Neurodegeneration

Lecture 17 The Aging Brain

Cognitive decline during normal aging: inductive reasoning; spatial orientation; perceptual speed; numeric ability; verbal ability; verbal memory.

Some cognitive abilities do not decline much, like autobiographical memory[自传记忆], emotional processing and some implicit (unconscious) memories[无意识记忆].

Even in non-disease cases, brains shrink with age:

Ventrical loss of neurons tissue, synapses degeneration.

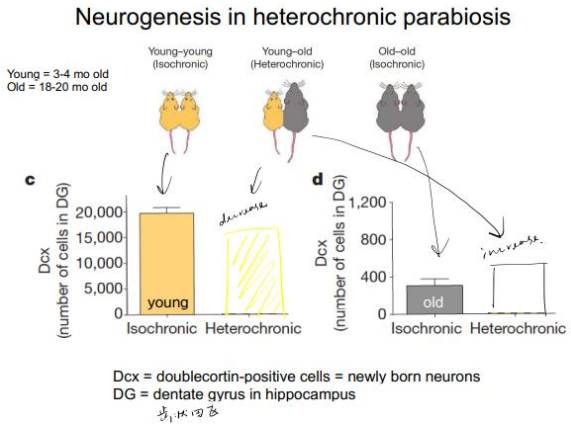
Some loss of neurons, but more loss of myelin[髓磷脂], dendrites[树突] and synapses[突触].

Aging synapses [突触老化]

Synapse morphology changes with age

Effects of aging on neurogenesis

Molecules involved in cognitive aging can be studied using **parabiosis[共生体]**



Certain molecules in young blood can **"rejuvenate[返老还童]"** old mice

Revitalized. Linking an old mouse to the circulation of a young mouse or injecting the animal with a protein called **growth differentiation factor 11** reversed signs of aging in muscle and the brain.

• There are some cognitive declines with age
• Some loss of **neurons** and **glia cells,** but also alterations to **synapses**
• Neurogenesis decreases with age
• Factors found in the blood can affect the rate of neurogenesis in young or old mice

Normal, healthy older people can still form new memories and learn new tasks

Neurogenesis and plasticity are still present, but at reduced rates

Pathological[病理] changes to the brain can lead to dementia in the elderly

Neurodegeneration

Neurodegeneration is defined by **progressive loss of neurons and/or neuronal function.**

Protein aggregation and prions

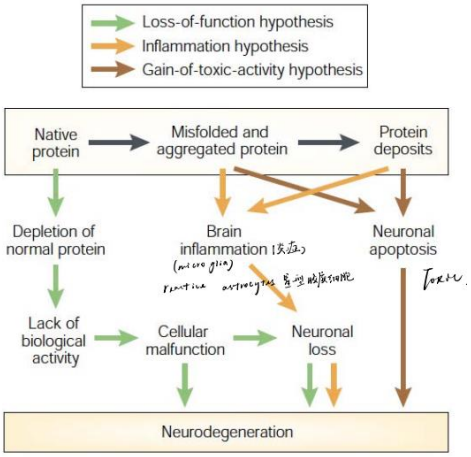
Protein aggregates in neurodegenerative diseases

Alzheimer's plaques and tangles
Parkinson's Lewy bodies
Huntington's intranuclear inclusions
Prion amyloid plaques
Amyotrophic lateral sclerosis aggregates

Misfolded proteins can aggregate together

Native protein monomer →Misfolded intermediate monomer
→soluble oligomers (2~10 proteins), β-sheets, toxic
→insoluble protofibrils[不溶性原纤维], not dyed by Congo Red
→fibrils, structure also known as amyloid fibrils or aggregates. Dyed by Congo Red, characterized by beta-sheets.

How does protein aggregation contribute to neurodegeneration?



LOF hypothesis:

Native protein → Depletion of protein → Lack of biological activity, cellular malfunction, neuronal loss → Neurodegeneration

Inflammation hypothesis:

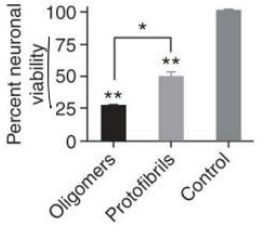
Misfolded and aggregated protein / protein deposits → Brain inflammation → Neuronal loss → Neurodegeneration

Gain-of-toxic-activity hypothesis:

Misfolded and aggregated protein / protein deposits → Neuronal apoptosis → neurodegeneration

Oligomers are thought to be the most toxic to neurons

Mouse cortical neurons in culture treated with b-amyloid oligomers for 48 hours:



Could an **antibody** bind and inhibit oligomers in multiple diseases?

Oligomers are the main toxic form and they share structural similarities, regardless of if it is Ab (Alzheimer), a-synuclein (Parkinson) or Htt (Huntington). Could you make an antibody that can bind all of these oligomers and help impair protein associations?

W20 is a small fragment of an antibody that binds **the beta sheets of many different oligomers**, W20 antibody improves memory impairments in mouse model of Alzheimer disease W20 antibody also improves motor impairments in mouse models of Huntington and Parkinson disease

Prions

Prions are proteins that are found in **two folded forms**

The abnormal, misfolded form can **cause the correctly folded versions to change conformations and aggregate**

In other words, prions are infectious proteins which lead to protein aggregation

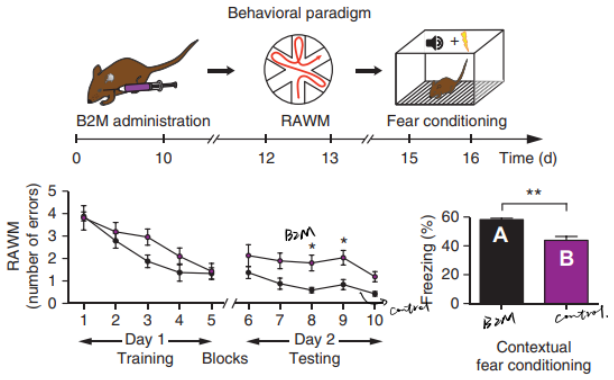
The first prion that was discovered (PrP) causes transmissible spongiform encephalopathy[传染性海绵脑病] (Mad cow disease, Creutzfeldt-Jakob disease in humans)

Can protein aggregates spread throughout the brain in a **prion-like mechanism**? Or are there other factors at play (inflammation, etc) that affect some neurons before others?

Practice problem

Using parabiosis, researchers found a protein B2M, which increases in the blood of old mice (and humans). B2M impairs neurogenesis in the dentate gyrus.

In this experiment, they injected B2M (or vehicle) into the blood of young mice and had them do the radial arm water maze (RAWM) and fear conditioning. Label the lines in the RAWM results: which mice were injected with B2M and which are the controls? Label the axes for the fear conditioning results: which received B2M?



Summary

- Old brains can still make new neurons and undergo synaptic plasticity, but not as much as young brains, some of this may have to do with **molecules circulating** in the blood **(parabiosis)**
- Changes associated with normal aging
  - Cognitive decline, slower response
  - Loss of neurons and glia
  - Altered synapses and decreased synaptic plasticity
  - Decreased neurogenesis
- Normal aging causes changes to synapse structure, loss of brain volume in some areas, decreased cognitive abilities Some of this may have to do with molecules circulating in the blood (parabiosis)
- Neurodegenerative diseases lead to major loss of neurons, leading to impaired cognitive function
- Neurodegeneration often involves protein aggregation

  Some proteins **aggregate** when they are misfolded (and non-functional). **Oligomers** are usually the most toxic. Aggregates/oligomers can lead to **inflammation and cell death**.
- Misfolded proteins might be able to spread from cell to cell, causing proteins in the native conformation to change to the misfolded version

  Example: Prion protein, which causes Creutzfeldt-Jakob disease

Lecture 18 Huntington Disease

- Age of onset usually 35-45 years old, but can start earlier or later
- Earliest physical signs of HD are difficulty maintaining grip and chorea – involuntary body movements
- Fine motor skills impaired and eye movements abnormal, slurred speech
- Motor impairments may be preceded by mild depression, anger, psychosis[精神病]
- Difficulty concentrating and multitasking, dementia and memory loss
- Fatal within 10-20 years

Neurodegeneration in striatum

Neuron loss starts in striatum in basal ganglia

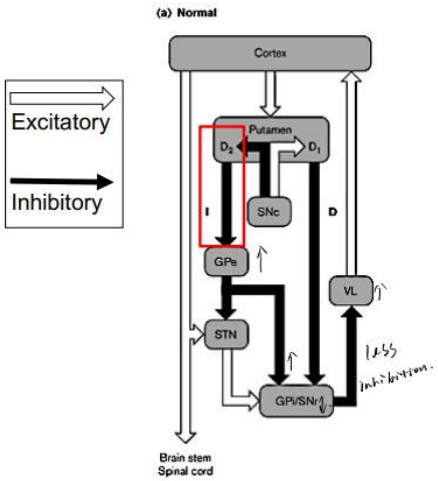
Neurodegeneration in **striatum** detectable years before symptoms start

Loss of **medium spiny GABAergic striatal neurons[中等多刺 GABA 能纹状体神经元]** causes increased output to motor cortex

Neurodegeneration "spreads" into cortex as disease progresses

Huntington disease affects the basal ganglia[基底神经节]

In HD, striatal D2R neurons in indirect pathway selectively die，When the D2 neurons die, neurons in GPe will be more active and neurons in the thalamus (VL) will be more active.



In HD, striatal neurons in indirect pathway die, so less inhibition of GPe, Less inhibition of thalamus, so more excitation to cortex → hyperkinetic disorder

Huntingtin gene and protein

Huntington disease history

The first comprehensive description of HD was published in 1872 by George Huntington who had three generations worth of studies

Described autosomal dominant inheritance[常染色体显性]

Many of the early studies on HD were conducted and funded by leaders of the eugenics movement[优生运动]

Finding the Huntingtin gene

The Wexler family was affected by HD and several biologists in the family worked to figure out the genetic cause of HD

Nancy Wexler studied families in Venezuela[委内瑞拉] where HD was more common and affected individuals earlier

Through genetic linkage analysis, they found the huntingtin (htt) gene on chromosome 4

Huntingtin (HTT) protein

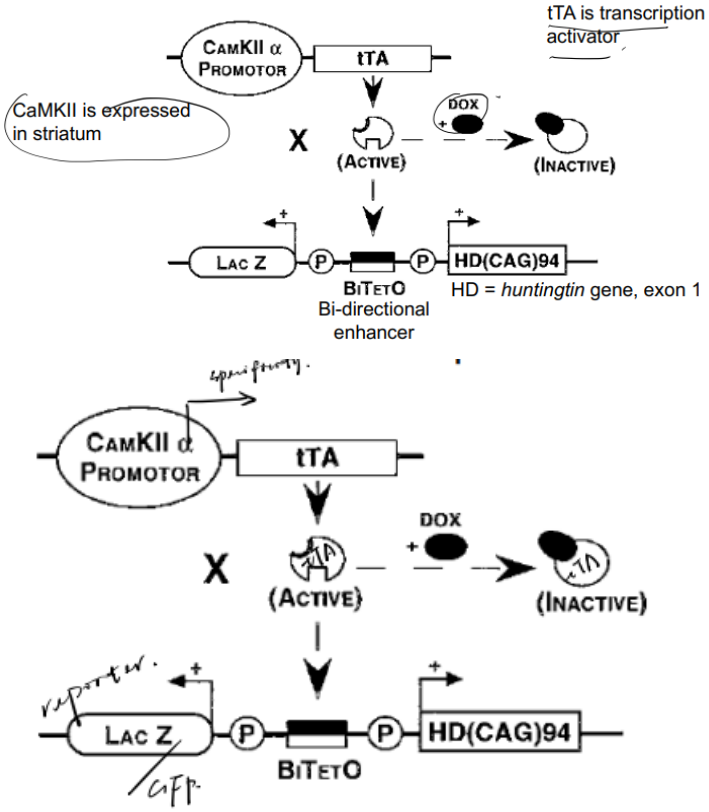
PolyQ = polyglutamine repeats encoded by CAG codon

If repeat number is > 40, then this is the mutant form of the protein which causes HD

CAG (glutamine) repeats cause protein aggregation

CAG repeats can expand during DNA replication (in spermatogonia [精母细胞]).
More CAG repeats → earlier onset

Testing connection between CAG repeats and HD pathology

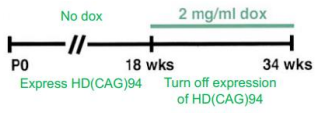


Explain the components of these transgenes. What will the researchers be able to do with the offspring of this cross?

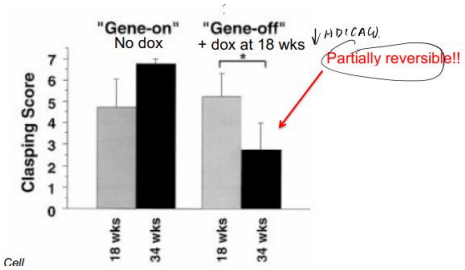
tTA is transcription activator, what is the purpose of tTA in this experiment? In other words, why not just directly attach the CaMKII promoter to HD(CAG)94?
tTA allows researchers to **turn on and off expression of HD(CAG)**

Staining for Htt shows aggregates in **nuclei and extracellularly** (brown spots)

CAG repeats are sufficient for HD neuronal pathology, smaller brains, loss of striatal neurons.
15s tail suspension test HD mice show **characteristic clasping phenotype**

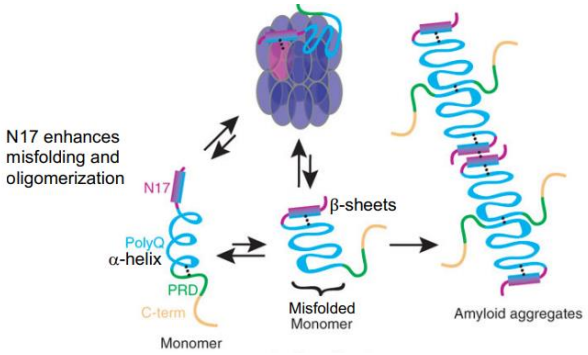


1) Mice express HD(CAG)94 for first 18 weeks
2) Give mice doxycycline to **turn off** expression of HD(CAG)94 from 18-34 weeks



Expanded polyQ increases chance of **misfolding and oligomerization**

N17 enhances misfolding and oligomerization



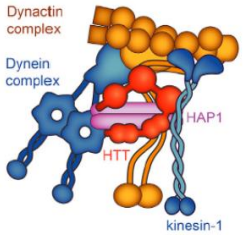
Like other neurodegenerative disorders, the oligomers are **more toxic** than the intranuclear inclusions (aggregates)

Pathogenic cellular pathways

Mutant Htt targeted for destruction by **proteasome**, but too much mutant Htt and the **proteasome is impaired**.

