MAYO CLINIC

Using Zebrafish to Develop the First Pharmacotherapy for the Treatment of Hearing Loss Caused by Usher Syndrome Type I due to Variants in MYO7A

Alaa Koleilat, Ph.D.

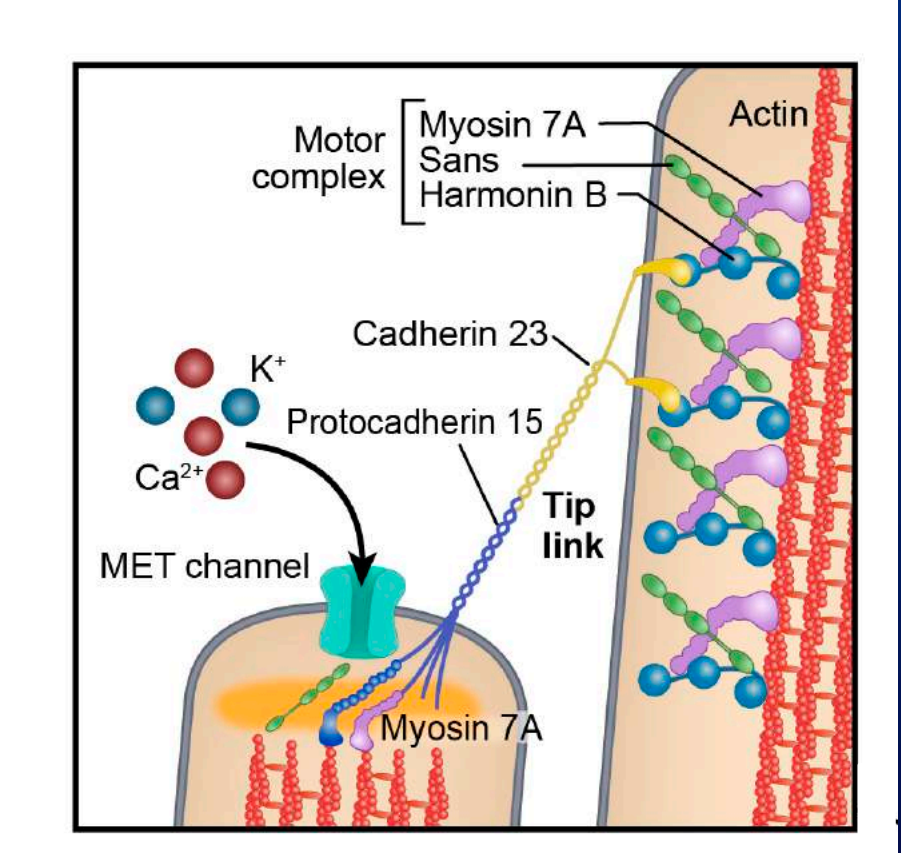
USH Connections Week 2020

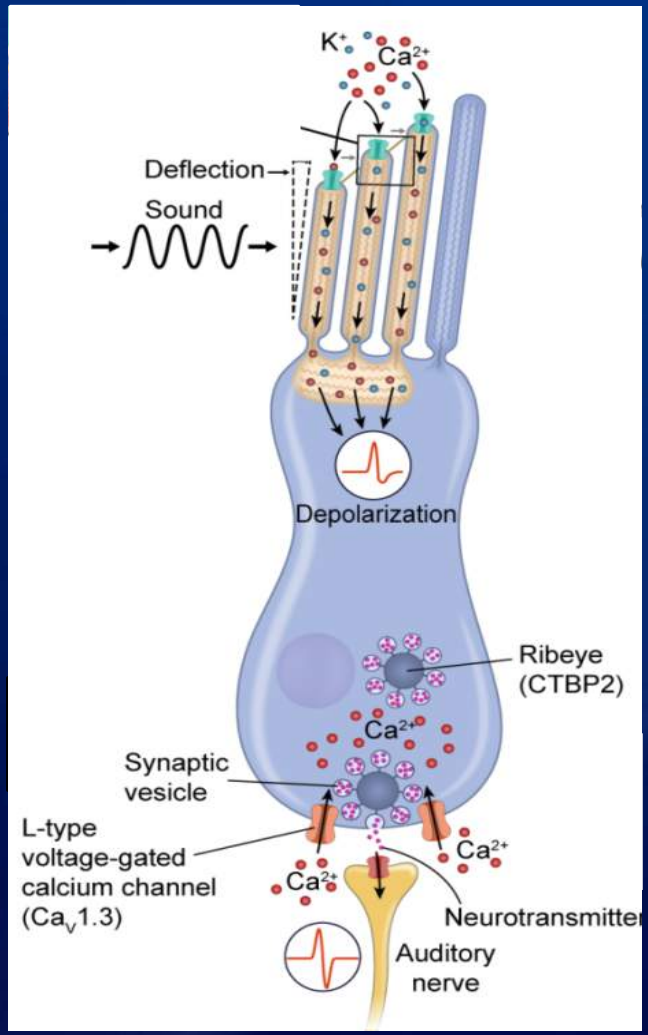
Usher Syndrome

Type	Hearing Impairment	Onset of Hearing Loss	Vestibular Impairment	Vision Loss	Genes
I	Severe to profound hearing loss	At birth	Severe (E.g. walk at a later age, etc.)	Onset in the first decade of life	***MYO7A*** *USH1C* *CDH23* *PCDH15* *SANS/USH 1G*
II	Moderate to severe hearing loss	At birth	none	Onset in first to second decade of life	*USH2A* *VLGR1* *WHRN*
III	Variable, progressive hearing loss	Adolescence	Variable	Variable	*CLRN1* *HARS*

Koleilat et al. Accepted *Pediatric Retina*

MYO7A and the inner ear hair cell

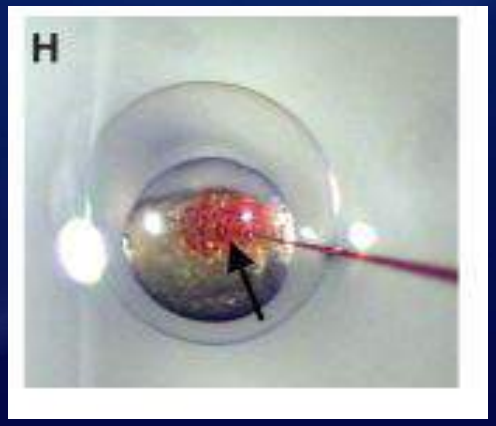




Koleilat et al. Under Review

Zebrafish as Model Organism to Study Human Disease

- Availability of a zebrafish genome
- Ease of gene editing to model human diseases
- Produce hundreds of embryos in one day
- Ease of administering drugs

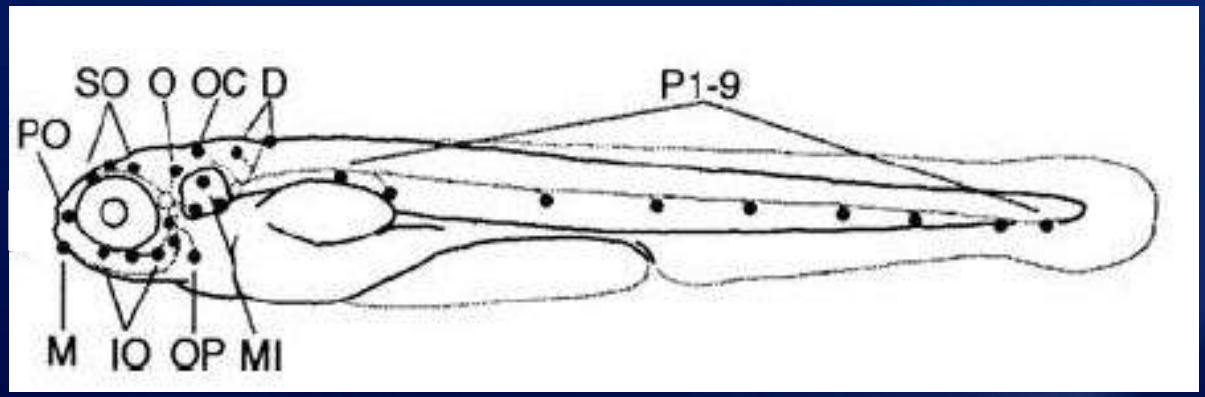


Bill et al. 2009 *Zebrafish*

MAYO CLINIC

Zebrafish as Model Organism to Study Genetic Hearing Loss

- Acoustic startle response present at 5 days post fertilization
- External sensory organ→ lateral line



Harris et al. 2003 *JARO*

MAYO CLINIC

Neuron, Vol. 20, 271–283, February, 1998, Copyright ♥1998 by Cell Press

Genetic Analysis of Vertebrate Sensory Hair Cell Mechanosensation: the Zebrafish Circler Mutants

Teresa Nicolson,*# Alfons Rüsch,‡
Rainer W. Friedrich,† Michael Granato,*‖
Johann Peter Ruppersberg,‡§
and Christiane Nüsslein-Volhard*

Table 1. Mutations Affecting Larval Vestibular Function

Gene	Abbreviation
sputnik	*spu*
mariner	*mar*
orbiter	*orb*
mercury	*mrc*
gemini	*gem*
skylab	*skb*
astronaut	*asn*
cosmonaut	*csm*

