What is a cell?

By BilZ0r

A cell is "the lowest level of structure capable of performing all the activities of life" (Campbell et al., 1996). Physically, a cell is a collection of molecules contained within a membrane of some kind, capable of reproducing itself, energy utilization and other hallmarks of life. While some forms of life exist as single cells, so-called "higher life forms" can only sustain life as a collection of cells; they are multi-cellular. In many multi-cellular life forms groups of cells have undergone some form of specialization and aggregated into tissue so that the individual cells (and hence the tissue) are well suited to a particular task. Hepatocytes are the primary cells of the liver, and contain a huge array of metabolic enzymes, allowing the liver to degrade potentially dangerous chemicals and create complex molecules. Myocytes are the primary cells of muscles, and contain specially produced protein fibers which can change their length, allowing the muscle to contract and produce movement. Neurons are the cells of the nervous system, these unique cells allow information to be quickly sent from one part of the body to another. Neurons make rapid sensation and reaction possible and allow animals to change and learn new behaviors so that they are better suited to their environment.

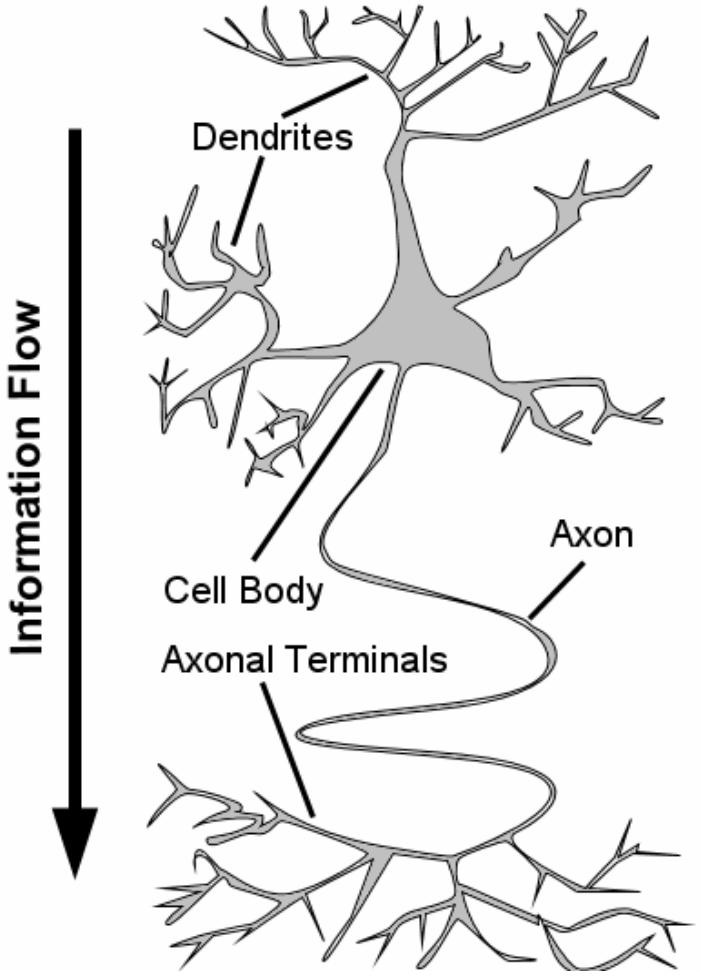

Figure 1. Schematic of a neuron

What is a neuron?

By BilZ0r

The most important properties of a neuron is its ability to fire an action potential and to release neurotransmitters. Anatomically, a neuron is analogous to a tree: it has roots in the form of a huge number of of branching dendrites (the receiving end of a neuron), it has a trunk, in the form of an axon (the transmitting section of a neuron) and a branches,

in the form of axonal arborizations or terminals (Fig 1 and 2). Generally, a neuron can be seen as an integrator and disseminator of information. Figure 2 shows a real image of a single neuron filled so it can be visualize independently of the hundreds of neurons surrounding it. This image shows the dendritic spines of a dendrite (the small dots along the length of many of the dendrites). Each of those these spines will make at least one connection (or synapses) with a neighboring neuron, there will also be many synapses which we can not see, this means that this neuron makes thousands of connections with other neurons. Although we can not see it's axonal terminals, it is safe to assume that this cell then makes thousands of connections with other neurons. Hence a neuron both receives inputs from a huge number of neurons, as well as giving inputs to a large number of neurons.

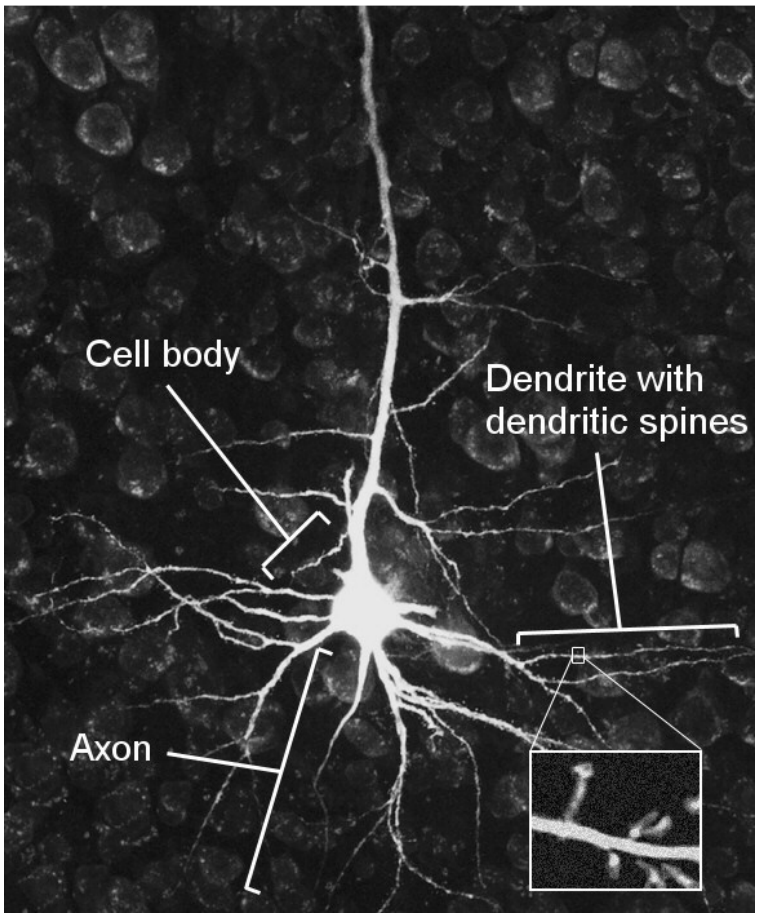

Figure 2. Micrograph of a filled neuron, probably a hipocampal pyramidal cell. Neuron image thanks to www.lebenswissen.de/pix/ Dendritic spine image thanks to tonto.stanford.edu/~viktor/

Functionally, a neuron is similar to a piece of wire, with a few changes. For one, information is generally only sent in one direction. Neurotransmitters are chemicals that are released by neurons in order to send signals to other neurons. Neurotransmitters are released by the axonal terminals of one cell, and diffuse across the synapse to the dendrites of another cell. Here neurotransmitters can bind to "receptors" and effect the neuron in many ways, but importantly they can alter the probability of it firing an "action potential", the electrical signal which neurons send over long distances. These principles are discussed further in the "electrical properties of the neuron" and the "chemical properties of the neuron" chapters.

Another difference between a neuron and a piece of wire, is that a neuron can alter the nature of the information it is going to transmit depending on previous signals it has

received, that is to say, it is not a passive conductor but a small processor, capable of making decisions. There are some 100 billion neurons in the brains of humans. Each one making and receiving thousands of connections. This results in an unfathomable number of connections and pathways, signals can move through the brain in. Integrative processing allows the overwhelming depth of information received by the sensory organs to be processed into discrete, meaningful perceptions. Conversely, the dissemination of information allows for associative processes to occur. These principles are discussed further in the "signalling properties of neurons"

What is a Protein?

By ksi and BilZ0r

Proteins are a certain type of biopolymers/biomacromolecules (molecules that are made out of very many components and therefore have a large molecular weight).

Proteins are made out of amino acids, joined together in a generally linear chain. There are 20 different amino acids that proteins can be made from. The length of those "polypeptid chains" can be as short as less than 20 amino acids or as long as tens of thousand amino acids. This enables the proteins to have a large variety of functions. To mention just a few of them, they can function as neurotransmitters, enzymes, receptors or ion channels, and in fact mediate and control near every function of the body.

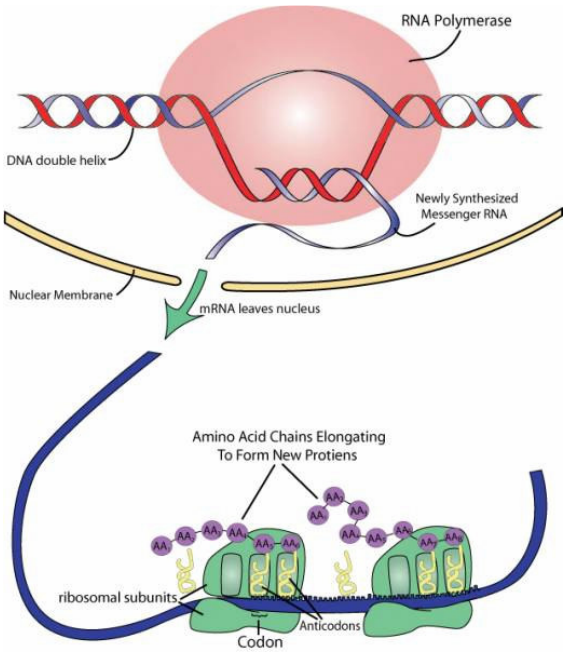

Figure 3. The cascade of transcription to translation. Thanks to moracca

Every amino acid has slightly different proterties: some are water soluble while others are hydrophobic, some easily function as catilytic centers (that is, they are places for chemical reactions to take place), some can easily be joined to other amino acids, forming bonds between distant places on the protein, or even other proteins. The sequence of amino acids which make up the protein are tightly regulated by messanger ribonucleic acid (mRNA), another biopolymer which is copied from, and is essentially a mirror image of DNA. If the body was a construction projection, DNA can be thought of as the manual, mRNA is a photocopy of the manual, and proteins are the finished projected.

The important thing about proteins is that they hold their shape. Although proteins are produced like long rope, they fold up as they are produced into shapes dictated by the amino acids that make them up, hence all proteins of the same structure should form into the same shape. Hydrophilic amino acids will form to face into the aqueous composition

of the cell, while hydrophobic amino acids will stick next to other hydrophobic amino acids, often forming the interior of the protein. Some proteins are produced to be partially buried in, or transit the membrane, here, hyrdophobic amino acids will form the part of the protein which sit in the membrane.

While proteins are rigid, they can subtly change their shape, or conformation. These changes may happen spontaneously, may be induced by chemical changes to the protein, or even by the distribution of electrical charge which surrounds the protein.

What is a Receptor?

By BilZ0r

Pharmacologically speaking, a receptor is a structure where a chemical/drug binds with some kind of specificity, and produces some kind of biological effect. A chemical that binds to a receptor is called a ligand. Receptors are usually locations on proteins where ligand binding can cause a change in the shape (conformation) and cause the protein to become 'active' in some way. Ligands basically come in two main types: ones that bind to a receptor and activate it, or ones that bind to the receptor and do not activate it, these are called agonists and antagonists respectively. Ligand binding also happens in two main ways, **reversibly** and **irreversibly**. Simply a ligand may either approach, bind to and dissociate from a receptor, in a fully reversibly manner, or when a ligand binds to a receptor chemical bonds (covalent) form between them, effectively locking the ligand onto the receptor. Most recreational drugs are reversible ligands, though there are some exceptions (deprenyl).

The reversible binding of ligands is caused by an electrostatic attraction between the ligand and the receptor. Parts of the ligand that may be positively charged might have corresponding negatively charged areas on the receptor, and the converse for negatively charged parts of the ligand to positively charged sections of the receptor. This is why ligands that bind to the same receptors often have similar structures: they must all fit into the same location.

Generally speaking, neurotransmitters and hormones are agonists at receptors. However many drugs are antagonists at receptors. Although strictly speaking a receptor is simply a location on a protein, with the exception of enzymes, often the entire protein is labelled as the receptor for its most famous ligand e.g. the entire protein which is activated by nicotine is called the nicotinic receptor. This double use of the word receptor can become confusing when a protein has many receptor sites on it e.g. the GABA-A receptor protein not only has a receptor for the neurotransmitter GABA, but it also has independent receptors for benzodiazepines, barbiturates and thujone. These different ligands alter the activity of the same protein, but do not compete for the same receptor, hence one can describe them as **non-competitive** ligands, e.g. thujone is a non-competitive antagonist of the GABA-A receptor. On the other hand many drugs are **competitive**, for instance most antipsychotic drugs compete for the same binding site as dopamine on dopamine

receptors so it can be said that antipsychotics are competitive antagonists at the dopamine receptor. One can also have situations whether neither of these terms explain the drug in question. The classical example of this are the benzodiazepines, drugs which bind to, and modulate the GABA-A receptor. These drugs are neither truly agonists nor antagonists, as they have no affect on the receptor themselves; however, they massively potentiate the activity of GABA. This is often called **allosteric modulation**, specifically positive allosteric modulation.

The situation gets more complicated still, when ligands can be not just agonists or antagonists, but somewhere in-between, or so called **partial agonists**. Partial agonists bind to and activate receptors, but not to the same extent as full agonists. This ability of a ligand to activate a receptors is called it' **efficacy**, and is usually given as a percentage of a full agonist, so an antagonist has 0% efficacy, while a full agonist has 100%, and a partial agonist has somewhere in-between. LSD and most other tryptamine and phenethylamine hallucinogens are partial agonists at a subtype of serotonin receptors called 5-HT2A receptors.

Ligands also can bind to receptors with varying affinities. The affinity for a receptor is a ratio of the rate at which a ligand binds to a receptor to the rate at which it unbinds, but it is usually thought of as just the attraction a ligand has for the receptor. One can think of a ligands affinity and efficacy like a key in a lock. A keys ability to fit into the lock is its affinity, but its ability to open the lock is its efficacy. A drugs affinity is also sometimes called its **potency**.

