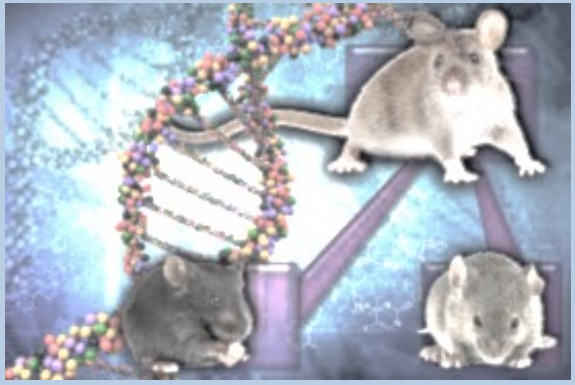
Genetically engineered mouse models with atypical auditory processing

UCONN

CONNECTICUT INSTITUTE FOR THE BRAIN AND COGNITIVE SCIENCES



Canadian Academy of Audiology

Oct 18, 2018

WHY SHOULD WE CARE ABOUT HOW MICE PROCESS SOUNDS?

CONSIDER THE GOAL OF UNDERSTANDING AND TREATING DISEASES LIKE TAY-SACHS…

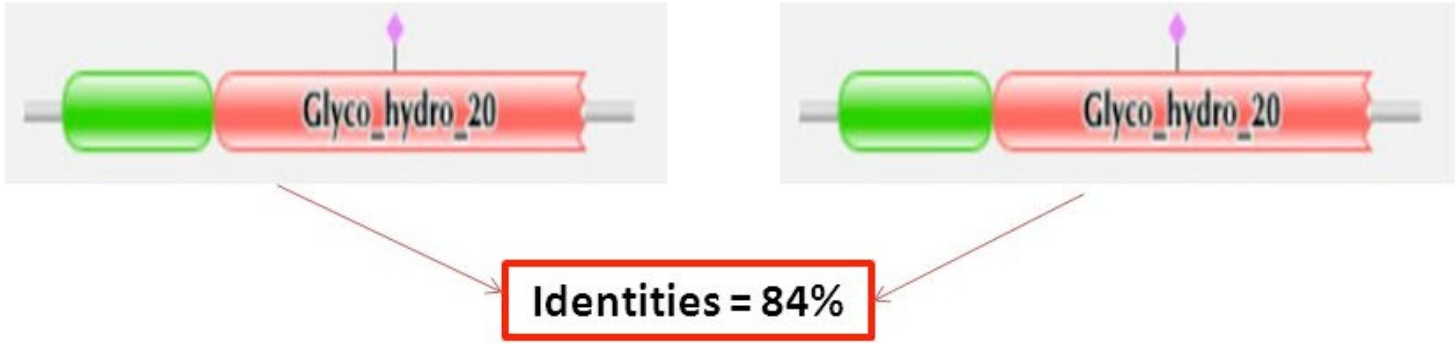
Tay-Sachs disease, or TSD, is a fatal genetic disorder that results in the destruction of the nervous system over time. It happens most often in children. TSD is caused by the lack of an important enzyme in the body. Without the enzyme, a fatty substance (lipid ganglioside) accumulates in neurons, causing cell death.

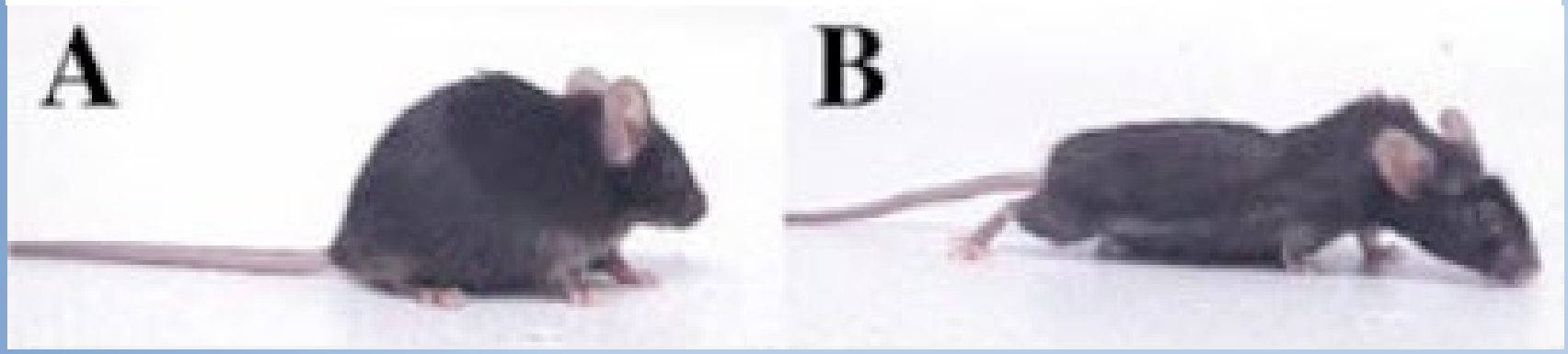
Tay-Sachs disease occurs most commonly in infancy, and is usually fatal by age 4.

…..TAY-SACHS MICE PROVIDE A MODEL WITH GENETIC, CELLULAR and PHENOYTPIC VALIDITY

(CH 15, Hexasominadase A)

- Human protein domain HEXA
- Mouse protein domain Hexa

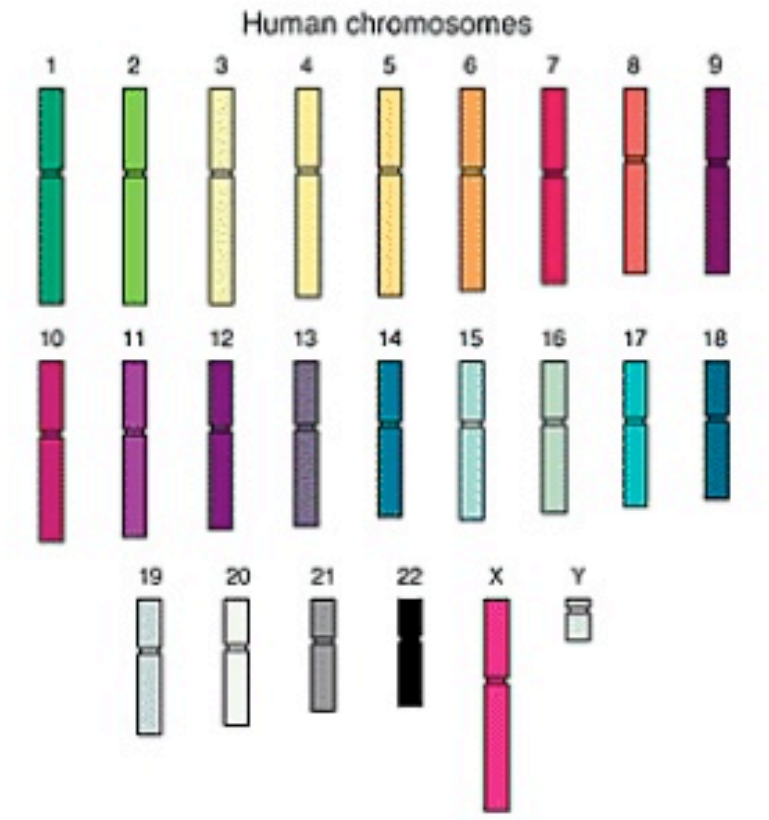




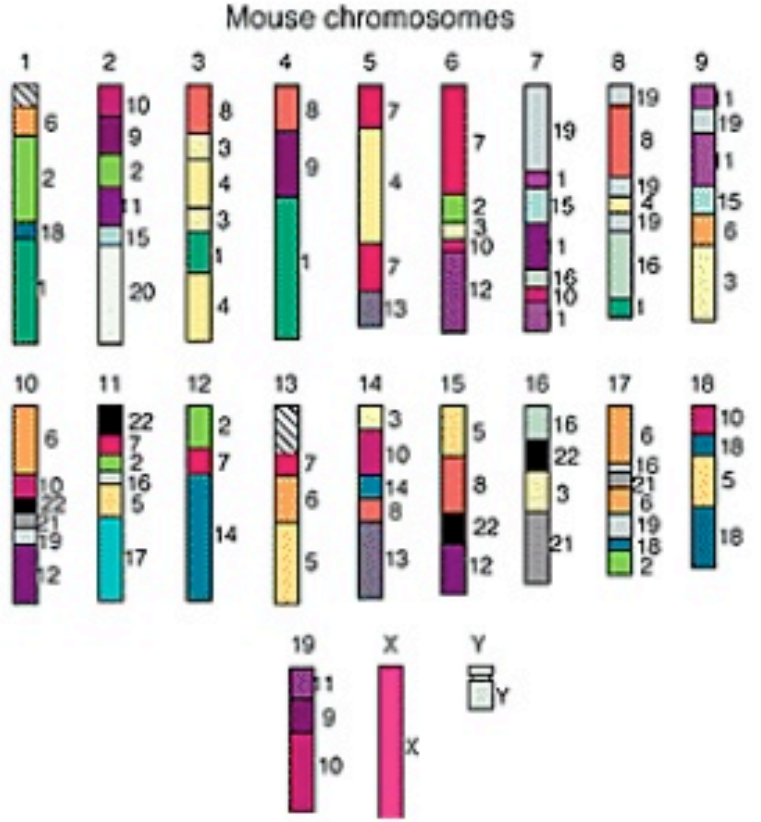
The genetic, cellular and phenotypic features make the HEXA KO TSD mouse model a *valuable tool to understand the etiology and test possible treatments for the disease.*

In fact, mice carry highly homologous **genes** for *most* of the human genes implicated in developmental sensory and cognitive disorders in humans.

23 pairs

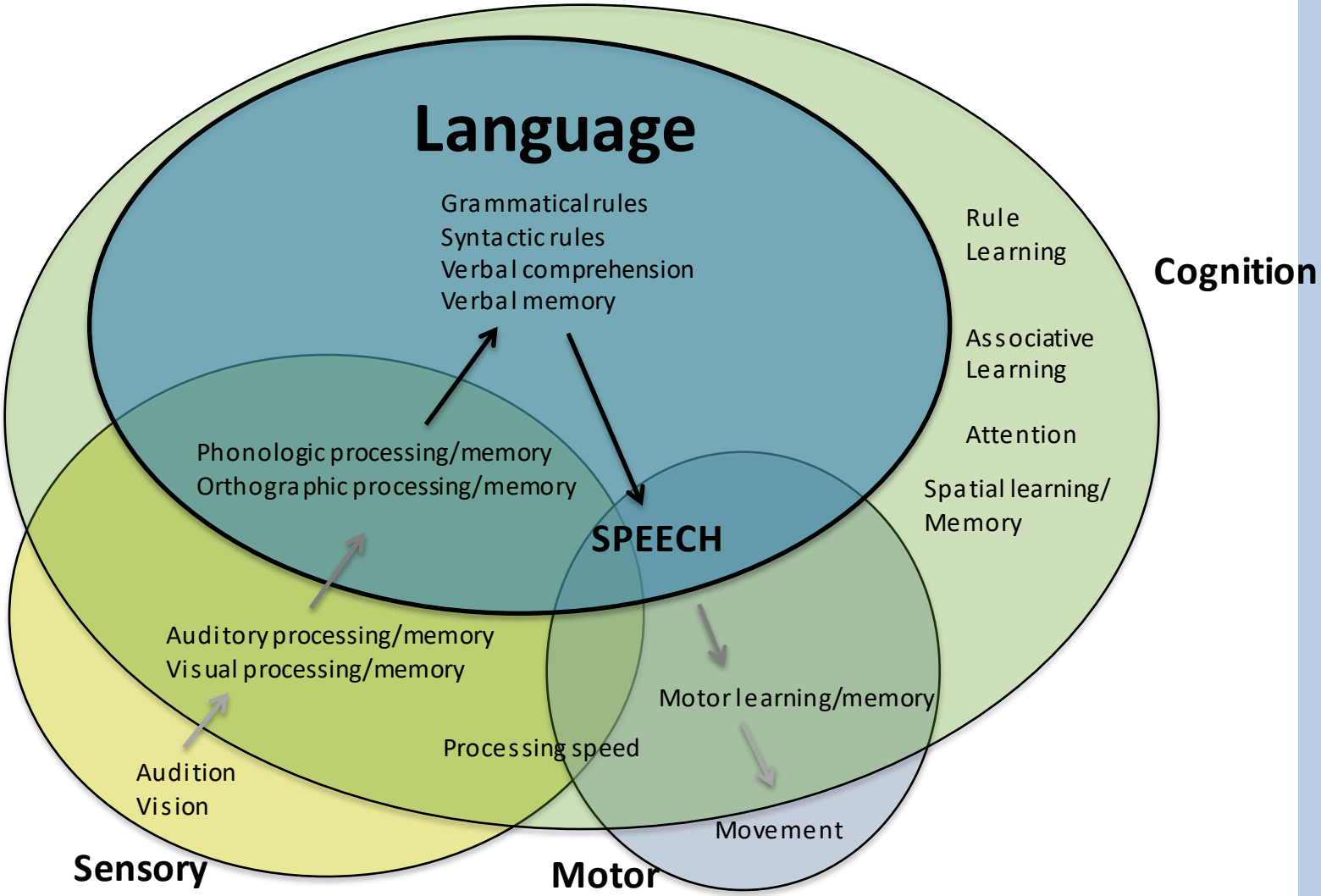


20

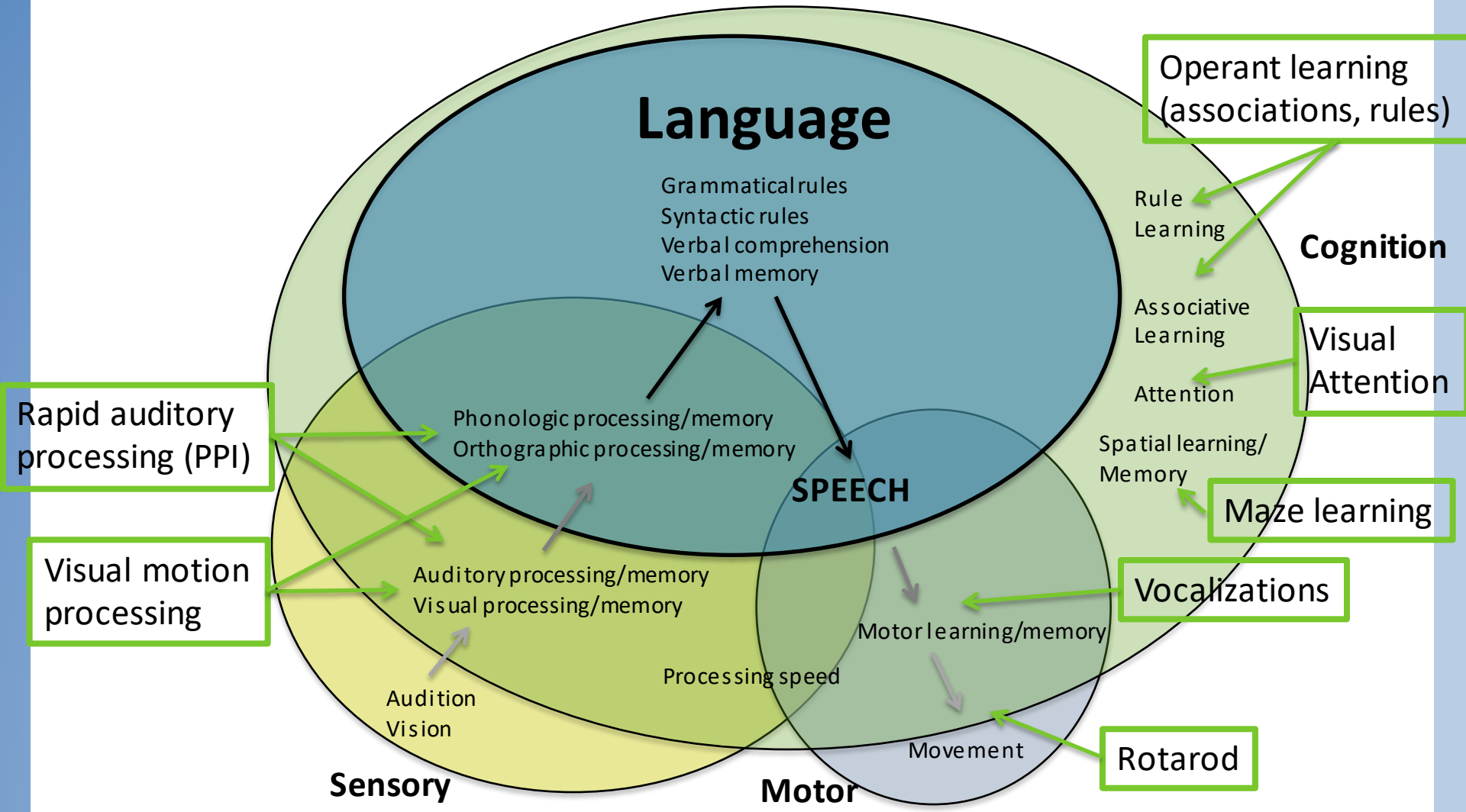


Cut human genome into ~150 pieces to piece together the mouse genome.
Location usually irrelevant, but clustering is often required.

