MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Human Brain Project
Unifying our understanding of the human brain.

Principles of Neuropharmacology

Understanding the brain

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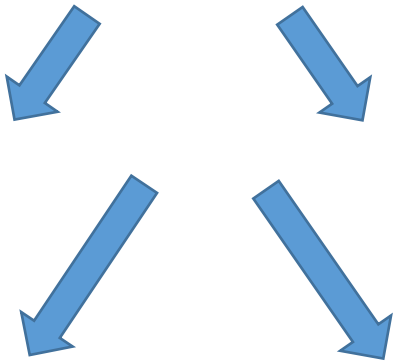
Contents

Neuropharmacology goal

„Apply information about **drugs and their mechanisms of action** to develop safer, more effective treatments and eventually curative and preventive measures for a host of **nervous system abnormalities**“

Nestler E.J., McGraw Hill

Neuropsychopharmacology:
how drugs that influence nervous system functioning



Psychopharmacology:
the effect of drugs on psychologic parameters, emotions and cognition

Behavioral Neuropharmacology:
how drugs affect human behavior, including drug dependence and addiction

Molecular Neuropharmacology:
study of neurons and chemical interactions to develop drugs for neurological function

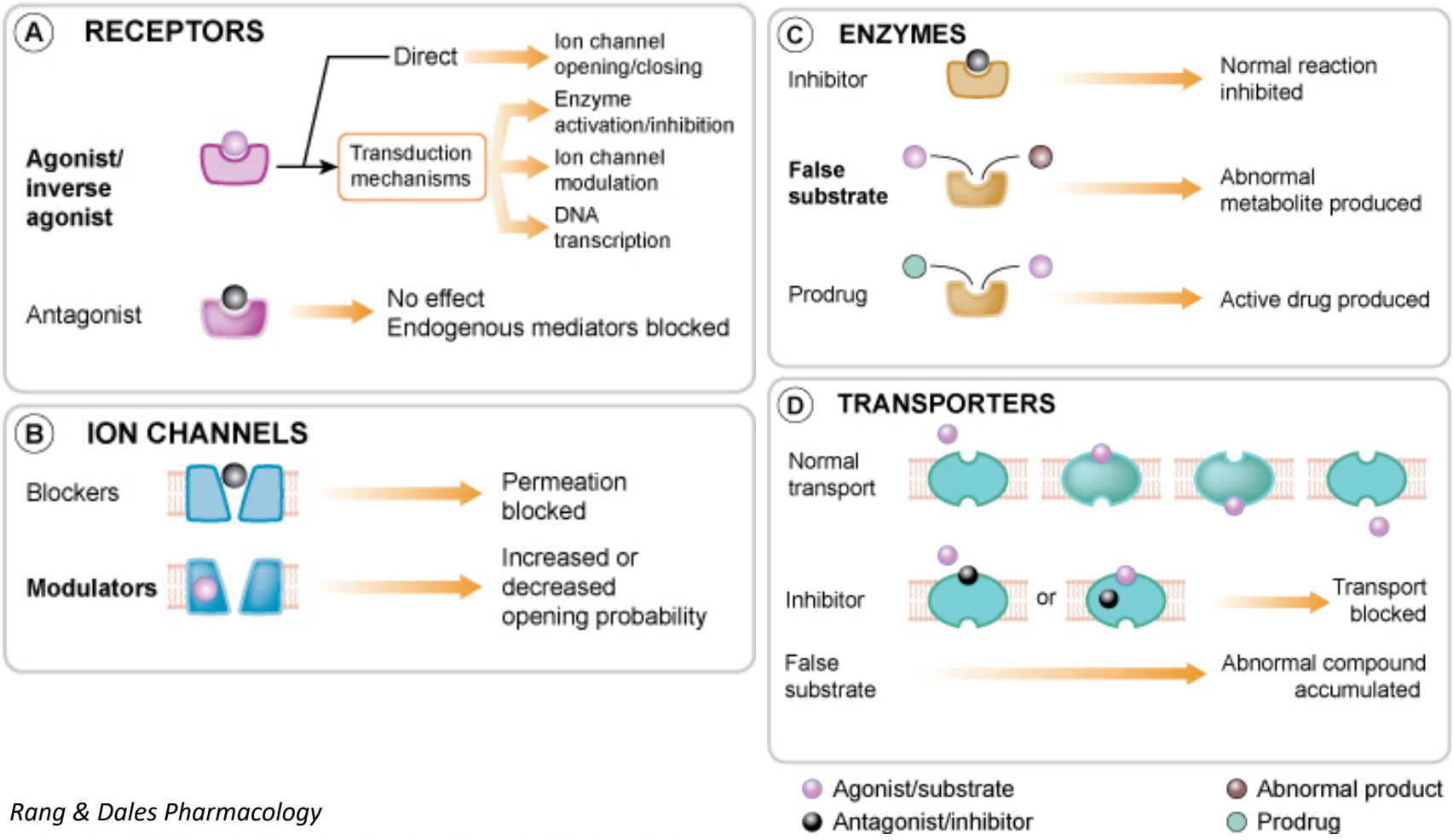
Pharmacodynamics (PD)

Pharmacokinetics (PK)

How drugs act on a living body, and which are the biochemical and physiological effects, dependent on drug concentration

Pharmacodynamics

Drug targets



Rang & Dales Pharmacology

Pharmacodynamics

Neuropharmacologic agents

Peripheral nervous system drugs

Central nervous system (CNS) drugs

Mechanism

Axonal conduction (non-selective drugs)

Synaptic transmission (highly selective)

Effect on synaptic transmission

Transmitter synthesis ②

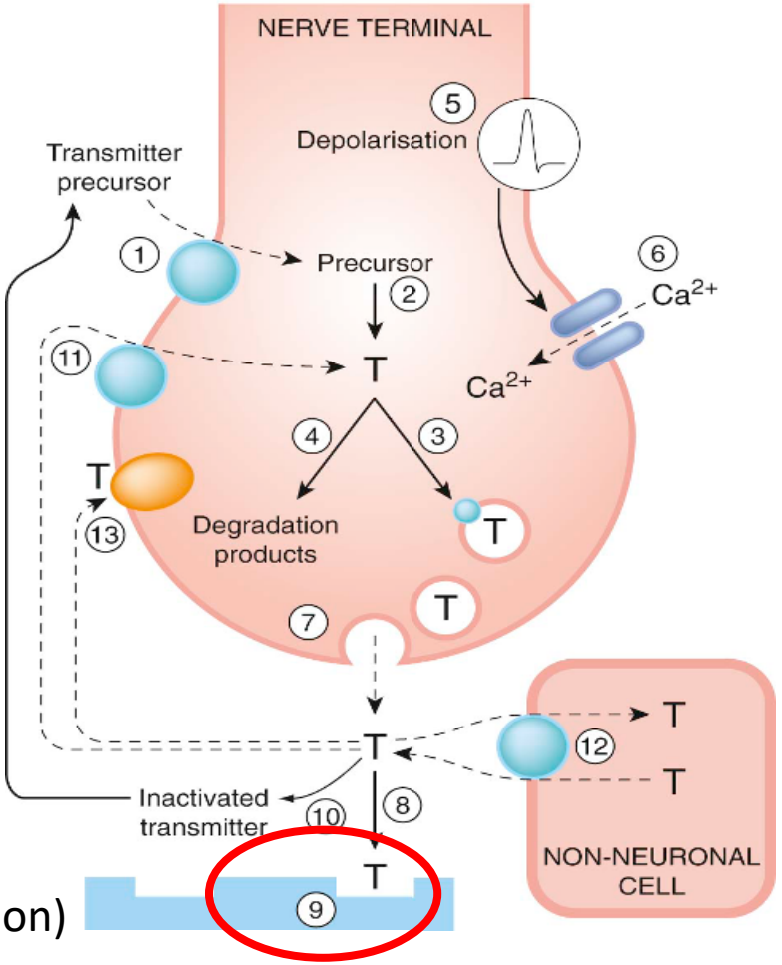
(increase, decrease or enhance)

Transmitter release ⑦

Receptor binding ⑨

(activation, enhancement or inhibition of activation)

Re-uptake ⑪



Rang & Dales Pharmacology,

Receptor binding

Specific, **saturable** and **stereoselective** reaction between a (**L**igand) and **R**eceptor

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} L\text{-}R$$ Equilibrium

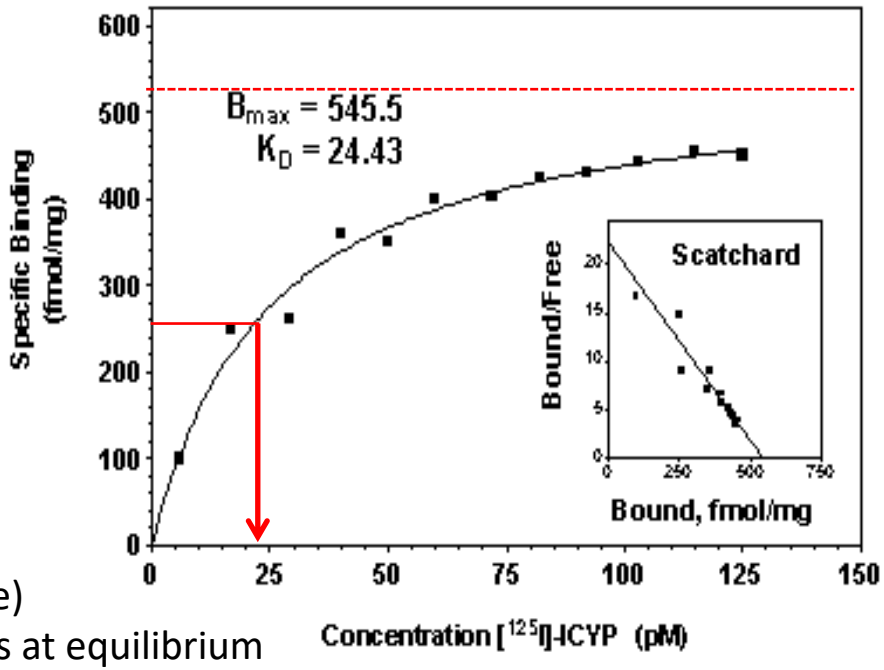
$K_D = k_{off}/k_{on} =$ [L][R]/ [L-R]

