Accelerating Anesthetic Drug Discovery and Mechanisms Research with Zebrafish

Stuart A. Forman, M.D., Ph.D.
DACCPM, Massachusetts General Hospital

ISAP, October 12, 2018

Conflict of Interest Statement

I am named as a co-inventor on several drug patents that are owned by MGH. I have no direct conflict of interest related to the content of this presentation.

The problem

60 Million General Anesthetics Annually in USA

Increasing demands for efficiency & safety.
Increasing age & risk in population.
Increasing outpatient procedures: low tolerance for toxicity.

General Anesthetic Goals:

Reversible Unconsciousness, Amnesia, & Immobilization.

Problems:

Pharmacodynamics: Side-effects include: cardiovascular depression, respiratory compromise, hypothermia, arrhythmias, adrenal suppression, post-operative nausea and vomiting, post-operative delirium, and neurodevelopmental effects in neonates.
Pharmacokinetics: Variable and unpredictable.

Risk Factors:

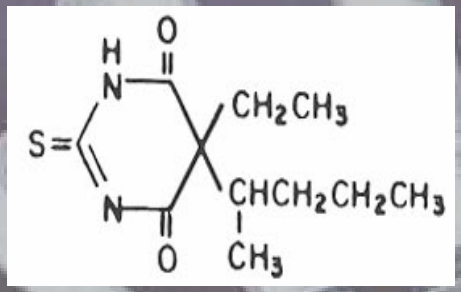
Extremes of age, systemic diseases.

Inhaled General Anesthetics

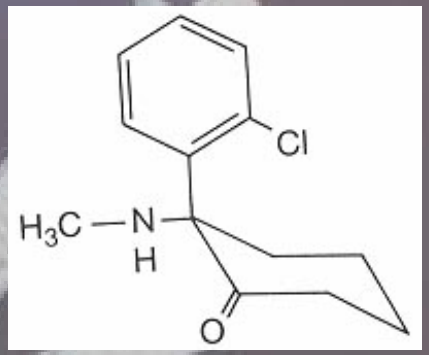
	Hydrocarbons	Ethers	Others
1840-1949	Chloroform Cyclopropane Ethylene	Diethyl Ether	Nitrous Oxide
1950-1989	Halothane	Enflurane Methoxyflurane Isoflurane Fluroxene	Xe Xenon
1990-		Desflurane US-1990 Sevoflurane US-1995	

Intravenous General Anesthetics

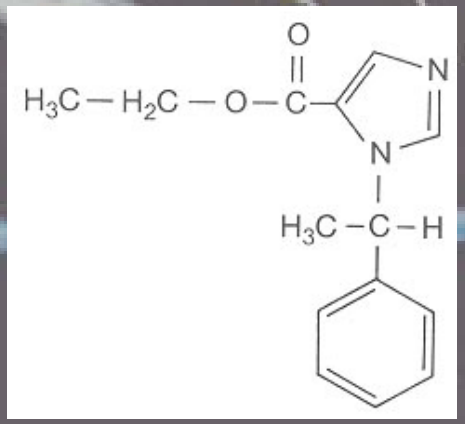
Barbiturates (1930s)



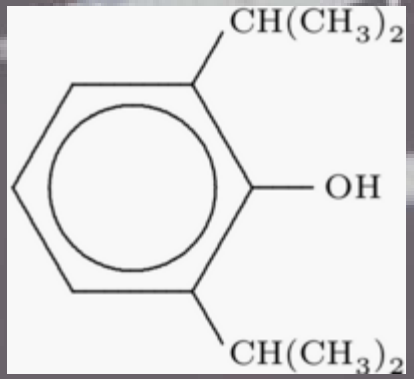
Ketamine (1960s)



Etomidate (1972)



Propofol (1977)



Neuronal Ion Channel Targets of General Anesthetics

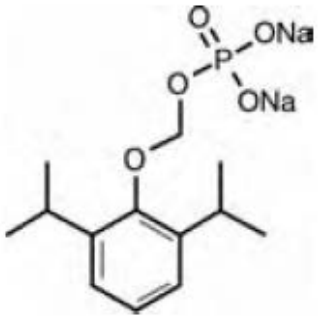
	Cys-Loop LGICs				Glu Receptors			K^+ Channels			Other
Anesthetic(s)	$GABA_A$	Glyc	nACh	$5HT_3A$	NMDA	AMPA	Kainate	K_{2P}	K_{IR}	K_V	HCN1
Etomidate	↑↑↑	0/↑	0	0/↓	0	0	0	0/↑	0/↓	ND	0
Alphaxalone	↑↑↑	0	0/↓	0/↓	0	0	0	ND	0/↓	0/↓	ND
Ketamine	0	0	↓↓↓	0/↑	↓↓	0	0	0	0/↓	↓	↓↓↓
Barbiturates	↑↑↑	0/↑	↓↓	0/↓	0	↓↓	↓↓	0	↓	ND	ND
Propofol	↑↑↑	↑	0	0/↓	0/ ↓	↓	0	0	0/↓	↓↓	↓↓
Volatiles	↑↑	↑↑	↓↓↓	↓↓	↓↓	↓↓	↓	↑↑	↑	↓	ND
N_2O, Xenon	0/↑	0	↓↓	↓	↓↓	↓	↓	↑	↑	0	ND

Therapeutic Index (LD50/ED50) range is 2 to 25

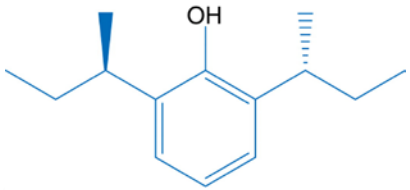
Correlates with Potency.

Inversely related to # of Ion Channel Targets.

