$GABA_A$ receptor pharmacology:

Alcohol, Benzodiazepines..and the rest

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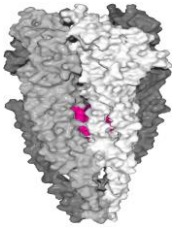
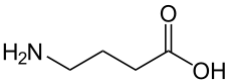


GABA is a simple amino acid

Electroneutral zwitterion (isoelectric point, 7.3)

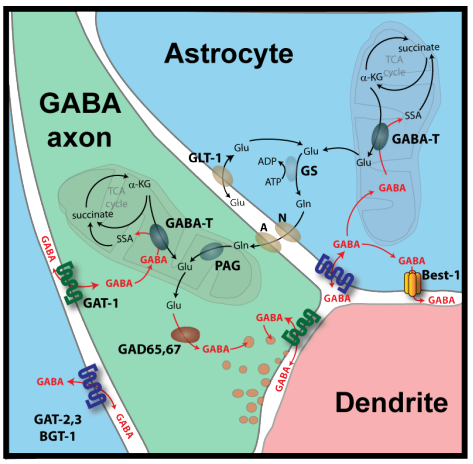


γ-**Aminobutyric acid in brain.** EUGENE ROBERTS AND SAM FRANKEL (introduced by C. CARRUTHERS). *Division of Cancer Research, Washington Univ., St. Louis, Mo.*
Relatively large quantities of an unidentified

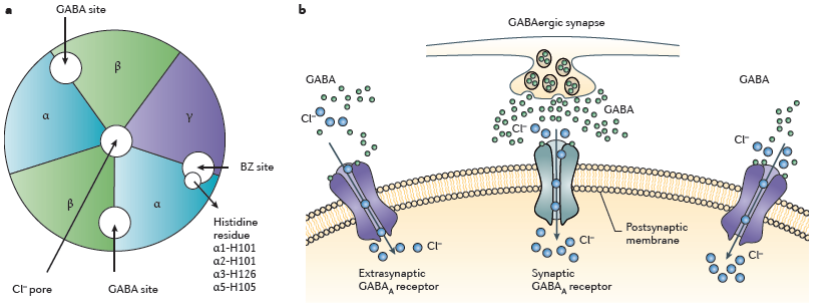
"enabling it, in "stealth" fashion, to escape the charged minefields encountered in passage through the dense extracellular environment lying between presynaptic sites of release and postsynaptic sites of action..... Try as one might, one cannot come up with a better choice for the job. "

Eugene Roberts

GABA is synthesized at nerve terminals where it is released into the synaptic cleft



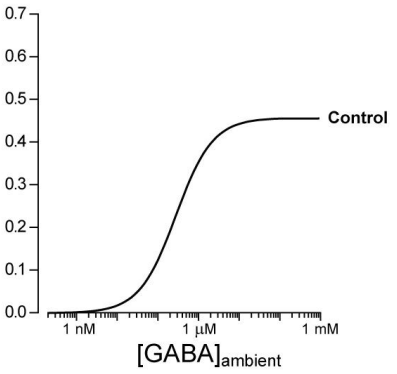
Where it binds the postsynaptic $GABA_A$ receptor



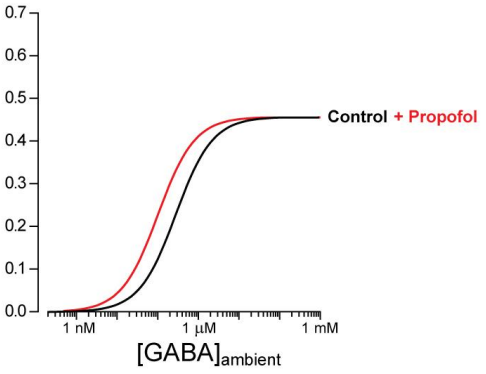
Enhancement of $GABA_A$ receptor function or an increase in the release of GABA can lead to sedation, anxiolysis and anaesthesia.

- Allosteric modulators – Benzodiazepines and neurosteroids
- Direct action and action on release – Alcohol
- Changes in GABA level (an action on transporters GABApentin, tiagabine)

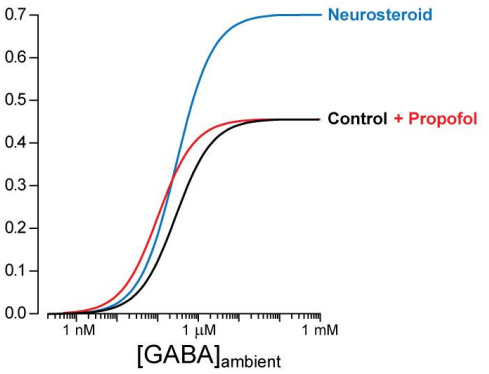
All of these drugs have potent actions on $GABA_A$Rs



Drugs like propofol and diazepam enhance $GABA_A$Rs By increasing apparent affinity



However, this is not the case for all allosteric modulators



Many clinically relevant drugs alter sleep due to their actions on GABA-A receptors

