

# *Anesthesia*



Christian Oswood  
MacMillan Lab group meeting  
April 27th, 2020

# Outline

## ■ Definitions and general principles

- What is anesthesia?
- History
- How do nerves transmit signals?

## ■ Local anesthesia

- Methods of administration
- Commonly-used compounds
- Mechanism of action

## ■ General anesthesia

- Methods of administration
- Commonly-used compounds
- Mechanism of action
  - Lipid hypothesis
  - Protein hypothesis

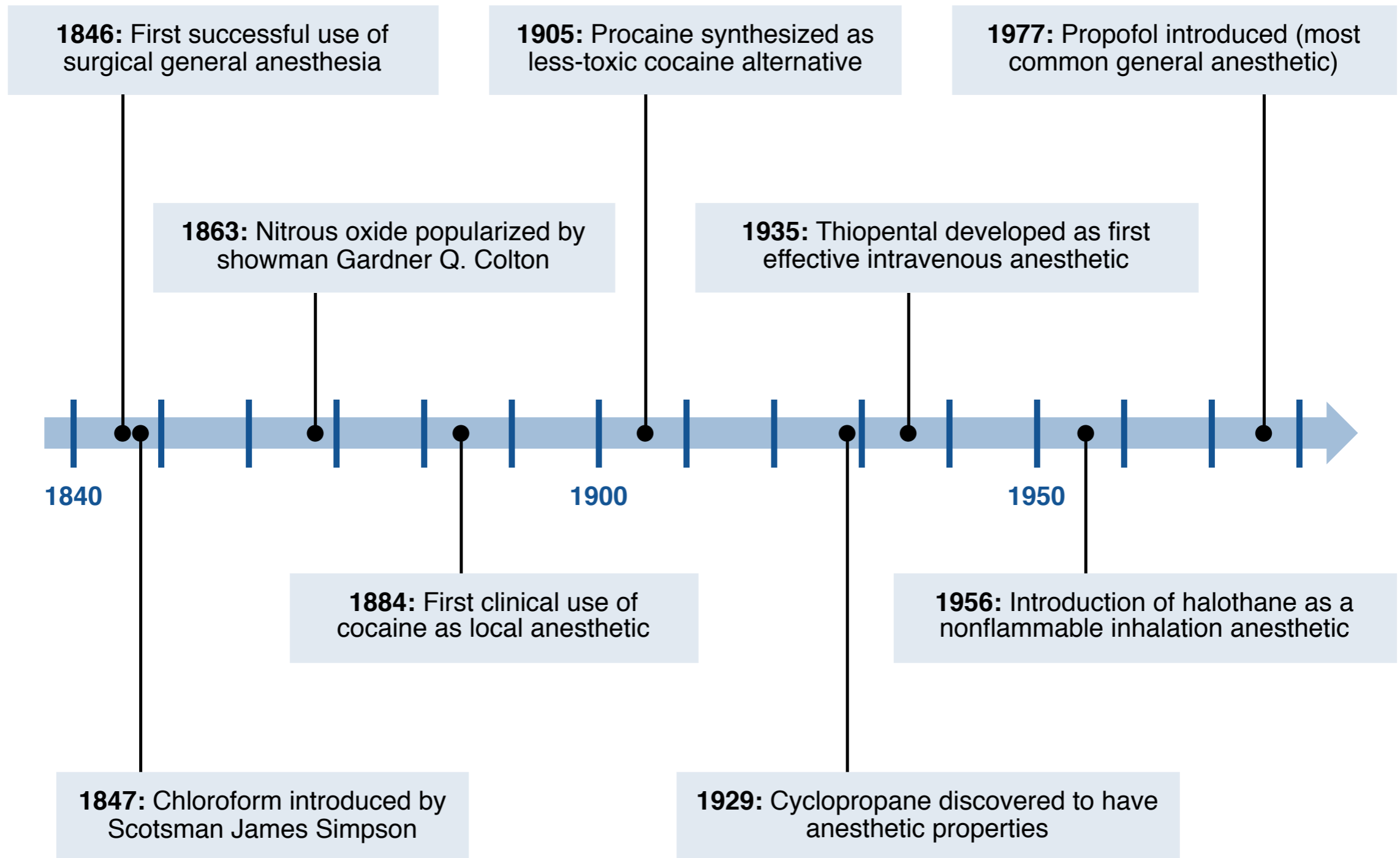
## *What is anesthesia?*

*Anesthesia is the loss of sensation or awareness of either the whole body or part of it, typically for purposes of performing an otherwise painful or unpleasant procedure.*

### ***Two main categories:***

- Local anesthesia: affects only part of the body, local nerves
- General anesthesia: full body, depresses CNS and consciousness

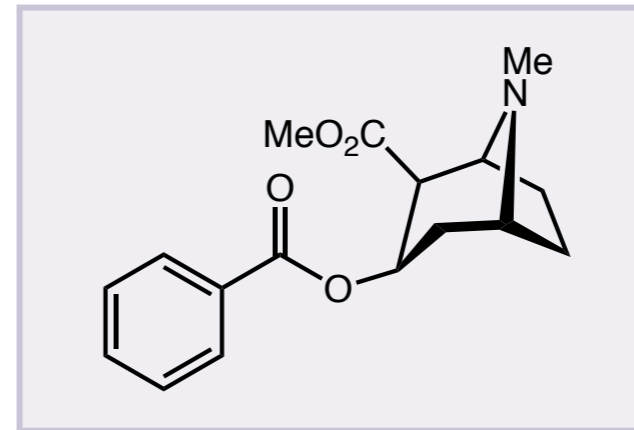
# History of anesthesia



## Freud and cocaine



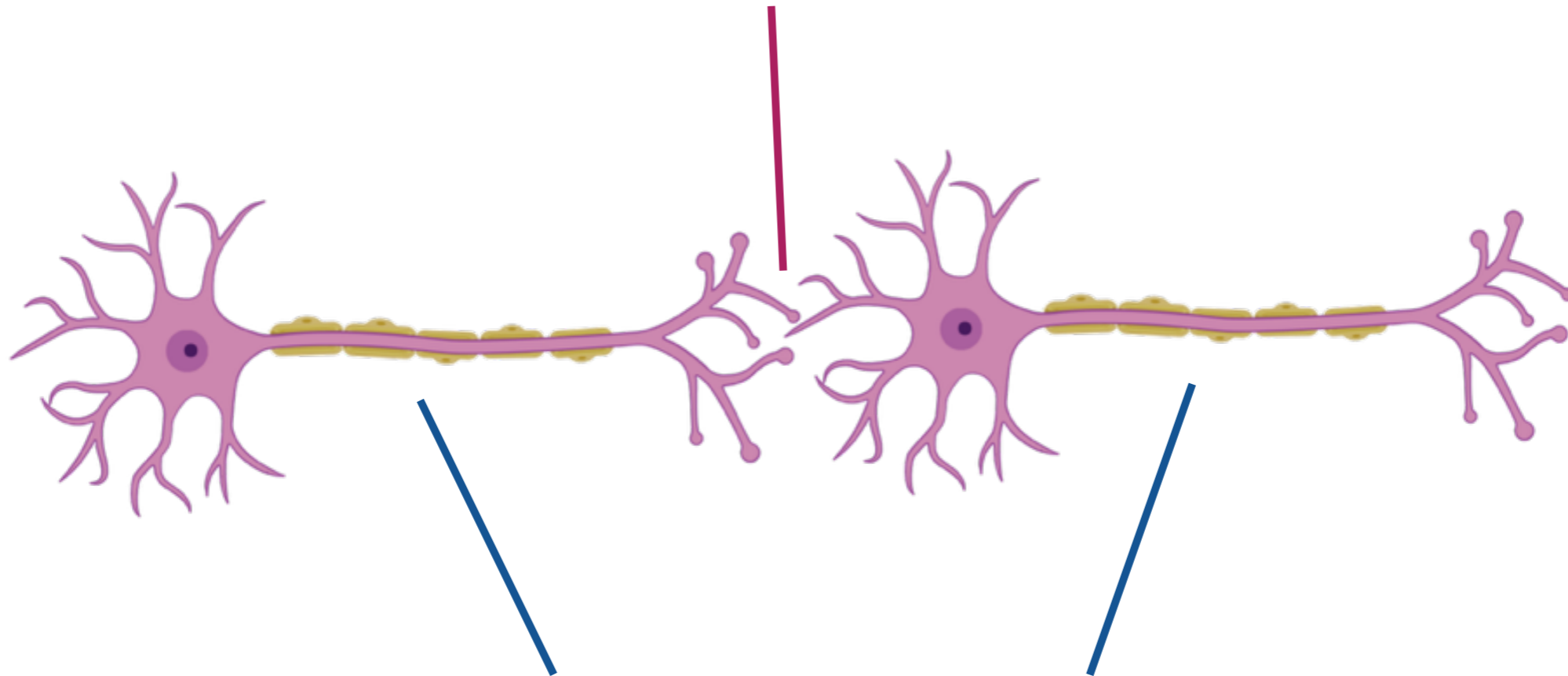
Sigmund Freud  
(1856-1939)



- German neurologist and founder of psychoanalysis
- Less well known: major proponent of cocaine for anesthetic and other uses
  - Also recommended as a stimulant, a treatment for indigestion, depression, morphine withdrawal, asthma, and an aphrodesiac
- First noted its effectiveness in anesthesia, but was beaten to publication by Karl Koller
- Therapeutic use strongly opposed by Erlenmeyer: called it “the third scourge of humanity”

# *How do nerves transmit signals?*

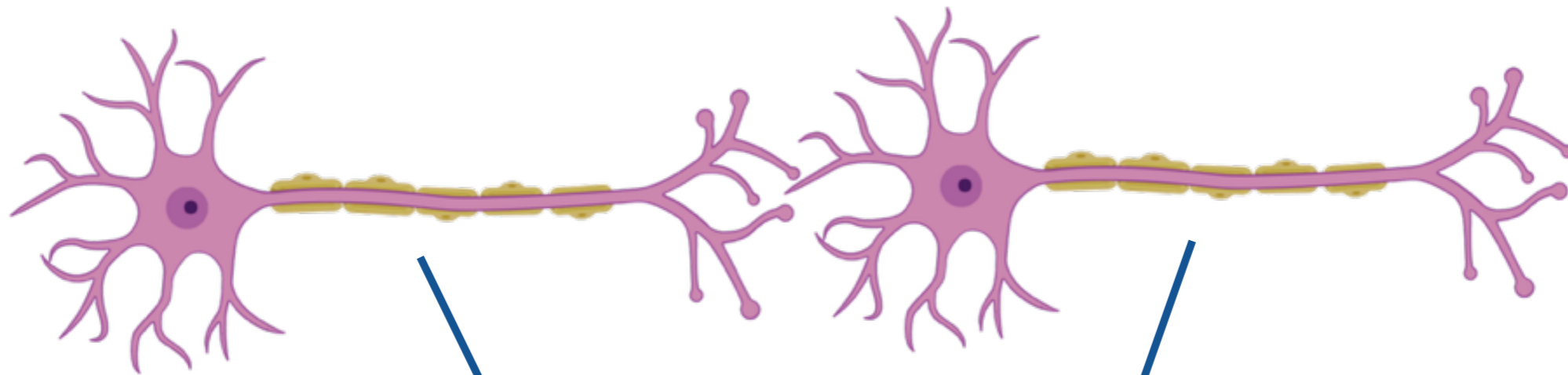
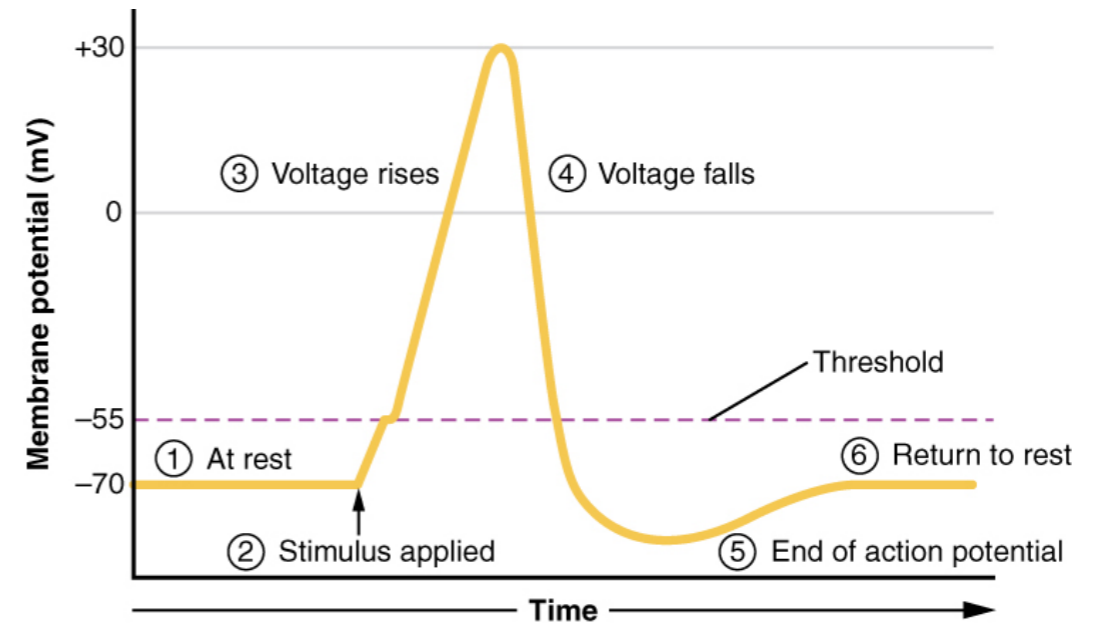
**Signals are transmitted between neurons by release and reception of neurotransmitters in synapses**



**Signals are transmitted along axons by changes in the voltage gradient across the membrane**

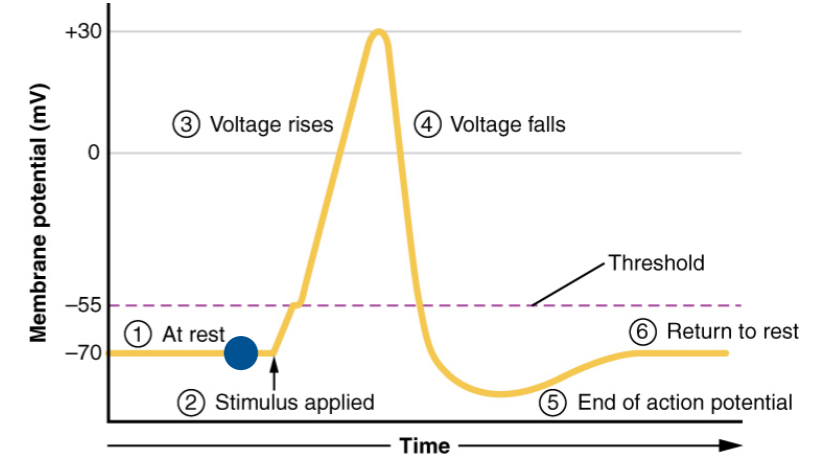
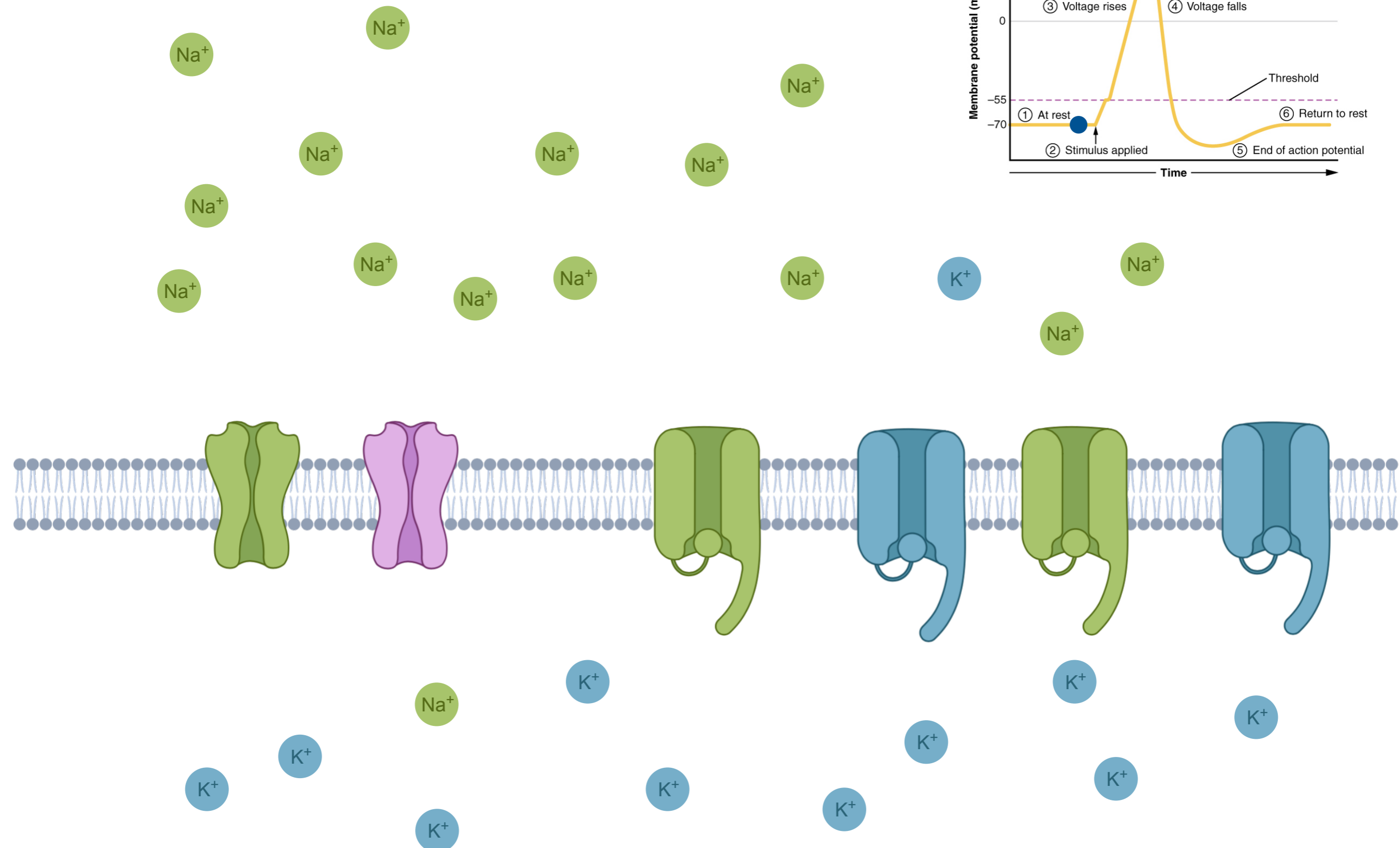
# How do nerves transmit signals?