Mechanism-Based Drug Improvements Have Not Yielded a New Clinical General Anesthetic

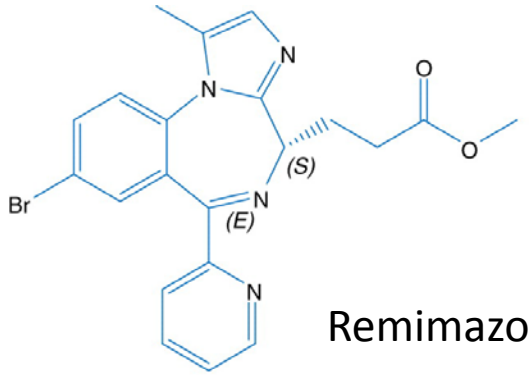


Phos-Propofol



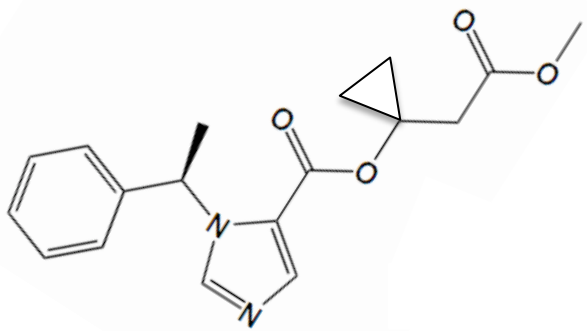
PF0713

Butyfol

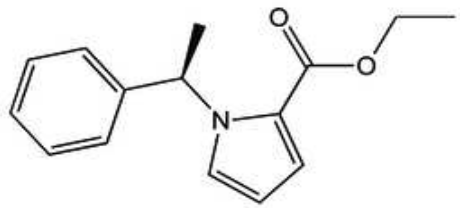


CNS7056

Remimazolam



CPMMetomidate



Carboetomidate

Most Hypnotic Screening Strategies Have Been Based on $GABA_A$ Receptors

Heusser et al. Functional validation of virtual screening for novel agents with general anesthetic action at ligand-gated ion channels. Mol. Pharmacol. 2013;84(5):670-8.

Middendorp et al. Accelerated discovery of novel benzodiazepine ligands by experiment-guided virtual screening. ACS Chem. Biol. 2014;9(8):1854-9.

McKinstry-Wu et al. Discovery of a novel general anesthetic chemotype using high-throughput screening. Anesthesiology. 2015;122(2):325-33

Our Strategy

1) Develop an un-biased high-throughput screen for potent reversible sedative-hypnotic drug activity in aquatic vertebrates (zebrafish larvae).

2) Screen drug libraries to identify novel potent hypnotics (active at 10 µM or lower).

3) Characterize hits in other animals to assess translational potential.

4) Characterize hits in molecular targets to learn about mechanisms.

5) Genetically modify zebrafish to investigate importance of possible molecular targets.

Key Collaborators:

Eric Liao, MD-PhD (MGH Plastic & Reconstructive Surgery)– CRISPR in zebrafish.
John Porco, PhD & Scott Schaus, PhD (BU Center for Molecular Discovery)– drug library.
Joe Cotten, MD-PhD (MGH Anesthesia Critical Care & Pain Med)– rat studies.

Zebrafish as an Experimental Model

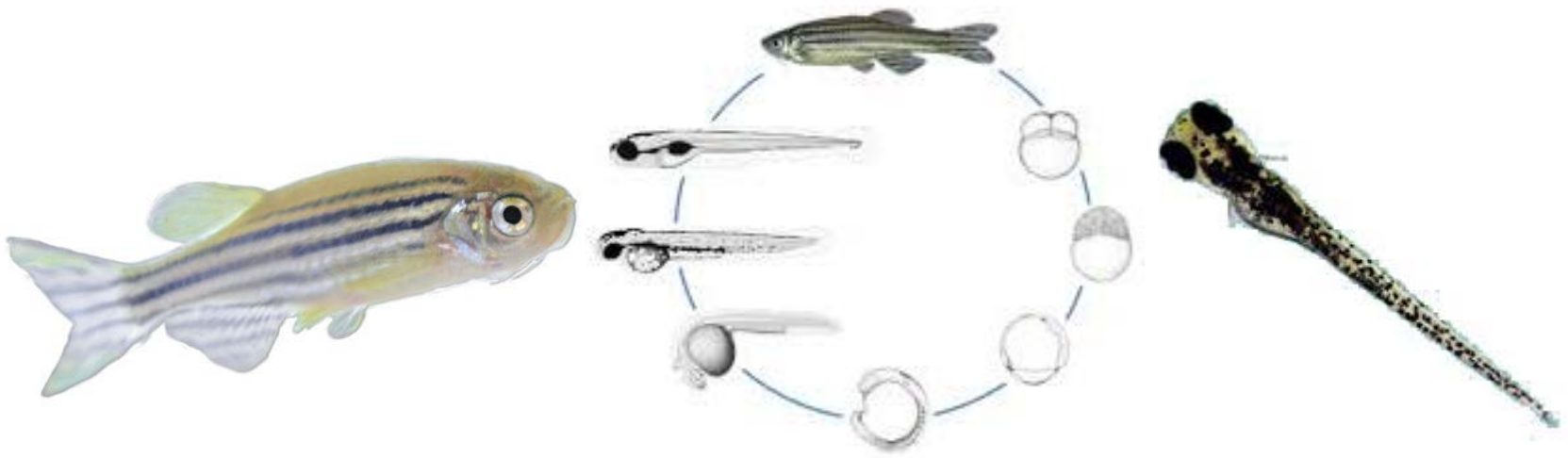
1) Inexpensive to maintain, easy to breed, reach sexual maturity in 2 months.

2) Embryos and larvae require no feeding and are small enough to study in 96-well plates.

3) Video behavioral analyses of many animals in parallel, with rapidly evolving sophistication.