Mice can be tested on **behaviors** and domain-general skills that form a *critical foundation* for functional and academic skills including language and reading.



Mice can be tested on **behaviors** and domain-general skills that form a *critical foundation* for functional and academic skills including language and reading.

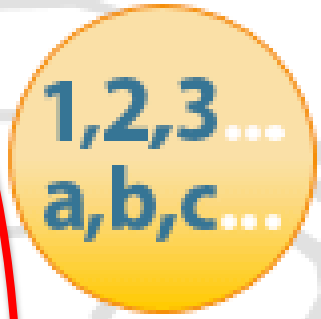


Foundational reading skills

Memory
Important for learning new sounds and words, comprehension - remembering sentences we've read and connecting them together.

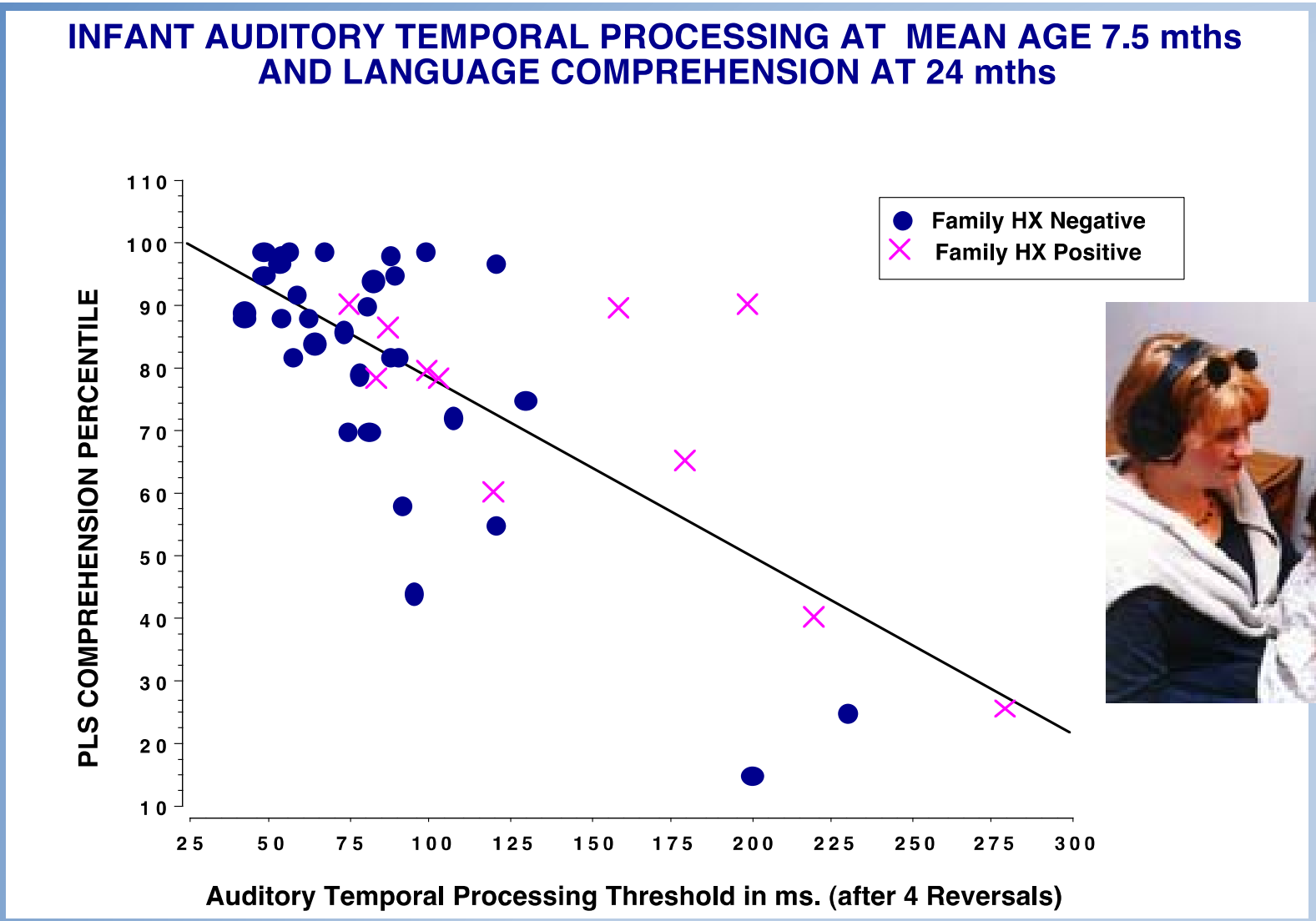
Attention
Important for learning new sounds and words, sticking with a reading task

Auditory Processing
Important for perceiving sounds and words correctly. If the sounds are not processed correctly, the word will be misread



Sequencing
Important for ordering letters within words, words within sentences, sentences within paragraphs

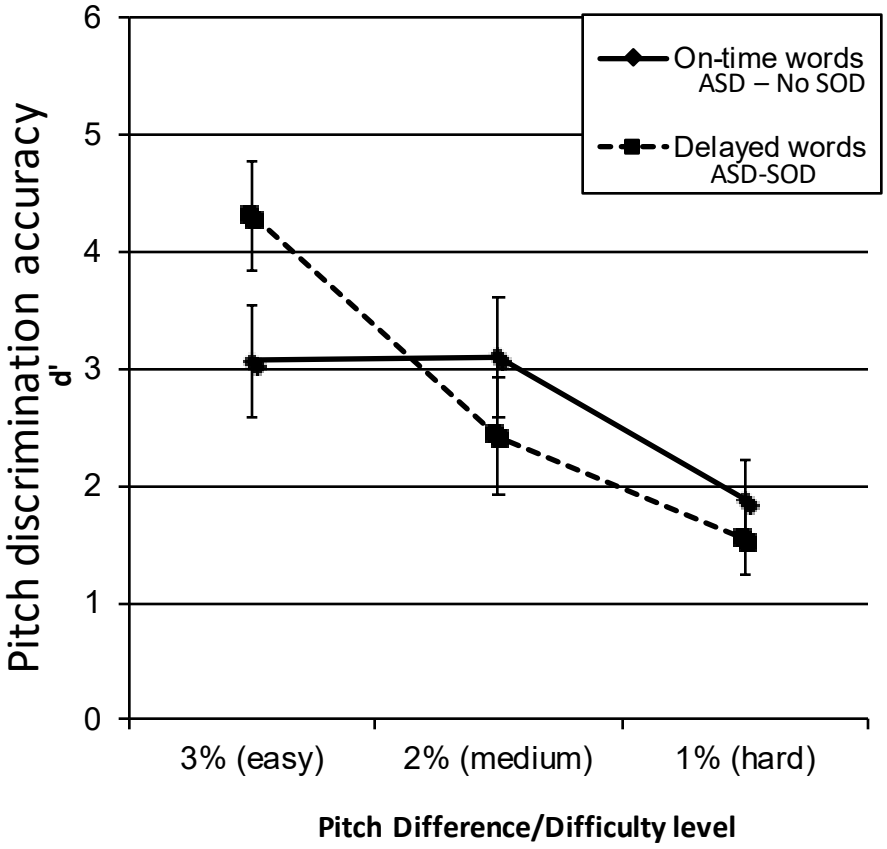
Low level *temporal* acoustic processing deficits are associated with impaired speech and language development



From *Benasich et al., Dev Psychobiol, 2002*

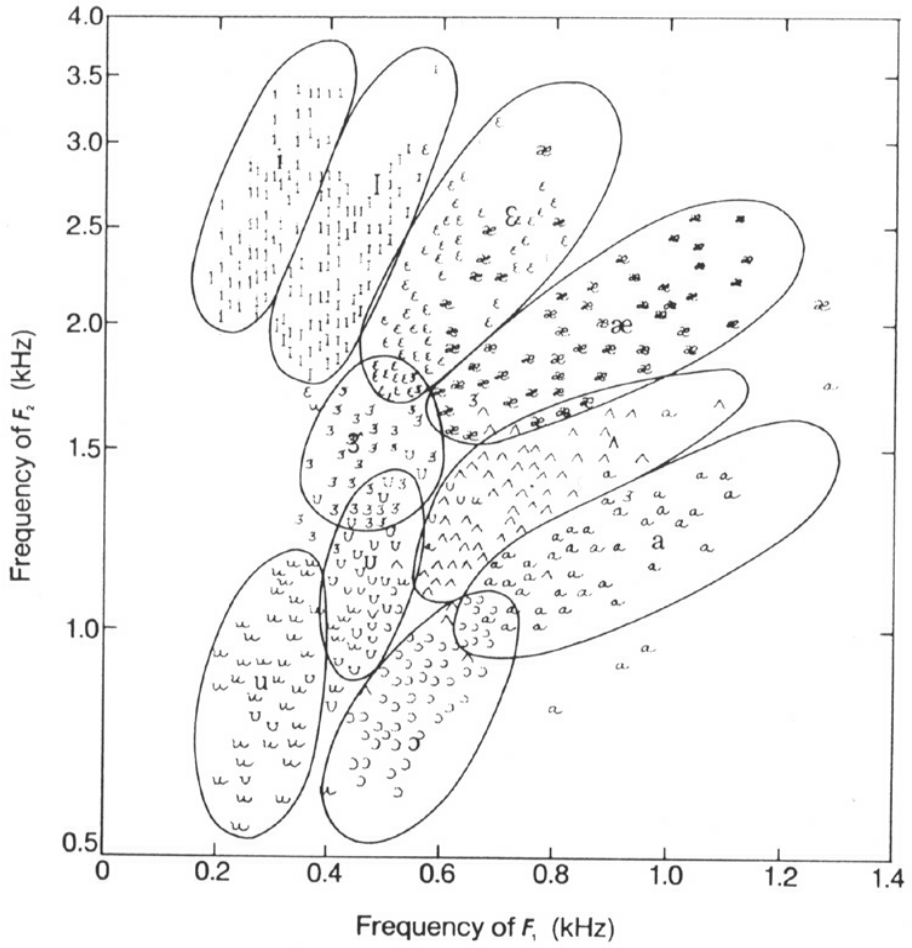
Perceptual pitch processing *enhancements* may also impair speech and language development.

1. ASD = ***better*** pitch discrimination
2. Symptom severity and pitch discrimination (β = 0.292**)
3. ***Better*** pitch discrimination = Language delay (ASD-SOD)

