

‘Alive but not Kicking’:
The Molecular Neurobiology of Anesthesia.



Pain.

Sculpture by Dr. R. Tait Mackenzie

Before anesthesia.....



Mural of Dr. Villander, Hôtel de Dieu, Paris.

A

From *Behind the Doctor*, by Logan Clendenning, published by Alfred A. Knopf.

B

From *Devils, Drugs and Doctors*, by Howard W. Haggard, M.D., published by Harper and Brothers.

C

PICTORIAL RECORDS OF THE AGONY ENDURED IN OPERATIONS BEFORE THE ADVENT OF ANESTHESIA

- A. A surgeon cutting with his big saw.
- B. A very painful operation of the seventeenth century.
- C. A surgeon torturing his patient.

Early anesthetic use:

In surgery



[FIG. 1] Early use of alcohol for anesthetic purposes in a monastic hospital. From Diebold Schilling's *Swiss Chronicle*, 1513.

In recreation

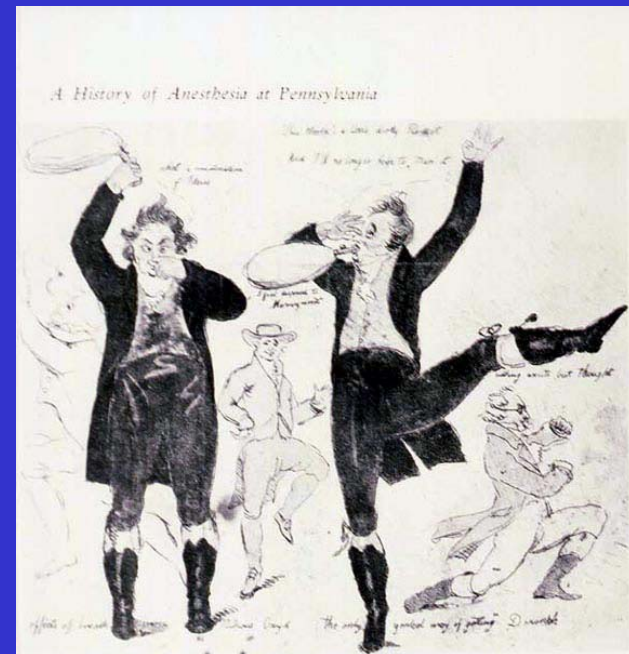
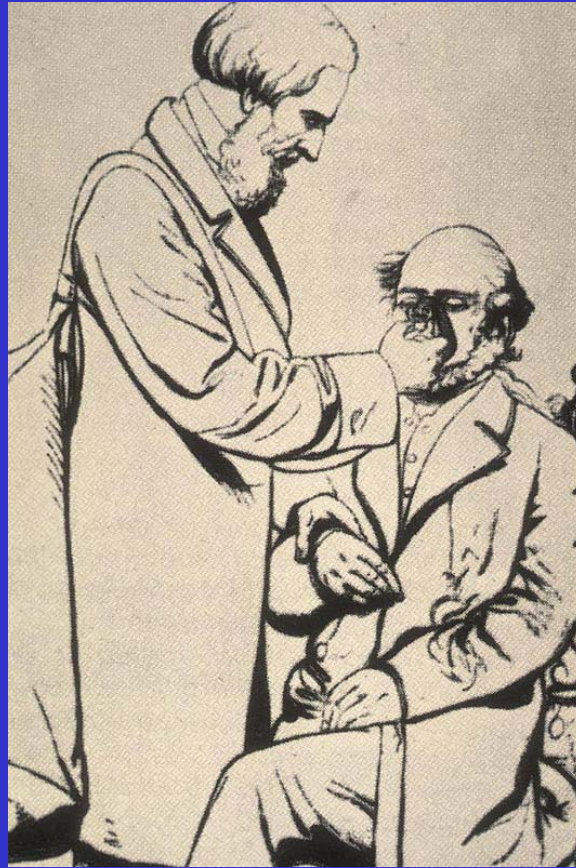


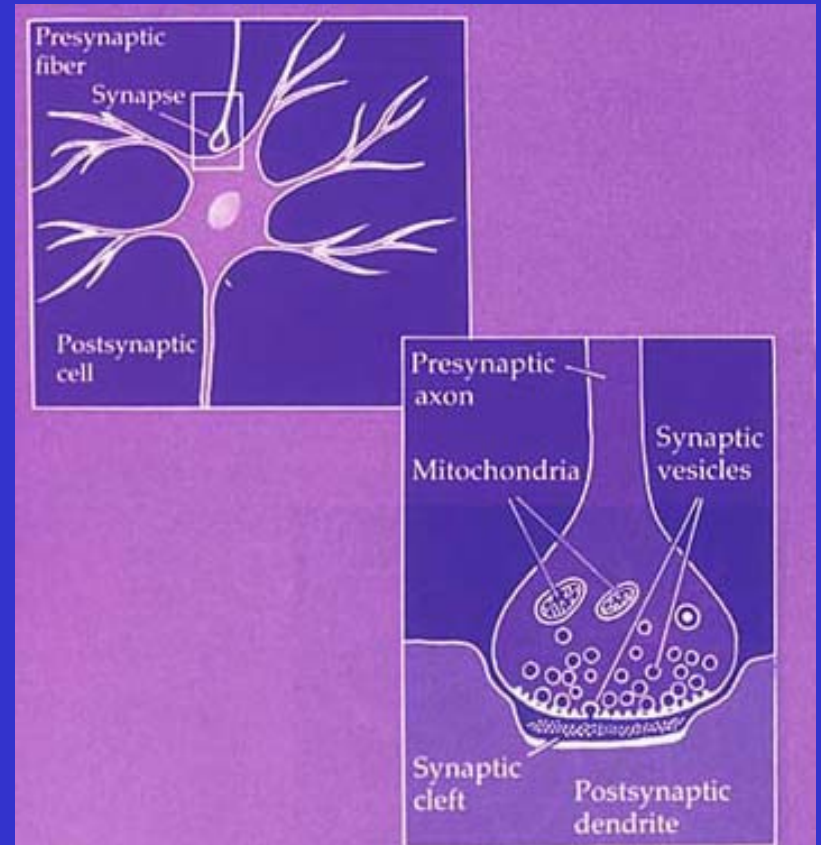
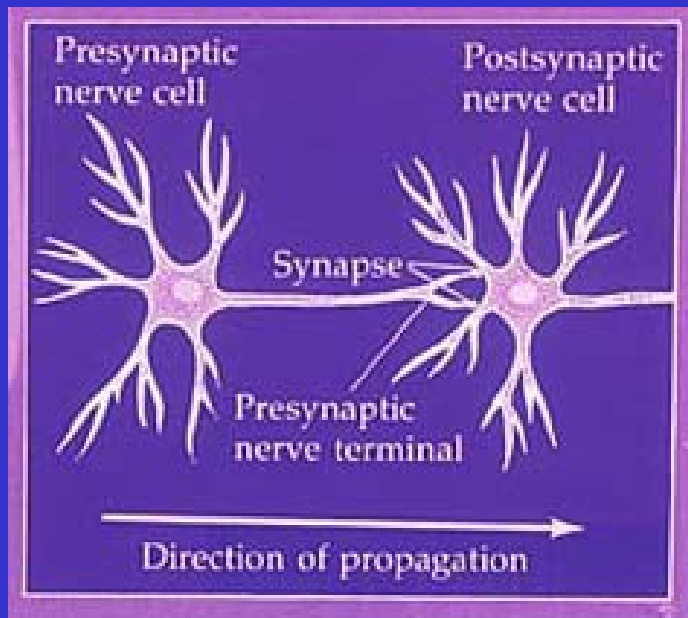
FIGURE 3. Caricature published in 1808 satirizing the work and thoughts of W. P. C. Barton on Nitrous Oxide. (Reproduced with the permission of the Edgar Fabs Smith Memorial Collection, University of Pennsylvania.)

