytosis.

This study is the first showing free structurally intact mitochondria in plasma.



The presence of mitochondria in unicellular to mammalian organisms originates from an ancient symbiosis between primitive eukaryotic cells and free-living aerobic prokaryotes.

Mitochondria are crucial organelles for central cell functions, and they are the principal nutrient-up-taking and energy-producing cell organelle; they also take part in calcium signaling, reactive oxygen species (ROS) production, cell death, and diverse cell signaling events.

Cell-derived mitochondrial components, including mitochondrial DNA, have been found in the extracellular space.

Mitochondrial DNA has a small circular genome, without protective histones, and thus is more sensitive to degradation in the circulation.

There are approximately 50,000-fold more copies of the mitochondrial genome than the nuclear genome in the plasma of healthy individuals.

Our work demonstrates for the first time the presence in blood of circulating cell-free respiratory competent mitochondria.

In view of the potential roles of mitochondria in cell to cell communication, immune response, and inflammation, our discovery has broad implications in homeostasis and disease, and paves the way for new paths toward the treatment and prevention of diseases.

Effects of Infrared Radiation and Heat on Human Skin

Aging *in vivo*

Soyun Cho^{1,2,3}, Mi Hee Shin^{1,2,3}, Yeon Kyung Kim^{1,2,3}, Jo-Eun Seo^{1,2,3}, Young Mee Lee^{1,2,3}, Chi-Hyun Park^{1,2,3} and Jin Ho Chung^{1,2,3}

Sunlight damages human skin, resulting in a wrinkled appearance. Since natural sunlight is polychromatic, its ultimate effects on the human skin are the result of not only the action of each wavelength separately, but also interactions among the many wavelengths, including UV, visible light, and infrared (IR). In direct sunlight, the temperature of human skin rises to about 40°C following the conversion of absorbed IR into heat. So far, our knowledge of the effects of IR radiation or heat on skin aging is limited. Recent work demonstrates that IR and heat exposure each induces cutaneous angiogenesis and inflammatory cellular infiltration, disrupts the dermal extracellular matrix by inducing matrix metalloproteinases, and alters dermal structural proteins, thereby adding to premature skin aging. This review provides a summary of current research on the effects of IR radiation and heat on aging in human skin *in vivo*.

Journal of Investigative Dermatology Symposium Proceedings (2009) **14**, 15–19; doi:10.1038/jidsymp.2009.7

INTRODUCTION

Infrared (IR) radiation consists of wavelengths from 760 nm to 1 mm, and it is subdivided into three regions of increasing wavelength, IR-A (760–1400 nm), IR-B (1400–3000 nm), and IR-C (3000 nm–1 mm). Given that almost half of the solar energy reaching the earth's surface is in the IR range, solar IR is expected to have significant biological effects on human skin.

Heat is a form of energy that may be transmitted by IR radiation, which results in raised skin temperature. Human skin is exposed daily to natural sunlight. We found that the temperature of human skin can increase to more than 40°C under direct IR irradiation (Lee *et al.*, 2006) due to the conversion of IR into heat. There is clinical evidence indicating that chronic heat exposure of human skin may cause alterations. The skin disease called erythema ab igne is

known to be caused by chronic heat exposure. It is characterized clinically by reticular pigmentation of the skin and histologically by the presence of solar elastosis in the dermis similar to what is seen in photoaged skin. Furthermore, severe skin aging may develop occasionally on bakers' arms, because of exposure to hot ovens, and on the faces of glass blowers.

However, the effects of IR radiation and heat on cutaneous aging are still largely unknown. This review provides an overview of the current knowledge of contributions of IR radiation and heat exposure to aging in human skin.

EFFECTS OF IR RADIATION ON AGING IN HUMAN SKIN *IN VIVO*

It has been reported that IR-A can penetrate epidermal and dermal layers and reach subcutaneous tissues without increasing the skin temperature significantly, whereas IR-B and IR-C are absorbed mostly in the epidermal layers and increase skin temperature significantly (Schieke *et al.*, 2003). The few studies conducted on IR collectively have concluded that IR radiation produces heat upon exposure. Considering the general principle that all biochemical processes are affected by temperature, the effects of IR on human skin can no longer be ignored.

Effects of IR on the expression of collagen and MMPs in human skin *in vivo*

Alterations and deficiencies of collagen, the major structural component of the skin, have been suggested to be a cause of the skin wrinkling observed in both photoaged and naturally aged skin (Fisher *et al.*, 1997; Varani *et al.*, 2000). Because collagen fibrils are responsible for the strength and resilience of skin, their disarrangement during photoaging causes the skin to appear aged. Excessive matrix degradation by UV-induced matrix metalloproteinases (MMPs) secreted by various cells, including keratinocytes, fibroblasts, and inflammatory cells, contributes substantially to the connective tissue damage that occurs during photoaging (Fisher *et al.*, 1996; Chung *et al.*, 2001, 2002). Although human skin is frequently exposed to IR radiation, little is known about the

¹Department of Dermatology, Seoul National University College of Medicine, Seoul, Korea; ²Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea and ³Institute of Dermatological Science, Seoul National University, Seoul, Korea

Correspondence: Dr Jin Ho Chung, Department of Dermatology, Seoul National University Hospital, 28 Yongon-dong, Chongno-Gu, Seoul 110-744, Korea. E-mail: jhchung@snu.ac.kr

Abbreviations: IR, infrared; MMP, matrix metalloproteinase; ROS, reactive oxygen species; TRPV, transient receptor potential vanilloid

Received 14 November 2008; accepted 8 January 2009



FULL TEXT LINKS



Skin Pharmacol Physiol. 2010;23(1):15-7. doi: 10.1159/000257259. Epub 2010 Jan 14.

Photoprotection beyond ultraviolet radiation--effective sun protection has to include protection against infrared A radiation-induced skin damage

P Schroeder ¹, C Calles, T Benesova, F Macaluso, J Krutmann

Affiliations

Affiliation

¹ Institut für umweltmedizinische Forschung (IUF) an der Heinrich-Heine-Universität Düsseldorf gGmbH, Düsseldorf, Deutschland.

PMID: 20090404 DOI: [10.1159/000257259](https://doi.org/10.1159/000257259)

Abstract

Solar radiation is well known to damage human skin, for example by causing premature skin ageing (i.e. photoageing). We have recently learned that this damage does not result from ultraviolet (UV) radiation alone, but also from longer wavelengths, in particular near-infrared radiation (IRA radiation, 760-1,440 nm). IRA radiation accounts for more than one third of the solar energy that reaches human skin. While infrared radiation of longer wavelengths (IRB and IRC) does not penetrate deeply into the skin, more than 65% of the shorter wavelength (IRA) reaches the dermis. IRA radiation has been demonstrated to alter the collagen equilibrium of the dermal extracellular matrix in at least two ways: (a) by leading to an increased expression of the collagen-degrading enzyme matrix metalloproteinase 1, and (b) by decreasing the de novo synthesis of the collagen itself. IRA radiation exposure therefore induces similar biological effects to UV radiation, but the underlying mechanisms are substantially different, specifically, the cellular response to IRA irradiation involves the mitochondrial electron transport chain. Effective sun protection requires specific strategies to prevent IRA radiation-induced skin damage.

