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# **Studying the neural correlates of upper aerodigestive tract functions under natural conditions: A protocol using functional near-infrared spectroscopy, cervical acoustics, and accelerometry**

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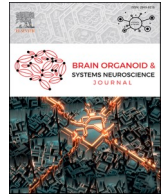


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## Method articles

## Studying the neural correlates of upper aerodigestive tract functions under natural conditions: A protocol using functional near-infrared spectroscopy, cervical acoustics, and accelerometry

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## ABSTRACT

**Background:** The upper aerodigestive tract (UADT) is a complex structure with multiple synchronized vital functions, including swallowing and breathing, that rely on central neurological controls and cervical effectors. Reference UADT assessments have questionable limitations in natural conditions. Here, we describe our new protocol addressing these limitations. Our protocol combines three non-invasive technologies to evaluate UADT functions in natural conditions. We aim to correlate the cortical and cervical activities of UADT functions in a real-world context.

**New method:** Healthy subjects perform speech, coughing, throat clearing, and swallowing tasks in a natural sitting position. Cervical evaluation uses acoustic and accelerometric measures that reflect the laryngeal movements and bolus progression. We manually segment the events of each task. Functional near-infrared spectroscopy measures the concurrent cortical activity from the bilateral inferior pericentral regions, including the laryngeal sensorimotor cortices. We statistically compare event signal duration and bolus types under volitional and spontaneous conditions across the three technologies.

**Comparison with existing methods:** Reference UADT evaluations show limitations: cervical assessment references are irradiating (videofluoroscopy) or invasive (flexible nasal laryngoscopy); neurological assessment with functional magnetic resonance imaging (fMRI) does not allow swallowing in sitting position.

**Expected results and perspectives:** We aim to validate this protocol in natural settings and correlate cervical activity with cortical responses. This protocol opens perspectives to investigations on UADT functions in subjects currently with reduced access to gold standards, including children and dysphagic subjects with neurological dystonia.

**Conclusion:** We propose an innovative protocol for UADT evaluation in non-invasive natural conditions.

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## 1. Introduction

### 1.1. Rationale and background

#### 1.1.1. The functions of the upper aerodigestive tract

The upper aerodigestive tract (UADT) is a complex structure incorporating the oral cavity, the throat, the larynx, and the upper esophageal sphincter. Suggested by its name, the UADT is the crossroads of both the airways and the digestive tract. Given these have different functions, the UADT relies on the perfect synchronization of over 30 neuromuscular structures (Ludlow, 2005). This synchronization is crucial for multiple antagonist functions, mainly breathing, swallowing, speech, and airway protection. Furthermore, this synchronization is critical under both reflective and volitional conditions.

The breathing function illustrates this synchronization well. The diaphragm is the primary muscle involved and its cyclic spontaneous contractions can be modulated at will according to various spontaneous or volitional conditions (physical activities, singing, coughing, hiccups). The automatic respiratory activity of the diaphragm has been shown to emanate from a central pattern generator (CPG) located in the Pre-Bötzinger and Böttinger Complexes of the medulla (Fogarty et al., 2018; Fogarty and Sieck, 2020). This activity is unconsciously modulated by brainstem chemoreceptors and lung mechanoreceptors.

If needed, diaphragm activity can be volitionally-controlled. The cortical correlates of this control appear to be located on the posterior primary motor cortex close to the midline, with superior control from the supplementary motor area (SMA) and premotor cortex (PMC) (Khedr and Trakhan, 2001; McKay et al., 2003). It should be noted that there is also evidence that different volitional tasks may involve different cortical networks (Helou and Dum, 2023). These cortical networks modulate the Pre-Bötzinger and Böttinger Complex activities, but could also modulate another specific CPG activity for forced expiration (Janczewski and Feldman, 2005).

Coughing is also an interesting mechanism coordinating respiration (through diaphragm contraction) and laryngeal glottal closure then release (Fogarty et al., 2018). On the one hand, in its reflexive form, coughing allows the tracheobronchial tree to clear itself from secretions and/or aspirated substances creating local stimulation. On the other hand, volitional coughing does not strictly need tracheal stimulation to be triggered. In both cases, coughing is also followed by a swallowing reflex to ensure pharyngeal clearance from secretions/substances (Pitts et al., 2013). Spontaneous and volitional coughing have been shown to clinically differ in terms of airflow (Brandimore et al., 2015). This difference could reflect the different neural pathways of the two coughing conditions (Mazzone et al., 2013).

Although phonation is not a vital function of the UADT, there is still a defined regulation of breathing and swallowing so they can perform their roles without hindering intelligibility. In healthy subjects, some studies have suggested that breath intake duration is correlated to the length of sentences within a speech (Fuchs et al., 2013; Whalen and Kinsella-Shaw, 1997). This mechanism suggests a role for speech motor planning, namely the role of the PMC. Other studies have demonstrated that the length of preceding sentences influences the duration and depth of breath intake, which strengthens the role of spontaneous respiration (Kallay et al., 2019). Although it is still unclear whether breathing prepares or corrects speech duration, or both, we can see how breathing coordinates both breath intake and depth accordingly. Interestingly, Huber et al. (2012) found data suggestive of impairment of both respiratory and cognitive mechanisms in subjects with Parkinson's Disease.

Swallowing has specific characteristics according to its spontaneous or volitional activities. The reflective synchronization of spontaneous swallowing with breathing is critical. For instance, our saliva is constantly produced throughout the day when we are awake, and one control mechanism of this flow is spontaneous swallowing. This happens during everyday life while talking and breathing (Fukuike et al., 2015). Similarly, synchronization of breathing and swallowing is still needed

while sleeping to ensure normal ventilation and prevent aspiration pneumonias. However, the frequency of breathing and swallowing is reduced according to the stage of sleep (Ertekin, 2011; Sato, 2020). This means that the CPGs for breathing and swallowing interact, as shown in cats by Dick et al. (1993). The authors showed that swallowing CPG activity may inhibit the respiratory CPG, but the respiratory CPG could also modulate swallowing CPG activity. Some physiological aspects of this coordination are well described, with spontaneous swallowing shown to occur mostly during the expiratory phase of the respiratory cycle. Indeed, observations have associated the swallow sequence with a pause during the respiratory cycle, hereafter called a "deglutitive apnea" by Shaker et al. and others (Hattori et al., 2022; Shaker et al., 1992; Yagi et al., 2017). During this apnea, the larynx is closed by both the epiglottic posterior tilt, the arytenoids anterior tilt, and glottis closure contraction (Abe and Tsubahara, 2011).

Nevertheless, this coordination must be capable of adapting to voluntary swallowing (i.e., during meals). When this "deglutitive apnea" occurs in young healthy subjects varies according to task conditions. Although it occurs 55 % of the time during the expiratory phase of the saliva swallowing cycle at rest, this rate increases to 90 % during tachypnea or water swallowing. In their review, Ertekin et al. (2011) illustrated that the critical cause for changes in "deglutitive apnea" timing is the presence of an oral phase during volitional swallowing, whereas this phase is inconsistent during spontaneous swallowing, or at least shorter. This led to measures showing that volitional swallowing is longer than spontaneous swallowing (Ertekin, 2011). The pharyngeal and esophageal phases appear similar in mechanism and length as they are more reflective phases dependent of a specific bulbar CPG (Ertekin, 2011). In all, the regulation and adaptation of each function contributes to the smooth running of all the other functions.

UADT dysfunctions can lead to vital and complex social complications that alter patient quality of life (Jones et al., 2018). It is therefore important to consider the deficiency of each function, both individually and when combined with other functions. Indeed, as in neurodegenerative disorders or respiratory diseases, accumulated deficiencies lead to rapid deterioration in subject well-being (Martin-Harris, 2008; Shaker et al., 1992). Dysphagia alone increases the risk of malnutrition and aspiration pneumonia, while it also alters breathing function. Ultimately, the onset of dysphonia reduces the possibility of receiving caregiver help and also leads to isolation of the subject, worsening the prognosis.

