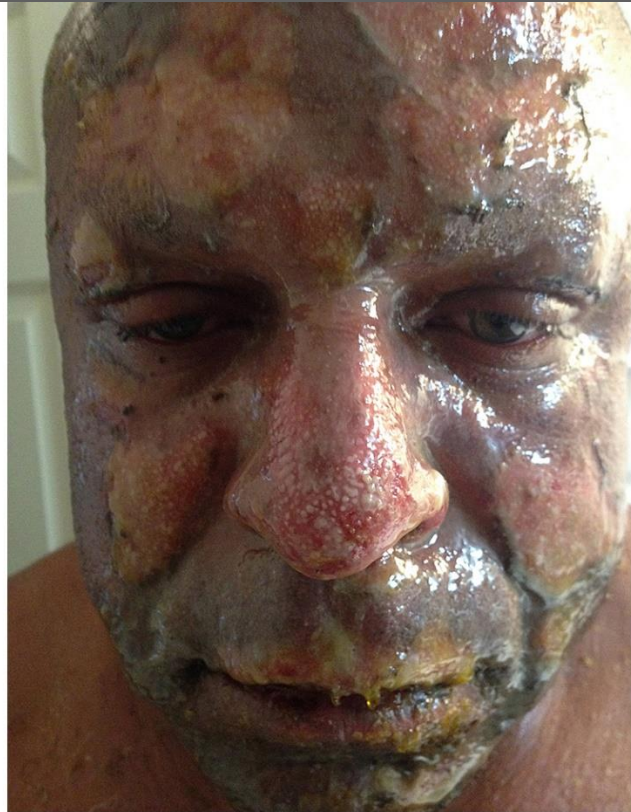


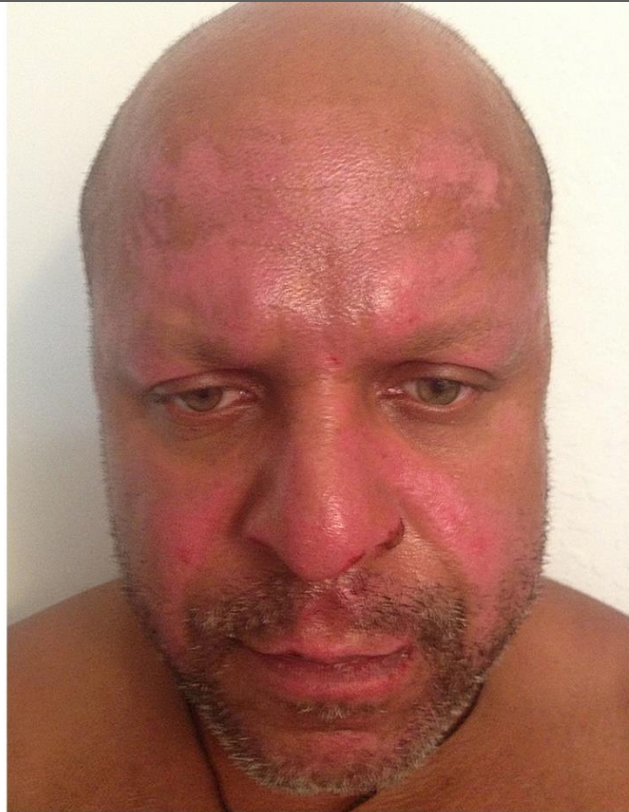
THERAPEUTIC APPLICATIONS OF MSC EXOSOMES

Douglas J. Spiel, MD

TOPICAL EXOSOMES



Day 0



Day 7



Day 60

MSC Exosomes

Arnold Caplan:

- 2006 – “The trophic effects of MSCs may have profound clinical use.”¹
- 2017 – “I now urge that we change the name of MSCs to Medicinal Signaling Cells ...”
- “It is, indeed, the patient’s own site-specific and tissue specific resident stem cells that construct the new tissue as stimulated by the bioactive factors secreted by the exogenously supplied MSCs”²

Revisionist Viewpoint!

- 1917 – Julius Wagner-Jauregg
 - Patients with dementia paralytica after febrile illness frequently become lucid, sane. He began treating dementia paralytica patients with malaria for 8-12 febrile paroxysms then rescued with Quinine Sulfate and they became lucid.
- 1927 – Nobel Prize in Medicine awarded to Julius Wagner-Jauregg for the healing properties of malarial fever
- 1928 – Alexander Fleming discovers Penicillin
- 1938 – Dr. Howard Florey reads Fleming's paper. He is joined by Dr. Ernst Chain and Dr. Norman Heatley
- 1942 – Mass production of Penicillin
 - WW I death rate from pneumonia: 18%
 - WW II death rate from pneumonia: <1%

TOMORROW'S REGENERATIVE MEDICINE TEXTBOOKS

- Bone Marrow Harvest MSCs

- Adipose Harvest MSCs



Malaria

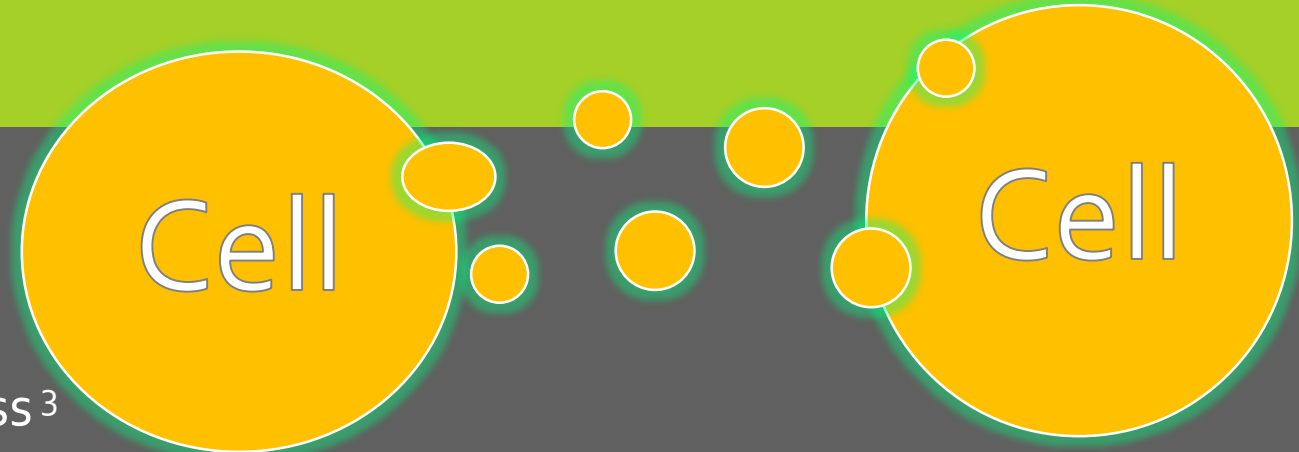
- Exosomes



Penicillin

WHAT ARE MSC EXOSOMES?

- Vesicles from 40-100 nm
- Formed by a two-step budding process³
 - Inward budding of membranous vesicles in a multivesicular-body
 - MVBs fuse with the plasma membrane to release their cargo
 - **Transmembrane proteins are conserved!**
- Composed of lipids, proteins, mRNA, and miRNAs



I SAID TRANSMEMBRANE PROTEINS ARE CONSERVED FROM MSCS. WHY?

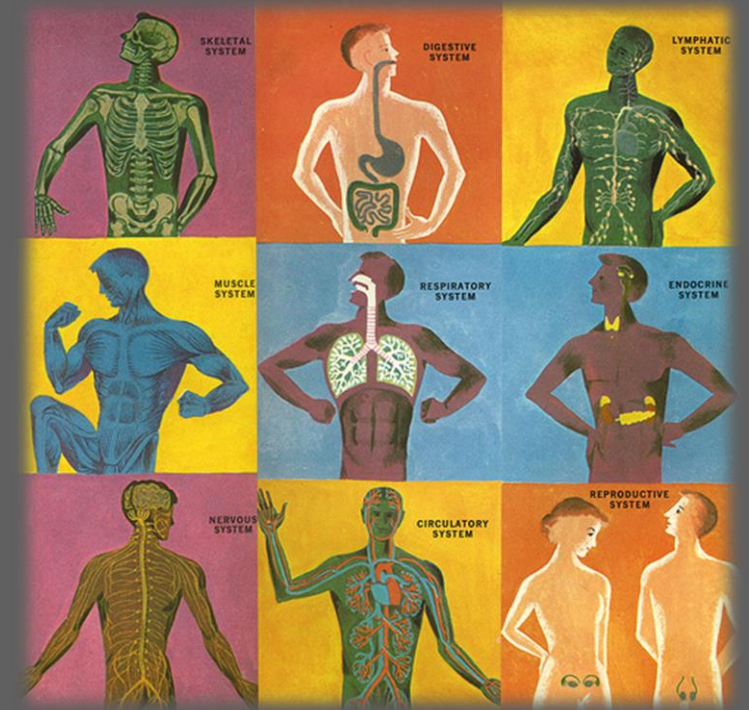
- MSCs are recruited at the site of injury by receptor-mediated interaction
- MSC-derived vesicles bear the same membrane receptors of MSCs and it is likely for this reason that they accumulate at the site of injury by the same mechanism ^{4,5}
- MSC exosomes via IV infusion were taken up by M2 Macrophages in spinal cord of injured rats ⁸

WHAT TYPES OF MEDICAL CONDITIONS MIGHT BE AIDED BY MSC EXOSOMES?

- Musculoskeletal – Joints, Discs, Muscles, Bones, Ligaments, Tendons
- Autoimmune – SLE (Lupus), RA, Dermatomyositis
- Neurodegenerative – Multiple Sclerosis, Alzheimers, Parkinsons, Spinal Cord Injuries
- Burns, Scars, Ulcers
- Heart Disease – MI, CHF
- Lung Disease – COPD, Pulmonary Fibrosis, Interstitial Lung Disease
- Liver Disease
- Kidney Disease
- Inflammatory Bowel Disease – Ulcerative Colitis, Crohn's Disease

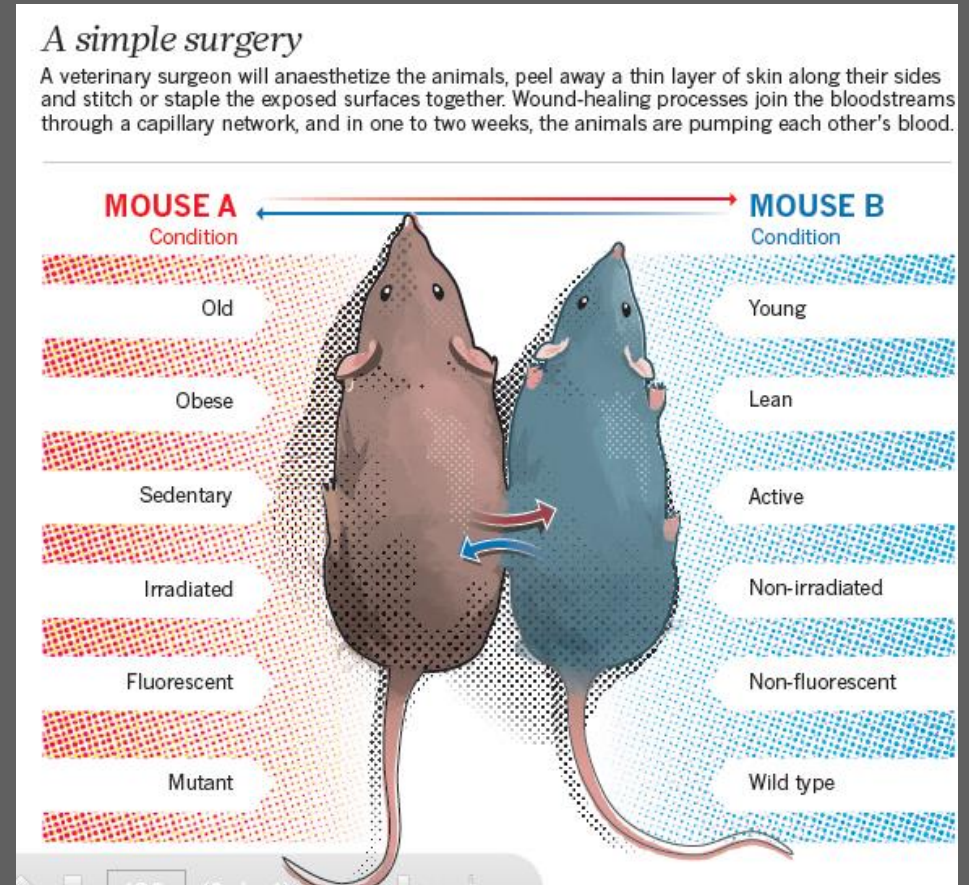
WHAT TYPES OF MEDICAL CONDITIONS MIGHT BE AIDED BY MSC EXOSOMES?(CONTINUED)

- Cerebral Palsy, Autism
- Neuropathy, CIDP
- Erectile Dysfunction
- Anorgasmia
- Urinary Incontinence
- Complex Regional Pain Syndrome
- Chronic Pelvic Pain
- Lyme Disease (Upregulate Macrophages)
- Numerous Aesthetic Applications



WHAT CAN SCIENCE TEACH US REGARDING AGING?

By splicing the blood circulation of 2 animals together we have shown that young blood rejuvenates old tissues, bones, muscles, brain, and nerve tissue.



Nik Spencer/Nature; Chart Data: A. Eggel & T. Wyss-Coray Swiss Med. Wkly 144, W13914 (2014) [Photograph]. Retrieved from <https://www.nature.com/news/ageing-research-blood-to-blood-1.16762>

WHAT CAN SCIENCE TEACH US REGARDING AGING?

A Hong Kong billionaire backed a scientist from Stanford (Wyss-Coray) to start Alkahest, Inc, in Menlo Park, California on 2014.

To Treat Alzheimers they are sharing plasma from adults < 30 and transfusing it into Alzheimers patients.

WHAT CAN SCIENCE TEACH US REGARDING AGING?

A second company, Ambrosia has started a similar trial in California, giving adults age 35+ transfusions from younger adults <25.

This one-time infusion of a 2L bag of plasma (no blood cells) is offered for \$8,000.

WHY DOES PLASMA TRANSFER WORK?

Are we transfusing stem cells?

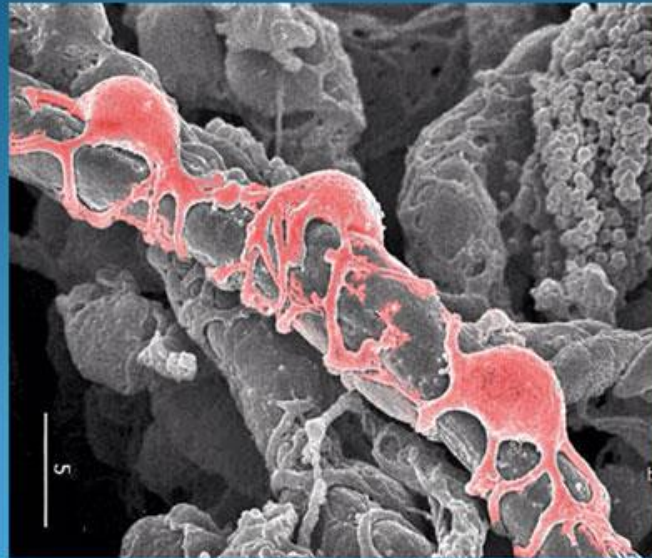
No. Simply, there are not enough stem cells in blood/plasma. In fact, factors present in the plasma seem to upregulate the older stem cells themselves.

WHAT MIGHT THESE FACTORS BE?

EXOSOMES

Pericytes: cells on capillaries and micro vessels.

ALL MSCs are PERICYTES!



modified by
BRUNO PFAULT from <http://www.geocities.co.jp/HeartLand-Suzuran/3389/kekka>

Mesenchymal Stem Cells

[Photograph] Retrieved from <http://stemcellorthopedic.com/mesenchymal-stem-cells/>

TRANSLATIONAL STUDIES EMPLOYING MSC-DERIVED EXOSOMES

- **TARGET TISSUES**

- Heart
- Brain
- Liver
- Lung
- Intestine
- Skin/Wound
- Limb Ischemia
- Skeletal Muscles
- Spinal Cord
- Immune System

These studies demonstrate that MSC-derived exosomes recapitulate the broad therapeutic effects previously attributed to MSCs via horizontal transfer of mRNAs, miRNAs, and proteins⁶

KEY ADVANTAGES OF EXOSOMES:

1. Can travel via systemic therapy without risk of clumping
2. Can travel via local therapy
3. Cross the "Blood-Brain Barrier"
4. Deliver miRNA and mRNA
5. Can home
6. Not perceived as foreign
7. No first-pass lung removal as in MSCs
8. Can not transdifferentiate into other cells or into malignant cells
9. Easy to administer, store, freeze (Out of the box stem cell therapy!)
10. Easily controlled dosage
11. Potency related to age of parent MSC

KEY THERAPEUTIC EFFECTS OF MSC EXOSOMES

1. Influence Growth of Target Cells
2. Influence Phenotype
3. Contribute to Cell Fate Decision
4. Promote Regeneration
5. Immunomodulation
6. Anti-Inflammatory
7. Anti-Fibrotic

SOME KEY IMMUNE AND GROWTH FACTORS PRESENT IN MSC EXOSOMES

- BMP-5 Stimulates bone growth
- GDF-15 Growth Differentiation Factor-15
- OPG Stimulates bone growth/blocks osteoclast precursor formation
- G-CSF Granulocyte Colony Stimulating Factor
- SCF Stem Cell Factor shown to be responsible for stem cell and melanocyte growth
- TGF- β_3 The most important anti-inflammatory protein. Converts inflammatory T-Cells to anti-inflammatory regulatory T cells.
- VEGF Vascular Endothelial Growth Factor
- ICAM-1 Binds inflammatory ligands on white cells

SOME KEY IMMUNE AND GROWTH FACTORS PRESENT IN MSC EXOSOMES

- IL-1 α Binds and sequesters the inflammatory cytokine IL-1
- IL-6 Responsible for macrophage activation
- IL-10 An anti-inflammatory cytokine responsible for immunomodulation and regulatory T cell conversion
- MCP-1 Monocyte Chemoattractant Protein-1 is a chemokine that recruits mononuclear cells to the treatment area
- MIP-1 Also known as CCL-4, this chemokine recruits mononuclear cells to the treatment area
- PDGF- $\beta\beta$ Platelet Derived Growth Factor Beta

SOME KEY IMMUNE AND GROWTH FACTORS PRESENT IN MSC EXOSOMES

- TIMP-1
 - TIMP-2
 - HGF
 - GDNF
 - BDNF
 - FGF
 - TNF-RI
- Tissue Inhibitor of Matrix Metalloproteinases, blocks collagen and extracellular matrix degradation. Important for cartilage repair
- Hepatocyte Growth Factor
- Glial-Derived Neurotrophic Factor
- Brain-Derived Neurotrophic Factor
- Fibroblast Growth Factor
- Binds and inactivates the inflammatory TNF- α

CONTRAINDICATIONS TO STEM CELLS/EXOSOMES

- Cancer
- Myeloproliferative Disease
 - Bone Marrow Dysplasia
 - Sickle Cell
- Primary Pulmonary Hypertension
- Acute Bacterial Infection
- Recent Dental Work
- Macular Degeneration with neovascularization
- Any abnormal neovascularization
- Immuno-compromised

PREOPERATIVE LABS

- CBC
- CMP
- UA
- CA-125 for females
- PSA for males over 40
- CEA for males and females over 40
- PT/INR
- EKG

IMPORTANT IMMUNOLOGICAL EFFECTS OF MSC EXOSOMES

- Downregulate TH1 and TH17 cells while upregulating T_{Reg} cells
- Upregulate M2 macrophages at expense of M1 Macrophages

AESTHETIC PROTOCOLS

- Hair
PRP + Exosomes
- Ulcers/Wounds/Scars/Venous Insufficiency Ulcers
Debride, Exosomes along periphery of wound and into wound base +/-
Tisseel covering
- Erectile Dysfunction
Exosomes +/- PRP
- Urinary Incontinence
PRP + Exosomes
- Arterial Insufficiency
Exosomes

Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodelling

Lu Wang, Li Hu, Xin Zhou, Zehuan Xiong, Chenguang Zhang, Hassan M. A. Shehada, Bo Hu, Jinlin Song ✉ & Lili Chen ✉

- 100 Million people annually suffer pain and discomfort caused by scarring.
- Scar is a complex process involving inflammatory, proliferative , and remodeling phases
- Fetuses retain the ability to heal regeneratively without scars
- Scarless wound tissue is marked by fine reticular collagen, less cross-linking, less inflammation, and fewer fibroblasts.
- The remodeling of extracellular matrix is one of the most important factors for scar formation.

