Glia and Inflammation

Team Hormonal
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Agenda

- Introduction
- Astrocytes
  - Synapses & plasticity
  - Calcium waves
  - BBB
- Oligodendrocytes
  - Myelination
- Microglia
  - TNF-α
  - C1q/Neurodegenerative disorders
- ALS & TDP-43
Introduction to Glia

- Types of Glial cells
  - Astrocytes
  - Oligodendrocytes/Schwann Cells
  - Microglia
  - Ependymal cells
Theme: Glia is a majorly underresearched area!
Glia Background

Main Ideas

1. Glia are not neurons (but that doesn’t mean there not worth studying)
   a. Do not generate action potentials
   b. Do not have same structure

2. They are more than just a “support system”, they have an active role

3. Different types of Glia in the CNS with different functions and structures

Fun Fact: About 90% cells in brain are glia

Sources:
**Astrocytes**

Protoplasmic Astrocytes

Fibrous/Fibrillary Astrocytes

**Gray Matter**

Ensheath synapses and blood vessels

**White Matter**

Contact nodes of Ranvier and blood vessels

Sources:
http://www.wesapiens.org/file/5874234454704128/Protoplasmatic+and+Fibrous+astrocytes+stained+with+GFAP
Mystery #1: Astrocytes Synaptic Activity & Synapses

What we know: Astrocytes are crucial players in controlling levels of ions and neurotransmitters in the extracellular space

Study 1: Synaptic activity increase by about 100-fold when retinal ganglion cells are cultured with astrocytes (Pfrieger & Barres 1997)

Study 2: When retinal ganglion cells are not cultured with astrocytes, few synapses form between retinal ganglion cells (Ullian et. al 2001)

But how do astrocytes influence synapses and synaptic activity??

Sources:
Mystery #1 cont...

Answer: Thrombospondins (TSP)

TSPs are proteins released by astrocytes that induce synaptogenesis (creation of synapses)

In fact, astrocytes secrete many signals that influence activity at the synapses:

- **Cholesterol** -> Enhances presynaptic function at synapses by 100-fold
- **Unidentified protein** -> Enhances postsynaptic function of synapses in conjunction with level of synaptic AMPA glutamate receptors
- **Unidentified signal** -> Control synapse elimination by inducing the expression and secretion of C1q

Sources:


Mystery #2: Astrocytes and Plasticity

What we know: Astrocytes have been implicated to have a role in brain plasticity

Study 1: Ocular dominance plasticity was restored when immature astrocytes cultured from visual cortex of newborn kittens were transplanted into adult primary visual cortex of cats (Muller & Best, 1989)

Study 2: Getting rid of cellular debris (which astrocytes are known for), such as removing inappropriate synaptic connections, is important for brain plasticity (Boulanger & Shatz, 2004)

Sources:
Interesting fact: TSPs are highly upregulated in human brain compared to primate brain? Does this perhaps contribute to increased brain plasticity in humans?

Quite possibly...Recent evidence...

Study 1: Mice w/ deficiency of TSP1 &2 showed impaired recovery after stroke, demonstrating the importance of TSP1 & 2 in synaptic plasticity (Liauw et. al 2008)

Study 2: Thrombospondins were critical in restoring presynaptic muting at glutamatergic synapses in rat hippocampal neurons (Crawford et. al 2012)

Source:
Glial Calcium Waves

Neural stimulation induces calcium waves in astrocytes

- Glutamate stimulated Calcium increased levels propagate across astrocytes (long-range signalling pathway between glia?)

Acting like neurons:

- Calcium waves demonstrate astrocytes respond to visual stimuli w/ spatial receptive fields, tune to visual features such as orientation

Sources:
Calcium Waves and Microvascular Blood Flow

What we know: Calcium waves correlate w/ increase of Microvascular blood flow

May suggest existence of glial circuits

Neurovascular unit: close coupling of neurons, glia, and blood vessels

May be implicated in neurodegenerative diseases like Alzheimer’s:

- Astrocytes may be important in microvasuclar blood circulation so..
- Abnormal astrocyte activity and abnormal calcium levels may contribute to vascular instability in AD (Takano et. al 2007)

Mystery #3: Astrocytes and Neuron Survival

What we know: Astrocytes release neurotrophic signals that lead to increased neuron survival

Buy why?

