Anxiolytics: mechanisms

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Conflict of interest

WHO expert for drug dependence (2000-2016)

Anxiety versus fear

- **ANXIETY**

-Anxious apprehension and worry that is a more general reaction that is out of proportion to threats in environment

- Future oriented

- Can be adaptive if not excessive

- **FEAR**

- Experienced when a person is faced with real and immediate danger.

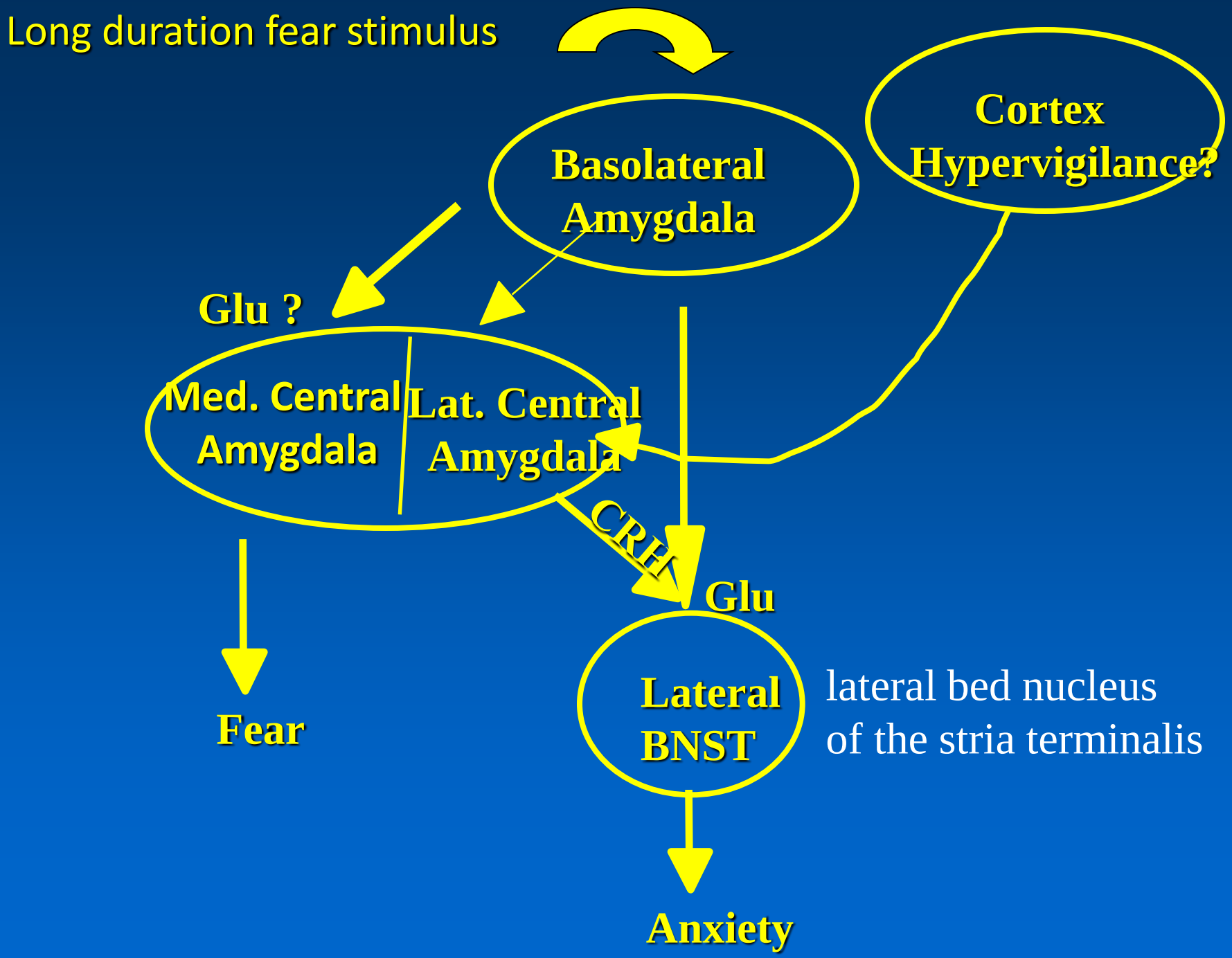
-Present-oriented

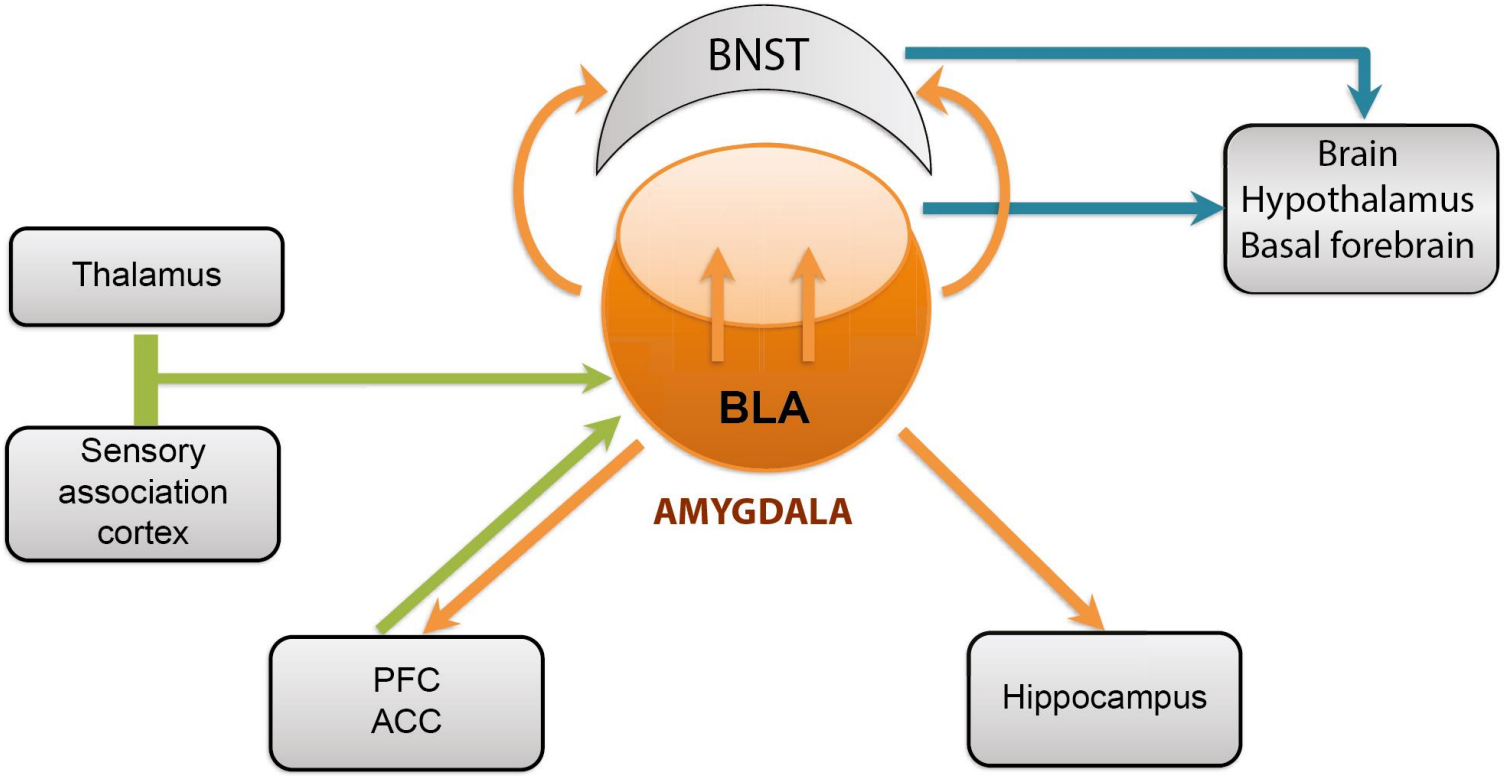
- Can be adaptive

Fear and anxiety

The prefrontal cortex regulates the expression of fear based on previously learned information.

Recently, this brain area has emerged as being crucial in the initial formation of fear memories, providing new avenues to study the neurobiology underlying aberrant learning in anxiety disorders.


Long duration fear stimulus
Basolateral Amygdala
Cortex Hypervigilance?
Glu ?
Med. Central Amygdala
Lat. Central Amygdala
CRH
Glu
Fear
Lateral BNST
lateral bed nucleus of the stria terminalis
Anxiety


BNST
Brain
Hypothalamus
Basal forebrain
Thalamus
BLA
Sensory
association
cortex
AMYGDALA
PFC
ACC
Hippocampus

Pathophysiology of anxious disorders

Abnormal regulation of neurobiological substrates :

- 5-HT, GABA, Glutamate
- Autonomic nervous system
- Hypothalamo- hypophysis axis
- Neuropeptides: CCK, P substance, galanin…..

