Craniofacial Muscles and the Pathology of Dysphagia in Canine Degenerative Myelopathy

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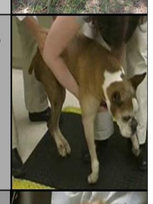
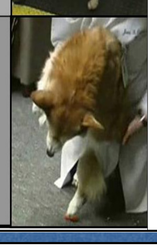
Introduction

Canine degenerative myelopathy (DM) is a neurodegenerative disease with similarities to some forms of amyotrophic lateral sclerosis (ALS), the most common motor neuron disease in humans. Canine DM and ALS share a mutation in the superoxide dismutase 1 gene (*SOD1*) resulting in protein aggregates within motor nuclei. Canine DM is characterized by progressive upper motor neuron (UMN) paresis and general proprioceptive ataxia of the pelvic limbs, which progresses to affect the thoracic limbs and develops to generalized flaccid weakness. In addition, DM and ALS are also associated with dysphagia. Research on canine DM has focused on the pathology of the spinal cord and limb muscles with its innervation, but is deficient in evidence for pathology of craniofacial swallowing musculature.

Table 1: Stage of neurological signs in DM dogs

Stage	Neurologic Signs	
1 Early	**UMN Paraparesis** • Progressive general proprioceptive ataxia • Asymmetric spastic paraparesis • Intact spinal reflexes	
2 Early	**Nonambulatory Paraparesis to Paraplegia** • Mild to moderate loss of muscle mass • Reduced to absent spinal reflexes in pelvic limbs • +/- urinary and fecal incontinence	
3 Late	**LMN Paraplegia to Thoracic Limb Paresis** • Signs of thoracic limb paresis • Flaccid paraplegia • Severe loss of muscle mass in pelvic limbs • Urinary and fecal incontinence	
4 Late	**LMN Tetraplegia and Brain Stem Signs** • Flaccid tetraplegia • Difficulty with swallowing and tongue movements • Reduced to absent cutaneous trunci reflex • Generalized and severe loss of muscle mass • Urinary and fecal incontinence	

Objectives

•Identify and correlate histopathology of the tongue muscle and hypoglossal nucleus.

Methods

1. Preparations:

•Collected tissue samples from dogs at euthanasia and immersion-fixed in 10% neutral buffered formalin (NBF)

*Brainstem medulla (n=28)

*Tongue to trachea tissue (n=4)

-Five additional tongues of young, unaffected dogs were used for project planning

•Matched samples of similar age, body size and fixation duration.

*14 DM-affected: dogs at stages 3 and 4 (14 Medulla, 2 Tongue)

*14 Controls (14 Medulla, 2 Tongue)

•Tongue regions of interest: genioglossus (protrusion) and hyoglossus (retraction), biopsy samples based on gross dissection and MRI study (Figure 2)

2. Tissue Processing: All brain and tongue samples were subjected to paraffin processing and embedding.

3. Sectioning- In progress: Paraffin embedded samples are being sectioned by microtome at 10uM thickness and mounted on slides.

