Molecular Targets of General Anaesthetics

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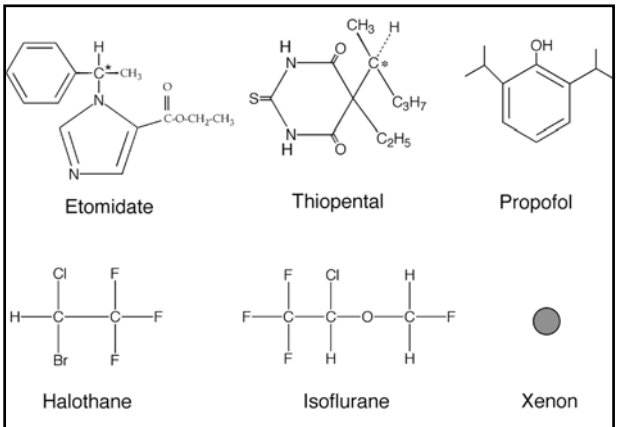
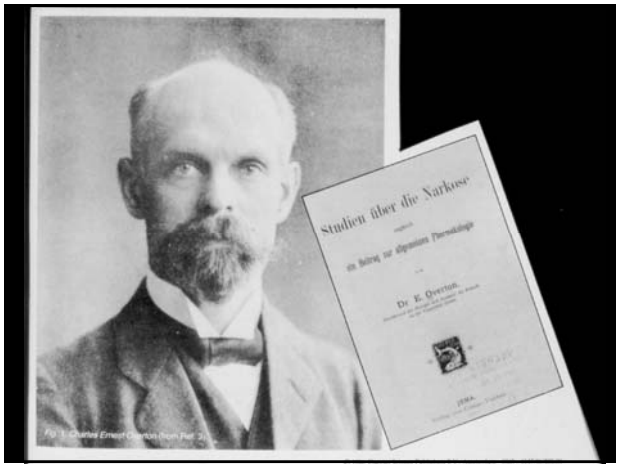
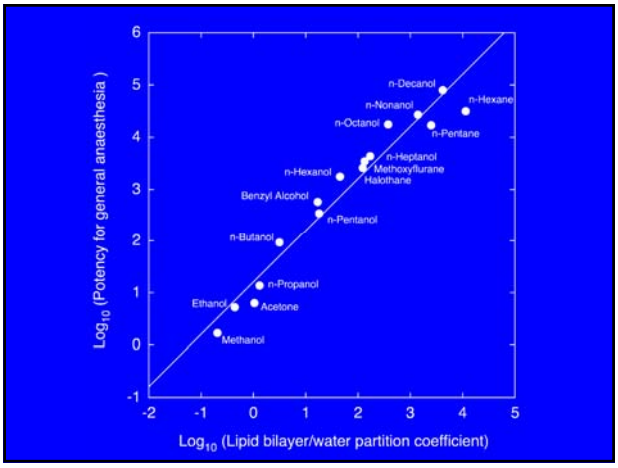
Molecular Targets of General Anaesthetics

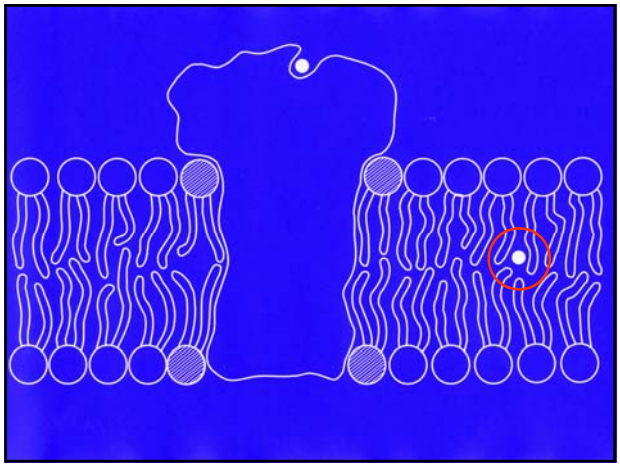
- Meyer-Overton correlation
- Theories of general anaesthesia
  - Lipid theories
  - Protein theories
- Molecular interactions with proteins
- Ion channel targets
  - Criteria for putative targets



General anaesthetics come in all shapes and sizes



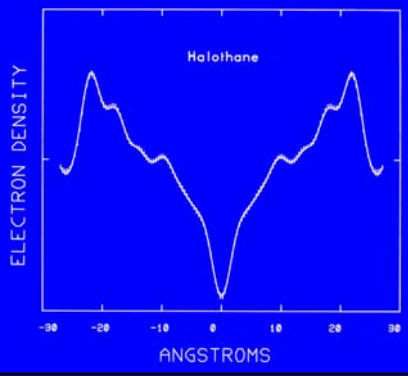




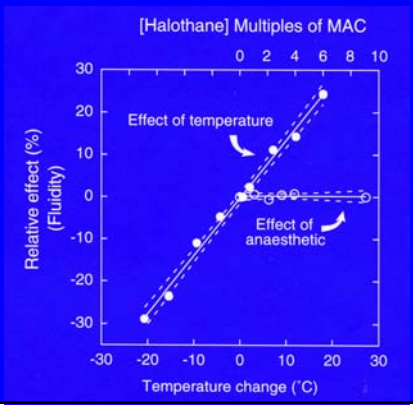
Lipid theories of anaesthesia

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

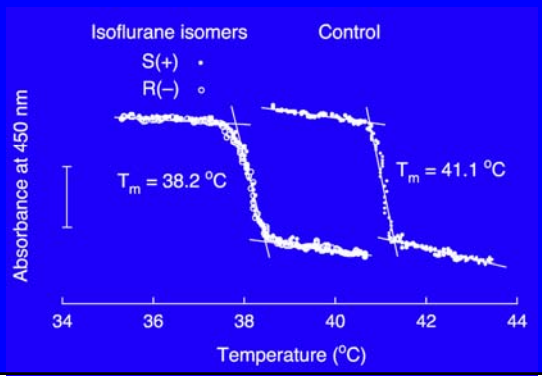
General anaesthetics do not change lipid bilayer dimensions