MAYO CLINIC

Neuron, Vol. 20, 271–283, February, 1998, Copyright ♥1998 by Cell Press

Genetic Analysis of Vertebrate Sensory Hair Cell Mechanosensation: the Zebrafish Circler Mutants

Teresa Nicolson,*# Alfons Rüsch,‡
Rainer W. Friedrich,† Michael Granato,*‖
Johann Peter Ruppersberg,‡§
and Christiane Nüsslein-Volhard*

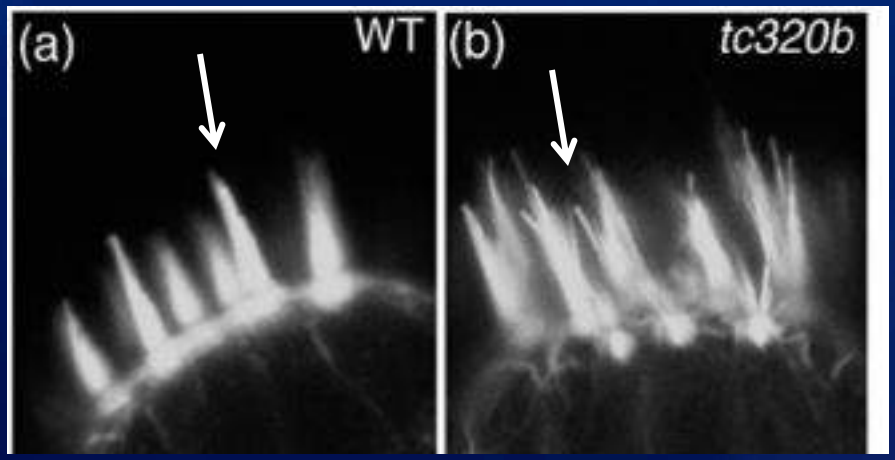
Table 2. Summary of the phenotypes of the vestibular mutants

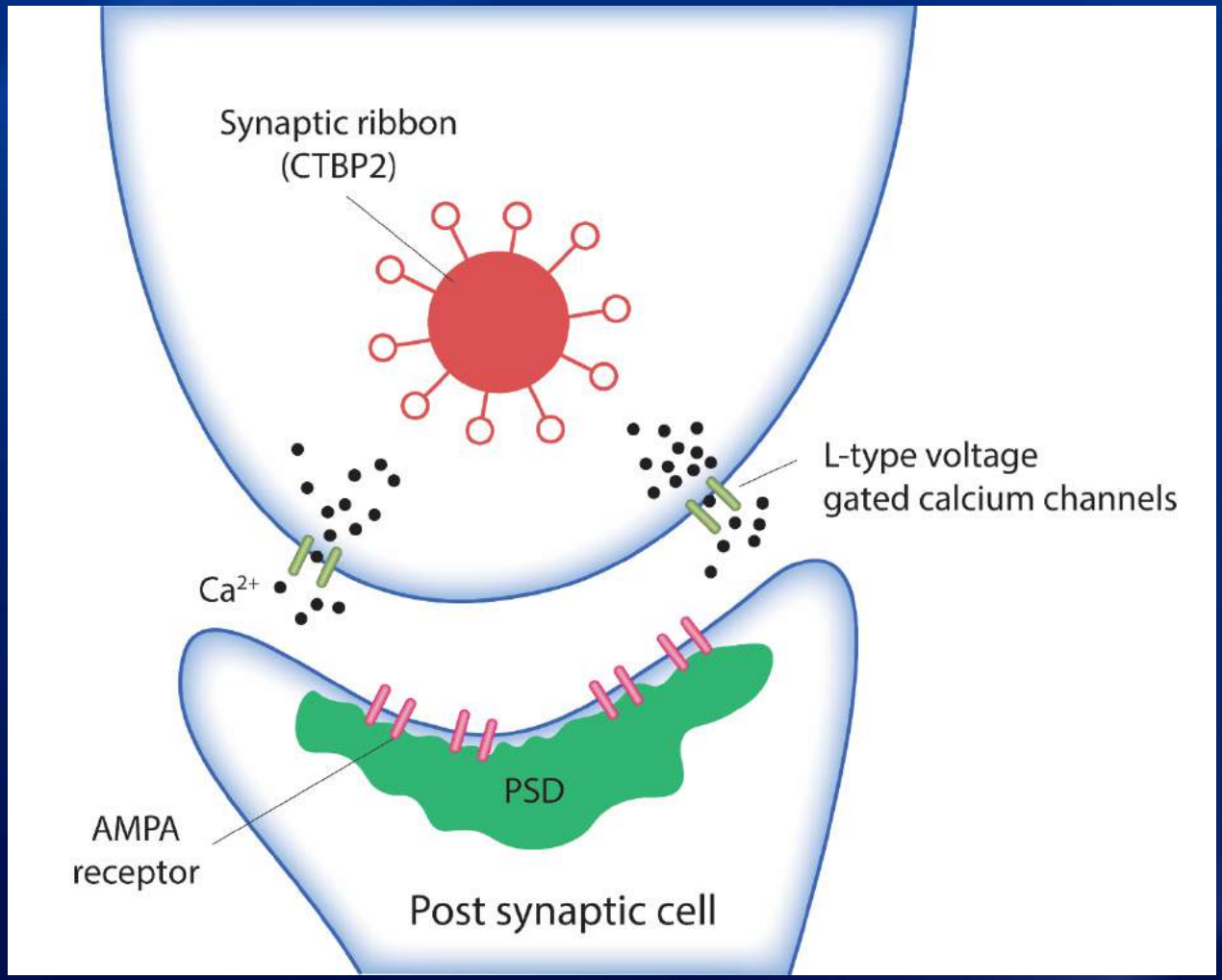
Strain	Allele	Hair cell morphology	Acoustic vibrational sensitivity	Startle reflex Ca^{2+} signal (%)
wildtype		Normal	Present	100 ± 56
mariner (mar)	*tc320b*	Bundle defect	Absent	9 ± 8

Human Molecular Genetics, 2000, Vol. 9, No. 14 2189–2196

Mariner is defective in *myosin VIIA*: a zebrafish model for human hereditary deafness

Sylvain Ernest, Gerd-Jörg Rauch[1], Pascal Haffter[1], Robert Geisler[1], Christine Petit and Teresa Nicolson[2+]