2010 S. Karger AG, Basel.

Related information

[MedGen](#)

LinkOut - more resources

Full Text Sources

[S. Karger AG, Basel, Switzerland](#)

Other Literature Sources

[The Lens - Patent Citations](#)

REVIEW ARTICLE

Open Access

Mitochondria in skin health, aging, and disease

Annapoorna Sreedhar¹, Leopoldo Aguilera-Aguirre¹ and Keshav K. Singh^{2,3}

Abstract

The skin is a high turnover organ, and its constant renewal depends on the rapid proliferation of its progenitor cells. The energy requirement for these metabolically active cells is met by mitochondrial respiration, an ATP generating process driven by a series of protein complexes collectively known as the electron transport chain (ETC) that is located on the inner membrane of the mitochondria. However, reactive oxygen species (ROS) like superoxide, singlet oxygen, peroxides are inevitably produced during respiration and disrupt macromolecular and cellular structures if not quenched by the antioxidant system. The oxidative damage caused by mitochondrial ROS production has been established as the molecular basis of multiple pathophysiological conditions, including aging and cancer. Not surprisingly, the mitochondria are the primary organelle affected during chronological and UV-induced skin aging, the phenotypic manifestations of which are the direct consequence of mitochondrial dysfunction. Also, deletions and other aberrations in the mitochondrial DNA (mtDNA) are frequent in photo-aged skin and skin cancer lesions. Recent studies have revealed a more innate role of the mitochondria in maintaining skin homeostasis and pigmentation, which are affected when the essential mitochondrial functions are impaired. Some common and rare skin disorders have a mitochondrial involvement and include dermal manifestations of primary mitochondrial diseases as well as congenital skin diseases caused by damaged mitochondria. With studies increasingly supporting the close association between mitochondria and skin health, its therapeutic targeting in the skin—either via an ATP production boost or free radical scavenging—has gained attention from clinicians and aestheticians alike. Numerous bioactive compounds have been identified that improve mitochondrial functions and have proved effective against aged and diseased skin. In this review, we discuss the essential role of mitochondria in regulating normal and abnormal skin physiology and the possibility of targeting this organelle in various skin disorders.

Facts

- Skin is the largest organ with high turnover rate in the human body.
- Mitochondria play a vital role in the skin.
- Mitochondrial dysfunction induces skin aging.
- Skin disorders manifest mitochondrial dysfunction.
- Targeting mitochondria may help rejuvenate skin.

Open Questions

- How do mitochondria regulate skin aging?
- Is mitochondrial dysfunction a primary or a secondary cause of skin aging?
- Does targeting mitochondria prevent or slow down skin aging?

Introduction

The skin is the outer sheath of the body, which interfaces with the external environment. It is a complex structure made of several tissue layers, each with a distinct cellular composition. It has crucial functions like sensation, heat insulation and prevention of water loss, and acts as a physical barrier to pathogens. The upper layer of the skin is the epidermis, a stratified structure interspersed with hair follicles. The outer epidermal surface is made of

Correspondence: Keshav K. Singh (kksingh@uab.edu)

¹Yuva Biosciences, 1500 1st Avenue N, Birmingham, AL 35203, USA

²Department of Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Full list of author information is available at the end of the article

Edited by E. Candi

© The Author(s) 2020



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

"In addition to the UV radiation, infrared (IR) rays have also been associated with photo-aging of human skin.

Schieke et al. showed that exposure to IR dysregulated the expression of metalloproteases in primary human fibroblasts.

Similar changes were observed in situ in human skin exposed to IR, while chronic exposure to IR also resulted in the typical clinical signs of photo-aging in animal models.

Schauen et al. detected a significant increase in the intra-mitochondrial levels of ROS in primary human skin fibroblasts exposed to IR, indicating a direct role of the mitochondria in IR-induced photoaging.

The underlying mechanism of IR-induced mitochondrial damage was elucidated by Karu et al. who showed that the IR rays are absorbed by the copper atoms of ETC complex IV, leading to disturbed electron flow and 'leakage,' and increased ROS production."

June 13, 2023

FROM: Chiropractor in Tennessee

TO: Michelle Schaer, DC and Dan Murphy, DC

Hello to both of you. I am a DC in ••••••••, TN. My father developed a massive blood clot in his basilar artery/brain stem in February that caused him to have repeated strokes over the course of several weeks. The doctors had no answer and he gradually got so bad that he was placed on hospice and death seemed near. I drove up to see him (for what appeared to be our final goodbyes). I left behind my Erchonia PLT red laser and showed my mom your brain protocol. She used to the laser 4-6 times per day on my dad for 2 min in each region (nasal, vertex, cerebellum and temporal). He has done nothing but improve since starting with the laser. He was released from hospice two weeks ago and his md's are shocked at his results . . . so are we. His medical doctors are all left scrambling to figure out what the laser is and how it works. Wanted to thank you for your guidance and help with this. If you'd like a more formal testimony to share I'm happy to write one up.

Unfortunately my PLT is now in Wisconsin with my dad so I'm going to need to invest in a new laser to replace it. I'm a little unsure of if I should get a red/violet (EVL) or the green/violet (GVL). I do not have another red laser here so I'm concerned that I won't have all three types of lasers to stimulate all 4 complexes in the electron transport chain as explained in your webinar. Is there an advantage to the green vs red in regards to mitochondrial health/function or ATP production? If I get the GVL will I be missing out a lot on not having that red laser to effect complex 4? Just not sure if there is an advantage to one over the other in terms of brain/mitochondrial function? I could really use help from the two of you . . . have tremendous faith in your clinical experience and research. Any thoughts or recommendations for me on this one?

Thank you so much for helping me with my dad! I really am so very thankful for any guidance you can give me on this.

The Key To Understanding Clinical Laser

Energy v. Power

Background

μ = "micro" = one millionth of the unit:

μ W = microwatt

m = "milli" = one thousandth of the unit.

mW = milliwatt

1 mW = 1000 μ W

Erchonia Handheld Lasers use 7.5 mW = 7500 μ W

<https://www.kmlabs.com/en/wavelength-to-photon-energy-calculator>

Wavelength nm	Power μ W	Electron Volt=eV = Photon Energy	Photons/Second = Flux
405 nm violet	7500 μ W (7.5 mW)	3.06 eV	15+ Quadrillion
520 nm green	7500 μ W (7.5 mW)	2.38 eV	19+ Quadrillion
635 nm red	7500 μ W (7.5 mW)	1.95 eV	23+ Quadrillion
830 nm IR	1,000,000 μ W (1W)	1.49 eV	4172+ Quadrillion

Energy does the work.

Energy is dependent on the wavelength.

Shorter wavelengths have more energy.

Shorter wavelengths have greater frequency.

Power controls the rate of the work

Power is dependent in watts, milliwatts.

Research Article

Assessing the Impact of High Photon Energy Wavelengths on the Treatment of Chronic Neck and Shoulder Pain

Travis Sammons ¹, Kirk Gair,² Robert G. Silverman,³ and Steve Shanks¹

¹Erchonia Corporation, Melbourne, FL, USA

²Clinical Study Site, Laser Chiropractic, West Covina, CA, USA

³Clinical Study Site, New York ChiroCare, White Plains, NY, USA

Correspondence should be addressed to Travis Sammons; tsammons@erchonia.com

Received 18 August 2023; Revised 18 September 2023; Accepted 26 September 2023; Published 4 October 2023

Academic Editor: B. O. Ajiboye

Copyright © 2023 Travis Sammons et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The effect of low-level laser therapy with high photon energy wavelengths, green and violet, for treating chronic musculoskeletal pain was examined in the first-ever clinical trial of its kind. Participants ($n = 43$) underwent a single 13-minute laser session. The primary measure of effectiveness was the change in initial visual analog pain (VAS) scores observed three minutes posttreatment. The success of a participant was defined in advance as a reduction of $\geq 30\%$ in VAS scores, while the success of the study was predetermined as achieving a $65 \pm 5\%$ success rate among individual participants. Results demonstrated subjects' VAS pain scores decreased from 71.79 to 34.02 ($p < 0.0001$), while most participants in the study (81.4%) achieved a $\geq 30\%$ decrease in pain scores. The findings from this clinical investigation provided substantial support for the first Food and Drug Administration clearance (K221987) for the combined application of green and violet lasers.

