Pharmacologic Reversal of General Anesthesia using Competitive Antagonists: Proof of Principle

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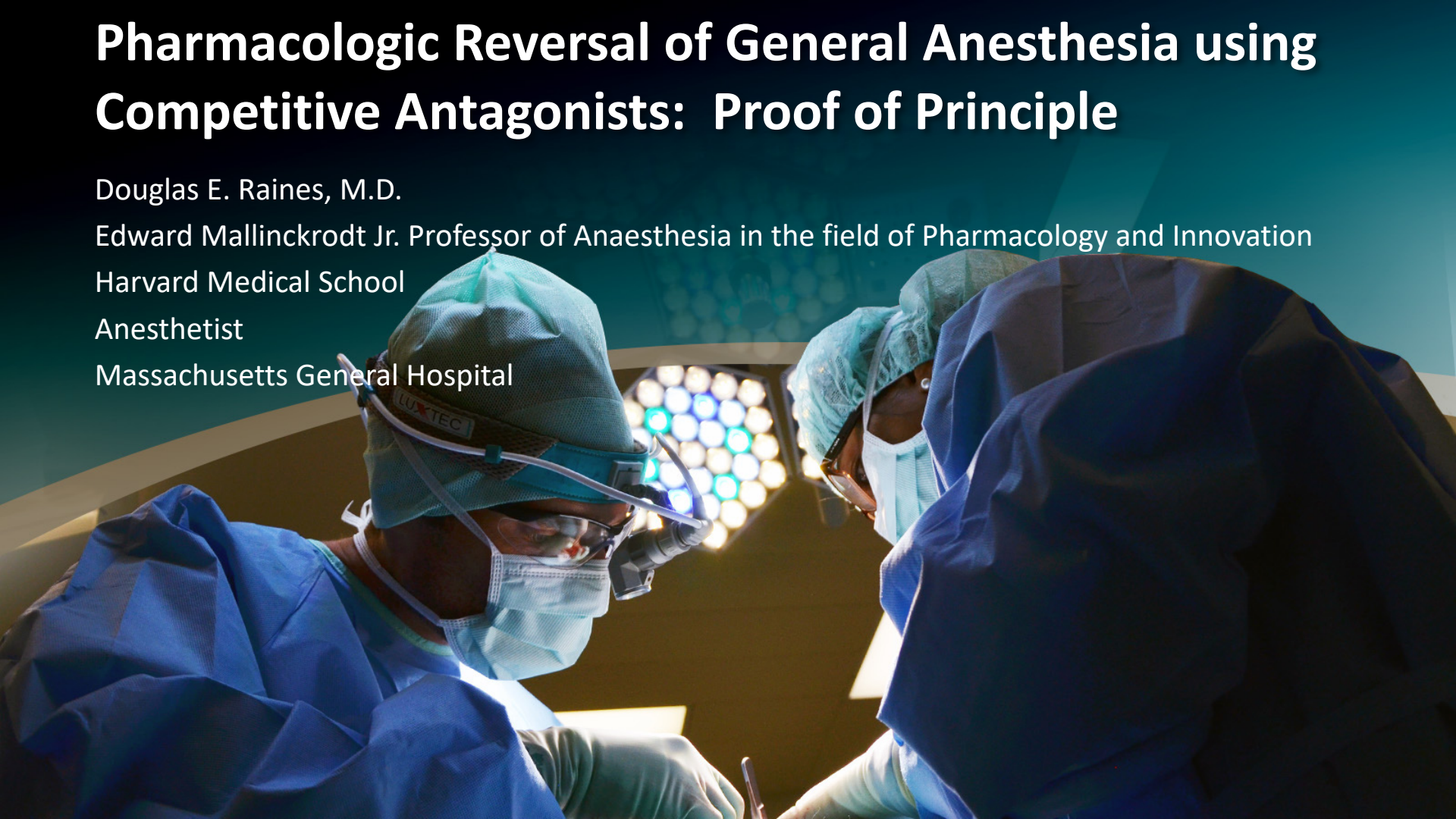


Outline

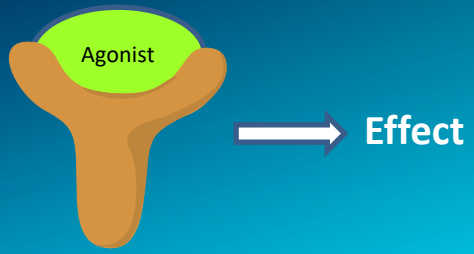
- Competitive Antagonists
- $GABA_A$ receptor structure and ligand binding sites
- Antagonism of anesthetic action on $GABA_A$ receptors *in vitro*
- Antagonism of anesthetic action *in vivo*

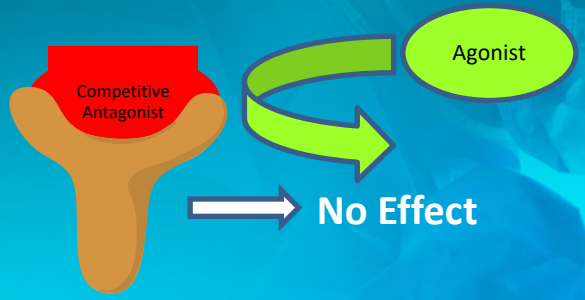
Disclosures

None

Competitive Antagonist Key Features

- Bind to the same molecular binding site as the active drug (i.e. the agonist)
- Have relatively low intrinsic efficacy (i.e. they minimally modulate target action)



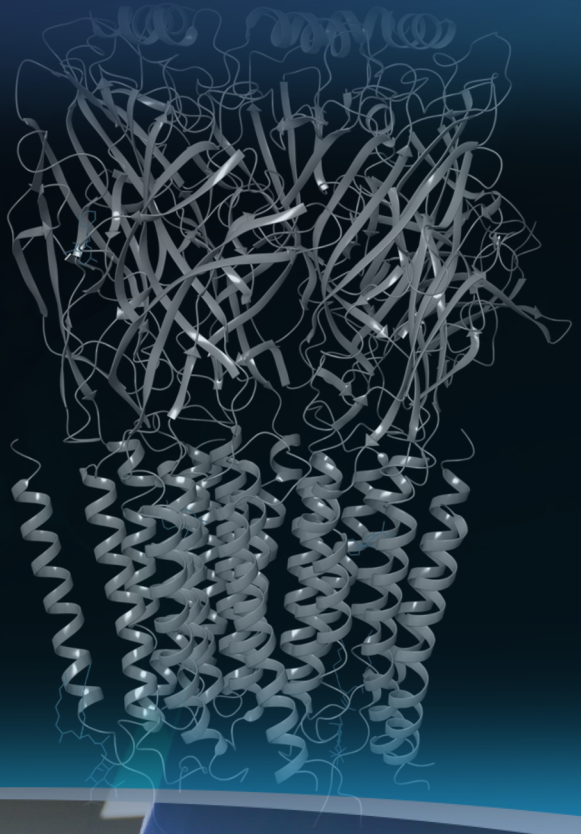


Clinically-Used Competitive Antagonists

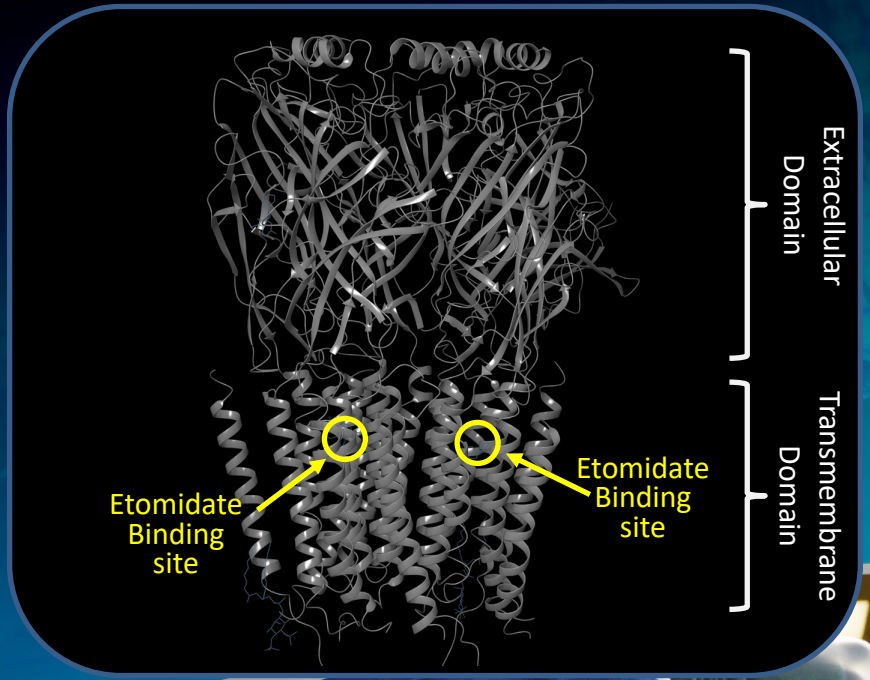
- Naloxone (Narcan®): Opiate antagonist
- Flumazenil: Benzodiazepine antagonist
- Rocuronium: Acetylcholine antagonist

