Examples of small-molecule and peptide neurotransmitters

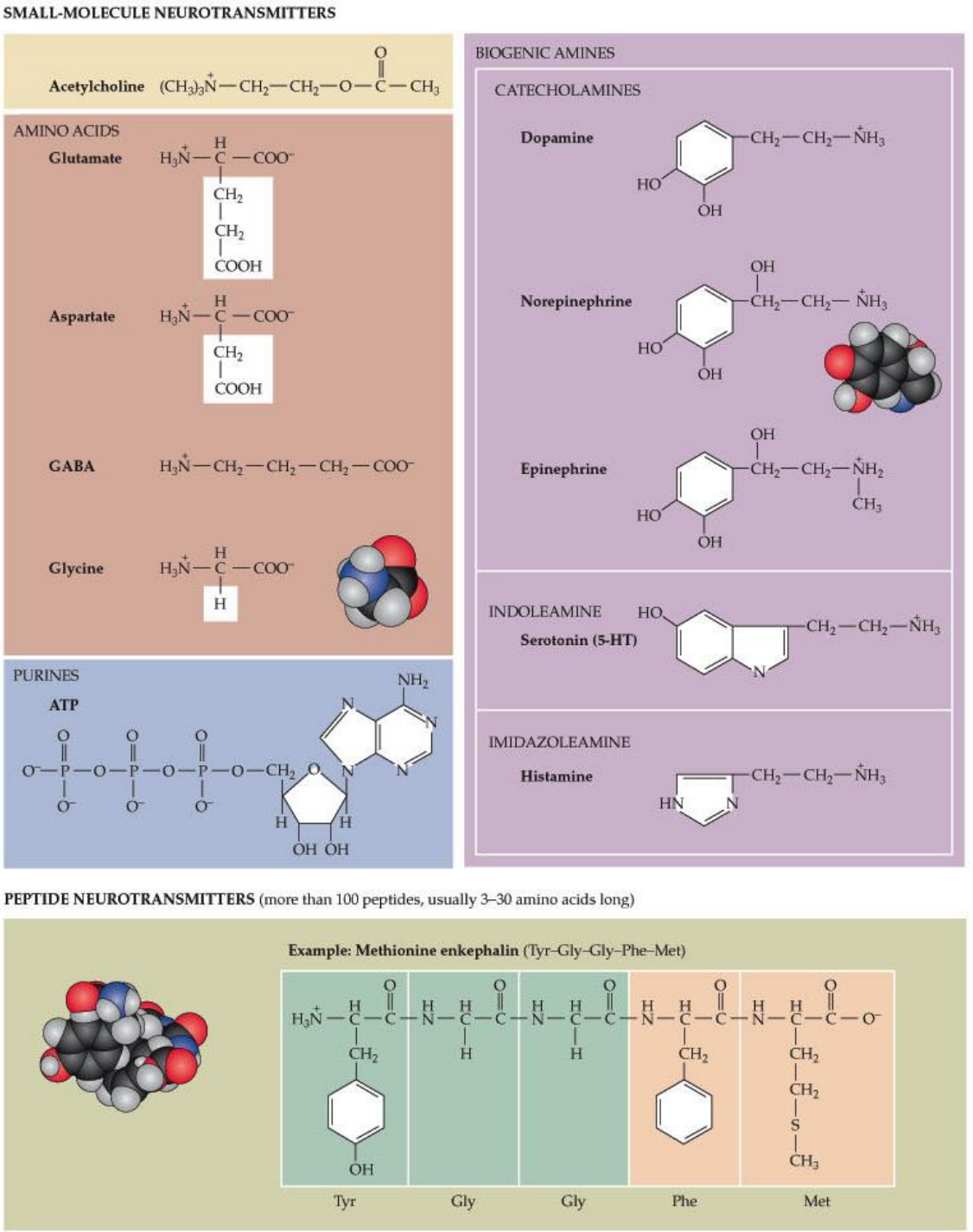


TABLE 6.1 Functional Features of the Major Neurotransmitters

Neurotransmitter	Postsynaptic effect[a]	Precursor(s)	Rate-limiting step in synthesis	Removal mechanism	Type of vesicle
ACh	Excitatory	Choline + acetyl CoA	CAT	AChEase	Small, clear
Glutamate	Excitatory	Glutamine	Glutaminase	Transporters	Small, clear
GABA	Inhibitory	Glutamate	GAD	Transporters	Small, clear
Glycine	Inhibitory	Serine	Phosphoserine	Transporters	Small, clear
Catecholamines (epinephrine, norepinephrine, dopamine)	Excitatory	Tyrosine	Tyrosine hydroxylase	Transporters, MAO, COMT	Small dense-core, or large irregular dense-core
Serotonin (5-HT)	Excitatory	Tryptophan	Tryptophan hydroxylase	Transporters, MAO	Large, dense-core
Histamine	Excitatory	Histidine	Histidine decarboxylase	Transporters	Large, dense-core
ATP	Excitatory	ADP	Mitochondrial oxidative phosphorylation; glycolysis	Hydrolysis to AMP and adenosine	Small, clear
Neuropeptides	Excitatory and inhibitory	Amino acids (protein synthesis)	Synthesis and transport	Proteases	Large, dense-core
Endocannabinoids	Inhibits inhibition	Membrane lipids	Enzymatic modification of lipids	Hydrolysis by FAAH	None
Nitric oxide	Excitatory and inhibitory	Arginine	Nitric oxide synthase	Spontaneous oxidation	None

[a]The most common postsynaptic effect is indicated; the same transmitter can elicit postsynaptic excitation or inhibition depending on the nature of

Small- molecule transmitters are transported from the cytosol into vesicles or from the synaptic cleft to the cytosol by TRANSPORTERS

