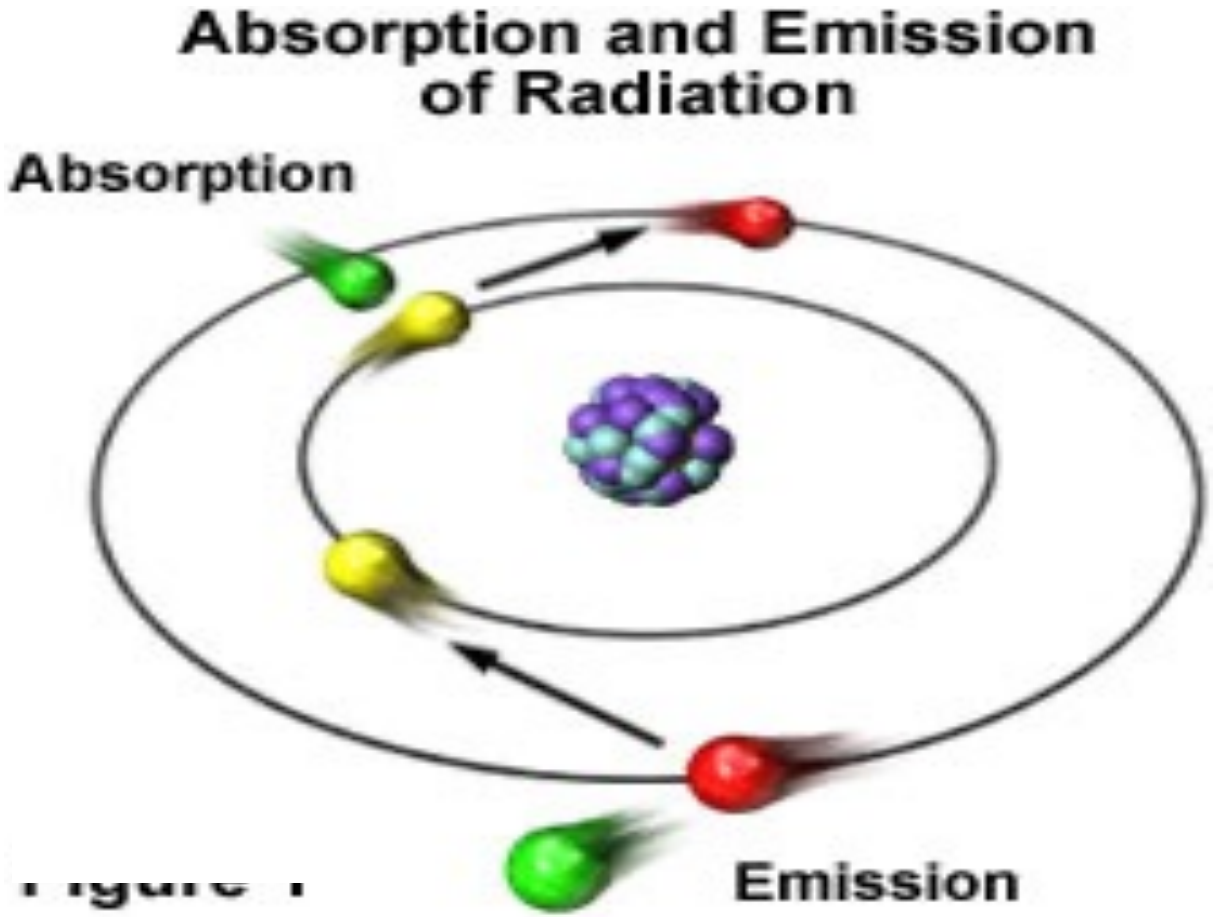
IT’S TIME FOR A NEW SYNTONIC PRINCIPLE

Ray Gottlieb, OD, PhD, Dean CSO



The Syntonic Principle: a Balanced Autonomic

After prolonged and demanding sympathetic effort (running away from a mass shooting), the parasympathetic goes to work to rebalance the autonomic. The faster it brings balance, the better one's health.

In some individuals, The parasympathic might overpower the sympathetic or the opposite might be the case.

They might both increase together, or both might be too weak and neither able to rise to the occasion. In any case unable to sustain a healthy balanced autonomic.

In Spitler's model, selected visible light frequencies (wavelengths) are able to stimulate or relax one or the other branch of the autonomic. Frequencies on the blue side of the visible spectrum boost the parasympathetic. Frequencies on the red side fire up the sympathetic. Green frequencies bring balance.

In the 80 years after Spitler's book was published, much has been discovered about how our bodies work.

It’s time for a new syntonic principle

Spitler’s book, *The Syntonic Principle* (1941), was more of a review of the science of how light might work to improve health. It was not a how-to, recipe book of which color(s) to use for what conditions. The ‘syntonic principle was based on rebalancing the two sides of the autonomic nervous system.

The sympathetic branch provides the energy to perform appropriate actions to reach our goals and overcome the dangers and challenges of life. (“Fight and Flight”)

The parasympathetic branch works to clean up and replenish the nutrients used-up by these efforts. (“Rest and Digest”)

These two sides were thought to be antagonistic rather than mutually supportive.

Successful Syntonic outcomes

Syntonic therapy often improves much more than the diagnosed visual/ocular function(s). Not only do poor accommodation, convergence insufficiency, reduced functional visual field, visual acuity, strabismus, etc. improve, but often after just a few weeks of syntonics brings lasting improvements in our patients' attention, memory, self-esteem, social attitude, happiness, academic success, reading, spelling, handwriting, energy, sleep, physical health, etc.

How can selected frequencies of light shined into the eyes cause such a wide variety of positive changes, sometimes for the very first time in a patient's life.

PHOTOBIOMODULATION
An Explosion of Research

While there is a rapidly expanding body of published work on these observed benefits, the molecular mechanisms governing the initiation of these effects are only superficially understood.

Therapeutic Effects of Photobiomodulation

These include

- tissue healing,
- reduce pain, swelling, inflammation,
- regenerate nerve and stem cells
- protection of tissues from poisons,
- protect from retinal damage due to high-intensity light or hyperoxia,
- ameliorate symptoms of traumatic brain injury
- protect or revitalize mitochondria

When applied correctly, PBM has an almost complete lack of reported adverse effects. The remarkable range of medical benefits provided by PBM, has led some to suggest that it may be “too good to be true”.

Advances in understanding of PBM mechanisms of action at a molecular and cellular level, are beginning to provide a scientific rationale for its use for multiple diseases.

One of the most general benefits of PBM that has recently emerged, is its anti-inflammatory effects. The local reduction of edema, and reductions in markers of oxidative stress and pro-inflammatory cytokines are well established.

There also appears to be a systemic effect whereby light delivered to the body, can positively benefit distant tissues and organs.

PBM, Inflammation and Stem Cell Therapy

Chronic diseases of the modern age involving systemic inflammation such as type II diabetes, obesity, Alzheimer's disease, cardiovascular disease and endothelial dysfunction are again worth investigating in the context of PBM.

The versatile benefits of PBM on the brain and the central nervous system, encourages further study of its ability to reduce neuroinflammation.

There may be some overlap between the ability of PBM to activate and mobilize stem cells and progenitor cells, and its anti-inflammatory action, considering that one of the main benefits of exogenous stem cell therapy has been found to be its anti-inflammatory effect.

MITOCHONDRIAL DISEASE SYMPTOMS
There is an urgent need for more effective treatments and therapies, as current therapies can only provide partial relief.

- Mitochondrial dysfunction is emerging as a causative factor for a wide range of human diseases both inherited and acquired, including cancer, cardiomyopathies, and neuro-degenerative (ND) diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and hereditary spastic paraplegia.
- It has been estimated that 1 in 5000 humans suffers from a mitochondrial disease and estimated to affect more than 50 million adults in the United States alone.

Light, Cytochrome C Oxidase, and Nitric Oxide.

Quirk, B. Whelan, H. (2020) *Photobiomodulation, Photomedicine, and Laser Surgery*: 38 (9) 527–530.

- After many decades of study, the underlying mechanisms of photobiomodulation (PBM) remain elusive. Although the most attractive hypotheses revolve around the role of cytochrome c oxidase (CCO) and cellular energetics, no reliable demonstration of any PBM-related light-induced mechanistic effect on CCO has been reported. Oxygen bound to the heme a3 site of CCO was photolabile using light of 532 nm.
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Gut-Brain

The enteric nervous system – a third branch of the autonomic nervous system

- The enteric nervous system (neurons and glial cells) of the gastrointestinal tract regulate all the functions of the gut. (the gut brain).
- The gut require the secretion of fluid into the lumen in a regulated manner.
- Fluid is controlled by the secretion or absorption of ions across the epithelium.
- Enteric glia work in concert with enteric nerves by using nitric oxide to regulate the movement of ions across the wall of the colon, thereby affecting water movement and hence digestion and host defense.

Vagal (parasympathetic) works by releasing NO in the gut.

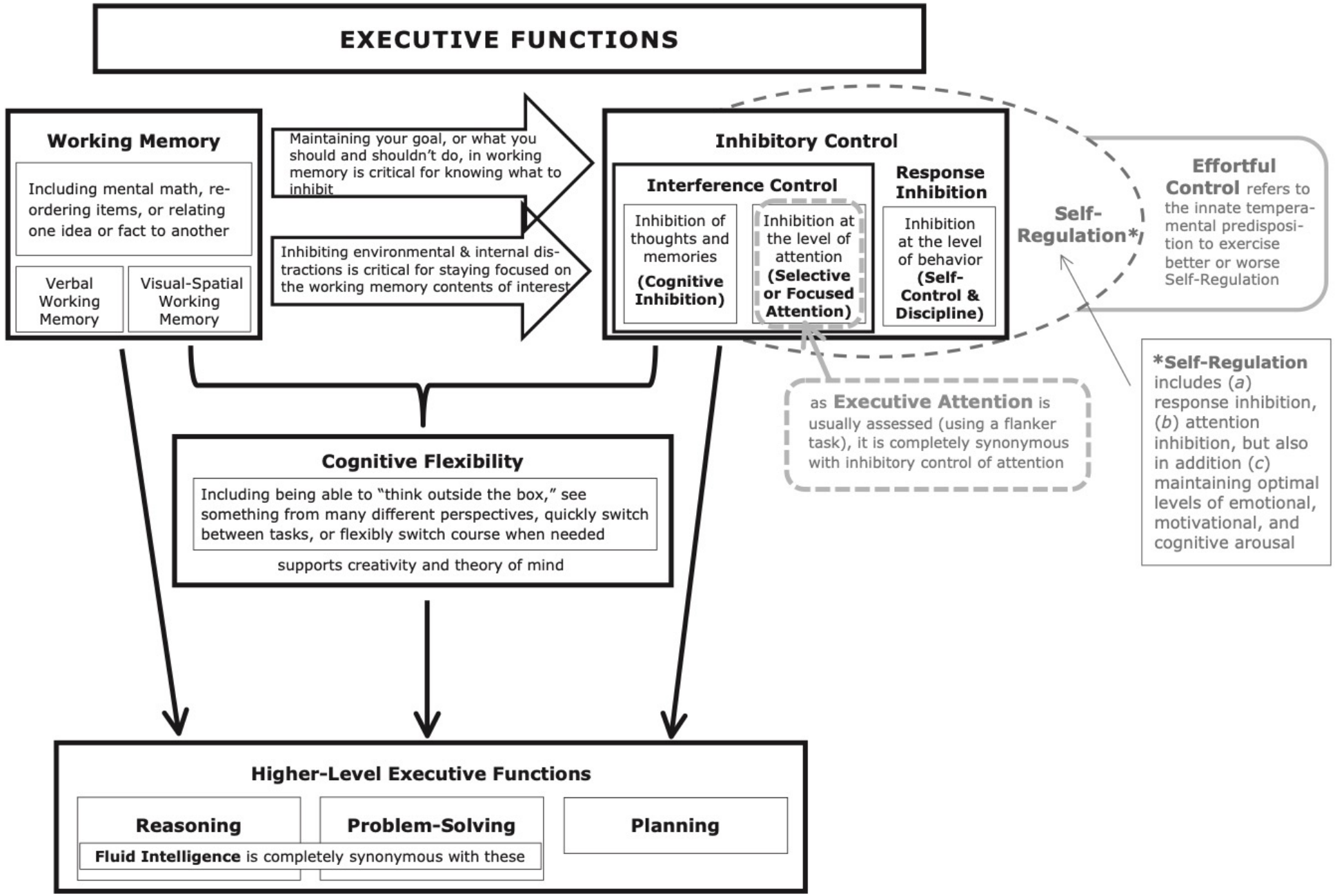
- The control of water movement across the epithelium of the gastrointestinal (GI) tract is central to health and well-being. Water movement is required to hydrate the surface of the epithelium for contact digestion and nutrient absorption, and as an essential component of the epithelial barrier and hence innate immunity. Neurons of the submucosal plexus of the enteric nervous system (ENS) represent the main physiological control mechanism regulating epithelial ion transport. In contrast, neurons of the myenteric plexus that are well known to control GI motility have been largely overlooked when considering the regulation of epithelial ion transport. Our understanding of the control of epithelial barrier function has taken on a new dimension recently because it was shown that not only were neurons of the ENS involved, but also the enteric glial cells. Enteric glia are analogous to astrocytes of the central nervous system, protecting and supporting enteric neurons (**Gabella, 1981**). In addition to regulating barrier function, enteric glia actively participate in neurotransmission within the ENS (**Gulbransen & Sharkey, 2009**; **Gulbransen *et al.* 2010**). Whether enteric glia play a role in the regulation of ion transport has yet to be determined.
- Under normal conditions nitric oxide (NO) is produced by the nitric oxide enzyme (NOS). During inflammation NO is released in higher amounts. Nitric oxide liberated from a variety of cell types including neurons can affect enteric epithelial ion transport by acting directly upon the epithelium and through the submucosal plexus of the ENS. modulated by NO-mediated cross-talk between neurons and enteric glia of the myenteric plexus. Vagal (parasympathetic) signaling results in the release of NO from neurons and enteric glia to modulate epithelial ion transport

TBI the Silent Epidemic

- Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide.
- TBI is called a “silent epidemic” because consequences of the trauma are not always immediately visible.
- The term “silent” furthermore reflects the common underestimation and unawareness of the impact of TBI by the society.
- This is particularly reflected by the fact that a clear definition of “TBI” has been lacking until recently.
- TBI can now be defined as “an alteration in brain function, or other evidence of brain pathology, caused by an external force.”

Traumatic brain injury has been called “the most complicated disease of the most complex organ of the body”

Despite increased awareness of TBI—and particularly concussion—in recent years, we still lack effective means of diagnosis, prognosis, and treatment. Over 30 clinical trials of pharmaceutical products to treat TBI have failed, and the U.S. Food and Drug Administration has yet to approve a single diagnostic test or therapy for the condition. The total cost of these failed clinical trials is estimated at 1.1 billion dollars. Research is hampered by imprecise classification, methodological inconsistencies, measurement issues, and uncertainty about underlying pathophysiology. (2017)


EXECUTIVE FUNCTIONS
Working Memory
Including mental math, re-ordering items, or relating one idea or fact to another
Verbal Working Memory
Visual-Spatial Working Memory
Maintaining your goal, or what you should and shouldn't do, in working memory is critical for knowing what to inhibit
Inhibiting environmental & internal distractions is critical for staying focused on the working memory contents of interest
Inhibitory Control
Interference Control
Inhibition of thoughts and memories (Cognitive Inhibition)
Inhibition at the level of attention (Selective or Focused Attention)
Response Inhibition
Inhibition at the level of behavior (Self-Control & Discipline)
Self-Regulation*
Effortful Control refers to the innate temperamental predisposition to exercise better or worse Self-Regulation
as Executive Attention is usually assessed (using a flanker task), it is completely synonymous with inhibitory control of attention
*Self-Regulation includes (a) response inhibition, (b) attention inhibition, but also in addition (c) maintaining optimal levels of emotional, motivational, and cognitive arousal
Cognitive Flexibility
Including being able to "think outside the box," see something from many different perspectives, quickly switch between tasks, or flexibly switch course when needed
supports creativity and theory of mind
Higher-Level Executive Functions
Reasoning
Problem-Solving
Planning
Fluid Intelligence is completely synonymous with these

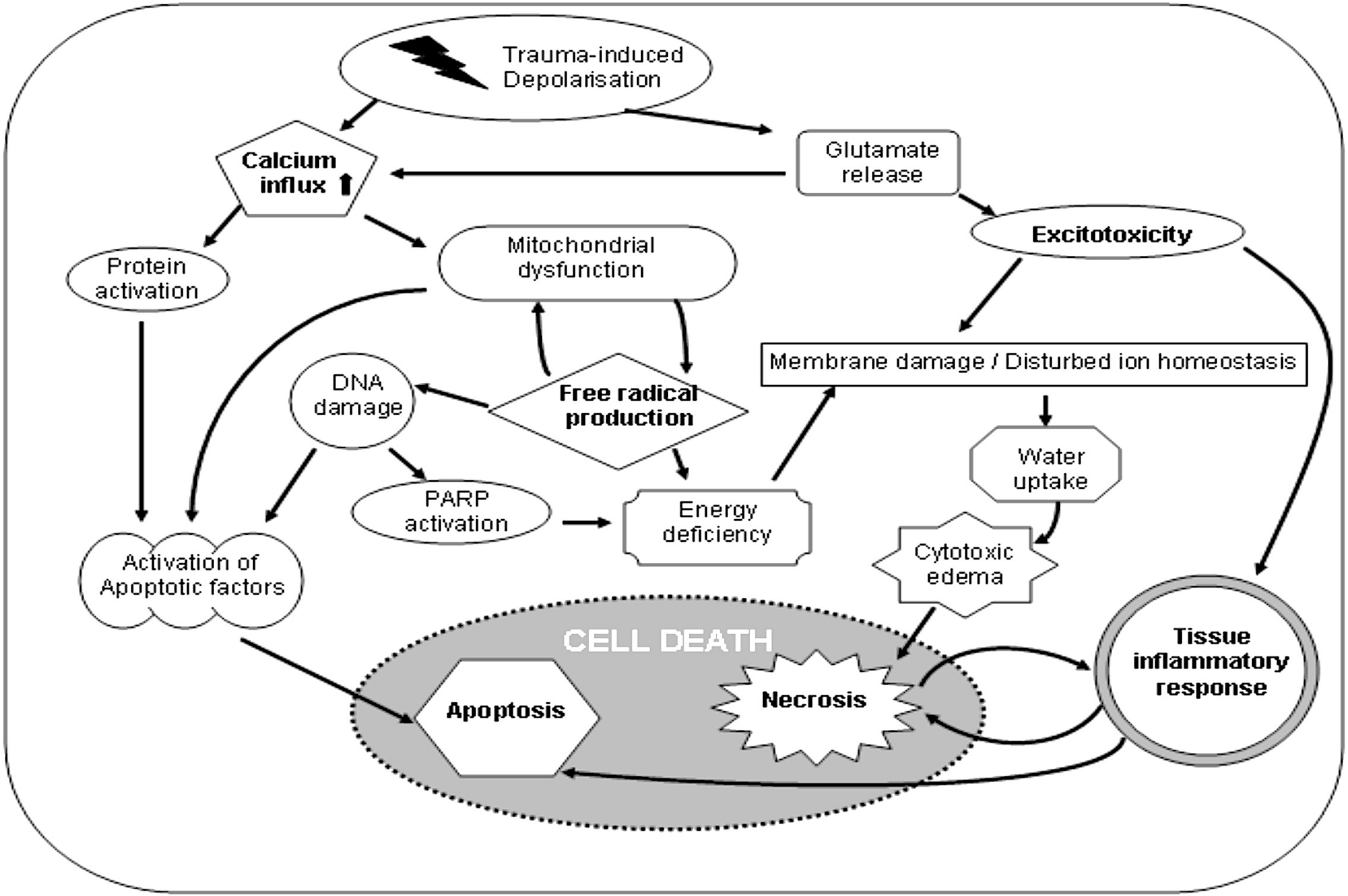
Brain Edema & TBI

Brain edema was initially defined as an increase in net brain water content leading to an increase in tissue volume. Extensive research has led to a more accurate definition, This includes pathophysiological characteristics such as molecular, cellular, structural, and functional changes in the blood–brain barrier (BBB), microcirculation, cell volume regulation, and cell death.

Given the fixed volume of the rigid skull even a small degree of cerebral edema can compromise cerebral blood flow, metabolism, compressed vital structures, and the secondary neuronal injury caused by swelling.

Impact due to brain edema is more severe and extensive damage of many pediatric neurological disorders.

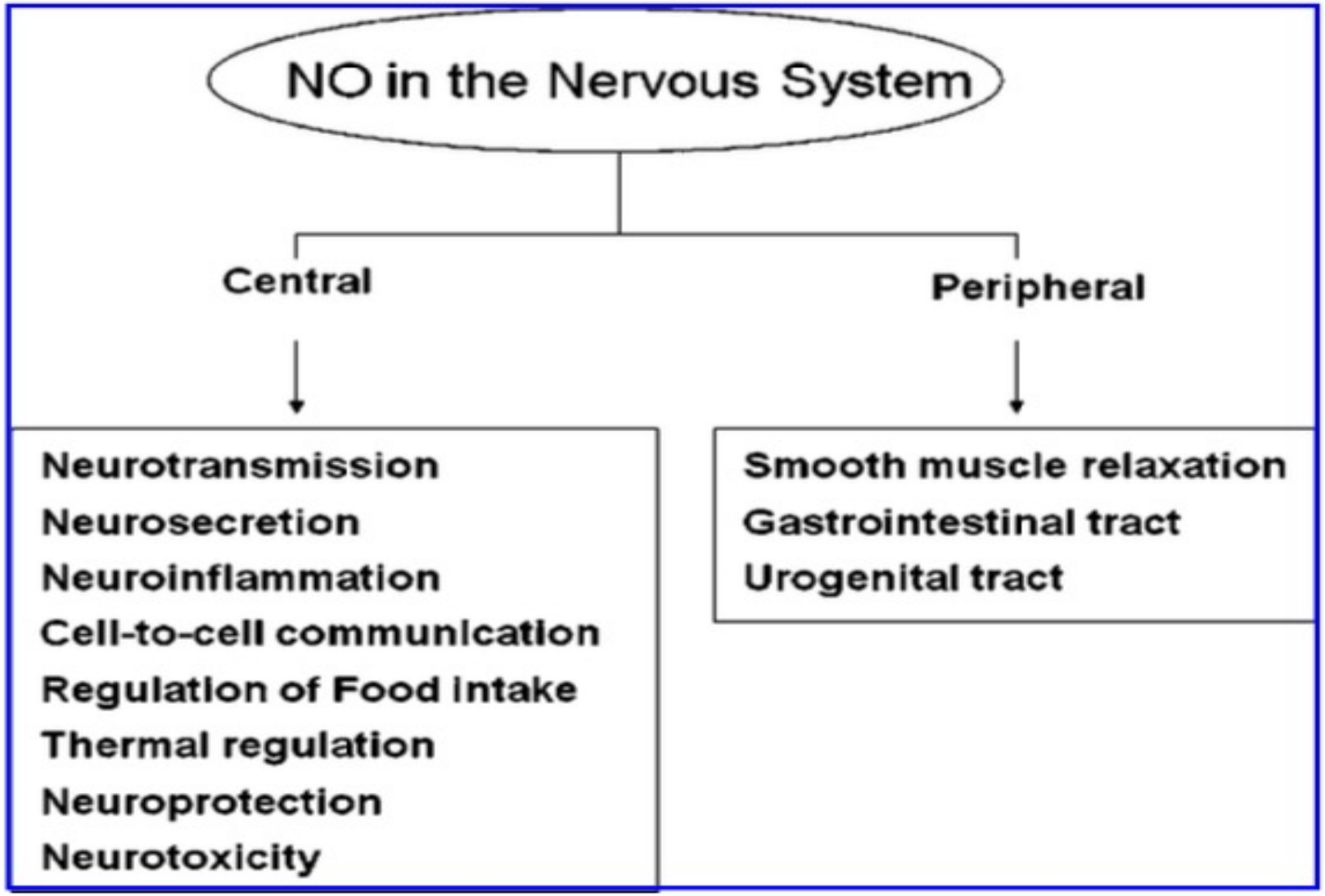
Secondary Neuronal Damage Following TBI



We propose that the effectiveness of photobiomodulation is under the control of tissue NO and oxygen levels.

