Cell & Molec. Neuro. Tools:

How to measure & localize gene/protein expression?

How to knock down/up specific gene?

How to identify/target neurons?

How to target a specific neuron type?

How to kill a neuron?

synthetic pathway?
degradation pathway?
where are components located?

Monoamines

1. Review Basic Monoamine Neurochemistry

- synthetic pathway?
- degradation pathway?
- where are components located?

2. Implications for gene expression/protein localization by monoamine neurons:

- Saporin-DBH antibodies to lesion norepi & epi cells
- Specific knockout of dopamine vs. norepi & epi
- Transcriptional control of serotonergic genes

Biogenic Amines

Catecholamines, Serotonin, & Histamine

Localization, Anatomy

Synthesis & Degradation

Regulation

Receptors

Drugs

Disorders/Model Systems

Monoamines

catecholamines:
- **dopamine**
- **norepinephrine**
- **epinephrine**

indolamines
- **serotonin**
- **melatonin**

modified amino acids (tyrosine, tryptophan) that act as neurotransmitters or hormones

Catecholamines

catechol group with an amine attached

dopamine

norepinephrine (noradrenaline)

epinephrine (adrenaline)

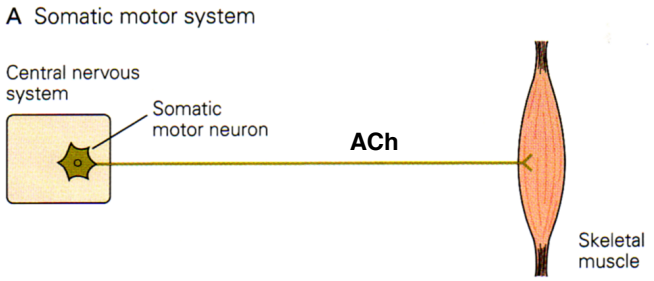
modified amino acids that act on 7-transmembrane domain G-protein-coupled receptors

Peripheral Anatomy of Catecholamines

Sympathetic nervous system

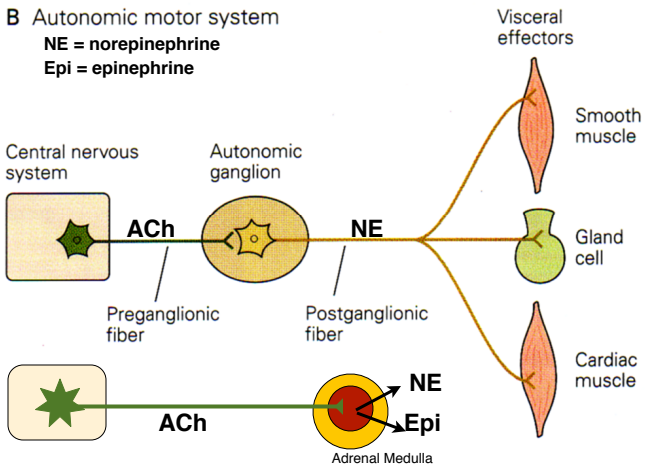
- **part of autonomic nervous system**
- **"fight or flight response" -- ability to expend energy**
- **preganglionic motor neurons in lateral horn of spinal cord**
- **short axons synapse onto pre- or para-vertebral column**
- **release acetylcholine onto postganglionic neurons**
- **postganglionic neurons release norepinephrine onto target tissues**
- **also release acetylcholine onto adrenal medulla of adrenal gland (a modified sympathetic ganglion) to cause release of norepinephrine and epinephrine into the bloodstream.**

(several notable exceptions to use of Norepi by SNS)



ACh = Acetylcholine

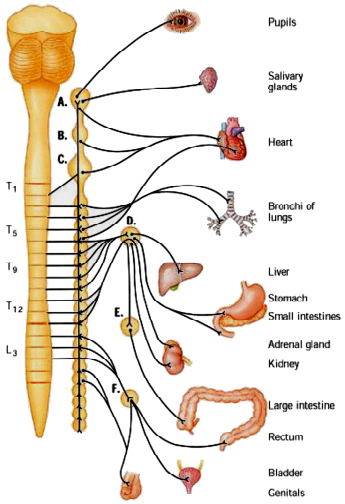
Sympathetic:

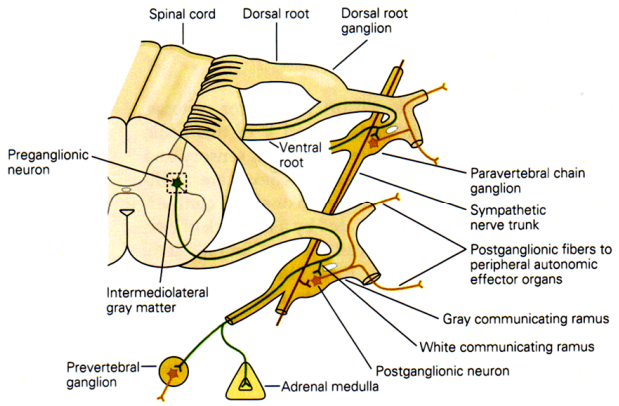


Sympathetic Nervous System

Nerves from spinal cord run to chain ganglia and then to glands and smooth muscle

mobilize energy
divert blood to muscle
prepare to fight/flee





Effects of Norepi/Epi on Peripheral Tissues

via adrenergic receptors

- **bronchial airways expand**
- **intestinal peristalsis halts**
- **heart rate and cardiac output increases**
- **vasodilation in striated muscle, heart, lung**
- **vasoconstriction in smooth muscle (gut, skin)**