*Change in voltage occurs in a predictable pattern called the action potential*



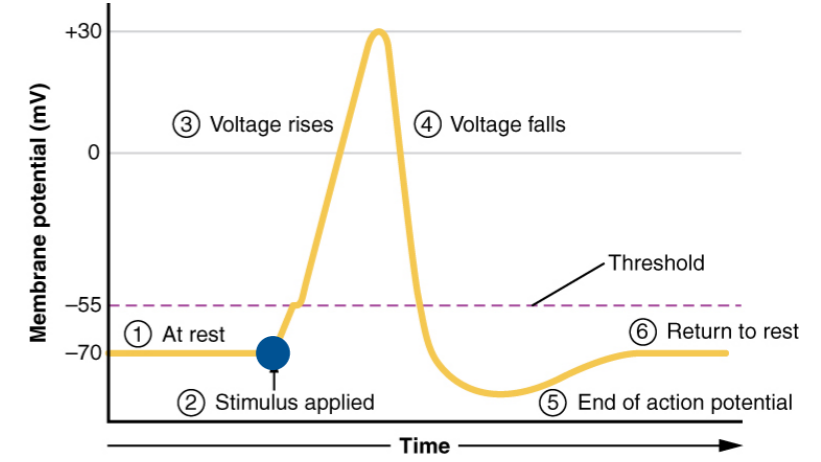
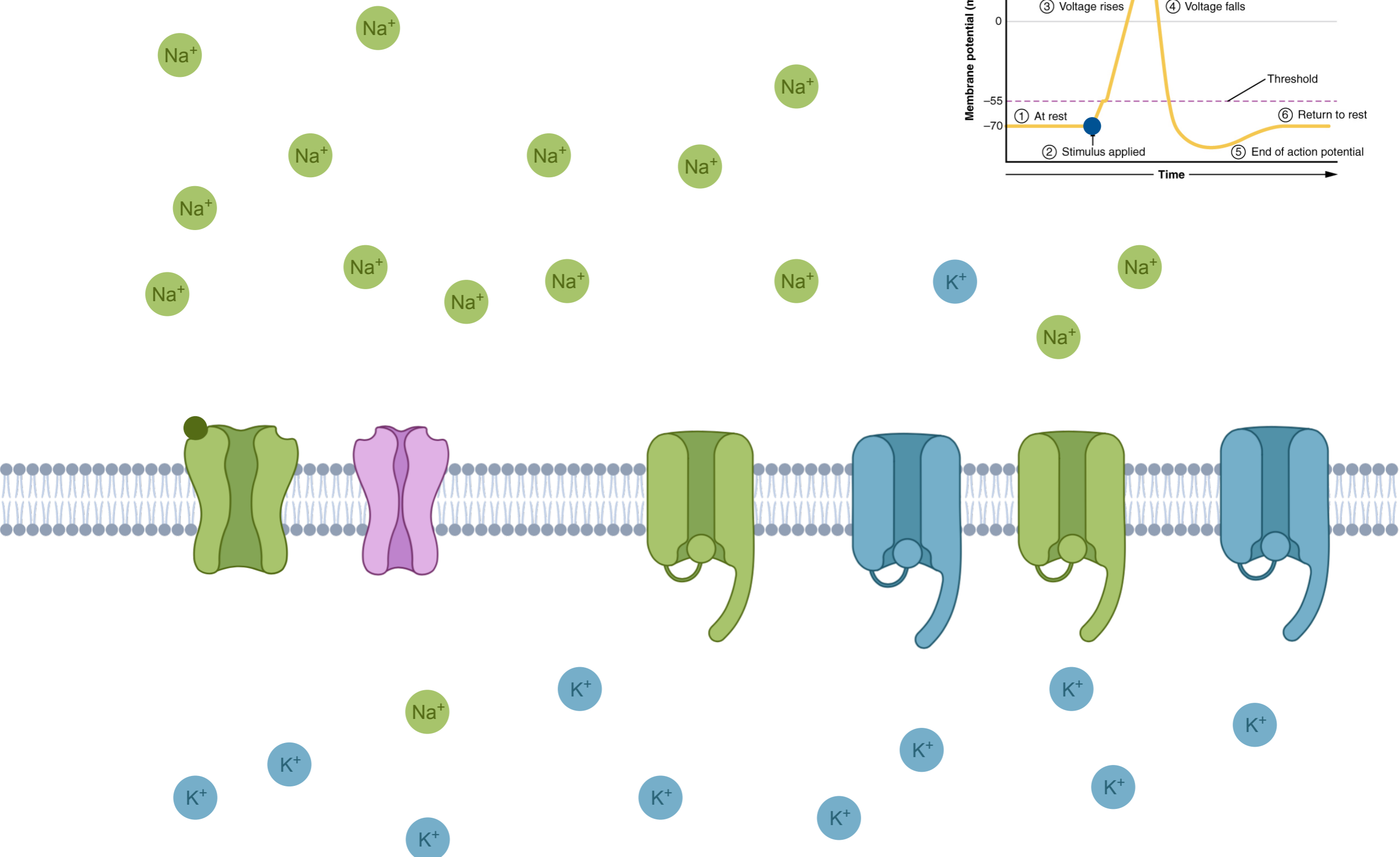
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# How do nerves transmit signals?

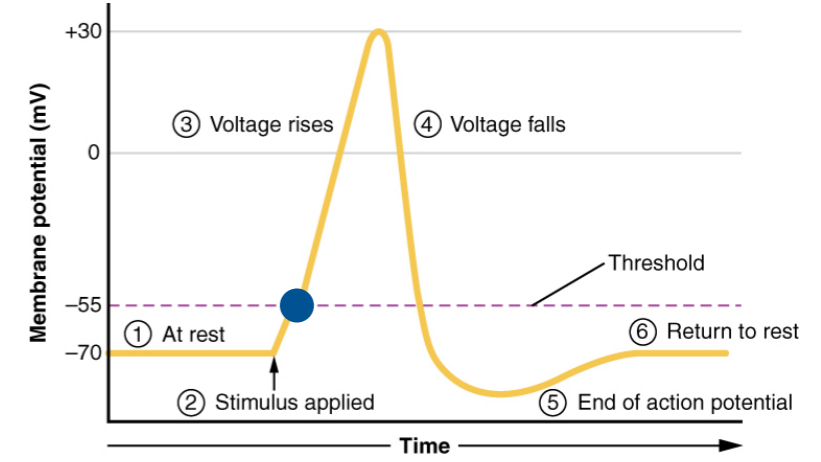
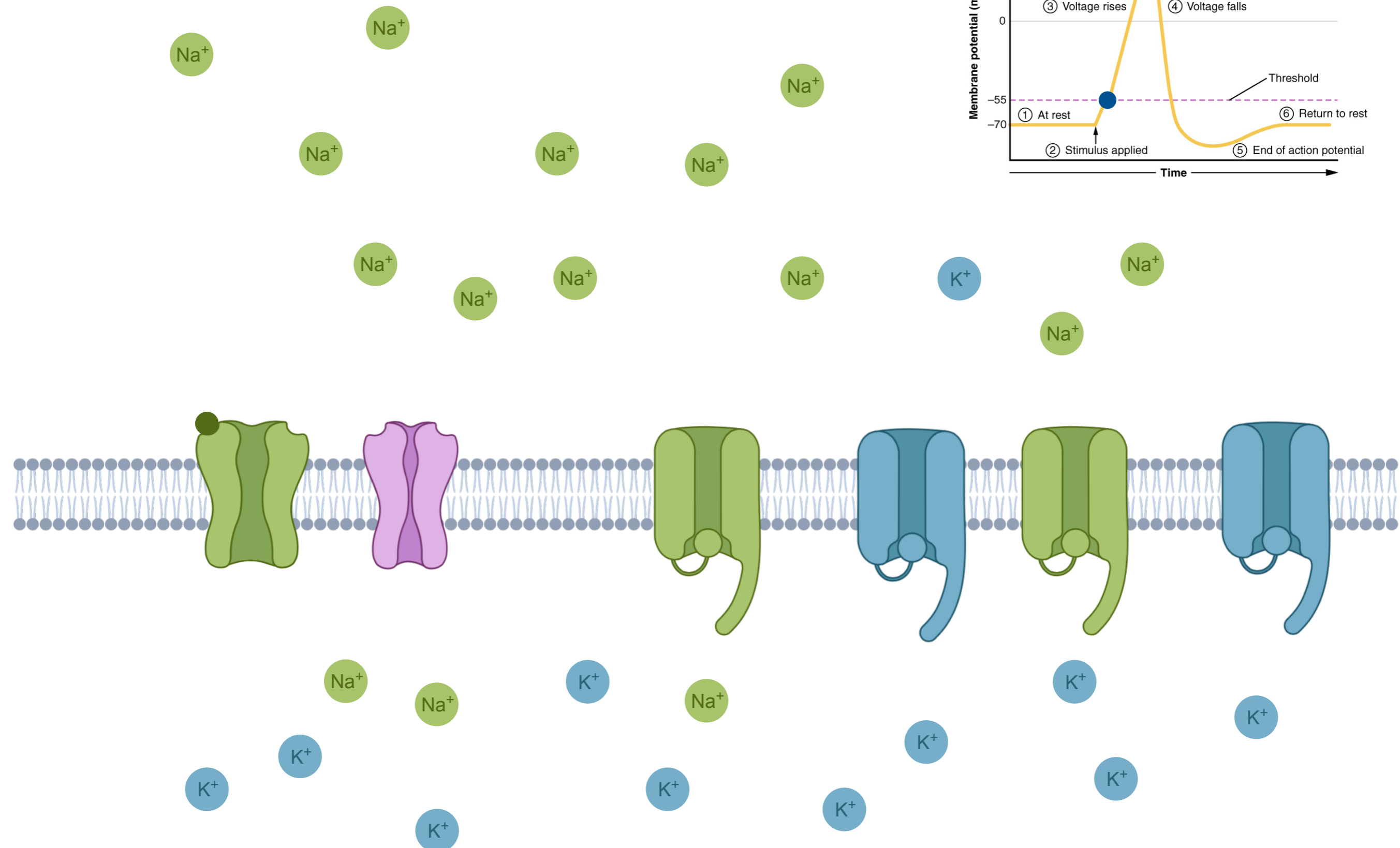




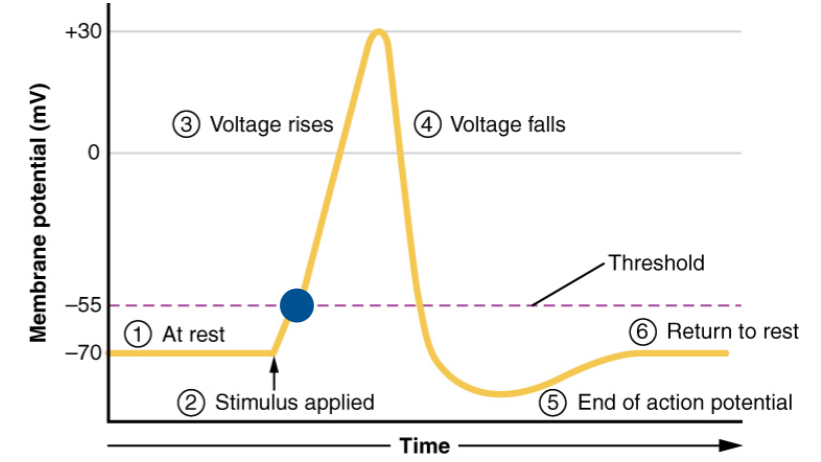
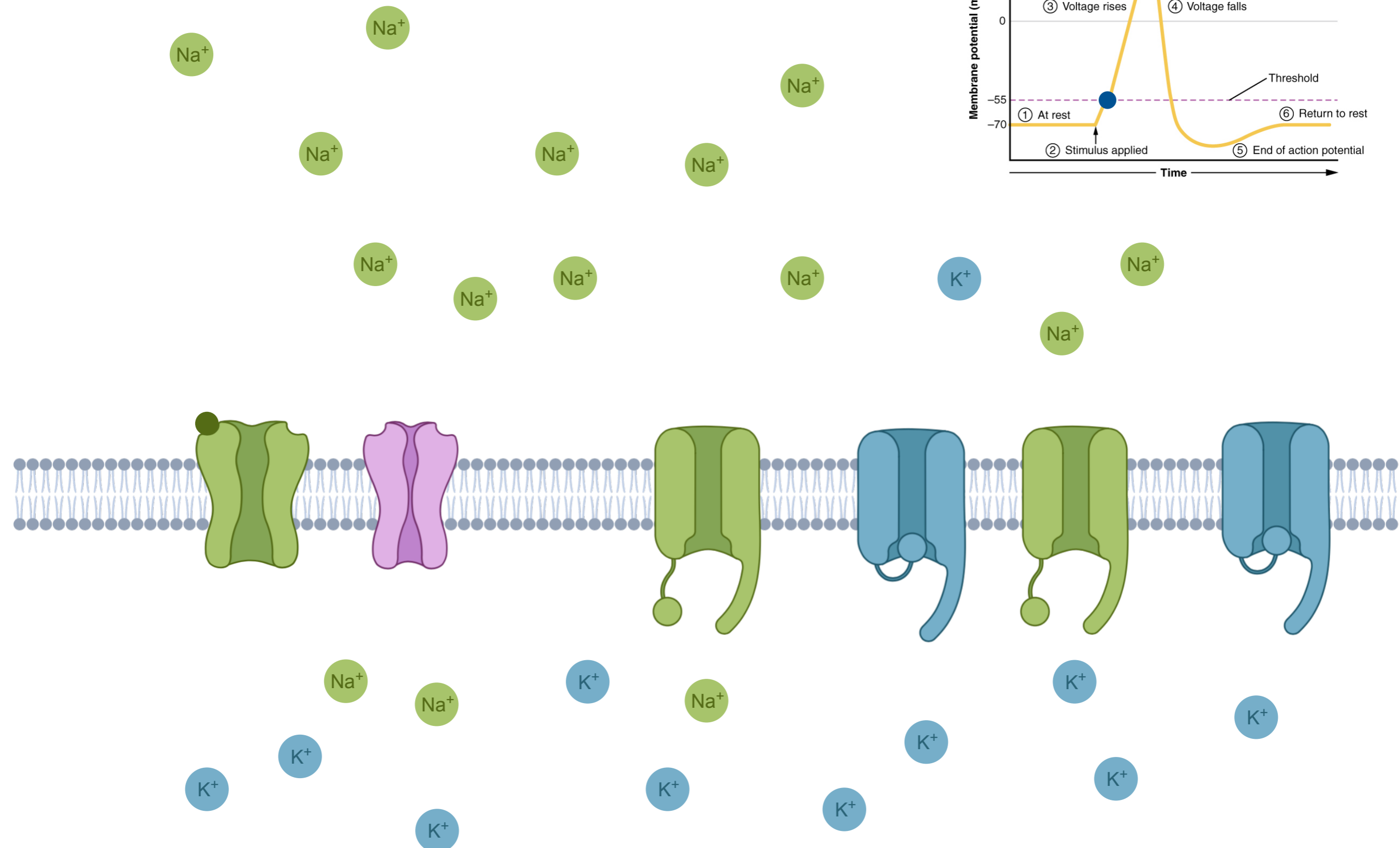
# How do nerves transmit signals?



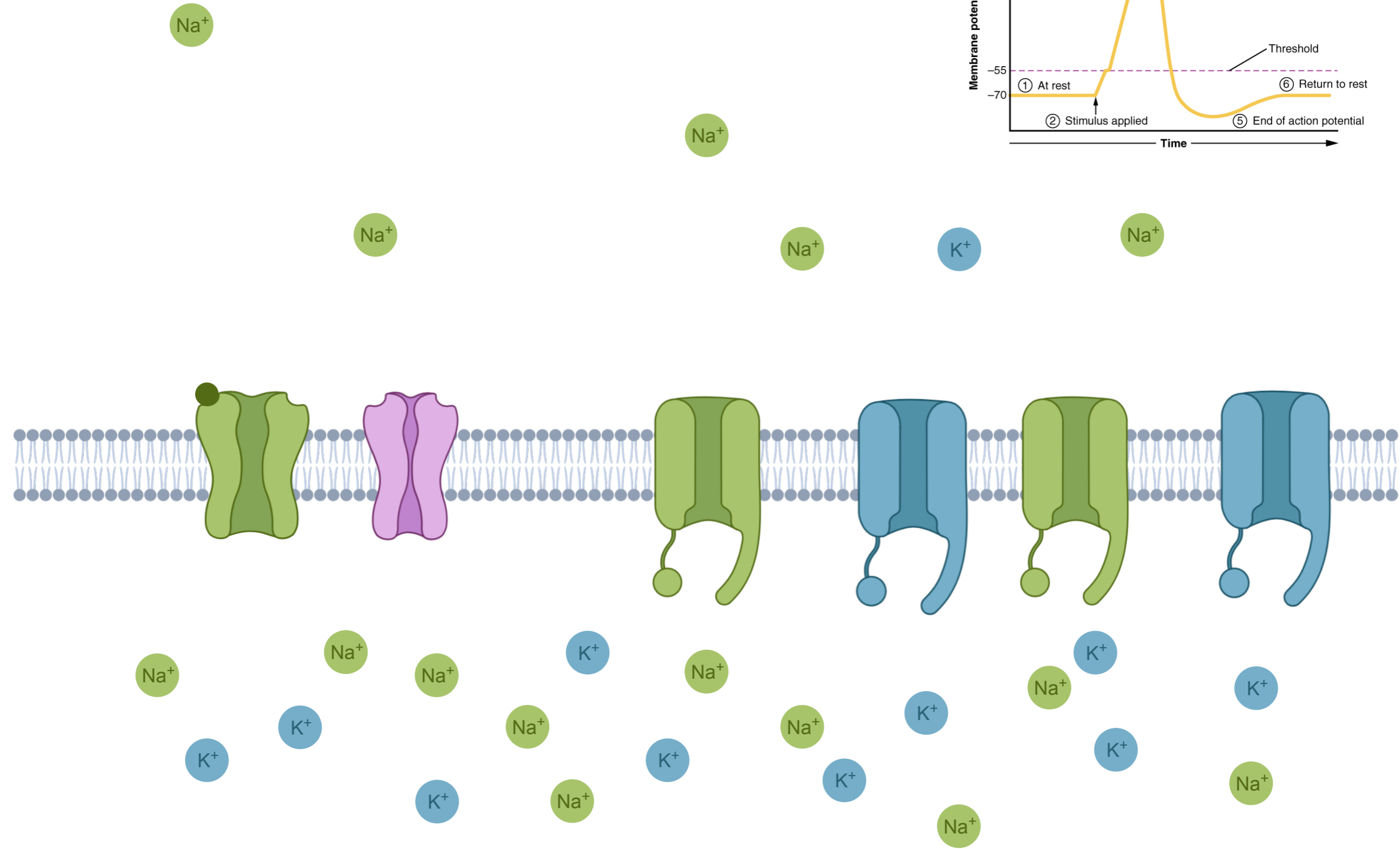
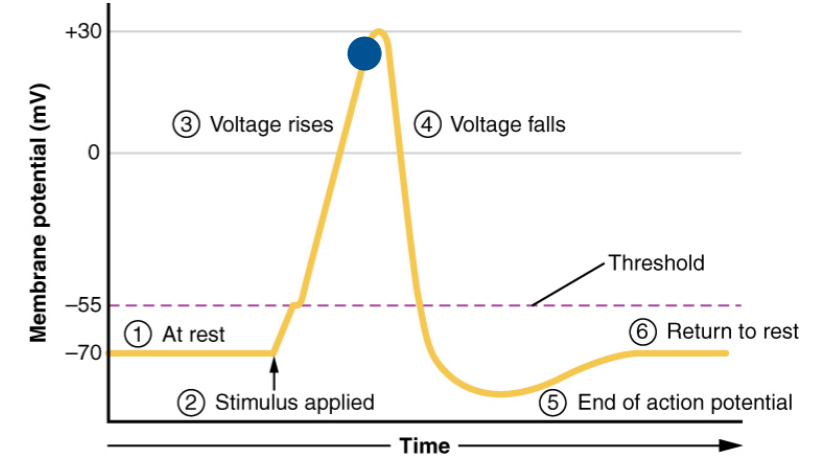
# How do nerves transmit signals?