- Although early studies identified mitochondrial cytochrome c oxidase as an endogenous photoreceptor for photobiomodulation, the cellular and molecular mechanisms underlying photobiomodulation have not been clear.
- Low intensity light has been found to enhance nitric oxide synthesis by cytochrome c oxidase without altering its ability to reduce oxygen.
- From these findings, we propose that cytochrome c oxidase functions in photobiomodulation by producing nitric oxide, a signaling molecule which can then function in both intra- and extracellular signaling pathways. [Discovery Medicine 11(57): February 2011]

Maybe NO is the Answer



Nitric Oxide NO

- NO is involved in several cellular functions, such as neurotransmission, regulation of vessel tone, and immune response . Intracellular signal-transduction pathways are involved in NO effects via it's interaction with other intracellular targets and a wide array of stimulatory or inhibitory signals. NO can travel freely, even through cell membranes to signal and coordinate neighboring cells
- In the central nervous system, NO production has been associated with cognitive function, because of its role in the induction and maintenance of synaptic plasticity, control of sleep, appetite, thermoregulation, and neurosecretion.
- NO also regulates the relaxation of the smooth muscle causing blood vessels to dilate in specific locations in the central nervous system thus bringing blood and oxygen to efficiently provide for the rapidly changing needs of different parts of the brain, and also to the body organs and the gastrointestinal tract.
- In contrast, the term, ‘‘nitrosative stress’’, has been used to indicate the cellular damage elicited by NO and its sequelae. Excess NO, can contribute to uncontrolled inflammatory response cytotoxicity causing acute and chronic neurodegenerative diseases including stroke, multiple sclerosis, Parkinson disease (PD), and Alzheimer disease (AD).

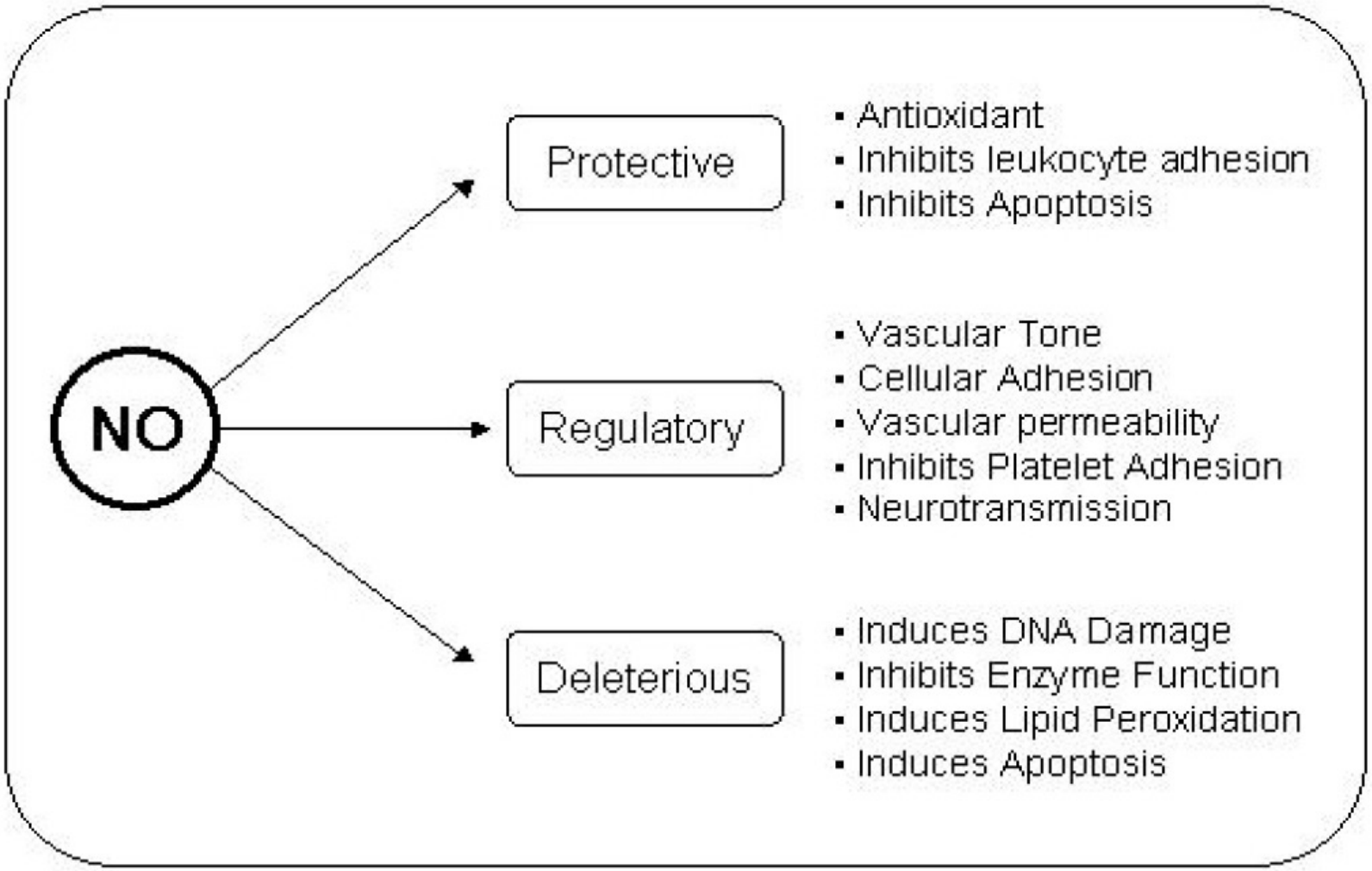
The NO produced by Cco/NO functions in a clinically beneficial way.

The NO generated by Cco/NO is an ideal candidate for initiating signaling pathways in response to the photobiomodulation of cytochrome c oxidase.

The NO produced by Cco/NO can be used both inside cells, where it functions in hypoxic signaling, and outside of cells where it may function in vasodilation and other signaling pathways. This is likely to have a multitude of effects.

Insofar as light may have differential effects under normoxic and hypoxic conditions, the challenge for the future will be to sort out the relationships between tissue oxygen levels and the beneficial effects of photobiomodulation.

The good and the ugly face of NO



NO is involved in neurotransmission, regulating blood vessel dilation, and immune action

NO in the central nervous system helps cognitive function, synaptic plasticity, sleep, appetite, thermoregulation, and neurosecretion.

In the peripheral nervous system, NO regulates the relaxation of smooth muscle in the corpora cavernosa, (penile erection), and in many aspects of the gastrointestinal tract.

NO interacts with Intracellular signal-transduction pathways to trigger other intracellular targets to stimulate or inhibit signals able to control a wide range of actions.

In contrast, high levels of NO can be toxic when released in an uncontrolled inflammatory response and can participate in acute and chronic neurodegenerative diseases including stroke, multiple sclerosis, Parkinson disease (PD), and Alzheimer disease.

Nitro- and Oxidant-Mediated Damage

- in circumstances associated with high NO production, nitrosative stress enhances the cellular susceptibility to oxidant-mediated damage, providing an important cycle of cytotoxic amplification in inflammatory conditions.
- In contrast, the beneficial influence of NO at low concentration on intracellular GSH, represents another aspect of NO acting as an antioxidant and cytoprotective molecule.
- “Nitrosative stress’’ refers the cellular damage of proteins elicited by NO and its sequelae, The substantially different roles of these different types of modification are implicated in various physiologic and pathologic processes. Numerous S-nitrosylated proteins have been identified in vivo, including blood serum albumin, hemoglobin and red blood cells.

Oxidative stress

Oxidative stress

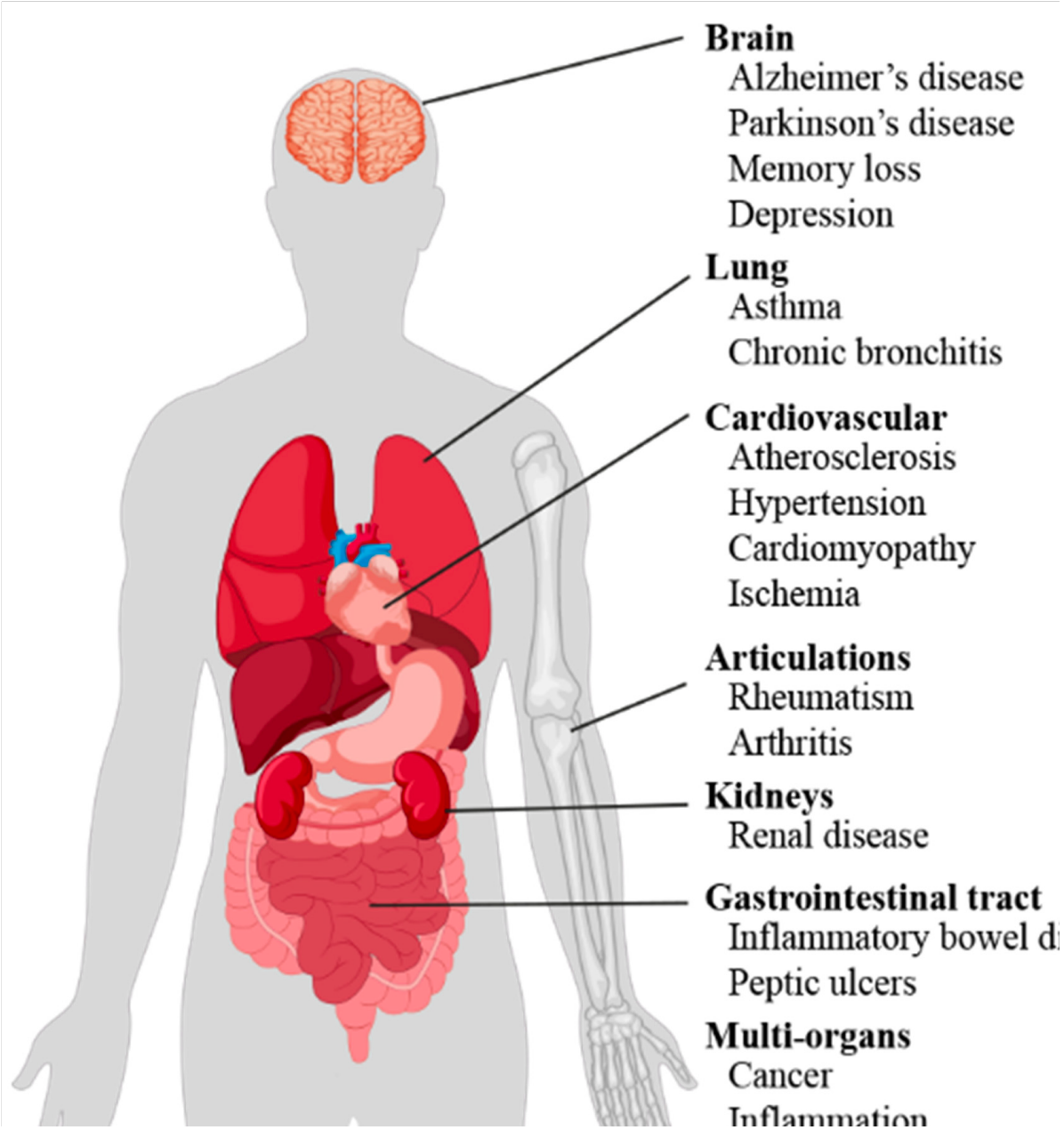
Oxidative stress in living organisms results from the imbalance between the production of reactive oxygen species (ROS) and the ability to neutralize them. The disparity between excessive reactive molecules and weak endogenous defense leads to damage to cell structures and molecules such as lipids, proteins, and DNA, ultimately contributing to the pathogenesis of a wide range of diseases.

ROS, when available in appropriate low amounts, act as signal transduction molecules driving cell activities and also provide cell protection. On the other hand, if generated in excess, as in inflammation, ROS can trigger the production of additional highly reactive species. Crucially is the oxidative modification of key enzymes or regulatory sites, whose redox modification triggers cell signaling alteration and programmed cell death.

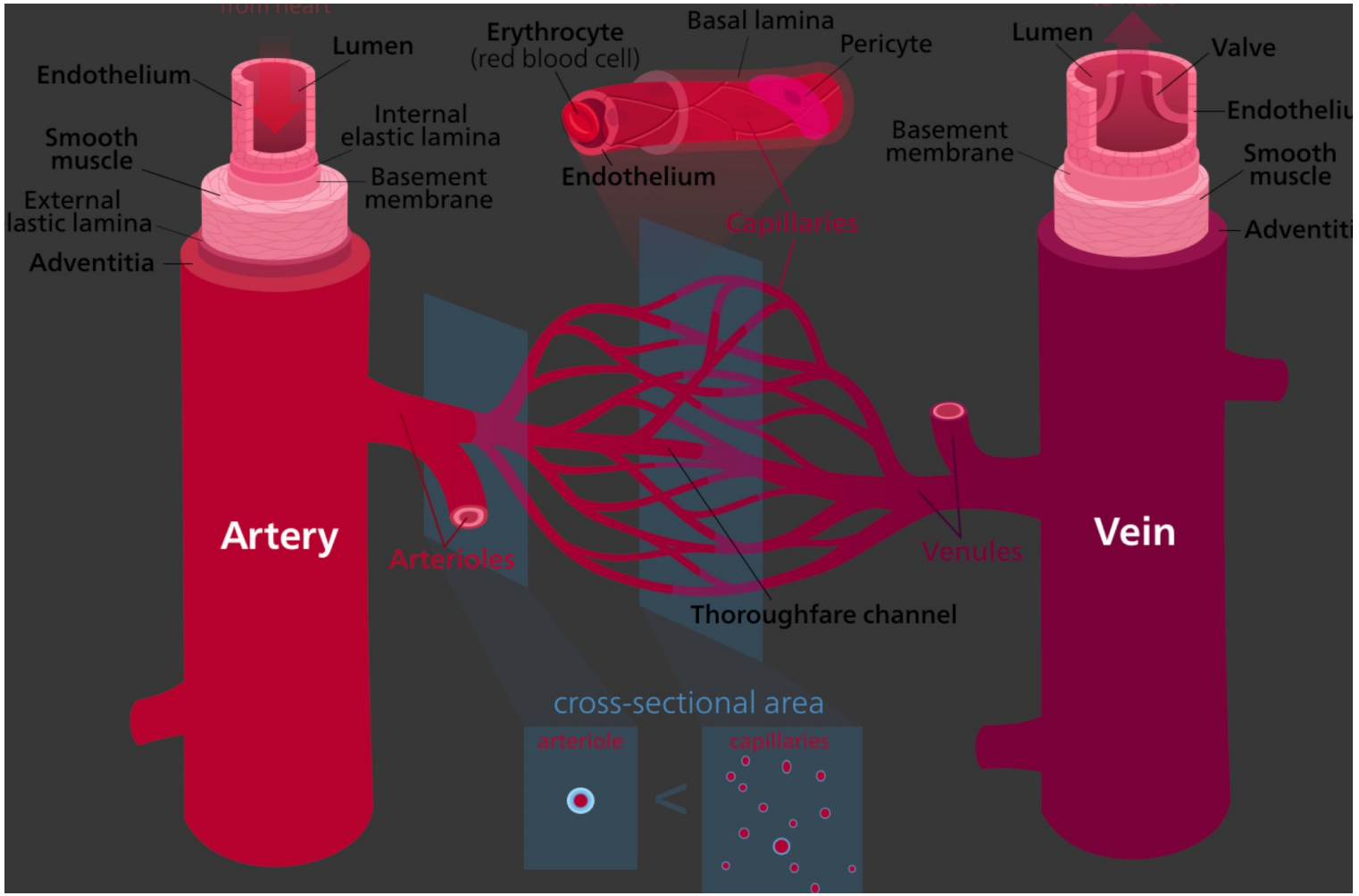
Oxidative stress and inflammation are closely linked. Oxidative stress can cause inflammation and this, in turn, induces oxidative stress generating a vicious circle that results in cell damage, which promotes a pro-inflammatory environment.

Literature data confirm the key role of oxidative stress in etiology of numerous and different diseases (Figure 1), including metabolic syndrome [6], atherosclerosis [7], cardiovascular disease [8,9], cancer [10,11], neurodegenerative disorders [12,13] diabetes [14], infertility [15], renal diseases [16], and gastrointestinal and hepatic diseases [17].

Oxidative Stress in Human Pathology



Blood


Endothelium
Lumen
Internal elastic lamina
Smooth muscle
Basement membrane
External lastic lamina
Adventitia
Erythrocyte (red blood cell)
Basal lamina
Pericyte
Endothelium
Capillaries
Lumen
Valve
Basement membrane
Smooth muscle
Artery
Arterioles
Venules
Vein
Thoroughfare channel
cross-sectional area
arteriole
capillaries
<

- Previously, red blood cells (RBCs) were considered exclusively as transporters of oxygen and nutrients to the tissues.
- More recent experimental evidence indicates that RBCs are important inter-organ communication systems with additional functions, including participation in control of systemic nitric oxide metabolism, redox regulation, blood rheology, and viscosity.

Retinal Vessel Caliber and the Long-Term Incidence of Age-Related Cataract

- We aimed to assess whether narrowed retinal vessel caliber predicted the long-term incidence of age-related cataract.
- Retinal vessel narrowing predicted greater risk of long-term incidence of cataract and cataract surgery.
- Retinal vessel narrowing could be a marker of age-related factors associated with risk of posterior subcapsular and nuclear cataract.
- If light can influence changes in blood and microcirculation, syntonics

Dan Oren

TBI

Cerebral Blood Flow

The vasculature in the brain is an actively regulated organ which, on the one hand, is influenced by global changes of systemic parameters (systemic blood pressure, blood gases, and blood pH).

On the other hand, is capable of locally directing the blood to the regions of demand with high spatial and temporal resolution.

Together with pericytes and astrocytes, as part of the neurovascular unit, cerebral vascular endothelium forms the tight blood-brain barrier and has an important immuno-regulatory function.

TBI Brain Edema & Ischemia

Brain edema was initially defined as an increase in net brain water content leading to an increase in tissue volume. Extensive research has led to a more accurate definition, including pathophysiological characteristics such as molecular, cellular, structural, and functional changes in the blood–brain barrier (BBB), microcirculation, cell volume regulation, and cell death. With even small changes in cellular and extracellular volume, cerebral edema can compromise regional or global cerebral blood flow and metabolism, or result in the compression of vital structures, given the fixed volume of the rigid skull and the secondary neuronal injury caused by swelling. Less is known about developing brain. Brain water content is higher in juvenile rats than in adults, and edema formation is more severe and extensive in many pediatric neurological disorders.

Cerebral ischemia is defined as a condition where the brain or its parts do not receive enough blood flow to maintain normal neurological function. This causes metabolic changes and possibly cellular death. Restoration of blood flow, although necessary for brain survival, could lead to excessive reactive oxygen species (ROS) formation and nitric oxide synthase (NOS) activation with resulting oxidative/nitrosative stress. Thus, cerebral ischemia and reperfusion can produce neuronal damage triggering a complex series of biochemical events that affect structure and function of brain.

There is an urgent need for more effective treatments and therapies, as current therapies can only provide partial relief of mitochondrial disease symptoms

- Mitochondria are complex, dynamic, and vital organelles that mediate several fundamental and critical cellular processes — including metabolism, respiration, ion homeostasis, and apoptosis.
- Mitochondrial dysfunction is emerging as a causative factor for a wide range of human diseases both inherited and acquired, including cancer, cardiomyopathies, and neuro-degenerative (ND) diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia, and Charcot–Marie–Tooth disease.
- It has been estimated that 1 in 5000 humans suffers from a mitochondrial disease and estimated to affect more than 50 million adults in the United States alone.

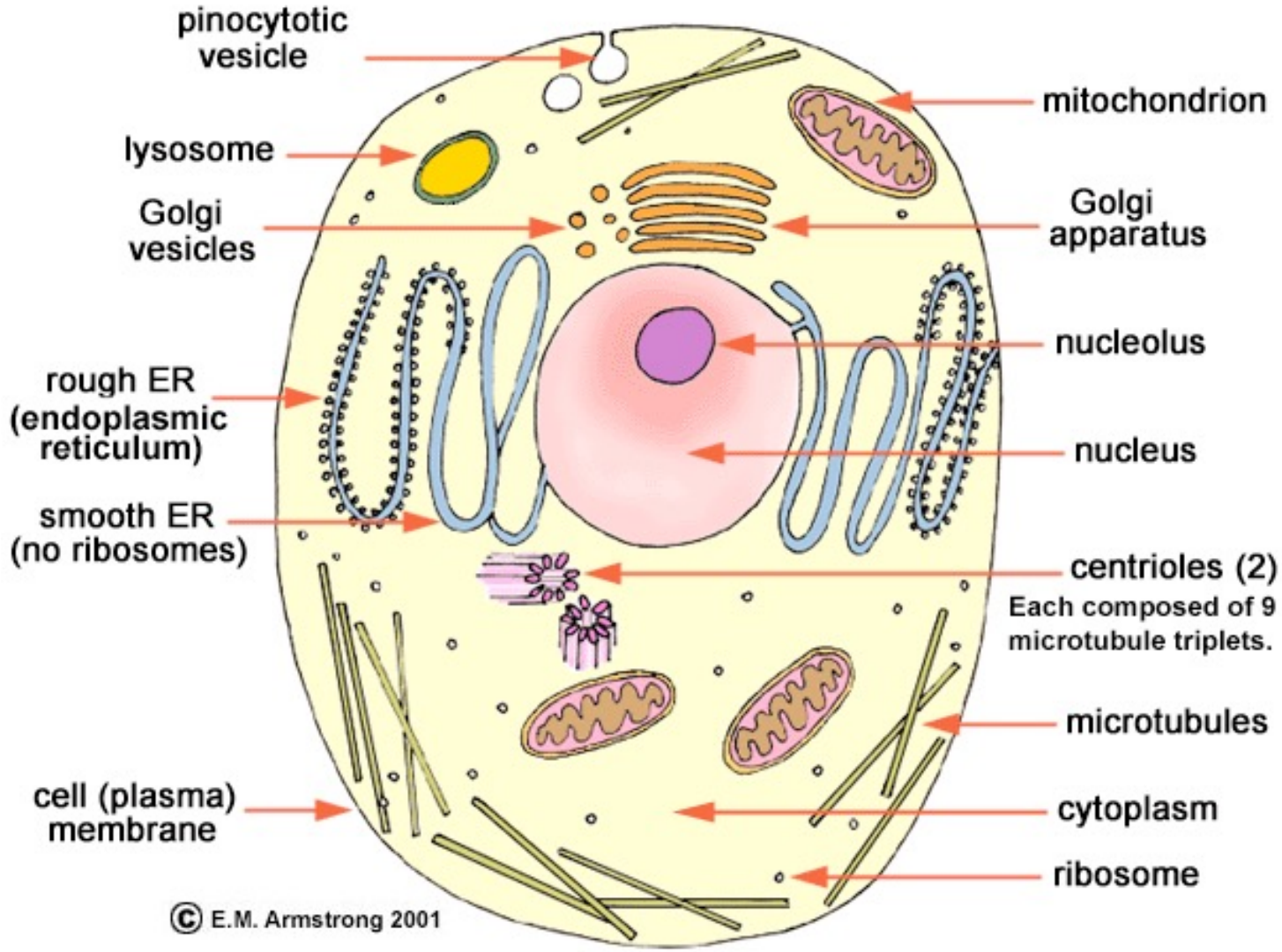
Mitochondria functions

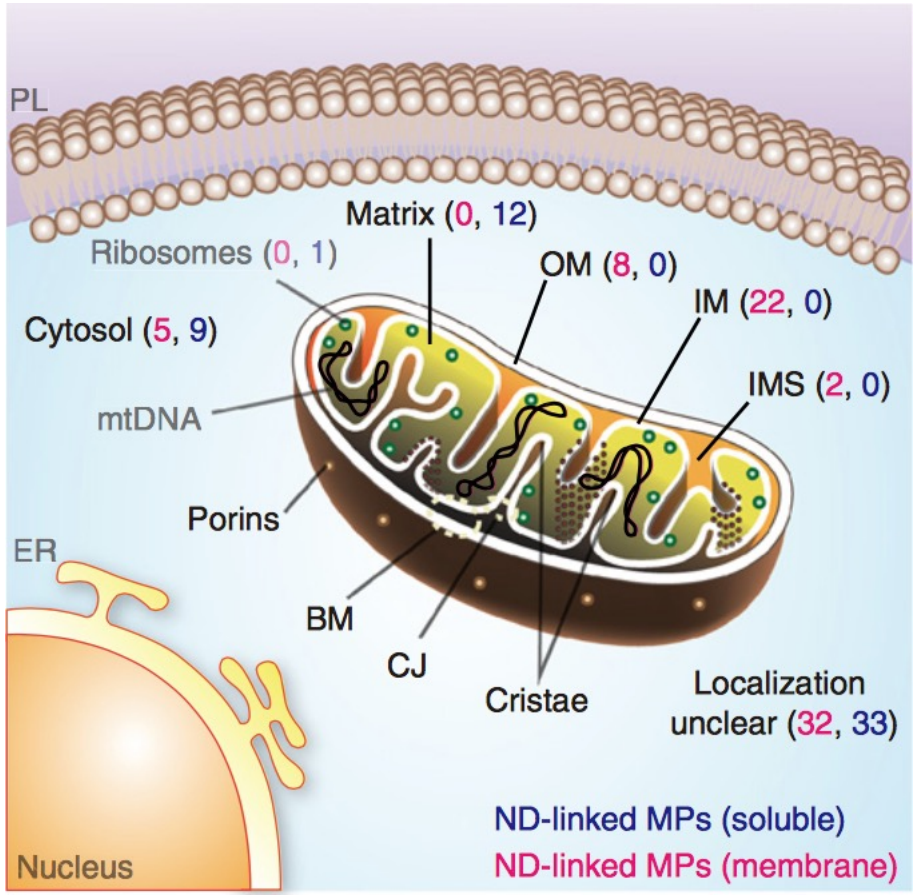
- Mitochondria are involved in many cellular functions, including the production of adenosine triphosphate (ATP), redox homeostasis, ROS and NADPH generation, calcium metabolism, and apoptosis [151]. Moreover, mitochondria can also detect warning signs and induce inflammation by activating and controlling the innate immune system [152]. Given the importance of mitochondria, alterations in their functions can have a profound effect on immunology and cell biology.
- For instance, abnormalities in mitochondrial function have been described in human airway smooth muscle (ASM) cells from asthmatic patients [153], and in bronchial epithelial cells from ex-smokers with chronic obstructive pulmonary disease (COPD) [154]. In both pathologies, there are excessive mitochondrial ROS production, damaged mitochondrial structures with depletion of cristae, increased branching, elongation, and swelling of the mitochondria. Moreover, ASM cells from severe asthmatic patients present also a lack in the NRF2 antioxidant system [153].
- Furthermore, it has been observed that oxidative damage can cause lesions of endothelial cells and deleterious vasodilatory effects, which could induce functional alterations in the smooth muscle cells of the vessel wall.

