Neurophysiology Notes

Lecture 1:

- The brain consists of **oligodendrocytes (**myelinating cell)**, axon initial segments, soma** (cell body)**, myelin sheath, microglia** (macrophage derived- immune cells of the brain-repair debris after damage)**, astrocytes, synapses** and **blood vessels** (supply of nutrition)**. Glia** is the global terminology of cells that exist to hold the neurons together (such as astrocytes).
- Astrocytes extend throughout the brain.
- The glial cells in the **PNS** include **satellite cells** (support cell bodies) and **Schwann cells** (which form myelin sheaths and secrete neurotrophic factors). The glia of the **CNS** includes **oligodendrocytes** which form myelin sheaths, **astrocytes** (support the nervous system, form the blood brain barrier, secrete neurotrophic factors and take up K+, neurotransmitters), **microglia** (act as scavengers) and **ependymal cells** (create barriers between compartments and is a source of neural stem cells-thus can make new neurons- such as in learning).
- Humans have more astrocytes than neurons (with increasing complexity- the number of astrocytes increases per neuron-thus astrocytes aren't just support cells). There are 100 billion neurons in the human brain estimated while there is 900 billion glia.
- The astrocytes are everywhere. The only part of a neuron that's not covered by an astrocytic process is the synapse (to allow for free and rapid movement of the neurotransmitter).
- Astrocytes have their own territory that its processes patrol. They are interested in their own little part of the brain with neurons squashed between them. The **siphon processes** of astrocytes follow a particular line (wrap around a blood vessel) to produce the blood-brain barrier (required to stop things we don't want getting into the brain-antibiotics, large molecules and peptides blocked by blood-brain barrier), most things are blocked out by the blood-brain barrier (don't take in glutamate from the blood stream from food as it's an excitatory neurotransmitter- if blood brain barrier wasn't present we would have an epileptic fit every time we ate).
- The **circumventricular organs** have a leaky blood-brain barrier which listen to what is going on around the body.
- Astrocytes also respond to transmitters and information processing within the nervous system. The astrocytes help to take up the neurotransmitter from the synapse such that we don't keep acting on the downstream neuron (fidelity of neuronal signalling).
- Sodium and potassium move around in the intracellular and extracellular space, the amount of potassium released into the ECF despite being infinitesimally small is enough to change the composition of the ECF as the ECF volume itself is low. In order for normal excitability to occur we need 3mM of K+. Lots of APs have the ability to increase the ECF concentration of the potassium which will change the excitability and information processing of the cell which is what we don't want. So in order to return conditions back to basal, we have astrocytes which take up K+ via various co-transporters (Na+/K+ antiporter or Na+/K+/Cl- symporter) such that the astrocyte concentration of potassium increases. To reduce the K+ concentration in the astrocytes we have **gap junctions** which pass the K+ from the localised area with a lot of activity to adjacent astrocytes, reducing the K+ concentration in the astrocytes. This can be seen experimentally with a calcium wave flowing along astrocytes.
- The **gap junctions** are a 4-transmembrane spanning domain protein that exists across the membrane of the astrocytes and also across some neurons. Each 4-transmembrane spanning domain protein is a **connexin** which come together in the membrane as a group of 6 to form a **connexon**. One side of the membrane will have a connexon and so will the other, which come together to form the gap junction for small molecules to travel via gap junctions to adjacent astrocytes. The gap junctions can be regulated (opened or closed) and they can be used as seen with K+ in order to maintain the ionic homeostasis of the ECF. Electrical activity can be passed between astrocytes as well (as movement of current is the movement of cations).
- Synaptic activity results in the extravasation of K+ (under physiological conditions-several mM change). For glia, under physiological conditions, the membrane potential is largely determined by K+ (small changes in ECF K+ produce changes in voltage). Depolarisation occurs wherever [K+] changes-different from localised nature of synaptic inputs (doesn't reflect excitation or inhibition, just altered

activity). Current will flow along a potential gradient. Inward current (due to increased ECF K+) induces local depolarisation that will spread throughout the glial cell and through gap junctions to neighbouring glia (so at other regions K+ is released to the ECF). This is called **spatial buffering.** In addition, astrocytes possess **Na+/K+ ATPase**, an **anion pump** and a **Na+/K+ symporter** to assist spatial buffering. Neuronal activity also induces considerable changes in ECF pH (increase). Astrocytes possess **Na+/H+ exchanger, Na+/HCO3- cotransporter** (bidirectional, electrogenic pump-changes electrical potential- which is unique to glial cells in the nervous system), **Cl-/HCO3- exchanger** and **active H+ ion extrusion mechanisms**.

