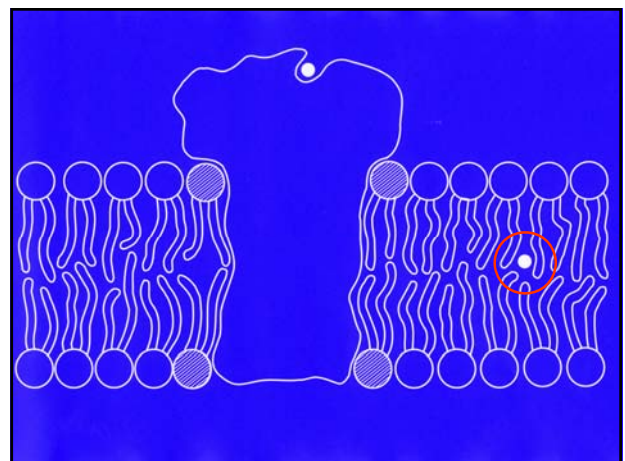
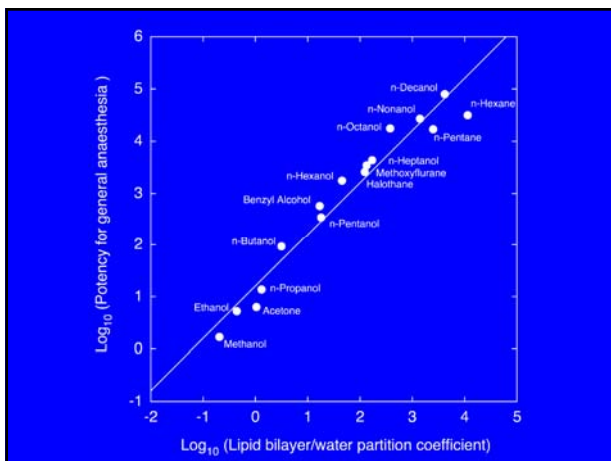
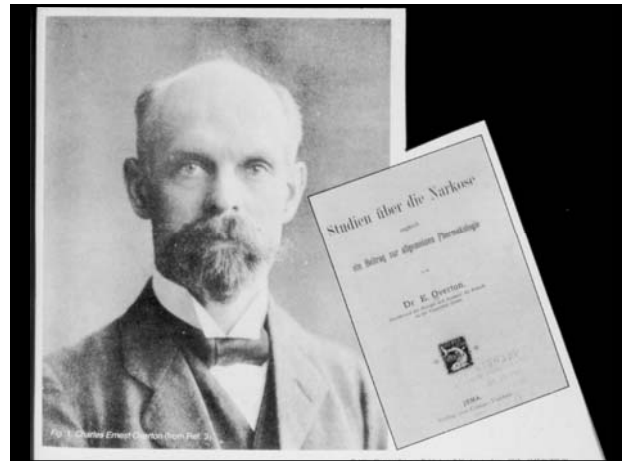
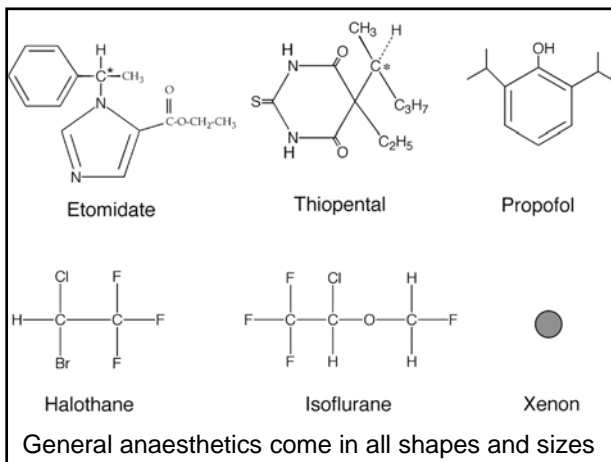


## Molecular Targets of General Anaesthetics

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 Intensive Care Section  
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 Biophysics Group  
 Blackett Laboratory  
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 r.dickinson@imperial.ac.uk

## Molecular Targets of General Anaesthetics

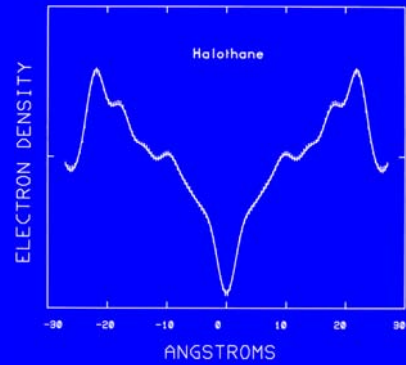
- Meyer-Overton correlation
- Theories of general anaesthesia
  - Lipid theories
  - Protein theories
- Molecular interactions with proteins
- Ion channel targets
  - Criteria for putative targets



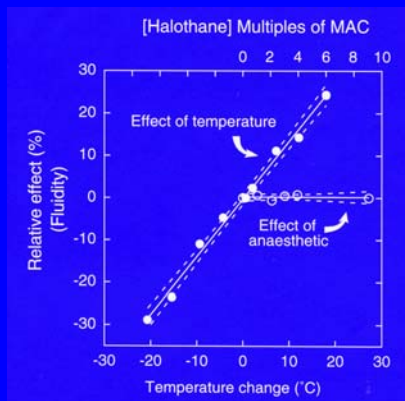
## Lipid theories of anaesthesia

- Unitary hypothesis
- Diversity of theories
  - membrane expansion
  - membrane fluidity
  - membrane phase transitions

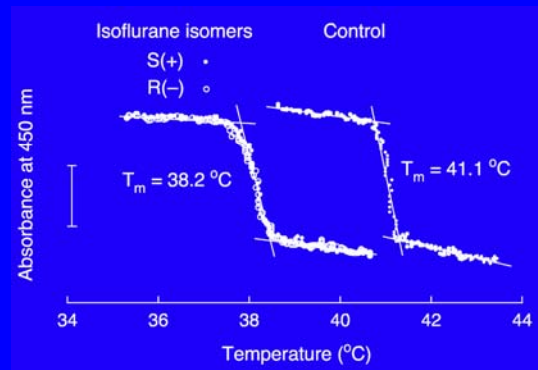
## General anaesthetics do not change lipid bilayer dimensions