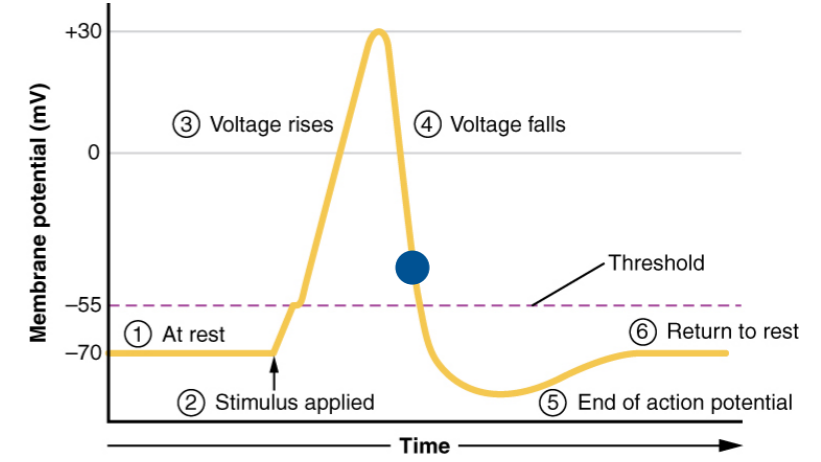
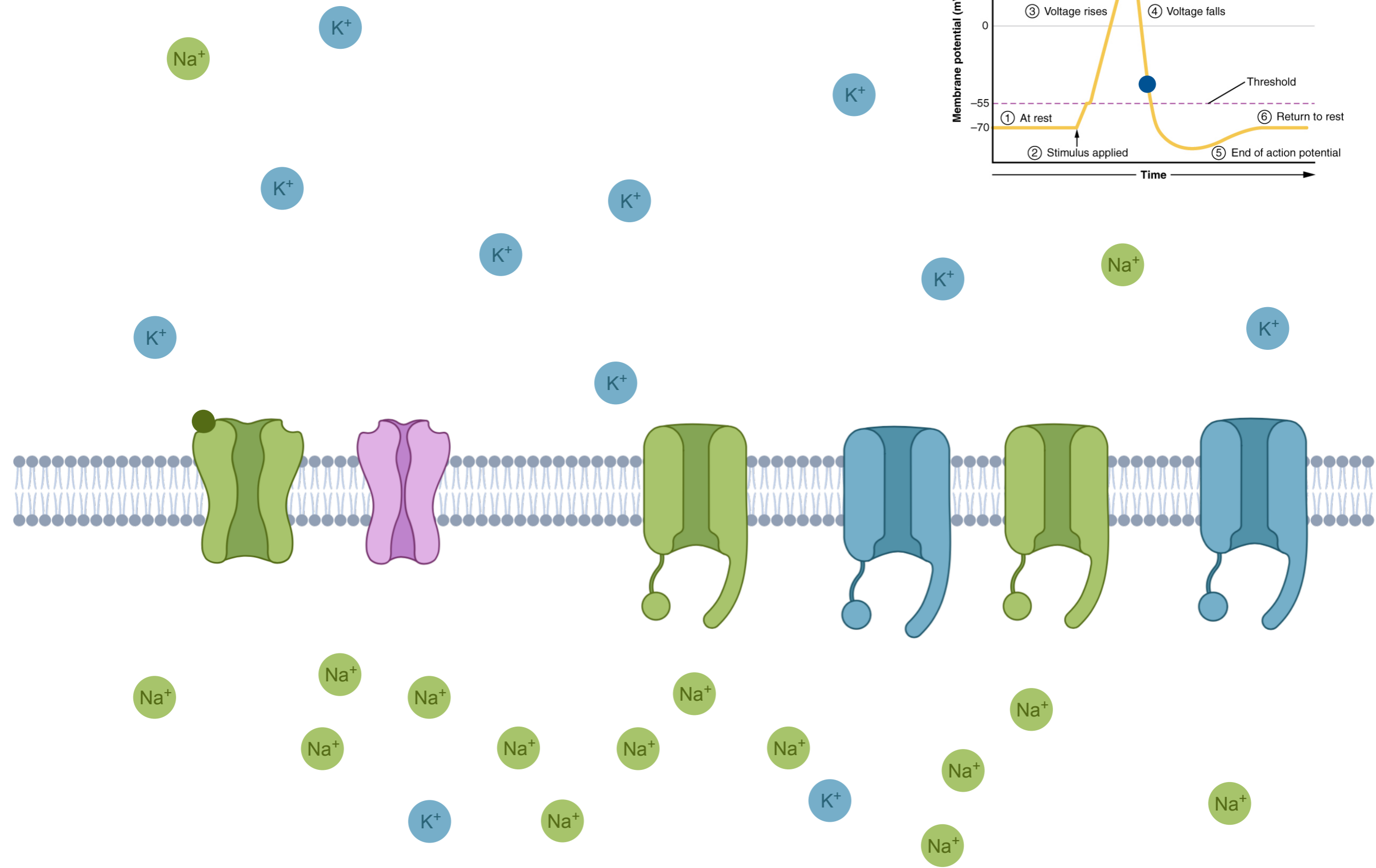
# How do nerves transmit signals?



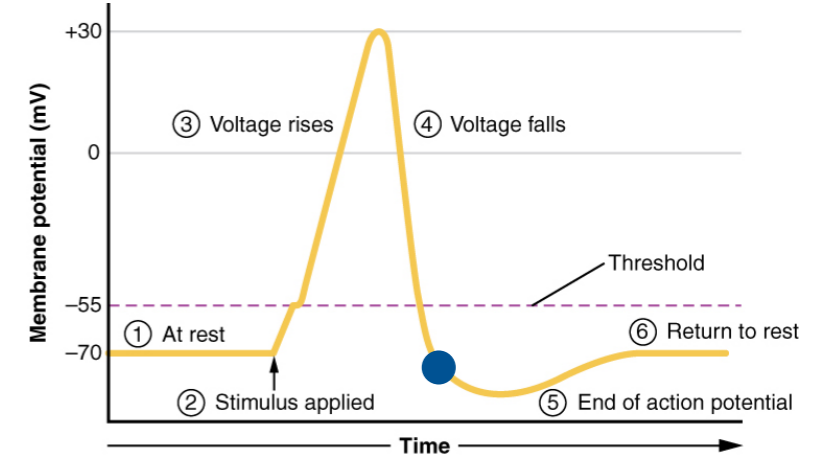
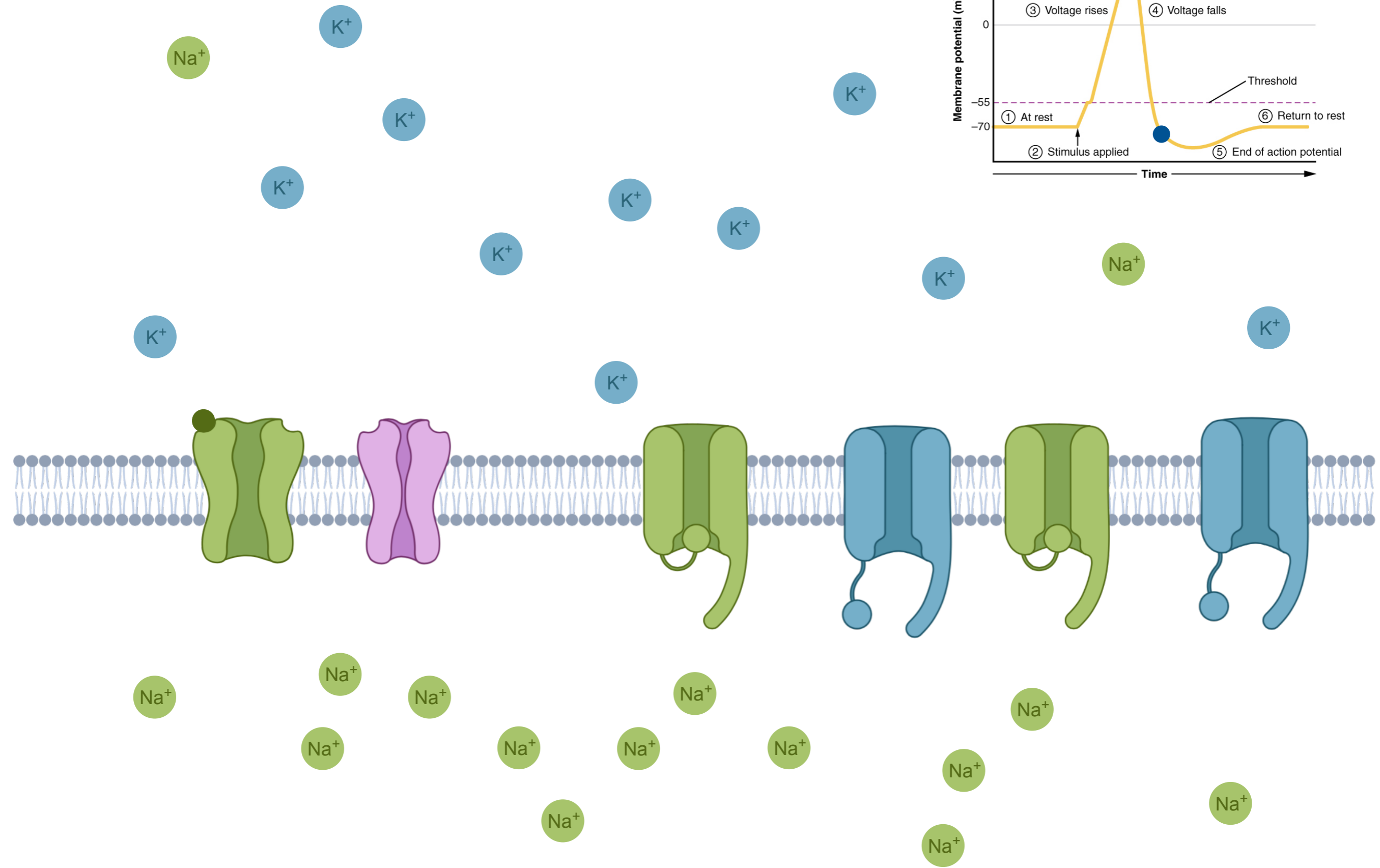
# How do nerves transmit signals?



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# How do nerves transmit signals?





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- What is anesthesia?
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- Methods of administration
- Commonly-used compounds
- Mechanism of action

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## *Local anesthesia*

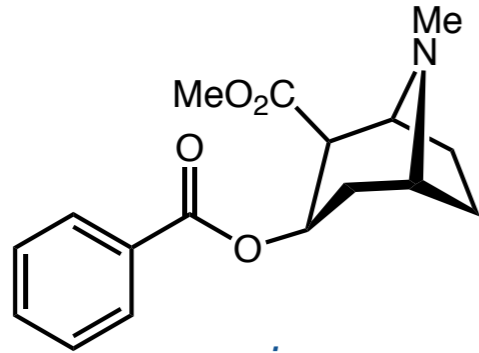
- State with no sensation in part of the body, with local nerves being affected
- Typically used for minor procedures or ones where consciousness should be maintained

### **Numerous methods of administration:**

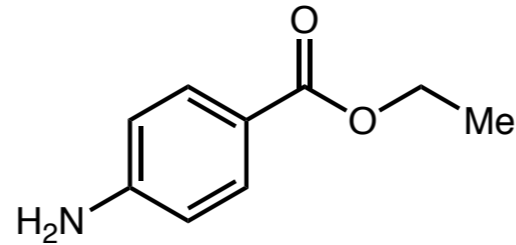
- Topical
- Infiltration
- Intravenous
- Nerve block
- Spinal anesthesia
- Epidural anesthesia
- Inhibiting a nerve also blocks all sensation downstream of the site of inhibition

# Common local anesthetics

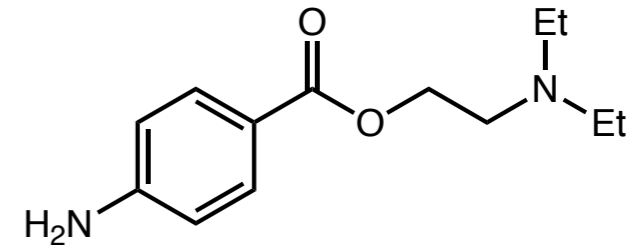
## Ester-type:



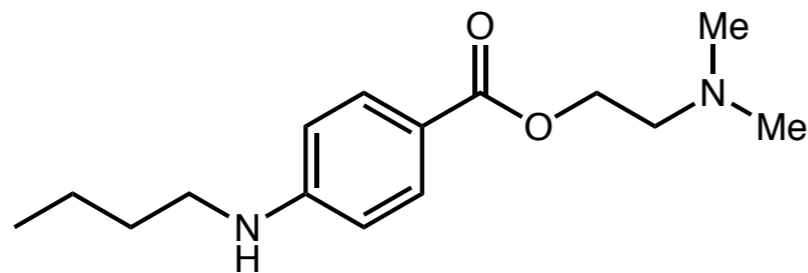
*cocaine*



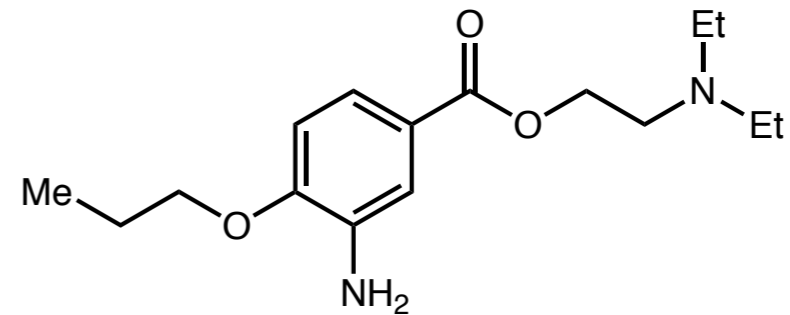
*benzocaine*



*procaine*

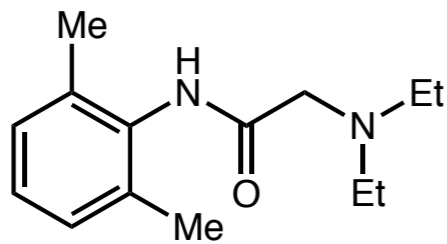


*tetracaine*

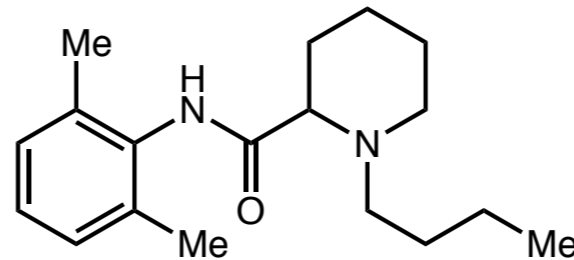


*proparacaine*

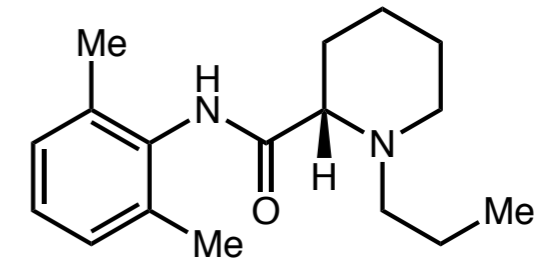
## Amide-type:



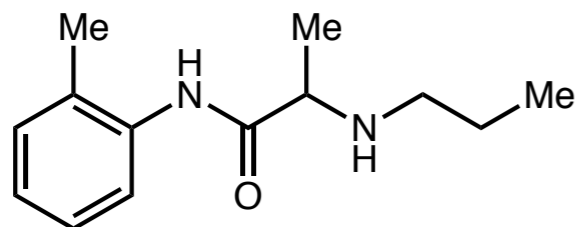
*lidocaine*



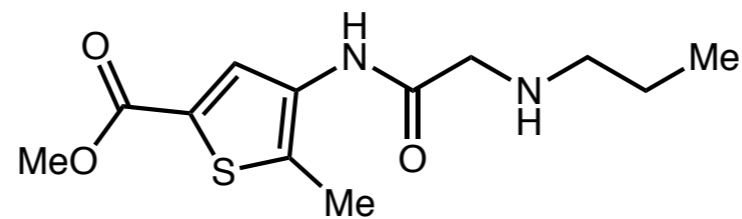
*bupivacaine*



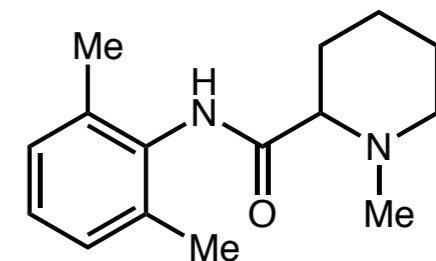
*ropivacaine*



*prilocaine*

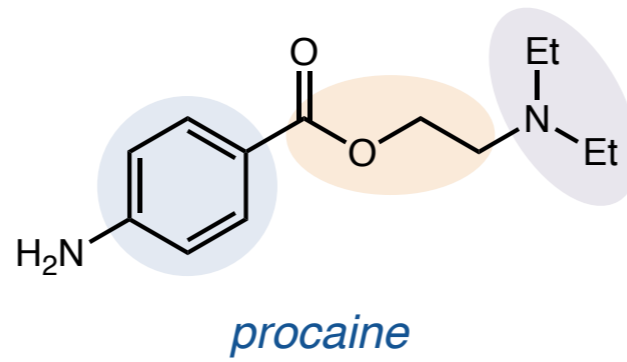


*articaine*



*mepivacaine*

## Common local anesthetics



■ Hydrophobic aryl group

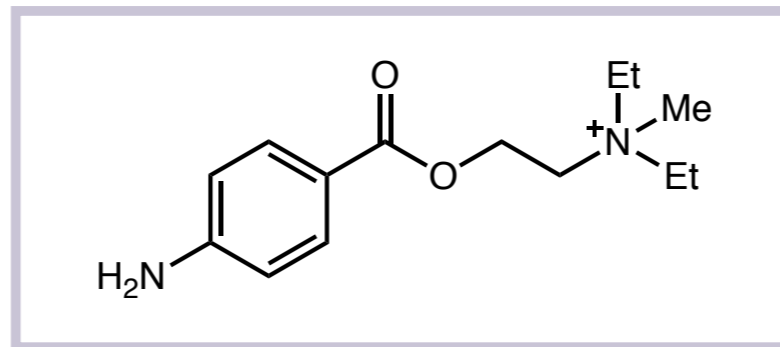
■ Ester or amide linker

■ Hydrophilic amine

## Mechanism of action

Experimental observations in the 70s:

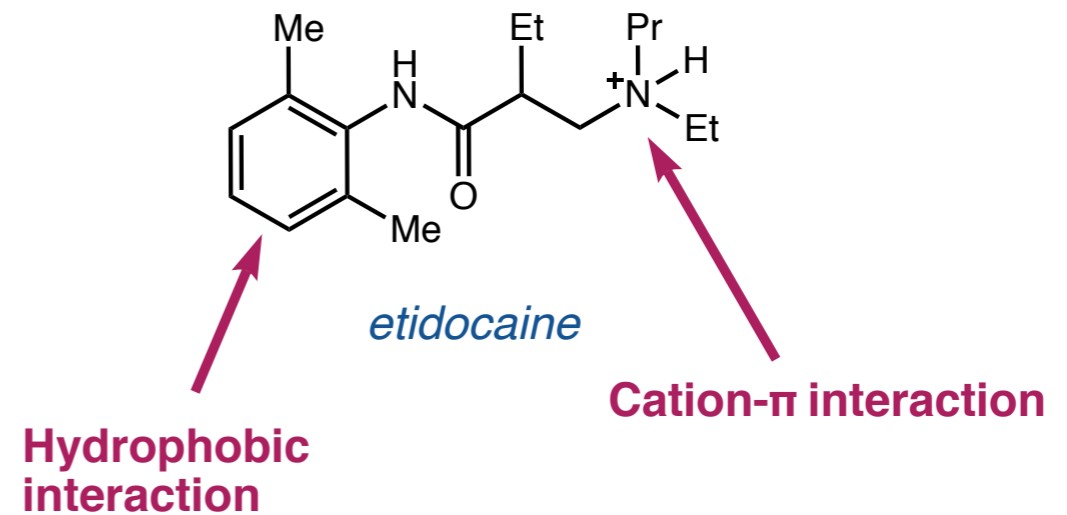
- Effectiveness is dependent on pH both inside and outside cell:
  - low pH inside cell, anesthesia more effective
  - low pH outside cell, anesthesia less effective
- Quaternized analogs produce anesthesia, but only when introduced intracellularly