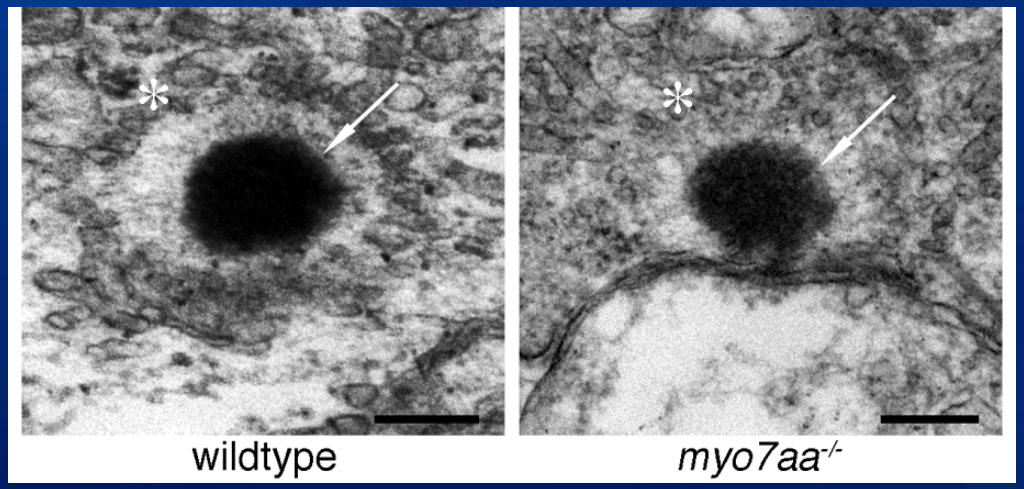


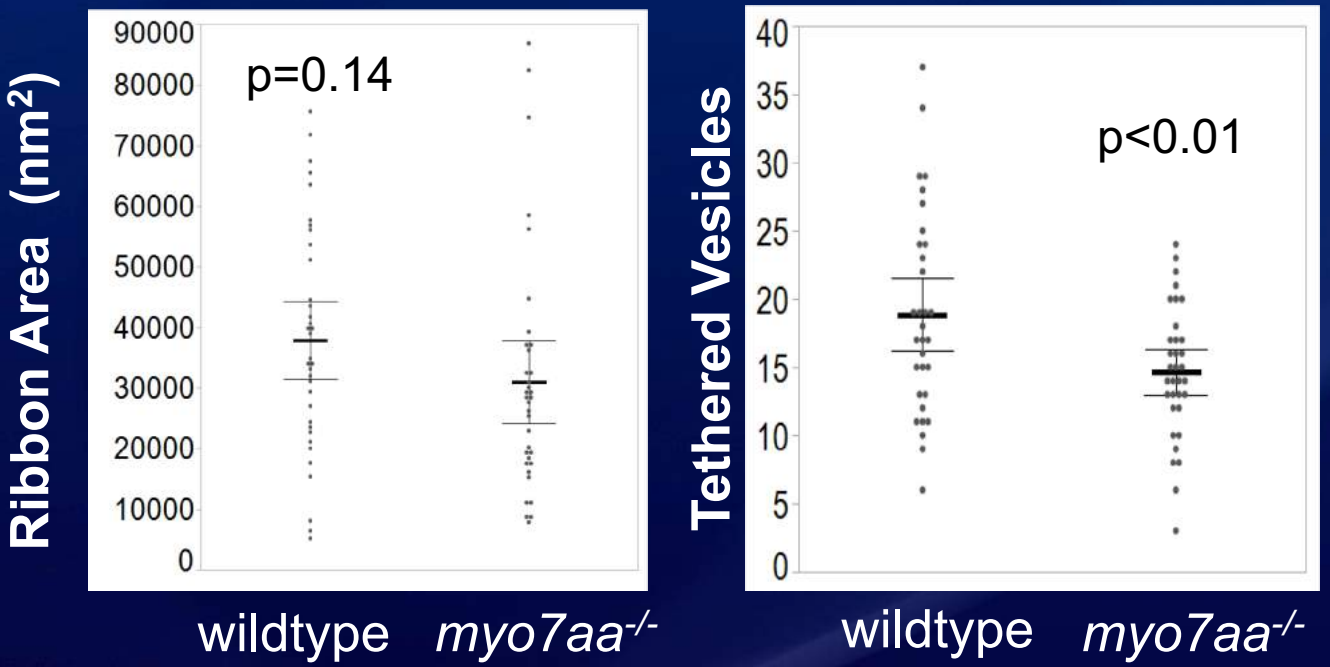


MAYO CLINIC

Koleilat et al. Under Review

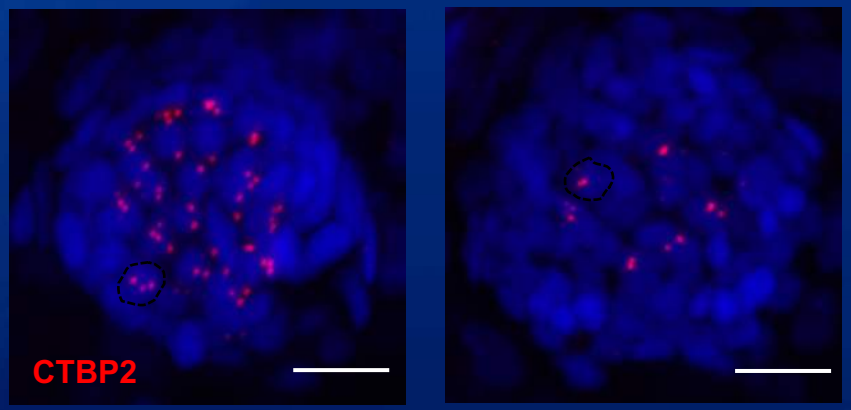
myo7aa$^{-/-}$ mutant zebrafish have comparable ribbon area, but fewer glutamatergic vesicles