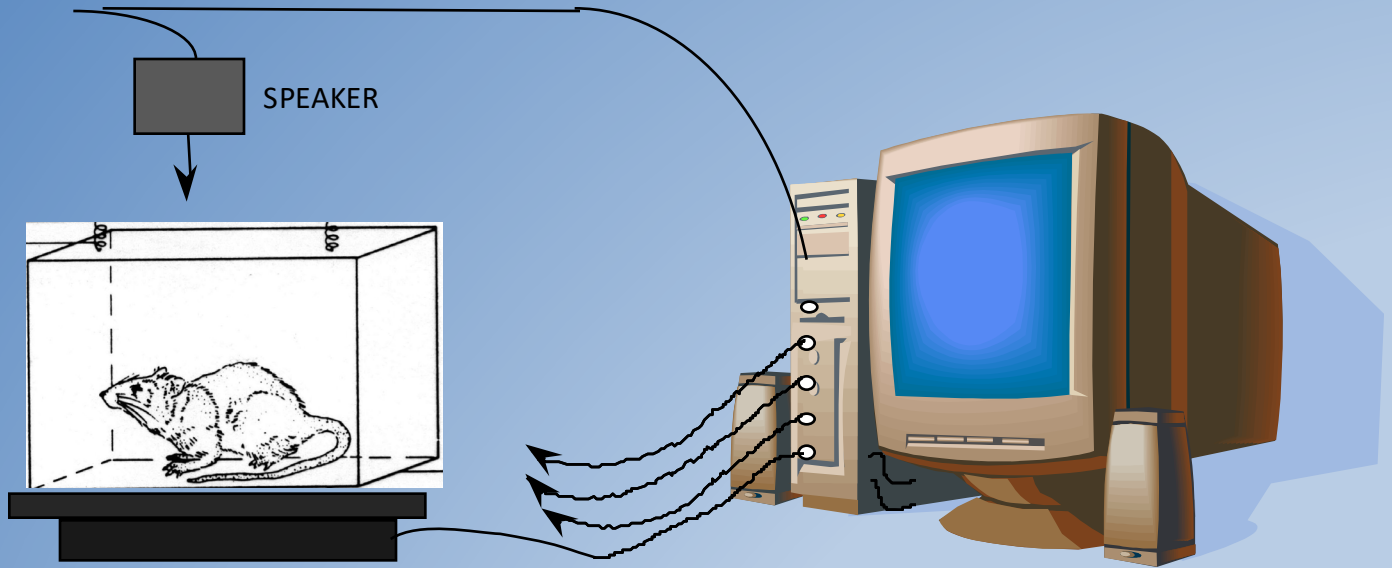


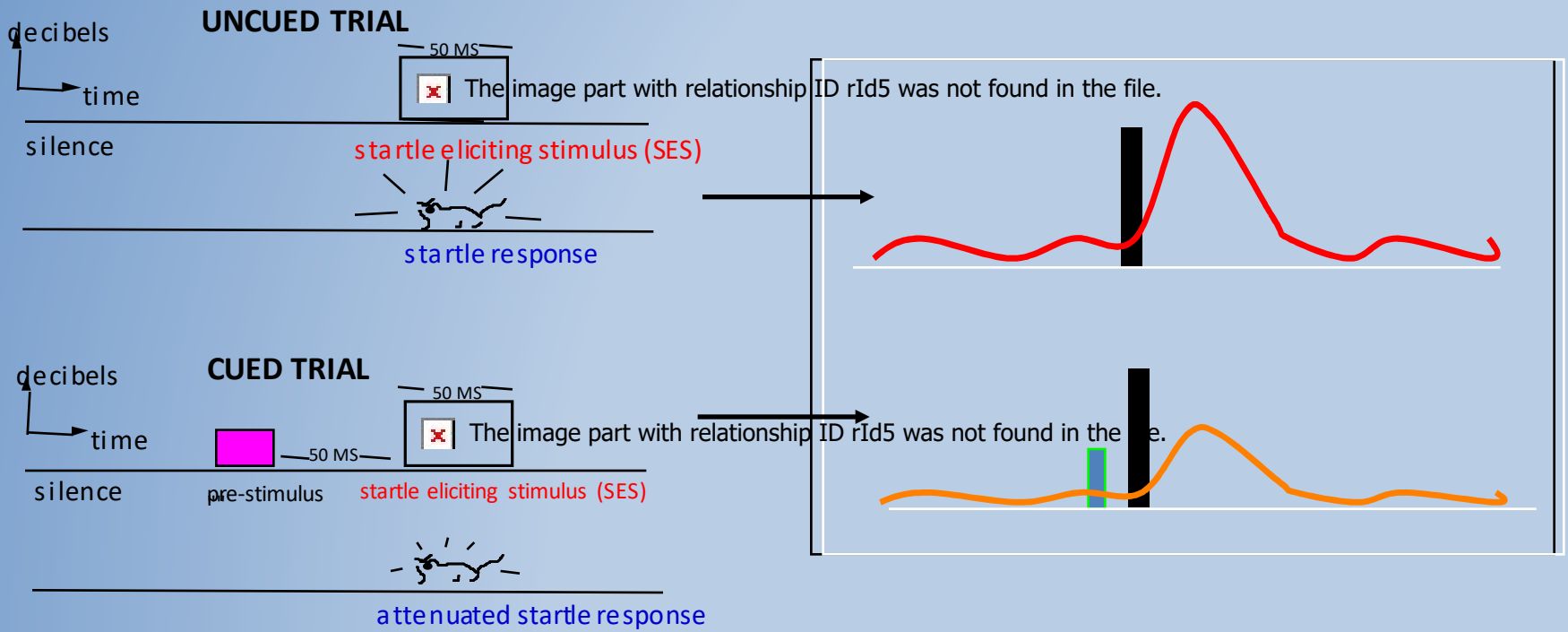
Fein et al., 2013, *JCPP*

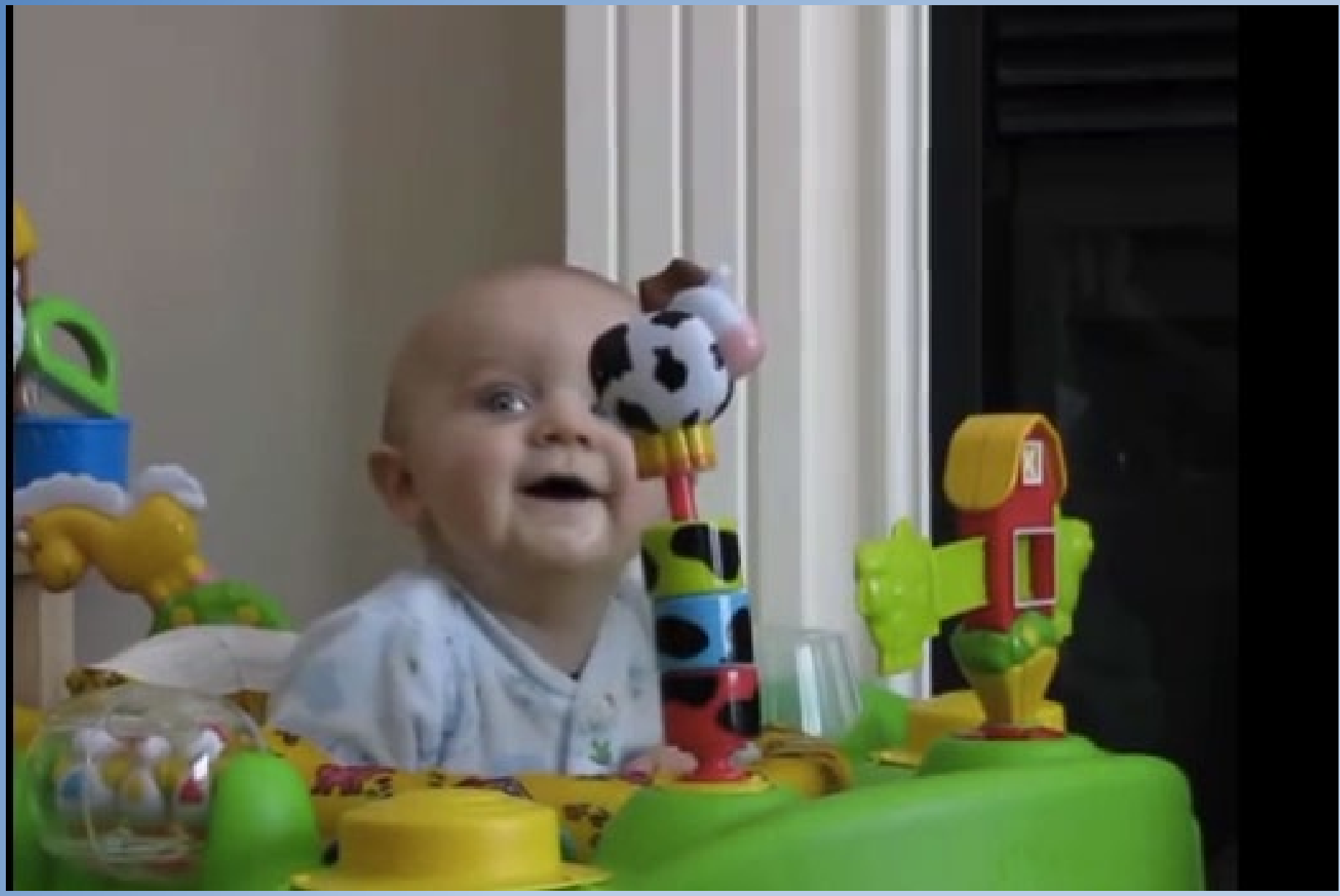
- Early in development: perceive *all* contrasts
- By 12 months: only hear contrasts that are present in native language
- Superior spectral acoustic discrimination may ***impede*** phonological category learning [in] ASD



Kuhl, 2006; Bortfeld & Morgan, 2010


SPEAKER

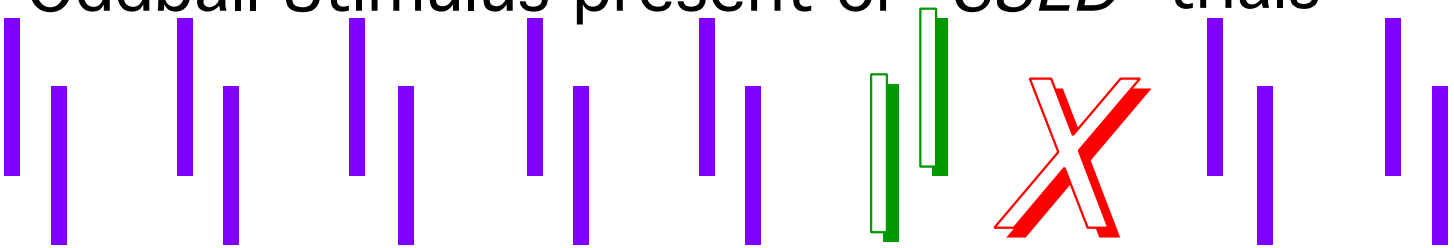

UNCUED TRIAL
decibels
time
silence
50 MS
The image part with relationship ID rId5 was not found in the file.
startle eliciting stimulus (SES)
startle response
CUED TRIAL
decibels
time
silence
50 MS
50 MS
The image part with relationship ID rId5 was not found in the file.
pre-stimulus
startle eliciting stimulus (SES)
attenuated startle response



Measures of rapid/complex auditory processing

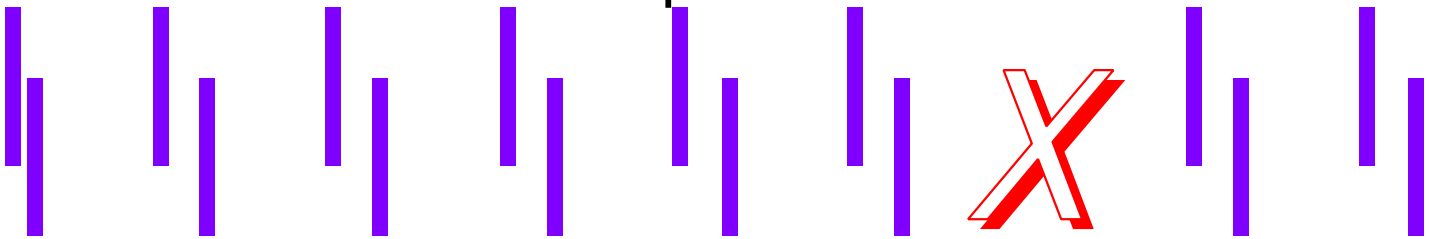
Modified auditory startle reduction, 2-tone oddball (after MMN designs)

Oddball Stimulus present or *CUED* trials



"Within-stimulus" ISI "Between-sequence" ISI

Oddball Stimulus NOT present or *UNCUED* trials



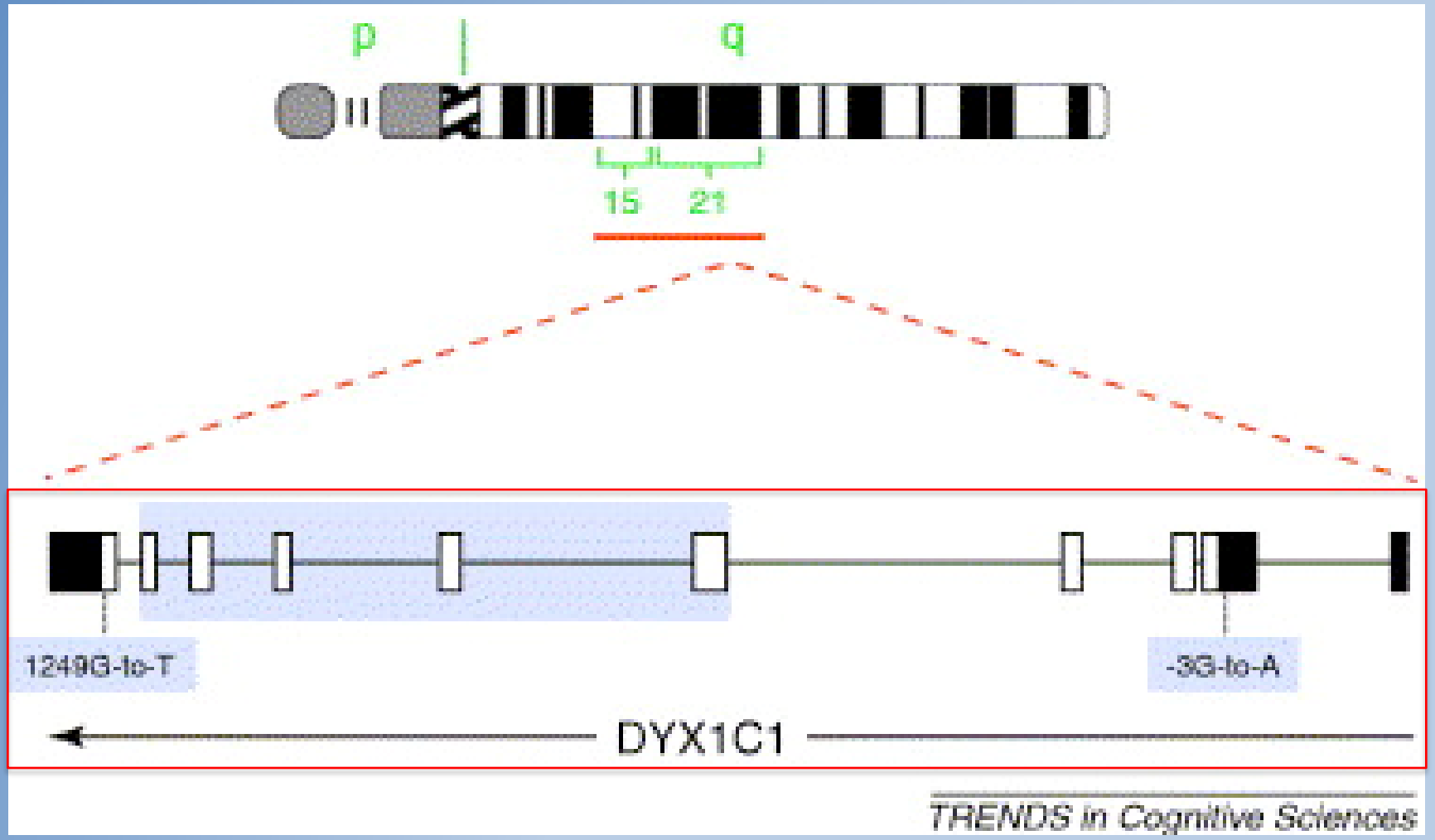
=75 dB, Hi-Low "Standard" Stimulus Pair

=75 dB, Low-Hi "Oddball" Stimulus Pair

=50 msec, 105 dB white noise SES burst

Time ➡

DYX1 (*dyslexia-risk*) region, Ch 15 (human)
DYX1C1 mutations - DYSLEXIA

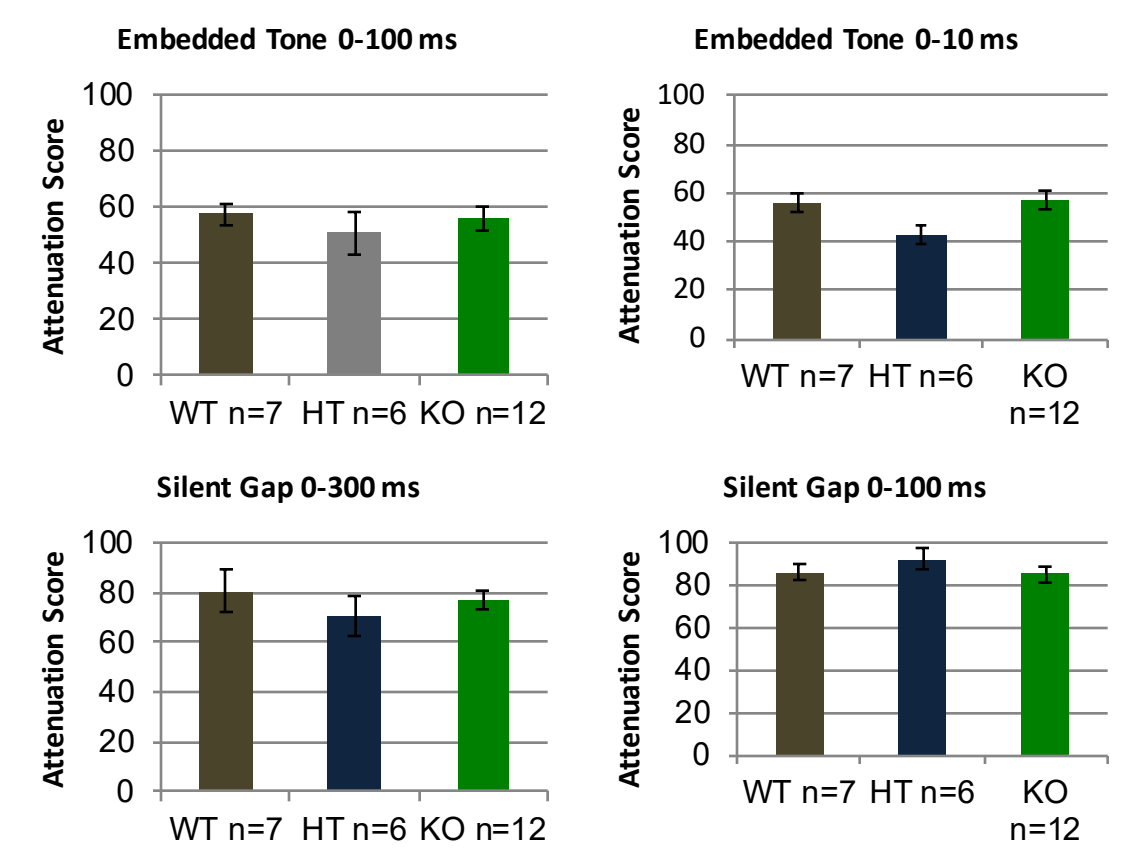


Dyx1c1 KO Mice - Auditory

No deficits were seen for *Dyx1c1* knock-out (KO) mice as compared to WTs on a silent gap detection or embedded tone detection task.

