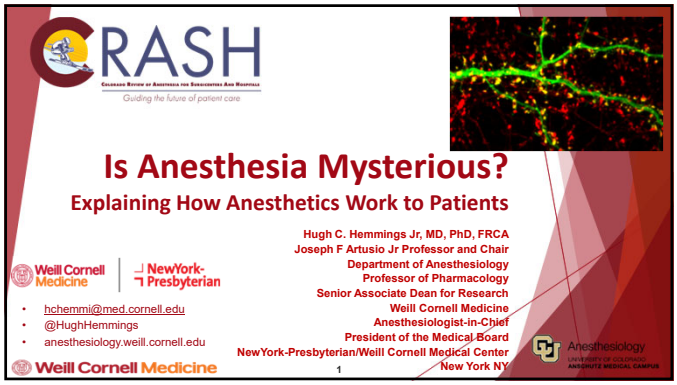
CRASH
Guiding the future of patient care
Is Anesthesia Mysterious?
Explaining How Anesthetics Work to Patients
Hugh C. Hemmings Jr, MD, PhD, FRCA
Joseph F Artusio Jr Professor and Chair
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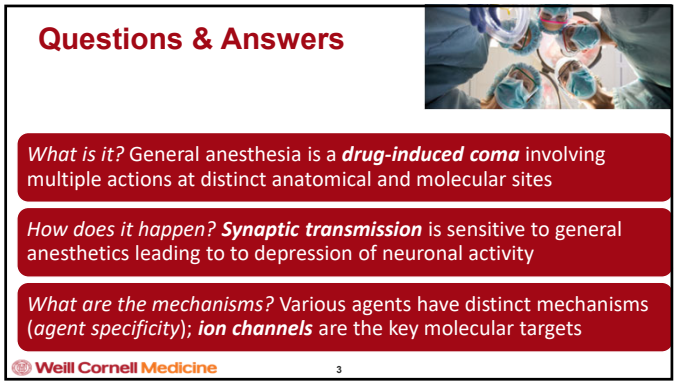
1

1


Declaration of interests
Editor-in-Chief of the British Journal of Anaesthesia
Research funding from the National Institutes of Health
BJA
Weill Cornell Medicine
Anesthesiology
NIH
National Institutes of Health
Turning Discovery Into Health

2


Questions & Answers
What is it? General anesthesia is a drug-induced coma involving multiple actions at distinct anatomical and molecular sites
How does it happen? Synaptic transmission is sensitive to general anesthetics leading to to depression of neuronal activity
What are the mechanisms? Various agents have distinct mechanisms (agent specificity); ion channels are the key molecular targets
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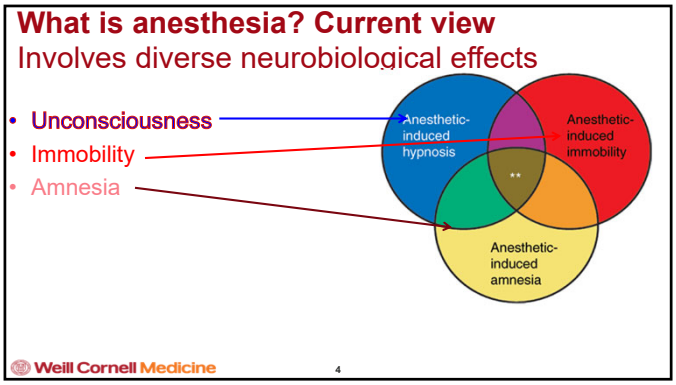
3


What is anesthesia? Current view
Involves diverse neurobiological effects
Unconsciousness
Immobility
Amnesia
Anesthetic-induced hypnosis
Anesthetic-induced immobility
Anesthetic-induced amnesia
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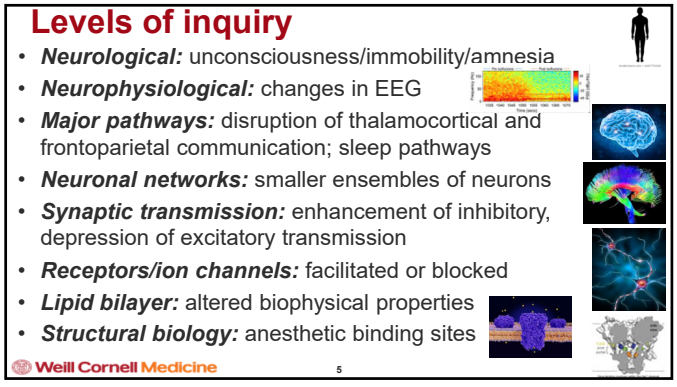
4


