Serotonin – receptors and the role

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History

- Monoamine neurotransmitter serotonin = 5-hidroksitriptamin (5-HT).
- In the mid 19th century the presence of vasoconstrictor substance in the blood was noticed
- In the beginning of 20th century platelets were recognized as the source of this substance.
- In 1940s Page et al. isolated and identified this substance and named it ***serotonin.***

Role – serotonin in the brain

- Mood
- Clinical depression, schizophrenia
- Sleep
- Apetite
- Descending pathways of endogenous analgesia
- Cardiovascular system
- Respiratory system

- disturbances in the metabolism of serotonin in Sudden Infant Death Syndrome (SIDS)
- Changes in 5-HT receptors in the brain of suicidal victims – reduced activity of the 5-HT neurons

Role – on the periphery

- *In Gastro-Intestinal System*: regulates local circulation, motility of the intestins , peristaltick reflex, secretion (5-HT_4 receptors)
- *In heart*: ablation of 5-HT_{2B} receptors leads to irregular development of the heart in the mouse
- *Mezenhimal organs*: mythosis, proliferation
- *Bone system*: inhibits osteoblasts, activates osteoclasts

- *Blood vessels:* Relaxation of smooth musculature – vazodilatation (5-HT_7 receptor)
- *Blood vessels :* vazoconstriction

- “carcinoid syndrome” – carcinoid tumor (EC intestinal cells) excessive level of 5-HT – flushing, asthma, diarrhea, fibrous thickening of the hearth valves

Location and synthesis

- Cental Nervous System- 1% of total serotonin
- Enterocromaphine intestinal cells (EC) - 90% of total serotonin
- Platelets – 2% of total serotonin
- Body: synthesis in EC, sometimes named “para-neuron”, ultrastructural similarity to the neurons, some markers of neuronal differentiation, byosinthesis of the neurotransmitter
- Platelets overtake serotonin from plasma using the special transport protein in their membrane.

- Romantic love and serotonin: subjects in early phase of love relationship and those with OCD have similar density of platelet 5-HT transporter (lower than controls)

- Serotonin does not cross blood-brain barrier
- 1953. Twarog and Page have proven that serotonin is localized in the brain – serotonin is synthetised in the brain!

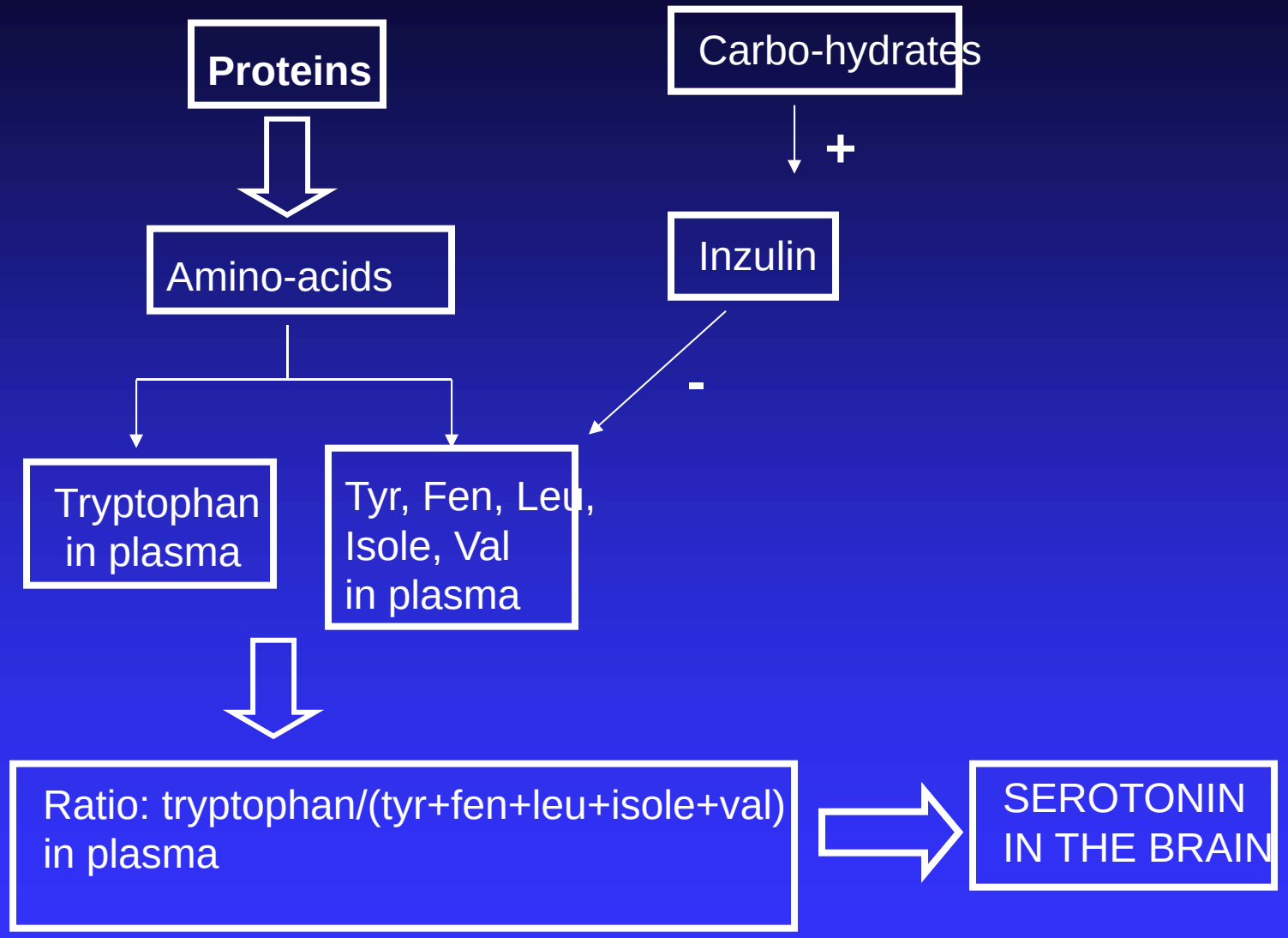
- Synthesis starts with the transport of L-tryptophan from blood to brain
- Source of the tryptophan is food
- Only 1% of tryptophan from food can cross blood-brain barrier

Transport of tryptophan depends on:

1. *Competition of other amino-acids* to the same carrier: tyrosine, phenylalanine, valine, leucine, isoleucine
2. *Ratio of concentrations of tryptophan and other AC in plasma :*

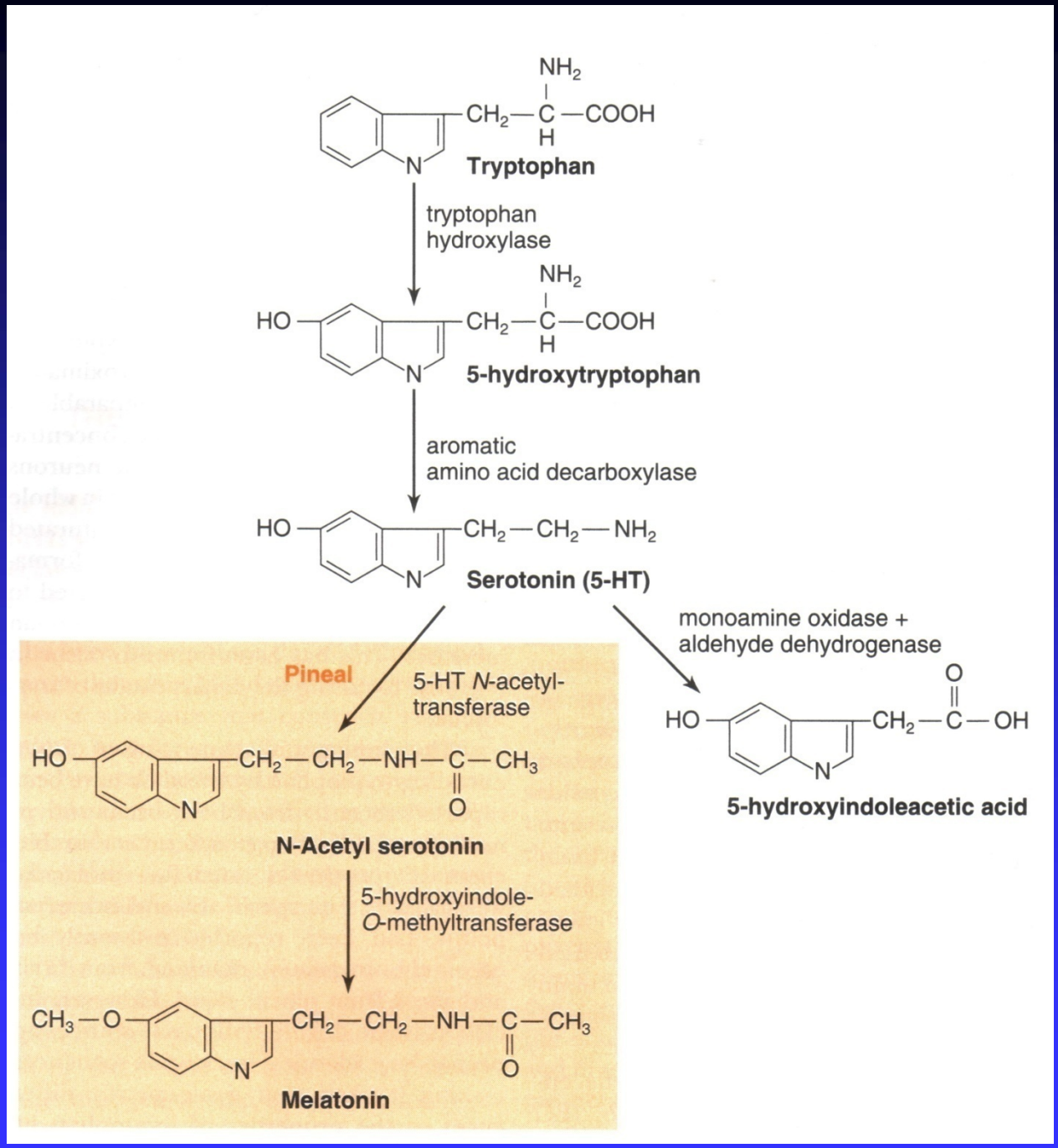
   a) Food rich in carbohydrate

   b) Food rich in tryptophan


Proteins
Carbo-hydrates
+
Amino-acids
Inzulin
-
Tryptophan in plasma
Tyr, Fen, Leu, Isole, Val in plasma
Ratio: tryptophan/(tyr+fen+leu+isole+val) in plasma
SEROTONIN IN THE BRAIN

