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Craniofacial neuroscience

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Craniofacial neuroscience

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Table of contents

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Editorial: Craniofacial neuroscience

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Editorial on the Research Topic
Craniofacial neuroscience

The 12 cranial nerves (CNs) are essential for life-sustaining behaviors (feeding, swallowing, coughing, chewing, and breathing) and are vital to communication (sight, speech, and facial expressions). These functions require precise and coordinated control of the nerves, muscles, and hard tissues of the craniofacial complex, which are influenced by sensory input. Craniofacial impairments, abnormalities, and disorders can result from genetic conditions, neurodegeneration, neurotrauma, and/or cranial nerve damage. They affect people of all age groups, sexes, and ethnicities. These impairments affect quality of life through decreased oral hygiene, psychosocial anxiety, chronic pain, speech and sight impairments, developmental delays, dysphagia, and/or dystussia (coughing reflex). These can lead to malnutrition, failure to thrive, and sometimes premature death. Scientific investigations of these impairments are often overlooked due to their complex nature, which spans across multiple fields and specialties such as genetics, physiology, neuroscience, critical care, speech and language pathology, physical and occupational therapy, and dentistry. In this editorial, we will briefly review the impact of the CNs on swallowing, breathing, pain, and expression.

Upper airway dysfunction continues to be detailed in a number of neurodegenerative and neurotraumatic diseases. Song et al. reported that the prevalence of post-stroke dysphagia is 47% with associated risk factors such as hypertension, previous stroke, and atrial fibrillation. The authors also reported the persistence of dysphagia at discharge and 1 month post to be 75% and 51%, respectively. Martinez-Peña et al. also reported a high prevalence of dysphagia in older individuals with mild cognitive impairment and dementia. This suggests that early identification and intervention are key to preventing serious health outcomes. Liu et al. reported an association between lower income levels and increased oropharyngeal dysphagia, highlighting the complex challenges of healthcare accessibility, nutritional adequacy and stress. The authors also called for a sex-specific approach to early intervention in the aging population.

Preclinical animal models are investigating several potential mechanisms of dysphagia and upper airway dysfunction. Motoneuron (MN) death associated with amyotrophic lateral sclerosis (ALS) disrupts orolingual and aerodigestive behaviors such as speech and swallowing, in addition to ventilatory and non-ventilatory behaviors, breathing, and coughing, respectively. These deficits also result in an increased risk of airway protection and aspiration pneumonia. Using a mouse model of ALS, Fogarty et al. investigated the timeline of size-dependent XII MN loss and tongue innervation in SOD1 mice. They observed a significant reduction in larger XII MNs at the mid- and end-stage of the disease with no difference at the pre-symptomatic or onset ages. Specific disruption to neuromuscular junctions in the tongue did not occur until the end-stage, leading the authors to conclude that denervation of neuromuscular junctions in the tongue may be a consequence, not a cause, of MN deficits in SOD1 mice. Meanwhile Keilholz et al. used a different rodent model to induce XII MN death and investigated the use of a strength endurance tongue exercise program as a potential therapy for patients with motoneuron disease and ALS. They found that tongue exercise mitigated airflow deficits and preserved the upper airway and ultrafine structures in the tongue, suggesting that a high-repetition, low-endurance tongue exercise program could be an effective therapy to maintain upper airway patency.

Patients with Down Syndrome experience dysphagia across all three swallowing phases: oral, pharyngeal, and esophageal. This is likely due to disordered tongue function, uncoordinated swallowing and breathing, and esophageal dysmotility. Glass et al. utilized clinical techniques with a mouse model of Down Syndrome (Ts65Dn). They found that adult Ts65Dn mice have significantly slower swallow rates and longer time intervals between consecutive swallows. Adult Ts65Dn mice also have slower rates of developing tongue force and a more rapid onset of tongue muscle fatigue. The authors concluded that heightened susceptibility to tongue muscle fatigue could contribute to an increased duration of the oral phase and decreased efficacy of deglutition (swallowing).

The use of opioids, specifically morphine and remifentanil, has been associated with aspiration and swallowing dysfunction. However, the influence of codeine, the most abused opioid drug worldwide, on swallowing is unknown. Bolser et al. reported that intravenous codeine induced spontaneous swallowing in vagally intact and vagally denervated cats, suggesting that the swallow-promoting actions of this drug do not require sensory feedback from the vagus nerve to occur. Although there was an increase in the amplitude of upper airway muscles during water-induced swallows, swallow frequency did not change. This finding continues to support the concept that swallow frequency and swallow amplitude are independently regulated.

While swallowing is thought to be predominantly controlled by the brainstem, there is strong evidence that the spinal cord also plays an important role. Kitamura et al. utilized Gaussian frequency stimulation applied to the skin surface of the back over the ribs in a rodent model to activate sensory spinal pathways as a possible therapy to improve swallowing activity. Stimulation at the T9-T10 level significantly increased swallowing-related muscle amplitudes of the mylohyoid, thyroarytenoid, and thyropharyngeus. The mylohyoid is a laryngeal elevator muscle, while the thyropharyngeus is a pharyngeal constrictor muscle that is crucial for bolus transfer during swallowing. The thyroarytenoid is a laryngeal adductor muscle that activates during swallowing to close the airway and prevent food or liquid from entering. Hashimoto et al. sought to develop a new dysphagia model with reduced pharyngeal constriction during the pharyngeal phase of swallowing in guinea pigs. Denervation of the pharyngeal branch of the vagus nerve significantly impacted the expiratory and swallowing-related activity of the thyropharyngeus and disrupted swallow function 1 month after injury. This new experimental model could provide insight into the development of dysphagia therapies and the mechanisms associated with cranial nerve injury, reinnervation, and regeneration. The motor neurons that regulate these pharyngeal and laryngeal muscles are located within the nucleus ambiguus. Fogarty characterized the dendritic morphology of MNs and non-MNs within the compact, semi-compact, and loose formations of the nucleus ambiguus in the brainstem. These findings provide valuable insight into the inter-network connections of these neurons, which must coordinate for swallowing to function properly.

Beyond ingestion, the trigeminal nerve is responsible for both sensory and motor functions of the face, by providing motor innervation to the muscles of mastication and sensory feedback from the jaws and teeth. The mesencephalic trigeminal nucleus, a key portion of the CN V, is activated during bruxism, which is the repetitive clenching or grinding of the teeth. This behavior can occur while a person is awake or asleep and affects up to 30% of the world population. Uchima Koecklin et al. described the current understanding of the brain regions and neurotransmitters involved in bruxism, along with the associated psychological traits and clinical implications. The authors encourage continued animal and human studies on bruxism, specifically of the trigeminal system, to improve treatment approaches and understanding of this multifactorial condition.

In dental procedures, damage to branches of the trigeminal nerve (inferior alveolar nerve and lingual nerve) is often the major complication following lower jaw surgeries and third molar extractions and can lead to temporary and/or permanent numbness in the lower lip and tongue. Facial Palsy refers to weakness or paralysis of the facial muscles caused by damage to the facial nerve. Facial synkinesis is a complication that can develop after facial palsy, which refers to the involuntary, simultaneous movement of the facial muscles. These conditions can affect facial expressions, eating, drinking, speech, and eye closure, hindering daily activities and quality of life. Machetanz et al. drew attention to the lack of specialized treatment options for facial palsy and the fact that the vast number of specialists required for its treatment contributes to treatment satisfaction and better quality of life. Di Stadio et al. presented the idea of pairing physical facial nerve rehabilitation with early lower eyelid surgery to improve or prevent synkinesis. They reported that patients who underwent eyelid surgery along with physical facial nerve rehabilitation experienced faster and better recovery of facial movements and had no synkinesis even 24 months after surgery. Conversely, 37% of patients who did not undergo surgery and only participated in physical facial nerve rehabilitation developed synkinesis.

While the primary focus of craniofacial neuroscience research is to support our understanding of human populations, this knowledge is also extremely valuable in the rehabilitation of other species, including harbor seals. Thousands of infant harbor seals have been admitted to the Vancouver Aquarium's Marine Mammal Rescue Center and other rehabilitation centers worldwide. The primary cause of death in seal pups is malnutrition, and in adults it is pneumonia. Skoretz et al. described the novel ability to use clinical Videofluoroscopic Swallow Studies (VFSs) in seal pups to identify four distinct swallowing phases, which vastly expands our understanding of airway protection in independently feeding seals.

This Research Topic of articles draws attention to the wide variety of specialties associated with the 12 cranial nerves and their role in many different behaviors and conditions. Continued collaboration between basic and clinical scientists is necessary to improve our understanding of craniofacial neuroscience.

Author contributions

AH: Writing – original draft, Writing – review & editing. KK: Writing – review & editing. TP: Writing – original draft, Writing – review & editing.

Conflict of interest

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Outpatient care for facial palsy—a survey on patient satisfaction in uni- and interdisciplinary approaches

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Objective: The various causes of facial palsy, diagnostic methods and treatment approaches frequently involve different medical specialities. Nevertheless, there exist only few specialized consultation and therapy services for patients with facial palsy (FP) in Germany. The aim of the present study was to evaluate factors affecting quality of life (QoL) and treatment satisfaction of patients presenting to an interdisciplinary facial nerve outpatient clinic.

Methods: The study analyzed patients presenting to the interdisciplinary facial palsy outpatient clinic in Tuebingen between February 2019 and December 2022. General satisfaction and QoL was estimated by numerous self-rating questionnaires: ZUF-8, SF-36, FDI, FaCE, PHQ-9. An ANOVA was performed to analyze determinants affecting the ZUF-8. Correlation analyses between cause and regeneration of FP as well as questionnaire scores were performed. Results were compared with a group of patients who were managed in an unidisciplinary setting.

Results: In total, 66 patients with FP were enrolled. FP patients showed increased levels of depression (PHQ-9: 14.52 ± 3.8) correlating with recovery of the palsy ($p = 0.008$), FaCE ($p < 0.001$) and FDI ratings ($p < 0.001$). There was a high level of satisfaction with the services provided during the uni-and interdisciplinary consultation (ZUF-8: 24.59 ± 6.2), especially among the 12/66 patients who received reconstructive, surgical treatment. However, some patients requested more psychological and ophthalmological support.

Conclusion: High levels of treatment satisfaction can be achieved in both an uni-and interdisciplinary setting. However, multimodal therapy approaches should be applied, considering physical and psychological aspects. In the absence of recovery, surgical interventions must be considered as treatment options. Further studies should continue to investigate potential differences between uni-and interdisciplinary treatment.

KEYWORDS

Bell's Palsy, facial palsy, patient satisfaction, quality of life, vestibular schwannoma

Introduction

Temporary and permanent facial palsy (FP) might be induced by a broad spectrum of disorders, including stroke, tumor, trauma, iatrogenic (e.g., resection of vestibular schwannomas or tumors of the parotid gland) or idiopathic paralysis (i.e., Bell's Palsy) (1, 2). It is a debilitating and highly visible condition that can lead to physical complaints (e.g., corneal irritation, problems with drinking) and psychological problems due to appearance-affecting facial asymmetry and resulting stigmatization (3–7). In skull base surgery, the current EANO guidelines for vestibular schwannomas therefore more often recommend irradiation or—in case of large tumors—partial resection followed by irradiation to prevent FP (8, 9). However, previous studies also demonstrated that residual tumor can affect patient's quality of life (QoL) or increase the risk of recurrence (10–12). Recurrence surgery, in turn, increases the risk of FP in comparison to primary resection. Furthermore, potential recovery of the facial nerve should not be underestimated. Improvement of facial function may occur up to approximately 1.5 years after the onset of FP, and depends on, e.g., the cause and severity of the palsy as well as effectiveness of medical support (13–18). Consequently, in addition to preventing the occurrence of FP, there should be an increased effort to encourage specialized treatment options for facial rehabilitation.

FP patients are likely to have contact with numerous medical specialties and disciplines. Neurologists are often the initial patient contact in new, non-iatrogenic FP. Conservative therapy is often guided by speech therapists and physiotherapists. Neurosurgeons and otolaryngologists are involved in the treatment of iatrogenic FP after, e.g., the resection of parotid tumors or vestibular schwannomas. In case conservative treatment is not satisfactory, ophthalmologists, maxillofacial and plastic surgeons are involved, e.g., in the treatment of lagophthalmos. However, therapists and physicians are often not specialized in FP, as it is only a minor topic of their specialty. Moreover, a standardized therapy does not exist: There are numerous physiotherapeutic approaches (e.g., Bobath, Proprioceptive Neuromuscular Facilitation) (19), that often originate from extremity rehabilitation and are adapted to the face. For surgical procedures, ENT surgeons mainly use direct (facio-facial and hypoglossal-facial) extracranial reconstruction techniques, whereas neurosurgeons are more familiar with intracranial facio-facial and/or hypoglossal-facial nerve reconstruction (20). In contrast, maxillofacial and plastic surgeons mostly use masseteric-facial and cross-face reconstructions in FP (21). This unidisciplinary segregation can imply that patients (i) receive a therapy that is not the most suitable for them, but the one that their physician is familiar with, or (ii) that they have to pass through a series of medical appointments and travel long distances to get there. A previous study analyzing the health service situation for FP patients in Great Britain demonstrated that in total 10% of the patients had to travel >115 miles to receive specific FP therapy (22). In Germany, there are also only a few interdisciplinary healthcare services that are specifically established for patients with FP.

In this context, the present survey study evaluated uni-and interdisciplinary outpatient services for patients with facial palsy. A set of self-rating questionnaires—which in the past have shown high reliability in predicting generic QoL (i.e., SF-36), depression (i.e., PHQ-9), facial function-associated QoL (i.e., FaCE and FDI), and treatment satisfaction (i.e., ZUF-8) (23–27), were used to investigate factors impacting QoL and treatment satisfaction in patients with FP. The objective of these analyses is to improve future patient management of patients with FP and, thus, contribute to an improvement in facial recovery and quality of life for patients.

Methods

Patients

In this prospective study 108 patients with FP were contacted and invited to participate by answering standardized questionnaires on QoL, demographics and treatment satisfaction. All adult and native German speaking patients who were treated in the interdisciplinary facial outpatient clinic (INTER; conducted by the Department of Neurosurgery and Plastic Surgery Tuebingen) and a control group of patients who were treated due to FP at the University Hospital of Tuebingen in an unidisciplinary setting (UNI; e.g. at the Department of Neurology or Neurosurgery) were contacted. The latter were identified using a computer-based search of the hospital system (i.e., SAP) based on the ICD-10 code G.51.0 and assessment of the medical reports. Inclusion criteria were (i) age over 18 years, and (ii) facial palsy as reason for presentation to the hospital. Exclusion criteria were (i) the presence of central FP (i.e., stroke, multiple sclerosis), (ii) recurrent occurrence of FP, and (iii) documentation of manifest cognitive impairment. The study was carried out in accordance with the recommendations of the ethics committee of the Eberhard Karls University Tuebingen.

Questionnaires

Several questionnaires were completed by the participants to examine demographics, treatment satisfaction and QoL of patients with FP: a self-designed questionnaire on patient characteristics and general aspects of therapy, the adapted Client Satisfaction Questionnaire (ZUF-8) to evaluate treatment satisfaction, the general Short-Form Health Survey (SF-36) and Patient Health Questionnaire-9 (PHQ-9) to analyze general QoL and depression, and finally the Facial Disability Index (FDI) and Facial Clinimetric Evaluation (FaCE) for evaluation of facial function specific QoL.

The ZUF-8 is a German adaptation of the Client Satisfaction Questionnaire (CSQ) and evaluates subjective patient satisfaction through 8 items (28). It is often used for evaluation and quality assurance of treatments. The response options are classified on a Likert scale from 1 to 4. During the collection 4/8 items are negatively poled and have to be reversed during the evaluation, so that patients can award overall a minimum of 8 (low satisfaction) and a maximum of 32 (high satisfaction) points. In somatic health services, a cut-off value of 24 points was identified for a *"satisfactory treatment"* (29).

The SF-36 is a very common and extensively researched instrument designed to measure health-related QoL (30, 31). Grouped

Abbreviations: CFNG, cross-facial nerve grafting; ENT, ear, nose and throat surgery; FaCE, Facial Clinimetric Evaluation; FDI, Facial Disability Index; FP, facial palsy; HFGN, hypoglossal-facial nerve graft; MFNT, masseteric-facial nerve transfer; PHQ-9, Patient Health Questionnaire-9; QoL, quality of life; SF-36, Short-Form Health Survey; ZUF-8, Client Satisfaction Questionnaire.

into the two main categories "mental health" and "physical health," its 36 items can be divided into eight distinct health domains: vitality/energy (SF36-VT), social functioning (SF36-SF), role limitations due to emotional problems (SF36-RE) and mental health/psychological distress (SF36-MH), physical functioning (SF36-PF), role limitation due to physical health problems (SF36-RP), bodily pain (SF36-BP), general health perceptions (SF36-GH) (6). Each of the eight domain is scored from 0 to 100, with a lower score corresponding to a lower QoL.

The PHQ-9 consists of nine items and is a well-validated instrument for identifying and assessing the severity of depression in individuals (24). PHQ-9 items are rated based on frequency of occurrence in the past 2 weeks with 0: not at all, 1: several days, 2: more than half of the days or 3: nearly every day. Item scores are summed to a total score ranging from 0 to 27 with higher scores indicating a more severe depression.

The FDI is a patient-reported assessment tool to evaluate the impact of FP on QoL including physical limitations and emotional wellbeing (25). It contains of 10 items with response options from 1 to 5, which can be grouped into the two main categories physical function (−25 = worst to 100 = best function; FDI-PF) and social function (0 = worst to 100 = best function; FDI-SF).

The FaCE is an outcome measure consisting of 15 questions which are answered by the patient on a 5-point Likert scale (1-lowest level of function, 5-highest level of function) (32, 33). It evaluates the facial function in 6 domains: facial movement, facial comfort, eye comfort, oral function, lacrimal control and social function. Domain scores are transformed to a total score from 100 (best) to 0 (worst).

Statistics

Statistical tests were performed using SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). Group differences of clinical characteristics (e.g., sex, patient's age, distance to home) were evaluated by Chi-squared or Kruskal-Wallis tests. An univariate ANOVA was performed to analyze potential determinants affecting the ZUF-8. Correlation analyses were performed by Spearman's correlation. Data are shown as mean ± SD. Statistical significance was considered at $p < 0.05$ for each statistical test.

Results

Patient characteristics—INTER vs. UNI

A total of 108 questionnaires were submitted. Overall, 66/108 (61.1%) patients returned completed surveys, resulting in 39/66 (59.1%) questionnaires from the INTER group (response rate: 39/56, 69.6%) and 27/66 (40.9%) from the UNI group (response rate: 27/52, 51.9%) (Table 1). The mean age of the total cohort was 54.2 ± 13.5 years and did not differ between the two groups. However, there were differences in sex distribution and education level between the groups, with more women and significantly more university degrees in the INTER group than in the UNI group. The majority (21/39, 53.8%) of the INTER group had already been treated in Tuebingen and had thus become aware of the facial outpatient service, while 3/39 (7.7%) had been informed about the consultation by their neurologist or general practitioner and 5/39 (12.8%) by the Internet. However, 10/39 (25.6%) patients did not provide information about their source of information. The catchment area for the INTER consultation was significantly larger than for the UNI group (Figure 1).

The most frequent cause of FP was iatrogenic with 35/66 (53%, mainly after resection of vestibular schwannomas), followed by idiopathic FP (19/66, 28.1%). Less frequently infections (4/66, 6.1%) or a tumor itself were the cause of FP (5/66, 7.6%). However, the INTER and UNI groups differed significantly in the distribution of causes. While idiopathic causes were much more frequent in the UNI group, facial palsy of iatrogenic cause was leading in the INTER group (25/39, 64.1%), due to the also frequent resections of vestibular schwannomas performed in the local neurosurgical department. In *VS* patients, large *VS* (Koos 3 and 4: 36/40, 90%) clearly exceeded small tumors (Koos 2: 4/40, 10%). In addition, differences in the duration and recovery of FP emerged. Although patients in the INTER group had FP for longer on average, recovery was significantly worse than in the UNI group. Patients who were treated in the interdisciplinary consultation received significantly more often a surgical treatment regarding the facial palsy and more often botulinum toxin injections.

Satisfaction with treatment did not differ between the groups (Figure 2). A total of 18.1% of patients were dissatisfied with the local physiotherapeutic/logopedic therapy. Similarly, 11/66 (16.7%; 7/39 INTER and 4/27 UNI) stated to be dissatisfied with the care at the university hospital. The ZUF-8 correlated significantly with the FDI-PF ($r = 0.34$, $p = 0.011$, Spearman's), i.e., physical impairment due to FP. A correlation with patient age, degree of regression of the paresis, FaCE, FDI-SF or PHQ-9 could not be detected. However, an ANOVA revealed none of the above factors as predictive for the ZUF-8, including the FDI-PF. Also, satisfaction with local physiotherapeutic services did not correlate with the treatment satisfaction in Tuebingen measured by the ZUF-8 ($r = 0.15$, $p = 0.292$, Spearman's). 3/11 patients (27.3%) were dissatisfied with the therapy in Tuebingen mentioned that they would have appreciated additional psychotherapy, 3/11 (27.3%) would have welcomed ophthalmologic care, and 7/11 (63.6%) gave other reasons (e.g., more information). Dissatisfied patients in the UNI group stated that they had only become aware of the facial outpatient service through this study and would have liked to have known about it earlier. Furthermore, some of the patients expressed the wish for better instructions for the training at home.

There were no differences between the groups in QoL and level of depression as measured by the SF-36 and PHQ-9. However, an overall mild to moderate depression was observed in patients with FP (Figure 3). The degree of depression correlated with the FDI-PF ($r = -0.45$, $p < 0.001$), the FDI-SF ($r = -0.70$, $p < 0.001$), the FaCE ($= -0.50$, $p < 0.001$) and the degree of regression of the paresis ($r = 0.33$, $p = 0.008$). In the group of *VS* patients, no correlation was found between QoL or depression scores and the size of VS.

Idiopathic vs. iatrogen

Comparing patients with idiopathic and iatrogen FP, we found a significant difference for sex, distance to home, FP improvement, FaCE, and the frequency of FP-related surgeries and botulinum toxin injections. Notably, patients with idiopathic FP were more likely to

TABLE 1 Patient characteristics.

	Total	INTER	UNI		Idiopathic	Iatrogen	
	n = 66	*n* = 39	*n* = 27		*N* = 19	*N* = 35	
Sex							
Male	23/66 (34.8%)	8 (20.5%)	15 (55.6%)	$X^2 = 8.63$	10 (52.6%)	9 (25.7%)	$X^2 = 3.91$
Female	43/66 (65.2%)	31 (79.5%)	12 (44.4%)	***p* = 0.003***	9 (47.4%)	26 (74.3%)	***p* = 0.048***
Age	54.2 ± 13.5	52.7 ± 12.4	56.3 ± 14.6	$p = 0.418$	58.63 ± 14.5	54.66 ± 11.5	$p = 0.441$
Marital status							
Single	8 (12.1%)	4 (10.3%)	4 (14.8%)	$X^2 = 1.90$	1 (5.3%)	4 (11.4%)	$X^2 = 2.55$
Married/ partnership	50 (75.8%)	31 (79.5%)	19 (70.4%)	$p = 0.592$	14 (73.7%)	27 (77.1%)	$p = 0.467$
Divorced	7 (10.6%)	4 (10.3%)	3 (11.1%)		3 (15.8%)	4 (11.4%)	
Widowed	1 (1.5%)	0 (0%)	1 (3.7%)		1 (5.3%)	0 (0%)	
Education							
Secondary school[#]	13 (19.7%)	7 (17.9%)	6 (22.2%)	$X^2 = 12.32$	3 (15.8%)	7 (20.0%)	$X^2 = 8.70$
High school[##]	19 (28.8%)	7 (17.9%)	12 (44.4%)	***p* = 0.015***	10 (52.6%)	7 (20.0%)	$p = 0.069$
A-level	11 (16.7%)	5 (12.8%)	6 (22.2%)		4 (21.1%)	6 (17.1%)	
University	17 (25.8%)	15 (38.5%)	2 (7.4%)		2 (10.5%)	12 (34.3%)	
Other	6 (9.0%)	5 (12.9%)	1 (3.7%)		0 (0%)	3 (8.6%)	
Distance to home (km)	151.9 ± 175.8	189.5 ± 182.4	97.5 ± 153.1	$H = 9.96$	39.6 ± 54.0	207.8 ± 185.0	$H = 17.00$
				p* = 0.002			***p* < 0.001***
VS surgery							
No	26 (39.4%)	11 (28.2%)	15 (55.6%)	$X^2 = 4.99$			
Yes	40 (60.6%)	28 (71.8%)	12 (44.4%)	***p* = 0.025***			
Outpatient clinic							
INTER					3 (15.8%)	25 (71.4%)	$X^2 = 15.27$
UNI					16 (84.2%)	10 (28.6%)	***p* < 0.001***
FP side							
Left	29 (43.9%)	20 (51.3%)	9 (33.3%)	$X^2 = 3.21$	5 (26.3%)	18 (51.4%)	$X^2 = 3.18$
Right	36 (54.5%)	19 (48.7%)	17 (63%)	$p = 0.201$	14 (73.7%)	17 (48.6%)	$p = 0.204$
Bilateral	1 (1.5%)	0 (0%)	1 (3.7%)		0 (0%)	0 (0%)	
FP cause							
Idiopathic	19 (28.8%)	3 (7.7%)	16 (59.3%)	$X^2 = 22.90$			
Infection	4 (6.1%)	3 (7.7%)	1 (3.7%)	***p* < 0.001***			
Tumor	5 (7.6%)	5 (12.8%)	0 (0%)				
Iatrogen	35 (53.0%)	25 (64.1%)	10 (37.0%)				
Other	3 (4.5%)	3 (7.7%)	0 (0%)				
FP duration							
≤1 year	20 (30.3%)	3 (7.7%)	17 (63.0%)	$X^2 = 34.05$	9 (47.4%)	9 (25.7%)	$X^2 = 8.41$
1–2 years	12 (18.2%)	5 (12.8%)	7 (25.9%)	***p* < 0.001***	5 (26.3%)	4 (11.4%)	$p = 0.135$
2–3 years	10 (15.2%)	10 (25.6%)	0 (0%)		1 (5.3%)	7 (20.0%)	
3–4 years	5 (7.6%)	5 (12.8%)	0 (0%)		0 (0%)	4 (11.4%)	
4–5 years	6 (9.1%)	4 (10.3%)	2 (7.4%)		2 (10.5%)	3 (8.6%)	
>5 years	13 (19.7%)	12 (30.8%)	1 (3.7%)		2 (10.5%)	8 (22.9%)	
FP improvement							
100%		0 (0%)	10 (37.0%)	$X^2 = 24.77$	8 (42.1%)	1 (2.9%)	$X^2 = 16.38$
>75%		8 (20.5%)	10 (37.0%)	***p* < 0.001***	6 (31.6%)	10 (28.6%)	***p* = 0.006***

(Continued)

TABLE 1 (Continued)

	Total	INTER	UNI		Idiopathic	Iatrogen	
	n = 66	*n* = 39	*n* = 27		*N* = 19	*N* = 35	
50-75%		10 (25.6%)	1 (3.7%)		1 (5.3%)	7 (20.0%)	
<50%		13 (33.3%)	4 (14.8%)		2 (10.5%)	13 (37.1%)	
No improvement		7 (17.9%)	2 (7.4%)		2 (10.5%)	4 (11.4%)	
Missing data		1 (2.6%)	0 (0%)		0 (0%)	0 (0%)	
FDI							
Physical function	66.67 ± 19.3	66.92 ± 17.0	66.30 ± 23.0	$p = 0.906$	70.0 ± 25.3	68.71 ± 14.4	$p = 0.343$
Social function	70.61 ± 20.6	69.44 ± 22.1	72.30 ± 18.5	$p = 0.734$	74.74 ± 17.7	70.63 ± 19.9	$p = 0.450$
FaCE	62.50 ± 24.8	57.07 ± 21.5	70.29 ± 27.4	**$p = 0.023$***	74.17 ± 27.6	60.75 ± 20.3	**$p = 0.031$***
SF36							
Physical function	78.91 ± 27.3	81.84 ± 23.6	74.62 ± 32.0	$p = 0.537$	79.72 ± 30.8	81.03 ± 22.9	$p = 0.695$
Role physical	67.58 ± 41.0	72.37 ± 39.3	60.58 ± 43.1	$p = 0.247$	69.44 ± 37.9	63.97 ± 43.2	$p = 0.975$
Bodily pain	73.02 ± 30.8	73.84 ± 28.9	71.81 ± 34.0	$p = 0.965$	80.11 ± 25.7	69.65 ±32.8	$p = 0.289$
General health	61.44 ± 21.3	62.08 ± 21.1	60.50 ± 21.9	$p = 0.763$	64.72 ± 15.9	62.00 ± 22.7	$p = 0.862$
Vitality	54.45 ± 18.5	54.21 ± 18.6	54.81 ± 18.7	$p = 0.885$	55.00 ± 19.1	53.53 ± 18.5	$p = 0.780$
Social function	76.95 ± 29.0	75.99 ± 26.2	78.37 ± 33.1	$p = 0.306$	81.25 ± 30.7	75.74 ± 26.8	$p = 0.228$
Role emotional	74.48 ± 38.8	71.05 ± 41.1	79.49 ± 35.4	$p = 0.491$	74.07 ± 38.9	73.53 ± 39.2	$p = 0.911$
Mental health	68.94 ± 19.4	67.47 ± 20.4	71.08 ± 18.0	$p = 0.519$	73.33 ± 18.0	67.41 ± 18.8	$p = 0.243$
PHQ-9	14.52 ± 3.8	14.39 ± 3.6	14.70 ± 4.1	$p = 0.904$	13.68 ± 3.0	14.62 ± 4.1	$p = 0.456$
ZUF-8	24.59 ± 6.2	24.09 ± 6.1	25.38 ± 6.29	$p = 0.383$	24.53 ± 6.7	24.41 ± 6.6	$p = 0.971$
Satisfied with local physical therapy							
Definitely not actually	3 (4.5%)	2 (5.1%)	1 (3.7%)	$X^2 = 1.84$	1 (5.3%)	1 (2.9%)	$X^2 = 4.18$
Not	9 (13.6%)	6 (15.4%)	3 (11.1%)	$p = 0.765$	2 (10.5%)	5 (14.3%)	$p = 0.382$
In general yes definitely	26 (39.4%)	14 (35.9%)	12 (44.4%)		6 (31.6%)	18 (51.4%)	
Yes	21 (31.8%)	14 (35.9%)	7 (25.9%)		6 (31.6%)	9 (25.7%)	
No statement	7 (10.6%)	3 (7.7%)	4 (14.8%)		4 (21.1%)	2 (5.7%)	
FP surgery							
No	53 (80.3%)	27 (69.2%)	26 (96.3%)	$X^2 = 7.39$	19 (100%)	25 (71.4%)	$X^2 = 6.66$
Yes	13 (19.7%)	12 (30.8%)	1 (3.7%)	**$p = 0.006$***	0 (0%)	10 (28.6%)	**$p = 0.009$***
Botulinum toxin							
No	51 (77.3%)	26 (66.7%)	25 (92.6%)	$X^2 = 13.27$	16 (84.2%)	26 (74.3%)	$X^2 = 6.62$
Yes	13 (19.7%)	13 (33.3%)	0 (0%)	**$p = 0.001$***	1 (5.3%)	9 (25.7%)	**$p = 0.036$***
Missing data	2 (3.0%)	0 (0%)	2 (7.4%)		2 (10.5%)	0 (0%)	
Conservative therapy							
Physical therapy	11 (16.7%)	4 (10.3%)	7 (25.9%)	$X^2 = 8.48$	7 (36.8%)	2 (5.7%)	$X^2 = 18.50$
Logopedics	17 (25.8%)	13 (33.3%)	4 (14.8%)	$p = 0.132$	2 (10.5%)	10 (28.6%)	**$p = 0.002$***
Occupational therapy	1 (1.5%)	1 (2.6%)	0 (0%)		0 (0%)	0 (0%)	
No therapy	6 (9.1%)	2 (5.1%)	4 (14.8%)		4 (21.1%)	1 (2.9%)	
Several methods	30 (45.5%)	19 (48.7%)	11 (40.7%)		5 (26.3%)	22 (62.9%)	
Unknown	1 (1.5%)	0 (0%)	1 (3.7%)		1 (5.3%)	0 (0%)	

HB, House Brackmann score; FaCE, Facial Clinimetric Evaluation; FDI, facial disability index; FP, facial palsy; PHQ-9, Patient Health Questionnaire-9; SF-36, Short-Form Health Survey; VS, vestibular schwannoma; #german Hauptschule, ##german; Realschule; *p-values indicate significance comparing pre- and postoperative patients by a Chi-square (X^2) or Kruskal-Wallis test (H). Bold values are significant p-values.

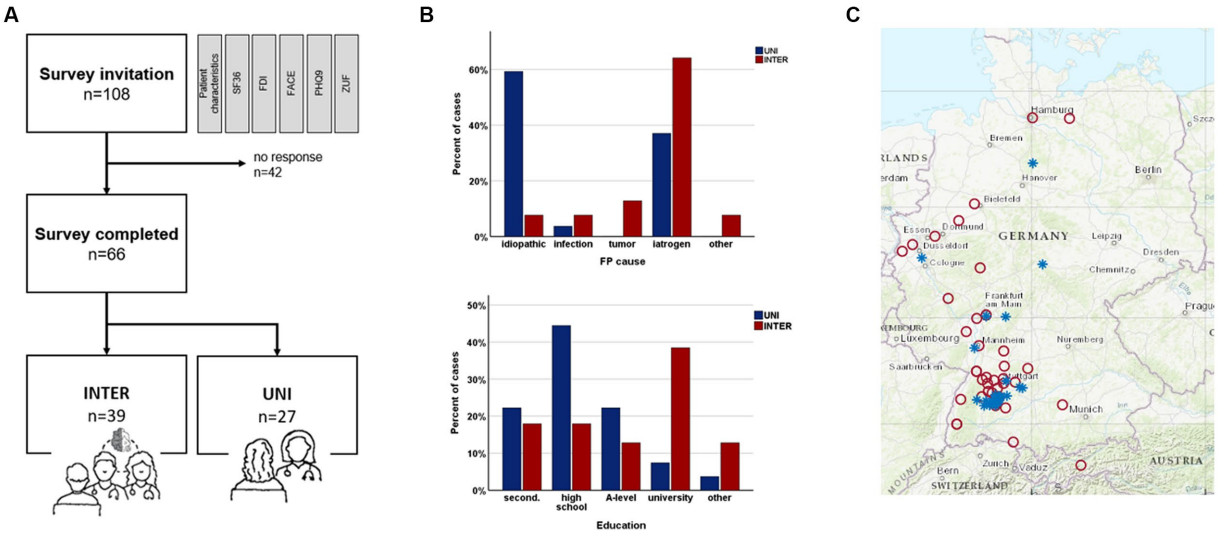

FIGURE 1
Overview about differences between INTER und UNI. **(A)** Flow chart about the surveys and the number of patients included into the groups. **(B)** Group differences of education level and cause of facial palsy. **(C)** Distribution of patients' places of residence; blue, UNI; red, INTER.

come from the immediate vicinity of the university hospital, whereas patients with iatrogenic causes lived much more distantly. In contrast to the UNI/INTER comparison, there was no difference between the groups in the level of education or the duration of FP. However, analyses indicated a group difference in conservative therapies (e.g., logopedics) that was not found in the UNI/INTER comparison. Patients with iatrogenic FP were significantly more likely to use multiple therapies than patients with idiopathic FP.

Reconstructive surgeries in facial palsy

A total of 13/66 (19.7%, 11 female) patients received surgical intervention due to FP, although one patient underwent an external procedure and did not provide any information about the procedure when surveyed. Therefore, this patient is not listed in Table 2 and will not be included in further analysis.

The most common procedure among patients was implantation of a lid chain (9/12, 75%) (Table 2). Nerve reconstructive procedures included masseteric facial nerve transfer (MFNT; 4/12, 33.3%) most frequently, followed by cross-facial nerve grafting (CFNG; 3/12, 25%). Only one patient (8.3%)—who underwent external surgery—received reconstruction using a hypoglossal-facial nerve anastomosis (HFGN) in combination with CFNG. Overall, 11/12 (91.7%) patients were satisfied or very satisfied with the outcome of the procedure. Only one patient (8.3%) was dissatisfied after lid loading, as she developed a temporary hypospagma.

Discussion

The occurrence of facial palsy is an impactful life event compromising patients' mental and physical quality of life (4–6, 34). Treatment objectives should provide patients with the most holistic care possible, exhausting the full regenerative potential of the facial nerve. However, while many medical disciplines get in touch with FP patients, the disorder *"facial palsy"* usually represents only a minor topic in which only a few therapists specialize. This leads to an inadequate supply and possibly unspecific therapy (22). The present study examined treatment satisfaction and QoL in patients with FP in uni-and interdisciplinary treatment settings.

While overall satisfaction with FP treatment reached the ZUF-8 cut-off level of 24 points (29), no significant difference of satisfaction was found between UNI and INTER. This is contrary to the expectation that an interdisciplinary approach results in higher level of satisfaction (35). However, several factors may contribute to this discrepancy and suggest that interdisciplinary care could nevertheless preferable for the patient (20, 35, 36): (i) We found a significant difference between UNI and INTER regarding the cause of FP, duration and degree of recovery. While the UNI group exhibited a proportion of causes similar to that described in the literature (2), iatrogenic FP causes were overrepresented in the INTER group. This can be attributed to the direct affiliation of the interdisciplinary facial outpatient service with the Department of Neurosurgery in Tuebingen, which is a center for vestibular schwannomas. However, previous studies have shown that idiopathic FP generally recover better than palsies of iatrogenic origin and that improvement and chances for reinnervation may differ (14, 15), which could affect QoL and patient satisfaction. Vicente-Ruiz and Hontanilla (37) found that a longer duration of FP prior to reconstructive treatment was associated with decreased treatment satisfaction. Accordingly, it should further be evaluated whether different types of care are required for different causes of FP or whether interdisciplinary care should be offered in a standardized manner, e.g., after a certain period of time when the paresis has not improved (38, 39); (ii) While the overall response rate of the study was in good average compared to other questionnaire studies, there was a significantly better response rate in the INTER compared to the UNI group. This may be an indirect sign of patient satisfaction. However, a higher response rate due to the patient's relationship with the researcher (KM), who also attends the

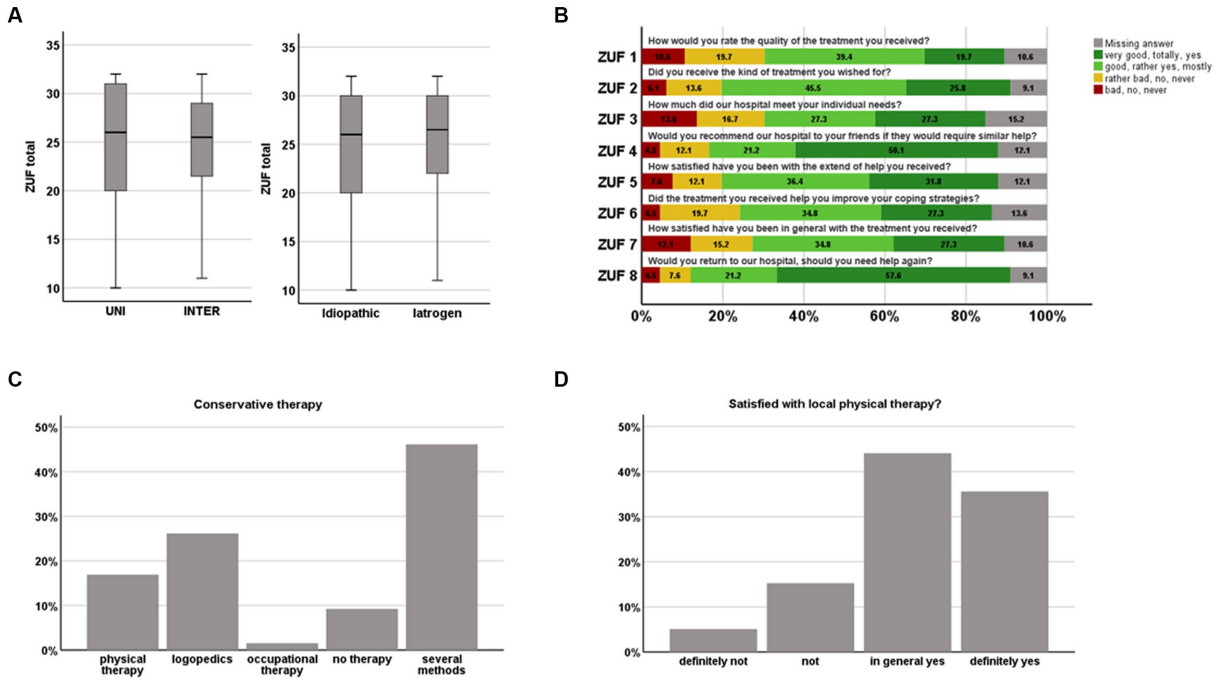

FIGURE 2
Patients satisfaction with facial palsy treatment. **(A,B)** Treatment in Tuebingen measured by the ZUF-8; and **(C,D)** of local physiotherapeutic/logopedic services.

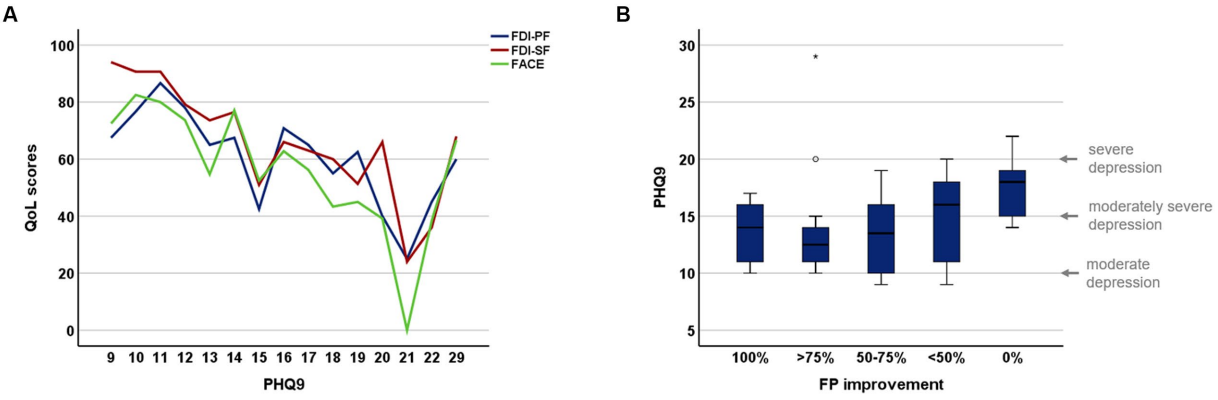

FIGURE 3
Depression scores in patients with facial palsy. **(A)** Correlation between PHQ-9 and facial specific QoL scores; **(B)** relation between PHQ-9 and facial palsy improvement.

interdisciplinary consultation, cannot be completely excluded (40); (iii) The distance to home was larger in the INTER group, suggesting that patients were willing to travel a greater distance for this treatment; (iv) Patients in the INTER group who were not completely satisfied with the treatment did not indicate a preference for an UNI setting, but wished the involvement of other disciplines (especially psychological and ophthalmological care). On the contrary, dissatisfied patients in the UNI group emphasized that they would have liked to have known about the option of specialized interdisciplinary consultation earlier.

Interdisciplinary treatment in this context offers the chance to treat a patient more holistically, as unidisciplinary treatment result in a narrower perspective on patient care. This has already been demonstrated in numerous oncological studies (41). With regard to the postoperative treatment of vestibular schwannomas, e.g., the control of the tumor as well as the treatment of FP can be performed simultaneously. Neurosurgeons are principally familiar with nerve surgery through the specialty of peripheral nerve surgery. However, the expertise in the area of the face is low. While HFGN are still performed, MFGN and CFNG are less common (20). Furthermore, there is often a lack of expertise in static procedures. Seeberger et al. (42) demonstrated in a nationwide study how often various types of procedures were preferred by different disciplines in FP. Interventions in the area of the mouth or eye were rarely performed by

TABLE 2 Plastic and reconstructive surgeries of the study cohort.

	Procedure											
	CFNG (VII-VII)	HFGN (XII-VII)	MFGN (V-VII)	Lid chain	Static methods	Muscle transfer	Satisfaction	FDI-PF	FDI-SF	FACE	ZUF-8	PHQ9
1								65	52	50	28	15
2								75	84	56.7	11	12
3								70	48	41.7	24	19
4								55	32	16.7	21	19
5								70	76	68.3	25	16
6								75	84	68.3	30	13
7								60	92	80	24	9
8								50	32	31.67	24	19
9								65	20	-	-	18
10								95	96	85	31	17
11								45	56	23.3	19	16
12								55	60	43.3	31	18
Total	3	1	4	9	4	1		65.0 ± 13.5	61.0 ± 25.4	51.4 ± 22.6	24.2 ± 6.0	15.9 ± 3.2

CFNG, cross-facial nerve grafting; FDI-PF, Facial disability index physical function; FDI-SF, Facial disability index social function; HFGN, hypoglossal-facial nerve graft; MFGN, masseteric facial nerve transfer; dark green, very satisfied with procedure, green, satisfied with procedure, yellow, not satisfied with procedure.

neurosurgeons. Through a joint consultation, the inhibition threshold in patients for plastic or maxillofacial surgery treatment can be lowered. While many patients may feel ashamed or embarrassed about seeking help from a plastic surgeon or there is a general societal stigma associated with cosmetic surgery, treatment by a "nerve surgeon" is more recognized; also by the health insurance companies, which are more often reluctant to cover costs of plastic surgery treatment although plastic surgery can be a valuable tool for improving the QoL of patients with FP, helping to restore their confidence and self-esteem (43, 44). In this context, our results show that significantly more patients in the INTER group received surgical treatment or injection with botulinum toxin compared with the UNI group. Almost all of these patients were satisfied with the surgical intervention. Similarly, Bradbury et al. found high levels of patient satisfaction after reconstructive surgery in FP (45).

Our results indicated that PHQ-9 scores were elevated in FP compared with the average normal population (46). In this context, previous studies showed that not only physical but also mental QoL was impaired. An U.S. study showed that patients with FP had depression in 47.7%, while only 5.1% of the control group had depression. Further investigations confirm considerable psychological distress in patients with facial palsy (47, 48). Though, there is disagreement whether the degree of FP determines the extent of QoL or depression (4, 5, 7, 48). While some studies have failed to demonstrate a correlation between these factors, other studies have shown a significant correlation, consistent with our findings. Regardless of this, psychological/psychiatric co-care of FP patients seems to be useful and was explicitly requested by patients. The effect of psychological counseling of FP patients to improve QoL and mental health has rarely been systematically investigated. However, a pilot study by Siemann et al. with a small number of cases suggests a positive effect of psychological counseling (49). Moreover, a significant reduction in anxiety and depression has been demonstrated as a result of psychological co-care in other medical conditions with a high psychosocial burden, such as oncological diseases (50, 51). Corresponding studies should also be performed for patients with FP in the future.

Limitations

While the present study provides valuable insights into the outpatient treatment of FP patients, it is tempered by the single-center design which compromises generalizability of the results. A small cohort size reduces statistical power, precludes subgroup analyses and, therefore, reduces the informative value. The results may be influenced by the fact that only 66/108 patients participated in the survey and that the questionnaires were completed by the participants at different times after the therapy, so that there may be a recall bias. There is no information available on the extent to which the patients received psychological support (e.g., from relatives or friends) and whether antidepressant medication was taken. Finally, the absence of a matched UNI control group limits group comparability. In the future, these limitations should be considered in multi-center studies and should also take into account the composition of interdisciplinary teams.

Conclusion

Treatment satisfaction of patients with FP depends on multifactorial aspects, whereby the present study could not demonstrate a difference in satisfaction between uni-and interdisciplinary consulting. However, in addition to treatment of

physical impairments, psychological support should be provided. Surgical interventions should not be underestimated as potential treatment options in the absence of recovery, as they can improve quality of life and treatment satisfaction. Finally, the aspect of uni-and interdisciplinarity should be verified in future studies by addressing described limitations and using more homogeneous study groups.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors on reasonable request without undue reservation.

Ethics statement

The studies involving humans were approved by ethical board of the University Hospital Tuebingen. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KM: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft. LO: Data curation, Investigation, Methodology, Writing – review & editing. SW: Data curation, Writing – review & editing. EW: Data curation, Writing – review & editing. MG: Data curation, Writing – review & editing. HL: Methodology, Writing – review & editing. AD: Conceptualization, Resources, Writing – review & editing. MT: Conceptualization, Resources, Writing – review & editing. GN: Conceptualization, Resources, Writing – review & editing. RS: Conceptualization, Data curation, Investigation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Hohman MH, Hadlock TA. Etiology, diagnosis, and management of facial palsy: 2000 patients at a facial nerve center. *Laryngoscope*. 124:E283–93. doi: 10.1002/lary.24542

2. Palsy Facial: *Techniques for reanimation of the paralyzed face: Tzou, Chieh-Han John, Rodríguez-Lorenzo, Andrés: Amazon.de: Bücher*. Available at: https://www.amazon.de/-/en/Chieh-Han-John-Tzou/dp/3030507831 (Accessed June 4, 2023).

3. Hotton M, Huggons E, Hamlet C, Shore D, Johnson D, Norris JH, et al. The psychosocial impact of facial palsy: a systematic review. *Br J Health Psychol*. (2020) 25:695–727. doi: 10.1111/bjhp.12440

4. Nellis JC, Ishii M, Byrne PJ, Boahene KDO, Dey JK, Ishii LE, et al. Association among facial paralysis, depression, and quality of life in facial plastic surgery patients. *JAMA Facial Plast Surg*. (2017) 19:190–6. doi: 10.1001/jamafacial.2016.1462

5. Verhoeff R, Bruins TE, Ingels KJAO, Werker PMN, van Veen MM. A cross-sectional analysis of facial palsy-related quality of life in 125 patients: comparing linear, quadratic and cubic regression analyses. *Clin Otolaryngol*. (2022) 47:541–5. doi: 10.1111/coa.13934

6. Machetanz K, Lee L, Wang SS, Tatagiba M, Naros G. Trading mental and physical health in vestibular schwannoma treatment decision. *Front Oncol*. (2023) 13:1152833. doi: 10.3389/fonc.2023.1152833

7. Cross T, Sheard CE, Garrud P, Nikolopoulos TP, O'Donoghue GM. Impact of facial paralysis on patients with acoustic neuroma. *Laryngoscope*. (2000) 110:1539–42. doi: 10.1097/00005537-200009000-00024

8. Goldbrunner R, Weller M, Regis J, Lund-Johansen M, Stavrinou P, Reuss D, et al. Eano guideline on the diagnosis and treatment of vestibular schwannoma. *Neurooncology*. (2020) 22:31–45. doi: 10.1093/neuonc/noz153

9. Kondziolka D, Mousavi SH, Kano H, Flickinger JC, Lunsford LD. The newly diagnosed vestibular schwannoma: radiosurgery, resection, or observation? *Neurosurg Focus*. (2012) 33:E8. doi: 10.3171/2012.6.FOCUS12192

10. Wang SSY, Machetanz K, Ebner F, Naros G, Tatagiba M. Association of extent of resection on recurrence-free survival and functional outcome in vestibular schwannoma of the elderly. *Front Oncol*. (2023) 13:1153698. doi: 10.3389/fonc.2023.1153698

11. Vakilian S, Souhami L, Melançon D, Zeitouni A. Volumetric measurement of vestibular schwannoma tumour growth following partial resection: predictors for recurrence. *J Neurol Surgery B Skull Base*. (2013) 73:117–20. doi: 10.1055/s-0032-1301395

12. Link MJ, Lund-Johansen M, Lohse CM, Driscoll CLW, Myrseth E, Tveiten OV, et al. Quality of life in patients with vestibular schwannomas following gross total or less than gross total microsurgical resection: should we be taking the entire tumor out? *Clin Neurosurg*. (2018) 82:541–7. doi: 10.1093/neuros/nyx245

13. Troude L, Boucekine M, Montava M, Lavieille JP, Régis JM, Roche PH. Predictive factors of early postoperative and long-term facial nerve function after large vestibular schwannoma surgery. *World Neurosurg*. (2019) 127:e599–608. doi: 10.1016/j.wneu.2019.03.218

14. Urban E, Volk GF, Geißler K, Thielker J, Dittberner A, Klingner C, et al. Prognostic factors for the outcome of Bells' Palsy: a cohort register-based study. *Clin Otolaryngol*. (2020) 45:754–61. doi: 10.1111/coa.13571

15. Hayler R, Clark J, Croxson G, Coulson S, Hussain G, Ngo Q, et al. Sydney facial nerve clinic: experience of a multidisciplinary team. *ANZ J Surg*. (2020) 90:856–60. doi: 10.1111/ans.15782

16. Rivas A, Boahene KD, Bravo HC, Tan M, Tamargo RJ, Francis HW. A model for early prediction of facial nerve recovery after vestibular schwannoma surgery. *Otol Neurotol*. (2011) 32:826–33. doi: 10.1097/MAO.0b013e31821b0afd

17. Adegbite AB, Khan MI, Tan L. Predicting recovery of facial nerve function following injury from a basilar skull fracture. *J Neurosurg*. (1991) 75:759–62. doi: 10.3171/jns.1991.75.5.0759

18. Volk GF, Klingner C, Finkensieper M, Witte OW, Guntinas-Lichius O. Prognostication of recovery time after acute peripheral facial palsy: a prospective cohort study. *BMJ Open*. (2013) 3:e003007. doi: 10.1136/bmjopen-2013-003007

19. Silva MC, Oliveira MT, Azevedo-Santos IF, DeSantana JM. Effect of proprioceptive neuromuscular facilitation in the treatment of dysfunctions in facial paralysis: a systematic literature review. *Braz J Phys Ther*. (2022) 26:100454. doi: 10.1016/j.bjpt.2022.100454

20. Lassaletta L, Morales-Puebla JM, González-Otero T, Moraleda S, Roda JM, Gavilán J. The experience of a facial nerve unit in the treatment of patients with facial paralysis following skull base surgery. *Otol Neurotol*. (2020) 41:E1340–9. doi: 10.1097/MAO.0000000000002902

21. Aronson S, Applebaum SA, Kelsey LJ, Gosain AK. Evidence-based practices in facial reanimation surgery. *Plast Reconstr Surg*. (2023) 152:520e–33e. doi: 10.1097/PRS.0000000000010539

22. Szczepura A, Holliday N, Neville C, Johnson K, Khan Khan AJ, Oxford SW, et al. Raising the digital profile of facial palsy: national surveys of patients' and clinicians' experiences of changing UK treatment pathways and views on the future role of digital technology. *J Med Internet Res*. (2020) 22:e20406. doi: 10.2196/20406

23. Györi E, Przestrzelski C, Pona I, Hagmann M, Rath T, Radtke C, et al. Quality of life and functional assessment of facial palsy patients: a questionnaire study. *Int J Surg*. (2018) 55:92–7. doi: 10.1016/j.ijsu.2018.04.061

24. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. (2001) 16:606–13. doi: 10.1046/j.1525-1497.2001.016009606.x

25. Vanswearingen JM, Brach JS. The facial disability index: reliability and validity of a disability assessment instrument for disorders of the facial neuromuscular system. *Phys Ther*. (1996) 76:1288–98. doi: 10.1093/ptj/76.12.1288

26. Sun Y, Kong Z, Song Y, Liu J, Wang X. The validity and reliability of the PHQ-9 on screening of depression in neurology: a cross sectional study. *BMC Psychiatry*. (2022) 22:98. doi: 10.1186/s12888-021-03661-w

27. Brazier JE, Harper R, Jones NMB, O'Cathain A, Thomas KJ, Usherwood T, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *Br Med J*. (1992) 305:160–4. doi: 10.1136/bmj.305.6846.160

28. Schmidt J, Lamprecht F, Wittmann WW. Zufriedenheit Mit Der Stationaren Versorgung. Entwicklung Eines Fragebogens Und Erste Validitatsuntersuchungen. *Psychother Psychosom Med Psychol*. (1989) 39:248–55.

29. Kriz D, Nübling R, Steffanowski A, Wittmann WW, Schmidt J. Patientenzufriedenheit in der stationären rehabilitation: psychometrische Reanalyse des ZUF-8 auf der Basis multizentrischer Stichproben verschiedener Indikation. *Z Med Psychol*. (2008) 17:67–79.

30. Tarlov AR. The medical outcomes study: an application of methods for monitoring the results of medical care. *JAMA J Am Med Assoc*. (1989) 262:925–30. doi: 10.1001/jama.262.7.925

31. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (sf-36): I. Conceptual framework and item selection. *Med Care*. (1992) 30:473–83. doi: 10.1097/00005650-199206000-00002

32. Kahn JB, Gliklich RE, Boyev KP, Stewart MG, Metson RB, McKenna MJ. Validation of a patient-graded instrument for facial nerve paralysis: the FaCE scale. *Laryngoscope*. (2001) 111:387–98. doi: 10.1097/00005537-200103000-00005

33. Volk GF, Steigerwald F, Vitek P, Finkensieper M, Kreysa H, Guntinas-Lichius O. Facial disability index and facial clinimetric evaluation scale: validation of the German versions. *Laryngorhinootologie*. (2015) 94:163–8. doi: 10.1055/s-0034-1381999

34. Vargo M, Ding P, Sacco M, Duggal R, Genther DJ, Ciolek PJ, et al. The psychological and psychosocial effects of facial paralysis: a review. *J Plast Reconstr Aesthetic Surg*. (2023) 83:423–30. doi: 10.1016/j.bjps.2023.05.027

35. Butler DP, Grobbelaar AO. Facial palsy: what can the multidisciplinary team do? *J Multidiscip Healthc*. (2017) 10:377–81. doi: 10.2147/JMDH.S125574

36. Steinhäuser J, Volk GF, Thielker J, Geitner M, Kuttenreich AM, Klingner CM, et al. Multidisciplinary Care of Patients with facial Palsy: treatment of 1220 patients in a German facial nerve Center. *J Clin Med*. (2022) 11:427. doi: 10.3390/jcm11020427

37. Vicente-Ruiz M, Hontanilla B. Measuring patient-reported outcomes after facial paralysis reconstruction surgery using the FACE-Q. *Facial Plast Surg Aesthetic Med*. (2023). doi: 10.1089/fpsam.2023.0022

38. Glass GE, Tzafetta K. Optimising treatment of Bell's Palsy in primary care: the need for early appropriate referral. *Br J Gen Pract*. (2014) 64:e807–9. doi: 10.3399/bjgp14X683041

39. Butler DP, Morales DR, Johnson K, Nduka C In: C-HJ Tzou and A Rodríguez-Lorenzo, editors. *Facial palsy*. Cham: Royal College of General Practitioners (2019)

40. Holtom B, Baruch Y, Aguinis H, Ballinger GA. Survey response rates: trends and a validity assessment framework. *Hum Relat*. (2022) 75:1560–84. doi: 10.1177/00187267211070769

41. Pillay B, Wootten AC, Crowe H, Corcoran N, Tran B, Bowden P, et al. The impact of multidisciplinary team meetings on patient assessment, management and outcomes in oncology settings: a systematic review of the literature. *Cancer Treat Rev*. (2016) 42:56–72. doi: 10.1016/j.ctrv.2015.11.007

42. Seeberger S, Schlattmann P, Guntinas-Lichius O. Surgery for patients with facial palsy in Germany: a diagnosis-related-groups-based nationwide analysis, 2005–2019. *Eur Arch Otorhinolaryngol*. (2023) 281:451–9. doi: 10.1007/s00405-023-08259-4

43. Díaz-Aristizabal U, Valdés-Vilches M, Fernández-Ferreras TR, Calero-Muñoz E, Bienzobas-Allué E, Aguilera-Ballester L, et al. Effect of botulinum toxin type a in functionality, synkinesis and quality of life in peripheral facial palsy sequelae. *Neurologia*. (2021) 38:560–5. doi: 10.1016/j.nrl.2021.01.015

44. Liu RH, Yau J, Derakhshan A, Xiao R, Hadlock TA, Lee LN. Facial filler in facial paralysis: a prospective case study and multidimensional assessment. *Facial Plast Surg Aesthetic Med*. (2023). doi: 10.1089/fpsam.2023.0048

45. Bradbury ET, Simons W, Sanders R. Psychological and social factors in reconstructive surgery for hemi-facial palsy. *J Plast Reconstr Aesthetic Surg*. (2006) 59:272–8. doi: 10.1016/j.bjps.2005.09.003

46. Tomitaka S, Kawasaki Y, Ide K, Akutagawa M, Ono Y, Furukawa TA. Stability of the distribution of patient health Questionnaire-9 scores against age in the general population: data from the National Health and nutrition examination survey. *Front Psych*. (2018) 9:390. doi: 10.3389/fpsyt.2018.00390

47. Pouwels S, Beurskens CHG, Kleiss IJ, Ingels KJAO. Assessing psychological distress in patients with facial paralysis using the hospital anxiety and depression scale. *J Plast Reconstr Aesthetic Surg*. (2016) 69:1066–71. doi: 10.1016/j.bjps.2016.01.021

48. Fu L, Bundy C, Sadiq SA. Psychological distress in people with disfigurement from facial palsy. *Eye*. (2011) 25:1322–6. doi: 10.1038/eye.2011.158

49. Siemann I, Sanches EE, de Jongh FW, Luijmes R, Ingels KJAO, Beurskens CHG, et al. Psychological counselling in patients with a peripheral facial palsy: initial experience from an expert Centre. *J Plast Reconstr Aesthetic Surg*. (2022) 75:1639–43. doi: 10.1016/j.bjps.2021.11.079

50. Newell SA, Sanson-Fisher RW, Savolainen NJ. Systematic review of psychological therapies for cancer patients: overview and recommendations for future research. *J Natl Cancer Inst*. (2002) 94:558–84. doi: 10.1093/jnci/94.8.558

51. Trijsburg RW, Van Knippenberg FCE, Rijpma SE. Effects of psychological treatment on cancer patients: a critical review. *Psychosom Med*. (1992) 54:489–517. doi: 10.1097/00006842-199207000-00011

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Altered tongue muscle contractile properties coincide with altered swallow function in the adult Ts65Dn mouse model of down syndrome

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Purpose: Down syndrome (DS) is a developmental disability associated with difficulties in deglutition. The adult Ts65Dn mouse model of DS has been previously shown to have differences in measures of swallowing compared with euploid controls. However, the putative mechanisms of these differences in swallowing function are unclear. This study tested the hypothesis that the Ts65Dn genotype is associated with atypical measures of tongue muscle contractile properties, coinciding with atypical swallow function.

Methods: Adult (5-month-old) Ts65Dn ($n = 15$ female, 14 male) and euploid sibling controls ($n = 16$ female, 14 male) were evaluated through videofluoroscopy swallow studies (VFSS) to quantify measures of swallowing performance including swallow rate and inter-swallow interval (ISI). After VFSS, retrusive tongue muscle contractile properties, including measures of muscle fatigue, were determined using bilateral hypoglossal nerve stimulation.

Results: The Ts65Dn group had significantly slower swallow rates, significantly greater ISI times, significantly slower rates of tongue force development, and significantly greater levels of tongue muscle fatigue, with lower retrusive tongue forces than controls in fatigue conditions.

Conclusion: Tongue muscle contractile properties are altered in adult Ts65Dn and coincide with altered swallow function.

KEYWORDS

Down syndrome, Ts65Dn, mouse, tongue, swallow, adult

1 Introduction

Dysphagia, or a disorder in swallowing, can be associated with critical problems in the safety and efficiency of eating and drinking that may lead to malnutrition, dehydration, aspiration pneumonia, and increased risk of death (1, 2). Risks for these problems increase with age. Adults with Down syndrome (DS) may experience dysphagia across oral, pharyngeal,

and esophageal phases of swallowing (3) along with gastro-esophageal reflux disease, choking, and aspiration (2, 3). These issues can contribute to behavioral differences such as food refusal. Factors that may contribute to problems at the oral preparatory stage of the swallow in DS may include atypical tongue protrusion from the mouth during eating and drinking, inefficient mastication, and dental differences (4–7). Hypotonicity is often believed to be present and may be implicated in oromotor discoordination, inadequate lip sealing, and impaired tongue movement (8, 9). Pharyngeal phase swallowing problems include timing delays, which lead to a high risk of aspiration (4). In addition, uncoordinated patterns of breathing and swallowing can expose some individuals to higher risk of aspiration, presumably due to temporal incongruities that may result in presence of the bolus near an open airway (4). DS is also associated with primary or secondary esophageal motor disorders (10, 11). As adults with DS age, dysphagia might also be observed at earlier time points than in the general aging population (3). Accordingly, studies of putative mechanisms of dysphagia associated with adulthood and aging in DS are critical to development of effective management of care for individuals with DS experiencing signs of dysphagia.

Dysphagia in adults with DS may involve many underlying anatomical and functional factors including sensorimotor, orofacial, and developmental differences. Anatomically, craniofacial differences including an underdeveloped maxilla and shorter length and width of the palate in adults with DS may impact the safety and efficiency of feeding and swallowing (8, 12). People with DS may have differences in palatal morphology compared with people without DS, and it has been proposed that neuromotor dysfunction incurred by DS may result in less capacity to adjust tongue movement to accommodate these differences (8). For these and other reasons, studies of dysphagia in adults without DS cannot easily generalize to adults with DS. This population must be studied directly.

Mouse models of DS provide a means to study biomedical problems through experimental paradigms that are highly controlled for many variables, and that may involve mechanistic hypotheses and methodology that are not possible to perform in studies with human subjects. The Ts65Dn mouse model of DS displays a remarkable number of phenotypes applicable to characteristics of individuals with DS (13). The craniofacial phenotype of Ts65Dn has been extensively studied and has been reported to include relative reductions in size of the palate, as well as reduced size of the mandibles, which parallels aspects of craniofacial differences in many humans with DS (14). Applicable phenotypes also include traits indicative of muscle differences associated with DS, such as reduced grip strength, altered motor coordination, and reduced running and swimming speeds (15, 16). Genotype-specific differences have also been reported in a variety of measures of muscle biology (17–19). This suggests Ts65Dn is an appropriate model for studying DS-related craniofacial muscle impairments. However, the Ts65Dn mouse model does not entirely replicate the trisomy in humans with DS. The Ts65Dn partial trisomy is comprised of a portion of mouse chromosome 16 (Mmu16) and a section of chromosome 17, leading to a failure to model triplication of some of the genes found on Hsa21, in addition to including the triplication of other genes that are not applicable to DS (13, 20). Consequently, while this mouse model displays numerous phenotypes of interest, it may not encompass all of the genetic and molecular aspects of specific DS features. While work is on-going to compare phenotypes of Ts65Dn to phenotypes of newer mouse models that are anticipated to have superior molecular verisimilitude to DS, Ts65Dn is presently the only mouse model that has been both characterized for feeding and swallowing phenotypes, as well as having been found to demonstrate feeding and swallowing phenotypes that are translationally applicable to DS (21, 22). Ts65Dn exhibits differences from controls in body weight, chewing rates under some conditions, and some biological measures of specific muscles involved in swallowing and jaw movement (21, 23).

Although muscle weakness and hypotonia are typically assumed to be present in adults with DS and are often believed to impact muscles of the tongue, there are relatively few clinical studies and quantitative measures available for this area of study (9). Evaluation of pressure exerted by the tongue during swallowing in adults with DS has been studied through intra-oral pressure sensors (8). This has demonstrated that DS is associated with reductions in the duration and magnitude of tongue pressure on the median palate during swallowing, and that some of these reductions can be explained by differences of palate anatomy that are unique to DS. However, while some DS-specific tongue function phenotypes may occur as adaptations to anatomical differences of the palate, tongue function phenotypes may also be partly or wholly attributable to physiological, sensorimotor, or neuromotor differences of the tongue muscle system (8). Further, despite the fact that tongue strength is believed to be relevant to some aspects of swallowing ability under some circumstances, it is unclear if tongue muscle weakness coincides with significant swallowing phenotypes in DS. Murine models of DS provide access to a wide range of options for investigation of tongue muscle function that can circumnavigate some of these challenges, and which may provide some indication of whether differences in tongue muscle function coincide with differences in swallow function in DS.

The Videofluoroscopic Swallow Study (VFSS) is one of the gold standard diagnostic tests in humans for assessing oropharyngeal stages of swallowing. To adapt this method for mice and small rodents, a murine VFSS protocol was developed (24). This includes a reliable step-by-step test protocol for quantifying swallow metrics (24, 25). While VFSS has been used to identify significant differences in swallowing in the Ts65Dn model (21), VFSS alone gives incomplete information about muscle function during swallowing. Tongue muscle contractile properties can be directly evaluated in murine models as a function of neuromuscular electrical stimulation (NMES), either directly to muscle or with stimulation of the hypoglossal nerves (26). These methods allow quantification of twitch and tetanic forces, rate of force development, and half-decay times. These measures, indicative of tongue muscle force, temporal features of muscle contraction, and tongue muscle fatigue resistance can offer valuable insights into tongue muscle function. Thus, NMES and VFSS methods complement each other to evaluate structure and function of swallowing more precisely. The present study used VFSS and hypoglossal nerve stimulation in the adult Ts65Dn model of DS to test the hypothesis that the Ts65Dn genotype is associated with atypical measures of tongue muscle contractile properties, as well as atypical swallow function.

2 Methods

2.1 Mice

Mice for this study were generated from Ts65Dn breeding pairs comprised of one of the following: (1) Ts65Dn female (JAX 005252) and euploid F1 male (JAX 003647) purchased from the Jackson

Laboratory, or (2) Ts65Dn females (JAX 005252) and euploid F1 males which were produced by mating C57BL/6JEiJ (JAX 000924) with C3Sn.BLiA-Pde6b+/Dn (JAX 003648). All breeding mice with the JAX strain numbers indicated were purchased directly from the Jackson Laboratory. Mice were maintained on the Harlan Teklad diet #7913, were genotyped for the presence or absence of the partial trisomy through Transnetyx®, or by our own team members using JAX Protocol 24762, and were genotyped for the Clcc1$^{<m1J>}$ using JAX protocol 20574. Mice were analyzed at the age of 5 months (145–160 days of age). All procedures were conducted in accordance with an IACUC protocol approved through the University of Wisconsin, Madison, School of Medicine and Public Health Institutional Animal Care and Use Committee and the Roswell Park Comprehensive Cancer Center Institutional Animal Care and Use Committee.

2.2 Videofluoroscopy swallow studies

Mice were maintained on a reverse light cycle (27), and all behavioral experiments were performed between 9 am and 12 pm during the animals' dark cycle.

2.2.1 VFSS acclimation

Three days prior to the scheduled videofluoroscopy acquisition, mice were acclimated to Fritos™ mild cheddar cheese dip and Varibar® Thin Honey Barium sulfate oral suspension 40% w/v barium. Days one and two of acclimation consisted of presenting the cheese to the mice in their home cages as they would encounter it during the videofluoroscopy session. Mice were allowed access to cheese until all cage mates had examined the cheese and attempted to feed, after which the cheese was removed from the cage. Day three of acclimation consisted of making a mixture of cheese and barium at a ratio of 2 mL cheese:1 mL barium suspension, which generated an extremely thick puree of IDDSI level 4. After mixing thoroughly, the cheese was presented as on days one and two. If mice exhibited signs of neophobia or disinterest on day one or two of acclimation, a food pellet was dipped into the cheese and left to remain in the cage overnight. The day prior to acquisition between 3:00 pm and 6:00 pm food was removed from the cages and mice were transferred to single housing. Food was withheld overnight for no more than 18 h, and *ad libitum* access to water was maintained.

2.2.2 VFSS acquisition

A Genoray fluoroscopic x-ray system model ZEN-7000 was used to image mice in a lateral view. Images were captured in the Photon Fast Camera Viewer (PRV4) software with the following settings: frame rate 60fps, shutter speed 2.5 ms, resolution 1024×1024, Def 2 LUTS. On the C-ARM touchscreen the following settings were used: 55kVp, 3.0 mA, fluoroscopy mode, collimator 9-inch, hand and foot mode, dynamic noise reduction high mode. Once the settings were verified, footage comprised of a minimum of 7 s of continuous eating was acquired. Mice sometimes paused while eating, in which case acquisition was paused, and resumed when mice resumed eating. The field of view included the mouse's entire head and, at minimum, upper body cavity with a view of the stomach, or the entire mouse body.

2.2.3 VFSS analysis

Four parameters were evaluated in video acquisitions for each mouse: swallow rate (SR) which is defined as the number of swallows during two consecutive seconds of continuous, uninterrupted eating, inter-swallow interval (ISI) which is defined as the time between two successive, uninterrupted swallows (24), jaw excursion rate (JER), and jaw cycle-swallow ratio (JSR). All of these measures were quantified as in previous research (21, 24, 25). A minimum of three and up to five different instances of each measure were analyzed for each mouse, and then averaged to generate one data point for each mouse. JSR, or jaw cycle-swallow ratio is analogous to the lick-swallow ratio, and is defined as the number of jaw excursion cycles that occur during each ISI.

To verify appropriate inter-rater reliability of VFSS analysis, no fewer than 20% of VFSS videos in the study, arbitrarily selected and distributed across both sexes and both genotypes, were independently analyzed by two or more workers. Inter-class correlation coefficients (ICC) at or above 0.8, or Pearson correlation coefficients at or above 0.8 were confirmed for results from independent raters for all measures of analysis in this study.

2.3 Tongue muscle contraction studies

After VFSS acquisition, mice were weighed and anesthetized with isoflurane and an I.P. injection 40–50 mg/kg sodium pentobarbital. Through close monitoring over the subsequent two-hour procedure, supplemental 5 mg/kg doses of sodium pentobarbital were administered intermittently to preserve a deep plane of anesthesia. Mice were placed in a supine position beneath an operating microscope. Following a midline incision of the skin over the location of the larynx and tongue base, salivary glands and the posterior digastric muscle were gently retracted, adjacent vessels were gently repositioned to preserve them, and a 5-mm section of the nerve was visualized. Custom-built silastic nerve cuffs were placed on the right and left hypoglossal nerves (Figure 1).

Following placement of hypoglossal nerve cuffs, cuff wires were connected to stimulus isolators through small alligator clips. A 10 mm needle and 6–0 or 7–0 silk suture were used to connect the tip of the tongue to an external force transducer to record elicited tongue muscle forces. Optimal line tension to yield maximum tongue muscle twitch forces was identified by stimulating the nerves with supramaximal stimulation (between 200 and 400 μA) and adjusting the length until the maximum force was obtained.

Measures of retrusive tongue properties were recorded during whole bilateral hypoglossal nerve stimulation using a data acquisition system (Aurora Scientific) as previously described (28), which generated the following experimental measures:

1) Maximum twitch force (mN) (the peak force generated from a single electrical stimulus), and the rate of force development (RFD) of twitch.
2) The relationship between muscle force and stimulation frequency (force-frequency curve) was demonstrated through stimulus frequencies of 1, 20, 40, 60, 80, 120, 160, 250, and 300 Hz. Maximum tetanic tension (mN) was the fused maximum tetanic force elicited from a range of stimulation frequencies.
3) Rate of force development from baseline to 50% of peak force (RFD1, Δ nM/s), rate of force development from 50% of peak

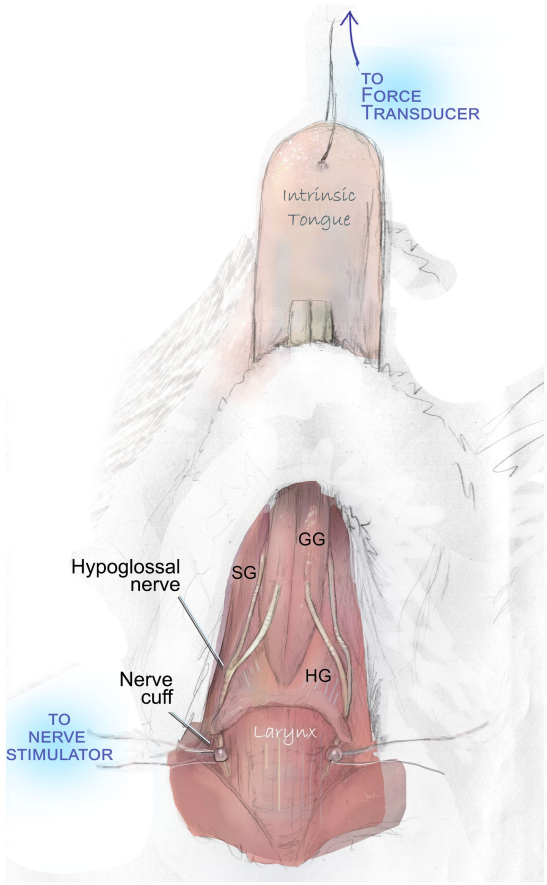

FIGURE 1
Hypoglossal nerve stimulation to evaluate tongue muscle contractile properties. With the exception of a midline incision and gentle retraction to permit access to the hypoglossal nerves, all overlying muscles and adjacent vessels are preserved intact during this procedure. They are omitted in this figure to demonstrate the course of the nerve and key tongue muscles. Bilateral stimulation of the hypoglossal nerves elicits contraction of the genioglossus (GG), hyoglossus (HG), styloglossus (SG), and intrinsic tongue muscles. This produces a net retrusive action. Contraction force (mN) is quantified with a force transducer.

force to maximum (RFD2, Δ nM/s), and rate of force loss (RFL, Δ nM/s) were normalized to maximum force values prior to analysis.

4) Data acquisition ended with a muscle fatigue protocol which consisted of a 1 s 80 Hz stimulus frequency (1:1 duty cycle) with force measurements collected at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 s, followed by a recovery period with data collection at 30, 60, 120, 300, 600, and 900 s. Results were calculated as the average of tetanic force (mN) relative to the initial tetanic tension (mN), expressed as a percentage of initial tension.

After completion of data collection, mice were euthanized through an injection of Euthasol.

2.4 Statistics

A target sample size of 14 mice per group was anticipated to provide sufficient power to reveal biologically meaningful differences in measurement variables based on effect sizes of the ISI and JSR measures in a prior study of swallow function in Ts65Dn (21). However, group sizes for some measures in some groups varied slightly due to experimental factors such as incidental attrition or sporadic technical artifact. Data were analyzed by 2-way ANOVA to interrogate main effects for the Ts65Dn genotype and sex, as well as interaction effects. Data that failed to conform to the assumptions of ANOVA were log transformed prior to analysis to comply with requirements for heteroscedasticity and normality. Because JAX recommends evaluating Clcc1$^{<m1J>}$ genotype in Ts65Dn, exploratory analysis of the impact of the Clcc1$^{<m1J>}$ genotype on the ISI measure of swallow function was performed through 2-way ANOVA in data pooled from male and female mice. Significance was set at $\alpha = 0.05$.

3 Results

3.1 Videofluoroscopy swallow studies

During continuous eating of a puree texture, Ts65Dn showed significantly slower swallow rate than euploid ($F(1,54) = 9.79$, $p = 0.003$). Ts65Dn also showed significantly longer inter-swallow intervals, or the amount of time elapsing between consecutive swallows ($F(1, 53) = 17.71$, $p < 0.0001$). Ts65Dn showed significantly larger JSR values, indicating a greater number of jaw cycles per swallow ($F(1,52) = 11.25$, $p = 0.0015$). However, there were not significant genotype-specific differences in the jaw excursion rate (JER), which reflects the rate of jaw movements during eating (Figure 2). For all VFSS measures, there were no significant effects for sex, and no significant interactions between genotype and sex. Exploratory analysis suggested ISI measures in Ts65Dn were not significantly impacted by Clcc1$^{<m1J>}$ genotype (Supplementary Figure S1).

3.2 Tongue muscle contraction studies

There were significant differences in body weight associated with both sex and genotype, in the absence of interaction between genotype and sex. Ts65Dn weighed significantly less than euploid (F (1,44) = 13.37, $p < 0.001$) and females weighed significantly less than males (F (1,44) = 22.547, $p < 0.001$) (Figure 3A). Analysis through ANOVA suggested that Ts65Dn had significantly lower maximum forces than euploid (F (1,44) = 5.772, $p = 0.021$) and females had significantly lower maximum forces than males (F (1,44) = 5.847, $p = 0.020$). However, there was a moderate and significant relationship between body weight and maximum retrusive tongue force in both euploid ($p = 0.03$, r = 0.444) and Ts65Dn ($p = 0.0003$, r = 0.677) (Figure 3B). Therefore, it is likely that these significant differences in tongue force are attributable to overall animal size, and by extension, overall muscle size, rather than to physiological differences between groups in these comparisons. To accommodate the possibility that differences in animal size could confound group comparisons of tongue force measures, maximum retrusive tongue force was analyzed with body weight as a covariate. Analysis with a covariate of weight indicated no significant differences in maximum retrusive tongue force due to genotype or sex, and an absence of significant interactions between genotype and sex (Figure 3C). Similarly, there was a moderate and significant relationship between body weight and maximum twitch force in both Ts65Dn ($p = 0.022$, r = 0.464) and euploid ($p = 0.013$, r = 0.500). However, analysis with a co-variate of weight indicated no significant differences in maximum twitch force due to genotype or

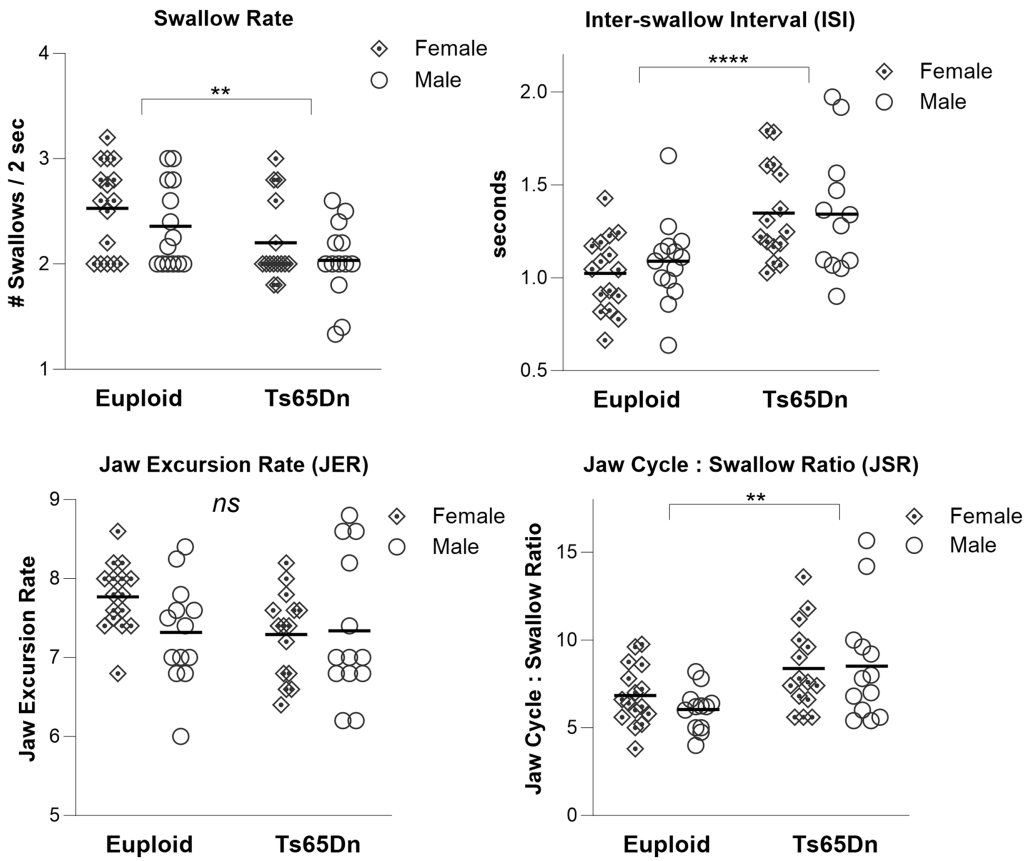

FIGURE 2
Swallow phenotypes in Ts65Dn at 5 months of age. Ts65Dn have significantly reduced swallow rates compared to euploid control. Ts65Dn have significantly increased inter-swallow intervals compared to euploid control. Ts65Dn have significantly greater number of jaw cycles preceding each swallow compared to euploid control. No significant differences between groups were detected in jaw excursion rates. *N*=12–16 mice per group. **=$p \leq 0.01$, *** = $p \leq 0.001$, ****=$p \leq 0.0001$.

sex, and an absence of significant interactions between genotype and sex.

There were no significant differences due to genotype or sex in measures of the force-frequency relationship when forces were normalized to each individual's maximum retrusive tongue force (Figure 4).

In the measures of the rates of force development for tetanus normalized to the maximum force, Ts65Dn showed significantly lower values for RFD1 than euploid control (F (1,44)=7.709, $p=0.008$), in the absence of differences due to sex or significant interactions between sex and genotype. However, there were no significant differences between groups in RFD2 (Figure 5), and there were no significant differences due to genotype or sex in RFL measures. Ts65Dn also showed significantly lower values than euploid for the RFD of twitch (F (1,44)=6.915, $p=0.012$). Females showed lower values than males for the RFD of twitch (F (1,44)=5.749, $p=0.021$), in the absence of significant interactions between genotype and sex.

In evaluation of muscle fatigue elicited by repeated hypoglossal nerve stimulation, Ts65Dn showed significantly lower levels of retrusive tongue force than euploid after 140 s of stimulation (F (1,44) = 4.473, $p=0.040$), 180 s of stimulation (F (1,44) = 4.283, $p=0.044$), and 200 s of stimulation (F (1,44) = 4.133, $p=0.048$), which suggests that onset of tongue muscle fatigue occurs more quickly with hypoglossal nerve stimulation in Ts65Dn than euploid (Figure 6). In analysis of muscle fatigue there were no significant differences due to sex, and no significant interactions between genotype and sex. There were also no significant differences between groups at timepoints during the recovery phase.

4 Discussion

The goal of this work was to test the hypothesis that the Ts65Dn genotype is associated with atypical measures of swallow function and tongue muscle contractile properties. Our findings were compatible with this hypothesis. We found that during continuous eating of a puree texture, adult Ts65Dn demonstrated significantly slower swallow rates and significantly longer time intervals between consecutive swallows. In addition, Ts65Dn demonstrated slower rates of tongue force development and a more rapid onset of tongue muscle fatigue during evoked retrusive actions. While Ts65Dn had lower values of retrusive tongue forces than euploid control, there was also a significant relationship between animal size and tongue force in both Ts65Dn and control groups, such that presumably smaller muscles generated commensurately lower forces. This is an important factor to consider because DS is often associated with slower growth and thus smaller body size than euploid. Also, sex-specific differences in body size occur in DS as well as in those without DS (29–31). When tongue forces were normalized to body size for analysis, such that tongue forces were analyzed in the context of body weight, no significant

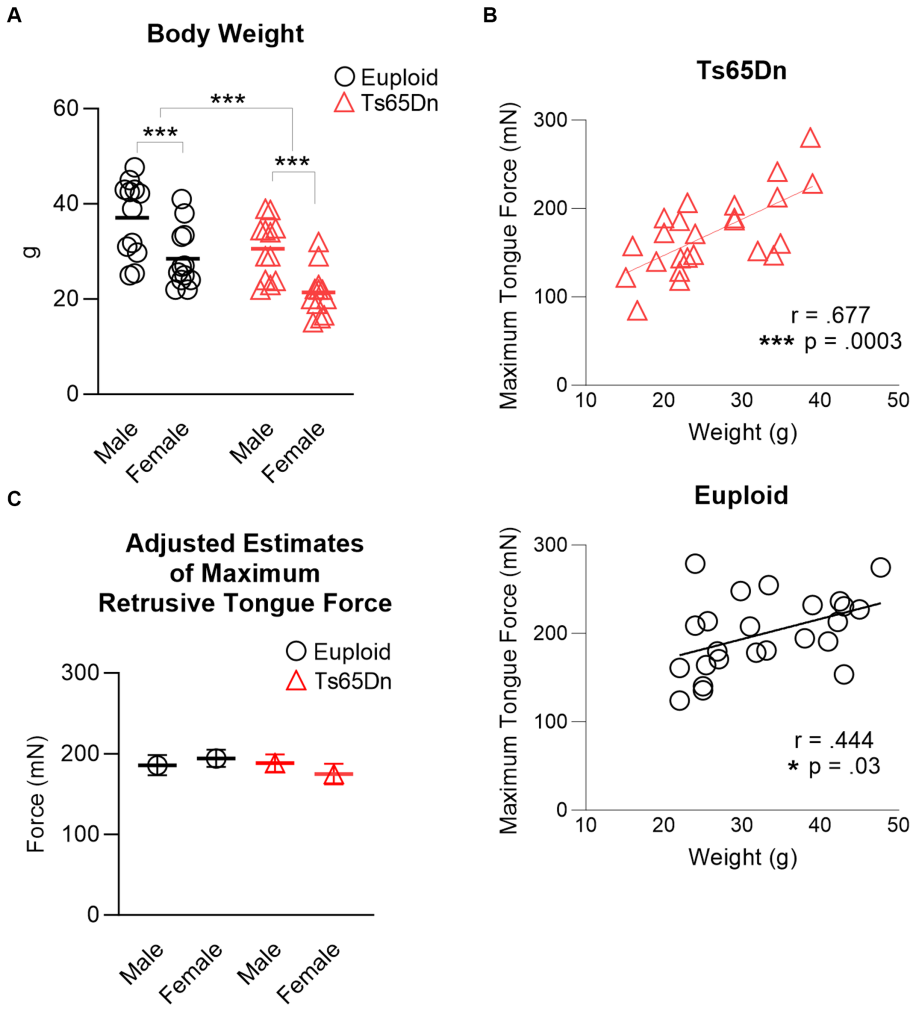

FIGURE 3
Maximum retrusive tongue muscle force. **(A)** Animal sizes were significantly different between groups. Each data point indicates one mouse. **(B)** Significant moderate correlations between animal size and maximum retrusive tongue force suggest that force differences between Ts65Dn and euploid are attributable to animal size. Each data point indicates one mouse. **(C)** Adjusted estimates of maximum retrusive tongue forces resulting from analysis covarying by animal weight were not significantly different between groups. Each data point indicates the group mean. Standard error is shown. *N*=12 mice per group. *** = $p \leq 0.001$.

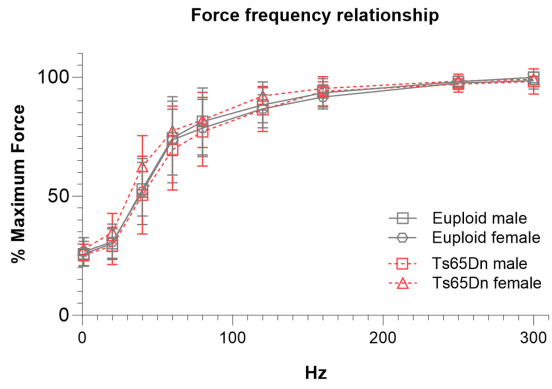

FIGURE 4
Ts65Dn show no significant differences in force frequency measures. Each data point indicates the group mean. Error bars indicate SD. *N*=11–12 mice per group.

differences in tongue force were observed between Ts65Dn and control. Collectively, these findings suggest that while tongue forces are appropriate to animal size in Ts65Dn, Ts65Dn have significant increases in fatiguability of tongue muscles and are slower to develop force in tongue muscles. While it is plausible that these muscle contraction phenotypes may be implicated in increased ISI and decreased swallow rates in Ts65Dn, future studies are required to determine whether there is a causal relationship between these tongue muscle contractile phenotypes and swallowing phenotypes in Ts65Dn.

Properties of swallowing characterized in this study independently replicated previous findings of the adult Ts65Dn swallowing phenotype (24). While the causes of this phenotype remain unknown, slower swallow rates do not coincide with significantly slower jaw movements during food procurement, as evaluated through the JER measure. Compared to controls, Ts65Dn had significantly more jaw excursion cycles prior to each swallow as demonstrated in the JSR measures. A possible explanation is that despite maintaining typical jaw cycle rates, Ts65Dn may have reduced

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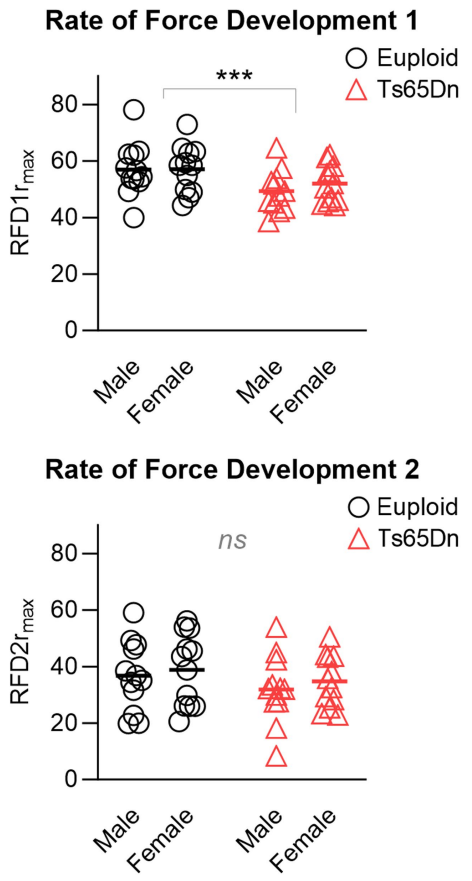

FIGURE 5
Ts65Dn show significantly lower values for RFD1 than euploid control, however, there are no significant genotype-specific differences in RFD2. RFD = Rate of force development. r_{max} indicates relative to maximum force. *N* = 12 mice per group. Each data point indicates one mouse. Bars indicate the group mean. ***=$p \leq 0.001$.

efficiency of oral processing for collection and transfer the bolus prior to initiation of the swallow. Oral processing depends upon sensorimotor coordination of the tongue muscle system. To examine the possibility that function of the tongue muscle system is altered in Ts65Dn, a hypoglossal nerve stimulation paradigm was used to quantify muscle contractile properties during experimentally elicited tongue retrusion. While there was no evidence of genotype-specific weakness in the tongue muscle system upon initial stimulation, Ts65Dn showed significant reductions in the rates of retrusive tongue muscle force development. In addition to having slower rates of force development, Ts65Dn also showed significant increases in fatiguability of tongue retrusion upon repeated stimulation, manifested as significant reductions in the ability to maintain retrusive tongue muscle force upon repeated muscle contraction demands. Heightened susceptibility to muscle fatigue in the tongue may be particularly concerning for deglutition efficacy in Ts65Dn because the swallowing phenotype in this model entails significantly greater oral processing times prior to each swallow than for controls. In some older human populations the consumption of even a typical meal may cause subsequent delays in swallowing initiation, possibly due to fatigue incurred by consuming the meal (32). However, it is worth noting that tongue fatigue in our experimental system did not occur immediately upon stimulation, but rather, occurred only after stimulation for over 2 minutes, which may not have been entirely applicable to the duration of tongue activity elicited during VFSS sessions which typically lasted only several seconds. Therefore, one may speculate that findings of precocious tongue fatigue in Ts65Dn may be more applicable to more naturalistic deglutition settings, such as in *ad libitum* feeding occurring in standard husbandry conditions. In those settings, more naturalistic eating bouts may unfold over much long periods of time. It is also reasonable to consider that longer oral processing times in Ts65Dn may exacerbate tongue muscle fatigue during eating. In humans, it has been reported that oral processing demands of consuming meals may elicit fatigue-induced reductions in tongue strength in some populations (33, 34). From the standpoint of translational DS research, future work to better understand the causes and the consequences of tongue muscle fatigue in DS may support improved understanding of deglutition phenotypes in this syndrome.

In both humans and murine models, tongue protrusion and potentially co-activation of tongue muscles are involved in successful deglutition (35–37). The puree food used in this study was acquired by mice through licking, which requires at least protrusion of the tongue from the oral cavity to procure the food, retrusion of the tongue into the oral cavity, and collection of a cohesive food bolus followed by a number of key muscle actions that alter pressures within the upper aerodigestive tract for posterior transport of the bolus leading the swallow. Tongue protrusion is accomplished through the genioglossus (an extrinsic tongue muscle), and may be aided through activity of the transverse and verticalis (intrinsic tongue muscles), which are thought to promote elongation of the intrinsic tongue (36). Conversely, tongue retrusion is accomplished through the styloglossus and hyoglossus (extrinsic tongue muscles), and contraction of the superior longitudinal and inferior longitudinal (intrinsic tongue muscles) that may act to shorten the intrinsic tongue when contracted (37, 38). Because of the complex interdigitation of these tongue muscles and the fact that they work synergistically during tongue movement and shape changes of the intrinsic tongue, it is not uncommon for physiological studies of the tongue to interrogate the tongue muscle system intact. This strategy was used in the present study, in which bilateral stimulation of the hypoglossal nerves in the intact tongue muscle system elicited contraction of both protrusive and retrusive tongue muscles, ultimately resulting in net retrusive forces, which were then quantified. The benefit of this approach is it generates information about the physiological properties of the intact muscle system in the mice. The substantial limitation of this approach is that it is unable to clearly identify which components of the tongue muscle system may be primarily responsible for contractile phenotypes of interest. Therefore, the precise muscular etiologies of the genotype-specific differences in muscle fatigue in tongue retrusion identified in this study remain somewhat unclear, as described below.

It is possible that susceptibility to muscle fatigue in Ts65Dn may be related to differences of basic muscle biology associated with this syndrome, including phenotypes related to constitutive oxidative stress, energy metabolism, and muscle bioenergetics, as characterized previously (19, 39, 40). A prior study of the soleus muscle in Ts65Dn identified typical contraction forces in Ts65Dn soleus without fatigue, but found reduced muscle forces during recovery from fatigue (19). Since both a prior study of limb muscle as well as the current study of

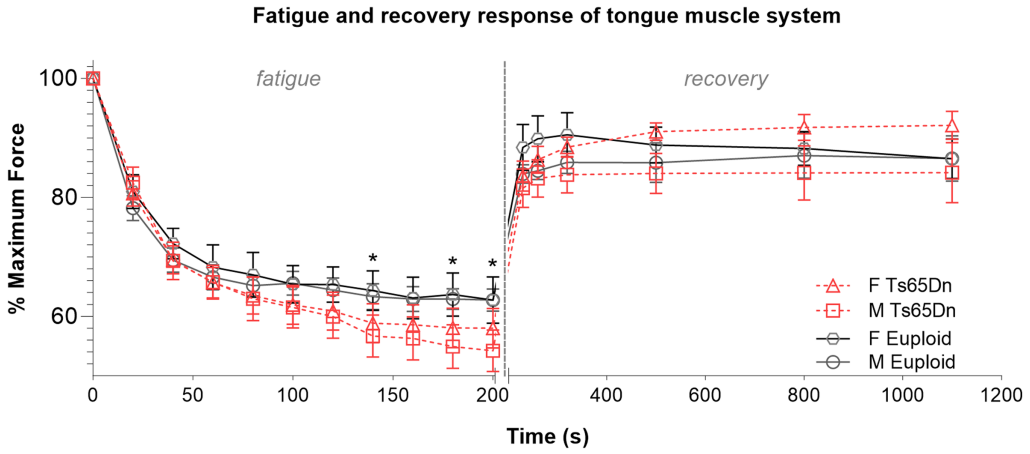

FIGURE 6
Ts65Dn show significantly increased retrusive tongue muscle fatiguability. *N* = 11–13 mice per group. Each data point indicates the group mean. Error bars indicate SEM. *=$p<0.05$.

tongue muscle found no evidence of muscle weakness under typical conditions, but did find genotype-specific reductions in force related to fatigue conditions, it can be speculated that Ts65Dn may have global muscle phenotypes specific to fatigue.