Levels of inquiry
Neurological: unconsciousness/immobility/amnesia
Neurophysiological: changes in EEG
Major pathways: disruption of thalamocortical and frontoparietal communication; sleep pathways
Neuronal networks: smaller ensembles of neurons
Synaptic transmission: enhancement of inhibitory, depression of excitatory transmission
Receptors/ion channels: facilitated or blocked
Lipid bilayer: altered biophysical properties
Structural biology: anesthetic binding sites
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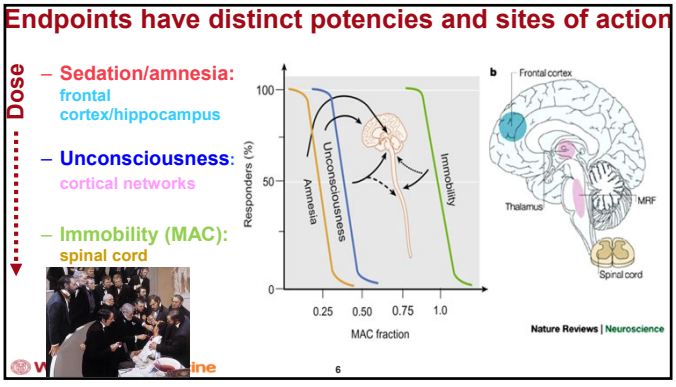
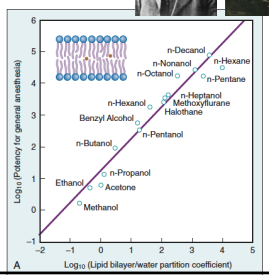

Endpoints have distinct potencies and sites of action
Dose
Sedation/amnesia:
frontal cortex/hippocampus
Unconsciousness:
cortical networks
Immobility (MAC):
spinal cord
Responders (%)
100
50
0
Amnesia
Unconsciousness
Immobility
0.25
0.50
0.75
1.0
MAC fraction
Frontal cortex
Thalamus
MRF
Spinal cord
Nature Reviews | Neuroscience
6

6

Meyer-Overton Correlation

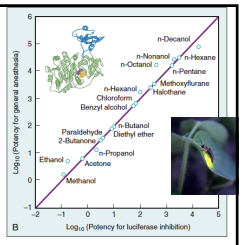
- Early concepts dominated by Meyer-Overton correlation of anesthetic potency with oil solubility/lipophilicity (1899/1901)
- Led to nonspecific lipid-based hypothesis
- Accounts for chemical diversity, ***but***…
- Not supported by biophysical studies seeking changes in membrane properties

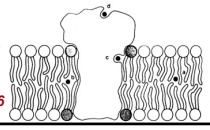


7

Protein target hypothesis

- Similar correlation of luciferase inhibition with lipid solubility
- Shifted focus to protein sites of anesthetic action in 1980s
- Anesthetics bind in water-filled hydrophobic protein cavities
- Selective stabilization of specific protein conformations to alter function
- Focus on receptors/ion channels





Franks, 2006

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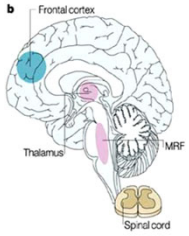
Pharmacological criteria for identification of relevant targets

- Low anesthetic potency (mM) results in low specificity; necessitates criteria to distinguish relevant targets



- Acute and ***reversible*** alteration of target function – ***reversibility***
- ***Clinically relevant concentrations*** – pharmacological ***sensitivity***
- Target is expressed in ***appropriate anatomical locations*** to mediate the specific behavioral effect – ***plausibility***