B (fractional occupancy) =

Bound receptors/total receptors=

[L-R]/[R]+[L-R] **= [L] / [L] +K_D**

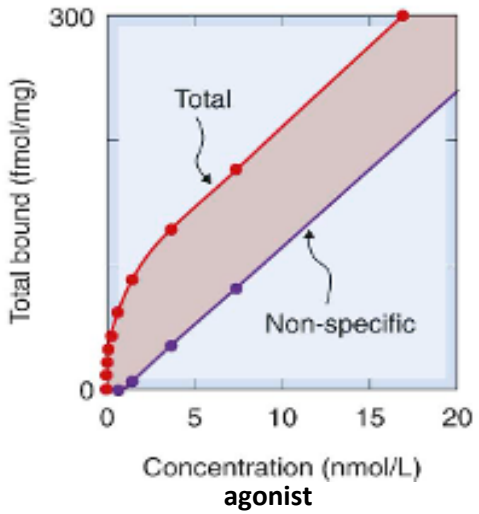
When [L] = K_D then B is 0.5



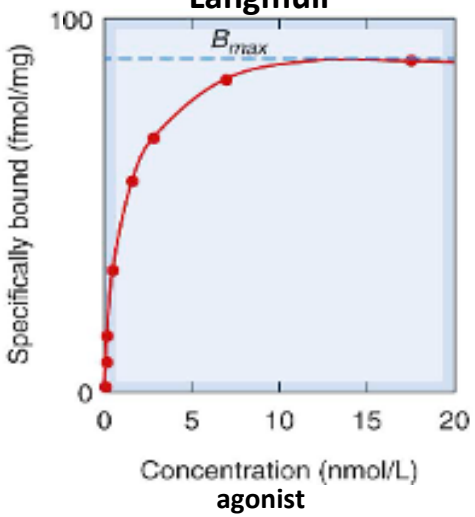
K_D (1/**affinity** = ability of a drug to bind a receptor site)
concentration of drug required to occupy 50% of sites at equilibrium
Bmax total amount of binding (extrapolation)

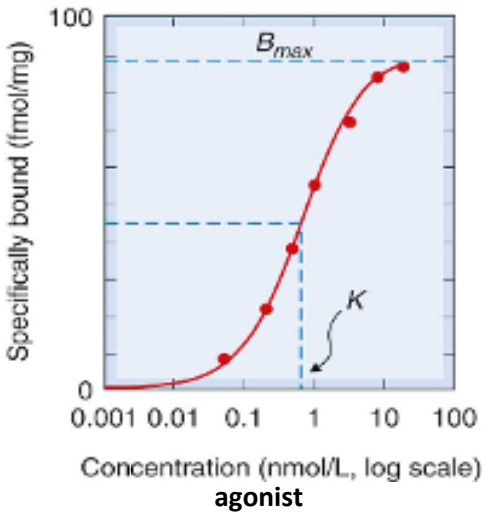
Binding studies do not give information on Receptor function

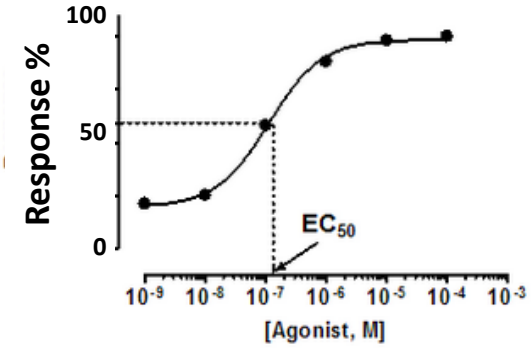
Receptor binding

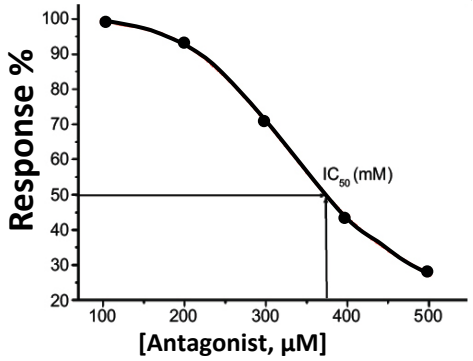


Langmuir



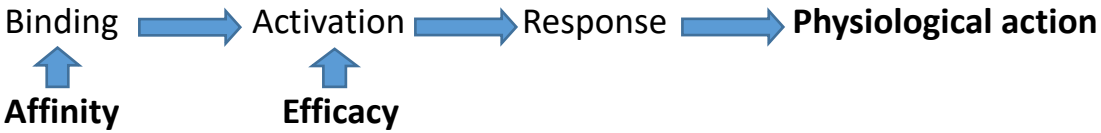




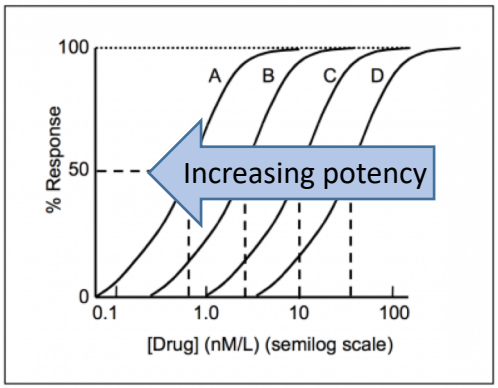


Rang & Dales Pharmacology

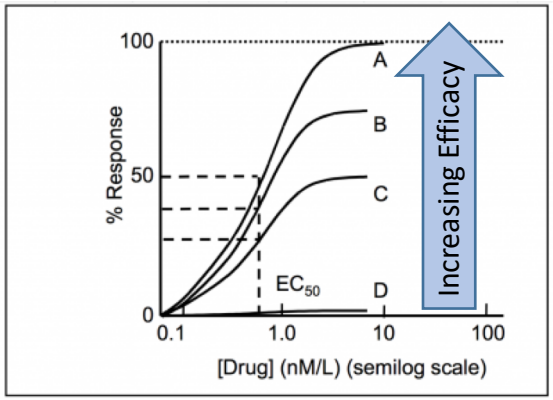
Receptor binding



Potency:
strength of binding drug-target
(comparison of drugs A...D effects)



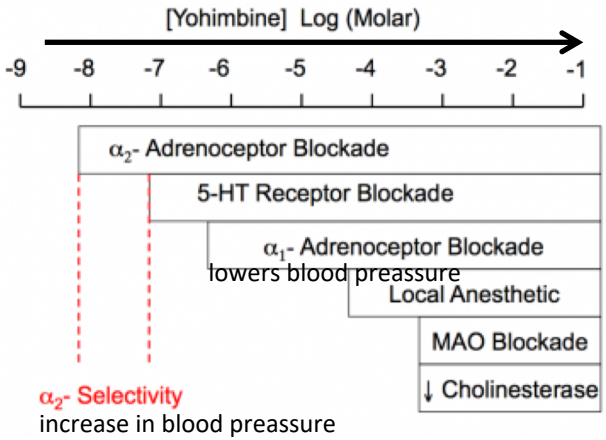
Efficacy:
maximal biological effect of an agonist



Selectivity:
if a drug binds only one receptor (**therapeutic window**)

Specificity:
able to alter a disease process while leaving other processes unaffected

Ex: Therapeutic window of Yohimbine

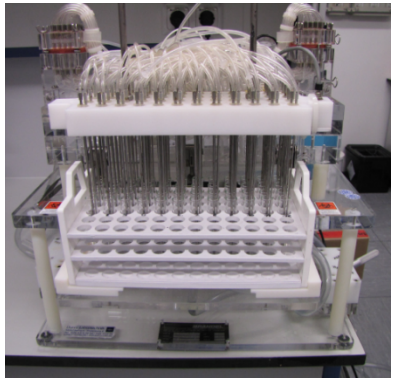


Taylor & Francis 1997; Lippincott-Raven, 1997; Churhill LIvingston, 1990

Receptor binding

In vitro:

Radioactive:
Cell harvester for
radioligand binding assays



Non-radioactive:

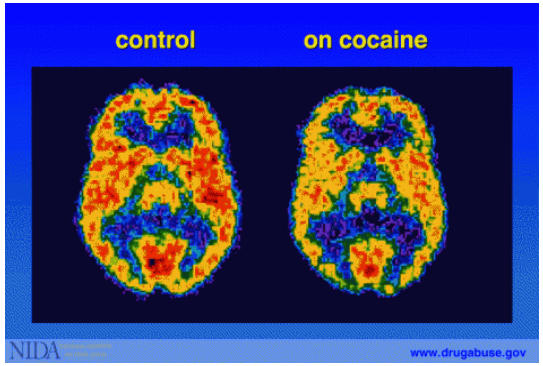
- Surface Plasmon Resonance

(SPR, Biacore) binding in real time

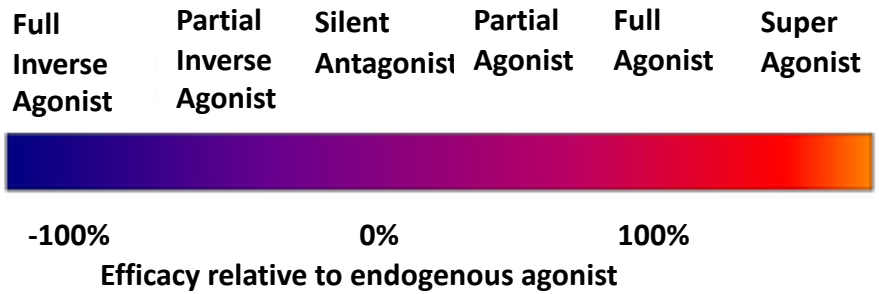
Biacore X100 GE Healthcare

- Fluorescence resonance energy transfer (FRET)
- Fluorescence polarization (FP)....

