2 Types of neurotransmitters

	Classical small molecules	Neuropeptides
Size	small (like single amino acid)	large (4-100 a.a. polypeptide)
Synthesis	uptake or enzymes	protein synthesis
Vesicles	small, filled by transporters	large secreted proteins from RER
Duration of action	fast but short	slow & long

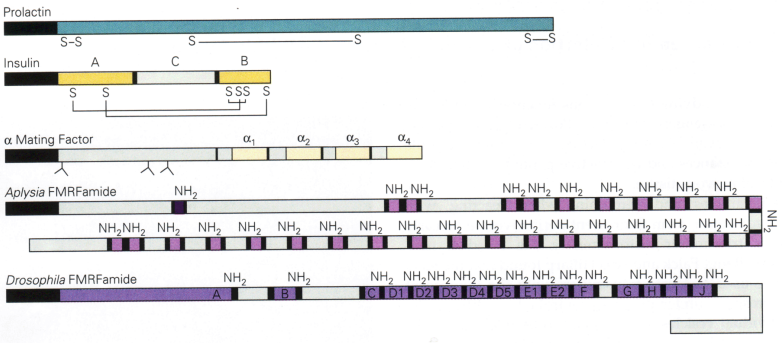
Neuropeptides

Category	Peptide
Hypothalamic releasing hormone	Thyrotropin-releasing hormone
	Gonadotropin-releasing hormone
	Somatostatin
	Corticotropin-releasing hormone
	Growth hormone-releasing hormone
Neurohypophyseal hormones	Vasopressin
	Oxytocin
Pituitary peptides	Adrenocorticotropic hormone
	β-Endorphin
	α-Melanocyte-stimulating hormone
	Prolactin
	Luteinizing hormone
	Growth hormone
	Thyrotropin
Invertebrate peptides	FMRFamide[1]
	Hydra head activator
	Proctolin
	Small cardiac peptide
	Myomodulins
	Buccalins
	Egg-laying hormone
	Bag cell peptides

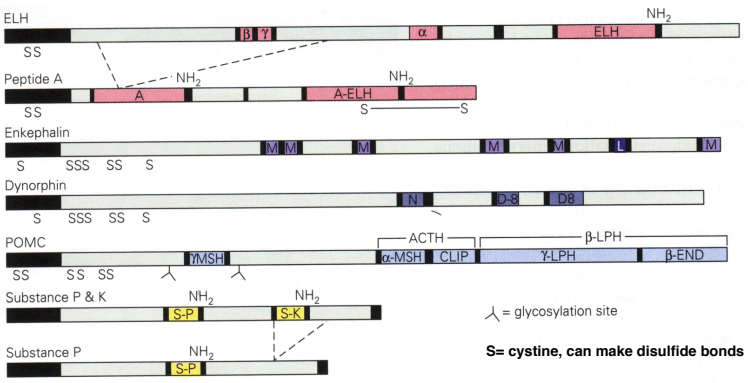
Category	Peptide
Gastrointestinal peptides	Vasoactive intestinal polypeptide
	Cholecystokinin
	Gastrin
	Substance P
	Neurotensin
	Methionine-enkephalin
	Leucine-enkephalin
	Insulin
	Glucagon
	Bombesin
	Secretin
	Somatostatin
	Thyrotropin-releasing hormone
	Motilin
Heart	Atrial naturetic peptide
Other	Angiotensin II
	Bradykinin
	Sleep peptide(s)
	Calcitonin
	CGRP[2]
	Neuropeptide Y
	Neuropeptide Yy
	Galanin
	Substance K (neurokinin A)

more variety, because combination of 4 to 100 a.a.
(similar variety of receptors!)

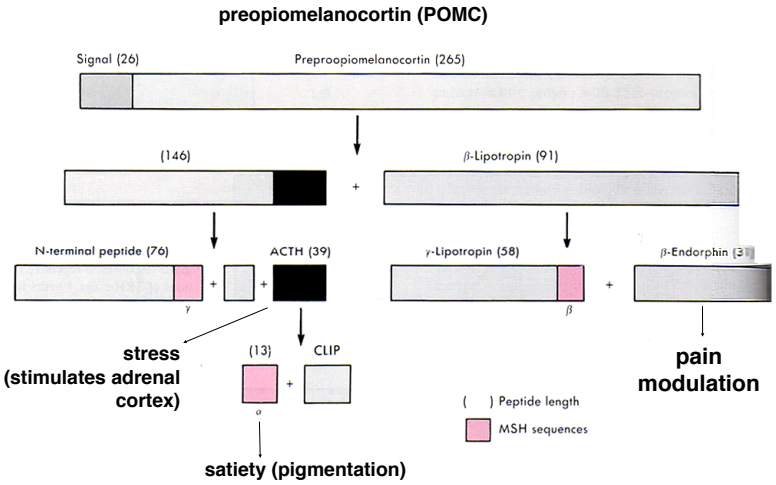
Neuropeptides are cleavage products of prepropeptides



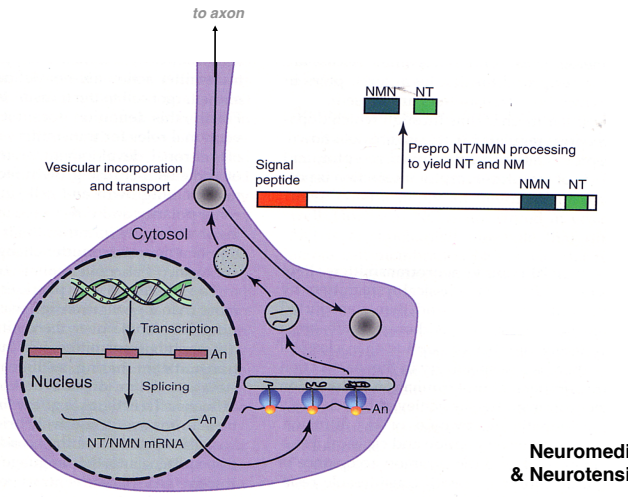
Neuropeptides are cleavage products of prepropeptides



POMC -> ACTH

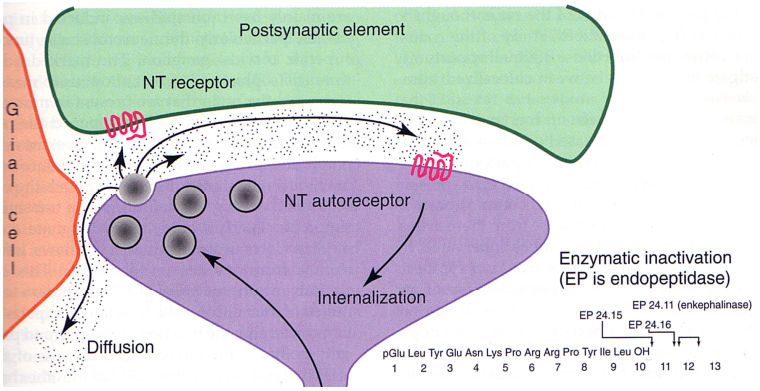


Neuropeptide Synthesis



Neuromedin & Neurotensin

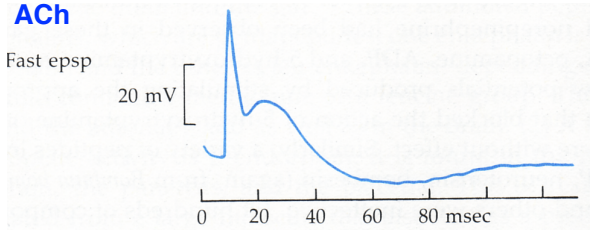
Neuropeptide Release and Degradation



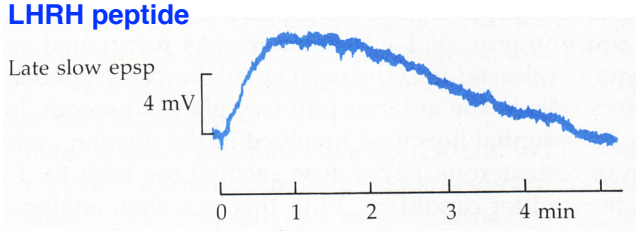
inhibit endopeptidase to increase NP effect
inject endopeptidase to attenuate NP effect

Neuropeptides & classical NTs in same synapse, but different effects

ACh



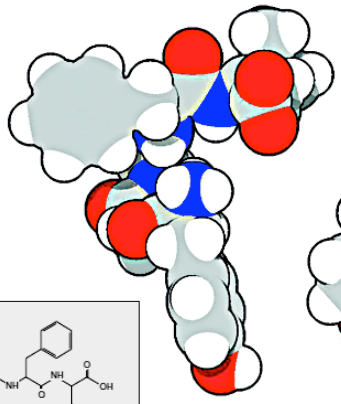
LHRH peptide

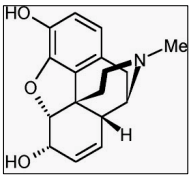


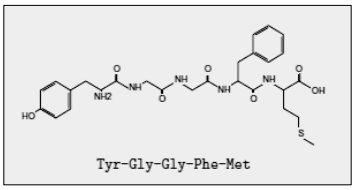
Neuropeptide Receptors

- Usually G-protein-coupled receptors; may be multiple subtypes
- Agonists & antagonists derived by shuffling amino acid sequence
- Inhibition of specific endopeptidases can prolong synaptic activity
- Oral peptides digested; systemic peptides cannot cross blood brain barrier (but may act on circumventricular organs)
- non-peptide agonists and antagonists desirable

