Models of ALS in zebrafish, flies and worms

MVIMG: Neuromuscular Biology and Disease

February 19, 2015

Each model system has its own strengths and weaknesses:

C. elegans

Strengths:

- Short life span (2-3 weeks), 4 day generation time.
- Breed at will
- Large numbers, easy to manipulate
- First multicellular organism to have its genome sequenced
- 302 neurons defined as is the connectome
- Ease of genetic manipulations
- Genetic screens

Weaknesses:

- Neural anatomy and neural circuitry different than in vertebrates (eg. no cortex, no spinal cord)
- Motoneurons are gaba-ergic (as opposed to cholinergic)
- Limited number of glia and no microglia
- Lack blood brain barrier

Drosophila

Strengths:

- Short life span (1 month), 7 day generation time.
- Breed at will
- Large numbers, easy to manipulate
- Strong genetic tools
- Ease of genetic manipulations
- Genetic screens

Weaknesses:

- Neural anatomy and neural circuitry different than in vertebrates (eg. no cortex, no spinal cord)
- Motoneurons are glutamatergic (as opposed to cholinergic)
- Limited number of glia and no microglia
- Lack blood brain barrier

zebrafish

Strengths:

- Vertebrate
- Longer lifespan (~2 yrs in the lab setting)
- Breed at will
- Large numbers, easy to manipulate
- Genetics. Genome sequenced, transgenics, knockouts
- Vertebrate genetic and chemical screens
- Vertebrate neuroanatomy
- Have robust glia and microglia
- Have a blood brain barrier
- Behavior

Weaknesses:

- ~3 month generation time
- No cortex
- Yes a vertebrate, but not a mammal

Strengths:

- Mammal
- Longer lifespan (~2 yrs in lab setting)
- Genetics. Genome sequenced, transgenics, knockouts
- Vertebrate genetic screen and chemical screens
- Vertebrate neuroanatomy and a cortex
- Microglia and a blood brain barrier
- Robust behavior

Weaknesses:

- 21 day gestation period and an ~2 month generation time
- Low numbers/small litters
- Not great for genetic or large drug screens
- Too inbred? Doesn’t represent human population

Questions to ask when deciding on a model system

- Does your model system have the cells that are affected in the disease?

The entire system/circuit may not be present.

- What are the genetics of the disease?

In many cases it is not know whether ALS is caused by a lack of function or gain of function.

- How will you generate the model?

If making a transgenic what promoter will be used?

- Is this an adult onset or embryonic/early disease?

Model organisms have shorter lifespans than humans.

- What is your plan to assess the phenotype?

The phenotype may look different in a model than in humans

- If your goal is to do a drug screen, do you have a readout?

- Does your plan play to the strengths of that model organism?

Genetics, imaging, cell biology, drug screens

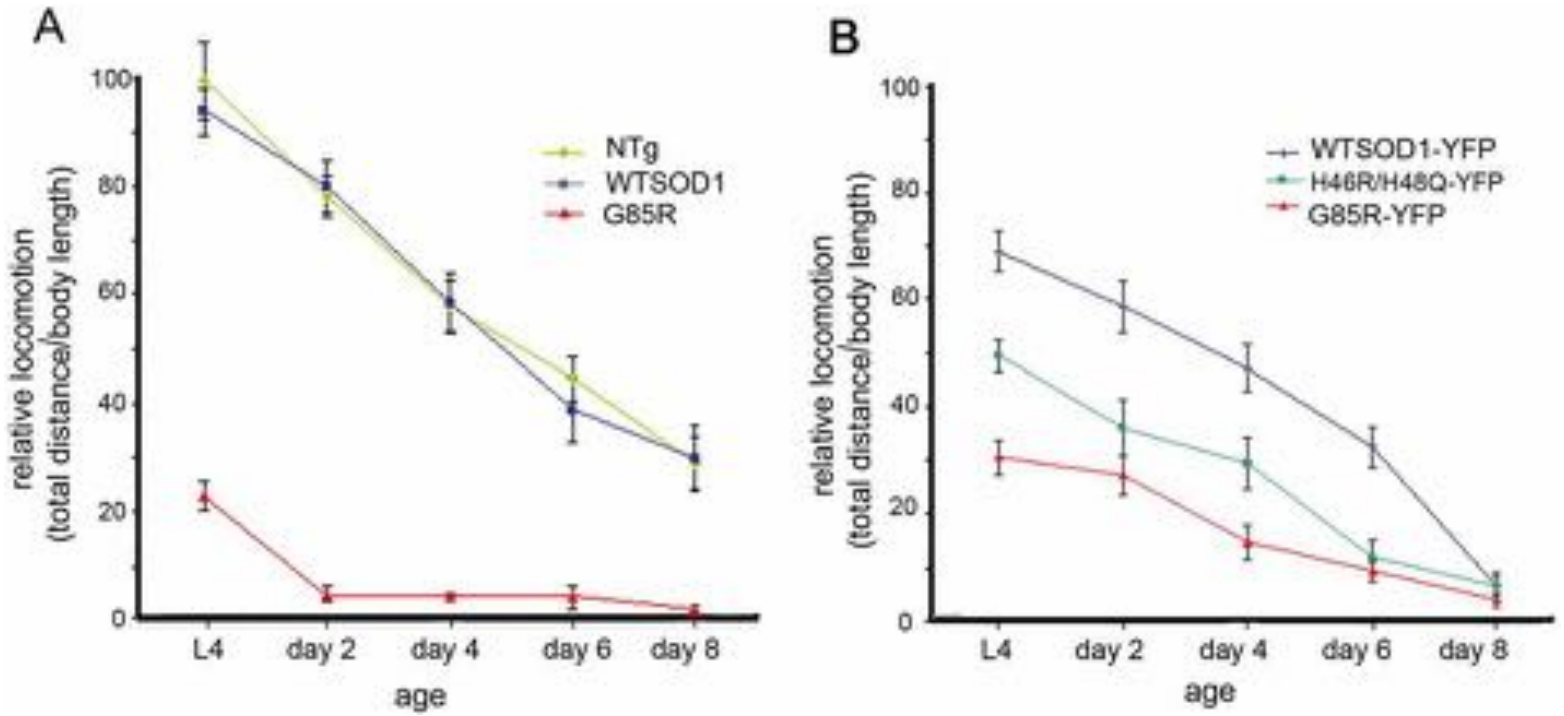
Look at SOD mutant over-expression in these 4 systems.

Look at phenotypes
Compare across models
What can we learn?

C. Elegans

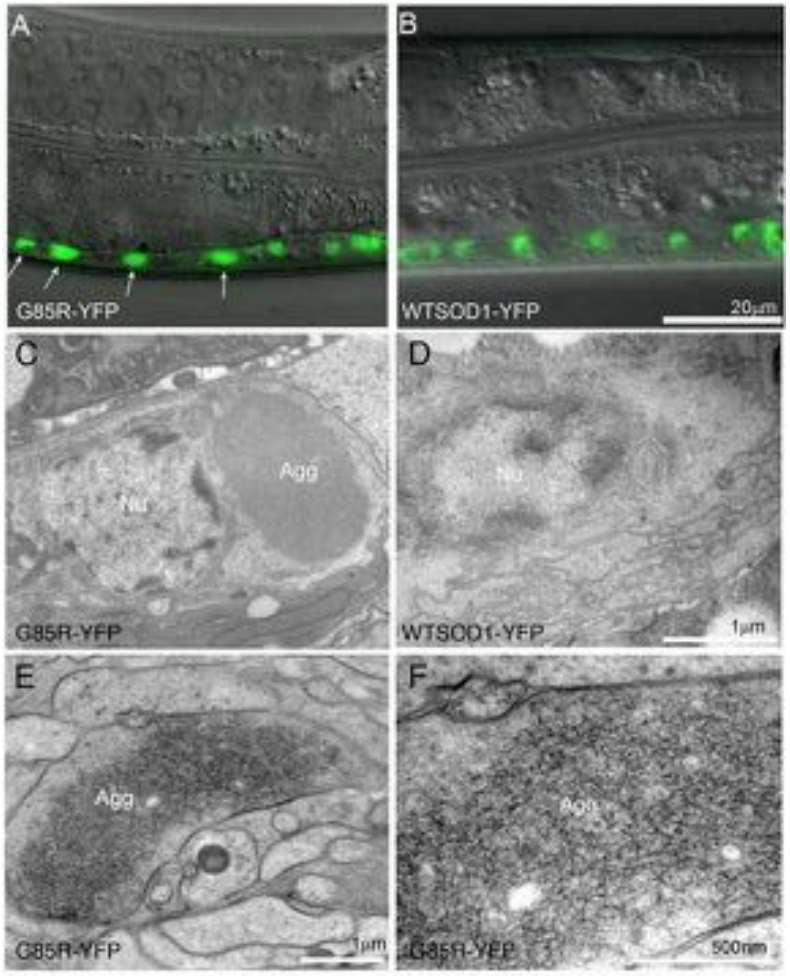
Drove mutant human SOD pan-neuronally (worm synaptobrevin promoter)