ADIPOSE MSC EXOSOMES

- Increase ratio of Collagen III to Collagen I
- Prevent fibroblast differentiation into myofibroblasts
- Inhibit granulation tissue formation
- Increase ratio of TGF- β_3 to TGF- β_1
- After IV administration accumulate in wound area
- Increase ratio of MMP₃ to TIMP₁

MSK PROTOCOLS

- Joints
 - PRP + Exosomes
 - PRP + Exosomes

Day 0
Day 14
- Muscles
 - Direction Injection Exosomes
- Tendons/Ligaments
 - PRP + Exosomes (+ Tenotomy for tendons)
- Bones
 - BMAC + Exosomes +/- DBM vs. other scaffold
- Nerves
 - Exosomes +/- PRP
- Discs
 - Exosomes + PRP
 - Exosomes + PRP

Day 0
Day 14

MECHANISMS OF MSC TROPHIC ACTIVITY WITHIN THE JOINT SPACE

- Trophic factors influence anabolic tendencies of:
 - chondrocytes
 - chondrocyte progenitor cells (CPCs)
 - cartilage-derived stem/progenitor cells (CSPCs)
 - synovium-resident multipotent progenitor cells
 - osteoblasts/osteoclasts/resident MSCs within the subchondral bone
 - chondrogenic cells within the infrapatellar fat pad

MSC TROPIC ACTIVITIES RELEVANT TO MSK THERAPY

- Angiogenesis
- Neurogenesis
- Osteogenesis
- Musculogenesis

Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis

Stella Cosenza, Maxime Ruiz, Karine Toupet, Christian Jorgensen & Danièle Noël 

Microparticles and exosomes exerted similar chondroprotective and anti-inflammatory function *in vitro* and protected mice from developing osteoarthritis *in vivo*.

AUTO-IMMUNE DISEASE

Rheumatoid Arthritis, Psoriasis, Thyroid, Ulcerative Collitis/Crohn's, Autoimmune Encephalitis, MS, etc.

VITILIGO

Review Article

Trends in Regenerative Medicine: Repigmentation in Vitiligo Through Melanocyte Stem Cell Mobilization

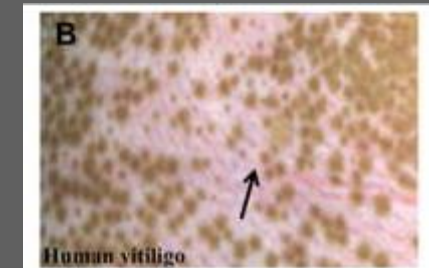
Stanca A. Birlea✉, Gertrude-E. Costin, Dennis R. Roop, David A. Norris✉

First published: 28 December 2016 | <https://doi.org/10.1002/med.21426> | Cited by:1

- Vitiligo most frequent human pigmentary disorder characterized by progressive autoimmune destruction of mature epidermal melanocytes.
- Affects 0.3-0.5% of the population.
- Melanocytes are generally completely affected in the epidermis but melanocyte precursors persist in the hair follicle bulb.
 - Therefore these precursors are essentially immune privileged!
 - Depigmented areas where hair follicles are absent (palms, soles, mucous membranes) are more refractory to treatment.

CLINICAL PATTERNS OF REPIGMENTATION

- Peritolicular
 - Most Prevalent
 - Indicates hair follicle is main source
- Marginal
 - Suggests functional epidermal melanocytes at lesional borders
- Diffuse
 - Generalized darkening across the patches from dermal/epidermal melanocyte precursors that persist
- Combined
 - Includes more than one pattern



Berlea SA. *Figure 1.*
Medicinal research
reviews; 2017.



4 Weeks after IV MSC Exosomes



Vitiligo before and 3 weeks after IV MSC Exosomes

ROSACEA



Day 0

Intradermal
Injection MSC
Exosomes



Day 2

LUMBAR DISC DISEASE

WHEN TO CHOOSE SURGERY AND WHEN TO CHOOSE REGENERATIVE MEDICINE

Low back pain annual cost is US \$500 Billion

Will affect 75-80% of population

Prevalence 15-45%

DISC ANATOMY – INTERVERTEBRAL DISC

- Fibrocartilaginous articulation between adjacent vertebrae composed of:
 - Nucleus Pulposus (NP)
 - Annulus Fibrosis (AF)
 - Hyaline Cartilage Endplates

NUCLEUS PULPOSUS

- Proteoglycans – Glycosaminoglycans (GAG), Aggrecan
- Type II Collagen
- No Nerve Fibers
- Immune Privileged
- Cells:
 - Chondrocyte like
 - Notochordal Cells
 - Early “Organizers”
 - Stem Cell Progenitor Cells
 - 1-3% in youth, declines with age

ANNULUS FIBROSIS

- Type I Collagen predominates
- Elastin Fibers
- Concentrically arranged lamellae
- Cells mostly fibroblastic-like
- Nerve Fibers (Free unmyelinated nerve endings) in external 1/3

HYALINE CARTILAGE ENDPLATES

- Thin – Thinnest at midpoint
- Porous
- Allows for “Imbibition”

WHY DO DISCS HURT? AUTOIMMUNE

- Nuclear Tissue – Immune Privileged
 - Similar to lens of the eye
 - Prior to immune system being fully functional, the complex proteins of the nucleus are sequestered away from immunosurveillance
 - Annular tears, disc herniations, and endplate fractures subject these proteins to the circulating immune system
 - Inflammation ensues – Metalloproteinases (enzymes) begin to digest disc. Vicious Cycle

WHY DO DISCS HURT? CHEMICAL NOCICEPTION

- Phospholipase A2 (PLA2) – Enzyme.
 - Exponentially higher after disc herniations
 - Converts arachidonic acid into proinflammatory mediators such as leukotrienes
- Local Nociceptors
 - Outer 1/3 of annulus
 - Anterior dura
 - VB Periosteum
 - Nerve Root sheath
 - Dorsal Root Ganglion

WHY DO DISCS HURT? MECHANICAL COMPRESSION

- Disc herniations can directly compress both traversing and exiting nerves
- Some believe this requires a “Double Crush”

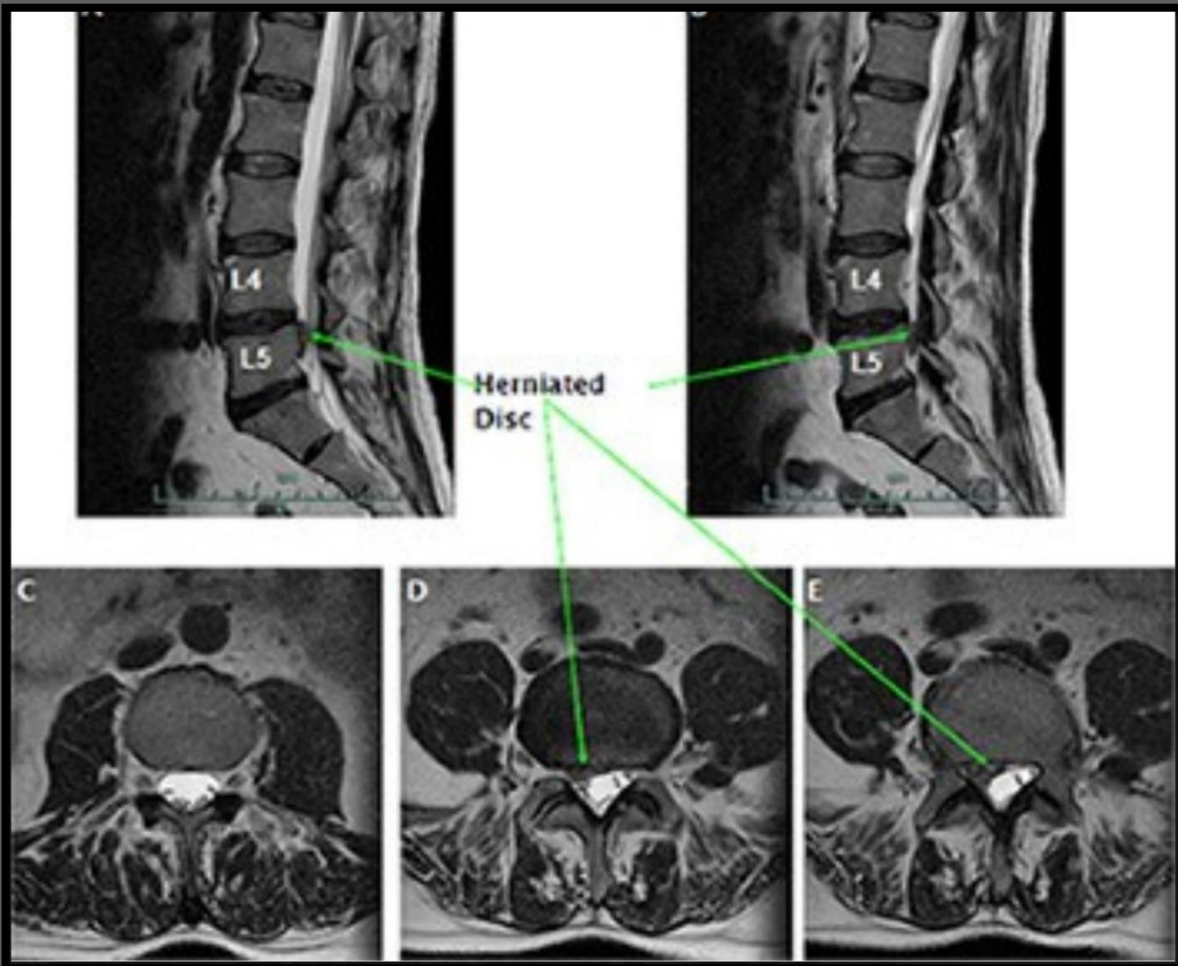
MORPHOLOGICAL HALLMARKS OF PAINFUL DISCS

- Reduced Disc Height
- Lack of distinction of annulus and nucleus
- Decrease T2 signal of nucleus
- **Disc Herniations/Extrusions/Sequestrations**
- **Annular Tear**
- **Modic Changes**
- **Schmorl's Nodes (Acute)**
 - Scheuermann's Disease

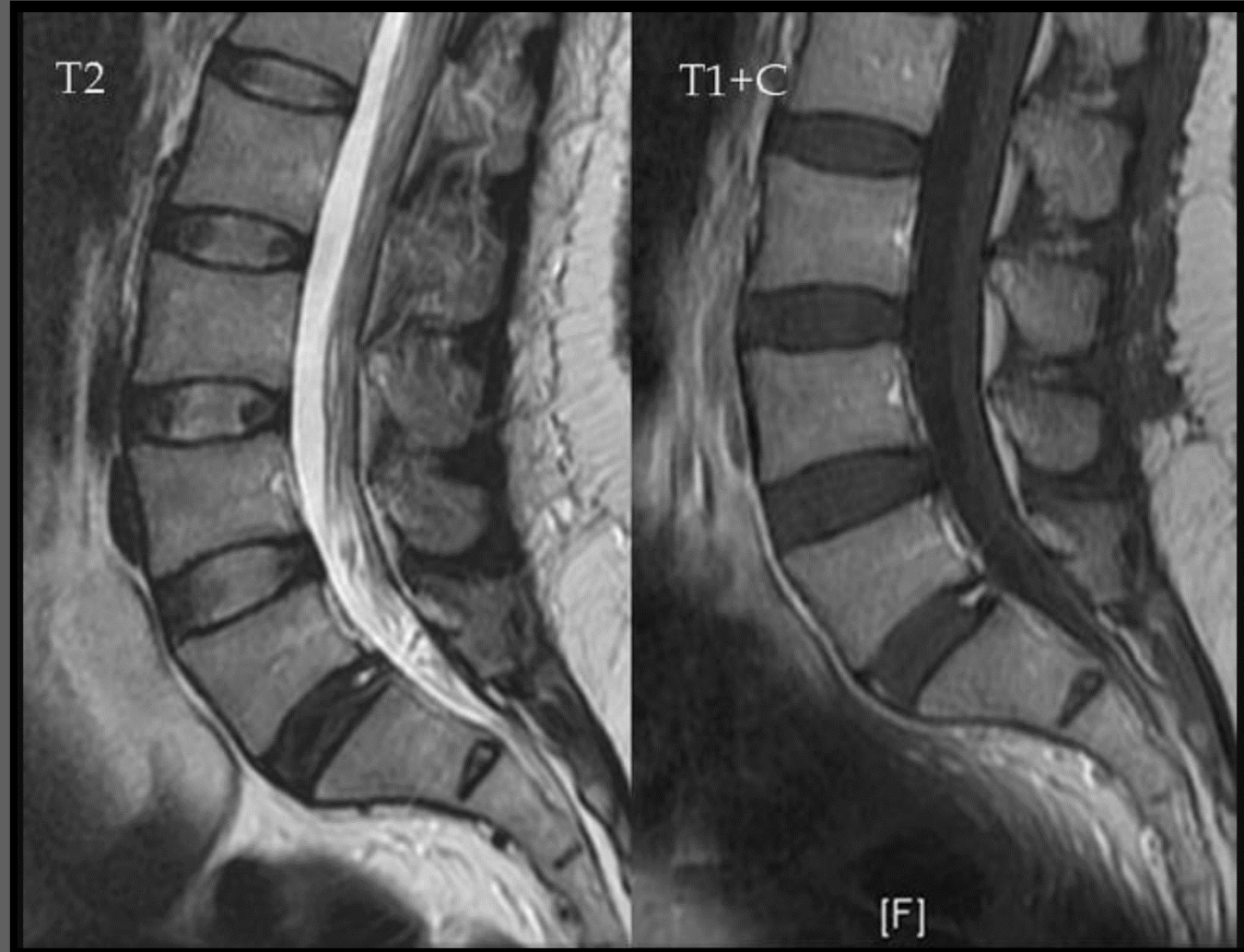


STRONGLY ASSOCIATED

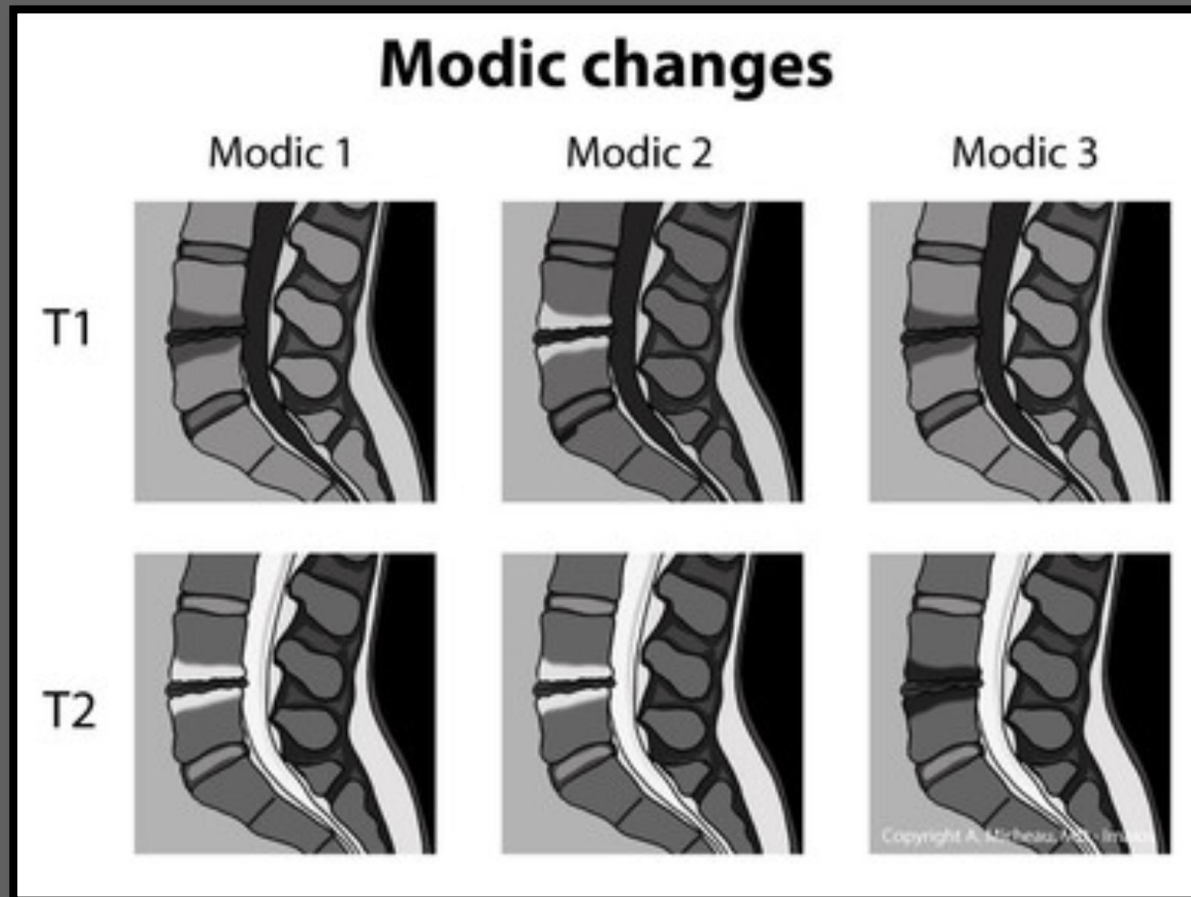
DISC HERNIATION/EXTRUSION/SEQUESTRATION



ANNULAR TEAR



MODIC CHANGES



MODIC CHANGES TYPE I

- T₁ Low
- T₂ High
- T₁ + Contrast shows enhancement
- Represents BM Edema, inflammation, infection



Modic Changes Type II

- T1 High
- T2 Iso to High
 - Represents fatty degeneration



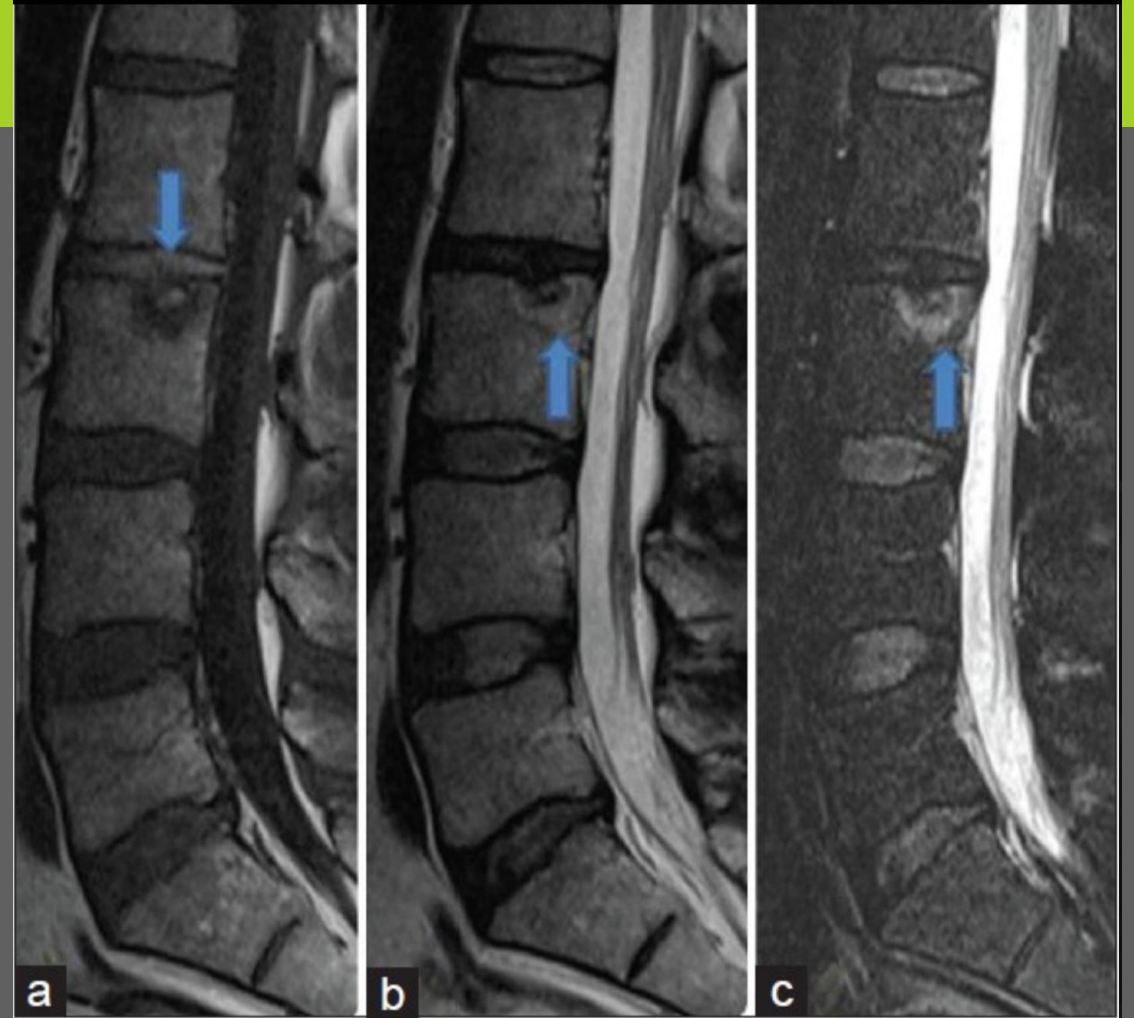
MODIC CHANGES TYPE III

- T1 Low
- T2 Low
- Represents bony sclerosis



SCHMORL'S NODES/ENDPLATE FRACTURES

ACUTE VS. CHRONIC



WHAT CAN GO WRONG IN THE DISC?

