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SPINAL MUSCULAR ATROPHY IN DROSOPHILA

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INTRODUCTION

Spinal Muscular Atrophy (SMA) is the most commonly inherited form of motor neuronal disease in humans and a leading cause of infant mortality. SMA is characterized by a loss of motor neurons which lead to muscle degeneration, paralysis, and eventual death by respiratory failure. More than ninety-five percent of the children born with this disease die; One in forty people are carriers and one in six thousand live births is an SMA child. SMA is a result of a loss of a gene known as Survival of Motor Neurons (SMN).

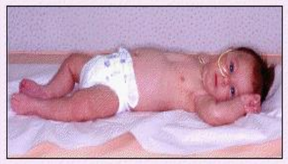
We hypothesize that SMN is required in motor neurons for proper axonal extension and synapse formation/maintenance, and in muscles to ensure innervation. Using *Drosophila melanogaster,* we examined the requirements for SMN in motor neuronal development using RNAi. Fly embryos were collected and injected with varying concentrations of dsRNA that specifically targets the *Drosophila* SMN transcript for degradation. Fly embryos were allowed to develop to late stages of development and were then filleted, fixed, stained and imaged on a confocal microscope. Using an open-source software Image J (http://rsbweb.nih.gov/ij/), measurements could be collected for each image.

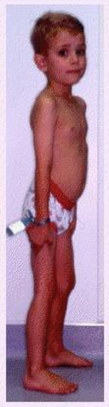
With this information, opportunities to develop effective drugs to treat different types of genetic mutations can be developed in the future. We believe that a study of SMN in motor neurons will lead to an understanding of motorneuron homeostasis that is currently lacking. Models of neurodegeneration as they apply to SMA might also apply to ALS and other diseases of motor neurons.

Spinal Muscular Atrophy

SMA derives from the loss of a gene known as SMN, an acronym for Survival of Motor Neurons.

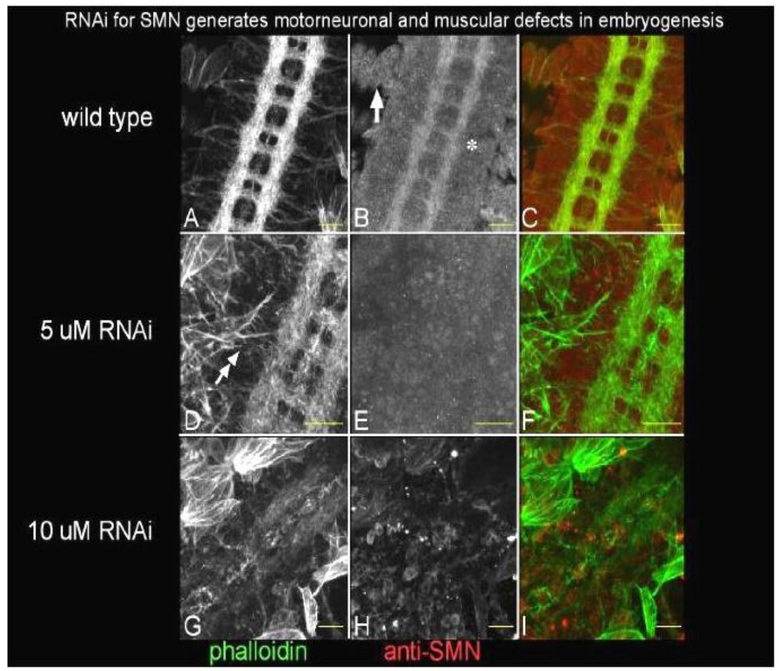
Motor neuronal degeneration is the hallmark of this disease and loss of motor neurons leads to death by respiratory failure---just like ALS.





SMA type	Severity	Age of onset	Genotype	Phenotype
I	Severe	< 6 months	SMN1$^{-/-}$; SMN2$^{+/+}$	Unable to sit, severe respiratory dysfunction
II	Intermediate	< 18 months	SMN1$^{-/-}$; SMN2$^{+/+}$; SMN2$^{+/-}$	Able to sit but unable to walk
III	Mild	> 18 months	SMN1$^{-/-}$; SMN2$^{+/+}$; SMN2$^{+/+}$; (SMN2$^{+/-}$....)	Able to stand and walk unaided

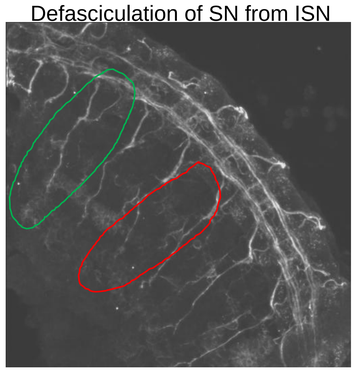
RNAi for SMN generates motorneuronal and muscular defects in embryogenesis