Because the present study measured net retrusive forces that occurred as a result of co-activation of tongue protrusors and retrusors, an alternative possibility to consider is that precocious fatigue in tongue retrusion in this context could also result from an imbalance in the contribution of tongue protrusor muscles relative to retrusor muscles, or a larger proportion of force generated by tongue protrusors in Ts65Dn relative to controls. There are presently few, if any, studies elucidating the relative contributions of tongue protrusor muscles and tongue retrusor muscles to the anatomy and function of the tongue muscle system in DS. However, there is extensive information in the literature that DS may entail atypical co-activation of agonist and antagonistic muscles, and it is possible to speculate that this may occur in the tongue muscle system (9). In conjunction with further studies of tongue physiology, future work may consider avenues through which such an imbalance between tongue protrusors and tongue retrusors could occur in DS. One example of an avenue for future investigation may be the somatotopic organization of the hypoglossal motor nucleus, the anatomical region in which motor neurons in the brainstem responsible for the control of intrinsic and extrinsic tongue muscles of tongue protrusion and tongue retrusion. This somatotopic organization is directed in part through expression of the transcription factor *Runx1*, which is located on the 21st human chromosome and has been reported to have atypical expression levels in DS (39). *Runx1* has been reported to promote expansion of hypoglossal motor neurons controlling tongue protrusor muscles specifically (41). Several studies have characterized a preponderance of tongue protrusion phenotypes in DS, or tendencies to protrude the tongue in situations that would not typically elicit tongue protrusion in individuals without DS (7, 42, 43). Historically, it has been widely recognized that many differences in tongue movement and positioning in DS may be at least partly attributable to craniofacial etiologies, including differences in palate morphology and a disproportionately smaller oral cavity, which could impose substantial anatomical constraints on tongue movement (8, 44). However, the present study evaluated retrusion of the tongue muscle system through a nerve stimulation paradigm in which the tongue was not constrained within the oral cavity, and in which there were neither behavioral nor volitional influences on tongue movement. Therefore, it is possible that the retrusive phenotype reported here could be attributable to differences of neuromuscular anatomy or physiology of the tongue in Ts65Dn.

Using information gained from basic studies of this area to ultimately improve outcomes for individuals with DS will require engagement with a variety of challenges. The clinical study of tongue muscle function and swallowing disorders in adults with DS has some logistical limitations. These include limited geographical availability and capacities of specialized multidisciplinary clinical care centers for DS, which creates barriers for some adults with DS to receive health care services aligned with best practices (45, 46), accessibility barriers that disproportionately disenfranchise individuals with communication support needs who may require special accommodation in order to communicate with medical providers (47), which may be applicable to many individuals with DS (44, 48, 49), and risks for disconnects between researcher expectations and the lived experiences of some disabled study participants (50). In light of these systemic challenges, the continued use of animal models of DS for basic discovery of underlying mechanisms of dysphagia is one of many strategies that can be used to ultimately advance more equitable inclusion of people with DS in the benefits of basic dysphagia research.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by University of Wisconsin, Madison, School of Medicine and Public Health Institutional Animal Care and Use Committee and the Roswell Park Comprehensive Cancer Center Institutional Animal Care and Use Committee. The

study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. EF: Data curation, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. MO: Validation, Writing – original draft, Writing – review & editing. NA: Investigation, Writing – original draft, Writing – review & editing. YY: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. NC: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2024.1384572/full#supplementary-material

References

1. Jensen KM, Bulova PD. Managing the care of adults with Down's syndrome. *BMJ*. (2014) 349:g 5596. doi: 10.1136/bmj.g5596

2. Chenbhanich J, Wu A, Phupitakphol T, Atsawarungruangkit A, Treadwell T. Hospitalisation of adults with down syndrome: lesson from a 10-year experience from a community hospital. *J Intellect Disabil Res*. (2019) 63:266–76. doi: 10.1111/jir.12572

3. Lazenby T. The impact of aging on eating, drinking, and swallowing function in people with Down's syndrome. *Dysphagia*. (2008) 23:88–97. doi: 10.1007/s00455-007-9096-1

4. Smith CH, Teo Y, Simpson S. An observational study of adults with down syndrome eating independently. *Dysphagia*. (2014) 29:52–60. doi: 10.1007/s00455-013-9479-4

5. Limbrock GJ, Fischer-Brandies H, Avalle C. Castillo-Morales' orofacial therapy: treatment of 67 children with down syndrome. *Dev Med Child Neurol*. (1991) 33:296–303. doi: 10.1111/j.1469-8749.1991.tb14880.x

6. Faulks D, Collado V, Mazille MN, Veyrune JL, Hennequin M. Masticatory dysfunction in persons with Down's syndrome. Part 1: aetiology and incidence. *J Oral Rehabil*. (2008) 35:854–62. doi: 10.1111/j.1365-2842.2008.01877.x

7. Gisel EG, Lange LJ, Niman CW. Tongue movements in 4-and 5-year-old Down's syndrome children during eating: a comparison with normal children. *Am J Occup Ther*. (1984) 38:660–5. doi: 10.5014/ajot.38.10.660

8. Hashimoto M, Igari K, Hanawa S, Ito A, Takahashi A, Ishida N, et al. Tongue pressure during swallowing in adults with down syndrome and its relationship with palatal morphology. *Dysphagia*. (2014) 29:509–18. doi: 10.1007/s00455-014-9538-5

9. Chu SY, Barlow SM. A call for biomechanics to understand Hypotonia and speech movement disorders in down syndrome. *Adv Commun Disord*. (2016) 16:2–40.

10. Zárate N, Mearin F, Gil-Vernet JM, Camarasa F, Malagelada JR. Achalasia and Down's syndrome: coincidental association or something else? *Am J Gastroenterol*. (1999) 94:1674–7. doi: 10.1111/j.1572-0241.1999.01161.x

11. Zárate N, Mearin F, Hidalgo A, Malagelada JR. Prospective evaluation of esophageal motor dysfunction in Down's syndrome. *Am J Gastroenterol*. (2001) 96:1718–24. doi: 10.1111/j.1572-0241.2001.03864.x

12. Kaczorowska N, Kaczorowski K, Laskowska J, Mikulewicz M. Down syndrome as a cause of abnormalities in the craniofacial region: a systematic literature review. *Adv Clin Exp Med*. (2019) 28:1587–92. doi: 10.17219/acem/112785

13. Rueda N, Flórez J, Martínez-Cué C. Mouse models of down syndrome as a tool to unravel the causes of mental disabilities. *Neural Plast*. (2012) 2012:584071. doi: 10.1155/2012/584071

14. Hill CA, Reeves RH, Richtsmeier JT. Effects of aneuploidy on skull growth in a mouse model of down syndrome. *J Anat*. (2007) 210:394–405. doi: 10.1111/j.1469-7580.2007.00705.x

15. Costa AC, Walsh K, Davisson MT. Motor dysfunction in a mouse model for down syndrome. *Physiol Behav*. (1999) 68:211–20. doi: 10.1016/S0031-9384(99)00178-X

16. Costa AC, Stasko MR, Schmidt C, Davisson MT. Behavioral validation of the Ts65Dn mouse model for down syndrome of a genetic background free of the retinal degeneration mutation Pde 6b (rd 1). *Behav Brain Res*. (2010) 206:52–62. doi: 10.1016/j.bbr.2009.08.034

17. Cisterna B, Costanzo M, Scherini E, Zancanaro C, Malatesta M. Ultrastructural features of skeletal muscle in adult and aging Ts65Dn mice, a murine model of down syndrome. *Muscles Ligaments Tendons J*. (2013) 3:287–94.

18. Cisterna B, Sobolev AP, Costanzo M, Malatesta M, Zancanaro C. Combined microscopic and Metabolomic approach to characterize the skeletal muscle Fiber of the Ts65Dn mouse, a model of down syndrome. *Microsc Microanal*. (2020) 26:1014–23. doi: 10.1017/S143192762002437X

19. Cowley PM, Keslacy S, Middleton FA, DeRuisseau LR, Fernhall B, Kanaley JA, et al. Functional and biochemical characterization of soleus muscle in down syndrome mice: insight into the muscle dysfunction seen in the human condition. *Am J Physiol Regul Integr Comp Physiol*. (2012) 303:R1251–60. doi: 10.1152/ajpregu.00312.2012

20. Duchon A, Raveau M, Chevalier C, Nalesso V, Sharp AJ, Herault Y. Identification of the translocation breakpoints in the Ts65Dn and Ts1Cje mouse lines: relevance for modeling down syndrome. *Mamm Genome*. (2011) 22:674–84. doi: 10.1007/s00335-011-9356-0

21. Glass TJ, Valmadrid LCV, Connor NP. The adult Ts65Dn mouse model of down syndrome shows altered swallow function. *Front Neurosci*. (2019) 13:906. doi: 10.3389/fnins.2019.00906

22. Kim HN, Kim JY. A systematic review of oropharyngeal dysphagia models in rodents. *Int J Environ Res Public Health*. (2021) 18:4987. doi: 10.3390/ijerph18094987

23. Glass TJ, Twadell SL, Valmadrid LC, Connor NP. Early impacts of modified food consistency on oromotor outcomes in mouse models of down syndrome. *Physiol Behav*. (2019) 199:273–81. doi: 10.1016/j.physbeh.2018.11.031

24. Lever TE, Braun SM, Brooks RT, Harris RA, Littrell LL, Neff RM, et al. Adapting human videofluoroscopic swallow study methods to detect and characterize dysphagia in murine disease models. *J Vis Exp*. (2015) 97:52319. doi: 10.3791/52319

25. Lever TE, Brooks RT, Thombs LA, Littrell LL, Harris RA, Allen MJ, et al. Videofluoroscopic validation of a translational murine model of Presbyphagia. *Dysphagia*. (2015) 30:328–42. doi: 10.1007/s00455-015-9604-7

26. Connor NP, Ota F, Nagai H, Russell JA, Leverson G. Differences in age-related alterations in muscle contraction properties in rat tongue and hindlimb. *J Speech Lang Hear Res*. (2008) 51:818–27. doi: 10.1044/1092-4388(2008/059)

27. Peirson SN, Brown LA, Pothecary CA, Benson LA, Fisk AS. Light and the laboratory mouse. *J Neurosci Methods*. (2018) 300:26–36. doi: 10.1016/j.jneumeth.2017.04.007

28. Connor NP, Russell JA, Jackson MA, Kletzien H, Wang H, Schaser AJ, et al. Tongue muscle plasticity following hypoglossal nerve stimulation in aged rats. *Muscle Nerve*. (2013) 47:230–40. doi: 10.1002/mus.23499

29. Myrelid A, Gustafsson J, Ollars B, Annerén G. Growth charts for Down's syndrome from birth to 18 years of age. *Arch Dis Child*. (2002) 87:97–103. doi: 10.1136/adc.87.2.97

30. Zemel BS, Pipan M, Stallings VA, Hall W, Schadt K, Freedman DS, et al. Growth charts for children with down syndrome in the United States. *Pediatrics*. (2015) 136:e1204–11. doi: 10.1542/peds.2015-1652

31. Group WMGRS. WHO child growth standards based on length/height, weight and age. *Acta Paediatr Suppl*. (2006) 95:76–85. doi: 10.1111/j.1651-2227.2006.tb02378.x

32. Hiramatsu T, Kataoka H, Osaki M, Hagino H. Effect of aging on oral and swallowing function after meal consumption. *Clin Interv Aging*. (2015) 10:229–35. doi: 10.2147/CIA.S75211

33. Brates D, Molfenter S. The influence of age, eating a meal, and systematic fatigue on swallowing and mealtime parameters. *Dysphagia*. (2021) 36:1096–109. doi: 10.1007/s00455-020-10242-8

34. Kays SA, Hind JA, Gangnon RE, Robbins J. Effects of dining on tongue endurance and swallowing-related outcomes. *J Speech Lang Hear Res*. (2010) 53:898–907. doi: 10.1044/1092-4388(2009/09-0048)

35. Gestreau C, Dutschmann M, Obled S, Bianchi AL. Activation of XII motoneurons and premotor neurons during various oropharyngeal behaviors. *Respir Physiol Neurobiol*. (2005) 147:159–76. doi: 10.1016/j.resp.2005.03.015

36. McClung JR, Goldberg SJ. Functional anatomy of the hypoglossal innervated muscles of the rat tongue: a model for elongation and protrusion of the mammalian tongue. *Anat Rec*. (2000) 260:378–86. doi: 10.1002/1097-0185(20001201)260:4<378::AID-AR70>3.0.CO;2-A

37. Gilbert RJ, Napadow VJ, Gaige TA, Wedeen VJ. Anatomical basis of lingual hydrostatic deformation. *J Exp Biol*. (2007) 210:4069–82. doi: 10.1242/jeb.007096

38. Napadow VJ, Chen Q, Wedeen VJ, Gilbert RJ. Biomechanical basis for lingual muscular deformation during swallowing. *Am J Phys*. (1999) 277:G695–701. doi: 10.1152/ajpgi.1999.277.3.G695

39. Lyle R, Gehrig C, Neergaard-Henrichsen C, Deutsch S, Antonarakis SE. Gene expression from the aneuploid chromosome in a trisomy mouse model of down syndrome. *Genome Res*. (2004) 14:1268–74. doi: 10.1101/gr.2090904

40. Lott IT. Antioxidants in down syndrome. *Biochim Biophys Acta*. (2012) 1822:657–63. doi: 10.1016/j.bbadis.2011.12.010

41. Chen X, Wang JW, Salin-Cantegrel A, Dali R, Stifani S. Transcriptional regulation of mouse hypoglossal motor neuron somatotopic map formation. *Brain Struct Funct*. (2016) 221:4187–202. doi: 10.1007/s00429-015-1160-2

42. Limbrock GJ, Hoyer H, Scheying H. Regulation therapy by Castillo-Morales in children with down syndrome: primary and secondary orofacial pathology. *ASDC J Dent Child*. (1990) 57:437–41.

43. Limbrock GJ, Castillo-Morales R, Hoyer H, Stöver B, Onufer CN. The Castillo-Morales approach to orofacial pathology in down syndrome. *Int J Orofacial Myology*. (1993) 19:30–8. doi: 10.52010/ijom.1993.19.1.6

44. Kent RD, Vorperian HK. Speech impairment in down syndrome: a review. *J Speech Lang Hear Res*. (2013) 56:178–210. doi: 10.1044/1092-4388(2012/12-0148)

45. Joslyn N, Berger H, Skotko BG. Geospatial analyses of accessibility to down syndrome specialty care. *J Pediatr*. (2020) 218:146–150.e1. doi: 10.1016/j.jpeds.2019.10.058

46. Santoro SL, Campbell A, Balasubramanian A, Haugen K, Schafer K, Mobley W. Specialty clinics for adults with down syndrome: a clinic survey. *Am J Med Genet A*. (2021) 185:1767–75. doi: 10.1002/ajmg.a.62169

47. Morris MA, Dudgeon BJ, Yorkston K. A qualitative study of adult AAC users' experiences communicating with medical providers. *Disabil Rehabil Assist Technol*. (2013) 8:472–81. doi: 10.3109/17483107.2012.746398

48. Vorperian HK, Kent RD, Lee Y, Buhr KA. Vowel production in children and adults with down syndrome: fundamental and formant frequencies of the corner vowels. *J Speech Lang Hear Res*. (2023) 66:1208–39. doi: 10.1044/2022_JSLHR-22-00510

49. Wild A, Vorperian HK, Kent RD, Bolt DM, Austin D. Single-word speech intelligibility in children and adults with down syndrome. *Am J Speech Lang Pathol*. (2018) 27:222–36. doi: 10.1044/2017_AJSLP-17-0002

50. Shariq S, Cardoso Pinto AM, Budhathoki SS, Miller M, Cro S. Barriers and facilitators to the recruitment of disabled people to clinical trials: a scoping review. *Trials*. (2023) 24:171. doi: 10.1186/s13063-023-07142-1

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Dendritic morphology of motor neurons and interneurons within the compact, semicompact, and loose formations of the rat nucleus ambiguus

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Introduction: Motor neurons (MNs) within the nucleus ambiguus innervate the skeletal muscles of the larynx, pharynx, and oesophagus. These muscles are activated during vocalisation and swallowing and must be coordinated with several respiratory and other behaviours. Despite many studies evaluating the projections and orientation of MNs within the nucleus ambiguus, there is no quantitative information regarding the dendritic arbours of MNs residing in the compact, and semicompact/loose formations of the nucleus ambiguus..

Methods: In female and male Fischer 344 rats, we evaluated MN number using Nissl staining, and MN and non-MN dendritic morphology using Golgi–Cox impregnation Brightfield imaging of transverse Nissl sections (15 μm) were taken to stereologically assess the number of nucleus ambiguus MNs within the compact and semicompact/loose formations. Pseudo-confocal imaging of Golgi-impregnated neurons within the nucleus ambiguus (sectioned transversely at 180 μm) was traced in 3D to determine dendritic arbourisation.

Results: We found a greater abundance of MNs within the compact than the semicompact/loose formations. Dendritic lengths, complexity, and convex hull surface areas were greatest in MNs of the semicompact/loose formation, with compact formation MNs being smaller. MNs from both regions were larger than non-MNs reconstructed within the nucleus ambiguus.

Conclusion: Adding HBLS to the diet could be a potentially effective strategy to improve horses' health.

KEYWORDS

Golgi–Cox, brainstem, swallow, vocalisation, convex hull

Introduction

The generation of aerodigestive behaviours such as swallowing and vocalisation must be coordinated with respiration and involve a highly orchestrated activation of skeletal muscle within the larynx, pharynx, and oesophagus, whose motor neurons (MNs) reside within the nucleus ambiguus (Lawn, 1966; Holstege et al., 1983; Davis and Nail, 1984; Bieger and Hopkins, 1987; Portillo and Pasaro, 1988a,b; Altschuler et al., 1991; McGovern and Mazzone, 2010). A somatotopic organisation of the rat nucleus ambiguus has been shown to prevail, with the more rostral compact formation innervating oesophageal MNs, with the more caudal semicompact and loose formations innervating the palatopharyngeal

and laryngeal MNs, respectively (Fryscak et al., 1984; Bieger and Hopkins, 1987; Portillo and Pasaro, 1988b). Intense investigation of swallow and airway defence has identified a host of local brainstem patterns and premotor centres having projections to and from the nucleus ambiguus (Pitts et al., 2012; Bolser et al., 2015; Pitts and Iceman, 2023).

There are a variety of neural subtypes within the nucleus ambiguus, including cardiopulmonary neurons (McAllen and Spyer, 1978; Nosaka et al., 1979; Mazzone and Canning, 2013; Veerakumar et al., 2022) and extensive intermingling with the neurons of the ventral respiratory group (Ellenberger and Feldman, 1990a,b; Monnier et al., 2003; Neuhuber and Berthoud, 2022). Despite some strides being made in the molecular characterisation of various neurons within the nucleus ambiguus (Coverdell et al., 2022; Veerakumar et al., 2022), there is little quantified regarding the dendritic trees of the neuronal subtypes within the nucleus ambiguus. Indeed, extant data concern the general anatomical orientations of unspecified nucleus ambiguus MNs (Sun et al., 1995) or oesophageal MNs (Bieger and Hopkins, 1987; Altschuler et al., 1991; Kruszewska et al., 1994) and laryngeal MNs (Bieger and Hopkins, 1987), with no quantification of the dendritic or axonal processes, with all but one study in males alone and the outlier (Sun et al., 1995) having unspecified sexes.

In this study, we present the first detailed quantitative morphology (besides a limited Sholl analysis of seven neurons (Sun et al., 1995)) of MNs and interneurons within the rat nucleus ambiguus in male and female rats. We present these results stratified into anatomical locations within the rostral compact formation or more caudal semicompact and loose formations.

Methods

Ethical approval and experimental animals

All protocols were approved by the Mayo Clinic Institute Animal Care and Use Committee (IACUC #A57714) and complied with National Institutes of Health (NIH) and American Physiological Society guidelines. We used 10 female (five) and male (five) Fischer 344 rats of 6 months old obtained from Charles River. Rats were housed two rats per cage were housed under a 12 h: 12 h light–dark cycle with *ad libitum* access to food and water. The animals were allowed at least 1 week to acclimatise to these conditions before experiments were performed.

Nissl terminal procedures, processing, imaging, and stereological counting

A subset ($n = 6$, 3 females, 3 males) of Fischer 344 rats were deeply anaesthetised with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) and intracardially perfused with saline (euthanised via exsanguination) followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The fixed brainstem was then excised and post-fixed in 4% PFA in PBS overnight and then immersed overnight in 25% sucrose in PBS. Serial transverse 15 µm cryosections of the brainstem were cut and stained with 0.1% cresyl violet (v/v) in an acetic acid buffer using previously established methods (Fogarty, 2023). Brainstem images were created in a manner identical to previous studies (Fogarty, 2023) on a Zeiss Axioskop II equipped with a motorised stage 4x and 20x air objective (1.0 NA, Zeiss, Gottingen, Germany).

MN numbers were quantified unilaterally in the nucleus ambiguus, with landmark identification aided by a rat brain atlas (Paxinos, 1999; Paxinos and Watson, 2014) and past examples of retrogradely labelled rat laryngeal, pharyngeal, and oesophageal MNs (Wetzel et al., 1980; Hinrichsen and Ryan, 1981; Bieger and Hopkins, 1987; Portillo and Pasaro, 1988a; Altschuler et al., 1991; Flint et al., 1991; Sun et al., 1995; Hernandez-Morato et al., 2013). More specifically, the rostral boundary of the nucleus ambiguus comprised sections rostral to the hypoglossal nucleus, with the presence of the more rostral portion of the facial nucleus (Figure 1), consistent with reports from the cat (Holstege et al., 1983), and Sprague Dawley (Altschuler et al., 1991) and Wistar (Bieger and Hopkins, 1987; Paxinos and Watson, 2014) rats. The caudal extent of the nucleus ambiguus was approximated by that of the hypoglossal nucleus, within ~1 mm of the obex (consistent with the cat (Holstege et al., 1983; Holstege, 1989), and Sprague Dawley (Wetzel et al., 1980), Wistar (Hinrichsen and Ryan, 1981; Paxinos and Watson, 2014), and albino (Portillo and Pasaro, 1988a) rats) and complete enclosure of the central canal (Figure 1). As there was no readily identifiable demarcation between the rostral compact formation and the more caudal semicompact and loose formations, we approximated this border to be the halfway point between the most rostral extent of the nucleus ambiguus and the obex, consistent with reports in Sprague Dawley (Altschuler et al., 1991) and Wistar rats (Bieger and Hopkins, 1987; Paxinos and Watson, 2014). Occasionally (two of six rats used for Nissl), we would observe a cluster of larger neurons slightly medial (~10 degrees) and dorsal (~0.5–1 mm) from the nucleus ambiguus proper, usually in the section ~1 mm rostral to immediately after the obex in the longitudinal axis (Figure 1). These clusters are consistent with the dorsolateral group observed in rats (Wetzel et al., 1980; Hinrichsen and Ryan, 1981); however, we did not include these neurons in our quantifications as they do not project to laryngeal muscles (Hinrichsen and Ryan, 1981). We did not observe a dorsomedial cluster of neurons in these sections (Hinrichsen and Ryan, 1981).

Nucleus ambiguus MN counts were performed on every 10th section in a manner identical to previous nucleus ambiguus histological studies (Sturrock, 1990) on a Zeiss Axioskop II equipped with a motorised stage 20x air objective (1.0 NA, Zeiss, Gottingen, Germany). To qualify for counting, MNs had to be large cells (the mean of the long and short axis > 15 µm) (Odutola, 1976; Dobbins and Feldman, 1995; Fogarty, 2023), have a dark cytoplasm, and have a distinct pale nucleus and dark nucleoli, as outlined previously (Lance-Jones, 1982; Fogarty et al., 2013, 2015). Note that despite our sectioning of Nissl material in an axis (transverse) non-parallel to the axis of the maximum somal projection of nucleus ambiguus, a prior study using retrograde approaches and transverse sectioning observed major diameters of MN somas of >30 µm (Hernandez-Morato et al., 2013), consistent with our >15 µm radii classification. Neuronal fragments, without a nucleus and nucleoli, are not counted in order to avoid "double counting," consistent with stereological principles (Slomianka, 2020).

To estimate the MN surface area, the x and y radii of every hypoglossal MN counted were measured using ImageJ (Schneider et al., 2012), with surface areas calculated using the prolate spheroid approximation (Ulfhake and Cullheim, 1988).

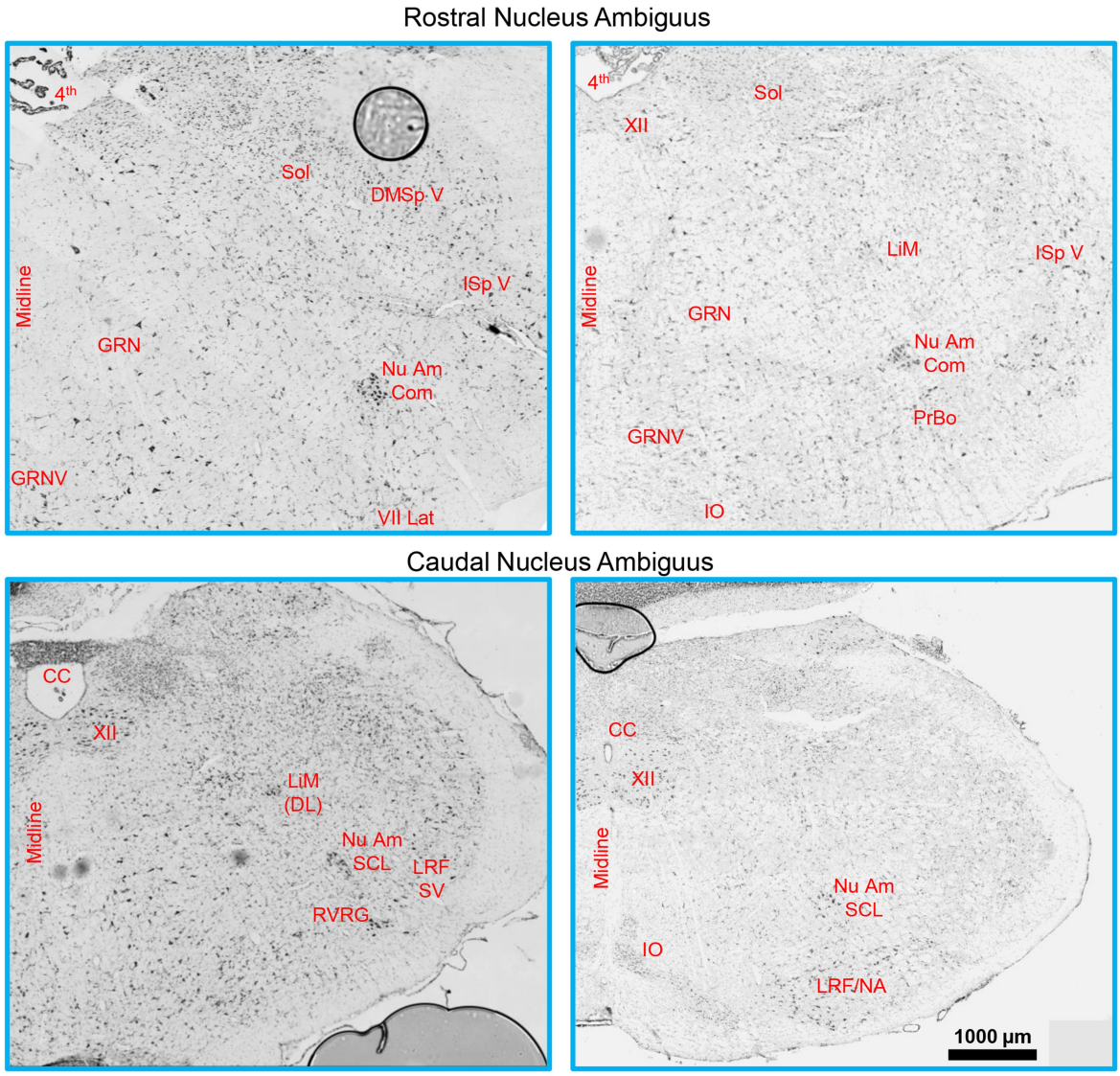

FIGURE 1
A Brainstem Nissl staining showing the rostral (top row) and caudal (bottom row) nucleus ambiguus identified. The compact formation of the nucleus ambiguus was relatively circular and started at the very caudal end of the lateral portion of the facial nucleus and extended to ~0.5–1 mm prior to the closure of the central canal. The semicompact/loose formations of the nucleus ambiguus were more ovoid in shape, commencing ~0.5–1 mm rostral to the formation of the central canal, and were immediately dorsal to the rostroventral respiratory group. 4th, fourth ventricle; CC, central canal; DMSp V, dorsomedial/spinal trigeminal nucleus; GRN, gigantocellular reticular nucleus; GRNV, ventral gigantocellular reticular nucleus; IO, inferior olive; ISp V, interpolar spinal trigeminal nucleus; LiM, linear nucleus of the medulla; LiM (DL), linear nucleus of the medulla (dorsolateral to main nucleus ambiguus); LRF/NA, lateral reticular formation noradrenaline cells; LRF SV, lateral reticular formation subtrigeminal region; Nu Am Com, compact formation of the nucleus ambiguus; Nu Am SCL, semicompact/loose formation of the nucleus ambiguus; PrBo, pre-pre-Bötzinger complex; RVRG, rostral ventral respiratory group; Sol, solitary tract; VII lat, lateral portion of the facial nucleus; XII, hypoglossal nucleus.

Golgi–Cox terminal procedures and processing

At the terminal experiment, a subset of animals ($n = 4$, 2 females, 2 males) were deeply anesthetised with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) and euthanised via exsanguination. Following euthanasia, the brainstem was removed and processed for Golgi–Cox impregnation (FD Rapid Golgi, FD NeuroTechnolgies) (Glaser and Van der Loos, 1981).

Following dissection, the brainstem was placed in Golgi–Cox impregnation solution for 16–18 days and changed once after 24 h. Following impregnation, the brainstem was frozen in melting isopentane and prepared for cryosectioning at 180 µm. Transverse-sectioned brainstem slices were left on slides to dry overnight at 24°C and then developed, dehydrated with ethanol and xylene, and coverslipped.

Region selection, microscopy, and neuronal dendritic evaluation

The compact, semicompact, and loose regions of the nucleus ambiguus were located in transverse brainstem sections with the aid of

aforementioned brain atlases and publications. Regions were also cross-referenced with brainstem histology from Fischer 344 rats in the present study (Figure 1). The nucleus ambiguus was imaged under brightfield illumination with a 40X oil objective (1.3 NA, 1600x 1,600 pixel array) using a pseudo-confocal (2 µm step size, 200 nm pinhole) method (Williams et al., 1994), allowing for sufficient illumination (non-fluorescent) of the Golgi-impregnated material. We used mosaic imaging to visualise multiple neurons within the region at high magnification, at the expense of some tessellating stitching artefacts. Despite Golgi–Cox assessments not being particularly amenable to stereological approaches, we endeavoured to adhere to the stereological principles, including systematic random sampling (Slomianka, 2020). Here, we ensured an equal sampling throughout the rostrocaudal axis of the compact, semicompact, and loose divisions. To aid consistency in the locations assessed, and the necessity for clustering of MNs to readily identify the nucleus ambiguus, interneuron dendritic assessments were only done in regions containing MNs.

Interneurons and MNs were assessed in a three-dimensional manner using Neurolucida 11 Desktop Edition (MBF Bioscience) in a manner identical to our past efforts using Golgi–Cox (Klenowski et al., 2016; Fogarty et al., 2016b, 2017b, 2019b, 2020b), with the dorsal or ventral and medial or lateral anatomical projections of the dendrites noted, similar to past studies in mice and rats (Kruszewska et al., 1994; Kanjhan et al., 2016). Neurons with multi-polar dendrites were classified as MNs only if they had a somal long radius of >15 µm, in accordance with prior histological studies and with retrograde approaches in rats (Bieger and Hopkins, 1987; Altschuler et al., 1991; Hayakawa et al., 1996; Fogarty et al., 2018; Williams et al., 2019; Fogarty, 2023). Thus, MNs and interneurons were distinguished based on somal size alone, not dendritic size. Dendrites were distinguished from axons via their tapering with branch order (Kruszewska et al., 1994; Kanjhan et al., 2016).

Statistical methods

We used Prism 9 for all data analyses (GraphPad, Carlsbad, CA). Each data set was assessed for normality with D'Agostino and Pearson tests. *A priori* it was determined that within a particular data set, any data point outside 2.5 standard deviations from the mean was excluded from further analysis. Fortunately, we did not observe any outliers in the main outcome measures in the current study. Paired Student's *t*-tests or unpaired or paired two-way ANOVA, with Bonferroni *post-hoc* tests, were performed, where appropriate. Statistical significance was established at the $p < 0.05$ level, with all *p*-values reported to four decimal places in the text. All data are reported as the mean ± 95% confidence intervals unless otherwise noted.

Results

Region selection and motor neuron quantification in transverse sections

The compact, semicompact, and loose regions of the nucleus ambiguus were readily observed in female and male rats with the aid of a brainstem atlas (Paxinos, 1999; Paxinos and Watson, 2014) using Nissl staining (Figures 1, 2). These regions were matched to thicker Golgi–Cox-impregnated sections, where neurons meeting assessment criteria were traced and reconstructed in 3D.

The number of compact formation nucleus ambiguus MNs (1723 ± 277) exceeded that of semicompact/loose formation MNs (974 ± 205) by almost 2-fold ($p = 0.0014$, Student's paired *t*-test; Figure 2C). Similarly, the percentage of total nucleus ambiguus MN within the compact formation (64 ± 5%) exceeded that of semicompact/loose formation MNs (36 ± 5%) by almost 2-fold ($p = 0.0011$, Student's paired *t*-test; Figure 2D). There was no difference in the somal surface areas between nucleus ambiguus MNs form the compact formation (1,541 ± 127 µm^2) compared to MNs for the semicompact/loose formations (1,502 ± 171 µm^2, $p = 0.6392$, Student's paired *t*-test; Figure 2E).

Dendritic tree lengths in nucleus ambiguus motor neurons and interneurons

Golgi–Cox impregnation readily labelled neurons within the nucleus ambiguus (Figure 3). Interneurons within the compact or semicompact/loose formations and MNs within the compact or semicompact/loose formations of the nucleus ambiguus were traced in 3D for morphometric quantifications (Figure 3). The total length of the dendritic arbour of nucleus ambiguus MNs and interneurons was dependent on the neuronal type ($F_{(1,103)} = 81.6$, $p < 0.0001$) and region ($F_{(1,103)} = 6.9$, $p = 0.010$, two-way ANOVA; Figure 4A). MNs within the compact formation (2027 ± 200 µm) had ~29% smaller total arbour lengths than MNs from the semicompact/loose formations (2,787 ± 468 µm; $p = 0.0016$, Bonferroni post-test; Figure 4A). Compact formation interneurons (1,107 ± 208 µm) exhibited ~half the total dendritic arbour length of compact ($p = 0.0002$) and semicompact/loose MNs ($p < 0.0001$, Bonferroni post-tests; Figure 4A). Similarly, semicompact/loose formations interneurons (1,107 ± 197 µm) exhibited ~half the total dendritic arbour length of compact ($p = 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 4A). There was no difference in total dendritic arbour length between interneurons of either region ($p > 0.99$, Bonferroni post-test; Figure 4A).

The mean length of each individual dendritic tree (tree length) of nucleus ambiguus MNs and interneurons was dependent on the neuronal type ($F_{(1,103)} = 88.5$, $p < 0.0001$) and region ($F_{(1,103)} = 10.7$, $p = 0.0014$; two-way ANOVA; Figure 4B). MNs within the compact formation (525 ± 47 µm) had ~25% smaller mean tree lengths than MNs from the semicompact/loose formations (718 ± 101 µm; $p = 0.005$, Bonferroni post-test; Figure 4B). Compact formation interneurons (290 ± 51 µm) exhibited ~45–60% the mean dendritic tree length of compact ($p < 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 4B). Similarly, semicompact/loose formations interneurons (318 ± 57 µm) exhibited ~50–65% the mean dendritic tree length of compact ($p = 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 4B). There was no difference in mean dendritic tree length between interneurons of either region ($p > 0.99$, Bonferroni post-test; Figure 4B).

When analysed with respect to neural branch order (from first to eighth and beyond), the total dendritic arbour length within a branch order was dependent on the branch order ($F_{(7,721)} = 28.4$, $p < 0.0001$), neuronal type ($F_{(1,103)} = 42.9$, $p < 0.0001$), and region*type interaction ($F_{(1,103)} = 0.5$, $p = 0.4525$; three-way ANOVA; Figure 4C). Bonferroni *post-hoc* tests show reduced dendritic length in nucleus ambiguus

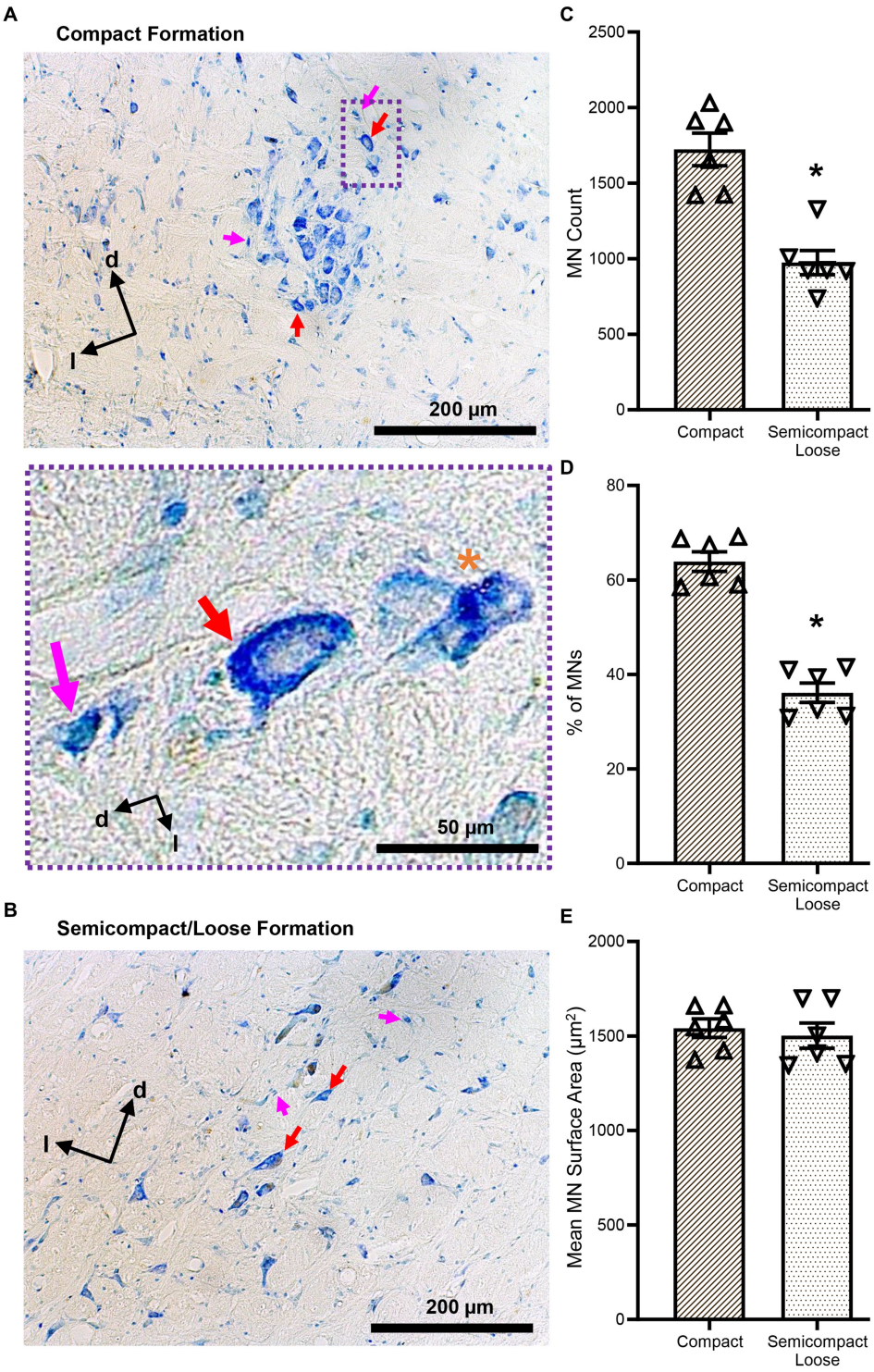

FIGURE 2
(A,B) Show brightfield images of Nissl-stained MNs within the compact and semicompact/loose formations, respectively, exhibiting classical histological characteristics of large cytoplasm and prominent nucleoli (red arrows). Non-MNs (putative interneurons), excluded from MN counts, are identified by pink arrows. Dorsal (d) and lateral (l) are also indicated on the images. The inset (purple dashed area) shows a higher powered image of an excluded non-MN (pink arrow) an example MN (red arrow), and a fragmented cell excluded from counting (orange asterisk). **(C)** Plots of reduced MN counts (mean ± 95% CI) in the semicompact/loose formations of the nucleus ambiguus compared to the compact formation. **(D)** Plots of reduced % of total MN (mean ± 95% CI) in the semicompact/loose formations of the nucleus ambiguus compared to the compact formation. **(E)** Plots of unchanged mean MN surface areas (mean ± 95% CI) between semicompact/loose formations and the compact formation of the nucleus ambiguus. All analyses were carried out using paired Student's *t*-tests, *denotes statistical differences between groups (i.e., $p < 0.05$).

interneurons compared to MNs at second ($p < 0.0001$), third ($p < 0.0001$), fourth ($p < 0.0001$), fifth ($p < 0.0001$), and eighth and beyond ($p = 0.0211$) branch orders, regardless of region (Figure 4C). In addition, semicompact/loose formation MNs had more total dendritic arbour length at the eighth and beyond branch orders than compact formation MNs ($p = 0.0130$; Figure 4C).

When analysed with respect to neural branch order (from first to eighth and beyond), the mean dendritic tree length within a branch order was dependent on the branch order ($F_{(7,721)} = 26.4$, $p < 0.0001$), neuronal type ($F_{(1,103)} = 42.9$, $p < 0.0001$), and a region*type interaction ($F_{(1,103)} = 4.3$, $p = 0.041$; three-way ANOVA; Figure 4D). Bonferroni *post-hoc* tests show reduced mean tree dendritic length in nucleus ambiguus interneurons compared to MN at the at the 2nd ($p = 0.0025$), 4th ($p = 0.0002$), 5th ($p < 0.0001$), 6th ($p = 0.0037$), and 8th and beyond ($p = 0.0004$) branch orders, regardless of region (Figure 4D). In addition, semicompact/loose formation MNs had more mean dendritic tree arbour length at the 8th and beyond branch orders than compact formation MNs ($p < 0.0001$; Figure 4D).

Dendritic tree surface areas in nucleus ambiguus motor neurons and interneurons

Dendritic diameters can be readily determined from Golgi–Cox-impregnated material (Figure 3A), with dendritic surface areas closely related to passive MN capacitance and intrinsic excitability (Rall, 1959, 1960, 1977; Ulrich et al., 1994). The total surface area of the dendritic arbour of nucleus ambiguus MNs and interneurons was dependent on the neuronal type ($F_{(1,103)} = 163.9$, $p < 0.0001$), but not region ($F_{(1,103)} < 0.01$, $p = 0.8235$, Figure 5A). MNs within the compact formation ($10{,}111 \pm 978\,\mu m^2$) had greater surface areas than interneurons within the compact ($4{,}700 \pm 1{,}081\,\mu m^2$; $p < 0.0001$) and semicompact/loose formations ($3{,}330 \pm 725\,\mu m^2$, $p < 0.0001$, Bonferroni post-test; Figure 5A). Similarly, MNs within the semicompact/loose formations ($11{,}733 \pm 1{,}516\,\mu m^2$) had greater surface areas than interneurons within the compact ($p < 0.0001$) and semicompact/loose formations ($p < 0.0001$, Bonferroni post-test; Figure 5A). There was no difference in the total dendritic surface area between MNs from different regions ($p = 0.2085$) nor between interneurons of different regions ($p = 0.4717$, Bonferroni post-test; Figure 5A). In particular, the range (semicompact/loose: $14369\,\mu m^2$; compact: $9494\,\mu m^2$) and interquartile range (semicompact/loose: $5938\,\mu m^2$; compact: $2546\,\mu m^2$) of the total dendritic surface area were markedly increased in semicompact/loose formation compared to compact formation MNs.

The mean dendritic tree surface area of nucleus ambiguus MNs and interneurons was dependent on the neuronal type ($F_{(1,103)} = 109.9$, $p < 0.0001$) but not region ($F_{(1,103)} < 0.01$, $p = 0.9293$, Figure 5B). MNs within the compact formation ($2{,}936 \pm 356\,\mu m^2$) had greater mean tree surface areas than interneurons within the compact ($1{,}309 \pm 361\,\mu m^2$; $p < 0.0001$) and semicompact/loose formations ($1{,}001 \pm 232\,\mu m^2$; $p < 0.0001$, Bonferroni post-test; Figure 5B). Similarly, MNs within the semicompact/loose formations ($3{,}212 \pm 514\,\mu m^2$) had greater mean tree surface areas than interneurons within the compact ($p < 0.0001$) and semicompact/loose formations ($p < 0.0001$, Bonferroni post-test; Figure 5B). There was no difference in the mean tree surface area between MNs from different regions ($p > 0.99$), nor between interneurons of different regions ($p > 0.99$, Bonferroni post-test; Figure 5B). In particular, the range (semicompact/loose: $14369\,\mu m^2$; compact: $9494\,\mu m^2$) and interquartile range (semicompact/loose: $1957\,\mu m^2$; compact: $1111\,\mu m^2$) of the mean tree dendritic surface area was markedly increased in semicompact/loose formation compared to compact formation MNs.

Dendritic tree complexity in nucleus ambiguus motor neurons and interneurons

Sholl analysis of dendritic arbours provides an estimate of the complexity of a dendritic arbour (Sholl, 1953; Bird and Cuntz, 2019). We evaluated the number of intersections per Sholl radii at 10 µm intervals from the soma out to 200 µm and farther away from the soma (Figure 6). The number of intersections was dependent on the branch order ($F_{(19,1957)} = 40.0$, $p < 0.0001$), neuronal type ($F_{(1,103)} = 54.3$, $p < 0.0001$), and region*type interaction ($F_{(1,103)} = 23.9$, $p = 0.0194$; three-way ANOVA; Figure 6). Nucleus ambiguus MNs from the compact and semicompact/loose formations had greater interactions than compact formation interneurons and semicompact/loose formation interneurons from the 80th to the 120th µm, and the 200 µm and beyond radii ($p < 0.04$ in all cases, Bonferroni post-tests; Figure 6). Additionally, nucleus ambiguus MNs from the semicompact/loose formations had greater intersections than compact MNs at 200 µm and beyond radii ($p < 0.0001$, Bonferroni post-test; Figure 6).

Dendritic tree convex hull assessments in nucleus ambiguus motor neurons and interneurons

Convex hull assessments are highly correlated with the overall field of inputs various neural types receive and thus an estimate of the receptive area (synapses and dendro–dendro contacts) of an individual neuron (Malmierca et al., 1995; Rojo et al., 2016; Bird and Cuntz, 2019) as opposed to an indication of passive computational properties (Marks and Burke, 2007). Convex hull structures were readily differentiated in nucleus ambiguus MNs and interneurons (Figure 7A). The total dendritic convex hull surface area of the dendritic arbours of nucleus ambiguus MNs and interneurons was dependent on the neuronal type ($F_{(1,103)} = 128.0$, $p < 0.0001$) and region ($F_{(1,103)} = 4.7$, $p = 0.0332$, Figure 7B). MNs within the compact formation ($128{,}128 \pm 18{,}564\,\mu m^2$) had ~25% smaller total dendritic convex hull surface areas than MNs from the semicompact/loose formations ($168{,}764 \pm 28{,}687\,\mu m^2$, $p = 0.0135$, Bonferroni post-test; Figure 7B). Compact formation interneurons ($43{,}718 \pm 12{,}017\,\mu m^2$) exhibited ~24–35% of the total dendritic convex hull surface area of compact ($p < 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 7B). Similarly, semicompact/loose formations interneurons ($43{,}158 \pm 10{,}563\,\mu m^2$) exhibited ~25–35% of the total dendritic convex hull surface area of compact ($p < 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 7B). There was no difference in the total dendritic convex hull surface area between interneurons of either region ($p > 0.99$, Bonferroni post-test; Figure 7B).

The mean dendritic tree convex hull surface area of each individual dendritic tree of nucleus ambiguus MNs and interneurons was dependent on the neuronal type ($F_{(1,103)} = 98.1$, $p < 0.0001$) and region ($F_{(1,103)} = 6.2$, $p = 0.00144$, Figure 7C). MNs within the compact formation ($33{,}240 \pm 4{,}638\,\mu m^2$) had ~27% smaller mean dendritic tree convex hull

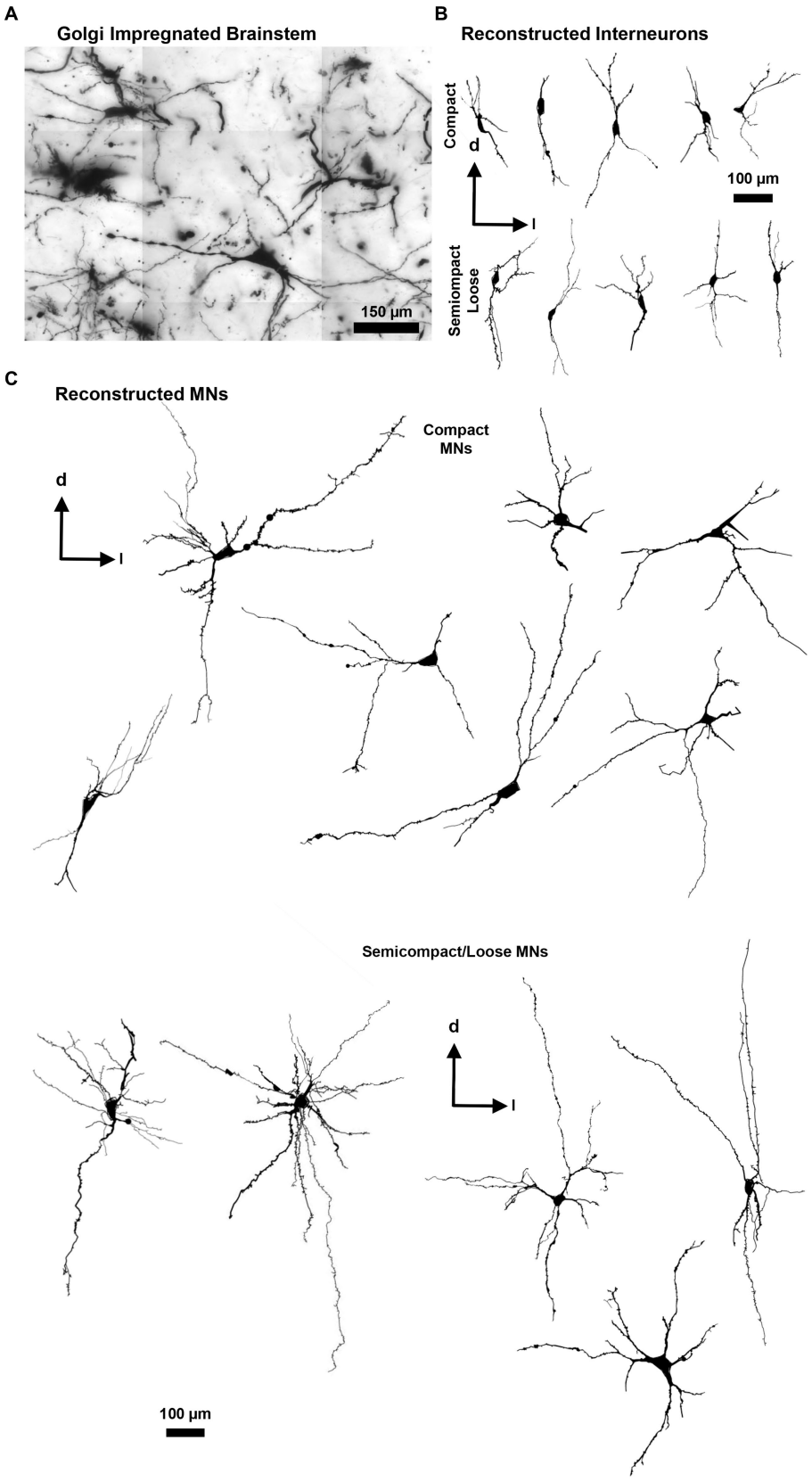

FIGURE 3
(A) Golgi-impregnated brainstem showing staining of nucleus ambiguus neurons. The tessellations are an artefact of the mosaic imaging process, this image is a minimum-intensity projection of five optical slices. **(B)** Representative Neurolucida tracings of nucleus ambiguus interneurons within the complex formation (top row) and the semicomplex/loose formations (bottom row). **(C)** Representative Neurolucida tracings of nucleus ambiguus MNs within the complex formation (top group) and the semicomplex/loose formations (bottom group). Dorsal (d) and lateral (l) are also indicated on the images.

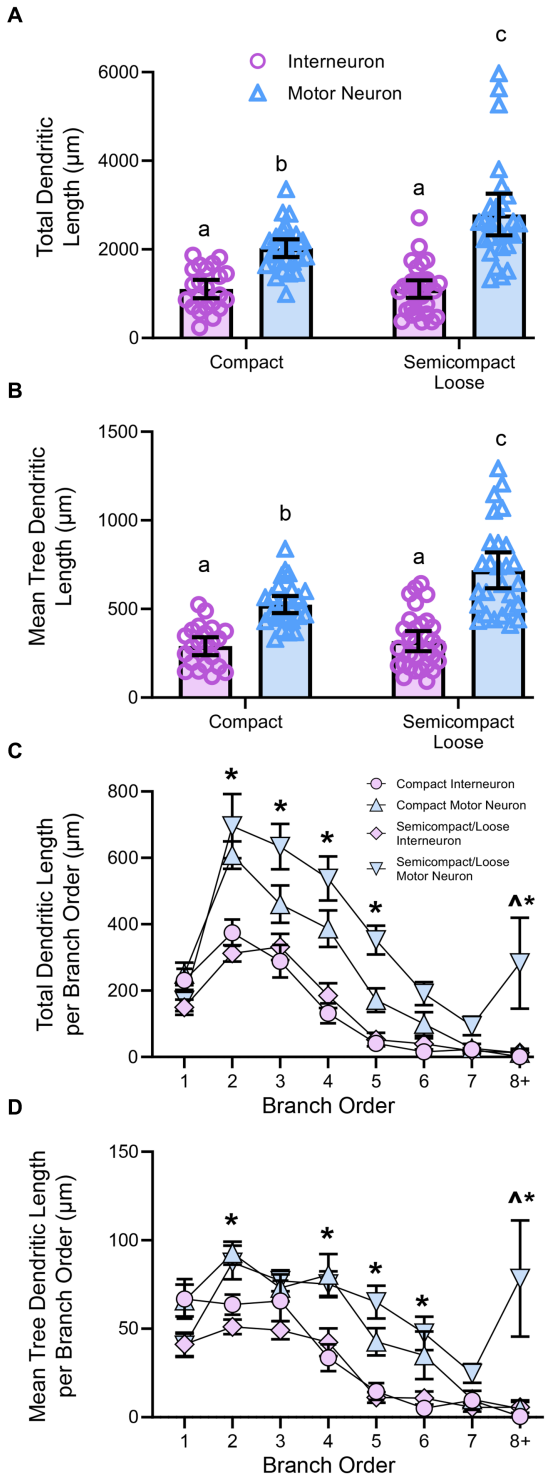

FIGURE 4
(A) Plots showing smaller total dendritic tree length (± 95% CI) in nucleus ambiguus interneurons compared to MNs, with semicompact/loose formation MN larger than compact formation MNs (two-way ANOVA with Bonferroni post-tests, with different letters denoting statistical differences between groups [i.e., $p < 0.05$]). **(B)** Plots showing smaller mean tree dendritic length (± 95% CI) in nucleus ambiguus interneurons compared to MNs, with semicompact/loose formation MN larger than compact formation MNs (two-way ANOVA with Bonferroni post-tests, with different letters denoting statistical differences between groups [i.e., $p < 0.05$]). **(C)** Plots of reduced total dendritic length per branch order of nucleus ambiguus interneurons compared to MNs; and reductions in compact MNs compared to semicompact/loose MNs at the eighth branch order and greater (three-way ANOVA with Bonferroni post-tests, *denotes the statistical difference between MNs and interneurons, ^denotes the statistical difference between compact MNs and semicompact/loose MNs [i.e., $p < 0.05$]). **(D)** Plots of reduced mean tree dendritic length per branch order of nucleus ambiguus interneurons compared to MNs; and reductions in compact MNs compared to semicompact/loose MNs at the eighth branch order and greater (three-way ANOVA with Bonferroni post-tests, *denotes statistical difference between MNs and interneurons, ^denotes statistical difference between compact MNs and semicompact/loose MNs [i.e., $p < 0.05$]). Dorsal (d) and lateral (l) are also indicated on the images.

surface area than MNs from the semicompact/loose formations (45,265 ± 8,479 µm^2; $p = 0.0114$, Bonferroni post-test; Figure 7C). Compact formation interneurons (11,820 ± 3,398 µm^2) exhibited ~25–35% of the mean dendritic tree convex hull surface area of compact ($p < 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 7C). Similarly, semicompact/loose formations interneurons (13,235 ± 3,689 µm$_2$) exhibited ~30–40% the mean dendritic tree convex hull surface area of compact ($p = 0.0001$) and semicompact MNs ($p < 0.0001$, Bonferroni post-tests; Figure 7C). There was no difference in mean dendritic tree convex hull surface area between interneurons of either region ($p > 0.99$, Bonferroni post-test; Figure 7C).

Discussion

Our study is the first to quantify the dendritic morphologies of MNs and interneurons within the compact, semicompact, and loose formations of the nucleus ambiguus. Our study had six major findings: (i) the number of MNs in the compact formation of the nucleus ambiguus exceeds those of the semicompact/loose regions; (ii) there were no systematic differences in MN size between different regions of the nucleus ambiguus; (iii) the dendritic lengths were greatest in MNs of the semicompact/loose regions than compact formation MN, with interneurons from both regions smaller than MNs; (iv) dendritic surface areas were greater in MNs than interneurons; (v) dendritic complexity was greatest in MNs of the semicompact/loose regions than compact formation MNs, with interneurons from both regions less complex than MNs; and (vi) dendritic convex hull area was greatest in MNs of the semicompact/loose regions than compact formation MNs, with interneurons from both regions less complex than MNs. These findings are interpreted in the context of the functional roles of the pharynx, larynx, and oesophagus during aerodigestive behaviours.

Delineation of the formations within the nucleus ambiguus is not straightforward, particularly in cases such as ours where retrograde labelling was not used; thus, we did not attempt to separate the semicompact from the loose formation. Nonetheless, given the inability of intramuscular approaches to quantify MN pools in their entirety (Mantilla et al., 2009), we are confident that our stereological approach provides a robust and reliable estimate of total nucleus ambiguus MNs (Sturrock, 1990; Slomianka, 2020) and a reasonable estimate of rostrocaudal regional differences. A major limitation of the present study is that our approach was not sensitive enough to observe MN somal size differences previously reported across regions (Hernandez-Morato et al., 2013). This is likely due to our underestimation of somal surface areas due to our sectioning being in the transverse, as opposed

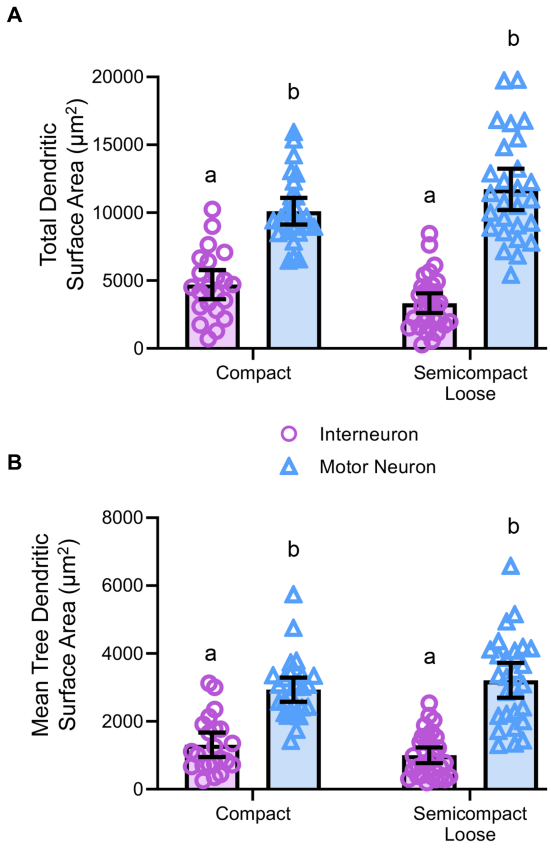

FIGURE 5
(A) Plots of smaller total dendritic surface area (± 95% CI) in nucleus ambiguus interneurons compared to MNs. **(B)** Plots of smaller mean tree surface areas (± 95% CI) in nucleus ambiguus interneurons compared to MNs. All analyses are two-way ANOVAs with Bonferroni post-tests, where appropriate. Different superscript letters denote statistical differences between groups (i.e., $p < 0.05$).

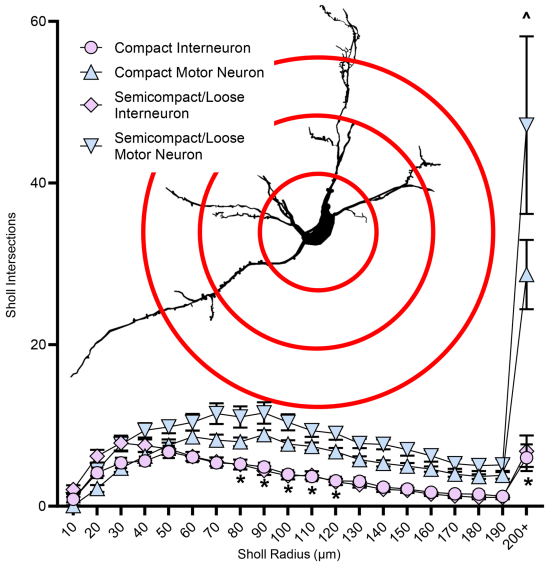

FIGURE 6
Plots of reduced dendritic Sholl interactions (mean ± SEM) in nucleus ambiguus interneurons compared to MNs, with semicompact/loose formation MNs having more Sholl interactions at very distal regions compared to compact formation MNs. Analysis three-way ANOVA with Bonferroni post-tests, *denotes statistical difference between MNs and interneurons, ^ denotes statistical difference between compact MNs and semicompact/loose MNs (i.e., $p < 0.05$). Insets show examples of Sholl analysis of a nucleus ambiguus MN; Sholl radii (red) are separated by 75 µm.

to the sagittal plane, where MN somal projections are greatest (Bieger and Hopkins, 1987). A reduced proportion of total nucleus ambiguus MNs within the semicompact/loose formation compared to the compact formation is consistent with prior reports (Sturrock, 1990; McGovern and Mazzone, 2010), and our total numbers resemble similar multiples (i.e., 2–2.5 times the MN number) of other brainstem nuclei in mice (Sturrock, 1990), previously observed in facial and hypoglossal MNs (Johnson and Duberley, 1998; Fogarty, 2023).

Overall surface areas of nucleus ambiguus MNs of Fischer 344 rats were slightly smaller than other brainstem (i.e., hypoglossal) and spinal cord (i.e., phrenic) MNs in Fischer 344 rats (Fogarty and Sieck, 2023). Although larger MNs are more likely to comprise fast fatiguable motor units compared to smaller MNs, comprising slow or fast fatiguable MNs (Burke et al., 1973; Dick et al., 1987; Heckman and Enoka, 2012; Fogarty and Sieck, 2019). This premise holds within rather than between motor pools, given the difference in MN sizes between different motor pools with muscles exhibiting mixed skeletal muscle fibre types (Brandenburg et al., 2018, 2020), particularly evident in brainstem MNs innervating orofacial muscles (Hinrichsen and Dulhunty, 1982; Rhee et al., 2004; Fogarty and Sieck, 2021; Sieck et al., 2023). Indeed, despite being smaller than hypoglossal MNs, laryngeal, pharyngeal, and oesophageal muscles express palpable levels of the myosin heavy chain type IIx ($MyHC_{2X}$) (Rhee et al., 2004) or lower succinate dehydrogenase and mitochondrial volume density (Hinrichsen and Dulhunty, 1982), consistent with being fast intermediate (fatiguable) motor units (Brown et al., 2021b). Based on the past findings in aged Fischer 344 rats (Hashizume et al., 1988; Jacob, 1998; Kanda and Hashizume, 1998; Fogarty et al., 2018; Fogarty, 2023; Fogarty and Sieck, 2023) and facial (Johnson and Duberley, 1998) and findings in Fisher–Brown Norway crosses (Basken et al., 2012), we would expect nucleus ambiguus MNs to be vulnerable to age-associated death. This vulnerability would be consistent with age-associated dysphonias and dysphagias (Turley and Cohen, 2009; Basken et al., 2012; Marino and Johns, 2014; Thiyagalingam et al., 2021).

Our findings show that MNs within the nucleus ambiguus had much larger dendritic arbours than interneurons, regardless of whether they resided within the compact or semicompact/loose formations. This is not merely a function of somal size as interneurons in a variety of regions possess dendritic arbours that approach the lengths and projection of pyramidal neurons (Porter et al., 2001; Kecskes et al., 2013). These differences can be largely attributed to the more elaborate distal branches of brainstem MNs (Kruszewska et al., 1994; Sun et al., 1995; Hayakawa et al., 1996; Kanjhan et al., 2016; Fogarty et al., 2016a, 2017a,b, 2020b) compared to more simplistic interneurons (Hayakawa et al., 1996; Kubota et al., 2011; Klenowski et al., 2015). In addition, branch structure analyses showed an increased dendritic length of semicompact/loose MNs compared to compact formation MNs. These patterns of very large semicompact/loose formation MNs, large compact formation MNs and smaller interneurons were consistent with the dendritic complexity as evidenced by Sholl analysis. In addition to these passive properties,

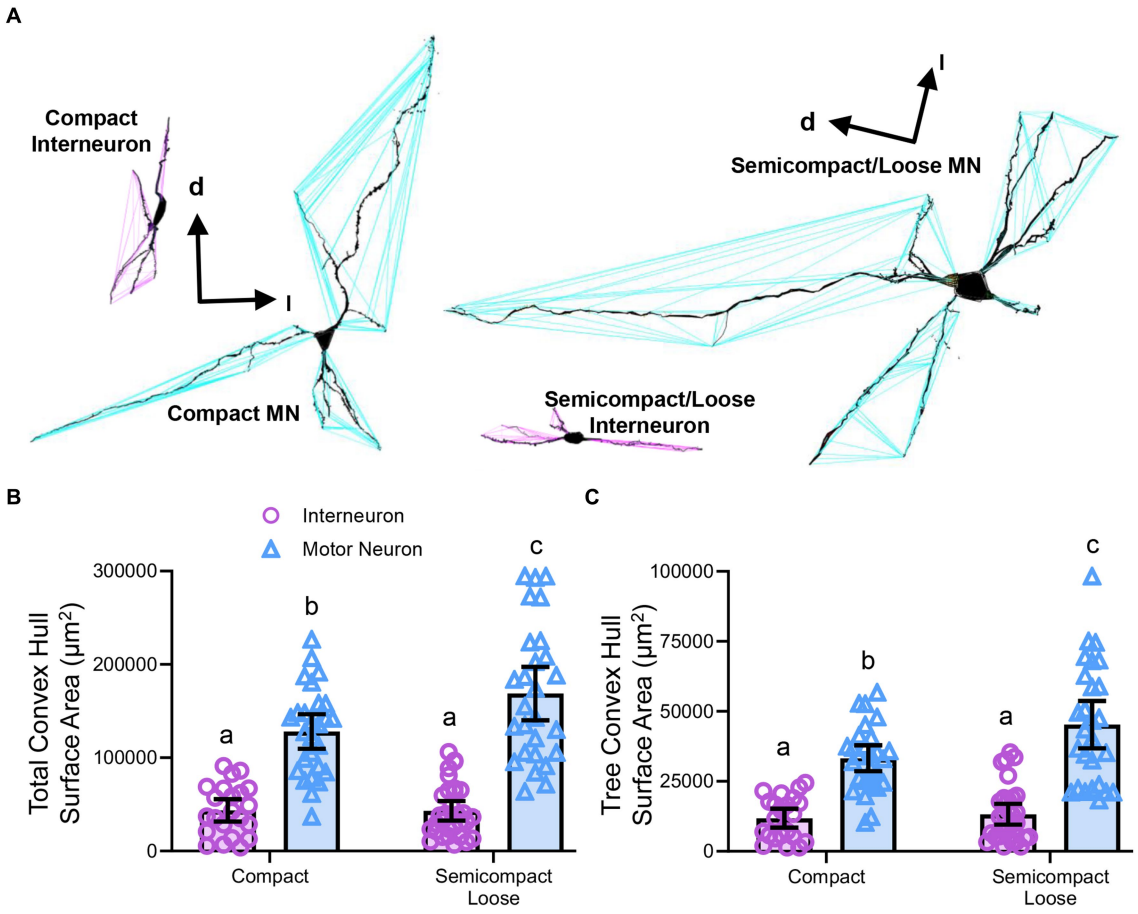

FIGURE 7
(A) 3D reconstruction of convex hulls (purple and blue polygons) of compact, semicompact/loose MNs and interneurons. **(B)** Plot shows smaller total dendritic tree convex hull surface areas (± 95% CI) in nucleus ambiguus interneurons compared to MNs, with semicompact/loose formation MN larger than compact formation MNs. **(C)** Plot shows smaller mean tree convex hull surface areas (± 95% CI) in nucleus ambiguus interneurons compared to MNs, with semicompact/loose formation MN larger than compact formation MNs. All analyses two-way ANOVAs with Bonferroni post-tests, with different letters denoting statistical differences between groups (i.e., $p < 0.05$). Dorsal (d) and lateral (l) are also indicated on the images.

the receptive fields of neurons within the nucleus ambiguus held to an identical outline, with convex hull surface areas extremely large in semicompact/loose formation MNs, large in compact formation MNs, and smallest in the interneurons. Moreover, our quantitative data are consistent with qualitative data in transverse brainstem slices of adult Sprague Dawley rats labelled with neurobiotin (Sun et al., 1995), where dendritic restrictions within the compact formation MNs were noted, compared to the more elaborate projections of laryngeal MNs within the semicompact formation.

In the present study, we did not examine the activities of the individual neurons, nor did we examine circuit activation and motor or cardiorespiratory responses. However, an abundance of prior retrograde tracing studies provides us with a reasonable idea of the normal physiological role of each neural population. These studies provide some clues as to why the morphologies of the MNs differ across regions within the nucleus ambiguus. As aforementioned, the MNs within the compact formation innervate the oesophagus (Holstege et al., 1983; Fryscak et al., 1984; Bieger and Hopkins, 1987; Altschuler et al., 1991), whilst the more caudal nucleus ambiguus MNs of the semicompact and loose formations innervate the palate and larynx (Hinrichsen and Ryan, 1981; Holstege et al., 1983; Flint et al., 1991; Hayakawa et al., 1996; Basken et al., 2012). Despite eschewing retrograde labelling, being largely incompatible with our Golgi approach, we used these extensive prior characterisations, the first-rate transverse-orientation rat brain atlas (Paxinos and Watson, 2014), and our cross-matching of histology in the Fischer 344 rat (this study). We therefore suggest that anatomical variability does not affect our study to a greater extent than the widely appreciated intermingling of neurons (particularly the ventrolateral respiratory group) within the semicompact/loose formations previously identified (Ellenberger and Feldman, 1990b; Sun et al., 1995). Although the nucleus ambiguus MN dendrites are reported to project in a longitudinal (parasagittal) manner (Bieger and Hopkins, 1987; Altschuler et al., 1991; Kruszewska et al., 1994; Hayakawa et al., 1996), two of these papers illustrate large transverse projections (Bieger and Hopkins, 1987; Altschuler et al., 1991), and one paper shows non-compact formation neuron, consistent with a semicompact/loose MN having extensive dorso-ventral projections (consistent with being oriented in the transverse plane) (Kruszewska et al., 1994). In addition, a prior study using intracellular labelling showed extensive arbourisation of nucleus ambiguus MNs in the transverse plane (Sun et al., 1995). Thus, given the advantages of anatomic orientation provided by transverse

sectioning (Figure 1), we proceeded with this approach—although our neuronal arbourisation quantifications may be an underestimate, particularly of total arbours as some trees of the compact formation MNs may have been obscured in the z-axis. Our final major limitation is the shrinkage of brainstem sections during processing (Kruszewska et al., 1994), which may hinder the classification of MNs, although this likely puts MNs in the interneuron category, due to reductions in size below the 30-μm-diameter MN threshold (Bieger and Hopkins, 1987; Altschuler et al., 1991; Hayakawa et al., 1996; Fogarty et al., 2018; Fogarty, 2023). Thus, our interneuron groups within the nucleus ambiguus dendritic assessments may include some of the smaller MNs. Indeed, these interneurons may be parasympathetic neurons, visceral and branchial efferents, gamma MNs, or shrunken small alpha MNs (Kalia and Mesulam, 1980; Hinrichsen and Ryan, 1981; Stuesse and Fish, 1984; Portillo and Pasaro, 1988a). Despite gamma MNs being important in other MN pools, it is unlikely that the smaller neurons in the population within the nucleus ambiguus contain gamma MNs as proprioception and muscle spindles are not readily identified in rat laryngeal muscles (Andrew, 1954, 1955, 1956a). So more correctly, the interneurons may be considered putative interneurons or non-MNs.

In the MNs of the compact formation, there is an overall need to coordinate oesophageal functions with ventilation. The close proximity of the compact region MNs and the relative restriction of their length and convex hull surface areas compared to semicompact/loose MNs is consistent with the necessity for synchronous activation of the cervical oesophagus and coordinated propagation in the thoracic and abdominal regions of the oesophagus (Altschuler et al., 1991; Kruszewska et al., 1994). The lack of extensive dendritic lengths at eighth and beyond branch orders is also consistent with this premise. This oesophageal activation in swallow is likely highly stereotyped in action [i.e., a reflex binary, initiated synchronously by distension or by the initiation of the swallow pattern (Andrew, 1956b; Meyer and Castell, 1982; Paterson, 1999; Broussard and Altschuler, 2000)], similar to the laryngeal functions in ventilation and swallow (Andrew, 1955; Fregosi and Ludlow, 2014; Huff et al., 2022; Pitts and Iceman, 2023). By contrast, laryngeal muscles operate over a wider range of behaviours (notably changes in pitch during vocalisation) (Wetzel et al., 1980; Bartlett and Knuth, 1984; Jurgens, 2009; Riede et al., 2020) and emotional states (Geyer et al., 1978; Geyer and Barfield, 1978; McIntosh et al., 1978; Barfield et al., 1979; Vanderhorst et al., 2000; Holstege, 2014), particularly in humans (Levin, 2006; Pisanski et al., 2022). Synchronous activation of MNs and their innervated muscle can be facilitated via: (i) gap junctions, which are not prevalent in oesophageal nucleus ambiguus MNs (Kruszewska et al., 1994); (ii) homogeneous high intrinsic excitability (i.e., less dendritic length/surface area leading to reduced neural capacitance), evident in findings of reduced dendritic surface areas and narrower ranges dendritic surface area in compact, compared to semicompact/loose MNs; and (iii) constrained dendritic projections, integrating inputs from fewer brainstem areas, evident in the reduced Sholl and dendritic convex hull surface areas of compact, compared to semicompact/loose MNs.

What remains evolutionarily peculiar is the observation that these oesophageal-innervating MNs are rostral to both pharyngeal MNs and the disparate brainstem swallow centres within the nucleus of the solitary tract and the dorsal and ventral swallow groups (Holstege et al., 1983; Kessler and Jean, 1985a,b; Zheng et al., 1997; Jean, 2001; Huff et al., 2022; Pitts and Iceman, 2023). Second, these neurons are activated last in the swallow phase—first oral, then pharyngeal, then oesophageal (Pitts and Iceman, 2023). Oesophageal activity seems impervious to unilateral dysfunction, due to a bilateral spread of innervated fibres within the oesophagus (Gruber, 1978). Moreover, in these rats, biochemical analysis of fibres is consistent with type IIa fibres, relatively resilient to age-associated weakness and denervation (Fogarty et al., 2019a, 2020a; Brown et al., 2021a; Sieck et al., 2023). Thus, the oesophageal functions and phase of swallow may be less vulnerable to perturbations of age and degeneration than those involved in the pharyngeal and oral phases. In particular, the resilience of compact formation nucleus ambiguus is evident in aged mice, where ~20% death of compact formation retrofacial nucleus ambiguus MNs compared to an overall loss of ~35% (Sturrock, 1990). In brainstem and spinal MNs of rats and humans, there are known motor unit type-dependent differences in vulnerability, with slow and fatigue-resistant MNs resilient and fatigueable MNs prone to death in old age (Hashizume et al., 1988; Kanda and Hashizume, 1998; Fogarty et al., 2018; Fogarty, 2023) and neurodegenerative conditions (Kiernan and Hudson, 1991; Pun et al., 2006; Dukkipati et al., 2018). Type-dependent differences in dendritic arbours, with larger MNs having larger arbours MNs (Cullheim et al., 1987; Ma and Vacca-Galloway, 1991; Leroy et al., 2014; Fogarty et al., 2019b, 2020b) and synaptic inputs, larger MNs having more excitatory inputs (Kellerth et al., 1979; Brannstrom, 1993) exist in MNs and may underpin differential susceptibility to degeneration and death (Ahmed et al., 2016; Fogarty, 2018; Kiernan et al., 2019). Our current observation of relatively restricted dendritic arbour size and previous quantifications of less synaptic inputs compared to pharyngeal MNs (Hayakawa et al., 1996) is consistent with a propensity of this region to harbour MNs comprising fatigue-resistant (slow or fast) motor units. It remains to be determined whether compact formation MNs and their dendritic arbours are conserved with age and neurodegeneration in the rat. We would expect that in old age, compact formation MNs would be spared in number and degenerative phenotype.

The MNs of the semicompact/loose formations, innervating the palate and laryngeal muscles, are activated during vocalisation and the oral and pharyngeal phases of swallow (Wetzel et al., 1980; Holstege et al., 1983; Holstege, 1989; Vanderhorst et al., 2000; Jurgens, 2009). Indeed, interactions between emotional processing centres of the brainstem and the muscles of speech/vocalisation are becoming increasingly studied (Vanderhorst et al., 2000; Holstege, 2014; Subramanian et al., 2018, 2021). Similarly to oesophageal MNs, these MNs must coordinate their activation with the control of breathing as well as vomiting, cough, and a host of other behaviours (Kessler and Jean, 1985b; Car and Amri, 1987; Tomomune and Takata, 1988; Amri et al., 1991; Nishino and Hiraga, 1991; Dick et al., 1993; Umezaki et al., 1998; Jean, 2001; Sawczuk and Mosier, 2001; Roda et al., 2002; Pitts et al., 2012; Fregosi and Ludlow, 2014; Huff et al., 2022). Thus, compared to oesophageal MNs, palate and laryngeal MNs must integrate their activities across a wider range of behavioural states. The exaggerated length and convex hull surface area to provide the substrate for synaptic inputs from diverse sources is evident in the current study and is consistent with synaptic inputs quantified by electron microscopy and qualitative assessments in Sprague Dawley rats (Sun et al., 1995; Hayakawa et al., 1996). The larger range of dendritic sizes is consistent with a more diverse motor unit population, confirmed by the mixed myosin heavy chain expressions confirming type I, IIa, IIx, IIb, and mixtures of IIx and IIb with extraocular fibres in cricothyroid, cricoarytenoid, and thyroarytenoid muscles (Rhee et al., 2004). Thus, within the semicompact/loose formations of the

nucleus ambiguus there resides age- and neurodegeneration-vulnerable and resilient MNs innervating the larynx, similar to other mixed motor unit population motor pools (Fogarty et al., 2018; Fogarty, 2023; Fogarty and Sieck, 2023). This is borne out in observations of dysphagia and dysphonia in the elderly, in Alzheimer's disease and amyotrophic lateral sclerosis sufferers (Turley and Cohen, 2009; Humbert et al., 2010; Christmas and Rogus-Pulia, 2019; Garand et al., 2022), alongside rodent studies of aged Fischer 344–Brown Norway crosses (Basken et al., 2012; Krekeler et al., 2020). In particular, the study by Basken showed a specific loss of retrogradely labelled laryngeal MNs (Basken et al., 2012), although it remains unconfirmed whether these findings hold in Fischer 344 rats. It is likely that vulnerable MNs in this population will exhibit distal dendritic pathology consistent with degeneration.

Conclusion

In conclusion, this study presents the first comprehensive quantitative evaluation of the dendritic morphologies of MNs and non-MNs within the compact, semicompact, and loose formations of the nucleus ambiguus. Our findings in MNs are consistent with motor unit type-specific differences between the different regions. Our findings are also consistent with differences in the number of different behaviours each motor pool must coordinate its activities with. These findings provide a solid basis with which to assess various developmental, ageing, and disease states.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Mayo Clinic Institute Animal Care and Use Committee (IACUC approval #A57714). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ahmed, R. M., Devenney, E. M., Irish, M., Ittner, A., Naismith, S., Ittner, L. M., et al. (2016). Neuronal network disintegration: common pathways linking neurodegenerative diseases. *J. Neurol. Neurosurg. Psychiatry* 87, 1234–1241. doi: 10.1136/jnnp-2014-308350

Altschuler, S. M., Bao, X. M., and Miselis, R. R. (1991). Dendritic architecture of nucleus ambiguus motoneurons projecting to the upper alimentary tract in the rat. *J. Comp. Neurol.* 309, 402–414. doi: 10.1002/cne.903090309

Amri, M., Lamkadem, M., and Car, A. (1991). Effects of lingual nerve and chewing cortex stimulation upon activity of the swallowing neurons located in the region of the hypoglossal motor nucleus. *Brain Res.* 548, 149–155. doi: 10.1016/0006-8993(91)91116-I

Andrew, B. L. (1954). Proprioception at the joint of the epiglottis of the rat. *J. Physiol.* 126, 507–523. doi: 10.1113/jphysiol.1954.sp005225

Andrew, B. L. (1955). The respiratory displacement of the larynx: a study of the innervation of accessory respiratory muscles. *J. Physiol.* 130, 474–487. doi: 10.1113/jphysiol.1955.sp005421

Andrew, B. L. (1956a). A functional analysis of the myelinated fibres of the superior laryngeal nerve of the rat. *J. Physiol.* 133, 420–432. doi: 10.1113/jphysiol.1956.sp005597

Andrew, B. L. (1956b). The nervous control of the cervical oesophagus of the rat during swallowing. *J. Physiol.* 134, 729–740. doi: 10.1113/jphysiol.1956.sp005679

Barfield, R. J., Auerbach, P., Geyer, L. A., and Mcintosh, T. K. (1979). Ultrasonic vocalizations in rat sexual-behavior. *Am. Zool.* 19, 469–480. doi: 10.1093/icb/19.2.469

Bartlett, D. J., and Knuth, S. L. (1984). Human vocal cord movements during voluntary hyperventilation. *Respir. Physiol.* 58, 289–294. doi: 10.1016/0034-5687(84)90005-7

Basken, J. N., Connor, N. P., and Ciucci, M. R. (2012). Effect of aging on ultrasonic vocalizations and laryngeal sensorimotor neurons in rats. *Exp. Brain Res.* 219, 351–361. doi: 10.1007/s00221-012-3096-6

Bieger, D., and Hopkins, D. A. (1987). Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. *J. Comp. Neurol.* 262, 546–562. doi: 10.1002/cne.902620408

Bird, A. D., and Cuntz, H. (2019). Dissecting Sholl analysis into its functional components. *Cell Rep.* 27:e3085, 3081–3096.e5. doi: 10.1016/j.celrep.2019.04.097

Bolser, D. C., Pitts, T. E., Davenport, P. W., and Morris, K. F. (2015). Role of the dorsal medulla in the neurogenesis of airway protection. *Pulm. Pharmacol. Ther.* 35, 105–110. doi: 10.1016/j.pupt.2015.10.012

Brandenburg, J. E., Fogarty, M. J., Brown, A. D., and Sieck, G. C. (2020). Phrenic motor neuron loss in an animal model of early onset hypertonia. *J. Neurophysiol.* 123, 1682–1690. doi: 10.1152/jn.00026.2020

Brandenburg, J. E., Gransee, H. M., Fogarty, M. J., and Sieck, G. C. (2018). Differences in lumbar motor neuron pruning in an animal model of early onset spasticity. *J. Neurophysiol.* 120, 601–609. doi: 10.1152/jn.00186.2018

Brannstrom, T. (1993). Quantitative synaptology of functionally different types of cat medial gastrocnemius alpha-motoneurons. *J. Comp. Neurol.* 330, 439–454. doi: 10.1002/cne.903300311

Broussard, D. L., and Altschuler, S. M. (2000). Central integration of swallow and airway-protective reflexes. *Am. J. Med.* 108, 62–67. doi: 10.1016/S0002-9343(99)00340-X

Brown, A. D., Davis, L. A., Fogarty, M. J., and Sieck, G. C. (2021a). Mitochondrial fragmentation and dysfunction in type IIx/IIb diaphragm muscle fibers in 24-month old Fischer 344 rats. *Front. Physiol.* 12:727585. doi: 10.3389/fphys.2021.727585

Brown, A. D., Fogarty, M. J., and Sieck, G. C. (2021b). Mitochondrial morphology and function varies across diaphragm muscle fiber types. *Respir. Physiol. Neurobiol.* 295:103780. doi: 10.1016/j.resp.2021.103780

Burke, R. E., Levine, D. N., Tsairis, P., and Zajac, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* 234, 723–748. doi: 10.1113/jphysiol.1973.sp010369

Car, A., and Amri, M. (1987). Activity of neurons located in the region of the hypoglossal motor nucleus during swallowing in sheep. *Exp. Brain Res.* 69, 175–182. doi: 10.1007/BF00247040

Christmas, C., and Rogus-Pulia, N. (2019). Swallowing disorders in the older population. *J. Am. Geriatr. Soc.* 67, 2643–2649. doi: 10.1111/jgs.16137

Coverdell, T. C., Abraham-Fan, R. J., Wu, C., Abbott, S. B. G., and Campbell, J. N. (2022). Genetic encoding of an esophageal motor circuit. *Cell Rep.* 39:110962. doi: 10.1016/j.celrep.2022.110962

Cullheim, S., Fleshman, J. W., Glenn, L. L., and Burke, R. E. (1987). Membrane area and dendritic structure in type-identified triceps surae alpha motoneurons. *J. Comp. Neurol.* 255, 68–81. doi: 10.1002/cne.902550106

Davis, P. J., and Nail, B. S. (1984). On the location and size of laryngeal motoneurons in the cat and rabbit. *J. Comp. Neurol.* 230, 13–32. doi: 10.1002/cne.902300103

Dick, T. E., Kong, F. J., and Berger, A. J. (1987). Correlation of recruitment order with axonal conduction velocity for supraspinally driven diaphragmatic motor units. *J. Neurophysiol.* 57, 245–259. doi: 10.1152/jn.1987.57.1.245

Dick, T. E., Oku, Y., Romaniuk, J. R., and Cherniack, N. S. (1993). Interaction between central pattern generators for breathing and swallowing in the cat. *J. Physiol.* 465, 715–730. doi: 10.1113/jphysiol.1993.sp019702

Dobbins, E. G., and Feldman, J. L. (1995). Differential innervation of protruder and retractor muscles of the tongue in rat. *J. Comp. Neurol.* 357, 376–394. doi: 10.1002/cne.903570305

Dukkipati, S. S., Garrett, T. L., and Elbasiouny, S. M. (2018). The vulnerability of spinal motoneurons and soma size plasticity in a mouse model of amyotrophic lateral sclerosis. *J. Physiol.* 596, 1723–1745. doi: 10.1113/JP275498

Ellenberger, H. H., and Feldman, J. L. (1990a). Brainstem connections of the rostral ventral respiratory group of the rat. *Brain Res.* 513, 35–42. doi: 10.1016/0006-8993(90)91086-V

Ellenberger, H. H., and Feldman, J. L. (1990b). Subnuclear organization of the lateral tegmental field of the rat. 1: nucleus ambiguus and ventral respiratory group. *J. Comp. Neurol.* 294, 202–211. doi: 10.1002/cne.902940205

Flint, P. W., Downs, D. H., and Coltrera, M. D. (1991). Laryngeal synkinesis following reinnervation in the rat. Neuroanatomic and physiologic study using retrograde fluorescent tracers and electromyography. *Ann. Otol. Rhinol. Laryngol.* 100, 797–806. doi: 10.1177/000348949110001003

Fogarty, M. J. (2018). Driven to decay: excitability and synaptic abnormalities in amyotrophic lateral sclerosis. *Brain Res. Bull.* 140, 318–333. doi: 10.1016/j.brainresbull.2018.05.023

Fogarty, M. J. (2023). Loss of larger hypoglossal motor neurons in aged Fischer 344 rats. *Respir. Physiol. Neurobiol.* 314:104092. doi: 10.1016/j.resp.2023.104092

Fogarty, M. J., Kanjhan, R., Bellingham, M. C., and Noakes, P. G. (2016a). Glycinergic neurotransmission: a potent regulator of embryonic motor neuron dendritic morphology and synaptic plasticity. *J. Neurosci.* 36, 80–87. doi: 10.1523/JNEUROSCI.1576-15.2016

Fogarty, M. J., Kanjhan, R., Yanagawa, Y., Noakes, P. G., and Bellingham, M. C. (2017a). Alterations in hypoglossal motor neurons due to GAD67 and VGAT deficiency in mice. *Exp. Neurol.* 289, 117–127. doi: 10.1016/j.expneurol.2016.12.004

Fogarty, M. J., Mantilla, C. B., and Sieck, G. C. (2019a). Impact of sarcopenia on diaphragm muscle fatigue. *Exp. Physiol.* 104, 1090–1099. doi: 10.1113/EP087558

Fogarty, M. J., Marin Mathieu, N., Mantilla, C. B., and Sieck, G. C. (2020a). Aging reduces succinate dehydrogenase activity in rat type IIx/IIb diaphragm muscle fibers. *J. Appl. Physiol.* 128, 70–77. doi: 10.1152/japplphysiol.00644.2019

Fogarty, M. J., Mu, E. W. H., Lavidis, N. A., Noakes, P. G., and Bellingham, M. C. (2017b). Motor areas show altered dendritic structure in an amyotrophic lateral sclerosis mouse model. *Front. Neurosci.* 11:609. doi: 10.3389/fnins.2017.00609

Fogarty, M. J., Mu, E. W., Lavidis, N. A., Noakes, P. G., and Bellingham, M. C. (2019b). Size-dependent vulnerability of lumbar motor neuron dendritic degeneration in SOD1G93A mice. *Anat Rec (Hoboken).* 303:1455–1471. doi: 10.1002/ar.24255

Fogarty, M. J., Mu, E. W. H., Lavidis, N. A., Noakes, P. G., and Bellingham, M. C. (2020b). Size-dependent dendritic maladaptations of hypoglossal motor neurons in SOD1(G93A) mice. *Anat Rec (Hoboken).* 303, 1455–1471. doi: 10.1002/ar.24255

Fogarty, M. J., Mu, E. W., Noakes, P. G., Lavidis, N. A., and Bellingham, M. C. (2016b). Marked changes in dendritic structure and spine density precede significant neuronal death in vulnerable cortical pyramidal neuron populations in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *Acta Neuropathol. Commun.* 4:77. doi: 10.1186/s40478-016-0347-y

Fogarty, M. J., Omar, T. S., Zhan, W. Z., Mantilla, C. B., and Sieck, G. C. (2018). Phrenic motor neuron loss in aged rats. *J. Neurophysiol.* 119, 1852–1862. doi: 10.1152/jn.00868.2017

Fogarty, M. J., and Sieck, G. C. (2019). Evolution and functional differentiation of the diaphragm muscle of mammals. *Compr. Physiol.* 9, 715–766. doi: 10.1002/cphy.c180012

Fogarty, M. J., and Sieck, G. C. (2021). Tongue muscle contractile, fatigue, and fiber type properties in rats. *J. Appl. Physiol.* 131, 1043–1055. doi: 10.1152/japplphysiol.00329.2021

Fogarty, M. J., and Sieck, G. C. (2023). Aging affects the number and morphological heterogeneity of rat phrenic motor neurons and phrenic motor axons. *Physiol. Rep.* 11:e15587. doi: 10.14814/phy2.15587

Fogarty, M. J., Smallcombe, K. L., Yanagawa, Y., Obata, K., Bellingham, M. C., and Noakes, P. G. (2013). Genetic deficiency of GABA differentially regulates respiratory and non-respiratory motor neuron development. *PLoS One* 8:e56257. doi: 10.1371/journal.pone.0056257

Fogarty, M. J., Yanagawa, Y., Obata, K., Bellingham, M. C., and Noakes, P. G. (2015). Genetic absence of the vesicular inhibitory amino acid transporter differentially regulates respiratory and locomotor motor neuron development. *Brain Struct. Funct.* 220, 525–540. doi: 10.1007/s00429-013-0673-9

Fregosi, R. F., and Ludlow, C. L. (2014). Activation of upper airway muscles during breathing and swallowing. *J. Appl. Physiol.* 116, 291–301. doi: 10.1152/japplphysiol.00670.2013

Fryscak, T., Zenker, W., and Kantner, D. (1984). Afferent and efferent innervation of the rat esophagus. A tracing study with horseradish peroxidase and nuclear yellow. *Anat Embryol (Berl)* 170, 63–70. doi: 10.1007/BF00319459

Garand, K. L. F., Bhutada, A. M., Hopkins-Rossabi, T., Mulekar, M. S., and Carnaby, G. (2022). Pilot study of respiratory-swallow coordination in amyotrophic lateral sclerosis. *J. Speech Lang. Hear. Res.* 65, 2815–2828. doi: 10.1044/2022_JSLHR-21-00619

Geyer, L. A., and Barfield, R. J. (1978). Influence of gonadal hormones and sexual behavior on ultrasonic vocalization in rats: I. Treatment of females. *J. Comp. Physiol. Psychol.* 92, 438–446. doi: 10.1037/h0077480

Geyer, L. A., Barfield, R. J., and Mcintosh, T. K. (1978). Influence of gonadal hormones and sexual behavior on ultrasonic vocalization in rats: II. Treatment of males. *J. Comp. Physiol. Psychol.* 92, 447–456. doi: 10.1037/h0077487

Glaser, E. M., and Van Der Loos, H. (1981). Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. *J. Neurosci. Methods* 4, 117–125. doi: 10.1016/0165-0270(81)90045-5

Gruber, H. (1978). Motor innervation of striated oesophageal muscle. Part 2. Characteristics of the oesophagomotor fibres in the rat studied by implanting the vagus nerve into a skeletal muscle. *J. Neurol. Sci.* 36, 171–186. doi: 10.1016/0022-510X(78)90081-3

Hashizume, K., Kanda, K., and Burke, R. E. (1988). Medial gastrocnemius motor nucleus in the rat: age-related changes in the number and size of motoneurons. *J. Comp. Neurol.* 269, 425–430. doi: 10.1002/cne.902690309

Hayakawa, T., Yajima, Y., and Zyo, K. (1996). Ultrastructural characterization of pharyngeal and esophageal motoneurons in the nucleus ambiguus of the rat. *J. Comp. Neurol.* 370, 135–146. doi: 10.1002/(SICI)1096-9861(19960624)370:2<135::AID-CNE1>3.0.CO;2-3

Heckman, C. J., and Enoka, R. M. (2012). Motor unit. *Compr. Physiol.* 2, 2629–2682. doi: 10.1002/cphy.c100087

Hernandez-Morato, I., Pascual-Font, A., Ramirez, C., Matarranz-Echeverria, J., Mchanwell, S., Vazquez, T., et al. (2013). Somatotopy of the neurons innervating the cricothyroid, posterior cricoarytenoid, and thyroarytenoid muscles of the rat's larynx. *Anat Rec (Hoboken)* 296, 470–479. doi: 10.1002/ar.22643

Hinrichsen, C., and Dulhunty, A. (1982). The contractile properties, histochemistry, ultrastructure and electrophysiology of the cricothyroid and posterior cricoarytenoid muscles in the rat. *J. Muscle Res. Cell Motil.* 3, 169–190. doi: 10.1007/BF00711941

Hinrichsen, C. F., and Ryan, A. T. (1981). Localization of laryngeal motoneurons in the rat: morphologic evidence for dual innervation? *Exp. Neurol.* 74, 341–355. doi: 10.1016/0014-4886(81)90174-6

Holstege, G. (1989). Anatomical study of the final common pathway for vocalization in the cat. *J. Comp. Neurol.* 284, 242–252. doi: 10.1002/cne.902840208

Holstege, G. (2014). The periaqueductal gray controls brainstem emotional motor systems including respiration. *Prog. Brain Res.* 209, 379–405. doi: 10.1016/B978-0-444-63274-6.00020-5

Holstege, G., Graveland, G., Bijker-Biemond, C., and Schuddeboom, I. (1983). Location of motoneurons innervating soft palate, pharynx and upper esophagus. Anatomical evidence for a possible swallowing center in the pontine reticular formation. An HRP and autoradiographical tracing study. *Brain Behav. Evol.* 23, 47–62. doi: 10.1159/000121488

Huff, A., Karlen-Amarante, M., Pitts, T., and Ramirez, J. M. (2022). Optogenetic stimulation of pre-Botzinger complex reveals novel circuit interactions in swallowing-breathing coordination. *Proc. Natl. Acad. Sci. USA* 119:e2121095119. doi: 10.1073/pnas.2121095119

Humbert, I. A., Mclaren, D. G., Kosmatka, K., Fitzgerald, M., Johnson, S., Porcaro, E., et al. (2010). Early deficits in cortical control of swallowing in Alzheimer's disease. *J. Alzheimers Dis.* 19, 1185–1197. doi: 10.3233/JAD-2010-1316

Jacob, J. M. (1998). Lumbar motor neuron size and number is affected by age in male F344 rats. *Mech. Ageing Dev.* 106, 205–216. doi: 10.1016/S0047-6374(98)00117-1

Jean, A. (2001). Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol. Rev.* 81, 929–969. doi: 10.1152/physrev.2001.81.2.929

Johnson, I. P., and Duberley, R. M. (1998). Motoneuron survival and expression of neuropeptides and neurotrophic factor receptors following axotomy in adult and ageing rats. *Neuroscience* 84, 141–150. doi: 10.1016/S0306-4522(97)00500-9

Jurgens, U. (2009). The neural control of vocalization in mammals: a review. *J. Voice* 23, 1–10. doi: 10.1016/j.jvoice.2007.07.005

Kalia, M., and Mesulam, M. M. (1980). Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. *J. Comp. Neurol.* 193, 467–508. doi: 10.1002/cne.901930211

Kanda, K., and Hashizume, K. (1998). Effects of long-term physical exercise on age-related changes of spinal motoneurons and peripheral nerves in rats. *Neurosci. Res.* 31, 69–75. doi: 10.1016/S0168-0102(98)00026-1

Kanjhan, R., Fogarty, M. J., Noakes, P. G., and Bellingham, M. C. (2016). Developmental changes in the morphology of mouse hypoglossal motor neurons. *Brain Struct. Funct.* 221, 3755–3786. doi: 10.1007/s00429-015-1130-8

Kecskes, S., Koszeghy, A., Szucs, G., Rusznak, Z., Matesz, C., and Birinyi, A. (2013). Three-dimensional reconstruction and quantitative morphometric analysis of pyramidal and giant neurons of the rat dorsal cochlear nucleus. *Brain Struct. Funct.* 218, 1279–1292. doi: 10.1007/s00429-012-0457-7

Kellerth, J. O., Berthold, C. H., and Conradi, S. (1979). Electron microscopic studies of serially sectioned cat spinal alpha-motoneurons. *J. Comp. Neurol.* 184, 755–767. doi: 10.1002/cne.901840408

Kessler, J. P., and Jean, A. (1985a). Identification of the medullary swallowing regions in the rat. *Exp. Brain Res.* 57, 256–263

Kessler, J. P., and Jean, A. (1985b). Inhibition of the swallowing reflex by local application of serotonergic agents into the nucleus of the solitary tract. *Eur. J. Pharmacol.* 118, 77–85. doi: 10.1016/0014-2999(85)90665-X

Kiernan, J. A., and Hudson, A. J. (1991). Changes in sizes of cortical and lower motor neurons in amyotrophic lateral sclerosis. *Brain* 114, 843–853. doi: 10.1093/brain/114.2.843

Kiernan, M. C., Ziemann, U., and Eisen, A. (2019). Amyotrophic lateral sclerosis: origins traced to impaired balance between neural excitation and inhibition in the neonatal period. *Muscle Nerve* 60, 232–235. doi: 10.1002/mus.26617

Klenowski, P. M., Fogarty, M. J., Belmer, A., Noakes, P. G., Bellingham, M. C., and Bartlett, S. E. (2015). Structural and functional characterization of dendritic arbors and GABAergic synaptic inputs on interneurons and principal cells in the rat basolateral amygdala. *J. Neurophysiol.* 114, 942–957. doi: 10.1152/jn.00824.2014

Klenowski, P. M., Shariff, M. R., Belmer, A., Fogarty, M. J., Mu, E. W., Bellingham, M. C., et al. (2016). Prolonged consumption of sucrose in a binge-like manner, alters the morphology of medium spiny neurons in the nucleus Accumbens Shell. *Front. Behav. Neurosci.* 10:54. doi: 10.3389/fnbeh.2016.00054

Krekeler, B. N., Weycker, J. M., and Connor, N. P. (2020). Effects of tongue exercise frequency on tongue muscle biology and swallowing physiology in a rat model. *Dysphagia* 35, 918–934. doi: 10.1007/s00455-020-10105-2

Kruszewska, B., Lipski, J., and Kanjhan, R. (1994). An electrophysiological and morphological study of esophageal motoneurons in rats. *Am. J. Phys.* 266, R622–R632. doi: 10.1152/ajpregu.1994.266.2.R622

Kubota, Y., Karube, F., Nomura, M., Gulledge, A. T., Mochizuki, A., Schertel, A., et al. (2011). Conserved properties of dendritic trees in four cortical interneuron subtypes. *Sci. Rep.* 1:89. doi: 10.1038/srep00089

Lance-Jones, C. (1982). Motoneuron cell death in the developing lumbar spinal cord of the mouse. *Brain Res.* 256, 473–479. doi: 10.1016/0165-3806(82)90192-4

Lawn, A. M. (1966). The localization, in the nucleus ambiguus of the rabbit, of the cells of origin of motor nerve fibers in the glossopharyngeal nerve and various branches of the vagus nerve by means of retrograde degeneration. *J. Comp. Neurol.* 127, 293–305. doi: 10.1002/cne.901270210

Leroy, F., Lamotte D'incamps, B., Imhoff-Manuel, R. D., and Zytnicki, D. (2014). Early intrinsic hyperexcitability does not contribute to motoneuron degeneration in amyotrophic lateral sclerosis. *eLife* 3:e04046. doi: 10.7554/eLife.04046

Levin, R. J. (2006). Vocalised sounds and human sex. *Sex. Relatsh. Ther.* 21, 99–107. doi: 10.1080/14681990500438014

Ma, W. Y., and Vacca-Galloway, L. L. (1991). Reduced branching and length of dendrites detected in cervical spinal cord motoneurons of wobbler mouse, a model for inherited motoneuron disease. *J. Comp. Neurol.* 311, 210–222. doi: 10.1002/cne.903110204

Malmierca, M. S., Seip, K. L., and Osen, K. K. (1995). Morphological classification and identification of neurons in the inferior colliculus: a multivariate analysis. *Anat Embryol (Berl)* 191, 343–350. doi: 10.1007/BF00534687

Mantilla, C. B., Zhan, W. Z., and Sieck, G. C. (2009). Retrograde labeling of phrenic motoneurons by intrapleural injection. *J. Neurosci. Methods* 182, 244–249. doi: 10.1016/j.jneumeth.2009.06.016

Marino, J. P., and Johns, M. M. (2014). The epidemiology of dysphonia in the aging population. *Curr. Opin. Otolaryngol. Head Neck Surg.* 22, 455–459. doi: 10.1097/MOO.0000000000000098

Marks, W. B., and Burke, R. E. (2007). Simulation of motoneuron morphology in three dimensions. II. Building complete neurons. *J Comp Neurol* 503, 701–716. doi: 10.1002/cne.21417

Mazzone, S. B., and Canning, B. J. (2013). Autonomic neural control of the airways. *Handb. Clin. Neurol.* 117, 215–228. doi: 10.1016/B978-0-444-53491-0.00018-3

Mcallen, R. M., and Spyer, K. M. (1978). Two types of vagal preganglionic motoneurones projecting to the heart and lungs. *J. Physiol.* 282, 353–364. doi: 10.1113/jphysiol.1978.sp012468

Mcgovern, A. E., and Mazzone, S. B. (2010). Characterization of the vagal motor neurons projecting to the Guinea pig airways and esophagus. *Front. Neurol.* 1:153. doi: 10.3389/fneur.2010.00153

Mcintosh, T. K., Barfield, R. J., and Geyer, L. A. (1978). Ultrasonic vocalisations facilitate sexual behaviour of female rats. *Nature* 272, 163–164. doi: 10.1038/272163a0

Meyer, G. W., and Castell, D. O. (1982). Physiology of the oesophagus. *Clin. Gastroenterol.* 11, 439–451. doi: 10.1016/S0300-5089(21)00539-3

Monnier, A., Alheid, G. F., and Mccrimmon, D. R. (2003). Defining ventral medullary respiratory compartments with a glutamate receptor agonist in the rat. *J. Physiol.* 548, 859–874. doi: 10.1113/jphysiol.2002.038141

Neuhuber, W. L., and Berthoud, H. R. (2022). Functional anatomy of the vagus system: how does the polyvagal theory comply? *Biol. Psychol.* 174:108425. doi: 10.1016/j.biopsycho.2022.108425

Nishino, T., and Hiraga, K. (1991). Coordination of swallowing and respiration in unconscious subjects. *J. Appl. Physiol.* 70, 988–993. doi: 10.1152/jappl.1991.70.3.988

Nosaka, S., Yamamoto, T., and Yasunaga, K. (1979). Localization of vagal cardioinhibitory preganglionic neurons with rat brain stem. *J. Comp. Neurol.* 186, 79–92. doi: 10.1002/cne.901860106

Odutola, A. B. (1976). Cell grouping and Golgi architecture of the hypoglossal nucleus of the rat. *Exp. Neurol.* 52, 356–371. doi: 10.1016/0014-4886(76)90211-9

Paterson, W. G. (1999). Alteration of swallowing and oesophageal peristalsis by different initiators of deglutition. *Neurogastroenterol. Motil.* 11, 63–67. doi: 10.1046/j.1365-2982.1999.00131.x

Paxinos, G. (1999). *Chemoarchitectonic Atlas of the Rat Brainstem*. San Diego: Academic Press.

