Neuro-glial interaction in modulating the neuronal connectom

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If “the connectome” represents a complete map of anatomical and functional connectivity in the brain, it should also include glia!

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Grey matter might be imagined more like an astrocytic Christmas tree farm superimposed on a neural rainforest.
Glial Cells – Non-neuronal Cells

- It is now a myth that glial cells make up about 90% of the cells in the nervous system, even if the statement is written in the textbooks…
- The number of glial and neuronal cells depends on the nervous system region
- Recent studies suggests that the glial cell : neuron ratio can be 1:1 rather than 10:1… → the brain could contain about 1 billion glial cells along with the 1 billion neurons…
The Glia/Neuron Ratio: How it Varies Uniformly Across Brain Structures and Species and What that Means for Brain Physiology and Evolution

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It is a widespread notion that the proportion of glial to neuronal cells in the brain increases with brain size, to the point that glial cells represent “about 90% of all cells in the human brain.” This notion, however, is wrong on both counts: neither does the glia/neuron ratio increase uniformly with brain size, nor do glial cells represent the majority of cells in the human brain. This review examines the origin of interest in the glia/neuron ratio; the original evidence that led to the notion that it increases with brain size; the extent to which this concept can be applied to white matter and whole brains and the recent supporting evidence that the glia/neuron ratio does not increase with brain size, but rather, and in surprisingly uniform fashion, with decreasing neuronal density due to increasing average neuronal cell size, across brain structures and species. Variations in the glia/neuron ratio are proposed to be related not to the supposed larger metabolic cost of larger neurons (given that this cost is not found to vary with neuronal density), but simply to the large variation in neuronal sizes across brain structures and species in the face of less overall variation in glial cell sizes, with interesting implications for brain physiology. The emerging evidence that the glia/neuron ratio varies uniformly across the different brain structures of mammalian species that diverged as early as 90 million years ago in evolution highlights how fundamental for brain function must be the interaction between glial cells and neurons.

Key words: brain size, brain metabolism, cell size

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Oligodendrocytes

- Form myelin electrical insulation, increasing conduction velocity by at least 50 times.
- Provide vital metabolic support for axons
- Save energy consume in transmitting the nerve impulse
Microglia

Highly motile and responsive to nervous-system injury and infection.
Monitor electrical activity in neurons and prune synaptic connections.
Involved in almost all nervous-system diseases and in certain psychiatric conditions.

Microglia - 10-15% of all cells found within the brain

- the immune cells/resident macrophages of the brain and spinal cord (main form of active immune defense in the central nervous system, constantly scavenging the CNS for damaged neurons and infectious agents and decreasing inflammation).
- release substances that stimulate repair.
- prune back synapses and rewire neural connections in a healthy brain
Microglia

• ‘Synaptic pruning’ vs. apoptosis (PCD)
• Synaptic Pruning: the process by which extra neurons and synaptic connections are eliminated in order to increase the efficiency of neuronal transmissions.

SYNAPTIC DENSITY: Synapses are created with astonishing speed in the first three years of life. For the rest of the first decade, children’s brains have twice as many synapses as adults’ brains.

Drawings supplied by H.T. Chugani.
Astrocytes

Influence nervous-system communication and plasticity

Primarily responsible for homeostasis of the central nervous system.

Ensheathe synapses, regulate neuronal excitability and synaptic transmission.

Respond to injury by secreting extracellular matrix proteins.

Implicated in neurogenesis, cell migration, many neurological and psychiatric disorders.
Astocytes

- Produce **growth factors** → regulate morphology, proliferation, differentiation or survival of neurons and glial cells

- Role in regulation of synaptic function, volume transmission, Neuron-glia connection, Network signalling

- Can also undergo remodeling (**Plasticity**); astrogliosis in injury, neurodegeneration.

- The fine distal processes are interposed between all neuronal elements. Create a kind of synaptic island defined by its ensheathing processes.