1. Introduction

Extensive documentation supports the effectiveness of low-level laser therapy (LLLT) in reducing pain and inflammation associated with various musculoskeletal disorders [1]; nonetheless, there remains limited investigation of high photon energy wavelengths, such as green light (495–570 nm) and violet/blue light (400–495 nm), for pain-related musculoskeletal ailments. Earlier research involving green and violet/blue wavelengths has largely focused on acne [2, 3], cellulite [4], fungi [5], and viruses [6, 7]. This clinical trial stands as the first known study to examine the specific effects of combining green and violet lasers on chronic musculoskeletal pain. Its findings mark a significant milestone in the field of research related to pain management.

Chronic neck and shoulder pain is widely recognized as one of the most common musculoskeletal disorders [8]. Neck pain is considered the fourth leading cause of disability, with a prevalence rate exceeding thirty percent on an annual basis [9]. While certain instances of neck pain may resolve spontaneously or with minimal treatment, studies

show that nearly fifty percent of individuals will continue to experience varying degrees of pain or frequent recurrences.

The Food and Drug Administration (FDA) granted low-level laser market clearance for the reduction of neck and shoulder pain in 2002 for red laser diode alone [10] and in 2018 for the combination of red and violet laser diodes [11]. A previous comparative study analysed the difference between a red laser diode alone and a red/violet laser diode combination [12]. The primary objective of this nonrandomized non-inferiority design trial was to determine whether the combination of violet and green lasers is as effective as the previously FDA-cleared treatments using either the red diode alone or the combination of red and violet laser diodes.

2. Materials and Methods

2.1. Study Subjects. The study recruited participants aged 18 years and older from two sources: patients seeking treatment for neck and shoulder pain within the investigators' patient pool and individuals who responded

BIPHASIC DOSE RESPONSE IN LOW LEVEL LIGHT THERAPY

Ying-Ying Huang □ Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, **Harvard** Medical School, Boston, MA; Aesthetic and Plastic Center of Guangxi Medical University, Nanning, P.R. China

Aaron C.-H. Chen □ Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; **Boston University** School of Medicine, Graduate Medical Sciences, Boston, MA

James D. Carroll □ THOR Photomedicine Ltd, 18A East Street, Chesham, HP5 1HQ, UK

Michael R. Hamblin □ Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA; Department of Dermatology, **Harvard** Medical School, Boston, MA; Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA

□ The use of low levels of visible or near infrared light for reducing pain, inflammation and edema, promoting healing of wounds, deeper tissues and nerves, and preventing cell death and tissue damage has been known for over forty years since the invention of lasers. Despite many reports of positive findings from experiments conducted in vitro, in animal models and in randomized controlled clinical trials, LLLT remains controversial in mainstream medicine. The biochemical mechanisms underlying the positive effects are incompletely understood, and the complexity of rationally choosing amongst a large number of illumination parameters such as wavelength, fluence, power density, pulse structure and treatment timing has led to the publication of a number of negative studies as well as many positive ones. A biphasic dose response has been frequently observed where low levels of light have a much better effect on stimulating and repairing tissues than higher levels of light. The so-called **Arndt-Schulz curve** is frequently used to describe this biphasic dose response. This review will cover the molecular and cellular mechanisms in LLLT, and describe some of our recent results in vitro and in vivo that provide scientific explanations for this biphasic dose response.

1. INTRODUCTION

1.1. Brief history

Low level laser therapy (LLLT) is the application of light (usually a low power laser or LED in the range of **1mW – 500mW**) to a pathology to **promote tissue regeneration, reduce inflammation and relieve pain**. The light is typically of narrow spectral width in the red or near infrared

Address correspondence to Professor Michael R. Hamblin, BAR 414, Wellman Center for Photomedicine, Massachusetts General Hospital, 40 Blossom Street, Boston, MA 02114; Phone: 617-726-6182, Fax: 617-726-8566, E-mail: hamblin@helix.mgh.harvard.edu

(NIR) spectrum (600nm – 1000nm), with a power density (irradiance) between 1mw-5W/cm². It is typically applied to the injury for a minute or so, a few times a week for several weeks. Unlike other medical laser procedures, LLLT is not an ablative or thermal mechanism, but rather a photochemical effect comparable to photosynthesis in plants whereby the light is absorbed and exerts a chemical change.

The phenomenon was first published by Endre Mester at Semmelweis University, Budapest, Hungary in 1967 a few years after the first working laser was invented (Mester *et al.* 1967). Mester conducted an experiment to test if laser radiation might cause cancer in mice. He shaved the hair off their backs, divided them into two groups and irradiated one group with a low powered ruby laser (694-nm). The treatment group did not get cancer and to his surprise, the hair grew back more quickly than the untreated group. He called this “Laser Biostimulation”.

1.2. Evidence for effectiveness of LLLT

Since 1967 over 100 phase III, randomized, double-blind, placebo-controlled, clinical trials (RCTs) have been published and supported by over 1,000 laboratory studies investigating the primary mechanisms and the cascade of secondary effects that contribute to a range of local tissue and systemic effects.

RCTs with positive outcomes have been published on pathologies as diverse as osteoarthritis (Bertolucci and Grey 1995; Ozdemir *et al.* 2001; Stelian *et al.* 1992), tendonopathies (Bjordal *et al.* 2006b; Stergioulas *et al.* 2008; Vasseljen *et al.* 1992), wounds (Caetano *et al.* 2009; Gupta *et al.* 1998; Ozelik *et al.* 2008; Schubert *et al.* 2007), back pain (Basford *et al.* 1999), neck pain (Chow *et al.* 2006; Gur *et al.* 2004), muscle fatigue (Leal Junior *et al.* 2008a; Leal Junior *et al.* 2008b), peripheral nerve injuries (Rochkind *et al.* 2007) and strokes (Lampl *et al.* 2007; Zivin *et al.* 2009); nevertheless results have not always been positive. This failure in certain circumstances can be attributed to several factors including dosimetry (inadequate or too much energy delivered, inadequate or too much irradiance, inappropriate pulse structure, irradiation of insufficient area of the pathology), inappropriate anatomical treatment location and concurrent patient medication (such as steroidal and non-steroidal anti-inflammatories which can inhibit healing) (Aimbire *et al.* 2006; Goncalves *et al.* 2007).

1.3. The medicine and the dose

As with other forms of medication, LLLT has its active ingredients or “medicine” (irradiation parameters) and a “dose” (the irradiation time). Table 1 lists the key parameters that define the medicine and Table 2 defines the dose. It is beyond the scope of this paper to exhaustively list and discuss every conceivable aspect of laser radiation or other light

7/29/2023

One photon kicks off photosynthesis

Laboratory experiments confirm a long-held hypothesis

BY EMILY CONOVER

For photosynthesis, one photon is all it takes.

Only a single particle of light is required to spark the first steps of the biological process that converts light into chemical energy, scientists report June 14 in *Nature*.

Researchers have long assumed that the reactions of photosynthesis begin upon the absorption of just one photon, but it had never been demonstrated, says physical chemist Graham Fleming of the University of California, Berkeley. He and colleagues decided “we would just look to see was it really true that one photon was enough to start the whole thing off.”

