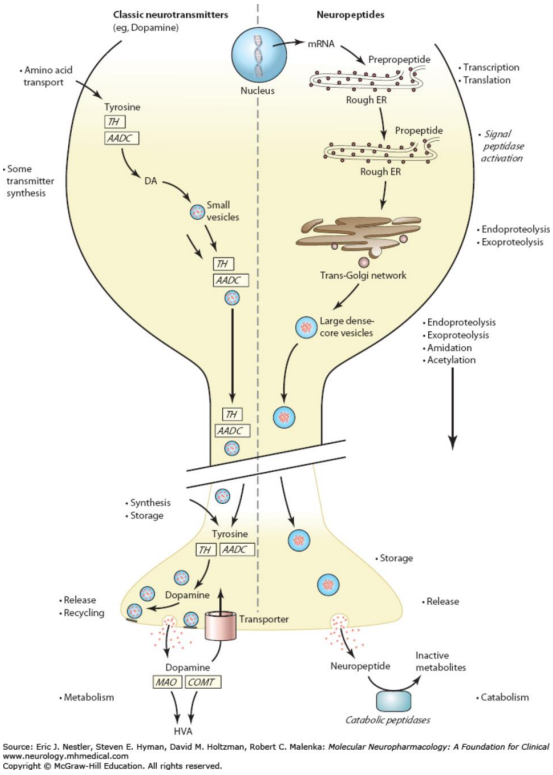
Lecture 10 – Neuropeptides

Describe the synthesis, storage, release and inactivation of neuropeptides and how they differ from classical neurotransmitters

- What are they
  - Small proteins or polypeptides with NT-like roles that **are released similarly to NTs** to activate post-synaptic receptors → most often to GPCRs
  - Secreted into blood by neurones (oxytocin + vasopressin) or hormones secreted by endocrine gland (pituitary = ACTH and growth hormone) → act within peripheral organ (GIT by CCK)
- Roles
  - Sleep, reward, energy balance, pain, memory in both CNS and PNS
- Synthesis
  - Gene → transcription and splicing that can produce alternately spliced RNA and therefore variants → translated to large propeptide that can have multiple peptides within it → cleavage and stored in vesicles (LDCV within golgi and modified further) → modifications can change function or bio-compatability → effect
  - **POMC (proopiomelanocortin)** → undergoes step by step process to release these peptides → involves multiple enzymatic proteolytic cleavages (i.e. pro-hormone convertase 1/2)
  - **Peptide diversity** → Relative few genes leading to large amount of proteins being synthesised → Different function, tissue distribution, receptors as well are diverse because they're proteins → **have tissue specific proteolytic cleavage and translational modifcations**
- Storage
  - Can have multiple peptides in same neuron but in different LDCV
- Release
  - Ca^{2+} increase **of lesser magnitude but longer duration**
  - **Bind to GPCRs mostly with multiple receptor subtypes**
- Inactivation
  - By endopeptidases and exopeptidases that cleave peptides to terminate action → **no reuptake**
- Differences
  - Classical synthesised and stored in small vesicles mostly in pre-terminal before hanging **closer to presynaptic membrane before release** (need Ca^{2+} influx) → turned off through uptake or AChE
  - Neuropeptides start at nucleus and transcribed before storage in **LDCVs** that travel on axons to synaptic release sites (not close) and **need a longer Ca^{2+} influx (train of APs) to release** → never inactivated by uptake, but **metabolised by enzymes located on EC membranes**



Outline the difficulties encountered in designing safe and effective peptide drugs

- **Big size = interact multiple sites = complex and hard to predict**
- **Poorly absorbed when orally = gut metabolism** so need nasal or injection
- **Rapidly degraded = short action duration** (hydrolysis by peptidases)
- **Unpredictable ability to cross BBB**
- **Expensive, immunogenic, lack of pharmacological tools** (for some drugs, might have a tracer (radio labelled(and administer this to use PET scan to see where it has gone in the brain - see whether the drug is going to the right place, these tools are rare), **redundancy** (receptors alternately spliced - drug that blocks one receptor, quite often - other receptors or drugs kick in and compensate for you blocking that subtype), **few peptide drugs exist**