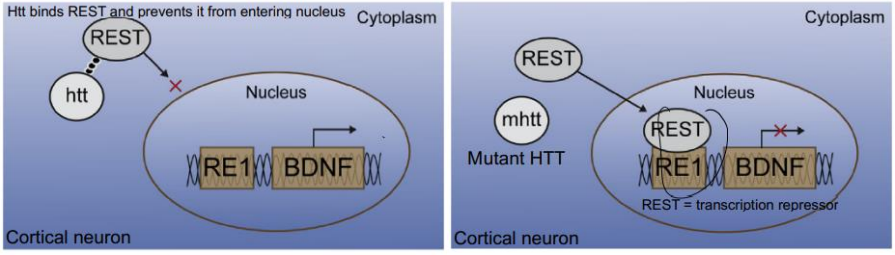
Normal functions of Huntingtin – BDNF signaling

Wildtype Huntingtin interacts with **motor proteins (transportation)**

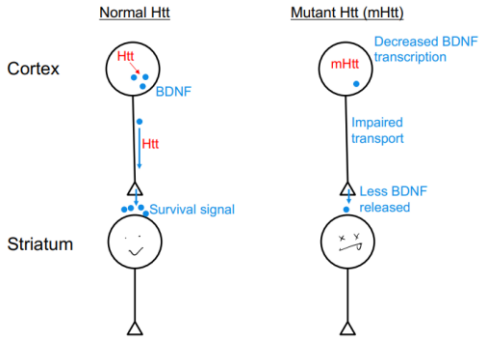


Wildtype HTT regulates **transcription factors**

BDNF = brain derived neurotrophic factor, important for neuron survival and plasticity, 50% reduction of BDNF in brains of HD patients.



HTT important for delivery of BDNF from cortex → striatum. Striatal neurons don't receive the pro-survival[促生存] signal, so this could partially explain why striatal neurons die off



Summary of cellular defects

- PolyQ expansion (encoded by CAG repeats) produces a mutant Huntingtin protein that forms oligomers and intranuclear inclusions
- Oligomers are toxic to cells, and aggregates may bind up important proteins that cells need
- Mutant Huntingtin is acting as dominant negative, so it somehow impairs wildtype HTT (dominant inheritance)
- Transcription regulation disrupted
- Axonal transport impaired
- Less BDNF to striatal neurons, which contributes to cell death
- Many other cellular functions are impaired in cells with the mutant HTT

Potential treatments – Anti-sense oligonucleotides (ASOs)

All you need to do is remove mutant Htt (patients still have a good copy). How can we do this in a patient?

Antisense oligos (ASO) are complementary to mRNA. They bind mRNA and prevent translation.

- ASO for mutant Htt improves HD phenotypes in mouse models
- ASO for wildtype Htt can reduce Htt levels in rhesus monkey brain

Currently in clinical trials (with ASO that targets both mutant and wildtype genes), Results of phase 1/2a showed **reduced levels of mutant Htt**.

Double setback for ASO trials in Huntington disease

Clinical failures of antisense candidates from two companies highlight the challenges for huntingtin-lowering approaches, but a diverse pipeline could yet provide a disease-modifying therapy.

• One trial was stopped because it made symptoms worse for patients (probably from the extra high dose that was given to get ASO to striatum)

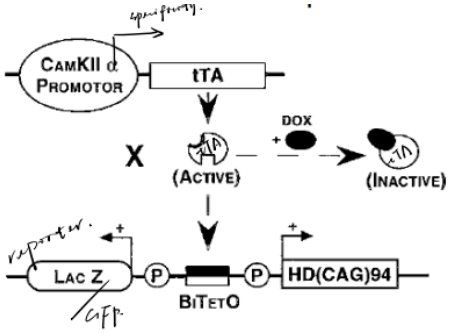
• Challenges include how to administer ASO, how often and at what doses

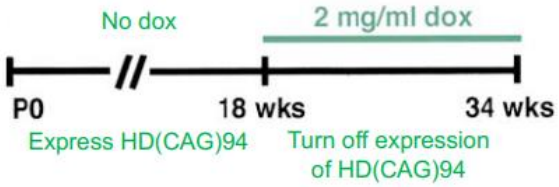
• It may also be better to start the ASO treatment before patients show symptoms (you would know they have it from a simple genetic test)

Practice problem

You want to see if antisense oligonucleotides (ASO) will help “cure” a mouse model of Huntington’s disease.

a) How would you create the mouse model? It may be similar to the experiment we discussed in class, but it doesn’t have to be exactly the same.





b) After 18 weeks, the mice are showing signs of the disease, so you inject them with the ASO. How would you make sure that the ASO only targets the mutant form of Htt? Is it important to just target the mutant form?

c) What phenotypes will you measure to test if you have cured the mice? What would be a good control?

Summary of Lecture 18

- Huntington disease causes loss of **striatal neurons**, which decreases signaling in the indirect pathway → too much excitation to motor cortex
- Hyperkinetic motor disorder – major symptom is chorea (uncontrolled movements), fine motor skills are impaired, speech difficult, depression, psychosis and eventually death
- Caused by increased CAG repeats in first exon of huntingtin gene
- CAG encodes for glutamines (Q). A long polyQ region causes protein aggregation, neuron loss and motor impairments.
- Mutant Huntingtin protein forms oligomers, and aggregates in the nucleus along with other proteins
- Wildtype Huntingtin protein helps transcribe and transport BDNF, so in mutant cells there is less release of BDNF from cortex to striatum à neuron death
- Inhibitory oligonucleotides (ASOs) might be a good way to knock down expression of the mutation mRNA.

Lecture 19 Alzheimer Disease I

AD epidemiology

- More Alzheimer's patients as population ages
- 6th leading cause of death in the United States (not including COVID)
- Physical, emotional and financial toll on caregivers
- $271 billion spent to take care of AD patients in the US
- By 2050, these costs are estimated to rise to $1.1 trillion
- It is the only cause of death in the top 10 that cannot be cured, prevented or even slowed

Alzheimer Disease (AD) is characterized by **widespread neurodegeneration**, leading to progressive deficits in memory and cognition

Typical progression of AD neurodegeneration:
Preclinical AD:
Recent episodic memory loss
Mild to Moderate AD:
Persistent memory loss
Forget personal history, friends, family
Changes in mood
Severe AD:
Confuse past and present
Lose ability to communicate

Motor impairment

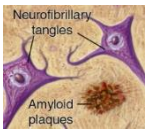
Hallucinations

Given this information, does it make sense that the ability to form new memories is lost first before long-term memories?

Traditional Diagnosis of AD

It requires: cognitive impairment & dementia

In addition, a histopathologic[组织病理学] confirmation including a microscopic examination of brain tissue is required for a definitive diagnosis.



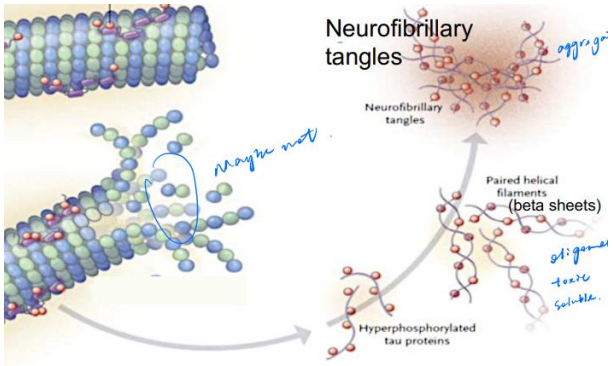
- Neurofibrillary tangles (NFT)
- Amyloid plaques

New imaging techniques can be used to visualize plaques and tangles in living patients

Neurofibrillary tangles (NFT)

- In normal neurons, **Tau protein** is phosphorylated a few times, and binds microtubules (exact function unknown)
- In the disease state, Tau is **hyperphosphorylated**.
- Hyperphosphorylated Tau releases **microtubules and forms insoluble aggregates (neurofibrillary tangles)** inside neurons

Hyperphosphorylation of Tau

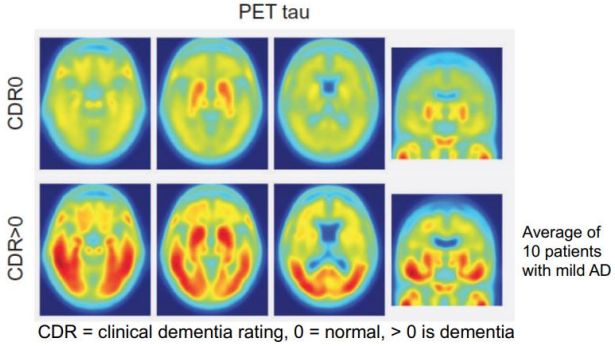


Tauopathies[陶氏病]

- Other neurodegenerative diseases show NFTs
- Tau oligomers (paired helical fibers) are taken up by human neurons in vitro
- Oligomers cause neurite retraction, loss of synapses, imbalanced neurotransmitter release and neuron death

Imaging Tau using PET scan

Inject patients with 18F-AV-1451, which binds paired-helical Tau filaments (hyperphosphorylated oligomers)



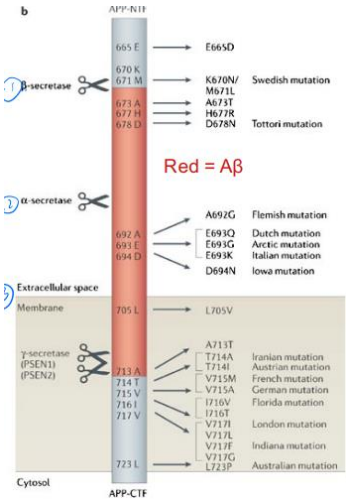
Amyloid plaques (APP and Aβ)

- Neuritis/senile[神经炎/老年] **plaques** are aggregates of insoluble proteins that are extracellular
- In AD, plaques are made up predominantly of **beta amyloid (Aβ)**
- Aβ is made by cleavage of a large transmembrane protein called **Amyloid Precursor Protein (APP)**

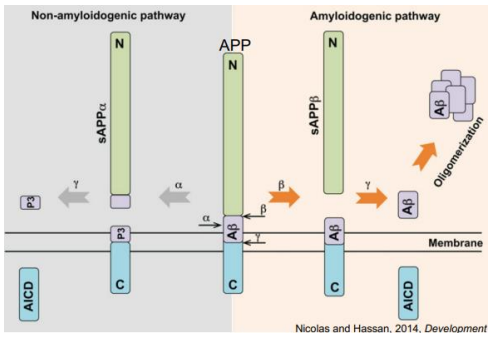
What is the normal function of APP? We don't know exactly, but seems to play a role in neural stem cell development, neural differentiation, and synaptic homeostasis/plasticity

Genetic risk factors – APP and PSEN1/2

- Amyloid precursor protein
- APP is cleaved by a number of enzymes
  - β-secretase (**BACE1**)
  - α-secretase (ADAM10)
  - γ-secretase (**PSEN1, PSEN2** + others)
- APP is normally cleaved and most amyloid beta peptides are harmless (and probably functional)
- The problem is when there is a mutation or some imbalance that results in too much **Aβ42** formation



Formation of Aβ42 fragment



Aβ fragment is different lengths (37-43 amino acids) and it is the Aβ42 form that is the most toxic (most likely to form oligomers)

Forms of Alzheimer Disease

- Familial Alzheimer Disease (aka "early onset")
  - Less than 10% of cases
  - If one parent has FAD, each child has a 50% chance of inheriting the disease (mutations in **APP**, **PSEN1/2**)
- Sporadic Alzheimer Disease (aka "late onset")
  - Most common
  - Not well understood
  - Genetic risk factors: **ApoE4**

Mutations in Familial AD

Based on the 50% risk value, what is the genetic nature of these mutations? **Autosomal Dominant**

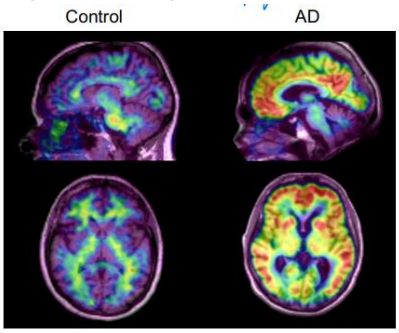
How do these mutations (**APP, PSEN1/2**) affect Aβ42 formation? More chances to make Aβ42, rather than Aβ42.

The APP gene is on chromosome 21, so what does this mean for someone with Down's Syndrome who does not have any of the known Familial AD mutations?

They will develop amyloid plaques early in life. Down's Syndrome patients have 3 copies of chromosome 21, generating more APP protein, more Aβ42, more aggregation.

Imaging amyloid using PET scan

PiB (Pittsburg compound) binds to insoluble Aβ but not oligomers



Are pathogenic Aβ oligomers transmissible?

Prions in contaminated growth hormone extracts can cause Creutzfeldt-Jakob disease (CJD, a form of prion disease) in other people. At least 226 people have died from CJD contracted from contaminated growth hormone extracts. Some of the younger patients who died from CJD also had more Aβ accumulation than you would expect at that age. At least some of the extracts did contain Aβ-42.

Ongoing questions about Alzheimer Disease

Can Aβ oligomers move from cell to cell within a patient?

Can they move from a medical instrument into a patient? Does it matter?

1. Do the Aβ oligomers/aggregates **initiate** Alzheimer disease? This is known as the Amyloid Cascade Hypothesis.
2. Or are the Aβ oligomers and the NFTs **a result** of some other widespread disease? Perhaps they are a cellular response to inflammation.

Practice problems

1. How would you make a mouse model for AD? (Focus on the Aβ)

Overexpression of mutant APP; Overexpression of mutant PSEN; Up-expression of Tau

2. What behavior impairments should the AD model mice have? How would you test for these impairments?

Memory test water maze, novel object test, fear conditioning.

socialization? movement impairment?