This complex interweaving of functions means neural network planning is required for each different function when one single function is performed. This involves neural networks specific for each task and their coordination, with different neural networks for volitional and reflective conditions. Indeed, different dysfunctions lead to different clinical presentations and different neurological adaptations. This complexity justifies the need for specific protocols for measuring cerebral activity in each condition, and for examining the correlations between cerebral activity and the quality of function execution. Given that the literature to date focuses on a single function and condition, and in order to explore the synchronized cervical and cortical activities, we sought to set up a protocol investigating the different integrated functions in a non-invasive and real-world context. For clarity, we focus on swallowing and dysphagia. However, we propose a protocol recording continuous activity, thus including non-swallowing functions. This protocol aims to better decipher the pathophysiology underlying the different UADT dysfunctions and opens perspectives in the rehabilitation of these disorders.

#### 1.1.2. Cervical evaluation of UADT functions

At the cervical level, UADT functions can be explored through multiple technologies. In clinical practice, the current gold standard methodologies remain flexible laryngoscopy (FLS) of the nose and videofluoroscopy (VFS). FLS allows observation of pharyngolaryngeal movements during breathing, speech, and swallowing (Stachler et al.,

2018). VFS can follow bolus transit while swallowing, and allows observation of muscle movement during swallowing and other functions (Helliwell et al., 2023). However, these methods either use radiation or are invasive (thus potentially painful) and alternatives are needed, especially for children.

Other technologies have thus been developed, such as surface electromyography (sEMG) (Ding et al., 2002; Dziewas et al., 2003; Teismann et al., 2007), accelerometry (Jestrović et al., 2013), and microphonics (Hammoudi et al., 2014; Jestrović et al., 2013; Morinière et al., 2006). Electromyography explores muscle contractions, accelerometry measures laryngeal movements, and microphonics collect the acoustics of bolus and air flows through the UADT when performing each of its functions. Each technique has been used to explore multiple UADT functions with specific signals. For instance, it has been shown that swallowing strength is correlated to the amplitude and duration of sEMG signals (Ng et al., 2022) and with some properties of bolus intake (Kohyama et al., 2015). Alfonsi et al. used sEMG for dysphagia evaluation in subjects with neurological diseases. The authors showed an increased motor sequence duration in subjects with Parkinson's disease and multiple sclerosis (Alfonsi et al., 2013, 2007), as well as a lack of coherence of muscular activities in subjects with amyotrophic lateral sclerosis. Similarly with acoustics, Morinière et al. (2006) illustrated that swallowing sounds follow a three-component pattern in healthy subjects. This pattern was found modified in patients with laryngeal cancer after surgery, with the post-operative pattern being correlated to the type of surgery (Morinière et al., 2011). In case of partial laryngectomy, the three components persisted with a prolonged total swallowing time and reduced upper esophageal sphincter opening sound. In case of total laryngectomy, the third component (the laryngeal release sound) was either absent in half of the cases or replaced by a pharyngeal opening sound in half of the cases.

These different technologies can now be used to automatically detect UADT events. Using the different signal characteristics specific to the bolus or task, and the indices of specific diseases identified through deep learning, we can now automatically detect normal UADT events (Dudik et al., 2018; Mao et al., 2019) as well as their abnormal counterparts. For instance, the detection of silent aspiration (Shu et al., 2022) without any risk to the subjects. Such methodologies setup in natural conditions are critical for exploring the neurological correlates of UADT functions with reduced methodological bias, especially in subjects with medical conditions or after surgery.

### 1.1.3. Neurological correlates of UADT functions and their evaluation

Neurologic control of the UADT has been shown to emanate from both the brainstem and cerebral cortex through multiple anatomical and neurofunctional studies (Jean, 2001; Ludlow, 2015, 2005). Early anatomical studies have suggested that the nucleus ambiguus and retroambiguus, located in the medulla, collects multiple reflexive CPGs for UADT functions, such as speech, coughing, and swallowing. This theory was further supported by neurofunctional studies (Ludlow, 2015, 2005; Zhang et al., 1995). In a review of the literature – focusing on animal anatomical studies, functional magnetic resonance imaging (fMRI), and human functional positron emitting tomography (fPET) studies – Ludlow (2015) has described brainstem control as a CPG located in the nucleus ambiguus (in the ventral swallowing group). This activates the superior laryngeal nerve. The cortical areas that influence this CPG were described as the "cortical swallowing area", referring to the association of the cingulate cortex, insula, SMA, and the inferior pericentral sensorimotor cortex. The "cortical swallowing area" corresponds to the pharyngolaryngeal area according to the Penfield's homunculi (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950). These neurological centers activate the pharyngeal and laryngeal muscles, which then sequentially propel the bolus from the mouth to the esophagus.

One limitation of the aforementioned studies is that fPET uses radiation. Furthermore, both fPET and fMRI are performed in lying position, which is not always the normal conditions for UADT functioning and

thus limits applicability of these findings to real-life. A non-radiative and real-world alternative for studying the cerebral correlates of UADT functions would overcome these limitations.

It was found in a previous review of the literature (Gallois et al., 2022) that multiple studies have tried to overcome these limitations by using three techniques for cerebral swallowing evaluation: electroencephalography (EEG), magnetoencephalography (MEG), and functional near-infrared spectroscopy (fNIRS). On the one hand, EEG and MEG measure neuronal cellular activity, either electrical (EEG) or electromagnetic (MEG). These techniques can precisely locate the swallowing cortical activity on the inferior pericentral cortex, similar to the cortical swallowing area described by Ludlow (2015). Using EEG, Hiraoka (2004) studied the movement-related cortical potential (MRCP) associated with swallowing. The authors showed that the MRCP is located on the inferior pericentral cortex and the vertex, and MRCP amplitude is greater with swallowing saliva than swallowing water. Nonaka et al. (2009) then observed that the amplitude of swallowing-associated MRCPs were increased for supervised swallowing tasks compared to self-paced volitional swallowing tasks. Using MEG, Dziewas et al. showed the implication of the pericentral inferior cortex for the swallowing function (Dziewas et al., 2003; Teismann et al., 2009). The authors also showed modifications of pericentral inferior cortex activity in dysphagic patients compared to healthy subjects (Dziewas et al., 2009; Teismann et al., 2011a, 2011b, 2008). After, the authors further explored the effect of therapies on pericentral inferior cortex activity, such as transcranial direct-current stimulation (Suntrup-Krueger et al., 2018). Briefly, fNIRS measures the variations in oxyhemoglobin and deoxyhemoglobin, similarly to blood oxygen level-dependent (BOLD) signals measured with fMRI. These signals (fNIRS and BOLD) reflect the hemodynamic blood flow variations associated with a task and are considered an indirect measure of cellular activity. From these principles, studies using fNIRS found results showing hemodynamic responses over the inferior pericentral cortex with less spatial precision (Kober, 2018; Kober et al., 2015a).

These three methods (EEG, MEG, fNIRS) are able to measure differences according to the type of function, the type of bolus (quantity and viscosity), and the difficulty of the task (Gallois et al., 2022). For instance, Jestrović et al. have shown through EEG that micro-architectural structural differences of the cerebral networks are associated with swallowing, and variations are due to the effects of texture (Jestrović et al., 2014), head position (Jestrović et al., 2016, 2015), or the presence of a cognitive distractor during the task (Jestrović et al., 2018). Nonaka et al. (2009) showed different cortical EEG activity between swallowing naturally and "with effort". Using fNIRS, Kober et al. showed the different cortical activities of swallowing water versus swallowing saliva (Kober, 2018), and the different cortical activities of motor execution and motor imagery of swallowing (Kober et al., 2015a). Suntrup et al. (2013b) have studied the effect of a "challenging" swallow versus a "normal" swallow effort using MEG. However, none of the studies compared swallowing function to other functions in real-world contexts (i.e. similar to during a meal).

