Neurophysiology Lecture 1 – Introduction to the Course

- How does a neurone generate activity
- Brain isn't all about neurones – lots of other cellular elements that play an important role
- Neurone as the main player, connect with another neurone via a synapse from its axon terminal to a dendrite
- Neurone's myelinated to change the speed of conduction via oligodendrocytes (CNS) and Schwann cells (PNS) – differences in the way these operate
- Blood cells are important, brain reliant upon it for oxygen and nutrients

Glia

- German word for glue – first discovered as cells that were thought to hold the brain together in a structural role
- Multiple types – eg. astrocytes, oligodendrocytes
- Astrocyte – reasonably large cell body (compared to most neurones, quite small, about a quarter of the size)
    - Complex web of processes that eminate from the cell body and extend quite a distance from cell body into the substance of the brain
- Within the CNS we have glial cells (also found in PNS)
- Oligodendrocytes for myelination in CNS, Schwann cells in PNS
- Microglia – modified macrophages, not from a neuronal linage, migrate through brain to act as immune cells/surveyors of the brain
- Ependymal cells – make barriers, make cerebral spinal fluid and have cilia that move it around through the brain, also a source of neural stem cells (exist within different parts of ventricles)
- Satellite cells in PNS

Astrocytes

- Form support for CNS, ie. glue
- Form blood brain barrier – way in which the brain is protected from what's happening in the systemic circulation
- Brain is like an apex predator, only takes what it needs – blood brain barrier enables this as it has lots of transporters that allow lots of substances to come across from blood to the brain
- Tight barriers to prevent things from just diffusing across
- Glutamate primary excitatory transmitter in the brain – it is an amino acid, if you eat a meal with protein you end up with quite a lot of glutamate in the circulation
    - If this got straight into the brain, every time you eat you'd get massive excitation of all the neurones in your brain – not good!
    - Brain stops that glutamate from coming across the blood brain barrier, it stays within the systemic circulation and the brain *chooses* how much glutamate it will take
- Secrete factors and maintain extracellular environment – reduce the amount of K+ that builds up within the extracellular space
- All information up until 15 years ago said that astrocytes were support cells
    - Began to realise this is a limited view of what astrocytes do
    - Number of astrocytes per neurone – relative to each neurone, a leech doesn't have many astrocytes… increase in complexity of the nervous system (ie. moving from leech to higher mammals such as cats/humans), the number of astrocytes per neurone is dramatically increased
        - If all they're doing is providing support, why would you need more astrocytes per neurone? You'd have the same amount if this was the case.
        - This alerts us to the fact that astrocytes are doing something more than just support
- Support: neuronal areas with dendrites, axons, etc. and astrocytic elements surrounding them
    - Everywhere that's not a close apposition between 2 neuronal elements is surrounded by astrocytic processes

    - Astrocytes have filled everything that's not neurone – no spaces in the brain
- We know astrocytes are filling the space, but each astrocyte has its own territory within the brain
    - Territories don't overlap all that much
- Astrocytes are in control of a particular space within the brain
- Have very long processes, and also have a xiphoid process that goes up and contacts the blood vessel – interacting with endothelial cells to form the blood brain barrier, also maybe for changing blood flow to a particular area in response to a change in activity
- Glutamatergic synapse within the brain – synaptic/axon terminal, releasing glutamate via vesicles into the synaptic space… post-synaptic side with receptors for glutamate (NMDA/AMPA)
    - Glutamate released, goes across synapse and interacts with the receptors causing a change in the activity of this
    - In order for this to have some time-based fidelity (ie. an on/off signal) you have to get rid of the glutamate somewhat quickly
    - In order for it to have some spatial relevance, you have to make sure the glutamate doesn't go everywhere otherwise the pre-synpatic space wouldn't have any relevance in relation to the post-synpatic space
    - Astrocytes at the edge of the synapse with transporters that rapidly pick up the glutamate and take it out of the synaptic space to stop the interaction of one neurone to the other and to also stop that glutamate from spilling over and effecting other synapses
- Astrocytes play an important role in maintaining the fidelity of synaptic interactions between neurones
- Just outside the synapse are glutamate receptors, if this synapse becomes very active that it overwhelms the ability of the astroyctes to remove the glutamate, it starts to spill over and have effects outside of the synapse – extrasynaptic glutamate receptors
    - This has impact for the post-synaptic glutamate receptor as well
        - Memory processing, excitotoxicity
- Astrocytes try to maintain fidelity of signalling but sometimes activity rates are too high and that has effects as well
- Every time an AP comes along to a synaptic terminal, we're gonna have release/movement of K+ out of that axon and movement of Na+ in
    - Extracellular space changes its K+ concentration with every AP
    - Sits somewhere around 3mM (extracellular K+ conc)
    - But one AP within that very confined space can lead to an increase of about 3.75-4mM K+
    - Train of AP, then the K+ conc can increase dramatically
    - If you change the extracellular K+ conc, you're going to change the gradient for movement of K+ and in doing that you change excitability of the neurone
    - Have to keep extracellular K+ at a constant low level in order to maintain the ability of neurones to work properly
    - Astrocytes come into this via the NaKATPase, different co-transporters, etc.
    - Any build up of K+ within the extracellular space is channelled into the astrocyte such that it can increase its extracellular K+ concentration
- Astrocytes are a bit like a K+ electrode, as the extracellular K+ increases, the intracellular K+ changes as well
- What do you do with intracellular K+ once it's in the astrocytes?
    - They are connected in a network that enables them to communicate over very large areas and dissipate an increase in K+ in one space to another area so it can go back out into the extracellular space where it doesn't cause a problem
- Calcium wave within some cultured astrocytes…
    - Astrocytes are connected up one to the other
    - Ions like Ca+ and K+ can dissipate between astrocytes over very long distances
    - Changes in concentration within one point can be moved away
        - Happens via gap junctions
- Gap junctions between astrocytes are incredibly important
    - Made up of particular transmembrane spanning proteins