decidedly of opinion with Mr. Davy, that this gas has the power of removing intense physical pain." In his thesis, Barton explained Woodhouse's confusion, caused by the sensations experienced by students breathing atmospheric air from closed containers. Carbon dioxide accumulation was responsible. Barton conceived nitrous oxide of use in inhalation-therapy, for the treatment of mental

Technology for administration of volatile anesthetics is developed for major surgery.



How do anesthetic agents affect neuronal function?



4 Questions , 4 Techniques:

1) How is neuronal communication affected by anesthetic agents?

Technique: Electrophysiology using isolated neurons

2) What family of neuronal receptors is modulated by anesthetics?

Technique: Xenopus oocyte expression system

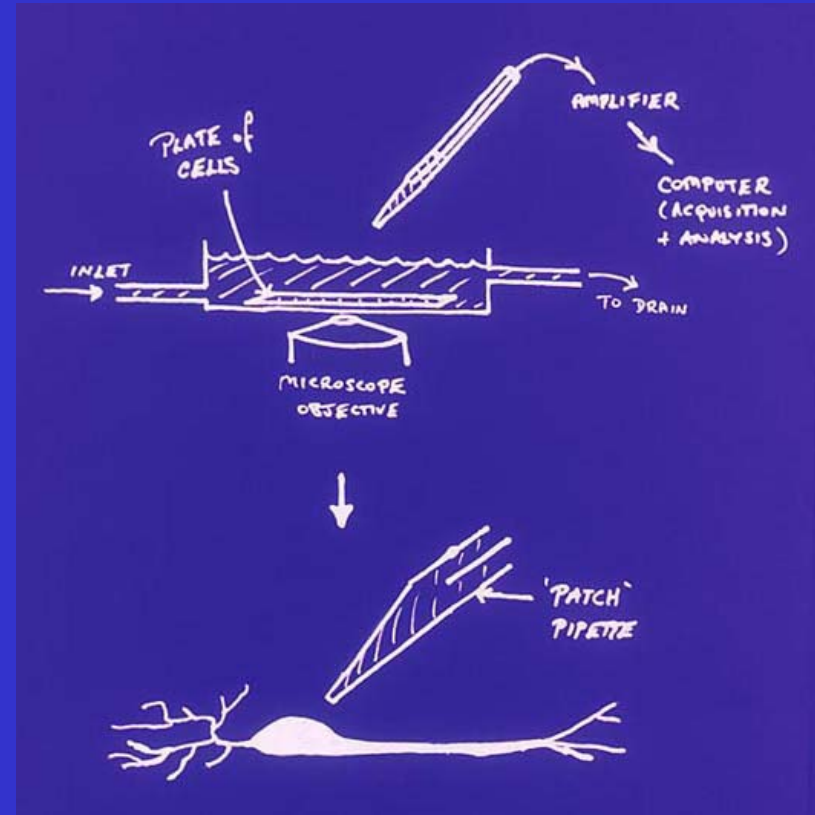
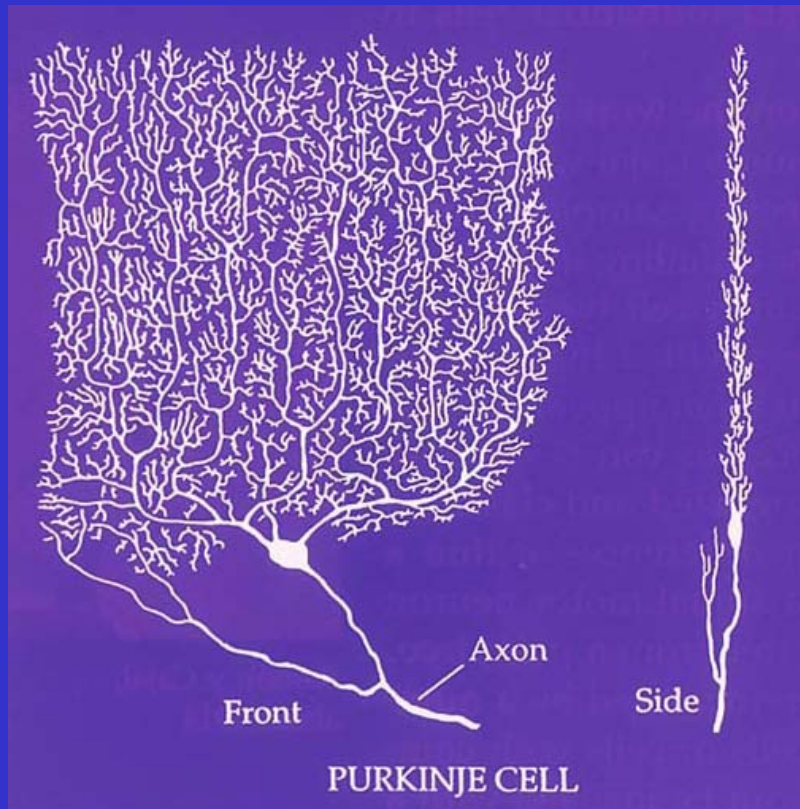
(3) Where do anesthetic molecules bind?