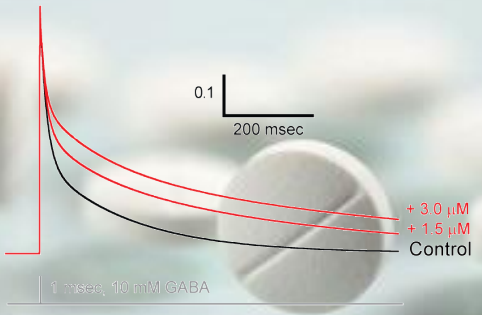
Benzodiazepines

- Alprazolam (Xanax, Xanor, Kalma, Tafil, Alprox, Frontal)
- Bromazepam (Bromam, Compendium, Creosedin, Calmepam, Durazanil, Lectopam, Lexaurin, Lexilium, Lexomil, Lexotan, Lexotanil, Normoc, Novepam, Somalium)
- Chlordiazepoxide (Librium, Tropium, Risolid, Klopoxid)
- Cinolazepam (Gerodorm)
- Clobazam (Frisium)
- Clonazepam (Klonopin, Klonapin, Rivotril, Rivatril)
- Clorazepate (Tranxene)
- Cloxazolam (Olcadil, Sepazon)
- **Diazepam (Valium, Apzepam, Stesolid, Vival, Apozepam, Hexalid, Valaxona, Ducene, Antenex)**
- Estazolam (ProSom)
- Flurazepam (Dalmane, Dalmadorm)
- Flunitrazepam (Rohypnol, Fluscand, Flunipam, Hynodorm, Ronal, Rohydorm)
- Halazepam (Paxipam)
- Ketazolam (Anseren, Ansieten, Ansietil, Marcen, Sedatival, Sedotime, Solatran, Unakalm)
- Loprazolam (Dormonoct)
- Lorazepam (Ativan, Temesta, Lorabenz)
- Lormetazepam (Loramet, Nictamid, Pronoctan, Ergocalm, Dilamet, Sedaben, Stilaze, Nocton, Noctamid, Noctamide, Loretam, Minias, Methyllorazepam)
- Meprobamate (Meprospan , Miltown, Equanil)
- Midazolam (Versed, Hypnovel, Dormicum)
- Nitrazepam (Mogadon, Alodorm Pacisyn, Dumolid)
- Nordazepam (Calmday, Stilny, Madar, Vegesan, Desoxydemoxepam, Nordiazepam, Desmethyldiazepam)
- Oxazepam (Serax, Seresta, Serenid, Sobril, Oxascand, Alopam, Oxabenz, Oxapax, Murelax, Alepam)
- Quazepam (Doral)
- Temazepam (Restoril, Normison, Euhypnos, Temaze, Temtabs, Remestan, Tenox, Norkotral)
- Triazolam (Halcion, Rilamir)

Many clinically relevant drugs alter sleep due to their actions on $GABA_A$ receptors

non-Benzodiazepines or Z drugs (sleeping pills)

- Zopiclone (Lunesta)
- Zaleplon (Sonata)
- Zolpidem (Ambien)
- Alpidem

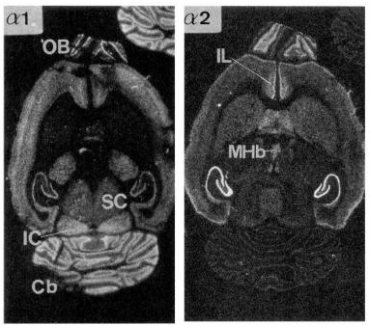


$GABA_A$ receptor subtypes

Multiple subunit isoforms form distinct subtypes of $GABA_A$ receptor.

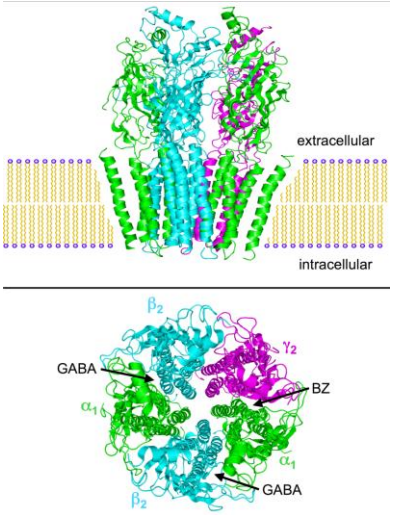
Different subtypes are targeted to different brain regions.

The α2 subunit is linked to anxiety and a genetic predisposition to alcohol dependence. (Enoch et al 2006 American Journal of medical genetics 141B; 599)

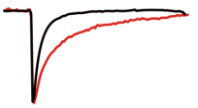


Wisden et al 1992 J Nsci 12; 1040

BDZs are non-selective

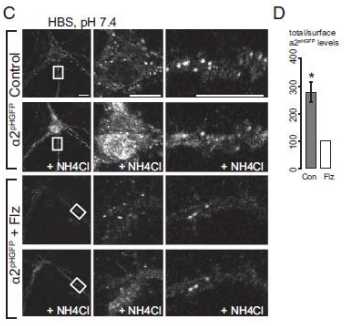


- Benzodiazepines bind a modulatory binding site on all $GABA_A$ receptors that contain a γ subunit (synaptic).
- They do not open the channel alone and there is no increase in the maximal response.
- The time-course (and amplitude) of IPSCs is increased. Neurons become less excitable.
- BDZs non-selectively enhance α1, α2 α3 and α5 containing $GABA_A$ receptors.

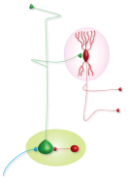


BDZs are non-selective – side effects and tolerance

- The anxiolytic effects occur at lower doses than sedative but sedation can still occur. Day-time treatment of anxiety comes with some sedation.
- BDZs have addictive properties. Long-term use can lead to tolerance physical dependence and addiction and as such there use is limited to short periods of time.
- Tolerance and physical dependence leads to withdrawal symptoms on removal – anxiety and insomnia (similar to alcohol withdrawal).