What is an enzyme?

By Anonymous

Enzymes are proteins that could be simply described as molecular catalysts; that is to say, they massively increase the rate of specific chemical reactions. Enzymes generally have a small cleft or crevice in their surface where the chemicals they act on (substrates) can bind; this is referred to as the enzyme's "**active site**". Importantly, the activity of most enzymes can be regulated, either by chemicals that reversibly bind to receptors on the enzyme, or by the action of other enzymes that can bond small chemicals to the enzyme. For instance, the dopamine precursor L-DOPA inhibits the enzyme that produces it, tyrosine hydroxylase. Also dopamine receptors alter the activity tyrosine hydroxylase by covalent bonding or removing of phosphate molecules through activating other enzymes call protein kinases or protein phosphatases respectively. In the case of tyrosine hydroxylase the addition of a phosphate (phosphorylation) increases the rate at which it forms L-DOPA, while dephosphorylation slows it down. This is not the case with all enzymes, but generally, phosphorylation/dephosphorylation alters the rate of enzymes and also affects the behaviour of receptors (discussed further in **G-protein coupled receptors and signalling networks**). Indeed, this kind of enzyme cascade, where one enzyme activates another enzyme, which activates another enzyme etc. is a very common theme in neurons and other cells in the body.

Enzymes are not a particularly common target for recreational drugs. Monoamine oxidase (MAO) is an enzyme that breaks down both natural (endogenous) neurotransmitters but also chemicals which are ingested. Several antidepressants block MAO that inhibits its normal function of breaking down dopamine, serotonin and norepinephrine (also called noradrenaline). Beta carbolines from Banisteriopsis caapi or syrian rue are also MAO inhibitors, and are vital components of ayahuasca, because they stop the breakdown of DMT caused by MAO in the gut.

The most important thing to appreciate about enzymes is that they cause a selected chemical process, and that their activity can be modulated in many ways.

For more information, the interested reader should consult: Campbell, N.A. & Reece, J.B. (2002). Biology. pp. 24-103. San Francisco: Benjamin Cummings.

Electrical properties of the Neuron

By BilZ0r

As already mentioned, one of the most distinctive and functionally important property of a neuron is that it is electrically excitable. This excitability is an emergent property of the neurons ability to alter its **membrane potential** (the word potential can be used interchangeably with voltage). All cells have a membrane potential, and it is generated by the uneven distribution of charged atoms (ions) across the cells membrane, which is impermeable to these ions. The most important ions for generating and altering the membrane potential are the positively charged sodium (Na+) and potassium ions (K+), and the negatively charged chloride ions (Cl-). In general, Na+ and Cl- is found at a higher concentration outside the cell, while K+ is found at high concentration inside the cell. The distribution is found because a protein, usually called the Na+-K+ pump (or ATPase), swaps three intracellular Na+ ions for two extracellular K+ ions. Not only does this action produce a **chemical gradient** of high extracellular Na+ and intracellular K+, but it also produces a **electrical gradient** because it swaps three intracellular positive charges, for two extracellular positive charges i.e. a net movement of one positive charge out of the cell. The eventually leads to a difference of charges, i.e. a voltage, of somewhere around –50 to –80mV. This is called the **resting potential.**

Because particles have a natural urge to equally distribute themselves (2nd law of thermodynamics), it can be said that there is a chemical driving force on these unevenly spread ions. Na+ wants to flow into the cell, and K+ wants to flow out of the cell (i.e. into the areas where the particular ion is at low concentration). Because charged particles are attracted to areas of opposite charge, there is also an electrical driving force on the ions. Na+ wants to flow into the negatively charged cell, which would make the cell more positive, and if this was allowed to happen, it would make the cell increasing positive until the cell became so positive it began to repel the positively charged Na+. Eventually, the electrical force pushing Na+ out would become equal to the chemical force drawing it in. The voltage at which a cell would usually reach this Na+ equilibrium is around +55mV (called the Na+ **equilibrium potential** or reversal potential).

K+ wishes to leave the cell because of its high intracellular concentration and if it did so, it would make the cell increasing negative, until the electrical force drawing K+ back into the cell caused K+ flow to reach equilibrium. This K+ equilibrium potential is around –75mV. Because Cl- is a negative ion, it is repelled from entering the negative cell, even though there is a chemical force drawing it in (because of the high extracellular concentration). So Cl- has its equilibrium potential around –60mV, or very close to the membrane potential. This means that if the cell at resting potential became permeable to Cl-, not much Cl- would flow. Ca2+ is another important ion, which is distributed nearly exclusively extracellularly, and has an equilibrium potential of around +60mV. Importantly, you can see that *the distribution of a particular ion, and the charge of the cell, dictates that ions equilibrium potential, which is the voltage that ion is trying to pull the cell towards.*

This brings us back to the important property of the neuron: it is excitable. A neuron's cell membrane can rapidly change its permeability to particular ions, by opening **ion channels**. Ion channels are pores formed by proteins that allow the flow of ions (usually a particular kind). Usually, these ion channels are can be opened (i.e. gated), by chemicals or by the cells voltage, which leads these kinds of ion channels to be called ligand, or voltage gated ion channels respectively. These channels are explained in more detail in the next chapters.

If the membrane of a cell were to suddenly become permeable to K+ ions due to potassium channels opening, potassium would flow out of the cell. This would make the cell more negative than its usual resting potential, down to a maximum of the K+ equilibrium potential of –75mV. When a cell becomes more negative than usual, it can be described as being **hyperpolarised**. If, on the other hand, the cell became permeable to Na+ ions, because of sodium channels opening, Na+ would flow into the cell, making the cell less negative, and up to a maximum of +55mV. When a cell becomes less negative than usual it can be described as being **depolarised**.

You can see that the neuron has a mechanism for changing its membrane potential. While it may not be obvious to you now why this is so important, it will be explained in the following chapters how this allows the neuron integrate as well as transmit information over long distances.

Voltage gated ion channels

By BilZ0r

As already mentioned ion channels are pores in the membrane of a cell which allow ions to pass through the otherwise impermeable membrane. These channels can be gated (i.e. opened) by various things. In this chapter we will look at ion channels which can be gated by the voltage across the cells membrane itself, or **voltage-gated ion channels**.

There are many individual kinds of voltage-gated ion channels, but all we will be concerned about are three large families, voltage-gated sodium, potassium and calcium ion channels; channels, which when open pass sodium, potassium and calcium respectively.

The most important function of two of these voltage gated ion channels is to generate the **action potential**. The action potential is often thought of as an electrical signal which passes down the axons of neurons, like current down a wire. In reality it is caused by a chain reaction of voltage gated ion channels opening. The third channel is responsible for converting the electrical nature of the action potential into chemical signals a neuron can deal with.

If a part of a neuron expressing voltage gated sodium and potassium channels (usually the axon and cell body) became depolarized (less positive) to around –50mV, voltage gated

ion channels start to become active i.e. they reach threshold. At the cell body, the fastest activating voltage gated ion channel is the sodium channel. The sodium channels start to open, allowing Na+ to enter the cell, further depolarizing the cell, encouraging more sodium channels to open. The Na+ passively diffuses down the axon of the neuron, causing neighbouring areas of neurons to become depolarized, where further voltage gated sodium channels open. This causes a chain reaction of Na+ entering the cell, depolarizing close-by areas of cell, opening further sodium channels, causing more Na+ to pour into the cell etc… If this were to happen unabated, the neuron would fire one action potential, Na+ would reach its equilibrium potential and the cell would become electrically dead. But two things happen to stop this, 1) sodium channels **inactivate** and 2) slower activating potassium channels being to open.

Inactivation of sodium channels occurs normally around 1 millisecond after they begin to open. Inactivation is a transient block of a channel, which in the case of voltage gated sodium channels is caused by a length of the protein which forms the channel, physically blocking the channel like a cork. This inactivation limits both the time and voltage of the action potential. As stated, inactivation is transient, and if the neuron wasn't returned to its resting potential, or at least below threshold, as soon as inactivation passed, the sodium channels would open again. This is when voltage gated potassium channels began to play their part. As potassium channels take about 1-2ms to open after they reach threshold, they are beginning to become fully activated when sodium channel inactivation is in full swing. K+ ions being to flood out of the cell, rapidly making the neuron more negative (repolarizing). Potassium channels do not show inactivation, but as they act to repolarize the cell the pull it below the threshold for sodium and potassium channel activation, which closes the potassium channels.

Importantly the action potential is **all-or-none**, that is to say, the body can't code information in the amplitude of the action potential, the action potential either happens or it doesn't. The body codes information in the frequency of action potentials. For instance, in neurons which transmit pain, more painful stimuli causes the neurons to fire more frequently, but with the same amplitude. Cocaine, apart from its well-known action of increasing dopamine, also blocks voltage gated sodium channels, which stops the formation and propagation of the action potential. This is why it causes numbness, by blocking the transmission in sensory neurons.

Finally, when the action potential has travelled the whole length of the axon, it depolarizes the ends of the neuron, (usually -synaptic terminals-), here voltage gated calcium channels can open, causing Ca2+ to enter the cell. This Ca2+ influx causes neurotransmitter release (as described in **the synapse**). Although this Ca2+ influx shares many properties with the sodium/potassium action potential, it is not all-or-none. Alcohol is believed to inhibit Ca2+ channel function directly (Hendricson et al., 2003), and many common drugs effects Ca2+ channel indirectly. For instance, D9-THC from cannabis and yohimbine from Yohimbe. By effecting Ca2+ influx, these drugs affect neurotransmitter release (discussed further in **the synapse** and **G-Protein Coupled Receptors, and signalling cascades**).

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Ligand gated ion channels

By BilZ0r

Ligand gated ion channels are, as their name suggests, channels in a cells membrane that are gated by ligands, i.e. drugs/chemicals. The physiological role of nearly all ligand gated ion channels is to receive chemical signals in the way of neurotransmitters (discussed further in **the synapse**), and to transduce them to electrical signals. In order for these ion channels to be gated by a neurotransmitter, they have a **receptor** for the specific neurotransmitter as part of the proteins that make up the receptors. Therefore the ion channel complex is often refereed to by the name of the neurotransmitter/chemical/drug which is has a receptor for, and for the rest of this chapter we will largely use this style.

The two most common types ligand gated ion channels (also called ionotropic receptors) are the ion channels that are opened by the neurotransmitters glutamate and GABA, or ionotropic glutamate and GABA receptors. The ionotropic glutamate receptors may be further divided up into AMPA, kainic acid and NMDA receptors (named after drugs that specifically activate these types). While it is possible to further subdivide these receptors based on the individual proteins that make them up, it is outside the scope of this text. AMPA and kainic acid receptors are generally similar; both are opened by glutamate and both are largely selective for the flow of Na+ ions, which in all physiological situations is into the neuron. This flow of Na+ depolarizes the cell, making it more positive and bringing it closer to the threshold for firing an action potential. Because of this, it can be said that AMPA and kainic acid receptors are “excitatory”. The NMDA receptor is an anomaly amongst ligand gated ion channels, in that it is also partially voltage gated. The channel of the NMDA receptor has a site in which Mg2+ ions can sit. This Mg2+ is much larger than the normal ions that flow through the NMDA receptor (Na+ and Ca2+) and hence blocks it. When the cell partially depolarized, positive Mg2+ ions begin to be pushed out of the NMDA receptor channel (presumably because of the positive charge inside the neuron repelling it). Also, because the NMDA receptor is very permeable to Ca2+ channels, not only does it depolarize (excite) the cell, it also can cause many of the chemical changes within the cell caused by Ca2+ (see **G-Protein Coupled Receptors, and signalling cascades**). Largely, it is the release of glutamate, and its action of ionotropic glutamate receptors that allow one cell to excite another cell into firing (although usually it requires 100s of cells to release glutamate onto a cell to cause this).

The most famous drugs which directly effect ionotropic glutamate receptors are the so called “anaesthetic dissociates”, e.g. ketamine, PCP and DXM. These drugs all block the NMDA receptors ion channel, i.e. they are NMDA channel antagonists. Alcohol's actions

are thought to be at least in part due to its ability to block NMDA receptor channels (Woodward, 2000).

The ligand gated ion channel that is gated by GABA is called the GABA-A receptor (to distinguish it from the non-ion channel GABA-B receptor). This channel is largely selective for the transit of Cl- ions. As stated before, Cl- ions have a reversal potential of around -60mV, so if a cell has a resting membrane potential of around –60mV GABA-A receptors do not cause much of an effect on membrane potential i.e. they neither hyperpolarise nor depolarise the cell. But if the cell is being depolarised by the action of ionotropic glutamate receptors, then GABA-A receptors strongly oppose this, and hence its action is often referred to as inhibitory).

A wealth of drugs directly effect GABA-A receptors, specifically benzodiazepines and barbiturates which bind to sites apart from the GABA binding site or the channel, to increase channel opening only when GABA normally opens the receptor. This is an example of **allosteric modulation**, and is a common feature of ligand gated ion channels. Muscimol is a direct agonist, acting like GABA. Alcohol is also though to stimulate GABA-A receptors, though whether this is a direct action is still debated (Aguaya et al., 2002)

There are other kinds of ligand gated ion channels, though the only ones which have much relevance to recreational drugs are the ionotropic acetylcholine and serotonin receptors, also called the nicotinic and 5-HT3 receptors. Both of these receptors are ligand gated sodium channels. Nicotine activates the nicotinic receptor, and serotonin, which could be released by the action of MDMA, can activate 5-HT3 receptors (which may cause MDMA-induced vomiting). There are also the glycine, P2X and VR1 ligand gated ion channels expressed in the central nervous system.

As you can see, ligand gated ion channels are an important (probably the most important) mechanism of neuron-to-neuron communication, and drugs acting on this form of chemical to electrical transmission have a powerful way to alter neuronal activity (discussed more in **Signalling properties of neurons**).