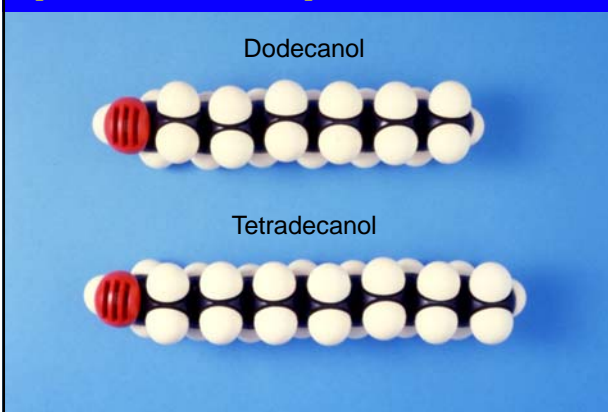
## Anaesthetics do not affect lipid bilayer fluidity



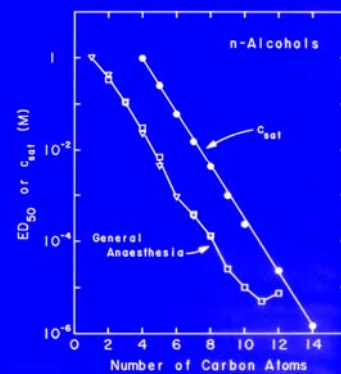
## Effect of anaesthetics on lipid phase transitions is not stereoselective



## Lipid theories cannot explain the “cutoff effect”

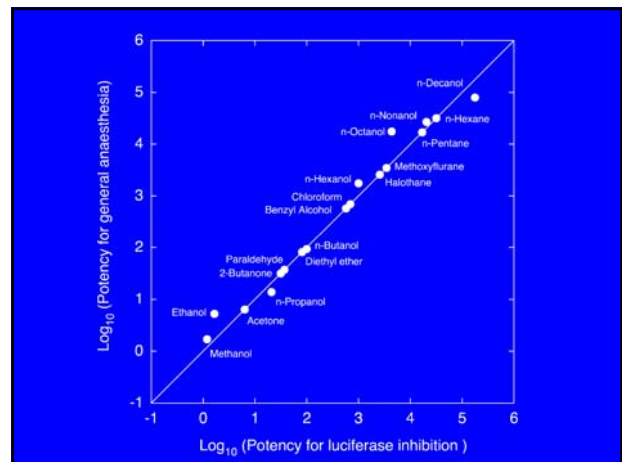


## Lipid theories cannot explain the “cutoff effect”

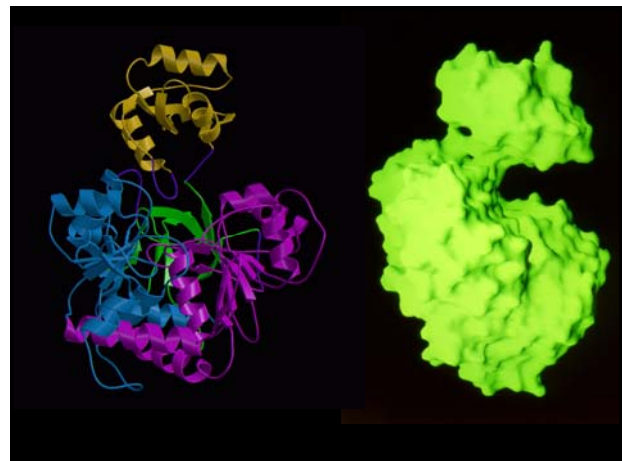
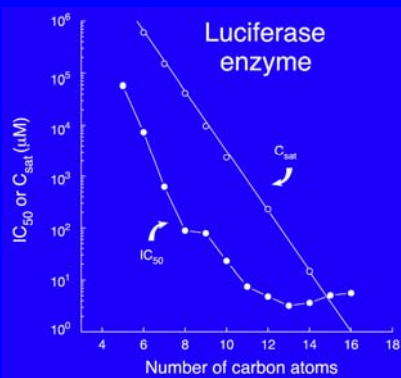


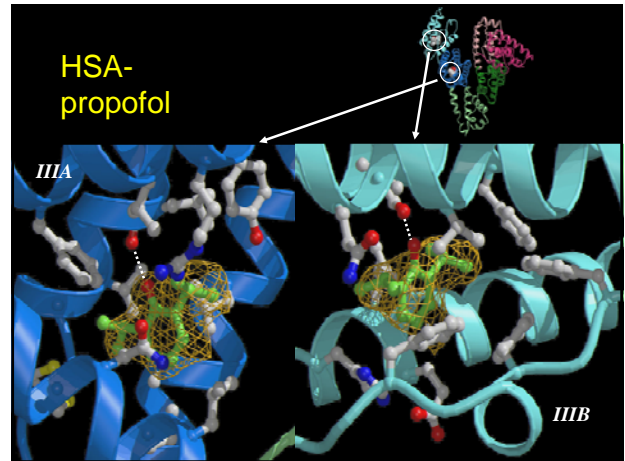
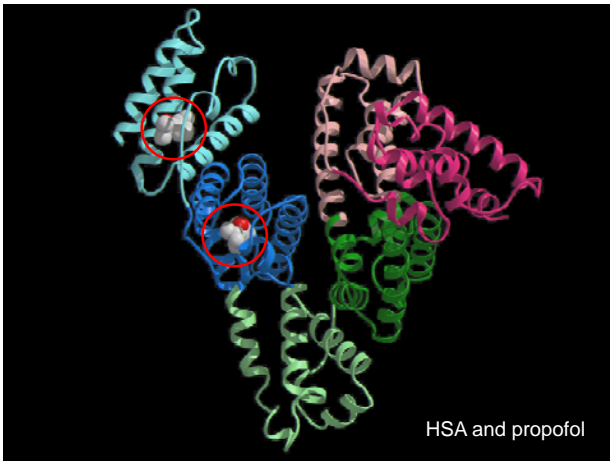
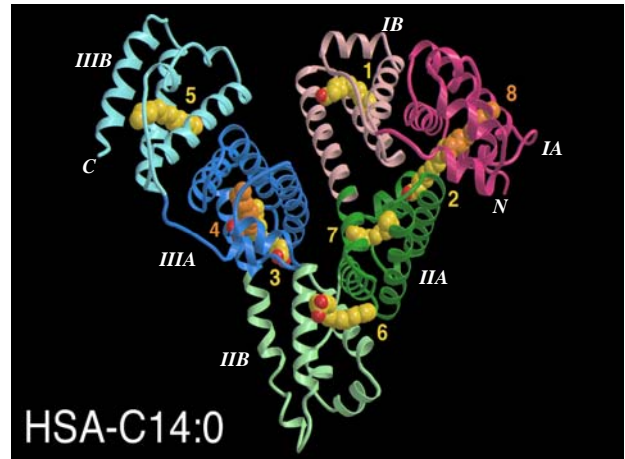
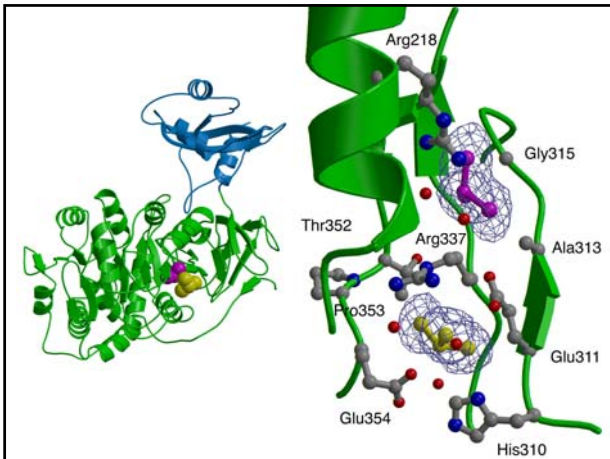
Anaesthetics do not act by disrupting lipid bilayers