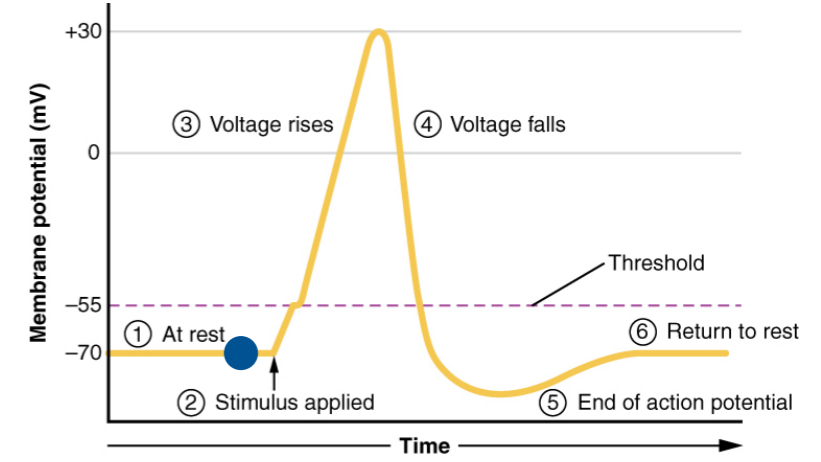
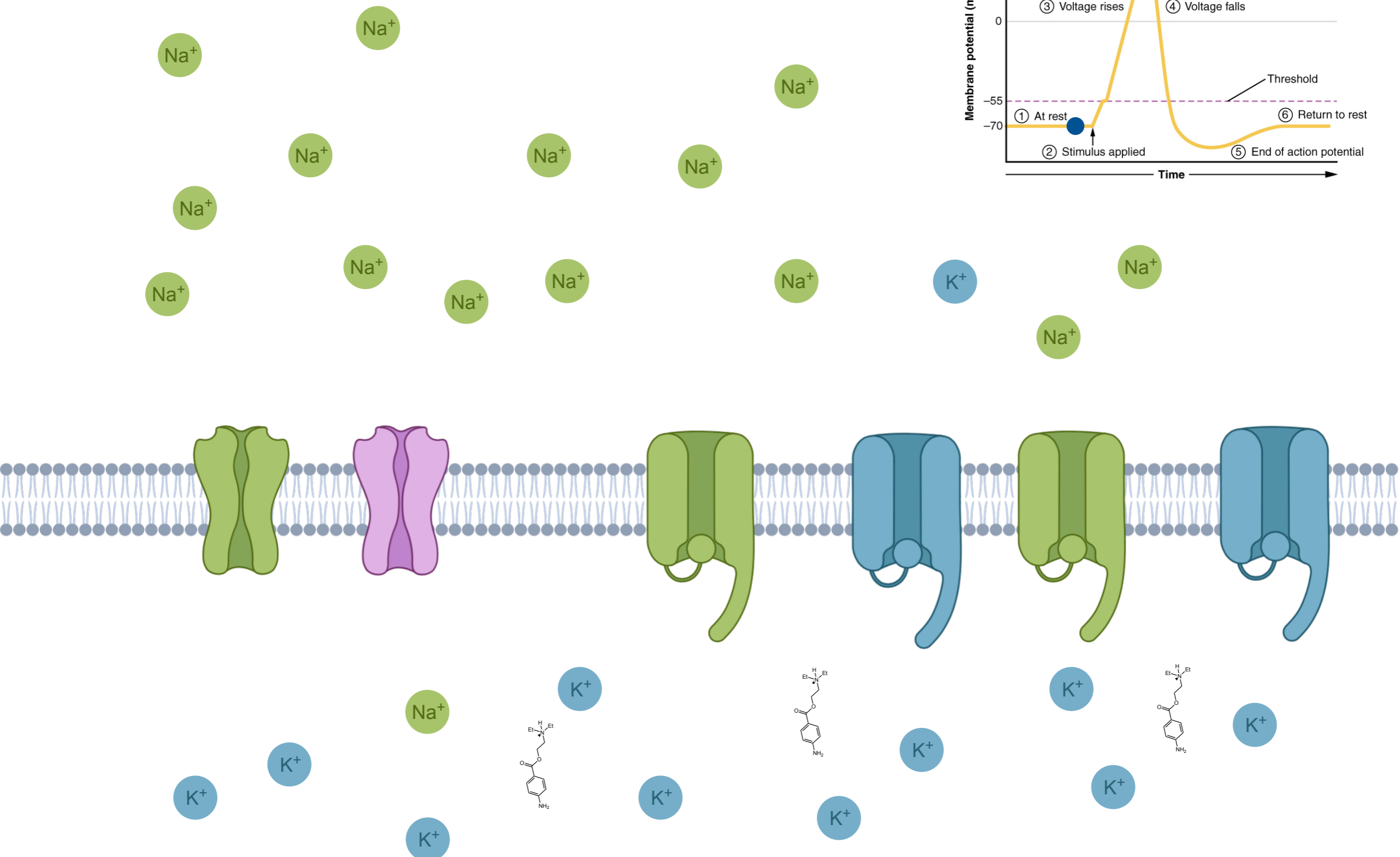
**Hypothesis: protonated local anesthetics bind to a protein inside the neuron**

## Mechanism of action

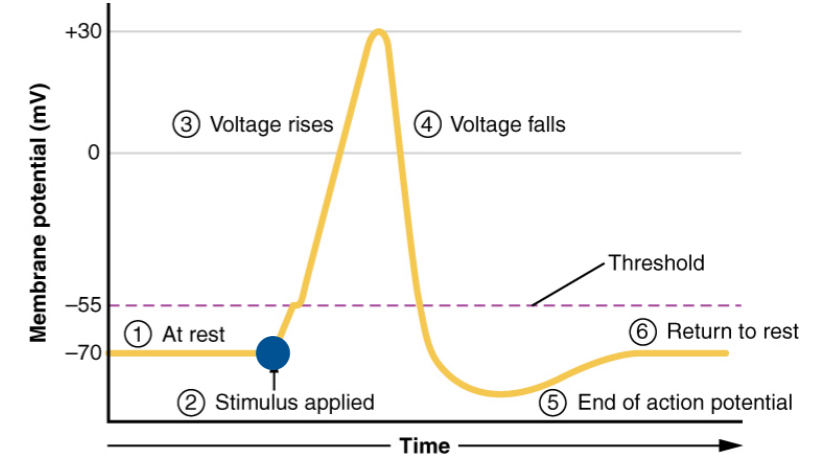
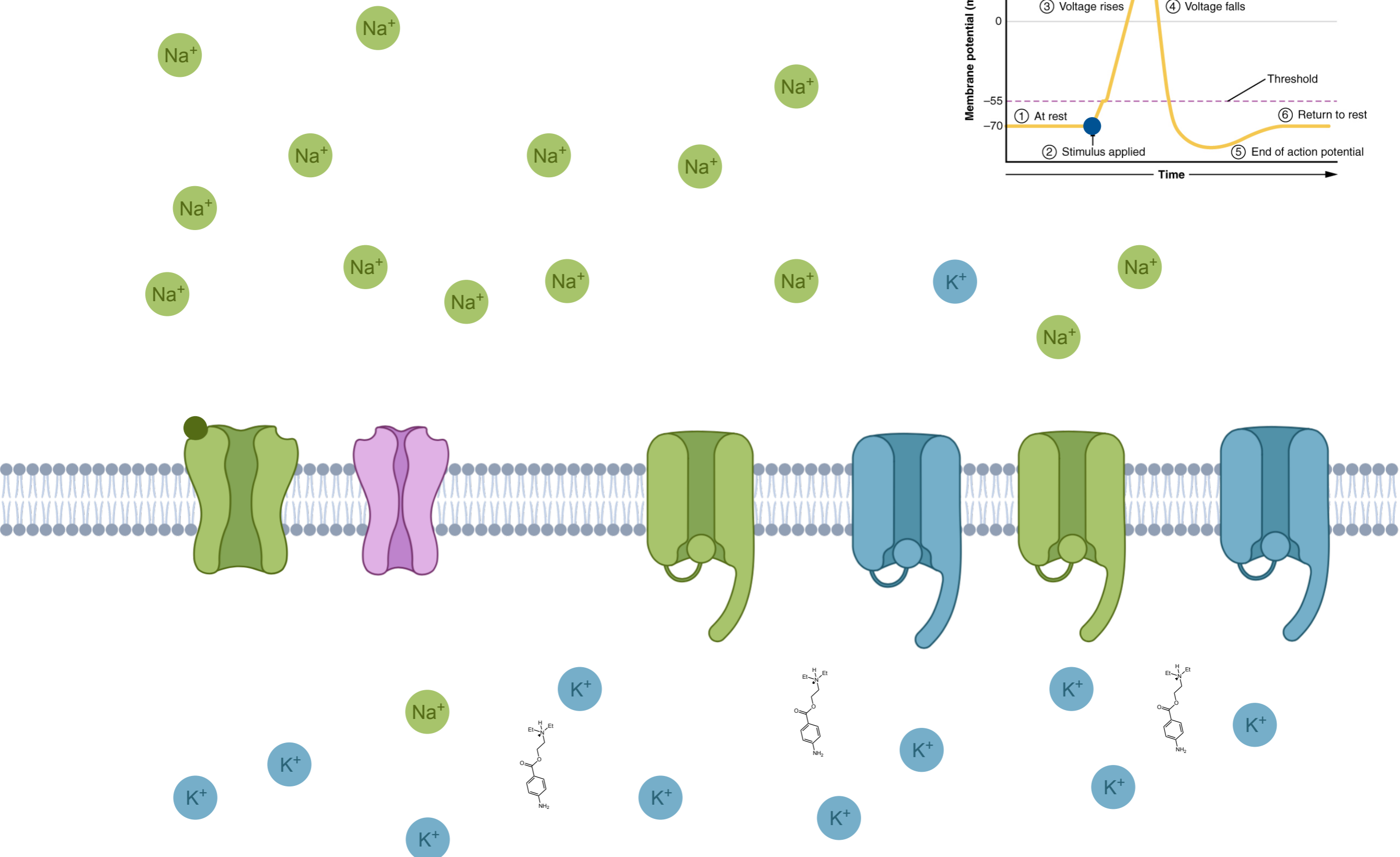
- Site-directed mutagenesis identifies binding site as inner pore of Na<sub>v</sub> channel
- Ammonium interacts with Phe, aryl ring interacts with Tyr
- Binding only occurs after channel is open



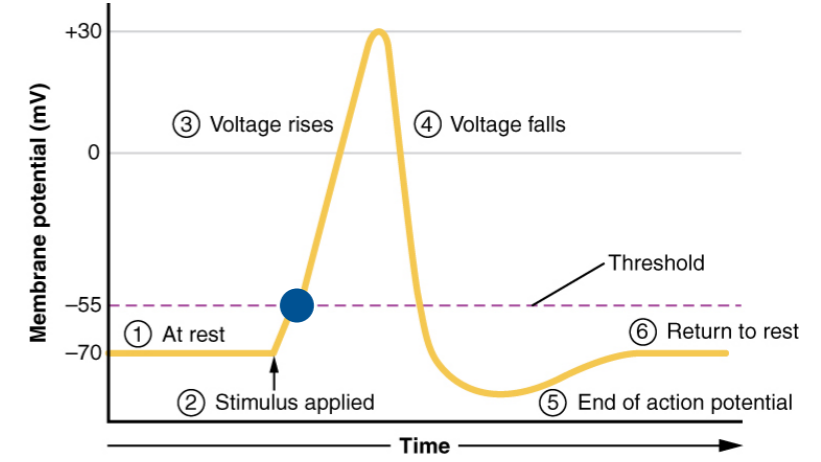
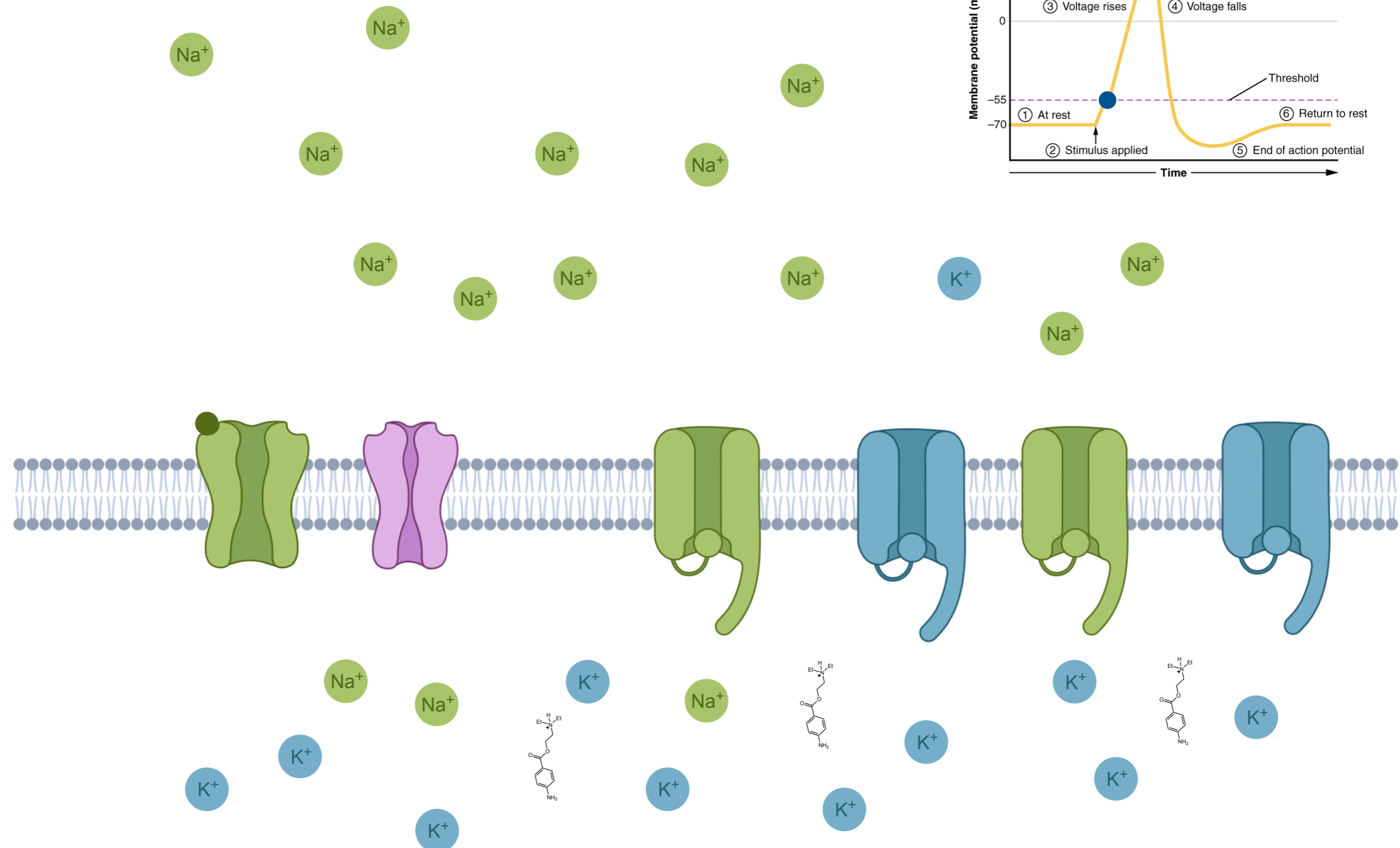
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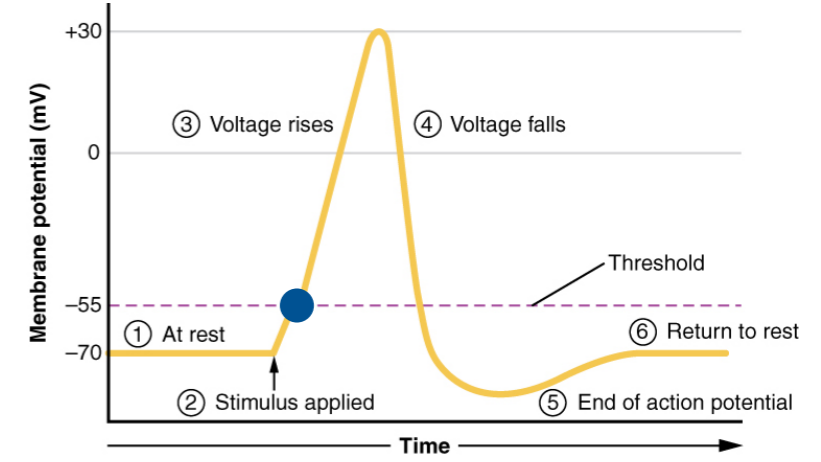
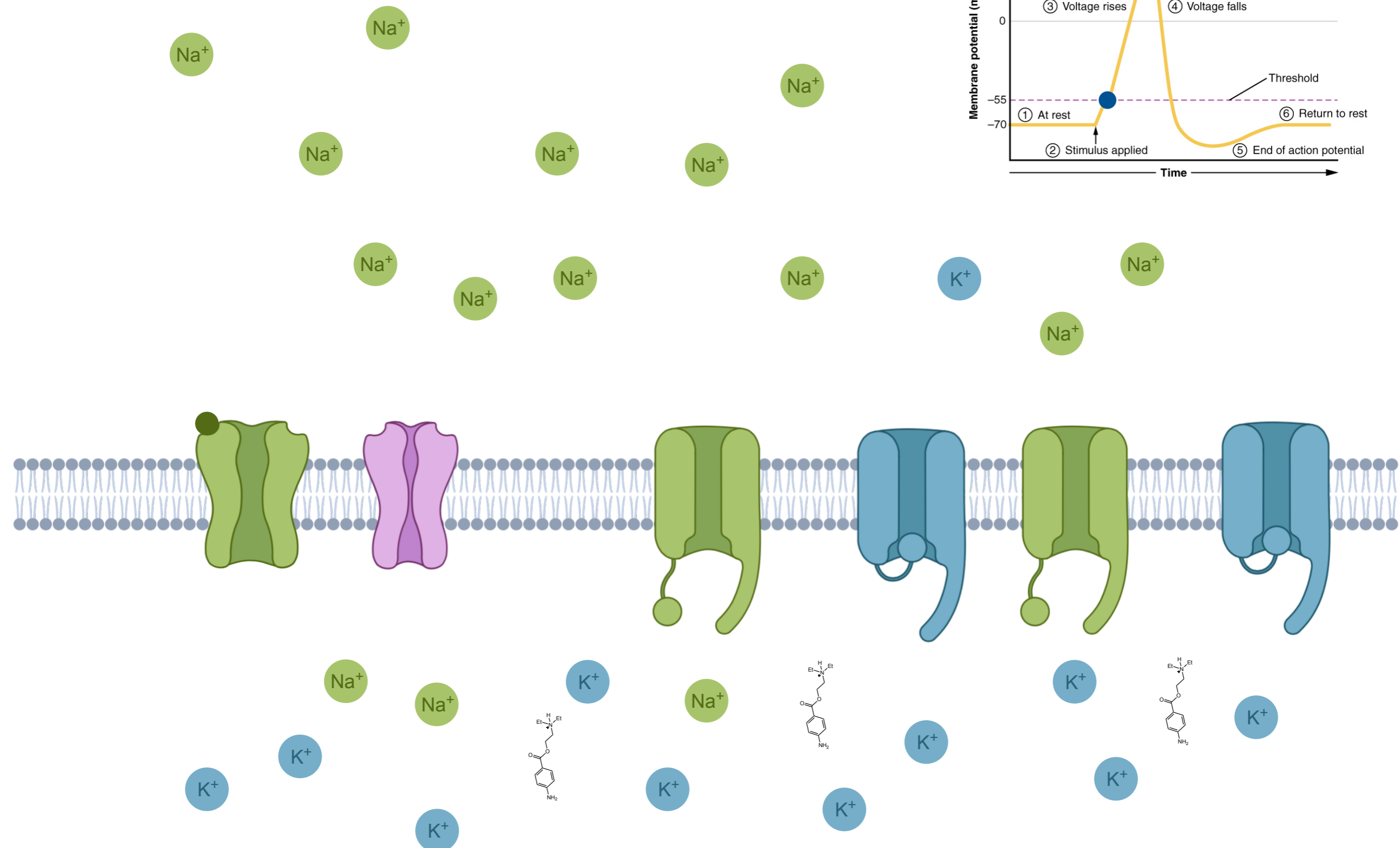