3. *Exercise:* concentration of tryptophan in the brain increases during running

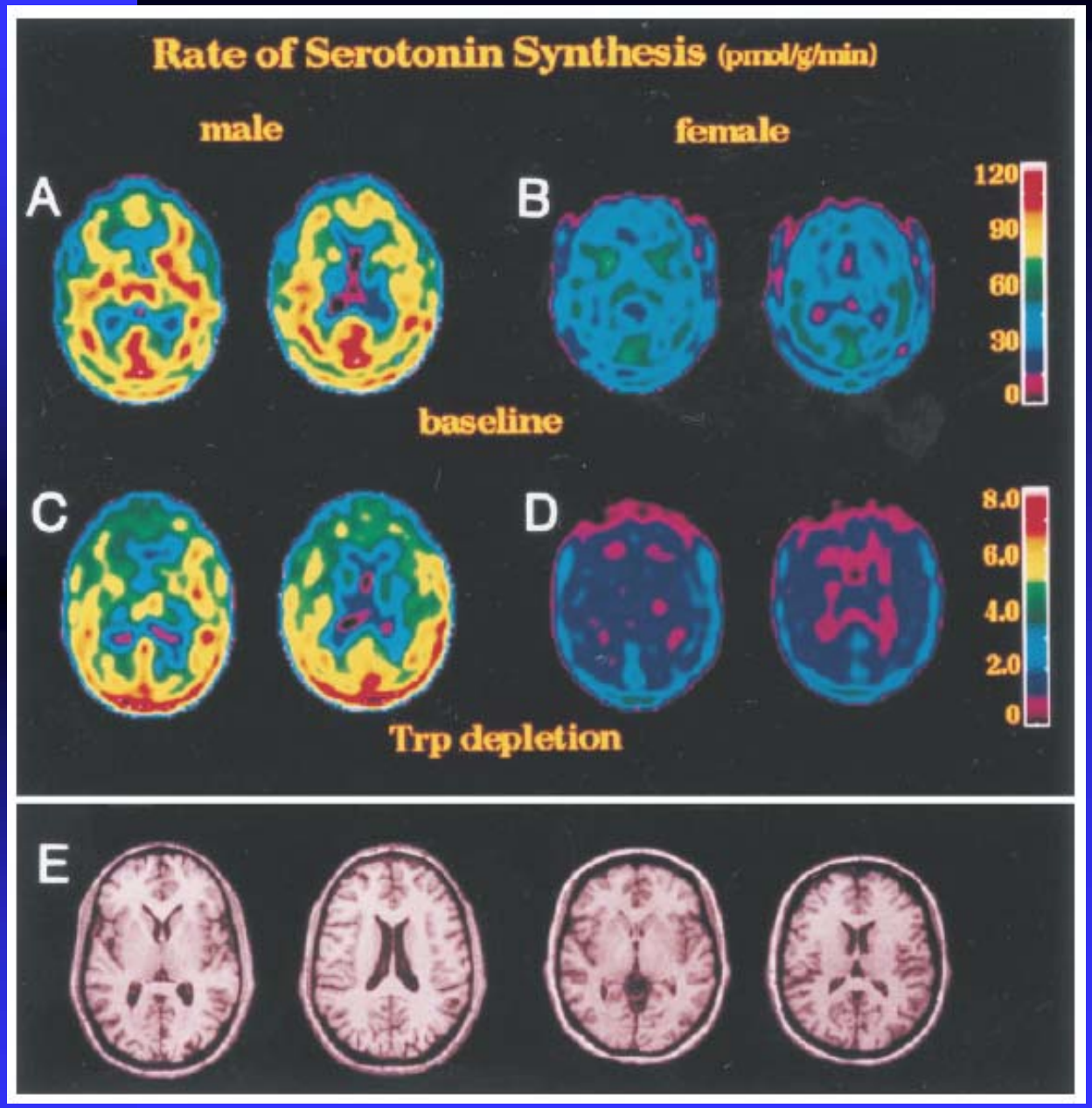
- Two enzymes play a role in serotonin synthesis:
  - Tryptophan hydroxylase (tryptophan into 5-hydroxytryptophan)
  - Aromatic amino-acids decarboxylase– AADC (5-hydroxytryptophan into serotonin), same enzyme catalyses L-DOPA into dopamine
- In epyphisis, retina:
  - 5-HT N-acetyltransferase turn serotonin into N-acetyl-serotonin and into **melatonin**


Tryptophan
tryptophan hydroxylase
5-hydroxytryptophan
aromatic amino acid decarboxylase
Serotonin (5-HT)
monoamine oxidase + aldehyde dehydrogenase
5-hydroxyindoleacetic acid
Pineal
5-HT N-acetyl-transferase
N-Acetyl serotonin
5-hydroxyindole-O-methyltransferase
Melatonin

Rate of Serotonin synthesis

- Positron emission tomography (PET)
- Rate in females: 47-55pmol/gmin
- Rate in males: 66-85 pmol/gmin

from: Nishizava, S. et al, *Proc. Natl. Acad. Sci.* USA, 1997



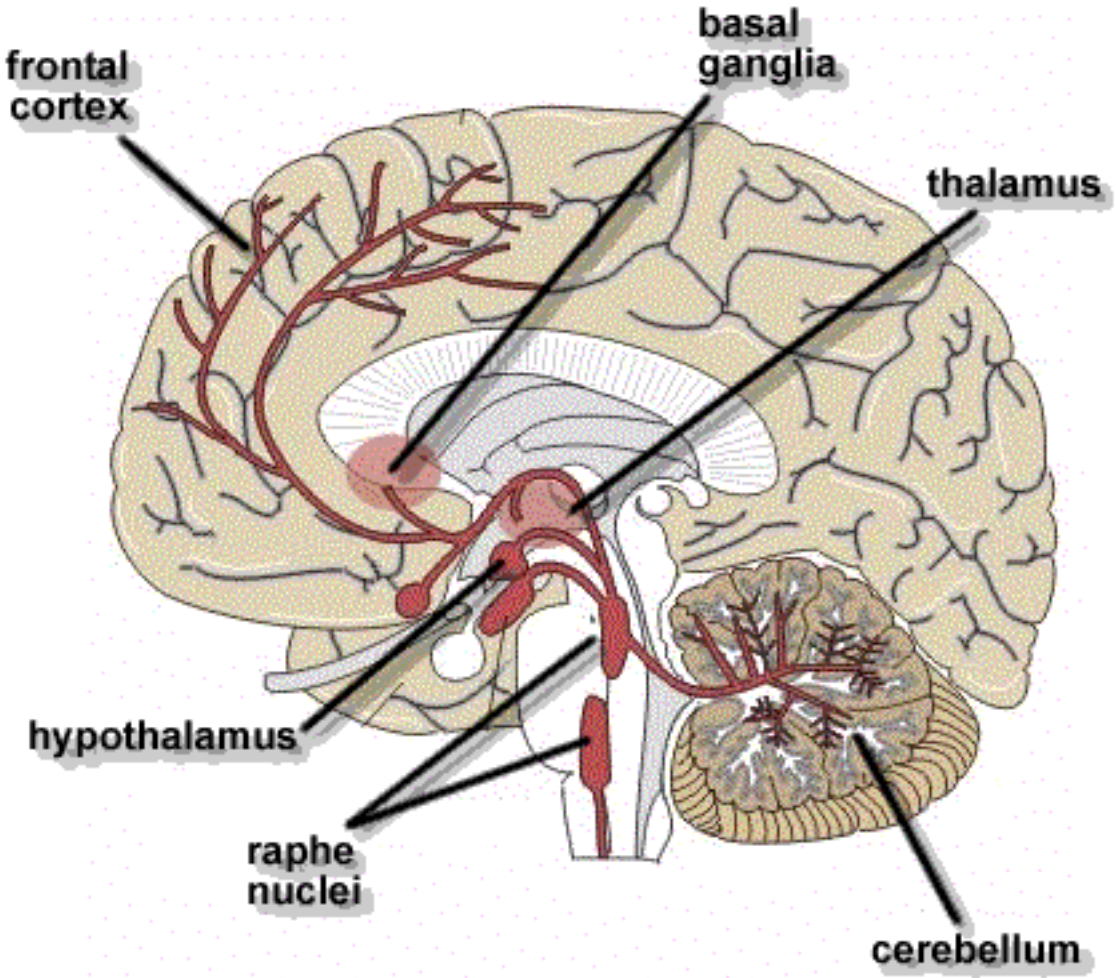
Rate of synthesis is 52% faster in men – better adjustment on stressful stimuli, smaller incidence of bipolar and depressive episodes.

from: Nishizava, S. et al, *Proc. Natl. Acad. Sci.* USA, 1997

Location of 5-HT neurons

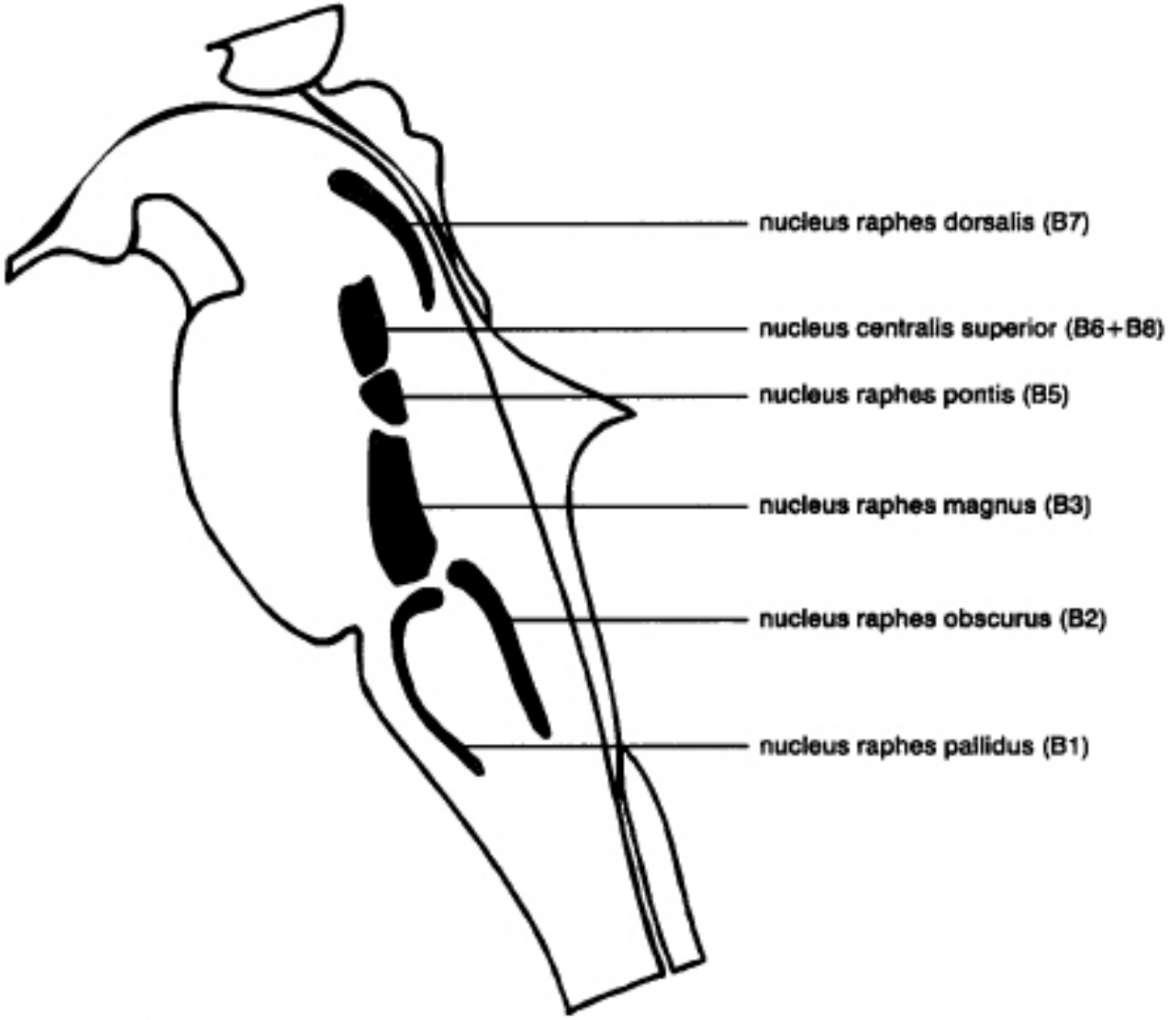
- In midline medulla – raphe nuclei (1964, Dalhstrome i Fuxe: B1-B9)
- Serotonergic neurons have been identified out of the raphe nuclei.
- Not all raphe neurons are serotonergic
- Their axons innervate many brain area