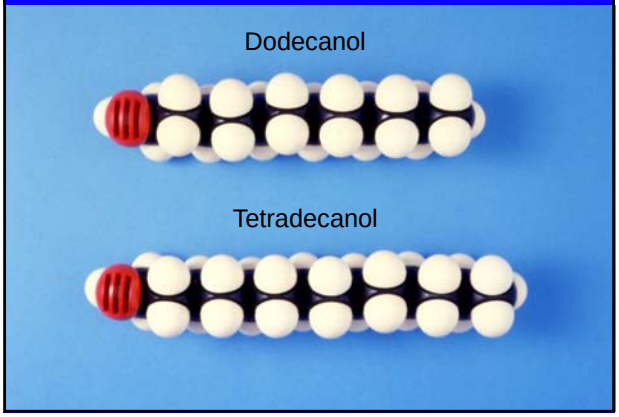
Anaesthetics do not affect lipid bilayer fluidity



Effect of anaesthetics on lipid phase transitions is not stereoselective



Lipid theories cannot explain the "cutoff effect"



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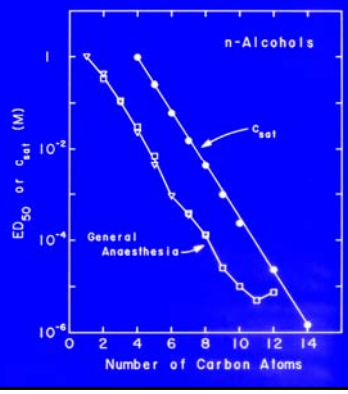
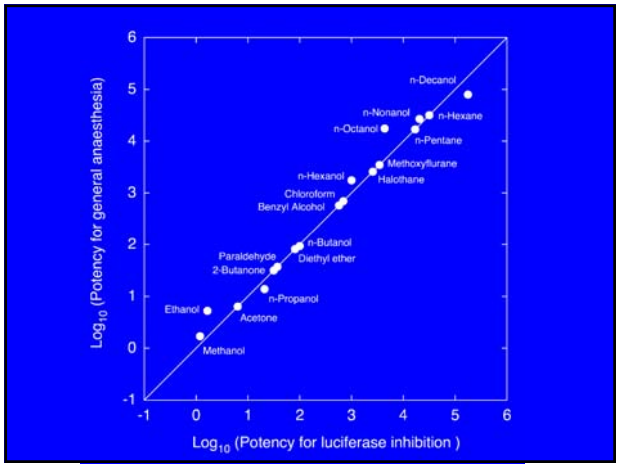
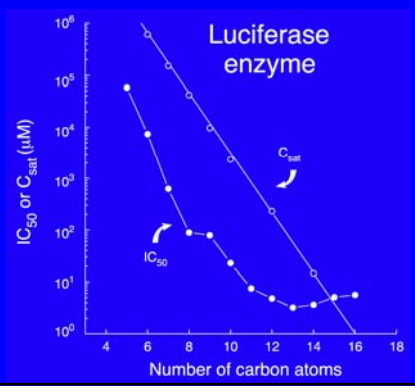


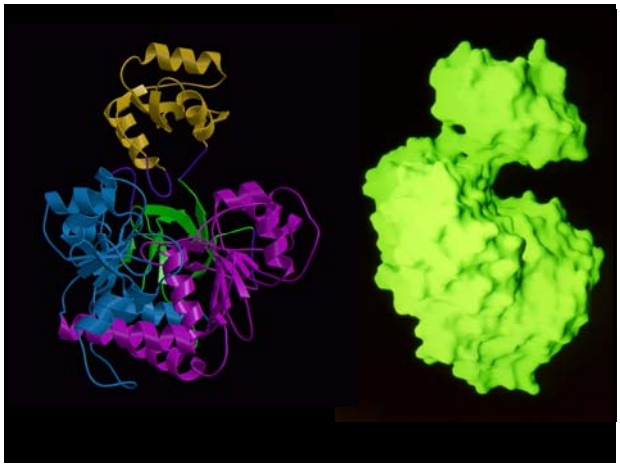
Anaesthetics do not act by disrupting lipid bilayers

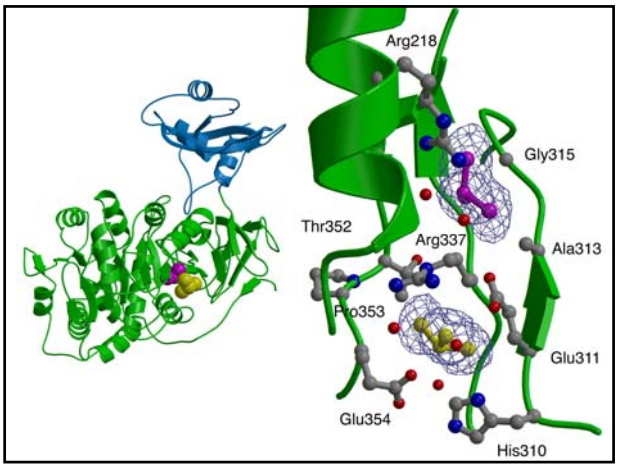
Do anaesthetics act by binding to proteins?

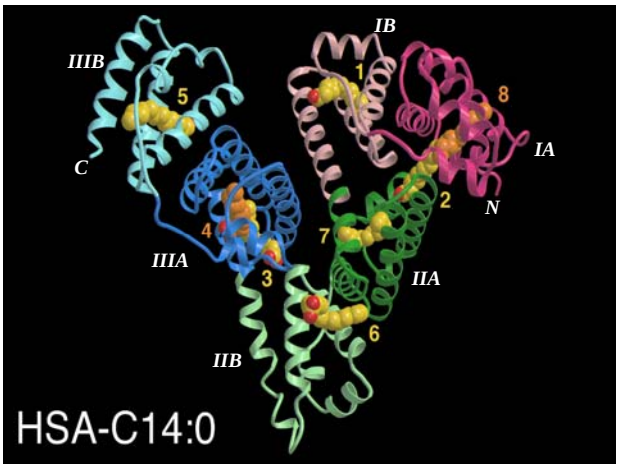


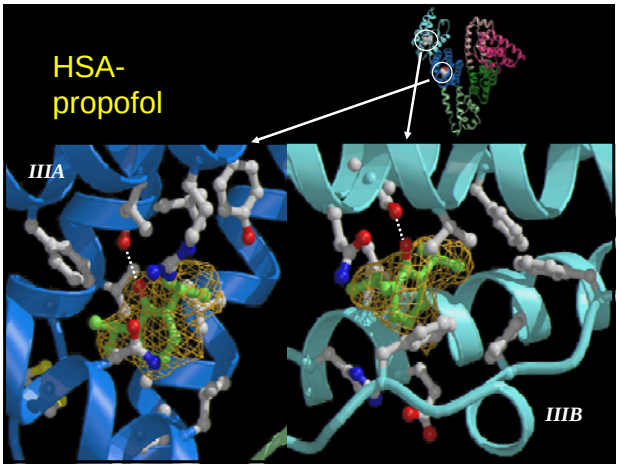
Firefly luciferase exhibits a “cutoff” effect