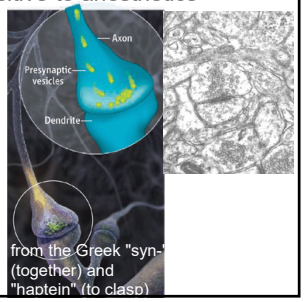
9

9

The **synapse:** principal neuronal site of anesthetic action

Synaptic transmission is more sensitive to anesthetics than is axonal conduction

- **Sherrington & Sowton (BMJ 1905)**
- **Larrabee & Posternak (1952)**



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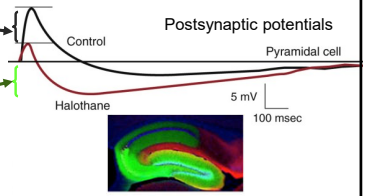
Opposing actions on *inhibitory* and *excitatory* synaptic transmission

Volatile anesthetics:

Depress excitatory synaptic transmission glutamatergic

Facilitate inhibitory synaptic transmission (and tonic inhibition) GABAergic



What are the mechanisms for these opposite effects?

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Nishikawa & MacIver 2000

11

Putative molecular targets for neurobiological effects

- $GABA_A$ receptors* (inhibitory)
- NMDA and AMPA glutamate receptors* (excitatory)
- Neuronal nicotinic acetylcholine receptors
- Two-pore domain (K_{2P}) and ATP-sensitive (K_{ATP}) K^+ channels
- Voltage-gated cation channels (Na^+, Ca^{2+}, K^+, HCN)

Ligand-gated and voltage-gated ion channels are the most important targets

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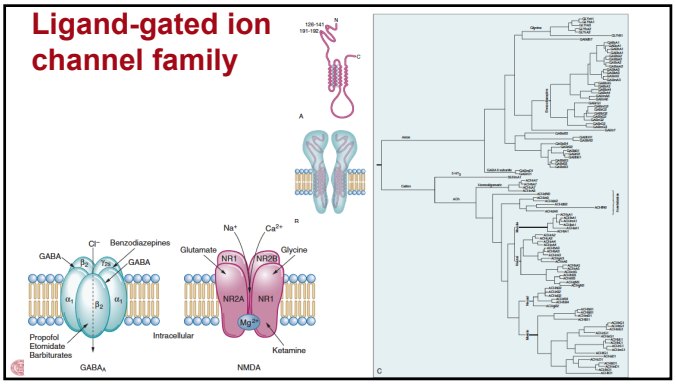
Ligand-gated ion channel family



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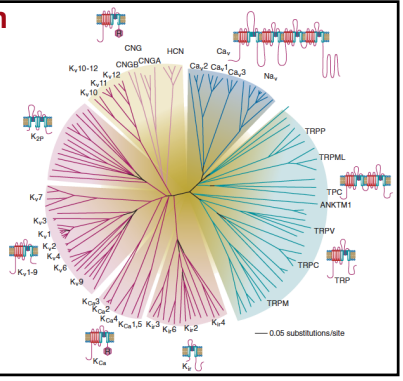
Voltage-gated ion channel family



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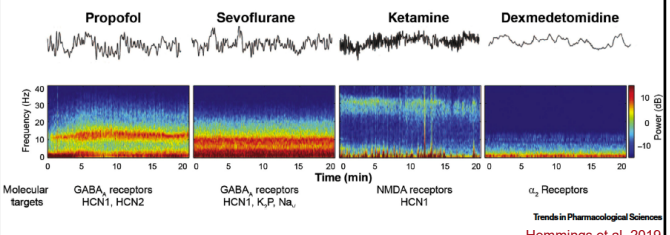
Agent specificity for molecular targets:

Different primary targets between major anesthetic classes



Hemmings et al. 2019

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Agent specificity for molecular targets:

Different primary targets between major anesthetic classes

Potent inhaled (volatile) anesthetics: promiscuous with multiple targets	• Ligand-gated ion channels: GABA$_A$, glycine, NMDA, AMPA, neuronal nicotinic acetylcholine • Ion channels: K$_{2P}$, HCN, Na$^+$, Ca^{2+}, K$^+$
Gaseous inhaled anesthetics: less promiscuous (N$_2$O, Xe, cyclopropane)	• Block NMDA and activate K$_{2P}$ channels • Inactive at GABA$_A$ receptors
Intravenous anesthetics: relatively selective	• Propofol and etomidate potentiate GABA$_A$ receptors • Ketamine blocks NMDA receptors and HCN1 channels

