# Neurology, Neuropsychiatry, and Infectious Disease

Dr. Jay Lombard

Website: https://www.drjaylombard.com/

Disclaimer: I have no conflicts of interest to declare

## Crucial questions regarding the etiology of neurodegenerative and neuropsychiatric conditions:

1. Are diseases of the brain - both psychiatric and neurologic - primarily based upon reactivation of chronic infectious disease?

2. What is the evidence in schizophrenia, autism, Alzheimer's, MS, PD and ALS are infectious diseases?

3. What are the diagnostic and treatment implications if this hypothesis is confirmed?

# The Koch Postulate Applied to Neurodegenerative Diseases

An assessment of whether microorganisms cause a particular disease:

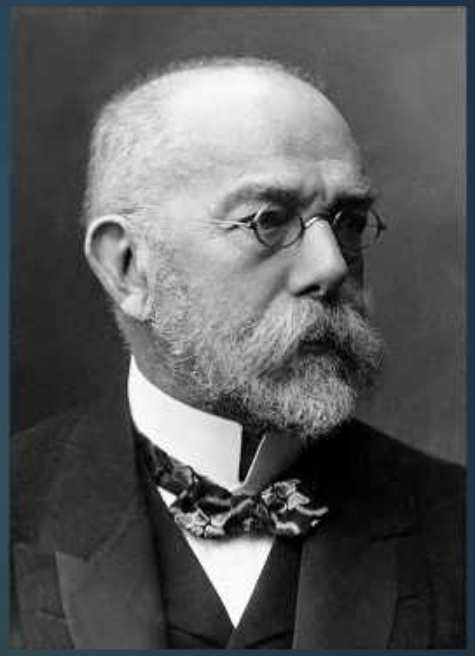
1. The microorganism must be found in diseased but not healthy individuals;
2. The microorganism must be cultured from the diseased individual;
3. the microorganism must recapitulated the disease; and finally;
4. The microorganism must be re-isolated from the diseased individual and matched to the original microorganism.

   However, The issue is that microbial pathogens **can lie dormant and resist** being positively cultured.

# The “Invisible Enemy”

‘These germs  are transparent bodies. Like glass. Like water. To make them visible you must stain them. Well, do what you will, some of them won't stain; they won't take any methylene blue, they won't take gentian violet, they won't take any colouring matter. Consequently, though we know as scientific men that they exist, we cannot see them’.

The Doctor’s Dilemma. George Bernard Shaw, 1906



Dr. Robert Koch

# The “Walking Dead” Theory of Neurodegeneration

“Many human pathogens respond to environmental stresses by entry into a novel physiological state as spores or biofilms where the cells remain viable, but are no longer culturable.”

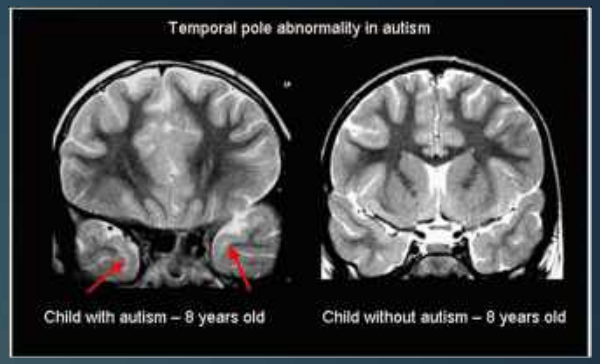
Neurodegeneration is a re-activation of microbes from this ‘viable but nonculturable’ state to cause infection.

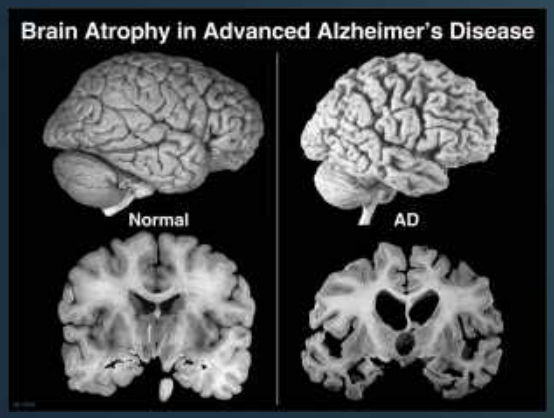
Ref: Oliver J.D. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol. Rev. 2010;34:415–425. doi: 10.1111/j.1574-6976.2009.00200.x.

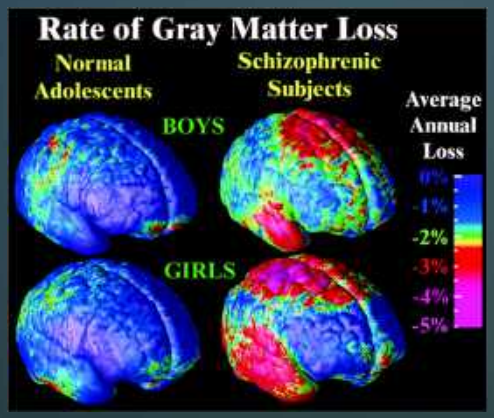
# CNS Disorders are Dimensional, not Categorical

What do diseases labeled “neuropsychiatric” and “neurodegenerative” have in common?

*Deterioration of CNS tissues!*




Multiple agents can cause brain degeneration:

- Genes
- Environmental toxins
- Pathogens

# Overlapping Neuropathological Features in CNS Disease

Common clinical features:

1. Immunosuppression
2. Chronic infection
3. Mitochondrial dysfunction

These pathways lead to protein aggregation, a common feature across diagnoses in the brain

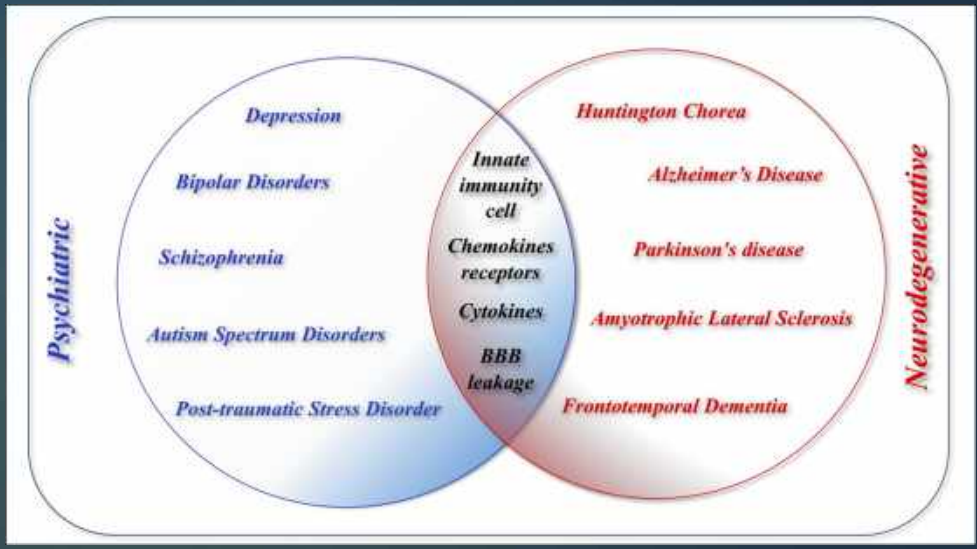
Refs:
(1) Polajnar M, Zerovnik E. Impaired autophagy: a link between neurodegenerative and neuropsychiatric diseases. J Cell Mol Med. 2014;18(9):1705-1711.
(2) Bradshaw, N.J., Korth, C. Protein misassembly and aggregation as potential convergence points for non-genetic causes of chronic mental illness. Mol Psychiatry 24, 936–951 (2019).

# Altered Immunity in Brain Disorders

Elevated cytokines including IL-6 in early stage disease

In later stages of disease, characteristic findings include:

- T-cell dysregulation
- Reduced Treg function
- Lymphopenia
- Reduced CNS iron metabolism



# Altered Sphingolipid Metabolism in Neuropsychiatric Disease

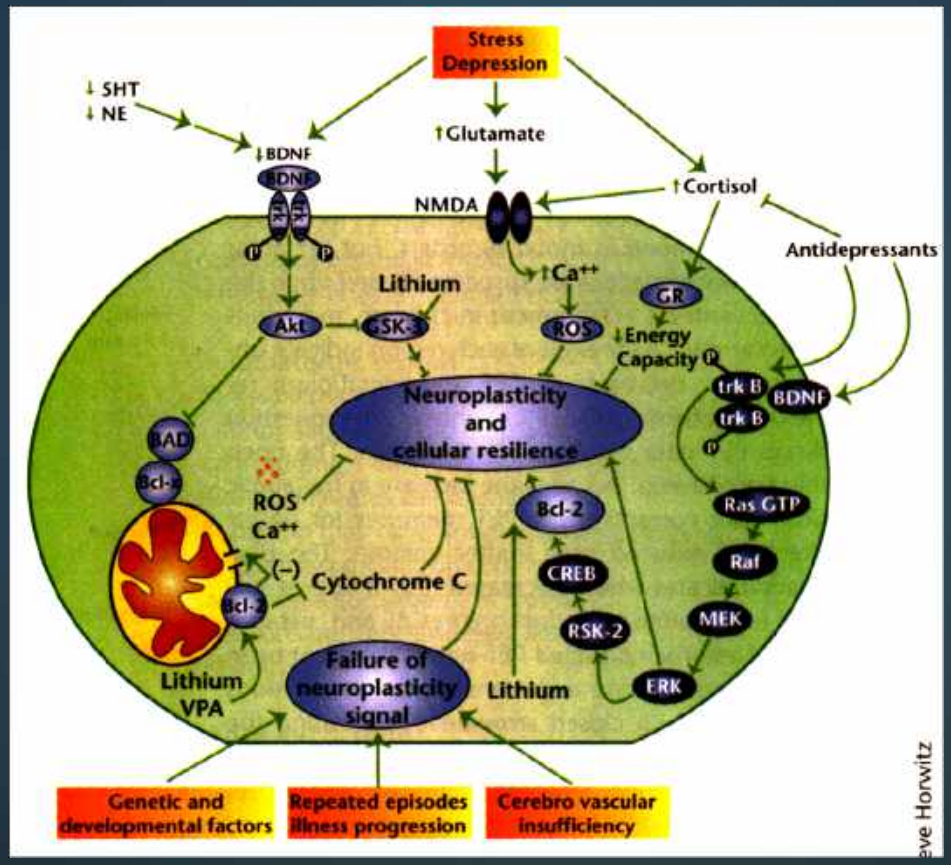
Sialic acid  can modify neural cell adhesion molecules (NCAM), thereby mediating neuronal outgrowth, synaptic connectivity and memory formation.

"... plasma SA in the control group was significantly higher than that in the ASD group ($p < .01$). Autistic children had higher positive rates of anti-GM1 antibodies (37.8%) than controls (21.67%, $P = .04$)." (1)

CD33, a sialic acid binding lectin has been demonstrated to be a risk factor in Alzheimer's disease (Tanzi)

Refs:
(1) Yang X, Liang S, Wang L, et al. Sialic acid and anti-ganglioside antibody levels in children with autism spectrum disorders. Brain Res. 2018;1678:273-277.
(2) Miles LA, Hermans SJ, Crespi GAN, et al. Small Molecule Binding to Alzheimer Risk Factor CD33 Promotes Aβ Phagocytosis. iScience. 2019;19:110-118.