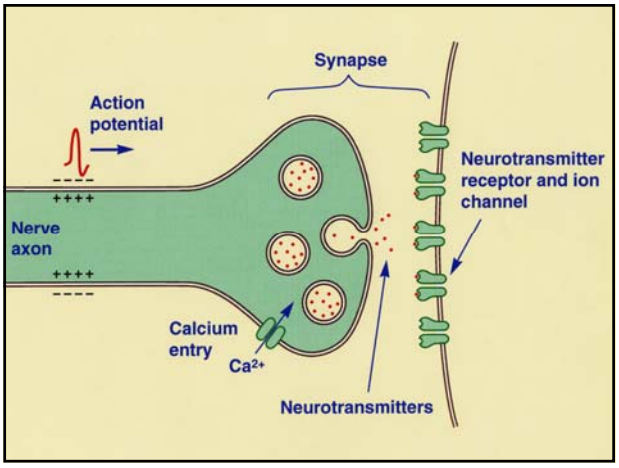






Anaesthetics act by binding directly to sensitive protein targets in pre-formed cavities or clefts

.....but which proteins are relevant?



Criteria for putative targets

- Plausibility
- Sensitivity
- Stereoselectivity

Anaesthetic endpoints & free aqueous concentrations for thiopental

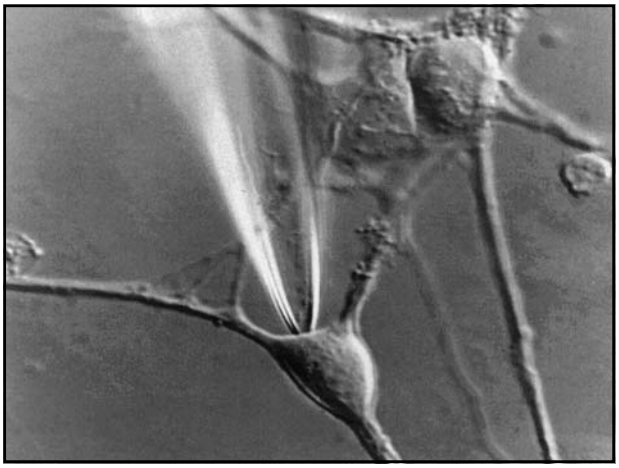
Rat		Human	
	Thiopental (μM)		Thiopental (μM)
Righting reflex	9	Response to verbal command	9
Response to painful stimulus (Tail clamp)	22	Response to painful stimulus (Surgical incision)	23
Tracheal intubation	39	Tracheal intubation	46

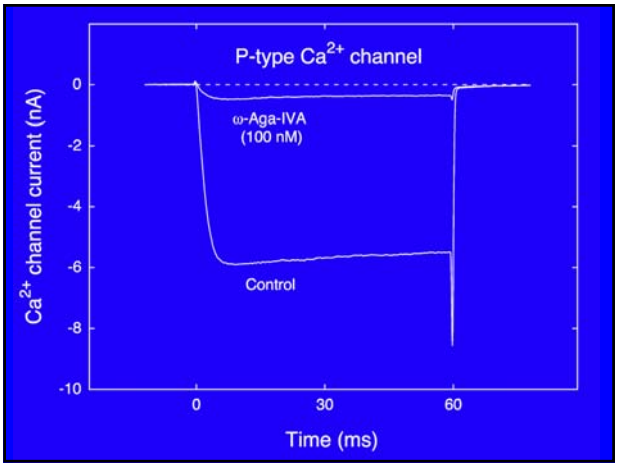
Data from Becker (1978) *Anesthesiology* **49,** 192-196, Hung *et al.* (1992) *Anesthesiology* **77,** 237-244 and Gustafsson *et al.* (1996) *Anesthesiology* **84,** 415-427.

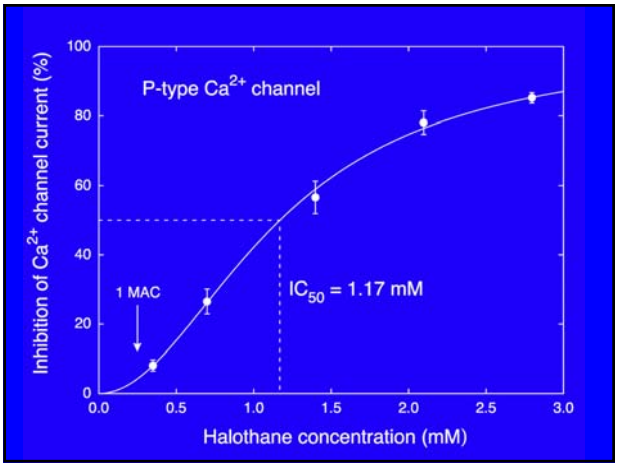
General anaesthetic EC_{50} concentrations for mammals

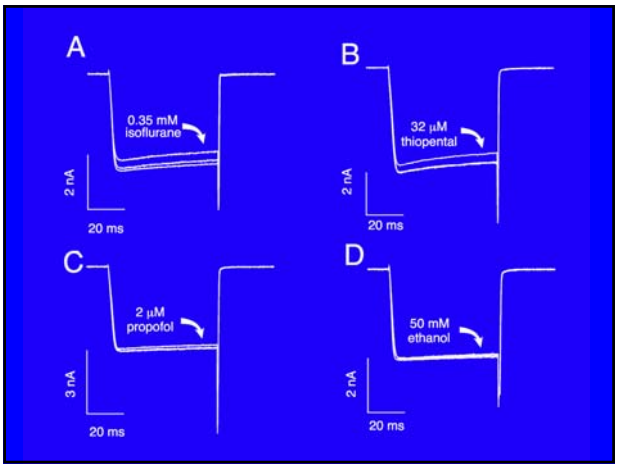
(inhibition of a response to a painful stimulus)