- Role in processing information …

- Through perivascular processes astrocytes contribute to blood-brain barrier and form "**glymphatic**" drainage system of the CNS.

- **Supplier of glutamine** (neurotransmitters precursor).

- Contribute to neuropathologies through mounting complex defensive programme generally known as **reactive astrogliosis**.
Synaptic astrocytes

1. regulate synaptic transmission by
   - responding to ATP and glutamate, released from the presynaptic neuron
   - uptake of glutamate from the synaptic cleft via membrane transporters (green arrow) or the release of glutamate upon reversal of the transporter induced by $\uparrow [\text{Na}^+]_i$
   - D-serine released from astrocyte strengthen synaptic transmission by coactivating NMDA receptors in the postsynaptic membrane, or reduce synaptic transmission by secreting transmitter-binding-binding proteins (TBP)

2. communicate with adjacent astrocytes via gap junctions and with distant astrocytes via extracellular ATP.

3. the rise in $\text{Ca}^{2+}$ causes release of glutamate from astrocytes, and ATP is released via an unknown mechanism, which propagates ATP signaling to adjacent cells.

An electron micrograph of a synapse surrounded by an astrocyte (yellow) from rat spinal cord.

GluR, glutamate receptor; Ado, adenosine; IP3, inositol trisphosphate; P1, adenosine receptor; P2, ATP receptor.

Amzica, 2000
Modulation and optimization of synapse: Regulation of chemical synapses function by neuron – astroglia connections

GLAST = glutamate/aspartate transporter; GLT-1 = glutamate transporter EEAT2 both Na+-dependent
Neuron to Astrocyte Signaling

1. Glutamate release from pre-synaptic neuron

2. Metabotropic receptors for Glutamate (mGluR) located on astrocyte bind synaptic Glutamate. Subsequent intracellular Phospholipase C release leads to Inositol Triphosphate (InsP3) production.

3. Ion channels open, allowing vesicular-bound pools of Ca2+ into the intracellular environment.

4. Intracellular levels of Ca2+ rise, free Ca2+ releases other pools of vesicular-bound Ca2+.
Astrocyte Role

- **Regulation of ion concentration in the ECS:**
  Ex: High number of K+ channels (high permeability).
  Transference of K+ to sites of lower accumulation. High levels of K+ in ECS would change neuronal excitability.

- **Clear neurotransmitters (glutamate and GABA):**
  Astrocytes have distal processes rich in transporters that remove excess neurotransmitters (especially glutamate)
  If Glutamate is not removed:
  Diffuses into the ECS. Presynaptic bind and inhibition of its own release.
  Influence other synapses - “Intersynaptic cross-talk”

- **Secrete large complex substances to the ECS:** Important as structural elements and cell to cell communication.
  Ex: Promotion of the myelinating activity of oligodendrocytes through release of cytokine leukemia inhibitory factor (LIF).

- **Nervous system repair:** upon injury to nerve cells within the central nervous system, astrocytes become phagocytic to ingest the injured nerve cells. The astrocytes then fill up the space to form a glial scar, repairing the area and replacing the CNS cells that cannot regenerate.
-Vasomodulation:
- Restrict access of neurosecretory terminals to perivascular basal lamina.
  (blood flow)
Control the effect of paracrine/autocrine secreted peptides.
Regulate neurosecretion.
Neurons alone provide only a partial explanation for complex cognitive processes, formation of memories. The complex branching structure of glial cells, integrating information from spatially distinct parts of the brain and their relatively slow chemical (as opposed to electrical) signaling in fact make them better suited than neurons to certain cognitive processes.