Paxinos, G., and Watson, C. (2014). *Paxino's and Watson's the Rat Brain in Stereotaxic Coordinates*. Amsterdam; Boston: Elsevier/AP, Academic Press Is an Imprint of Elsevier.

Pisanski, K., Bryant, G. A., Cornec, C., Anikin, A., and Reby, D. (2022). Form follows function in human nonverbal vocalisations. *Ethol. Ecol. Evol.* 34, 303–321. doi: 10.1080/03949370.2022.2026482

Pitts, T., and Iceman, K. E. (2023). Deglutition and the regulation of the swallow motor pattern. *Physiology (Bethesda)* 38, 10–24. doi: 10.1152/physiol.00005.2021

Pitts, T., Morris, K., Lindsey, B., Davenport, P., Poliacek, I., and Bolser, D. (2012). Co-ordination of cough and swallow in vivo and in silico. *Exp. Physiol.* 97, 469–473. doi: 10.1113/expphysiol.2011.063362

Porter, J. T., Johnson, C. K., and Agmon, A. (2001). Diverse types of interneurons generate thalamus-evoked feedforward inhibition in the mouse barrel cortex. *J. Neurosci.* 21, 2699–2710. doi: 10.1523/JNEUROSCI.21-08-02699.2001

Portillo, F., and Pasaro, R. (1988a). Location of bulbospinal neurons and of laryngeal motoneurons within the nucleus ambiguus of the rat and cat by means of retrograde fluorescent labelling. *J. Anat.* 159, 11–18

Portillo, F., and Pasaro, R. (1988b). Location of motoneurons supplying the intrinsic laryngeal muscles of rats. Horseradish peroxidase and fluorescence double-labeling study. *Brain Behav. Evol.* 32, 220–225. doi: 10.1159/000116549

Pun, S., Santos, A. F., Saxena, S., Xu, L., and Caroni, P. (2006). Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. *Nat. Neurosci.* 9, 408–419. doi: 10.1038/nn1653

Rall, W. (1959). Branching dendritic trees and motoneuron membrane resistivity. *Exp. Neurol.* 1, 491–527. doi: 10.1016/0014-4886(59)90046-9

Rall, W. (1960). Membrane potential transients and membrane time constant of motoneurons. *Exp. Neurol.* 2, 503–532. doi: 10.1016/0014-4886(60)90029-7

Rall, W. (1977). "Core conductor theory and cable properties of neurons," in handbook of physiology: the nervous system, ed. E.R. *Kandel* 1977, 39–97.

Rhee, H. S., Lucas, C. A., and Hoh, J. F. (2004). Fiber types in rat laryngeal muscles and their transformations after denervation and reinnervation. *J. Histochem. Cytochem.* 52, 581–590. doi: 10.1177/002215540405200503

Riede, T., Schaefer, C., and Stein, A. (2020). Role of deep breaths in ultrasonic vocal production of Sprague-Dawley rats. *J. Neurophysiol.* 123, 966–979. doi: 10.1152/jn.00590.2019

Roda, F., Gestreau, C., and Bianchi, A. L. (2002). Discharge patterns of hypoglossal motoneurons during fictive breathing, coughing, and swallowing. *J. Neurophysiol.* 87, 1703–1711. doi: 10.1152/jn.00347.2001

Rojo, C., Leguey, I., Kastanauskaite, A., Bielza, C., Larranaga, P., Defelipe, J., et al. (2016). Laminar differences in dendritic structure of pyramidal neurons in the juvenile rat somatosensory cortex. *Cereb. Cortex* 26, 2811–2822. doi: 10.1093/cercor/bhv316

Sawczuk, A., and Mosier, K. M. (2001). Neural control of tongue movement with respect to respiration and swallowing. *Crit. Rev. Oral Biol. Med.* 12, 18–37. doi: 10.1177/10454411010120010101

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. doi: 10.1038/nmeth.2089

Sholl, D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat.* 87, 387–406.

Sieck, G. C., Hernandez-Vizcarrondo, G. A., Brown, A. D., and Fogarty, M. J. (2023). Sarcopenia of the longitudinal tongue muscles in rats. *Respir. Physiol. Neurobiol.* 319:104180. doi: 10.1016/j.resp.2023.104180

Slomianka, L. (2020). Basic quantitative morphological methods applied to the central nervous system. *J. Comp. Neurol.* 529, 694–756. doi: 10.1002/cne.24976

Stuesse, S. L., and Fish, S. E. (1984). Projections to the cardioinhibitory region of the nucleus ambiguus of rat. *J. Comp. Neurol.* 229, 271–278. doi: 10.1002/cne.902290211

Sturrock, R. R. (1990). A comparison of age-related changes in neuron number in the dorsal motor nucleus of the vagus and the nucleus ambiguus of the mouse. *J. Anat.* 173, 169–176

Subramanian, H. H., Balnave, R. J., and Holstege, G. (2021). Microstimulation in different parts of the periaqueductal gray generates different types of vocalizations in the cat. *J. Voice* 35, 804.e9–804.e25. doi: 10.1016/j.jvoice.2020.01.022

Subramanian, H. H., Huang, Z. G., Silburn, P. A., Balnave, R. J., and Holstege, G. (2018). The physiological motor patterns produced by neurons in the nucleus retroambiguus in the rat and their modulation by vagal, peripheral chemosensory, and nociceptive stimulation. *J. Comp. Neurol.* 526, 229–242. doi: 10.1002/cne.24318

Sun, Q. J., Pilowsky, P., and Llewellyn-Smith, I. J. (1995). Thyrotropin-releasing hormone inputs are preferentially directed towards respiratory motoneurons in rat nucleus ambiguus. *J. Comp. Neurol.* 362, 320–330. doi: 10.1002/cne.903620303

Thiyagalingam, S., Kulinski, A. E., Thorsteinsdottir, B., Shindelar, K. L., and Takahashi, P. Y. (2021). Dysphagia in older adults. *Mayo Clin. Proc.* 96, 488–497. doi: 10.1016/j.mayocp.2020.08.001

Tomomune, N., and Takata, M. (1988). Excitatory and inhibitory postsynaptic potentials in cat hypoglossal motoneurons during swallowing. *Exp. Brain Res.* 71, 262–272. doi: 10.1007/BF00247486

Turley, R., and Cohen, S. (2009). Impact of voice and swallowing problems in the elderly. *Otolaryngol. Head Neck Surg.* 140, 33–36. doi: 10.1016/j.otohns.2008.10.010

Ulfhake, B., and Cullheim, S. (1988). Postnatal development of cat hind limb motoneurons. III: changes in size of motoneurons supplying the triceps surae muscle. *J. Comp. Neurol.* 278, 103–120

Ulrich, D., Quadroni, R., and Luscher, H. R. (1994). Electronic structure of motoneurons in spinal cord slice cultures: a comparison of compartmental and equivalent cylinder models. *J. Neurophysiol.* 72, 861–871. doi: 10.1152/jn.1994.72.2.861

Umezaki, T., Shiba, K., Zheng, Y., and Miller, A. D. (1998). Upper airway motor outputs during vomiting versus swallowing in the decerebrate cat. *Brain Res.* 781, 25–36. doi: 10.1016/S0006-8993(97)01145-1

Vanderhorst, V. G., Terasawa, E., Ralston, H. J. 3rd, and Holstege, G. (2000). Monosynaptic projections from the lateral periaqueductal gray to the nucleus retroambiguus in the rhesus monkey: implications for vocalization and reproductive behavior. *J. Comp. Neurol.* 424, 251–268. doi: 10.1002/1096-9861(20000821)424:2<251::AID-CNE5>3.0.CO;2-D

Veerakumar, A., Yung, A. R., Liu, Y., and Krasnow, M. A. (2022). Molecularly defined circuits for cardiovascular and cardiopulmonary control. *Nature* 606, 739–746. doi: 10.1038/s41586-022-04760-8

Wetzel, D. M., Kelley, D. B., and Campbell, B. A. (1980). Central control of ultrasonic vocalizations in neonatal rats: I. Brain stem motor nuclei. *J. Comp. Physiol. Psychol.* 94, 596–605. doi: 10.1037/h0077699

Williams, P. A., Bellinger, D. L., and Wilson, C. G. (2019). Changes in the morphology of hypoglossal motor neurons in the brainstem of developing rats. *Anat Rec (Hoboken)* 302, 869–892. doi: 10.1002/ar.23971

Williams, K. P. J., Pitt, G. D., Batchelder, D. N., and Kip, B. J. (1994). Confocal Raman microspectroscopy using a stigmatic spectrograph and Ccd detector. *Appl. Spectrosc.* 48, 232–235. doi: 10.1366/0003702944028407

Zheng, Y., Umezaki, T., Nakazawa, K., and Miller, A. D. (1997). Role of pre-inspiratory neurons in vestibular and laryngeal reflexes and in swallowing and vomiting. *Neurosci. Lett.* 225, 161–164. doi: 10.1016/S0304-3940(97)00208-5

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Evidence for peripheral and central actions of codeine to dysregulate swallowing in the anesthetized cat

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Systemic administration of opioids has been associated with aspiration and swallow dysfunction in humans. We speculated that systemic administration of codeine would induce dysfunctional swallowing and that this effect would have a peripheral component. Experiments were conducted in spontaneously breathing, anesthetized cats. The animals were tracheotomized and electromyogram (EMG) electrodes were placed in upper airway and chest wall respiratory muscles for recording swallow related motor activity. The animals were allocated into three groups: vagal intact (VI), cervical vagotomy (CVx), and supra-nodose ganglion vagotomy (SNGx). A dose response to intravenous codeine was performed in each animal. Swallowing was elicited by injection of 3 mL of water into the oropharynx. The number of swallows after vehicle was significantly higher in the VI group than in SNGx. Codeine had no significant effect on the number of swallows induced by water in any of the groups. However, the magnitudes of water swallow-related EMGs of the thyropharyngeus muscle were significantly increased in the VI and CVx groups by 2–4 fold in a dose-related manner. In the CVx group, the geniohyoid muscle EMG during water swallows was significantly increased. There was a significant dose-related increase in spontaneous swallowing in each group from codeine. The spontaneous swallow number at the 10 mg/kg dose of codeine was significantly larger in the CVx group than that in the SNGx group. During water-evoked swallows, intravenous codeine increased upper airway motor drive in a dose-related manner, consistent with dysregulation. The data support the existence of both central and peripheral actions of codeine on spontaneous swallowing. At the highest dose of codeine, the reduced spontaneous swallow number in the SNGx group relative to CVx is consistent with a peripheral excitatory action of codeine either on pharyngeal/laryngeal receptors or in the nodose ganglion itself. The higher number of swallows in the CVx group than the VI group supports disinhibition of this behavior by elimination of inhibitory vagal sensory afferents.

KEYWORDS

swallow, codeine, breathing, airway, vagal reflexes

1 Introduction

Swallow is a critically important behavior for protecting the airways from aspiration in addition to its role in feeding. Swallowing is produced by a complex neural circuit that is functionally identified as the swallow pattern generator. This circuit is located in the brainstem, but is subject to significant modulation by suprapontine pathways (1). Further, swallowing also is modulated by peripheral sensory afferents from the lungs and airway (2, 3), chest wall (3), mouth, pharynx, and esophagus (1, 4–6) and these afferents travel through the vagus, trigeminal, or hypoglossal nerves.

Administration of opioids, including morphine and remifentanyl is associated with significant dysphagia and aspiration syndromes (7–11). The site(s) of opioid actions on swallowing are not fully understood. It is well known that systemic administration of opioids can activate pulmonary vagal afferents resulting in perturbation of laryngeal function (12, 13). Further, microinjection of opioids into the nodose ganglion can induce effects that are similar to activation of peripheral vagal afferents (14). Savilampi et al. (10) found that subjective and objective metrics of swallow were not affected by pretreatment with a peripherally-restricted opioid antagonist. The results of this study were consistent with a peripheral action of opioids to influence swallowing.

Codeine is one of the most commonly abused opioid drugs worldwide and is likewise among the most common pharmaceutical opioids found in fatal poisonings (15, 16). Systemic administration of this drug is known to stimulate vagal C-fibers to influence breathing (17). Further, codeine is well known to alter breathing and inhibit cough via central action (18–24). However, the influence of this opioid on swallowing is unknown. We speculated that intravenous codeine would have suppressive effects on swallow frequency and upper airway muscle EMG magnitudes and that some of these effects would be mediated by vagal afferents.

2 Methods

Experiments were performed on 19 spontaneously breathing adult male cats with an average age of 1.05 ± 0.03 years and weight of 4.95 ± 0.12 kg. These cats were purchased from Marshall BioResources (North Rose, NY) and pair-housed in the University of Florida Animal Care Services on a 12-h light/12-h dark cycle with food and water *ad libitum*. The protocol was approved by the University of Florida Institutional Animal Care and Use Committee (IACUC) and all procedures were compliant with the Guide for the Care and Use of Laboratory Animals. Anesthesia was initially induced with sevoflurane (4.5%), then the animals were weaned onto sodium pentobarbital (Lundbeck, Inc., Deerfield, IL, 25 mg/kg IV). Based on forelimb withdrawal reflex and jaw tone, supplemental doses were given as needed in 0.1–0.3 mL IV boluses. A single dose of atropine sulfate (0.054 mg/kg IV, Patterson Veterinary Supply, Inc., Devens, MA) was given at the beginning of surgery to minimize airway secretions. The femoral vein and artery were cannulated to deliver drugs and record blood pressure, respectively. Arterial blood pressure and end-tidal CO_2 (30–38 mm Hg $ETCO_2$; Datax Engstrom; Datax Ohmeda, Inc.; Madison, WI) were continuously recorded, and arterial blood gas samples (epoc Blood Analysis System; Siemens Healthineers USA) were obtained every hour. Body temperature was maintained at 37.5°C with a TC-1000 homeothermic pad and rectal temperature probe (CWE Inc.; United States). Esophageal pressure was measured via a small balloon inserted through the mouth into the midthoracic esophagus. The cats were euthanized by an overdose of pentobarbital (IV) followed by 3 mL of a saturated potassium chloride solution (IV) (Thermo Fisher Scientific, Waltham, MA). Animals were tracheostomized and allowed to breathe spontaneously.

Electromyograms (EMGs) were recorded using bipolar insulated fine wire electrodes (A-M Systems stainless steel #791050) according to the technique of Basmajian and Stecko (25). Eight muscles were used to evaluate swallow: mylohyoid, geniohyoid, thyrohyoid, thyropharyngeus, thyroarytenoid, upper esophageal sphincter (UES), parasternal, and costal diaphragm. The digastric muscles were dissected away from the surface of the mylohyoid and electrodes were placed on the left mylohyoid. A small horizontal incision was made at the rostral end of the right mylohyoid followed by an incision following the midline for approximately 1 cm to reveal the geniohyoid underneath. Electrodes were placed 1 cm from the caudal insertion of the right geniohyoid muscle. The thyroarytenoid electrodes were inserted through the cricothyroid window into the anterior portion of the left vocal fold, which were visually inspected post-mortem. Rotation of the larynx and pharynx counterclockwise revealed the superior laryngeal nerve, which facilitated placement of the left thyropharyngeus muscle electrodes. The thyropharyngeus is a fan shaped muscle with the smallest portion attached to the thyroid cartilage; electrodes were placed in the ventral, caudal portion of the muscle overlaying thyroid cartilage within 5 mm of the rostral insertion of the muscle. To place the electrodes within the cricopharyngeus muscle, the larynx and pharynx were rotated counterclockwise to reveal the posterior aspect of the larynx. The tissue was palpated for the edge of the cricoid cartilage and electrodes were placed just cranial to the edge of this structure (for a bilateral recording). The left thyrohyoid electrodes were inserted approximately 1 cm rostral to the attachment to the thyroid cartilage. The sternal diaphragm was placed by elevation of the sternum and the electrodes placed along the dorsal surface. Swallow was operationally defined as a large ballistic-like increase in the mylohyoid, geniohyoid, thyrohyoid, thyropharyngeus, and thyroartytenoid muscle EMGs in response to water swallow or codeine administration in conjunction with no increase in esophageal pressure. This definition separated swallow from other airway protective behaviors such as cough, sneeze, or expiration reflex which require increased intrathoracic pressure to be executed (20).

The positions of all electrodes were confirmed by visual inspection (following electrode placement and post-mortem) and by EMG activity patterns during breathing and swallow (6, 26–28).

2.1 Stimulation of swallow

Swallow was induced by infusing 3 ccs of water into the oropharynx via a 1-inch long, thin polyethylene catheter (outer diameter 0.5–1.0 mm). For all time points at least three trials were completed, separated by a minimum inter-stimulus interval of one minute. All water trials for each animal were performed by the same researcher to maintain stimulus consistency. Swallow number is the swallow count within 1 min after the water swallow stimulus. For codeine induced swallowing, swallow number was the total swallows that occurred within 1 min after the completion of the administration of codeine.

2.2 Animal groups

Animals were randomly assigned to one of three groups: (A) vagus intact ($n = 5$), (B) mid-cervical vagotomy ($n = 7$), and (C) supra-nodose vagotomy ($n = 5$). For cervical vagotomy, the vagosympathetic trunk was isolated from the surrounding tissue and sectioned bilaterally with small scissors at the level of the larynx. This intervention was intended to eliminate vagal afferent feedback from thoracic and abdominal organs. In supra-nodose vagotomy animals, 3–4 mm of the vagal trunk was isolated rostral to the jugular/nodose ganglionic complex by blunt dissection of the surrounding tissue. The supra-nodose vagal trunk was cut bilaterally with small scissors. This intervention was intended to eliminate sensory feedback from the larynx in addition to that from thoracic and abdominal organs. Laryngeal nerves directly enter the jugular/nodose ganglion complex, and this level is rostral to the site of section of the cervical vagus in the cervical vagotomy group.

2.3 Protocols

Vagotomy. To understand the impact of bilateral vagotomy swallow trials were completed just prior to and within 15 min post-vagotomy (mid-cervical or supra-nodose). There was at least a 30-min interval between the vagotomy and the start of the pharmacological trials.

Codeine and vagotomy. To additionally understand the impact of codeine, swallow trials were performed with a cumulative dose response to codeine monohydrate ($C_{18}H_{21}NO_3 \cdot H_2O$; Sigma Aldrich Chemical Co. St. Louis, MO) infused intravenously. The protocol began a vehicle (0.9% saline) followed by a cumulative dose–response of 0.1, 0.3, 1, 3, 10 mg/kg controlling to total volume. All doses calculated as their free base and 10 min between each dose.

2.4 Analysis

EMG channels were rectified and smoothed with a 50 ms time constant prior to analysis.

The data was then normalized to the median amplitude following vehicle. All data are reported as a mean ± standard error (SEM) unless otherwise stated. Normality of the data was tested using the Kolmogorov–Smirnov normality test. For statistical analysis, two-way repeated-measures ANOVA (RM-ANOVA) was used to determine the effect of condition (vagotomy) and dose of codeine. Differences between swallow number pre- and post- cervical vagotomy and supra-nodose section were assessed with a paired *t*-test and a Wilcoxon matched-pairs signed-ranks test, respectively, using SigmaPlot (Grafiti LLC, Palo Alto, CA). Alpha was set with a *p*-value = 0.05.

3 Results

3.1 Swallow count

Swallows occurring without an oropharyngeal stimulus (i.e., spontaneous) are very rare in this preparation, however they were observed consistently in response to codeine (Figure 1A). A two-way repeated measures ANOVA was performed to evaluate the effects of vagotomy and codeine administration on number of spontaneous swallows (Figure 1B). The results indicated a significant main effect for vagal condition [RM-ANOVA: $F(2, 70) = 3.99$, $p = 0.04$] and dose of codeine [$F(5, 70) = 14.58$, $p < 0.001$]. Student–Newman–Keuls post-hoc testing revealed an effect condition at 3 and 10 mg/kg with significantly more spontaneous swallows in the mid-cervical group compared to vagal intact animals [3 mg/kg, $q = 3.33$, $p = 0.003$; 10 mg/kg, $q = 2.76$, $p = 0.02$] and animals with a supra-nodose vagotomy [3 mg/kg, $q = 3.42$, $p = 0.004$; 10 mg/kg, $q = 3.94$, $p < 0.001$].

In response to the water stimuli there was a significant reduction in average number of swallows produced following mid-cervical (*t*-test: 2.1 ± 0.4 pre to 1.1 ± 0.3 post; $p = 0.006$) and supra-nodose (Wilcoxen test: 4.0 ± 1.0 pre to 0.8 ± 0.5 post; $p = 0.03$) vagotomy (Table 1). Supra-nodose vagotomy resulted in so few swallows that our analysis could not reliably determine how the swallow motor pattern was affected by the procedure.

3.2 Swallow motor pattern

EMG amplitude from submental muscles (mylohyoid and geniohyoid), an extrinsic laryngeal muscle (thyrohyoid) and the inferior pharyngeal constrictor (thyropharyngeus) were compared (Figures 2A,B) to determine the effect of mid-cervical vagotomy and codeine using 2-way ANOVA. There was no significant change to the maximum EMG amplitude during water swallow of the *mylohyoid* [condition ($p = 0.2$) or dose ($p = 0.5$)] or *geniohyoid* [condition ($p = 0.1$) or dose ($p = 0.3$)] (Table 2).

For the *thyrohyoid* (Figure 2C) there was a significant effect of vagal condition [RM-ANOVA: $F(1, 57) = 4.87$, $p = 0.03$]. At 10 mg/kg thyrohyoid EMG amplitude was significantly greater following mid-cervical vagotomy ($q = 4.05$, $p = 0.006$) compared to vagus intact animals. For the *thyropharyngeus* (Figure 2D) there was a significant effect of vagal condition [$F(1, 57) = 8.09$, $p = 0.006$] and codeine dose [$F(5, 57) = 5.69$, $p < 0.001$]. For condition, at 10 mg/kg thyropharyngeus EMG amplitude was significantly greater following mid-cervical vagotomy (541%, $q = 6.82$, $p < 0.001$) compared to vagus intact animals. For dose, post-hoc testing revealed no change in EMG magnitude with codeine in vagally intact animals, however post mid-cervical vagotomy the EMG magnitude at 10 mg/kg was significantly greater than at 0.1 ($q = 8.47$, $p < 0.001$), 0.3 ($q = 7.83$, $p < 0.001$), 1 ($q = 7.13$, $p < 0.001$), and 3 ($q = 5.97$, $p < 0.001$) mg/kg.

4 Discussion

The major findings of this study were that intravenous codeine induced spontaneous swallowing in a dose-dependent manner in vagal intact and vagally denervated cats. This drug did not increase the frequency of water-induced swallows in any group. During water swallows, codeine increased the EMG magnitudes of selected upper airway muscles (geniohyoid, mylohyoid, thyrohyoid and thyropharyngeus) only in animals with mid-cervical vagotomy.

To our knowledge, this is the first report that the mu-opioid receptor agonist, codeine, alters swallow function in an animal model. In humans, the mu-opioid receptor agonist, remifentanil, altered pharyngeal function and esophageal motility during swallowing (7–11, 29). These effects included obvious aspiration (9), reductions in pharyngeal bolus movement (11), altered upper esophageal sphincter

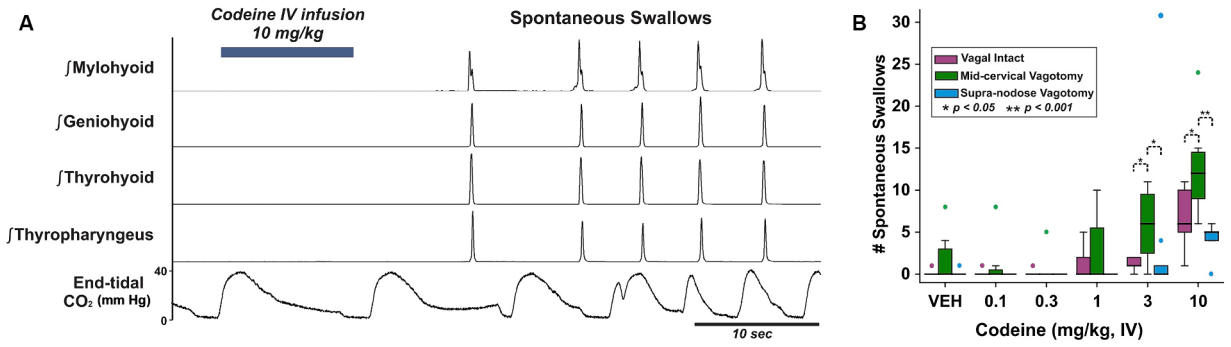

FIGURE 1
(A) Integrated upper airway muscle EMGs in a mid-cervical vagotomized cat after intravenous application of codeine and the resulting spontaneously produced swallows. Bottom, end-tidal CO_2 oscillates with the breathing rhythm and is disrupted by swallows. **(B)** Box-and-whisker plots show that spontaneously produced swallows increased with mid-cervical vagotomy but not supra-nodose vagotomy. VEH, vehicle; vagus intact ($n = 5$), mid-cervical vagotomy ($n = 7$), and supra-nodose vagotomy ($n = 5$). Colored markers are outliers for each condition.

TABLE 1 Swallow counts induced by water under vagus intact, cervical vagotomy, and supra-nodose vagotomy conditions.

	Codeine doses						
Vagus intact ($n = 5$)	Pre-cut	VEH	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	10.0 mg/kg
Water swallow count	n.a. ± n.a.	2.7 ± 0.7*,†	2.6 ± 0.7**	2.1 ± 0.5*	1.6 ± 0.3	1.4 ± 0.4	1.8 ± 0.3*
Cervical vagotomy ($n = 7$)	Pre-cut	VEH	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	10.0 mg/kg
Water swallow count	2.1 ± 0.4	1.1 ± 0.3	1.3 ± 0.3	1.7 ± 0.4*	1.5 ± 0.3	1.8 ± 0.3*	1.7 ± 0.4*
Supra-nodose Vagotomy ($n = 5$)	Pre-cut	VEH	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	10.0 mg/kg
Water swallow count	4.0 ± 1.0	0.8 ± 0.5	0.4 ± 0.3	0.2 ± 0.2	0.6 ± 0.5	0.3 ± 0.2	0.3 ± 0.1

Values reported are mean ± SEM. Statistical significance relative to SNVx at a given dose is indicated by $*p < 0.05$ or $**p < 0.01$. Statistical significance relative to CVx at a given dose is indicated by $^{\dagger}p < 0.05$. VEH, vehicle. SNVx, supra-nodose vagotomy. CVx, mid-cervical vagotomy. n.a., not applicable.

function (7), and a reduction in the strength of pharyngeal contractions (11). Similar effects were observed with morphine (11, 30). The effects of remifentanil on pharyngeal swallowing were not attenuated following administration of a peripherally acting derivative of the opioid antagonist naltrexone (7), suggesting a central action of the opioid. We are not aware of any report in the literature that codeine alters swallow function on the human. We note that we observed enhancement of pharyngeal swallow at doses of this drug (3 and 10 mg/kg IV) that were frankly respiratory depressant in this model (data not shown). In the human, prescription oral doses of codeine typically do not result in respiratory depression. It is possible that codeine in the human would have significant effects on swallow function at higher doses or if administered by the intravenous route.

We cannot attribute these effects of codeine solely to its actions at mu-opioid receptors given that it is a nonspecific drug. Codeine binds to an allosteric site on the central nicotinic receptor and enhances cholinergic transmission by a mechanism that is not sensitive to the alpha-7 nicotinic receptor antagonist methyllycaconitine (31–33). Therefore, the excitatory effects on pharyngeal swallowing observed in this study may be specific to codeine and not extendable to mu-opioid agonists in general.

Codeine induced swallowing in the absence of a peripheral stimulus, and swallow number was higher in vagotomized relative to vagal intact or SNGx animals. These observations support several different conclusions. First, codeine can induce swallowing in animals that have vagal axons and/or vagal axons plus ganglia sectioned, indicating that the swallow-promoting actions of this drug do not require sensory feedback from vagal sources to occur. Second, under vagus intact conditions sensory feedback blunted the swallow-inducing effects of codeine. Third, the differences between cervical vagotomy and section of the vagal trunk rostral to the nodose ganglion support the concept that ganglion cells and/or superior laryngeal nerve afferent pathways had a role in blunting the actions of codeine. The superior and inferior laryngeal nerves enter the vagal ganglion complex and this anatomical site is rostral to the typical location of cervical vagotomy. This afferent pathway was intact in our CVx animals, but was eliminated in the SNGx group.

While codeine is well-known to have central actions (18, 34), it and other opioids also alter the excitability of thoracic vagal and perhaps laryngeal afferents (13, 17, 35). The extent to which the swallow-inducing actions of codeine were due to solely central effects of this drug that were modified by spontaneous discharge of vagal sensory pathways or were due to both central and peripheral actions is unknown.

Codeine had differential actions on swallowing when actuated by increasing doses of the drug itself or a water stimulus. Spontaneous swallow count increased in a dose-dependent manner in response to the drug (Figure 1B), especially in CVx animals, yet the swallow count did not increase when the behavior was elicited by water (Table 1). First, codeine may have acted to decrease the threshold for actuation of swallow, presumably at the central level. Second, codeine may have acted on the swallow central pattern generator for swallowing if it is subordinate to a distinct threshold mechanism. Third, this drug may have increased the excitability of other circuits that stimulate swallowing through disinhibition. For example, Pitts and coworkers

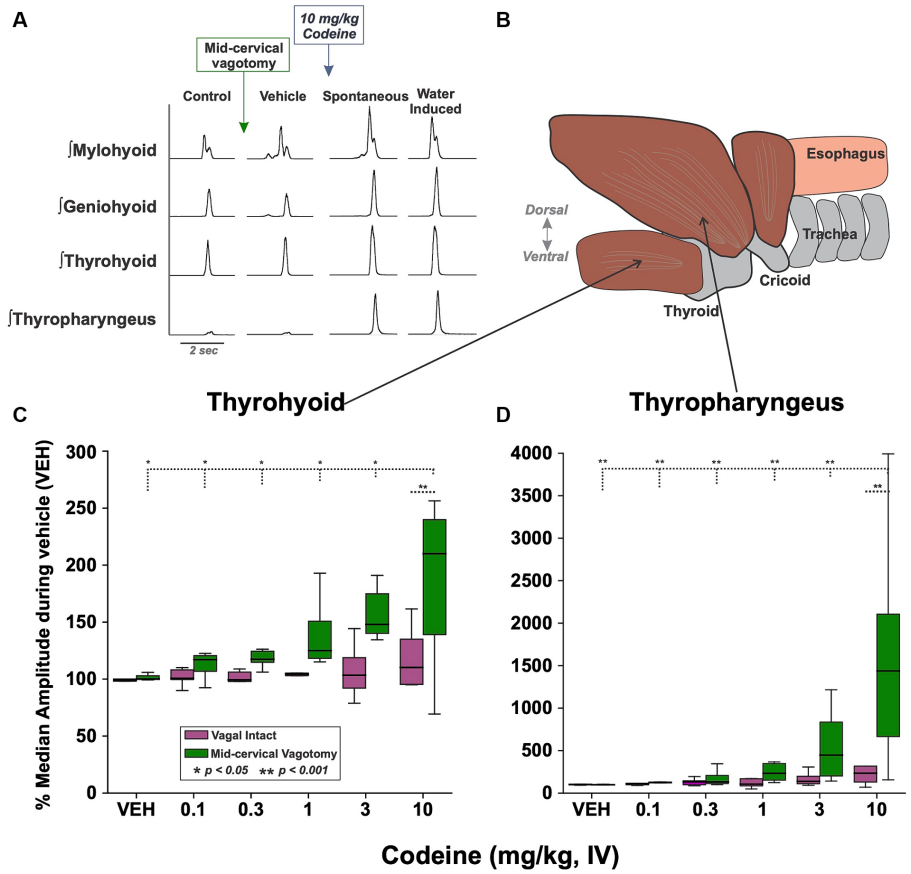

FIGURE 2
(A) Examples of upper airway muscle EMGs showing swallowing behavior before/after mid-cervical vagotomy (left) and before/after intravenous codeine application (middle). Approximately 1 min after the presentation of each codeine dose water trials were begun (right). **(B)** A sagittal view showing the muscle arrangement of the thyrohyoid **(C)** and thyropharyngeus **(D)** muscles. Box plots show that EMG amplitude increased with codeine doses under the mid-cervical vagotomy condition for both muscles. VEH, vehicle.

have shown that spinal pathways can have significant excitatory effects on swallowing (36). Our approach cannot separate between these three hypotheses and they are not mutually exclusive.

These findings also support the concept that swallow frequency can be controlled differently than motor drive to upper airway muscles. In response to water stimuli, swallow number did not change but the magnitudes of upper airway muscle EMGs significantly increased, especially in CVx animals. As noted above, enhancement of motor drive to selected upper airway muscles during swallowing can be observed following cervical spinal injury (36). The extent to which this mechanism can be actuated by drugs in the absence of spinal injury is unknown.

We conducted these experiments solely in male animals. Recently, Huff et al. (3) showed in a mouse model that there were no differences in swallow number or upper airway muscle EMG magnitudes between sexes under control conditions in vagal intact animals. However, male mice had 30–40% higher motor drive to geniohyoid and mylohyoid muscles after vagotomy. In our study, cervical vagotomy did not significantly alter motor drive to these two muscle groups or the thyrohyoid or thyropharyngeus muscles during water swallow but post vagotomy magnitudes were highly variable. The extent to which vagotomy alters upper airway muscle motor drive in female cats is unknown.

Bautista and co-workers have shown that swallowing can occur during autoresuscitation (37) and this behavior has been proposed to be important in the autoresuscitation process (38). It is likely that the role of swallowing in autoresuscitation is one of oropharyngeal clearance. Motor activation of pharyngeal muscles to move material out of the upper airway is an essential component of this behavior. Given that codeine initiated spontaneous swallowing but did not increase swallow number after water swallows, the action of this drug may be on neural elements that participate in non-ingestive functions of swallow control, such as autoresuscitation.

There were very large magnitude water swallows in our study for some upper airway muscle EMGs. This effect was magnified somewhat by the fact that vagotomy reduced EMG magnitudes for the thyropharyngeus in four animals. Although this effect was not statistically significant it did alter normalization. However, the largest water swallows in these animals were still several hundreds of percent larger than pre-vagotomy levels. As such, we chose not to remove these swallows from the dataset as outliers. It is our view that others who may choose to repeat our work be informed regarding the large range of swallow EMG magnitudes that can be observed after administration of codeine.

In conclusion, intravenous codeine induced spontaneous swallowing in the anesthetized cat. Further, the magnitudes, but not the frequency of occurrence of water-induced swallows were enhanced by this drug. These effects were larger in vagotomized animals. The

TABLE 2 Changes in EMG amplitudes for water-induced swallows relative to vehicle for upper airway muscles under vagus intact and cervical vagotomy conditions.

Vagus intact (n=5)	Codeine doses						
Water swallow amps. (% relative to VEH)	Pre-cut	VEH	0.1mg/kg	0.3mg/kg	1.0mg/kg	3.0mg/kg	10.0mg/kg
Hyoid/Laryngeal elevators							
Mylohyoid	n.a. ± n.a.	101 ± 4	100 ± 9	106 ± 10	98 ± 13	83 ± 11	108 ± 45†
Geniohyoid	n.a. ± n.a.	96 ± 2	93 ± 16	100 ± 19	95 ± 30	91 ± 41	121 ± 49††
Thyrohyoid	n.a. ± n.a.	106 ± 17	101 ± 8	98 ± 12	104 ± 15	107 ± 28	119 ± 29††
Pharyngeal							
Thyropharyngeus	n.a. ± n.a.	101 ± 5	118 ± 32	132 ± 43	117 ± 53	170 ± 96	294 ± 254†††
Cervical vagotomy (n=7)	Codeine doses						
Water swallow amps. (% relative to VEH)	Pre-cut	VEH	0.1mg/kg	0.3mg/kg	1.0mg/kg	3.0mg/kg	10.0mg/kg
Hyoid/Laryngeal elevators							
Mylohyoid	145 ± 148	106 ± 10	146 ± 83	132 ± 71	151 ± 84	202 ± 153	934 ± 1878†
Geniohyoid	88 ± 31	106 ± 12*	120 ± 53*	126 ± 45*	143 ± 40*	189 ± 99*	547 ± 802††
Thyrohyoid	126 ± 58	110 ± 25*	124 ± 36*	120 ± 12*	139 ± 31*	156 ± 70*	262 ± 243††
Pharyngeal							
Thyropharyngeus	151 ± 87	103 ± 8***	122 ± 14***	175 ± 96***	303 ± 221***	555 ± 445***	1,590 ± 1,305†††

Values reported are mean ± SEM. VEH, vehicle. n.a., not applicable. Statistical significance relative to 10 mg/kg codeine of other doses, under the given vagal condition, is indicated by *$p < 0.05$ or ***$p < 0.001$. Statistical significance for a muscle EMG between vagal conditions at a given dose is indicated by †$p < 0.05$, ††$p < 0.01$ or †††$p < 0.001$.

results support a central action of codeine to enhance swallowing in the anesthetized cat model. Mid-cervical vagotomy resulted in a greater stimulation of spontaneous swallowing than in vagal intact animals. We suggest that systemic administration of codeine also actuated vagal afferent reflexes that suppress the expression of centrally activated spontaneous swallowing. The receptor-based mechanism for this effect may be more related to binding of codeine to an allosteric site on nicotinic receptors than its binding affinity for mu-opioid receptors. The central neural elements responsible for these effects of codeine are unknown.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by University of Florida Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

DB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. TS: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. MM: Investigation, Methodology, Writing – review & editing. MR: Investigation, Methodology, Writing – review & editing. JH: Writing – review & editing. TP: Data curation, Formal analysis, Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev*. (2001) 81:929–69. doi: 10.1152/physrev.2001.81.2.929

2. Horton K-K, Segers LS, Nuding SC, O'Connor R, Alencar PA, Davenport PW, et al. Central respiration and mechanical ventilation in the gating of swallow with breathing. *Front Physiol*. (2018) 9:785. doi: 10.3389/fphys.2018.00785

3. Huff A, Reed MD, Iceman KE, Howland DR, Pitts T. Sex-specific vagal and spinal modulation of swallow and its coordination with breathing. *PLoS One*. (2020) 15:e0234194. doi: 10.1371/journal.pone.0234194

4. Frazure ML, Brown AD, Greene CL, Iceman KE, Pitts T. Rapid activation of esophageal mechanoreceptors alters the pharyngeal phase of swallow: evidence for inspiratory activity during swallow. *PLoS One*. (2021) 16:e0248994. doi: 10.1371/journal.pone.0248994

5. Shaker R, Hogan WJ. Reflex-mediated enhancement of airway protective mechanisms. *Am J Med*. (2000) 108:8–14. doi: 10.1016/S0002-9343(99)00289-2

6. Spearman DG, Poliacek I, Rose MJ, Bolser DC, Pitts T. Variability of the pharyngeal phase of swallow in the cat. *PLoS One*. (2014) 9:e106121. doi: 10.1371/journal.pone.0106121

7. Cajander P, Omari T, Cock C, Magnuson A, Scheinin M, Savilampi J. Effects of remifentanil on pharyngeal swallowing and esophageal motility: no impact of different bolus volumes and partial antagonism by methylnaltrexone. *Am J Physiol*. (2021) 321:G367–77. doi: 10.1152/ajpgi.00137.2021

8. Doeltgen SH, Omari TI, Savilampi J. Remifentanil alters sensory neuromodulation of swallowing in healthy volunteers: quantification by a novel pressure-impedance analysis. *Am J Physiol*. (2016) 310:G1176–82. doi: 10.1152/ajpgi.00138.2016

9. Savilampi J, Ahlstrand R, Magnuson A, Geijer H, Wattwil M. Aspiration induced by remifentanil: a double-blind, randomized, crossover study in healthy volunteers. *Anesthesiology*. (2014) 121:52–8. doi: 10.1097/ALN.0000000000000202

10. Savilampi J, Ahlstrand R, Magnuson A, Wattwil M. Effects of remifentanil on the esophagogastric junction and swallowing. *Acta Anaesthesiol Scand*. (2013) 57:1002–9. doi: 10.1111/aas.12134

11. Savilampi J, Omari T, Magnuson A, Ahlstrand R. Effects of remifentanil on pharyngeal swallowing: a double blind randomised cross-over study in healthy volunteers. *European J Anaesthesiol*. (2016) 33:622–30. doi: 10.1097/EJA.0000000000000461

12. Miner NB, Schutzer WE, Zarnegarnia Y, Janowsky A, Torralva R. Fentanyl causes naloxone-resistant vocal cord closure: a platform for testing opioid overdose treatments. *Drug Alcohol Depend*. (2021) 227:108974. doi: 10.1016/j.drugalcdep.2021.108974

13. Willette RN, Krieger AJ, Sapru HN. Opioids increase laryngeal resistance and motoneuron activity in the recurrent laryngeal nerve. *Eur J Pharmacol*. (1982) 80:57–63. doi: 10.1016/0014-2999(82)90177-7

14. Zhang Z, Zhang C, Zhou M, Xu F. Activation of opioid μ-receptors, but not δ- or κ-receptors, switches pulmonary C-fiber-mediated rapid shallow breathing into an apnea in anesthetized rats. *Respir Physiol Neurobiol*. (2012) 183:211–7. doi: 10.1016/j.resp.2012.06.032

15. Fountain JS, Reith DM, Tomlin AM, Smith AJ, Tilyard MW. Deaths by poisoning in New Zealand, 2008–2013. *Clin Toxicol*. (2019) 57:1087–94. doi: 10.1080/15563650.2019.1582777

16. Häkkinen M, Launiainen T, Vuori E, Ojanperä I. Comparison of fatal poisonings by prescription opioids. *Forensic Sci Int*. (2012) 222:327–31. doi: 10.1016/j.forsciint.2012.07.011

17. Gruhzit CC. Chemoreflex activity of dextromethorphan (romilar), dextrorphan, codeine and morphine in the cat and the dog. *J Pharmacol Exp Ther*. (1957) 120:399–407.

18. Bolser DC. Mechanisms of action of central and peripheral antitussive drugs. *Pulm Pharmacol*. (1996) 9:357–64. doi: 10.1006/pulp.1996.0047

19. Chou DT, Wang SC. Studies on the localization of central cough mechanism; site of action of antitussive drugs. *J Pharmacol Exp Ther*. (1975) 194:499–505.

20. Korpáš J., Tomori Z. (1979). *Cough and other respiratory reflexes, Progress in Respiration Research*.

21. May AJ, Widdicombe JG. Depression of the cough reflex by pentobarbitone and some opium derivatives. *Br J Pharmacol Chemother*. (1954) 9:335–40. doi: 10.1111/j.1476-5381.1954.tb01689.x

22. Mutolo D. Brainstem mechanisms underlying the cough reflex and its regulation. *Respir Physiol Neurobiol*. (2017) 243:60–76. doi: 10.1016/j.resp.2017.05.008

23. Mutolo D, Bongianni F, Cinelli E, Fontana GA, Pantaleo T. Modulation of the cough reflex by antitussive agents within the caudal aspect of the nucleus tractus solitarii in the rabbit. *Am J Phys Regul Integr Comp Phys*. (2008) 295:R243–51. doi: 10.1152/ajpregu.00184.2008

24. Poliacek I, Wang C, Corrie LW-C, Rose MJ, Bolser DC. Microinjection of codeine into the region of the caudal ventral respiratory column suppresses cough in anesthetized cats. *J Appl Physiol*. (2010) 108:858–65. doi: 10.1152/japplphysiol.00783.2009

25. Basmajian JV, Stecko G. A new bipolar electrode for electromyography. *J Appl Physiol*. (1962) 17:849–9. doi: 10.1152/jappl.1962.17.5.849

26. Pitts T, Gayagoy AG, Rose MJ, Poliacek I, Condrey JA, Musslewhite MN, et al. Suppression of abdominal motor activity during swallowing in cats and humans. *PLoS One*. (2015) 10:e0128245. doi: 10.1371/journal.pone.0128245

27. Pitts T, Poliacek I, Rose MJ, Reed MD, Condrey JA, Tsai H-W, et al. Neurons in the dorsomedial medulla contribute to swallow pattern generation: evidence of inspiratory activity during swallow. *PLoS One*. (2018) 13:e0199903. doi: 10.1371/journal.pone.0199903

28. Pitts T, Rose MJ, Mortensen AN, Poliacek I, Sapienza CM, Lindsey BG, et al. Coordination of cough and swallow: a meta-behavioral response to aspiration. *Respir Physiol Neurobiol*. (2013) 189:543–51. doi: 10.1016/j.resp.2013.08.009

29. Cock C, Doeltgen SH, Omari T, Savilampi J. Effects of remifentanil on esophageal and esophagogastric junction (EGJ) bolus transit in healthy volunteers using novel pressure-flow analysis. *Neurogastroenterol Motility*. (2018) 30:e13191. doi: 10.1111/nmo.13191

30. Hårdemark Cedborg AI, Sundman E, Bodén K, Hedström HW, Kuylenstierna R, Ekberg O, et al. Effects of morphine and midazolam on pharyngeal function, airway protection, and coordination of breathing and swallowing in healthy adults. *Anesthesiology*. (2015) 122:1253–67. doi: 10.1097/ALN.0000000000000657

31. Atkinson AP, Baguet E, Galland N, Le Questel J-Y, Planchat A, Graton J. Structural features and hydrogen-bond properties of Galanthamine and codeine: an experimental and theoretical study. Chemistry – a. *European J*. (2011) 17:11637–49. doi: 10.1002/chem.201100475

32. Maelicke A, Sclattenholz A, Storch A, Schröder B, Gutbrod O, Methfessel C, et al. Minireview: noncompetitive Agonism at nicotinic acetylcholine receptors; functional significance for CNS signal transduction. *J Receptors Signal Transduct*. (1995) 15:333–53. doi: 10.3109/10799899509045225

33. Storch A, Schrattenholz A, Cooper JC, Ghani EMA, Gutbrod O, Weber K-H, et al. Physostigmine, galanthamine and codeine act as 'noncompetitive nicotinic receptor agonists' on clonal rat pheochromocytoma cells. *Eur J Pharmacol Mol Pharmacol*. (1995) 290:207–19. doi: 10.1016/0922-4106(95)00080-1

34. Bolser DC, Hey JA, Chapman RW. Influence of central antitussive drugs on the cough motor pattern. *J Appl Physiol*. (1999) 86:1017–24. doi: 10.1152/jappl.1999.86.3.1017

35. Willette RN, Evans DY, Doorley BM. The in situ isolated larynx for evaluating peripheral opiate receptor antagonists. *J Pharmacol Methods*. (1987) 17:15–25. doi: 10.1016/0160-5402(87)90033-7

36. Pitts T, Iceman KE, Huff A, Musselwhite MN, Frazure ML, Young KC, et al. Laryngeal and swallow dysregulation following acute cervical spinal cord injury. *J Neurophysiol*. (2022) 128:405–17. doi: 10.1152/jn.00469.2021

37. Bautista TG, Fong AY, Dutschmann M. Spontaneous swallowing occurs during autoresuscitation in the in situ brainstem preparation of rat. *Respir Physiol Neurobiol*. (2014) 202:35–43. doi: 10.1016/j.resp.2014.07.015

38. Khurana A, Thach BT. Effects of upper airway stimulation on swallowing, gasping, and autoresuscitation in hypoxic mice. *J Appl Physiol*. (1996) 80:472–7. doi: 10.1152/jappl.1996.80.2.472

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A dysphagia model with denervation of the pharyngeal constrictor muscles in guinea pigs: functional evaluation of swallowing

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Introduction: Swallowing impairment is a crucial issue that can lead to aspiration, pneumonia, and malnutrition. Animal models are useful to reveal pathophysiology and to facilitate development of new treatments for dysphagia caused by many diseases. The present study aimed to develop a new dysphagia model with reduced pharyngeal constriction during pharyngeal swallowing.

Methods: We analyzed the dynamics of pharyngeal swallowing over time with the pharyngeal branches of the vagus nerve (Ph-X) bilaterally or unilaterally transected, using videofluoroscopic assessment of swallowing in guinea pigs. We also evaluated the detailed anatomy of the pharyngeal constrictor muscles after the denervation.

Results: Videofluoroscopic examination of swallowing showed a significant increase in the pharyngeal area during swallowing after bilateral and unilateral sectioning of the Ph-X. The videofluoroscopy also showed significantly higher pharyngeal transit duration for bilateral and unilateral section groups. The thyropharyngeal muscle on the sectioned side was significantly thinner than that on the intact side. In contrast, the thickness of the cricopharyngeal muscles on the sectioned and intact sides were not significantly different. The mean thickness of the bilateral thyropharyngeal muscles showed a linear correlation to the pharyngeal area and pharyngeal transit duration.

Discussion: Data obtained in this study suggest that denervation of the Ph-X could influence the strength of pharyngeal contraction during pharyngeal swallowing in relation to thickness of the pharyngeal constrictor muscles, resulting in a decrease in bolus speed. This experimental model may provide essential information (1) for the development of treatments for pharyngeal dysphagia and (2) on the mechanisms related to the recovery process, reinnervation, and nerve regeneration following injury and swallowing impairment possibly caused by medullary stroke, neuromuscular disease, or surgical damage from head and neck cancer.

KEYWORDS

dysphagia, swallowing, guinea pig, pharyngeal branch of the vagus nerve, videofluoroscopy

Introduction

Early and proper assessment of swallowing impairment is crucial in preventing aspiration. However, dysphagia is more likely to occur in the elderly population due to age-related diseases such as cerebrovascular disorders, neurodegenerative diseases, and malignant tumors (1–4). It is a critical issue that can lead to aspiration, pneumonia, and malnutrition. Swallowing is a complex behavior that involves the sequential contraction of many swallowing-related muscles (5, 6). It is controlled by neural pathways called the swallowing central pattern generators (CPG) in the brainstem (6–9). Because of this complexity, it is essential to comprehend the pathophysiology of dysphagia. Spatiotemporal stereotyped movements during the pharyngeal stage of swallowing include laryngeal elevation, pharyngeal constriction, glottal adduction, and cricopharyngeal opening; these movements are driven by the swallowing CPG, and activated by peripheral afferent signals from the pharynx and larynx. The reflexibility of swallowing can also be mediated by descending signals from the higher brain centers, as well as by other afferents in the spinal cord (10–15). In particular, the sequential activation of pharyngeal constrictor muscles (e.g., thyropharyngeal muscle) is critical for efficient bolus transfer during swallowing. Deteriorated movement of these muscles can cause attenuated pharyngeal contraction, possibly resulting in severe pharyngeal dysphagia. These movements involve well-coordinated swallow-breathing coordination to prevent aspiration (16).

Animal models have proven useful in revealing the pathophysiology and to facilitate development of new treatments for dysphagia caused by many diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, and stroke (17–21). To understand the mechanisms for various patterns of dysphagia and to develop effective treatments for dysphagia, animal models are still necessary to test the causal mechanisms that produce swallowing disorders (22–24). A sensory deficit, which results from inadequate sensory information from the bolus passage into the pharynx to the swallowing CPG, can cause a delayed incidence of swallowing (2, 6, 25–27). To examine swallowing function, the animal model for this sensory deficit has already been developed by transecting the superior laryngeal nerve, a significant afferent pathway to evoke pharyngeal swallowing (28, 29).

However, the most essential factor that renders a patient incapable of swallowing is the reduction of the swallowing-related muscle activity during swallowing, which can result in severe aspiration pneumonia. Thus, assessing swallowing function for dysphagia caused by the impairment of swallow motor activity in relation to larynx excursion and hyoid movement in animal models is crucial (18, 30). King et al. (31) have developed a dysphagia animal model with attenuated jaw excursion produced by injury of the mylohyoid muscle. While the immobility of the pharynx and larynx due to bulbar paralysis, possibly caused by medullary stroke (e.g., Wallenberg syndrome), leads to severe pharyngeal dysphagia, animal models with attenuated pharyngeal contraction during swallowing have not been well developed (32–36).

On the other hand, ethical concerns should be considered when developing an animal model with chronic impaired swallowing function. The animals with severe dysphagia may suffer from an intolerable condition, such as chronic aspiration, likely leading to continuous body weight loss. As such, a suitable animal model representing swallowing motor impairment must be developed without any chronic distress during the course of a long-term study.

The present study was aimed to establish a new dysphagia model with reduced pharyngeal constriction during pharyngeal swallowing. We also purposed development of an animal model of a specific motor deficiency related to pharyngeal swallowing, which does not suffer severe complications, such as chronic aspiration, severe body weight loss, and the lack of oral intake. In guinea pigs, using videofluoroscopic assessment of swallowing, we analyzed the dynamics of pharyngeal swallowing over time with the pharyngeal branches of the vagus nerve (Ph-X) bilaterally or unilaterally transected. We also evaluated the anatomy of the pharyngeal constrictor muscles after the denervation.

Methods

All experimental procedures, approved by the local Universal Committee for the Use of Animals in Research (M2022-314), were performed on 10 guinea pigs (Hartley, male, Shimizu Laboratory Supplies, Kyoto, Japan) weighing 450–520 g and were confirmed by the Physiological Society of Japan Principles for the Care and Use of Animals.

Surgery and recording procedures

Eight animals were prepared for the surgery groups, whereas the remaining two animals were used as radiographic and histological control groups. Prior to the inclusion of the study, animals were gently handled for several days to be compliant in the recording apparatus of the videofluoroscopy to immobilize the animals for a restraint period of at least 30 min without any indicators of distress. Initially, the videofluoroscopic examination of swallowing was performed in all animals using an X-ray fluoroscopy system (SXT-9000A, Toshiba Medical Manufacturing Co. Ltd., Tochigi, Japan) (44 mV, 0.4 mA, 30 frames/s). The animal was placed in a polyethylene terephthalate cylindrical tube with a small iron ball (10 mm in diameter) to stabilize the animal for subsequent swallowing sessions and to standardize the images obtained from the X-ray system (Figure 1A). Sequential pharyngeal swallowing was induced by the infusion of contrast medium (Iotrolan, 270 mg/mL, viscosity of approximately 8.6 mPa·s, Bayer Yakuhin, Ltd., Osaka, Japan) into the oral cavity using a 1 mL syringe. A series of two or three trials was performed at each time point for all animals tested.

Surgeries were conducted using aseptic procedures in the dedicated operating room, and anesthesia was initiated and maintained with a constant infusion of isoflurane (4% for induction, 1–2.5% for maintenance) following intramuscular injections of dexamethasone (1 mg/kg) and atropine sulfate (0.1 mg/kg) to minimize laryngeal edema and secretion. The level of anesthesia was carefully maintained without any reflexive movement during the surgery, and without a decrease in breathing rate below 20 cycles/min. A midline incision of the cervical skin was made and exposed the larynx. The superior laryngeal nerve was bilaterally identified to avoid mechanical damage during the surgery. The pharyngeal branch of the vagus nerve (Ph-X) was then identified alongside the lateral border of the thyroid cartilage (Figure 1B). We divided all animals into four groups in order to compare the denervation effect on swallowing function. Group 1

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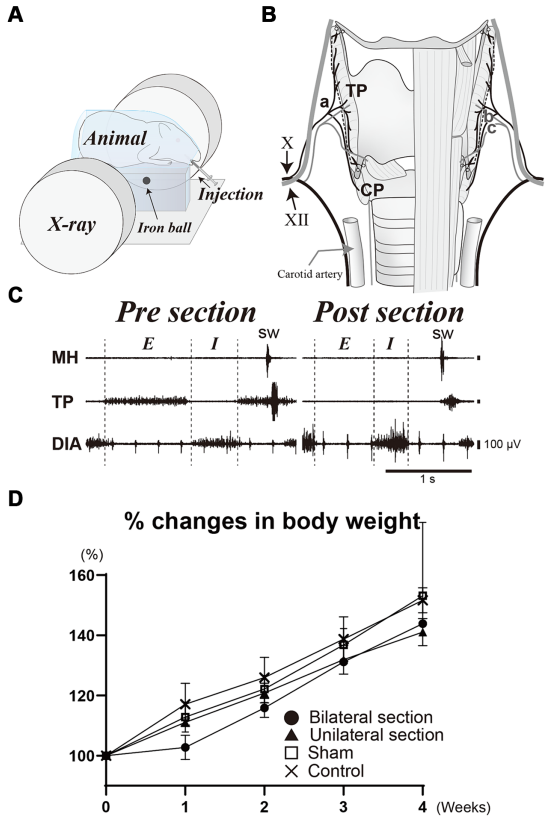

FIGURE 1
(A) Illustration of videofluoroscopic examination of swallowing. **(B)** Schema of surgical procedure for the denervation dysphagia model. Bilateral sectioning of the pharyngeal branch of the vagus nerve (Ph-X) (a) is performed (dashed line) while preserving the internal (b) and external (c) branches of the superior laryngeal nerve. **(C)** A representative recording of activities of the mylohyoid (MH), thyropharyngeal (TP), and diaphragm (DIA) muscles during breathing and swallowing before and after the section of the Ph-X. Body weight changes for bilateral (closed circle), unilateral Ph-X section (closed triangle), sham (open square), and control groups (cross) are shown in panel **(D)**. X, vagus nerve; XII, hypoglossal nerve; E, expiratory phase; I, inspiratory phase; CP, cricopharyngeal muscle; sw, swallowing.

($n=3$) was animals whose Ph-X was bilaterally cut (bilateral section), while Group 2 ($n=3$) animals underwent unilateral Ph-X section (unilateral section), Group 3 ($n=2$) were sham-operated animals, and Group 4 ($n=2$) were intact animals (control). We used two animals each for sham and control groups, according to the animal reduction approach. For injured animals, three animals were tested to investigate the effects of the nerve denervation, due to the variation in experimental variability within each group. The activities of mylohyoid, thyropharyngeal (TP), and diaphragm muscles were recorded under anesthesia using bipolar hooked stainless steel wire electrodes (insulated except for the tips) during breathing and swallowing; swallowing was evoked by infusion of a small amount of water into the oral cavity. The denervation procedure was deemed to be adequate as respiratory- and swallowing-related activities of the TP muscle were substantially attenuated after the denervation of the Ph-X (Figure 1C). To minimize possible damage caused by the electrodes on the TP muscles, we tested the effects of the section of the Ph-X only in representative animals for each group. After the skin incision was closed by suturing, anesthesia was gradually terminated, and the animals were observed in the recovery cage with a heat lamp for 1 h. Videofluoroscopic examination of swallowing was performed before the surgery and once each week after the surgery. Approximately 1 month after the surgery, animals were sacrificed by using an overdose of pentobarbital administration (intraperitoneally) and were perfused transcardially with 4% paraformaldehyde following physiological saline.

Histological and data analysis procedures

Consecutive sagittal sections of the larynx and pharynx (10 μm thickness) were made using a freezing microtome and stained with hematoxylin and eosin (Merck KGaA, Darmstadt, Germany) and photographed using a Biorevo BZ-X700 microscope (Keyence, Osaka, Japan). The thickness of the thyropharyngeal (TP) and cricopharyngeal (CP) muscles was measured at four and three different rostrocaudal levels in the sections at approximately 600 μm lateral from the midline, respectively. The body weight of each animal was measured every week.

Muscle activities were amplified and filtered (MEG-5200; Nihon Kohden, Tokyo, Japan) and input into a computer through the analog-digital converter (Power 1401 mk2 data collection system), and the signals were sampled at 5 kHz using Spike2 software (Cambridge Electronic Design, Cambridge, UK).

A video editing system (Premiere Pro, Adobe Systems, San Jose, CA, USA) was used to measure the temporal parameters. The dye areas of the contrast solution before swallowing (Figures 2A,Bi), during the pharyngeal stage of swallowing (Figures 2A,Bii), and during the esophageal stage of swallowing (Figures 2A,Biii) were delineated using images extracted from the videos (in three representative swallows). The actual areas were then calculated with reference to the iron ball attached to the recording apparatus. The pharyngeal area at the maximal contraction time of the pharynx during the pharyngeal stage of swallowing was measured [shaded areas in Figures 2A,Biv]. The esophageal area was also measured to evaluate bolus volume for each swallowing. The pharyngeal area was dependent upon the bolus volume and the force of the pharyngeal constriction (37, 38). The pharyngeal transit duration was defined as the period from the initiation of pharyngeal swallowing to the time when the bolus tail passed through the esophageal entrance and was measured in more than three episodes.

Statistical analyses were performed using Prism 10 software (GraphPad Software, San Diego, CA, USA). *t*-Test and single regression analysis were applied to determine the influences of the Ph-X sections on the swallowing-related muscles and swallowing function. Pooled data are presented as means ± standard error. Statistical significance was assumed for $p<0.05$.

Results

The present study was aimed to investigate the effects of sectioning the Ph-X on the swallowing function of guinea pigs. All animals tested tolerated well without lethal conditions with the protocols in this study. Consistent increases in body weight were observed in all animals sampled during the course of this study, although a slight decline in their typical trend of weight increase was noted 1 week after the surgery in the bilateral section group (within less than 5%). Aspiration was not observed on videofluoroscopy before or after the

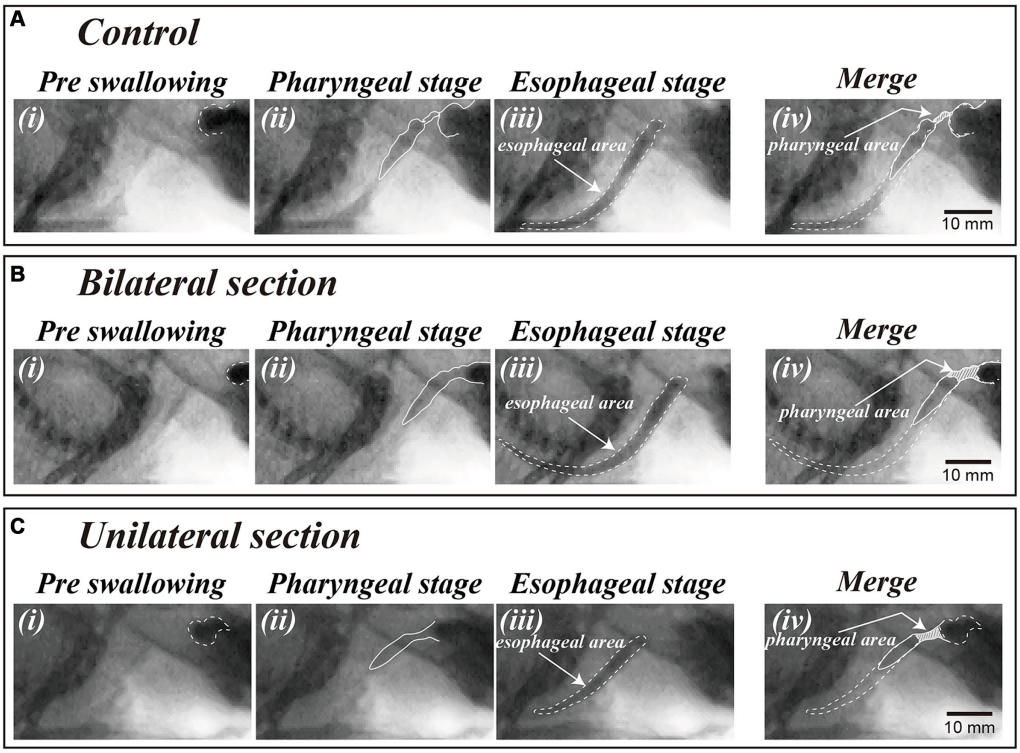

FIGURE 2
Video-fluoroscopic examination of swallowing in a control animal **(A)** and a dysphagia model 1 month after bilateral **(B)** and unilateral **(C)** sectioning of the Ph-X during oral **(i)**, pharyngeal **(ii)**, and esophageal stages of swallowing **(iii)**. The areas of contrast medium during the oral and esophageal stage of swallowing are depicted by dashed lines in panels **(i,iii)** [i.e., esophageal area in panel **(iii)**]. The dye areas in the pharyngeal cavity during bolus transfer in the pharyngeal stage of swallowing are shown by shaded areas in panel **(iv)** (i.e., pharyngeal area) delineated with reference to the oral **(i)** and esophageal area **(iii)**.

surgery, and the percent increases in body weight were not statistically different among all groups tested (One-way ANOVA) (Figure 1D).

Videofluoroscopic examination of swallowing for the bilateral and unilateral section group showed significant increases in the pharyngeal area after the surgery (One-way ANOVA, Tukey's multiple comparisons test, $p < 0.01$), and the areas for bilateral section group were significantly higher compared with those for unilateral section group (One-way ANOVA, Tukey's multiple comparisons test, $p < 0.01$) (Figures 2, 3A and Table 1). There was no statistical significance between sham and control groups (One-way ANOVA, Tukey's multiple comparisons test, $p = 0.9998$) (Figure 3A and Table 1).

The esophageal areas for the bilateral section group did not show a significant difference after the surgery (One-way ANOVA) (Figure 3B and Table 1). Although denervation of unilateral Ph-X in the unilateral section group elicited a transient decrease in the esophageal area, the reduction of the areas reached a stable state 1 month after the surgery; statistical significance was not apparent compared among all groups tested (One-way ANOVA).

Videofluoroscopic examination of swallowing also showed significantly higher pharyngeal transit duration after the surgery for bilateral and unilateral section groups (One-way ANOVA, Tukey's multiple comparisons test, $p < 0.01$, <0.05) (see Figure 3C and Table 1). In addition, the pharyngeal transit duration for bilateral section group was statistically higher compared with that for unilateral section group at 3 weeks after the surgery (One-way ANOVA, Tukey's multiple comparisons test, $p < 0.05$).

The time course of changes in the pharyngeal area, the esophageal area, and the pharyngeal transit duration are almost identical (repeated measures one-way ANOVA), and these areas and duration are not significantly different from sham and control groups (*t*-test) (Figures 3A–C). Figure 3D represents scatter plotting showing relationships between the pharyngeal area and the pharyngeal transit duration for all animals tested. A good linear correlation existed between these parameters (simple linear regression, $p < 0.0001$).

The TP muscle on the sectioned side (0.53 ± 0.02 mm on average) was significantly thinner than that on the intact side (0.78 ± 0.02 mm on average) (*t*-test, $p < 0.0001$) (Figures 4A–C). In contrast, the thickness of the CP muscles on the sectioned and intact sides did not show a significant difference (0.66 ± 0.02 mm for the sectioned side, 0.65 ± 0.03 mm for the intact side) (*t*-test) (Figures 4A,B,D). The mean thickness of the bilateral TP muscles for each animal was significantly correlated with the pharyngeal area and pharyngeal transit duration using simple linear regression analysis ($p < 0.01$) (Figures 4E,F and Table 1). However, the mean thickness of the CP muscles showed no correlation to the pharyngeal area or pharyngeal transit duration.

Discussion

The current study introduces a novel animal model that represents the dysfunction of pharyngeal contraction during pharyngeal swallowing. This model is produced by bilateral or unilateral

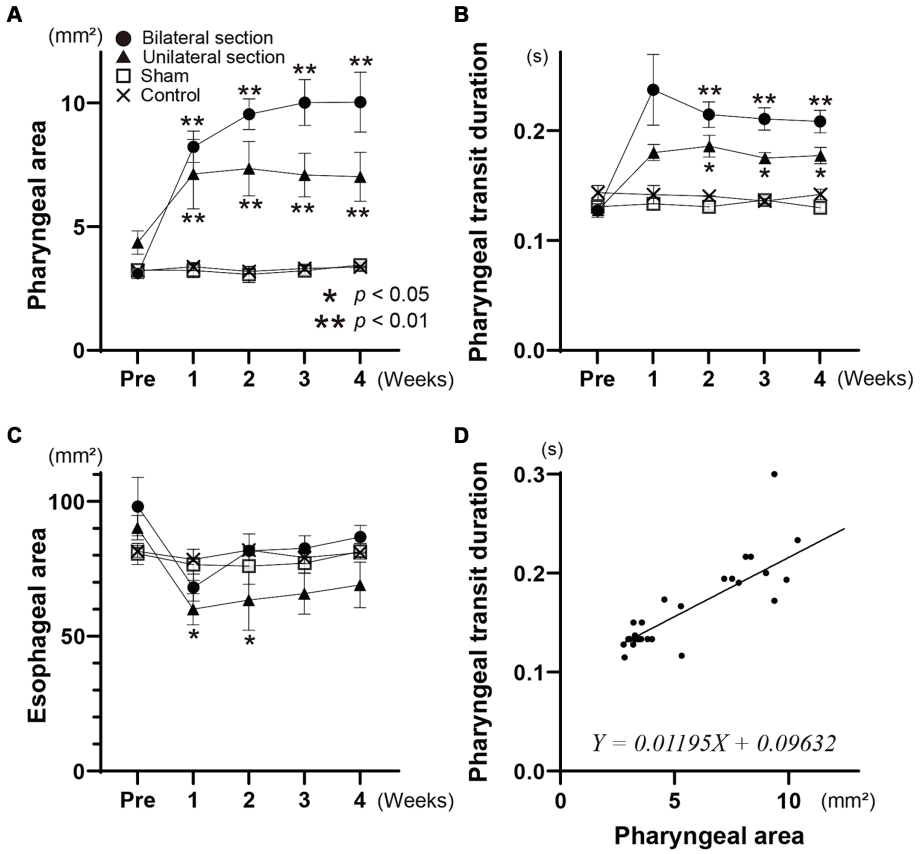

FIGURE 3
Changes in mean areas of the contrast dye in the pharynx **(A)** and esophagus **(B)** are indicated during the pharyngeal and esophageal stages of swallowing for all groups, respectively. The time course of the pharyngeal transit duration for each group is shown in panel **(C)**. Scatter plots, and relationships between the pharyngeal area and pharyngeal transit duration are represented in panel **(D)**. The formula of the linear regression line is given in panel **(D)**. Different symbols are used to indicate each group in panels **(A–C)**. Closed circle, bilateral section group; closed triangle, unilateral section group; open square, sham-operated group; cross, control group.

transection of the Ph-X in guinea pigs; its characteristics were investigated using videofluoroscopy and histological verification. Data obtained suggest that denervation of the Ph-X could reduce the strength of pharyngeal contraction during pharyngeal swallowing possibly attributing to atrophy of the denervated TP muscles, resulting in a decrease in bolus speed.

The nerve transections were made alongside the lateral edge of the thyroid cartilage, resulting in a marked thinning of the TP muscle, and, thereby, attenuation of the pharyngeal constriction during swallowing (39–41). Meanwhile, the CP muscle functions as a sphincter muscle whose activity is inhibited in accordance with pharyngeal swallowing, determining the resistance of bolus passage at the esophageal entrance (38, 42–44). As such, the timing of the CP activity may influence bolus speed and pharyngeal residue during swallowing. The dysphagia model provided in this study could provide a suitable platform for pharyngeal dysphagia caused by diminished pharyngeal contraction forces.

Nevertheless, even with the transient decreases in bolus volume for each swallow after the injury, feeding behavior was maintained in all groups over time. The pharyngeal area and pharyngeal transit duration reached a stable level as their weight consistently increased 1 month after the surgery, probably allowing the support of swallowing movements from the glossopharyngeal nerve region to remain functional. Regarding histological and functional changes after nerve and muscle injury, denervation of the Ph-X is sufficient to cause changes in swallowing function 1 month after the surgery (18, 21, 31). The animal model created in this study therefore provides a non-lethal dysphagia model that can be used for pathological analysis and the development of new treatments for dysphagia caused by poor pharyngeal contraction.

The Ph-X nerve fibers mainly consist of motor efferent fibers projecting to striated muscles in the pharynx, including the TP and CP muscles (8, 41, 45–47). These fibers drive not only sequential contraction of the pharyngeal cavity during swallowing but also other respiratory and non-respiratory-related behaviors such as coughing (41, 47).

The motoneurons of the Ph-X are topographically distributed in the nucleus ambiguus (45). Previous studies reported that the membrane potentials of these neurons predominantly showed expiratory-related change during respiration (41, 46). These neurons also showed swallowing-related depolarization following transient hyperpolarization, which determines the timing of muscle contraction

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TABLE 1 Spatiotemporal and histological analyses regarding swallowing in different experimental groups.

Groups	Videofluoroscopic examination of swallowing						Tissue	
		Pre-surgery	Post-surgery (weeks)				Mean TP thickness (mm)	Mean CP thickness (mm)
			1	2	3	4		
Bilateral section ($n=3$)	Pharyngeal area (mm^2)	3.11 ± 0.20	8.22 ± 0.64**	9.55 ± 0.62**	10.0 ± 0.92**	10.0 ± 1.21**	0.55 ± 0.03*	0.64 ± 0.03
	Esophageal area (mm^2)	98.1 ± 10.8	68.0 ± 4.98	81.6 ± 6.26	82.5 ± 4.64	86.8 ± 4.26		
	Pharyngeal transit duration (s)	0.13 ± 0.01	0.24 ± 0.03	0.21 ± 0.01**	0.21 ± 0.01**	0.21 ± 0.01**		
Unilateral section ($n=3$)	Pharyngeal area (mm^2)	4.38 ± 0.47	7.15 ± 1.40**	7.37 ± 1.10**	7.12 ± 0.88**	7.05 ± 0.99**	0.63 ± 0.04	0.64 ± 0.03
	Esophageal area (mm^2)	90.2 ± 4.51	60.0 ± 5.76*	63.4 ± 11.1*	65.8 ± 7.67	69.0 ± 8.46		
	Pharyngeal transit duration (s)	0.13 ± 0.01	0.18 ± 0.01	0.19 ± 0.01*	0.18 ± 0.00*	0.18 ± 0.01*		
Sham ($n=2$)	Pharyngeal area (mm^2)	3.27 ± 0.08	3.26 ± 0.28	3.09 ± 0.32	3.24 ± 0.15	3.47 ± 0.11	0.77 ± 0.00	0.64 ± 0.02
	Esophageal area (mm^2)	80.5 ± 3.91	76.5 ± 5.73	76.0 ± 6.76	77.0 ± 3.76	81.5 ± 2.88		
	Pharyngeal transit duration (s)	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.14 ± 0.00	0.13 ± 0.00		
Control ($n=2$)	Pharyngeal area (mm^2)	3.23 ± 0.03	3.41 ± 0.16	3.21 ± 0.09	3.34 ± 0.11	3.39 ± 0.03	0.79 ± 0.03	0.71 ± 0.00
	Esophageal area (mm^2)	81.5 ± 2.21	78.5 ± 0.78	82.0 ± 1.16	79.3 ± 2.28	81.1 ± 0.22		
	Pharyngeal transit duration (s)	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00		

This table presents the results of spatiotemporal analyses of videofluoroscopic examination of swallowing and histological analyses, indicating the pre-and post-surgery values of the pharyngeal, esophageal areas, and the pharyngeal transit duration in different experimental groups. The experimental groups include those with bilateral and unilateral sectioning of the pharyngeal branch of the vagus nerve (bilateral and unilateral section groups), a sham-operated group (sham group), and a control group. The mean thickness of the thyropharyngeal (TP) and cricopharyngeal (CP) muscles for all groups is also noted. The data for the control group are shown in accordance with the respective time course of the study.
*$p<0.05$, **$p<0.01$.

of pharyngeal constrictor muscles (47). Indeed, the expiratory-related and swallowing-related activities of the TP muscle were markedly decreased after the nerve transection.

Concerning the motor innervation in the pharyngeal region from the glossopharyngeal and vagal efferent through the pharyngeal plexus, massive damage of the pharyngeal plexus rostrocaudally along the pharynx may cause dysfunction of other swallowing-related muscles, such as the stylopharyngeal muscle controlled by the glossopharyngeal nerve (41, 45, 48). Further studies exploring the degeneration of the neuromuscular component caused by the Ph-X transaction may be necessary to investigate the detailed mechanisms of the muscle atrophy.

Although the nerve contains afferent fibers through which pharyngeal sensory signals are conveyed to the nucleus tractus solitarius, the superior laryngeal and glossopharyngeal nerves are significant afferent nerves that trigger pharyngeal swallowing (6, 26, 49–55). Thus, the influence of sensory denervation due to the transection of the Ph-X on swallowing function is likely to be minimal, which is in contrast to the dysphagia animal model produced by the transection of the superior laryngeal nerve.

In this animal model, pharyngeal contraction insufficiency induced the changes in the spatiotemporal parameters using videofluoroscopy. In this protocol for assessing swallowing function, fluid was directly infused into the oral cavity through a catheter to minimize the influence on oral movement, including licking and mastication. This testing procedure may facilitate evoking of sequential pharyngeal swallowing and may clarify the influence of the Ph-X section on pharyngeal swallowing. In the previous studies in the dysphagia animal models in rodents, swallowing function was assessed by videofluoroscopy using various parameters, such as inter-swallow intervals, swallow rate, pharyngeal and esophageal transit time, pharyngeal residue area,

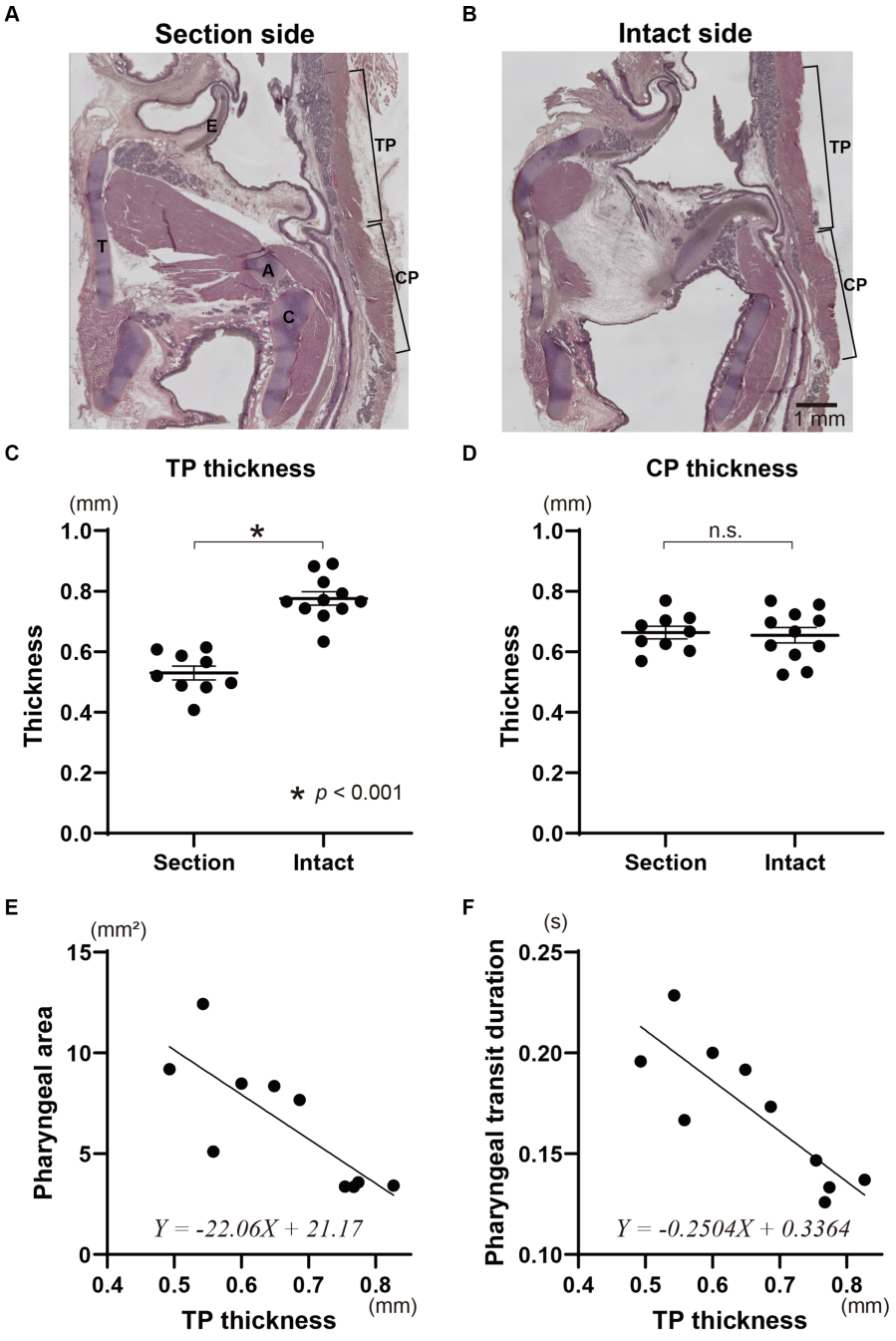

FIGURE 4
Histological evaluation of the pharyngeal constriction muscles due to the sectioning of the Ph-X and influence of the denervation on swallowing function. Representative sagittal tissue sections on the sectioned and intact sides of the pharynx in an animal with unilateral Ph-X section are shown in panels **(A,B)**, respectively. The thickness of the thyropharyngeal (TP) and cricopharyngeal (CP) muscles on the section and intact sides are represented in panels **(C,D)**. Relationships between pharyngeal area and the mean thickness of the TP muscle are shown in panel **(E)**. The pharyngeal transit duration and the thickness of the TP muscle for each animal are plotted in panel **(F)**. The linear regression lines and their formulae are provided in panels **(E,F)**. A, arytenoid cartilage; C, cricoid cartilage; E, epiglottis; T, thyroid cartilage.

and bolus speed (18, 21, 23, 24, 28, 31, 56). In the present study, we focused on the evaluation of the motor performances caused by nerve denervation-induced pharyngeal constrictor muscle atrophy and the development of a suitable model for pharyngeal muscle incompetence. Therefore, initiation timing and likelihood of swallowing triggered by oropharyngeal bolus transition was not assessed. Additional behavioral testing for self-feeding circulation pattern to examine compensatory orofacial movement against feeding and swallowing difficulties will be necessary.

The study has limitations, including difficulty detecting the difference in the two-dimensional analysis of the pharyngeal passage area, particularly in the unilateral amputation model.

However, the spatiotemporal and anatomical measures difference between animal groups of the Ph-X section and Sham-operated/control groups imply that swallowing dysfunction in the cranial motor denervation model could be appropriately evaluated by videofluoroscopic analyses. Although data obtained in this study provided significant information regarding swallowing function caused by Ph-X section, comparative studies with a larger sample size may be better powered to minimize the margin of error to confirm the efficacy of a new treatment for injured animals. Furthermore, additional studies are required including quantitative analyses of the electromyographic activities and immunohistochemical analyses for swallowing-related muscles before and at 1 month after the surgery to further evaluate functional and histological alteration after the denervation.

Perspectives and conclusion

In this study, we developed the animal model that produced reduced pharyngeal contraction during swallowing by unilateral or bilateral transection of the Ph-X and assessed swallowing function using videofluoroscopy. This experimental model may provide essential information for the development of treatments for pharyngeal dysphagia and mechanisms related to the recovery process after injury, reinnervation, and nerve regeneration by swallowing impairment possibly caused by medullary stroke, neuromuscular disease, and surgical damage from head and neck cancer. This model organism also holds excellent promise for further research, as it could lead to significant advancements in diagnosing and treating swallowing disorders.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the local Universal Committee for the Use of Animals in Research (M2022-314). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

KH: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft, Funding acquisition. YS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MK: Formal analysis, Investigation, Project administration, Writing – review & editing, Funding acquisition. SK: Investigation, Writing – review & editing. RY: Investigation, Writing – review & editing. TI: Formal analysis, Writing – review & editing. TU: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. SH: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Ertekin C, Aydogdu I, Yüceyar N, Kiylioglu N, Tarlaci S, Uludag B. Pathophysiological mechanisms of oropharyngeal dysphagia in amyotrophic lateral sclerosis. *Brain*. (2000) 123:125–40. doi: 10.1093/brain/123.1.125

2. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. *Stroke*. (2005) 36:2756–63. doi: 10.1161/01.STR.0000190056.76543.eb

3. Nguyen NP, Moltz CC, Frank C, Vos P, Smith HJ, Karlsson U, et al. Dysphagia following chemoradiation for locally advanced head and neck cancer. *Ann Oncol*. (2004) 15:383–8. doi: 10.1093/annonc/mdh101

4. Walther EK. Dysphagia after pharyngolaryngeal cancer surgery. Part I: pathophysiology of postsurgical deglutition. *Dysphagia*. (1995) 10:275–8. doi: 10.1007/BF00431422

5. Doty RW, Bosma JF. An electromyographic analysis of reflex deglutition. *J Neurophysiol*. (1956) 19:44–60. doi: 10.1152/jn.1956.19.1.44

6. Jean AA. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev*. (2001) 81:929–69. doi: 10.1152/physrev.2001.81.2.929

7. Huff A, Karlen-Amarante M, Pitts T, Ramirez JM. Optogenetic stimulation of pre–Bötzinger complex reveals novel circuit interactions in swallowing–breathing coordination. *Proc Natl Acad Sci U S A*. (2022) 119:e2121095119. doi: 10.1073/pnas.2121095119

8. Sugiyama Y, Shiba K, Nakazawa K, Suzuki T, Umezaki T, Ezure K, et al. Axonal projections of medullary swallowing neurons in guinea pigs. *J Comp Neurol*. (2011) 519:2193–211. doi: 10.1002/cne.22624

9. Umezaki T, Matsuse T, Shin T. Medullary swallowing-related neurons in the anesthetized cat. *Neuroreport*. (1998) 9:1793–8. doi: 10.1097/00001756-199806010-00022

10. Fuse S, Sugiyama Y, Hashimoto K, Umezaki T, Oku Y, Dutschmann M, et al. Laryngeal afferent modulation of swallowing interneurons in the dorsal medulla in perfused rats. *Laryngoscope*. (2020) 130:1885–93. doi: 10.1002/lary.28284

11. Jean A. Control of the central swallowing program by inputs from the peripheral receptors. A review. *J Auton Nerv Syst*. (1984) 10:225–33. doi: 10.1016/0165-1838(84)90017-1

12. Kaneko M, Sugiyama Y, Munekawa R, Kinoshita S, Mukudai S, Umezaki T, et al. Sustained effects of capsaicin infusion into the oropharynx on swallowing in perfused rats. *Laryngoscope*. (2024) 134:305–14. doi: 10.1002/lary.30918

13. Pitts T, Iceman KE, Huff A, Musselwhite MN, Frazure ML, Young KC, et al. Laryngeal and swallow dysregulation following acute cervical spinal cord injury. *J Neurophysiol*. (2022) 128:405–17. doi: 10.1152/jn.00469.2021

14. Takemura A, Sugiyama Y, Yamamoto R, Kinoshita S, Kaneko M, Fuse S, et al. Effect of pharmacological inhibition of the pontine respiratory group on swallowing interneurons in the dorsal medulla oblongata. *Brain Res*. (2022) 1797:148101. doi: 10.1016/j.brainres.2022.148101

15. Tsuji K, Tsujimura T, Sakai S, Suzuki T, Yoshihara M, Nagoya K, et al. Involvement of capsaicin-sensitive nerves in the initiation of swallowing evoked by carbonated water in anesthetized rats. *Am J Physiol Gastrointest Liver Physiol*. (2020) 319:G564–72. doi: 10.1152/ajpgi.00233.2020

16. Huff A, Day TA, English M, Reed MD, Zouboules S, Saran G, et al. Swallow-breathing coordination during incremental ascent to altitude. *Respir Physiol Neurobiol*. (2019) 265:121–6. doi: 10.1016/j.resp.2018.06.005

17. Ciucci MR, Russell JA, Schaser AJ, Doll EJ, Vinney LM, Connor NP. Tongue force and timing deficits in a rat model of Parkinson disease. *Behav Brain Res*. (2011) 222:315–20. doi: 10.1016/j.bbr.2011.03.057

18. Cullins MJ, Connor NP. Reduced tongue force and functional swallowing changes in a rat model of post stroke dysphagia. *Brain Res*. (2019) 1717:160–6. doi: 10.1016/j.brainres.2019.04.023

19. Lever TE, Simon E, Cox KT, Capra NF, O'Brien KF, Hough MS, et al. A mouse model of pharyngeal dysphagia in amyotrophic lateral sclerosis. *Dysphagia*. (2010) 25:112–26. doi: 10.1007/s00455-009-9232-1

20. Russell JA, Ciucci MR, Hammer MJ, Connor NP. Videofluorographic assessment of deglutitive behaviors in a rat model of aging and Parkinson disease. *Dysphagia*. (2013) 28:95–104. doi: 10.1007/s00455-012-9417-x

21. Sugiyama N, Nishiyama E, Nishikawa Y, Sasamura T, Nakade S, Okawa K, et al. A novel animal model of dysphagia following stroke. *Dysphagia*. (2014) 29:61–7. doi: 10.1007/s00455-013-9481-x

22. German RZ, Crompton AW, Gould FDH, Thexton AJ. Animal models for dysphagia studies: what have we learnt so far. *Dysphagia*. (2017) 32:73–7. doi: 10.1007/s00455-016-9778-7

23. Lever TE, Braun SM, Brooks RT, Harris RA, Littrell LL, Neff RM, et al. Adapting human videofluoroscopic swallow study methods to detect and characterize dysphagia in murine disease models. *J Vis Exp*. (2015) 97:52319. doi: 10.3791/52319-v

24. Lever TE, Brooks RT, Thombs LA, Littrell LL, Harris RA, Allen MJ, et al. Videofluoroscopic validation of a translational murine model of presbyphagia. *Dysphagia*. (2015) 30:328–42. doi: 10.1007/s00455-015-9604-7

25. Aviv JE, Kaplan ST, Thomson JE, Spitzer J, Diamond B, Close LG. The safety of flexible endoscopic evaluation of swallowing with sensory testing (FEESST): an analysis of 500 consecutive evaluations. *Dysphagia*. (2000) 15:39–44. doi: 10.1007/s004559910008

26. Jafari S, Prince RA, Kim DY, Paydarfar D. Sensory regulation of swallowing and airway protection: a role for the internal superior laryngeal nerve in humans. *J Physiol*. (2003) 550:287–304. doi: 10.1113/jphysiol.2003.039966

27. Miyaji H, Umezaki T, Adachi K, Sawatsubashi M, Kiyohara H, Inoguchi T, et al. Videofluoroscopic assessment of pharyngeal stage delay reflects pathophysiology after brain infarction. *Laryngoscope*. (2012) 122:2793–9. doi: 10.1002/lary.23588

28. Mok A, Allen J, Haney MM, Deninger I, Ballenger B, Caywood V, et al. A surgical mouse model for advancing laryngeal nerve regeneration strategies. *Dysphagia*. (2020) 35:419–37. doi: 10.1007/s00455-019-10045-6

29. Stevens M, Mayerl CJ, Bond L, German RZ, Barkmeier-Kraemer JM. Pathophysiology of aspiration in a unilateral SLN lesion model using quantitative analysis of VFSS. *Int J Pediatr Otorhinolaryngol*. (2021) 140:110518. doi: 10.1016/j.ijporl.2020.110518

30. Pollard RE. Videofluoroscopic evaluation of the pharynx and upper esophageal sphincter in the dog: a systematic review of the literature. *Front Vet Sci*. (2019) 6:117. doi: 10.3389/fvets.2019.00117

31. King SN, Fletcher B, Kimbel B, Bonomo N, Pitts T. Adaptations to oral and pharyngeal swallowing function induced by injury to the mylohyoid muscle. *Dysphagia*. (2020) 35:814–24. doi: 10.1007/s00455-019-10087-w

32. Aydogdu I, Ertekin C, Tarlaci S, Turman B, Kiylioglu N, Secil Y. Dysphagia in lateral medullary infarction (Wallenberg's syndrome): an acute disconnection syndrome in premotor neurons related to swallowing activity? *Stroke*. (2001) 32:2081–7. doi: 10.1161/hs0901.094278

33. Fang TJ, Tam YY, Courey MS, Li HY, Chiang HC. Unilateral high vagal paralysis: relationship of the severity of swallowing disturbance and types of injuries. *Laryngoscope*. (2011) 121:245–9. doi: 10.1002/lary.21342

34. Holcombe SJ, Derksen FJ, Stick JA, Robinson NE. Effect of bilateral blockade of the pharyngeal branch of the vagus nerve on soft palate function in horses. *Am J Vet Res*. (1998) 59:504–8. doi: 10.2460/ajvr.1998.59.04.504

35. Mok P, Woo P, Schaefer-Mojica J. Hypopharyngeal pharyngoplasty in the management of pharyngeal paralysis: a new procedure. *Ann Otol Rhinol Laryngol*. (2003) 112:844–52. doi: 10.1177/000348940311201004

36. Nilsson H, Ekberg O, Sjoberg S, Olsson R. Pharyngeal constrictor paresis: an indicator of neurologic disease? *Dysphagia*. (1993) 8:239–43. doi: 10.1007/BF01354545

37. Dantas RO, Kern MK, Massey BT, Dodds WJ, Kahrilas PJ, Brasseur JG, et al. Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing. *Am J Physiol Gastrointest Liver Physiol*. (1990) 258:G675–81. doi: 10.1152/ajpgi.1990.258.5.G675

38. Hoffman MR, Ciucci MR, Mielens JD, Jiang JJ, Mcculloch TM. Pharyngeal swallow adaptations to bolus volume measured with high-resolution manometry. *Laryngoscope*. (2010) 120:2367–73. doi: 10.1002/lary.21150

39. Ekberg O, Ekman M, Eriksson LI, Malm R, Sundman E, Arner A. An in vitro model for studying neuromuscular transmission in the mouse pharynx. *Dysphagia*. (2009) 24:32–9. doi: 10.1007/s00455-008-9168-x

40. Sundman E, Ansved T, Margolin G, Kuylenstierna R, Eriksson LI. Fiber-type composition and fiber size of the human cricopharyngeal muscle and the pharyngeal constrictor muscle. *Acta Anaesthesiol Scand*. (2004) 48:423–9. doi: 10.1111/j.1399-6576.2004.00364.x

41. Zoungrana OR, Amri M, Car A, Roman C. Intracellular activity of motoneurons of the rostral nucleus ambiguus during swallowing in sheep. *J Neurophysiol*. (1997) 77:909–22. doi: 10.1152/jn.1997.77.2.909

42. Bonington A, Whitmore I, Mahon M. A histological and histochemical study of the cricopharyngeus muscle in the guinea-pig. *J Anat*. (1987) 153:151–61.