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Chemical Properties of the neuron

By BilZ0r

Aside from having electrical properties that give a neuron it's ability to process information in a rapid manner, neurons express a wide variety of molecular componentry that allow these electrical machines to me modulated. This biochemical soup allows neurons to be dynamic, so that they can change both the signalling properties over the course of seconds and minutes, but also change structurally, to respond to completely novel situations.

The synapse

By BilZ0r

As already mentioned in the "**What is a neuron**", the synapse is junction between axonal terminals and another cell (nearly always a neuron, but sometimes a muscle, or a cell specialized hormone release cell) what has been modified for the release and effect of neurotransmitters. There are also so called "**electrical synapses**", where neurons are electrically coupled by channels (gap junctions) that pass through both of the cells' membranes and allow the passage of ions and small organic molecules, but these synapses are poorly understood and are outside the scope of this text.

The action at the synapse in its simplest form is easy to understand. When an action potential invades the axonal terminal, it causes voltage sensitive calcium channels to open (see **Voltage Gated Ion Channels**) and Ca2+ floods into the channel. The Ca2+ influx causes the neurotransmitter containing vesicles to fuse with the membrane of the cells, and to release their contents into the synapse. Here the neurotransmitter diffuses across the synapse and can interact with its appropriate receptor and depending on the neurotransmitter and the receptor, this can have any of the myriad of effects that receptors are capable of inducing in a cell (See **Ligand Gated Ion Channels** and **G-Protein Coupled Receptors**). The neurotransmitter could also diffuse back and activate presynaptic receptors.

As stated above, the Ca2+ influx caused by the action potential invading the presynaptic terminal and opening voltage sensitive calcium channels is the signal for neurotransmitter release. Not only can drugs directly effect calcium channels, like alcohol, which inhibits them, and hence decrease Ca2+ influx and neurotransmitter release, but presynaptic receptors can effect their activity. For instance, when the CB1 receptor is activated, it causes the activation of a multi-subunit protein called a G-protein (discussed further in **G-protein coupled receptors and signalling networks**). The particular type of G-protein which CB1 receptors activates binds to and inhibits calcium channels, which inhibits the release of neurotransmitter. That G-protein also activates a potassium channel, which causes potassium to leave the presynaptic terminal that lowers the presynaptic depolarization and reduces the number of open Ca2+ channels, and neurotransmitter release.

You can see that the important role of the synapse is a place to release neurotransmitters in order to transmit signals from one cell to the other. However, just as important as the release of neurotransmitter is the termination of their action, because if neurotransmitters weren't cleared they would continue to act indefinitely. Also, in order for any neuronal signals to have any degree of temporal, spatial or amplitudinal resolution they must be able to be discerned from each other, i.e. they can not 'blur' together. Neurotransmitters are cleared by the action of enzymes and/or by molecular carriers (generally called transporters). The enzymes metabolize the neurotransmitter to inactive compounds (i.e. they do not act at receptors) and the transporters carry the neurotransmitter from the extracellular fluid to the intracellular compartment, so that they can not act on receptors any more. Drugs that effect neurotransmitter transporters or enzymes that break down neurotransmitters increase the action of the appropriate neurotransmitter. Cocaine is the classic example of a transporter inhibitor (aka a reuptake inhibitor), it inhibits the uptake of dopamine by the dopamine transporter. The enzyme which breaks down monoamine neurotransmitters (dopamine, serotonin, noradrenaline and adrenaline) monoamine oxidase (MAO), is the target of many pharmaceutical drugs like the antidepressant, MAO inhibitors (MAOIs), and most amphetamines have some action as MAOIs.

So, the synapse is place where two neurons connect and signal to each other. By effect release, reuptake or degradation of neurotransmitters, drugs have a powerful way of modulating synaptic transmission. Indeed, it would be safe to say that the vast majority of psychoactive drugs act directly at the synapse.

G-protein coupled receptors and signalling pathways

By BilZ0r

G-protein coupled receptors (GPCRs) are found in all animals so far investigated and even many plants and make up the single largest gene family in the human genome, indicating their importance as mediators of cell signalling. GPCRs should be of great interest to anyone investigating recreational drugs, as, for instance all of the opioid receptors, all of the 5 dopamine receptors, all of the 9 adrenoreceptors and all but 1 of the 13 or more serotonin receptors are GPCRs. GPCRs are large proteins that exist in the outer membrane of cells, with part of their protein exposed to the extracellular side (so that ligands can bind) and part of the protein facing the intracellular side (so that the receptor can effect the cell). GPCRs gain their name from their ability to bind to and activate guanine nucleotide-binding protein (G-proteins). These G-proteins allow the receptor to amplify the initial signal and effect many intracellular systems.

G-proteins are a complex of three separate subunits, called alpha, beta and gamma. The alpha subunit of the G-protein binds a guanine nucleotide: guanosine triphosphate (GTP) when the subunit is active and guanosine diphosphate (GDP) when the subunit is inactive. When an GPCR is in it's neutral, non-active, agonist free state, it is not associated with a G-protein, however, when the GPCR becomes active because of agonist binding, the conformation of the GPCR is such that a G-protein can bind to its intracellular side. Once a G-protein binds to a GPCR it enhances a conformational change

in it alpha-subunit, which causes the G-protein to release its molecule of GDP and bind a molecule of GTP. Now the G-protein dissociates from the GPCR and splits in two: into the alpha subunit and a beta-gamma subunit complex. These activated subunits can now alter the activity of many "effector systems", for instance the GTP containing alpha subunit can effect many enzymes and proteins, while the beta-gamma complex often directly affects the activity of many ion channels and enzymes (fig. 4). If a G-protein activated effector system produces a molecule which continues the signaling cascade, then the molecule is called a 2nd messanger. The alpha subunit catalyses the breakdown of GTP of GDP, when this occurs the alpha subunit rebinds to the beta-gamma complex, ceasing both of their abilities to activate effector systems.

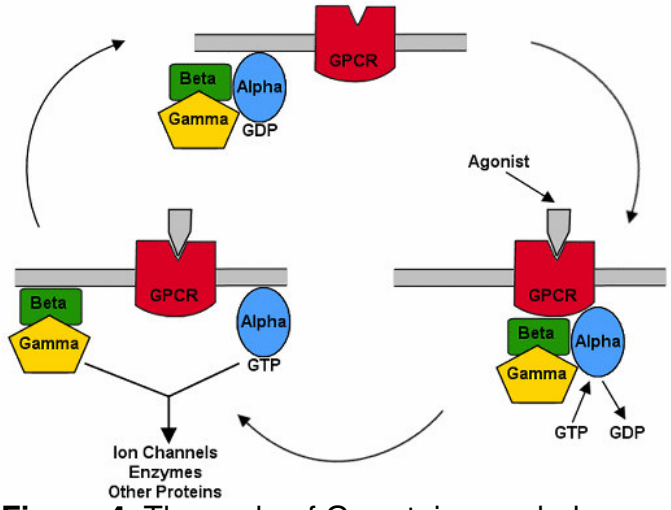

Figure 4. The cycle of G-protein coupled receptors and their assocaited G-proteins

When a GPCR is activated by an agonist, as mentioned, G-proteins can bind, but this process is not limited to a single G-protein, indeed, as long as an agonist remains bound to GPCR it can activate many G-proteins. Likewise, the active forms of the alpha and beta-gamma subunits of the G-protein can activate many effector proteins. Furthermore, if the effector protein is an enzyme, each enzyme can produce a huge number of products while it is being activated by the G-protein subunits. This means that for a single agonist binding, a huge amplification of the signal can be transduced into the intracellular environment.

G-proteins and hence GPCRs can effect a huge variety of proteins, but there is specificity in their actions. The alpha subunits of G-proteins are not always the exact same kind of protein, indeed, there are over 20 varieties of alpha subunit (and there is a growing body of literature about multiple subtypes of beta and gamma subunits). The alpha subunits are usually sorted into 4, functionally different families, with each family containing anywhere from 2 to 9 different alpha subtypes. The families are alpha-s, alpha-i, alpha-q and alpha-12. G-proteins are generally named after the alpha subunit they contain, so that a G protein containing alpha-s is called G-alpha-s, or just G-s. These alpha families generally effect the same effector systems, alpha-s subtypes generally stimulate the enzyme "adenylyl cyclase", alpha-i subtypes inhibit adenylyl cyclase and inhibit presynaptic Ca2+ channels, alpha-q subtypes stimulate the enzyme phospholipase C and alpha-12 effects various novel intracellular targets.

Different GPCRs have different affinities for G-proteins made up of different alpha

subunits, so that some GPCRs will only couple to a particular G-protein. On the other hand many GPCRs couple to several kinds of G-proteins. Furthermore, in receptors that couple to two or more kinds of G-proteins, different agonists can cause the GPCR to activate a particular G-protein over other kinds, a processes called "agonist-directed trafficking". For instance the serotonin 5-HT2A receptor has been shown to couple to G-alpha-q, G-alpha-12 and possibly the novel G-alpha-13. When serotonin binds to the 5-HT2A receptor it causes a roughly even activation of G-alpha-q and G-alpha-12, but psilocin activates G-alpha-12 roughly 25 times more readily.

Adenylyl cyclase (AC) is a very common enzyme, which converts the ubiquitous energy currency of the cell "ATP" into the 2nd messanger cyclic adenosine monophosphate (cAMP). cAMP activates kinases (enzymes which phosphorylate proteins); kinases which are activated by cAMP fall into the protein kinase A (PKA) family, and regulate the activity of a huge number of receptors and ion channels especially (fig. 5). The effect of PKA on ion-channels can have profound effects on neuronal activity, for instance when a neuron is strongly excited (depolarized) it will generally fire action potentials in rapid succession, but the rate of firing will slow and after about 2 seconds firing will stop completely. This "accommodation" is due to the Ca2+ which enters the cell due to depolarization, activating calcium-activated potassium channels and hence positively charged potassium will leave the cell and attempt to repolarize the cell, preventing action potential formation. In certain cells, noradrenaline binding to beta-adrenoreceptors actives G-alpha-s G-proteins, which activates AC, which causes cAMP build up and activates PKA. PKA phosphorylates calcium-activated potassium channels, preventing accommodation. This means that cells which are strongly depolarized and exposed to noradrenaline (for instance, released by arousal or amphetamines) will continue to fire at a high frequency for a long time, where normally, they would fall silent.

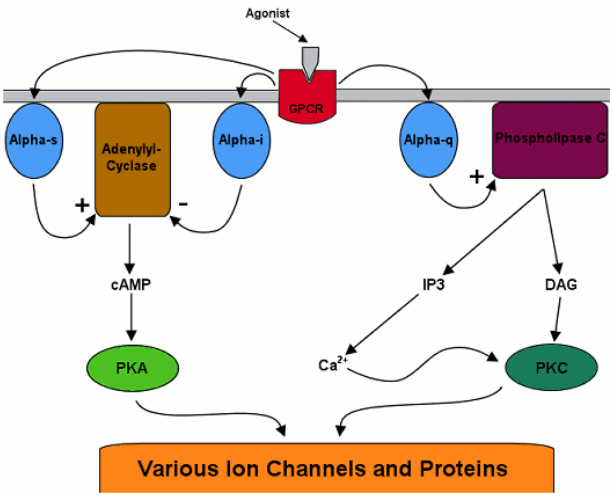

Figure 5. The signaling casaced of the three classical G-alpha subunits

Phospholipase C (PLC) is activated by G-alpha-q and it breaks down particular fats in the membranes of neurons into two 2nd messenger products: inositol triphosphate (IP3) and diacylglycerol (DAG). These two molecules affect two different, but often-complimentary systems; IP3 binds to intracellular IP3 receptors on compartments within the cell (i.e. endoplasmic reticulum) and cause them to release Ca2+. DAG on the other hand activates protein kinase C (PKC) a kind of Ca2+ dependent protein kinase. You can see then that the two signalling molecules produced by the action of G-alpha-q on PLC work synergistically to increase the activity of PKC, although the Ca2+ released by IP3 can have many other effects. PKC (and other

Ca2+-dependent protein kinases) affect a huge number of protein targets, but of special interest to neuropharmacologists is their effects on ligand gated ion channels (fig. 5). One of the classical effects of PKC is to phosphorylate the NMDA glutamate receptor (See ligand gated ion channels), and this phosphorylation can enhance the effect of the NMDA receptor or paradoxically increase its rate of inactivation depending on the particular nature of the PKC cascade. PKC can also enhance or depress AMPA glutamate receptors and GABA-A receptors.

As mentioned, the beta-gamma subunit complex can directly affect ion channels, the classic target being the so-called G-protein-coupled inwardly rectifying potassium (GIRK) channel. This class of potassium channel only opens when the cell is held at below –70mV, which stabilize the membrane potential by cancelling any depolarizing (excitatory) currents by the opposing flow of K+ ions. This means that small excitatory inputs have no effect, and that larger currents are needed to raise the cell above –70mV, at which point excitatory inputs are much more efficient at exciting the cell. When the beta-gamma subunit complex binds to the GIRK channel it massive increase the current which can flow through them, meaning that an even larger excitatory current is needed to get the cell above the –70mV threshold needed to close the GIRK. Both the cannabinoid CB1 and Mu opioid receptor activate GIRK, though as both receptors act presynaptically, their effect is to reduce the Ca2+ influx at presynaptic terminals which induce transmitter release. In combination with the fact that both of these receptors couple to G-alpha-i containing G-proteins that directly inhibit Ca2+ channels, the end result of activation of these receptors is to reduce the amount of neurotransmitter released.

GPCRs also have many mechanisms for signalling to the nucleus of a cell, and hence to control gene expression, this area is still poorly understood, but probably affects many properties of the brain, such as receptor expression, propensity for learning and memory and cell division.

Homeostatis in neuronal signalling

By BilZ0r

Homeostatis is the body's tendency to keep everything the same, like a thermostat, but not just for temperature, but nearly every physiological parameter. Simple homeostatic controls exist for things like hormones, for instance the adrenal medullary cells which releases adrenaline, will have receptors for adrenaline, which slow adrenal release. This process is known as negative feedback. More complicated homeostatic controls exist for things such as body weight, blood pressure and water intake, however the general theme is the same, if a change takes the body away from its homeostatic set point, then negative feedback will try and restore this.