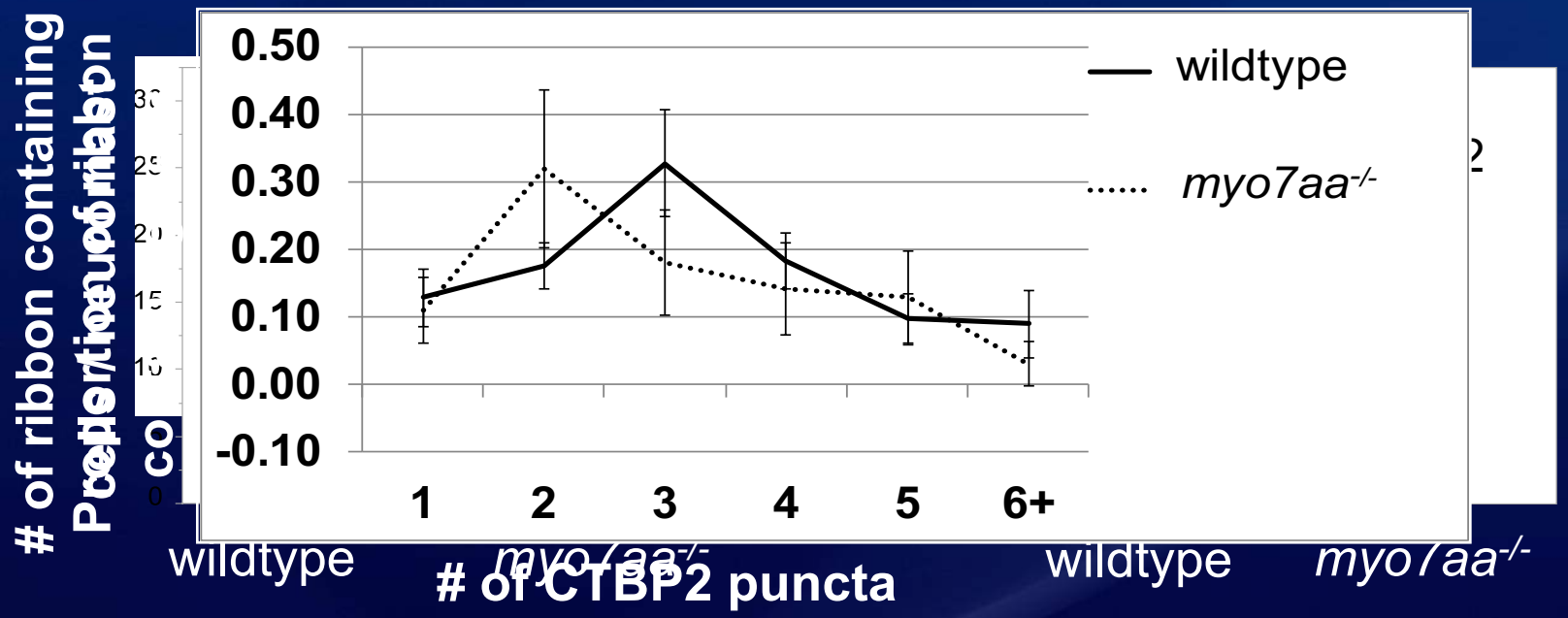


Koleilat et al. Under Review

myo7aa$^{-/-}$ mutant zebrafish have different distribution of CTBP2 puncta

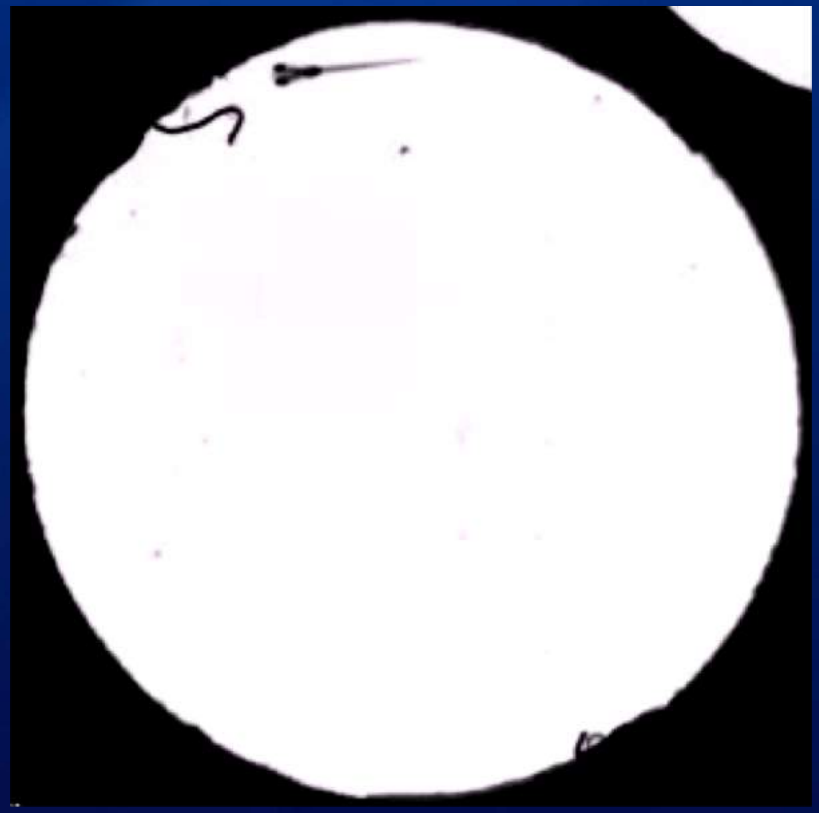


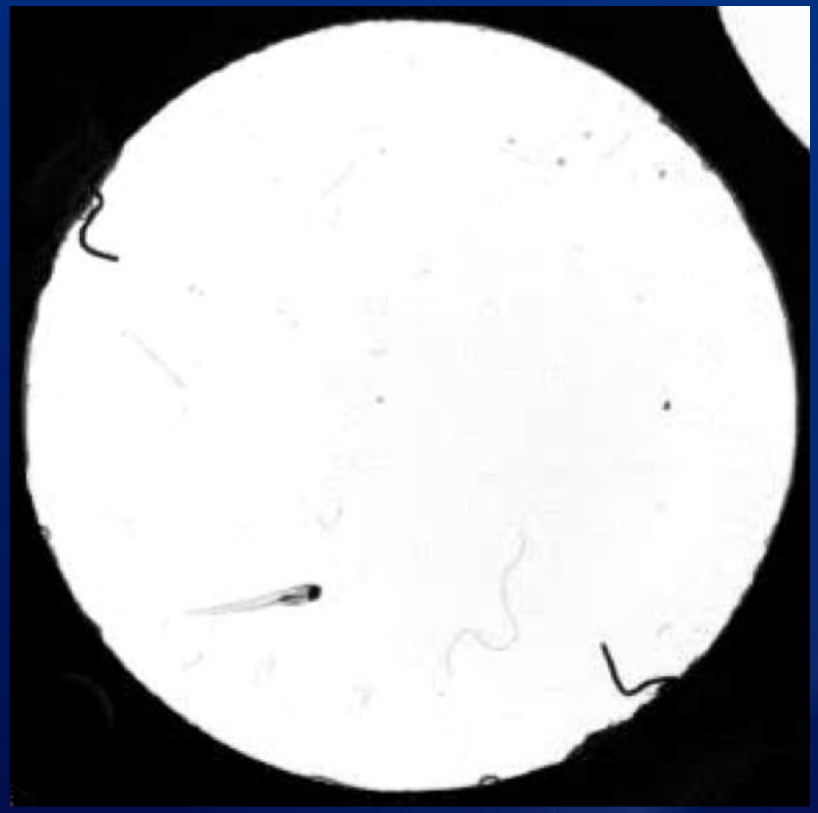
wildtype *myo7aa*$^{-/-}$



Koleilat et al. Under Review

Quantification of behavior between the wildtype and *myo7aa*$^{-/-}$ mutant





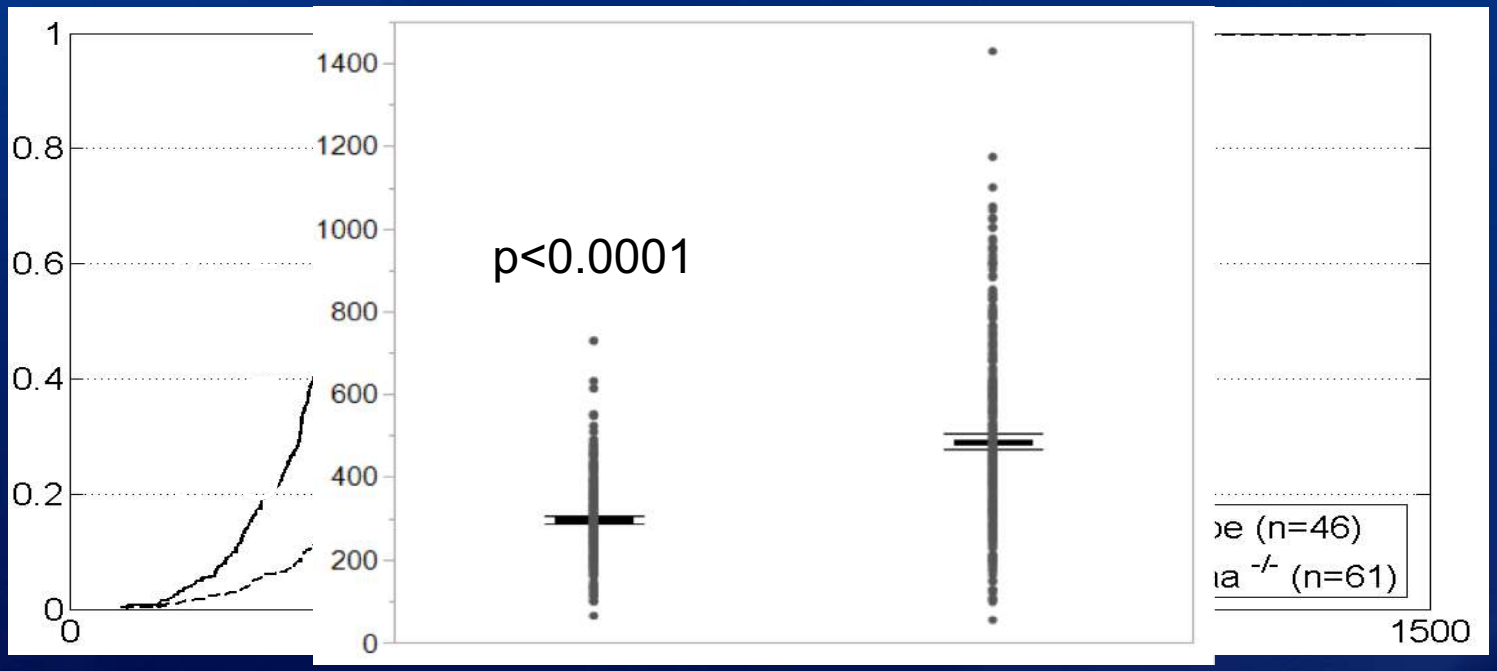
wildtype

myo7aa$^{-/-}$

MAYO CLINIC

Koleilat et al. Under Review

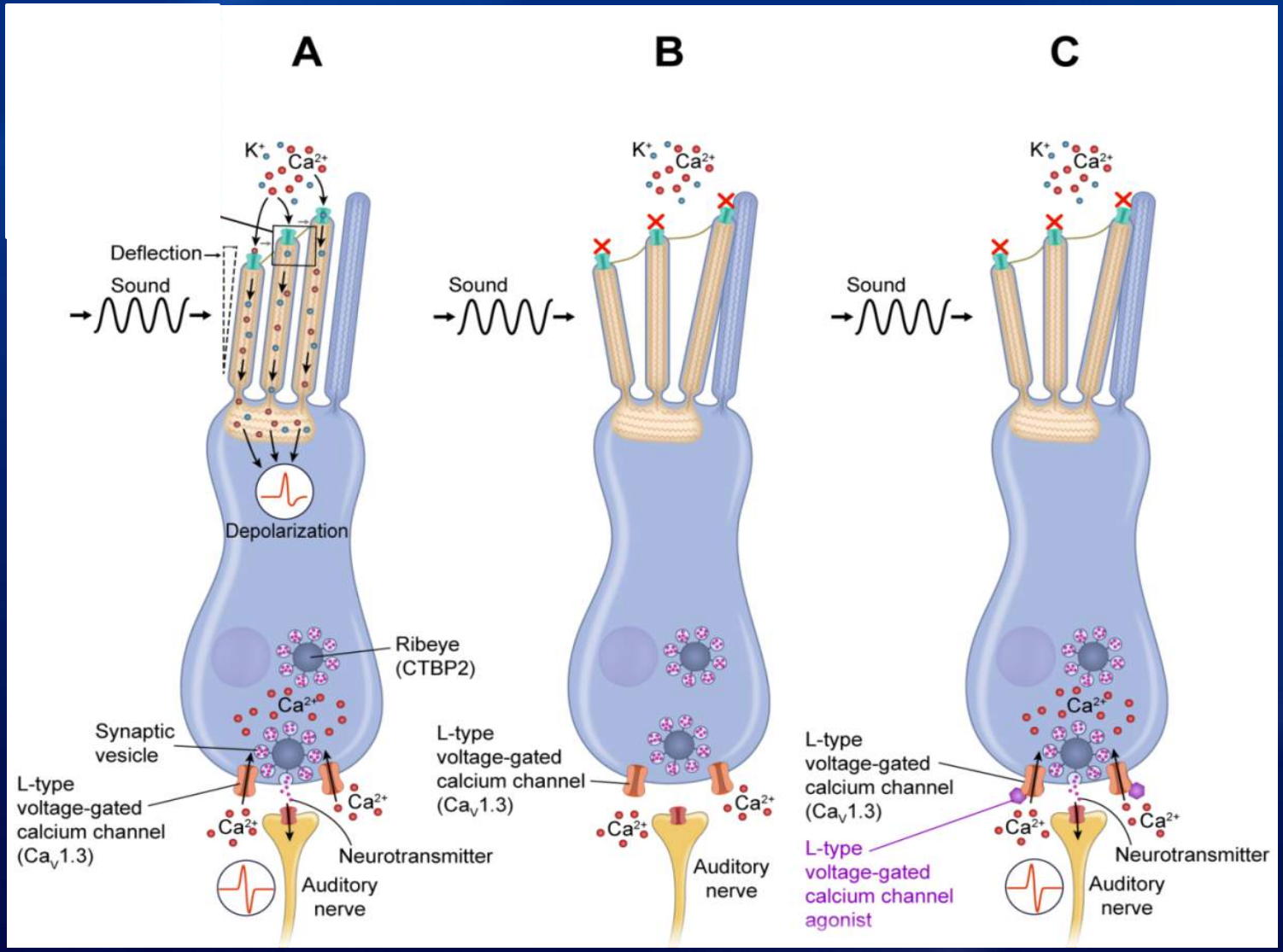
myo7aa$^{-/-}$ mutant zebrafish have larger turning angles