Halothane	230 μM
Isoflurane	280 μM
Thiopental	25 μM
Propofol	1.5 μM

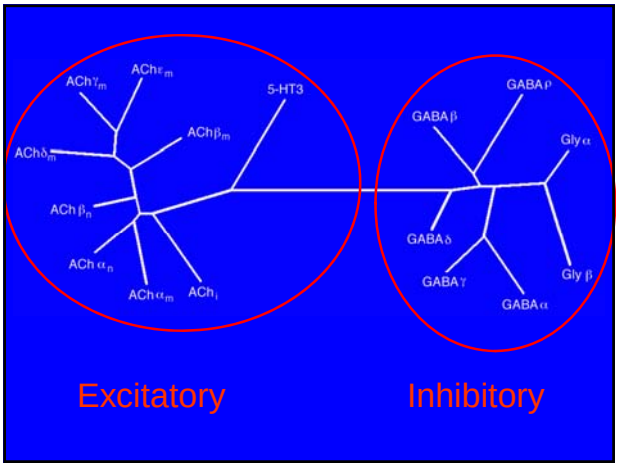


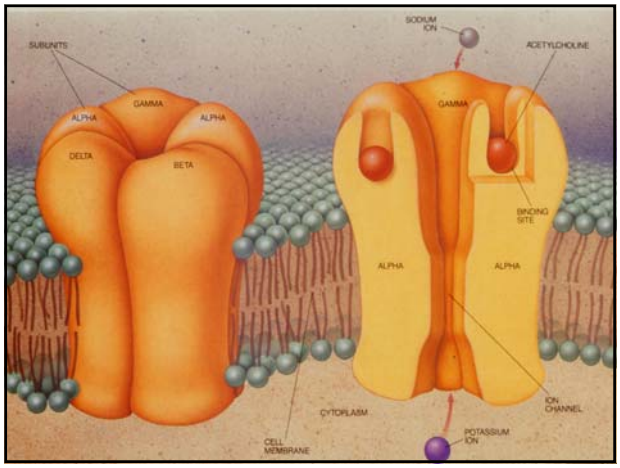


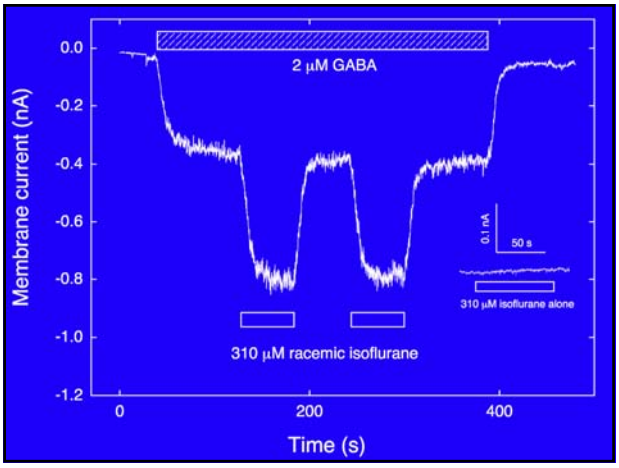


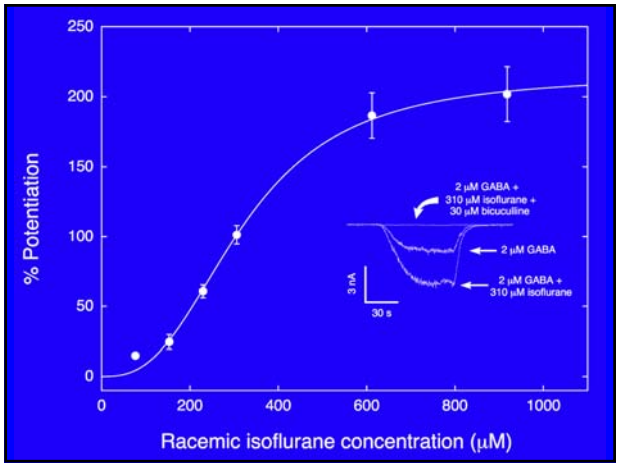


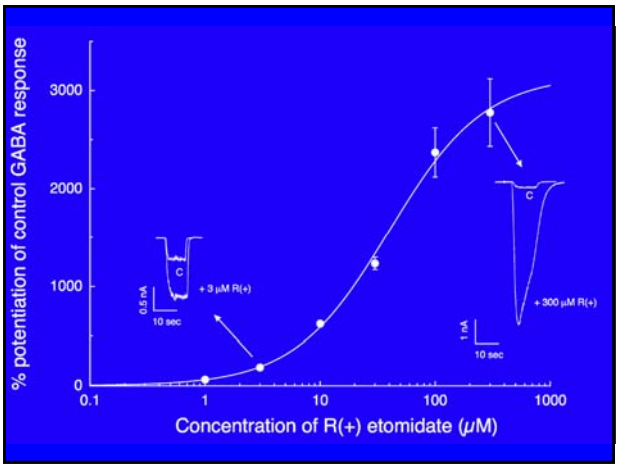
Most voltage-gated ion channels are insensitive to anaesthetics