Do anaesthetics act by binding to proteins?



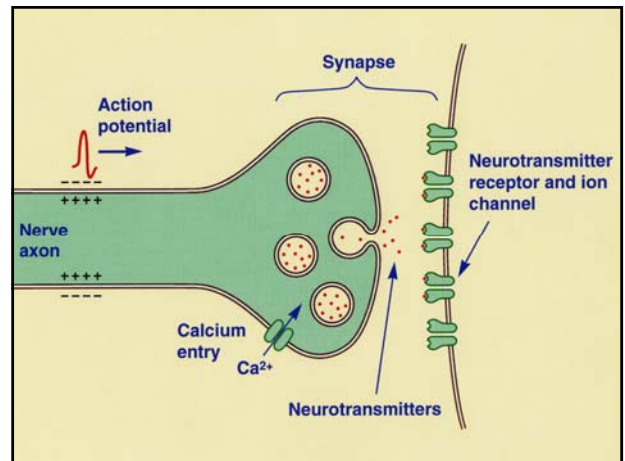
Firefly luciferase exhibits a "cutoff" effect





Anaesthetics act by binding directly to sensitive protein targets in pre-formed cavities or clefts

.....but which proteins are relevant?



### Criteria for putative targets

- Plausibility
- Sensitivity
- Stereoselectivity

### Anaesthetic endpoints & free aqueous concentrations for thiopental

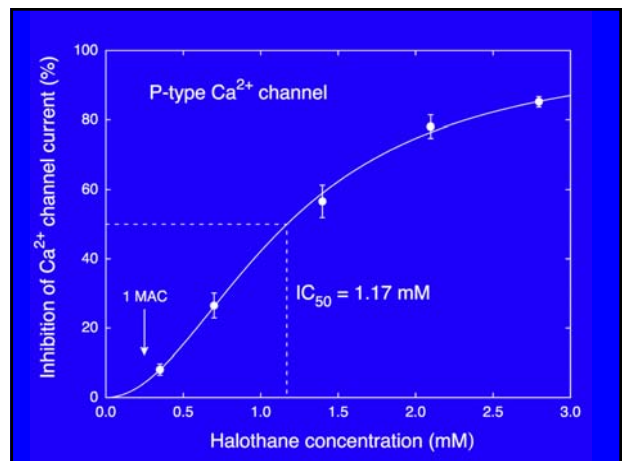
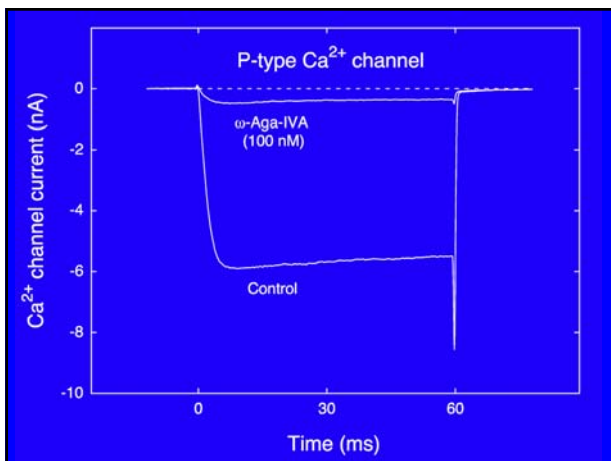
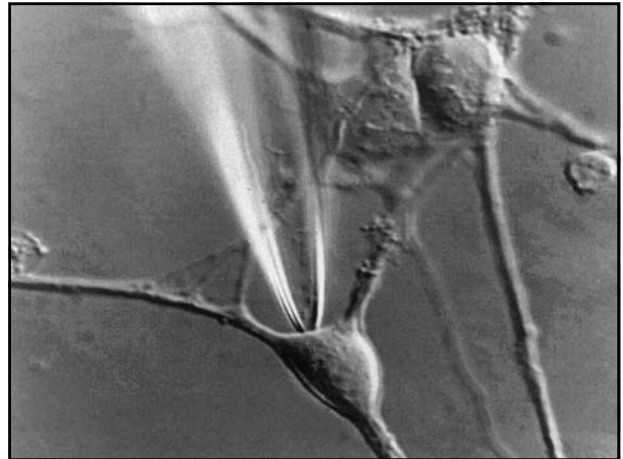
| Rat                                       |                              | Human  |                              |
|---|------------------------------|--|------------------------------|
|   | Thiopental ( $\mu\text{M}$ ) |  | Thiopental ( $\mu\text{M}$ ) |
| Righting reflex                           | 9                            | Response to verbal command                       | 9                            |
| Response to painful stimulus (Tail clamp) | 22                           | Response to painful stimulus (Surgical incision) | 23                           |
| Tracheal intubation                       | 39                           | Tracheal intubation                              | 46                           |