The sunlight that falls on Earth's surface seems brilliant to human eyes. But on small scales, that light translates to a dribble of photons. Every second, only a few tens of photons of the appropriate wavelengths of sunlight fall on a square nanometer, the scale of the tiny chlorophyll and bacteriochlorophyll molecules that are central to photosynthesis in plants and bacteria.

Many experiments on photosynthesis use lasers, much more powerful light sources, to kick off the reactions. Instead, Graham and colleagues used a source of light that produces just two photons at a time. One photon served as a herald, going off to a detector to let researchers know when the photons were released. The other photon went into a solution containing light-absorbing structures from the bacterium *Rhodobacter sphaeroides*. These structures—called light-harvesting 2 complexes, or LH2—are made up of two rings of bacteriochlorophyll and other molecules.

In a normal photosynthesis reaction, it's thought that LH2 absorbs a photon and passes its energy to another LH2 complex, and then another, like a game

of hot potato. Eventually, the energy reaches another type of ring, LH1, which then passes the energy to the reaction center where it is finally converted into a form that the bacterium can use.

In the experiment, the solution contained no LH1, so LH2 emitted a photon instead of passing its energy on to LH1. The emitted photon had a different wavelength than the absorbed photon, a sign that energy had been transferred between LH2's two rings, a first step of photosynthesis. The team detected that second photon and, by comparing the detection time with that of the initial herald photon, confirmed that LH2 needed to absorb only one photon to kick things off.

Though plants and bacteria use different processes for photosynthesis, the initial steps are similar enough that a single photon would kick off the process in plants as well, Fleming says. However, plants require multiple independently

absorbed photons to complete the reactions.

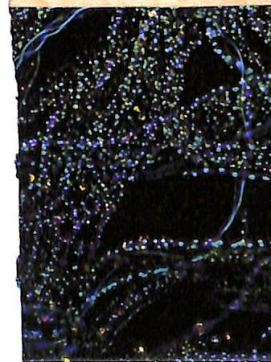
The role of single photons isn't surprising, says Richard Cogdell, a biochemist at the University of Glasgow in Scotland who was not involved in the research. The important thing, he says, is the new technique. With it, “you're able to essentially interrogate what will be happening in nature,” Cogdell says.

Some researchers suspect that photosynthesis relies on quantum physics (SN: 2/27/10, p. 10). While it's not clear

In *Rhodobacter sphaeroides* bacteria (shown fluorescing in this microscope image), one particle of light can initiate photosynthesis.

whether the new technique could resolve the role of quantum effects, it could help researchers disentangle natural effects from artifacts of using intense sources of light in studies of photosynthesis.

“You can really work out what's happening in the early reactions,” Cogdell says, “[as if] you could shrink yourself down and watch these photons moving around.” ■



NASA, ESA AND J. OLIMSTED/STSCI

RESEARCH ARTICLE

Light stimulation of mitochondria reduces blood glucose levels

Michael B. Powner¹  | Glen Jeffery² 

¹Department of Optometry and Visual Science, Centre for Applied Vision Research, School of Health and Psychological Sciences, City, University of London, London, UK

²Department of Visual Neuroscience, UCL Institute of Ophthalmology, University College London, London, UK

Correspondence

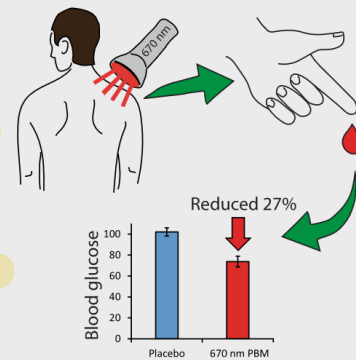
Michael B. Powner, Department of Optometry and Visual Science, Centre for Applied Vision Research, School of Health and Psychological Sciences, City, University of London, London, UK.
Email: michael.powner@city.ac.uk

Funding information

Sight Research UK

Abstract

Mitochondria regulate metabolism, but solar light influences its rate. Photobiomodulation (PBM) with red light (670 nm) increases mitochondrial membrane potentials and adenosine triphosphate production and may increase glucose demand. Here we show, with a glucose tolerance test, that PBM of normal subjects significantly reduces blood sugar levels. A 15 min exposure to 670 nm light reduced the degree of blood glucose elevation following glucose intake by 27.7%, integrated over 2 h after the glucose challenge. Maximum glucose spiking was reduced by 7.5%. Consequently, PBM with 670 nm light can be used to reduce blood glucose spikes following meals. This intervention may reduce damaging fluctuations of blood glucose on the body.



KEYWORDS

670 nm, glucose, human, mitochondria, oral glucose tolerance test, photobiomodulation, red light



Review

A Translational Review of Mechanisms of Effectiveness of Photobiomodulation on Somatosensory Neurons and the Peripheral Nervous System—From Molecular Mechanisms to Clinical Applications in Medicine and Dentistry

Roberta Chow * and Patricia Armati 

Brain and Mind Centre, Faculty of Medicine and Health, University of Sydney, 94 Mallet Street,

July 9, 2026; Vol. 48; No. 7; Article 695

This review aims to provide a translational link between clinical evidence in the application of photobiomodulation for treatment of painful conditions in medicine and dentistry and neurophysiological effects of photobiomodulation therapy (PBMt).

Central to the therapeutic effects of PBMt for pain is its interaction with neurons.

PBM influences cytoskeletal organization, mitochondrial structure and function, and intracellular signaling pathways, with downstream effects on neuronal excitability and nerve conduction.

Pain remains a problematic and universally important clinical challenge with treatments for acute and chronic pain varied as to their effectiveness.

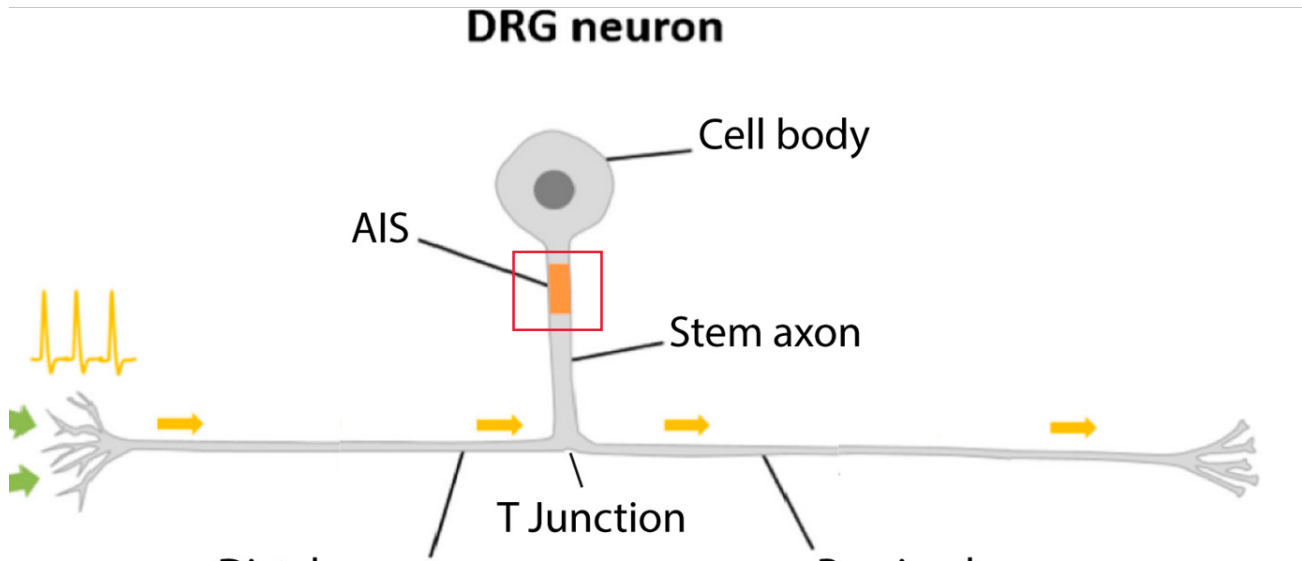
The idea of using light for pain remains outside mainstream medicine's accepted practice.