See movement defects

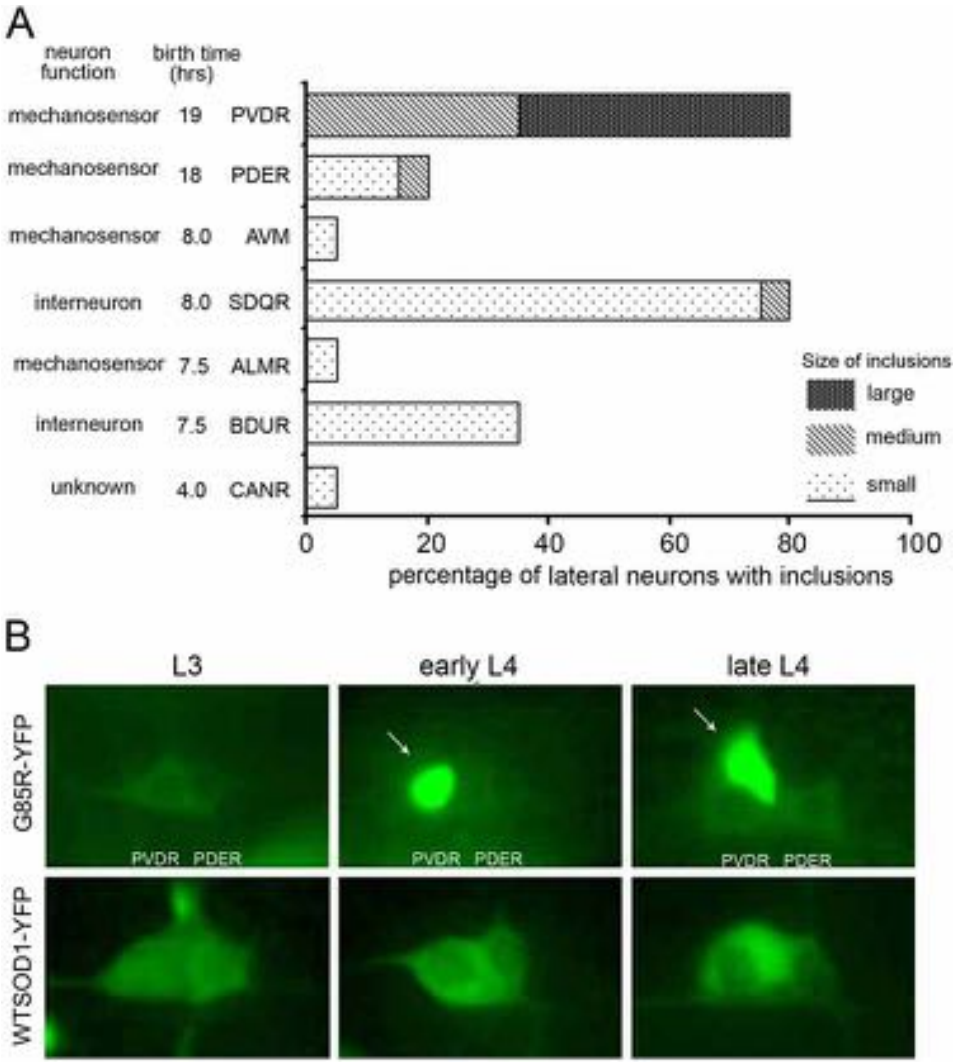


Wang et al (2009) PLoS Genetics: 5, e1000350

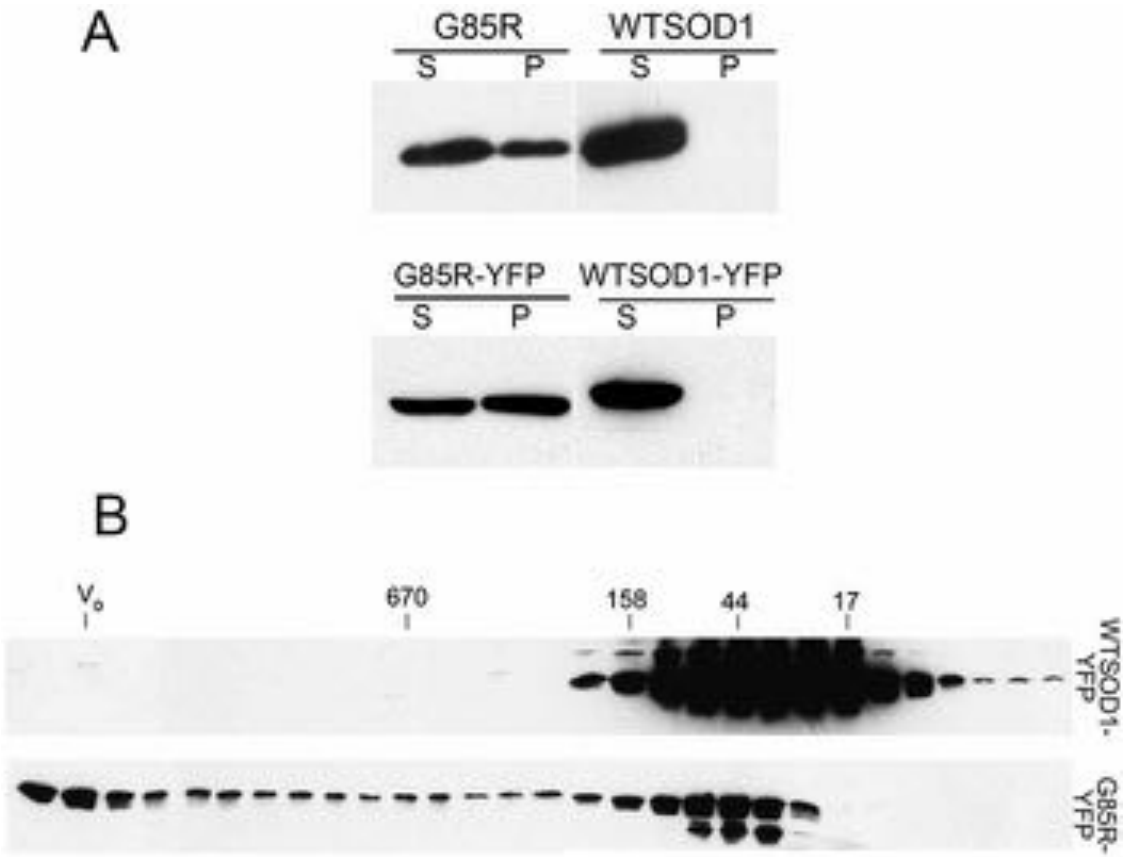
Neuronal aggregates form in transgenics expressing mutant SOD



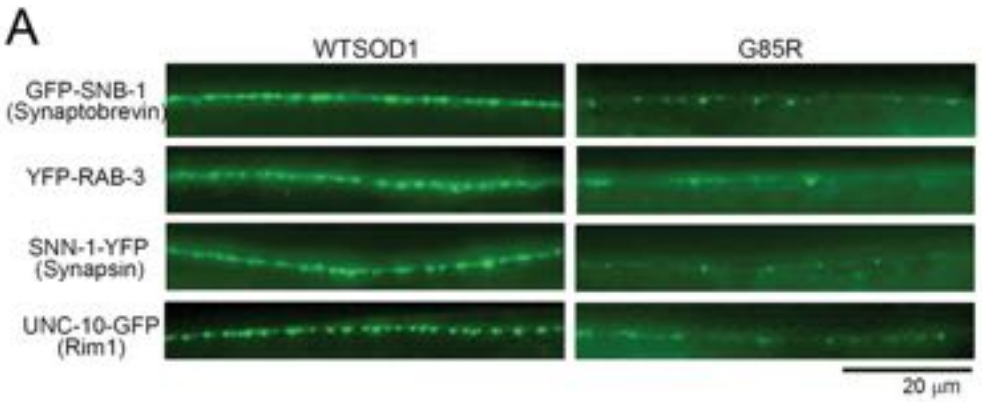
Neuronal birthdate and type do not correlate with aggregates

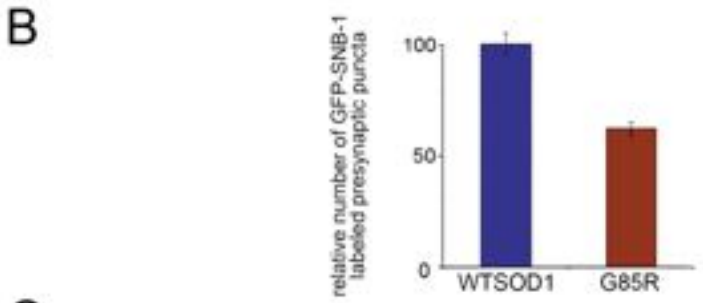


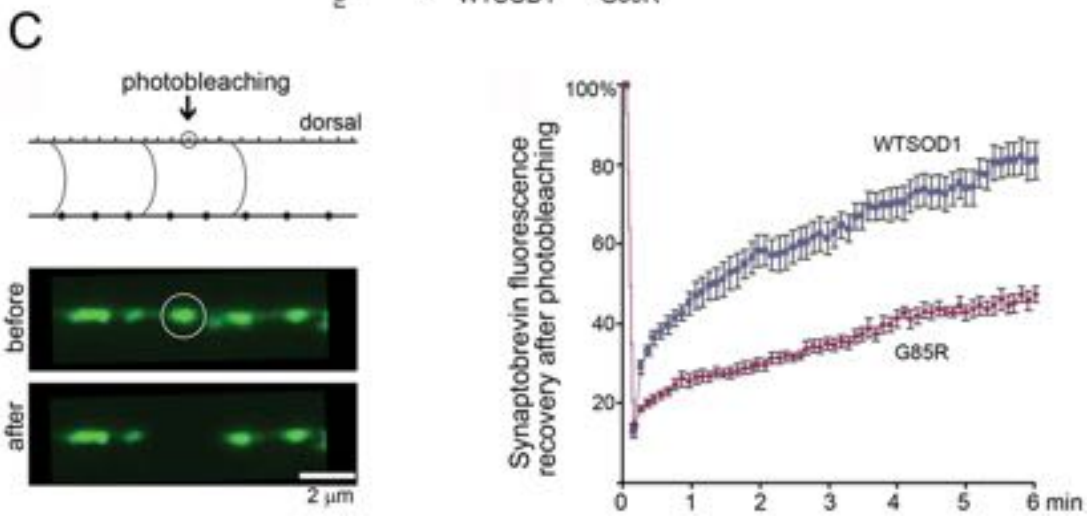
Biochemical analysis revealed insoluble SOD protein in Tgs