Nearly all aspects of signalling in the synapse are homeostatically controlled. The release of nearly all neurotransmitters are controlled by an **autoreceptor**, a presynaptic receptor which receives the type of neurotransmitter which the neuron it is located on releases, and feedbacks to inhibit the release of this neurotransmitter. Noradrenaline release is controlled by the alpha2 adrenoreceptor, GABA by the GABAB receptor, serotonin by

the 5HT1A/B receptor, dopamine by the D2 receptor, glutamate by mGluRIII. When neurotransmitters bind to their autoreceptors, they usually activate a G-protein coupled cascade which leads to the inhibition of calcium channels and/or activation of potassium channels, which leads to a reduction in neurotransmitter release and presynaptic depolarization respectively. Many autoreceptors also inhibit the synthesis of their respective neurotransmitter. For instance the dopamine D2 receptor leads to phosphorylation and inhibition of tyrosine hydroxylase, the rate limiting enzyme in dopamine production. It is worth noting that these receptors are usually expressed in non-presynaptic locations, on other neuronal types, where they have a different function. Though sometimes they maintain their function as inhibitors of neurotransmitter release, for instance the histamine autoreceptor, the H3 receptor, is found presynaptically on nearly every neurochemical class of neuron, and has been shown to inhibit the release of every neurotransmitter so far investigated. When a receptor is acting like this it is referred to as **heteroreceptor**, the 5-HT1A is another common heteroreceptor. In most native systems, neurotransmitters are being released most of the time, and hence the autoreceptors are tonically (i.e. continuously) active, constantly damping the release of neurotransmitter. Therefore, drugs which block autoreceptors, (e.g. yohimbine, and alpha2 adrenoreceptor antagonist) lead to a large increase in released neurotransmitter.

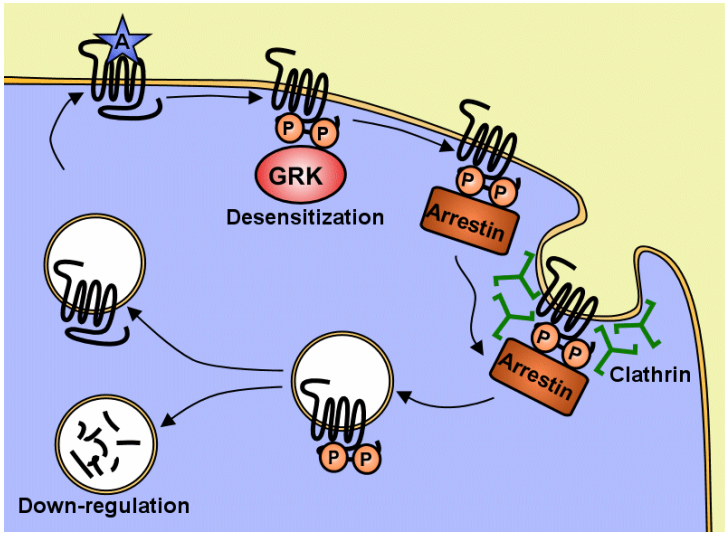

Figure 6. Downregulation of a receptor. If a receptor is occupied by an agonist (A), then it may be phosphorylated (P) by GPCR Kinase (GPK), which can lead to arrestin binding, and internalization.

Autoreceptor action is exclusively a presynaptic method of homeostastis. Receptors themselves are capable of gating hyperactivity. Many ligand gated ion channels **desensitize** (i.e. they enter a state of low efficacy) in response to the application of an agonist, often on an extremely rapid time scale, the AMPA glutamate receptor desensitizes to 10% of maximum current within 10ms of saturating concentrations of agonist. Benzodiazepines rapidly induce tolerance to their behavioural effects. These drugs, which potentiate the action of GABA at the GABA-A receptor, produce molecular changes which mirror this tolerance. The exact mechanism remains unclear, but evidence shows that it is likely that prolonged benzodiazepine treatment renders GABA-A receptors insensitive to benzodiazepine modulation. It seems likely that this is primarily due to the receptor being pulled into an intracellular vesicle (**internalization**), presumably after the action of a kinase. The receptor is then modulated in some way,

possibly by removing benzodiazepine sensitive subunits, so that it is insensitive to benzodiazepines and returned to the membrane surface. It seems that only very high doses or very long treatments with benzodiazepines lead to a total decrease in GABA-A receptor number, and this may be through reduction in GABA-A receptor subunit mRNA expression (Bateson, 2002).

G-protein coupled receptors (GPCR) are also subject to desensitization. Activation of GPCRs increases the activity of G-protein receptor kinases, which phosphorylate the receptor and decrease their signalling efficacy (usually through a decrease in ligand or G-protein affinity). This phosphorylation is reversible, but also allows the binding of proteins called arrestins to the intracellular side of the GPCR. Arrestins not only completely cut the GPCR off from activating G-proteins but they also allows the binding of other molecules, classical the clathrins, which internalize the receptor. Once internalized, (where receptor is separated from interactions with ligands) it awaits one of two fates, reinsertion back into the membrane, or degradation by protiolytic enzymes. Hence long term treatment with agonists lead to a long-term depletion in receptor number (**down-regulation**), which only the synthesis of new receptors can resolve. It is worth noting that GPCRs can also interact with genes, altering expression and after chronic agonist application sufficient to induces internalization it is common to note a decrease in the production no that receptors mRNA, which will also reduce receptor numbers and further slow recovery times.

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Special Topics

Pharmacokinetics

By BilZ0r

Pharmacokinetics is often differentiated from the rest of pharmacology, as it is the study of what the body does to drugs as opposed to what drugs do to the body. For the aid of pharmacology students, it is usually broken down into four stages, mirroring the drugs passage through the human body: absorption, distribution, metabolism and excretion.

Absorption

The classical route of drug administration is orally. There are many factors altering the absorption of orally administered drugs: gastric contents, intestinal pH and most importantly, the physiochemistry of the drug. Drugs which are most rapidly absorbed are drugs which can easily pass through the membrane of cells which make up the drug wall. This means the drug needs to be partially lipophillic, that is, soluble in fats, to be rapidly absorbed. Drugs also can pass between cells, as opposed to through them, so lipophobic chemicals can also be absorbed though this is a limited pathway. Conversely, the drug also needs to be partially hydrophilic, because it needs to be able to dissolve in the watery (aqueous) environment of both the gut and the blood. That means drugs like ethanol are very rapidly absorbed from the gut (it rapidly passes through cell membranes, being both lipophillic, hydrophilic and physically small) while hydrophobic benzodiazepines often take hours to reach the maximum plasma concentration after a single dose (though the water soluble benzodiazepine midazolam is absorbed over twice as fast as it's more hydrophobic cousins, such as diazepam and alprazolam).

The lipophillicity of drugs can also be effected by the pH of the gastric contents. Chemicals which are either basic (like amphetamines) or acidic (like barbiturates) become more fat soluble (and hence more easily absorbed) in acidic or basic solutions. So theoretically, once could eat something acidic or basic and enhance the absorption of drugs from the gut (though in practice this has an equal an opposite reaction in regards to excretion). Also, most absorption happens in the small intestine, where pH is kept below 7 (~pH 5) by bile secretions, and hence any attempts to manipulate gastric pH are probably pointless.

As any drinker knows, one can also alter the absorption of drugs by filling the stomach with food, which slows the movement of drugs form the stomach, to the small intestine. However, one can find reports of fatty foods increasing the absorption of drugs, specifically highly lipophillic compounds or compounds where are extended release formulations (XR). This is because these drugs do not dissolve in aqueous environments, and essentially stay in a big clump, slowing their transit out of the gut and into the blood, and increasing the fat content of the gastric canal allows them to dissolve. There is evidence that some benzodiazepines may be fat soluble enough for this effect to come into play.

Distribution

Once drugs are absorbed, either through oral, intravenous or any other way, they are distributed throughout the body via the blood. Orally administered drugs are absorbed through the intestinal wall, where they dissolve into the blood in the hepatic-portal vein which travels directly to the liver. From there the blood travels to the heart and is pumped around the body. What is of most interest to the users of recreational drugs is getting the drug distributed into the brain. The brain is unlike any other organ in the body, and it uniquely protected by the "blood-brain-barrier" (BBB) which is a conceptual term for the nature of the blood vessels which permeate the brain. The cells which make up these blood vessels are tightly bound together, so that drugs can not move in between cells as they can in other tissue types, and must pass through the cells. They are also bristling with so-called "multi-drug transporters" (like P-glycoprotein), molecular pumps which actively extrude drugs back into the blood. While these transporters can not pump all drugs out of the brain, they can certainly effect the brain permeation of a lot of chemicals.

Because drugs MUST be lipophillic to pass through the BBB some drugs are excluded from the brain. The classical example of BBB impermeable drugs are the new generate of "non-sedating antihistamines". Old antihistamines were fat soluble, and could enter the brain, blocking histamine receptors, and causing sleeplyness. 2nd generation antihistamines were generally made by adding lipophobic groups such carboxycylic acids and alcohols to the structure of first generation antihistamines, making the whole molecule fat insoluble and preventing their passage into the brain; stopping them causing sedation. Another example which frustrates some recreational drug users is the potent opioid loperamide, which although fat-soluble, is a high affinity substrate for P-glycoprotein transporter, and is essentially excluded from the brain (indeed, P-glycoprotein transporters in the gut wall prevent it from getting much further than the intestines). Cannabinoids are also distributed in an interesting way. They are in generally, extremely fat soluble and water insoluble, which lends them to dissolving in fat tissue in the body. Because of this cannabinoids can take weeks to clear from the body after a single dose, while most, more water soluble drugs, are nearly completely cleared from the body after 3-5 days.

Metabolism

Drugs which are taken orally are taken directly to the liver via the hepatic portal vein. The liver is a densely infused with blood vessels, and metabolic enzymes for various classes. Because nearly all chemicals absorbed from the gut needs to pass through the liver, the metabolism that takes place there, before the chemical enters the rest of the body is called "first pass metabolism". Metabolism in the liver can be broadly split into two catagories, phase I and phase II metabolism. Phase I metabolism evolves breaking down chemicals. The classical phase I enzymes are the super family of enzymes know as the cytochrome P450 enzymes (CYP), which has about 50 subtypes, in 17 families, each with several subfamilies. Each enzyme has a name like CYP2D6, which means it's the 6th subtype in the D subfamily in the 2 family. Most drugs are metabolised by drugs in the 1,2 and 3 families, specifically, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5. Furthermore, drugs can be metabolized by a whole range of

hepatic enzymes, like monoamine oxidase, alcohol dehydrogenase, flavin-containing monooxygenases and many others. It is worth noting that the majority of these enzymes are expressed in the gut wall, and begin to metabolize drugs well before they get into the blood stream.

Phase II enzymes add things to the drugs, usually to make them highly water soluble, so they are excreted by the kidneys into the urine. Phase II enzymes conjugate large molecules like glutathione and glucuronide or small sulphate molecules to drugs. Phase II reactions are generally subsequent to phase I reactions, though not exclusively.

The activity and quantity of phase I enzymes are plastic, that is to say, the amount of enzymes and the speed at which the break down drugs can be increased by chemicals known as “inducers” and decreased by drugs called “inhibitors”. This means that coadministration of drugs with enzyme inhibitors leads to an increased amount of drug which make it into the systemic blood supply and ultimately to the brain. Conversely, coadministration of inducers with a drug means that less of the drug gets into the brain. Enzyme inhibitors and inducers usually only effect a small number of enzymes. Enzyme inhibitors can work via a number of mechanisms. Suicide inhibitors work by being a substrate for the enzyme, but undergo an irreversible reaction once in contact with enzyme, covalently binding to the active site of the enzyme, rendering it useless. Other inhibitors bind to allosteric sites on the enzyme, slowing its activity. One needs to be careful about mixing drugs and chemicals which inhibit their metabolic enzymes. More than one death is attributed to mixing MDMA and the anti-HIV drug ritonavir which potently inhibits CYP2D6, the primary enzyme responsible for MDMA metabolism. The danger appears because it adds two more levels of variability. Drugs are absorbed variably from person to person, and enzyme inhibitors act to varying amounts from people to people. Hence a dose of inhibitor which might reduce the activity of an enzyme by 20% in one person, might reduce it by 80% in another, making a normally safe dose of drug lethal. One can also essentially inhibit an enzyme by taking two drugs at the same time which are metabolized by the same enzyme. Here you get “enzyme competition”. For enzyme competition to work, one needs to nearly saturate the metabolic capacity of an enzyme, and this can usually only be achieved when there is only a limited amount of the enzyme. For instance administering two drugs which are metabolized by CYP3A4 are unlikely to compete, because CYP3A enzymes make up 40-60% of the total amount of

CYP450 in the liver, however two drugs which are metabolized by CYP2D6 are likely to compete as they make up only 2% of liver CYP450s. Hence it can be dangerous to mix drugs which use the same metabolic enzymes.

Administering drug via routes other than oral skips first pass metabolism, though of course, the drug will pass through the liver eventually, where metabolism will begin. The differences in metabolism and distribution between intravenous and oral administration produces some interesting effects. If a drug is administer intravenously (IV) one measures the plasma concentration of the drug over time, you get will a graph as shown in figure 7. If one administers the same dose of the drug orally and measures the same properties, you will get a similar curve, though shifted to the right and far more squat. This should be obvious to anyone who has used intravenous drugs. The peak of the drug effect, which corresponds (roughly) to the plasma peak happens essentially instantaneously with IV administration, though when the same amount of drug is administered orally, the maximum effect (and plasma peak) happens much later and is much less. If one measures the area under the curve (AUC) of the plasma concentration x time graph one can judge the amount of metabolism a drug undergoes in the body. For example, if a drug is in no way metabolized during oral administration, then the AUC is equal to the AUC during intravenous use. However, if the drug is metabolized, the AUC decreases below than of the IV graph. If one takes the AUC of the oral dose and divide it by the AUC of the intravenous dose, you get a value known as the "bioavailability" which is essentially the percentage of the drug which escapes first pass metabolism, e.g. a non metabolized drug has a bioavailability of 100% while an extremely metabolized drug would have a bioavailability of 1%. Not only do different drugs have different bioavailabilties but so do different routes of administration, e.g. smoking has 100% bioavailability but intramuscular or subcutaneous administration usually have bioavailabilties below 100% as there are metabolic enzymes in the skin and muscle.