43. Perlman AL, Palmer PM, Mcculloch TM, Vandaele DJ. Electromyographic activity from human laryngeal, pharyngeal, and submental muscles during swallowing. *J Appl Physiol*. (1999) 86:1663–9. doi: 10.1152/jappl.1999.86.5.1663

44. Shaw DW, Cook IJ, Gabb M, Holloway RH, Simula ME, Panagopoulos V, et al. Influence of normal aging on oral-pharyngeal and upper esophageal sphincter function during swallowing. *Am J Physiol Gastroint Liver Physiol*. (1995) 268:G389–96. doi: 10.1152/ajpgi.1995.268.3.G389

45. Altschuler SM, Bao XM, Miselis RR. Dendritic architecture of nucleus ambiguus motoneurons projecting to the upper alimentary tract in the rat. *J Comp Neurol*. (1991) 309:402–14. doi: 10.1002/cne.903090309

46. Grélot L, Barillot JC, Bianchi AL. Pharyngeal motoneurones: respiratory-related activity and responses to laryngeal afferents in the decerebrate cat. *Exp Brain Res*. (1989) 78:336–44. doi: 10.1007/BF00228905

47. Umezaki T, Shiba K, Sugiyama Y. Intracellular activity of pharyngeal motoneurons during breathing, swallowing, and coughing. *J Neurophysiol*. (2020) 124:750–62. doi: 10.1152/jn.00093.2020

48. Bieger D, Hopkins DA. Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. *J Comp Neurol*. (1987) 262:546–62. doi: 10.1002/cne.902620408

49. Altschuler SM, Bao X, Bieger D, Hopkins DA, Miselis RR. Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. *J Comp Neurol*. (1989) 283:248–68. doi: 10.1002/cne.902830207

50. Kinoshita S, Sugiyama Y, Hashimoto K, Fuse S, Mukudai S, Umezaki T, et al. Influences of GABAergic inhibition in the dorsal medulla on contralateral swallowing neurons in rats. *Laryngoscope*. (2021) 131:2187–98. doi: 10.1002/lary.29242

51. Kitagawa J, Shingai T, Takahashi Y, Yamada Y. Pharyngeal branch of the glossopharyngeal nerve plays a major role in reflex swallowing from the pharynx. *Am J Physiol Regul Integr Comp Physiol*. (2002) 282:R1342–7. doi: 10.1152/ajpregu.00556.2001

52. Miyazaki J, Shin T, Murata Y, Masuko S. Pharyngeal branch of the vagus nerve carries intraepithelial afferent fibers in the cat pharynx: an elucidation of the origin and central and peripheral distribution of these components. *Otolaryngol Head Neck Surg*. (1999) 120:905–13. doi: 10.1016/S0194-5998(99)70335-9

53. Ootani S, Umezaki T, Shin T, Murata Y. Convergence of afferents from the SLN and GPN in cat medullary swallowing neurons. *Brain Res Bull*. (1995) 37:397–404. doi: 10.1016/0361-9230(95)00018-6

54. Sang Q, Goyal RK. Swallowing reflex and brain stem neurons activated by superior laryngeal nerve stimulation in the mouse. *Am J Physiol Gastroint Liver Physiol*. (2001) 280:G191–200. doi: 10.1152/ajpgi.2001.280.2.G191

55. Sugimoto T, Umezaki T, Takagi S, Narikawa K, Shin T. Crossing inputs of the superior laryngeal nerve afferents to medullary swallowing-related neurons in the cat. *Neurosci Res*. (1998) 30:235–45. doi: 10.1016/S0168-0102(98)00004-2

56. Welby L, Ukatu CC, Thombs L, Lever TE. A mouse model of dysphagia after facial nerve injury. *Laryngoscope*. (2021) 131:17–24. doi: 10.1002/lary.28560

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Stochastic electrical stimulation of the thoracic or cervical regions with surface electrodes facilitates swallow in rats

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Introduction: Aspiration pneumonia, a leading cause of mortality, poses an urgent challenge in contemporary society. Neuromuscular electrical stimulation (NMES) has been commonly used in dysphagia rehabilitation. However, given that NMES at motor threshold targets only specific muscles, it carries a potential risk of further compromising functions related to swallowing, respiration, and airway protection. Considering that the swallow motor pattern is orchestrated by the entire swallow pattern generator (the neural mechanism governing a sequence of swallow actions), a rehabilitation approach that centrally facilitates the entire circuit through sensory nerve stimulation is desirable. In this context, we propose a novel stimulation method using surface electrodes placed on the back to promote swallowing.

Methods: The efficacy of the proposed method in promoting swallowing was evaluated by electrically stimulating sensory nerves in the back or neck. Probabilistic stimulus was applied to either the back or neck of male and female rats. Swallows were evoked by an oral water stimulus, and electromyographic (EMG) activity of the mylohyoid, thyroarytenoid, and thyropharyngeus muscles served as the primary outcome measure.

Results: Gaussian frequency stimulation applied to the skin surface of the thoracic back elicited significant increases in EMG amplitude of all three swallow-related muscles. Neck stimulation elicited a significant increase in EMG amplitude of the thyroarytenoid during swallow, but not the mylohyoid or thyropharyngeus muscles.

Discussion: While the targeted thoracic spinal segments T9-T10 have been investigated for enhancing respiration, the promotion of swallowing through back stimulation has not been previously studied. Furthermore, this study introduces a new probabilistic stimulus based on Gaussian distribution. Probabilistic stimuli have been reported to excel in nerve stimulation in previous research. The results demonstrate that back stimulation effectively facilitated swallow more than neck stimulation and suggest potential applications for swallowing rehabilitation.

KEYWORDS

swallow, dysphagia, spinal cord stimulation, central pattern generator, rehabilitation

1 Introduction

Dysphagia refers to the difficulty in safely forming or transferring food boluses from the oral cavity to the esophagus. Untreated dysphagia can lead to the aspiration of food particles and saliva containing bacteria into the lungs, resulting in inflammation and infection. Aspiration pneumonia constitutes almost 70% of pneumonia cases (1), ranking as the third leading cause of death among elderly individuals in Japan (2). Additionally, the prevalence of dysphagia among the elderly has been reported as high as 33% in the United States (3), 23% in Europe (4), 33% in individuals aged 80 and above in Europe (4), 14% in Japan (5), and 34% in South Korea (6). Therefore, the development of rehabilitation techniques for dysphagia is of utmost urgency in contemporary aging societies.

An established rehabilitation technique for dysphagia is neuromuscular electrical stimulation (NMES). This often involves stimulating the muscles in the anterior neck region through surface electrodes. However, this therapy does not directly induce swallow and is limited in its effectiveness as it primarily targets specific muscles rather than facilitation of the central swallow pattern generator. Moreover, there are concerns that electrical stimulation therapy may lead to rapid muscle fatigue, potentially further compromising functions related to swallow, respiration, and airway protection with stimulation (7). Since the sequence of swallow muscle activities is not solely dependent on specific muscles but also on the activation of the entire swallow reflex circuit, it is logical to explore rehabilitation methods to activate the entire swallow reflex circuit through sensory nerve system stimulation (8).

As a method to activate sensory nerves, it has been proposed that dysphagia can be treated by directly applying magnetic stimulation to the areas near the primary somatosensory cortex, as a large portion of the primary somatosensory cortex is associated with swallow (9–12). While this approach holds promise for improving dysphagia, there are challenges associated with the enlargement of stimulation devices, resulting in high implementation costs for rehabilitation equipment (13–15).

Respiration and swallow are closely related, with both behaviors relying on signals from various sensory nerves. Generally, the respiratory cycle can be divided into inspiration, early expiration (also known as post-inspiration), and late expiration phases. Swallow is inhibited during the inspiration phase (16, 17). Based on this fact and considering the desirability of the expiration phase for swallow timing, we proposed a method that involves electrical stimulation of sensory nerves which travel through pathways within the spinal cord. We used Gaussian frequency stimulation applied through surface electrodes placed either near the thoracic spine T9-T10 on the dorsal side, or on the anterior lateral region of the neck to test the hypothesis that this novel stimulus would facilitate swallow.

2 Materials and methods

2.1 Animals

The experiments were conducted using spontaneously breathing retired breeder Sprague Dawley rats [$N = 16$, 8 male (0.63 ± 0.11 kg), 8 female (0.26 ± 0.02 kg); 9–12 months of age]. Ethical approval for the protocol was obtained from the Institutional Animal Care and Use Committee (IACUC) at the University of Louisville.

The animals were initially anesthetized through inhalation of gaseous isoflurane (1.5–2%). Subsequently, pentobarbital sodium was administered at a dosage of 25 mg/kg via a femoral vein catheter (IV). Thereafter, isoflurane was ceased, and supplementary doses of pentobarbital sodium (ranging from 1 to 4 mg/kg via IV injection) were administered. To reduce tracheal secretions, atropine sulfate was administered at the beginning of the experiment at a dose of 0.01 mg/kg via IV injection. The depth of anesthesia was assessed every 15 min by evaluating withdrawal reflexes, blinking, and jaw tone. At the same intervals, body temperature, respiratory rate and heart rate were monitored. If the anesthetic level was deemed insufficient based on these evaluations, supplemental doses of pentobarbital sodium (ranging from 1 to 4 mg/kg via IV injection) were administered as required (18). Prior to initiating the stimulation protocol, anesthetic depth was confirmed, and then no additional doses were administered during the experiment. Body temperature was maintained using a heating pad.

2.2 Electrophysiology recording

Electromyographic (EMG) recordings of all muscle activities were obtained using bipolar insulated fine-wire electrodes (AM Systems stainless steel No. 791050) placed on each muscle following the technique described by Basmajian and Stecko (19). Three swallow muscles were evaluated: mylohyoid, thyroarytenoid, and thyropharyngeus muscles. The costal diaphragm was recorded to evaluate breathing. Figure 1 illustrates the anatomical locations of each muscle and typical traces of muscle activity during pharyngeal swallow.

The recording electrodes were surgically placed as follows: The mylohyoid muscle electrodes were inserted into both the right and left mylohyoid muscles after making a small horizontal incision along the midline of the chin to expose the surface of the digastric muscle. The thyroarytenoid muscle electrodes were inserted anteriorly through the window of the cricothyroid membrane, reaching the front of the vocal cords. For the thyropharyngeus muscle, the electrodes were inserted into the posterior part of the thyropharyngeus muscle by curving the insertion needle. The diaphragm electrode was positioned by inserting the injection needle caudally, guided by palpating and elevating the xiphoid process. Electrocardiogram (ECG) activity was recorded by placing electrodes on the left pectoralis major and right caudal gastrocnemius muscles. ECG was used for measuring heart rate and removing cardiac artifacts from the EMG traces.

2.3 Experimental protocol

Our objective was to investigate the effects on swallow of electrical stimulation via surface electrodes placed on the back (T9-T10) or neck (Lateral side of the neck) regions. In this study, we introduce a novel probabilistic stimulus based on Gaussian distribution (Figure 2B). In the figure, t_c denotes a pulse width, and V denotes a voltage of the pulse waveform. Let $N(\mu,\sigma)$ be a Gaussian distribution with mean $\propto$ and standard deviation σ (Figure 2A). For each pulse, the value of the interval between pulses are set as $t_{N(\mu,\sigma)}$, which is the inverse of the value $x_{N(\mu,\sigma)}$ realized value from $N(\mu,\sigma)$. The electrical stimulator used in this experiment was the Grass Stimulator Model S88 (RRID:SCR_016192) (Grass, Inc., Warwick, RI). The waveform

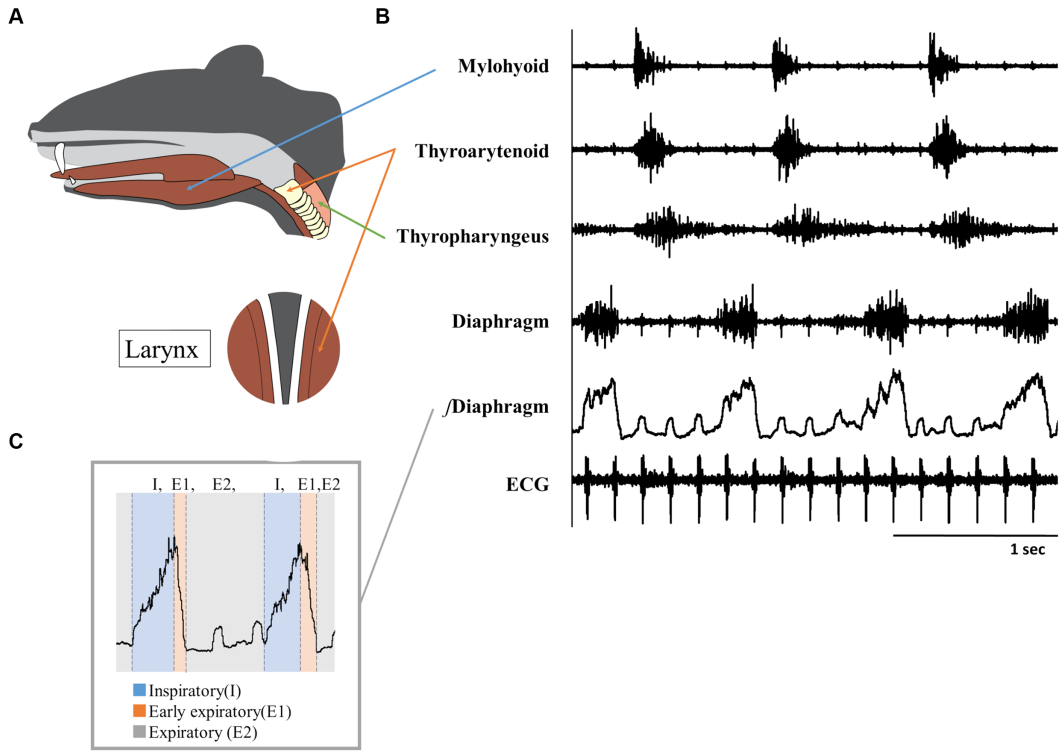

FIGURE 1
(A) Diagram illustrating the anatomical positions of the muscles targeted for EMG recording, **(B)** Representative EMG trace during swallows, **(C)** Methodology for segmenting respiratory phases using diaphragm EMG.

generation program was implemented using custom scripts in Spike2 software (CED, Cambridge Electronic Design). In this experiment, the parameters for the stimulation waveform were set with a pulse width of 15 ms and an output voltage of 5 V. Surface electrodes were placed as follows: in the back stimulation group, surface electrodes were positioned on the dorsal thoracic area (T9-T10) of 8 rats [4 males (0.64 ± 0.14 kg) and 4 females (0.26 ± 0.02 kg)]; in the neck stimulation group, surface electrodes were positioned bilaterally near the larynx on the cervical area of 8 rats [4 males (0.61 ± 0.06 kg) and 4 females (0.26 ± 0.02 kg)] (Figure 3). Surface electrodes with a diameter of 25 mm were used for female rats, while electrodes with a diameter of 30 mm were used for male rats. The electrodes were fixed in place with tape after shaving the hair on the surface of the application site. Using a probabilistic paradigm, pulses were applied at each site for 120 s in four repetitions (designated as S1-S4) with a frequency randomly generated according to the Gaussian distribution set with a mean of 30 Hz and a standard deviation of 10 Hz (Figure 2C). Previous studies on spinal stimulation in rats have demonstrated that muscle activity can be induced with low current intensities of approximately 1 mA (20, 21). The current applied in this study ranged from 0.75 mA to 1.1 mA, which was lower than the current threshold (14 mA) required to induce muscle contraction (22). It has been found that when high-amplitude stimulation is used peripherally, exceeding the optimal amplitude may block orthodromic action potentials generated within the spinal cord (23).

We recorded EMG activity from three swallow-related muscles (mylohyoid muscle, thyroarytenoid muscle, thyropharyngeus muscle), as well as the diaphragm, along with heart rate and respiratory rate. Oral water administration was performed by injecting 1 cc of room temperature water into the oropharynx through a thin polyethylene catheter (0.5–1.0 mm outer diameter, 0.5 inches in length) placed at the base of the tongue. Three oral water administrations were conducted immediately before stimulation (control) and immediately after each of the four stimulation trials (S1-S4) (Figure 2D). At the conclusion of the experimental protocol, euthanasia was induced through an overdose of pentobarbital sodium followed by intravenous administration of 1 cc of saturated potassium chloride. Pneumothorax was also induced as a secondary euthanasia method.

2.4 Analysis

The EMG signals were amplified using Grass P511 AC Amplifiers (Natus Neurology), bandpass-filtered (200–5,000 Hz), recorded at a sampling rate of 10 KHz (1,401 Power3 + ADC16 Expansion, Cambridge Electronic Design), and analysed using Spike2 Software (RRID:SCR_000903) (v8, Cambridge Electronic Design). EMG signals were rectified and integrated with a time constant of 20 ms. Swallows were identified by coordinated bursts in EMG activity of the mylohyoid, thyroarytenoid, and thyropharyngeus muscles. Swallow events were included if they were induced within 30 s after oral water infusion. To normalize EMG values across animals, the percent change was calculated by dividing the average maximum EMG amplitude during post-stimulation swallows (S1-S4) by the average maximum EMG amplitude during control pre-stimulation swallows.

Changes in respiration were evaluated by determining the phase based on the diaphragmatic EMG. Inspiration (I) was defined as the period from the onset of diaphragmatic activity to the peak of

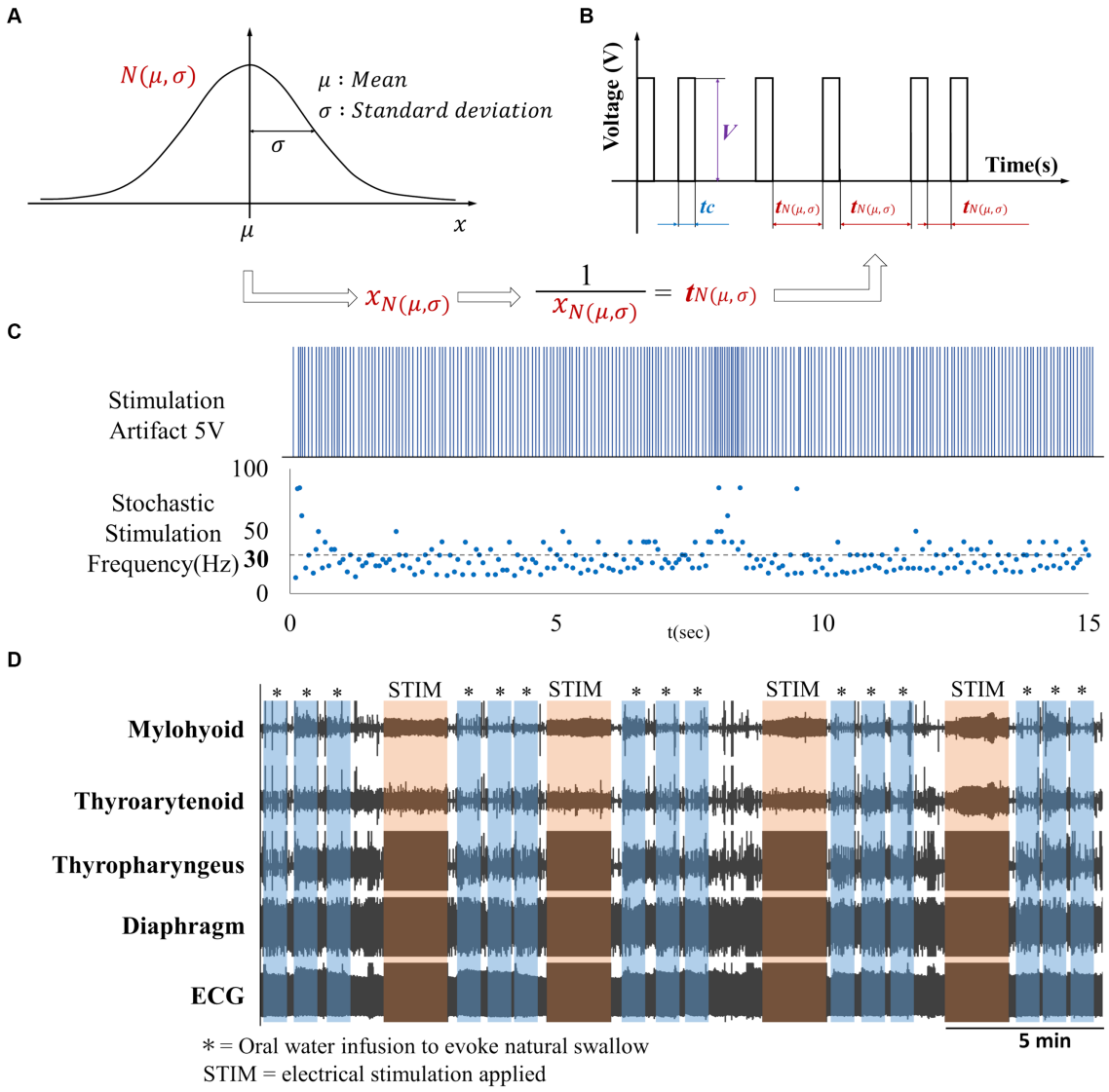

FIGURE 2
(A) Gaussian distribution, **(B)** Gaussian frequency stimulation waveform. Waveform with intermittent single-pulse stimulation is applied based on the reciprocal of the frequency $^{x}N(\mu,\sigma)$ randomly generated according to the Gaussian distribution depicted in **(A,C)** Artifact produced by Gaussian frequency electrical stimulation with a mean frequency of 30 Hz, **(D)** Overview of EMG activity throughout the experiment.

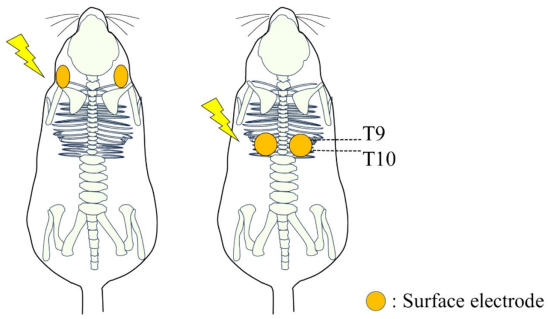

FIGURE 3
Diagram illustrating the placement of wet electrodes for electrical stimulation on the neck and back of rats.

diaphragmatic burst. Expiration (E) was defined as the period from the peak of diaphragmatic activity to the onset of the subsequent diaphragmatic activation. Furthermore, expiration was subdivided into early expiration (E1; from the peak of diaphragmatic amplitude to diaphragmatic quiescence) and late expiration (E2; from the offset of diaphragmatic activity to the onset of the subsequent diaphragmatic activity) (24) (Figure 1C). To assess the variations in the three phases (I, E1, E2) within a single breath, we conducted comparisons to normalize by dividing the time duration of each phase by the total duration of one respiratory cycle. This approach enables the elimination of differences in respiratory cycles between measurement trials, allowing for the observation of changes in the proportion of each respiratory phase. Swallow duration was defined as the interval from the onset of activity in the mylohyoid muscle to the cessation of thyropharyngeus muscle activity.

All measures are reported as mean ± standard deviation (SD). The following parameters were measured and compared between the swallows that were induced in the pre-stimulus (control) and post-stimulus (S1-S4) time periods: amplitudes of the three swallow-related muscles, diaphragm amplitude, duration of each swallow, respiratory rate per minute, heart rate per minute, and proportion of each respiratory phase within one respiratory cycle. Statistical analysis was performed using Prism (GraphPad) software. Normality tests were conducted, and then either analysis of variance (ANOVA) or Friedman tests were performed as appropriate to test for main effects. *Post hoc* multiple comparison tests were performed (Dunnet's for one-way parametric, Fisher's LSD for two-way parametric, and Dunn's for non-parametric) to assess specific differences between the control and stimulus groups. Differences with *p*-values below 0.05 were considered statistically significant.

3 Results

First, we evaluated changes in maximum EMG amplitude of three swallow-related muscles (mylohyoid, thyroarytenoid, and thyropharyngeus) (Figure 4B) before and after the application of Gaussian frequency electrical stimulation (four stimulation trials each, labelled S1-S4). The stimulating electrodes were placed bilaterally on the surface of the skin either at the posterior T9-T10 thoracic spine region, or at the anterior cervical region bilateral to the larynx. We compared EMG amplitudes during swallow induced by oral water infusion before stimulation (control) and immediately after each electrical stimulation (S1 to S4). For the back stimulation groups (T9-T10 region), ANOVA detected a significant effect of stimulus on EMG amplitude of the mylohyoid [$F_{(4,28)} = 6.219$, $p = 0.006$], thyroarytenoid [$F_{(4,28)} = 4.798$, $p = 0.02$], and thyropharyngeus [$F_{(4,28)} = 5.909$, $p = 0.009$] muscles during swallow (Figure 4A). *Post hoc* comparisons showed that mylohyoid EMG amplitude increased following the second ($p = 0.02$) and fourth ($p = 0.02$) trials of back stimulation. Thyroarytenoid EMG amplitude increased following the first ($p = 0.005$) and third ($p = 0.04$) trials of back stimulation. Thyropharyngeus EMG amplitude increased following the fourth ($p = 0.04$) trial of back stimulation. For the anterior neck stimulation groups, ANOVA detected a significant effect of stimulus on EMG amplitude of the thyroarytenoid [$\chi^2(5) = 10.70$, $p = 0.03$], but not the mylohyoid or thyropharyngeus muscles during swallow. *Post hoc* comparisons showed that thyroarytenoid EMG amplitude increased following the fourth ($p = 0.02$) trial of neck stimulation. An ANOVA omnibus test detected a significant interaction effect between sex and stimulus treatment [$F_{(4,24)} = 3.19$, $p = 0.03$] and a significant effect of sex [$F_{(1,6)} = 6.92$, $p = 0.04$] for the thyropharyngeus EMG amplitude following back stimulation.

We then evaluated the effects of the back and neck Gaussian frequency electrical stimulation on swallow duration, respiratory rate, and heart rate (Figure 5). Swallow duration increased in the back stimulation group [$\chi^2(5) = 12.50$, $p = 0.01$], and *post hoc* comparisons showed a significant effect following the second ($p = 0.03$), third ($p = 0.045$), and fourth ($p = 0.01$) back stimulus trials. While omnibus ANOVA did not detect an overall difference in swallow duration for the neck stimulation group [$F_{(4,28)} = 2.177$, $p = 0.2$], *post hoc* tests detected a significant increase in swallow duration following the second ($p = 0.009$) and fourth ($p = 0.02$) neck stimulus trials (Figure 5i). There were no differences detected for the effect of back stimulation on respiratory rate. However, respiratory rate was increased following neck stimulation [$F_{(4,28)} = 4.113$, $p = 0.04$], and *post hoc* tests detected a significant increase in respiratory rate following the third ($p = 0.03$) neck stimulus trial (Figure 5ii). While omnibus ANOVA did not detect an overall difference in heart rate for the back stimulation group [$F_{(4,28)} = 0.925$, $p = 0.4$], *post hoc* tests detected a significant increase in heart rate following the first ($p = 0.01$) back stimulus trial. There were no differences detected for the effect of neck stimulation on heart rate (Figure 5iii).

Finally, we assessed whether electrical stimulation at the neck or back affects diaphragm amplitude during breathing or modulates respiratory phase. There were no significant changes in the maximum EMG amplitude of the diaphragm for the back or neck stimulus groups (Figure 6A). Omnibus F-tests conducted for each group revealed no significant changes in the proportions of the I (inspiratory), E1 (early expiratory), and E2 (expiratory) phases of respiration, and post-hoc multiple comparisons similarly indicated no significant differences in any of the groups (Figure 6B).

4 Discussion

This study aimed to investigate whether a novel Gaussian frequency stimulation paradigm applied to skin of the back could facilitate swallow. Gaussian frequency stimulation applied to the skin surface of the thoracic back (at the T9-T10 level) elicited significant increases in EMG amplitude of all three swallow-related muscles at a variety of timepoints (S1-S4), indicating that this stimulation method facilitates swallow. This effect on thyropharyngeus EMG activity was more pronounced in the male group compared to the female group. Swallow duration was also increased after stimulation of the back or the neck.

Swallow preferentially occurs in the expiratory phase of breathing. While swallow occurs during post-inspiration in rats and mice (25, 26), it is most likely to occur later in expiration in cats and humans (27–29). While swallow is mediated by cranial nerves, it is impaired by spinal cord injury (18), and is influenced by afferent feedback from pulmonary stretch receptors that are activated during inspiration (16, 25). Activation of spinal afferents in the chest wall also affects swallow (25), and rib vibration increases laryngeal tone, which is important during respiration and for airway protection during swallow (30). Swallow also influences the phase coordination of the respiratory cycle, including inspiration and expiration (16, 31, 32). Indeed, the swallow and respiratory pattern generators are both situated in the brainstem, adjacent to each other and overlapping at least somewhat.

Spinal pathways are also involved in respiration. The load compensation reflex that occurs during airway occlusion is spinally mediated (33), and stimulus of the chest wall by vibration or electrical stimulation induces significant changes to respiration (34–41). The muscles that we recorded are representative of key movements that occur during the pharyngeal phase of swallow. The mylohyoid represents laryngeal elevation, the thyroarytenoid represents laryngeal adduction and the thyropharyngeus represents pharyngeal constriction. Each of these components is necessary for airway protection and bolus passage through the pharynx and into the

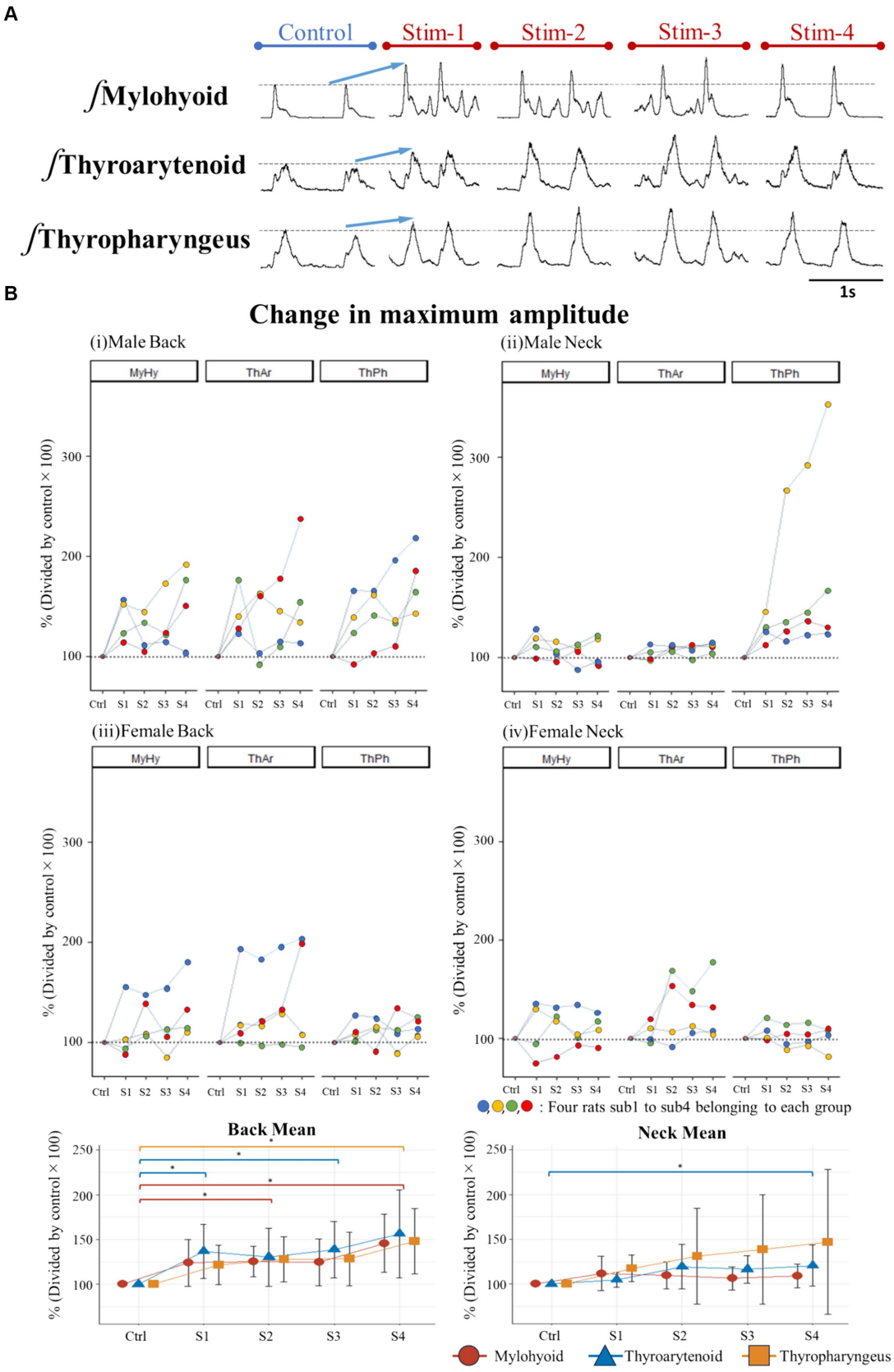

FIGURE 4
(A) Example of changes in EMG amplitude of swallow muscles when stimulating the back of male rats, **(B)** Percent change in maximum amplitude of swallow EMG potentials immediately after stimulation (S1-S4) relative to control (set as 100%) for each rat and for the back and neck group means.

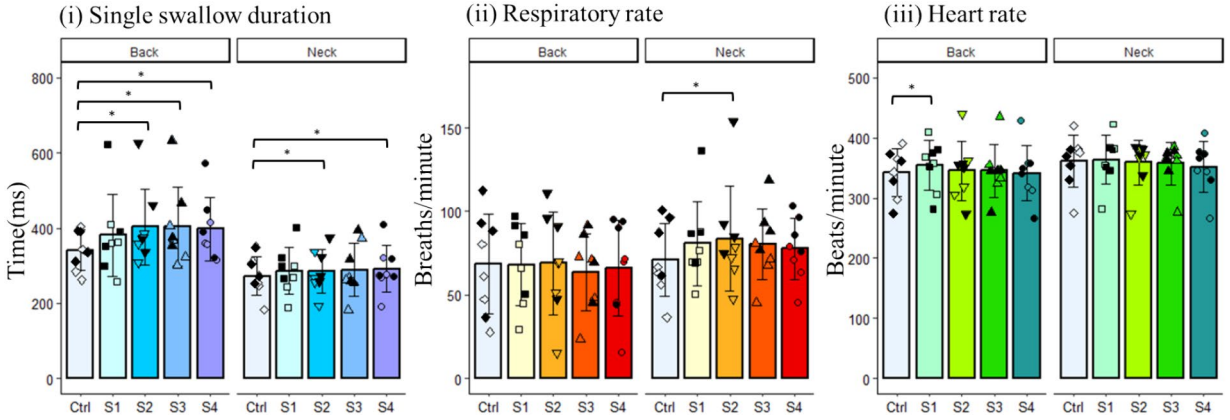

FIGURE 5
Changes in various physiological parameters of the back and neck groups. Filled black symbols represent male data points; open symbols represent female data points.

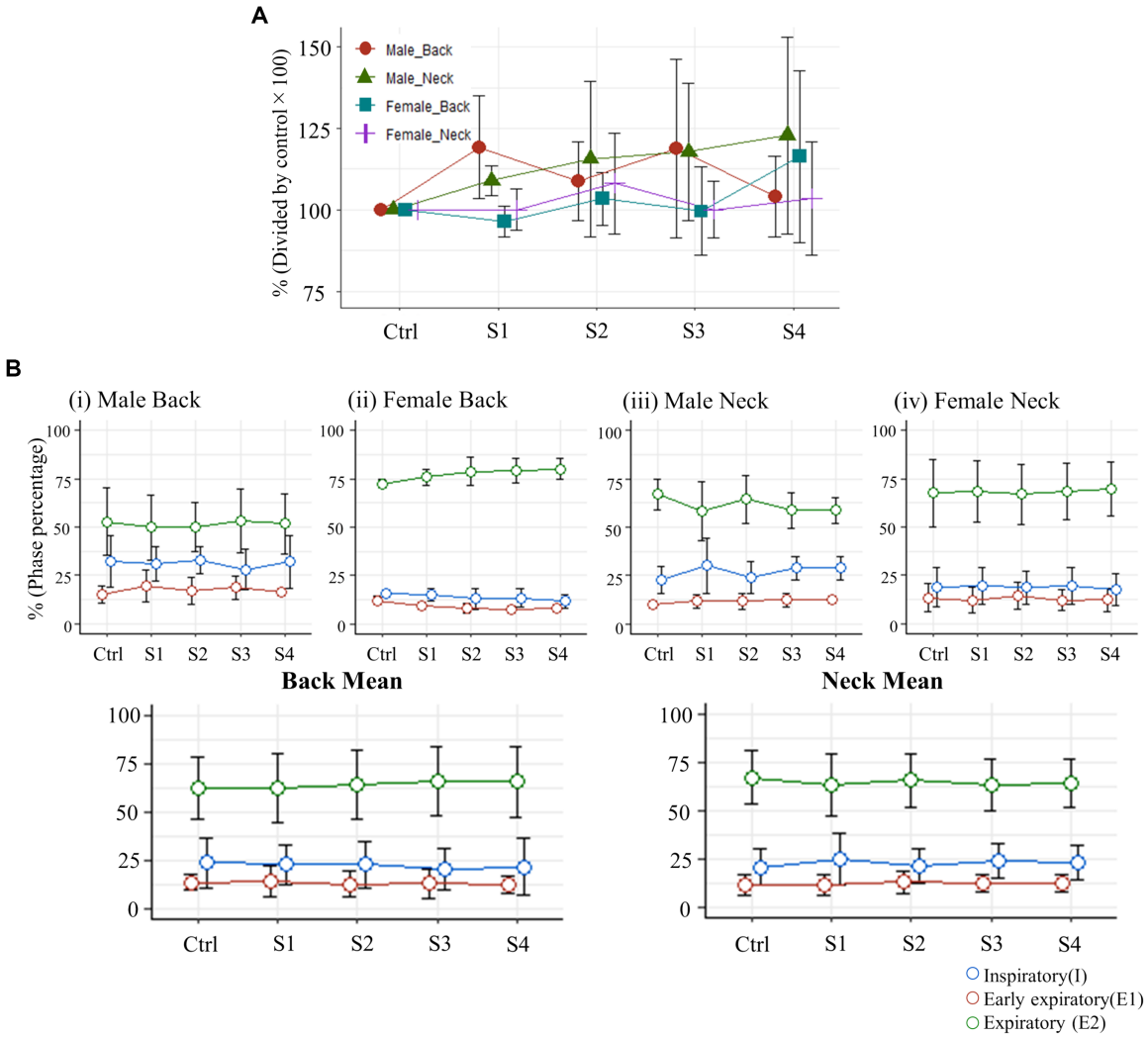

FIGURE 6
(A) Changes in the maximum amplitude of diaphragm muscle EMG activity (S1-S4) immediately after each stimulation trial, expressed as a percent change relative to the control diaphragm EMG activity (normalized to 100%). **(B)** Variations in the proportion of each respiratory phase within one respiratory cycle, expressed as a percentage.

esophagus during swallow. Each muscle is innervated by different nerves and/or branches, suggesting that a stimulus which would affect all these muscles would act centrally. Sensory nerves involved in respiration or swallowing are likely to influence both functions. The precise mechanisms by which spinal afferent information influences the swallow and respiratory central pattern generators, differentially or in concert, to produce changes in swallow and breathing remain to be discovered.

The Gaussian frequency stimulation used in this study differs from the conventional fixed-frequency single-pulse waves commonly used in therapeutic devices. Applying stimulation with Gaussian frequency can enhance the detection of weak signals in sensory signal transmission (42), and varying the applied frequency is expected to prevent adaptation to the stimulation (43). NMES using commercially available hardware stimulate with a fixed frequency of 70 Hz (44), investigations using *in vivo* animal models traditionally use fixed frequency stimulation between 5 and 30 Hz. Stimulation of the SLN with 20–30 Hz fixed frequency evokes rapid continuous swallow with short latencies and low threshold (45–49). Recent research has reported that probabilistic frequency stimulation led to a more significant swallow response compared to fixed-frequency stimulation of the superior laryngeal nerve (SLN) (50). In that study, stochastic SLN stimulation decreased swallow latency when compared to fixed-frequency stimulation, and increased swallow number and swallow EMG amplitudes; thus it appeared to increase excitability of the swallow pattern generator. Some of these same effects are observed when the frequency is increased in the setting of SLN stimulation with a fixed frequency (51). Adding stochastic variance to the electrical stimulation signal may work similarly to increasing the fixed frequency, while maintaining the overall stimulation delivered. Furthermore, since the swallow-related muscle groups are not directly stimulated to contract during swallow, the method we used in this study importantly does not act as an inhibitory factor during natural swallow.

The therapeutic effects of spinal cord stimulation have been demonstrated in various medical conditions, improving sensory-motor function and autonomic nervous system functions (52, 53). Two approaches for spinal cord stimulation are epidural spinal cord stimulation (eSCS), which requires surgical intervention, and non-invasive stimulation techniques, such as magnetic or electrical stimulation applied to the skin surface over the spinal cord. In epidural SCS, studies have explored the selective stimulation of different segments of the cervical, thoracic, and lumbar spine to treat disorders related to cardiorespiratory, motor function, and excretion (54–56). For instance, stimulation at the C4 level in a rat model with spinal cord injury at C3-C5 improved normal breathing and blood oxygen concentration, induced diaphragmatic nerve response time, and produced frequency-dependent changes (short-term facilitation) (57). Furthermore, in the case of patients with chronic paralysis, voluntary leg movements recovery has been observed through electrical stimulation by implanting electrodes at the T11-T12 level (58).

In previous research on transcutaneous electrical stimulation using surface electrodes, electrical stimulation near the thoracic spine (T11-L1) with surface electrodes induced an increase in sensation and contraction pressure in the anal-rectal region in patients with neurogenic bowel dysfunction following spinal cord injury (59). Additionally, for individuals with idiopathic lower urinary tract dysfunction after spinal cord injury, high-frequency biphasic burst wave transcutaneous spinal cord electrical stimulation at T11 and L1 resulted in immediate effects such as decreased overactivity of voiding muscles and improved incontinence (60).

While research on the effects of spinal cord stimulation on swallow movements remains limited, significant progress has been made in studying how spinal cord stimulation influences respiratory regulation. DiMarco and colleagues demonstrated that stimulation at the thoracic spine levels T9-T11 facilitates laryngeal and abdominal expiratory muscle activity (41, 61–63). In one study, patients with cervical C5-C6 injuries who were unable to cough normally had electrodes implanted and stimulation was applied to either T9, T11, or L1 of the thoracic spine, either individually or in combination. The results showed an increase in airway pressure and expiratory flow, allowing patients to induce coughing (62). Other areas of the thoracic spine are recognized as sites to modify ongoing inspiratory drive (64–66). In another study on spinal cord stimulation in dogs, stimulating at T2-T3 allowed for the maintenance of 6 h of respiration. Analysis of inspiratory muscles revealed firing frequencies of motor units equivalent to spontaneous breathing, leading to the activation of both the diaphragm and inspiratory intercostal muscles (64). In contrast, in the current study, stimulation of the T9-T10 region on the back did not produce significant differences in any respiratory phases. The disparity in these results is believed to stem from the fact that, in prior studies, high-intensity direct stimulation of the spinal cord (T9-T10) was employed, rather than the transcutaneous low-intensity stimulation via surface electrodes conducted in this study. This distinction implies a significant difference in the actual current flowing through the spinal cord. It is best if any therapeutic stimulation targeting swallow does not affect respiration, and it is plausible that higher-intensity stimulation than what was performed in the current protocol is necessary to induce the off-target effects on expiration. However, because we calculated respiratory parameters directly after, rather than during, the electrical stimulation, changes in airway pressure and respiratory muscle activity during stimulation as reported in prior studies cannot be discounted. In addition, in contrast to the experimental model of spinal cord injury utilized in prior research, the present study employed a healthy rat model. Prior to experimentation, stable spontaneous respiration was confirmed, and no decline in respiratory capacity was observed.

Possible therapeutic applications for Gaussian electrical stimulation include stabilizing breathing (67, 68) and infant suck-swallow patterns (69), and decreasing tremor and bradykinesia using deep brain stimulation in patients with Parkinson's disease (70). In clinical dysphagia therapy, an 80 Hz fixed frequency has been successfully used (71) in the short term (7, 72). However, in several studies long-term therapeutic use has not been successful (73–77).

In the present study we found that surface probabilistic electrical stimulation of sensory nerves in the back or neck of rats can enhance activation of certain muscles during swallow in rats immediately after stimulation. Probabilistic electrical stimulation should be explored further in future experiments to test the sustained effectiveness of facilitating swallow over longer time courses. Previous studies have shown long-term brain plasticity after sensory nerve stimulation (78). Additionally, although the stimulation waveform in this study differs, experiments using

transcranial random noise stimulation (tRNS) have suggested the rapid modulation of voltage-dependent sodium channels (79), and the application of tRNS to the pharyngeal cortex has demonstrated a sustained two-hour increase in pharyngeal motor-evoked potentials (80). In conclusion, the results from the current study suggest that stochastic electrical surface stimulation, especially to the thoracic T9-T10 region, may show promise for improving swallow dysfunction. This stimulation method should be explored for therapeutic potential in further studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by Institutional Animal Care and Use Committee (IACUC) at the University of Louisville. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

IK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MF: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. KI: Conceptualization, Formal analysis, Investigation, Supervision, Validation, Writing – review & editing. TK: Supervision, Writing – review & editing. TP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Teramoto S, Fukuchi Y, Sasaki H, Sato K, Sekizawa K, Matsuse T. High incidence of aspiration pneumonia in community and hospital acquired pneumonia in hospitalized patients: a multicenter, prospective study in Japan. *J Am Geriatr Soc*. (2008) 56:577–9. doi: 10.1111/j.1532-5415.2008.01597.x

2. Statistics and Information Department. 2016 analysis by cause of death, abridged life tables for Japan 2016, life tables 2016. Tokyo: Ministry of Health, Labor and Welfare (2023).

3. Roy N, Stemple JC, Merrill RM. Dysphagia in the elderly: preliminary evidence of prevalence, risk factors, and socioemotional effects. *Ann Otol Rhinol Laryngol*. (2007) 116:858–65. doi: 10.1177/000348940711601112Thomas, l

4. Serra-Prat M, Hinojosa G, Lopez D, Juan M, Fabré E, Voss DS, et al. Prevalence of oropharyngeal dysphagia and impaired safety and efficacy of swallow in independently-living older persons. *JAGS*. (2011) 59:186–7. doi: 10.1111/j.1532-5415.2010.03227.x

5. Kawashima K, Motohashi Y, Fujishima I. Prevalence of dysphagia among community-dwelling elderly individuals as estimated using a questionnaire for dysphagia screening. *Dysphagia*. (2004) 19:266–71. doi: 10.1007/s00455-004-0013-6

6. Yang EJ, Kim MH, Lim JY, Paik NJ. Oropharyngeal dysphagia in a community-based elderly cohort: the Korean longitudinal study on health and aging. *J Korean Med Sci*. (2013) 28:1534–9. doi: 10.3346/jkms.2013.28.10.1534

7. Ludlow CL, Humbert I, Saxon K, Poletto C, Sonies B, Crujido L. Effects of surface electrical stimulation both at rest and during swallowing in chronic pharyngeal dysphagia. *Dysphagia*. (2007) 22:1–10. doi: 10.1007/s00455-006-9029-4

8. Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev*. (2001) 81:929–69. doi: 10.1152/physrev.2001.81.2.929

9. Tamura Y, Shibukawa Y, Shintani M, Kaneko Y, Ichinohe T. Oral structure representation in human somatosensory cortex. *NeuroImage*. (2008) 43:128–35. doi: 10.1016/j.neuroimage.2008.06.040

10. Brown S, Ngan E, Liotti M. A larynx area in the human motor cortex. *Cereb Cortex*. (2008) 18:837–45. doi: 10.1093/cercor/bhm131

11. Aziz Q, Rothwell JC, Hamdy S, Barlow J, Thompson DG. The topographic representation of esophageal motor function on the human cerebral cortex. *Gastroenterology*. (1996) 111:855–62. doi: 10.1016/S0016-5085(96)70053-7

12. Dziewas R, Soros P, Ishii R, Chau W, Henningsen H, Ringelstein EB. Cortical processing of esophageal sensation is related to the representation of swallowing. *Neuroreport*. (2005) 16:439–43. doi: 10.1097/00001756-200504040-00005

13. Simons A, Hamdy S. The use of brain stimulation in dysphagia management. *Dysphagia*. (2017) 32:209–15. doi: 10.1007/s00455-017-9789-z

14. Gow D, Rothwell J, Hobson A, Thompson D, Hamdy S. Induction of long-term plasticity in human swallowing motor cortex following repetitive cortical stimulation. *Clin Neurophysiol*. (2004) 115:1044–51. doi: 10.1016/j.clinph.2003.12.001

15. Wei K.C., Wang T.G., Hsiao M.Y. (2023). The cortical and subcortical neural control of swallowing: A narrative review. *Dysphagia*. (2024) 39:177–97. doi: 10.1007/s00455-023-10613-x16

16. Nishino T, Sugimori K, Kohchi A, Hiraga K. Nasal constant positive airway pressure inhibits the swallowing reflex. *Am Rev Respir Dis*. (1989) 140:1290–3. doi: 10.1164/ajrccm/140.5.1290

17. Takemura A, Sugiyama Y, Yamamoto R, Kinoshita S, Kaneko M, Fuse S, et al. Effect of pharmacological inhibition of the pontine respiratory group on swallowing interneurons in the dorsal medulla oblongata. *Brain Res.* (2022) 1797:148101. doi: 10.1016/j.brainres.2022.148101

18. Pitts T, Iceman KE, Huff A, Musselwhite MN, Frazure ML, Young KC, et al. Laryngeal and swallow dysregulation following acute cervical spinal cord injury. *J Neurophysiol.* (2022) 128:405–17. doi: 10.1152/jn.00469.2021

19. Basmajian J, Stecko G. A new bipolar electrode for electromyography. *J Appl Physiol.* (1962) 17:849. doi: 10.1152/jappl.1962.17.5.849

20. Dietz BE, Mugan D, Vuong QC, Obara I. Electrically evoked compound action potentials in spinal cord stimulation: implications for preclinical research models. *Neuromodulation.* (2022) 25:64–74. doi: 10.1111/ner.13480

21. Hogan MK, Barber SM, Rao Z, Kondiles BR, Huang M, Steele WJ, et al. A wireless spinal stimulation system for ventral activation of the rat cervical spinal cord. *Sci Rep.* (2021) 11:14900. doi: 10.1038/s41598-021-94047-1

22. Honda Y, Tanaka N, Kajiwara Y, Kondo Y, Kataoka H, Sakamoto J, et al. Effect of belt electrode-skeletal muscle electrical stimulation on immobilization-induced muscle fibrosis. *PLoS One.* (2021) 16:0244120. doi: 10.1371/journal.pone.0244120

23. Arpin DJ, Ugiliweneza B, Forrest G, Harkema SJ, Rejc E. Optimizing neuromuscular electrical stimulation pulse width and amplitude to promote central activation in individuals with severe spinal cord injury. *Front Physiol.* (2019) 10:1310. doi: 10.3389/fphys.2019.01310

24. Richter DW. Generation and maintenance of the respiratory rhythm. *J Exp Biol.* (1982) 100:93–107. doi: 10.1242/jeb.100.1.93

25. Huff A, Reed MD, Iceman KE, Howland DR, Pitts T. Sex-specific vagal and spinal modulation of swallow and its coordination with breathing. *PLoS One.* (2020) 15:e0234194. doi: 10.1371/journal.pone.0234194

26. Huff A, Karlen-Amarante M, Pitts T, Ramirez JM. Optogenetic stimulation of pre-Bötzinger complex reveals novel circuit interactions in swallowing-breathing coordination. *Proc Natl Acad Sci USA.* (2022) 119:e2121095119. doi: 10.1073/pnas.2121095119

27. Horton KK, Segers LS, Nuding SC, O'Connor R, Alencar PA, Davenport PW, et al. Central respiration and mechanical ventilation in the gating of swallow with breathing. *Front Physiol.* (2018) 9:785. doi: 10.3389/fphys.2018.00785

28. Martin-Harris B, McFarland DH. Coordination of deglutition and respiration. In: *Princip Deglut Springer.* eds. Shaker, R., Belafsky, P., Postma, G., Easterling, C. (New York, NY: Springer) (2013):25–34.

29. Pitts T, Rose MJ, Poliacek I, Condrey J, Davenport PW, Bolser DC. Effect of laparotomy on the swallow–breathing relationship in the cat. *Lung.* (2015) 193:129–33. doi: 10.1007/s00408-014-9662-x

30. Bolser DC, Remmers JE. Synaptic effects of intercostal tendon organs on membrane potentials of medullary respiratory neurons. *J Neurophysiol.* (1989) 61:918–26. doi: 10.1152/jn.1989.61.5.918

31. Oku Y, Dick TE. Phase resetting of the respiratory cycle before and after unilateral pontine lesion in cat. *J Appl Physiol.* (1992) 72:721–30. doi: 10.1152/jappl.1992.72.2.721

32. Paydarfar D, Gilbert RJ, Poppel CS, Nassab PF. Respiratory phase resetting and airflow changes induced by swallowing in humans. *J Physiol.* (1995) 483:273–88. doi: 10.1113/jphysiol.1995.sp020584

33. Corda M, Eklund G, Von E. External intercostal and phrenic alpha-motor responses to changes in respiratory load. *Acta Physiol Scand.* (1965) 63:391–400. doi: 10.1111/j.1748-1716.1965.tb04079.x

34. Homma I, Eklund G, Hagbarth KE. Respiration in man affected by TVR contractions elicited in inspiratory and expiratory intercostal muslces. *Respir Physiol.* (1978) 35:335–48. doi: 10.1016/0034-5687(78)90007-5

35. DiMarco AF, Romaniuk JR, Supinski GS. Electrical activation of the expiratory muscles to restore cough. *Am J Respir Crit Care Med.* (1995) 151:1466–71. doi: 10.1164/ajrccm.151.5.7735601

36. DiMarco AF, Romaniuk JR, Kowalski KE, Supinski G. Pattern of expiratory muscle activation during lower thoracic spinal cord stimulation. *J Appl Physiol.* (1999) 86:1881–9. doi: 10.1152/jappl.1999.86.6.1881

37. DiMarco AF, Onders RP, Kowalski KE, Miller ME, Ferek S, Mortimer JT. Phrenic nerve pacing in a tetraplegic patient via intramuscular diaphragm electrodes. *Am J Respir Crit Care Med.* (2002) 166:1604–6. doi: 10.1164/rccm.200203-175CR

38. DiMarco AF, Kowalski KE, Romaniuk JR. Effects of diaphragm activation on airway pressure generation during lower thoracic spinal cord stimulation. *Respir Physiol Neurobiol.* (2007) 159:102–7. doi: 10.1016/j.resp.2007.06.007

39. Kowalski KE, Romaniuk JR, Brose SW, Richmond MA, Kowalski T, DiMarco AF. High frequency spinal cord stimulation-new method to restore cough. *Respir Physiol Neurobiol.* (2016) 232:54–6. doi: 10.1016/j.resp.2016.07.001

40. Kowalski KE, Romaniuk JR, Kowalski T, DiMarco AF. Effects of expiratory muscle activation via high-frequency spinal cord stimulation. *J Appl Physiol.* (2017) 123:1525–31. doi: 10.1152/japplphysiol.00402.2017

41. DiMarco AF, Kowalski KE, Supinski G, Romaniuk JR. Mechanism of expiratory muscle activation during lower thoracic spinal cord stimulation. *J Appl Physiol.* (2002) 92:2341–6. doi: 10.1152/japplphysiol.01231.2001

42. Bloch-Salisbury E, Indic P, Bednarek F, Paydarfar D. Stabilizing immature breathing patterns of preterm infants using stochastic mechanosensory stimulation. *J Appl Physiol.* (2009) 107:1017–27. doi: 10.1152/japplphysiol.00058.2009

43. Dimitrijević MR, Faganel J, Gregorić M, Nathan PW, Trontelj JK. Habituation: effects of regular and stochastic stimulation. *J Neurol Neurosurg Psychiatry.* (1972) 35:234–42. doi: 10.1136/jnnp.35.2.234

44. Humbert IA, Michou E, MacRae PR, Crujido L. Electrical stimulation and swallowing: how much do we know? *Semin Speech Lang.* (2012) 33:203–16. doi: 10.1055/s-0032-1320040

45. Kessler J, Jean A. Identification of the medullary swallowing regions in the rat. *Exp Brain Res.* (1985) 57:256–63. doi: 10.1007/BF00236530

46. Ezure K, Oku Y, Tanaka I. Location and axonal projection of one type of swallowing interneurons in cat medulla. *Brain Res.* (1993) 632:216–24. doi: 10.1016/0006-8993(93)91156-M

47. Oku Y, Tanaka I, Ezure K. Activity of bulbar respiratory neurons during fictive coughing and swallowing in the decerebrate cat. *J Physiol.* (1994) 480:309–24. doi: 10.1113/jphysiol.1994.sp020361

48. Ootani S, Umezaki T, Shin T, Murata Y. Convergence of afferents from the SLN and GPN in cat medullary swallowing neurons. *Brain Res Bull.* (1995) 37:397–404. doi: 10.1016/0361-9230(95)00018-6

49. Kitagawa J, Nakagawa K, Hasegawa M, Iwakami T, Shingai T, Yamada Y, et al. Facilitation of reflex swallowing from the pharynx and larynx. *J Oral Sci.* (2009) 51:167–71. doi: 10.2334/josnusd.51.167

50. King SN, Shen TY, Nicholas M, Huff A, Reed MD, Poliacek I, et al. Swallow motor pattern is modulated by fixed or stochastic alterations in afferent feedback. *Front Hum Neurosci.* (2020) 14:112. doi: 10.3389/fnhum.2020.00112

51. Beyak M, Collman P, Valdez D, Xue S, Diamant N. Superior laryngeal nerve stimulation in the cat: effect on oropharyngeal swallowing, oesophageal motility and lower oesophageal sphincter activity. *Neurogastroenterol Motil.* (1997) 9:117–27. doi: 10.1046/j.1365-2982.1997.d01-22.x

52. Laskin JJ, Waheed Z, Thorogood NP, Nightingale TE, Noonan VK. Spinal cord stimulation research in the restoration of motor, sensory, and autonomic function for individuals living with spinal cord injuries: a scoping review. *Arch Phys Med Rehabil.* (2022) 103:1387–97. doi: 10.1016/j.apmr.2022.01.161

53. Tator CH, Minassian K, Mushahwar VK. Spinal cord stimulation: therapeutic benefits and movement generation after spinal cord injury. *Handb Clin Neurol.* (2012) 109:283–96. doi: 10.1016/B978-0-444-52137-8.00018-8

54. Darrow D, Balser D, Netoff TI, Krassioukov A, Phillips A, Parr A, et al. Epidural spinal cord stimulation facilitates immediate restoration of dormant motor and autonomic Supraspinal pathways after chronic neurologically complete spinal cord injury. *J Neurotrauma.* (2019) 36:2325–36. doi: 10.1089/neu.2018.6006

55. Boakye M, Ball T, Dietz N, Sharma M, Angeli C, Rejc E, et al. Spinal cord epidural stimulation for motor and autonomic function recovery after chronic spinal cord injury: a case series and technical note. *Surg Neurol Int.* (2023) 14:87. doi: 10.25259/SNI_1074_2022

56. Chang HH, Yeh JC, Ichiyama RM, Rodriguez LV, Havton LA. Mapping and neuromodulation of lower urinary tract function using spinal cord stimulation in female rats. *Exp Neurol.* (2018) 305:26–32. doi: 10.1016/j.expneurol.2018.03.007

57. Bezdudnaya T, Lane MA, Marchenko V. Paced breathing and phrenic nerve responses evoked by epidural stimulation following complete high cervical spinal cord injury in rats. *J Appl Physiol.* (2018) 125:687–96. doi: 10.1152/japplphysiol.00895.2017

58. Angeli CA, Edgerton VR, Gerasimenko YP, Harkema SJ. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain.* (2014) 137:1394–409. doi: 10.1093/brain/awu038

59. Kreydin E, Zhong H, Lavrov I, Edgerton VR, Gad P. The effect of non-invasive spinal cord stimulation on anorectal function in individuals with spinal cord injury: a case series. *Front Neurosci.* (2022) 16:816106. doi: 10.3389/fnins.2022.816106

60. Kreydin E, Zhong H, Latack K, Ye S, Edgerton VR, Gad P. Transcutaneous electrical spinal cord neuromodulator (TESCoN) improves symptoms of overactive bladder. *Front Syst Neurosci.* (2020) 14:1. doi: 10.3389/fnsys.2020.00001

61. Remmers JE. Extra-segmental reflexes derived from intercostal afferents: phrenic and laryngeal responses. *J Physiol.* (1973) 233:45–62. doi: 10.1113/jphysiol.1973.sp010296

62. DiMarco AF, Kowalski KE, Geertman RT, Hromyak DR. Spinal cord stimulation: a new method to produce an effective cough in patients with spinal cord injury. *Am J Respir Crit Care Med.* (2006) 173:1386–9. doi: 10.1164/rccm.200601-097CR

63. DiMarco AF, Kowalski KE. Effects of chronic electrical stimulation on paralyzed expiratory muscles. *J Appl Physiol.* (2008) 104:1634–40. doi: 10.1152/japplphysiol.01321.2007

64. DiMarco AF, Kowalski KE. High-frequency spinal cord stimulation of inspiratory muscles in dogs: a new method of inspiratory muscle pacing. *J Appl Physiol.* (2009) 107:662–9. doi: 10.1152/japplphysiol.00252.2009

65. DiMarco AF, Altose MD, Cropp A, Durand D. Activation of the inspiratory intercostal muscles by electrical stimulation of the spinal cord. *Am Rev Respir Dis.* (1987) 136:1385–90. doi: 10.1164/ajrccm/136.6.1385

66. DiMarco AF, Kowalski KE. Activation of inspiratory muscles via spinal cord stimulation. *Respir Physiol Neurobiol*. (2013) 189:438–49. doi: 10.1016/j.resp.2013.06.001

67. Paydarfar D, Eldridge FL, Kiley JP. Resetting of mammalian respiratory rhythm: existence of a phase singularity. *Am J Physiol Regul Integr Comp Physiol*. (1986) 250:R721–7. doi: 10.1152/ajpregu.1986.250.4.R721

68. Paydarfar D, Forger DB, Clay JR. Noisy inputs and the induction of on–off switching behavior in a neuronal pacemaker. *J Neurophysiol*. (2006) 96:3338–48. doi: 10.1152/jn.00486.2006

69. Finan DS, Barlow SM. Intrinsic dynamics and mechanosensory modulation of non-nutritive sucking in human infants. *Early Hum Dev*. (1998) 52:181–97. doi: 10.1016/S0378-3782(98)00029-2

70. Brocker DT, Swan BD, So RQ, Turner DA, Gross RE, Grill WM. Optimized temporal pattern of brain stimulation designed by computational evolution. *Sci Transl Med*. (2017) 9:eaah3532. doi: 10.1126/scitranslmed.aah3532

71. Poorjavad M, Talebian Moghadam S, Nakhostin Ansari N, Daemi M. Surface electrical stimulation for treating swallowing disorders after stroke: a review of the stimulation intensity levels and the electrode placements. *Stroke Res Treat*. (2014) 2014:1–7. doi: 10.1155/2014/918057

72. Humbert IA, Fitzgerald ME, Mclaren DG, Johnson S, Porcaro E, Kosmatka K, et al. Neurophysiology of swallowing: effects of age and bolus type. *NeuroImage*. (2009) 44:982–91. doi: 10.1016/j.neuroimage.2008.10.012

73. Shaw GY, Sechtem PR, Searl J, Keller K, Rawi TA, Dowdy E. Transcutaneous neuromuscular electrical stimulation (VitalStim) curative therapy for severe dysphagia: myth or reality? *Ann Otol Rhinol Laryngol*. (2007) 116:36–44. doi: 10.1177/000348940711600107

74. Bülow M, Speyer R, Baijens L, Woisard V, Ekberg O. Neuromuscular electrical stimulation (NMES) in stroke patients with oral and pharyngeal dysfunction. *Dysphagia*. (2008) 23:302–9. doi: 10.1007/s00455-007-9145-9

75. Christiaanse ME, Mabe B, Russell G, Simeone TL, Fortunato J, Rubin B. Neuromuscular electrical stimulation is no more effective than usual care for the treatment of primary dysphagia in children. *Pediatr Pulmonol*. (2011) 46:559–65. doi: 10.1002/ppul.21400

76. Langmore SE, McCulloch TM, Krisciunas GP, Lazarus CL, Van Daele DJ, Pauloski BR, et al. Efficacy of electrical stimulation and exercise for dysphagia in patients with head and neck cancer: a randomized clinical trial. *Head Neck*. (2016) 38:E1221–31. doi: 10.1002/hed.24197

77. Krisciunas GP, Castellano K, McCulloch TM, Lazarus CL, Pauloski BR, Meyer TK, et al. Impact of compliance on dysphagia rehabilitation in head and neck Cancer patients: results from a multi-center clinical trial. *Dysphagia*. (2017) 32:327–36. doi: 10.1007/s00455-016-9760-4

78. Hamdy S, Rothwell JC, Aziz K, Singh KD, Thompson DG. Long-term reorganization of human motor cortex driven by short-term sensory stimulation. *Nat Neurosci*. (1998) 1:64–8. doi: 10.1038/264

79. Potok W, van der Groen O, Bächinger M, Edwards D, Wenderoth N. Transcranial random noise stimulation modulates neural processing of sensory and motor circuits, from potential cellular mechanisms to behavior. *Scop Rev eNeuro*. (2022) 9:ENEURO.0248–21.2021. doi: 10.1523/ENEURO.0248-21.2021

80. Zhang M., Cheng I., Sasegbon A., Dou Z., Hamdy S. (2021). Exploring parameters of gamma transcranial alternating current stimulation (tACS) and full-spectrum transcranial random noise stimulation (tRNS) on human pharyngeal cortical excitability. Neurogastroenterol Motil. 33:e14173. doi: 10.1111/nmo.14173

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Prevalence, risk factors, and outcomes of dysphagia after stroke: a systematic review and meta-analysis

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Background: Dysphagia is a common complication after stroke, which not only brings adverse outcomes but also greatly affects the quality of life of patients. At present, there is no systematic review or meta-analysis to comprehensively evaluate the epidemiological characteristics of post-stroke dysphagia (PSD). A systematic review of the prevalence, risk factors, and prognosis of PSD is essential.

Methods: Through 31 December 2022, a comprehensive literature search was performed for observational studies related to PSD. Five databases were retrieved. Random-effects models were used to estimate the pooled prevalence, odds ratio (OR), and 95% CIs.

Results: A total of 34 studies were included, and the results showed that the overall prevalence of PSD was 46.6% (95% CI, 0.405–0.528). The prevalence of dysphagia in ischemic stroke and hemorrhagic stroke was 43.6% (95% CI 0.370–0.501) and 58.8% (95% CI 0.519–0.654), respectively. The prevalence of PSD in Africa was 49.4% (95% CI, 0.196–0.792), in Asia was 40.1% (95% CI, 0.348–0.454), in Europe was 45.8% (95% CI, 0.327–0.590), in North America was 44.3% (95% CI, 0.370–0.517), in South America was 57.5% (95% CI, 0.441–0.708), and in Oceania was 64.1% (95%CI, 0.558, 0.724). In risk factor analysis, hypertension, previous stroke, and atrial fibrillation were significantly associated with the occurrence of PSD, pooled OR = 1.179 [(95% CI, 1.002–1.386), $p < 0.05$], pooled OR = 1.514 [(95% CI, 1.204–1.905), $p < 0.001$], and pooled OR = 1.980 [(95% CI, 1.580–2.481), $p < 0.001$]. In outcome studies, the prevalence of aphasia and dysarthria in PSD was 35.6% (95% CI, 0.213–0.499) and 54.5% (95% CI, 0.293–0.798), respectively. The prevalence of respiratory tract infection was 27.1% (95%CI, −0.038–0.579), and the prevalence of pneumonitis was 32.1% (95% CI, 0.224–0.418). Persistence of dysphagia at discharge and at 1 month was 74.5% (95% CI, 0.621–0.869) and 50.9% (95% CI, 0.142–0.876), respectively. Mortality rates for PSD patients during admission and discharge at 1 month, 3 months, and 1 year were 11.8% (95% CI, 0.083–0.152), 26.5% (95% CI, 0.170–0.359), 25.7% (95% CI, 0.19–0.324), and 31.3% (95% CI, 0.256–0.369), respectively.

Conclusion: This study found that the overall prevalence of PSD was 46.6%. Prevalence is most influenced by the diagnosis method. Hypertension, history of stroke, atrial fibrillation, patient age, and stroke severity were risk factors significantly associated with PSD. The prevalence of aphasia, dysarthria, respiratory tract infection, and pneumonitis in PSD patients is 2–4 times that of patients without PSD.

Systematic review registration: www.crd.york.ac.uk/PROSPERO, PROSPERO, CRD42021252967.

KEYWORDS

dysphagia, stroke, post-stroke dysphagia, systematic review, meta-analysis, prevalence

1 Introduction

Stroke is the leading cause of death and disability worldwide, and the 2010 global estimates ranked stroke as the second most common cause of death worldwide (1). Studies have shown that while the incidence of stroke has decreased in high-income countries, it is still increasing in low-income and middle-income countries (2). Dysphagia is a common post-stroke complication and one of the first obstacles to recovery after a stroke. Dysphagia not only increases mortality after stroke but also greatly affects the patient's quality of life, and it leads to asymmetry of the swallowing musculature in both motor cortices. Stroke affects major swallowing projections of the cerebral hemispheres, leading to dysphagia. This asymmetrical bilaterality may explain why several stroke patients have dysphagia. Recovery from post-stroke dysphagia (PSD) is associated with compensatory changes in the unaffected cerebral hemispheres, which explains the ability of PSD patients to regain safe swallowing function in a relatively short time (3, 4). However, if dysphagia is not detected early and the patient continues to eat, the results can be life-threatening, and serious complications such as pneumonia, dehydration, malnutrition, and asphyxia can occur (5). Moreover, the ability of stroke patients to eat and drink through the mouth has become a key factor in discharge (6).

The prevalence of PSD ranges from 18 to 81% (7–10), which is varied. The reasons for such inconsistencies in prevalence may include different locations of stroke lesions and study areas, and further discussions on these inconsistencies or potential contributing factors are needed. Although several studies have analyzed the epidemiology of PSD, most did not report or explain the factors that may affect the prevalence of PSD, which may further explain the inconsistencies in prevalence. Moreover, there is no consensus on factors that may influence the development of PSD (11, 12), and there has been no comprehensive systematic review of PSD outcomes that included large sample studies. Therefore, we aimed to systematically assess the actual prevalence of PSD and investigate the impact of other factors on PSD and patient outcomes to provide a better and more comprehensive understanding of the epidemiological features of PSD, which can provide a basis for clinical practice.

2 Method

2.1 Data sources and search strategy

This systematic review and meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (13) (PRISMA; Supplementary Table 1) and the Meta-Analyses of Observational Studies in Epidemiology guidelines (14) (MOOSE; Supplementary Table 2). The protocol was registered *post-hoc* in the International Prospective Register of Systematic Reviews (PROSPERO CRD42021252967 at www.crd.york.ac.uk/PROSPERO).

A literature search was performed using five electronic medical databases, including Embase, PubMed, Cochrane Library, Web of Science, and MEDLINE (via Web of Science), to identify all articles published on the prevalence of PSD and its related factors or outcomes from their inception to 31 December 2022. Furthermore, to ensure that no relevant studies were missed, we traced references to the full text that had been identified. We used a search strategy that combines subject terms with free words. The detailed literature search strategy for every database is shown in Supplementary Appendix 1.

2.2 Inclusion and exclusion criteria

Only articles that used an observational design, including a cross-sectional, case–control, or cohort design, and reported the prevalence or incidence of PSD or had sufficient data to allow the calculation of the prevalence of PSD in the general population or in clinical patients were included in this study. We included only articles published in the English language. Regarding the inclusion of stroke patients who had a clear hospital-based diagnosis of ischemic or hemorrhagic stroke, we excluded studies on patients with transient ischemic attacks (TIAs) and on screening for dysphagia without a clear diagnosis method and the time of diagnosis. Considering that PSD may be related to the recurrence of stroke, type, and location of stroke, we only included studies that reported on ischemic or hemorrhagic stroke and included patients with both hemorrhagic and ischemic strokes and studies in which the prevalence of swallowing disorders could be calculated separately from the given data for patients with a first-time or recurrent stroke. We excluded studies that included only ischemic or hemorrhagic stroke at specific sites, such as stroke in the brainstem only, and studies on first-time stroke only or recurrent stroke only. We excluded review articles, case reports, protocols, brief communications, personal opinions, letters, posters, conference abstracts, or laboratory studies as well as literature for which the prevalence of PSD could not be calculated from the given data or were not available after attempts to contact the authors. If there were duplicate articles on the same cohort within the same period, we included only the articles with the most data. There was no restriction on the year of publication.

2.3 Study selection and data extraction

After excluding all duplicates, two researchers (WS and HW) independently screened the titles and abstracts of the articles and

subsequently screened the full texts for relevance to the topic. If any article met the inclusion criteria, they were included in the final meta-analysis. Two investigators (WS and MW) independently retrieved information from the included studies and cross-checked the information to ensure the integrity of the content. If inconsistencies or disagreements arose during the screening process or information retrieval, they were resolved by consensus between the two investigators or by consultation with a third and senior investigator (LZ). Data extracted included the name of the first author, year of publication, country, continent, time of study, participants' age, population origin, type of stroke, stroke lesions, stroke severity, number of dysphagia patients, total study population, diagnosis method and time of PSD diagnosis, severity of dysphagia, risk factors (diseases mentioned in the literature that may be associated with PSD), and outcomes (complications or prognosis of PSD). When multiple time points of dysphagia were diagnosed in the same study, only the time of the first diagnosis was used for the calculation of the main prevalence, and the other diagnosis times were analyzed as the outcomes of dysphagia. We inserted the data into two tables (Table 1 and Supplementary Table 3).

2.4 Quality assessment

Since three types of observational studies were included, we assessed them separately. We used the Newcastle–Ottawa scale (NOS) (49) to assess the risk of bias in the included case–control or cohort studies. The scale was divided into three main parts, including the quality of the selected cohort, the comparability of the cohort, and the adequacy of the outcome or follow-up. The maximum score for each study was 9. Study quality was divided into the following three categories based on quality scores: high (0–4), moderate (5–6), and low risk of bias (7–9). We used the Agency for Healthcare Research and Quality (AHRQ) (50) Scale to evaluate the quality of cross-sectional studies. The AHRQ checklist for evaluation consists of 11 items. Each item is marked as "1" when the answer is "yes" and "0" when the answer is "unclear" or "no." Studies were rated as high, moderate, and low risk of bias when the quality scores were 0–3, 4–7, and 8–11, respectively. Quality assessments were conducted independently by two researchers (WS and RP), and when inconsistencies or disagreements were encountered, they were resolved in consultation with a third researcher (LZ). The details of the evaluation scale are presented in Supplementary Appendix 2.

2.5 Data analysis

We used the logit method to transform the reported prevalence in each study due to the heterogeneity of the studies and subsequently performed an inverse-variance-weighted random-effects meta-analysis using the method by DerSimonian and Laird (51). Next, we obtained the pooled prevalence (95% CI) of PSD in the overall population. Between-study heterogeneity was assessed using the I^2 statistic and the p-value for heterogeneity (Cochran's Q statistic). The range of the I^2 statistic is between 0 and 100%; $I^2 \geq 50\%$ indicates significant heterogeneity (52). The results are displayed using forest plots. To explore the sources of heterogeneity and to obtain the effects of different regions, different stroke types, sex, method and time of diagnosis, and first or recurrent stroke on the prevalence of dysphagia, we first explored the prevalence of PSD separately using subgroup analysis of these variables. If heterogeneity was still not found, we performed a meta-regression analysis using study characteristics, including year of publication, continents, type of study, and method of PSD diagnosis, as moderating variables. To explore exposure factors other than the above subgroups that may influence the occurrence of swallowing disorders after stroke, we extracted data on the effect of other possible exposure factors such as hypertension and diabetes on the prevalence of swallowing disorders recorded and used these data to calculate the crude OR of 2×2 tables for each study and provided its 95% CI and a combination of random-effects heterogeneity models to generate pooled estimates to determine the exposure factors for PSD. If the OR could not be calculated, we performed a systematic descriptive analysis. Regarding the outcomes of swallowing disorders, we performed a pooled analysis of the outcomes reported in the literature. We used a random-effects model to pool patients' mortality, incidence of pneumonia, and other reported comorbidities. Funnel plots and Egger's test were used to assess publication bias for the outcomes. To ensure that the study results are credible and to eliminate the effects of small population, heterogeneity, or study quality on outcomes, sensitivity analysis was performed to assess the stability of the results in the included literature. The Stata 16 software (Stata Statistical Software: College Station, TX: Stata Corp LP) was used to Meta-analyze the extracted data.

3 Results

3.1 Literature search and features of included literature

We retrieved 14,938 studies from the five databases. After removing 4,572 duplicates, the titles and abstracts of the remaining 10,366 studies were checked, and 10,251 studies were excluded. The full texts of the remaining 115 studies were screened, and 34 studies that met the inclusion criteria were identified (15–48). The detailed screening process is shown in Figure 1.

The detailed study characteristics are shown in Table 1 and Supplementary Table 3. Overall, all included studies were hospital-based studies with publication years ranging from 1998 to 2021, study sample sizes ranging from 29 to 12,276, and a total study population of 25,022 across 17 countries, including Canada, the USA, the UK, Belgium, Spain, Nigeria, Brazil, Sweden, Germany, Italy, Iraq, Egypt, Switzerland, Iran, Korea, Malaysia, and Australia, and 6 continents, of which 7 studies (16, 19, 23, 29, 31, 33, 37) were conducted in Asia, 14 (17, 22, 24, 27, 28, 32, 38–43, 45, 48) in Europe, 6 (15, 20, 25, 30, 36, 44) in North America, 4 (34, 35, 46, 47) in South America, 2 (18, 26) in Africa, and 1 in Oceania (25). All studies reported the prevalence of PSD; 22 studies (15, 18–20, 22, 24–26, 28, 36, 38–48) documented risk factors of PSD, and 23 studies (21, 23, 24, 26, 30–37, 39–52) documented the complications of PSD or mortality of PSD patients. One was a case–control study, 26 were cohort studies, and 7 were cross-sectional studies. The quality assessment of each article included in this study is shown in Supplementary Tables 4, 5. The average NOS score was 5.4, and the average AHRQ score was 6.7, indicating that most studies were of moderate quality.

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TABLE 1 General characteristics of the included literature.

Author and year	Continents	Study design	Type of stroke	No. of PSD	No. of total	Diagnose method
Marlis 2008 (15)	North America	Case–control	Ischemic	14	29	Clinical
Zahra 2021 (16)	Asia	Cross-section	Ischemic and hemorrhagic	36	100	Clinical
Antia 2019 (17)	Europe	Cohort	Ischemic and hemorrhagic	60	106	Clinical
Eman 2021 (18)	Africa	Cross-section	Ischemic and hemorrhagic	98	250	Clinical
Hamidon 2006 (19)	Asia	Cohort	Ischemic	55	134	Clinical
Michael 2013 (20)	North America	Cohort	Ischemic	25	67	Clinical
Giselle 2000 (21)	Oceania	Cohort	Ischemic and hemorrhagic	82	128	Instrument
Maurizio 2004 (22)	Europe	Cohort	Ischemic and hemorrhagic	141	406	Clinical
Mahsa 2021 (23)	Asia	Cross-section	Ischemic and hemorrhagic	136	349	Clinical
Anna 2018 (24)	Europe	Cohort	Ischemic	81	140	Clinical
Heather 2017 (25)	North America	Cohort	Ischemic	76	160	instrument
Sani 2017 (26)	Africa	Cohort	Ischemic and hemorrhagic	32	94	Clinical
SK 2017 (27)	Europe	Cross-section	Ischemic and hemorrhagic	165	200	Instrument
Bendix 2018 (28)	Europe	Cohort	Ischemic and hemorrhagic	571	687	Instrument
Zeki 2010 (29)	Asia	Cohort	Ischemic and hemorrhagic	41	72	Clinical
Kaila 2019 (30)	North America	Cohort	Ischemic	32	100	Clinical
Shiva 2019 (31)	Asia	Cohort	Ischemic	29	88	Clinical
Elien 2020 (32)	Europe	Cohort	Ischemic	35	151	Clinical
Nayeon 2021 (33)	Asia	Cohort	Ischemic	1940	5,740	Clinical
Danielles 2016 (34)	South America	Cross-section	Ischemic and hemorrhagic	32	42	Clinical
Juli 2019 (35)	South America	Cohort	Ischemic and hemorrhagic	86	201	Clinical
Heather 2013 (36)	North America	Cohort	Ischemic	98	221	Clinical
Shiva 2016 (37)	Asia	Cross-section	Ischemic and hemorrhagic	545	113	Clinical
Smithards 2007 (38)	Europe	Cohort	Ischemic and hemorrhagic	567	1,188	Clinical
Avinash 2019 (39)	Europe	Cohort	Ischemic	81	340	Clinical
Polo 2009 (40)	Europe	Cohort	Ischemic and hemorrhagic	62	151	Clinical
Rofes 2018 (41)	Europe	Cohort	Ischemic and hemorrhagic	178	395	Clinical
Mohamed 2016 (42)	Europe	Cohort	Ischemic	3,083	12,276	Clinical
Carlo 2019 (43)	Europe	Cohort	Ischemic and hemorrhagic	94	249	Clinical
Sachiyo 2021 (44)	North America	Cohort	Ischemic and hemorrhagic	233	427	Clinical
Hakan 1998 (45)	Europe	Cohort	Ischemic and hemorrhagic	14	72	Clinical
Anna 2012 (46)	South America	Cohort	Ischemic and hemorrhagic	134	212	Clinical
Aline 2016 (47)	South America	Cohort	Ischemic and hemorrhagic	50	100	Clinical
Felix 2021 (48)	Europe	Cohort	Hemorrhagic	84	132	Clinical

3.2 Prevalence of PSD

The meta-analysis of 34 studies showed that the random-effects pooled overall prevalence of PSD was 46.6% (95% CI, 0.405–0.528), with high heterogeneity (I^2 = 58.76%; p < 0.001) (Figure 2 and Supplementary Figure 1). Figure 3 shows the prevalence of dysphagia among individuals with stroke for all countries with at least one study.

These studies were analyzed in subgroups according to the different stroke types (Figure 2 and Supplementary Figure 2). In 26 studies (15–20, 22, 24, 25, 28–44, 46) that reported dysphagia after ischemic stroke, the pooled prevalence was 43.6% (95% CI, 0.370–0.501), with high heterogeneity (I^2 = 98.71%; p < 0.001). In 15 studies (17, 18, 22, 28, 29, 31, 35, 37, 38, 40, 41, 43, 44, 46, 48) that reported dysphagia after a hemorrhagic stroke, the pooled prevalence was 58.8% (95% CI, 0.519–0.654), which was significantly higher than that after ischemic stroke, and the results were highly heterogeneous (I^2 = 58.76%; p < 0.001).

We performed a subgroup analysis by sex (Figure 2 and Supplementary Figure 3). Of the 34 studies, 28 (15–18, 20, 22, 24–26, 28, 29, 31–33, 35–48) documented the prevalence of PSD by sex. The random-effects pooled prevalence of PSD in men was 42% (95% CI, 0.357–0.484) and in women was 46.2% (95% CI, 0.382–0.542), both

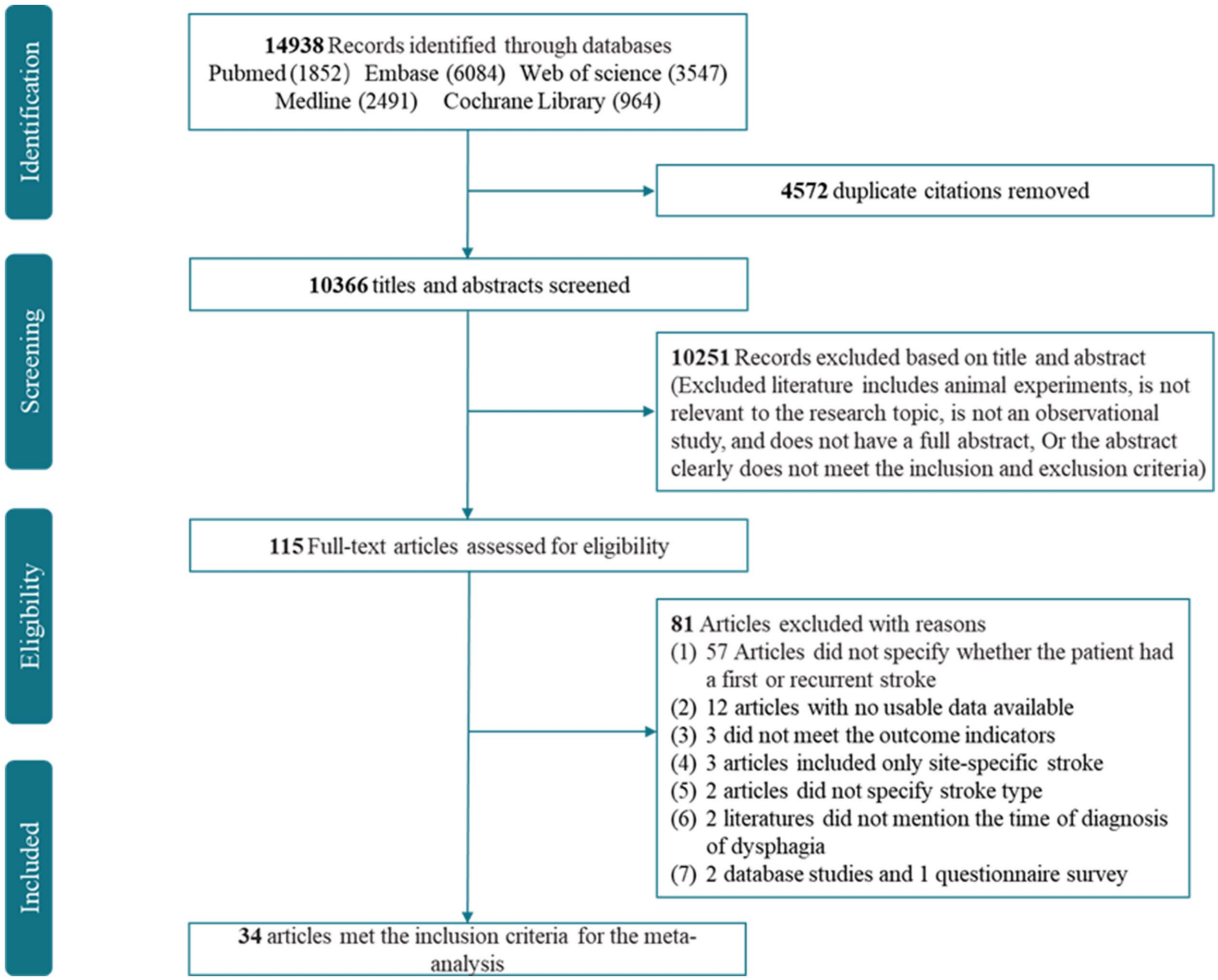

FIGURE 1
Flowchart of the identification of eligible studies.

with high heterogeneity ($I^2 = 98\%$; $p < 0.001$ and $I^2 = 97.95\%$; $p < 0.001$, respectively).

To investigate whether the prevalence of PSD differed between first and recurrent strokes, we performed a subgroup analysis according to the presence or absence of a stroke history (Figure 2 and Supplementary Figure 4). All 34 studies documented the prevalence of PSD after the first stroke, with a random-effects pooled prevalence of 45.1% (95% CI, 0.374–0.527) and high heterogeneity ($I^2 = 98.72\%$; $p < 0.001$). Ten of these studies (43–52) documented the prevalence of PSD in patients with a history of stroke. The random-effects pooled prevalence was 49.9% (95% CI, 0.361–0.637), with high heterogeneity. ($I^2 = 95.04\%$; $p < 0.001$).

We performed a subgroup analysis based on the time until the first dysphagia diagnosis after the stroke (Figure 2 and Supplementary Figure 5). We found that all 34 studies recruited patients in the acute phase of stroke (≤2 weeks). One study (46) evaluated within 60 days of stroke onset, but no time-specific assessment results were recorded. The remaining 33 were evaluated within 14 days of stroke onset. We stratified the prevalence of PSD in the acute phase according to the specific time of assessment. Seven studies (19, 26, 27, 43, 44, 46, 49) evaluated dysphagia within 24 h after stroke onset; the prevalence was 32.1% (95% CI, 0.272–0.370), with high heterogeneity ($I^2 = 85.85\%$; $p < 0.001$). Seven studies (20, 24, 32, 35, 41, 47, 48) evaluated dysphagia within 48 h after stroke onset; the prevalence was 45.6% (95% CI, 0.358–0.555), with high heterogeneity ($I^2 = 91.74\%$; $p < 0.001$). Seven studies (15, 17, 18, 26, 29, 30, 42) evaluated dysphagia within 72 h after stroke onset; the prevalence was 43.4% (95% CI, 0.368–0.500), with high heterogeneity ($I^2 = 75.36\%$; $p < 0.001$). In 11 studies, dysphagia was assessed within 7 days after stroke onset (15, 19, 27, 28, 31, 33, 34, 37, 38, 43, 44); the prevalence was 53.8% (95% CI, 0.399–0.676), with high heterogeneity ($I^2 = 99.22\%$; $p < 0.001$). In four studies, swallowing disorders were assessed within 7 days after stroke onset (16, 21, 25, 36). The prevalence was 48% (95% CI, 0.374–0.587), with high heterogeneity ($I^2 = 86.24\%$; $p < 0.001$).

We performed a subgroup analysis on the included studies according to the location of the stroke lesion (Figure 2 and Supplementary Figure 6). We found that most studies that documented stroke lesion sites of ischemic stroke patients reported these sites as follows and calculated the prevalence of PSD: total anterior circulation (TAC) infarct, partial anterior circulation (PAC) infarct, lacunar (LAC) infarct, and posterior circulation (POC) infarct. Due to the small number of studies on hemorrhagic stroke and the limited number of studies that documented the lesion sites, a pooled subanalysis could not be performed for hemorrhagic stroke. The results of the subgroup

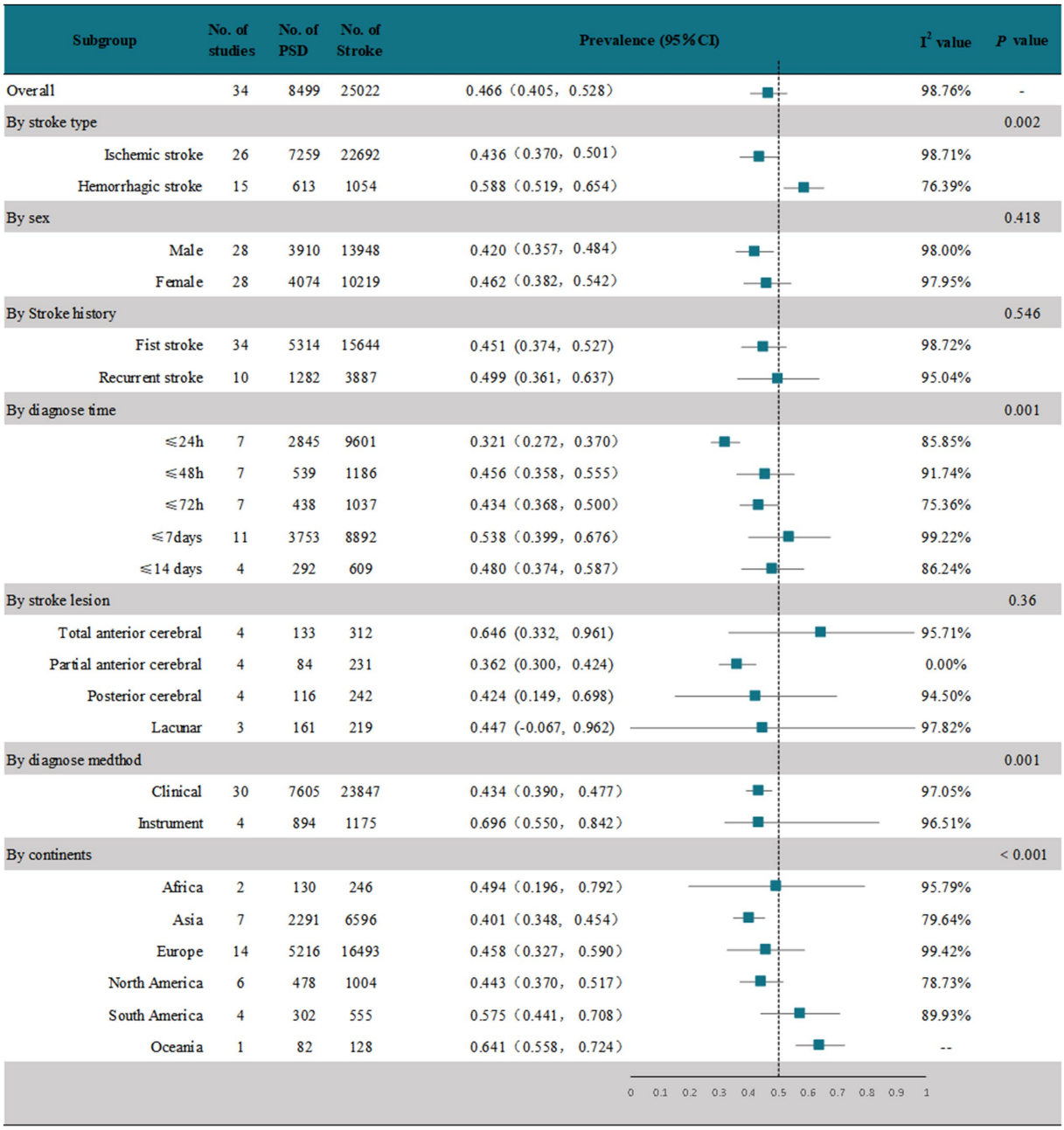

FIGURE 2
Overall prevalence and subgroup analysis of PSD.

analysis showed that there were four studies on TAC (20, 29, 38, 47). The random-effects pooled prevalence of PSD was 64.6% (95% CI, 0.399–0.676), with high heterogeneity ($I^2 = 91.74\%$; $p < 0.001$). There were four studies on PAC (20, 29, 38, 47), with a random-effects pooled PSD prevalence of 36.2% (95% CI, 0.300–0.424); the results showed 0% heterogeneity. There were four studies (29, 38, 39, 47) on POC. The random-effects pooled prevalence of PSD was 42.4% (95% CI, 0.149–0.698), with high heterogeneity ($I^2 = 94.50\%$; $p < 0.001$). There were three studies (20, 38, 47) on LAC, and the prevalence of PSD was 44.7% (95% CI, −0.067–0.962), with high heterogeneity ($I^2 = 97.82\%$; $p < 0.001$).

We performed subgroup analyses according to the method of dysphagia diagnosis after stroke, clinical diagnosis, or instrumental diagnosis (Figure 2 and Supplementary Figure 7). Thirty studies used the clinical method to diagnose PSD (15–20, 22–24, 26, 29–48); the remaining four studies (21, 25, 27, 28) used the instrumental method. The subgroup analysis showed that the random-effects pooled prevalence of clinically assessed PSD was 43.4% (95% CI, 0.390–0.477), with high heterogeneity ($I^2 = 97.05\%$; $p < 0.001$). The random-effects pooled prevalence of instrumentally assessed PSD was 69.6% (95% CI, 0.550–0.842), and the prevalence was significantly higher than that of the clinically assessed PSD ($p < 0.01$), with high heterogeneity ($I^2 = 96.51\%$; $p < 0.001$).

We performed subgroup analyses according to the six continents covered in the study (Figure 2 and Supplementary Figure 8). Two

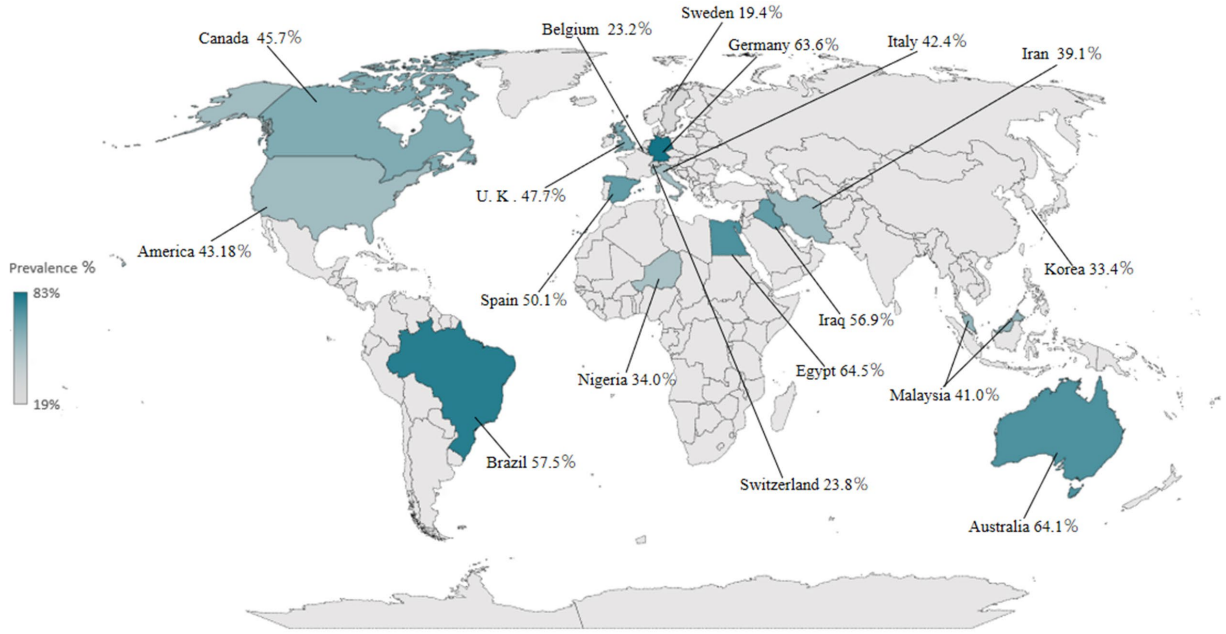

FIGURE 3
Global prevalence of PSD.

studies (22, 30) conducted in Africa reported a PSD prevalence of 49.4% (95% CI, 0.196–0.792), with high heterogeneity (I^2 = 95.7%; p < 0.001). Seven studies (16, 19, 23, 29, 31, 33, 37) in Asia reported a PSD prevalence of 40.1% (95% CI, 0.348–0.454), with high heterogeneity (I^2 = 79.64%; p < 0.001). Fourteen studies in Europe (17, 22, 24, 27, 28, 32, 38–43, 45, 48) reported a PSD prevalence of 45.8% (95% CI, 0.327–0.590), with high heterogeneity (I^2 = 99.42%; p < 0.001). The random-effects pooled prevalence of PSD based on six studies in North America (15, 20, 25, 30, 36, 44) was 44.3% (95% CI, 0.370–0.517), with high heterogeneity (I^2 = 78.73%; p < 0.001). The random-effects pooled prevalence of PSD based on four studies in South America (34, 35, 46, 47) was 57.5% (95% CI, 0.441–0.708), with high heterogeneity (I^2 = 89.93%; p < 0.001). The prevalence of only one study (21) in Oceania was 64.1% (95%CI, 0.558–0.724).