Tg animals have reduced presynaptic puncta and indicate less vesicle content

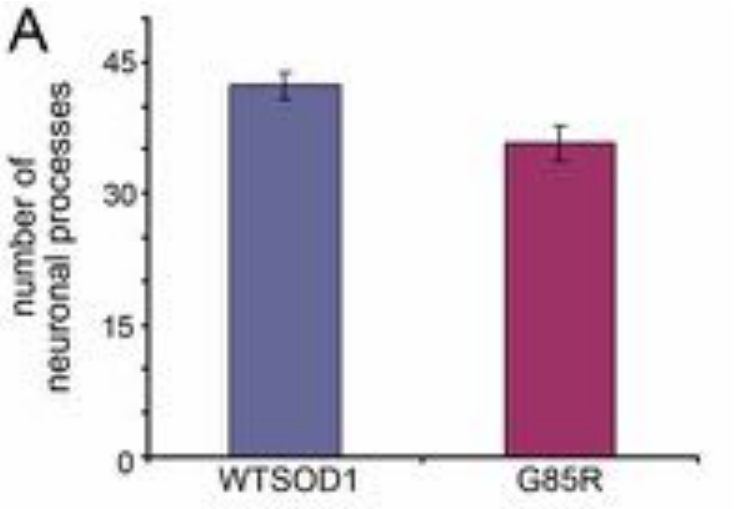




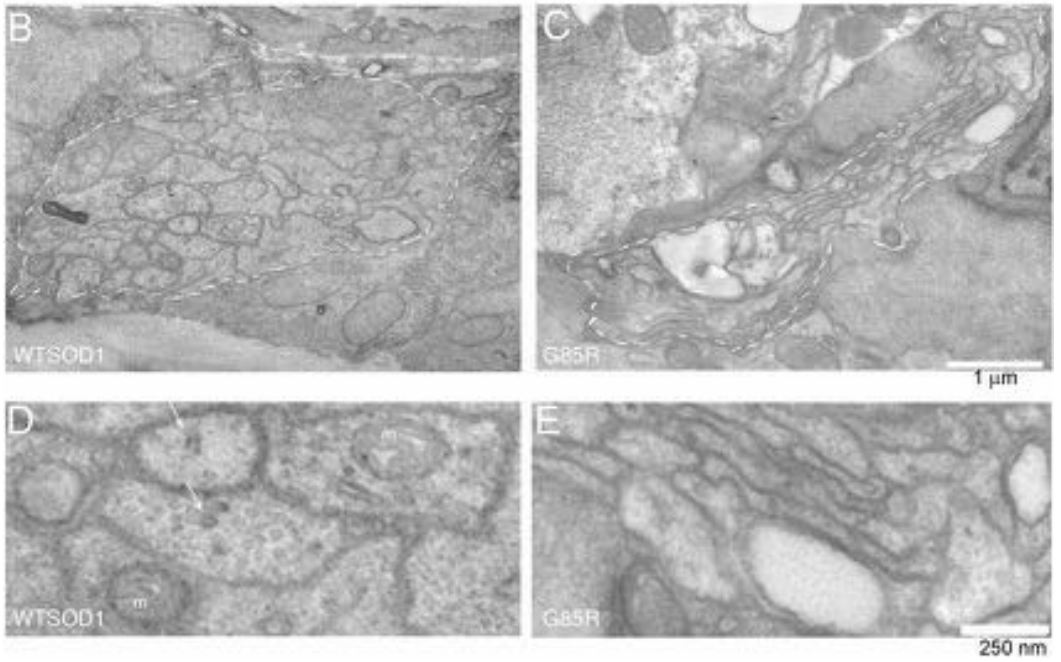


Decreased recovery after photobleaching suggests defects in axonal transport.

The number of neuronal processes is mildly reduced



EM reveals:

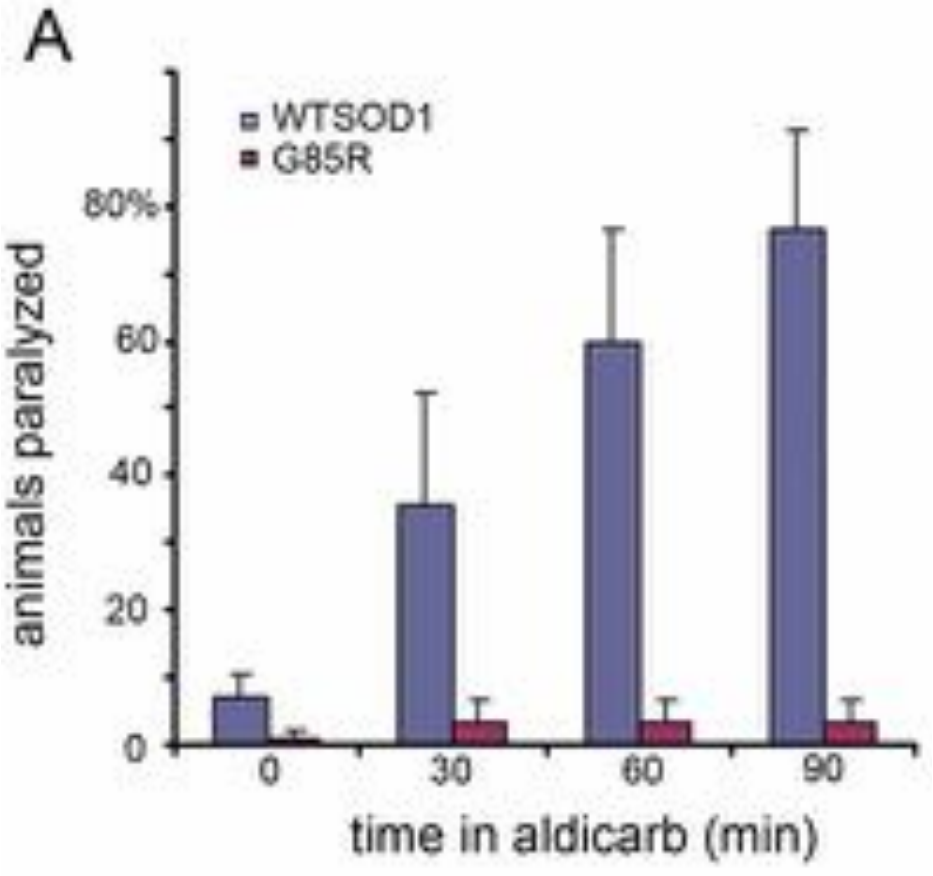


decreased axonal bundle

decreased mitochondria and synaptic vesicles

To assess synaptic function, put embryos in a cholinesterase inhibitor (aldicarb) which paralyzes wt worms.

Had no effect in Tg worms suggesting loss of cholinergic synaptic transmission.



Did an RNAi screen to look for genetic modifiers of aggregation

Just feed the worms bacteria expressing RNAi constructs (!)
Look 3-6 days later for changes in the number/intensity of the aggregates

Table 1. Summary of 81 RNA interference hits that worsened aggregation.

Protein chaperones, turnover, and modification	22	27.2%
Redox	3	3.7%
Signal transduction	7	8.6%
Transcription, RNA processing	6	7.4%
Metabolism	6	7.4%
DNA replication and repair	4	4.9%
Extracellular matrix	2	2.5%
Translation	2	2.5%
Intracellular trafficking	2	2.5%
Uncategorized	27	33.3%

Took candidate genes from RNAi screen and tested them genetically

Table 2. Selected genes whose inactivation strongly aggravates formation of SOD-YFP neuronal inclusions[1].

CATEGORY	GENE	FUNCTION	[2]RNAI SCORE	[3]ALLELE SCORE
Chaperone/quality control	*hsf-1* (Y53C10A.12)	Heat shock transcription factor	5	*sy441* ++
	C30C11.4	homolog to human apg-1 (a heat shock 110 kDa protein)	3	*gk533* ++
	dnj-19 (T05C3.5)	homolog to DnaJ subfamily A member 2	3	*gk649* ++
	F08H9.4	neuron-specific HSP16	3	*ok1976* ++
	stc-1 (F54C9.2)	member of HSP70 superfamily (microsome associated)	3	
Protein turnover	*sel-10 (F55B12.3)*	member of the CDC4/CUL-1 family of ubiquitin ligases	3	
	rbx-1 (ZK287.5)	RING box protein RBX1, a subunit of the SCF ubiquitin-ligase complex	2	ok782 +
	W07G4.4	Predicted aminopeptidase	4	
Protein modification	*uba-2 (W02A11.4)*	sumo activating enzyme	5	
	ubc-9 (F29B9.6)	sumo conjugating enzyme	4	
	gei-17 (W10D5.3)	Homologous to E3 SUMO-protein ligase PIAS1	3	
Redox	*bli-3 (F56C11.1)*	dual oxidase	3	*e767* ++
	pdi-2 (C07A12.4)	Protein disulfide isomerase	2	*gk375* ++
	C30H7.2	thioredoxin domain-containing protein precursor	2	
Signal transduction	*dbl-1 (T25F10.2)*	member of the TGFβ superfamily	3	*nk3* +
Dopamine metabolism	*dat-1 (T23G5.5)*	plasma membrane dopamine transporter	3	*tm903* +
Dna replication & repair	*top-1 (M01E5.5)*	DNA topoisomerase I	5	
	div-1 (R01H10.1)	homolog of the B subunit of the DNA polymerase alpha-primase complex	3	*or148* ++
Transcription	*H43I07.2*	RNA polymerase I and III, subunit RPA40/PRC40	3	
Longevity factor	*pha-4 (F38A6.1)*	FoxA transcription factor	4	

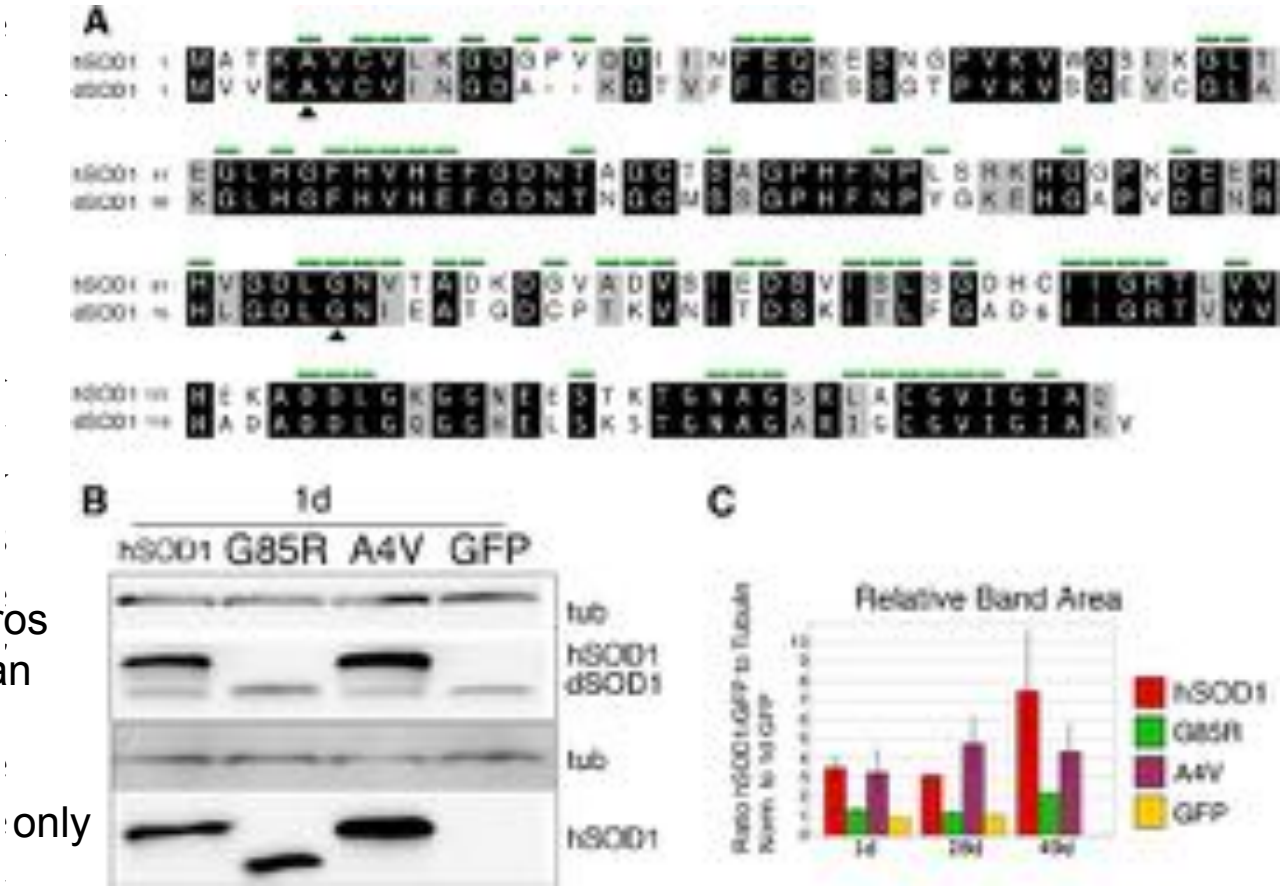
Summary of worm model:

Neuronal aggregates
Movement defect
Synapse defect (find link to aggregates- ie lines with more aggregates had a stronger presynaptic defect)
Ability to do a large scale RNA interference screen (16,757 RNAi clones screened)

Model only expressed mutant SOD in neurons
Did not see any evidence of neuronal cell death

Drosophila:

Used Gal4:UAS system to drive human SOD wt and mut in motoneurons (D42-larval-adult) and eye
hSOD and dSOD differ in 49/153 residues so also used dSOD



Ab both dros and human

Ab human only

Relative expression levels

Watson et al., (2008) JBC 283: 24972-24981

Lifespan was not affected by expressing mutant SOD in motoneurons

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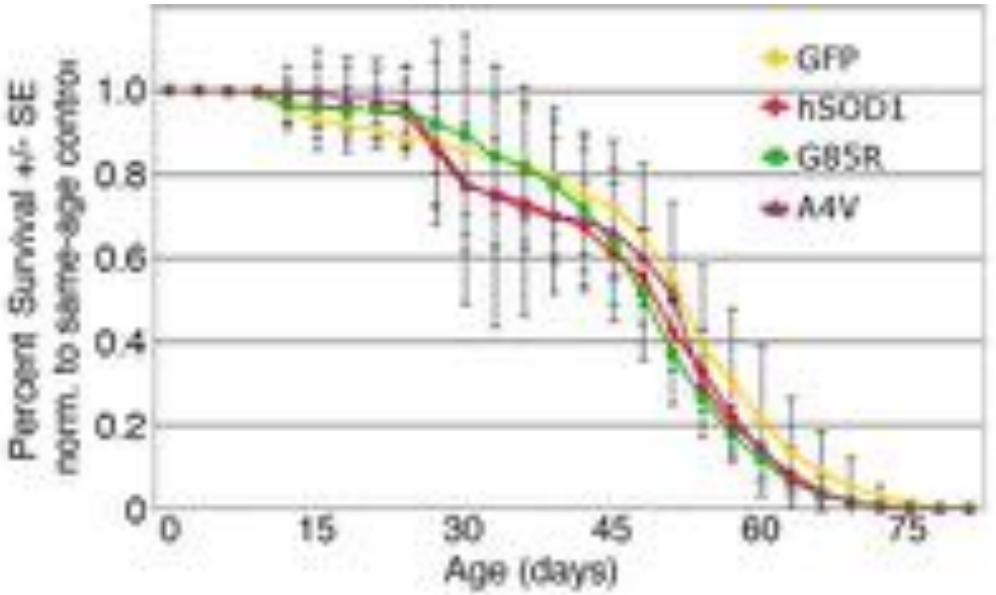

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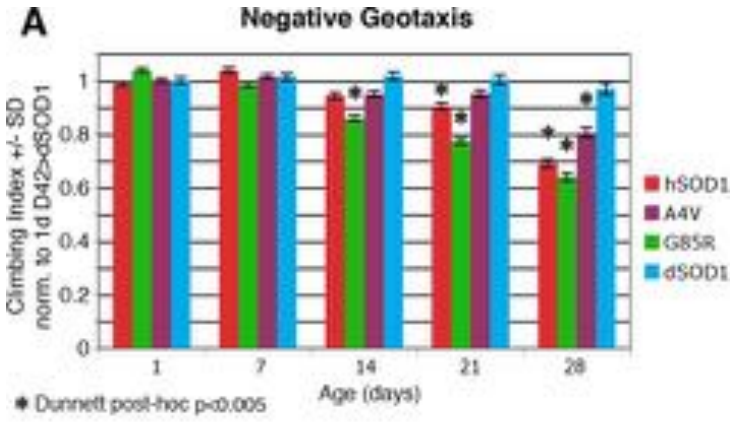
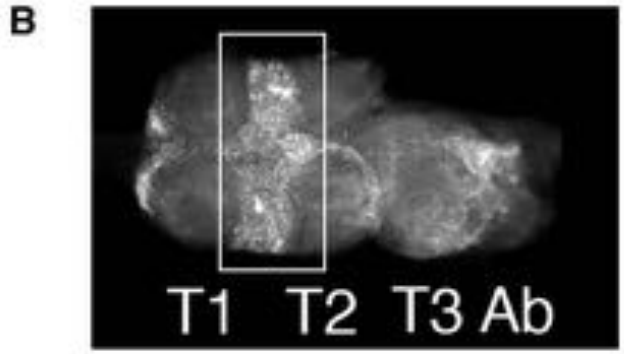
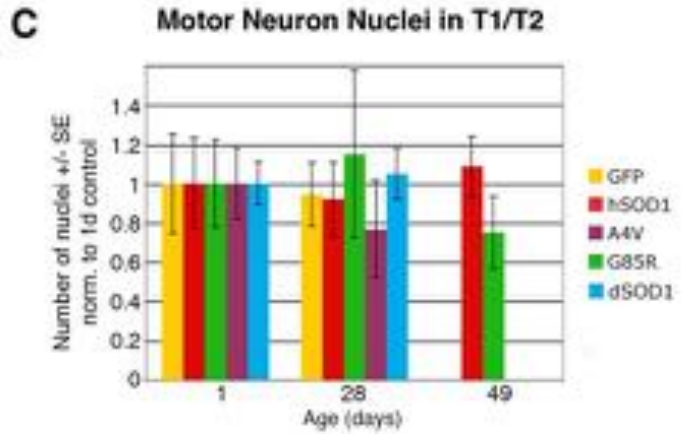
Genotype	Experiments (n per Experiment)	Mean Lifespan +/- SE (days)	Median Lifespan 95% CI (days)
D42>GFP	3 (399, 231, 399)	48.3 +/- 0.52	51 51-51
D42>wt hSOD1	3 (199, 361, 400)	42.6 +/- 0.49	48 45-48
D42>G85R	3 (394, 221, 281)	46.9 +/- 0.47	48 48-48
D42>A4V	2 (241, 198)	45.1 +/- 0.67	51 48-51



Flies expressing WT or mutant hSOD1 showed progressive loss of climbing when compared with dSOD1 controls, starting at 14 days (G85R) or 21 days (WT)



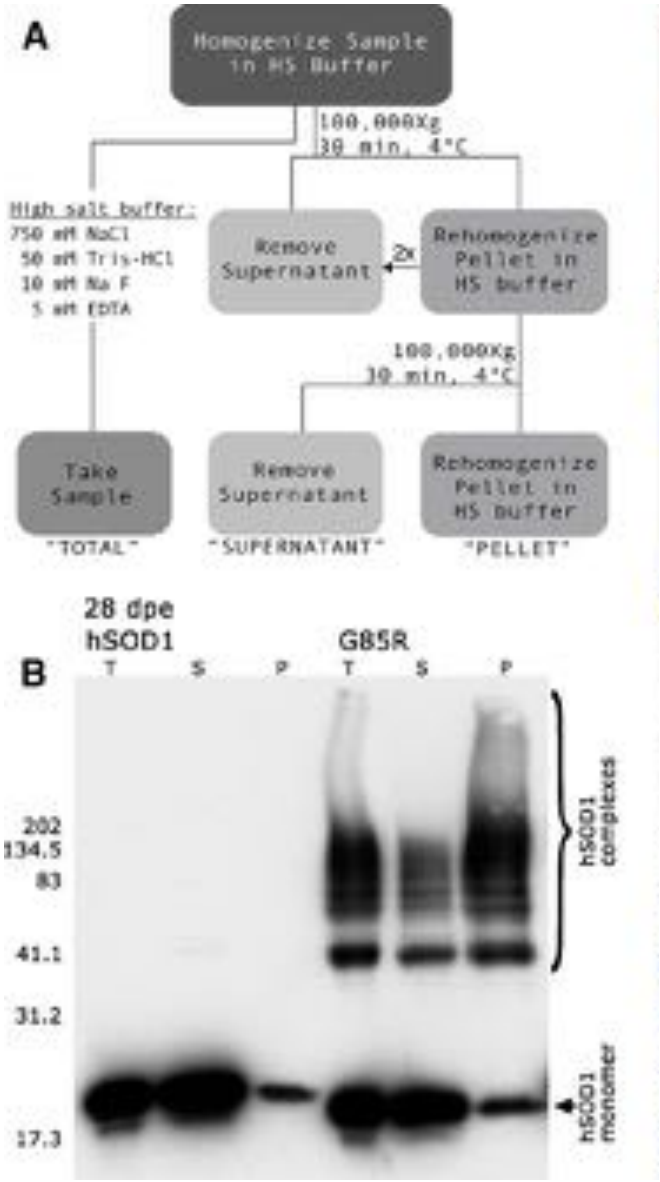
Counted nuclei in the T1/T2 thoracic ganglia

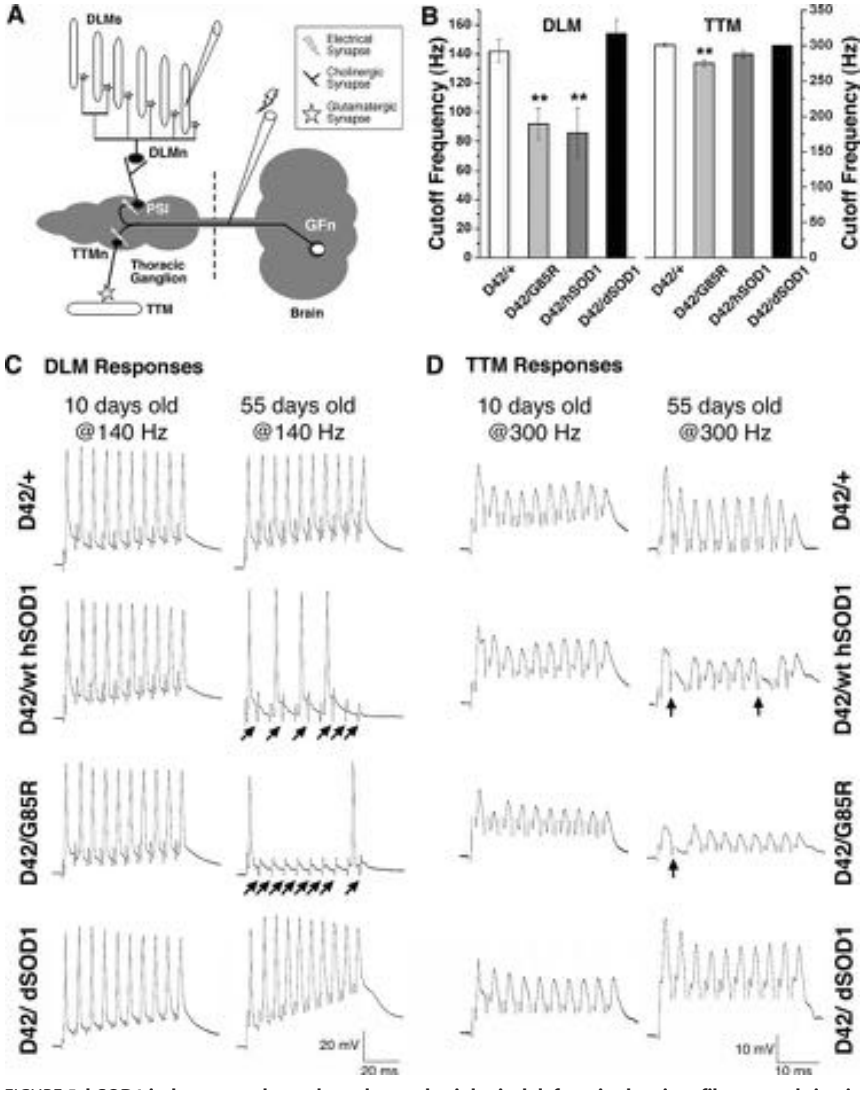


Large scale motor neuron loss did not occur

Climbing loss may reflect motor dysfunction.