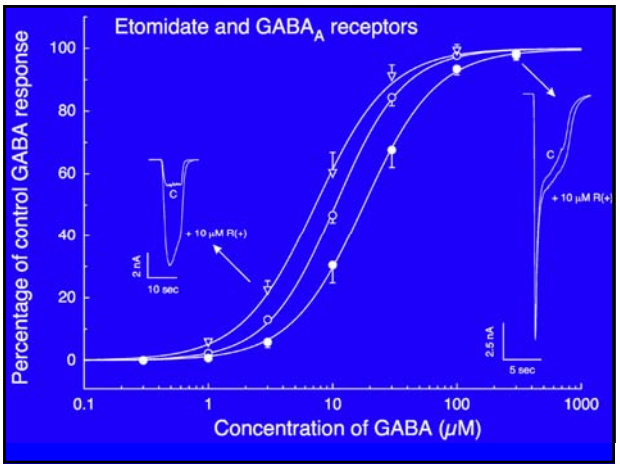


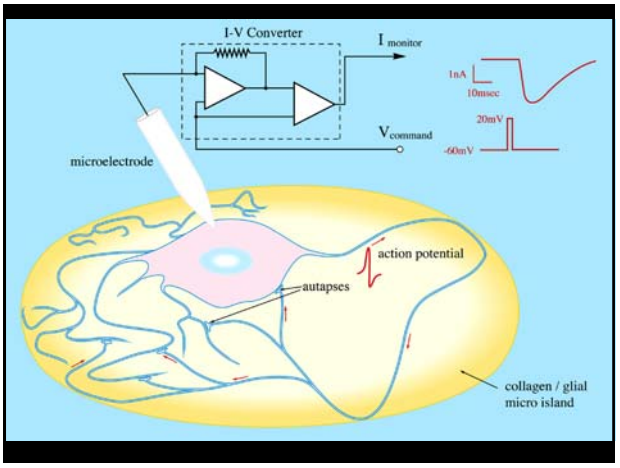


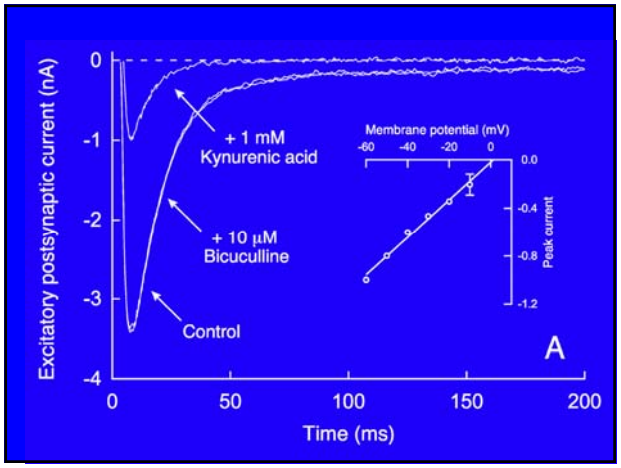


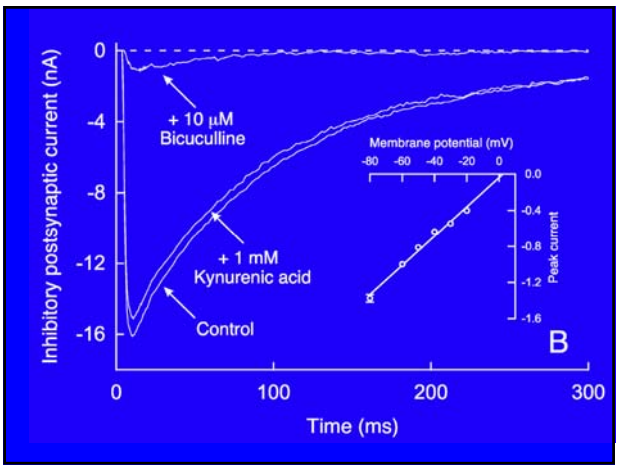


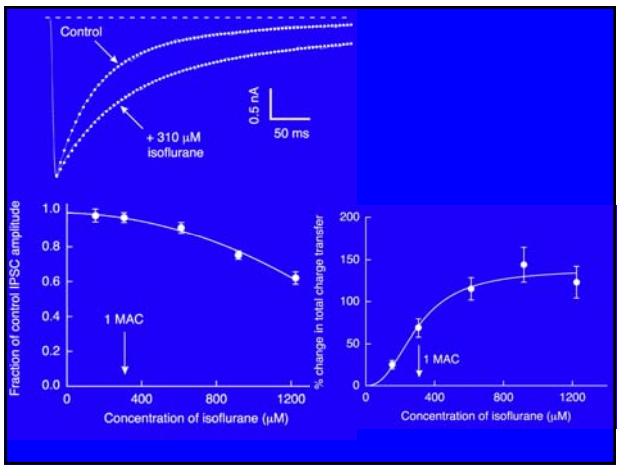

% potentiation of control GABA response
3000
2000
1000
0
0.1
1
10
100
1000
Concentration of R(+) etomidate (μM)
C
+ 3 μM R(+)
0.5 nA
10 sec
+ 300 μM R(+)
1 nA
10 sec

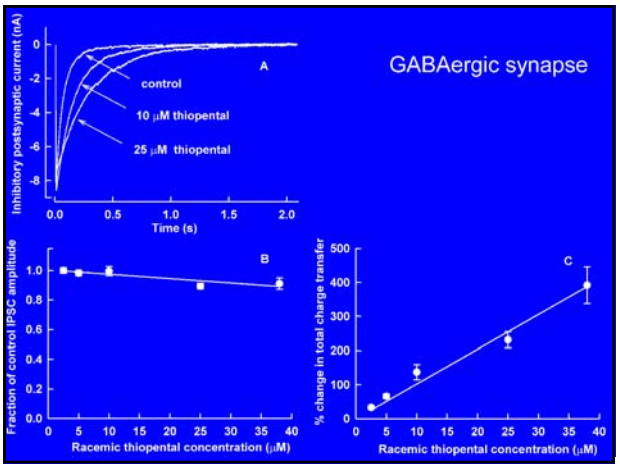

Etomidate and GABA$_A$ receptors
Percentage of control GABA response
100
80
60
40
20
0
0.1
1
10
100
1000
Concentration of GABA (μM)
C
+ 10 μM R(+)
2 nA
10 sec
2.5 nA
5 sec

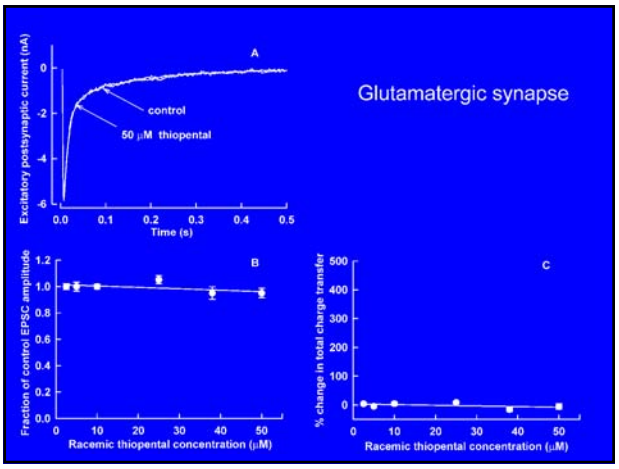

I-V Converter
I monitor
V command
1nA
10msec
20mV
-60mV
microelectrode
action potential
autapses
collagen / glial
micro island