3.3 Risk factors

We assessed crude ORs for risk factors of PSD documented in the studies using a random-effects model. A meta-analysis of 15 studies (22, 24, 26, 28–30, 32, 40, 42, 43, 45, 46, 49, 51) showed that hypertension was associated with the development of PSD (pooled OR = 1.179, [95% CI, 1.002–1.386], p < 0.05) (Figure 4 and Supplementary Figure 9). In another 15 studies (18, 20, 22, 24–26, 36, 38, 39, 41, 42, 44, 45, 47, 48) that assessed the association of diabetes with PSD, the meta-analysis showed that diabetes was not associated with the development of PSD (pooled OR = 0.940 [95% CI, 0.763–1.157], p < 0.05) (Figure 4 and Supplementary Figure 10). A meta-analysis of eight studies (18, 22, 25, 36, 39, 44, 45, 47) showed that smoking was not associated with the occurrence of PSD (pooled OR = 0.781, [95% CI, 0.580–1.052], p < 0.05) (Figure 4 and Supplementary Figure 11). A meta-analysis of 10 studies (39–48) showed that a history of stroke was significantly associated with the occurrence of PSD (pooled OR = 1.514, [95% CI, 1.204–1.905], p < 0.001) (Figure 4 and Supplementary Figure 12). Three studies (22, 25, 36) recorded the association of previous TIA with PSD, and the results showed no association (pooled OR = 0.707 [95% CI, 0.437–0.142], p > 0.05) (Figure 4 and Supplementary Figure 13). A meta-analysis of nine studies (18, 22, 24, 36, 38, 39, 42, 44, 45) showed that atrial fibrillation was significantly associated with the occurrence of PSD (pooled OR = 1.980 [95% CI, 1.580–2.481], p < 0.001) (Figure 4 and Supplementary Figure 14). A meta-analysis of five studies (18, 22, 41, 45, 47) showed that heart disease was not associated with the occurrence of PSD (pooled OR = 1.201 [95% CI, 0.753–1.918], p > 0.05) (Figure 4 and Supplementary Figure 15). A meta-analysis of nine studies (18, 20, 22, 24, 25, 36, 39, 41, 44) showed that dyslipidemia was not associated with the occurrence of PSD (pooled OR = 0.891 [95% CI, 0.718–1.105], p > 0.05) (Figure 4 and Supplementary Figure 16). A meta-analysis of three studies (18, 42, 44) showed that hypercholesterolemia was not associated with the occurrence of PSD (pooled OR = 0.771, [95% CI, 0.507–1.171], p > 0.05) (Figure 4 and Supplementary Figure 17). A meta-analysis of two studies (18, 22) showed that obesity was not associated with the occurrence of PSD (pooled OR = 0.923, [95% CI, 0.334–2.552], p > 0.05) (Figure 2 and Supplementary Figure 18).

In most studies, age and stroke severity were not reported as dichotomous or stratified variables, so we were unable to calculate the crude ORs; therefore, we performed a systematic descriptive analysis (Supplementary Table 3). Comparing the ages of patients with and without PSD in the included studies, whether recorded as mean or as median, PSD patients were older than those without PSD. The included studies used different scales to assess stroke severity. Fourteen studies (15, 17, 18, 20, 22, 24, 26, 28, 32, 33, 35, 42, 44, 48) used and documented in detail the National Institutes of Health Stroke Scale (NIHSS) scores of patients with and without PSD. Regardless of whether the mean or median was recorded, the NIHSS scores of

Risk factors	No. of studies	No. of Dysphagia (Yes/no)	No. of dysphgia (Yes/no)	Calculated crude OR (95% CI)	I^2 value	*P* value for effect estimate
Hypertension	15	3828/5328	9025/11495	1.179 (1.002, 1.386)	37.50%	0.047
Diabetes	15	1253/4841	2745/11316	0.940 (0.763, 1.157)	58.80%	0.558
Smoking	8	176/791	301/1185	0.781 (0.580, 1.052)	37.70%	0.104
Previous stroke	10	1282/4013	2605/10406	1.514 (1.204, 1.905)	38.90%	< 0.001
Previous TIA	3	36/315	66/472	0.707 (0.437, 1.142)	8.40%	0.156
Atrial fibrillation	9	1717/4396	2617/10924	1.980 (1.580, 2.481)	52.30%	< 0.001
Heart disease	5	116/481	151/742	1.201 (0.753, 1.918)	51.20%	0.442
Dyslipidemia	9	391/1011	614/1395	0.891 (0.718, 1.105)	22.50%	0.293
Hypercholesterolemia	3	1471/3414	5043/9539	0.771 (0.507, 1.171)	30.10%	0.223
Obesity	2	30/239	50/417	0.923 (0.334, 2.552)	72.40%	0.878

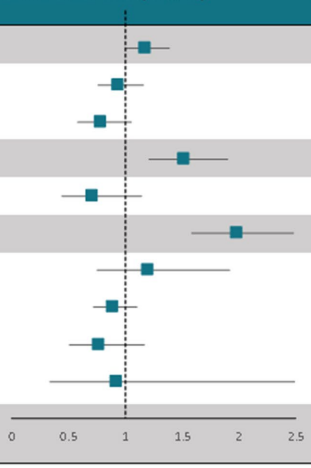

FIGURE 4
Assessment of possible risk factors for PSD.

patients with PSD were greater than those of PSD patients. The analysis of four studies (25, 28, 36, 48) that assessed and recorded the modified Rankin Scale (mRS) scores of patients with or without PSD in detail showed that the scores of PSD patients were higher than those of patients without PSD. The analysis of two studies (25, 36) that used and recorded the Canadian Neurological Scale (CNS) scores of patients with and without PSD showed that the CNS scores of PSD patients were lower than those of patients without PSD. Two studies (15, 28) recorded the volume of stroke lesions in patients with and without PSD, and the analysis showed that the volume of lesions in patients with PSD was significantly larger than that in PSD patients. The above analysis shows that the patient's age and stroke severity may be risk factors for PSD. The older the patient, the more likely the patient is to develop dysphagia after stroke, and the more severe the stroke, the more likely the patient is to develop dysphagia.

3.4 Outcomes

A random-effects analysis of seven studies (17, 28, 37, 40, 42, 47, 48) showed that the prevalence of aphasia in PSD patients was 35.6% (95% CI, 0.213–0.499), with high heterogeneity (I^2 =97.6%; p<0.001). However, only 17% (95% CI, 0.07–0.27) of stroke patients without PSD had aphasia (Figure 5 and Supplementary Figure 19). Six studies (17, 28, 38, 40, 42, 47) reported the prevalence of dysarthria after PSD, and the analysis showed that 54.5% (95% CI, 0.293–0.798) of PSD patients had dysarthria, with high heterogeneity (I^2 =99.53%; p<0.001), whereas 29%(95%CI, 0.17–0.41) of stroke patients without PSD had dysarthria, with high heterogeneity (I^2 =98.01%; p<0.001) (Figure 5 and Supplementary Figure 20). Four studies (17, 28, 40, 41) recorded the prevalence of respiratory tract infection after PSD, and the analysis showed that 27.1% (95% CI, −0.038–0.597) of stroke patients with PSD had respiratory tract infection, with high heterogeneity (I^2 =99.06%; p<0.001), whereas only 7% (95% CI, 0.01–0.12) of those without PSD had respiratory tract infection (Figure 5 and Supplementary Figure 21). Six studies (26–28, 35, 45, 48) recorded the prevalence of pneumonitis after PSD, and the analysis showed that 32.1% (95% CI, 0.224–0.418) of the stroke patients with PSD had pneumonitis, with high heterogeneity (I^2 =88.54%; p<0.001). Conversely, only 7% (95% CI, 0.02–0.12) of stroke patients without PSD had pneumonitis (Figure 5 and Supplementary Figure 22). Of the studies that reported the persistence of dysphagia in PSD patients (Figure 5 and Supplementary Figure 23), 74.5% (95% CI, 0.621–0.869) in two (33, 44) of these studies had persistent dysphagia at discharge, with high heterogeneity (I^2 =93.64%; p<0.001). Of these two studies, we found only one study (44) that screened for factors that still had dysphagia at discharge, and the significant correlation was NIHSS, which was higher in patients with dysphagia at discharge (p<0.001). While three studies (19, 29, 43) recorded the persistence of dysphagia in patients at 1 month, the random-effects pooled prevalence was 50.9% (95% CI, 0.142–0.876), with high heterogeneity (I^2 =97.00%; p<0.001). One of the studies found that dysphagia (43), which persisted up to 1 month after stroke, was significantly associated with moderate or high dependence (mRS≥3, p<0.001) and a BMI≥20 (p<0.001) as protective factors. Some studies recorded the mortality rate of PSD patients at different periods (Figure 5 and Supplementary Figure 24). Four studies (36, 39, 41, 42) recorded the mortality of PSD patients from admission to discharge, and the pooled prevalence from the random-effects model was 11.8% (95% CI, 0.083–0.152), with high heterogeneity (I^2 =65.25%; p=0.03). The pooled prevalence of three studies (19, 26, 29, 43) that recorded the mortality rate of patients with PSD at 1 month was 26.5% (95% CI, 0.170–0.359), with low heterogeneity (I^2 =35.22%; p=0.21). The random-effects pooled mortality prevalence of PSD patients from five studies (17, 22, 35, 41, 46) at 3 months was 25.7% (95% CI, 0.190–0.324), with high heterogeneity (I^2 =72.1%; p=0.01). The random-effects pooled mortality prevalence of PSD patients recorded in two studies (39, 41) at 12 months was 31.3% (95% CI, 0.256–0.369), with no heterogeneity (I^2 =0%).

Other outcomes mentioned in the included studies were not reported as stratification variables (Supplementary Table 3), preventing any inferential analysis; therefore, we performed a systematic descriptive analysis for those variables. Nine studies (20, 28, 33, 35, 36, 40–42, 45) recorded the hospitalization period of patients with or without PSD;

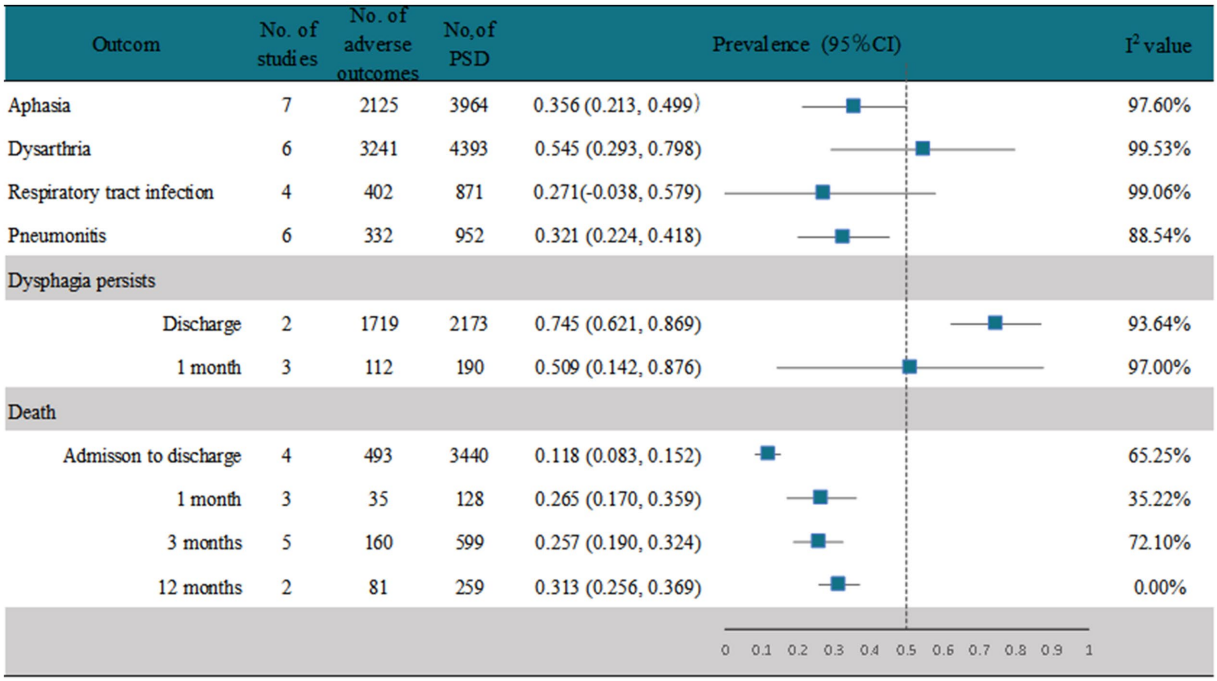

FIGURE 5
Outcome of PSD.

whether the data are expressed as mean or median, the hospitalization period of PSD patients was longer than that of patients without PSD. One study (20) used mini-nutritional assessment (MNA) to assess the nutritional state. The results showed that although patients with and without PSD were at risk of malnutrition, PSD patients had higher MNA scores than those of patients without PSD. Another study (44) also showed that the risk of malnutrition in patients without PSD was 82%, while the risk of malnutrition in PSD patients was as high as 95%. Another study (35) used the Nutritional Risk Screening tool to evaluate the nutritional status of patients, and the results showed that the probability of obtaining a Nutritional Risk Screening score of >3 (severe malnutrition) in patients with or without PSD was 23 and 14%, respectively.

3.5 Publication bias

We used funnel plots and Egger's test to assess publication bias in the 34 included studies. The funnel plots showed a seemingly symmetrical distribution, while Egger's test indicated that there was no statistically significant publication bias ($p > 0.05$; Supplementary Appendix 3). Therefore, publication bias was not the source of heterogeneity.

3.6 Meta-regression analysis

Because of the extremely high heterogeneity among the prevalence rates of the 34 studies and because no source of heterogeneity was found after subgroup analysis, the characteristics of the included articles, including publication year, continents, study design, and method of PSD diagnosis, were inputted as covariates in the meta-regression analysis (Supplementary Table 6). The analysis showed that publication year, continents, and study design were not the source of heterogeneity ($p > 0.05$). However, with the method of dysphagia diagnosis as a covariate, the p-value was <0.001, indicating that it was the main source of article heterogeneity and explaining the main reason for the inconsistencies in the prevalence of PSD.

3.7 Sensitivity analysis

Thirty-four studies were included in this meta-analysis. After excluding one, the combined results of the remaining studies (33) were within a 95% CI of 0.41 to 0.53, indicating stable results (Supplementary Figure 25).

4 Discussion

To the best of our knowledge, this is the first comprehensive meta-analysis of PSD prevalence, risk factors, and outcomes. After excluding studies with site-specific stroke, our results showed that the overall prevalence of PSD was 46.6%, which is similar to the midpoint of the prevalence range reported in previous studies (7–10). We calculated the prevalence of PSD according to the type of stroke and found that the prevalence of dysphagia after hemorrhagic stroke was higher than that after ischemic stroke (58.8% vs. 46.6%). PSD in women with stroke was more prevalent than that in men with stroke (46.2% vs. 42%). Patients with a history of stroke have a higher prevalence than those with first-time stroke (49.9% vs. 45.1%). We analyzed the location of stroke lesions and found that patients with TAC had the highest prevalence of PSD at 64.4%. Since the 34 included studies found that the study time was in the acute phase of stroke, we analyzed PSD according to the time of first diagnosis, and after excluding a

study that assessed PSD within 60 days, we found that the prevalence of PSD within 24 h after stroke was not high, but gradually an upward trend was observed in 2, 3, or even 7 days, followed by a gradual decline. The prevalence of PSD was the highest in 7 days, reaching 53.8%. Overall, the prevalence of PSD in the acute phase is characterized first by an increase, a peak on day 7, and a gradual decline thereafter. Among the six continents, one study performed in Australia had the highest prevalence of PSD (64.1%), followed by four studies in South America with a prevalence of 57.9%. In the rest of the continents, the overall prevalence was between 40 and 50%. A subgroup analysis of diagnostic methods for PSD was the one that led to the largest differences in the prevalence of PSD in the current analysis. After the meta-regression analysis, the method of PSD diagnoses was the main source of heterogeneity in this study. We found that the prevalence of clinically assessed PSD was 43.4%, while the prevalence of instrumentally assessed PSD was 69.6%, which showed a statistically significant difference. This indicates that the method of diagnosis is the main reason for the inconsistency in the prevalence of PSD. The clinical evaluation methods of PSD reported in this study mainly included the Mann Assessment of Swallowing Ability, Gugging Swallowing Screen, Volume-Viscosity Swallow Test, Water Swallowing Test, the Burke dysphagia screening test (39), and Repetitive Oral Suction Swallow test (45). However, clinical assessments are always highly subjective, and most speech and language pathologists apply their clinical reasoning to customize their bedside assessments rather than using standardized assessments. Although some studies used standardized assessments, the results are mixed because the assessors were not professionals or systematically trained. Moreover, dysphagia screening protocols vary widely, and there is no consensus on the best assessment protocol (53, 54). Instrumental evaluation using video fluoroscopy remains the gold standard due to its better sensitivity and specificity. However, it requires considerable expertise and is inconvenient for routine practice, which poses great limitations for economically deprived areas and regions. Furthermore, speech therapists in different units use different food textures, doses, and sequences in the videofluoroscopy assessment process, and the evaluation and termination criteria also vary from person to person; therefore, the reliability between experts is still low (55). Research is still needed regarding the selection and application of PSD evaluation methods, and professional training of evaluators is also required to ensure that the screening of PSD is more accurate and uniform and heterogeneity is eliminated.

In this study, the possible risk factors reported in the included studies were analyzed, including hypertension, diabetes, smoking, previous stroke, previous TIA, atrial fibrillation, heart disease, dyslipidemia, hypercholesterolemia, obesity, age, and severity of stroke. Among them, hypertension was associated with the occurrence of PSD. We speculate that this result is because hypertension can cause hemorrhagic stroke, and the prevalence of PSD after hemorrhagic stroke is higher than that after ischemic stroke. Moreover, previous stroke and atrial fibrillation were significantly associated with the occurrence of PSD. Although stratified information on age and stroke severity was not available, the studies examined showed that PSD patients were older and had more severe strokes than observed in patients without PSD, so this better explains why recurrent stroke and atrial fibrillation are risk factors for PSD as they can lead to the aggravation of stroke, which makes them prone to dysphagia.

Regarding the accompanying symptoms of dysphagia, we analyzed the common complications reported in the included studies, including aphasia, dysarthria, respiratory tract infection, and pneumonitis. The most common accompanying symptoms of PSD were dysarthria, followed by aphasia, respiratory tract infection, and pneumonitis (27.1 and 32.1%, respectively). The prevalence of the above symptoms in patients with PSD was 2–4 times higher than that in patients without PSD. Our study showed that dysphagia persisted in 74.5% of the patients with PSD at discharge and in 50.9% of patients with PSD 1 month later. Moreover, patients with PSD have longer hospital stays and more severe malnutrition than that observed in patients without PSD. The mortality rate of patients with PSD reached 31.3% within 1 year and increased from admission to discharge and even to 1 month. Although the mortality rate at 3 months was slightly lower than that at 1 month, it was still high.

Although this is the first comprehensive meta-analysis of PSD prevalence, risk factors, and outcomes, shortcomings remain. First, although we found different diagnostic methods as the source of heterogeneity in PSD prevalence, we could not further classify and analyze the source of heterogeneity due to the limited number of articles. Our conclusion may not be completely plausible because of the high heterogeneity. Second, because all the included studies assessed patients in the acute phase, we did not analyze the epidemiological characteristics of PSD in the other phases, and the conclusions of this study are only applicable to the acute phase. Finally, our analysis of PSD outcomes was limited because the included studies did not have long-term follow-up cohorts.

Based on these limitations, first, there should be a more standardized and unified evaluation method for PSD and more professional evaluators involved in PSD diagnosis, as it had the greatest impact on the prevalence of PSD in this study. When assessing the prevalence of dysphagia, the method of assessment of dysphagia is important, as is the detection of aspiration, so that the importance of PSD is not underestimated. Many low-sensitivity dysphagia screens focus only on dysphagia without considering inhalation and vice versa. Dysphagia may occur in the absence of inhalation and vice versa, so using only low-sensitivity dysphagia screening has the potential to mislead results (54). Conversely, highly sensitive dysphagia screenings designed to detect aspiration and tested against FEES are more likely to depict the real situation in terms of dysphagia prevalence and risk factors (56, 57). Patients with dysphagia may have an 11-fold higher risk of developing pneumonia than non-dysphagic patients, depending on whether the dysphagia assessment method can also detect aspiration and silent aspiration (58–60). Failing to detect silent aspirators could be of particular relevance both when assessing dysphagia prevalence and for pneumonia, since a large part of pneumonia may be due to silent aspiration. In fact, stroke patients who passed low-sensitive screening for dysphagia reported higher stroke-associated pneumonia compared to those who passed high-sensitive screening, which can also detect silent aspiration (58). It can be seen that the use of some low-sensitivity assessment methods and inconsistent assessment methods will bring different results to the incidence of PSD and the occurrence and development of pneumonia after stroke. Furthermore, future studies on PSD can control for the risk factors that have a significant impact on PSD, such as hypertension, stroke history, and atrial fibrillation, develop detailed inclusion and exclusion criteria, or strictly document patients with these risk factors to ensure more accurate and reliable research conclusions and guide clinical practice more precisely. Finally, cohort studies with long-term follow-up of patients with PSD can determine their long-term outcomes and provide a better management plan in the future.

5 Conclusion

The overall prevalence of PSD was 46.6%, and the prevalence of dysphagia after hemorrhagic stroke was higher than that after ischemic stroke, higher in women than in men, higher in patients with a history of stroke than in patients with the first stroke, highest within 7 days of the acute phase than within other time frames, highest in patients with ischemic stroke with TAC lesions compared to the lesion sites, and highest in Australia and South America than in other continents. The prevalence of PSD was influenced most by the method of diagnosis, with the instrumental diagnosis being significantly higher than the clinical diagnosis. Hypertension, history of stroke, atrial fibrillation, patients' age, and stroke severity were significant risk factors associated with PSD. The prevalence of aphasia, dysarthria, respiratory tract infection, and pneumonitis was 2–4 times higher in patients with PSD than in those without PSD. Our findings should be used with caution because further restrictions on the source of heterogeneity could not be made in this study. We hope that, in the future, there will be more professional personnel and a more unified evaluation method for PSD diagnosis. Furthermore, studies should be conducted with strict control or detailed documentation of risk factors, and long-term follow-up should be conducted.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

WS: Conceptualization, Formal analysis, Methodology, Software, Validation, Writing – original draft. MW: Formal analysis, Methodology, Writing – review & editing. HW: Data curation, Investigation, Writing – review & editing. RP: Data curation, Investigation, Writing – review & editing. LZ: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2024.1403610/full#supplementary-material

References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. *Lancet*. (2012) 380:2095–128. doi: 10.1016/S0140-6736(12)61728-0

2. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol*. (2009) 8:355–69. doi: 10.1016/S1474-4422(09)70025-0

3. González-Fernández M, Ottenstein L, Atanelov L, Christian AB. Dysphagia after stroke: an overview. *Curr Phys Med Rehabil Rep*. (2013) 1:187–96. doi: 10.1007/s40141-013-0017-y

4. Singh S, Hamdy S. Dysphagia in stroke patients. *Postgrad Med J*. (2006) 82:383–91. doi: 10.1136/pgmj.2005.043281

5. Ickenstein GW, Stein J, Ambrosi D, Goldstein R, Horn M, Bogdahn U. Predictors of survival after severe dysphagic stroke. *J Neurol*. (2005) 252:1510–6. doi: 10.1007/s00415-005-0906-9

6. Maeshima S, Osawa A, Miyazaki Y, Seki Y, Miura C, Tazawa Y, et al. Influence of dysphagia on short-term outcome in patients with acute stroke. *Am J Phys Med Rehabil*. (2011) 90:316–20. doi: 10.1097/PHM.0b013e31820b13b2

7. Barer DH. The natural history and functional consequences of dysphagia after hemispheric stroke. *J Neurol Neurosurg Psychiatry*. (1989) 52:236–41. doi: 10.1136/jnnp.52.2.236

8. Daniels SK, Ballo LA, Mahoney MC, Foundas AL. Clinical predictors of dysphagia and aspiration risk: outcome measures in acute stroke patients. *Arch Phys Med Rehabil*. (2000) 81:1030–3. doi: 10.1053/apmr.2000.6301

9. Meng NH, Wang TG, Lien IN. Dysphagia in patients with brainstem stroke: incidence and outcome. *Am J Phys Med Rehabil*. (2000) 79:170–5. doi: 10.1097/00002060-200003000-00010

10. Maeshima S, Osawa A, Yamane F, Ishihara S, Tanahashi N. Dysphagia following acute thalamic haemorrhage: clinical correlates and outcomes. *Eur Neurol*. (2014) 71:165–72. doi: 10.1159/000355477

11. Kojima A, Imoto Y, Osawa Y, Fujieda S. Predictor of rehabilitation outcome for dysphagia. *Auris Nasus Larynx*. (2014) 41:294–8. doi: 10.1016/j.anl.2013.12.009

12. San Luis CO, Staff I, Fortunato GJ, McCullough LD. Dysphagia as a predictor of outcome and transition to palliative care among middle cerebral artery ischemic stroke patients. *BMC Palliat Care*. (2013) 12:21. doi: 10.1186/1472-684X-12-21

13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 372:n71. doi: 10.1136/bmj.n71

14. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA*. (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008

15. Gonzalez-Fernandez M, Kleinman JT, Ky PK, Palmer JB, Hillis AE. Supratentorial regions of acute ischemia associated with clinically important swallowing disorders: a pilot study. *Stroke*. (2008) 39:3022–8. doi: 10.1161/STROKEAHA.108.518969

16. Ghoreyshi Z, Nilipour R, Bayat N, Nejad SS, Mehrpour M, Azimi T. The incidence of aphasia, cognitive deficits, apraxia, dysarthria, and dysphagia in acute post stroke Persian speaking adults. Indian. *J Otolaryngol Head Neck Surg*. (2021) 74:5685–95. doi: 10.1007/s12070-021-03006-9

17. Fernández-Pombo A, Seijo-Raposo IM, López-Osorio N, Cantón-Blanco A, González-Rodríguez M, Arias-Rivas S, et al. Lesion location and other predictive factors of dysphagia and its complications in acute stroke. *Clin Nutr ESPEN*. (2019) 33:178–82. doi: 10.1016/j.clnesp.2019.05.019

18. Khedr EM, Abbass MA, Soliman RK, Zaki AF, Gamea A. Post-stroke dysphagia: frequency, risk factors, and topographic representation: hospital-based study. *Egypt J Neurol Psychiatry Neurosurg*. (2021) 57:1–8. doi: 10.1186/s41983-021-00281-9

19. Hamidon BB, Nabil I, Raymond AA. Risk factors and outcome of dysphagia after an acute ischaemic stroke. *Med J Malaysia*. (2006) 61:553–7.

20. Crary MA, Humphrey JL, Carnaby-Mann G, Sambandam R, Miller L, Silliman S. Dysphagia, nutrition, and hydration in ischemic stroke patients at admission and discharge from acute care. *Dysphagia*. (2013) 28:69–76. doi: 10.1007/s00455-012-9414-0

21. Mann G, Hankey GJ, Cameron D. Swallowing function after stroke: prognosis and prognostic factors at 6 months. *Stroke*. (1999) 30:744–8. doi: 10.1161/01.str.30.4.744

22. Paciaroni M, Mazzotta G, Corea F, Caso V, Venti M, Milia P, et al. Dysphagia following stroke. *Eur Neurol*. (2004) 51:162–7. doi: 10.1159/000077663

23. Mahmoudinezhad M, Khalili M, Rezaeemanesh N, Farhoudi M, Eskandarieh S. Subjective global assessment of malnutrition and dysphagia effect on the clinical and Para-clinical outcomes in elderly ischemic stroke patients: a community-based study. *BMC Neurol*. (2021) 21:466. doi: 10.1186/s12883-021-02501-4

24. Losurdo A, Brunetti V, Broccolini A, Caliandro P, Frisullo G, Morosetti R, et al. Dysphagia and obstructive sleep apnea in acute, first-ever, ischemic stroke. *J Stroke Cerebrovasc Dis*. (2018) 27:539–46. doi: 10.1016/j.jstrokecerebrovasdis.2017.09.051

25. Flowers HL, AlHarbi MA, Mikulis D, Silver FL, Rochon E, Streiner D, et al. MRI-based neuroanatomical predictors of dysphagia, dysarthria, and aphasia in patients with first acute ischemic stroke. *Cerebrovasc Dis Extra*. (2017) 7:21–34. doi: 10.1159/000457810

26. Abubakar SA, Jamoh BY. Dysphagia following acute stroke and its effect on short-term outcome. *Niger Postgrad Med J*. (2017) 24:182–6. doi: 10.4103/npmj.npmj_96_17

27. Suntrup-Krueger S, Kemmling A, Warnecke T, Hamacher C, Oelenberg S, Niederstadt T, et al. The impact of lesion location on dysphagia incidence, pattern and complications in acute stroke. Part 2: oropharyngeal residue, swallow and cough response, and pneumonia. *Eur J Neurol*. (2017) 24:867–74. doi: 10.1111/ene.13307

28. Labeit B, Mueller H, Muhle P, Claus I, Warnecke T, Dziewas R, et al. Predicting dysphagia with National Institute of Health Stroke Scale: distinction between infra-and Supratentorial region is essential. *Cerebrovasc Dis*. (2018) 46:150–8. doi: 10.1159/000493371

29. Hasan ZN, Al-shimmery EK, Taha MA. Evaluation of neurogenic dysphagia in Iraqi patients with acute stroke. *Neurosci J*. (2010) 15:90–6.

30. Stipancic KL, Borders JC, Brates D, Thibeault SL. Prospective investigation of incidence and co-occurrence of dysphagia, dysarthria, and aphasia following ischemic stroke. *Am J Speech Lang Pathol*. (2019) 28:188–94. doi: 10.1044/2018_AJSLP-18-0136

31. Ebrahimian Dehaghani S, Yadegari F, Asgari A, Bagheri Z. The mediator effect of cognition on the relationship between brain lesion location and dysphagia in patients with stroke: applying a structural equation model. *J Oral Rehabil*. (2019) 46:33–9. doi: 10.1111/joor.12722

32. De Cock E, Batens K, Hemelsoet D, Boon P, Oostra K, De Herdt V. Dysphagia, dysarthria and aphasia following a first acute ischaemic stroke: incidence and associated factors. *Eur J Neurol*. (2020) 27:2014–21. doi: 10.1111/ene.14385

33. Ko N, Lee HH, Sohn MK, Kim DY, Shin YI, Oh GJ, et al. Status of dysphagia after ischemic stroke: a Korean nationwide study. *Arch Phys Med Rehabil*. (2021) 102:2343–2352.e3. doi: 10.1016/j.apmr.2021.07.788

34. Otto DM, Ribeiro MC, Barea LM, Mancopes R, Almeida ST. Association between neurological injury and the severity of oropharyngeal dysphagia after stroke. *Codas*. (2016) 28:724–9. doi: 10.1590/2317-1782/20162015139

35. Souza JT, Ribeiro PW, de Paiva SAR, Tanni SE, Minicucci MF, Zornoff LAM, et al. Dysphagia and tube feeding after stroke are associated with poorer functional and mortality outcomes. *Clin Nutr*. (2020) 39:2786–92. doi: 10.1016/j.clnu.2019.11.042

36. Flowers HL, Silver FL, Fang J, Rochon E, Martino R. The incidence, co-occurrence, and predictors of dysphagia, dysarthria, and aphasia after first-ever acute ischemic stroke. *J Commun Disord*. (2013) 46:238–48. doi: 10.1016/j.jcomdis.2013.04.001

37. Dehaghani SE, Yadegari F, Asgari A, Chitsaz A, Karami M. Brain regions involved in swallowing: evidence from stroke patients in a cross-sectional study. *J Res Med Sci*. (2016) 21:45. doi: 10.4103/1735-1995.183997

38. Smithard DG, Smeeton NC, Wolfe CD. Long-term outcome after stroke: does dysphagia matter? *Age Ageing*. (2007) 36:90–4. doi: 10.1093/ageing/afl149

39. Beharry A, Michel P, Faouzi M, Kuntzer T, Schweizer V, Diserens K. Predictive factors of swallowing disorders and bronchopneumonia in acute ischemic stroke. *J Stroke Cerebrovasc Dis*. (2019) 28:2148–54. doi: 10.1016/j.jstrokecerebrovasdis.2019.04.025

40. Falsetti P, Acciai C, Palilla R, Bosi M, Carpinteri F, Zingarelli A, et al. Oropharyngeal dysphagia after stroke: incidence, diagnosis, and clinical predictors in patients admitted to a neurorehabilitation unit. *J Stroke Cerebrovasc Dis*. (2009) 18:329–35. doi: 10.1016/j.jstrokecerebrovasdis.2009.01.009

41. Rofes L, Muriana D, Palomeras E, Vilardell N, Palomera E, Alvarez-Berdugo D, et al. Prevalence, risk factors and complications of oropharyngeal dysphagia in stroke patients: a cohort study. *Neurogastroenterol Motil*. (2018) 30:e13338. doi: 10.1111/nmo.13338

42. Al-Khaled M, Matthis C, Binder A, Mudter J, Schattschneider J, Pulkowski U, et al. Dysphagia in patients with acute ischemic stroke: early dysphagia screening may reduce stroke-related pneumonia and improve stroke outcomes. *Cerebrovasc Dis*. (2016) 42:81–9. doi: 10.1159/000445299

43. Gandolfo C, Sukkar S, on the behalf of the Pre DyScore GroupCeravolo MG, Cortinovis F, Finocchi C, et al. The predictive dysphagia score (pre DyScore) in the short-and medium-term post-stroke: a putative tool in PEG indication. *Neurol Sci*. (2019) 40:1619–26. doi: 10.1007/s10072-019-03896-2

44. Hota S, Inamoto Y, Oguchi K, Kondo T, Otaka E, Mukaino M, et al. Outcomes of dysphagia following stroke: factors influencing oral intake at 6 months after onset. *J Stroke Cerebrovasc Dis*. (2021) 30:105971. doi: 10.1016/j.jstrokecerebrovasdis.2021.105971

45. Nilsson H, Ekberg O, Olsson R, Hindfelt B. Dysphagia in stroke: a prospective study of quantitative aspects of swallowing in dysphagic patients. *Dysphagia*. (1998) 13:32–8. doi: 10.1007/PL00009547

46. Baroni AFFB, Fábio SRC, Dantas RO. Risk factors for swallowing dysfunction in stroke patients. *Arq Gastroenterol*. (2012) 49:118–24. doi: 10.1590/s0004-28032012000200005

47. Mourão AM, Lemos SM, Almeida EO, Vicente LC, Teixeira AL. Frequency and factors associated with dysphagia in stroke. *Codas*. (2016) 28:66–70. doi: 10.1590/2317-1782/20162015072

48. Hess F, Foerch C, Keil F, Seiler A, Lapa S. Association of lesion pattern and dysphagia in acute intracerebral hemorrhage. *Stroke*. (2021) 52:2921–9. doi: 10.1161/STROKEAHA.120.032615

49. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z

50. Rostom A, Dubé C, Cranney A, Saloojee N, Sy R, Garritty C, et al. Celiac disease. *Evid Rep Technol Assess*. (2004) 104:1–6.

51. Holden BA, Fricke TR, Wilson DA, Jong M, Naidoo KS, Sankaridurg P, et al. Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. *Ophthalmology*. (2016) 123:1036–42. doi: 10.1016/j.ophtha.2016.01.006

52. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. (2002) 21:1539–58. doi: 10.1002/sim.1186

53. McAllister S, Kruger S, Doeltgen S, Tyler-Boltrek E. Implications of variability in clinical bedside swallowing assessment practices by speech language pathologists. *Dysphagia*. (2016) 31:650–62. doi: 10.1007/s00455-016-9724-8

54. Daniels SK, Anderson JA, Willson PC. Valid items for screening dysphagia risk in patients with stroke: a systematic review. *Stroke*. (2012) 43:892–7. doi: 10.1161/STROKEAHA.111.640946

55. Stoeckli SJ, Huisman TA, Seifert B, Martin-Harris BJ. Interrater reliability of videofluoroscopic swallow evaluation. *Dysphagia*. (2003) 18:53–7. doi: 10.1007/s00455-002-0085-0

56. Toscano M, Viganò A, Rea A, Verzina A, Sasso D'Elia T, Puledda F, et al. Sapienza global bedside evaluation of swallowing after stroke: the GLOBE-3S study. *Eur J Neurol*. (2019) 26:596–602. doi: 10.1111/ene.13862

57. Martino R, Silver F, Teasell R, Bayley M, Nicholson G, Streiner DL, et al. The Toronto bedside swallowing screening test (TOR-BSST): development and validation of a dysphagia screening tool for patients with stroke. *Stroke*. (2009) 40:555–61. doi: 10.1161/STROKEAHA.107.510370

58. Jannini TB, Ruggiero M, Viganò A, Comanducci A, Maestrini I, Giuliani G, et al. The role of the Sapienza GLObal bedside evaluation of swallowing after stroke (GLOBE-3S) in the prevention of stroke-associated pneumonia (SAP). *Neurol Sci*. (2022) 43:1167–76. doi: 10.1007/s10072-021-05449-y

59. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. *Stroke*. (2005) 36:2756–63. doi: 10.1161/01.STR.0000190056.76543.eb

60. Suntrup-Krueger S, Minnerup J, Muhle P, Claus I, Schröder JB, Marian T, et al. The effect of improved dysphagia care on outcome in patients with acute stroke: trends from 8-year data of a large stroke register. *Cerebrovasc Dis*. (2018) 45:101–8. doi: 10.1159/000487811

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Videofluoroscopy of the aerodigestive tract in *Phoca vitulina*: reshaping perspectives on translational medicine

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Thousands of rescued harbor seals (*Phoca vitulina*) require rehabilitation worldwide. Many require resource intensive gavage feeding due to abandonment soon after birth. Little is known about seal swallowing, therefore, our primary objective was to determine the feasibility of conducting videofluoroscopic swallowing studies (VFS) on seal pups prior to their release. Secondarily, we propose swallowing phase descriptions. We adapted a VFS approach used in humans and our feasibility parameters included: bolus detection and consumption, and number of analyzable swallowing events. Unrestrained seals were imaged in a dry environment using a Siemens mobile c-arm fluoroscopy unit. Oral boluses were thawed herring injected with liquid barium suspension (105% w/v). Two independent raters described swallows using a standardized approach with results summarized descriptively. We successfully completed freely-behaving VFS with two infant seals (1 male: 8 wks, 3 d; 1 female: 5 wks, 3 d). Both consumed five boluses with six fully analyzable swallowing events. We describe four swallow phases: preparatory, prehension, oropharyngeal and esophageal. Airway protection likely occurs in two ways: (1) during the preparatory phase through modified corniculate cartilage contact with the glottis and (2) with soft palate contact to the base of tongue prior to swallow initiation. We have conducted a unique VFS approach on rehabilitated seals, prior to their release. We have described airway protection and suggest that swallowing is initiated earlier in the feeding process than described previously. This protocol success will afford: (1) collection of normative swallowing data, and (2) future knowledge translation from humans to seals.

KEYWORDS

***Phoca vitulina*, videofluoroscopy, swallowing, rehabilitation, upper aerodigestive tract, airway protection**

1 Introduction

Over the past decade, thousands of infant harbor seals have been admitted to the Vancouver Aquarium's Marine Mammal Rescue Centre (MMR) and other rehabilitation facilities worldwide (1). From 2012 to 2020, the MMR successfully rehabilitated and released 1,017 individuals – 80% of their harbor seal admissions (1). In a retrospective review of necropsies following natural death after stranding, the highest cause of mortality in seal pups was a lack of nutrition, with sub-adults/adults dying primarily from infections secondary to bacterial pneumonia (2). With the ultimate goal to return to the wild (3), stranded seals receive life sustaining medical care, management, and rehabilitation. Successful rehabilitation efforts are a vital component of population recovery across many locations (4), contributing to endangered seal species recovery (5, 6), as in the Mediterranean and Hawaiian monk seal, and environmental stabilization (4). As many rescued seal pups and neonates cannot feed on their own, they require frequent gavage (tube feeding) to ensure adequate nutrition and efficient medication delivery (7). To safeguard seal and rehabilitation team welfare, gavage requires animal restraint during tube placement and throughout the feeding process. Once permanent dentition is fully functional at the age of 4–6 weeks (8, 9), reliance on tube feeding is decreased through gradual weaning and introduction of a whole fish (oral) diet.

Weight gain is a key predictor of survival both after weaning and on release, thus transition to oral intake (weaning) must be efficient and effective (7, 10). To maintain seal health, the progression to independent oral intake occurs over several weeks in a multi-step, gradual process. At the MMR, rehabilitation staff engage in a systematic step-wise program where harbor seals are hand-fed herring with varying degrees of oral and tactile stimulation used to support its retention within the oral cavity (7). Based on the progression of the seal, the weaning/feeding approach is adjusted according to the animal need. For example, early stages may involve inserting a small herring directly into the oral cavity while holding the muzzle closed until a swallow is triggered a few times a day, while optimizing nutrition by tube. Later stages may involve simulating water movement in the tank to draw attention to herring presence, mirroring naturally occurring circumstances in which prey would appear in the wild. Once the seals are able to feed independently, they are transitioned to communal tanks feeding with others prior to release. While this conventional rehabilitation approach has proved effective for many centers over the years (7, 11–13), it is invasive and resource intensive. Adaptations to the rehabilitation process (14) may be informed by thorough investigation of upper aerodigestive tract (UAT) anatomy and physiology, specifically understanding clinically relevant events during the oral, pharyngeal and esophageal swallowing phases.

The respiratory tract (larynx, trachea, bronchi, and lungs) evolved as an outgrowth of the foregut, and develops embryologically as a diverticulum from the endodermal gut tube. The larynx is the cranial most part of the lower airway and exists at the interface between the pharynx and trachea. It is not surprising that the major function of the larynx is to act as a valve, or sphincter, that prevents food and liquid from entering the airway. Closure of the laryngeal inlet and apposition of mucosal folds within the laryngeal cavity effectively block the entrance to the airway during swallowing. In mammals generally, the vocal folds on the lateral walls of the laryngeal inlet have been further modified to produce sounds as air courses between them. These sounds can be modulated by changes in the position of the larynx in the neck (15–17), by skeletal muscle activity that changes the length and tension of the folds, by the addition of accessory tissues that change the mass of the folds (18), and by structures in and around the oral cavity which modify resonance. In general, the larynx demarcates the transition from an oral (19) to a respiratory tract (20). In harbor seals, the larynx is positioned high in the neck (21) relative to humans, however the superior edge of the structure is not anchored into the nasopharynx by a skeletal muscle sphincter like it is in odontocete cetaceans (22). In our earlier work (21), we described potential laryngeal modifications which may play a role in swallowing in the harbor seal – "the arytenoid and corniculate cartilages together form prong-like projections directed forward to form the posterior aspects of the lateral margins of the laryngeal inlet (p. 3)." As a result, further analyses of our *in vivo* imaging was conducted to better understand how the larynx and other UAT structures function during swallowing in harbor seals.

Previous work in pinnipeds has focused on feeding methods (23–25), ways in which the prey is moved from the environment to the oral cavity (e.g., filter, biting, and suction). Often, swallowing physiology in the harbor seal has been inferred through external observation of anatomical movements (26). In order to develop innovative feeding and swallowing rehabilitation, regardless of species, understanding UAT swallowing physiology according to clinically relevant phases is imperative. For some mammals, swallowing research has focused on acquired dysphagia due to pathology (27, 28), animal models for human applications (29) and/or establishing species specific imaging protocols (30). In the area of feeding and swallowing rehabilitation, studies including harbor seals have explored environmental enrichment (31) with limited work on adaptive feeding devices (14). To the best of our knowledge, interventions focusing on bridging therapies when transitioning to oral intake from tube feeding, have yet to be systematically explored in this species. In human pediatric rehabilitative medicine, well-developed programs exist where bridging therapies are systematically used during tube feeding, and as the infant transitions to oral intake (32) – these approaches have yet to be investigated in pinnipeds but require an understanding of their swallow early in development. To the best of our knowledge, *in vivo* radiological, dynamic imaging of oral, pharyngeal and esophageal function has not yet been investigated in seals. As a result, how to optimize tube feeding and/or weaning processes are empirical and whether approaches used in human medicine could be leveraged for use in seals remains unknown. To explore this hypothesis and to further report on our innovative upper aerodigestive tract anatomical (21) and physiological (33) line of inquiry in harbor seals, our primary objective was to determine the feasibility of conducting videofluoroscopic swallowing studies on seal pups. Our secondary objective was to develop swallowing phase descriptions in the harbor seal through observation of the oral, pharyngeal and esophageal swallowing phases during videofluoroscopy.

2 Materials and methods

2.1 Animals

We conducted this study at the University of British Columbia (UBC) Centre for Comparative Medicine (CCM, Vancouver, BC,

Canada) in collaboration with the Vancouver Aquarium's Marine Mammal Rescue Centre (MMR). Prior to release to the wild, we imaged a convenience sample of rehabilitated harbor seal pups (*Phoca vitulina*) using videofluroscopy. These seals were able to self-feed and had no evidence of previous clinical history of feeding and/or swallowing difficulties. In addition to the principal investigator (SAS), other attending personnel included those for animal health and welfare: a veterinarian (MH), two veterinary technicians, and an imaging technician. This study was approved by the UBC Animal Care Committee (A18-0252) and personnel conducting the protocol completed animal welfare training and ethical research approaches as per UBC requirement.

2.2 Videofluoroscopy

A videofluroscopic swallowing study (VFS) is a dynamic imaging technique used in standard human clinical practice to assess swallowing (from oral cavity to the esophagus) and diagnose dysphagia (34). We adapted a VFS approach used in humans for use in seals. A Siemens mobile c-arm fluoroscopy unit and plastic translucent tub were utilized for imaging. Upon arrival at the CCM, the seals were kept in kennels typically used for their transport and in a quiet, dark space. Seals were lifted into the imaging tubs by their attending veterinarian team. Imaging was conducted in the lateral plane and the seals were unrestrained and able to behave freely. They were not anaesthetized or sedated. Once the seal was placed in the tub and moving freely, a 10-min period was afforded prior to imaging so that the seal could acclimatize and investigate their surroundings (Supplementary Figure A: Figure S1). Veterinarian staff monitored the seals for any signs of distress or discomfort throughout the imaging. All personnel conducting imaging were wearing protective lead equipment not limited to lead aprons, thyroid shields, gloves (MH), and eyewear.

The food boluses consisted of whole (previously flash frozen) herring, prepared with a barium suspension. Barium is a radiopaque contrast agent conventionally employed during videofluoroscopy in human (34, 35) and animal medicine (27, 30). After thawing but before presentation to the seal, we injected the herring gills and swim bladders with liquid barium suspension (Liquid Polibar Plus® Barium Sulfate Suspension, 105% w/v, E-Z-EM Canada Inc.). Excess barium on the surface of the fish was removed by disposable towels. The attending veterinarian (MH), presented the food bolus to the seal by hand at muzzle level. Throughout each videofluoroscopy, five boluses were delivered. The swallow was imaged at 30 frames per second with lossless digital capture. The view for each analyzable swallow event included the anterior lips to the proximal esophagus with panning to the lower esophageal sphincter as able.

2.3 Analysis

Two raters (HS, JV) blinded to each other described the structural movements and major deglutition events across four proposed phases adapted from human imaging: preparatory, oral, pharyngeal and esophageal. They evaluated swallow physiology frame-by-frame using TIMS™ DICOM review software (TIMS Medical and Foresight Imaging LLC). Following their rating, the two raters were unblinded and disagreements regarding interpretation were resolved by consensus. Confirmation of all findings was conducted by a third expert rater (SAS). Collectively, these raters have extensive experience with videofluoroscopic evaluation of swallowing including standardized rating training (35). We summarized the results descriptively.

3 Results

We successfully conducted freely-behaving videofluoroscopic swallowing studies on two infant seals (1 male: 8 wks, 3 d; 1 female: 5 wks, 3 d). Prior to videofluoroscopy, the male was in rehabilitation for 8 weeks and the female for 2 weeks and 3 days. For each seal, five boluses were administered (67–90 g each), with 10 (N) recorded swallowing events. Total fluoroscopy exposure time for each individual study was approximately 3 min which included lower gastrointestinal imaging (not reported here). Because of seal movement, six swallowing events were fully analyzable (three for each seal), with four partially analyzable. To meet our criteria for full analyses, the radiographic image had to contain a full view of the head (including lips) and proximal esophagus during the bolus presentation and swallow. Following our analyses, we delineated the following distinct but overlapping swallowing phases: preparatory, prehension, oropharyngeal and esophageal. Our phase titles were modified from that which we originally proposed based on the observed swallowing events.

3.1 Preparatory phase

The preparatory phase is characterized by two main events: (1) early airway closure and (2) base of tongue (BOT) positioning. Airway closure likely occurred as the boluses were brought toward the seal but prior to the bolus being secured and/or entering into the oral cavity (prehension – please see Supplemental B (Video S1) for slow motion video: Airway closure in seal during preparatory phase). The airway appeared to valve (close) at multiple anatomic levels prior to bolus entry into the oral cavity (Figure 1A). This closure occurred as the: (1) proximal aspect of modified corniculate cartilages appeared to deflect/invert and move rostrally to meet the glottal folds, (2) glottis and corniculates presumably medialize/close as both are bilateral structures (not directly observable in lateral radiographic imaging), and (3) base of tongue (BOT) retracts with depression of the soft palate, closing the caudal aspect of the oral cavity and the narrowing pharyngeal space (Figures 1A–C). Throughout this phase the epiglottis remains upright.

3.2 Prehension phase

In order to properly position the fish for subsequent swallowing phases, the bolus/prey must be secured and positioned for transport. Please see Supplementary Figure C: Figure S2 for a structural schematic overlaid on a fluoroscopic image from this phase. While the airway remains closed and the head angled toward the prey (Figure 2A), the jaw closes, securing the bolus (Figures 2B,C). No mastication occurs however biting occurs in order to position the

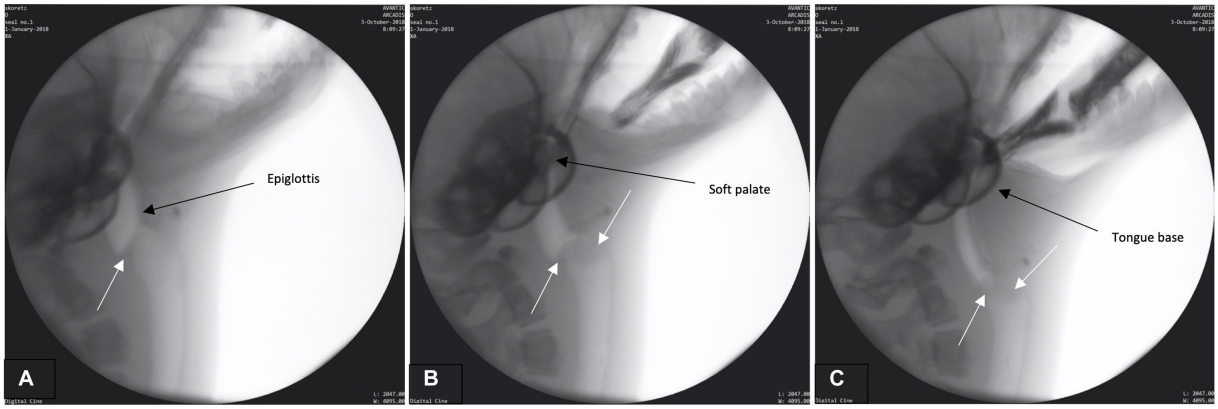

FIGURE 1
(A–C) Fluoroscopic images from the preparatory phase. White arrows indicate modified corniculate cartilage shown moving rostrally **(A)** and approximating glottis **(B,C)**. Other structures (i.e., epiglottis, soft palate, tongue base) also labeled.

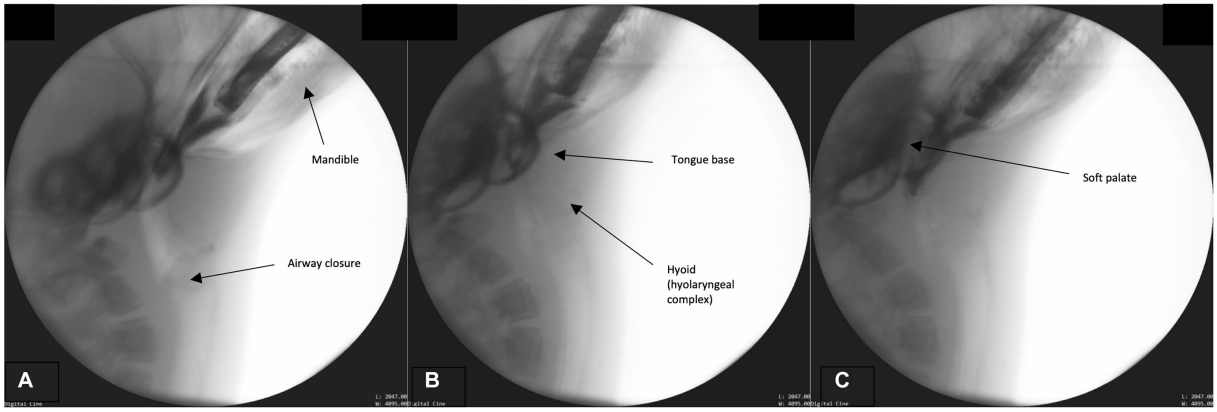

FIGURE 2
(A–C) Fluoroscopic images from the prehension phase. Key events for this phase include continued airway closure (approximation of the modified corniculate cartilages and glottis), mandible elevation, tongue base descent, hyoid (hyolaryngeal complex ascent), and soft palate elevation.

bolus head first in the oropharynx and contain it in the oral cavity. The BOT retracts fully into the pharynx and the lateral and posterior pharyngeal wall contracts resulting in maximum circumferential pharyngeal constriction as observed through the contact of the pharyngeal walls on the bolus (Figures 2B,C). Simultaneously, the hyoid bone, along with the larynx (hyolaryngeal complex), begins a rostral ascent under the tongue base, the soft palate begins elevation, and the epiglottis begins to retroflex or invert over the larynx.

3.3 Oropharyngeal phase

This phase primarily involves bolus transport from the oral cavity, through the pharynx and into the upper esophagus. Bolus transport occurs following BOT retraction, repeated rostral-caudal hyolaryngeal excursion, and pharyngeal wall contraction. To accomplish these maneuvers, the head tends to lower to a neutral rather than extended position. The hyoid and head engage in repeated rhythmical motion which cycles from maximal rostral-caudal hyolaryngeal ascent (Figure 3A) and then returns to rest in a more ventral position (Figure 3B). Simultaneously, pharyngeal constriction and BOT retraction are maintained, as evidenced by the lack of air space between these structures and the bolus (Figure 3C). This likely exerts force and pressure on the bolus propelling it toward the upper esophageal sphincter. As this occurs, the upper esophageal sphincter relaxes in order to accommodate the oncoming bolus (Figure 3C). Airway closure is maintained throughout this phase.

3.4 Esophageal phase

We marked the initiation of this phase when maximum upper esophageal sphincter distention occurs. The bolus passes whole through the distended sphincter into the esophagus, while the hyolaryngeal complex continues its cyclical ascent and descent (Figure 4A). Throughout, the pharynx remains contracted around the bolus until it passes through pharynx and fully enters the esophagus. As the bolus continues to move caudally through the esophagus and the fish tail leaves the sphincter (Figure 4B), the airway continues to remain closed and the

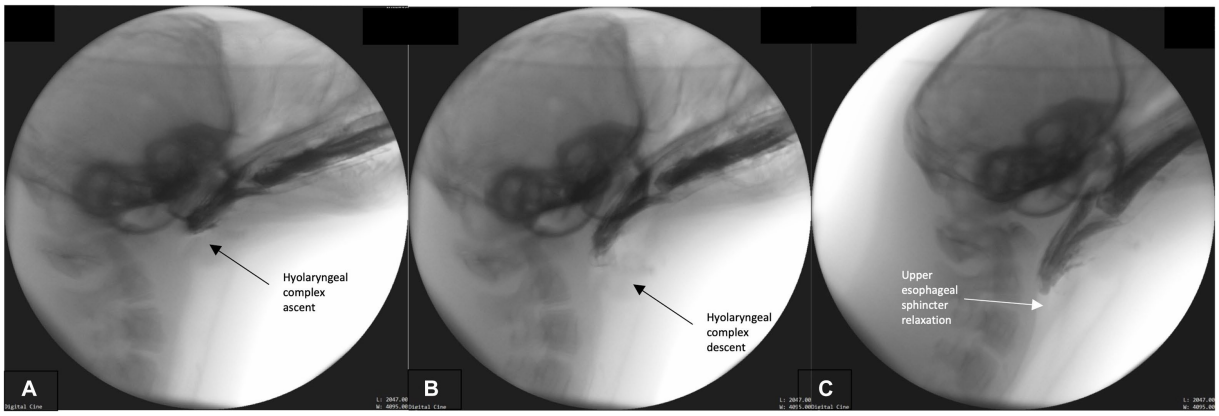

FIGURE 3
(A–C) Fluoroscopic images from the oropharyngeal phase. Rhythmical hyolaryngeal ascent **(A)** and descent **(B)** occur during this phase supporting pharyngeal transport of the bolus. Pharyngeal and tongue base contact on bolus is continual throughout this phase and upper esophageal sphincter relaxation commences **(C)**.

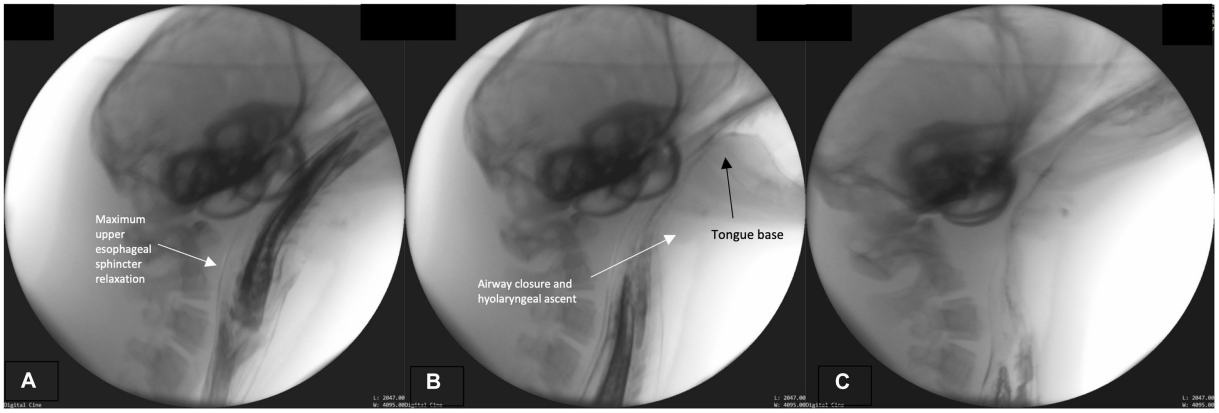

FIGURE 4
(A–C) Fluoroscopic images from the esophageal phase. The upper esophageal sphincter maximally relaxed (distended; **A**). The airway remains closed and tongue base retracted (on bolus tail; **B,C**).

epiglottis retroflexed (Figures 4A–C). Upper aerodigestive tract structures return to their rest position after the bolus has entered the distal esophagus. See Supplemental D (Video S2) for a sample harbor seal videofluoroscopic swallowing study.

4 Discussion

We have successfully conducted videofluoroscopic swallowing studies on soon-to-be released rehabilitated seal pups, offering a novel glimpse of swallowing physiology. In doing so, we have determined that *in vivo* swallowing imaging is feasible using our protocol, reporting our findings regarding seal swallowing physiology for the first time in their entirety. Furthermore, our preparation of whole herring injected with the barium contrast made the bolus readily observable throughout the seal digestive tract, allowing for deglutition event observations relative to bolus position. Not only was the oral bolus easily visible into the distal esophagus and stomach, the boluses were readily consumed by the seals. Additionally, we are the first to describe four functionally distinct swallow phases, furthering our understanding of airway protection during swallowing in independently feeding seals. In this paper, we have proposed how the paired, modified corniculate cartilages described anatomically in our earlier work (21) may be involved in airway protection during swallowing-as an auxiliary valving mechanism of the seal larynx during swallowing. It remains to be determined if this potential valving adaptation is unique to harbor seals, whether it is crucial in diving and/or if it is a common feature among pinnipeds.

We have taken an innovative perspective, elucidating similarities between harbor seal and human deglutition during swallowing. These include: (1) tongue base retraction and soft palate depression supporting bolus entry strictly to the oral cavity while closing off the pharynx, (2) pharyngeal constriction enabling bolus propulsion to the esophagus, and (3) hyolaryngeal complex elevation supporting airway protection and bolus transition from the oropharynx into the esophagus. While well known in other mammals (36), we are also the first to describe positional changes of the soft palate in harbor seals during swallowing – where the soft palate moves from contact with

the tongue base to an elevated position closing off the nasopharynx. With age-appropriate adaptations, this protocol would likely be suitable to gather the first normative swallowing data for neonatal, infant and juvenile seals. This information would enhance our understanding of swallowing physiology in seals, support the development of a cross species swallowing framework, and also provide opportunities to improve diagnostic capabilities and rehabilitation for these animals. Translational research involves bridging basic science to clinical research in order to advance human medicine (37). Although the contemporary focus is shifting from the use of animal models to leveraging biobanking and analysis of human tissue and other samples in "bench" science (38), the use of animal models to inform medical advances in animal (39) and human medicine is longstanding (37). While it is still unknown whether advances made in human medicine could be used in seals, we have emerging information to support a "reversal" in knowledge translation. Not only was this imaging approach successfully used for seals, but once more information is gathered on seal swallowing physiology, intervention approaches used in human medicine may also be translatable.

Although we are the first to describe swallowing in harbor seals using real time, *in vivo* radiographic imaging, others have: described swallowing in other mammals using videofluoroscopy (27–30) and developed predatory aquatic mammal feeding frameworks, with a primary focus on processes related to securing prey (23, 25) and generally applicable mammalian swallowing models (e.g., the Process Model) (40). Hocking and colleagues' proposed framework included (25): prey capture, prey manipulation and transport, prey processing, water removal, and swallowing. In this work, swallowing is described as the passage of food through the digestive tract, and specific to seals, transported by serial "gulping" (p. 5). Kienle and colleagues proposed a modified version of this framework (23) to provide more flexible application across aquatic mammals when compared to other tetrapods. The phases were as follows: ingestion, intraoral transport, processing, water removal, and swallowing. In this adapted framework, "ingestion encompasses all behaviors used to capture, subdue, kill and process prey before it enters the oral cavity" (p. 1). This previously published work also suggests that these phases may be modulated according to feeding event. Similarly, human swallowing also modulates, despite being described as partially reflexive (41, 42). Based on our current observations, we propose that swallowing events are initiated much earlier within these frameworks than previously described, and therefore, feeding and swallowing processes should be described as overlapping synergistic processes occurring simultaneously. Specifically, we observed airway "valving" (through the approximation of the modified corniculate cartilages and glottis; tongue base and soft palate contact) well before the prey was secured. This adaptation is crucial to the protection of the airway from aspiration, and may contribute to differential pressure generation to facilitate feeding processes (e.g., suction) (26) and swallowing processes (e.g., bolus transport). Specific to bolus transport, we recognize that prehension may vary depending on feeding circumstances, as a result, we propose a broad prehension definition to include specific feeding and/or positioning methods (e.g., grasping, crushing and/or suction) even though some actions were not directly observed (i.e., crushing) or measured (i.e., suction) during our study. The Process Model was designed to explain a variety of mammalian feeding behaviors, specifically for those who consume a variety of bolus texture types (i.e., liquid, semisolid, and solid), and assuming that solids need to be altered in order to be swallowed (40). Although harbor seals primarily consume their solid boluses whole, our observations align with the two transport types proposed in this model (40). Specifically, Stage I transport is described as when the bolus is "moved from the incisal area to the postcanine region (p. 417)" – which was observed during our prehension phase. Stage II transport occurs when the bolus is positioned and the swallow is initiated – aligning with our oropharyngeal phase.

In all terrestrial vertebrates, the lower respiratory tract develops early in gestation as an outgrowth of the primitive 'gut tube'. As a result, mammalian breathing and swallowing share a common pathway – the pharynx – which predisposes them to adverse outcomes (e.g., airway invasion/aspiration) should a food/liquid bolus be misdirected away from the esophagus and into the airways (43). The larynx and other AT structures engage in "valve-like" movements to facilitate this separation as the bolus leaves the oral cavity (44). In this study, we have observed the potential function of paired modified corniculate cartilages at the laryngeal inlet, which when oral intake is anticipated, approximate the glottis or vocal folds. This airway "valving" or covering of the laryngeal inlet was observed early in the "preparatory phase" during which the oral boluses were transported (or proffered) toward the seal and imaging equipment. Although our study was conducted using dry conditions without submersion of either the seal or oral bolus, we hypothesize that this adaptation, along with valving by the soft palate to tongue base, are early and pre-emptive mechanisms to protect the airway during submerged feeding, reaffirming our earlier anatomical descriptions (21). During the "prehension phase" of underwater feeding, if the seal opens the oral cavity without these protectionary valving mechanisms, the airway would be exposed to water influx. This same valving mechanism may also be engaged during deep dives as an adaptation to prevent aspiration of water into the airway under pressure. In humans, these movements include similar protective mechanisms: (1) tongue base to soft palate contact and then soft palate elevation/tongue base depression, protecting the airway and nasal passages, respectively, from oncoming bolus, and (2) anterior/superior hyolaryngeal displacement for airway closure including vocal fold and aryepiglottic folds medialization and epiglottic retroflection (44).

Although outside of the scope of our feasibility study, some investigators have described morphological adaptations for feeding (45, 46) while others have explored feeding kinematics including pressure measurements (26, 47, 48). Harbor seals have been reported to engage in both pierce (46) and suction feeding (26). Harbor seals also share cranial morphologic characteristics with other pinnipeds including harp seals, ribbon seals, and Ross seals (49), particularly "short (rostro-caudally) and narrow (medio-laterally) caudal portions of the skull, wide (medio-laterally) rostra, thickened (dorso-ventrally) palates" p. 402 (49). Mandibular girth (49) and specialized dentition (45) enable prey piercing or biting (49) however, harbor seals are also able to engage in suction feeding without specialized skull morphology or ability to generate relatively high subambient pressures as compared to other pinnipeds (50). In humans, pressure differentials occur during the swallow where the bolus moves from areas of higher pressure (e.g., in the oral cavity, pharynx) to areas of relatively lower (or subambient) pressure (e.g., the esophagus) (51). These pressure differentials are generated even out of the context of volitional sucking, in part due to increased lingual pressure placed on bolus and "valving"

of the base of tongue to soft palate during bolus preparation (52). When the bolus is compressed and ready for transport into the pharynx, the base of tongue lowers and soft palate raises, creating an area of lower pressure relative to that of the oral cavity, with pressure on the bolus increased again with pharyngeal contraction (51). Harbor seals engage in suction feeding despite the previously reported lack of unique cranial adaptations to do so (50). We hypothesize that suction may also be supported by "valving" through the creation of an area of relatively lower pressure, or subambient pressure specifically during: (1) early "valving" of the modified corniculate cartilage and glottis, and (2) soft palate depression and tongue base retraction closing off the pharynx. Once the prey is secured and oral transport initiated, the tongue base to palate "valve" is opened and an area of subambient pressure is generated – potentially creating, or supporting, suction.

Current interventions for feeding and swallowing in seals and humans have had different foci. In seals, feeding enrichment intervention supports foraging development (e.g., underwater feeding boxes) and swimming skills (e.g., floating kelp) (53). We are aware of only one controlled study (54) where clinical outcomes were compared between seals randomized to enrichment versus standard rehabilitation care. In that study, progression to independent feeding was not expedited in those receiving enrichment however, improved foraging was observed (31). Human infant feeding and swallowing rehabilitation include enrichment but also bridging therapies for those transitioning from tube feeding to oral intake (55). In a metanalyses of randomized controlled trials (56), preterm human infants receiving oral motor intervention had shorter transition time to full oral intake, shorter hospitalizations, more weight gain, and improved feeding efficiency. The incorporation of non-nutritive sucking along with oral motor interventions has also improved outcomes (32, 57). While facilitating spontaneous sucking is not a typical intervention focus in seals, some engage in spontaneous sucking behavior using their flipper or tub wall (58). Similarly some rooting behaviors were noted in the seals with stimulation to the external buccal area, as observed in human neonates (59). While investigations focused on developing oral feeding devices to support seal feeding and swallowing rehabilitation are few (11, 31) and their effectiveness is emerging, given the promising results in human preterm infants, translating these advances should be explored for seal rehabilitation.

By virtue of its feasibility design, this study has inherent limitations. We conducted the protocol with only two seals, and while we are confident that the observations are generalizable to other seals of similar age, we were not able to confirm its application to younger seals and/or those who are not feeding independently. Additionally, this study did not explore feeding strategies and/or kinematics of aerodigestive tract structures. Because of the novelty of this endeavor, we did not attach a known scalar to the seals during imaging. Doing so would have afforded us the opportunity to conduct structural movement analyses, documenting individual variations during swallowing. This information would not only contribute normative group data but also provide information on swallowing modulation in response to changes in environmental conditions (e.g., bolus size). Additionally, we did not conduct durational measures due to our small sample size as a larger data set would provide a more in-depth characterization of swallowing events. Our dry environment and the few analyzable swallows available also limited our study. As this study was to determine the feasibility of performing a videofluoroscopic swallowing study in seals, we elected not to include a submerged condition thereby limiting what we could explore about: (1) natural head positioning during prehension, (2) feeding or "ingestion" strategies, (3) the influence of suction on the swallow, and (4) how water is evacuated from the oropharynx prior to movement of the bolus into the esophagus. We are pursuing measures to explore an aquatic environment for videofluoroscopy as well as improve our movement tracking to increase the number of analyzable events. Finally, this study was completed on seal pups who were successfully rehabilitated and ready for release to the wild. It remains to be determined whether or not our physiological findings would be generalizable to free ranging seals. With the successful completion of this study and exploration of seal imaging techniques, we are well positioned to continue our mechanistic work.

Understanding how seals swallow while integrating airway protection provides further insight into mechanisms, adaptations, and biological modulation while determining translatable applications between terrestrial mammal and seal species. Future work should focus on detailed laryngeal imaging, kinematic movements of seal upper aerodigestive tract structures, comparisons of these structural movements during oral intake on land and underwater, and whether swallowing changes in seals during early development or at the time of weaning. This information will contribute to the development of dynamic multi-system assessment approaches and determine feasible rehabilitation translation between species, specifically compensatory processes to support an individual's resumption of 'natural' ways of eating. With thousands of abandoned seals admitted annually around the world for rehabilitation, future work will inform biomechanical interventions and determine optimal timing for their use. Regardless of species, protecting the lower airway from food/liquid will reduce adverse events and system burden while improving life quality – important during this time of limited health and human resources.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the University of British Columbia Animal Care Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. AA: Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. AV: Formal analysis, Methodology, Validation, Writing – review & editing. SR: Methodology, Validation, Writing – review & editing. MH: Investigation, Methodology, Project administration, Supervision, Writing – review & editing. HS: Formal analysis, Validation, Writing – review & editing. CD: Conceptualization, Formal analysis, Methodology, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1412173/full#supplementary-material

References

1. Pace CN, Haulena M, Drumm HE, Akhurst L, Raverty SA. Causes and trends of harbor seal (*Phoca vitulina*) mortality along the British Columbia Coast, Canada, 2012-2020. *J Wildlife Dis*. (2023) 59:629–39. doi: 10.7589/JWD-D-22-00172

2. Ashley EA, Olson JK, Adler TE, Raverty S, Anderson EM, Jeffries S, et al. Causes of mortality in a harbor seal (*Phoca vitulina*) population at equilibrium. *Front Mar Sci*. (2020) 7:319. doi: 10.3389/fmars.2020.00319

3. Osinga N. Harbour seals (*Phoca vitulina*) and rehabilitation. *NAMMCO Sci Publ*. (2010) 8:355–72. doi: 10.7557/3.2699

4. Reijnders P, Brasseur S, Ries EH, editors. The release of seals from captive breeding and rehabilitation programs: a useful conservation management tool. Rescue, rehabilitation, and release of marine mammals: an analysis of current views and practices. Proceedings of a workshop held in Des Plaines, Des Plaines, IL. (1996).

5. Lowry LF, Laist DW, Gilmartin WG, Antonelis GA. Recovery of the Hawaiian monk seal (*Monachus schauinslandi*): a review of conservation efforts, 1972 to 2010, and thoughts for the future. *Aquat Mamm*. (2011) 37:397–419. doi: 10.1578/AM.37.3.2011.397

6. Karamanlidis AA. Current status, biology, threats and conservation priorities of the vulnerable Mediterranean monk seal. *Endanger Species Res*. (2024) 53:341–61. doi: 10.3354/esr01304

7. MacRae A, Haulena M, Fraser D. The effect of diet and feeding level on survival and weight gain of hand-raised harbor seal pups (*Phoca vitulina*). *Zoo Biol*. (2011) 30:532–41. doi: 10.1002/zoo.20356

8. Meyer W, Matzke T. On the development of the deciduous teeth in the common seal (*Phoca vitulina*). *Mamm Biol*. (2004) 69:401–9. doi: 10.1078/1616-5047-00162

9. Kahle P, Ludolphy C, Kierdorf H, Kierdorf U. Dental anomalies and lesions in eastern Atlantic harbor seals, *Phoca vitulina vitulina* (Carnivora, Phocidae), from the German North Sea. *PLoS One*. (2018) 13:e0204079. doi: 10.1371/journal.pone.0204079

10. Zatrak M, Brittain S, Himmelreich L, Lovick-Earle S, Pizzi R, Shaw KJ, et al. Factors affecting the survival of harbor (*Phoca vitulina*) and gray seal (*Halichoerus grypus*) juveniles admitted for rehabilitation in the UK and Ireland. *Mar Mamm Sci*. (2023) 39:462–80. doi: 10.1111/mms.12983

11. MacRae A. Hand-rearing harbour seal pups (*Phoca vitulina*): The effect of diet and supplementary heat on growth and survival. Vancouver, BC: University of British Columbia (2009).

12. Cole J, Fraser D. Sink or swim: risk stratification of preweaning mortality in harbor seal pups (*Phoca vitulina richardii*) admitted for rehabilitation. *Mar Mamm Sci*. (2021) 37:807–25. doi: 10.1111/mms.12777

13. Hörst D. Caring for seals and the Wadden Sea: multispecies entanglements in seal rehabilitation. *Marit Stud*. (2021) 20:305–16. doi: 10.1007/s40152-021-00235-0

14. MacRae A. Investigating an alternative to gavage feeding for harbour seal pups (*Phoca vitulina*). Vancouver, Canada: International Association for Aquatic Animal Medicine (2010).

15. Weissengruber GE, Forstenpointner G, Peters G, Kubber-Heiss A, Fitch WT. Hyoid apparatus and pharynx in the lion (*Panthera leo*), jaguar (*Panthera onca*), tiger (*Panthera tigris*), cheetah (Acinonyxjubatus) and domestic cat (*Felis silvestris* f. catus). *J Anat*. (2002) 201:195–209. doi: 10.1046/j.1469-7580.2002.00088.x

16. Frey R, Volodin I, Volodina E, Soldatova NV, Juldaschev ET. Descended and mobile larynx, vocal tract elongation and rutting roars in male goitred gazelles (*Gazella subgutturosa* Guldenstaedt, 1780). *J Anat*. (2011) 218:566–85. doi: 10.1111/j.1469-7580.2011.01361.x

17. Fitch WT, Reby D. The descended larynx is not uniquely human. *Proc Biol Sci*. (2001) 268:1669–75. doi: 10.1098/rspb.2001.1704

18. Klemuk SA, Riede T, Walsh EJ, Titze IR. Adapted to roar: functional morphology of tiger and lion vocal folds. *PLoS One*. (2011) 6:e27029. doi: 10.1371/journal.pone.0027029

19. Brizuela M, Winters R. Histology, oral mucosa. Treasure Island, FL: StatPearls Publishing (2023).

20. Herbert RA, Janardhan KS, Pandiri AR, Cesta MF, Miller RA. Nose, larynx, and trachea. in ed. AW Suttie Boorman's pathology of the rat. Elsevier, Academic Press. (2018). 391–435.

21. Adams A, Vogl W, Dawson C, Raverty S, Haulena M, Skoretz SA. Laryngeal and soft palate valving in the harbour seal (*Phoca vitulina*). *J Exp Biol*. (2020) 223:jeb230201. doi: 10.1242/jeb.230201

22. Reidenberg JS, Laitman JT. Position of the larynx in odontoceti (toothed whales). *Anat Rec*. (1987) 218:98–106. doi: 10.1002/ar.1092180115

23. Kienle SS, Law CJ, Costa DP, Berta A, Mehta RS. Revisiting the behavioural framework of feeding in predatory aquatic mammals. *Proc Biol Sci*. (2017) 284:20171035. doi: 10.1098/rspb.2017.1035

24. Werth AJ. Adaptations of the cetacean hyolingual apparatus for aquatic feeding and thermoregulation. *Anat Rec*. (2007) 290:546–68. doi: 10.1002/ar.20538

25. Hocking DP, Fitzgerald EM, Salverson M, Evans AR. Prey capture and processing behaviors vary with prey size and shape in Australian and subantarctic fur seals. *Mar Mamm Sci*. (2016) 32:568–87. doi: 10.1111/mms.12285

26. Marshall CD, Wieskotten S, Hanke W, Hanke FD, Marsh A, Kot B, et al. Feeding kinematics, suction, and hydraulic jetting performance of harbor seals (*Phoca vitulina*). *PLoS One*. (2014) 9:e86710. doi: 10.1371/journal.pone.0086710

27. Pollard RE, Marks SL, Cheney DM, Bonadio CM. Diagnostic outcome of contrast videofluoroscopic swallowing studies in 216 dysphagic dogs. *Vet Radiol Ultrasound*. (2017) 58:373–80. doi: 10.1111/vru.12493

28. Lever TE, Braun SM, Brooks RT, Harris RA, Littrell LL, Neff RM, et al. Adapting human videofluoroscopic swallow study methods to detect and characterize dysphagia in murine disease models. *J Vis Exp*. (2015) 97:52319. doi: 10.3791/52319

29. Gould FDH, Mayerl CJ, Adjerid K, Edmonds C, Charles N, Johnson M, et al. Impact of volume and rate of milk delivery on coordination of respiration and swallowing in infant pigs. *J Exp Zool A Ecol Integr Physiol*. (2023) 339:1052–8. doi: 10.1002/jez.2754

30. Pollard RE. Videofluoroscopic evaluation of the pharynx and upper esophageal sphincter in the dog: a systematic review of the literature. *Front Vet Sci*. (2019) 6:117. doi: 10.3389/fvets.2019.00117

31. Chudeau KR, Johnson SP, Caine NG. Enrichment reduces stereotypical behaviors and improves foraging development in rehabilitating eastern Pacific harbor seals (*Phoca vitulina richardii*). *Appl Anim Behav Sci*. (2019) 219:104830. doi: 10.1016/j.applanim.2019.07.001

32. Li L, Liu L, Chen F, Huang L. Clinical effects of oral motor intervention combined with non-nutritive sucking on oral feeding in preterm infants with dysphagia. *J Pediatr*. (2022) 98:635–40. doi: 10.1016/j.jped.2022.02.005

33. Skoretz S, Haulena M, Akhurst L, Johnson E, Dawson C. *Phoca vitulina* swallowing physiology using videofluoroscopy: creating a comparative rehabilitation model. *FASEB J*. (2019) 33:33. doi: 10.1096/fasebj.2019.33.1_supplement.lb417

34. Logemann JA, Rademaker AW, Pauloski BR, Ohmae Y, Kahrilas PJ. Normal swallowing physiology as viewed by videofluoroscopy and videoendoscopy. *Folia Phoniatr Logop*. (1998) 50:311–9. doi: 10.1159/000021473

35. Martin-Harris B, Brodsky MB, Michel Y, Castell DO, Schleicher M, Sandidge J, et al. MBS measurement tool for swallow impairment—MBSImp: establishing a standard. *Dysphagia*. (2008) 23:392–405. doi: 10.1007/s00455-008-9185-9

36. Matsuo K, Palmer JB. Anatomy and physiology of feeding and swallowing: normal and abnormal. *Phys Med Rehabil Clin N Am*. (2008) 19:691–707, vii. doi: 10.1016/j.pmr.2008.06.001

37. Proudfoot AG, McAuley DF, Hind M, Griffiths MJ. Translational research: what does it mean, what has it delivered and what might it deliver? *Curr Opin Crit Care*. (2011) 17:495–503. doi: 10.1097/MCC.0b013e32834a4b19

38. Kinter LB, DeHaven R, Johnson DK, DeGeorge JJ. A brief history of use of animals in biomedical research and perspective on non-animal alternatives. *ILAR J*. (2021) 62:7–16. doi: 10.1093/ilar/ilab020

39. Quimby F. Contributions to veterinary medicine from animal research. *Appl Anim Behav Sci*. (1998) 59:183–92. doi: 10.1016/S0168-1591(98)00132-4

40. Hiiemae KM. CHAPTER 13-feeding in mammals In: K Schwenk, editor. Feeding. San Diego: Academic Press (2000). 411–48.

41. Martin-Harris B, Brodsky MB, Michel Y, Ford CL, Walters B, Heffner J. Breathing and swallowing dynamics across the adult lifespan. *Arch Otolaryngol Head Neck Surg*. (2005) 131:762–70. doi: 10.1001/archotol.131.9.762

42. Paydarfar D, Gilbert RJ, Poppel CS, Nassab PF. Respiratory phase resetting and airflow changes induced by swallowing in humans. *J Physiol*. (1995) 483:273–88. doi: 10.1113/jphysiol.1995.sp020584

43. Altman KW, Yu GP, Schaefer SD. Consequence of dysphagia in the hospitalized patient: impact on prognosis and hospital resources. *Arch Otolaryngol Head Neck Surg*. (2010) 136:784–9. doi: 10.1001/archoto.2010.129

44. Shaw SM, Martino R. The normal swallow: muscular and neurophysiological control. *Otolaryngol Clin N Am*. (2013) 46:937–56. doi: 10.1016/j.otc.2013.09.006

45. Churchill M, Clementz MT. Functional implications of variation in tooth spacing and crown size in pinnipedimorpha (mammalia: carnivora). *Anat Rec*. (2015) 298:878–902. doi: 10.1002/ar.23082

46. Adam PJ, Berta A. Evolution of prey capture strategies and diet in the Pinnipedimorpha (Mammalia, Carnivora). *Oryctos*. (2002) 4:3–27.

47. Marshall CD, Kovacs KM, Lydersen C. Feeding kinematics, suction and hydraulic jetting capabilities in bearded seals (*Erignathus barbatus*). *J Exp Biol*. (2008) 211:699–708. doi: 10.1242/jeb.009852

48. Marshall CD, Rosen DA, Trites AW. Feeding kinematics and performance of basal otariid pinnipeds, Steller Sea lions and northern fur seals: implications for the evolution of mammalian feeding. *J Exp Biol*. (2015) 218:3229–40. doi: 10.1242/jeb.126573

49. Kienle SS, Berta A. The better to eat you with: the comparative feeding morphology of phocid seals (Pinnipedia, Phocidae). *J Anat*. (2016) 228:396–413. doi: 10.1111/joa.12410

50. Kienle SS, Hermann-Sorensen H, Costa DP, Reichmuth C, Mehta RS. Comparative feeding strategies and kinematics in phocid seals: suction without specialized skull morphology. *J Exp Biol*. (2018) 221:jeb179424. doi: 10.1242/jeb.179424

51. Jones CA, Ciucci MR, Abdelhalim SM, McCulloch TM. Swallowing pressure variability as a function of pharyngeal region, bolus volume, age, and sex. *Laryngoscope*. (2021) 131:E52–8. doi: 10.1002/lary.28667

52. Hori K, Taniguchi H, Hayashi H, Magara J, Minagi Y, Li Q, et al. Role of tongue pressure production in oropharyngeal swallow biomechanics. *Physiol Rep*. (2013) 1:e00167. doi: 10.1002/phy2.167

53. Hunter SA, Bay MS, Martin ML, Hatfield JS. Behavioral effects of environmental enrichment on harbor seals (*Phoca vitulina concolor*) and gray seals (*Halichoerus grypus*). *Zoo Biol*. (2002) 21:375–87. doi: 10.1002/zoo.10042

54. Chudeau K. The physical and behavioral effects of enrichment in rehabilitating eastern Pacific harbor seals (*Phoca vitulina richardii*). San Marcos, CA: California State University San Marcos. (2017).

55. Mahoney AS, O'Donnell M, Coyle JL, Turner R, White KE, Skoretz SA. Non-pharmacological and non-surgical feeding interventions for hospitalized infants with pediatric feeding disorder: a scoping review. *Dysphagia*. (2023) 38:818–36. doi: 10.1007/s00455-022-10504-7

56. Chen D, Yang Z, Chen C, Wang P. Effect of oral motor intervention on oral feeding in preterm infants: a systematic review and meta-analysis. *Am J Speech Lang Pathol*. (2021) 30:2318–28. doi: 10.1044/2021_AJSLP-20-00322

57. Thabet AM, Sayed ZA. Effectiveness of the premature infant oral motor intervention on feeding performance, duration of hospital stay, and weight of preterm neonates in neonatal intensive care unit: results from a randomized controlled trial. *Dimens Crit Care Nurs*. (2021) 40:257–65. doi: 10.1097/DCC.0000000000000475

58. Dawson C, Haulena M, Skoretz SA. Creating a cross-species conceptual framework: an analysis of spontaneous sucking in wild seal pups following feeding and swallowing rehabilitation. *Dysphagia*. (2019) 34:996.

59. Rochat P, Hespos SJ. Differential rooting response by neonates: evidence for an early sense of self. *Infant Child Dev*. (1997) 6:105–12. doi: 10.1002/(SICI)1099-0917(199709/12)6:3/4<105::AID-EDP150>3.0.CO;2-U

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Timeline of hypoglossal motor neuron death and intrinsic tongue muscle denervation in high-copy number SOD1^{G93A} mice

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In amyotrophic lateral sclerosis (ALS) *postmortem* tissue and the SOD1 mouse model at mid-disease, death of hypoglossal motor neurons (XII MNs) is evident. These XII MNs innervate the intrinsic and extrinsic tongue muscles, and despite their importance in many oral and lingual motor behaviours that are affected by ALS (e.g., swallowing, speech, and respiratory functions), little is known about the timing and extent of tongue muscle denervation. Here in the well-characterised SOD1^{G93A} (high-copy) mouse model, we evaluated XII MN numbers and intrinsic tongue muscle innervation using standard histopathological approaches, which included stereological evaluation of Nissl-stained brainstem, and the presynaptic and postsynaptic evaluation of neuromuscular junctions (NMJs), using synapsin, neurofilament, and α-bungarotoxin immunolabelling, at presymptomatic, onset, mid-disease, and endstage timepoints. We found that reduction in XII MN size at onset preceded reduced XII MN survival, while the denervation of tongue muscle did not appear until the endstage. Our study suggests that denervation-induced weakness may not be the most pertinent feature of orolingual deficits in ALS. Efforts to preserve oral and respiratory functions of XII MNs are incredibly important if we are to influence patient outcomes.

KEYWORDS

amyotrophic lateral sclerosis, motor neurons, neuromuscular junctions, hypoglossal, tongue

Introduction

Death of corticospinal neurons and the motor neurons (MNs) that innervate skeletal muscle is the pathognomic feature of amyotrophic lateral sclerosis (ALS) (1, 2). In ALS, disruption of the neuromotor system and muscle denervation leads to weakness and eventual death by respiratory insufficiency within 3 years of diagnosis (3, 4). Hence, respiratory neuromotor impairments have a strong association with disease morbidity and mortality, with ALS having effects on ventilatory (breathing) and non-ventilatory (cough and sneeze) respiratory behaviours, as well as orolingual and aerodigestive behaviours including speech and swallowing (3, 5). All of these behaviours involve some aspects of tongue musculature (6–8). These deficits (including insufficient cough and dysphagia) result in an increased risk of airway obstruction and aspiration pneumonia (4).

In patients, hypoglossal (XII) MN loss is apparent in human postmortems (2). In the most widely used murine model of ALS, hSOD1^{G93A} (SOD1) hypoglossal MN death is observed at postnatal (P) day 90 (early-disease) and endstage (~P140) in SOD1 mice (9) and rats (10). Across multiple avenues of investigation, fast fatigueable (type FF) motor units, comprising larger MNs and higher force-producing type IIx/IIb muscle fibres they innervate are vulnerable to ALS (11–14). By contrast, slow and fast fatigue-resistant (type S and FR units, respectively) motor units, comprising smaller MNs innervating low-force producing type I and IIa muscle fibres, are relatively resilient. These findings are consistent with the survival of smaller XII MNs in *postmortem* patients (2) and murine brainstem (9). The pattern of FF-type MN death and muscle fibre denervation underpins the specific behavioural vulnerability of higher-force requiring behaviours (e.g., cough) observed in ALS (4).

At presymptomatic ages [prior to MN death (9)], XII MNs of SOD1 mice have altered intrinsic and extrinsic excitability (15) and dendritic abnormalities (16) that were exclusive to larger XII MNs (17). However, it is unknown whether this early pathophysiology is matched by presymptomatic changes in XII MN survival or tongue muscle innervation, as evident in *Tibialis anterior* motor units in these SOD1^{G93A} mice (18). In this study, we assessed XII MN survival and intrinsic tongue muscle innervation at presymptomatic, onset, mid-disease, and endstage. We hypothesise that there will be a loss of larger XII MN concomitant with tongue NMJ denervations at disease onset in SOD1^{G93A} mice.

Methods

Ethics approval and experimental animals

All procedures were performed in accordance with national and international guidelines and were approved by the University of Queensland Animal Ethics Committee. Female and male age and litter-matched wild-type (WT; $n = 25$ for the fixed brainstem histology and $n = 16$ for frozen tongue evaluations) and high-copy number heterozygote hSOD1^{G93A} (SOD1; $n = 24$ for the fixed brainstem histology and $n = 16$ for frozen tongue evaluations) were used at four pre-defined disease stages; presymptomatic (P30), onset (P70), mid-disease (P100–120), and endstage (P130–150) (16, 19). Endstage and humane endpoints for euthanasia were determined by a paralysis of the hindlimbs and the lack of righting reflex. Animals were obtained from our female WT x male SOD1 heterozygote breeding scheme, with pups genotyped and then randomly assigned to each experimental group. Animals were maintained in filtered cages under a 12:12-h light–dark cycle with *ad libitum* access to chow and water. In all experiments, mice were deeply anaesthetised with 60–80 mg/kg of pentobarbitone (Vetcare), with levels verified by the loss of palpebral and pain reflexes.

XII MN histology

Following deep anaesthesia, mice were intracardially perfused with phosphate-buffered saline pH 7.4 (PBS, 0.1 M) and then 4% paraformaldehyde in PBS. The brainstem was removed, sunk in 30% sucrose, subsequently frozen in liquid nitrogen, sectioned into 16 µm serial transverse cryosections, stained with 0.1% thionin (v/v in an acetic acid buffer), and imaged using a Zeiss Axioskop II microscope. XII MNs were quantified bilaterally with counts of every 5th section (20–22) with anatomical regions located with the aid of a brain atlas (23) and the 4th ventricle and central canal used as key landmarks. To estimate the XII MN volumes, the *x*, *y*, and *z* diameter of every 10th XII MN was provided for the volume of an ellipsoid.

NMJ assessment

Following deep anaesthesia, the tongue blade was removed, and 40 µm transverse vibratome sections were cut and stained for presynaptic and postsynaptic elements of the NMJ (24). Acetylcholine receptors (AChRs) were located using Alexa-555 conjugated α-bungarotoxin [BTX; diluted 1:500 in PBS; Sigma MO, USA], and motor nerve endings were located with rabbit anti-SV2 1 [1:50, Sigma] and anti-neurofilament [1:200, Sigma], with secondary Alexa-488 goat anti-rabbit [1:500, Invitrogen] as previously described (25). All antibodies were diluted in a blocking buffer. Intrinsic tongue muscles were imaged using a Zeiss LSM 900 confocal microscope using Airyscan detectors in multiplex 4y mode using a 63x oil objective N.A. 1.4. Approximately 25 tongue NMJs were imaged per mouse, across the proximal middle and distal regions of the tongue blade. The percentage overlap between presynaptic synapsin-NF and BTX-labelled postsynaptic AChRs was used to classify innervated, partially, or fully denervated muscle fibres (18).

Statistical analysis

Prism 9 was used for all analyses (GraphPad, Carlsbad, CA), which were performed blind to genotype. For all comparisons, two-way ANOVAs with Bonferroni *post-hoc* tests were performed, with statistical significance established at a p-value of <0.05. All data were reported as the mean ± 95% CI of the mean.

Results

Loss of bodyweight by endstage in SOD1 mice

Both female and male mice gained weight until mid-disease, where endstage SOD1 mice (19.3 ± 1.2 g, $n = 13$) weighed ~12% less than WT controls (22.1 ± 1.3 g, $n = 13$; $p = 0.0123$, unpaired t-test).

Loss of larger XII MNs in SOD1 mice

XII MN numbers were evaluated bilaterally in the entire XII nucleus in WT and SOD1 mice (Figure 1). XII MN loss was dependent on genotype ($F_{(1.41)} = 16.5$, $p = 0.0002$) and genotype–age interactions ($F_{(3.41)} = 4.0$, $p = 0.014$; two-way ANOVA), with significant reductions (~25%) at mid-disease (WT: 2247 ± 330, $n = 7$; SOD1: 1678 ± 336, $n = 6$; $p = 0.023$) and endstage (~41%; WT: 2236 ± 304, $n = 9$; SOD1: 1328 ± 294, $n = 8$; $p < 0.0001$) in SOD1 mice (Bonferroni post-tests; Figure 1B). No differences in XII MN number were apparent at

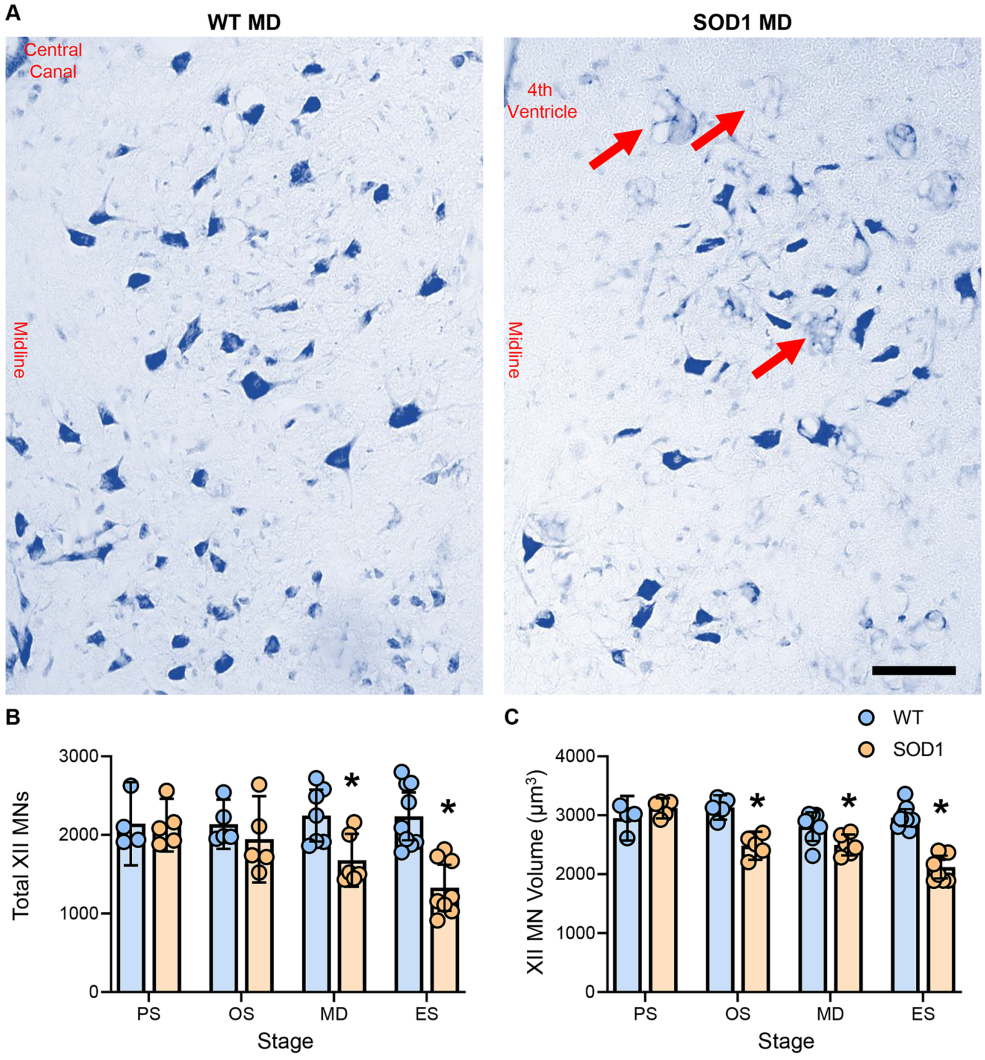

FIGURE 1
(A) Photomicrographs of the Nissl-stained brainstem and XII MNs in WT and SOD1 mice at mid-disease (MD). **(B)** Plot showing a reduced number of XII MNs in SOD1 mice at mid-disease and endstage (ES) but not presymptomatic (PS) or onset (OS). Note that the XII MN number is relatively stable until mid-disease, where ~25% of XII MNs have perished. Red arrows indicate vacuolated regions within the hypoglossal nucleus from mid-disease in SOD1 mice. **(C)** Scatterplot showing reduced XII MN volume in SOD1 mice from the onset. These somal changes are not the earliest alterations in XII MN morphology, but they are consistent with the resilience of type S and FR motor units. Two-way ANOVAs with Bonferroni post-tests, * indicates $p < 0.05$ between genotypes within an age group. Each symbol represents one mouse (i.e., the *n*). Scale bar = 100 µm.

presymptomatic (WT: 2144 ± 429, $n = 4$; SOD1: 2123 ± 336, $n = 5$; $p > 0.99$) or onset ages (WT: 2136 ± 314, $n = 5$; SOD1: 1945 ± 550, $n = 5$; $p > 0.99$; Bonferroni post-tests; Figure 1B). In SOD1 mice at mid-disease stages, extensive vacuolation of the hypoglossal nucleus was apparent, similar to that previously observed at endstage (26).

XII MN volume was dependent on genotype ($F_{(1.41)} = 45.0$, $p < 0.0001$) and genotype–age interactions ($F_{(3.41)} = 12.4$, $p < 0.0001$; two-way ANOVA), with significant reductions at onset (~21%; WT: 3132 ± 207 µm², $n = 5$; SOD1: 2484 ± 238 µm², $n = 5$; $p < 0.0001$), mid-disease (~11%; WT: 2808 ± 244 µm², $n = 7$; SOD1: 2498 ± 178 µm², $n = 6$; $p = 0.0386$), and endstage (~28%; WT: 2960 ± 145 µm², $n = 9$; SOD1: 2121 ± 194 µm², $n = 8$; $p < 0.0001$) in SOD1 mice (Bonferroni post-tests; Figure 1C). No difference in XII MN volume was apparent in presymptomatic mice (WT: 2950 ± 389 µm², $n = 4$; SOD1: 3120 ± 171 µm², $n = 5$; $p > 0.99$; Bonferroni post-test; Figure 1C).

Tongue NMJ denervation occurs subsequent to XII MN loss in SOD1 mice

Intrinsic tongue muscle NMJs were evaluated whole-mount within 40-µm transverse cryosections of the tongue blade (Figure 2). The % of NMJ endplates that were innervated was dependent on genotype ($F_{(1.24)} = 17.6$, $p = 0.0003$) and genotype–age interactions ($F_{(3.24)} = 7.1$, $p < 0.0014$; two-way ANOVA). We did not observe any denervated fibres in WT mice, and denervation was rare in SOD1 mice at presymptomatic (100% innervated), onset (~99% innervated), and mid-disease (~97% innervated). However, at endstage, there was an 11% reduction of innervated fibre SOD1 mice, compared to WT (SOD1: 89 ± 10%; $p < 0.0001$, Bonferroni post-test; $n = 4$ all ages/groups; Figure 2B).