In vivo:
Positron Emission Tomograpy (PET)



Receptor binding ligands



Full agonist: binds to receptor and initiates or alters a cellular activity

Gamma-aminobutyric acid (GABA) to GABA-receptors

Superagonist: higher efficacy than endogenous agonist

Synthetic superagonists of Vit D nuclear receptor

Partial agonist: binds to receptor site but induces less celullar activity than agonist

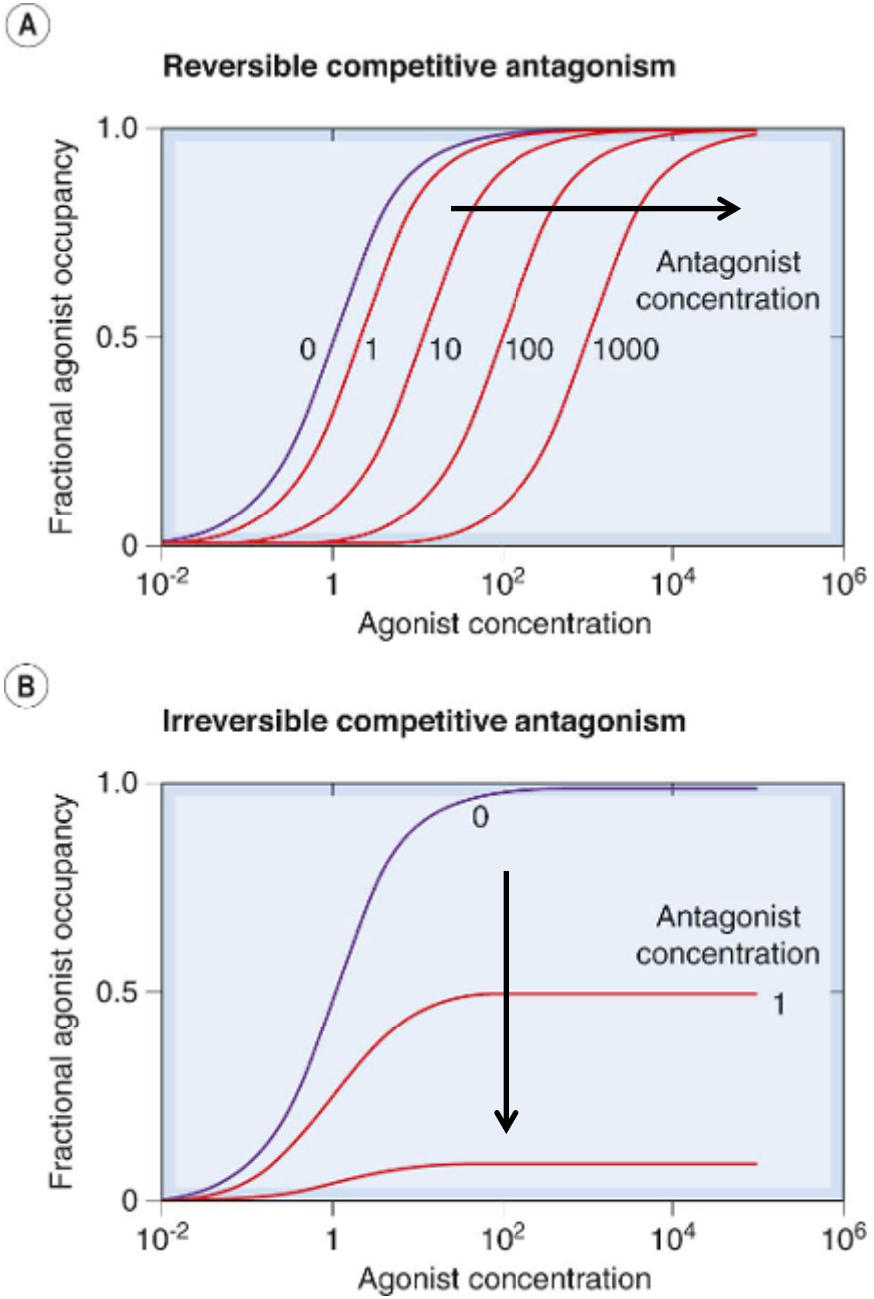
Gaboxadol (hypnotic), $GABA_A$-receptor

Antagonist: affinity for the receptor but does not stimulate it

Competitive: competes for agonist binding site. **Reversible** or **Irreversible**.

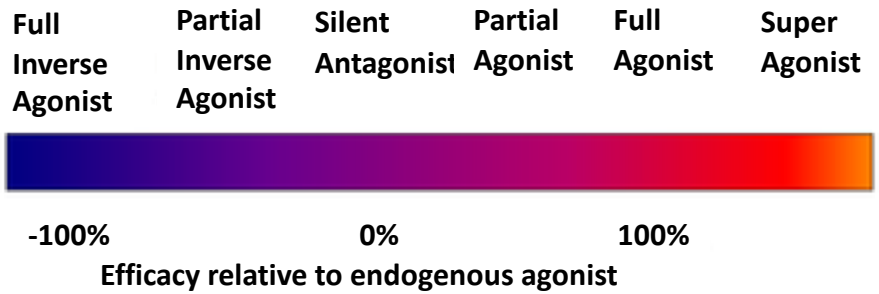
Non competitive: binds reversibly to another site which reduces the binding of the agonist (**allosteric binding**)

Receptor binding
Antagonism



Rang & Dales Pharmacology

Receptor binding ligands



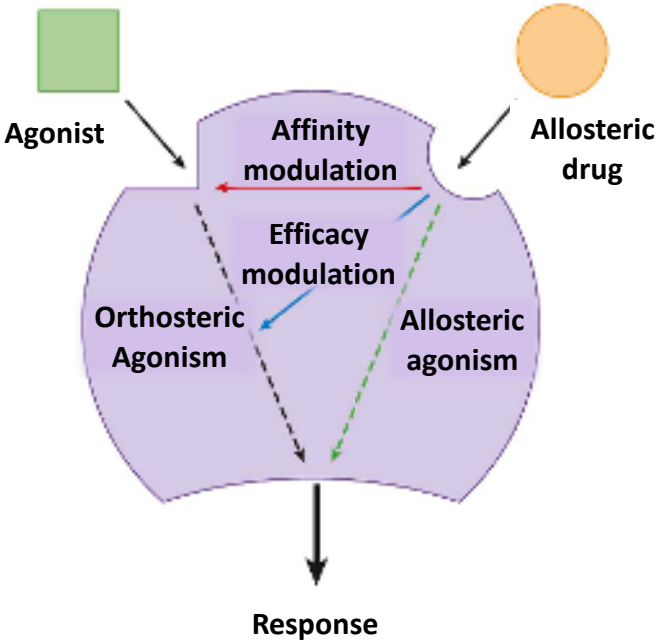
Inverse agonist: opposite effect to agonist, negative efficacy (there has to be some constitutive receptor activation)

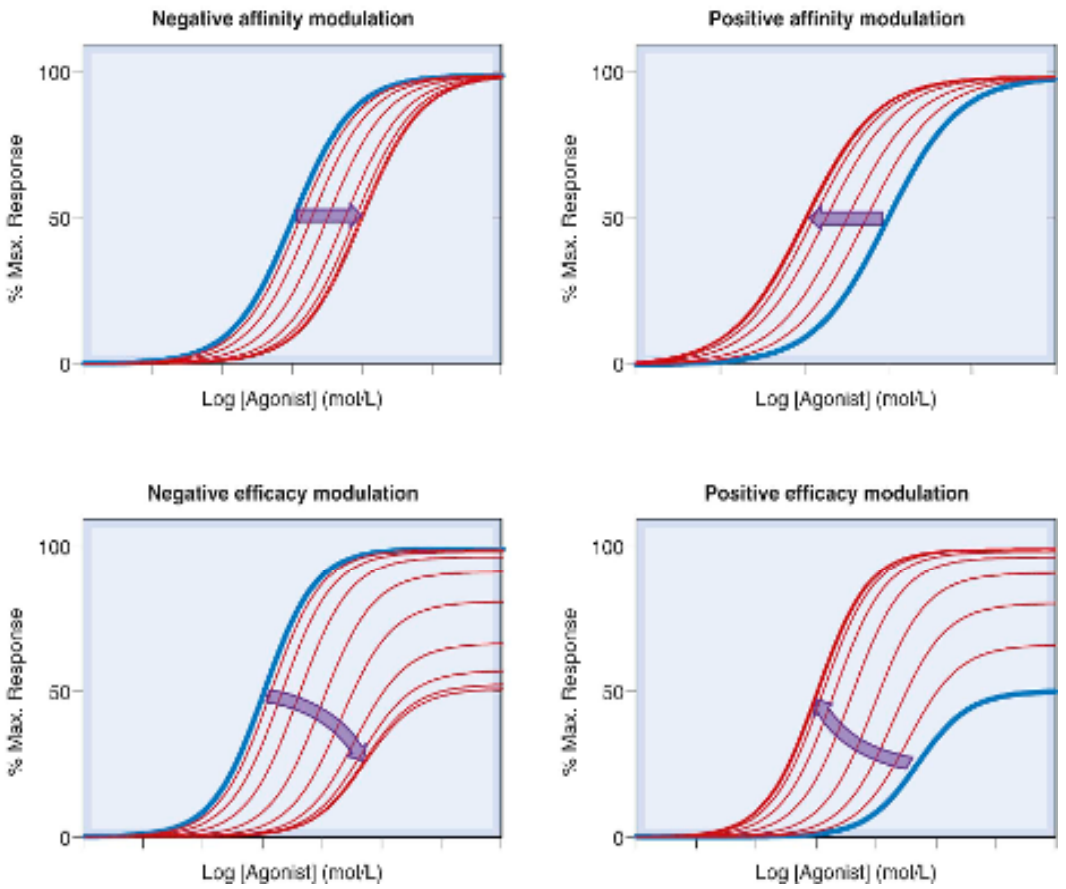
Convulsant benzodiazepines (RY023), $GABA_A$ receptor, no clinical use

