An Experimental Swallow Evoked Potential Protocol to Investigate the Neural Substrates of Swallowing

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Abstract

Advancement in dysphagia intervention is hindered by our lack of understanding of the neural mechanisms of swallowing in health and disease. Evoking and understanding neural activity in response to normal and disordered swallowing is essential to bridge this knowledge gap. Building on sensory evoked potential methodology, we developed a minimally invasive approach to generate swallow evoked potentials (SwEPs) in response to repetitive swallowing induced by citric acid stimulation of the oropharynx in lightly anesthetized healthy adult rats. The SwEP waveform consisted of 8 replicable peaks within 10 milliseconds immediately preceding the onset of electromyographic swallowing activity. Methodology refinement is underway with healthy rats to establish normative SwEP waveform morphology before proceeding to models of advanced aging and age-related neurodegenerative diseases. Ultimately, we envision that this experimental protocol may unmask the pathologic neural substrates contributing to dysphagia to accelerate the discovery of targeted therapeutics.

Dysphagia is a debilitating comorbidity of advanced aging and age-related neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. In these cases, dysphagia often leads to malnutrition and aspiration pneumonia, 2 independent predictors of early mortality.[1-4] Unfortunately, treatment is predominantly palliative because the affected neurologic regions and pathophysiologic mechanisms contributing to dysphagia onset and progression are largely unknown and are likely different for each disease. To address this clinical need, we propose to adapt the fundamentals of sensory evoked potential testing to investigate dysphagia in rat models.

Sensory evoked potential testing is commonly performed in clinical and surgical settings to detect pathology of the auditory[5-8] and somatosensory[9-13] pathways. Delivering an acoustic stimulus into the ear canal stimulates the auditory nerve, whereas applying mechanical, chemical, thermal, or electrical stimulation to the skin of the limbs or face stimulates the corresponding spinal and cranial nerves, respectively. The evoked response, which is time locked to the stimulus, is a compound action potential that can be extracted from background electroencephalography (EEG) recordings via noninvasive scalp electrodes. Signal-to-noise ratio is improved by averaging hundreds of evoked responses to produce a stereotypical waveform consisting of several distinct peaks occurring within milliseconds of stimulation. Based on experimental lesion studies, each peak corresponds to ≥ 1 neuroanatomic components (eg, cranial or spinal nerves, brainstem nuclei, and cortical/subcortical regions) of the associated pathway. Alterations in peak amplitude and/or latency signify pathology of the corresponding neuroanatomic sites, thereby facilitating diagnosis and guiding treatment planning.

Utilizing this concept, we explored the feasibility of generating swallow evoked potentials (SwEPs) in response to repetitive swallowing induced by chemical stimulation of the oropharynx in lightly anesthetized rats. Here, we describe our minimally invasive methodology with healthy rats in

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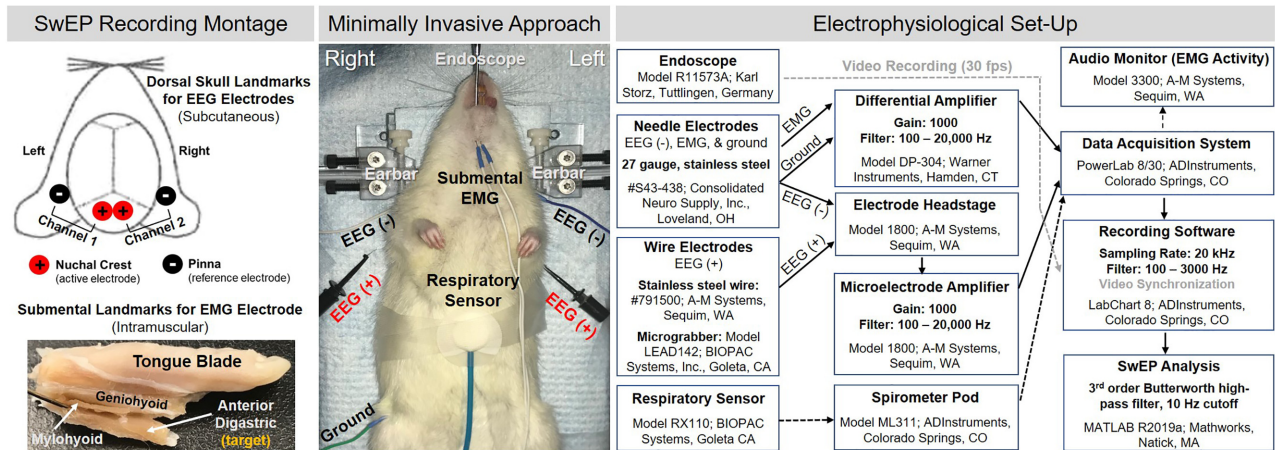Email: levert@health.missouri.edu