- Decrease in large proteoglycans (Aggrecan, Versican) to smaller proteoglycans (Biglycan, Decorin) which means less water retention
- More Type I collagen in NP yielding more fibrosis
- Imbalance of homeostatic levels of anabolism and catabolism
 - Production of matrix metalloproteinases (MMPs) and similar enzymes decrease matrix production
- Dehydration leads to loss of structural integrity
- Inflammatory mediators, like proinflammatory cytokines and interleukins further increase MMPs (Catabolic Factors) suppressing proteoglycan synthesis

WHAT CAN GO WRONG IN THE DISC?

- These proinflammatory cytokines may sensitize nerves in outer 1/3 of annulus (“Peripheral Sensitization”)
 - Damage to endplates – Smoking, Aging, Trauma – Negatively affects internal vertebral disc microenvironment
 - Decreased O₂
 - Decreased Nutrition affects
 - Increased Acidity
- } Decreased viability of NP cells (Apoptosis) and adversely matrix production

WHAT CAN GO WRONG IN THE DISC?

- Inappropriate Disc Stressors
 - Static unloading of disc > Degenerative/Catabolic
 - Dynamic loading of disc > Anabolic
 - Overloading of disc > Catabolic
- Genetic predisposition for developing symptomatic discs

Unloading

Dynamic

Overloading



CATABOLISM

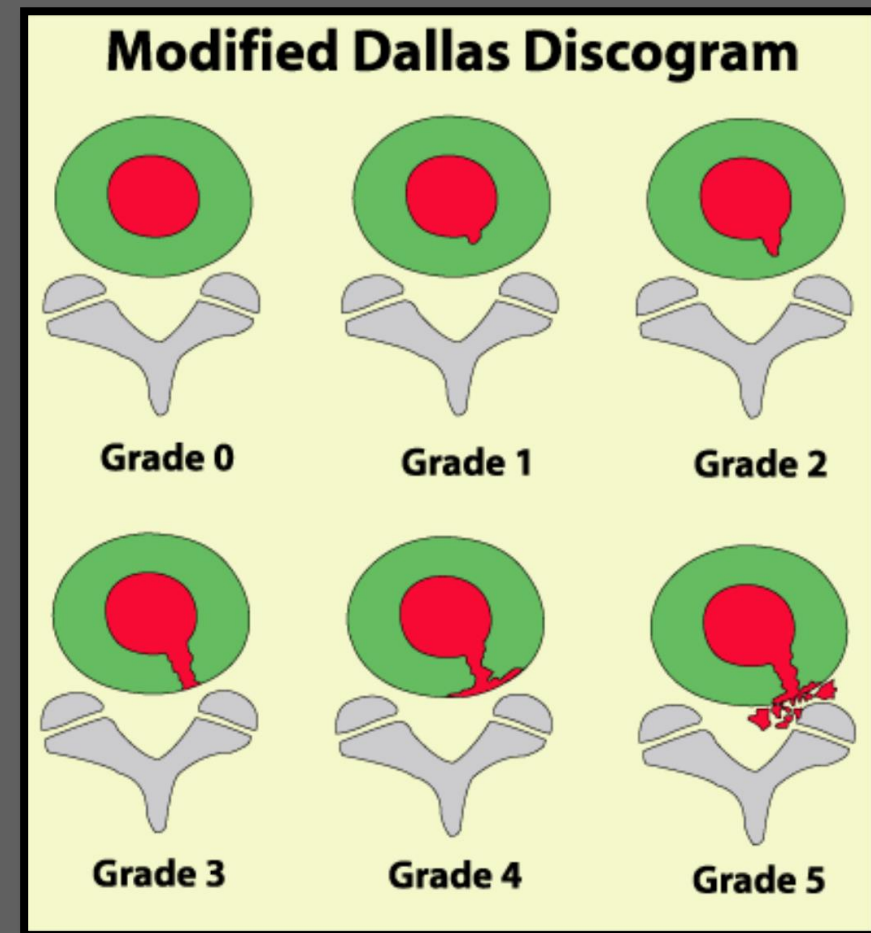
ANABOLISM

CATABOLISM

ARTIFICIAL DESIGNATIONS OF DISC PATHOLOGY

INTERNAL DISC DISRUPTION

- Focal radial and circumferential fissures
- Nuclear degeneration minimal early
- Most common cause of detectable lower back pain
 - Prevalence of 39% as per Bogduk



ARTIFICIAL DESIGNATIONS OF DISC PATHOLOGY DEGENERATIVE DISC DISEASE

- Diffuse progressive age-related phenomenon defined by nuclear dehydration and fibrosis
- Mechanical, biochemical, nutritional and genetic factors contribute to a catabolic metabolism
 - MMP Activation, decreased cell viability, decreased proteoglycan synthesis
- Resultant disc space narrowing causes a redistribution of forces, compromises disc integrity and predisposes disc to additional injury
- Superimposed endplate spurring, facet overgrowth, tissue irritation and degeneration

ARTIFICIAL DESIGNATIONS OF DISC PATHOLOGY

DISC HERNIATION, EXTRUSION, AND SEQUESTRATION

- Displacement of nuclear disc material beyond normal contours
- Most common etiology of radicular leg pain via chemical radiculitis or mechanical compression
- May cause axial lower back pain as well
- Decreased disc integrity yields accelerated degenerative disc disorder

ARTIFICIAL DESIGNATIONS OF DISC PATHOLOGY

SCHMORL'S NODES

- ▶ Intravertebral herniations with associated endplate fractures

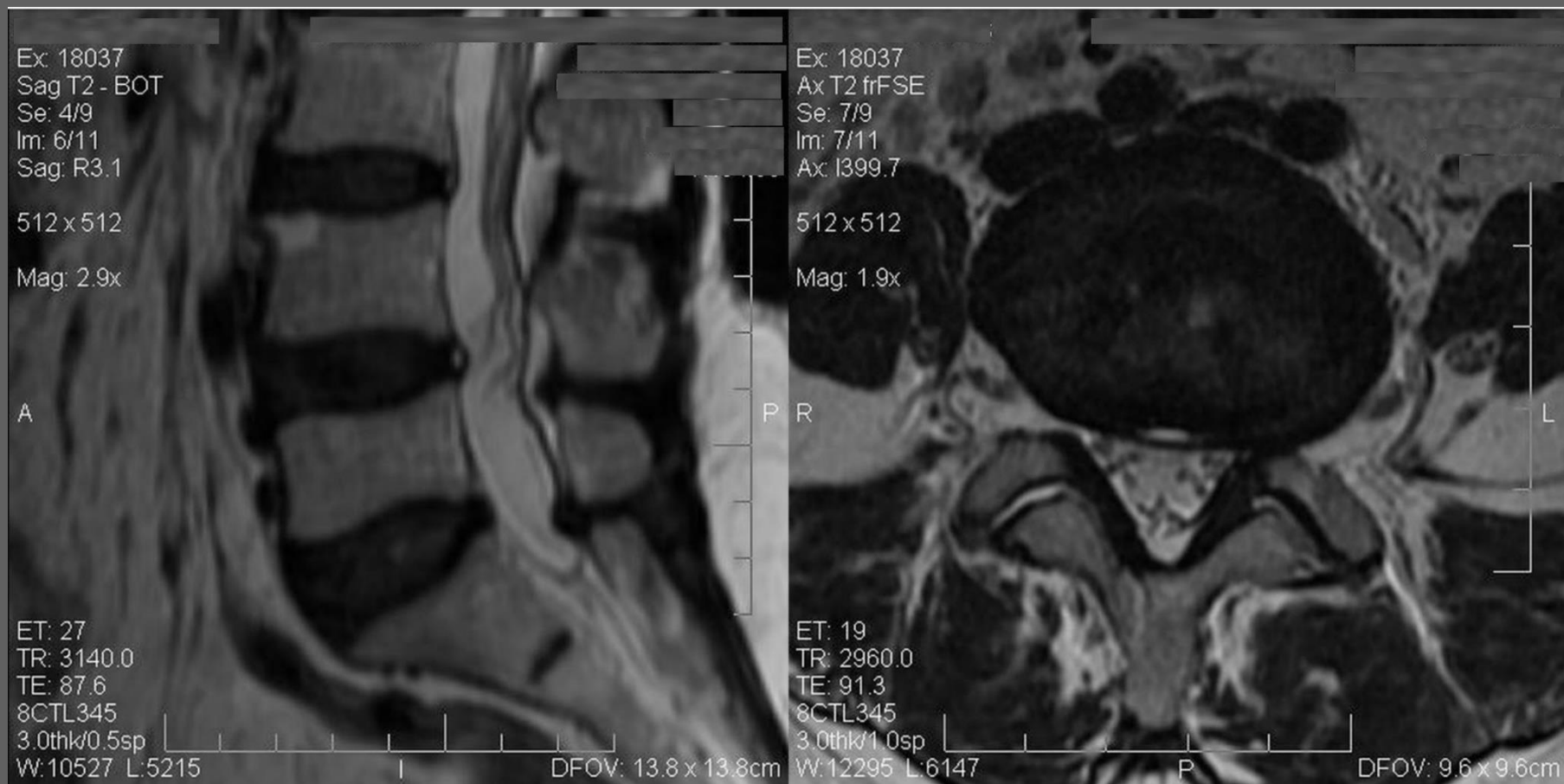
WHEN TO CHOOSE SURGERY

- Large Protrusions
- Extrusions/Sequestrations
- Instability Complicating Disc Pathology

WHEN TO CHOOSE REGENERATIVE THERAPY

- Internal Disc Disruption
- Degenerative Disc Disease
- Small Protrusions –
Non-compressive
- Schmorl's Nodes

INTERNAL DISC DISRUPTION



DEGENERATIVE DISC DISEASE



SMALL PROTRUSIONS NON-COMPRESSIVE



SCHMORL'S NODES



REGENERATIVE THERAPY

WHAT MAKES SENSE?

Scaffold

- Aid in cell delivery, orientation, differentiation, growth, replication, cellular metabolism, sustained viability, and cell retention.
 - Hyaluronan-based hydrogels
 - Fibrin
 - Platelet Rich Plasma (PRP)
 - TGF- β
 - PDGF
 - EGF
 - VEGF
 - IGF-1

REGENERATIVE THERAPY

WHAT MAKES SENSE?

Mesenchymal Stem Cells

- Easy to isolate – Bone marrow, adipose
- Suitable for regeneration of NP and differentiate into NP-like phenotype (*Chun, 2009*)
- Viable for 1-6 months after injection
- Have a profound effect on immune function and inflammation
 - Secrete IL-10, IL-17, LIF, IL1 receptor antagonist
- Shift T_H cell to T_{REG} Cells
- Shift M₁ macrophages to M₂
- Inhibit production of TNF- α

REGENERATIVE THERAPY

WHAT MAKES SENSE?

Growth Factors

Anabolic

*TGF- β
*BMP
*GDF
IGF
*IGF-BP

Catabolic

IL-1
TNF- α
ADAMTS
MMPs

Anti Catabolic

*TIMP-1
*IL-1- α

- No specific factors have proven sufficient to mediate the therapeutic effects of MSCs.
- Factors with an asterisk have been found in MSCs and MSC-derived Exosomes.

REGENERATIVE THERAPY

WHAT MAKES SENSE?

Exosomes

- Extracellular vesicles released via exocytosis
- Nano-sized membrane vesicles with a diameter between 40-120 nm
- Released by all kinds of cells into extracellular environment
- Contain cytokines, proteins, lipids, mRNAs, miRNAs, non-coding RNAs, and ribosomal RNAs
- Bilayer membranous shell prevents dehydration
- Appealing candidates as vectors of MSC efficacy
- Contain no live cells

EXOSOMES

Exosomes as potential alternatives to stem cell therapy for intervertebral disc degeneration: in-vitro study on exosomes in interaction of nucleus pulposus cells and bone marrow mesenchymal stem cells

Kang Lu, Hai-yin Li, Kuang Yang, Jun-long Wu, Xiao-wei Cai, Yue Zhou and Chang-qing Li ✉

Stem Cell Research & Therapy 2017 8:108 | <https://doi.org/10.1186/s13287-017-0563-9> | © The Author(s). 2017

Received: 31 July 2016 | Accepted: 26 April 2017 | Published: 10 May 2017

- NP Cells and Bone Marrow MSCs (BM-MSCs) secrete exosomes which undergo uptake by the corresponding cells.
- BM-MSC derived exosomes promote NP cell proliferation and healthier matrix production in degenerated NP cells.
- Reciprocal uptake of exosomes by BM-MSCs and NP cells.
- BM-MSC exosomes upregulate anabolic/matrix protective genes (TIMP-1, aggrecan, collagen II, sox-9) and decrease matrix degrading genes MMP-1 and MMP-3 in NP cells leading to a more balanced level of homeostasis.

EXOSOMES

Exosomes as potential alternatives to stem cell therapy for intervertebral disc degeneration: in-vitro study on exosomes in interaction of nucleus pulposus cells and bone marrow mesenchymal stem cells

Kang Lu, Hai-yin Li, Kuang Yang, Jun-long Wu, Xiao-wei Cai, Yue Zhou and Chang-qing Li ✉

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Received: 31 July 2016 | Accepted: 26 April 2017 | Published: 10 May 2017

- Recruitment of cells from the surrounding environment around intervertebral discs are an important aspect of the regenerative process.
- Micro-particles can activate MSC migration and cytokine secretion
- Exosomes may be a more appropriate candidate than MSCs in stem-cell based therapy of intervertebral discs as the disc itself is a hostile environment (decreased glucose, increased osmolarity, decreased pH, decreased O₂, increased mechanical variations) and exosomes are more stable and will yield better survivability.

Mesenchymal stem cells deliver exogenous miR-21 *via* exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration

Xiaofei Cheng, Guoying Zhang, Liang Zhang, Ying Hu, Kai Zhang, Xiaojiang Sun, Changqing Zhao, Hua Li, Yan Michael Li, Jie Zhao ✉

- Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration

EXAMPLE

JH, 23 YEARS OLD

5 Years of Low Back Pain L4-5 and L5-S1

BMAC + Exosomes
2ccs/disc

10 Days



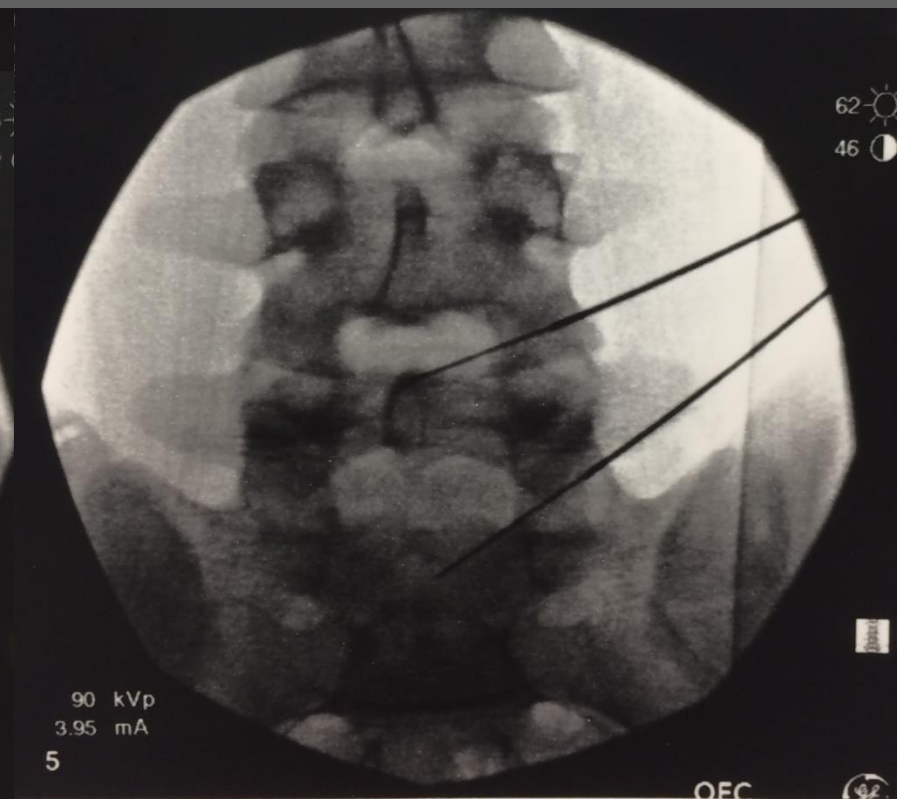
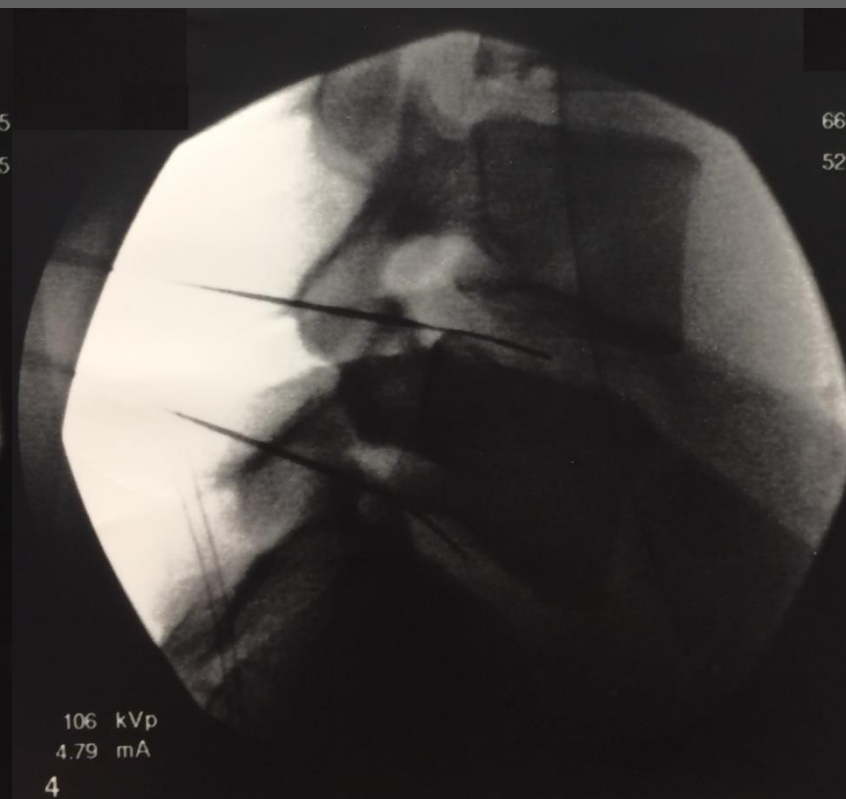
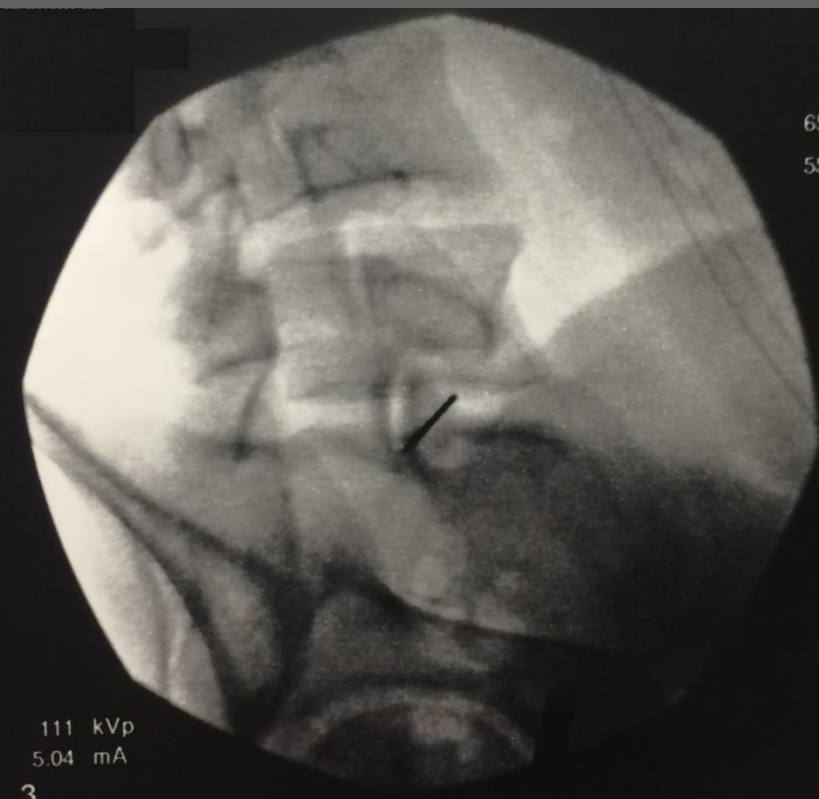
Fibrin + Exosomes
2ccs/disc

WBC 5.3
CRP <0.1
ESR 3

(Normal <0.5)
(Normal <21)

EXAMPLE

JH, 23 YEARS OLD



EXAMPLE

JH, 23 YEARS OLD

Pre-Op



4 Months Post-Op



5:07 PM ✓✓

UNRESOLVED QUESTIONS

- BMAC vs. Adipose?
 - Younger patients may choose either
- Stem Cells + Exosomes vs. Exosomes alone?
- Best quality scaffold?
 - Must allow for cell retention and cellular migration
- Long term follow up?
- Need for reinjection?