The serotinergic system consists of ascending axons from cell bodies in the raphe nuclei



Serotonergic neurons (B1-B9) – n. raphe

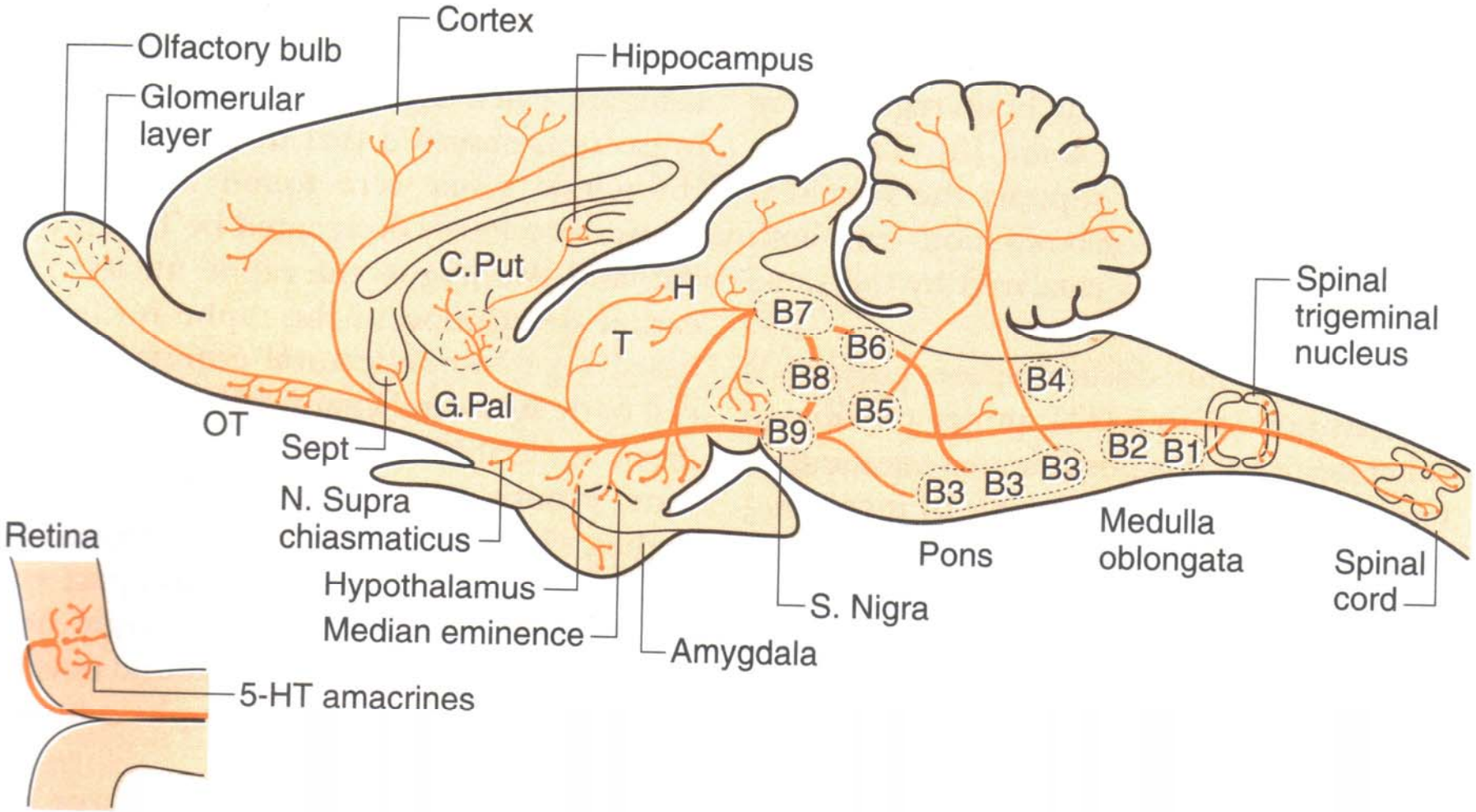
- ***Nuclei raphes*** - in the midline
  - ***Nucleus raphe pallidus*** ***(B1)***
  - ***Nucleus raphe obscurus*** ***(B2)***
  - ***Nucleus raphe magnus*** ***(B3)***
  - ***Nucleus raphe pontis*** **(B5)**
  - ***Nucleus centralis superior*** **(B6)**
  - ***Nucleus raphe dorsalis*** ***(B7)***



Slika 17-7. Serotoninske skupine neurona (B1 – B7) u moždanom deblu čovjeka. Za pojedinosti vidi tekst.

Ascending and Descending projections

- Ascending projection from rostral raphe nuclei into the cortex (B6, B7, B8, B9)
- Descending projection from caudal raphe nuclei into the spinal cord (B1 - B5)


Olfactory bulb
Glomerular layer
Cortex
Hippocampus
C.Put
H
T
G.Pal
OT
Sept
N. Supra chiasmaticus
Hypothalamus
Median eminence
Amygdala
S. Nigra
B7
B6
B8
B9
B5
B4
B3
B3
B3
B2
B1
Pons
Medulla oblongata
Spinal trigeminal nucleus
Spinal cord
Retina
5-HT amacrines

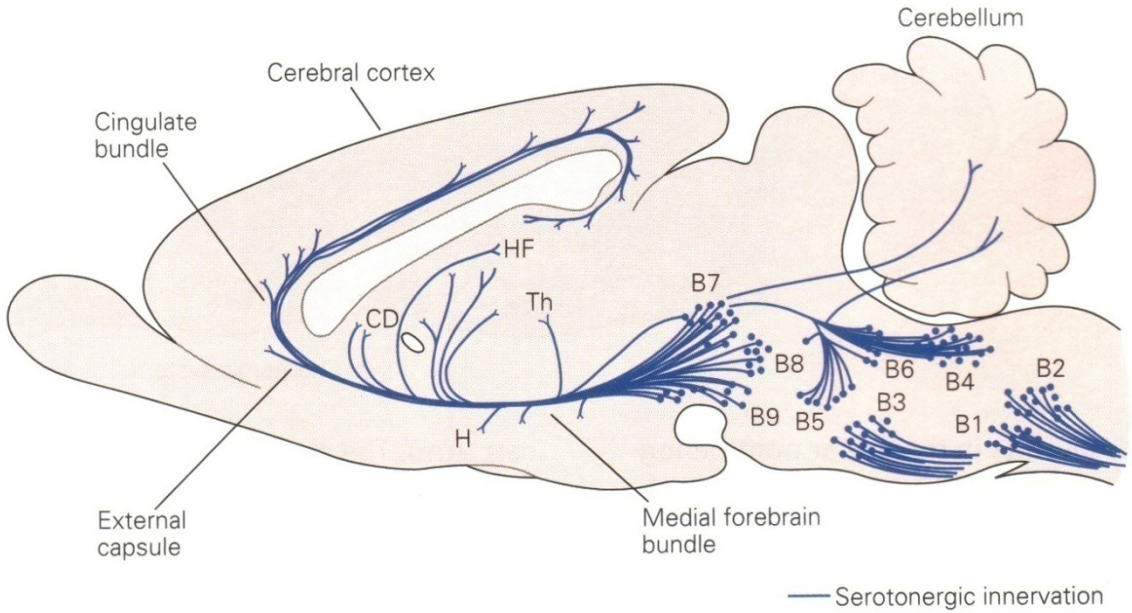
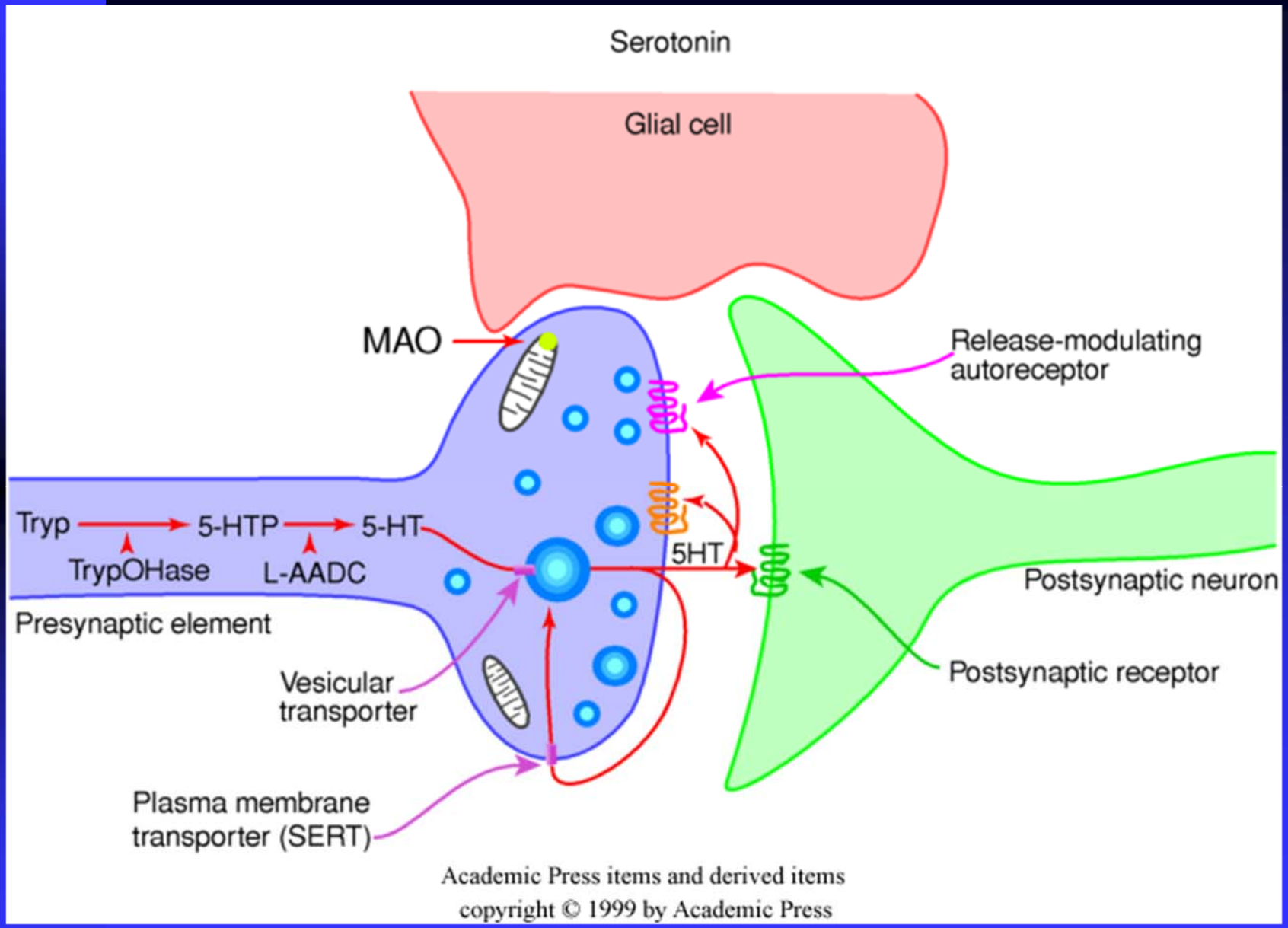


Figure 45-4 Serotonergic neurons along the midline of the brain stem. Neurons in the B1–3 groups, corresponding to the raphe magnus, raphe pallidus, and raphe obscurus nuclei in the medulla, project to the lower brain stem and spinal cord. Neurons in the B4–9 groups, including the raphe pontis, median raphe, and dorsal raphe nuclei, project to the upper brain stem, hypothalamus, thalamus, and cerebral cortex. **CD** = caudate nucleus; **HF** = hippocampal formation; **H** = hypothalamus; **Th** = thalamus.