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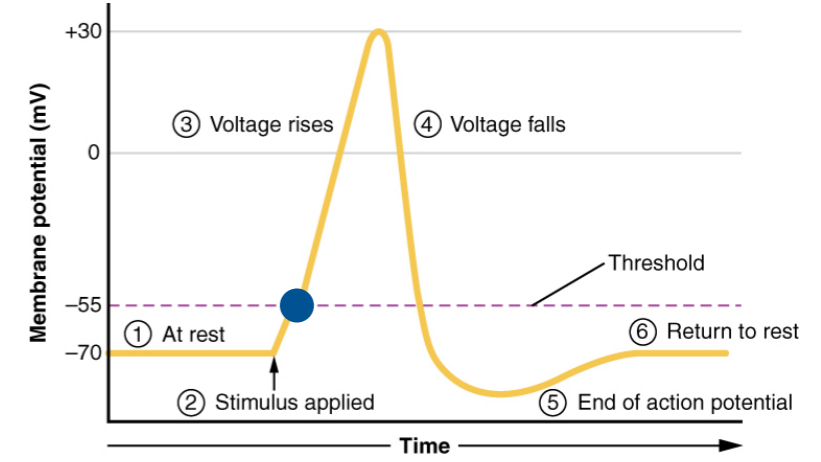
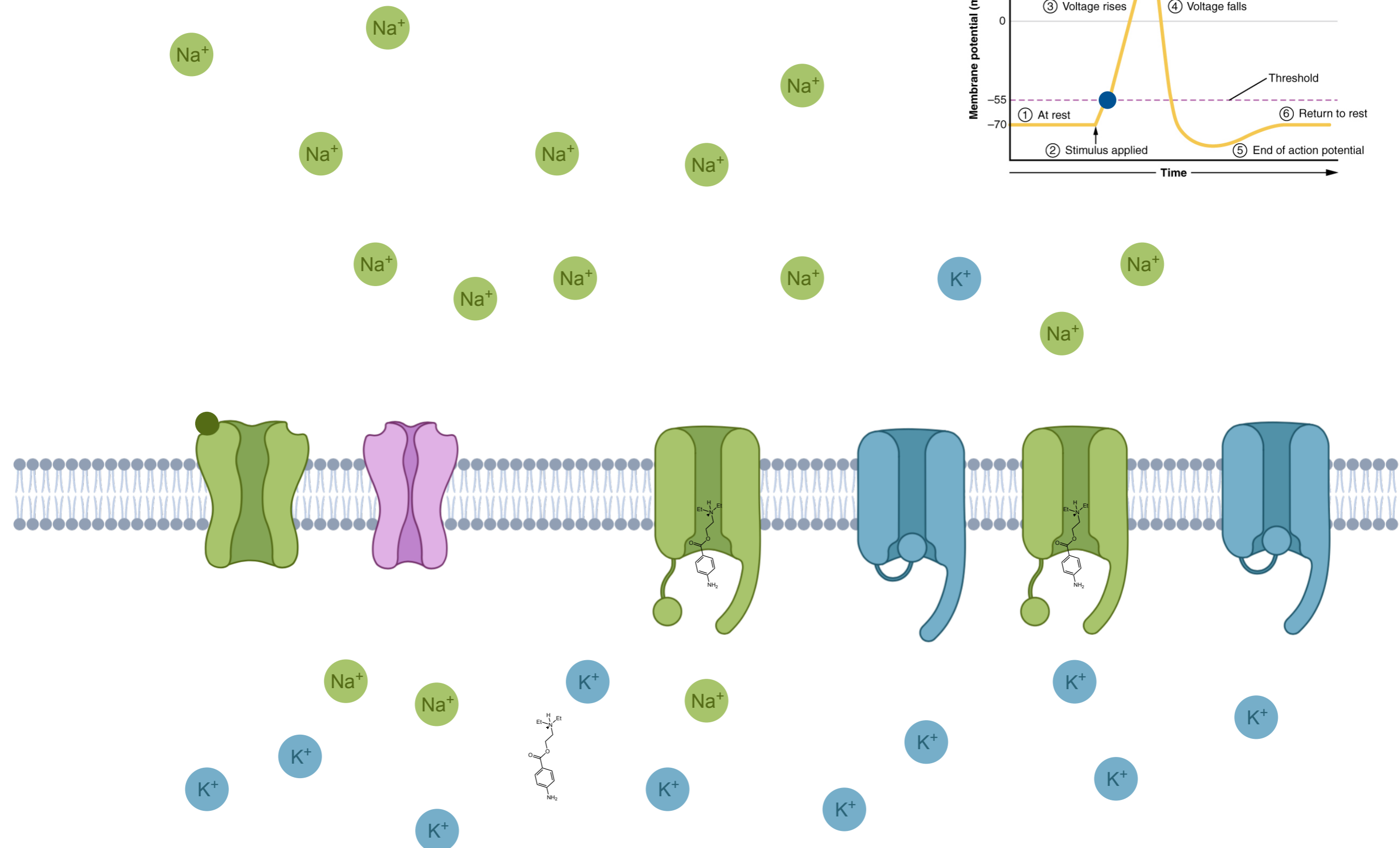




# Mechanism of action



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# *General anesthesia*

**Goal is depression of the CNS: induction of the anesthetic state**

**Used for surgical procedures where unconsciousness is desirable**

## ***The anesthetic state***

- Amnesia: no memories formed during procedure
- Sedation and hypnosis: unconsciousness, slow or no response to stimuli
- Immobility
- Determined by lack of response to painful stimulus

# Methods of administration

**Two primary administration methods:**

## **Inhalation**

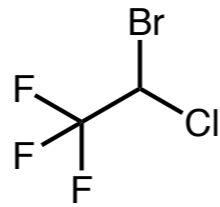
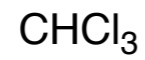
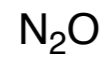
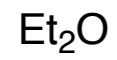
- Introduced as gas or nebulized liquid
- Minimum Alveolar Concentration: fraction of compound in lungs needed for lack of response to pain

## **Intravenous**

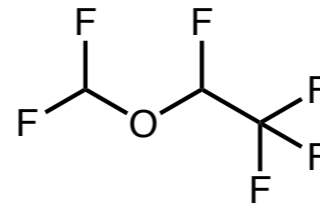
- Introduced as solution/suspension
- Typically administered with adjuvants for pain, blood pressure, etc.
- Often used for induction

# Common general anesthetics

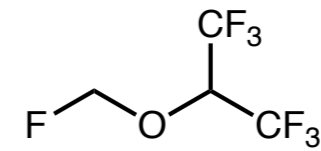
## Inhaled anesthetics



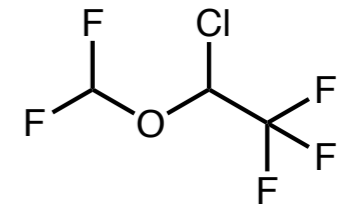
*halothane*



*desflurane*

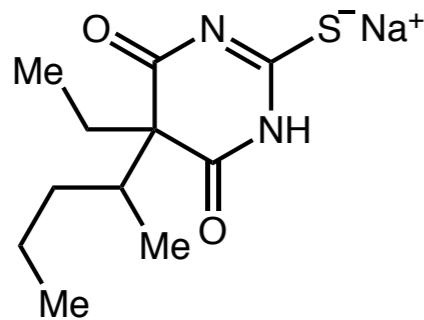


*sevoflurane*

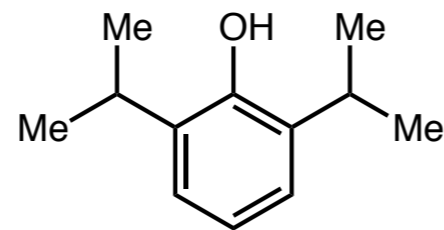


*isoflurane*

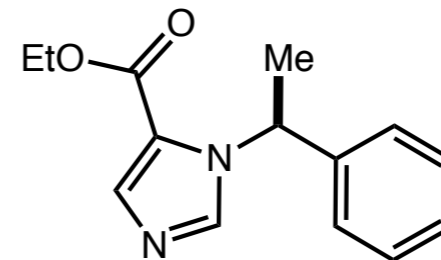
## Intravenous anesthetics



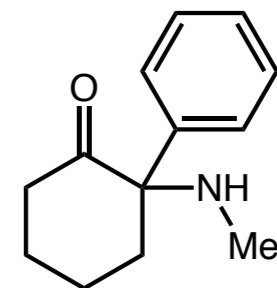
*thiopental*



*propofol*



*etomidate*



*ketamine*

# Characteristics of inhaled anesthetics

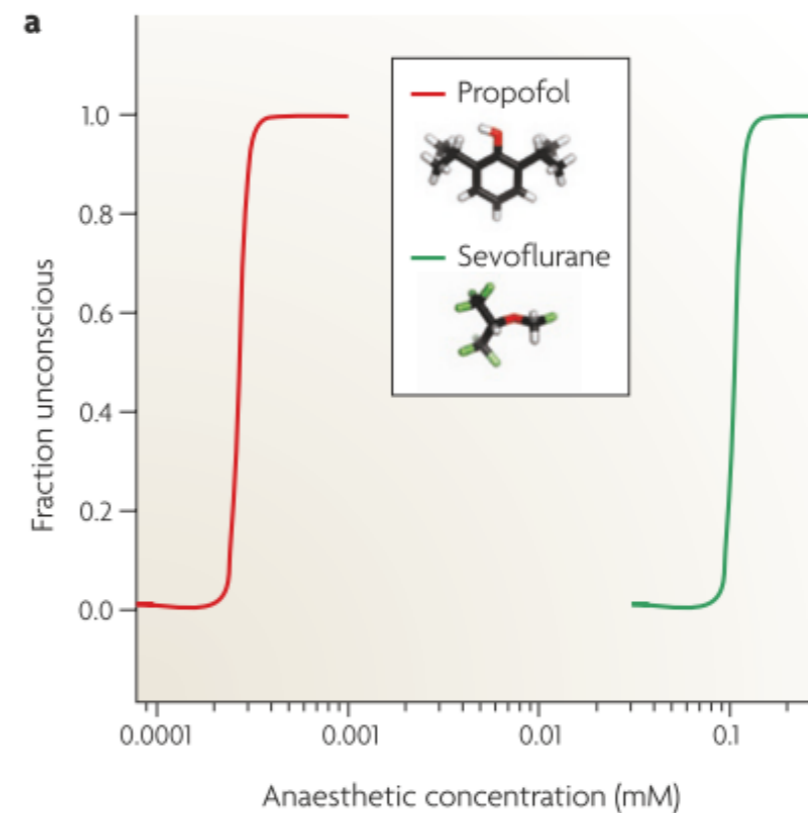
## Variable potency:

Compound	MAC
N <sub>2</sub> O	105%
halothane	0.8%
desflurane	6.0%
sevoflurane	2.0%
isoflurane	1.2%

## Effects are purely additive:

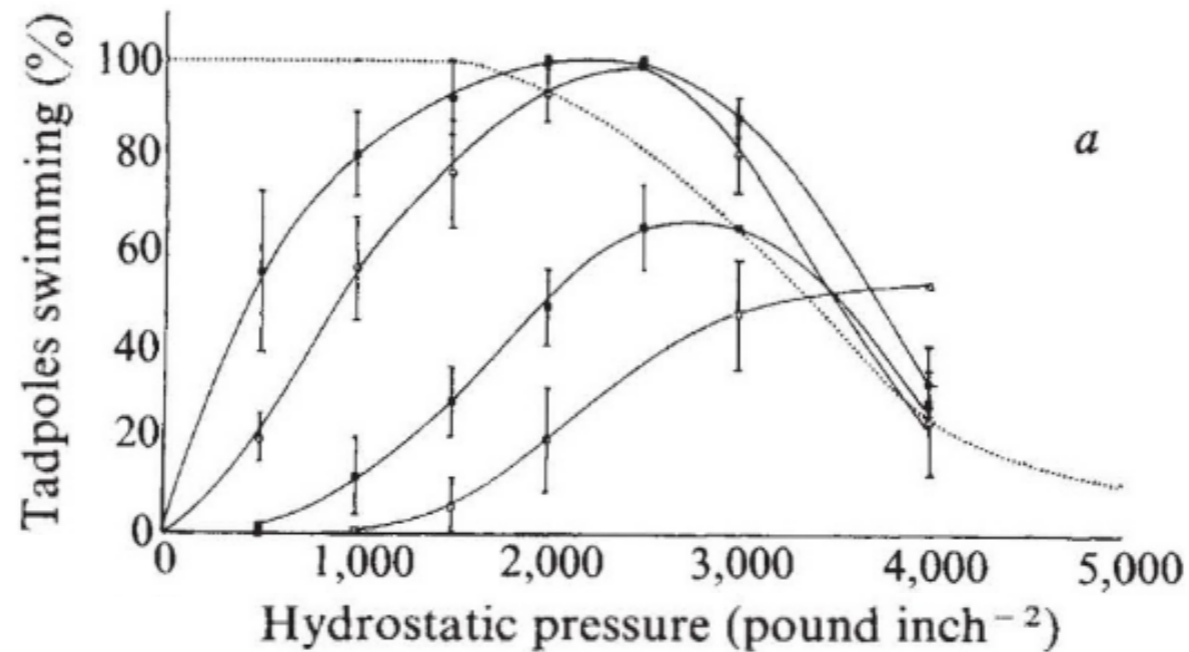
0.5 MAC N<sub>2</sub>O + 0.5 MAC isoflurane = 1.0 MAC

## Very sharp dose-response:



# Characteristics of inhaled anesthetics

*Can be reversed under increased pressure:*



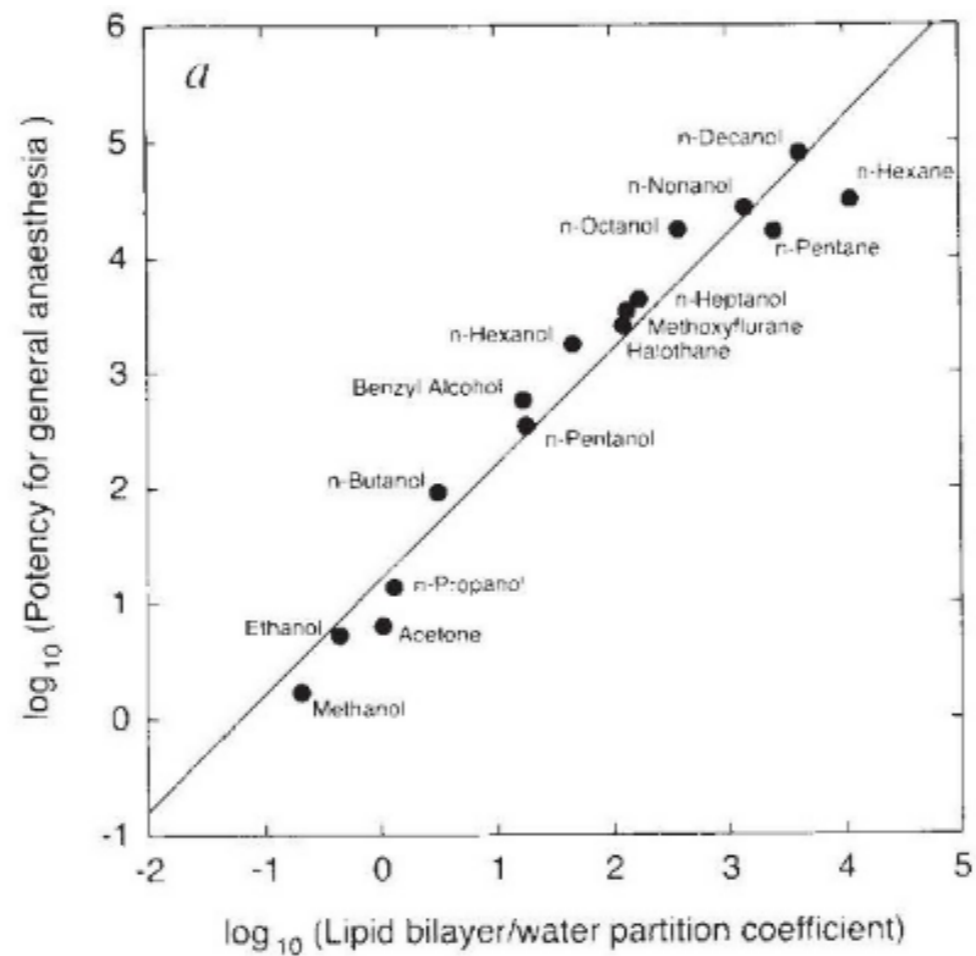
**Fig. 1 a**, Effect of pressure on tadpoles swimming in water equilibrated with different concentrations of halothane. ●, 0.005 atm; ○, 0.0075 atm; ■, 0.01 atm; □, 0.015 atm. Error bars indicate  $\pm 1$  s.e.m. calculated from five groups of five tadpoles. Dotted line shows effect of pressure alone.

*"We thank Mrs. F. J. Nunn and the Misses Halsey for the sustained supply of tadpoles"*



# The Meyer-Overton correlation

**Independent discovery by Hans Meyer and Charles Overton around 1900:**



*Anesthetic potency strongly correlated with lipid solubility*

# *The lipid hypothesis*

***Hypothesis: general anesthetics function by altering the properties of the cell membrane***

*Possible changes to membrane:*

- Increased lateral membrane pressure
- Membrane fluidization
- Increased membrane thickness



***Disruption of proteins  
critical to signaling***

## *The lipid hypothesis: negative evidence*

**Evidence contradicting cell membrane involvement in general anesthesia appeared over time:**

- Temperature effects on anesthetic potency
- Failures of Meyer-Overton correlation
- Relevance of stereoisomers to anesthetic effects
- Ability of general anesthetics to bind to proteins

## Temperature and anesthetic effectiveness

- Membrane fluidity increases with higher temperatures
- Increased body temperature should increase anesthetic potency
- Change in membrane fluidity due to anesthetics can be mimicked by 1 °C increase in temperature

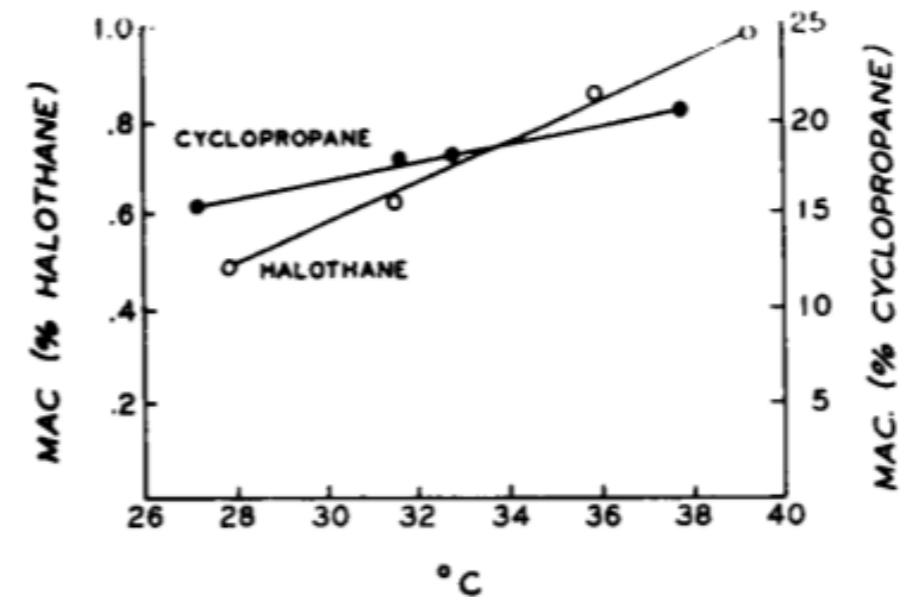


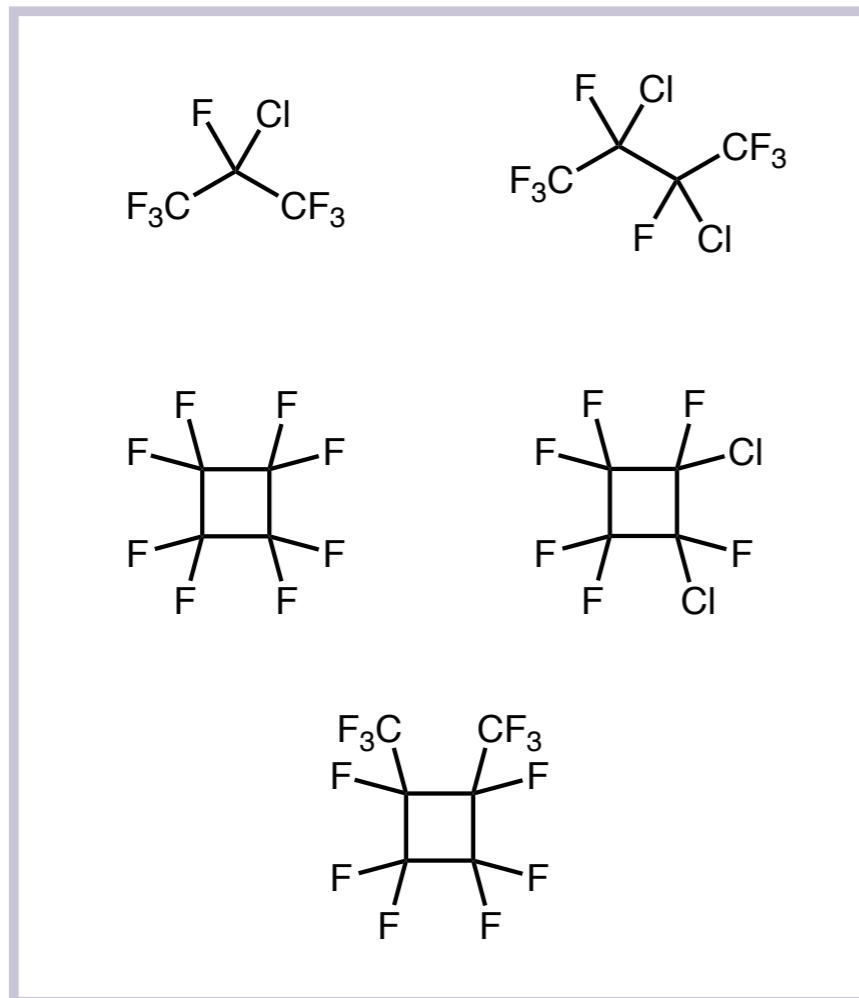
FIG. 3. A summation of the effect of temperature change on the MAC for halothane and for cyclopropane. The MAC scale for halothane is on the left while that for cyclopropane is on the right.