3. What cellular signs of AD should the AD model mice have?

NTF/ Aβ plagues

4. How would you set up a parabiosis experiment to test if circulating pathogenic Aβ can cause AD in a normal mouse?

Summary of Lecture 19

- AD is characterized by progressive cognitive impairment and dementia, as neurodegeneration spreads **from the hippocampus to the frontal lobe and rest of cortex**
- There are two cellular hallmarks of AD brains:
  - Neurofibrillary tangles (intracellular) which consist of hyperphosphorylated Tau. This prevents Tau from stabilizing microtubules, impairing axonal transport. Tau oligomers are also toxic in various ways to neurons.
  - Amyloid plaques (extracellular) which consist mostly of Aβ42, which is produced by cleavage of APP by enzymes. The Aβ42 oligomers are most toxic to cells.
- Both the tangles and plaques can be imaged using PET
- Early onset AD is rare and caused by mutations in APP and the proteases that cleave it, resulting in more Aβ42 accumulation
- Late onset AD is more common and the cause is less understood

- Some evidence that Aβ can be transmitted from outside body to brain

Lecture 20 Alzheimer Disease II

Amyloid cascade model

Practice question
Imagine you generate a very good mouse model of Alzheimer disease (i.e. high validity). Which of the following would be part of this excellent AD model?

A) Generated by expressing human APP with mutations associated with familial AD
B) Hyperphosphorylated Tau
C) Neurodegeneration in brain stem
D) Impaired memory in a water maze
E) Hindlimb clasping in tail suspension test
F) Increased Aβ42 expression

Two cellular hallmarks of Alzheimer Disease

- β-amyloid plaques made of Aβ42 from APP cleavage
- Neurofibrillary tangles made of hyperphosphorylated Tau

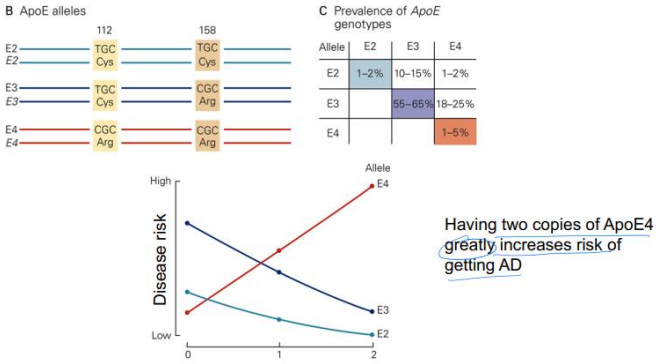
5XFAD mouse model
Overexpress human APP with 3 FAD mutations and overexpress human PSEN1 with 2 FAD mutations.
Increase in Aβ aggregates over time, progressive neurodegeneration (including hippocampus), hyperphosphorylated Tau, motor impairments (or maybe hyperactive), cognitive impairment, impaired LTP.
reactive astrocytes & microglia → inflammation

The amyloid cascade hypothesis
AD starts with the build up of Aβ oligomers → Leads to NFTs → Aβ oligomers and NFTs cause neurodegeneration
Evidence in favor:
Gene for FAD; Can detect Aβ42 early; Mouse model; ApoE4 risk allele (↑Aβ)
Evidence against:
Mouse model not exactly like human AD
PET scan: Aβ plagues not great predictor, some elder people plagues not related to dementia.
Plagues + NFT from injuries cannot be explained.
All medicines that targeted Aβ failed.

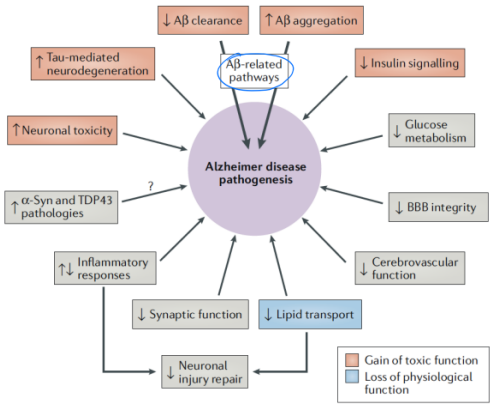
ApoE4 genetic risk factor in sporadic AD

- ApoE is a protein found in **lipoproteins**
- ApoE plays a role in **cholesterol and lipid homeostasis**[胆固醇和脂质稳态] in the CNS
- ApoE consistently is the most significant association with AD in GWAS

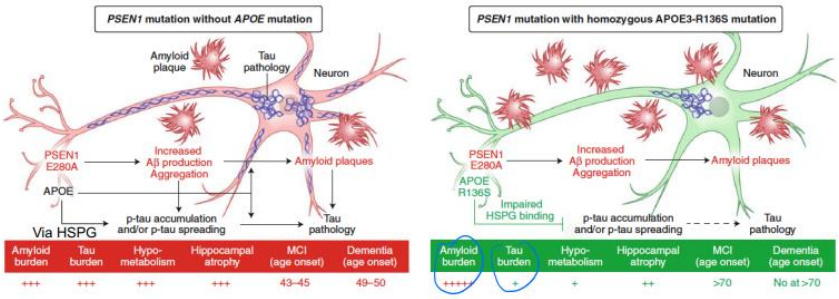
Three common alleles of ApoE defined by changes in just two amino acids



Effects of ApoE4 on AD pathogenesis



ApoE3 mutation protects one patient with **PSEN1** FAD mutation



Greater amyloid builds up than in relatives, but reduced NFTs and mild symptoms.

This mutation in ApoE3 prevents it from binding HSPG (a proteoglycan[蛋白聚糖]), which normally promotes neuronal uptake of extracellular Tau.

Neuroinflammation model

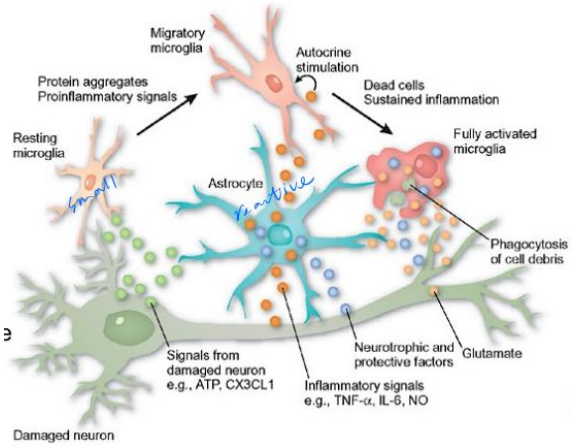
Normal functions of microglia[小胶质细胞]

Microglia are the resident immune cells in the CNS

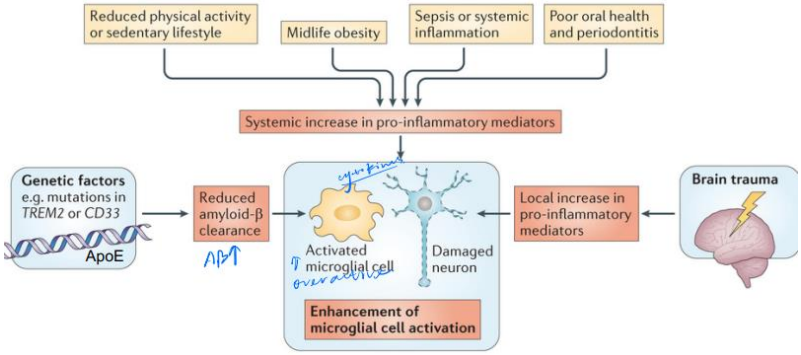
- Activated microglia have larger cell body and fewer/shorter branches
- Microglia clear out cellular debris
- Reactive astrocytes also help with immune functions

Microglia help remove amyloid beta deposits

- Overactivation of microglia can result in neuron death
- Need microglia to be just the right amount of active



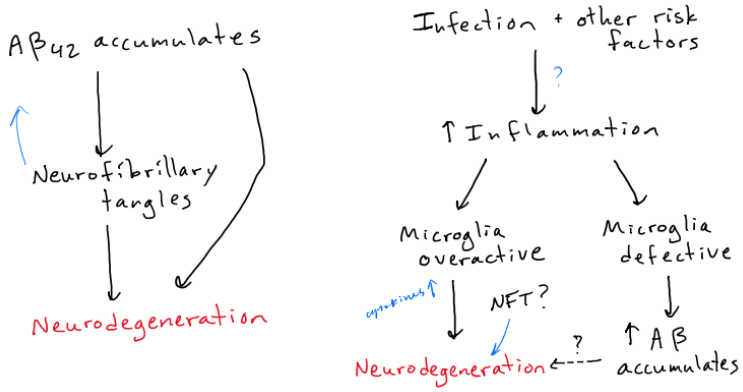
Neuroinflammation model of AD



Inflammation in the brain causes neurodegeneration.
Microglia can act as macrophages to clear away debris (like plaques), but if they are too active they cause neurodegeneration

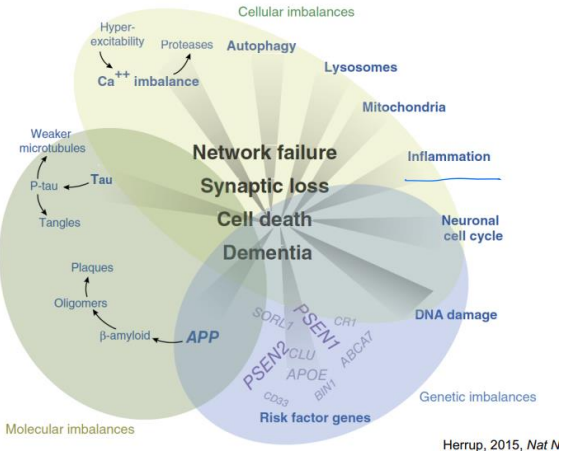
Summary of the two main models for Alzheimer disease

Amyloid cascade Vs. Neuroinflammation model



Treatments

AD is a complex disease caused by many different factors



Drugs in development for treating AD have focused on three main aspects of the disease

1. Drugs to reduce neurofibrillary tangles (NFT): Immunotherapy (Tau antibodies)
2. Drugs that reduce inflammation: Steroids and NSAIDs[类固醇和非甾体抗炎药] (ibuprofen and aspirin)
3. Drugs to decrease formation of Aβ42 or to clear it out of brain:

Targets of anti-Aβ therapy: Aβ immunotherapy

Clinical trials for immunotherapy to increase Aβ clearance: No encouraging results so far. Should we start treatment before there are symptoms? Should we combine with Tau treatments? Are we totally wrong to target these two proteins?

Monthly intravenous infusion of antibody that binds Aβ oligomers, $56,000/year and not approved by Medicare yet

Two large clinical trials demonstrated that Aduhelm lowered Aβ plaques in brain (via PET scanning). One study showed no effect on cognition, the other showed mild beneficial effects.

Common side effects of mild bleeding in brain and brain swelling

The FDA gave accelerated approval based only on the fact that it lowered amyloid burden, and did not give guidance on what stage of AD to treat

Approval opposed by the FDA independent advisory committee

Diet, exercise and cognitive training intervention

Double-blind randomized controlled trial with people 60-77 years old ~ 600 people in treatment and control groups each

Gamma stimulation (paper 8 preview)

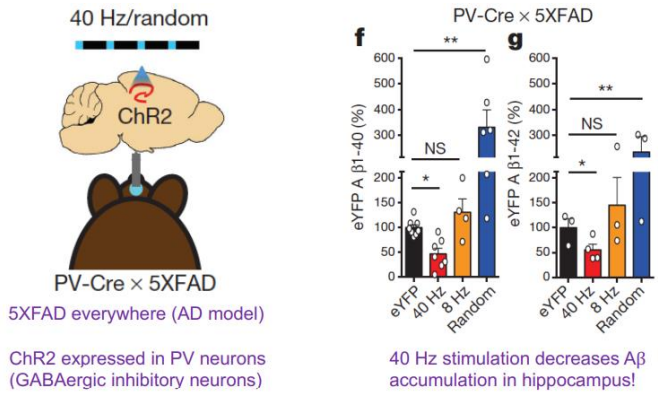
Gamma waves are reduced in AD patients and mouse models

- Gamma waves are high frequency oscillations that occur in different parts of brain (around 20-50 Hz)
- Occurs when local circuits of excitatory and GABAergic inhibitory neurons are active
- Gamma waves are reduced in AD patients and mice even **before** amyloid pathology

What will happen to AD mice if you force their cells to do gamma waves?

Use optogenetics to drive activity of neurons at 40 HZ

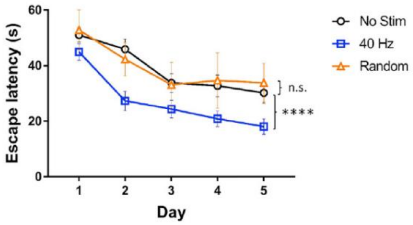
Stimulation of neurons at gamma frequency reduces Aβ production and accumulation



** Listen to Radiolab "Bringing Gamma Back" episode before reading paper: http://www.radiolab.org/story/bringing-gamma-back/

40 Hz auditory stimulation improves cognition in FAD mice, Two weeks of 40 Hz auditory stimulation for 1 hour/day

- Activates CA1 in hippocampus at 40 Hz
- Reduced Aβ-42 in hippocampus
- Improved novel object recognition and performance in water maze



Practice problem

A 70 year old woman finds out she has the genotype ApoE3/ApoE4 from a consumer gene sequencing company. There is no history of AD in her family. She demands a PET scan for beta-amyloid. Her results are shown here (colors are relative to an average of healthy controls).

You conduct a series of cognitive tests on her and she scores average compared to other healthy women in their 70s.
Will you treat her for AD? Why or why not?
Would she be a good candidate for a Aβ immunotherapy clinical trial?