There are well-designed clinical trials showing PBMt's effectiveness for painful musculoskeletal conditions.

The Peripheral Nervous System (PNS) is made up of somatosensory first-order neurons however peripheral nerves include not only afferent axons transmitting sensory information to the Central Nervous System (CNS) but also motor axons arising from ventral horn cells of the spinal cord and sympathetic axons.

The sensory nerve cell bodies lie within the DRG- and PNS-related ganglia such as the trigeminal nerve ganglia in humans and are of particular interest in understanding how noxious and other PNS stimuli are transmitted and perceived as pain.

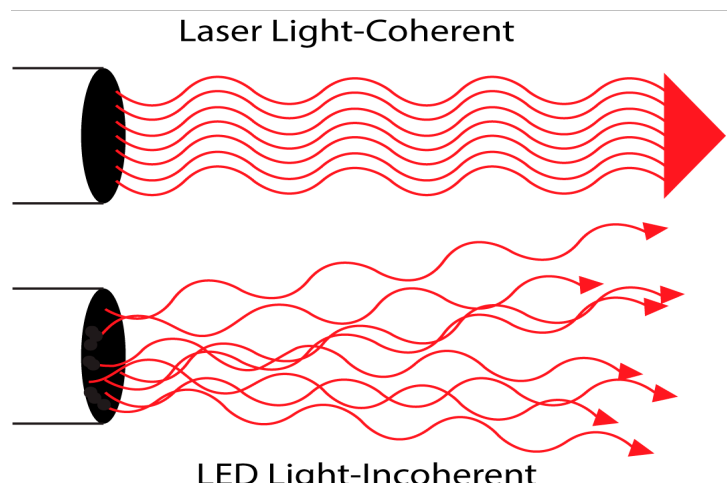
AIS axon initial segment is essential for propagation, integration and modulation of the action potential at the bifurcation of the axon into the proximal and distal branches.



The importance of the AIS within the DRG neuron, which plays a major role in the propagation, integration and modulation of the action potential, has been emphasized previously. It therefore presents as a potential target for PBMt as a logical research step.

Photobiomodulation Therapy (PBMt) describes the interaction between photons and cells at sub-ablative fluences to modulate cell physiology to achieve therapeutic effects and pain modulation.

There are significant differences between laser and light emitting diodes (LEDs) which can alter therapeutic outcomes.



Coherence is one of the unique properties of laser radiation.

Lasers diffuse and scatter very differently from LEDs within biological tissues.

Coherence may influence light distribution in tissue, and it can be demonstrated that lasers produce light speckles or pockets of intense light within tissues. Lasers therefore have the potential for a greater depth of penetration than LEDs.

From a clinical perspective, the use of LED can be effective in treating conditions such as diabetic neuropathy which involves damaged C neurons which lie only a few microns under the keratin in skin and does not require significant penetration.

Variables critical to "dose" and application include wavelength (λ), power output (W), time of exposure (seconds or minutes), "spot size" and mode (pulsed or continuous wave).

There are now several significant systematic reviews and randomized controlled trials of PBM for multiple painful medical conditions, including neck pain, knee osteoarthritis, diabetic peripheral neuropathy, shoulder tendinopathy, neuropathic pain, chemotherapy-related neuropathy and musculoskeletal pain.

Other clinical trials and reviews have reported favorable outcomes in PBM delivered transcutaneously in back pain, trigeminal neuralgia, post-herpetic neuralgia, fibromyalgia, and crystalline arthropathies.

The complex cascade of intra- and extracellular signaling initiated by photon absorption by cytochrome c oxidase in mitochondria in multiple compact cell types, such as fibroblasts, macrophages and neutrophils, has been well described and underpins many other diverse applications of PBMt such as wound healing and sports performance.

The collective body of evidence demonstrates biological plausibility and internal consistency across multiple levels of investigation. They reflect a systems-level neurophysiological basis for pain modulation and support the hypothesis that PBM modulates the somatosensory nervous system as an independent mechanism for pain modulation in medicine and dentistry and provide a platform for further research.

This study cites 134 references.

Take a flashlight – the one on your phone will do – turn it on and place the pad of your index finger over the bulb. See that red glow? You might think it's something to do with blood, but you'd be wrong. It's photons of red light bouncing around inside your finger, looking for somewhere to land.

This simple experiment is a doorway to one of the most profound and surprising biological discoveries of recent years. "Light is a nutrient," says Bob Fosbury, who was an astrophysicist at the European Southern Observatory before he turned his attention to the biology of light. That's obviously true for plants, which use light to make carbohydrates. But it turns out it's also true for animals and fungi, all of which exploit light in a hitherto unappreciated way. All living organisms – including us – are solar-powered.

Sunlight has long been understood to be critical to life on Earth. But the fact that light is also used to oil the wheels of metabolism is only just, er, coming to light. Long wavelengths, which come from red and infrared (IR) light, are particularly beneficial for creating energy, and are readily available from sources including sunlight and incandescent bulbs.

But changes in lighting intended to help save energy have inadvertently deprived us from this crucial supply. The irony is that just as we have discovered the benefit of red light, we are unintentionally starving ourselves of it.

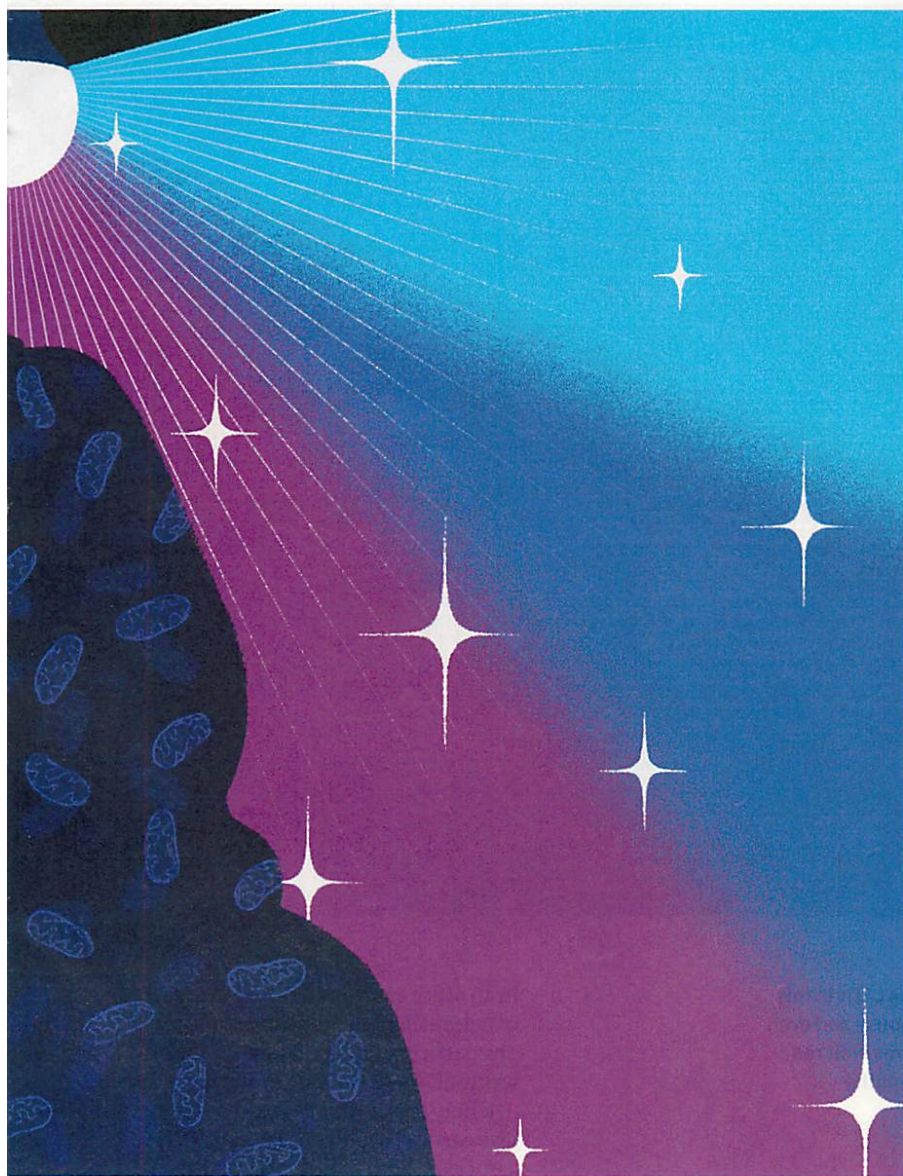
The story begins in the early 2000s with the adoption of two now-ubiquitous energy efficiency technologies. The first was the light-emitting diode (LED), which largely replaced energy-guzzling incandescent bulbs. Although this change helped conserve energy, it had other unforeseen consequences. The second was window glass that filters out IR, designed to prevent heat leaking into or out of buildings. Together, these dramatically narrowed the



