

THE EFFECTS OF CARBONATED FLUIDS ON THE HUMAN CORTICAL SWALLOWING MOTOR SYSTEM

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Words: 44829

List of Abbreviations

ACE	Angiotension converting enzyme
ANOVA	Analysis of variance
BSA	Bedside assessment
BOLD	Blood oxygen level dependent
Car 4	Carbonic anhydrase 4
CN	Cranial nerve
CNS	Central nervous system
CO ₂	Carbon dioxide
CPG	Central pattern generator
CT	Computerised tomography
CTL	Carbonated thin liquid
CVA	Cerebrovascular accident
DPNS	Deep pharyngeal neuromuscular stimulation
DVT	Deep venous thrombosis
EEG	Electroencephalography
EMG	Electromyography
ES	Electrical stimulation
FEES	Flexible endoscopic examination of swallowing
FLI	Fos-like-immunoreactivity
fMRI	Functional magnetic resonance imaging
FP	Faucial pillar
ICC	Intra-class correlation coefficients
IPS	Initiation of pharyngeal swallow
IV	Intravenous line
LHD	Left side hemisphere damage
LMS	Labelled magnitude scale
mA	Milliamps

MEG	Magnetoencehalography
MEP	Motor Evoked potentials
MRI	Magnetic resonance imaging
ms	Millisecond
MT	Motor threshold
μV	Microvolt
NA	Nucleus ambiguus
NaCl	Sodium chloride
NCTL	Non-carbonated thin liquid
NGT	Nasogastric tube feeding
NHS	National health service
NTS	Nucleus tractus solitarius
OFC	Orbitofrontal cortex
OTT	Oral transit time
PAS	Paired associative stimulation
PEG	Percutaneous endoscopic gastrostomy
PENASP Scale	Penetration-aspiration scale
PES	Pharyngeal electrical stimulation
PET	Positron emission tomography
PMEP	Pharyngeal motor evoked potential
PR	Pharyngeal retention
PTT	Pharyngeal transit time
rMT	Resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation
SAD	Swallowing apnoea duration
SD	Standard deviation
SEM	Standard error of the mean
SI	Swallowing interval

sEMG	Surface electromyography
SRT	Swallowing reaction time
TMSPs	Thenar motor evoked potentials
TMS	Transcranial magnetic stimulation
TPN	Total parental nutrition
TRCs	Taste receptor cells
TRP	Transient receptor potential
TRPV1	Transient receptor potential vanilod 1
TTS	Thermal tactile stimulation
UK	United kingdom
USO	Upper oesophageal sphincter
VFS	Videofluoroscopy
VR1	Vanilod receptor 1

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Abstract

Swallowing is a complex neurophysiological process involving the activation of several components of the central nervous system with bilateral but asymmetric representations of swallowing musculature in the motor cortex. Difficulty in swallowing (dysphagia) in stroke patients has been reported by up to 50% of victims, and can increase morbidity and mortality in this population due to the development of aspiration pneumonia and malnutrition. One of the common factors that predispose patients to dysphagia after a stroke is believed to be the reduced sensory awareness in the oropharyngeal area, which affects the swallowing process. The uses of diet modification to reduce thin liquid aspiration have gained interest but are often unpalatable or have limited success. Carbonated liquid have shown some beneficial effects in swallowing behaviour. However, there is very little evidence to support this intervention. Therefore, the aim of this thesis is to investigate the neurophysiological and behavioural effects of carbonated liquids on swallowing in healthy volunteers.

The effects of carbonated solutions on swallowing performance compared to non-carbonated solutions (still water) was investigated in a pilot study and (still water and citric acid) in the main study using reaction time task (chapter 2). Carbonation appears to alter swallowing performance compared to other liquids by improving complex tasks. In addition, beneficial neurophysiological effects of carbonated liquids were evident after 10 minutes of carbonated liquid swallowing compared to still water and citric acid solution in healthy volunteers (chapter 3).

In chapter 4, the response of the healthy swallowing motor cortex to carbonated liquids following application of a virtual lesion compared to still water and saliva swallowing, was investigated. Carbonated liquids were able to reverse the inhibitory effect induced by 1 Hz rTMS to the dominant pharyngeal motor representation. Moreover, the beneficial effects of carbonated liquids on swallowing performance, measured with a swallowing reaction times task after application of a virtual lesion was observed in a pilot investigation in healthy volunteers (chapter 5).

These data demonstrate that carbonated liquids have beneficial neurophysiological and swallowing performance effects and support notion that the chemical properties of carbonated liquids may provide the required peripheral sensory information that alter the brain swallowing function, which leads to an improvement in the swallowing performance of stroke dysphagic patients. These data lay the foundation for considering the use of carbonation as facilitating stimuli in dysphagic patients.

Declaration

Data for the pilot study has been submitted as part of the projection options degree of Manchester University by Aliya Mastan, who initiated the project and assisted me during the data collection process. Analysis and interpretation was under taken independently

No other portion of the work referred to in the thesis has been subitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Dedication

I dedicate this thesis to the soul of my father, who passed away very recently without seeing his dream come true. He was my inspiration that kept me going and allowed me to overcome difficulties. I know he would have been proud of me reaching this step.

I would like to dedicate this work to my mother, who contributed to my efforts and my ambitions, and who missed my presence during these years as much as I have.

I dedicate this work to all my lovely family (brothers and sisters), and especially to my brother, Mohammad. No single word could describe the effort and support that he has always given me, from my initial stages at University until now. He is truly the Unknown Soldier behind my success. I will never forget his help in my life.

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CHAPTER 1

Introduction

1.1 Normal swallowing

Swallowing is a complex neuromuscular process that leads to successful bolus ingestion. Normal swallowing includes three different phases: oral, pharyngeal and oesophageal [1]. Each phase is dependent on the other. Therefore, all three phases are necessary to complete the swallowing process. In general, the oral phase is voluntary, whereas the two other phases are involuntary. However, it has recently become apparent that higher inputs also influence the pharyngeal and oesophageal phases, possibly through increased modulatory capacity [2].

1.1.1 Anatomic structures

There are many of anatomical structures that are involved in the deglutition process including the oral cavity, pharynx, larynx and oesophagus. The tongue is the main structure in the oral cavity that plays an important role in the deglutition process. The anterior two thirds of the tongue lie in the mouth and the posterior third lies in the pharynx [3]. The tongue has two types of muscles: intrinsic muscles which are not attached to bone and extrinsic muscles such as the genioglossus, hypoglossus, styloglossus and palatoglossus which are attached to the mandible, hyoid bone, styloid process and soft palate respectively. The intrinsic muscles alter the shape of the tongue and the extrinsic muscles alter the tongue's position. They move the tongue in different directions: protrusion, retraction, depression and elevation. The facial nerve (CN VII) supplies the sensory part of the anterior two thirds of the tongue, whereas the posterior third is supplied by the glossopharyngeal nerve (CN IX) [3].

The floor of the mouth is formed by the mylohyoid muscles, which stretch as a diaphragm, the anterior belly of the digastrics muscle, the submandibular gland and submandibular lymph nodes are covered by deep fascia and platysma [3]. The roof of the mouth is formed by the palate which is divided into two parts. The hard palate separates the oral and nasal cavities and the soft palate separates the nasopharynx from the oropharynx [4]. Anteriorly, it is attached to the posterior border of the hard palate as a mobile fold and posteriorly it gives rise to a midline projection called the uvula [5]. Five pairs of muscles are attached to

the soft palate and their contraction leads to the closing off of the nasopharynx from the buccal cavity by the soft palate which occurs during speaking, swallowing and blowing. The maxillary division of the trigeminal nerve (CN V) supplies the most sensory part of the palate; however, the posterior part is supplied by the glossopharyngeal nerve.

The pharynx is a muscular tube, which extends from the base of the skull to the oesophagus at the level of the sixth cervical vertebra. It acts as a gate for both the alimentary and respiratory tracts. The pharynx is divided into three portions from above downwards: nasopharynx, oropharynx and laryngopharynx. The nasopharynx lies behind the nasal cavities and above the soft palate which prevents regurgitation of food through the nose during deglutition by shutting off the nasal portion from the oral portion of the pharynx.

The oropharynx lies behind the mouth's cavity and begins from the soft palate to the level of the upper border of the epiglottis. The laryngopharynx lies behind the larynx and extends from the upper border of the epiglottis to the lower border of the cricoid cartilage [3-5]. The muscles of the pharynx are divided into two types: circular and longitudinal. Circular muscles consist of the superior, middle, and inferior constrictor muscles. The contraction of these muscles pushes the bolus of food down into the oesophagus. The inferior constrictor muscle, which is known as the cricopharyngeus muscle, acts as a sphincter and prevents air entry into the oesophagus between swallowing. The longitudinal muscles during swallowing raise the pharynx and larynx [5]. The pharyngeal plexus is responsible for the nerve supply of the pharynx. This plexus is formed from branches of glossopharyngeal, vagus (CN X) and sympathetic nerves.

The larynx lies in the neck and extends from the laryngopharynx superiorly to the trachea inferiorly. The skeletal structures of the larynx are the epiglottis and the hyoid bone, thyroid, cricoid and arytenoids cartilages. The larynx has a function during deglutition, phonation and coughing. The muscles of the larynx are responsible for these functions: opening the glottis during inspiration, closing the vestibule and glottis during deglutition, and changing the tone of the vocal cords in phonation [4]. Superior and recurrent laryngeal branches of vagus supply the larynx [4].

The innervation patterns and coordination of the described anatomical structures in the process of deglutition lead to the safe ingestion of bolus from mouth to stomach.

1.1.2 The physiology of swallowing

Swallowing is a complicated process involving the activation of several parts of the central nervous system (CNS) to maintain an adequate intake of food and liquid for the body to stay healthy [6]. Both hunger (a desire for food that determines the amount of food to be consumed) and appetite (a desire for a specific type of food) mechanisms are important automatic regulatory systems to maintain nutrition. Swallowing occurs approximately once a minute in an individual who is awake and a thousand times every day [7, 8]. Saliva elicits the high swallowing rate because it is produced at a rate of approximately 0.5 ml/min [9, 10]. Most swallows occur under subconscious control; however, during the eating process the swallowing process occurs under conscious control. Swallowing is almost completely stopped during deep sleep but does occur during rapid eye movement sleep [2] and swallows increase in number at arousal from sleep [7].

1.1.2.1 Oral phase

With solid or semisolid food ingestion, the oral phase is preceded by a preparatory phase. During the preparatory phase of deglutition, sensory information from the oral cavity is relayed to the brainstem and cortex, which creates a critical environment that, allows the mouth to prepare the bolus [11]. Anteriorly, this is achieved with a good lip seal by orbicularis oris, and posteriorly, this is performed by elevation of the posterior part of the tongue and lowering of the soft palate which prevents premature escape into the pharynx. Solid ingested foods accumulate on the depression of the anterior surface of the tongue, before masticating and pulling back into the mouth by the tongue to reach to the molar regions where it is divided into small particles. This process is repetitive to achieve many small food particles. The masticator muscles involved in this process include: tongue, lip, cheeks, palate and jaw muscles (temporalis, masseters, medial and lateral pterygoids) [12]. In addition to mastication, food mixes with saliva which contains enzymes resulting in the breakdown of the bolus to form a cohesive size bolus ready for swallowing. Moreover, the

chewing process leads to a higher salivary flow rate compared to stimulation by liquids [13] which moistens the bolus.

Liquid food differs from solid food since it does not require a preparatory phase and does not enter the pharynx during the oral phase. Also, multiple swallows are needed to clear a solid bolus from the mouth compared to a liquid bolus [12]. The oral phase starts once the bolus is adequately prepared. The soft palate is elevated by the levator veli palatine and palatopharyngens (CN X) muscles and the hyoid bone is elevated by contraction of the suprahyoid muscles and relaxation of the infrahyoid muscle, with a mild elevation of the pharyngeal tube entrance. Then, the tongue moves upwards and backwards towards the posterior wall of the pharynx against the hard palate to reach the oral pharynx [11]. The oral stage is finished in approximately 1-1.5 seconds but for a solid bolus this time is increased significantly due to the action of mastication and bolus preparation [14].

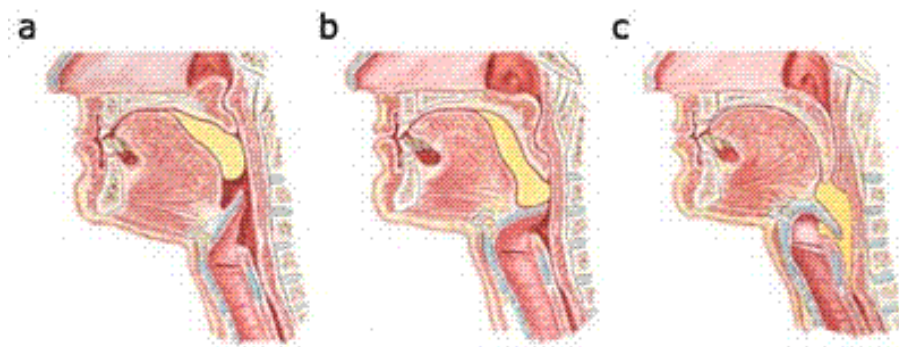


Figure 1.1: Schematic representation of oral (A, B) and pharyngeal phases (C). Copy from John. T. Hansen, Netter's Clinical Anatomy, 2nd edition, Saunders Elsevier, (2010) with requested permission.

1.1.2.2 Pharyngeal and oesophagus phases

During the pharyngeal phase the bolus is transferred from the oral pharynx into the oesophagus. As the bolus enters the oropharynx, it stimulates all swallowing receptors in the mucosal layers around the pharynx opening, which transfers impulses through CN IX and CN X to the brainstem to start numerous pharyngeal muscle contractions [15]. The soft palate is elevated to seal off the nasopharynx. The hyoid and larynx begin their elevation due to the contraction of the neck and superior constrictor muscles, which pull the lateral

walls medially [11]. The palatopharyngeal folds are approximated to each other to allow food to pass into the posterior pharynx through a sagittal slit formed by this fold [15]. The vestibule closes during elevation of the larynx and the aryepiglottic folds form a membrane of pyriform recesses due to the elevation of arytenoid cartilage [11]. Both vocal cords (false and true) are tightly approximated and the epiglottis begins to fold down over the opposed cords and cover the vestibule due to increased pressure at its base and elevation of the larynx from below upwards [11]. This mechanism helps to protect the airway during swallowing.

The bolus is transmitted through the pharynx due to the contraction of constrictor muscles from top to bottom. The anterior and upward movement of the larynx, elevation of the hyoid bone and pressure of the swallowed bolus leads to relaxation of the pharyngoesophageal sphincter or cricopharyngeus to allow the movement of food from the posterior pharynx into the upper oesophagus [15]. This sphincter remains closed during the previous stages of swallowing to prevent the entering of air into the oesophagus during respiration. The pharyngeal phase lasts for less than 0.65 seconds [2]. After the bolus passes into the oesophagus safely, the upper oesophageal sphincter (UOS) closes again, and the larynx moves downward, with the glottis reopening (epiglottis moves upwards) with the abduction of vocal cords. The bolus is transferred through the oesophagus by peristaltic movements under the influence of contracting muscles at a speed of approximately 2-4cm/second from the upper part of the oesophagus into the stomach [16, 17]. The oesophageal phase takes between 6-10 seconds [2].

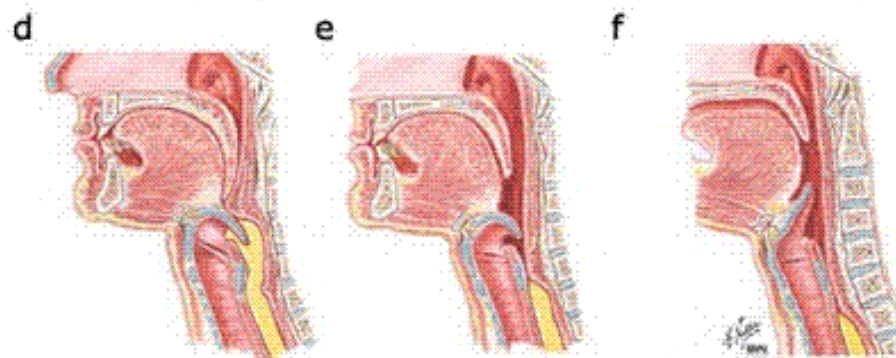


Figure 2: Schematic representation of pharyngeal (D) and oesophagus phases (E, F). Copy from John. T. Hansen, Netter's Clinical Anatomy, 2nd edition, Saunders Elsevier, (2010) with requested permission

1.1.3 Variations of normal swallowing

Recent published reports have shown that normal swallowing physiology can be affected by methods of bolus administration and characteristics of the swallowed material.

The most important factors which provide a description of these changes include: bolus volume, bolus viscosity, single bolus swallows, continuous or sequential cup drinking or drinking through a straw.

1.1.3.1 Bolus volume

Many studies report that the timing of normal physiological swallows is affected by the bolus volume [18-21]. Both oral and pharyngeal structures change their cavities according to the size of the bolus to accommodate it. There are also a number of physiological changes associated with increasing bolus volume during the oral phase, such as increased closure time of the velopharyngeal [20], different types of tongue movement [19, 20] with increased speed [22], change in elevation of the hyoid bone and anterior movements of the hyolaryngeal complex [19, 23]. During the pharyngeal phase, the velocity of bolus increases [24] and a large bolus moves faster than a small one [25, 26]. Additionally, arytenoid elevation increases with shortening of laryngeal length [27]. The increase in bolus volume leads to the opening of the UOS for a longer period of time [19, 28]. Also, a large volume of bolus leads to an increased closure time of the laryngeal vestibule and to longer apneic intervals [29, 30].

1.1.3.2 Bolus viscosity

Evidence from many studies has shown that the normal physiological swallow is affected by bolus viscosity. Thick fluid requires more tongue movement and transport time to the posterior oral cavity. Also, increased viscosity of food leads to increased tongue force and pharyngeal contraction [31-33]. Oral and pharyngeal transit times are influenced by viscosity [33, 34], and a thick fluid leads to an increased UOS opening time [19]; however, Robbins et al. found that UOS opening time was longer for thin fluids rather than thick [34]. The swallowing duration for thick fluids is longer than that of thin fluids [34, 35].

1.1.4 Imaging methods for swallowing physiology

There are many assessment tools that are used by speech and language therapists and clinicians to evaluate swallowing function. In hospital settings, this process takes many steps ranging from screening to instrument evaluation. Clinical examination includes a bedside assessment (BSA) which contains many assessment measurements, and instrumental examination which includes a non-imaging examination such as pharyngeal manometry or surface electromyography, and imaging examination such as barium swallow videofluoroscopy (VFS) which is recognised as the gold standard for the assessment of swallowing function, flexible endoscopic examination of swallowing (FEES), ultrasound, computerised tomography (CT) scanning and magnetic resonance imaging (MRI). The most common imaging method used to examine swallowing function is VFS and FEES.

1.1.4.1 Videofluoroscopy

Videofluoroscopy (modified barium swallows) is a dynamic imaging study which is able to show the different stages of swallowing (oral, pharyngeal and oesophageal stages) in real time or by playing back the recorded data to assess different details (Figure 1.3). Small quantities of barium sulphate are used to decrease the risk of aspiration, either mixed with water in liquid form or mixed with food of varying consistencies, prior to ingestion. This technique allows for an accurate assessment of penetration and aspiration of the airway at all swallowing stages and helps to assess bolus modification and compensatory techniques in preventing aspiration in dysphagic patients [17, 36]. The Rosenbeck penetration-aspiration scoring scale is widely used to assess the airway protection. It is divided into eight points; 1=normal swallow and 8=gross aspiration into the lungs, with scores between them (2-7) indicating an additional range of degree of penetration of material in the airway and aspiration [37]. There are some disadvantageous points of VFS such as exposure to radiation and the requirement of the patient to be in an upright position which may be difficult for patients that are unwell, especially immediately after stroke. Also, there is a small well defined risk of aspiration pneumonitis after aspiration of large amounts of barium [38, 39]; however, the strict guidelines for termination of investigation in high risk dysphagic patients usually prevent this side effect.

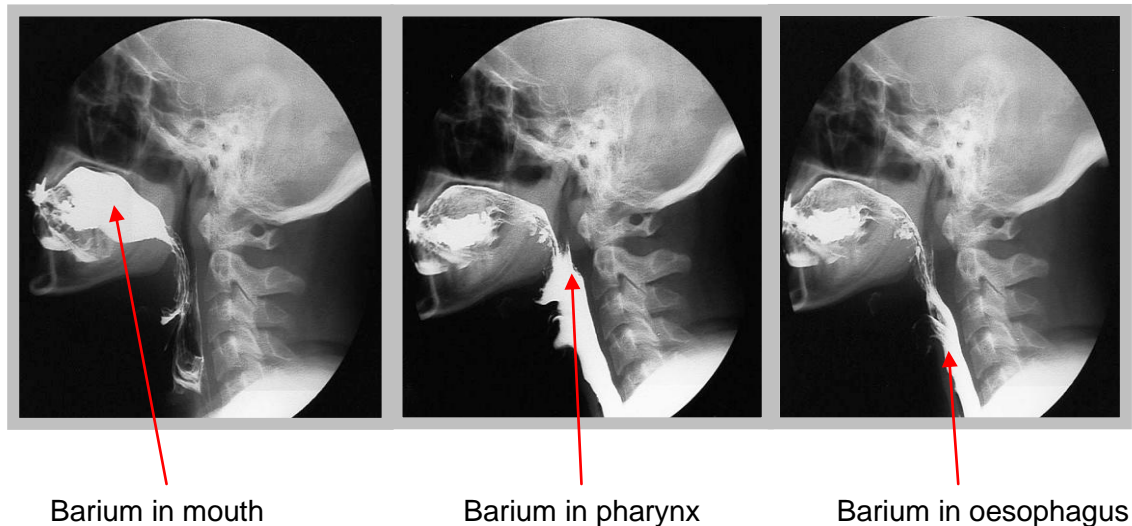


Figure 1.3: Series of videofluoroscopic images showing the bolus of liquid barium.

This bolus appears in white and moves sequentially from the oral cavity to the pharynx and then into the oesophagus.

1.1.4.2 Flexible endoscopic examination of swallowing (FEES)

This is a new technique that has been developed to visualise the pharynx and larynx before and after swallowing. Occasionally, a food dye is added to ingested food and drink to make them easier to see by the examiner. It can be used at the bedside in poorly mobile patients without exposing them to radiation. FEES has the ability to provide information regarding the timing and direction of movement of the food through the hypopharynx, anatomy of the laryngeal pharyngeal structures and the ability to clear the food bolus [17, 40]. FEES is unable to provide adequate information on the oral stage and additionally the pharyngeal stages cannot be observed due to contraction of the pharyngeal muscles. Therefore, aspiration determination is limited due to pre swallow spillage and aspiration is only determined by the presence of food residue post swallow [41]. According to a recent study, FEES has an excellent endoscopic scoring system [42] and can be used as an independent predictor for pneumonia in dysphagic stroke patients [43].

1.1.5 Neurophysiology of swallowing

The swallowing neural control centre is defined as “multidimensional in nature” [44]. All levels of the nervous system are involved in this multidimensional network and their connections. Sensory receptors in muscles, peripheral nerves, cerebellum, brainstem, subcortical and cortical regions are included under these levels. In general, the control of swallowing is described as: a voluntary process that starts under the control of the cerebral cortex. The corticobulbar tracts descend from the cortex to brainstem centres. These tracts contain cranial nerve motoneurons which supply the bulbar muscles and regulate the swallowing process. Also, peripheral afferent impulses ascend from the brainstem to higher centres to transfer the sensory information related to the bolus characteristics [45].

1.1.5.1 The role of sensation in the swallowing process

Food preparation depends on sensory feedback from the bolus characteristics, such as size and texture received from the sensory receptors in the tongue, soft palate, floor of mouth and tooth pulp. This information determines the activity of mastication muscles to complete the chewing process [46] [47]. The sensory impulses from the posterior mouth and pharynx initiate the pharyngeal phase [33]. Additionally, the duration and the activity of the pharyngeal muscles are not fixed because they depend on the sensory feedback from afferent receptors via bolus characteristics [23]. If the mucosal layers of the posterior portion of the mouth and pharynx are anaesthetised locally, the time for repetitive swallowing can be increased leading to disruption of the swallowing process although this does not stop the swallowing process completely [48-50].

1.1.5.2 Oropharyngeal sensory system

Sensation is classified into somatic, visceral, and special types, which provide all the information relating to the internal and surrounding environment. Somatic type sensors include two types: cutaneous and deep somatic sensors. Cutaneous sensors arise from skin epithelial tissues and respond to fine touch, pressure, temperature and vibration, whereas deep sensors arise from the muscles, tendons and joints and respond to pain and position. Visceral senses include nausea, gastrointestinal pain and other visceral sensations. Special type sensors respond to smell, taste, hearing dynamics and static equilibrium and sight.