Jacob et al (2012) PNAS

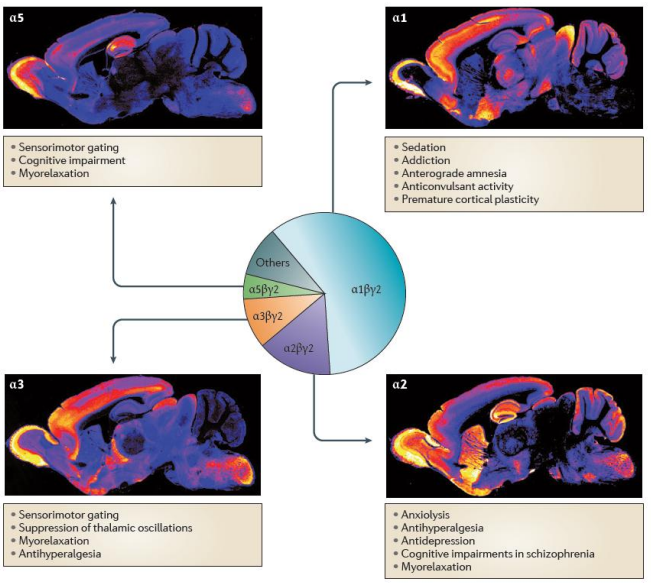


Different $GABA_A$ receptor subtypes mediate different physiological effects

- Role of different α subunit containing $GABA_A$ receptors revealed using transgenic mice (Low et al. (2000) Science; Rudolph and Knoflach (2011) Nat Rev Nsci).
- α1 – sedative, anterograde amnesic actions and anticonvulsant actions of Diazepam and (importantly) addictive properties.

  α2 – anxiolytic actions and myorelaxant

  α5 – linked to development of tolerance and sedation – learning and memory.
- Zolpidem – α1 selective - Used for sedation
- Different subtypes have been shown to mediate different physiological effects of drugs like anaesthetics. (Reynolds et al 2003 J Nsci 23; 8608)



Rudolph and Knoflach (2011) Nat Rev Nsci 10: 685

Development of α2 selective BDZs may allow anxiolysis without sedation and addiction

Table 1 | **Subtype selective compounds for $GABA_A$ receptors**

Compound	Receptor subtype	Binding/functional selectivity	Indication	Development status
L-838417	Partial agonist at α2, α3, α5	Functional	Anxiety disorders	Preclinical
TPA023 (MK-0777)	Partial agonist at α2, α3	Functional	Anxiety disorders, schizophrenia	Phase II
TPA023B	Partial agonist at α2, α3	Functional	Anxiety disorders, schizophrenia	Phase I
TPA123	Partial agonist at α1, α2, α3, α5	Functional	Anxiety disorders	On hold
MRK-409 (MK-0343)	Partial agonist at α2, α3	Functional	Anxiety disorders	Phase I, halted
TP003	Agonist at α3	Functional	Anxiety disorders	On hold
Ocinaplon (DOV-273547)	Partial agonist at α2, α3, α5. Full agonist at α1	Functional	Anxiety disorders	On hold
NS11394	Agonist at α5. Partial agonist at α3, α5	Functional	Anxiety disorders	Preclinical
MRK-016	Full inverse agonist at α5	Functional	Cognitive impairment	Phase I, halted
α5IA	Partial inverse agonist at α5	Functional	Cognitive impairment	Phase I, halted
RO4938581	Full inverse agonist at α5	17–40-fold binding selectivity for α5	Cognitive impairment	Preclinical
L-655708 (FG8094)	Very weak inverse agonist at α5	30–70-fold binding selectivity for α5	Cognitive impairment	Preclinical
SH-053-2′F-R-CH3	Full agonist at α5. Partial agonist at α1, α2, α3	8–10-fold binding selectivity for α5	Schizophrenia?	Preclinical
Gaboxadol	Supra-maximal agonist at α4β3δ	>Tenfold binding selectivity for α4	Insomnia	Phase III, halted

Worries about alcohol

Alcohol 'is to blame for most weekend casualty admissions'

By Daily Mail Reporter

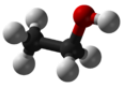
Rising alcohol abuse among middle-class pensioners as hospital admissions soar

By Daniel Martin

Drinking and obesity fuel surge in liver disease among middle-age Britons

Daily Mail

Sites of Alcohol action in the brain

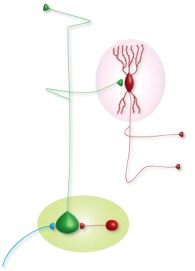


- Weak interactions with a large number of targets (high concentrations 10-100mM)

-Ligand-gated receptors, excitatory and inhibitory (NMDA and $GABA_{A,}$ nAchRs)

- Changes to GABA release

- Voltage-gated ion channels (K^+)



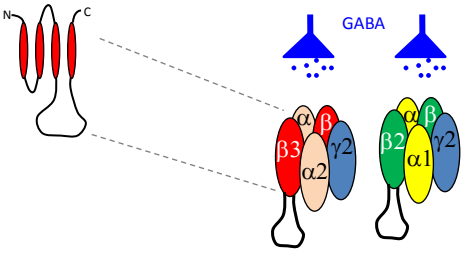
- Enzymes and intracellular signalling pathways. alcohol dehydrogenase, adenylyl cyclase (enhancement of cAMP production)

- Stimulation of neurosteroid production

Results in overall shift in balance between inhibitory and excitatory drive within the brain such that inhibition dominates.

Harris et al 2008 Sci signal.15; 1-5

Alcohol and the $GABA_A$ receptor



GABA and the $GABA_A$ receptor have been implicated in mediating both the acute and chronic effects of alcohol consumption.

- Alcohol consumption has similar physiological effects to known $GABA_A$ receptor modulators (BDZs and barbiturates)

- Anxiolysis, sedation, hypnosis, anti-convulsant, motor and cognitive impairment.

