

Unsilencing

Silent synapses = no release

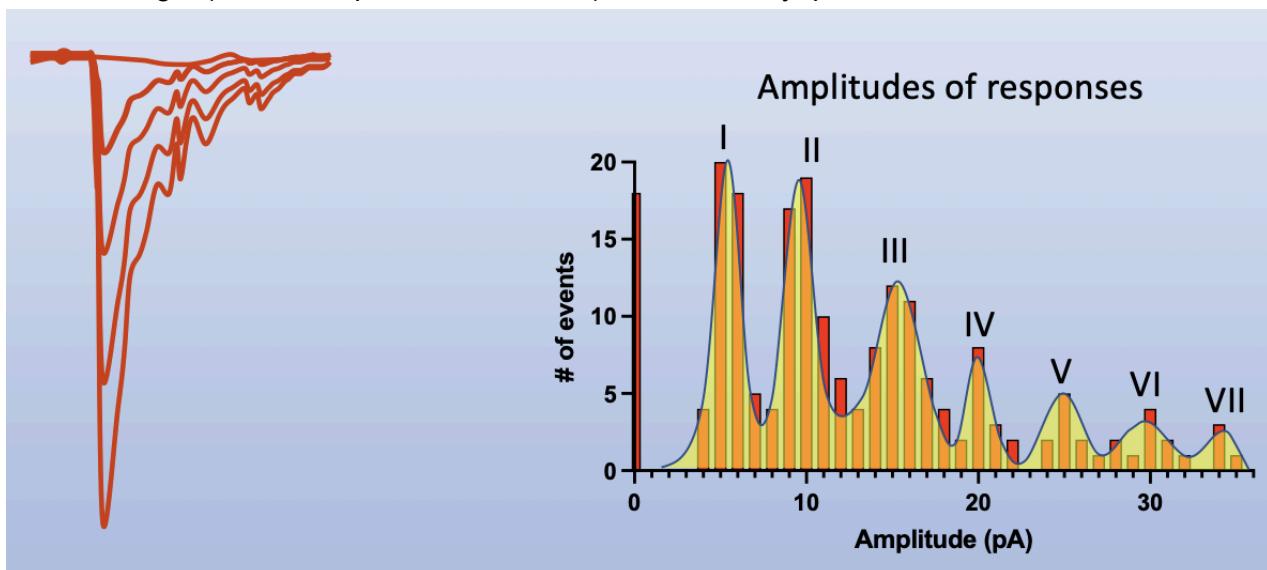
Postsynaptic silent synapse: No AMPAR only NMDAR, thus at basal level of activity, only slow NMDA (not enough depolarisation to cause fast NMDA response)

Unsilencing → Inserted AMPA receptors → each response increase (the graph will shift to the right similar to increased q)

Quantal Analysis and Unsilencing

Position of peaks determined by: q

Relative height (number of peaks and failures) determined by: p and n



I - amplitude when 1 synapse activated

II - amplitude when 2 synapses activated

III - 3 synapses activated

etc...

Increase q : Shift peaks to the right (peaks have larger amplitudes due to larger single synapse amplitudes)

Increase p: Increase peaks at right end, more likely to get release from multiple sites than 1 site, hence increase in peaks for the greater amplitudes (which indicate that more than 1 synapse are activated)

Increase n: Increase peaks at right end

Retrograde messengers can act as signals from post-synaptic to presynaptic to influence % of release

Late Phase LTP

Late LTP → protein synthesis

Synaptic tagging important = highlights that the synapse has undergone LTP and highlights for late LTP

Neuromodulation

Neurotransmission vs Neuromodulation

- Neurotransmission
 - Precise; Point to point
 - Direct gating of ion channels
 - Direct postsynaptic effects lasting tens of ms
 - No secondary effects
 - Postsynaptic electrical effects are fast and strong
- Neuromodulation
 - Diffuse transmission
 - Effects mediated by second messengers
 - Postsynaptic effects lasting several hundreds of ms to hours
 - **Typically involve a G-protein**
 - Postsynaptic electrical effects are slow and weak

Transmitters responsible for neuromodulation:

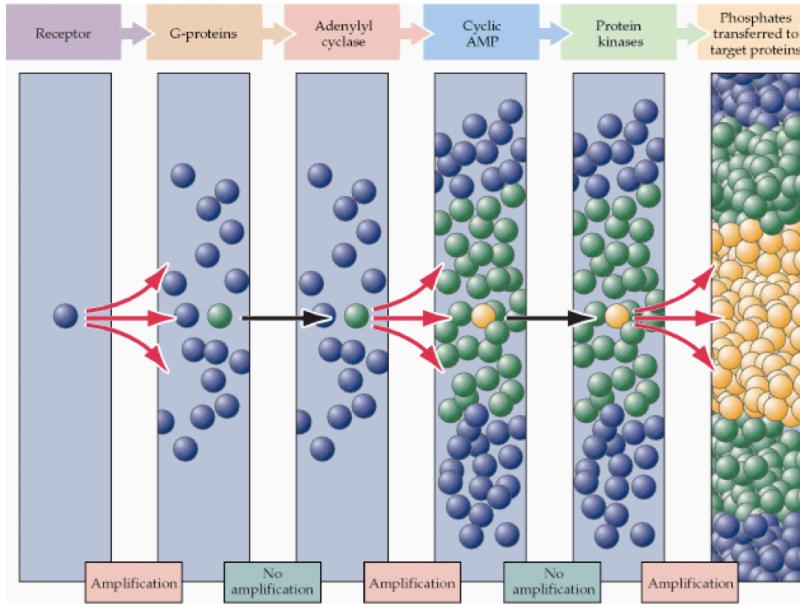
- Monoamine: Effect over large area
 - Noradrenaline → maybe responsible for global functional state change
 - Dopamine → wide range of function (e.g. executive functions, reward)
 - Serotonin → maybe responsible for global functional state change (arousal e.g. switch from sleep to arousal)
 - Histamine
- Acetylcholine
- Glutamate
- GABA
- ATP
- Neuropeptides
- Opioids
- Cannabinoids