CENTRAL NERVOUS SYSTEM INJURIES

PATHOLOGY OF SPINAL CORD INJURY

Current Concept of Stem Cell Therapy for Spinal Cord Injury: A Review

[Sun Kyu Oh](#), MD and [Sang Ryong Jeon](#), MD, PhD✉

- 29.5 cases per million per year
 - Greater than 1 million patients suffer paralysis secondary to spinal cord injury
 - Causes: MVAs, Falls, Violence, Sports Injuries, and Industry-related Injuries
 - Increased incidence in men in their 20's
-
- No Sovereign Treatment Options *

Exosomes: Origins and Therapeutic Potential for Neurodegenerative Disease

[Diana K. Sarko](#)¹ and [Cindy E. McKinney](#)^{2,*}

The Role of microRNA Markers in the Diagnosis, Treatment, and Outcome Prediction of Spinal Cord Injury.

[Martirosyan NL](#)¹, [Carotenuto A](#)², [Patel AA](#)², [Kalani MY](#)³, [Yagmurlu K](#)³, [Lemole GM Jr](#)⁴, [Preul MC](#)³, [Theodore N](#)³.

- 180,000 Spinal Cord Injuries per year
- Two phases:
 - Primary Phase
 - Immediate loss of sensory, motor, and autonomic functions after a sheer, lacerating, impact or compression injury
 - Primarily disrupts gray matter and microvasculature
 - Minimal damage of white matter
 - Secondary Phase (Primary target for clinical therapeutics)
 - Begins seconds after injury and lasts for months
 - Within seconds to minutes, vascular and metabolic disturbances leading to biochemical changes, altered lipid peroxidation, and neurotransmitter accumulation
 - Within hours to weeks, cascades of inflammatory cells arrive and apoptosis occurs
 - Within weeks to months, fiber tract disturbances occur along with demyelination and glial scar

PRIMARY DAMAGE

- Compressive-contusive type injury:

- ➔ Axon severing, membrane rupture, death of neurons, glia, and endothelial cells.
- ➔ Surviving neurons fire action potentials causing a spike in ion levels and accumulation of neurotransmitters yielding additional neuron and glial cell death
- ➔ Mechanical trauma causes intra-parenchymal hemorrhage damaging gray matter, disrupting blood-spinal cord barrier with resultant edema and swelling
 - ➔ Vasospasm and thrombosis of superficial vessels yield additional hypoxia, ischemia and increasing neural cell death
 - ➔ Systemically, transient hypertension gives way to hypotension causing additional hypoxia and subsequent shut off of spinal cord function below the injury – “Spinal Shock”

SECONDARY DAMAGE

- In the minutes to months that follow the initial damage, the secondary phase of SCI takes place leading to an inflammatory response and cell death that significantly expands the area of damage.

SECONDARY DAMAGE

- Vascular alterations from hemorrhage and ischemia lead to vasospasm and hypoperfusion of the spinal cord.



Followed by a period of reperfusion yielding oxygen and nitrogen free radicals contributing to oxidative stress and exacerbated damage and cell death.



Alterations of the blood-spinal cord barrier extend beyond the injury site for days to weeks after injury



Inflammatory cytokines like IL-1, TNF α , matrix metalloproteinases, reactive oxygen species further contribute to enhanced vascular permeability.



Immune cells are recruited and begin to infiltrate the injury

SECONDARY DAMAGE IMMUNE CELLS

- Neutrophils (Hours-Days)
 - Remove debris
 - Release proteolytic enzymes which extend tissue damage
- Macrophages (Days)
 - From activated microglia or blood monocytes
 - Remove debris
 - Present antigens
 - Release pro-inflammatory cytokines
- T-Lymphocytes (1 Week)
 - Cell-mediated adaptive immunity

Anti-inflammatory effect of stem cells against spinal cord injury via regulating macrophage polarization

Authors Cheng ZJ, He XJ

Received 24 June 2016

Accepted for publication 5 September 2016

Published 13 February 2017 [Volume 2017:5](#) Pages 31—38

DOI <https://doi.org/10.2147/JN.S115696>

- Two types of macrophages participate in the inflammatory response after SCI
 - Monocyte derived
 - Microglia derived
- Macrophages are the major inflammatory effector cells
- Microenvironment after SCI induced M1 macrophages
- Myelin debris may switch M2 to M1 macrophages
- M2 macrophages promote myelination, neurogenesis, and axonal regeneration
- MSC exosomes have regulatory abilities and induce anti-inflammatory M2

SECONDARY DAMAGE

- ➔ Apoptosis of white matter cells (oligodendrocytes and microglia)
- ➔ Astrocytosis – Hypertrophy leads to hyperplasia
- ➔ Months to years later, cell death, scarring, gliosis, and other local alterations of the tissue lead to the formation of cavities filled with fluid and surrounded by glial scar which can extend several segments above or below the injury

ASTROGLIOSIS

- Astrocytes regulate CNS homeostasis by maintaining blood-brain barrier, directing neuronal migration, differentiation and development, and providing materials for axonal growth and regeneration.
 - After Spinal Cord Injury → Astrogliosis
 - Acute astrogliosis (about 3rd day after injury) = Hypertrophic astrocytes
 - Later stages astrogliosis (about 4-6 weeks after injury) hyperplastic astrocytes form glial scar.

Acute stages are protective and beneficial while later stages are detrimental

miRNA 21 shifts hypertrophic astrocytes to hyperplastic astrocytes but prevents apoptosis

Apoptosis: Programmed Cell Death

SECONDARY DAMAGE: FREE RADICALS

- Another secondary effect of spinal cord injury, traumatic brain injury, or ischemia is **reactive oxygen and nitrogen species** which destroy cell and DNA structure, interfere with important cellular precursors and cause cell death.

Exp Neurol. 2014 Aug;258:121-129. doi: 10.1016/j.expneurol.2014.04.023.

The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury.

Schwab JM^{#1,2}, Zhang Y^{#3}, Kopp MA¹, Brommer B¹, Popovich PG³.

- During the transition from acute to chronic stages of SCI, there is an evolving state of immunologic dysfunction which exacerbates problems associated with the original neurologic deficit.
 - The immune system is hardwired into the nervous system
 - What starts as intraspinal inflammation affects the entire immune system.

Intraspinal inflammation caused by trauma persists indefinitely

- It may be that within a privileged site like the spinal cord the extent of tissue damage exceeds the resolving ability of the intrinsic resolution pathways.
- In fact, activated microglia are frequently found remote from site of injury. This chronic remote activation of microglia has been implicated in the onset and progression of neuropathic pain.

Exp Neurol. 2014 Aug;258:121-129. doi: 10.1016/j.expneurol.2014.04.023.

The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury.

Schwab JM^{#1,2}, Zhang Y^{#3}, Kopp MA¹, Brommer B¹, Popovich PG³.

- Another cause of persistent inflammation is poor clearance of apoptotic cells which eventually lose membrane integrity and release immunogenic self antigens.
 - In humans and in mouse models this failed clearance has been the major proposed cause of Systemic Lupus Erythematosus (SLE)
 - Yields autoimmunity against CNS

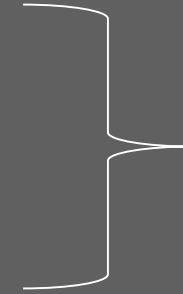
CROSSTALK/INTERACTIONS BETWEEN THE NERVOUS AND IMMUNE SYSTEMS, AND NEUROGENIC REGULATION OF IMMUNE SYSTEM

- Cytokines released by leukocytes can cross the BBB and can affect the survival and functions of neurons and glia.
- Primary and secondary lymphoid tissues are innervated by the autonomic nervous system and cells of the immune system show surface receptors for neurotransmitters, hormones, and neuropeptides associated with the sympathetic nerve system and the hypothalamic-pituitary-adrenal axis.

Spinal Cord Injury induced Immune Depression Syndrome (SCI-IDS)

CNS Injury induced Immune Deficiency Syndrome (CIDS)

- After TBI or CVA



Develops quickly after injury within 24 hours, and affects both the innate and adaptive immune system

- SCI-IDS and CIDS both increase susceptibility to infection which is leading cause of morbidity/mortality in SCI.
- Both SCI-IDS and CIDs are associated with Autonomic Dysreflexia
- Therefore, after SCI both autoimmunity and immune deficiency may be present together or one may supercede the other.

NEURODEGENERATIVE DISEASE AND STROKE

T cells from patients with Parkinson's disease recognize α -synuclein peptides

David Sulzer , Roy N. Alcalay, Francesca Garretti, Lucien Cote, Ellen Kanter, Julian Agin-Liebes, Christopher Liong, Curtis McMurtrey, William H. Hildebrand, Xiaobo Mao, Valina L. Dawson, Ted M. Dawson, Carla Oseroff, John Pham, John Sidney, Myles B. Dillon, Chelsea Carpenter, Daniela Weiskopf, Elizabeth Phillips, Simon Mallal, Bjoern Peters, April Frazier, Cecilia S. Lindestam Arlehamn & Alessandro Sette 

Nature **546**, 656–661 (29 June 2017)

doi:10.1038/nature22815

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[Immunology](#)

[Neuroimmunology](#)

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Accepted: 03 May 2017

Published online: 21 June 2017

Erratum: 13 September 2017

- Two fragments of α -synuclein, a protein that accumulates in PD, can activate T-Cells involved in autoimmune attacks
- The immune response to α -synuclein may be the initial cause of PD, or may contribute to neuronal death after the onset.

Associations between specific autoimmune diseases and subsequent dementia: retrospective record-linkage cohort study, UK

Clare J Wotton, Michael J Goldacre

- Analysis of 1.8 million cases in England between 1999 and 2012
- In general, having an autoimmune disease increases your risk by 20% of having dementia
 - MS = 2X Risk
 - Psoriasis = 29% Increased Risk
 - Lupus Erythematosus = 46% Increased Risk
 - Rheumatoid Arthritis = 10% decreased risk (Likely secondary to treatment with NSAIDs and Aspirin)

Autoimmune Disease = 53% increased risk of hospital admission for cardiovascular disease

Autoimmune Disease = 46% increased risk of stroke

Nat Immunol. 2017 Dec;18(12):1342-1352. doi: 10.1038/ni.3867. Epub 2017 Oct 23.

Metabolic shift induced by systemic activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional behavior.

Miyajima M¹, Zhang B^{1,2}, Sugiura Y³, Sonomura K⁴, Guerrini MM¹, Tsutsui Y¹, Maruya M¹, Vogelzang A¹, Chamoto K², Honda K³, Hikida T⁵, Ito S¹, Qin H⁶, Sanuki R⁷, Suzuki K¹, Furukawa T⁷, Ishihama Y⁸, Matsuda F⁴, Suematsu M³, Honjo T², Fagarasan S¹.

- Augmented T-Cell activation yields a system decrease in amino acids
- Decreased tryptophan and tyrosine led to decreased serotonin and dopamine (monoamine neurotransmitters) in the brain, which led to behavioral changes such as increased anxiety and fear.
- This systemic drop in amino acid availability downstream of strong activation of the immune system affected biochemical pathways which depend on free amino acids as precursor metabolites.
- Other published *in vitro* studies show that T-Cell activation causes increased metabolism of glutamine and glucose required for proliferation, survival, and cytokine production.
- T-Cells are essential providers of adaptive immunity, but as they monopolize the systemic pool of amino acids to support their own needs, they do this at the cost of diminished brain function.

FLAME STUDY

- In patients with ischemic stroke and moderate to severe motor deficit, early prescription of **Fluoxetine** enhanced motor recovery after 3 months.
- Demonstrates modulation of spontaneous brain plasticity
- Serotonin reuptake inhibitors exert neuroprotection, promote hippocampal neurogenesis, and activate motor cortices.

Stem Cells. 2007 May;25(5):1307-16.

Bone marrow-derived mesenchymal stem cells promote neuronal networks with functional synaptic transmission after transplantation into mice with neurodegeneration.

Bae JS¹, Han HS, Youn DH, Carter JE, Modo M, Schuchman EH, Jin HK.

- Study provides the first in vivo evidence that upregulation of neurotransmitter receptors may contribute to synapse formation (via AMPA receptor upregulation)

Review Article

Potential Therapies by Stem Cell-Derived Exosomes in CNS Diseases: Focusing on the Neurogenic Niche

Alejandro Duarte,¹ Luis Federico Bátiz,^{2,3,4} Ursula Wyneken,¹ and Carlos Lafourcade¹

- Highly prevalent CNS disorders associated with degeneration
 - Parkinson's (PD)
 - Alzheimer's (AD)
 - Huntington Disease (HD)
 - Stroke
 - Epilepsy
- Based on clinical manifestations or site of brain affected



NEED FOR NEW THERAPIES

- Animal models of chronic stress decrease neurogenesis
- Some antidepressants seem to involve modulation of the neurogenic niche
- In Parkinson's Disease:
 - There is a reduction of proliferating cells in SGZ and SVZ
 - α -synuclein accumulation disrupts adult neurogenesis
- In stroke –Recovery is dependent on neurogenesis
- MSCs exert their effects via upregulation of the neurogenic niche and this is accomplished via release of extracellular vesicles not engraftment
 - Also promote angiogenesis and reduce neuroinflammation

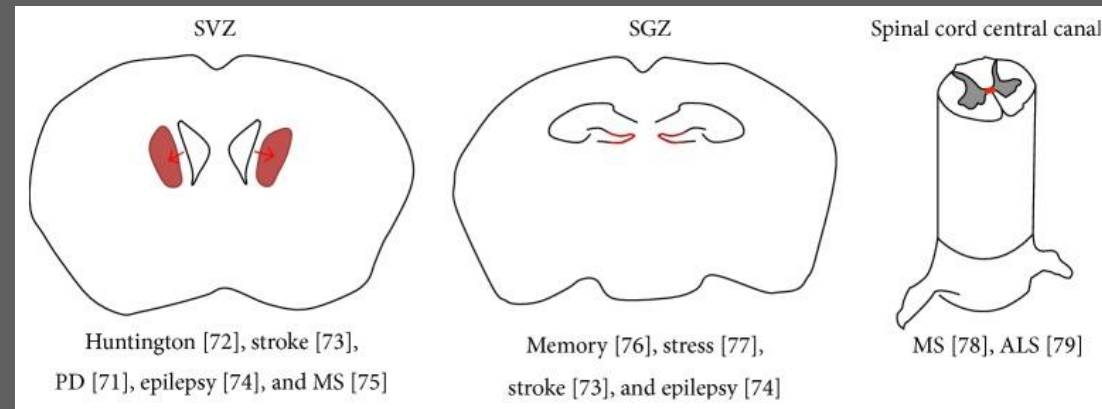
NEED FOR NEW THERAPIES

- For example, Parkinson's Disease:
- L-dopa, a precursor of L-dopamine (neurotransmitter)
 - Improves symptoms but does not replace or improve survival of degenerating neurons.
 - Side effects -> dyskinesias
 - Unable to alleviate non-dopaminergic symptoms (i.e. dementia and psychiatric disorders)
 - These associated neurotransmitter systems involve noradrenaline, acetylcholine and serotonin which affect cognition and neuropsychiatric presentation

Multipotent stem cells are found in neurogenic niche of adult CNS in mammalian brains:

Subventricular Zone of Lateral Ventricles

Subgranular Zone of Dentate Gyrus of the hippocampus



Luarte, A. (2016). *Figure 1. Potential Therapies by Stem Cell-Derived Exosomes in CNS Diseases: Focusing on the Neurogenic Niche. Stem Cells International, 2016.*

J Clin Invest. 2016 Apr 1;126(4):1190-7. doi: 10.1172/JCI81133. Epub 2016 Apr 1.

Exosomes in stroke pathogenesis and therapy.

Zhang ZG, Chopp M.

- Present treatment for ischemic stroke:
 - TPA (Tissue Plasminogen Activator) can help treat ischemic strokes if utilized within 4.5 hours.
 - Endovascular thrombectomy extends therapeutic window to 12 hours
- Occlusion of a major vessel triggers secondary downstream thrombosis yielding dysfunction of endothelial cells, pericytes, and astrocytes as well as disruption of the blood-brain barrier and ischemic cell damage.
 - MSC-Derived Exosomes and Therapies
 - MSC-derived vesicles improve neurological outcomes in ischemic mice

POTENTIAL OF STEM CELL BASED THERAPIES

- Ischemic Stroke = 80% CVAs
- More than 53 clinical trials on the use of stem cell based therapy for stroke

INDUCTION OF NEURONAL REGENERATION

- Most neurodegenerative disease demonstrate progressive neuronal loss.
 - PD – Loss of dopaminergic neurons in Substantia Nigra
 - ALS – Degeneration of motor nerves in brainstem/spinal cord
 - AD – Global loss of neurons in cerebral cortex and hippocampus
 - HD - Degeneration of projection neurons in dorsal striatum
- Do introduced MSCs differentiate into neuronal tissues? Unlikely!
- Most likely mechanism is to encourage endogenous neural stem cells to generate appropriate neurons

INDUCTION OF NEURONAL REGENERATION

- Neurotrophic Factors (NTFs) implicated in induction of Neurogenesis
 - VEGF – Vascular Endothelial Growth Factor
 - BDNF – Brain-Derived NTF
 - GDNF – Glial Cell-Derived NTF
 - FGF2 – Fibroblast Growth Factor
 - NT-3 – Neurotrophin-3

NEUROMODULATION AND INFLAMMATION

- Inflammation plays a role in various neurodegenerative diseases.
 - AD – Activated microglia and astrocytes
 - PD – Enhanced microglia activation, astrogliosis, and lymphocyte infiltration, pro-inflammatory cytokines in blood and CSF
 - ALS – Accumulation of activated microglia and macrophages, multiple pro-inflammatory compounds. Up regulation of COX-1 and COX-2
- **What is inflammation in Central Nervous System?**
 - Mediated by activation of microglia cells (Specialized macrophages)
 - Normally, deactivated microglia supports homeostasis via neurotrophic and anti-inflammatory mediators
 - When activated they recruit circulating immune cells from periphery through Blood-Brain Barrier (BBB), perform phagocytosis, secrete pro-inflammatory cytokines, chemokines, and reactive oxygen species
 - This leads to chronic inflammation and accelerated neuronal death

IMMUNOMODULATION AND MS

- MS is chronic autoimmune and neurodegenerative disease of CNS in which T_H cells infiltrate the CNS and promote inflammation resulting in myelin injury and axonal loss.
- Treatment strategies aim at modulating immune response



Review Article

Potential Therapies by Stem Cell-Derived Exosomes in CNS Diseases: Focusing on the Neurogenic Niche

Alejandro Duarte,¹ Luis Federico Bátiz,^{2,3,4} Ursula Wyneken,¹ and Carlos Lafourcade¹

▪ Neurodegenerative Diseases

- Common trait is accumulation of insoluble proteins extra- or intra- cellular
 - AD – β -Amyloid, Tau
 - PD – α -Synuclein
 - ALS – TDP₄₃, superoxide dismutase 1
 - HD – Huntingtin protein

PROTEIN AGGREGATE CLEARANCE

- Abnormal aggregation of proteins and formation of inclusion bodies is a major hallmark of neurodegenerative diseases.
 - Parkinson's Disease: Intraneuronal inclusions in Substantia Nigra (Lewy Bodies) composed of α -synuclein
 - Huntington's Disease: Protein polyglutamination leading to fibers with β -Sheet structure
 - Alzheimer's Disease: Extracellular amyloid plaques containing $\alpha\beta$ peptide, intracellular neurofibrillary tangles containing phosphorylated tau proteins
 - ALS: Intraneural inclusions of TDP-43, FUS, and SOD1 proteins
 - Prion Diseases: Creutzfeldt-Jakob Disease and Gerstmann-Sträussler-Scheinker Disease. Intra- and extracellular deposition of abnormally folded proteins

Two Major Methods of Protein Aggregate Clearance

Microglia and proteolytic enzyme secretion

Autophagy

- MSCs have a soluble intracellular adhesion molecule which causes secretion of neprilysin from microglia
- Increased Neprilysin reduces $\alpha\beta$ burden of plaques in hippocampus of AD mouse models
- Therefore MSCs may enhance the endogenous proteolytic pathway

- A cellular pathway involved in protein and organelle degradation through formation of autophagic vacuoles that fuse with lysosomes.
 - A key role in aggregate clearance in neurodegenerative disease models.
- MSCs given to mice with increased $\alpha\beta$ in hippocampus increased survival of these neurons and decreased α -synuclein in a mouse model of PD
- Therefore MSCs appear to increase protein aggregate clearance through an increase in autophagy

KEY OBJECTIVES TOWARDS IMPROVING OUTCOMES

- Decrease Cell Death
- Reduce scarring and cavitation
- Regain healthy neural cells
- Stimulate functional axonal regeneration
- Remodeling of the injury niche

Although MSCs can home, minimal will engraft, and present data supports that the efficacy secondary to paracrine and neuroprotective functions.