- Cross-tolerance of BDZs and barbiturates (also used to alleviate the symptoms of withdrawal).

Alcohol and inhibitory transmission

GABAergic transmission :

- Enhanced during intoxication
- Altered by chronic consumption
- Therapeutic target for withdrawal and abstinence

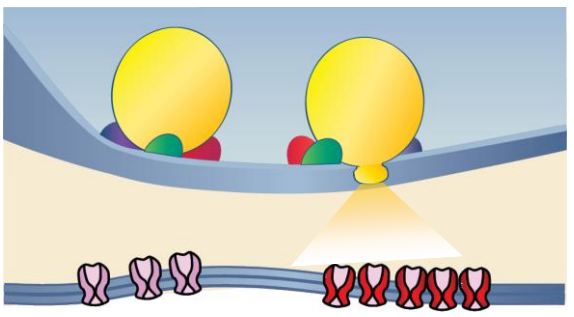
- Risk factor: polymorphisms in $GABA_A$ receptor genes linked to alcoholism

Alcohol enhancement of $GABA_A$ receptors

Mechanism is controversial

Direct action

Increased GABA release

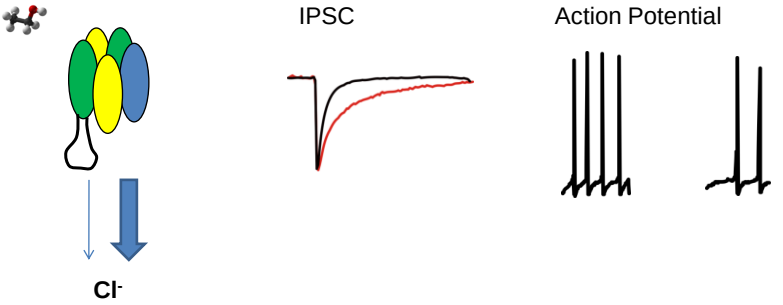


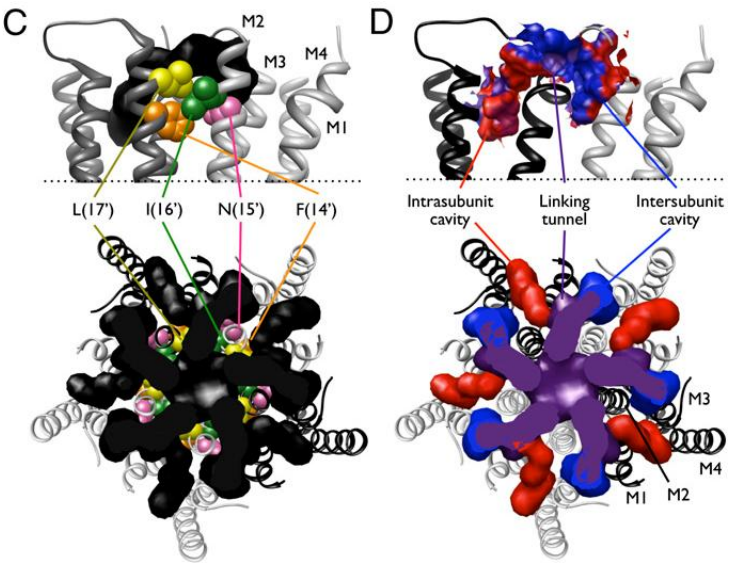
Indirect action e.g. Neurosteroid production

Alcohol and inhibitory transmission - direct action

Direct binding to the $GABA_A$ receptor – enhancement of function.

Proposed site of direct binding on the alpha subunit (TM2-3) – lining of channel pore.

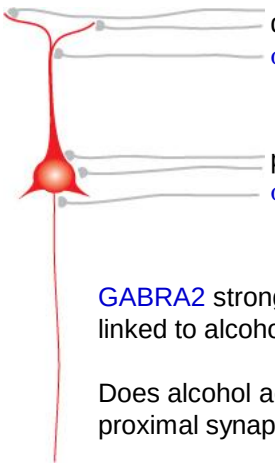




Jung et al 2005 JBC 280; 308-16 Howard et al 2011 PNAS 108; 12149

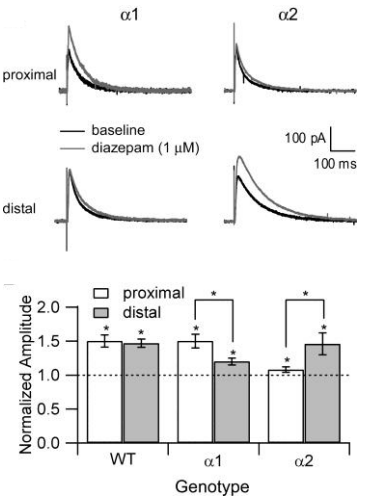
Subcellular targeting of $GABA_AR$ isoforms

CA1 pyramidal neurons



GABRA2 strongly linked to alcoholism

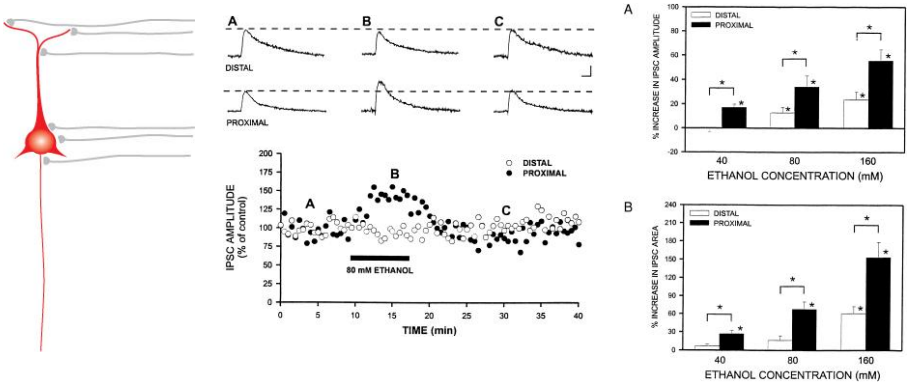
Does alcohol act on proximal synapses?