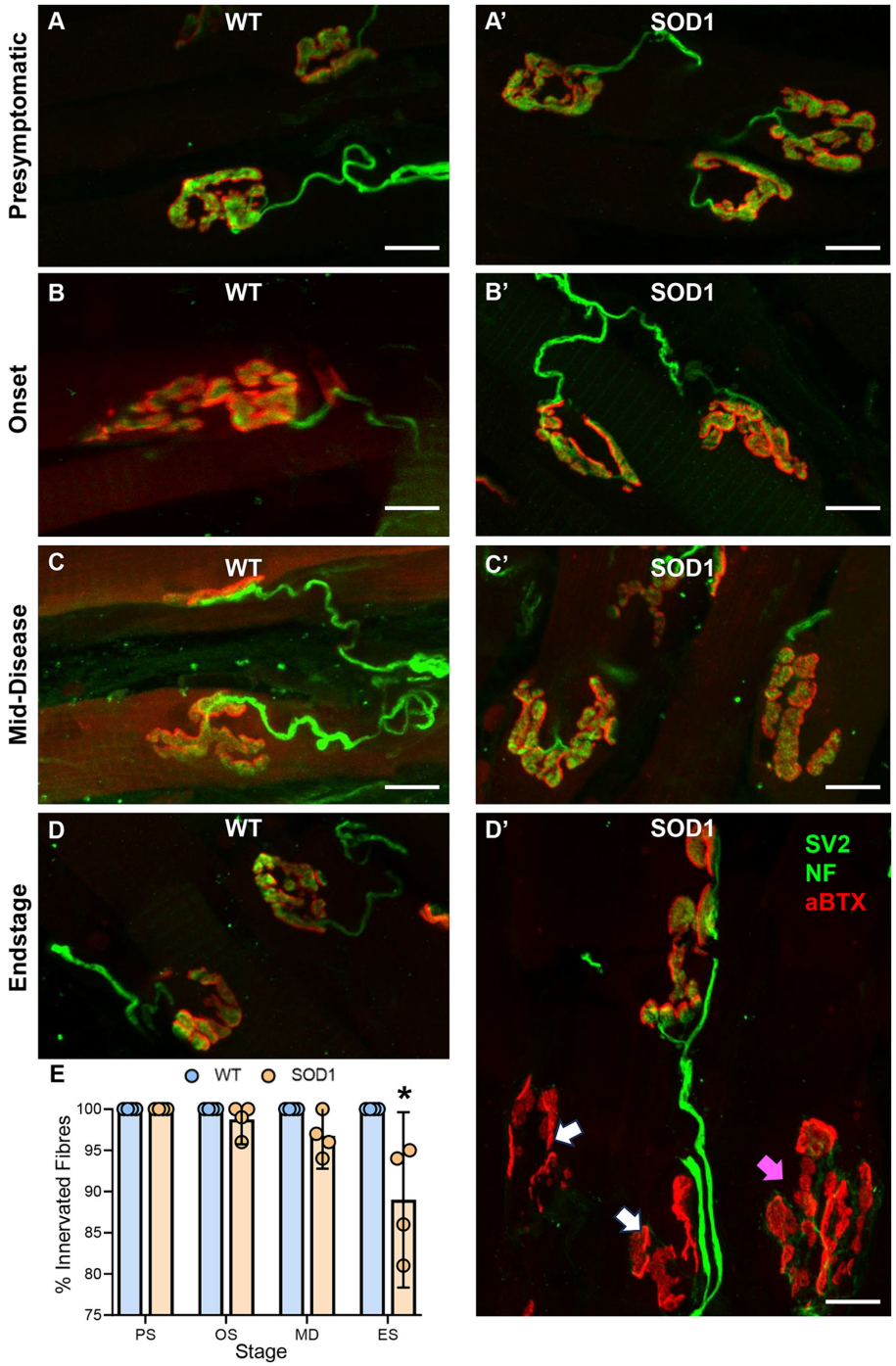

FIGURE 2
(A–D') Show flattened *z*-stack projections of NMJs from wild-type (WT, *left column* **A–D**) to aged match SOD1^{G93A} (SOD1, *right column*, **A'–D'**), at all stages of ALS-like disease progression, including, presymptomatic (PS), onset (OS), mid-disease (MD), and end-stage (ES). In panel **(D')**, white arrows indicate denervated endplates at ES, and purple arrows indicate partially denervated endplates. In all panels, presynaptic components of the NMJ (green) are labelled with antibodies to SV2 and neurofilament (NF), while postsynaptic acetylcholine receptors (red) are located with αBTX.
(E) Scatterplot shows a reduction in the percentage (%) of innervated muscle fibres in SOD1^{G93A} mice at endstage, following XII MN loss, with innervation unchanged at presymptomatic, onset, and mid-disease. Two-way ANOVA with Bonferroni post-tests. * indicates $p < 0.05$ between genotypes within an age group. Each symbol represents one animal (i.e., the *n*) with data based on ~25 NMJs per tongue. Scale bars = 10 μm.

Discussion

In the present study, we report the first longitudinal study of XII MN size-dependent survival and tongue blade innervation in SOD1 mice. This study has important implications regarding the interpretation of pathophysiological changes in young SOD1 XII MNs, and in relation to the natural history of MN death and denervation in SOD1 mice more generally. Notably, our current findings suggest that in the case of orolingual and aerodigestive behaviours, disordered neural networks altering the pattern of MN

activation may be a more salient feature of dysfunction than muscle denervation and weakness *per se,* as maximal tongue strength seems maintained in SOD1 rats (27) and when corrected for baseline values in SOD1 mice (28). For behaviours such as swallowing, where multiple brainstem MN pools are activated, swallow deficits occur in SOD1 mice (26, 29) and rats (27) in the absence of reduced nucleus ambiguus MN density counts (10), although stereological assessments of MN numbers and detailed assessment of the computational capacity of these nucleus ambiguus MNs have not been comprehensively assessed [see (30)]. How aberrant brainstem neural circuitry and synaptic transmission very early in the pathogenesis of SOD1 mice (15–17) influences orolingual and aerodigestive activities at later disease stages (26, 29) remains unclear. Exploring these nuances may yield sensitive diagnostic hallmarks and widen therapeutic windows.

Our findings of XII MN loss from mid-disease are not surprising given the prior studies in P90 and endstage SOD1 mice (9, 26). Here, we extend that finding by showing that XII MN loss is not apparent at younger ages, despite widespread presymptomatic functional and morphological changes (15–17). In a major advance, we show that at onset, the surviving XII MNs of SOD1 mice are smaller than their WT comparators, consistent with reduced volumes at mid-disease and endstage. This observation suggests that the larger MNs, likely comprising type FF motor units, are vulnerable to loss (2, 9, 12–14). Indeed, the major differences in XII MNs compared to lumbar MNs in SOD1 mice seem to be their capacity to compensate for their altered synaptic milieu which is evident in neonates (15).

In XII MNs, morphological changes consistent with lowered intrinsic MN activity (i.e., dendritic expansion) are present presymptomatically in the larger more vulnerable XII MNs (16, 17). By contrast, in the more rapidly degenerating lumbar MNs and corticospinal neurons, neuronal death is apparent presymptomatically or at onset (18, 31). Notably, corticospinal neurons and lumbar MNs do not exhibit neuroplastic changes consistent with compensation but display degenerative morphologies (16, 32, 33). This compensation is not related to the respiratory neuromotor system in general, as there are marked deficits in respiratory MNs and muscles in SOD1 models (34), including the tongue (35).

Despite evidence of XII MN loss (2, 9), degeneration (26), and a plethora of early pathophysiological changes (15–17), the integrity of tongue NMJs has not been evaluated in ALS. Here, we show that gross NMJ morphology is relatively well preserved in SOD1 mice, with pathology largely absent until endstage, similar to SOD1 rats (10, 27). Importantly, our NMJ assessments do not discriminate the dynamic re-innervation of tongue muscle fibres, where formerly denervated fibres are innervated by nascent axonal projections from surviving XII MNs or fibres that have been totally unaffected. This is a key caveat to our interpretations as it has been shown that dynamic structural and functional NMJ remodelling precedes MN death in the limb muscles of SOD1^{G93A} mice (36, 37). Perhaps most relevant to ALS are recent observations in muscle from early staged ALS patients who show type 1 fibre type grouping is evident during the onset of leg muscle weakness in ALS patients, supporting the idea of re-innervation by resilient type S MNs (11). These observations, along with the inclusion of some type I and IIa fibres in the mouse tongue (38), may confound our NMJ evaluations. Regardless, the timing of pathology, including XII MN loss occurring just prior to NMJ denervation, suggests that the complete denervation of NMJs may be a consequence, not a cause of MN deficits in SOD1^{G93A} mice, consistent with neonatal XII MN dysfunction in this ALS model (15).

In the respiratory neuromotor system, expulsive behaviours, such as cough and sneeze, requiring the activation of type FF motor units, are impaired in ALS patients (4), consistent with ALS pathology selectively afflicting larger MNs (2). For the tongue muscles, ballistic-type activations occur during swallowing, which is impaired in ALS (3). There are few ways to directly assess the maximal activations of individual intrinsic and extrinsic tongue muscles. Reduced uniaxial approaches (complicated by the complex interdigitation of tongue musculature) give unequivocal maximum specific forces at the cost of being *ex vivo*, while *in vivo* nerve stimulation may be confounded by NMJ or axonal pathology (6). Alternatively, behavioural tasks, such as licking and swallowing, altered in low-copy SOD1^{G93A} mice (29, 39), can be evaluated, although interpretations are couched, as the involvement of other muscle groups is required for these activities (particularly the nucleus ambiguus for swallowing). Regardless, these tongue deficiencies are also a likely cause of the poor grooming and coat conditions of SOD1 mice from both strains.

As ALS is an age-associated neurodegenerative condition, separating the ravages of age from the effects of the disease, particularly in animal models, is often overlooked. In rodents (20, 40–43) and humans (44, 45), ageing-related XII MN loss, tongue weakness, and impaired orolingual and aerodigestive behaviours are readily observed. An important goal is to uncover the precise pathophysiological phenomena differentiating ALS from old age sarcopenia (*cf* old age vigour, or healthy ageing). To this end, the low-copy number SOD1^{G93A} mice have been employed, with their onset of symptoms is delayed compared to the high-copy number strain used in the present study (46). Although swallowing and licking behavioural deficits are identified (29, 39) and smaller genioglossal muscle fibre cross-sectional areas are evident at endstage (39), corticospinal involvement has not been clarified [see (47)], and thus, the validity of this strain is unverified. Notably, the endstage in the low-copy strain occurs at ~240 days (29, 46), barely aged enough to be considered "young" in rodent ageing studies (48). Efforts to create animal models where the timing of abnormalities can be controlled (i.e., where MN death can commence in ages approaching higher risk in humans) are likely to be highly informative and are within our grasp (49–51).

In conclusion, we have novel results showing a relative resilience of tongue muscle to denervation in SOD1^{G93A} mice, and XII MN loss consistent with prior neural (9) and behavioural (26, 29, 52) assessments. These results are also largely in agreement with unaltered maximal licking force (27) and absent (27) to mild (10) evidence of tongue denervation in SOD1 rats. In addition, we show altered morphology of XII MNs, consistent with the vulnerability of type FF motor units occurring prior to statistically robust XII MN death and tongue denervation. Whether the relative delay in XII MN and tongue denervation compared to limb MNs and muscle denervation is related to the increased capacity of the XII MNs to effect adaptive compensatory changes, rather than early pathophysiological degenerations, is an exciting new avenue to pursue (53, 54). Therapeutic assessments designed to preserve MNs would do well to include aerodigestive behaviours, particularly of swallows evoked

using naturalistic methods [see (55, 56)], as there is major brainstem sensory degeneration in SOD1 mice (26). Due to their highly relevant implications for both disease morbidity and mortality, efforts assessing the bulbar manifestations of ALS, which occur even in limb-onset patients, are of great importance.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the University of Queensland Animal Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JD-T: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. MB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. PN: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Hardiman O, Van Den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat Rev Neurol*. (2011) 7:639–49. doi: 10.1038/nrneurol.2011.153

2. Kiernan JA, Hudson AJ. Changes in sizes of cortical and lower motor neurons in amyotrophic lateral sclerosis. *Brain*. (1991) 114:843–53. doi: 10.1093/brain/114.2.843

3. Garand KLF, Bhutada AM, Hopkins-Rossabi T, Mulekar MS, Carnaby G. Pilot study of respiratory-swallow coordination in amyotrophic lateral sclerosis. *J Speech Lang Hear Res*. (2022) 65:2815–28. doi: 10.1044/2022_JSLHR-21-00619

4. Tabor-Gray LC, Gallestagui A, Vasilopoulos T, Plowman EK. Characteristics of impaired voluntary cough function in individuals with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*. (2019) 20:37–42. doi: 10.1080/21678421.2018.1510011

5. Borges ALF, Velasco LC, Ramos HVL, Imamura R, Roldao P, Petrillo MVB, et al. Association between dysphagia and tongue strength in patients with amyotrophic lateral sclerosis. *Braz J Otorhinolaryngol*. (2022) 88:752–7. doi: 10.1016/j.bjorl.2020.10.015

6. Fogarty MJ, Sieck GC. Tongue muscle contractile, fatigue, and Fiber type properties in rats. *J Appl Physiol*. (2021) 131:1043–55. doi: 10.1152/japplphysiol.00329.2021

7. Sawczuk A, Mosier KM. Neural control of tongue movement with respect to respiration and swallowing. *Crit Rev Oral Biol Med*. (2001) 12:18–37. doi: 10.1177/10454411010120010101

8. Wealing JC, Cholanian M, Flanigan EG, Levine RB, Fregosi RF. Diverse physiological properties of hypoglossal Motoneurons innervating intrinsic and extrinsic tongue muscles. *J Neurophysiol*. (2019) 122:2054–60. doi: 10.1152/jn.00478.2019

9. Haenggeli C, Kato AC. Differential vulnerability of cranial Motoneurons in mouse models with motor neuron degeneration. *Neurosci Lett*. (2002) 335:39–43. doi: 10.1016/S0304-3940(02)01140-0

10. Kashlan ON, Kashlan BN, Oh SS, Mcginley LM, Chen KS, Kupfer R, et al. Histological bulbar manifestations in the ALS rat. *Neurodegener Dis*. (2015) 15:121–6. doi: 10.1159/000377725

11. Ding Q, Kesavan K, Lee KM, Wimberger E, Robertson T, Gill M, et al. Impaired signaling for neuromuscular synaptic maintenance is a feature of motor neuron disease. *Acta Neuropathol Commun*. (2022) 10:61. doi: 10.1186/s40478-022-01360-5

12. Dukkipati SS, Garrett TL, Elbasiouny SM. The vulnerability of spinal Motoneurons and Soma size plasticity in a mouse model of amyotrophic lateral sclerosis. *J Physiol*. (2018) 596:1723–45. doi: 10.1113/JP275498

13. Kaplan A, Spiller KJ, Towne C, Kanning KC, Choe GT, Geber A, et al. Neuronal matrix Metalloproteinase-9 is a determinant of selective neurodegeneration. *Neuron*. (2014) 81:333–48. doi: 10.1016/j.neuron.2013.12.009

14. Pun S, Santos AF, Saxena S, Xu L, Caroni P. Selective vulnerability and pruning of phasic Motoneuron axons in Motoneuron disease alleviated by CNTF. *Nat Neurosci*. (2006) 9:408–19. doi: 10.1038/nn1653

15. Van Zundert B, Peuscher MH, Hynynen M, Chen A, Neve RL, Brown RH Jr, et al. Neonatal neuronal circuitry shows Hyperexcitable disturbance in a mouse model of the adult-onset neurodegenerative disease amyotrophic lateral sclerosis. *J Neurosci*. (2008) 28:10864–74. doi: 10.1523/JNEUROSCI.1340-08.2008

16. Fogarty MJ, Mu EWH, Lavidis NA, Noakes PG, Bellingham MC. Motor areas show altered dendritic structure in an amyotrophic lateral sclerosis mouse model. *Front Neurosci*. (2017) 11:609. doi: 10.3389/fnins.2017.00609

17. Fogarty MJ, Mu EWH, Lavidis NA, Noakes PG, And Bellingham MC. Size-dependent dendritic Maladaptations of hypoglossal motor neurons in SOD1(G93A) mice. *Anat Rec*. (2020) 303:1455–71. doi: 10.1002/ar.24255

18. Ngo ST, Baumann F, Ridall PG, Pettitt AN, Henderson RD, Bellingham MC, et al. The relationship between Bayesian motor unit number estimation And histological measurements of motor neurons in wild-type And Sod1(G93A) mice. *Clin Neurophysiol*. (2012) 123:2080–91. doi: 10.1016/j.clinph.2012.01.028

19. Lee JD, Kamaruzaman NA, Fung JN, Taylor SM, Turner BJ, Atkin JD, et al. Dysregulation of the complement Cascade in the hSOD1G93A transgenic mouse model of amyotrophic lateral sclerosis. *J Neuroinflammation*. (2013) 10:119. doi: 10.1186/1742-2094-10-119

20. Fogarty MJ. Loss of larger hypoglossal motor neurons in aged Fischer 344 rats. *Respir Physiol Neurobiol*. (2023) 314:104092. doi: 10.1016/j.resp.2023.104092

21. Fogarty MJ, Smallcombe KL, Yanagawa Y, Obata K, Bellingham MC, Noakes PG. Genetic deficiency of GABA differentially regulates respiratory and non-respiratory motor neuron development. *Plos One*. (2013) 8:E56257. doi: 10.1371/journal.pone.0056257

22. Fogarty MJ, Yanagawa Y, Obata K, Bellingham MC, Noakes PG. Genetic absence of the vesicular inhibitory amino acid transporter differentially regulates respiratory and locomotor motor neuron development. *Brain Struct Funct*. (2015) 220:525–40. doi: 10.1007/s00429-013-0673-9

23. Paxinos G., And Franklin K.B.J. (2001). The mouse brain in stereotaxic coordinates. Academic Press. San Diego, CA, USA

24. Johnson A.M., And Connor N.P. (2011). Effects of electrical stimulation on neuromuscular junction morphology in the aging rat tongue. *Muscle Nerve* 43, 203–211, doi: 10.1002/mus.21819

25. Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie JP, et al. Defective neuromuscular synaptogenesis in Agrin-deficient mutant mice. *Cell*. (1996) 85:525–35. doi: 10.1016/S0092-8674(00)81253-2

26. Lever TE, Simon E, Cox KT, Capra NF, O'brien KF, Hough MS, et al. A mouse model of pharyngeal dysphagia in amyotrophic lateral sclerosis. *Dysphagia*. (2010) 25:112–26. doi: 10.1007/s00455-009-9232-1

27. Smittkamp SE, Spalding HN, Brown JW, Gupte AA, Chen J, Nishimune H, et al. Measures of bulbar and spinal motor function, muscle innervation, and mitochondrial function in ALS rats. *Behav Brain Res*. (2010) 211:48–57. doi: 10.1016/j.bbr.2010.03.007

28. Smittkamp S.E., Brown J.W., And Stanford J.A. (2008). Time-course and characterization of Orolingual motor deficits in B6SJL-Tg(SOD1-G93A)1Gur/J mice. *Neuroscience* 151, 613–621, doi: 10.1016/j.neuroscience.2007.10.017

29. Osman KL, Kohlberg S, Mok A, Brooks R, Lind LA, Mccormack K, et al. Optimizing the translational value of mouse models of ALS for dysphagia therapeutic discovery. *Dysphagia*. (2020) 35:343–59. doi: 10.1007/s00455-019-10034-9

30. Fogarty MJ. Dendritic morphology of motor neurons and interneurons within the compact, Semicompact, and loose formations of the rat nucleus Ambiguus. *Front Cell Neurosci*. (2024) 18:1409974. doi: 10.3389/fncel.2024.1409974

31. Ozdinler PH, Benn S, Yamamoto TH, Guzel M, Brown RH Jr, Macklis JD. Corticospinal motor neurons and related subcerebral projection neurons undergo early and specific neurodegeneration in hSOD1g(9)(3)A transgenic ALS mice. *J Neurosci*. (2011) 31:4166–77. doi: 10.1523/JNEUROSCI.4184-10.2011

32. Fogarty MJ, Noakes PG, Bellingham MC. Motor cortex layer V pyramidal neurons exhibit dendritic regression, spine loss, and increased synaptic excitation in the Presymptomatic hSOD1(G93A) mouse model of amyotrophic lateral sclerosis. *J Neurosci*. (2015) 35:643–7. doi: 10.1523/JNEUROSCI.3483-14.2015

33. Genc B, Jara JH, Lagrimas AK, Pytel P, Roos RP, Mesulam MM, et al. Apical dendrite degeneration, a novel cellular pathology for Betz cells in ALS. *Sci Rep*. (2017) 7:41765. doi: 10.1038/srep41765

34. Seven YB, Nichols NL, Kelly MN, Hobson OR, Satriotomo I, Mitchell GS. Compensatory plasticity in diaphragm and intercostal muscle utilization in a rat model of ALS. *Exp Neurol*. (2018) 299:148–56. doi: 10.1016/j.expneurol.2017.10.015

35. Smittkamp SE, Spalding HN, Brown JW, Yeh HW, Stanford JA. Relationships between tongue motility, grip force, and survival in SOD_1-G93A rats. *Physiol Behav*. (2014) 125:17–20. doi: 10.1016/j.physbeh.2013.11.010

36. Dobrowolny G, Martini M, Scicchitano BM, Romanello V, Boncompagni S, Nicoletti C, et al. Muscle expression of Sod1(G93A) triggers the dismantlement of neuromuscular junction via PKC-Theta. *Antioxid Redox Signal*. (2018) 28:1105–19. doi: 10.1089/ars.2017.7054

37. Rocha MC, Pousinha PA, Correia AM, Sebastiao AM, Ribeiro JA. Early changes of neuromuscular transmission in the SOD1(G93A) mice model of ALS start long before motor symptoms onset. *PLoS One*. (2013) 8:E73846. doi: 10.1371/journal.pone.0073846

38. Abe S, Maejima M, Watanabe H, Shibahara T, Agematsu H, Doi T, et al. Muscle-Fiber characteristics in adult mouse-tongue muscles. *Anat Sci Int*. (2002) 77:145–8. doi: 10.1046/j.0022-7722.2002.00019.x

39. Mueller M, Thompson R, Osman KL, Andel E, Dejonge CA, Kington S, et al. Impact of limb phenotype on tongue denervation atrophy, dysphagia penetrance, and survival time in a mouse model of ALS. *Dysphagia*. (2022) 37:1777–95. doi: 10.1007/s00455-022-10442-4

40. Glass TJ, Figueroa JE, Russell JA, Krekeler BN, Connor NP. Progressive protrusive tongue exercise does not alter aging effects in retrusive tongue muscles. *Front Physiol*. (2021) 12:740876. doi: 10.3389/fphys.2021.740876

41. Kletzien H., Hare A.J., Leverson G., And Connor N.P. (2018). Age-related effect of cell death on Fiber morphology and number in tongue muscle. *Muscle Nerve* 57, E29–E37, doi: 10.1002/mus.25671

42. Nagai H., Russell J.A., Jackson M.A., And Connor N.P. (2008). Effect of aging on tongue protrusion forces in rats. *Dysphagia* 23, 116–121, doi: 10.1007/s00455-007-9103-6

43. Sieck G.C., Hernandez-Vizcarrondo G.A., Brown A.D., And Fogarty M.J. (2023). Sarcopenia of the longitudinal tongue muscles in rats. *Respir Physiol Neurobiol* 319:104180. doi: 10.1016/j.resp.2023.104180

44. Crow H.C., And Ship J.A. (1996). Tongue strength and endurance in different aged individuals. *J Gerontol A Biol Sci Med Sci* 51, M247–M250, doi: 10.1093/gerona/51A.5.M247

45. Youmans SR, Youmans GL, Stierwalt JA. Differences in tongue strength across age and gender: is there a diminished strength reserve? *Dysphagia*. (2009) 24:57–65. doi: 10.1007/s00455-008-9171-2

46. Alexander GM, Erwin KL, Byers N, Deitch JS, Augelli BJ, Blankenhorn EP, et al. Effect of transgene copy number on survival in the G93A SOD1 transgenic mouse model of ALS. *Brain Res Mol Brain Res*. (2004) 130:7–15. doi: 10.1016/j.molbrainres.2004.07.002

47. Fogarty MJ. Driven to decay: excitability and synaptic abnormalities in amyotrophic lateral sclerosis. *Brain Res Bull*. (2018) 140:318–33. doi: 10.1016/j.brainresbull.2018.05.023

48. Sturrock RR. Stability of motor neuron and interneuron number in the hypoglossal nucleus of the ageing mouse brain. *Anat Anz*. (1991) 173:113–6.

49. Fogarty M.J., Dasgupta D., Khurram O.U., And Sieck G.C. (2023). Chemogenetic inhibition of TrkB Signalling reduces phrenic motor neuron survival and size. *Mol Cell Neurosci*, 125:103847, doi: 10.1016/j.mcn.2023.103847

50. Lind L.A., Murphy E.R., Lever T.E., And Nichols N.L. (2018). Hypoglossal motor neuron death via Intralingual CTB-Saporin (CTB-SAP) injections mimic aspects of amyotrophic lateral sclerosis (ALS) related to dysphagia. *Neuroscience* 390, 303–316, doi: 10.1016/j.neuroscience.2018.08.026

51. Nichols N.L., Vinit S., Bauernschmidt L., And Mitchell G.S. (2015). Respiratory function after selective respiratory motor neuron death from intrapleural CTB-Saporin injections. *Exp Neurol* 267, 18–29, doi: 10.1016/j.expneurol.2014.11.011

52. Lever TE, Gorsek A, Cox KT, O'Brien KF, Capra NF, Hough MS, et al. An animal model of Oral dysphagia in amyotrophic lateral sclerosis. *Dysphagia*. (2009) 24:180–95. doi: 10.1007/s00455-008-9190-z

53. Filipchuk A, Pambo-Pambo A, Gaudel F, Liabeuf S, Brocard C, Patrick Gueritaud J, et al. Early hypoexcitability in a subgroup of spinal motoneurons in superoxide dismutase 1 transgenic mice, a model of amyotrophic lateral sclerosis. *Neuroscience*. (2021) 463:337–53. doi: 10.1016/j.neuroscience.2021.01.039

54. Fogarty MJ. Neuronal Hypoexcitability and dendritic overbranching - the case for failed compensatory mechanisms in ALS Aetiology. *Neuroscience*. (2021) 465:231–2. doi: 10.1016/j.neuroscience.2021.02.034

55. Bolser DC, Shen TY, Musselwhite MN, Rose MJ, Hayes JA, Pitts T. Evidence for peripheral and central actions of codeine to dysregulate swallowing in the anesthetized cat. *Front Neurol*. (2024) 15:1356603. doi: 10.3389/fneur.2024.1356603

56. Frazure M, Morimoto I, Fielder N, Mellen N, Iceman K, Pitts T. Serotonin therapies for opioid-induced disordered swallow and respiratory depression. *J Appl Physiol*. (2024) 136:821–43. doi: 10.1152/japplphysiol.00509.2023

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Tongue exercise ameliorates structural and functional upper airway deficits in a rodent model of hypoglossal motor neuron loss

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Introduction: Tongue weakness and atrophy can lead to deficits in the vital functions of breathing and swallowing in patients with motor neuron diseases (MNDs; e.g., amyotrophic lateral sclerosis (ALS) and pseudobulbar palsy), often resulting in aspiration pneumonia, respiratory failure, and death. Available treatments for patients with MNDs are largely palliative; thus, there is a critical need for therapies targeting preservation of upper airway function and suggesting a role for tongue exercise in patients with MNDs. Here, we leveraged our inducible rodent model of hypoglossal (XII) motor neuron degeneration to investigate the effects of a strength endurance tongue exercise program on upper airway structure and function. Our model was created through intralingual injection of cholera toxin B conjugated to saporin (CTB-SAP) into the genioglossus muscle of the tongue to induce targeted death of XII motor neurons.

Methods: Rats in this study were allocated to 4 experimental groups that received intralingual injection of either CTB-SAP or unconjugated CTB + SAP (i.e., control) +/- tongue exercise. Following tongue exercise exposure, we evaluated the effect on respiratory function (via plethysmography), macrostructure [via magnetic resonance imaging (MRI) of the upper airway and tongue], and ultrafine structure [via ex vivo magnetic resonance spectroscopy (MRS) of the tongue] with a focus on lipid profiles.

Results: Results showed that sham exercise-treated CTB-SAP rats have evidence of upper airway restriction (i.e., reduced airflow) and structural changes present in the upper airway (i.e., airway compression) when compared to CTB-SAP + exercise rats and control rats +/- tongue exercise, which was ameliorated with tongue exercise. Additionally, CTB-SAP + sham exercise rats have evidence of increased lipid expression in the tongue consistent with previously observed tongue hypertrophy when compared to CTB-SAP + exercise rats or control rats +/- tongue exercise.

Conclusion: These findings provide further evidence that a strength endurance tongue exercise program may be a viable therapeutic treatment option in patients with XII motor neuron degeneration in MNDs such as ALS. Future directions will focus on investigating the underlying mechanism responsible for tongue exercise-induced plasticity in the hypoglossal-tongue axis, particularly inflammatory associated factors such as BDNF.

KEYWORDS

motor neuron disease (MND), breathing, respiration, dysphagia, degeneration, rat model

Introduction

Movement of the tongue is important for the vital functions of breathing and swallowing. The genioglossus muscle of the tongue is the primary active dilator of the upper airway during normoxia, as anterior movement of the genioglossus at the level of the epiglottis during inspiration helps maintain airway patency (1). Upper airway dysfunction in motor neuron diseases (MNDs; e.g., amyotrophic lateral sclerosis (ALS), spinobulbar muscular atrophy/Kennedy's disease and progressive bulbar palsy) involve complex processes leading to the loss of motor neurons that innervate the muscles of mastication, facial muscles, and/or muscles of the palate and pharynx/larynx (e.g., cranial nerves V, VII and IX/X respectively) (2). However, upper airway dysfunction in MNDs is predominantly thought to be attributed to degeneration of motor neurons (MNs) in the hypoglossal (XII) axis, including XII lower motor neurons (LMNs) that innervate the genioglossus muscle causing extensive tongue weakness, leading to impaired breathing and swallowing functions (3–6). Breathing and swallowing require reciprocal roles of the tongue during swallowing, and impaired control and coordination of these opposing behaviors can result in aspiration pneumonia, respiratory failure, and ultimately death (2, 7–9). Few studies have investigated therapeutic strategies to preserve breathing and/or swallowing function in MNDs. Thus, existing treatments are palliative and do not significantly improve functional outcomes and quality of life, which highlights the critical need for therapies aimed at preserving upper airway function. Studies focused on early detection have identified impaired tongue strength as an independent prognostic indicator of shorter survival in ALS (10, 11), suggesting that tongue exercise may play an essential therapeutic role in patients with MND.

Upper airway dysfunction in ALS models is difficult to study since the rate which hypoglossal motor neuron death occurs cannot be controlled, and degeneration is not limited to the hypoglossal nucleus. Thus, to study this, we utilized our validated rodent model with targeted loss of XII motor neurons, which provide the sole motor innervation to the tongue via the hypoglossal nerve. This model was created by intralingual injection of cholera toxin B conjugated to saporin (CTB-SAP) into the genioglossus muscle of the tongue base, which is transported retrogradely to XII motor neurons. Upon entering the XII LMN cell bodies, CTB-SAP dissociates and SAP is free to bind to ribosomes and inhibit protein synthetic machinery, resulting in apoptotic cell death (12, 13). Our previous investigations with this model revealed degenerative changes in the XII nerve (i.e., denervation atrophy) and genioglossus muscle [i.e., myofiber atrophy; (14)]. Additionally, *in vivo* magnetic resonance imaging (MRI) in this model revealed evidence of macrostructural changes in the brainstem (e.g., a trend for 4th ventricle enlargement) and tongue (e.g., significantly increased tongue volume and thickness, and marked hyperintensity of the tongue) consistent with potential muscle fiber inflammation, fatty replacement (possibly due to increased lipid concentration) of atrophied muscle fibers, and/or edema which were somewhat mitigated via tongue exercise in CTB-SAP rats (15).

The goal of the current study was to investigate whether tongue weakness and hypertrophy observed in this inducible model would result in evidence of decreased airflow (i.e., decreased peak inspiratory flow and increased inspiratory time) consistent with upper airway restriction, upper airway degenerative changes (i.e., compressed airway lumen), and ultrafine structural changes in the tongue (e.g., increased lipid profiles) that could be prevented by implementing targeted tongue exercise. To test this, we utilized our previously defined high-repetition/low-resistance (i.e., strength endurance) exercise paradigm designed for muscle growth (15), which consists of two non-consecutive overnight training sessions (Days 4 and 6 after intralingual injections).

We hypothesized that: (1) sham exercise-treated CTB-SAP rats would develop evidence of decreased airflow consistent with upper airway resistance (e.g., decreased peak inspiratory flow and mean inspiratory flow, and increased inspiratory time) during normoxic conditions at endline; (2) upper airway restriction in CTB-SAP rats during normoxia would be mitigated by tongue exercise; (3) lipid profiles in the tongue of CTB-SAP sham-exercise treated rats would be increased, consistent with fatty deposition of atrophied muscle fibers; (4) macrostructural and ultrafine structural degenerative changes in the tongue would be mitigated by tongue exercise in CTB-SAP rats; and (5) sham exercise-treated CTB-SAP rats would have degenerative changes in their upper airway (i.e., compressed airway lumen) consistent with evidence of upper airway restriction. These complementary studies may provide translationally relevant insight for early clinical indication of XII LMN degeneration. Overall, these findings help us understand the potential benefits of tongue exercise as a therapeutic strategy in patients with MND to preserve life-sustaining upper airway function and structure.

Materials and methods

Animals

Experiments were conducted on adult (3–4 months old) male Sprague Dawley rats (Envigo Colony 208a; Indianapolis, IN, United States). Rats were pair-housed in standard vivarium conditions (ambient temperature 20–26° C, humidity 30–70%, and standard 12:12 light:dark cycle). Animals had access to a standard commercial pelleted diet and water *ad libitum*, except during experimental testing (described below). Daily health monitoring and routine surveillance

for common rodent illnesses were performed by veterinary staff. All experimental procedures were approved by our Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals within our USDA-licensed and AAALAC-accredited academic institution.

Experimental procedures

Rats ($N = 50$) were randomly allocated to four experimental groups to study the effects of tongue resistance exercise on tongue-related structure and function. All rats received intralingual injection of either unconjugated CTB + SAP (i.e., control) or conjugated CTB-SAP, followed by exposure to either tongue exercise (i.e., treatment) or sham exercise (i.e., sham treatment), as described in detail below. Before tongue injections and after exercise/sham exercise exposure (i.e., 8 days after tongue injection), rats underwent behavioral testing to evaluate respiratory function via whole body plethysmography. At the study endpoint (i.e., 9 days after tongue injection), a subset of 13 rats underwent *in vivo* MRI of the upper airway to investigate corresponding structural changes under normoxic conditions that may correlate with behavioral findings. Additionally, *ex vivo* MRI and MR spectroscopy were performed on the tongues of a subset of rats ($N = 24$) to study macrostructural and ultrafine structural changes in the tongue, which included studying the metabolites of the tongue with a focus on lipid profiles. This study endpoint is the same as our previous studies with this model (14, 16), which was determined based on pilot data showing that other time points either did not result in dysphagia (4 days post tongue injection) or resulted in severe dysphagia (11–14 days post tongue injection) compromising animal welfare. All rats were euthanized on Day 9 using American Veterinary Medical Association (AVMA) approved methods. Specifically, rats were euthanized via exsanguination under deep isoflurane anesthesia by transcardial perfusion with cold PBS/saline (0.9% NaCl in 0.01 M sodium phosphate buffer, pH 7.0) followed by paraformaldehyde (in 0.1 M sodium phosphate buffer, pH 7.4) for collection of tissue for future histological analysis. In all cases, death was confirmed via bilateral pneumothorax where the chest cavity was opened, and lack of respiration and heartbeat were confirmed.

Intralingual injections to create an inducible rat model of selective hypoglossal motor neuron degeneration

Our rat model was created as previously described (14, 16). In brief, rats were anesthetized via 5% isoflurane in an induction chamber and immobilized in ear bars in the supine position on a custom-built tilt table to stabilize the head during tongue injections. For the remainder of the procedure, isoflurane (2–3% via 1–1.5 L/min oxygen) was delivered via nose cone to extinguish hindlimb and jaw reflexes. The jaw was gently held open by a custom-built weighted pulley-mechanism looped around the mandibular incisors for unobstructed access to the tongue. Under light guidance (LED Stereotactic Light #59290, Stoelting; Wood Dale, IL, United States), fine forceps were used to gently grasp and lift the tongue for visualization of the frenulum, which was the anatomical landmark for targeted injection into the midline genioglossus muscle in the tongue base. Each rat received a single "control" injection (20 µg CTB + 25 µg SAP; unconjugated CTB + SAP) or CTB-SAP injection (25 µg of CTB conjugated to SAP) using a 50 µl Luer tip syringe (Microliter #705, Hamilton; Reno, NV, United States) and 26-gauge Luer lock needle (26G 3/8 Becton, Dickinson and Company, Franklin Lakes, NJ, United States). The needle was angled at 45-degrees during insertion into the midpoint of the frenulum, with half of the bolus delivered at ~8 mm depth (i.e., near maximum needle insertion) and the remainder at ~4 mm (i.e., half the needle insertion depth) during needle retraction. All injections were performed by the same investigator for replicability of results. Rats were recovered from anesthesia (typically within 10 min after induction) and closely monitored for several hours to ensure resumption of food and water intake before being returned to standard vivarium conditions and daily health monitoring.

Whole-body plethysmography to assess respiratory function

Respiratory function was evaluated in CTB-SAP rats and controls rats (CTB + SAP) +/− tongue exercise via plethysmography by placing the rats in a whole-body plethysmograph (Data Sciences International/Harvard Bioscience, Holliston, MA) and altering the mixture of inspired gases (gas concentrations controlled by a gas mixer, CWe, Inc., Ardmore, PA). Testing was conducted on all rats prior to tongue injections (i.e., baseline) and on day 8 following tongue injections (i.e., endline). Baseline respiratory function was established by first exposing the animals to 30 min of normoxia. All rats were then challenged by exposing them to 5 min. of a hypoxic + hypercapnic gas mixture (10.5% O_2, 7% CO_2, balanced N_2; maximum chemoreceptor stimulation, max). A pressure calibration signal, ambient pressures, and chamber pressures were utilized for automated calculation of breath-by-breath respiratory parameters [frequency (f), inspiratory time (TI), expiratory time (TE), tidal volume (VT), minute ventilation ($\dot{V}E$), peak inspiratory flow (PIF) and peak expiratory flow (PEF)] at 10-s intervals using FinePointe Software (Data Sciences International/Harvard Bioscience, Holliston, MA). VT, $\dot{V}E$, and mean inspiratory flow (VT/TI) were normalized to body weight (per 100 g). Data were rejected in rare instances of pressure fluctuations caused by gross body movements. Additionally, the apnea detection function within FinePointe software was used to identify an additional outcome measure, total number of apneas, that were defined as the absence of at least two inspirations (i.e., a pause in breathing 2x the normalized breath duration threshold). The automatically detected apneas were manually reviewed to verify accuracy and exclude rare instances of automated event detection errors, for example, if two shallow breaths were detected as an apnea rather than two individual breaths.

Tongue exercise paradigm

Prior to tongue injections, force-lickometer testing was performed to determine the baseline maximum voluntary lick force (MVLF) and corresponding personalized tongue exercise intensity level for each rat (15). Testing entailed individually enclosing rats in a custom lickometer chamber (clear polycarbonate, 25 cm long × 8 cm wide × 15 cm high) positioned on a custom vertical lift platform within a modified force-lickometer system (Force Lickometer for Rat, Med Associates; Fairfax, VT, United States). A custom lickometer spout (i.e., a funnel with an adjustable force, double ball-bearing spout) was filled with a 30% sucrose solution to motivate participation, and the lick-force threshold to obtain liquid from the spout was set to

≤4 grams to mimic the negligible lick-force requirement of our vivarium's standard double ball-bearing waterspouts. Additionally, a webcam (V-u0018, Logitech; Newark, CA, United States) was positioned inside the lickometer system for continuous close-up visualization of the spout. As each rat drank, tongue contact against the spout was captured (via PowerLab data acquisition device; ADInstruments; Colorado Springs, CO, United States) for real-time visual display and recording (via LabChart software; ADInstruments) of tongue force in grams (g) in synchrony with the webcam video stream. Each rat was recorded for approximately 5 min to obtain multiple drinking bouts for MVLF analysis. Offline, the best representative 3–5 drinking bouts (i.e., continuous drinking with visible tongue contact against the spout) per rat were manually identified for automated quantification (via LabChart) of peak-to-peak amplitude (g) for individual licks. The 10 highest lick-force values across the multiple bouts were averaged to obtain each rat's MVLF, which was used to determine the individualized exercise program for each rat as described below.

All rats participated in our established strength endurance tongue exercise paradigm on Day 4 and Day 6 post-tongue injection (15), which necessitated single housing for the remainder of the study to ensure a personalized medicine approach. On both days, a custom exercise spout (i.e., resisto-spout) containing 30% sucrose solution was placed in the home cage of individually housed rats for 12 h overnight (i.e., 8:00 PM–8:00 AM), coinciding with peak activity (including food/water consumption) in these nocturnal rodents. The spout was customized with a manually adjustable tension spring mechanism with a force range of ~2–50 g to accommodate sham exercise (≤4 g) and exercise (~20–35 g, based on unpublished pilot testing) conditions. Each resisto-spout force setting was manually calibrated immediately prior to use with one of two analog tension force meters: low range (0–10 g; model GD-1, Jonard Tools, Elmsford, NY United State) for sham exercise or high range (0–50 g; model GD-5, Jonard Tools) for exercise.

For rats in the two exercise groups (i.e., control + exercise and CTB-SAP + exercise), the resisto-spout was set to 50% greater than baseline MVLF (i.e., 50% > MVLF) throughout the overnight exercise period. For example, a rat with a MVLF of 20 g would have the resisto-spout set to 30 g during exercise. For the two sham exercise groups (i.e., control + sham exercise and CTB-SAP + sham exercise), the resisto-spouts were set to ≤4 grams, consistent with the negligible force setting used during force-lickometer testing, as conducted previously. Rats had free access to standard food pellets and enrichment materials during exercise/sham exercise training in the home cage; thus, the only cage-level difference was the substitution of the resisto-spout bottle (containing 30% sucrose solution) for the standard vivarium water bottle.

Ex vivo magnetic resonance imaging and spectroscopy of the tongue

Ex vivo magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) was performed on a subset of rat tongues ($N = 19$) using a 7 T Bruker AVANCE III BioSpec MRI scanner (Bruker BioSpin Inc., Billerica, MA) equipped with a mouse brain CryoProbe (Bruker Biospin). On day 9 following tongue injections, rats were transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH~7.4) and the tongues were harvested. *Ex vivo* tongue T2-weighted rapid acquisition relaxation enhanced (RARE) sequence was performed with the following acquisition parameters: 29 axial slices, TE/TR: 23/1,784.52 ms, 256 × 256 image size, 14 × 14 field of view (FOV), and 0.7 mm slice thickness with 0.055 × 0.055 mm resolution. Single voxel ($2.73 \times 1.33 \times 3\,\text{mm} = 10\,\text{mm}^3$) localized MR spectroscopy was performed using a Point RESolved Spectroscopy (PRESS) sequence with TE/TR: 18/2,500 ms, 64 averages and 0.81 Hz/points spectral resolution in the lymphoid nodules in the tongue. Data was analyzed using Paravision7.0 (Bruker) and LCModel (17). All lipid peaks were fitted using the LCModel and normalized to the lipid peak at 1.3 ppm (Lip 1.3). We choose to normalize to Lip 1.3 because it is the most abundant and stable peak among the lipid contents.

In vivo magnetic resonance imaging of the upper airway

In vivo MRI was performed on a subset of rats ($N = 18$) using a 7 T Bruker AVANCE III BioSpec MRI scanner (Bruker BioSpin Inc., Billerica, MA) and a four-element phased-array radiofrequency (RF) rat cardiac coil. Rats were anesthetized with 1.0–3.5% isoflurane (1–1.5 L/min in oxygen) via a nose cone, placed in ventral recumbency, and studied under eupneic conditions. A physiological monitoring and gating system (SA Instruments, Inc.) was used to monitor vital signs and for triggering respiratory-gated MRI scans. Body temperature was maintained at 36–37°C with warm air circulating in the magnet bore. T1-weighted MRI sagittal and axial scans of the upper airway lumen were performed with fat-suppression using a FLASH (Fast Low Angle Shot) sequence. Respiratory gating was used to trigger each scan to acquire images at the expiratory or inspiratory phase, respectively. Other imaging parameters included: 5 sagittal slices and 21 axial slices, slice thickness – 0.8–1.0 mm, in-plane resolution 111 × 156 μm, TR (repetition time) – 158.6 ms, TE (echo time) – 1.482 ms, and 6 averages. Image analysis and processing were performed using ParaVision 7 software (Bruker Biospin Corporation, 2020). Axial slices were used to measure the cross-sectional areas of the upper airway lumen. Both axial and sagittal slices were utilized for airway lumen cavity comparisons. Segmentation of the upper airway volume was performed using Segment (Medviso AB, Lund, Sweden), starting from one slice below the junction of the hard and soft palate to the slice at the root of the tongue.

Statistical analysis

Investigators involved with data collection were blinded to experimental group assignment. Data outliers for each variable were identified and re-checked for accuracy, but not removed from the dataset. The following dependent variables were assessed: (1) Plethysmography: frequency (f; breaths/min.), inspiratory time (TI; sec), expiratory time (TE; sec), tidal volume (VT/100 g; ml/100 g), minute ventilation ($\dot{V}$E/100 g; ml/min./100 g), mean inspiratory flow (VT/TI/100 g; ml/s/100 g), peak inspiratory flow (PIF; ml/s), peak expiratory flow (PEF; ml/s), and number of apneas (#); (2) MR spectroscopy: calculated lipid ratio (Lip2.1 + Lip2.3) normalized to the most abundant and stable peak among the lipid concentrations (Lip1.3); and (3) *in vivo* MRI of the upper airway: airway volume (mm^3). Averaged values for each rat were used for plethysmography, and raw/unaveraged data were used for MR spectroscopy (i.e., single value per rat) and *in vivo* MRI of the upper airway. For plethysmography, separate two-way repeated measures ANOVAs were performed using SigmaPlot 15.0 (Systat Software, San Jose, CA) to compare the: (1) effect of time (repeated measure at baseline and endline), treatment groups (Control + sham exercise,

Control + exercise, CTB-SAP + sham exercise, and CTB-SAP + exercise) and time×treatment group interactions on each outcome measure at each gas level (normoxia and max); and (2) effect of gas level (repeated measure at normoxia and max), treatment groups and level×treatment group interactions on each outcome measure at each timepoint (baseline and endline). For MR spectroscopy and *in vivo* MRI studies, a Students *t*-test was used to compare the relative concentration of Lip2.1 + Lip2.3 with respect to the Lip1.3 peak and differences in airway volume (mm^3) between groups, respectively. If significant differences were indicated, multiple comparisons were made using a Fisher's LSD *post hoc* test. Differences between groups were considered significant if $p < 0.05$, and all values were expressed as means ± 1 SEM.

Results

Intralingual CTB-SAP causes upper airway restriction that is decreased with tongue exercise

Figure 1 depicts differences in breathing parameters [e.g., inspiratory time (TI), mean inspiratory flow (VT/TI/100 g), peak inspiratory flow (PIF), and peak expiratory flow (PEF)] for the four experimental groups at baseline and endline timepoints during normal breathing conditions (normoxia; 21% O_2, balanced N_2) and during max (hypercapnia + hypoxia; 7% CO_2 + 10.5% O_2, balanced N_2). As hypothesized, all breathing parameters in Figure 1 were significantly decreased (TI) or increased (VT/TI/100 g, PIF and PEF) when comparing max to normoxia during both baseline [TI: control + sham exercise ($N = 15$; $p = 0.003$), control + exercise ($N = 15$; $p < 0.001$), and CTB-SAP + exercise ($N = 7$; $p < 0.001$); VT/TI/100 g: all experimental groups ($p < 0.001$); PIF: all experimental groups ($p < 0.001$); and PEF: all experimental groups ($p < 0.001$)] and endline timepoints [TI: control + sham exercise ($p = 0.003$), control + exercise ($p = 0.009$), and CTB-SAP + sham exercise ($N = 13$; $p = 0.001$); VT/TI/100 g: all experimental groups ($p < 0.001$); PIF: all experimental groups ($p < 0.001$); and PEF: all experimental groups ($p < 0.001$)]. As hypothesized, breathing parameters shown in Figure 1 were significantly different when comparing endline to baseline timepoints during normoxia for CTB-SAP + sham exercise (TI: increased, $p < 0.001$; VT/TI/100 g: decreased, $p = 0.001$; and PIF: decreased, $p < 0.001$) and CTB-SAP + exercise groups (TI: decreased, $p = 0.013$; VT/TI/100 g: increased, $p = 0.010$; PIF: increased, $p = 0.002$; and PEF: increased, $p = 0.010$). In addition, CTB-SAP + sham exercise rats had a significantly decreased TI vs. CTB-SAP + exercise rats ($p = 0.004$) and control + exercise rats ($p = 0.032$), and significantly increased VT/TI/100 g and PIF vs. CTB-SAP + exercise rats (VT/TI/100 g, $p = 0.017$; PIF, $p = 0.026$) and control + exercise rats (VT/TI/100 g, $p = 0.018$; PIF,

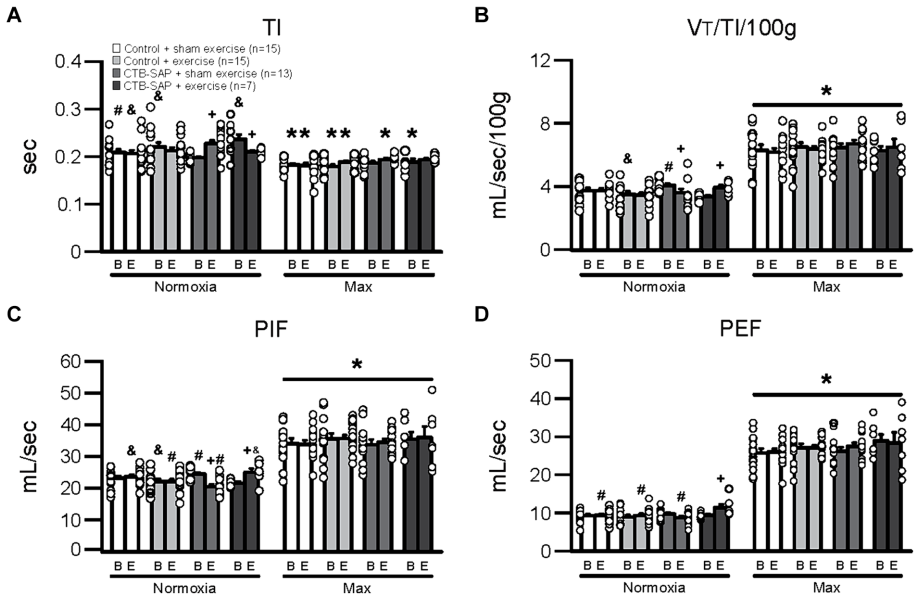


FIGURE 1
Breathing parameters [inspiratory time (TI), mean inspiratory flow (VT/TI/100 g), peak inspiratory flow (PIF), and peak expiratory flow (PEF)] at baseline (B) and endline (E) timepoints during normoxia and maximum chemoreceptor stimulation (max) for all groups (control + sham exercise, control + exercise, CTB-SAP + sham exercise, and CTB-SAP + exercise). All breathing parameters (TI, VT/TI/100 g, PIF and PEF) were significantly impacted when comparing max to normoxia during both baseline and endline timepoints (denoted by *). Some breathing parameters were significantly impacted in CTB-SAP + sham exercise rats vs. CTB-SAP + exercise rats (TI) and control + exercise rats (TI, VT/TI/100 g, and PIF) during normoxia at the baseline timepoint and vs. control + sham exercise rats (TI and PIF) and CTB-SAP + exercise rats (PIF) during normoxia at the endline timepoint (denoted by &). In addition, breathing parameters were significantly affected in CTB-SAP + exercise rats vs. control + sham exercise rats (TI) and CTB-SAP + sham exercise rats (VT/TI/100 g and PIF) during normoxia at the baseline timepoint and vs. CTB-SAP + sham exercise rats (PIF and PEF), control + exercise rats (PIF and PEF), and control + sham exercise rats (PEF) during normoxia at the endline timepoint (denoted by #). Lastly, breathing parameters (TI, VT/TI/100 g, PIF, and PEF) were significantly different when comparing baseline to endline timepoints during normoxia for CTB-SAP + sham exercise (TI, VT/TI/100 g, and PIF) and CTB-SAP + exercise groups (TI, VT/TI/100 g, PIF, and PEF; denoted by +). Values are expressed as means ±1 S.E.M., and differences were considered significant if $p < 0.05$. The adjacent dots to the left or right of each bar represent individual animal values.

$p = 0.022$) during normoxia at the baseline timepoint. In comparison, CTB-SAP + exercise rats had a significantly increased TI vs. control + sham exercise rats ($p = 0.008$) during normoxia at the baseline timepoint. During normoxia at the endline timepoint, CTB-SAP + sham exercise rats had a significantly increased TI vs. control + sham exercise rats ($p = 0.029$), a significantly decreased PIF vs. control + sham exercise rats ($p = 0.001$) and CTB-SAP + exercise rats ($p < 0.001$), and a significantly decreased PEF vs. CTB-SAP + exercise rats ($p = 0.001$). In comparison, CTB-SAP + exercise rats had a significantly increased PIF vs. control + exercise rats ($p = 0.017$) and increased PEF vs. control + exercise rats ($p = 0.010$) and control + sham exercise rats ($p = 0.009$) during normoxia at the end endline timepoint. There were no significant differences between experimental groups during max at the baseline or endline timepoints for TI, VT/TI/100 g, PIF, and PEF.

Summarized in Table 1 are the remaining outcome measures [e.g., frequency, expiratory time (TE), tidal volume (VT/100 g), and minute ventilation ($\dot{V}E$/100 g)] between the four experimental groups. Similar to the outcome measures in Figure 1, all breathing parameters were significantly increased (frequency, VT/100 g, and $\dot{V}E$/100 g) or decreased (TE) as hypothesized for all experimental groups when comparing max to normoxia during both baseline and endline timepoints (all $p < 0.001$). Only $\dot{V}E$/100 g was significantly different when comparing endline to baseline timepoints during normoxia for the CTB-SAP + sham exercise group (decreased, $p = 0.026$), while only VT/100 g was significantly different when comparing endline to baseline during max for the CTB-SAP + sham exercise group (increased, $p = 0.019$). There were no significant differences between experimental groups during normoxia at the baseline timepoint, and only $\dot{V}E$/100 g was significantly increased in CTB-SAP + exercise rats vs. control + exercise rats ($p = 0.046$) during normoxia at the endline timepoint but this small difference (64.7 ± 4.6 vs. 56.1 ± 2.4) is likely not biologically significant. During max at the baseline timepoint, CTB-SAP + sham exercise rats had a significantly decreased frequency vs. control + exercise rats ($p = 0.011$). In addition, during max at the endline timepoint, CTB-SAP + sham exercise rats had a significantly decreased frequency vs. control + sham exercise rats ($p = 0.001$) and increased VT/100 g vs. control + sham exercise rats ($p < 0.001$) and control + exercise rats ($p = 0.048$); CTB-SAP + exercise rats had a significantly decreased frequency ($p = 0.012$) and increased VT/100 g ($p = 0.020$) vs. control + sham exercise rats; and control + exercise rats had a significantly decreased frequency vs. control + sham exercise rats ($p = 0.034$). Lastly, the total number of apneas detected are summarized in Table 2. Total number of apneas were significantly decreased for all experimental groups when comparing max to normoxia during both baseline and endline timepoints (Table 2, all $p < 0.001$). Additionally, CTB-SAP + sham exercise treated rats had a significantly decreased number of total apneas vs. control + exercise rats ($p = 0.044$) during normoxia at the endline timepoint.

Increased lipid profiles in the tongue in CTB-SAP rats are mitigated by tongue exercise

Ex vivo T2-weighted (T2W) MRI was performed on the rat tongues to study the ultrafine structures of the tongue tissues and the lipid

TABLE 1 Breathing parameters [frequency, expiratory time (TE), tidal volume (VT/100 g), and minute ventilation ($\dot{V}E$/100 g)], at baseline and endline timepoints during normoxia and maximum chemoreceptor stimulation (max) for all groups (control + sham exercise, control + exercise, CTB-SAP + sham exercise, and CTB-SAP + exercise).

Experimental Groups	Frequency (breaths/min)		TE (sec)		VT/100 g (ml/100 g)		$\dot{V}E$/100 g (ml/min/100 g)	
	Normoxia	Max	Normoxia	Max	Normoxia	Max	Normoxia	Max
Baseline								
Control + sham exercise	83.1 ± 3.1	159 ± 3.6*	0.56 ± 0.02	0.21 ± 0.00*	0.77 ± 0.03	1.14 ± 0.06*	61.2 ± 2.4	178 ± 9.0*
Control + exercise	78.0 ± 3.5	165 ± 4.2*&	0.59 ± 0.02	0.20 ± 0.00*	0.76 ± 0.04	1.16 ± 0.05*	57.6 ± 3.0	183 ± 7.3*
CTB-SAP + sham exercise	80.0 ± 2.6	151 ± 3.0*	0.59 ± 0.03	0.22 ± 0.00*	0.81 ± 0.03	1.20 ± 0.04*	62.8 ± 1.9	179 ± 6.3*
CTB-SAP + exercise	75.8 ± 5.3	157 ± 9.8*	0.61 ± 0.05	0.21 ± 0.01*	0.79 ± 0.03	1.19 ± 0.09*	57.9 ± 2.6	180 ± 8.3*
Endline								
Control + sham exercise	82.5 ± 3.2	165 ± 6.8*&#^	0.56 ± 0.03	0.20 ± 0.01*	0.76 ± 0.02	1.11 ± 0.04*&#	61.0 ± 2.3	175 ± 6.4*
Control + exercise	80.7 ± 2.6	153 ± 3.1*	0.58 ± 0.02	0.21 ± 0.00*	0.73 ± 0.03	1.20 ± 0.03*&	56.1 ± 2.4#	180 ± 4.2*
CTB-SAP + sham exercise	75.5 ± 3.5	146 ± 1.9*	0.61 ± 0.03	0.22 ± 0.00*	0.77 ± 0.02	1.30 ± 0.04*+	57.2 ± 2.5+	189 ± 5.9*
CTB-SAP + exercise	80.6 ± 3.4	148 ± 3.9*	0.58 ± 0.04	0.22 ± 0.01*	0.83 ± 0.04	1.25 ± 0.10*	64.7 ± 4.6	183 ± 16*

All breathing parameters (frequency, TE, VT/100 g, and $\dot{V}E$/100 g) were significantly impacted when comparing max to normoxia during both baseline and endline timepoints (denoted by *). Some breathing parameters were significantly impacted in CTB-SAP + sham exercise rats vs. control + exercise rats (frequency) during max at the baseline timepoint and vs. control + sham exercise rats (frequency and VT/100 g) and control + exercise rats (VT/100 g) during max at the endline timepoint (denoted by &). Breathing parameters were significantly affected in CTB-SAP + exercise rats vs. control + exercise rats ($\dot{V}E$/100 g) during normoxia at the endline timepoint and vs. control + sham exercise rats (frequency and VT/100 g) during max at the endline timepoint (denoted by #). Additionally, frequency was significantly different in control + exercise rats vs. control + sham exercise rats at max during the endline timepoint (denoted by ^). Lastly, breathing parameters were significantly impacted when comparing baseline to endline timepoints for the CTB-SAP + sham exercise group during normoxia ($\dot{V}E$/100 g) and during max (VT/100 g; denoted by +). Values are expressed as means ± 1 S.E.M., and differences were considered significant if $p < 0.05$.

TABLE 2 Apnea detection (total apneas) at baseline and endline timepoints during normoxia and maximum chemoreceptor stimulation (max) for all groups (control + sham exercise, control + exercise, CTB-SAP + sham exercise, and CTB-SAP + exercise).

Experimental Groups	Total Apneas (#)	
	Normoxia	Max
Baseline		
Control + sham exercise	12.9 ± 0.9*	0
Control + exercise	13.1 ± 1.6*	0
CTB-SAP + sham exercise	12.2 ± 0.8*	0.08 ± 0.1
CTB-SAP + exercise	13.9 ± 1.5*	0
Endline		
Control + sham exercise	11.8 ± 0.9*	0
Control + exercise	13.0 ± 1.1*&	0
CTB-SAP + sham exercise	10.5 ± 1.58*	0
CTB-SAP + exercise	10.9 ± 1.7*	0.14 ± 0.1

Number of total apneas were significantly impacted when comparing max to normoxia during both baseline and endline timepoints (denoted by *). Total apneas were significantly decreased in CTB-SAP + sham exercise rats vs. control + exercise rats during normoxia at the endline timepoint (denoted by &). Values are expressed as means ± 1 S.E.M., and differences were considered significant if $p < 0.05$.

profiles of the lymphoid nodules in the tongue. The lymphoid nodules in the tongue showed hyper-intense signals as opposed to the tongue muscle on T2W MRI (Figure 2). Further, Figure 2 depicts the larger dimensions of the lymphoid nodules in the CTB-SAP + sham exercise rats as compared to the control + sham exercise rats. Further studies of the lymphoid nodules were carried out by MRS in a single voxel size ($2.73 \times 1.33 \times 3\,mm = 10\,mm^3$). The lipid profiles were shown with the assigned lipid peaks and their respective chemical shifts in Figure 3. The spectra were fitted using LC Model software to obtain the relative concentrations of the lipid components. As shown in Figure 4, the ratio of Lip2.1 + Lip2.3 with respect to Lip1.3 was significantly higher in the CTB-SAP + sham exercise rats as compared to the control + sham exercise rats ($p = 0.036$). The CTB-SAP + exercise rats showed a decreased trend in the ratio of Lip2.1 + Lip2.3 to Lip1.3 as compared to the CTB-SAP + sham exercise rats but was not significant ($p = 0.17$).

Intralingual CTB-SAP appears to induce structural changes in the upper airway of CTB-SAP rats that are mitigated by tongue exercise

In vivo airway MRI (Figure 5) suggests that upper airway volume is significantly decreased in CTB-SAP + sham exercise rats ($37.4 \pm 3.1\,mm^3$; $n = 4$) as compared to control + sham exercise rats ($47.0 \pm 2.2\,mm^3$; $n = 4$; $p = 0.04$). However, after exercise ($n = 5$) we observed a significant expansion in upper airway volume for CTB-SAP rats ($47.2 \pm 0.3\,mm^3$; $n = 5$), as compared to the CTB-SAP + sham exercise rats ($p = 0.009$), which is in close proximity to the average volume for control + sham exercise and control + exercise rat rats ($47.4 \pm 1.0\,mm^3$; $n = 5$).

Discussion

The main findings from this study were that: (1) There is evidence of upper airway restriction (i.e., reduced peak inspiratory flow and increased inspiratory time) in sham exercise-treated CTB-SAP rats vs. controls when studying breathing under normoxic conditions; (2) tongue exercise mitigated airflow deficits in CTB-SAP rats during normoxic conditions; (3) sham exercise-treated CTB-SAP rats have increased lipid expression in the tongue via *ex vivo* MR spectroscopy, consistent with previously observed tongue hypertrophy (15); (4) structural changes were present in the upper airway in sham exercise-treated CTB-SAP rats via *in vivo* MRI; and (5) strength endurance tongue exercise preserves upper airway structure and ultrafine structures in the tongue in CTB-SAP rats. Our previous studies have shown that ~60% of the hypoglossal motor neuron pool is lost due to intralingual injection of CTB-SAP, which results in hypoglossal motor deficits such as decreased motor output and motor neuron survival similar to what is seen in ALS mouse models (16, 18). This study provides novel evidence consistent with our hypothesis that tongue weakness in CTB-SAP treated rats results in restriction of the upper airway (e.g., decreased flow and increased inspiratory time) that can be mitigated with strength endurance tongue exercise, providing further evidence that it may be a viable therapeutic treatment for patients with hypoglossal-tongue axis degeneration in motor neuron diseases such as ALS and progressive bulbar palsy. Additionally, these studies reveal that lipid expression, as well as changes in upper airway diameter, are consistent with our previous findings of tongue hypertrophy (i.e., increased tongue volume and thickness, and marked hyperintensity of the tongue) due to hypoglossal motor neuron degeneration via intralingual injection of CTB-SAP (14).

Tongue exercise has a beneficial therapeutic effect on respiratory function

We hypothesized CTB-SAP rats would show evidence of upper airway restriction compared to control rats during normoxic conditions. Here, CTB-SAP sham exercise-treated rats had significantly decreased indicators of airflow (i.e., peak inspiratory flow, peak expiratory flow, and mean inspiratory flow; surrogates for measuring potential changes in upper airway restriction) and significantly increased inspiratory time compared to CTB-SAP

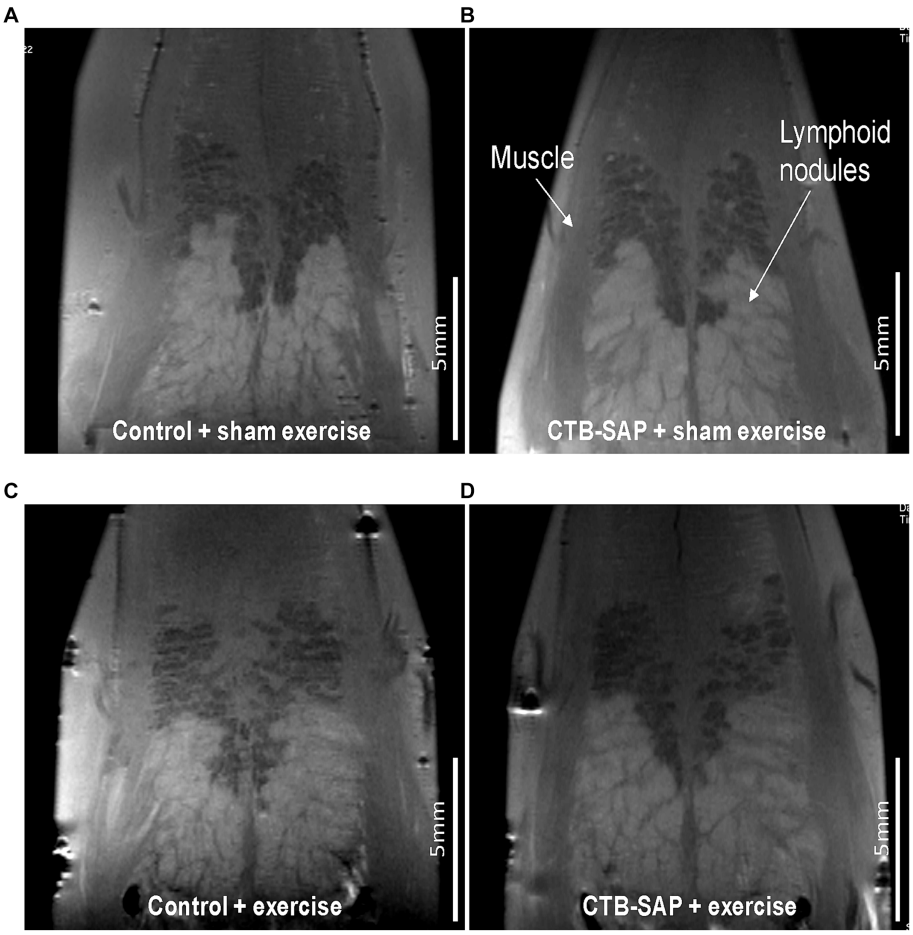


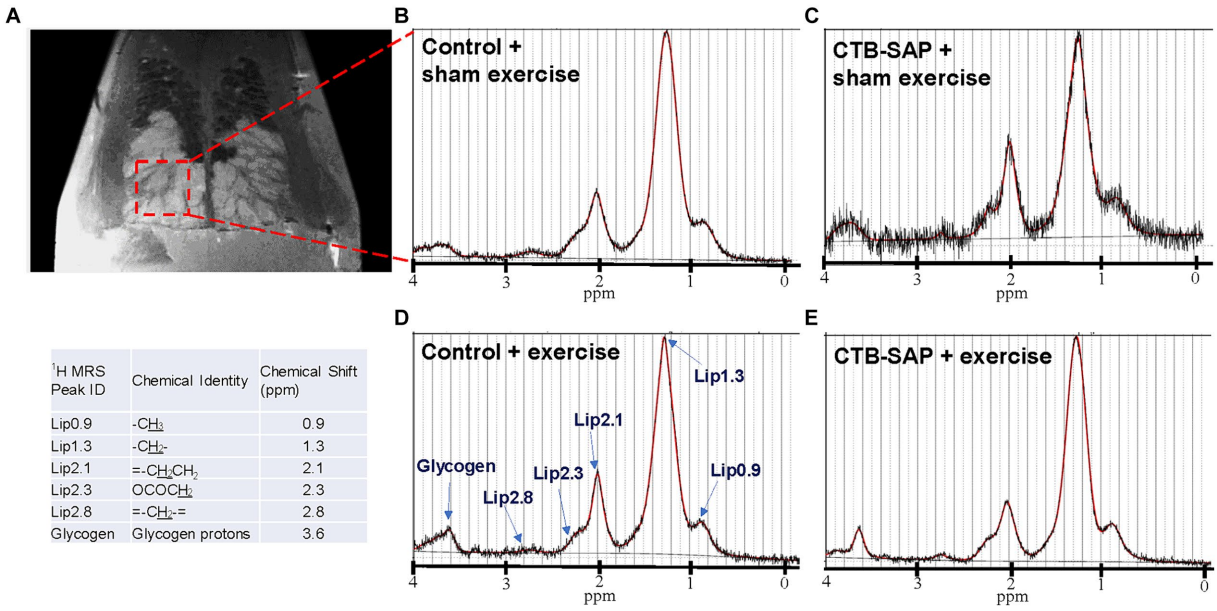
FIGURE 2
Ex vivo T2 weighted MRI of the tongue. Tongues from control + sham exercise **(A)**, control + exercise **(C)**, CTB-SAP + sham exercise **(B)**, and CTB-SAP + exercise treated rats **(D)**. Representative coronal slices are shown with lymphoid nodules and muscle indicated with white arrows **(B)**. Lymphoid nodules in the tongue in CTB-SAP + sham exercise rats **(B)** appear more extensive vs. control + sham exercise rats **(A)**.

exercise-treated rats and control rats +/− exercise during normoxic conditions (Figure 1). These data provide further evidence that our strength endurance tongue exercise paradigm may restore airflow or prevent airflow deficits in CTB-SAP treated rats back towards control levels by preventing the characteristic weakened state of the tongue and limited tongue motion caused by degeneration, as observed in MNDs such as ALS (1).

As hypothesized, we did not anticipate evidence of upper airway restriction in CTB-SAP sham exercise-treated rats when challenged with hypercapnic + hypoxic conditions (10.5% O_2 + 7% CO_2; Figure 1; Tables 1, 2), providing evidence that CTB-SAP sham exercise-treated rats are somehow able to compensate and increase their respiratory function when challenged. This is consistent with studies involving *in vivo* neurophysiology utilizing intermittent or sustained hypoxia, which have shown evidence of respiratory plasticity known as hypoglossal long term facilitation (XII LTF) as a potential mechanism to increase upper airway tone and preserve upper airway patency (19–21). Additionally, this upper airway compensation could be due to the coactivation of muscles that work to protrude (e.g., genioglossus muscle), and retract (e.g., styloglossus and hyoglossus muscles) the tongue in response to a hypoxic + hypercapnic challenge (22, 23). However, whether XII LTF and/or muscle coactivation occurs during a hypoxic + hypercapnic challenge following CTB-SAP induced XII motor neuron loss remains unknown.

Ex vivo MRI and MR spectroscopy of the tongue reveals altered lipid metabolism in CTB-SAP rats

Tongue fat is increased in patients with obstructive sleep apnea, suggesting that there are regional differences in fat distribution at the base of the tongue in apneic vs. non-apneic patients, which may affect the ability of the genioglossus to properly position the tongue away from the airway, causing restriction and loss of airway patency (24). Our previous *in vivo* MRI studies in this model revealed evidence of macrostructural changes in the tongue (e.g., significantly increased tongue volume and thickness, and marked hyperintensity of the tongue) consistent with potential muscle fiber inflammation, fatty replacement (possibly due to increased lipid concentration) of atrophied muscle fibers, +/− edema, which were somewhat mitigated via tongue exercise in CTB-SAP rats (15). Human ALS studies utilizing *in vivo* MRI reveal conflicting results as most studies have shown long-lasting disease characterized by decreased size (i.e.,



^{1}H MRS Peak ID	Chemical Identity	Chemical Shift (ppm)
Lip0.9	-CH_3	0.9
Lip1.3	-CH_2-	1.3
Lip2.1	=-CH_2CH_2	2.1
Lip2.3	$OCOCH_2$	2.3
Lip2.8	=-CH_2-=	2.8
Glycogen	Glycogen protons	3.6

FIGURE 3
Ex vivo magnetic resonance spectroscopy (MRS) of the tongue. MRS of the lymphoid nodules in the tongue was performed in a single voxel (2.73 × 1.33 × 3 mm = 10 mm^3), indicated by red dashed box on T2 weighted MRI **(A)**. Acquired MRS spectrum (black traces) and LC Model fitted lipid peaks (red traces) for each treatment group [control + sham exercise **(B)**, control + exercise **(D)**, CTB-SAP + sham exercise **(A,C)**, and CTB-SAP + exercise rats **(E)**] are shown. The major lipid peaks with their chemical shifts are indicated in **D** and the provided table, respectively.

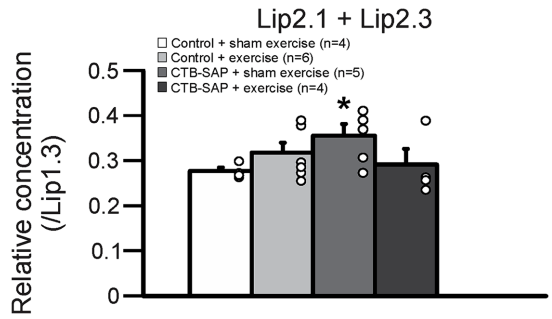


FIGURE 4
Relative lipid composition of the tongue lymphatic nodule tissue for all groups (control + sham exercise, control + exercise, CTB-SAP + sham exercise, and CTB-SAP + exercise). The relative concentration of Lip2.1 + Lip2.3 with respect to the Lip1.3 peak was significantly higher in CTB-SAP + sham exercise rats vs. control + sham exercise rats (denoted by *, $p < 0.05$). Values are expressed as means ±1 S.E.M. The adjacent dots to the right of each bar represent individual animal values.

atrophic muscle fibers, increased connective tissue and fatty replacement) (25), while only a few describe enlarged tongues characterized as pseudohypertrophy due to denervation atrophy with fatty replacement caused by venous or lymphatic obstruction (26). Lipids have various roles, such as energy storage, membrane structure, and hormone synthesis. Due to metabolic disorders, inflammation, or injury, lipids accumulate in various tissues and can affect the function and viability of cells by changing their membrane properties, signaling pathways, and oxidative stress (27). Here, we showed an increase in the relative levels of the unsaturated fatty acid lipids (Lip2.1 + Lip2.3) to the saturated fatty acid (Lip1.3) in the rat tongue in CTB-SAP + sham exercise rats (Figures 2–4). The relative higher composition of the unsaturated fatty acid may indicate the activation of lymphatic macrophages in the regional tongue tissue. Importantly, macrophages can be pro-inflammatory in nature, secreting inflammatory cytokines such as IL-1β or IL-6 leading to host tissue damage (28), or anti-inflammatory in nature, secreting BDNF and supporting tissue remodeling (27). The role these potential lymphatic macrophages may play in regional tongue tissue of CTB-SAP rats is currently unknown but may contribute to inflammation and concurrent upper airway restriction. Future directions will focus on investigating the underlying mechanism responsible for tongue exercise-induced plasticity in the hypoglossal-tongue axis, particularly inflammatory associated factors such as BDNF.

Tongue exercise preserves upper airway patency in CTB-SAP rats

An important factor that influences upper airway resistance is the diameter of the upper airway (29), and patients with MNDs such as ALS often have decreased muscle tone and strength in their upper airway that results in reduction of the airway lumen (30). We hypothesized CTB-SAP sham exercise-treated rats would have degenerative changes in their upper airway (i.e., compressed airway lumen) via *in vivo* MRI consistent with our plethysmography evidence of reduced airflow compared to CTB-SAP exercise-treated rats and control rats +/− tongue exercise. Our MRI results revealed significant upper airway constriction (i.e., decreased volume, mm^3) in the oropharynx between the base of the tongue and the junction of the hard and soft palate in CTB-SAP + sham exercise-treated rats vs.

control rats +/− tongue exercise, and CTB-SAP exercise-treated rats, suggesting that degenerative changes appear to be prevented by tongue exercise in CTB-SAP exercise-treated rats (Figure 5). Furthermore, our data are consistent with small changes in airway diameter leading to drastic changes in flow rate (29). Collectively, these findings suggest that a high-repetition/low endurance tongue exercise program could be used as an effective therapeutic to maintain upper airway patency in CTB-SAP rats.

Limitations

Our studies involving *in vivo* MRI of the upper airway reveal a significant upper airway constriction in CTB-SAP + sham exercise rats (Figure 5). However, due to the narrow time window (less than 100 ms) that *in vivo* MRI images were taken, we were not consistently able to capture high quality images of the oropharynx at peak inspiration. Thus, our measurements of upper airway volume (mm^3) may not consistently be representative of the upper airway at its peak capacity. The current *in vivo* MRI was performed using a heart imaging coil, which was not optimal for imaging the oropharynx. We expect to obtain higher quality images of the oropharynx at peak inspiration in future studies by using a new dedicated rat brain/neck RF coil and/or a higher field strength MRI, such as 9.4 T.

Significance

Tongue exercise as a therapeutic option for patients with MNDs remains highly controversial in the absence of high rigor investigations (31–34). Low-repetition/high-resistance tongue exercise programs have been tested in a variety of rat models (e.g., primary aging, Parkinson's disease, and ALS), where beneficial effects were shown for rat models of primary aging (35–37) and Parkinson's disease (38, 39). However, an investigation using animal models of ALS concluded that tongue force training could result in detrimental effects on some measures of bulbar function (32), suggesting that low-repetition/high-resistance tongue exercise may prove to be a suitable therapeutic treatment for only some patients with ALS and other MNDs, but not in all cases. Thus, we chose to investigate a high-repetition/low-resistance exercise program that is tailored to prevent weakness, fatigue, and limited tongue movement caused by MNDs. Our current studies provide further evidence in support of high-repetition/low-endurance tongue exercise as a treatment option to preserve upper airway patency in patients with MNDs through improving airflow and preventing structural changes in the tongue and oropharynx. Therefore, we will continue leveraging our CTB-SAP rodent model to investigate the underlying mechanism of action responsible for tongue exercise-related treatment effects, and to optimize tongue exercise dosing parameters that will be translatable to humans. Further, these studies suggest MRI could offer a modality to detect and track degenerative/regenerative changes in the upper airway that could serve as a clinical tool to facilitate early diagnosis and treatment monitoring (and perhaps disease staging) in patients with MNDs such as ALS. MRI is widely available in human hospitals and non-invasive, providing an opportunity for earlier diagnosis and intervention when effective treatments become available.

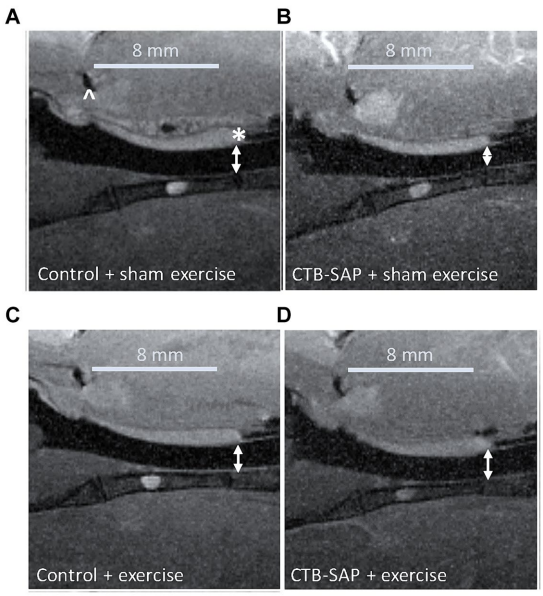


FIGURE 5
In vivo sagittal airway MRI. Airway volume (denoted by double-sided white arrows) evaluated from the junction of the hard and soft palate (denoted by a white * in **A**) through the slice representing the tongue root (denoted by a white ^ in **A**). The CTB-SAP + sham exercise treated rat **(B)** appears to show evidence of a compressed airway when compared to rats from the other treatment groups [i.e., control + sham exercise **(A)**, control + exercise **(C)**, and CTB-SAP + exercise rats **(D)**].

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by our University of Missouri Institutional Animal Care and Use Committee and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals within our USDA-licensed and AAALAC-accredited academic institution. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AK: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. IP: Data curation, Formal analysis, Investigation, Writing – review & editing. CS: Data curation, Formal analysis, Investigation, Writing – review & editing. KO: Data curation, Investigation, Writing – review & editing. LS: Data curation, Formal analysis, Investigation, Writing – review & editing. GO: Data curation, Formal Analysis, Investigation, Writing – review & editing. MG: Formal analysis, Writing – review & editing. LM: Formal analysis, Writing – review & editing, Conceptualization, Data curation, Investigation,

Methodology. TL: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – review & editing, Funding acquisition, Supervision. NN: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Methodology, Supervision, Writing – review & editing, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Cheng S, Butler JE, Gandevia SC, Bilston LE. Movement of the tongue during normal breathing in awake healthy humans. *J Physiol*. (2008) 586:4283–94. doi: 10.1113/jphysiol.2008.156430

2. Hadjikoutis S, Wiles CM. Respiratory complications related to bulbar dysfunction in motor neuron disease. *Acta Neurol Scand*. (2001) 103:207–13. doi: 10.1034/j.1600-0404.2001.d01-22.x

3. Fenik VB, Rukhadze I, Kubin L. Antagonism of alpha1-adrenergic and serotonergic receptors in the hypoglossal motor nucleus does not prevent motoneuronal activation elicited from the posterior hypothalamus. *Neurosci Lett*. (2009) 462:80–4. doi: 10.1016/j.neulet.2009.06.083

4. Fung SJ, Yamuy J, Sampogna S, Morales FR, Chase MH. Hypocretin (orexin) input to trigeminal and hypoglossal motoneurons in the cat: a double-labeling immunohistochemical study. *Brain Res*. (2001) 903:257–62. doi: 10.1016/S0006-8993(01)02318-6

5. Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci*. (1998) 18:9996–10015. doi: 10.1523/JNEUROSCI.18-23-09996.1998

6. Stettner GM, Kubin L. Antagonism of orexin receptors in the posterior hypothalamus reduces hypoglossal and cardiorespiratory excitation from the perifornical hypothalamus. *J Appl Physiol (1985)*. (2013) 114:119–30. doi: 10.1152/japplphysiol.00965.2012

7. Corcia P, Pradat PF, Salachas F, Bruneteau G, Forestier N, Seilhean D, et al. Causes of death in a post-mortem series of ALS patients. *Amyotroph Lateral Scler*. (2008) 9:59–62. doi: 10.1080/17482960701656940

8. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, et al. Amyotrophic lateral sclerosis. *Lancet*. (2011) 377:942–55. doi: 10.1016/S0140-6736(10)61156-7

9. Kurian KM, Forbes RB, Colville S, Swingler RJ. Cause of death and clinical grading criteria in a cohort of amyotrophic lateral sclerosis cases undergoing autopsy from the Scottish Motor Neurone Disease Register. *J Neurol Neurosurg Psychiatry*. (2009) 80:84–7. doi: 10.1136/jnnp.2008.149708

10. Umemoto G, Furuya H, Tsuboi Y, Fujioka S, Arahata H, Sugahara M, et al. Characteristics of tongue and pharyngeal pressure in patients with neuromuscular diseases. *Degener Neurol Neuromuscul Dis*. (2017) 7:71–8. doi: 10.2147/DNND.S132745

11. Weikamp JG, Schelhaas HJ, Hendriks JC, de Swart BJ, Geurts AC. Prognostic value of decreased tongue strength on survival time in patients with amyotrophic lateral sclerosis. *J Neurol*. (2012) 259:2360–5. doi: 10.1007/s00415-012-6503-9

12. Llewellyn-Smith IJ, Martin CL, Arnolda LF, Minson JB. Tracer-toxins: cholera toxin B-saporin as a model. *J Neurosci Methods*. (2000) 103:83–90. doi: 10.1016/S0165-0270(00)00298-3

13. Lujan HL, Palani G, Peduzzi JD, DiCarlo SE. Targeted ablation of mesenteric projecting sympathetic neurons reduces the hemodynamic response to pain in conscious, spinal cord-transected rats. *Am J Phys Regul Integr Comp Phys*. (2010) 298:R1358–65. doi: 10.1152/ajpregu.00755.2009

14. Lind LA, Lever TE, Nichols NL. Tongue and hypoglossal morphology after intralingual cholera toxin B-saporin injection. *Muscle Nerve*. (2021) 63:413–20. doi: 10.1002/mus.27131

15. Murphy ER, Thompson R, Osman KL, Haxton C, Brothers M, Lee L, et al. A strength endurance exercise paradigm mitigates deficits in hypoglossal-tongue axis function, strength, and structure in a rodent model of hypoglossal motor neuron degeneration. *Front Neurosci*. (2022) 16:869592. doi: 10.3389/fnins.2022.869592

16. Lind LA, Murphy ER, Lever TE, Nichols NL. Hypoglossal motor neuron death via intralingual CTB-saporin (CTB-SAP) injections mimic aspects of amyotrophic lateral sclerosis (ALS) related to dysphagia. *Neuroscience*. (2018) 390:303–16. doi: 10.1016/j.neuroscience.2018.08.026

17. Provencher SW. Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR Biomed*. (2001) 14:260–4. doi: 10.1002/nbm.698

18. Silva-Hucha S, Fernandez de Sevilla ME, Humphreys KM, Benson FE, Franco JM, Pozo D, et al. VEGF expression disparities in brainstem motor neurons of the SOD1(G93A) ALS model: correlations with neuronal vulnerability. *Neurotherapeutics*. (2024) 21:e00340. doi: 10.1016/j.neurot.2024.e00340

19. Baker-Herman TL, Strey KA. Similarities and differences in mechanisms of phrenic and hypoglossal motor facilitation. *Respir Physiol Neurobiol*. (2011) 179:48–56. doi: 10.1016/j.resp.2011.06.022

20. Fuller DD. Episodic hypoxia induces long-term facilitation of neural drive to tongue protrudor and retractor muscles. *J Appl Physiol (1985)*. (2005) 98:1761–7. doi: 10.1152/japplphysiol.01142.2004

21. Wilkerson JER, Devinney M, Mitchell GS. Intermittent but not sustained moderate hypoxia elicits long-term facilitation of hypoglossal motor output. *Respir Physiol Neurobiol*. (2018) 256:15–20. doi: 10.1016/j.resp.2017.10.005

22. Fregosi RF, Fuller DD. Respiratory-related control of extrinsic tongue muscle activity. *Respir Physiol*. (1997) 110:295–306. doi: 10.1016/S0034-5687(97)00095-9

23. Mateika JH, Millrood DL, Kim J, Rodriguez HP, Samara GJ. Response of human tongue protrudor and retractors to hypoxia and hypercapnia. *Am J Respir Crit Care Med*. (1999) 160:1976–82. doi: 10.1164/ajrccm.160.6.9903001

24. Kim AM, Keenan BT, Jackson N, Chan EL, Staley B, Poptani H, et al. Tongue fat and its relationship to obstructive sleep apnea. *Sleep*. (2014) 37:1639–48. doi: 10.5665/sleep.4072

25. Hensiek N, Schreiber F, Wimmer T, Kaufmann J, Machts J, Fahlbusch L, et al. Sonographic and 3T-MRI-based evaluation of the tongue in ALS. *Neuroimage Clin*. (2020) 26:102233. doi: 10.1016/j.nicl.2020.102233

26. McKee HR, Escott E, Damm D, Kasarskis E. Macroglossia in amyotrophic lateral sclerosis. *JAMA Neurol*. (2013) 70:1432–5. doi: 10.1001/jamaneurol.2013.3138

27. Rosa Neto JC, Calder PC, Curi R, Newsholme P, Sethi JK, Silveira LS. The immunometabolic roles of various fatty acids in macrophages and lymphocytes. *Int J Mol Sci*. (2021) 22:8460. doi: 10.3390/ijms22168460

28. Komatsu R, Okazaki T, Ebihara S, Kobayashi M, Tsukita Y, Nihei M, et al. Aspiration pneumonia induces muscle atrophy in the respiratory, skeletal, and swallowing systems. *J Cachexia Sarcopenia Muscle*. (2018) 9:643–53. doi: 10.1002/jcsm.12297

29. Campbell M, Sapra A. Physiology, Airflow Resistance. Tampa, FL: StatPearls Publishing (2023). Available at: https://www.ncbi.nlm.nih.gov/books/NBK554401/

30. Quaranta VN, Carratu P, Damiani MF, Dragonieri S, Capozzolo A, Cassano A, et al. The prognostic role of obstructive sleep apnea at the onset of amyotrophic lateral sclerosis. *Neurodegener Dis*. (2017) 17:14–21. doi: 10.1159/000447560

31. Dworkin JP, Hartman DE. Progressive speech deterioration and dysphagia in amyotrophic lateral sclerosis: case report. *Arch Phys Med Rehabil*. (1979) 60:423–5.

32. Ma D, Shuler JM, Kumar A, Stanford QR, Tungtur S, Nishimune H, et al. Effects of tongue force training on bulbar motor function in the female SOD1-G93A rat model of amyotrophic lateral sclerosis. *Neurorehabil Neural Repair*. (2017) 31:147–56. doi: 10.1177/1545968316666956

33. Plowman EK. Is there a role for exercise in the Management of Bulbar Dysfunction in amyotrophic lateral sclerosis? *J Speech Lang Hear Res*. (2015) 58:1151–66. doi: 10.1044/2015_JSLHR-S-14-0270

34. Watts CR, Vanryckeghem M. Laryngeal dysfunction in amyotrophic lateral sclerosis: a review and case report. *BMC Ear, Nose and Throat Disord*. (2001) 1:1. doi: 10.1186/1472-6815-1-1

35. Cullins MJ, Krekeler BN, Connor NP. Differential impact of tongue exercise on intrinsic lingual muscles. *Laryngoscope*. (2018) 128:2245–51. doi: 10.1002/lary.27044

36. Kletzien H, Russell JA, Leverson GE, Connor NP. Differential effects of targeted tongue exercise and treadmill running on aging tongue muscle structure and contractile properties. *J Appl Physiol (1985)*. (2013) 114:472–81. doi: 10.1152/japplphysiol.01370.2012

37. Krekeler BN, Leverson G, Connor NP. Tongue exercise and ageing effects on morphological and biochemical properties of the posterior digastric and temporalis muscles in a Fischer 344 Brown Norway rat model. *Arch Oral Biol*. (2018) 89:37–43. doi: 10.1016/j.archoralbio.2018.02.002

38. Ciucci MR, Russell JA, Schaser AJ, Doll EJ, Vinney LM, Connor NP. Tongue force and timing deficits in a rat model of Parkinson disease. *Behav Brain Res*. (2011) 222:315–20. doi: 10.1016/j.bbr.2011.03.057

39. Ciucci MR, Schaser AJ, Russell JA. Exercise-induced rescue of tongue function without striatal dopamine sparing in a rat neurotoxin model of Parkinson disease. *Behav Brain Res*. (2013) 252:239–45. doi: 10.1016/j.bbr.2013.06.004

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Combining early lower eyelid surgery with neuromuscular retraining for synkinesis prevention after facial palsy: the role of the eye in aberrant facial nerve regeneration

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Background: Facial synkinesis (FS) is a distressing sequela of facial palsy (FP) characterized by involuntary, simultaneous movements of facial muscles occurring during voluntary facial expressions. Treatment of synkinesis is challenging, and preventive methods are needed.

Aim: This study evaluated the efficacy of physical facial nerve rehabilitation (PFNR) therapy alone vs. PNFR with eyelid surgery to correct lagophthalmos and prevent the onset of synkinesis.

Methods: Twenty five outpatients were randomized to receive either PFNR alone (neuromuscular retraining and Kabat proprioceptive neuromuscular facilitation) or PNFR and early (90 days after FP onset) eyelid surgery (involving a conservative oculoplastic correction for lagophthalmos with epiphora or ectropion). Comprehensive otolaryngological assessments and Magnetic Resonance Imaging (MRI) were conducted. Synkinesis progression was measured using Another Disease Scale (ADS) at baseline, 3-, 6-, 12-, and 24-months post-treatment. The data were analyzed with ANOVA, τ-test, Chi-Square analyses.

Results: Patients undergoing eyelid surgery with PFNR showed faster ($p < 0.001$) and better recovery of facial movements ($p < 0.05$) than patients receiving PFNR alone comparing T0 and T12 ($p < 0.0001$). No synkinesis were observed in the PFNR plus surgery group while 37% of patients in PFNR alone had synkinesis ($p = 0.03$). At 24months, none of the patients in the surgery group presented synkinesis.

Conclusion: Combining early surgical treatment of paralytic lagophthalmos or epiphora with PFNR accelerated functional recovery and reduced synkinesis in patients with FP compared to facial rehabilitation alone. Further investigations in larger populations with long-term follow-up are needed.

Clinical trial registration: https://clinicaltrials.gov/study/NCT06538103, NCT06538103.

KEYWORDS

facial paralysis, synkinesis, paralytic ectropion, lagophthalmos, oculoplastic surgery, aberrant facial reinnervation syndrome

Introduction

Facial paralysis results in functional impairments that affect facial expression, eating, speaking, and eye closure, collectively impairing daily activities and quality of life. The disfigurement associated with facial paralysis can lead to altered self-perception, social stigma, withdrawal, depression, and anxiety. Bell's palsy, the most common cause of facial paralysis, has an annual incidence of 23–53 per 100,000 individuals (1, 2). Facial paralysis ranges in severity and can involve dynamic and static function.

Synkinesis, a manifestation of aberrant facial nerve reinnervation syndrome, is a common and distressing sequela of facial paralysis. Most often affects eye closure (2) chewing and drinking, due to impairment of orbicularis oculi and oris muscles (3). Patients often adapt their eating habits to compensate for masticatory deficits (3). The disorder is characterized by involuntary simultaneous movement of different facial muscle groups and can be accompanied by abnormal facial tone and spasm; it occurs in up to 78% of patients during the recovery of facial movement (4). Synkinesis often develops after stroke-associated facial paralysis (5). It is attributed to misdirected regeneration of injured facial nerve fibers, aberrant muscle reinnervation, and cortical rearrangement (6). Oculo-oral synkinesis refers to synkinesis principally affecting the orbicularis muscles of the eyes and mouth (i.e., orbicularis oculi and the orbicularis oris muscles) (6).

We hypothesized that early intervention to prevent eyelid dysfunction might reduce patients' risk of developing synkinesis. The eye secretes through the retina nerve growth factors (NGF) (7, 8), especially when cornea surface becomes dry. In facial paralysis, impaired eyelid function predisposes to inadequate eyelid closure (lagophthalmos), tearing (epiphora), and eyelid eversion (ectropion) (2, 9). Lack of eye protection can cause exposure keratitis, vision loss, and increased light exposure to the retina that alters retinal signaling. Current interventions for ocular synkinesis aim at symptomatic relief, rather than prevention. Temporary solutions like bandaging, gel drops and adhesive palpebral weight but provide interim relief until eyelid motility is recovered (10). Although Botulinum toxin affords temporary chemodenervation, synkinesis inevitably returns. Neurectomy or myectomy may allow for longer-term effects; but there are higher risk of complications and benefits are often temporary (6, 11).

Non-invasive strategies aiming to reorganize the facial motor cortex, such as neuromuscular retraining, Kabat technique (also known as Proprioceptive Neuromuscular Facilitation), or biofeedback require ongoing commitment and typically result in incomplete recovery (5, 12). Although preclinical studies have studied neuro-inhibition in preventing synkinesis (13), clinical studies on synkinesis prevention are lacking.

On the other side, several of oculoplastic interventions have been studied for eyelid dysfunction (14), including eyelid weights (15, 16), wedge excision (16), lateral canthoplasty (17), and other approaches (18) but nobody evaluated the effect of these surgeries on synkinesis onset, nor the combination of surgery with facial therapy to prevent this disfiguring condition. All such procedures have variable success in controlling symptoms and intermittent complications. All such procedures have variable success in controlling symptoms and intermittent complications (14–17). In addition, some of these surgical interventions offer poor esthetic results (16, 17).

Di Stadio proposed a minimally invasive technique to treat eyelid dysfunction (18), the main benefits of this technique are (i) being specific for the eye concern (different approaches for lagophthalmos, epiphora, or ectropion) and (ii) offering excellent esthetic results (18). This minimal eyelid surgery use musculocutaneous flap to support the eyelid and is less invasive than shortening the lower placing eyelid weights, tarsal plate repositioning, or canthopexy (18). This office-based eyelid surgery might be useful to treat deficit in eye closure, reducing the risk of corneal desiccation, and excessive retinal light exposure which might be implicated in the onset of synkinesis.

By the way, the effect of this technique as well as overmentioned other ones (14–17) has never been evaluated on synkinesis prevention, not alone nor associated to physical rehabilitation. Furthermore, it was not compared with traditional physical rehab as preventor of synkinesis.

This study aimed at investigating if minimally invasive eyelid surgery for patients with facial paralysis can reduce the onset of synkinesis. We therefore explored whether this early, pre-emptive eyelid surgery could improve the recovery trajectory of facial paralysis, alleviate risk of developing periorbital complications, and prevent the onset of synkinesis.

Materials and methods

This study was conducted at a tertiary referral hospital from January 2022 to November 2023. Patients with facial paralysis (FP) were consecutively recruited from the otolaryngology department upon presentation. The hospital's Internal Review Board (IRB) approved the study with number CT X06, which adhered to the Helsinki Declaration for human rights. The study was registered on Clinicaltrial.gov with identification number NCT06538103. After being informed about the study's objectives and procedures, all participants provided written consent, which included permission to share their anonymized data for scientific purposes.

The otolaryngologist research team collected patient histories and data on age, sex, known systemic diseases, and family history of neurological conditions. A comprehensive otolaryngological examination was performed, including head and neck exam, microscope exam of bilateral ears, equilibrium testing, and facial nerve scoring using the Another Disease Scale (ADS) scale (9) and a validated new assessment. This assessment evaluates the movement of the facial muscles both analyzing the superior, middle, and inferior segment of the face singularly and the entire facial expression. Patients are invited to perform some expressions, i.e., smiling or kissing, so that physician can understand which muscle has a functional deficit. Moreover, the ADS scale integrates synkinesis into the scoring scheme and segments the face into upper, middle, and lower regions, facilitating a nuanced evaluation of facial paralysis and synkinesis. Computer tomography (CT) scan was performed to rule out stroke or other vascular pathology. An internal medicine consultation was also sought to corroborate

cause of facial paralysis. Patients with systemic or neurological diseases potentially causing facial paralysis were referred to specialists and excluded from the study.