4. Staining- In progress: All samples will undergo histochemical and immunohistochemical techniques.(Figure 3)

Acknowledgements

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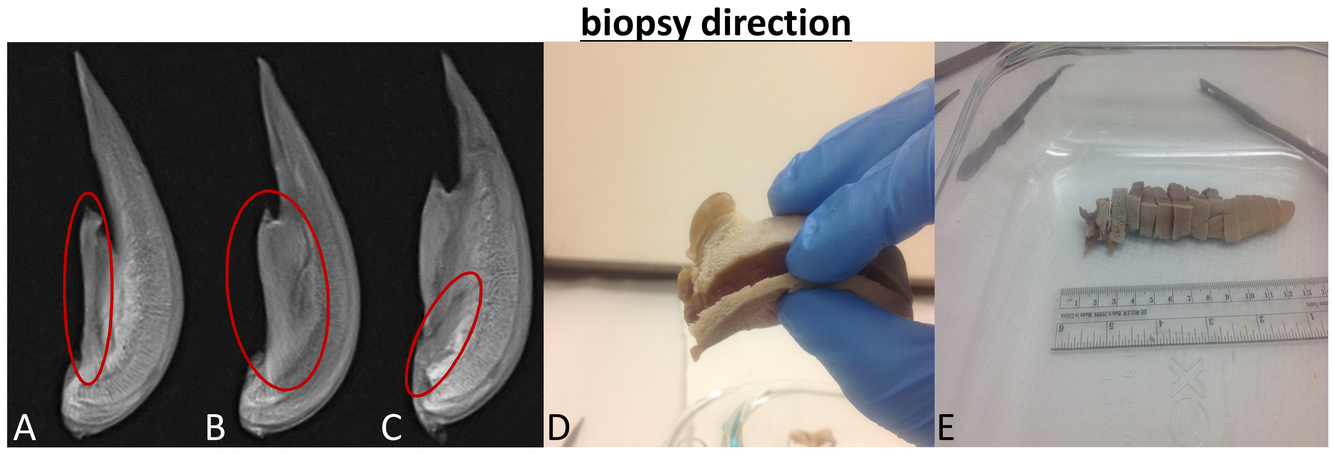
•This project would not have been possible with out the Lever Lab Histology team: Iyas Daghlas, Haley Martin and Grace Goding as well as Research Specialist Kate Robbins

Table 2: Pictures to understand muscle fiber organization of tongue and determine biopsy direction

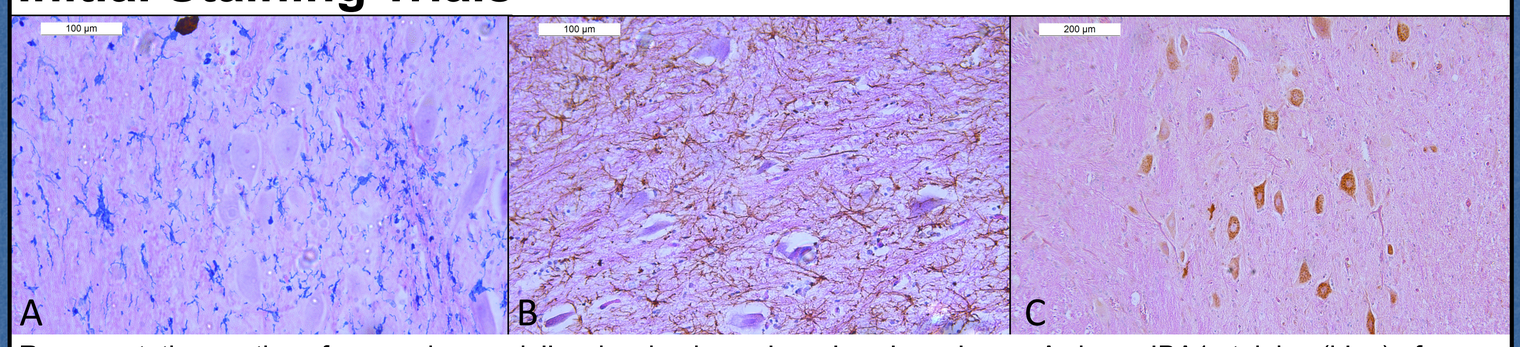


(A-C: 3T MRI images of fixed tongue) A. horizontal genioglossus muscle, B. oblique genioglossus muscle, C. styloglossus or hyoglossus muscles, D. sagittal sections made during gross dissection, E. coronal sections made during gross dissection

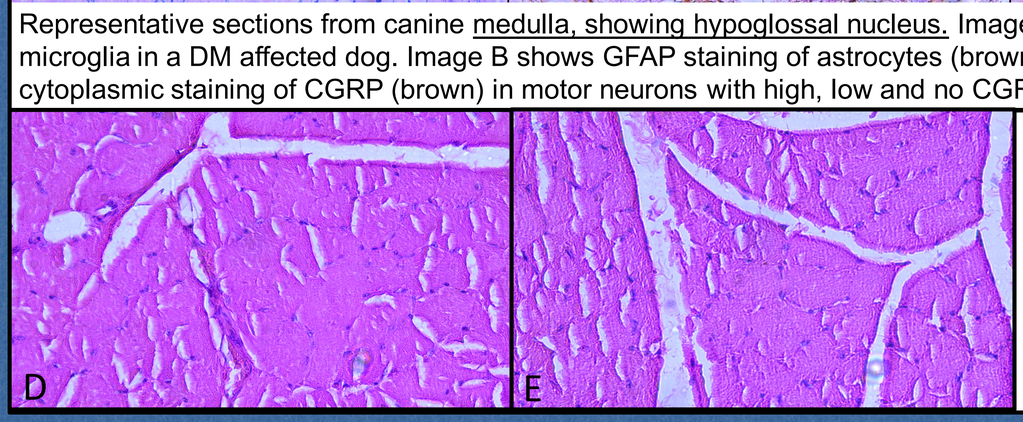
Table 3: Immunohistochemistry (IHC) Staining Methodology