Koleilat et al. Under Review

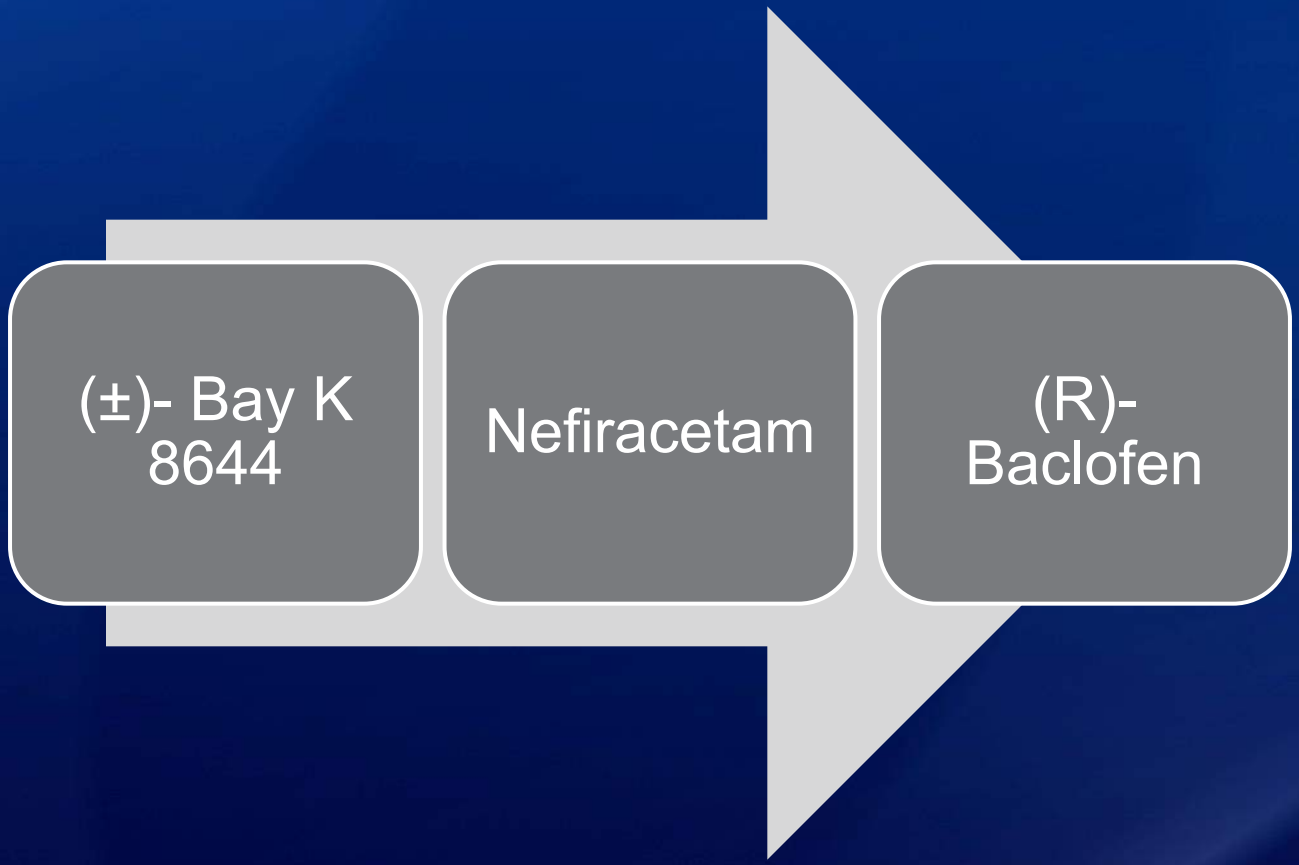
Can modulating the $Ca_V1.3a$ channel using drugs provide a therapeutic effect in the *myo7aa*$^{-/-}$ mutant zebrafish?

MAYO CLINIC



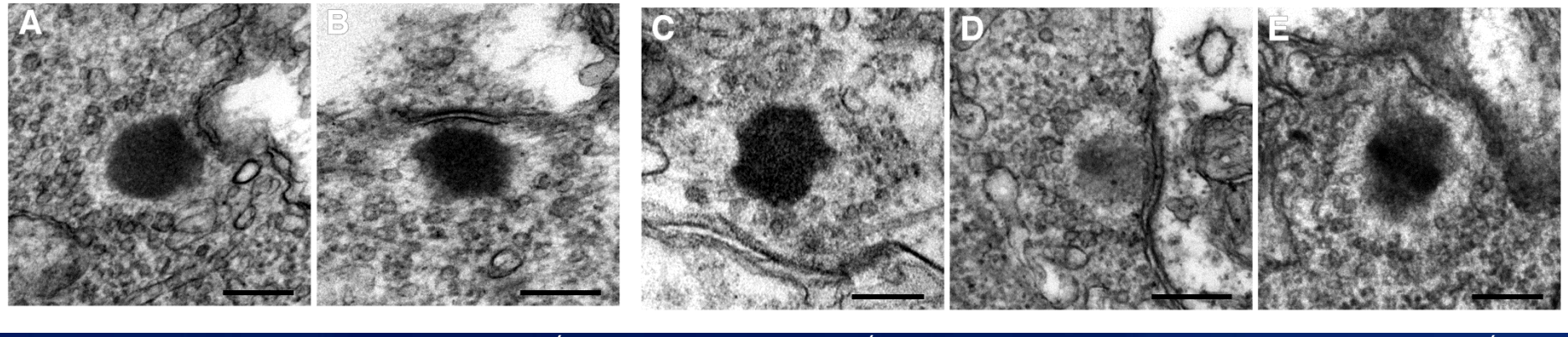
Koleilat et al. Under Review

L-type voltage gated calcium channel agonists tested in this study:



MAYO CLINIC

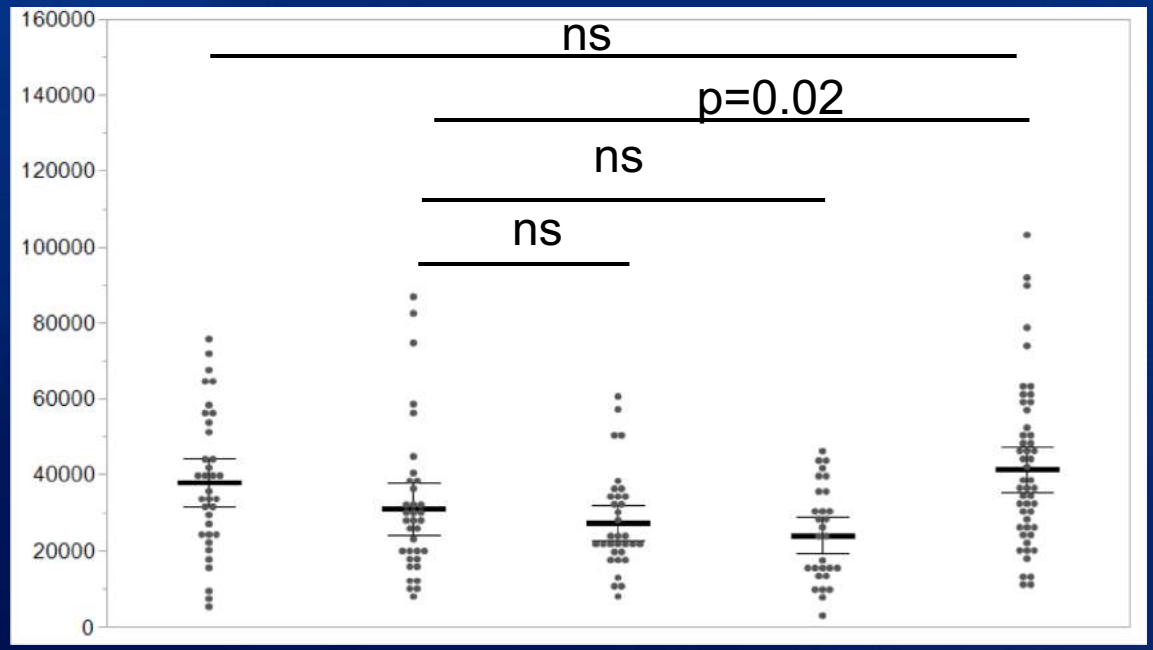
(R)-Baclofen increases ribbon area and number of tethered vesicles in *myo7aa*$^{-/-}$ mutant zebrafish



wildtype untreated	*myo7aa*$^{-/-}$ untreated	*myo7aa*$^{-/-}$ (±)-Bay K 8644	*myo7aa*$^{-/-}$ Nefiracetam	*myo7aa*$^{-/-}$ (R)-Baclofen