Describe the important characteristics of the tachykinin and opioid families of neuropeptides

- Tachykinin
  - Produces response slower than bradykinin family
  - Caused powerful contractions of arteries and gut
  - **3 tachykinin genes encode a family of neuropeptides → complexity in predicting (Tac2 = Tac1, also have Tac3/4)**
  - **Similar in sequence (with amidated C-terminal required for biological activity)** → Large intracellular tail with different functions
  - Despite the variety of receptors and substances → SSP (depression and vomiting) for NK1R really only found use so far

Lecture 13 – Blood Brain Barrier 1 → What, Where and How?

Learning objectives

- The blood brain barrier are multiple barrier interfaces are present between the blood and CNS
- A combination of structural, functional and enzymatic barrier mechanisms restrict entry of compounds into the CNS
- Physicochemical properties of compounds determine ability to passively enter the CNS
- Assisted transfer mechanisms deliver essential compounds into the CNS
- Assisted and passive transfer mechanisms export compounds from the CNS
- The blood-CNS barriers are functionally effective from the earliest stages of brain development.

Blood-CNS barriers

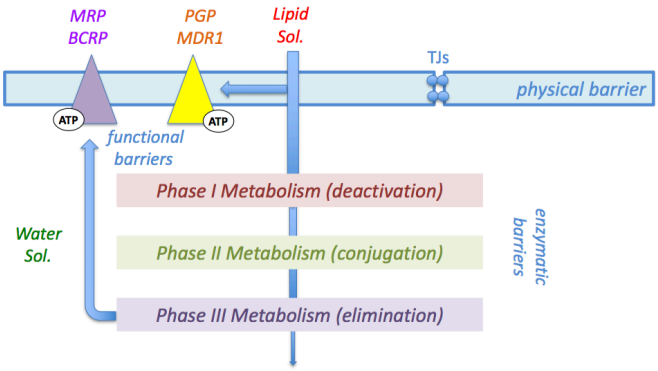
- A series of cellular interfaces that combine structural (passive) and functional (active) elements to regulate exchange of compounds to and from the systemic blood supply to maintain the composition of the internal environment of the CNS
  - **Can transfer IN things that are needed and RESTRICT entry of things not needed (drugs)**
- Multiple barriers
  - Blood-Brain → cerebral endothelium
    - Highly vascularised mean drugs can get to location but cannot actually enter due to pericytes, astrocytes that wrap around capillaries as well as the **capillary endothelial cell → SQUAMOUS** tubular sheets that form cellular interface between blood and brain → **use tight junctions**
    - **Lack of pinocytosis and fenestrations = no aqueous pathway across cells**
  - Blood-CSF → choroid plexus epithelium, arachnoid
  - CSF-Brain → neuroependyma, pia mater
    - Cells are born at the centre phase and migrate upwards and have tight junctions that form barrier and fetal CSF contains for protein (problem here = turn into glial cells not neurones)
    - Adult one doesnt have block

Structural components that allow things to cross

- **Tight junctions** →hold cell membranes together with EC domains maintain cell-cell contact and IC domains anchor to cytoskeleton (actin) → **restricts passive paracellular entry, hold adjacent cell membranes together, force transcellular route taking**
- **Plasma membrane** → restricts passive entry depending on **lipid solubility**
  - Dependent on H-bonds they can form → fewer H-bonds = less polar = easier to cross, so drugs that can shake off water molecules = better penetration
  - ALSO, rest of molecular influences strength of H bonds they form (i.e. ratio of polar:non-polar atoms)

Functional components that allow things to cross

- Active influx mechanisms → import water soluble compounds
- CSF sink effect → bulk flow removes compounds from CSF & brain
- Metabolising enzymes → limit penetration beyond barrier interface
  - Cytochrome P450 → change functional groups and stop drugs doing what they do (make water soluble so cant leave)
  - Sulfur transferees etc. in order to expel things that the cell doesn't want → MRP proteins transporters - which transport motif and move it out → so get rid of compound by putting a particular sulphate or glutathione etc.