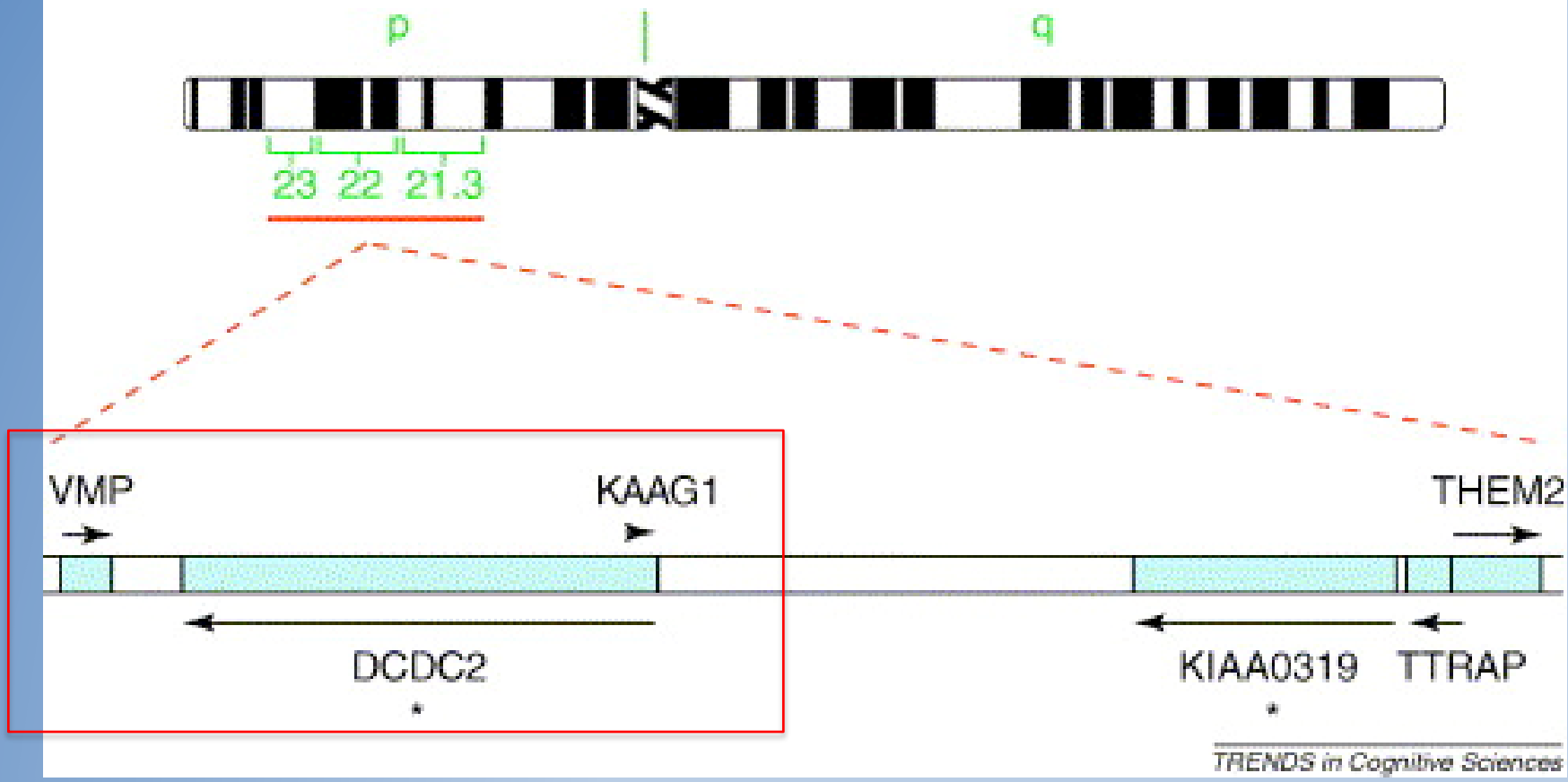
However, we did see *learning, memory* and *motivational* impairments in the KOs.

BETTER ←----------→ WORSE



Rendall, A., Tarkar, T., Contreras-Mora, H.M., LoTurco, J.J. & Fitch, R.H. 2015. Deficits in learning and memory in mice with a mutation of Dyx1c1. *Brain and Language, Special Issue*, S0093-934X(15)00102-9.

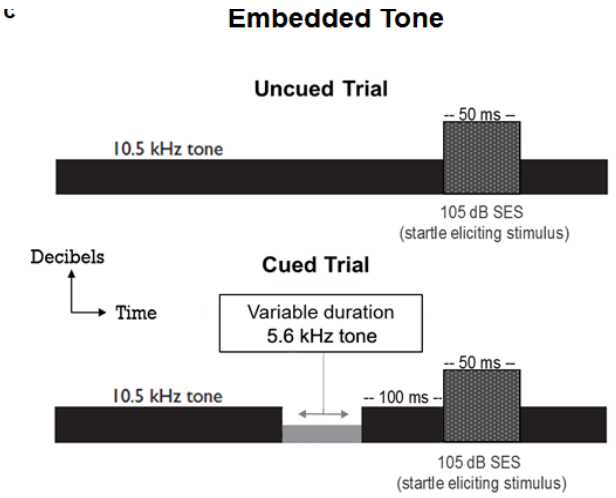
DYX2 region, Ch 6 (human)
DCDC2 mutations and allelic variants – DYSLEXIA, READING

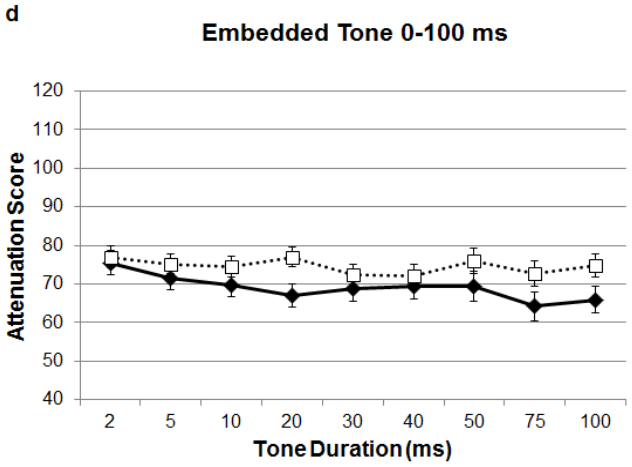


Dcdc2 KO Mice - Auditory

Significant deficits were seen in mice with constitutive KO for *Dcdc2* as compared to WTs on the detection of short embedded tones.

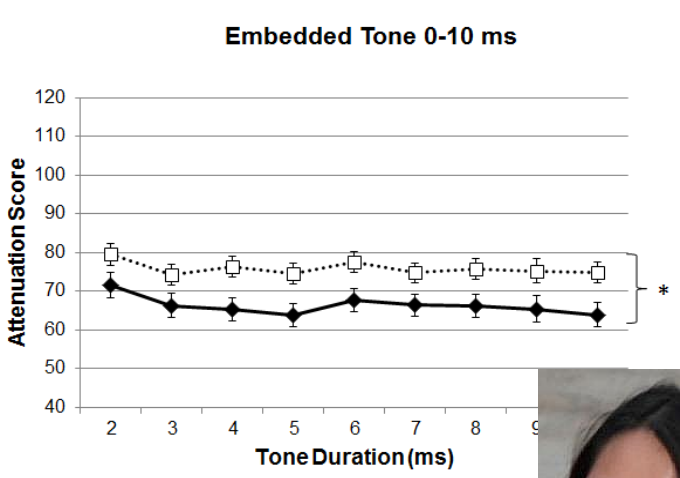
We also saw *learning, and tactile and visual motion discrimination deficits* in the KOs.





WORSE

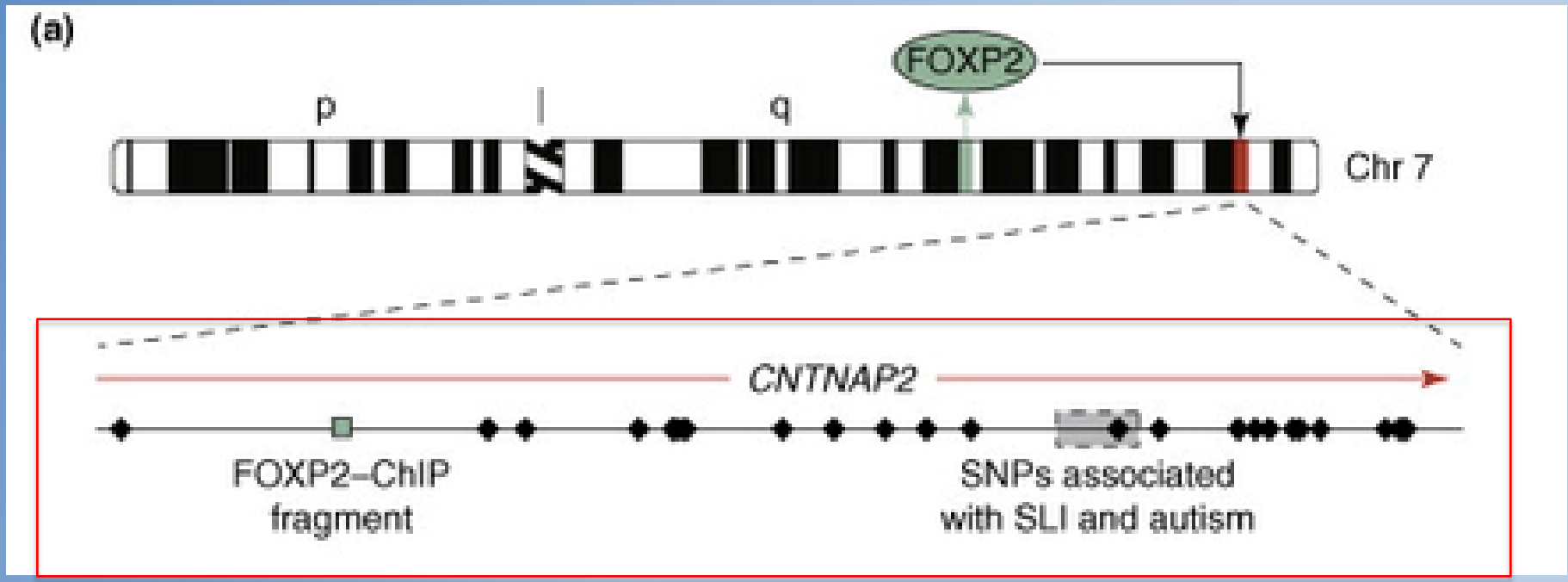
BETTER



WT n=17 Dcdc2$^{del2/del2}$ n=22

Truong, DT, Che, A, Rendall, AR, Szalkowski, CE, LoTurco, JJ, Galaburda, A. & Fitch, RH. 2014. Mutation of *Dcdc2* in mice leads to impairments in auditory processing and memory ability, *Genes, Brain and Behavior*, 13 (8), 802 - 811.

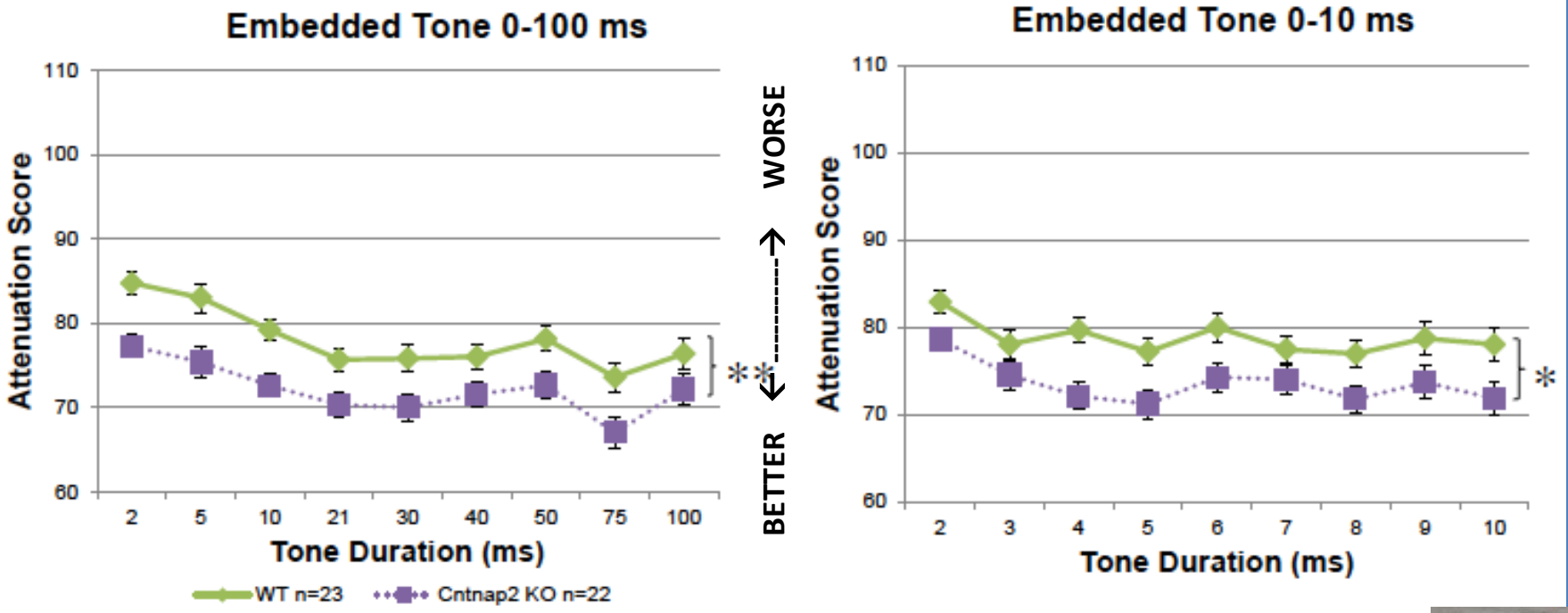
CNTNAP2, Ch 7 (human)
CNTNAP2 mutations – ASD, SLI



CNTNAP2 (contactin-associated protein-like 2) is associated with a number of language-related neurodevelopmental disorders including dyslexia, specific language impairment (SLI), and autism spectrum disorder (ASD), and has also been implicated in language acquisition within the general population (Rodenas-Cuadrado, Ho & Vernes, 2013; Whitehouse et al., 2011).