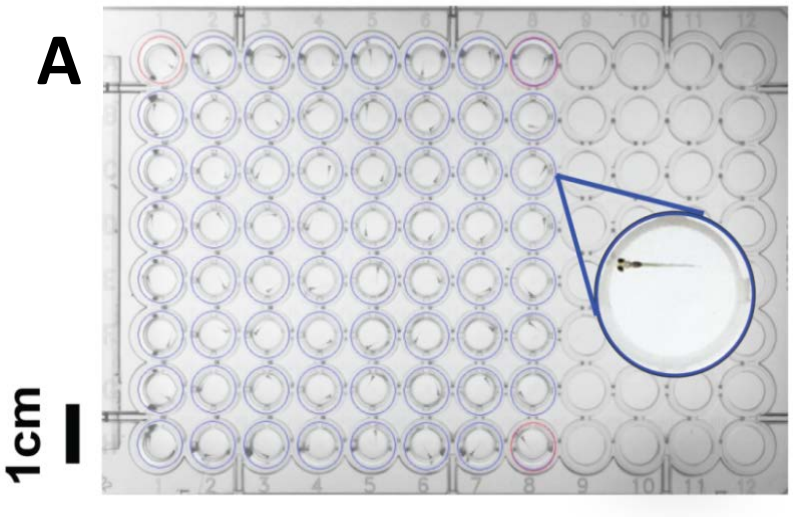
4) Methods for targeted gene mutations (KO or KI) established.

5) Methods for electrophysiology in adults and larvae established.

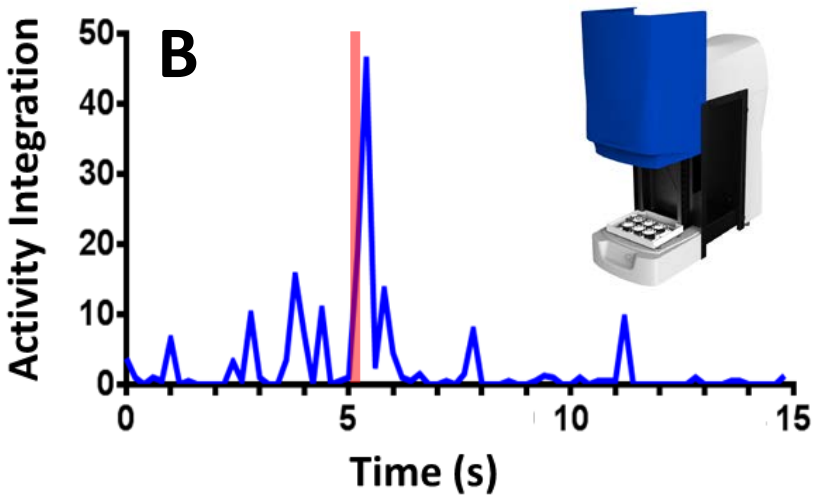


Zebrafish (7 dpf) Photomotor Responses

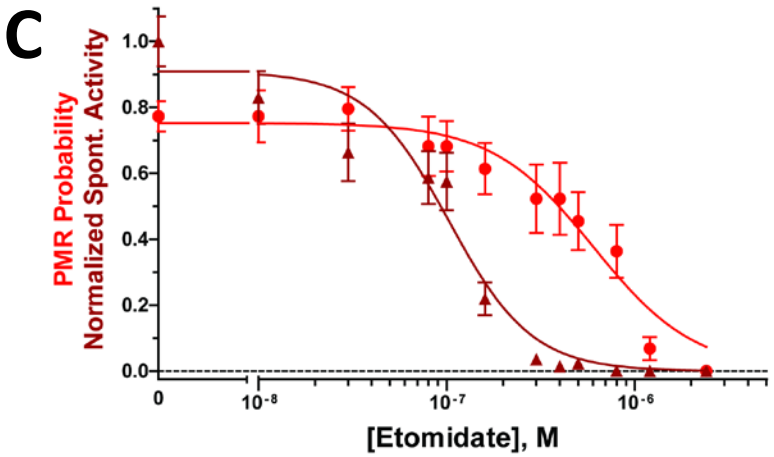
MASSACHUSETTS GENERAL HOSPITAL
RESEARCH INSTITUTE



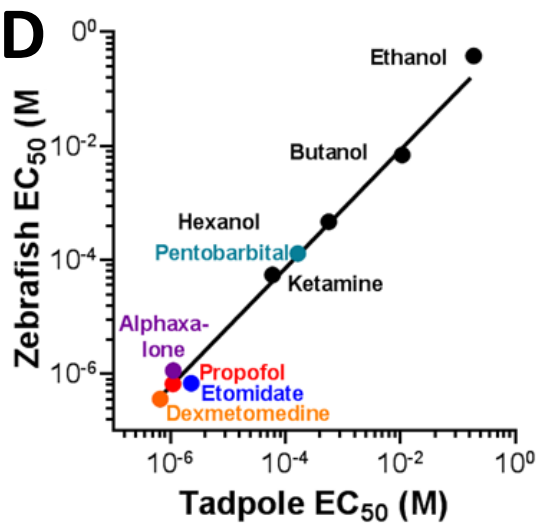
Up to 96 larvae can be studied at a time.



PMR assays are automated, rapid, robust.



Concentration-responses for both sedation and hypnosis can be established in one expt.