- Astrocytes respond to specific inputs via **neurotransmitter receptors.** This response includes activation of intracellular calcium via **IP3**. Activation of neighbouring astrocytes occurs via gap junction communication (discriminate on the basis of size-allow Na+, K+ and other ions to flow through freely-such that it dissipates from the synapse) and **gliotransmitters** (for transmission of signals along non-adjacent astrocytes)**.**
- In response to a stimulus astrocytes can send information between themselves via these gap-junctions. The stimulus in this case increases Ca2+ (key intracellular messenger) concentrations intracellularly via IP3 which then sends the signal through gap junctions to adjacent astrocytes. Changes in intracellular calcium will change the function.
- In response to the stimulation, there is an intracellular calcium response as well as release of transmitters to affect neighbouring astrocytes (in this case by ATP-for wider spread of signal). Astrocytes have all the machinery for vesicular release and a large number of signalling molecules. They contain the key molecules required for tethering the vesicle to the membrane for vesicular release (**synaptobrevin, syntaxin** and **SNAP-23**). Unlike neurons, astrocytes don't have long projections for long distance signalling and hence their signalling is more confined.
- The types of gliotransmitters include glutamate, ATP, adenoside, D-serine, eicosanoids, cytokines, proteins and peptides.
- Gliotransmitter release occurs via exocytosis where synaptic-like microvesicles form, glutamate appears to have **VGLUT** for loading and these microvesicles cluster at release sites associated with exocytotic proteins such as **SNAP**, **SNARE** and **VAMP**. Dense core secretory granules are also present (nucleotides and peptides). The process of exocytosis is slower than in neurons where it's triggered by IP3 induced changes in intracellular calcium. Various channels may also play a role in release such as the **hemichannel, anion channel** and **P2X7 receptor.**
- Neuronal transmission is seen when there is transmission between the pre-and postsynaptic neurons via neurotransmitters. These neurotransmitters are taken up by the glia for fidelity of the signalling such that more information stimuli can be received and acted upon. Glia also remove K+ from the extracellular space. The glia will also respond to the neurotransmitter (in this case glutamate-metabotropic receptor) leading to signalling via IP3 releasing intracellular calcium sending calcium between the gap junctions such that glia can modulate transmission of neighbouring synapses (both by gap junctions and gliotransmitters).
- Astrocytes receive inputs and respond, release neuroactive substances and are positioned as a bridge between many elements in the CNS (neurons, blood vessels and neighbouring domains).
- The glia have a complex structure, they cover the surface of neurons with the exception of the synapse, contact blood vessels (to form the blood brain barrier) and are an interface between neurons and blood and they fill the space of the nervous system making small intracellular spaces of ~20nm. The glia outnumbers neurons (10:1) with an increased number per neuron with increasing nervous system complexity. The glia is present in different types there are astrocytes (many functions) including **fibrous** (among bundles of myelinated axons) and **protoplasmic** (mostly in grey matter) astrocytes. The oligodendrocytes and Schwann cells have roles in myelination, the **radial glia** have roles in development (traffic neurons to the right place), the **ependymal cells** line the ventricles and the microglia are part of the injury response.

Lecture 2: Resting membrane potential

- Neurons are excitable cells that sit in a positon of imbalance of charge across the cell membrane such that they have a membrane potential difference (this difference allows us to generate APs). Resting membrane potential is around -60mV. The movement of positive current determines the direction in which the membrane potential will change.