Allosteric modulators: bind to sites in the receptor other than the agonist site, modify agonist activity

Benzodiazepines potentiate GABA effect

Receptor binding
Allosteric modulation





Rang & Dales Pharmacology

Receptor binding Complexity

$GABA_A$ receptor

Ligand-gated ion channel, postsynaptic, pentameric

GABA (gamma-aminobutyric acid) endogenous agonist (reduces excitability)

Muscimol agonist from mushroom (sedative-hypnotic)

Bicuculline antagonist (convulsant, epilepsy)

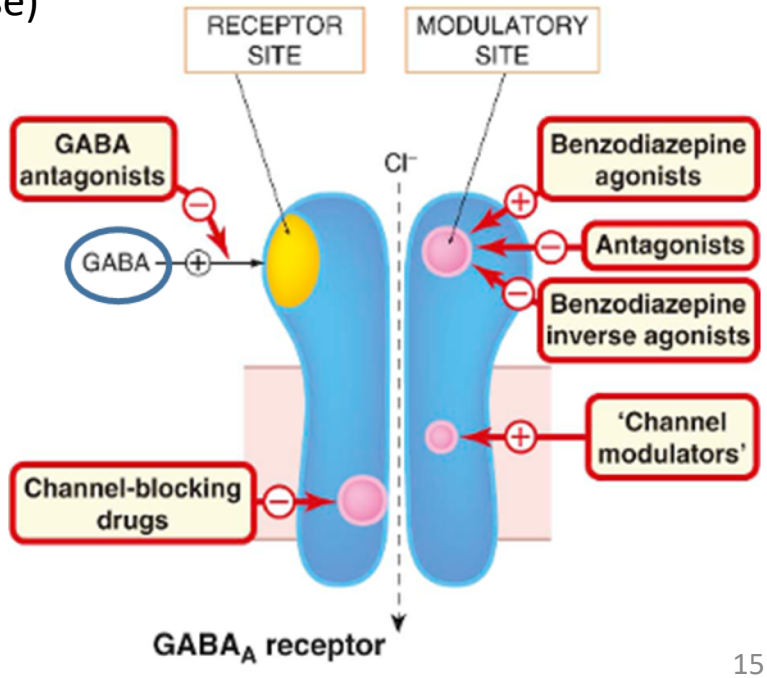
Benzodiazepines positive allosteric (anticonvulsant, sedatives)

Flumazenil benzodiazepine antagonist (overdose)

ß-carboline inverse agonist intensifies anxiety

Picrotoxin channel blocking (convulsant)

Others: ETOH, barbiturates, anaesthetic agents, neurosteroids, potentiate GABA effect



Rang & Dales Pharmacology

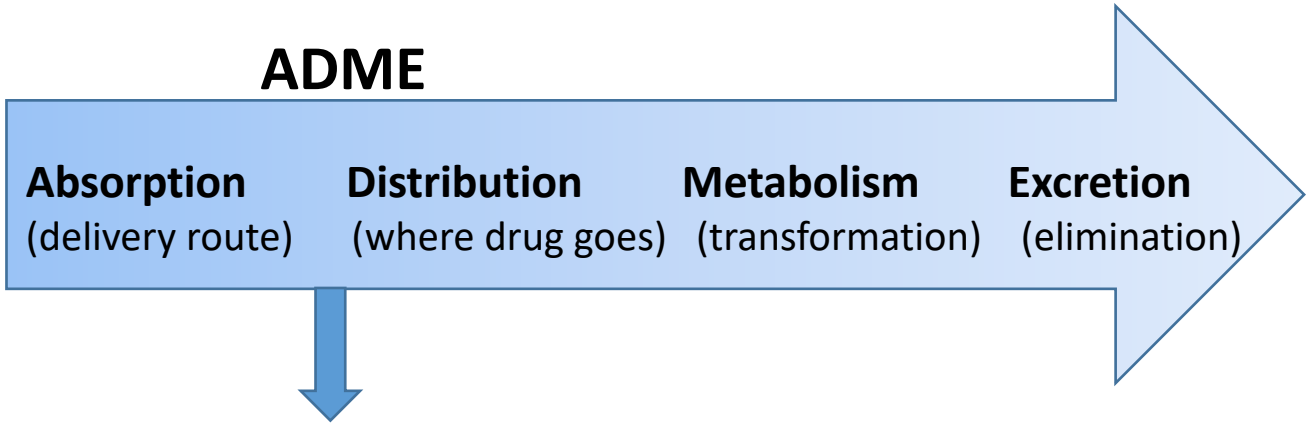
Pharmacodynamics (PD)

Pharmacokinetics (PK)

How a living **body acts on drugs**

“measurement of changes of drug concentrations in blood plasma along time in relation to dose as a result of absortion, metabolism, distribution and excretion”

Pharmacokinetics (PK)



Bioavailability: How much of the administered drug reaches its target

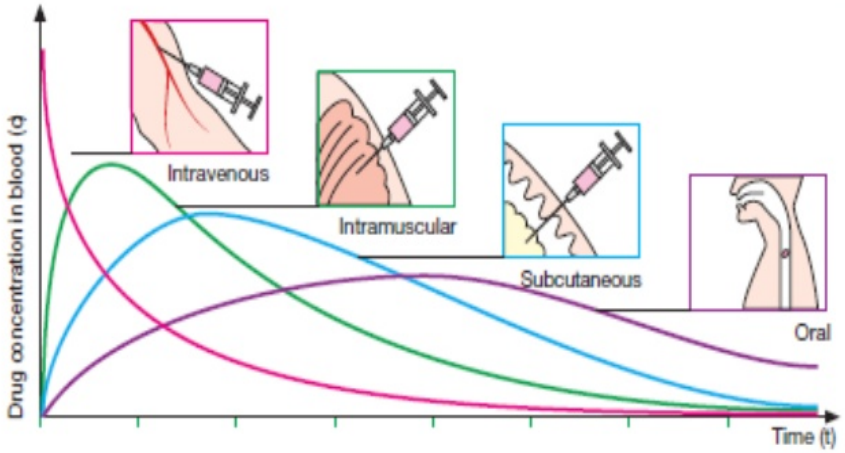
Pharmacokinetics (PK)

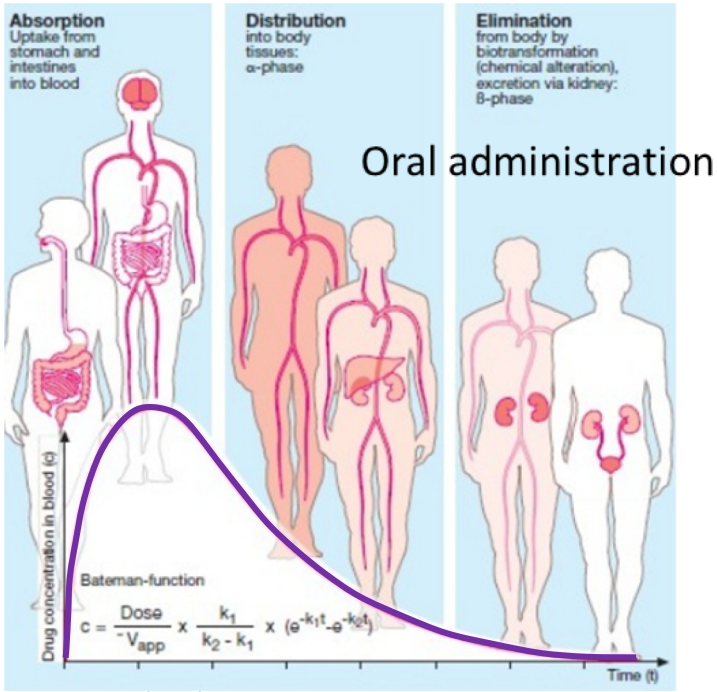
Bioavailability:

- Route of administration
- Dose
- Onset of action
- Peak action time and duration
- Frequency of dosing...