NB: same transmitter can have opposite effects on different target tissues

Epinephrine more potent than Norepi

Epinephrine causes glucose release from liver

Norepinephrine

Adrenergic α_1	Contractile effects of NE on smooth muscle, especially blood vessels, urogenital, and sphincter muscles	↓ cAMP
α		Blocker: Yohimbine
Adrenergic α_2	Presynaptic control (inhibitory) of release of NE, ATP, and ACh from nerve terminals	
Adrenergic β_1	Stimulatory effects of NE and circulating epinephrine on heart	
Adrenergic β_2	Relaxant effects of NE on smooth muscle in gastrointestinal tract, urinogenital system, and airways	↑ cAMP
β		Blocker: Propanolol Agonist: Isoproterenol
Adrenergic β_3	Stimulate release of free fatty acids from adipose tissue	

Pheochromocytoma model system for catecholamines

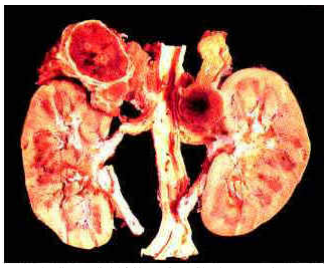
tumor of adrenal medulla

oversecretion of norepinephrine and epinephrine

hypertension, headache, diabetes mellitus, panic attacks

rare in humans, common in rats

PC12 cells are an immortalized adrenal cell line for studying catecholamines



http://www.medical-definitions.net/images/Pheochromocytoma.jpg

Central Anatomy of Catecholamines

Limited to discrete nuclei ("A" and " C" groups) with long projection axons

(different from Glutamate and GABA, which are ubiquitous)

Dopamine

- **Ventral Tegmental Area = reward, cognition**
- **Substantia Nigra = motor control**
- **hypothalamus = pituitary control**

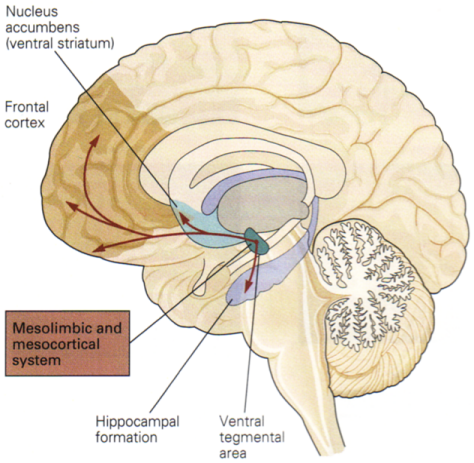
Norepinephrine

- **brainstem = cardiovascular, autonomic nuclei**
- **Locus Ceuruleus = altertness and stress**

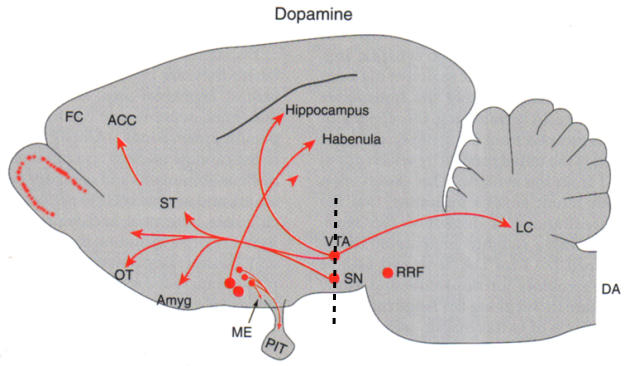
Epinephrine *C1 & C2*

- **brainstem = cardiovascular nuclei**

Human Ventral Tegmental Area



Rat Midbrain Dopaminergic System

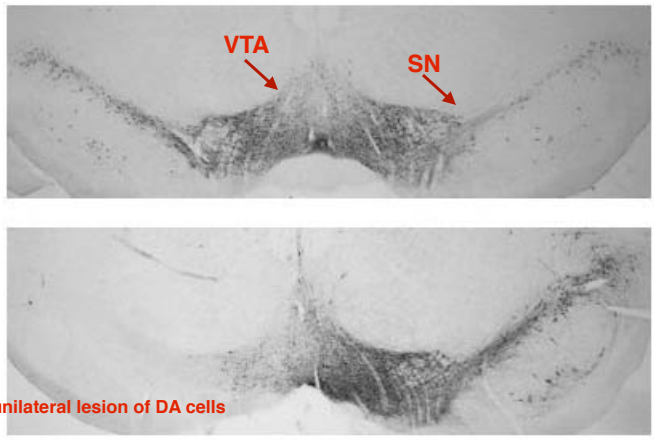


Ventral Tegmental Area (VTA) = reward & motivation
Substantial Nigra (SN) = movement regulation

Parkinsons Disease

Degeneration of dopaminergic cells in Substantia Nigra

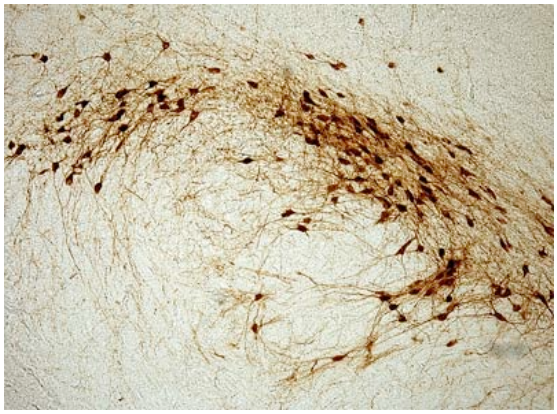
http://www.urmc.rochester.edu/neuroslides/slide199.html



unilateral lesion of DA cells

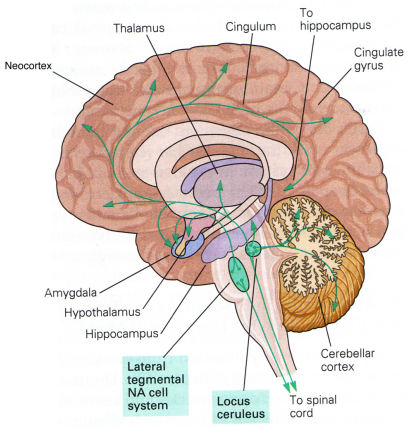
http://www.psy.herts.ac.uk/res/an-models.html