**Opposite trend observed!**

# Failures of Meyer-Overton

Some lipid-soluble compounds have no anesthetic effect

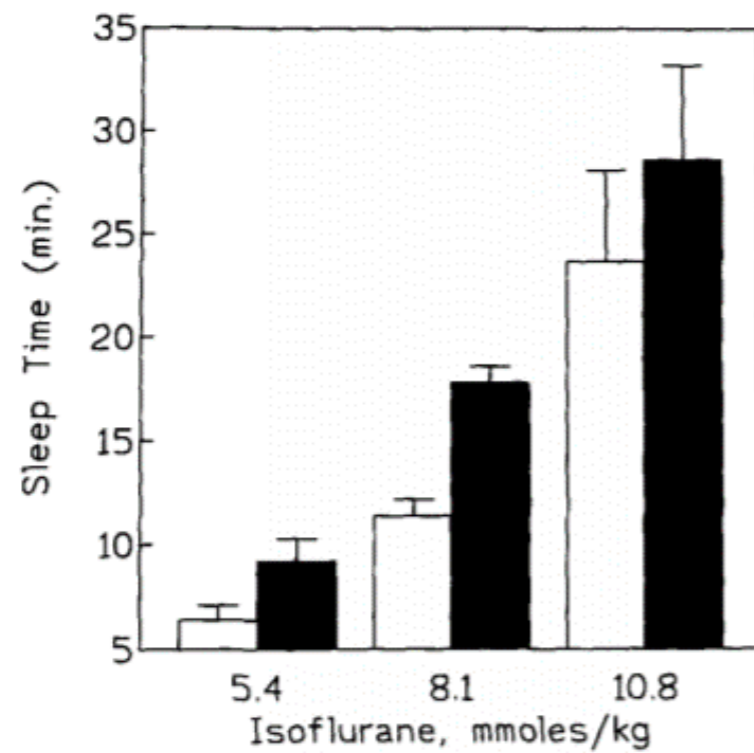
Increasing chain length results in eventual falloff of activity



Alcohol	EC <sub>50</sub> ± s.e.
Methanol	590 ± 41 mM
Ethanol	190 ± 16 mM
Propanol	73 ± 2.4 mM
Butanol	10.8 ± 0.77 mM
Pentanol	2.9 ± 0.11 mM
Hexanol	570 ± 37 μM
Heptanol	230 ± 11 μM
Octanol	57 ± 2.5 μM
Octanol <sup>a</sup>	55 ± 3.1 μM
Octanol <sup>b</sup>	59 ± 3.1 μM
Nonanol	37 ± 2.4 μM
Decanol <sup>a,b</sup>	12.6 ± 0.48 μM
Undecanol <sup>a</sup>	8.1 ± 0.81 μM
Dodecanol <sup>a,b</sup>	4.7 ± 0.33 μM
Tridecanol <sup>a</sup>	Not anaesthetic
Tetradecanol <sup>a</sup>	Not anaesthetic

# Stereoselectivity of anesthetics

Stereoisomers of isoflurane have modest but significant potency differences:



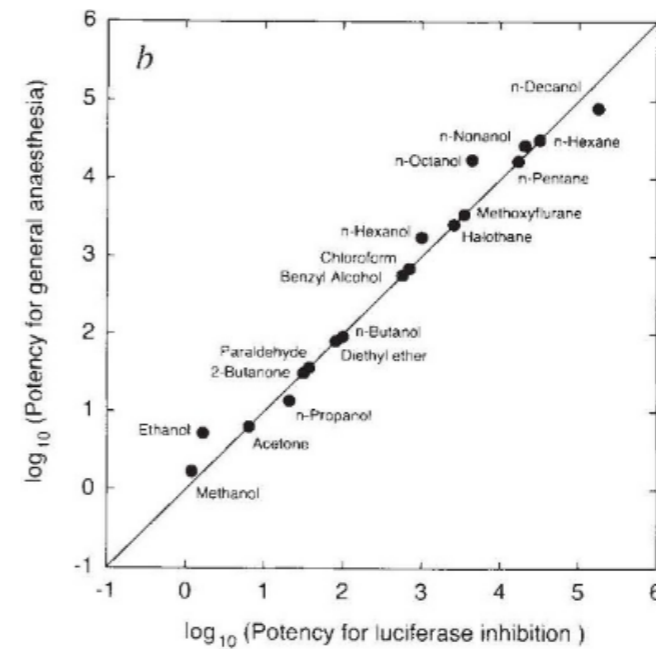
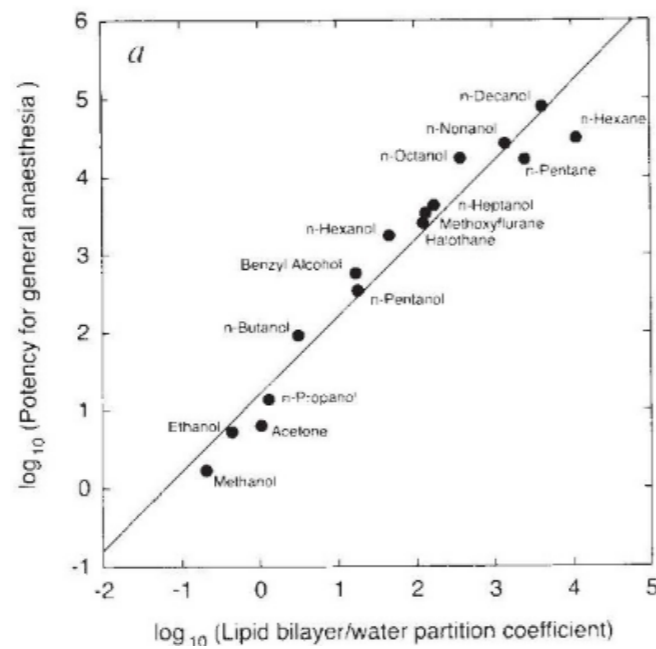
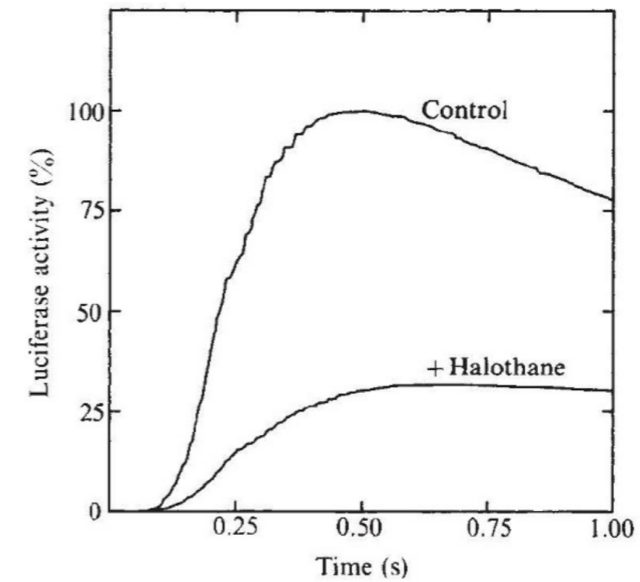
● (+)-isoflurane

○ (-)-isoflurane

*Target of anesthetic is likely chiral, not largely achiral lipid membrane*

# General anesthetic effects on protein function

General anesthetics are capable of inhibiting luciferase function without any involvement of lipid membranes



Potency for luciferase inhibition also correlates well with lipophilicity

# *The protein hypothesis*

## ***Protein hypothesis of anesthetic function***

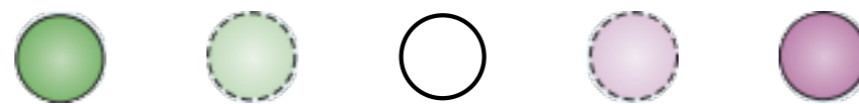
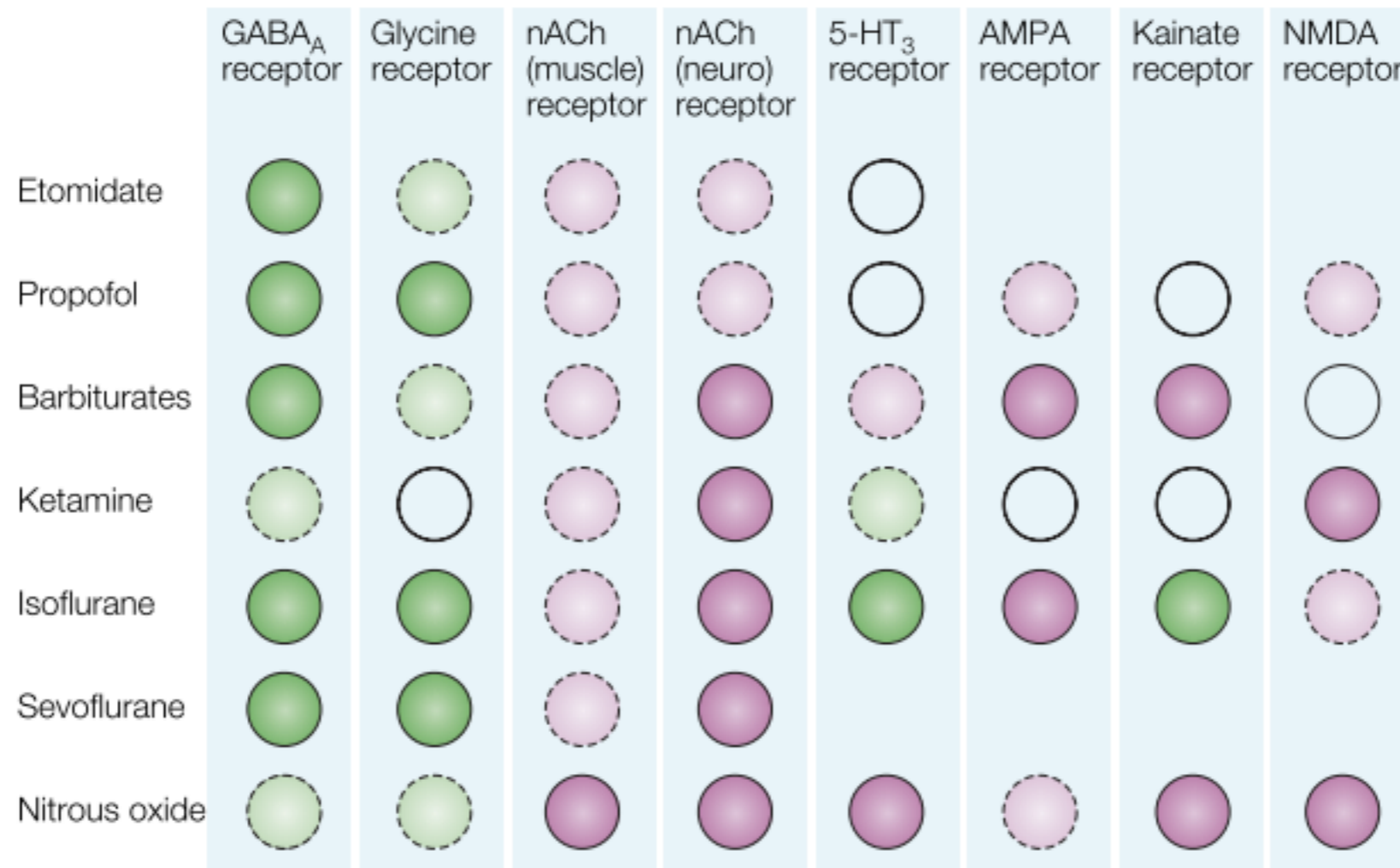
*Despite their unusual structures, general anesthetics function by binding directly to proteins and altering their properties*

- Binding is typically allosteric, with anesthetics occupying small hydrophobic pockets
- Stability of conformations is altered, rather than causing significant shape changes



# Protein targets of general anesthetics

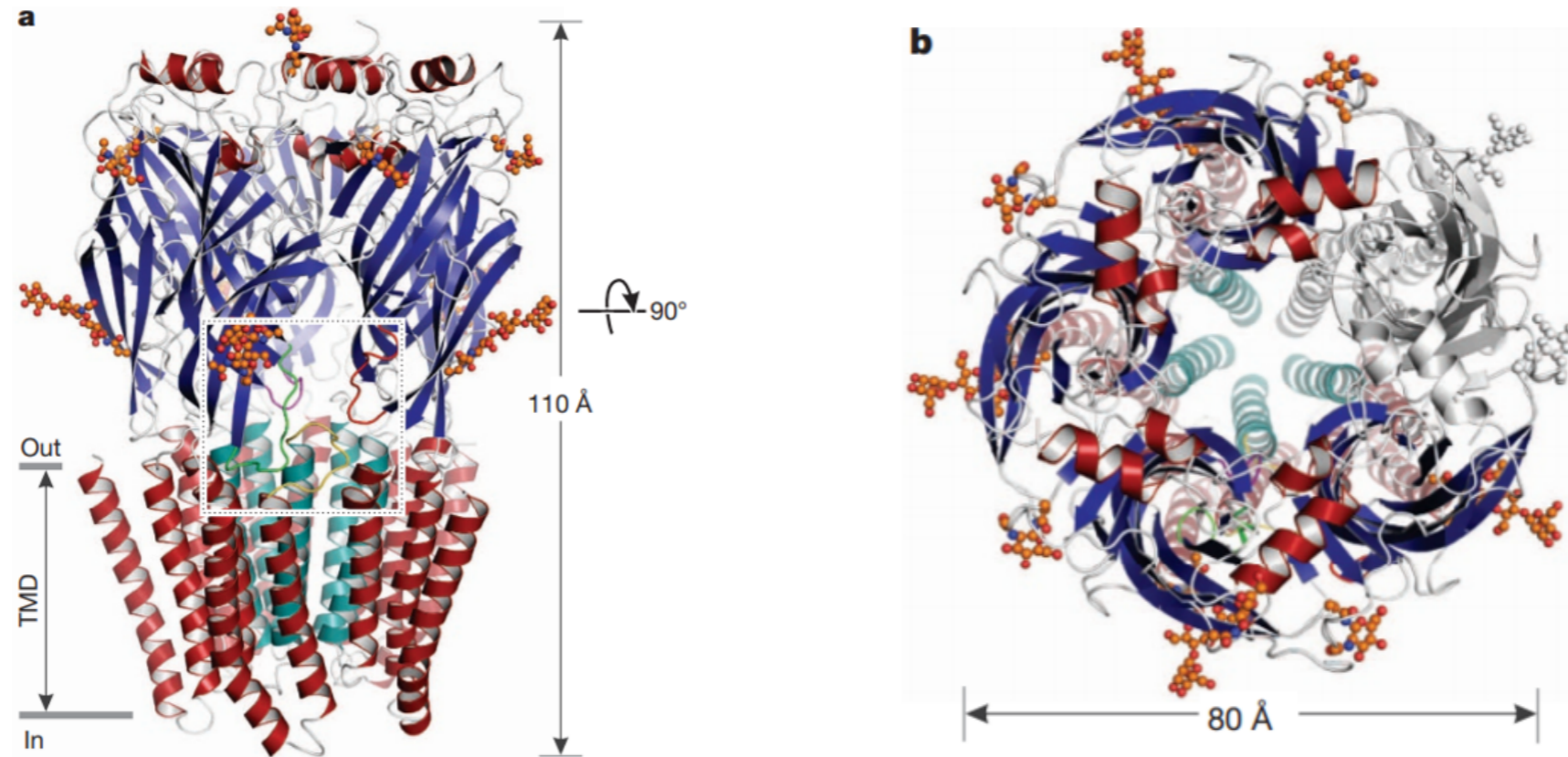
Many classes of ligand-gated ion channels are modulated by general anesthetics