Only the cells of the patient can heal the patient.

- No physician in the history of humanity has ever healed a patient. Only cells know how to close wounds, understand what to do with insulin and how to destroy pathogens.
- The best a physician can do is to: move obstacles out of the way of cells (e.g. by surgery), supply materials and weapons to the cells (e.g. drugs and building blocks of life) and leave the fight against disease to the cells.

“AN ORGANISM IS A**"WHIRLWIND OF CELLS,"MADE UP OF WHIRLWINDS OF ATOMS**.”— Vernadsky





mitochondrial subcellular compartments

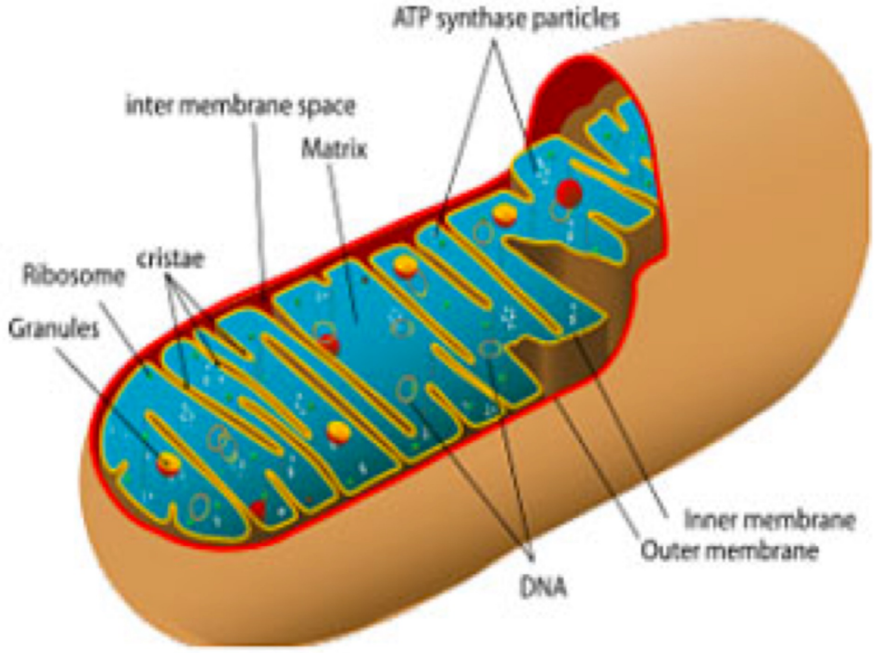
Mitochondria

There are four mitochondrial sub-compartments: outer membrane, inter-membrane space, inner membrane, and inner membrane space or the matrix.

Each of these sub-compartments is further organized into regions containing specific functions and unique biochemical capabilities. These processes and functions are highly interconnected and integrated with extra-mitochondrial pathways.

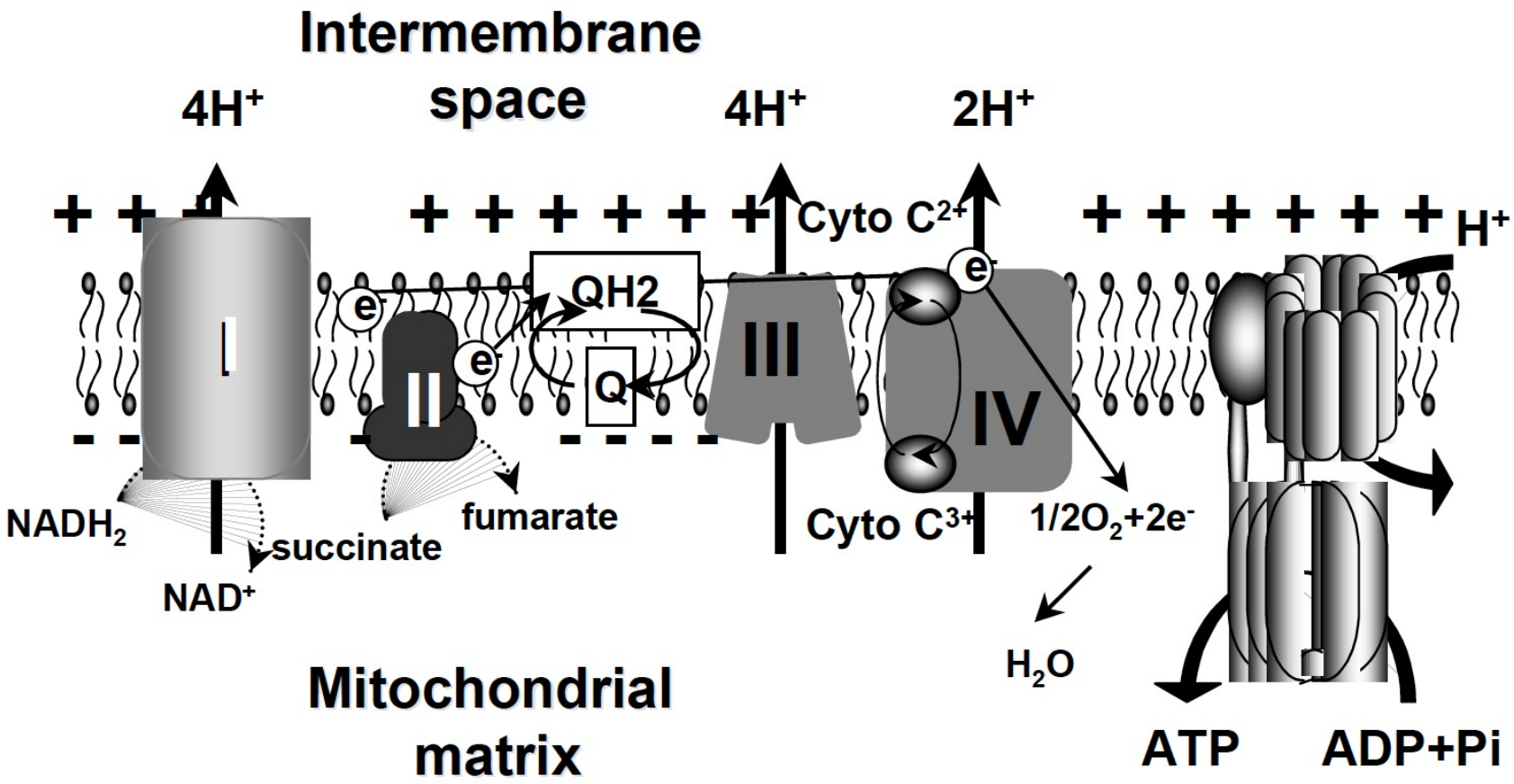
Active mechanisms coordinate the assembly of nuclear- and mitochondrial-encoded proteins.

Like any other modular biological subsystem, cellular processes in mitochondria are mediated by extensive and complex networks of physical (protein–protein) and functional (gene–gene, or genetic) interactions.



Mitochondrial machineries function together in pathways and complexes, particularly within the mitochondria but also between mitochondria and extra-mitochondrial proteins. There is a strong global association between protein connectivity and disease activity.

Mitochondria COXC



The nitric oxide generated by Cco/NO is an ideal candidate for initiating signaling pathways in response to the photobiomodulation of cytochrome c oxidase.

Recently, mitochondrial cytochrome c oxidase was discovered to have a second enzymatic activity: the reduction of nitrite to nitric oxide. This activity has been called Cco/NO. The rate of the Cco/NO reaction increases with nitrite concentration and with decreasing pH, findings that are consistent with the following reaction: (NO_2^- + Fe(II) + H^+ →Fe(III) + OH^-).

Cco/NO activity is operative at physiological nitrite concentrations and has been detected in a variety of eukaryotes, including yeast, rat liver, human endothelial cells, bovine heart and calf liver, plants, and algae.

Some of the nitric oxide produced by Cco/NO acts inside of cells and some is released from cells. nitric oxide produced by Cco/NO can be used both inside cells, where it functions in hypoxic signaling, and outside of cells where it may function in vasodilation and other signaling pathways. it is likely to have a multitude of effects.

Insofar as light may have differential effects under normoxic and hypoxic conditions the challenge for the future will be to sort out the relationships between tissue oxygen levels and the beneficial effects of photobiomodulation, and to elucidate how the nitric oxide produced by Cco/NO functions in a clinically beneficial way.

Nitric oxide (NO) is a powerful regulator of circulation.

Nitric oxide (NO), a free radical gas that is a powerful regulator of circulation (it is an endogenous vasodilator) and a neurotransmitter (it helps in the processing of nerve signals as they cross synapses).

The Nobel Prize was awarded to three Americans in 1998 for their work on discovering NO and clarifying its role in health. Their most important contributions lay in describing the effect of NO on the circulation. The blood flow and nerve responses are rapid. Small increases in NO lead to both vasodilation and to better sensory perception. NO metabolism is necessary for normal circulation (venous, arterial, and lymph flows) and for the ability to sense pain, temperature, and pressure.

NO and a Novel Function of Mitochondrial Cytochrome C Oxidase

Although early studies identified mitochondrial cytochrome c oxidase as an endogenous photoreceptor for photobiomodulation, the cellular and molecular mechanisms underlying photobiomodulation have not been clear.

Three recent findings provide important new insight.

First, nitric oxide has been implicated.

Second, cytochrome c oxidase, an enzyme known to reduce oxygen to water at the end of the mitochondrial respiratory chain, has been shown to have a new enzymatic activity — the reduction of nitrite to nitric oxide. This nitrite reductase activity is elevated under hypoxic conditions but also occurs under normoxia.

And third, low intensity light enhances nitric oxide synthesis by cytochrome c oxidase without altering its ability to reduce oxygen.

From these findings, we propose that cytochrome c oxidase functions in photobiomodulation by producing nitric oxide, a signaling molecule which can then function in both intra- and extracellular signaling pathways. We also propose that the effectiveness of photobiomodulation is under the control of tissue oxygen and nitrite levels.

[Discovery Medicine 11(57):n-n, February 2011]

NO

- NO is implicated in numerous physiological and
- pathophysiological neurodegenerative diseases and injuries,
- such as stroke, epileptic seizures, Alzheimer’s
- disease, Huntington’s disease, Parkinson’s disease,
- amyotrophic lateral sclerosis (ALS), traumatic brain
- injury, aging and AIDS dementia. NO is a key modulator
- in physiological processes such as memory, long term
- potentiation, and long-term depression (Tabuchi
- et al., 1996; Fitzsimonds and Poo, 1998; Hawkins et
- al., 1998; Tao and Poo, 2001).

Light effects on NO and TBI

Cerebral Ischemia

- Cerebral ischemia is defined as a condition where the brain or its parts do not receive enough blood flow to maintain normal neurological function. This causes metabolic changes and possibly cellular death. Restoration of blood flow, although necessary for brain survival, could lead to excessive reactive oxygen species (ROS) formation and nitric oxide synthase (NOS) activation with resulting oxidative/nitrosative stress. Thus, cerebral ischemia and reperfusion can produce neuronal damage triggering a complex series of biochemical events that affect structure and function of brain.

NO is involved diverse range of cellular functions

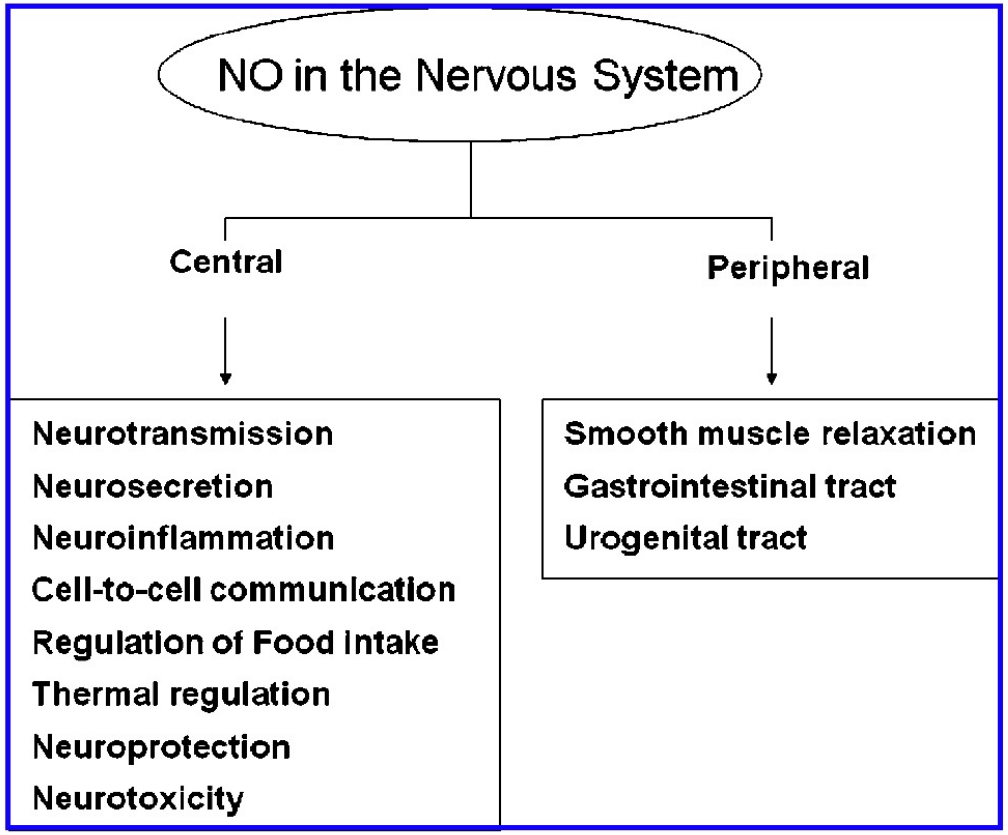


FIG. 2. Some of the main effects of nitric oxide (NO) in the central and peripheral nervous system.

Wavelength- and irradiance-dependent changes in intracellular nitric oxide level

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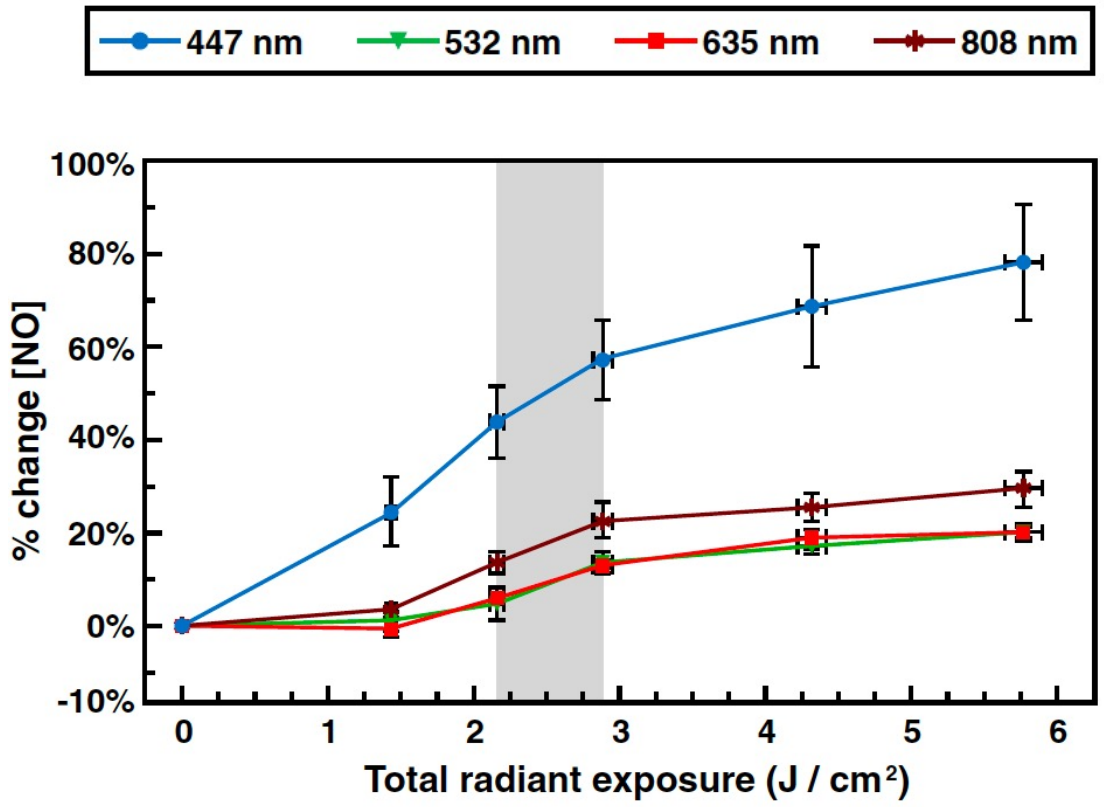
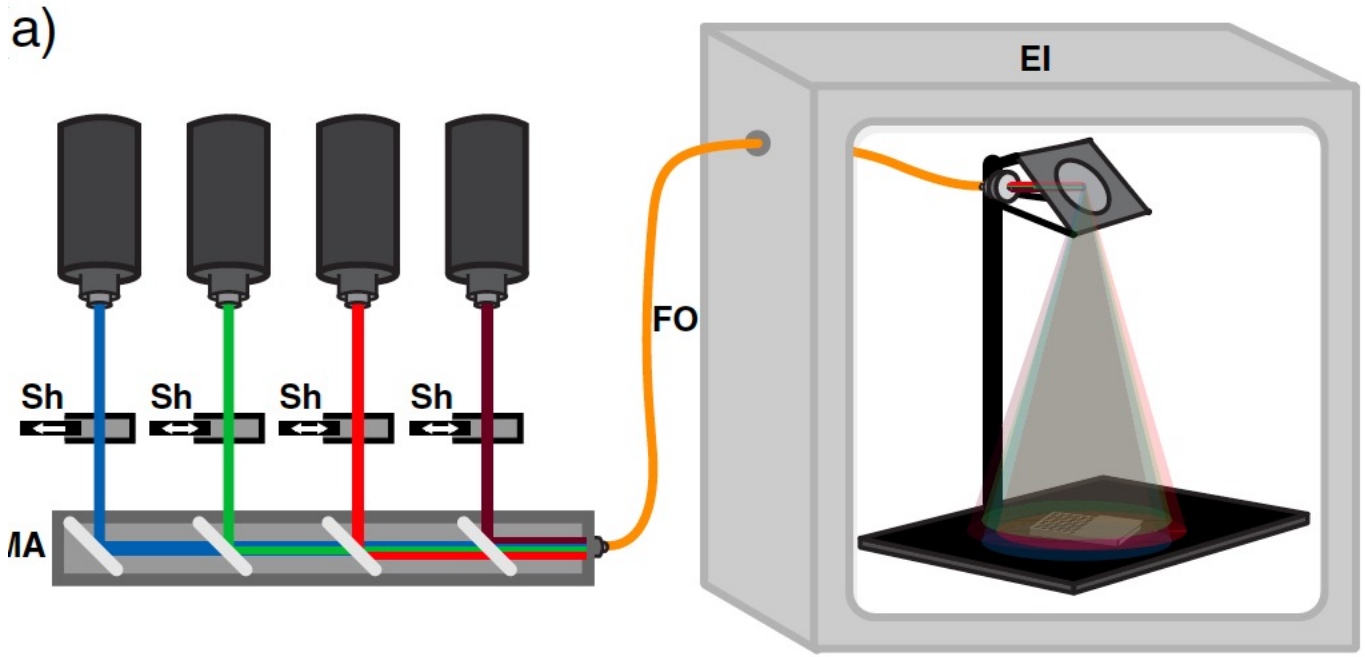
Abstract

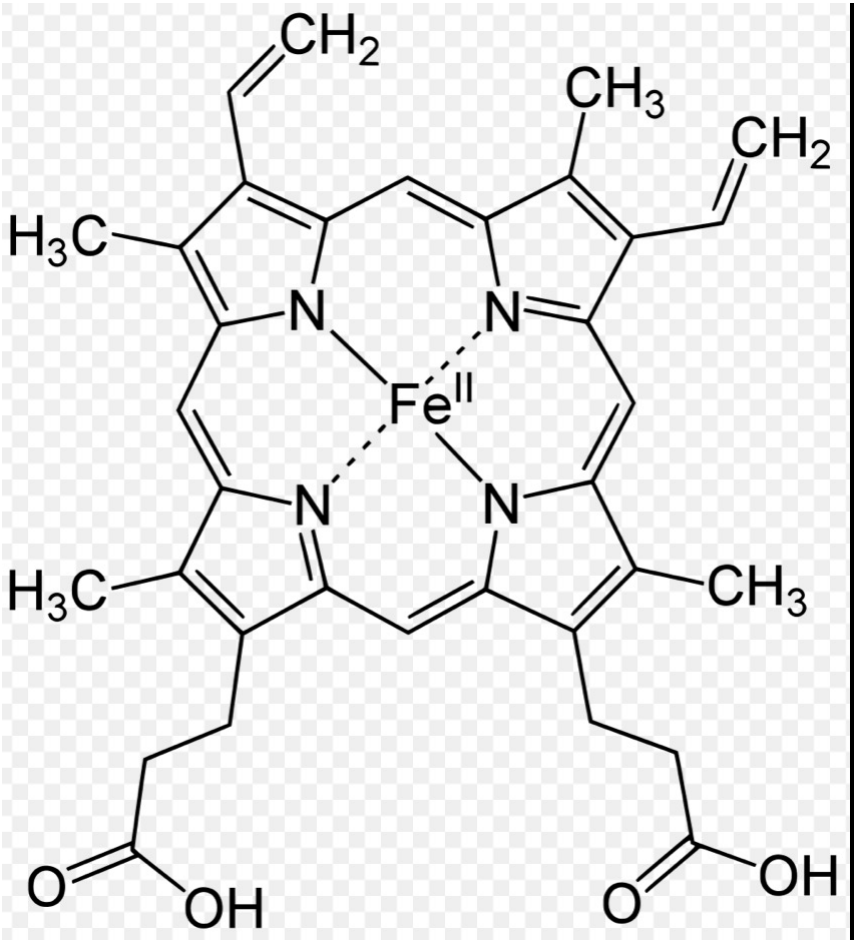
Significance: Photobiomodulation (PBM) refers to the beneficial effects of low-energy light absorption. Although there is a large body of literature describing downstream physiological benefits of PBM, there is a limited understanding of the molecular mechanisms underlying these effects. At present, the most popular hypothesis is that light absorption induces release of nitric oxide (NO) from the active site of cytochrome *c* oxidase (COX), allowing it to bind O_2 instead. This is believed to increase mitochondrial respiration, and result in greater overall health of the cell due to increased adenosine triphosphate production.