Technique: Cultured cells transfected with relevant receptors

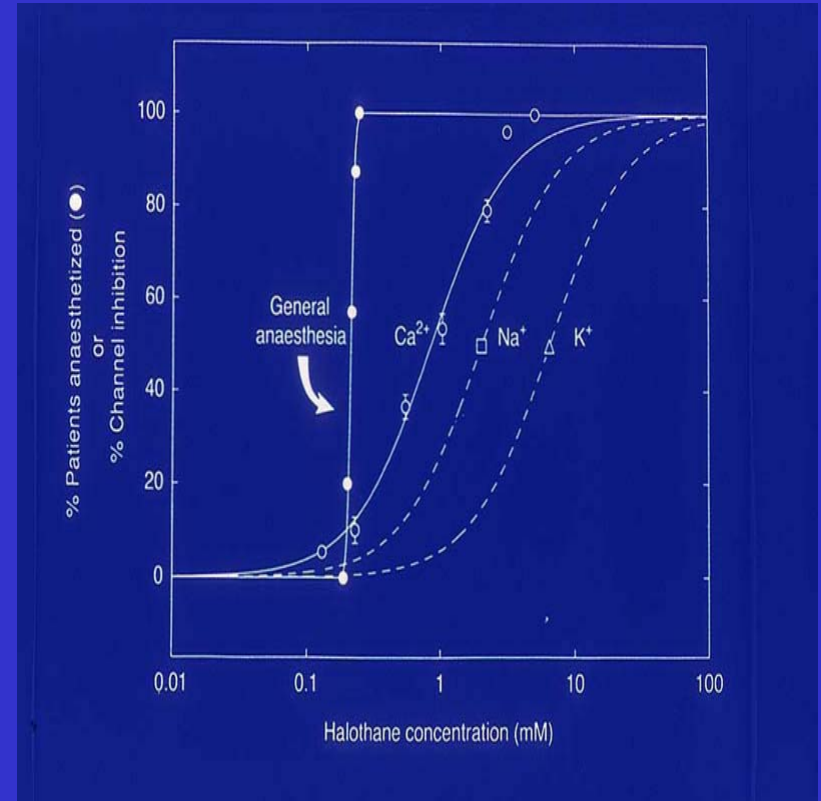
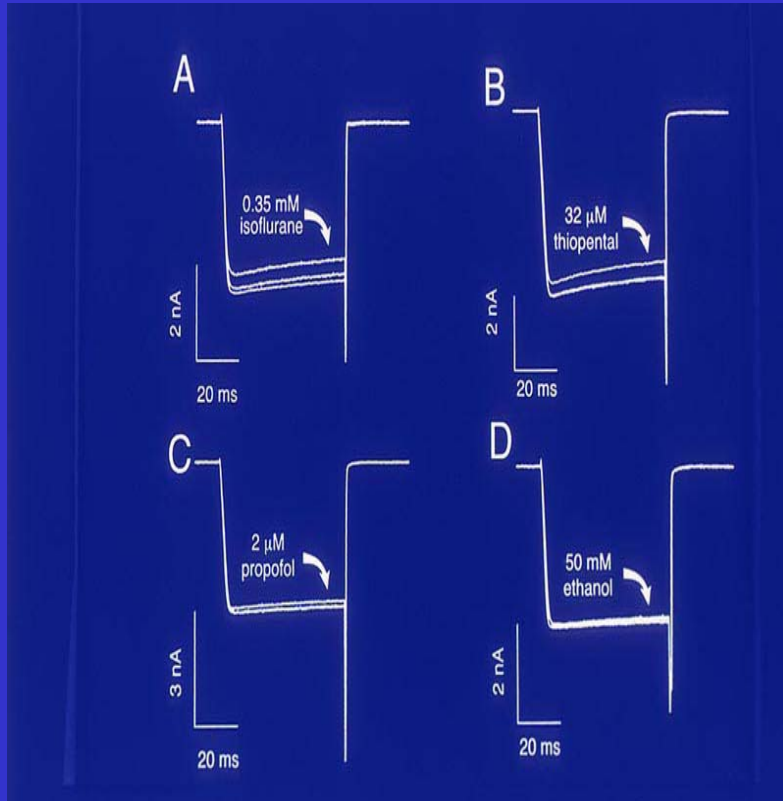
(4) What are long-term side effects of anesthetics on neuronal tissue?

Technique: DNA microarray technology

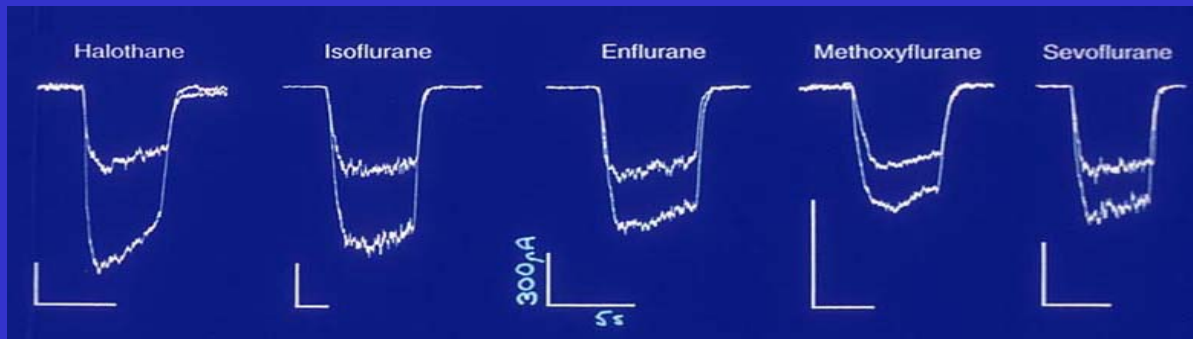
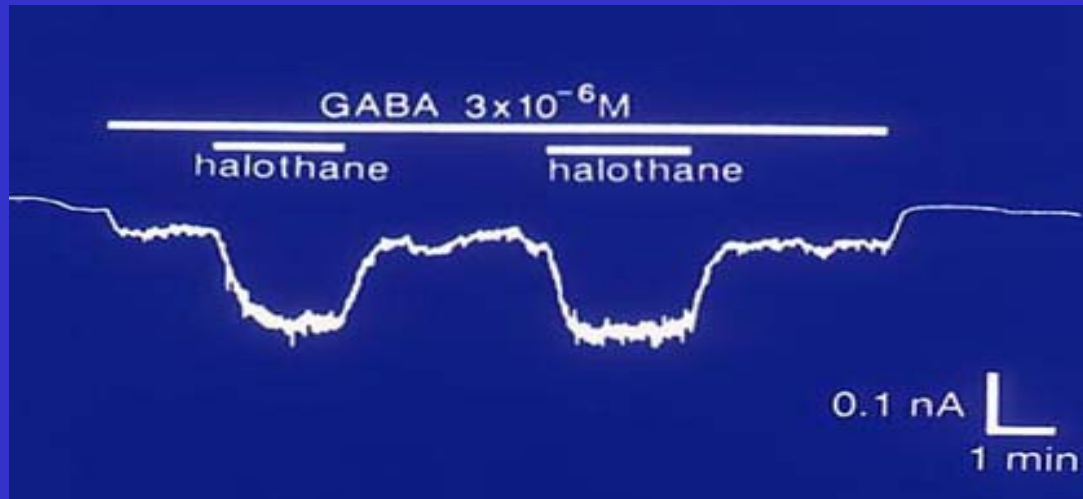
1) Whole-cell patch-clamp recordings from isolated mammalian neurons.



Clinical concentrations of anesthetics do not affect Na⁺, Ca²⁺ and K⁺ voltage-gated currents in native membranes.



At clinical concentrations, volatile anesthetics enhance currents evoked by the inhibitory neurotransmitter, GABA.