**Why are astrocytes important?**

Different spatial and temporal scales for neuronal and glial activity…
Neuron-glia connections

Synchronous Firing Groups:

Astrocytic regulation of neural networks
Premises for neuron - glial cell - cerebral capillary unit

- Nervous system function ↔ cellular energetic status ↔ aerobic metabolism ↔ blood perfusion

- Brain vulnerability to hypoxia/ischemia
  
  brain receives 15%-20% from CO
  
  $O_2$ brain consume – 20% from the whole body
  
  consume (250 ml $O_2$/min)
  
  glucose brain consume – 25% from the whole body
Brain Vulnerability

• Aerobic metabolism:
  -95% of brain ATP derive from cerebral oxidative phosphorilation
  -No energy stores in the brain (low glycogen…)

• Facts - blockage of cerebral blood flow results in:
  - loss of consciousness in 10-20 sec
  - irreversible cerebral changes in 3-5 min
Physiological coupling of brain metabolism and neuronal activity: Glutamate-induced glycolysis in astrocytes

As Neural activity ↑ there is an ↑ Energy requirement

To solve this...

↑ Astrocytic uptake of Glutamate leads to ↑ ADP leads to ↑ Glycolysis within Astrocytic endfeet which finally leads to ↑ Lactate delivered to neuron
Physiological changes linking neural and vascular responses

BBB & Neurovascular coupling

neurotransmitter release (e.g., glutamate, GABA)

ATP consumption

Oxygen consumption Glucose consumption

vasoactive chemical agents, metabolites (e.g., K+, NO, adenosine)

Cerebral Blood Flow (CBF)

Typical CBF response to brief neural activation.
Blood Brain Barrier functions

- **BBB** = *structural and functional barrier* which impedes and regulates the influx of most compounds from blood to brain

- BBB formed by
  - *brain microvascular endothelial cells*
  - *astrocyte end feet*
  - *pericytes*

- **BBB is essential** for normal function of CNS
  - Regulates passage of molecules in and out of brain to maintain *neural environment*.
  - Responsible for *metabolic activities* such as the metabolism of L-dopa to regulate its concentration in the brain.
Neuroglia in neurovascular coupling

- an important glial function - *isolation of the nervous tissue from the rest of the body by the blood–brain barrier (BBB)*

- *The barrier function of the cerebral endothelial cells is under astrocytic control.*
Astrocyte end feet

- Provides biochemical support for cerebral endothelial cells
- Influence of morphogenesis and organization of vessel wall
- Factors released by astrocytes involved in postnatal maturation of BBB
- **Direct contact between endothelial cells and astrocytes necessary to generate BBB**

- Co-regulate function by
  - secretion of soluble cytokines
  - \( \text{Ca}^{2+} \) dependent signals triggered by intracellular IP-3 consecutive to ATP binding on P2Y receptors
  - gap junction dependent pathways \( \rightarrow \) form a syncytium able to propagate signals (as **Ca waves**) for large distances
Blood brain barrier selectivity

• Free permeability (passive diffusion):
  – small molecules: H$_2$O, O$_2$, CO$_2$, NH$_3$, ethanol
  – lipid soluble molecules: steroid hormones

• **Carrier mediated transport** (apical-basal polarity):
  – glucose: GLUT-1 (insulin independent)
  – amino acids
  – nucleosides, nucleobases

• Pinocytosis

• **Aquaporin-4** is the main channel through which water enters and leaves the CNS

• Physical *blockage* to paracellular diffusion (ions, peptides, immune cells)
Figure 11-8  BBB function of brain capillaries. A, Capillaries from most other organs often have interendothelial clefts or fenestrae, which makes them relatively leaky. B, Brain capillaries are not leaky and have reduced transcytosis. C, Continuous tight junctions connect the endothelial cells in the brain, making the capillaries relatively tight. GLUT1, glucose transporter 1.
Neuron-glia communication by volume transmission - quadrupartite synapse

Neurons-to-neurons and neurons to glia communication by extrasynaptic “volume transmission”, which is mediated by diffusion in the extracellular space (ECS) of the CNS = the microenvironment of neurons and glial cells. Composition & size of ECS change dynamically during neuronal activity and during pathological states.