Summary of Lecture 20

• Amyloid cascade model states that formation of excess Aβ42 is central to cellular pathology → causes NFTs and neurodegeneration
• Supported by the main genetic risk factor for sporadic AD: ApoE4 allele
• ApoE is found in lipoproteins and seems to play a role in clearing out amyloid oligomers and plaques (but also spreading Tau)
• Neuroinflammation model states that excess inflammation in brain causes neurodegeneration. Aβ may contribute to inflammation and impaired microglia signaling, but the main neuron killer is the immune system.
• There is not just one clear explanation for sporadic AD: there are lots of risk factors that contribute to neuron death
• Researchers are working on drugs that target Tau accumulation and decrease inflammation.
• Many companies are working on immunotherapy against Aβ but the results have been disappointing so far
• A totally different approach is using gamma wave stimulation (paper 8)

Lecture 21 Parkinson Disease

L-DOPA (levodopa): precursor for dopamine synthesis

Major motor symptoms of Parkinson Disease:

Rigidity and trembling of head

Forward tilt of trunk

reduced arm swinging

Rigidity and trembling of extremities

Shuffling gait with short steps

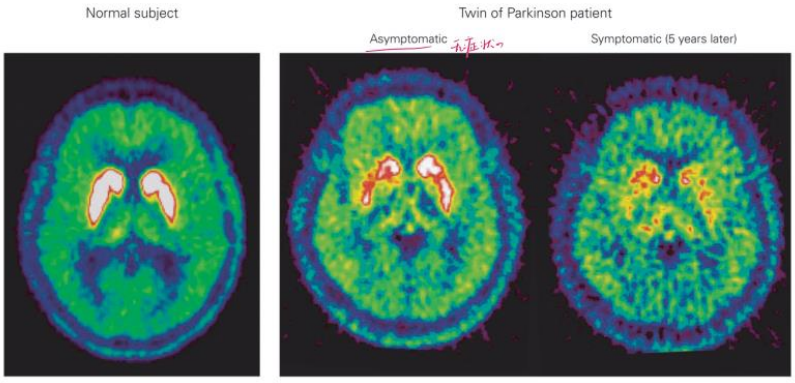
Hypokinetic disorder

Difficulty initiating movement (akinesia)

Slower movement (bradykinesia)

Loss of dopaminergic neurons in substantia nigra

Loss of dopaminergic neurons seen in PET scan

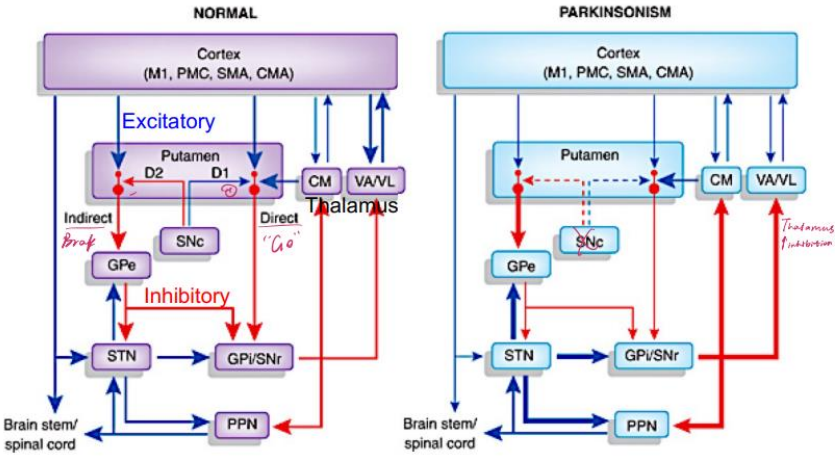


[^{18}F]Fluorodopa uptake labels dopaminergic synapses in striatum

Basal ganglia[基底节] output to thalamus is altered in Parkinson Disease

Increased inhibition to thalamus → less stimulation of motor areas in cortex

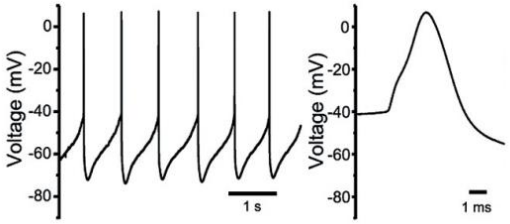
Opposite of Huntington Disease

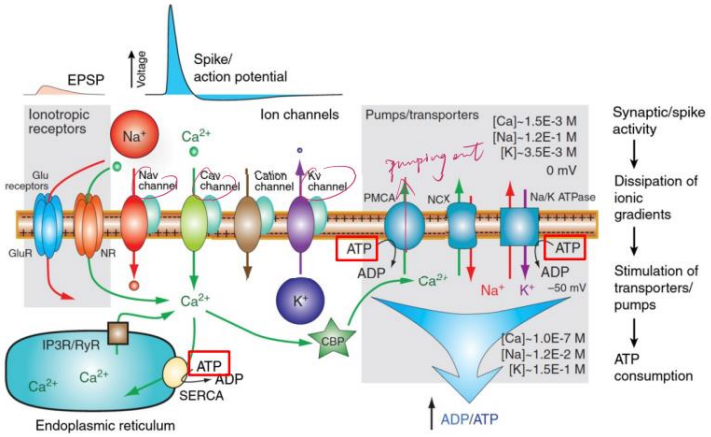


• ***Neurons in substantia nigra***

Neurons in SN are more vulnerable

Neurons in SN have intrinsic rhythmic electrical activity (i.e. pacemakers), requiring a great deal of ATP



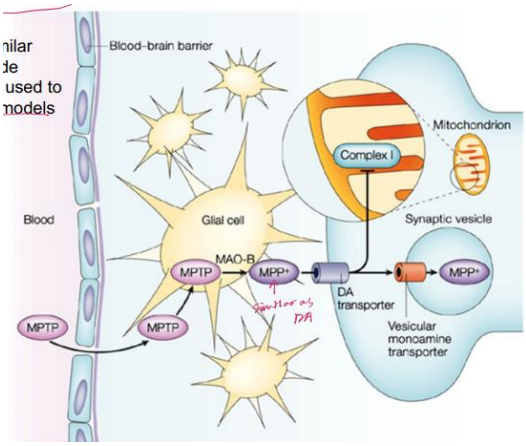


Think about what you know about making ATP. Why are the neurons in the substantia nigra particularly vulnerable to degeneration?

MPTP induces Parkinson-like symptoms

MPTP and similar drugs (pesticide rotenone) are used to **make animal models of PD**

Astrocytes convert MPTP into MPP+, which inhibits complex I in electron transport chain → ATP depleted, build up of ROS



Genetic factors: Parkin, PINK1, α-Syn

Parkinson disease involves environmental and genetic factors

environmental factors: oxidative stress, mitochondrial damage, excitotoxicity inflammation

A-synuclein, Parkin, PINK, LRRK2

Familial PD caused by genetic mutations

Cause early-onset PD (age 30-40)

Protein	Gene	Heritance	Function
α-Synuclein	SNCA	Dominant	Synaptic vesicle release
LRRK2	PARK8	Dominant	Synaptic transmission
Parkin	PARK2	Recessive	E3 ubiquitin ligase
PINK1	PARK6	Recessive	Targets malfunctioning mitochondria
DJ-1	PARK7	Recessive	Cytoplasmic sensor for oxidative stress
ATP13A	ATP13A2	Recessive	Lysosomal membrane protein

Most PD is spontaneous and not inherited

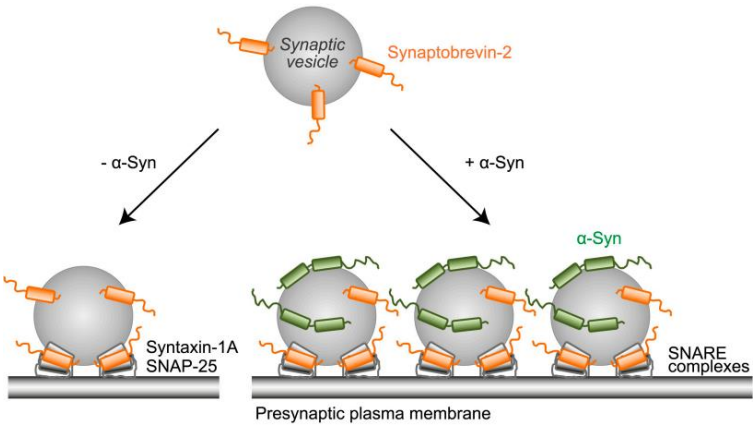
PINK1 and Parkin help cells **destroy damaged mitochondria**

- PINK1 is in mitochondria membranes
- Healthy mitochondria cleave off PINK1
- PINK1 accumulates on damaged mitochondria
- PINK1 recruits Parkin
- Parkin ubiquitinates substrates in mitochondria membrane
- Mitochondria marked for destruction by autophagosome and lysosome

Mutation in **α-synuclein** causes dominant form of familial PD

- Italian family has early onset PD (onset ~ 46 years old)
- Caused by single base pair change in SNCA that changes one amino acid
- Other mutations in SNCA have been found, and duplications of SNCA also cause early onset PD
- Genome wide association studies for sporadic PD have also pulled out SNPs in α-synuclein that are associated with disease

α-Synuclein interacts with SNARE complex to cluster synaptic vesicles at presynaptic membrane

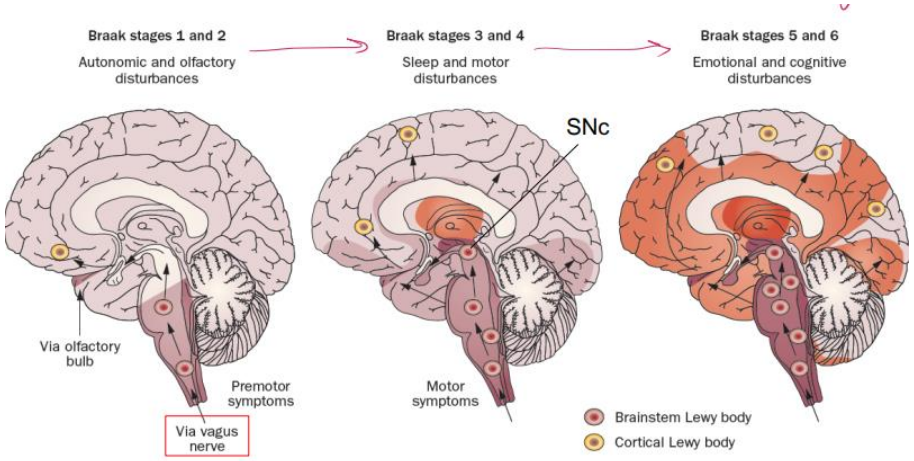


Lewy bodies

α-Synuclein aggregates into Lewy bodies

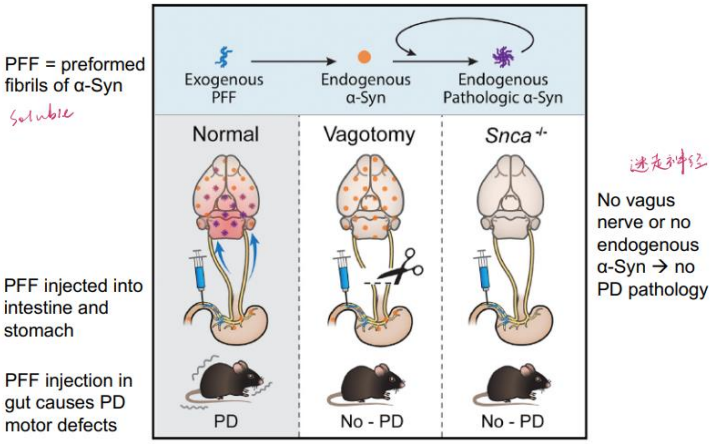
- Lewy bodies are known as “intracellular inclusions”
- Also contain other proteins like ubiquitin, Tau, and even some organelles
- As with other neurodegenerative diseases, the Lewy body aggregates may be protective to remove toxic α-synuclein oligomers

Spread of Lewy bodies correlates with severity of disease



Prion nature of α-synuclein (paper 9 preview)

Under pathological conditions, small α-synuclein fibrils can move from neuron to neuron, by exosome, direct penetration, endocytosis, trans-synaptic, membrane receptor...
α-Syn fibrils spread through nervous system from gut



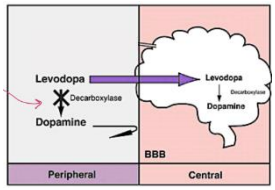
Treatments

Levodopa (L-Dopa) and carbidopa: dopamine replacement

- L-Dopa is often taken with carbidopa
- Carbidopa inhibits the enzyme decarboxylase
- L-Dopa can cross the blood brain barrier, but carbidopa and dopamine cannot

Why is L-Dopa taken with carbidopa?
Carbidopa prevents **premature dopamine synthesis** in the periphery.

Carbidopa inhibits decarboxylase in the periphery, so only convert levodopa to dopamine in the brain.



Crucial to get the dose correct, so don't have too much dopamine → dyskinesia[运动障碍]
Levodopa/carbidopa works really well to improve symptoms, but only works for 5-10 years

Deep brain stimulation: electrodes placed in midbrain nuclei

- Chronic and steady high frequency stimulation to GPi and STN seems to decrease inhibition to cortex
- Exact mechanism not understood
- Used in patients after levodopa stops working well to relieve motor symptoms

Cell based therapies

cell types trialed in humans: Retinal pigment epithelial cells; human and porcine fVM tissue; Fetal ventral mesencephalic cells; Adrenal medullary cells; carotid body cells;

stem cell sources for dopaminergic neuron differentiation: preimplantation embryo, ESCs, iPSCs, somatic cells, mesenchymal stem cells, expanded fVM tissue.

Practice problems

1) There are other drug treatments besides levodopa. Explain how these other medications could help PD patients:
   a) Dopamine receptor agonists (should they be **D1** or D2 agonists or both?)
   b) MAO inhibitors
2) A side effect of these medications, especially the receptor agonists, is impulse control disorders (like compulsive gambling). Explain why this side effect occurs with these medications.

Summary of Lecture 21

- PD is a hypokinetic disorder: slow movement, hard to initiate movement, resting tremor
- Other symptoms include impaired olfaction, constipation, sleep disturbances, ANS impairment, depression, dementia sometimes
- Progressive loss of neurons, especially dopaminergic neurons in substantia nigra (SN) in basal ganglia
- SN neurons are vulnerable to cell death: fire rhythmically → use mitochondria a lot to make ATP → produce ROS → damage to mitochondria, damage to cell
  These neurons might be more vulnerable, because they are pacemakers that use Ca+2 and they need to make a lot of ATP, which causes oxidative stress
- There are familial and spontaneous forms of PD
  Familial form caused by mutations in PINK1, Parkin, SNCA and other genes
- α-Synuclein forms oligomers and aggregates called Lewy bodies inside neurons
- Some evidence shows that α-synuclein can spread from cell to cell
- There are decent treatments that alleviate the motor symptoms
  1) Levodopa increases dopamine in brain
  2) Deep brain stimulation in basal ganglia
- Cell transplants have been tried in the past to mixed results. Dopaminergic neurons derived from stem cells in clinical trials.

Lecture 22 Multiple sclerosis

Multiple sclerosis is a demyelinating disease, what do you already know about myelin[髓磷脂]?