Neuromodulation speed depends on receptor mediating action of neurotransmitter

- Ionotropic = fast
- Metabotropic = slow
 - Receptor and G-protein are two different things for a GPCR
 - G protein alpha subunit types

- Gs
 - Activate adenylyl cyclase
 - Increase cAMP levels
 - Activate protein kinase A (PKA)
 - Increase in protein phosphorylation
- Gq
 - Activate phospholipase C
 - Increase in diacylglycerol (DAG) and IP₃ concentration
 - DAG level increase activate protein kinase C
 - IP₃ level increase lead to Ca²⁺ release
 - Increase in protein phosphorylation and activate calcium binding proteins
- Gi
 - Inhibit adenylyl cyclase
 - Decrease in cAMP levels
 - Inhibit protein kinase A
 - Decrease protein phosphorylation
- Different types of G-protein types formed by different alpha, beta and gamma subunits
 - Beta-gamma subunit can also interact with final protein
- Most neurons express more than 1 G-alpha subunit type

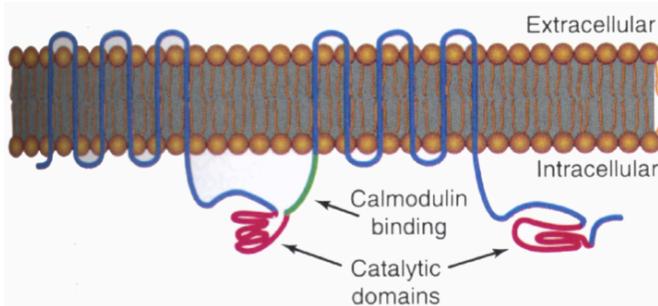
GPCR cascade = good for signal amplification



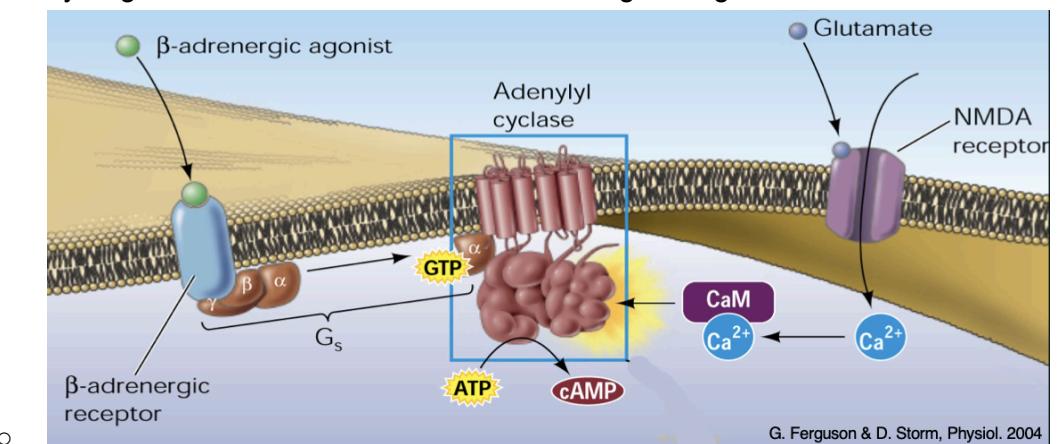
Convergence or divergence of signals mediated by G-protein

- 1 receptor may activate several G-proteins / 1 receptor activate 1 G protein which activates multiple effectors → divergence
- Many receptors may activate 1 G-protein / Many receptors activate different G-proteins but all activate 1 effector → convergence

Adenylyl cyclase structure



- Adenylyl cyclase can be co-stimulated or inhibited by other signals
 - AC 1 can be stimulated by calcium level increase as it **could be stimulated by Ca^{2+} -calmodulin**
- Therefore, these complex signal transduction cascades are useful in signal integration
 - Coincidence detectors
 1. Effectors in neurons receive signals from 2 stimuli from 2 activated metabotropic receptors within reasonable time window
 2. Enzyme will sum up and produce effect that is a result of combined action of both pathways inside cells.
 - Synergistic effect, increased effect, more long-lasting.



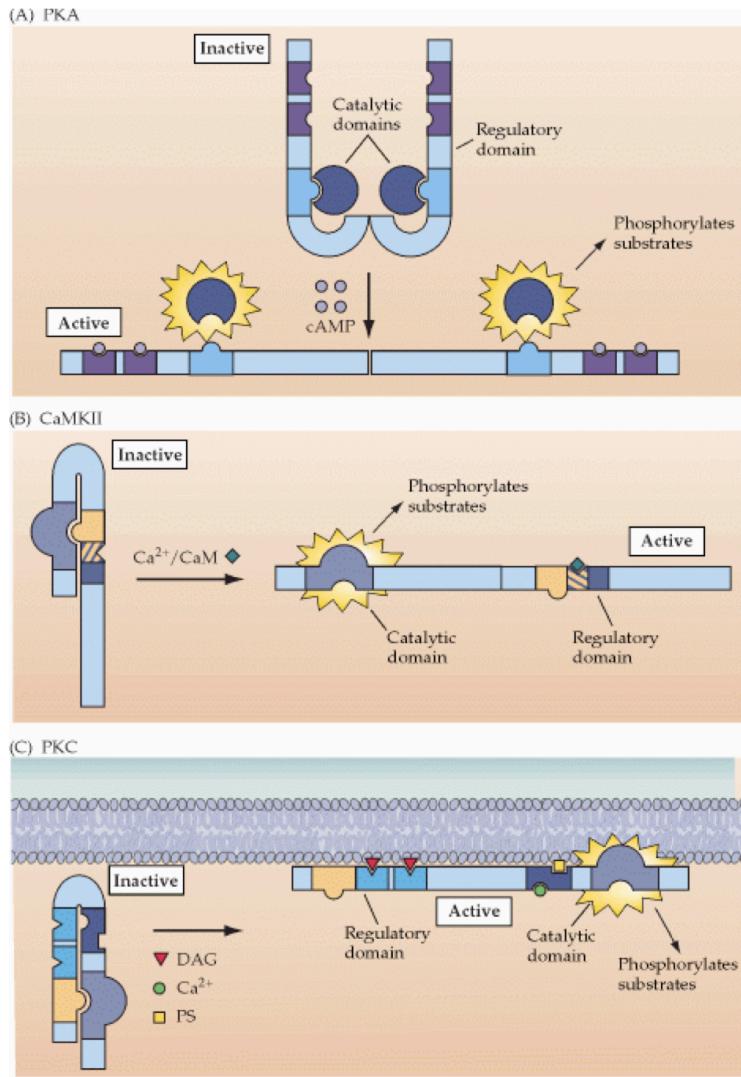
Second messengers

- Small diffusible molecules that are produced in response to receptor activation
- Bind and activate effector molecules such as protein kinases, ion channels and a variety of other proteins, thus continuing the signalling cascade
- Some are stored in special organelles and released when needed (e.g. Ca^{2+})
- Production and release can be localised to limit space and time of signal activity
- Hydrophobic molecules: Diffuse from plasma membrane into intermembrane space to reach and regulate membrane associated effectors