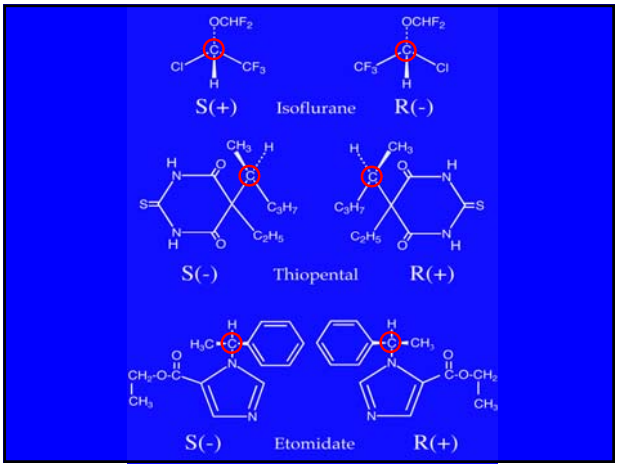

Excitatory postsynaptic current (nA)
0
-1
-2
-3
-4
0
50
100
150
200
Time (ms)
+ 1 mM
Kynurenic acid
+ 10 μM
Bicuculline
Control
Membrane potential (mV)
-60
-40
-20
0
0.0
-0.4
-0.8
-1.2
Peak current
A

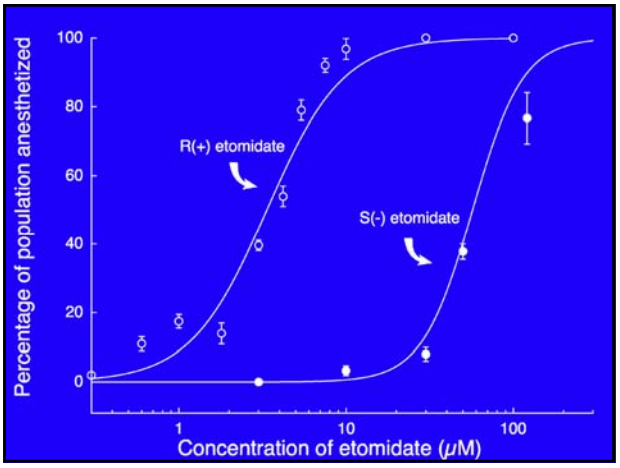

Inhibitory postsynaptic current (nA)
0
-4
-8
-12
-16
0
100
200
300
Time (ms)
+ 10 μM
Bicuculline
+ 1 mM
Kynurenic acid
Control
Membrane potential (mV)
-80
-60
-40
-20
0
0.0
-0.4
-0.8
-1.2
-1.6
Peak current
B


Control
+ 310 μM
isoflurane
0.5 nA
50 ms
Fraction of control IPSC amplitude
1.0
0.8
0.6
0.4
0.2
0.0
1 MAC
0
400
800
1200
Concentration of isoflurane (μM)
% charge in total charge transfer
200
150
100
50
0
1 MAC
0
400
800
1200
Concentration of isoflurane (μM)



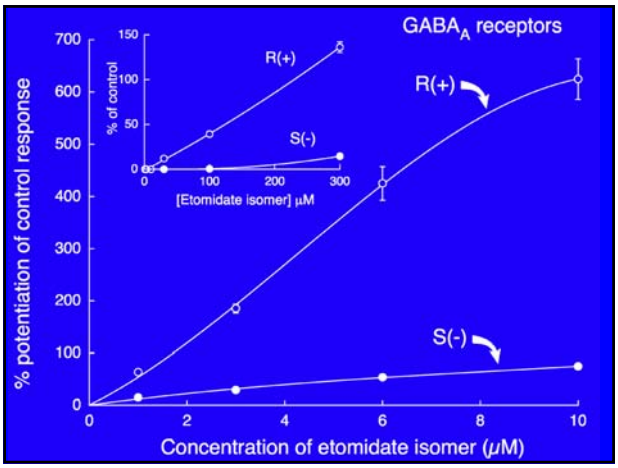


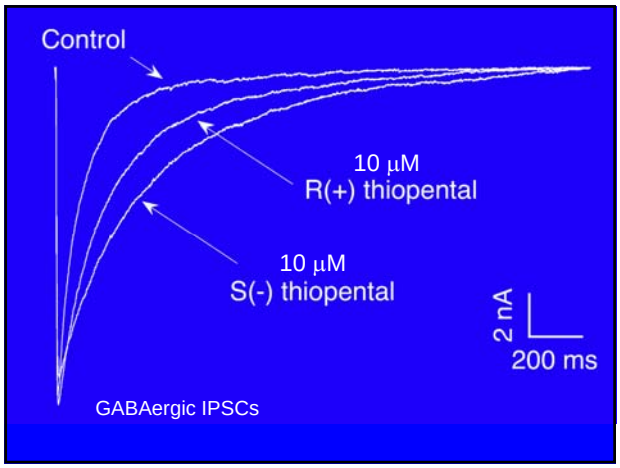




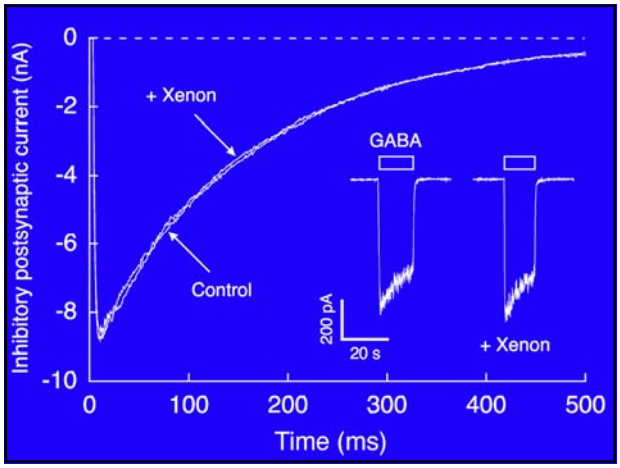
Stereoselectivity for general anaesthesia