A) One oligodendrocyte wraps around multiple axons in the brain

B) One Schwann cell wraps around one axon in the brain (PNS)

C) Axons make the myelin and glia cells stabilize the myelin (myelin is made by glia cells)

D) Myelin prevents the leakage of ions through voltage-gated Na+ channels located under the myelin (at the nodes)

E) Voltage-gated ion channels are primarily found in the Nodes in between myelin

Review of myelin structure/function

- Oligodendrocytes[少突胶质细胞] are glia cells in the brain that myelinate axons
- Myelin speeds up action potential propagation

- Myelin made up of plasma membrane of oligodendrocyte as well as myelin-specific proteins

Epidemiology and symptoms

Multiple sclerosis basics

- Autoimmune disease[自身免疫疾病] causes chronic inflammation in CNS: B&T cells getting into central neuron system.
- Demyelination and neural degeneration
- Characterized by multiple sclerotic plaques (lesions) visualized in MRI
- Variety of symptoms: depending on the area
- Genetic and environmental factors contribute to disease Age of onset ~20-40 years old

Rates of MS are higher in the north: more vitamin D may ameliorate?

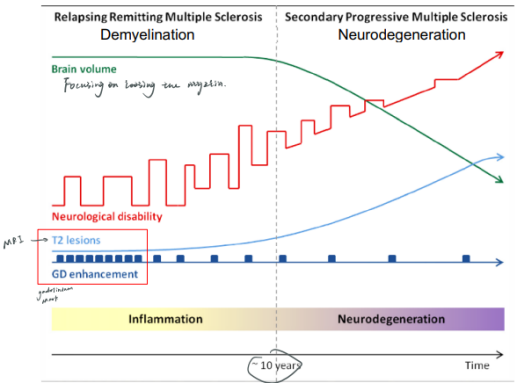
Wide variety of symptoms: numbness, tingling; cognitive dysfunction; depression; fatigue; dizziness; walking difficulty...

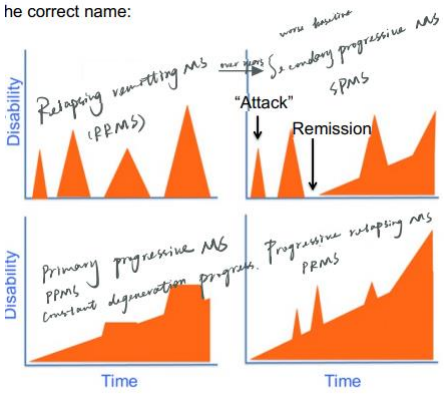
Sclerotic lesions

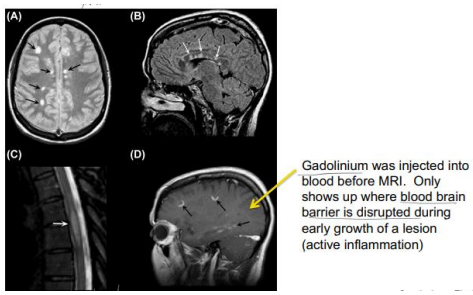
Four clinical patterns of MS progression

- Primary progressive MS[原发性进展型]
- Progressive relapsing MS[进发行复发]
- Relapsing remitting MS[复发缓解型]
- Secondary progressive MS[二级进发型]

RRMS often progresses into SPMS



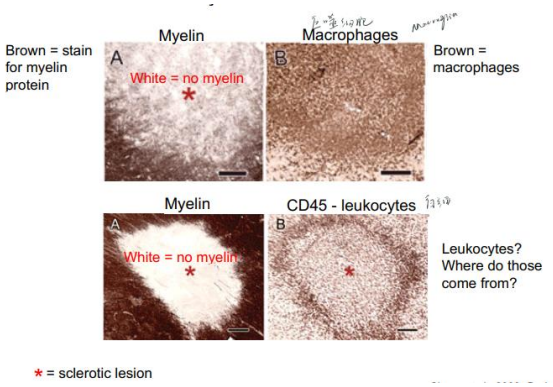




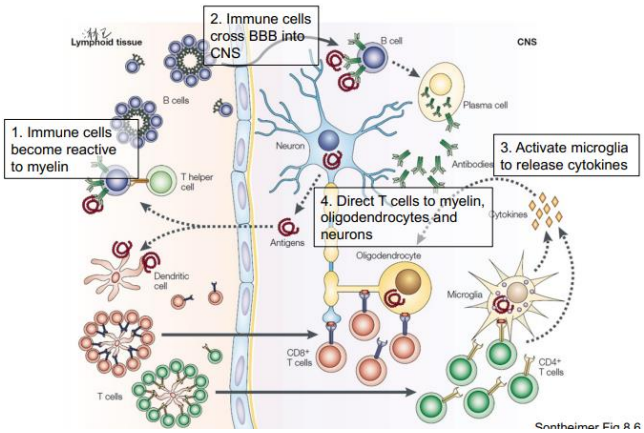
Diagnosis

1) Two incidences of neurological symptoms spaced by a few months (RRMS)
2) Multiple lesions in white matter visible in MRI (> 5mm)

Lesions contain demyelinated neurons and immune cells

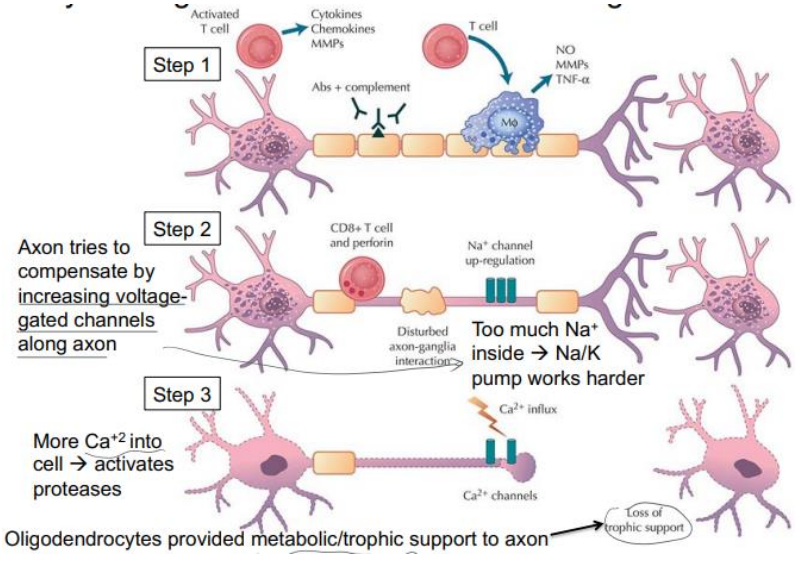


Autoimmune response

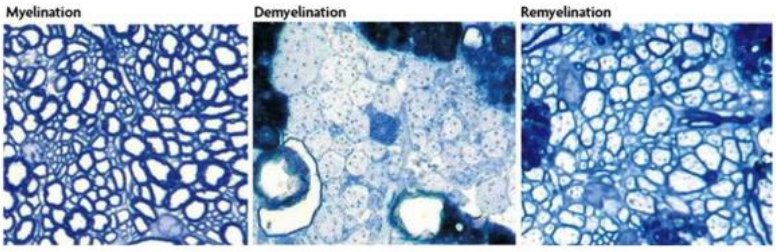


Myelin degeneration and regeneration

Myelin degeneration can lead to axon degeneration

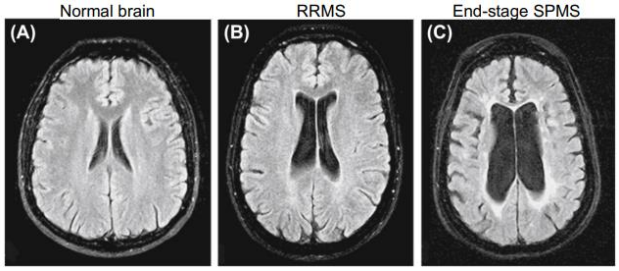


Axons can be remyelinated[髓鞘再生]

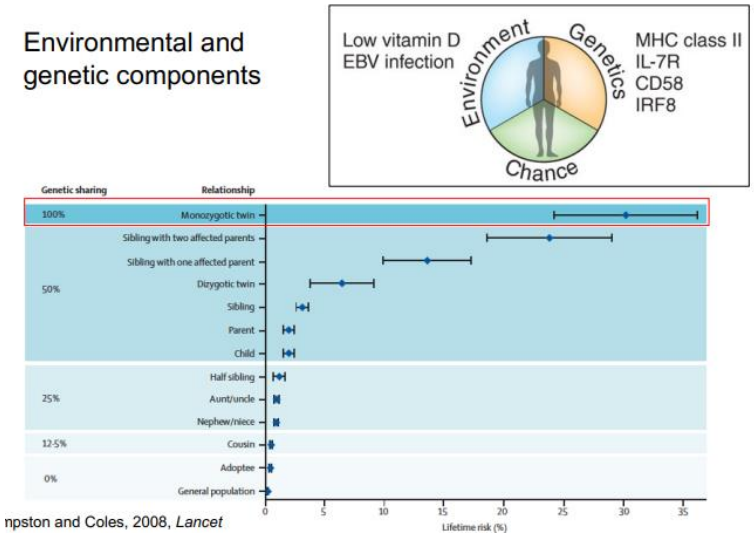


Oligodendrocyte precursor cells are recruited to damage site where they differentiate and rewrap the axon
Ability to remyelinate decreases with age
What stage of multiple sclerosis likely corresponds to the remyelination?: remitting phase

In later stages of MS, there is major axon loss. Up to 70% of axons in corticospinal tract are lost à paralysis.

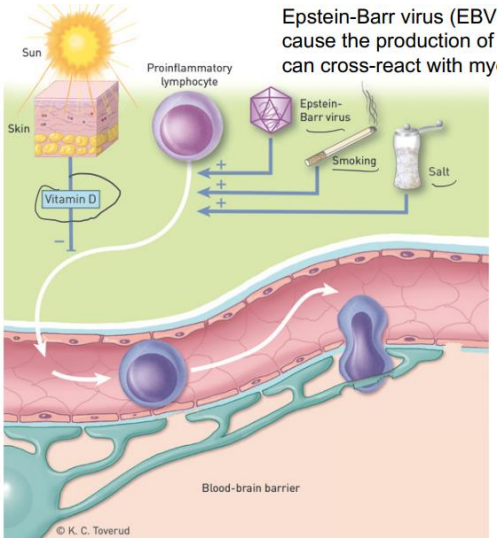


Genetic and environmental factors



HLA genes highly associated with MS

Environmental factors that are associated with multiple sclerosis: Low vitamin D associated with MS; Metabolite of vitamin D suppresses immune system.



Epstein-Barr virus (EBV) infection may cause the production of antibodies that can cross-react with myelin proteins.
EBV infection necessary, but not sufficient for MS.

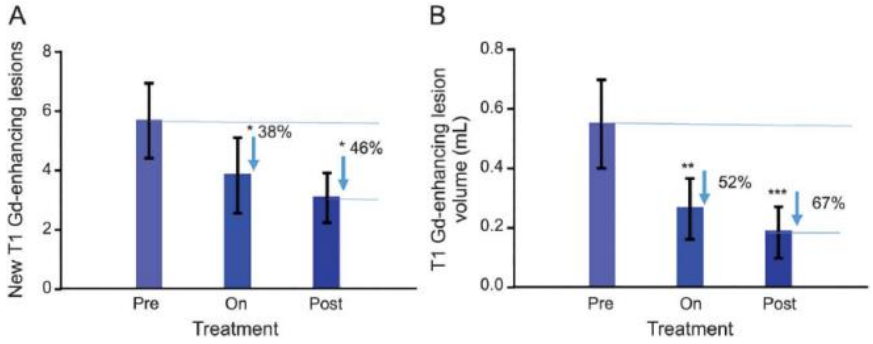
- Risk of MS increased 32x after infection with EBV, but not other herpes viruses
- 800 out of 801 MS cases were EBV-positive and had seroconverted (making antibodies against EBV)
- But 95% of people have been infected with EBV, so there are obviously other factors

Treatments

Disease-modifying treatments
Disease-modifying drugs decrease immune response and inflammation
Most target the RRMS stage

Immunotherapy for MS

- Inject a mixture for 4 small peptides from myelin basic protein under the skin
- Start with a small amount and work way up to higher concentration
- It causes **tolerance** in the immune system, so the T-cells stop attacking those epitopes



Hematopoietic stem cell (HSC)[造血干细胞] transplant

- Patient's HSCs removed, then they are given chemotherapy to kill off all white blood cells (immunoablation)
- The HSCs are grown outside the body and then transplanted back
- They will give rise to new T-cells and B-cells that hopefully are not autoreactive

Practice problem
A 35 year old woman (in good health, non-smoker) goes to the optometrist, complaining of a partial loss of vision in the right eye. She has blurred vision through the right eye, which came on suddenly in the last few days. In addition, she has lost some color vision in that eye.

Upon further examination, we learn that she had a similar problem in her left eye about a year ago, but it resolved itself within a few days and she didn't go to the doctor.
You suspect she has MS, so you ask about her family medical history and learn she has an aunt with MS. The aunt was raised in **Canada** and our patient was raised in **Florida.**
1) What are the next steps you would do to see if her vision problems are caused by multiple sclerosis?
MRI for sclerotic lesions enough.
2) How could MS cause these kinds of problems?
Optic neurons demyelination & inflammation in optic nerve.

Summary of Lecture 22

- Multiple sclerosis is an autoimmune disorder characterized by demyelination and neuron loss in the CNS
- Genetic risk factors and environmental factors both contribute to disease
  - Genetics: HLA and other immune genes
  - Environment: Vitamin D deficiency? EBV infection
- Patients produce lymphocytes[淋巴细胞] that are auto-reactive to something in myelin. These immune cells disrupt the BBB and pass into the brain.
- This leads to inflammation and myelin degeneration which causes a lesion in the brain and a physical "attack"
- Myelin can then regenerate and cover the axons again, which leads to the **remitting phase** of the disease
- Eventually, the brain is not able to remyelinate the axons as well, so axons degenerate, leading to secondary progressive MS and worsening symptoms
- Standard treatments try to decrease immune response and prevent the lymphocytes from getting into the brain.