Storage

- In small and medium vesicles of dense core
- Reserpine, tetrabenazine – inhibit transport protein in vesicular membrane.
- Leads to decreased concentration of 5-HT and CA in the brain
- Storage in the vesicules protects serotonin from intraneuronal enzymatic degradation



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Exocytosis

- Electric stimulation of serotonin neurons leads to egzocytosis of the serotonin
- active zone, cycle of synaptic vesicles, 3 groups of proteins
- Egzocytosis starts wit influx of Ca^{2+} into the presinaptic ending

Receptors

- 15 different types of receptor
- Metabotropic all except 5-HT_3

5-HT receptors

Receptor *	***Distribution***	***Effector mechanism***
5-HT_{1A}	Hippocampus, amygdala, septum, entorhinal cortex, hypothalamus, raphe nuclei	Inhibition of adenylyl cyclase, opening of K^+ channels
5-HT_{1B}		Inhibition of adenylyl cyclase
5-HT_{1Da}	Not distinguishable from 5-HT_{1Db}	Inhibition of adenylyl cyclase
5-HT_{1Db}	Substantia nigra, basal ganglia, superior colliculus	Inhibition of adenylyl cyclase
5-ht_{1E}	?	Inhibition of adenylyl cyclase
5-ht_{1F}	Cerebral cortex, striatum, hippocampus, olfactory bulb	Inhibition of adenylyl cyclase
5-HT_{2A}	Claustrum, cerebral cortex, olfactory tubercle, striatum, nucleus accumbens	Stimulation of phosphoinositide-specific phospholipase C (IP3/DAG), closing of K+ channels
5-HT_{2B}	?	Stimulation of phosphoinositide-specific phospholipase C (IP3/DAG)
5-HT_{2C}	Choroid plexus, globus pallidus, cerebral cortex, hypothalamus, septum, substantia nigra, spinal cord	Stimulation of phosphoinositide-specific phospholipase C (IP3/DAG)

5-HT receptors

Receptor	Distribution	Effector mechanism
5-HT_3	Hippocampus, entorhinal cortex, amygdala, nucleus accumbens, solitary tract nerve, trigeminal nerve, motor nucleus of the dorsal vagal nerve, area postrema, spinal cord	Ligand-gated cation channel
5-HT_4	Hippocampus, striatum, olfactory tubercle, substantia nigra	Stimulation of adenylyl cyclase
5-ht_{5A}	?	Inhibition of adenylyl cyclase
5-HT_{5B}	?	?
5-ht_6	?	Stimulation of adenylyl cyclase
5-HT_7	Cerebral cortex, septum, thalamus, hypothalamus, amygdala, superior colliculus	Stimulation of adenylyl cyclase

*Lower-case appellations are used in some cases because the functions mediated by these receptors in intact tissue are presently unknown.

Autoreceptors

- Serotonergic neurons have somatodendritic autoreceptors – mainly 5-HT_{1A} receptors
- Agonist of 5-HT_{1A} receptors: 8-OH-DPAT injected in Dorsal raphe nucleus decreases activity of serotoergic neurons!
- 5-HT_{1B} and 5-HT_{1D} autoreceptors – modulate release of serotonin
- 5-HT_{1B} and5-HT_{1D} on a postsynaptic membrane modulate release of Ach and DA

Mechanizms of inactivation

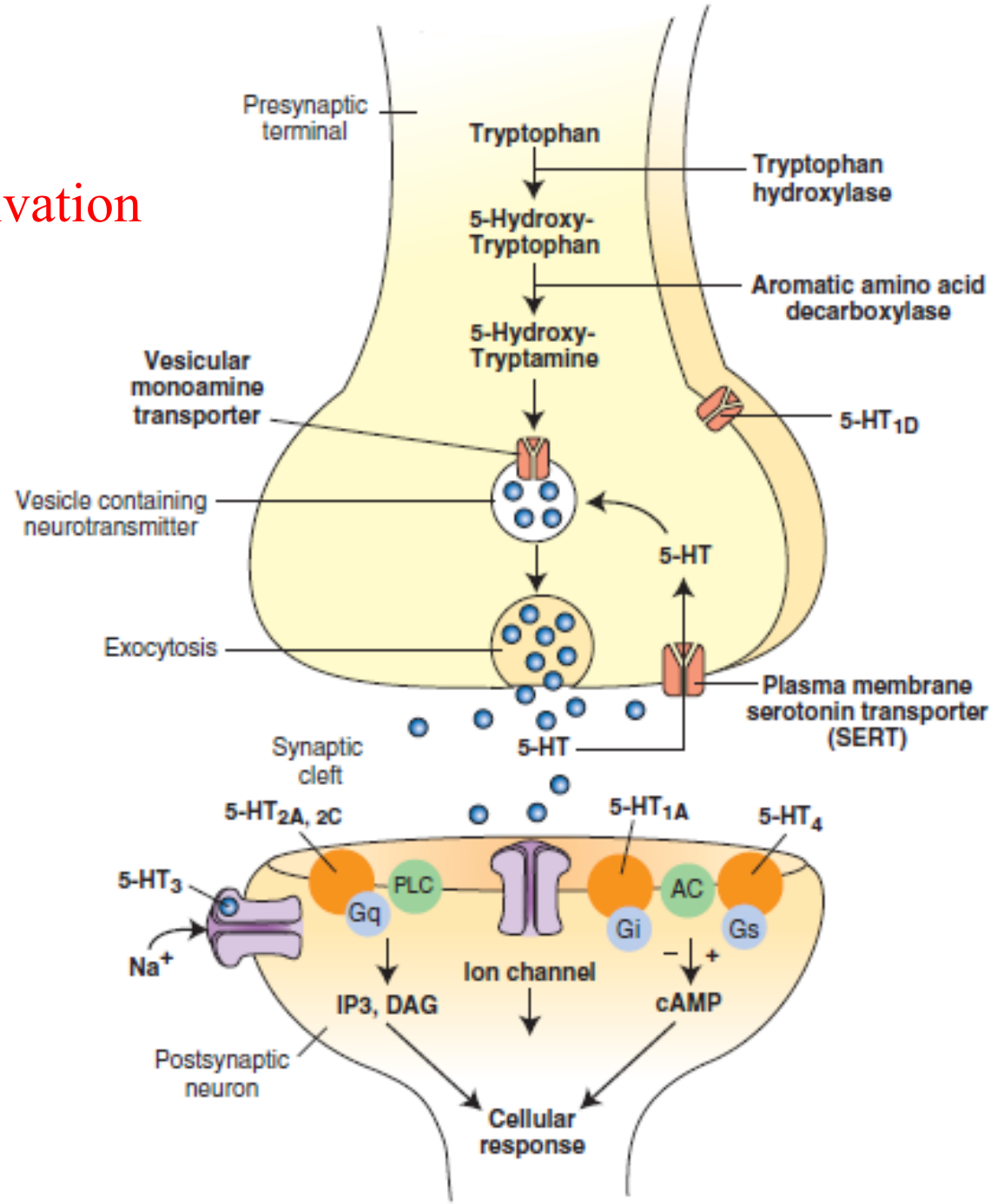


FIGURE 8–14 Steps involved in the synthesis and release of serotonin. The distribution of some 5-HT receptors on different components of the serotonergic synapse is also shown. AC = adenylyl cyclase; cAMP = cyclic adenosine monophosphate; DAG = diacylglycerol; Gi, Go, Gq = different G-proteins; IP3 = inositol triphosphate; 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{3}, 5-HT_{4} = different 5-HT receptors.

Mehanisms of inactivation

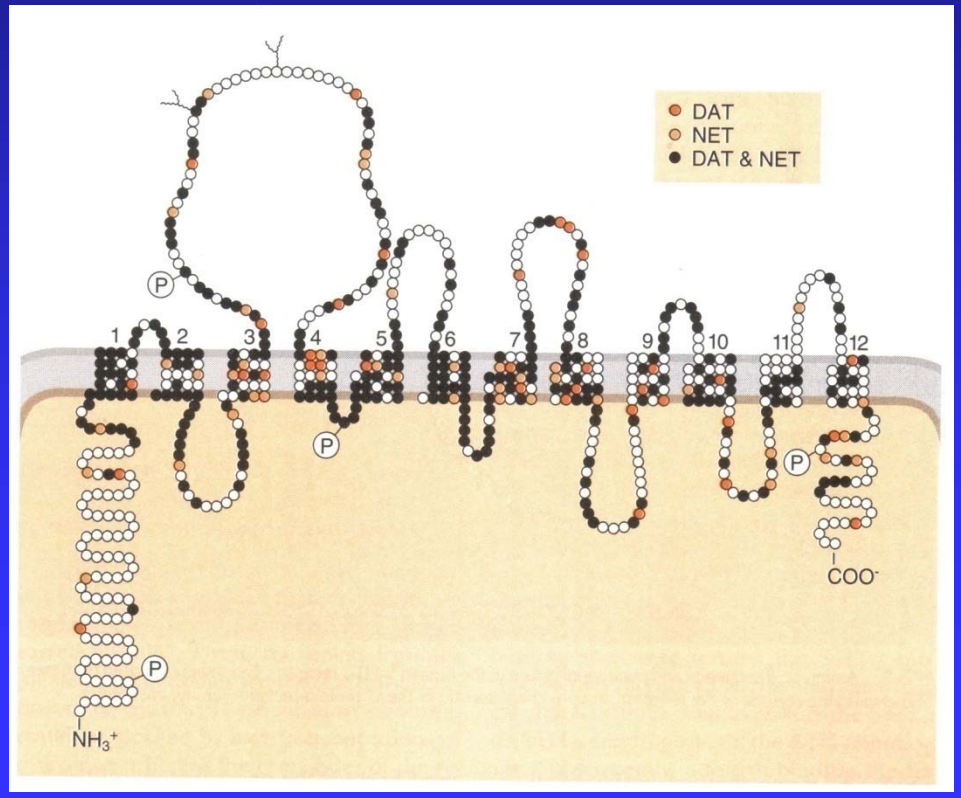
- *Intracellular degradation* of serotonin with the enzyme monoamine oksidase-MAO
- *Re–uptake from the synapse* transport protein located in the membrane of the serotonergic neuron

Degradation of serotonin

- Monoamine oksidase (MAO)
- 2 izoenzymes: MAO type A and MAO type B
- 5-HT neurons contain more MAO B
- catecholamine neurons contain more MAO A
- Deprenyl - inhibitor MAO B
- Clorgyline i moclobemide – inhibitors of MAO A

Reuptake of serotonin

- Serotonin reuptake transporter (SERT) has 12 transmembrane domains



Reuptake of serotonin

- Depends on extracellular Na^+ and Cl^-
- Depends on intracellular K^+
- Energy needed for concentration gradient
- Na^+, 5-HT and Cl^- bind from the outside
- Protein changes its conformation, brings serotonin into the neuron
- K^+ binds on the inner side of the membrane

Inhibition of serotonin reuptake

- Competitive inhibitors of serotonin reuptake – Selective Serotonin Reuptake Inhibitors (SSRI)
- Antidepressives:
  - SSRI - *fluoxetine*, *sertraline*
  - Tricyclic antidepressant drugs –inhibitors of the uptake of NE: imipramine, amitriptyline, klomipramine
- *sibutramine* – simpatomimetic

