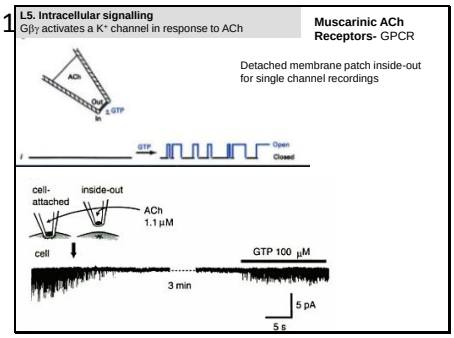
cAMP (messenger) shown by if you inject cAMP into cell in the absence of neurotransmitter, get same effect.

Slide 11

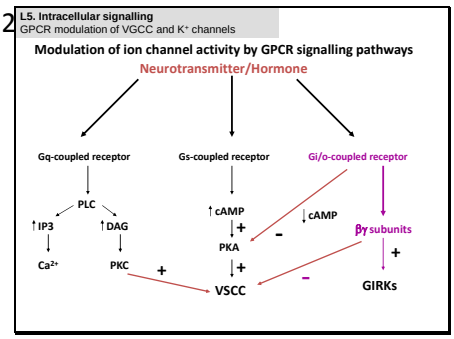


If you put neurotransmitter in patch can make case for direct signalling- they cant diffuse away from membrane.

AcH activates a muscarinic GPCR also activates a ligand gated ion channel nicotinic Ach receptor. Which allows us to show neurotransmitter is quantitative.

Put Ach inside the patch pippete and pull patch away from rest of cell, if you get activity under these conditions you know all the molcules neede to activate the receptors in the patch are already i the patch.
Recording potassium channels (reflections = channel opening). Put on cell attached patch, appl ACh and pull away from cell, the current runs dow because as the subunit is hydrolysing GTPase and theres no GTP available as you're pulling it away from cytoplasmic content. This is an inside out patch the inside the cell is facing out to the bathir solution. If you give GTP back you get the channel activity back. We know theres no secondary messenger involved!
Membrane delimited regulation- all the regulatory protein are in membrane!
If you apply beta gamma subunits get same effect.

Slide 12



VSCC voltage sensitive calcium channel
Increase cAMP increases PKA which phosphorylate channel.
Example: Gi/o coupled receptor (muscarinic receptor) which activating the ch__ channels whic are called G protein-coupled inwardly-rectifying **potassium channel** via a Gi coupled receptor alpha subunit usually cAMP.
So beta gamma subunits are activating calcium channels

Reverse can also happen the beta gamma subunits can activate K channels downstream of Gi/o