Strong potentiation

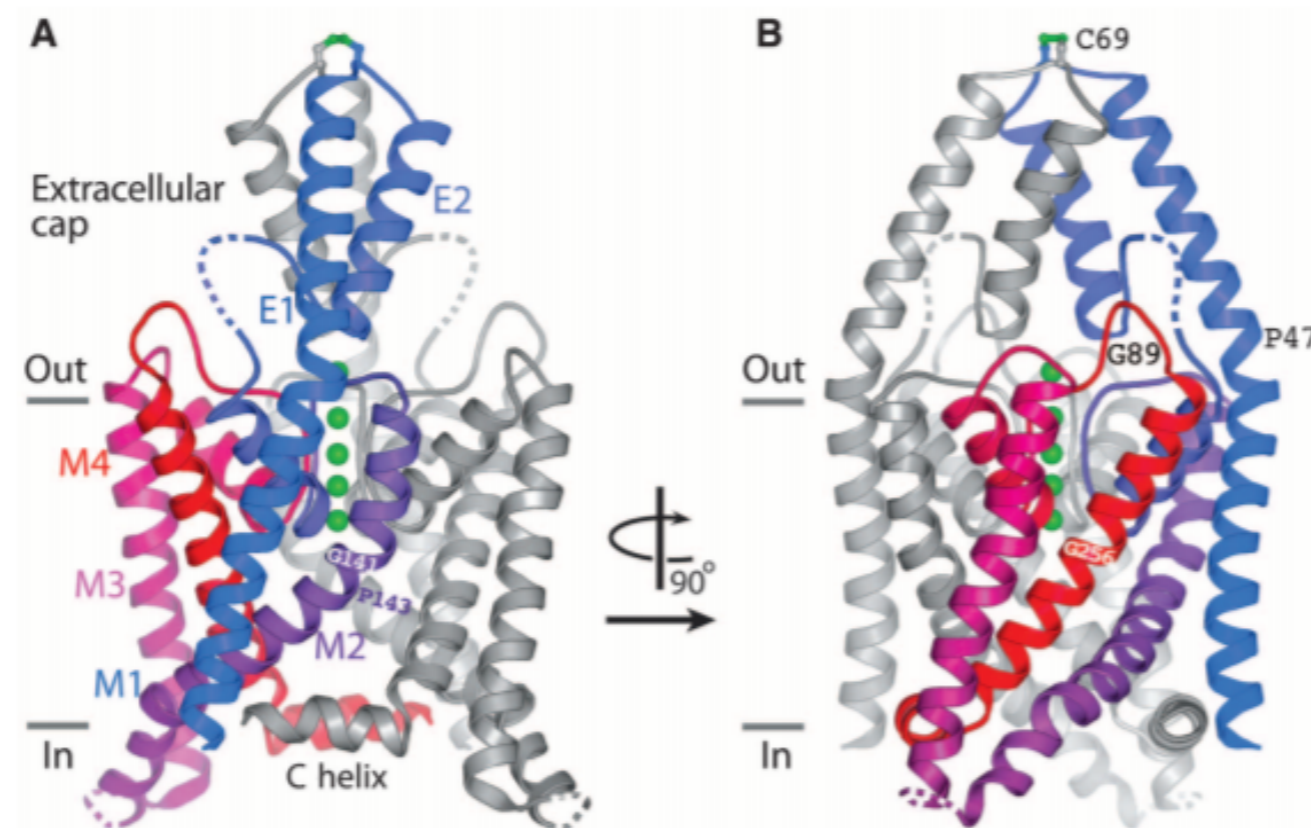
Strong inhibition

## Protein targets: GABA<sub>A</sub> receptors



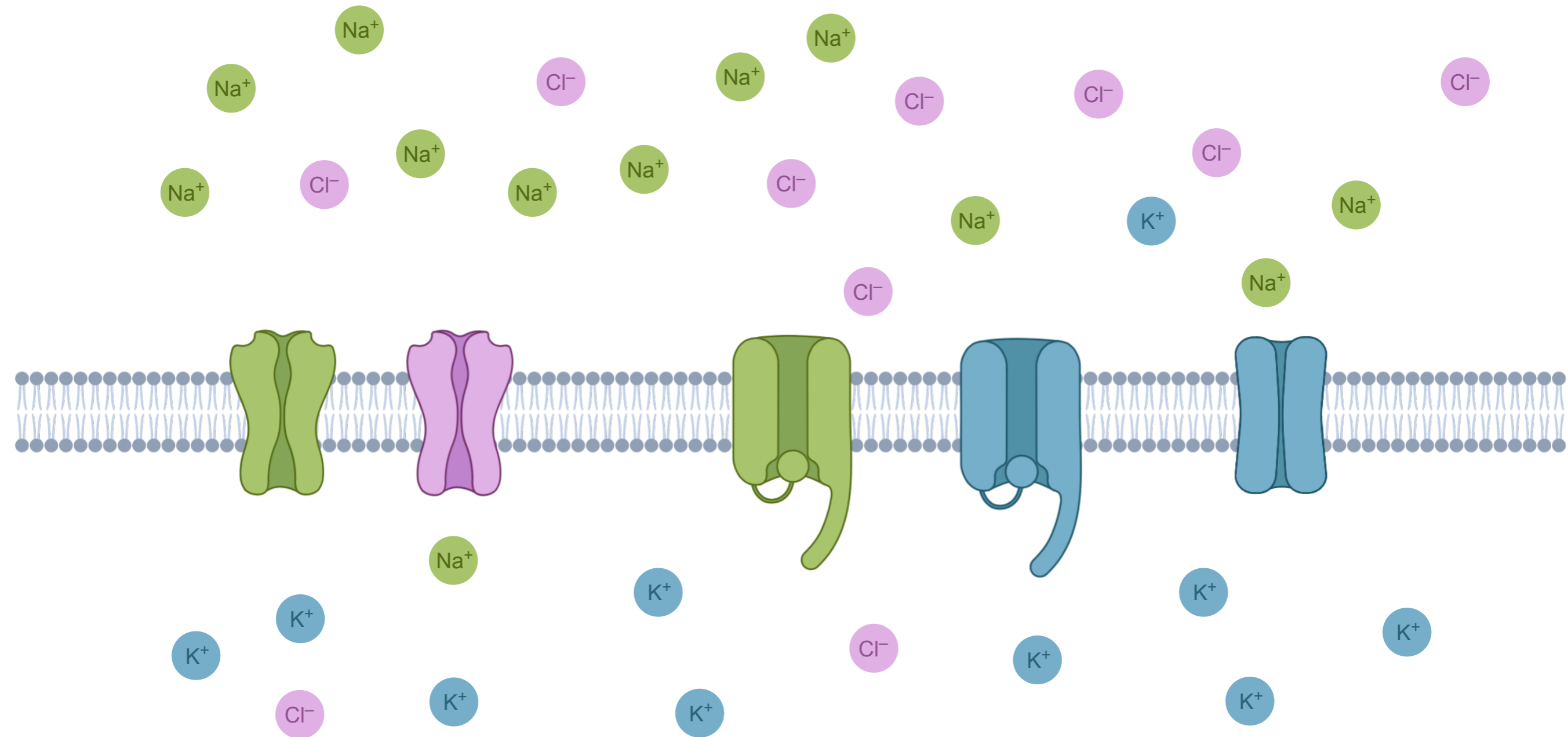
- Cl<sup>-</sup> channel activated by  $\gamma$ -aminobutyric acid (GABA)
- Involved in attention, anxiety, muscle tension, memory performance
- Pentamers with several types of subunits
- Most common subunit combination in brain is  $\alpha_1\beta_2\gamma_2$

## Protein targets: two-pore $K^+$ channels

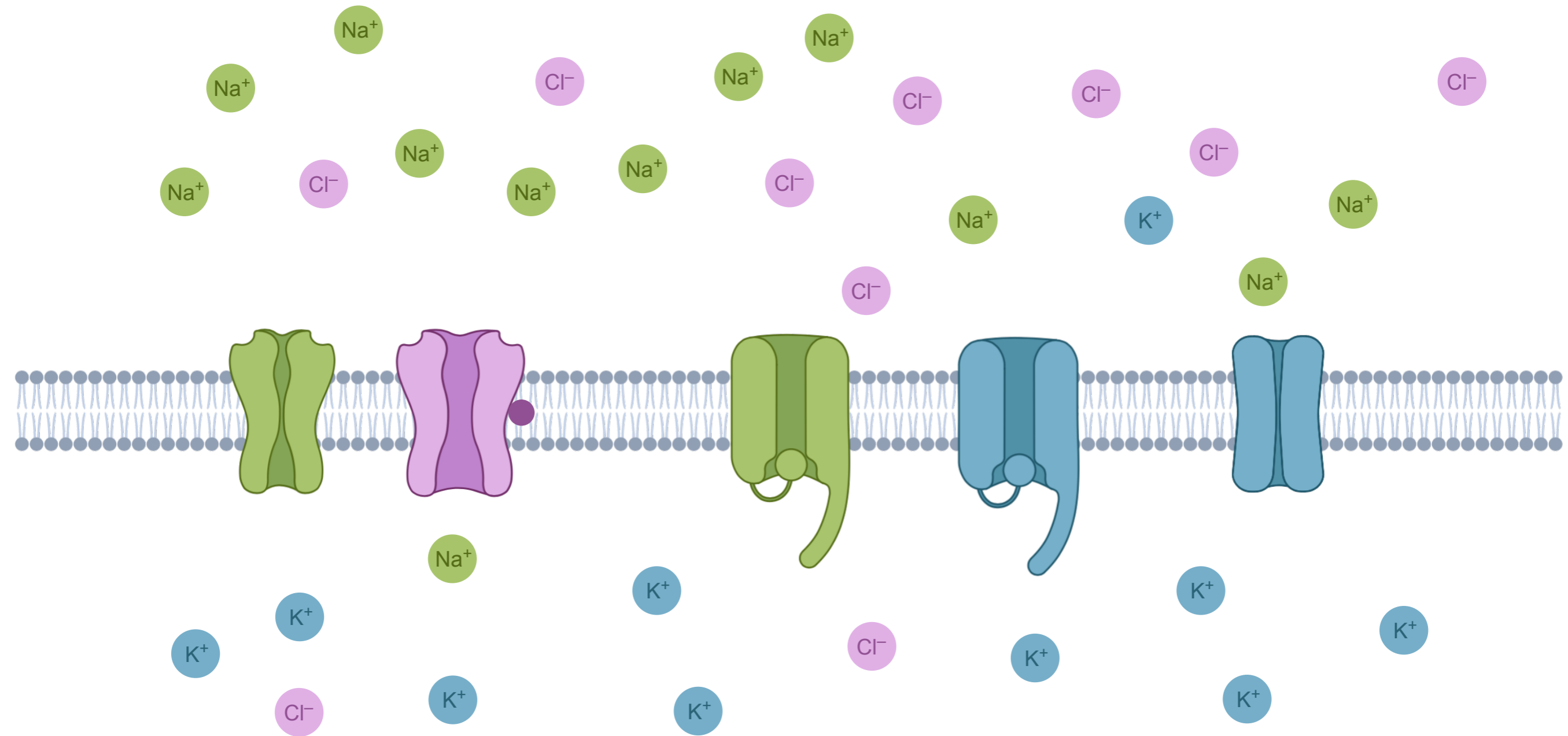


- “Leak” channel, opens and closes semirandomly
- Source of background  $K^+$  current, keeps resting potential stable
- Naturally regulated by temperature, pH, membrane stretch

# Why is neuron signaling inhibited?

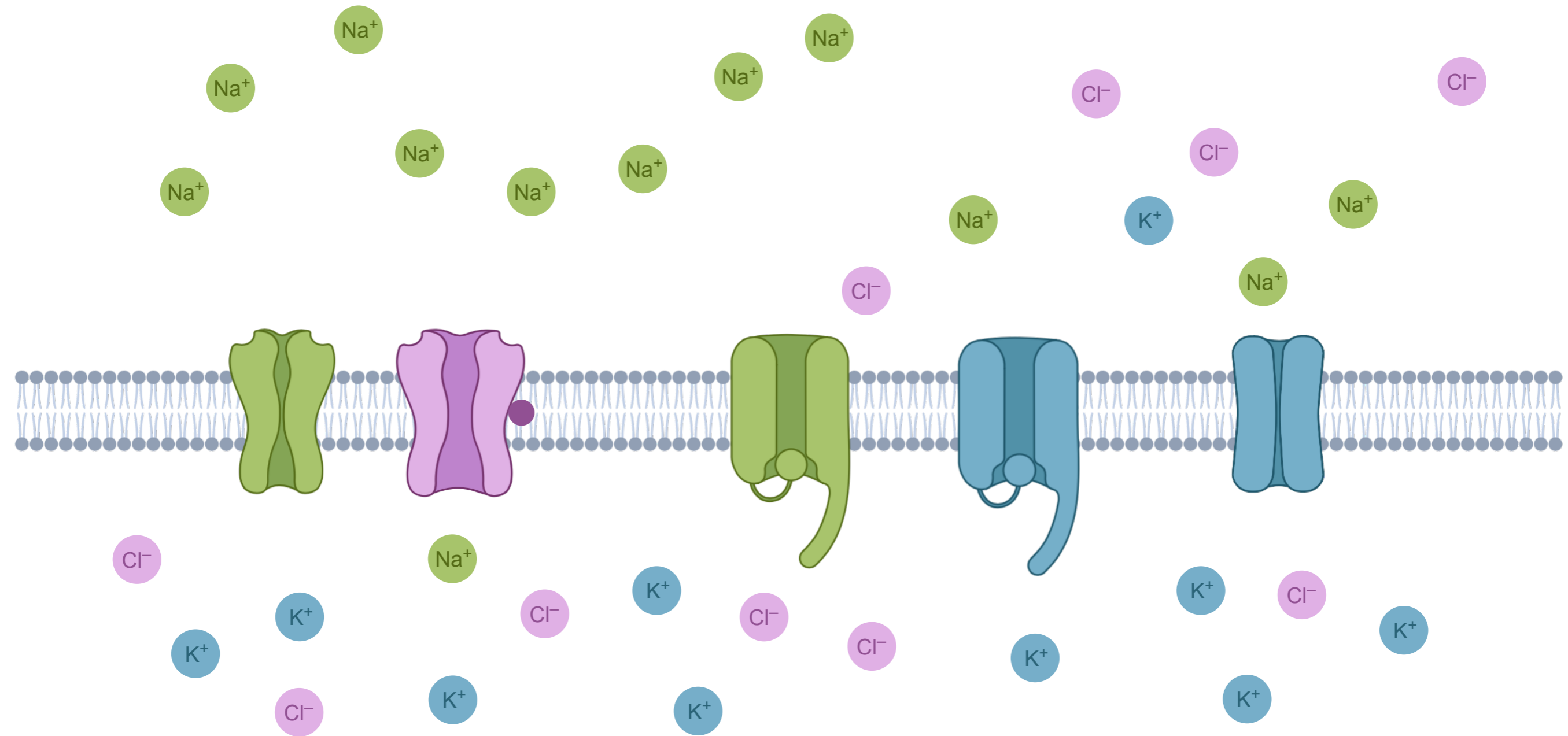


# Why is neuron signaling inhibited?

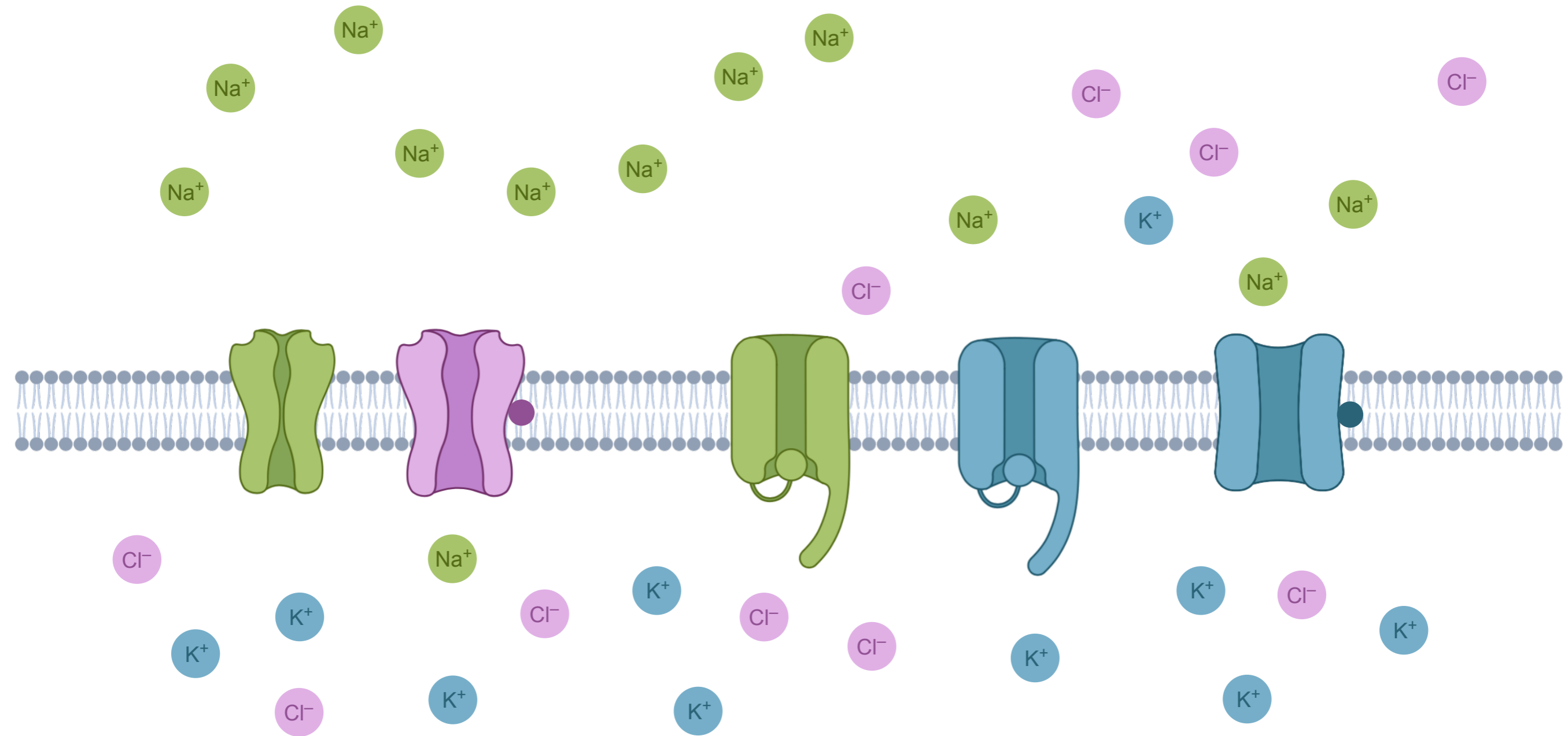




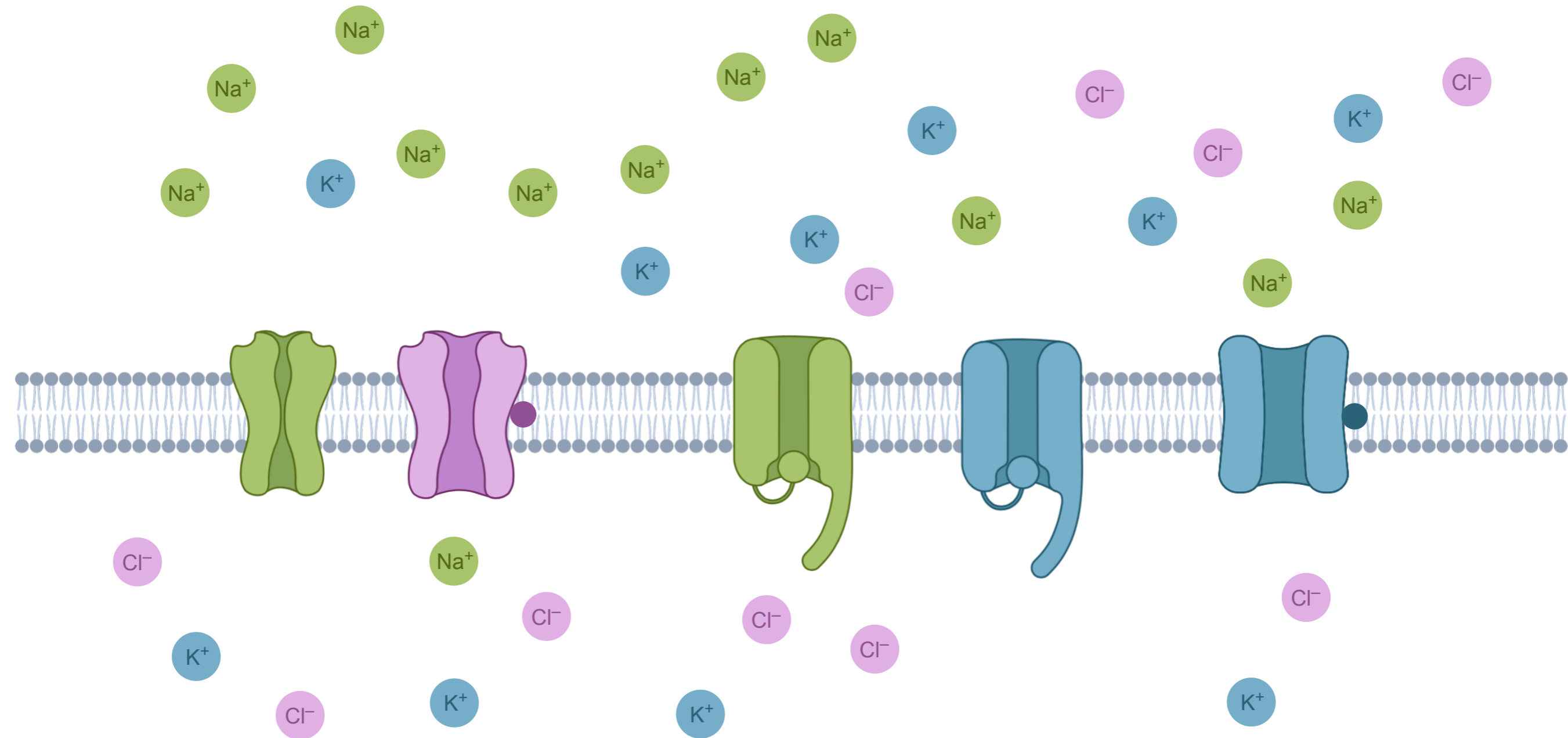
# Why is neuron signaling inhibited?



