EVALUATING ULTRASONIC VOCALIZATION IN A NOVEL RAT MODEL THAT MIMICS ASPECTS OF AMYOTROPHIC LATERAL SCLEROSIS

College of Veterinary Medicine
University of Missouri

Kristen D. Bagley, Nicole L. Nichols, and Teresa E. Lever

College of Veterinary Medicine, North Carolina State University, Raleigh, NC (Bagley)
Department of Biomedical Sciences, College of Veterinary Medicine (Nichols, Lever), Department of Otolaryngology—Head and Neck Surgery, School of Medicine (Lever), University of Missouri, Columbia, MO

NC STATE UNIVERSITY College of Veterinary Medicine

Introduction and Rationale

Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease in which the death of motor neurons leads to a loss of voluntary muscle control. In addition to global muscle atrophy, functional deficits of ALS include dysarthria (speech dysfunction) and dysphagia (swallowing dysfunction) due to a lack of tongue movement, which can lead to aspiration pneumonia[1].

1

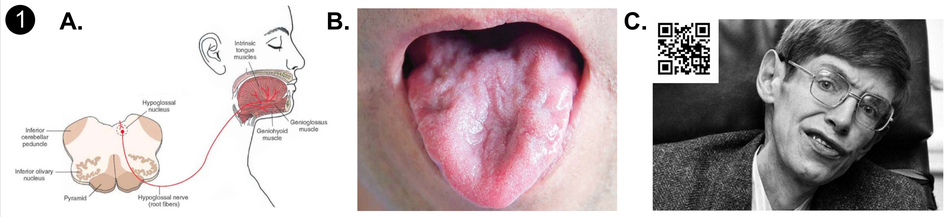

Figure 1. **A.** The tongue is innervated by the hypoglossal nerve, which degenerates in patients with ALS. **B.** Tongue muscle atrophy in a patient affected by ALS[2]. **C.** Prior to using a speech synthesizer, physicist Stephen Hawking communicated with the help of an interpreter (an example of Dr. Hawking's speech is available at the QR link)[3].

Most ALS patients will succumb to respiratory failure within 1.5 - 4 years[4]. Despite the grave prognosis and severe impact of this disease on patient quality-of-life, there are currently no effective treatments to preserve or restore these critical functions. SOD1 transgenic rodents are available for research but take months to develop ALS and are highly variable in the impairment shown[5,6].

Can we mimic human ALS symptoms to study only swallowing and speech dysfunction?

We recently developed a novel rat model of dysphagia by administering intralingual injections of cholera toxin B conjugated to saporin (CTB-SAP). These rats quickly display neural and swallowing deficits due to hypoglossal motor neuron death[7].

2

Neural deficits in CTB-SAP model

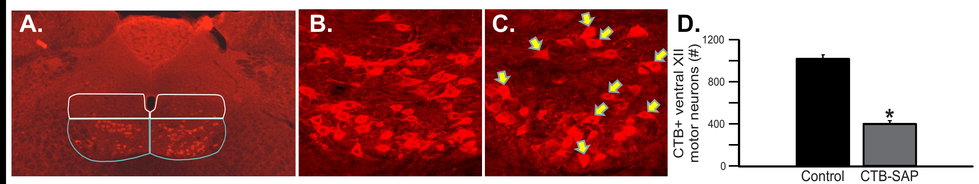

Figure 2. CTB-SAP treatment results in fewer surviving hypoglossal (XII) motor neurons. A. Immunohistochemistry using antibody for CTB labels motor neurons within the XII nucleus in the brainstem (white outline = dorsal XII nucleus; blue outline = ventral XII nucleus). B. Surviving neurons within the ventral XII nucleus of a control treated animal. C. Arrows indicate surviving ventral XII motor neurons in a CTB-SAP treated animal[5]. D. CTB+ ventral XII motor neurons in control *vs.* CTB-SAP treated animals (* = p<0.05).