Dopaminergic cells in rat Substantia Nigra (TH immunostaining)

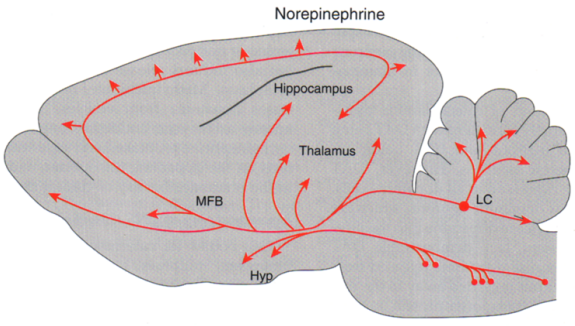


http://www.psy.herts.ac.uk/res/an-models.html

Human Noradrenergic System



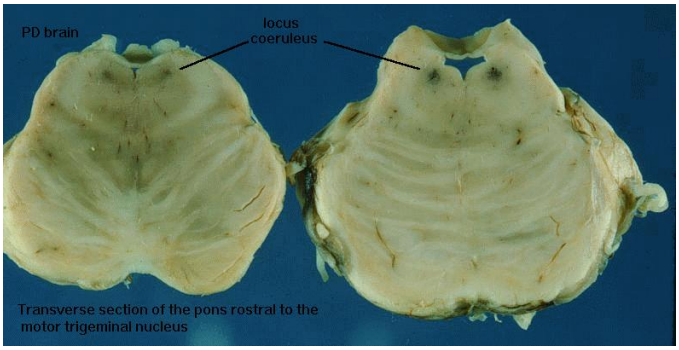
Rodent Noradrenergic System



Locus Coeruleus = arousal, attention, stress response

Parkinsons Disease

Degeneration of noradrenergic cells in Locus Coeruleus



http://medweb.bham.ac.uk/http/depts/clin_neuro/teaching/tutorials/parkinsons/coeruleus.jpg

Central Anatomy of Catecholamines

Limited to discrete nuclei ("A" and " C"groups) with long projection axons

(different from Glutamate and GABA, which are ubiquitous)

Note parallels to Sympathetic System

Norepinephrine

brainstem = cardiovascular nuclei

Locus Ceuruleus = altertness and stress

Epinephrine *C1 & 2*

brainstem = cardiovascular nuclei

Classical NTs & Synthetic pathways

enzyme 1 ***enzyme 2***

cytoplasmic precursor → **intermediate** → **neurotransmitter**

transporter

vesicles

Synthesis of Catecholamines

tyrosine

↓ **TH** tyrosine hydroxylase *rate limiting*

L-DOPA

↓ **AADC** L-aromatic acid decarboxylase

dopamine

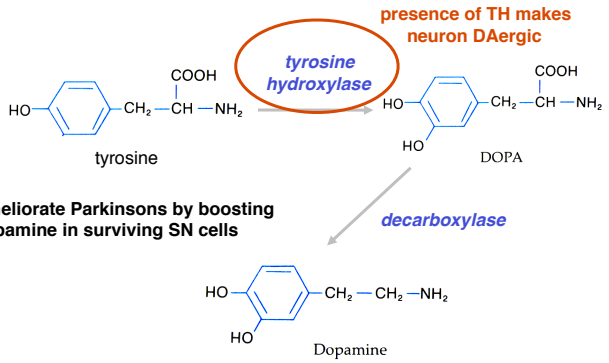
↓ **DBH** dopamine β hydroxylase

norepinephrine

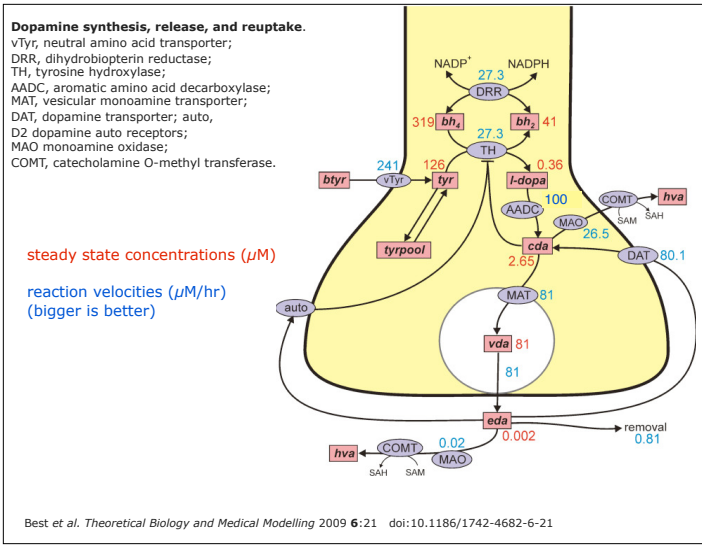
↓ **PNMT**
phenyl-ethanolamine-N-methyl-transferase

epinephrine

Amines: Catecholamines (DA, NE, Epi)