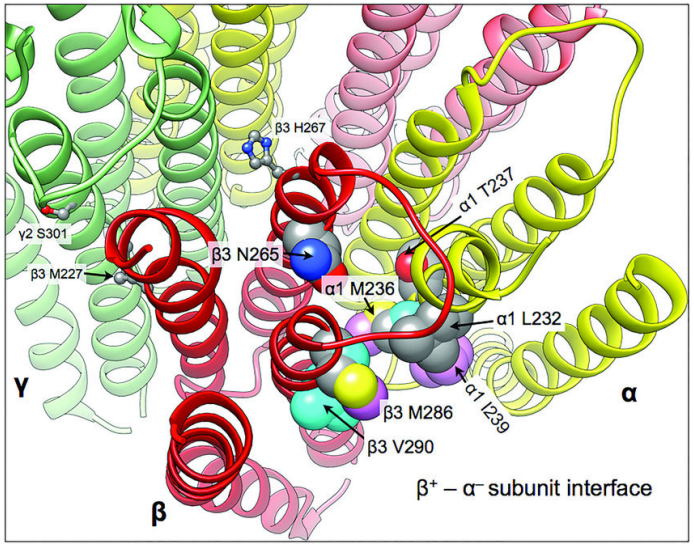
$GABA_A$ Receptor

- Etomidate
- Propofol
- Barbiturates
- Alcohols
- Volatile agents



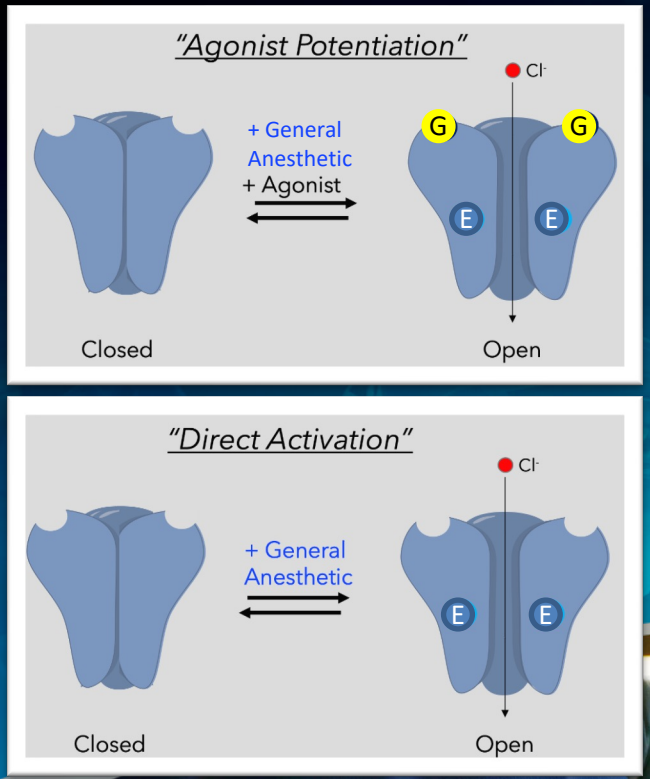
Etomidate Binding Site on the $\alpha_1\beta_3\gamma_2$ $GABA_A$ Receptor