Transcriptomes (gene profiles) may point to a possible explanation...

- Transcriptomes show many trophic factors are made by astrocytes
- Highly expressed genes shown by the transcriptomes of astrocytes may play a functional role (ApoE, ApoJ, MFGE8, crystatin C)

Sources:
Astrocytes and the Blood Brain Barrier (BBB)

- **Common thought of role of astrocytes**: inducing the BBB
  - Formed at the same time as astrocyte generation
- **New Research**: BBB is intact before astrocyte generation
  - Due to Wnt signaling and neural stem cells
  - Postnatal development: astrocytes take over neural stem cells
  - Astrocytes serve as a *maintenance function*

Astrocytes and Neurological Disorders

- Astrocytes are involved in all CNS diseases
  - Make up 50% of cells in human brain
- Harmful component of brain injury = astrocyte swelling
- Reactive astrocytes upregulate thrombospondins
  - Helpful but also harmful
- Sick astrocytes give a neurotoxic signal

Figure: Rosado, Florian. “Researchers Find Molecular Trigger for Brain Inflammation.” ReliaWire, 4 Dec. 2017, reliawire.com/brain-inflammation-multiple-sclerosis/
Mystery #4: Astrocytes Role in Treating Neurological diseases?

What we DON’T know: Why astrocytes are more likely to swell than neurons and how to stop that swelling

Hypothesis: damage to oligodendrocytes $\rightarrow$ increased DMS production $\rightarrow$ inflammatory astrocyte response (only in sensory neuron sensitization)

-If we solve this problem, we can better understand neurological diseases and how to treat them

Mystery #5: How do oligodendrocytes myelinate?

Knowledge is still very limited

What we know:

- Oligodendrocytes are generated by OPCs
- Role of neuregulin-1 and gliomedin in Schwann Cells

What we don’t know: **Molecular mechanisms** that enable oligodendrocytes to recognize, ensheath and wrap axons
Mystery #5 cont.

Hypothesis

- Unidentified axonal signal triggers ensheathment by inhibiting gamma secretase activity within oligodendrocytes
- All ensheath in a brief time period: 12-18 hours
- White matter astrocytes → role unknown
Oligodendrocytes and role in MS and Major Depressive Disorder

Multiple Sclerosis

- Initial remyelination = generation of new oligodendrocytes and new myelin but the repair process fails
  - Not sure why

Major Depressive Disorders

- Massive loss of oligodendrocytes and myelin in temporal lobe
- Oligodendrocytes exhibit high levels of dopa decarboxylase (Serotonin synthetic enzyme) --> effect not cause?
Microglia and TNFα

- A pro-inflammatory cytokine involved in demyelination
- Microglial-derived TNFα play a role in promoting the generation of new oligodendrocytes
  - Weakens integrity of BBB during brain inflammation
- Controls normal function and plasticity of neural circuits
- Needs to be studied more!

Figure: structure of TNFα
Microglia, C1q and the development of CNS

- Initiating protein of classical complement cascade
- Secreted C1 binds to and tags developing synapses → synaptic deposition of complement C3
- Complement tagged synapses removed by microglia & phagocytosis by macrophages

Figure: rat microglia in green, nerve fiber processes in red
Application of microglia and C1q to neurodegenerative diseases

- Alzheimer’s Disease
  - C1q levels = 70-fold increase
- Inhibition of C1q can lead to a decrease in phagocytotic microglia
- Can also be found in ALS, MS, and glaucoma
- Drugs that block synaptic complement cascade activation → minimize neurodegeneration

Figure: Imbimbo, Bruno, et al. “Are NSAIDs Useful to Treat Alzheimer's Disease or Mild Cognitive Impairment?” Frontiers, Frontiers, 23 Apr. 2010.
Mystery #6: Role of Glia in Neurodegenerative Diseases

What we know: In multiple types of neurodegenerative diseases, the development of insoluble protein aggregates serves as a major hallmark ultimately leading neuronal cell death.