### 1.1.4. Multiple signal analysis and algorithmics

Some authors have found it valuable to associate neurological with cervical signal measures. Teismann et al. and Suntrup et al. synchronized sEMG with MEG signals in multiple studies on the swallowing cortical networks of subjects with neurological diseases (Suntrup et al., 2014; Teismann et al., 2011a). For their study of the micro-architectural structure of the swallowing network, Jestrović et al. associated accelerometric with EEG measures (Jestrović et al., 2016, 2015). Suchlike technique combinations allow for the precise measure of synchronization, thus unveiling the cortical correlates of the cervical sensorimotor activity. Identification of these cortical correlates would also highlight any correlations between cervical and cortical signals, allowing us in turn to question the differences in correlations between healthy subjects and subjects with a medical condition. However, in most of these

studies, the cervical signal was limited to an event marker. The exploration of correlations between the characteristics of swallowing cervical activity and its cortical correlates has remained limited. Indeed, studies have focused mostly on one UADT function, and the cervical signals were mostly used as a time reference to study the cortical signal. The cortical activity was never correlated to the cervical signal task-related characteristics. Thus, cervical- and cerebral-associated UADT physiology was never addressed, limiting the transfer of findings to pathophysiology.

## 1.2. Hypotheses

Our first hypothesis is that cervical signal labeling would allow us to measure more precisely the cortical activity associated with UADT tasks, and especially allow us to demonstrate differences in cortical activity according to each individual task.

Our second hypothesis focuses on the swallowing function itself. We hypothesize that the type of bolus and swallowing quality would correlate with and modify the associated cortical response (Jestrović et al., 2014; Kober, 2018). We expect to find correlations between the cervical and the cortical fNIRS parameters. We expect the accelerometer and acoustic characteristics of the events to align with temporal and amplitude patterns, as well as the models already described in the literature (Dudik et al., 2018; Mao et al., 2019; Morinière et al., 2006, 2011). These characteristics should be correlated to the cortical evoked hemodynamic responses (HDR).

## 1.3. Objectives

We first aim to create and validate a synchronized cervical and cortical measure protocol able to study the different UADT functions in both volitional and reflexive conditions under natural conditions in healthy subjects. For future applications, this protocol must be suitable for any subject; with or without a medical condition, adults or children. Secondly, we aim to correlate cortical activity with different cervical signal characteristics for the many different UADT functions. Thirdly, we aim to compare the results from manual versus an algorithmic segmentation of our signals. Lastly, we aim to validate the cortical patterns evoked by UADT tasks in healthy subjects. Overall, we sought to evaluate the feasibility of this protocol for use in determining swallowing pathophysiological profiles.

## 2. Materials and methods

### 2.1. Subject inclusion

According to a previous literature review and more recent reports in the field, the mean number of subjects included has been 17 [10–20] for the study of cortical-control of swallowing with sufficient statistical power to account for intra- and inter-subject differences between tasks (Chua and Chan, 2024; Gallois et al., 2022; Lee et al., 2018; Muhle et al., 2021; Restrepo et al., 2020; Suntrup-Krueger et al., 2021; Yuan et al., 2017). To reduce age-related variability, all subjects included are aged from 18 to 65 years, and are free of any neurological, muscular, or cervical diseases. We use the cognitive disorder examination (CODEX test) to ensure that subjects do not manifest any cognitive impairment. Only subjects with test scores of 4 or 5 will be included for study. Subjects with a score of  $\leq 3$  will be excluded. Subjects receive oral and written information about the study protocol, and only subjects giving oral and signed consent for participation can initiate the experimental protocol and be included for study.

The following protocol has been accepted by the Toulouse University Research Ethics Committee (international review board n°00011835–2023–0221–635).

### 2.2. Experimental conditions and tasks

Subjects sit in a chair at a table to perform the different tasks in a 2 m<sup>2</sup> room with no windows to reduce light noise for fNIRS. A single lamp is positioned on the floor to enable subjects to perform their tasks while limiting noise from other external light sources. Fig. 1a displays the installation and depicts the recording process.

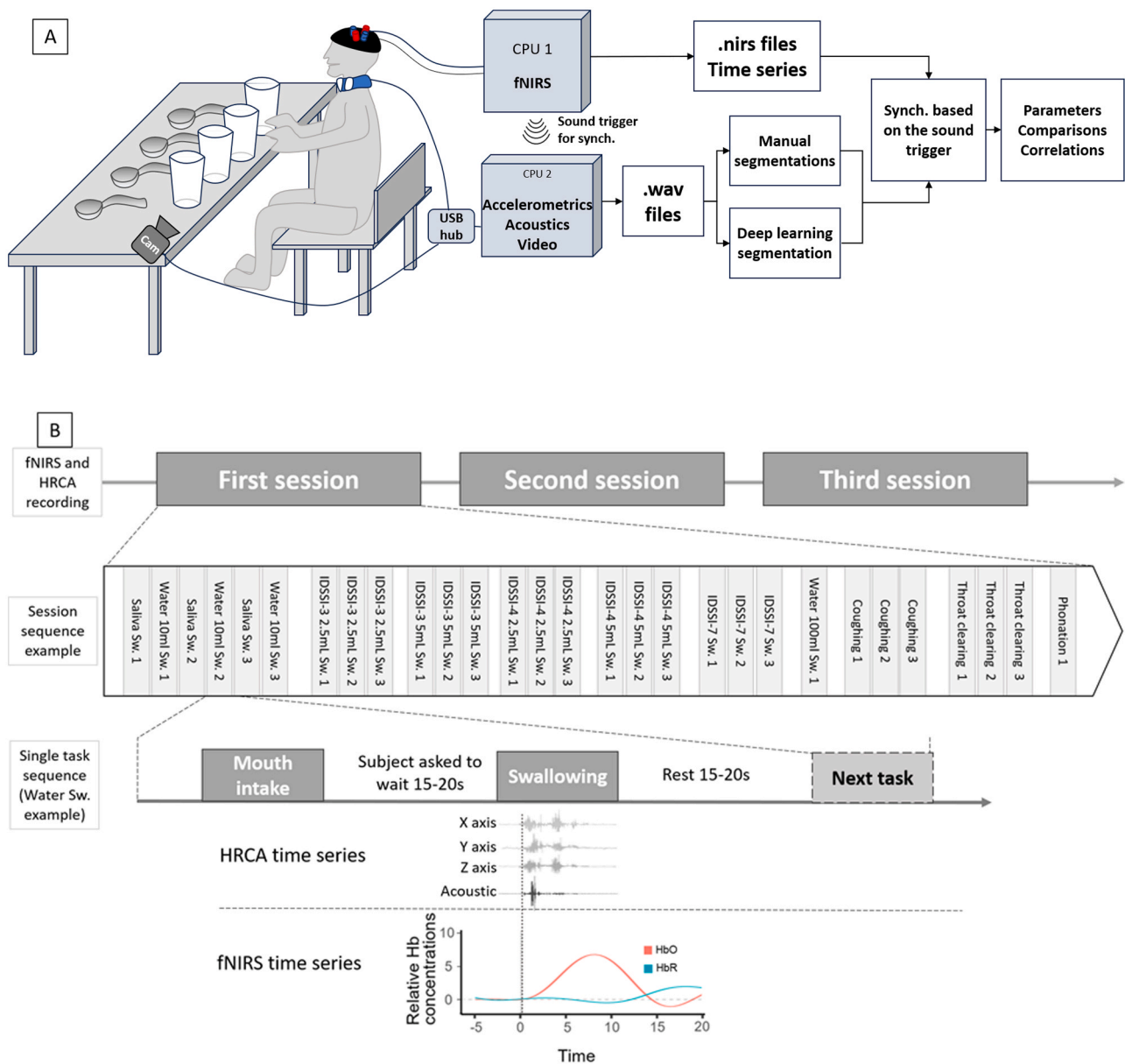
The subjects sequentially perform different tasks: swallowing, coughing, throat clearing, and phonation (see Table 1). Each task is performed 3–4 times in one session, and then each session is repeated 3 times, resulting in a total of 10 repetitions for each task. Note that the 100 ml liquid swallowing task takes a long time to perform and may be repeated less times given the good-quality cerebral signals that can be received. There is a break between each session given the numerous tasks to allow the subject to relax. Fig. 1b displays the course of the experimental phase and the tasks performed. We do not randomize tasks to avoid increasing inter-subject variability in task order performance. Introducing this variability would require the inclusion of more subjects given the high number of different tasks. We have made this choice at the cost of introducing a task order effect in future study cohorts.