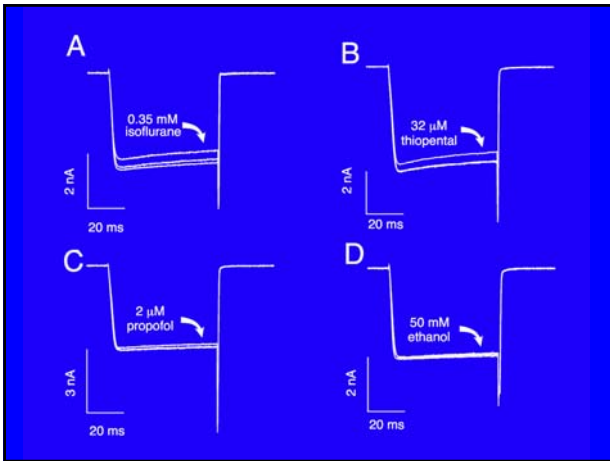
Data from Becker (1978) *Anesthesiology* 49, 192-196, Hung et al. (1992) *Anesthesiology* 77, 237-244 and Gustafsson et al. (1996) *Anesthesiology* 84, 415-427.

### General anaesthetic $\text{EC}_{50}$ concentrations for mammals

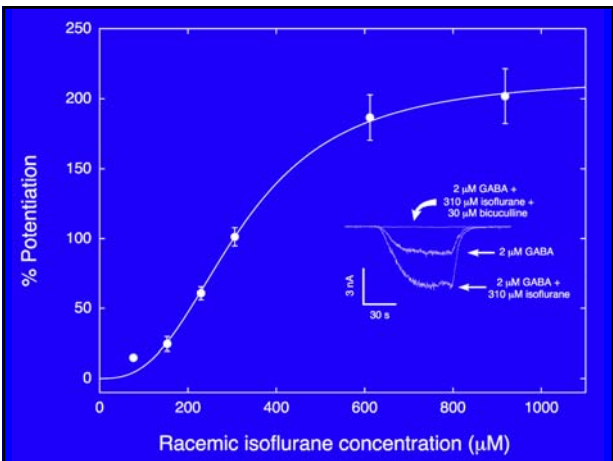
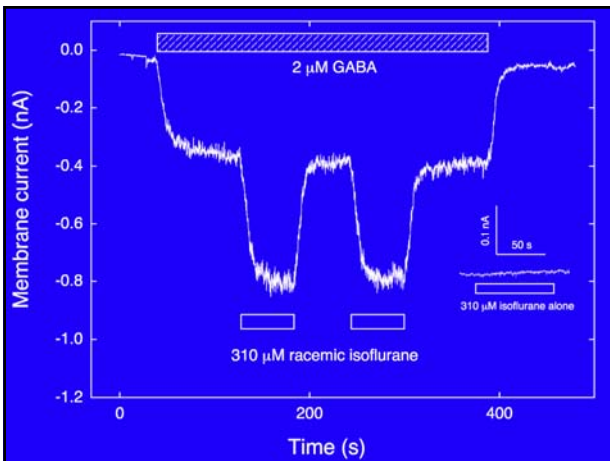
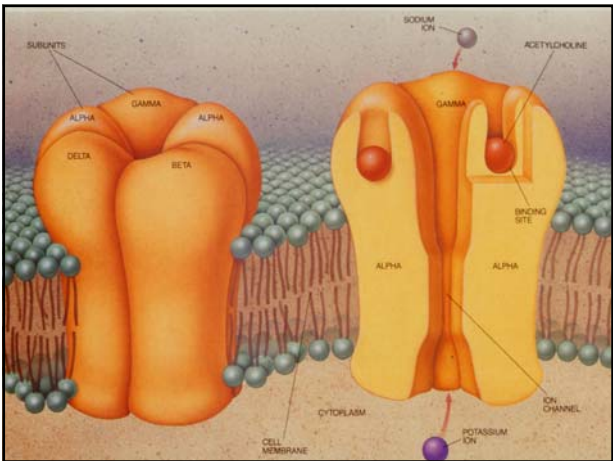
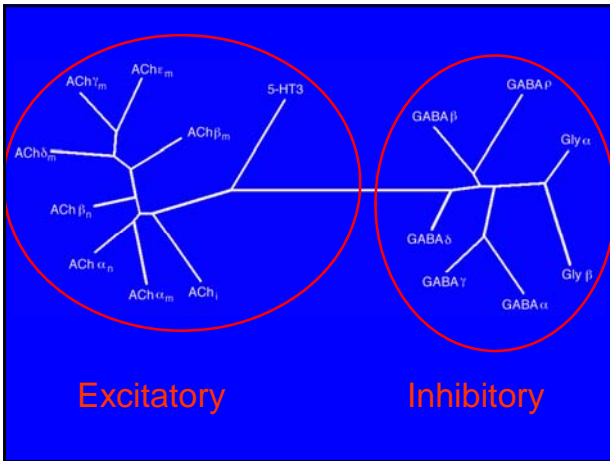
(inhibition of a response to a painful stimulus)

