ASTROCYTES AND MICROGLIA
Astrocytes........most abundant glial cell type

Form anatomical link between neurons and arterioles

Radial astrocytes: surround ventricles
Protoplasmic astrocytes: in gray matter
Fibrous astrocytes: in white matter
ASTROCYTE FUNCTIONS

Essential for normal neuronal activity

Glutamate uptake

Glutamate release

K$^+$ and H$^+$ buffering

Water transport

Controls environment
Provides metabolic support to neurons
   Neurons cannot survive without close interaction with astrocytes

Involved in synapse formation (development)
   Produce molecules necessary for neuronal growth  eg BDNF

Propagate calcium signals

Neurons – v. vulnerable to glutamate
Astrocytes – particularly vulnerable to acidosis & hypoxia
Both vulnerable to reactive oxygen species
Astrocytes can release gliotransmitters like glutamate.

Astrocytes form an anatomical link between neurons and arterioles.

Astrocytes do not overlap. They may interact with thousands of neurons.

"End-feet" connect to blood vessels. Astrocytes modulate blood vessels, regulating local blood flow.
Uptake of glutamate

Coupled with Na⁺-K⁺ transport down their concentration gradient

Membrane potential contributes ...net +ve influx with glutamate

Release of glutamate

- by ‘reverse’ action of transporter
eg ATP depletion/ischaemia
- by Ca²⁺-dependent mechanisms
eg in response to PGE₂
- by activation of P2X₇ receptors
- by activation of Volume-Sensitive Organic Anion Channels
- Calcium waves propagate through the syncytium GAP JUNCTIONS.
- Non-synaptic means of communication within the brain.
- Waves can be induced by mechanical stimulation and by glutamate.
- Modulates extracellular concentration of molecules.
- Influx of calcium leads to calcium-sensitive release and uptake of ions and neuromodulators.
Calcium waves induce release of glutamate from astrocytes.

Glutamate interacts with AMPA and NMDA receptors on nearby neurons, causing depolarization.
Astrocytes can release gliotransmitters like glutamate.

Astrocytes form an anatomical link between neurons and arterioles.

Astrocytes do not overlap. They may interact with 1000s of neurons.

"End-feet" connect to blood vessels. Astrocytes modulates blood vessels, regulating local blood flow.
Astrocytes form an anatomical link between neurons and arterioles

- Neuronal activity
  - Glutamate release
    - Activates astrocytic metabotropic glutamate receptors
    - Astrocytic calcium wave
      - Calcium wave invades astrocytic endfoot
      - Phospholipid $\xrightarrow{\text{PLA}_2}$ Arachidonic acid
      - Arachidonic acid $\xrightarrow{\text{COX-1}}$ Vasodilating PG

Vasodilation facilitated by $\text{Ca}^{2+}$-induced production of IP$_3$

Gap junctions trigger synchronous $\text{Ca}^{2+}$ waves. Ongoing cycle
PG induces vasodilation by acting in pericytes?
Potentiation of inhibitory synapses between GABA interneurons and PNs— an example of feedback synaptic modulation.

GABA (1) activates GABAB on a neighbouring astrocyte; astrocyte releases glutamate (2) onto interneuron, causing feedback potentiation of the interneuron's inhibitory drive (3).

Heterosynaptic depression (example of feedforward synaptic modulation)

Glu release (1) stimulates astrocyte, which releases ATP. ATP converts to adenosine (2), which activates presynaptic A1 adenosine receptor. Inhibits a different connection.

Excitation and synchronization of adjacent pyramidal neurons

Oscillations in Ca²⁺ in astrocyte triggers glutamate release activating synapses from 2 neurons.

Potentiation of inhibitory synapses between GABA interneurons and PNs— an example of feedback synaptic modulation.