IHC	Antigen	Purpose	Antibody Used
Calcitonin Gene Related Peptide (CGRP)	Motor neurons that express CGRP (a neuropeptide)	Pathological motor neurons should be CGRP-positive at early stages of disease and completely degenerate by disease end-stage. Those with the highest density will die first.	*Primary:* Anti CGRP (Abcam, Mouse Monoclonal, Primary Dilution 1:400) *Secondary:* Vectastain ABC Kit Mouse IgG
Choline Acetyltransferase (ChAT)	Motor neurons that express acetylcholine transferase	Pathological motor neurons should have high or moderate ChAT staining that is associated with cholinergic dysfunction, leading to motor neuron death at disease end-stage.	*Primary:* Anti ChAT (EMD Millipore Corp., Goat Whole Antisera, Primary Dilution 1:400) *Secondary*: Vectastain ABC Kit Goat IgG
Glial Fibrillary Acidic Protein (GFAP)	Astrocytes	Astrocyte activity is increased in ALS	*Primary:* Anti GFAP (Novus Bio. Goat Column Purified Polyclonal, Primary Dilution 1:150) *Secondary:* Vectastain ABC Kit Goat IgG
Ionized Calcium Binding Adaptor Molecule 1 (IBA1)	Microglia	Microglia activity is increased in ALS	*Primary:* Anti IBA-1 (Wako Pure Chem., Rabbit Column Purified Polyclonal, Primary Dilution 1:1000) *Secondary*: Vectastain ABC Kit Rabbit IgG
Superoxide Dismutase 1 (SOD1)	SOD1 aggregates in motor nuclei	SOD1 mutant protein aggregates are seen in DM and some forms of ALS	*Primary:* Anti SOD1 Cu/Zn ((Enzo Life Sci., Rabbit Column Purified Polyclonal, Primary Dilution 1:200) *Secondary:* Vectastain ABC Kit Rabbit IgG
Vimentin	Vimentin protein contained in glial cells and endothelial cells of CNS	Establish proper fixation levels of canine neural tissues. Overfixed tissue will not stain with Vimentin. Strong staining occurs in ALS-affected neural tissue compared to weaker staining in non-disease neural tissue..	*Primary:* Anti Vimentin (DAKO, Mouse Monoclonal, Primary Dilution 1:200) *Secondary:* Vectastain ABC Kit Mouse IgG

Initial Staining Trials



Representative sections from canine medulla, showing hypoglossal nucleus. Image A shows IBA1 staining (blue) of microglia in a DM affected dog. Image B shows GFAP staining of astrocytes (brown) in a control dog. Image C shows cytoplasmic staining of CGRP (brown) in motor neurons with high, low and no CGRP levels in a DM affected dog.



Representative sections from canine tongue muscle in cross section. Image D shows Hemotoxylin and Eosin (H&E) staining of the horizontal area of the genioglossus muscle. Image E shows H&E staining of the hyoglossus muscle.

Future Direction

•This study is a work in progress.

-Medulla and tongue muscle will continue to be processed. In addition medulla will be stained with SOD1, ChAT , Vimentin. Muscle will be stained with Masson's trichrome and antimyosin heavy chain type 1 antibodies

- The goal is to correlate histopathology of tongue muscles (genioglossus and hyoglossus) with the hypoglossal subnuclei that innervate them.

•Requests will also be made for bilateral medulla samples after euthanasia to better preserve the hypoglossal nucleus for histological processing

•We intend to use 7T MRI imaging of canine tongue to better identify regions for biopsy

•We also intend to study the same tissues of young control dogs to determine histopathology due to aging.