3

Swallowing deficits in CTB-SAP model

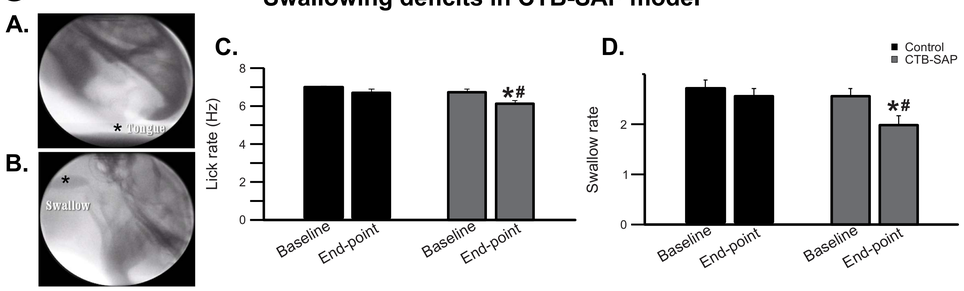

Figure 3. Videofluoroscopic swallow study analysis of CTB-SAP treated rats. A. Still image from video, where asterisk indicates tongue apex of rat. B. Still image from video, where asterisk indicates esophagus containing radio-opaque barium solution. C and D. CTB-SAP treated rats had a significantly decreased lick and swallow rate compared to its baseline values (# = p<0.05) and *vs.* end-point values for control rats (* = p<0.05)[5].

Since speech and vocalization are altered in ALS patients[8] and rodent models for neurodegenerative disease[9], we want to characterize vocalization in our CTB-SAP rodent model. We will examine rodent ultrasonic vocalization (USV) in this study, which can be analyzed as a translational analog to human speech.

Hypothesis

Following intralingual injection of CTB-SAP, we hypothesize that rats will display altered ultrasonic vocalizations that mimic dysarthric speech in human ALS.

Experimental Methods

4

Genioglossus injection of CTB-SAP or CTB+SAP (control)

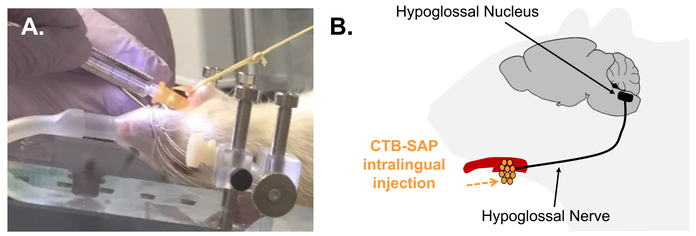

Figure 4: **A.** Isoflurane anesthetized adult male rats are given a midline injection into the genioglossus muscle with either 25 µg CTP-SAP treatment (n=2) or Control (CTB unconjugated to SAP (CTB+SAP); n=2). **B.** CTB-SAP is taken up by hypoglossal nerve axons and is retrogradely transported to the cell body in the hypoglossal nucleus. SAP will then bind to and inactivate ribosomes, leading to apoptosis.

5

Ultrasonic vocalization testing

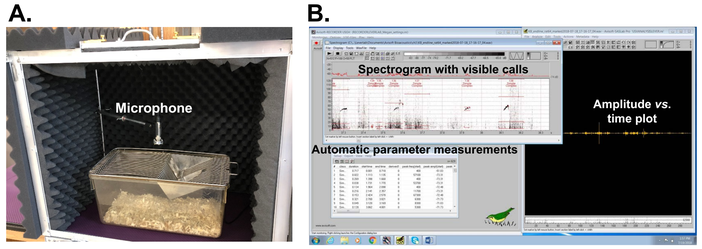

Figure 5: Ultrasonic vocalization (USV) testing is performed prior to the intralingual injection (baseline) and 8 days after the intralingual injection in CTB-SAP treated and control rats. **A.** Inside of the sound-attenuated chamber for USV recording. A female rat in her home cage is placed in the chamber. A male to be tested is placed in the cage with her and allowed to socialize for 5 minutes. At the end of the 5 minute period, the female is removed and a 5 minute USV recording of the male is conducted. **B.** Recordings are acquired (see amplitude *vs.* time plot and spectrogram with visible calls) and analyzed (see automatic parameter measurements above) using *Avisoft Bioacoustics* hardware and software.

Ultrasonic vocalization analysis

Rat ultrasonic calls will be sorted into different classes based on frequency (Hz) and duration patterns (Table 1 below)[10]. Other quantifiable call characteristics include: call rate, latency to call, intensity, and peak frequency.