- DAG
 - PIP2
- Hydrophilic molecules: located in cytosol
 - cAMP
 - cGMP
 - IP3
 - Ca^{2+}
- Gases: Can diffuse both through cytosol and across cellular membranes
 - NO
 - CO

Protein kinases

- Specialised domains with specific functions
- Each of the kinases has homologous catalytic domains responsible for transferring phosphate groups to substrate proteins
 - Catalytic domains inactive due to presence of autoinhibitory domains that occupy catalytic site
 - Binding of II messengers to appropriate regulatory domain of kinase removes autoinhibitory domain → catalytic domain activated
- Some kinases (e.g. PKC and CaMKII) the autoinhibition and catalytic domains are part of the same molecule, others (e.g. PKA) the autoinhibitory and catalytic domains are separate subunits



Loewi's experiment:

Connected 2 chambers with hearts inside, fluid between 2 chambers can be transferred

- 1 heart had vagus nerve attached to it
- Another heart had none

Heart 1 contraction force and rate decrease when the vagus nerve was stimulated

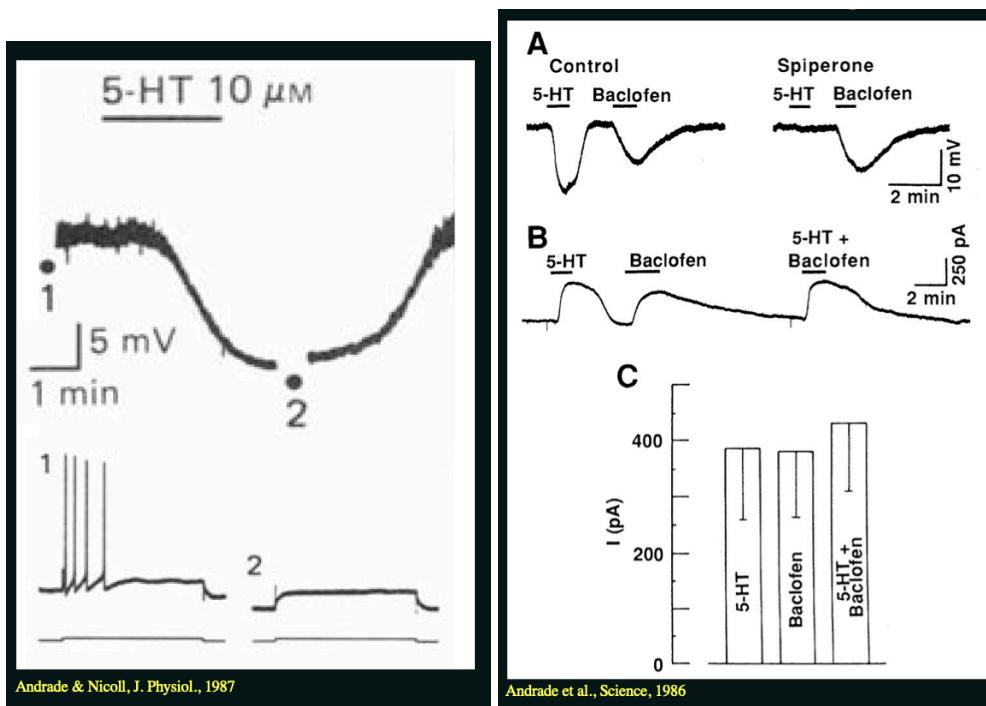
Heart 2 contraction force and rate also decreased → inhibitory effects of vagus transferred, must be some substance that could be transferred

1. Stimulation of the vagal nerve
2. Release of ACh
3. ACh binds to muscarinic AChR
4. Activation of G-proteins
5. Beta-gamma subunits binds to K^+ channels (GIRK)

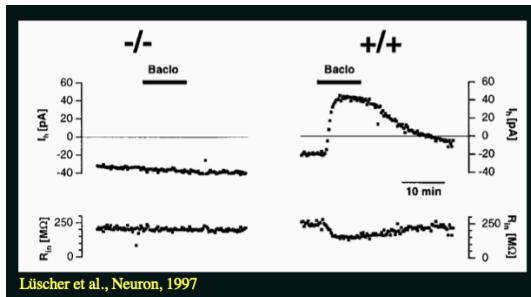
- a. GIRK: G-protein inward rectifying potassium channels
- 6. Membrane potential goes negative as it approaches equilibrium potential for potassium (K^+ flows out)
 - a. **Membrane hyperpolarisation**
- 7. Move away from threshold potential for AP generation
- 8. Heart beat rate slows down
 - a. Action is relatively fast (30-100msec) and localised

Postsynaptic Modulation

- Serotonin
 - o Membrane resting potential hyperpolarises when 5-HT binds to 5-HT_{1A} receptors
 - o Beta-gamma subunit bind to GIRK and leads to increase membrane permeability to potassium
 - o Reduce cell excitability
- GABA receptors exerts same effect as serotonin and shares same target
 - o Baclofen = GABA-B (metabotropic) agonist
- GABA + 5-HT = convergence of effect