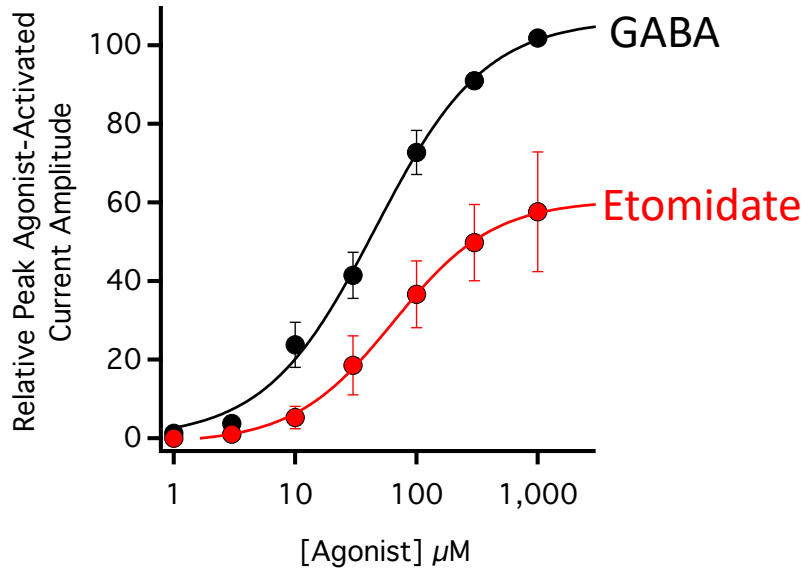




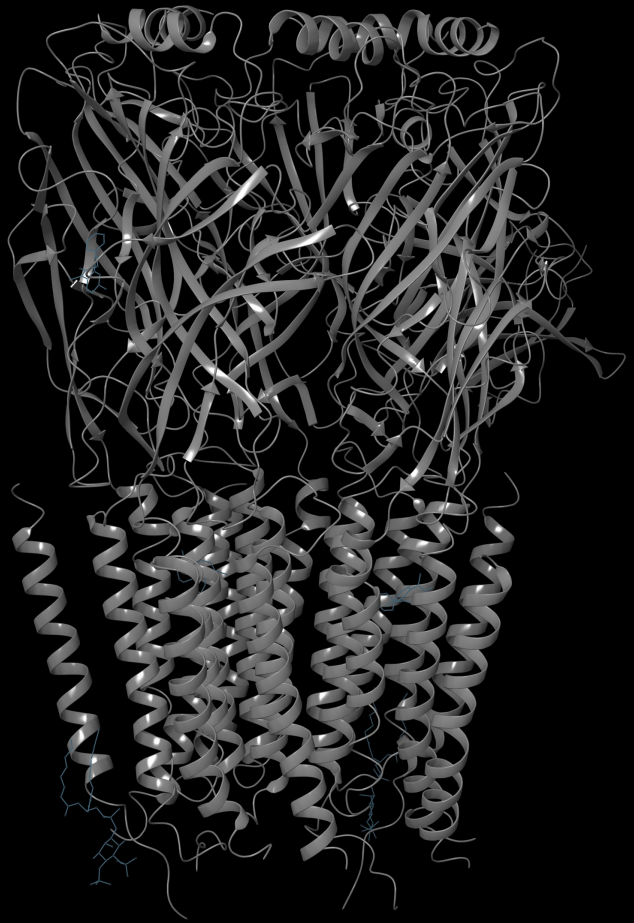
Transmembrane view

$GABA_A$ Receptor





$\alpha_1\beta_3\gamma_2$ $GABA_A$ Receptor



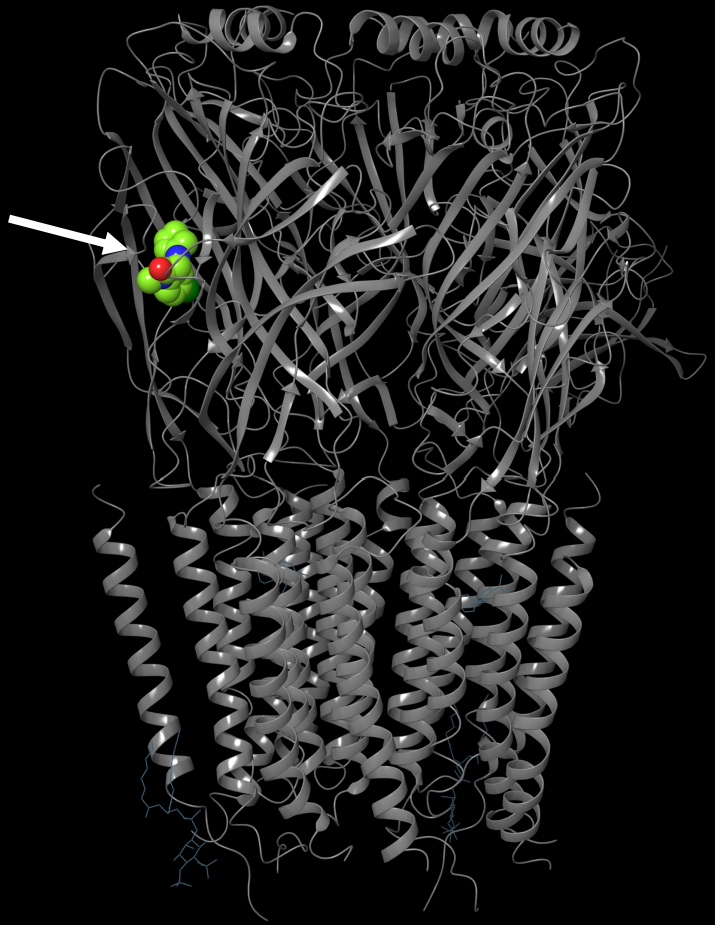
Extracellular Domain

Transmembrane Domain

Zivanov J et al.: GABAA receptor signalling mechanisms revealed by structural pharmacology. Nature 2019; 565: 454-459

$\alpha_1\beta_3\gamma_2$ $GABA_A$ Receptor

Diazepam
(at the classical benzodiazepine binding site)



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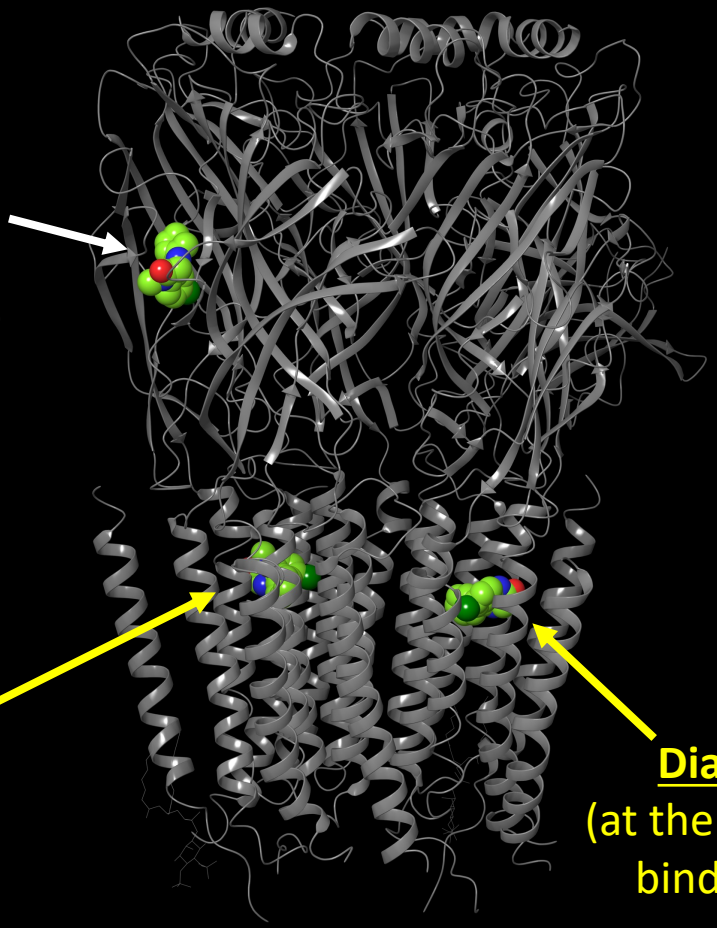

Diazepam
(at the classical benzodiazepine binding site)

Diazepam
(at the etomidate binding site)

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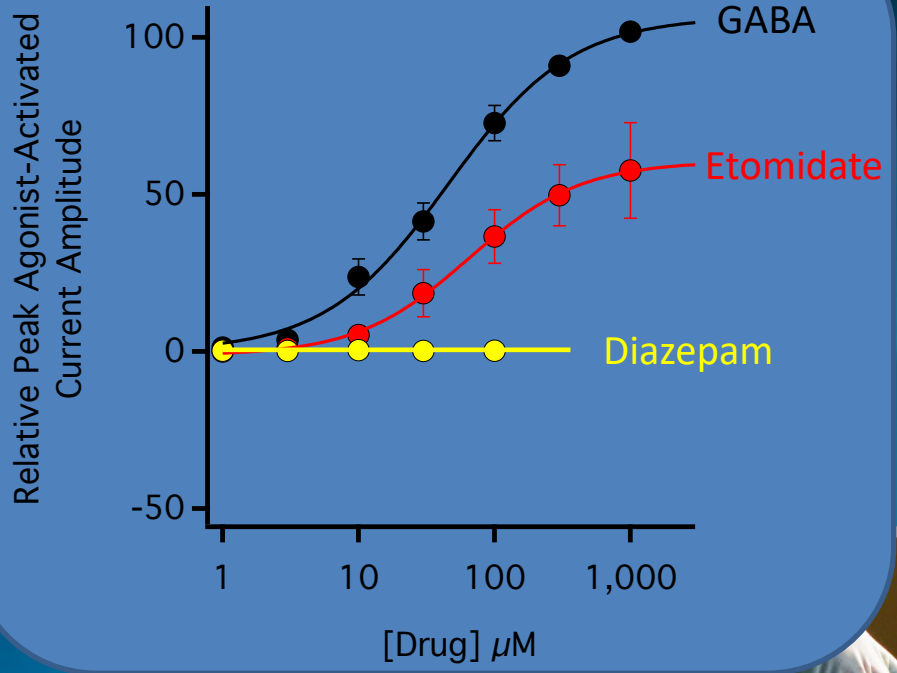
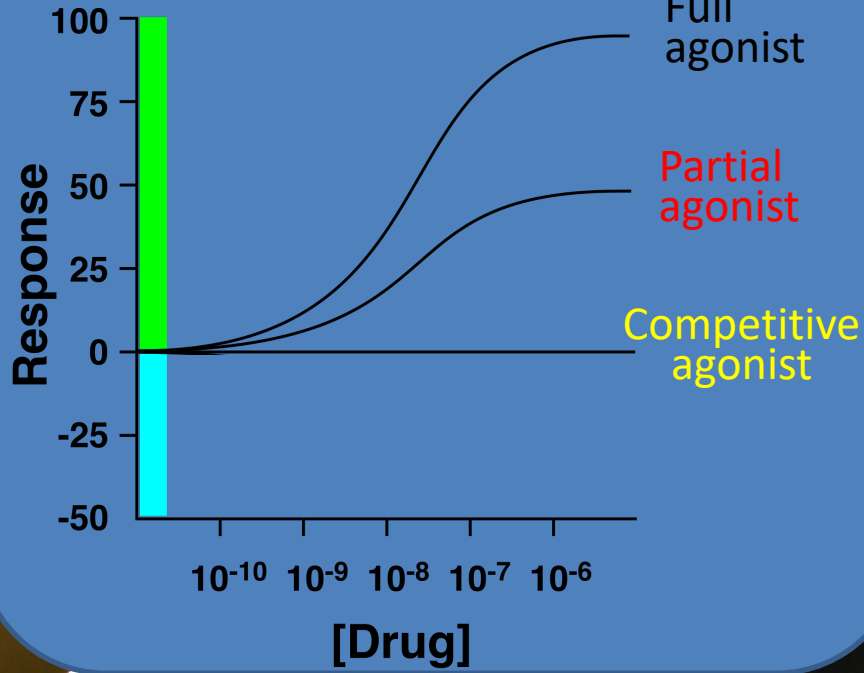
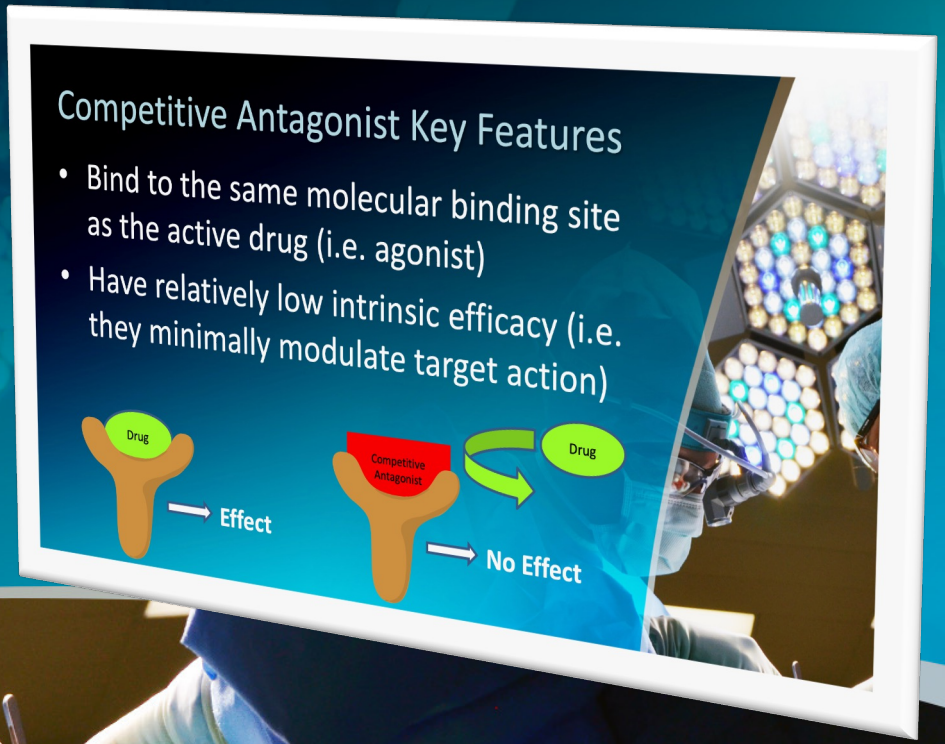
Direct Activation of $\alpha_1\beta_3\gamma_2$ $GABA_A$ Receptors