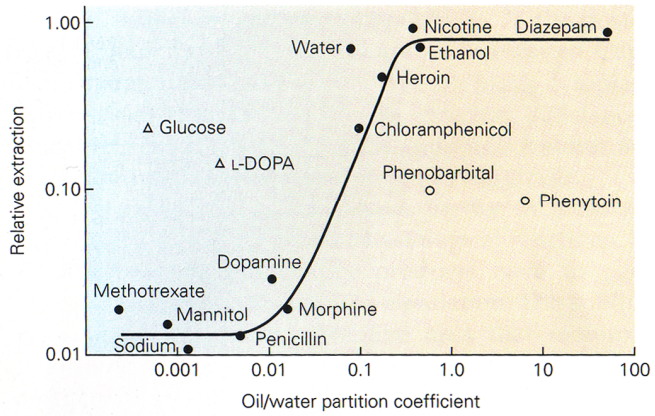


Ameliorate Parkinsons by boosting dopamine in surviving SN cells



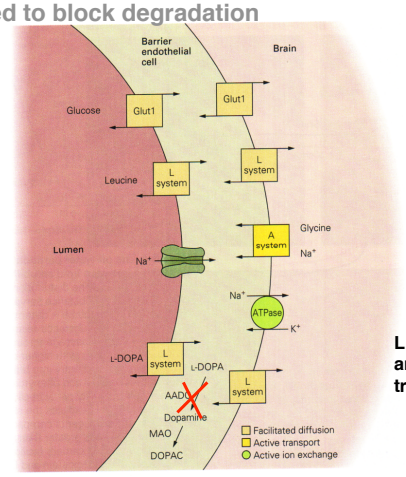
Best *et al. Theoretical Biology and Medical Modelling* 2009 **6**:21 doi:10.1186/1742-4682-6-21

L-DOPA relieves Parkinsons by crossing BBB and bypassing rate-limiting step of DA synthesis



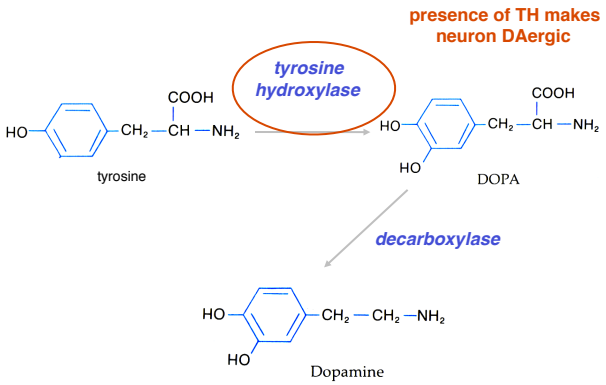
L-DOPA actively transported across BBB

but need to block degradation



L = large neutral amino acid transporter

Amines: Catecholamines (DA, NE, Epi)



PROTEIN KINASE	SERINE RESIDUE	RESULT OF PHOSPHORYLATION[a]
PKA	40	alleviation of feedback inhibition
ERK1&2	31, 8 to lesser extent	activation 2-fold
MAPKAPK-2	40, 19 to lesser extent	alleviation of feedback inhibition
Cdk5	31	unknown
CaMKII	19, 40 to lesser extent	binding of 14-3-3, activation (?)
PRAK	19	binding of 14-3-3, activation (?)

[a] *(13, 14)*

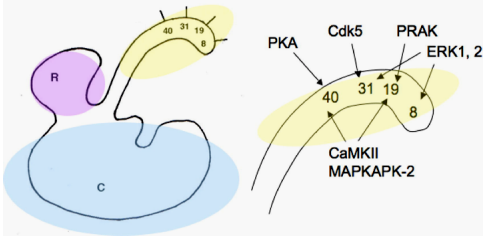
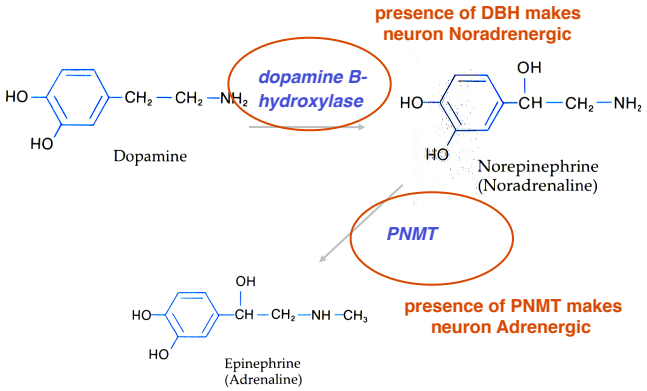


Fig. 6. Simplified map of the reactivity of some protein kinases with the serine residues of the R domain of TyrH. Ser40 is modified by PKA, CaMKII, and MAPKAPK-2. Ser31 is modified by ERK1 & 2 and Cdk5. PRAK labels ser19, as do CaMKII, and MAPKAPK-2. Ser8 is modified by ERK1 but it is not certain that the reaction has an effect on TyrH activity.