Neuromediators in the brain (µmol/g)

Amino Acides (70-90 %)

Glutamate	14×10^6	GABA	$2{,}5 \times 10^4$
Aspartate	4×10^6	Glycine	2×10^6

Amines (5-20 %)

Acetylcholine	25×10^3	Serotonin	$2{,}5 \times 10^3$
Dopamine	$6{,}5 \times 10^3$	Histamine	1×10^3

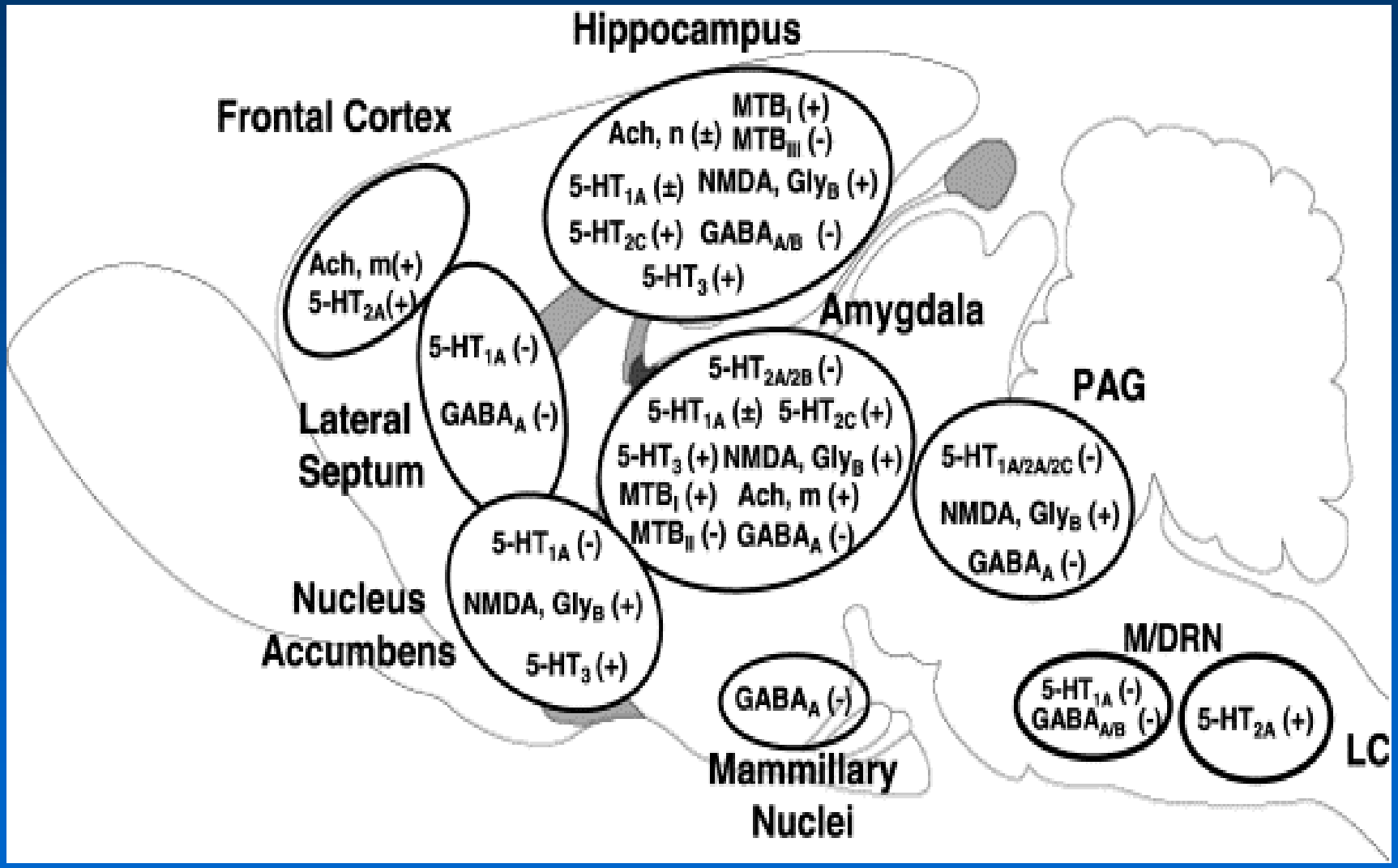
Neuropeptides (< 5 – 10 %)

CCK	470	Somatostatin	30
Met-enkephalin	350	Neurotensin	12
Substance P	100		
VIP	40		

Monoaminergic functions

NA	5-HT	DA
Vigilance	Impulsivity	Vigilance
Motivation	Appetite	Hyperactivity
	Sex	Dependence
	Agressivity	

SITES OF ACTION OF NEUROTRANSMITTERS



Anxiolytics

- Benzodiazepines and GABA active drugs
- Buspirone: 5HT1a partial agonist
- SSRIs
- Antipsychotics ?

Serotonin in anxiety

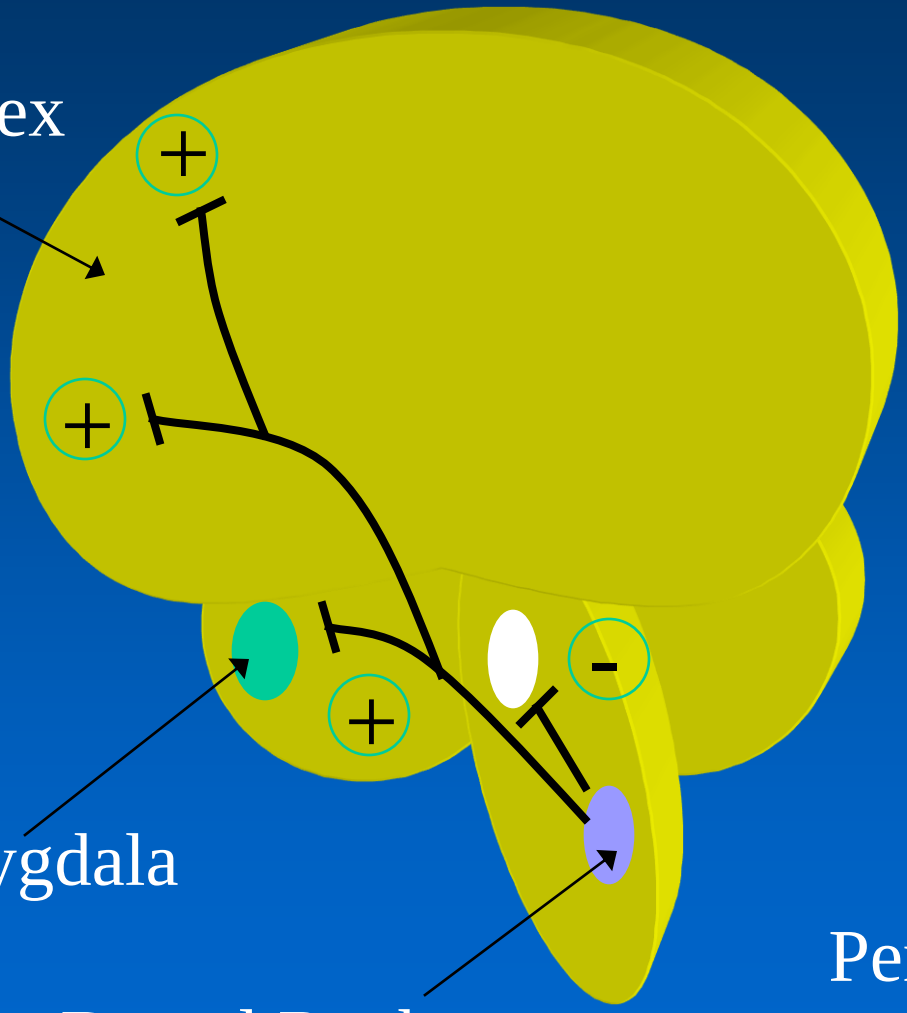
- Classical hypothesis of anxiety:
- $\Downarrow$ serotonin pathways $\Rightarrow\Rightarrow$ anxiolytic effect
- $\Uparrow$ serotonin pathways $\Rightarrow\Rightarrow$ anxiogenic effect

- Dual hypothesis of anxiety:
- Role of amygdala and peri-aqueductal gray

- Lesions to the amygdala disrupt the conditioned responses but do not affect the learning of relevant declarative facts

- Hippocampal lesions disrupt the learning of relevant facts but do not affect the acquisition of conditioned responses

Serotoninergic model of anxiety (Graeff et al 1996)