Large MW forms of mutant SOD present when driven in the eye





Flies expressing hSOD1 and dSOD1 in motor neurons were assessed for reduced or abnormal signaling at the neuromuscular junctions of the giant fiber system.

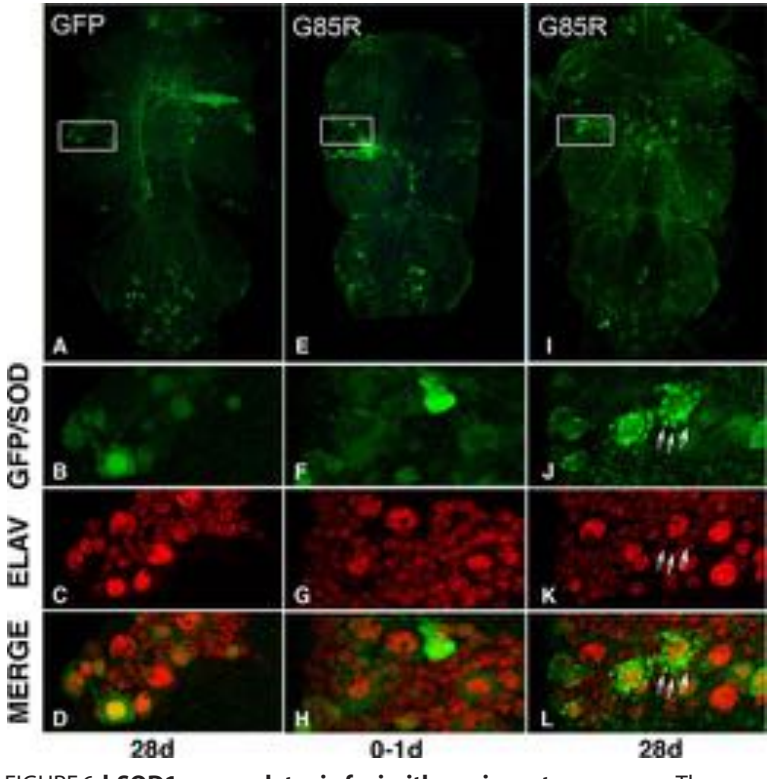
Looked at synaptic physiology of indirect flight muscles.

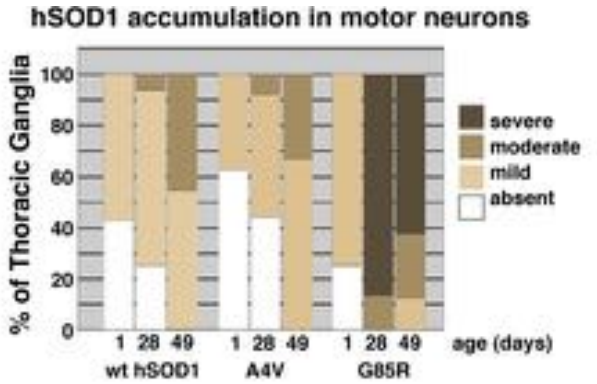
DLM motoneurons mediate wing depression during the escape response

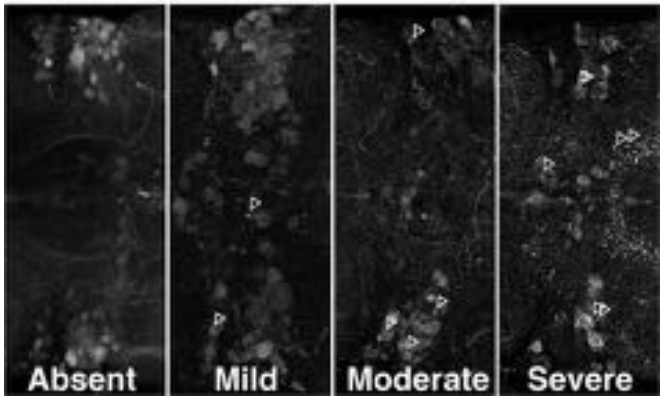
TTM motoneurons initiate leg extension

Both wt hSOD and mutant SOD show protein accumulations in motoneurons

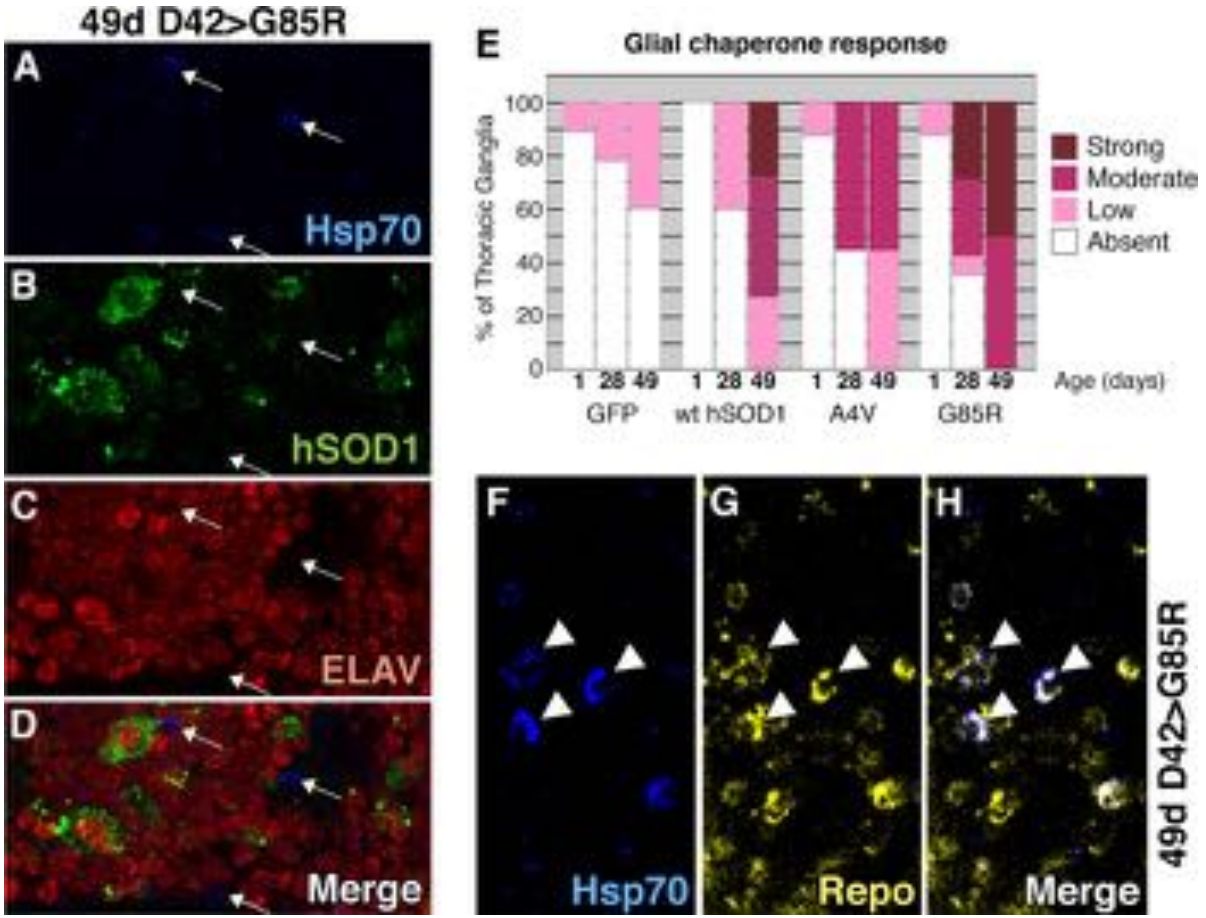
ELAV labels neurons







Expression of SOD in motoneurons is associated with a stress response in glia



HSP70 expressing cells are not neurons

Repo=glial cell marker

Summary of fly model:

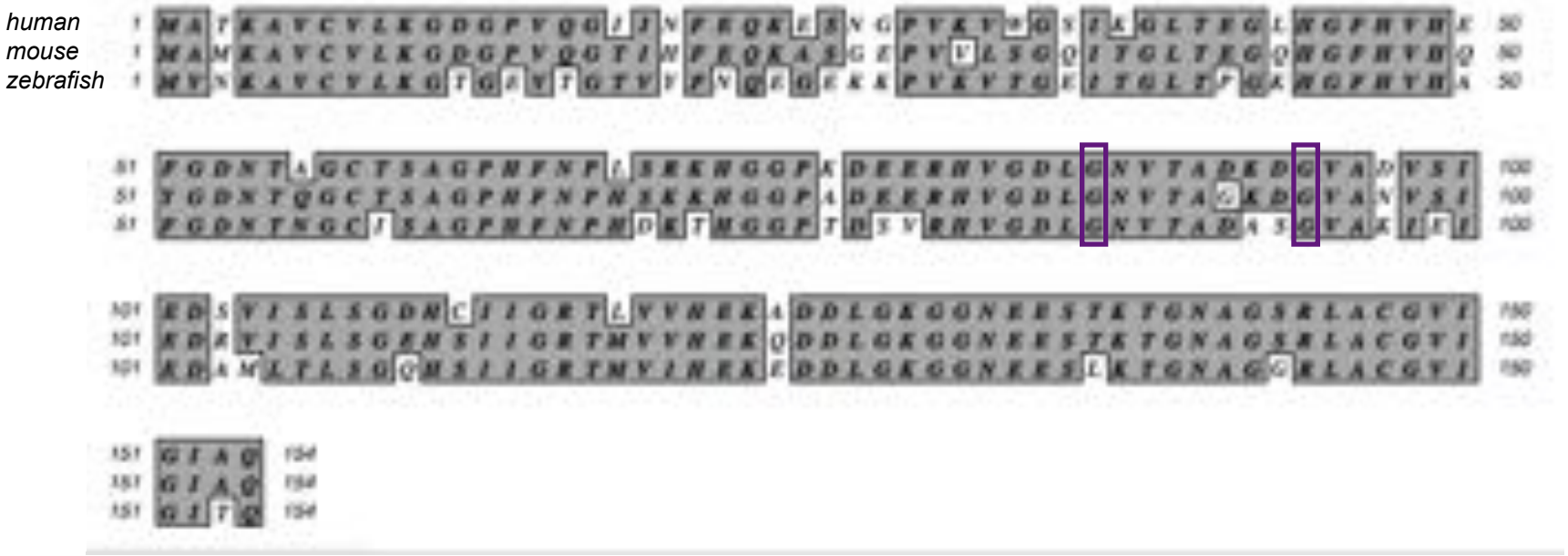
Motor dysfunction
Failure of high frequency synaptic transmission
Neuronal aggregates
Non-cell autonomous stress response in glia