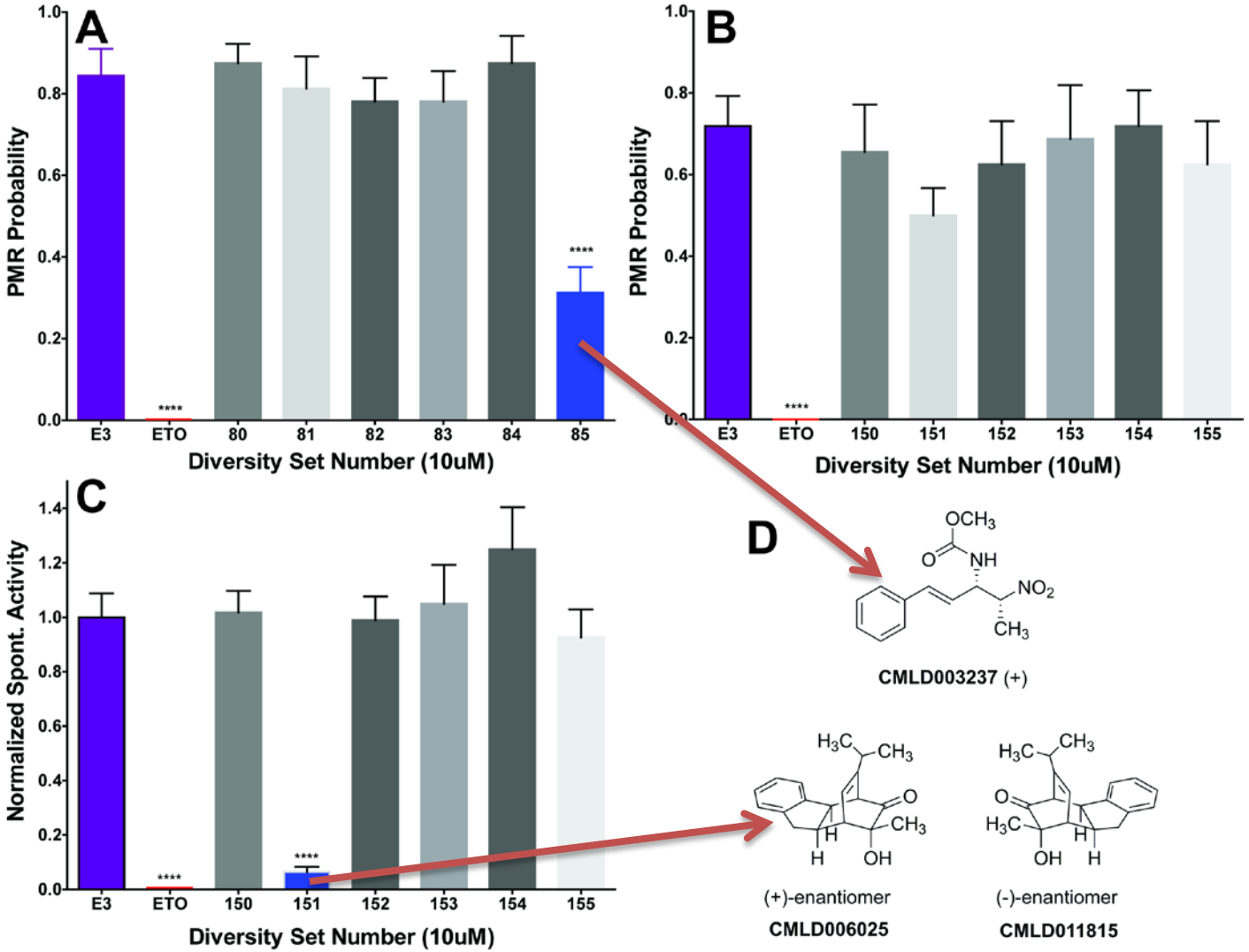


Results correlate well with older standard.