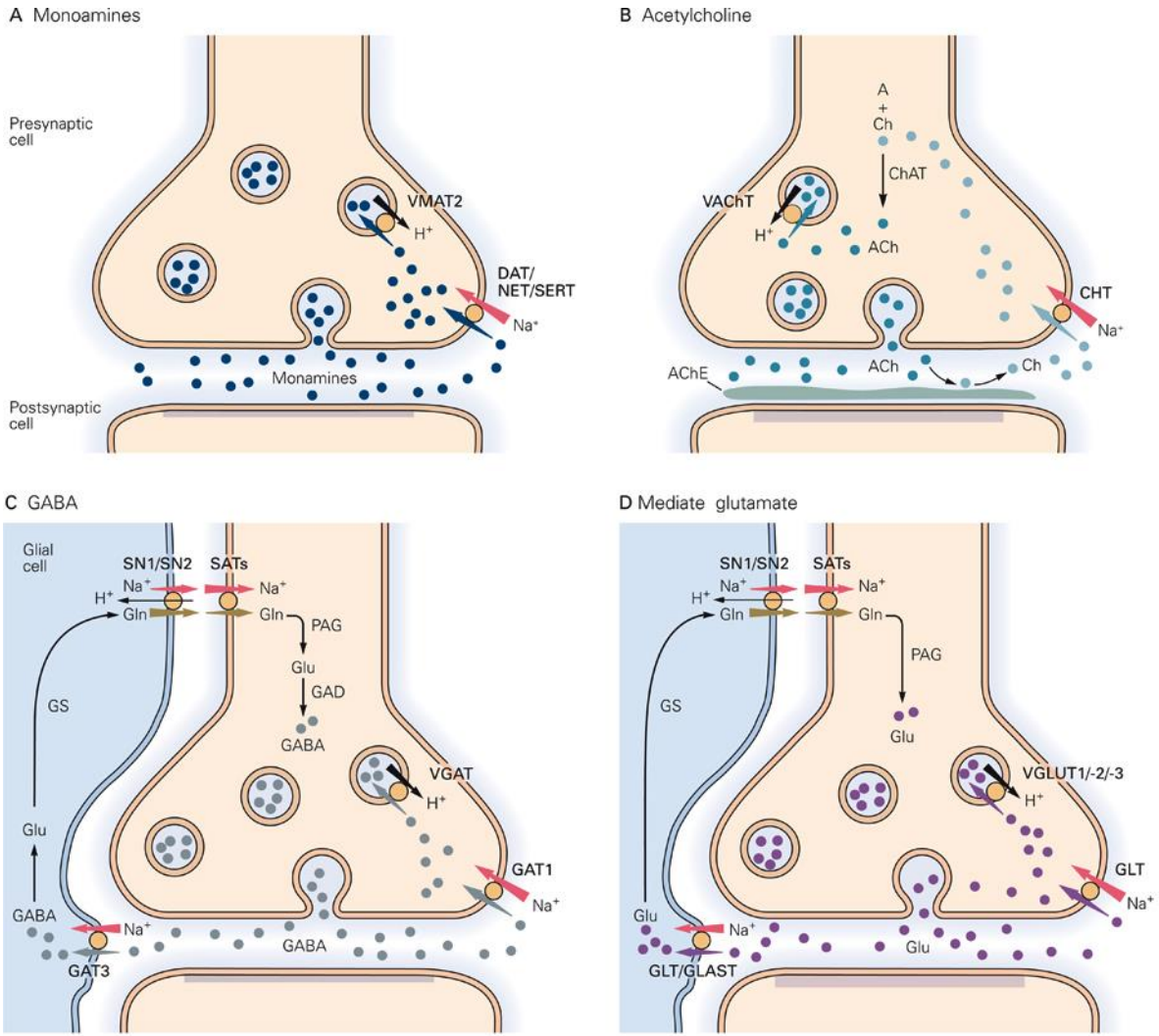


Figure 13–1 Small-molecule transmitters are transported from the cytosol into vesicles or from the synaptic cleft to the cytosol by transporters. Most small-molecule neurotransmitters are released by exocytosis from the nerve terminal and act on specific postsynaptic receptors. The signal is terminated and transmitter recycled by specific transporter proteins located at the nerve terminal or in surrounding glial cells. Transport by these proteins (**orange circles**) is driven by the H^+ (**black arrows**) or Na^+ (**red arrows**) electrochemical gradients. (Adapted, with permission, from Chaudhry et al. 2008.)

A. Three distinct transporters mediate reuptake of monoamines across the plasma membrane. The dopamine transporter (DAT), norepinephrine transporter (NET), and serotonin transporter (SERT) are responsible for the reuptake (**dark blue arrows**) of their cognate transmitters. The vesicular monoamine transporter VMAT2 transports all three monoamines into synaptic vesicles for subsequent exocytotic release.

B. Cholinergic signaling is terminated by metabolism of acetylcholine (ACh) to the inactive choline and acetate by acetylcholinesterase (AChE), which is located in the synaptic cleft. Choline (Ch) is transported back into the nerve terminal (**light blue arrow**) by the choline transporter (CHT), where choline acetyltransferase (ChAT) subsequently catalyzes acetylation of choline to reform ACh. The ACh is transported into the vesicle by the vesicular ACh transporter (VAChT).

C. At GABAergic and glycinergic nerve terminals the GABA transporter (GAT1) and glycine transporter (GLYT2, not shown) mediate reuptake of GABA and glycine (**gray arrow**), respectively. GABA may also be taken up by surrounding glial cells (eg, by GAT3). In the glial cells glutamate (Glu) is converted by glial glutamine synthetase to glutamine (Gln). Glutamine is transported back to the nerve terminal by the concerted action of the system N transporter (SN1/SN2) and system A transporter (SAT) (**brown arrows**). The glial transporter GLYT1 (not shown) also contributes to the clearance of glycine.

D. After release from excitatory neuronal terminals the majority of glutamate is taken up by surrounding glial cells (eg, by GLT and GLAST) for conversion to glutamine, which is subsequently transported back to the nerve terminals by SN1/SN2 and a type of SAT (SATx) (**brown arrows**). Reuptake of glutamate (**purple arrow**) at glutamatergic terminals also has been demonstrated for a GLT isoform.

Neuromodulatory neurons in the brainstem and basal forebrain project to sites throughout the forebrain

A group of neurotransmitters plays a predominantly modulatory role, i.e. **serotonin, dopamine, norepinephrine, histamine**. A striking feature of neuromodulatory systems is their broad reach. Although the cell bodies of these neurons are clusterd in discrete nuclei in the brainstem and basal forebrain, their axons reach much of the forebrain.