Figure 1. Schematic showing the anatomic landmarks for recording swallow evoked potentials (SwEPs) in rats (left), our minimally invasive approach with subcutaneous/intramuscular electrodes (middle), and the setup for electrophysiologic recording and analysis (right). EEG, electroencephalography; EMG, electromyography.

preparation for longitudinal investigations with rat models of neurogenic dysphagia. Our ultimate goal is to identify the pathologic components contributing to dysphagia in advanced aging and various neurodegenerative diseases, thus providing neuroanatomic targets for mechanistic investigations and therapeutic discovery.

Methods

Following Institutional Animal Care and Use Committee approval (Animal Welfare Assurance A3394-01), Sprague-Dawley rats (n = 20 males, 3-4 months; Envigo, Indianapolis, Indiana) were divided into 2 equal groups: 10 for protocol development to reliably evoke repetitive swallowing and 10 for SwEP proof of concept. Protocol development entailed optimization of the anesthesia regimen and citric acid delivery method. For anesthesia, we used our established murine protocol of ketamine:xylazine (90:11.25 mg/kg, subcutaneous injection), followed by one-fourth to one-half dose of ketamine as needed every 20 minutes to maintain a light anesthesia plane (ie, local limb movement in response to toe pinch) that prevented spontaneous body movement without abolishing swallowing.[14,15] Prior to anesthesia, rats underwent a 4- to 6-hour food restriction to prevent residual food in the throat that may interfere with testing.[14] Based on published work by our group[16,17] and others,[18,19] a 2.7% citric acid solution was used as the chemical stimulus to evoke repetitive swallowing. Under endoscope guidance, citric acid was delivered transorally via bolus injection into the vallecular space (100 μL/bolus via blunt-tip needle syringe; n = 5 rats) versus direct application to the vallecular mucosa (ie, tongue base and epiglottis) via a saturated cotton-tipped applicator (n = 5 rats). For electromyography (EMG) detection of swallowing, bipolar electrodes (spaced 2-3 mm apart) were inserted through the skin into the submental muscles, targeting the superficially located anterior digastric that contributes to hyolaryngeal excursion at the onset of swallowing.[20,21] Presumed EMG swallowing events were confirmed via

transoral videoendoscopy,[22,23] which permitted visualization of pharyngeal constriction in synchrony with EMG bursting activity.

Following optimization, the protocol for evoking/recording repetitive swallowing was used with the remaining 10 rats for SwEP feasibility testing (**Figure 1**). Rats were anesthetized with isoflurane (5% until nonambulatory), followed by ketamine-xylazine to maintain light sedation while being secured in ear bars in dorsal recumbency on a custom platform within a Faraday cage. Eyes were lubricated to prevent drying, and core body temperature was maintained at 37°C ± 0.2°C (DC Temperature Control System; FHC, Bowdoin, Maine). A pneumatic sensor taped to the abdomen permitted monitoring of respiratory rate and coordination of swallowing/breathing. Electrodes were inserted subcutaneously on the skull for bilateral EEG recording with the standard 2-channel montage for rodent brainstem auditory and vestibular evoked potential testing: midline between the ears (ie, nuchal crest) and adjacent to the pinna (ie, near the intratragal notch).[24,25] Swallow, respiratory, and SwEP recordings were acquired in response to 2 conditions: (1) with the endoscope inserted transorally to visualize the pharynx in preparation for citric acid delivery (5-minute baseline recording) and (2) immediately after citric acid delivery (10-minute recording). For SwEP averaging, EEG activity was time locked to EMG bursts coinciding with endoscopic pharyngeal constriction. To investigate swallow "stimulability," a subsample of 4 rats underwent an additional citric acid delivery (5-minute recording). Following testing, 5 rats were recovered for procedure-related morbidity assessment; the remaining rats were euthanized for postmortem identification of submental EMG electrode placement.