Clinical studies:

Therapeutic effect/adverse effects



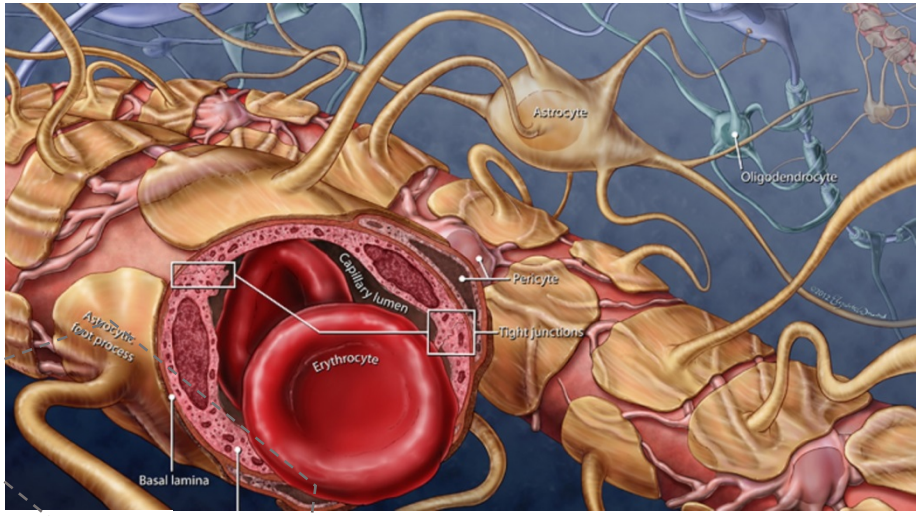


Modified from Cynthia Acosta, Abc.pharma

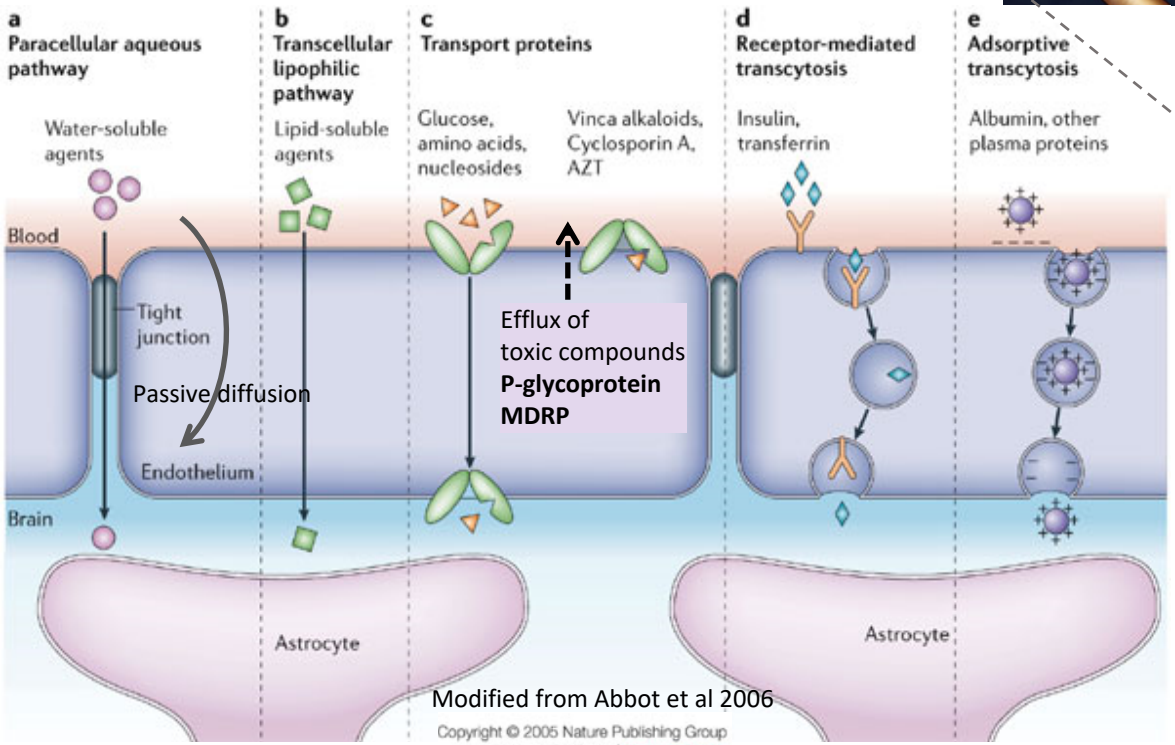
- Lipid: water partition coefficient
- Plasma protein binding
- Stability once absorbed...
- **Blood Brain Barrier (BBB)**

Blood Brain Barrier

"Highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the CNS"



loonylabs.org



Modified from Abbot et al 2006

Blood Brain Barrier
Drug delivery

> 98 % of small molecule drugs do not cross the BBB	~100 % of large molecule drugs do not cross the BBB	<1 % of drug companies have a BBB drug targeting program	<1 % of academic neuroscience programs emphasize BBB transport biology

> 1 KDa, peptides

Pardridge W. M., J. Amer. Soc. Exp. NeuroTherap., 2005

Physiological delivery

Modify drug that it may use endogenous nutrient transporters

L-DOPA transported by the Large-neutral amino acid carrier

Receptor-mediated transcytosis

ligand (or antibody against the receptor) conjugated to the drug

Transferrin receptor (e.g. transferrin coated nanoparticles)

Insulin receptor

LDL receptor

Blood Brain Barrier
Drug delivery

Pharmacological delivery

Drug modification

decreasing drug size
increasing lipophilicity
linking it to a lipophilic carrier (pro-drug)
Increasing stability and solubility: micelles, polymers

Blocking active efflux of P-glycoproteins: with certain L-type Ca^{2+} channel blockers

Disrupt BBB temporarily: Mannitol (change osmotic properties
e.g. chemotherapeutics; Oregon Univ. OHSU)

Neuropharmacology Trends
Drug development
FDA approvals

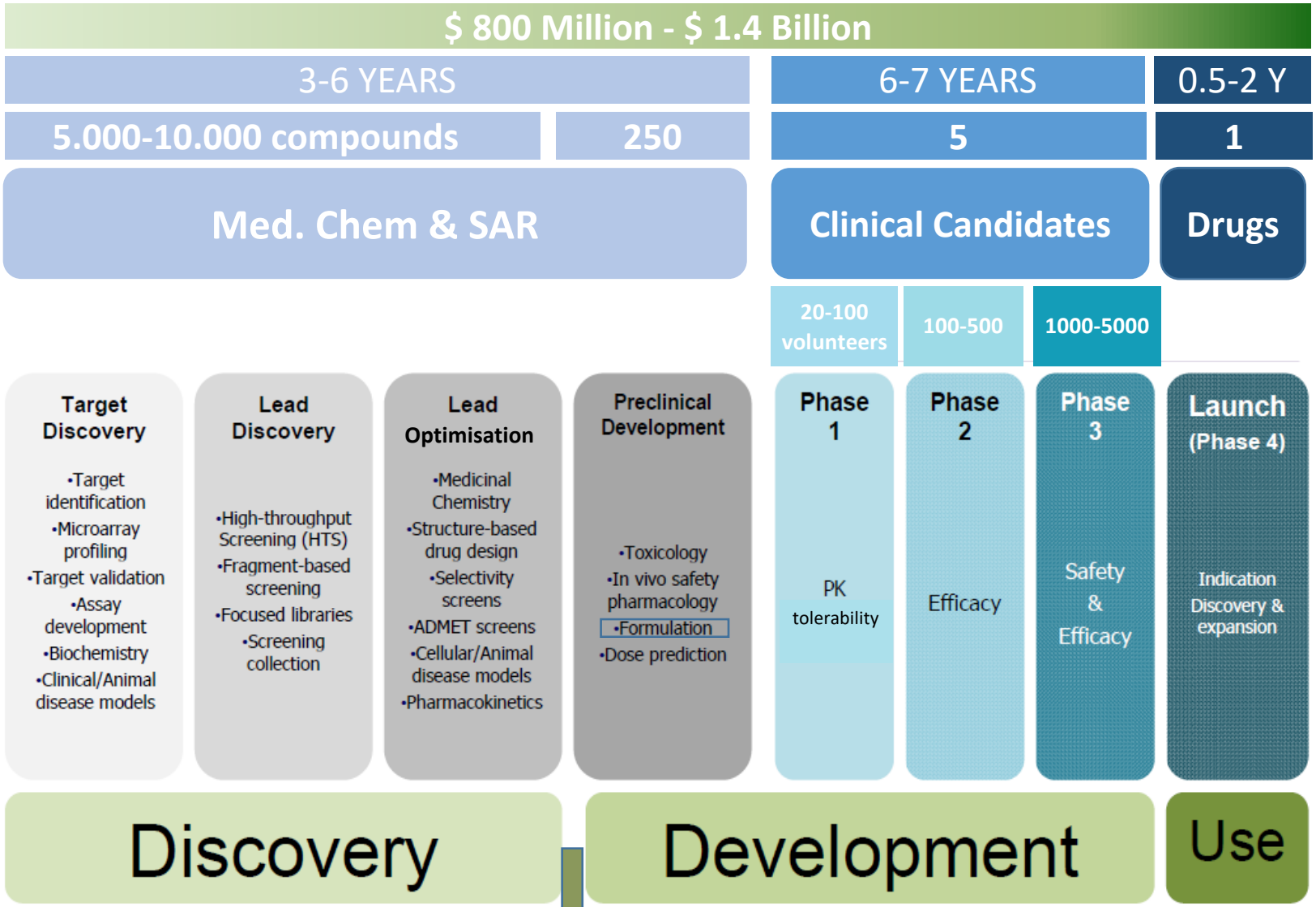
„In 2009, from 10.974 clinical trials, 250 targeted depression and more than 5.500 oncology indications “...