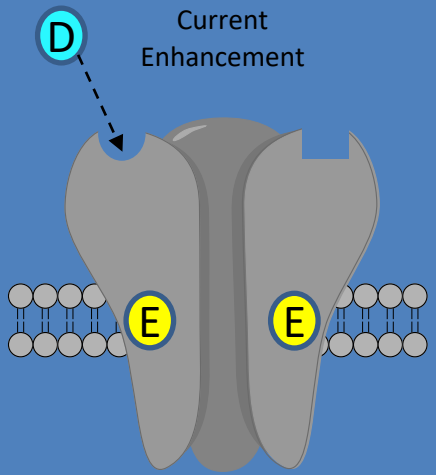


Diazepam has relatively low positive modulatory efficacy

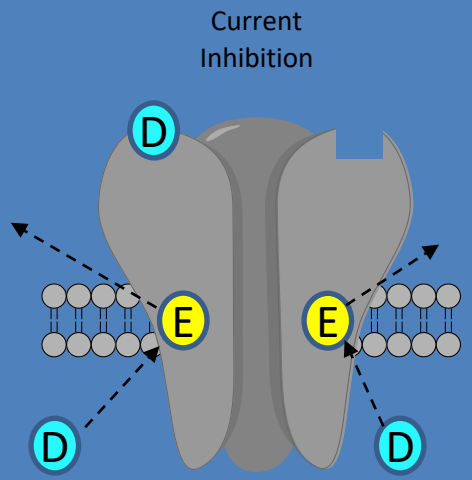
Could Diazepam Act as a Competitive Antagonist for Etomidate at the $GABA_A$ Receptor?



nM Diazepam Concentrations



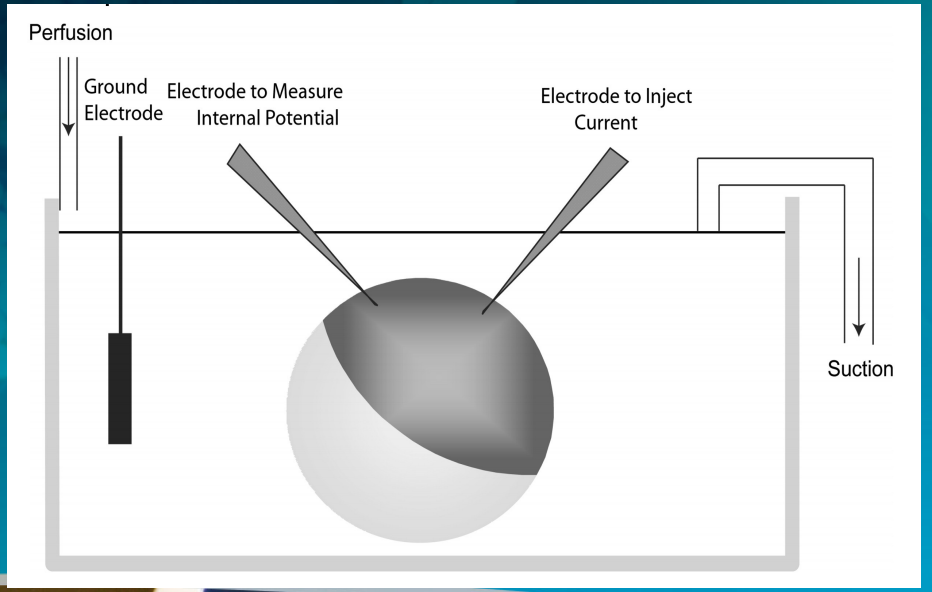
µM Diazepam Concentrations



At the etomidate binding site:
Diazepam efficacy << Etomidate efficacy

$GABA_A$ Receptor Electrophysiology

- Inject oocytes with RNA encoding for $GABA_A$ receptor subunits
- Clamp transmembrane potential at 50 mV.
- Directly activate receptors with etomidate (or GABA, as a control) +/- diazepam
- Record the electrophysiological response
- **Predictions:**
  - At concentrations where diazepam binds to the classical high affinity benzo site, it will potentiate both etomidate-activated and GABA-activated currents.
  - However, at concentrations where diazepam binds to the etomidate binding site, it will reduce etomidate-activated (but not GABA-activated) currents.