Oral chemesthesis is the sense of chemical stimulation that activates oropharyngeal mucosal receptors such as thermoreceptors (temperature), nociceptors (pain) and mechanoreceptors (touch). This activation is mediated by the trigeminal, facial, glossopharyngeal and vagus nerves [51, 52]. There are many different types of chemical stimuli that activate oral chemesthesis. For instance, a cold-sensitive nociceptor is stimulated by menthol in peppermint, and a heat-sensitive nociceptor is stimulated by capsaicin in chili peppers. Nociceptors and thermoreceptors are also stimulated by salts, bitters and acids. There is evidence, according to Green et al. [52], that mechanoreceptors can be stimulated by one class of chemicals called alkylamides.

1.1.5.2.1 Receptors

Oral pharyngeal receptors can be irritated by stimuli such as salt, acid or alcohols that might not irritate the other receptors on the skin because mucous membranes of the epithelium in oropharyngeal area lack to a substantial stratum corneum [52]. Oral pharyngeal receptors are especially sensitive, therefore, the information about the bolus characteristics guide the different stages of swallowing (preparatory and transport stages) and stimulate protective reflexes to prevent aspiration.

1.1.5.2.1.1 Thermoreceptors

Thermoreceptors are sensory receptors with free nerve endings that respond to heat and cold. The heat receptors are unmyelinated C-fibres (low conduction velocity), whereas cold receptors have both C-fibres and thinly myelinated A-delta fibres (faster conduction velocity) [53]. Cold receptors increase their firing rates with cooling, and decrease them with warmth and they respond to temperatures from 24 C° to 34 C°. They also respond to high temperatures between 45 C° to 55 C° which is referred to as a paradoxical response. This response is caused by the co-activation of nociceptors around cold thermoreceptors [54, 55]. Heat receptors respond to temperatures between 32 C° to 45 C° and their firing rates increase with an increase in temperature [54, 55]. In general, heating is an irritant stimulus which causes an increase in thermoreceptor irritation whereas cooling it result in decreasing irritation [56]. However, the effect of temperature on CO₂ irritation is the opposite, in that cooling enhances the irritation caused by carbonated water [57].

1.1.2.5.1.2 Nociceptors

Nociceptors are sensory receptors with unspecialized free nerve endings that are activated by noxious, temperature, chemical and mechanical stimuli. However, they are found in any area of the body, either internal or external, in order to detect sensation of pain, the numbers of nociceptors in the oral cavity and the nose are more than in other parts of the body. The fungiform papillae of the anterior tongue contains the highest density of nociceptors, but they are also located throughout the maxillary and mandibular branches of trigeminal nerve, the pharyngeal branch of the glossopharyngeal nerve, and the internal superior laryngeal branch of the vagus nerve [58].

There are two types of axons for nociceptors; A-delta axons (myelinated axons) which conduct at about 20m/s and the more slowly conducting C-fibres axons (unmyelinated) which conduct at velocities of less than 2m/s. Unmyelinated nociceptors are called poly modal receptors because they respond to mechanical, thermal and chemical stimuli.

The chemical stimulation of nociceptors is caused by different chemesthetic sensations (burning, coolness, to tingling, etc) and the perceptual responses to the chemical may differ according to the amount of chemical in the solution. For example, a higher concentration of menthol might be perceived as burning or stinging, whereas a low concentration is perceived as being cool [51], and certain chemicals in very small concentrations might not elicit a chemesthetic response at all [51, 58].

Chemical nociceptors respond to a wide variety of spices which are used in cooking such as piperine from black pepper and capsaicin from chilli peppers that elicit the most strongest response and are widely tested [59]. Also, they respond to organic acids such as citric acid and carbonic acid which stimulate heat-sensitive nociceptors [52]. However, the enhanced pungency of carbonated water by cooling suggests that there may also be another mechanism for transduction [52].

The transduction of oral chemesthesis for different chemicals is still not fully understood. The vanilloid receptor 1 (VR1) is a specific ion channel from the transient receptor potential (TRP) ion channels family which is thought to be important for the transduction of oral chemesthesis [52]. It is sensitive to capsaicin, low pH and heat [52]. An understanding of the effect of different food and beverages on the oropharyngeal mucosa

will be better defined, if the transduction mechanisms of different chemicals become clearer. In addition, according to Green [52], many chemical compounds that are considered to be traditional pure taste stimuli, also stimulate oral chemesthesis. He wrote that a “vanishingly small number of conventionally defined taste stimuli excite only the taste system, and (that) chemesthesis is therefore a much more common component of the flavour of foods and beverages than generally recognised”.

According to Yuanyuan [60], TRPA1 is an important and sufficient component of the nociceptive response to CO₂. Carbon dioxide diffuses into cells and produces intracellular acidification which causes the activation of TRPA1 ion channels by protons. Furthermore, they found the opposing effect of acidification on TRPA1. Internal exposure caused activation of TRPA1 whereas when applied externally, it blocked TRPA1 ion channels. TRPV1 showed the opposite effects in that, it is activated by intracellular alkalization and extracellular acidification [61].

1.1.2.5.1.3 Mechanoreceptors

Mechanoreceptors are sensory receptors that are represented in large numbers in the oral cavity and respond to touch, vibration, dental pressure and proprioception [54, 62, 63]. Mechanoreceptors are divided into categories in terms of their rate of adaptation whether they adapt slowly or rapidly. The anterior mechanoreceptors which lie near to the anterior two thirds of the tongue and the fungiform papillae are usually rapidly adapting [64] and are mostly innervated by the mandibular branch of the trigeminal nerve. During mastication and the oral preparation, the awareness of bolus characteristics such as size, texture, shape and position are transferred mainly through the mechanical receptors [63, 65]. The posterior mechanoreceptors located on the lingual surface of the epiglottis, the aryepiglottic folds and the ventricular folds of the laryngeal vestibules are usually slowly adapting in order to protect the larynx. They are supplied by the branches glossopharyngeal and vagus nerves [63, 66, 67].

1.1.2.5.1.4 Taste receptors

Taste receptor cells (TRCs) are located in the oral cavity and the pharynx and transmit information regarding the different gustatory stimuli [64, 68]. Taste receptor cells are present in groups of 50-120 in the form of so-called taste buds which contain structures

called papillae (fungiform papillae, foliate papillae and circumvallate papillae). Beside the papillae, taste receptors are also present in the palate, the larynx and the upper part of oesophagus. Each TRC has receptors on the apical end of its surface, and each taste bud has a pore that opens on the surface of tongue which allow molecules and ions taken into the mouth to reach the receptor cell inside [64, 69]. Particular chemical stimuli (tastants) are selected by TRCs including amino acids, fatty acids, ions, small organic molecules, and carbohydrates [70]. The transduction mechanisms which generate action potentials in afferent fibres for tastants are different. TRCs sensitive to salt and sour tastants use ion channels, whereas TRCs which are sensitive to sweet and bitter tastants use a second messenger system, as G protein to open an ion channels that allows sodium ion enter cell, calcium release and generated action potential. Other tastes, according to Green et al [51] may stimulate other nociceptors around taste buds which lead to the stimulation of chemesthesis as well as taste.

1.1.5.3 Peripheral control of swallowing

Five important cranial nerves (CN V, VII, IX, X, XII) are involved in the swallowing process. These cranial nerves supply the swallowing muscles by releasing acetylcholine at the neuromuscular junction at the motor end plate of the muscles, which is a highly excitable region to initiate the action potential of the muscle surface to contract the muscle.

- Cranial nerve V (trigeminal)

Supplies most of the sensory information (touch, temperature, pain, pressure) except for taste from the oral cavity, anterior two thirds of the tongue, mandible and face. It also provides the motor supply for the mastication muscles (temporalis, masseter, medial and lateral pterygoid) and several small muscles such as the tensor tympani, tensor veli palatine, mylohyoid and anterior belly of digastrics which push the bolus backward [11, 71].

- Cranial nerve VII (facial)

The motor part controls facial expressions and especially the muscles around the mouth which are responsible for lip seal during the oral phase of swallowing. The sensory part transfers the taste from the anterior two thirds of the tongue and soft palate through the chorda tympani and the great petrosal nerves. Also, it gives secretomotor branches to the

sublingual, submandibular, lacrimal glands and glands of the nasal and palatine mucosa [11].

- Cranial nerve IX (glossopharyngeal)

The sensory input conveys taste from the posterior third of the tongue and general sensory impulses from mucous membranes of the oropharynx, palatine tonsils and faucial pillars. It supplies the stylopharyngeus with vagus nerves. This muscle moves the larynx anterior and upward, which leads to relaxation of the UOS. Secretomotor branch supplies the parotid gland which stimulates saliva secretion [11]. The injury of this nerve leads to a defect in the gag reflex (absence) unilaterally [72].

- Cranial nerve X (vagus)

The pharyngeal branch of the vagus (with the cranial part of the accessory nerve XI) supplies all the muscles of the pharynx and soft palate except the stylopharyngeus which is the receiving branch of the glossopharyngeal nerve. Also, the pharyngeal branch of the vagus nerve and the glossopharyngeal nerve are responsible for relaxation of the UOS and adduction of vocal cords [71]. Superior and recurrent branches supply all the larynx muscles which protect the airway during swallowing. The internal branch of the superior laryngeal nerve receives sensory impulses from the mucous membrane of the epiglottic base of the tongue, aryepiglottic and the upper larynx above the vocal folds. The sensory impulses below the vocal folds are transmitted by the recurrent laryngeal nerve.

- Cranial nerve XII (hypoglossal)

All the intrinsic and extrinsic muscles of the tongue are supplied by the hypoglossal nerve except the palatoglossus [5].

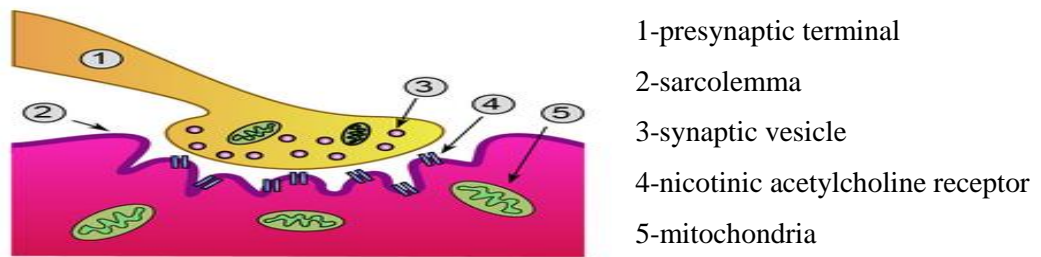


Figure 1.4: Schematic representation of a neuromuscular junction. Permission is granted to copy under terms of the GNU Free Documentation License, Version 1.2.

1.1.5.4 Brain stem control of swallowing

Many researchers have concentrated on the central regulation of swallowing and the brainstem regions with the use of different techniques for their studies. The brainstem swallowing centre is an important region to regulate the swallowing process in humans. It is distributed within the reticular formation in the upper medullary and pontine areas [73]. The neural system of the brainstem is made up of three components:

- An afferent component - Afferent inputs come from the sensory receptor of the periphery and afferent fibres of central swallowing.
- An efferent component - Efferent inputs come from the cortex and from the supra bulbar region and then to motor neurons of the cranial motor nuclei which supply muscles involved in swallowing.
- Central pattern generator (CPG) - Is an inter neuronal network which connects both afferent and efferent components and works as an organising system that determines the swallowing pattern according to incoming impulses (information) from the central and peripheral systems.

Although the initiation of swallowing is under the control of the cortex [44], the “sequential excitation of motor neurons” is the responsibility of the CPG control over the swallowing muscles [73]. In animal studies, researchers have found that defects in both

afferent and efferent fibres do not change the pattern of swallowing. This is an important finding that supports the presentation of CPG in the brainstem [73]. The interneurons of the CPG are divided into three types: early, late, and very late neurons. Each type of interneuron works at a specific muscular part during the swallowing process: early neurons work on the oral cavity, late neurons work on the pharynx and very late neurons work on the oesophagus [74].

The brainstem swallowing centre is separated into two regions: dorsal and ventral regions. The dorsal neurons are located within and around the nucleus tractus solitarius (NTS) and the ventral neurons are located within the reticular formation and around the nucleus ambiguus area. The dorsal neurons are described as “generating neurons” that initiate the swallowing sequence and modulate its pattern [73, 74]. Ventral neurons play a switching role that determine the output time of motor neuron activation during swallowing [48, 75]. There is a short interneuron network between the two medullary regions that transfers the excitatory or inhibitory effects between them. Also, the neurotransmitters between them play an important role as excitatory or inhibitory substances that regulate the deglutition process [2].

Beside the dorsal and ventral medullary centres, there is a small area in the pons that has ascending fibres directly to the cortex without passing through the NTS [2]. Therefore, sensory information includes: temperature, taste and touch, and the cranial nuclei of the cardiovascular and respiratory systems are fused in the NTS area with information passing from the NTS to the NA (nucleus ambiguus) and then to the muscles through the activation of the motor neurons of these cranial nerves (CN V, VII, IX, XI, XII) [16]. Also, the sensory impulses are transferred from the NA to the sensorimotor areas of the cortex.

1.1.5.5 Cortical and subcortical control of swallowing

According to animal studies, subcortical structures have a role in the control and modulation of swallowing [76]. The subcortical regions are divided anatomically into the hind brain (including the cerebellum), mid brain (including the substantia nigra and the ventral tegmentum) and the basal forebrain (including the hypothalamus, amygdala and basal ganglia). Stimulation of these sites by the superior laryngeal nerve or cortex stimulation leads to modulation of the swallowing process. Furthermore, in human studies,

there is evidence from patients suffering from Parkinson's disease, who have a problem with oropharyngeal swallowing coordination due to lesions of basal ganglia that leads to disturbance of normal swallowing and causes dysphagia [77].

According to Hamdy et al. [78], who utilised functional magnetic imaging in their study during volitional swallowing, cerebral blood flow is increased in the left amygdala, the left cerebellum and dorsal brainstem. The cerebral cortex has a significant role in the initiation and regulation of swallowing. This evidence has been present for nearly a century. In 1898, Henry and Charlton reported the first diagnosis of dysphagia after hemispheric stroke [79]. Animal studies have also played an important role in understanding the brain control of swallowing with stimulation of cortical swallowing areas. Different studies in different animals and in humans have demonstrated that electrical stimulation of each cortical hemisphere can induce the complete swallowing response/sequence.

This finding has provided evidence that swallowing muscles are controlled bilaterally [44, 80, 81]. Although the swallowing process is controlled equally by both hemispheres [44], one hemisphere appears more dominant than the other [82]. This evidence is supported by studies of swallowing after cerebral injury in stroke patients. The central regulation of swallowing is hypothesised to be organised in a hierarchical manner as illustrated in Figure (1.5).

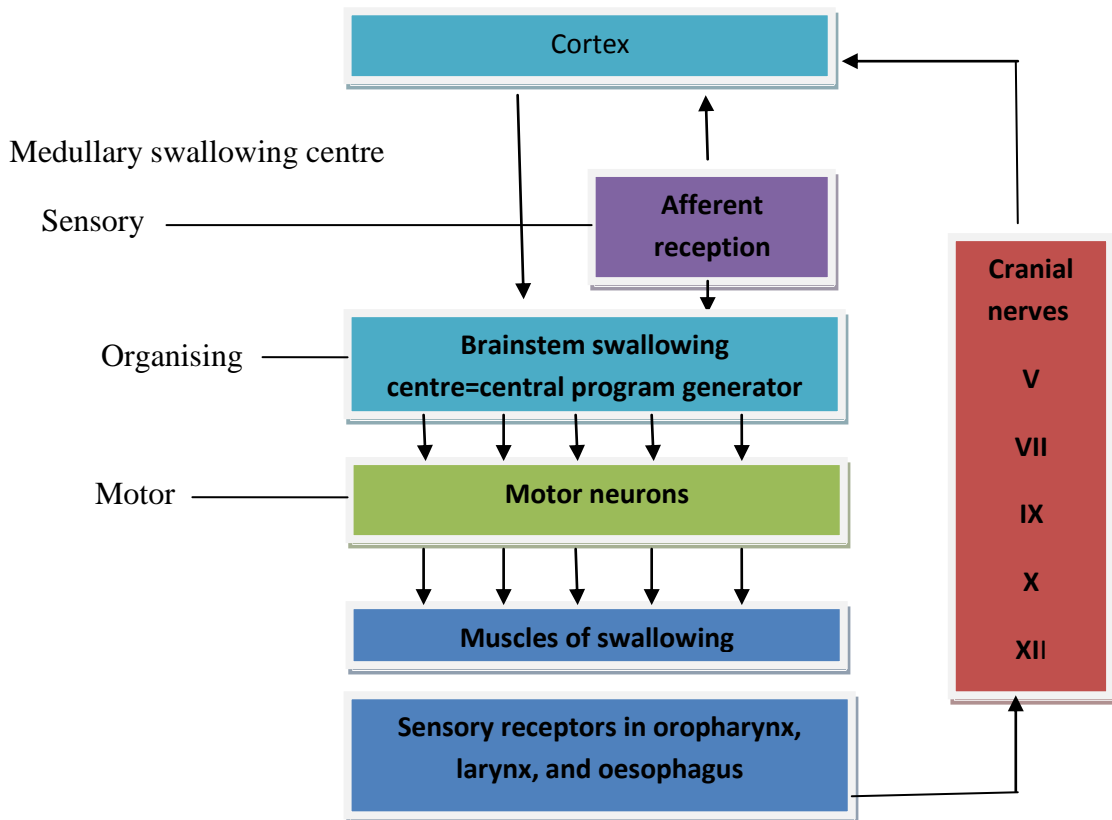


Figure 1.5: Diagram showing the hierarchical organisation of central neural control.

The input from the higher centre and peripheral meet at the CPG, which stimulates swallowing musculature contraction through bulbar motor nuclei.

Regarding the various studies that have been performed after cerebral injury, the different areas of the brain in human swallowing considered to be important and associated with dysphagia are the thalamus, pyramids tracts, frontal operculum and insula. In addition, the lateral sensorimotor cortex and especially the right insula have been described recently to play an important role in the swallowing process [78, 83]. Transcranial magnetic stimulation technique (TMS) has provided further information regarding the cortical control of swallowing especially in the human precentral gyrus [84-86]. Transcranial magnetic stimulation (TMS) mapping studies by Hamdy et al. have demonstrated that the projection of motor areas to oral muscles are arranged laterally, whereas the pharynx and oesophagus are arranged more medial [85]. Also, in another study that included a large

number of healthy subjects, it was found that the representation of the swallowing musculature in one hemisphere is larger than the other [85].

This evidence has also been demonstrated by other imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) [78, 83, 87]. Mosier and Bereznaya [88] reported that there are five functional clusters, independent brain regions that are involved in the control of the swallowing process. These clusters are:

(1) Primary motor cortex, primary sensory cortex, supplementary motor cortex and cingulated gyrus. (2) Inferior frontal gyrus, secondary sensory cortex, corpus callosum, basal ganglia and thalamus. (3) Premotor cortex, posterior parietal cortex. (4) Cerebellum. (5) Insula.

All of these cortical regions are involved in the voluntary or automatic swallowing process [89]. Many of them overlap and have a strong interrelation to areas responsible for taste, thirst, hunger, smell and flavour. Therefore, after cerebral injury in dysphagic patients, if the lesion is isolated, the application of treatments that stimulate taste, smell or flavour may play a role in swallowing rehabilitation [11].

1.1.6 Effect of taste, smell and texture on the swallowing process

The anatomy and physiology of both taste and smell are complex. The rate of swallowing is affected by the taste as well as food temperature and carbonation of liquids. Taste sensation has strong links with olfactory inputs (smell sensation) and both senses are required to perceive flavour [11]. Therefore, the perception of flavour is affected by a reduction of smell sensation. Generally in older individuals, both smell and taste sensations are decreased.

1.1.6.1 Taste sensation

The primary sensations of taste include sour, salty, sweet and bitter. There is a fifth unique taste called “umami taste” which is dependent on glutamic acid and is presented in different types of food and vegetables, and in breast milk. According to Kuribara and Kashiwayanagi [90], the umami substance does not affect pleasant taste but it increases flavour perceptions. Two thirds of taste buds are present in different areas of the tongue and the other third is present in the soft palate, posterior wall of the oropharynx and the epiglottis. The primary sensation of taste is distributed on different sites. On the tip of the tongue, the sweet and salty tastes are present, whereas the sour taste is located on the two lateral sides and the bitter taste is on the soft palate and posterior region of the tongue [11]. Taste sensation from the anterior two thirds of the tongue is transmitted via the fifth cranial nerve and chorda tymani into the facial nerve and then to the tractus solitarius, whereas the taste sensation from the posterior third of the tongue is through the glossopharyngeal nerve to the tractus solitarius. The epiglottis area is supplied by the vagus nerve and transmits its sensation through the same tracts.

The second-order neurons transmit these sensations to the thalamus and the post central gyrus. Therefore, taste sensation is passed to both the thalamus and the cortex [91]. Taste sensation information is transmitted to both the primary gustatory cortex (insula) and the secondary gustatory cortex (orbitofrontal cortex) [92]. Figure (1.6) illustrates the human taste system.

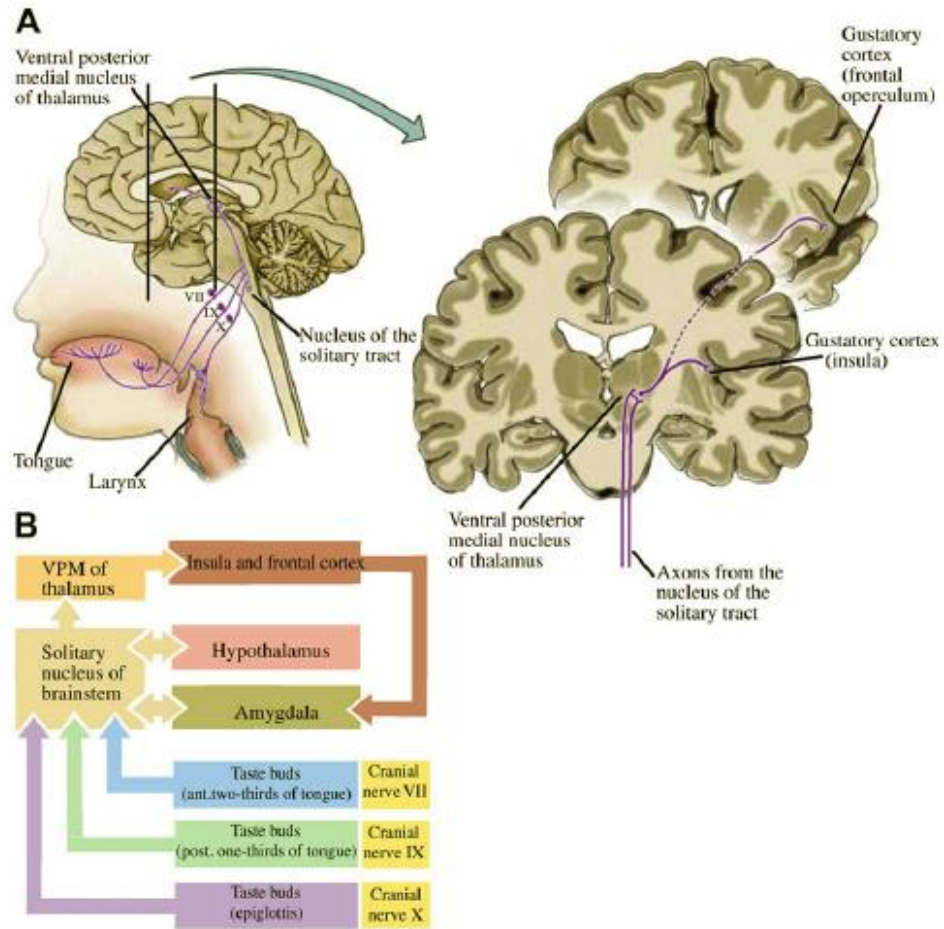


Figure 1.6: Illustration of the human taste system. A - (Left) shows the relationship between receptors in the mouth and upper alimentary canal with the nucleus tractus solitaries in the medulla. A - (Right) The coronal sections illustrate the ventral posterior medial nucleus of the thalamus and its connection with both primary and secondary gustatory regions of the cerebral cortex. B - Schematic representation of the basic pathways for processing taste information (copy from Purves, D, Augustine, GL, Fitzpatrick, D, *et al.* Neuroscience, Third edition. Sunderland (MA): Sinaure Associates Inc.; 2004.with permission).

According to Guinard et al. [13] acidity leads to an increase of the flow rate of saliva. This effect has been observed for citric acid, bitter and sweetness. In addition, the combination of chemical sensations, especially sour and tangy sensations, as in wine, leads to an increase of the saliva flow rate. Kaatzke-McDonald et al. [93] have demonstrated that glucose, distilled water and isotonic saline do not affect the swallowing response in healthy individuals. Also, they mention that there is no difference between cold and warm

chemical solutions. Swallowing initiation and the pharyngeal response are faster with a sour bolus in dysphagic individuals [94], but the therapeutic use of sour bolus is limited because people do not prefer it. However, mixing of both citric acid and sucrose (as sour bolus modifications) to improve palatability leads to an improvement of the flavour without changes in airway protection [95]. Also, they found that the number of spontaneous swallows is increased with flavoured substances, other than water. This finding helps dysphagic patients in the swallowing of saliva. The sweet tastes lead to increased salivary flow rates and stimulate lip closure, and its taste provides sensory feedback stimulation.