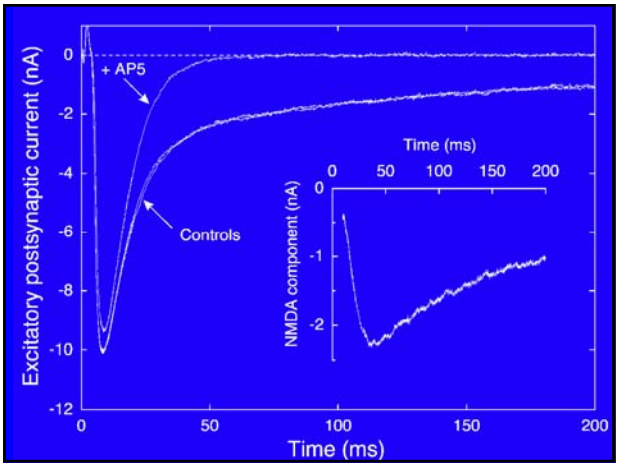
Isoflurane (Dickinson et al., 2000)	~1.5
Barbiturates (Andrews & Mark, 1982)	2-4
Ketamine (White et al., 1985)	2-4
Etomidate (Tomlin et al., 1998)	>10
Neurosteroids (Wittmer et al., 1996)	>10



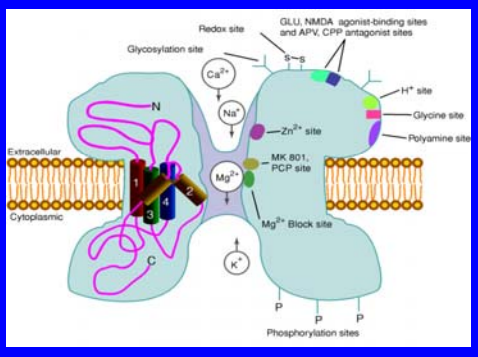


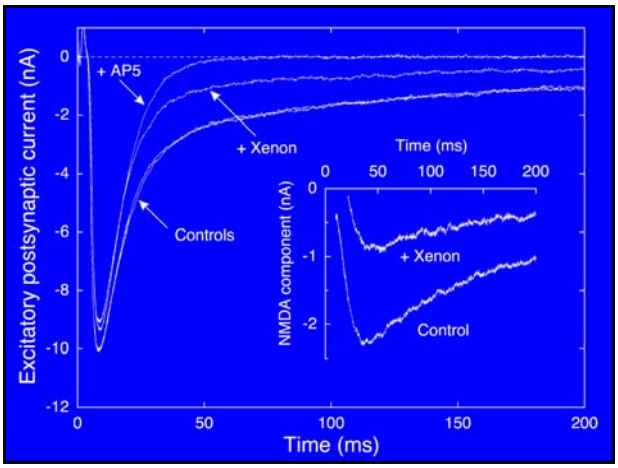
Not all anaesthetics act on GABA$_A$ receptors

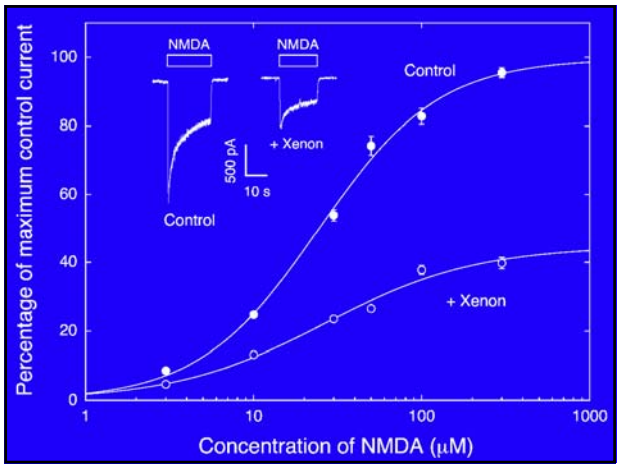


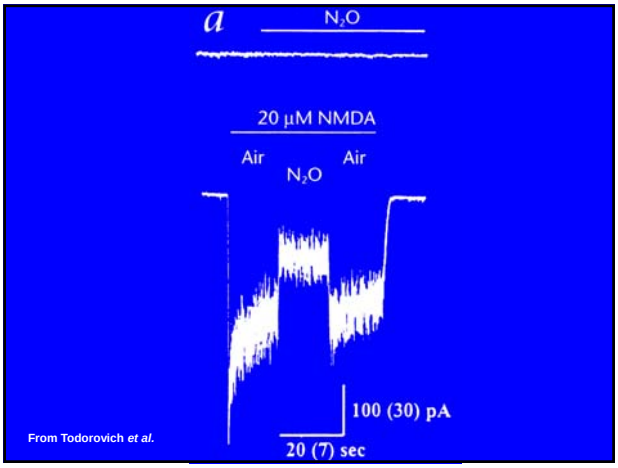


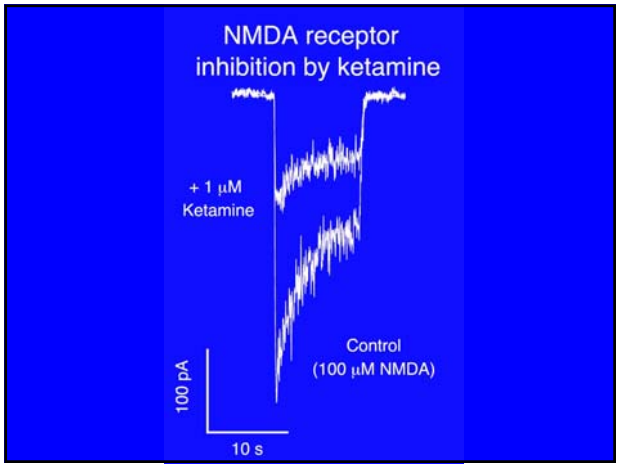
The NMDA receptor











Where does xenon act on NMDA receptors?

- Can molecular modelling combined with electrophysiology provide the answer?