Koleilat et al. Under Review

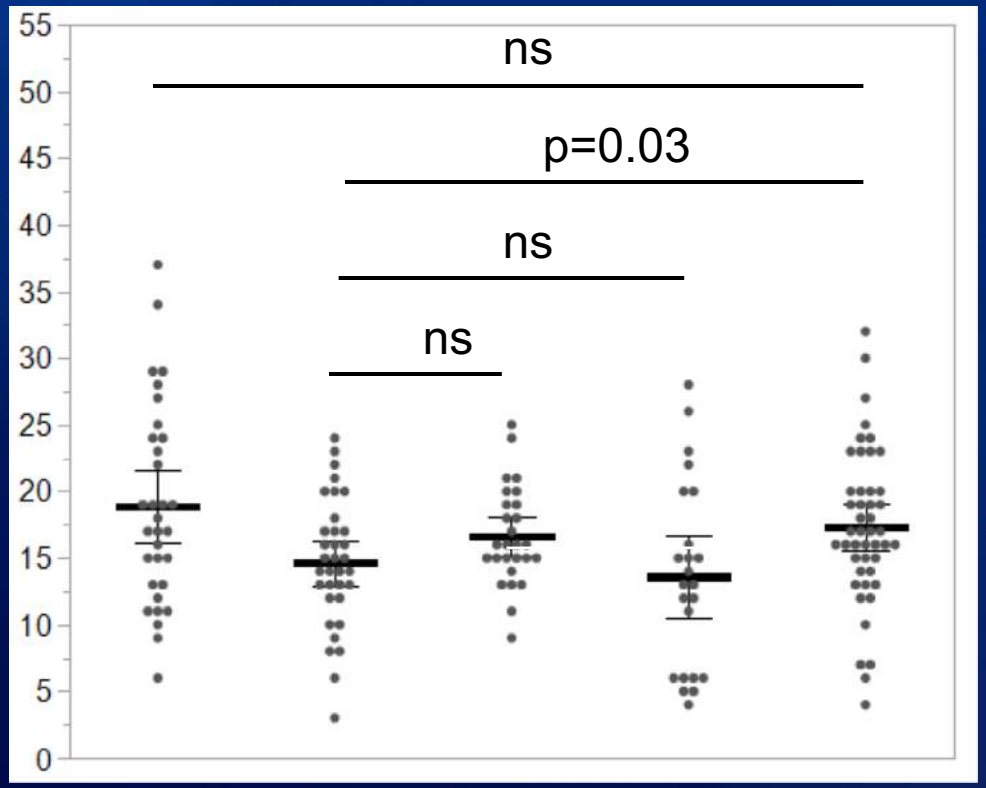
(R)-Baclofen increases ribbon area in *myo7aa*$^{-/-}$ mutant zebrafish



	wildtype	*myo7aa*$^{-/-}$			
(±)-Bay K 8644	-	-	+	-	-
Nefiracetam	-	-	-	+	-
(R)-Baclofen	-	-	-	-	+

Koleilat et al. Under Review

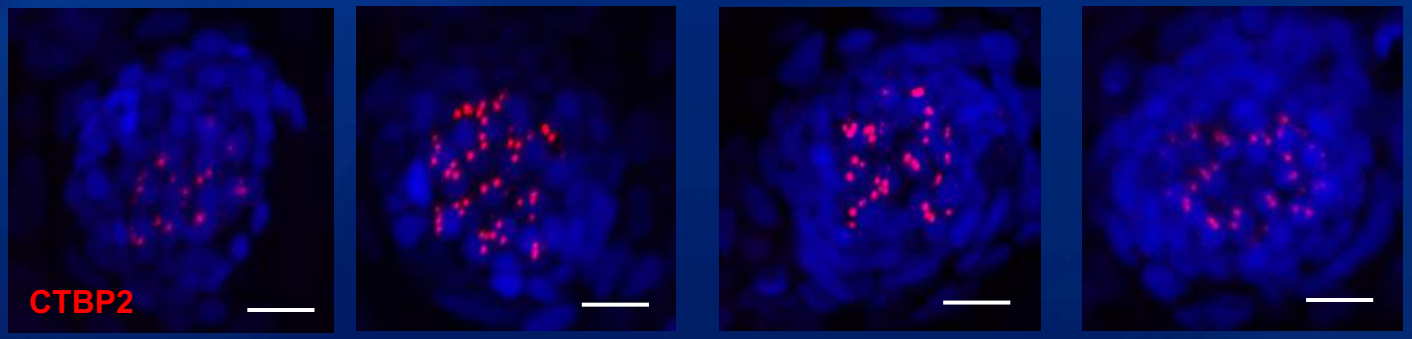
(R)-Baclofen increases number of tethered vesicles in *myo7aa*$^{-/-}$ mutant zebrafish



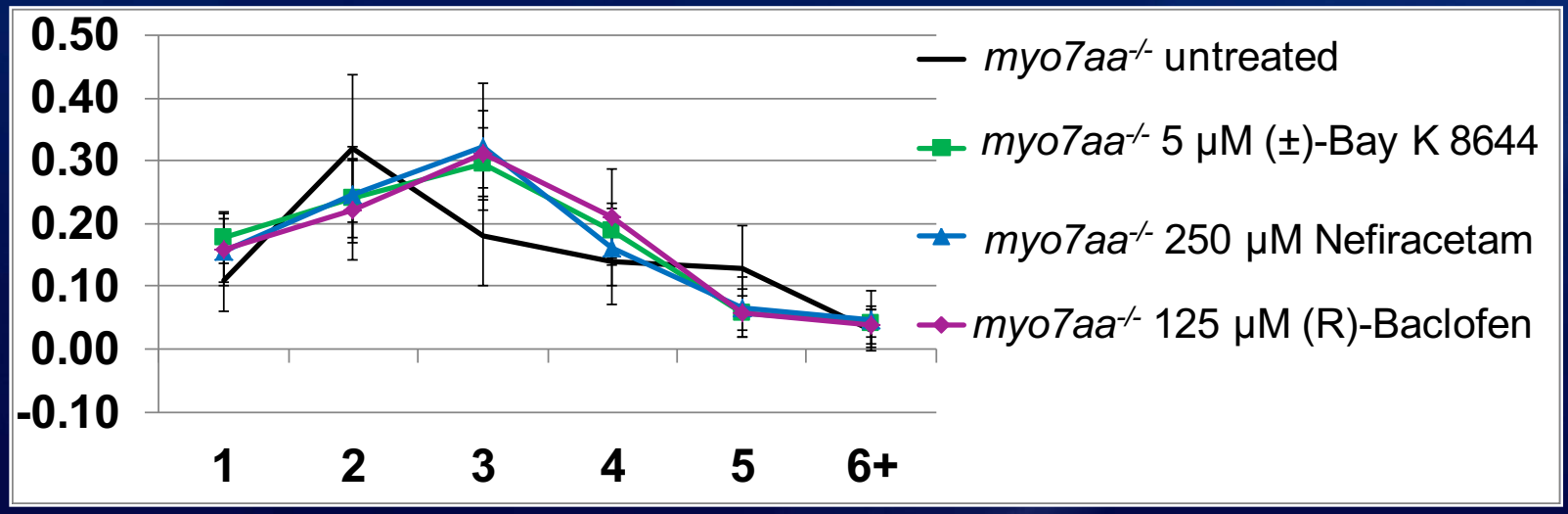
(±)-Bay K 8644	-	-	+	-	-
Nefiracetam	-	-	-	+	-
(R)-Baclofen	-	-	-	-	+

MAYO CLINIC

L-type voltage-gated calcium channel agonists alter CTBP2 distribution in *myo7aa*$^{-/-}$ mutant zebrafish



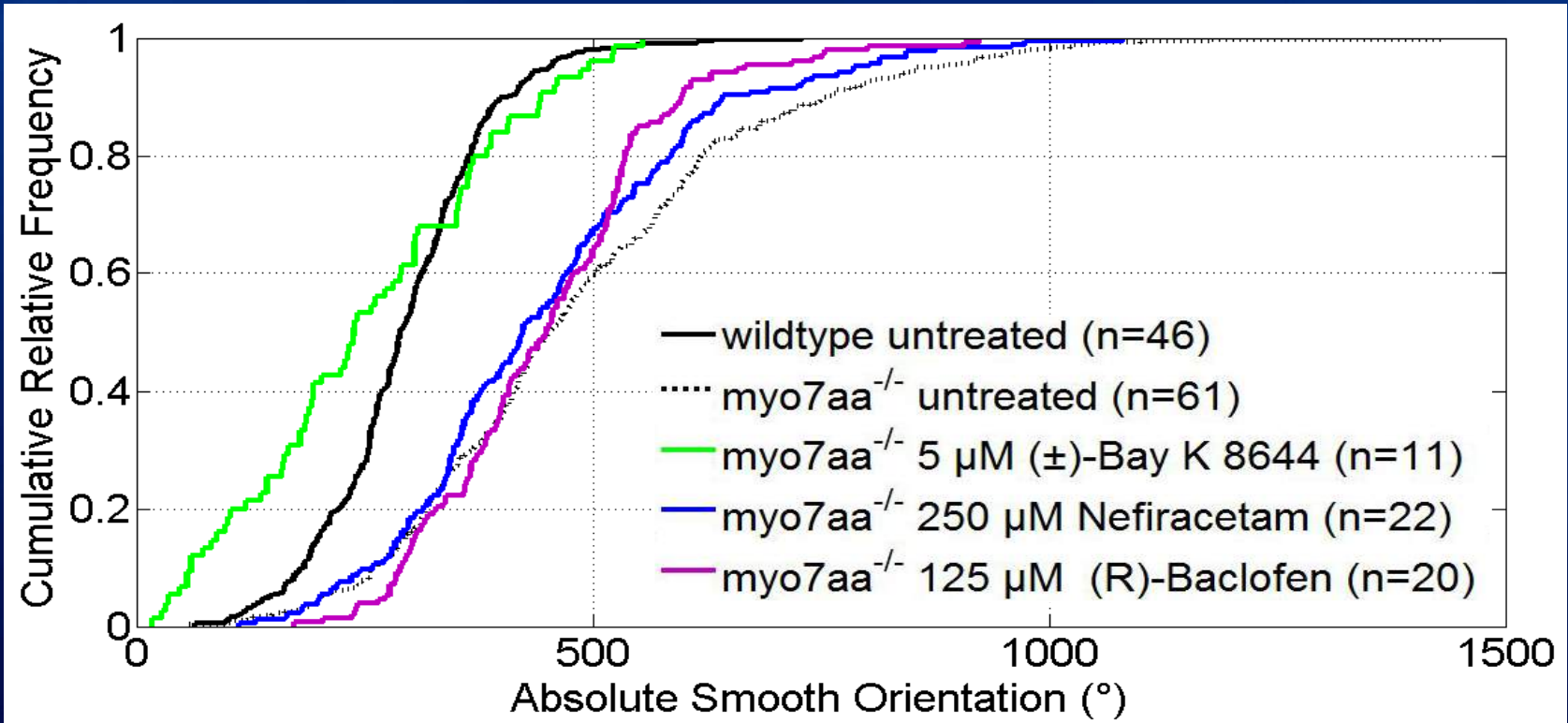
myo7aa$^{-/-}$ untreated | *myo7aa*$^{-/-}$ (±)-Bay K 8644 | *myo7aa*$^{-/-}$ Nefiracetam | *myo7aa*$^{-/-}$ (R)-Baclofen