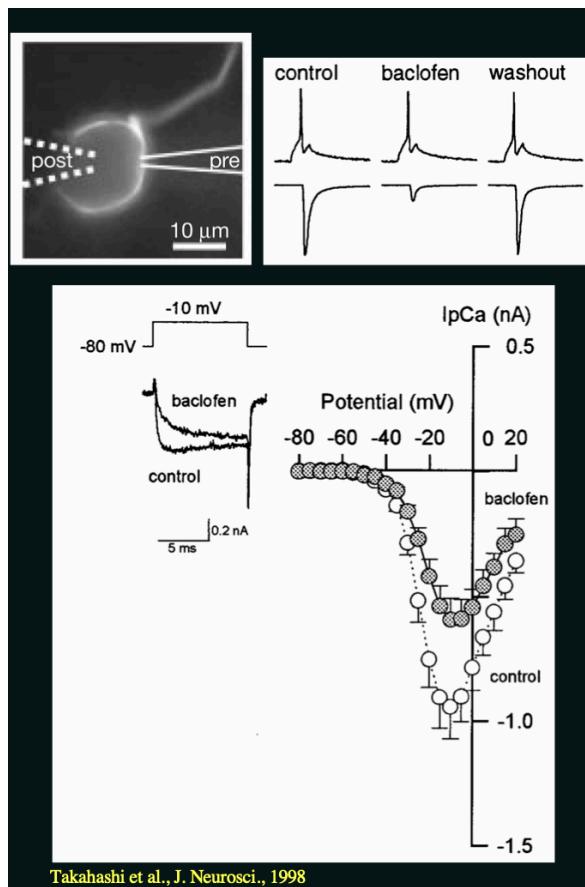
Mice lacking GIRK2 gene do not display K^+ current and a change in membrane resistance in response to GABA-B agonists



Presynaptic modulation

GABA

- Presynaptic terminal with GABA-B receptors and GABAergic neurons form axo-axonal synapse
- Release of GABA inhibits presynaptic calcium channels directly which is mediated by G-protein beta-gamma subunits

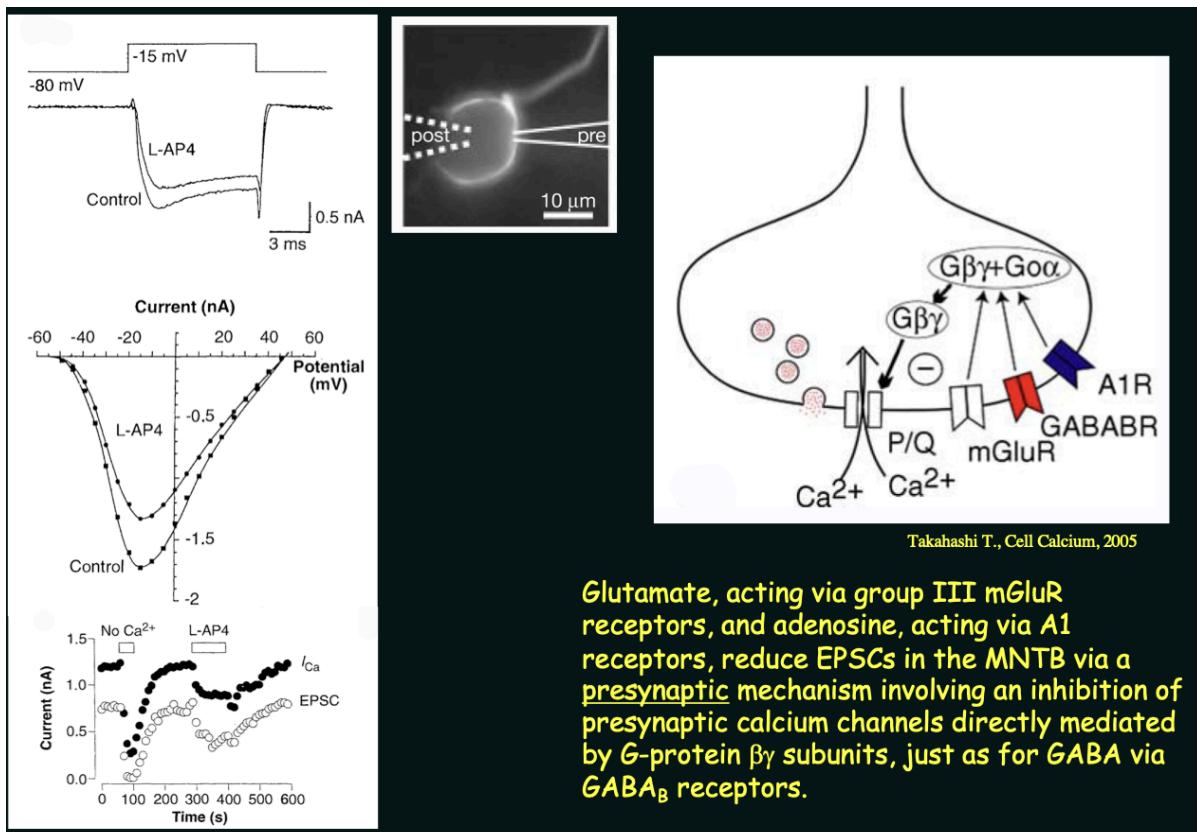


- Top: Presynaptic current is detected, but no postsynaptic current is detected in calyx of held (squid axons) injected with baclofen
- Bottom: Calcium current is smaller when cell is injected with baclofen (GABA-B agonist)