Results

Results from the first 10 rats revealed that ketamine-xylazine did not abolish swallowing; however, maintenance doses of ketamine consistently caused respiratory depression
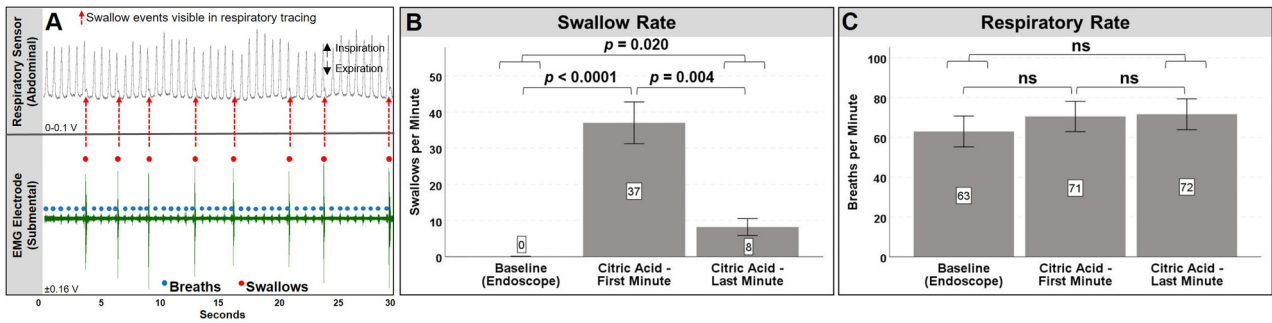
Figure 2. (A) Representative electrophysiologic recordings for quantification of (B) swallow and (C) respiratory rates immediately before and 10 minutes following a single citric acid application. EMG, electromyography; ns, nonsignificant ($P > .05$).
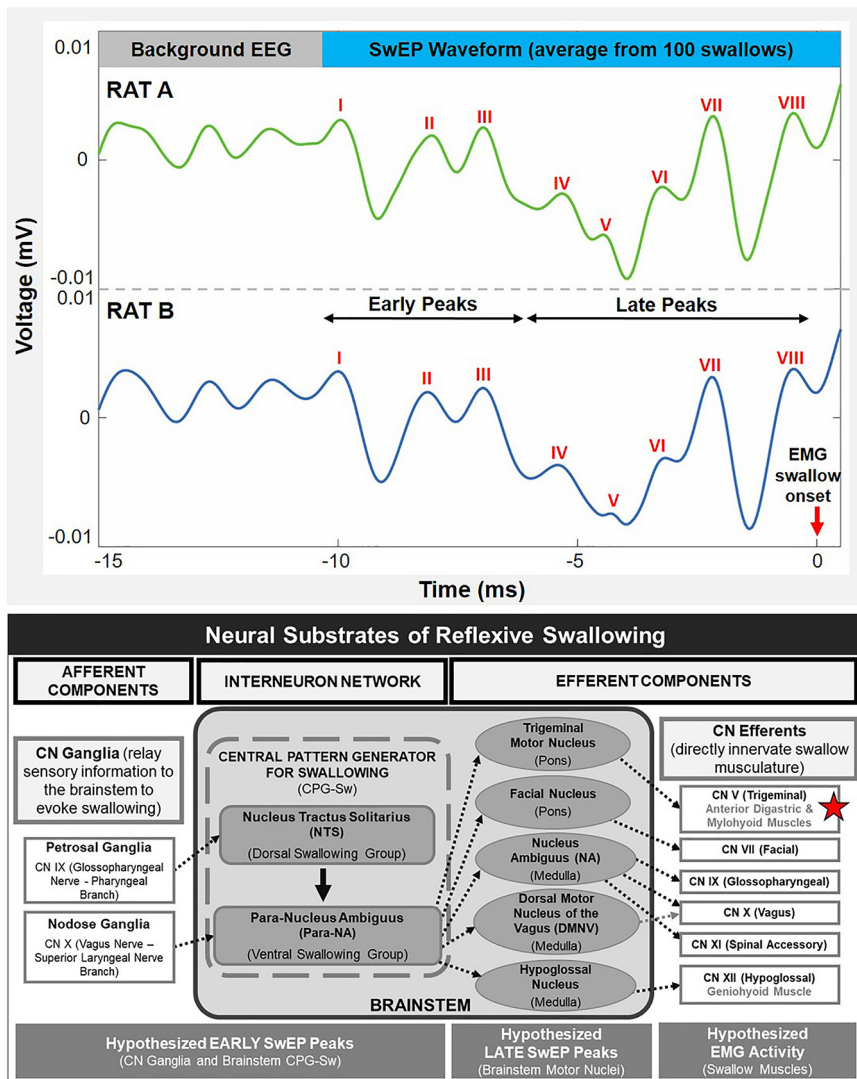