Stem Cells. 2017 Aug;35(8):1867-1880. doi: 10.1002/stem.2651. Epub 2017 Jun 30.

Concise Review: Mesenchymal Stem Cells in Neurodegenerative Diseases.

Volkman R¹, Offen D¹.

- Advantages for degenerative disease:
 - Neurotrophic factor-mediated protection (homeostasis and reduced oxidative stress)
 - Enhanced neurogenesis and neuronal survival
 - Modulation of inflammation
 - Abnormal protein aggregate clearance
- Translational studies:
- Human MSCs in murine models of neurodegeneration
 - Alzheimer's
 - Parkinson's
 - ALS
 - ASD
 - Huntington
 - Nerve Injury
 - Stroke
 - MS
 - Inflammation

According to translational studies, **intrathecal** administration is most effective route for local engraftment (compared with intravenous). Intramedullary administration poses some significant risks.

Pharmacol Ther. 2014 Nov;144(2):114-22. doi: 10.1016/j.pharmthera.2014.05.009. Epub 2014 May 20.

Intrathecal delivery of protein therapeutics to the brain: a critical reassessment.

Calias P¹, Banks WA², Begley D³, Scarpa M⁴, Dickson P⁵.

- Over 1 billion people worldwide suffer from neurological disorders:
 - Brain Tumors
 - Epilepsy
 - Cerebrovascular Diseases
 - Neurodegenerative Diseases
 - Depression
 - Multiple Sclerosis
 - Autoimmune Encephalopathy
 - Chronic Neuropathic Pain
- **Exosomes cross the blood brain barrier.**
 - Can be administered IV
 - Can be administered Intrathecal

CSF FLOW DYNAMICS

- Major direction of CSF Flow is between sites of production (Choroid Plexuses) and the site of reabsorption (arachnoid villi and primitive lymphatic system of the cribriform plate)
 - Total Volume 150 mL
 - Brain daily production 500 mg
 - Humans turnover rate 4.4 hours
- Brain-wide fluid transport is dependent upon astroglial water flux which is lymphatic-like in clearing solutes from the interstitial space = “Glymphatic Pathway”
 - This glymphatic pathway explains why intrathecal substances distribute more deeply into the brain parenchyma than could be predicted by diffusion alone.
 - Distribution into the deep brain after intrathecal delivery can also be aided by receptor mediated cellular uptake and intercellular transfer.
- Numerous studies in animal models of stroke and lysosomal storage diseases show that intrathecal delivery of protein therapeutics result in widespread penetration into the brain parenchyma, improved signs of neuropathology, and improved clinical outcomes.

PROCESSES OF SPINAL CORD INJURY REGULATED BY MIRNA

NEUROPLASTICITY

- With incomplete spinal cord injury, recovery takes place due to neuroplasticity of the cortical and subcortical neurons and glial cells
- **miRNA 133b** is associated with increased neuronal plasticity and neurite remodeling in the ischemic zone

AXON REGENERATION AND RE-MYELINATION

- *Why are PNS injuries more likely to repair than CNS injuries?*
- PNS axons have one Schwann Cell per myelin sheath (equaling many per axon)
- CNS axons have one oligodendrocyte per several sheaths

Therefore oligodendrocytes are far more indispensable. CNS is capable of regeneration but SCI, trauma, inflammation, and other factors destroy neurons and glial cells.

NEURON REGENERATION

- Multiple miRNA are responsible for neuron growth, regeneration, and re-myelination.

IMPORTANT MIRNA

- miRNA 21 -
against ischemia- Improved survival of intact neurons, neuroprotective
reperfusion injury
- miRNA 133b - Promotes neurite outgrowth, improves functional recovery
- miRNA 124 - Promote productivity of neurogenic cells
- miRNA 12 - Promote productivity of neurogenic cells

PAIN

- Increasing evidence suggests that miRNAs play a significant role in regulating both inflammatory and neuropathic pain following SCI.
- *The major problem after spinal cord injury is that harmful miRNAs are expressed and beneficial miRNA are inhibited.

Front Cell Neurosci. 2014 Feb 25;8:53. doi: 10.3389/fncel.2014.00053. eCollection 2014.

MicroRNA dysregulation in spinal cord injury: causes, consequences and therapeutics.

Nieto-Díaz M¹, Esteban FJ², Reigada D¹, Muñoz-Galdeano T¹, Yunta M³, Caballero-López M¹, Navarro-Ruiz R¹, Del Águila A¹, Maza RM¹.

Pediatr Res. 2012 Apr;71(4 Pt 2):474-81. doi: 10.1038/pr.2011.64. Epub 2012 Feb 8.

Mesenchymal stem cells as a treatment for neonatal ischemic brain damage.

van Velthoven CT¹, Kavelaars A, Heijnen CJ.

- The number of NPCs (Neural Progenitor Cells) is relatively high in the newborn brain
- Parenchymal astrocytes in neonatal brains maintain a higher level of “stemness”
- Microglia are the resident macrophages of the CNS
 - Two Type of Microglia
 - M1 = Classically activated
 - Pro-inflammatory cytokines, oxidative metabolites, neurotoxins
 - M2 = Alternatively activated
 - Neuroprotective, promote repair by release of IL-10, IGF-1, TGF- β and modulate the immune response.
 - Increase differentiation of neural precursor cells

Pediatr Res. 2012 Apr;71(4 Pt 2):474-81. doi: 10.1038/pr.2011.64. Epub 2012 Feb 8.

Mesenchymal stem cells as a treatment for neonatal ischemic brain damage.

van Velthoven CT¹, Kavelaars A, Heijnen CJ.

- Adding MSCs after ischemic brain injury –
 - M1→M2 thereby promoting regenerative growth.
 - Large increase in oligodendrocyte progenitor cells, mature oligodendrocytes and myelin formation
- Oligodendrocytes progenitor cells are especially vulnerable to hypoxic-ischemic brain injury which helps explain the characteristic WM Lesions
- A niche is created which is permissive to axonal sprouting, white matter remodeling, and synaptogenesis

- **Eden Carlson** from Arkansas (2 year old with severe anoxic brain injury)



- Prognosis: unable to walk, talk, eat, or react to surroundings



- 55 days later: hyperbaric oxygen therapy



- Able to walk, talk & brain reversed

HYPERBARIC OXYGEN INDUCES ENDOGENOUS NEURAL STEM CELLS TO PROLIFERATE AND DIFFERENTIATE IN HYPOXIC-ISCHEMIC BRAIN DAMAGE IN NEONATAL RATS

· Y-JYANG, X-L WANG, X-H YU, X. WANG, M. XIE, C-T LIU

MSC THERAPY IN HUMAN CLINICAL TRIALS

- MS

- MSA

- Parkinson's

- Stroke

- ALS

- Location: IT, IV, IA, Stereotactic, IM

- Type: UC, BM

- Improved:
 - Functional Performance
 - Clinical Stability

J Spinal Cord Med. 2016 Nov;39(6):655-664. Epub 2015 Jul 24.

Intrathecal transplantation of autologous adipose-derived mesenchymal stem cells for treating spinal cord injury: A human trial.

Hur JW¹, Cho TH¹, Park DH¹, Lee JB¹, Park JY¹, Chung YG¹.

- No adverse effects
- Mild Efficacy

MSC-DERIVED EXOSOMES AS A PROMISING THERAPEUTIC VESICLE AFTER SPINAL CORD INJURY

- About 857 unique gene products
- Greater than 150 kinds of miRNA
- Comparable therapeutic potential as cells themselves
- Protective effects on myocardial/reperfusion injury
- Reverse degeneration of neurons and astrocytes and synaptic loss in hippocampus of diabetic mice
- Promote axonal growth of cortical neurons
- Improve neurological function

J Neurotrauma. 2018 Feb 1;35(3):521-532. doi: 10.1089/neu.2017.5201. Epub 2017 Nov 20.

Effects of Intrathecal Injection of the Conditioned Medium from Bone Marrow Stromal Cells on Spinal Cord Injury in Rats.

Kanekiyo K¹, Wakabayashi T¹, Nakano N¹, Yamada Y², Tamachi M², Suzuki Y³, Fukushima M⁴, Saito F⁵, Abe S⁶, Tsukagoshi C⁶, Miyamoto C⁶, Ide C¹.

- Beneficial effects on locomotor improvements, tissue repair, and axonal regeneration

[Sci Rep](#). 2018 Jan 11;8(1):480. doi: 10.1038/s41598-017-18867-w.

Human Mesenchymal Stromal Cell-Derived Extracellular Vesicles Modify Microglial Response and Improve Clinical Outcomes in Experimental Spinal Cord Injury.

[Ruppert KA](#)¹, [Nguyen TT](#)¹, [Prabhakara KS](#)¹, [Toledano Furman NE](#)¹, [Srivastava AK](#)¹, [Harting MT](#)¹, [Cox CS Jr.](#)¹, [Olson SD](#)².

- MSC-derived extracellular vesicles exhibit stem cell-like regenerating ability with decreased malignant potential, are less immunogenic, and evade pulmonary first pass effect

After Administration in a rodent model of Spinal Cord Injury:

- Decrease in M1 Microglia
- Increase in locomotor Recovery
 - Decrease in hypersensitivity
- Decrease in neuroinflammation
 - Overall improved outcome

EXOSOMES AND CNS DISEASE

WHAT MAKES SENSE?

Most Direct: Intrathecal

C1/2 (Brain, Cervical, High Thoracic) + IV

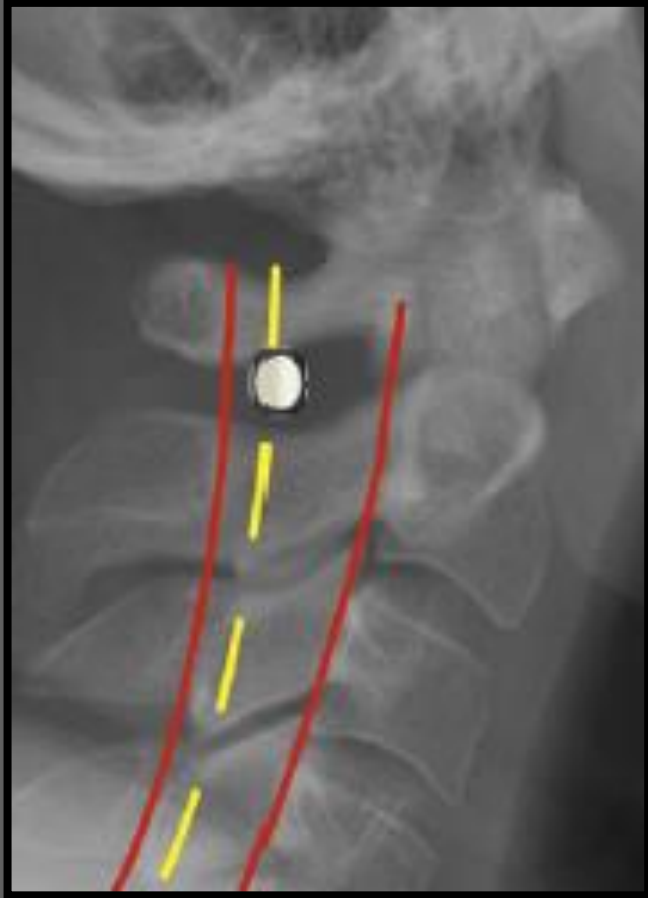
Lumbar (Low Thoracic and Lumbar Arachnoiditis) + IV

Indications: Chronic neurodegeneration, Stroke, Spinal Cord Injury, TBI

Less Invasive: Intranasal + IV

Indications: Autism, Cerebral Palsy, Chronic Migraine, Epilepsy, Acute CVA/TBI/SCI, Drug Addiction

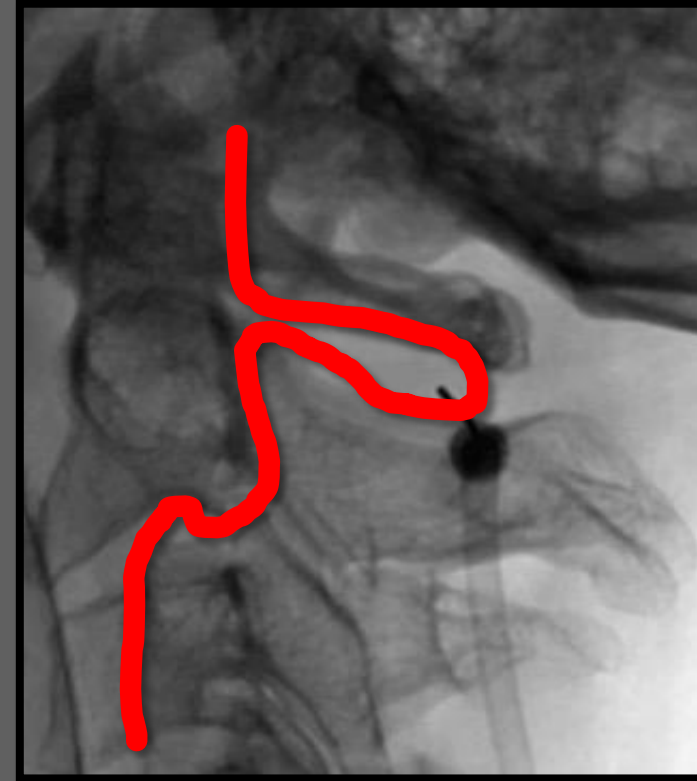
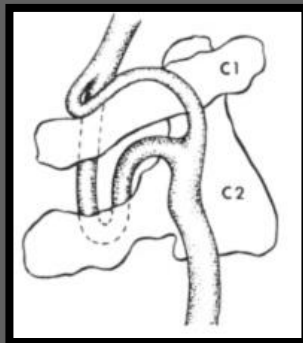
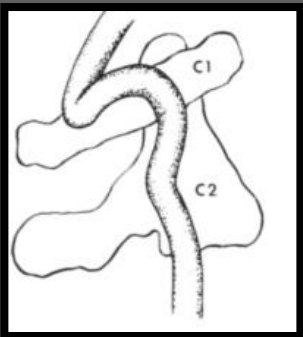
C₁/2 APPROACH



Gibbs, W. (2015). *Slide 16*. C₁-2 Puncture: A safe, efficacious, and potentially underutilized techniques [PowerPoint slides]

C₁/2 APPROACH: CONTRAINDICATIONS

- Chiari Malformation
- Mass at C₁/2
- Acquired or Congenital Stenosis at C₁/2
- Ectatic Vertebral Arteries extending into posterior 1/3 of canal (Up to 2%)
- PICA caudal loop extending to C₁/2 or origin below C₁ (Up to 1%)
- Increased Intracranial Pressure
- Coagulopathy



Gibbs, W. (2015). *Slides 30-31. C1-2 Puncture: A safe, efficacious, and potentially underutilized techniques* [PowerPoint slides]

C_{1/2} APPROACH: COMPLICATIONS

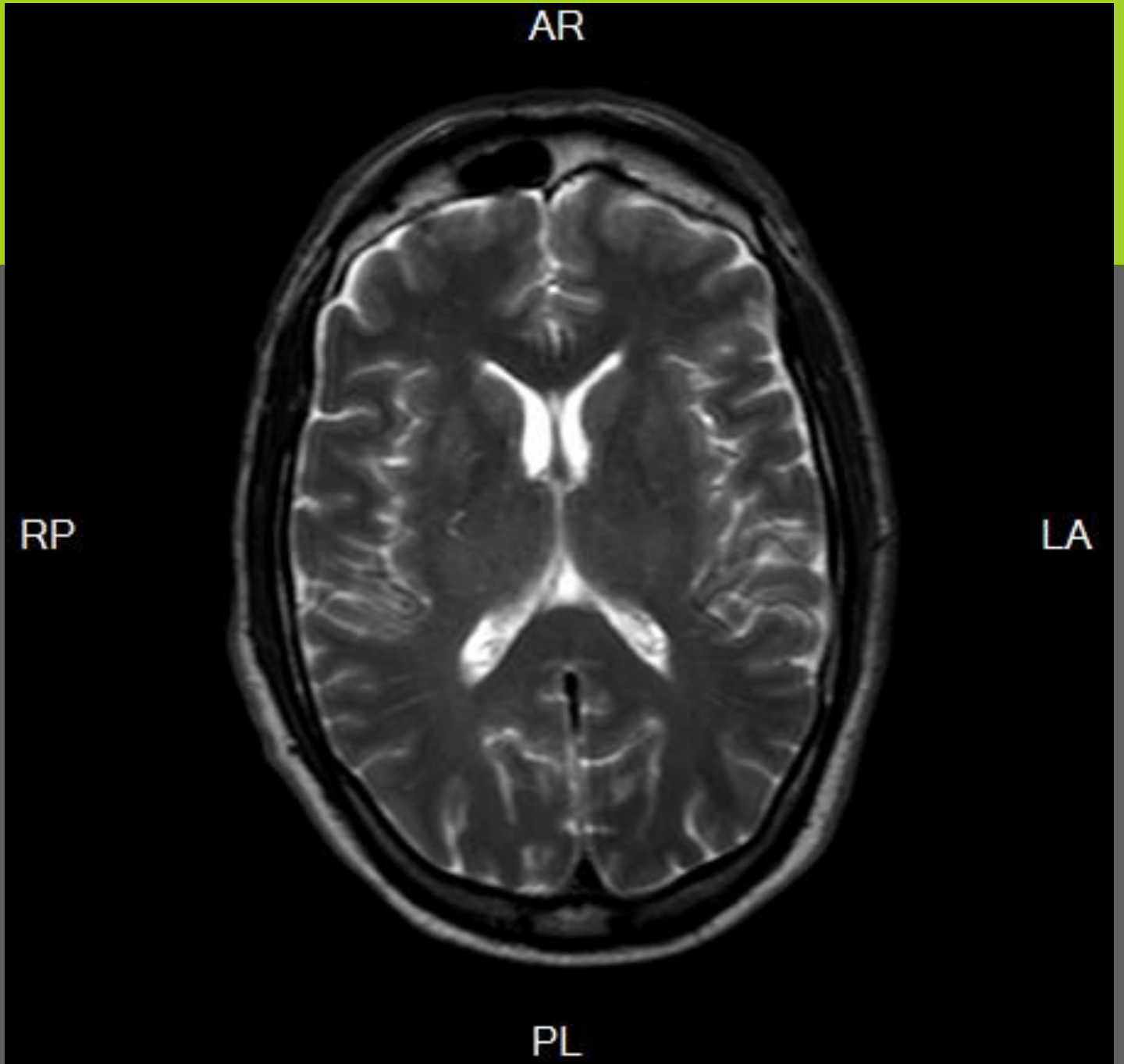
- Pain
- Anxiety
- Bleeding
- Infection
- Subarachnoid Hemorrhage
- Epidural or Subdural Hemorrhage
- Spinal Cord Puncture with or without Hemorrhage
- Vascular Injury (Vertebral or PICA)