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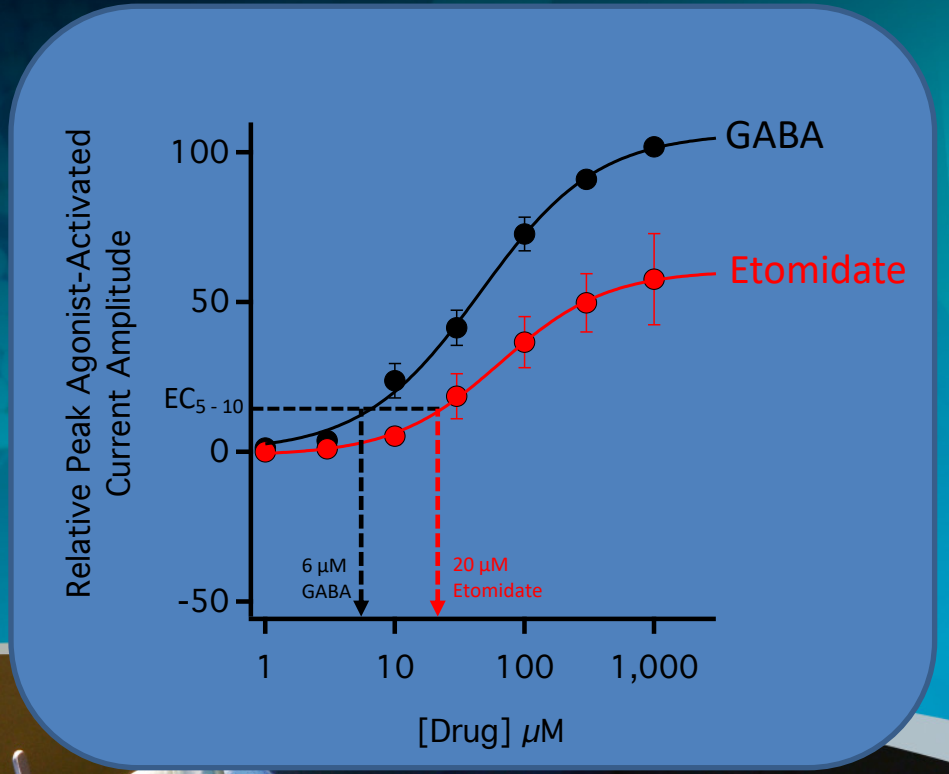
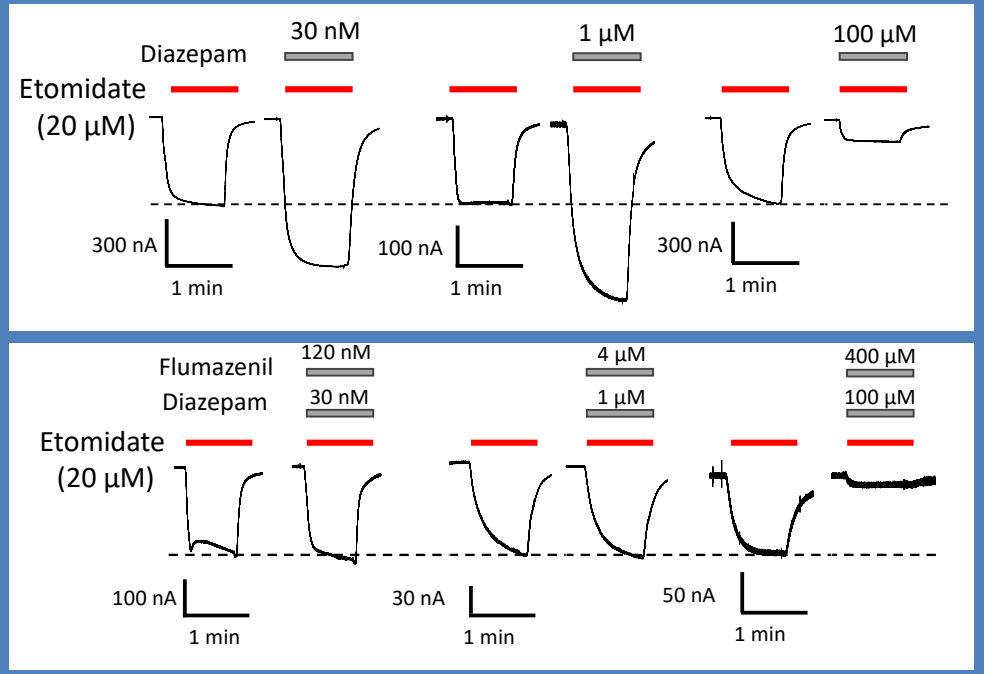
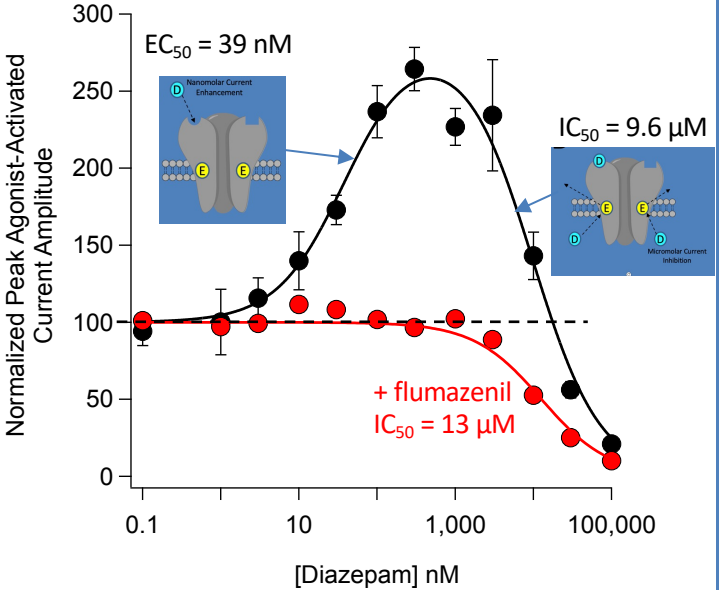
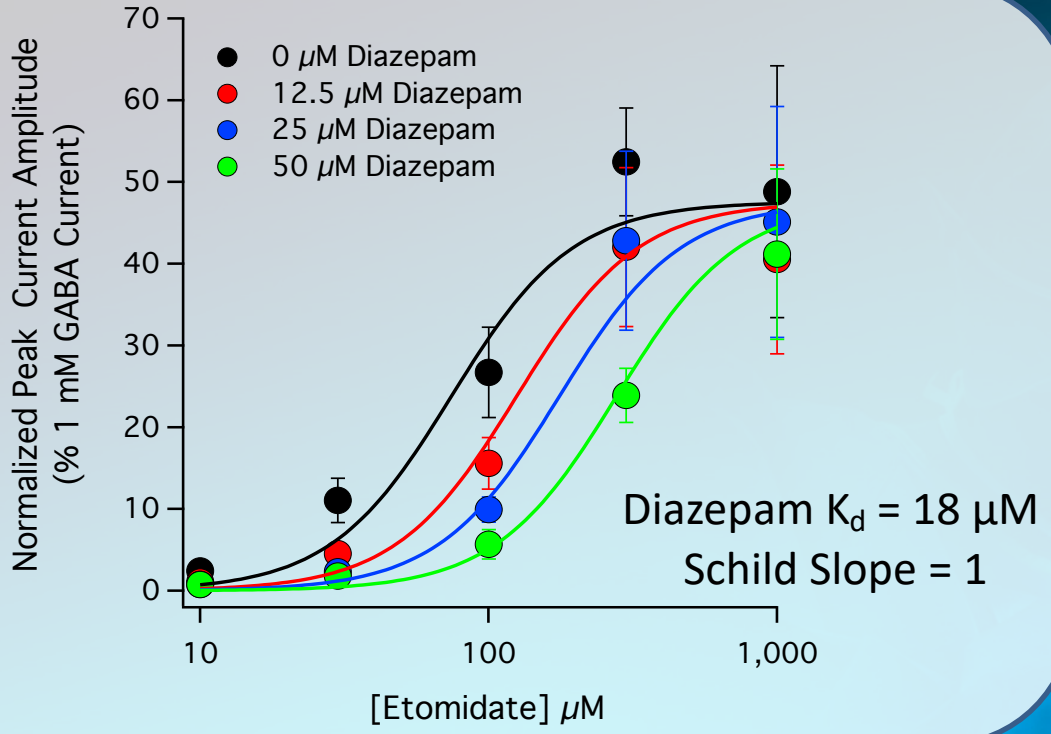