All patients in the study initiated facial nerve therapy (19, 20) and medical therapy as indicated. Individuals diagnosed with idiopathic facial paralysis (Bell's palsy) received steroid therapy in accordance with clinical practice guidelines (21). Individuals diagnosed with Ramsay Hunt syndrome (herpes zoster oticus) received acyclovir 800 mg, prescribed five times daily for 10 days. Individuals with post-surgical (e.g., post-vestibular schwannoma resection) or stroke-related facial paralysis underwent only physical therapy and supportive care.

After completing the acute phase of care (10 days), the patients who presented a total facial paralysis (0–3 ADS scores) were included in the controlled randomization process that assigned even numbers of patients with infectious-origin FP (suspected or confirmed) and odd numbers to those with stroke or previous surgery.

Randomization was conducted using a computer-generated random number sequence to assign participants to either the intervention or control group in a 1:1 ratio. Enrolled participant completed all baseline assessments and were assigned to their respective group. This process was designed to minimize selection bias and ensure the comparability of groups at baseline.

All patients were blinded to group assignment to minimize any psychological influences (positive or negative) that might bias the results.

The sample size was calculated considering a 95% confidence interval (CI), a 5% margin of error, and a 50% population proportion, requiring 28 subjects for the original enrollment. The chosen sample size aims for a precise estimation within the 95% CI, acknowledging the potential for minimal margin of error. For this reason, we decided to include 30 patients. A computerized system allocated 30 participants 1:1 to one to the following treatment regimens resulting in 15 participants per group:

- Treatment Group 1 (TG1): Kabat therapy (22) and neuromuscular reeducation (NMR) at home (23).
- Treatment Group 2 (TG2): Kabat therapy (22), home NMR, and eyelid surgery at 90 days (starting the count from day one of treatment) (if lagophthalmos with epiphora or ectropion was present) (18).

Kabat therapy involved thrice-weekly 35-min sessions. NMR was done twice weekly for 15 min, using a mirror. Patients were assessed at 3, 6, 9, and 12 months. Those with persisting deficits at 12 months received monthly video-call follow-ups until full recovery. Physical therapy lasted at least 12 months, except for those who recovered earlier. The study team performed video-call check-ins every 3 months for a year post-therapy, to assess facial function, with a final follow-up at 24 months post-facial paralysis onset.

We used physical rehabilitation in both groups because this approach followed the standard treatment for facial palsy as described by Robinson et al. (23). For ethical reasons, it was not possible to include different control groups, as for example patients treated with eye surgery only or untreated patients.

Early lower eyelid surgery was performed in accordance with the protocol previously described by Di Stadio (18), with medial or lateral eyelid lifting surgery as indicated. The indication for eye surgery were in case of ectropion (exposure of the conjunctiva due to a reduction in tension of the anterior compartment of the eye muscle) and lagophthalmos (incomplete/abnormal closure of the eye with eyelid in closed position) a lateral lower eyelid lifting surgery was performed (18); otherwise, in presence of epiphora (the eversion of the lachrymal point) and lagophthalmos medial lower eyelid lifting surgery was done (18) (Figure 1). We used ocular surface area (OSA) measurement to evaluate the exposure of sclera (24).

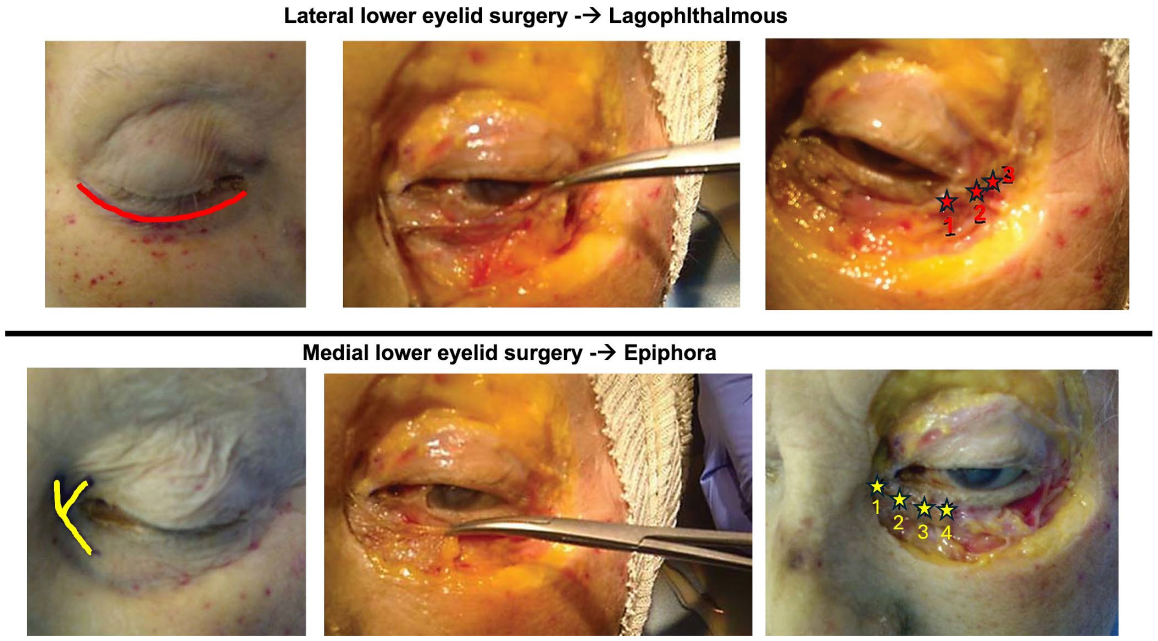

FIGURE 1
Image modified from Di Stadio (18). The upper row of images depicts the laterally based technique used for lagophthalmos. Using subciliary access incision, the surgeon elevates a lateral orbicularis muscle flap, taking care to preserve its neurovascular supply. The muscle flap is suspended laterally by anchoring it to the condensation of connective tissue comprising Whitnall's tubercle. The lower row of images demonstrates the medially based lower eyelid surgery used for treating epiphora. Using a y-shaped incision, the surgeon elevates the medial orbicularis muscle, preserving neurovascular supply, and anchoring the flap to the connective tissue and medical canthal tendon with four sutures, allowing more efficient drainage (red stars).

To reduce operator dependent bias, the eye surgery was always performed by the same surgeon with over a decade of experience in facial plastic surgery (ADS).

The study incorporated safety provisions. Lower eyelid surgery was the only "at risk" procedure; inflammation and/infection of the sutures could happen rarely. To minimize this risk, all patients were treated for 6 days by antibiotic for os (Amoxicillin 1 g each 12 h) associated to eye drops (Tobramicin 0.3%, one drop three times a day). Patients also received standard postoperative follow-up.

Inclusion criteria were being over 18 years old, willingness to participate, and acute/new onset of FP (less than 15 days).

Exclusion criteria included neuro-inflammatory diseases (e.g., multiple sclerosis), FP from systemic disorders (e.g., Guillain-Barré syndrome, Lyme disease, and encephalitis), FP from middle ear infections or untreated cholesteatoma, severe cognitive or psychological disorders, or non-consent to participate in the study. Patients who did not complete at least two follow-ups were excluded from the final analysis and considered dropouts.

The primary outcomes of this study were to evaluate the incidence of the facial synkinesis and the final score at the end of the therapy (best scores correspond to best recovery).

The secondary outcome was to evaluate the time to recover normal facial function.

Statistical analyses

We compared the changes of ADS scale scores within and between the two groups using One-way ANOVA and Bonferroni-Holmes (BH) *ad hoc* test at the baseline and at the end of the observation period (12 months). The differences in the months of recovery were analyzed by τ-test (τ). The comparison between the presence and not of synkinesis was done by using Chi-Square (χ) test. All analyses were performed using Stata ®. p was considered statistically significant <0.05.

Results

General

All patients included in the study were suffering from severe facial paralysis, 0–3 scores in according to ADS, without any movements. Of the 30 patients enrolled in the study, 25 (84%) completed at least two follow-ups, including the one at 12 months. These participants (11 male, 14 female) were age 46.5 ± 15.9 years (CI 95% 23–75). Of the 25 patients, 12 patients were diagnosed with Bell' palsy, 12 presented with a facial paralysis after surgery, one patient had a stroke, and there were no cases of Ramsey Hunt syndrome or other etiologies. Of the post-surgical patients, nine had a facial paralysis as consequence of vestibular schwannoma removal, two due to surgical removal of a VII schwannoma, and one case had facial paralysis after timpano-jugular paraganglioma surgery. None of the patients who underwent surgery had facial nerve sacrifice or reconstruction. Fourteen patients were in the facial nerve therapy group with only one patient lost (6.6% drop) and 11 in surgery plus facial nerve therapy group (26.6% drop; four patients did not complete the follow-ups).

During the study, none of the patients received botulinum toxin or underwent other procedures related to their facial paralysis. All patients had evidence of superior eyelid movement after 12 weeks but at least some residual eyelid dysfunction, involving mild to moderate lagophthalmos with either ectropion, epiphora, or a combination of these symptoms. In the surgery plus facial nerve treatment group, there was improvement of symptoms in all cases. Table 1 summarizes the characteristics of treatment group 1 (facial nerve therapy alone); nine patients were affected by a right facial paralysis (64.3%) and five by a left sided paralysis (35.7%). Table 2 summarizes characteristics of treatment group 2 (surgery plus facial nerve therapy); seven patients suffered from a right facial paralysis (63.6%) and four from a left sided paralysis (36.4%).

TABLE 1 Demographic of TG1.

Patients	Sex	Age	ADS score T0	Side and cause	Synkinesis	Recovery time months	Final ADS score
6	m	75	2.3	Right; Bell's	Yes	13	4.7
7	f	33	0	Right; Paraganglioma	Yes	12	5
8	m	22	2.7	Left; Bell's	No	7	9
10	f	29	0	Right; Bell's	No	16	3.1
11	f	25	1.8	Left; Vestibular Schwannoma	No	7	9
15	m	57	1.8	Right; Vestibular Schwannoma	Yes	14	5.6
16	f	45	1.8	Right; Bell's	No	6	9
18	f	63	3	Right; Bell's	No	11	8.1
19	f	60	0.9	Left; Vestibular Schwannoma	Yes	12	5.3
20	m	36	1.8	Right; Bell's	No	11	8.7
21	f	47	3	Right; Vestibular Schwannoma	No	12	8.4
22	f	52	3	Left; Bell's	Yes	14	5.2
23	m	51	2.7	Left; Facial Schwannoma	No	10	8.4
25	m	55	2.4	Right; Vestibular Schwannoma	No	11	8.4

Demographic characteristics of patients included in TG1 who performed only physical rehabilitation.

TABLE 2 Demographic of TG2.

Patients	Sex	Age	ADS score at T0	Side and cause	Synkinsis	Recovery time months from T0	Final ADS score
1	m	48	1.5	Right; Vestibular Schannoma	No	9	8.4
2	f	23	2.7	Left; Bell's	No	8	8.7
3	f	40	0	Left; Vestibular Schannoma	No	8	8.7
4	m	62	1.2	Right; Bell's	No	8	9
5	f	44	0	Left; Vestibular Schannoma	No	9	9
9	f	75	2.4	Right; Facial Schwannoma	No	7	9
12	f	20	1.5	Right Bell's	No	8	8.7
13	m	35	0	Right;Vestibular Schannoma	No	7	9
14	m	47	0.3	Right; Bell's	No	6	9
17	f	52	0	Right; Stroke	No	7	7.2
24	m	67	0.3	Left; Bell's	No	7	9

Demographic characteristics of patients included in TG2 who performed physical rehabilitation with Kabat and eye surgery.

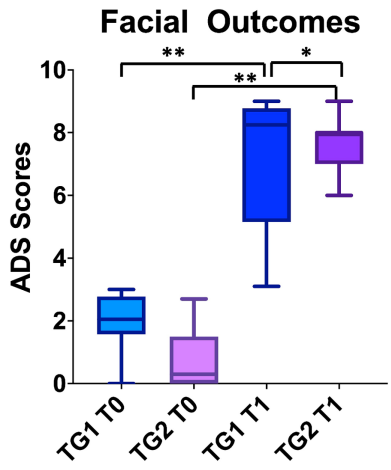

FIGURE 2
The graph bar compares the facial outcomes between the two groups at the baseline and at the end of the treatment. Higher the score better the facial motility. To note ADS scale considers synkinesis also, which presence decrease the final score. Despite both groups recovered from the baseline, the patients in TG2 group had better ADS scores. "*" $p < 0.05$, "**" $p < 0.01$.

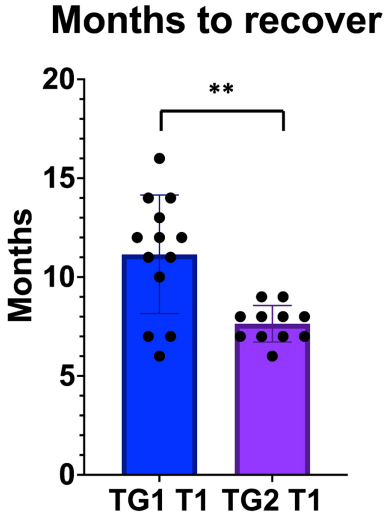

FIGURE 3
The patients in TG2, who underwent early eye surgery, recovered from FP earlier than TG1, treated by physical rehab only. "**" $p < 0.01$.

Two patients in the surgery plus facial nerve therapy group (TG2) had epiphora and lagophthalmos and were treated by medial lower eyelid surgery (n 9 and 24), the other nine were affected by ectropion and lagophthalmos for which lateral lower eyelid surgery was performed.

All patients improved their ADS scores after treatment (ANOVA: $p < 0.0001$) both in facial nerve therapy alone (TG1; average 6.9 ± 2.4; CI95%: 3.1–9) (BH: $p < 0.01$) and surgery plus facial nerve therapy (TG2; average 8.7 ± 0.5; CI95%: 7.2–9) (BH: $p < 0.01$) (Figure 1). No statistically significant differences were observed between TG1 (average 1.9 ± 1; CI95%: 0–2.7) and TG2 (average 0.9 ± 1; CI95%: 0–2.7) before treatment (BH: $p > 0.05$). Statistically significant differences in term of ADS scores were identified after treatment comparing TG1 and TG2 (BH: $p < 0.05$); TG2 had higher ADS scores corresponding to better recovery (Figure 2).

Statistically significant differences were observed between TG1 and TG2 at 3 months (τ: $p < 0.001$); TG2 recovered 11 ± 2.9 (average; CI95%: 6–13) and TG1 7.6 ± 0.9 (average; CI95%:7–9) (Figure 3). 100% of patients in TG2 stopped physical therapy before 12 months due to the recovery of their facial functions.

None of the patients in the surgery plus facial nerve therapy group had synkinesis after 12 months or 24 months from facial paralysis onset. In the facial nerve therapy alone group, five patients (35.7%) suffered from synkinesis on the side of the original paralysis, which was still present in all cases at the 24 months check. None of the other patients were affected by synkinesis at the last video-follow-up. The presence of synkinesis differed significantly between the two groups (χ: $p = 0.03$). The significant reduction in synkinesis in the surgery group thus supported a role for early eyelid surgery in enhancing facial nerve recovery outcomes.

Discussion

Our study revealed that corrective eyelid surgery for paralytic lagophthalmos with facial nerve therapy was associated with shorter recovery period, reduced likelihood of developing synkinesis, and enhanced facial function compared to facial nerve therapy alone, based on ADS scores. Although a variety of oculoplastic interventions have been studied for eyelid dysfunction, including eyelid weights (16, 17), wedge excision (16), lateral canthoplasty (17), and other approaches (18), these interventions have not been systematically studied in combination with facial therapy or with specific emphasis on synkinesis. Furthermore, their role in preventing or alleviating synkinesis is unknown. The present study highlights a novel consideration in management of eyelid disorders with emphasis on early intervention providing eyelid suspensory anchoring and its potential role in mitigating synkinesis.

Regarding the use of eyelid surgery after 3 months from the onset of paralysis, it is important to underline that we only performed the surgery on patients with total absence of any facial movements. Anyway, it is also true that the timing to recover the movement can change between patients even depending on age (19–22) and in some cases (patients 2 and 12) the surgery could be delayed waiting for spontaneous recovery. The latter could also have impacted on the recovery especially in younger patients. On the other hand, retina of young people might be more sensitive to the light exposure than the one of older people (some of them can suffer from cataract) so the neuroepithelium of the structure might hyperproduces NGF (7, 8) causing an earlier onset of synkinesis than the one observed in aging people.

Some monkey's studies should be performed to understand how long the surgery can be delayed at different ages. We suggested monkey because primates share 99% DNA with humans, and this could increase the accuracy of results avoiding controversies and doubts (25). However, because we only present a theoretic mechanism, preliminary studies using less expensive animals (mice and rats) might be considered. Once clarified this aspect, a tailor-made treatment could be suggested for each patient affected by FP.

Physical rehabilitation is a widely accepted treatment for facial paralysis as integral part of facial nerve rehabilitation and is often a first-line therapy for synkinesis (23). Various methods, including KABAT (19–21), Neuromuscular Training (NMR) (26), combinations thereof (27), and recently, KABAT with facial taping (28), have been described. In all studies, the use of facial rehabilitation was able to ameliorate the recovery in term of better facial motility and reduced time to recovery (21) independent from the primary cause of facial palsy (20, 21). In case of severe facial palsy, the benefit of rehabilitation was most apparent (19). Physical rehabilitation alone if using a single method (Kabat or NMR), a combination of methods, or associated with other technique commonly used in rehab (muscular taping) benefits patients when compared with control groups. Often, the eye is the portion of the face that tends to remain asymmetric or because of lack of complete closure or because of the onset of synkinesis (5). Despite the large success of physical rehabilitation on the restoration of facial motility, synkinesis sometimes occurs.

There are some cases, i.e., post angle ponto cerebellum schwannoma removal, in which physical rehabilitation cannot allow the recovery of facial movements causing invalidating sequela. In case of persistent facial palsy, there are several surgical procedures to use, surgical options include nerve transfers and free microvascular muscle transfer (29). However, these invasive procedures are complex (29) and to note, the long-term sequela, including late onset of synkinesis have never been investigated.

For less severe functional impairments, more typical of idiopathic facial paralysis or partial loss of function, a combination of minimally invasive techniques can be considered (30, 31). An example of minimally invasive techniques is the use of thread lifting (32). This method has been used to restore symmetry, by static suspension of the muscles on the side with facial palsy. The researchers combined static suspension on the affected side to botulin toxin on the healthy side. The combination of two techniques improved facial palsy both in static and in dynamic conditions; in fact, all patients improved Sunnybrook scores (37.4 at the baseline vs. 83.3 post treatment) and dynamic facial asymmetry ratios (0.58 at the baseline vs. 0.92 after treatment). Overall, 82.4% patients were satisfied with treatment (31).

Ozturan et al. in 2023 proposed the use of sutureless transconjunctival insertion of an eyelid gold weight to treat lagophthalmos in patients with facial palsy (33). The study was conducted on only six patients, who were followed for 6 months after treatment. The authors reported satisfactory results without patient discomfort or need for re-operation and concluded that their technique is practical, relatively easy and fast to perform. The preservation of the attachment of the elevator muscle to the tarsus allows the surgeon to obtain results like conventional methods. Sutureless method reduce the need for external wound care, burden of suture removal, and suture related complication, such as extrusion (31).

Our proposed technique could potentially be used in association with thread lifting, and it can be modified following Ozturan suggestions and performed with transconjunctival approach to reduce the risk related to the external suture (extrusion, infection, and patient's discomfort). The main advantage of our lower eyelid surgery (18) is that it can be modified (tailoring muscular flap sutures) depending on the patient's need and does not present the typical risks observed in case of gold weight or other weight used to support the closure of the superior eyelid (14–17). Moreover, the lower eyelid surgery treats the problem at the site of impairment (lower eyelid) and not indirectly as done by superior eyelid weight.

The combination between physical rehabilitation and minimal eyelid surgery, seems to be, based on our results a good combination to limit the onset of synkinesis, to improve the quality of recovery and to short the recovery times. A small temporizing measure, which can be done in office, may alleviate the difficulties of the patients whom suffer from synkinesis, including drinking and eating concerns.

The eye is consider the window to the brain (34), it is well known that ocular disorders often precede onset of full clinical manifestations neurological conditions, as in the optic neuritis and internuclear ophthalmoplegia of Multiple Sclerosis (MS). Based on this concept, it might be possible that in presence of excessive dryness of the eye and light overexposure (6), the retina, perhaps even under brain influence, starts to hyperproduce nerve growth factor (NGF) (7). NGF is crucial for ocular surface moisture, corneal integrity, and overall eye health (35, 36) (Figure 4A), and might be responsible of aberrant neuronal regeneration and reinnervation (2) on the base of synkinesis. The latter general couples of the orbicular muscles (eye and mouth) through the action of major and minor zygomatic muscles and superior elevator lip muscle (2) (Figure 4). Excess light penetration

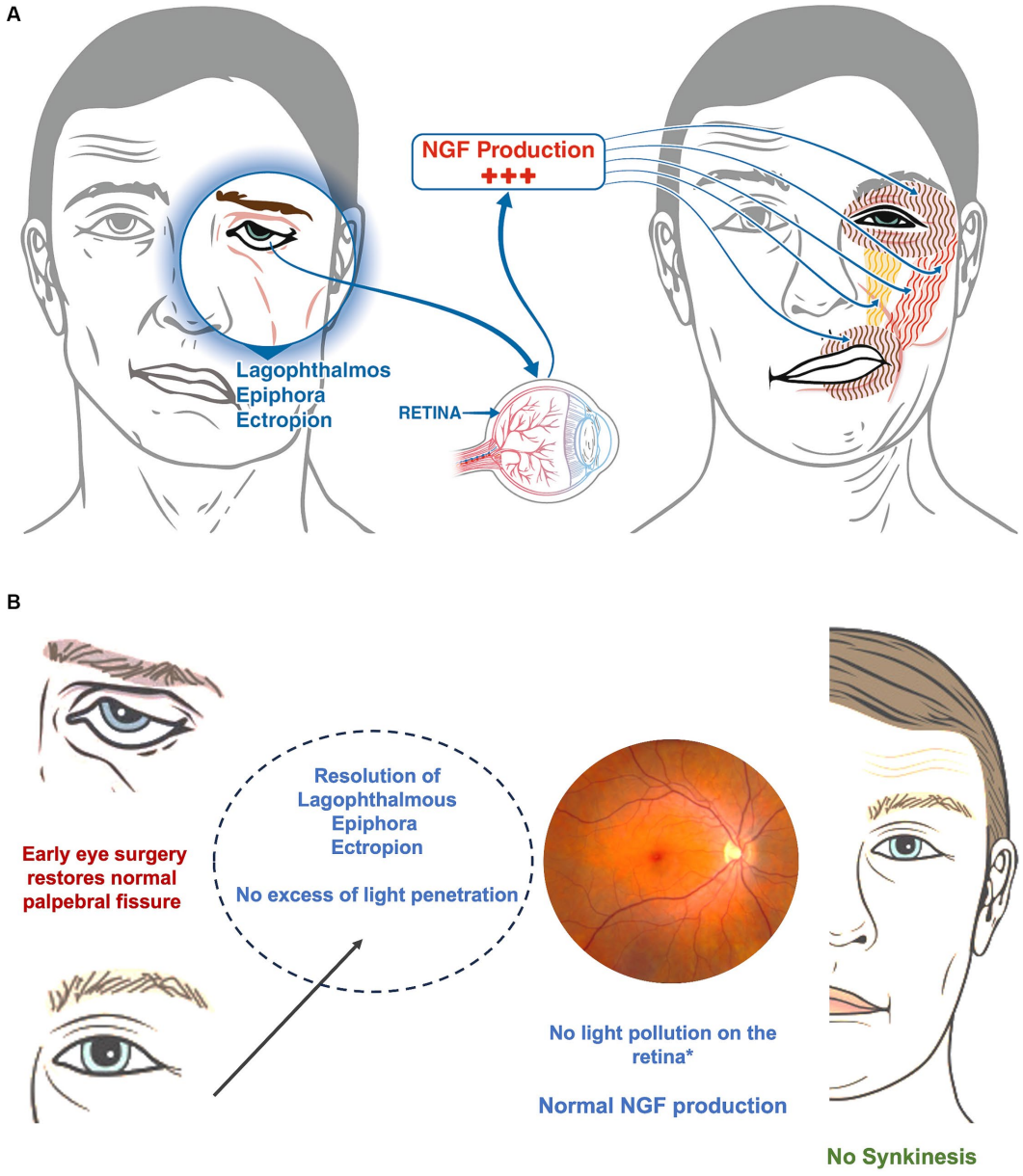

FIGURE 4
(A) In a patient with a facial palsy, the incapacity to close the eye causes both eye dryness and hyper stimulation of the retina because of an excess of light. The retina hyper produces the NGF that stimulate aberrant growth of the facial nerve (not illustrated in the image) with hyper stimulation of the orbicular muscles (eye and mouth-brown vertical line), the major and minor zygomatic muscles (red vertical lines) and the superior elevator lip muscle (yellow). **(B)** The image shows what could happen once soft eye surgery is performed. The resolution of the lagophthalmos and the dryness of the eye, as well as the reduction of the excess of light on the retina (called light pollution) could stop the hyperproduction of NGF avoiding the onset of synkinesis.

(37, 38) due to inadequate eye closure or absence of natural blinking, could induce over production of NGF, promoting aberrant facial nerve regeneration (38). The absence of synkinesis in patients undergoing early lower eyelid surgery might be attributable to the procedure's pre-emptive protection of the eye, inhibiting retinal overproduction of NGF and thus facilitating normal facial function restoration.

The eyelid surgery described herein, aimed at correcting lagophthalmos with ectropion or epiphora by supporting the eyelid, mitigates conjunctival irritation and eyestrain, potentially reducing the risk of developing synkinesis (Figure 4B). Furthermore, early eye closure provided by the surgery might prevent compensatory brain mechanisms typically leading to synkinesis (39–42) such as exaggerated compensation originating in the contralateral insula (40) and gray matter remodeling ipsilaterally (42). While animal studies, such as those on primates, are necessary to confirm the link between retinal NGF production and aberrant facial nerve regeneration, our findings align with the recognized effectiveness of Kabat therapy in restoring facial movements. Importantly, since eye closure significantly influences ADS scores, surgical support in this area could notably impact both the overall rehabilitation outcomes and the recovery timeline, as demonstrated by the

significant differences between the two study groups (Figures 1, 2). Further studies are needed to corroborate findings of the present study and to better understand associated mechanisms.

Future directions

The results of this study, provide an impetus for further investigation into the role of the eye in the regrowth of damaged facial nerves. The exposure of the sclera induces dryness that might stimulate hyperproduction of NGF and consequent synkinesis onset; on the other hand, over exposure of the retina to the light can also induce hyperproduction of NGF.

Early lower eyelid surgery could be valuable for patients with high risk of non-recovery, as for example aging patients or individuals who are post-stroke. Moreover, additional studies could explore the role for eye drops able to hydrate and create a film on the cornea to protect retina.

Study limitations

The study has several limitations including the small sample size, limited outcome measures, and heterogeneity, despite similarity in baseline severity of facial nerve impairment between groups. The small sample size precluded age-stratification or analysis of other covariates; elderly patients could be more at risk of epiphora and ectropion due to physiological laxity of eyelid or may differ in regenerative capacity and patterns of recovery. We did not perform electromyography because of limited access to this test. This electrophysiologic test is important to determine the severity of neuronal damage. The study also did not include an arm without facial nerve therapy, nor did it include other potential interventions. Due to the nature of the interventions, blinding of investigators was not feasible, which may introduce bias in outcome assessment. The findings should therefore be regarded as preliminary, and future studies with larger enrolment allowing for detailed analyses by etiology of facial paralysis are needed. Finally, the mechanistic explanation about the protective effect of the early eye surgery is only speculative and so animal studies to confirm the hypothesis are mandatory. However, these clinical results can be useful as preliminary data for further, even translational, studies.

Conclusion

Early intervention with conservative eyelid surgery to correct lagophthalmos and epiphora may have a role in promoting favorable outcomes after facial paralysis. In the present study, patients undergoing eyelid surgery combined with facial nerve therapy had swifter recovery and lower risk of synkinesis than patients receiving facial therapy alone. Physical rehabilitation remains a proven effective method for restoring normal facial motility and aiding functional recovery. Integrating eye surgery can potentially mitigate the development of synkinesis and expedite recovery, facilitating a quicker return to daily activities for patients. This minimally invasive procedure can be performed under local anesthesia, providing potential benefits with minimal intervention. However, while our findings are promising, they should be regarded as preliminary due to sample size and outcomes assessed. Future studies with larger sample sizes, diverse patient populations, and other therapies are needed to corroborate these preliminary findings and explore underlying mechanisms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ospedale Rodolico University of Catania. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ADS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. MR: Data curation, Writing – original draft. PL: Investigation, Writing – review & editing. JS: Writing – review & editing. TF: Data curation, Validation, Writing – review & editing. MA: Data curation, Investigation, Writing – review & editing. ILM: Investigation, Resources, Validation, Writing – review & editing. SF: Investigation, Validation, Writing – review & editing. EF: Data curation, Supervision, Validation, Writing – review & editing. MB: Data curation, Supervision, Validation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Marson AG, Salinas R. Bell's palsy. *West J Med*. (2000) 173:266–8. doi: 10.1136/ewjm.173.4.266

2. Monini S, Lazzarino AI, Iacolucci C, Buffoni A, Barbara M. Epidemiology of Bell's palsy in an Italian Health District: incidence and case-control study. *Acta Otorhinolaryngol Ital*. (2010) 30:198.

3. Sohrab M, Abugo U, Grant M, Merbs S. Management of the eye in facial paralysis. *Facial Plast Surg*. (2015) 31:140–4. doi: 10.1055/s-0035-1549292

4. Celik M, Forta H, Vural C. The development of synkinesis after facial nerve paralysis. *Eur Neurol*. (2000) 43:147–51. doi: 10.1159/000008154

5. Pu L, Wang L, Zhang R, Zhao T, Jiang Y, Han L. Projected global trends in ischemic stroke incidence, deaths and disability-adjusted life years from 2020 to 2030. *Stroke*. (2023) 54:1330–9. doi: 10.1161/STROKEAHA.122.040073

6. Guntinas-Lichius O, Prengel J, Cohen O, Mäkitie AA, Vander Poorten V, Ronen O, et al. Pathogenesis, diagnosis and therapy of facial synkinesis: a systematic review and clinical practice recommendations by the international head and neck scientific group. *Front Neurol*. (2022) 13:1019554. doi: 10.3389/fneur.2022.1019554

7. Fan B, Zhang C, Chi J, Liang Y, Bao X, Cong Y, et al. The molecular mechanism of retina light injury focusing on damage from short wavelength light. *Oxidative Med Cell Longev*. (2022) 2022:1–14. doi: 10.1155/2022/8482149

8. Rocco ML, Soligo M, Manni L, Aloe L. Nerve growth factor: early studies and recent clinical trials. *Curr Neuropharmacol*. (2018) 16:1455–65. doi: 10.2174/1570159X16666180412092859

9. Di Stadio A. Another scale for the assessment of facial paralysis? ADS scale: our proposition, how to use it. *J Clin Diagn Res*. (2015) 9:MC08–11. doi: 10.7860/JCDR/2015/15366.6953

10. de Swart BJ, Verheij JC, Beurskens CH. Problems with eating and drinking in patients with unilateral peripheral facial paralysis. *Dysphagia*. (2003) 18:267–73. doi: 10.1007/s00455-003-0011-0

11. Markey JD, Loyo M. Latest advances in the management of facial synkinesis. *Curr Opin Otolaryngol Head Neck Surg*. (2017) 25:265–72. doi: 10.1097/MOO.0000000000000376

12. Tikhtman R, Hsieh TY. Minimization of facial synkinesis. *Curr Opin Otolaryngol Head Neck Surg*. (2023) 31:293–9. doi: 10.1097/MOO.0000000000000920

13. Nakamura K, Toda N, Sakamaki K, Kashima K, Takeda N. Biofeedback rehabilitation for prevention of synkinesis after facial palsy. *Otolaryngol Head Neck Surg*. (2003) 128:539–43. doi: 10.1016/S0194-59980223254-4

14. Ali SA, Hanks JE, Stebbins AW, Cohen ST, Hunter DA, Snyder-Warwick AK, et al. Comparison of myelin-associated glycoprotein with vincristine for Facial nerve inhibition after bilateral Axotomy in a transgenic Thy1-Gfp rat model. *JAMA Facial Plast Surg*. (2019) 21:426–33. doi: 10.1001/jamafacial.2019.0398

15. Chepeha DB, Yoo J, Birt C, Gilbert RW, Chen J. Prospective evaluation of eyelid function with gold weight implant and lower eyelid shortening for facial paralysis. *Arch Otolaryngol Head Neck Surg*. (2001) 127:299–303. doi: 10.1001/archotol.127.3.299

16. Kinney SE, Seeley BM, Seeley MZ, Foster JA. Oculoplastic surgical techniques for protection of the eye in facial nerve paralysis. *Am J Otolaryngol*. (2000) 21:275–83. doi: 10.1016/S0196-0709(00)80022-8

17. Leatherbarrow B, Collin JR. Eyelid surgery in facial palsy. *Eye*. (1991) 5:585–90. doi: 10.1038/eye.1991.101

18. Di Stadio A. Eyelid lifting for ectropion and scleral show in facial palsy disease. *ORL J Otorhinolaryngol Relat Spec*. (2014) 76:329–35. doi: 10.1159/000369623

19. Barbara M, Monini S, Buffoni A, Cordier A, Ronchetti F, Harguindey A, et al. Early rehabilitation of facial nerve deficit after acoustic neuroma surgery. *Acta Otolaryngol*. (2003) 123:932–5. doi: 10.1080/00016480310000629

20. Barbara M, Antonini G, Vestri A, Volpini L, Monini S. Role of Kabat physical rehabilitation in Bell's palsy: a randomized trial. *Acta Otolaryngol*. (2010) 130:167–72. doi: 10.3109/00016480902882469

21. Baugh RF, Basura GJ, Ishii LE, Schwartz SR, Drumheller CM, Burkholder R, et al. Clinical practice guideline: Bell's palsy. *Otolaryngol Head Neck Surg*. (2013) 149:S1–S27. doi: 10.1177/0194599813505967

22. Monini S, Iacolucci CM, Di Traglia M, Lazzarino AI, Barbara M. Role of Kabat rehabilitation in facial nerve palsy: a randomised study on severe cases of Bell's palsy. *Acta Otorhinolaryngol Ital*. (2016) 36:282–8. doi: 10.14639/0392-100X-783

23. Robinson MW, Baiungo J. Facial rehabilitation: evaluation and treatment strategies for the patient with Facial palsy. *Otolaryngol Clin N Am*. (2018) 51:1151–67. doi: 10.1016/j.otc.2018.07.011

24. Cabuk KS, Karabulut GO, Fazil K, Nacaroglu SA, Gunaydin ZK, Taskapili M. 2D analysis of gold weight implantation surgery results in paralytic Lagophthalmos. *Beyoglu Eye J*. (2021) 6:200–5. doi: 10.14744/bej.2021.95866

25. Phillips KA, Bales KL, Capitanio JP, Conley A, Czoty PW, Hart BAT, et al. Why primate models matter. *Am J Primatol*. (2014) 76:801–27. doi: 10.1002/ajp.22281

26. Jeong J, Lee JM, Kim J. Neuromuscular retraining therapy combined with preceding botulinum toxin a injection for chronic facial paralysis. *Acta Otolaryngol*. (2023) 143:446–51. doi: 10.1080/00016489.2023.2207599

27. Di Stadio A. Combination of rehabilitation and eye soft surgery thought to reduce eye disease and the incidence of syncinesia. *Ital J Maxillofac Surg*. (2013) 24:69–75.

28. Di Stadio A, Gambacorta V, Ralli M, Pagliari J, Longari F, Greco A, et al. Facial taping as biofeedback to improve the outcomes of physical rehab in Bell's palsy: preliminary results of a randomized case-control study. *Eur Arch Otorrinolaringol*. (2021) 278:1693–8. doi: 10.1007/s00405-020-06193-3

29. Mehta RP. Surgical treatment of facial paralysis. *Clin Exp Otorhinolaryngol*. (2009) 2:1–5. doi: 10.3342/ceo.2009.2.1.1

30. Cohn J, Shokri T, Vincent AG, Hohman MH, Ducic Y, Sokoya F. Surgical rehabilitation of facial paralysis—eyelids and lower face In: S Wang and K Fung, editors. Principles of Lateral Craniofacial Reconstruction. Cham: Springer (2021)

31. Aguilar K. Facial paralysis. Speedy and easy techniques in facial palsy surgery. *NOVA Biomed*. (2016):61–85.

32. Choe WJ, Kim HD, Han BH, Kim J. Thread lifting: a minimally invasive surgical technique for long-standing facial paralysis. *HNO*. (2017) 65:910–5. doi: 10.1007/s00106-017-0367-3

33. Ozturan O, Yenigun A, Senturk E, Aksoy F. Sutureless transconjunctival insertion of eyelid gold weight. *Am J Otolaryngol*. (2023) 44:103874. doi: 10.1016/j.amjoto.2023.103874

34. Nguyen CTO, Acosta ML, Di Angelantonio S, Salt TE. Editorial: seeing beyond the eye: the brain connection. *Front Neurosci*. (2021) 15:719717. doi: 10.3389/fnins.2021.719717

35. Muzi S, Colafrancesco V, Sornelli F, Mantelli F, Lambiase A, Aloe L. Nerve growth factor in the developing and adult lacrimal glands of rat with and without inherited retinitis pigmentosa. *Cornea*. (2010) 29:1163–8. doi: 10.1097/ICO.0b013e3181d3d3f9

36. Lambiase A, Micera A, Pellegrini G, Merlo D, Rama P, De Luca M, et al. In vitro evidence of nerve growth factor effects on human conjunctival epithelial cell differentiation and mucin gene expression. *Invest Ophthalmol Vis Sci*. (2009) 50:4622–30. doi: 10.1167/iovs.08-2716

37. Lee AC, Yu VM, Lowe JB 3rd, Brenner MJ, Hunter DA, Mackinnon SE, et al. Controlled release of nerve growth factor enhances sciatic nerve regeneration. *Exp Neurol*. (2003) 184:295–303. doi: 10.1016/S0014-4886(03)00258-9

38. Contín MA, Benedetto MM, Quinteros-Quintana ML, Guido ME. Light pollution: the possible consequences of excessive illumination on retina. *Eye*. (2016) 30:255–63. doi: 10.1038/eye.2015.221

39. Benedetto MM, Contin MA. Oxidative stress in retinal degeneration promoted by constant LED light. *Front Cell Neurosci*. (2019) 13:139. doi: 10.3389/fncel.2019.00139

40. Wu JJ, Lu YC, Zheng MX, Hua XY, Shan CL, Ding W, et al. Structural remodeling in related brain regions in patients with facial synkinesis. *Neural Regen Res*. (2021) 16:2528–33. doi: 10.4103/1673-5374.313055

41. Wu JJ, Lu YC, Zheng MX, Hua XY, Xu JG, Ding W, et al. Motor control deficits in Facial Synkinesis patients: neuroimaging evidences of cerebral cortex involvement. *Neural Plast*. (2019) 2019:7235808. doi: 10.1155/2019/7235808

42. Zhang CH, Wang HQ, Lu Y, Wang W, Ma H, Lu YC. Exploration of rich-club reorganization in facial synkinesis: insights from structural and functional brain network analysis. *Cereb Cortex*. (2023) 33:11570–81. doi: 10.1093/cercor/bhad390

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The neural substrates of bruxism: current knowledge and clinical implications

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Bruxism is a complex orofacial behavior that can occur during sleep or wakefulness, characterized by the involuntary grinding or clenching of teeth, involving repetitive activity of the jaw muscles. Its etiology is multifactorial, influenced by genetic, psychological, physiological, and lifestyle factors. While the mild bruxism may not necessitate treatment, severe bruxism can lead to significant consequences, including tooth damage, jaw pain, fatigue, and headaches. The bruxism has been associated with medical conditions, such as stress, anxiety, sleep disorders, and various neurological disorders; however, the exact pathophysiology remains elusive. Although the central nervous system is strongly implicated in the development of bruxism, specific neural substrates have not yet been conclusively established. Furthermore, there is evidence to suggest that individuals with bruxism may exhibit neural plasticity, resulting in the establishment of distinct neural circuitry that control the jaw movements. The application of various neurophysiological techniques in both clinical and pre-clinical studies provides valuable insights into the neural mechanisms underlying bruxism. This review aims to comprehensively examine the current literature on the neural pathways involved in bruxism, with the goal of improving the clinical approach and therapeutics for this condition. A deeper understanding of the neural circuitry controlling bruxism holds the potential to advance future treatment approaches and improve the management of patients with bruxism.

KEYWORDS

brain, bruxism, neural substrates, trigeminal, jaw muscles, orofacial behavior, neuroimaging

1 Introduction

Bruxism is defined as repetitive masticatory muscle activity characterized by clenching or grinding of the teeth, and/or by bracing or thrusting of the mandible, according to an international consensus in 2013 (1). Bruxism is a parafunctional activity that is commonly observed, with a prevalence estimated to affect up to 30% of the population (2, 3). This condition can occur either during the day or at night, known as awake bruxism and sleep bruxism, respectively (4–6). While sleep bruxism is defined as a masticatory muscle activity during sleep, characterized as rhythmic (phasic), non-rhythmic (tonic), and is not a movement disorder or a sleep disorder in otherwise healthy individuals; awake bruxism is defined as a masticatory muscle activity during wakefulness, characterized by the repetitive or sustained tooth clenching and grinding, as well as mandibular bracing and thrusting, and is not a movement disorder in otherwise healthy individuals (5, 7). Current consensus considers bruxism a behavior that is a risk factor for many health conditions, including not only tooth

wear and prosthodontic complications, but also serious masticatory muscle pain and temporomandibular joint (TMJ) pain (5, 8–10).

Despite regular dental examinations aiding early detection and management of bruxism, current treatments are limited to reducing symptoms and preventing damage without targeting the underlying cause. For instance, occlusal splints or mouthguards are used to intervene symptomatically on sleep bruxism, but they may lead to an increase in clenching or grinding activity (11, 12). Medications, such as botulinum injections, can be prescribed to alleviate muscle activity and pain. However, these injections only decrease the strength of masticatory muscle contractions but do not decrease the incidence of sleep bruxism events (13). Therefore, it is essential to understand the mechanisms of bruxism to develop better treatments targeting the causes of this condition.

Bruxism presents a multifactorial etiology (14). The development of bruxism likely involves genetic, psychological, physiological, and lifestyle factors. Genetic predisposition may play a role, as bruxism can run in families, suggesting a hereditary component (15). Psychological conditions, such as stress and anxiety, have been highly related to bruxism (16). Physiological factors, including sleep disorders, abnormal neurotransmitter levels, and certain medications, can also contribute to the development of bruxism (17, 18). Lifestyle factors like the consumption of alcohol, caffeine, tobacco, and drug abuse have also been associated with bruxism (19). All of these factors converge on the excessive masticatory muscle activities, so it would be valuable to identify the neural pathways involved in bruxism (20). Although the prevalence of bruxism in children and adolescence is reported to be as high as 40–50%, these estimates often rely on parental reports and encompass a wide age range of participants (3, 21, 22). Furthermore, most studies on the neural circuitries involved in bruxism have been conducted in adults. Given that the adult brain's circuitry is fully mature and developed, focusing on adults allows for a clearer understanding of the neural pathways involved in this complex behavior.

The use of different modern tools for the evaluation of the neural pathways involved in bruxism in both clinical and animal studies can provide a deeper understanding in this topic. The aim of this review is to comprehensively examine the current literature on the neural pathways involved in bruxism. The uncovering of the neural control involved in this behavior can provide important information for the development of future therapeutic approaches and prevent the consequences of severe bruxism.

2 Methods

The following databases were searched for article selection: PubMed, Scopus, Science direct, and EBSCO host until December 2023. The search strategies are detailed in Table 1. The search strategy was modified for Science direct and EBSCO host to improve the number of articles obtained. Additionally, filters were applied to Science direct to narrow the search and avoid the search of tertiary sources and gray literature. To secure a deep search and retrieve more relevant articles related to the topic, a manual search was performed in Google scholar and the bibliographic references of the included studies. There were no restrictions on language or date of publication. Both human and animal studies related to the neural control of bruxism, muscle hyperactivity, tooth grinding or clenching were considered for this review, while duplicate studies and review papers were excluded. A total of 4,751 articles were found, with 837 duplicate articles excluded. Article selection by title and abstract included 179 articles. Finally, after reading the full-text articles, a total of 23 animal studies and 72 clinical studies were included in this review.

3 Brain regions involved in jaw movement and bruxism

3.1 Motor neurons

The masticatory muscles, vital for tasks like chewing and clenching, are governed by motor neurons in the trigeminal motor nucleus (Vmotor) within the pontine region of the brainstem. These muscles exhibit distinct patterns of activity depending on the function they perform, such as during normal chewing, forceful clenching, or bruxism (23). Studies have shown that the excitability of the temporalis muscle, a key masticatory muscle, can be assessed through H-reflex measurements. Svensson et al. demonstrated that H-reflex amplitudes increase with the intensity of clenching tasks, indicating heightened neural excitability during stronger muscle contractions (24). Clenching movements require isometric muscle contraction, while closing movements require isotonic muscle contraction. These different muscle contractions may produce more muscle effort during clenching, compared to jaw opening and closing tasks. The differences in motor control strategies during clenching could potentially lead to decreased inhibitory jaw reflexes specific to this task (23).

Motor neuron activity during voluntary clenching in patients with bruxism may differ from normal conditions. During voluntary contractions, trigeminal motoneurons activate Persistent Inward Currents (PICs). The onion skin effect, where higher threshold units fire at lower rates than lower threshold units, along with PIC activation, facilitates sustained activation of the masseter muscle. Evaluation of these parameters have shown that the motor neuronal activation during voluntary clenching in bruxism patients seemed to be similar to non-brux patients (25). However, nerve growth factor-induced sensitization associated with motor training task have been reported to have no significant effect in the central modulation of motor pathways in bruxers, as opposed to non-bruxers (26).

Sleep-bruxism patients present both lower levels of the masseter motor-evoked potentials and the masseter inhibitory reflex, with possible altered interneuronal activity necessary for the processing of non-nociceptive information (27, 28). This suggests that an abnormal motor control of the masticatory muscles during bruxism, due to disrupted interneuronal activity, could lead to a reduced excitability of the inhibitory circuitry in the jaw motor system.

3.2 Cerebellum

The cerebellum, a crucial brain structure for motor coordination and cognitive processing, has been shown to activate during clenching with natural teeth in functional MRI studies (29–31). Similarly, voluntary clenching induced by a visual stimulus has demonstrated either bilateral or unilateral cerebellar activation prior to clenching,

TABLE 1 Search strategy.

Article search	Search strategy	Filter	Number of articles
PubMed	(bruxism OR grinding OR clenching OR Brux) AND (neural plasticity OR functional MRI OR neural control OR neural changes OR brain OR neuroscience)	None	1,203
Scopus	(bruxism OR grinding OR clenching OR brux) AND ("neural plasticity" OR "functional mri" OR "neural control" OR "neural changes" OR brain OR neuroscience)	None	826
Science direct	(bruxism OR grinding OR clenching) AND ("neural plasticity" OR "neural control" OR neuroscience)	Review Research article	2,170
EBSCO host	(bruxism OR grinding OR clenching) AND ("neural plasticity" OR "neural control" OR neuroscience)	None	525
Manual selection			27

suggesting the involvement of the cerebellum in the cognition process of orofacial motor tasks (32). These findings suggest that the cerebellum plays a role not only in motor control but also in the higher-order cognitive processing required for complex motor behaviors like clenching.

In a study on mice utilizing Ca^{2+} imaging to identify the neuronal activity, researchers observed that the stellate cells (inhibitory interneurons of the cerebellum molecular layer) were active during bruxing behaviors in freely moving animals (33). These stellate cells were specifically activated in the Crus II area of the posterior lobe of the cerebellum. This area was also active during licking, suggesting a link between the Crus II area and orofacial muscle activity. However, while the stellate neurons activated during licking presented an organized spatial distribution along the parallel fiber axis, those activated during bruxing lacked spatial organization. Interestingly, the cerebellar basket cell interneurons activated during bruxing presented high levels of coordination, and their Ca^{2+} signal strength was similar to the that of the stellate neurons.

3.3 Higher brain areas

3.3.1 Higher brain areas in clenching and brux behaviors in human

The higher brain areas involve the parts of the brain that perform higher-level functions necessary for complex behaviors, including learning, memory, sensory integration, and decision-making activities (34). Involvement of higher brain areas, including the cerebral cortex, during clenching and bruxism behaviors underscores the complex neural processes associated with these parafunctional masticatory tasks (35). Functional MRI studies have revealed robust activation patterns across various cortical regions in response to different types of clenching stimuli, highlighting the intricate interplay between sensory, motor, and cognitive pathways (35–37).

The inferior frontal gyrus, including the prefrontal area and Brodmann's area (BA) 44 and 45, is activated during clenching with natural teeth (30). Interestingly, the visual cortex (BA 17, 18) is also active during clenching with soft splints. In contrast, clenching with a hard splint activates additional areas such as the supplementary motor area (SMA, BA 6) and the temporal association area (TAA, BA20, 37), leading to higher masseter activity compared to clenching with natural teeth (30). Increased clenching force in healthy subjects activates these areas and additional regions like the primary sensorimotor cortex (SMC, BA 1, 4), and the temporal association area (BA 20–22, 37) (29). These results suggest that the proprioceptive information from orofacial tissues may be involved in these differences, affecting the striate and parastriate areas.

Other cortical areas activated during teeth clenching include the pre-central gyrus, post-central gyrus, sensory cortex, motor cortex, pre-motor cortex, somatosensory cortex, prefrontal cortex, temporal cortex, frontal operculum, basal ganglia (putamen), parietal cortex, cingulate cortex, and insula (31, 38–40).

Although the motor and premotor cortex present high activation during clenching tasks, the activation pattern depends on the type of clenching task. Intercuspal clenching tasks show higher area and volume activation compared to incisor tooth clenching task (41). This suggests that the differences in proprioceptive information from the periodontal ligament of the anterior and posterior teeth could be involved. It is possible that the differences between the centric and eccentric movements in parafunctional jaw movements and bruxism correspond to different activation patterns in the brain.

Brain activity differences between left and right hemispheres during clenching tasks have been found in different subjects, with asymmetrical activations in the motor, premotor, and somatosensory cortices (37). Chewing-side preferences may be reflected in hemispheric dominance, and neural plasticity due to long term habits could involve both the primary sensorimotor cortex and primary motor cortex (42).

Pathological brain activity has also been associated with abnormal jaw activity. Tooth clenching has been linked to triggering migraine (43). Acquired brain injuries, such as traumatic brain injury, and sub arachnoid hemorrhage, are associated with increased parafunctional activity of the masticatory muscles (44). Neurological conditions like epilepsy have shown connections with bruxism as well (45), with bruxism episodes reported during temporal lobe seizures (46). However, the exact relationship between these pathological neural conditions and bruxism remains unclear.

3.3.2 Cortical involvement in the generation of brux-like behaviors in animals

Cortical stimulation can induce bruxism-like behaviors in animals (47, 48). In a study with rabbit, stimulation of the brain cortex surface around the chewing area under anesthesia elicited bruxing behaviors (47). Bruxing was induced by high-frequency electrical stimulations in the anteromedial region of the cortex, specifically the jaw motor cortical area. The regular rhythm of the bruxing behaviors were 3–4 cycles per second, regardless of the stimulation frequency,

suggesting that abnormal excitation of the jaw-motor cortical area plays a role in the development of bruxing behaviors. Other animal studies have showed that structural disease conditions in the forebrain can lead to awake bruxism in dogs (49).

Higher processing areas in the brain, such as the central nucleus of the amygdala (CeA), are connected to Vmotor in humans. The CeA is involved in the processing of stress, and it has been suggested that the CeA–Vmotor circuit may be related to aberrant jaw motor function in humans (50). Interestingly, the CeA also increases its activity while when performing masticatory behaviors to cope with stress in rodents (51).

3.3.3 Differences in neural activation of patients with bruxism

Repeated clenching tasks can trigger neural plasticity in the corticomotor circuitry involved in jaw-closing muscle activation, as evidenced by motor-evoked potential facilitation of the masseter muscle and increased cortical excitability (52). Similarly, sleep bruxism is associated with neural plasticity changes, affecting motor force control and masticatory motor learning (53). The installation of the aberrant orofacial movements may provide the basis for the neural adaptability changes in patients with bruxism.

Decreased cortical activation patterns have been reported in patients with bruxism during voluntary clenching tasks, particularly showing less activation in the right inferior parietal lobule and dorsal posterior cingulate area (54). This suggests that patients with bruxism may have less controlled movements of the masticatory muscles.

In addition to the cortical activation, subcortical activation pattern is also different in patients with parafunctional brux behaviors. Patients with parafunctional tooth grinding and clenching exhibit a narrower activation pattern in the SMA, pre-SMA (the most anterior section of SMA), and left inferior parietal lobule during voluntary clenching and grinding compared to normal subjects (55). Similarly, SMA, pre-SMA, proSMA (posterior part of SMA, or proper SMA), and SMC areas present lower activation during clenching tasks in subject with brux behaviors compared to normal subjects. During grinding tasks, SMA, pro-SMA, the Rolandic operculum, and the putamen, show lower activation in patients with brux behaviors (35). It is implied that patients with these pathological jaw movements have less extensive activity patterns due to the habitual execution of these over-learned tasks, resulting in more efficient and controlled jaw muscle movement (55).

Patients with sleep bruxism exhibit changes in the neuronal excitability in the corticomotor control of the jaw-closing muscles. Motor-evoked potentials of the masseter and cortical area activation are lower in patients with sleep bruxism, while motor force control and masticatory motor learning are also affected (53). Conversely, increased cortical activation in the primary sensory motor cortex (BA 4), has been reported during clenching and chewing in patients with confirmed sleep bruxism, compared to healthy patients. However, no changes in the masticatory muscle activity were found (56). The authors suggested that patients with sleep bruxism may not have special trained skills for these tasks, thus showing an increased cortical activation. Another explanation proposed was that enhanced parafunctional activity with more recruitment of the tongue muscles, accompanied by jaw movements, could correspond to the increase in cortical activation. The authors also recommended confirmatory diagnosis of sleep bruxism with polysomnography rather than self-reported or oral evaluation of bruxism.

Patients with sleep bruxism also present abnormal excitability in the neural masticatory pathways compared to control subject, with possible involvement of neurotransmitters such as serotonin. These changes in neuronal excitability may be influenced by brainstem area, rather than the cortex (57).

4 Somatosensations and bruxism

Bruxism involves complex interactions between sensory and motor pathways, including proprioception and nociception. Understanding these mechanisms can provide insights into the pathophysiology of bruxism and potential therapeutic targets.

4.1 Proprioception in bruxism

Jaw tremor at a frequency of 6–10 Hz has been identified as potential biomarker of bruxism in humans. This increased jaw tremor in patients with sleep bruxism may be attributed to increased afferent feedback from the mechanoreceptors in the periodontal ligament (58).

Proprioception information from the periodontal ligament has been linked to the development of bruxism-like behavior in animal models. Mechanoreceptors in the periodontal ligament transmit the sensory information during grinding movements, allowing molar teeth to sense the magnitude and direction of the forces generated during tooth grinding (48).

The mesencephalic nucleus of the trigeminal nerve (Vmes) is crucial for processing the proprioceptive information from muscle spindles and the periodontal ligament (59). Vmes is a premotor area of Vmotor and contains excitatory neurons expressing the vesicular glutamate transporter 1 (VGLUT1). It has been suggested that Vmes is involved in the generation of masseter hypercontraction in mice. Studies using malocclusion models, such as anterior crossbite in rats, have shown high levels of VGLUT1 expression in Vmes, the periodontal ligament, Vmotor, and the masseter muscle. Increased acetylcholinesterase, a marker for muscle activity, was also observed in the masseter (60). These findings suggest that Vmes-mediated VGLUT1 expression plays a role in masseter hypercontraction.

A model of chronic restraint stress in mice showed increasing anxiety-like behaviors accompanied by masseter hyperactivity and enhanced excitability of Vmes neurons. Downregulation of VGLUT1 in Vmes reduced acetylcholinesterase and creatine kinase muscle-type levels in the masseter muscle, indicating reduced masseter hyperactivity (61).

Another malocclusion study showed development of anxiety-like behaviors and increased activity levels in the lateral habenula (62), a brain area involved in the processing of stress, anxiety, depression, and aversive stimuli (63, 64). Most efferent neurons in the lateral habenula are glutamatergic, expressing the vesicular glutamate transporter 2 (VGLUT2) (65). Furthermore, the lateral habenula neurons sent projections to Vmes, and increased VGLUT2 synaptic vesicles were found around the caudal portion of Vmes neurons after inducing a unilateral anterior crossbite. Downregulation of VGLUT2 expression in the lateral habenula also decreased Vmes activity (62).

Vmes neurons also receive projections from neurotransmitter systems implicated in bruxism. Caudal and to a lesser extent, rostral Vmes neurons receive dopaminergic, noradrenergic, GABAergic, and serotonergic fibers (66). Additionally, Vmes premotor neurons of Vmotor receive dense tyrosine hydroxylase innervation, possibly from the locus coeruleus, suggesting its potential role in generating bruxism-like behaviors (67).

4.2 Nociception in bruxism

Nociceptive processing in masticatory muscles may differ between bruxers and non-bruxers. Patients with bruxism may not show changes in masseter corticomotor excitability after noxious stimuli combined with masticatory-muscle training tasks, unlike control subjects who exhibit changes in corticomotor excitability (26).

In a study in anesthetized rats, electrical stimulation of the masseter muscle induced teeth clenching, suggesting a role for sensory innervation from the muscle in teeth clenching. Moreover, administration of dantrolene, a muscle relaxant, decreased the force of the teeth clenching, indicating the involvement of sensory information from orofacial structures in the neuropathogenesis of brux behaviors (68).

5 Neurotransmitters involved in bruxism

5.1 Glutamate and gamma-aminobutyric acid (GABA)

Both glutamatergic (excitatory) and GABAergic (inhibitory) systems could be affected in patients with bruxism. A study on occlusal splint users with possible bruxism showed that lower levels of GABA is and higher levels on Glutamine metabolites are present in the dorsolateral prefrontal cortex, while only GABA is increased in the thalamus, compared to the control group (69). On the other hand, decreased levels of GABA were found in the brainstem of subject with possible diagnosis of sleep bruxism (70), suggesting that the inhibitory control of the different nuclei in brainstem could be affected by this condition.

Disturbances in glutamatergic and GABAergic systems have been related to sleep bruxism. A negative feedback circuit between hippocampus and dorsolateral prefrontal cortex (DLPFC) might play an important role in regulating bruxism behaviors (69). Lower levels of GABA+ have been reported in the brainstems of potential sleep bruxism patients, suggesting that sleep bruxism might be primarily influenced by brainstem networks rather than cortical networks. Additionally, brainstem GABAergic networks might be a potential treatment target for this condition (70). However, well conducted clinical trials are necessary to validate these findings.

5.2 Serotonin

Serotonin is another neurotransmitter associated with bruxism, known for its role in regulating muscle tone during sleep and maintaining arousal states (71). Genetic studies have linked polymorphisms in serotonergic neurotransmission to an increased risk of sleep bruxism, such as the C allele of the HTR2A rs6313 single nucleotide polymorphism (SNP), which is associated with a 4.25 times higher risk of sleep bruxism, due to reduced expression of 5-HT2A receptors (71). *In vitro* studies using human induced pluripotent stem cells-derived neurons carrying the HTR2A C allele further revealed altered membrane properties, including increased action potential frequency and decreased half-duration, indicating heightened neuronal excitability in sleep bruxism patients (72).

Selective serotonin reuptake inhibitors (SSRIs), commonly used for chronic depression, have also been linked to the development of sleep bruxism. SSRIs like paroxetine, citalopram, and fluvoxamine have been shown to associate with increased incidence of bruxism (73–78). Furthermore, experimental studies in mice with citalopram injections demonstrated alterations in masseter muscle activity during non-REM sleep, indicating a potential role of serotonin in motor control during this phase, though not affecting the overall sleep–wake cycle distribution (79).

5.3 Dopamine

The dopaminergic system has also been suggested to be involved in the pathophysiology of bruxism. Dopamine plays a crucial role in motor control, and its dysregulation is observed in pathological conditions affecting motor functions, such as Parkinson's disease (80, 81).

In sleep bruxism, an imbalance in striatal dopamine receptor-2 (D2R) expression has been observed, with a unilateral decrease in D2R levels (82). Treatment with the D2R agonist bromocriptine has been shown to reduce the number of sleep bruxism episodes and correct the imbalance in D2R distribution (83). For patients with sleep/awake bruxism, reduced frontal lobe activity has been noted. These patients respond to metoclopramide, a selective D2R antagonist, which decreases bruxism episodes. This suggests hypersensitivity to presynaptic D2R in the prefrontal cortex, indicating a distinct etiopathogenesis from sleep bruxism (84).

Genetic studies have also linked single nucleotide polymorphisms (SNPs) in dopaminergic pathway genes to bruxism. The G allele of the DRD2 rs1800497 polymorphism is associated with a decreased risk of awake-sleep bruxism, possibly due to enhanced dopaminergic function. Similarly, the C allele of the DRD5 rs6283 polymorphism is linked to a decreased risk of awake bruxism, potentially due to changes in D5 receptors. Conversely, the C allele of the DRD3 rs6280 polymorphism is associated with an increased risk of sleep bruxism, likely due to increased dopamine release (6). These findings suggest that variations in dopamine levels may influence whether bruxism occurs during sleep, wakefulness, or both.

Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to symptoms such as muscle rigidity and tremor (81). Patients with PD often experience increased episodes of bruxism during both sleep and wakefulness, suggesting a potential link between dopaminergic dysfunction and bruxism (85, 86).

The association between dopamine and bruxism has also been demonstrated in animal studies. In a study with rats, injection of the dopamine receptor agonist combination of SKF 38393 and quinpirole

into the ventral striatum induced periodic episodes of audible teeth grinding at the later phases of the drug action. This grinding was accompanied by continuous activity in both the masseter and anterior digastric muscles during the peak drug effect. Interestingly, the rats also exhibited varied oral movements, including jaw opening and closing, lateral movements, and frequent tongue protrusion. In contrast, injection of the acetylcholine receptor agonist carbachol did not produce any teeth grinding (87).

Although the association between bruxism and malocclusion in humans is not strong (88), occlusal disharmonies and malocclusion have been associated with bruxism behaviors in animal models. Acute occlusal disharmonies in the lower incisors of rats have shown increase of DOPA levels in the striatum, frontal cortex, and hypothalamus, with dopamine increase in the hypothalamus, and both dopamine and noradrenaline in the frontal cortex (89).

Stress-induced non-functional masticatory activity in rats resulted in higher levels of extracellular dopamine in the prefrontal cortex, correlating with the severity of the activity. Additionally, stress-induced non-functional masticatory activity led to increased 3,4-Dihydroxyphenylalanine (DOPA) accumulation and decreased dopamine breakdown in the striatum (90, 91).

6 Other behaviors associated to bruxism

6.1 Stress and anxiety

Bruxism is a multifactorial condition whose etiology remains unclear, involving various psychological and physiological factors (92). Psychological traits, such as depressive symptoms, manic tendencies, stress, and anxiety have been closely associated with bruxism (93). Patients with mood disorders exhibit significantly higher odds of developing bruxism, underscoring the role of mental health in its pathogenesis (94).

Stress, in particular, has been implicated in disrupting neural synapses and connectivity, potentially exacerbating bruxism symptoms (95). Specific anxiety symptoms such as panic, stress sensitivity, and coping capability deficits are also linked with bruxism (96, 97). Opipramol, a tricyclic antidepressant, has been shown to reduce episodes of sleep bruxism, highlighting the therapeutic relevance of managing underlying anxiety (98).

Experimental studies in rodents have demonstrated that increased masticatory muscle hyperactivity resembling brux behaviors. Tooth clenching has been observed to impact cardiac output and activate the sympathetic nervous system, potentially linking it to stress responses (99). Interestingly, administration of diazepam, an anxiolytic drug that enhances GABA-receptors, reduces bruxism-like behaviors in rats, suggesting a modulation of masticatory muscles hyperactivity by emotional state (100). Higher brain areas implicated in stress regulation, such as the amygdala or thalamus, along with changes in the GABAergic system, may be involved in the development of bruxism under stress conditions.

Chronic malocclusion, such as unilateral anterior crossbite, has been shown to increase anxiety-like behaviors in rodents. Excitatory neurons expressing VGLUT1 in the dorsomedial part of the principal sensory trigeminal nucleus (Vpdm), a key area in the trigeminal system and postsynaptic to Vmes, appear pivotal in the anxiety induced by malocclusion. Inhibition of these VGLUT1+ neurons in Vpdm alleviates malocclusion-induced anxiety, while their activation alone induces anxiety-like behaviors irrespective of malocclusion presence (101). Moreover, Vmes neurons transmit proprioceptive information to Vdpm, whereas Vpdm neurons project to the ventral posteromedial nucleus (VPM). Activation of VPM neurons, which receive innervation from Vpdm, correlates with anxiety generation, suggesting a neural pathway where VPM activation may contribute to anxiety-like behaviors induced by malocclusion. Given that both anxiety and malocclusion models in rodents demonstrate masseter muscle hyperactivity, proprioception processed in Vpdm could play a role in the development of bruxism-like behaviors.

Restraint stress models in rodents also induce bruxism behaviors, with behaviors like wood chewing and gnawing observed to alleviate these conditions (61, 102–104). The paraventricular hypothalamic nucleus (PVH) is a brain area involved in the regulation of trigeminal system neuronal activity during stress, with chronic restriction stress models showing PVH activation. PVH also shows projections to the trigeminal system brain areas, like Vmotor, Vmes and the peritrigeminal premotor area (Peri5). Studies suggest that PVH-Peri5 neural connections may contribute to bruxism-like behaviors following restraint stress in mice, with inhibition of PVH neurons projecting to Peri5 reducing gnawing behaviors (101).

Other brain areas associated with stress and emotional control, like CeA, lateral hypothalamic area, and parasubthalamic nucleus, directly or indirectly activate Vmotor by projecting to Peri5, Vmes, and the supratrigeminal nucleus (105).

6.2 Sleep–wake states and bruxism

6.2.1 Sleep–wake regulation of masticatory muscle activity

The sleep wake states play a role in the activity of the masticatory muscles. In a study in guinea pigs, the overall masseter and neck muscles activation were recorded during sleep–wake cycles. While the neck muscles activity was decreased during non-REM, and almost not present during REM sleep, the magnitude of the masseter activity during both REM and non-REM sleep was five times lower than during wakefulness. Overall, the activation patterns of the masseter muscles during sleep–wake cycles differ from those observed in the neck muscles, indicating that motor activation and inhibition in the masticatory muscles may be regulated distinctly during sleep (106).

This distinct regulation was also observed in mice, where the activity of the masseter and neck muscles activity showed correlation during non-REM sleep, but this correlation was absent during REM sleep. During REM sleep, phasic twitches are frequently found for the masseter muscle but not the neck muscles. Moreover, the masseter activity during non-REM sleep was bimodal, with higher activity during arousal, while the neck muscles only present unimodal firing activity. Similar patterns were observed during wakefulness, where bimodal activation of the masseter muscle reflected its dual role in fine jaw movement control with low-level contraction, as well as high-level contraction for tasks requiring more muscle force (107).

6.2.2 Bruxism and sleep architecture

Sleep bruxism episodes and tooth grinding during sleep have been proposed as manifestations of micro-arousal or awakening, due to a response to cortical arousal (108, 109). Rhythmic masticatory muscles activities (RMMA) episodes observed in patients with sleep bruxism are preceded by cortical EEG changes, followed by heart rate acceleration. These RMMA events have also been associated with concurrent movements of the legs and neck, indicating that these orofacial muscle activations may occur in response to micro-arousals (110). The study proposes that RMMA are preceded by increased brain activity and autonomic nervous system activation. Given the involvement of heart rate changes and the autonomic system activity in sleep bruxism, the use of clonidine, an antihypertensive drug, has been shown to reduce the episodes of sleep bruxism, possibly due to suppression of the sympathetic innervation (111).

Patients with severe sleep bruxism present a shortened rapid-eye movement (REM) sleep latency and percentage of occurrence, and an increase in the number of the sleep stage transitions (112). REM sleep and sleep stage N2 seem to be fragmented in patients with sleep bruxism however, this was not related to RMMA episodes. RMMA may be present during long runs of N3 sleep stage, suggesting that this muscle activation is related to dissipation of the sleep pressure (113). Furthermore, while sleep bruxism may be associated with insomnia, RMMA episodes do not seem to be the cause (114). These results may imply that patients with sleep bruxism present altered sleep architecture, possibly related to REM sleep, regardless of RMMA episode occurrence.

Sleep bruxism episodes are observed to be more frequent during the non-REM sleep stage 1 or 2 compared to REM sleep, suggesting a stage-specific occurrence pattern (109, 115). Patients with bruxism also exhibit altered theta wavelength activity during sleep (116). The microstructural aspect of sleep are affected in individuals with sleep bruxism, characterized by reduced K-complexes and K-alpha index during the non-REM stage 2 (115).

Interestingly, patients with severe sleep bruxism accompanied by temporomandibular joint and masticatory muscle pain, are more likely to present brux episodes during REM sleep, compared to patients without severe bruxism (117). Similarly, there are reports linking REM sleep behavior disorder with severe sleep bruxism, indicating a potential subclinical consequence of REM disturbances (118).

In patients with altered consciousness states such as coma, bruxism episodes can occur at varying levels of consciousness, and typically resolve with improvements of consciousness levels, coinciding with the return of the sleep–wake cycles (119). Studies on patients with acquired brain injury also exhibit a high incidence of bruxism, indicating its association with altered states of consciousness (120).

Overall, the sleep architecture of patients with sleep bruxism appears to be disrupted, with bruxism episodes potentially linked to disturbances in the sleep–wake cycle. However, the variability of these changes may depend on the severity of the condition and its interaction with other sleep disturbances.

6.3 Tooth clenching and limb muscle activity

Voluntary teeth clenching, also known as the Jendrassik maneuver, enhances tendon reflexes in the upper and lower extremities by facilitating peripheral monosynaptic reflexes (H-reflexes) (121). This facilitation is likely influenced by central motor control and sensory feedback from the muscle spindles and periodontal ligament (122), suggesting a possible role of orofacial proprioception in the regulation of limb muscle function. In humans, H-reflexes in muscles like soleus and the flexor carpi radialis are boosted during voluntary tooth clenching (122, 123). Conversely, tooth clenching may reduce deltoid muscle recruitment through presynaptic inhibition of muscle spindle inputs to the motor neurons of the deltoid muscles (124); while tooth grinding during sleep could inhibit the H-reflex of the gastrocnemius muscle (125).

The relationship between muscle reflexes and sleep–wake states remains unclear despite evidence suggesting a link to arousal states. During voluntary clenching, corticospinal pathways are implicated in increased recruitment of hand and leg muscles (121, 126). Additionally, teeth clenching neural circuitry seems to be more complex than the one involved in hand clenching (127). Muscle recruitment facilitation during teeth clenching may involve both cortical and subcortical areas for the upper extremities, while only subcortical areas are involved for the lower extremities (121). Motor-evoked response facilitation using transcranial magnetic stimulations have shown that an anterior-medially current direction facilitates the motor responses of the finger muscles at early stages of voluntary clenching task, possibly by influencing the motor cortical circuitry (128, 129). The motor cortex may regulate the hand muscles at the beginning of clenching, while the spinal cord may stabilize this activation at the late phase of clenching. Additionally, activation of both the hand motor area and greater hand muscle excitability during the execution phase of clenching (36). This may be explained as teeth clenching neural circuitry, possibly the primary motor cortex, cingulate motor area or supplementary motor area, may activate the hang region in the primary motor cortex (36, 130). The role of clenching in the control of limb muscles is suggested to be necessary for body stabilization, as in tasks such as weightlifting.

Sleep disorders involving motor activity, such as sleep bruxism and periodic limb movements during sleep, often co-occur and exhibit temporal concurrence with electroencephalogram arousals, indicating shared neurophysiological mechanisms (131–134). Patients with sleep bruxism frequently exhibit RMMA, accompanied by limb movements during sleep, indicating a response to cortical arousal (109, 135). These episodes, especially when accompanied by limb movements, suggest that sleep bruxism may be linked to cortical arousal and neural pathways interconnected with limb muscle facilitation.

7 Future insights and clinical implications

The understanding of the neural circuitry and neurotransmitters involved in bruxism holds the potential to develop future therapeutic strategies to effectively manage this condition.

While new technologies in both human and animal studies are shedding light on some aspects of the neural pathways involved in bruxism, many aspects remain unclear. This suggests the need for more studies to improve the treatment approach for this condition. Conducting more clinical studies that integrate neuronal activity, neuronal plasticity assessments, and electromyographic recording of the masticatory muscles would be valuable. Additionally, animal

studies utilizing genetic tools and advanced techniques in neuronal tracing and manipulation can provide crucial insights.

Priority should be given to investigating the function of the trigeminal system under stress and anxiety conditions to better comprehend the development of masticatory muscle hyperactivity and bruxism. Exploring the roles and neuronal connectivity of areas such as Vmes, Peri5, Vpdm, and Vmotor could significantly advance our understanding in this field.

8 Conclusion

Bruxism is a multifactorial condition that can be present during wakefulness, sleep, or wake–sleep cycles, potentially involving distinct neural pathways for each subset. Higher brain regions responsible for processing stress and anxiety, along with nuclei within the trigeminal system, exhibit extensive neural connectivity that likely contributes to the pathophysiology of bruxism behaviors. Disruptions in serotonin and dopamine systems are implicated in the development of this disorder, highlighting their potential roles in its pathophysiology.

Author contributions

KHUK: Writing – original draft, Writing – review & editing. AADC: Writing – original draft. PL: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, et al. Bruxism defined and graded: an international consensus. *J Oral Rehabil*. (2013) 40:2–4. doi: 10.1111/joor.12011

2. Manfredini D, Winocur E, Guarda-Nardini L, Paesani D, Lobbezoo F. Epidemiology of bruxism in adults: a systematic review of the literature. *J Orofac Pain*. (2013) 27:99–110. doi: 10.11607/jop.921

3. Melo G, Duarte J, Pauletto P, Porporatti AL, Stuginski-Barbosa J, Winocur E, et al. Bruxism: an umbrella review of systematic reviews. *J Oral Rehabil*. (2019) 46:666–90. doi: 10.1111/joor.12801

4. De Leeuw R, Klasser GDAmerican Academy of Orofacial Pain. Orofacial pain. Illinois, US: Guidelines for assessment, diagnosis, and management Quintessence Publishing Company, Incorporated (2013).

5. Lobbezoo F, Ahlberg J, Raphael KG, Wetselaar P, Glaros AG, Kato T, et al. International consensus on the assessment of bruxism: report of a work in progress. *J Oral Rehabil*. (2018) 45:837–44. doi: 10.1111/joor.12663

6. Oporto GH, Bornhardt T, Iturriaga V, Salazar LA. Single nucleotide polymorphisms in genes of dopaminergic pathways are associated with bruxism. *Clin Oral Investig*. (2018) 22:331–7. doi: 10.1007/s00784-017-2117-z

7. Murali RV, Rangarajan P, Mounissamy A. Bruxism: conceptual discussion and review. *J Pharm Bioallied Sci*. (2015) 7:265–S270. doi: 10.4103/0975-7406.155948

8. Abe S, Yamaguchi T, Rompré PH, De Grandmont P, Chen Y-J, Lavigne GJ. Tooth wear in young subjects: a discriminator between sleep bruxers and controls? *Int J Prosthodont*. (2009) 22:342–50.

9. Manfredini D, Lobbezoo F. Relationship between bruxism and temporomandibular disorders: a systematic review of literature from 1998 to 2008. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. (2010) 109:e26–50. doi: 10.1016/j.tripleo.2010.02.013

10. Manfredini D, Poggio CE, Lobbezoo F. Is bruxism a risk factor for dental implants? A systematic review of the literature. *Clin Implant Dent Relat Res*. (2014) 16:460–9. doi: 10.1111/cid.12015

11. Macedo CR, Silva AB, Machado MA, Saconato H, Prado GF. Occlusal splints for treating sleep bruxism (tooth grinding). *Cochrane Database Syst Rev*. (2007) 2010:CD005514. doi: 10.1002/14651858.CD005514.pub2

12. Okeson JP. The effects of hard and soft occlusal splints on nocturnal bruxism. *J Am Dent Assoc*. (1987) 114:788–91. doi: 10.14219/jada.archive.1987.0165

13. Tan EK, Jankovic J. Treating severe bruxism with botulinum toxin. *J Am Dent Assoc*. (2000) 131:211–6. doi: 10.14219/jada.archive.2000.0149

14. Lobbezoo F, Naeije M. Bruxism is mainly regulated centrally, not peripherally. *J Oral Rehabil*. (2001) 28:1085–91. doi: 10.1046/j.1365-2842.2001.00839.x

15. Lobbezoo F, Visscher CM, Ahlberg J, Manfredini D. Bruxism and genetics: a review of the literature. *J Oral Rehabil*. (2014) 41:709–14. doi: 10.1111/joor.12177

16. Flueraşu MI, Bocşan IC, Ţig IA, Iacob SM, Popa D, Buduru S. The epidemiology of bruxism in relation to psychological factors. *Int J Environ Res Public Health*. (2022) 19:691. doi: 10.3390/ijerph19020691

17. Aloé F. Sleep bruxism neurobiology. *Sleep Sci*. (2009) 2:40–8.

18. George S, Joy R, Roy A. Drug-induced bruxism: a comprehensive literature review. *J Adv Oral Res*. (2021) 12:187–92. doi: 10.1177/2320206821992534

19. Bertazzo-Silveira E, Kruger CM, Porto De Toledo I, Porporatti AL, Dick B, Flores-Mir C, et al. Association between sleep bruxism and alcohol, caffeine, tobacco, and drug abuse: a systematic review. *J Am Dent Assoc*. (2016) 147:859–866.e4. doi: 10.1016/j.adaj.2016.06.014

20. Boscato N, Exposto F, Nascimento GG, Svensson P, Costa YM. Is bruxism associated with changes in neural pathways? A systematic review and meta-analysis of clinical studies using neurophysiological techniques. *Brain Imaging Behav*. (2022) 16:2268–80. doi: 10.1007/s11682-021-00601-w

21. Machado E, Dal-Fabbro C, Cunali PA, Kaizer OB. Prevalence of sleep bruxism in children: a systematic review. *Dental Press J Orthod*. (2014) 19:54–61. doi: 10.1590/2176-9451.19.6.054-061.oar

22. Manfredini D, Restrepo C, Diaz-Serrano K, Winocur E, Lobbezoo F. Prevalence of sleep bruxism in children: a systematic review of the literature. *J Oral Rehabil*. (2013) 40:631–42. doi: 10.1111/joor.12069

23. Hück NL, Abbink JH, Hoogenkamp E, Van Der Bilt A, Van Der Glas HW. Exteroceptive reflexes in jaw-closing muscle EMG during rhythmic jaw closing and clenching in man. *Exp Brain Res*. (2005) 162:230–8. doi: 10.1007/s00221-004-2167-8

24. Svensson P, De Laat A, Graven-Nielsen T, Arendt-Nielsen L, Macaluso GM. Effect of clenching levels on heteronymous H-reflex in human temporalis muscle. *Exp Brain Res*. (1999) 126:467–72. doi: 10.1007/s002210050754

25. D'Amico JM, Yavuz SU, Saraçoğlu A, Atiş ES, Gorassini MA, Türker KS. Activation properties of trigeminal motoneurons in participants with and without bruxism. *J Neurophysiol*. (2013) 110:2863–72. doi: 10.1152/jn.00536.2013

26. Boscato N, Exposto FG, Costa YM, Svensson P. Effect of standardized training in combination with masseter sensitization on corticomotor excitability in bruxer and control individuals: a proof of concept study. *Sci Rep*. (2022) 12:17469–14. doi: 10.1038/s41598-022-21504-w

27. Gastaldo E, Quatrale R, Graziani A, Eleopra R, Tugnoli V, Tola MR, et al. The excitability of the trigeminal motor system in sleep bruxism: a transcranial magnetic stimulation and brainstem reflex study. *J Orofac Pain*. (2006) 20:145–55. doi: 10.11607/jofph.20145

28. İnan R, Şenel GB, Yavlal F, Karadeniz D, Gündüz A, Kızıltan ME. Sleep bruxism is related to decreased inhibitory control of trigeminal motoneurons, but not with reticulobulbar system. *Neurol Sci*. (2017) 38:75–81. doi: 10.1007/s10072-016-2711-x

29. Ariji Y, Kondo H, Miyazawa K, Sakuma S, Tabuchi M, Kise Y, et al. Study on regional activities in the human brain caused by low-level clenching and tooth separation: investigation with functional magnetic resonance imaging. *Oral Sci Int*. (2019) 16:87–94. doi: 10.1002/osi2.1020

30. Ariji Y, Koyama S, Sakuma S, Nakayama M, Ariji E. Regional brain activity during jaw clenching with natural teeth and with occlusal splints: a preliminary functional MRI study. *Cranio J Craniomandib Pract*. (2016) 34:188–94. doi: 10.1179/2151090315Y.0000000017

31. Iida T, Overgaard A, Komiyama O, Weibull A, Baad-Hansen L, Kawara M, et al. Analysis of brain and muscle activity during low-level tooth clenching – a feasibility study with a novel biting device. *J Oral Rehabil*. (2014) 41:93–100. doi: 10.1111/joor.12128

32. Iida T, Kawara M, Hironaga N, Ioannides AA. Cerebellar activity before teeth-clenching using magnetoencephalography. *J Prosthodont Res*. (2010) 54:48–52. doi: 10.1016/j.jpor.2009.09.003

33. Astorga G, Li D, Therreau L, Kassa M, Marty A, Llano I. Concerted interneuron activity in the cerebellar molecular layer during rhythmic oromotor behaviors. *J Neurosci*. (2017) 37:11455–68. doi: 10.1523/JNEUROSCI.1091-17.2017

34. Farris SM. Evolution of complex higher brain centers and behaviors: behavioral correlates of mushroom body elaboration in insects. *Brain Behav Evol*. (2013) 82:9–18. doi: 10.1159/000352057

35. Byrd KE, Romito LM, Dzemidzic M, Wong D, Talavage TM. FMRI study of brain activity elicited by oral parafunctional movements. *J Oral Rehabil*. (2009) 36:346–61. doi: 10.1111/j.1365-2842.2009.01947.x

36. Furubayashi T, Sugawara K, Kasai T, Hayashi A, Hanajima R, Shiio Y, et al. Remote effects of self-paced teeth clenching on the excitability of hand motor area. *Exp Brain Res*. (2003) 148:261–5. doi: 10.1007/s00221-002-1299-y

37. Iida T, Fenwick PBC, Ioannides AA. Analysis of brain activity immediately before conscious teeth clenching using magnetoencephalographic method. *J Oral Rehabil*. (2007) 34:487–96. doi: 10.1111/j.1365-2842.2007.01736.x

38. Romeo M, Vizioli L, Breukink M, Aganloo K, Lao J, Cotrufo S, et al. A functional magnetic resonance imaging paradigm to identify distinct cortical areas of facial function: a reliable localizer. *Plast Reconstr Surg*. (2013) 131:527e–33e. doi: 10.1097/PRS.0b013e3182818b68

39. Tamura T, Kanayama T, Yoshida S, Kawasaki T. Functional magnetic resonance imaging of human jaw movements. *J Oral Rehabil*. (2003) 30:614–22. doi: 10.1046/j.1365-2842.2003.01054.x

40. Wang Y, Ma X, Jin Z, Zhang L. Brain activities during maximum voluntary clenching with soft occlusal pad in healthy adults by functional magnetic resonance imaging. *Hua Xi Kou Qiang Yi Xue Za Zhi*. (2005) 23:57–9.

41. Watson C, Walshaw D, McMillan AS. Effect of motor tasks on the cortical topography of the human masseter muscle. *Arch Oral Biol*. (2000) 45:767–73. doi: 10.1016/S0003-9969(00)00051-0

42. Jiang H, Liu H, Liu G, Jin Z, Liu X. The effects of chewing-side preference on human brain activity during tooth clenching: an fMRI study. *J Oral Rehabil*. (2010) 37:877–83. doi: 10.1111/j.1365-2842.2010.02115.x

43. Zaproudina N, Rissanen APE, Lipponen JA, Vierola A, Rissanen SM, Karjalainen PA, et al. Tooth clenching induces abnormal cerebrovascular responses in migraineurs. *Front Neurol*. (2018) 9:1–8. doi: 10.3389/fneur.2018.01112

44. Kothari M, Madsen VLF, Castrillon EE, Nielsen JF, Svensson P. Spontaneous jaw muscle activity in patients with acquired brain injuries—preliminary findings. *J Prosthodont Res*. (2018) 62:268–72. doi: 10.1016/j.jpor.2017.05.004

45. Lavigne G, Manzini C. Bruxism In: MH Kryger, T Roth and WC Dement, editors. Principle and practice of sleep medicine. Philadelphia: WB Saunders (2000). 62: 773–85.

46. Meletti S, Cantalupo G, Volpi L, Rubboli G, Magaudda A. Rhythmic teeth grinding induced. *Neurology*. (2004) 62:3–6. doi: 10.1212/wnl.62.12.2306

47. Kawamura Y, Tsukamoto S, Miyoshi K. Experimental studies on neural mechanisms of bruxism. *J Dent Res*. (1961) 40:217. doi: 10.1177/00220345610400011601

48. Nagata K, Itoh S, Tsuboi A, Takafuji Y, Tabata T, Watanabe M. Response properties of periodontal mechanosensitive neurons in the trigeminal ganglion of rabbit and neuronal activities during grinding-like jaw movement induced by cortical stimulation. *Arch Oral Biol*. (2008) 53:1138–48. doi: 10.1016/j.archoralbio.2008.06.007

49. Liatis T, Madden M, Marioni-Henry K. Bruxism in awake dogs as a clinical sign of forebrain disease: 4 cases. *J Vet Intern Med*. (2022) 36:2132–41. doi: 10.1111/jvim.16570

50. Kaya B, Geha P, de Araujo I, Cioffi I, Moayedi M. Identification of central amygdala and trigeminal motor nucleus connectivity in humans: an ultra-high field diffusion MRI study. *Hum Brain Mapp*. (2023) 44:1309–19. doi: 10.1002/hbm.26104

51. Stalnaker TA, España RA, Berridge CW. Coping behavior causes asymmetric changes in neuronal activation in the prefrontal cortex and amygdala. *Synapse*. (2009) 63:82–5. doi: 10.1002/syn.20583

52. Iida T, Komiyama O, Obara R, Baad-Hansen L, Kawara M, Svensson P. Repeated clenching causes plasticity in corticomotor control of jaw muscles. *Eur J Oral Sci*. (2014) 122:42–8. doi: 10.1111/eos.12101

53. Ikuta M, Iida T, Kothari M, Shimada A, Komiyama O, Svensson P. Impact of sleep bruxism on training-induced cortical plasticity. *J Prosthodont Res*. (2019) 63:277–82. doi: 10.1016/j.jpor.2018.12.008

54. Yilmaz S. To see bruxism: a functional MRI study. *Dentomaxillofacial Radiol*. (2015) 44:20150019. doi: 10.1259/dmfr.20150019

55. Wong D, Dzemidzic M, Talavage TM, Romito LM, Byrd KE. Motor control of jaw movements: an fMRI study of parafunctional clench and grind behavior. *Brain Res*. (2011) 1383:206–17. doi: 10.1016/j.brainres.2011.01.096

56. Kervancioglu BB, Teismann IK, Rain M, Hugger S, Boeckmann JA, Young P, et al. Sensorimotor cortical activation in patients with sleep bruxism. *J Sleep Res*. (2012) 21:507–14. doi: 10.1111/j.1365-2869.2012.01005.x

57. Huang H, Song YH, Wang JJ, Guo Q, Liu WC. Excitability of the central masticatory pathways in patients with sleep bruxism. *Neurosci Lett*. (2014) 558:82–6. doi: 10.1016/j.neulet.2013.11.014

58. Laine CM, Yavuz U, D'Amico JM, Gorassini MA, Türker KS, Farina D. Jaw tremor as a physiological biomarker of bruxism. *Clin Neurophysiol*. (2015) 126:1746–53. doi: 10.1016/j.clinph.2014.11.022

59. Singh GP In: GP Rath, editor. Anatomy of trigeminal nerve BT – handbook of trigeminal neuralgia. Singapore: Springer Singapore (2019). 11–22.

60. Liu X, Zhang C, Wang D, Zhang H, Liu X, Li J, et al. Proprioceptive mechanisms in occlusion-stimulated masseter hypercontraction. *Eur J Oral Sci*. (2017) 125:127–34. doi: 10.1111/eos.12331

61. Zhao YJ, Liu Y, Wang J, Li Q, Zhang ZM, Tu T, et al. Activation of the mesencephalic trigeminal nucleus contributes to masseter hyperactivity induced by chronic restraint stress. *Front Cell Neurosci*. (2022) 16:1–13. doi: 10.3389/fncel.2022.841133

62. Liu X, Zhou KX, Yin NN, Zhang CK, Shi MH, Zhang HY, et al. Malocclusion generates anxiety-like behavior through a putative lateral Habenula–mesencephalic trigeminal nucleus pathway. *Front Mol Neurosci*. (2019) 12:1–15. doi: 10.3389/fnmol.2019.00174

63. Jacinto LR, Mata R, Novais A, Marques F, Sousa N. The habenula as a critical node in chronic stress-related anxiety. *Exp Neurol*. (2017) 289:46–54. doi: 10.1016/j.expneurol.2016.12.003

64. Yang SH, Yang E, Lee J, Kim JY, Yoo H, Park HS, et al. Neural mechanism of acute stress regulation by trace aminergic signalling in the lateral habenula in male mice. *Nat Commun*. (2023) 14:2435. doi: 10.1038/s41467-023-38180-7

65. Fremeau RT, Troyer MD, Pahner I, Nygaard GO, Tran CH, Reimer RJ, et al. The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron*. (2001) 31:247–60. doi: 10.1016/S0896-6273(01)00344-0

66. Copray JCVM, Ter Horst GJ, Liem RSB, Van Willigen JD. Neurotransmitters and neuropeptides within the mesencephalic trigeminal nucleus of the rat: an immunohistochemical analysis. *Neuroscience*. (1990) 37:399–411. doi: 10.1016/0306-4522(90)90410-6

67. Mascaro MB, Bittencourt JC, Casatti CA, Elias CF. Alternative pathways for catecholamine action in oral motor control. *Neurosci Lett*. (2005) 386:34–9. doi: 10.1016/j.neulet.2005.05.062

68. Takahata T, Shouji I, Maruyama S, Sato Y, Nishida Y, Ueno T. Teeth clenching and positive acceleration-induced cerebral arterial hypotension in rats. *Aviat Sp Environ Med*. (2011) 82:442–7. doi: 10.3357/ASEM.2741.2011

69. Dharmadhikari S, Romito LM, Dzemidzic M, Dydak U, Xu J, Bodkin CL, et al. GABA and glutamate levels in occlusal splint-wearing males with possible bruxism. *Arch Oral Biol*. (2015) 60:1021–9. doi: 10.1016/j.archoralbio.2015.03.006

70. Fan X, Qu F, Wang J-J, Du X, Liu W-C. Decreased γ-aminobutyric acid levels in the brainstem in patients with possible sleep bruxism: a pilot study. *J Oral Rehabil*. (2017) 44:934–40. doi: 10.1111/joor.12572

71. Abe Y, Suganuma T, Ishii M, Yamamoto G, Gunji T, Clark GT, et al. Association of genetic, psychological and behavioral factors with sleep bruxism in a Japanese population. *J Sleep Res*. (2012) 21:289–96. doi: 10.1111/j.1365-2869.2011.00961.x

72. Sarkar AK, Nakamura S, Nakai K, Sato T, Shiga T, Abe Y, et al. Increased excitability of human iPSC-derived neurons in HTR2A variant-related sleep bruxism. *Stem Cell Res*. (2022) 59:102658. doi: 10.1016/j.scr.2022.102658

73. Ak M, Gulsun M, Uzun O, Gumus HO. Bruxism associated with serotonin reuptake inhibitors. *J Clin Psychopharmacol*. (2009) 29:620–2. doi: 10.1097/JCP.0b013e3181c0e942

74. Kishi Y. Paroxetine-induced bruxism effectively treated with tandospirone. *J Neuropsychiatry Clin Neurosci*. (2007) 19:90–1. doi: 10.1176/jnp.2007.19.1.90

75. Lobbezoo F, van Denderen RJ, Verheij JG, Naeije M. Reports of SSRI-associated bruxism in the family physician's office. *J Orofac Pain*. (2001) 15:340–6. doi: 10.11607/jofph.15405

76. Miyaoka T, Yasukawa R, Mihara T, Shimizu Y, Tsubouchi K, Maeda T, et al. Successful electroconvulsive therapy in major depression with fluvoxamine-induced bruxism. *J ECT*. (2003) 19:170–2. doi: 10.1097/00124509-200309000-00010

77. Romanelli F, Adler DA, Bungay KM. Possible paroxetine-induced bruxism. *Ann Pharmacother*. (1996) 30:1246–8. doi: 10.1177/106002809603001107

78. Wise MEJ. Citalopram-induced bruxism. *Br J Psychiatry*. (2001) 178:182–2. doi: 10.1192/bjp.178.2.182

79. Ikawa Y, Mochizuki A, Katayama K, Kato T, Ikeda M, Abe Y, et al. Effects of citalopram on jaw-closing muscle activity during sleep and wakefulness in mice. *Neurosci Res*. (2016) 113:48–55. doi: 10.1016/j.neures.2016.07.004

80. Blum K, Cshen ALC, Giordano J, Borsten J, Chen TJH, Hauser M, et al. The addictive brain: all roads lead to dopamine. *J Psychoactive Drugs*. (2012) 44:134–43. doi: 10.1080/02791072.2012.685407

81. Kalia LV, Lang AE. Parkinson's disease. *Lancet*. (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3

82. Lobbezoo F, Soucy JP, Montplaisir JY, Lavigne GJ. Striatal D2 receptor binding in sleep bruxism: a controlled study with iodine-123-iodobenzamide and single-photon-emission computed tomography. *J Dent Res*. (1996) 75:1804–10. doi: 10.1177/00220345960750101401

83. Lobbezoo F, Soucy JP, Hartman NG, Montplaisir JY, Lavigne GJ. Effects of the D2 receptor agonist bromocriptine on sleep bruxism: report of two single-patient clinical trials. *J Dent Res*. (1997) 76:1610–4. doi: 10.1177/00220345970760091401

84. Chen WH, Lu YC, Lui CC, Liu JS. A proposed mechanism for diurnal/nocturnal bruxism: hypersensitivity of presynaptic dopamine receptors in the frontal lobe. *J Clin Neurosci*. (2005) 12:161–3. doi: 10.1016/j.jocn.2004.07.007

85. Verhoeff MC, Koutris M, van Selms MKA, Brandwijk AN, Heres MS, Berendse HW, et al. Is dopaminergic medication dose associated with self-reported bruxism in Parkinson's disease? A cross-sectional, questionnaire-based study. *Clin Oral Investig*. (2021) 25:2545–53. doi: 10.1007/s00784-020-03566-0

86. Verhoeff MC, Lobbezoo F, Wetselaar P, Aarab G, Koutris M. Parkinson's disease, temporomandibular disorders and bruxism: a pilot study. *J Oral Rehabil*. (2018) 45:854–63. doi: 10.1111/joor.12697

87. de Beltrán KK, Koshikawa N, Saigusa T, Watanabe K, Koshida Y, Kobayashi M. Cholinergic/dopaminergic interaction in the rat striatum assessed from drug-induced repetitive oral movements. *Eur J Pharmacol*. (1992) 214:181–9. doi: 10.1016/0014-2999(92)90117-M

88. Ribeiro-Lages MB, Martins ML, Magno MB, Masterson Ferreira D, Tavares-Silva CM, Fonseca-Gonçalves A, et al. Is there association between dental malocclusion and bruxism? A systematic review and meta-analysis. *J Oral Rehabil*. (2020) 47:1304–18. doi: 10.1111/joor.12971

89. Areso MP, Giralt MT, Sainz B, Prieto M, García-Vallejo P, Gómez EM. Occlusal disharmonies modulate central catecholaminergic activity in the rat. *J Dent Res*. (1999) 78:1204–13. doi: 10.1177/00220345990780060301

90. Gómez FM, Giralt MT, Sainz B, Arrúe A, Prieto M, García-Vallejo P. A possible attenuation of stress-induced increases in striatal dopamine metabolism by the expression of non-functional masticatory activity in the rat. *Eur J Oral Sci*. (1999) 107:461–7. doi: 10.1046/j.0909-8836.1999.eos107607.x

91. Gómez FM, Ortega JE, Horrillo I, Meana JJ. Relationship between non-functional masticatory activity and central dopamine in stressed rats. *J Oral Rehabil*. (2010) 37:827–33. doi: 10.1111/j.1365-2842.2010.02110.x

92. Maciejewska-Szaniec Z, Kaczmarek-Ryś M, Hryhorowicz S, Przystańska A, Gredes T, Maciejewska B, et al. Polymorphic variants in genes related to stress coping are associated with the awake bruxism. *BMC Oral Health*. (2021) 21:496. doi: 10.1186/s12903-021-01844-1

93. Manfredini D, Landi N, Romagnoli M, Bosco M. Psychic and occlusal factors in bruxers. *Aust Dent J*. (2004) 49:84–9. doi: 10.1111/j.1834-7819.2004.tb00055.x

94. John MT. Mood psychopathology is possibly associated with bruxism. *J Evid Based Dent Pract*. (2006) 6:189–90. doi: 10.1016/j.jebdp.2006.04.005

95. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med*. (2016) 22:238–49. doi: 10.1038/nm.4050

96. Manfredini D, Arreghini A, Lombardo L, Visentin A, Cerea S, Castroflorio T, et al. Assessment of anxiety and coping features in bruxers: a portable electromyographic and electrocardiographic study. *J Oral Facial Pain Headache*. (2016) 30:249–54. doi: 10.11607/ofph.1616

97. Manfredini D, Landi N, Fantoni F, Segù M, Bosco M. Anxiety symptoms in clinically diagnosed bruxers. *J Oral Rehabil*. (2005) 32:584–8. doi: 10.1111/j.1365-2842.2005.01462.x

98. Wieckiewicz M, Martynowicz H, Wieczorek T, Wojakowska A, Sluzalec-Wieckiewicz K, Gac P, et al. Consecutive controlled case series on effectiveness of opipramol in severe sleep bruxism management—preliminary study on new therapeutic path. *Brain Sci*. (2021) 11:1–14. doi: 10.3390/brainsci11020146

99. Zhang M, Hasegawa Y, Sakagami J, Ono T, Hori K, Maeda Y, et al. Effects of unilateral jaw clenching on cerebral/systemic circulation and related autonomic nerve activity. *Physiol Behav*. (2012) 105:292–7. doi: 10.1016/j.physbeh.2011.07.028

100. Rosales VP, Ikeda K, Hizaki K, Naruo T, Nozoe SI, Ito G. Emotional stress and brux-like activity of the masseter muscle in rats. *Eur J Orthod*. (2002) 24:107–17. doi: 10.1093/ejo/24.1.107

101. Ji YY, Liu X, Li X, Xiao YF, Ma T, Wang J, et al. Activation of the VpdmVGLUT1-VPM pathway contributes to anxiety-like behaviors induced by malocclusion. *Front Cell Neurosci*. (2022) 16:1–21. doi: 10.3389/fncel.2022.995345

102. Ono Y, Kataoka T, Miyake S, Cheng SJ, Tachibana A, Sasaguri KI, et al. Chewing ameliorates stress-induced suppression of hippocampal long-term potentiation. *Neuroscience*. (2008) 154:1352–9. doi: 10.1016/j.neuroscience.2008.04.057

103. Sato C, Sato S, Takashina H, Ishii H, Onozuka M, Sasaguri K. Bruxism affects stress responses in stressed rats. *Clin Oral Investig*. (2010) 14:153–60. doi: 10.1007/s00784-009-0280-6

104. Xiao F, Hu A, Meng B, Zhang Y, Han W, Su J. PVH-Peri5 pathway for stress-coping oromotor and anxious behaviors in mice. *J Dent Res*. (2023) 102:227–37. doi: 10.1177/00220345221130305

105. Mascaro MB, Prosdócimi FC, Bittencourt JC, Elias CF. Forebrain projections to brainstem nuclei involved in the control of mandibular movements in rats. *Eur J Oral Sci*. (2009) 117:676–84. doi: 10.1111/j.1600-0722.2009.00686.x

106. Kato T, Masuda Y, Kanayama H, Morimoto T. Muscle activities are differently modulated between masseter and neck muscle during sleep-wake cycles in guinea pigs. *Neurosci Res*. (2007) 58:265–71. doi: 10.1016/j.neures.2007.03.008

107. Katayama K, Mochizuki A, Kato T, Ikeda M, Ikawa Y, Nakamura S, et al. Dark/light transition and vigilance states modulate jaw-closing muscle activity level in mice. *Neurosci Res*. (2015) 101:24–31. doi: 10.1016/j.neures.2015.07.004

108. Satoh T, Harada Y. Electrophysiological study on tooth-grinding during sleep. *Electroencephalogr Clin Neurophysiol*. (1973) 35:267–75. doi: 10.1016/0013-4694(73)90238-1

109. Zhang Y, Lu J, Wang Z, Zhong Z, Xu M, Zou X, et al. Companion of oral movements with limb movements in patients with sleep bruxism: preliminary findings. *Sleep Med*. (2017) 36:156–64. doi: 10.1016/j.sleep.2017.05.015

110. Kato T, Rompré P, Montplaisir JY, Sessle BJ, Lavigne GJ. Sleep bruxism: an oromotor activity secondary to micro-arousal. *J Dent Res*. (2001) 80:1940–4. doi: 10.1177/00220345010800101501

111. Sakai T, Kato T, Yoshizawa S, Suganuma T, Takaba M, Ono Y, et al. Effect of clonazepam and clonidine on primary sleep bruxism: a double-blind, crossover, placebo-controlled trial. *J Sleep Res*. (2017) 26:73–83. doi: 10.1111/jsr.12442

112. Boutros NN, Montgomery MT, Nishioka G, Hatch JP. The effects of severe bruxism on sleep architecture: a preliminary report. *Clin EEG Neurosci*. (1993) 24:59–62. doi: 10.1177/155005949302400204

113. Kishi A, Haraki S, Toyota R, Shiraishi Y, Kamimura M, Taniike M, et al. Sleep stage dynamics in young patients with sleep bruxism. *Sleep*. (2020) 43:1–12. doi: 10.1093/sleep/zsz202

114. Kuang B, Aarab G, Wei Y, Blanken TF, Lobbezoo F, Someren EJWV, et al. Associations between signs of sleep bruxism and insomnia: a polysomnographic study. *J Sleep Res*. (2023) 32:e13827–9. doi: 10.1111/jsr.13827

115. Lavigne GJ, Rompré PH, Guitard F, Sessle BJ, Kato T, Montplaisir JY. Lower number of K-complexes and K-alphas in sleep bruxism: a controlled quantitative study. *Clin Neurophysiol*. (2002) 113:686–93. doi: 10.1016/S1388-2457(02)00037-8

116. Heyat MBB, Lai D, Akhtar F, Hayat MAB, Azad S. Short time frequency analysis of theta activity for the diagnosis of bruxism on EEG sleep record Switzerland. Switzerland: Springer International Publishing (2020).