GABA (1) activates GABA₉ on a neighbouring astrocyte; astrocyte releases glutamate (2) onto interneuron, causing feedback potentiation of the interneuron's inhibitory drive (3).
Astrocytes in brain pathology

**Ca^{2+} oscillations** (which stimulates glutamate release and synchronizes neuronal activity) are
- Decreased in peri-traumatic area after *mechanical insult*
- Stimulated by *epileptiform* activity
- Spreading depression associated with *migraine* involves astrocyte-neuron interaction

**Brain tumours (glioma)**
- Expansion may be due to excess glutamate/decreased uptake
- Gliomas express AMPA receptors that lack GluR2 subunit → permeable to Ca^{2+} → growth

**AIDS-related neuropathology**
- Microglia are infected by HIV (not neurons, but neuronal death occurs)
- Astrocytes express CXCR4 (used by HIV isolates to infect CD4 T cells)

\[
\text{HIV gp120} \rightarrow \text{CXCR4} \rightarrow \text{TNF} \alpha \rightarrow \text{Ca}^{2+}\text{-dep glu release} \rightarrow \text{excitotoxicity} \rightarrow \text{neuronal death}
\]

**Alzheimer’s Disease**
- Astrocytes are protective – they internalize Aβ (involves ApoE dependent signalling?)
- A defect in internalization/digestion switch astrocytes to promoters of Aβ accumulation
- ApoE is genetic risk factor for AD; it is produced by astrocytes

**Motor neuron disease (Amyotrophic lateral sclerosis – ALS)**
- Associated with mutation in SOD1 in neurons and astrocytes
- Astrocytes with mutated SOD1 are activated and damaged
- EAAT1 is decreased → glutamate is increased........... excitotoxicity
<table>
<thead>
<tr>
<th>Factor</th>
<th>Astrocytes</th>
<th>Microglia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokines</strong></td>
<td>IL-1, IL-6, IL-8, TNFα</td>
<td>IL-1, IL-6, IL-8, TNFα</td>
</tr>
<tr>
<td></td>
<td>MCP-1, MIP-1α</td>
<td>MCP-1, MIP-1α</td>
</tr>
<tr>
<td><strong>Growth Factors</strong></td>
<td>NGF, TGFβ, FGF, IGF, CNTF</td>
<td>NGF, TGFα, FGF,</td>
</tr>
<tr>
<td><strong>Eicosanoids</strong></td>
<td>VCAM, ICAM-1,-2, NCAM</td>
<td>PGD₂, Leukotriene C4,</td>
</tr>
<tr>
<td><strong>Adhesion</strong></td>
<td>VCAM, ICAM-1,-2, NCAM</td>
<td></td>
</tr>
<tr>
<td><strong>RNS</strong></td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td><strong>ROS</strong></td>
<td></td>
<td>Superoxide Ions</td>
</tr>
</tbody>
</table>

OTHERS: Protease, Protease inhibitors, Matrix proteins,
3 reviews

Neuron-astrocyte communication and synaptic plasticity
Paixao and Klein (2010)...

Astrocytes, from brain glue to communication elements:
the revolution continues (Volterra and Meldolesi, 2005)

Astrocytes and Brain Injury (Chen and Swanson, 2003)
Microglia

- Smallest and least abundant glial cell
- Phagocytic activity (Engulf invading microorganisms and dead neurons)
- Derive from monocytes
**Activation signals**
- Paraquat, rotenone
- Diesel exhaust particles
- Reactive oxygen species
- Thrombin
- Lipopolysaccharide
- Amyloid-β

**Responses**
- Cell surface markers (MHCII, CD11b, CD40)
- Release of inflammatory mediators (e.g., Cytokines, ROS, RNS)
- Increased expression of chemokines (chemoattractants)
- Altered morphology

**Functional consequences**
- Phagocytosis
- Interaction with other cells (T cells, neurons)
- Modulation of cell function (T cells, neurons, astrocytes)
Proposed sequence of reactive changes in microglia