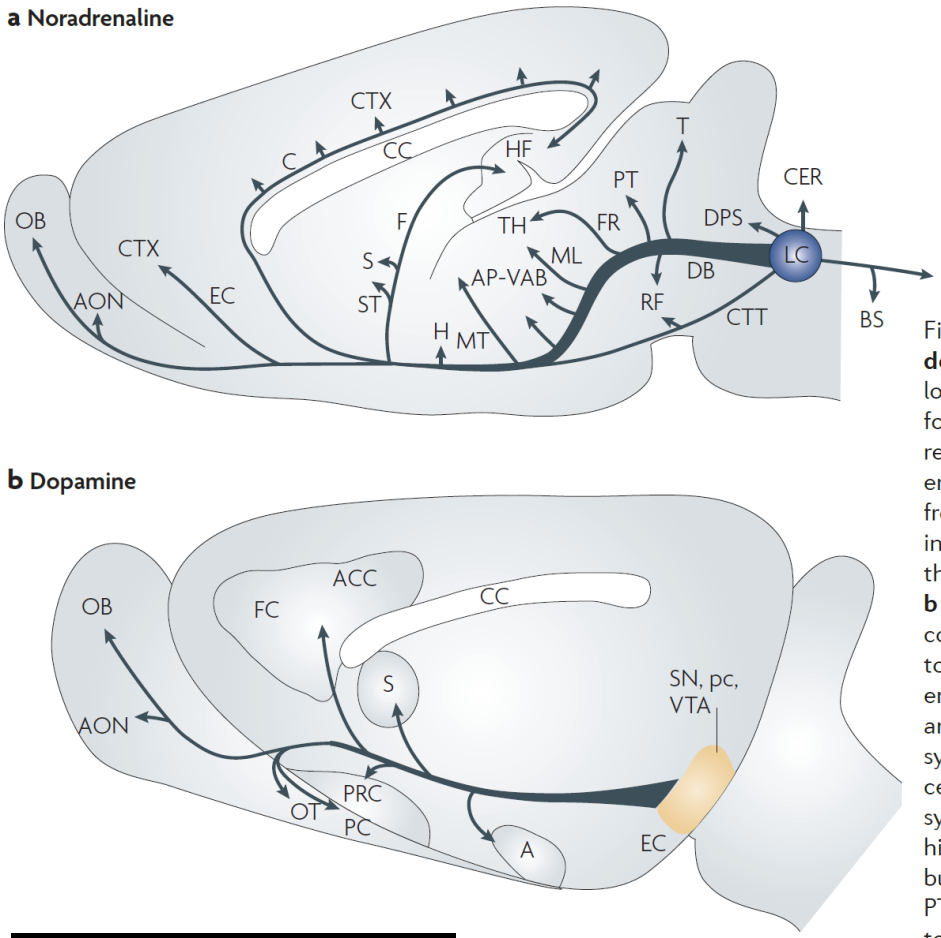
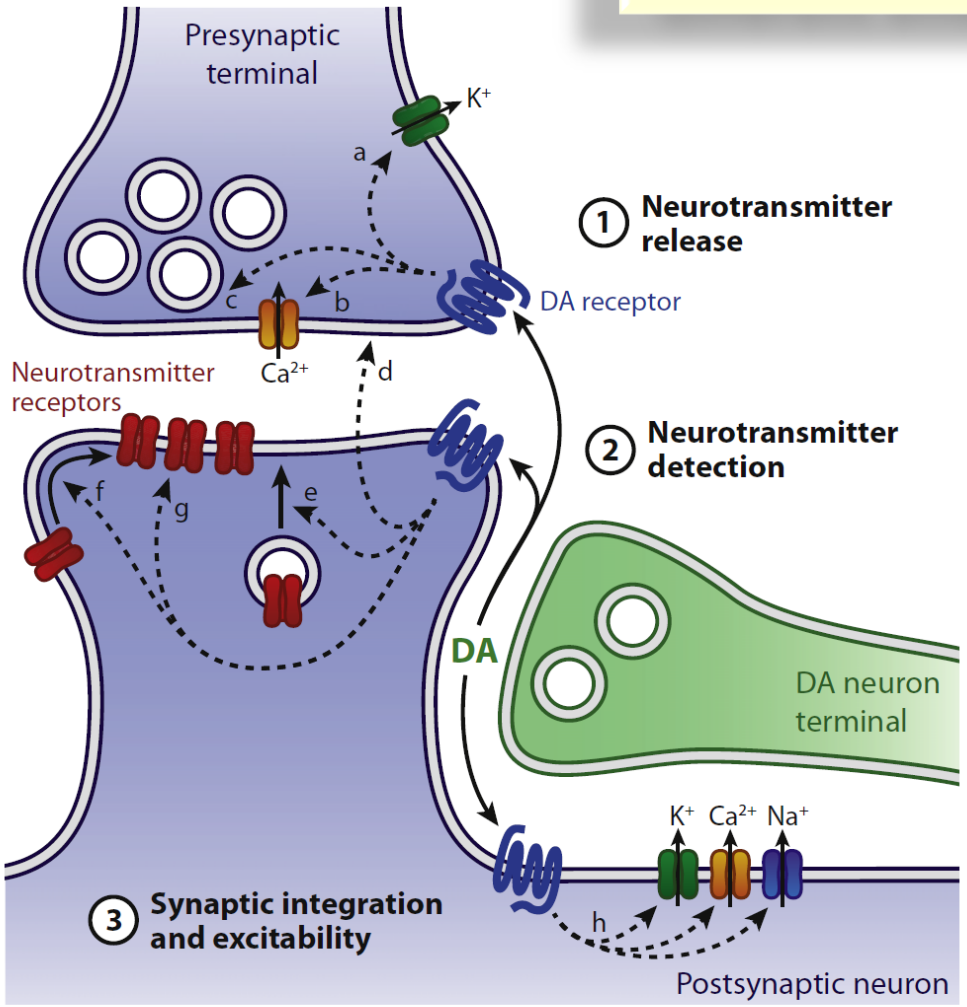


Figure 2 | **Comparative anatomy of the distribution of noradrenergic and dopaminergic projections in the rat brain. a** | Noradrenergic nuclei, including the locus coeruleus (LC), which contains ~1,500 cells that project mainly to the ipsilateral forebrain. Through extensive axonal branching, a single cell can have terminals in diverse remote brain regions, including the forebrain, the brainstem and the cerebellum. The entire cerebral cortex, including the frontal cortex and all sensory regions, receives input from the LC. The LC also sends projections to thalamic nuclei and limbic structures, including the amygdala (A), the hippocampus and the septum (S). The only major region that does not receive input from the LC is the area that contains the basal ganglia. **b** | Dopaminergic nuclei, including the ventral tegmental area (VTA), which is the main cortical input of the dopaminergic system, and the substantia nigra (SN), which projects to the striatum. Note that the cortical inputs are limited to the frontal regions — the entorhinal cortex (EC) and the piriform cortex (PC). ACC, anterior cingulate cortex; AON, anterior olfactory nucleus; AP-VAB, ansa peduncularis–ventral amygdaloid bundle system; BS, brainstem nuclei; C, cingulum; CC, corpus callosum; CER, cerebellum; CTT, central tegmental tract; CTX, cortex; DB, dorsal bundle; DPS, dorsal periventricular system; F, fornix; FC, frontal cortex; FR, fasciculus retroflexus; H, hypothalamus; HF, hippocampal formation; ML, medial lemiscus; MT, mamillothalamic tract; OB, olfactory bulb; OT, olfactory tract; pc, pars compacta; PC, piriform cortex; PRC, perirhinal cortex; PT, pretectal area; RF, reticular formation; S, septum; SC, spinal cord; ST, stria terminalis; T, tectum;TH, thalamus. Part **a** is modified, with permission, from REF. 121 © (1979) Annual Reviews, inc. Part **b** is modified, with permission, from REF. 122 © (1978) Annual Reviews, inc.

Sara, Nature Reviews Neurosci 2009
doi:10.1038/nrn2573

Neuromodulatory neurotransmitters (NEUROMODULATORS) exert several different effects on target neurons



Whereas neurotransmitters such as glutamate or GABA can elicit rapid excitation or inhibition of postsynaptic neurons by activating ionotropic receptors, neuromodulators act on a **slower time scale**, usually through GPCRs. Neuromodulators can have diverse effects on their targets depending on the location and downstream signalling events of their receptors.

Figure 1. Potential Sites of Modulation of Synaptic Transmission by DA

DA may affect neurotransmitter release by modulating axon terminal excitability (a), Ca^{2+} influx (b), or vesicular release machinery (c). This can occur directly, through activation of presynaptic DA receptors, or indirectly, after the recruitment of postsynaptic DA receptors and liberation of retrograde signaling molecules (d). Postsynaptic DA receptors may influence neurotransmitter detection by modulating the membrane insertion (e), synaptic recruitment (f), or properties (g) of neurotransmitter receptors. In addition, DA alters synaptic integration and the excitability of pre- and postsynaptic membranes by modulating ion channels that control resting potential, Ca^{2+} influx, and action potential threshold and waveform (h).