Prenosil, G. A. et al. J Neurophysiol 96: 846-857 2006

Isolating alcohol sensitive synapses

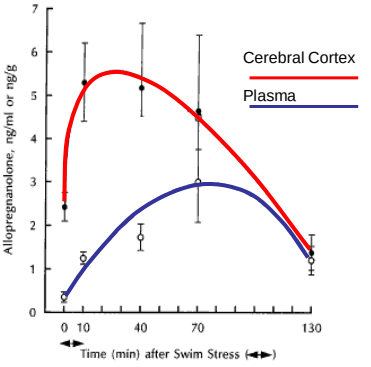
In hippocampal pyramidal cells, proximal synapses show greater alcohol sensitivity



Weiner, J. L. et al. J Neurophysiol 77: 1306-1312 1997

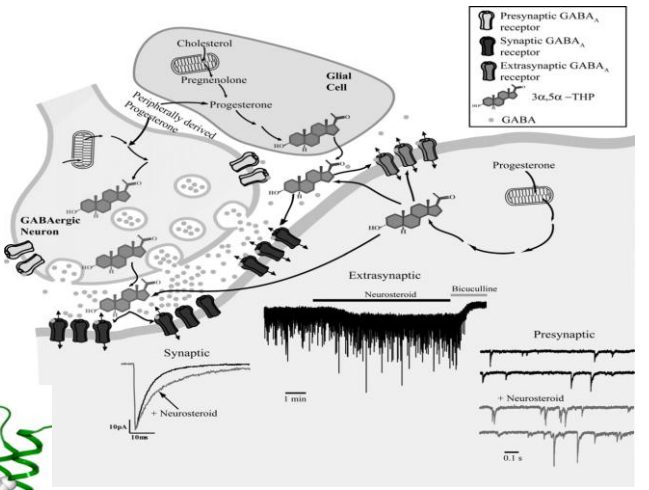
-indirect actions
Alcohol and neurosteroids

Neurosteroids are produced in response to stress

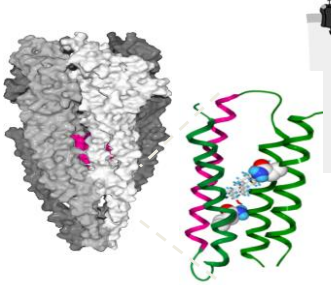


Alcohol consumption elicits neurosteroid production

Neurosteroid Modulation of $GABA_A$ receptors



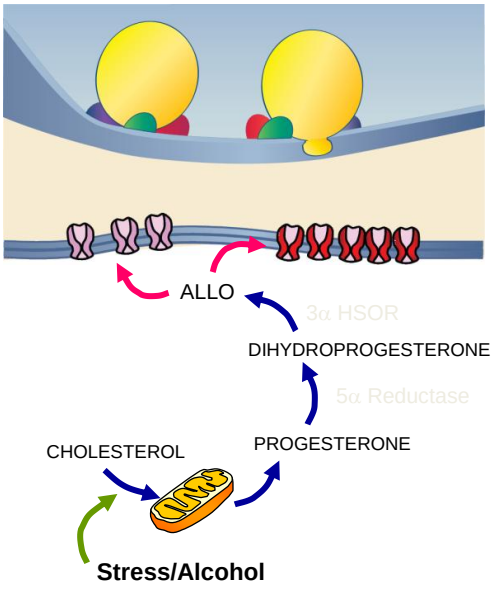
Hosie *et al*., Nature, 444, 468



Herd *et al* (2007) Pharmacol & Therapeutics

Hypothesis

- Stress actives ALLO synthesis in pyramidal neurons
- ALLO enters membrane binds to and enhances $GABA_AR$
- Alcohol hijacks this system



Alcohol, genetics and neurosteroids

In Humans

$GABA_A$ alpha2 alleles alter the subjective effects of alcohol

As did ingestion of finasteride a drug that blocks the synthesis of neurosteroids.

Pierucci-Lagha et al 2005 Neuropsychopharmacology 30; 1193

α2/3 selective agonists and schizophrenia

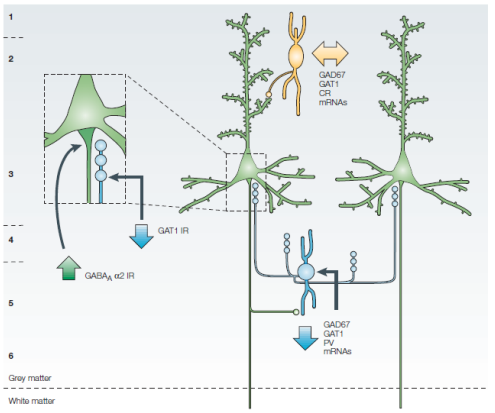


Figure 6 | **Schematic summary of alterations in GABA circuitry in the dorsolateral prefrontal cortex of individuals with schizophrenia.** Reduced levels of gene expression in chandelier neurons (blue) are associated with a decrease in immunoreactivity (IR) for GABA (γ-aminobutyric acid) transporter 1 (GAT1) in the axon cartridges of these neurons and an upregulation of $GABA_A$ (GABA type A) receptor α2 subunit immunoreactivity in the postsynaptic axon initial segment of pyramidal neurons (green). Gene expression in calretinin (CR)-expressing subpopulations of GABA neurons does not seem to be altered (yellow). GAD67, 67 kD isoform of glutamic acid decarboxylase; PV, parvalbumin; 1–6, layers of dorsolateral prefrontal cortex.