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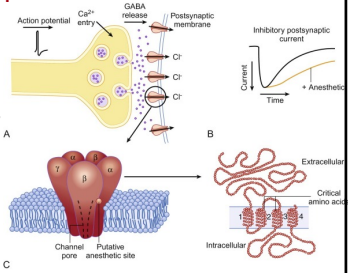
Synaptic GABA$_A$ receptors: potentiation of phasic and tonic inhibition

- Strongest evidence for any molecular target
- GABA is the major inhibitory neurotransmitter
- GABA$_A$ receptors are allosterically modulated by both volatile and intravenous anesthetics
- Potentiate fast inhibitory *phasic* transmission, prolongs the IPSC
- *Tonic* activation of highly sensitive extrasynaptic channels



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Hemmings et al., 2013

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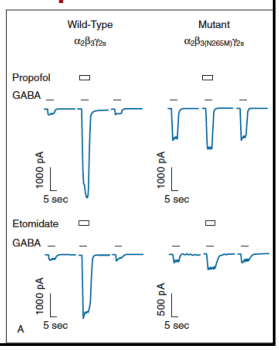
Pharmacology of GABA$_A$ receptor potentiation by propofol

- Site-directed mutagenesis used to create insensitive receptors
- Single point mutation in β$_3$ subunit (N265M) markedly reduces propofol sensitivity
- Suggests a specific binding site



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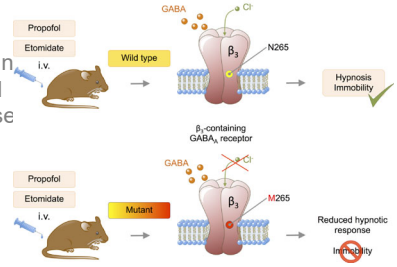
$GABA_A$ receptors are critical to intravenous anesthetic action – genetic evidence

- Knock-in mutation in $GABA_A$ receptor β_3 subunit abolishes *etomidate* and *propofol* induced increase in Cl^- flux *in vitro* **and**….
- Prevents immobilizing response in mice *in vivo*
- Reduced hypnotic response *in vivo*



Jurd et al., 2005

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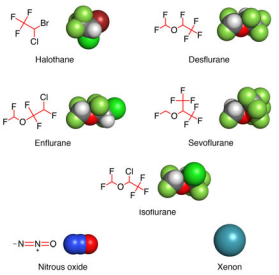
What are the target(s) for immobilization by volatile anesthetics



- **Not mediated by $GABA_A$ receptors**
  - No effect of anesthetic-resistant α_1 or α_2 knock-in mice
  - No effect of intrathecal $GABA_A$ receptor antagonists (gabazine, bicuculline)
- **Importance of other targets**
  - Reduction of excitatory transmission?

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Contrasting effects of anesthetics on excitatory *vs.* inhibitory transmission

- **Facilitate** inhibitory synaptic transmission
  - Postsynaptic and extrasynaptic actions at $GABA_A$ receptors (major for IV agents)
- **Depress** excitatory synaptic transmission
  (prominent for volatile anesthetics)…
  - *Presynaptic:* inhibition of neurotransmitter release
  - *Postsynaptic:* inhibition of NMDA and AMPA glutamate receptors

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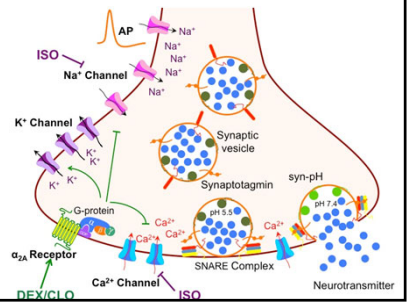
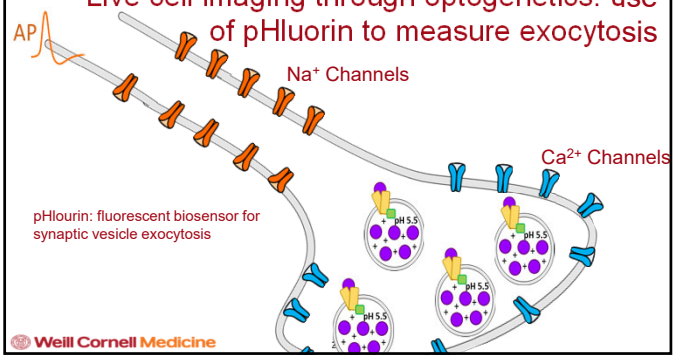
Multiple putative targets for anesthetic effects on excitatory transmission