What we DON’T know: Whether glial cells play a more central role in the progression of these diseases than previously thought.
Previous Studies on the Progression of ALS

- When ALS was first being studied, the first genetic lead that scientists discovered was the mutation of the **Cu/Zn super-oxide dismutase (SOD1) protein**
  - Responsible for destroying free superoxide radicals in the body

Previous Studies (cont.)

- Mice that expressed copies of this mutation demonstrated features indicative of motor neuron (MN) dysfunction and ALS pathology.

- In future experiments, it was found that nonneuronal cells (most likely glia) that contained mutant SOD1 and neighbored healthy MNs had a more detrimental effect in the progression of neurodegenerative pathologies.

*Led to the conclusion that glia must have some sort of impact in the overall health of MNs*
Recent Studies on the Progression of ALS

- A more prominent protein has been found to be directly responsible for the formation of hallmark protein aggregates found in ALS
  - TDP-43 (TAR-binding protein 43) → main component found in neuronal protein aggregates undergoing ALS pathology

Source: “Yuna Ayala, PhD.” The Hope Center, hopecenter.wustl.edu/?faculty=youna-ayala-phd.
More on TDP-43

- **What is it?**
  - An RNA-binding protein with 2 RNA recognition sites + a carboxyl-terminal domain

- **Function**
  - TDP-43 binds to mRNA + regulates splicing/turnover
  - Mainly found in nucleus, but can move to the cytoplasm

- In ALS, mutations cause the carboxyl terminus to cleave off, causing TDP-43 to create insoluble protein aggregates

Source: “Yuna Ayala, PhD.” *The Hope Center*, hopecenter.wustl.edu/?faculty=youna-ayala-phd.
More on TDP-43

- Once TDP-43 is cleaved, it is transported to the cytoplasm where it can then begin aggregate accumulation.
- An important question: Is this cytoplasmic displacement of TDP-43 directly cytotoxic to cells?

It has been found to be sufficient to cause cytotoxicity.

- This then raises the question if neighboring glial cells have a similar effect, since protein aggregates can also be found there.
Experiment by Serio et. al.

- **Purpose:** Determine if glial cells that express the TDP-43 mutation affects cellular longevity/health and whether cytoplasmic TDP-43 plays a more direct role
- **Methods:** Use **induced pluripotent stem cells (iPSCs)** to create human astrocytes with specific **TDP-43 mutations (M337V)**
- **Results:**
  - Turns out that M337V astrocytes accumulated 30% more than control astrocytes
  - Control astrocytes injected with M337V mutation also resulted in similar levels of cytoplasmic accumulation

*Demonstrates that this TDP-43 mutation directly causes cytoplasmic displacement*
Fig. 1. A model of astrocyte pathology in TDP-43 proteinopathies. Astrocytes derived from human iPSCs are used to examine the cell-autonomous effects of TDP-43 mislocalization. Astrocytes that are generated from patients harboring ALS-causing mutations in the TARDBP gene display cytoplasmic TDP-43 inclusions, mimicking the common neuronal and glial histopathologic findings. Mutant TDP-43 patient-derived astrocytes display significantly impaired survival after 10 d.

To determine the effects of cytoplasmic TDP-43 accumulation on cell longevity:

- **Fluorescent microscopy** used to observe potential cell death
- Serio et. al. observed a **223% increase in risk of cell death** in M337V astrocytes vs. control

Found that non-apoptotic mechanisms were the primary cause in this increased susceptibility:

- Possibly due to the release of **inflammatory cytokines** by both activated + dying astrocytes, which recruit/activate microglia

Cytoplasmic TDP-43 accumulation --> cell-autonomous glial pathology --> **direct glial activation**
Takeaways

- While the big question of whether glia play significant roles has been deduced, more questions must still be asked for further study.

- For instance, how does activation of astrocytes/microglia cause the activation of neuronal degenerative mechanisms? Additionally, what are these mechanisms?

These studies so far allow us to posit that proteinopathies involving mutations in glial TDP-43 cause activation of cell death mechanisms, which in turn cause the progression of multiple neurodegenerative diseases.
“Having 20,000 neuroscientists that study LTP while only 20 are studying glia simply makes no sense.”
“And please don’t forget the glia!”