Griffin, R., National Academies Press, 2010

....6.7% people affected by depression in the US
... 50 million americans affected by neurological dissorders
... 600 aprox. neurological disorders
... 8% global health burden

Medicines in Development, 2015 Report

Drug development



Modified from J. Overington
„Learning from historical drug discovery data“ EMBL presentation

Drug development

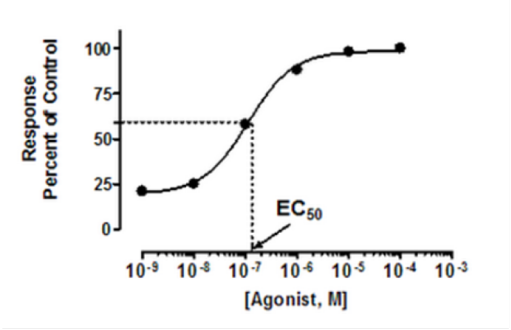
Discovery phase

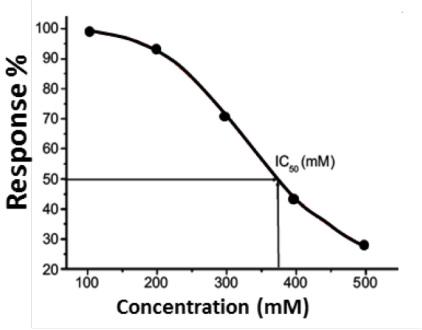
Target discovery and validation:

- Discovery of a biomolecule of interest and evaluate its potential as target
- Design a bioassay to measure biological activity (drug potency),
- Perform high-throughput screening to find hits
- Hit to lead (Medicinal Chemistry)

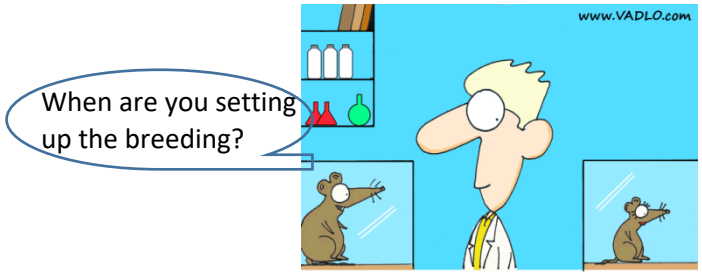
EC_{50} Concentration of agonist where 50% of its maximal effect is observed

IC_{50} Concentration of an antagonist where 50% of its maximal antagonistic effect is observed





Drug development
Pre-clinical Phase



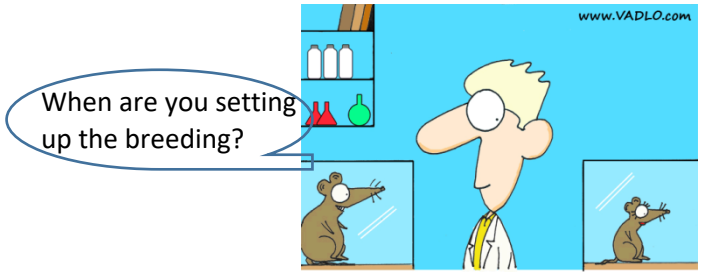
Non human studies necessary before getting **EMEA/FDA** permission for human studies

- **Pharmacological testing (safety pharmacology):** no cardiac dysrhythmias, blood pressure changes, ataxia
- **Preliminary toxicological testing:** maximum non toxic dose (for 28 days in two species)
  Post mortem examination: histological and biochemical signs of damage
- **PK testing:** To link pharmacological and toxicological effects to plasma concentration and drug exposure
- **Chemical and pharmaceutical development:** to assess feasibility of large-scale synthesis and purification, stability, formulation for clinical studies

EMEA: European Agency for the Evaluation of Medicinal Products
FDA: Food and Drug Administration

Drug development

Pre-clinical Phase



Parameters

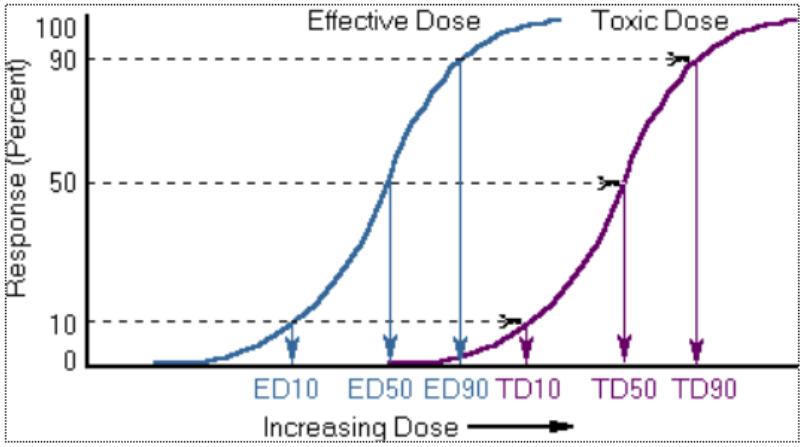
LD_{10} **Lethal** dose 10%: dose that causes death in 10% of animals tested (3R animal ethical use: **R**eplacement, **R**eduction and **R**efinement)

TD_{50} Median **toxic** dose: dose at which (or above) 50% of animals tested had toxic effects

ED_{50} Median **effective** dose: Minimum dose to show desired activity in half the members of a population after a specific duration of time

Therapeutic index It is a safety parameter
= LD_{10}/ED_{50} in animals
= TD_{50}/ED_{50} in humans

Large difference: <u>medication is safe</u>

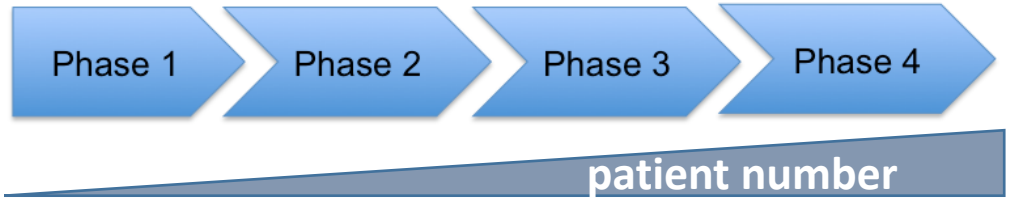


toxicologyschools.com

Drug development

Clinical Phase

Compounds are tested for efficacy, side effects and potential dangers in volunteers



Stage of Development	Phase 1 20-100 healthy/p	Phase 2 100-500 patients	Phase 3 10^3 patients	Phase 4 long term
End Point	Safety	Efficacy	Efficacy	Efficacy
Specific End Point	Safety Profile Tolerability	Cardiac Output	Reduction in Mortality Rate	Reduction in Mortality Rate
Types of Studies	Different Indications; Single or Multiple Dose	Placebo Controlled; Dose Escalation	Placebo Controlled; Long Term Follow Up	Comparative; New Indications

Open
Single-blind
Double-blind
Prevention
Unicenter
Multicenter
Parallel
Sequential....

Modified from M. Silverman
BioStrategics Consulting Ltd

General classification of drugs acting on the CNS

Class	Definition	Examples
General anaesthetic agents	Drugs used to produce surgical anaesthesia	Isoflurane, desflurane, **propofol**, etomidate
Analgesic drugs	Drugs used clinically for controlling pain	**Opiates** Neuropathic pain – carbamazepine, gabapentin, amitriptyline, duloxetine
Anxiolytics and sedatives	Drugs that reduce anxiety and cause sleep	**Benzodiazepines** (e.g. diazepam -Valium-, chlordiazepoxide, flurazepam, clonazepam)
Antiepileptic drugs Synonym: anticonvulsants	Drugs used to reduce seizures	**Carbamazepine**, **valproate**, lamotrigine
Antipsychotic drugs (antischizophrenic drugs)	Drugs used to relieve the symptoms of schizophrenic illness	Clozapine, **haloperidol**, risperidone
Antidepressant drugs	Drugs that alleviate the symptoms of depressive illness	**Selective serotonin reuptake inhibitors, tricyclic antidepressants**, monoamine oxidase inhibitors
Psychomotor stimulants (psychostimulants)	Drugs that cause wakefulness and euphoria	**Amphetamine, cocaine**, methylphenidate, **caffeine**
Psychotomimetic drugs (hallucinogens)	Drugs that cause disturbance of perception (particularly visual hallucinations) and of behaviour in ways that cannot be simply characterised as sedative or stimulant effects	Lysergic acid diethylamide (**LSD**), mescaline, MDMA (**ecstasy,** 3,4-methylenedioxymethamphetamine)
Cognition enhancers (nootropic drugs)	Drugs that improve memory and cognitive performance	Acetylcholinesterase inhibitors: donepezil, galantamine, **rivastigmine** NMDA receptor antagonists: **memantine** Others: **piracetam**, modafinil

