Molecular Pharmacology and Function of Drug Targets in Addiction and Depression

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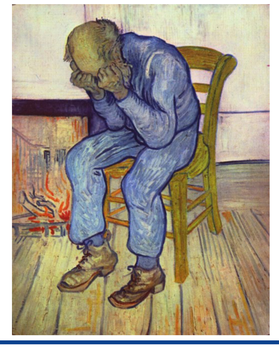
DEPT. OF FORENSIC MEDICINE

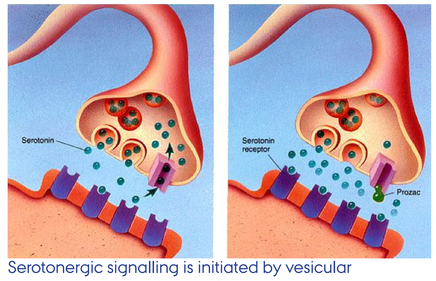
Depression

-300 million people worldwide are suffering from major depressive disorder (depression).
-Depression is the leading cause of diability worldwide.

-The serotonergic system is involved in mood regulation.
-Serotonergic signalling is terminated by the serotonin transporter (SERT) that mediates reuptake of serotonin into the preneuron.

-Antidepressants predominantly target SERT by inhibiting the transporter.
-MDMA ("ecstasy") causes release of serotonin by reversing the transport direction (efflux).





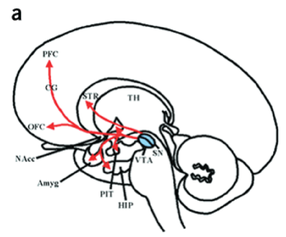
Serotonergic signalling is initiated by vesicular release of the neurotransmitter, serotonin. Signalling is terminated by reuptake via the serotonin transporter. Antidepressants inhibit the serotonin transporter.

Addiction

-247 million people worldwide are addicted to one or more drugs.
-In most developed countries addiction accounts for 5% of the total disease burden.

-The dopaminergic system is involved in reward.
-Dopaminergic signalling is terminated by the dopamine transporter (DAT) that mediates reuptake of dopamine into the preneuron.

-Cocaine predominantly targets DAT by inhibiting the transporter.
-Amphetamine causes release of dopamine by reversing the transport direction (efflux).

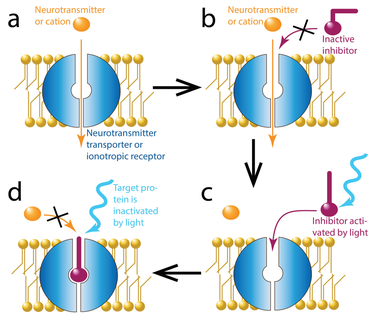


The neurobiological endpoint of all addictive drugs is release of dopamine in Nucleus Accumbens.

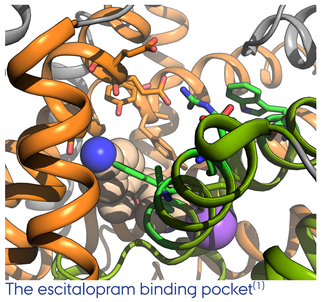
Photopharmacology - spatiotemporal control of drug action

-Photopharmacology utilizes the incorporation of lightswitchable chemical moieties in pharmacologically active compounds. If designed appropriately the ligand will undergo conformational changes in response to irradiation with light of a specific wavelength, switching between active and inactive states.

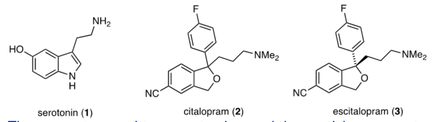
-Photopharmacological inhibitors can be activated at specific sites in the body at the desired time, which is useful to study the site of action and mechanism of drugs.
-Photopharmacological compounds can be developed to therapeutics that are only active at the desired site/organ/region to allow for higher local concentrations and fewer adverse effects.



The photopharmacology principle.

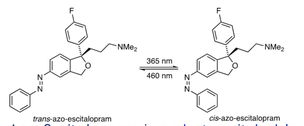


The escitalopram binding pocket[1] can be used for rational design of photoswitchable analogs.

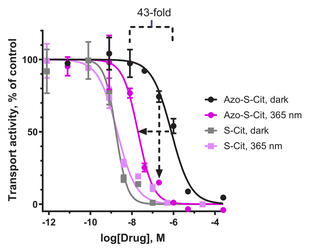


serotonin (**1**) citalopram (**2**) escitalopram (**3**)

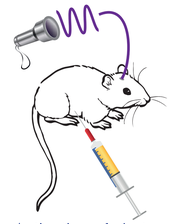
The neurotransmitter, serotonin, and the antidepressants citalopram and escitalopram.



Azo-S-citalopram is a photoswitchable derivative of escitalopram.



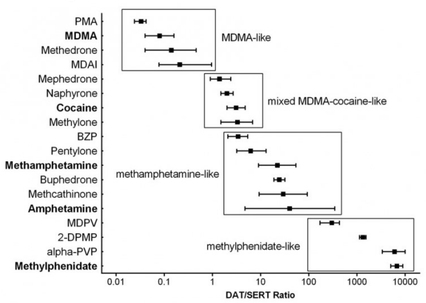
When photoswitched (365 nm) to the *cis*-conformation, Azo-S-citalopram achieves 43-fold more potent serotonin transporter inhibition.