- Active efflux pumps → ATP binding cassette using ATP hydrolysis to pump things out of the cell via conformational change → exports lipid soluble and modified compounds
  - Such as **PGP** (molecules intercepted and exported)

Barrier misconceptions

- Functional barriers present at all stages of development → philosophical, high CSF, leaky etc.
- No MW cut-off for passive entry. Small MW compounds (e.g. ions and water) ARE restricted → **Amino acid active transport into developing brain >> ADULT, amino acids are RESTRICTED and need a transporter**

- **Treatment for neuropathic/opioid-resistant pain**
  - **GABAPENTINOIDS** Pregabalin, gabapentin
    - Target the $\alpha_2\partial$ subunit on VG-Ca^{2+} channels which are accessory subunits in ECM responsible for modulating opening length, where it is upregulated in DRG and central terminals in neuropathic → ↑opening time → ↑Ca^{2+} current → excitation in neuropathic pain
      - Nerve injury induces ↑regulation of subunit → axonal transport to presynaptic terminal of injured sensory fibres in DH
      - Centrally, ↑subunit → promote excitatory NT release → ↑activation of post DH + C.S
    - In periphery, this subunit doesn't always exist → so drugs don't have same side effects sometimes
      - ↑subunit → Δcurrent of Ca^{2+} → ↑afferent activation and sensitisation of presynaptic terminals
    - Gabapentinoids **designed to mimic GABA but ARE NOT INVOLVED WITH. GABA** (receptors, metabolism, reuptake or metabolism at all)
      - **PREGABALIN → 1st LINE THERAPY, *more potent, faster, predictable bioav > gabapentin***
        - Amino acid, easily crosses BBB via L-AA transporter, **modulator NOT antagonist of VG Ca^{2+} channel binding to $\alpha_2\partial$ subunit SHORTENING OPENING TIME = DECREASING Ca^{2+}_{influx}** at presynaptic terminals in hyperexcited neurons **= ↓NT (Glu, SP, NA) release = analgesia BY SUPPRESSION OF ECTOPIC DISCHARGES (DRG, DH)**
        - Good for chronic pain with good 90% bioavailability, fast onset, twice daily, improves sleep/anxiety, well tolerated (**NO LIVER METABOLISM** so combination ok) but **weight gain, ataxia, dizziness**
          - ***COMBINED WITH MORPHINE SO LOWER DOSES OF EACH DRUG***

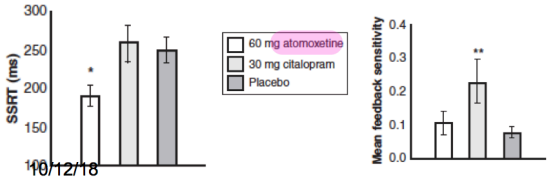
  - **N-TYPE Ca^{2+} CHANNEL ANTAGONISTS** Ziconotide (ω-conotoxin peptide)
    - *Conus* species each have a unique neurotoxin but have common elements and can be used as pharmacological targets
    - ω-conotoxin peptide are **highly selective blockers of PRESYNAPTIC N-type Ca^{2+} channels which are used by A-∂/C fibres → UPREGULATED in the DH** after peripheral tissue inflammation or nerve damage → reduces NT release and inhibits transmission in spinal cord
    - Must be via **intra-thecal** (mini pump into spinal cord) with LOW DOSES **otherwise i.v. = SEs (CVS -** inhibit SNS ability to regulate HR and BP etc because inhibition of N-type calcium channels)
      - **Can have CNS SE if too high dose** (dizziness, ataxia, confusion, memory impairment)
    - 10x potent than i.t. morphine with **efficacy** (use if other treatments don't work) in chronic severe pain and neuropathic pain → **additive analgesic effects observed** (+morphine)