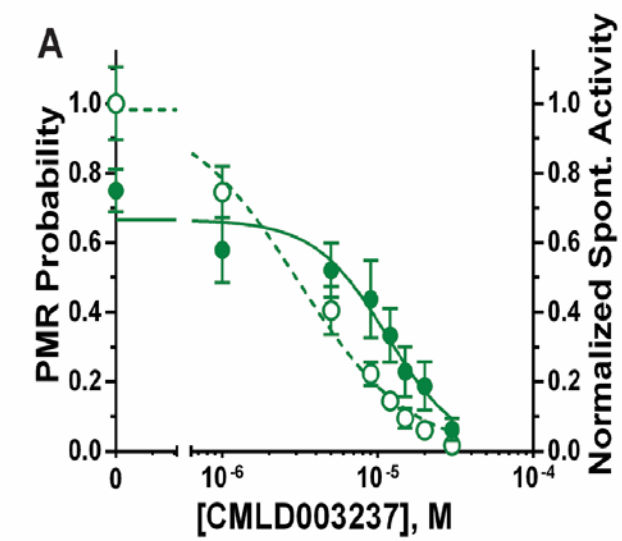
Discovery of New Sedative-Hypnotics

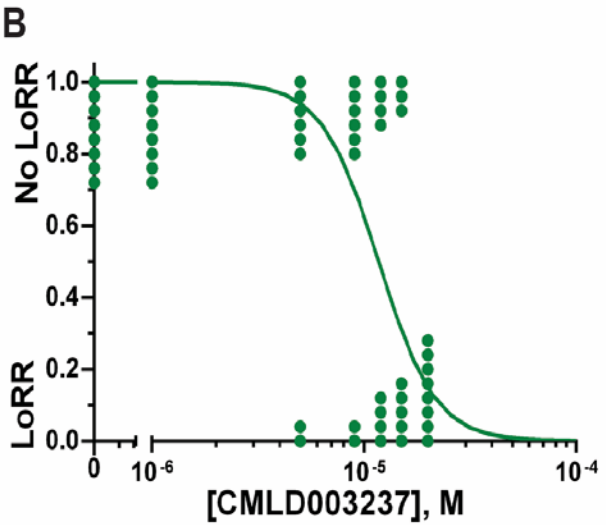
MASSACHUSETTS GENERAL HOSPITAL
RESEARCH INSTITUTE

2/350 Screened Compounds Show Sedative or Hypnotic Activity



Potency Characterization in Zebrafish and Tadpoles





Zebrafish

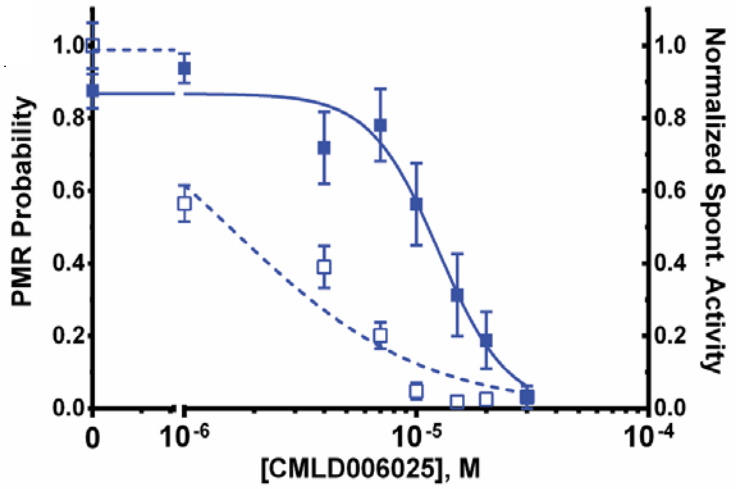
PMR IC50 = 11 µM

Spont Act. IC50 = 3 µM

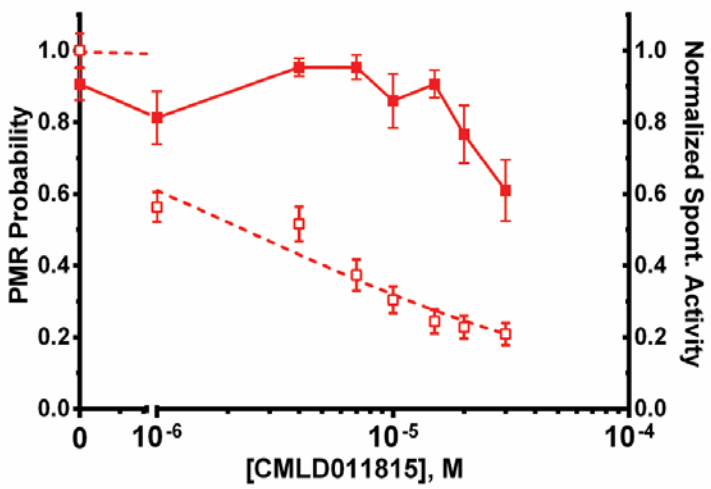
Tadpoles

LoRR IC50 = 12 µM

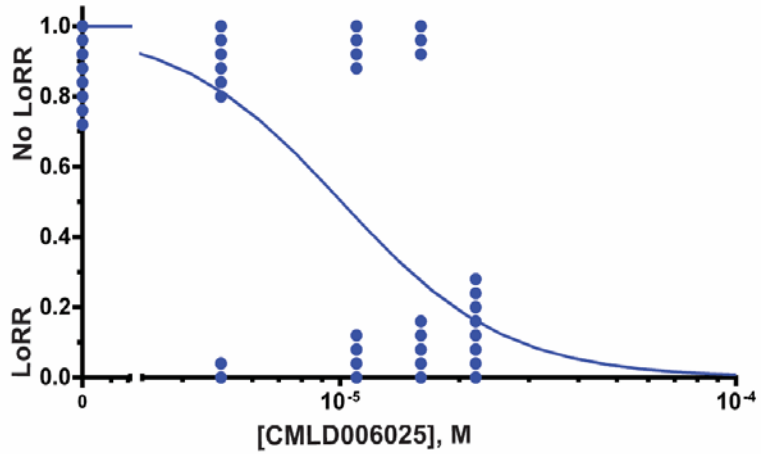
Potency Characterization in Zebrafish and Tadpoles