Morphological response to acute insult

Morphology of chronically-active microglia
Proposed response to an insult

Microglia may express cell surface markers and interact with other cells eg T cells

Microglia may become secretory

Microglia become phagocytic (eg UTP 'eat me' signal)

Microglia respond to chemotactic signal (eg ATP 'find me' signal)

Cytoskeletal changes

Microglia become motile

Activators
- Fractalkine
- Glutamate
- Complement (eg 5a)
- Neurotensin
- TGFβ

Inhibitor
- Morphine (MOR3)
Microglia can be neuroprotective

**Beneficial microglia functions**
- Controlled phagocytosis
- Directed migration
- Selective cytokine synthesis
- Neurotrophin release

**Detrimental microglia functions**
- Non-controlled phagocytosis
- Excessive cytokine synthesis
- Nitric oxide release
Innate immune response
Immediate de novo production of mediators to respond to pathogen

Driven by specific recognition systems ....LPS activating CD14 and TLR4

The key cell in the CNS is the microglial cell
**Toll-like Receptors (TLR)**

11 members of the TLR family in mammals; 10 in humans

<table>
<thead>
<tr>
<th>TLR</th>
<th>Pathogen</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1/2</td>
<td>Bacteria</td>
<td>Diacyl lipopeptides</td>
</tr>
<tr>
<td>TLR2/6</td>
<td>Bacteria</td>
<td>Triacyl lipopeptides</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>Zymosan</td>
</tr>
<tr>
<td>TLR3</td>
<td>Virus</td>
<td>dsRNA</td>
</tr>
<tr>
<td>TLR4</td>
<td>Bacteria</td>
<td>LPS</td>
</tr>
<tr>
<td>TLR5</td>
<td>Bacteria</td>
<td>Flagellin</td>
</tr>
<tr>
<td>TLR7</td>
<td>Virus</td>
<td>ssRNA</td>
</tr>
<tr>
<td>TLR8</td>
<td>Virus</td>
<td>ssRNA</td>
</tr>
<tr>
<td>TLR9</td>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virus</td>
<td>CpG DNA</td>
</tr>
<tr>
<td>TLR10</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>TLR11</td>
<td>Protozoa</td>
<td>Profilin-like molecule</td>
</tr>
</tbody>
</table>
Toll-like Receptors (TLR)

11 members of the TLR family in mammals; 10 in humans
Different activation states of macrophages

Classical
Alternative
Innate
Humoral
Deactivation

Innate activation
Humoral activation
Deactivation/Suppression
Classical and Alternative activation

Classical Activation

Alternative Activation
<table>
<thead>
<tr>
<th>Activation state</th>
<th>Induced by</th>
<th>Function</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innate immune response</td>
<td>Factors which act on pathogen recognition receptors (eg DAMP)</td>
<td>To fight infection in the periphery. Probably the same in CNS (eg in response to infection like meningitis)</td>
<td>Associated with production of inflammatory mediators. Needs to be controlled so some anti-inflammatory agents are also released</td>
</tr>
<tr>
<td>Classical activation</td>
<td>Interferon-γ</td>
<td>Causes inflammatory changes (but also attempts to fight infection)</td>
<td></td>
</tr>
<tr>
<td>Alternative activation</td>
<td>Antiinflammatory cytokines like IL-4</td>
<td>Protective and attempts to repair</td>
<td></td>
</tr>
<tr>
<td>Deactivation/suppression</td>
<td>Neuroimmune regulatory proteins like CD200</td>
<td>Helps to maintain microglia in a quiescent state</td>
<td></td>
</tr>
<tr>
<td>Humoral activation</td>
<td>Involves the complement system</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Microglia are very reactive – multiple pathogen recognition receptors (PRR) bind pathogen-associated molecular patterns (PAMPs).
Wyss-Coray et al. Neuron
Fibrillar Aβ