More recently, Humbert et al. [96] demonstrated that the swallowing of saliva, when compared to water, increases neural activation of cortical regions and BOLD (blood oxygen level dependent) response in the ventrolateral post central gyrus bilaterally. This study differs from the previous study of Martin et al. [97] who found that the neural activation of water swallows was four times more than that of saliva. Rika et al. [98] have demonstrated that water infusion into the posterior tongue leads to a prolonged swallowing interval (SI), whereas infusion into the pharyngeal region causes shorting in the swallowing interval. The opposite thing was found with 0.3 M NaCl solution and olive oil.

1.1.6.2 Smell sensation

The olfactory epithelium, olfactory bulb, olfactory tract and the cerebral olfactory areas form the olfactory system [91]. The olfactory epithelium covers a large area in the roof of the nasal cavity. It contains the olfactory sensory receptors that transmit information relating to different odours into the olfactory bulb via the olfactory nerve, then to the pyriform (olfactory) cortex in the temporal lobe, and to the other regions of the brain such as the hypothalamus, amygdala and insula [87, 92, 99-101]. Olfactory information from these regions is transmitted to other regions of the cerebral cortex through the thalamus. At this stage the odorant is identified and motor, visceral and emotional responses commence [92, 99, 100] (Figure 1.7).

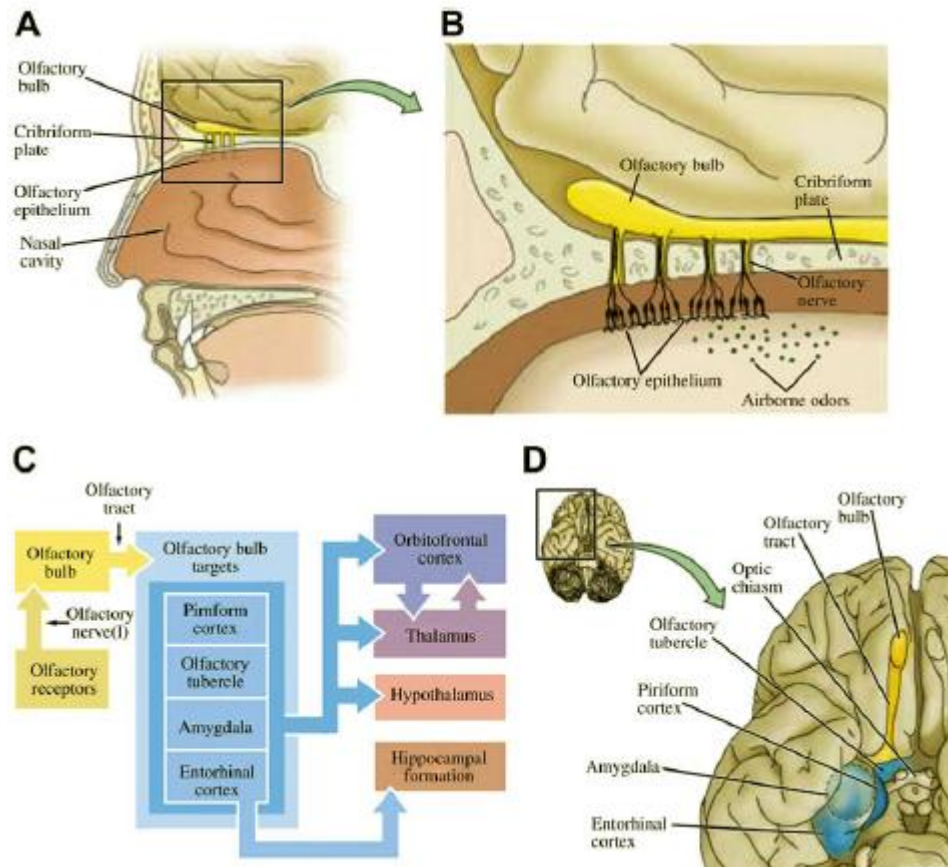


Figure 1.7: Illustration of the human olfactory system. A - Illustration of the peripheral and central components of the olfactory pathway. B - amplified section shows the relationship between the olfactory epithelium, containing the olfactory receptor neurons, and the olfactory bulb. C - Schematic representation of the basic pathways for processing olfactory information. D - Central compound of the olfactory system. Copy from Purves, D, Augustine, GL, Fitzpatrick, D, *et al.* Neuroscience, Third edition. Sunderland (MA): Sinaure Associates Inc.; 2004 with permission.

The detection of odour is most efficient between the age of 20 to 40 years. The general prevalence of impairment of olfaction is 24% in middle age, and reaches 70% in elderly individuals [102]. Also, calcification of the ethmoid bone and drying of the mucus layer leads to a decreased ability to perceive odour in older people but this decline occurs gradually. Therefore, older people are unaware of this change [103]. According to Paul Rozin [104], smell is unique since it has “dual nature” meaning it can sense odour from outside (orthonasal) and inside (retronasal) the body. Orthonasal stimulation refers to odorants that are sniffed via the external nares of the nose until they reach the olfactory cortex and then the primary olfactory cortex in the orbitofrontal cortex (OFC), whereas

retronasal stimulation is due to volatile molecules released during food ingestion from the back of the oral cavity to the olfactory epithelium via the nasopharynx [105].

Researchers have studied the function of both smell and taste pathways in humans using brain imaging, and have found that the stimulation of taste alone leads to activation of the tongue areas and insula, and stimulation of odour leads to activation of the primary olfactory area in the orbitofrontal region. However, the stimulation of taste and smell lead to enhanced activity in many regions with additional activity in the adjacent area around primary receiving areas [106]. Moore and Dulley [107] demonstrated that mechanical damage to the cribriform or olfactory bulbs causes complete loss of smell on the affected side. According to Kovacs [102], identification of odour in the early stage of Alzheimer's disease is altered; however, the detection of odour is impaired during the late stage of the disease. Also, the detection and identification of smell are damaged in patients with Parkinson's disease. The perception of smell is affected by the concentration of chemicals and by minimal changes of the chemosenses and affects the ability of the individual to enjoy food when they have lesions due to injury or ageing [103].

1.1.6.3 Food texture

The type or texture of food and/or fluid has an important role in the physiology of the normal swallow. For example, a thick fluid is more cohesive than a thin fluid bolus and travels more slowly than a thin liquid bolus [11]. The physiological changes that occur when swallowing a thickened fluid bolus have been mentioned on Page (26). Recent research has illustrated that the physiological response of discrete swallows is different from that of sequential swallows. For instance, oral transit duration, pharyngeal response duration, UOS opening duration and total swallow duration in sequential swallows are faster than discrete swallows, whereas sequential swallows show a slower oral to pharyngeal stage transition and pharyngeal transit duration [11]. For solid food, as previously mentioned, there are differences when compared to the swallowing of liquids because solid food requires a preparatory phase and enters the pharynx during the oral phase. Moreover, multiple swallows are required to clear a solid bolus from the mouth compared to a liquid bolus. Recently, Clave *et al.* [108] demonstrated that the safety of the oral and pharyngeal phases can be improved when bolus viscosity is increased. According to Lambert *et al.* [109], the thickened fluids and solid boluses could reduce aspiration

pneumonia in pseudobulbar patients. There are many examples of the effects of food texture, but one example is the effect of carbonated water.

1.1.6.3.1 Carbonation

The oral sensation evoked by carbonated drinks has emerged to be largely chemogenic in origin. According to Komai, et al [110], in a study performed on rats, acetazolamide (carbonic anhydrase blocker) leads to a depressed response of trigeminal neurons in the lingual nerve and chorda tympani, which is evoked by carbonated water. Simons et al. [111] investigated the origin of sensation elicited by carbonated water when applied on the tongue of rats and humans. They used three methodological approaches: electrophysiological methods to record responses of single units in trigeminal subnucleus caudalis elicited by carbonated water in rats, c-Fos immunohistochemistry and human psychophysics. They reported in each case that dorzolamide significantly reduced neural activity or perception induced by carbonated water.

Dessirier et al. [112] tested the effect of acetazolamide on the intensity of sensation in humans, or c-Fos expression of trigeminal neurons in rats, before applying carbonated water to the tongue. They found that acetazolamide reduced the magnitude of sensation induced by carbonated water, whereas it did not reduce the citric acid sensation. In a complementary animal study, acetazolamide was found to reduce Fos-like-immunoreactivity (FLI) in the dorsomedial trigeminal nucleus caudalis evoked by the application of carbonated water to the tongue. These data indicate that the sensation evoked by carbonated water is partly chemogenic in origin and the residual perceived sensation after acetazolamide pre-treatment could be elicited by a mechanical or osmotic component of carbonated water. In addition, they examined the effect of capsaicin on the sensation elicited by carbonated water and it was found that the pre-treatment of the tongue with capsaicin reduced the oral sensation evoked by carbonated water and citric acid. Therefore, capsaicin-sensitive nerve fibres partly conveyed irritation produced by CO₂ and citric acid. This finding was supported by another study of Dessirier et al. [113] who tested the effect of higher capsaicin concentration and amiloride on the oral sensation elicited by the application of carbonated water on the tongue. They reported that pre-treatment with capsaicin decreased the intensity of sensation evoked by a carbonated drink while

amiloride caused a small but significant increase in the intensity of sensation elicited by carbonated water.

Chandrashekar et al. [114] tested the responses and the detection of taste receptor cells (TRCs) to carbonation using electrophysiological methods that record the tastant induced action potential from chorda tympani in mammals. They reported that carbonation is a combination of multiple sensory inputs and has the ability to elicit a somatosensory as well as a chemosensory response including taste neurons. Also, they demonstrated that sour cells provided the taste sensor for carbonation and carbonic anhydrase 4 (Car4), a glycosylphosphatidylinositol-anchored is a specific cellular (taste) sensor for carbonation.

Moreover, there is an interaction between carbonation and thermal perception. Yau et al. [115] investigated the effect of temperature on carbonation perception using different temperatures of 3°C, 10°C, 16°C and 22°C and two carbonated levels of 2.4 and 3.0 volumes of CO₂. They reported a significant increase in the intensity of carbonation at the lower temperature in both trained and untrained panellists but this difference was clearer at the 3.0 volume level than at the 2.4 volume level. Therefore, this effect may also be dependent on the level of carbonation. Harper and McDaniel [116] used lexicon to describe sensory perception of carbonated water which included sour, bitter, salty, bubbly, sound and size of bubbles, gas expansion feeling, numbing, burn, and bite. They used four CO₂ levels (carbonated: 1.69, 2.75 and 4.63 volumes and non-carbonated) and two different temperatures (3°C and 10°C). They reported that an increased temperature of carbonated water leads to an increased perceived bubble size and bubble sound, and that a decreased temperature causes a decrease in cooling, bite, burn and numbing. Green et al. [57] investigated the interactions between CO₂ and temperature using different CO₂ concentrations and different temperatures. They found that the rating of oral irritation increased significantly by cooling carbonated solutions and the addition of CO₂ to the solution resulted in an increase in the perception of cooling of the solutions especially at very low temperatures.

In addition, carbonation has the ability to alter the quality and intensity of different tastes. McLellan et al. and Yau and McDaniel [117, 118] found a significant increase in sourness and a significant decline in sweetness in the presence of carbonation compared to sour and sweet tastes in aqueous solution. Also, an increase in the saltiness stimuli, in the presence

of carbonation, at the lowest concentration of NaCl presented (0.086 M) and a mixed effect on the bitterness of quinine were observed according to Cometto-Muniz et al. [119]. Beverly et al. [120] reported small increases in the total taste intensity but a high increase in the quality of the sweet and salty taste after carbonation when examining the total taste intensity of sweetness, sourness, bitterness and their binary combinations with or without carbonation. According to Green et al. [121] carbon dioxide has the ability to enhance sourness and saltiness and may be able to inhibit other tastes or flavours. Lederer et al. [122] reported that the aroma and flavour of cooked milk are significantly suppressed by high and low levels of carbonation, whereas sourness and astringency are significantly enhanced by high levels of carbonation. Also, they found a significant increase in chalkiness and bitterness with an increase in the concentration of carbon dioxide.

Different outcome measures have been used to examine the effect of carbonation on the swallowing mechanism, such as electromyography (EMG) and videofluoroscopy. Ding et al. [123] applied sEMG on the neck musculature to test swallow timing and muscle contraction intensity during the swallowing of liquids (distilled water, sweet water, sour water, salty water, and carbonated water) and cottage cheese with three taste conditions (sour, salt, sweet). They found that submental muscle contraction for salt bolus was stronger compared to sweet and sour bolus and a shorter EMG submental start time in the three taste conditions and in the two taste conditions (sweet and sour) for the infrahyoid muscle. However, they did not find any effect of carbonation on the amplitude or duration of submental sEMG. Miura et al. [124] examined the effects of five taste solutions (sucrose, sodium chloride, citric acid, sodium glutamate and caffeine) versus water and the effects of carbonation and cold stimulus on the power frequency content of swallowing submental sEMG. They demonstrated that sour taste, carbonated and cold stimuli, with an increase in high frequency content of swallowing submental sEMG, led to more organisation of submental muscle activity that provided a more efferent input to NTS.

Bulow et al. [125] examined 40 patients (36 patients had neurological impairment) during a therapeutic videoradiographic swallowing study using three different consistencies: thin liquid, thick liquid and carbonation. They analysed their swallowing according to three parameters: penetration/aspiration, pharyngeal transit time and pharyngeal retention. They reported that carbonated liquid had the ability to reduce pharyngeal retention, reduce penetration/aspiration and cause a shortening in pharyngeal transit time compared to

thickened thin liquid. Nixon [126] studied the effects of carbonated liquid and thin barium liquid on swallowing in four groups of patients/participants under videofluoroscopy. Group one included patients who aspirated on thin liquids. Group two included patients who required modified and non-oral feeding. Group three included patients who required non-oral feeding. Group four included healthy volunteers. Nixon reported a shortening of oral transit time (OTT), pharyngeal transit times and reduced aspiration in group one and group two. Another study conducted by Newman et al. [127] examined the effect of carbonation on swallowing physiology of 24 dysphagic infants and children. The authors reported that carbonated thin liquids (CTL) compared to non-carbonated liquids caused a significant reduction in the incidence of spill over, delayed pharyngeal response and laryngeal penetration, and they recommended the use of carbonation as a therapeutic option in dysphagic children. However, they did not find any significant effects on aspiration which might be due to the small number of participants who aspirated (n=7).

More recently, Sdravoy et al. [128] investigated the effect of carbonation on swallowing safety in 17 dysphagic patients using videofluoroscopy (VFSS). Temporal and descriptive measurements of bolus flow were used: oral transit time (OTT), pharyngeal transit time (PTT), stage transition duration, initiation of the pharyngeal swallow (IPS), penetration-aspiration scale (PENASP) and pharyngeal retention (PR). Carbonated thin liquids showed a significant reduction in the penetration and aspiration in neurogenic dysphagic patients compared to non-carbonated thin liquids (NCTL) in both bolus volumes (5ml and 10ml). However, carbonated thin liquids had no significant effect on other measurements of bolus flow.

Krival et al. [129] measured the differences between three types of liquids: water, carbonation (in Schweppes[®] Club Brew) and carbonation+gingerol (in Reed's Extra Ginger Brew) in peak linguapalatal pressure, linguapalatal pressure duration, and adjustments in the development of linguapalatal pressure in 20 healthy volunteers. They reported that carbonation and gingerol influenced greater neuromotor activity compared to water during the oral stage of swallowing. Also, they observed greater pressure phase durations and swallowing peak pressure with Ginger Brew compared to Club Soda which might show some benefit from adding chemosensory agents to carbonation.

1.2-Dysphagia

Dysphagia is defined as the difficulty in swallowing food and/or fluids, which can occur at any phase of deglutition [16]. The term dysphagia is of Greek origin that is divided into two parts. The first part “dys” means ‘difficulty’ and the second part “phagia” means ‘to eat’. Dysphagia is classified into oropharyngeal, pharyngeal or oesophageal dysphagia. Oropharyngeal dysphagia refers to when the transfer of food bolus from the mouth to the upper oesophagus is impaired, whereas oesophageal dysphagia is a problem involving the transfer of the bolus through the body of the oesophagus. Many mechanical and neuromuscular conditions cause dysphagia but the highest prevalence of dysphagia has been found in patients with neurological diseases such as stroke, head injury and Parkinson’s disease [16]. Dysphagia causes many problems such as chest infection, malnutrition and dehydration, which lead to an increase in morbidity and mortality in dysphagic patients.

1.2.1 Symptoms and signs of dysphagia

A variety of signs and symptoms of dysphagia are presented in dysphagic patients. Coughing, choking or the abnormal sensation of food sticking in the throat or chest is usually reported; however, some of these presentations can be absent as in people with “silent aspiration”. Silent aspiration is the term used to describe materials or saliva entering the trachea without coughing or showing any other signs of aspiration [130, 131]. The signs and symptoms of oropharyngeal dysphagia include difficulty in chewing and initiation of swallowing, coughing, choking, nasal regurgitation, food sticking in the throat, weight loss, change in dietary habits, recurrent pneumonia and wet voice [17]. Oesophageal symptoms include sensation of food sticking in the chest or throat, regurgitation and recurrent pneumonia.

1.1.2.2 The causes of dysphagia

1.1.2.2.1 Causes of oropharyngeal dysphagia

The causes of oropharyngeal dysphagia [132] can be divided as follows:

1-Neurological disorders such as stroke, multiple sclerosis, Guillain-Barré syndrome, Parkinson's disease, pseudobulbar palsy, amyotrophic lateral sclerosis and others.

2-Muscular and rheumatologic disorders such as myopathy, polymyositis and Sjogren's syndrome.

3-Metabolic disorders such as thyrotoxicosis, amyloidosis, side effects of medication and other causes.

4-Infectious diseases such as Lyme disease, diphtheria, polio and others.

5-Structural disorders such as oesophageal web, cricopharyngeal bar, radiation and postsurgical changes, oropharyngeal tumours, and other causes.

6-Motility disorders such as upper oesophageal sphincter dysfunction.

1.2.3 Assessment of oropharyngeal dysphagia in a stroke population

The prevalence of dysphagia at the time of admission to hospital and the risk of aspiration are high so dysphagia assessment should be undertaken at the time of admission at the bedside. This assessment involves a range of clinical techniques used as preliminary steps in the assessment of swallowing functions. These steps are dependent on the condition of the patients since some of them may require a range of assessment measures whereas others may require only a few steps.

1.2.3.1 Clinical assessment (bed side assessment BSA)

The bedside test allows the examination of oromotor function to determine who is suitable for oral feeding and who is not and to make a decision for other necessary assessments as needed for the diagnosis and treatment of the problem. Although the bedside assessment has the ability to diagnose the majority of oral stage disorders, it has a poor ability to characterise the laryngeal phase of swallowing [11]. The clinical bedside assessment includes background history and full physical examination, cervical auscultation pulse oximetry and a water swallowing test. A simple water swallowing test has been used to assess the swallowing ability in many studies and has been correlated to videofluoroscopy findings. In many cases, the water swallowing test has been able to detect dysphagia and in a more recent study, aspiration has been detected by a combination of these signs including a weak cough, dysphonia and coughing after drinking 5mls of water [133]. The presence of two or more from six clinical features (dysarthria, dysphonia, post swallow cough, abnormal volitional cough, changing of voice post swallow, and abnormal gag reflex) is considered as a high risk of dysphagia on VFS [134]. However, other studies have shown the sensitivity and specificity of the bedside swallowing test are variable and usually underestimate aspiration in comparison to VFS and FEES assessment of swallowing [135, 136]. Therefore, the requirement for using instruments in the examination of swallowing is increased.

1.2.3.2 Instrument examination

Videofluoroscopy and FEES are the two current standard investigations into the difficulty of swallowing. The description of both techniques has been detailed on page (27, 28).

1.2.3.2.1 Scintigraphy

This is an expensive and dynamic assessment of swallowing. The evaluation value of pharyngeal swallowing disorders by scintigraphy is limited. It is useful in the evaluation of both the quantity and quality of subglottic aspiration, gastroesophageal reflex and oesophageal motility disorder [137].

1.2.3.2.2 Ultrasound

This is a non-invasive technique that visualises the oral cavity and hypopharynx during deglutition. Ultrasound of swallowing requires good skills to interpret the results.

1.2.3.2.3 Computed tomography (CT) scanning and magnetic resonance imaging (MRI)

This is a widely used technique after stroke to determine structural abnormalities. It provides excellent findings regarding the localisation of a lesion (stroke hemisphere, brainstem, thalamus, etc.) in stroke patients with dysphagia.

1.2.3.2.4 Manometry

This has been commonly used in the assessment of motility disorders of the oesophagus but if the probes are placed in the oropharyngeal area, the propulsive forces of the oral cavity and pharynx that lead to the opening of UOS can be studied [138]. The use of manometry in oropharyngeal dysphagia is limited because it detects non obstructive abnormalities only in 25% of patients [137].

1.2.3.2.5 Electromyography (EMG)

1.2.3.2.5.1 Swallowing electromyography

Mechanical upwards–downwards movements of the larynx are detected and recorded during swallowing. Also, EMG can record the activity of the cricopharyngeal muscles of UOS.

1.2.3.2.5.2 Laryngeal electrography

The diagnosis of oropharyngeal dysphagia due to peripheral nerve injury such as superior laryngeal or recurrent laryngeal nerves can be detected by laryngeal EMG [137].

1.2.4 Complications of dysphagia

There are many complications of dysphagia ranging from aspiration and chest infection to malnutrition, dehydration and death.

1.2.4.1 Aspiration

Aspiration refers to the entering of food or liquid material into the airway below the true vocal cord [17], which leads to an increased risk of pneumonia. The effect of aspiration depends on the quantity, depth, physical properties of the aspirated material and the presence or absence of the clearance mechanisms of the pulmonary area.

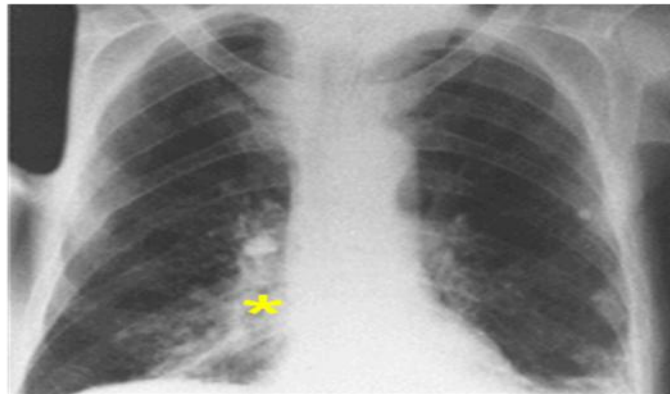


Figure 1.8: Chest image showing aspiration of liquid barium into the distal bronchus. Salford Royal NHS Trust.

1.2.4.2 Aspiration pneumonia

Many studies have demonstrated that the risk of chest infection increases from two to eight-fold in the presence of dysphagia [139, 140]. About 6% of the mortality rate after acute stroke is attributed to aspiration pneumonia [141] and post-mortem detection of aspiration pneumonia in stroke patients rises from 5% to 16%. Additionally, aspiration pneumonia is present in 95% of live stroke patients with dysphagia [142]. Aspiration pneumonia could also be due to other causes rather than neurological disorders such as gastric reflux [143] or infected saliva due to bad oral hygiene [144]. The diagnosis of

aspiration pneumonia is difficult since it may be present only with mild infiltration as determined by chest radiography but it is important as it has a high mortality rate [145].

1.2.4.3 Malnutrition

The decrease in dietary intake in dysphagic patients leads to an increased risk of malnutrition. Malnutrition is frequently reported in stroke patients with approximately 49% of stroke patients being reported as malnourished and 65% of patients being diagnosed with dysphagia [137]. The risk of pneumonia increases with malnutrition due to many causes: reduced resistance, lethargy and weakness, and a reduced strength of coughing and mechanical clearance in the lungs [137]. In stroke patients, nitrogen levels also appear to be below a normal level [146, 147]. The low nitrogen level during acute stroke may be related to stress response [148]. Also, the poor management of dysphagic patients with only intravenous fluids and the delay of enteral feeding may lead to malnutrition [149].

1.2.4.4 Dehydration

Dysphagic patients are at risk of dehydration, which in turn is a risk factor for pneumonia, due to many causes such as decrease of salivary flow, (which further causes the colonisation of the oropharynx), lethargy and mental confusion, and depression of the immune system [137]. Also, it may lead to death due to azotemia, hypernatraemia, hyperkalaemia and hypercalcemia [150].

1.3. Stroke and oropharyngeal dysphagia

1.3.1 Stroke

Stroke is a medical term meaning cerebrovascular accident (CVA) and is characterised by a focal cerebral neurologic deficit of acute onset that lasts longer than 24 hours, whereas a transient ischemic attack lasts less than 24 hours (usually resolved within an hour) [151]. Stroke is the third most common cause of death in the UK [152, 153] and is a major cause of long term disability. It commonly affects people over 50 years of age [151]. Stroke can be subdivided pathologically into infarction stroke (thrombotic or embolic), which

accounts for 85% of all cases, and haemorrhagic stroke, which accounts for 15% of cases [151]. Intracerebral haemorrhage is often caused by the rupture of arteries due to high blood pressure or cerebral aneurysm or arteriovenous malformation [151]. The clinical assessment for distinguishing between these types of stroke is often difficult. Therefore, computerised tomography scanning is used to differentiate between ischemic and haemorrhagic strokes. The mortality rate of individuals who have had an ischemic stroke is about 40% and for haemorrhagic stroke is 30-50% during the first 30 days [154]. Another type of stroke is called lacunar stroke which is caused by a small infarction involving deep penetrating branches of a large cerebral artery. Lacunar infarctions are associated with high blood pressure and uncontrolled diabetes. Lacunar infarctions have been found in several clinical syndromes but the most common syndrome is pure motor hemiparesis [151].