Wavelength- and irradiance-dependent changes in intracellular nitric oxide level at 447-, 532-, 635-, or 808-nm light




a)
EI
FO
Sh
Sh
Sh
Sh
MA

Haemeglobin



General Findings

- Significant wavelength-dependent elevations in intra cellular NO levels follow individual laser exposures (up to 30%) at all four wavelengths 447, 532, 635, and 808 nm.
- Sequential or simultaneous exposures to light at two different wavelengths enhanced the NO modulation up to 50% greater than unexposed controls.
- The immediate increases in cellular NO levels were an increased source of electrons entering the electron transport chain, that served to increase mitochondrial health and ATP production.

Single Wavelength Results

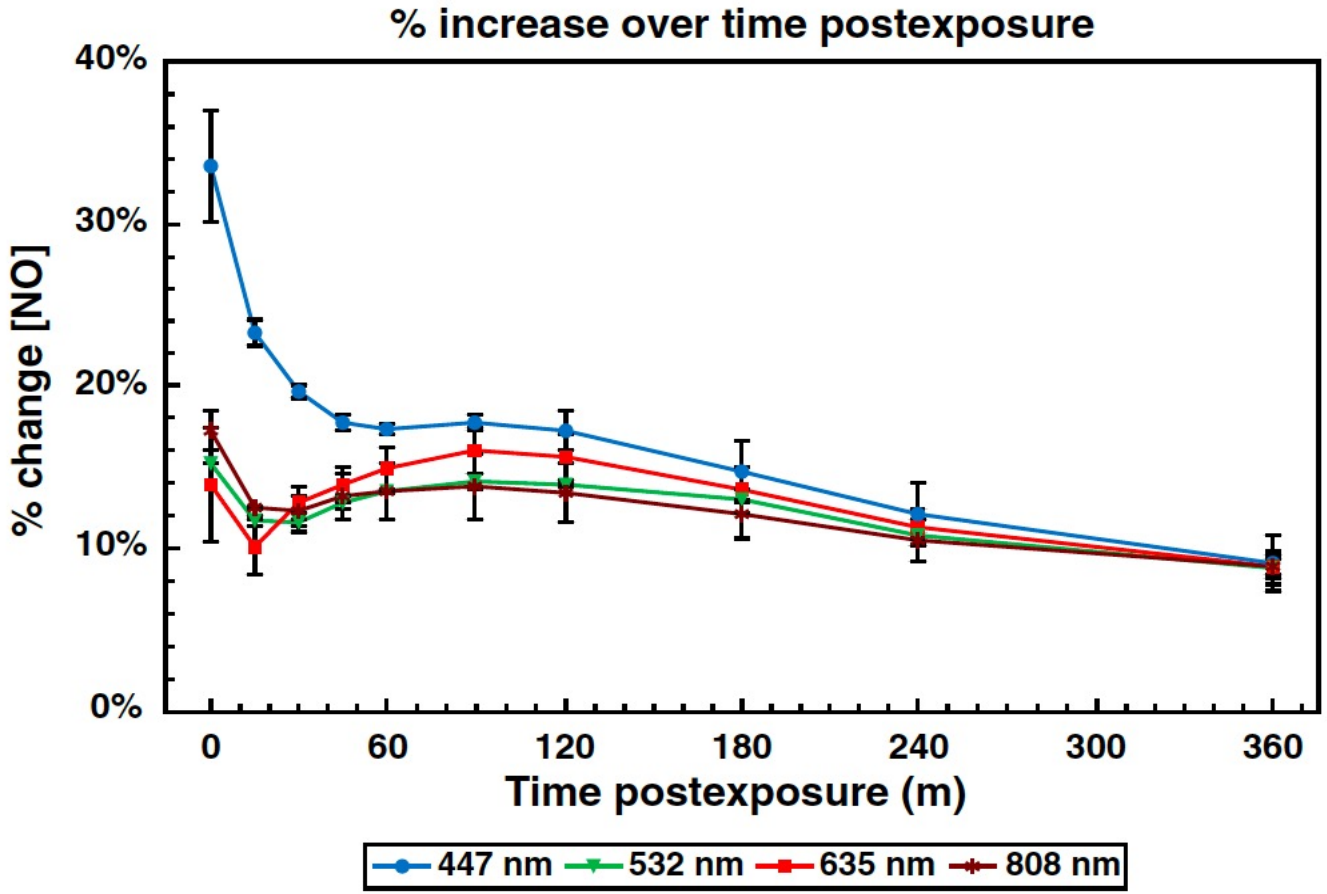
Exposures to 635-nm red, 447-nm blue, 532-nm green, and 808-nm for 30, 45, 60, 90, and 120 min. All four wavelengths, exposed cells exhibited statistically increased levels of free NO compared to control cells at all time points after 30 min.

Initial NO levels rose with radiant exposure until an optimal exposure time after which additional applied radiant exposure had minimal effect. This was particularly evident for 635-nm red, 532-nm green, and 808-nm NIR exposures; however, 447-nm blue exposures appear to continue to rise, though at a reduced rate.

The increase in NO associated with 447-nm blue light was far greater level than any of the other wavelengths measured across the entire exposure times.

For all wavelengths, the degree of increase in NO levels dropped within 15 min of the end of the exposure, even though they remained elevated ($p < 0.001$) compared to unexposed controls.

For all wavelengths, the
degree of increase in NO levels dropped within 15 min of the end of the exposure but remained elevated compared to unexposed controls.



NO levels when cells exposed to combinations of light frequencies

- If certain wavelengths were absorbed at different molecular chromophores, various combinations of wavelengths might interfere with, have no effect or enhance the response of another.
- Additionally, if multiple wavelengths were applied serially, would the order of exposure change the degree of NO elevation?
- If combinations of wavelengths have synergistic (or detrimental) effects on NO release, would indicate substantial implications for clinical applications.

Increases in NO for combinations revealed interesting results.

The **red and green** combinations were less effective than simultaneous red and green, green followed by red, and red followed by green.

Red and NIR Red followed by NIR and NIR followed by red were similar to red by itself. Simultaneous Red and NIR result in a significantly higher increase than red or NIR alone.

Blue and red Serial exposures of red followed by blue were indistinguishable from blue alone (very high).

Blue followed by red was not greater than red alone (not so high). This difference indicates that the order of the exposure is indeed important between these wavelengths. Simultaneous exposure of red and blue result in an intermediate value between the two single exposures.

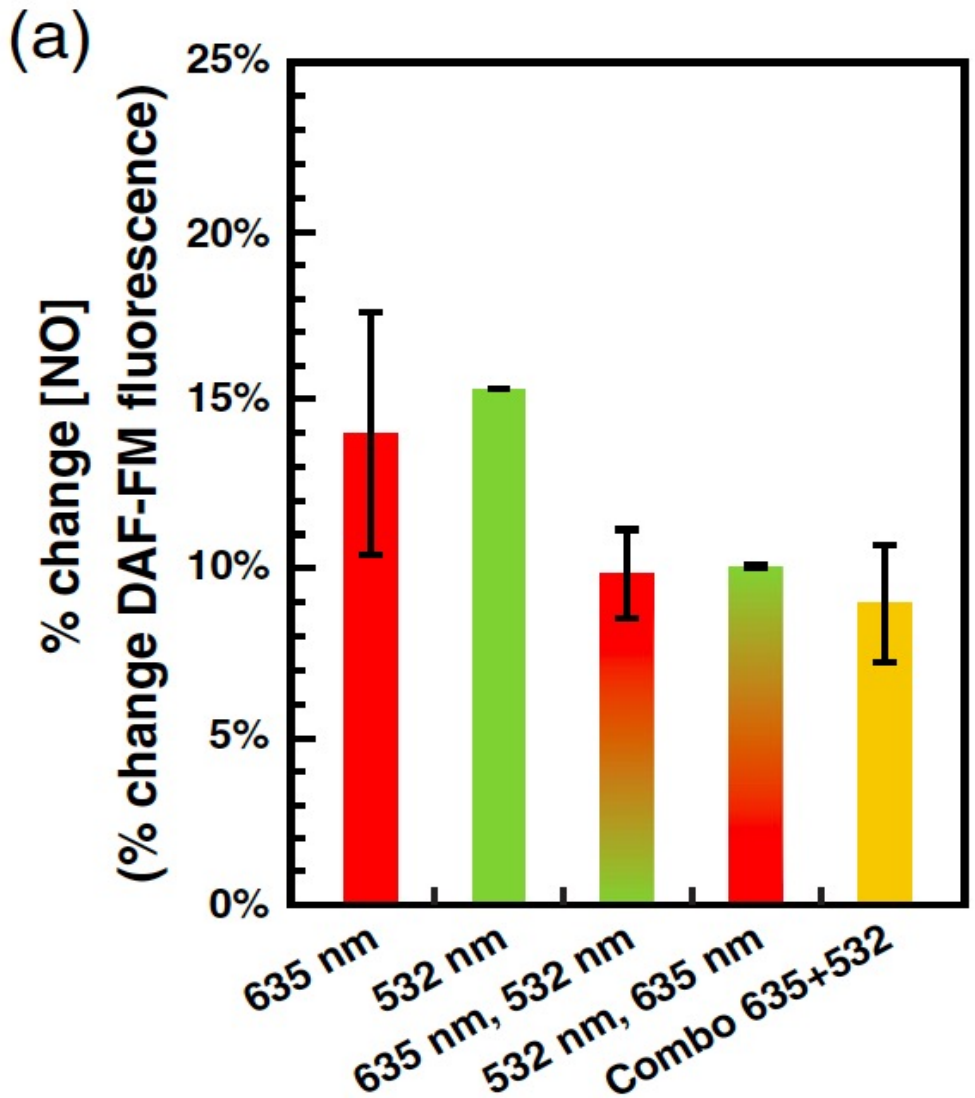
Blue and Near Infra-Red

- **Blue and NIR** combinations result in very different outcomes depending on the exposure paradigm.
- **Blue followed by NIR** results were similar to NIR only, but significantly lower than blue alone.
- **NIR followed by blue** results in a significantly greater NO increase than blue alone. **NIR followed by blue** was also greater than blue alone.
- **NIR and blue simultaneous** exposures were also higher than blue alone.

In summary

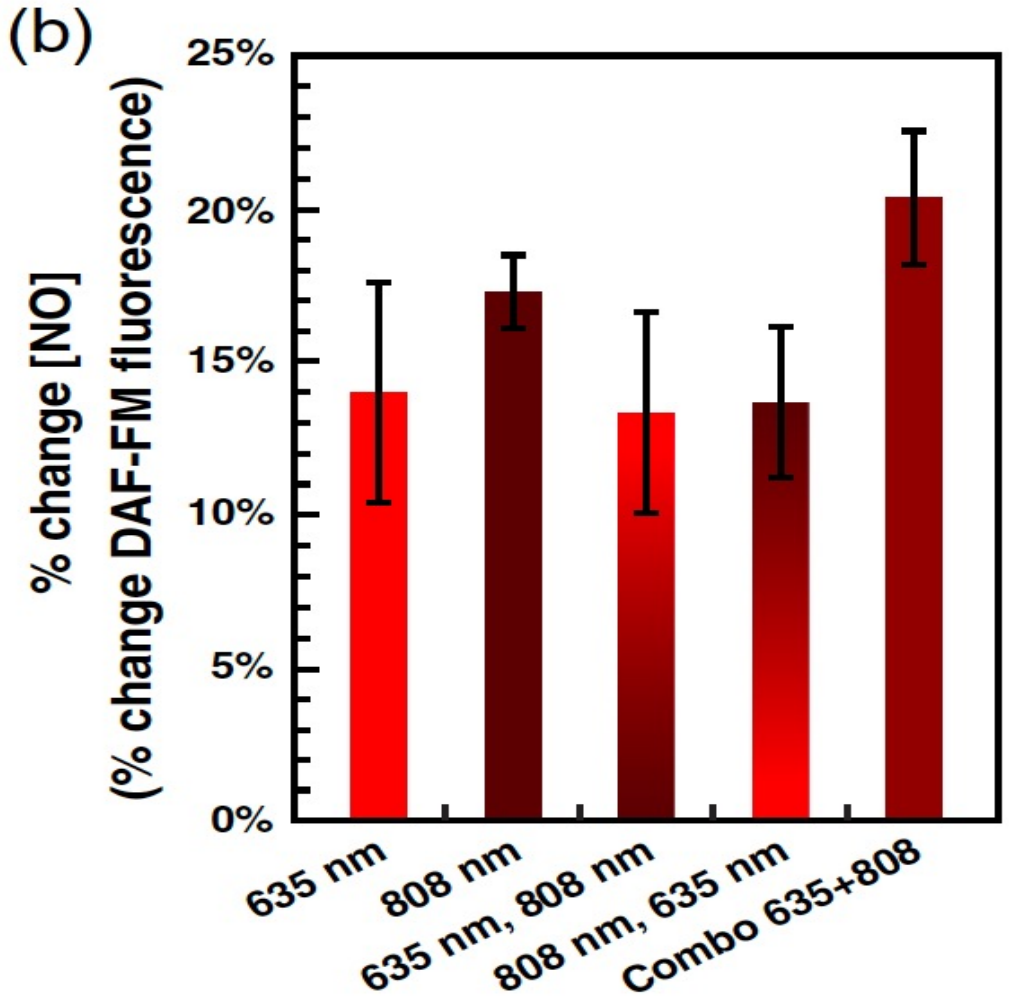
- In summary, we have found that exposure to different wavelengths of light (red, blue, green,
- and NIR) result in dose-dependent increases in free intracellular NO, with varying magnitudes
- and efficiencies, depending on the wavelength applied. Furthermore, when multiple wavelengths
- were combined, either in serial or simultaneous fashion, further differences were revealed.

Red and Green



- In contrast, other wavelengths worked synergistically and produced
- a greater NO response than either individually. For example, NIR and red combined exposures
- liberated significantly more NO than NIR (p . 0.005) or red ($p < 0.001$) individually.

Red and Infra-Red



- Likewise, NIR and blue combined exposures liberated significantly more NO than NIR or blue (both
- $p < 0.001$) individual exposures. We also determined that nNOS activity was not required for
- NO in an irradiance-dependent manner as well, with lower irradiances resulting in more efficient
- per-joule increases in NO. Figure 7 shows a 70% reduction in the cell's basal level (no laser
- exposure) of NO in the absence of pyruvate. When replacing pyruvate with succinate, NO basal
- levels were reduced by ~85%. More important, light-mediated increases in NO were not
- observed in the absence of pyruvate, or when pyruvate was replaced with succinate. As shown
- in Fig. 8, we found that the increase in NO did not result in a subsequent increase in cGMP.

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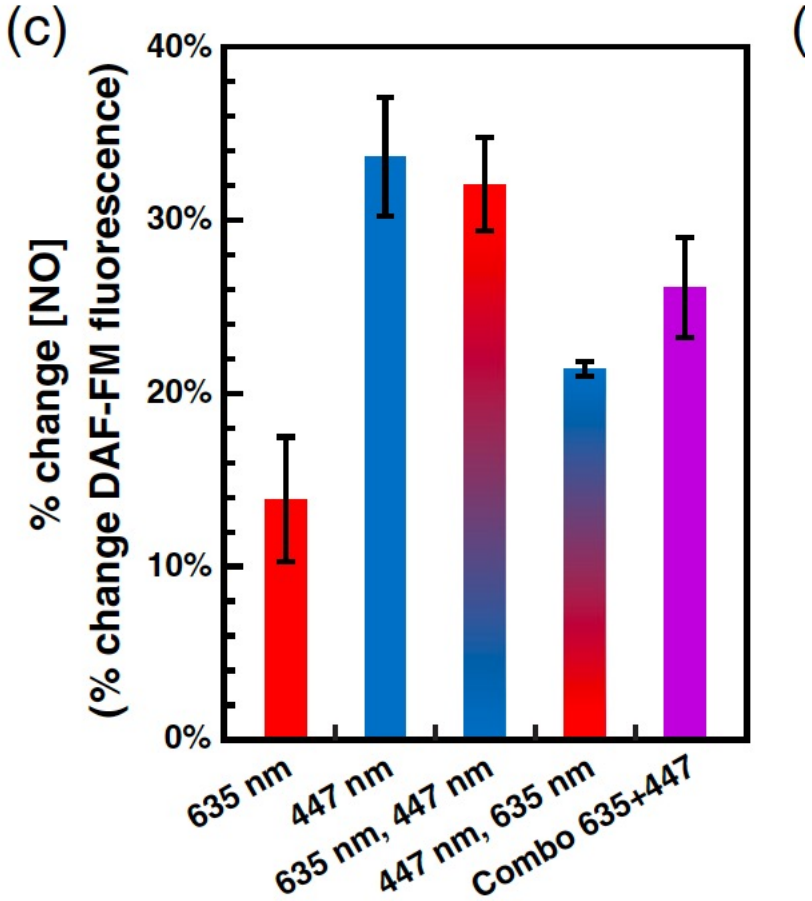
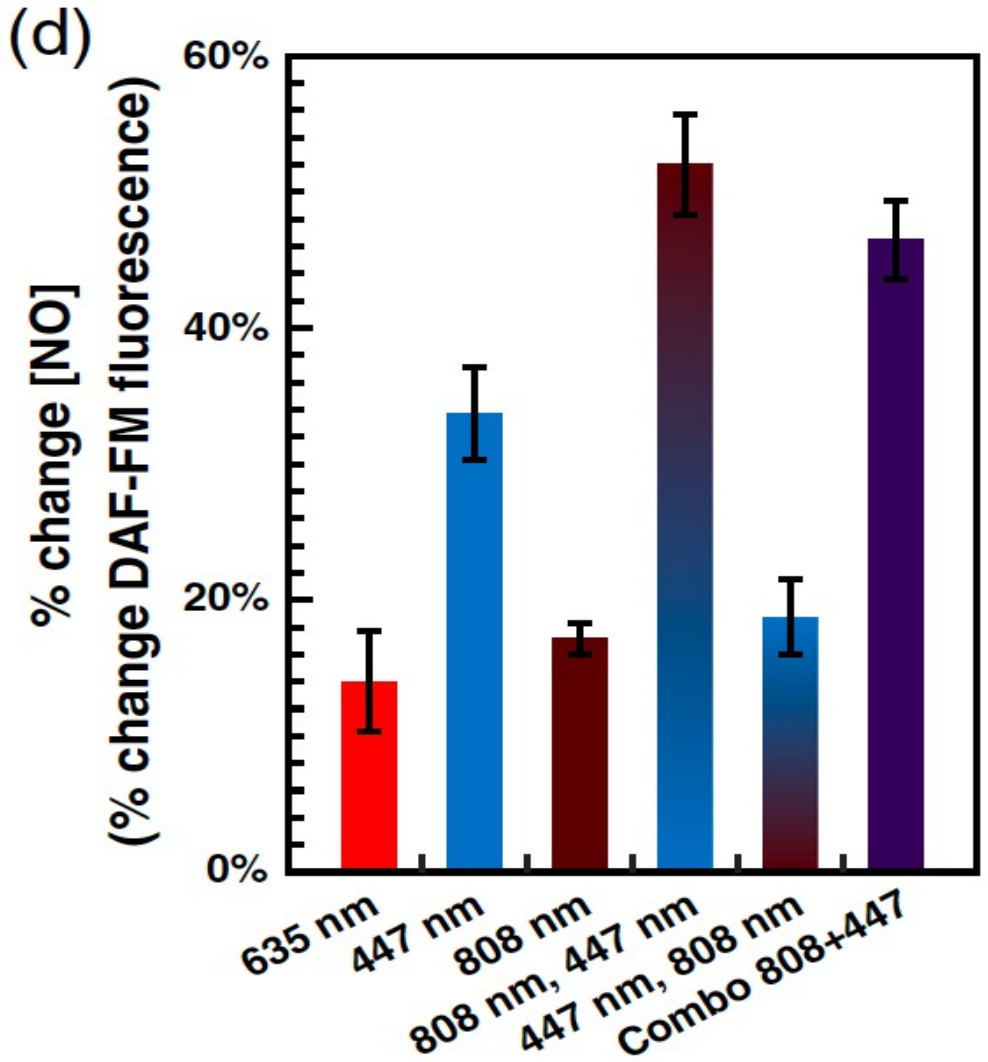
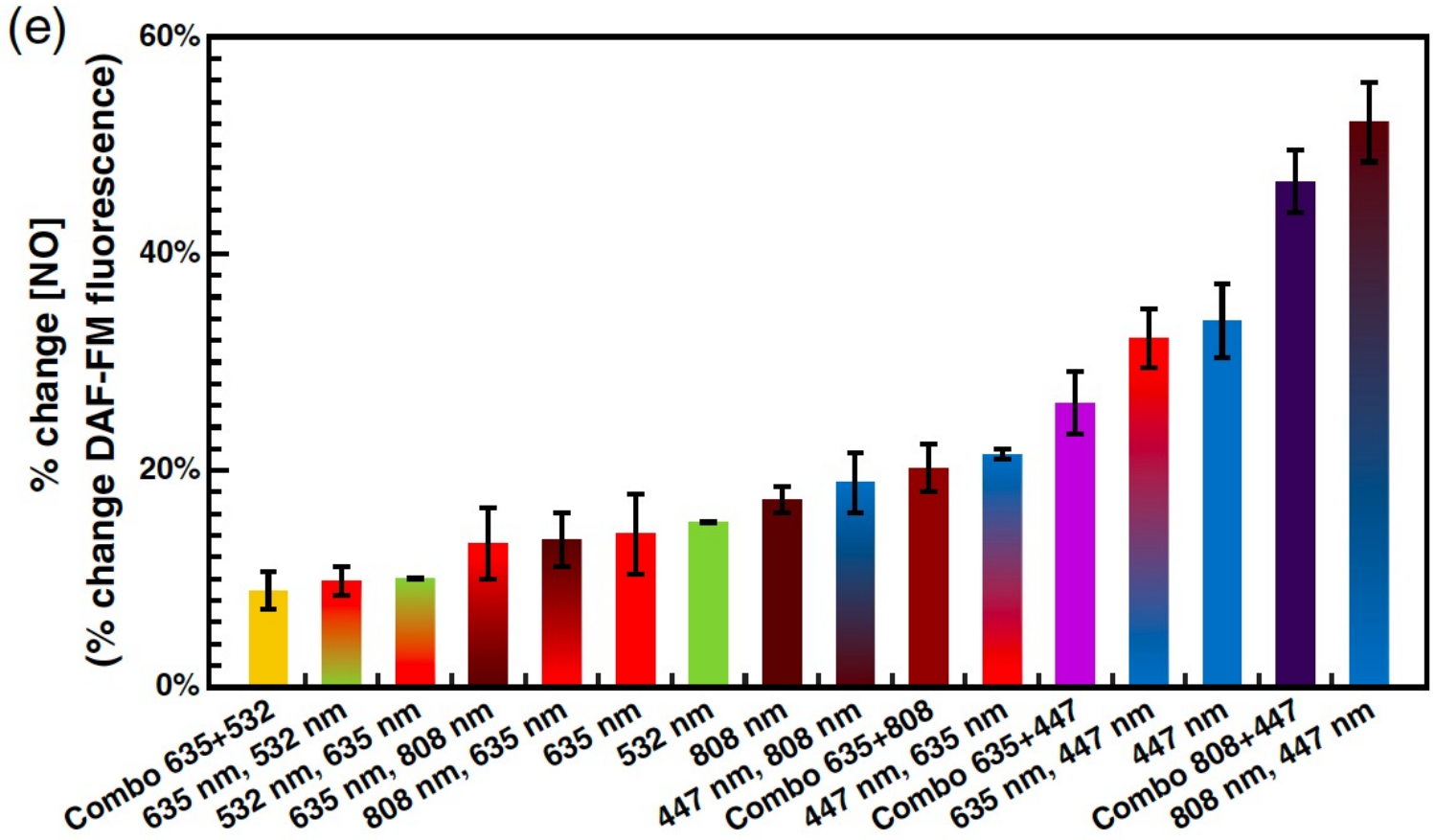
Red and Blue