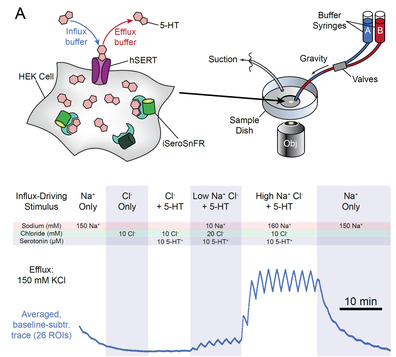
Activation of photoswitchable antidepressants by implanted optic fibres in specific brain regions can help identify the optimal site of action.

Pharmacology of novel amphetamine analogs

-To evade law enforcement and circumvent lists for controlled substances several designer drug variants of cathinones ("bath salts") and amphetamines ("ecstasy" variants) enter the illicit drug market.
-The pharmacological effects of these new designer amphetamines is generally poorly described.
-A good description of their pharmacology can be achieved by
1) describing their SERT/DAT inhibition profile
2) describing their ability to cause reverse transport (efflux) through SERT/DAT/NET.
-It has proven difficult to reliably quantify efflux with existing methods.We have developed a fluorescent neurotransmitter sensor protein that has allowed the establishment of a novel technological platform for characterization and classification of amphetamine pharmacology.

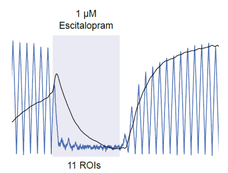


The DAT/SERT ratio is a key determinant for the psychotropic profile of amphetamine analogs.

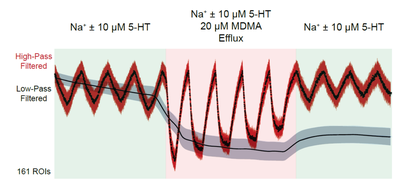


The Oscillation Stimulation Transporter Assay (OSTA)[2] relies on an intracellular fluorescence sensor protein to report on influx and efflux of serotonin (5-HT) through the co-expressed serotonin transporter (hSERT). Especially the efflux process is difficult to monitor reliably with existing methods.

Upward phase in the oscillations represent the influx process and downward phase represents efflux in OSTA. The ionic conditions to achieve influx and efflux reflects the ionic dependencies of the Na^+/Cl^--dependent 5-HT transporter, hSERT.



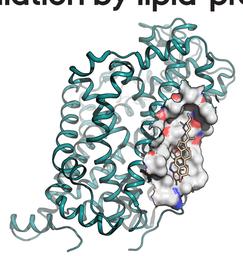
Extinction of oscillations (middle phase) is the hallmark signature of an inhibitor, here exemplified by the antidepressant Escitalopram. Oscillations recover after washout of the inhibitor.



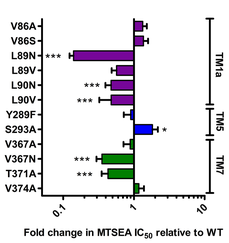
Contrary to inhibitors, releasers like MDMA ("ecstasy") enhance oscillations (middle phase) by causing efflux of 5-HT. Oscillations recover to normal levels after washout of the releaser.

Functional and pharmacological modulation by lipid-protein interactions

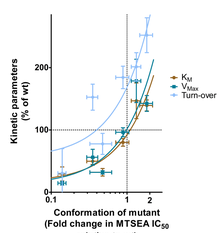
-Membrane protein function and pharmacology is shaped by specific interactions with the surrounding membrane environment.
-Specific lipids interact with specific binding sites on the membrane proteins to shape their conformational dynamics, activity and pharmacology.
-Lipids are heterogeneously distributed in the cell membrane and certain lipids are highly enriched in synapses. Lipid interactions may be a method to activate membrane proteins at the desired location.
-PIP_2 has been shown to modulate serotonin transporter response to amphetamine[3].
-We have shown that cholesterol binding to a specific site modulates the function of monoamine transporters[4,5,6].



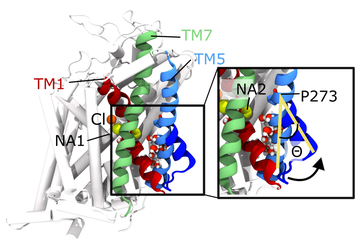
A putative cholesterol site is identified on hSERT between the flexible bundle domain and the scaffold domain[6].



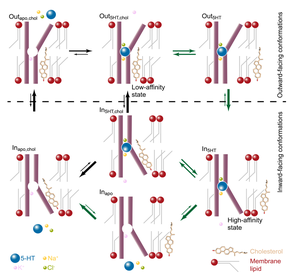
Mutation of the cholesterol site affects overall transporter conformation[6].



The conformational effect of mutating the cholesterol site translates to changes in key functional parameters[6].



CG-MD simulations show that without cholesterol bound the intracellular pathway is more prone to opening by kinking of TM5[5].



Model for how cholesterol binding and unbinding accelerates the conformational changes in transport (6).

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