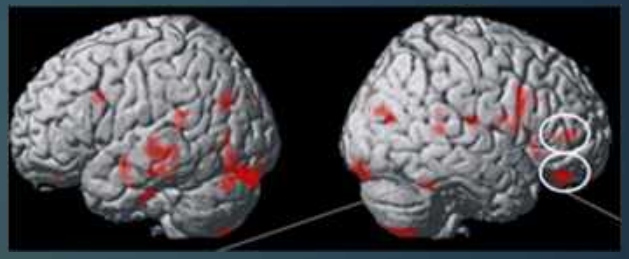
# Apoptosis in Neuropsychiatry

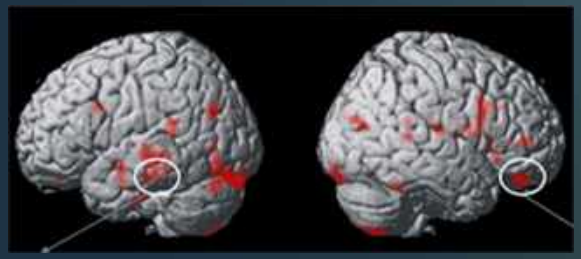


# Meta analysis: volumetric studies of other prefrontal and cortical structures

Results: Red highlighted areas have significant gray matter thinning in depressed subjects correlating with:

Cognition: circled areas-- where gray matter reduction correlated with performance on the Wisconsin Card Sorting Test





Severity: circled areas -- where gray matter reduction correlated with severity of depression by MADRS

Ref: Vasic N, et al.. J Affective Disorders epub 10 Jan 2008

# Hippocampal atrophy: a highly replicated finding

Degree of atrophy in depression correlated with:

- Duration of current episode
- Duration of depressive illness
- Duration untreated depression

First episode depression atrophy correlates with:

- Number of stressful experiences prior to 1st episode


Cognition negatively affected:

- Impaired cognition on Wisconsin Cart Sorting Test (WCST correlatates with reduced hippocampal volume

<u>Key points:</u>

- Reduced hippocampal volume appears to result from both stress and episodes of depression, and negatively impacts mood/cognition
- Untreated depression may allow for progressive neurodegenerative changes

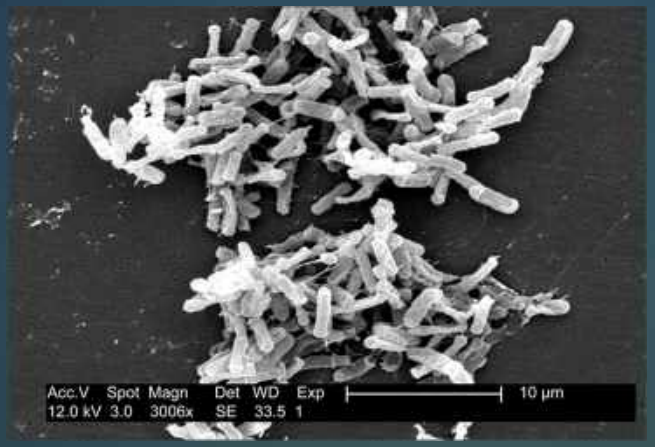
Refs:
(1) Vasic N, et al. Affective Disorders epub 10 Jan 2008. Sheline Y, et al. Proc Natl Acad Sci 83(9):3908-13 1996
(2) Sheline Y, Mokhtar H, Gado M. et al. Am J Psychiatry 160:1516-18 2003. Kronmuller KT, et al. J Affective Disorders epub Mar 5 2008;

What's linking these conditions?

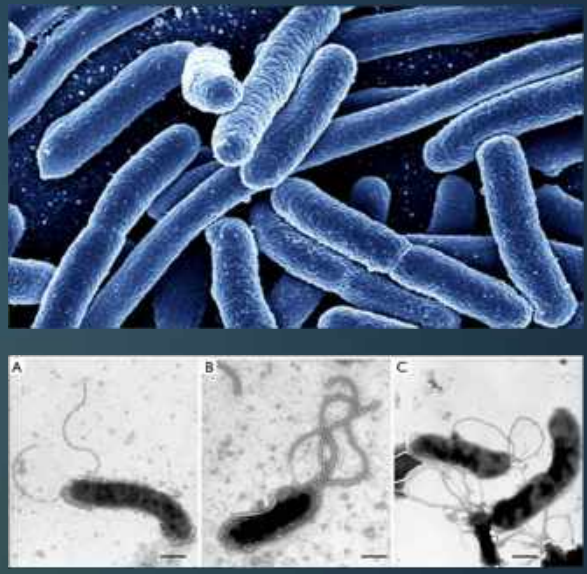
Is there a smoking gun?

# Enteropathogens associated with CNS disease

Campylobacter




Clostridium




E. Coli, H. Pylori

Refs:
(1) Dardiotis E, Tsouris Z, Mentis AA, et al. H. pylori and Parkinson's disease: Meta-analyses including clinical severity. Clin Neurol Neurosurg. 2018;175:16-24.
(2) Wagley S, Bokori-Brown M, Morcrette H, et al. Evidence of Clostridium perfringens epsilon toxin associated with multiple sclerosis. Q ypÅWgpiv 2019;25(5):653-660.
(3) Nyati KK, Nyati R. Role of Campylobacter jejuni infection in the pathogenesis of Guillain-Barré syndrome: an update. Biomed Res Int. 2013;2013:852195.

# What are their common cytopathic effects?

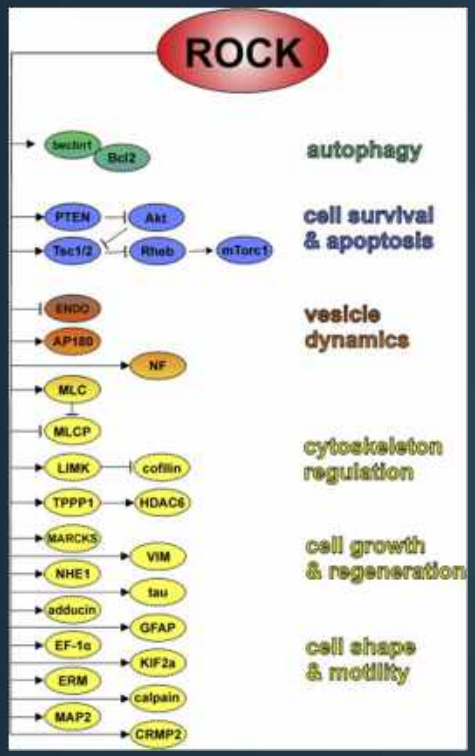
Toxins bind to subunits of GM1 gangliosides which may account for elevated GM1 antibodies in ALS

These toxins result in defects in ADP-ribosyltransferase (mRNA proteins)

A critical enzyme affected is Rho Kinase (ROCK). Overactivation disrupts actin cytoskeleton dynamics and nitric oxide production.

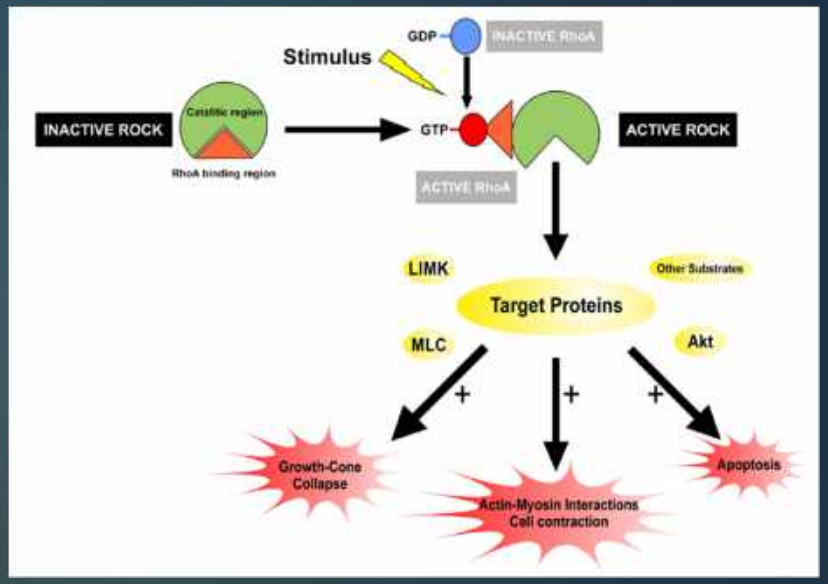
Refs:
(1) Popoff M.R. Bacterial factors exploit eukaryotic Rho GTPase signaling cascades to promote invasion and proliferation within their host. Small GTPases. 2014;5:e983863. doi: 10.4161/sgtp.28209.
(2) Li J. C., Tatenhorst L., Roser A.-E., Saal K.-A., Tönges L., Lingor P. Rock inhibition in models of neurodegeneration and its potential for clinical translation. Pharmacology & Therapeutics. 2018;189:1–21. doi: 10.1016/j.pharmthera.2018.03.008.

# Rho Kinase and Dopaminergic Degeneration




**Left:** Downstream targets of rho kinase activation.

**Right:** Schematic of ROCK activation via GTPase RhoA. Bacterial toxins can disrupt RhoA inhibition, causing an "always on" state.




Ref: Labandeira-Garcia J. L., Rodríguez-Perez A. I., Villar-Cheda B., Borrajo A., Dominguez-Meijide A., Guerra M. J. (2015). Rho kinase and dopaminergic degeneration: a promising therapeutic target for Parkinson's disease. Neuroscientist 21, 616–629. 10.1177/1073858414554954

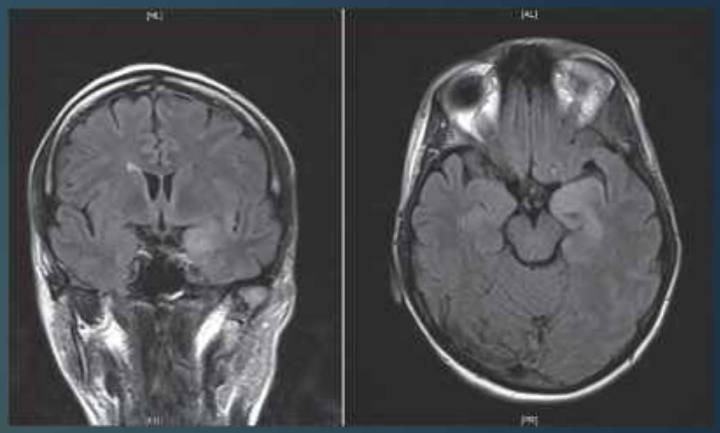
# Viral Infections in CNS disorders

Herpes Simplex Virus - Encephalitis

HIV, polio - In association with motor neuron disease

EBV, HSV- MS

COVID-19  seizures and encephalopathy

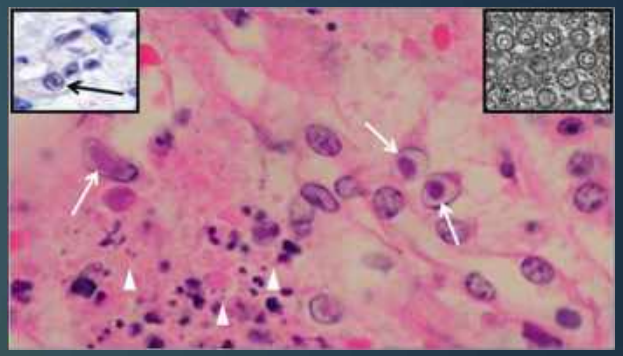


Brain images of HSV encephalitis


Refs:
(1) Helms J, et al. Neurologic Features in Severe SARS-CoV-2 Infection. N. Engl. J. Med. 2020;382:2268–2270.
(2) Acharya JN, Pacheco VH. Neurologic complications of hepatitis C. The Neurologist. 2008;14(3):151–156.
(3) Bowen LN, Tyagi R, Li W, Alfahad T, Smith B, Wright M, Singer EJ, Nath A. HIV-associated motor neuron disease: HERV-K activation and response to antiretroviral therapy. Neurology. 2016;87(17):1756–1762.
(4) Kennedy PGE, Chaudhuri A. Herpes simplex encephalitis. Journal of Neurology, Neurosurgery & Psychiatry 2002;73:237-238.