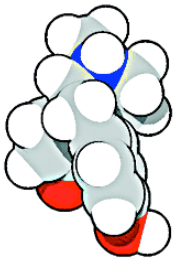
Neuropeptide Structure







Enkephalin



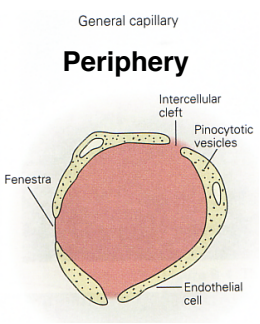
Morphine

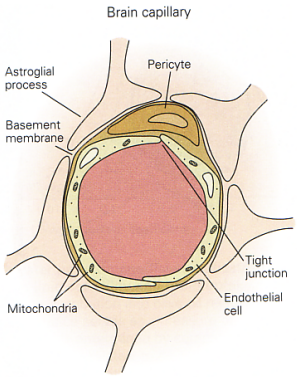
Goodsell, DS The Oncologist, 9 (2004) 717

Blood Brain Barrier

Tight junctions and pericytes seal off brain capillaries.

Circumventricular organs are an exception.

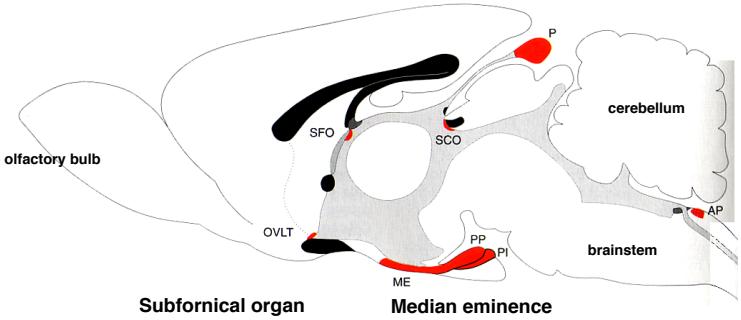




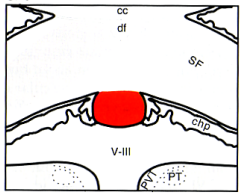
CVOs

Brain

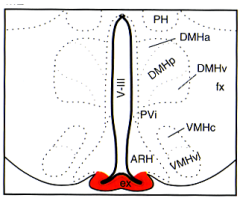
Circumventricular Organs



Subfornical organ



Median eminence

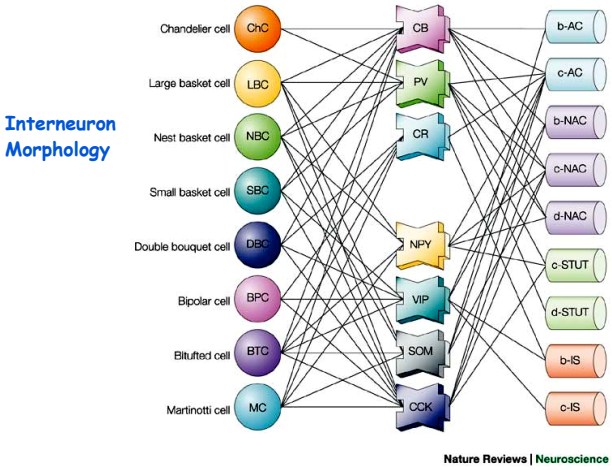


Squire F34.4

Colocalization (or not) with classical transmitters

NPY with NE in adrenal medulla, sympathetic nerves, locus coeruleus (but NE not in NPYergic cells of cortex, hypothalamus, etc.)

CCK in endocrine cells of the gut, and DA cells of the midbrain (but DA not in CCKergic cells of cortex, etc.)



Nature Reviews | Neuroscience

Expression profiles of the CBPs calbindin (CB), parvalbumin (PV) and calretinin (CR) and the neuropeptides neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), somatostatin (SOM) and cholecystokinin (CCK) by different morphological and electrophysiological classes of interneuron. AC, accommodating; b, burst subtype; c, classic subtype; d, delay subtype; IS, irregular spiking; NAC, non-accomodating; STUT, stuttering.

Interneurons of the neocortical inhibitory system,
Nature Reviews Neuroscience 5, 793-807 (October 2004)

How to detect Neurotransmitters

	Classical small molecules	Neuropeptides
histochemical	Neurotransmitter itself	Neuropeptide itself
proteins	transporter or enzymes	Neuropeptide itself
mRNA	enzyme, transporter gene expression	Neuropeptide gene expression

Nuclear Receptor Hormones

1. Lipophilic molecules that pass through membranes
2. Coordinate peripheral and central neural response
3. Release regulated by synthesis
4. Bind to cytoplasmic/nuclear receptors
5. Receptors bind to DNA, affecting gene transcription
6. Can have non-genomic (immediate) effects

1. Lipophilic Hormones

made up of sterol ring structures or long-chain hydrocarbons that easily cross lipid bilayers

Steroids:

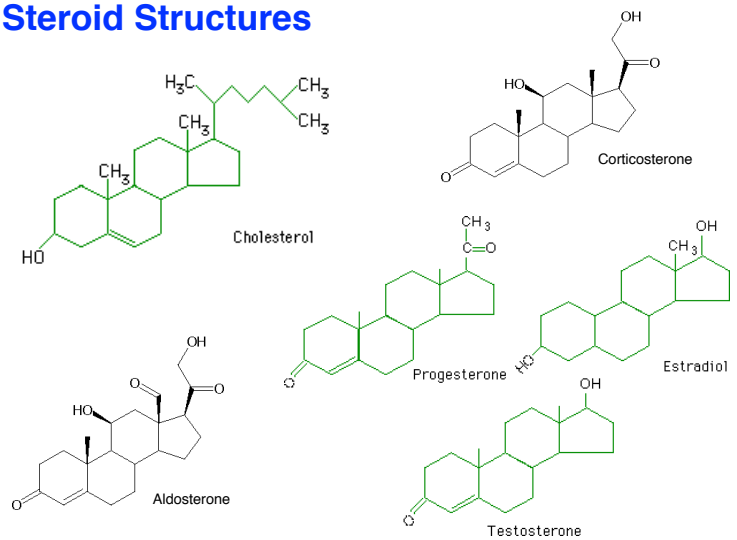
corticosterone - stress, mobilization of glucose
aldosterone - sodium homeostasis
estrogen - reproductive organs, sex. characters
progesterone - reproductive organs
testosterone - sex. characters

Lipophilic hormones:

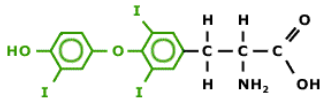
thyroid hormone - metabolism by tissues
retinoic acid - development

steroid/ thyroid/retinoid/sterol/xenobiotic/orphan receptor superfamily (48 family members in man, 49 in mouse).

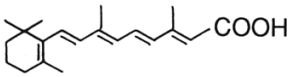
Steroid Structures



Thyroid Hormone and Retinoic Acid



3', 3, 5-triiodothyronine
T3



all-*trans*-retinoic acid

2. Peripheral Communication to Brain

Steroids:

corticosterone - adrenal gland
aldosterone - adrenal gland
estrogen - ovaries
progesterone - ovaries
testosterone - testes

Lipophilic hormones:

thyroid hormone - thyroid gland
retinoic acid - diet (Vitamin A), liver

Central Effects on Behavior parallel Peripheral Physiological Effects

Steroids:

corticosterone - feedback on stress system
aldosterone - induces salt appetite
estrogen - reproductive behavior
progesterone - reproductive behavior
testosterone - reproductive behavior

Lipophilic hormones:

thyroid hormone - GI, temperature, feeding
retinoic acid - neural development, (adult?)