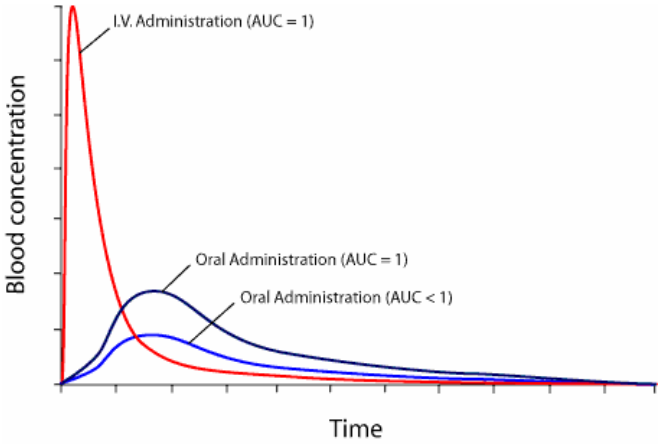

Figure 7. Blood concentration vs time of a hypothetical drug given IV (red) or orally. If the drug is not subject to first pass metabolism it will have an area under the curve (AUC) equal to the IV graph (dark blue). If the drug is subject to first pass metabolism it will have an AUC less than the IV curve (light blue)

Metabolism doesn't always reduce the effectiveness of drugs. Many drugs need to be metabolized to work, these drugs are called "pro-drugs". A classical pro-drug is codeine, it is metabolized by CYP2D6 into morphine. Codeine itself it virtually inactive at any of the opioid receptors, how morphine is a potent mu-opioid receptor agonist (it is worth

noting that the phase II metabolite of morphine, morphine-6-glucuronide, is far more potent than morphine, and there has been considerable debate as to whether morphine is a morphine-6-glucoronide prodrug). Because codeine needs hepatic enzymes to be active, it is most potent via oral administration, as it the case with all prodrugs dependent on hepatic enzymes. The CYP2D6 mediated conversion of codeine to morphine can be saturated by codeine doses of 200-400mg, and hence doses higher than this have no effect.

Drug	Primary Metabolic enzyme/clearance mechanism
Alcohol	Ethanol Dehydrogenase
Amphetamine	Renal>CYP2D6
Cocaine	Butyrylcholinesterase
D9-THC	CYP2C9
MDMA	Renal>CYP2D6
Methamphetamine	Renal>CYP2D6
PEA Hallucinogens	Renal>MAO?CYP
Morphine, Oxycodone, Hydrocodone	CYP3A4

Figure 8. The main metabolic enzymes or clearance mechanisms for common recreational drugs

Excretion

The final pathway for nearly all drugs is excretion via the kidneys. The kidneys work by essentially filtering all components of the blood out apart from very large very large molecules and blood cells, and then transporting all the useful components back into the blood, like salts, water, glucose, amino acids etc. As the kidney is designed to remove noxious chemicals, this is a cleaver system; as the body can't know what poisons it could face, a system designed to actively remove toxins wouldn't work, so the system instead removes everything, then keeps only that what it needs.

Unfortunately for the kidney, this system is not fool proof, as the kidney has difficulty excreting lipophilic chemicals, as they can permeate back through the walls of the kidney (you now see why phase II enzymes are important, by making noxious chemicals water soluble chemicals from escaping the kidney). As already mentioned certain chemicals can change their fat solubility depending on the pH of the solution they are dissolved in. Basic chemicals like amphetamines become fat soluble in basic environments and water soluble in acidic environments. Indeed, it has been shown that in subjects who have had treatments to make their urine more basic, they excrete amphetamine at a rate 10x slower than subjects who were treated to produce hyper-acidic urine.

Learning and Memory on a cellular level

By BilZ0r

Even some of the most simple animals with neural structures too small to call brains, can learn from their experiences. Sea snails (animals with only a few thousand neurons) can associate neutral stimuli (those which produce no response) with noxious ones, if they repeatedly happen together and learn to respond to the neutral one as if it were the noxious one. Importantly, they can also unlearn the response, if a previously neutral stimulus is no longer paired with the noxious stimulus. Brains can also learn to pair neutral stimuli with rewarding stimuli, the classical example being Pavlov's dogs, who learned to pair the sound of Pavlov entering his lab, with the food he gave them (he was studying the effects of food on salivation, however to his annoyance, the dogs began to salivate BEFORE he gave them the food). Interestingly, this so called Pavlovian conditioning is dependent on how well the neutral stimulus predicts the rewarding stimuli. If Pavlov had entered his lab regularly without feeding the dogs, it is likely that the dogs would have never learned to salivate to the sound of his footsteps.

Believe it or not, these learning situations are the comparatively complicated one as far as the neuroscientists is concerned. Imagine the situation where an experimental animal is exposed to a light that consistently precedes (and hence predicts) a tone, this is called sensory preconditioning. Following a Pavlovian style learning situation, a puff of air into the eye produces a blink, and if the puff is consistently paired with a tone, the subject will learn to blink just to the tone. Now if the animal is exposed to the light, it will blink, even though the tone has never been paired with the puff.

All of these experimental results may seem obvious, but they show some very important things. Firstly, it was proposed by Hebb that if a neuron A consistently takes part in firing (i.e. fires at the same time as, and is connected to) neuron B, then the connectivity between neuron A and B will increase. What is interesting about sensory preconditioning is that obvious connectivity between the two neutral sensory stimuli formed just by pairing them together, supporting Hebbs theory. Hebbian principles were further engrained when it was shown that simply by strongly exciting a set of neuronal inputs into a population of neurons, the neuronal inputs became more efficient at this excitation, importantly this potentiation lasts for extremely long period (recorded for over 1 year). This is due a series of changes in the synapse, both pre- and postsynaptically, including an increase in glutamate release and an increase in AMPA receptors in the post-synaptic membrane. This long term potentiation (LTP) has been used as a model for memory since it was discovered over 30 years ago, and has stood robustly against most challenges. Specifically drugs and genetic modulation which prevent the formation or maintenance of LTP also prevent learning.

Reward relating learning (like a rat pressing a lever for food) also has interesting cellular mechanisms, animals will not learn to pair a neutral stimulus with a food reward if their dopamine receptors are blocked, likewise, if dopaminergic neurons are destroyed the association can not be made. Dopamine cells are active by natural and drug induced

rewards. Interestingly, animals will learn to do almost anything for direct stimulation of dopamine neurons. Some people see this as evidence that dopamine directly mediates pleasure, but experiments in the 1960s where humans were given the ability to directly stimulate their own dopamine neurons didn't report extreme pleasure, though the would constantly activate their dopaminergic neurons (Heath, 1972). Furthermore, if an animal is trained to press a lever to stimulate their dopaminergic neurons, and another animal receives dopaminergic stimulation when the first does, the second animal does not show signs of pleasure, and can even show signs on distress. If this leaves one a bit confused about the role of dopamine, consider this, there are many places in the brain where it has been reported that dopamine massively facilitates the induction of LTP, indeed, in projections from the cortex to the striatum, LTP style stimulation actually produces a suppression in the power of the input neurons from the cortex into the striatum except when dopamine is present . While in the presence of dopamine, a large increase in synaptic strength in generated (Reynolds et al., 2001). The striatum, and especially the ventral striatum (AKA the nucleus accumbens) has been highly associated with the "rewarding" (i.e. reinforcing) properties of natural stimuli and addicting drugs.

This allows us to construct a model of the plasticity of synapses (at least in the striatum), if neuron A (cortical) and neuron B (striatal) are active out of synchrony then there is no change in synaptic strength while if they are active together, without dopamine the synaptic strength decreases. Finally, if neuron A and B are active together in the presence of dopamine, the synaptic strength increases. Although the exact functional role of this corticostriatal dopamine-dependent synaptic plasticity is unclear one can form a reasonable hypothesis. The cortex is activated by sensory stimuli while the striatum (which receives the majority of its input from the cortex) is involved in, and active during, movement. Hence a particular sensory stimuli activates a particular area of cortex and cortico-striatal projections, while, a particular behaviour leads to certain striatal neurons being active. If this combination of cortical activity (stimuli input) and striatal activity (behavioural output) produces a reward (dopamine) then the corticostriatal system that was active during this state is strengthened. Addictive drugs, which cause an inappropriate release of striatal dopamine lead to an aberrant corticostriatal state, where cortical neurons which code for drug associate stimuli lead to drug taking behaviour.

This becomes even more interesting when one more closely considers the activity of dopaminergic neurons in an awake behaving animal. While on the surface dopaminergic neurons seem to respond to rewarding stimuli, careful examination shows the more closely reflect the expectancy (or lack thereof) of reward. Dopamine release is most strongly induced by unexpected primary rewards (e.g. food, water), however, if the reward is preceded by a predictive stimuli (e.g. a tone), dopamine release will be shifted to being released by the tone. However, if the tone continually predicts the reward, eventually dopamine release will wane. Likewise, if an unpredicted reward happens regularly, the animal will cease to release dopamine to its presentation. You can see that if an animal expects a reward, dopamine will not heavily release. However, there is usually a basal level of dopamine being released and if an expected reward is denied, this basal level will drop to zero. Hence, you can see that dopamine release acts as prediction error signal, if the animal receives an unexpected reward, a large increase in dopamine is

induced while if it correctly or incorrectly predicts a reward, dopamine stays at the basal level or decreases respectively.

This shows us how reward related learning can be unlearned, that a pause in dopamine causes corticostriatal synapses to weaken, and the behaviour that was not rewarded is lessened. (theory reviewed by Contreras-Vidal and Schultz, 1999)

While this hypothesis is certainly a vast over simplification, the basic rules are probably true. It is worth noting that not all research groups show that dopamine increase the strength of synapses, and that it can also decrease them (probably dependent on which dopamine receptors are activated (Centonze et al., 2001). However still, dopamine is modulating synaptic plasticity in a reward dependent fashion. This dopamine-mediated synaptic remodelling helps associations form between stimuli and behaviour that lead to reward. When this system is hijacked by addictive drugs an aberrant association which encourages drug taking is formed, and it is formed as concretely as any other memory.

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Amphetamines and the monoamine transporter

By BilZ0r

Introduction

"Amphetamine" is a term given to a structurally related class of compounds sharing the alpha-methyl phenethylamine backbone. Although the amphetamines contain an array of pharmacologically distinct molecules, the classical amphetamine action is to raise the extracellular concentration of monoamine neurotransmitters (dopamine, serotonin and noradrenaline). Exactly how amphetamines cause the increase in monoamines is a complex, probably multifactorial mechanism, which will be reviewed here.

Facilitated exchange diffusion

Amphetamines cause a massive increase in extracellular monoamines. Unlike reuptake inhibitors such as cocaine, which might cause a 200-400% increase in free monoamines, amphetamines can cause increase up to and over 1000%. This increase seems largely to be dependent on a group of integral membrane proteins collectively called the monoamine transporters. The monoamine transporter comes in four flavours, the dopamine, serotonin, noradrenalin and vesicular monoamine transporters, which are selectively expressed on dopaminergic, serotonergic, noradrenergic neurons and monoamine containing vesicles selectively. Each transporter is relatively selective for the monoamine that it gets it name from, though not exclusively. Exactly how monoamine transporters take monoamines from the extracellular space and into the neuron is unclear. Monoamine transporters are 12 transmembrane spanning proteins which are believed to function a homomultimers, and as well as their neurotransmitter binding site, they have Na+ and Cl- ion binding sites (fig. 10). They transporter is generally believed to function by Na+ and Cl- first binding to the extracellular face of the protein, and then the neurotransmitter binds extracellularly. This binding somehow causes the protein to change conformation so that the extracellular binding sites are facing into the cell, the ions and neurotransmitter dissociate and hence are transported into the cell (fig. 10).

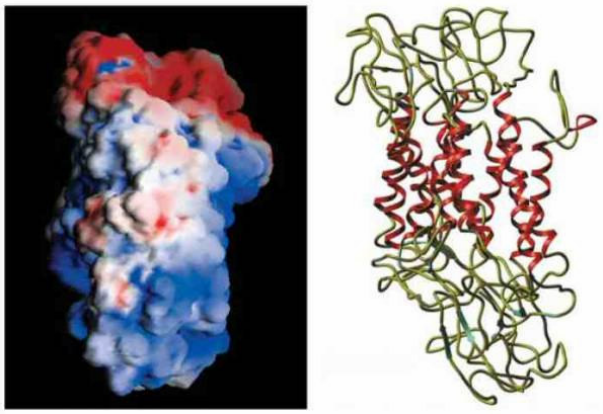

Figure 9. Structure of the monoamine transporter, Red reflects negative region, and blue positive. Intracellular face at the bottom of the figure. From Ravna et al., 2003

The first mechanism used to explain amphetamine function was the so-called "facilitated exchange diffusion" model by Paton, 1973. In this model, amphetamine binds to extracellular neurotransmitter binding site of the transporter, causing the transporter to move the amphetamine molecule into the intracellular space, leaving the neurotransmitter

binding site open in the intracellular face (for at least as long as it takes the transporter to flip back to facing extracellularly). Over the entire cell, amphetamine should cause an increase in the proportion of intracellular facing transporters, and on the whole increase the rate of reverse transporter (that is transport of neurotransmitter out of the cell). The source of the monoamines which are susceptible to reverse transport is unclear. Obviously, these monoamines must be in the cytosol of the cell, and not contained in vesicles, however, whether these neurotransmitters must first be displaced from vesicles by facilitated exchange diffusion through the vesicular monoamine transporter, or whether free neurotransmitter levels inside the cell are enough to support reverse transporter is unknown.