Daubner 2011

Amines: Catecholamines (DA, NE, Epi)



Sites of Synthesis

tyrosine

↓ TH cytoplasm

L-DOPA

↓ AADC cytoplasm

dopamine

transport in ↓ DBH inside vesicle

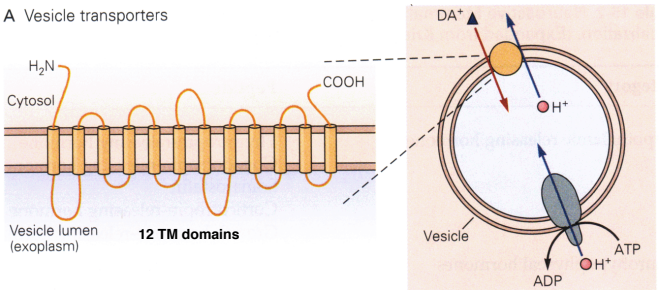
norepinephrine

transport out ↓ PNMT cytoplasm

epinephrine

transport back in

Packaging classical Catecholamines into vesicles



Vesicular Monoamine Transporter (VMAT)

blocked by reserpine

Dopamine Receptors

D1-like: D1, D5
D2-like: D2, D3, D4

Identified in 2 ways:

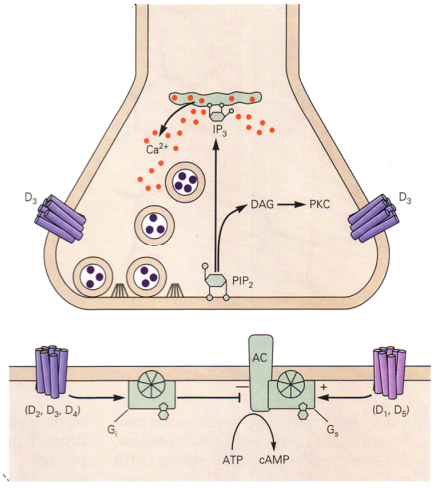
action of specific drugs on subsets of receptors

cloning of unique receptor genes

screening of orphan receptors

Dopamine Receptors

D3 is presynaptic autoreceptor that regulates DA release



D1-like and D2-like receptors have opposing effects on cAMP

Table 60-3 Five Major Types of Known Postsynaptic Dopamine Receptors

	D_1	D_2	D_3	D_4	D_5
Molecular structure	Seven membrane-spanning regions	Seven membrane-spanning regions	Seven membrane-spanning regions	Seven membrane-spanning regions	Seven membrane-spanning regions
Effect on cyclic AMP	Increases	Decreases	Decreases	Decreases	Increases
Agonists	SKF 38393	Bromocriptitine	7-OH-DPAT	?	SKF 38393
Antagonists	SCH 23390 Phenothiazines Thioxanthenes Butyrophenones	Sulpiride Phenothiazines Thioxanthenes Butyrophenones Clozapine	UH232 Clozapine	Clozapine	SCH 23390

AMP = adenosine monophosphate.
SKF 38393 = Smith Kline French compound no. 38393.
7-OH-DPAT = 7-hydroxy-dipropylaminotetralin.
SCH 23390 = Scherring A. G. compound no. 23390.
UH232 = U. Hacksell compound no. 232.

Termination of Catecholamine Signal

1. **Re-uptake into presynaptic cell**

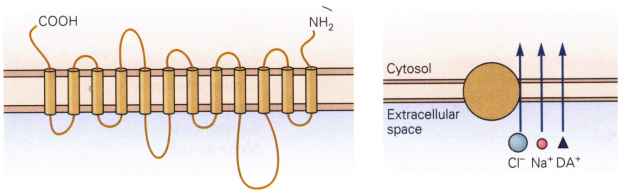
2. **Degradation by COMT in synapse**
   catechol-O-methyl transferase
   DA-> DOPAC and HVA

3. **Degradation by MAOs on mitochondria inside cells**
   monoamine oxidase A or B
   DA-> DOPAC

MAO inhibitors + cheese (tyramine) can lead to hypertensive crisis

Clearing Catecholamines from the synapse

C Uptake of other transmitters



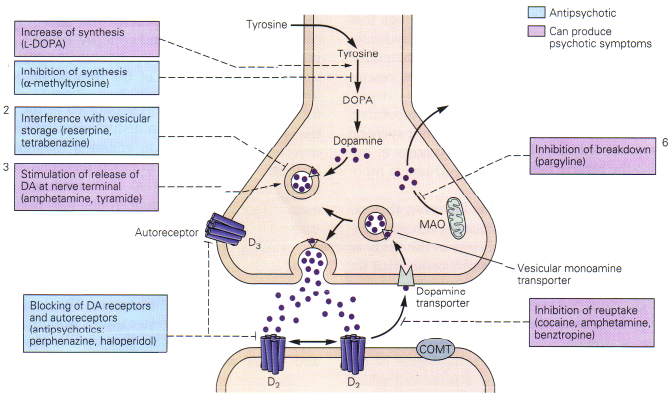
Dopamine transporter (DAT) -- target of cocaine
Norepi transporter (NET) -- target of some antidepressants

MPTP-induced Parkinsonism

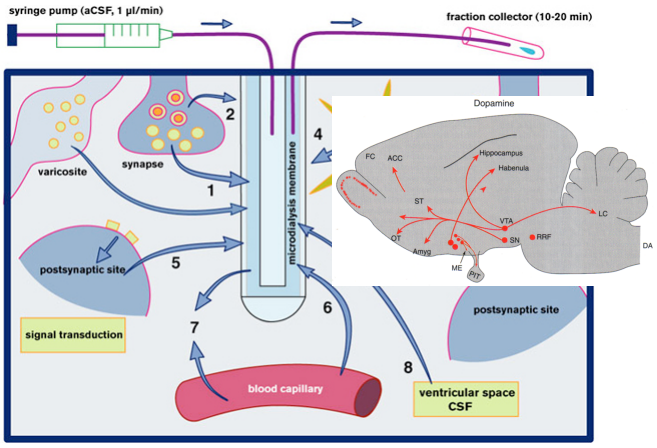
(1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine)