Tritsch & Sabatini, Neuron 2012
http://dx.doi.org/10.1016/j.neuron.2012.09.023

Unconventional transmitters (eCBs, NO): retrograde signalling

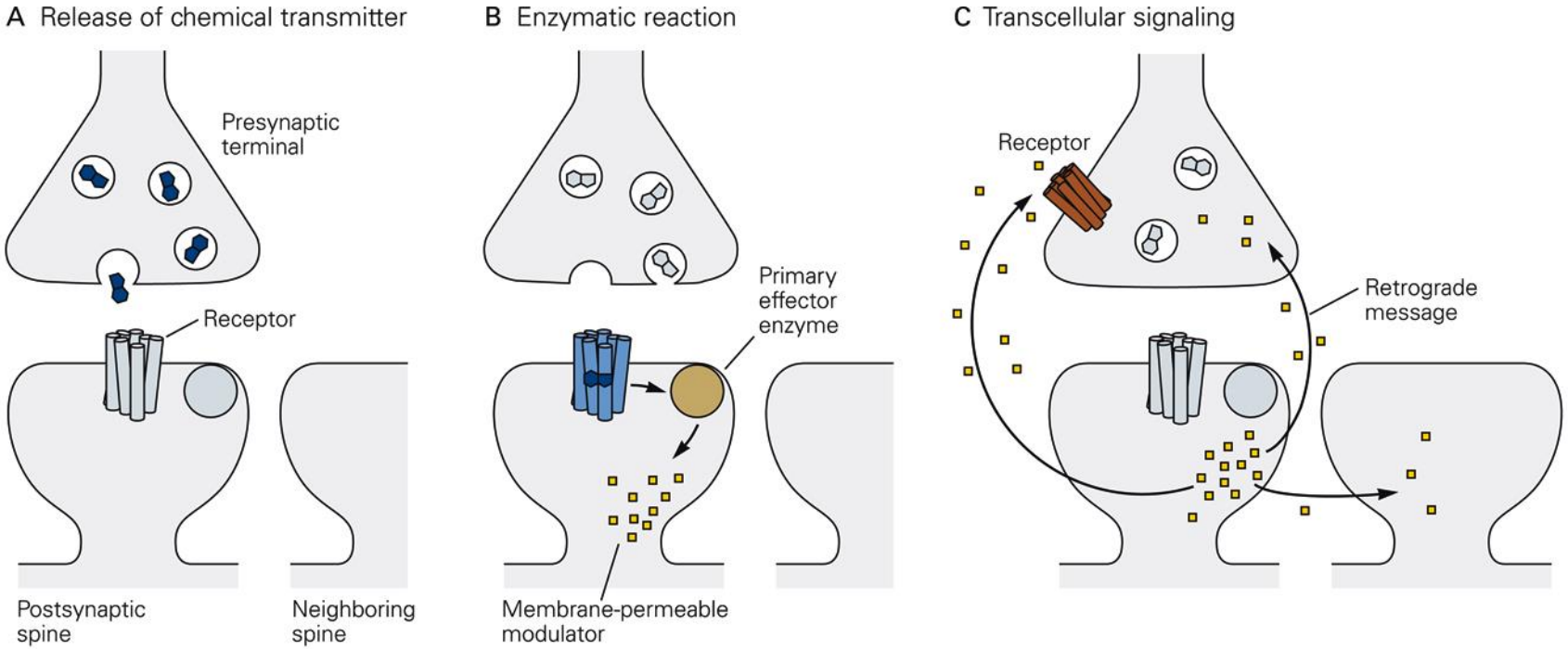
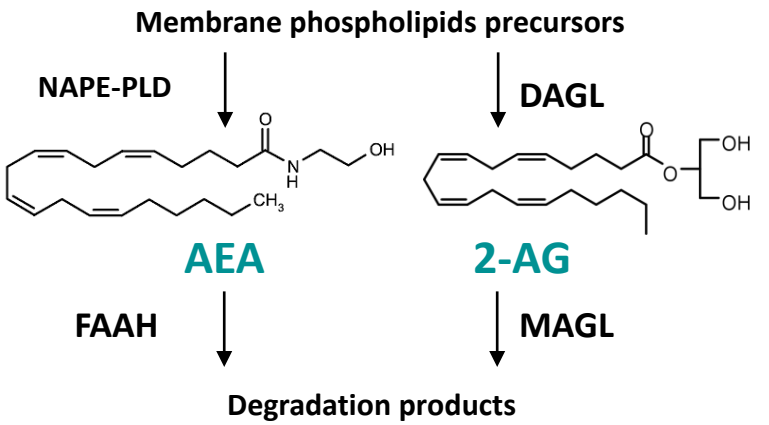


Figure 11–8 Transcellular signaling can occur from the postsynaptic neuron to the presynaptic neuron (retrograde transmission) and between postsynaptic cells. Until recently, synaptic signaling was thought to occur only from the presynaptic neuron to the postsynaptic cell. Transcellular signaling is initiated by a presynaptic signal. A presynaptic terminal releases a neurotransmitter at the synapse and that transmitter reacts with a G protein-coupled receptor in a postsynaptic dendritic spine **(A)**. The receptor activates enzymes that produce a membrane-permeable modulator **(B)**. The modulator is released from the postsynaptic spine and diffuses to neighboring postsynaptic spines as well as presynaptic terminals **(C)**. There it can produce either first-messenger effects, by acting on G protein-coupled receptors in the surface membrane, or second-messenger-like effects, by entering the cell to act within. This kind of modulator of the presynaptic terminal is called a *retrograde messenger* rather than a second messenger, and its action is called *transcellular signaling*.

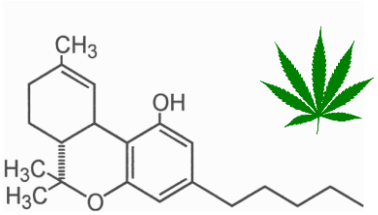
Endocannabinoid system

- **eCBs** (AEA = Anandamide; 2-AG = 2-Arachinodoyl glycerol)
- **Receptors** (CB1, CB2, …)
- **Enzymatic machinery for eCB synthesis and degradation**

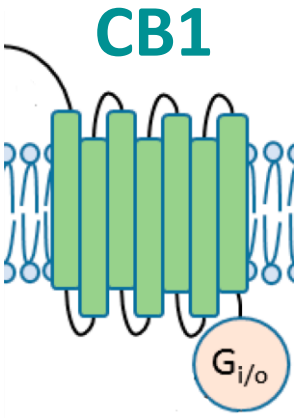
Endocannabinoids (eCBs)



Plant-derived cannabinoids (phytocannabinoids)



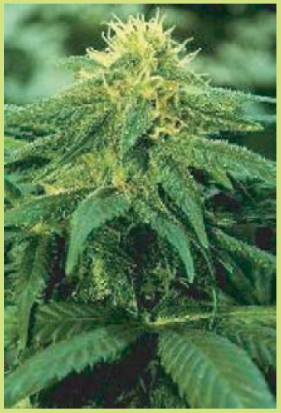
Δ^9 – THC
(Δ-9-tetrahydrocannabinol)

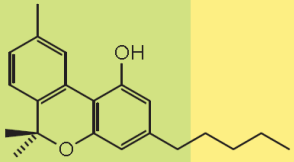


AEA and 2-AG are unsaturated fatty acids, derivatives of arachidonic acid.

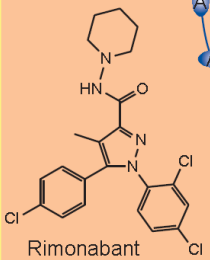
Their precursors are present in lipid membranes

A brief history of cannabinoid and endocannabinoid research

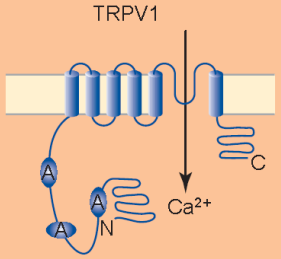




Cannabinol



Rimonabant (SR141716A)



New drugs

200 — The therapeutic properties of cannabis are described in Chinese pharmacopoeia

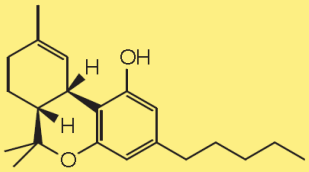
1838–1840 — Sir W.B. O'Shaughnessy assesses methodically the medicinal properties of cannabis

1932 — Cahn elucidates part of the structure of cannabinol

Wood *et al.* isolate cannabinol from cannabis resin 1899

Todd *et al.* (and Adams *et al.* in the USA) fully elucidate and synthesize cannabinol 1940