Model only expressed mutant SOD in motoneurons
Did not see any evidence of neuronal cell death

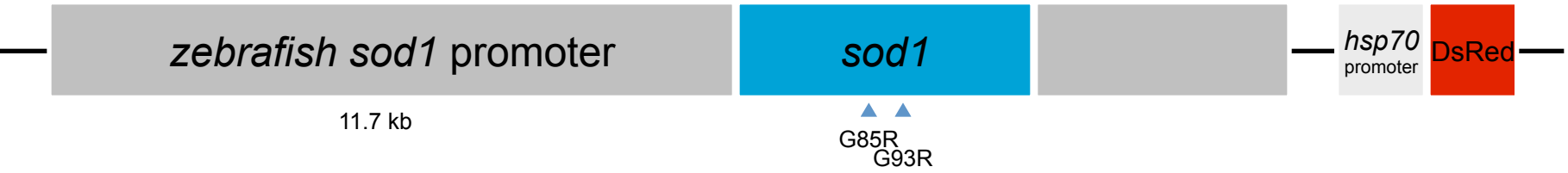
Zebrafish

Conservation of *SOD1*

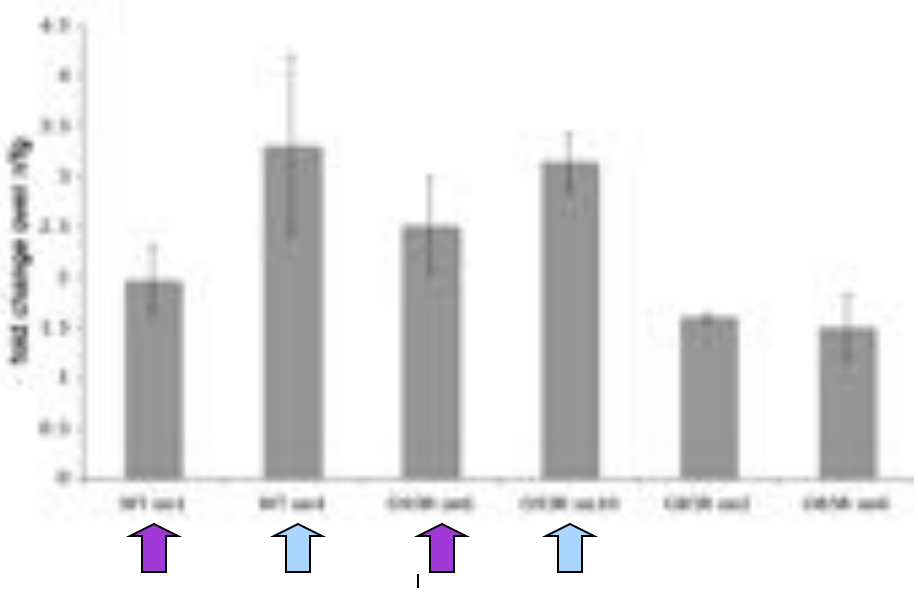
- Human & mouse sod1: 83% identical, 88% similar
- Human & fish sod1: 71% identical, 81% similar



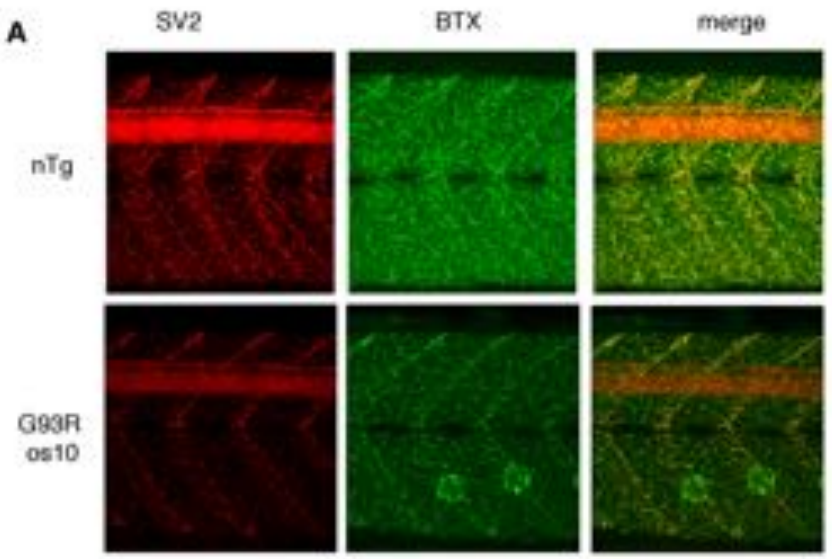
Generation of transgenic mutant *sod1* zebrafish

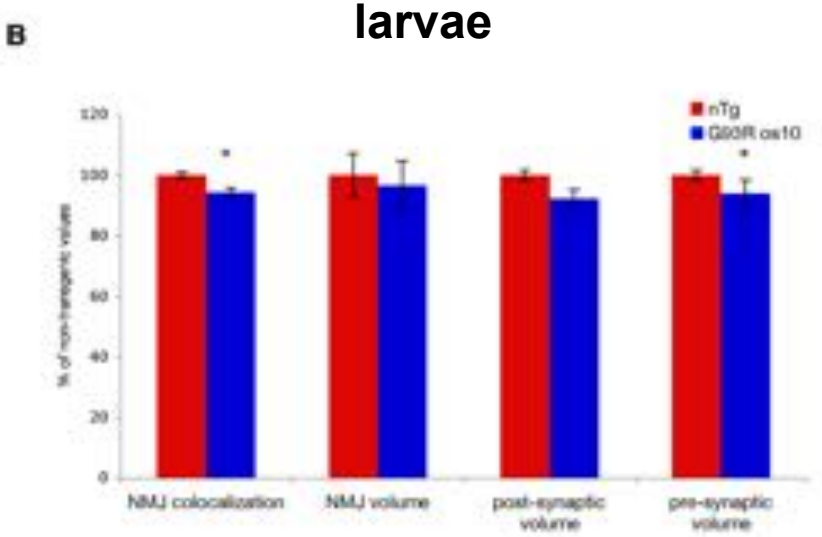


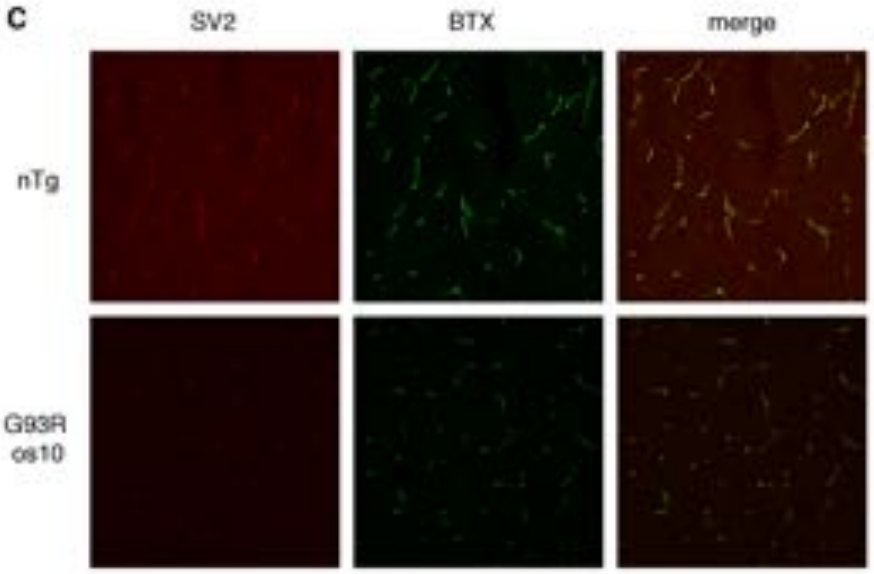
line	approximate *sod1* copies	transgene copy number
nTg	2 ± 0.3	0
WT os1	3.9 ± 0.6	2
WT os4	44.1 ± 8.9	42
G93R os6	10.7 ± 1.2	9
G93R os10	166.9 ± 20.3	165
G85R os2	3.27 ± 0.2	1
G85R os6	13.9 ± 2.3	12