There are many observed neurological signs of stroke including: hemiplegia, hemianopia, aphasia, agnosia involving visual, auditory, olfactory, etc. depending on the site of the lesion, visuospatial disorder, unilateral neglect and apraxia involving limb, oral and speech [155]. The complications after stroke include: dysphagia, aspiration, hemiparesis, shoulder subluxation, deep venous thrombosis (DVT), pneumonia, apathy, depression, fatigue and emotional lability which is presented in the first days of acute stroke and then progressively disappears over the next six months. Ischemic attack of the basilar artery, posterior inferior cerebral arteries or vertebral artery leads to dysphagia since these arteries have effects on the brainstem, cerebellum, lateral medulla, internal capsule and pons [11]. Also, lesions to the hypothalamus, the limbic system and basal ganglia, frontal cortex and corticobulbar tracts may lead to dysphagia [139].

1.3.2 Oropharyngeal dysphagia in a stroke population

Stroke disturbs oral and pharyngeal stages of swallowing. Dysphagia is a complication following unilateral cortical as well as bilateral stroke, brainstem or any other diffuse brain lesion [156]. In these stroke patients, either unilateral or bilateral, the sensory sensation in the laryngopharynx is decreased [157]. In one third of patients who have suffered unilateral hemiplegic stroke, according to Hamdy et al. [84], oropharyngeal dysphagia has been demonstrated. Also, Paciarani et al. [158] reported that dysphagia was present in

approximately 34.7% of stroke patients and more frequently in patients who had suffered a haemorrhagic stroke. Recent research has reported that the premotor and motor cortex of both hemispheres are responsible for dysphagia in some patients but more importantly it was reported that there is asymmetric representation of swallowing independent of handedness between the hemispheres [85]. Also, it has been proposed that the size of lesion is more important than location in the presentation of dysphagia [158]. The risk of aspiration and lingual discoordination are more associated with the lesions in the anterior and subcortical periventricular white matter rather than the posterior sites or subcortical grey matter [159]. Also, lesions of the middle cerebral artery can be responsible for dysphagia after stroke [158].

There are many symptoms of dysphagia after stroke but the most common symptoms, which usually occur in combination, include: delay in or an absent swallow reflex, a decrease in the tongue's control, reduction of pharyngeal contraction and reduction in hyolaryngeal excursion. According to Robbins et al. [82], left side hemisphere damage (LHD) leads to disturbances in lingual, labial and mandibular coordination and they suggested that the left side hemisphere is responsible for the oral phase of swallowing and they also observed that difficulty in swallowing in the pharyngeal phase is associated with right-sided stroke. Hamdy et al. [86] reported that there is a strong relation between recovery of swallowing after stroke and increased pharyngeal representation in an unaffected hemisphere. In addition, subcortical stroke leads to a delay in both the oral and pharyngeal phases of deglutition since it has effects on both the motor and sensory pathways to and from the cortex [24].

Both the medullary and pontine regions of the brainstem can be affected by stroke. The medullary region is responsible for swallowing coordination and organisation and therefore stroke affecting this region leads to a significant difficulty of swallowing in the pharyngeal phase rather than the oral phase [24]. Defects in the pharyngeal phase cause weak bolus propulsion through the pharynx, reduced hyolaryngeal excursion and residue in the vallecular space. Moreover, stroke in medullary areas causes loss of swallow-respiratory coordination. Therefore, dysphagia after medullary stroke is very severe during the first two weeks.

Logemann [24] reported that spasticity of the pharyngeal musculature can be caused by pontine stroke, which causes difficulty in moving the bolus, pharyngeal pooling and slow recovery. Generally, swallowing function changes over time after stroke, some swallowing problems resolve over the first week whereas others are persistent for six months or more [160].

1.3.3 Treatment of oropharyngeal dysphagia

The main goal for dysphagia therapy is to reduce morbidity and mortality associated with chest infection and nutritional status. A good swallowing therapy provides safe and adequate nutritional and hydration needs without complications. There are many types of treatment that have been suggested for oropharyngeal dysphagia. Therapy of dysphagia can be divided into compensatory and rehabilitative strategies. Compensation can be utilised in the early stages to keep patients safe when eating, whereas rehabilitation can be utilised both in the acute and the later stages to speed up the recovery process.

Although both compensatory and rehabilitation techniques play important roles in the recovery of swallowing function, most patients still rely on compensatory techniques before they are transferred to rehabilitation [11].

1.3.3.1 Compensation management for oropharyngeal dysphagia after stroke

1.3.3.1.1 Behavioural compensatory management

A large number of techniques and manoeuvres are included in behavioural therapy.

Posture and exercise:

Posture changes can affect deglutition in individuals with swallowing disorders to redirect the flow of food (body posture) or change pharyngeal dimensions (head postures). Although efficacy data of body posture changes is limited to small groups of patients with various diagnosis (neurological, neuromuscular, and head and neck cancers), there is the suggestion that postural changes can reduce aspiration, improve transit times in both oral

and pharyngeal phases and decrease residual amount after swallowing. There are many types of exercise such as:

- Lip exercises - To prevent leaking of food or liquid from the oral cavity.
- Tongue-strengthening exercises - To facilitate bolus manipulation, tongue base retraction and movement of the bolus through the oral cavity.
- Jaw exercises - To facilitate mastication movements.

1.3.3.1.2 Bolus modification and management

Diet modification becomes one of the first compensatory managements to improve the ability to swallow, maintain adequate nutrition status and general health. Alteration of food consistency, size of bolus and rate of delivery are affected by the ability to swallow in the different phases [16]. For instance, the control of a liquid bolus becomes difficult when the tongue and soft palate are paralysed. This problem leads to early entering of the liquid bolus into the pharynx with increased risk of penetration before the swallow has been triggered. Therefore, thick fluids and purées should be used to manage problems of the oral stage and delay triggering of the swallow. In contrast, thick purées and solid foods are preferred in pharyngeal weakness and bolus retention [161].

Various studies have tried to identify the appropriate consistency and specific nutrients to swallow. The effect of unlimited oral intake of water (in a small group known as thin liquid aspirators) was studied by Garon and Colleagues [162]. They divided the patients into two groups, whereby each group included ten patients known to have thin liquid aspirations. The first group (study group) received a thickened fluid and water at any time they requested, whereas the other group (control group) received a thickened fluid only. The outcome measure for this study was no patient in either group developing dehydration, pneumonia or any complication during the study. They did not find any patients that developed pneumonia by this way of management. Therefore, the authors recommended this type of management with careful follow up.

Also, recently, the effect of bolus viscosity and volume modification on swallowing physiology in both healthy subjects and patients with VFS was studied by Clave et al. [108]. They demonstrated that the safety of oral and pharyngeal phases is improved with

an increase in bolus viscosity and that swallowing function in neurogenic dysphagic patients is also improved with a reduction of bolus volume (10ml per swallow).

1.3.3.1.3 Non oral feeding of dysphagic stroke patients

Non oral feeding is essential in any patient who does not have the ability to achieve adequate supplement from food or water by mouth. Enteral feeding includes nasogastric tube feeding (NGT), percutaneous endoscopic gastrostomy (PEG) and oesophageal tube feeding. Total parenteral nutrition (TPN) and an intravenous line (IV) can also be used for non oral feeding. Figure (1.9) shows the different types of feeding tubes.

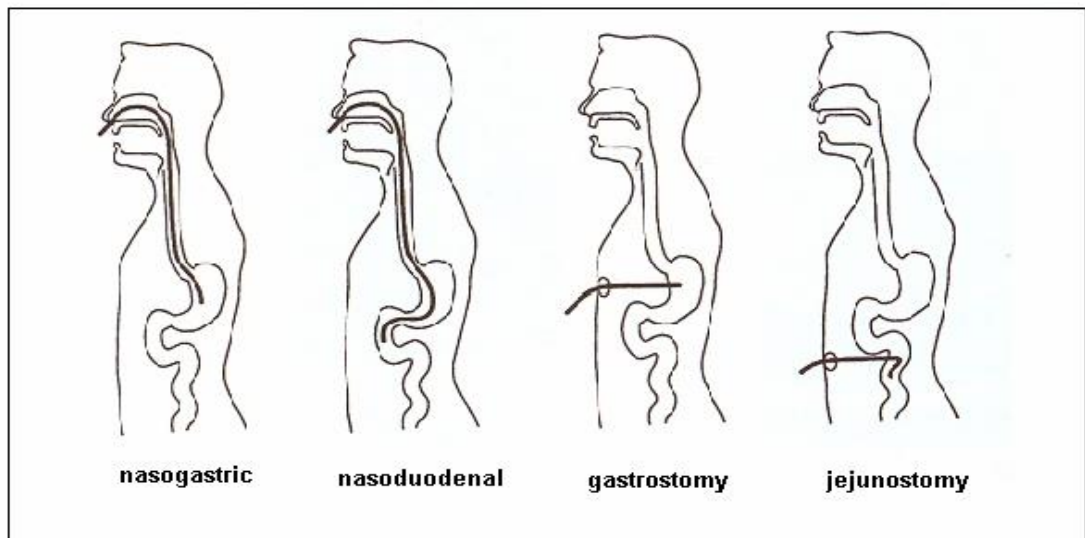


Figure 1.9: Schematic representation of different feeding tubes.

1.3.3.2 Rehabilitation management of oropharyngeal dysphagia after stroke

1.3.3.2.1 Medical management of dysphagia

A number of drugs have been used in the treatment of neurogenic dysphagia including nifedipine and ACE (angiotensin converting enzyme) inhibitors. However, evidence in the literature is limited to support their use at present. According to Perez et al. [163], a group treated with nifedipine improved pharyngeal transit time and swallowing delay compared to an untreated group.

1.3.3.2.2 Surgical treatment

Surgical intervention is required in certain situations and in selected dysphagic patients to achieve an adequate nutrient supplement. Surgical intervention includes surgical gastrostomy and cricopharyngeal myotomy to relax the upper oesophageal sphincter, and tracheostomy, medialisation, laryngeal suspension, laryngeal closure and laryngotracheal separation-diversion for chronic aspiration.

1.3.3.2.3 Stimulation techniques

A variety of oral stimulation techniques have been used as a part of the swallowing therapeutic procedures; however, evidence of their efficacy in treatment remains controversial. There are some simple techniques that have been used in dysphagic stroke patients such as increased pressure on the tongue by a spoon during feeding or using a sour bolus (containing lemon juice) to stimulate swallowing [94]. Also, another therapeutic program called deep pharyngeal neuromuscular stimulation (DPNS) has been used to improve pharyngeal swallow. It concentrates on the stimulation of three sites with frozen lemon-glycerin swabs. These sites are:

- The taste bud of bitter and base of tongue - to improve tongue retraction.
- Soft palate - to improve palate elevation.
- The superior and medial pharyngeal constrictor muscles - to improve peristalsis movement of pharyngeal and opening of the UOS [137].

Thermal tactile stimulation (TTS) technique to the area in the oral cavity which contains the sensory receptors involved in triggering the pharyngeal swallow [17, 164] is used in many research studies. Cold is the best stimulus to evoke a swallow [93]. TTS can be used to increase sensory awareness in the mouth before swallowing and to reduce delay between oral and pharyngeal phases (increase swallowing speed). According to Lazzara et al. [165], swallowing is improved in stroke patients after thermal stimulation which sensitises the base of the anterior faucial arches with a cold stimulus; however, mixed stroke types were included in this study and the limited reported data caused difficulty in the interpretations. Another study has reported that swallow improvement has occurred with repeat sensitisation [166]. Another study investigated the relationship between different intensities of TTS and reduced pharyngeal delay time. It demonstrated that no specific intensity of TTS was recognised as the most therapeutic [166]. Power and colleagues [167] demonstrated that the cortical motor excitability for pharyngeal swallow is inhibited, as recorded by EMG, following sensory stimulation of the anterior faucial pillar to the focal TMS of the precentral cortex [167]. Freed et al. [168] conducted a study which aimed to compare the efficacy of surface electrical stimulation (ES) and thermal tactile stimulation. Swallowing function was scored from zero to six (0=patient aspirates own saliva and 6=normal swallowing) before and after both treatments based on the substances which could be used during a modified barium swallow. Although the improvement in swallowing score was found in both groups, the swallowing score for the group treated with ES was higher than the other group

Recently, Fraser et al. [169] conducted a study which aimed to investigate the effects of pharyngeal electrical stimulation in healthy individuals with TMS and in stroke patients. They found that motor cortex excitability could be achieved at specific parameters. This study was followed by another study which included 16 dysphagic stroke patients, six of them received sham stimulation and another 10 patients received 5Hz of Pharyngeal Electrical Stimulation (PES) for 10 minutes. Videofluoroscopy (VFS) was used before and after stimulation to assess any changes in swallowing safety and performance. The outcome of this study was a 30% reduction in aspiration in the intervention group, whereas there was no change in aspiration for the sham group.

1.4. Neurostimulation and the treatment of dysphagia after stroke

1.4.1 Plasticity

The ability and capacity of the human adult cortex to adapt or reorganise to changes in the surrounding environment, either morphological or functional, is referred to as plasticity [170]. The cortical map, under different physiological conditions, can be modulated by the alteration of the sensory input experience and learning in adults [171]. Additionally, after injury to central or peripheral neuron systems, there are organisational changes of the primary motor cortex. These plasticity changes have occurred in many pathological conditions such as limb amputations, transection of the peripheral nerve and spinal cord injury [172]. According to Buonomano and Merzenich [173], plasticity changes can be studied on three levels:

- At the level of the synapse - by changing parameters such as “excitatory post synaptic potential amplitudes”.
- At a cellular level - following short-term conditioning protocols, the change in the single neurons response is detected.
- At a regional level - changes in the physiological brain response due to changes of inputs induced by training, lesions or other stimulation.

There have been many animal studies that have been performed for the investigation of the mechanisms of plasticity that mention that cortical maps are not fixed but are dynamic in nature. Cortical plasticity could be represented in body regions. At the first level of the representational cortical map (sensory maps), plasticity is expressed with alterations in cortical receptive areas such as reorganisation of the cortical representation of the skin, cornea and retina due to loss of afferent input. Also, reorganisation after peripheral nerve injury and limb amputation in the somatosensory system; however, plasticity of the motor map appears as changes in cortical outputs after amputation, stroke and specific stimulation tasks [174]. It is now generally known that plasticity changes may play a role in the recovery process of motor function. Therefore, many investigations have been

utilised to assess plasticity changes at different levels in animal studies, such as the reorganisation of representational maps and the change in activity of neurons (single or small groups), synaptic function and anatomic changes in vitro, and changes in the structure of neurons, including anatomy and synaptic function. In humans, TMS is used as a suitable tool to explore the mechanism of plasticity changes in the primary motor cortex [175].

1.4.2 Techniques to study plasticity

Non-invasive techniques to study neuroplasticity in humans are available. These techniques include functional magnetic resonance imaging (fMRI), multichannel electroencephalography (EEG), positron emission tomography (PET) magnetoencephalography (MEG) and TMS. TMS has been used as a suitable tool in the clinical neurosciences field for the study of plasticity, neuroplasticity and rehabilitation [176].

1.4.2.1 Transcranial magnetic stimulation

Magnetic stimulation was discovered in 1831 by Michael Faraday [177]. In 1985, cortical magnetic stimulation was described for the first time by Barker who performed direct stimulation of the human motor cortex using a magnetic coil which resulted in movement of the opposite hand and leg [178]. Over the last twenty years, the number of laboratories using TMS for therapeutic or neuroscientific research has increased dramatically and larger numbers of healthy subjects and patients have been involved in these studies [179].

1.4.2.1.1 The magnetic stimulation equipment and process

TMS is a non-invasive tool, which consists of a capacitor discharge system connected with switching elements to a coil [180]. The coil is composed of tightly copper wire encased in moulded plastic covers. Spatial characteristics of the induced magnetic field are determined by the size and shape of the coil. A circular coil produces a very high magnetic field strength below the ring, but a weak magnetic field at the centre. On the other hand, a figure of eight shape induces a higher current under the mid region (double), compared to

the current induced under its edges. Therefore, this coil allows a more focused stimulation of the brain [181] as demonstrated by Figure (1.10).

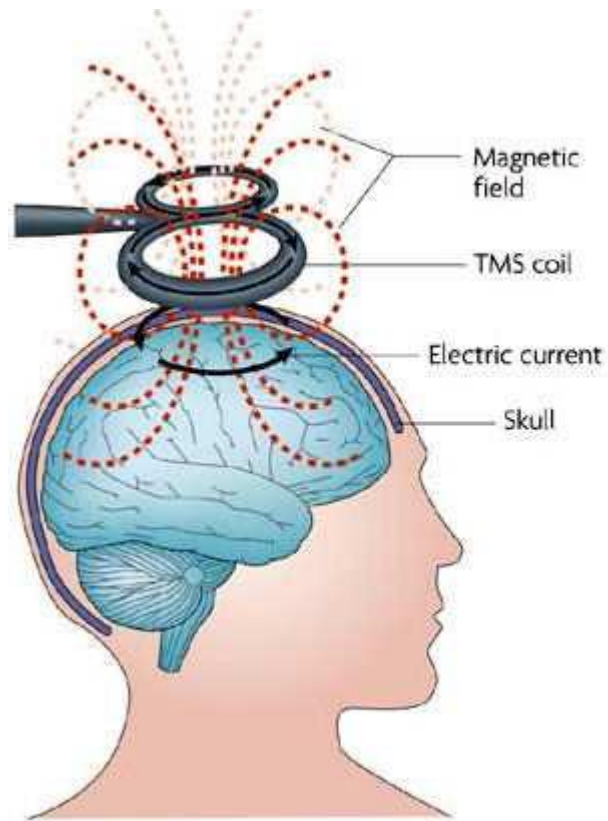


Figure 1.10: Schematic representation of the generation of electrical current in the brain induced by TMS.

Magnetic field intensity is dependent on the flow of current around the coil and the number of wires that turn within the coil as well as the dimensions. Charging of the magnetic field leads to an electrical current induced within the brain. The intensity of the magnetic stimuli decreases by increasing the distance from the coil. For example, the strength of coil decreases by half at a distance of 4-5cm from the coil surface in a typical 12cm diameter round coil.

Administration of magnetic stimulation is performed by activation of a stimulating coil after placing it on the scalp, followed by an electrical current running through the coil which induces a magnetic field that passes parallel to the central axis of the coil in its direction. Immediately, the magnetic stimulation passes through the bones and tissues to stimulate the brain without interruption. If the current amplitude, direction and duration are suitable, cortical neurons will depolarise and generate an action potential which can excite the cortical neurons [182]. The electrical field induced by the magnetic field causes a secondary ionic current in the brain by activation of the sensitive ion channels. In the motor system, this leads to depolarisation of neuronal membranes and produces an action potential which will transfer along a nerve to an effector such as muscle. The electrical response of the muscle is referred to as motor evoked potentials (MEP). In humans, TMS of the motor cortex can evoke an initial single volley of direct current (D-wave) and later repetitive indirect volleys (I-waves). D-waves are produced due to direct stimulation of the corticospinal axon and I-waves are considered to arise from transynaptic activation of pyramidal tract neurons, with I-waves having a greater variability in latency than D-waves [183]. The activation or inhibition of nervous tissues is dependent on the intensity, duration and frequency of the magnetic stimulation. Slow TMS (≤ 1 HZ) causes an inhibitory effect, however, fast TMS (≥ 1 HZ) leads to excitatory effects [169]. Magnetic stimulation could be administered in a single manner, in pairs or in a series with different durations. Single pulses and paired are used for neurodiagnosis, and administration of repetitive TMS (rTMS) is frequently used for research and therapeutic purposes.

1.4.2.1.2 Recording motor responses evoked by TMS

The motor evoked potentials (MEPs) can be recorded from muscles within the electromyographic activity (EMG) after application of a single pulse of TMS to the motor cortex. After an artefact stimulus, a period of time is taken for the development of the

motor evoked potentials, called the response latency. Another measurement is the response amplitude (peak to peak changes in positive and negative deflections of MEP) which reflects the level of excitability in the pathway. The information regarding the conductive properties of the pathway is provided by the response latency. The morphological pattern of pharyngeal MEP is shown in Figure (1.11).

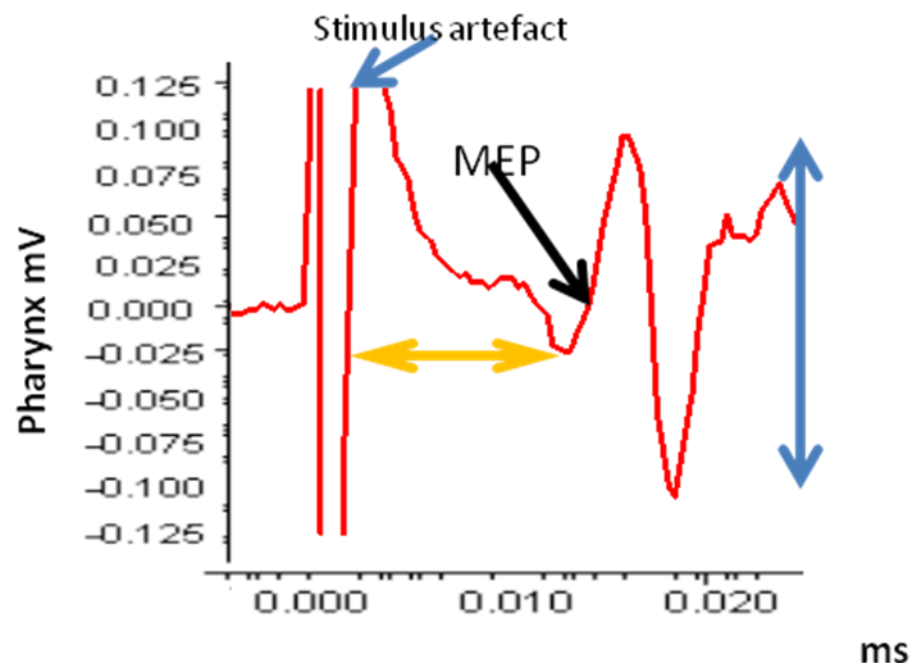


Figure 1.11: Schematic illustration of atypical pharyngeal MEP. Short blue arrow shows the artefact generated by the TMS pulse. MEP is the negative and positive deflection from the baseline. The orange arrow refers to response latency in milliseconds. The long blue arrow refers to response amplitude in microvolts.

The lowest magnetic intensity required to induce motor movement due to cortical stimulation is referred to as the motor threshold (MT) and varies from one individual to another [177]. Generally, MT is accepted as 50 μV in peripheral muscles and 20 μV in pharyngeal muscles. TMS has been widely used in the clinical neurosciences field to study brain reorganisation by temporarily and partially disrupting activity in a specific area of the cortex (virtual lesion), pathway excitability, and providing electrical stimulation of neural tissue from the cerebral cortex, spinal roots, cranial and peripheral nerves [176, 184-186].

1.4.2.2 Repetitive transcranial magnetic stimulation

When the TMS is delivered in a series of pulses this is referred to as repetitive transcranial magnetic stimulation (rTMS). Repetitive TMS is a non-invasive technique which has been used to modify neural activity; locally and distantly [186]. The capacity of rTMS to stimulate the motor cortex is dependent on frequency; higher frequencies (above 1 Hz) will increase brain excitability, whereas a lower frequency (1 Hz or below) will have an inhibitory effect on the brain site that is being stimulated [187]. Repetitive TMS has been used in a variety of clinical applications including psychiatric disorders (schizophrenia and depression), epilepsy, Parkinson's disease and others. In stroke, where the recovery of stroke patients is dependent on plasticity changes of the CNS, rTMS applications have been used in neurorehabilitation programs to enhance cortical reorganisation and to induce neuroplasticity [188]. It has been found that functional recovery might be obtained by applying a low frequency (1 Hz) over the normal (unaffected) hemisphere to restore defective inhibition or by applying high frequency (5 Hz or more) on the affected hemisphere to reactivate hypoactive regions [188]. The inhibitory effect of rTMS has been recently used to induce virtual lesion. Virtual lesion (focal cortical suppression) is a temporally and reversible interruption of a focal area of the brain and has been used to test the efficacy of neurostimulation and gain further information regarding the underlying mechanisms of beneficial plasticity in healthy volunteers. Using 1 Hz rTMS (600 single pulses in total) to the dominant swallowing motor cortex in healthy volunteers for 10 minutes, focal suppression (decrease in MEP amplitude) was observed immediately and continuously for up to 45 minutes after application [189].