α3 knockout mice show some behavioural characteristics consistent with schizophrenia models.

Schizophrenia is associated with increased excitability in VTA dopaminergic neurons (which express α3). More on this later....

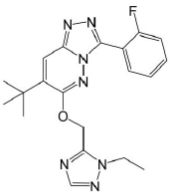


Fig. 1. Structure of TPA023.

TPA023 [7-(1,1-Dimethylethyl)-6-(2-ethyl-2*H*-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-*b*]pyridazine], an Agonist Selective for α2- and α3-Containing $GABA_A$ Receptors, Is a Nonsedating Anxiolytic in Rodents and Primates

John R. Atack, Keith A. Wafford, Spencer J. Tye, Susan M. Cook, Bindi Sohal, Andrew Pike, Cyrille Sur, David Melillo, Linda Bristow, Fran Bromidge, Ian Ragan, Julie Kerby, Les Street, Robert Carling, José L. Castro, Paul Whiting, Gerard R. Dawson, and Ruth M. McKernan

Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, United Kingdom

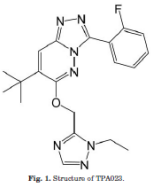
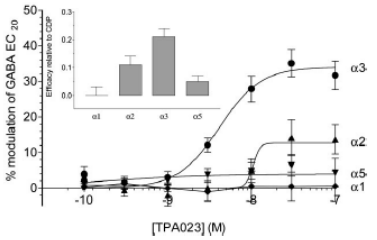


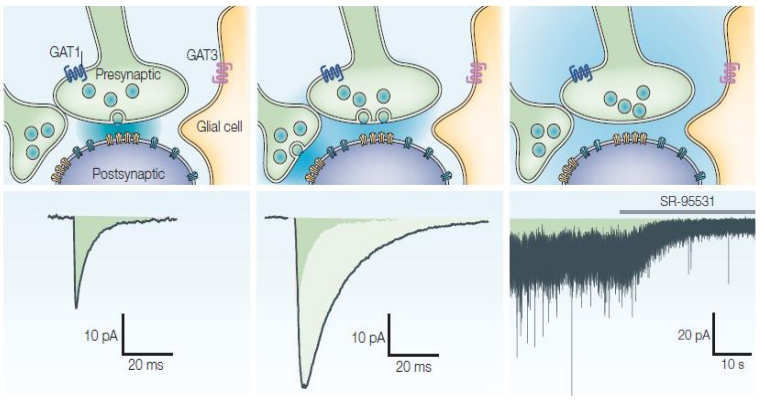
Fig. 1. Structure of TPA023.



Small clinical trial with TPA023 (α2/3) partial agonist showed some improvement in cognitive function in schizophrenic patients.

EEG showed increased γ oscillations controlled by GABAergic interneurons and thought to underlie cognitive symptoms of schizophrenia.

Extrasynaptic $GABA_A$ receptors mediate tonic inhibition



- **Phasic Inhibition**: Mediated by synaptic αβγ-containing receptors
- **Tonic Inhibition**: Mediated by extrasynaptic αβδ-containing receptors

GABA transporters are principally involved in setting the ambient GABA concentration

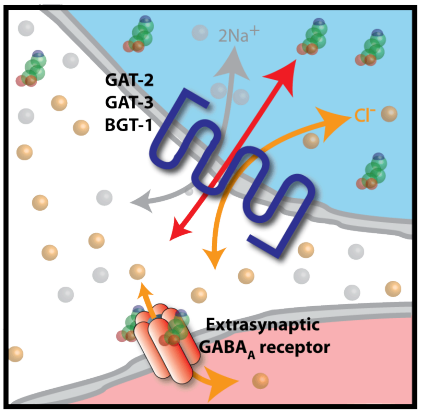


Table 1. Summary of Some Clinically Relevant Drugs that Can Alter Tonic Inhibition within the Brain