Steroids often potentiate neuropeptide systems

examples:

glucocorticoids and NPY on feeding

aldosterone and Ang II on salt appetite

Hypothalamic Pituitary Adrenal (HPA) Axis & Stress

1. Feedback loops of the HPA regulating cortisol release
2. Classic disorders of the HPA: synthetic enzyme disorders, Addisons, Cushings.
3. Use of feedback loops for diagnosis of HPA problems
4. Psychological Stress: limbic activation and disregulated stress models

Hypophyseal connections & circulation

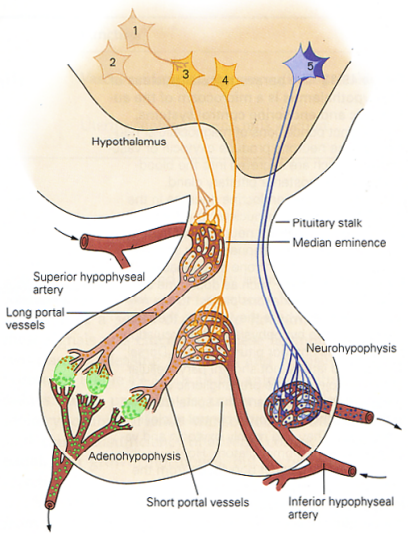


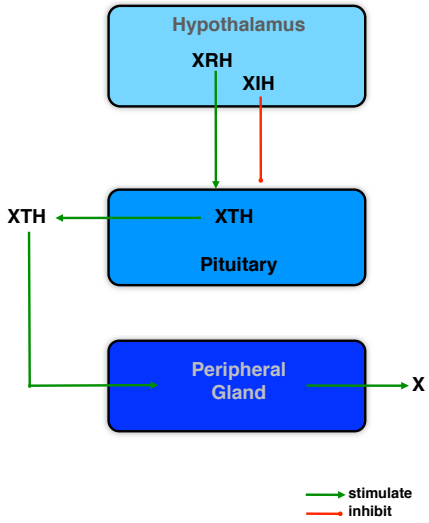
Figure 49-12 The hypothalamus controls the pituitary gland both directly and indirectly through hormone-releasing neurons.

Feedforward Loop

Releasing hormone:
hypothalamus –> pituitary

Trophic hormone:
pituitary –> target gland

target gland –> secretes X



Examples of Hypothalamic Pituitary Axes

	H-P-Adrenal Axis	H-P-Gonadal Axis	H-P-Thyroid Axis
Hypothal:	Corticotropin-releasing hormone (CRH)	Gonadotropin-releasing hormone (GnRH)	Thyrotropin-releasing hormone (TRH)
Anterior Pituitary:	Adrenocorticotropic hormone (ACTH)	Luteinizing hormone (LH)	Thyroid-Stimulating hormone (TSH)
Target Organ:	Adrenal Cortex	Ovaries & Testes	Thyroid Gland
Function:	Stimulate Corticosteroid Synthesis (Stress)	Stimulate Estrogen & Testosterone Synthesis (Reproduction)	Stimulate Thyroxine synthesis (Metabolism)

see Fox Table 11.6 & Table 11.7

Hypothalamic Pituitary axes

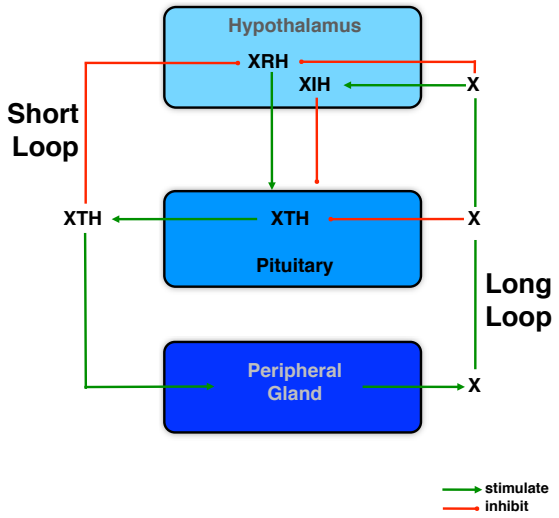
Hypothalamus regulates pituitary function with releasing and release-inhibitory hormones

releasing hormones –> pituitary to cause release of stimulatory hormones –> target glands activity

inhibitory hormones –> pituitary to suppress release of stimulatory hormones –> target gland activity *(esp. dopamine -> less prolactin)*

Transection of infundibulum –> decrease of all pituitary hormones except increased prolactin.

Feedback Loops



Hypothalamic-Pituitary pathologies:

Hypersecretion due to

- tumors
- lack of negative feedback
- inappropriate synthesis/degradation

Real or Functional Hyposecretion due to

- lack of releasing/trophic hormones
- lack of synthetic enzymes
- lack of receptors

Definition of Stress

Perturbation from homeostasis (maintenance of the constant internal environment)

"Fight or Flight" defined in 1900s by Cannon

Defined in 1930s as general response to "stress" by Selye in war veterans.

- increase in gastric secretion
- increase in adrenal secretion
- suppression of immune system

The Stress Response and the Hypothalamic-Pituitary-Adrenal Axis

stress (neural input, disease, learned response)

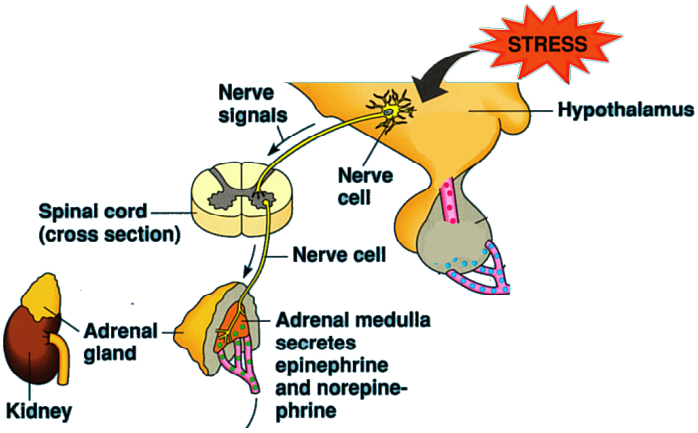
-> parvocellular PVN of hypothalamus

-> corticotropin-releasing hormone (CRH) cells

-> immediate response & long-term response

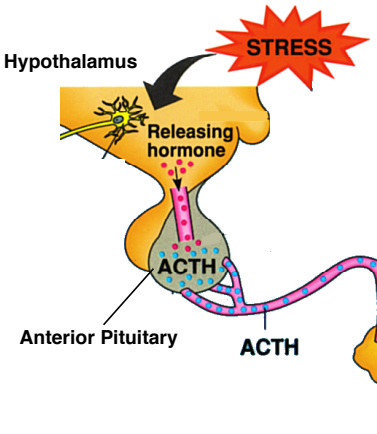
CRH also known as corticotropin-releasing factor (CRF)

Immediate, Neural Response to Stress:



Long-Term Response to Stress:

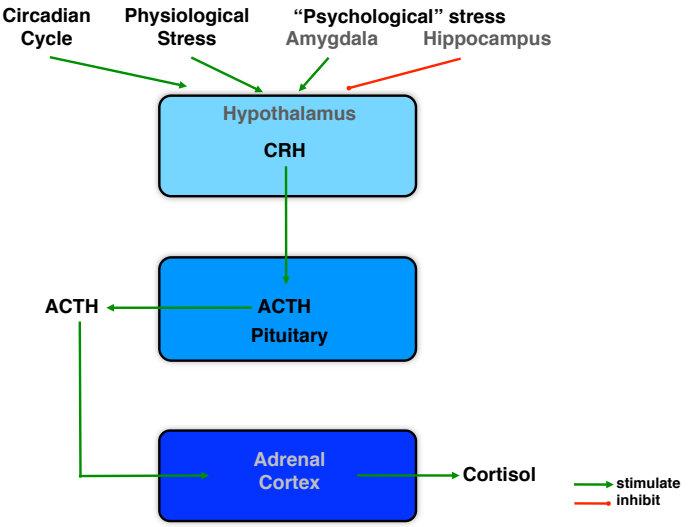
secretion of mineralo- & glucocorticoids



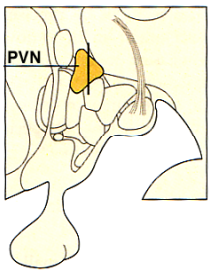
Mineralocorticoids (aldosterone):
retention of Na+ and H20 by kidneys
increased blood volume and pressure

Glucocorticoids (cortisol):
convert protein and fat to glucose
suppress immune system

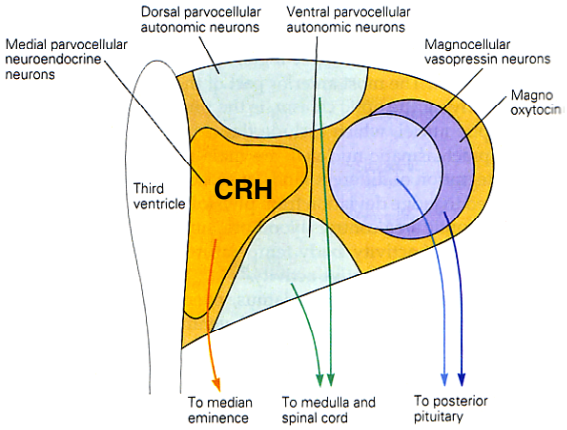
HPA axis: Positive Feed forward



Paraventricular Nucleus (PVN)



