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Tips and Practice Questions:

- Tips for MSTs and Final Exam.
- 30 self-made questions (for the two MSTs and the Final Exam).

W3-2: Presynaptic Processes

- Definition of a neurotransmitter.
- Major types of neurotransmitters.

Neurotransmitter:

- ➢ Soup (chemical transportation) vs. Spark (electrical signals).
- ➢ Otto Loewi (1921) – demonstrate that stimulating the vagus nerve in frogs slowed the heart by releasing "vagusstoff" (ACh), proving that neurotransmitters are chemical signals released from presynaptic nerve terminals into the synaptic cleft.

Neurotransmitter Release:

1. When an action potential reaches an axon terminal the signal needs to pass to the next cell, if not, nothing is achieved.

2. The signal transmission usually involves the release of one (or more) chemicals from the axon terminal.

3. The released chemical acts on receptors of the target cell to temporarily change the properties of that cell.

4. In terms of synaptic transmission we see a change in membrane potential or conductance.

Neurotransmitter Criteria:

1. Synthesis: The chemical must be synthesized within a neuron (in cell body or in terminal).
2. Storage: The chemical must be found within a neuron (packaged into vesicles).
3. Release: When stimulated (depolarized) a neuron must release the chemical (by fusing vesicles with membrane).
4. Receptor: When the chemical is released it must act on a post-synaptic receptor (by binding and activating) and cause a biological effect.
5. Inactivation: After the chemical is released, there must be a mechanism for inactivation (uptake, degradation, metabolism).
6. Same effect: If chemical is applied on post-synaptic membrane, it should have the same effect as when released by a neuron (like Loewi's experiment on ACh).

Classical vs. Non-classical Neurotransmitter:

Classical (Small Molecules)	Non-classical (Large Peptides)
➢ Amino Acids: ○ Glutamate.	➢ Peptides: ○ Substance P.

- GABA.
- Glycine.

➢ Monoamines:
- Noradrenaline.
- Dopamine.
- Adrenaline
- 5-hydroxytryptamine (serotonin).

➢ Acetylcholine (ACh).

- Vasoactive intestinal peptide (VIP).
- Somatostatin.
- Neuropeptide Y (NPY).
- Enkephalin (Enk).
- Kisspeptin.

➢ Gases (travel via diffusion):
- Nitric oxide (NO) → unconventional neurotransmitter, synthesize de novo and without storage (in vesicles).
- Carbon monoxide (CO) → not sure.

➢ Lipids.
- Anandamide.

	Classical (small molecules)	Neuropeptides
Size	Small (amino acid)	Large (4-100 amino acid)
Synthesis	Uptake or enzymes (synaptic terminal)	Protein synthesis (cell body)
Vesicles	Small (filled by transporters)	Large (secreted proteins from RER)
Duration of action	Fast and short	Slow and long

Classical:

1. Synthesis of enzymes in cell body.
2. Slow axonal transport of enzymes.
3. Synthesis and packaging of neurotransmitter.
4. Release and diffusion of neurotransmitter.
5. Transport of precursors into terminal.

→ neurotransmitters are synthesized by uptake or enzymes and packaged (stored) into small clear vesicles (normally filled by transporters), both process happened at the nerve terminal, which the process are fast and short acting.

Non-Classical:

1. Synthesis of neurotransmitter precursors and enzymes.
2. Transport of enzymes and pre-peptide precursors down microtubule tracks.
3. Enzymes modify pre-peptides to provide neurotransmitter.
4. Neurotransmitter diffuses away and is degraded by proteolytic enzymes.

→ neurotransmitters are synthesized by protein synthesis and packaged (stored) into large dense vesicles (normally secreted proteins from rough ER), both processes happened at the cell body, then transport down to the nerve terminal through microtubules, which the process are slow and long acting.

Co-transmitters:

Low frequency stimulation:	High frequency stimulation:
→ preferentially raises the Ca2+ concentration close to the membrane favoring the release of transmitter from small clear-core vesicles.	→ leads to more general increase in Ca2+ concentration causing the release of neuropeptides from large dense core vesicles as well as small molecule neurotransmitters. *peptides are stored in large dense vesicles, and are further away from the synapse, thus needed more calcium for SNARE complex to catalyze the reaction (releasing neurotransmitters).