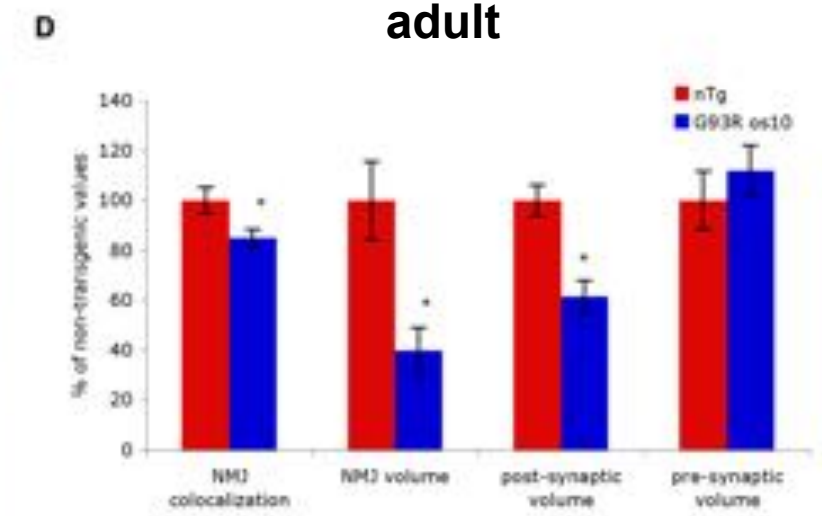


Progressive pre-synaptic defects



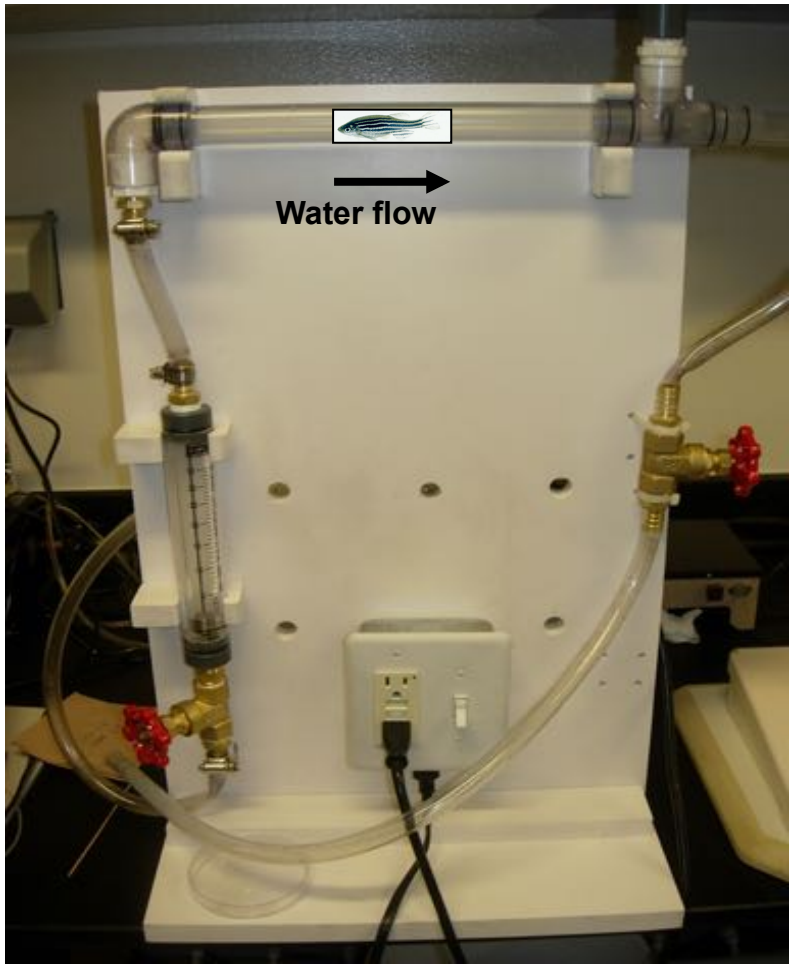






Analysis using Image J

Testing swimming strength in zebrafish



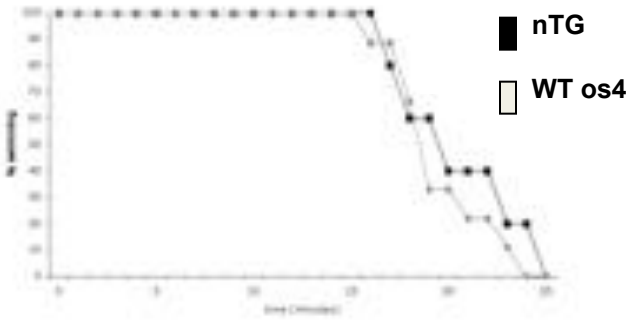
Fish swim against the water current

Flow rate is low initially and rate is increased by 3.25 cm/sec every 5 min

Fish drop out at specific flow rates

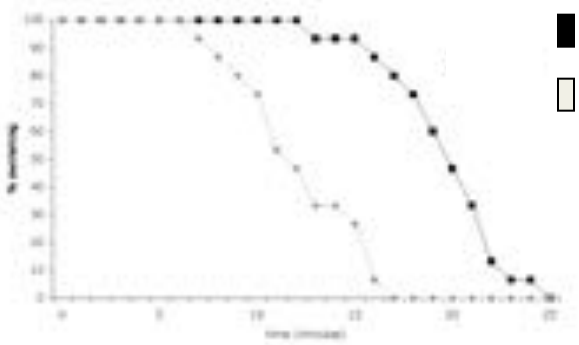
Critical swimming speed (U^{crit}) is calculated

A



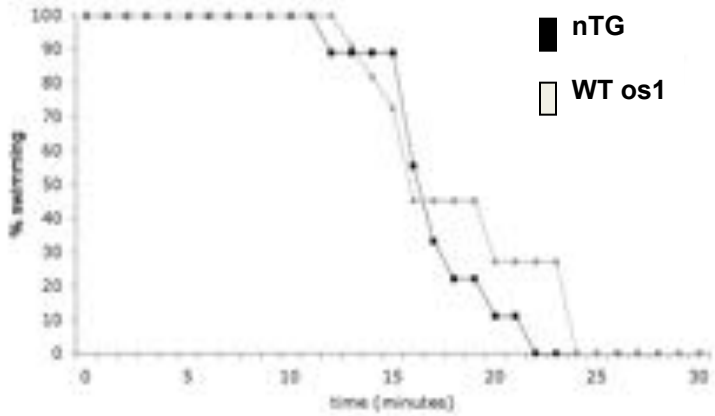
	nTg	WT os 4
10 months	16.2 ± 2.8	15.6 ± 2.1

B



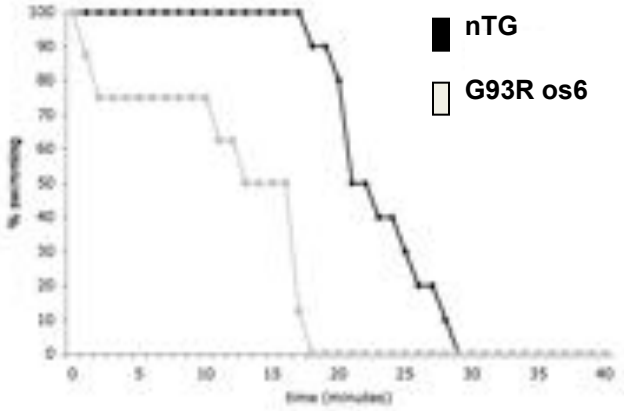
	nTg	G93R os10
12 months	15.7 ± 2.5	9.6 ± 2.6

C



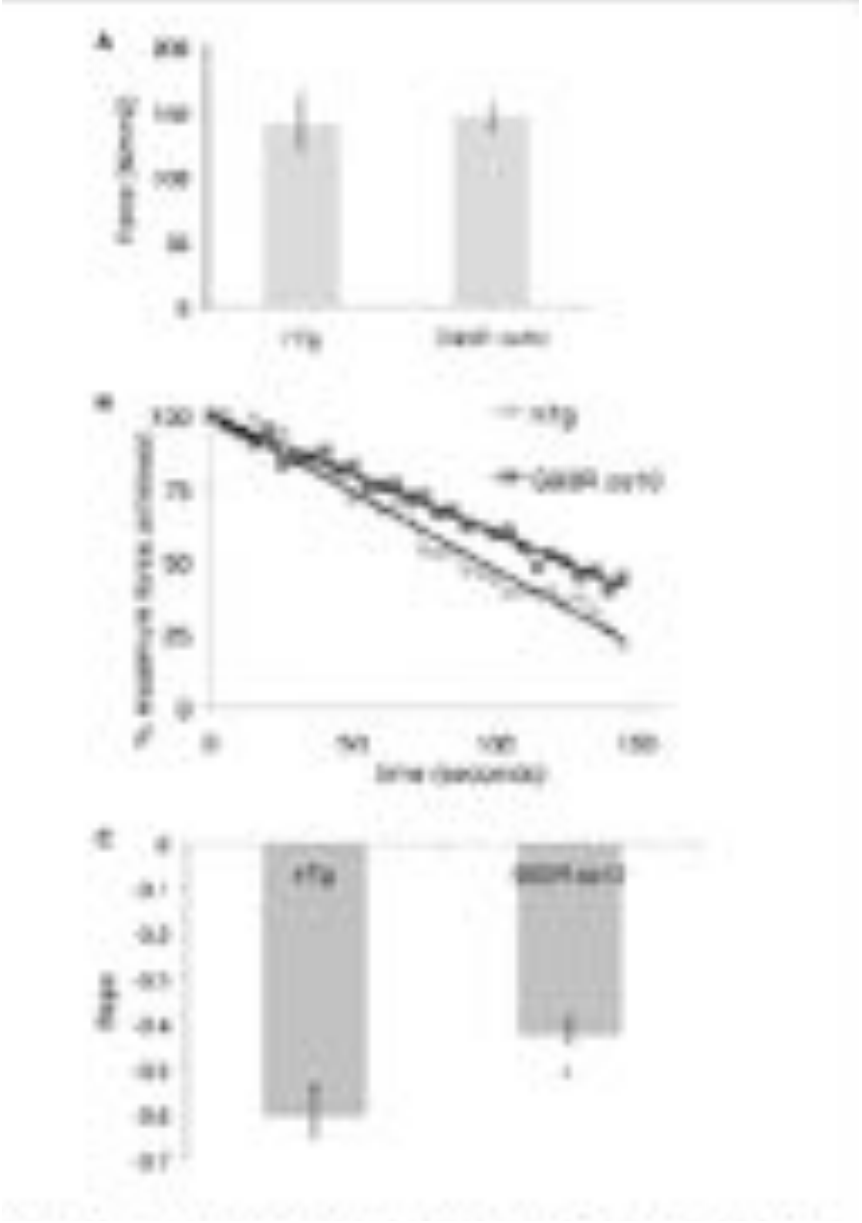
	nTg	WT os1
24 months	13.0 ± 2.1	14.2 ± 3.1

D



	nTg	G93R os6
16 months	14.7 ± 2.2	7.2 ± 4.4

Muscle physiology



No difference in maximal twitch force. Indicates that muscle contractile properties are intact.