4 Questions , 4 techniques:

1) **How is neuronal communication affected by anesthetic agents?**

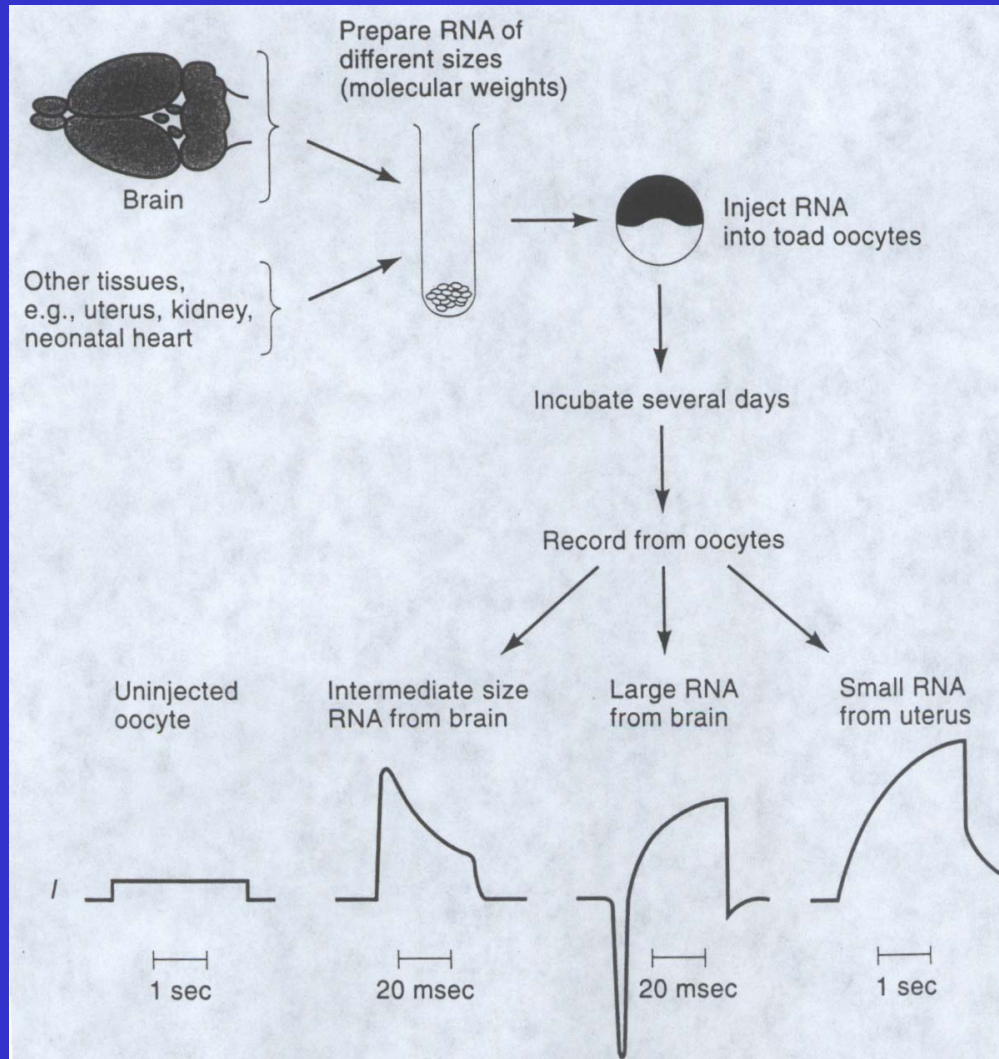
-Anesthetics modulate postsynaptic neurotransmitter-gated currents.

2) What family of neuronal receptors is modulated by anesthetics?

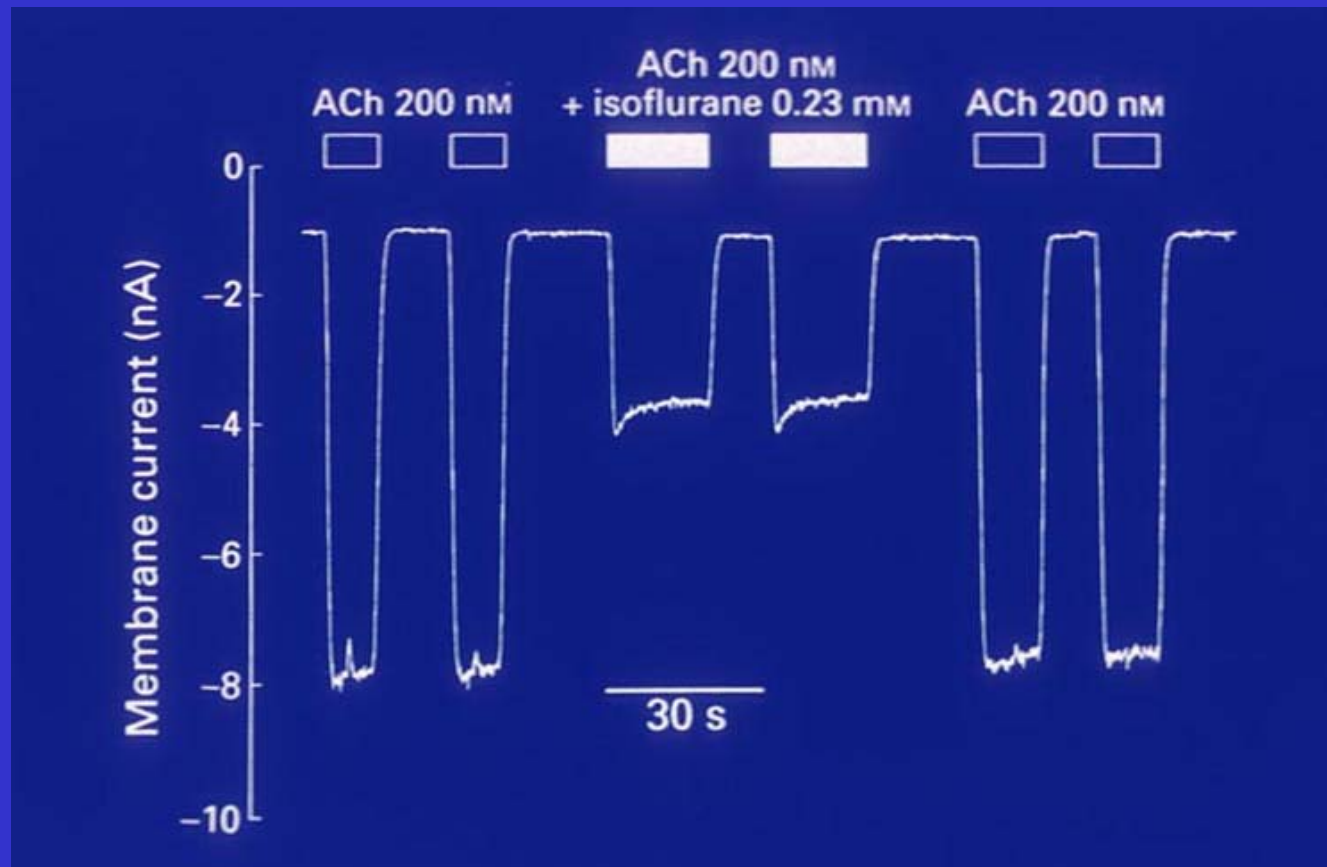
3) Where do anesthetic molecules bind?

4) What are long-term side effects of anesthetics on neuronal tissue?