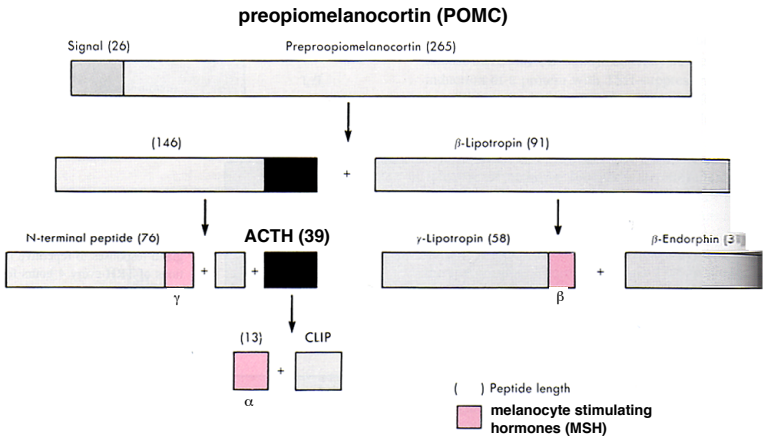
sagittal section



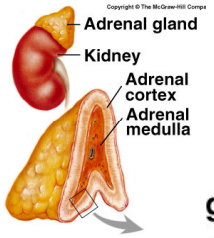
coronal section

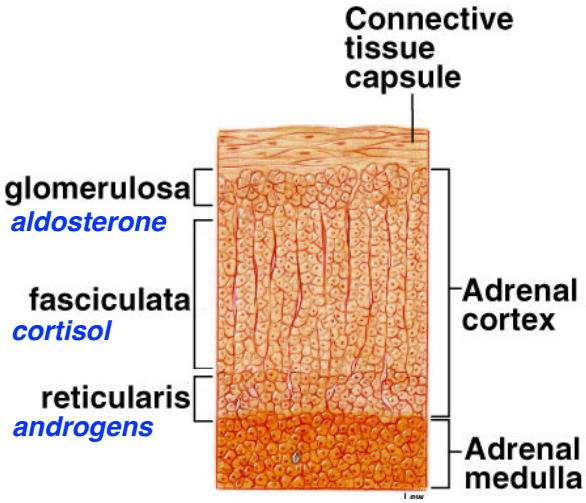
Corticotrophes in Pituitary

Synthesize POMC -> ACTH

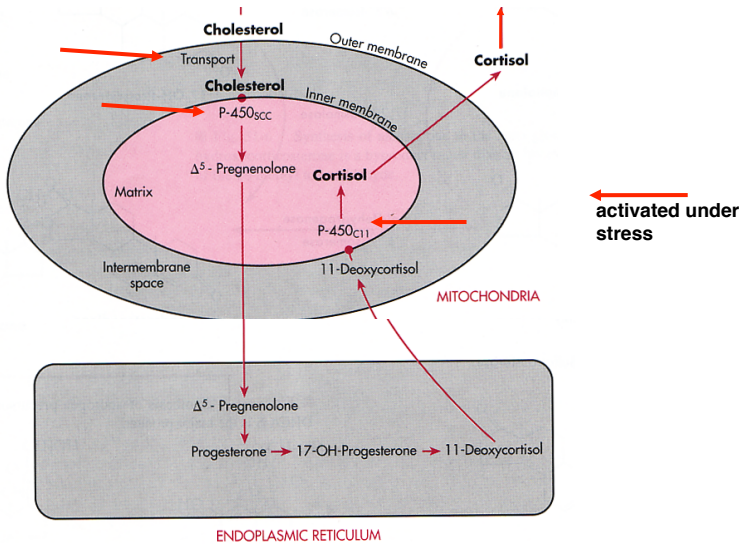


Adrenal gland anatomy

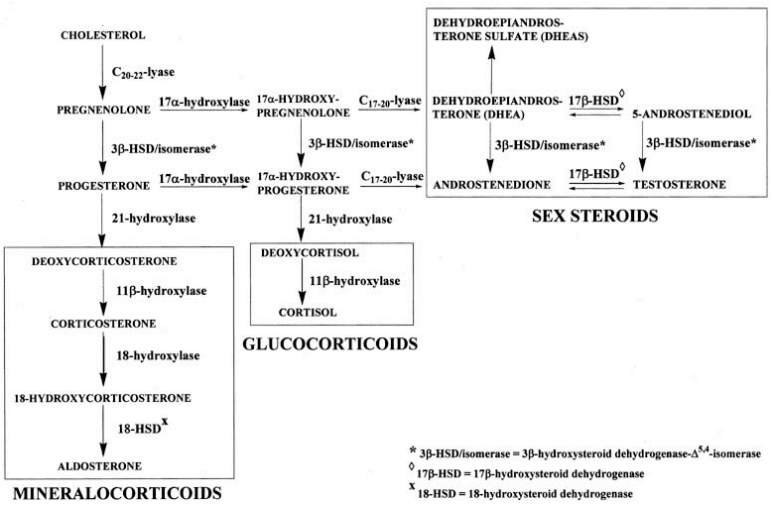




Cortisol Synthesis in Adrenal Gland



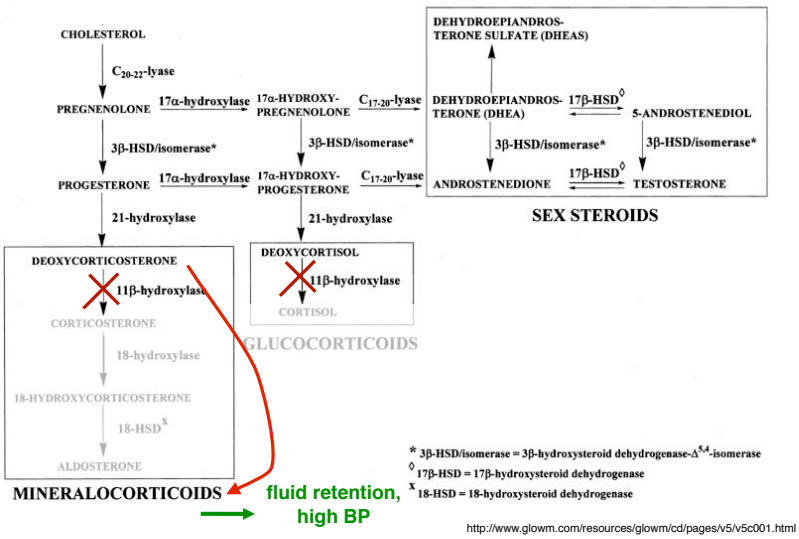
Steroid Synthesis in the Adrenal Gland



http://www.glowm.com/resources/glowm/cd/pages/v5/v5c001.html

Steroid Synthesis in the Adrenal Gland

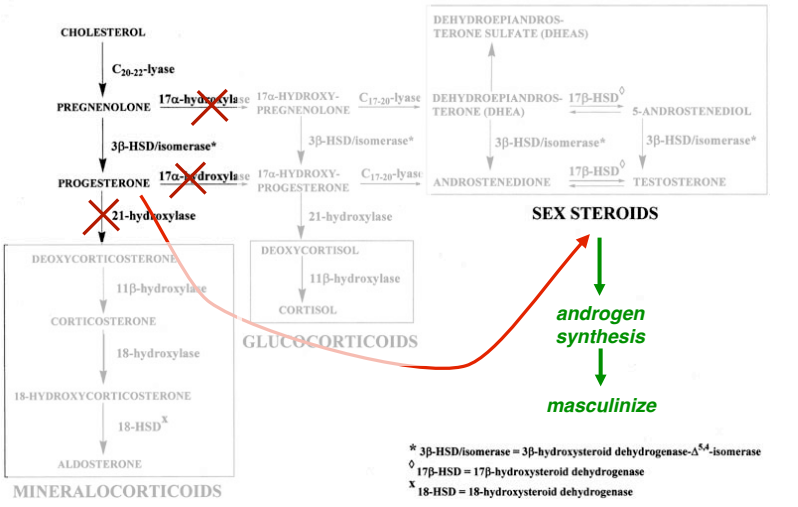
defect in 11-hydroxylase



http://www.glowm.com/resources/glowm/cd/pages/v5/v5c001.html

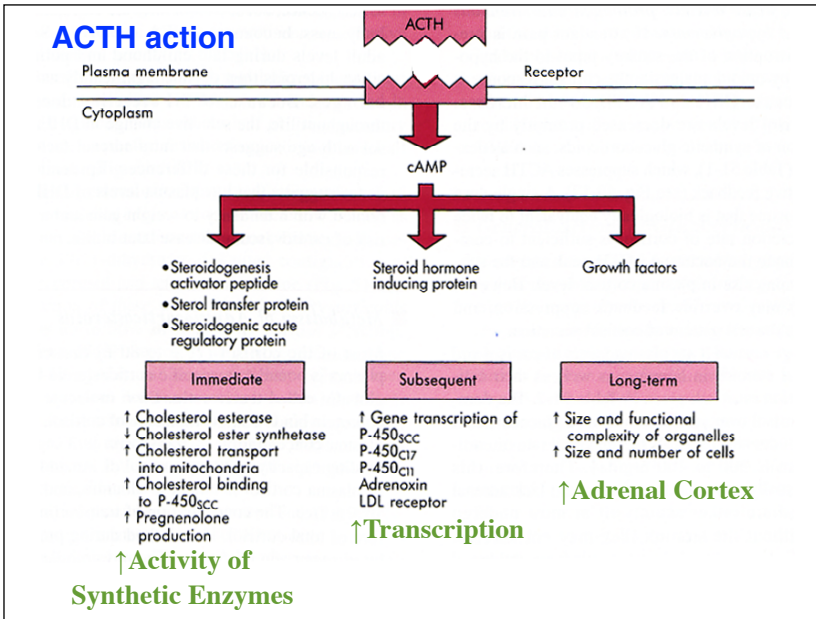
Steroid Synthesis in the Adrenal Gland

defect in 17- or 21-hydroxylase



http://www.glowm.com/resources/glowm/cd/pages/v5/v5c001.html

ACTH action



3. Release regulated by synthetic enzymes

Because lipophilic, can't store in vesicles.