Molecular Modelling

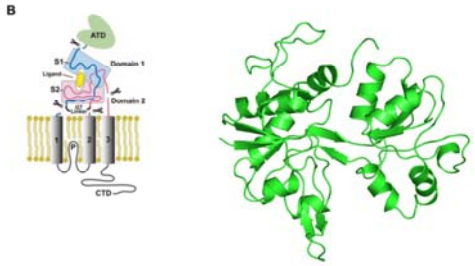
- Why might molecular modelling work for Xe binding?
- Xe is simple “noble” gas with only two relevant force/energy terms
  - van de Waals
  - charge-induced dipole
- Use GCMC simulations

The EMBO Journal Vol. 22 No. 12 pp. 2873–2885, 2003

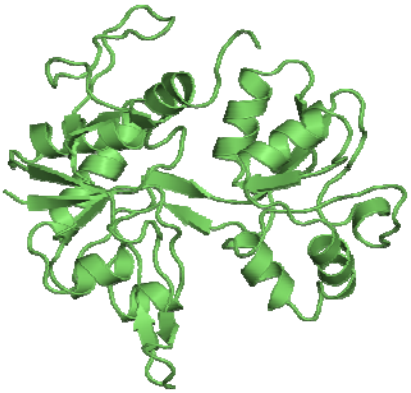
Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core

Hiroyasu Furukawa and Eric Gouaux[1]

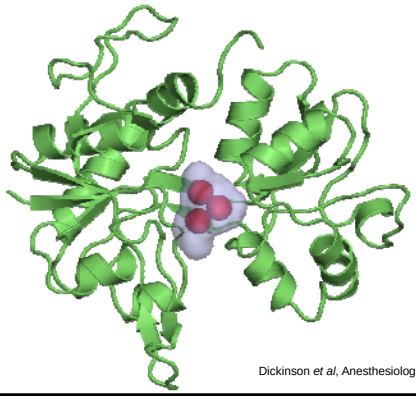
Department of Biochemistry and Molecular Biophysics and Howard Hughes Medical Institute, Columbia University, 650 West 168th Street, New York, NY 10032, USA



Structure of NMDA receptor NR1 subunit

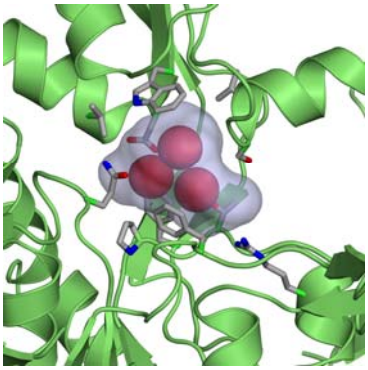


Modelling predicts xenon binds at glycine site



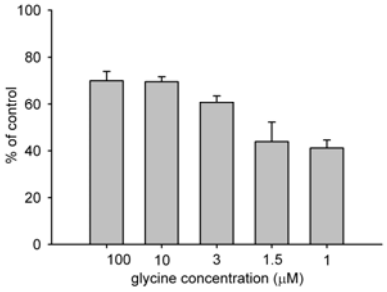
Dickinson *et al*, Anesthesiology, Nov 2007

Modelling predicts xenon binds at glycine site



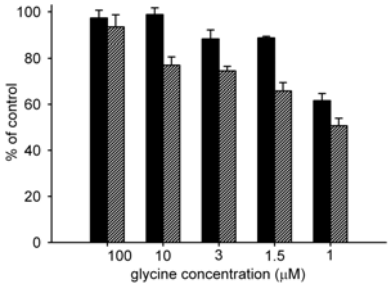
Dickinson *et al*, Anesthesiology, Nov 2007

Xenon inhibition increases at low glycine concentration



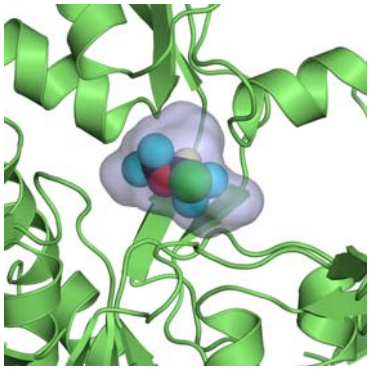
Dickinson *et al*, Anesthesiology, Nov 2007

Isoflurane inhibition increases at low glycine concentration



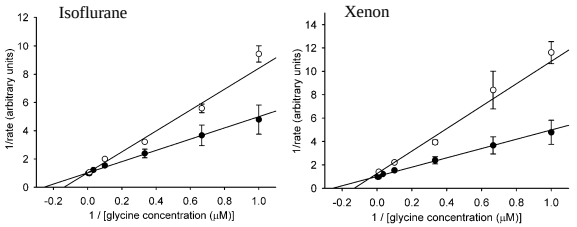
Dickinson *et al*, Anesthesiology, Nov 2007

Isoflurane fits in same site as xenon



Dickinson *et al*, Anesthesiology, Nov 2007

Lineweaver-Burk plots: quantitative analysis of competitive inhibition

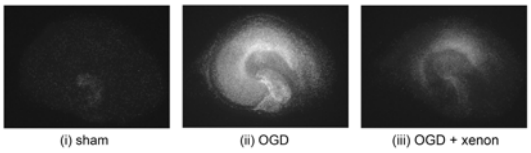


Dickinson *et al*, Anesthesiology, Nov 2007

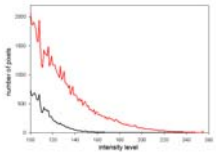
Clinical implications/Neuroprotection

- 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

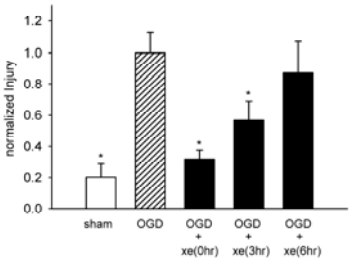


- inflict ischemic injury by OGD
- measure cell death by quantitative propidium iodide (PI) fluorescence



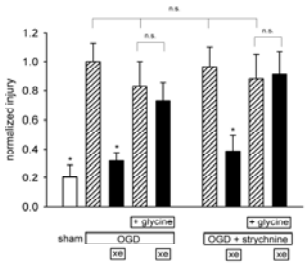
Banks, Franks & Dickinson, *Anesthesiology*, 2010

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 ⇒rapid onset
  - Therapeutic window up to 3hrs post-insult
  - Neonatal asphyxia, perioperative stroke, cardiac arrest

Ion channels sensitive to general anaesthetics

- $GABA_A$ receptor
- NMDA receptor
- 2 pore K^+ channels
- glycine receptor – spinal chord (immobility)
- neuronal nACh receptor – function unclear (amnesia?)

Further reading:

see reference list on handout