1964 — Gaoni and Mechoulam elucidate the structure of THC



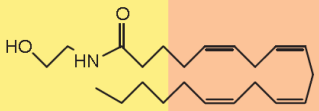
THC

Cannabinoid pharmacology is thoroughly investigated 1970–1990

1988 — Howlett's group identifies specific THC binding sites in the brain

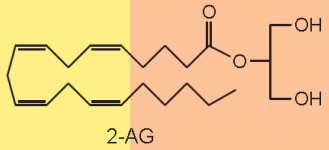
Matsuda *et al.* clone the CB_1 receptor 1990

1992 — Mechoulam's group in collaboration with Pertwee's group identify the first endocannabinoid, anandamide



Anandamide

Munro *et al.* clone the CB_2 receptor 1993



2-AG

1995 — Mechoulam's group and Waku's group identify the second endocannabinoid, 2-AG

Rinaldi-Carmona *et al.* at Sanofi develop the first CB_1 receptor antagonist 1994

1998 — House of Lord's report on medical cannabis; Di Marzo *et al.* propose the existence of interactions between endocannabinoids and vanilloid receptors

Cravatt *et al.* clone the first endocannabinoid-degrading enzyme, FAAH 1996

2003 — Bisogno *et al.* clone the first endocannabinoid-biosynthesizing enzymes

Zygmunt *et al.* and Smart *et al.* show that anandamide activates vanilloid receptors 1999–2000

???? — Cloning of new cannabinoid receptors; identification of other endocannabinoid enzymes; cloning of the endocannabinoid transporter; more endocannabinoid-based therapies

Sativex ® approved for sale in Canada; regulatory approval is filed to sell rimonabant in the USA; the Aberdeen group discovers an allosteric site on CB_1 receptors 2005

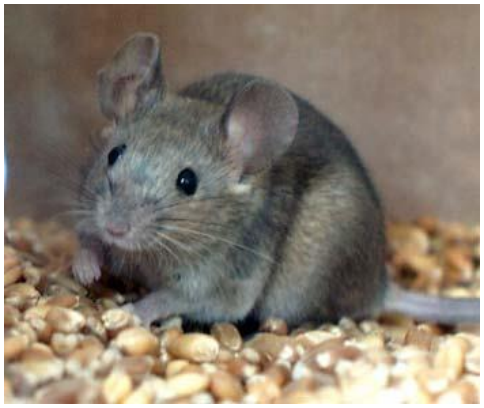
Cannabis research

Cannabinoid research

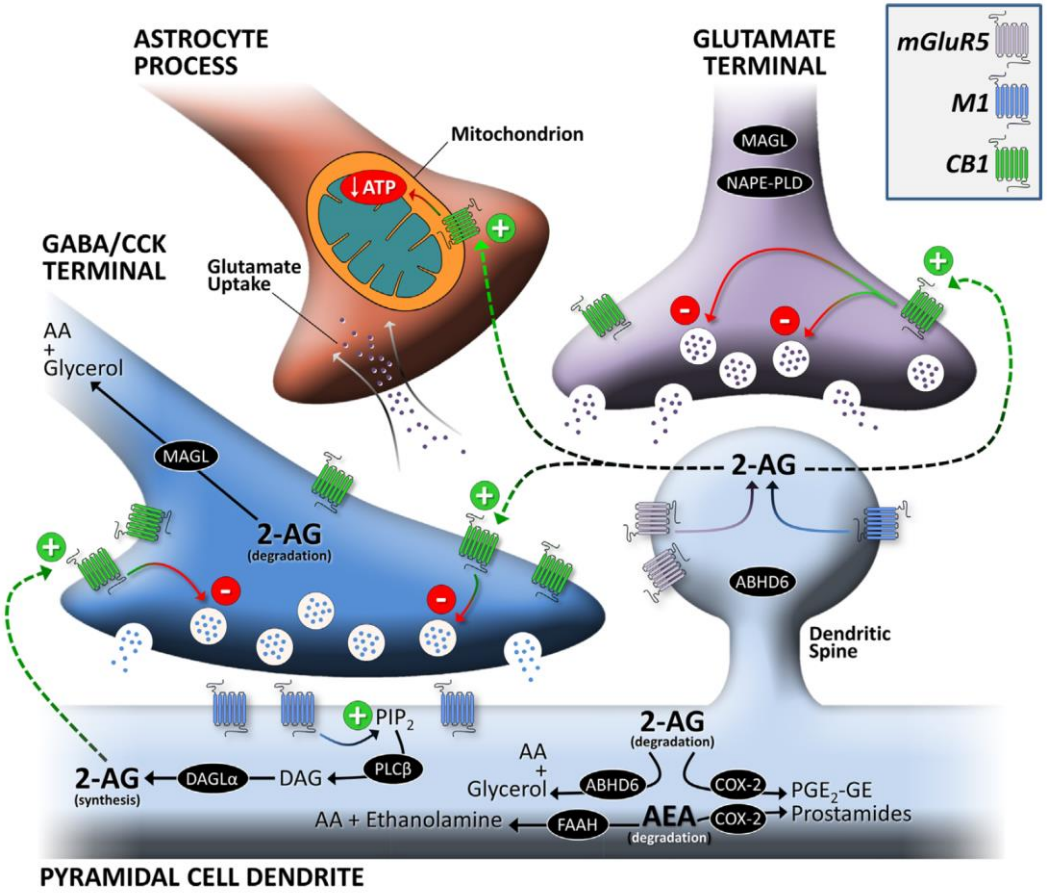
Endocannabinoid research

Phylogenesis of the Endocannabinoid System

- CB1/CB2-type receptors are unique to chordates (CiCBR in the urochordates *Ciona intestinalis*, BfCBR in the cephalochordate *Branchiostoma floridae*)
- Enzymes involved in biosynthesis/inactivation of eCBs occur throughout the animal kingdom.



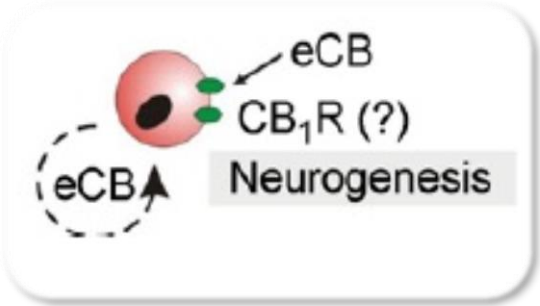
In the mature brain endocannabinoids (eCBs) inhibit neurotransmitter release by acting as retrograde messengers

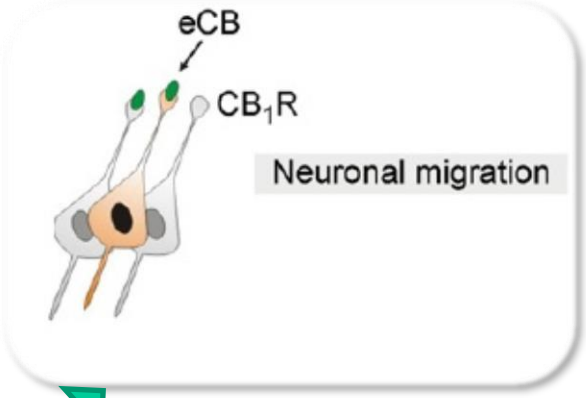


- most endocannabinoid receptors (CB1) are present on axon terminals and preterminal axon segments
- eCBs precursors are present in lipid membranes
- **on demand** (by activation of certain G protein–coupled receptors [mGluRs, mAChRs] or by depolarization) eCBs are liberated in one or two rapid enzymatic steps and released into the extracellular space.