- Graded/passive potentials are potential changes (either direction-magnitude determined by amount of current) that don't reach threshold to initiate an AP. In response to a decrease of inwards current the cell will hyperpolarise while if you increase the inwards current the cell will depolarise. Threshold being reached generates an action potential which is always at the same magnitude (fired or not-information processing unit of the nervous system).
- The RMP is setup by concentration gradients where K+ is higher intracellularly while Cl-, Na+ and Ca2+ are higher extracellularly. Thus, there is stored potential energy across membranes (as the ions want to move along their concentration gradients).
- The concentration gradients are setup by ion transporters which include **pumps** (use energy in the form of ATP to actively move ions against their concentration gradient - binds ion and with energy changes conformation to move ion against concentration gradient-Na+/K+ ATPase-consumes 70% of brains energy) and **ion exchangers**. The **Ca2+ pump** uses energy to pump H+ in and Ca2+ out against their concentration gradients.
- The Na+/K+ ATPase has an **α subunit** (main action occurs here) and a **β subunit**. The α subunit is a multiple transmembrane spanning domain but a single polypeptide chain (comes from one gene), it has a binding site for Na+ and K+, a binding site for ATP, a **phosphorylation site** (for modulation) and a **Ouabain** binding site (for modulation). The β subunit has roles in trafficking and modulation of the α subunit but aren't involved in the main function of the pump.
- The **ion exchangers** include the **Na+/Ca2+ exchanger**, the **Na+/K+/Cl- cotransporter**, the **K+/Cl-cotransporter**, the **Na+/H+ exchanger** and the **Na+/neurotransmitter transporter** (such as removing them from the extracellular space so don't continue stimulating their respective receptors)**.** These exchangers use the energy created by the concentration gradients to move other molecules against their gradients across the membrane (couple movement of an ion down its gradient with a movement of molecule up its gradient-don't use ATP).
- The cell membrane forms a barrier to the movement of ions. Transporters use energy to establish concentration gradients across the neuronal membrane. At rest, the neuronal membrane is selectively permeable to potassium. Potassium is close to equilibrium with little net movement due to a balance between the concentration gradient and the electrical gradient forces. This results in a membrane potential of approximately -65mV with the interior of the neuron negative. This is close to the K+ equilibrium potential (the potential difference required for K+ to not move along its concentration gradient- electrochemical gradient has been reached-negative interior attracts K+ into the cell, such that there is a dynamic equilibrium reached at the equilibrium potential- about -80mV for potassium- not reached as K+ conductance is not 100% and there is small conductance of Na+). The resting membrane of a neuron is selectively permeable to K+ (via leak channel K+ conductance out of the cell).
- The amount of K+ that moves is very low-only the paint on the membrane moves across to change membrane potential as charge across membrane is defined by the difference in charge across membrane only- not in the middle of the cell (attraction still occurs across the membrane of the cell-lining up of charges against the membrane of the cell).
- Ion selectivity is seen in K+ channels. Both K+ and Na+ are surrounded by H2O molecules (hydrated) with K+ being selected based on size. In the potassium ion channel (made up of multiple subunits-tetramer from 4 amino acids sequences- transmembrane spanning) the ion selectivity is seen in the pore helix of the channel. As K+ enters the channel, it loses its H2O and four K+ ions line up in the channel. This environment allows for selection of K+ and not Na+. The amino acid structure lining the pore contains oxygen molecules which perfectly replace the positions of water around the un-hydrated K+ ion such that the K+ is in its most relaxed state in the channel. 4 K+ line up and when one more enters the channel, the K+ at the top is knocked out into the extracellular space. The Na+ ions don't exist at rest in this channel and are therefore selected against.
- The gating of a channel is the ability to change the conformation of a channel such that they open or close. There are **voltage-gated** (respond to potential difference)**, ligand-gated** (respond to ligands) and **mechanically-gated channels** (respond to deformation).
- In the potassium channel, there is a **selectivity filter** and a **gating hinge** which responds to whatever stimuli gates the channel, causing a change in conformation of the channel and hence opening it.
- There is a vast array of ion channels with differing characteristics. The main voltage-gated ion channels are **Na+, Ca2+, K+** and **Cl-.** There are also ligand-gated ion channels such as those with **neurotransmitters**, **Ca2+-activated K+ channels** and **cyclic nucleotide gated channels**. The driver of a

non-selective ion channel is the electrochemical gradient. They just contain pores for ions to flow through.