- **Parkinson's Disease: tremor and rigidity due to death of dopamine cells in substantia nigra**
- **Demerol (opiate) addicts screwed up synthesis, produced MPTP**
- **MPTP taken up by dopamine cells via DAT, metabolized by MAOB to form MPP+**
- **MPP+ very toxic, kills cells (but only cells with DAT)**
- **Drug addict gets Parkinsons Disease**

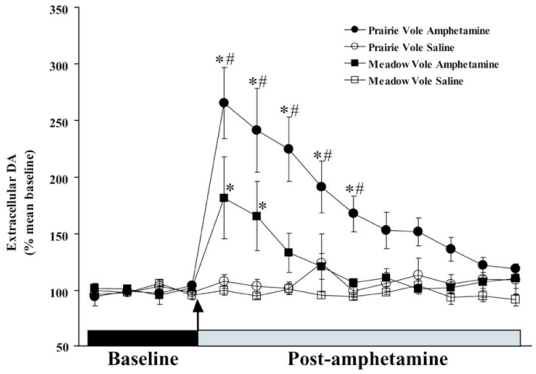
Dopamine Synapse



Amphetamine -> Increase DA in n. accumbens

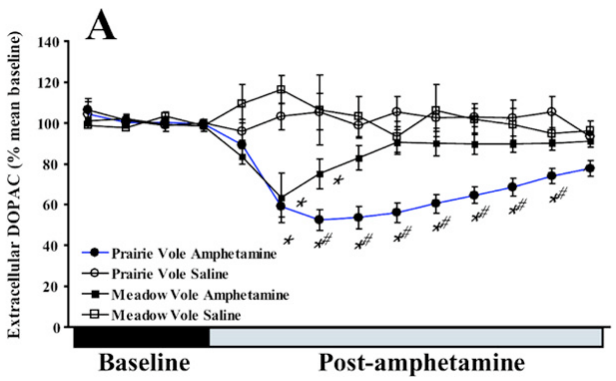


http://www.labautopedia.com/mw/index.php/Microdialysis:_An_introduction



Peripheral administration of amphetamine increased extracellular dopamine within the nucleus accumbens This response was greater in monogamous prairie voles (filled circles) than in promiscuous meadow voles (filled squares). Saline injections had no effect in either species (open symbols). Arrow indicates time of injection. * indicates a significant difference from the within-species baseline. # indicates sample periods for which there are significant species differences.

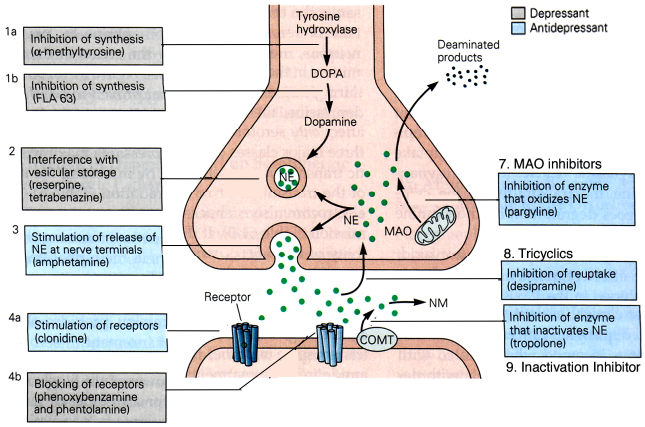
Curtis 2007



Amphetamine effects on extracellular levels of DOPAC
Peripheral administration of 3 mg/kg amphetamine significantly reduced NAcc DOPAC in both species, although the effect was greater in prairie voles (A).

Curtis 2007

Norepi Synapse



Identification of Catecholamine cells

AADC very common
MAOs very common

TH - DA, NE, Epi cells
DBH - NE, Epi cells
PNMT - Epi cells

DAT - DA cells
NET - NE cells
VMAT - DA, NE, Epi, and serotonin cells

All of these genes are being investigated for polymorphisms in psychiatric illnesses

Identification of Catecholamine cells

Cell Type	TH	AADC	DBH	PNMT	VMAT	DAT	NET	MAO
Non-catechol		+						+
Dopamine Cell	+	+			+	+		
Noradrenergic	+	+	+		+		+	
Adrenergic	+	+	+	+	+			

Expression profile of genes determines neuronal phenotype.

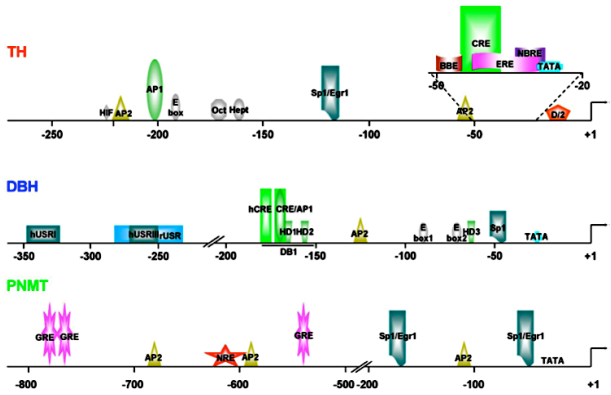


FIG. 6. Diagram of regulatory elements on proximal TH, DBH, and PNMT promoters. See text for description of the promoter elements. The elements implicated in the response to stress are enlarged.

KVETNANSKY 2009.

Some catecholamine drugs to know:

L-DOPA - enhance DA synthesis

reserpine - deplete catecholamines

amphetamine - stimulate DA release, block uptake

cocaine - block DA uptake

6-OH-dopamine - lesions DA terminals

haloperidol - dirty D2 blocker

clozapine - more specific D2 blocker

Monoamines

catecholamines:
- **dopamine**
- **norepinephrine**
- **epinephrine**

indolamines
- **serotonin**
- **melatonin**

modified amino acids (tyrosine, tryptophan) that act as neurotransmitters or hormones

Peripheral Anatomy of Serotonin (5HT)

blood-borne regulation of vasoconstriction, blood pressure, and gut motility

Synthesized by enterochromaffin cells of gut and mast cells.