- Less release of NT
- EPSCs reduced in postsynaptic membrane

Glutamate

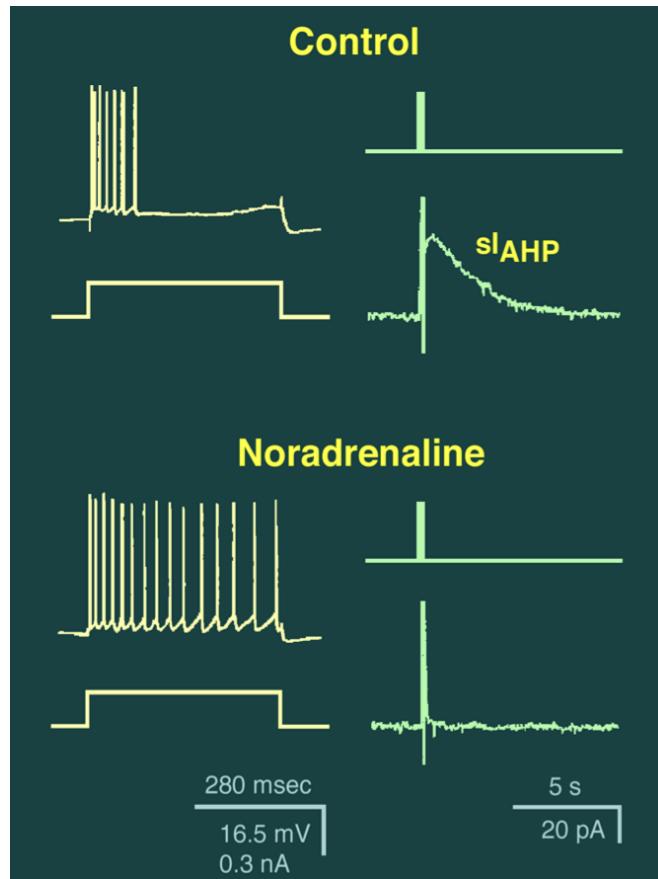
- Autoinhibition: glutamate released from the presynaptic terminal
- Glutamate act via group III mGluR receptors and adenosine act via A1 receptors
- Inhibit presynaptic voltage-gated Ca^{2+} channels directly which is mediated by G-protein beta-gamma subunit binding to channel
- Less release of NT
- EPSCs reduced in postsynaptic membrane



Modulation of signal encoding

- Serotonin and noradrenaline make neurons more excitable
- Modulation of spike frequency adaptation and of a Ca^{2+} -activated K^+ current by NA in hippocampal pyramidal neurons
 - Normal neuron → has adaptation
 - Extended excitation in postsynaptic terminal activated Ca^{2+} channels
 - Increase in Ca^{2+} concentration
 - Activate Ca^{2+} -dependent K^+ channels

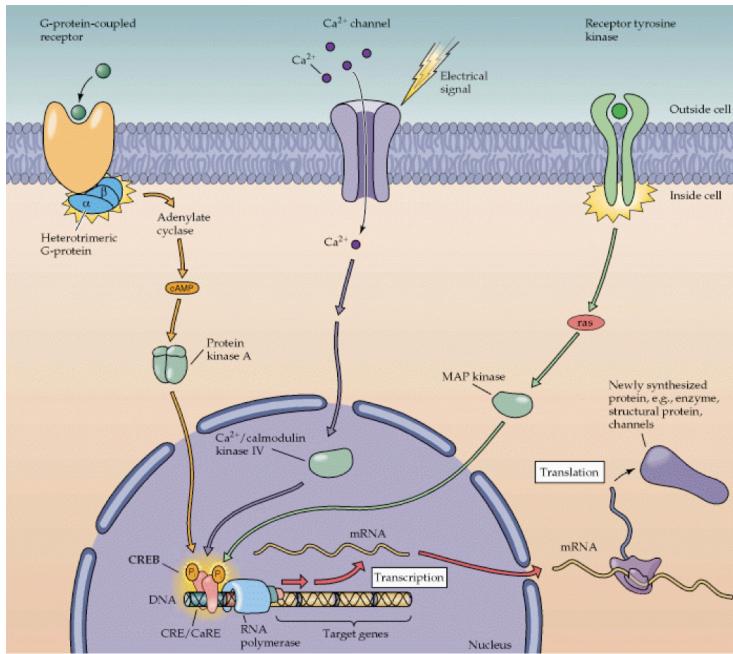
- Increase in K^+ levels → hyperpolarisation → voltage of membrane shifted away from threshold needed to AP firing and adaptation
- Inject noradrenaline → adaptation inhibited
 - NA abolish K^+ currents
 - NA bind to receptor
 - NA activate Gs alpha subunit
 - Gs increase cAMP → PKA activated
 - PKA phosphorylate for calcium-activated potassium channel
 - Lower open probability
 - Less K^+ current
 - Neuron stays depolarised for the whole fo the voltage step



- NA may underlie attention → enhanced state of excitability and reduced adaption

Neuromodulation may have long-term effects due gene expression changes → remodelling of neurons

- Long term LTP



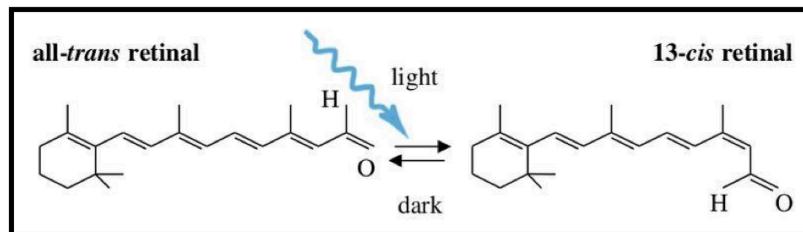
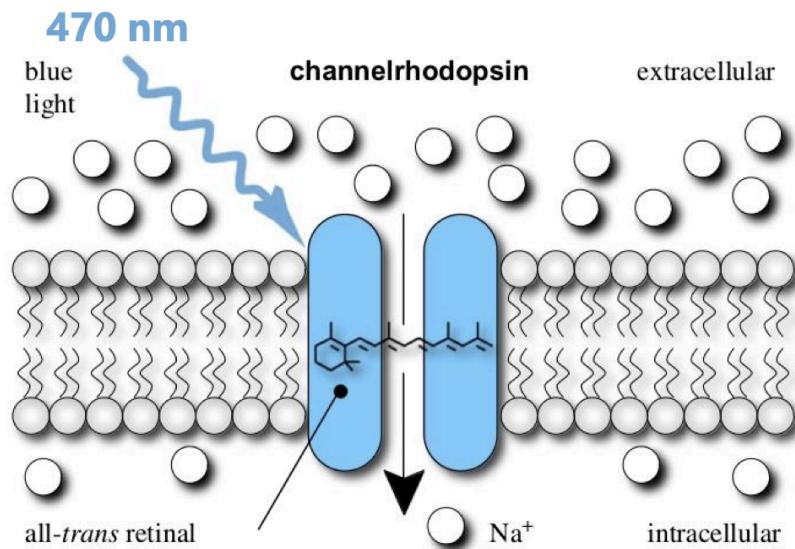
Optogenetics