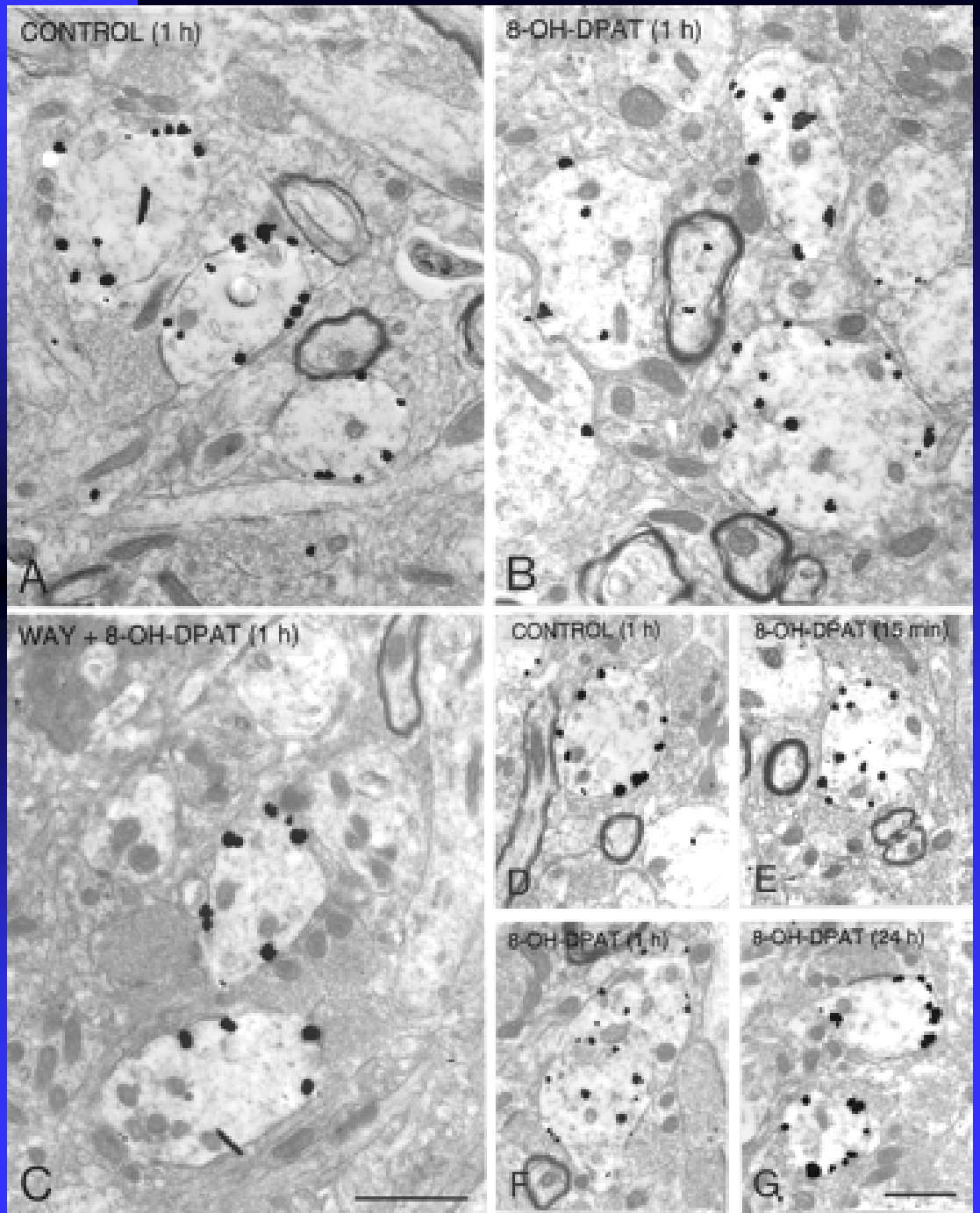
Desenzitisation of receptors

- Prolonged exposure of a receptor to endogenous or exogenous agonist reduces the responsivines of the receptor.
- NE + β adrenoreceptor G-protein is stimulated
- cAMP is formed protein kinaze A is stimulated
- β adrenorecptor is phosphorylated and uncoupled from the G-protein
- The receptor no longer responds to the agonist

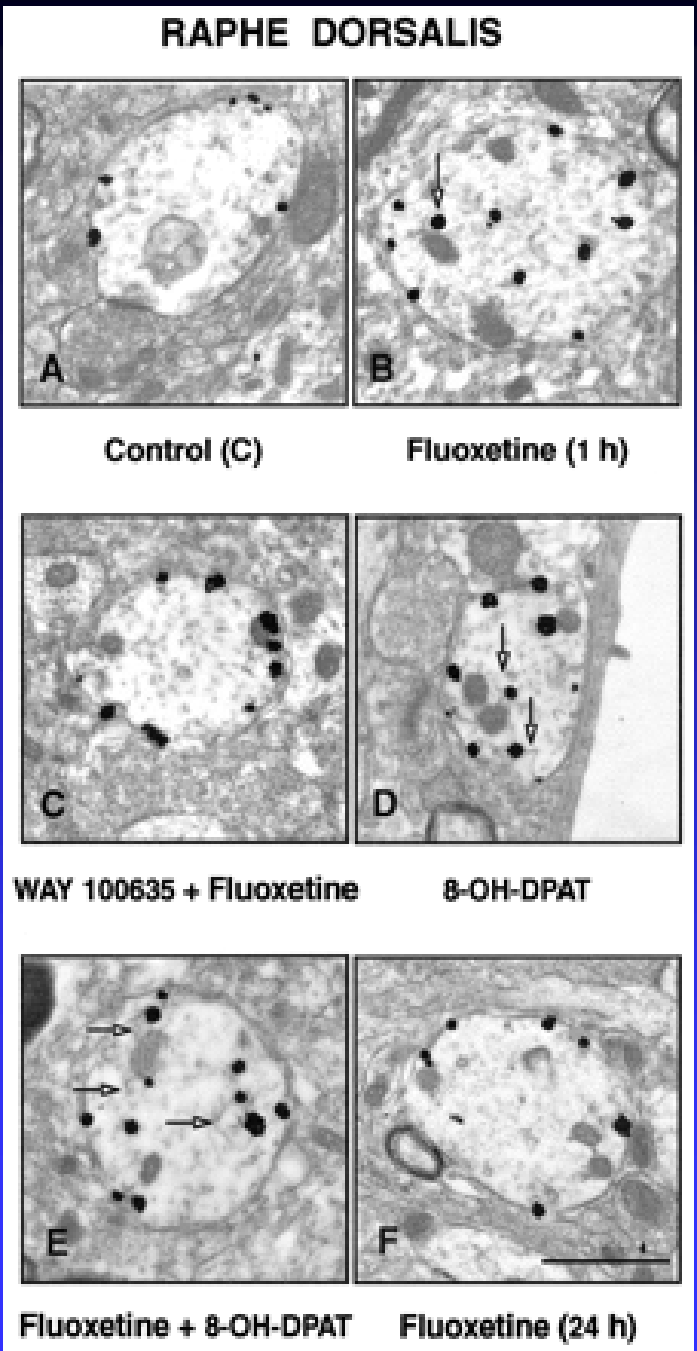
Down-Regulation

- When the number of receptors decreases.
- Receptors are internalized and sequestered inside the cell.
- Example: 5-HT receptors

INTERNALISATION 5-HT rec (8-OH-DPAT)



-Immunogold labeling of 5-HT_{1A} receptors in dendrites from the NRD - Riad et al:Internalization of 5-HT_{1A} receptors, JNeuoscience, 2001



INTERNALIZATION5-HT rec (fluoxetine)

-Immunogold labeling of 5-HT_{1A} receptors in dendrites from the NRD- Riad et al: Internalization of 5-HT_{1A} receptors

HETEROLOG DESENSITISATION OF 5-HT receptora

- 5-HT_{1A} and 5-HT_{2A} receptors are colocalized on neurosecretive neurons (oxytocine, CRF) in paraventricular nucleus of the hypothalamus
- Injection of the 5-HT_{2A} receptor agonist in paraventricular nucleus of the hypothalamus decreases sensitivty of the neurons on 5-HT_{1A} receptors agonist (decreased release of oxytocin)
- Mechanism of desensitisation is not known

Zhang et al, 2004

Medications

- Antidepressant drugs
- Anxiolytic drugs
- Antipsychotic drugs
- Antiemetic drugs (against vomiting and nausea)
- Hallucinogens

Antidepressant drugs

- Tricyclic antidepressant (TCA)
  - inhibit reuptake of NA and 5-HT
  - imipramine, amitriptyline, klomipramine
- Selective Serotonin Reuptake Inhibitors (SSRI)
  - fluoxetine (Prozac), sertraline (Zoloft)
  - Fluoxetine side-effects:
    - stimulation of 5-HT_{1A} autoreceptors – decreased endogenous production end secretion of serotonin in the first 2-3 weeks
    - Stimulation of 5-HT_2 and 5-HT_3 postsynaptic receptors – decreased secretion of DA u SN – parkinsonism, reproductive disfunction

- Inhibitors of monoamine-oksidaze (MAOI)
  - phenelzine, isocarboxazid, klorgiline

Anxiolitics

- TCA
  - klomipramine
- Benzodiazepines
  - increase the activity of GABA via $GABA_A$ receptors
  - diazepam, lorazepam
- Agonists of $5\text{-}HT_{1A}$ receptors
  - buspirone, gepirone

- Wilken et al, 1997: buspirone used in the treatment of respiratory disturbance (apneusis) after neurosurgical procedure.

Antipsychotic drugs

- Typical antipsychotic
  - antagonists of dopamine D1 receptors
  - chlorpromazine, haloperidol
- Atypical antipsychotic drugs
  - antagonists of 5-HT_2 and 5-HT_{1C} receptors
  - clozapine, ritanserine

- Agonist of 5-HT_{1D} and 5-HT_3 *sumatriptan* – effective in the acute treatmant of migraine headaches
- Antagonists of 5-HT_3 receptors such as *ondansetron* and *granisetron* drugs for treatment of nausea and emesis (vomiting) in cancer patients receiving chemotherapy.