Lecture 23 Traumatic brain injury

TBI and CTE introduction

Terminology
Catastrophic brain injury: intracranial bleeding + cerebral contusions → death
Mild traumatic brain injury (TBI) = concussion[脑震荡]
Multiple TBIs: Long-term effect
Chronic traumatic encephalopathy (CTE):

- NFTs in brain, neurodegeneration
- Impaired balance, impaired coordination, hypokinetic
- Attention and memory disturbances, depression, disinhibition, suicidal, aggression

Immediate response of concussion

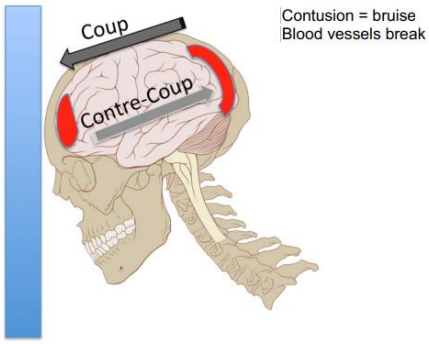
- Unconsciousness if severe TBI
- Dizziness, nausea, memory problems, headache
- Symptoms may go away after a week, or months later

TABLE 16.2 Categories of Neurological Damage following Traumatic Brain Injury

Injury mechanism	Focal	Diffuse
Primary	Skull fracture	Diffuse axonal injury
Result directly from mechanical force on brain	Cortical contusion	Petechial hemorrhage
	Focal hemorrhage	Blast injury
	Intracranial hematoma	Excitotoxicity
	Focal axonal injury	
Secondary	Microvascular injury	
	Hypoxic–ischemic injury	
Complications from primary damage	Neuroinflammation	
	Hypometabolism	
	Edema and herniation	
	Excitotoxicity	

Cortical contusion can occur at site of injury or on opposite side

Contusion = bruise Blood vessels break



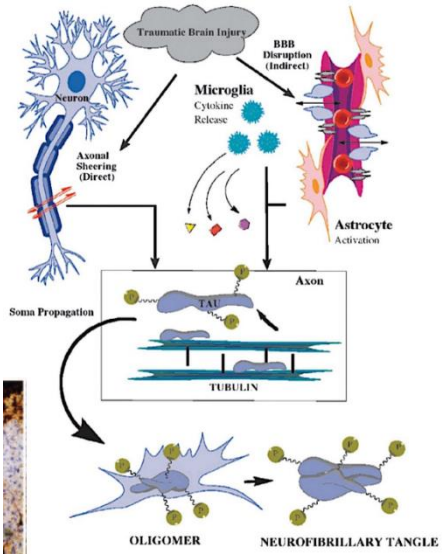
Neuropathologies after TBI

Tau pathology; inflammation; axon degeneration

Diffuse axonal injury (DAI)

Disconnection within white matter tract

Tau and neurofibrillary tangles



Primary injuries of neurons and glia lead to hyperphosphorylated Tau

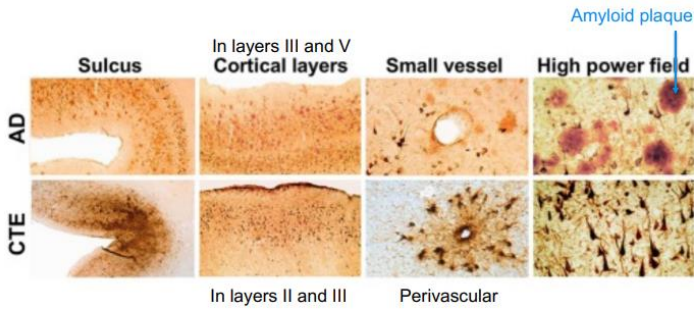
Injury causes inflammation

Neurofibrillary tangles in both neurons and astrocytes

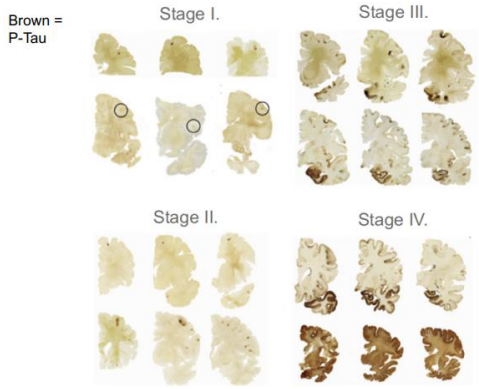
Diagnosis of chronic traumatic encephalopathy

AT: astrocytic tangle; NFT: neurofibrillary tangle

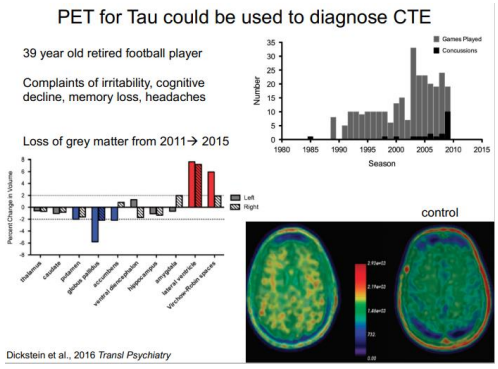
- Perivascular foci of p-tau immunoreactive ATs and NFTs
- Irregular cortical distribution of p-tau immunoreactive NFTs and ATs with a predilection for the depth of cerebral sulci
- Clusters of subpial and periventricular ATs in the cerebral cortex, diencephalon, and brainstem
- NFTs in the cerebral cortex located preferentially in the superficial layers.
  - *Tau tangles in astrocytes are rarely found in AD patients*
  - *Spread of NFTs through brain is different in AD vs CTE*



Stages of chronic traumatic encephalopathy (CTE)

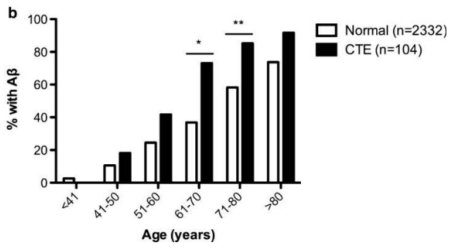


PET for Tau could be used to diagnose CTE

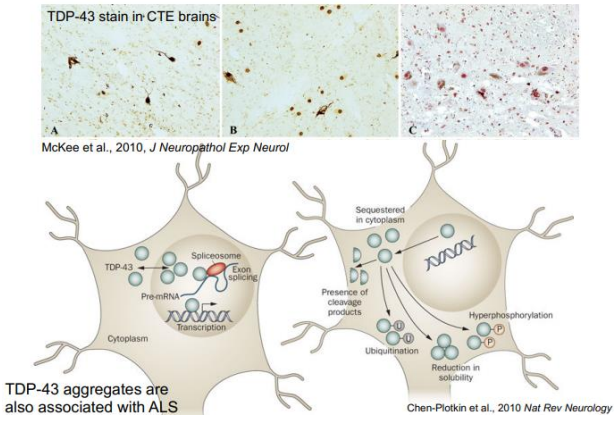


Beta-amyloid accumulation

CTE accelerates plaque formation. Associated with having ApoE4 allele.



TDP-43 aggregation



TDP-43 aggregates are also associated with ALS

How are CTE and Alzheimer disease similar? (select all the correct answers)

A. Build up of Aβ42 plaques

B. Hyperphosphorylated tau in neurons and astrocytes

C. TDP-43 aggregates in neurons

D. Later stages are characterized by dementia

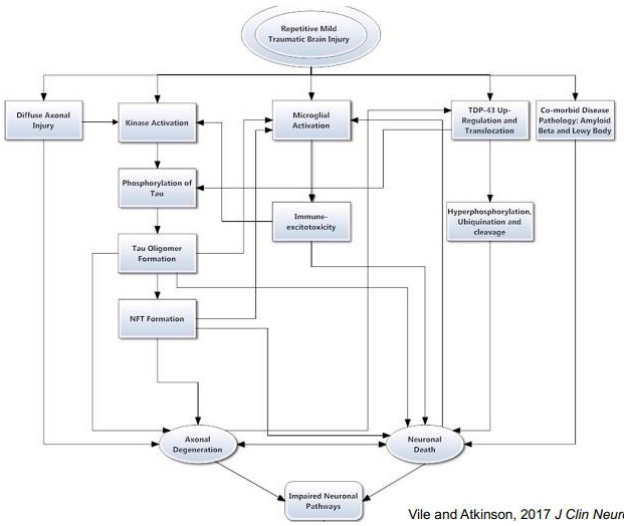
E. ApoE4 is a risk factor

F. Neurodegeneration occurs in the hippocampus first

G. Neurofibrillary tangles build up in cortical sulci

Summary of TBI and CTE mechanisms

- Severe brain injury can cause blood vessels to break open, intracranial bleeding and increased pressure, which is bad
- Even mild TBIs, though, can cause damage to axons
- Damaged axons cause hyperphosphorylation of Tau and formation of neurofibrillary tangles in neurons and astrocytes → blocks axon transport and damages cells
- Other defects associated with neurodegeneration like amyloid plaques, TDP-43 aggregates, microglia activation, excitotoxicity, etc
- Multiple mild TBIs can cause chronic traumatic encephalopathy (CTE)
- The severity of CTE is determined by the amount and location of NFTs



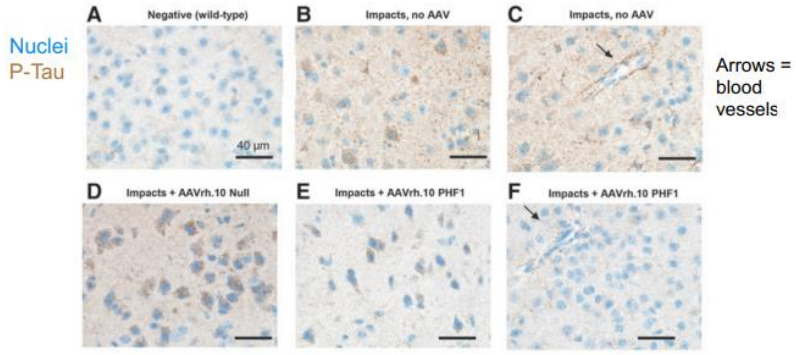
Treatments for TBI and CTE

PREVENTION!

- Wear a helmet, drive safely, don't become a professional boxer or football player (or soccer, hockey, etc)
- If you do get a concussion, take it easy and recover

Tau immunotherapy

- Mouse model of multiple TBIs → hyperphosphorylated Tau
- 3 weeks after impact, injected virus into hippocampus containing gene for anti-P-Tau antibody
- 6 weeks later look at P-Tau in cortex



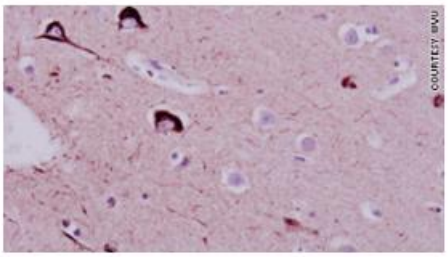
The link between NFL and CTE

Of 202 donated brains from American football players, 177 had CTE. Including 110 of 111 former National Football League players

- The NFL denied any connection between football, concussions and CTE for years
- Finally in 2016 an executive from the NFL publicly admitted that there is a link between football and CTE
- The repeated mild traumatic injuries during practices and games for years and years can lead to brain degeneration later in life
- The NFL has been sued by over 4000 former players and agreed to pay $1 billion to help former players
- They initially used a race-based scoring system for dementia that made it harder for Black players to be diagnosed with dementia, so the NFL conveniently did not have to pay them the settlement

Practice problem

Chris Henry was a wide receiver for the Cincinnati Bengals. Chris Henry died in 2009 at the age of 26 when he fell out of the back of a moving pick-up truck. This is an image of part of his cortex. What are we seeing in the image?



Chris Henry had CTE. It's unusual to see CTE in such a young person. What genetic factor(s) might have led to such an early onset?

He did hit his head in the car accident, so how could researchers determine the pathology was from CTE and not the accident?

He did hit his head in the car accident, so how could researchers determine the pathology was from CTE and not the accident?

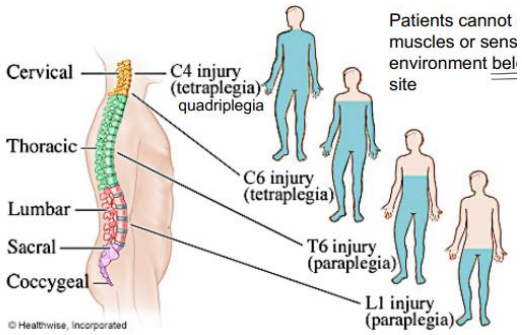
- Severe brain injuries can cause blood vessels to break open and can cause major damage to the brain
- Concussions, or traumatic brain injuries, even if they are mild, can shear axons, as seen in DTI MRI
- Damaged axons produce neurofibrillary tangles with hyperphosphorylated Tau and sometimes Aβ42 plaques, similar to Alzheimer Disease, but not exactly the same
  - The spread of NFT and Aβ42 are different in CTE and AD
  - NFTs are found in astrocytes in CTE, but not AD
  - Presence of TDP-43 aggregates in CTE
- Repetitive mild TBIs can cause widespread neurodegeneration, perhaps caused by NFTs and amyloid plaques, though the exact mechanism of CTE is just as complex and unknown as AD.

- Potential treatment could be to stop the production and spread of P-Tau. The best way to not get CTE, though, is prevention.

Lecture 24 Spinal cord injury[脊髓受伤]

Introduction to spinal cord injuries

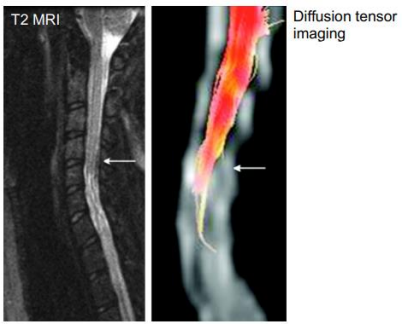
Degree of disability depends on location of damage: Patients cannot move muscles or sense the environment below injury site.



Remember that the axons of sensory neurons send signals up the spinal cord (ascending tract)
Axons from the motor cortex send signals down the spinal cord (descending tract)

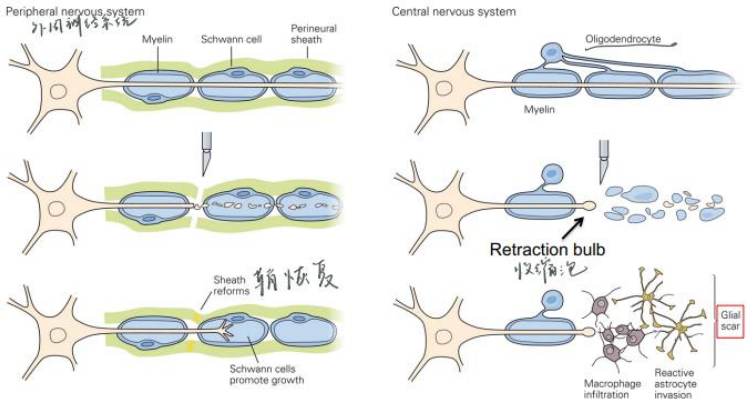
Broken vertebrae can damage spinal cord
Hyperextension of head on the neck causes cervical vertebrae to break. But it is possible to break your back (vertebrae) without damaging spinal cord. Broken vertebrae will heal.