For the swallowing tasks, we describe the textures according to the International Dysphagia Diet Standardization Initiative (IDDSI) food texture classification. This is currently used by both the European Society for Clinical Nutrition and Metabolism (ESPEN) and the Academy of Nutrition (Cichero et al., 2017; Thibault et al., 2021; Academy of nutrition and dietetics, 2021). This is despite debate in recent years (Pedrolli, 2022; Rothenberg et al., 2022). The classification extends from "thin liquids" like water (IDDSI 0) to "regular" solid food (IDDSI 7). For our protocol, the subjects process their own saliva, IDDSI-0 liquid, IDDSI-3 and IDDSI-4 preparations, and IDSSI-7 cookies.

We use either 10 ml or 100 ml of tap water in a glass for IDDSI-0. For IDDSI-3 and IDDSI-4 preparations, we use thickening powder from a commercial kit (Magic Mix, Laboratoire Picot®, Calais, France) added to water with syrup (either lemon, mint, or strawberry according to subject choice). We use 1.4 scoops of thickening powder in 100 ml of water with syrup for IDDSI-3, and 1.7 scoops in 100 ml of water with syrup for IDDSI-4. To avoid variations due to daily fluctuations of room temperature, we verify the final texture just before administration to the subject using a 10 ml syringe and the syringe-based flow test from the IDDSI Framework (Cichero et al., 2017). Powder is added if needed. IDDSI-3 and 4 were given by spoon at a volume of either 2.5 ml or 5 ml. IDDSI-7 consisted of half a biscuit (approximately 3–4 g). The tasks and volumes are presented in Table 1.

It is difficult to test more IDDSI textures given the subjects perform many tasks. We chose IDDSI-3 and IDDSI-4 as they are "boundary" liquids separating liquidized/moderately thick textures from pureed/extremely thick textures.

The oral intake steps before each swallow are used as a control sensorimotor task. It is defined as the period from mobilizing the hand to grab the glass/spoon to placing the glass/spoon back on the table. We suspect that this task activates different cortical areas according to the Penfield's homunculi (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950). For the 10 ml of water, IDDSI-3 and IDDSI-4 bolus, and the cookie, the subjects are asked to orally intake the bolus and to wait approximately 15–20 s before swallowing (or chewing and swallowing the cookie). Comments on the timing of this specific step are only given if the subject is really too fast or really too slow; in this case the subject is recommended to slightly speed up or slow down accordingly. This 15–20 s delay was chosen to allow reduction of food/drink-handling-evoked HDRs before swallowing and to avoid an overly long experimental phase. For other tasks, subjects were asked to wait for approximately 15–20 seconds before starting a new task session for similar reasons. As for the tasks themselves, the subjects completed them at their own pace and without pace correction.



**Fig. 1.** Recording paradigm. A) Diagram illustrating the subject and recording setups with the analyzes performed. B) Diagram representing the task flow paradigm. Each task sequence within a session was repeated three times. The fNIRS and HRCA records constantly. We use the HRCA signal to define the beginning of the accelerometric and acoustic event, and use it to define the HRCA and fNIRS time series. Cam: camera; fNIRS: functional near infra-red spectroscopy; HRCA: high-resolution cervical auscultation; Sw.: swallowing; Synch.: synchronization.

### 2.3. Cervical evaluation

We used a non-invasive high-resolution cervical auscultation (HRCA) method for collecting both acoustics and three-axis accelerometric recordings. This method has been found more efficient for swallowing detection than using acoustics and three-axis accelerometry separately. It has also shown promising results for dysphagia screening (Movahedi et al., 2017; Dudik et al., 2018; Yu et al., 2019; Coyle and Sejdic, 2020; Khalifa et al., 2020). We used the Swallis Device for Swallowing Analysis (DSA)<sup>TM</sup> (Swallis Medical® Colomiers, France) to explore cervical acoustics and movements during UADT functions. This device consists of a silicone collar with both a microphone and a 3-axis accelerometer (20 kHz sampling frequency), and a camera linked to a USB hub that can be connected to any computer. The USB hub allows for the perfect synchronization of both collar signals and the camera.

The three-dimensional accelerometer is placed at the anterior side of

the thyroid and cricoid cartilage, and the microphone is fixed laterally using a 1 cm round-shaped double-sided piece of tape to reduce cutaneous friction. These positions have been shown to be the optimal locations for swallowing detection (Takahashi et al., 1994a; Mamun et al., 2015). The collar is then fixed behind the subject's neck by the investigator, taking care to not alter subject cervical position and motility (Fig. 2). The subject wears the Swallis DSA<sup>TM</sup> constantly, breaks included, to prevent recording errors due to collar mispositioning.

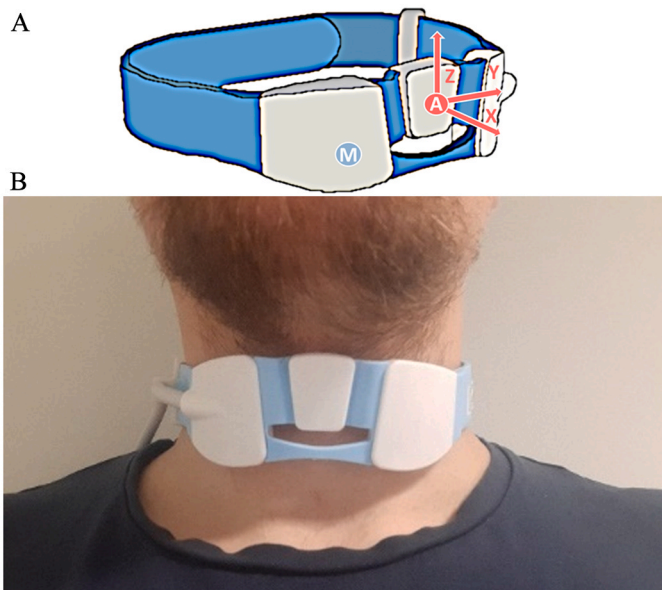
### 2.4. Neurofunctional evaluation

The first step was to choose the most appropriate tool for swallowing neuro-evaluation. According to our previous review (Gallois et al., 2022), MEG and EEG show a critical limitation in their use; the required number of repetitions of a same one task must be high to allow for good signal measurements. We showed that previous reports on MEG and EEG

**Table 1**  
Description of the different tasks.

Tasks	IDDSI	Type/Task Description	Volume	Total expected repetitions
Swallows	na	Voluntary saliva	na	10
	na	Spontaneous saliva	na	Variable
	0	Water	10 ml	10
			100 ml	3 glasses (multiple swallows)
	3	Water + Syrup + Thickening powder (about 1.4 gsp)	2.5 ml	10
			5 ml	10
	4	Water + Syrup + Thickening powder (about 1.7 gsp)	2.5 ml	10
	5 ml		10	
Cough Throat clearing	na	Voluntary coughs	na	10
	na	Voluntary throat clearings	na	10
Phonation		"The earth goes around the sun in 365 days" (in French)		3
Control motor task: Oral intake		For each swallow, the subject has to take the spoon/glass and bring it to their mouth. This is used as a control motor task		70

gsp: graduated spoon sold within the commercial kit; na: not applicable.