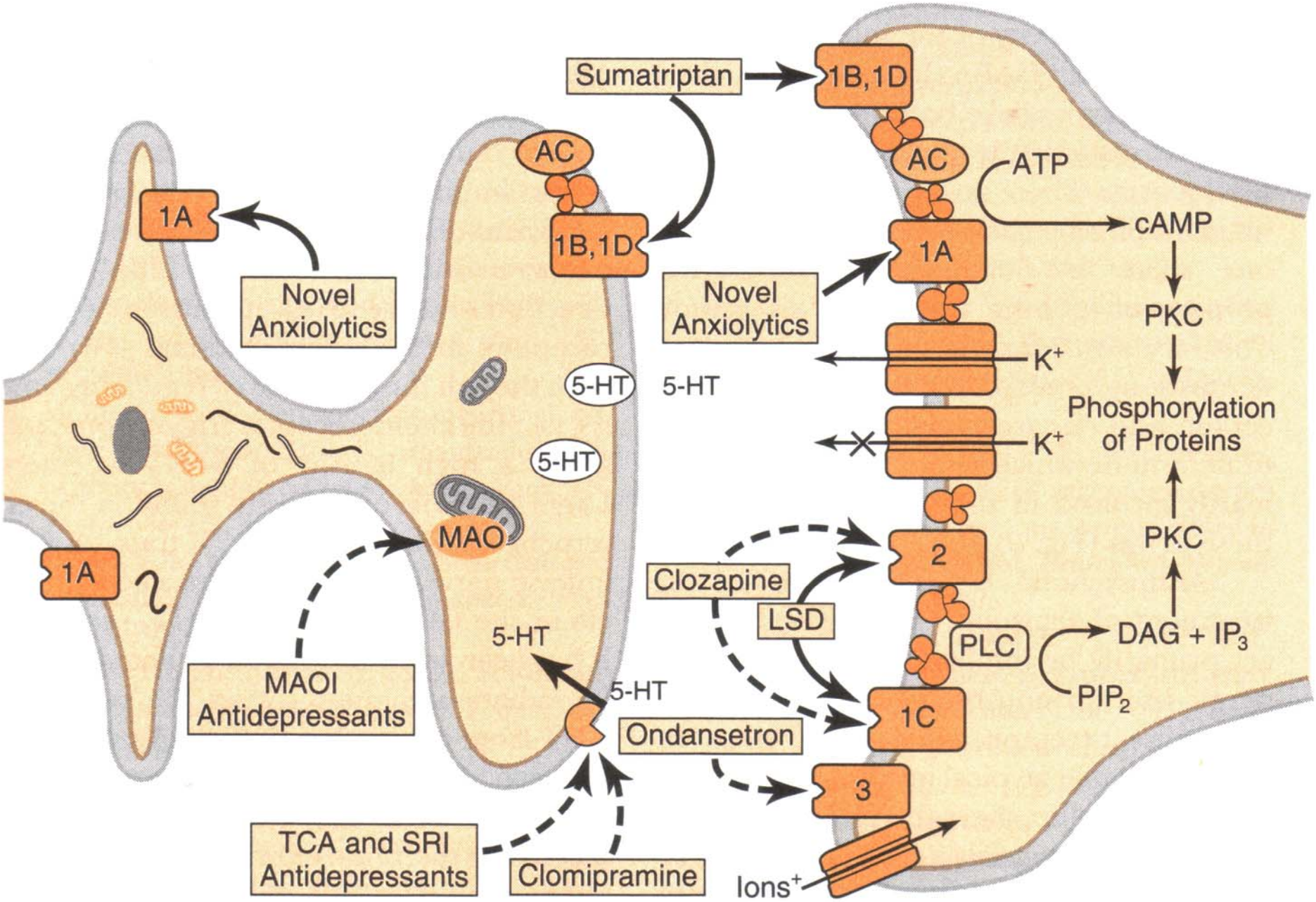
Serotonin and Hallucinogens

- LSD
  - agonist of 5-HT_2 and 5-HT_{1C} receptors
  - in parietofrontal cortex, the limbic system, and brainstem
  - agonist of 5-HT_{1A} 5-HT_{1B}/5-HT_{1D} autoreceptors in raphe nucleus

Serotonin and Hallucinogens

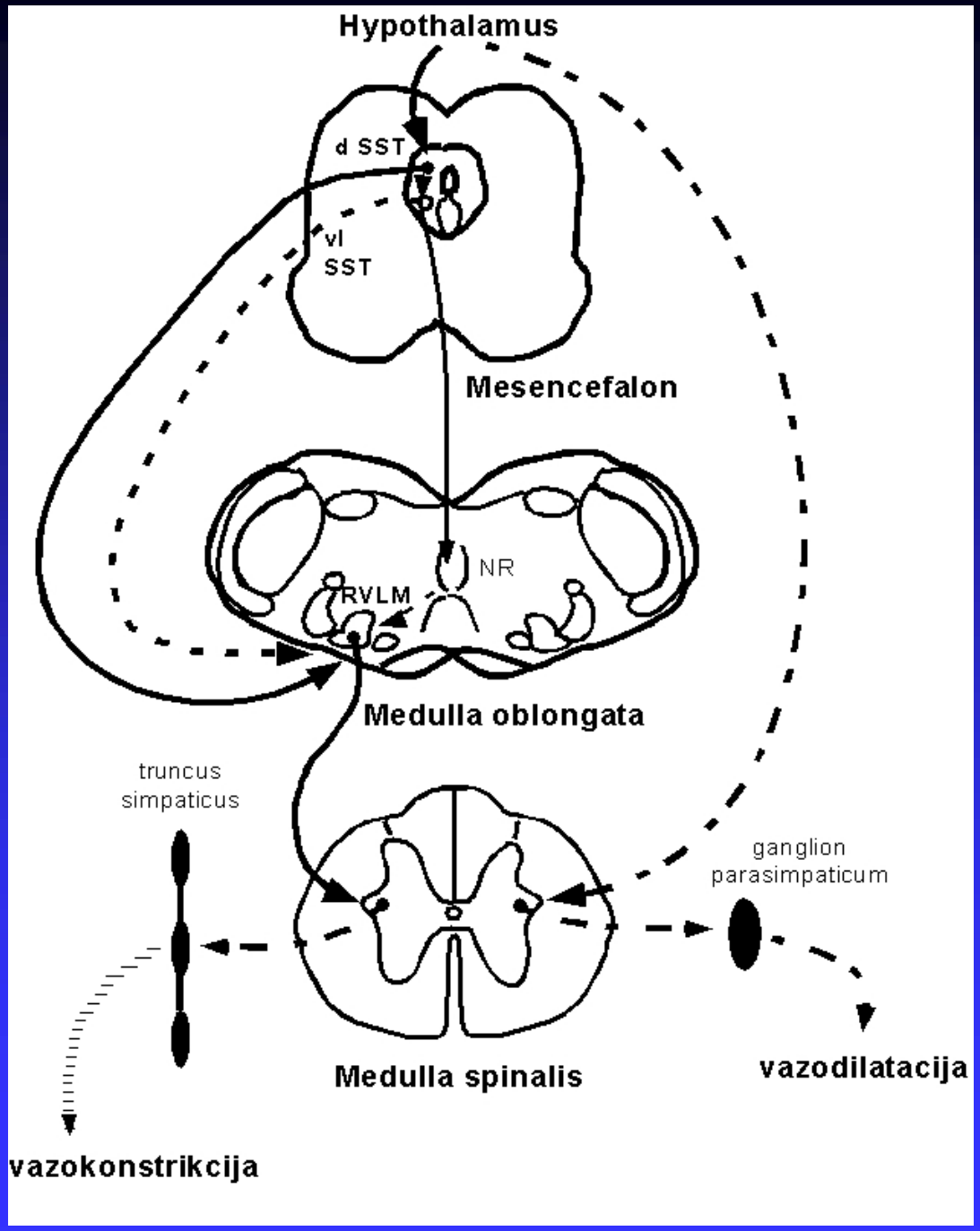
- MDMA (3,4- methylendioxy-methamphetamine, "ecstasy")
  - increases the activity of 5-HT and DA
  - positive effects are followed by "rebound effect" within the few next days, along with cognitive impairments.
  - neurotoxic – degeneration of 5-HT pathways

- Ecstasy – axons from dorzal raphe nucleus are more sensitive to the toxic effects of MDMA than axons from median raphe nucleus.
- Damage of the 5-HT projection to the frontal and pre-frontal cortices – impairment of current planning, coordination and executing commands
- Possible damage of hippocampal complex


Sumatriptan
1B,1D
AC
ATP
cAMP
1A
Novel Anxiolytics
PKC
K+
5-HT
5-HT
Phosphorylation of Proteins
K+
5-HT
MAO
PKC
2
Clozapine
LSD
PLC
DAG + IP3
5-HT
5-HT
PIP2
1C
MAOI Antidepressants
Ondansetron
3
TCA and SRI Antidepressants
Clomipramine
Ions+