Lu and Mackie, Biological Psychiatry 2016
http://dx.doi.org/10.1016/j.biopsych.2015.07.028

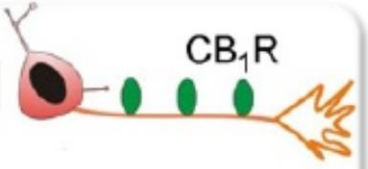
CB1 activation influences varoius aspects of neural development

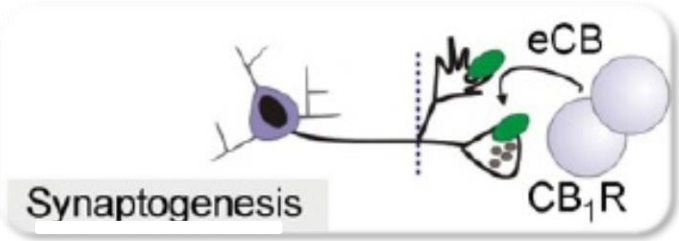




ENDOCANNABINOIDS

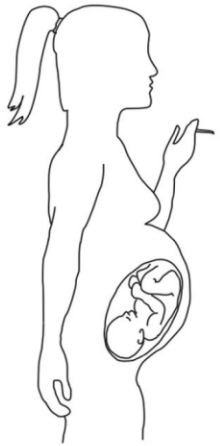
Axonal growth





What are the consequences of prenatal cannabis exposure?

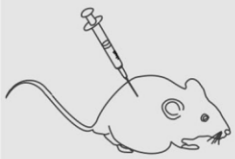
A



prenatal	neonatal	infant	child	adult
Reduced fetal growth[1]	**Decreased birth weight**[1]	Impaired mental development[3] (9 months)	Increased externalizing behaviour[2,3] (hyperactivity; 6 and 10 years)	Altered functioning in visuo-spatial memory[2] (18-22 years)
Reduced head circumference[1]	Altered gestational lenght[1]	Increased aggression and inattention in girls[1] (18 months)	Impaired abstract and visual reasoning[3] (10 years)	
Increased pulsatility and resistence index of uterine artery[1]	Increased startles and tremors[2]	**Impaired memory function**[2,3] (36-48 months)	Impaired visuoperceptual functioning[2] (9-12 years)	
Decreased inner diameter of aorta[1]	Reduced abituation to light[2]	Decreased verbal scores[2,3] (36-48 months)		
Placental resistance[1]	Altered EEG sleeping recordings[3]	**Increased anxiety and depression**[3]		

B

THC or cannabinomimetic

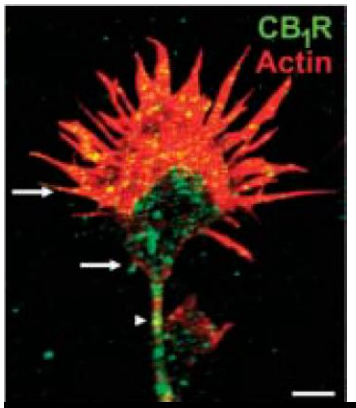


prenatal	neonatal	postnatal	adolescent	adult
Axonal bundle malformation (Tortoriello, 2014)	**Decreased birth weight** (Fried, 1976)	Increased rearing and locomotor activity at P15-20 (Navarro, 1994)	Altered open field performance (Fride, 1996)	**Memory impairment** at P40-80 (Mereu, 2003)
		Hyperactivity at P12 (Mereu, 2003)	Impaired consolidation of long-term memory at P22 (Silva, 2012)	**Reduced synaptic plasticity** (Tortoriello, 2013)
		Learning impairment at P10-12 (Antonelli, 2005)	Inhibited social interaction and play behaviour (Trezza, 2008)	**Cognitive impairment** (Campologno, 2007)
		Increased ultrasonic vocalization at P10 (Antonelli, 2005)		Altered social behaviour (Navarro, 1995)
		Impaired synapse formation (Tortoriello, 2014)		**Anxiogenic-like profile** (Trezza, 2008)

Fig. 1 Main physiological effects of in utero cannabis exposure in human and animal studies. **a** Overview of the major physiological complications found in prenatal cannabis exposure from human longitudinal studies: (1) the Generation "R" study, (2) the OPPS study and (3) the MHPC study. **b** List of comparable animal studies directly (*bold*) or indirectly, reflecting physiological findings from human studies

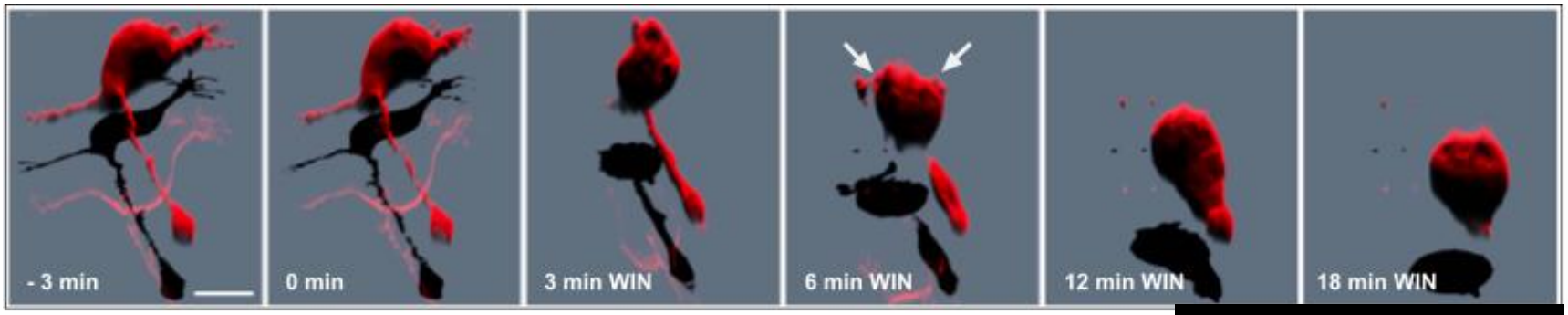
CB1 activation regulates neurite outgrowth in rodents

CB1 and DAGLα both accumulate in the central domain and actin-rich filopodia of growth cones (arrows)