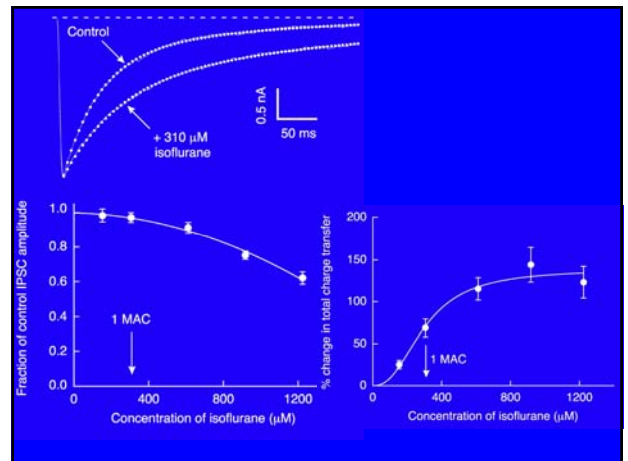
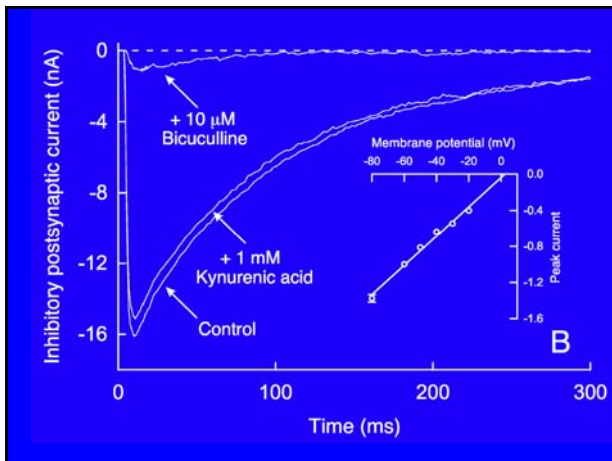
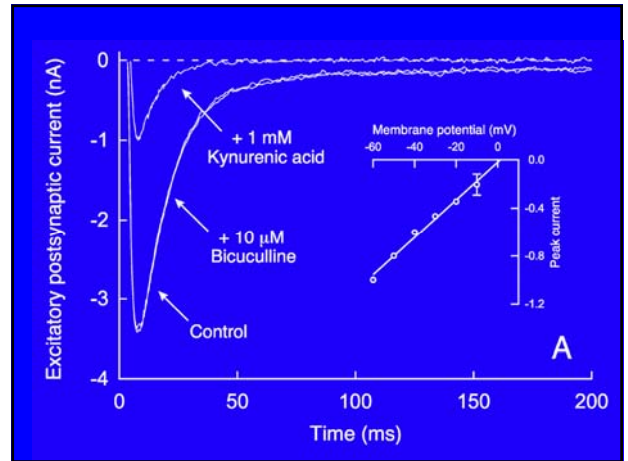
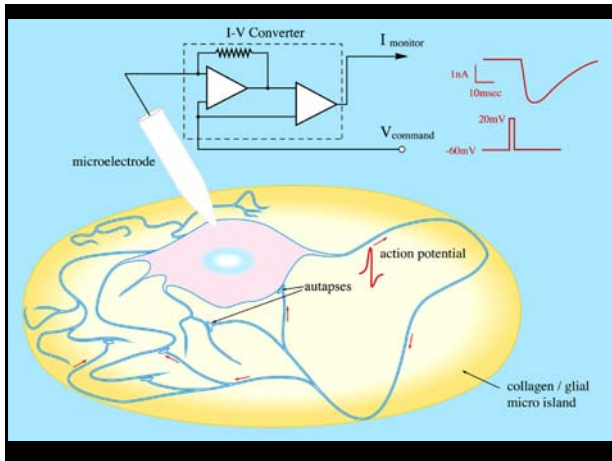
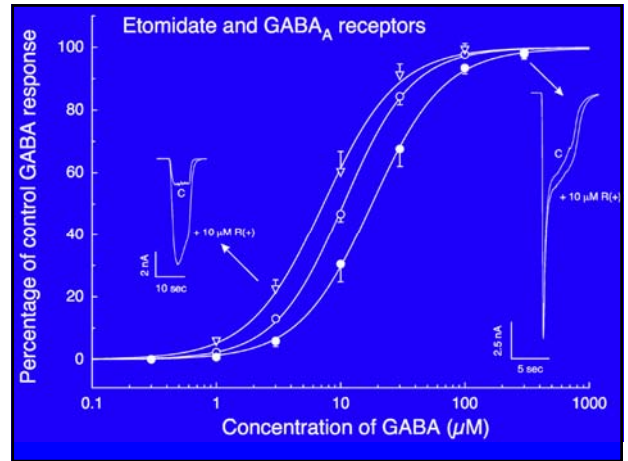
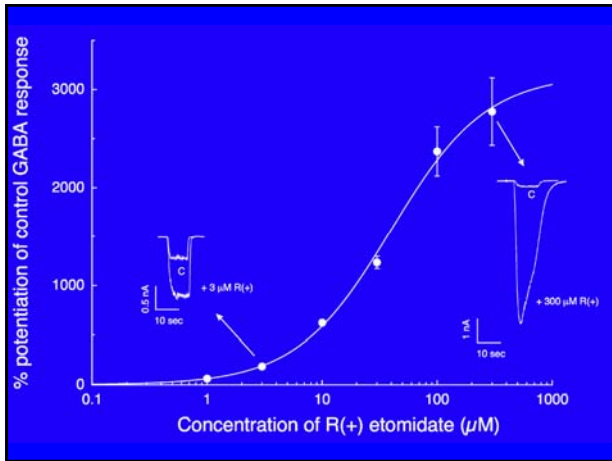
|            |                   |
|------------|-------------------|
| Halothane  | 230 $\mu\text{M}$ |
| Isoflurane | 280 $\mu\text{M}$ |
| Thiopental | 25 $\mu\text{M}$  |
| Propofol   | 1.5 $\mu\text{M}$ |