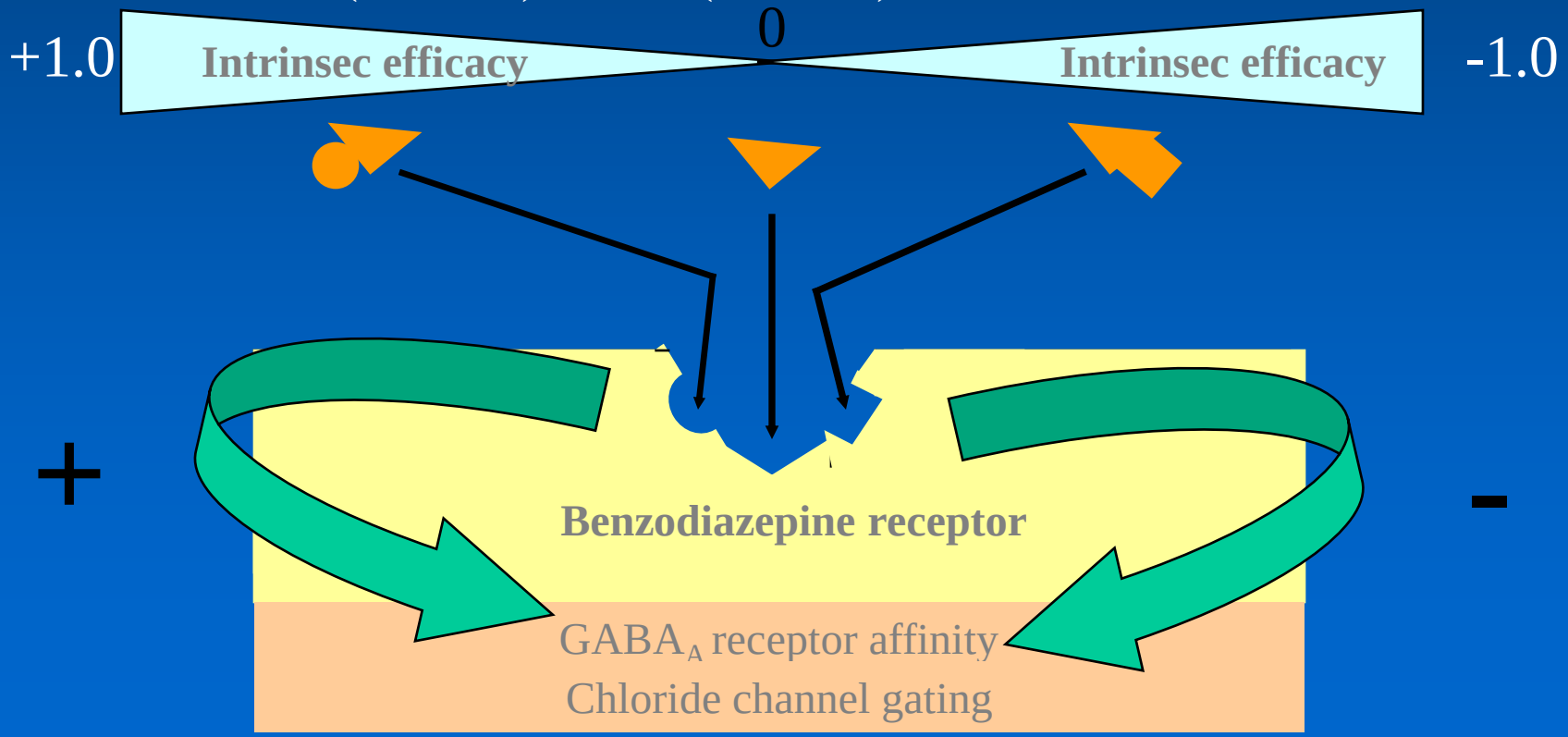


Benzodiazepines

- BZDs: Anxiety-Reducing Activity by Reduction of Serotonin Turnover in the Brain (**Wise CD**, **Berger BD**, **Stein L** Science. 1972)
- Anxiolytic effects of BZDs are neutralized by intravenous 5-HT
- BZDs: Allosteric modulators of $GABA_A$ receptors

SPECTRUM OF BENZODIAZEPINE RECEPTOR LIGANDS

Agonists	Partial Agonists	Antagonists	Partial Inverse Agonists	Inverse Agonists
midazolam	bretazenil (Ro 16-6028)	flumazenil (Ro 15-1788)	Ro 15-4513	Ro 19-4603



GABA

- ✓ g aminobutyric Acid (GABA): one of the most abundant neurotransmitters in the CNS
- ✓ All the brain structures contain GABAergic neurons
- ✓Discovered 50 years ago that GABA is an inhibitor neurotransmitter in the CNS
  - ➢ 30% of synapses in verterbrates

GABAergic system (1)

- ✓ **GABA: gamma-aminobutyric acid**
  - ➢ **inhibitor activity**
  - ➢ **1st amino acid which the neurotransmission role has been recognised**
  - ➢ **[GABA]: more 200 -1000 times than Ach. or 5-HT**

✓ **3 types of receptors: $GABA_A$ (ionotropic: coupled to ion channel), $GABA_B$ (metabotropic: coupled to G protein) and $GABA_c$ linked to chloride channels mainly in the retina.**

GABAergic system (2)

✓ $GABA_A$ receptor is sensitive to muscimol (agonist), bicuculline and picrotoxin (antagonists)

- GABA binding leads to opening of Cl^- channel followed by hyperpolarisation of the target cell

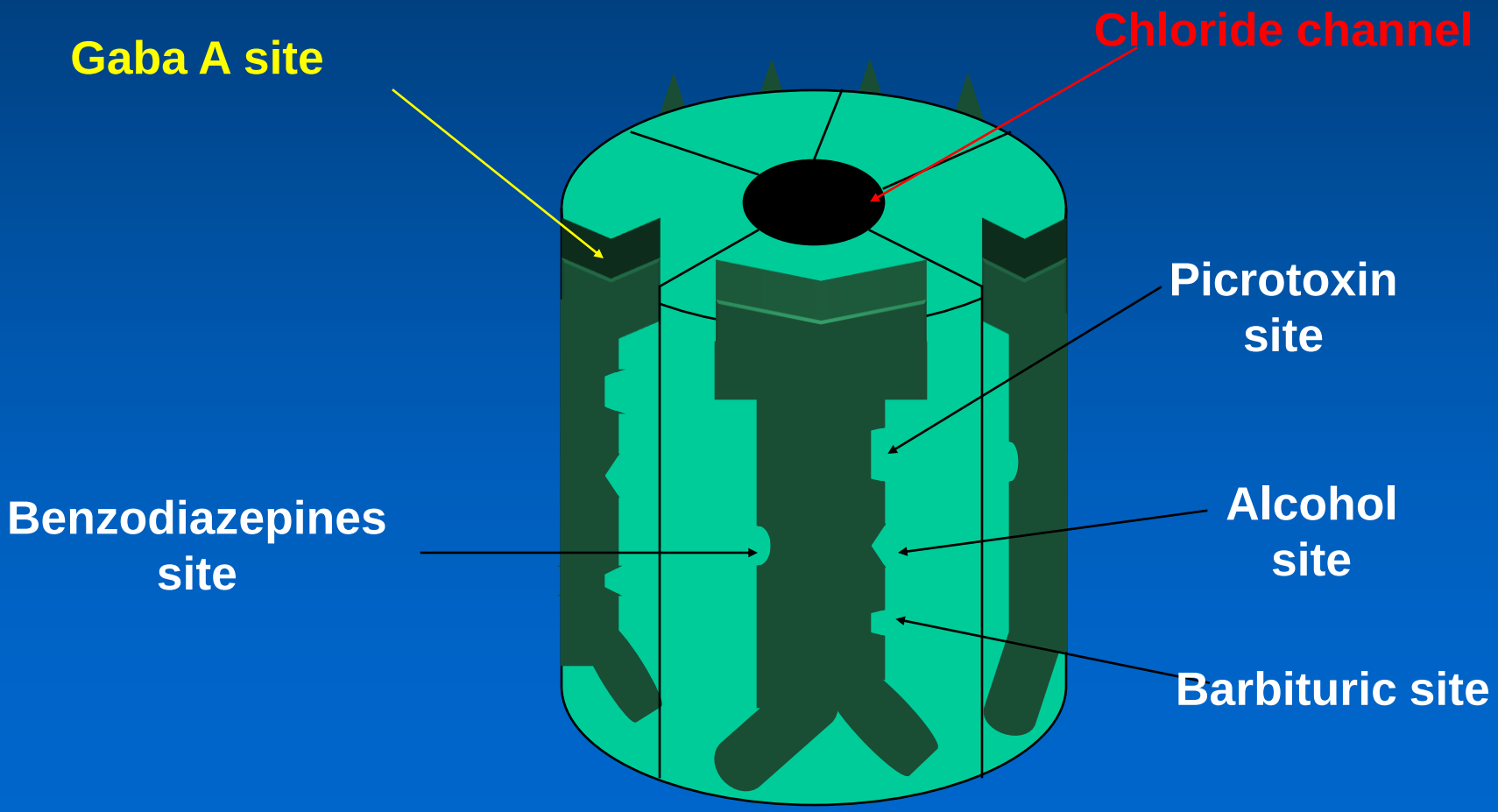
✓ $GABA_B$ receptor is sensitive to baclofen (agonist) and CGP 56119 (antagonist)

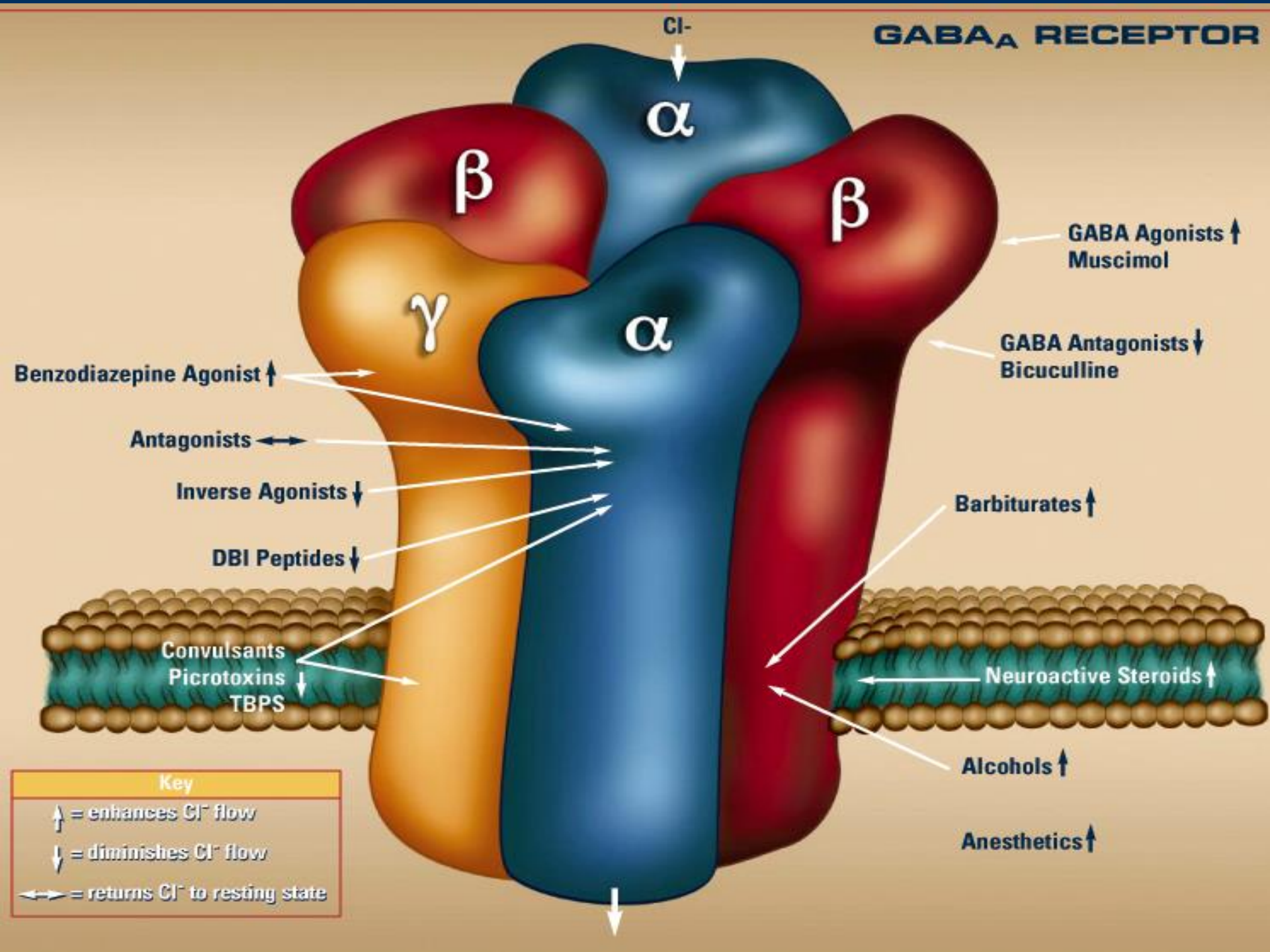
- receptor coupled to G protein G0 or Gi
- G0 protein can be coupled with Ca^{2+} ou K^+ channel