Repetitive TMS effects on the pharyngeal motor cortex have been studied. For example, Gow et al. [190] observed that 5 Hz rTMS leads to an optimal increase in excitability of the corticobulbar projections to the pharynx and the effect continues for up to an hour in healthy volunteers. Jefferson et al. [191] investigated the effect of high frequency rTMS (5 Hz rTMS) on the contralesional hemisphere following an induced unilateral virtual lesion in the pharyngeal motor cortex in healthy volunteers. They found that active rTMS (5 Hz rTMS) reversed the cortical suppression induced by a virtual lesion and led to an increase in pharyngeal cortical excitability and improved swallowing behaviour compared to sham treatment measured with SRT. Another group has used a 1 Hz inhibitory protocol to the

unaffected hemisphere in an attempt to inhibit transcallosal inhibition in chronic dysphagia stroke patients [192].



Figure 1.12: Repetitive transcranial magnetic stimulation (r TMS). rTMS can transiently excite or inhibit the motor cortex, ≥ 5 Hz are excitatory and ≤ 1 Hz are inhibitory .Medical illustration, Salford Royal NHS Trust.

1.4.2.3 Safety of magnetic stimulation

According to most studies, single pulse TMS is a safe and useful technique in neurophysiological investigations. Additionally, repetitive TMS appears to be safe and well tolerated. The long term risks of both single pulse TMS and rTMS in adults according to available data are not significant, whereas both single and paired pulses have a minimal risk in children [193]. Although there have been many investigators working with TMS over many years (and exposure to 100 pulses a day on average) they have not recorded any cases to date of developing epilepsy, cancer, cognitive impairment or any other adverse side effects [193, 194]. Low rate repetitive TMS is safe with very few side effects;

however, the rapid rate of repetitive TMS is not safe and should be applied with caution since it has been reported to cause seizures [195]. The careful choosing of frequencies, duration and amplitudes of TMS stimulation leads to decreased side effects [193].

1.4.2.4 Precautions of magnetic stimulation

The International Society for Transcranial Stimulation Consensus on rTMS [196] recommends that:

1-Family history or past medical history of epilepsy in healthy volunteers or patients is an indication to exclude them.

2-Risk factors and side effects of TMS should be discussed with participant individuals.

3-Both healthy volunteers and patients must complete a signed consent form.

4-Transcranial magnetic stimulation, especially rTMS, should be applied under supervision and be performed under appropriate surroundings close to medical facilities.

5-Risk of scalp burning should be avoided.

6-During stimulation of the motor cortex by rTMS, the spread of excitability should be considered as an early sign in the development of seizures. Therefore, the stimulation must be stopped.

7-All the subjects should be monitored during and after procedures.

8- All subjects and investigators must wear earplugs when rTMS frequencies ≥ 1 Hz.

1.4.2.5 TMS in studies of healthy swallowing

The use of TMS to study the neurophysiological properties of swallowing in humans has led to the discovery of a number of key findings regarding cortical control of swallowing. For instance, single pulse TMS has been used to create maps of the cortical areas dedicated to motor representation of swallowing [85, 197]. The application of TMS over the motor and premotor cortex of either the dominant or non-dominant cerebral hemisphere has the ability to produce MEP arising from the pharyngeal and oesophageal muscles [85, 197, 198]. According to the results from these studies, the swallowing is bilaterally represented,

the pharyngeal response maps are larger in one hemisphere than the other and this functional lateralisation is independent of handedness. This asymmetrical representation may explain the variability in the degree and duration of dysphagia following stroke. According to the direct electrical stimulation study, which was conducted by Penfield and Boldrely [199], the cortical control site of swallowing in humans was originally localised to an area antero-caudal to the primary motor cortex and this finding was confirmed by the TMS study [85]. TMS mapping has been used to identify the arrangement of other swallowing muscles such as the oesophageal, mylohyoid and pharyngeal muscles by Hamdy et al [200]. Furthermore, in comparison to single hemisphere stimulation, the stimulation of both hemispheres at the same time with TMS tends to enlarge the size of the pharyngeal response with shortening of the latency response. This finding is likely to suggest summation of input of both hemispheres at shared interneurons within the brainstem [200].

1.4.2.6 Therapeutic application of TMS in stroke patients

TMS rehabilitation techniques in stroke patients can be used to investigate plasticity of the human cortex. Firstly, to describe changes in motor excitability (cortico-cortical and cortico-subcortical connection), single or paired pulse TMS techniques are used. Secondly, TMS is applied to disturb activity in any cortical area (virtual lesion), to explore the functional relevance of cortical reorganisation. Thirdly, repetitive TMS can cause changes in cortical circuits which open the possibility of directly intervening with the mechanisms of cortical plasticity in the intact human cortex [201].

Pharyngeal MEP responses to TMS in patients with unilateral hemispheric stroke have shown different. This difference is dependent on whether they have normal or difficult swallowing. Dysphagic stroke patients have a small response in the pharynx compared to non-dysphagic patients during the stimulation of the unaffected hemisphere [202]. On the other hand, stimulation of the effected hemisphere of both groups (dysphagic and non dysphagic) leads to produce small responses than the unaffected hemisphere. Accordingly, dysphagia is more likely to occur after unilateral hemispheric stroke if the injury happens in the hemisphere with the large cortical projection [190].

TMS has shown interesting findings regarding recovery mechanisms of dysphagia after stroke. The size of the pharyngeal motor map in the undamaged hemisphere is markedly increased over time. This increase in map size is associated with an improvement of swallowing function (Figure 1.13). This neuroplasticity form is observed in the undamaged hemisphere and is not consistently found on the effected hemisphere. Furthermore, the functional improvement of dysphagia is preceded by an increase in cortical excitability so this suggests that the recovery of swallowing is dependent on cortical reorganisation. Moreover, these changes do not appear in non-dysphagic patients and patients who show no improvement in their dysphagia [202, 203].

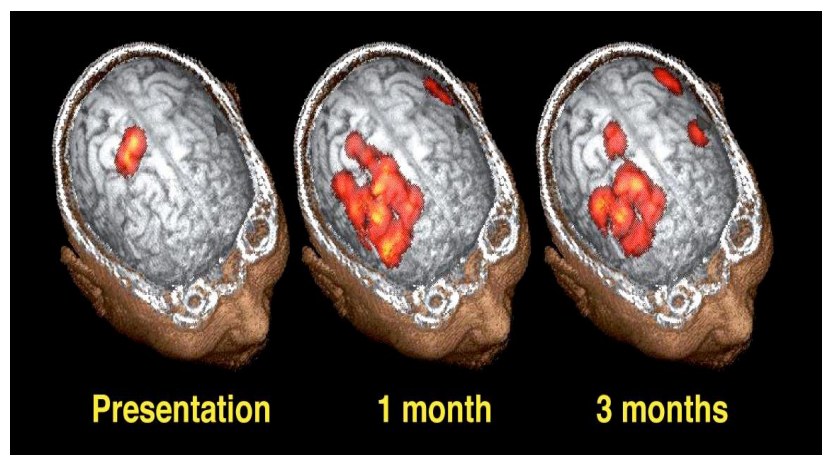


Figure 1.13: Pharyngeal cortical representation during recovery of a dysphagic patient.

The patient had a left hemisphere cortical stroke and functional swallowing recovered within 1 month. There are small changes of the pharyngeal representation in the affected hemisphere, whereas the pharyngeal cortical representation of the unaffected hemisphere appeared to be expanding anteriorlaterally during recovery (1 and 3 months). (With kind permission by Elsevier Ltd)

In contrast, the improvements of limb function in stroke patients are associated with increased cortical representation of the affected site (damaged hemisphere). This interesting finding may be explained as the swallowing muscles are controlled by a bilateral hemisphere, whereas limb function is controlled by a single hemisphere [200]. In addition, the response to TMS stimulation demonstrated a larger increase in the unaffected

hemisphere than the damaged hemisphere after applying pharyngeal electrical stimulation; therefore, peripheral stimulation has the ability to stimulate both cortices [169].

1.5-Aim and hypotheses

Swallowing and dysphagia in stroke patients is an important and unique research field. Dysphagia, especially persisting dysphagia after stroke, may have deleterious consequences for the patient that might increase mortality and morbidity. The stimulation with carbonated liquids may play a significant role in this respect, since it has been observed that such liquids may play a role in the reduction of airway penetration and pharyngeal transit time compared to thin or thick liquids. Therefore, the application of carbonated liquids could have a role in the change of the sensory perception, which leads to the activation of higher nervous centres.

The aim of this study is to therefore ascertain whether the carbonation of liquids enhances cortical swallowing and alters the behavioural swallowing function compared to non-carbonated solutions.

CHAPTER 2

Effects of Carbonated Solutions on Human Swallowing Performance

2.1 Introduction

Swallowing is a complex sensori-motor process which is initiated by the cerebral cortex and followed by a series of neuromuscular interactions between the brainstem swallowing centre, five of the cranial nerves (trigeminal, facial, glossopharyngeal, vagus and hypoglossal) and 26 pairs of muscles [204]. This process leads to the safe and coordinated transfer of ingested substances from the mouth to the stomach whilst providing protection of the air way.

Difficulty in swallowing (dysphagia) in stroke patients has been assessed by video-fluoroscopy [205]; it was found to affect between 50% and 78% of patients and up to 40% of these remained dysphagic a year later [206]. According to Martino, Foley et al. [205], the risks of aspiration pneumonia and malnutrition were increased in dysphagic patients and many of them required nasogastric tube feeding (NGT), percutaneous endoscopic gastrostomy (PEG) or total parenteral nutrition (TPN). Most of these procedures required special care and long hospital stays.

Despite the increased incidence of aspiration pneumonia after stroke, the treatments available to aid swallowing recovery by improving physiology and reducing the risk of aspiration are still limited. Sensory information from the upper gastrointestinal tract is transferred via cranial nerves through multiple levels of the CNS and interacts with the swallowing network. Therefore, the sensory input is dependent on stimulus characteristics which can change the physiology of swallowing, either through inhibition or facilitation of the cortical control of the swallowing musculature. For example, bitter and sweet tastes showed a reduction in PMEP with TMS due to a reduction of activity in the NTS, which may cause a reduction in the cortical activity associated with swallowing [207].

In the literature, it has been shown that carbonated water may activate lingual nociceptors which, in turn, excite the trigeminal neurons that are involved in signalling oral irritation. This is due to the conversion of CO₂ to carbonic acid in carbonated water [112]. There is some recent clinical evidence supporting the use of carbonated liquids to reduce penetration of the airway and pharyngeal transit time during transportation of the bolus [125] but there is limited literature looking into the effects of carbonation on the complex swallowing behaviours or neurophysiology. Also, it is not clear if the action of carbonated

solutions is due to its physical properties, i.e. its fizziness (the mechanical pressure of carbonated bubbles) or due to the acidity of carbonated solutions.

One of the common factors that predispose patients to dysphagia after stroke is believed to be reduced sensory awareness in the oropharyngeal area, which affects the swallowing process. Therefore, the aim of this study is to investigate the effects of carbonated solutions compared to non-carbonated solutions on swallowing performance in healthy volunteers using a swallowing reaction time task, prior to its therapeutic application in stroke patients.

2.2 Methods

2.2.1 Participants

Healthy volunteers were recruited through adverts placed around Salford Royal Hospital and through a departmental database of volunteers who have expressed an interest in participating in future research. My study was divided into two sections: first, a preliminary study of carbonation to assess feasibility and to obtain a power calculation and, secondly, a full study, comparing carbonation against other solutions with an equal pH value.

In the preliminary study, twenty-two healthy volunteers (eight female, mean age 26 ± 3.25 (\pm SEM), age range from 18 to 56) participated. In the main study, following a power calculation, sixteen healthy volunteers (eight male, mean age 33 ± 3.65 (\pm SEM), age range from 20 to 61) participated. All subjects were in good health and received an information sheet prior to obtaining written informed consent. Also, they had the opportunity to discuss any queries before and during the study. The volunteers were invited to attend the laboratory, prior to starting the study, in order to familiarise themselves with the equipment. All participants met the study inclusion criteria. Exclusion criteria included: a history of epilepsy, previous brain or throat surgery, cardiac pacemaker, prior history of swallowing difficulty, neurological disease, pregnancy, the presence of metal implants in eyes or head, or intake of any medication which acts on the central nervous system or gastrointestinal tract. For the pilot study, subjects attended for one visit lasting for not more

than 1.5 hours, while, for the main study, the subjects attended on three separate occasions, with each study taking approximately 2 to 2.5 hours and with at least three to four days between each visit. The approval for this study was granted by Great Manchester (Central North West 7) Research Ethic Committee (10/H1008/61) and all studies were performed in the clinical laboratory of the Gastrointestinal Physiology department at Salford Royal NHS Foundation.

2.2.2 Sample size

According to a power calculation following the preliminary study, the number of volunteers needed to participate in the main study was decided to be sixteen, in order to obtain 12 full data sets, with expected subject dropout.

2.2.3 Procedures

2.2.3.1 Pharyngeal Electrical Stimulation (PES)

Subjects were required to swallow a 3.2 mm diameter intraluminal catheter (Gaeltec Ltd, Dunvegan, Isle of Skye) which contained a pair of bipolar platinum ring electrodes and also housed a pressure transducer for the reaction times (see 2.2.6 below). According to the subject's preference, the catheter could be inserted orally or nasally. The catheter's position was adjusted according to visual inspection of EMG traces on the computer screen. An earth wire was connected to a skin electrode sited over the upper part of one of the sternocleidomastoid muscles in the neck.

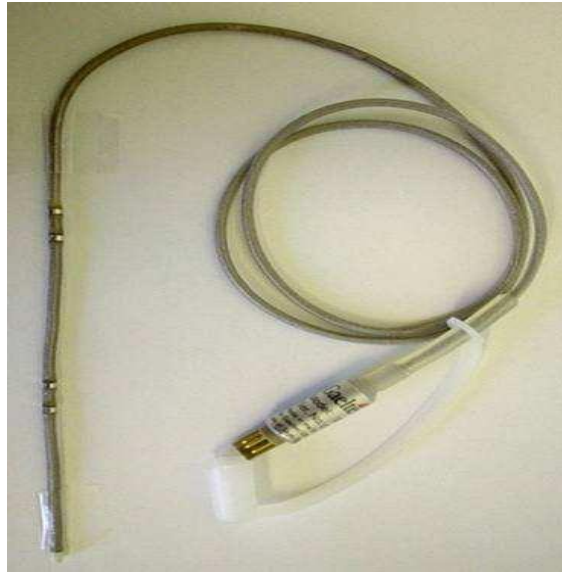


Figure 2.1: Gaeltec Catheter

The catheter houses a pair of bipolar platinum ring electrodes.

The same catheter, used for EMG recordings, was also used for pharyngeal stimulation when connected to an electrical stimulator (Digitimer model DS7, Welwyn-Garden City, Herts, United Kingdom) and a trigger generator (Digitimer Neurology system), allowing measurements of an individual's sensory and maximal pharyngeal electrical thresholds. With this technique we can record the sensory and maximal thresholds.

The sensory threshold was defined as the first perceptible sensation of electrical stimulation and was calculated as the average of three trials. In each trial, stepwise increments of approximately 0.1 mA per second were instigated starting at zero stimulator output. The maximum tolerated intensity was determined in an identical manner but this time the subject was asked to identify the point when the stimulation became uncomfortable.

2.2.4 Boluses for swallowing paradigm

In the main study, three different liquid boluses were used as a “sensory bolus stimulus intervention” all at 6 °C: a) a carbonated solution, b) a non-carbonated solution (mineral water) and c) a solution with weak acidity (citric acid). These were used in a randomised manner across each set of the volunteers’ visits. However, in the preliminary study, only still water and carbonated solutions were used in a randomised manner across the same set of the volunteers.

To determine whether the acidity of the solutions was consistent and standardised across the studies, I tested the pH values of the solutions prior to the start of each experiment. The pH level of all the solutions was measured with the pH-meter (Jenway model 3310/Jenway, Lts, Gransmore Green, UK) available in our laboratory. The 3ml boluses of each solution were delivered into the subject’s mouth manually using a single-use plastic syringe and a small plastic single-use tube. A straw was attached to the tube and the subjects were asked to hold the straw between the lips, approximately in the midline of the mouth.

2.2.4.1 Citric acid solution

The citric acid solution was prepared by adding 100% pure natural citric acid powder 50 mg (0.05 g) to 1000 ml water, in order to get a citric acid solution with a pH level of 4.1. This pH level is equivalent to the pH level of the carbonated solution. This amount of citric acid powder was determined by gradually adding citric acid powder (commercially available from Westmill foods, Enfield, Middlesex, UK) to water and checking the pH level of the solution until the desirable pH level was reached.

2.2.4.2 Carbonated solution

Carbonated water was prepared in a Soda Siphon maker (commercially available from iSi North America, Inc., West Fairfield, USA) which kept the water under constant pressure and temperature (Figure 2.2). The lid of the soda siphon was removed and the bottle was filled with one litre of cold water (6°C) before the lid was secured on top. The cap of the soda siphon was removed and replaced by the charger holder with iSi soda charger and this charger holder was screwed onto the siphon head (which contains an aperture with a sharp

piercer). As the iSi soda charger was pierced, the CO₂ contents of the charger were released into the bottle and dissolved in the water. The soda siphon was shaken vigorously at least five times, according to the manufacturer's instructions, and then the lever of the lid was pressed to allow carbonated water to flow through the nozzle.

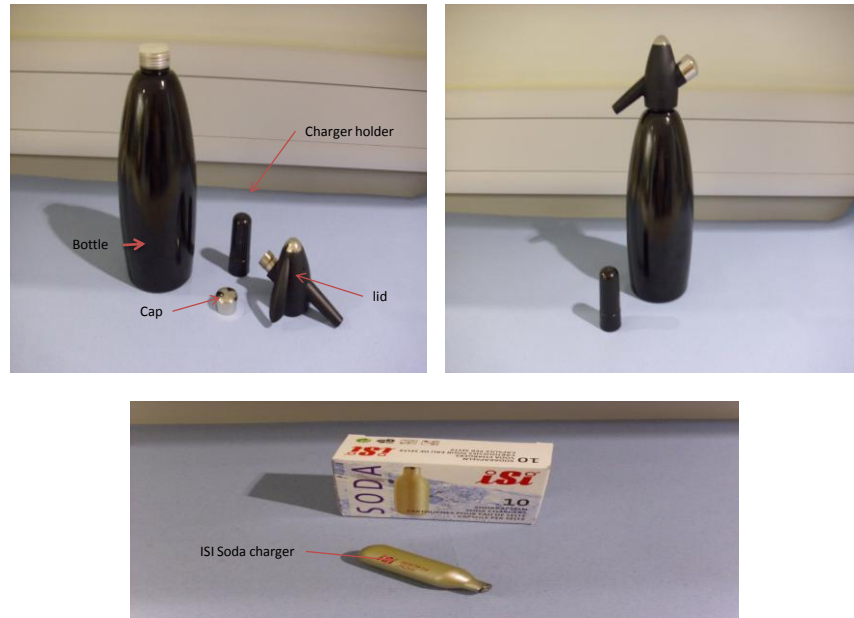


Figure 2.2: Soda siphon and ISI soda charger.

2.2.5 Taste intensity and difference scales

In the main study, each subject was tested individually in three sessions for the different solutions. To assess taste, each subject held each bolus in their mouth for three seconds to taste the sample and then rinsed his or her mouth twice before the next bolus. After each swallow, both the Labelled Magnitude Scale (LMS) and difference test chart were presented to the volunteers to identify taste, difference degree and its intensity. The Labelled Magnitude Scale (Green et al., 1996) includes seven verbal labels arranged according to their magnitude (nothing, barely detectable, weak, moderate, strong, very strong and strongest imaginable sensation). Expressing these labels as a percentage of the full LMS length is:

- Strongest imaginable: 100%
- Very strong: 53.3%
- Strong: 35.4%
- Moderate: 17.2%
- Weak: 6.1%
- Barely detectable: 1.4%
- Nothing: 0%

The difference test chart was divided into five levels (no difference=1, very mild difference=2, mild difference=3, moderately difference=4, extremely different=5).

2.2.6 Swallowing Reaction Timings (SRT)

The intraluminal catheter 3.2 mm (Gaeltec Ltd, Dunvegan, Isle of Skye) with built-in pressure transducer was used to record the pharyngeal pressure. The catheter was connected to a 'Pressure Box' (Department of Medical Physics, Salford Royal NHS Foundation Trust) which was then connected to a desktop computer running the customized software. A pair of electrodes was placed on the back of the subject's hand. These were used to deliver a weak electrical pulse through an electronic pulse generator (Digitimer model DS7; Welwyn-Garden City, Herts, UK) which cued the subject to swallow. A tube connected to a syringe was placed on the subjects lips and used to infuse carbonated water or other solutions (3 ml per swallow) to facilitate swallowing. Subjects were asked to perform up to 30 swallows a) 10 normally b) 10 as fast as they can and c) 10 swallows within a pre-determined time window (challenged swallow) (calculated from their own normal and fast swallowing physiology).

Swallowing reaction time (SRT) is defined as the delay time from the time receiving the electrical cue to the onset of the pharyngeal swallow that is determined when the pressure signal crosses the baseline pressure threshold (Figure 2.3).

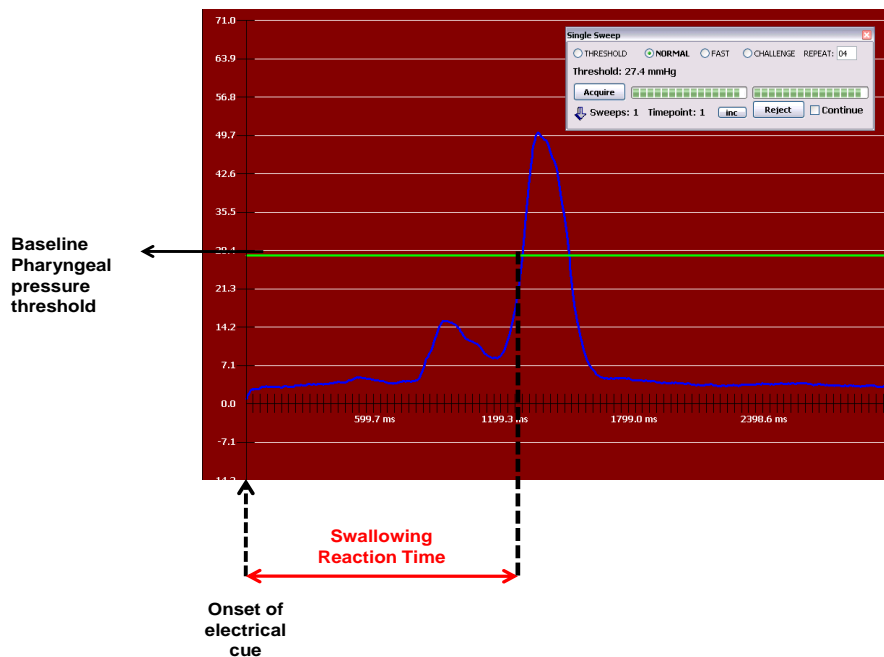


Figure 2.3: The pressure signal from the pharyngeal intraluminal catheter during a study with arrows showing the swallowing reaction time, the onset of electrical cue and the green line showing the baseline pharyngeal pressure threshold.

Challenged time window is a time window of 150 ms which was visually presented on a screen. This technique has been recently developed within our department as a reliable and accurate means to measure swallowing reaction times [189].

The time window was calculated from 10 normal swallows and 10 fast swallows based on this formula:

$$\text{Challenge Time Window} = \text{Fast Swallowing Reaction Time} + \left(\left\{ \begin{array}{l} \text{Normal Swallowing Reaction Time} \\ \text{Fast Swallowing Reaction Time} \end{array} \right\} / 2 \right) + 75\text{ms}$$

For the normal and fast swallows, volunteers were asked to perform the swallows after the stimulus was delivered to their hands. For the challenged swallows, they were instructed to

do 10 challenged swallows within the calculated time windows. The swallow was correct if they swallowed within the time window and incorrect if they swallowed outside the time window (Figure 2.4).