Cntnap2 KO Mice- Auditory

Mice with constitutive KO for *Cntnap2* showed an unexpected ***significant enhancement*** in frequency discrimination (Purple= KO) (better performance than wild-type controls). We also saw significant deficits in *learning* and *memory*.



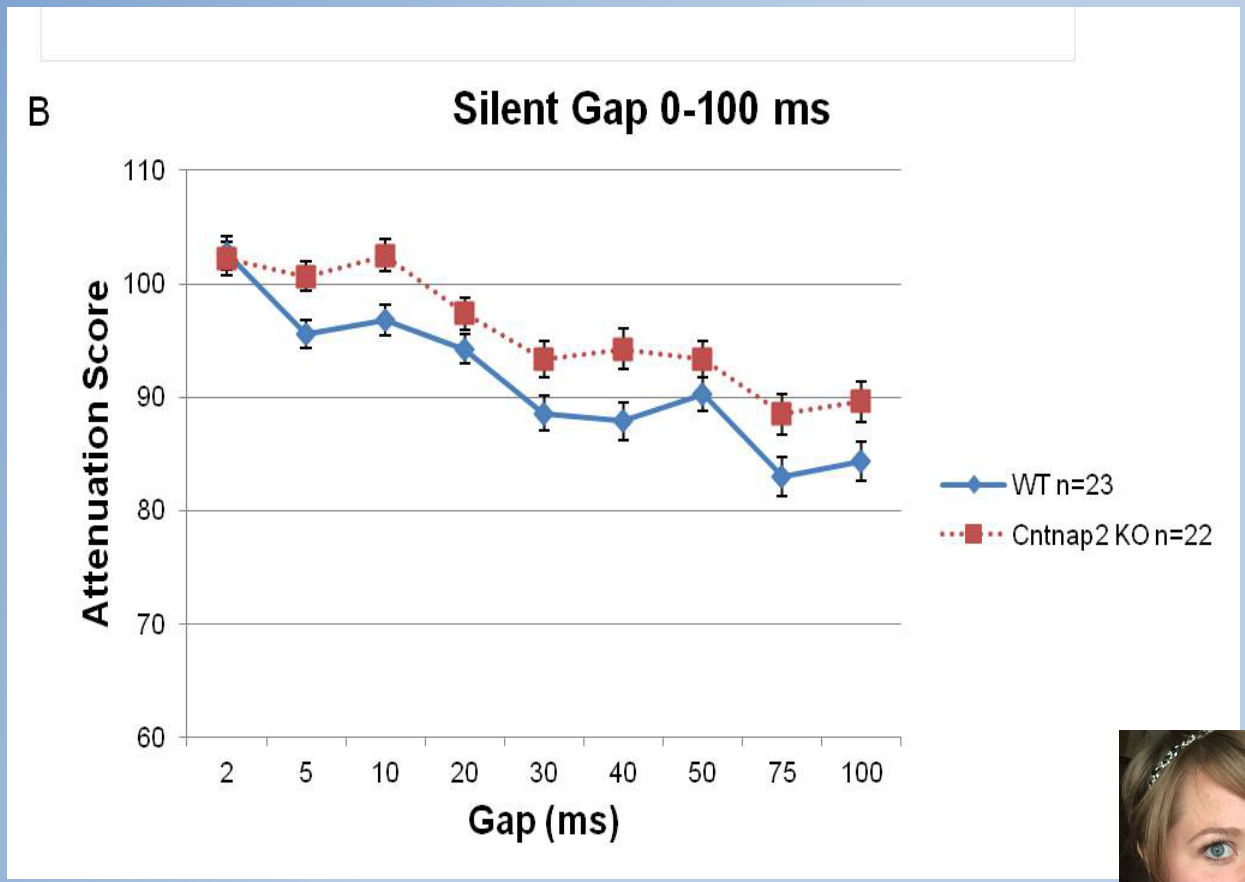
Truong, DT, Rendall, A., Castellucio, B., Eigsti, IME, Fitch, R.H. 2015. Auditory processing anomalies in Cntnap2 mutant mice. *Behavioral Neuroscience,* 129 (6), 731 – 743.

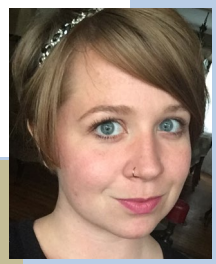
Cntnap2 KO Mice- Auditory

Cntnap2 KOs showed a ***significant impairment*** in spectro-temporal processing (silent gaps, broadband white noise) relative to wild-types.

We also saw *learning* and *memory impairments* in *Cntnap2* KO mice.

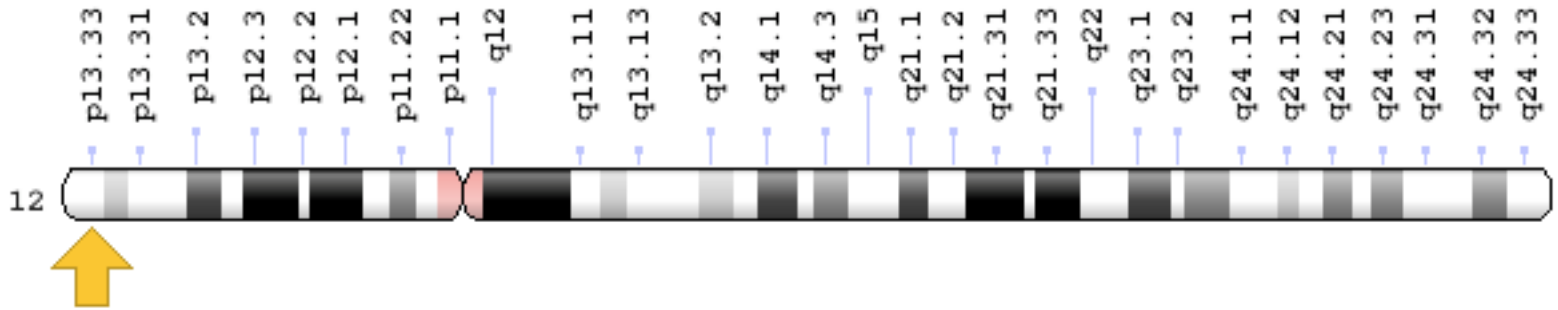
BETTER ←----------→ WORSE





Truong, DT, Rendall, A., Castellucio, B., Eigsti, IME, Fitch, R.H. 2015. Auditory processing anomalies in Cntnap2 mutant mice. *Behavioral Neuroscience,* 129 (6), 731 – 743.

CACNA1C, Chromosome 12 (human)
CACNA1C Mutations – Timothy Syndrome/ASD

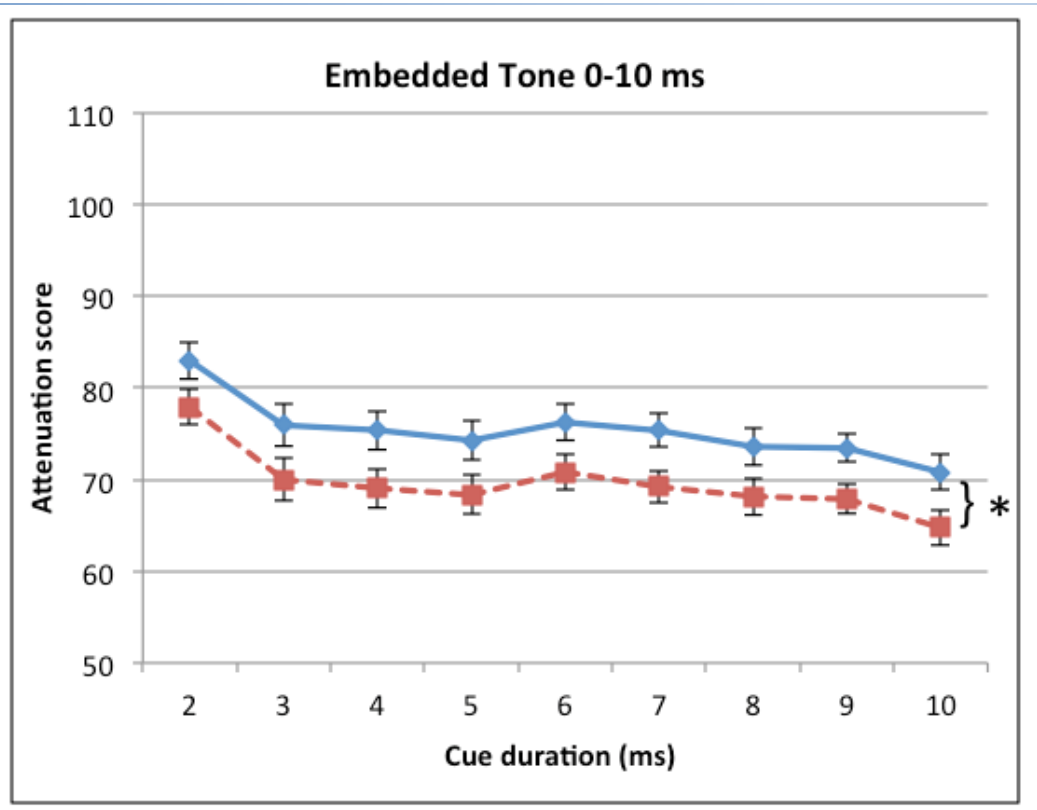


CACNA1C (calcium voltage-gated channel subunit alpha1 C) codes for calcium channel CaV1.2, and is associated with bipolar disorder, schizophrenia, major depression, and ASD. A single *de novo* missense mutation of the 8A exon reduces calcium channel inactivation and increases neuronal Ca2+ influx, resulting in a rare multisystem disorder known as **Timothy syndrome (TS)**. TS is strongly associated with cardiac arrhythmias, ASD, and neurological dysfunctions that include language impairments, seizures and intellectual disability.

TS2-Neo/CACNA1C KI Mice - Auditory

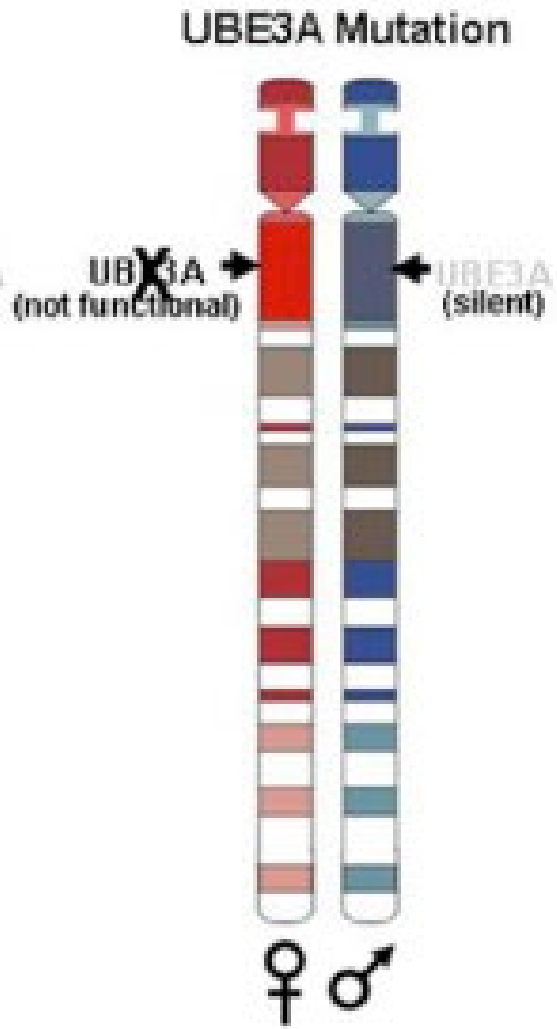
TS2-Neo mice (with a knock-in (KI) of the human *CACNA1C* exon 8A mutation) show atypical auditory processing behaviors, including a ***significant enhancement*** in frequency discrimination (better performance than wild-type controls). *No SG deficits, no effects on working memory*.

BETTER ← ---------→ WORSE



Rendall, A.R., Ford, A.L., Perrino, A.P. & Fitch, R.H. 2017. Auditory processing enhancements in the Ts2-neo mouse model of Timothy Syndrome, a rare genetic disorder associated with autism spectrum disorders. *Advances in Neurodevelopmental Disorders,* 1 (3), 176 - 189.

UBE3A, Ch 15 (human)
UBE3A mutations –
Maternal, Angelman. Paternal, Prader-willi syndrome

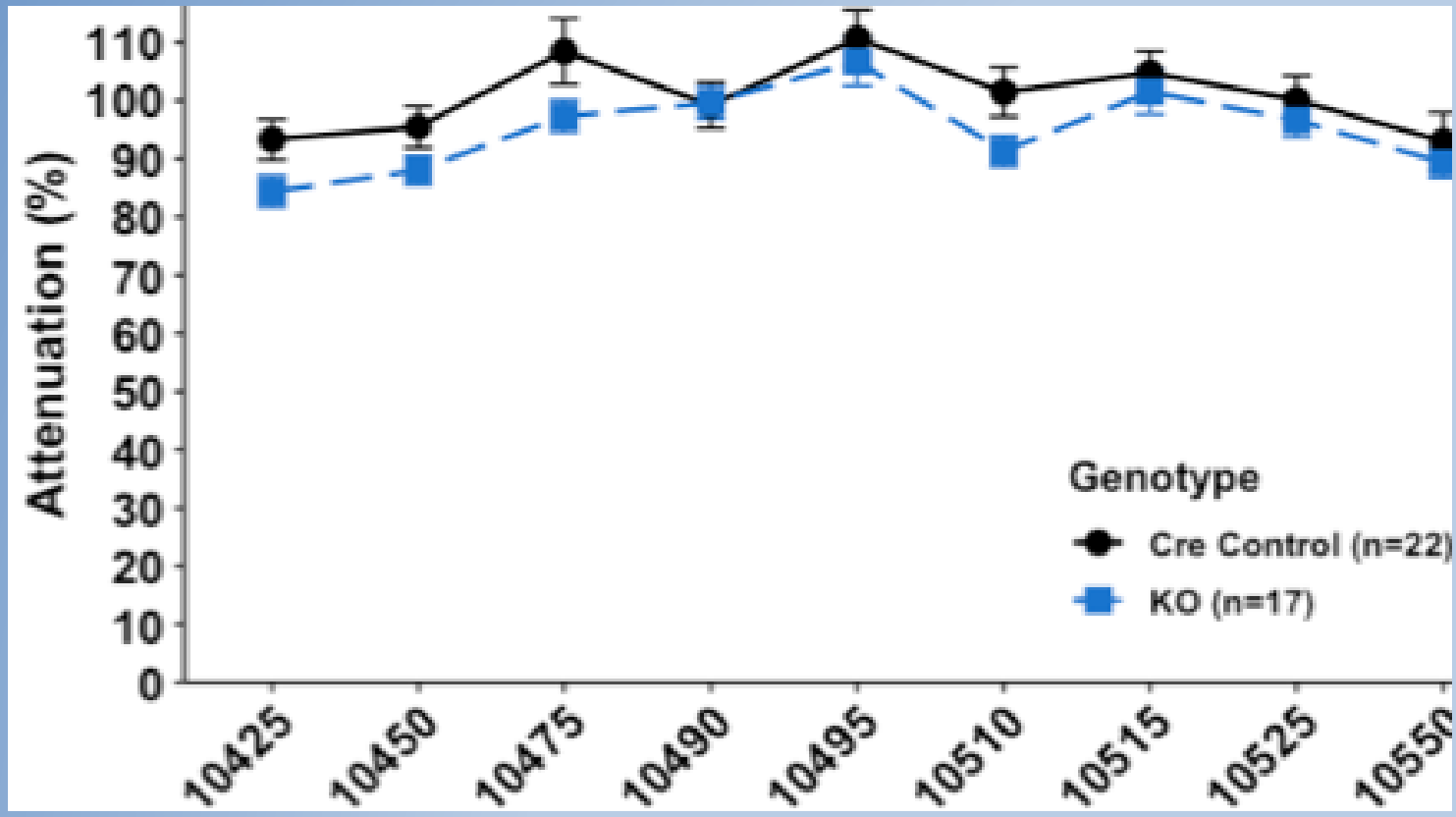


Angelman Syndrome (AS) is a congenital disorder characterized by developmental delays, seizures, and language impairments with reduced/absent speech. AS affects approximately 1 in 15,000 live births, and results from a lack of expression of the maternally-imprinted *UBE3A* gene on chromosome 15q11.2.