117. Ware JC, Rugh JD. Destructive bruxism: sleep stage relationship. *Sleep*. (1988) 11:172–81. doi: 10.1093/sleep/11.2.172

118. Tachibana N, Yamanaka K, Kaji R, Nagamine T, Watatani K, Kimura J, et al. Sleep bruxism as a manifestation of subclinical rapid eye movement sleep behavior disorder. *Sleep*. (1994) 17:555–8. doi: 10.1093/sleep/17.6.555

119. Pratap-Chand R, Gourie-Devi M. Bruxism: its significance in coma. *Clin Neurol Neurosurg*. (1985) 87:113–7. doi: 10.1016/0303-8467(85)90107-6

120. Kothari SF, Devendran A, Sørensen AB, Nielsen JF, Svensson P, Kothari M. Occurrence, presence and severity of bruxism and its association with altered state of consciousness in individuals with severe acquired brain injury. *J Oral Rehabil*. (2023) 51:143–9. doi: 10.1111/joor.13540

121. Boroojerdi B, Battaglia F, Muellbacher W, Cohen LG. Voluntary teeth clenching facilitates human motor system excitability. *Clin Neurophysiol*. (2000) 111:988–93. doi: 10.1016/S1388-2457(00)00279-0

122. Miyahara T, Hagiya N, Ohyama T, Nakamura Y. Modulation of human soleus H reflex in association with voluntary clenching of the teeth. *J Neurophysiol*. (1996) 76:2033–41. doi: 10.1152/jn.1996.76.3.2033

123. Sugawara K, Kasai T. Facilitation of motor evoked potentials and H-reflexes of flexor carpi radialis muscle induced by voluntary teeth clenching. *Hum Mov Sci*. (2002) 21:203–12. doi: 10.1016/S0167-9457(02)00099-4

124. Sato H, Kawano T, Saito M, Toyoda H, Maeda Y, Türker KS, et al. Teeth clenching reduces arm abduction force. *Exp Brain Res*. (2014) 232:2281–91. doi: 10.1007/s00221-014-3919-8

125. Satoh T, Harada Y. Depression of the H-reflex during tooth grinding in sleep. *Physiol Behav*. (1972) 9:893–4. doi: 10.1016/0031-9384(72)90072-8

126. Komeilipoor N, Ilmoniemi RJ, Tiippana K, Vainio M, Tiainen M, Vainio L. Preparation and execution of teeth clenching and foot muscle contraction influence on corticospinal hand-muscle excitability. *Sci Rep*. (2017) 7:1–9. doi: 10.1038/srep41249

127. Iida T, Kato M, Komiyama O, Suzuki H, Asano T, Kuroki T, et al. Comparison of cerebral activity during teeth clenching and fist clenching: a functional magnetic resonance imaging study. *Eur J Oral Sci*. (2010) 118:635–41. doi: 10.1111/j.1600-0722.2010.00784.x

128. Sugawara K, Furubayashi T, Takahashi M, Ni Z, Ugawa Y, Kasai T. Remote effects of voluntary teeth clenching on excitability changes of the human hand motor area. *Neurosci Lett*. (2005) 377:25–30. doi: 10.1016/j.neulet.2004.11.059

129. Takahashi M, Ni Z, Yamashita T, Liang N, Sugawara K, Yahagi S, et al. Excitability changes in human hand motor area induced by voluntary teeth clenching are dependent on muscle properties. *Exp Brain Res*. (2006) 171:272–7. doi: 10.1007/s00221-006-0430-x

130. Kawakubo N, Miyamoto JJ, Katsuyama N, Ono T, Honda E, Kurabayashi T, et al. Effects of cortical activations on enhancement of handgrip force during teeth clenching: an fMRI study. *Neurosci Res*. (2014) 79:67–75. doi: 10.1016/j.neures.2013.11.006

131. Bader G, Lavigne G. Sleep bruxism; an overview of an oromandibular sleep movement disorder. *Sleep Med Rev*. (2000) 4:27–43. doi: 10.1053/smrv.1999.0070

132. Lavigne GJ, Montplaisir JY. Restless legs syndrome and sleep bruxism: prevalence and association among Canadians. *Sleep*. (1994) 17:739–43.

133. van der Zaag J, Naeije M, Wicks DJ, Hamburger HL, Lobbezoo F. Time-linked concurrence of sleep bruxism, periodic limb movements, and EEG arousals in sleep bruxers and healthy controls. *Clin Oral Investig*. (2014) 18:507–13. doi: 10.1007/s00784-013-0994-3

134. Walters AS. Clinical identification of the simple sleep-related movement disorders. *Chest*. (2007) 131:1260–6. doi: 10.1378/chest.06-1602

135. Han K, Wang C, Zhong Z, Xu M, Zou X, Yu B, et al. Characterisation of the relationships between rhythmic masticatory muscle activities and limb movements in patients with sleep bruxism. *J Oral Rehabil*. (2019) 46:399–408. doi: 10.1111/joor.12760

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Dysphagia in older adults with mild cognitive impairment and dementia through fluoroscopic study with barium swallow in a memory clinic

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Introduction: Dysphagia and cognitive impairment are prevalent in older individuals. This study aimed to understand the characteristics of dysphagia through fluoroscopy in older adults with mild cognitive impairment (MCI) and dementia.

Methods: A cross-sectional study was conducted at a memory clinic in a tertiary hospital in Mexico City. A total of 158 patients were included, of whom 86 (54.4%) showed a risk of dysphagia, and 84 underwent barium swallow fluoroscopy.

Results: An association was observed between MCI and alteration in the oral phase (OR 0.33, 95% CI 0.12, 0.92, p = 0.034). Compared to patients with dementia, patients with MCI showed greater alteration in protection against regurgitation (OR 3.19, 95% CI: 1.05 to 9.72, p = 0.042) and in the contraction of the laryngeal muscles (OR 3.54, 95% CI: 1.30 to 9.62, p = 0.013).

Discussion: Our findings highlight the altered phases of swallowing in patients with dementia. Additionally, we found a high prevalence of dysphagia in older adults with MCI, underscoring the importance of early detection and intervention.

KEYWORDS

older adults, mild cognitive impairment, dementia, dysphagia, modified barium swallow study

Introduction

The aging of the population is a global phenomenon that has significant implications for healthcare. Among the conditions affecting older adults, dysphagia and cognitive impairment are particularly prevalent (1). Patients with dysphagia may exhibit cognitive disorders or language impairments (aphasia) resulting from underlying diseases such as dementia and stroke. Moreover, dysphagia is a geriatric syndrome that affects between 10 and 33% of older adults in general (2). In contrast, the prevalence of dysphagia in patients with dementia can range from 13 to 57% in advanced stages.

Dysphagia occurs due to difficulties in attention, initiative, planning, and execution of daily activities related to the feeding process. These difficulties are reflected in the deterioration of the oral, pharyngeal, and esophageal phases of swallowing and are associated with anatomical and functional alterations in cortical and subcortical regions related to swallowing and feeding (3). It is worth noting that swallowing changes have been identified in patients with Mild Cognitive Impairment (MCI) (4) with an estimated prevalence of 38–63% (5). Even

in patients with early Alzheimer's disease (6) where dysphagia is not clinically evident, it has been demonstrated that oxygen-dependent brain activity is reduced during swallowing compared to healthy controls, suggesting that changes in cortical control of swallowing may begin long before dysphagia becomes apparent (7). In the Mexican population, data are limited. The association between cognition and swallowing disorders suggests the involvement of multiple neuroanatomical systems (8). Dysphagia causes various health problems such as dehydration, malnutrition, and aspiration pneumonia, additionally affecting the patient's quality of life (9). Many dysphagia patients go undiagnosed and adapt through behavioral changes, while others may experience silent aspiration, it is defined as the entry of food, liquids, or saliva into the airways without the individual showing obvious signs of coughing or discomfort (10).

Some functional Magnetic Resonance Imaging (fMRI) studies have identified the involvement of cortico-subcortical areas in swallowing, particularly related to the activity of the insula, the inferior frontal gyrus, and the anterior cingulate cortex (11). Dysfunctions in these brain areas may contribute to motor impairments during swallowing, leading to difficulties in chewing and bolus formation (11).

The diagnosis and treatment of dysphagia in the early stages of cognitive impairment pose a challenge. From early stages, clinical symptoms of dysphagia may include difficulty initiating swallowing, deviation of the tongue or soft palate, which can lead to problems with bolus formation, cyanosis, unusual respiratory noises after eating, such as wheezing or stridor, persistent coughing after eating, increased gag reflex, delayed swallowing, dry mouth or difficulty forming the bolus, and difficulty coordinating breathing and swallowing (12). There are some clinical assessment scales that attempt to identify symptoms associated with dysphagia. Moreover, diagnostic methods such as video fluoroscopic swallowing studies are considered the gold standard, with a sensitivity of 80% and a specificity of 93%, as they allow the observation of the structural and anatomical stages of swallowing, providing qualitative data on the swallowing motor pattern (11). The objective of this study was to understand the characteristics of dysphagia through fluoroscopy in older adults with MCI and dementia.

Materials and methods

Participants

This was a cross-sectional, observational study that included outpatient participants aged 60 and older. All participants underwent clinical and cognitive evaluations at a tertiary care hospital in Mexico City between June 2022 and April 2023. The sample size was calculated based on an estimated dysphagia prevalence of 40%, using the absolute precision method. Sensitivity and specificity were set at 80%, with a precision of 10%. As a result, it was determined that 36 patients per group were needed to test the statistical hypothesis (12).

A total of 463 participants with a diagnosis of MCI and dementia were recruited. Of these, 305 were excluded based on specific criteria, which included a previous diagnosis of dysphagia, diabetic dysautonomia, scleroderma, structural pathology, or a history of neck surgery (tumors, polyps). Additional exclusion criteria encompassed uncontrolled psychiatric disorders (depression, anxiety, and/or delirium), neurological diseases (such as Parkinson's disease, myopathies, myasthenia gravis, and amyotrophic lateral sclerosis), orotracheal intubation in the last 6 months, and the use of medications that could affect swallowing (e.g., benzodiazepines, dopaminergic antagonists, antiepileptics, anticholinergics, antispasmodics, prokinetics, mucolytics, antihistamines, antibiotics, antineoplastics, and anti-inflammatories) (Figure 1).

Finally, 158 participants (66 men, 92 women) were included in the study. The diagnosis and type of MCI were established according to Petersen et al.'s criteria (4), which include memory loss reported by the patient or an informant and a memory score that is 1.5 standard deviations or more below the mean for their age. While cognitive screening tests such as the Mini-Mental State Examination (MMSE) (13) and the Montreal Cognitive Assessment (MoCA) (14) are used to assess general cognition, the standard deviations are calculated from more specific memory tests, such as the California Verbal Learning Test (CVLT), the Wechsler Memory Scale (WMS), and the Rey-Osterrieth Complex Figure Test.

Additionally, participants were required to maintain relatively intact overall cognitive functioning despite specific deficits, which was assessed using an MMSE score of ≥24 or a MoCA score of ≥26, indicating normal global cognitive functioning. Other inclusion criteria included the ability to perform normal activities of daily living and the absence of dementia diagnostic criteria, defined according to the National Institute on Aging and Alzheimer's Association (NIA-AA) clinical guidance (6). Furthermore, patients had to be able to follow verbal instructions and express a desire to participate in the study. Dysphagia risk was determined using a score of ≥3 on the Eating Assessment Tool-10 (EAT-10) (15, 16).

Evaluation and assessments

All participants were evaluated by geriatrics and/or neurology specialists. Sociodemographic and health information was obtained from electronic records and categorized based on sex, age, and years of education, as well as the presence or absence of common comorbidities. The functional status for Basic Activities of Daily Living (ADL) was assessed using the KATZ scale, while Instrumental Activities of Daily Living (IADL) were measured with the Lawton and Brody scale. Individuals were considered dependent if they scored less than 5 on the KATZ scale and less than 7 on the Lawton and Brody scale (17, 18). All participants accepted and signed informed consent.

Modified barium swallow study

The modified barium swallow study was performed on 84 patients at risk of dysphagia according to the EAT-10 (score > 3). A single expert radiologist, who was blinded to clinical evaluation, conducted the assessment and study. The swallowing evaluation included information from the oral (preparatory and transport), pharyngeal, and esophageal phases. Key points assessed included lip closure, bolus retention, bolus propulsion, tongue elevation and posterior displacement, soft palate elevation, upper esophageal sphincter contraction, protection against nasal regurgitation, bolus propulsion by the base of the tongue, epiglottis horizontalization, laryngeal elevation, pharyngeal constrictor muscle contraction, oral and hypopharyngeal cleaning, upper esophageal sphincter relaxation, the bolus entry into the esophagus and return to the initial position of the structures. Additionally, to complete the study, a

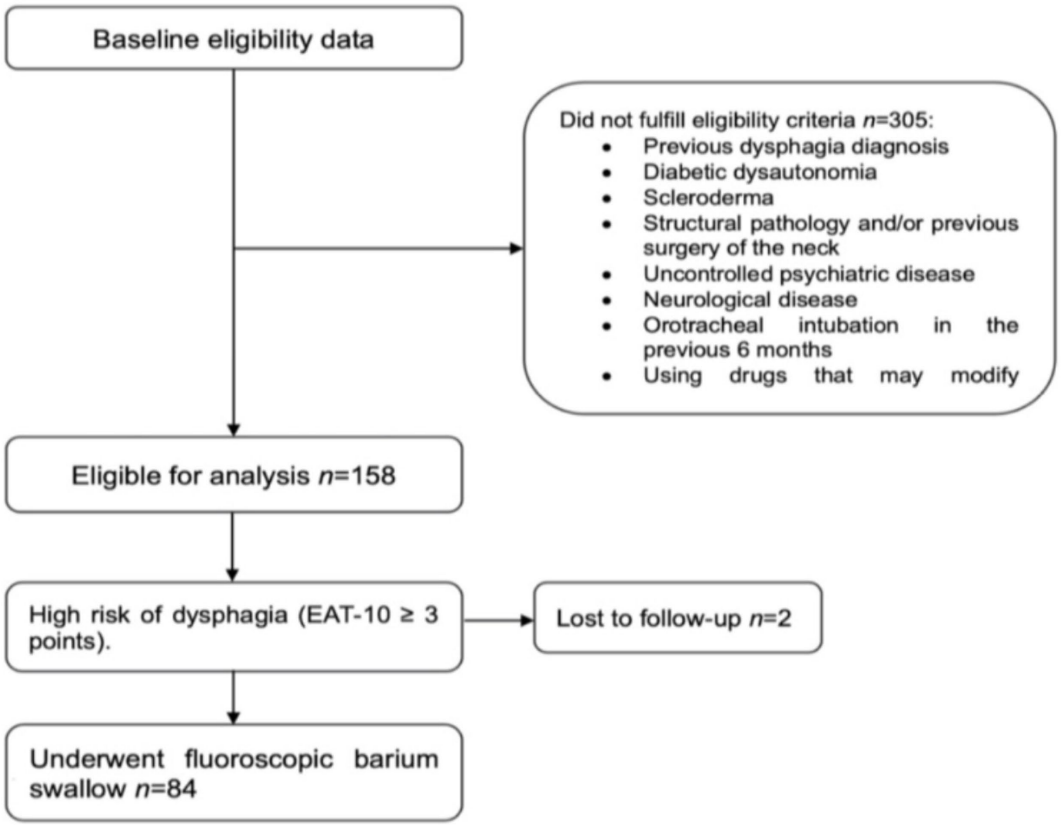

FIGURE 1
Flow diagram of the participants.

modified barium swallow study was conducted using three consistencies with contrast medium: solid consistency, prepared by mixing 30 grams of high-density barium sulfate with 5 grams of thickener, diluted with 2.5 mL of drinking water at a temperature of 30 degrees Celsius, and left to rest for 5 min; semisolid consistency, prepared by mixing 30 grams of high-density barium sulfate with 5 grams of thickener diluted with 1 mL of drinking water, left to rest for 5 min; liquid consistency, prepared by mixing 340 grams of high-density barium sulfate diluted with 65 mL of drinking water (19).

With the patient standing or sitting, a lateral and anteroposterior projection was requested, and solid consistency was initially administered in a maximum of 2 mL, with adequate tolerance. If no impairment in the swallowing mechanism was observed in any of the phases, a larger amount of the same consistency was administered. The same steps were repeated for semisolid and liquid consistencies. Each consistency was assessed for the different swallowing phases, and the presence or absence of impairment was defined (20).

Statistical analysis

A descriptive analysis was conducted to characterize differences between the groups (MCI vs. Dementia). The Chi-square test was used to test differences between categorical variables, and the Mann–Whitney U test was applied for continuous variables. A statistically significant value was considered at $p < 0.05$ for all analyses. Simple and multiple logistic regression analyses were performed to test the probability of having alterations in swallowing phases (dependent variable) based on the presence of cognitive impairment vs. dementia (independent variable with the main effect), adjusting for covariates (age, sex, Lawton scale). Odds ratios (ORs) were obtained from the exponential of the regression coefficients, along with 95% confidence intervals and p-values (using the Wald Chi-square test). Statistical analysis was conducted using SPSS software version 25 (SPSS Inc., Chicago, IL, USA).

The protocol was reviewed and approved by the ethics committee and the human research committee of the hospital. Approval code: GER-4207-22-23-1.

Results

A total of 158 participants were included, of whom 66 (41.8%) were diagnosed with MCI, and 92 patients (58.2%) had dementia (34.8% mild, 17.1% moderate, 6.3% severe). Females constituted 58.2% ($n = 92$) of the sample. The average years of education were 9.2 (5.4), with 39% having 1–6 years of education, 37% having 7–14 years, and 23% having more than 15 years of education. No statistically significant differences were found in terms of comorbidities between the groups. However, 12% of dementia patients had a Body Mass Index (BMI) less than 18.5 kg/m^2 ($p = 0.012$) (Table 1).

TABLE 1 Socio-demographic and health characteristics.

Characteristics	Cognitive diagnosis		*p* value*
	MCI *n* = 66	Dementia *n* = 92	
Age (years) $\bar{X}$ (SD)	74.8 (7.9)	81.5 (8.5)	<0.001
>75 years n (%)	31 (48%)	69 (74%)	<0.001
Sexo n (%)			0.174
Female	42 (65%)	50 (54%)	
Dysphagia n (%)	35 (54%)	51 (55%)	0.902
Body Mass Index (BMI) $\bar{X}$ (SD)	26.5 (4.6%)	23.9 (4.7%)	<0.001
BMI levels n (%)			
<18.5	3 (5%)	11 (12%)	0.012
18.5–24.9	22 (34%)	47 (51%)	
25–29.9	23 (35%)	25 (27%)	
≥30	17 (26%)	10 (11%)	
Social status n (%)			0.701
Single	7 (11%)	7 (8%)	
Married	29 (45%)	47 (51%)	
Widowed	24 (37%)	33 (35%)	
Divorced	4 (6%)	6 (6%)	
Common law marriage	1 (2%)	–	
Education n (%)			0.075
1–6 years	19 (29%)	43 (46%)	
7–14 years	30 (46%)	29 (31%)	
>15 years	16 (25%)	21 (23%)	
MMSE $\bar{X}$ (SD)	26.3 (2.5)	19.6 (5.8)	<0.001
MoCA $\bar{X}$ (SD)	20.9 (3.8)	13.3 (5.8)	<0.001
Katz			
Dependent (<5 ADL) n (%)	–	33 (35%)	
Independent n (%)	66 (100%)	60 (65%)	<0.001
Lawton			
Dependent (<7 AIVD) n (%)	16 (25%)	64 (69%)	<0.001
Independent n (%)	49 (75%)	29 (31%)	<0.001
Diabetes mellitus n (%)	25 (38%)	38 (41%)	0.762
Hypertension n (%)	38 (58%)	55 (59%)	0.932
Dyslipidemia n (%)	20 (31%)	29 (31%)	0.956
Atrial fibrillation n (%)	4 (6%)	10 (11%)	0.317

Means and standard deviations for numerical variables. ADL, Basic Activities of Daily Living; IADL, Instrumented Activities of Daily Living. The asterisk (*) next to the *p*-value indicates statistical significance.

Patients at risk of dysphagia based on EAT-10 scores showed 5–16 swallowing abnormalities on the barium swallow fluoroscopy. The distribution of abnormalities included 0.6% with 5 alterations, 1.3% with 6 alterations, 1.3% with 8 alterations, 1.3% with 10 alterations, 2.5% with 11 alterations, 5.7% with 12 alterations, 12.7% with 13 alterations, 19.6% with 14 alterations, 5.7% with 15 alterations, and 2.5% with 16 alterations in the swallowing process.

Simple and multiple logistic regression analyses were conducted to analyze the probability of having swallowing alterations (dependent variable) based on the presence of Mild Cognitive Impairment (MCI) versus dementia (independent variable with the main effect). Adjusting for covariates such as age, sex, and Lawton's functionality scale, the results specifically for the MCI group indicated the following probabilities of altered function: an OR of 0.33 (95% CI 0.12, 0.92) with $p = 0.034$ for alterations in the oral phase, an OR of 3.19 (95% CI 1.05, 9.72) with $p = 0.042$ for protection against regurgitation, and an OR of 3.54 (95% CI 1.30, 9.62) with $p = 0.013$ for contraction of laryngeal muscles (2.54 times associated with the presence of MCI compared to the dementia group). The reciprocal value was 0.22 (Figure 2).

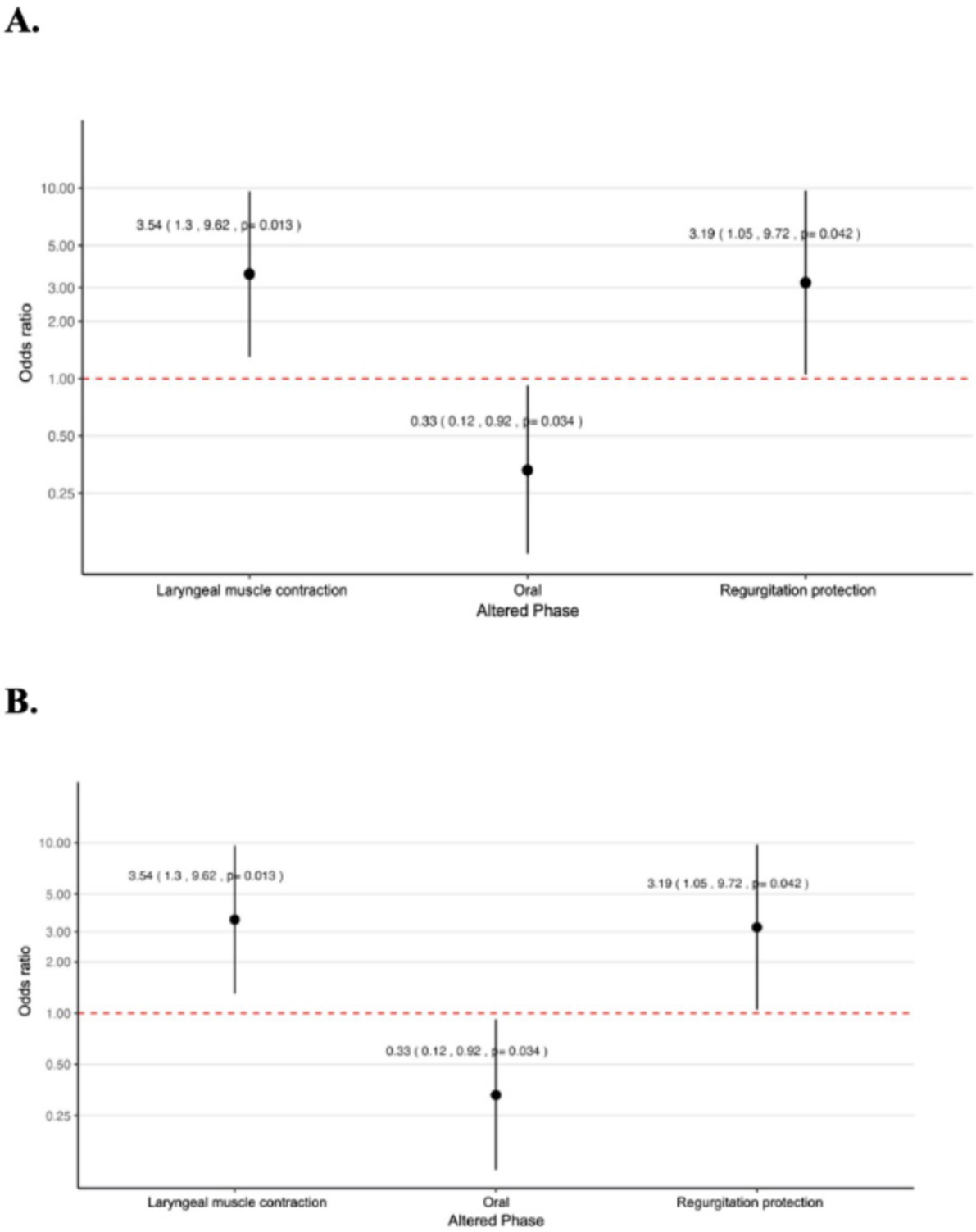

FIGURE 2
Logistic regression models showing the probability of developing alterations in swallowing phases in mild cognitive impairment versus dementia. OR and CI are shown. **(A)** Simple logistic regression. **(B)** Logistic regression adjusted for age, sex, and Lawton scale score for activities of daily living.

Discussion

The results of our study demonstrate a high prevalence of dysphagia in older patients with MCI and dementia. These findings are consistent with those reported by Seçil et al., who found that patients with Alzheimer's disease (AD) in their study had swallowing problems from early stages of the disease. They observed that 75% of patients did not report swallowing complaints; however, they noted that swallowing issues form a continuum, starting from "subclinical dysphagia" to "manifest dysphagia" and ultimately to "swallowing

apraxia" (21). Reaffirming this, in some patients, swallowing problems were noted even in the absence of overt complaints.

It appears that eating and swallowing difficulties in people with Alzheimer's disease (AD) are less severe than in those with frontotemporal lobar degeneration and dementia with Lewy bodies (DLB). Previous studies have looked for the association between eating problems and each of the multiple symptoms, including neuropsychiatric problems in subjects with DLB, and found that the presence of eating behaviors was strongly related to depression and apathy, whereas worsening eating behavior was related to apathy and nocturnal behavior. Importantly, physicians had relatively little knowledge about eating problems in these individuals (22). Our study showed that patients with dementia frequently present dysphagia and, although it was not possible to specify the type of dementia at the time of performing the barium swallow study, our results are consistent with those of previous studies, including the findings of Lages et al. (23). These authors analyzed the relationship between dysphagia and various clinical and cognitive aspects in older adults with dementia. While their study emphasized the influence of external factors, such as place of residence, on dysphagia, the consistency we highlight lies in the association between cognitive impairment and swallowing difficulties. Specifically, Lages et al. observed that dysphagia was more frequent in institutionalized older individuals, which they attributed to a combination of age-related changes and clinical and social aspects of dementia (23).

Another relevant aspect of our study is that patients with dementia presented a low body mass index (BMI), possibly related to dysphagia, which is closely associated with sarcopenia in older adults. The relationship between sarcopenia and dysphagia is bidirectional: sarcopenia can impair feeding capacity and nutrient absorption, potentially leading to food or liquid aspiration into the trachea, resulting in aspiration pneumonia or even death by suffocation in severe cases. In turn, dysphagia limits adequate nutrient intake, contributing to the loss of muscle mass and strength, thus exacerbating sarcopenia. Sarcopenic dysphagia is defined as dysphagia associated with generalized sarcopenia of skeletal muscles (throughout the body) and the muscles involved in swallowing (it is ruled out if there is no sarcopenia in the body) (24).

It is important to consider the effect of sarcopenia as a cause or cofactor of oropharyngeal dysphagia. While retrospective studies, such as one conducted on 109 patients in a nursing home, have not demonstrated a direct association between oropharyngeal dysphagia and sarcopenia, evidence suggests that these factors may be interrelated. The role of sarcopenia as a cause or cofactor needs to be further clarified, particularly to determine whether this condition is an epiphenomenon of severe disease or plays a causal role in the development of oropharyngeal dysphagia. Additionally, prospective studies are needed to explore the specific mechanisms of this bidirectional interaction and provide a better understanding of its clinical impact (25).

In addition, older individuals with dementia, whether mild, moderate, or advanced, often experience dysphagia. This difficulty in swallowing is not only attributable to the dementia itself but also to anorexia and various other associated pathophysiological and behavioral problems (24–26). In our study, a subset of patients underwent barium swallow fluoroscopy. According to the findings, more than half of the patients with mild cognitive impairment had dysphagia, with many swallowing abnormalities. Oszurecki et al. also demonstrated something similar in early stages of the disease (27). As reported by De Stefano et al., patients with MCI, despite having apparent good swallowing or chewing capacity, also presented oral diadochokinesis impairment and food spillage (28). This is primarily due to deteriorated oral motor skills and poor lip function (29). The underlying neural mechanisms are still unclear; however, it has been established that frontal regions are crucial aspects of executive function and eating and swallowing are actions that require cognitive awareness, visual recognition of food, physiological response, planning, and motor execution, as well as structured sensorimotor responses that decrease with the severity of cognitive impairment (31).

Likewise, we also observed a variety of swallowing alterations depending on the stage of cognitive impairment, as the group of patients with MCI had a lower probability of alteration in the oral phase compared to the dementia group. This is a result of swallowing apraxia, lack of coordination in lingual, labial, and mandibular functioning during the oral phase (30). In this phase, the bolus is mixed with saliva, reshaped, and chewed as needed to prepare it for movement to the pharynx. An important finding is that the alteration in protection against regurgitation was 2.19 times more associated with having MCI. On the other hand, we observed that the alteration in laryngeal muscle contraction was 2.54 times associated with the presence of MCI compared to the dementia group.

This finding may be surprising, given that patients with MCI are generally considered to be more neurologically intact than those with dementia. One possible explanation is that compensatory mechanisms within the central nervous system may not be fully effective in preventing laryngeal dysfunction in MCI, even though more generalized cognitive functions may still be preserved. Additionally, early neural damage in MCI could selectively affect the neural pathways involved in laryngeal contraction and airway protection. Although these individuals are in an earlier stage of cognitive decline, specific brain regions responsible for motor control of swallowing may be compromised earlier in the course of the disease.

Another factor to consider is cognitive fatigue, which could disproportionately affect the laryngeal phase of swallowing. Given the complexity of coordinating breathing and swallowing, cognitive fatigue may lead to greater variability or inconsistency in laryngeal muscle contraction and airway protection in MCI patients, even in the absence of other motor deficits. This could explain why the MCI group showed a higher probability of laryngeal dysfunction than the dementia group.

Finally, variability in the progression of neurological damage between individuals with MCI and dementia may account for this finding. While dementia involves widespread cognitive and functional decline, MCI may present with more focal neurological impairments, particularly in areas critical for the fine motor control required for airway protection.

This is a result of the fact that successful swallowing requires a proper connection from the cortex, subcortex, brainstem, and cranial nerves. Initially, it was believed that damage to both hemispheres and the brainstem was necessary for an individual to have dysphagia (5). However, with the advent of neuroimaging techniques, it was demonstrated that dysphagia can result from unilateral lesions of the cerebral cortex (10). Both reflex and voluntary swallowing activate the precentral gyrus, postcentral gyrus, insula, and anterior cingulate gyrus. Additionally, activation has also been observed in the basal ganglia, although their contributions to swallowing are less noticeable than in other mentioned areas (5, 7).

The greater probability of altered laryngeal contraction and protection against regurgitation in MCI highlights the importance of early screening for dysphagia in this population. Clinicians should be aware that patients

with MCI, despite their milder cognitive decline, may be at higher risk of developing swallowing-related complications, such as aspiration or pneumonia. Early identification and intervention could be key to preventing serious health outcomes and maintaining quality of life in this group. Furthermore, this finding suggests that dysphagia in MCI may be more complex and nuanced than previously thought, requiring tailored therapeutic approaches to address both the cognitive and motor aspects of swallowing dysfunction.

The results of our study have important clinical implications. Early identification of dysphagia in older individuals with MCI and dementia is crucial to implement appropriate interventions, such as swallowing therapy and dietary modifications, which could improve quality of life and prevent complications related to swallowing and nutritional status, such as aspiration pneumonia. Furthermore, we demonstrated that patients present dysphagia from early stages (MCI); therefore, dysphagia should be systematically sought through clinical or imaging studies, which undoubtedly could contribute to improving comprehensive patient care and their quality of life. We acknowledge that the use of different tests to establish a diagnosis of MCI is a limitation of our study. We also recognize that not having the specific subtype of dementia and/or a control group for each patient in this study is another limitation that can be addressed in future studies.

Conclusion

Our findings underscore the high prevalence of dysphagia in older individuals with MCI and dementia, as well as its association with greater cognitive impairment. The evidence suggests that patients may experience dysphagia from the early stages of cognitive decline. These results highlight the need to implement effective strategies for the detection and management of dysphagia in the geriatric population to optimize their quality of life and overall well-being.

Data availability statement

The data used in this study are available upon request. Please contact the corresponding author SA-N for access to the data.

Ethics statement

The study was reviewed and approved by the National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico Ethical Committee and the Committee on Research in Humans (approval code: GER-4207-22-23-1). All participants provided written informed consent.

Author contributions

GM-P: Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. AM-A: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. SA-N: Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Barczi SR, Sullivan PA, Robbins J. How should dysphagia care of older adults differ? Establishing optimal practice patterns. *Semin Speech Lang*. (2000) 21:0347–64. doi: 10.1055/s-2000-8387

2. Thiyagalingam S, Kulinski AE, Thorsteinsdottir B, Shindelar KL, Takahashi PY. Dysphagia in older adults. *Mayo Clin Proc*. (2021) 96:488–97. doi: 10.1016/j.mayocp.2020.08.001

3. Mourão LF, Xavier DAN, Neri AL, Luchesi KF. Estudo da associação entre doenças crônicas naturais do envelhecimento e alterações da deglutição referidas por idosos da comunidade. *Audiol Commun Res*. (2016) 21:e1657. doi: 10.1590/2317-6431-2015-1657

4. Tangalos EG, Petersen RC. Mild cognitive impairment in geriatrics. *Clin Geriatric Med*. (2018) 34:563–89. doi: 10.1016/j.cger.2018.06.005

5. Alagiakrishnan K, Bhanji RA, Kurian M. Evaluation and management of oropharyngeal dysphagia in different types of dementia: a systematic review. *Arch Gerontol Geriatric*. (2013) 56:1–9. doi: 10.1016/j.archger.2012.04.011

6. Jack CR, Andrews JS, Beach TG, Buracchio T, Dunn B, Graf A, et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association workgroup. *Alzheimers Dement*. (2024) 20:5143–69. doi: 10.1002/alz.13859

7. Humbert IA, McLaren DG, Kosmatka K, Fitzgerald M, Johnson S, Porcaro E, et al. Early deficits in cortical control of swallowing in Alzheimer's disease. *J Alzheimer's Dis JAD*. (2010) 19:1185–97. doi: 10.3233/JAD-2010-1316

8. Tagliaferri S, Lauretani F, Pelá G, Meschi T, Maggio M. The risk of dysphagia is associated with malnutrition and poor functional outcomes in a large population

of outpatient older individuals. *Clin Nutr*. (2019) 38:2684–9. doi: 10.1016/j.clnu.2018.11.022

9. Ekberg O, Hamdy S, Woisard V, Wuttge Hannig A, Ortega P. Social and psychological burden of dysphagia: its impact on diagnosis and treatment. *Dysphagia*. (2002) 17:139–46. doi: 10.1007/s00455-001-0113-5

10. Simpelaere IS, Vanderwegen J, Wouters K, De Bodt M, Van Nuffelen G. Feasibility and psychometric properties of the adjusted DSWAL-QoL questionnaire for Dysphagic patients with additional language and/or cognitive impairment: part I. *Dysphagia*. (2017) 32:401–19. doi: 10.1007/s00455-016-9770-2

11. Jo SY, Hwang JW, Pyun SB. Relationship between cognitive function and dysphagia after stroke. *Ann Rehabil Med*. (2017) 41:564–72. doi: 10.5535/arm.2017.41.4.564

12. Available at: http://www.sergas.es/Saude-publica/EPIDAT (Accessed June 6, 2022).

13. Folstein MF, Robins LN, Helzer JE. The Mini-mental state examination. *Arch Gen Psychiatry*. (1983) 40:812. doi: 10.1001/archpsyc.1983.01790060110016

14. Navarro SGA, Alvarado AJM, García AAP, Cruz AS, Gutiérre LAG, Funes JAÁ. Validez y confiabilidad del MoCA (Montreal Cognitive Assessment) para el tamizaje del deterioro cognoscitivo en México. *Rev Colomb Psiquiatr*. (2018) 47:237–43. doi: 10.1016/j.rcp.2017.05.003

15. Belafsky PC, Mouadeb DA, Rees CJ, Pryor JC, Postma GN, Allen J, et al. Validity and reliability of the eating assessment tool (EAT-10). *Ann Otol Rhinol Laryngol*. (2008) 117:919–24. doi: 10.1177/000348940811701210

16. Burgos R, Sarto B, Segurola H, Romagosa A, Puiggrós C, Vázquez C, et al. Traducción y validación de la versión en español de la escala EAT-10 (Eating Assessment Tool-10) para el despistaje de la disfagia. *Nutr Hosp*. (2012) 27:2048–54. doi: 10.3305/nh.2012.27.6.6100

17. Katz S. Assessing self-maintenance: activities of daily living, mobility, and instrumental activities of daily living. *J Am Geriatr Soc*. (1983) 31:721–7. doi: 10.1111/j.1532-5415.1983.tb03391.x

18. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist*. (1969) 9:179–86.

19. Clavé P, Arreola V, Velasco M, Quer M, Castellvi JM, Almirall J, et al. Diagnóstico y tratamiento de la disfagia orofaríngea funcional. Aspectos de interés para el cirujano digestivo. *Cir Esp*. (2007) 82:62–76. doi: 10.1016/S0009-739X(07)71672-X

20. Seçil Y, Arıcı Ş, İncesu TK, Gürgör N, Beckmann Y, Ertekin C. Dysphagia in Alzheimer's disease. *Neurophysiol Clin Neurophysiol*. (2016) 46:171–8. doi: 10.1016/j.neucli.2015.12.007

21. Affoo RH, Foley N, Rosenbek J, Kevin Shoemaker J, Martin RE. Swallowing dysfunction and autonomic nervous system dysfunction in Alzheimer's disease: a scoping review of the evidence. *J Am Geriatr Soc*. (2013) 61:2203–13. doi: 10.1111/jgs.12553

22. Shinagawa S, Hashimoto M, Yamakage H, Toya S, Ikeda M. Eating problems in people with dementia with Lewy bodies: associations with various symptoms and the physician's understanding. *Int Psychogeriatr*. (2024) 36:1194–204. doi: 10.1017/S1041610224000346

23. Lages DRP, Fonseca LC, Tedrus GMAS, De Oliveira IB. The relationship between dysphagia and clinical and cognitive aspects in elderly patients presented with dementia. *Rev CEFAC*. (2020) 22:e5719. doi: 10.1590/1982-0216/20202225719

24. Fujishima I, Fujiu-Kurachi M, Arai H, Hyodo M, Kagaya H, Maeda K, et al. Sarcopenia and dysphagia: position paper by four professional organizations. *Geriatr Gerontol Int*. (2019) 19:91–7. doi: 10.1111/ggi.13591

25. Calles M, Wirth R, Labeit B, Muhle P, Suntrup-Krueger S, Dziewas R, et al. Sarcopenic dysphagia revisited: a cross-sectional study in hospitalized geriatric patients. *Nutrients*. (2023) 15:1–11. doi: 10.3390/nu15122662

26. Rommel N, Hamdy S. Oropharyngeal dysphagia: manifestations and diagnosis. *Nat Rev Gastroenterol Hepatol*. (2016) 13:49–59. doi: 10.1038/nrgastro.2015.199

27. Wieseke A, Bantz D, Siktberg L, Dillard N. Assessment and early diagnosis of dysphagia. *Geriatr Nurs N Y N*. (2008) 29:376–83. doi: 10.1016/j.gerinurse.2007.12.001

28. Özsürekci C, Arslan SS, Demir N, Çalışkan H, Şengül Ayçiçek G, Kılınç HE, et al. Timing of dysphagia screening in Alzheimer's dementia. *JPEN J Parenter Enteral Nutr*. (2020) 44:516–24. doi: 10.1002/jpen.1664

29. De Stefano A, Di Giovanni P, Kulamarva G, Gennachi S, Di Fonzo F, Sallustio V, et al. Oropharyngeal dysphagia in elderly population suffering from mild cognitive impairment and mild dementia: understanding the link. *Am J Otolaryngol*. (2020) 41:102501. doi: 10.1016/j.amjoto.2020.102501

30. Delwel S, Scherder EJA, Perez RSGM, Hertogh CMPM, Maier AB, Lobbezoo F. Oral function of older people with mild cognitive impairment or dementia. *J Oral Rehabil*. (2018) 45:990–7. doi: 10.1111/joor.12708

31. Cristofori I, Cohen-Zimerman S, Grafman J. Executive functions. *Handb Clin Neurol*. (2019) 163:197–219. doi: 10.1016/B978-0-12-804281-6.00011-2

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Identify predictive factors for the emergence of self-reported oropharyngeal dysphagia in older men and women populations: a retrospective cohort analysis

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Background and objectives: Oropharyngeal dysphagia (OD) is an emergent health concern in older adults, with incidence rates escalating due to age-related and various neurological and physical conditions. This study identifies risk and protective factors for new-onset OD, with an emphasis on gender differences.

Methods: Utilizing data from the National Health and Aging Trends Study (NHATS), this study analyzed 6,360 participants (58.1% women) across 2011–2014 and 2015–2018 periods. Employing a random forest feature selection, specifically recursive feature elimination and mean decrease impurity algorithm, we assessed 128 variables to identify critical factors including demographics, health, physical and neurological functionality, and environmental conditions. The study further applied logistic regression and explored factor interactions using restricted cubic splines, streamlining the analysis to focus on key determinants of oropharyngeal dysphagia.

Results: Initial findings show a decrease in new-onset OD from 15.62% in 2011 to 14.49% in 2015, with women more frequently affected. The analysis elucidates a constellation of highly predictive factors for OD, encompassing extremes of body mass index (BMI), socioeconomic challenges (as indicated by low income), diminished physical conditioning, and adverse emotional states. Notably, gender-specific disparities emerged, highlighting the critical role of cognitive function and mood in men, whereas in women, the overarching influence of general health status and comorbidities was more pronounced.

Conclusion: This condensed examination highlights the complex, multifactorial nature of OD in older adults, influenced by sociodemographic, physical, and psychological factors, and underscores the need for gender-specific approaches in predicting, preventing, and managing OD.

KEYWORDS

oropharyngeal dysphagia, gender difference, machine learning, older adult, ML

Introduction

In the domain of geriatric healthcare, oropharyngeal dysphagia (OD) emerges as a paramount concern, with its prevalence intricately linked to the demographic shifts toward an older population. Scholarly investigations reveal a notable escalation in the incidence of OD, with the prevalence rates among older adults ranging from 10 to 33%, as documented in seminal studies (1, 2). This prevalence is alarmingly higher, oscillating between 8.1 and 80%, within cohorts suffering from neurological impairments or conditions, including but not limited to cerebrovascular accidents, Alzheimer's disease, and Parkinson's disease (3, 4). The ramifications of oropharyngeal dysphagia on the health and well-being of the aging population are profound and multifarious. The condition is a harbinger of grave health complications such as malnutrition (5), dehydration (6), and aspiration pneumonia (7), each significantly exacerbating the mortality risk associated with the affected demographics (8, 9). Moreover, the impact of OD transcends the physical domain, casting a long shadow over the mental health and overall quality of life of those afflicted (10, 11).

Within the United States, the economic repercussions of OD are profound, with estimations suggesting an annual fiscal impact exceeding $7 billion due to both direct and indirect costs (12). Notably, individuals hospitalized with OD face an augmented inpatient expense, exceeding those without OD by approximately $4,282 annually (13), underscoring its considerable contribution to global disability. The societal and economic ramifications of OD are poised for amplification in tandem with escalating healthcare costs and the anticipated surge in the demographic segment aged 60 and above (14), expected to rise from 12 to 22% by the year 2050 (15). Consequently, the formulation of cost-effective and efficacious intervention methodologies emerges as imperative, necessitating a comprehensive understanding of the myriad risk factors for OD within the aging population.

Extant literature delineates a variety of factors implicated in the etiology of OD, ranging from neurological afflictions (16, 17) and physical trauma (18) to the impacts of certain medications (19). Moreover, OD's association with individual characteristics—such as age (20), obesity (21), lifestyle habits like smoking (22), and even emotional and partner employment status (23)—underscores the intricate web of determinants influencing its development. This intricacy mandates a sophisticated and multifaceted research approach to elucidate the nuances of OD in aging individuals, thereby refining prevention and management paradigms for this prevalent disorder.

A particularly promising avenue for exploration resides in the gender-specific aspects of oropharyngeal dysphagia. Initial investigations reveal marked disparities in the incidence and clinical manifestation of OD between men and women cohorts (24), hinting at the potential for gender-differentiated protective and risk factors. This observation suggests a requisite for bespoke research methodologies and clinical interventions. The etiological complexity of oropharyngeal dysphagia challenges conventional research modalities, with the majority of studies adopting cross-sectional designs (25–27). The reliance on subjective criteria for feature selection in traditional approaches hampers the exhaustive examination of the multifactorial relationships between potential predictors and OD incidence, particularly in analyses incorporating a broad array of predictor variables.

In light of these considerations and acknowledging the absence of comprehensive comparative analyses of sociodemographic, health, cognitive, and functional predictors of dysphagia risk with a focus on gender disparities, our study endeavors to bridge this knowledge gap. By leveraging a vast publicly accessible dataset and integrating traditional statistical techniques with advanced machine learning algorithms, we aim to dissect the occurrence of OD through a gender-stratified lens, assessing 128 potential risk and protective factors to illuminate the path forward in understanding and combating this condition.

Methods

This study is characterized as a retrospective cohort analysis, underpinned by the utilization of data from the National Health and Aging Trends Study (NHATS). NHATS stands as a publicly accessible, nationally representative longitudinal survey specifically targeting Medicare beneficiaries aged 65 and older residing within the United States. Its primary objective is to delineate the risk factors associated with late-life disability among the aging demographic, thereby augmenting the corpus of knowledge pertaining to the trajectories of functional decline and resilience observed in older adults (28). Initiated in 2011, the initial cohort has been subjected to annual follow-up assessments, facilitating a comprehensive longitudinal examination of the dynamic changes and continuities experienced by this population over time.

Participants

We analyzed data across eight rounds of NHATS, spanning from 2011 through 2018. This encompassed an extensive review of participant data commencing with their initial interview and extending through the subsequent 3 years, offering a longitudinal perspective on their health trajectories. Within the baseline years of 2011 and 2015, a total of 12,437 NHATS participants engaged in the survey. From this initial cohort, exclusions were applied judiciously to ensure the integrity of the study's focus on new-onset OD. Specifically, 30 participants (20 in 2011 and 10 in 2015) were excluded due to the absence of baseline interviews pertaining to swallowing disorders. Furthermore, an additional 630 participants (400 in 2011 and 230 in 2015) were excluded for having pre-existing swallowing disorders at baseline. The meticulous exclusion criteria extended to 4,988 participants (3,508 between 2012 and 2014 and 1,480 between 2016 and 2018) who were unable to complete follow-up interviews, alongside 406 individuals who failed to provide specific OD outcomes in the subsequent three-year periods. An additional 30 participants were excluded due to having more than 20% missing data on independent variables. This rigorous selection process culminated in a refined final sample of 6,360 participants, of whom 58.1% were women.

Measures

New-onset of OD

OD is typically defined as difficulty in the process of initiating a swallow and moving food or liquid from the mouth to the esophagus.

In this study, we operationalized new-onset OD based on self-reported difficulties with chewing or swallowing. Participants were systematically queried during the follow-up phases (2012–2014 and 2016–2018) regarding their recent experiences with mastication or deglutition difficulties, specifically inquiring, "In the past month, have you experienced any chewing or swallowing problems leading to difficulty eating?" Responses were binary, encapsulating either an affirmative or negative experience. New-onset dysphagia was rigorously defined by the absence of such swallowing difficulties at the initial survey point (either 2011 or 2015) juxtaposed with the emergence of dysphagia within the subsequent 36-month observational window.

Demographics and daily activity

The demographic canvas included an array of variables such as age, stature, mass, body mass index (BMI), ethnoracial identity, educational attainment, income bracket, domestic arrangements (solo living or cohabitation with a spouse/partner), familial size, and progeny count, as recorded in the years 2011 and 2015. Lifestyle inquiries probed smoking habits, manual dominance, preferred pastimes, frequency of entertainment engagements in the preceding month, participation in ambulatory exercises, valuation of outdoor ventures, and proficiency in activities of daily living (e.g., transitional movements, ambulation, attire management, personal hygiene, and lavatory use).

Health condition

The health dimension encompassed self-assessments of overall health and detailed medical histories inclusive of cardiovascular incidents, heart conditions, hypertension, arthritic conditions, osteoporosis, diabetes mellitus, pulmonary diseases, cerebrovascular accidents, cognitive degeneration, oncological diagnoses, and skeletal fractures, alongside a tally of comorbid conditions. This was supplemented with details on surgical interventions, pain experiences, functional limitations of the appendages, and sensory impairments affecting vision and audition, in addition to sleep-related disorders.

Physical and neurological function

Measures of physical functioning included recording participants' total and sub-scores for performing the three tasks of the SPPS (Short Physical Performance Battery) including Balance Test, Walking Test and Repeat Chair Test (29). Additional inclusion was made of participants' one-legged eyes-open/eyes-closed standing test scores, mean vs. best grip/breath test scores, and self-reported walking ability (whether they could walk three blocks/ten steps).

Neurological functioning recorded participants' cognitive functioning as well as mood status. Among the measures of cognitive functioning were participants' self-reported memory status, Clock Drawing Test scores and Immediate/Delayed Word Recall Test scores, and also Date Recall and Presidential Recall Tests. Mood status was screened for the presence of depression/anxiety symptoms in participants using the PHQ4 (30).

Environment condition

Recognizing the potential influence of adverse living conditions on swallowing disorders, an examination of the participants' residential and surrounding environments was conducted. This entailed an inspection of dwelling and entryway structures (e.g., stairs, ramps), interior and exterior safety risks (e.g., structural integrities, neighborhood cleanliness, and security concerns), and bathroom configurations, aiming to establish a correlation with the prevalence of OD.

Data analysis

The analysis commenced with the initial processing of data and the application of fundamental statistical methodologies, including T-tests, via the SPSS platform. The subsequent analysis into a more sophisticated computational realm, utilizing the Python programming environment within PyCharm (version 2022.1.3) and leveraging the advanced capabilities of the scikit-learn library (version 1.3.2) for feature selection, recursive feature elimination (RFE), and the construction and nuanced interpretation of both univariate and multivariate analytical models.

In 2011, the prevalence of OD initiation within a triennial span was quantified at 15.62%, whilst a slight diminution was observed in 2015, with the incidence rate adjusting to 14.49%. A gender-stratified analysis revealed a discernible disparity, with women exhibiting a higher incidence rate of 15.8% in comparison to men at 14.38%. The median age bracket of participants situated between 75 and 79 years and the racial composition was predominantly non-Hispanic white, comprising 70.9% (4,458 individuals) of the sample cohort (Table 1).

Gender stratification of the dataset served as the initial step, allowing for the nuanced examination of categorical and continuous variables, presented as counts with percentages and means with standard deviations, respectively. The exclusion of individuals presenting with more than 20% missing data in crucial variables ensured the analytical purity of the study. The remaining gaps in the dataset were adeptly bridged via the iterative imputation prowess of the Missforest algorithm, thereby preserving the dataset's completeness for subsequent analysis. The process began by separating categorical and numerical variables, with categorical variables undergoing one-hot encoding to facilitate imputation and numerical variables were processed directly. A variable type indicator was created to distinguish between continuous (0) and categorical (1) variables, ensuring appropriate treatment during imputation. The MissForest algorithm was then applied with the following parameters: maximum iterations set to 10, number of trees in each forest set to 100, and all available processor cores utilized. Post-imputation, one-hot encoded categorical variables were converted back to their original form by selecting the category with the highest probability.

The random forest methodology, a machine learning algorithm that constructs and merges multiple decision trees for more accurate and stable predictions, was employed in our study to select the most important predictors of oropharyngeal dysphagia (OD) from an initial pool of 128 candidate variables. We specifically utilized the random forest feature recursive elimination (RFE) method, which iteratively constructs models, evaluates feature importance, and removes less significant features until reaching the desired number. To ensure the robustness and stability of our feature selection process, we implemented a cross-validation approach. Specifically, we used the GridSearchCV function from scikit-learn with 5-fold cross-validation to optimize the random forest parameters. The hyperparameters tuned included the number of estimators (100, 200, 300), maximum depth (None, 5, 10), and minimum samples split (2, 5, 10) (31). This cross-validation process not only helped in selecting stable features but also in identifying the optimal model parameters. The RFE process

TABLE 1 Demographic information stratified by gender.

Feature		Men (*N* = 2,664)		Women (*N* = 3,696)	
		Onset of OD	None-OD	Onset of OD	None-OD
Age	65–69	86(12.78%)	587(87.22%)	112(14.27%)	673(85.73%)
	70–74	75(12.36%)	532(87.64%)	88(11.62%)	669(88.38%)
	75–79	75(13.91%)	464(86.09%)	115(15.35%)	634(84.65%)
	80–84	59(12.58%)	410(87.42%)	113(16.07%)	590(83.93%)
	85–89	59(22.43%)	204(77.57%)	81(19.66%)	331(80.34%)
	90-	29(25.66%)	84(74.34%)	75(25.86%)	215(74.14%)
Education	Below high school	195(16.64%)	977(83.36%)	341(17.93%)	1,561(82.07%)
	Above high school	188(12.6%)	1,304(87.4%)	243(13.55%)	1,551(86.45%)
Living arrangement	Alone	84(14.63%)	490(85.37%)	242(15.57%)	1,312(84.43%)
	With partner	202(13.27%)	1,320(86.73%)	134(11.72%)	1,009(88.28%)
	With partner and others	52(15.29%)	288(84.71%)	35(16.36%)	179(83.64%)
	With others only	45(19.74%)	183(80.26%)	173(22.04%)	612(77.96%)
Height		1.74(0.11)	1.76(0.11)	1.63(0.09)	1.64(0.10)
Weight		83.70(16.81)	87.31(16.71)	72.91(17.59)	72.432(16.36)
BMI		27.71(6.16)	28.56(6.40)	27.53(6.94)	27.23(6.65)
Income		50,664(46091)	70,599(61055)	33,688(32770)	45,114(48,350)
Kid number		0.21(0.56)	0.17(0.53)	0.34(0.60)	0.23(0.58)
Smoke regularly	Yes	250(14.73%)	1,447(85.27%)	216(14.89%)	1,235(85.11%)
	No	133(13.75%)	834(86.25%)	368(16.39%)	1877(83.61%)
Disease sum		2.63(1.63)	2.21(1.48)	3.09(1.59)	2.47(1.46)
Visual impairment	Yes	80(15.63%)	432(84.38%)	141(18.63%)	616(81.37%)
	No	303(14.08%)	1849(85.92%)	443(15.07%)	2,496(84.93%)
Hearing impairment	Yes	78(17.14%)	377(82.86%)	80(19.85%)	323(80.15%)
	No	305(13.81%)	1904(86.19%)	504(15.31%)	2,789(84.69%)
Health status	Excellent	38(9.11%)	379(90.89%)	44(8.76%)	458(91.24%)
	Very good	104(12.46%)	731(87.54%)	132(11.72%)	994(88.28%)
	Good	127(14.65%)	740(85.35%)	191(15.37%)	1,052(84.63%)
	Fair	83(19.21%)	349(80.79%)	165(25.27%)	488(74.73%)
	Poor	31(27.43%)	82(72.57%)	52(30.23%)	120(69.77%)

was set to select the top 20 features. To further refine our variable selection, we incorporated the Mean Decrease Impurity (MDI) algorithm, an integral component of the random forest methodology. This step quantified each feature's ability to reduce impurity, providing an empirical basis for their inclusion in the predictive model.

Subsequently, we conducted univariate logistic regression analyses to elucidate the relationship between these chosen variables and the development of OD. Variables demonstrating statistical significance ($p < 0.05$) were then incorporated into a multivariate logistic regression model. This final phase allowed us to delineate the statistical significance and potential predictive utility of these variables through calculated *p*-values and confidence intervals. To address potential multicollinearity issues, we performed a Variance Inflation Factor (VIF) analysis on the selected variables. The VIF matrix were calculated to assess the degree of correlation between predictors. The results of this analysis are presented in Supplementary Tables S1, S2.

Moreover, the incorporation of Restricted Cubic Splines (RCS) facilitated the intricate analysis of non-linear relationships between variables and the onset of oropharyngeal dysphagia. This advanced statistical technique provided a nuanced lens through which the complex interplay of predictive factors could be interpreted, significantly enhancing the study's analytical rigor and the interpretability of its findings.

Results

Analytical comparisons conducted via T-tests illuminated significant contrasts between individuals with and without one-set OD. Notably, those afflicted with OD demonstrated lower educational levels (men: 16.64% without one-set OD vs. 12.6% one-set OD; women: 17.93% without one-set OD vs. 13.55% one-set OD), diminished

average income (men: $70,599.21 without one-set OD vs. $50,662.94 one-set OD; women: $45,114.28 without one-set OD vs. $33,688.12 with one-set OD), and a heightened burden of comorbidities (men: 2.21 without one-set OD vs. 2.63 one-set OD; women: 2.47 without one-set OD vs. 3.09 one-set OD) as delineated in Figure 1.

Subsequent to the execution of 10 iterations of RFE averaged for analytical rigor, Figures 2A,B elucidate the principal 20 variables forecasting OD in older adults of both genders, adjudged by the MDI criterion. This nuanced analysis, with exhaustive rankings available in Supplementary Table S1, showcased a substantial overlap in the key predictive variables across genders. For the demographics, "weight" emerged as the paramount predictor, succeeded by "BMI" and "income." Conversely, for women, "income" was identified as the foremost predictor, followed by scores on the Short Physical Performance Battery (SPPB) and "BMI." Within the intermediary tier of predictors (ranks 4–10), physical performance metrics notably influenced OD risk for both genders. However, the men group also highlighted cognitive and emotional dimensions—specifically "self-reported memory status" and "lack of interest or pleasure"—as lower-contribution predictors.

In alignment with the RFE analysis outcomes, the subsequent univariate analyses across both genders delineated a notable association between economic status and cognitive self-assessment with the incidence of OD. Specifically, among male participants, the odds ratio (OR) for economic status was precisely 1.000 [95% Confidence Interval (CI): 1.000–1.000, $p < 0.001$], while the OR for self-reported cognitive status stood at 1.467 (95% CI: 1.313–1.638, $p < 0.001$). Correspondingly, for female participants, the OR for economic status mirrored that of their male counterparts at 1.000 (95% CI: 1.000–1.000, $p < 0.001$), and the OR for cognitive self-assessment was observed at 1.384 (95% CI: 1.258–1.522, $p < 0.001$). The VIF matrix were calculated to assess the degree of correlation between predictors. The results of this analysis are presented in Supplementary Tables S2, S3.

In the men cohort, stature was significantly correlated with one-set OD in univariate analysis (OR: 0.364, 95% CI: 0.136–0.974, $p = 0.044$); however, this correlation dissipated in the multivariate analysis (OR: 0.201, 95% CI: 0.003–13.034, $p = 0.451$). Conversely, variables pertaining to self-rated cognitive performance and anhedonia retained their significance in multivariate analysis, suggesting a profound linkage with one-set OD manifestation (self-rated memory status OR: 1.266, 95% CI: 1.123–1.427, $p < 0.001$; anhedonia OR: 1.261, 95% CI: 1.120–1.421, $p < 0.001$). For the women, the physical capacity to traverse three city blocks exhibited a significant univariate correlation (OR: 2.503, 95% CI: 2.092–2.995, $p < 0.001$), which did not persevere in multivariate analysis (OR: 1.179, 95% CI: 0.924–1.504, $p = 0.185$). Nonetheless, the apprehension regarding potential falls persisted as a significant multivariate correlate (OR: 0.693, 95% CI: 0.567–0.846, $p < 0.001$), evidencing a robust, independent influence on OD incidence (refer to Tables 2, 3).

RCS analysis underscored that superior physical fitness parameters, such as SPPB scores, grip strength, and respiratory function, inversely correlated with dysphagia risk (illustrated in Figures 3A–C). Similarly, enhancements in cognitive faculties were linked with diminished dysphagia occurrence (depicted in Figure 3D). Moreover, an escalation in chronic disease count was directly proportional to increased NI (Figure 3E). Notably, both the lower and upper extremes of Body Mass Index (BMI) were implicated in elevated dysphagia risk (Figure 3F). The risk of OD decreased as the repeat

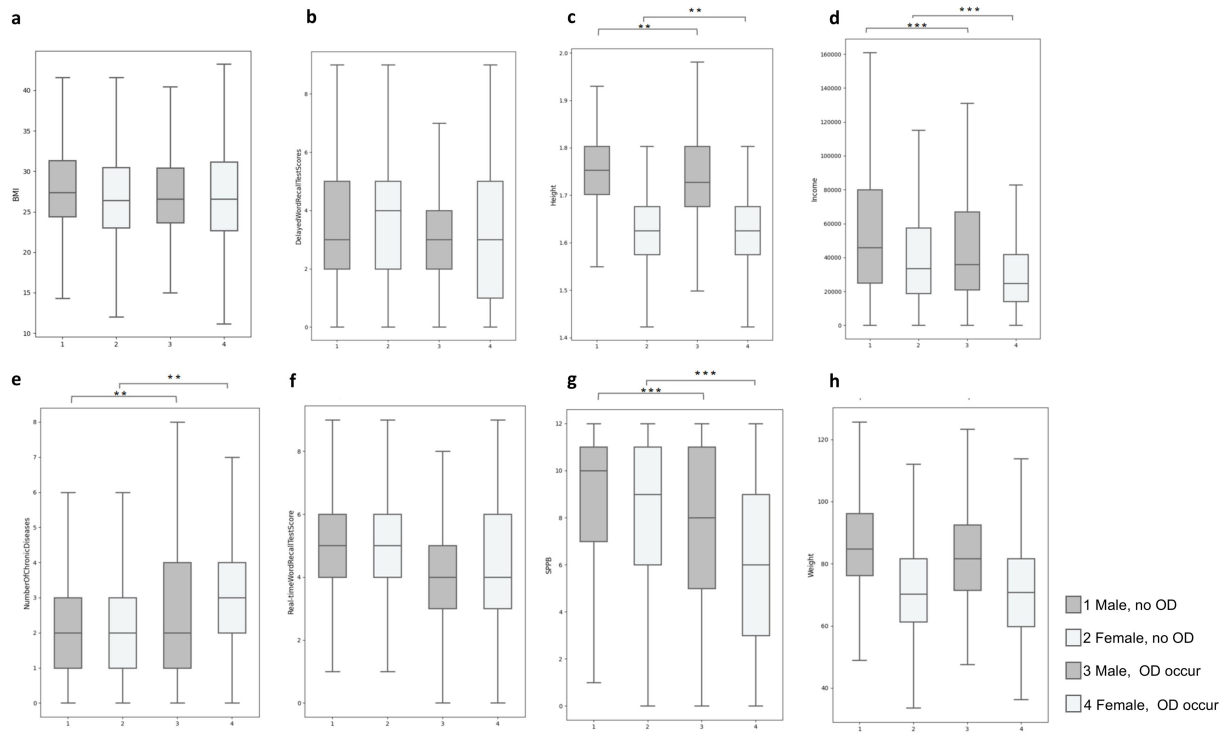

FIGURE 1
Quartile plots of the distribution of differences in typical characteristics of occurring and non-occurring OD in male versus female populations: **(A)** BMI, **(B)** Delay words recall test scores, **(C)** Height, **(D)** Income, **(E)** Number of chronic diseases, **(F)** Real time words recall test scores, **(G)** SPPB score, **(H)** Height $^{**}p < 0.01$; $^{***}p < 0.001$.

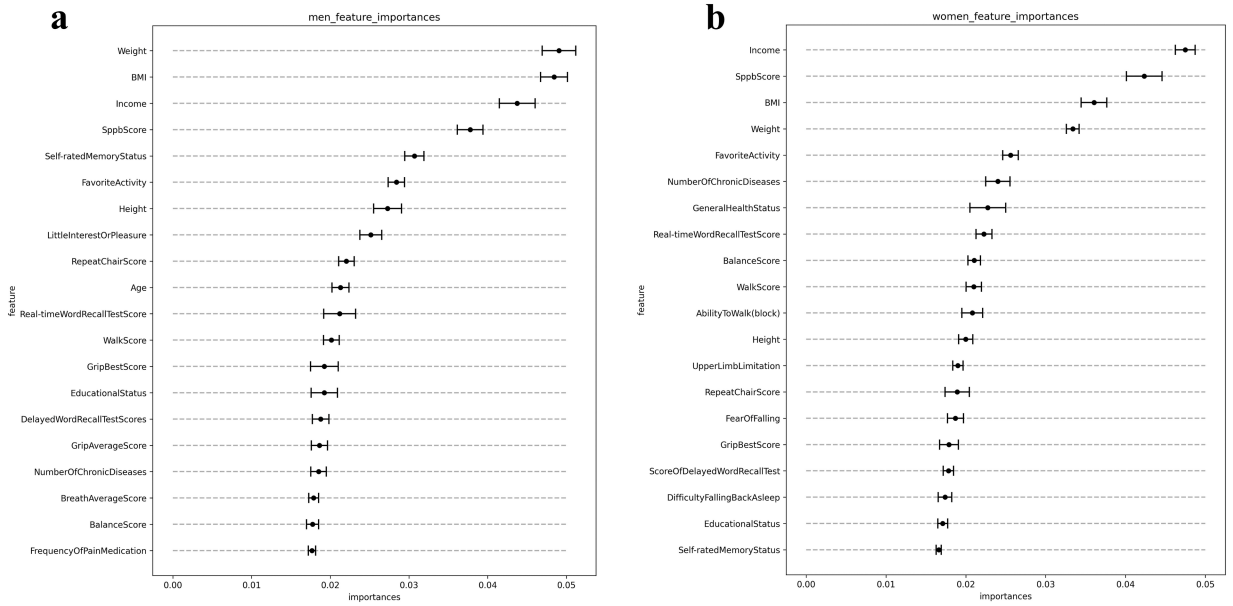

FIGURE 2
Top 20 features filtered by RFE and MDI calculations. **(A)** Top 20 features for men, **(B)** top 20 features for women. SPPB score: Short Physical Performance Battery score (0–12), assessing lower extremity function through balance, gait speed, and chair stand tests; Delayed Word Recall Test Scores: Number of words correctly recalled after a delay, from a list of 10 words presented earlier. Real-time Word Recall Test Score: Number of words immediately recalled from a list of 10 words. Grip Best Score: Highest recorded grip strength measurement using a dynamometer, best of two trials per hand. Grip Average Score: Average grip strength (kg) across all trials. Repeat Chair Score: Time to complete five consecutive chair stands without using arms (0–4). Walk Score: Time to walk 3 meters at usual pace (0–4). Balance Score: Ability to maintain balance in side-by-side, semi-tandem, and full tandem positions (0–4). Breath Average Score: Peak expiratory flow rate (L/min), average of two measurements using a peak flow meter.

chair score, realtime words recall score, and Grip average score increased (Figures 3G, H, J). As the number of chronic diseases increased, their risk of OD increased (Figure 3I).

Discussion

This retrospective cohort investigation employed a comprehensive analytical approach to elucidate the relative contribution of 128 potential protective and risk factors in the pathogenesis of one-set OD. We utilized a synthesis of Random Forest based RFE and MDI analysis to identify the most significant predictors. Subsequently, we applied both univariate and multivariate logistic regression modeling techniques to quantify the associations between these predictors and OD incidence.

Our findings revealed a notable prevalence of new-onset OD within a three-year span, with rates of 15.62% in 2011 and 14.49% in 2015. This slight decrease over time may reflect improvements in healthcare practices or increased awareness of OD prevention strategies. Importantly, we observed a gender disparity in OD prevalence, with women exhibiting a higher rate (15.8%) compared to men (14.38%).

The observed gender disparities in OD prevalence and associated risk factors reflect a complex interplay of biological, physiological, and sociocultural factors, underscoring the need for gender-specific approaches in OD prevention and management. Our comprehensive analysis reveals distinct patterns of risk factors between men and women, highlighting the importance of tailored interventions. In women, we found a stronger association between comorbidities and OD risk, which may be attributed to physiological differences such as lower muscle mass, especially in the oropharyngeal region, and a higher comorbidity burden including conditions like osteoporosis and autoimmune disorders that can indirectly impact swallowing mechanics. Additionally, women's increased susceptibility to malnutrition and vitamin deficiencies in older age groups may further contribute to their OD risk. Conversely, in men, our analysis revealed a more pronounced correlation between psychological factors, particularly anhedonia, and OD risk. This gender-specific pattern may be explained by neurobiological differences in stress responses, the impact of age-related testosterone decline on both muscle maintenance and mood regulation, and sociocultural factors such as traditional masculinity norms that may delay healthcare-seeking behaviors. Furthermore, men's higher likelihood of engaging in behaviors like excessive alcohol consumption or smoking may exacerbate both psychological distress and physical risk factors for OD.

Notably, the prevalence of chronic diseases emerged as a significant predictor of health outcomes irrespective of gender, signifying a ubiquitous health impact. Yet, the nuanced multivariate analysis among women participants accentuated the pronounced influence of comorbid conditions, suggesting a gender-disparate impact on health outcomes. Additionally, the role of psychological factors, particularly the manifestation of anhedonia, delineated notable gender-specific differences. While the association between psychological health dimensions and OD incidence was markedly significant in both univariate and multivariate analyses within the men cohort, such associations did not materialize within the female subset. This observation intimates that gender-specific mental health responses might pivotally influence the examined health outcomes, necessitating gender-tailored preventive and intervention strategies.

TABLE 2 Results of univariate logistic regression tests and multivariate logistic regression tests on the occurrence of OD in men.

Variable	OR	95% CI	OR	95% CI	*p*-value
	Univariate		Multivariate		
Height	0.364	(0.136, 0.974)	0.201	(0.003, 13.034)	0.451
Weight	0.986	(0.979, 0.993)	0.997	(0.957, 1.040)	0.903
BMI	0.977	(0.959, 0.995)	0.966	(0.855, 1.092)	0.582
Income	1.000	(1.000, 1.000)	1.000	(1.000, 1.000)	0.155
Sppbscore	0.894	(0.868, 0.921)	0.928	(0.835, 1.033)	0.172
DelayedWordRecallTestScores	0.921	(0.870, 0.975)	1.109	(1.015, 1.211)	**0.023**
Real-timeWordRecallTestScore	0.843	(0.788, 0.902)	0.907	(0.815, 1.008)	0.071
GripBestScore	0.797	(0.734, 0.866)	0.800	(0.498, 1.284)	0.354
GripAverageScore	0.800	(0.737, 0.869)	1.210	(0.752, 1.946)	0.432
RepeatChairScore	0.773	(0.713, 0.839)	0.990	(0.842, 1.164)	0.902
WalkScore	0.770	(0.705, 0.840)	1.035	(0.879, 1.217)	0.682
BalanceScore	0.774	(0.710, 0.844)	1.013	(0.862, 1.189)	0.879
Age	1.159	(1.078, 1.246)	1.029	(0.943, 1.123)	0.517
NumberOfChronicDiseases	1.194	(1.114, 1.280)	1.079	(0.999, 1.166)	0.053
FrequencyOfPainMedication	0.847	(0.790, 0.909)	0.900	(0.835, 0.971)	**0.006**
EducationalStatus	0.928	(0.887, 0.972)	1.024	(0.967, 1.084)	0.421
BreathAverageScore	0.797	(0.727, 0.873)	1.021	(0.908, 1.149)	0.729
Self-ratedMemoryStatus	1.467	(1.313, 1.638)	1.266	(1.123, 1.427)	**P < 0.001**
LittleInterestOrPleasure	1.413	(1.267, 1.576)	1.261	(1.120, 1.421)	**P < 0.001**

SPPB score: Short Physical Performance Battery score (0–12), assessing lower extremity function through balance, gait speed, and chair stand tests; Delayed Word Recall Test Scores: Number of words correctly recalled after a delay, from a list of 10 words presented earlier. Real-time Word Recall Test Score: Number of words immediately recalled from a list of 10 words. Grip Best Score: Highest recorded grip strength measurement using a dynamometer, best of two trials per hand. Grip Average Score: Average grip strength (kg) across all trials. Repeat Chair Score: Time to complete five consecutive chair stands without using arms (0–4). Walk Score: Time to walk 3 meters at usual pace (0–4). Balance Score: Ability to maintain balance in side-by-side, semitandem, and full tandem positions (0–4). Breath Average Score: Peak expiratory flow rate (L/min), average of two measurements using a peak flow meter. Bold values indicate $p < 0.05$.

TABLE 3 Results of univariate logistic regression tests and multivariate logistic regression tests on the occurrence of OD in women.

Variable	OR	95% CI	OR	95% CI	*p*-value
	Univariate		Multivariate		
SppbScore	0.880	(0.860, 0.900)	0.947	(0.866, 1.035)	0.227
Income	1.000	(1.000, 1.000)	1.000	(1.000, 1.000)	0.056
NumberOfChronicDiseases	1.307	(1.233, 1.384)	1.108	(1.035, 1.185)	0.003
Real-timeWordRecallTestScore	0.814	(0.773, 0.857)	0.851	(0.784, 0.923)	**P < 0.001**
UpperLimbLimitation	0.435	(0.363, 0.522)	0.716	(0.582, 0.881)	**0.002**
BalanceScore	0.710	(0.659, 0.764)	0.999	(0.862, 1.158)	0.989
WalkScore	0.690	(0.639, 0.745)	0.981	(0.852, 1.131)	0.796
RepeatChairScore	0.742	(0.694, 0.794)	1.077	(0.938, 1.235)	0.292
DelayedWordRecallTestScores	0.910	(0.873, 0.948)	1.088	(1.018, 1.162)	**0.012**
GripBestScore	0.672	(0.610, 0.741)	0.890	(0.797, 0.993)	**0.038**
GeneralHealthStatus	1.517	(1.393, 1.652)	1.073	(0.963, 1.196)	0.204
EducationalStatus	0.896	(0.857, 0.936)	1.015	(0.962, 1.072)	0.585
AbilityToWalk(block)	2.503	(2.092, 2.995)	1.179	(0.924, 1.504)	0.185
DifficultyFallingBackAsleep	0.834	(0.776, 0.897)	0.920	(0.854, 0.991)	**0.028**
FearOfFalling	0.449	(0.375, 0.538)	0.693	(0.567, 0.846)	**P < 0.001**
Self-ratedMemoryStatus	1.384	(1.258, 1.522)	1.080	(0.971, 1.201)	0.157

*SPPB score: Short Physical Performance Battery score (0–12), assessing lower extremity function through balance, gait speed, and chair stand tests. Real-time Word Recall Test Score: Number of words immediately recalled from a list of 10 words. Balance Score: Ability to maintain balance in side-by-side, semi-tandem, and full tandem positions (0–4). Repeat Chair Score: Time to complete five consecutive chair stands without using arms (0–4). Walk Score: Time to walk 3 meters at usual pace (0–4). Delayed Word Recall Test Scores: Number of words correctly recalled after a delay, from a list of 10 words presented earlier. Grip Best Score: Highest recorded grip strength measurement using a dynamometer, best of two trials per hand. Bold values indicate $p < 0.05$.

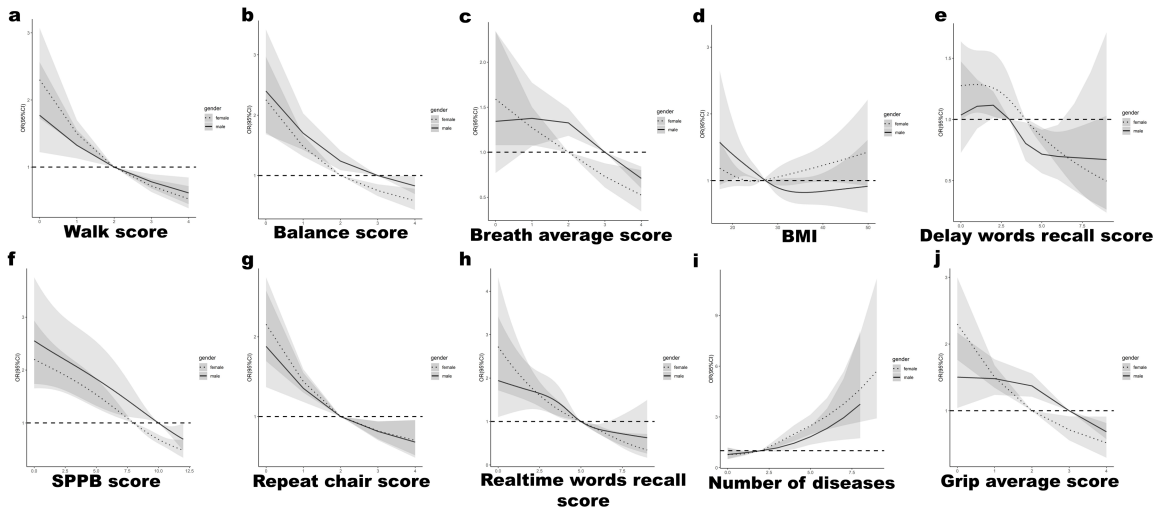

FIGURE 3
RCS curve analysis for male vs. female for top characteristics: **(A)** Walk score, **(B)** Balance score, **(C)** Breath average score, **(D)** BMI, **(E)** Delay words recall test scores, **(F)** SPPB score, **(G)** Repeat chair score, **(H)** Real time words recall test scores, **(I)** Number of chronic diseases, **(J)** Grip average score.

Our analysis revealed complex relationships between physical fitness, cognitive function, socioeconomic factors, and OD risk, with notable gender similarities and differences. T-tests revealed that individuals without OD, regardless of gender, had significantly higher income levels ($p < 0.001$), fewer comorbidities ($p = 0.01$), and higher SPPB scores ($p < 0.001$) compared to those who developed OD. Our findings both substantiate and extend the corpus of existing literature on the multifaceted etiology underpinning OD, highlighting the interplay of sociodemographic, physical, and mental health determinants (32). The delineation of both low and high BMI as pivotal indicators underscores the dual threats posed by malnutrition and obesity. Malnutrition, through its debilitative impact on pharyngeal muscle strength, directly impinges on the swallowing mechanism (33), whereas obesity predisposes individuals to conditions such as gastroesophageal reflux disease (GERD), a known precipitant of swallowing disorders (34).

Furthermore, the correlation between physical fitness, as assessed by the SPPB, and OD risk accentuates the criticality of maintaining robust physical health in ameliorating OD risk, spotlighting the essential role of muscle strength and mobility in effective deglutition (35). The SPPB score, a measure of physical fitness, showed a significant inverse relationship with OD risk in univariate analyses for both men and women. This finding underscores the complexity of OD etiology and the need for a multifaceted approach to prevention and management (35). Concurrently, the association of lower income levels with increased OD incidence highlights the intertwined challenges of healthcare accessibility, nutritional adequacy, and stress, elucidating a comprehensive framework for understanding and mitigating OD risk among older adults (36).

Cognitive impairment, with a particular emphasis on memory deficits, emerged as a salient predictive marker for one-set OD. The decrement in cognitive faculties is posited to compromise the coordination and safety of deglutition, thereby amplifying the susceptibility to aspiration and ancillary complications endemic to swallowing disorders (37). This underscores the imperative of integrating cognitive evaluations within the therapeutic framework for dysphagia management. It is worth noting that active participation in physical activities, especially outdoor activities (38), is closely related to the physical function (39) and cognition (40) of older adults, and should be identified as a protective factor for OD. Moreover, the investigation delineated a pronounced vulnerability to OD precipitated by negative affective states—specifically anhedonia—among the male geriatric cohort, a phenomenon less pronounced in their female counterparts. Chronic emotional distress is conjectured to catalyze a cascade of somatic and psychological responses, inclusive of detrimental effects on neurological integrity. The protracted ramifications of emotional stress may engender cognitive deterioration in older adults (41), potentially disrupting the neural circuits underpinning swallowing coordination (42). Additionally, the adverse emotional states are conjectured to modulate individual behavioral paradigms, such as dietary preferences and consumption patterns, further impinging upon swallowing functionality. For instance, depressive affective states may culminate in diminished appetite or disregard for nutritional adequacy, thereby exacerbating dysphagic conditions (43).

The findings from this research advocate for the adoption of a comprehensive, gender-specific approach to the early intervention and prophylaxis of one-set OD in the aging demographics. The institution of regular cognitive evaluations and rehabilitative measures is paramount for bolstering swallowing integrity and efficiency, particularly among individuals exhibiting cognitive impairment. Given the tangible impact of negative affective states on physiological well-being and deglutition dynamics, the provision of psychological assessments, emotional support mechanisms, and stress mitigation strategies is deemed essential. Behavioral and lifestyle modifications, encompassing nutritional guidance and the advocacy of salubrious eating behaviors, constitute pivotal components in the dysphagia management spectrum. For the male contingent, therapeutic strategies should

particularly accentuate psychological welfare and cognitive diagnostic evaluations, reflecting their heightened susceptibility to emotion-driven OD risks. Conversely, for the female cohort, the emphasis should pivot toward physical health optimization and comorbidity management. Additionally, the sensitization of caregivers and the broader community regarding the significance of early symptomatic recognition and holistic health stewardship is crucial for the timely identification and efficacious management of OD among the aging populace.

In this investigation, leveraging data spanning from 2011 to 2018, we prognosticated the incidence of new cases of OD within a triennial framework. While the forecasting methodology employed was rigorous, it was not without its limitations. The reliance on self-reported diagnoses, as opposed to the utilization of direct clinical diagnostic methodologies such as Video fluoroscopic Swallow Study (VFSS) or Fiberoptic Endoscopic Evaluation of Swallowing (FEES), introduces potential inaccuracies stemming from subjective biases. Furthermore, NHATS dataset's paucity of clinical measurements, including fluid analyses or imaging assessments, constrained the analytical depth, possibly eclipsing intricate facets of dysphagia's etiology. Additionally, the dataset lacked comprehensive information on certain diseases highly relevant to OD, such as amyotrophic lateral sclerosis (ALS) and gastroesophageal reflux disease (GERD), which could have provided valuable insights into the condition's development and progression. Moreover, the study's demographic skew toward a predominantly non-Hispanic white cohort may circumscribe the extrapolation of these findings to more heterogenous populations. Nonetheless, a pivotal strength of this research lies in its longitudinal design, which meticulously tracks temporal changes over a three-year period, offering invaluable insights into the evolution and determinants of OD. This longitudinal perspective significantly augments the study's contribution toward elucidating the dynamic trajectories of OD among older adults.

Looking forward, it is imperative for future inquiries to embrace a more inclusive demographic representation and to integrate clinical assessments of dysphagia. Specifically, future studies should consider employing objective diagnostic methods such as VFSS or FEES to validate self-reported results and reduce potential biases. Additionally, expanding the diversity of the sample through targeted recruitment strategies would enhance the generalizability of findings across different populations. Such enhancements will not only refine the precision of one-set OD diagnoses but also enrich our comprehension of its underpinnings, facilitating the development of more effective interventions and management strategies for this condition among aging populations.

Conclusion

In conclusion, this study illuminates the profound impact of one-set OD on older adults' quality of life and highlights the critical need for proactive prevention and timely intervention. Our analysis, employing random forest machine learning, has advanced the prediction of OD onset within a three-year timeframe, revealing complex interactions among socioeconomic, physical, cognitive, and emotional factors. Notably, we uncovered gender-specific risk patterns, with men showing greater vulnerability to cognitive and emotional factors, while women's risk is more closely tied to overall health and comorbidities. These findings underscore the necessity for gender-tailored approaches in OD management. We recommend that clinical applications focus on cognitive stimulation and emotional support for men, and comprehensive health management for women. Implementation strategies should include integrating gender-specific OD risk assessments into routine geriatric care, conducting targeted community screening programs, and launching public health campaigns to raise awareness. By adopting these personalized, evidence-based approaches, we can significantly enhance OD prevention and management, ultimately improving the quality of life for diverse older adult populations.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://www.nhats.org/researcher/nhats.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

SL: Conceptualization, Funding acquisition, Investigation, Validation, Writing – original draft, Writing – review & editing. L-JF: Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. HT: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. S-SW: Funding acquisition, Visualization, Writing – review & editing. S-JZ: Investigation, Writing – review & editing. MH: Supervision, Writing – original draft, Writing – review & editing. J-HW: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2025.1439579/full#supplementary-material

References

1. Clavé P, Shaker R. Dysphagia: current reality and scope of the problem. *Nat Rev Gastroenterol Hepatol*. (2015) 12:259–70. doi: 10.1038/nrgastro.2015.49

2. Thiyagalingam S, Kulinski AE, Thorsteinsdottir B, Shindelar KL, Takahashi PY. Dysphagia in older adults. *Mayo Clin Proc*. (2021) 96:488–97. doi: 10.1016/j.mayocp.2020.08.001

3. Meng P, Zhang S, Han C, Wang Q, Bai GT, Yue SW. The occurrence rate of swallowing disorders after stroke patients in Asia: a PRISMA-compliant systematic review and meta-analysis. *J Stroke Cerebrovasc Dis*. (2020) 29:105113. doi: 10.1016/j.jstrokecerebrovasdis.2020.105113

4. Takizawa C, Gemmell E, Kenworthy J, Speyer R. A systematic review of the prevalence of oropharyngeal dysphagia in stroke, Parkinson's disease, Alzheimer's disease, head injury, and pneumonia. *Dysphagia*. (2016) 31:434–41. doi: 10.1007/s00455-016-9695-9

5. Tagliaferri S, Lauretani F, Pelá G, Meschi T, Maggio M. The risk of dysphagia is associated with malnutrition and poor functional outcomes in a large population of outpatient older individuals. *Clin Nutr*. (2019) 38:2684–9. doi: 10.1016/j.clnu.2018.11.022

6. Stanga Z, Aubry E. Dehydration in dysphagia. In: O. Ekberg editor. Dysphagia. Springer. (2019) 859–71.

7. Carrión S, Costa A, Ortega O, et al. Complications of oropharyngeal dysphagia: malnutrition and aspiration pneumonia. In: O. Ekberg editor. Dysphagia. Springer, (2019) 823–57.

8. Regala M, Marvin S, Ehlenbach WJ. Association between postextubation dysphagia and long-term mortality among critically ill older adults. *J Am Geriatr Soc*. (2019) 67:1895–901. doi: 10.1111/jgs.16039

9. Banda KJ, Chu H, Chen R, Kang XL, Jen HJ, Liu D, et al. Prevalence of oropharyngeal dysphagia and risk of pneumonia, malnutrition, and mortality in adults aged 60 years and older: a meta-analysis. *Gerontology*. (2022) 68:841–53. doi: 10.1159/000520326

10. Doruk C, Mocchetti V, Rives H, Christos P, Rameau A. Correlations between anxiety and/or depression diagnoses and dysphagia severity. *Laryngoscope*. (2023) 134:2115–20. doi: 10.1002/lary.31164

11. Alentado VJ, Bisson EF, Potts EA. Dysphagia after cervical spine surgery: a review of risk factors and preventative measures. *J Neurosurg Spine*. (2022) 1:1–7. doi: 10.3171/2022.11.SPINE221247

12. Patel DA, Krishnaswami S, Steger E, Conover E, Vaezi MF, Ciucci MR, et al. Economic and survival burden of dysphagia among inpatients in the United States. *Dis Esophagus*. (2018) 31:dox131. doi: 10.1093/dote/dox131

13. Westmark S, Melgaard D, Rethmeier LO, Ehlers LH. The cost of dysphagia in geriatric patients. *Clin Econ Outcomes Res*. (2018) 10:321–6. doi: 10.2147/CEOR.S165713

14. Wirth R, Dziewas R, Beck AM, Clave P, Heppner HJ, Langmore S, et al. Oropharyngeal dysphagia in older persons–from pathophysiology to adequate intervention: a review and summary of an international expert meeting. *Clin Interv Aging*. (2016) 11:189–208. doi: 10.2147/CIA.S97481

15. The World Health Organization. Fact sheet: ageing and health The World Health Organization (2021). Available at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

16. Panebianco M, Marchese-Ragona R, Masiero S, Restivo DA. Dysphagia in neurological diseases: a literature review. *Neurol Sci*. (2020) 41:3067–73. doi: 10.1007/s10072-020-04495-2

17. Daniels SK. Neurological disorders affecting oral, pharyngeal swallowing GI Motility (2006). doi: 10.1038/gimo34

18. Schechter GL. Systemic causes of dysphagia in adults. *Otolaryngol Clin N Am*. (1998) 31:525–35. doi: 10.1016/S0030-6665(05)70068-2

19. Desai N, Hunold T, Kaperak C, Wang W, Kavitt R. Pharmacologic causes of dysphagia Dysphagia Academic Press (2023).

20. Christmas C, Rogus-Pulia N. Swallowing disorders in the older population. *J Am Geriatr Soc*. (2019) 67:2643–9. doi: 10.1111/jgs.16137

21. Fujita Y. Effects of developmental failure of swallowing threshold on obesity and eating behaviors in children aged 5–15 years. *Nutrients*. (2022) 14:2614. doi: 10.3390/nu14132614

22. Aly NAM, Ezzat WI, Fathi SM, Fayoumy N, Futooh SAA. Effect of smoking on multiple sclerosis related dysphagia. *Mult Scler Relat Disord*. (2020) 37:101531. doi: 10.1016/j.msard.2019.11.006

23. Namasivayam-MacDonald AM, Ayub A, Najeeb H, Shune SE. Understanding the independent predictors of dysphagia-related quality of life in stroke survivors. *J Speech Lang Hear Res*. (2022) 65:1697–723. doi: 10.1044/2022_JSLHR-21-00502

24. Dumican M, Watts C, Drulia T, Zhang Y. Dysphagia presentation, airway invasion, and gender differences in a clinically based sample of people with Parkinson's disease. *Dysphagia*. (2023) 38:353–66. doi: 10.1007/s00455-022-10472-y

25. Kenny C, Regan J, Balding L, Higgins S, O'Leary N, Kelleher F, et al. Dysphagia prevalence and predictors in cancers outside the head, neck, and upper gastrointestinal tract. *J Pain Symptom Manag*. (2019) 58:949–958.e2. e2. doi: 10.1016/j.jpainsymman.2019.06.030

26. Hägglund P, Gustafsson M, Lövheim H. Oropharyngeal dysphagia and associated factors among individuals living in nursing homes in northern Sweden in 2007 and 2013. *BMC Geriatr*. (2022) 22:1–11. doi: 10.1186/s12877-022-03114-3

27. Jung S, Kim JS, Jang I, Kim H. Factors related to dysphagia-specific quality of life in aged patients with neurologic disorders: a cross-sectional study. *Geriatr Nurs*. (2022) 43:159–66. doi: 10.1016/j.gerinurse.2021.11.016

28. Montaquila J, Freedman VA, Edwards B, Kasper JD. National Health and aging trends study round 1 sample design and selection: NHATS technical paper 1. (2012). Available at: https://www.nhats.org/sites/default/files/2021-01/NHATS%20Round%201%20Sample%20Design%2005_10_12_2.pdf (Accessed April 20, 2022).

29. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol*. (1994) 49:M85–94. doi: 10.1093/geronj/49.2.M85

30. Kroenke K, Spitzer RL, Williams JBW, Löwe B. An ultra-brief screening scale for anxiety and depression: the PHQ–4. *Psychosomatics*. (2009) 50:613–21. doi: 10.1176/appi.psy.50.6.613

31. Fan L, Zhao J, Hu Y, Zhang J, Wang X, Wang F, et al. Predicting physical functioning status in older adults: insights from wrist accelerometer sensors and derived digital biomarkers of physical activity. *J Am Med Inform Assoc*. (2024) 31:2571–82. doi: 10.1093/jamia/ocae224

32. Igarashi K, Kikutani T, Tamura F, Yajima Y, Tohara T. Factors predicting the effects of dysphagia rehabilitation on multidimensional functional status in elder outpatients: a prospective cohort study. *Gerodontology*. (2020) 37:271–8. doi: 10.1111/ger.12476

33. Veldee MS, Peth LD. Can protein-calorie malnutrition cause dysphagia? *Dysphagia*. (1992) 7:86–101. doi: 10.1007/BF02493439

34. Hampel H, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. *Ann Intern Med*. (2005) 143:199–211. doi: 10.7326/0003-4819-143-3-200508020-00006

35. Woods JL, Iuliano-Burns S, King SJ, Strauss BJ, Walker KZ. Poor physical function in elderly women in low-level aged care is related to muscle strength rather than to measures of sarcopenia. *Clin Interv Aging*. (2011) 6:67–76. doi: 10.2147/CIA.S16979

36. Brinkman HJ, de S, Sanogo I, Subran L, Bloem M. High food prices and the global financial crisis have reduced access to nutritious food and worsened nutritional status and health. *J Nutr*. (2010) 140:153S–61S. doi: 10.3945/jn.109.110767

37. Castagna A, Ferrara L, Asnaghi E, Rega V, Fiorini G. Functional limitations and cognitive impairment predict the outcome of dysphagia in older patients after an acute neurologic event. *Neurorehabilitation*. (2019) 44:413–8. doi: 10.3233/NRE-182635

38. Fan L, Zhang J, Wang F, Liu S, Lin T. Exploring outdoor activity limitation (OAL) factors among older adults using interpretable machine learning. *Aging Clin Exp Res*. (2023) 35:1955–66. doi: 10.1007/s40520-023-02461-4

39. Fan L, Zhang J, Zhao J, Wang F, Luo T, Lin T. PFIMPA: a multimodal approach to predict physical function impairment in older adults using physical activity from wrist-worn accelerometer. *IEEE Trans Instrum Meas*. (2024) 73:1–13. doi: 10.1109/TIM.2024.3470051

40. Fan L, Wang F, Zhao J, Zhang J-J, Li Y-A, Tang J, et al. From physical activity patterns to cognitive status: development and validation of novel digital biomarkers for cognitive assessment in older adults. *Int J Behav Nutr Phys Act*. (2025) 22:11. doi: 10.1186/s12966-025-01706-x

41. Lupien SJ, Juster RP, Raymond C, Marin MF. The effects of chronic stress on the human brain: from neurotoxicity, to vulnerability, to opportunity. *Front Neuroendocrinol*. (2018) 49:91–105. doi: 10.1016/j.yfrne.2018.02.001

42. Porges SW. The polyvagal theory: phylogenetic substrates of a social nervous system. *Int J Psychophysiol*. (2001) 42:123–46. doi: 10.1016/S0167-8760(01)00162-3

43. Massa L, Fattori B, Nacci A, Santoro A, Palagini L, Abelli M, et al. Psychopathological aspects of dysphagia: a systematic review on correlations with eating disorders and other psychiatric conditions. *Eat Weight Disord*. (2022) 27:881–92. doi: 10.1007/s40519-021-01227-z

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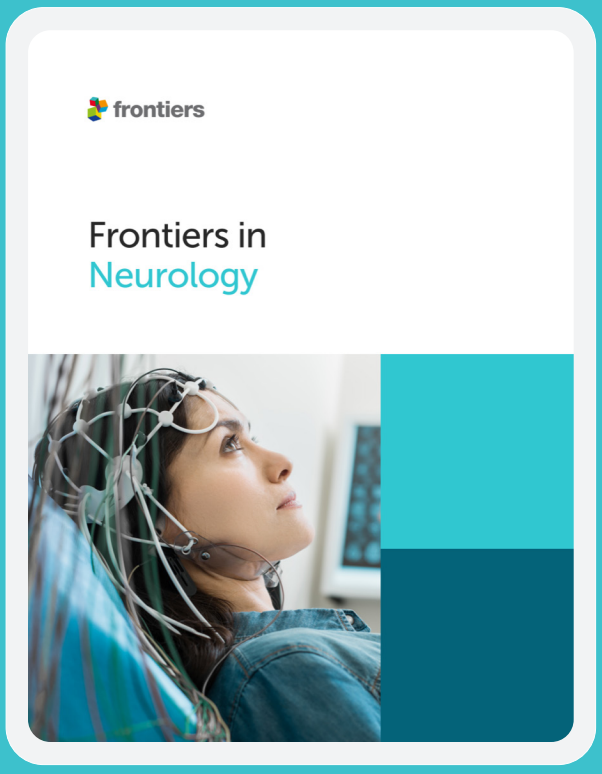

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