Neuron

Activated Microglia

Complement
ApoE
ATP

Cytokines (IL-1β, IL-6, TNFα, TGF-β)
Chemokines (MCP-1, MIP-1α, RANTES)
Complement Factors (C1q, C3a, C5a)
COX, Prostaglandins
ROS, NO, OHOO
Acute Phase Proteins (α1-ACT)
Microglial activation precedes neuronal loss
Does activation of microglia trigger neurodegenerative changes?
November 3, 1906

Alois Alzheimer described Auguste D’s symptoms
(Meeting of the Psychiatrists of SW Germany)

Clinical
Progressive dementia
Memory loss
Language difficulties

Pathology
Plaques and tangles.
EC, Hippocampus
Shrinking begins
Nerve cell death
Memory loss is first symptom

Cerebral cortex
Shrinking continues
Cell death continues
Memory loss
Confusion
Mood change
Anxiety

Widespread
Severe AD
Extreme shrinkage
4 genes: Amyloid precursor protein (APP)
  Presenilin 1 (PS1)
  Presenilin 2 (PS2)
  Apolipoprotein E (ApoE)

Autosomal dominant/Familial early-onset AD (FAD)

Late-onset AD (Sporadic AD)
  ApoE4 is a risk factor

STATISTICS
Dementia affects ~ 44,000 people in Ireland; 66% AD, remainder vascular dementia (preventable)
Prediction: 104,000 people with dementia by 2036
Aβ stimulates cells to release **cytokines, chemokines and ROS**

- **IL-1** increases & processing of APP
- **Chemokines recruit immune cells**
- **ROS cause degenerative changes**
Healthy nerve cell

Microtubule-bound tau

Unbound phosphorylated tau species

Phosphorylated tau aggregation intermediates

Stable hyperphosphorylated tau filaments (64 kDa)

NEUROFIBRILLARY TANGLE

Glyco__('Glyco')

Hyperphosphorylated Tau Protein

Tau Protein

Ubiquinated
PROPOSED SEQUENCE OF EVENTS

Sporadic AD

Failure of $\text{A}\beta$ clearance

Gradual increase in $\text{A}\beta$

Oligomerization of $\text{A}\beta$

Activation of inflammatory cells

Inflammatory stress

Formation of tangles

Loss of brain tissue

Enhance clearance

Reduce $\text{A}\beta$ production

Reduce inflammation

Change enzyme activity
Neutralize production
THERAPY

ADAPT (AD Anti-inflammatory prevention trial)

Trial stopped - cardiotoxicity - celecoxib

Immunotherapy
Development of Immunotherapy

1996: Antibodies blocks formation of fibrils (Solomon et al)

1999: Aβ vaccine reduces amyloid deposition (Schenk et al.)

2000: Passive immunization with Aβ antibodies reduces amyloid deposition promotes Aβ clearance (Bard et al) prevents memory deficits (Janus et al. Morgan et al.)

2001 Antibodies remove plaques (Bacskai et al), removes Aβ from brain (DeMattos et al.)

2002 Antibodies reverse memory deficits (Dodart et al; Kotilinek et al.)
2001: (Elan and Wyeth) Phase 1 trial
   Good immunological response
   Good tolerability

2001: Phase 2a study; 375 patients
   6% of patients developed meningoencephalitis (T cell infiltration?)

2002: Trial halted prematurely

2003: Improved cognition with vaccination - associated with high titers of antibodies benefit (Hock et al.)