$GABA_A$ receptor (1)

- 5 trans-membrane glycoprotein subunits arranged round central chloride channel - ‘ligand-gated ion channel’
- activation ➔ chloride influx ➔ hyperpolaristion ➔ neuronal inhibition
- may have multiple allosteric modulating sites as part of the receptor complex (e.g. benzodiazepines, barbiturates, alcohol)

$GABA_A$ receptor (2)




GABA$_A$ RECEPTOR
Cl-
α
β
β
γ
α
GABA Agonists
Muscimol
GABA Antagonists
Bicuculline
Benzodiazepine Agonist
Antagonists
Inverse Agonists
DBI Peptides
Barbiturates
Convulsants
Picrotoxins
TBPS
Neuroactive Steroids
Alcohols
Anesthetics
Key
= enhances Cl⁻ flow
= diminishes Cl⁻ flow
= returns Cl⁻ to resting state

$GABA_A$ receptor (3)

✓ **Allosteric regulation is operated by two different classes of compounds:**

- **Those that act on the extracellular domain**
- **Those that act on the channel domain of the receptor**
- **Both classes include positive and negative allosteric modulators**

$GABA_A$ receptor (4)

✓ **Drugs which bind within the channel domain can acts as**

- **Positive modulators (barbiturates and steroid hormone derivates)**
- **Negative modulators (pregnenolone sulfate and picrotoxin)**

$GABA_A$ sub-units

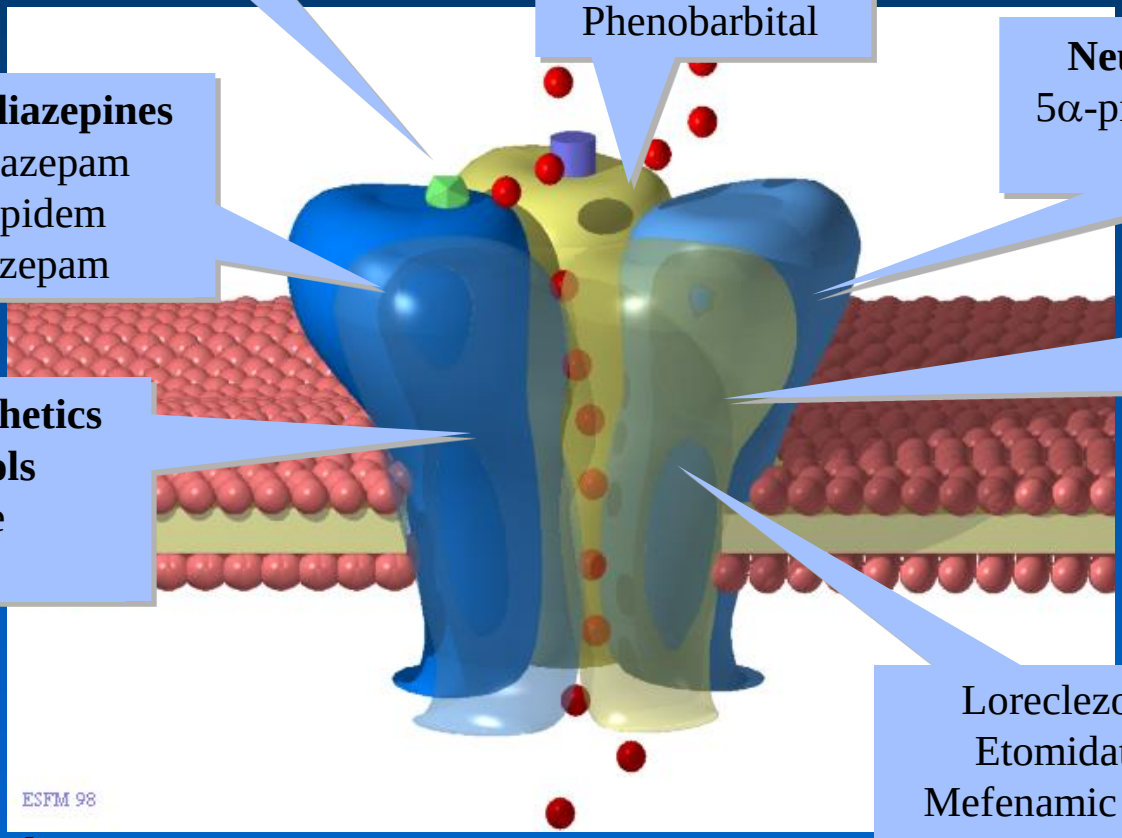
- 19 different sub-units, classified into 6 major classes
- composition determines pharmacological characteristics
- Function depends on subunits of pentameric complex
- $GABA_A$ ∝1 (60% of all $GABA_A$ receptors):
  - sedative, amnestic, anticonvulsant
- $GABA_A$ ∝ 2 (15% of all $GABA_A$ receptors):
  - anxiolytic, muscle relaxant
- $GABA_A$ ∝ 3 (15% of all $GABA_A$ receptors):
  - unknown

Korpi, Sinkkonen (2005). Pharmacol Ther.

Differential modulation of $GABA_A$-receptor subtypes

- classic benzodiazepines (e.g. diazepam, temazepam)
  - non-specific activation of all ∝ subtypes
- 'non-benzodiazepine' BZD-receptor agonists (e.g. zolpidem, zaleplon)
  - high affinity for ∝1 subtype
- GABA reuptake inhibitors (tiagabine)
  - enhanced activity at all ∝ subtypes

$GABA_A$ Receptors – A Rich Pharmacology



Propofol

Barbiturates
Pentobarbital
Phenobarbital

Neuroactive Steroids
5α-pregnan-3α-ol-20-one
5α-THDOC

Benzodiazepines
Clonazepam
Zolpidem
Diazepam

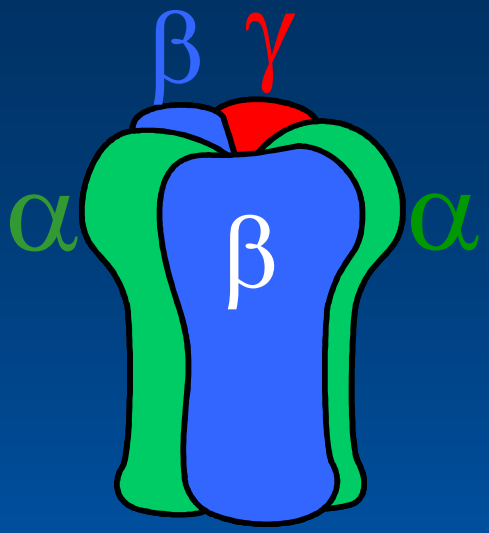
GABA
muscimol
Bicuculline
THIP

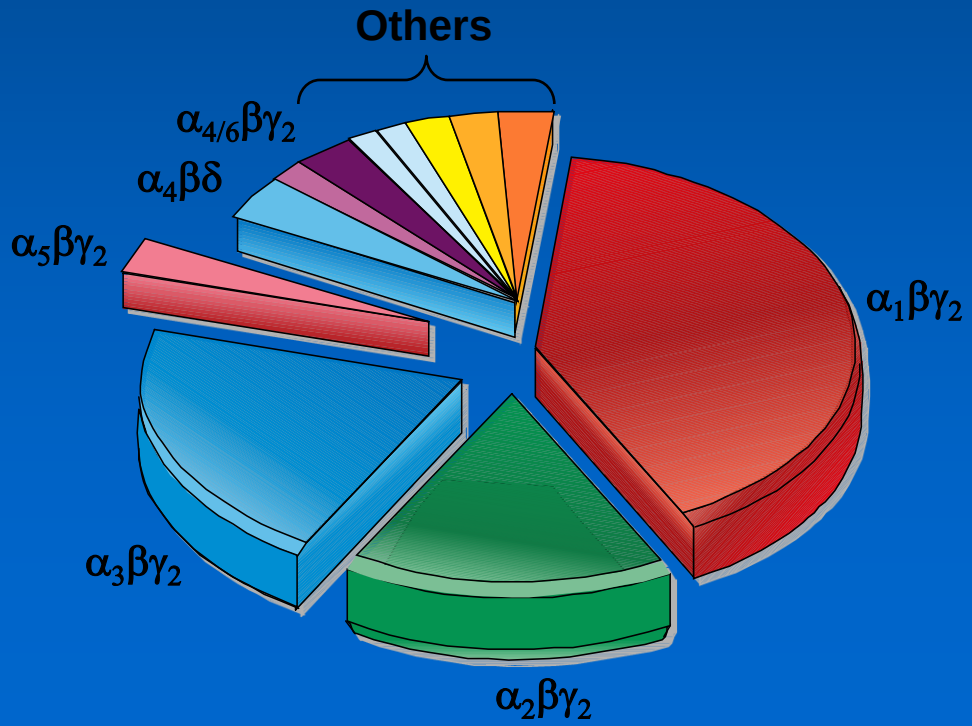
Volatile anaesthetics and alcohols
Halothane
Ethanol