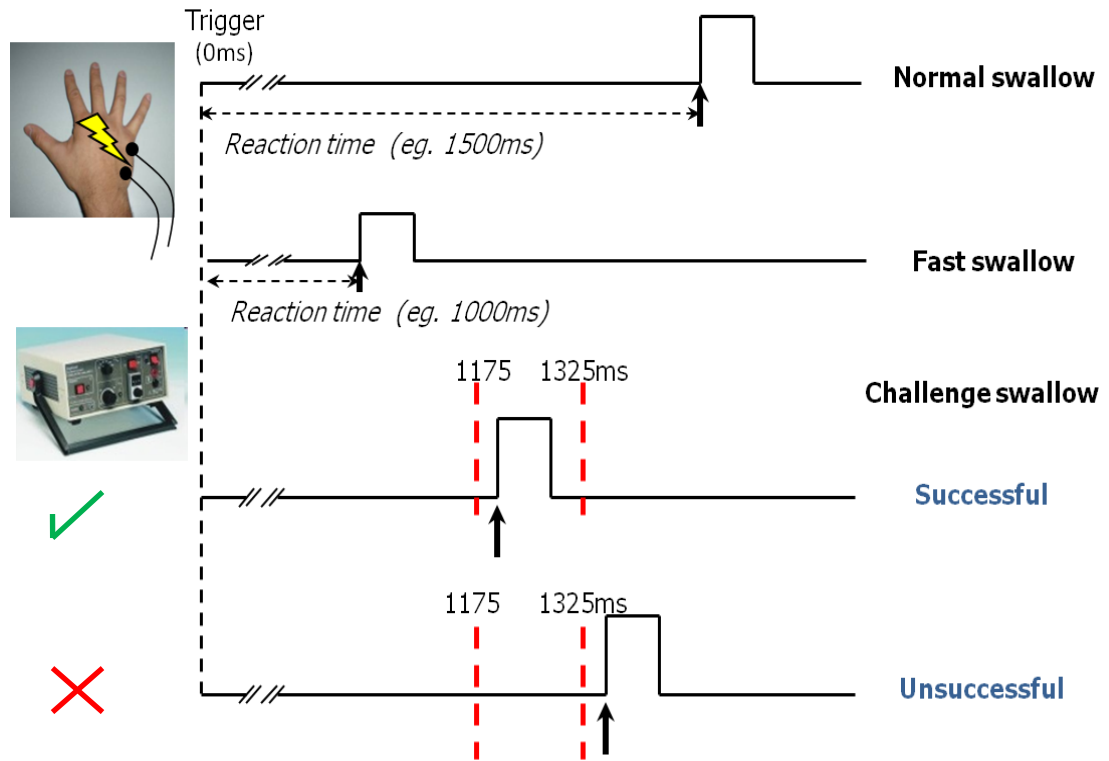


Figure 2.4: Experimental design of the reaction times tasks and challenged swallowing tasks. The subjects were instructed to swallow within the calculated time window. If they swallowed within the time window (between the red lines) the swallow was correct (marked with a tick) and if outside of the time window, the swallow was incorrect (marked with an X).

The calculated time window was visually presented on the computer screen as a vertical white line. In Figure (2.5), the left image shows a successful swallow: the swallow happened within the target window (150ms) and the blue line changed to green. The right image shows an unsuccessful swallow, since the subject swallowed before the target window and the line changed to red (Figure 2.5). The Swallow Splash Software (Department of Medical Physics, Salford Royal NHS Foundation Trust) was used to record and calculate the swallowing reaction time tasks.

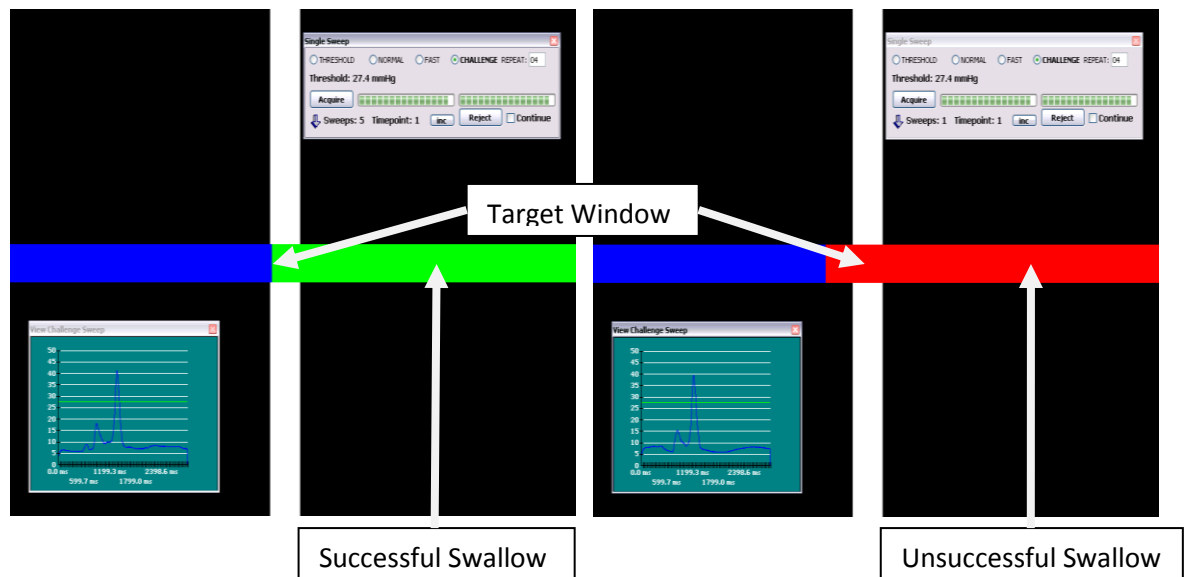


Figure 2.5: The computer screen for the challenge swallows task; a successful swallow (left) and an unsuccessful swallow (right).

2.2.7 Experimental Procedures

2.2.7.1 Protocol 1 (Pilot study)

With the catheter in site, sensory thresholds for each participant were recorded using PES, prior to any swallowing. The pair of electrodes was then placed on the back of the subject's hand. This was used to deliver an electrical pulse which was used to cue the subject to swallow. Subjects were asked to perform 30 swallows: 10 normal, 10 fast and 10 challenged swallows within a predetermined time window. Each swallow consisted of 3 ml of liquid of either still water or carbonated water. Swallowing reaction times and challenged swallows were performed four times (two times for carbonated solution and two for still water) in a randomised order.

2.2.7.2 Protocol 2 (Main study)

Subjects attended on three separate days with at least three to four days apart. On each occasion, they received three different solutions (carbonated solutions, non-carbonated solutions (mineral water) and a solution with equal pH acidity (citric acid) in a randomised order. On each visit they were asked to swallow the pharyngeal EMG catheter and then the sensory thresholds were recorded using PES, before performing the swallowing tasks, as in protocol 1. A pair of electrodes was placed on the back of the subject's hand. This was used to deliver an electrical pulse which cued the subject to swallow. Subjects were asked to perform baseline measurements of swallowing behaviour (30 swallows, 10 normal, 10 fast and 10 challenged swallows within a pre-determined time window). Swallowing reaction times and challenged swallows were performed at 15, 30, 45 and 60 minutes after the baseline recordings with each solution (carbonated and citric acid) and still water on each assigned randomised visit. Also, each volunteer was asked to attend the laboratory for an additional 15 minutes to complete the taste questionnaires.

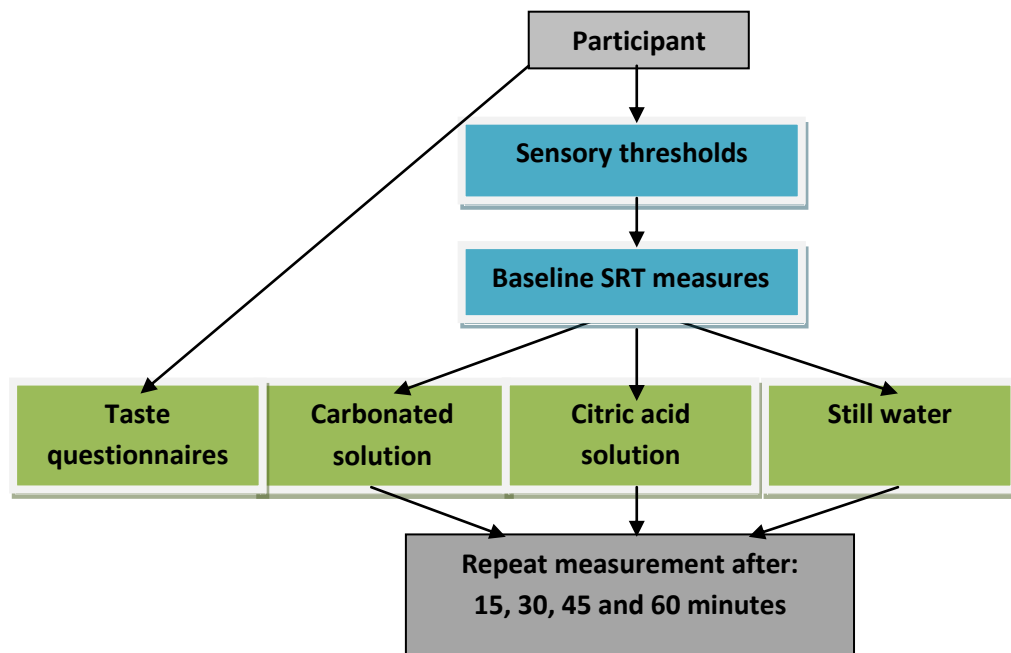


Figure 2.6: The diagram of the main study protocol

2.2.8 Data analysis

2.2.8.1 Protocol 1 (Pilot study)

Twenty-two subjects were involved in this study and data of 20 subjects was used for analysis. The data from two of the subjects was not analysed because technical difficulties occurred during the data capture. The variability of pharyngeal manometry within the two different conditions was assessed using SPSS 16.0 by calculating the intra-class correlation coefficients (ICC) for two runs of still water and two runs of carbonated solution. The data was presented as the mean (\pm SEM) and a Wilcoxon's signed-ranks test was applied to the two-sample designs to see the difference between still water and carbonated solution for each of the three types of swallow (normal, fast and challenged). P value of less than 0.05 was taken as a measure of statistical significance.

2.2.8.2 Protocol 2 (Main study)

Sixteen subjects were involved in this study and data of 12 subjects was used for analysis. The data from four of the subjects was not analysed because there were technical difficulties in obtaining artefact free data. The variability of pharyngeal manometry within the three different conditions was assessed using SPSS 16.0 by calculating the intra-class correlation coefficients (ICC) for five runs of still water, five runs of carbonated solution and five runs of citric acid solution. The data is presented as the mean (\pm SEM) and a Friedman's test was applied to test the repeatability within arms for each solution across five runs. Also, Friedman's test was applied to check difference in distribution between three solutions for each of the three types of swallow (normal, fast and challenged). A significant result followed by Wilcoxon's signed-ranks test which was applied to the three-sample designs to see the difference between still water, carbonated solution and citric acid solution for each of the three types of swallow. Also, a Wilcoxon's test was used to assess the taste intensity difference between still water, carbonated solution and citric acid solution. P value of less than 0.05 was taken as a measure of statistical significance.

2.9 Results

2.9.1 Protocol 1 (Pilot study)

All subjects completed the study without adverse effects, with those whose data were useable comprising seven female and thirteen male. The mean age of these subjects was 25.7 ± 5.87 (mean \pm SEM).

2.9.1.1 Normal Swallowing Reaction Time

All raw data was analysed and the average for each normal swallows run was calculated. The following table (2.1) shows the average and standard error of means for each run of normal swallows.

Swallow Type	Still water (1)	Still water (2)	Carbonated solution (1)	Carbonated solution (2)
Mean(ms)	1967.24	1990.89	2039.44	1998.05
SEM	102.51	110.58	106.64	105.72

Table .2.1: The mean and SEM for the two runs of still water and carbonated solution.

Intra-class correlation (ICC) was calculated to check the variability of the two data sets for normal swallow with still water and carbonated solution using SPSS 16.0. Intra-class correlation for still water is 0.962 and for carbonated solution is 0.953. This indicates a high level of agreement. The means of normal swallows is represented in the following figure (2.7). Wilcoxon's test showed no significant difference between still water and carbonated solutions (Z value=-0.649, p=0.522).

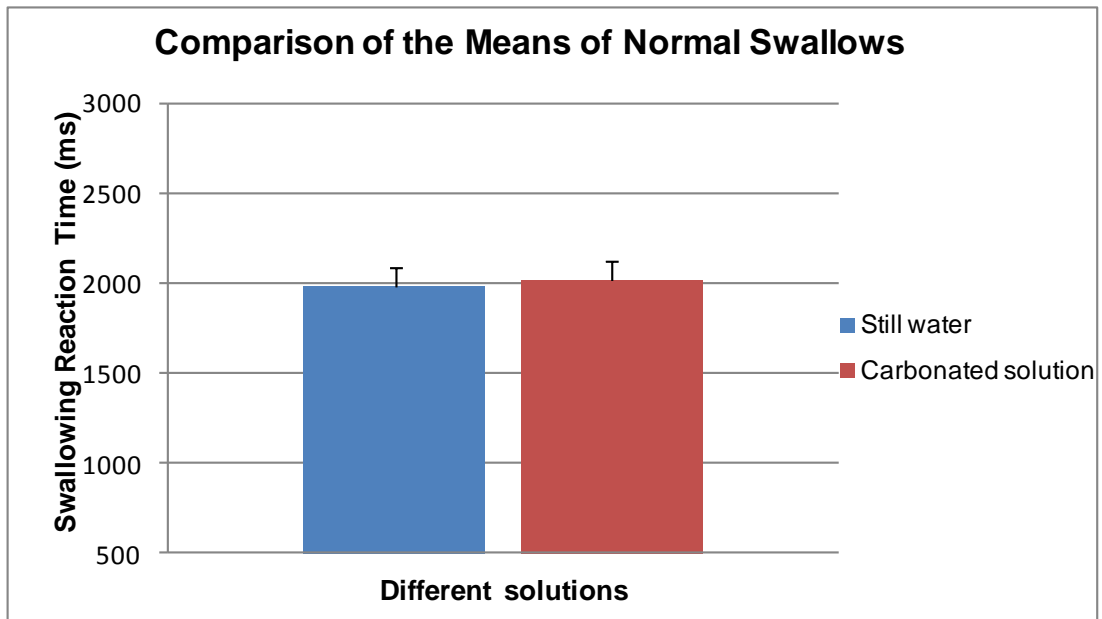


Figure 2.7: The means and SEM of normal swallows for still water and carbonated solution. Wilcoxon’s test showed no significance difference between the two variables ($p=0.522$).

2.9.1.2 Swallowing reaction time of fast swallows

All raw data was analysed and the mean for each fast swallows run was calculated. The intra-class correlation (ICC) was calculated for the two runs, for still water was 0.863 and for carbonated solution was 0.871. These results showed that there was slightly more variability in swallows with a good agreement. The following table (2.2) shows the average and standard error of means for each run of fast swallows.

Swallow Type	Still water (1)	Still water (2)	Carbonated solution (1)	Carbonated solution (2)
Mean(ms)	956.92	849.65	897.77	891.67
SEM	63.8	67.04	69.54	64.9

Table 2.2: The mean and SEM for the two runs of still water and carbonated solution.

Figure (2.8) for fast swallowing showed little difference between still water and carbonated solution. Wilcoxon's test showed no significant difference between the two variables (Z value=-0.447, p=0.648).

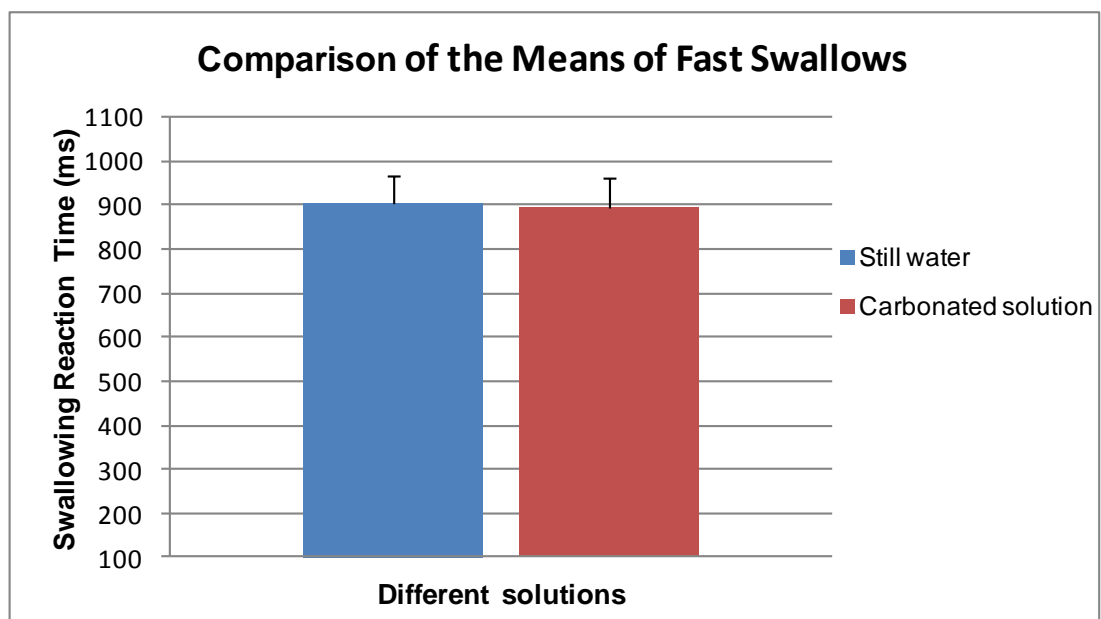


Figure2.8: The means and SEM of fast swallows for still water and carbonated solution.

2.9.1.3 Challenged swallows

The number of successful swallows was recorded and the percentage of correct swallows was then calculated. The following table (2.3) shows the average and standard error of means for each run of challenged swallows.

Swallow Type	Still water (1)	Still water (2)	Carbonated solution (1)	Carbonated solution (2)
Mean	29%	34%	34.5%	44%
SEM	3.24	5.05	4.13	4.5

Table 2.3: The mean and SEM for the two runs of still water and carbonated solution.

Challenged swallow performance for carbonated solutions was better (39.25 %) compared to still water (31.5%) (Figure.2.9). Wilcoxon's test showed a significant difference between two variables (Z value=-2.47, p=0.0237).

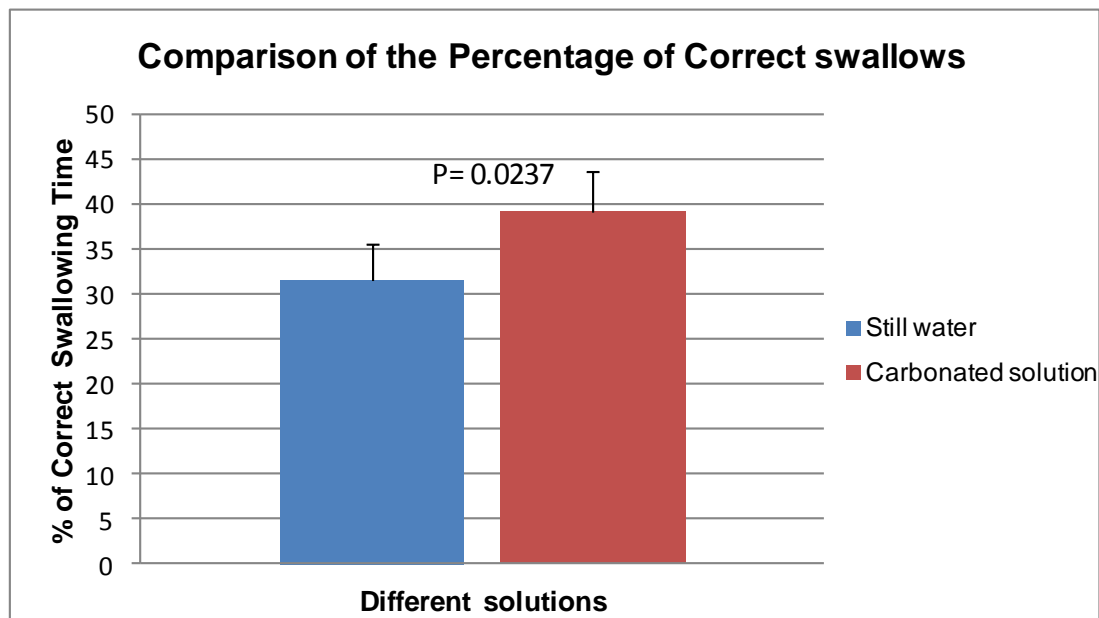


Figure 2.9: The means and SEM of percentage of correct swallows for still water and carbonated solution

2.9.2 Protocol 2

All participants completed the study without any side effects in three different days, with those whose data were useable comprising six male and six female. The mean age of these subjects was 33.92 ± 4.25 (mean \pm SEM).

2.9.2.1 Pharyngeal sensory thresholds

The average of both sensory and tolerance pharyngeal thresholds are represented in table (2.4) below. All participants completed the studies with no adverse events.

Subject NO	Minimum (Sensory) Threshold (mA \pm SEM)	Maximum (pain) Threshold (mA \pm SEM)
1	3.52 ± 1.42	5.01 ± 1.39
2	3.51 ± 1.24	7.07 ± 1.97
3	3.01 ± 0.63	7.71 ± 0.55
4	1.29 ± 0.1	3.95 ± 0.76
5	1.15 ± 0.51	2.72 ± 0.57
6	3.22 ± 0.13	7.25 ± 1.11
7	1.64 ± 0.50	4.98 ± 0.20
8	1.11 ± 0.21	3.86 ± 0.23
9	2.66 ± 0.91	4.14 ± 1.81
10	2.01 ± 0.14	13 ± 1.82
11	2.09 ± 0.39	3.41 ± 0.35
12	1.55 ± 0.16	5.64 ± 0.95

Table 2.4: Average of the pharyngeal sensory and maximum tolerated electrical thresholds, shown in mA with SEM.

The baseline sensory and tolerance thresholds during the three studies were similar for each participant [Friedman test (sensory threshold: Chi squares: 0.667, $p=0.717$, tolerance threshold Chi squares: 3.500, $p=0.174$)]. The minimum (sensory) threshold ranged from 1.11 to 3.52 mA, whereas the maximum (tolerance) threshold ranged from 2.72 to 7.71 mA

2.9.2.2 Normal Swallowing Reaction Time

All raw data was analysed and the grand mean for each normal swallow solution was calculated. The following table (2.5) shows the grand mean and standard error of means for each solution of normal swallows. Also, it shows the mean for each volunteer for the different three solutions.

Swallow Type	Still water	Carbonated solution	Citric acid solution
Volunteer No			
1	1543.48	1908.91	1471.77
2	2034.18	1835.78	1701.92
3	1045.23	1138.95	628.56
4	1665.6	1740	1638.38
5	1589.34	1710.06	1439.74
6	1343.59	1239.67	1368.79
7	1212.06	1328.72	1229.03
8	2610.1	1976.24	2604.56
9	1092.82	1162.52	1232.36
10	1343.58	1787.31	1422.44
11	1727.27	1683.02	1759.81
12	1641.01	1487.67	1485.95
Mean (ms)	1570.69	1583.24	1498.61
SEM	103.86	74.73	113.81

Table 2.5: The response latencies for normal swallowing reaction time. The table includes the mean response latencies in milliseconds (ms) for baseline and four follow-up time-points for each volunteer for the three different solutions and the grand mean and standard error of the normal swallows for each solution.

A) Repeatability dependency of normal Swallowing arms across time (within arms): Using the Friedman's test within each arm (still water, carbonated solution and citric acid solution), for the five time-points, the response latencies for the normal swallowing tasks were similar (still water: Chi squares: 1.133, p=0.889, carbonated solution: Chi squares: 2.733, p=0.603, citric acid solution: Chi squares: 4.655, p=0.325). Each time-point was

compared to the baseline which was performed with the same solution under investigation. In addition, ICCs were calculated to check the variability of the five time points for each solution during the normal swallowing. The result for still water was 0.986, for carbonated solution was 0.942 and for citric acid solution was 0.980. This shows a high level of agreement for each of the three solutions. The following figure (2.10) represents the grand means of normal swallows for the three different solutions.

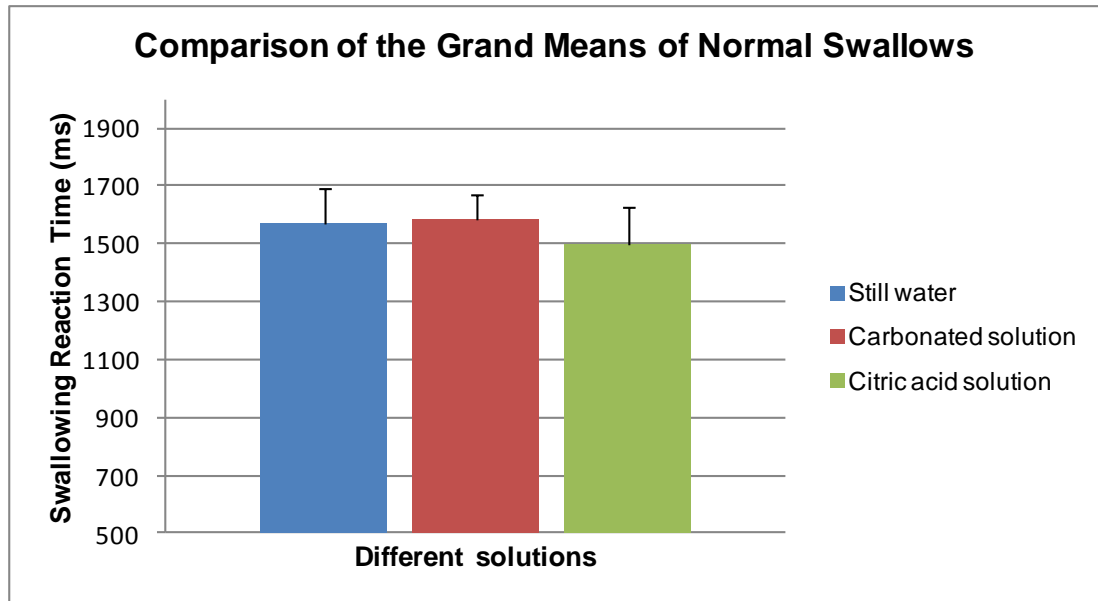


Figure 2.10: Grand means and SEM of normal swallows for still water, carbonated solution and citric acid solution. This figure shows the grand means of response latencies of normal swallows in millisecond for three different solutions, each grand mean calculated from the mean for 12 subjects, and each mean calculated from the response latency of the five time points for each volunteers.