Co-release vs. Co-transmission:

Co-release:	Co-transmission:
→ Both neurotransmitters are packaged into the same set of synaptic vesicles. When the presynaptic terminal is depolarized it causes the release of both neurotransmitters.	→ Requires transmitters to be packaged into distinct synaptic vesicles with differential release mediated by differential Ca2+ sensitivity. → Alternatively, it can rely on spatial segregation of vesicle populations to different terminal buttons to different synaptic targets.

- Vesicles and their roles.
- Key types of synaptic proteins.

Synaptic Vesicles:

- ➢ Cytoplasmic surface of the vesicle membrane is densely covered in proteins.
- ➢ These proteins (and their binding partners on the presynaptic membrane) act at one or more steps in the synaptic vesicle cycle (vesicle recycling).
    - Synapsin – keep vesicles tethered / anchored within the reserve pool by crosslinking vesicles to each other and to actin filaments in the cytoskeleton.

Proteins in Vesicle Recycling:

- ➢ Docking: GTP-binding protein, SNAREs
- ➢ Priming: SNAREs, NSF, SNAPS
- ➢ Fusion: Synaptotagmin 1; SNAREs
- ➢ Coating: Clathrin, synaptotagmins, synaptobrevin, NSF, SNAP
- ➢ Budding: Dynamin, clathrin, actin
- ➢ Uncoating: Clathrin, Hsc-70, auxilin, synaptojanin

Structure of a SNARE Complex:

- ➢ SNARE = “SNAP Receptor”
- ➢ Found in yeast and mammalian cells, important for vesicle fusion.
- ➢ Proteins on Vesicles:
  - ○ Synaptobrevin (vesicular SNARE)
  - ○ Synaptotagmin (vesicular Ca2+ binding protein)
- ➢ Proteins on Plasma Membrane:
  - ○ Syntaxin (plasma membrane SNARE)
  - ○ Snap25 (plasma membrane SNARE)
- ➢ Process:
  - ○ 1. Vesicle docks.
  - ○ 2. SNARE complexes form to pull membranes together.
  - ○ 3. Entering Ca2+ binds to synaptotagmin.
  - ○ 4. Ca2+ -bound synaptotagmin catalyzes membrane fusion.

3 Models of Synaptic Vesicle Recycling:

Kiss-and-run model:	Clathrin-mediated endocytosis model:	Ultrafast endocytosis and endosomal budding:
Vesicles transiently fuse with the plasma membrane. After the neurotransmitter release, the fusion pore is closed and the vesicles are recovered.	A synaptic vesicle fuses and collapses into the membrane. A new vesicle is formed in a region distant from the fusion site.	After a rapid internalization of the membrane via ultrafast endocytosis, the vesicle membrane is delivered to an endosome. Clathrin-mediated regeneration of synaptic vesicles occurs at the endosome.

➢ Primary neuronal culture:

Strengths	Limitations
● Relatively inexpensive. ● Can be used in a high throughput manner. ● Neurons mature quickly – 2-3 weeks in culture. ● Highly tractable – easy to control + modify the biology of the system, introduce genes of interest. ● Can be cultured from transgenic rodents. ● Can culture cells from different brain regions. ● Easy to assess synaptic function.	● Simplified system – generally a monolayer. ● Neurons lose their normal interconnectivity to different brain regions – normal circuits are disrupted. ● Cannot assess neural circuits (but can look at network activity).

➢ iPSC:

Strengths	Limitations
● Human neurons in culture. ● Can be programmed into many different types of neurons. ● Amenable to high throughput screens. ● Can be used for modelling human disease – fibroblasts taken from patients. ● Can be gene-edited to study gene function, make disease-relevant models (or isogeneic controls). ● Can assess synaptic physiology.	● Expensive. ● Takes a long time to mature (months in culture) and to develop mature synapses. ● Simplified system - neurons lose their normal interconnectivity and circuitry. ● Cannot assess neural circuits (but can look at network activity).

➢ Organoids:

Strengths	Limitations
● 3D human "mini-brains" in culture. ● Can be used to develop distinct brain structures which possess some of the layering that is found in human brain. ● Huge potential for modelling human disease and neurodevelopment – fibroblasts taken from patients. ● Can be gene-edited to study gene function, make disease-relevant models (or isogeneic controls). ● Can assess network activity and simple circuits.	● Very expensive. ● Takes a very long time to develop (several months in culture). ● Have a limited maturation. ● Significant issues with cell death due to limited oxygen and nutrient diffusion → 3D. ● Batch effects and heterogeneity - variation in efficiency of differentiation, morphology and variability in cell composition across different batches of organoids. ● Cannot assess complex neural circuits.