EMANUEL SANTOS

Red-light starvation

Since the 2000s, the spectrum of light we are exposed to has drastically shrunk. Now, we're learning how this can wreak havoc with our metabolism, finds **Graham Lawton**



spectrum of light we are exposed to indoors, an environment where most humans spend up to 90 per cent of their time. "We lost about 95 per cent of the spectrum," says neuroscientist Glen Jeffery at University College London. "Suddenly, without warning, boom, the long wavelengths go. And that's a problem."

For the vast majority of human existence, those wavelengths were unavoidable. Daylight is rich in visible red light, which covers about 650 to 750 nanometres. It also emits vast and invisible (to us, at least) quantities of IR, which extends out to about 2500 nanometres.

People who spend much of the day outside are naturally exposed to these

long wavelengths, even when fully clothed. When long-wavelength light hits a human body, it sails through clothing and penetrates deep into tissues (short-wavelength light such as UV, in contrast, is absorbed by clothing, which is why covering up helps prevent sunburn). Red and IR are also abundant in moonlight and in the glow of a campfire. Indoor light, too, used to be full of these wavelengths, either from sunlight streaming through windows or light sources such as open fires, candles and oil and gas lamps. The invention of the electric light didn't change that, as the spectrum of an incandescent light bulb closely matches that of the sun.

But the adoption of energy-efficient lighting and windows robbed us of longer parts of the spectrum. Glass that filters out IR obviously blocks that portion of sunlight, and while LEDs throw out a lot of visible light, they produce nothing beyond about 750 nm. "LEDs are completely dark in the infrared," says Fosbury. Scott Zimmerman, founder of the lighting company Silas Inc. in New Jersey who collaborates with Fosbury and Jeffery, calls LED light "ultra-processed", an analogy with ultra-processed food. The combined effect of ultra-processed light and modern glass means that the average human today receives much less long-wavelength light than their ancestors did.

21st-century scurvy

At the same time, conditions such as obesity, type 2 diabetes, dementia and early-onset cancers are on the march. The usual suspects for this are poor diet and lack of exercise. But according to Fosbury, Jeffery and others, these are only a piece of the puzzle; another reason, perhaps the principal one, is red-light deprivation. "There's not a single cause, but I do increasingly think that light is the most fundamental and dominant one," says Fosbury. "And it's probably the easiest one to solve."

To understand why light is so vital, we have to go back in time again, to the Soviet Union of the late 1980s, where, at the USSR Academy of Sciences in Moscow, a biophysicist called Tiina Karu was experimenting with red and near-infrared light as a therapy. She discovered that they were useful in wound healing and pain management and wondered what the mechanism was. Eventually she hit on energy metabolism, specifically the pace at which mitochondria inside the body's cells produce a molecule called ATP. Red light, she found, accelerated this process.

Karu's work never found a wide audience, mainly because she came from the wrong side of the Iron Curtain, according to Jeffery. But she was right, and today red-light therapy is everywhere, used to promote wound healing and treat skin conditions, hair loss, chronic pain and much more. The evidence of its efficacy is patchy, but growing, says John Mitrofanis, a neurologist at the University of Grenoble Alpes in France. There is much debate over the correct doses and wavelengths, but researchers agree that its effects are mediated by mitochondria.

The key to this is the electron transport chain, a complex and highly orchestrated metabolic process that takes in energetic



New Scientist audio

You can listen to many articles – look for the headphones icon in our app [newscientist.com/app](https://www.newscientist.com/app)

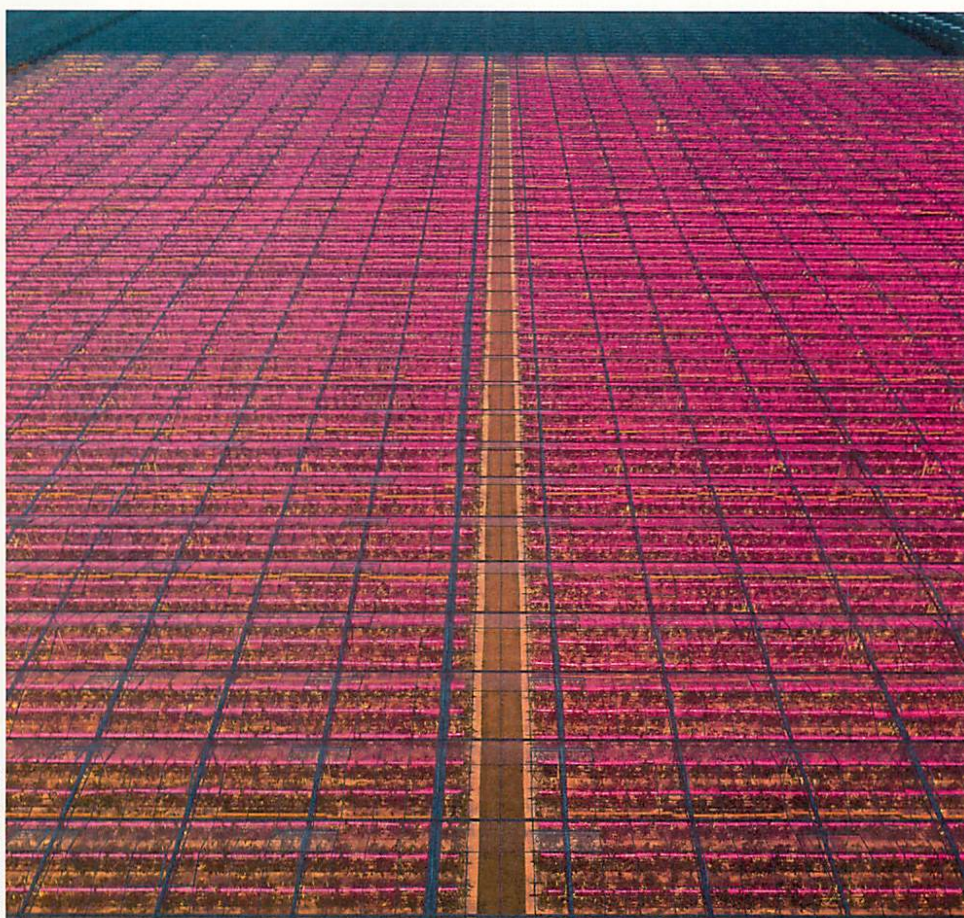
electrons and passes them down a chain of proteins, creating ATP in the process. ATP is biology's principal energy currency, analogous to cash in an economy. When a cell needs energy to perform a task – say, building new proteins – it uses ATP.