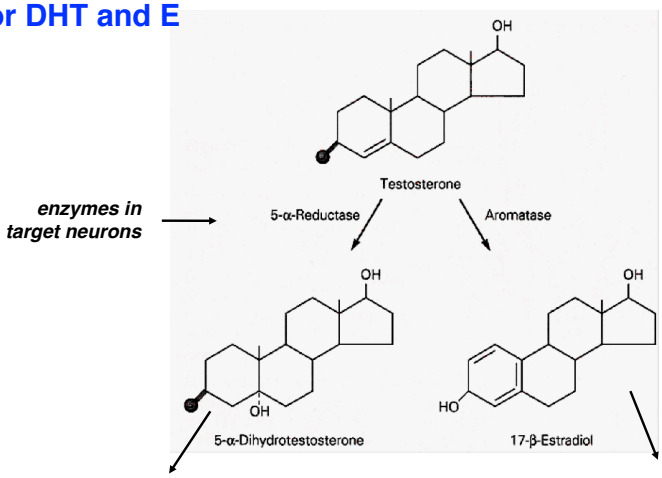
So, release is generated by "production on demand"

e.g. stress -> increased conversion of cholesterol to corticosterone.

Local effects can be regulated by converting enzymes in the target tissue.

e.g. testosterone -> converted to estrogen in target neurons.

Testosterone as prohormone for DHT and E

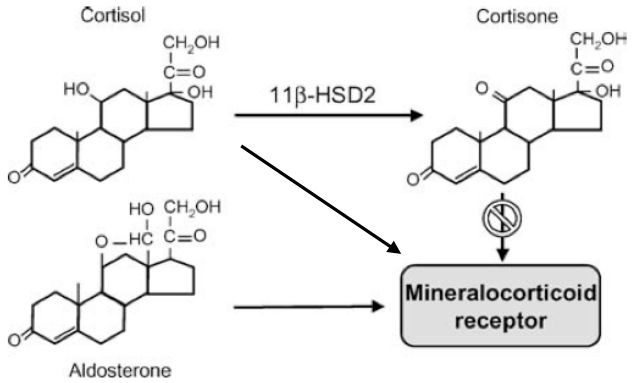


acts on androgen receptors

acts on ERs to organize "male-like" brain

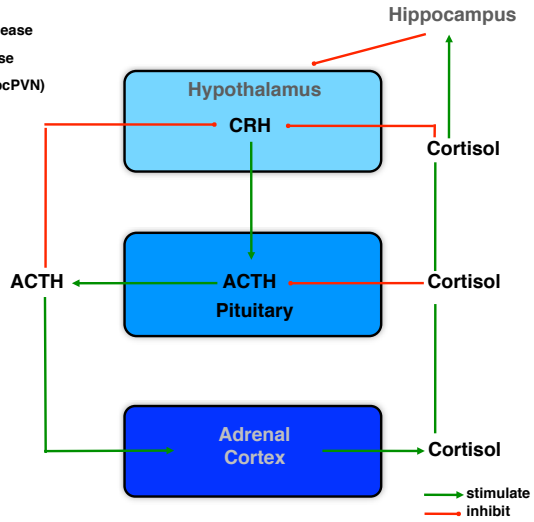
Tissue specific targeting of Cortisol vs. Aldosterone to Mineralcorticoid Receptors

11β hydroxysteroid dehydrogenase Type 2



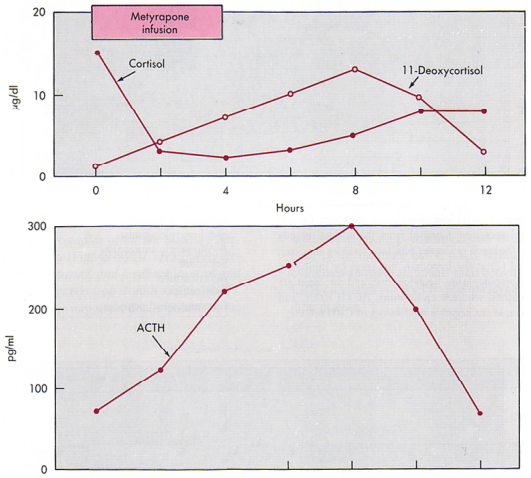
HPA axis: Negative Feedback

Cortisol feeds back to:
pituitary –> inhibit ACTH release
pcPVN –> inhibit CRH release
hippocampus –> fornix –> pcPVN)

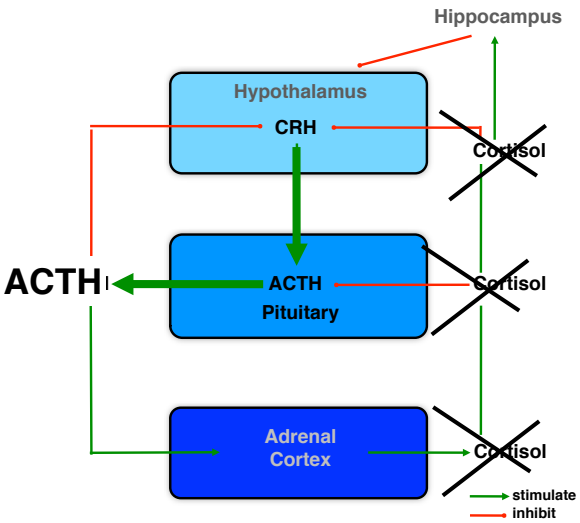


Feedback Loops: block negative feedback

metyrapone blocks conversion of 11-deoxycortisol -> cortisol;
so cortisol levels fall



HPA axis: Remove Negative Feedback ACTH & CRH levels increase

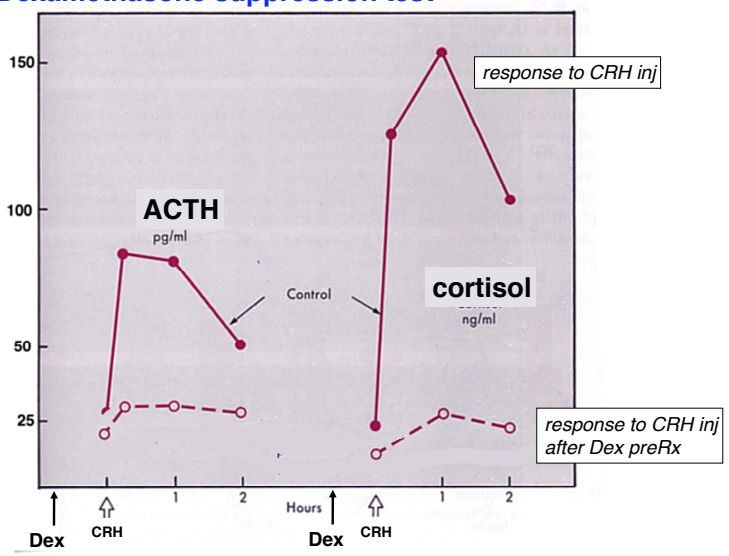


Dexamethasone suppression test

preRX with artificial GC (dexamethasone) suppresses cortisol response to CRH injection

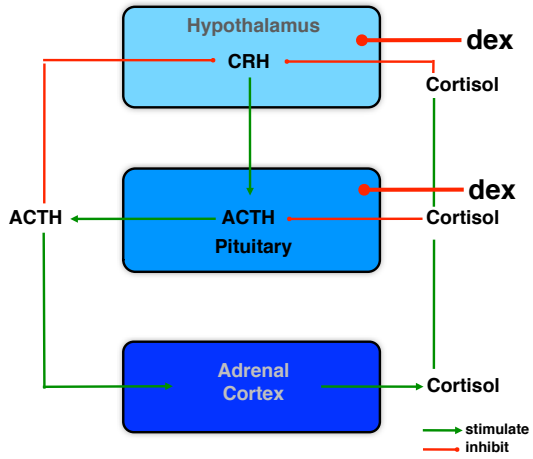
note:
can use suppression test to assay functioning of internal feedback loops

Dexamethasone suppression test



HPA axis: Enhanced Negative Feedback

Dex pretreatment -> blunted ACTH response to CRH



Psychological Stress

No immediate threat to homeostasis

Hard-wired and Learned Sensory stimuli
-> Amygdala, Limbic systems
-> Fear & Stress Responses

Chronic stress
-> increased GC
-> cell death in hippocampus, cortex, amygdala
-> impaired memory, depression
-> disregulated stress response.