Figure 3. Top: SwEP waveforms from 2 rats show 8 replicable peaks preceding EMG swallow onset. Bottom: Hypothesized sources for early versus late SwEP peaks and EMG activity (red star). CN, cranial nerve; EEG, electroencephalography; EMG, electromyography; SwEP, swallow evoked potential.

that resulted in mortality in 1 rat (10%). Furthermore, citric acid reliably evoked repetitive swallowing only when applied to the vallecular mucosa with a saturated cotton-tipped applicator; bolus injections were inconsistently effective. Thus, SwEP feasibility testing with the remaining 10 rats entailed a single anesthesia dose, followed by citric acid

delivery via a saturated cotton-tipped applicator. With this protocol, rats swallowed on average 174 times within 10 minutes following a single application of citric acid. EMG swallow events coincided with endoscopic pharyngeal constriction 100% of the time.

Repeated measures analyses of variance with Bonferroni pairwise comparisons revealed that swallow rate significantly increased after citric acid delivery and then significantly decayed over time, whereas respiratory rate remained unchanged (**Figure 2**). After a second application of citric acid, the average swallow rate increased to 34 per minute, which was not significantly different from the initial rate of 37 per minute ($P = .229$; paired samples t test); this finding suggests the experimental procedure could be extended to obtain considerably more swallows, if needed.

To extract SwEP responses from the EEG recordings, we used a 30-millisecond window immediately preceding EMG swallow activity. As shown in **Figure 3**, averaging 100 swallows per rat was sufficient to produce a replicable SwEP waveform consisting of 8 peaks within a 10-millisecond window in the right-sided EEG recording; left-sided EEG noise contamination prevented bilateral SwEP extraction. Hypothesized generator sources for early (sensory and interneuron) versus late (motor) peaks are based on the generally accepted neural substrates of reflexive swallowing.[26-29]

The 5 rats selected for procedure-related morbidity assessments recovered without adverse events, suggesting that longitudinal SwEP testing may be feasible. The 5 rats selected for postmortem dissection confirmed EMG electrode placement within the anterior digastric and mylohyoid muscles.

Discussion

We developed a minimally invasive experimental protocol for generating SwEPs in response to repetitive swallowing induced by citric acid stimulation of the oropharynx in lightly anesthetized rats. For improved repeatability, we are exploring alternative approaches (eg, electrode materials, anatomical sites, electrophysiological equipment, post-processing algorithms) to markedly improve the signal-to-noise ratio to permit consistent extraction of the relatively tiny SwEP responses from EEG recordings. Subsequently, replication with a larger sample size of healthy rats will be essential to establish normative SwEP waveform morphology before proceeding to disease models. Furthermore, additional studies will be needed to identify the corresponding neural generator source(s) for each peak (positive and negative) with electrical, biochemical, and/or optogenetic approaches.

Once fully optimized, we envision SwEP testing may be of value in longitudinal studies to detect and monitor real-time functional changes in the neural swallowing circuit in response to various diseases and treatment interventions in rats and other experimental animal species. Our ultimate goal is for this experimental protocol to provide much-needed translational insight into the pathophysiology of dysphagia in advanced aging and neurodegenerative diseases, thereby providing neuroanatomic targets for mechanistic investigations and therapeutic discovery.

Author Contributions

Ashley Kloepper, acquisition and analysis of the data; drafting and revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Joseph Arnold**, acquisition and analysis of the data; drafting and revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Alexis Ruffolo**, acquisition and analysis of the data; drafting and revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Brian Kinealy**, acquisition and analysis of the data; drafting and revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Chandler Haxton**, acquisition and analysis of the data; drafting and revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Nicole Nichols**, analysis of the data; revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Kazutaka Takahashi**, analysis of the data; revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work, **Teresa E. Lever**, conception and design of the work; acquisition, analysis, and interpretation of the data; drafting and revising the manuscript; final approval of the manuscript; and agreement to be accountable for all aspects of the work.

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