2003-05: Autopsies vaccinated patients have reduced Aβ (Nicoll et al; Ferrer et al; Masliah et al.)
PARKINSON'S DISEASE AND INFLAMMATION
Terminology

• NEURODEGENERATION
  The loss of neuronal processes (axons and dendrites) and death of nerve cells

• NEURODEGENERATIVE DISORDER
  A type of neurological disease marked by the loss of nerve cells: e.g. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD)
Parkinson’s disease

Degenerative disorder of the CNS - impairment of motor skills and speech

SYMPTOMS
Muscle rigidity
Tremor
Poverty of movement
Muscle weakness

Treatment: L-DOPA

PD patient (sketch, 1886)
**Key pathology**
Loss of DA-ergic neurons in SN

**Treatment:** L-DOPA

**PET scan**
Reduced (dopamine) activity in
Basal ganglia
Putamen
Caudate
Treatment: L-DOPA
Stem cells for transplantation in PD

- Embryonic stem cells
- Embryonic midbrain
- Subventricular zone
- Bone marrow

In vitro stem cells differentiation into dopaminergic neurons

Transplantation into Parkinson’s disease patients
Present limitations in the development of the human embryonic stem cell-based therapy for PD

1. Ethical issues raised by the use of human blastocysts

2. Xeno-free systems to derive, expand and differentiate the hESCs

3. Presence of undifferentiated hESCs that leads to teratoma formation and very mature neurons that do not survive to the transplantation

4. Teratoma formation, graft immune rejection and survival/phenotype maintenance of the DA neurons
Generation of dopamine neurons from autologous human mesenchymal stem cells (MSCs)
Evidence for a role for neuroinflammation in PD

**Serum** - High plasma [IL-6] associated with increased risk of PD
Increased IL-1, IL-6, TNFα, IL-2

**CSF** - Increased IL-1, IL-6, TNFα, IL-2

**Genetic analyses** -
Polymorphisms in TNFα and IL-1β associated with increased risk
Allele 122 of IFNγ is less common in early-onset than in late-onset PD
Increased frequency of GG phenotype of IL-6 at position 174 in PD

**Epidemiological studies**
Decreased risk of PD in people who take NSAID

- Markers of microglial activation
  - Increased inflammatory cytokines
  - Increased NOS, COX
  - Increased astrocytic activation

Substantia nigra
Striatum
Locus coeruleus

PET scan: $^{11}$C-(R)-PK-11195 binding
Risk Factors
- Genetic alterations
- Head traumas
- Transient ischemias
- Microvasculature defects
- Environmental insults

Increased Brain Inflammatory Processes

Induction of "Protective Genes"

Reactive Oxygen Species
- $O_2^-$
- $H_2O_2$
- $\cdot OH$

Reactive Nitrogen Species
- NO, $\text{ONO}_2^-$

Induction of "Toxic Genes"
- iNOS, COX-II

Neurodegenerative Diseases

Time
**BLUE: Neuroinflammation in PD**

IL-1β, TNFα, IFNγ induce CD23
iNOS expression increased
NO released

NO diffuses to DA-ergic neurons

Role of infiltrating T cells?

**RED: Inflammation–induced cytotoxicity**

NO reacts with $O_2^-$
Forms peroxynitrite (ONOO$^-$).....oxidative stress
This damages proteins
NO releases iron from ferritin.....oxidative stress

TNFR1 activates death signaling pathways
.........Caspases, ceramide, NFκB
Animal model of PD identifies a role for glia and T cells
Activated microglia release NO

\[
(O_2^- + NO) \rightarrow ONOO^- \rightarrow \text{oxidative damage to proteins in DA neurons}
\]

\[
O_2^- \rightarrow SOD \rightarrow H_2O_2 \rightarrow Fe^{2+} \rightarrow OH^- \]

Reactive astrocytes have increased myeloperoxidase

\[
H_2O_2 + Cl^- \rightarrow HOCl
\]

\[
HOCl + O_2^- \rightarrow OH^- \]
Activated microglia release inflammatory cytokines

- TNFα interacts with TNFR1 and induces COX2 in neurons...cell death pathways
- TNFα induces activation of iNOS in microglia by activating the low-affinity receptor of immunoglobulin E (CD23).

NO is released
Cascade is initiated
Cytokines/chemokines recruit activated T cells
T cells release cytokines and Fas ligand
Fas L interacts with Fas R and initiates activation of glia
Cascade of damage

Death of DA neurons triggers glial activation