When mitochondria are bathed in red or IR light, the electron transport chain runs a little faster and more ATP is produced – a process Fosbury calls “photometabolism”. The exact mechanism by which ATP production is accelerated remains a point of contention, but what's certain is that diseased cells are often short of ATP, so a boost can make up the deficit. That, says Jeffery, explains why red-light therapy is beneficial.

It also explains why taking away red light can be harmful. Jeffery points out that all life on Earth evolved in an environment rich in red light and IR, but humans now mostly live in one stripped of those wavelengths. The result is more sluggish ATP production. To add insult to injury, we have also flooded our indoor environment with shorter wavelengths in the form of blue light from screens and LEDs. Blue light inhibits ATP production by slowing down the electron transport chain. Lose, lose.

The effects are insidious. Fosbury likens long-wavelength light to a vitamin and calls the effects of deprivation “21st-century scurvy”, a nod to the disease of vitamin C deficiency that killed and sickened tens of thousands of sailors in the 18th century. Like scurvy, it creeps up on you. “It's something that accumulates over a period of time,” says Jeffery.

It isn't just the general benefits of being outside, but red light specifically that is proven to have benefits. One of the symptoms of 21st-century scurvy is elevated blood sugar, which, if left unchecked, can progress to type 2 diabetes. This is due to the slowing down of the electron transport chain, which burns glucose as its primary fuel. A couple of years ago, Jeffery showed that a 15-minute burst of red light blunts the blood sugar spike after a large dose of glucose. He has also done real-world experiments in windowless, LED-illuminated offices at University College London. When he added long-wavelength light from a dimmed incandescent bulb, he found that the office occupants had lower blood sugar, on average, than their colleagues labouring under pure LED light. “Their mitochondria are being kick-started by the red light,” says Jeffery. A study published in January found something similar; researchers at Maastricht University in the Netherlands exposed 13 individuals with type 2 diabetes to 4.5 solid working days of LED light



GETTY IMAGES/WESTEND61

The pink LEDs in this greenhouse and even campfires emit red wavelengths

“The success of red-light therapy may be down to the correction of a deficiency”

in an office environment, then 4.5 days of natural light in the same location. The participants' blood sugar control was much better during the daylight days.

Jeffery also has concerns about other diseases and health problems usually associated with ageing, especially neurodegenerative conditions. One of the causes of these, he says, is mitochondrial dysfunction, which is effectively induced by red-light deficiency. Clinical trials have found that near-infrared light can slow down age-related cognitive impairment. A recent study of almost 88,000 people in their sixties found that, over the course of eight years, those who spent more time in daylight were significantly less likely to develop dementia, though the underlying causes of this relationship aren't yet fully understood. Mitrofanis, meanwhile, has shown that red light can prevent Parkinson's in a primate model of the disease.

Cancer and cardiovascular diseases, too, have been linked to a lack of daylight. When dermatologist Richard Weller at the University



CLASSIC STOCK/ALAMY

of Edinburgh, UK, and his colleagues analysed data from more than 400,000 adults in a yet-to-be-published study, they found that people with higher habitual UV exposure – a proxy for sunlight exposure in general – were less likely to die from cancer and cardiovascular disease.

Indeed, says Mitrofanis, the success of red-light therapy may simply be down to correction of a red-light deficiency. “Maybe all this is doing is rescuing processes that have gone awry because we’re inside all the time, getting illuminated with blue light.”

The fact that longer-wavelength light speeds up energy production isn’t a happy accident. Fosbury and Jeffery argue that it is a design feature of living systems. Life, they say, evolved to feed off this once-abundant resource to maximise the efficiency of ATP production. “Life on Earth has evolved for 4 billion years in sunlight and it’s adapted exquisitely to the properties of sunlight,” says Fosbury.

One of those adaptations is the ability to harvest and store photons. “The body literally is designed to absorb as much as possible in the infrared,” says Zimmerman. The amount of light energy we take in every day is staggering, he says. “Sunlight is the number one energy input into the body. It’s at least twice, if not three times, as much as food.”

Once sunlight enters the body, it scatters, bouncing off cells and organelles repeatedly – perhaps tens or hundreds of times, says Fosbury – until it hits a molecule that can absorb it, dumping energy in the process. This scattering means that a photon of red or IR light remains inside the body for much longer than it would if it passed straight through. This is what you see when you press your fingertip to a flashlight.

The photon’s final destination is somewhat random, but occasionally it is absorbed by a component of the electron transport chain.

Scientists are currently evaluating whether water molecules in the mitochondria are key to converting light into energy. When they absorb energy from a photon, they vibrate faster and lubricate the passage of electrons down the chain. Remarkably, says Fosbury, each step in the chain requires the electrons to overcome an energy barrier of roughly 0.75 electron volts, which is almost precisely what a photon of long-wavelength light delivers. Without the supply of red light we evolutionarily developed for, we’re getting far less energy.

Fortunately, the solution to this modern-day scourge is simple: recharge your light battery whenever you can, either with daylight or an incandescent bulb. There are red-light therapy devices, but some of these are overpowered, which can send cellular energy production into overdrive and lead to inflammation. Of course, exposing yourself to sunlight also carries its own risk. But clothing and sunscreen help block cancer-causing UV, while letting red and IR through.

For people stuck in modern offices, Jeffery recommends buying a light fitting with a dimmer switch and putting it on your desk with an incandescent bulb in it. “Even when it [the incandescent bulb] is very, very, very dim, it still produces a vast amount of IR,” he says. There’s no specific magic wavelength and so a red LED light would likely be better than no red light at all, but nowhere near as effective

A London hospital has created an outdoor ward to help patients and staff get more red light exposure



KING'S COLLEGE HOSPITAL NHS FOUNDATION TRUST

as full-spectrum red light from incandescent light bulbs or the sun.

What would really make a difference, says Jeffery, is a change in attitude among the people who design and manage buildings. The pursuit of energy efficiency is laudable but has unintended consequences. And in any case, energy efficiency need not be sacrificed. True, incandescent bulbs are extremely inefficient as a source of visible light, producing mostly infrared, aka heat. But the energy consumption of a heavily dimmed incandescent bulb is no more than an LED, so there are no extra costs – and shedloads of savings in the form of a healthier workforce. “We can do masses for public health just by bringing back the long wavelengths of light you’re being denied in your office environment,” says Jeffery.

“There’s a real need for change,” says Alistair Nunn, director of science at the Guy Foundation in London, which supports research on quantum biology and its medical applications. “At the moment, everybody’s going down this road of ‘let’s get lots of LEDs’, but it’s not doing us any good at all.”