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# Why is neuron signaling inhibited?



**Activating either class of proteins causes hyperpolarization (more negative voltage gradient)**



## *Case study: identifying a general anesthetic binding site*

***Target: propofol binding site of human GABA<sub>A</sub> receptor***

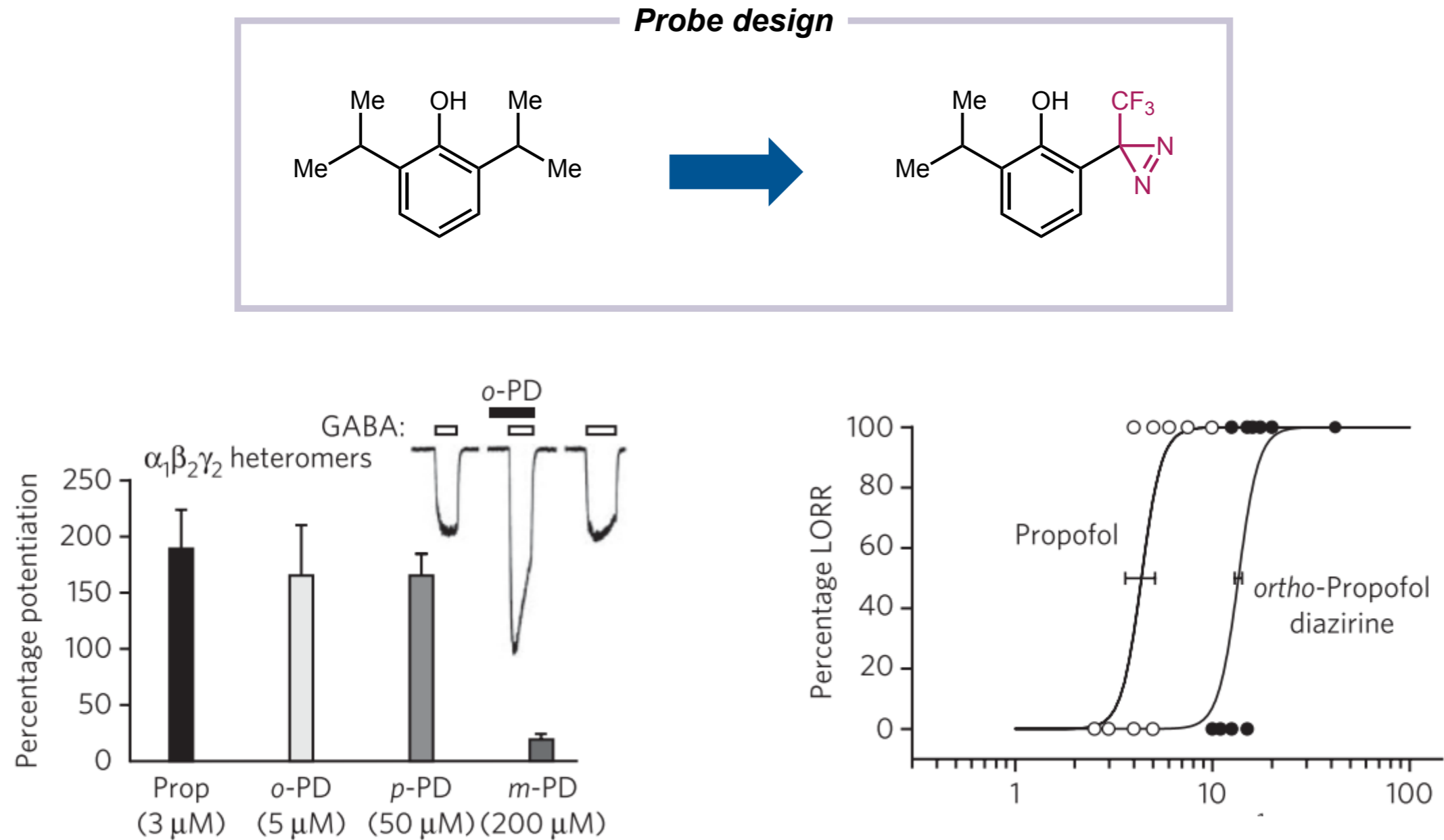
### **Challenges of finding a general anesthetic binding site:**

- Site-directed mutagenesis can cause small changes to conformation
- May alter other allosteric binding sites

### **A possible solution: photoaffinity labeling**

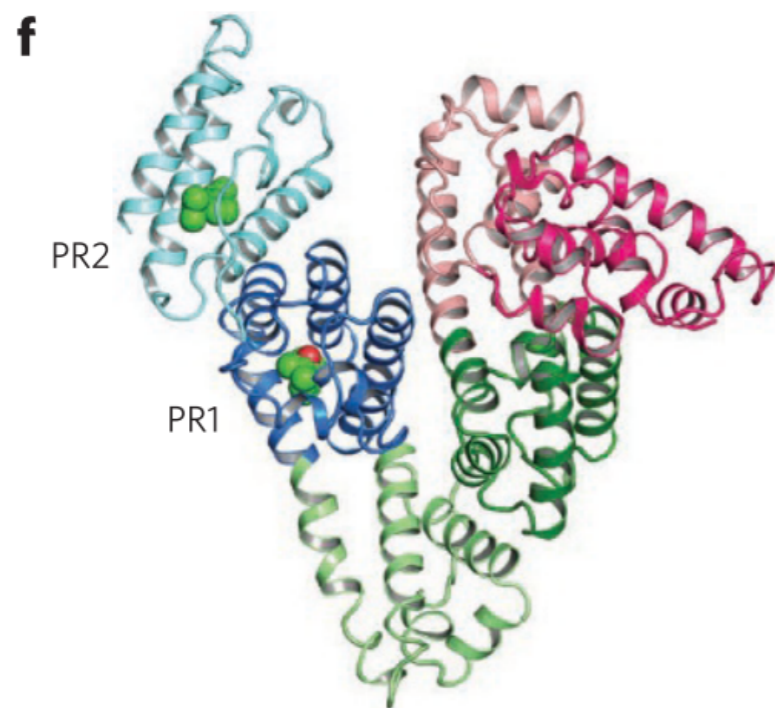
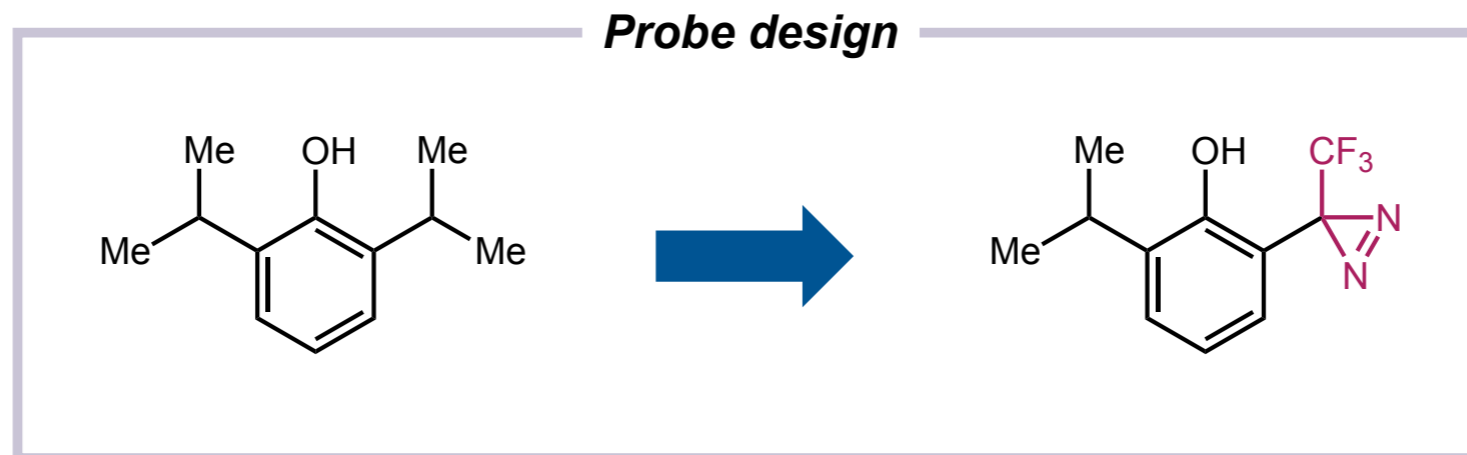
- No alteration of protein structure required
- However, only small modifications to ligand will be tolerated

## Case study: identifying a general anesthetic binding site



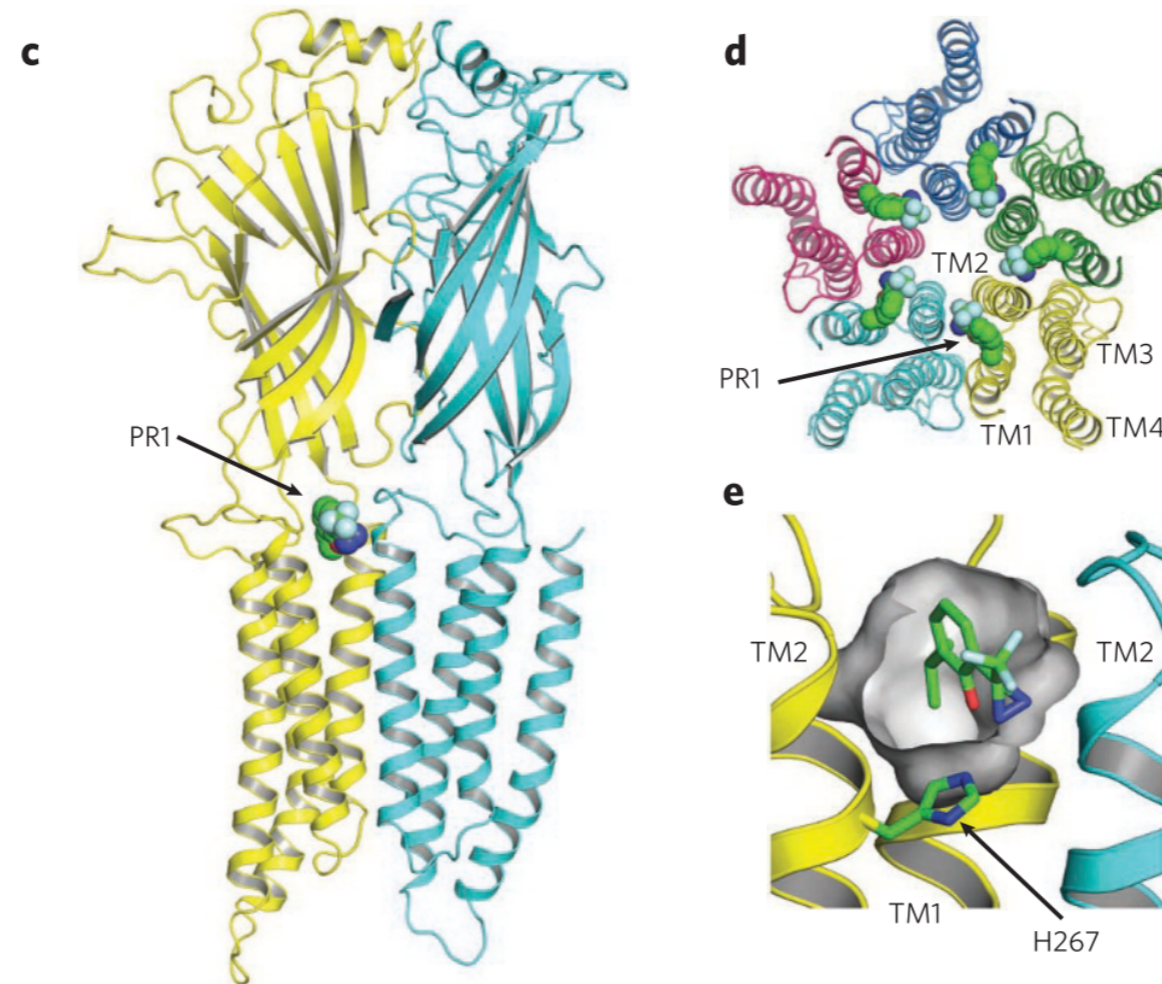
**Ortho-diazirine analog potentiates GABA<sub>A</sub> receptors and causes unconsciousness in animal models**

## Case study: identifying a general anesthetic binding site



**Further validation provided by identification of known binding sites in human serum albumin**

## Case study: identifying a general anesthetic binding site



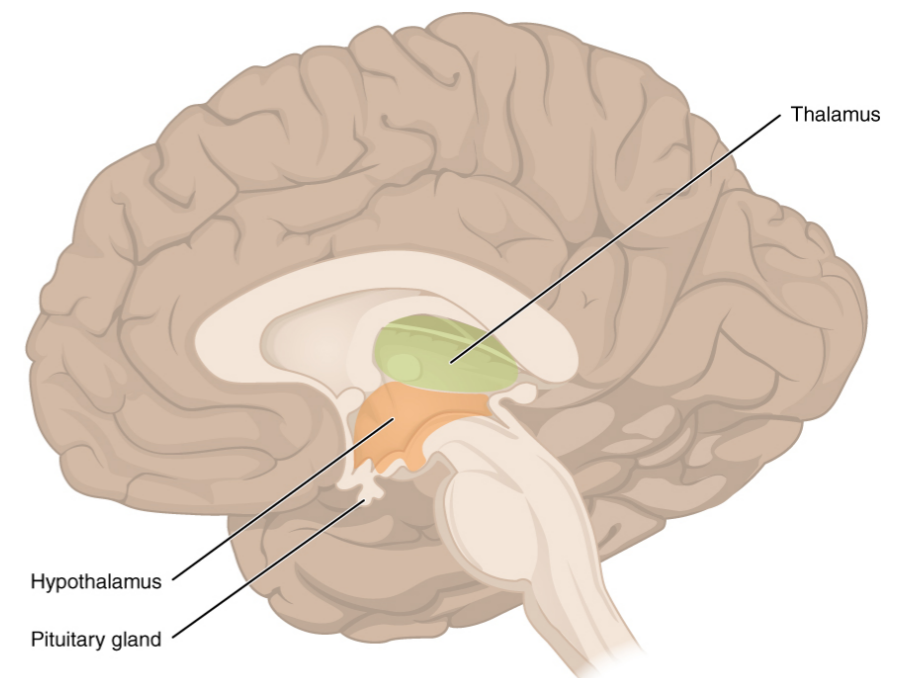
- Irradiation of probe and GABA<sub>A</sub> receptor in-membrane labels a single amino acid
- Binding site is a hydrophobic pocket accessible from the pore
- Mutation of residues in pocket alters propofol binding and protein activity

## Why does LOC occur?

**Why does inhibiting proteins critical to neural function cause reversible unconsciousness, rather than decreasing function while awake?**

Targets of local anesthetics are concentrated in the thalamus:

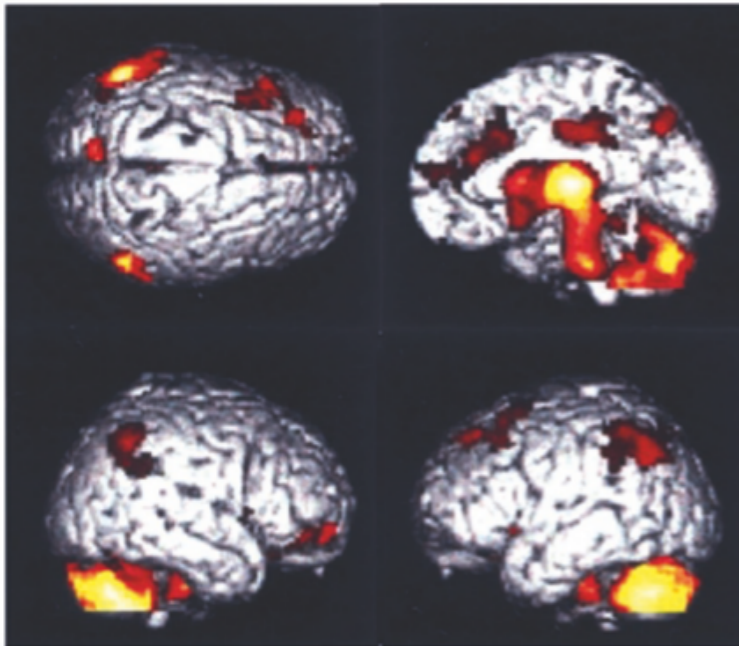
- Grey-matter section of the brain located in the forebrain
- Routes sensory signals to the cortex, does initial processing
- Regulates consciousness, alertness, and sleep



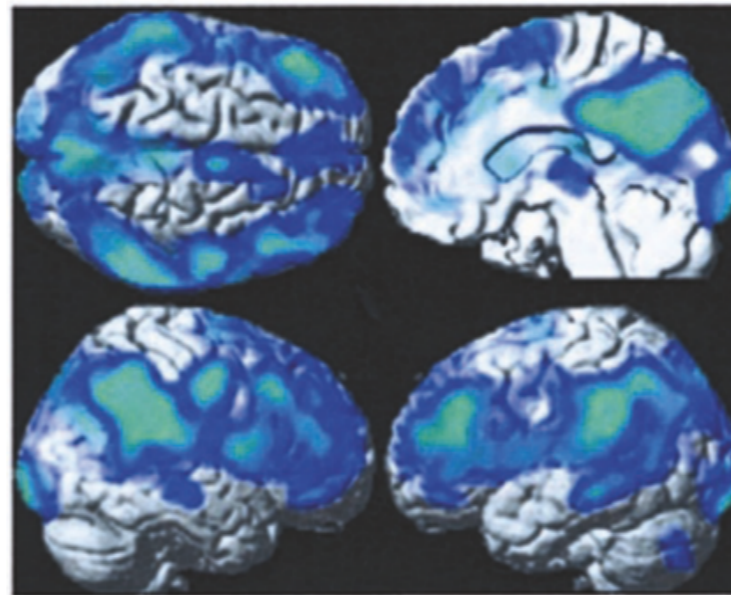


## Why does LOC occur?

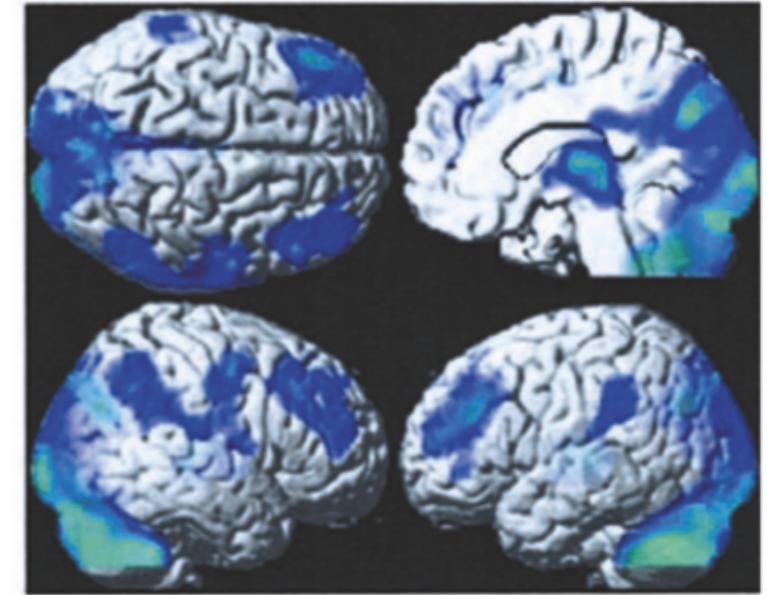
**b** NREM sleep



**c** Propofol LOC



**d** Sevoflurane LOC



*Decreased brain bloodflow is similar in deep sleep and anesthetic-induced unconsciousness*

## *Conclusion: it's complicated*

***“Discovery of a single fundamental mechanism, which seeks to explain the whole of anesthesia... has become less and less likely in recent years. The state of anesthesia is more likely to be based on many different effects and on multiple molecular biological targets.*”**