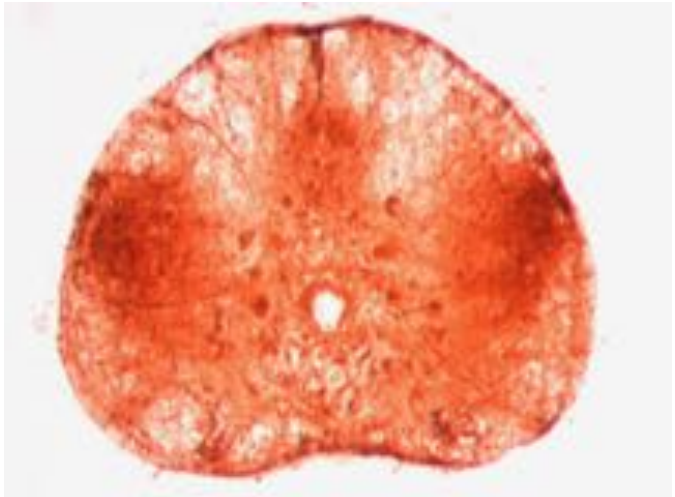
Was a difference in response to fatigue due to repeated stimulations (4 ms at 0.2 htz)

Sod mut had better fatigue resistance (this was also found in SOD mut mice)

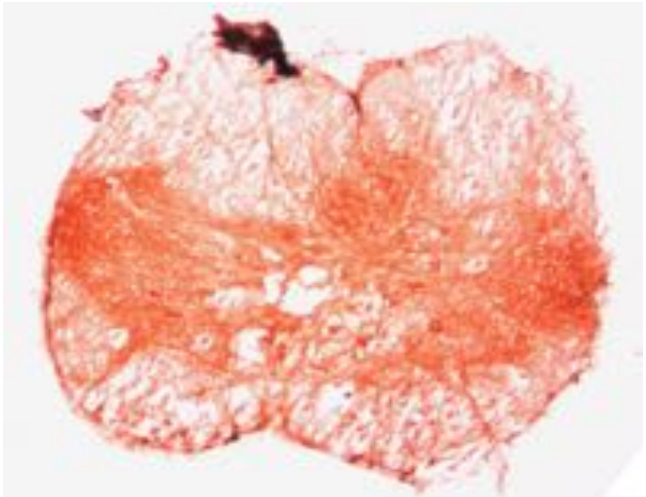
Late/end stage behavior in adult *Tg(sodG93R)os10*

Endstage fish overexpressing mutant Sod1 show motoneuron loss

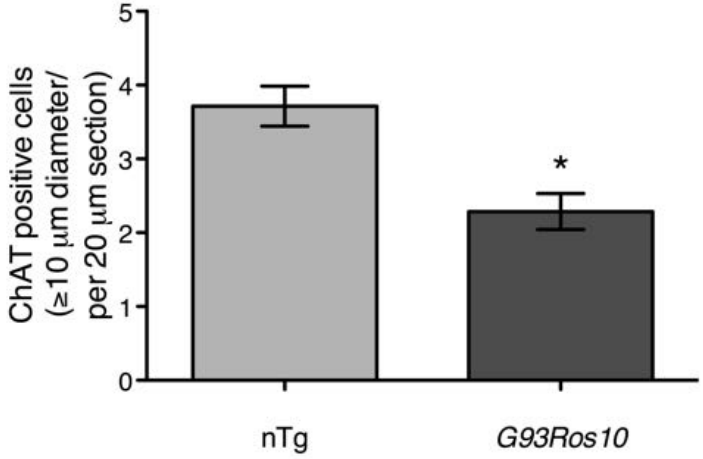
nTg



Tg(sodG93R)os10



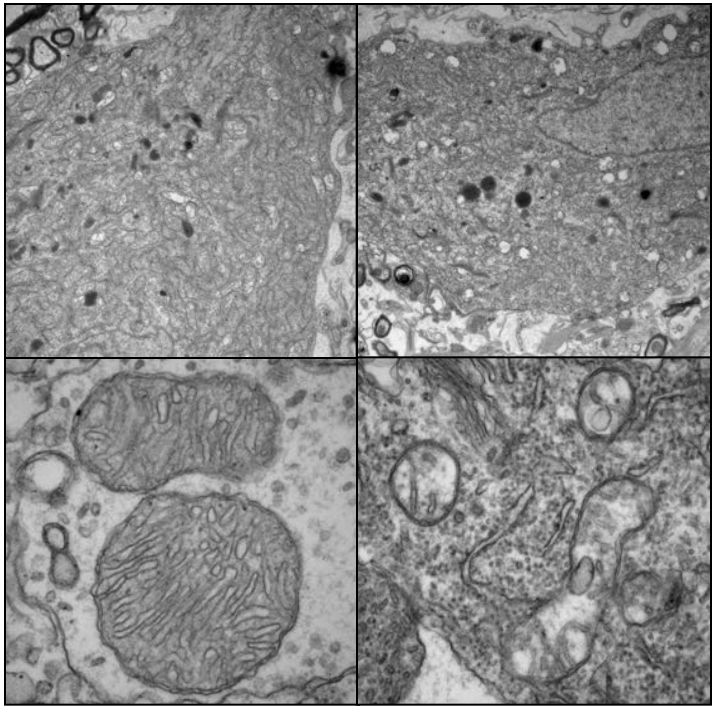
ChAT antibody labeling



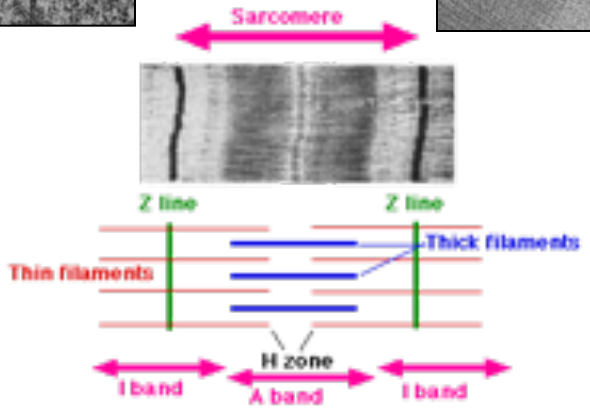
EM reveals spinal cord and muscle degeneration in endstage fish

Spinal Cord

nTg | *Tg(sodG93R)os10*

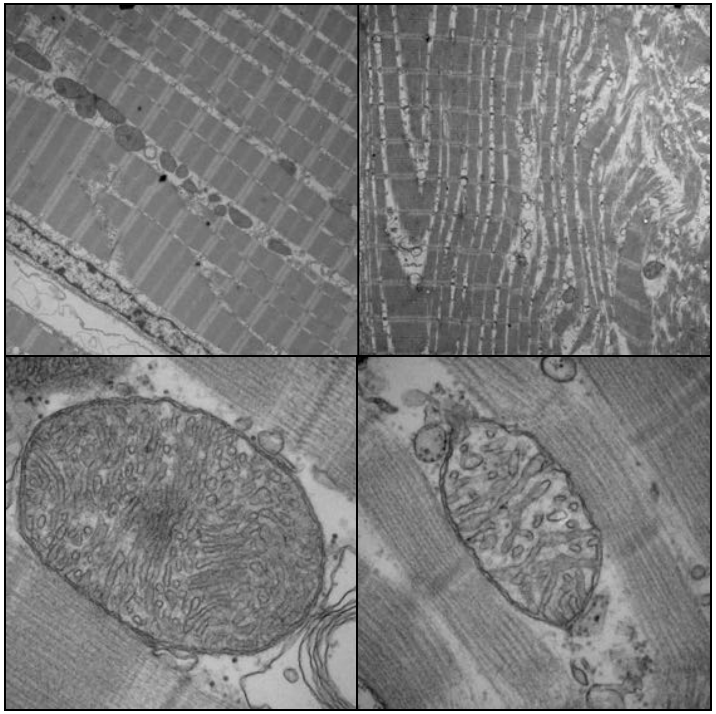


Shrunken mns
Vacuolated mitochondria
with disintegrating cristae



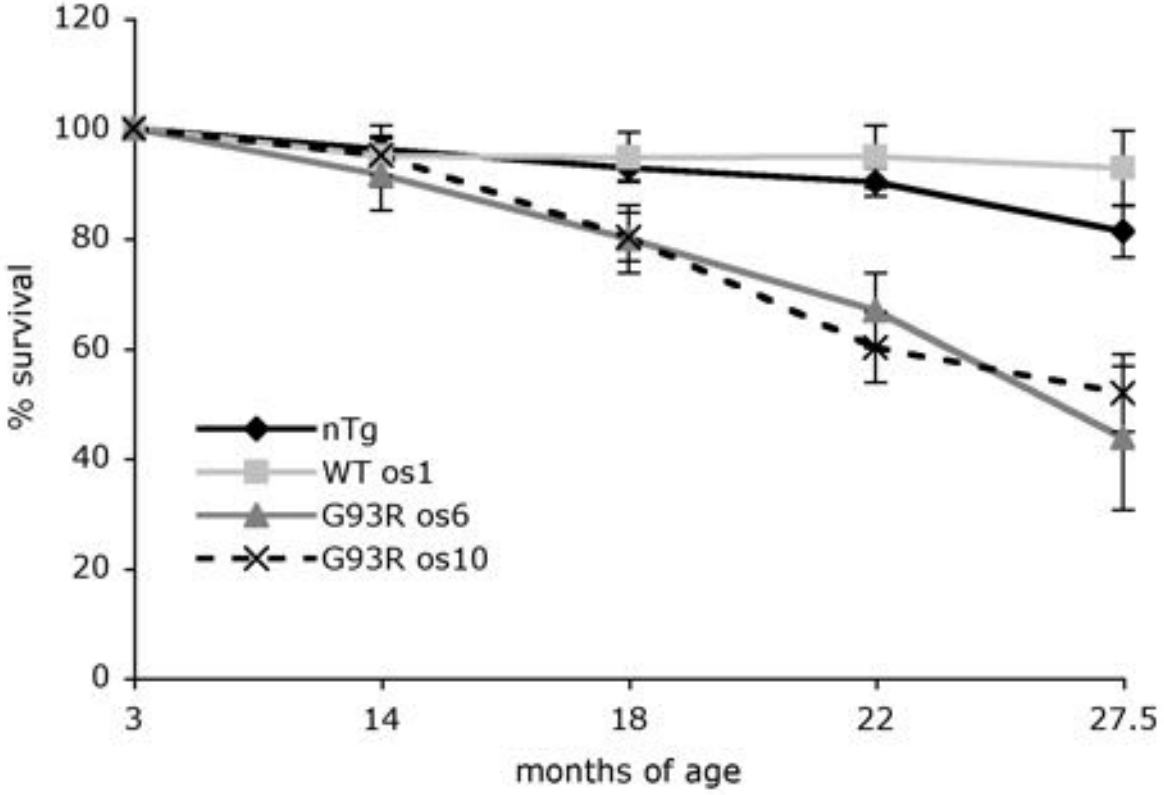
muscle

nTg | *Tg(sodG93R)os10*



Reduced myofibril size
Smaller mitochonidria
Collagen deposition

Premature death in fish expressing mutant Sod



Summary of zebrafish model:

Presynaptic defects
Movement defect
Motoneuron cell loss
Premature death

No aggregates found

Mouse model:

Used endogenous promoter
Neuronal aggregates
Loss of motoneurons
Decreased movement followed by paralysis
Axon sprouting and reinnervation

A commonly used model has 17-20X protein expression
Inbreed lines- will small changes translate to outbreed systems?
Sick animals make drug/genetic screens difficult

Discussion points:

What are the common themes? Is this telling us something?

Is it relevant to express mutant protein in only selected cell types?

The strength of non-mammalian models for genetic and drug screens

Is it useful to have non-mammalian models?