Koleilat et al. Under Review

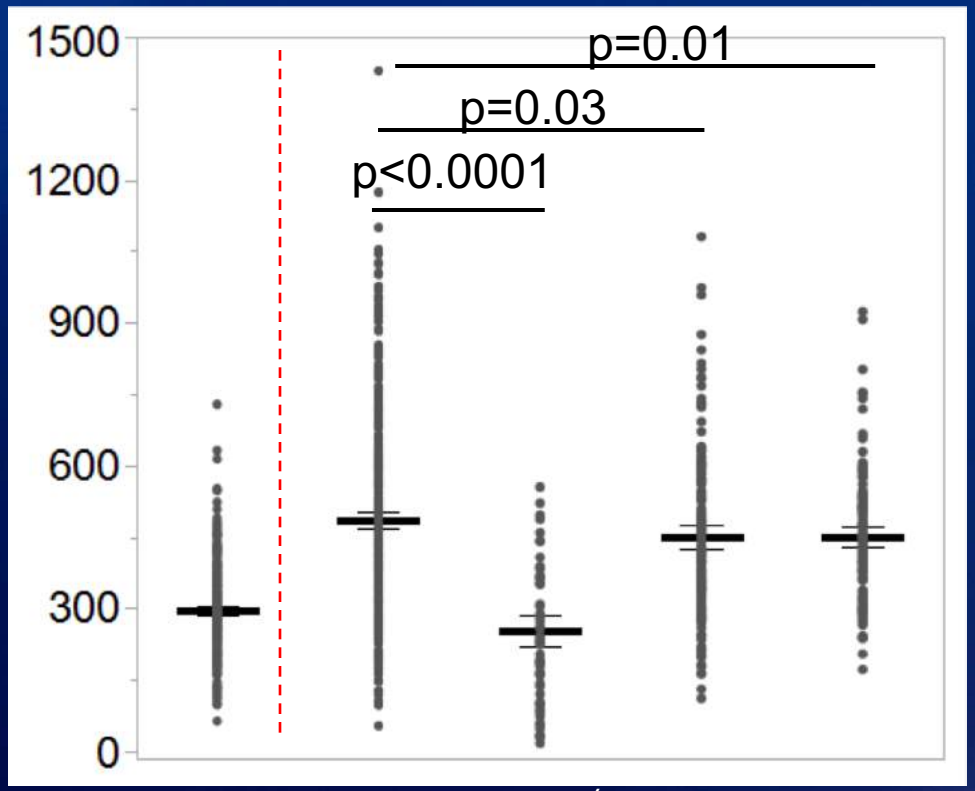
MAYO CLINIC

L-type voltage-gated calcium channel agonists decrease turning angle in *myo7aa*$^{-/-}$ mutant zebrafish



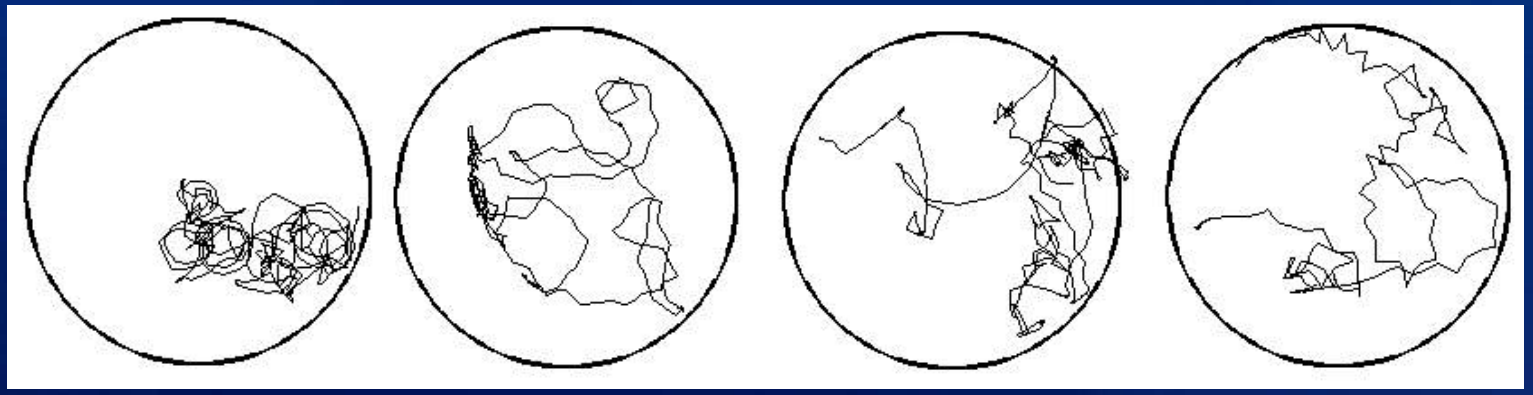
Koleilat et al. In Preparation

L-type voltage-gated calcium channel agonists decrease turning angle in *myo7aa*$^{-/-}$ mutant zebrafish



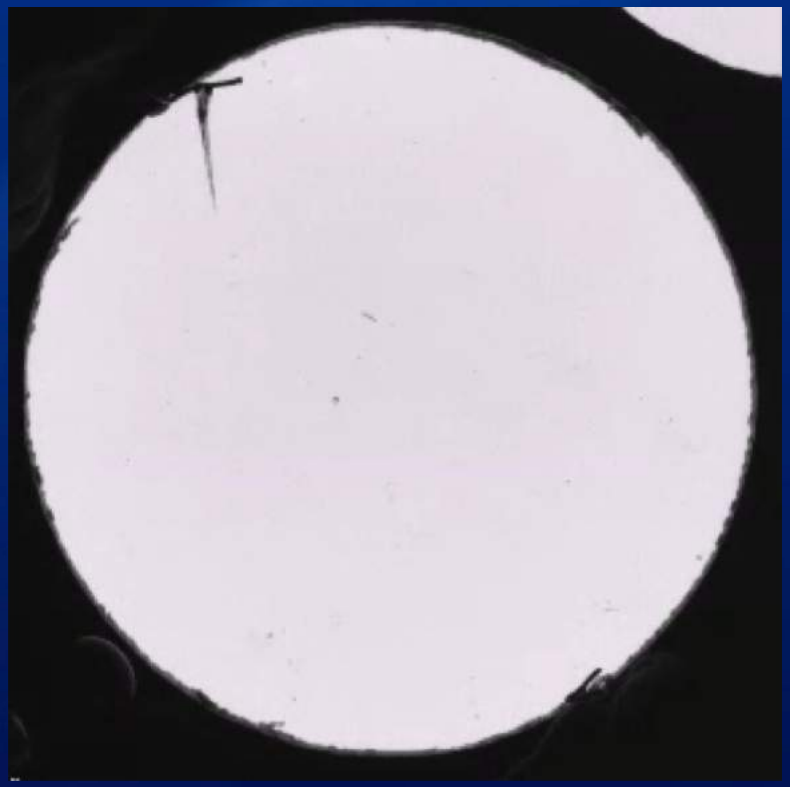
L-type voltage-gated calcium channel agonists decrease turning angle in *myo7aa*$^{-/-}$ mutant zebrafish