# Common pathogenic mechanism of CNS toxicity

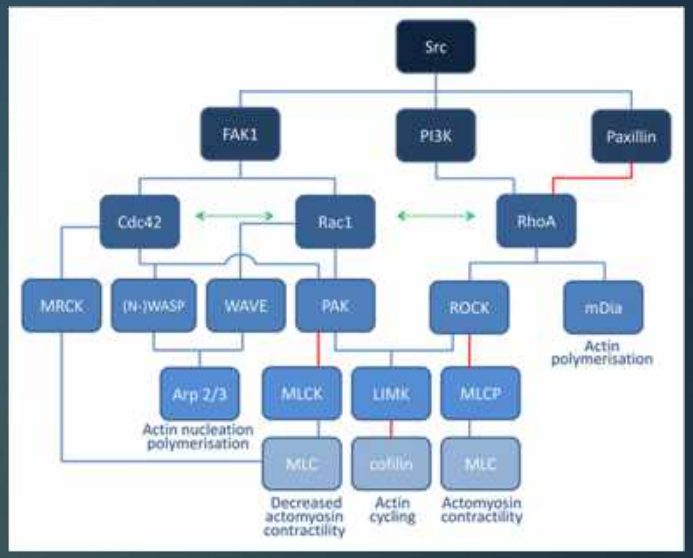
1. Lipid containing membrane envelopes derived from modified cell membranes borrowed from the host via serine proteases
2. The virus attaches and fuses to the host by breaking down phospholipids to produce inclusion bodies (as seen in ALS, AD, etc.)
3. Primary pathology for kidnapping host DNA and RNA involve specific viral polymerases that affect host mRNA including Rho Kinase



HSV-associated inclusion bodies

# Viral infections trigger rho GTPase dysfunction

- Disruption of rho kinase leads to microtubule and mitochondrial pathology
- Viruses alter host rho GTPase signaling to hasten endocytosis and promote nuclear delivery (1)
- Rho signaling inhibitors show potent antiviral effects (2)




Downstream effects of virus-mediated rho signaling (1)


Refs:

(1) Céline Van den Broeke, Thary Jacob & Herman W Favoreel (2014) Rho'ing in and out of cells, Small GTPases, 5:1

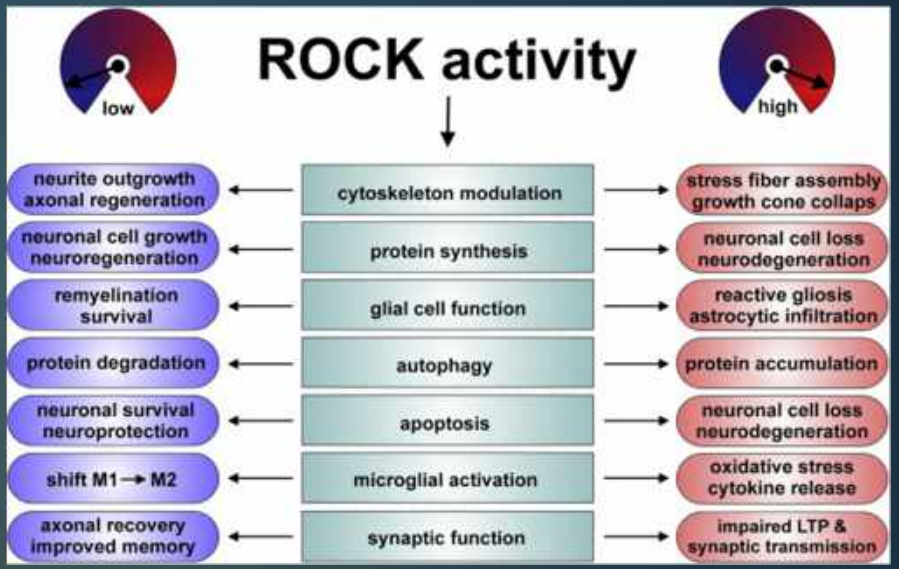
(2) del Real G, Jiménez-Baranda S, Mira E, et al. Statins inhibit HIV-1 infection by down-regulating Rho activity. J Exp Med. 2004;200(4):541-547.

(3) Lucera, M.B., Fleissner, Z., Tabler, C.O. ix&p2HIV signaling through CD4 and CCR5 activates Rho family GTPases that are required for optimal infection of primary CD4+ T cells. Vixszmspsk} 14, 4 (2017).

# Rho Kinase, Chronic Infections, and Neurodegeneration

Overactive rho kinase inhibits neuronal growth, structural stability, and metabolism

Rho kinase inhibitors have improved survival in animal models of: Parkinson's, ALS, Alzheimer's, and Huntington's




Neurological effects of Rho Kinase activity

Ref: Stankiewicz TR, Linseman DA. Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. Front Cell Neurosci. 2014;8:314. Published 2014 Oct 7. doi:10.3389/fncel.2014.00314

# What was going on in Salem?

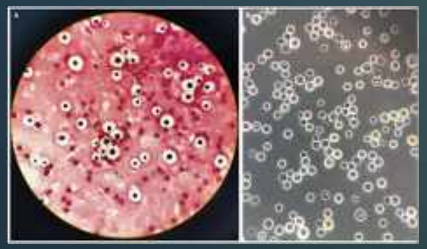
# Is *fungus* to blame???

"In 1976 Linnda Caporael offered the first evidence that the **Salem witch trials** followed an outbreak of rye ergot. Ergot is a **fungus** blight that forms hallucinogenic drugs in bread. Its victims can appear bewitched when they're actually stoned. Ergot thrives in a cold winter followed by a wet spring."

# Fungal infections in the CNS

**YEASTS**

Candida



Cryptococcus

**MOLDS**

Fusarium

Aspergillius

# Fungal Pathogen Spread and Effects

- Airborne with hematogenous spread
- CNS effects:
  - Aseptic meningitis
  - Encephalitis
  - Microabscesses
  - CNS vasculitis

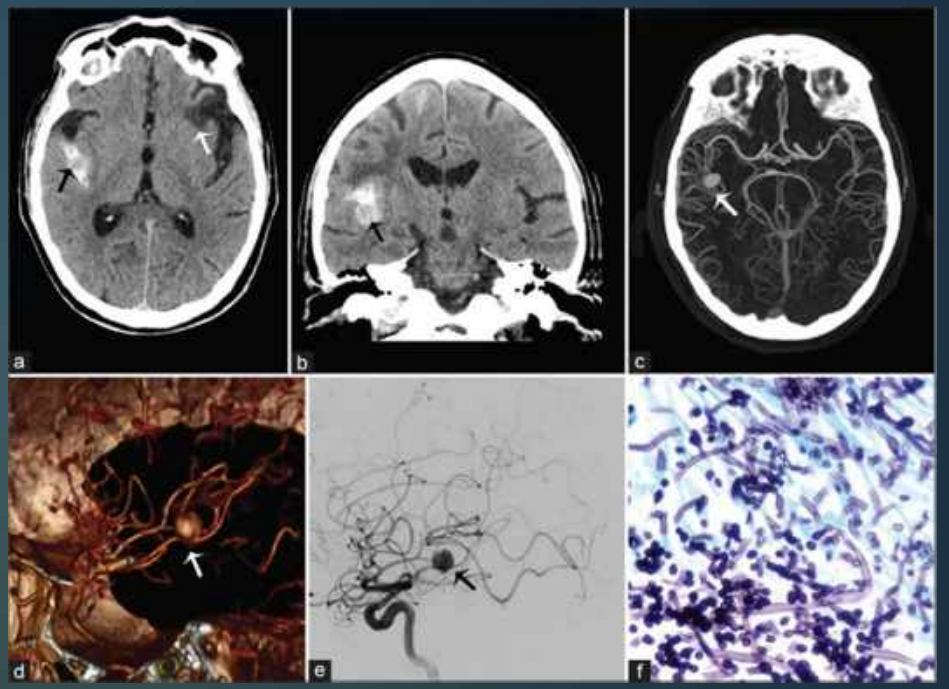



Figure: Computed tomography images of the brain showing subarachnoid hemorrhage and encephalomalacia in a patient with severe Candida Albicans infection in the CNS (A-C). Angiogram images show saccular aneurysm (D-E). Candida Albicans culture from patient (F).

Ref: Gavito-Higuera J, Mullins CB, Ramos-Duran L, Olivas Chacon CI, Hakim N, Palacios E. Fungal Infections of the Central Nervous System: A Pictorial Review. J Clin Imaging Sci. 2016;6:24. Published 2016 Jun 17. doi:10.4103/2156-7514.184244

# Insidious Symptoms Which May Precede CNS Fungal Infection

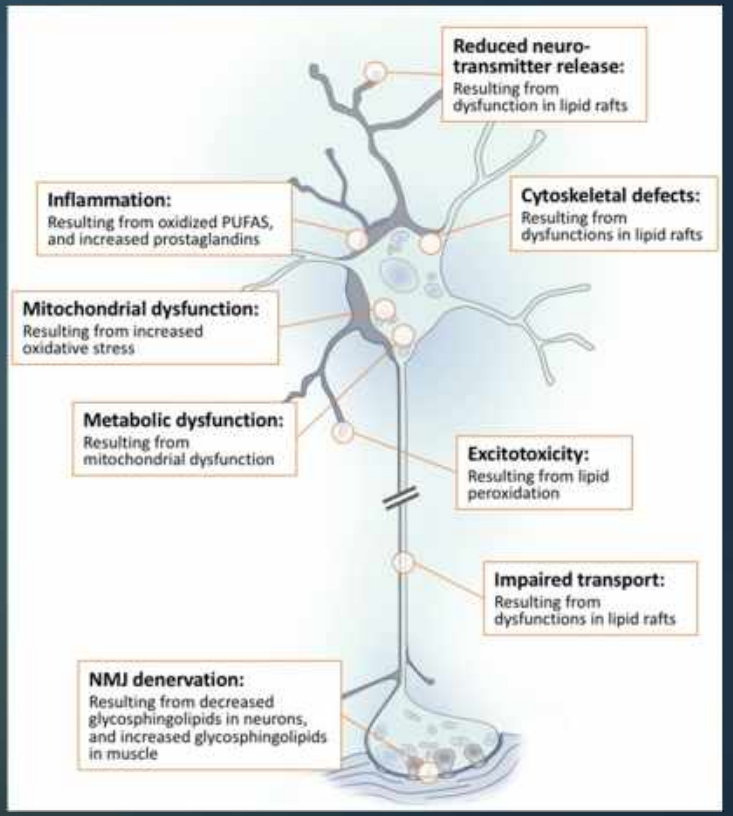
- Chronic asthma (particularly steroid induced)
- Chronic allergic rhinitis and sinusitis
- Productive cough

Refs:
(1) Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. The European Respiratory Journal. 2006 Mar;27(3):615-626.
(2) Góralska K, Blaszkowska J, Dzikowiec M. Neuroinfections caused by fungi. Infection. 2018;46(4):443-459.

# Fungal Etiology of Neurodegeneration

Fungal toxins including fumonisins alter sphingolipid metabolism which affects phospholipid metabolism

Increased production of excitatory neurotransmitters (ex: glutamate in ALS)





Refs:

(1) Merrill AH Jr, Sullards MC, Wang E, Voss KA, Riley RT. Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environ Health Perspect. 2001;109 Suppl 2(Suppl 2):283-289.