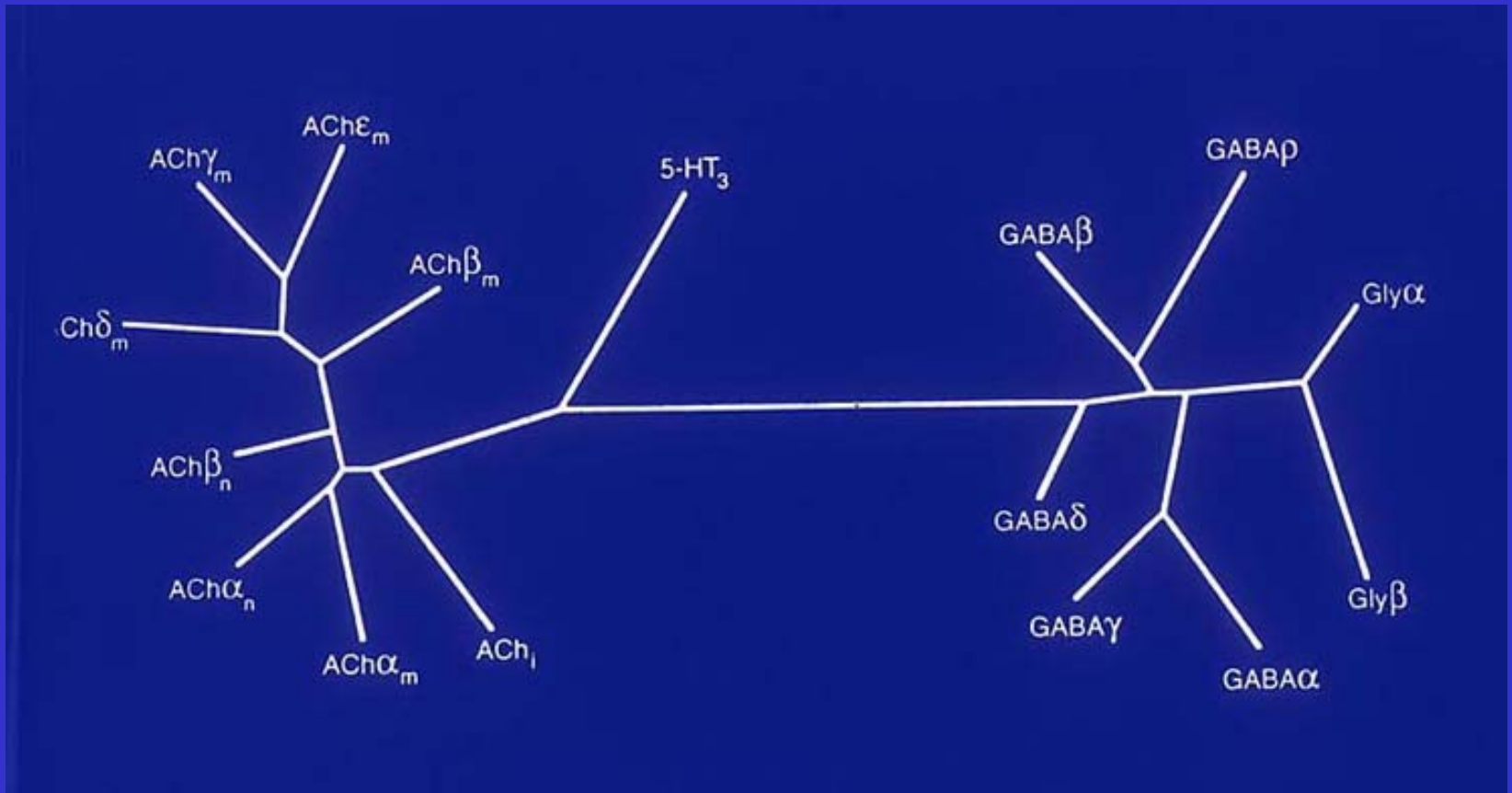
2) *Xenopus* oocyte expression system



From oocyte recordings, the nicotinic acetylcholine receptor channel is shown to be modulated by anesthetic agents.



A whole family of neurotransmitter-gated channels are modulated by the action of anesthetic agents



4 Questions , 4 techniques:

1) How is neuronal communication affected by anesthetic agents?

-Anesthetics modulate postsynaptic neurotransmitter-gated currents.

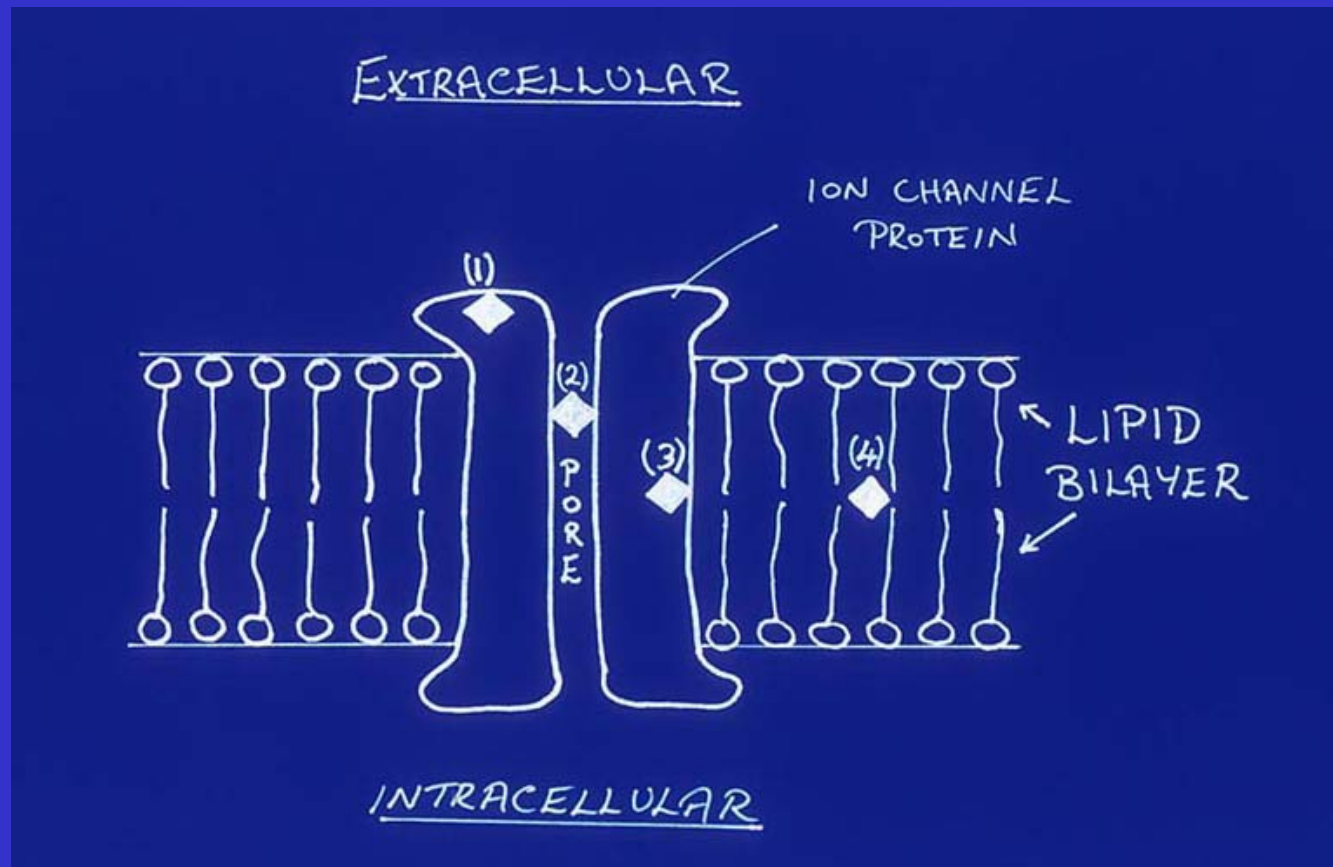
2) What family of neuronal receptors is modulated by anesthetics?