Loreclezole
Etomidate
Mefenamic acid

However, $GABA_A$ receptors are ubiquitously expressed in the CNS therefore – side effects

$GABA_A$ receptor isoforms



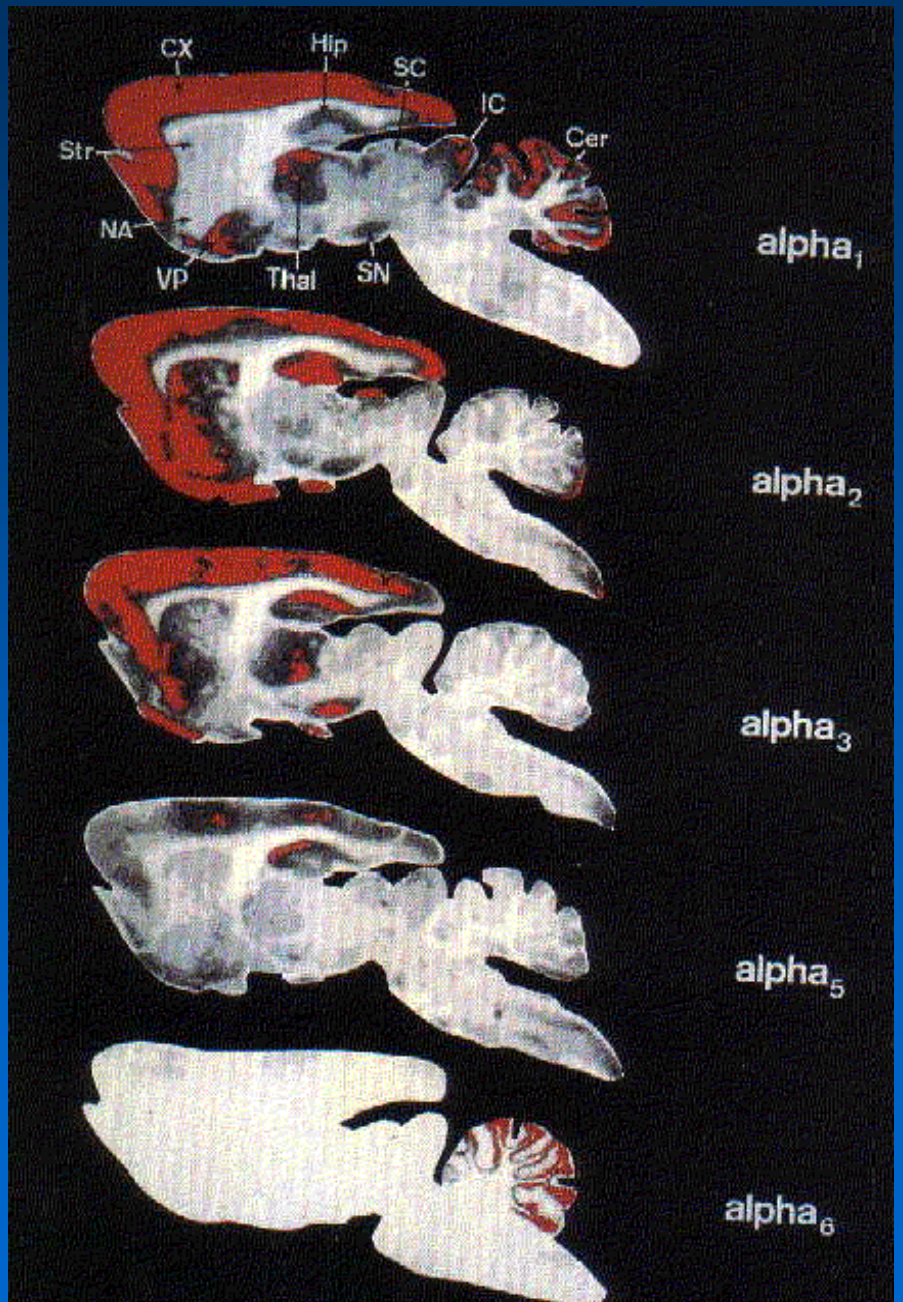


Most current therapeutics interact with many of the ~20 $GABA_A$ receptor subtypes causing adverse effects.

The modulatory site
BZD RECEPTOR CLASSIFICATION

Type I	α1, β2, gamma2
Type II	α1ou α3, β2, gamma2
Type III	α5, β3, gamma2
Type IV	α6, β2, gamma2
Type V	α1, β1, gamma1

IMMUNOHISTOCHEMICAL MAPPING OF $GABA_A$



Global distribution in rat of alpha-subunit immunoreactivity. The sites of the most abundant immunoreaction (red > grey > white)

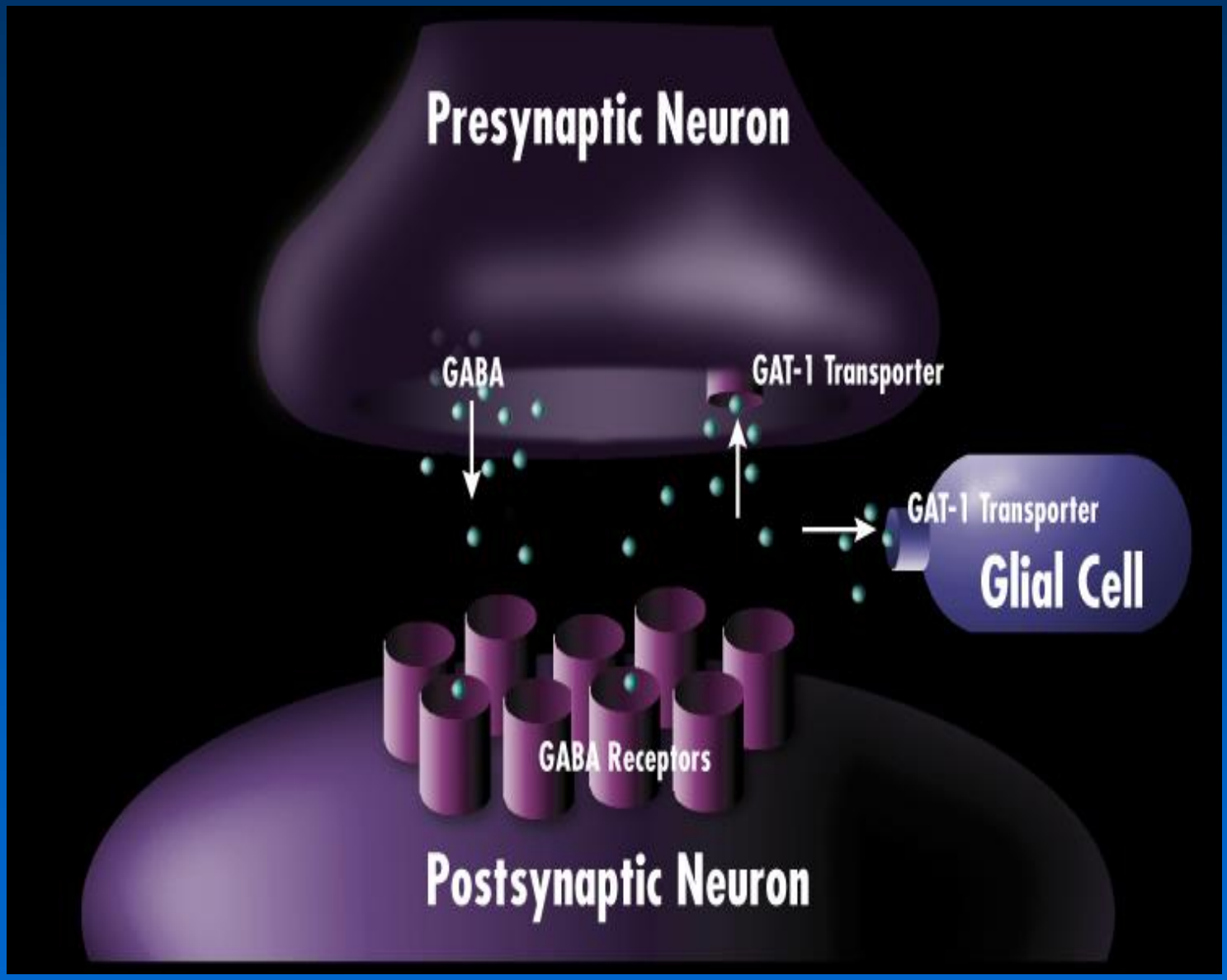
BENZODIAZEPINE PHARMACOLOGY OF $GABA_A$ RECEPTORS SUBTYPES

Pharmacological effect	Receptor involved
Anxiolysis	α2 containing
Sedation	α1 containing and those not containing α1
Anterograde amnesia	α1 containing