(2) Tracey TJ, Steyn FJ, Wolvetang EJ, Ngo ST. Neuronal Lipid Metabolism: Multiple Pathways Driving Functional Outcomes in Health and Disease. Front Mol Neurosci. 2018;11:10. Published 2018 Jan 23. doi:10.3389/fnmol.2018.00010

(3) French PW, Ludowyke R, Guillemin GJ. Fungal Neurotoxins and Sporadic Amyotrophic Lateral Sclerosis. Neurotox Res. 2019;35(4):969-980. doi:10.1007/s12640-018-9980-5

# Fungal Involvement in Neurodegenerative Disease

- A variety of fungal species has been found in the CSF of neurodegenerative disease patients
- Epidemiological data suggests populations with high rates of neurodegeneration may have been exposed to fungi. For example, in ALS:
  - Gulf war veterans: Potential exposure to fungal species used as biological weapons
  - Communities using well water: infectious agents in the water supply
  - Guam islanders: BMAA toxin exposure
  - Farmers: Increased fungal exposure from constant contact with agricultural products

Refs:
(1) Alonso R, Pisa D, Marina AI, et al. Evidence for fungal infection in cerebrospinal fluid and brain tissue from patients with amyotrophic lateral sclerosis. Int J Biol Sci. 2015;11(5):546-558. Published 2015 Apr 2.
(2) Alonso R, Pisa D, Carrasco L. Searching for Bacteria in Neural Tissue From Amyotrophic Lateral Sclerosis. Front Neurosci. 2019;13:171. Published 2019 Feb 26.
(3) French PW, Ludowyke R, Guillemin GJ. Fungal Neurotoxins and Sporadic Amyotrophic Lateral Sclerosis. Neurotox Res. 2019;35(4):969-980.
(4) Pisa, D., Alonso, R., Rábano, A. et al. Different Brain Regions are Infected with Fungi in Alzheimer's Disease. Sci Rep 5, 15015 (2015).

# What about fungal infections in autism and schizophrenia?

"Autistic children (r = 52) and healthy children [r = 58 (31 siblings and 27 unrelated subjects)] were recruited and body fluids and clinical data collected... Ochratoxin A (OTA), gliotoxin, zearalenone, and sphingosine/sphinganine ratio were determined by LC analysis in sera and urines... **Results:** By comparing the results of autistic patients with those of unrelated controls, a significant association was found for OTA levels in urines (T = 0.0002) and sera (T = 0.0017), and also comparing patients with siblings and unrelated controls together (T = 0.0081)"

Refs:
(1) De Santis B, Brera C, Mezzelani A, et al. Role of mycotoxins in the pathobiology of autism: A first evidence. Nutritional Neuroscience. 2019 Feb;22(2):132-144.
(2) Severance, E., Gressitt, K., Stallings, C. et al. Candida albicans exposures, sex specificity and cognitive deficits in schizophrenia and bipolar disorder. npj Schizophr 2, 16018 (2016).
(3) Schoental R. Fusarial mycotoxins and behaviour: possible implications for psychiatric disorder. Br J Psychiatry. 1985;146:115-119.

# Infections: Fungal, Bacterial, Viral

They share toxin-based homology through plasmid mediated transfer

CNS infections diseases must break down the BBB via MMP-9 in a manner similar to leaky gut

Pathogenic processes begins with penetration in lipid rich host cells

This unholy trinity kidnaps host RNA metabolism leading to protein aggregation

# How Do We Track Down These “Outlaws” in the CNS?

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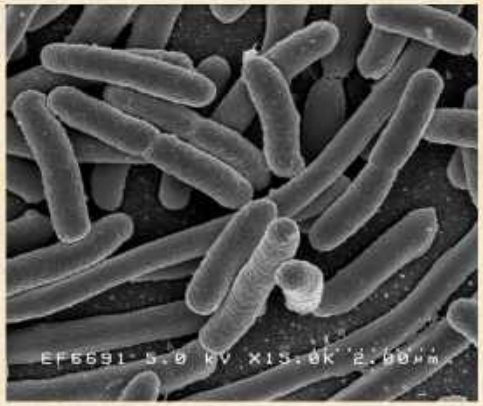


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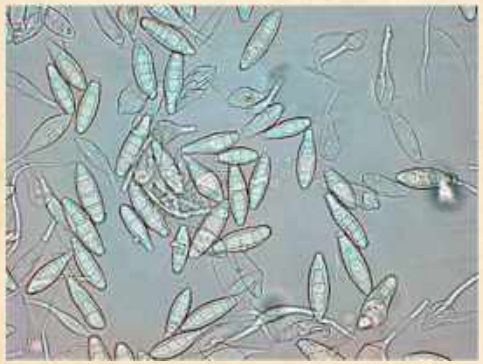

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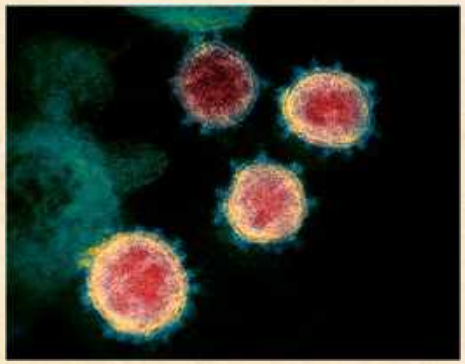

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# Diagnostics Evaluations In Suspected CNS Infections

**Risk of CNS Infection**

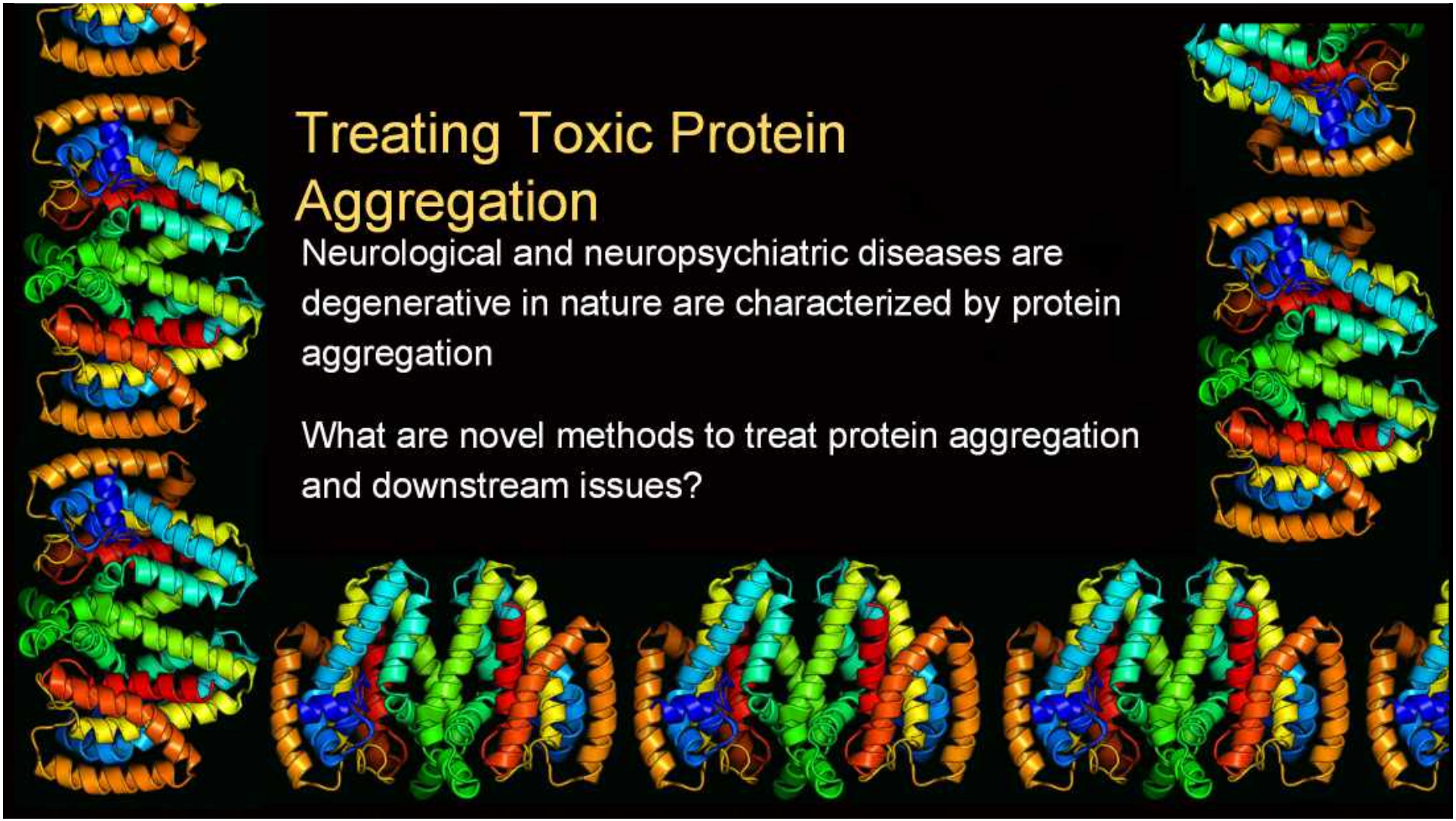
| Suggested | Increased | Definite |
|---|---|---|
| - Sinusitis or rhinitis<br>- Chronic asthma<br>- Low grade fever<br>- Temperature dysregulation<br>- Sleep abnormalities<br>- History of severe head injury | - Urine mycotoxin test<br>- Immunosuppression<br>- Sputum and fecal cultures<br>- Transcriptome analysis<br>- Family history of neurodegenerative disease | - CSF elevated protein with pleocytosis<br>- PCR or metagenomic analysis of CSF<br>- MRI enhancement of meninges |

# Summary:

- Increasing evidence that neurodegenerative and neuropsychiatric disorders may be causally associated with chronic infections
- Bacteria, viruses, and fungi share common pathogenic mechanisms presumed secondary to plasmid exchange
- Cytopathic effects include disrupted microtubules and mitochondria leading to protein aggregation
- Protein aggregation is secondary to increased production of pathological proteins and reduced autophagy for degradation
- Targeted therapies include the use of near-infrared (NIR) mitochondrial augmentation and immunopotentiation

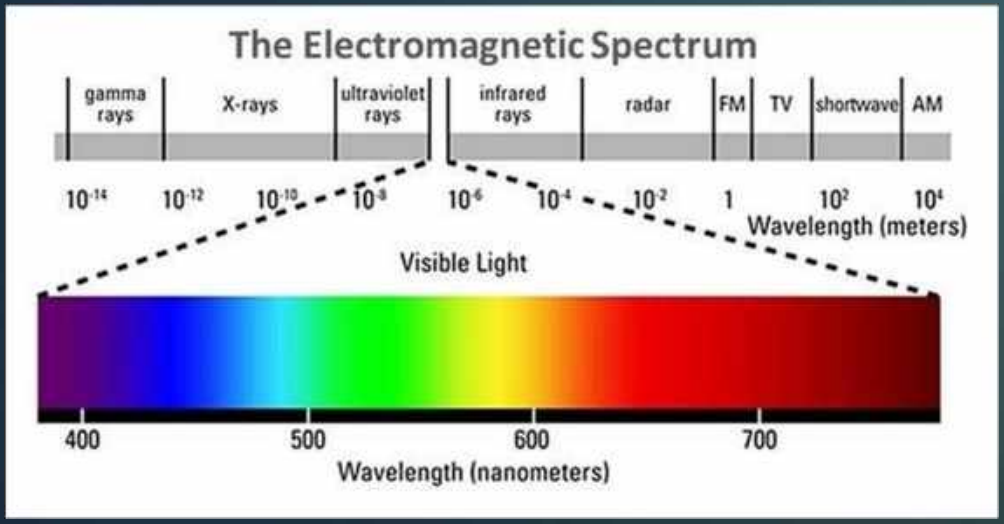
# Light Therapy for Brain Disease

Dr. Jay Lombard with Neil Olson, BSEE


Treating Toxic Protein Aggregation
Neurological and neuropsychiatric diseases are degenerative in nature are characterized by protein aggregation
What are novel methods to treat protein aggregation and downstream issues?