Call Category	Call type	Description
Simple	Flat	Flat, unmodulated with not more than a .2 kHz/second rate of frequency change
	Step	Two adjoining frequencies with a jump (or step) between them
	Short	Very brief, unmodulated call
	Ramp	Slow change in frequency of at least .2 kHz/second
Complex	Trill	Rapid frequency modulations
	Harmonic	Very intense flat call that breaks into the upper and lower harmonic frequencies
	Frequency Modulated	Call consisting of at least 3 frequency changes.
Compound	Compound	Two or more calls combined. (ex, Flat + Trill, Ramp + Trill)

Table 1: Descriptions of Call Categories from Grant et al. study[10].

Representative calls from a CTB-SAP treated and control rat

6

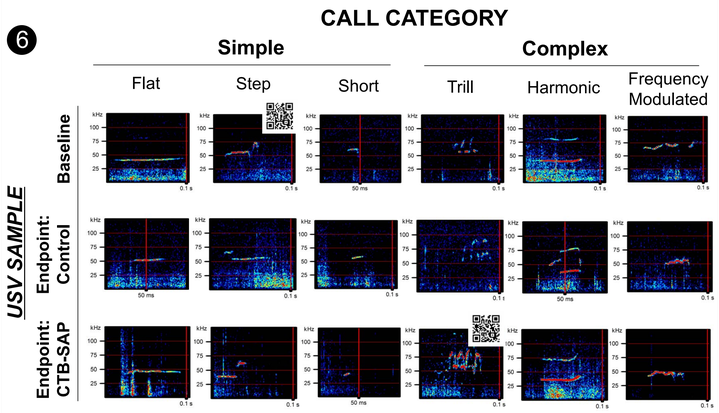

Figure 6: Spectrogram examples of ultrasonic calls fitting the categories defined by Grant et al[10]. Acoustic playback of calls is available at the QR link. Call frequency is modified to bring it into the human hearing range.

Expected Results

We expect statistical analysis to reveal differences in the call quality between endpoint USV recordings of CTB-SAP treated rats and controls, as well as between endpoint and baseline recordings of CTB-SAP treated rats. We do not expect the overall number of calls to be affected between groups, since the motivation to call is the same. We do not expect to see differences between endpoint and baseline USV recordings of controls.

Implications

Upon identification of USV differences, this study will provide a new biometric for further evaluation of translational therapies for dysarthria in ALS. More broadly, this study would also be the first to demonstrate that the tongue is an essential component of rodent communication, thus expanding translational potential of our CTB-SAP model.

Future Directions

1) What are the quantitative differences in USV call rate, call classifications, latency to call, intensity and peak frequency of the calls between CTB-SAP treated rats and control treated rats, as well as the differences between baseline and endpoint calls of the CTB-SAP treated rats?
2) Can we correlate altered USV calls with lick and swallow deficits?
3) Are there any therapeutic measures that would preserve or restore USV function of the CTB-SAP treated rats? Can these measures then be translated to generate novel treatments for human ALS symptoms?

References

1. Kühnlein, P. et al. *Nature Clinical Practice Neurology. 2008*
2. Toro, J. et al. *New England Journal of Medicine.* 2014
3. HawkingVideos. *YouTube.* 2010
4. Chio, A. et al. *Amyotroph Lateral Scler.* 2009
5. Gurney, M.E. et al. *Science.* 1994.
6. Lever, T.E. et al. *Dysphagia. 2010.*
7. Lind, L.A. et al. under review at *Neuroscience.*
8. Shellikeri, S. et al. *J Speech Lang Hear Res.* 2016
9. Kelm-Nelson, C.A. *Behav Brain Res.* 2016
10. Grant, L.M. et al. *Behav Neurosci.* 2018

Acknowledgements

Research Grant: **MU School of Medicine Bridge funding for USV research equipment, MU Research Board Grant, and private funding**

Student Support: **ASLAP Foundation**

Special thanks to: Vincent Pizzo, Kate Osman, Megan Haney, and Ian Deninger for their assistance with USV recording and Jeremy Rine for USV chamber construction.

Veterinary Research Scholars Program
University of Missouri