B) Difference in normal swallowing reaction tasks latencies between arms: Although the mean swallowing time for the carbonated solution appeared visibly higher compared to still water and the citric acid solution, a Friedman’s test showed no significant difference between three solutions (Chi squares: 0.913, $p= 0.633$.)

2.9.2.3 Fast Swallowing Reaction Time

The grand mean for each fast swallowing task within each arm was calculated after the analysis of all the raw data. The following table (2.6) shows the mean for each volunteer for the different three solutions and the grand mean and standard error of the means for each solution of fast swallows.

Swallow Type	Still water	Carbonated solution	Citric acid solution
Volunteers No			
1	1128.3	1097.54	1140.49
2	1637.71	1462.67	1376.82
3	652.61	630.98	557.06
4	876.53	863.22	918.03
5	877.26	936.25	936.47
6	963.15	564.89	643.54
7	1059.34	1157.88	926.59
8	903.43	938.01	953.77
9	782.54	872.23	824.38
10	1119.2	1230.93	999.15
11	543.47	731.52	695.92
12	732.77	807.31	844.91
Mean (ms)	939.69	941.12	901.43
SEM	70.95	64.75	55.08

Table 2.6: The response latencies for fast swallowing reaction time. The table includes mean response latencies in milliseconds (ms) of the baseline and follow-up time points for each volunteer for each of the different three solutions, the grand mean and standard error of means of the fast swallows for each solution.

A) Repeatability dependency of fast swallowing arms across time (within arms): Using the Friedman's test, the distribution of the response latencies in the five time-points with the three different solutions were not different (still water: Chi squares: 3.400, $p=0.493$, carbonated solution: Chi squares: 6.933, $p=0.139$, citric solution: Chi squares: 4.655,

p=0.325). Intra-class correlation for still water swallow reaction time was 0.984, for carbonated solution was 0.990 and for citric acid solution was 0.966. These results indicate a high agreement for three solutions. The figure (2.11) illustrates the grand means of fast swallows in three different solutions.

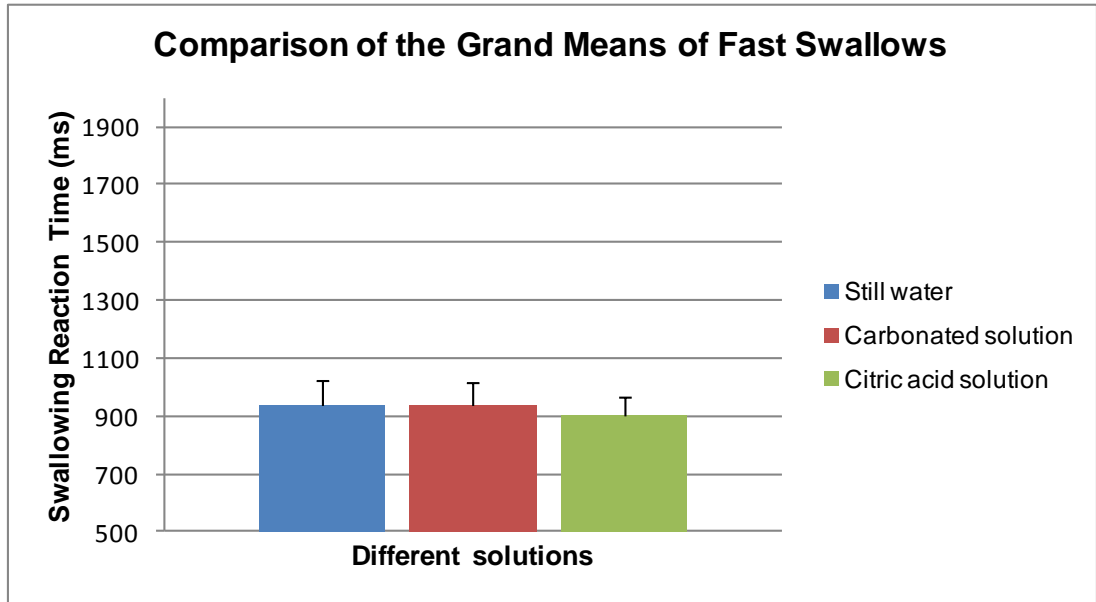


Figure 2.11: The grand means and SEM of fast swallows for still water, carbonated solution and citric acid solution. This figure shows the grand means of response latencies of fast swallows in milliseconds for three different solutions, each grand mean calculated from the mean for 12 subjects and each mean calculated from the response latency of the five time points for each volunteer.

B) Difference in fast swallowing reaction tasks latencies between arms: Friedman's test showed no significant difference between the mean of three solutions (Chi squares: 0.500, p=0.779).

2.9.2.4 Challenged Swallows

The number of successful swallows for each run (10 swallows) was recorded and the percentage of the grand mean (average of five repeated runs) of correct swallows for each volunteer and for each solution was then calculated.

The following table (2.7) shows the grand mean percentage of correct swallows and standard error of means for each solution of challenged swallows. Also it shows the mean percentage of correct swallows for each volunteer for the different three solutions.

Swallow Type	Still water	Carbonated solution	Citric acid solution
Volunteers NO			
1	50	46	48
2	30	28	20
3	30	54	24
4	60	56	56
5	80	52	42
6	40	60	28
7	40	44	52
8	50	50	12
9	60	62	64
10	0	28	34
11	50	44	38
12	60	44	52
Mean (%)	31.98	47.33	39.17
SEM	5.05	2.72	3.99

Table 2.7: The percentage of correct swallows for challenged swallowing reaction time. The table shows the means of the percentage of correct swallows (over five repeated runs) for each volunteer with each of the three different solutions and the grand mean percentage of correct swallows and standard error of percentage of correct swallows for each solution.

A). *Repeatability dependency of challenged swallowing arms across time (within arms):*

Using the Friedman's test, the number of successful swallows for the five time-points for each of the three solutions (still water, carbonated solution and citric acid solution) for the challenged swallowing tasks were similar (still water: Chi squares: 3.136, $p=0.698$, carbonated solution: Chi squares: 7.165, $p=0.127$, citric acid solution: Chi squares: 2.744, $p=0.602$). Intra-class correlation for still water swallow reaction time was 0.732, for carbonated solution was 0.599 and for citric acid solution was 0.808. These results indicate a good agreement for three solutions.

B). *Difference in challenged swallowing reaction tasks latencies between arms:*

The mean swallowing percentage of successful swallows for the carbonated solution was higher than that of still water and citric acid solution. The using of Friedman's test showed a significant difference between the mean of three solutions (Chi squares: 6.054, $p=0.048$) But the Wilcoxon's test showed a significant difference between the mean of carbonated and still water, but no significant difference between carbonated solution and citric acid solution: carbonated solution versus still water (Z value= -2.044, $p=0.041$), citric acid solution versus still water (Z , value=-0.045, $P= 0.964$) and citric acid solution versus carbonated solution (Z value=-1.293, $P= 0.196$ }).

The following figure (2.12) represented the grand means of percentage of correct swallowing time in different solutions.

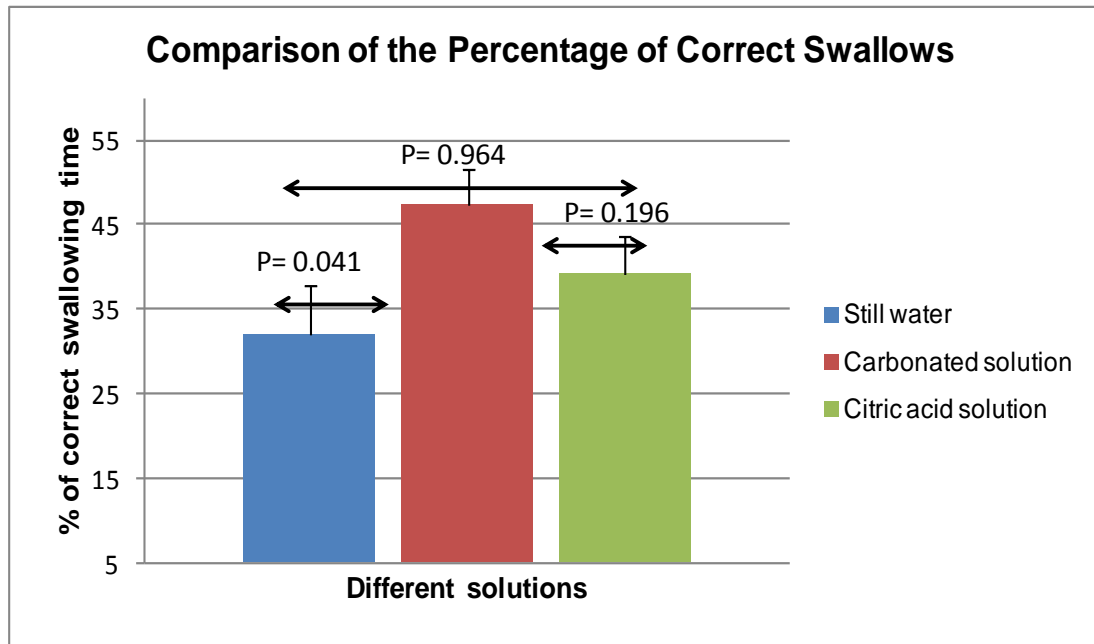


Figure 2.12: The grand means and SEM of challenged swallows for still water, carbonated solution and citric acid solution. This figure shows the grand means of percentages of correct swallows for three different solutions, each grand mean calculated from the mean for 12 subjects and each mean calculated from the number of correct swallows of the five time points for each volunteer.

2.9.2.5 Intensity and difference rating

The mean intensity rating for each taste on the intensity labelled Magnitude Scale (Green et al, 1996) are shown in Figure (2.13), with the highest intensity for carbonated solution followed by citric acid solution. The mean intensity rating for water was closed to zero.

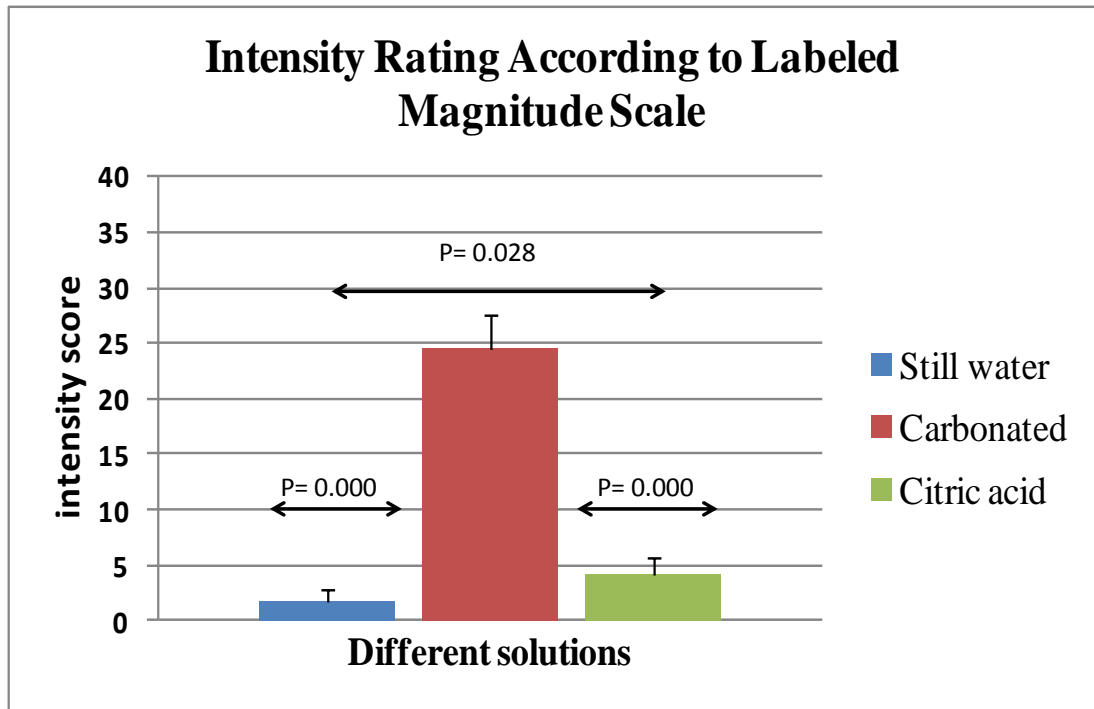


Figure 2.13: Mean intensity scores for each different stimulus according to Labelled Magnitude Scale. This figure shows the mean and SEM of intensity for 16 subjects according to the Green Scale, which ranges from 0 to 100. Carbonated liquid swallows had the highest intensity rate (24.53) and the control, still water, showed a mean intensity rating closed to 0.

Wilcoxon’s test showed a significant difference between the intensity score of carbonated solution and the other two solutions (water and citric acid) and significant difference between still water and citric acid solution {carbonated solution versus still water (Z value= -3.517, $p=0.000$), carbonated solution versus citric acid solution (Z value= -3.520, $P=0.000$) and citric acid solution versus still water (Z value=-2.201, $P= 0.028$)}.

Figure (2.14) shows the results from the “difference” scale. The carbonated solution was mostly perceived as “extremely different” compared to still water and citric acid solution and all participants perceived the difference between carbonation and other solutions ranging from extreme to mild. However, twelve subjects commented that they did not perceived any difference between water and the citric acid solution, while three subjects scored this difference as very mild and one subject found only a mild difference.

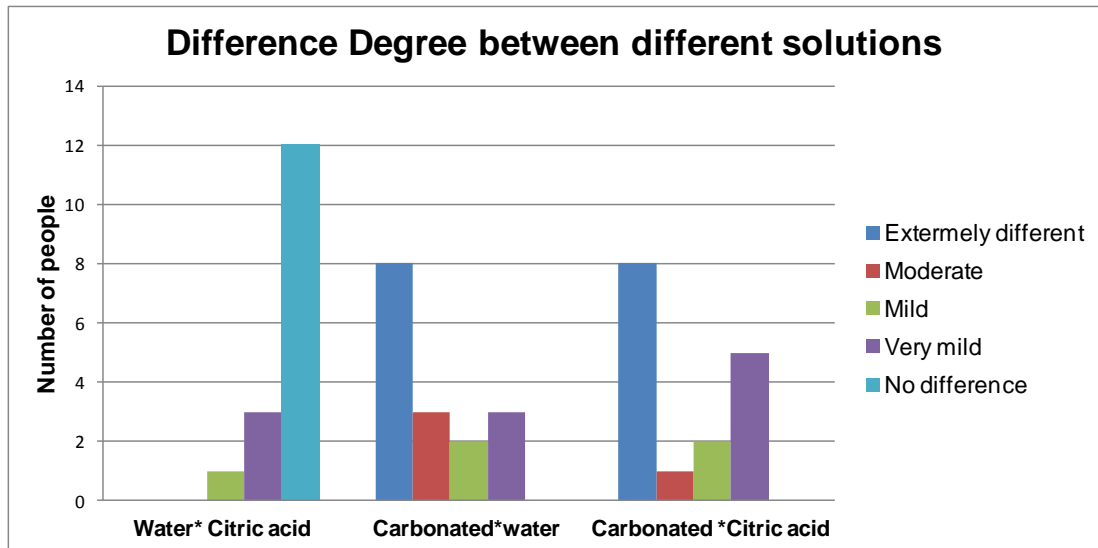


Figure 2.14: The difference degree for each solution compared to other solutions according to the Difference Taste Chart. The difference between the carbonated solution and citric acid solution and between carbonated solution and still water was very obvious compared to the difference between the citric acid solution and still water.

2.10 Discussion

2.10.1 Pilot study

This study examined the effect of carbonated solutions on swallowing performance with a swallowing reaction time paradigm compared to non-carbonated solutions. Our hypothesis was that carbonation would alter swallowing performance.

2.10.1.1 Normal swallowing reaction time

My data showed that there was no significant difference for the swallowing latencies of normal swallowing between still water and carbonated solutions. This finding is somewhat against the finding presented by Bulow et al [125] and Nixon et al [126] who showed that carbonated liquids decreased pharyngeal transit time. However, the methodologies used in these studies and current study were different; Bulow and Nixon studies using VFS and this study using manometry measurements. Therefore, it is possible that pharyngeal transit time and any change measured with pharyngeal pressure (using the current methodology)

are not directly correlated. In contrast, these findings are supported by Parker et al [208] who reported a slowing of water drinking with small volume resulting in a good control of the bolus in dysphasic patients. The inter-subject correlations of both water and carbonated swallowing runs were in close agreement so the rate of swallows was dependent mainly on the participant rather than other factors.

2.10.1.2 Fast swallowing reaction time

There was no significant difference between the swallowing latencies for fast swallowing between still water and carbonated solutions. The ICC between the two water runs and the two carbonated runs was strong; therefore, the rate of swallows was dependent on the participant more than other factors studied in this assessment. This finding supports the likelihood that this test is a sensitive measure of performance.

2.10.1.3 Challenged swallows

Subjects performed better with carbonated solution compared to still water during challenged swallows. This finding may be due to conversion of CO₂ to carbonic acid in carbonated water leading to activation of lingual nociceptors which excite the trigeminal neurons involved in signalling oral irritation [112]. However, other causes should be considered such as the acidity in the carbonated solution, the taste of the solution, temperature and inter-subject differences. The pH level of the carbonated solution was measured before the study and kept constant over the study time at 4.1. This acidity of the carbonated solution could lead to its enhanced performance compared to still water, which had a pH of 7.3. As mentioned before, a sour taste in Pelletier & Lawless et al [94] study caused increased muscle contraction, decreased aspiration and penetration and facilitated uniform swallows.

This pilot study acted as a prelude to designing the main study. Taste perception should be taken into consideration. Therefore, in the main study, taste questionnaires were given, regarding the difference in taste between the liquids and the intensity of the taste. Further, because of the uncertainty of whether effects were being driven by Ph/chemical vs. physical properties of carbonation, citric acid solution was used with the same pH level of the carbonated solution.

2.10.2 Main study

This study examined the effects of carbonated solutions on swallowing performance compared to non-carbonated solutions (still water and citric acid). Our hypothesis was that the carbonated taste would alter swallowing performance.

The use of Friedman's test across different time points (within arms) for each solution (still water, carbonated and citric acid solutions) and for each time task (normal, fast, challenged) showed no significant difference. Therefore, the repetition of tasks did not change the result and this means that there is no role for training or learning effect on our results.

2.10.2.1 Normal swallowing reaction time

There was no significant difference between swallowing latencies for the normal swallows between the three different solutions (still water, carbonated solution and citric acid solution). The mean swallow reaction time with carbonated solution is slightly longer compared to still water and citric acid solution swallows.

This result is consistent with the pilot study and again against the findings presented by Bulow et al [125] and Nixon et al [126] that show that carbonated liquids decrease pharyngeal transit time. However, there may be some explanations which contrast our study and Bulow study: Firstly, videofluoroscopy was used for measuring PTT which allowed Bulow et al. to get a different measurement of PTT compared to our methodology. The methodology used in this study was designed to measure reaction time. In addition, Bulow's participants swallowed the carbonated bolus immediately to prevent gas loss, however, other bolus stimuli were held in the mouth until the participant was directed to swallow. But in our study, all the liquid bolus swallowing occurred immediately after the electric cue. Furthermore, the viscosities of the boluses tested were different from our experiment, as they were all blended with barium sulphate powder. In addition, another study was done by Krival et al. [129] which supports our findings. They compared the carbonated water with the thin, nectar-like, and honey-like thickened liquids and reported that there was no significant differences for the PTT between carbonated and thin liquids but there was a significant shorter PTT difference between the carbonated water and other thick liquids.

Another possibility that might explain why the SRT was not shorter with carbonated solution compared to other solutions may be related to the “noxious taste” of the carbonated solution. Our questionnaires regarding taste intensity shows that participants perceived the carbonated solution as extremely different compared to citric acid solution and still water. Also, they did not perceive a big difference between the water and citric acid solution. This indicates that the results are not mediated by the effects of the flavour of “acidic” liquids.

2.10.2.2 Fast swallowing reaction time

There was no significant difference between swallowing latencies for the fast swallows between the three different solutions (still water, carbonated solution and citric acid solution).

The agreement between the baseline and four follow-up time points for still water, carbonated solution and citric acid solution was very strong. This finding supports the sensitivity of swallowing reaction task to show any differences in performance.

2.10.2.3 Challenged swallows

Subjects performed better with carbonated solution compared to still water and citric acid solution during challenged swallows. This finding may be due to many reasons. First point is that carbonation has the ability to change the texture of liquid. According to McLellan et al and Yau and McDaniel [117, 118], there is a significant increase in sourness and a significant decline in sweetness in the presence of carbonation compared to sour and sweet tastes in aqueous solution. Also, there may be some increase in the saltiness of the stimuli in the presence of carbonation, even with the lowest concentration of NaCl being presented (0.086 M). Further, there might be a mixed effect on the bitterness of quinine was observed by Cometto-Muniz et al [119]. Furthermore, Beverly et al [120] reported that there is a small increase in the total taste intensity but there is a high increase in the quality of sweet and salty taste after carbonation, when they examined the total taste intensity of sweetness, sourness, bitterness and their binary combinations with or without carbonation. In addition, according to Miura et al [124] sour taste, carbonated and cold stimuli increased the high frequency content of swallowing submental sEMG leading to more organization of submental muscle activity that provided a more efferent input to the NTS. Another point is

that the conversion of CO₂ to carbonic acid in carbonated water leading to activation of lingual nociceptors which excite the trigeminal neurons involved in signalling oral irritation [112]. This finding is supported by the results of Bulow et al [125] and, Nixon et al [126] that reported that carbonated solutions caused a reduction in pharyngeal retention, penetration and aspiration to the airway.

Krival et al [129] investigated the difference between three kinds of liquids: water, carbonation (in Schweppes[®] Club Brew) and carbonation + gingerol (in Reed's Extra Ginger Brew) on peak linguapalatal pressure, linguapalatal pressure duration and adjustments in the development of linguapalatal pressure in 20 healthy volunteers. They reported that carbonation and gingerol had a greater influence on neuromotor activity compared to water during the oral stage of swallowing. Another study conducted by Newman et al [127] examined the effects of carbonation on the swallowing physiology of 24 infants and children with dysphagia. The authors reported that carbonated thin liquids (compared to non-carbonated liquids) caused a significant reduction in the incidence of spill-over, delayed pharyngeal response and laryngeal penetration and they recommended the use of carbonation as a therapeutic option in children with dysphagia. However, they did not find any significant effect on the risk of aspiration, which might be due to the small number who aspirated (n=7). Moreover, according to Sdravou et al [128], carbonated thin liquids significantly reduced the penetration and aspiration incidence in a small group of stroke patients with neurogenic dysphagia compared to non-carbonated thin liquids.

This finding is against the finding presented by Ding et al [123] that showed strong muscle contraction for the salt bolus and did not find any effect of carbonation on the amplitude and duration of submental sEMG. This difference may be due to different experimental conditions, such as the use of small volume boluses (5ml stimulus bolus), so the carbon dioxide bubbles may have disappeared very quickly before the participant performed his/her swallows.

Moreover, my results are not in keeping with some other published data regarding the effect of citric acid solution. For instance, Pelletier and Lawless [95] found a significant reduction in penetration and aspiration with citric acid due to increase gustatory and chemesthetic stimulation to the NTS. Logemann et al [94] reported a shortening of swallowing onset time, oral transit time, pharyngeal transit time and reduction in aspiration

in stroke patients when a sour bolus was used. Another study was done by Steele et al. [209], who examined two different citric acid concentrations and moderately sweet and sweet-sour solutions in 16 healthy volunteers using electromagnetic articulography to test tongue movement during swallowing. They reported an increase in amplitude and peak velocity of tongue movements during swallowing with a high citric acid concentration solution compared to other solutions. Also, Chee et al [210] reported that sour, sweet and salty tastes caused a decrease in swallowing speed compared to water swallows.

In contrast, Hamdy et al [211] examined the effect of a sour bolus versus water in groups of young and old healthy volunteers and in patients with dysphagia using a timed water test. They reported no effect of a sour bolus alone in both groups, except when it was combined with cold, where a slower swallowing speed was observed. This result corroborates the results by Palmar et al [212], who compared a sour bolus (50% citric acid) to water at room temperature using intramuscular EMG. They found the strong submental muscles contraction with the sour bolus but they did not find any difference in muscle contraction duration, muscle activation time and swallow onset time. The effect of citric acid remains controversial.

In conclusion, my results show that the effect of carbonated solution is greater than citric acid solution. Therefore, the effect of carbonation solution (“referred to its’ fizz sensation”) may provide a strong stimulation to oropharyngeal receptors resulting in an increased number of correct swallows and consequently a presumed effect on better protecting the upper airway. Therefore, the results provide evidence for the benefits of using carbonated solution on swallowing performance and provide the basis for the next study, which looks into the effects of carbonation on the swallowing cortical network using transcranial magnetic stimulation.