**Fig. 2.** Illustration of the Swallis Device for Swallowing Analysis (DSA)<sup>™</sup>. Left) Schematic representation of the Swallis DSA<sup>™</sup>. M: Microphone, A: Accelerometer with X, Y, and Z axes. Right) Cervical positioning of the Swallis DSA<sup>™</sup> collar on the neck of a subject.

used approximately 50 task repetitions. Firstly, we expect this high repetition number to be difficult to achieve among healthy subjects, and even more so for subjects with medical conditions. Secondly, this high number of repetitions would limit the number of different swallowing tasks performed (Nonaka et al., 2009; Cuellar et al., 2016; Suntrup-Krueger et al., 2018, 2021; Muhle et al., 2021). In addition, MEG and EEG are often described as highly sensitive to movement artifacts, meaning the techniques are likely unsuitable for investigating real-world activities, such as use during a meal. Given we aimed to reduce the number of repetitions, and that fNIRS appears to offer good-quality signals with only 10 repetitions (Inamoto et al., 2015; Kamarunas et al., 2018; Kober et al., 2019, 2015a), we chose this technique for neurofunctional evaluation. This choice was made at the

expense of the lower spatial and temporal resolutions and the limited number of optodes, which reduces the cortical coverage of the montage (Nishiyori, 2016). However, the reduced number of repetitions required for fNIRS appears more suitable for the high number of tasks we aim to study (see Tables 1. and 2.2 Experimental conditions and tasks).

#### 2.4.1. The principles of fNIRS

fNIRS is an optical technology that measures variation in the cortical absorption of photons travelling through the skull bone. This absorption is related to variations in cortical hemoglobin concentrations, which have been shown to reflect cortical neuronal activity, similarly to the BOLD signal with fMRI. Cortical photon absorption is a measure of the cortical HDR associated with a task in the active region.

We used the Scout system 8/8 (8 light sources and 8 detectors) from NIRx Medical Technologies (LLC, USA). This system measures both relative oxyhemoglobin and deoxyhemoglobin concentrations via two LED light wavelengths of 750 nm ( $\lambda_1$ ) and 850 nm ( $\lambda_2$ ). Each source emits photons that travel through the skull and cortex, where they undergo absorption. The absorbed photons are detected, giving information about hemodynamic changes. The basic principle is that the fluctuation of the cortical blood flow (from the HDR) modifies the proportion of photons that undergo cortical absorption, thus inversely modifying the quantity of detected photons as a function of the HDR (see Delpy et al. 1988 and Scholkmann et al. 2014 for further details on fNIRS).

#### 2.4.2. fNIRS montage

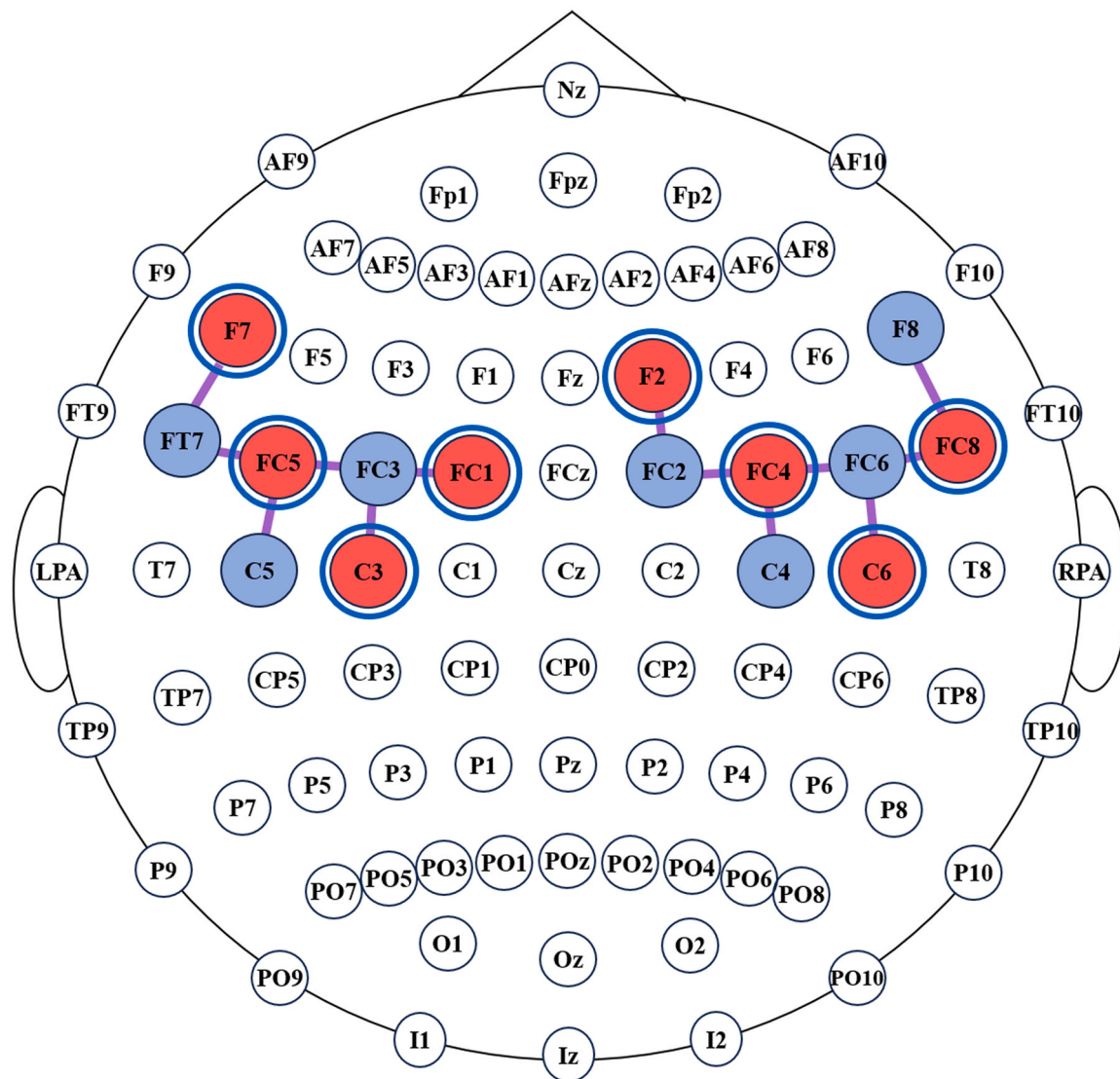
Considering previous studies and the Penfield's Homunculi, we focused on the central swallowing area in the inferior pericentral cortex, and both the SMA and PMC. We created an optode montage using the fOLD Toolbox from Zimeo Morais et al. (2018). We focused our optodes on the inferior pericentral cortical regions with 50 % specificity. We also ensured to include the F7-Ft7, Ft7-Fc5, Fc6-Ft8, and Ft8-F8 channels that have been found highly responsive in swallowing tasks in a previous study using fNIRS by Kober et al. (2015a). We replaced one of the detectors with seven short channels to measure scalp blood flow. A distance of 8 mm is considered optimum for such measures (Brigadoi and Cooper, 2015). It has been shown that scalp blood flow can elicit false HDRs in fNIRS measurements. The specific measure of this cutaneous blood flow can then be extracted from standard channel measures. The final montage shown in Fig. 3 is mounted on a NIRScap from NIRx<sup>®</sup> adapted to the head circumference of each subject. The subject wears the fNIRS cap during the whole experimental phase (breaks included) to prevent recording errors due to cap mispositioning.