(c)
% change [NO]
(% change DAF-FM fluorescence)
40%
30%
20%
10%
0%
635 nm
447 nm
635 nm, 447 nm
447 nm, 635 nm
Combo 635+447

Blue and Infra-Red



Summary of all Wavelengths and Combinations



SYNTONIC BASIC FILTERS

αδ
Red-Orange

Alpha Delta – "Lazy Eye Syndrome"
amblyopia, eso, poor accommodation

μδ
Lemon

Mu Delta – "Chronic Syndrome"
physiological, toxic, neuroendocrine

μυ
Turquoise

Mu Upsilon - "Acute Syndrome"
recent head trauma, high fevers, inflammation, swelling, pain, HA, monocular diplopia

υω
Indigo

Upsilon Omega – "Pain Reliever"
headaches, asthenopia

αω
Ruby

Alpha Omega – "Emotional Fatigue"
αω pupil, adrenal fatigue, poor coping, mood swings, frustration

 Alpha **α** = red

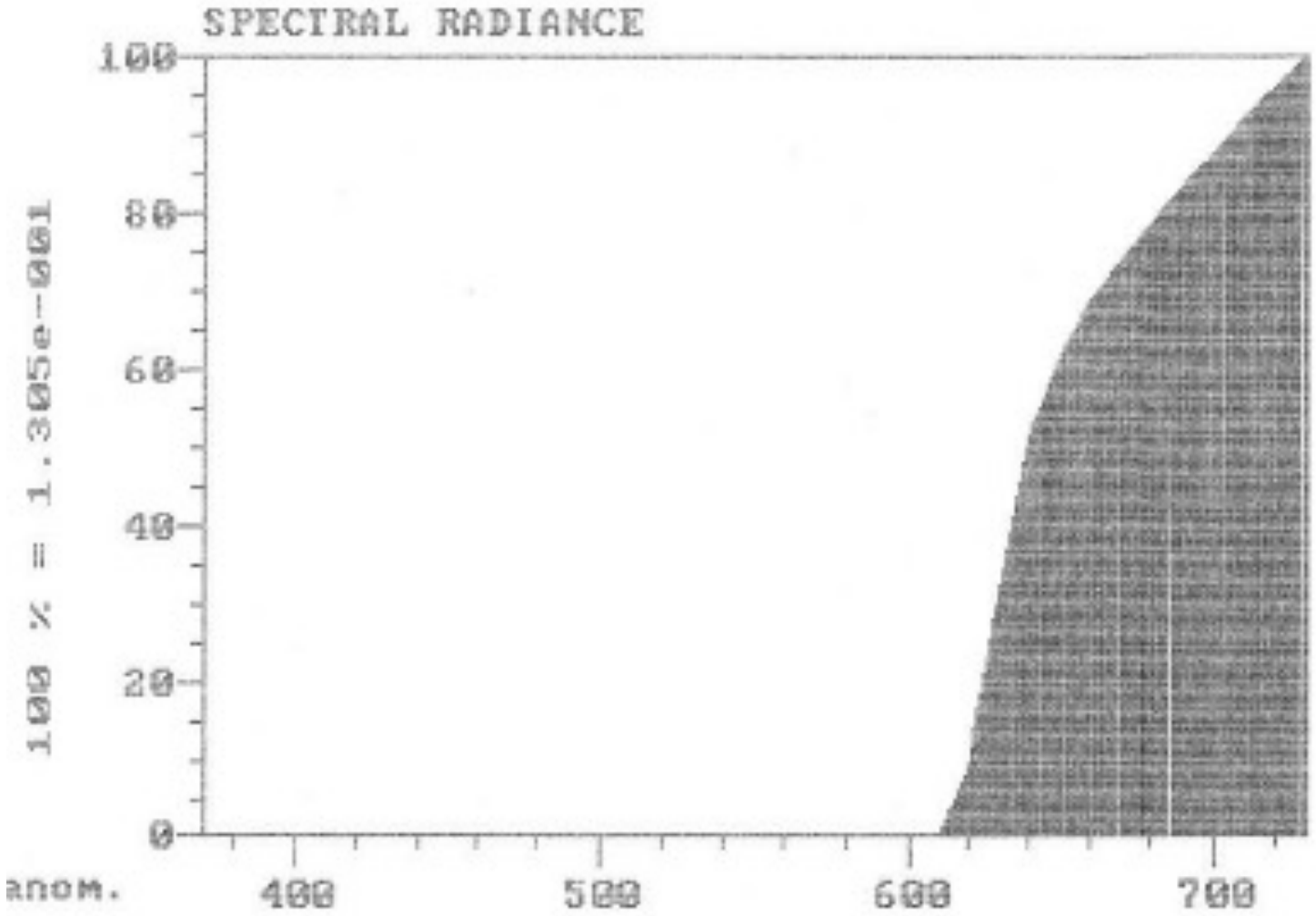
 Delta **δ** = amber

 Mu **μ** = green

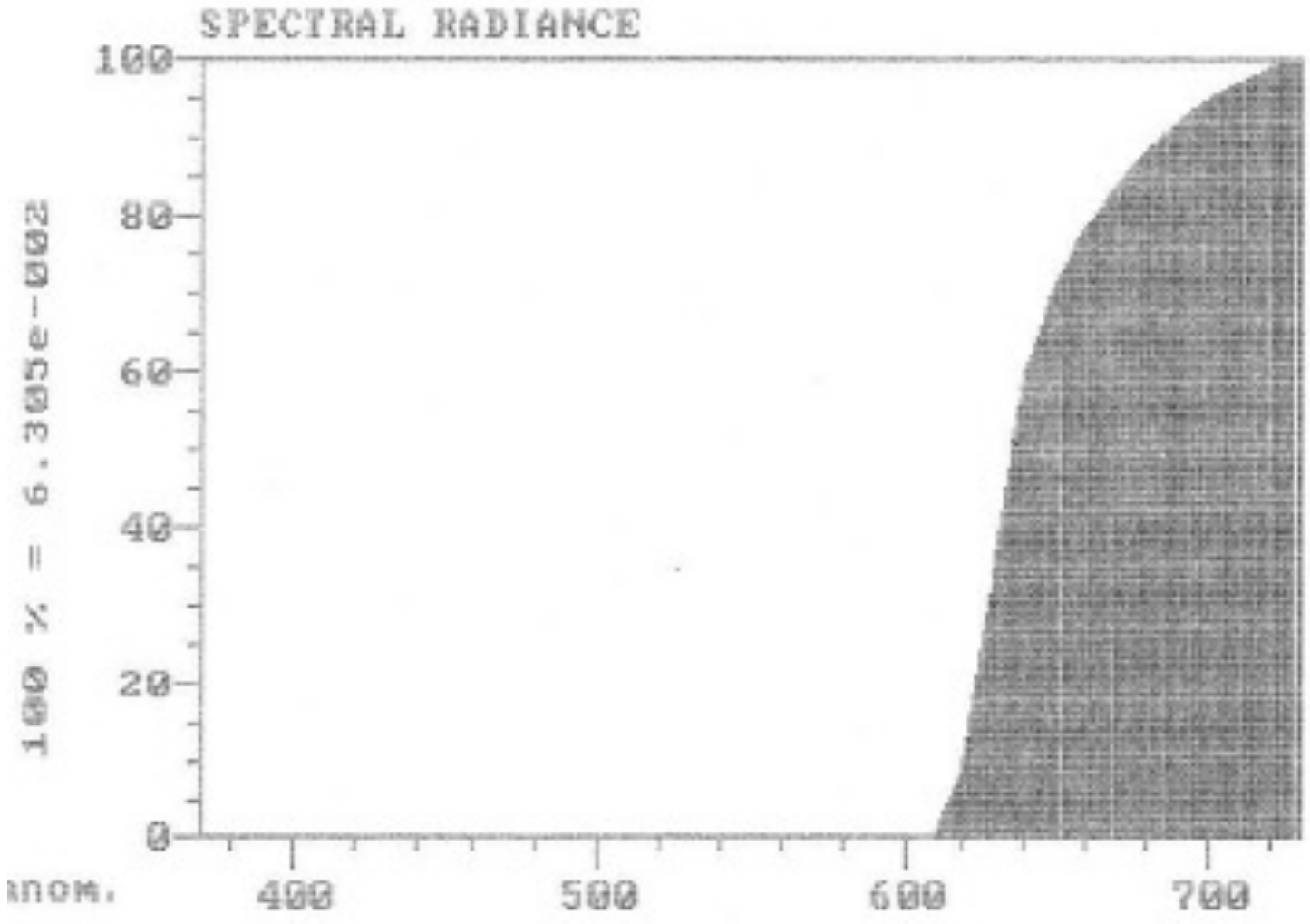
 Upsilon **υ** = blue

 Omega **ω** = cobalt

College of Syntonic Optometry


SPECTRAL RADIANCE
100
80
60
40
20
0
100 % = 1.305e-001
anom.
400
500
600
700

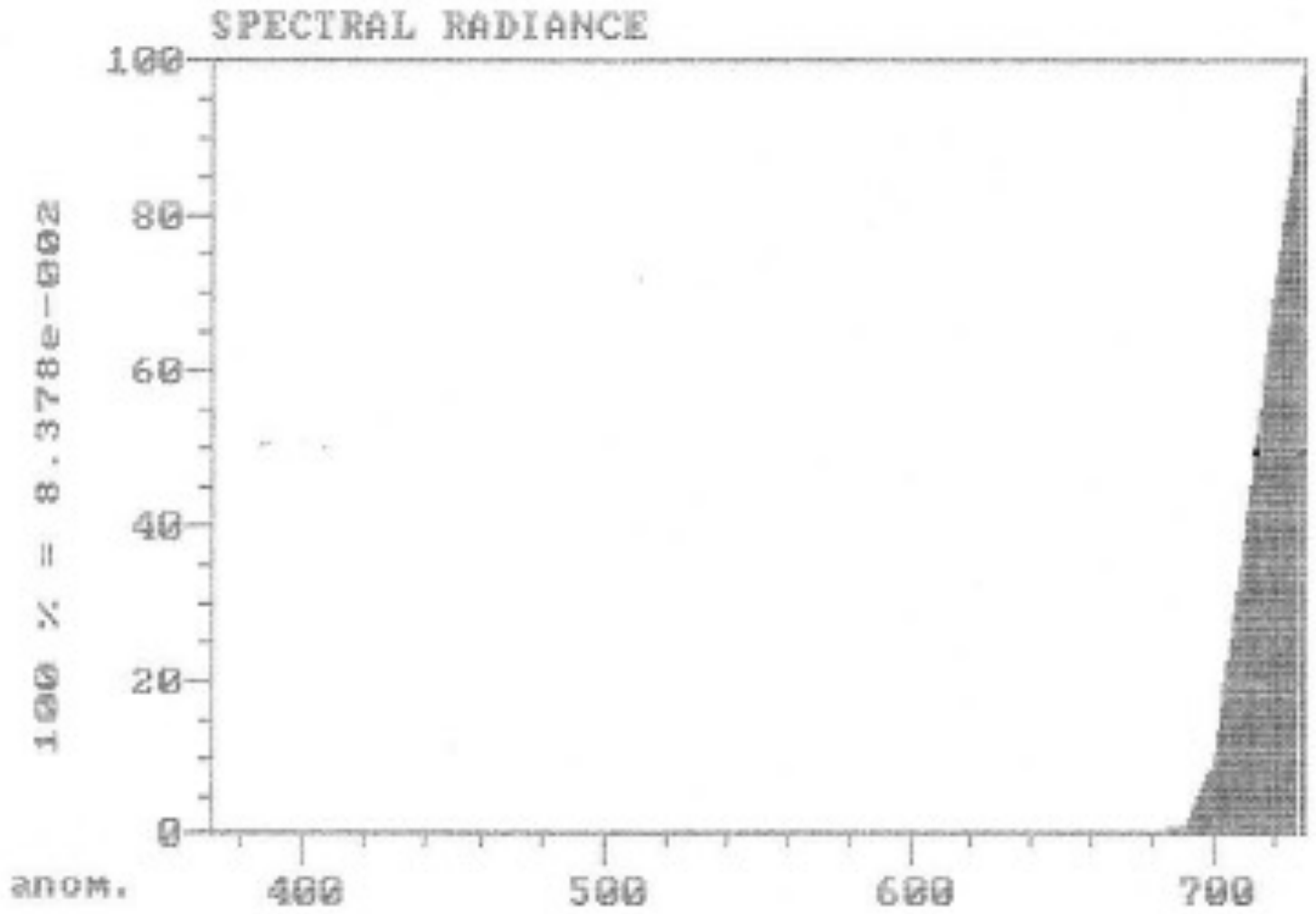
ALPHA



ALPHA DELTA

αδ **Alpha Delta – "Lazy Eye Syndrome"**

Red-Orange amblyopia, eso, poor accommodation



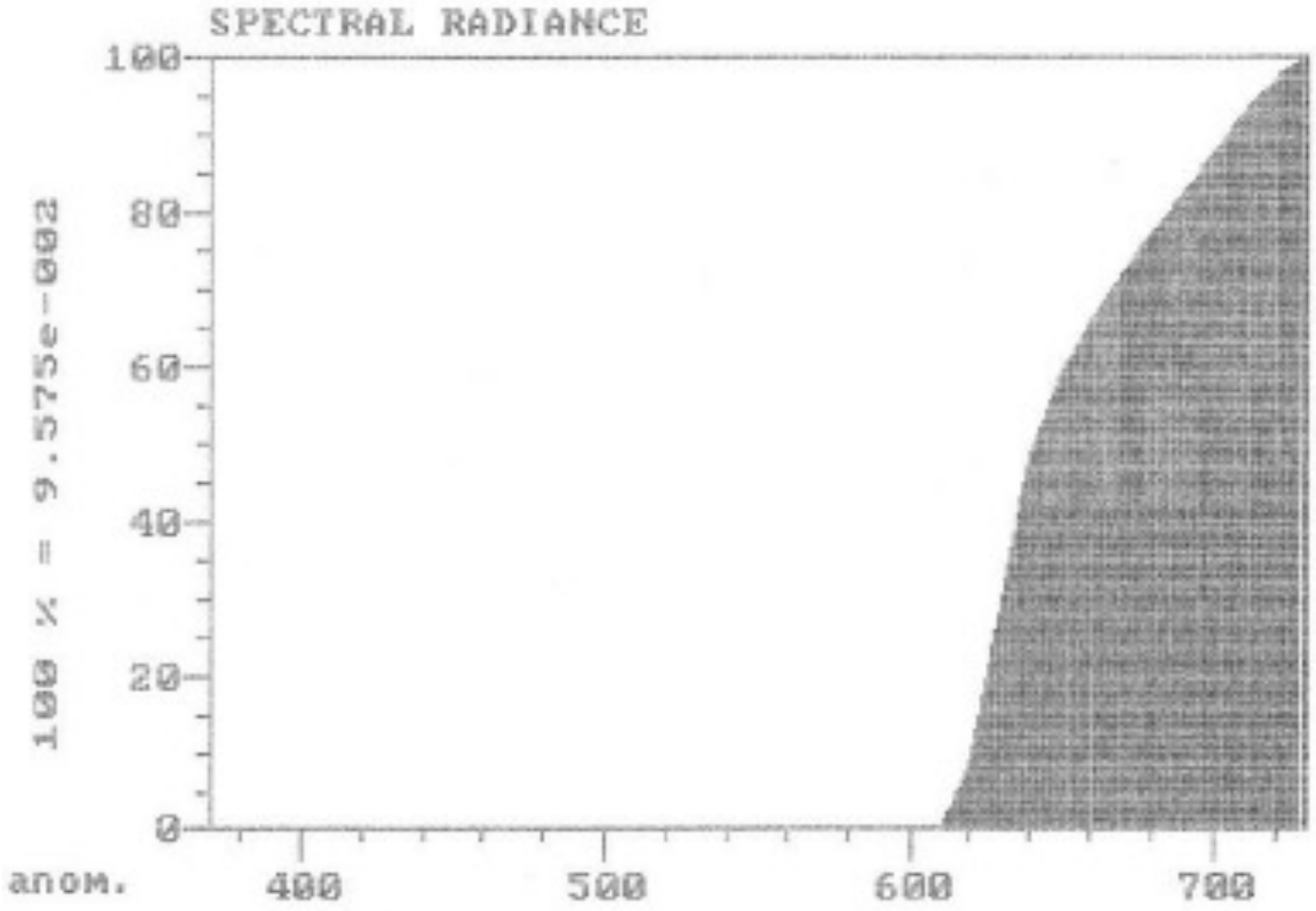
ALPHA OMEGA

αω

Ruby

Alpha Omega – "Emotional Fatigue"

αω pupil, adrenal fatigue, poor coping, mood swings, frustration


SPECTRAL RADIANCE
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80
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20
0
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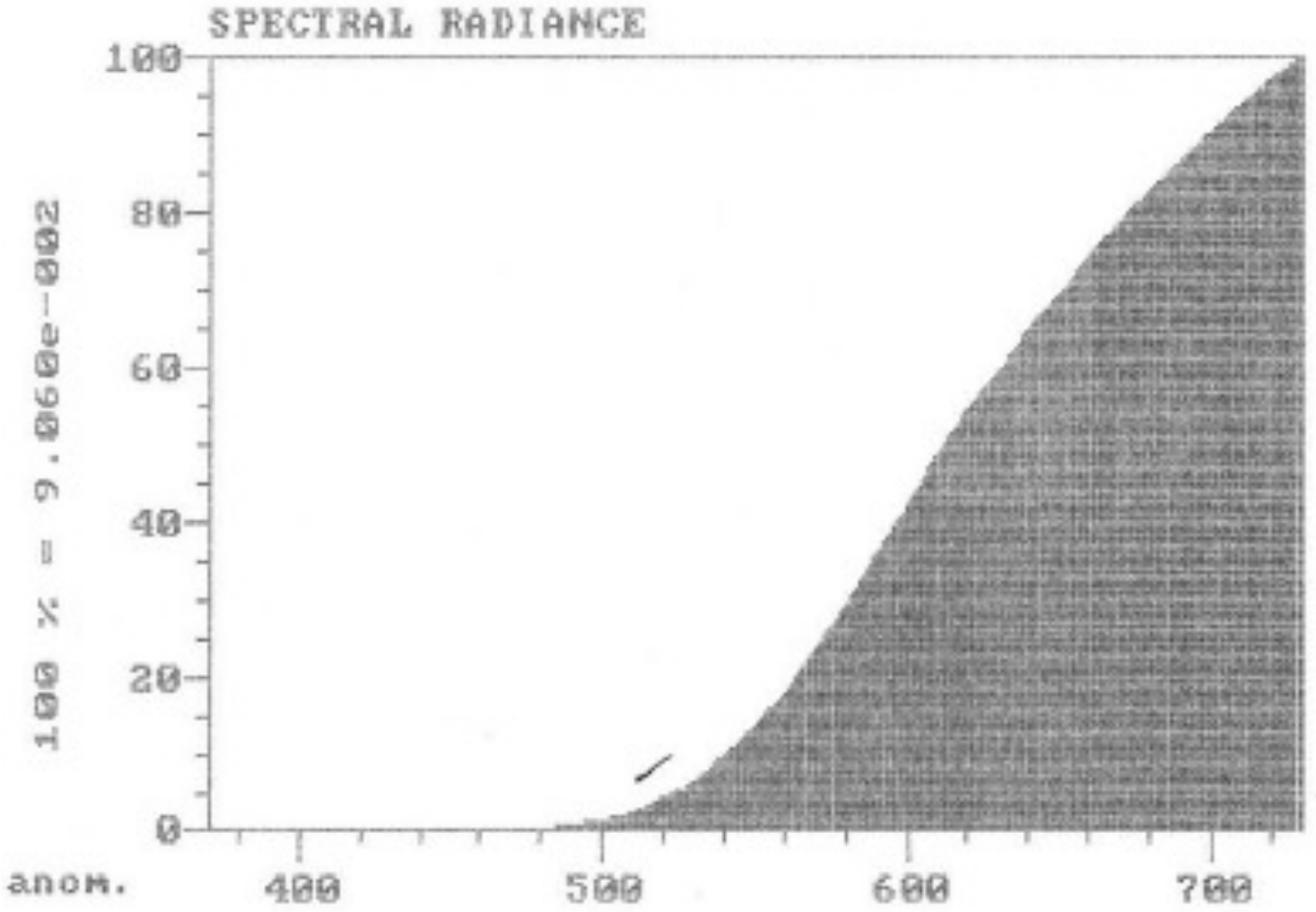
anom.
400
500
600
700

ALPHA THETA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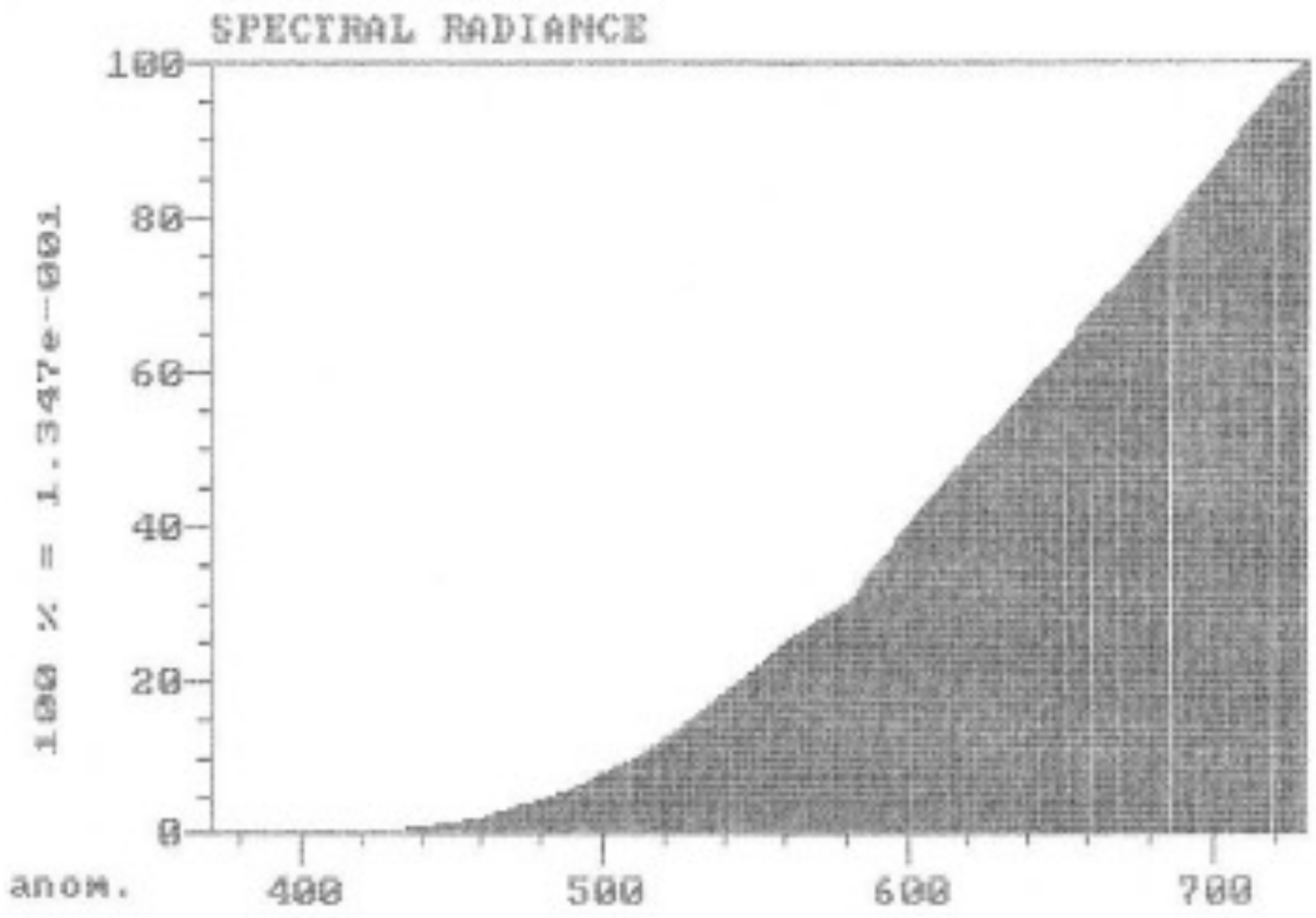
anom.
400
500
600
700