SURGICAL PLANNING

- Cervical Spine MRI or Cervical Spine Myelogram
 - Brain MRI or CT Scan
 - Neck CTA or MRA
 - Bloodwork/UA

SURGICAL PLANNING

Brain MRI or CT
Scan



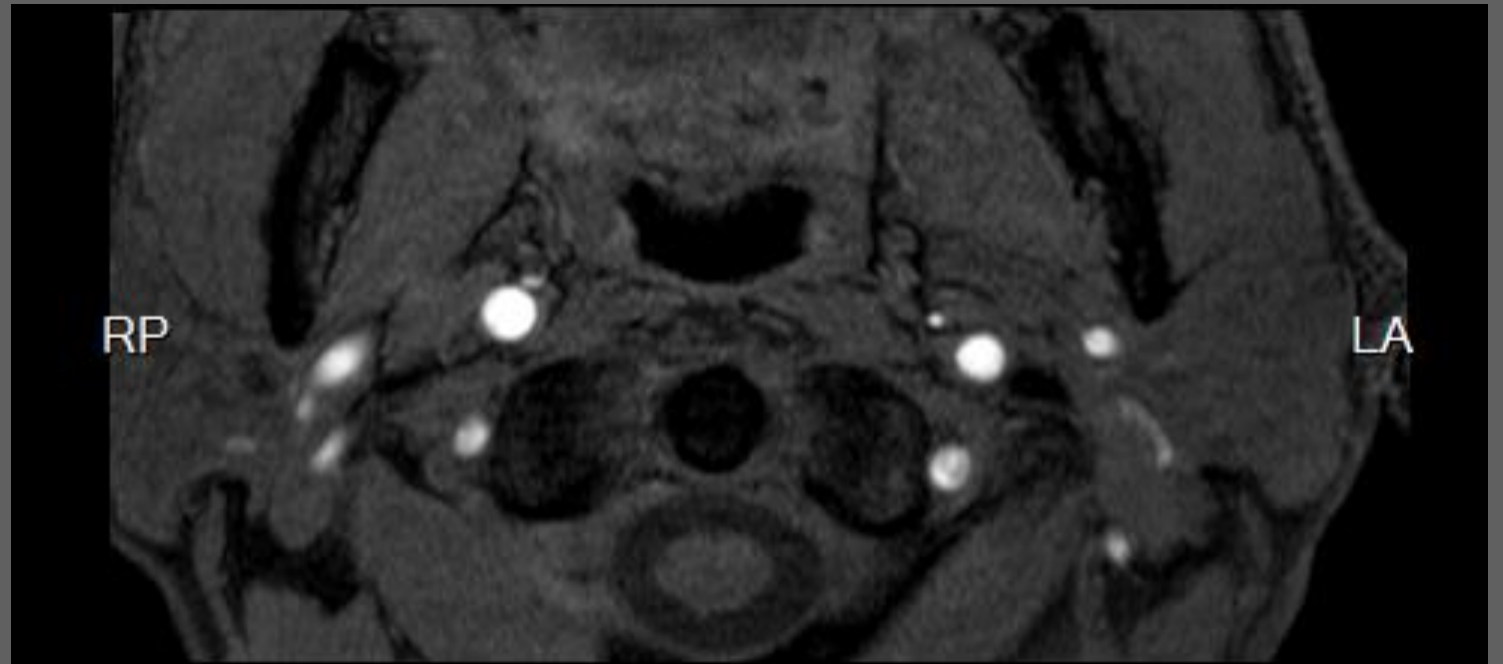
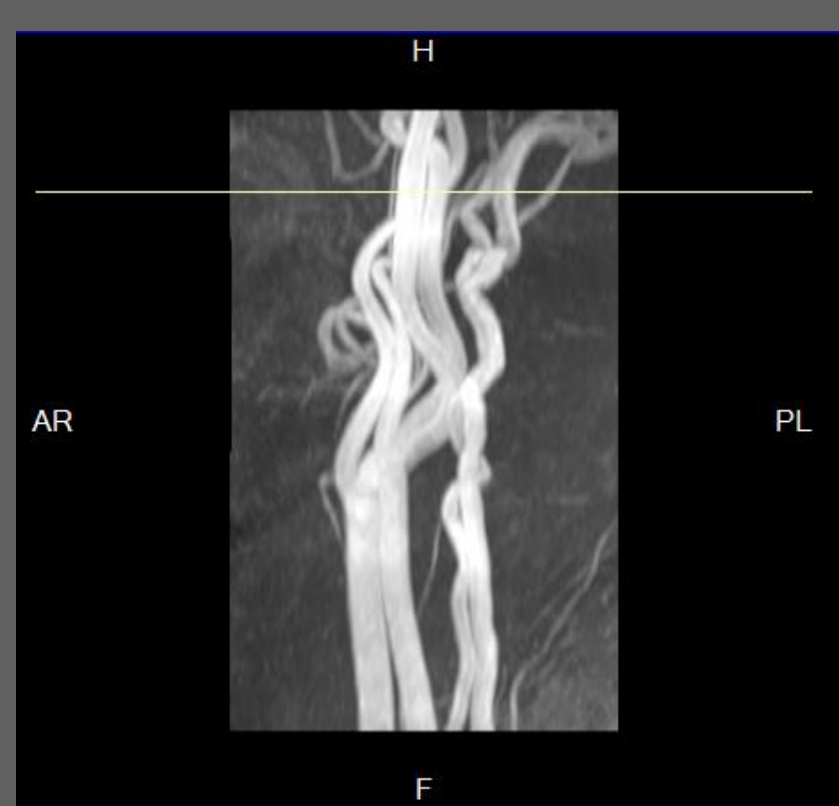
SURGICAL PLANNING

Cervical Spine MRI or
Cervical Spine
Myelogram



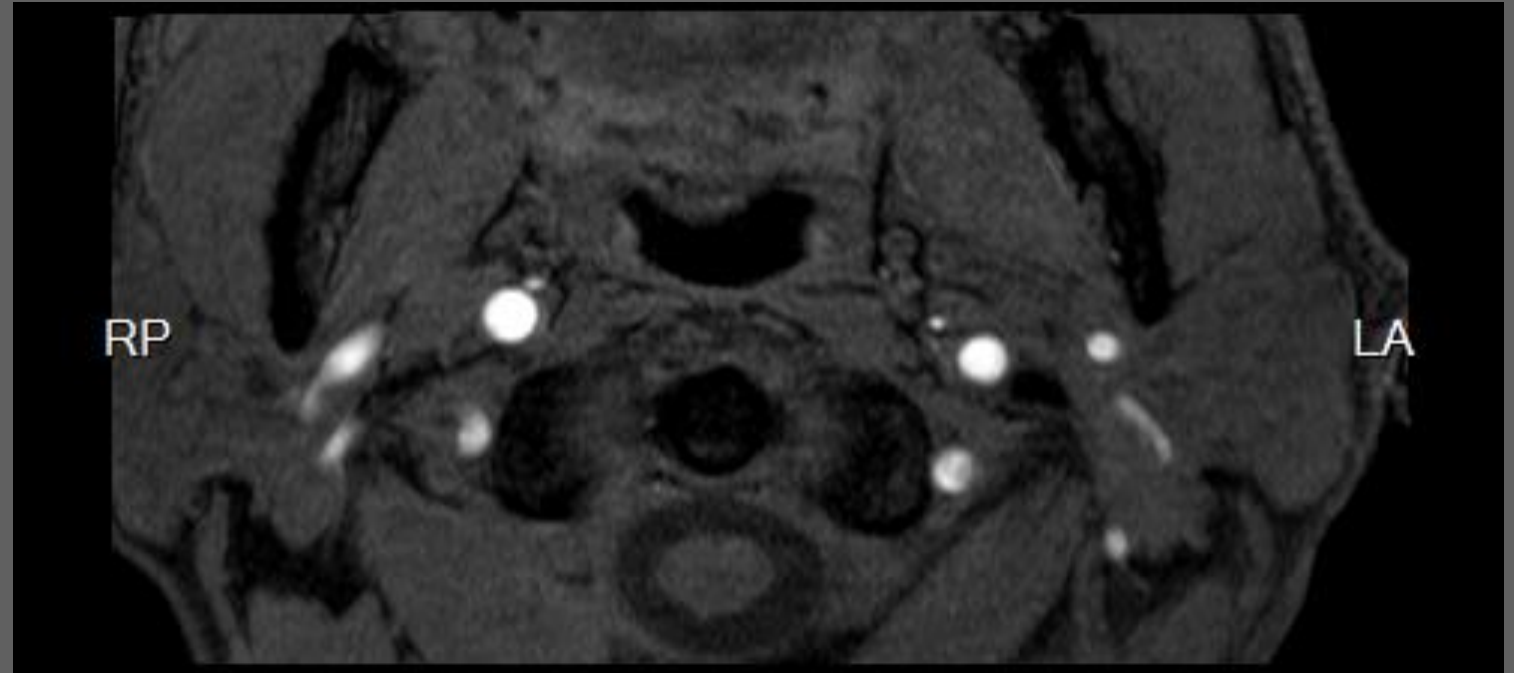
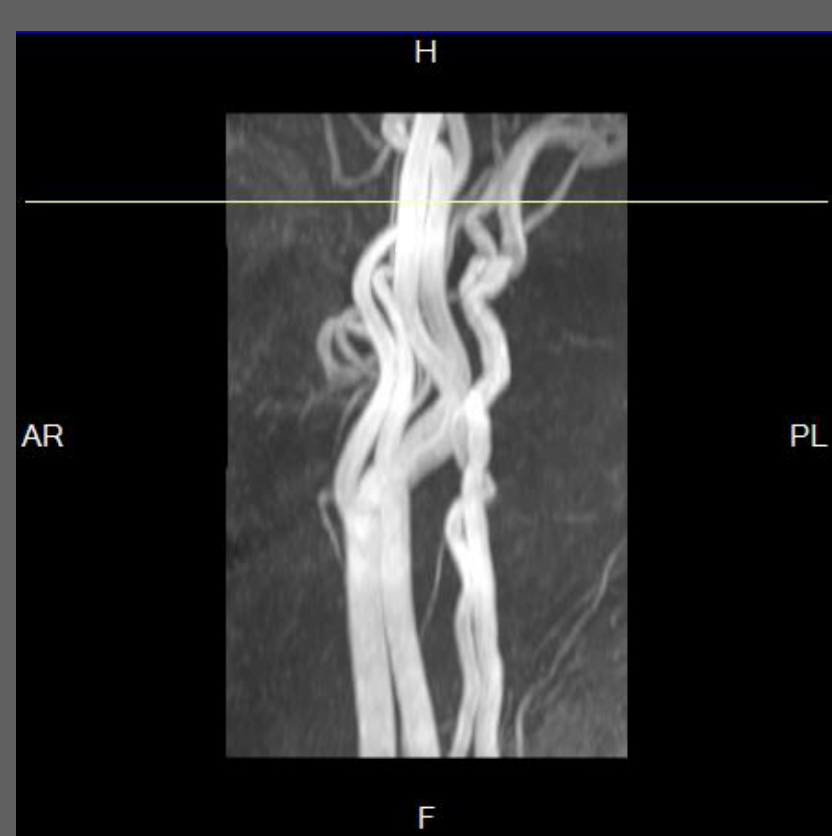
SURGICAL PLANNING

Neck CTA or
MRA



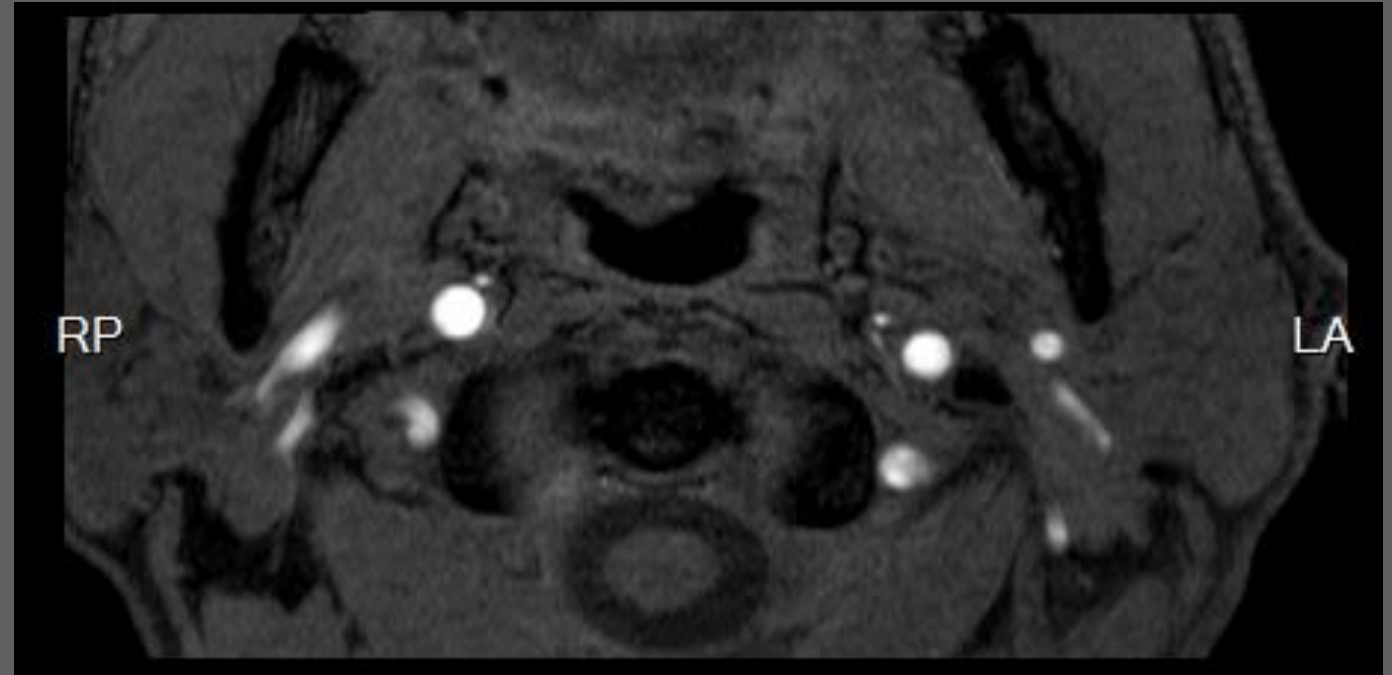
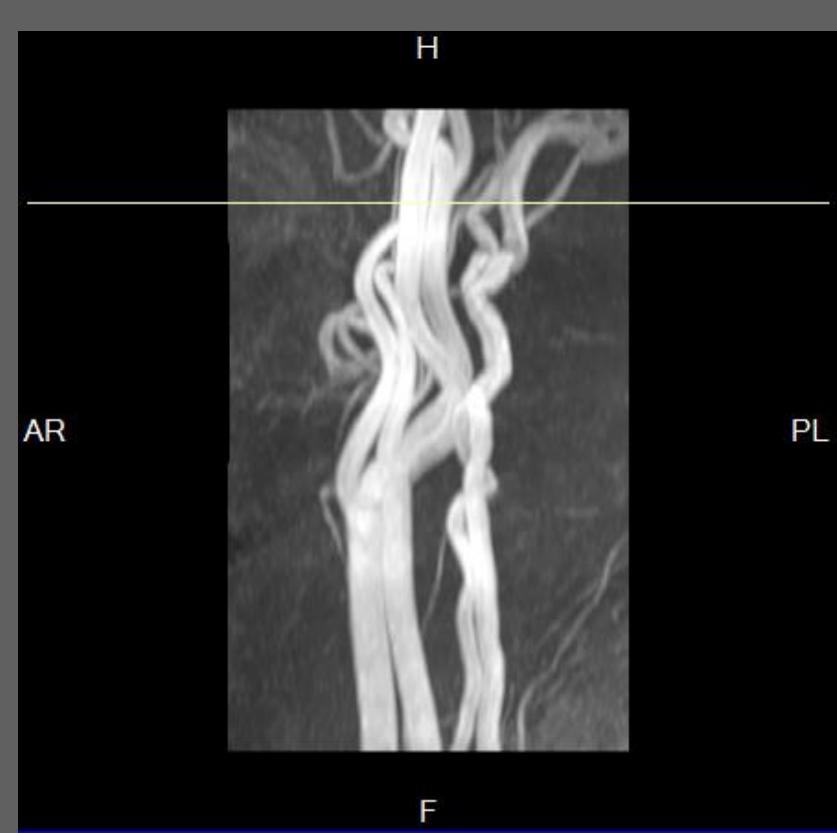
SURGICAL PLANNING

Neck CTA or
MRA



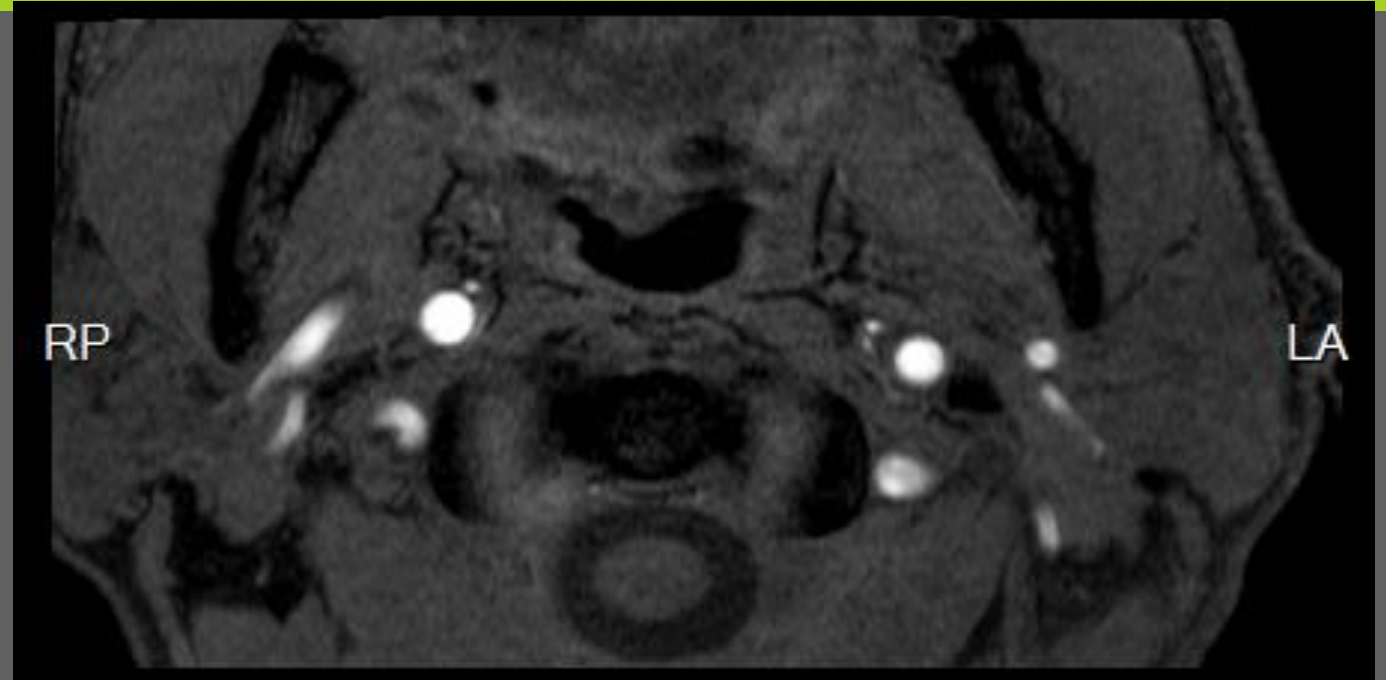
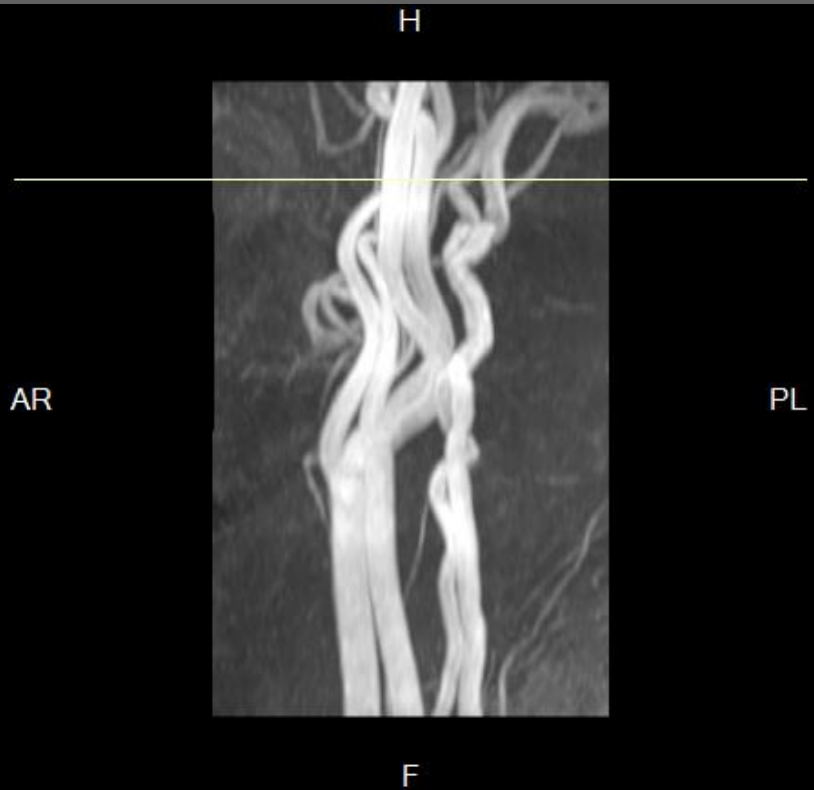
SURGICAL PLANNING