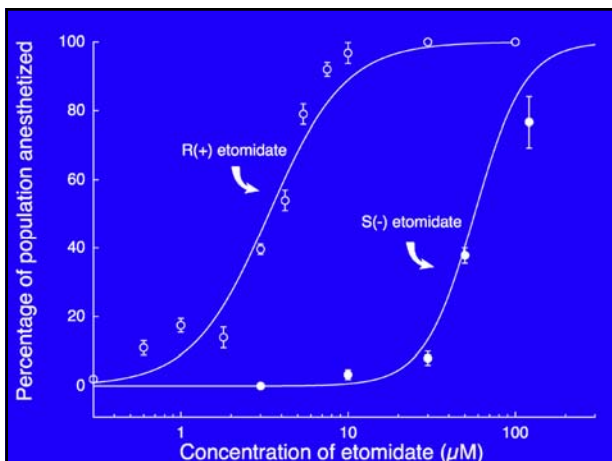
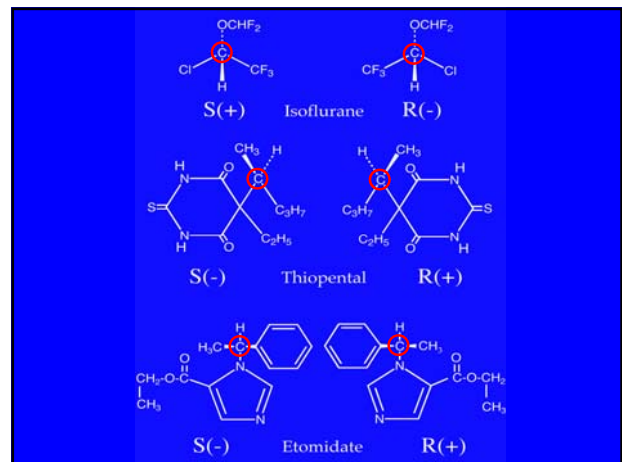
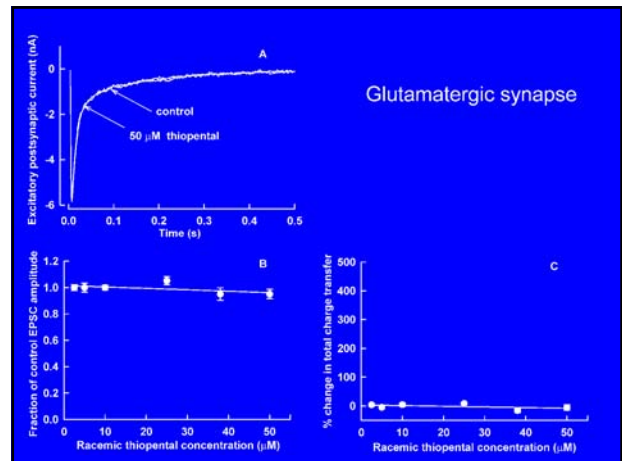
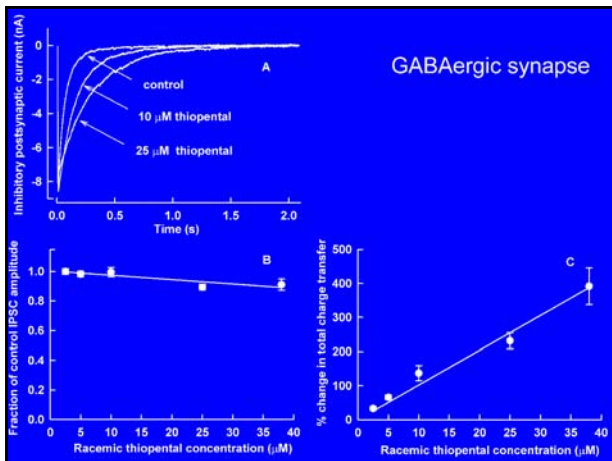




Most voltage-gated ion channels are insensitive to anaesthetics



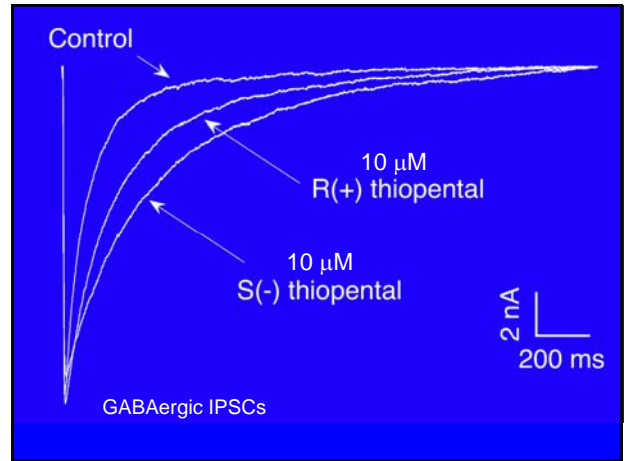
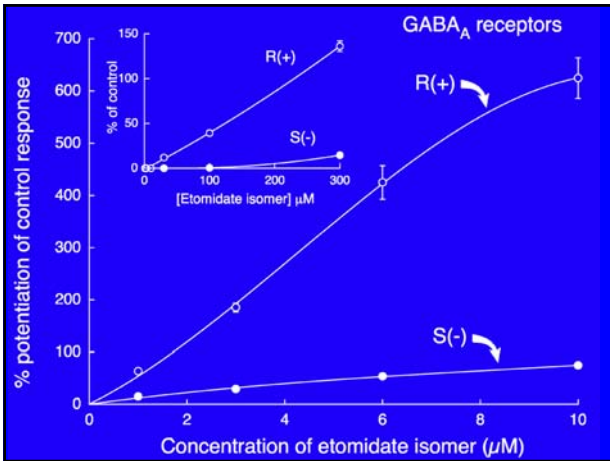




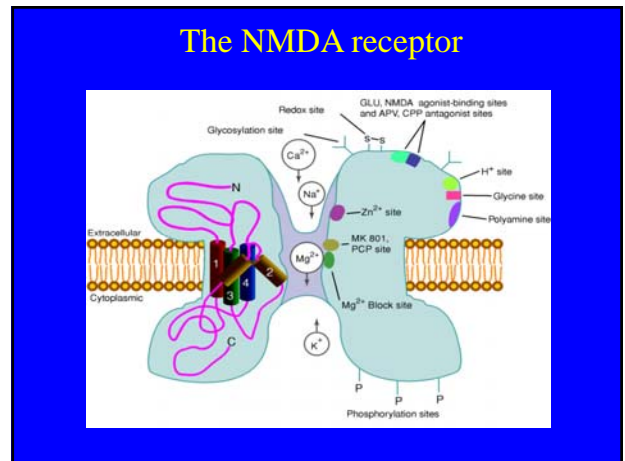
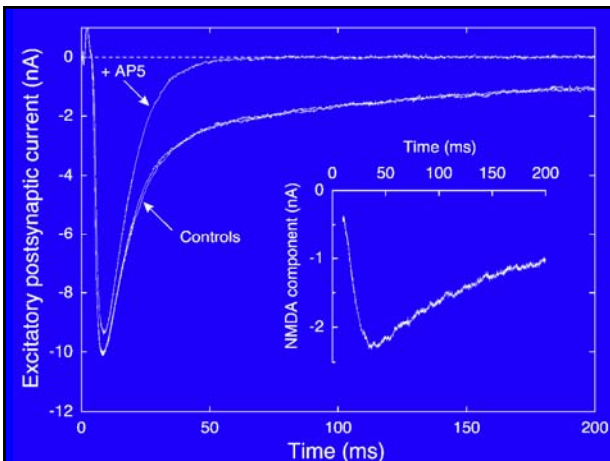
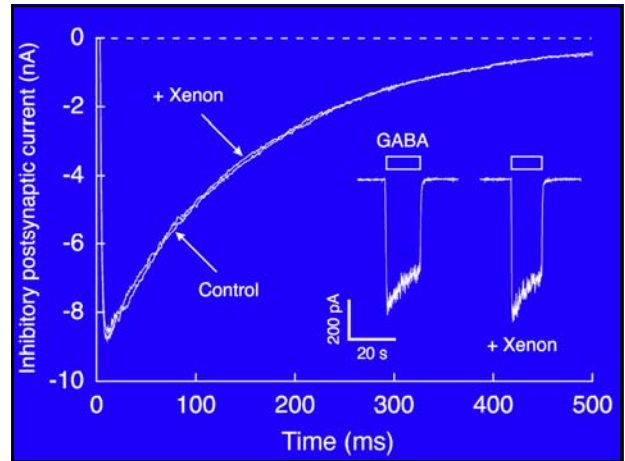
### Stereoselectivity for general anaesthesia

