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Low Copy Number SOD1-G93A Mice are Better Suited for Dysphagia Research Compared to the High Copy Number Model

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INTRODUCTION

Background: Our lab investigates dysphagia in amyotrophic lateral sclerosis (ALS), predominantly utilizing the high copy number (HCN) expressing SOD1-G93A transgenic mouse model. We have previously observed that these mice have dysphagia upon weaning, without other clinical signs of ALS. Therefore, we are investigating the low copy number (LCN) SOD1-G93A transgenic mouse model that has delayed onset of limb dysfunction and extended survival compared to HCN mice. Furthermore, LCN mice have forelimb and bulbar involvement that more closely resembles human ALS.

Objectives: The goal of this study was to use a videofluoroscopic swallow study (VFSS) protocol developed in our lab to characterize dysphagia in HCN and LCN mice at disease end-stage. Our ultimate goal is to identify a set of functional biomarkers that could facilitate early detection of dysphagia in ALS and serve as outcome measures to quantify treatment efficacy in clinical trials.

Hypothesis: Both models (HCN and LCN) have dysphagia at disease end-stage; however, dysphagia is more severe in HCN mice.

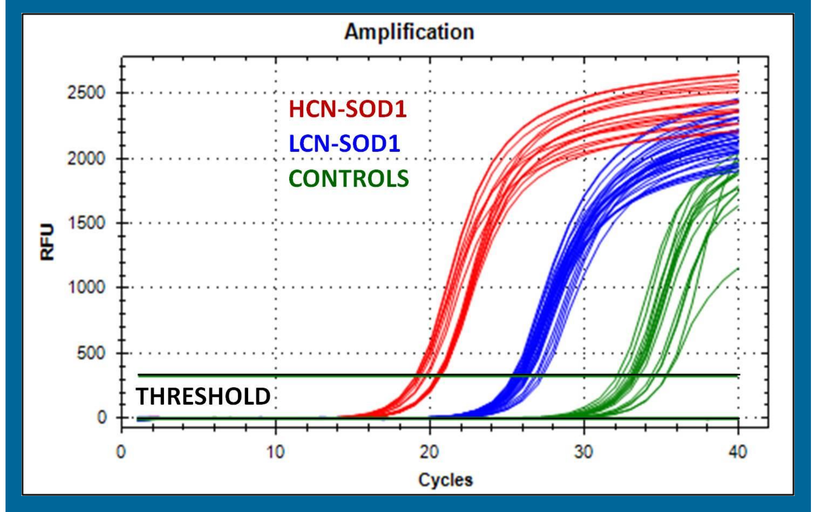
METHODS

Copy number was determined using quantitative PCR (qPCR) to clearly distinguish HCN, LCN and control mice from one another.

Behavioral testing was carried out to characterize various phenotypes among mice.

VFSS was performed on freely-behaving, disease end-stage LCN (n=24) and HCN (n=23) mice and age-matched nontransgenic littermate controls (n=44) of either sex. VFSS videos (recorded at 30 frames per second) were analyzed frame-by-frame to quantify 15 swallow metrics.

COPY NUMBER DETERMINATION



Quantitative PCR (qPCR). Genotype is determined by quantifying the number of copies of the human SOD1 gene for each mouse. Mice carrying a high copy number of the mutated gene are identified early in the assay as it proceeds in real time; followed by mice carrying a low-copy number of the gene and lastly nontransgenic control mice which do not harbor any copies of the mutated gene.