Diazepam Potentiates at Nanomolar Concentrations But Inhibits at Micromolar Ones

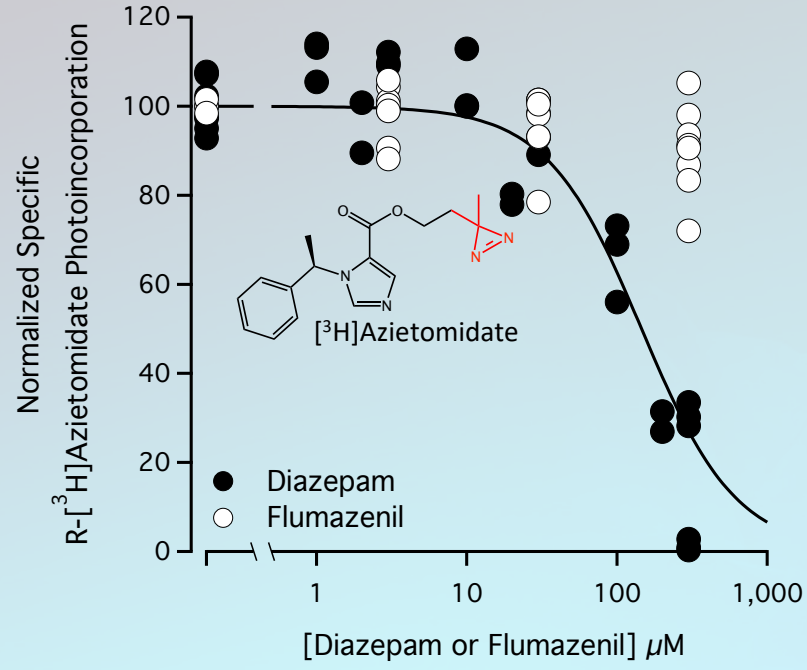




Schild Analysis Indicates Competition



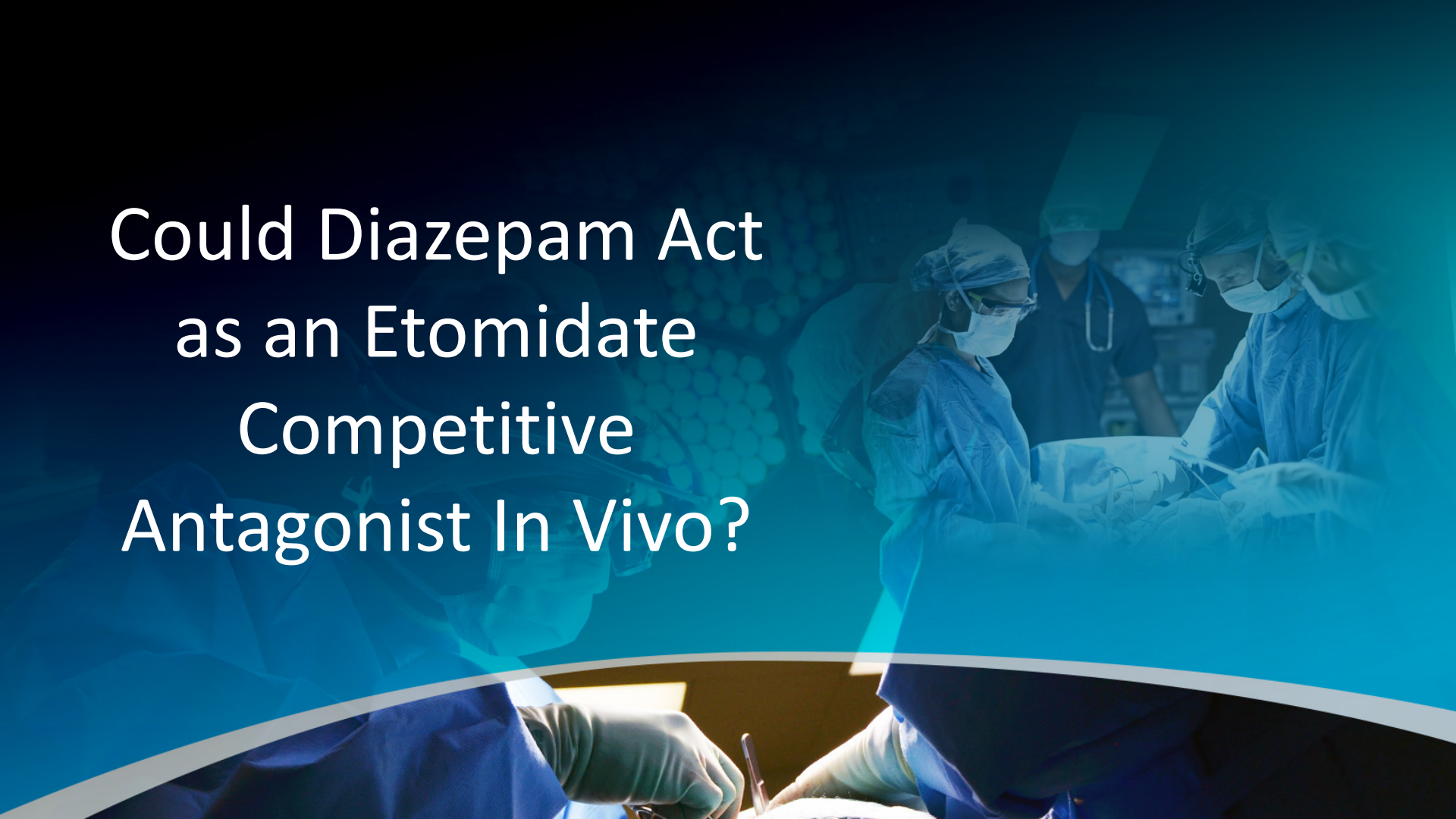
Diazepam Inhibits Photoaffinity Labeling of Purified $GABA_A$ Receptors by [^{3}H]Azietomidate



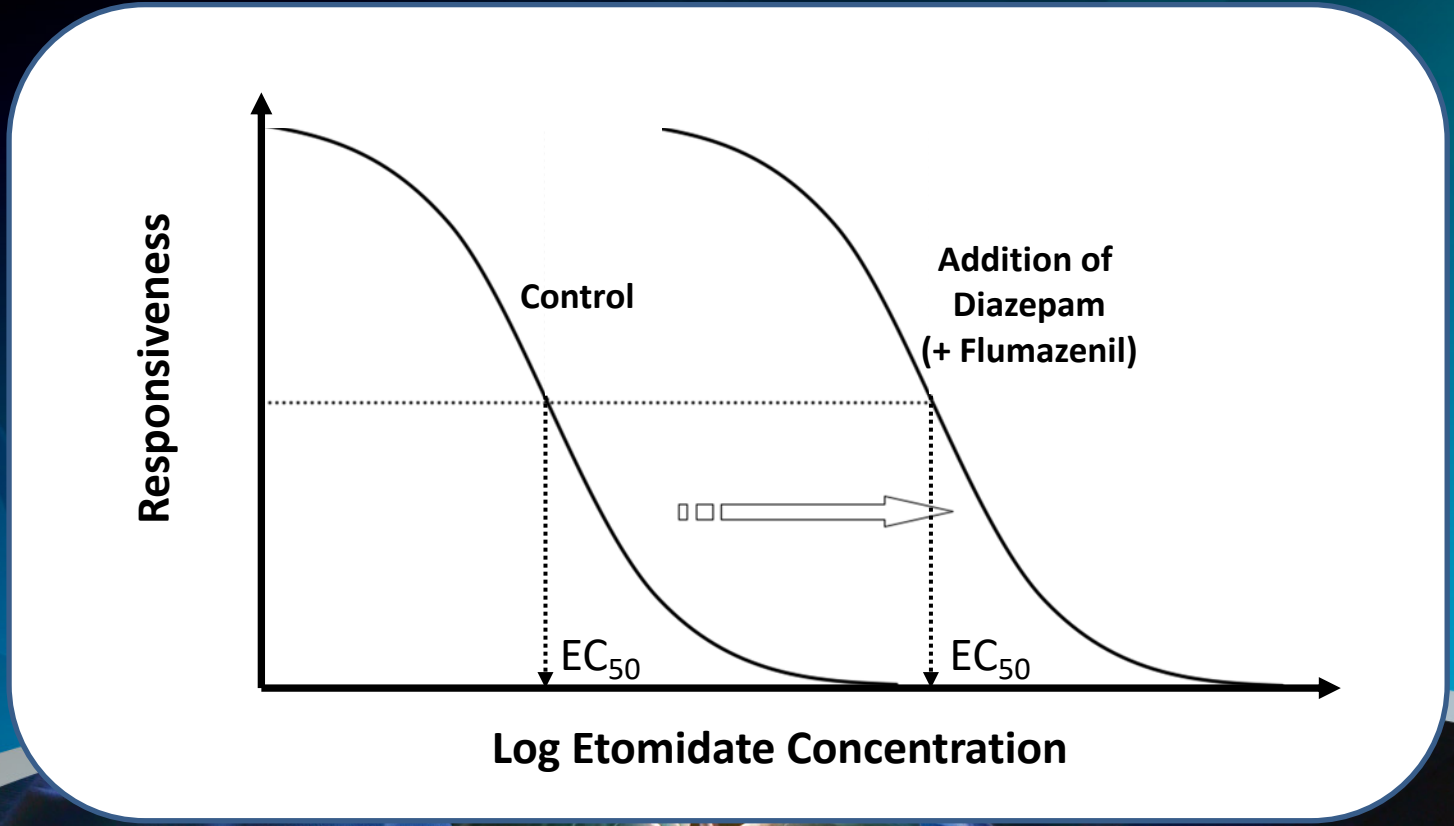
$GABA_A$ Receptor Summary: Diazepam Acts as a Competitive Etomidate Antagonist

- Binds to the etomidate binding site (K_d: ~10 – 20 µM)
- Low intrinsic efficacy at the $GABA_A$ receptor relative to etomidate
- Selectively antagonizes etomidate-activated currents
- Shifts the etomidate concentration-response curve for direct activation rightward
- Inhibits photolabeling of $GABA_A$ receptors by R-[^{3}H]azietomidate.