Optogenetics is the delivery of light to neurons expressing light-sensitive ion channels/pumps. It targets specific neurons with genes coding for light-sensitive ion channels

Alters cell function

Development of optogenetics

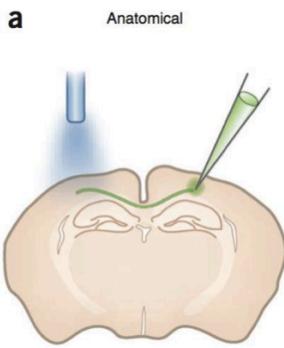
- Channel rhodopsins function as “visual” proteins direction the algae towards or away from light
 - These algae rely on light to move around → phototaxis
- Channel rhodopsins are a subfamily of **retinylidene** proteins that function as light-gated ion channels
- Light absorption triggers a subsequent conformational change of the protein and gating of the channel



- Open of channel allows influx of sodium and some calcium (cation influx) → more positive membrane potential
- Optogenetic control in vitro
 - Neurons grown in culture were transduced with ChR2
 - YFP (yellow fluorescent protein) was expressed under same promoter as ChR2
 - Effectively tagged neurons expressing ChR2 → to monitor expression
 - Activation of ChR2 by blue light induced a depolarising current in these cells
 - Short pulses of blue light were sufficient to induce AP
- Mice experiment, optogenetic control ex vivo (functional organ removed from animal)
 - A viral construct containing ChR2 was infused into hippocampi of adult mice
 - ChR2 was under control of EF-1 alpha promoter
 - ChR2 was expressed in adult mouse hippocampus
 - Blue light could drive spiking in hippocampal neurons in slice
 - Can time spiking
- Mice experiment, optogenetic control in vivo
 - Transgenic mouse line was generated to express ChR2 in cortical neurons
 - Hole in skull and fibre optic cable inserted
 - Reliably caused spiking in living animal
- Different optogenetic actuators
 - ChR2
 - Excite cells in response to light
 - NpHR
 - Inhibit target cells in response to light by pumping Cl^-

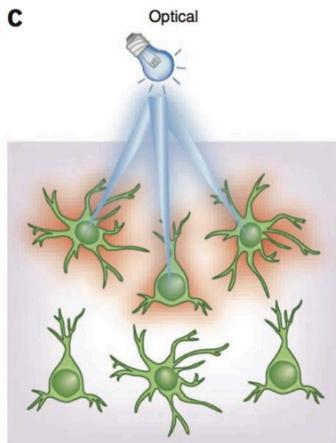
- Arch
 - Inhibit target cells in response to light
 - Arch lets H^+ out, hyperpolarization
- OptoXR → range of function depending on G-protein coupled to it

Precision



Physical delivery of virus to a given anatomical location; uncovers **circuit connectivity patterns** by making use of axonal projections.

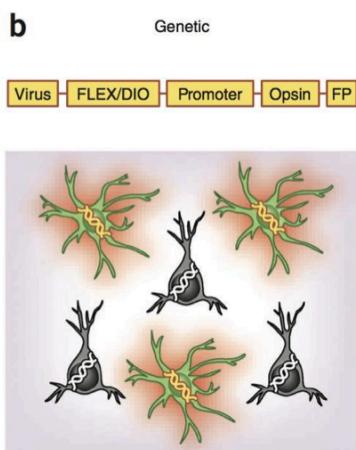
Delivering light to target location which the cells project to
 Uncover which cells in the given anatomical location projects to the other area that you shine light on



Directing the illumination source to a given set of cells or even individual neurones; useful when the targets of interest are **separated in space**.

High spatial precision with optic genetics through laser targeting

- Broad light activation
- Focal activation with 2-P

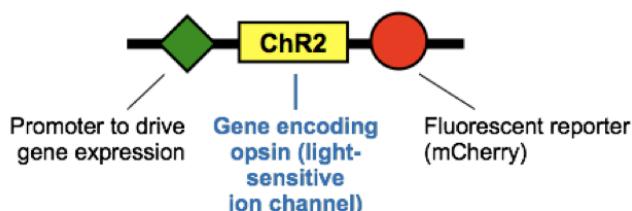


Cell types can be addressed if the cell type of interest has a **known genetic identity**.

- Express ChR2 only in cells with a certain gene (e.g. cre/lox method)

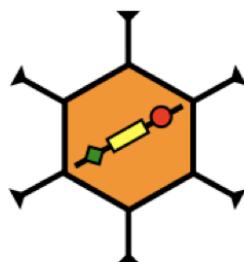
Delivering Genetic Construct

1. Piece together genetic construct (Transgene)



On its own, AAV is biologically inert (no chance of causing infection or disease) and non-replicating

2. Package construct into virus



Adeno-associated virus (AAV) is a small virus which can be used as a **vector** to deliver target genes into mammals