22

Live cell imaging through optogenetics: use of pHluorin to measure exocytosis



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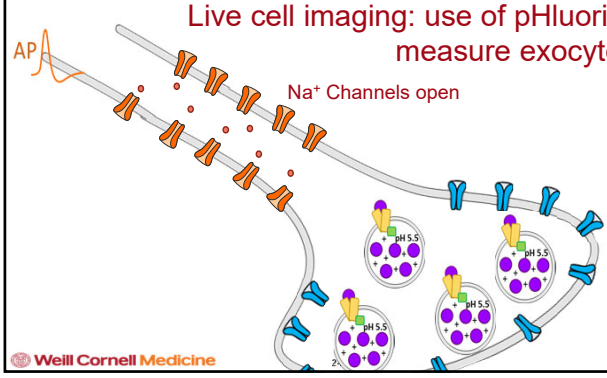
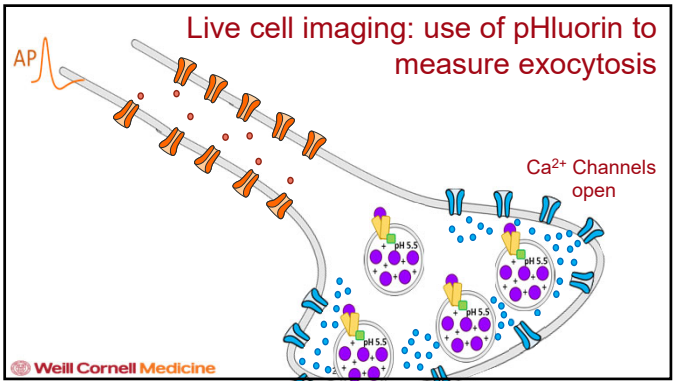
Live cell imaging: use of pHluorin to measure exocytosis



24

Live cell imaging: use of pHluorin to measure exocytosis



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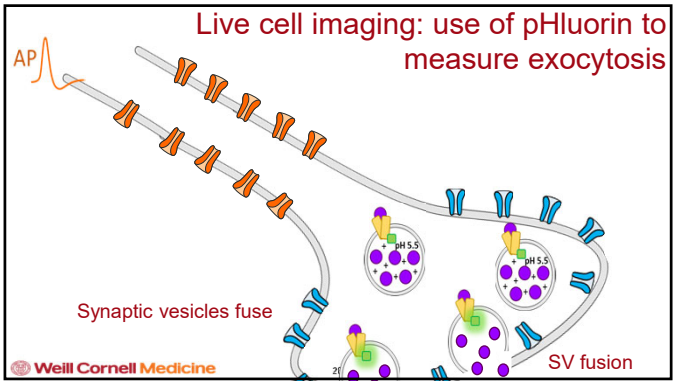
Live cell imaging: use of pHluorin to measure exocytosis



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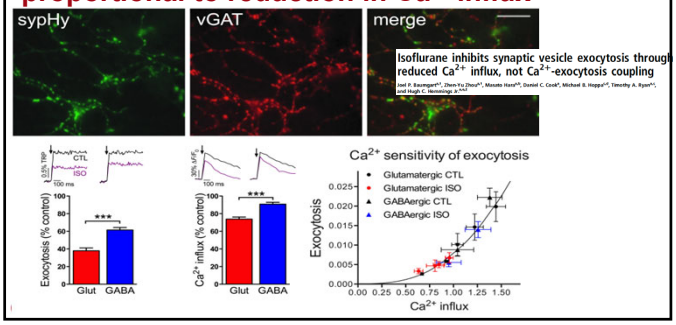
Isoflurane inhibition of exocytosis is proportional to reduction in Ca^{2+} influx



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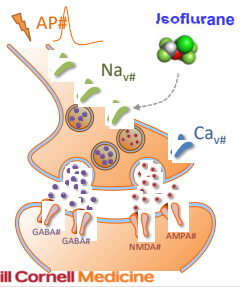
Volatile anesthetic effects on voltage-gated sodium channels