Spinal cord injuries can be visualized using MRI



After the initial injury, secondary effects can lead to more neuron death
Primary injury: loss-of Neurons/Axons; Demyelination
Secondary injury: loss-of Neurons/Axons; Demyelination; Inflammation; Reactive Oxidative Damage and the Astrocytic Glial Scar; Cyst Formation

Axons in the CNS cannot regrow past injury
Glia in the PNS release growth promoting signals to injured neurons
Glia in the CNS inhibit axon regeneration



Reactive astrocytes and formation of glia scar in the CNS

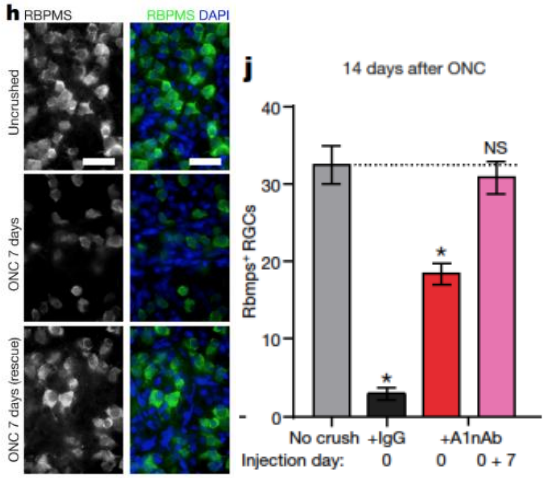
Astrocytes become "reactive" by signals released from active microglia

Reactive astrocytes are produced after a spinal cord injury. What other diseases/injuries likely cause reactive astrogliosis?

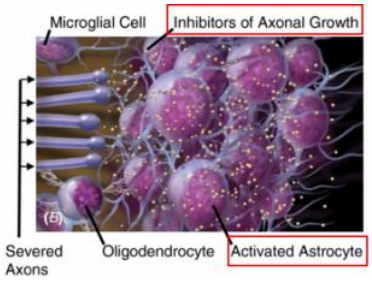
A) Alzheimer disease
B) Huntington disease
C) Parkinson disease
D) Multiple sclerosis
E) Traumatic brain injury

Inhibiting reactive astrocytes improves neuron survival after injury

- Crush optic nerve in rats
- Injected antibodies against inflammatory signals that activate astrocytes
- Fewer reactive astrocytes and more retinal ganglion neurons survive (RBPMS is RGC marker)

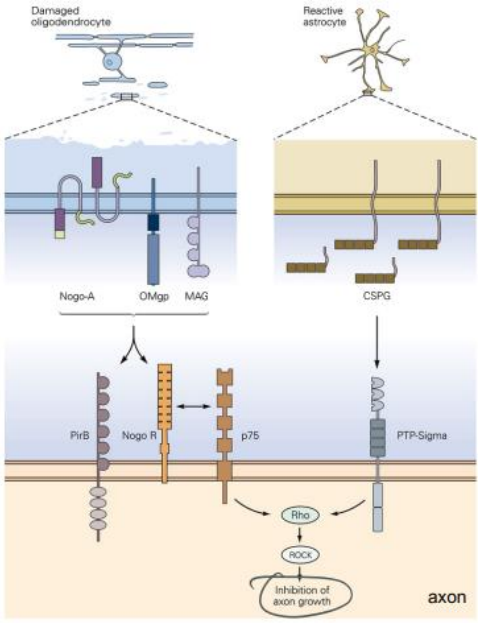


Glia and immune cells form glial scar around injury, There are both permissive and inhibitory signals in the glial scar, so it isn't all bad

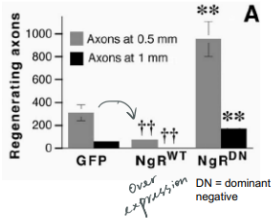


Inhibitory signals from myelin and glia

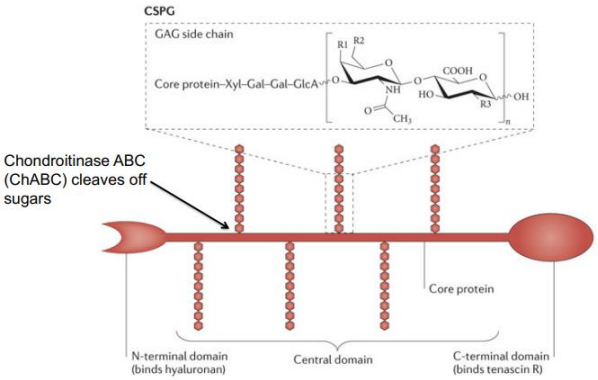
Damaged oligodendrocyte exposes myelin proteins to axon
Reactive astrocytes (and/or maybe other glia or fibroblasts) produce CSPGs



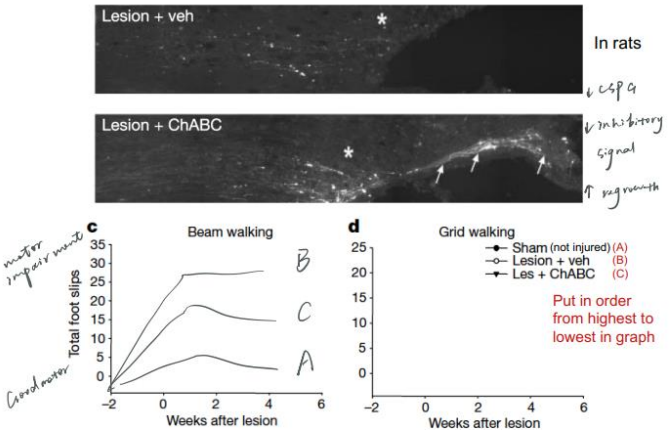
Axons regenerate more when Nogo receptor is impaired
Optic nerve regrowth after injury



Chondroitin sulphate proteoglycan



Physical and functional recovery after SCI treated with ChABC



Potential treatments to remove inhibition of axon regeneration

- Tip of nerve fibre with growth cone and exploring filopodia
- Chondroitinase ABC prunes CSPG

Clinical trials for some of these “anti-inhibition” drugs are in early stages.

Summary of SCI response

- Axons in the central nervous system have limited regenerative potential
- Astrocytes become “reactive” after injury and may secrete growth inhibitory signals like CSPGs (CSPGs may also come from other cells in the scar)
- Damaged oligodendrocytes also inhibit axons via myelin proteins, like Nogo
- The reactive astrocytes and immune cells make the glia scar, which seals off the lesion, but also creates a physical impediment to axon regrowth
- Future drug treatments for SCI will target these inhibitory signals
- Other treatments for SCI include stem cells (lecture 25), brain machine interfaces, and electrical stimulation of spinal cord

Brain machine interface (BMI), and spinal cord stimulation

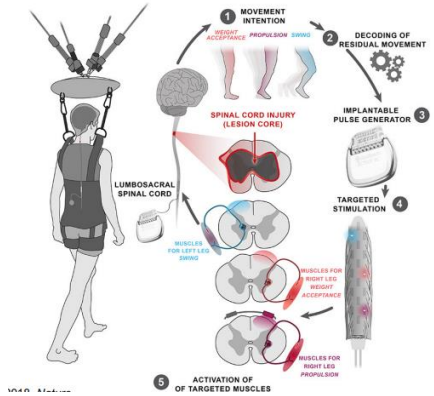
- After a spinal cord injury, the connections between the brain and muscles are broken, leading to paralysis
- Brain machine interfaces bypass the spinal cord
- Electrodes in the brain record signals from the motor cortex
- A computer decodes the intended motor output and sends signals to muscle stimulator around arm, causing arm movement

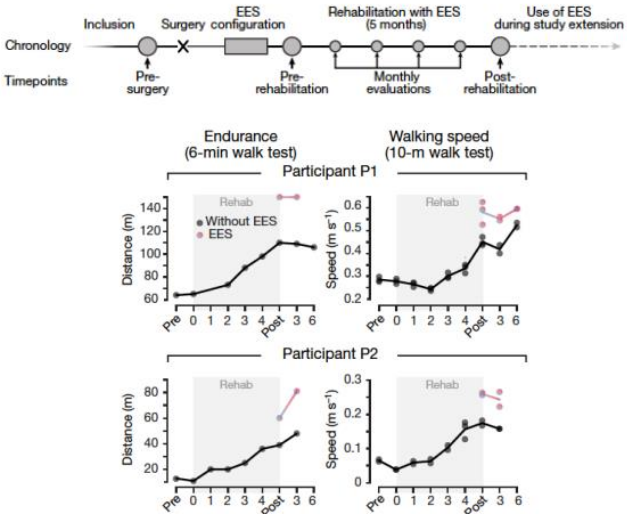
fMRI to determine which neurons control arm movement

Muscle stimulator

Intracortical electrode array

Restoring walking with epidural electrical stimulation





Practice problem

1) This patient has had a hemorrhagic stroke in Broca's area and part of the motor cortex

After the stroke, the patient is completely paralyzed on the left side of the body below the waist

Would this patient be a good candidate for a BMI?

No, because of damage to motor cortex.

What about for epidural electrical stimulation of the spinal cord?

Maybe, help walk.

2) In the last lecture, we will learn more about stem cell treatments for spinal cord injuries. If stem cells do not actually differentiate into neurons, then how could stem cells improve the symptoms of SCI patients? What might the stem cells be doing?

Summary of Lecture 24

- Injuries to the spinal cord can lead to paralysis and sensory loss below the lesion site
- SCIs cause astrocytes to become reactive, forming a glial scar
- Increased secretion of CSPGs, which inhibit axon regrowth
- Demyelination exposes the neurons to myelin proteins they don't normally "see", which activate inhibitory receptors like Nogo
- Inhibition of the inhibitors of axon regeneration could be a potential treatment for spinal cord injury
- Brain machine interface: bypass the spinal cord using a computer and stimulate muscles to make them contract
- Epidural electrical stimulation: electrodes implanted in spinal cord stimulate motor neurons in same pattern as walking

Lecture 25 Stem cells and regeneration

Neurogenesis in adults

If adults are able to make new neurons, then why can't we replace the neurons that die in neurodegenerative diseases?

Only two main sites of neurogenesis in adult brain

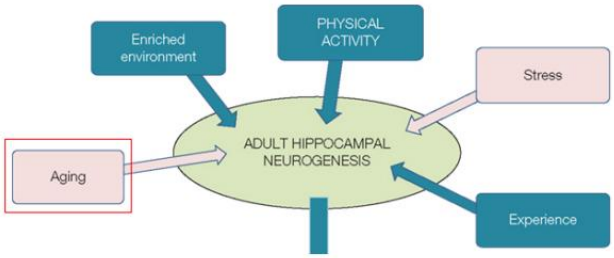
1) Subventricular zone makes neurons for olfactory bulb[嗅球]

2) Dentate gyrus[齿状回区] makes neurons in hippocampus

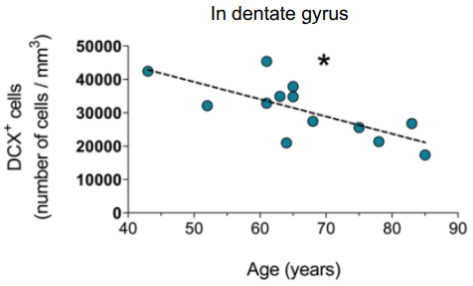
How to find the sites:

- Cancer patients injected with BrdU before they died
- BrdU incorporates into DNA during DNA replication, so it is a marker for new cells
- Saw BrdU staining in hippocampus and subventricular zone

Rate of neurogenesis affected by many factors: Enriched environments, physical activity and novel experiences increase neurogenesis, stress and aging decrease neurogenesis

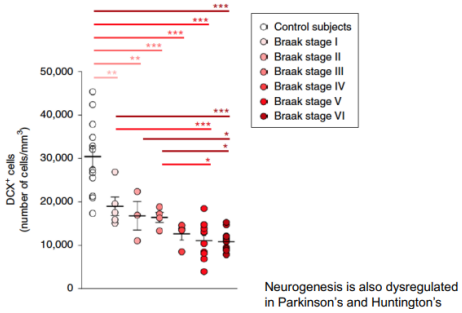


Neurogenesis decreases with age (in humans)



DCX = Doublecortin is expressed in neuronal precursors and for about 2-3 weeks as neurons mature.

Neurogenesis impaired in Alzheimer disease
DCX+ cells in Alzheimer patient brains. Classified by Braak stage – the more Aβ and NFTs, the higher the Braak stage



Potential therapeutics to boost neurogenesis

- Animal studies have shown some improvements when growth factors are injected directly into the brain
- Alternatively, could use virus to carry genes into brain
- In early phases of clinical trials

Summary of endogenous regeneration[内源再生]

- Brain can make new neurons from neural stem cells located in subventricular zone and hippocampus
- However, there are at least three reasons why neurogenesis can't prevent neurodegeneration. What are they?
  1. Rate of neurogenesis is low and only in two locations in brain
  2. Neurogenesis decreases with age
  3. Neurodegenerative diseases impair neurogenesis by impair neurogenesis
- What else can we do to boost the nervous systems' ability to regenerate? → Stem cell therapy

Can neurogenesis be used to treat neurodegeneration?

Stem cell treatments for neurodegenerative diseases
Stem cells are undifferentiated cells that can divide to make more stem cells, or they can differentiate into progenitor cells, which differentiate into neurons.