Enhancement of GABA

Mechanism	Examples
Increased synthesis	Topiramate, valproate
Inhibit breakdown	Valproate, vigabatrin
Inhibit reuptake	Tiagabine
Allosteric $GABA_A$ modulation	BZDs, barbiturates, neurosteroids, topiramate
Direct agonism	Alcohol, high-dose barbiturates, chloral hydrate, abecarnil, pagoclone
GABA analogues	Gabapentin, pregabalin

Mechanism of action of tiagabine



Non-GABA-ergic targets for anxiolytic drugs

- 5-HT_1 and 5-HT_2 receptor antagonists
- melatonin receptor agonists
- antagonists at the substance P (NK-1) receptor
- metabotropic glutamate receptor antagonists
- cholecystokinin antagonists
- neuropeptide Y agonists
- adenosine A_1 and A_{2A} receptor agonists

5-HT targets for novel anxiolytic drugs

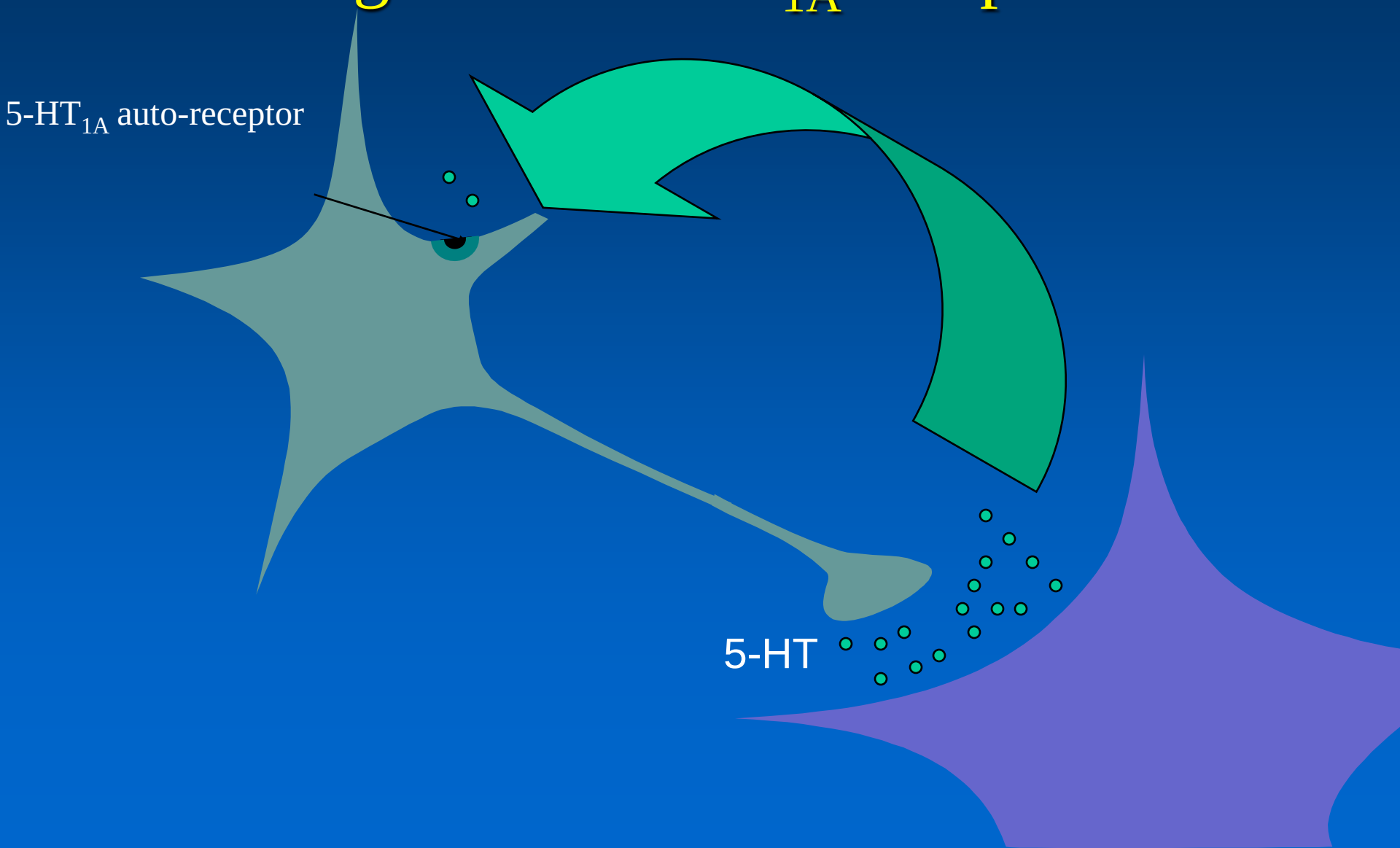
Approach	Examples
5-HT_{1A} agonist	Buspirone patch to avoid first-pass hepatic metabolism.
SSRI plus 5-HT_2 antagonism	Nefazodone. Relief of anxiety symptoms in major depression and efficacy in panic disorder.
5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists	May increase 5-HT availability in synaptic cleft and advance onset of action of SSRIs.
5-HT_{2C} antagonist	Deramciclane. Proven efficacy in Phase II study not confirmed in subsequent Phase III studies.
5-HT_3 antagonist	Claims of efficacy in GAD and panic disorder not supported by subsequent studies.
5-HT_{2C} antagonist and melatonin agonist	Agomelatine. Proven efficacy in Phase II and III studies in depression. Relief of anxiety symptoms.
SSRI plus norepinephrine reuptake inhibition	Venlafaxine, milnacipran, duloxetine. Venlafaxine has proven efficacy in GAD, social phobia, panic and PTSD

AGONISM AT THE 5-HT1A RECEPTOR

A. Activation of presynaptic 5-HT1A receptor may reduce 5-HT release

B. Activation of postsynaptic 5-HT1A may explain paradoxical anxiety after acute administration

Limiting role of 5-HT_{1A} receptors



Modulating role of 5-HT_{1B} receptors

- Activation of pre- synaptic 5-HT_{1B} autoreceptors seems to limit the effects of SSRIs on [5-HT], mainly in hippocampus

- Activation of post- synaptic 5-HT_{1B} receptors is necessary to the antidepressant activity of SSRIs

SSRIs (acute) in mice

+	PAROXETINE CITALOPRAM FLUVOXAMINE
-	FLUOXETINE
0	SERTRALINE

- They are not all active on several anxiety tests

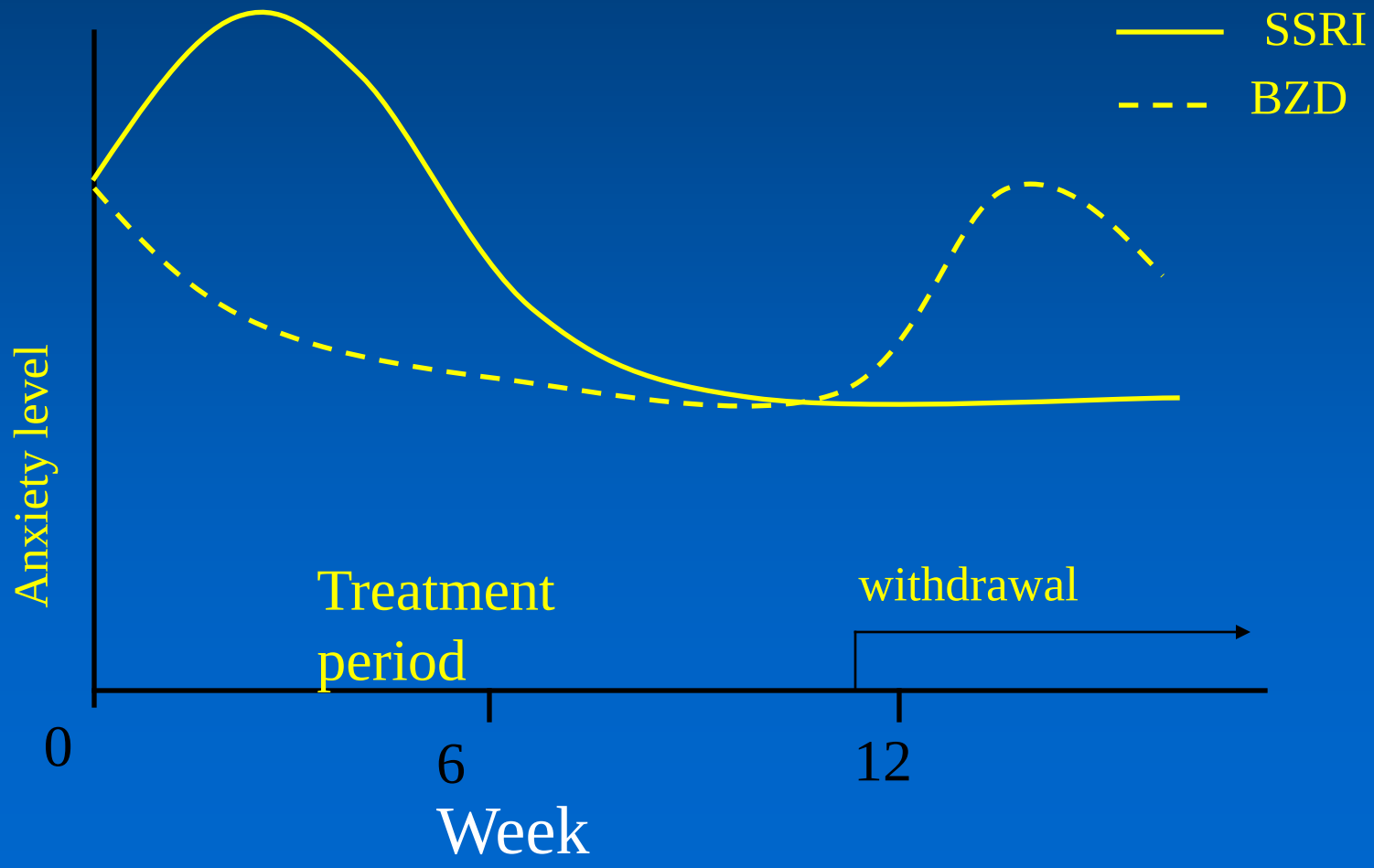
- + anxiolytic, - anxiogenic, 0 = no effect