Ube3a KO Mice- Auditory

Mice with constitutive KO for *Ube3a* showed an unexpected ***significant enhancement*** in frequency discrimination (blue = KO) (better performance than wild-type controls). Similar effects seen on FM sweep detection.

BETTER ←----------→ WORSE



Perrino, P., Chamberlain, S. & Fitch, R.H. 2018. Language-related assessments in a mouse AS model. SFN Abstracts.

Ube3a KO Mice- Vocalizations

Mice with constitutive KO for *Ube3a* made ***fewer vocalizations***. These vocalizations were significantly *longer, louder and higher in frequency*.

Figure 1D. KO females vocalizations were about 5,000 Hz higher in frequency (on average) than control females (p< .001).

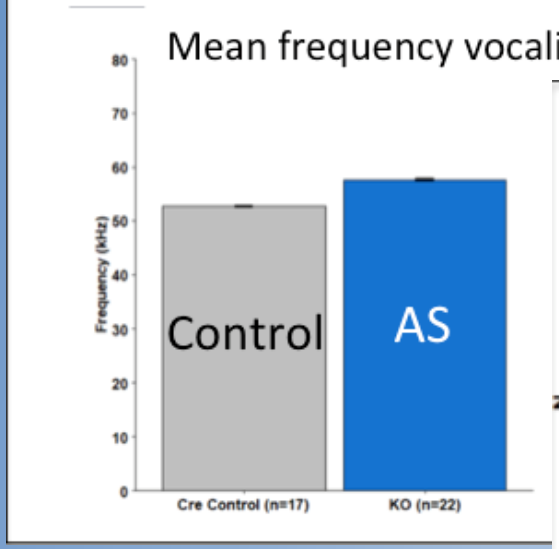
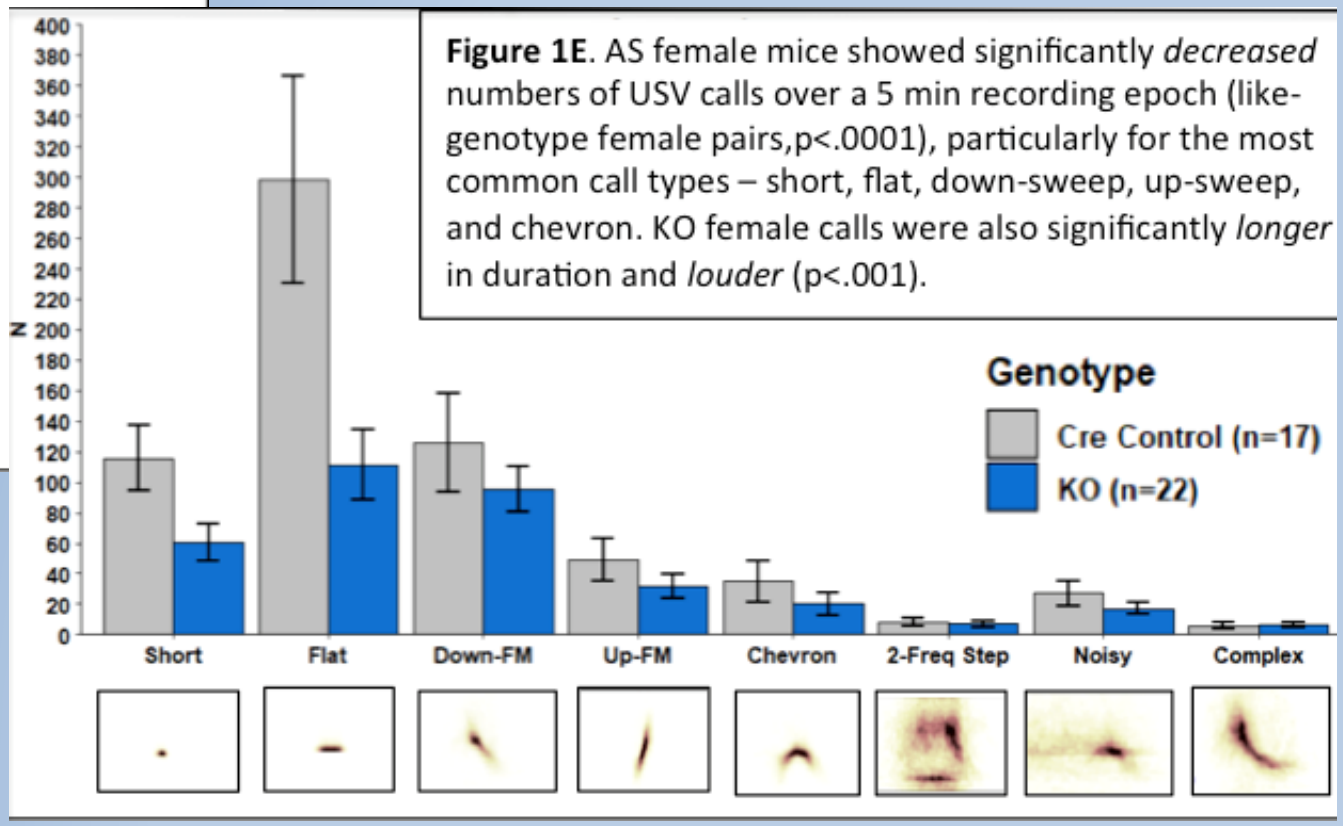


Figure 1E. AS female mice showed significantly *decreased* numbers of USV calls over a 5 min recording epoch (like-genotype female pairs,p<.0001), particularly for the most common call types – short, flat, down-sweep, up-sweep, and chevron. KO female calls were also significantly *longer* in duration and *louder* (p<.001).



Engineered mice allow us to study links between *atypical auditory processing* (deficits/enhancements), and *gene mutations* associated with developmental language disorders.

Our findings suggest *atypical low-level sound processing is associated with mutations in many of the genes linked to language disability.*

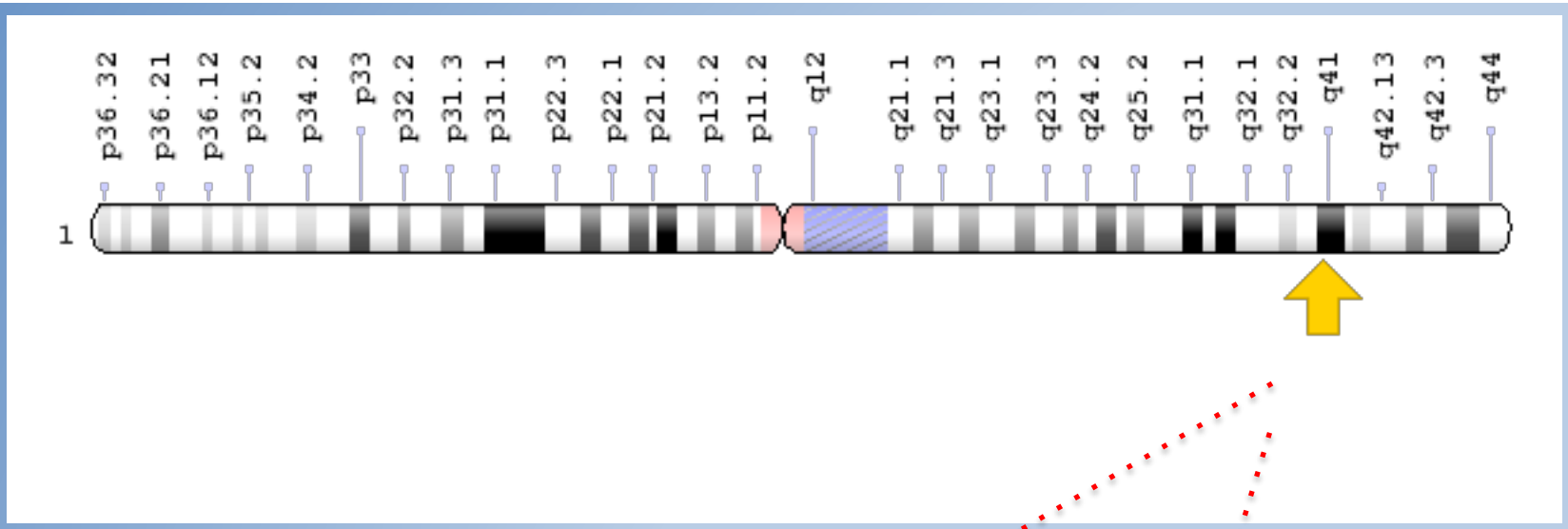
Causal or co-morbid? Mice cannot answer this.

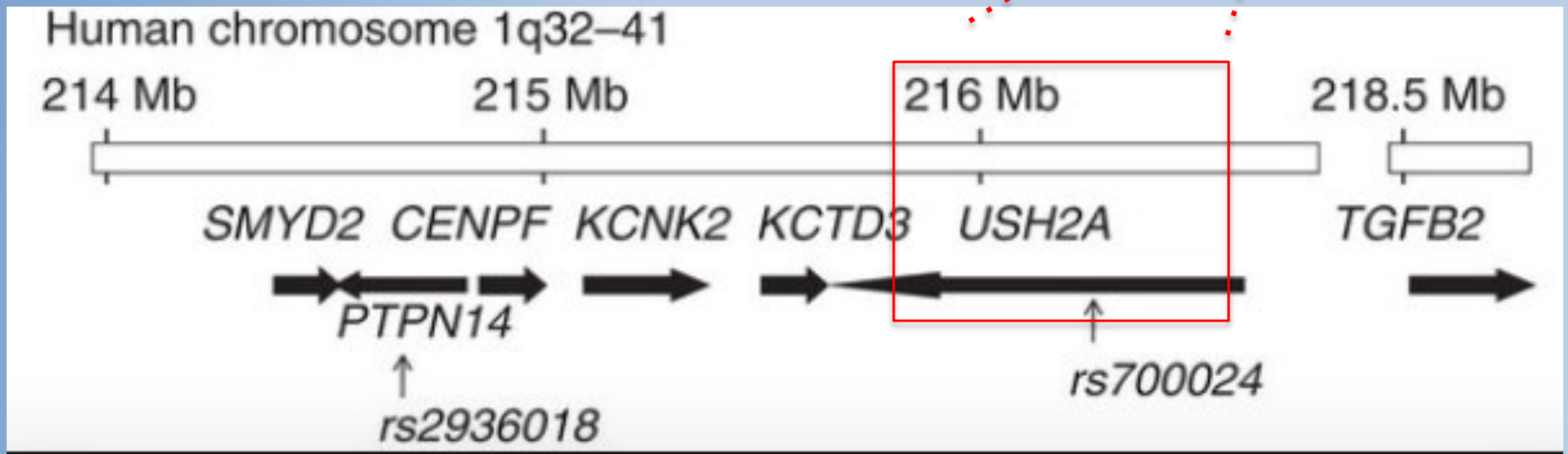
Gene	Clinical Association	Acoustic -Pitch	Acoustic-Rapid	Acoustic-Complex	Learning & memory	Visual Motion	USV Production
Dyx1c1	Dyslexia	=	=	–	–		
Kiaa0319	Dyslexia	=	–	=	=		
Dcdc2	Dyslexia	=	–	–	–	–	
Cntnap2	ASD, SLI	+	+	–	–		–
Cacna1c	ASD	+	+	=	=		–
Shank3	ASD	+	=	–	–		–
Ube3a	**AS**	+					–

USHER2A, Chromosome 1 (human)
USHER2A homozygous mutations – Usher syndrome

congenital/prelingual bilateral sensorineural hearing loss mild/moderate in low frequencies and severe/profound in higher frequencies, intact vestibular, retinitis pigmentosa.

Usher Syndrome is considered a sensory and NOT intellectual condition.

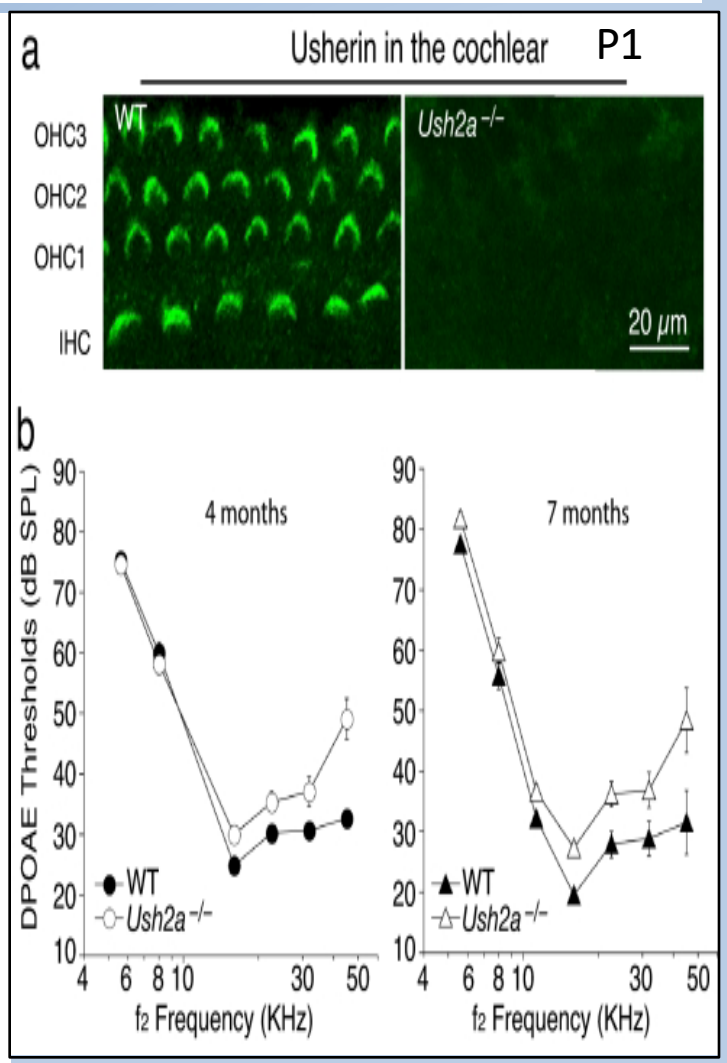




Ush2a KO Mice - Auditory

IHC = Inner Hair Cell, OHC = Outer Hair Cell

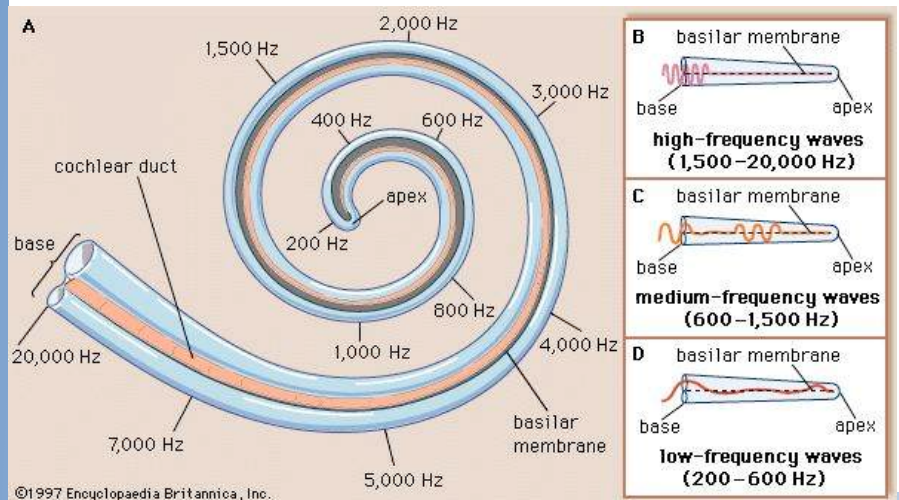
- *Ush2a* KO mice develop a spectrum of retinal and hearing deficits that closely resemble those of *USH2A* human patients
  - Photoreceptor degeneration
  - Moderate, nonprogressive hearing loss – especially at high frequencies (>10-20 kHz)