Early stress
-> disregulation of stress response
-> reversal by environmental enrichment

Limbic stimulation -> cortisol release

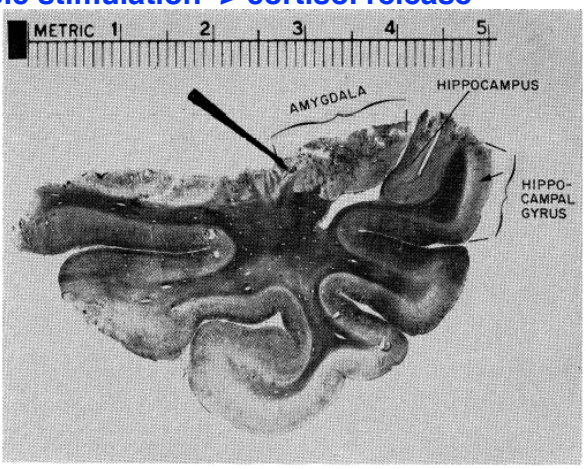


Fig. 1. Section of right temporal lobe, showing marker lesion (electrode tip) in basolateral amygdala. Lesion is the darker area at the immediate right of the pointer tip; Weil stain.

Science. 1966 Aug 12;153(737):767-8.

Amygdala stimulation -> cortisol release

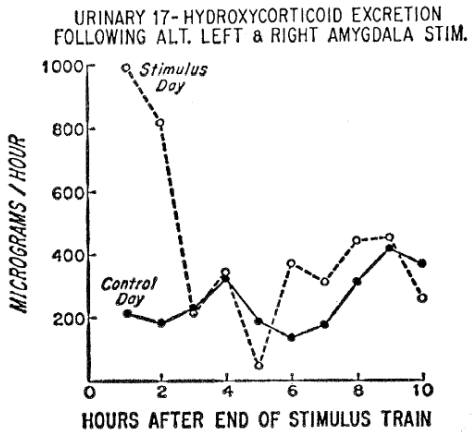
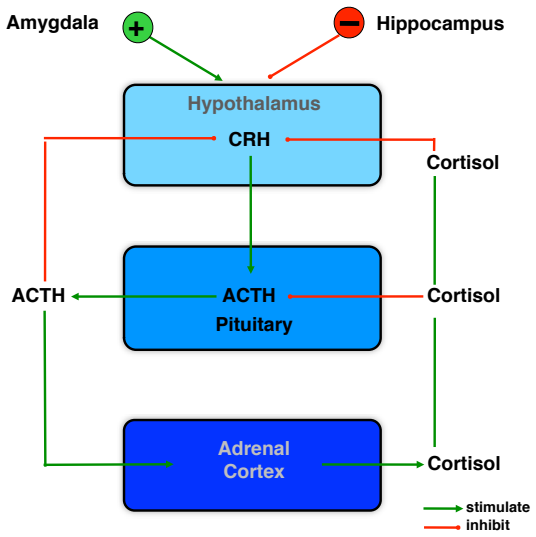


Fig. 2. Urinary 17-hydroxycorticoids after amygdala stimulation in patient 5.

Hippocampal Stimulation -> cortisol decrease
Amygdalar Stimulation -> cortisol increase

Patient	Location of stimulation	17-Hydroxy-corticoids in plasma: maximum change from control amount (%)
1	R-CA 2 hippocampus (histopath.)	−28
2	R-basolateral amygdala (histopath.)	+12
3	R-hippocampus (stereotaxic)	−88
3	L-subiculum hippocampus (histopath.)	−90
3	R-basolateral amygdala (stereotaxic)	+360
3	L-basolateral amygdala (histopath.)	+232
4	Anterior to L-amygdala (histopath.*)	+415
4	L-anterior hippocampus (histopath.*)	−18
4	L-CA 1 hippocampus (histopath.)	−100

* Unverified.



HPA Disregulation

Chronic mild stress as in poverty
-> increased stress response

Genetic differences as in rat strains
-> increased stress response

Clinical relevance:
Post-traumatic stress disorder (PTSD) & childhood abuse

Site of disregulation: hippocampus
in the limbic system, not hypothalamus or pituitary

Features of Stress

1. During acute stress, stress response declines in amplitude over time
2. After repeated stress, stress response adapts and stressor becomes less stressful
3. Both these characteristics can become disregulated.

Animal Model of HPA maladaption

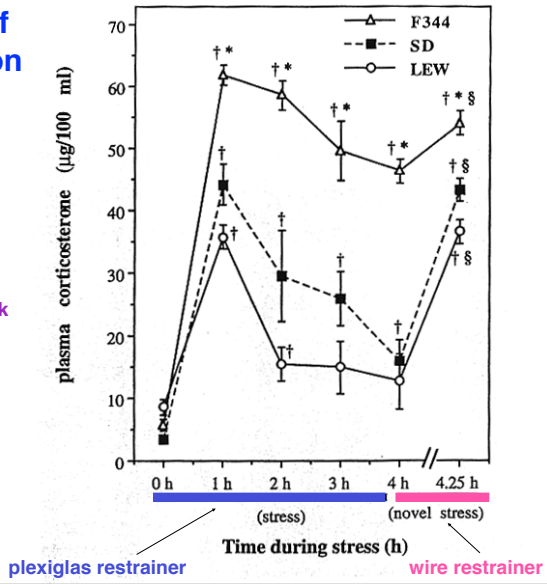
3 strains of rats:

Fisher 344

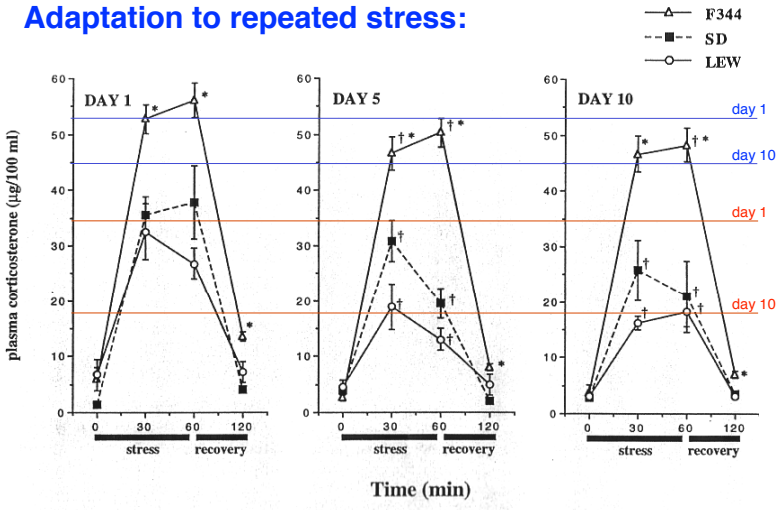
vs. Sprague Dawley

vs. Lewis

Fisher rats appear to lack appropriate negative feedback, because they have larger and longer stress response



Adaptation to repeated stress:

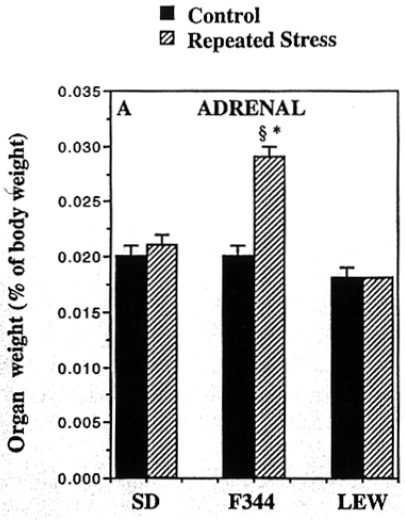


SD & Lew rat strains adapt to stress (day 10 < day 1), but F344 rats do not

Adaptation to repeated stress

Sprague Dawley & Lewis rats adapt to repeated stress (their adrenal glands remain normal size).

Fisher rats show increase in size of adrenal gland with repeated stress (i.e. stress continutes to elevate ACTH).