It takes at least two weeks for ChR2 to be expressed fully in transduced neurons

3. Inject virus into brain structure

AAV will be infused bilaterally into the **entorhinal cortex**

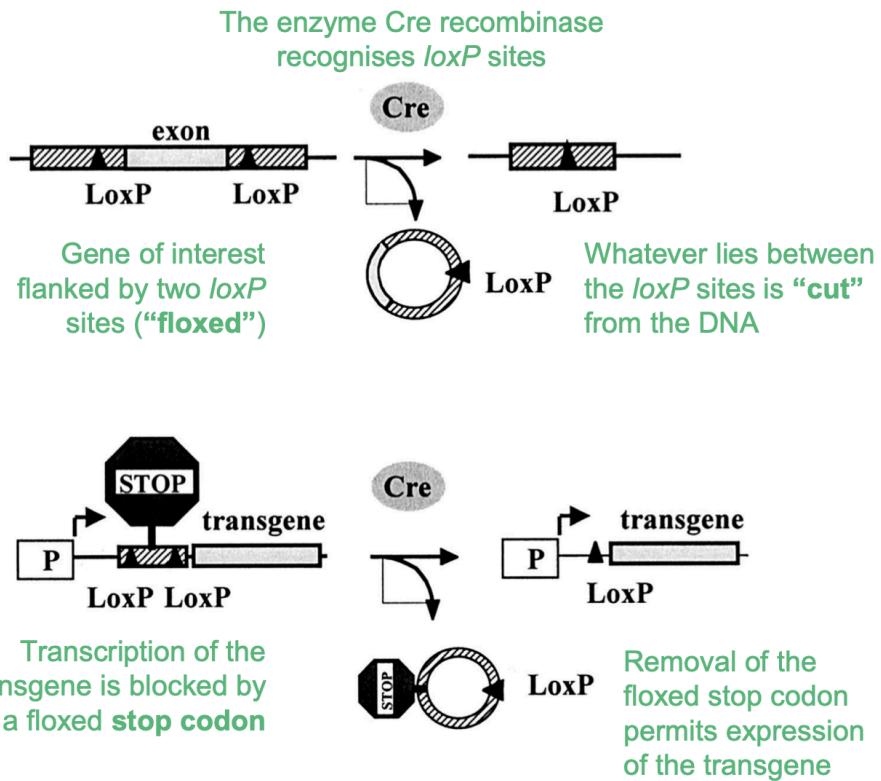


Cell type specific promoter → target a particular cell

Transduction is the process by which foreign DNA is introduced into another cell via viral transducer

Cre/Lox Recombination Method

- Cre gene encodes enzyme Cre recombinase
 - Cre protein recombines DNA when it locates specific sites in DNA molecules, the loxP sites (cleaves loxP sequences)
 - Knock-out NMDAR in hippocampus
- When cells that have loxP sites in their genome also express Cre, the enzyme catalyses a recombination event between the loxP sites
- Promoter followed by STOP codon flanked by 2 loxP sites, which is then followed by ChR2 gene (floxed-stop ChR2)
- When virus containing the transgene encoding ChR2 transduces a cell expressing cre, the loxP sites + the STOP codon they flank are cut out, gene expressed
 - Only get ChR2 in cells expressing cre enzyme
 - Virus is basically non-specific
- Cell specificity achieved



- Deliver ChR2 to target site, deliver cre gene to projection / source site, this way only the pathway you want to target will express ChR2

Targeting specific compartments of cell:

- Short peptides encoding intracellular retention signal, targeting or anchor signals can be used to target optogenetic actuators to specific subcellular compartments (localise expression to a specific subcellular compartment)
 - Myosin-binding domain of melanophilin or the C-terminus of neuroligin-1 → ChR2 in soma and dendrites
 - C terminus of Kv2.1 subunit restricts expression to soma and proximal dendrites
 - Ankyrin G-binding domain of intracellular loop II-III of voltage-gated sodium channels → localise ChR2 to axon initial segment
- Want to be specific in targeting single cell
 - For example: localise ChR2 to cell body → Selectively activate single cell

Application

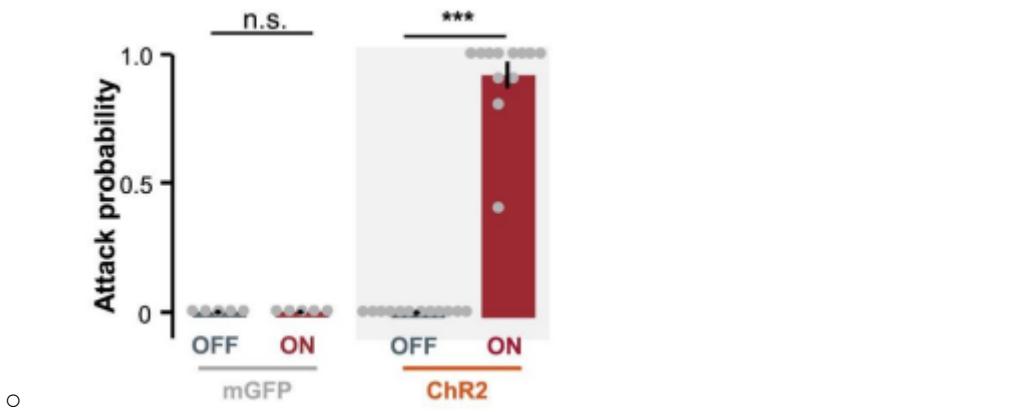
Optogenetics permits the activation or inhibition of specific sub-populations of neurons using light, and does so without affecting surrounding neurons or fibers

Used to study causal relationships between activity of targeted brain pathways and behaviour they may regulate

Hypothalamus

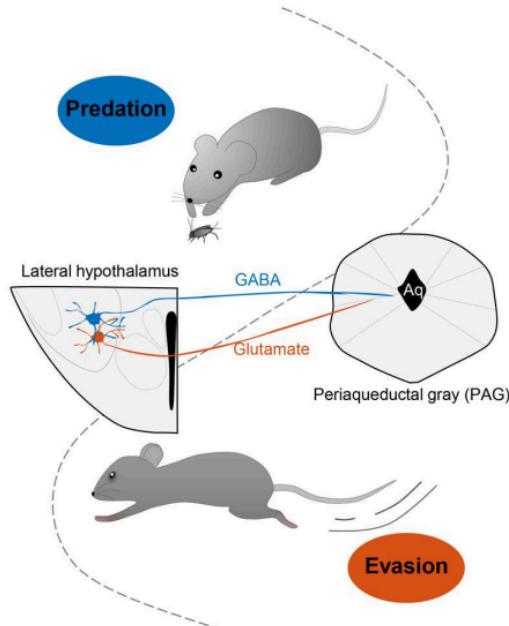
Hypothalamic circuit is important for predation and evasion

- PAG can elicit biting and attack responses in mice (electrical stimulation of LH and PAG shown to elicit biting attack responses)
- ChR2 inserted into lateral hypothalamus-PAG pathway and effects observed on predation assay
 - Inject RetroAAV which affects synaptic terminals
 - Gene product is transported back up the axon into cell body
 - Label all cells projecting to PAG with cre
 - Insert ChR2 gene floxed by loxP sites into cell
 - ChR2 only expressed in cells with cre (aka those projecting to PAG)
- Experiment: Put mice with crickets
 - Projection activated → mice attacks crickets
 - Aggressive attack behaviour triggered when light switched on