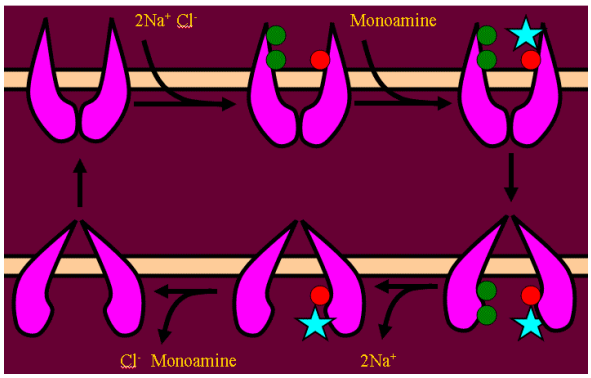

Figure 10. Model of monoamine transporter function

Facilitated exchange diffusion is supported by a large number of relatively correlative observations. In order for the neurotransmitter to bind to the transporter, sodium must bind as well and in accordance with that amphetamine-induced reverse transport depends on both extracellular Na+ (so that amphetamine can be transported into the cell) and intracellular Na+ (so that the neurotransmitter can be transporter out) (Schmitz et al., 2001) and in fact an increase in intracellular sodium is enough to cause reverse transport (Khoshbousie et al., 2003). Another observation that this author makes that at the very least doesn't disprove facilitated exchange diffusion is that the affinity of various amphetamines for the extracellular binding site is tightly correlated with their ability to release monoamines (fig. 11) (this seems to indicate that binding to the extracellular binding site, and hence probably transport into the cell, is all that is required to induce reverse transport). Another consequence of the facilitated exchange diffusion model is that amphetamines would compete for neurotransmitters at the transporter, and as a result inhibit monoamine uptake. Indeed, it is often sited that amphetamines work by inhibiting reuptake, but the actual contribution of amphetamine-induced reverse transport and reuptake inhibition in regards to the increase in free monoamines is hard to calculate but it has been estimated that the majority of the increase in monoamines is due to reverse transport (Schmitz et al., 2001). This conclusion can be easily seen to be true as the maximum increase in free monoamines in the brain caused by amphetamines is well over 1000%, while cocaine causes a maximum increase in extracellular dopamine around 500%.

There are however several observations that bring the simplicity of facilitated exchange diffusion, which have produced other theories, discussed below.

Channel Mode

Interestingly, it has been shown, that under the right conditions, cells expressing monoamine transporters can display large current events which are blocked by drugs

which block monoamine transporters and are coincident with very large effluxes of monoamine neurotransmitters. These channel like events have been shown to contain on the order of 10,000 molecules of neurotransmitter, released over a few milliseconds at the most (Kahlig et al., 2005). In order to give this number some scale, this is approximately the same number of neurotransmitter molecule inside a vesicle. Amphetamine drastically increases the rate of these channel like events. It is worth noting that Kahlig et al., reports that these events only happen when neurons are held at massively depolarized potentials (>+40mV), so these channel like events are only likely to happen during the peak of an action potential.

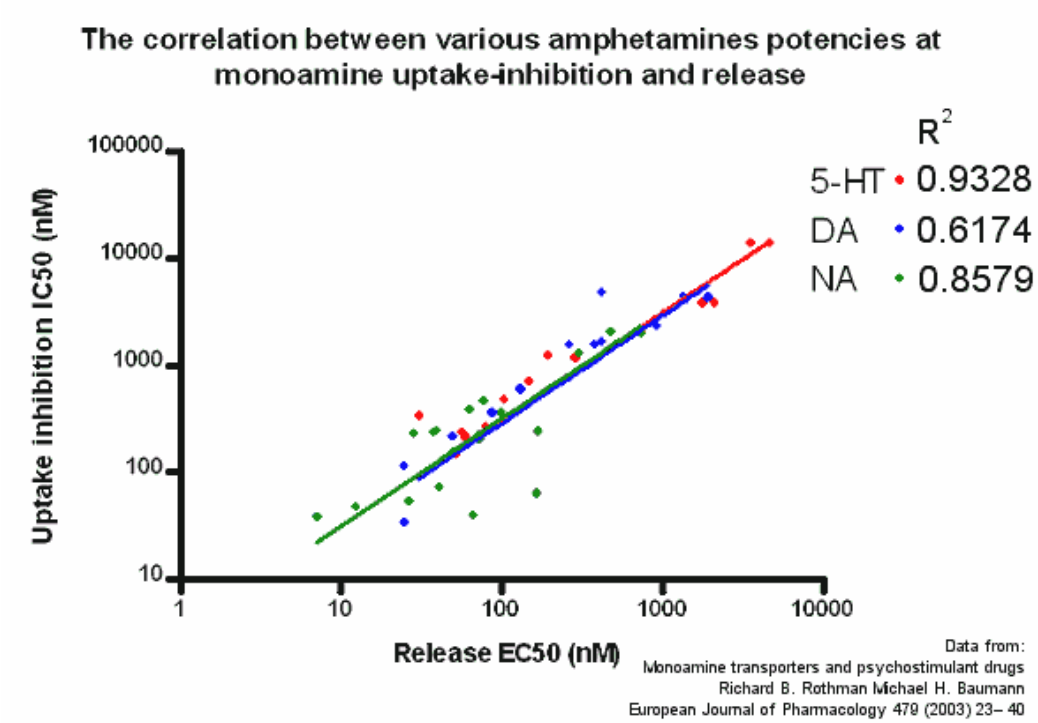

Figure 11. A tight correlation between uptake inhibition and release between various amphetamines

2nd Messanger systems

Monoamine transporters have numerous sites which can be phsophorylated and this phosphorylation seems to be play an important role in amphetamine-mediated reverse transport. Specifically the activity of protein kinase C (PKC) seems to regulate transporter activity, for instance activating PKC is enough to induce monoamine transporter-dependent monoamine release, and it has been reported that PKC inhibition blocks amphetamine mediated dopamine release (Kantor et al., 2001). Furthermore, removal of a small section of the N-terminus of the dopamine transporter, or modification of this sections of the amino acid sequence so that it can not be phsophorylated (serine residues replaced with alanine) reduced amphetamine mediated dopamine release by 80% while leaving dopamine uptake unchanged. (Khoshbouei et al., 2004). Exactly how amphetamine lead to an activation of PKC is unclear, but may revolve around an

alteration of intracellular Na+ homeostasis (due to Na+/amphetamine co-transport) and an influx of Ca2+ through the Na+/Ca2+ co-transporter.

Monoamine Oxidase Inhibition

It is often claimed that amphetamines work, at least in part, by inhibiting monoamine oxidase (MAO), the intracellularly expressed enzyme responsible for the break down of the monoamine neurotransmitters. The importance of this effect in general is probably minimal at best, as MDMA, methamphetamine and amphetamine are usually reported to need a concentration of 10-100µM to inhibit MAO-A 50% (most amphetamines are relatively MAO-A selective). However these drugs need concentrations 1000x lower than that to significantly effect monoamine release. While some people suggest that as MAO is an intracellular enzyme, and amphetamines are probably highly concentrated inside cells due to their transport by the monoamine transporters (though this has never been directly measured), monoamine oxidase inhibitors only cause a modest increase in free monoamine levels (~200%), and hence at best it is the MAOI effects of amphetamines must be minimal.

Other mechanisms

Many other mechanisms have been used to explain amphetamine actions, such as the weak base effect. In this theory the accumulation of amphetamines (which are weak bases) in synaptic vesicles, increases the vesicular pH to a point where the vesicular matrix, which holds neurotransmitters in a stable state breaks down, and causes neurotransmitters to leak into the cytosol of the cell. This increase of free intracellular monoamines favours reverse transport and leads to monoamine release. Another idea is that inward amphetamine transport which is driven by Na+ leads to a substantial inward Na+ current, depolarizing the cell, and leading to classical vesicular release. The contribution of this effect is largely unknown, but seems unlikely as vesicular release has largely been disproved as a mechanism of amphetamine-induced monoamine release (amphetamine-induced release is independent of extracellular Ca2+ and is insensitive to toxins which disrupt vesicular exocytosis).

Conclusion

Exactly how amphetamines cause monoamine release is still not clear. It seems to be caused by a reversal of monoamine transport through monoamine transporters. This amphetamine-induced transporter-mediated reverse transport has been shown to be largely dependent on PKC-mediated phosphorylation of the N-terminus of the transporter, and facilitated exchange diffusion. Other effects, such as reuptake inhibition, MAO inhibition and a facilitation of the channel like mode of the transporter may play small roles in mediating amphetamine-induced increase in monoamine neurotransmitter levels.

Further Reading

An excellent, complete historic review: Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. Prog Neurobiol. 2005 Apr;75(6):406-33

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G-protein coupled receptors: part of a network of signalling machinery

By mitogen

The section on "G-protein coupled receptors" (GPCRs) in the popular undergraduate level text book "Pharmacology" by Rang, Dale, Ritter & Moore [1], presents a relatively one-dimensional view of GPCR signalling. A neat diagram sums up the events that occur on binding of a ligand to its cognate GPCR: ligand binding attracts a GTPase ('g'-) protein to the receptor, and the GDP bound to the g-protein is swapped for a GTP. The now-activated subunits of this heterotrimeric g-protein then dissociate and activate another molecule in the signalling cascade, such as adenylyl cyclase, which subsequently produces 'second messenger' molecules. These second messengers are responsible for activation of other downstream effectors, and the signal that began with a ligand binding to the GPCR is propagated.

In contrast, even a cursory glance at any recent review [2,3,4] on the subject of GPCR signalling will reveal to the reader that the textbook model of GPCR signalling is woefully inadequate. Rather, these reviews present the concept that a whole network of interacting proteins and biomolecules nucleated around scaffolding proteins are involved in what was once thought to be a relatively simple, linear transduction process. These protein networks are involved with fine-tuning and regulation of every facet of GPCR function. The 'receptosome' concept, that whole networks of molecules are spatially compartmentalised into plasma membrane microdomains such as caveolae and other lipid rafts is integral to all recent models of how GPCR signalling is effected.

In this review I will discuss how each receptosome exists as a self-contained, functional signalling unit, and the importance of spatial compartmentalisation of GPCR signalling machinery. Important experimental observations which lead to the invalidation of older GPCR signalling dogma and forced reconsideration of the whole signalling paradigm will be included. Attention will be paid to the roles that particular groups of proteins play in receptosomes, and the modality of their interactions with other receptosome proteins. Finally I will discuss some of the methodology that is currently being used to determine these interactions and their importance to aspects of GPCR signalling.

The old GPCR signalling dogma:

The older "1-dimensional" (meaning that signal transduction follows a defined, stepwise path, as opposed to three dimensional networks of interactions) model of GPCR signalling has been superceded by models like the 'receptosome' model described in this review. Essentially, too many contradictions of and paradoxes in the old model arose from experimental observations. Some of the observations that forced the creation of a newer more comprehensive model are detailed below.

Old dogma states that a specific ligand binds to its cognate GPCR, which then undergoes a conformational change, such that its cytosolic domain develops high affinity for one particular G-protein family and subtype, which it recruits. The activated GPCR activates the recruited G-protein by functioning as a guanine nucleotide exchange factor, exchanging G-protein bound GDP for GTP. The activated G-protein then splits into its

alpha and beta-gamma subunit components, which activate secondary targets. G-alpha usually modulates the activity of a second messenger producing enzyme, such as activation of phospholipase C in the case of Gq-alpha, or activation of adenylyl cyclase by Gs-alpha. The second messenger activates second messenger dependent kinases which proliferate the signalling cascade. Additionally, there is room in the one-dimensional model for G-protein Receptor Kinase (GRK) mediated phosphorylation and arrestin mediated desensitisation, which is why although these molecules were discovered relatively early on, they did not push for creation of a new model. [1,3,4]

Organisation of GPCR signalling machinery

One of the fundamental concepts of biochemistry is that the proteins that comprise the majority of cellular machinery interact with each other as huge networks of multiprotein complexes, with specific chemical affinities determining the strengths of these interactions [1,5]. Thus when a ligand binds to its cognate GPCR, a conformational change is induced in the GPCR which creates a chemical site for which specific G-protein(s) have high affinity. Additionally, according to the laws of mass action, the magnitude and rate of chemical interactions and reactions are heavily dependent on the concentrations of the reactants. This raises a fundamental problem with the classical one-dimensional theory of GPCR signalling as can be found in most generic pharmacology textbooks: if one looks at the average concentration of each protein component involved in GPCR signalling, it is far too low to possibly account for the rapidity of the biochemical response to receptor agonism [5]. Kinetics of the protein-protein interactions required to form a signalling cascade must therefore be simply too unfavourable for any agonist directed response to occur if these proteins were randomly or even uniformly distributed across the plasma membrane or cytosol. This appears to be a massive flaw in the old model of GPCR signalling.
Several more flaws can be identified when the old model is compared with recent observations about the characteristics of GPCR signalling.

The old dogma of “1 g-protein couples to one GPCR” has been comprehensively disproved – in fact GPCRs more often than not couple to more than one G-protein [6]. This has significant ramifications for the signalling pathways activated by a particular GPCR. One particularly illuminating example of this observation is that the thyrotropin receptor is able to couple to all four major G-protein families [6]. Other experiments have shown that the majority of GPCRs have at least some affinity for each type of G-protein: therefore the preference for activation of a particular G-protein subtype actually lies on a continuum. In light of this concept, it is possible to infer that it is possible to describe GPCR interactions with particular G-proteins statistically: for example, a particular GPCR may interact with G-protein X 90% of the time, G-protein Y 9.99% of the time and G-protein Z a biochemically negligible 0.01% of the time. These statistics would be based on the chemistry of the interaction sites on the GPCR and G-proteins X, Y & Z. Interaction of the GPCR with G-protein X is obviously the most thermodynamically favourable binding interaction in a mixture of the four proteins at equilibrium.