There are signs of institutional change, with hospitals at the forefront. Studies have shown that infrared light in hospital wards leads to improved clinical outcomes, including shorter stays. And it has long been known that inpatients who have access to daylight are discharged sooner, on average, than those kept in windowless rooms. Now, some hospitals are taking action. Jeffery and his team recently did a light survey of the critical care unit at King’s College Hospital in London. “It’s a beautiful building, but there’s absolutely no long-wavelength light in there,” he says. “I said to them, ‘obviously I’m concerned about your patients, but I’m concerned about your staff too.’” The hospital managers took heed and, in May, opened a new six-bed ward on the roof. Two other London hospitals are considering outside space for their critical care patients, says Jeffery. “I think they’ll follow because they can’t afford not to.”

And even if you aren’t a patient in a critical care ward, you can’t afford to risk 21st-century scurvy through a steady diet of ultra-processed light. “You can live your life without red light,” says Jeffery, “but you cannot live healthily without it.”



Graham Lawton is a consultant for New Scientist. He is the author of *Mustn’t Grumble: The surprising science of everyday ailments*

Research Article

Stimulation of TRPV1 by Green Laser Light

Quanbao Gu,¹ Lina Wang,^{1,2} Fang Huang,³ and Wolfgang Schwarz^{1,4}

Low-level laser irradiation in the mW/cm² range of visible and near-infrared (NIR) light had been introduced as a medical treatment already in the late 60s, but its medical application still remains controversial.

Photo biostimulation particularly in the red and NIR range have been attributed to absorption by cytochrome oxidase C.

The stimulation by the green light is dose-dependent, but after about 2min a steady state is reached.

Detailed investigations have elucidated that irradiation of peripheral nerve with red or NIR laser light may also be of relevance for analgesic effects. In particular, decreased conduction velocities have been reported in experiments on humans and animals with involvement of A δ and C fibres.

In our experiments, we could demonstrate the effectiveness of green laser light on TRPV1. Interestingly, TRPV1 is highly expressed in peripheral A δ and C fibers.

We, therefore, like to suggest that in addition to red and blue laser light also green light with its intermediate penetration depth might be a useful tool in medical treatment.



Mesenchymal Stem Cells Synergize with 635, 532, and 405 nm Laser Wavelengths in Renal Fibrosis: A Pilot Study

Megan O'Connor, MS,¹ Rachana Patil, MS,¹ Jiangzhou Yu, MD, PhD,¹
Richard Hickey, BS,¹ Kavitha Premanand, MS,¹ Andre Kajdacsy-Balla, MD, PhD,²
Enrico Benedetti, MD,^{1,3} and Amelia Bartholomew, MD, MPH, FACS¹

Abstract

Objective: To address whether a single treatment of one of three visible light wavelengths, 635, 532, and 405 nm (constant wave, energy density 2.9 J/m²), could affect the hallmarks of established renal fibrosis and whether these wavelengths could facilitate mesenchymal stem cell (MSC) beneficence. **Background Data:** Chronic kidney disease is a global health problem with only 20% receiving care worldwide. Kidneys with compromised function have ongoing inflammation, including increased oxidative stress and apoptosis, peritubular capillary loss, tubular atrophy, and tubulointerstitial fibrosis. Promising studies have highlighted the significant potential of MSC-based strategies to mitigate fibrosis; however, reversal of established fibrosis has been problematic, suggesting that methods to potentiate MSC effects require further development. **Laser treatments at visible wavelengths have been reported to enhance mitochondrial potential and available cellular ATP, facilitate proliferation, and inhibit apoptosis.** We hypothesized that laser-delivered energy might provide wavelength-specific effects in the fibrotic kidney and enhance MSC responses. **Materials and Methods:** Renal fibrosis, established in C57BL6 mice following 21 days of unilateral ureter obstruction (UUO), was treated with one of three wavelengths alone or with autologous MSC. Mitochondrial activity, cell proliferation, apoptosis, and cytokines were measured 24 h later. **Results:** Wavelengths 405, 532, and 635 nm all significantly synergized with MSC to enhance mitochondrial activity and reduce apoptosis. Proliferative activity was observed in the renal sections following combined treatment with the 532 nm laser and MSC, and the 405 nm laser and MSC.

Acknowledgments

This study was supported by the University of Illinois, Department of Surgery. We thank Steve Shanks for the use of research-grade Erchonia lasers for this study.

Dan Murphy, DC
PO Box 7044
Auburn, CA 95604
Phone: (530) 878-6869
Fax: (530) 878-6559
www.danmurphydc.com
Email: dan@danmurphydc.com

Seminar Coordinator
General Contact and
Professional Assistant:
Michelle Schaer DC
(602) 826-2277
michelle@danmurphydc.com

Life Chiropractic College West
(510) 780-4500
Bookstore Extension: 4502

Dan Murphy's

Article Review Service

Studies are reviewed and summarized and the Key Points pertinent to chiropractic are listed

**The Most Relevant Studies Pertaining to
Chiropractic Clinical Practice**

**Adjusting / Neurology / Orthopedics / Whiplash
/ Posture / Headaches / Pediatrics / Vaccines /
Nutrition / Exercise / Risks / Outcomes /
Benefits /
Maintenance / and More**

**52 Articles Every Year
\$120.00 Per Year**

**24 Years of Archives
\$200.00**

**Sign Up At
www.danmurphydc.com**

**I am very grateful for keeping me in the loop of
knowledge. These article reviews are priceless.**

KB, San Francisco, March 15, 2023

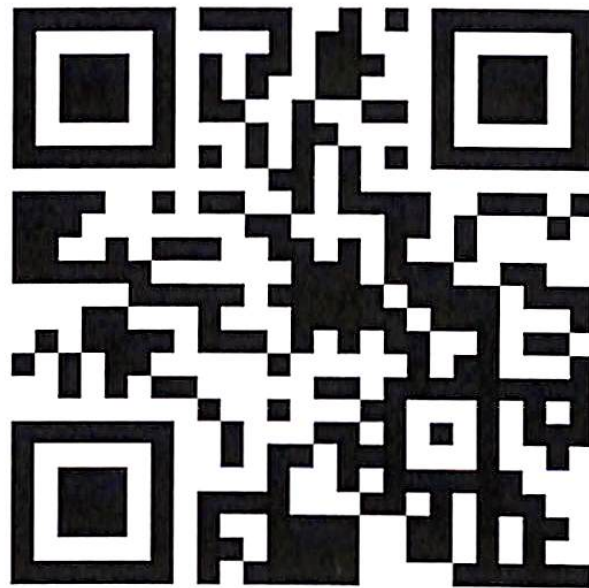
Stay Informed



Practice Guidelines: Fatty Acid Testing

What are your metrics?

Learn more and receive your
free trial opportunity by
scanning the image below.



If you have any difficulty,
simply go to
www.brainspan.com/murphy

DAN MURPHY DC
KNOWLEDGE FOR LIFE SET 5
DVD or Thumb Drive
\$299.00 plus \$10.00 s&h

Disc 1

Levers
Children and Phones
Ultraprocessed Foods and Health
Scientific Evidence

Disc 2

Plastic Brains
Glymphatic Brain Wash
Aggression Violence Homicide
Chiropractic Basic Neurology

.....

Disc 3

Chiropractic Basic Orthopedics
Exercise
Children's Health
Tylenol Autism Controversy

Disc 4

Whiplash 101
Olive Oil
Magnesium and Aging
Aspartame

VACCINATION
\$100.00 + \$10.00 s&h
DVD or Thumb Drive

.....

Name	Phone	Email	
Address	City	State	Zip
Credit Card #	Exp. Date	CCV Code	
Signature	Date	Representative	

COPYRIGHT 2024 by Mesa Chiropractic & Rehabilitation Center PC all rights reserved

DVD or Thumb Drive (Circle One)

Call 602-826-2277 or email to:

drmschaer@cox.net or michelle@danmurphydc.com