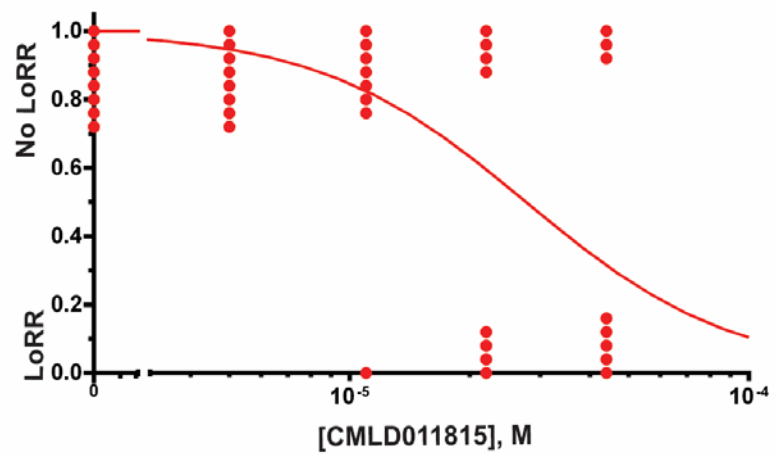
Zebrafish PMR IC50 = 13 µM



Zebrafish PMR IC50 > 30 µM

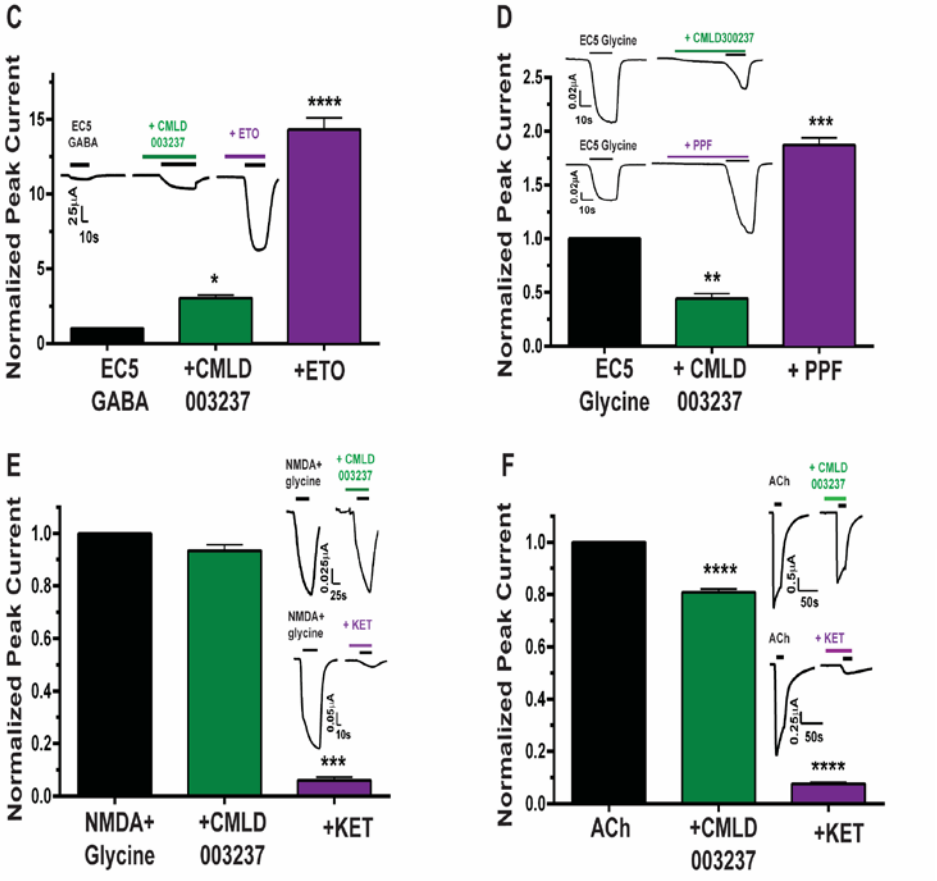


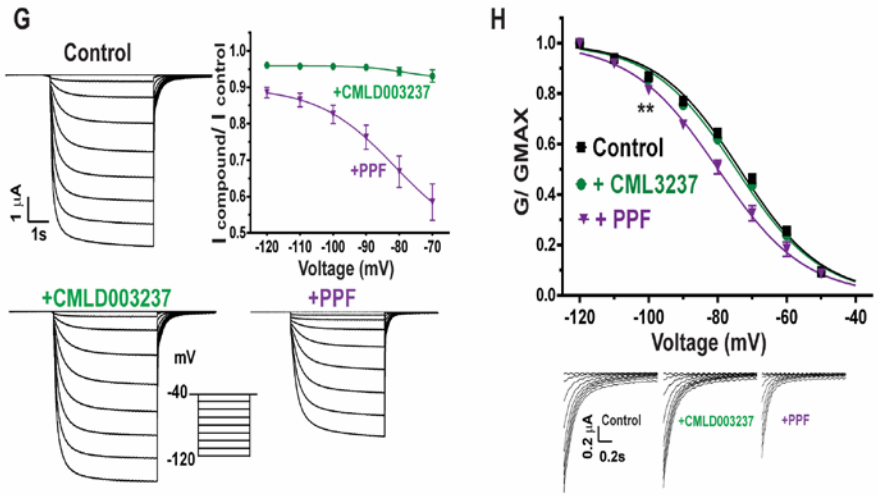
Tadpole LoRR IC50 = 10 µM

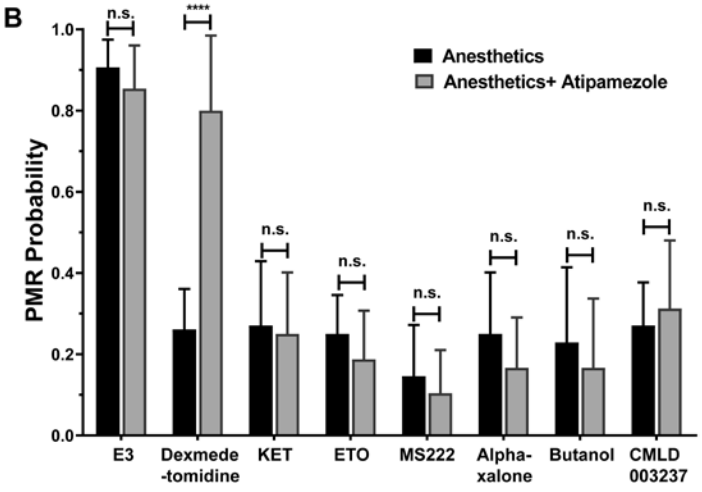


Tadpole LoRR IC50 = 28 µM

CMLD003237

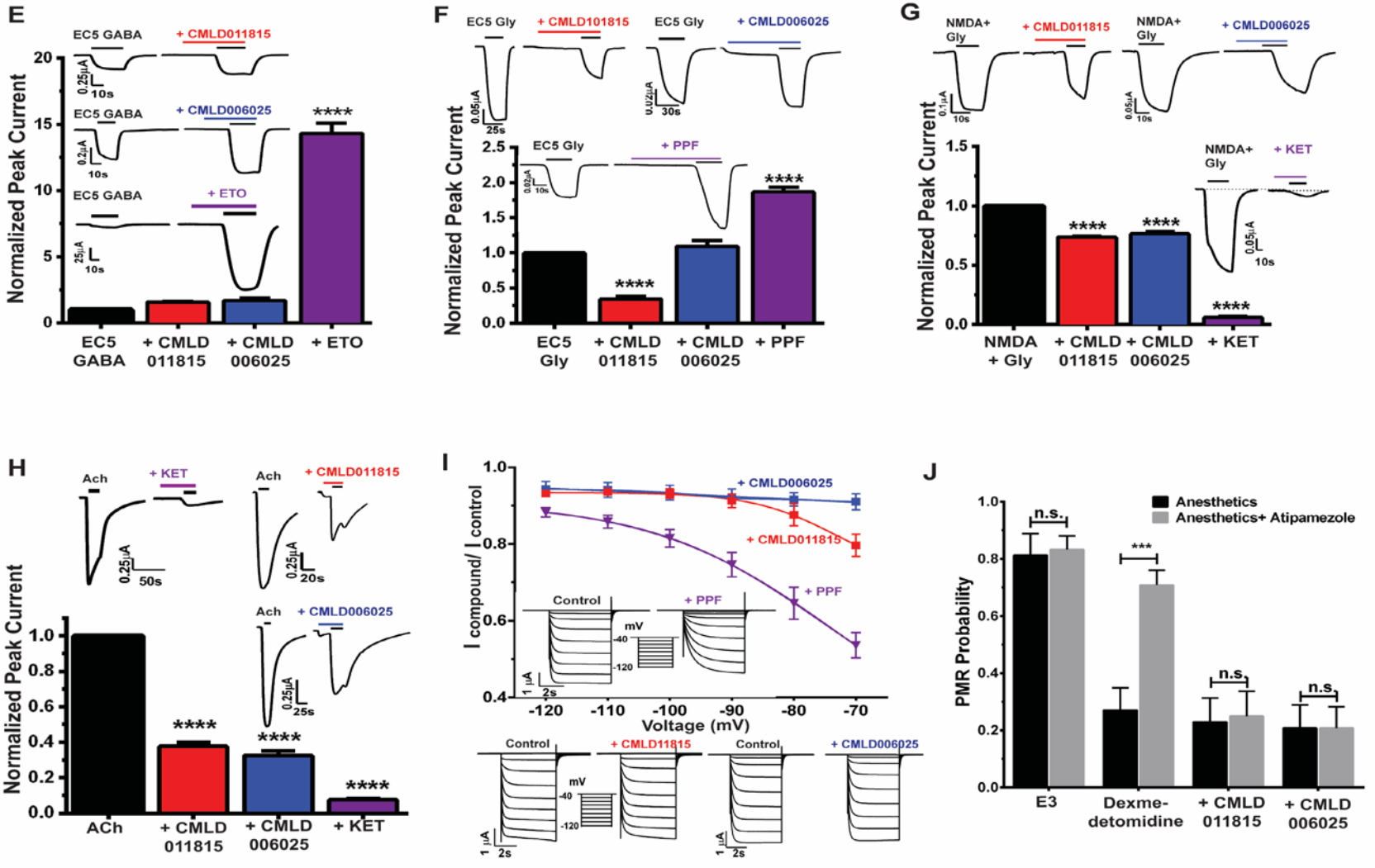






CMLD003237 affects $GABA_A$Rs, GlyRs, and neuronal nAChRs.

CMLD006025/CMLD011815



CMLD006025 affects NMDARs and neuronal nAChRs.