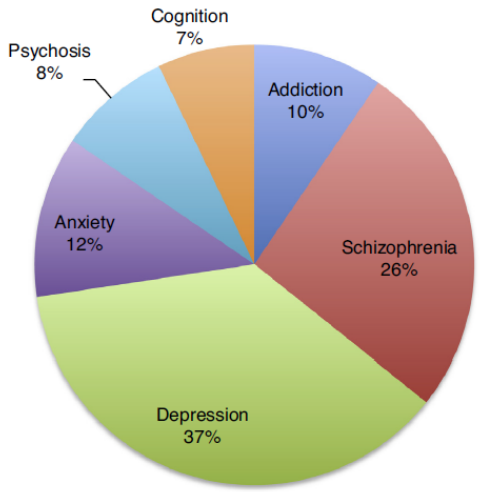
Modified from Rang & Dales Pharmacology

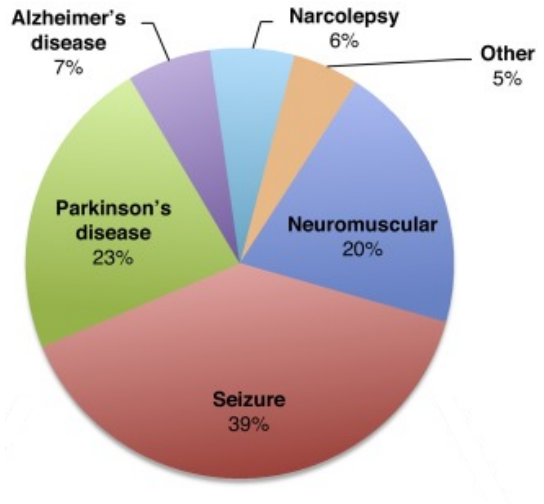
FDA approvals since 1950s

Psychiatric disorders: 78 NME

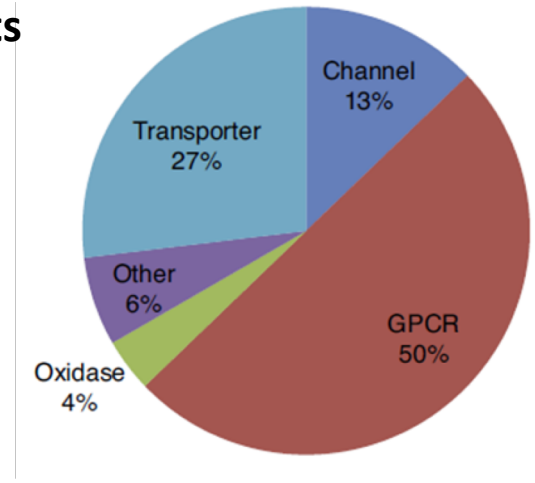
Neurological disorders: 79 NME

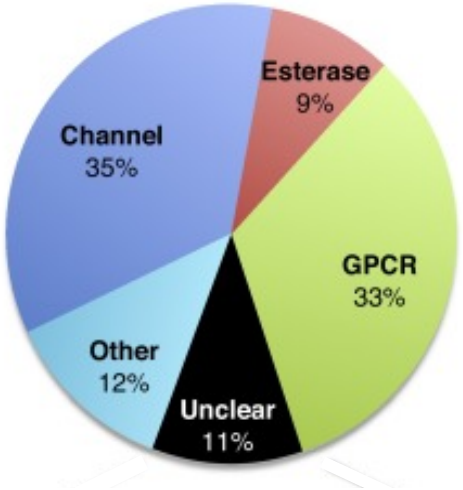
Prevalent indications





Targets

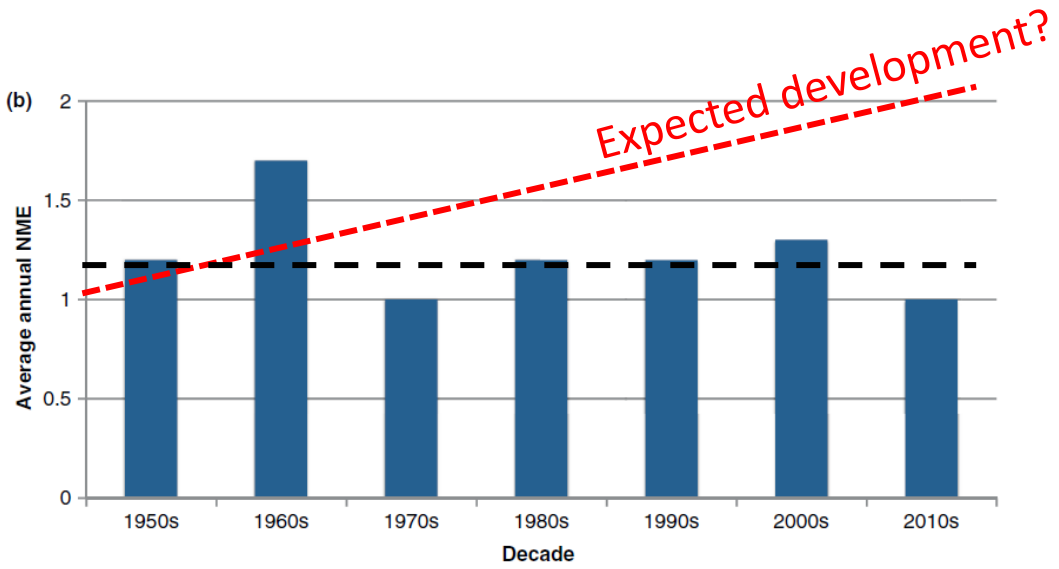




Kinch MS, Drug Discovery Today, 2015

NME: New molecular entities

FDA approvals in psychiatric disorders



Shortcomings:

- Small-molecule libraries composition biased (e.g. GPCRs, ion channels)
- More inhibitors and antagonists than agonists
- 7 of 15 agonist impact GABA-A receptor
- 13 of 20 reuptake inhibitors target the Serotonin transporter
- Narrow subset of existing animal models....

Modified from Kinch MS, Drug Discovery Today, 2015

Neurodegenerative Diseases

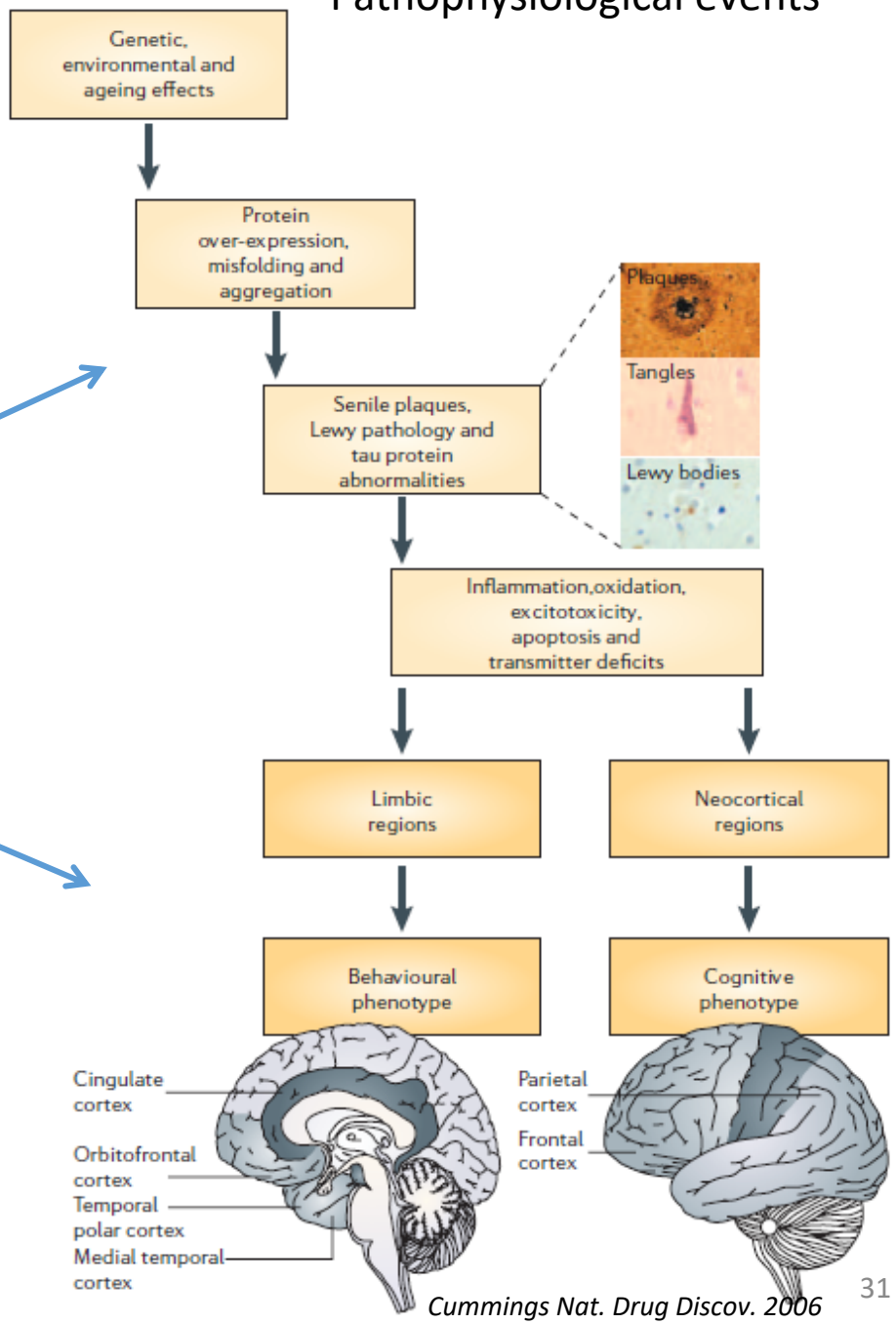
„Conditions characterised by progressive cell loss with abnormal production, accumulation or misfolding of proteins“

- **Disease-modifying drugs**

None

- **Symptomatic antidementia agents**
  cholinesterase inhibitors (galantamine)
  NMDA receptor antagonist (memantine)

- **Psychotropic drugs**



Cummings Nat. Drug Discov. 2006

Difficulties in drug discovery for CNS diseases

Complex diseases, single targets?
CNS disorders are **polygenic** with **environmental** and **epigenetic** components, **animal models** to validate drug discovery are difficult, **tissue availability**

...drugs that modulate more than one target (**non-selective**) are better?