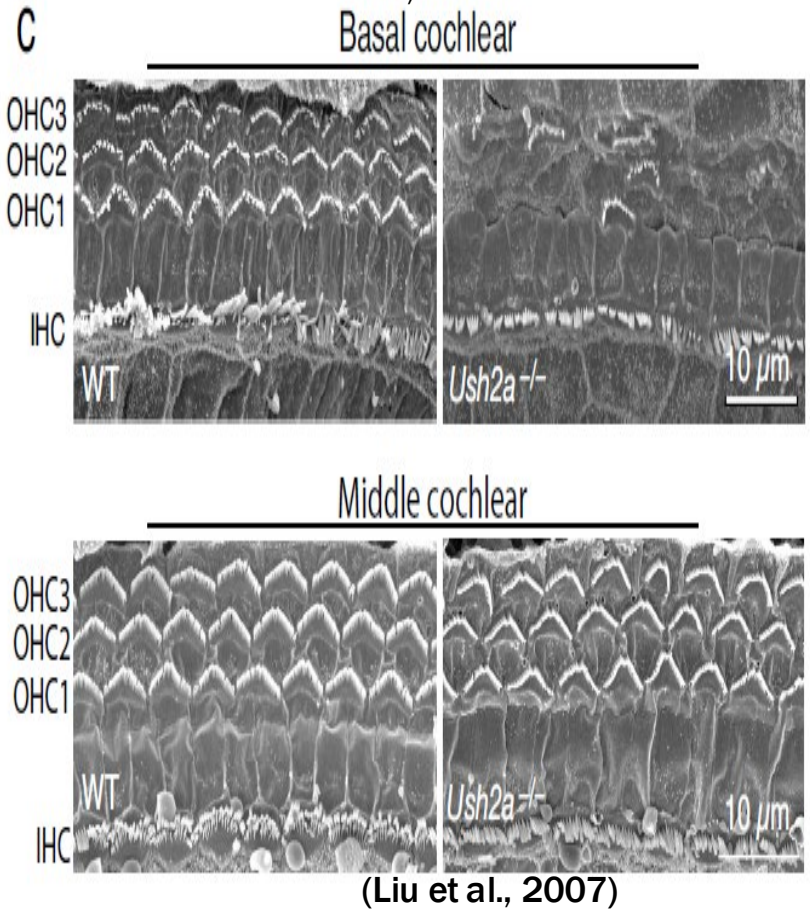
(Liu et al., 2007)

Ush2a KO Mice - Auditory

- *Ush2a* KO mice show structural changes in cochlea
  - Outer hair cells of **basal** cochlea **absent** in *Ush2a* KO mice
  - Outer hair cells of middle cochlea present in *Ush2a* KO mice
- Consistent with findings of high frequency hearing deficits



IHC = Inner Hair Cell, OHC = Outer Hair Cell



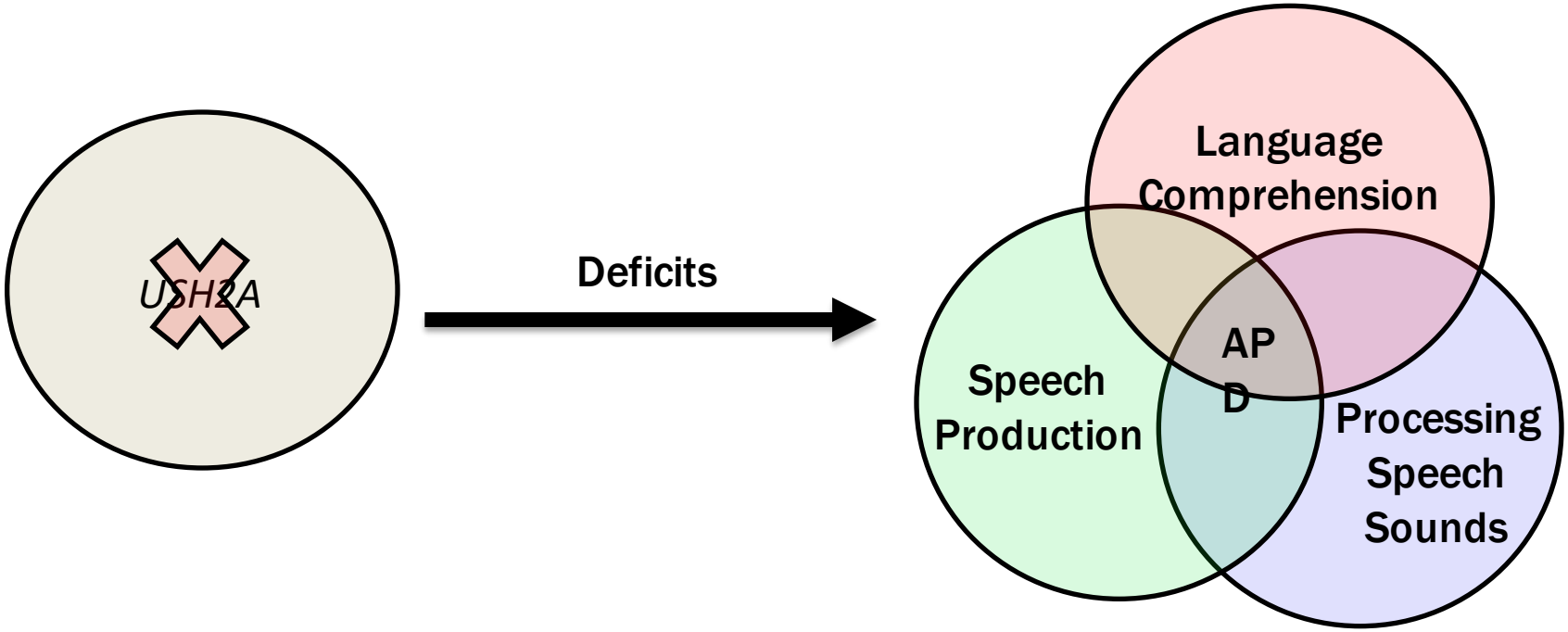
(Liu et al., 2007)

*Fixed cochlea from wt, -/- **and +/-** tested mice were recently sent to Newbury et al. for EM analysis.*

From NIH NCBI GeneReviews Database:

Genetic counseling. Usher syndrome type II is inherited in an autosomal recessive manner. Each subsequent pregnancy of a couple who has had a child with Usher syndrome type II has a 25% chance of resulting in an affected child, a 50% chance of resulting in an unaffected child who is a carrier, and a 25% chance of resulting in an unaffected child who is not a carrier. Prenatal testing is possible for pregnancies at increased risk if the pathogenic variants have been identified in the family.

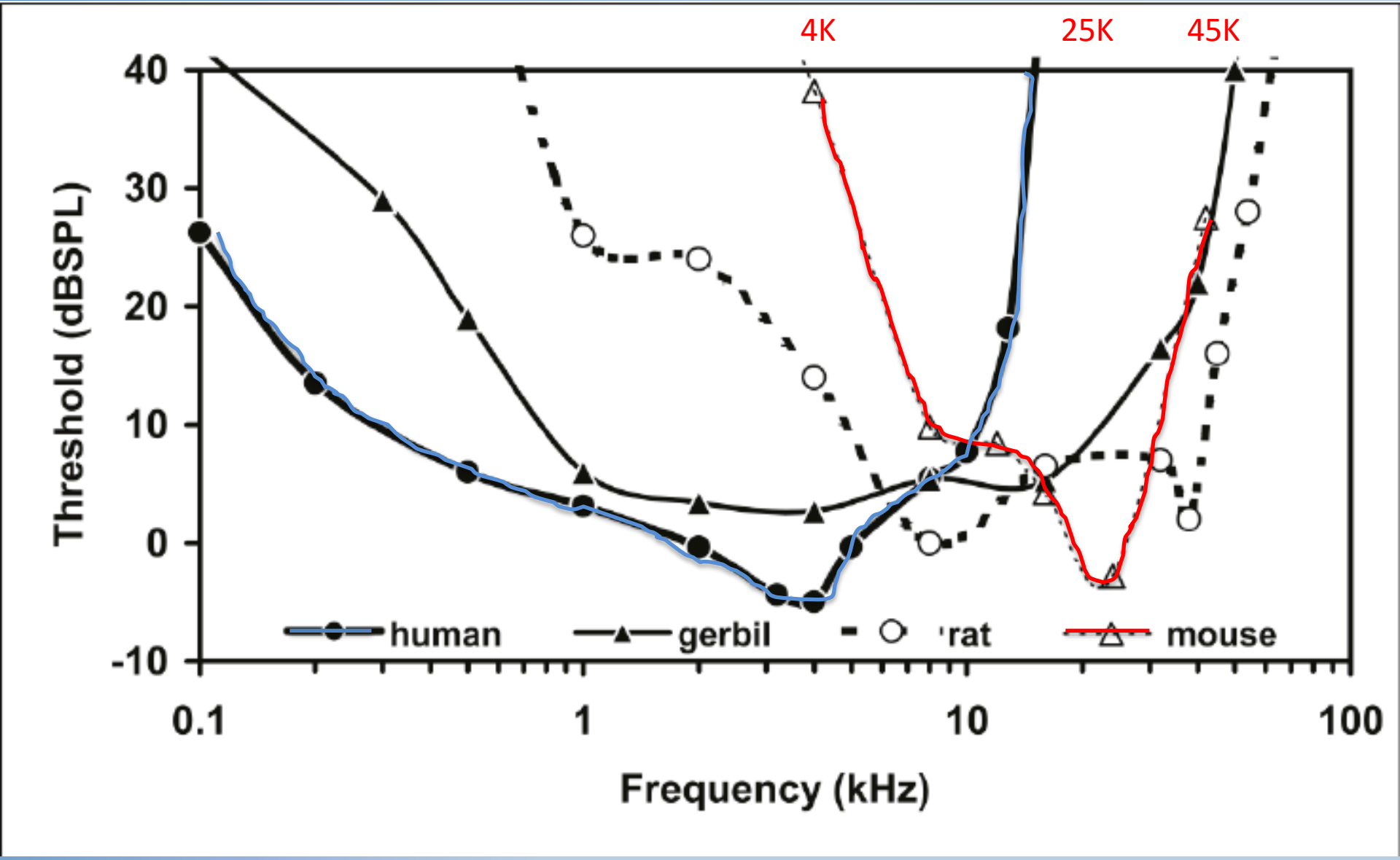
Whole genome sequencing identified a heterozygous mutation in the *USH2A* gene that co-segregated with auditory processing disorder in an extended family (APD; Newbury et al., in prep)



- distinct deliberate style of speech
- severe language comprehension deficits
- problems hearing speech sounds in the presence of background noise (e.g. on the telephone)
- all have normal hearing thresholds

We decided to evaluate heterozygous *Ush2a* mutant mice

Mouse audiogram (**red**) is much higher than human (**blue**)

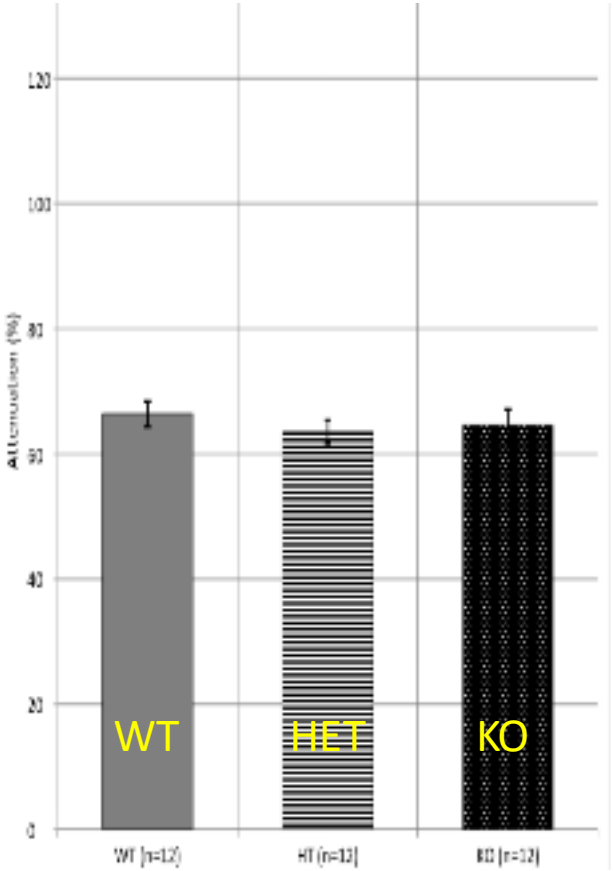


Otto & Jurgen, 2012

Ush2a KO and Hets - Auditory

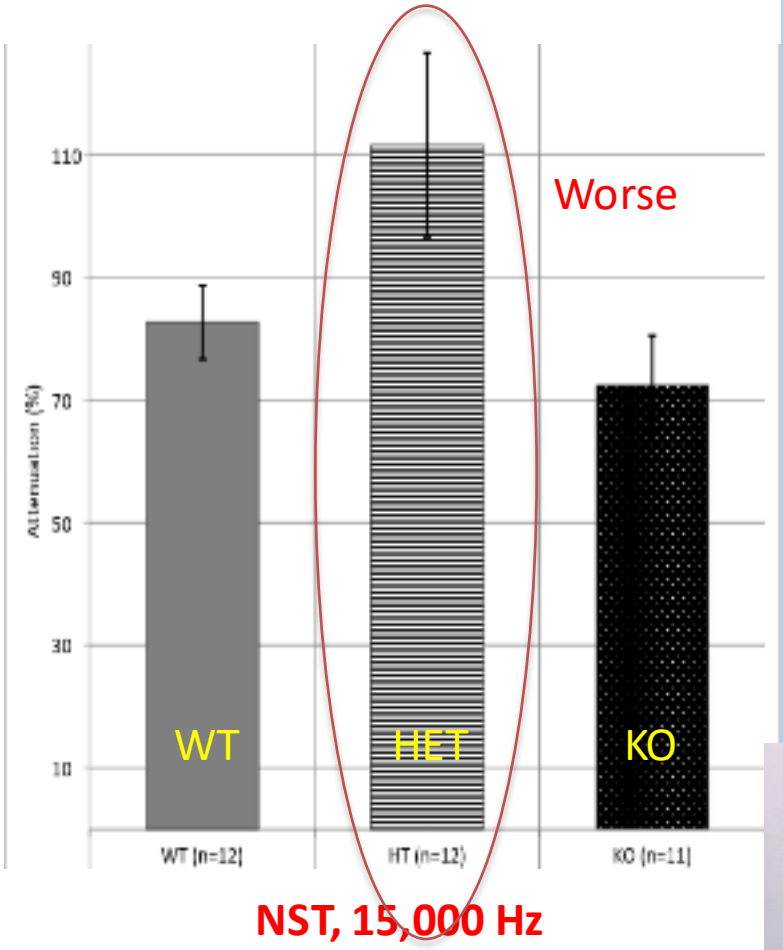
BETTER ← ---------- → WORSE

$p = 0.644$

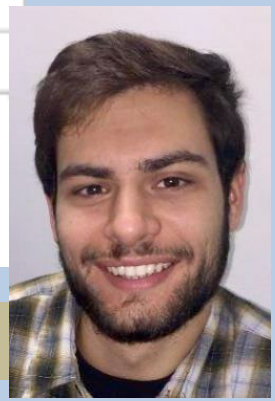


NST, 8,000 Hz
(LOW)