Could Diazepam Act as an Etomidate Competitive Antagonist In Vivo?

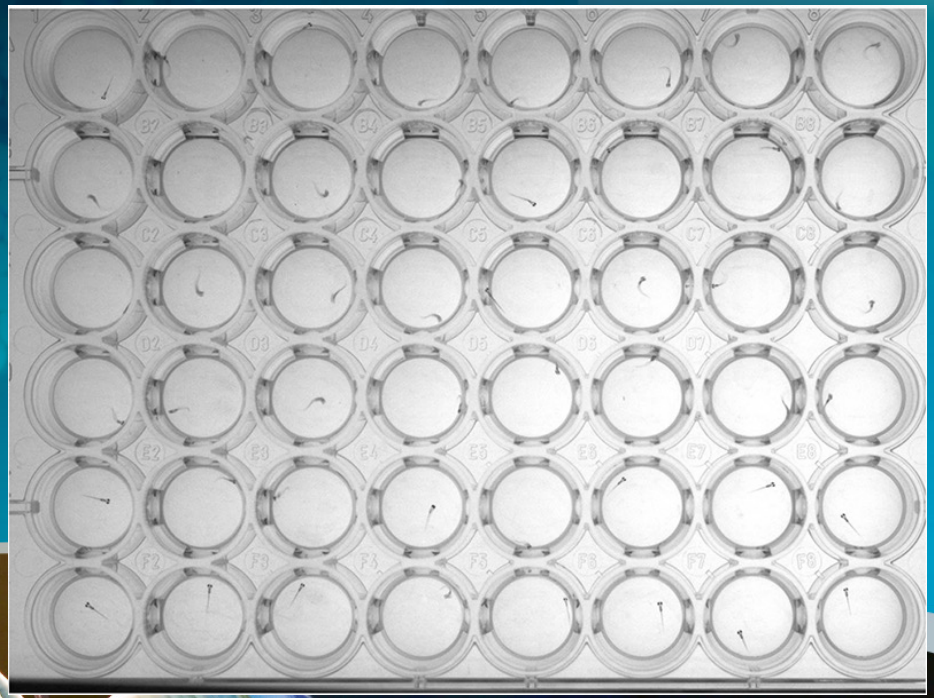


Competitive Antagonism of Etomidate Anesthesia



Zebrafish Larvae Photomotor Response

- Steady-state drug concentrations
- Minimal effect of protein binding on free-aqueous drug concentrations
- Large number of animals can be easily studied
- Fully automated



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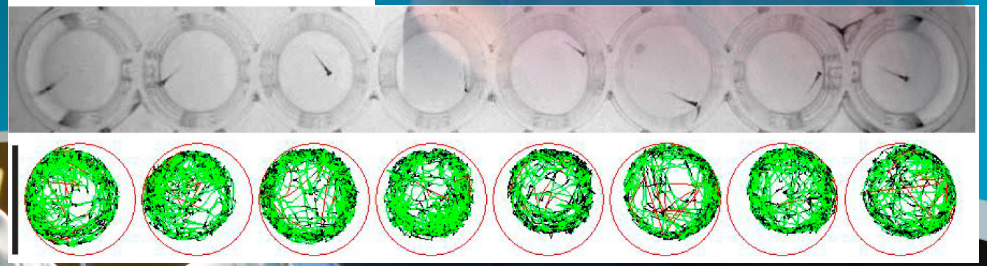


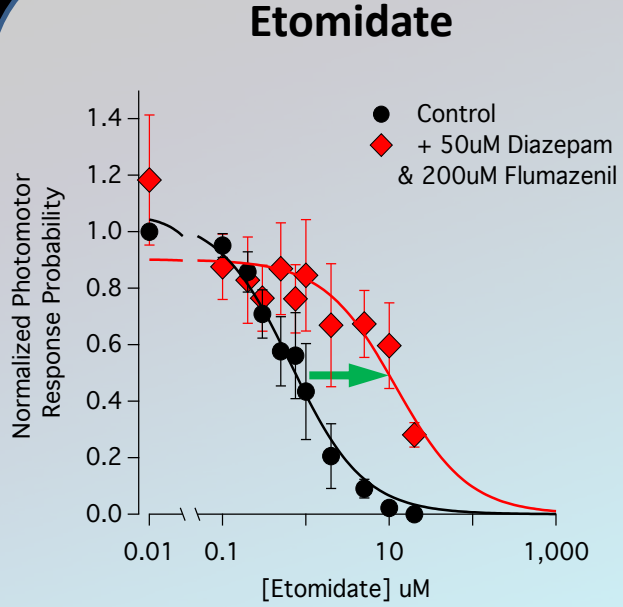
Zebrafish Larvae Photomotor Response

Etomidate (5 µM) alone

Etomidate (5 µM) + Flumazenil (200 µM)

Etomidate (5 µM) + Flumazenil (200 µM) + **Diazepam (50 µM)**

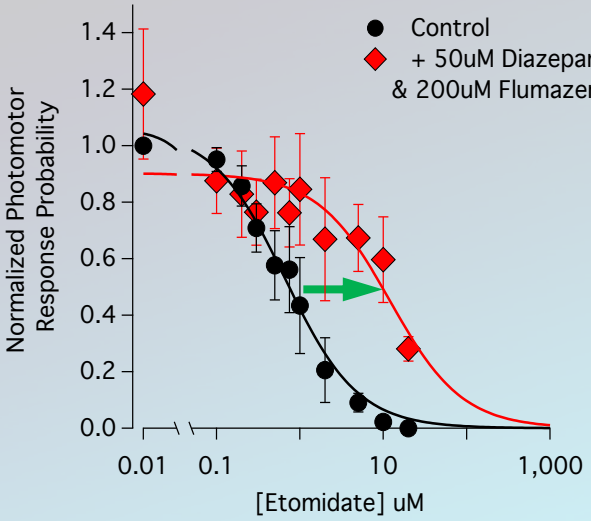
Competitive Antagonism of Etomidate Anesthesia



EC_{50} = 0.66 µM ➞ 12 µM
($p < 0.0001$)

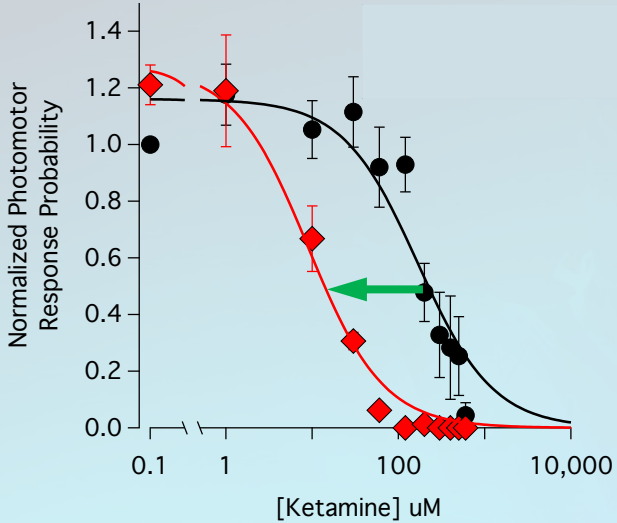
Competitive Antagonism of Etomidate Anesthesia

Etomidate



EC_{50} = 0.66 µM ➞ 12 µM
($p < 0.0001$)