Stem cell terminology

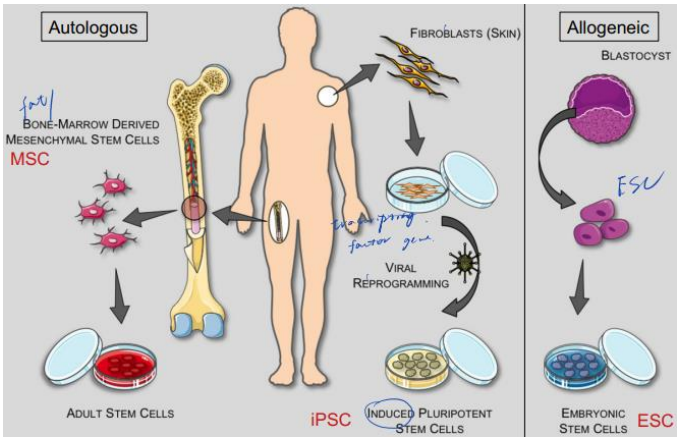
Autologous – made from patient, so don't need to worry about immune rejection

Allogeneic – from someone else (different genetic material than patient)

Pluripotent cells (ESCs and iPSCs) can become any kind of cell

Multipotent cells (adult stem cells like MSCs) can differentiate into a limited number of cell types

Sources of stem cells

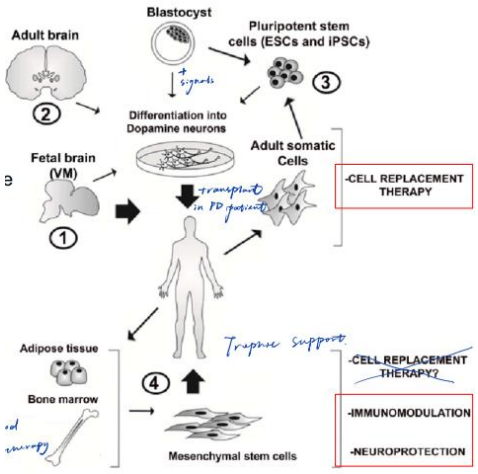


Note that all of these cells can be genetically engineered to enhance abilities.

Stem cell	Advantages	Disadvantages
ESC	pluripotent	Allogeneic potential ethical issues
iPSC	pluripotent autologous can make from stem cell	• New technology. need to study. • Epigenetic memory. (gene expression different from ESC) • time consuming for each patient.
Adult stem cells (MSC)	• Easy to get MSCs from fat) • Autologous	• Multipotent (probably ----)

Cell replacement vs Trophic support

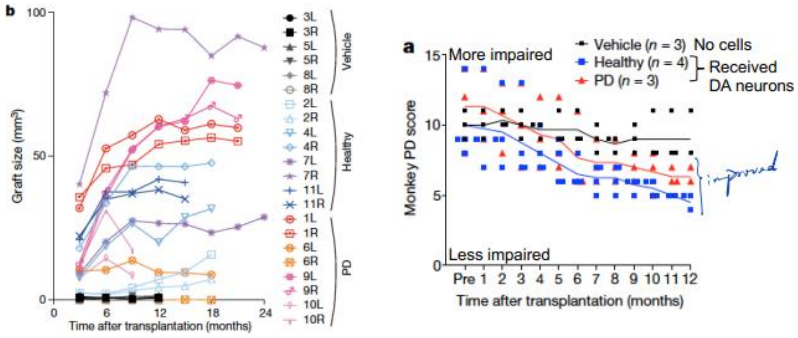
Are we trying to replace the neurons that have died off? Or do we want to protect the remaining cells?



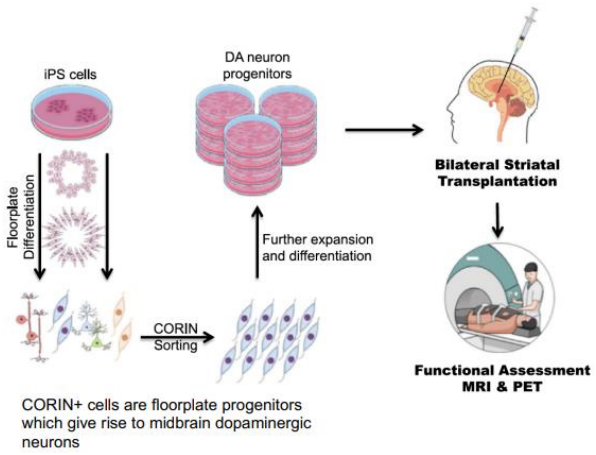
Besides Parkinson's, HTT disease would be a good candidate for cell replacement therapy (for specific neuron type death)

Cell replacement in PD monkey model

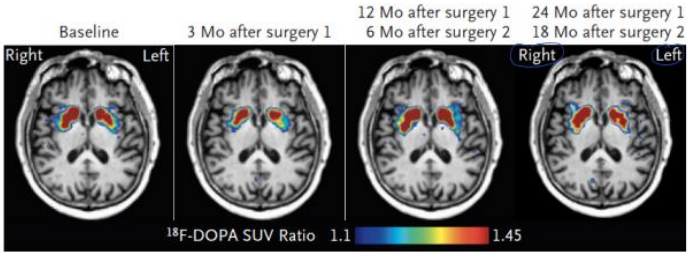
Model: Monkeys given MPTP → kills off all dopaminergic neurons in SN
Made iPSCs from Parkinson's patients and healthy controls then converted them into DA neurons
Transplanted human DA neurons into monkey striatum



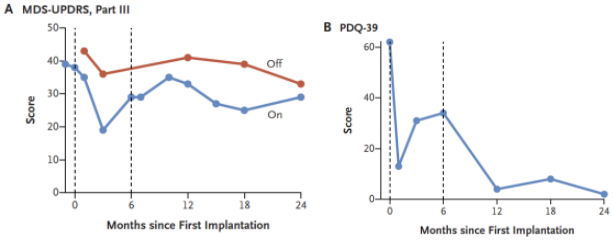
Clinical trial for Parkinson disease



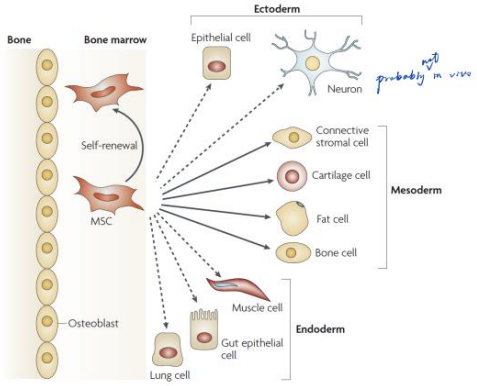
- 69-year-old patient with PD for 10 years
- Made midbrain dopaminergic progenitor cells from patient's iPSCs (autologous)
- Implanted into left putamen, then right putamen 6 months later



Improvement in motor symptoms (range: 0-132, with higher scores indicating worse parkinsonian motor signs)
Improved quality of life (Scores range from 0 to 156, with higher scores indicating worse quality of life)



Mesenchymal stem cells(MSC) repair mesoderm-derived tissue



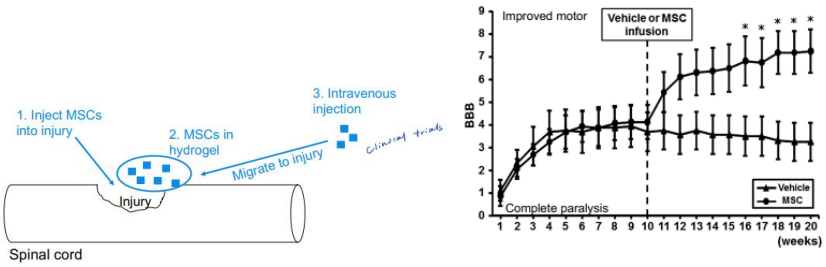
Trophic effects of MSCs on neural cells

- Release growth factors Prevent apoptosis
- Anti-inflammatory
- Support neuroblast proliferation and differentiation

MSC treatment for spinal cord injury

Rat model of spinal cord injury 10 weeks after injury, intravenous infusion of MSCs

- Improved locomotion (BBB is 21 point scale of motor behavior)
- Increased remyelination of remaining axons
- Increased regeneration of axons



Initial results from clinical trials show that MSC transplant is safe and tumors do not form.

The results of transplants are variable – sometimes symptoms of the injury improve and sometimes they do not.

Summary of Lecture 25

• Neurogenesis likely occurs in the mature brain in the subventricular zone and the dentate gyrus

• Neurogenesis decreases with age and can be impaired in neurodegenerative diseases

• Potential therapies for neurodegenerative diseases and injuries will try to boost the patient's own neurogenesis

• We use three main sources of stem cells for therapies: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs)

• Embryonic stem cells: pluripotent (can become any type of cell), easy to grow in the lab, but cells would be allogeneic (different DNA than patient)

• Induced pluripotent stem cells: pluripotent and can be made from patient's cells, so autologous, but involves viral reprogramming and may have epigenetic changes from original cell

• Mesenchymal stem cells: multipotent (limited potential), can be isolated from patient. Improve regeneration environment, but probably not becoming neurons.

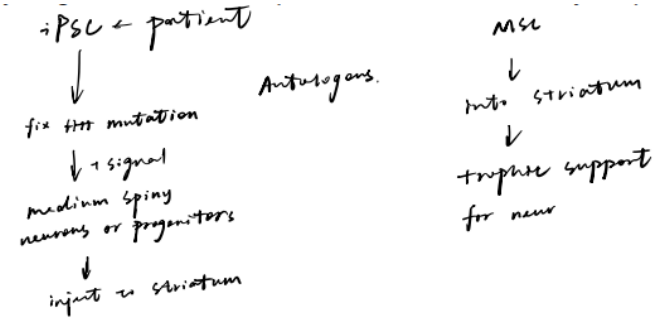
Lecture 26

Practice problem-0

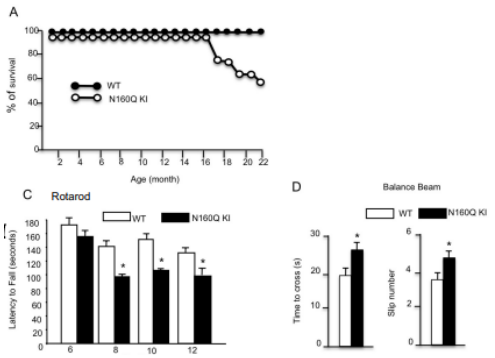
Your 30 year old patient just got a genetic test that indicates that she is carrying the mutant allele of *huntingtin*

She is not showing any obvious symptoms yet, but her dad's age of onset was 35 years old She wants to try an experimental stem cell therapy before she has symptoms
Which stem cell therapy would you suggest? What types of cells? How would you get them into the patient? How would they help her?

There is no right or wrong answers here, but you should justify your choice



Practice problem-1



This is a figure from a paper describing a new Htt mouse model.
a) What do you think N160Q KI (knock-in) means? How did they make this mouse line?
b) Does this mouse model show construct and face validity? Explain.

Practice problem-2

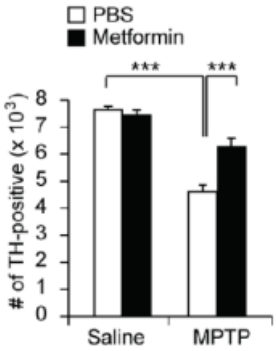
A. What are microglia – where are they found and what is their function?

B. What do active microglia look like? Specifically, what happens to their cell body diameter and the number of branches when they are active?

C. We learned that it is important to maintain a balance in microglia activation. In fact, in Alzheimer disease we discussed examples of how active microglia can be "good" and "bad". Explain how active microglia helped the AD mice in paper #8 by Iaccarino et al. (2016).

D. Explain one way that active microglia can contribute to neurodegeneration in AD.

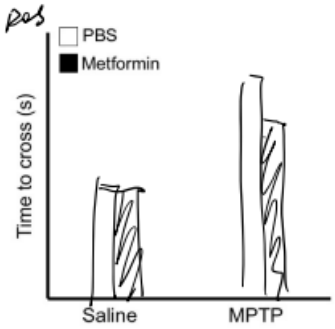
Practice problem-3



In the experiment to the right, mice were injected with MPTP or saline. The mice were then fed a chemical called metformin (which is actually a diabetes medicine) for one week. The researchers then measured the number of TH-positive cells in the brain.
A. What are TH-positive cells?

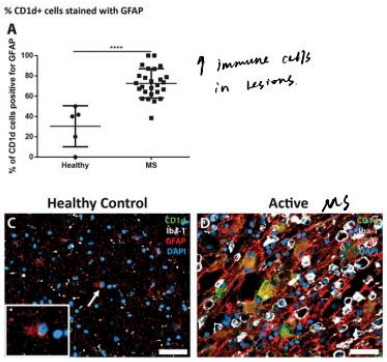
B. What is MPTP and why does it cause decreased TH-positive cells?

C. The researchers then measured how long it would take the same experimental groups to walk across a small beam. Draw the expected results in the graph to the right based on the TH data above. Saline and MPTP should have two bars each: first the PBS bar, then the metformin bar. Briefly explain your reasoning.



Practice problem-4

This figure is from a paper where they took samples from healthy human brains and from active lesions in MS patients
CD1d is a protein usually in membrane in antigen-presenting cells Iba-1 is a marker for microglia
What is the main finding from this data? Explain it in the context of what you know about MS



Practice problem-5

After graduating from college, you join a research lab studying new methods for treating spinal cord injuries. In particular, they are working on ways to improve axon regrowth through the injury site.
Your first project is to develop a gel-like patch that contains anti-sense oligonucleotides (ASO) for the Nogo receptor that can be applied directly onto the injury.
a) Explain why you're targeting the Nogo receptor with the ASO. How could that help with spinal cord regeneration?

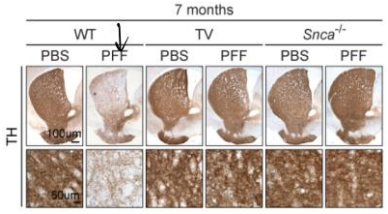
b) You suggest to your boss that you also try electrically stimulating the spinal cord daily for several months after you apply the gel onto the injury. What evidence did we discuss in class that supports this treatment using spinal cord stimulation?

Practice problem-6

Researchers have tried treating a mouse model of multiple sclerosis (MS) with neural stem cells derived from induced pluripotent stem cells (iPSC).
a) The researchers generated neural stem cells from mouse induced pluripotent stem cells. Explain how iPSCs are generated and how neural stem cells could be made from them. A diagram could be helpful here.
b) After transplanting the neural stem cells into the cerebrospinal fluid in the brain, symptoms improved. When the researchers looked at the fate of the implanted neural stem cells, they saw that the stem cells did not differentiate into neurons or glia cells. How could the transplanted stem cells have helped without actually forming differentiated cells?

Practice problem-7

This is a supplemental figure from paper 9 (Kim et al., 2019). The images are of the striatum stained in brown for TH. The bottom row of images is just a zoomed in view.



a) Explain the main result in this figure.

b) Similar images of the substantia nigra were shown in the main paper. Why look at the striatum?