myo7aa $^{-/-}$



untreated (±)-Bay K 8644 Nefiracetam (R)-Baclofen

Koleilat et al. Under Review



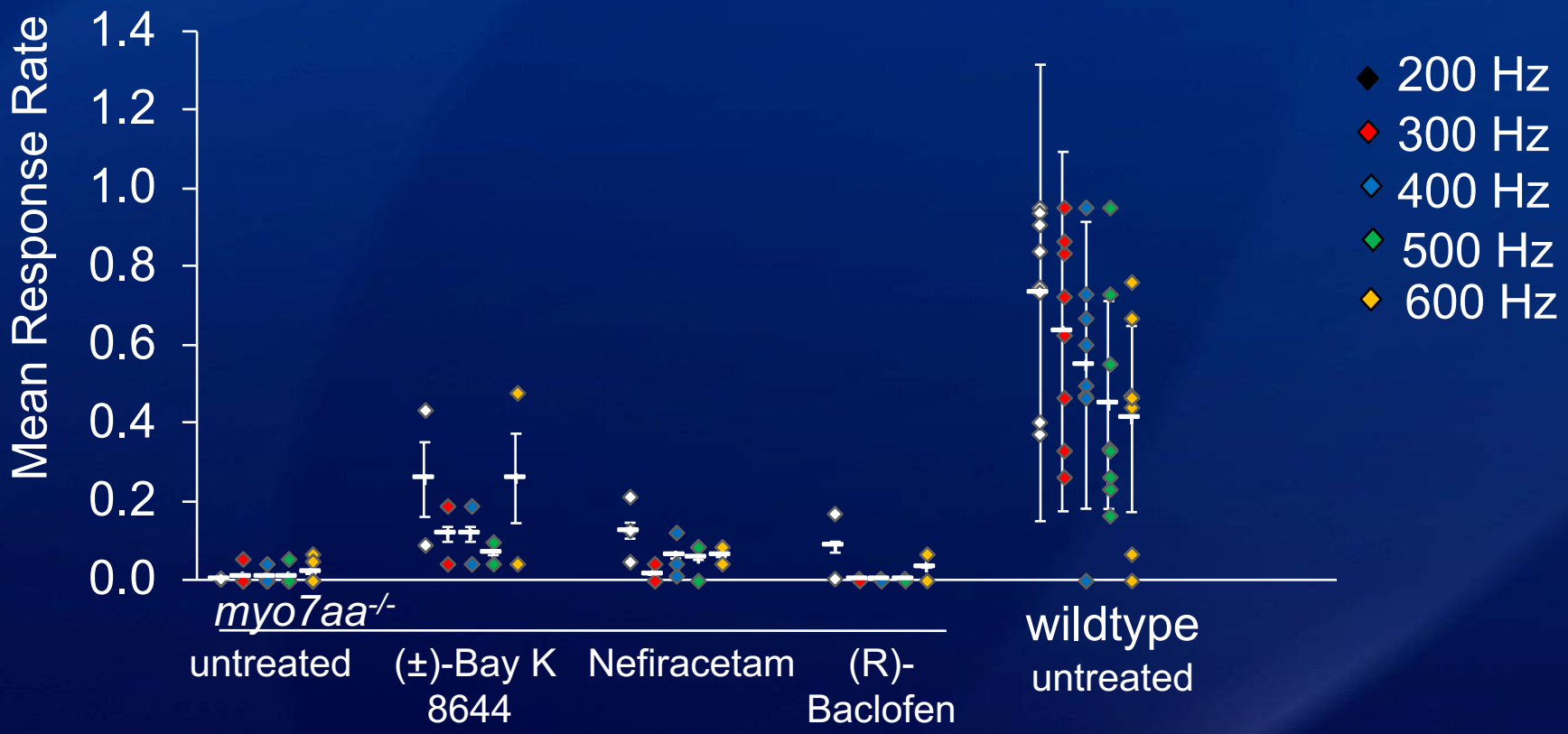
myo7aa$^{-/-}$
Nefiracetam

myo7aa$^{-/-}$
(R)-Baclofen

MAYO CLINIC

Koleilat et al. Under Review

L-type voltage-gated calcium channel agonists increase acoustic startle response in *myo7aa*$^{-/-}$ mutant zebrafish



Koleilat et al. Under Review

Summary and Conclusions

- *myo7aa*$^{-/-}$ mutants have a different synaptic morphology:
  - decreased number of vesicles tethered to ribeye
  - decreased number of ribbon containing cells
  - decreased total CTBP2 puncta per neuromast
  - different distribution of CTBP2 puncta

- *myo7aa*$^{-/-}$ mutants have larger turning angles as part of their swimming behavior

MAYO CLINIC

Summary and Conclusions

- Behavioral and synaptic morphological differences can be modified by drugs with L-type voltage-gated calcium channel activity
  - (R)-Baclofen increases ribbon area and number of tethered vesicles
  - L-type voltage-gated calcium channel agonists shift distribution of CTBP2 puncta to more closely resemble wildtype
  - L-type voltage-gated calcium channel agonists decrease turning angles in swimming behavior and increase acoustic startle response

MAYO CLINIC

Future Directions

- Identify exact mechanism of action of Nefiracetam and (R)-Baclofen
- Assess calcium signal in hair cells with and without L-type voltage-gated calcium channel agonists

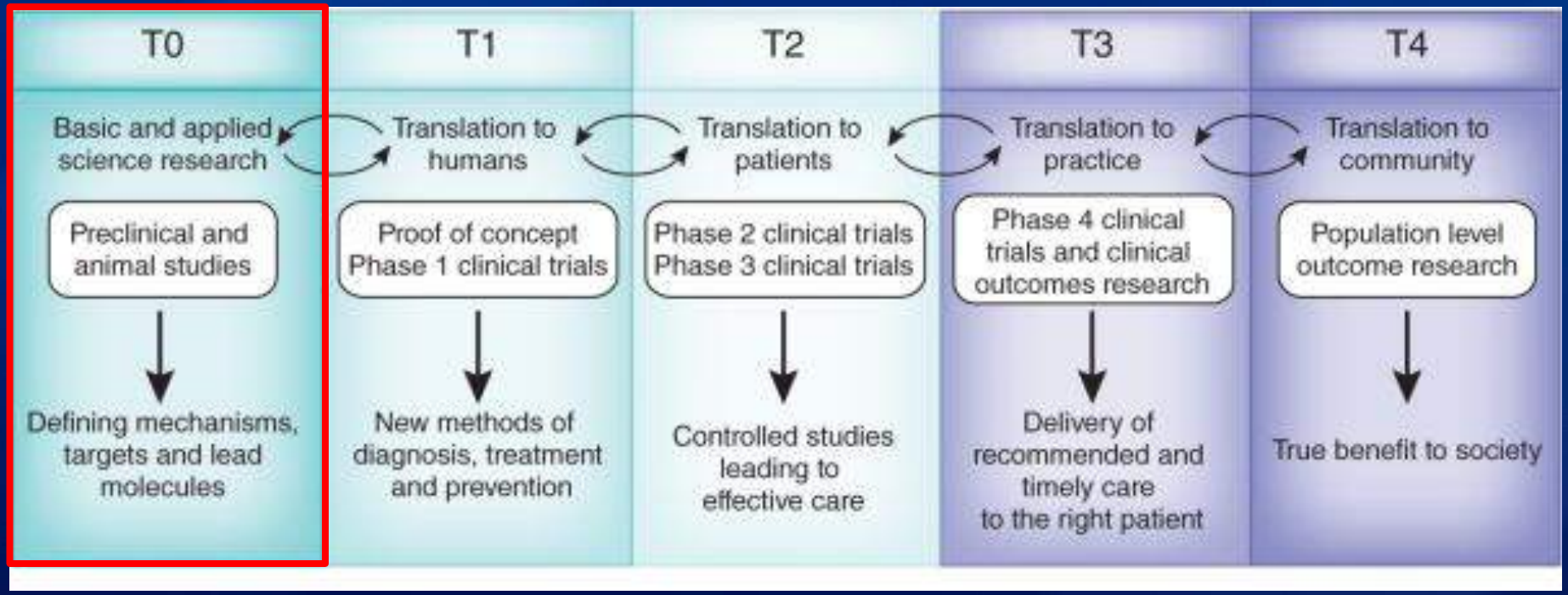
MAYO CLINIC

Future Directions

- Move to the mouse model of USH1 (*shaker-1)* and assess hearing thresholds through auditory brainstem responses upon injection with L-type voltage-gated calcium channel agonists.

MAYO CLINIC

Translational Research



Blumberg et al. 2012 Nat. Med

Acknowledgements

Schimmenti Laboratory- Mayo Clinic
Dr. Lisa Schimmenti, M.D.
Joseph Dugdale

Ekker Laboratory-Mayo Clinic
Dr. Stephen Ekker, Ph.D.
Gabriel Martínez Gálvez, Ph.D. Candidate

Masino Laboratory-University of Minnesota
Dr. Mark Masino, Ph.D.
Dr. Aaron Lambert, Ph.D.
Dr. Timothy Wiggins, Ph.D.

Clark Laboratory- Mayo Clinic
Dr. Han Lee, Ph.D.

Mayo Clinic Microscopy and Cell Analysis Core
Dr. Jeffrey Salisbury
Trace Christensen
Adam Jaspersen

Mayo Clinic Media Services
Mark Curry
Patrick Jochim

Columbia University
Jeff Bellah, Ph.D. Candidate

University of Minnesota and Mayo Clinic zebrafish core facility and staff members

CTSA Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH).

MAYO CLINIC