$p = 0.036$



NST, 15,000 Hz
(MED LOW)

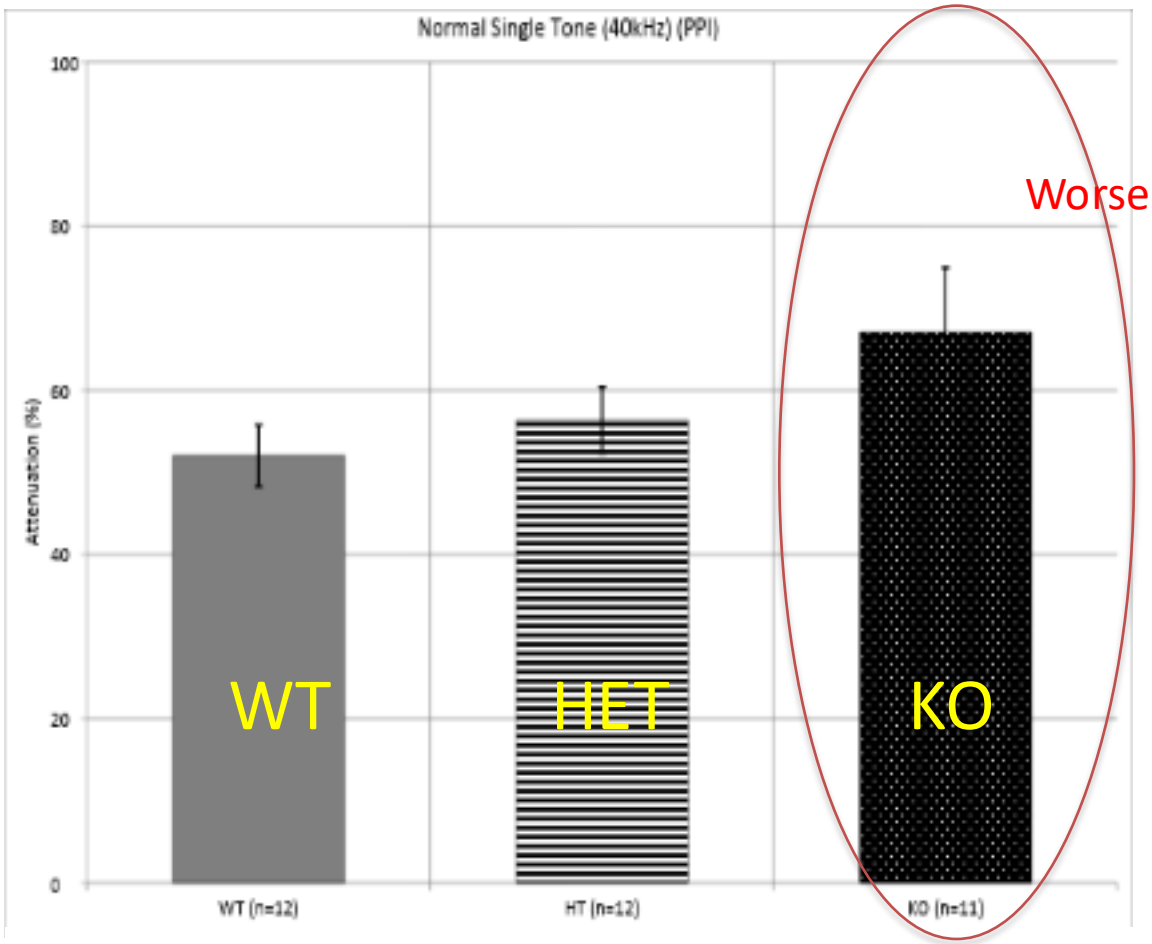


Perrino et al., *in prep*

Ush2a KO and Hets - Auditory

$p = 0.153$

BETTER ←----------→ WORSE



NST, 40,000 Hz (HIGH)

Perrino et al., *in prep*

Ush2a – Auditory Summary

- Genotype differences depend on task and frequency
- *Ush2a* HETs showed auditory deficits on tasks using lower frequency range (5-15kHz)
- *Ush2a* KOs showed auditory deficits on tasks a using higher frequency range (10-40kHz)

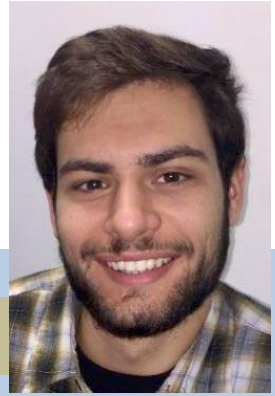
Task	Frequency	HET	KO
NST	8kHz	=	=
NST	15kHz	#	=
EBT 100	10.5kHz	*	=
EBT 10	10.5kHz	*	*
PD	10.5kHz	=	=
NST	40kHz	=	#
EBT 100	40kHz	=	=
PD	40.5kHz	=	*

Similar to WT (=)
Better than WT (*, #)
Worse than WT (*, #)

*Fixed cochla from wt, -/- **and** +/- tested mice were recently sent to Newbury et al. for EM analysis.*

NST = Normal Single Tone
EBT = Embedded Tone
PD = Pitch Discrimination

$p < 0.05$, # $p < 0.10$

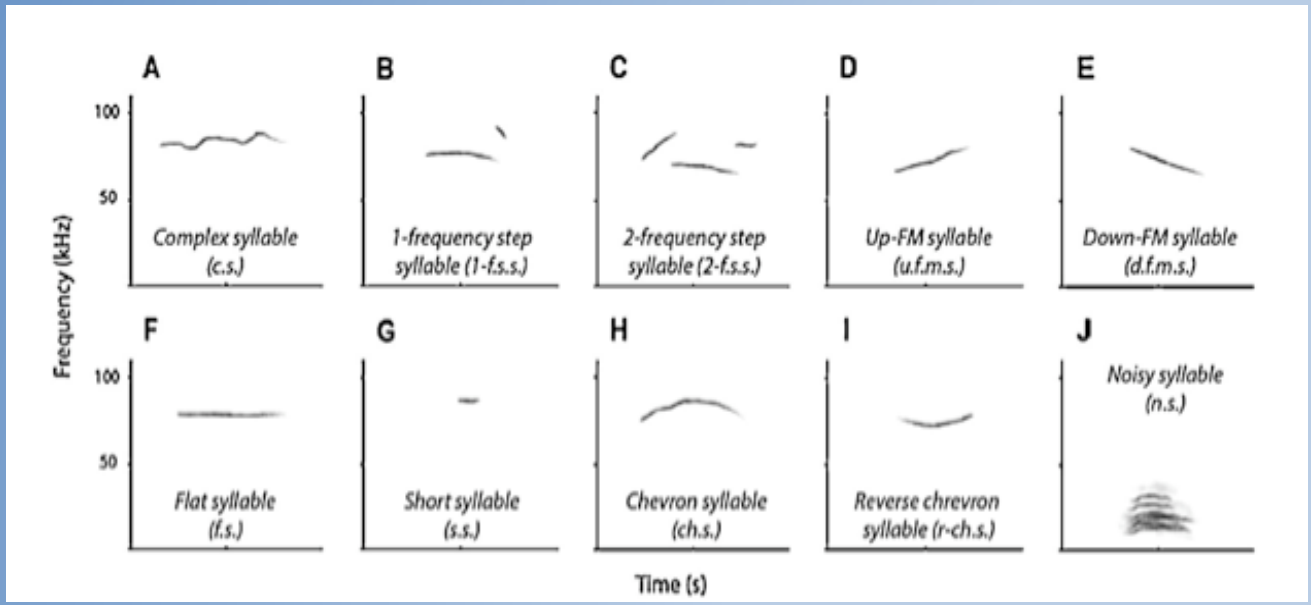


Perrino et al., *in prep*

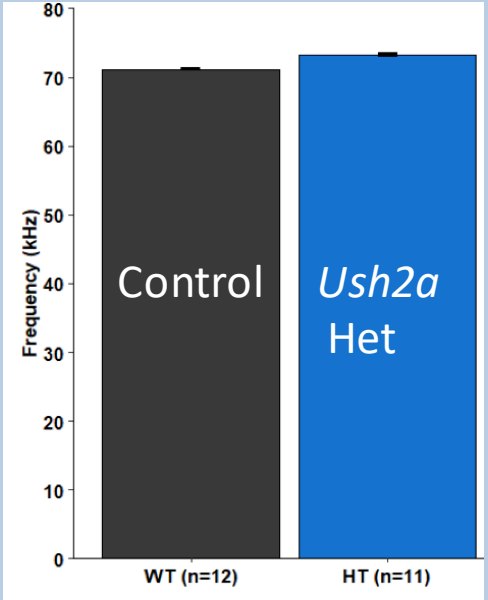
Ush2a KO & Het Mice - Vocalizations

Mice with a homozygous mutation in *Ush2a* (high freq hearing loss) showed atypically structured ultrasonic vocalizations that were longer and louder than controls.

Mice with a heterozygous mutation in *Ush2a* (low freq hearing loss) *vocalized at higher frequencies than controls.* **Heterozygous carriers are supposed to be phenotype free.**



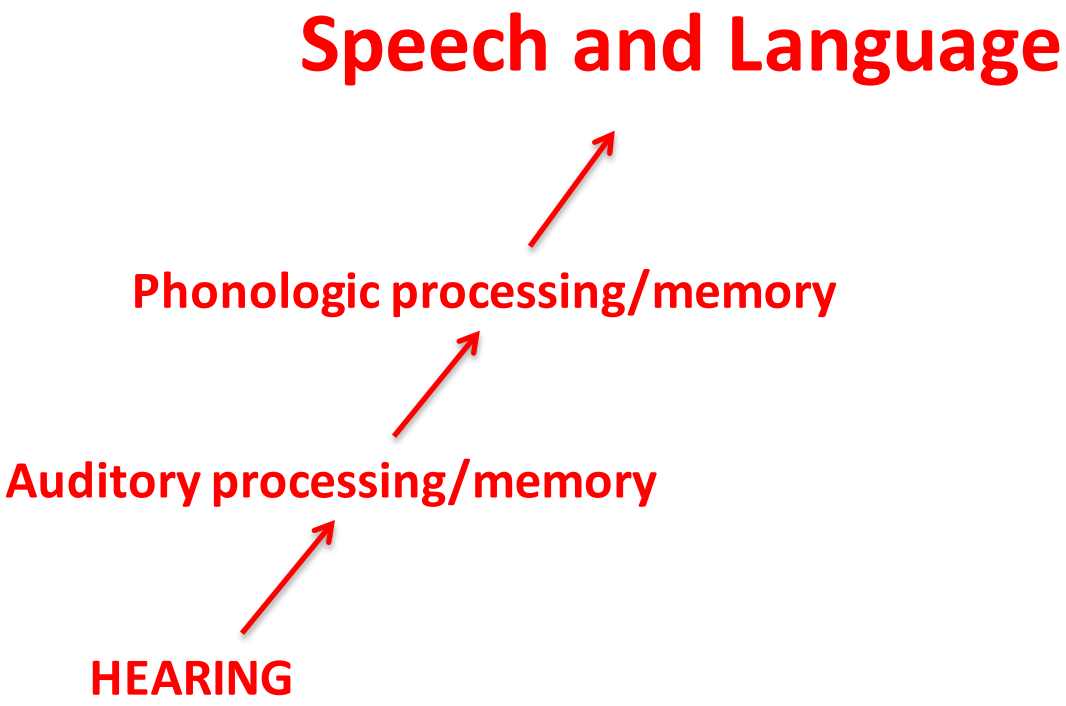
Typical mouse vocalizations



Mean vocalization frequency

CONCLUSIONS

•Some of the genetic contribution to developmental disabilities characterized by speech and language disability *may be mediated through auditory problems*.



CONCLUSIONS Cont.

- Different types of auditory processing anomalies (impairments, enhancements) may lead to *similar* patterns of speech and language deficits in humans.

- Although mice do not have language, they can be used to link specific genetic mutations to specific functional anomalies. This makes them a powerful tool to study intermediary developmental mechanisms, including links between *receptive* auditory problems and abnormalities in *expressive* vocalization.

- For heterozygous mutations in *Ush2a*, effects on higher order processing (in humans, speech and language) appear to be mediated through changes in *peripheral hearing* (at least initially), rather than higher order central auditory processing. This may inform genetically mediated conditions like dyslexia, ASD, and SLI where central effects (learning, memory, attention) co-occur with auditory problems, making it hard to dissociate auditory contributions to language deficits.

- Mouse models have the benefit of experimental control, allowing for study of precise gene-behavior associations. Results can be extrapolated to at-risk human populations to inform genetic screening and guide clinical intervention.

Acknowledgements

Faculty collaborators
April Benasich, PhD
Glenn Rosen, PhD
Joe LoTurco, PhD
Al Galaburda, MD/PhD
John Salamone, PhD
Ted Rosencrantz, MD
Inge-Marie Eigsti, PhD
Jim Magnuson, PhD
Gerry Altmann, PhD
Dianne Newbury, Ph.D.
Dr. Jun Yang, PhD

Current Students
Peter Perrino
Ruth McLeod

Former Students
Ann Peiffer, PhD
Melissa McClure, PhD
Steve Threlkeld, PhD
Courtney Hill, PhD
Caitlin Szalkowski, PhD
Michelle Alexander, PhD
Nhu Truong, PhD
Amanda Smith, PhD
Amanda Rendall, PhD

Supported by NIH, NSF IGERT, IBACS, and UConn ***Murine Behavioral Neurogenetics Facility***

Threlkeld
Regis College, Boston

Szalkowski
Canisius College, Buffalo NY

Truong
Yale University

Rendall
Yale University

Perrino
Uconn

Dedicated to the Fitch Lab Babies……

Engineered mice allow us to study links between *atypical auditory processing* (deficits/enhancements), and *clinical gene mutations* associated with developmental language disorders.

Our findings suggest *atypical low-level sound processing is associated with mutations in many of the genes linked to language disability.*

Findings also suggest atypical auditory processing may play a much greater role in higher order speech/language disability than once thought.

Gene	Clinical Association	Acoustic -Pitch	Acoustic-Rapid	Acoustic-Complex	Learning & memory	Visual Motion	USV Production
Dyx1c1	Dyslexia	=	=	–	–		
Kiaa0319	Dyslexia	=	–	=	=		
Dcdc2	Dyslexia	=	–	–	–	–	
Cntnap2	ASD, SLI	+	+	–	–		–
Cacna1c	ASD	+	+	=	=		–
Shank3	ASD	+	=	–	–		–
Ube3a	**AS**	+					–
Ush2A	Usher Syndrome	--- (low)	---- (low)				---