DELTA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anom.
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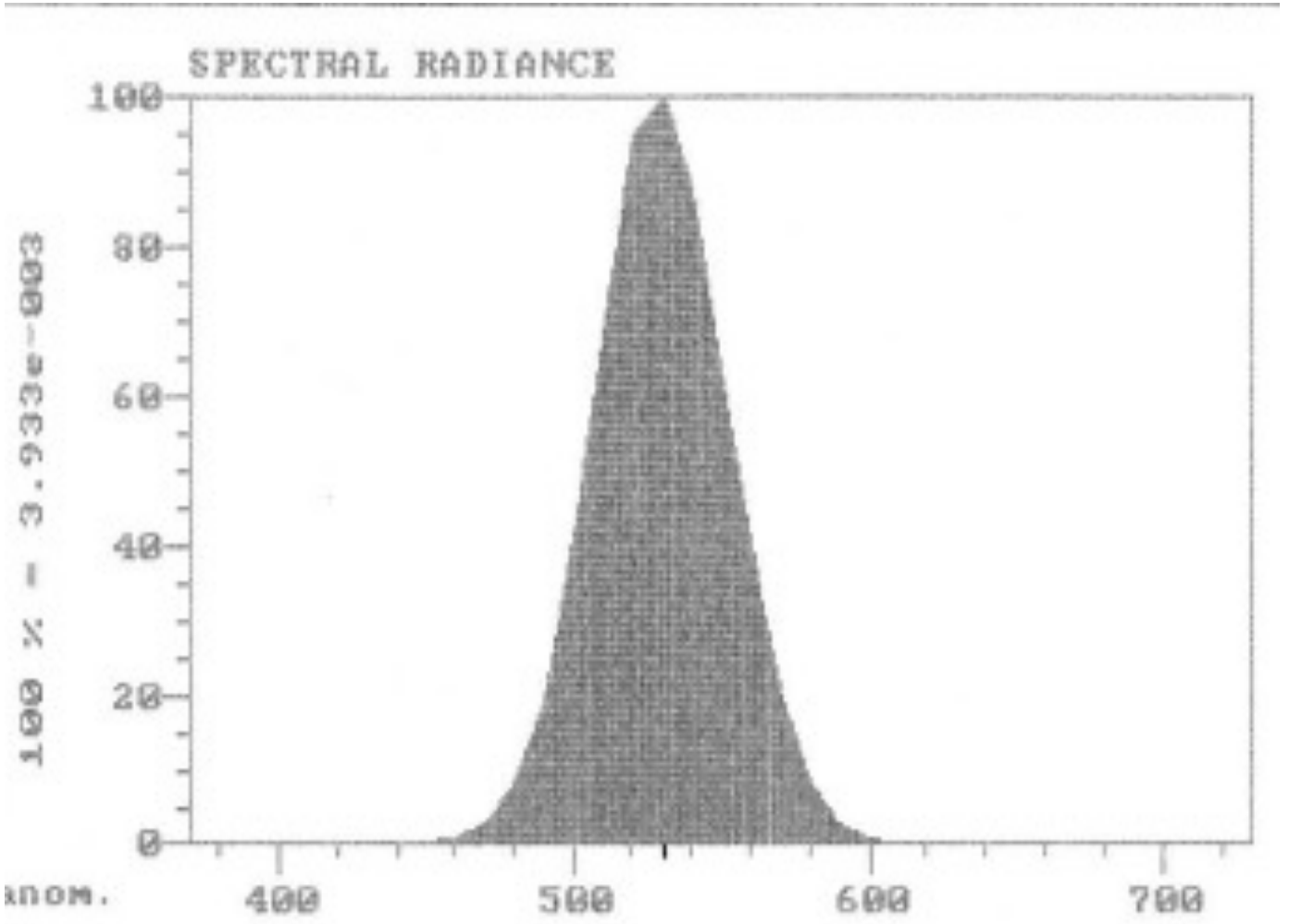
500
600
700
THETA


SPECTRAL RADIANCE
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80
60
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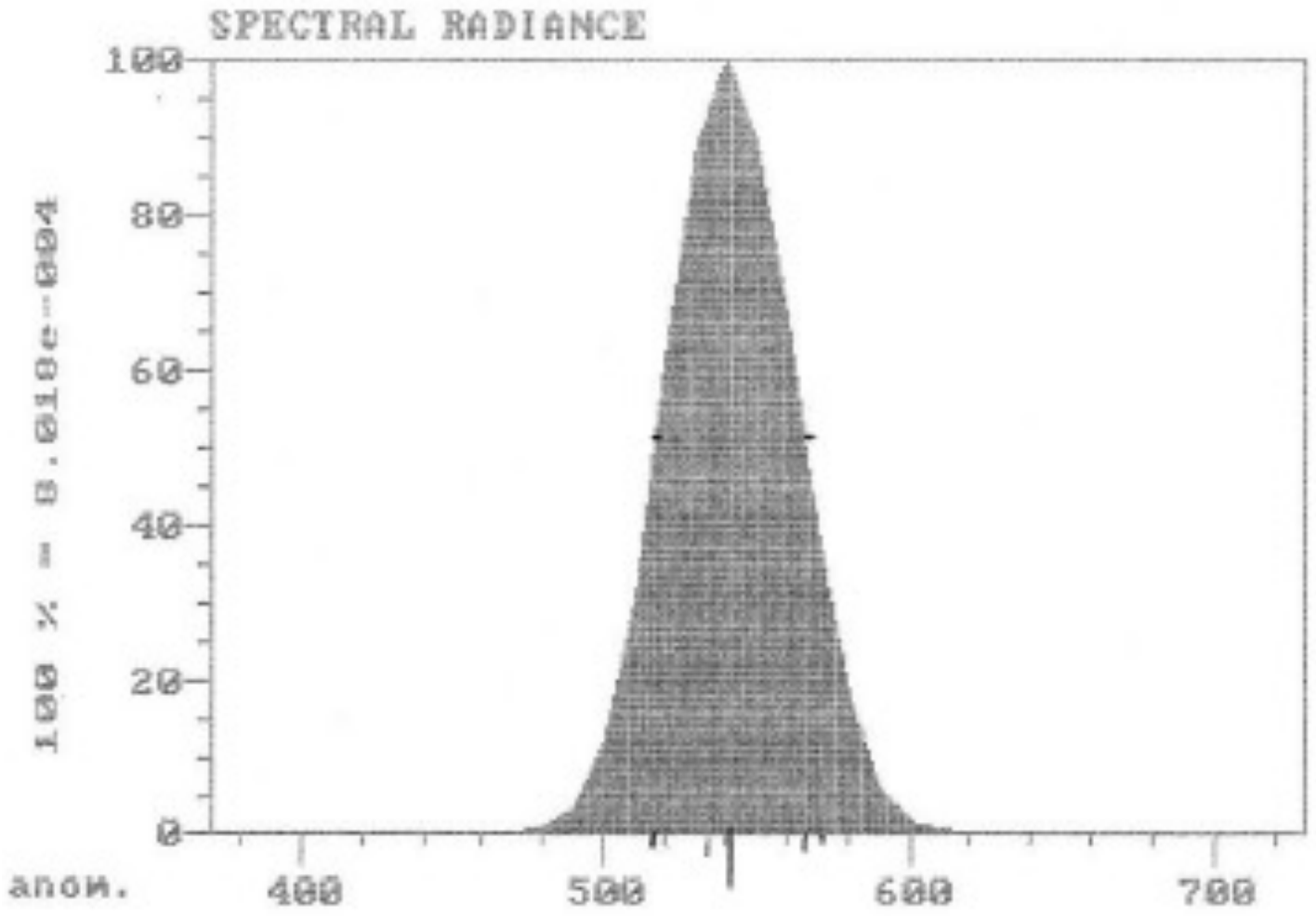
20
0
100 % = 3.933e-003
λnom.
400
500
600
700

MU



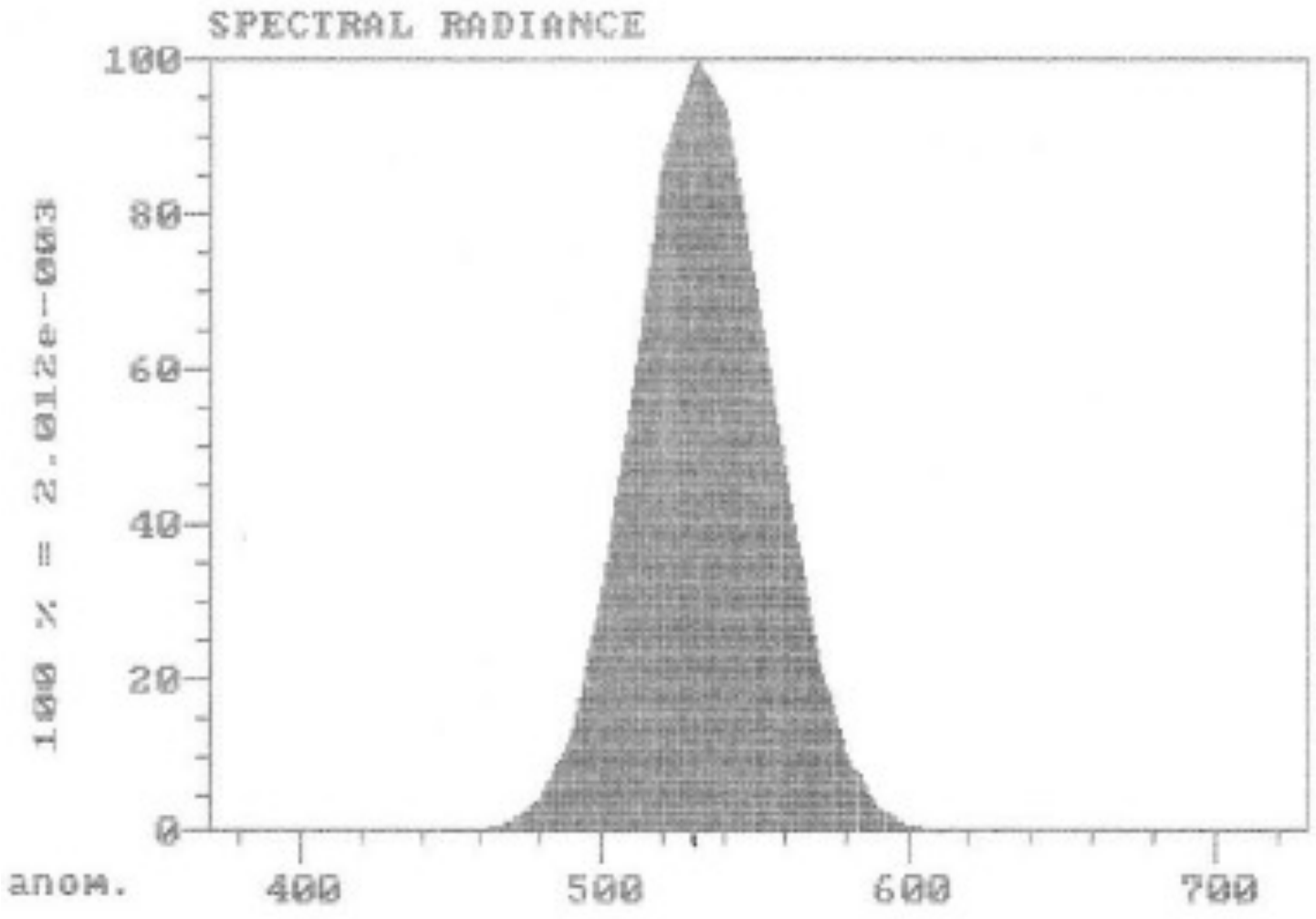
MU DELTA

μδ

Lemon

Mu Delta – "Chronic Syndrome"

physiological, toxic, neuroendocrine


SPECTRAL RADIANCE
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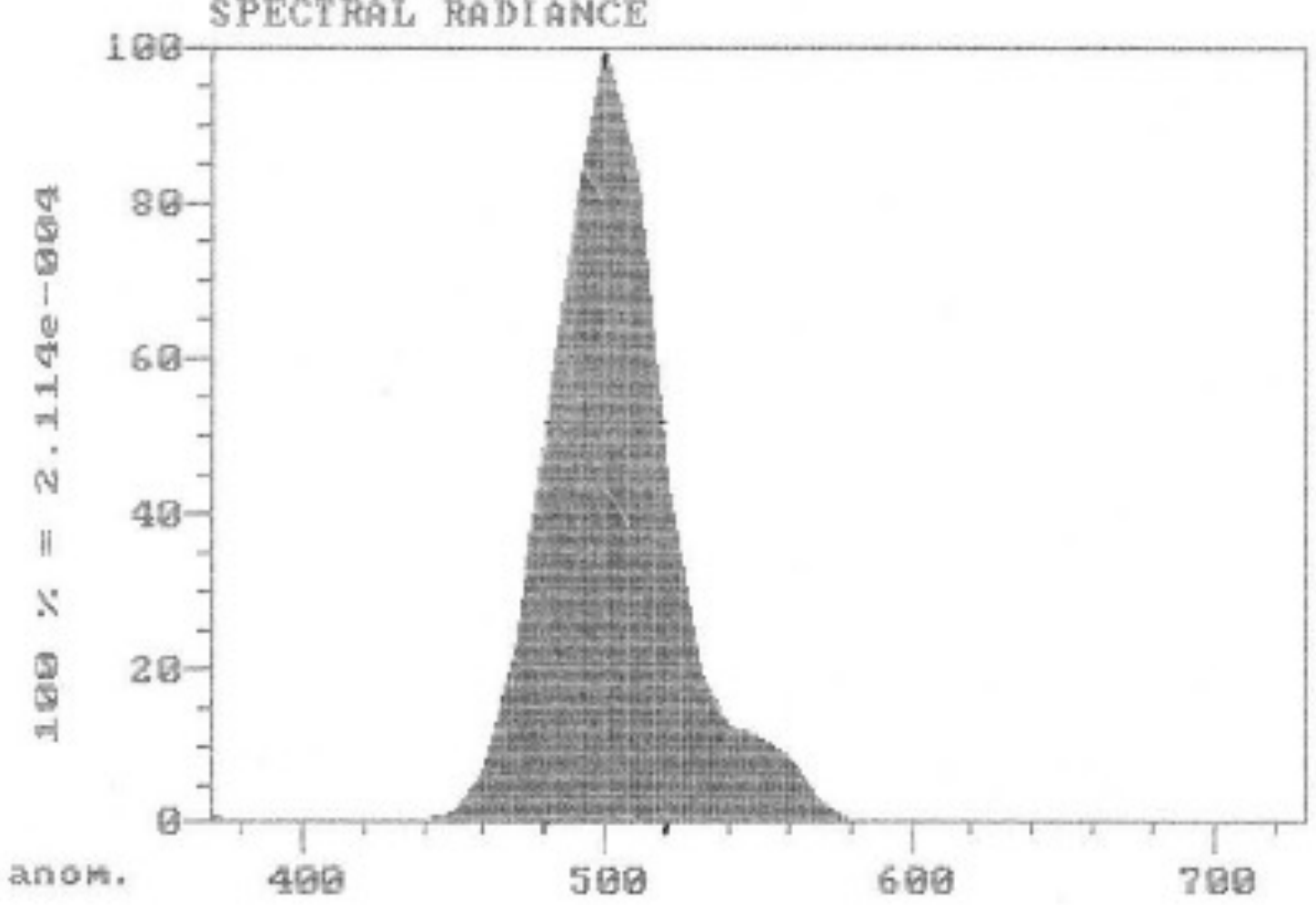
80
60
40
20
0
100 % = 2.012e-003
anom.
400
500
600
700

MU THETA



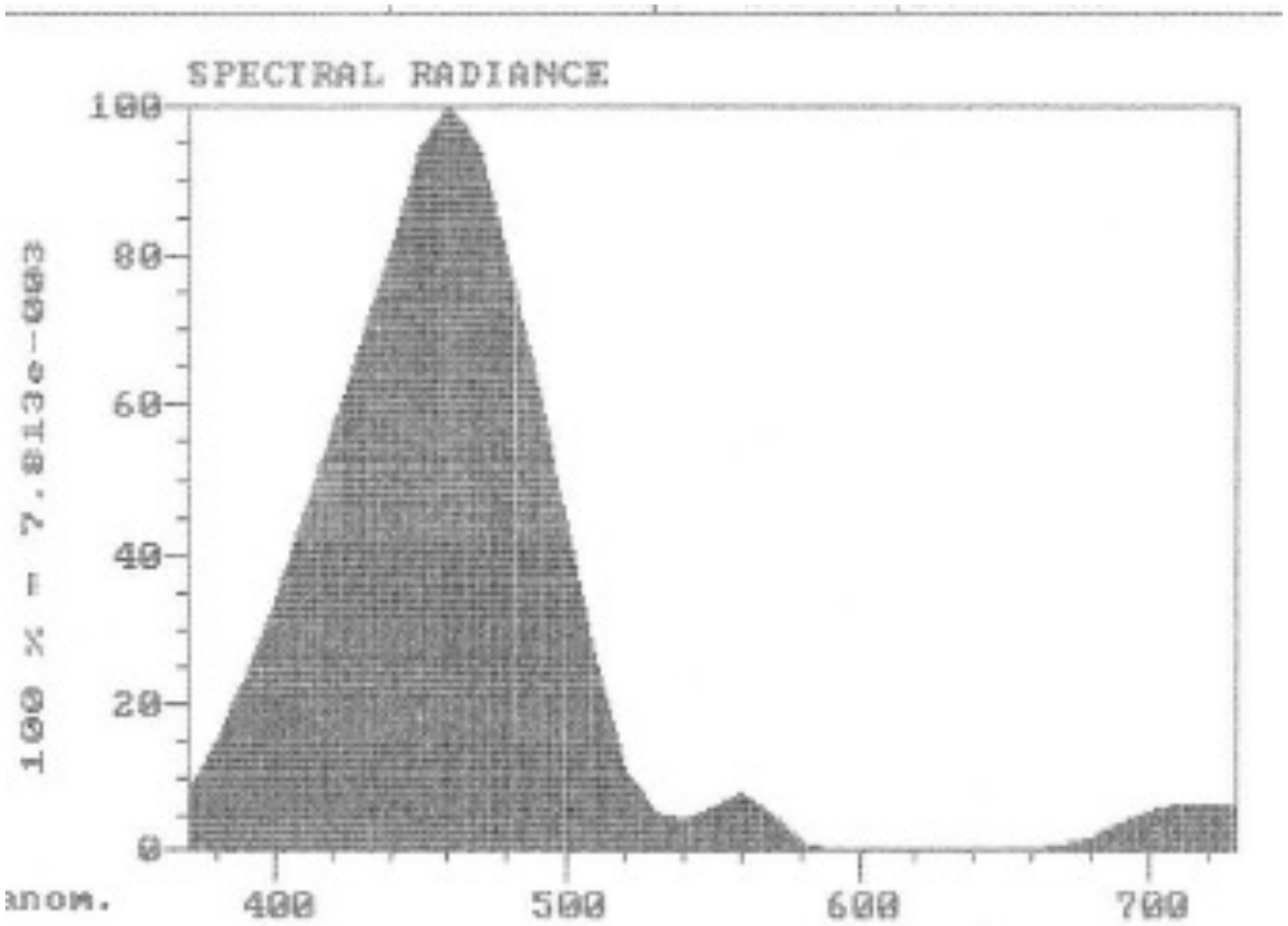
MU UPSILON

μυ

Turquoise

Mu Upsilon - "Acute Syndrome"

recent head trauma, high fevers, inflammation, swelling, pain, HA, monocular diplopia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60
40
20
0
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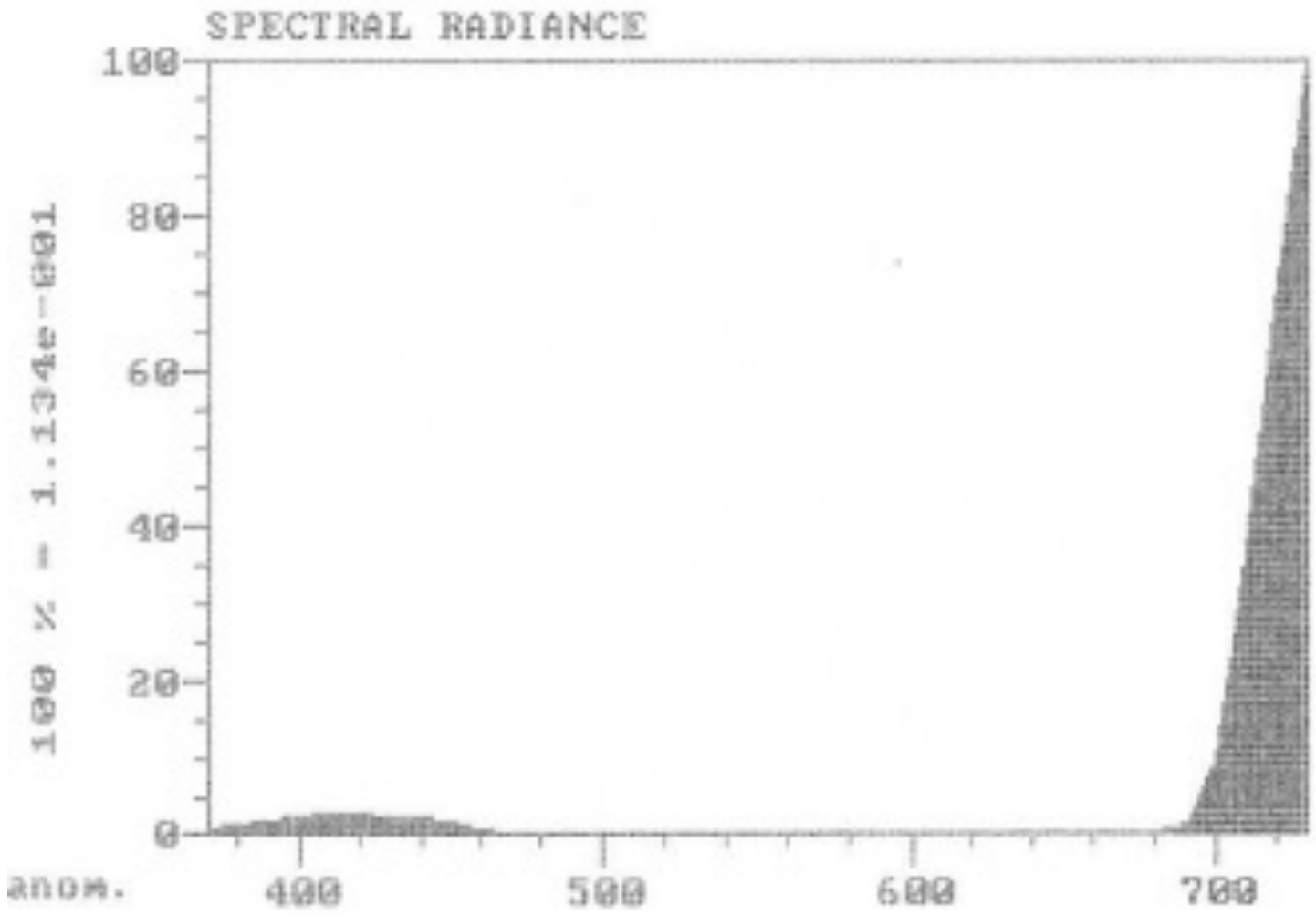
anom.
400
500
600
700