# “Let there be light!”

The human body utilizes light energy to catalyze chemical reactions. This is referred to as “*photobiomodulation*”

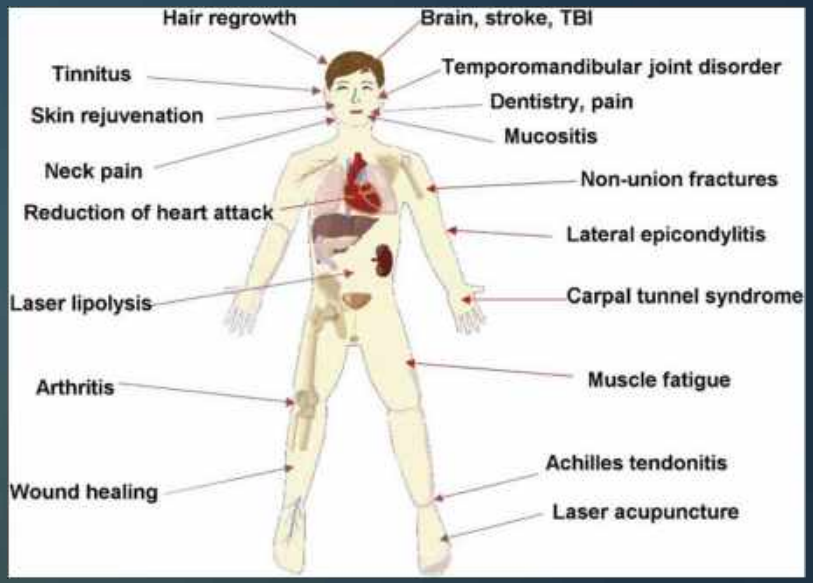



Endogenous molecules have different absorption peaks throughout the electromagnetic spectrum, resulting in different physiological effects:

- Melanin (~340nm)
- Unbound hemoglobin (~430nm)
- Water (~1900nm)
- Reduced mitochondrial Cytochrome C (~605nm)

# Medical Applications

- Altering human physiology with light is a potent therapeutic for numerous conditions
- Medical use of lasers particularly is known as “low-level laser therapy” (LLLT)
- The common “therapeutic range” of laser wavelengths for treating disease is from ~600nm to 1000nm. These wavelengths readily penetrate tissues while hitting disease-relevant biological targets.

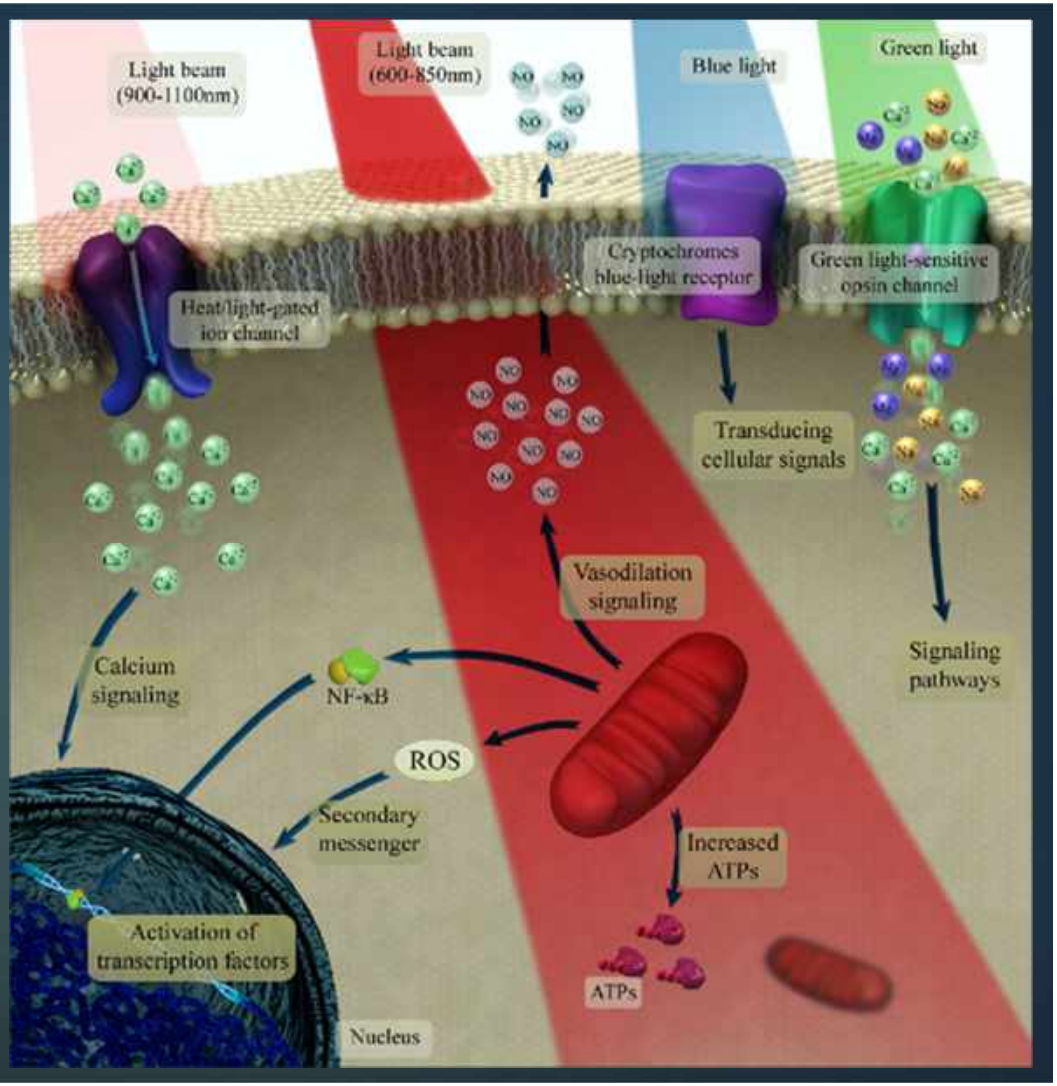



Ref: Hashmi JT, Huang YY, Osmani BZ, Sharma SK, Naeser MA, Hamblin MR. Role of low-level laser therapy in neurorehabilitation. PM R. 2010;2(12 Suppl 2):S292-S305. doi:10.1016/j.pmrj.2010.10.013

# Mechanisms at cellular and molecular levels: common understanding

- Increased ATP production
- Nitric oxide release
- Gene expression changes (NF-kB, VEGF)
- Normalization of membrane potentials via activation of light-sensitive ion channels
- Heat shock protein induction

Ref: de Freitas LF, Hamblin MR. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J Sel Top Quantum Electron*. 2016;22(3):7000417. doi:10.1109/JSTQE.2016.2561201



# Intercellular and Systemic Changes

- Angiogenesis and increased blood flow
- Reduced pain and inflammation
- Increased tissue growth
- Increased stem cell proliferation
- Reduced tissue atrophy (due to lower apoptosis rate)
- Improvement in motor function in skeletal muscle


Refs:

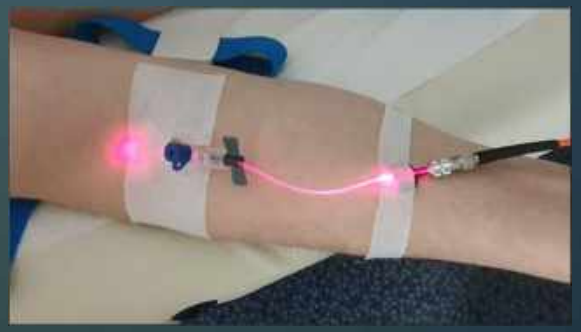
1. Ihsan FR. Low-level laser therapy accelerates collateral circulation and enhances microcirculation. Tlsxsq ih$Pewiv$ Wyvk. 2005;23(3):289-294.
1. Cotler HB, Chow RT, Hamblin MR, Carroll J. The Use of Low Level Laser Therapy (LLLT) For Musculoskeletal Pain. MOJ Orthop Rheumatol. 2015;2(5):00068.
1. Ferraresi C, Hamblin MR, Parizotto NA. Low-level laser (light) therapy (LLLT) on muscle tissue: performance, fatigue and repair benefited by the power of light. *Photonics Lasers Med*. 2012;1(4):267-286.
1. Hawkins D, Houreld N, Abrahamse H. Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. Annals of the New York Academy of Sciences. 2005 Nov;1056:486-493.
1. Ginani F, Soares DM, Barreto MP, Barboza CA. Effect of low-level laser therapy on mesenchymal stem cell proliferation: a systematic review. Lasers Med Sci. 2015;30(8):2189-2194.

# Common Delivery Methods

Hand-held lasers

LED panels/beds

Intracranial & intranasal

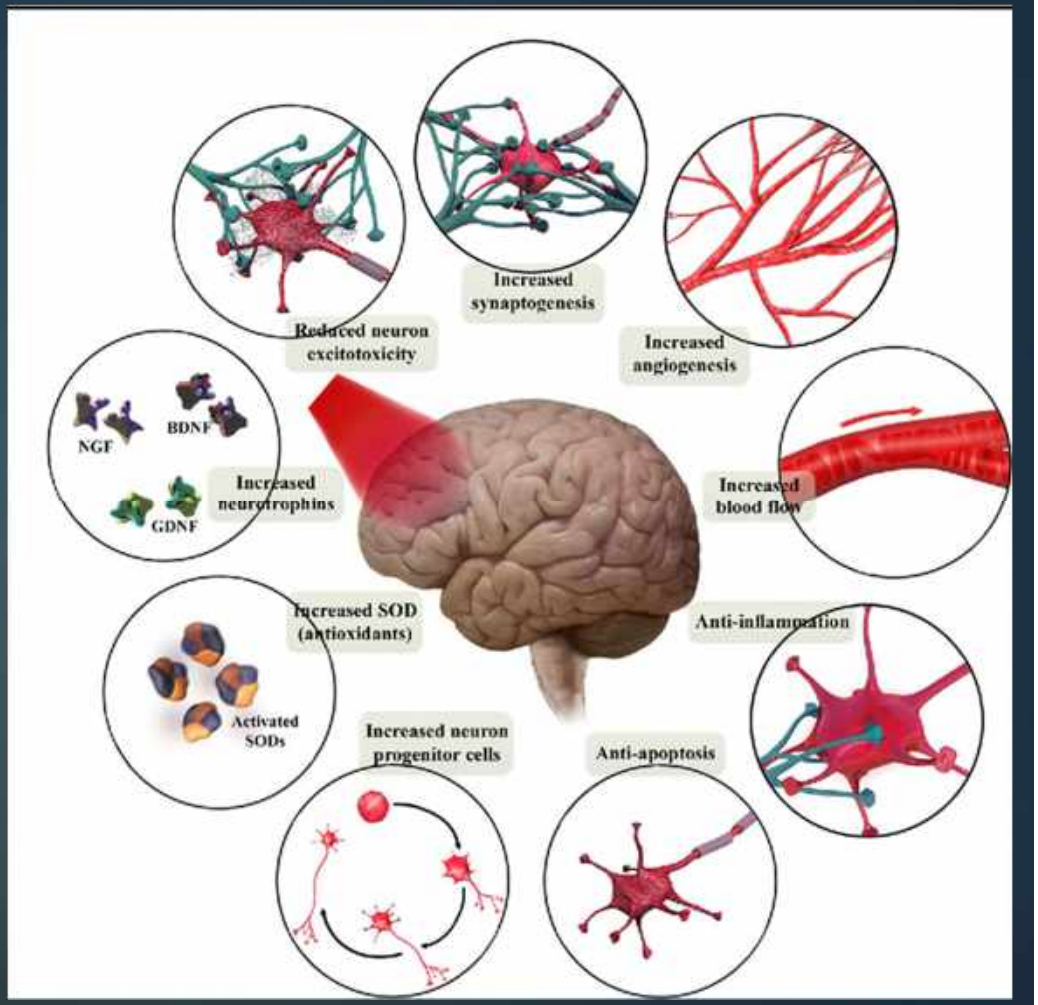


Intravenous

# What About the Brain?

Functional brain changes from photobiomodulation

It's not all about increased blood circulation




Ref: Salehpour F, Mahmoudi J, Kamari F, et al. Brain Photobiomodulation Therapy: a Narrative Review. Molecular Neurobiology 2017.