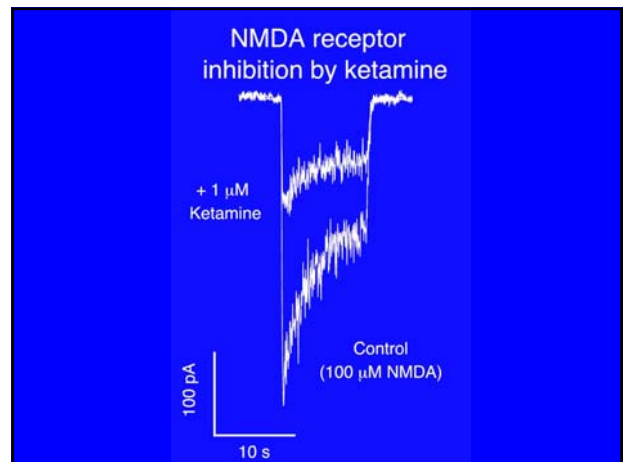
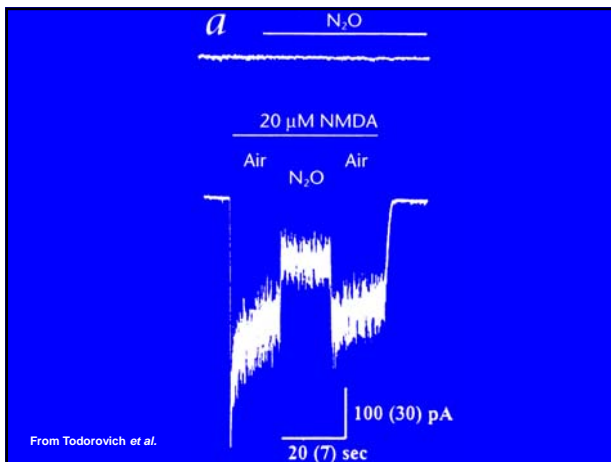
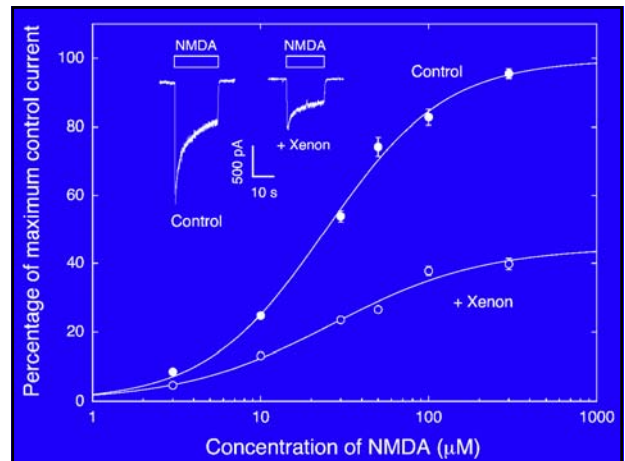
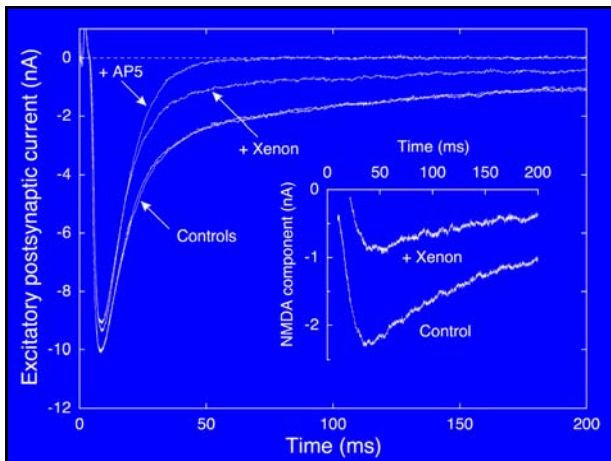
|   |      |
|---|------|
| Isoflurane<br>(Dickinson et al., 2000)  | ~1.5 |
| Barbiturates<br>(Andrews & Mark, 1982)  | 2-4  |
| Ketamine<br>(White et al., 1985)        | 2-4  |
| Etomidate<br>(Tomlin et al., 1998)      | >10  |
| Neurosteroids<br>(Wittmer et al., 1996) | >10  |





Not all anaesthetics act on GABA<sub>A</sub> receptors





### Where does xenon act on NMDA receptors?

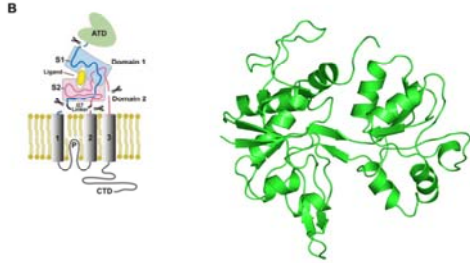
- Can molecular modelling combined with electrophysiology provide the answer?