#### 2.5. Data acquisition and synchronization

To capture the fNIRS signals, we use the NIRStar<sup>®</sup> software (Version 15–3, Build 06, 2020–04–10, NIRx Medical Technologies, LLC, USA) with a scan rate of 7.8125 Hz on a dedicated computer. Signals are encoded as.nirs files for compatibility with Matlab, the Nirs-Toolbox, and the Homer 2 software.

We use the Swallis DSA<sup>™</sup> with the Swallis App<sup>®</sup> (Swallis Medical<sup>®</sup>, Colomiers, France) on a second computer to record the acoustic and accelerometric data, at 20 kHz sampling frequency for acoustic data and with a scan rate of 20 kHz for accelerometric data. This software allows for real-time recordings and annotations of many events: i.e. swallows, speech, coughs, but also custom annotations (See Supplementary Materials 1). For swallowing, the texture can also be reported according to the IDDSI Framework classification (<http://iddsi.org>). We then extract "wav" files with 4 tracks containing one acoustic and three accelerometric time series, and an event file containing the marked events.

Given the Swallis DSA<sup>™</sup> cannot yet synchronize with neurofunctional tools, we used the NIRStim<sup>®</sup> 4–0 software to emit the start of each recording; both a specific start trigger for NIRStar<sup>®</sup> and a custom-made concurrent single-sound trigger that is captured by the Swallis DSA<sup>™</sup>. The acoustic recording then allow us to hear and recognize the



**Fig. 3.** fNIRS montage. fNIRS optodes cover both the pericentral cortex, the supplementary motor area, and the premotor cortex. Red circles denote the photon sources, the blue circles are the detectors. Purple lines represent a measure channel. All emitters are associated with a short channel (indicated by the blue circle surrounding the red circles) to detect the underlying cutaneous blood flow. Every position is named after the EEG 10–10 system and placed on a NIRSCap from NIRx®.

spectroscopic pattern of the sound trigger. Delays should be approximately 9 ms given the signal source and the Swallis DSA™ were 3 m apart. This delay is negligible compared to the duration of a UADT function (>500 ms [Morinière et al., 2006](#)) and the duration of a HDR (approximately 15 s [Pinti et al., 2020](#)). The NIRStar® trigger and the Swallis DSA™ sound trigger serve as start timestamps for synchronization of our signals.

## 2.6. Annotation, signal pre-processing, and parameters of interest

### 2.6.1. Event annotation method

The labeling of the events is performed manually with the Swallis App®. This software allows direct event annotation on the interface showing the signal. We annotate the "start" and the "end" of every event. We annotate any signals of interest between these periods. Two investigators manually annotate the signal after auditory impressions through headphones and visual appreciation of the raw signal on the interface. As the Swallis App® does not display all accelerometric signals from the 3-axis, signals can be extracted as a 4-track wav file collecting the 3 axes and the acoustic signal. This wav file can then be imported in

Audacity® Version 3.5.1 (MuseCY Holdings Ltd, Limassol, Cyprus, available at: <https://www.audacityteam.org>) to verify the quality of the annotations if needed.

We segment the pharyngeal phase specifically for the swallow tasks (See [Supplementary Materials 1](#) for an example). Indeed, the oral phase being voluntary, its duration is highly dependent of the bolus used, but also of its oral manipulation. Variability is thus introduced throughout same task repetitions. However, the pharyngeal phase being more reflexive, we expect it to be only affected by the bolus characteristics. [Mackowiak et al. \(1967\)](#), [Cichero and Murdoch \(1998\)](#) and [Morinière et al. \(2008\)](#) have studied the acoustic characteristics of this pharyngeal phase. The authors describe reproducibility, with the existence of a central phase, that we also annotate here. All consecutive swallows for a same task are segmented and numbered.

We also annotate non-volitional events. These are mostly spontaneous swallows, but also spontaneous throat clearings and coughs, these latter being annotated as aspiration if they immediately follow a swallow. We also annotate regurgitations and belching. These events are further analyzed if at least 5 events are recorded for a single subject.

### 2.6.2. Cervical evaluation parameters

We compute multiple parameters from both the acoustic and accelerometric signal segments using Matlab (Mathworks, Natick, Massachusetts) and the PRAAT software (University of Amsterdam, Netherlands, [Boersma and Weenink, 2024](#)). Two approaches are used for further analyzes and are intended for segmentation of known intra-and inter-subject variability of the acoustic and accelerometric measures ([Movahedi et al., 2017](#); [Takahashi et al., 1994b](#)). The first approach is based on auditory and visual signal inspection. The second approach is performed systematically according to previous literature reports.

From the visuo-auditory inspection of the signals, we focus on temporal events, the rhythms, length, and intensity of the emergence of events. Each event is expected to be too short to allow for processing of any relevant spectrums to detect frequency characteristics. However, a waveform with a spectrogram can facilitate the detection of the swallowing sound peak, as well as the temporal regularity and the duration of swallowing sound ([Cichero and Murdoch, 2002](#)). Moreover, we perform an auditory analysis of the spectral pitch of the events. This appears to be a relevant auditory characteristic for differentiating between events. We measure the duration of each movement from "start" to "end". In the event of, we also measure the duration between either the "start" or the "end" and the labeled central "peak" phase of the event as previously described ([Cichero and Murdoch, 1998](#); [Mackowiak et al., 1967](#); [Morinière et al., 2008](#)). The analysis of the accelerometric signal is visual, similarly to the work from Celeste et al. The authors computed time domain and frequency domain-specific features according to visual examination of the signals ([Celeste et al., 2012](#)). The time domain features collected the median, the absolute difference between mean and median, the p-value of the chi-squared test for normality, the total area under the maximum hyolaryngeal excursion lobe as well as the width of this lobe. The frequency domain-specific features may collect the maximum spectral density frequency over all time of the short-time Fourier transform spectrogram, the difference between frequency values corresponding to 75 % and 25 % of maximum spectral density, and the other features computed by the summation of power spectral density values within a 10 Hz range, from 0 to 200 Hz (calculated in unit of power per radian per sample using Welch's averaged modified periodogram method of spectral estimation).

Many features from the literature have been of interest and can be extracted systematically from both signals. These features emanate from time, frequency, and time-frequency domains as previously reported ([Celeste et al., 2012](#); [Dudik et al., 2015](#); [Lee et al., 2011, 2009](#); [Zoratto et al., 2010](#)). We compute the mean relative amplitude, duration, peak Fast Fourier Transform (FFT) magnitude, frequency at spectral peaks for each sound, and accelerometric components of the events. We also compute the maximum hyolaryngeal excursion, the cross-entropy rate, and the cross-correlation of the signals between the 3-axis signals for accelerometric signals.