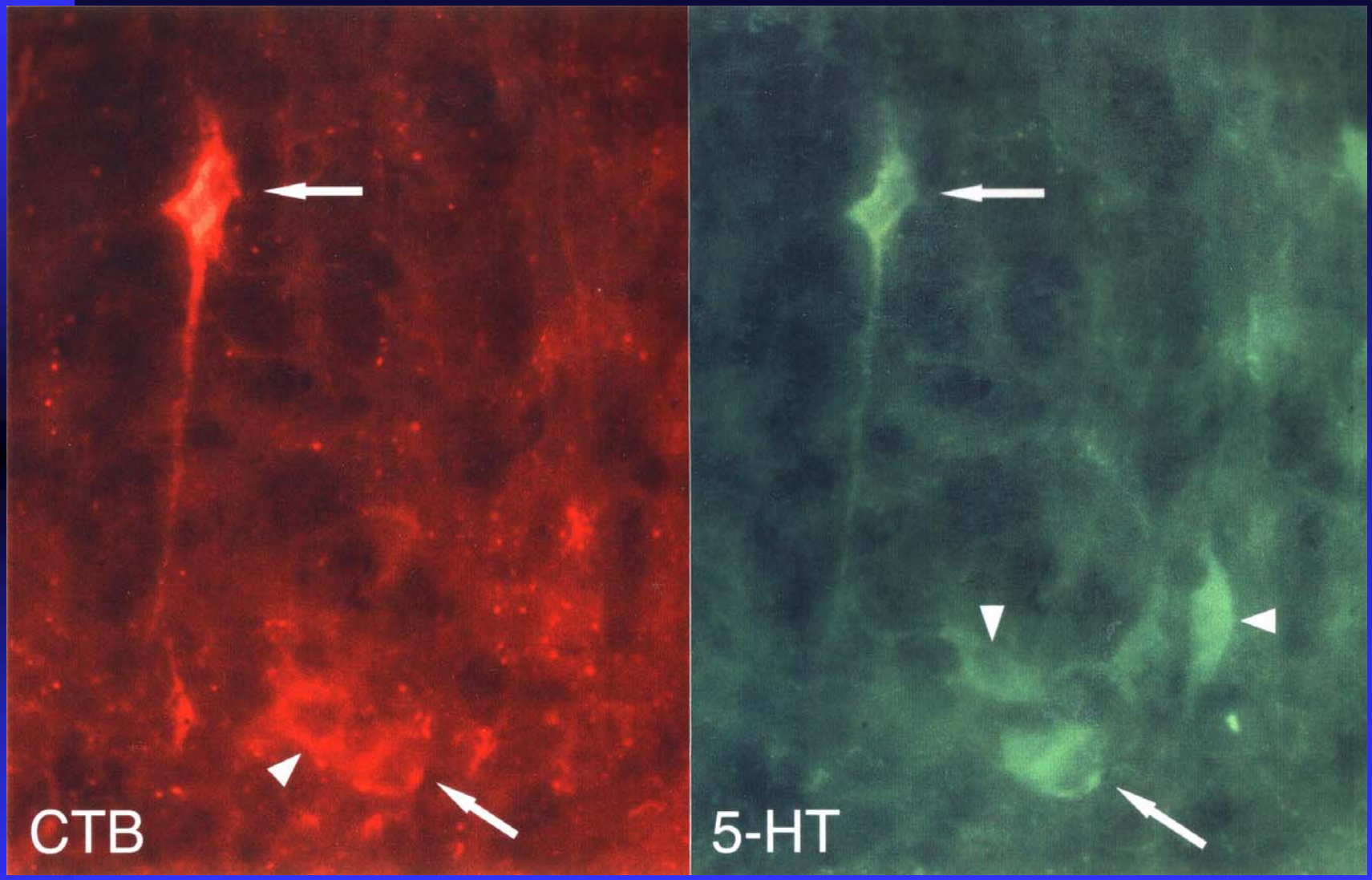
Serotonin and cardiovascular regulation

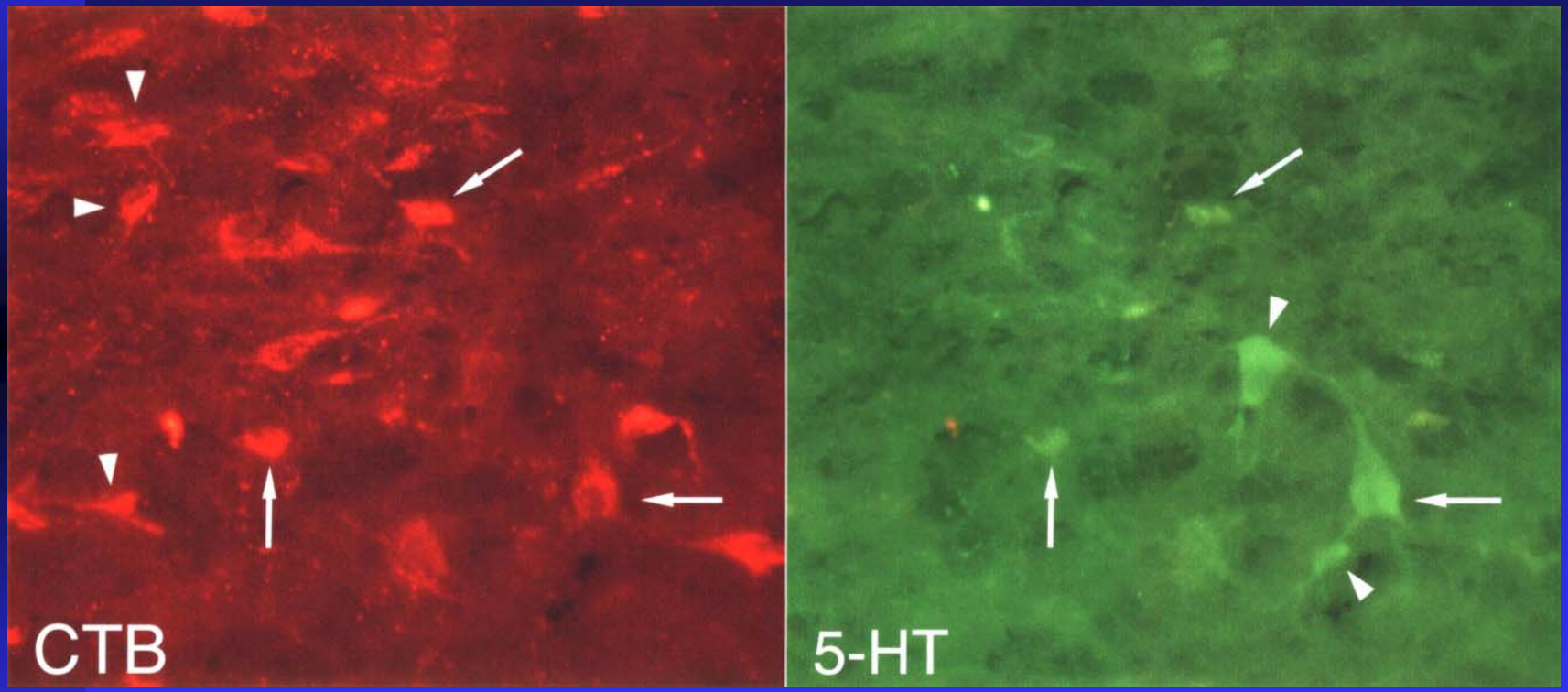
- Intravenous application of serotonin gives contradictory results.
- Central (into the brain) injections of selective agonists or antagonists offer more consistent results.
- Role of 5-HT_{1A} receptora in the medulla:
  - inhibition of sympathetic nervous system
  - hypotension
  - acute hemorrhage


Hypothalamus
d SST
vl
SST
Mesencefalon
NR
RVLM
Medulla oblongata
truncus
simpaticus
ganglion
parasimpaticum
Medulla spinalis
vazodilatacija
vazokonstrikcija

Where is the source of serotonergic projection into the RVLM?

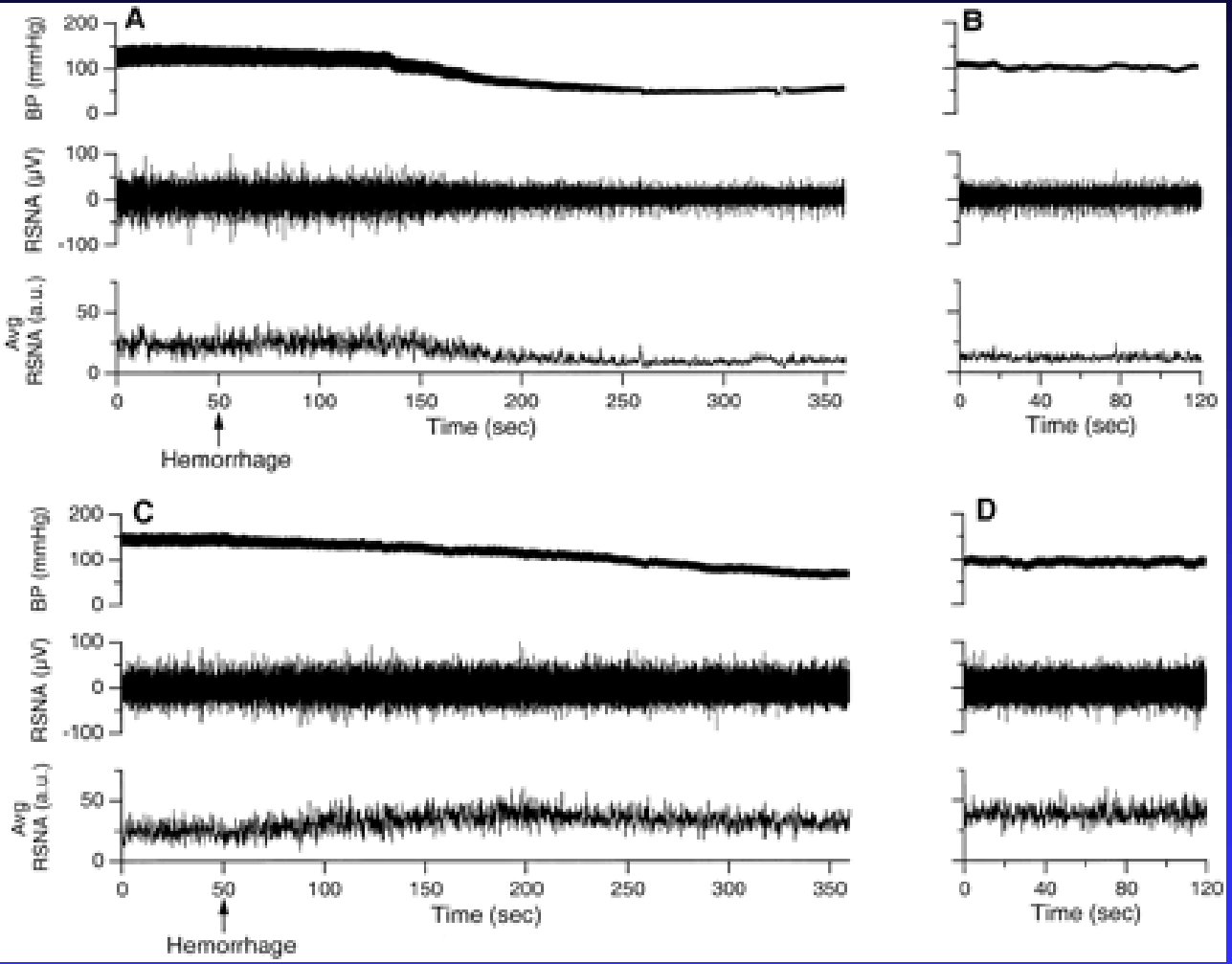
- Retrograde labeling can determine the source – usage of Cholera toxin B
- Double immuno–labeling to determine if these projections contain serotonin


CTB
5-HT


CTB
5-HT

Hemorrhage

- Acute hemorrhage leads to hypotension and sympathoinhibition
- Antagonists of serotonin given iv and blockade of serotonin synthesis diminish hypotension produces by acute hemorrhage.
- Antagonosts of 5-HT_{1A} receptors into the RVLM decrease hypotension and sympathoinhibition evoked by acute hemorrhage


A
B
C
D
BP (mmHg)
200
100
0
RSNA (μV)
100
0
-100
Avg RSNA (a.u.)
50
0
0
50
100
150
200
250
300
350
Time (sec)
Hemorrhage
0
40
80
120
Time (sec)

Serotonin and respiration

- serotonergic neurons as CO_2 sensor
- “central respiratory chemoreceptors”
- role in acidosis
- role in hypoxia
- 5-HT neurons stimulate breathing
- Buspiron - Agonist of 5-HT_{1A} receptors

Serotonin and feeding

- stronger in females
- agonist fenfluramin
  - decreases appetite
- agonist of 5-HT_{1C} and 5-HT_{1B} receptors
  - decreases appetite
- agonist of 5-HT_{1A} receptors
  - increases appetite
  - via autoreceptors – decreased secretion of serotonin

Serotonin and sleep-wake cycle

- 5-HT was first associated with the initiation and maintenance of sleep:
  - destruction of the raphe nuclei leads to insomnia
  - inhibition of 5-HT synthesis leads to insomnia

- **Jouve (1983): 5-HT released during wake might induce and/or liberation of hypnogenic factors that would be secondarily responsible for sleep.**

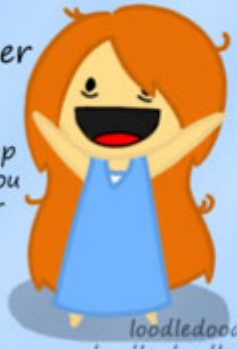
Stages of Sleepiness

Tired
Where you yawn occasionally, but you still have enough energy to stay up.

Slightly Sleepy
Where most people can tell you're tired, and you yawn constantly.

Extremely Hyper
(Only for some people)

Where your body somehow gathered up enough energy for you to abuse, which later ends up in a crash.



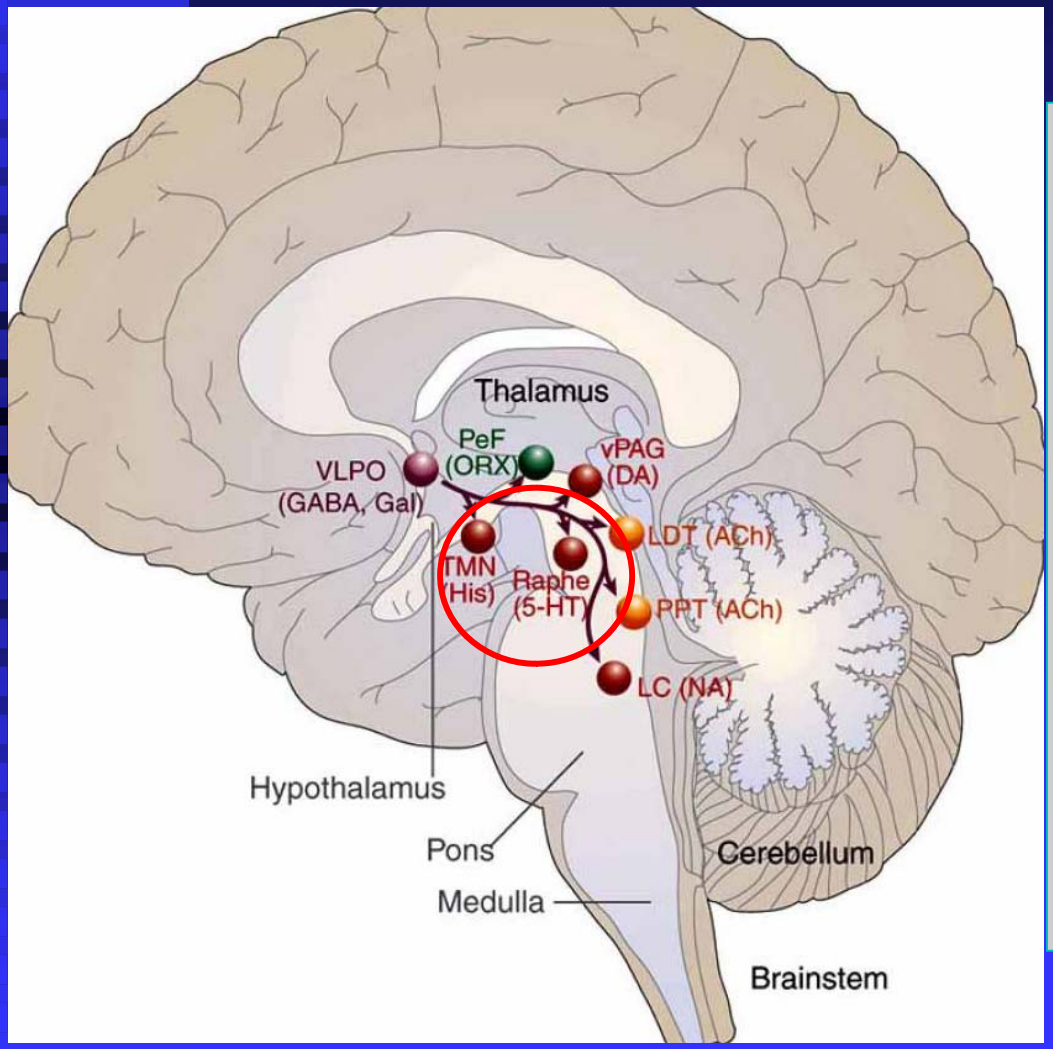
Super Sleepy
Where you could barely keep your eyes open, and it's obvious that you're extremely tired.