Berghuis et al., Science 2007
DOI: 10.1126/science.1137406

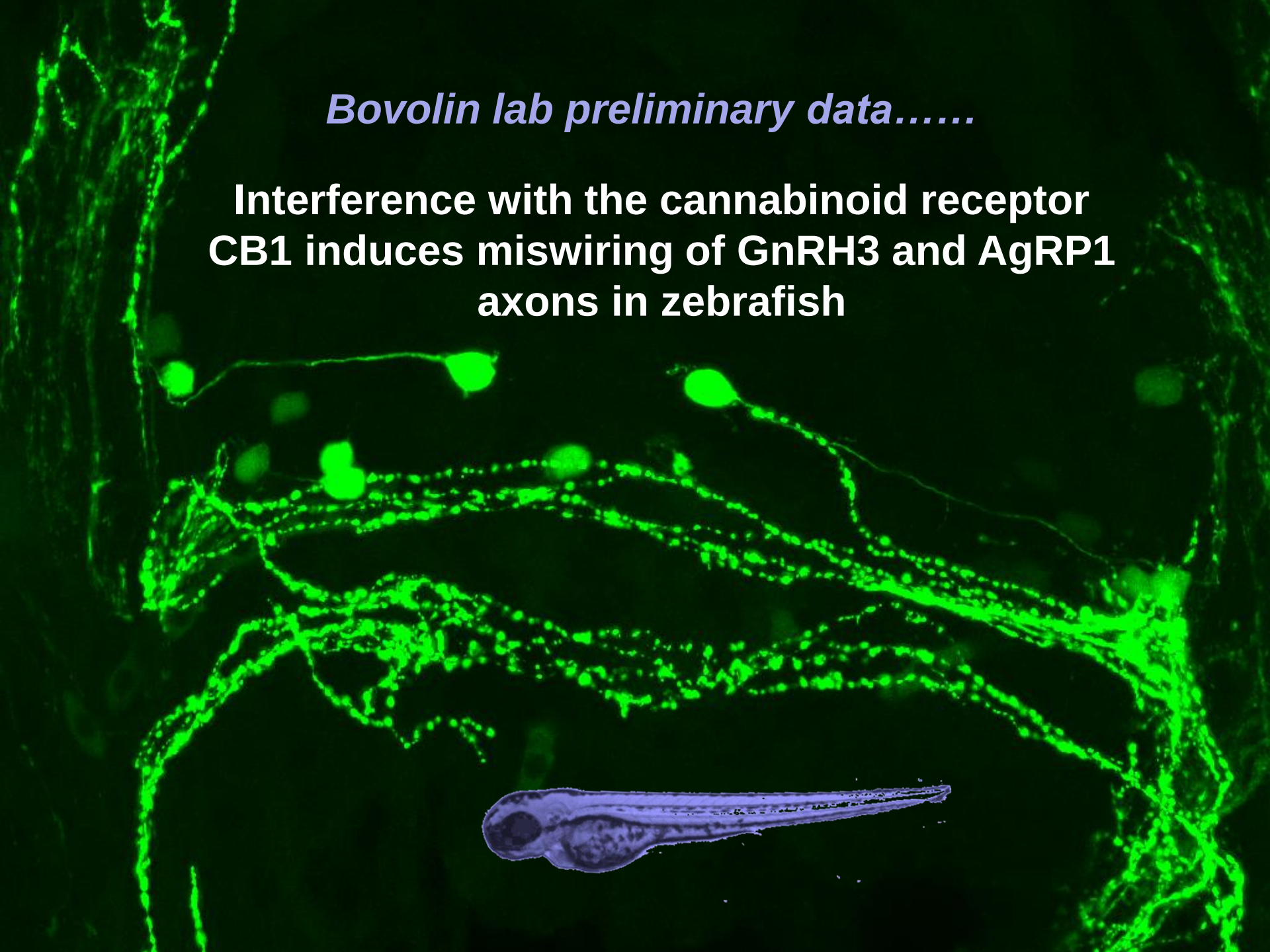
CB1-induced actomyosin contraction results in neurite retraction:



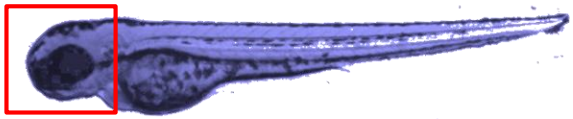
from Roland et al, eLIFE 2014
DOI: 10.7554/eLife.03159.001

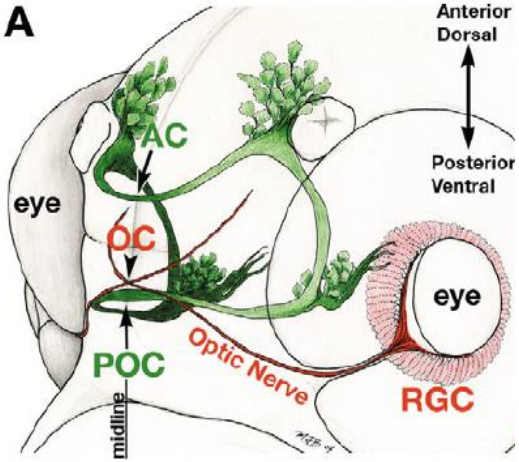
Bovolin lab preliminary data……

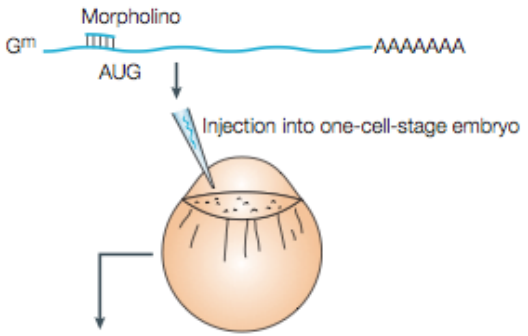
Interference with the cannabinoid receptor CB1 induces miswiring of GnRH3 and AgRP1 axons in zebrafish

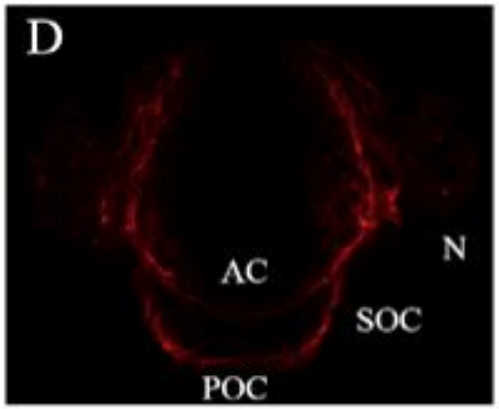


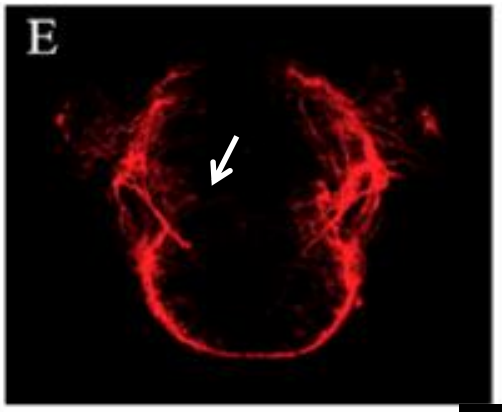
Zebrafish larvae injected with CB1 morpholino at 72 hours post fertilization (hpf) showed defects in fasciculation of the anterior commissure

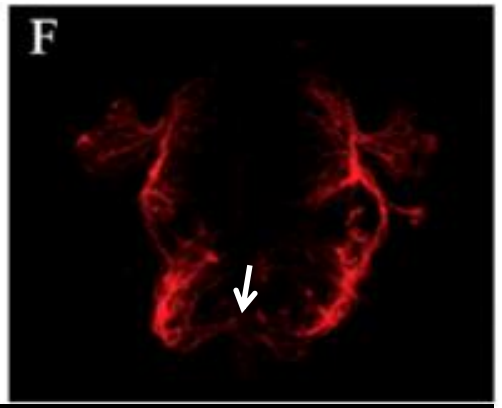












Watson et al, Mol & Cell Neurosci 2008
doi:10.1016/j.mcn.2008.02.001

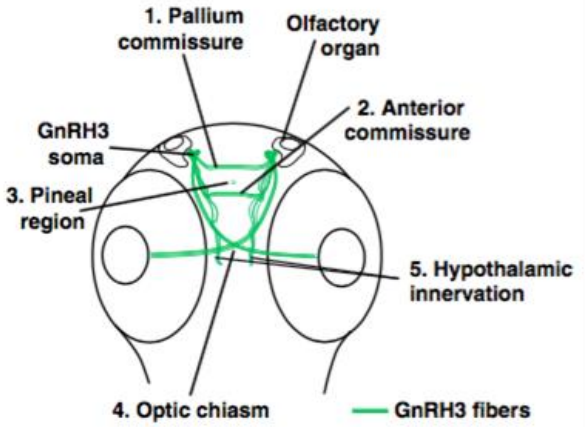
Experimental design:

Pharmacological modulation and downregulation of **CB1**

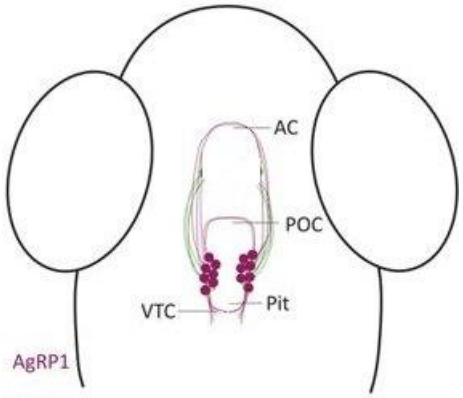
axonal pathfinding and **fasciculation** of **GnRH3** and **AgRP1 fibers**

Zebrafish transgenic lines:

gnrh3:EGFP



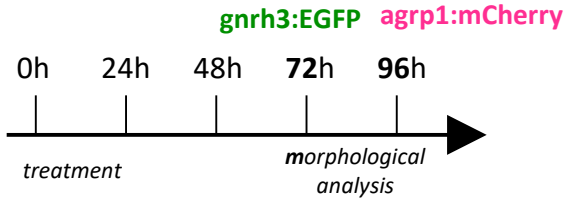
agrp1:mCherry



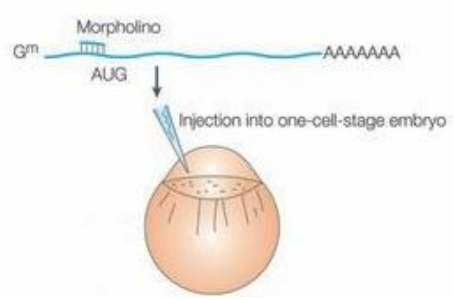
Abraham et al. 2009

Shainer et al. 2017

In collaboration with prof. Gothilf lab

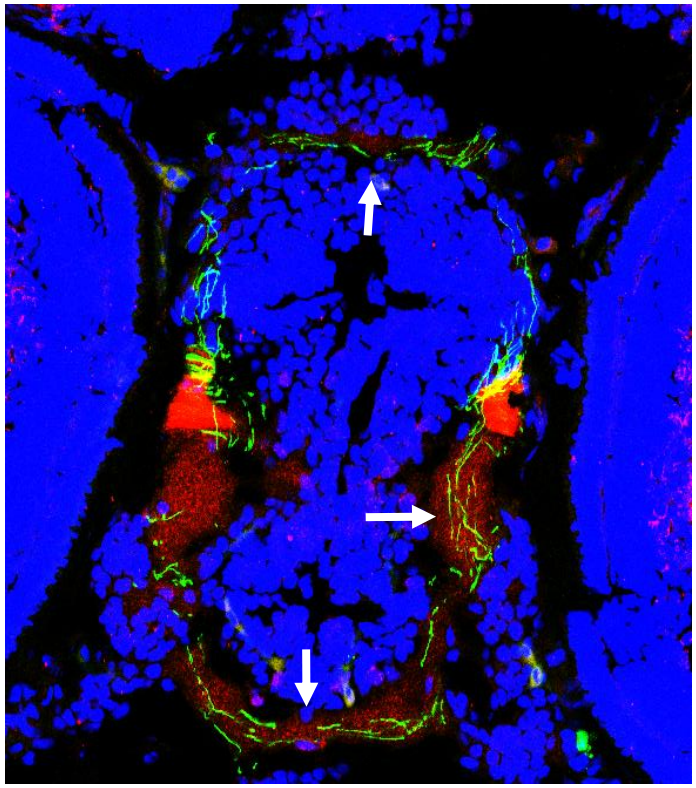


CB1 agonist/reverse agonist/antagonist



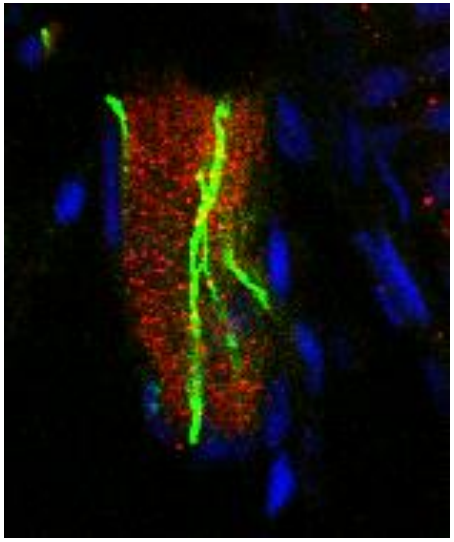
Morpholino-mediated knockdown

CB1 receptors are expressed in forebrain axonal fibers, including anterior commissure, postoptic commissure and optic chiasm, in close proximity to GnRH+ fibers



Immunocytochemical staining with anti-CB1 antibody (red fluorescence) on horizontal section of a 72 hpf zf embryo.
Green = GnRH3+ fibers; Blu = DAPI

Colocalization of GnRH3- and CB1-positive fibers in the brain of GnRH3::EGFP zf embryo



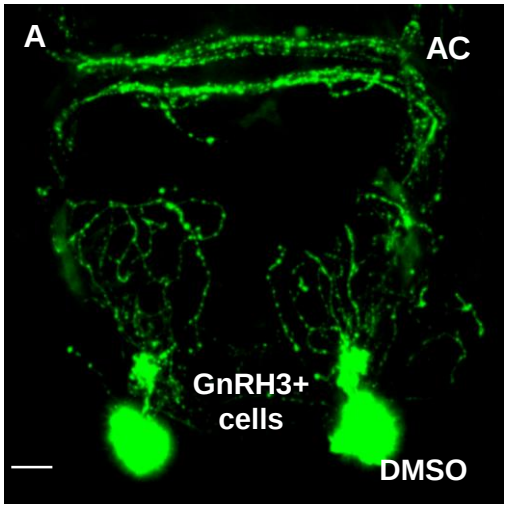
High magnification of GnRH3+ fibers in close proximity to CB1+ punctate staining

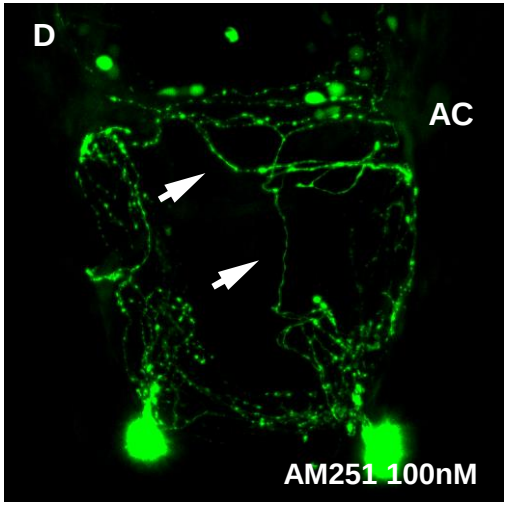
Pharmacological inhibition of CB1 receptors affects the normal pathfinding of GnRH3 neurons in zebrafish embryos

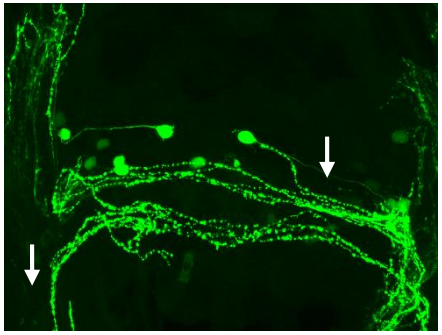
Control

CB1-antagonist

gnrh3:EGFP
larvae 72hpf







agrp1:mCherry
larvae 96hpf