-A homologous family of neurotransmitter-gated ion channels are all affected by anesthetic agents.

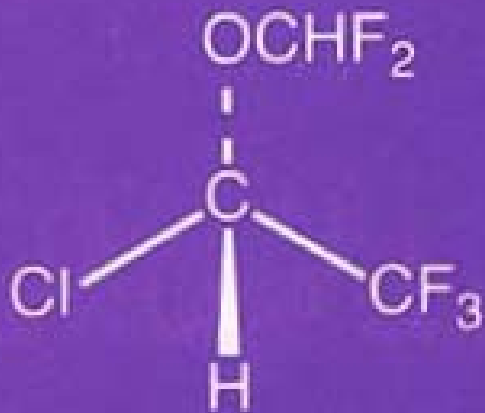
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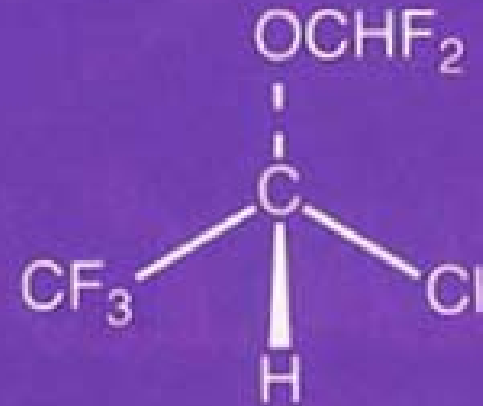


Stereoisomers of Isoflurane



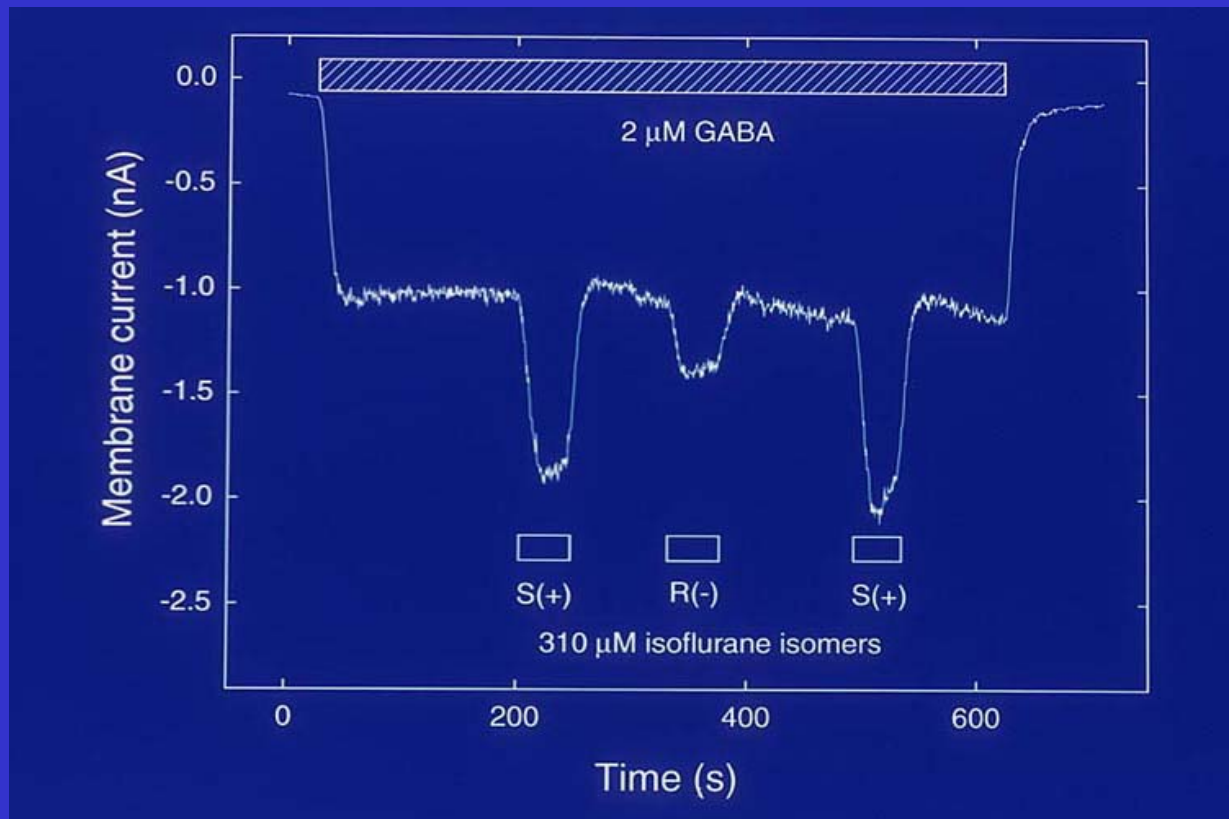
S(+)

Isoflurane



R(-)

Current recordings from cultured cells transfected with GABA receptors demonstrate stereoselectivity of anesthetic action.