# Useful mechanisms for neurodegenerative diseases

- Improved mitochondrial metabolism: improved energy production will augment all other cellular processes
- Normalization of badly imbalanced cellular redox potentials
- Upregulated autophagy and antioxidants to clear harmful compounds
- Reduction in oxidative stress
- Nitric oxide release by mitochondria may help oxygenation of hypoxic tissues

Experimental evidence suggests that photobiomodulation can save damaged neurons under stress


Refs:

(1) Johri A, Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. J Pharmacol Exp Ther. 2012;342(3):619-630.

(2) Sharma SK, Kharkwal GB, Sajo M, et al. Dose response effects of 810 nm laser light on mouse primary cortical neurons. Lasers Surg Med. 2011;43(8):851-859.

(3) Liang HL, Whelan HT, Eells JT, et al. Photobiomodulation partially rescues visual cortical neurons from cyanide-induced apoptosis. Riyvswgmirgi. 2006;139(2):639-649.

(4) Hamblin MR. Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. Photochem Photobiol. 2018;94(2):199-212.

(5) Djavid GE, Bigdeli B, Goliaei B, Nikoofar A, Hamblin MR. Photobiomodulation leads to enhanced radiosensitivity through induction of apoptosis and autophagy in human cervical cancer cells. J Biophotonics. 2017;10(12):1732-1742.

# Saving Damaged Neurons

“Excitotoxicity contributes to brain damage after stroke, traumatic brain injury, and neurodegenerative diseases... We tested whether low level laser (light) therapy (LLLT) at 810-nm could protect primary murine cultured cortical neurons against excitotoxicity in vitro… LLLT (3 J/cm2 delivered at 25 mW/cm2 over 2 min) gave highly significant benefits in increasing ATP, raising mitochondrial membrane potential, reducing intracellular calcium concentrations, reducing oxidative stress and reducing nitric oxide.” (1)

Refs:
(1) Huang YY, Nagata K, Tedford CE, Hamblin MR. Low-level laser therapy (810 nm) protects primary cortical neurons against excitotoxicity in vitro. J Biophotonics. 2014;7(8):656-664.
(2) Huang YY, Nagata K, Tedford CE, McCarthy T, Hamblin MR. Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. J Biophotonics. 2013;6(10):829-838. doi:10.1002/jbio.201200157
(3) Meng C, He Z, Xing D. Low-level laser therapy rescues dendrite atrophy via upregulating BDNF expression: implications for Alzheimer's disease. J Neurosci. 2013;33(33):13505-13517.

# Publications Regarding Specific Diseases:

## Alzheimer’s, Dementia, Parkinson’s:


- Hamblin MR. Photobiomodulation for Alzheimer's Disease: Has the Light Dawned?. Photonics. 2019;6(3):77.
- Saltmarche AE, Naeser MA, Ho KF, Hamblin MR, Lim L. Significant Improvement in Cognition in Mild to Moderately Severe Dementia Cases Treated with Transcranial Plus Intranasal Photobiomodulation: Case Series Report. Photomed Laser Surg. 2017;35(8):432-441.
- Salehpour F, Hamblin MR. Photobiomodulation for Parkinson's Disease in Animal Models: A Systematic Review. Biomolecules. 2020;10(4):610. Published 2020 Apr 15.


## Traumatic Brain Injury & Stroke:


- Hamblin MR. Photobiomodulation for traumatic brain injury and stroke [published correction appears in J Neurosci Res. 2019 Mar;97(3):373]. J Neurosci Res. 2018;96(4):731-743.
- Huang YY, Gupta A, Vecchio D, et al. Transcranial low level laser (light) therapy for traumatic brain injury. J Biophotonics. 2012;5(11-12):827-837.

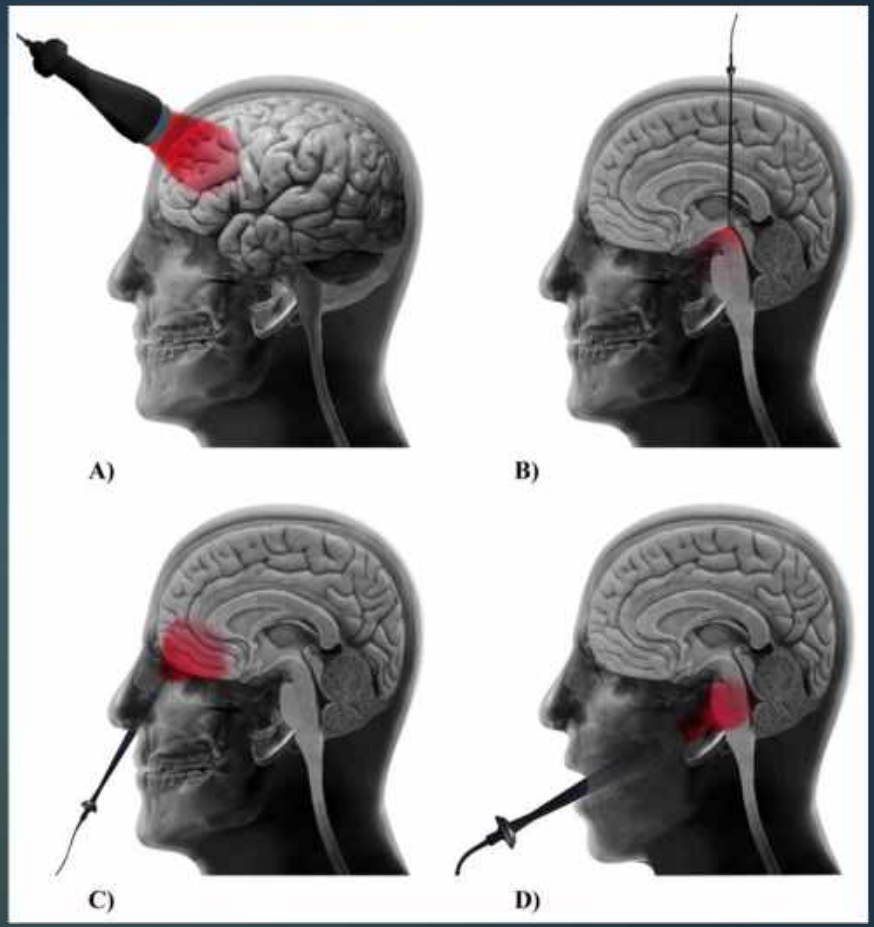

## Depression & Psychiatric Conditions:


- Cassano P, Petrie SR, Hamblin MR, Henderson TA, Iosifescu DV. Review of transcranial photobiomodulation for major depressive disorder: targeting brain metabolism, inflammation, oxidative stress, and neurogenesis. *Neurophotonics*. 2016;3(3):031404.
- Schiffer F, Johnston AL, Ravichandran C, et al. Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. *Behav Brain Funct*. 2009;5:46. Published 2009 Dec 8.

# Methods for Delivering Light to the Brain

Equipment used in several of the Boston University TBI studies (refs ibid)

(A) Transcranial
(B) intracranial
(C) intranasal
(D) brain photobiomodulation via oral cavity




Ref: Salehpour F, Mahmoudi J, Kamari F, Sadigh-Eteghad S, Rasta SH, Hamblin MR. Brain Photobiomodulation Therapy: a Narrative Review. Mol Neurobiol. 2018;55(8):6601-6636.

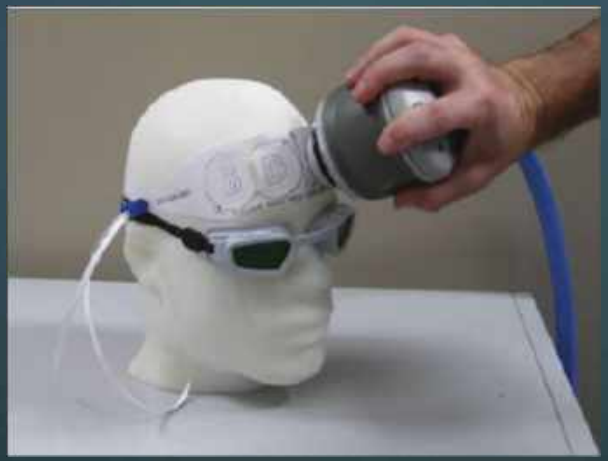
# Brain PBM Devices

## FDA Approved

VIELIGHT

## In Clinical Trials

NeuroThera
Transcranial Photobiomodulation



weber medical

# Cognitive Enhancement

LLLT has been investigated for cognitive enhancement due to its ability to increase oxygenation, blood flow, and energy production in the brain.

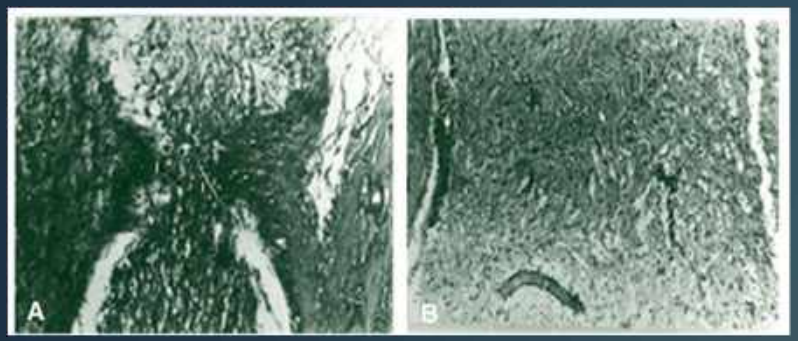
VGX$sj$xerwgvermep$PPPX - “Participants were tested for prefrontal measures of sustained attention with the psychomotor vigilance task (PVT) and working memory with the delayed match-to-sample task (DMS) before and after the treatments. As compared to CON, both LLLT and exercise reduced reaction time in the PVT [F(1.56) = 4.134, p = 0.01, η (2) = 0.181] and increased the number of correct responses in the DMS [F(1.56) = 4.690, p = 0.005, η (2) = 0.201], demonstrating a significant enhancing effect of LLLT and exercise on cognitive performance.” (3)

Refs:
(1) Gonzalez-Lima F, Barrett DW. Augmentation of cognitive brain functions with transcranial lasers. Front Syst Neurosci. 2014;8:36. Published 2014 Mar 14.
(2) Holmes E, Barrett DW, Saucedo CL, O'Connor P, Liu H, Gonzalez-Lima F. Cognitive Enhancement by Transcranial Photobiomodulation Is Associated With Cerebrovascular Oxygenation of the Prefrontal Cortex. Front Neurosci. 2019;13:1129. Published 2019 Oct 18.
(3) Hwang J, Castelli DM, Gonzalez-Lima F. Cognitive enhancement by transcranial laser stimulation and acute aerobic exercise. Lasers Med Sci. 2016;31(6):1151-1160.