Taken up (via 5HT transporter) into platelets and other cells.

Also taken up by NE transporter into NE nerve terminals.

carcinoid tumors:
GI tumors which oversecrete 5HT and other peptides
-> hypertension, nausea, & high levels of 5HIAA in urine

Central Anatomy of Serotonin

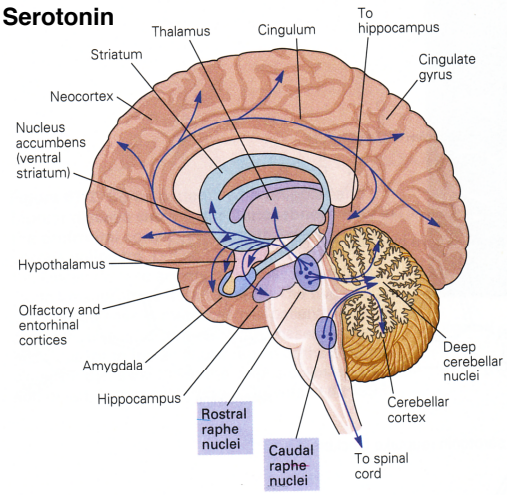
Limited to discrete nuclei ("B" groups) with long projection axons
Synthesized by TPH2 enzyme (TPH1 in periphery)

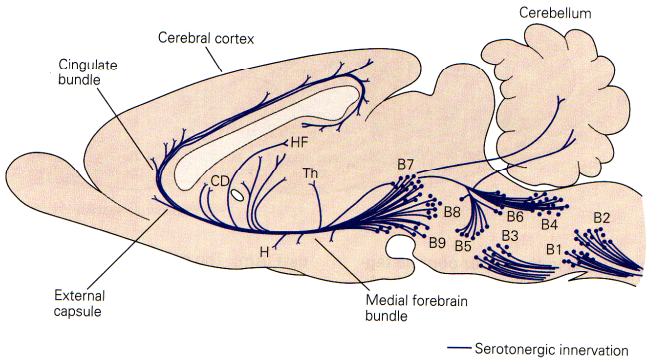
Brainstem = descending motor, pain, and autonomic modulation

Pons and Midbrain = forebrain projections
dorsal and median raphe

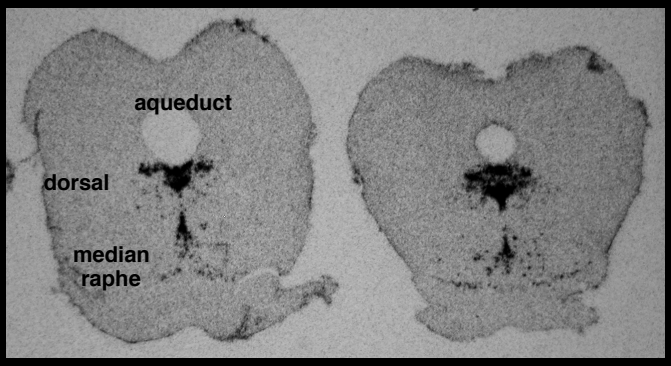
alertness, mood, hypothalamic regulation

Human Serotonin

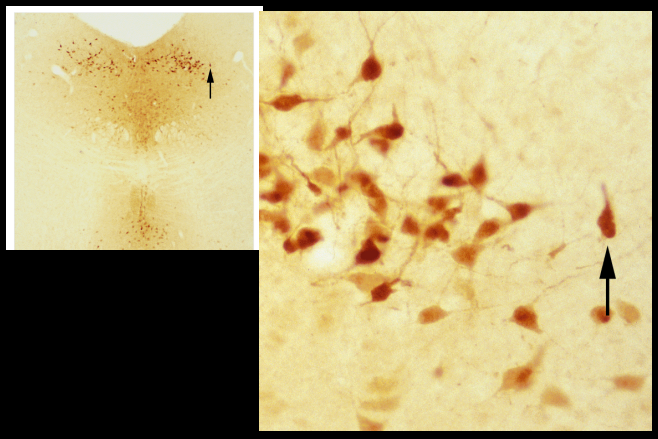




Cell bodies that express mRNA for serotonin transporter



Cell bodies that synthesize serotonin



Synthesis of Serotonin

tryptophan *supply limited*

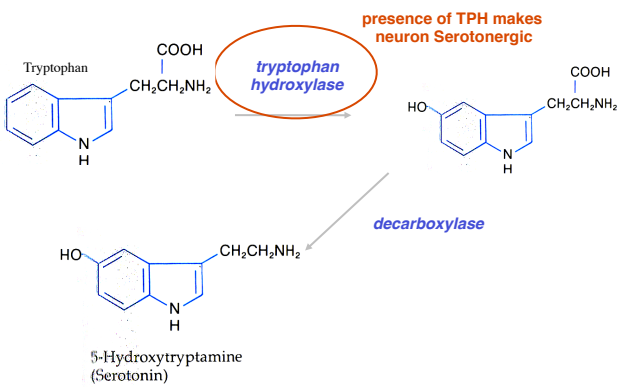
↓ TPH2 tryptophan hydroxylase *rate limiting*