    - Individual component = connexon, 4 transmembrane spanning domain protein
    - Each connexon joins with another 5 to form 6 connexins called a connexon
    - Forms a channel that can be regulated – opened and closed so connection between astrocytes can be modulated
    - Neighbouring membrane also has an arrangement of these gap junctions as well so they line up to form pores in the membranes
    - Between normal membrane there's a gap of about 20nM but gap junctions between astrocytes which form with ion channels can bring the membrane very very close together (3.5nM)
    - Enables ions to move readily between 2 astrocytes
    - Some transmitters also move through these channels, as well as larger second messenger molecules
- Stimulate one astrocyte and record membrane potential in another astrocyte, can see that electrical charge can also move between them through the gap junctions – can go in both directions
- Electrical charge, ions, messengers can all move between neighbouring astrocytes
- Synaptic activity changes extracellular ion concentrations
    - Glia involved in maintaining extracellular space
    - Depolarise when K+ channel changes – receiving signal and remove that via current flow
    - Process is called spatial buffering
- Astrocytes posses the NaKATPase, energy dependent movement of ions, symporters and other ion transporters to assist with maintaining the extracellular environment
- Astrocytes play a bigger role than just this
- Cultured astrocytes – calcium signal, but what's going to happen is some glutamate is going to be put onto them… no neurones in this field, just astrocytes… glutamate = principle excitatory transmitter that works through AMPA, NMDA and metabotropic receptors
    - Increase in Ca+ signal and that moves along through the entire group of astrocytes
    - Astrocytes depolarised by change in extracellular concentration of K+
    - Also responding to not just the changes in the extracellular ions but also to the transmitters
    - Astrocytes have receptors for a whole lot of transmitters and respond with calcium signals to transmitters that have been released in their territory and when they do that, the Ca+ signal can work its way through the syncytium of the astrocytes and have effects through quite wide areas
- Culture of astrocytes – gauged out astrocytes in one area so that there was no connection between astrocytes on either side of the gap
    - Then they stimulated one side and saw that astrocytes on the other side of the gap also responded to the stimulus!
    - Thus this cannot have been via a gap junction as there is no connection there
- Astrocytes respond but also release transmitters that have effects on neighbouring astrocytes and also potentially neurones
- Astrocytes have all of the machinery for vesicular release of transmitters
- Everything we know about in neurones for releasing neurotransmitters is present in astrocytes, different levels in different populations
    - Ability to package transmitters within vesicles so they're then released
- Now think of astrocytes as communication elements, using neurotransmitters to communication within the CNS
- Glia are now know to have gliotranmitters – glutamate, ATP, adenosine, etc.
    - Lots of the peptides we know as communicators between neurones are also being released by astrocytes to communicate
    - Not just maintenance but response and signalling
- In response to stimulation, glia release neuroactive substances called gliotransmitters
    - Have synaptic like microvesicles, dense core secretory granules and various channels that also play a role in release

- Not just neurone to neurone that's controlling communication, but also presynaptic neurone, post synaptic neurone and astrocytes as a maintenance role (uptake of glutamate, maintenance of extracellular ion concentration, etc.) but also the glutamate that is released at the synapse is having an effect on, and signalling to, the astrocytes
    - In response to that the astrocytes are having a calcium response
    - This is involved in vesicular release (gliotransmitter release)
    - Glutamate, ATP and other gliotransmitters can then have an effect on both the pre- and post-synaptic neurone to change the fidelity of information transport across the synapse
    - Influencing the way that one neurone talks to another, not just by maintenance but by actively being involved in the signalling process
    - This is called the <u>tripartite synapse</u>
        - Not just a pre and post synaptic neurone, but an astrocyte as well
    - Via gap junctions (via ability of astrocytes to communication) a change that's occurring in the synapse between pre- and post-synaptic neurones can then have an effect via the release of gliotransmitters on a neighbouring synapse
        - Spatial effects via which one synapse can start to talk to and influence another synapse within a neighbouring area
    - Groups of neurones can be synchronised via small numbers of inputs
    - Starts to explode the options in talking about the way neuronal information is processed and integrated within a part of the brain
- Astrocytes influence, receive inputs and response, release neuractive substances, positioned as a bridge between many elements within the CNS

<u>Neurophysiology Lecture 2 – Resting Membrane Potential</u>

- In this cartoon, you can see a neurone (stylised neurone), some recording electrodes and a stimulating electrodes
- When you see recording electrodes, it's always depicted as the potential relative to something else, always a differential
- Electrode inside the cell (recording electrode), measuring difference in potential between inside of cell and the reference electrode in the bath outside of cell
- Reference electrode taken as a 0 and everything in the recording electrode taken in relation to this
- Membrane potential = -65mV
- Ion concentrations, potassium permeability – key factors that determine RMP
- Deviations in RMP, information processing and transfer between neurones
- Response of neurones, integrated response, can be nothing or an action potential
- Stimulating electrode used to eject positive or negative current, to change membrane potential of cell
- What determines RMP? Ion concentrations, neurones exist in a state of ion imbalance between the interior and exterior
- Intracellular concentration of potassium much larger than that of extracellular
- Extracellular conc of sodium larger than intracellular
- Chloride lower intracellular
- Calcium higher extracellular and vanishing low in the cell, all sorts of mechanisms that mop it up
- Ion concentration gradient maintained? Neurones use a lot of energy to maintain it, 70% of ATP used by brain goes into maintainance of ion conc gradients
- Ion transporters use energy to actively move ions against their conc gradient