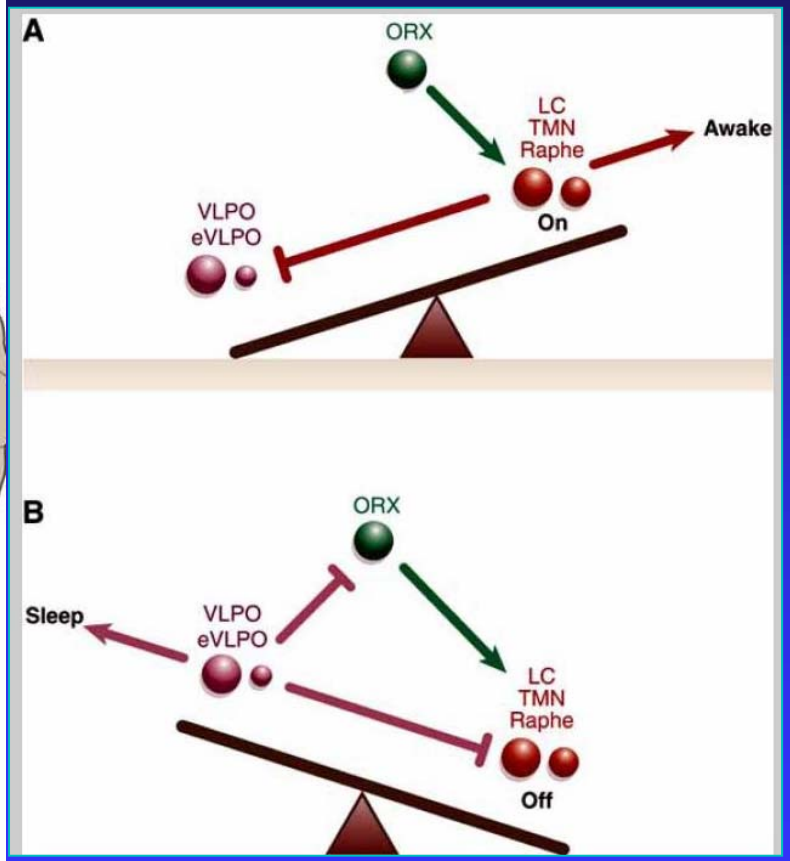
loodledoodles.tumblr
doodle: loodledoodlez.tumblr

Cranky
Where you're beyond tired and could probably kill someone if you don't get your sleep.

Knock Out
Where you fall asleep almost instantly and almost anywhere.

“Flip-flop” mehanizam

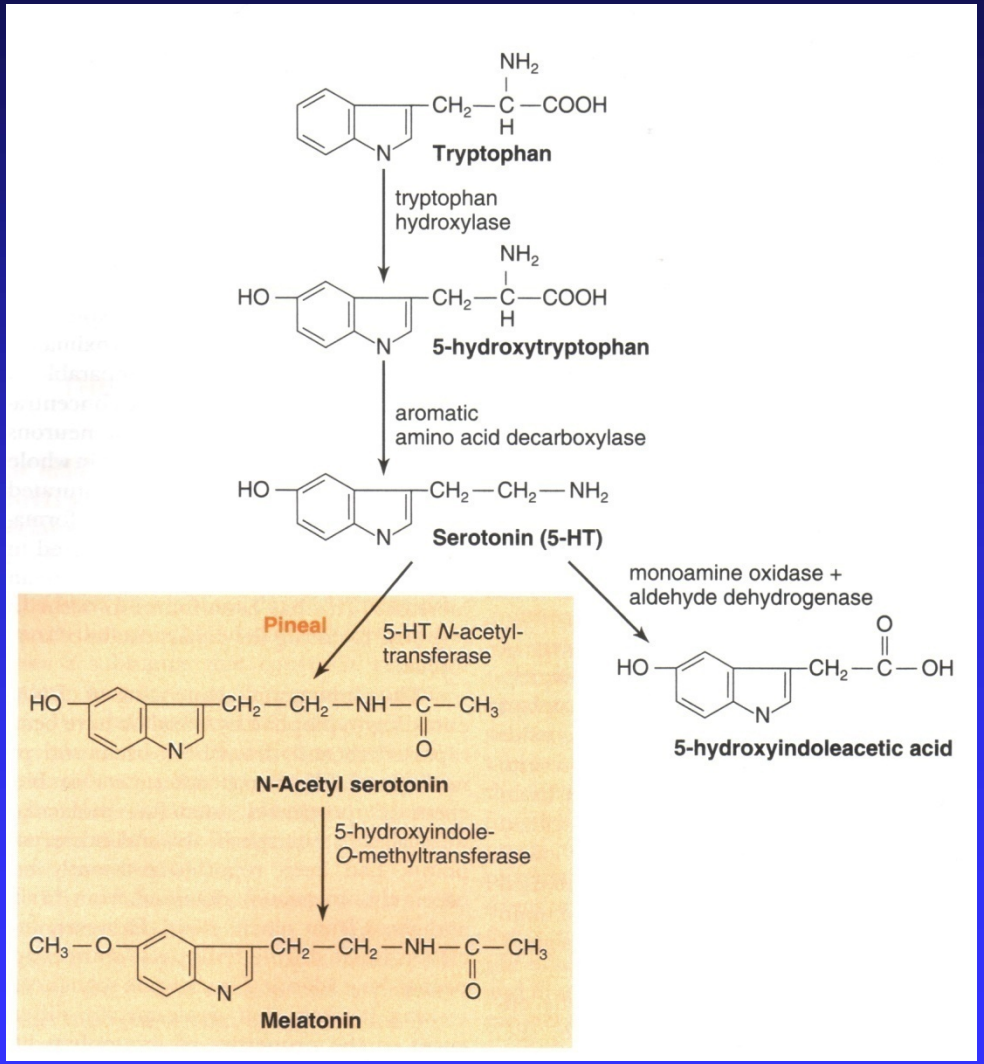


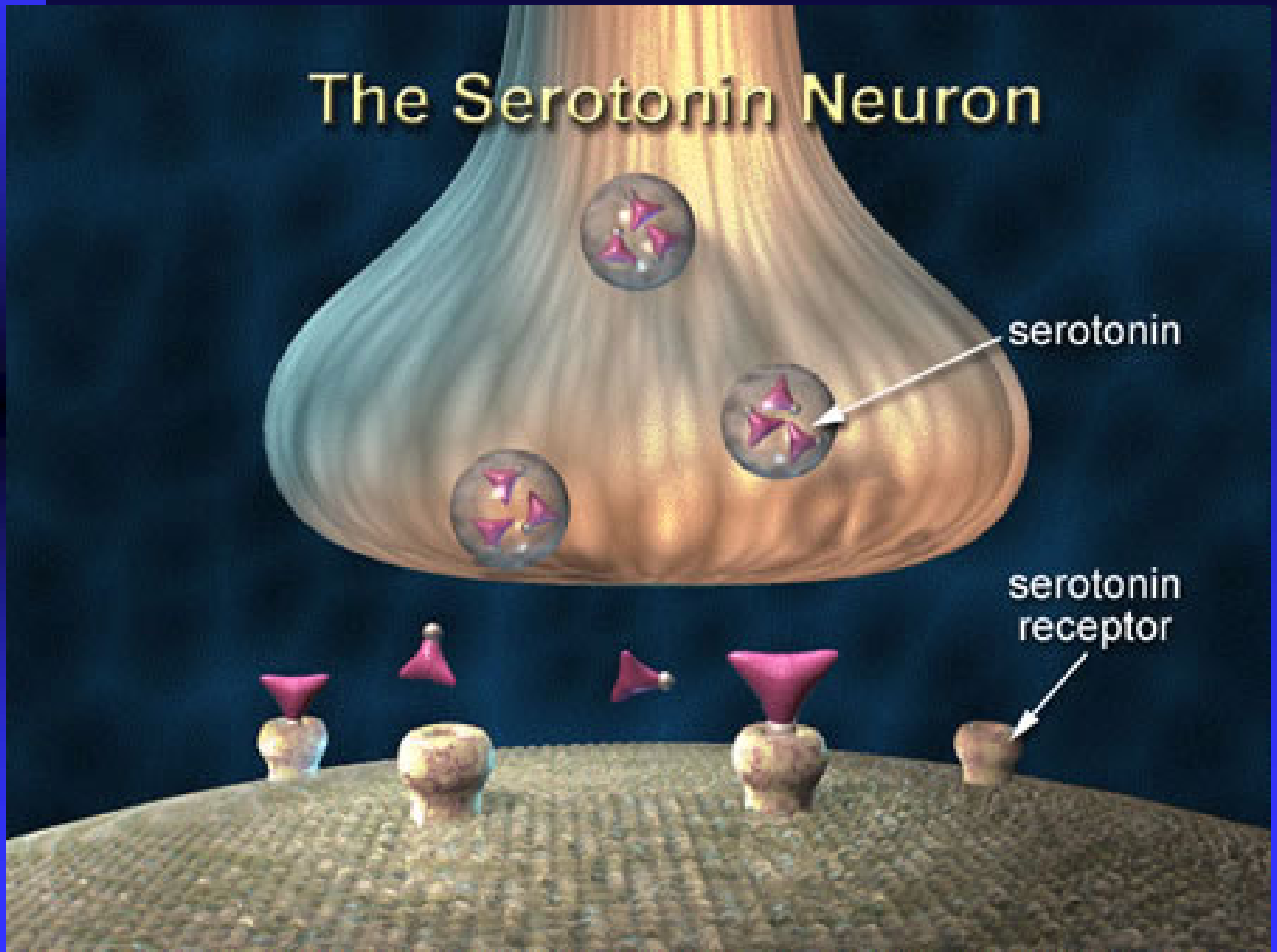


- SSRI – affect sleep:
  - Fluoxetin - reduces REM sleep, prolonges sleep latency, shortening of slow-wave sleep

Melatonin

- produced by the pineal gland
- Production of melatonin by the pineal gland is inhibited by light
- onset each evening is called the dim-light melatonin onset
- sleep




The Serotonin Neuron
serotonin
serotonin receptor