- Multiple target hypothesis of volatile anesthetics
  - Enhance ***inhibitory***
  - Depress ***excitatory*** neurotransmission
- Block Na_v at clinical concentrations
  - Isolated nerve terminal preparations
  - Recombinant Na_v in mammalian cell lines
- **What about *in vivo*?**

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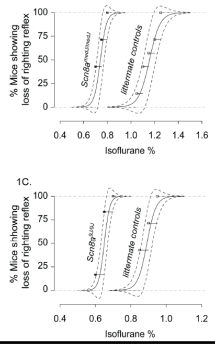
Is there a role of Na^+ channels in general anesthesia *in vivo*?

- Mutant mice with reduced expression of $Na_v1.6$
- Show increased sensitivity to isoflurane and sevoflurane

Reduced $Na_v1.6$ Sodium Channel Activity in Mice Increases *In Vivo* Sensitivity to Volatile Anesthetics

Dinesh Pal[1,2], Julie M. Jones[3], Stella Wisidagamage[1], Miriam H. Meisler[3]*, George A. Mashour[1,2,4]*



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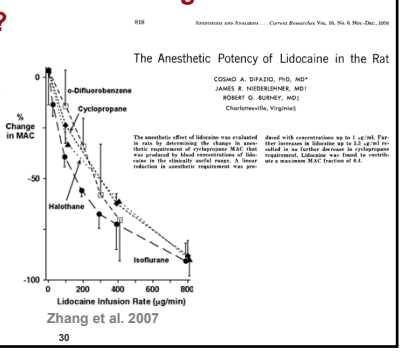
Is there a role of Na^+ channels in general anesthesia *in vivo*?

- Lidocaine reduces MAC for inhaled anesthetics up to 90% in vivo (Eger, DiFazio)
- MAC, a measure of the immobilization, is mediated in the spinal cord (Antognini, Rampil)
- Are spinal cord Na^+ channels involved in immobilization?



Zhang et al. 2007

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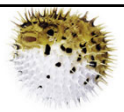
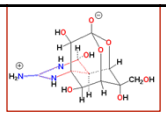
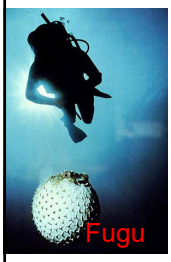
Tetrodotoxin







- Extremely selective and potent Na^+ channel blocker toxin from *Fugu*
- 1200 times more potent than cyanide
  - **One fish has enough toxin to kill 30 people**
- Cannot be served to Japanese Emperor
- Predicted to ***reduce*** MAC

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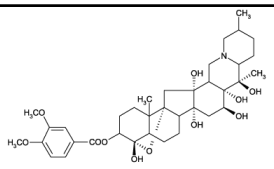
Veratridine



Hellebore

- Selective and potent Na^+ channel activator
- Used by Native Americans for poison arrows
- Predicted to ***increase*** MAC

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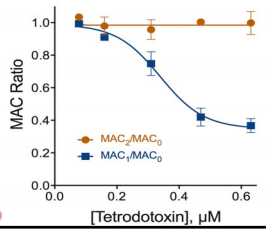
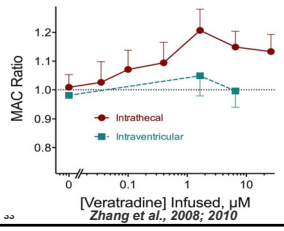
Opposing effects of Na^+ channel modulators on MAC

- Intrathecal tetrodotoxin reduces isoflurane MAC
- Intrathecal, *not* intracerebro-ventricular, veratradine increases isoflurane MAC





Zhang et al., 2008; 2010

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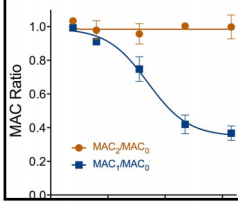
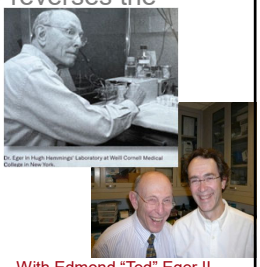
Opposing effects of Na^+ channel modulators on MAC

- Intrathecal veratridine completely reverses the effect of intrathecal tetrodotoxin