Selective serotonin reuptake inhibitors: Increase the extracellular level of serotonin by limiting reabsorption into the presynaptic cell

Table 2 | **Antidepressants with complex modes of action are superior to single-action antidepressants**

Prototypical drug	Class	Mode*	Molecular target(s)	Phase of testing	Efficacy vs SSRI	Company
Electro-convulsive therapy	Somatic therapy	C	Undefined	In use for decades	Greater efficacy[58]	None
Imipramine	Tricyclic antidepressant	C	NET, SERT, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, α_1-adrenergic, muscarinic	In use for decades	Slight advantage[41]	Generic
Fluoxetine	Serotonin-selective reuptake inhibitor	S	SERT	In use for >10 years	N/A	Eli Lilly
Venlafaxine	Dual serotonin/ norepinephrine reuptake inhibitor	C	NET; SERT	In use 10 years	Slight advantage[41,42,59]	Wyeth
Pindolol	5-HT_{1A} partial agonist/SSRI combination	C	5-HT_{1A}	Several double-blind, placebo controlled clinical trials completed; both drugs approved for use	Combination > than SSRI alone in uncomplicated depression[60] but not in refractory or chronic depression[61]	Generic
Duloxetine	Dual serotonin/ norepinephrine reuptake inhibitor	C	NET; SERT	NDA submitted	Unknown; predicted to be >than SSRIs	Eli Lilly

Roth B. L., Nature Rev. Drug Discov., 2004 http://kidb.cwru.edu/

How to develop best „non-selective“ drugs?

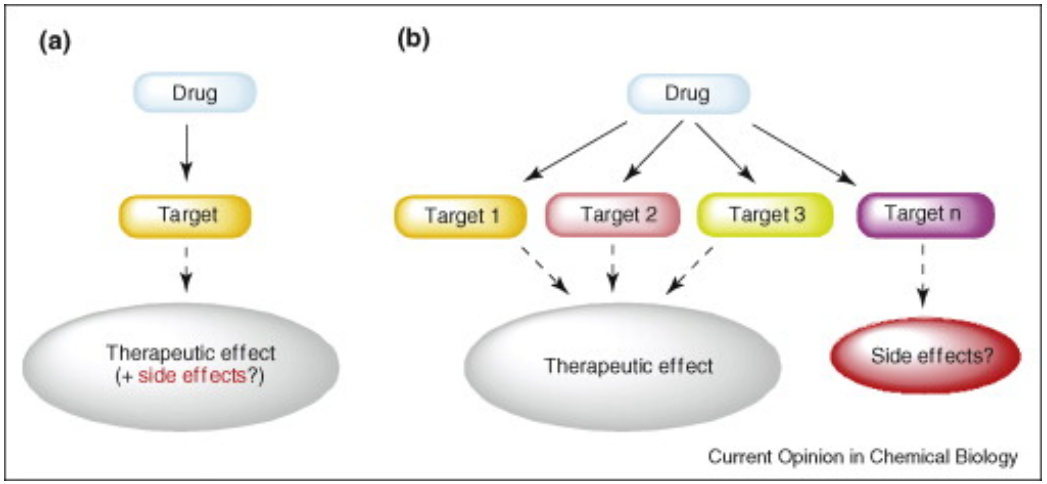
1- High-throughput screening: single molecular targets

2- Behaviour-based screening: Drug-responses in entire organisms (2 antidepressants with no affinity for known targets: YKP10A, INN00835)

3- Genomic approaches: Ability to modify the coordinated expression of gene families

4- Structure-based drug design: many molecular targets high degree of structural similarity

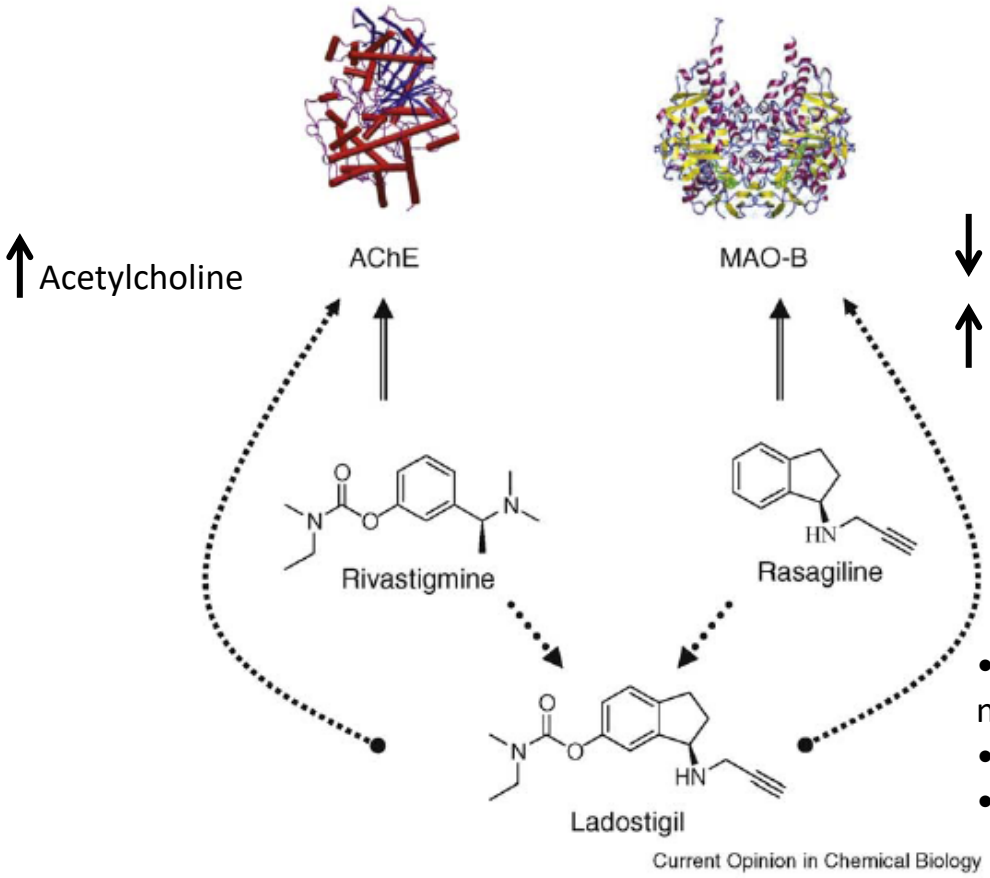
Multitarget drug discovery



Bolognesi, Current Op in Chem Biol, 2009

Example: Ladostigil for Alzheimer´s disease

in Phase II Clinical Trials

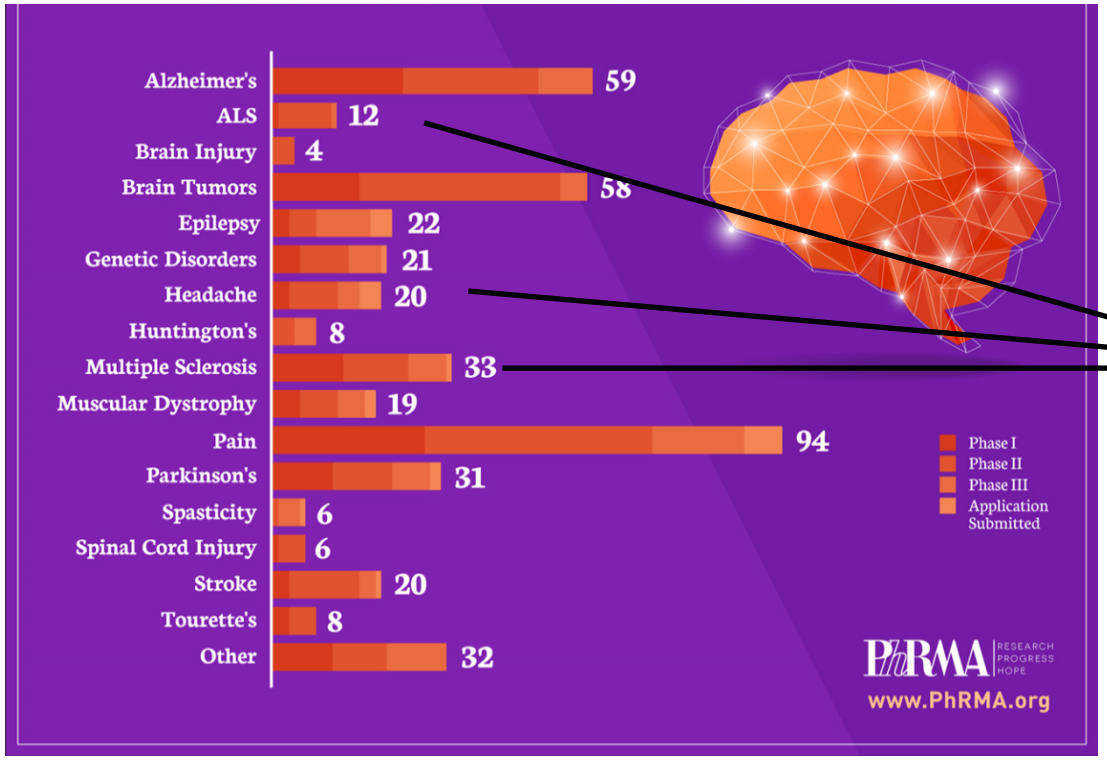


- Protective against oxidative stress-induced neuronal apoptosis
- Increase brain-derived nerve factor expression
- Neutroprotective

Modified from Bolognesi, Current Op in Chem Biol, 2009

Conclusions:

- Drug discovery: multidisciplinary enterprise (*in silico, in vitro, in vivo*)
- New targets in neuropharmacology
- New chemical libraries
- New animal models
- New approaches which integrate behavioural, genomic, and medicinal chemistry studies..... **INNOVATION**



420 Medicines in pipeline for neurological disorders

Monoclonal antibodies

http://phrma.org/sites/default/files/pdf/2015-neurological-disorders_drug-list.pdf
Adapted from Medicines in Development, 2015 Report

Thank you