Animal Model: Early Maternal Separation

-> enhanced stress response

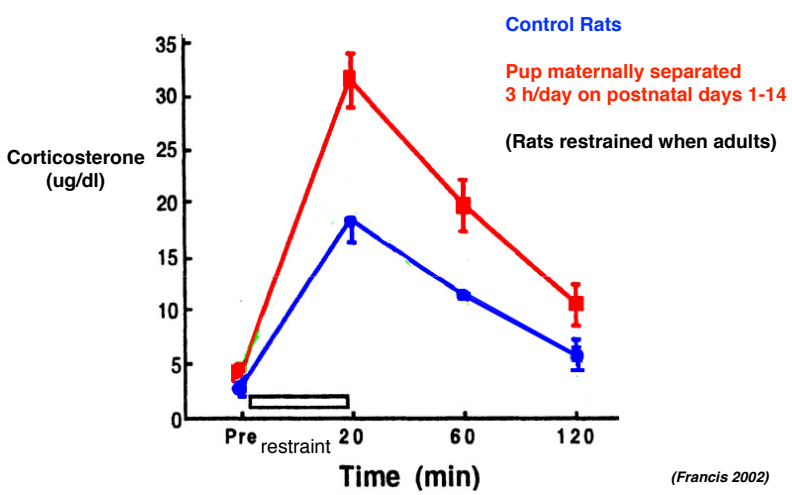
Maternal Separation = pups removed from mom for 3 h/day on PN days 1-14

Adults have increased positive feedback, decreased negative feedback

HPA Component	Adult after neonatal handling	Adult after neonatal separation
CRH mRNA (PVN)	↓	↑
CRH mRNA (Amygdala)	↓	↑
CRH mRNA (locus ceruleus)	↓	↑
CRH Receptor Binding	↓	↑
Glucorticoid Receptors (Hippocampus)	↑	↓
Glucorticoid Receptors (PVN)	No effect	↓
GC feedback inhibition of CRH	↑	↓

Separation-induced Hyperstress Response

Reversed by Enriched Environment



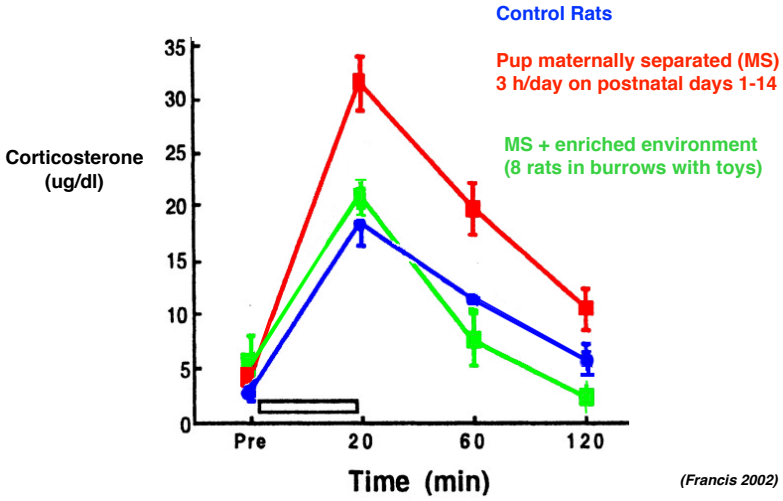
Control Rats

Pup maternally separated
3 h/day on postnatal days 1-14

(Rats restrained when adults)

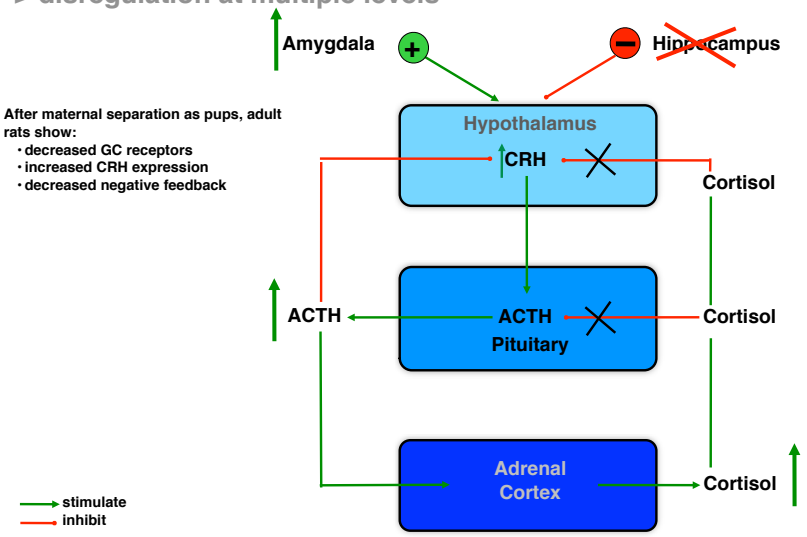
(Francis 2002)

Separation-induced Hyperstress Response
Reversed by Enriched Environment



(Francis 2002)

Maternal Separation or Early Abuse
-> disregulation at multiple levels



Addison's disease

Extreme adrenal steroid deficiency

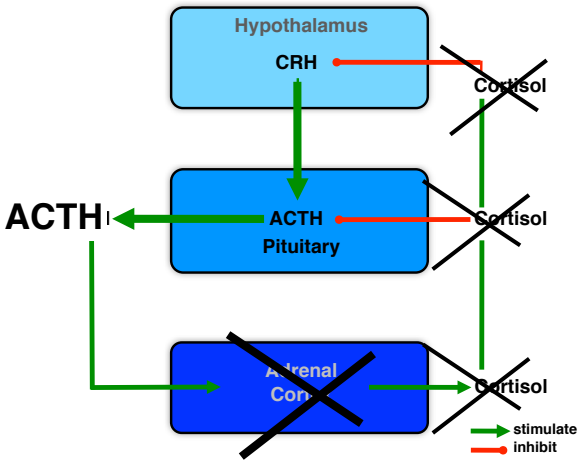
Caused by autoimmune or infectious **destruction of adrenal cortex**.

Extreme intolerance of stress, loss of appetite, malaise, fasting hypoglycemia, low blood pressure, salt craving

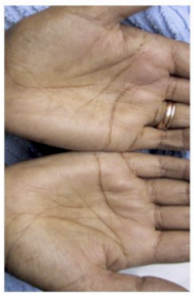
No glucocorticoids, so:
–> no negative feedback
–> hypersecretion of ACTH
–> hyperpigmentation of skin (because ACTH acts as melanocyte-stimulating hormone)

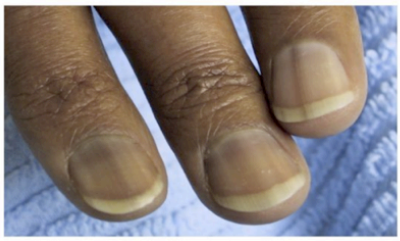
Treatment: administer exogenous corticosteroids to replace function of adrenal cortex

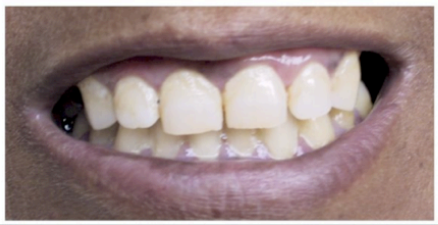
Addison's disease - low corticosteroids, elevated ACTH



Addison's disease - low corticosteroids, elevated ACTH



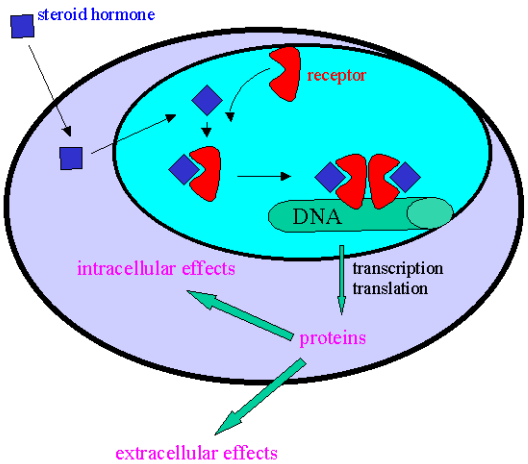


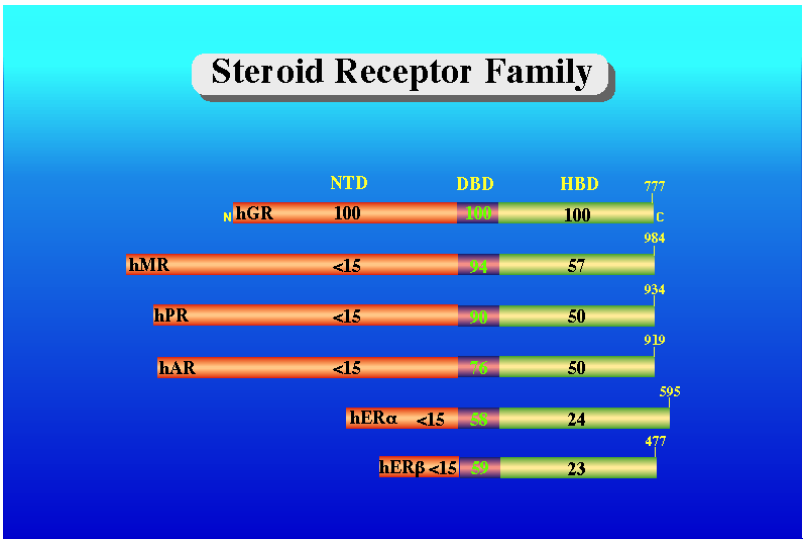


- Note the generalised skin pigmentation (in a Caucasion patient) but especially the deposition in the palmer skin creases, nails and gums.