Drug (*Trade Names*)	Mechanism of Action	Current Drug Indications
Gabapentin (*Fanatrex*, *Gabarone*, *Gralise*, *Neurontin*)	Originally thought to be a GABA mimetic, but mechanism of action is now unclear. Possible enhancement of GABA synthesis could explain why ambient GABA levels in the brain are raised (Maneuf et al., 2003).	Partial-onset seizures in adults and the elderly (Beghi, 2010); alcohol withdrawal as a combination therapy (Anton et al., 2011); sleep disorders (Ehrenberg, 2000).
Vigabatrin (*Sabril*)	Irreversible block of GABA transaminase to interfere with GABA cetabolism and, therefore, raise ambient GABA levels.	Refractory complex partial seizures and infantile spasms (Tolman and Faulkner, 2009). Not favored due to visual field loss in some adults and children (Chiron and Dulac, 2011).
Tiagabine (*Gabitril*)	Blockade of GABA transporters on nerve terminals (predominantly GAT-1) leads to raised ambient GABA levels.	Partial seizures; generalized anxiety disorders/panic disorders (Pollack et al., 2005).
Pregabalin (*Lyrica*)	Enhances the activity of glutamic acid decarboxylase (GAD) leading to increased GABA synthesis and, therefore, raised ambient GABA levels.	Partial seizures with or without secondary generalization (Tassone et al., 2007); neuropathic pain in diabetese, postherpetic neuralgia, and fibromyalgia (Tassone et al., 2007); generalized anxiety disorder (Tassone et al., 2007).
Gaboxadol	Selective orthosteric agonist at δ-$GABA_A$Rs leading to specific enhancement of the tonic conductance.	Sleep enhancer, but withdrawn from Phase III clinical trials due to poor risk-to-benefit ratio (Saul, 2007).
L-655,708	High-affinity negative allosteric modulator of α5-$GABA_A$Rs that will reduce tonic conductances.	Cognitive enhancer but not thought to be suitable for human use due to anxiogenic properties (Navarro et al., 2002).
Ganaxolone	Positive allosteric modulator of most $GABA_A$Rs with greater potency at δ-$GABA_A$Rs leading to selective enhancement of the tonic conductance.	Catemenial epilepsy (Biagini et al., 2010).
Alphaxalone (*Althesin*, *Saffan*)	Positive allosteric modulator of most $GABA_A$Rs with greater potency at δ-$GABA_A$Rs leading to selective enhancement of the tonic conductance.	Anesthetic (Winter et al., 2003) and sedative in long-term intensive care patients (Stewart et al., 1983). Was withdrawn from clinical practice due to complications with the vehicle, Cremophor EL. Rebranded as *Saffan* and widely used as an anesthetic in veterinary surgery.
Propofol (*Diprivan*)	Positive allosteric modulator of most $GABA_A$Rs including α5 and δ-$GABA_A$Rs leading to enhanced tonic conductance.	Widely used as an intravenous anesthetic.

Cellular/Molecular

Are Extrasynaptic $GABA_A$ Receptors Important Targets for Sedative/Hypnotic Drugs?

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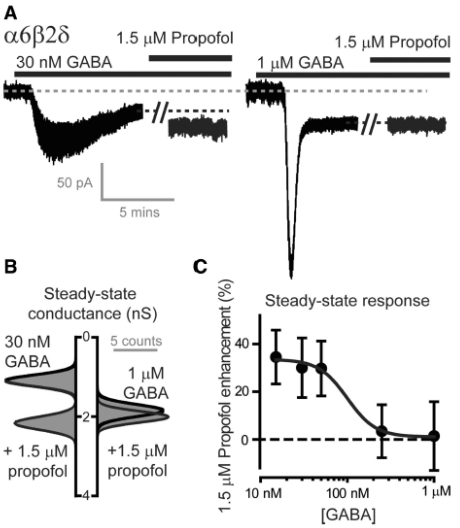
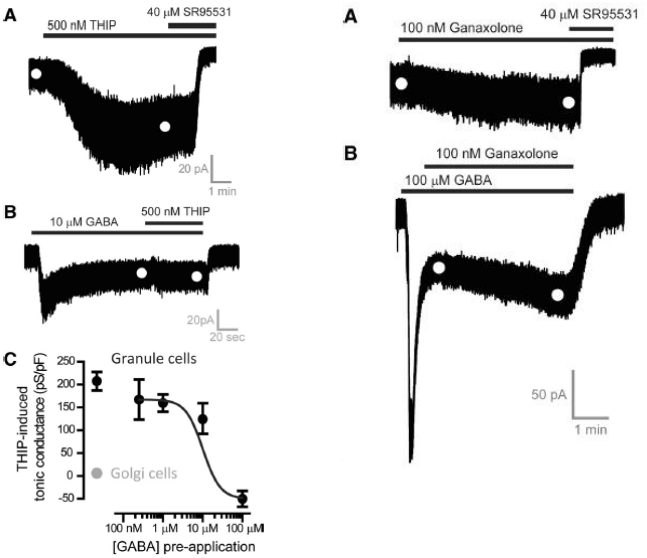
The concentration of ambient GABA may influence drug modulation.

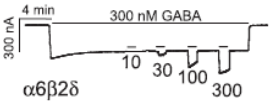
- THIP and GABA compete for the same binding site. THIP has a lower affinity and so cannot displace GABA if already bound.

- Propofol is an allosteric modulator that increases the apparent affinity of GABA


A
α6β2δ
1.5 μM Propofol
30 nM GABA
1.5 μM Propofol
1 μM GABA
50 pA
5 mins
B
Steady-state conductance (nS)
30 nM GABA
5 counts
1 μM GABA
+ 1.5 μM propofol
+1.5 μM propofol
C
Steady-state response
1.5 μM Propofol enhancement (%)
[GABA]
10 nM
100 nM
1 μM

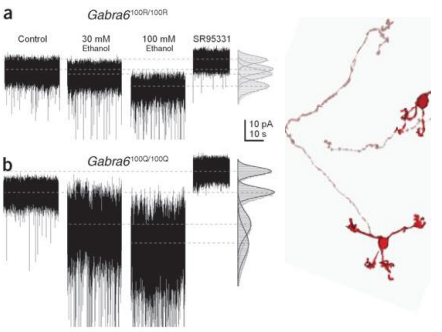

A
40 μM SR95531
500 nM THIP
20 pA
1 min
B
10 μM GABA
500 nM THIP
20pA
20 sec
C
Granule cells
Golgi cells
THIP-induced tonic conductance (pS/pF)
[GABA] pre-application
A
40 μM SR95531
100 nM Ganaxolone
B
100 nM Ganaxolone
100 μM GABA
50 pA
1 min

Extrasynaptic $GABA_A$ receptors as a target for ethanol



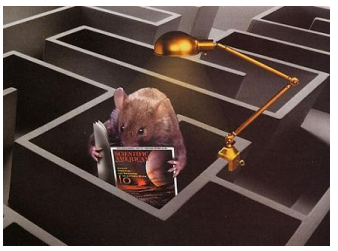
Hanchar et al 2005 Nature Nsci 8; 339

Naturally occurring polymorphism in rats confers increased EtOH induced motor impairment.