  - **CANNABINOID AGONISTS** CB_1 and CB_2 receptor agonists
    - **CB_1 receptors**
      - Dense in hypothalamus, cortex, hippocampus, cerebellum
      - **LOW** in **brainstem** → lack of respiratory depression or cardiotoxicity
      - **Part of pain pathways in CNS** (except brainstem), **1˚ afferents,** vasculature, urogenital tissues, gut, skin
      - **Analgesia, motor coordination, CV, memory disruption, anti-emesis, ↑appetite**
    - **CB_1 agonists**
      - **INHIBIT**
        - Peripheral/1˚ nerve transmission
        - Spinal relay/2˚ neurons via **pre** (G_i/N-type Ca^{2+} channels), **post** (↑K^+ channels)
      - **ENHANCE**
        - Via α_2-adrenoceptor - ↑activity of descending inhibitory pathway
    - **Cannabinoid agonists – could also be used for mood, epilepsy**
      - **THC + CBD, 2-AG (endogenous), Nabilone → lipophilic = slow residual elimination** so drugs given periphery will easily cross BBB so if you don't want psychotropic effects – need to be careful
      - **SATIVEX (THC + CBD 50:50) → oromucosal spray** (self-dose control, well tolerated, ↓SE)
        - THC – **analgesic**, muscle relaxant, antiemetic, ↑appetite + **psychoactive**
        - CBD – **analgesic**, anti-convulsant, anxiolytic, neuroprotective, antipsychotic
        - Adjunctive treatment for symptom relief (MS, cancer + AIDS neuropathy)

- Risk
  - PFC dysfunction in children of drug-dependence family history → poor cognitive control in these children predicts risk for developing drug addiction
    - **Binge drinking prediction from 14 years old assessing 16 year old (PFC activity)**
- Drug attentional bias
  - Drug-related stimuli → drug dependent users had bias towards drug stimuli and **predicts poorer treatment outcomes**

Treatment and Recovery

- Cognitive impairment = poorer treatment retention (relapse prediction), less power of interventions working
- Longer-term cognitive effects → **mixed study results**
  - Abstinence = ↓cognitive possibly or possibly returning to healthy control levels but **domain specific** (i.e. only motor abilities and information processing speed improved, whereas learning/memory/executive function didn't)
- **Can we improve/treat control?** Is there a neurochemical basis underlying cognitive control?
  - **NA improves SST performance NOT 5-HT**
  - **5-HT improves reward learning performance NOT NA**



- **Improving control in disease**
  - Atomoxetine and ritalin → ADHD children improved on performance on attention tasks and right inferior frontal gyrus during stop trials increased activity → **Inferior frontal gyrus important for cognitive control and impaired in drug dependence**
    - **Psychostimulants may have short term benefits for cocaine users ↑ventro-medial PFC activity during stop trials and cognitive benefits for MA and cocaine dependence users**
  - **Cognitive enhancers** have NOT generally improved treatment outcomes (none-slight improvement)
    - Models used mimic methadone - replace drug with substitution therapy - seems unlikely it is going to show positive benefits
    - Question whether we can use it early adjunct therapy - thats when cognition drops early (2 weeks) maybe use this in this period for short period - to improve cognition → get them to point where they can benefit from other interventions (CBT)
    - Gold standard for meth use - **cognitive behaviour therapy** - very effective and evidence based - requires you to be able to contemplate own cognitions and how you are modify it
    - Hard to do if youre withdrawal symptoms etc.