www-clinpharm.medschl.cam.ac.uk

4. bind to cytoplasmic/ nuclear receptors

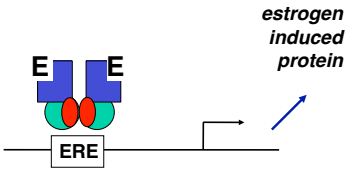




5. Nuclear receptors bind to target genes and regulate transcription

Glucocorticoid R
Mineralcorticoid R
Estrogen R α and β
Progesterone R
Androgen R

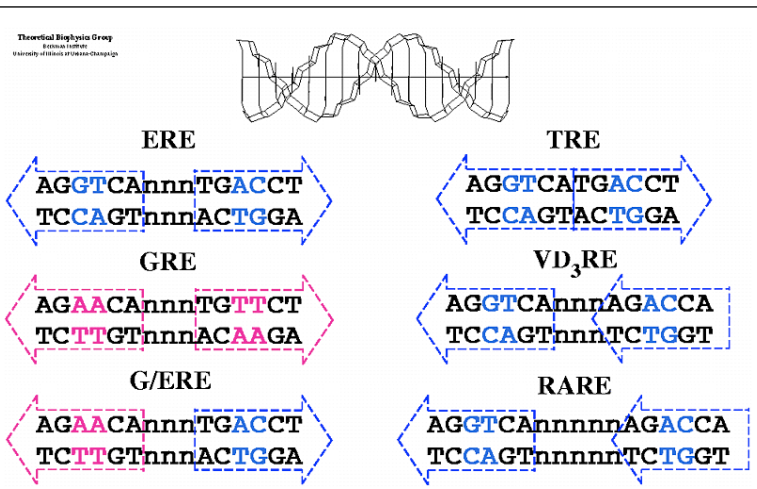
Thyroid R α and β, RX R
RA R α, β, γ



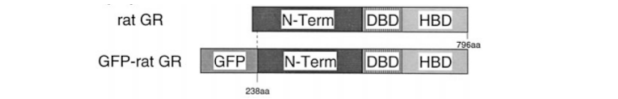
estrogen induced protein

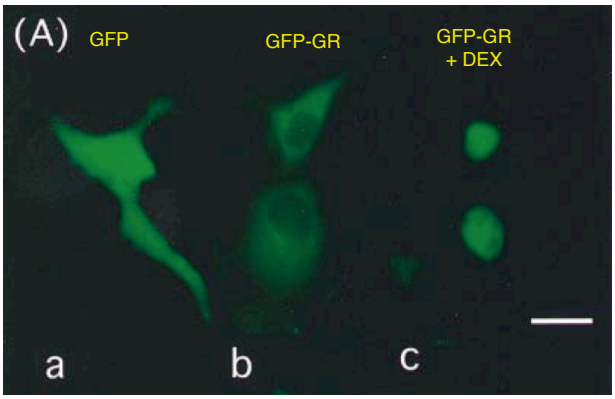
estrogen response element

Have ligand-binding domain, DNA-binding domain, and homomer binding domain
(so can transgenically mix and match ligands and induced genes)



http://www.ks.uiuc.edu/Research/pro_DNA/ster_horm_rec/

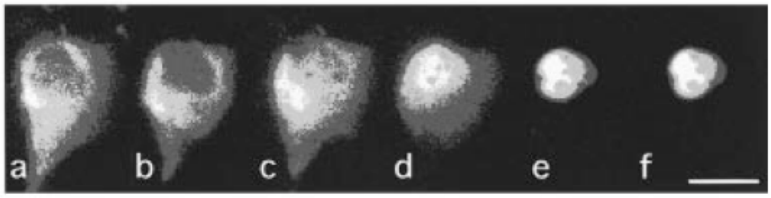




(a) GFP-transfected COS-1 cells.
(b) GFP-GR-transfected COS-1 cells without DEX.
(c) GFP-GR-transfected COS-1 cells with DEX.

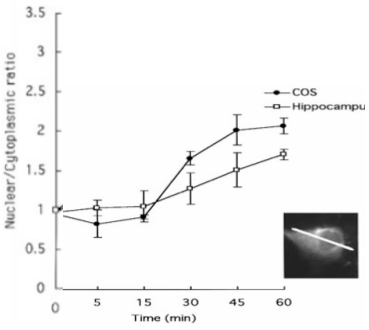
Nishi et al., European Journal of Neuroscience, Vol. 11, pp. 1927±1936, 1999

Movement of GRs into nucleus of Hippocampal Neurons after Dexamethasone Administration

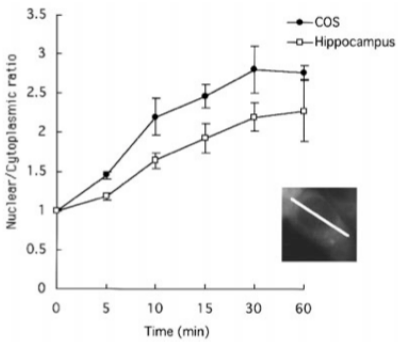


0 min 5 min 15 min 30 min 45 min 60 min

10^{-9} M Dex



10^{-7} M Dex



Long-term, genomic effects of N.R. hormones

Because the nuclear receptors bind to DNA, their effects are necessarily genomic (e.g. not directly ionotropic or metabotropic)

They induce protein synthesis.

It can take hours or days before an effect is seen.

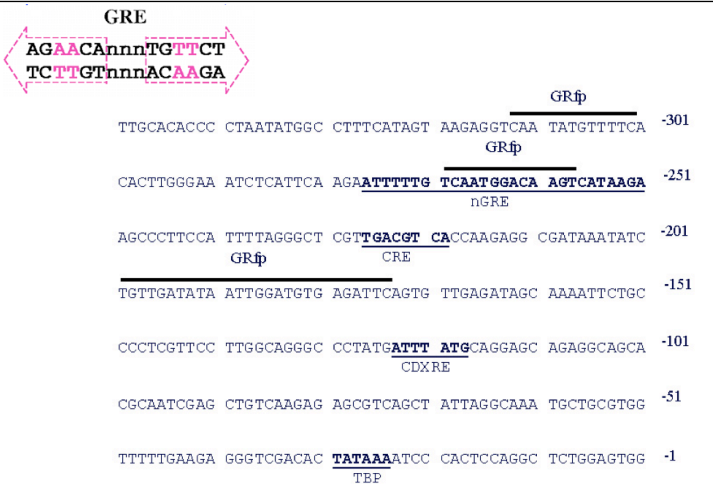


Figure 1. The Human CRH promoter DNA sequence and regulatory elements. The first 350 bp of the human CRH promoter is shown with several regulatory sites discussed in the text highlighted in bold-underlined font. The areas discussed in the text as GR-footprint (GRfp) regions on indicated by overlining the sequence.

Nicholson, Frontiers in Bioscience 9, (2004) 32-39

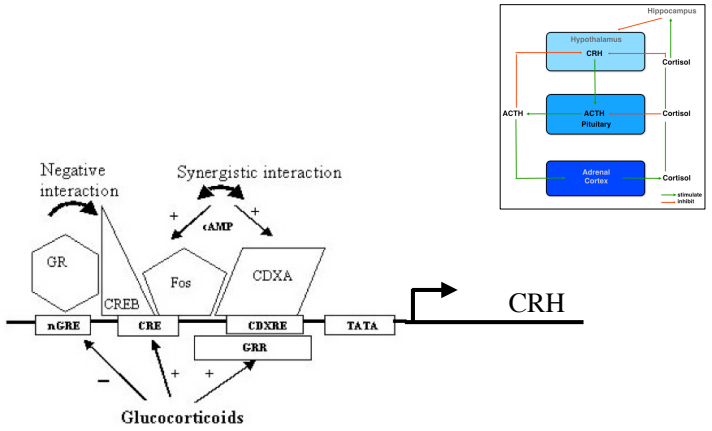
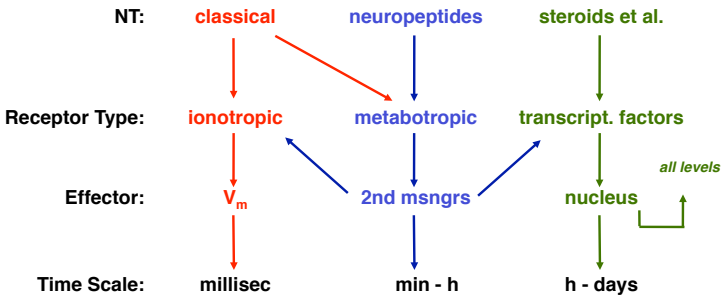


Figure 6. A schematic model of CRH promoter regulation in the hypothalamus. The nGRE is a negati glucocorticoid regulatory element, CRE is the cAMP regulatory element, GRR represents the region located between –213 to –99 bps that is stimulated by glucocorticoids, CDXRE is caudal type homeol response element and TATA is the TATA box. Stimulatory (+) and inhibitory (-) regulatory effects by cAMP and glucocorticoids through the different elements (thin arrows), negative (thick arrow) and synergistic stimulatory (double headed arrow) interactions between sites is shown.

Integrated actions of Classical, Neuropeptide, and Nuclear Receptor Transmitters



6. Short-term, non-genomic actions of steroids

1. **steroid receptor can have non-genomic effect**

   e.g. ERa ligand-binding domain can activate MAP kinase in cytoplasm

2. **steroid can directly modulate membrane receptor**

   e.g. progesterone can modulate GABA-A ionotropic receptor via membrane-spanning domains

   e.g. estrogen can bind GPCR GPR30 to stimulate cAMP

Test by using membrane-impermeant form of steroid,
e.g. estrogen-albumin conjugate