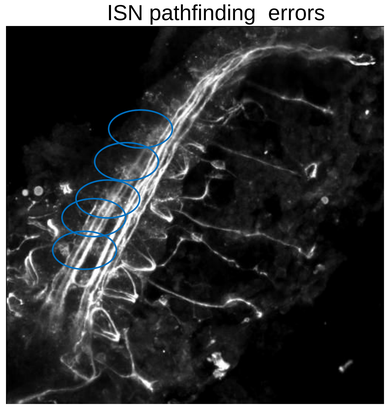
20 µM RNAi produced embryonic lethality in 100% of the embryos (A. Schmid)
10 µM RNAi produced gross defects in surviving embryos
5 µM RNAi produced subtle and varying defects in surviving embryos

Observed effects of 5uM RNAi injections

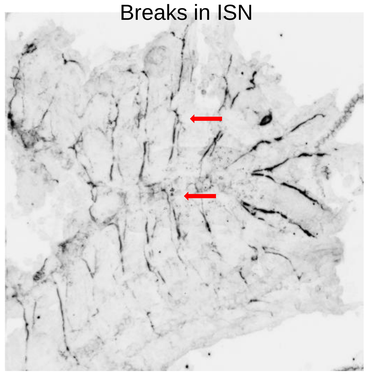
Defasciculation of SN from ISN



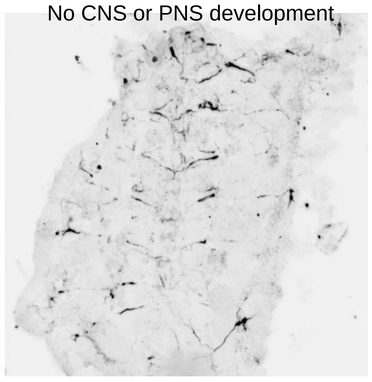
ISN pathfinding errors



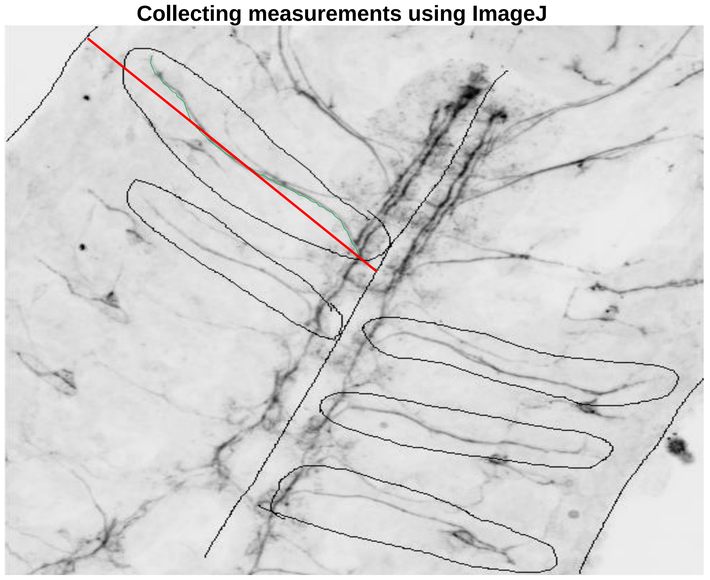
Breaks in ISN



No CNS or PNS development



Collecting measurements using ImageJ



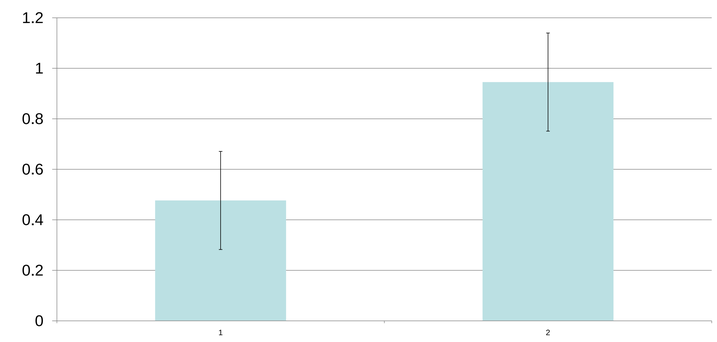
	Mean	Min	Max	Length	Average	% Body Wall
1	191.916	6	233.111	270.705		
2	178.652	67.136	236.724	270.307		
3	178.668	91.866	228.818	270.679	270.564	0.906068
4	203.209	73.011	232.355	298.613		

Average of the three ISN measurements is blue
Straight line distance from midline to edge of the body wall is yellow
Length of the ISN is expressed as the percentage of the distance to the body wall in green

Intersegmental Nerve Summary

Length	<50%	51-70%	71 – 90%	>91%
#	16	10	41	31
% of total	16.30%	10.20%	41.80%	31.60%

Axon length short vs long



A total of 98 ISNs were measured in 13 embryos