Neck CTA or
MRA



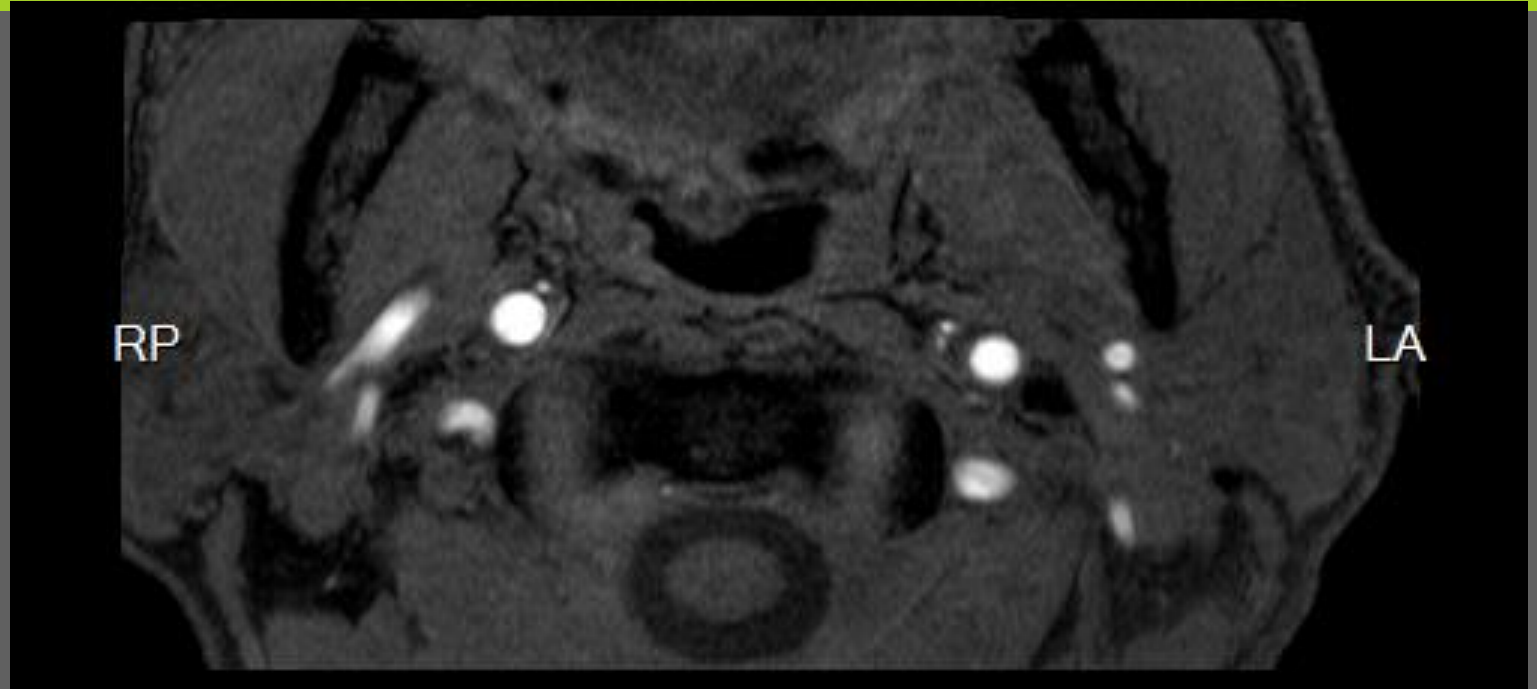
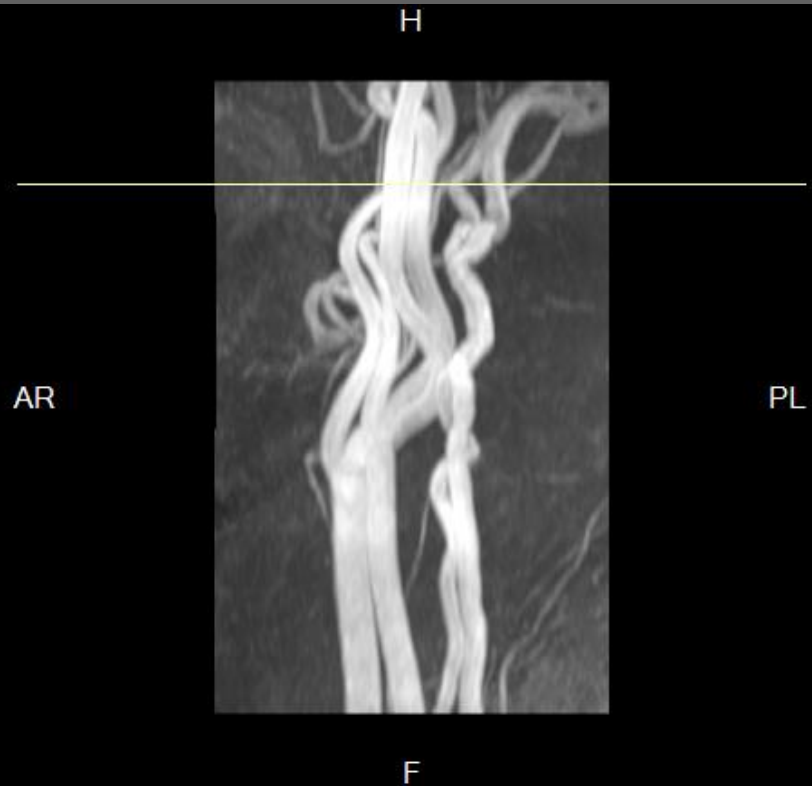
SURGICAL PLANNING

Neck CTA or
MRA



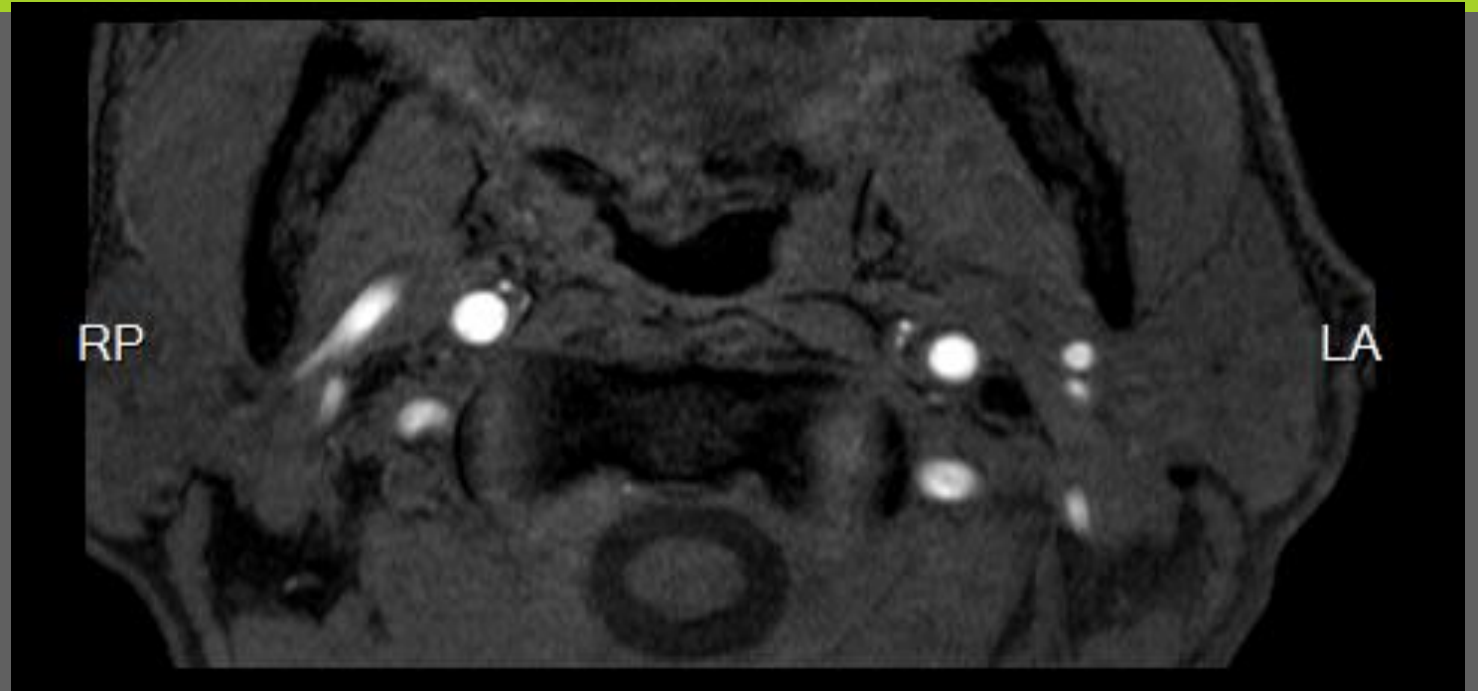
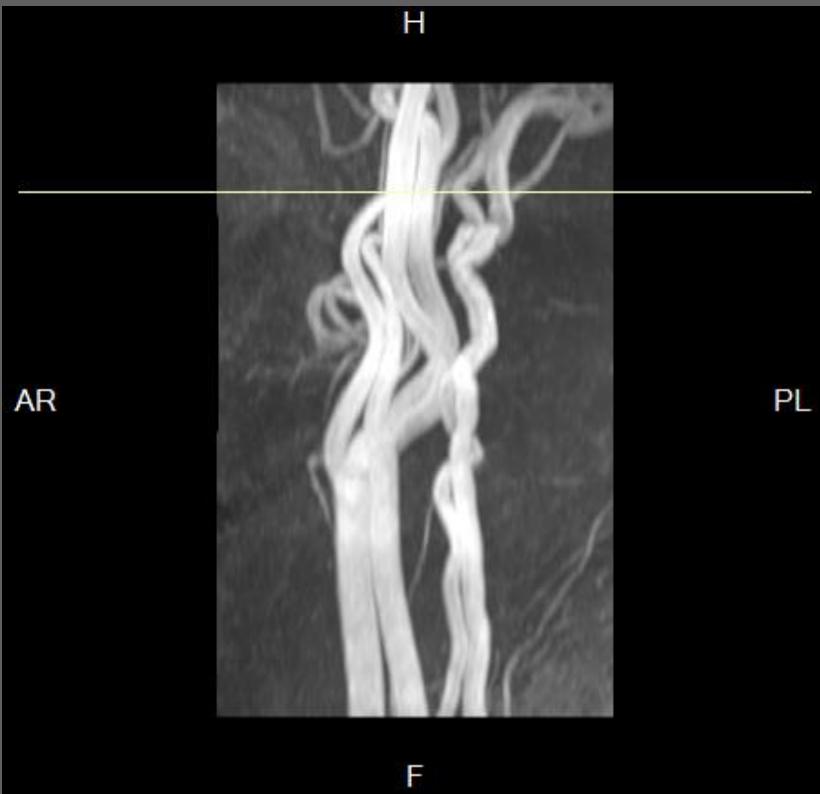
SURGICAL PLANNING

Neck CTA or
MRA



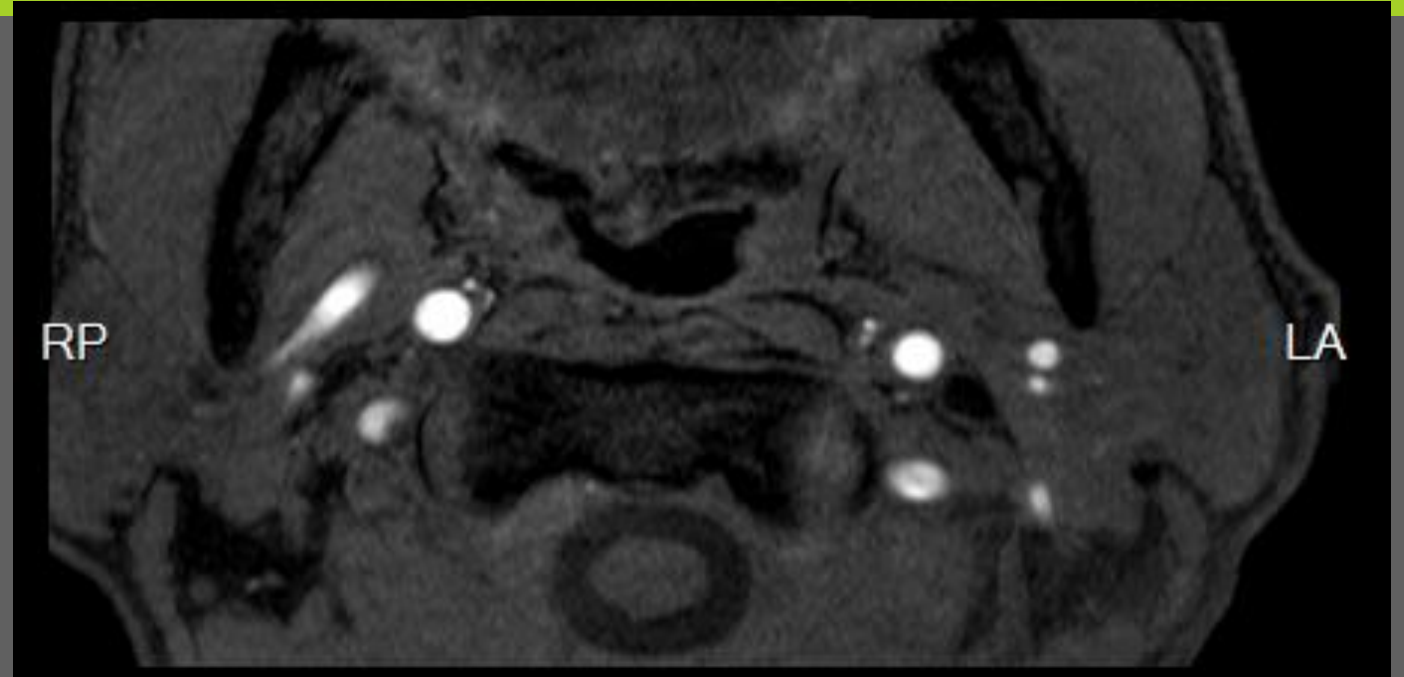
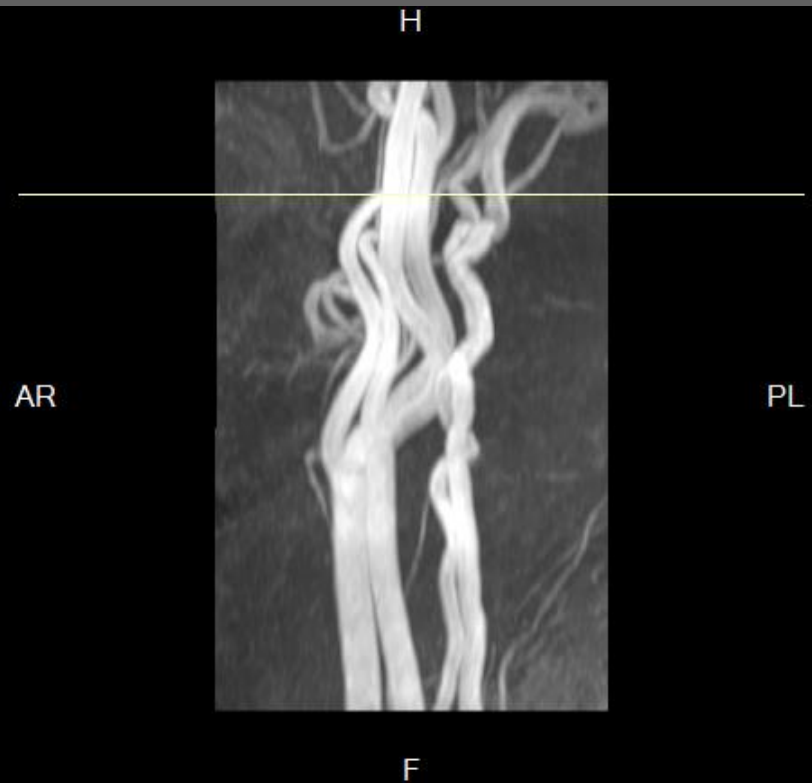
SURGICAL PLANNING

Neck CTA or
MRA



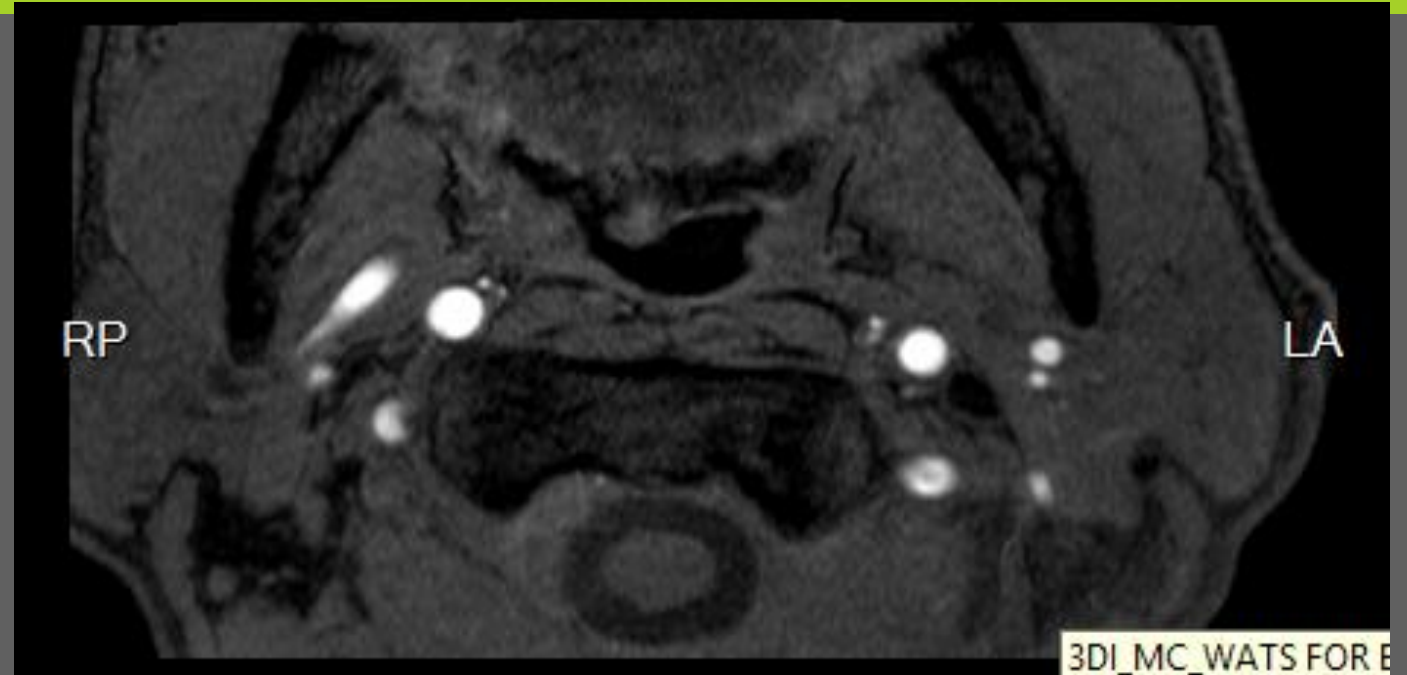
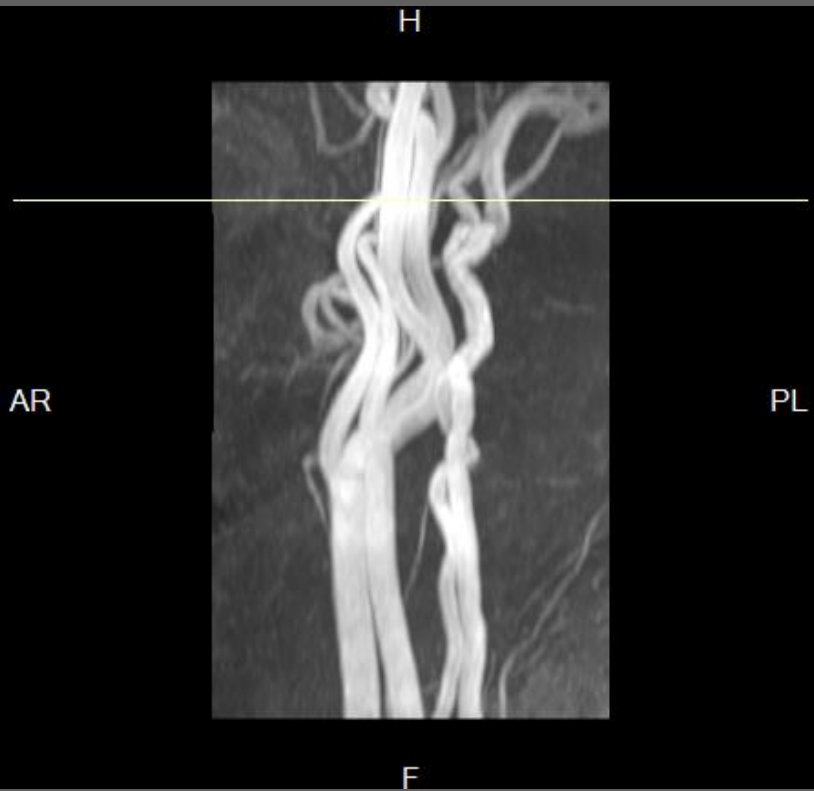
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Neck CTA or
MRA