DRUG EFFECTS IN ANIMAL MODELS OF ANXIETY

Animal models	BZD agonists	5-HT1A agonists	5-HT2A/2C antagonists	5-HT3 antagonists	CCK-2 antagonists	AD agents	GLU	CRF
EPM	++	+	+	+	+	++		++
L/D	++	++	+	+	+	-		++
FP	++	++	++	0	NA	++	++	

++: anxiolytic-like effect; +: anxiolytic-like effect or no effect, anxiogenic effect, 0:no effect

Action of SSRIs in GAD



Antipsychotics as anxiolytics

Atypical antipsychotics such as quetiapine, aripiprazole, olanzapine and risperidone have been shown to be helpful in addressing a range of anxiety symptoms in individuals with schizophrenia and schizoaffective disorders, and have since been used in the treatment of anxiety

5HT2c and anxiety

As a therapeutic purpose several 5HT2c antagonists were developed for the treatment of several nervous system disorders including anxiety

Potential impact of melatonin receptor agonists

- Agomelatine: melatonin agonist and 5-HT_{2C} antagonist
- superior to placebo in at least 2 placebo-controlled trials [1,2]
- side effect profile similar to placebo at 5-25 mg/day
- no discontinuation symptoms[3]

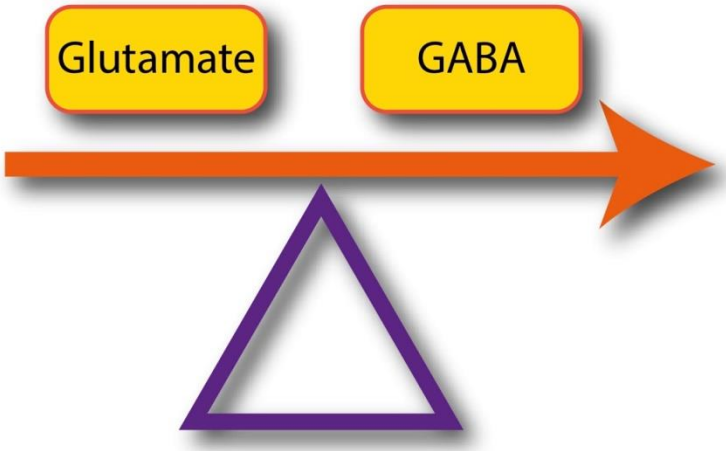
[1]Loo *et al*., 2002; [2]Loo *et al*., 2003; [3]Montgomery *et al*., 2004

Glutamate receptors as targets for anxiolytics

Receptor type	Role
Ionotropic (iGlu)	Ligand-gated ion channels. Mediate synaptic excitability and plasticity
Metabotropic G-protein coupled receptors ($mGlu_{1-8}$)	Regulate glutamate release and modify postsynaptic excitability
Plasma membrane glutamate transporters ($EAAT_{1-5}$)	Clear synaptic space of released glutamate and other excitatory amino acids
Vesicular glutamate transporters ($vGluT_1$ and $vGluT_2$)	Package glutamate for exocytotic release

Swanson *et al*. (2005) Nature Rev Drug Discov 4: 131-144

GABAergic-glutamatergic balance



Abnormal hyperexcitabilty

Normal physiologic range

Abnormal hypoexcitability

Metabotropic glutamate receptors and anxiety

Group	Receptor	Excitatory	Effects of receptor ligands
I	$mGlu_1$	Yes	Agonist (*trans*-ACPD) enhances startle response.
	$mGlu_5$	Yes	Antagonist (MPEP) exerts anxiolytic effects in animals.
II	$mGlu_2$	No	Agonist (LY354740) limits Glu release and demonstrates anxiolytic profile in animal models. Effects reversed by flumazenil. Prevents CO2 induced anxiety in panic patients and reduced HAMA score in GAD patients.
	$mGlu_3$	No	
III	$mGlu_4$	No	Few selective ligands available.
	$mGlu_6$	No	Agonist (MSOP) exerts anxiolytic effects in animals.
	$mGlu_7$	No	$mGlu_7$ knock-out mice show decreased freezing behaviour and decreased condition taste aversion.
	$mGlu_8$	No	$mGlu_8$ knock-out mice show increased anxiety behaviour in elevated plus maze.

Swanson *et al*. (2005) Nature Rev Drug Discov 4: 131-144

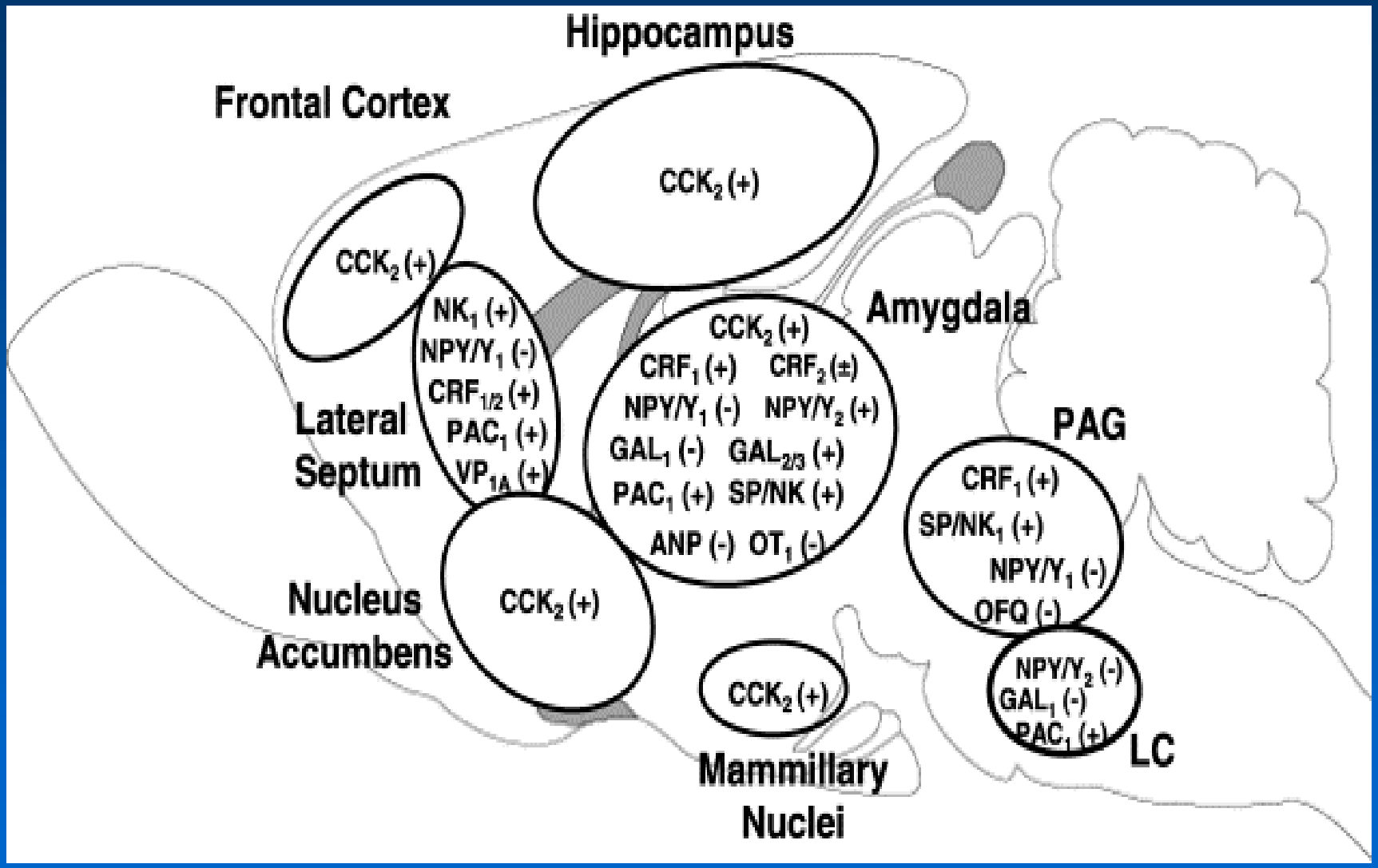
Adenosine receptors - a role in anxiety?

