PHYS30008 Course Summary Notes

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Lecture block 1 (L7-9) – Neuro

Viruses

- Two main classes (based on their genetic material):
    - RNA viruses – undergo reverse transcription (in host cell cytoplasm)
    - DNA viruses – replicate in host cell nucleus
- Generally **species**- (or even **cell**-) **specific**– "tropisms" (multiple species) are quite rare
    - Enables specific tissue targeting of therapeutic viruses
    - Can track cells effected using Green Fluorescent Protein
- Types:
    - Lentivirus – RNA virus, responsible for HIV
        - GAG – encodes proteins which assemble HIV
        - Pol – makes reverse transcriptase, and integrase (which catalyses insertion of pro-viral DNA into the host genome)
        - Env – encodes proteins that make surface glycoproteins (cell entry with gp120 and CD4 cells)
        - Rev – encodes RNA binding protein that exports viral RNA form nucleus
        - Tat – encodes viral transactivator that binds in the LTS to regulate viral transcription
    - Adenovirus – dsDNA (e.g. common cold)
    - Adeno-associated virus – requires adenovirus to replicate (cannot by itself)
        - ssRNA or ssDNA
        - E.g. pavovirus (deadly to dogs)
- Uses in treatment
    - Use "inactivated virus" to enter cell and produce proteins
        - Genes that code for pathogenic proteins and are removed (e.g. genes that enable replication)
        - Replace with genes for wanted proteins
    - Produced a synthetic promoter (8x stronger) – called 'PRS X8 promoter' (successfully produced more NA cells)

Optogenetics

- Channelrhodopsins (GPCR) – in the eyes
    - Responsive to/activated by specific wavelength of light (470 = blue light (can be red-shifted however))
    - Relatively slow (as GPCR)
- Halorhodopsins
    - Cl pump channels (pump in in response to photon of yellow light)
        - One photon = one ion (not a flood like channelrhodopsins)
    - Hyper-polarisation = inhibition
- Archaerhodopsins and bacteriorhodopsins (fungi)
    - Ion channel – relatively fast
    - Proton pump – one photon releases one H^+ ion (hyper-polarisation = inhibition)
        - One photon = one ion (not a flood like channelrhodopsins)

Nucleus accumbens (nAc)

- Key for reward and motivation
- Different responses to stimuli in **depressed** and **healthy** mice (as a model of people)
    - Measured by "paired pulse ratio" (PPR) – how much transmitter is released in first pulse (higher in susceptible mice)
        - First pulse – affected by: pre-synaptic Ca^{2+} store, neurotransmitter level, pot-synaptic receptor number and conductance)

        - Second pulse (immediately after) – Ca^{2+} level increases in resilient mice (hasn't dropped after first pulse), but not in susceptible mice (takes longer to reload vesicles)
    - Can also test by injecting channelrhodopsins (via virus) into **pre-frontal cortex** (PFC)
        - Enables specific activation of PFC (measured via patch clamp post-surgical removal)
- Results: showed "resilient" mice have:
    - **Extra glutamate** released from **PFC**
    - **Less glutamate** released from **ventral hippocampus**

<u>Chemogenetics</u>

- Modulates activity via ligand-gated ion channels and GPCR in which either:
    - The **ligand doesn't exist in mammals**
    - The **binding site is modified** to not bind the native ligand and bind an otherwise inactivated ligand: DREADDs (Designer Receptors Exclusively Activated by Designer Drugs)
        - Generally slower response/effect than optogenetics
    - Eg: CNO – inert ligand, but can be used to activate modified human muscarinic receptors (hM_3D) that no longer responds to ACh (until 10,000x higher ACh level, which is unrealistic of biological conditions)
        - Can do this for excitatory or inhibitory receptors
- Able to track changes in response via inserting GFPs (or other markers) along with the new receptors in the viral vectors

<u>Neurological Control of Respiration</u>

Traube-Herring Waves – bursts of sympathetic activity after each breath that slightly constricts blood vessels

- Exaggerated in hypertensive people
    - If "normotensive" but have exaggerated Traube-Herring Wave could indicate future hypertension
        - Lesions in C1 neurons (found in rostral-ventral lateral medulla) **prevents** this future hypertension
- NK-1 receptor – receives substance P, responsible for generating breathing ability and has connection with sympathetic modulatory activity of breathing with C1 neurons and hypertension (blood pressure)

<u>Neurotropic Viruses</u> – taken up by terminals, replicated (amplified) in nucleus, projected to next neuron, etc

- Often "lytic" – kills the neuron
- E.g. 'Pseudo-rabies virus' (EnvA-SADB19-delta G-rabies)
    - Has 'G-protein' removed (so prevents it moving between neurons)
        - Produces **EnvA** instead (**no mammalian receptors** for it (only in birds), so cannot infect cells)
        - Alter C1 neurons to enable EnvA-receptor (TVA) production – DNA required transmitted to C1neurons by AAV
            - Also make this transmit a GFP receptor – to track uptake
        - Now inside, virus can reform with G-protein on outside
    - Now able to be transmitted neurotropically from this "seed neuron" to infect other cells
        - This is the "modified rabies" that gives immunity