ECS size, geometry, and composition, together with pre- and postsynaptic terminals and glial processes, form the so-called “quadrupartite synapse”.

ECS diffusion parameters affect neuron-glia communication, ionic homeostasis and the movement and/or accumulation of neuroactive substances in the brain → plays an important role in extrasynaptic transmission, transmitter spillover, cross-talk between synapses, and in vigilance, sleep, depression, chronic pain, memory formation and other plastic changes in the CNS.
Figure 11-5  Tight packing of neurons and astrocytes. This is an electron micrograph of a section of the spinal cord from an adult rat showing the intermingling and close apposition of neurons and glial cells, mainly astrocytes. Neurons and glial cells are separated by narrow clefts that are ~20 nm wide and not visible at this magnification. The BECF in this space creates a tortuous path for the extracellular diffusion of solutes. Astrocyte processes are colored. As, astrocytes; At, en passant synapses; Ax, unmyelinated axons; Ax₁ and Ax₂, myelinated axons; Den, dendrites; f, astrocytic fibrils; nf, neurofilaments; S, synapses; SR, smooth endoplasmic reticulum. (Modified from Peters A, Palay SL, Webster H: The Fine Structure of the Nervous System. Philadelphia, WB Saunders, 1976.)
Retraction of glial processes in rat supraoptic nucleus (SON) and consequences for diffusion and synaptic crosstalk.

Reduced astrocytic coverage of SON neurons in lactating rats leads to deficient glutamate clearance, resulting in increased glutamate concentration in the ECS, increased crosstalk between synapses and increased activation of either presynaptic or postsynaptic receptors.
In cerebral edema, the brain fluid that accumulates comes from the vascular compartment.
- Cell swelling due to the mere shift of fluid from the extracellular to the intracellular fluid is not cerebral edema.
Astrocytes are connected by gap junctions thereby forming a syncytium that is able to propagate signals for large distances. Can be also caused by increased extracellular K+ levels. Modify gene expression and consequent morphological changes. Maintenance of microvascular tone. Cause own release of glutamate. Further adjacent neuron activation (not confirmed)

Ca2+ Increase cause...
- Wave propagation signal
- Mechanism of wave propagation via release of ATP to ECS > Activates neighboring cells.
- Thigh junctions. Not certain. Observed only in intense electrical stimulation

Astrocytes are connected by gap junctions thereby forming a syncytium that is able to propagate signals for large distances
**ATP and adenosine:**

ATP - P2Y receptors in astrocytes. Triggers intracellular Ca2+ release and wave propagation. > Glutamate
Signal neighboring neurons by pre/post synaptic purinergic receptors.

Converted to adenosine by ectonucleotidases in ECS. Suppression of synaptic transmission.
A1/A2 receptors activation leads to positive action of K+ channels and negative action of Ca2+ channels.
Neuropathological conditions

Any structural change in astrocyte environment should affect properties of ECS.

Epilepsy - accompanied by astrocyte hypertrophy and hyperplasia
- Elevated baseline \([K^+]_o\) has been directly correlated with the likelihood of transition from interictal to ictal epileptiform activity (Jensen et al. 1994)
- Pharmacological block of \(K^+\) influx through \(K^+\) channels into glia causes an abnormal accumulation of \(K^+\) in the extracellular space and an increase in neuronal excitability (Ballanyi et al. 1987; D'Ambrosio et al. 1998; Gabriel et al. 1998)

Astrocytes activated by injury - regulation of synaptic activity and strength. Importance in development of inflammatory pain.
The role of astrocytes in Epilepsy

– In astrocytes from epileptic foci mGluRs are overexpressed by a factor of about 20 (rat models and human)

Increased \( \text{Ca}^{2+} \) spikes during epileptic seizure
Ong et al. J. Neurochem. 72, 1574 (1999)

– More spontaneous astrocytic calcium spikes in epileptic foci
Tashiro et al., J. Neurobiol. 50, 45 (2002)