PRE-SYMPTOMATIC

HCN | LCN | Control

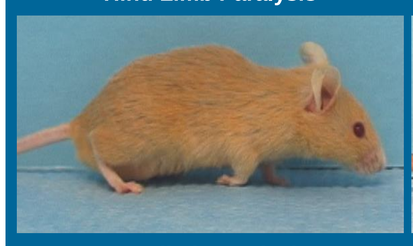
END-STAGE

HCN | LCN | Control

PHENOTYPES OF LOW-COPY MICE

Hind Limb Paralysis

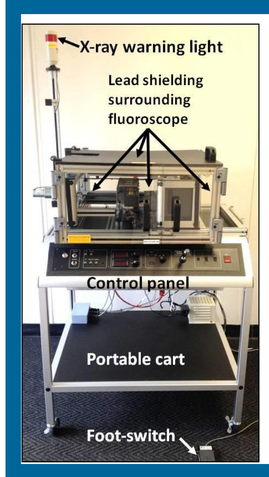
Hind Limb & Forelimb Paralysis

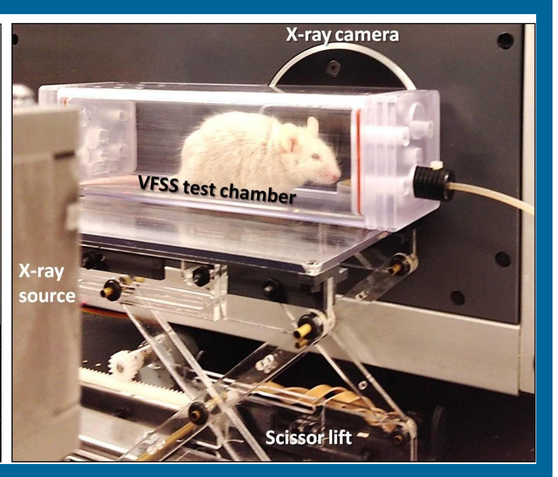


Forelimb Paralysis

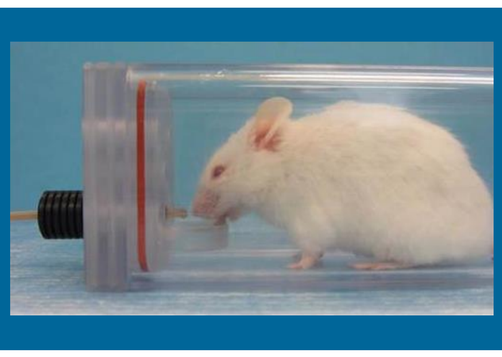
Forelimb Paralysis & Head Drop

VIDEO FLUOROSCOPY

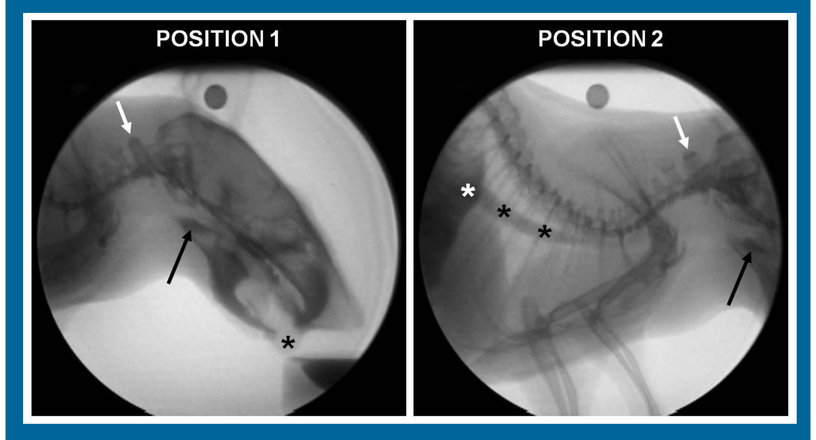




Low Energy Fluoroscopy System. A desktop fluoroscope for visualizing swallow function in mice. *Left* Front view of the fluoroscope showing labeled components. *Right* VFSS test chamber with a mouse positioned in lateral view within the system. A motorized scissor lift table permits remote positioning of the test chamber from a distance.



Mouse drinking from bowl within observation chamber. A syringe delivery system is attached to the bowl and observation tube to allow remote delivery of contrast solution to the fluoroscope system during testing.