- caffeine is non-selective adenosine receptor (A_1 and A_{2A}) antagonist
- induces wakefulness in wild and A_1 receptor knockout mice, with no induction in A_{2A} knockout mice
- wakefulness therefore arises through effects on A_{2A} receptor[1]
- A_{2A} agonists have potential anxiolytic properties
- possible polymorphism of A_{2A} receptor in panic disorder[2]

[1]Huang *et al* (2005) Nature Neurosci 8: 858-859

[2]Hamilton *et al* (2004) Neuropsychopharmacol 29: 558-565

SITES OF ACTION OF NEUROPEPTIDES



From Millan, 2003

CHOLECYSTOKININ

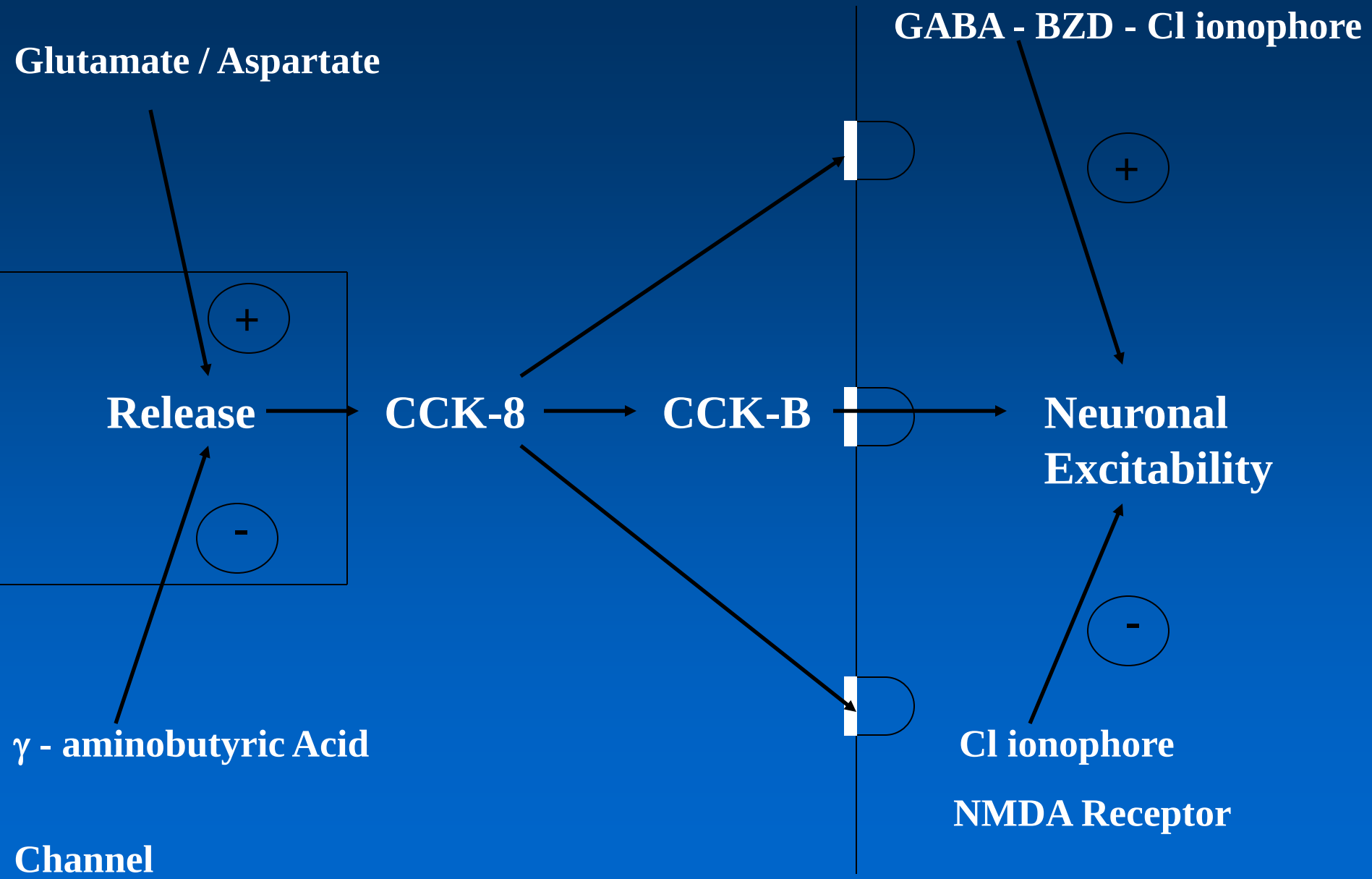
- Cholecystokinin is a neuropeptide discovered in the gastrointestinal tract
- Probably the most abundant neuropeptide in the brain
- Two subtypes of receptors are existing: CCK_1 receptors are widely distributed in the periphery and they are established in the distinct brain nuclei, whereas CCK_2 receptors are present in the brain and stomach

CHOLECYSTOKININ

- Cholecystokinin is involved in the regulation of various physiological functions, including pain, feeding and emotional behaviour
- Cholecystokinin is implicated in the regulation of anxiety. The administration of CCK-4 induced panic attacks in humans and anxiety-like state in animals
- Anxiogenic-like action of CCK is mediated via CCK_2 receptors
- Cholecystokinin is co-mediator of GABA in the neurons of hippocampus and cerebral cortex

Cholecystokinin antagonists

- CCK-4 can provoke anxiety in healthy volunteers
- Effects of CCK-4 are attenuated by anti-panic medications
- CCK-4 antagonists block anxiogenic effects of CCK
- No proven efficacy in anxiety disorders



From Bourin 1995

Corticotrophin-releasing factor (CRF) is an neuropeptide that plays a prominent role in the endocrine, autonomic, behavioural and immune responses to stress

CRF injected into brain of rats produced many of the signs and symptoms seen in patients with anxiety disorders. (Arborelius et al., 1999)

CRF receptor type 1 (but not type 2) located within the amygdala plays a role in the modulation of anxiety in mice exposed to the elevated plus maze.

Cipriano et al. Horm Behav. 2016;81:59-67.

CRF Human studies

Mainly in depression but disapointing

Anxiolytic effect of neurotensin microinjection into the ventral pallidum

Ollmann et al 2016

Behav Brain Res. 2015 ;294:208-14.

GALANIN

- Administred directly into the central nucleus of the amygdala blocked the anxiogenic effect of yohimbine
- GAL-R1 deficient mice show increased anxiety like behaviour
- Behavioural reponse to stress may depend on the balance between NA, NPY and galanin

- Modification of Anxious Behavior after Psychogenic Trauma and Treatment with Galanin Receptor Antagonist.

- Lyudyno VI, Tsikunov SG, Abdurasulova IN, Kusov AG, Klimenko VM.

- Bull Exp Biol Med. 2015 ;159(3):344-7

ATRIAL NATRIURETIC PEPTIDE AND PANIC DISORDER

- Pretreatment of 150 microg of atrial natriuretic peptide protected against CCK-4 induced panic in PD patients. Strohle et al Am J Psychiatry 2001, 158, 1514-1516
- As well in healthy volunteers Wiedmann et al Arch Gen Psychiatry 2001, 58, 371-377

ATRIAL NATRIURETIC PEPTIDE

Intraperitoneal atrial natriuretic peptide attenuates anxiety-related behaviour during alcohol withdrawal in mice.

von der Goltz C, Jahn H, Mutschler J, Wiedemann K, Kiefer F.

Pharmacopsychiatry. 2014;47:97-100

Vasopressin Antagonist

- The Vasopressin V1b Receptor Antagonist SSR149415 in the Treatment of Major Depressive and Generalized Anxiety Disorders: Results From 4 Randomized, Double-Blind, Placebo-Controlled Studies

- Guy Griebel; Sandra Beeské, and Stephen M. Stahl,

Vasopressinergic control of stress-related behavior: studies in Brattleboro rats.

- CsikotaP, Fodor A, Balázsfi D, Pintér O, Mizukami H, Weger S, Heilbronn R, Engelmann M, Zelena D

- Stress. 2016 17:1-13

- .

Neuropeptides in anxiety

- Less important than GABA and Serotonin
- They play like a light dimmer not like light producers

Conclusion of anxiolytics

CLASSES OF ANXIOLYTICS	USES
Benzodiazepines	Generalized anxiety disorders, OCD, phobia, panic attack
SSRIs	Generalized anxiety disorders, OCD, phobia, panic attack
Tricyclic antidepressants (doxepin, imipramine)	Anxiety with depression. panic attacks
5HT1A agonists (Buspirone)	Mild anxiety Not effective in panic attack
Beta blockers (propranolol, atenolol)	Social anxiety
MAO inhibitors Phenelzine	Panic attack, phobia

CONCLUSION

- The neurocircuitry of fear and anxiety is complex
- Neuropeptides seems to be regulators of the monoamine transmitters

- There is no yet drug acting on neuropeptides receptors active as anxiolytic

Most highly cited journal for drug discovery
Peer reviewed for authoritative coverage

20th Anniversary of Combinato

- Dynamic combinatorial chemistry
- Combinatorial chemistry in anti-inf
- Statistical design in combinatorial c
- Predictive ADME simulation in drug

Case vignette 1

Man 32 year old sailor who was previously a carpenter.

Secondary school level, he was adopted in early age

Presenting with anxiety and difficulties of concentration and obsessive ideas

Taking cannabis sometimes

Case vignette 2

A 72 yo man retired (He was previously M.D.radiologist) presenting with anxiety.

His wife told me that he began to be anxious when he stopped to work one year ago

He was not presenting with mood disorder. He has no lost of memory.

He plays music in a band (old jazz) and love gardening

Case vignette 3

Female 68 yo presenting with Crohn disease since she was 18 yo treated with azathioprine.

Separated from her husband 4 years ago

Agoraphobia , difficulty of concentration

Sometimes aggressive treated by general practionner by venlafaxine 75 mg a day.

No sign of dementia even if she is desinhibited

Case vignette 4

Female 56 yo presenting numerous depressive since she was 16.

Two children : daughter 30 yo, son 26 yo.

After each pregnancy, she presented with post-partum depression.

Recently hospitalized during 3 weeks for anxiety and depression.

Case vignette 5

Male 29 yo, 65 m / 60 kg soccer player during several years

Vertigo during 2 months.

2 years ago , panic attack then depressive episode during 6 months

He is presenting with lost of energy working now on business