Potential for Translation

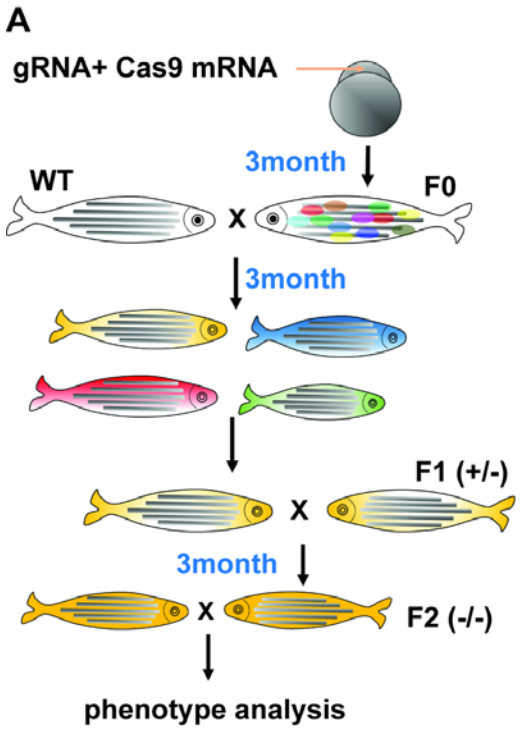
CMLD003237: IV injection in SD rats produces LoRR at 25 mg/kg and higher doses. One structural modification improves potency in zebrafish larvae.

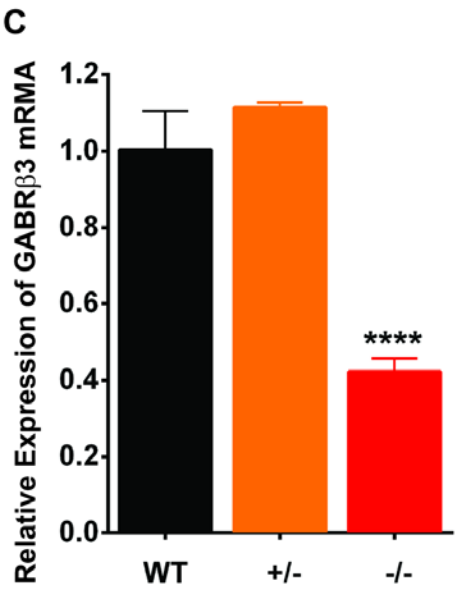
CMLD006025: No LoRR in rats observed after 40 mg/kg IV injection. Structural modifications tested to date eliminate activity in zebrafish larvae.

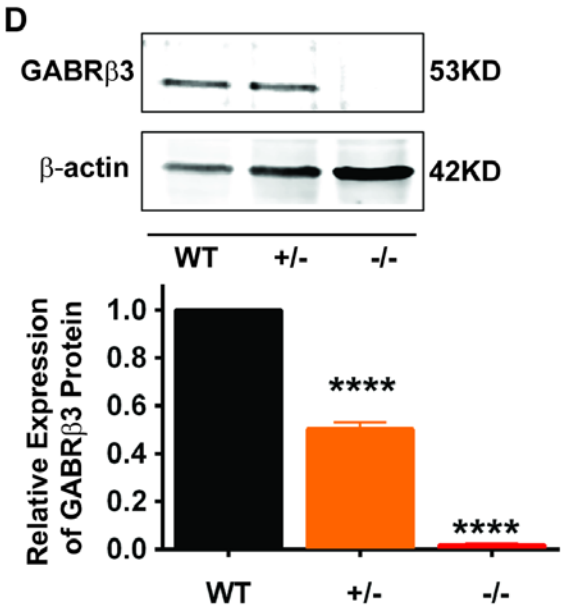
Note: These are preliminary results.