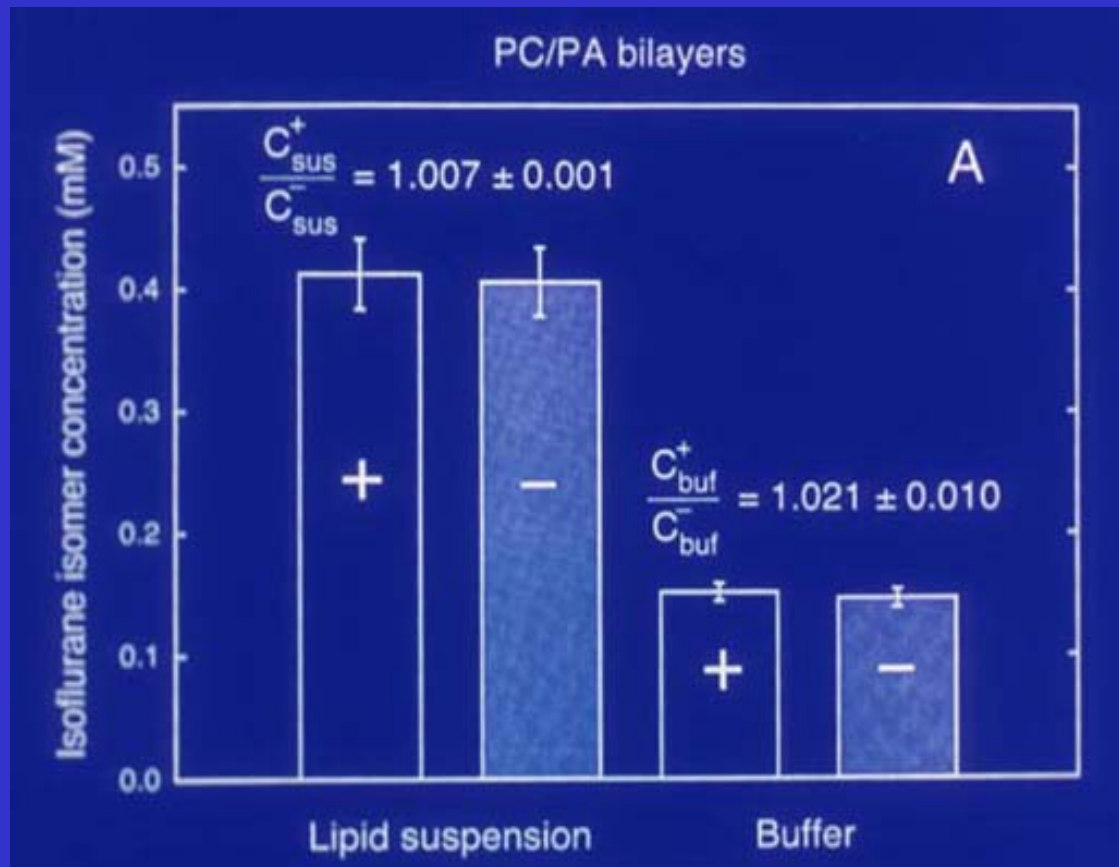
Same stereoselectivity is observed *in vivo*.

In vivo data on isoflurane isomers*

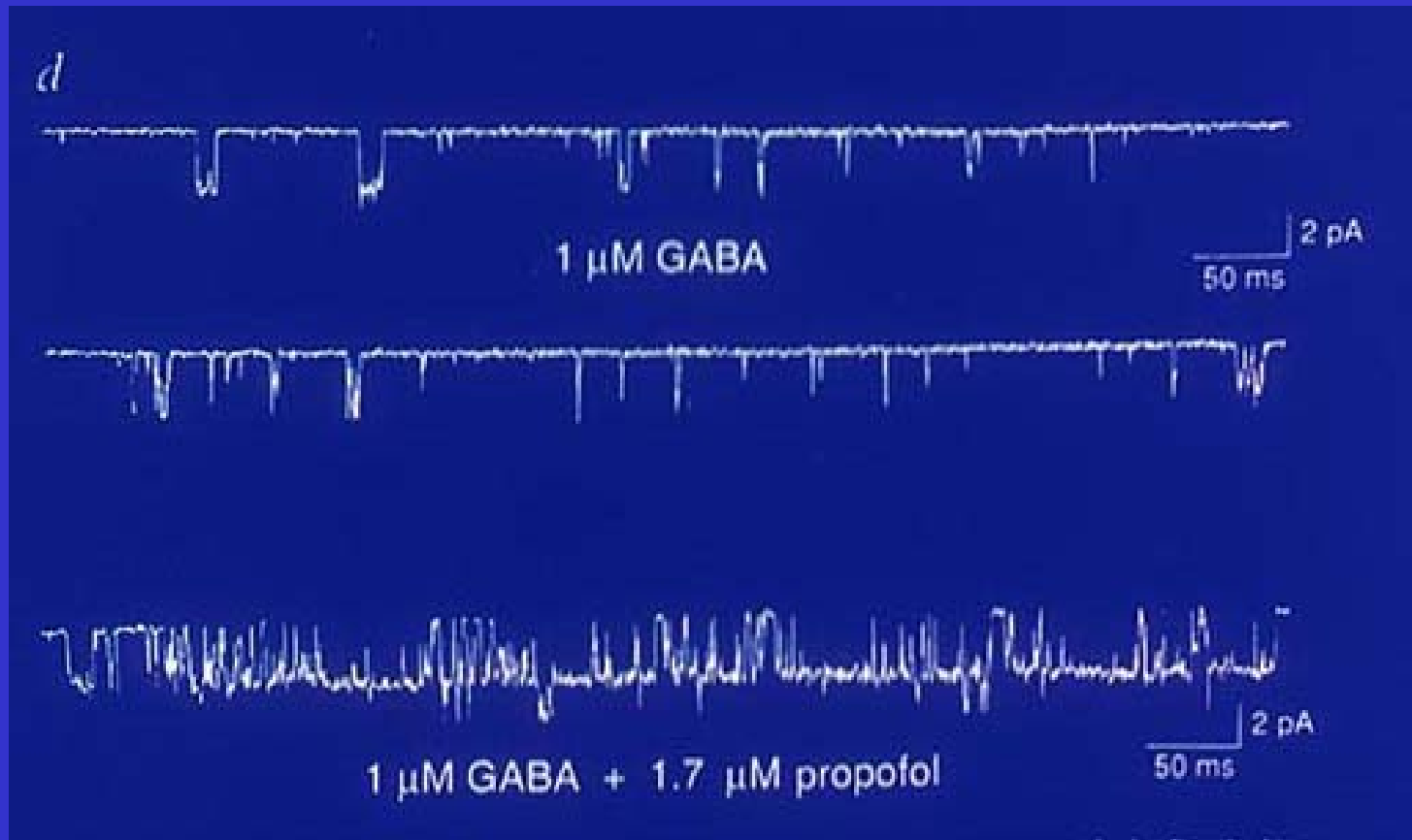
	Racemate	R-(-)	S-(+)
Rat MAC (v/v %)	1.32 ± 0.03	1.62 ± 0.07	1.06 ± 0.02
Mean Arterial Pressure @ MAC (%change)	15 ± 6	32 ± 3	18 ± 3
Heart Rate (%change)	12 ± 1	19 ± 1	9 ± 2

* Data of G. S. Lysko, J. L. Robinson, R. Casto and R. A. Ferrone (Anaqest, BOC Group Inc.)

Stereoselectivity was not observed in anesthetic binding to lipids.