UPSILON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20
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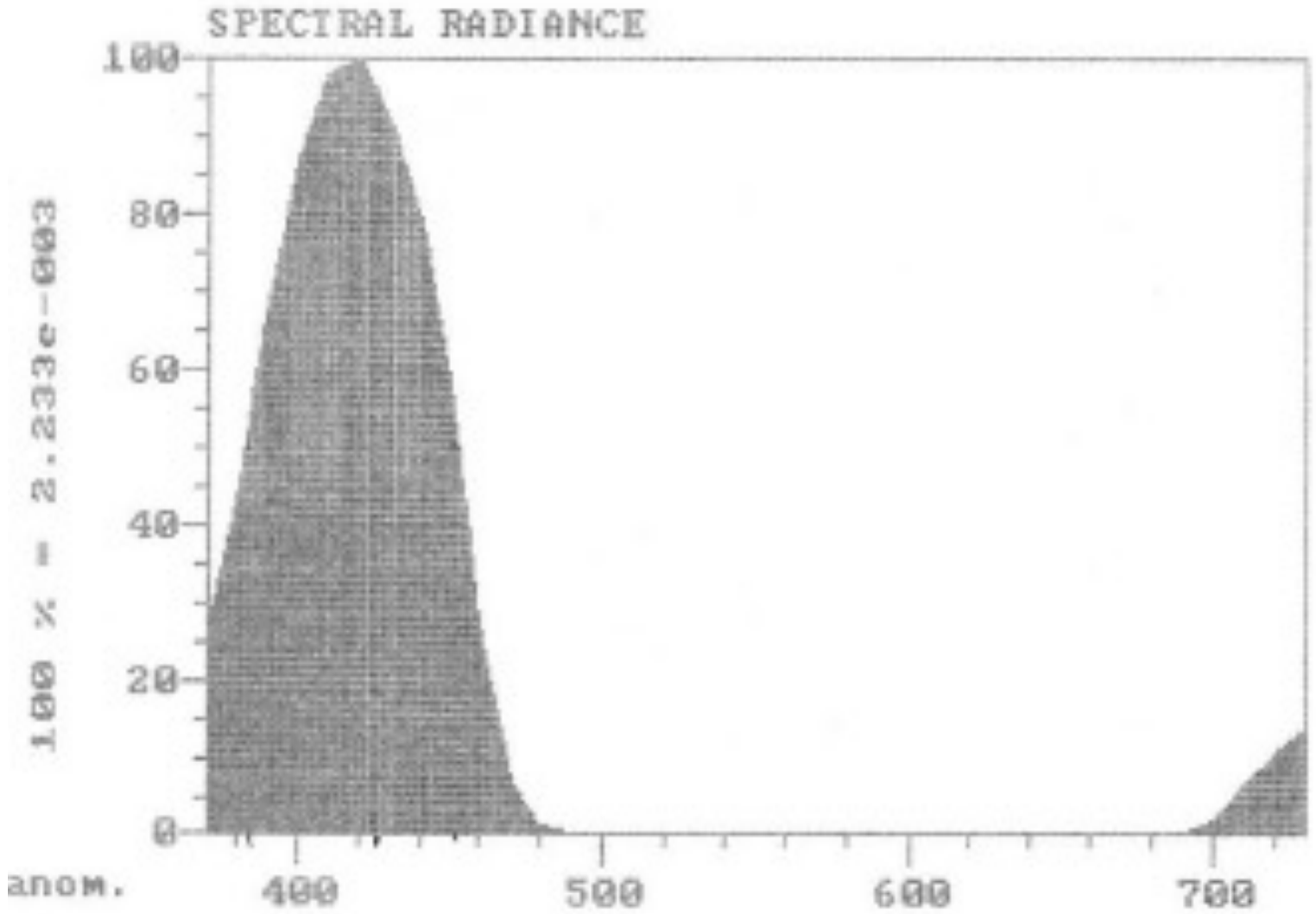
anom.
400
500
600
700

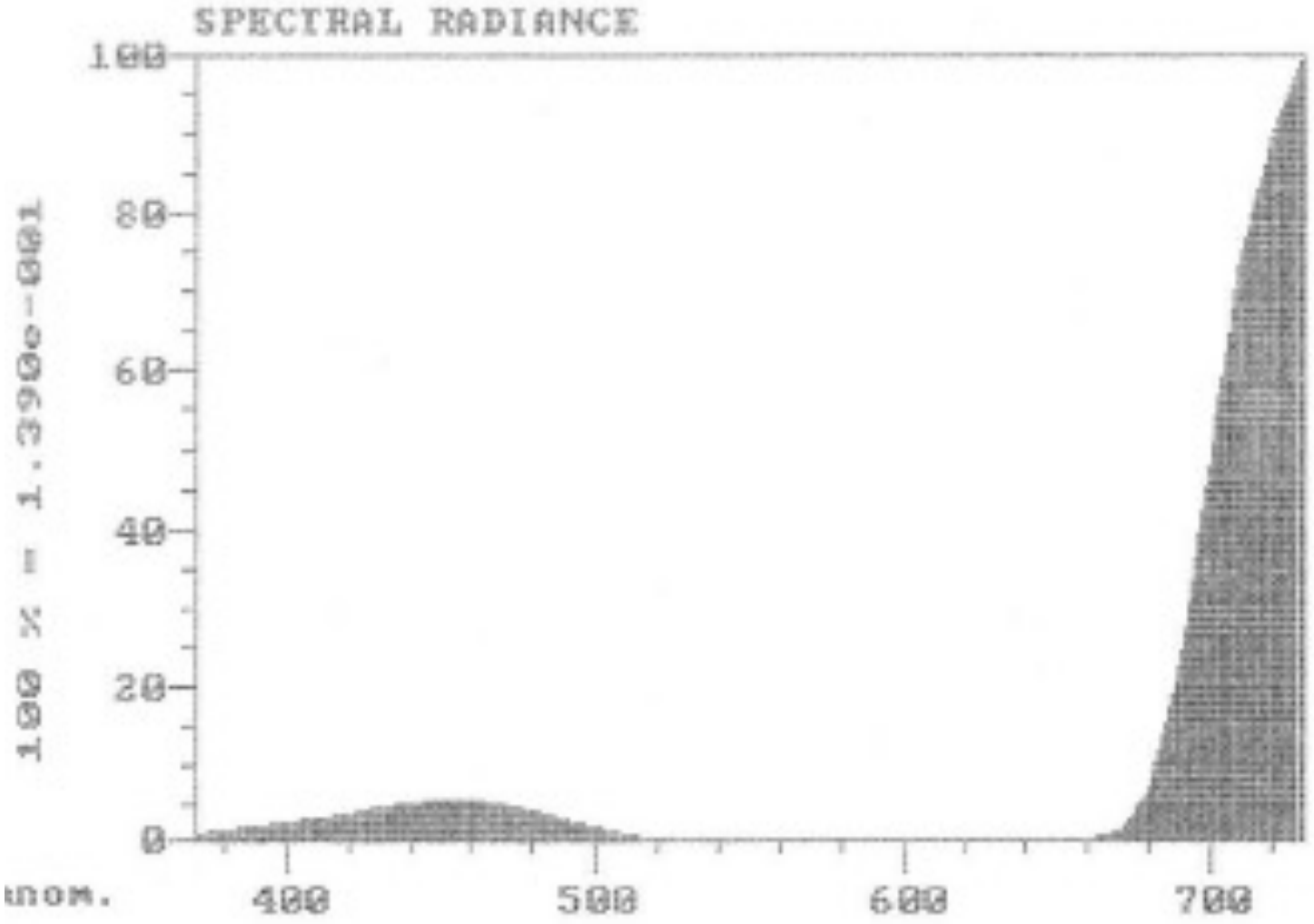
OMEGA



UPSILON OMEGA

υω **Upsilon Omega – "Pain Reliever"**

Indigo headaches, asthenopia


SPECTRAL RADIANCE
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40
20
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100 % = 1.390e-001
nom.
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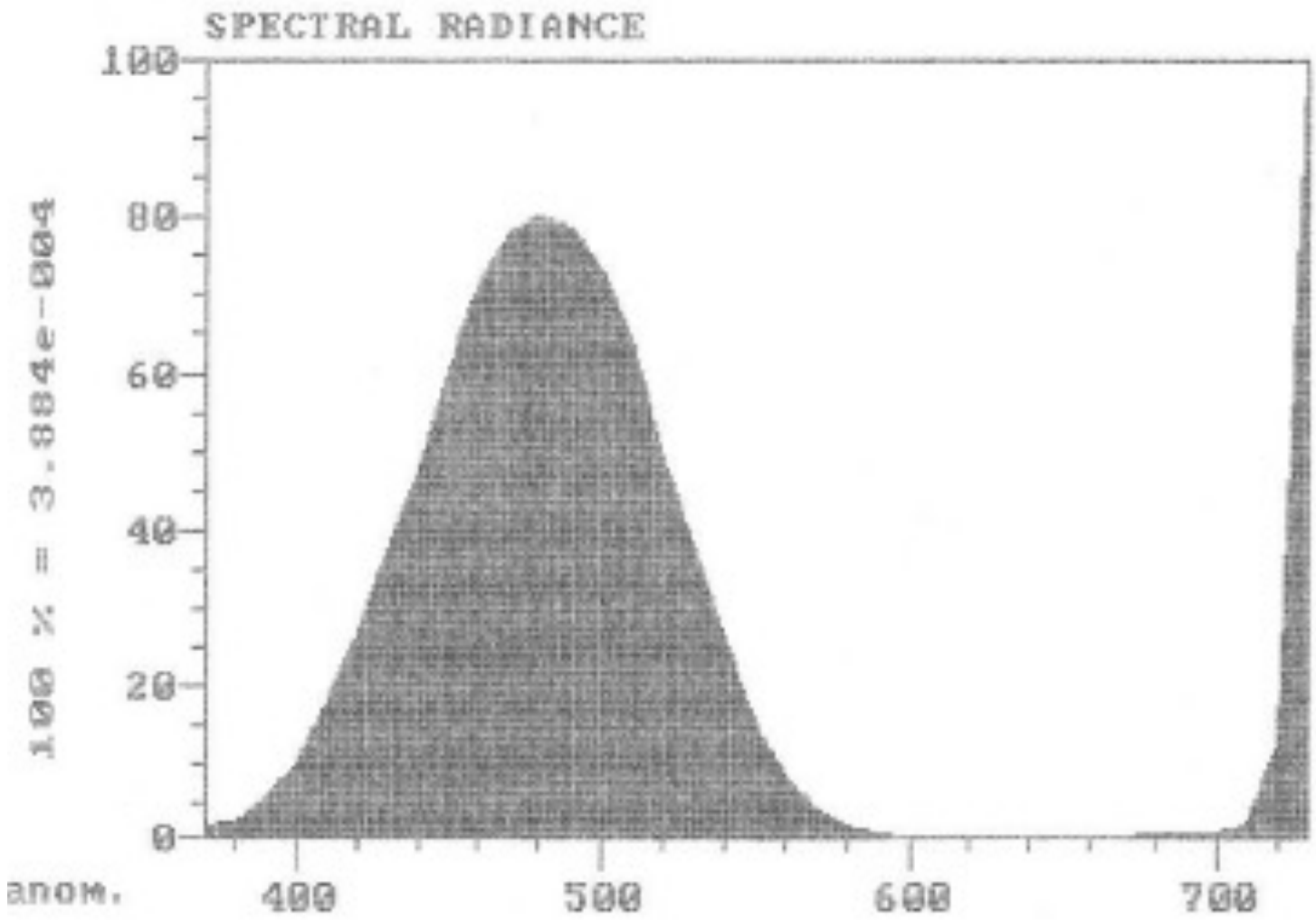
500
600
700
LAMBDA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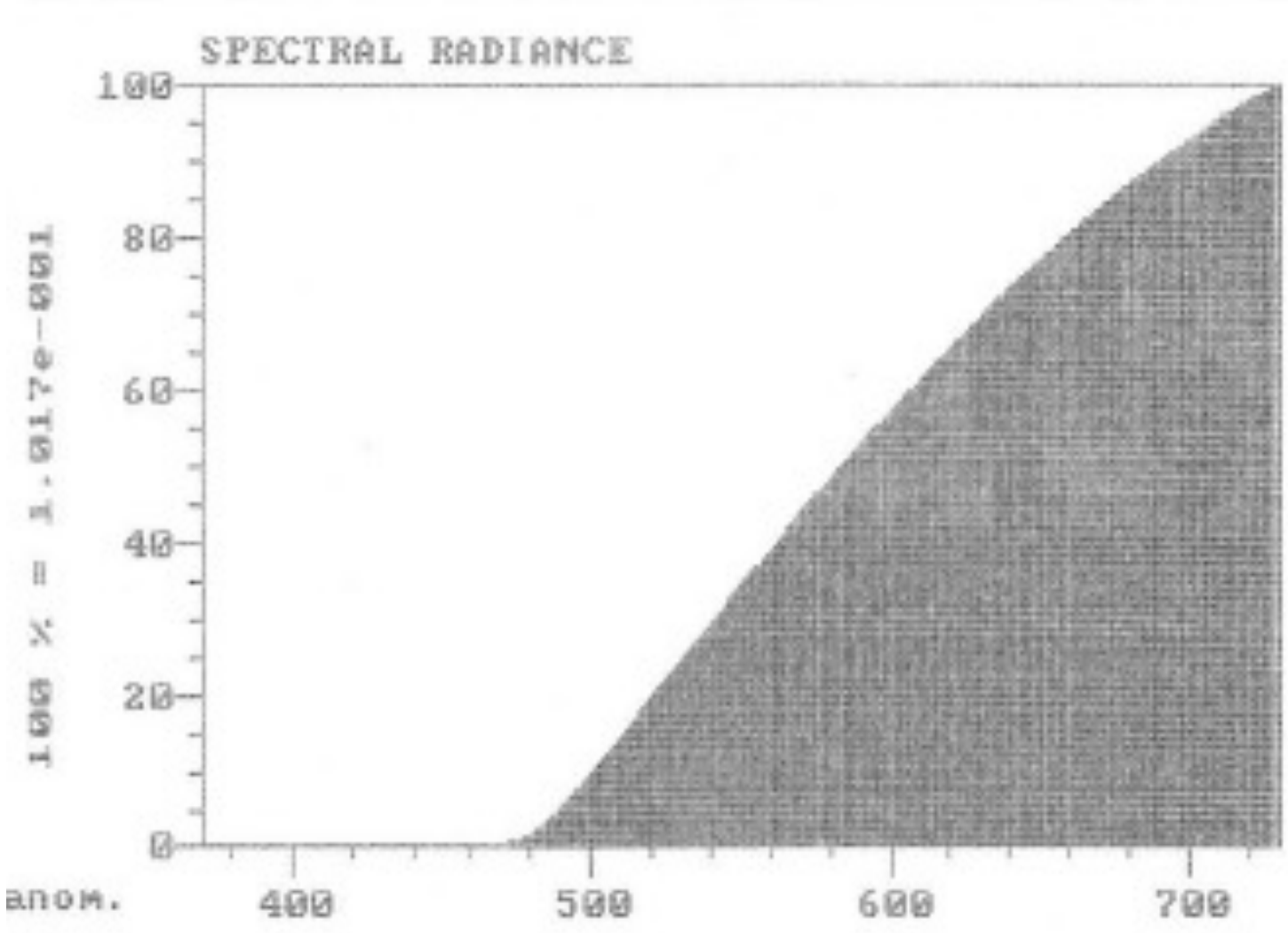
600
700

PI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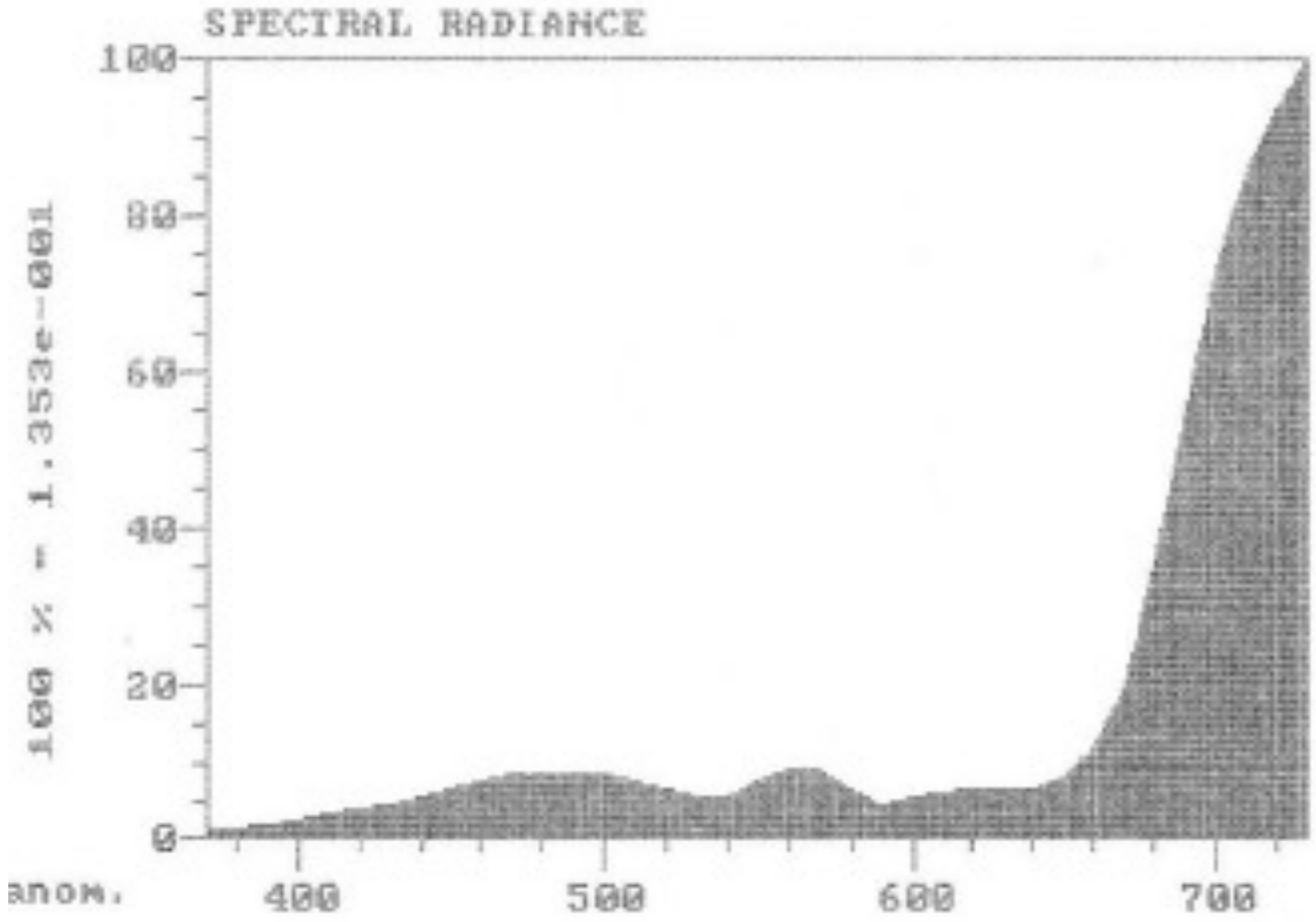
20
0
100 % = 1.917e-001
anom.
400
500
600
700

S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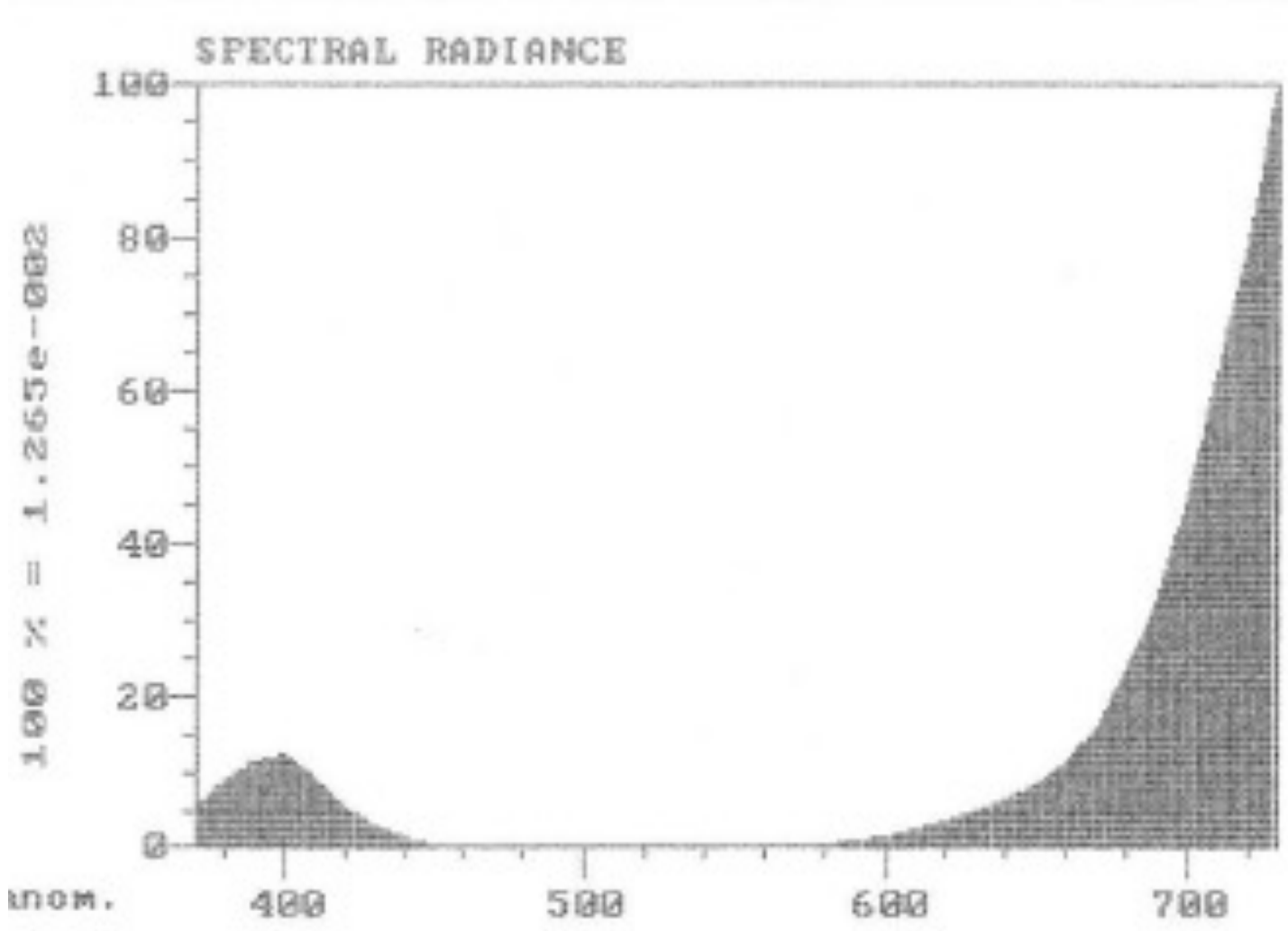
100 % = 1.353e-001
anom.
400
500
600
700

D


SPECTRAL RADIANCE
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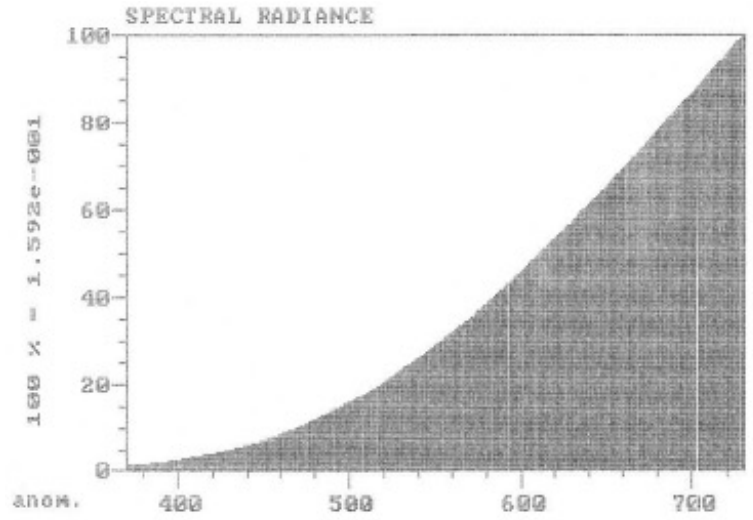
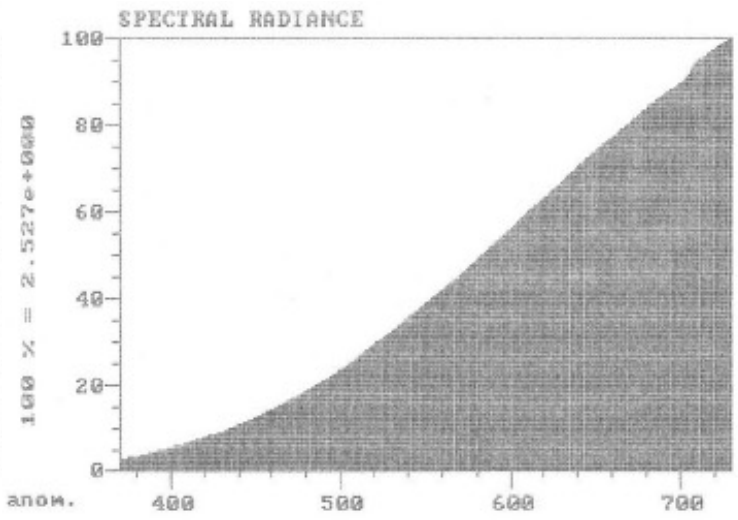
40
20
0
100 % = 1.265e-002
nom.
400
500
600
700

N





BULB 110 V **BULB 140 V**

How can this work through the eyes?