### 2.6.3. Cortical activity features

To our knowledge, the swallowing-specific scientific literature is not completely consensual regarding the analysis method and the features of interest from fNIRS signals. Firstly, some studies present only HbO data ([Inamoto et al., 2015](#); [Matsuo et al., 2021](#); [Mulheren et al., 2016](#)). However, some studies suggest that both HbO and HbR time series should be recorded, as their time series are classically negatively correlated during a classical canonical HDR ([Cui et al., 2010](#); [Nishiyori, 2016](#); [Scholkmann et al., 2014](#)). According to Cui et al., a positive correlation could evoke signal noise contamination, questioning the validity of the measurement ([Cui et al., 2010](#)). Secondly, while a time series reflects the quality of the signal, the parameters of interest are numerous and varied. Kober et al. computed the mean amplitude in HbO and HbR concentrations from multiple studies, using different time windows according to the reported activity peak: from 5 s to 15 s ([Kober and Wood, 2014](#)), 10 s to 20 s after task onset ([Kober et al., 2015a](#)), or even

comparing both the 0–5 s, 5–10 s, 10–15 s, and 15–20 s time windows ([Kober, 2018](#)). Ludlow's team focused on single swallows. They used relative concentration Z-scores. They focused on the mean Z-score in different time windows, with two activity peaks: between 2 s to 7 s and 17 s to 22 s ([Mulheren et al., 2016](#)), between 4 s to 7 s and 14 s to 17 s ([Mulheren and Ludlow, 2017](#)), and between 2 s to 8 s and 8 s to 32 s ([Kamarunas et al., 2018](#)). They also focused on the timing of the peak ([Kamarunas et al., 2018](#)). [Chua and Chan \(2024\)](#) used the peak Z-score (the maximum value from baseline) for their study. Another team used the area under the curve to examine cortical activity ([Knollhoff et al., 2022](#)). Basically, there is no consensus in the literature to date, and so any parameter reflecting activity could be used. It is difficult to anticipate the ideal time window for a specific task, as the adequate time window is highly dependent on the task modalities and duration, the region of interest, and the subjects as previously demonstrated in reports on other motor tasks ([Kamran et al., 2015](#)). Given this lack of consensus and given this is an exploratory protocol, the method proposed below could be adapted to our data and our tasks.

### 2.6.4. Cortical signal preprocessing and parameters

We use the open-source HomER 2 software (Neurophotonic Center, Boston University, Massachusetts, USA) and Matlab with the NIRS Toolbox (University of Pittsburgh, Pennsylvania, USA) for data preprocessing and analyzes (available at <https://openfnirs.org/software/>). These are standard tools for fNIRS analysis. Both HbO and HbR signals are considered. The fNIRS signal pre-processing consists in artifact correction and data filtering with a 0.01 Hz high pass filter and a 0.5 Hz low pass filter to remove physiological signals ([Scholkmann et al., 2014](#)). We then average 10 measured signal epochs during a time window extending from 5 s before the beginning of the event to 20 seconds after the end of the event. The interval adds 25 seconds to event duration. We use the longest interval to average the time series given that events may not be even in duration. A similar delay is used in many studies in the field according to our previous review ([Gallois et al., 2022](#)), and we expect the peak of activity to occur between 5 and 10 seconds after the end of the swallowing task, similarly to findings from [Kamarunas et al. \(2018\)](#). This allows us to present the time series of the signal. Then, these average time series are transformed to Z-scores relative to the mean and standard deviation of the baseline signal, similarly to previous works ([Chua and Chan, 2024](#); [Mulheren et al., 2016](#); [Mulheren and Ludlow, 2017](#)). This reduces inter-subject measure variability related to non-hemodynamic differences:

$$Z\text{-score} = (\text{concentration value} - \text{mean baseline}) / \text{SD baseline} \quad (1)$$

The resulting Z-score time series could be subject to jaw movement artifacts due to temporal muscle contractions, particularly during speech and chewing functions. We use multiple tools to reduce such artifacts ([Yücel et al., 2021](#)). Firstly, the fNIRS signal is inspected and clearly contaminated blocks are excluded ([Schecklmann et al., 2017](#)). Secondly, we use short-channel signals across the entire montage and particularly over the temporal muscle region ([Brigadoi and Cooper, 2015](#)). Short-channel regression from the native signal is through a principal component analysis with general linear models (PCA-GLM), as described in previous studies ([Brigadoi et al., 2014](#); [Zhang et al., 2023](#)). Lastly, we remove motion artifacts from fNIRS signals through spline interpolation and wavelet filtering ([Novi et al., 2020](#)).

From a qualitative standpoint, we analyze these averaged time series to check if signals correspond to canonical HDR or not. We collect data on the following criteria to determine a typical canonical HDR detection from various reviews ([Nishiyori, 2016](#); [Scholkmann et al., 2014](#); [Cui et al., 2010](#); [Kamran et al., 2015](#)): both HbO and HbR start at baseline, increase (or decrease) with a peak between 5 and 15 s, and then return to baseline; HbO and HbR should be negatively correlated; variation should begin in a time window from -2s to +2s around the start of the HRCA signal; increasing HbO and decreasing HbR defines activation,

whereas decreasing HbO and increasing HbR defines deactivation. The signal corresponds to a relative Hb concentration and so there are no amplitude criteria, and it has shown fluctuation according to the task, region of interest, and the subjects (Kamran et al., 2015). From these criteria, two authors evaluate HDR validity using a Lickert scale from 0 (not at all a HDR) to 4 (a typical HDR). Some examples are given in Fig. 4.

From a quantitative standpoint, we calculate the max value, the mean amplitude, and the peak response time for each epoch. The max value is the maximum amplitude value of the relative concentration of either HbO (usually positive) or HbR (usually negative). The peak response time is the delay to obtain the max value. The mean amplitude is the average of the amplitude value over a defined time window. The time window used depends on the task duration. Given that swallowing is a short task (Morinière et al., 2011, 2006), the activity peak usually occurs at approximately 5 s after swallow onset. We chose a 5-second window from 2 s to 7 s for averaging after the onset of swallowing recorded by the HRCA, similarly to previous works from Ludlow's team (Kamarunas et al., 2018; Mulheren et al., 2016; Mulheren and Ludlow, 2017). Indeed, the authors report that the first HDR peak occurs during this window. Our window can be adapted according to the different tasks.

### 2.7. Statistical analyzes

We first aim to compare the cortical activity according to the different tasks: swallowing, coughing, throat clearing, speech, and mouth intake. We aim also to compare all the different parameters between each task, and specifically for the swallowing function we aim to explore the effects of bolus type (saliva, water, IDDSI-3 and 4), texture (IDDSI-3 and IDDSI-4), and the different volumes (2.5 ml and 5 ml). We aim to compare volitional to spontaneous saliva swallowing. The activity parameters are compared through the different channels and the

cortical side, based on either the suspected neurological role of the underlying cortex and their similarities in activities.

For the swallowing function, we will analyze both the cervical and the cortical signals. We will compare the duration and the relative amplitudes of the events (acoustic, accelerometric, and hemodynamic) between the different bolus textures (saliva, water, IDDSI-3, IDDSI-4), volumes (2.5 ml and 5 ml), and between the volitional and spontaneous saliva swallows.