- The pump and ion exchangers are proteins that sit in the membrane, have binding sites for ions (limited spots for ions to sit), change their conformation in response to stimulus to take the ion against its concentration gradient.
- There is a larger amount of complexity in the potassium channels, with different isoforms and different constructs. Some potassium channels are involved in the excitability of the cell, some are involved in the RMP and some involved in repolarisation after an AP.
- The four main types of K+ channels include the **Kv** and **HERG** (have 7 transmembrane spanning domains and a re-entrant loop- intracellular C and N tails. In most cases, each of these subunits make a tetramer channel- α subunit. The β subunit is responsible for trafficking and modulation- varying properties), the **inward rectifier** (2 membrane spanning domains and a re-entrant pore), the **Ca2+ activated channel** (multiple subunits with an extension of the intracellular length of the polypeptide to given an equivalent of the β-subunit that is still part of the main polypeptide) and the **2-pore channel** (come together as groups of 4 transmembrane domains to make 2 ion channels).
- The following channels properties have been determined from an experiment on a cell without ion channels (we added specific ion channels to it) that starts at rest (-60mV) and then we either hyperpolarise it or depolarise it to see the response from the particular K+ ion channels.
- At rest we have K+ leak channels that allow K+ to flow out which contributes to RMP.
- The **Kv2.1 channel** shows movement of ions when the cell is depolarised but not hyperpolarised such that the channels allows K+ to move out of the cell. The cell doesn't open to the hyperpolarisation event as the channel is voltage-gated and hasn't reached threshold to stimulate the opening of the ion channel and thus it has zero conductance after the hyperpolarisation event (this channel will not have the characteristics to contribute to resting membrane potential). The same is the case for the **Kv4.1 channel** except that the period of time upon which it is open is much shorter.
- The **calcium activated channel** responds to the depolarisation event, but if you increase the calcium concentration the conductance and opening properties are increased such that calcium acts to modulate the action of the channel (still opens at low calcium concentration via voltage).
- The **2-pore channel** responds to pH where at a basic pH (8) it opens but at an acidic pH (6) it closes.
- The **HERG (Human etherogogo related gene) channel** also acts in a similar way to the Kv4.1 channel except that it takes longer to open, but closes quickly.
- The **inward rectifier** responds to voltage, but when the cell is hyperpolarised rather than depolarised and stays open for longer, it is therefore able to allow K+ back into the cell for repolarisation (along its electrical but not chemical gradient). They act as such that they help to keep the cell at around the RMP (such that the membrane potential won't get too negative).
- The channels that contribute to RMP include the inwards rectifier and potentially the HERG channel.
- The different types of channels include **nonselective cation channels, delayed rectifiers**, **slow-delayed rectifiers, classical inward rectifiers**, **ATP-sensitive channels, 2-pore channels** and **GPCR.**
- The 2-pore potassium channels respond to a range of stimuli such as CO2 or may be mechanically gated.
- A general **anaesthetic** stops the sensory cortex from working to lose perception and pain without losing other functions of the brain (hence need selectivity of action). Alcohol is a class of anaesthetic that inhibits the activity of some neurons (unclear mechanism of action). Some anaesthetics increase the activity of **GABAergic neurons** (inhibitory cells of the brain). **Chloroform** is a gaseous anaesthetic (not used anymore-use isoflurane). The **loss of righting reflex** (**LORR**) is the inability for an animal that has been flipped over to get back to its normal side. If we give an animal a general anaesthetic, it loses this righting reflex and we can thus use the animal for surgery. The latency (time) for LORR increases in the knockout (removal of certain channels) compared to the wild-type- takes more anaesthetic to induce LORR on the knockout than the wildtype. **Pentobarbital** (opioid) is unaffected by the knockout (not affected by loss of the gene) and the gene that is lost is one of the 2-pore channels. Therefore in response to the loss of one of the 2-pore channels we lose the efficacy of a gaseous anaesthetic as these channels are critical for maintaining RMP and the leaking K+ conductance that determines the RMP (the GABAergic neurons have become more excitable). These 2-pore channels are responsive to anaesthetics, lipids, heat, stretch, cytoskeleton arrangement changes, voltage, pH and various secondary messengers (PKA, cAMP, DG, PKC). These cells are thus highly regulatable and critical for setting the excitability of the cell.

- Ionic concentration gradients exist across the neuronal membrane (key role of ion pumps-energy dependent- and ion exchangers-use ion gradient to drive movement). These together with K+ permeability determine the resting membrane potential. Inward rectifier and **K2P** channels have characteristics that enable them to contribute to RMP. Ion channels are selective, have differing characteristics and are gated.