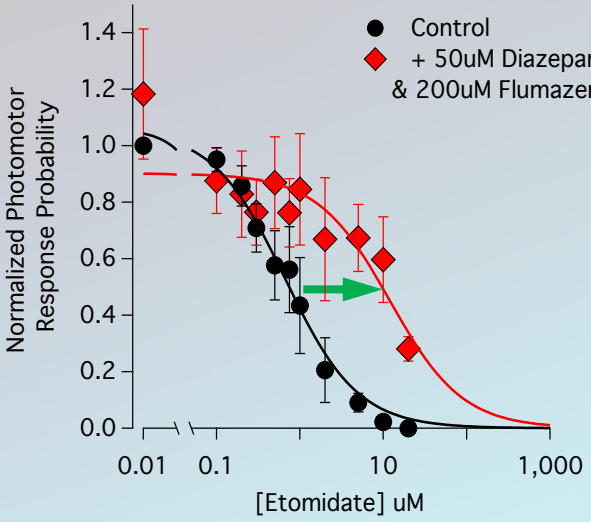
Ketamine



EC_{50} = 170 µM ➞ 9 µM
($p < 0.0001$)

Competitive Antagonism of Etomidate Anesthesia

Etomidate



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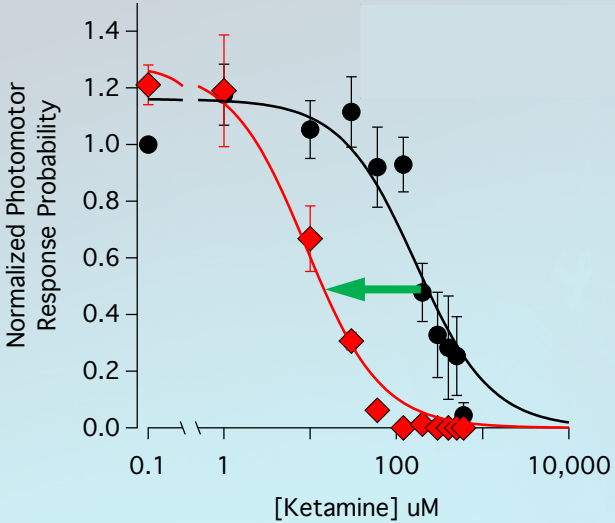
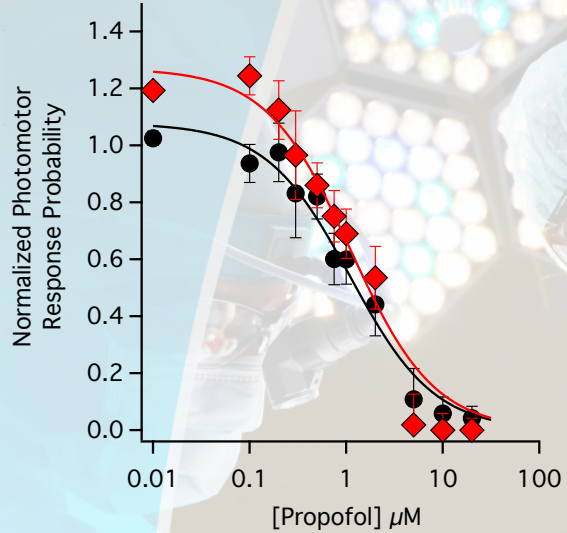


EC_{50} = 170 µM ➞ 9 µM
($p < 0.0001$)

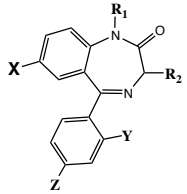
Propofol



EC_{50} = 0.75 µM ➞ 1.1 µM
(NS)

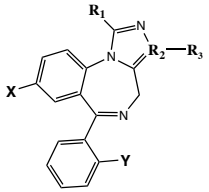
Do Other Benzodiazepines Act Similarly?

A 5-Aryl-1,4-benzodiazepines



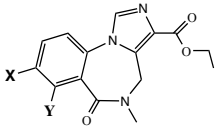
Name	X	Y	Z	R_1	R_2
Diazepam	Cl	H	H	CH_3	H
Nordiazepam	Cl	H	H	H	H
Nitrazepam	NO_2	H	H	H	H
Lorazepam	Cl	Cl	H	H	OH
Fludiazepam	Cl	F	H	CH_3	H
1-Me	H	H	H	CH_3	H
7-Me	CH_3	H	H	H	H
1,4-Me	H	H	CH_3	H	H
1,7-Me	H	H	CH_3	CH_3	H

B Diazolo- and triazolo-benzodiazepines

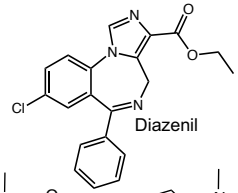


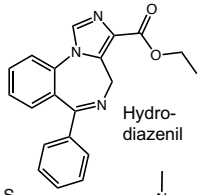
Name	X	Y	R_1	R_2	R_3
Midazolam	Cl	F	CH_3	C	H
Alprazolam	Cl	H	CH_3	N	-
Estazolam	Cl	H	H	N	-
Imidazenil	H	Br	H	C	$CONH_2$

C Benzodiazepine antagonists



Name	X	Y
Flumazenil	F	H
Bromazenil	Br	H
Iomazenil	H	I





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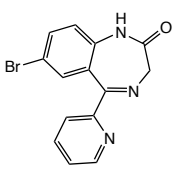
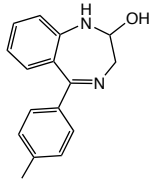
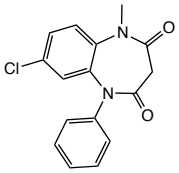
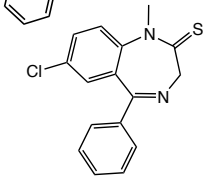
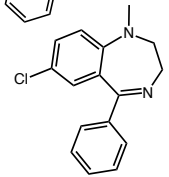


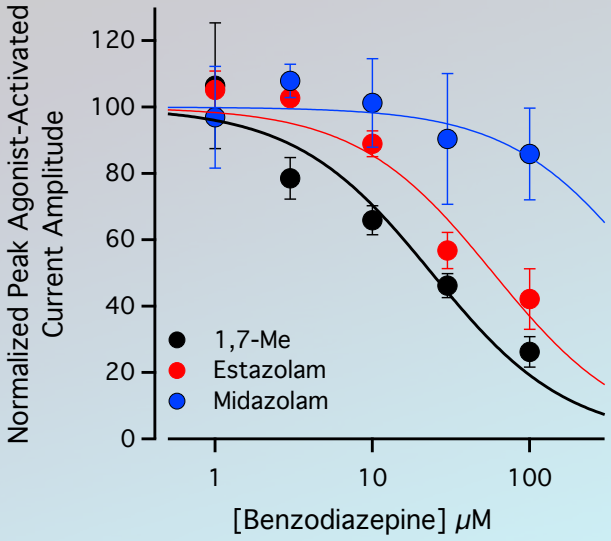


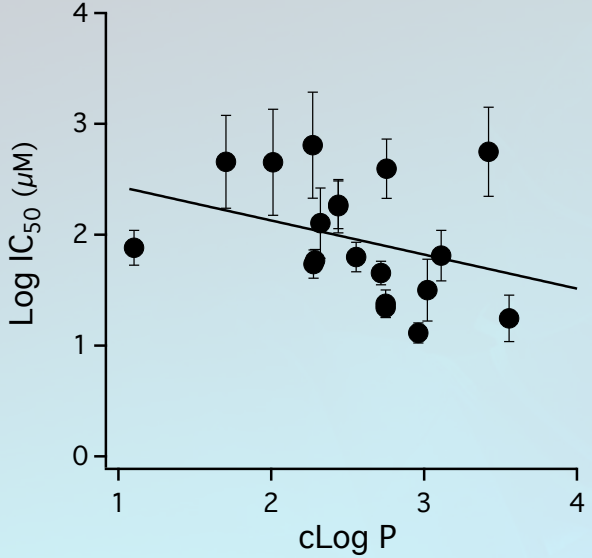






Do Other Benzodiazepines Act Similarly?





Summary

- Diazepam binds to the etomidate binding sites of the $GABA_A$ receptor
- Such binding competitively displaces etomidate from this site
  - Antagonizes etomidate action at the $GABA_A$ receptor *in vitro*
  - Antagonizes etomidate action *in vivo*
- Other benzodiazepines also bind to the etomidate binding site with ranging apparent affinities
- Benzodiazepines may represent a chemical template upon which $GABA_A$ receptor-selective anesthetic reversal agents can be designed

Thank You