Anesthetics prolong ion channel opening of GABA receptors

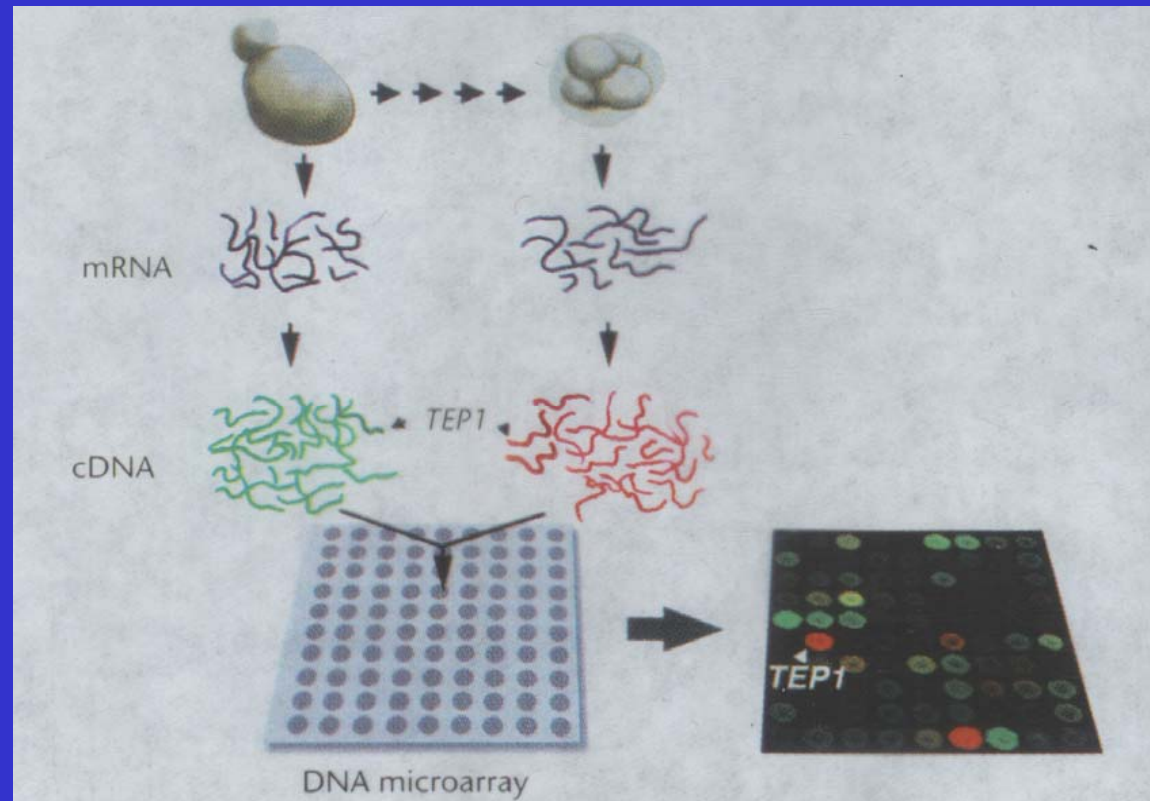
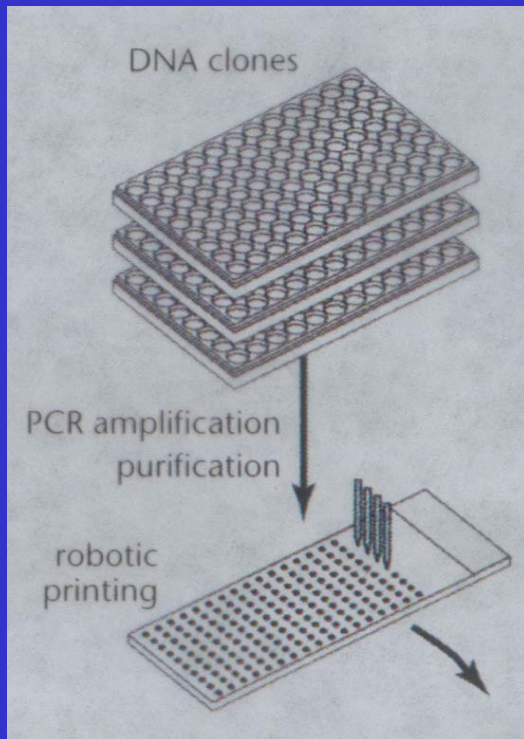


4 Questions , 4 techniques:

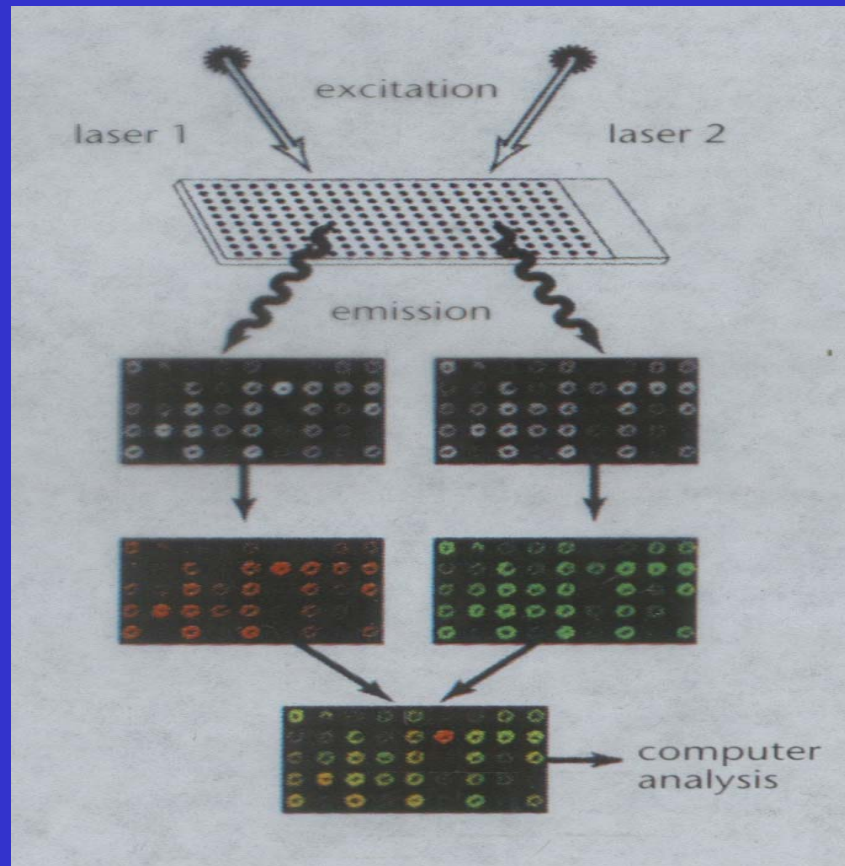
- 1) How is neuronal communication affected by anesthetic agents?
-Anesthetics modulate postsynaptic neurotransmitter-gated currents.
- 2) What family of neuronal receptors is modulated by anesthetics?
-A homologous family of neurotransmitter-gated ion channels are all affected by anesthetic agents.
- 3) **Where do anesthetic molecules bind?**
-**Anesthetic molecules modulate channel activity by binding directly to channel proteins not to the lipid bilayer.**
- 4) What are long-term side effects of anesthetics on neuronal tissue?

4) Microarray Technology

Screening of whole or partial genomes for differential gene expression between tissues.



Laser scanners read relative levels of fluorochromes (dyes) for differential gene expression analysis.



Future Questions for Research at Smith College:

1) What neuronal genes are differentially expressed after exposure to anesthetic/sedative/anxiolytic agents?

Use DNA microarray experiments on custom-made or neurobiology-focused arrays to monitor long-term changes in neuronal gene expression.

2) What receptor subunits are important for anesthetic action?

Measure anesthetic sensitivities in various cultured cell-lines transfected with different combinations of receptor subunits.

3) What specific regions on channel proteins enable anesthetic binding?

Record from mutant channels (e.g. site-directed mutagenesis) expressed in *Xenopus* oocytes.

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