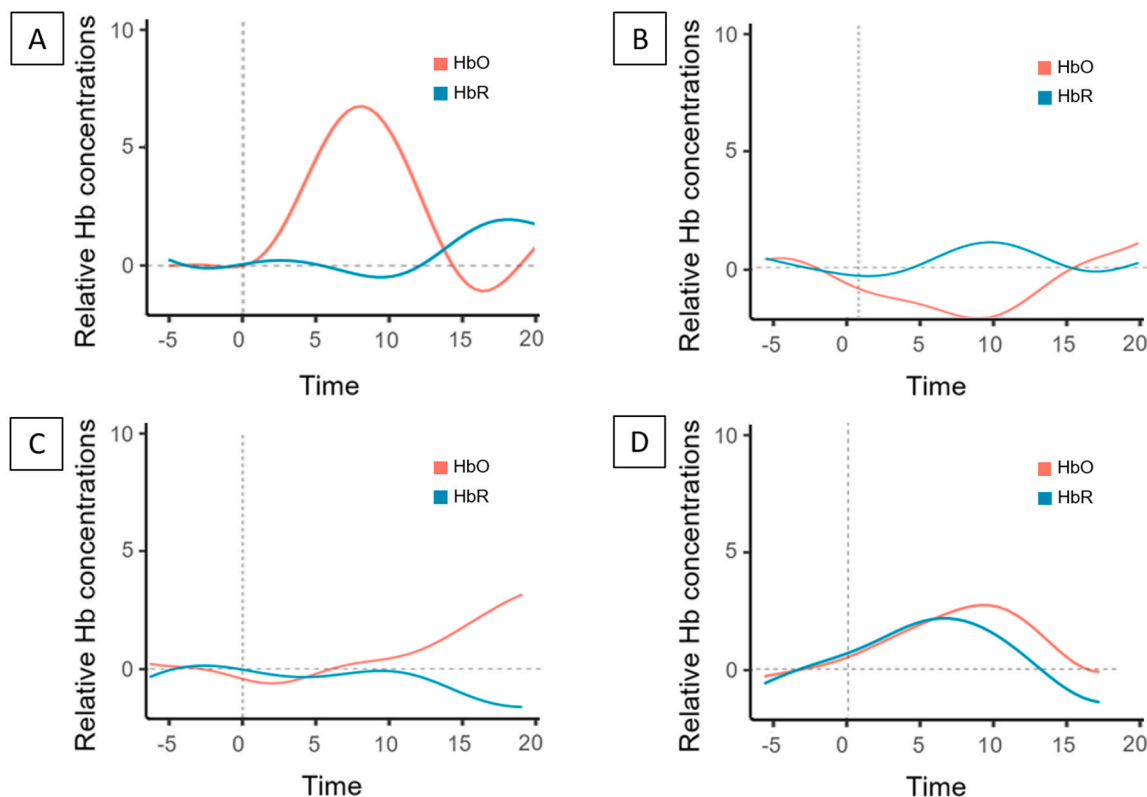
We will compare the timings and relative amplitudes of the activity through ANOVA. In case of significant differences, post-hoc analyzes will be performed using Tukey's test as we have multiple tasks.

We also aim to explore correlations between fNIRS parameters using the Swallis DSA™ parameters. For this, we will compute Spearman's  $\rho$  coefficients for non-normally distributed data and Pearson's  $r$  coefficients for normally distributed data (mean HbR and HbO concentrations, timings for acoustic and accelerometric events, and relative amplitudes).

### 3. Expected results

We believe the labeling of the cervical signal will allow us to measure both cervical and cortical activities of the tasks, and show the differences between each task. The correlations of these activities will help us to better understand the physiology of UADT functions. Given we perform continuous recordings, we may also capture function errors (i.e. aspirations) and put forward specific patterns as signs of pathophysiology in comparison with normal patterns.

Focusing on the swallowing function, we expect the type of bolus and the characteristics of the swallows to correlate with the cortical response. We also expect to find correlations between the parameters of the cervical signals and the cortical fNIRS parameters. We expect the accelerometric and acoustic characteristics of the events to follow models described in the literature; models that we should retrieve via



**Fig. 4.** Example of fNIRS curves and their interpretation. A: Typical activation curve; B: Possible deactivation curve; C: No activity; D: Although HbO increases, it is positively correlated to HbR; thus this is likely contaminated by noise.

the many observations made throughout our protocol. We expect to identify the 3-component pattern of swallowing as described by [Morinière et al. \(2006\)](#) in healthy subjects. We will correlate the parameters of these patterns (duration and amplitude). Those models should be correlated to the parameters of the HDR measured over the inferior pericentral cortex similarly to previous works ([Kamarunas et al., 2018](#); [Kober et al., 2015a](#)).

#### 4. Discussion and perspective

Setting-up a protocol for tracking swallowing function from a neural and cortical point of view, especially in natural and non-invasive conditions, presents several challenges. Notably, the transfer of such a protocol from the research domain to clinical practice is difficult, especially for use with children or dysphagic patients. This protocol has been designed to be adaptable to such subjects, without being invasive or painful, and free of irradiation. Our protocol is also more in line with the natural conditions of UADT functions.

The synchronization of the different signals allows us to study all cervical- and cortical-associated UADT functions. This will allow us to describe the activity patterns for each function. Furthermore, we will be able to compare different modalities of a single function, and examine modalities beyond the characteristics given in [Table 1](#) with the same recording setup. Indeed, the major advantage of our setup is it can ultimately be used for any event, during a normal meal for example. The validation of this protocol with healthy subjects is the first step for future studies.

The principal limitation is the labeling of the cervical signals. It is time consuming to manually label every cervical event. This is the reason why some authors have already used deep learning algorithm methods to differentiate the swallowing events from accelerometric data ([Mao et al., 2019](#); [Sejdic et al., 2009](#)) or from acoustic signals ([Mao et al., 2019](#)). For instance, [Mao et al. \(2019\)](#) used their accelerometric and acoustic data to train a system through deep learning with a stacked recurrent neural network. The authors were able to automatically differentiate the hyoid bone movement associated with swallowing from other movements. Using suchlike trained algorithms, it should be possible to avoid VFS and FLS for UADT evaluation, thus reducing the need for invasive examination in more real-world and natural settings. This will enhance our signal treatment for further studies.

Our protocol has the potential to differentiate healthy subjects from dysphagic subjects. Indeed, it has been shown that cervical acoustic signals from total laryngectomy subjects differ from healthy subjects ([Morinière et al., 2011](#)). In children, [De Almeida et al. \(2018\)](#) showed differences in cervical acoustic signals between children with tracheal aspirations and non-dysphagic children.

The association of both cervical and cerebral signals opens the possibility of direct meal observation as well as the detection of aspirations, with an insight on cortical sensitive-motor correlates. For example, [Kober et al. \(2015a\)](#) presented a case series of fNIRS analysis of swallowing among subjects after cerebral stroke and with dysphagia. The authors illustrated that two subjects with cortical lesions showed reduced cortical blood flow over the inferior pericentral region, whereas two subjects with brainstem lesions showed an increased response over these same regions.

Similarly, association of cervical and cortical activity could also promote the early diagnosis of neurodegenerative disorders, illustrating both the cervical dysfunction and the cortical anomalies induced by the disease. Many research teams have illustrated cortical modifications during swallowing tasks performed by subjects with neurological diseases: acute stroke ([Kober et al., 2015a](#); [Teismann et al., 2011a](#)), amyotrophic lateral sclerosis ([Teismann et al., 2011b](#)), Parkinson's disease ([Suntrup et al., 2013a](#)). Our protocol will improve the investigation of UADT functions and their cerebral patterns, in turn allowing for the detection of pattern modifications in neurologic diseases. These patterns could help in the understanding of the pathophysiology of the

related dysphagia, dysarthria, etc.

Lastly, our protocol could guide the future rehabilitation of dysphagia patients. fNIRS has been used in neurofeedback research protocols ([Kober et al., 2019, 2015b](#); [Kohl et al., 2020](#)). Preliminary results are promising but have yet to be confirmed through more robust studies. For instance, dysphagia rehabilitation could be improved using neurofeedback from cervical signals, with correction of the observed anomalies resulting in better swallowing quality. This would help clinicians in improving the overall management of dysphagia by reducing complications, such as aspiration pneumonia and malnutrition.

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#### CRediT authorship contribution statement

**Gravellier Lila:** Writing – original draft, Methodology, Conceptualization. **Lemaire Yann:** Visualization, Methodology, Formal analysis. **Souche Jeanne:** Writing – original draft, Visualization, Formal analysis. **Gaillard Pascal:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization. **Farinas Jérôme:** Methodology, Conceptualization. **Nicolini Linda:** Resources. **Barone Pascal:** Resources. **Gallois Yohan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Woisard Virginie:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bosn.2025.04.002](https://doi.org/10.1016/j.bosn.2025.04.002).

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