With Edmond "Ted" Eger II

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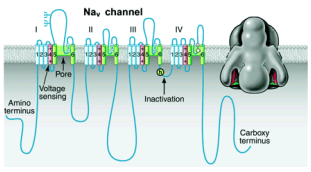
In vivo pharmacological evidence for a role of Na^+ channels in anesthesia

- Specific Na^+ channel blocker (tetrodotoxin) reduces MAC
- Specific Na^+ channel activator (veratridine) increases MAC
- Role for volatile anesthetic block of spinal Na^+ channels in immobilization



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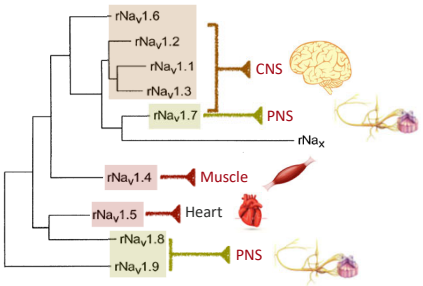
Mammalian Na^+ channel subtypes



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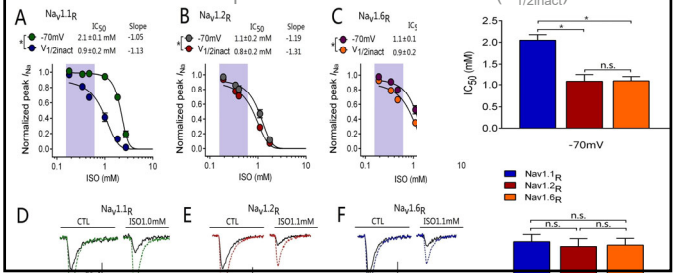
Inhibition of peak Na⁺ current by isoflurane is subtype dependent

- Higher IC_{50} value of $Na_V1.1$ at normal resting membrane potential
- No difference at equivalent levels of inactivation ($V_{1/2inact}$)



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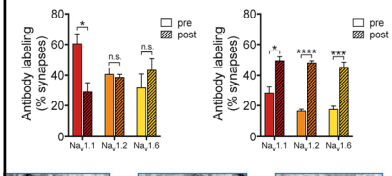
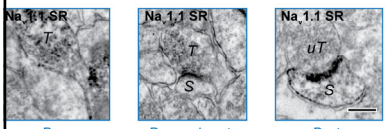
Asymmetric distribution of Na_V subtype immunoreactivity in rat hippocampal synapses



Na_V 1.1 immunoreactivity is more presynaptic in central hilum, and all Na_V subtypes are more postsynaptic in stratum radiatum



Pre-synaptic | Pre- and post-synaptic | Post-synaptic

RESEARCH ARTICLE

WILEY The Journal of Comparative Neurology

Sodium channel subtypes are differentially localized to pre- and post-synaptic sites in rat hippocampus

Kenneth W. Johnson[1] | Karl F. Herold[2] | Teresa A. Milner[2,4]

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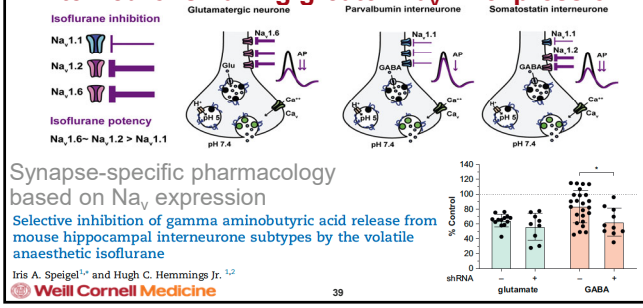
Isoflurane selectively inhibits GABAergic interneurons having greater $Na_V1.2$ expression



Synapse-specific pharmacology based on Na_V expression

Selective inhibition of gamma aminobutyric acid release from mouse hippocampal interneurone subtypes by the volatile anaesthetic isoflurane

Iris A. Speigel[1,*] and Hugh C. Hemmings Jr. [1,2]

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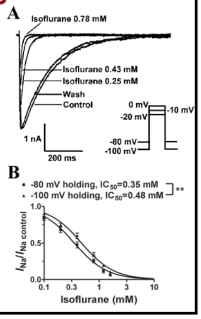
Isoflurane inhibits the bacterial channel NaChBac at clinical concentrations