Summary

- Neurobiology of Addiction has broadly demonstrated:
  - **(1) Genetic predisposition**
  - **(2) Neuroanatomical relationship between sensitivity to drug euphoria and the transition to dependence**
  - **(3) Alterations in neural mechanisms underlying reward and control**
    - That sensitise the individual to seeking the drug in their environment
    - Limit their ability to control their response once encountered
    - These changes may be permanent or at least long lasting
  - **(4) Available treatment are effective for individuals but with varying degrees and there is no evidence that it provides a 'long-lasting' cure**
    - Some treatments may maintain problems with cognition
- **Provides evidence of neuronal mechansims, heritability, treatment responses and progressive clinical course considered evidence for a DISEASE concept of addiction**

Policy Implications

- If drug use **hijacks the brain** such that **addicted individuals lack autonomy/wilful decision making** about their drug of addiction
  - **Can they provide informed consent for clinical trials/treatments** that provide a replacement
  - **Can they be compelled to undergo treatment** if they are unable/unwilling to participate/comply
- Genetic tests – if able to identify addiction heritable traits
  - **Handling this information to individual, family, insurers**
  - **Influence on drug control policies that aim to reduce availability of drugs** → would make economic sense that drug control measures focussed on highest risk (drug dependence)

Ethosuximide	anti-epileptic drug for absence seizures (3Hz spike and wave patterns, thalamo-cortical axons, active like sleep, lower threshold channel), blocks T-type Ca2+ channels, general SEs
Vigabatrin	anti-epileptic drug irreversibly inhibits GABA transaminase (last resort) with visual field constriction irreversible in 20-40% of patients, hyperpolarises via increasing GABA presence = harder to fire
Tiagabine	anti-epileptic drug inhibits GABA reuptake used with focal seizures, inhibit GAT and GABA transaminase to ↑ GABA in pre and cleft = more effect
Topiramate	anti-epileptic drug for generalised tonic-clonic seizures with MULTIMODAL - 4 targets - (1) enhances GABA action, (2) inhibits VG-Na+ channels, (3) activates K+ current = hyperpol., (4) blocks GluAMPA receptors
Morphine	naturally occurring opioid (opiate), μ opioid agonist to cause analgesia, euphoria via descending inhibitory system and many places on neurons, but sedation and respiratory depression via decreased sensitivity of medullary respiratory centre to CO2 - dose limiting, SE (nausea, vomiting, pinhole pupil, GIT (constipation), urinary retention/urgency, CNS (bradycardia), histamine release (bronchoconstriction, vasodilatation decreasing BP), tolerance with decreasing effectiveness at intracellular and organ level, with different tolerance rates
Naloxone	general opioid receptor antagonist, confused with naltrexone, rapid hepatic metabolism (short 1hr half-life), treat/reverse opioid overdose
Pregabalin	1st line therapy, Gabapentinoid that targets the α2ð subunit on VG-Ca2+ channels responsible for modulating opening length (upregulated in DRG, central terminals in neuropathic pain, less in periphery), potent, faster, predictable, shortening opening time and Ca2+ influx to suppress ectopic discharges (DH, DRG) and reduce NT release and induce analgesia, combined with morphine (lower doses), weight gain, ataxia, dizziness, no liver metabolism and good bioavailability and tolerance
Ziconotide	N-type Ca2+ channel antagonist derived by conus species of neurotoxins, selective blocker on að and C fibres (upregulated in DH after inflammation/nerve damage), intra-thecal must with extremely lose doses other peripheral SE (CVS and brain effects), 10x potent that morphine treating chronic severe pain and neuropathic pain, can be combined with morphine
Sativex	THC + CBD (2.7mg + 2.5mg) in oromucosal spray (individual dosing, self-control), analgesic (THC psychoactive, CBD opposite)
Belsomra	Orexin receptor antagonist induces sleep without motor SE, only once per night as long half-life means shouldn't take it within 7hrs of awakening
Orexin	neuropeptide produced in lateral hypothalamus response for acting on Orexin A and B GPCRs involved with sleep/wake cycle, feeding and reward, ACTIVE during active wake stage, silent during NREM and REM