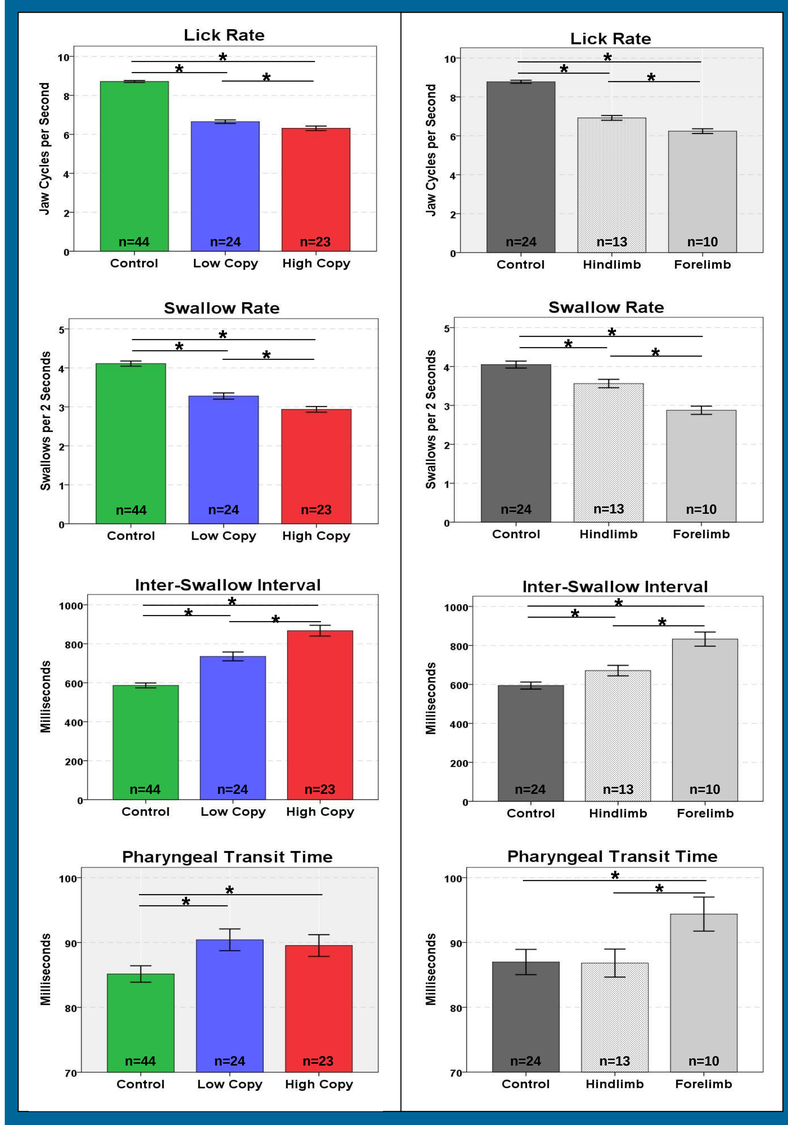
VFSS Test Positions. Lateral radiographic images of a mouse, demonstrating two test positions used to visualize the entire swallowing mechanism.

Position 1: The entire head and proximal thoracic region is in the field of view, with the swallow trigger point (black arrow) positioned in the center. The tongue (black asterisk) is visible as the mouse drinks from the bowl.

Position 2: The fluoroscopy field of view spans from the swallow trigger point (black arrow) to the stomach (white asterisk). Note the bolus (black asterisks) passing through the distal esophagus as the vallecular space (black arrow) fills in preparation for the next swallow.

White arrows in both frames: 2nd cervical vertebra; black circles centered at the top of each field of view are radiopaque markers for scaling the fluoroscopic image magnification.

ANALYSIS OF SWALLOW FUNCTION



Analysis of VFSS Metrics. A characteristic dysphagia profile is differentiated between ALS and nontransgenic mice. Fifteen metrics were analyzed and four are shown here. For each of the metrics shown here, ALS mice exhibit significantly impaired swallow function compared to control mice; and HCN mice display a more severe phenotype compared to LCN mice, as shown by the significant differences between lick rate, swallow rate and inter-swallow interval. Upon further investigation into the sub-phenotypes of the LCN mice we observed that mice with forelimb paralysis exhibit a significantly higher degree of impairment compared to those that develop hind limb paralysis alone. Error bars: ±1 SEM; asterisks: $p<0.05$.

CONCLUSIONS

Quantification of VFSS metrics generates a distinct dysphagia profile between ALS and control mice.

HCN and LCN mice share the same dysphagia profile at disease end-stage; however HCN mice have a higher degree of dysphagia severity.

HCN mice exhibit dysphagia at weaning, making the LCN model more suitable for research of dysphagia in ALS due to the extended onset period with which therapeutic interventions can be investigated.

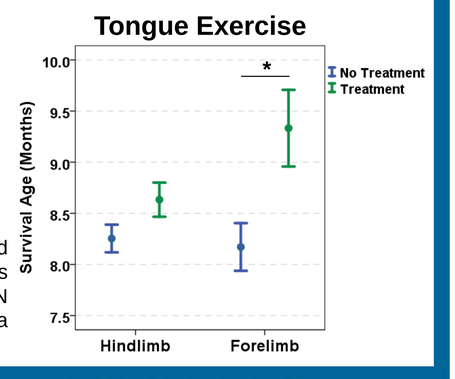
FUTURE DIRECTIONS

Determine the timing of dysphagia onset in LCN mice.

Investigate individual therapeutic strategies (exercise, serotonin, hypoxia) and a combinatorial approach.

Preemptively determine phenotype severity based on genetics.

Generate a bulbar ALS model.



Tongue Exercise Program. Targeted tongue exercise significantly extends survival for forelimb affected LCN mice with the more severe dysphagia phenotype.

ACKNOWLEDGEMENTS

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