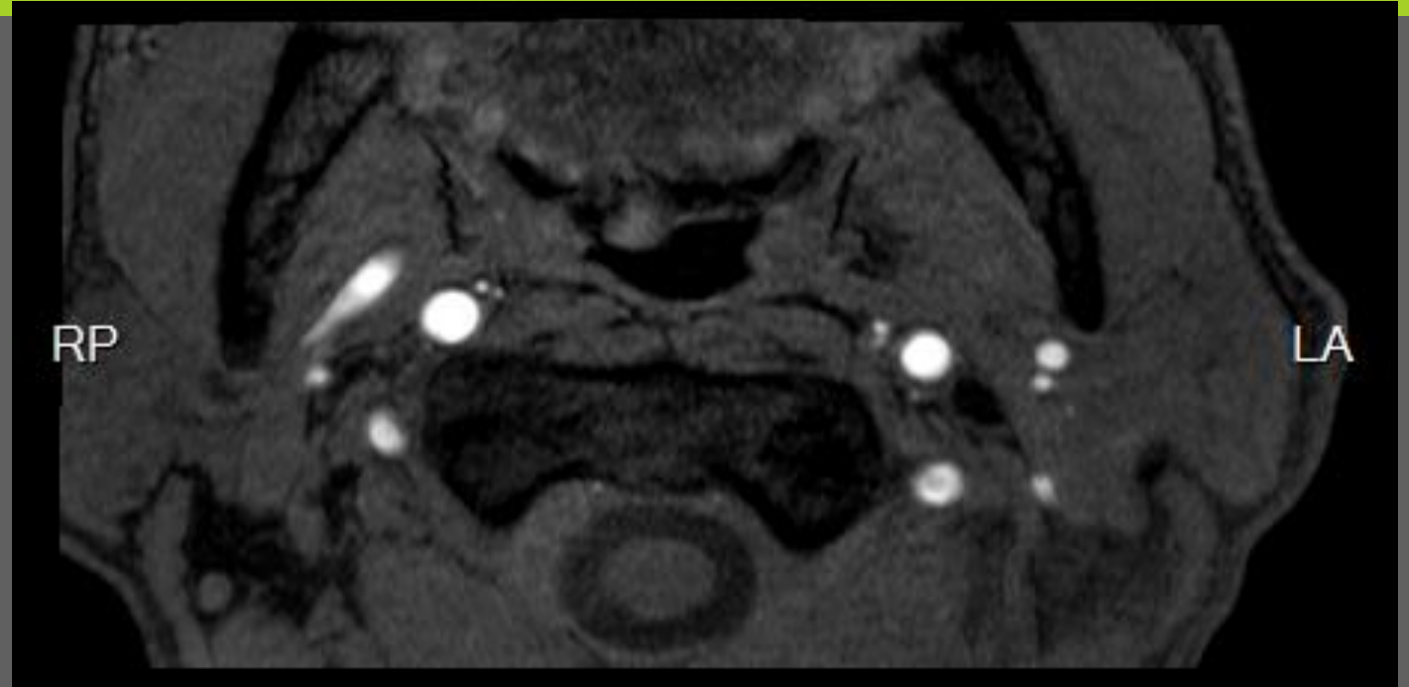
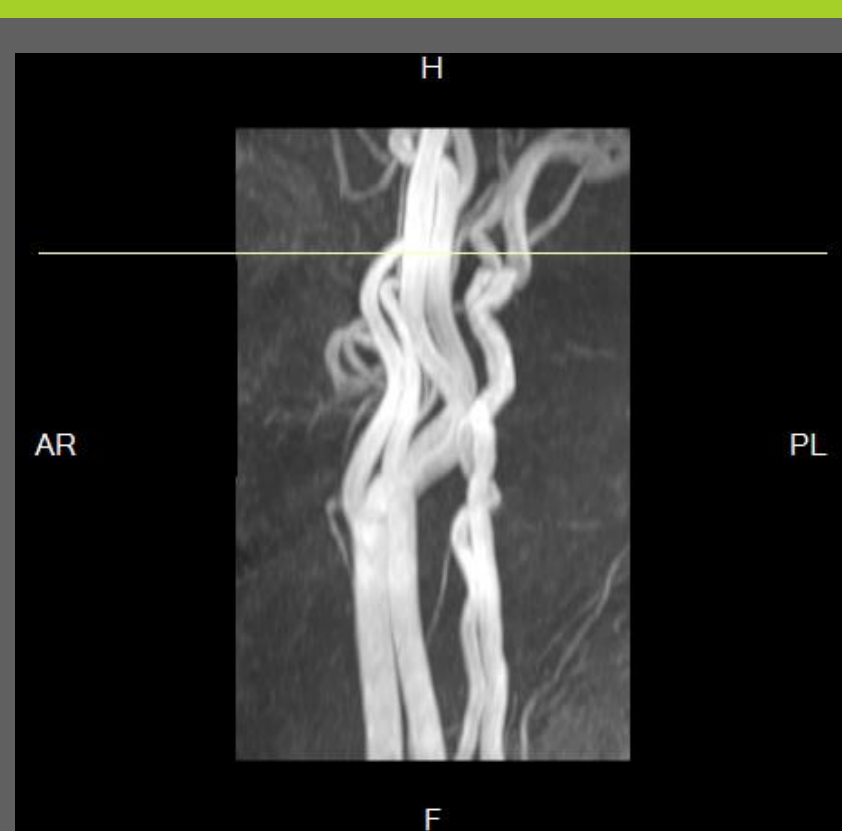
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Neck CTA or
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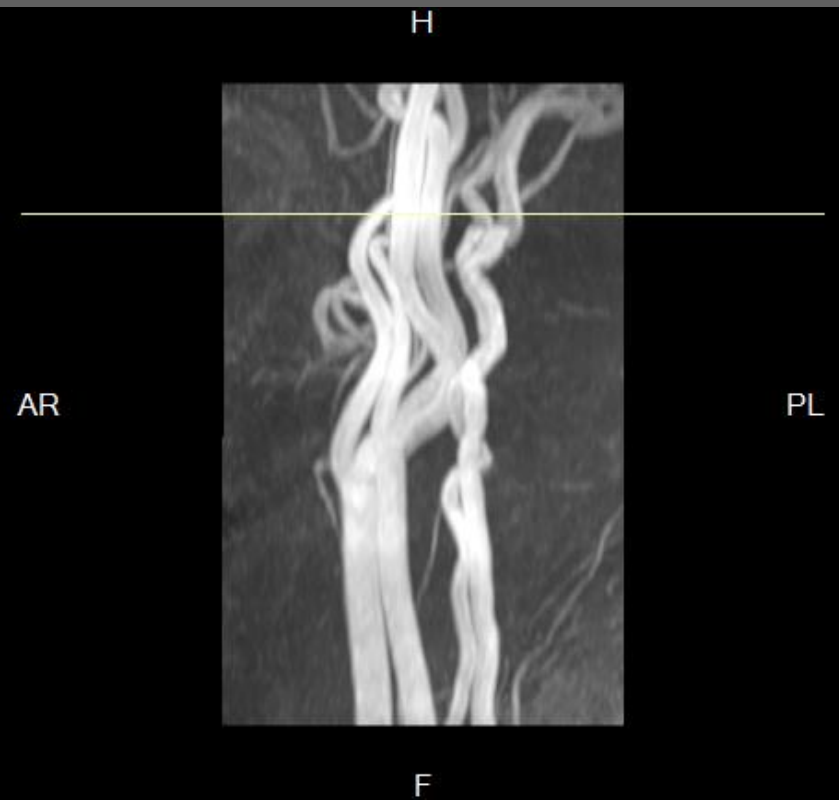
SURGICAL PLANNING

Neck CTA or
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SURGICAL PLANNING

Neck CTA or
MRA



CASE #1

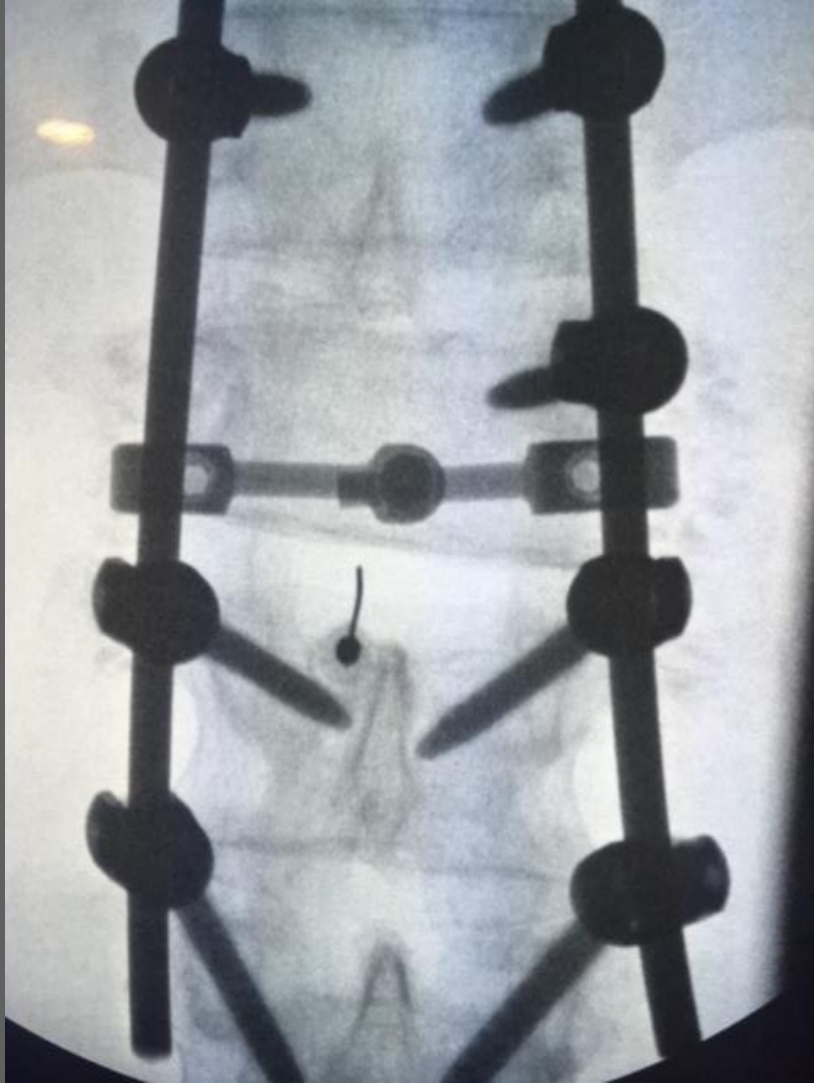
44 year old healthy male.
Parachuting accident 10/15/2017.

T11/12 fracture, left tibia/fibula
fracture, open left femur fracture,
pelvic fracture. Dense
motor/sensory deficits to T10/11.

- First injection 12/6/17 with 3 vials 5x concentrate MSC exosomes.
 - 3 Weeks post injection severe burning pain in lower extremities. Responded well to Gabapentin 600 mg TID
 - 4 Weeks post injection sensory returning in thighs along with pressure and proprioception down through lower lumbar segments
- Second dose of exosomes 1/25/18, 3 vials 5x concentrate.
 - Within 7 days his burning pain improved and motor function dramatically began improving. Particularly hip flexors/adductors and quadriceps

10/16/2017 5:56:23









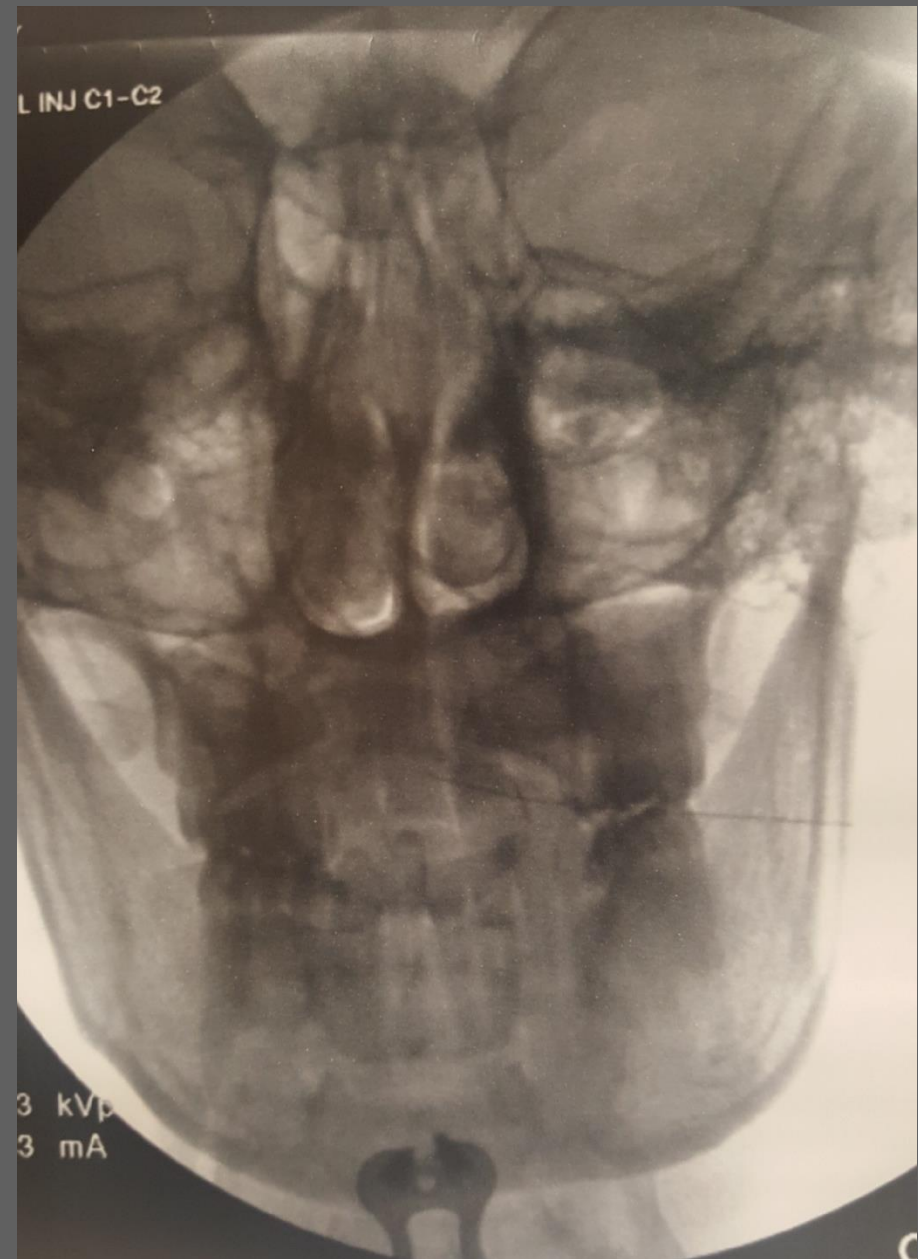
CASE #2

19 Year old male. Boating accident 8/2015. Spinal Cord Injury.

Multiple prior stem cell injections with bone marrow. Status post cervical fusion C4-C6. All previous injections in the lumbar cistern.

Patient had some improvement including new bladder continence with limited ability to ambulate within an exceptional assistance for few steps.







CASE #3

28 year old male. Spinal cord injury
C₄ to C₆ on 3-25-16 secondary to
motor vehicle accident.

C₄ Asia C

3/21/18 C₁/2 Intrathecal and IV
Exosomes



- Status post scalp injection
with PRP and exosomes
- Major complaints:
Allodynia
Extreme spasticity/Tremors
Dysautonomia with bladder
catheterization
Poor Mobility – Motorized
Wheelchair



Pre



5 Weeks Post



CASE #4

59 year old male

Parkinson's Disease for 11 years

2/22/18 – C1/2 Intrathecal and IV Exosomes

Previously: 11-12 pills Carbidopa/Levodopa a day

Now: 5-6 pills Carbidopa/Levodopa a day

Decreased usage of cannabis (augments dopamine)

INTRANASAL DELIVERY – A NEW FRONTIER

- Using the intranasal delivery system, researchers have reversed neurodegeneration and rescued memory in a mouse model of Alzheimer's Disease.
- Intranasally administered therapeutics reach the CNS via the olfactory and Trigeminal neural pathways.
- Cerebral neurogenesis was induced in the subventricular zone of adult mice after intranasal administration of FGF-2

NANOSUSPENSION – A NOVEL DRUG DELIVERY

- Following intranasal application studies in rodents show:
 - NGF
 - Insulin-like Growth Factor – I (IGF-I)
 - Insulin
 - Cytokine Interferon – β -1b

Are rapidly transported to the olfactory bulb, brainstem, brain, and spinal cord.

INTRANASAL DELIVERY

- Intranasal Fibroblast Growth Factor 2 increases neurogenesis in the olfactory bulb and subventricular zone of normal mice and in the subventricular zone and hippocampus of rats subjected to transient focal ischemia.
- In adult cynomolgus monkeys interferon β -1b is transported to many different brain areas within an hour.
- Intranasal insulin and melanocortin are detectable in human CSF less than 30 minutes following administration.
- The olfactory neural pathway provides both an intraneuronal and extraneuronal pathway into the brain.
 - Intraneuronal – Axon Transport, hours to days = Slow
 - Extraneuronal – Perineural Channels, minutes = Fast
- Important formulation considerations:
 - Therapeutic Dose <25 mg/dose
 - Dose volume 0.05-0.15 mL/dose

THE DOPAMINE HYPOTHESIS OF DRUG ADDICTION

- Dopamine Transmission is deeply affected by drugs of abuse.
- In humans dependent on cocaine, heroin, and alcohol:

Decreased Dopamine Receptors

Decreased Release of Endogenous Dopamine in Ventral Striatum

= Visual proof of the “dopamine-impoverished” addicted human brain.

Therefore the mesolimbic dopamine system is hypofunctional in addicted brains.

- Yielding decreased interest in non-drug related stimuli
- Increased sensitivity to drug of choice
- Restoring dopaminergic function may be therapeutically advantageous.

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