# LLLT in Neurosurgery

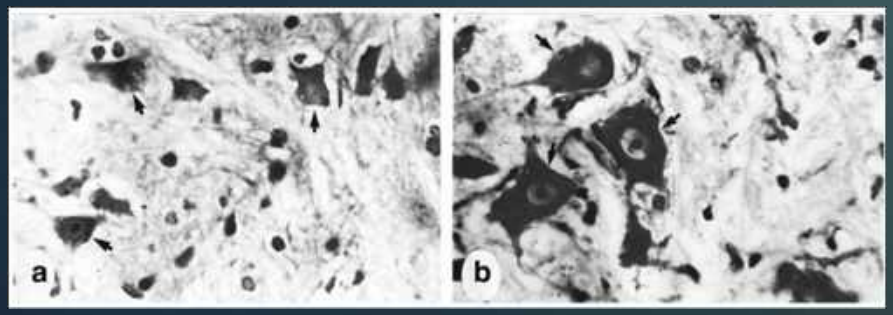
Ref: Rochkind S. Photobiomodulation in Neuroscience: A Summary of Personal Experience. Photomed Laser Surg. 2017;35(11):604-615.

## Crush injury model of peripheral nerve injury




"Histological section of the crush area of the rat sciatic nerve showing the response of the nerve to laser phototherapy. H&E stain, original magnification ·150.
(A) Non-irradiated nerve. Note the scar in the crush area.
(B) Laser-treated nerve shows no visible scar."

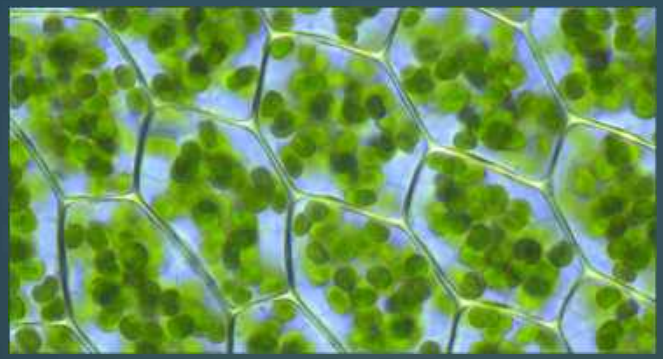
## Treatment of peripheral nerves saves motor neurons




"... Segments of a rat spinal cord 14 days after crush injury to the sciatic nerve, showing the spinal cord response to laser treatment of the injured peripheral nerve. (a) Section from a control animal shows extensive chromatolysis and cytoplasmic atrophy found in 40% of the motor neurons (arrows). (b) Section from a laser-treated animal shows minimal degenerative changes found in 20% of the motor neurons (arrows)"

# Photoactivation of Ingested Chromophores: The “Rainbow Effect”


Methylene Blue

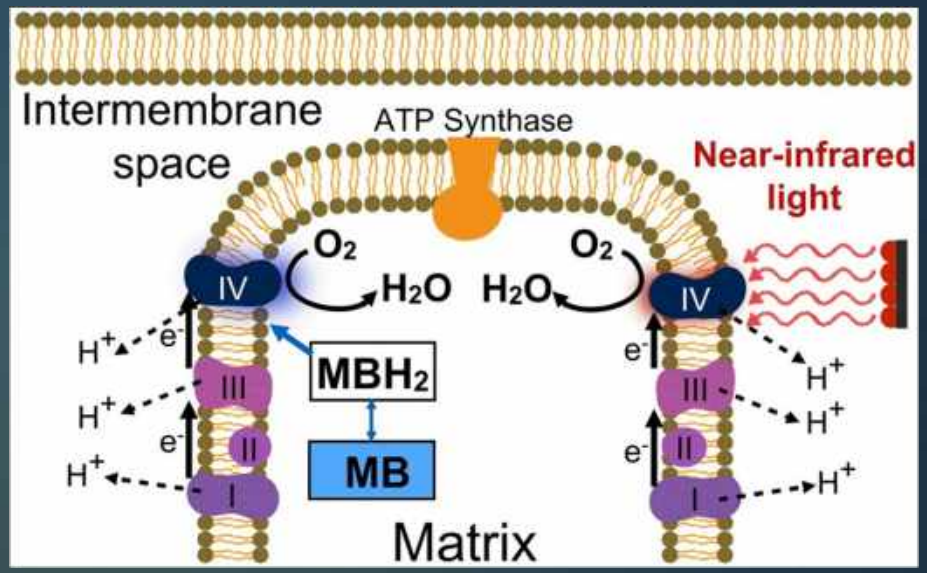


Chlorophyll

Riboflavin

# Methylene Blue

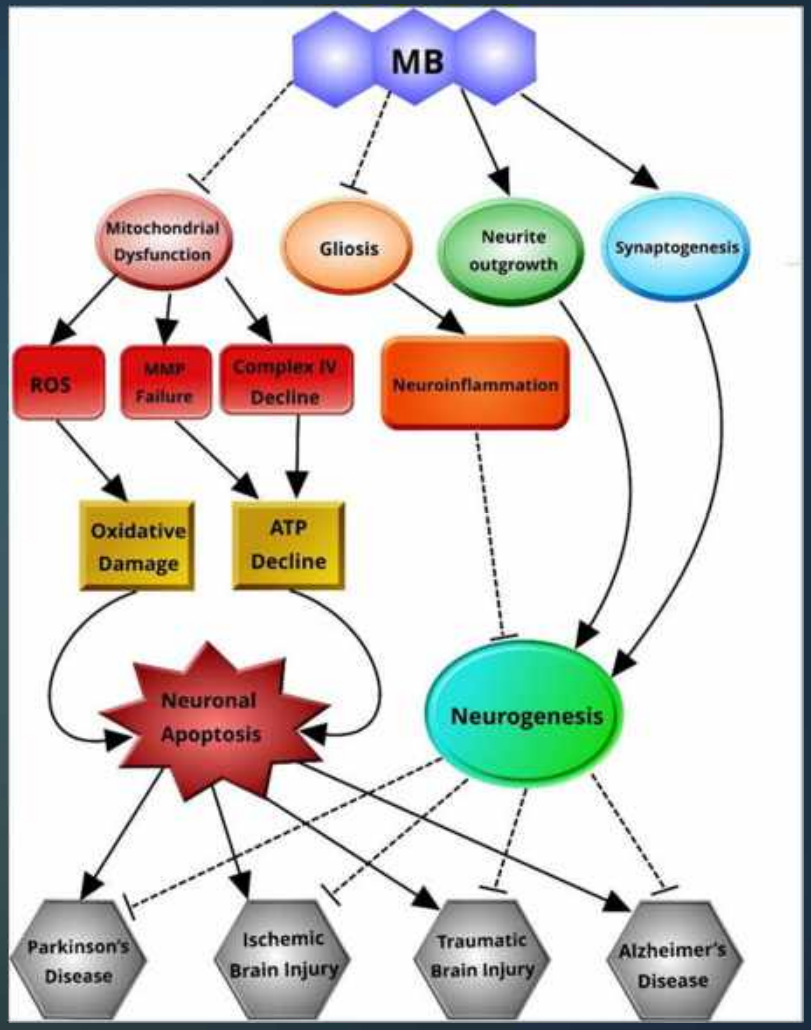
- Multi-use dye historically used for malaria and methemoglobinemia
- Acts as an electron donor for ETC Complex IV. Can absorb light like endogenous cytochrome oxidase to increase catalysis.
- Boosts oxygen consumption and ATP output




Ref: Gonzalez-Lima F, Auchter A. Protection against neurodegeneration with low-dose methylene blue and near-infrared light. Front Cell Neurosci. 2015;9:179. Published 2015 May 12.

# Methylene Blue for Neurodegeneration

- Can help combat impaired mitochondrial metabolism
- MB has a antioxidant effect
- Potent stimulator of neurogenesis via epigenetic changes
- Crosses the blood-brain barrier





Refs:
(1) Tucker D, Lu Y, Zhang Q. From Mitochondrial Function to Neuroprotection-an Emerging Role for Methylene Blue. *Mol Neurobiol*. 2018;55(6):5137-5153.
(2) Yang SH, Li W, Sumien N, Forster M, Simpkins JW, Liu R. Alternative mitochondrial electron transfer for the treatment of neurodegenerative diseases and cancers: Methylene blue connects the dots. Prog Neurobiol. 2017;157:273-291.

# Direct Inhibition of Protein Aggregation

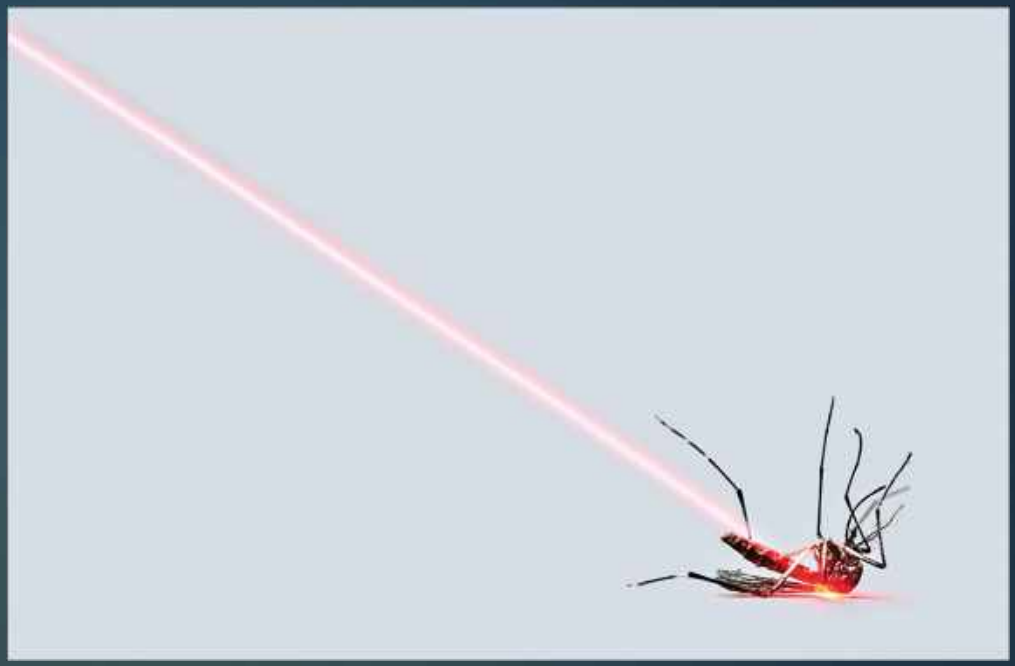
“MB inhibited recombinant protein aggregation in vitro, even when added to preformed oligomers and fibrils… In functional assays, MB increased survival of primary cortical neurons transduced with mutant Htt, reduced neurodegeneration and aggregation in a Drosophila melanogaster model of HD” (1)

“...dietary MB treatment reduces Aβ levels and improves learning and memory deficits in the 3xTg-AD mice. The mechanisms underlying the effects of MB on Aβ pathology appears to be mediated by an increase in Aβ clearance as we show that MB increases the chymotrypsin-and trypsin-like activities of the proteasome in the brain.” (2)

Refs:
(1) Sontag EM, Lotz GP, Agrawal N, et al. Methylene blue modulates huntingtin aggregation intermediates and is protective in Huntington's disease models. J Neurosci. 2012;32(32):11109-11119.
(2) Medina DX, Caccamo A, Oddo S. Methylene blue reduces aβ levels and rescues early cognitive deficit by increasing proteasome activity. *Brain Pathol*. 2011;21(2):140-149. doi:10.1111/j.1750-3639.2010.00430.x
(3) Yamashita M, Nonaka T, Arai T, et al. Methylene blue and dimebon inhibit aggregation of TDP-43 in cellular models. FEBS Lett. 2009;583(14):2419-2424.

Could light also help kill the bugs contributing to neurodegeneration?