- - Shining light on mGFP → control
 - Demonstrate that light itself has no effect on animal behaviour
- Slice PAG, record from neurons in PAG, but stimulate lateral hypothalamus
 - Mixed GABA and glutamate projections from LH to PAG
- Experiment: GABAergic pathway sufficient to trigger predation
 - V-gat (particular GABA transporter) cre mouse → all GABAergic neurons will express cre
 - Couple with viral injection of ChR2
 - Just GABAergic express ChR2
 - Light stimulation of GABAergic neurons → trigger attack behaviour
- Experiment to show circuit is necessary for predation
 - V-gat cre animal
 - Virus with GtACR1 delivered into lateral hypothalamus
 - GtACR1 = Light gated chloride channel (inhibitory)
 - Light stimulates channel → inhibition of GABAergic neuron → loss of hunting behaviour

- Experiment demonstrating glutamatergic neurons important for evasion
 - V-glut2 cre mouse
 - Virus with GtACR1 delivered into lateral hypothalamus
 - Blue light activates channel → inhibition of glutamatergic neurons → no preemption of evasion behaviour (only run away when robot hits the mouse)
- Experiment with glutamatergic neurons #2
 - V-glut2 cre mouse
 - Deliver ChR2 into glutamatergic LH neurons
 - Activate neuron → trigger escape behaviour
 - Activate neuron → mice in process of hunting switches to evasion behaviour

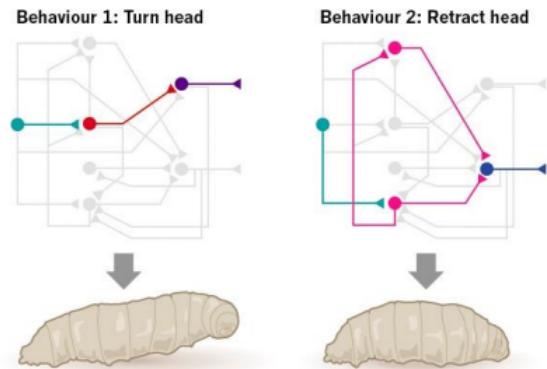


- Limitation
 - Studies are not specific in the population of neurons they activate
 - Other pathways from LH may be important not the LH-PAG specifically
 - Only first experiment targeted LH-PAG specifically

Functional Connectome

Optogenetics used to activate single neurons and in combination with other techniques (e.g serial electrophysiology) can be used to create functional map of brain

- 1 neurons may have several pathways



Smith 2017

- Activating single neuron may not lead to single response
- Serial EM to assess connectivity of neurons → reconstruct connectome (complete map of neuron connections)
- Chettih and Harvey experiment
 - Express ChR2 in cell bodies of single pyramidal neurons in V1 (expression is sparse)
 - Express calcium indicator (fluoresce in presence of calcium)
 - Useful because when neuron activates, calcium influx
 - Mice watch video
 - Periodically stimulating 1 neuron of interest, look at how stimulated neuron impacts function of other neurons
 - Neuron surrounds stimulated neuron = excited
 - Further away = inhibition (may be due to inhibitory interneuron)
 - Effect also dependent on functional properties of neuron
 - Neurons with same response properties to stimulated neuron = excited
 - Clarify signal
 - Neurons which are quite similar = inhibited more strongly
 - Sensory discrimination → eliminate nearby possibilities to reduce confusion, precision

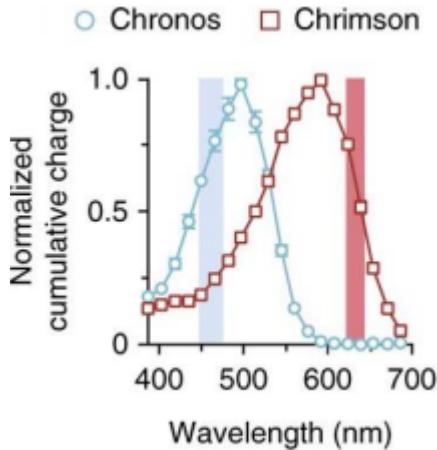
Limitations and Modifications

Limitations

- Insertion of fibre optical cables damage brain (limit therapeutic potential)
- Light cause heating → affect brain function
- Channels open stochastically, new conductance may affect functional properties of cell
- Depolarisation caused by alien channels may affect integration and plasticity
 - Pattern of release not naturalistic

Modifications

- Optogenetic actuators are being developed with modified kinetics and/or activation wavelength



- ○ Can activate both at same time as chronos and chrimson (2 types of optogenetic actuators) have different sensitivities to different wavelengths
- ○ Target 2 population of neurons at the same time → high degree of control
- Far-red shifted opsins may negate need for fibre optic cables
 - Light in far-red is less defracted by tissues → penetrate more easily
 - ChRmine opens in response to far-red wavelengths
 - ChRmine expressing parvalbumin neurons
 - Light travels through skull and brain
 - Epileptic mouse
 - AMplifier detects seizure activity
 - 50% of red light activated during epilepsy
 - Trials with red light activated → seizure duration shorter
 - Terminate / shorten seizures
- Vision restoration
 - Retinitis pigmentosa → loss of photoreceptors = complete blindness
 - Virus expressing ChrimsonR injected into remaining retinal circuitries, express ChrimsonR in ganglion cells
 - Goggles scan environment and recode light into appropriate wavelength and intensity and yield partial restoration (e.g. detect object, grasp object)
- New actuators
 - Opto-XR
 - Modify intracellular domain of rhodopsin and replace it with other components to trigger different pathways (e.g. G-protein)
 - Opto-XR → Rhodopsin molecules
 - Activate opto-beta2-adrenal receptor → Gi signalling
 - Activate opto-alpha1-adrenal receptor → Gq signalling
 - Opto-RTK
 - Dimerise → autophosphorylation
 - Remove lox domain
 - Attach lox (light sensing) domain in intracellular part
 - RTK pathway now under control of light

Revision & Exam Techniques

Section A is a single question, all subsections must be answered

Section B - pick 1 of the 3 questions

Use separate answer books for Sections A and B