- Bacterial ion channels are more amenable to structural studies
- Potency (IC_{50}) comparable to mammalian Na_V
- Potential model for structure-function studies



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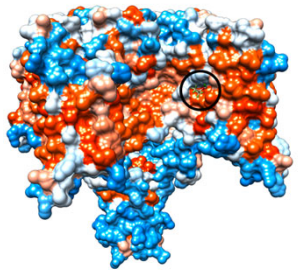
Binding site for sevoflurane in NavMs by x-ray crystallography



- Studies underway with NMR and x-ray crystallography of NavMs
- Crystal structure showing bound sevoflurane (black circle) in the S5 helix region between the voltage sensor and pore domains

With Bonnie Wallace and Dave Hollingworth, Birkbeck College, UCL

Weill Cornell Medicine

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Anesthesia-induced unconsciousness?

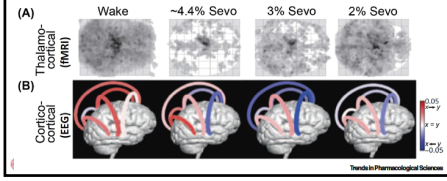
- Understanding limited by consciousness itself being an unsolved problem
- Reduction in thalamocortical *connectivity*
- Reduction in anterior-posterior cortico-cortical information transfer

Feature Review

Towards a Comprehensive Understanding of Anesthetic Mechanisms of Action: A Decade of Discovery

Hugh C. Hemmings Jr.[1,2] Paul M. Riegelhaupt,[1] Max B. Kelz,[3] Ken Solt,[4,5] Roderic G. Eckenhoff,[3] Beverley A. Orser,[6] and Peter A. Goldstein[7,8,*]

Trends in Pharmacological Sciences, July 2019, Vol. 40, No. 7 https://doi.org/10.1016/j.tips.2019.05.001



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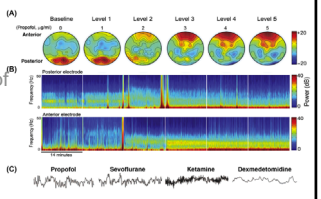
EEG correlates of loss of consciousness

- EEG a biomarker of unconsciousness
- Similarities between agents in unprocessed EEG (C)
- Occipital to frontal anteriorization of alpha frequencies (8-12 Hz) with increasing doses (A-B)
- Due to increased inhibitory conductance anteriorly and reduced thalamocortical input
- Inhibition of sodium channels could disrupt neuronal communication



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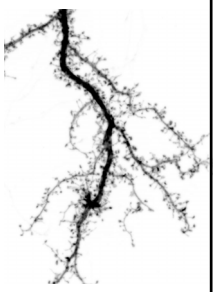
Summary points

- General anesthesia is a composite of pharmacological effects involving distinct sites and molecular targets
- General anesthetics interact with ion channels to enhance inhibition/depress excitation
- Synaptic actions reduce connectivity to alter integration of higher-level network functions to produce unconsciousness



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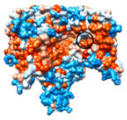
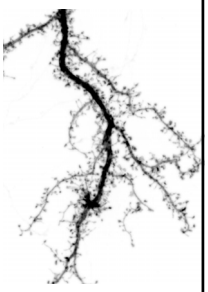
Summary points

- Isoflurane has neurotransmitter selective presynaptic effects on SV exocytosis
- Voltage-gated Na^+ channels are plausible presynaptic targets for volatile anesthetics
- Structural models are beginning to reveal binding sites on Na^+ channels





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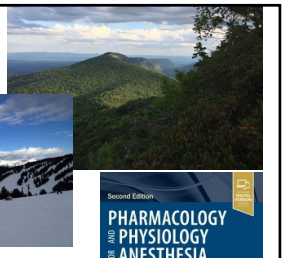

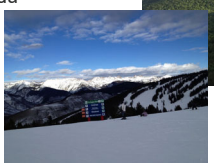

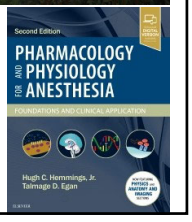

Trends in Pharmacological Sciences

Feature Review

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Highly Commended BMA Award 2019

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