A further observation that can not be integrated with the old model of GPCR signalling is that agonism by different ligands induces GPCRs to have different affinities for particular G-proteins. The paper "Opioid agonists differentially regulate Mu-opioid receptors and trafficking proteins in vivo" [7] is a good example of how different agonists can induce different biochemical responses in the cell. It is likely that the mechanism for this involves the two agonists used, morphine and etorphine, inducing different receptor conformations, and therefore recruiting different groups of G-proteins to the Mu-opioid receptor.
It is also possible, however, that this effect is not actually mediated by G-proteins at all, and involves direct interaction of other non-G-protein signalling machinery at the Mu receptor. Models have been suggested in which particular receptors may have a number of different conformations which they can assume, and different agonist ligands thermodynamically stabilise particular conformations, which each have a set of G-proteins they activate to different extents. This is a discrete model – there are a defined number of conformations that a receptor can take, and the potency of the agonist to induce that conformation and therefore the overall activity of the drug at the receptor, depends on the degree of thermodynamic stabilisation of that conformation [8]. Alternately, it is possible to imagine a continuous model, where each agonist induces an individual receptor conformation, which alters the G-protein coupling of the receptor and therefore the properties of the signal induced by that agonist. It is even possible to amalgamate these two theories, and conceptualise a model where each different agonist does continuously induce a different receptor conformation, but there are 'peaks' in agonist affinity and efficacy which correspond to stabilisation of particular, discrete conformations [9]. Needless to say, a "one GPCR binds to one G-protein" model is completely unable to account for any of these ideas.

Another observation that has forced progression from the older GPCR signalling dogma is that G-protein coupling is not necessarily required for biochemical responses to receptor agonism [10]. Following receptor activation and subsequent G-protein activation, the GPCR is often phosphorylated by a G-protein coupled receptor kinase (GRK,) [10] (or sometimes a by second messenger dependent kinases,) [11] and it is this chemical modification that creates a binding site on the GPCR for a group of proteins called arrestins, which attache to the GPCR and blocks any further coupling to G-proteins, in effect causing the cessation of G-protein mediated signalling [10]. It has been shown, however, that Beta-arrestin may act as a scaffolding molecule and serve to recruit other non-G-protein related signalling machinery. Experiments have shown that arrestin-2 can recruit the tyrosine kinase Src by binding to its SH3 domain, and can also activate MAP kinase pathways. Other experiments showed binding of JNK3 and ASK1, which is a JNK kinase kinase. Thus GPCRs can activate MAPK and tyrosine kinase pathways via their interaction with arrestin proteins [11]. Recent studies have shown an interaction between activated beta2adrenoreceptors (B2AR) and Src which is increased by overexpression of beta-arrestin. Additionally, inhibition of beta-arrestin binding to either B2AR or Src attenuates B2AR mediated activation of MAPK. [10]
Beta-arrestin has also been implicated in regulation of receptor trafficking and endocytosis by its interaction with the heavy chain of clathrin and the clathrin adaptor protein AP2 [10]. These observations of arrestin molecules as scaffolds that nucleate

assembly of non-G-protein mediated signalling processes add further detract from old GPCR signalling dogma.

Described above is a series of experimental observations that obligatorily invalidate the one-dimensional model of GPCR signalling, while at the same time building the concept of GPCR signalling as involving a whole network of interacting proteins, with some acting as nodes and scaffolds onto which other proteins nucleate, while others are involved in fine tuning and regulation of the signalling machinery, and still others involved in the trafficking and regulation of the receptors themselves. The most important feature of a newer model of GPCR signalling would have to incorporate the principles of biochemical kinetics and concepts such as collision theory. If all the protein components required for GPCR signalling were to be randomly or even uniformly distributed throughout the cytosol and plasma membrane, the observed rapid response of GPCRs to agonism could not possibly occur. Thus, a new model must include a spatial dimension. The components must be spatially organised such that the biochemistry is actually possible. In answer to this requirement, the literature is packed with reviews and papers documenting the existence of membrane microdomains, or lipid rafts, such as caveolae, in which many of the signalling components and receptors are often congregated.

Caveolae are small (50-100nm) invaginations in the plasma membrane of cells, and are considered to be a subclass of lipid rafts. The lipid composition of caveolae includes characteristically high levels of cholesterol and sphingolipids, along with caveolins, a group of proteins which comprises three isoforms: cavelolin-1, caveolin-2 and caveolin-3. It is generally accepted that caveolae will form if a cell expresses caveolin-1, or in the case of striated muscle myocytes, caveolin-3. Thus, while the plasma membranes of most or all cells contain lipid rafts, only some cells contain caveolae. A 2003 paper in the Journal of Neurochemistry [12] gives a good example of a GPCR being localised to caveolin membrane fractions, and shows “molecular and functional association of mGluR1a receptors with caveolins.” The study demonstrates that agonistic activation of mGluR1a receptors increased ERK phosphorylation in low density caveolin enriched membrane fractions, but not in high density membrane fractions containing no caveolins. Also mentioned in the study was the observation that mGluR1 heterodimerizes with adenosine A1 receptor and calcium sensing receptor; all three of these proteins localise to caveolin rich membrane microdomains [12].

Another example of the role of localisation of receptors and signalling machinery to caveolae is the comparison of Beta1-adrenoreceptor (B1AR) and B2AR signalling in cardiac myocytes. B2ARs activate adenylyl cyclase 6 (AC6) with a lower efficacy than B1ARs, and it appears that this is due to rapid translocation of B2ARs out of caveolae and into clathrin coated pits after receptor activation. AC6 is localised strictly to caveolae, and as such when the B2AR is translocated, it can no longer physically contact AC6 to activate it [5].

A concept that is integral to the model of spatial compartmentalisation of signalling proteins into regions such as caveolae and other lipid rafts is the selective expression of certain isoforms of G-proteins and second messenger synthesising enzymes such as adenylyl cyclase (AC) to particular types of raft, or not to any raft at all. There are nine

AC subtypes, but not all of them localise to lipid rafts [5]. Therefore, the fact that many different subtypes exist of G-proteins, second messenger synthesising enzymes and other signalling proteins such as Regulators of G-protein Signalling (RGS,) is a way of increasing the diversity of plasma membrane domains and microdomans.
Many proteins which associate with caveolin proteins contain a caveolin- or caveolin-like- binding motif [13]. Le Clerc et al. published a study in 2002 in the journal Endocrinology examining the effect of Angiotensin II Receptor Type 1's (AIIR1) caveolin-like binding motif (CLBM) (_X_XXXX_XX_, where _ represents an aromatic amino acid residue) on AIIR1's signalling and trafficking properties. They mutated this binding motif by replacing each aromatic residue with alanine, a small, sterically unintrusive molecule. The mutated receptor was shown to be four-fold less effective at activating phospholipase C, indicating that the functional CLBM is required for proper signalling. The authors proposed that the CLBM could be acting as a site for nucleation of proteins involved in the regulation of function of AIIR1 [13]. A similar study by Tomohiro Yamaguchi and colleagues [14] examined the effects of interaction of endothelin type A and B (ETaR & ETbR, respectively) receptors with caveolin-1. It was found that ETbR only interacted with caveolin-1 in the absence of an agonist, or bound to the antagonist RES-701-1. When endothelin-1 or another antagonist BQ788 were added, the complex dissociated. ETaR, however, bound to caveolin-1 irrespective of whether a ligand was bound or not. Additionally, overexpression of caveolin-1 dramatically increased the amount of ETbR localised to caveolae, while addition of endothelin-1 reduced caveolar localisation. Disruption of caveolae by filipin reduced the effect of endothelin-1 agonism on ERK1/2 phosphorylation [14].

Taken together, the concepts and experimental observations described here provide the framework for a GPCR signalling platform that is heavily based around spatial compartmentalization of a network of interacting components. This has been called a 'receptosome' in some publications [4], and it is quite possible that these receptosomes are the functional unit of plasma membrane receptor signalling, like a cell is the functional unit of a tissue. Agnati et al. in a review publication called "On the molecular basis of the receptor mosaic hypothesis of the engram," suggest that signalling units such as receptosomes form mosaics on the pre- and post-synaptic membranes of synapses, and that these mosaics are the computational entity that actually decodes the neurochemical messages. They move on to suggest that the arrangement of these mosaics of receptosomes could form 'supramolecular networks' that store information about the previous activity patterns of the synapse. While it is important to note that not all GPCR related signalling machinery is congregated into lipid rafts, it is likely that the receptosome theory applies to the majority of GPCR signalling, principally because compartmentalisation of signalling proteins makes such good sense kinetically.

Componentry and organisation of the receptosome: GPCRs & GPCR Interacting Proteins

Having identified general features of the receptosome and the logic behind organising signalling machinery this way, this section of the review will discuss the main groups of proteins that are likely to be part of the receptosome network and their functions.

I will address the questions of what these proteins are, where and how they interact with each other, and why these interactions are fundamental to GPCR / heptahelical transmembrane receptor signalling. Several protein-protein interaction domains such as PDZ, SH2 and SH3 domains are common in receptosome proteins, and the roles of these domains in protein interactions will be highlighted where appropriate.

G-protein Receptor Kinases:

It has been observed experimentally for decades that GPCRs undergo desensitisation and subsequent internalisation under repeated agonist stimulation. The first event in this process is usually phosphorylation of the receptor. There are at least two methods which the cell uses to perform this function: phosphorylation by second messenger activated kinases such as Protein Kinase A (PKA), and phosphorylation by non-second messenger dependent G-protein coupled Receptor Kinases, which are specific to activated GPCRs. The former is an example of 'heterologous' desensitisation, whereby agonism of one receptor can result in activation of PKA and subsequent phosphorylation and desensitisation of another receptor [1]. This effect is usually weak and short lasting, and the phospho-residue is not a target for arrestin binding. GRK mediated desensitisation is termed 'homologous,' since agonism of a receptor induces desensitisation of the same receptor [1]. Unlike phosphoresidues created by PKA or other second messenger activated kinases, GRK will phosphorylate different sites, and these phosphoresidues are targets for arrestin binding. Once arrestin is bound, various events occur, most importantly blockade of GPCR access to G-proteins. It is not the actual phosphorylation event that desensitises the GPCR in this case, but arrestin binding. GRK mediated phosphorylation was first discovered in the context of rhodopsin-dependent visual signalling, and later, beta2adrenergic receptor signalling. Since then, it has been established that the majority of GPCRs are desensitised in this way [15].

Arrestins:

Arrestins have been known to interact with GPCRs for a relatively long time, and their function was not particularly difficult to fit into the classical GPCR signalling dogma. Arrestins bind, as described previously, to GRK-phosphorylated GPCRs, and for a long time it was thought that arrestins were only involved in desensitisation and internalisation [4]. While some GPCRs internalise independent of arrestins, the usual scenario involves the bound arrestin attaching to clathrin – one of the major components of endocytotic machinery. Follow clathrin binding, arrestin acts as a scaffold protein and nucleates several other proteins to form the multiprotein complex that will effect receptor endocytosis. Other proteins identified in this complex include: AP2 (assembly particle-2,) a large (340kDa) protein that binds to the globular domain at the end of each clathrin heavy chain and function to promote clathrin triskelion formation and oligomerization into the cage that coats membrane invaginations to form clathrin coated pits [16], NSF (n-ethylamide sensitive factor), an intracellular trafficking protein, ARF6, an ADP-ribosylation factor and its exchange factor ARNO, which together regulate vesicle budding. Additionally, arrestin-2 can act as signalling intermediates, and attaches to multiple of the tyrosine kinase c-Src, including its SH3 and SH1 domains to activate

MAPK pathways [11]. Arrestin-2 also has an ERK1/2 phosphorylation dependent regulation site at residue Ser-412 which modulates c-SRC and GPCR binding [11]. There are three beta-arrestin subtypes: arrestin-1, 2 & 3, each with different binding specificities and signalling functions. Arrestin-1 is specific to the visual GPCR rhodopsin, while arrestin-2 has a much wider GPCR specificity, and while arrestin-1 is dimeric, arrestin-2 exists as a monomer in solution [11]. These varying characteristics of arrestins add to the overall specificity and complexity of GPCR signalling.

RGS's:

Regulators of G-protein Signalling, or 'RGS' proteins play a crucial role in regulating the function of G-proteins, and therefore in the signalling efficacy of the receptor system. There are, like many other GIPs examined in this review, a number of members of the RGS family, each with their own G-protein subunit specificity. The mammalian RGS family comprises several subfamilies, termed: Rz, R4, RA, R12 and R7, which are classified on the basis of structural and sequence homology. RGS proteins contain an RGS box which allows them to interact with activated G-alpha subunits and increase the rate that the G-alpha subunit hydrolyses GTP to GDP. The net effect of this interaction is to reduce the time that the G-alpha subunit actively signals to other proteins. As well as their characteristic RGS box domains, RGS proteins often contain other protein-protein interaction domains such as PDZ domains on RGS-R12 members. These protein-protein interaction domains make RGS proteins the target of considerable research efforts because of the implication that RGS proteins can, like arrestins, act as signalling intermediates as well as their role in regulating G-alpha signalling. For example, RGS proteins containing the RBD domain have been shown to initiate MAPK signalling [17]
The roles of RGS proteins in mu-opioid receptor signalling have been quite extensively studied, and examples of these studies are demonstrative of general RGS function. RGS2 and RGS3, for example, increase opioid agonist potency, while RGS4 and RGS16 reduce the potency of agonists. It is not known whether RGS2 and RGS3 actively reduce the rate of G-alpha GTP hydrolysis, or whether their effect is mediated by one of their other protein-protein interactions [18]. Experiments in which RGS9-2 is knocked out show increased response to Mu-opioid agonists and impaired desensitisation [18]. Garzon et al. in a 2004 paper [17] demonstrated that morphine "alters the selective association between mu-opioid receptors and specific RGS proteins in mouse periaqueductal gray matter," and in pull-down assays, they noted that certain proteins increased or decreased in their association with mu opioid receptors. It is possible that this may be something to do with morphine altering the receptor conformation and subsequently the network of proteins, particularly G-proteins, which interact with it. RGS proteins have selectivity for specific G-proteins, and if the group of G-proteins present in the network changes, then the group of RGS proteins present would also be likely to change.