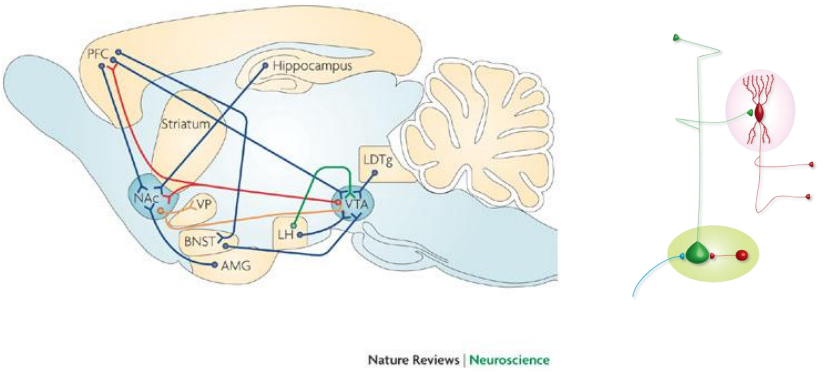


Cognitive enhancement through α5?

- Classical BDZs impair learning and memory. Negative allosteric modulators (inverse agonists) at the BDZ site have been proposed as possible memory enhancers.
- Non-selective inverse agonists have multiple side-effects (anxiogenesis. convulsant activity).
- In the hippocampus a region important for learning and memory there is a high level of α5 expression mediating both phasic and tonic inhibition.
- α5 selective compounds are in development.
- α5IA was found to reverse memory deficits induced by alcohol consumption with no sign of anxiogenesis.
- Possible use in Alzheimer's but...renal toxicity.



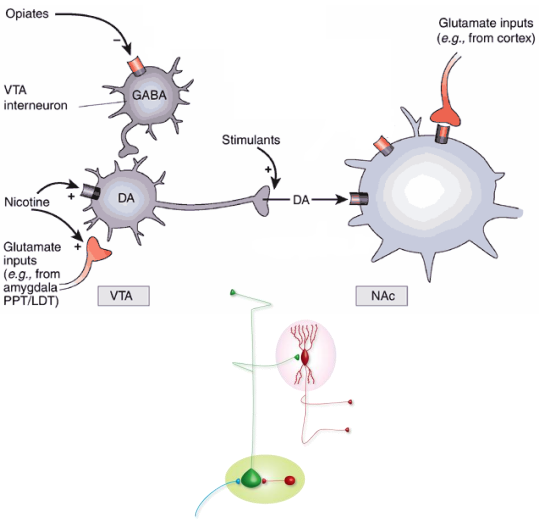
Reward: the mesolimbic dopamine system



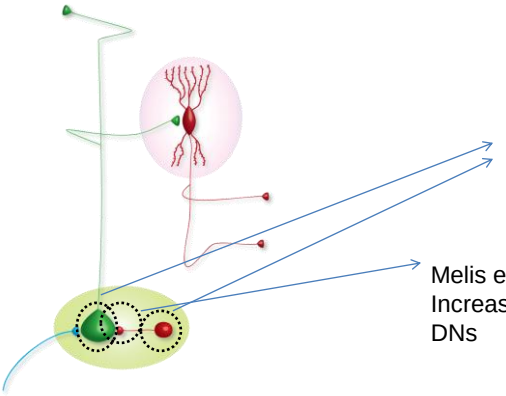
Dopamine neurons from Ventral Tegmental Area project to:
Nucleus Accumbens (NAc), pre- and orbito-frontal cortex (PFC, OFC)

Reward: the VTA-NAc

All addictive drugs increase dopamine levels in the Nucleus Accumbens (NAc) through an increase in activity of ventral tegmental area (VTA) dopaminergic neurons



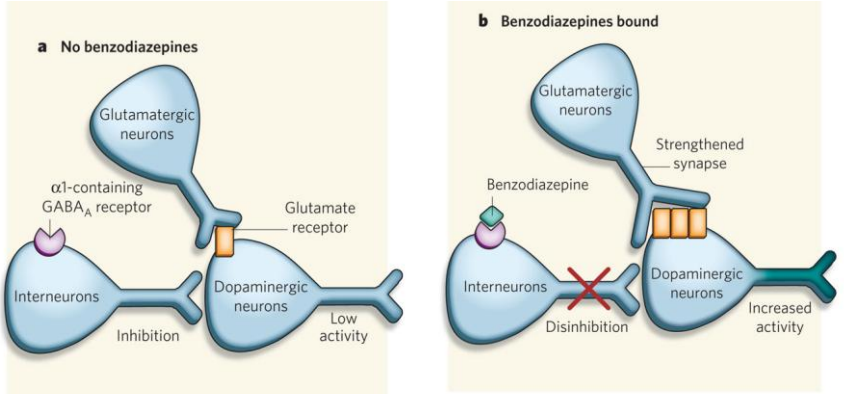
Actions of EtOH in the VTA



Xiao et al., 2007 Alcohol Clin Exp Res 31; 1106
Direct excitation of DNs and inhibition of INs

Melis et al., 2002
Increased IPSC frequency onto DNs

Actions of Benzodiazepines in the VTA



Tan et al., 2010 Neural bases for addictive properties of benzodiazepines. Nature 463, 769-74

In summary...

- Understanding the physiological role of different $GABA_A$ receptor subtypes in different regions of the brain is key to understanding pharmacology.
- The dream....
  Subtype selective BDZs
- Anxiolysis without sedation or dependence
- A new class of hypnotics