Our First Transgenic Zebrafish Line: $GABA_A$ $\beta3^{0/0}$

MASSACHUSETTS GENERAL HOSPITAL
RESEARCH INSTITUTE







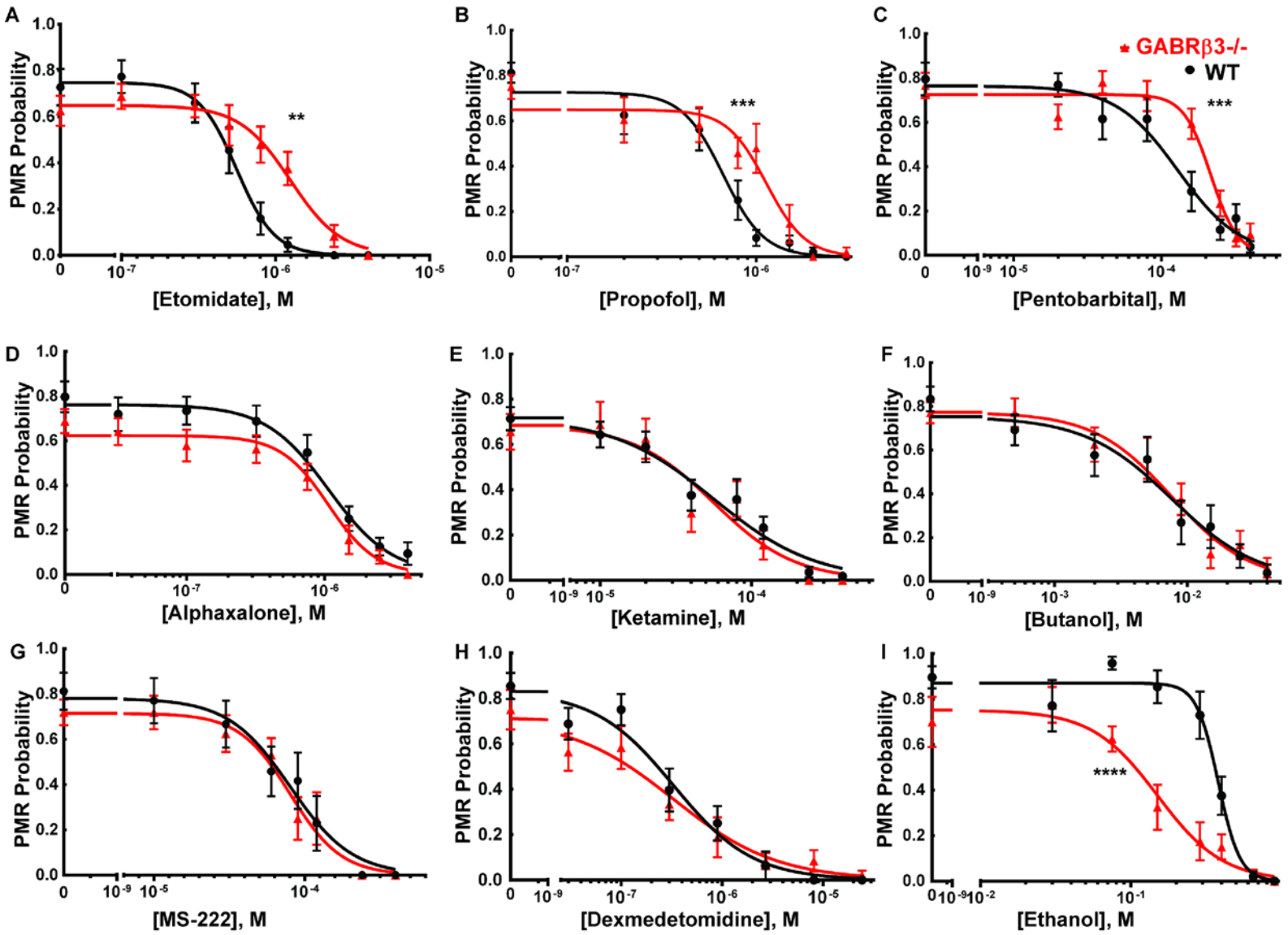
B

WT	AAACCGCAG - - - - - - - - - - - TGACGGGTGTGTCACGCATCGAGCTCCCGCAGTTCTCCATCGTTGACTA
+10 bp	AAACCGCAGCTACAACACCTGACGGGTGTGTCACGCATCGAGCTCCCGCAGTTCTCCATCGTTGACTA
gRNA	AAACCGCAG - - - - - - - - - - - TGA

in Silico Translation

WT	GYTTDDIEFYWKGGETAVTGVSRIELPQFSIVDYKLVSRNVV...
+10 bp	GYTTDDIEFYWKGGETAATTPDGCVTHRAPAVLHR*

Our First Transgenic Zebrafish Line: $GABA_A$ $\beta3^{0/0}$



Significance

1. **We discovered two new compounds with potent sedative-hypnotic activity in a 350-drug library. Screening larger libraries may identify many more.**
2. **Our new drugs apparently act through different mechanisms than currently used intravenous anesthetics, which may provide advantages in clinical application.**
3. **Transgenic zebrafish represent a potentially informative system for studying anesthetic effects on neural circuits.**

Acknowledgments

Forman Lab (EDR5)

Cindy Yang, MD-PhD (Shanghai, PRC)
Youssef Jounaidi, PhD (MGH)
Jennifer Dai, BS (NYU Medical) , Francisco Marte, BS (UPR SoM)
Elizabeth Halpin, BS, Jennifer Park, BS (Virginia Tech Carillon SoM),
Ryan Fantasia, BS

Joseph Cotten, MD-PhD

MGH Center for Regenerative Medicine

Eric Liao, MD-PhD (Plastic & Reconstructive Surgery)
Renee Daigle, BS, Kusumika Mukherjee, PhD

BU Center for Medical Discovery

John Porco Jr., PhD
Scott E. Schaus, PhD
Lauren Brown, PhD
Richard Trilles, BA
Wenqing Xu, PhD

$upport

DACCPM Innovation Fund
DACCPM Scholar Fund
NIGMS (R01-GM128989)

Questions?