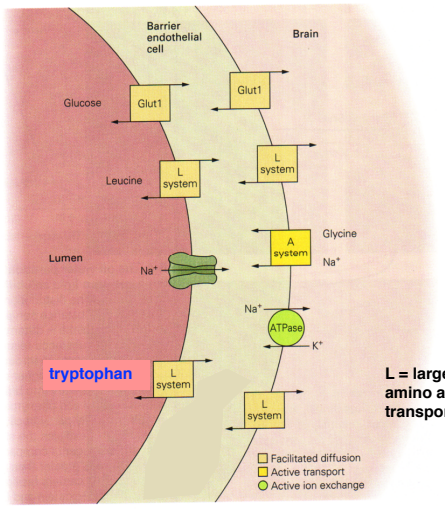
5-hydroxytryptophan (5HTP)

↓ AADC L-aromatic acid decarboxylase

serotonin (5HT)

Amines: Serotonin (5HT)





L = large neutral amino acid transporter

Synthesis of Melatonin in Pineal Gland

tryptophan

↓ **TPH** tryptophan hydroxylase

5-hydroxytryptophan

↓ **AADC** L-aromatic acid decarboxylase

serotonin

↓ **SNAT** serotonin N-acetyltransferase

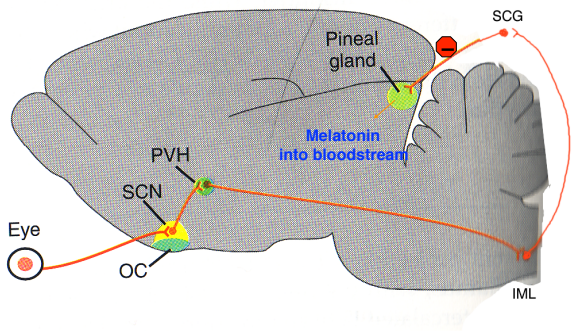
activity increased at night

N-acetylserotonin

↓ **Hydroxyindole-o-methyltransferase**

melatonin

Melatonin secretion is high at night; suppressed by light & biological clock in SCN



SCN = suprachiasmatic nucleus; biological clock
SCG = superior cervical ganglion; sympathetic N.S.

Serotonin Receptors

Receptors linked to second-message systems

-	5-HT_{1A} linked to inhibition of adenylyl cyclase	autoreceptors that modulate release
+	5-HT_{1B} linked to inhibition of adenylyl cyclase	
	5-HT_{1D} linked to inhibition of adenylyl cyclase	
	5-HT_{1E} linked to inhibition of adenylyl cyclase	
	5-HT_{1F} linked to inhibition of adenylyl cyclase	
	5-HT_{2A} linked to phospholipase and PI turnover	
	5-HT_{2B} linked to phospholipase and PI turnover	
	5-HT_{2C} linked to phospholipase and PI turnover	
	5-HT_{4} linked to stimulation of adenylyl cyclase	
	5-HT_{5} unknown linkage	
	5-HT_{6} linked to stimulation of adenylyl cyclase	
	5-HT_{7} linked to stimulation of adenylyl cyclase	

Receptors linked to an ion channel

5-HT_{3}

Behaviors mediated by Serotonin Receptors

5HT-1a knockout - less reactive, more anxious, and possibly less aggressive

5HT-1b knockout - more aggressive, more reactive, and less anxious

5HT-2c knockout - obese

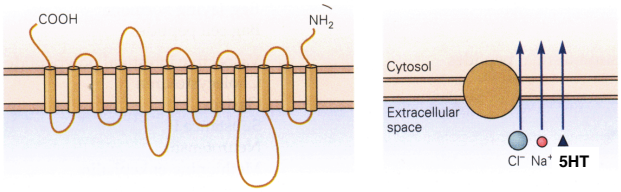
5HT-5a knockout - enhanced exploration

Termination of Serotonin Signal

1. Re-uptake into presynaptic cell via SERT

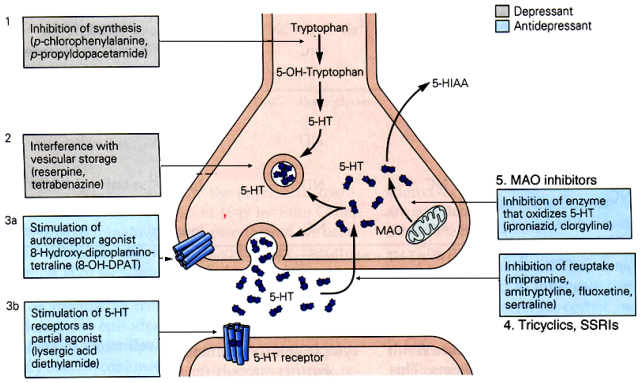
2. Degradation by MAOs on mitochondria inside cells
   monoamine oxidase A
   5HT-> 5HIAA

Clearing Serotonin from the synapse



SERT (5HTT)
very specific for 5HT
target of some antidepressants
can be blocked (fluoxetine) or run backwards (MDMA, fenfluramine)

The 5HT synapse



Some Serotonergic Drugs

PCPA - blocks TPH

reserpine - blocks vesicular transporter

6-OH-DPAT - stimulates release presynaptically

LSD - stimulates postsynaptic receptors

fluoxetine - (Prozac) selective serotonin reuptake inhibitor (SERT)

MAOIs - blocks MAO

fenfluramine - SERT releaser (norfenfluramine = 5HT2C agonist)

MDMA (ecstasy) - SERT releaser

	Catecholamines	Serotonin
Anatomy	A,C nuclei with long projections	B nuclei with long projections
Synthesis	TH rate-limiting	tryptophan supply, TPH rate-limiting
Receptors	D1-5, α1-3,β1&2	14 subtypes
Drugs	reuptake inhibitors	reuptake inhibitors
Models	reward, movement	mood, obesity