### Molecular Modelling

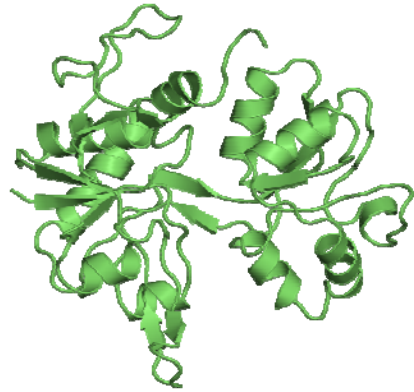
- Why might molecular modelling work for Xe binding?
- Xe is simple “noble” gas with only two relevant force/energy terms
  - van de Waals
  - charge-induced dipole
- Use GCMC simulations

### Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core

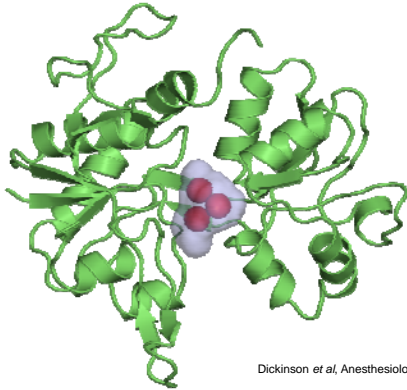
Hiroyasu Furukawa and Eric Gouaux<sup>1</sup>  
Department of Biochemistry and Molecular Biophysics and Howard Hughes Medical Institute, Columbia University, 650 West 168th Street, New York, NY 10032, USA



### Structure of NMDA receptor NR1 subunit

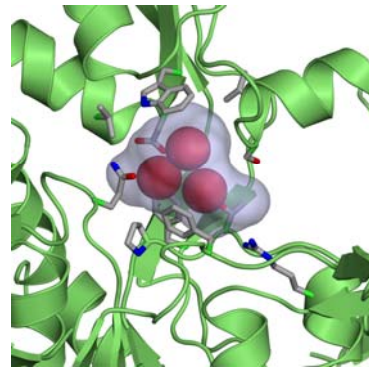


### Modelling predicts xenon binds at glycine site



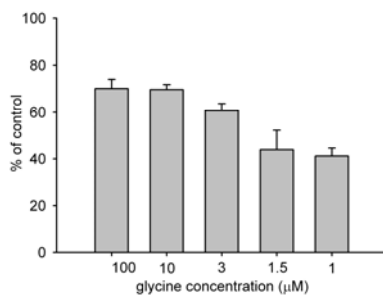
Dickinson et al, Anesthesiology, Nov 2007

### Modelling predicts xenon binds at glycine site



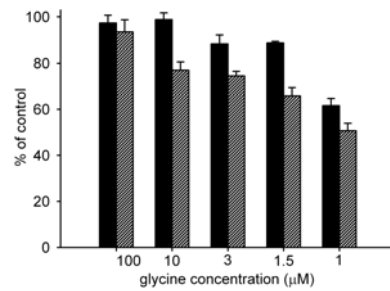
Dickinson et al, Anesthesiology, Nov 2007

### Xenon inhibition increases at low glycine concentration



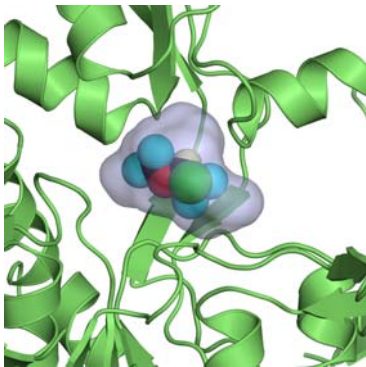
Dickinson et al, Anesthesiology, Nov 2007

### Isoflurane inhibition increases at low glycine concentration



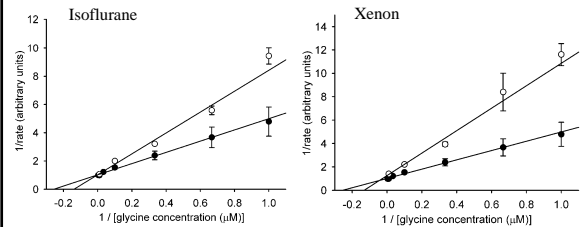
Dickinson et al, Anesthesiology, Nov 2007

Isoflurane fits in same site as xenon



Dickinson et al, Anesthesiology, Nov 2007

Lineweaver-Burk plots: quantitative analysis of competitive inhibition

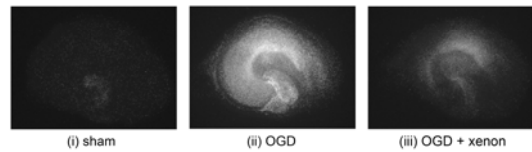


Dickinson et al, Anesthesiology, Nov 2007

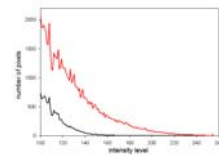
### Clinical implications/Neuroprotection

- NMDA receptors critical in signalling pathways involved in cell death & neuronal injury in stroke, neonatal asphyxia & head trauma.
- NMDA receptor glycine site antagonists (e.g gavestinel) well tolerated & devoid of psychotomimetic side effects.

### In-vitro neuroprotection studies

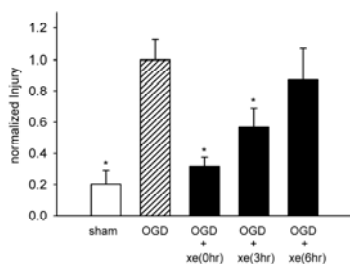


- inflict ischemic injury by OGD
- measure cell death by quantitative propidium iodide (PI) fluorescence



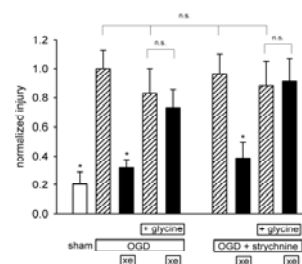
Banks, Franks & Dickinson, Anesthesiology, 2010

50% xenon protects up to 3hr post insult



Banks, Franks & Dickinson, Anesthesiology, 2010

xenon neuroprotection is reversed by adding glycine



Banks, Franks & Dickinson, Anesthesiology, 2010

- Xenon neuroprotection against ischemia mediated by glycine-site inhibition
- Identifies NMDA receptor as target for xenon neuroprotection against ischemia
- Clinical implications
  - Glycine-site antagonists well tolerated in patients
  - Low blood/gas coefficient  $\Rightarrow$  rapid onset
  - Therapeutic window up to 3hrs post-insult
  - Neonatal asphyxia, perioperative stroke, cardiac arrest

#### Ion channels sensitive to general anaesthetics

- GABA<sub>A</sub> receptor
- NMDA receptor
- 2 pore K<sup>+</sup> channels
- glycine receptor – spinal chord (immobility)
- neuronal nACh receptor – function unclear (amnesia?)

Further reading:

see reference list on handout