Homer:

Several metabotropic glutamate receptors, such as mGluR1a and mGluR5a & b, along with Ca++ permeable IP3 receptors, ryanodine receptors, TRP channels, dynamin II and

shank proteins contain the sequence (-PPxxFR-) which is a binding sequence for 'Homer' proteins. These Homer proteins, contain an enabled "VASP homology-like" domain which binds to the Homer binding sequence, and a C-terminal coiled coil domain which allows them to homo- and heteromultimerise. It is Homer's coiled coil interactions that allow the above proteins to form large complexes. A complex containing mGluR's, Homer proteins, TRP channels, ryanodine receptors and P/Q Ca++ channels, according to Bockaert et al. [4] would "constitute an ideal machinery for intracellular Ca++ release." Homer proteins act primarily as scaffolding for protein complex formation, but experiments inhibiting Homer activity by using Homer1a, which lacks the coiled coil domain and acts as a dominant negative form of Homer, have shown that Homer has regulatory effects on mGluR signalling and ryanodine channel function [4].

GPCR-GPCR Interactions:

GIPs are essential to the function of a receptosome, but it is important to note that GPCRs do not just interact with non-GPCR proteins: the recent literature [19] documents many experiments exploring GPCR-GPCR interactions, including homo- and hetero-oligomerisation. Oligomerisation of GPCRs can affect many properties of GPCR function or sometimes only one or none, depending on the particular oligomer. For example, heteromeric complexes of B2ARs and delta or kappa opioid receptors doesn't affect the pharmacology of either the adrenergic or opioidergic units, but profoundly alters the trafficking properties of the heteromer [20]. Again a familiar concept can be found in the nature of GPCR oligomerisation: signalling specificity and complexity are increased by a further level.

RAMPs:

The discovery of Receptor Activity Modifying Proteins or RAMPs revolutionised the field of GPCR signalling, because it demonstrated that not only could GIPs fine tune GPCR signalling, modulate trafficking and activate secondary signalling pathways, they could also turn a receptor into a completely different entity, with a totally different cognate ligand. There are three members of the RAMP family that have been identified so far, designated RAMP1, 2 & 3. As an example of RAMP function, RAMP1 can bind to the calcitonin receptor-like receptor (CL,) and convert it into a 'high affinity calcitonin gene-related peptide receptor." Alternatively, interaction of CL with RAMP2 or 3 produces an adrenomedullin receptor. RAMPs are now known to interact with the majority of GPCR Class II receptors, and are regulated heavily by physiological and pathophysiological processes. For example, RAMP2 and adrenomedullin mRNA are elevated in models of cardiac hypotrophy, and during pregnancy, progesterone causes upregulation of all three RAMPs. It is also thought that many of the orphan ligands which have been found (i.e. no receptor has been identified,) are ligands to GPCR-RAMP complexes, when the GPCR already has a cognate ligand in its non-RAMP complexed state [20].

'Magic tail' interacting proteins & PDZ Domains:

The C-terminal tail of many GPCRs contains a PDZ ligand, to which proteins with the PDZ protein-protein interaction domain common to many proteins involved in receptor signalling bind [21]. The protein PICK1 (Protein Interacting with C-kinase 1) is one such protein: by binding to the PDZ ligand motif of mGluR7a, PICK1 induces clustering of these receptors at presynaptic terminals. It is also proposed that PICK1 interaction with mGluR7a receptors mediates coupling to Ca++ channels [22].
The protein NHERF (Na+/H+ exchange regulatory factor) also contains PDZ domains, and controls the signalling properties of parathyroid hormone receptor, which binds to NHERF by its PDZ ligand. PDZ-ligand mediated coupling of NHERF to B2AR's and kappa opioid receptors is likely the way that NHERF controls the Na+/H+ exchanger protein [21,23].
PDZ-ligand interactions between the PDZ domain of cyclic nucleotide Ras guanine exchange factor and the PDZ ligand on B2A enables B2AR to activate Ras and the associated MAPK pathway [21].
PDZ-ligand interactions also play an important role in receptosome scaffolding. The protein Shank spatially organises receptors and ion channels and provides interaction between receptors and the cytoskeleton [21]

Methods: Proteomics and experimental determination of protein-protein interactions

The dawn of the new millennium has seen the development of high throughput methods which generate vast amounts of novel data on protein-protein interactions. A number of different methods have been used to generate this data, all with their respective advantages and limitations. Use of different methods, or even variations of conditions within methods, can produce conflicting data sets. Appropriate synthesis of data sets produced by different methods is required to produce a coherent 'map' of interactions.

Researchers studying protein-protein interactions have a large toolbox of methodologies at their disposal. These include complementation assays, mass spectrometric approaches, chip based methods and bioinformatic analysis. The nature of the data produced by various methods differs: data can be qualitative or quantitative, and can describe pairwise interactions between two interaction partners, or can describe grouped interactions within a complex. The inability of most methods used to investigate large scale interactomes to measure quantitative information about interactions such as kinetics raises an important question: what exactly constitutes an interaction? Some biologically relevant interactions may occur on short timescales with very low affinity, but might be considered irrelevant by, or be below the sensitivity of such methods [25].

One issue that is particularly applicable to the study of protein-protein interactions occurring in receptosomes, and particularly interactions with membrane bound proteins, is the difficulty of resolving hydrophobic proteins in 2D gels [25,26]. Modern two-dimensional liquid chromatographic techniques have been able to provide improved resolution of hydrophobic proteins but preparation of pull-down assay experiments still proves difficult with membrane proteins [26]. One of the other important problems in GPCR and GIP interaction analysis is the low cellular concentrations of these proteins. If

the experimenter chooses to overexpress a particular GPCR or GIP, they run the risk of ruining the stoichiometry of the interaction network [26].

One particularly effective method of analysing protein complexes is called SEAM, which stands for Sequential Epitope tagging, Affinity tagging and Mass spectrometry. In this process, a protein is selected and epitope tags such as Myc are fused to one of its termini. It is then overexpressed in a cellular system of choice and the cell lysate is run through an affinity column where anti-Myc antibodies are attached to the beads. A second mixture of proteins is then run through the column, and those proteins that are bound to the epitope tagged proteins are resolved by 2D liquid chromatography and fed into a mass spectrometer for identification. Subsequently, one of the MS identified proteins is then Myc tagged and the procedure run again. In this way, it is possible to build up complexes of proteins [27].

Obviously a vast amount of information has to be gathered regarding protein-protein interactions between components of signalling machinery before any kind of mathematical modelling process can be applied to these networks. First, it is necessary to determine the stoichiometry of each complex, and the precise interactions of each protein with each other. It would also be exceptionally useful to have the crystal or NMR structures of each protein involved. Additionally, having identified the qualitative aspects of the system, quantitative biophysical data would be needed concerning the strength and kinetics of interactions. This task will be a massive undertaking, but eventually researchers will be able to build these mathematical models of GPCR signalling and incorporate them into pre-existing models of human brain function, such as the Blue Brain project that is being run on IBM's Blue Gene supercomputer ([url]http://bluebrainproject.epfl.ch/[/url]). Once GPCR and GIP interactions can be comprehensively modelled, the potential for drug design targeted to, and therapeutic intervention of these systems will be unprescendented.

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Drug Glossary

14BD: A GHB pro-drug, largely devoid of intrinsic activity.

2C-X: Chemicals such as 2C-B, 2C-I, 2C-E and 2C-T-7. These substitued phenethylamine hallucinogens have had little research devoted to them and nothing is known about their binding properties, though it is almost certain that they will be potent 5-HT2A receptor agonists. There is one paper show that 2C-I/B/D/H are 5-HT2A receptor antagonists, but this was demonstrated in a system which has little relevance to the complex nature of 5-HT2A receptor activation in the brain.

5-MeO-DMT: A tryptamine hallucinogen. It's hallucinogenic properties are attributed to its 5-HT2A receptor agonist effects though it is also a potent 5-HT1A receptor agonist.

Alcohol: See ethanol

Amphetamine: Cause the noradrenaline, dopamine and serotonin reuptake transporters to work in reverse (in that order of potency). Theses transporters normally take their respective neurotransmitter out of the extracellular fluid surrounding neurons and prevent them from binding to receptors, amphetamines cause the transporters to work in reverse, and move their neurotransmitters out of the cell, into the extracellular fluid. The action of amphetamines is dependent on them amphetamine passing through the transporter, hence the action of amphetamine is blocked reuptake inhibitors (like some antidepressants)

Benzodiazepines: A collection of pharmacologically and chemically related compounds which bind to the GABA-A ion channel, but at a site seperate to the GABA binding site. Benzodiazepines increase the affinity of the GABA-A receptor for GABA, and hence potentiate GABAs action.

Cannabidiol: Although often refered to as "non-psychoactive", cannabindiol is definately active. It has been shown to inhibit the anxiety inducing effects of THC [1] and be neuroprotective in many models of neurodegeneration. It has been consistantly shown that cannabidiol does not act on the CB1 receptor. It has been shown that cannadidiol acts by inhibiting the uptake and breakdown of the endogenous cannabinoid anandamide, but even if this action is replicated in vitro, the excess anandamide can not be acting on CB1 receptors. It has been hypothesised that Cannabidiol acts on the as yet uncharacterised cannabinoid receptor(s) which are speculated to exsist.

Cannabinoids: Cannabinoids are any of the chemically unique components in cannabis, though generally refers to psychoactive components. The classical cannabinoids are Ä9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabadiol (CBD).

Cannabinol: Another so called "non-psychoactive" cannabinoid. Reports show that it has very little or no psychoactive effects, though it definately has some physiological effects through unknown, non-CB1 receptor mechanisms. One report indicates it potentiates

some of the effects of THC in humans[2].

Cocaine: Inhibitor of Dopamine, Serotonin and noradrenaline reuptake transporters, probably in that order of potency, leading to a higher level of these neurotransmitters in the extracellular fluid. Also blocks voltage sensitive sodium channels at low potency, which causes its local anaethetic action.

Codiene: A metabolic precursor of morphine (see opioids), converted to morphine by the liver enzyme CYP2D6.

DMT: One of the simplest members of the hallucinogenic tryptamine family. It's hallucinogenic activity is due to its agonist activity at 5-HT2A receptors, though it also has high affinity actions at 5-HT1A/D and 5-HT6 receptors, though not 5-HT1B receptors. It may have actions at other receptors, but these have not been studied at this time.

Ethanol: Alters the function of several ligand gated and voltage gated ion channels, including potentiating certain GABA-A receptors, certain nicotinic receptors, 5-HT3 receptors and glycine receptors while inhibiting NMDA receptors, voltage gated Ca2+, Na+ and K+ channels and certain nicotinic receptors. The most potent (and hence probably most important) actions of ethanol are potentiation of GABA-A receptor actions, inhibition of NMDA receptors, inhibition of voltage gated calcium channels and possibley potentiation of Nicotinic receptors.

Dextromethorphan: Classically known for its NMDA-receptor antagonist effects, it is actually a more potent serotonin reuptake transporter inhibitor. It also has significant potency for the sigma receptor. It is converted in the body, into dextrophan, which has a significantly higher NMDA receptor affinity.

GHB: Both an endogenous neurotransmitter and a recreational drug. GHBs highest affinity action is as an agonist at the GHB receptor, while it has a lower affinity action as an agonist at the GABA-B receptor. A lot of experimental results have indicated that the GABA-B receptor is the pharmacologically important target of GHB, but this is generally because the experimenters have used high doses of GHB and have recorded GABA-B dependent measures. It is likely that low doses of GHB in humans act primarily via the GHB receptor while higher, hynotic doses act via GABA-B.

GBL: A GHB prodrug, but as well is a more potent GABA-B receptor agonist

Heroin: See opioids

LSD: A prototypic indole hallucinogen. It's recreational, hallucingenic effect is largley due to its 5-HT2A receptor (partial) agonist effect. It also has marked affinity for 5-HT1A/B/E/F, 5-HT2B/C, 5-HT5A/B, 5-HT6, 5-HT7, D1, D2, D3, D4, D5, and alpha1A/B receptors. These effects on other receptors may explain LSDs unique potency and nature.

Ketamine: A dissociative anaethetic best known for its potent non-competative NMDA receptor antagonist effects. It has also been shown to be a sigma, 5-HT2[3], D2[3,4] and a relatively very weak (~20μM) kappa opioid receptor agonist (PDSP data)

MDMA: An amphetamine which is about ten times more specific for releasing serotonin and noradrenaline than dopamine, though it probably still causes significant dopamine release through the serotonin it releases activating 5-HT2 receptors on dopaminergic cells or cells which control the firing of dopaminergic cells. MDMA itself is relatively weak at the 5-HT2A receptor (100x weaker than its actions at monoamine transporters)[5]

Methamphetamine: An amphetamine which causes the release of noradrenaline, dopamine and serotonin (in that order of potency). Methamphetamine is probably more potent that amphetamine because it is less suceptable to metabolism and more rapidly pentrates into the brain.

Morphine: See opioids

Nicotine: Active chemical in tobacco. Nicotinic binds to a wide variety of nicotinic acetylcholine receptors (ligand gated Na+ channels). Nicotine binds with high affinity to α4β2 nicotinic receptor although evidence indicates that it is the (α4)2α5(β2)2 and α4α6α5(β2)2 nicotinic on dopaminergic neurons that causes the addictive profile of nicotine [6].

Nitrous Oxide: Like most gaseous anaethetics, it's actions are somewhat of a mystery. It is likely that nitrous oxide's analgesic effects are somehow caused by the release of endogenous opioids, though its dissociative action are probably a mix of actions on ion channels (like those mentioned for ethanol).

Opioids: Any drug which shares a significant pharmacological similarity with morphine. Distinguished from "opiates" which are chemicals found in opium. Pharmacologically, opioids which are used recreationally have potent Mu-opioid receptor agonist effects, however most are non-specific agonists are all opioid receptor subtypes.

Oxymorphone/oxycodone: See opioids

Salvia/Salvinorin: Salvia is a plant containing a large number of alkaloids and non-alkaloids, the most famous of which is Salvinorin A, which is a selective Kappa opioid agonist. It is believed that this is the mechanism for salvias psychedelic action.

THC: The architypal cannabinoid. A potent agonist at CB1 and CB2 cannabinoid receptors as well as actions which can not be atributed to either of those receptors.

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