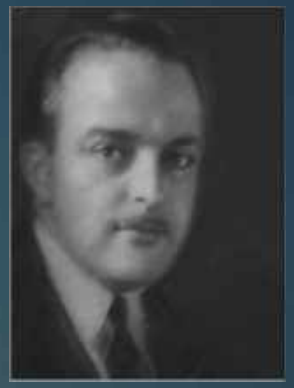
Yes!



# UV Blood Irradiation - “The Cure that Time Forgot”

“Ultraviolet blood irradiation (UBI) was extensively used in the 1940s and 1950s to treat many diseases including septicemia, pneumonia, tuberculosis, arthritis, asthma and even poliomyelitis. The early studies were carried out by several physicians in USA and published in the American Journal of Surgery. However with the development of antibiotics, UBI use declined and it has now been called “the cure that time forgot”

- Treatment involves circulating blood under UV lamps outside the body
- Early experiments suggest irradiating 5-7% of blood volume is optimal



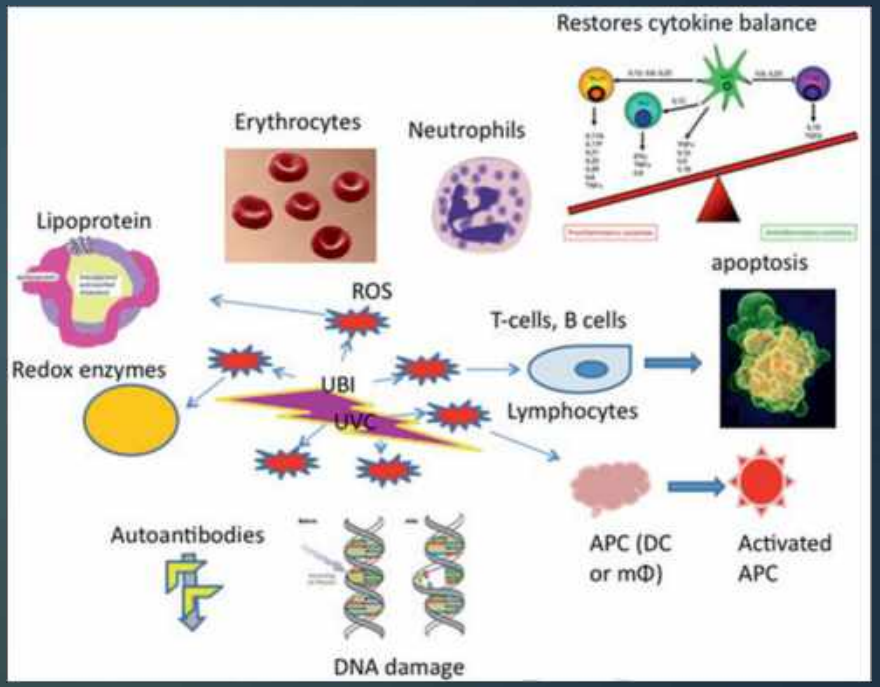
Early UBI pioneer Dr. Emett Knott and his “hemo-irradiator”

A contemporary machine for UV apheresis

Refs: Hamblin MR. Ultraviolet Irradiation of Blood: "The Cure That Time Forgot"?. Adv Exp Med Biol. 2017;996:295-309. doi:10.1007/978-3-319-56017-5_25

# Mechanism of UV Blood Irradiation (UBI)

- Contrary to intuition, there is little evidence that UBI kills pathogens directly
- Results are thought to come from effects on the immune system:
  - Increased phagocytosis
  - Increases secretion of NO and reactive nitrogen species
  - Deactivation of circulating lymphocytes may reduce inflammation
  - Transient increase in ROS activates antioxidant defenses

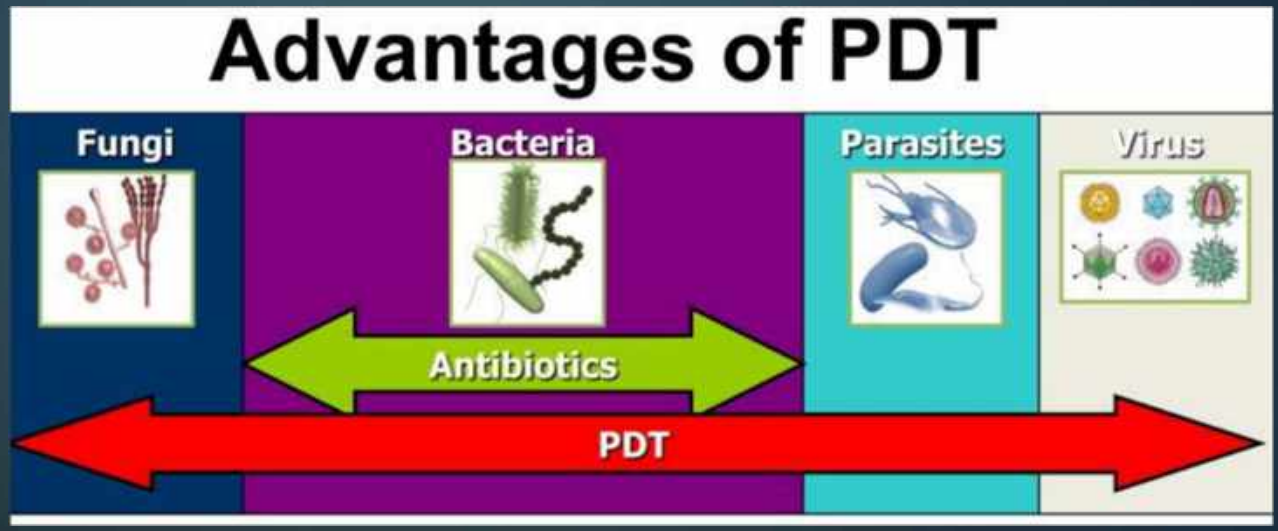


Is that the *best* light therapy can do?

# Antimicrobial Photodynamic Therapy (aPDT)

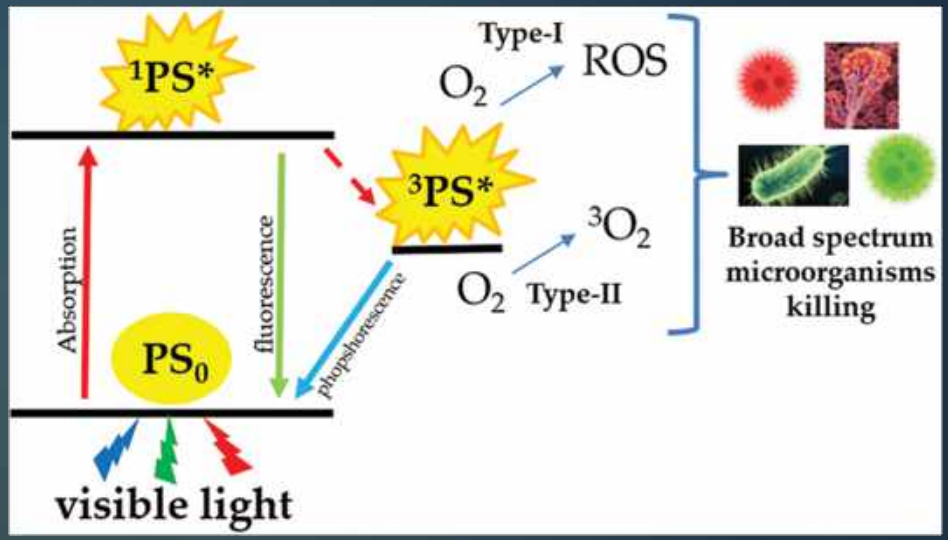
**Photodynamic therapy** is a therapeutic approach that can kill microorganism by combining light (of a specific wavelength), a light-sensitive substance (photosensitizer) and oxygen

- Effective against drug-resistant pathogens
- Few side effects
- Cost efficient



# aPDT: Mechanism of Action

1. Photosensitizer binds to microbes
2. “Light Activation”: Photosensitizer absorbs photons and is excited to highly reactive state
3. Reaction with ambient oxygen to generate reactive oxygen species
4. ROS causes irreparable damage to microbe DNA and cell walls -> microbial death




Ref: Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease?. Photochem Photobiol Sci. 2004;3(5):436-450.

# aPDT: Overview of Photosensitizers

**Desirable properties:**

- Selectivity for microbial cells over host mammalian cells
- Low toxicity
- Good quantum yields of ROS (particularly singlet oxygen)
- Kills a broad range of pathogens

**Common Photosensitizers:**

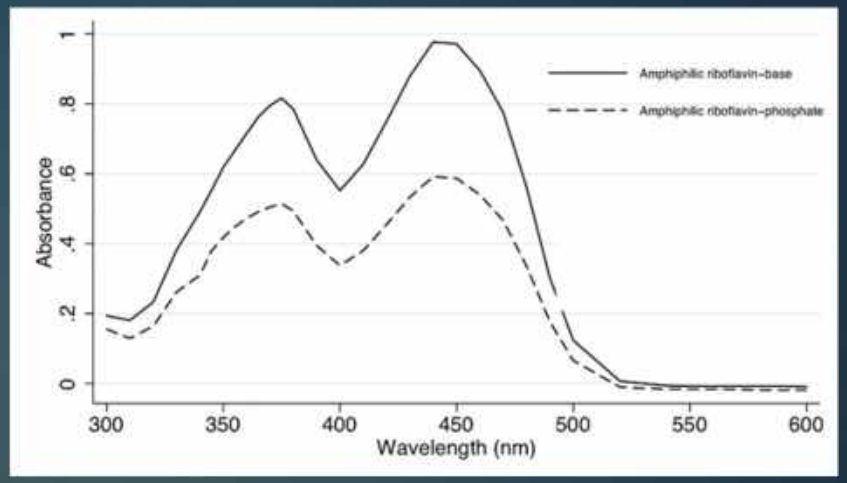
- Riboflavin
- Methylene blue
- Porphyrins
- Curcumin
- Chlorins
- Hypericin

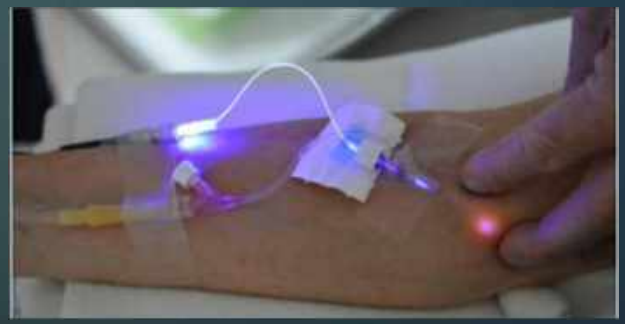
Ref: Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. *Biochem J*. 2016;473(4):347-364.

# Example: Riboflavin (vitamin B2)

- Activated by blue (450nm) and UV light (370nm)
- Extremely low toxicity
- Can be administered through intravenous application
- Effective against viruses including: malaria, HIV
- Currently being explored for COVID-19

Absorption spectrum of riboflavin



Ref: Yin R, Dai T, Avci P, et al. Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. Curr Opin Pharmacol. 2013;13(5):731-762.

# In Conclusion

- Light therapies have broad application to neuropsychiatric and neurodegenerative diseases
- LLLT has strong regenerative effects:
  - Improving neuronal metabolism
  - Saving neurons under stress
- Research has shown the potential efficacy of LLLT for: Alzheimer's, dementia, TBI, Parkinson's, mood disorders, cognitive enhancement, peripheral nerve injury, and more!
- Ultraviolet blood irradiation and antimicrobial photodynamic therapy may be useful tools to deal with enteropathogens causing CNS diseases
- Light absorbing porphyrins may prove to be promising pharmaceutical agents for reducing the effects of protein aggregation

## SPECIAL THANKS TO THE FOLLOWING


The “light project” team:

Neil Olson

Tom Radcliff

Jim Radcliff