A large percentage of our therapy patients have a history of neonatal, chronic or acute, often multiple, TBI. Syntonic treatments are generally amazingly successful in reversing post TBI symptoms.

Often the symptoms of a mild TBI (mTBI) do not present until days, weeks or months after the event. Sometimes they resolve and often they persist.

Usually, Syntonics treatments bring quick, permanent and life-changing recoveries (miracles).

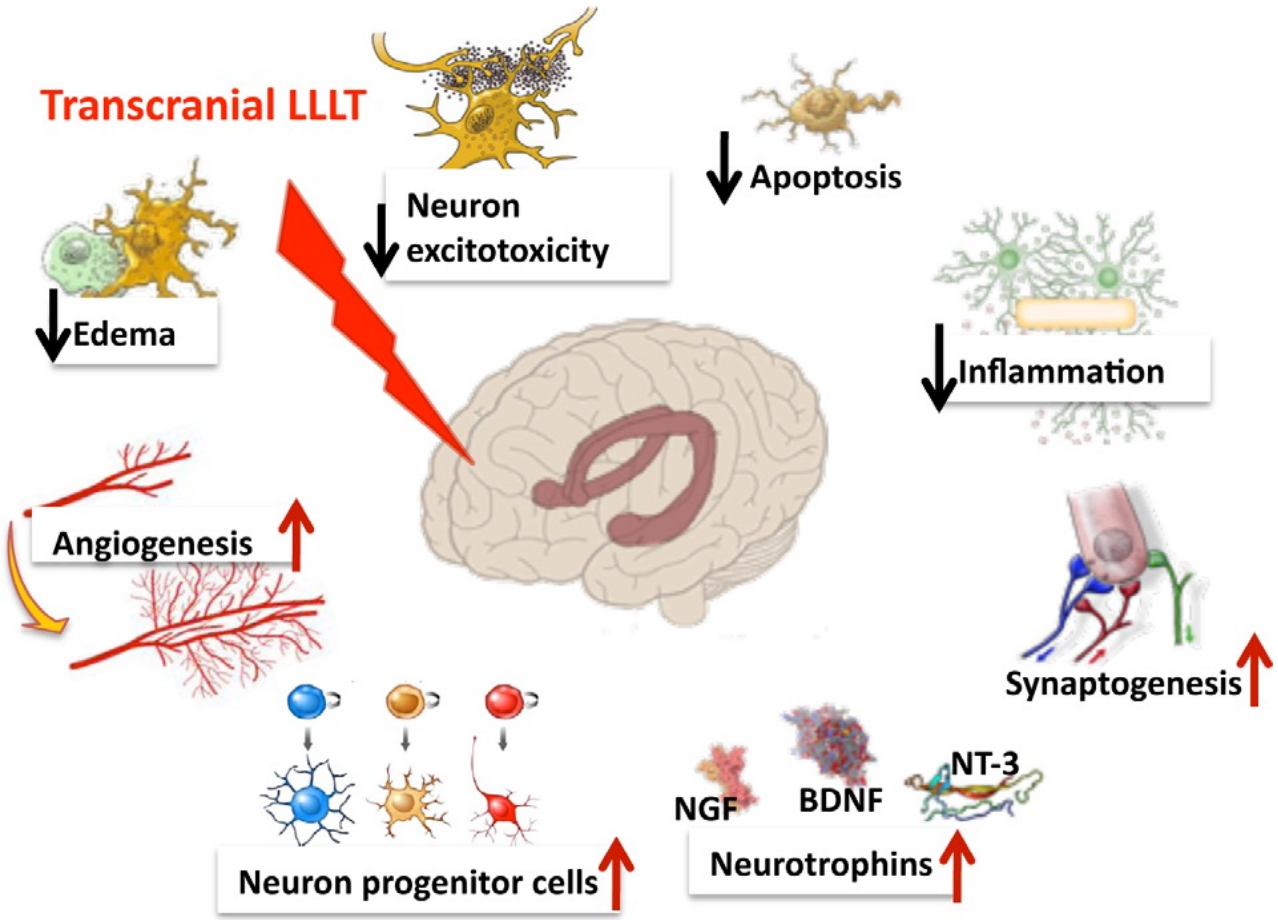
There is a paucity of proven therapies for post TBI sequela.

- most of the trials that have been performed in recent years have failed to demonstrate any significant improvement in outcomes.

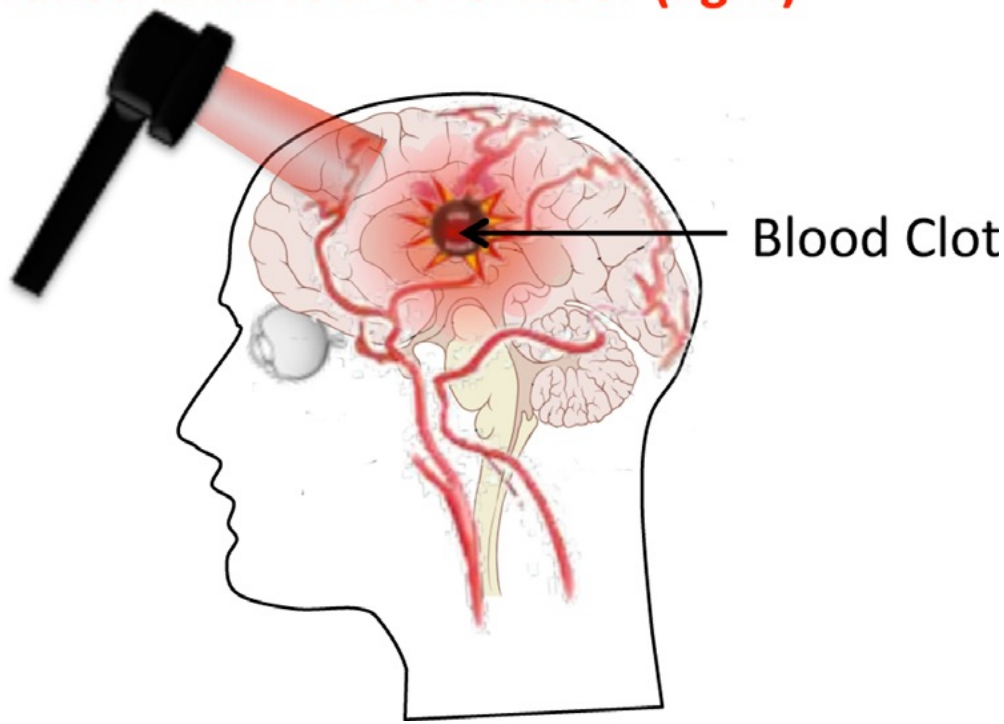
Physiology of TBI

- iNOS induction and NO played important roles in the development of secondary injury following TBI and that NO may be detrimental to the injured brain, due to formation of peroxynitrite.
- Posttraumatic pharmacological modulation of NOS may improve the outcome.

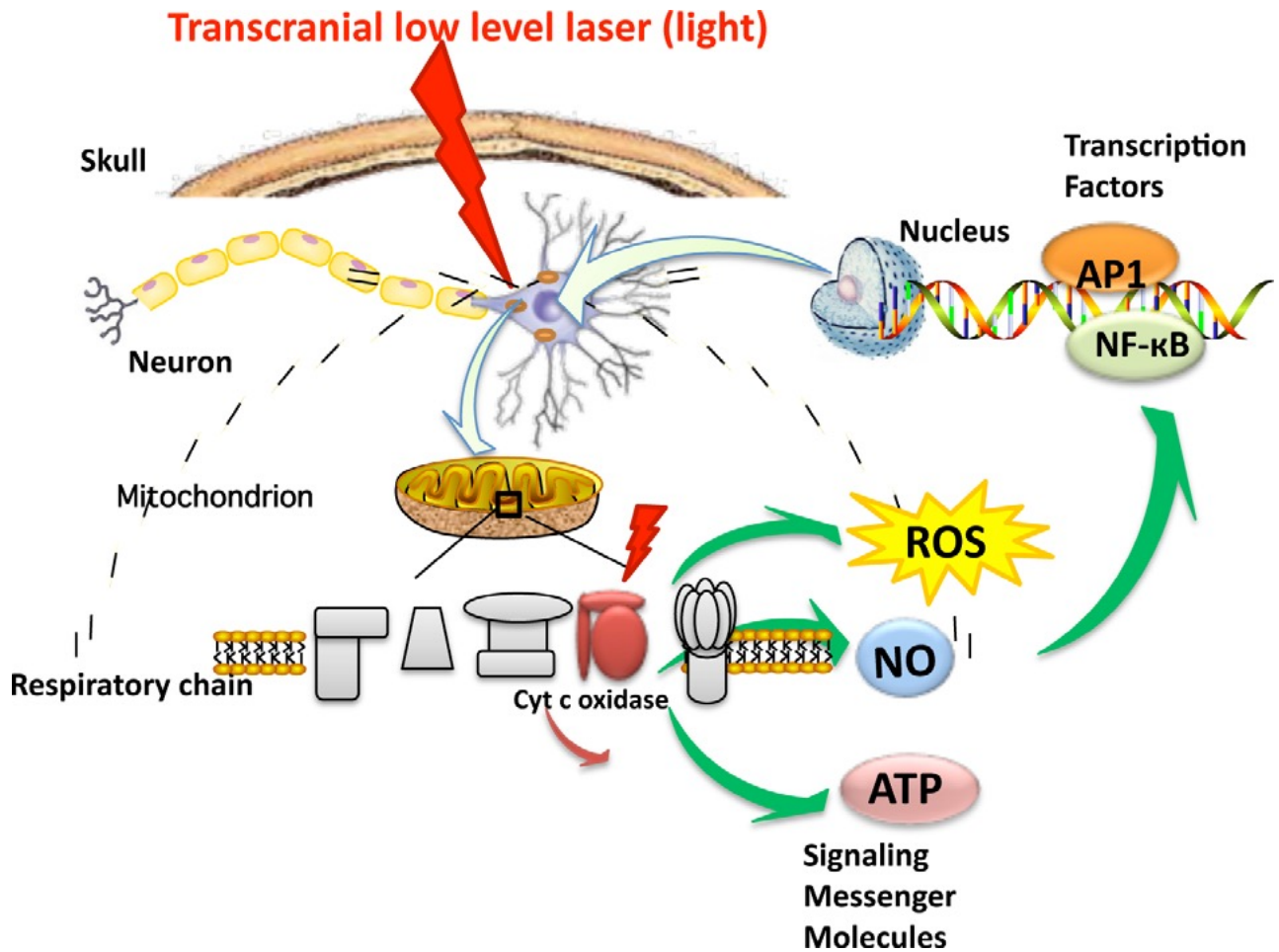
TBI


Transcranial LLLT
Neuron excitotoxicity
Apoptosis
Edema
Inflammation
Angiogenesis
Synaptogenesis
NGF
BDNF
NT-3
Neurotrophins
Neuron progenitor cells

Transcranial low level laser (light)



- Clinical trial for stroke Number of subjects
- Eligibility Criteria Parameters of treatment Effect References NEST-1 120 Patients: between 40 to 85 years of age; clinical diagnosis of ischemic stroke; measurable neurological deficit; NeuroTera Laser System (NST) within 24 hours of stroke onset 808 nm; 700 mW/cm2 on shaved scalp with cooling; 1 J/cm2 at cortical surface; 20 predermined location 2 min each study demonstrated the safety and effectiveness of infrared laser therapy within 24 hours of stroke onset. [49] NEST-2 660 Patients: between 40 to 90 years of age; clinical diagnosis of ischemic stroke within 24 hours of onset; NIH stroke scale 722 808 nm; 700 mW/cm2 on shaved scalp with cooling; 1 J/cm2 at cortical surface; 20 predermined location 2 min each
- In this second clinical trial transcranial laser therapy (TLT) within 24 hours of stroke onset demonstrated safety but the efficacy did not statistical significance.
- Mortality and adverse event rate were not adversely


Transcranial low level laser (light)
Skull
Transcription
Factors
Nucleus
AP1
NF-κB
Neuron
Mitochondrion
ROS
NO
Respiratory chain
Cyt c oxidase
ATP
Signaling
Messenger
Molecules

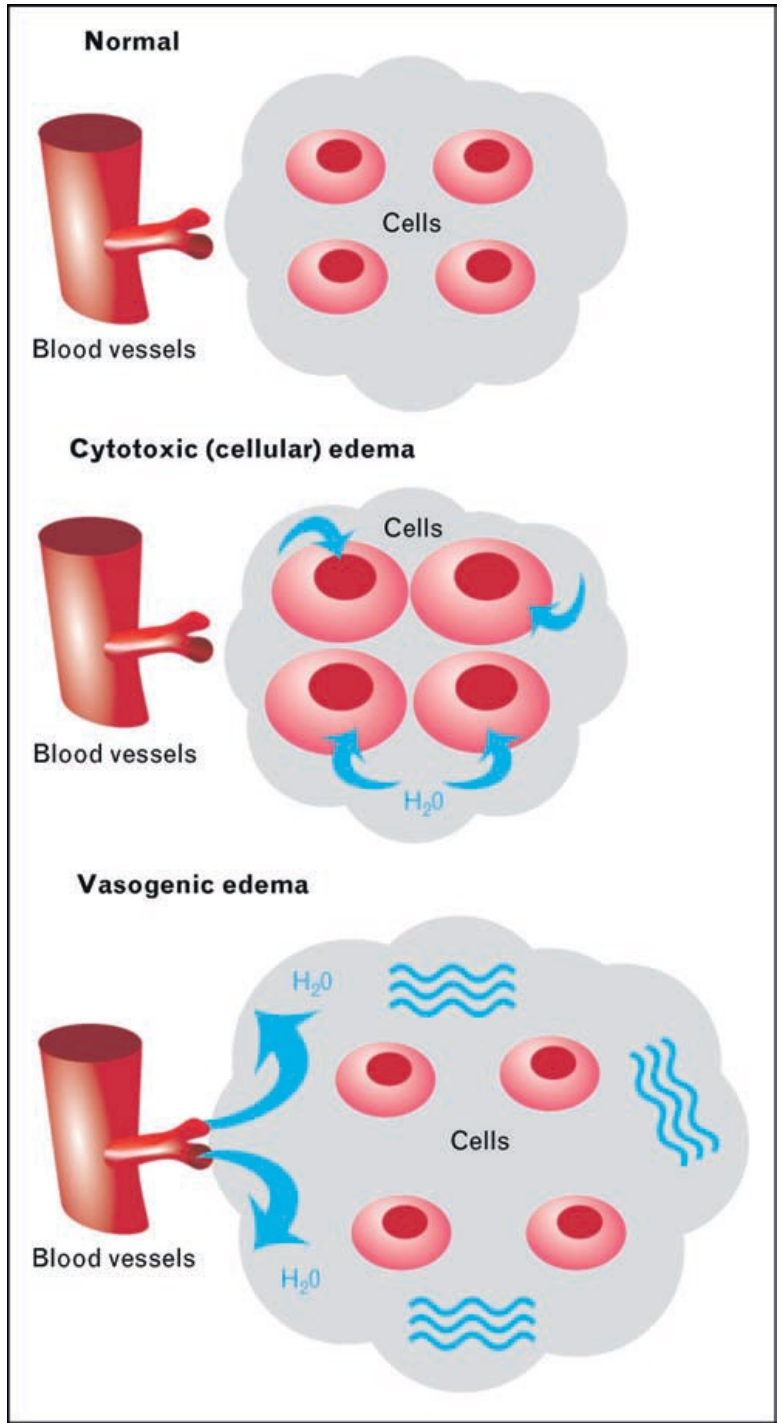
Evidence that transcranial LLLT is a beneficial treatment for acute TBI is rapidly accumulating.

- The large number of published studies that transcranial LLLT is effective for acute stroke suggested that the same approach would also be effective for acute TBI which shares many of the pathophysiological features found in ischemic stroke.
- The benefits of transcranial LLLT appear to be based on many different biological mechanisms.
- Neuroprotection or the ability of the laser to prevent the spread of brain cell death that occurs in the hours and days after a brain lesion is formed, is shown by the smaller size of the lesion area in LLLT treated mice.
- Anti-inflammatory, anti-edema and pro-angiogenic effects of LLLT may also have roles to play in the beneficial effects.
- Perhaps the most exciting possible beneficial mechanism is that LLLT may stimulate neurogenesis or increase the ability of the brain to repair itself.
- Not only may new brain cells be formed after LLLT but the existing brain cells may be encouraged to form new synaptic connections in the process known as synaptogenesis or synaptic plasticity.
- If these processes can be reliably shown to occur after transcranial LLLT it opens the door to the treatment being applied to neurodegenerative diseases such as Alzheimer’s and many diverse psychiatric disorders.

Mechanisms of cerebral edema in traumatic brain injury

- Cerebral edema may account for up to half of the mortality in all victims of TBI [1], and in younger victims of TBI, up to half of all mortality and morbidity [2].
- Edema is harmful because it causes cell swelling that alters cellular metabolite concentration, cellular physiology, biochemistry and function.
- When the swelling involves not only the cells themselves but also the surrounding tissue, causing a rapid increase in intracranial pressure (ICP), that can compress blood vessels, reduce tissue blood flow, reduce oxygenation and can eventually pressure tissue gradients (herniations) that can crush vital brain centers involved in respiration and cardiac function.

Cerebral edema with brain swelling remains the most significant predictor of outcome.



The blood–brain barrier

Mitochondrial Neuroprotection in Severe Traumatic Brain Injury

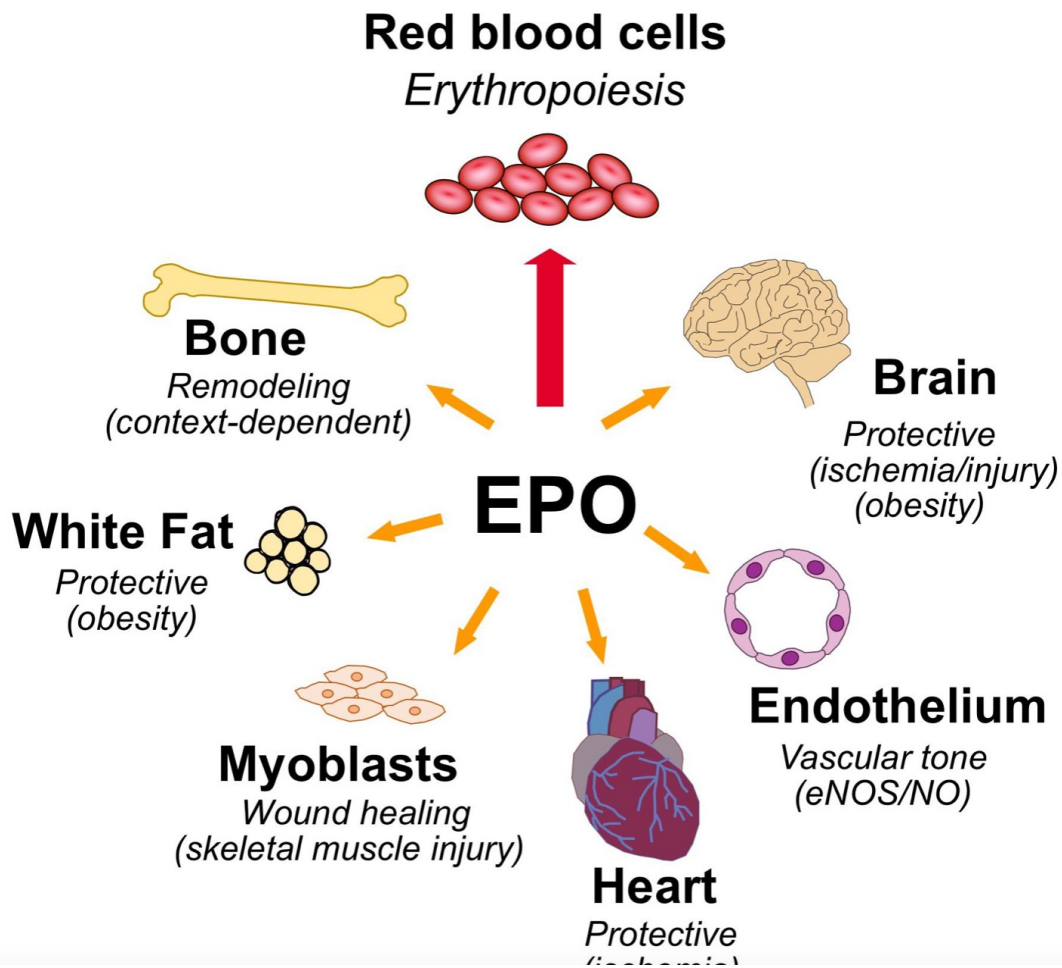
Reduced cerebral oxygenation may arise from at least four mechanisms:

1. Reduced oxygen delivery due to impaired cerebral blood flow.
2. Reduced oxygen delivery by diminished hemoglobin content or hemoglobin function (e.g., carbon monoxide poisoning or anemia)
3. Reduced oxygen uptake from the lungs (e.g., Acute Respiratory Distress Syndrome (ARDS), severe lung disease, or pulmonary contusions).
4. Reduced oxygen unloading into the tissue (e.g., if mitochondrial damage incapacitates aerobic metabolism).

Nitric oxide, vasodilation and red blood cell

At the tissue level light can influence blood flow, following release of the vasodilator, NO

- Nitric oxide (NO) has also been observed to be released from cells during LLLT.
- It is possible that LLLT may cause photodissociation of NO from CCO [20, 21].
- Cellular respiration is down-regulated by the production of NO by mitochondrial NO synthase (mtNOS, a NOS isoform specific to mitochondria), that binds to CCO and inhibits it.
- The NO displaces oxygen from CCO, inhibiting cellular respiration and thus decreasing the production of ATP [22].
- By dissociating NO from CCO, LLLT prevents this process from taking place and results in increased ATP production [23, 24].
- Therefore, [24]. Enhanced perfusion will facilitate improved oxygenation and recruitment of inflammation cells to the areas undergoing repair as well as further re-vascularization and proliferation of cells to achieve systemic effect. Figure 1 graphically illustrates some of the intracellular signaling pathways that are proposed to occur after LLLT.


Red blood cells
Erythropoiesis
Bone
Remodeling
(context-dependent)
Brain
Protective
(ischemia/injury)
(obesity)
EPO
White Fat
Protective
(obesity)
Endothelium
Vascular tone
(eNOS/NO)
Myoblasts
Wound healing
(skeletal muscle injury)
Heart
Protective

- To meet the rapidly changing metabolic needs of neurons, blood vessels in the CNS must be capable of supplying oxygen and nutrients in a flexible manner [60,62].
- When neurons in a specific brain region are activated, blood flow in this region will increase in a temporarily and spatially coordinated manner [64].
- Several cell types of the neurovascular unit contribute to this regulation.
- Indeed, neurons release various types of vasodilators [8], whereas astrocytes act as sensors of neuronal activity and are key regulators of the cerebral microcirculation [64]. They can induce vasodilation or vasoconstriction depending on the metabolic needs of the brain and the pre-existing vascular tone of arterioles

NO is involved in neurotransmission, regulating blood vessel dilation, and immune action

- NO in the central nervous system helps cognitive function, synaptic plasticity, sleep, appetite, thermoregulation, and neurosecretion.
- In the peripheral nervous system, NO regulates the relaxation of smooth muscle in the corpora cavernosa, (penile erection), and in many aspects of the gastrointestinal tract.
- NO interacts with Intracellular signal-transduction pathways to trigger other intracellular targets to stimulate or inhibit signals able to control a wide range of actions.
- In contrast, high levels of NO can be toxic when released in an uncontrolled inflammatory response and can participate in acute and chronic neurodegenerative diseases including stroke, multiple sclerosis, Parkinson disease (PD), and Alzheimer disease.
- “Nitrosative stress’’ refers the cellular damage of proteins elicited by NO and its sequelae, The substantially different roles of these different types of modification are implicated in various physiologic and pathologic processes. Numerous S-nitrosylated proteins have been identified in vivo, including blood serum albumin, hemoglobin and red blood cells.