CHAPTER 3

Effects of Carbonation on Excitability in the Human Pharyngeal Motor Cortex

3.1 Introduction

In chapter two, the effect of three different solutions (carbonated solution, mineral water and citric acid) on swallowing behaviour were investigated in healthy volunteers. The pilot study showed that there was a significant increase in the percentage of successful swallows with carbonated water in the challenge swallowing task compared to still water. This finding was supported by the result of the main study which also showed that the percentage of successful challenged swallows with carbonated solution was significantly increased compared to still water, while there was no significant difference between swallowing latencies for the normal and fast swallows for the three different solutions. Therefore, it seems appropriate to investigate the effect of these solutions on the cortical excitability using the well-established technique of TMS.

The cerebral cortex plays an important role in the initiation and regulation of the swallowing function, which is affected by the oropharyngeal sensation. Sensory feedback from the different bolus characteristics plays an important role in swallowing process [213]. There is much evidence to support the role of sensory stimulation in changing motor cortical excitability. For example, according to Hamdy et al [84], cranial nerves stimulation led to facilitation of the motor pathways of the cortical swallowing area. Also, topical anaesthesia of oropharyngeal mucosa led to laryngeal aspiration and oropharyngeal dysphagia in healthy volunteers [214], indicating that the absence or disruption of the sensory input for the mucosal receptors causes disturbances in the voluntary swallowing which may lead to dysphagia.

Furthermore, multiple bolus characteristics, such as taste, touch, pressure and temperature may lead to initiation of pharyngeal swallowing by exciting different types of sensory receptors in the oral cavity [215]. In addition, according to Power et al [216], there was an altering effect of human oral stimulation on pharyngeal corticobulbar excitability and swallowing behaviour. Intriguingly, they found that high frequency (5 Hz) faucial pillar (FP) produced cortical inhibition and had lengthened swallowing response time whereas low frequency (0.2 Hz) stimulation caused a modest late increase in cortical excitability without change in swallowing behaviour. All these observations give guidance for the therapeutic role of oral stimulation in treatment of dysphagic patients.

TMS application has shown that the recovery after stroke is associated with an increase in cortical excitability in the undamaged hemisphere [203]. Moreover, previous studies in the literature have shown a strong relationship between taste and swallowing cortical excitability. For example, bitter and sweet tastes have detectable effects on corticobulbar excitability [204]. Also combined thermal (cold) and chemical modification water (citrus) led to altered swallowing behaviour in both healthy population and patients with cerebral injuries [211]. Swallowing initiation and pharyngeal swallowing response are faster with sour bolus in dysphagic patients [94]. Furthermore, using carbonated solutions led to reduction of aspiration/penetration into airways, reduced pharyngeal retention and shortening in the pharyngeal transit time (the time that the bolus is transferred from oral pharynx to oesophagus) compared to other liquids (thin and thick) [125]. This means that, carbonated solutions may contribute to aiding rehabilitation of dysphagia after stroke. However, the underlying mechanisms for these effects remain unclear.

Therefore, the aim of this study was to investigate the effects of carbonated solutions on cortical swallowing function compared to non-carbonated solutions in healthy volunteers using non-invasive neurostimulation technique, Transcranial Magnetic Stimulation (TMS).

3.2 Methods

3.2.1 Participants

Healthy volunteers were recruited through advertisements placed around Salford Royal Hospital and through a departmental database of volunteers who have expressed the interest to participate in future research.

Sixteen healthy volunteers (8 female, mean age 33 ± 3.65 (\pm SEM) years, age range from 21 to 60) participated in this study. All subjects were in good health and received the information sheet prior to obtaining written informed consent. Also they had the opportunity to discuss any queries before and during the study. The volunteers were invited to attend the laboratory prior to starting the study in order to familiarise themselves with the equipment. All participants met the study inclusion criteria. Exclusion criteria included:

a history of epilepsy, previous brain or throat surgery, cardiac pacemaker, prior history of swallowing difficulty, neurological disease, pregnancy, the presence of metal implants in eyes or head, or intake of any medication which acts on the central nervous system or gastrointestinal tract. The subjects attended on three separate visits, with each study taking approximately 2-2.5 hours and with at least 3-4 days between each visit. The approval for this study was granted by Great Manchester (central North West 7) Research Ethic Committee (10/H1008/61) and all studies were done in the clinical laboratory of the Gastrointestinal Physiology department at Salford Royal NHS Foundation.

3.2.2 Sample size

Power calculations performed by the Medical Statistics department at Salford Royal Foundation Trust using information obtained from Chapter 2 indicated that the number of participants required to allow appropriate statistical power would be 12, hence 16 participants were written into the protocol in order to obtain 12 full data sets with expected subject dropout. This sample would be sufficient for obtaining pilot data in order to illustrate any significant effects of an intervention according to results from previous work with TMS at the department of GI Sciences.

3.2.3 Procedures

The methods utilized for PES and different boluses for mineral water, citric acid solution and carbonated solution swallowing for these studies remained identical to that used in Chapter 2, (Chapter two, page 76 and 78)

3.2.3.1 Transcranial Magnetic Stimulation

TMS was delivered using a Magstim 200 stimulator and a 70 mm figure of eight coil (Magstim Company, Whitland, Wales). Subjects were seated on a comfortable chair and a surgical cap was placed over their head so that the cranial vertex could be marked. Magnetic stimulation was discharged over both sides to identify the site producing the

greatest response on each hemisphere for the pharyngeal musculature and hand motor cortex which was also marked over the head. For pharyngeal motor evoked potentials, each hemisphere was identified. The stronger pharyngeal projection (termed as the “dominant hemispheric” hotspot) was the hemispheric site evoking the greatest pharyngeal response at the lowest threshold and the weaker pharyngeal projection (“non dominant” hotspot) was the hemispheric hotspot for pharyngeal musculature on the contralateral cortex. Resting motor threshold (rMT) is defined as the minimum intensity of stimulator output to achieve motor evoked potentials (MEP) of at least 20 μ V in five out of ten trials. MEP amplitude was assessed by giving TMS at 110% and 120% of pharyngeal motor threshold, with 20 stimuli was being given at these intensities and repeated over both hemispheres after the intervention.

For thenar (hand), resting motor threshold (rMT) is defined as the minimum intensity of stimulator output to achieve motor evoked potentials (MEP) of at least 50 μ V in five out of ten trials. Motor evoked potentials were recorded using disposable electrodes placed over the hand opposite the side of the brain evoking the largest pharyngeal response and then the optimal site and motor threshold for thenar stimulation was identified as described above.

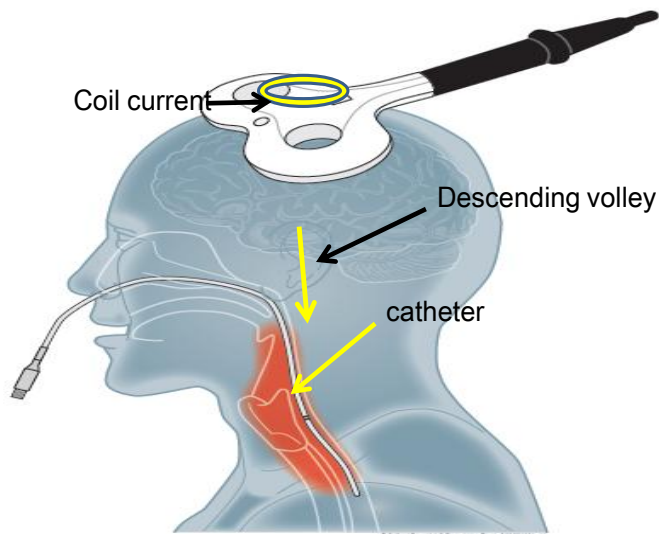


Figure 3.1: Transcranial magnetic stimulation (TMS). TMS is a safe and non-invasive technique that uses a magnetic pulse to excite the neurons of the cortex on the area of interest. When a magnetic coil (70 mm diameter, shaped in a figure eight) was activated the electrical current running through the coil induced a rapidly change of magnetic field. This magnetic field penetrated the skull and the scalp and generated a secondary volley of potentials. With the electrodes housed in the catheter placed in the pharynx I can record the typical EMG response and I term change in cortical excitability, the change in the peak-to- peak amplitude of the EMG response.

3.2.3.2 Electromyographic (EMG) measurements

Subjects were required to swallow a 3.2 mm diameter intraluminal catheter (Gaeltec Ltd, Dunvegan, Isle of Skye) contains a pair of bipolar platinum ring electrodes to recorded electromyographic (EMG) traces from the pharynx. According to subjects' preference, the catheter was inserted either orally or nasally. The position of the catheter was adjusted upon visual inspection of subject EMG traces on the computer screen. An earth wire was connected to a skin electrode sited over the upper part of one of the sternocleidomastoid muscles in the neck. This catheter was connected via a preamplifier (Cambridge Electronic Design Ltd, Cambridge), amplifier (CED 1902) and interface (CED 1401) to a laboratory computer which was using Signal Application Program (Cambridge Electronic

Design Ltd, Cambridge), which allowed real time visualization and recording of the traces. This had filters set at 200Hz to 2 kHz and allowed a sampling rate of 4-8 kHz. Signal software (Cambridge Electronic Design Ltd) was used for analysis of the amplitude and latencies of the traces.

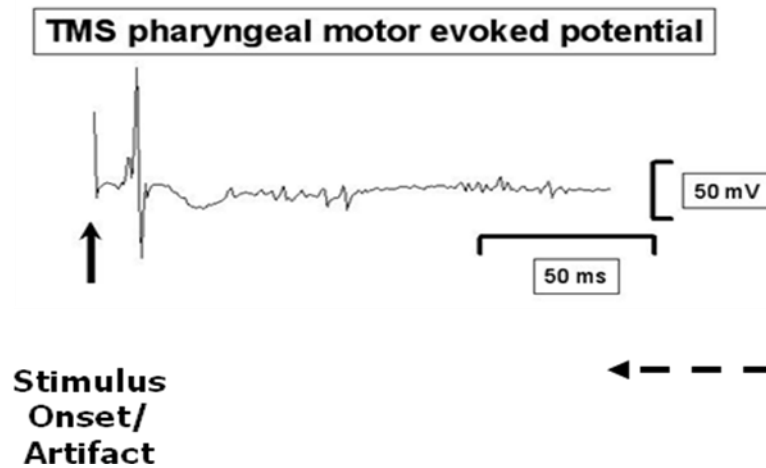


Figure 3.2: Shows typical pharyngeal electromyography (EMG). An Intraluminal catheter with a pair of electrodes was utilized to record motor evoked potentials (MEPs) from the pharynx. Each EMG activity in each individual muscle consists of phasic discharges in the form of a burst of EMG spikes whose range depends on the muscle.

3.2.3.3 Thenar EMG measurements

Disposable skin electrodes were placed over the thenar eminence muscle opposite to the dominant hemisphere (greatest pharyngeal response) to record the thenar MEPs (TMEPs). An earth wire was connected to a skin electrode sited over the wrist on the anterior surface. The same equipment and software which was used for the pharyngeal measurements were used for the thenar MEPs.

3.2.4 Experimental Procedures

3.2.4.1 Protocol

Subjects attended on three separate days with at least 3-4 days apart. On each occasion they received one of the following three different solutions in a single-blinded randomised manner: carbonated solutions, non-carbonated solutions (mineral water) and solutions with equal pH acidity (citric acid). On each visit they sat in a comfortable chair and a pharyngeal EMG catheter was passed down according to their preference either orally (13-16 cm) or nasally (15-17 cm). The electrodes of the catheter were positioned in the pharynx and the correct placement was verified, dependent on the baseline pharyngeal EMG. A disposable surgical cap was put on the head and taped. Then the cranial vertex was identified and marked on the cap according to Jasper [217]. With this catheter, I was able to record sensory and tolerance thresholds with PES and baseline and follow-up MEPs were obtained using single pulse TMS as detailed above.

They then received either the carbonated solutions, the non-carbonated solutions (mineral water) or the solutions with weak acidity. The water swallowing tasks comprised delivering 3 ml boluses intra-orally via a plastic catheter connected to a hand held syringe, one every 15 seconds (40 swallows). A power-point presentation was presented to them on a personal computer to direct them to swallow. Following the interventions the neurophysiological measurements were repeated. They received single pulse TMS immediately, and after 15, 30, 45 and 60 minutes, to measure any change in MEP amplitude and latency.

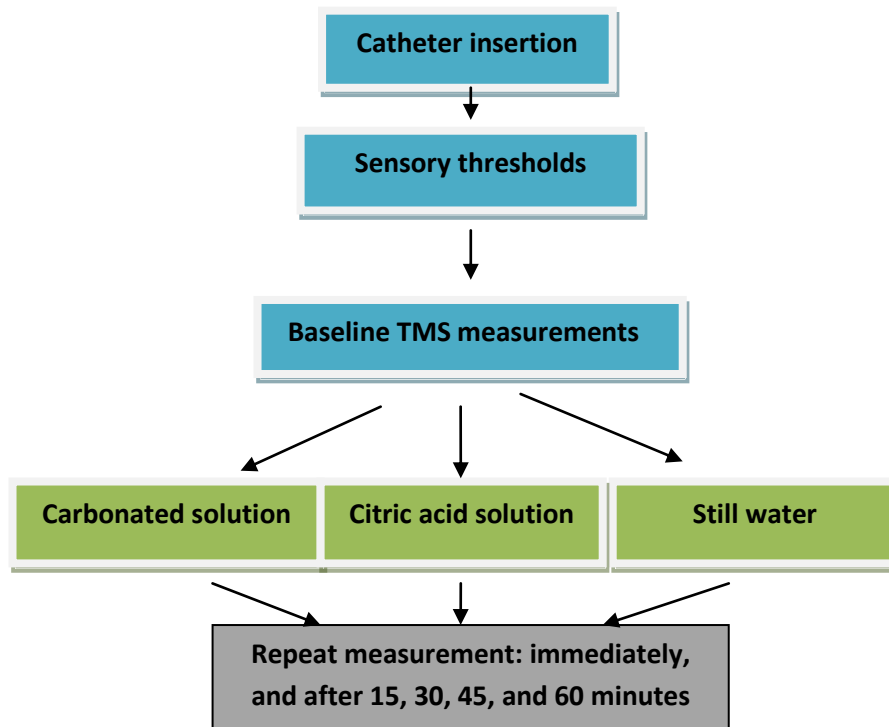


Figure 3.3: Diagram of the study protocol

3.2.5 Data analysis

The peak-to-peak amplitude of MEPs evoked by magnetic stimulation was used as a measure of motor cortex excitability. Signal software was used to review individual MEPs in micro Volts (μV) and to identify the amplitude of each trace. Ten EMG traces for each hemispheric hotspot: dominant (stronger projection), non-dominant (weaker projection) and thenar muscle were used for analysis. The average amplitude and latencies were calculated for each intensity and each time-interval. The average of both intensities (MT+10% and MT+20%) for each time-interval was calculated. After that, grand mean amplitudes for each time point were calculated for each individual and normalised to the baseline and were shown as a percentage of change from baseline.

3.2.6 Statistical Methods

SPSS 16 (SPSS Inc., Chicago, USA) was used for the statistical analysis of the normalised data. Comparison was made between the different interventional groups for the cortical excitability changes for each hemispheric hotspot. The GLM repeated measures ANOVA was used, including each time point except the baseline. The data are expressed as mean (\pm SEM) unless stated otherwise and P values of less than 0.05 were taken as a measure of statistical significance. Across the different study days, the reproducibility of baseline raw data was investigated using non-parametric tests (Friedman's test).

3.3 Result

3.3.1 Pharyngeal sensory threshold

The average of both sensory and tolerance pharyngeal threshold are represented in table (3.1) below.

Subject NO	Minimum (Sensory) Threshold (mA ± SEM)	Maximum (Pain) Threshold (mA ± SEM)
1	1.74 ± 0.44	16.73 ± 1.54
2	2.17 ± 1.3	5.66 ± 3.51
3	4.92 ± 1.28	7.13 ± 1.84
4	7.75 ± 1.46	22.33 ± 2.02
5	7.04 ± 2.38	23.32 ± 2.96
6	1.71 ± 0.27	4.62 ± 0.34
7	1.32 ± 0.16	2.54 ± 0.1
8	1.54 ± 0.02	9.67 ± 1.15
9	3.96 ± 1.0	8.76 ± 3.12
10	1.74 ± 0.64	9.73 ± 2.60
11	1.63 ± 0.76	10.53 ± 3.36
12	2.39 ± 0.78	5.93 ± 0.45
13	2.17 ± 0.46	4.87 ± 1.26
14	1.98 ± 0.58	10.07 ± 3.85
15	2.55 ± 0.21	3.6 ± 0.27
16	4.86 ± 0.7	12.35 ± 1.42

Table 3.1: Average of the pharyngeal sensory and maximum tolerated electrical thresholds for each subject (mA and SEM).

The baseline sensory and tolerance thresholds during the three studies were similar for each participant (Friedman test: sensory threshold: Chi squares: 4.500, p=0.105, tolerance threshold Chi squares: 1.625, p=0.444). The minimum (sensory) threshold ranged from 1.3 to 7.8 mA whereas the maximum (tolerance) threshold ranged from 2.5 to 23.3 mA

3.3.2 Localisation of cortical representations

Right hemispheric dominance was observed in 12 out of 16 participants. The hemispheric site evoking the greatest pharyngeal response at the lowest threshold (hotspot) was

between 5.1 ± 0.5 cm (mean \pm SD) anterior to the vertex and 2.3 ± 0.4 cm (mean \pm SD) lateral to the midline for the right hemisphere. In subjects with left hemispheric dominance, the optimal site for stimulation was between 4.7 ± 1.1 cm (mean \pm SD) anterior to vertex and 2.5 ± 0.4 cm lateral to the midline. The optimal sites for stimulation are shown in Figure (3.4).

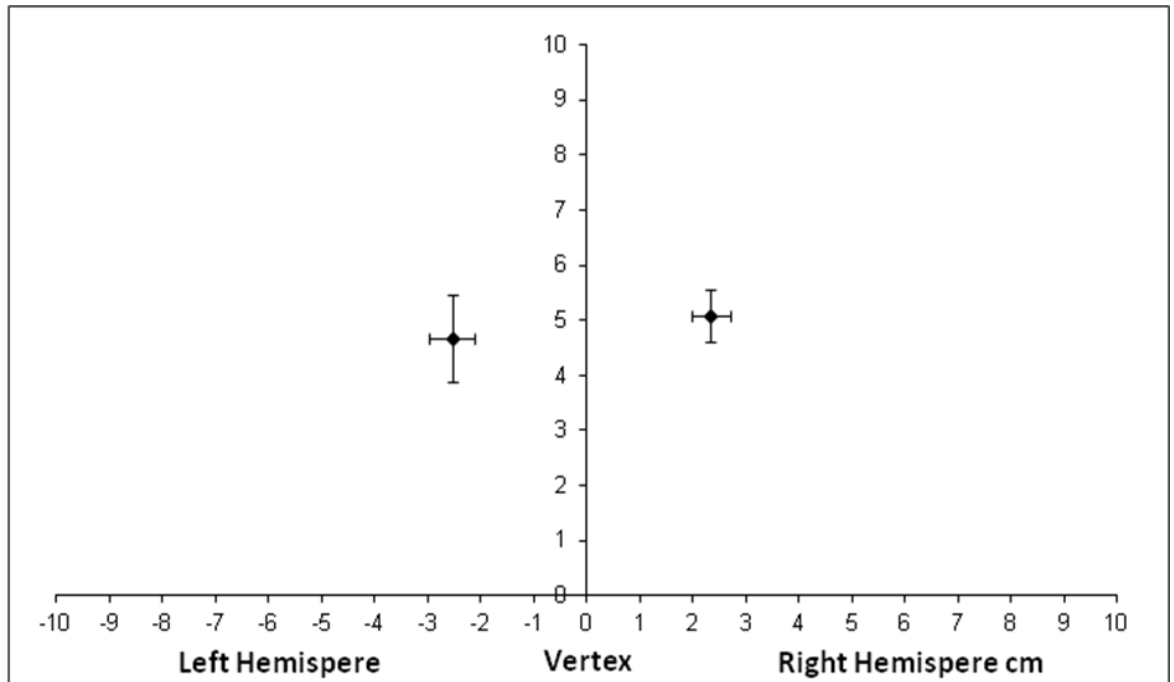


Figure 3.4: Plot illustrating the sites of maximum PMEPs in response to TMS. The right and left hemispheric sites for stimulation are shown in this figure, with horizontal and vertical distance measured in centimetres from the vertex (mean \pm SD) for each hemisphere

The average TMS intensity used for elicitation of PMEPs (Pharyngeal EMGs after the application of single TMS pulses) from the dominant hemispheric was $61.9 \pm 1.2\%$ (mean \pm SEM) whereas from the non-dominant hemisphere it was $63.5 \pm 1.2\%$ (mean \pm SEM). The average of TMS intensity from the thenar representation was $39.5 \pm 1.2\%$ (mean \pm SEM).

3.3.3 Amplitude

PMEPs traces from one volunteer before (baseline) and after intervention with still water, carbonated solution and citric acid solution for the dominant hemisphere are shown in Figure (3.5).

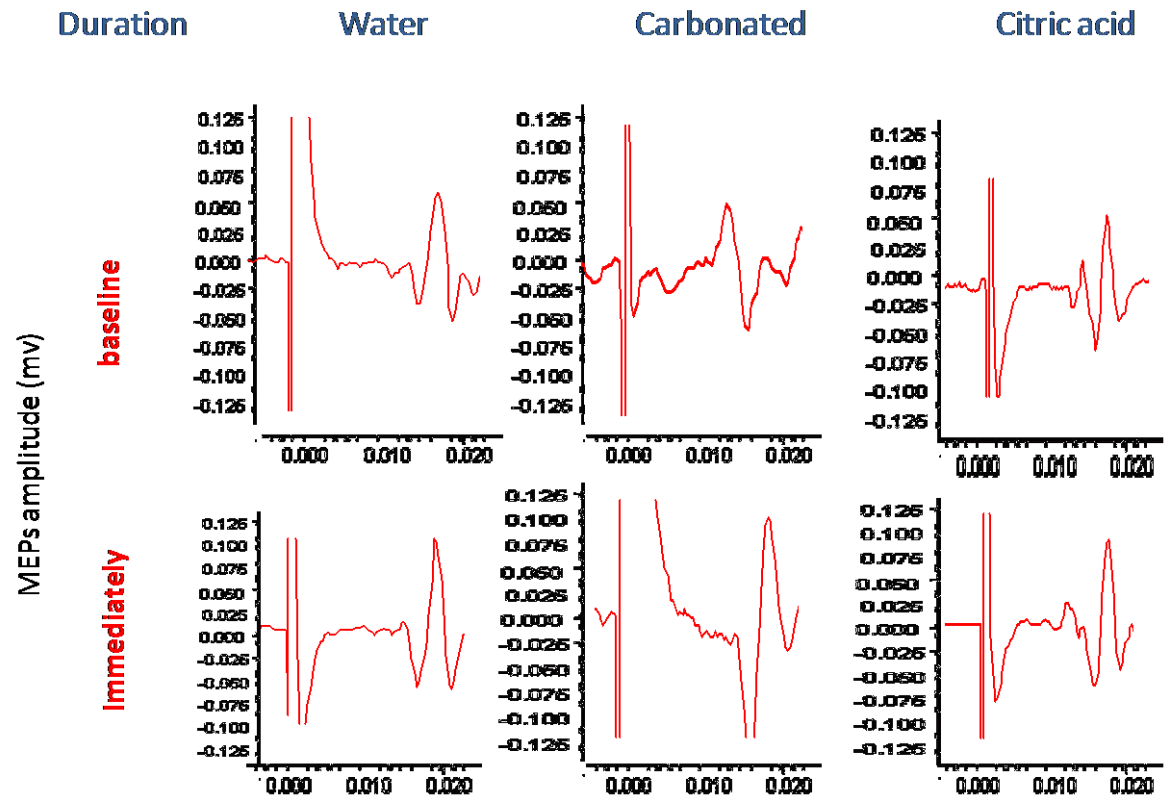


Figure 3.5: represents amplitudes of PMEPs of the dominant hemisphere projection at baseline and immediately after still water, carbonated solution and citric acid solution swallowing intervention.

Baseline cortical excitability prior to the swallowing interventions with the different solutions was similar across all six arms for each site: the dominant and non dominant pharyngeal representation and thenar cortical representation (Friedman's test, dominant cortical representation: Chi squares: 2.000, $p=0.3$, non-dominant cortical representation: Chi squares: 1.625, $p=0.444$, thenar cortical representation: Chi squares: 0.875, $p=0.646$).

Three-way ANOVA was used to analyse data with factors of INTERVENTION (carbonated, water and citric acid), TIME (immediately, 15 minutes, 30 minutes, 45 minutes and 60 minutes) and SITE (dominant hemisphere, non-dominant hemisphere and thenar) then separate two-way ANOVAs were used to analyse data with factors of INTERVENTION (carbonated, water and citric acid) and TIME (immediately, 15 minutes, 30 minutes, 45 minutes and 60 minutes) and SITE (dominant hemisphere, non-dominant hemisphere and thenar).

3.3.3.1 Pharynx

The group mean percentage changes in PMEPs amplitude of dominant pharyngeal motor projection from the baseline for the 16 subjects after the different types of swallowing intervention at the different time points are shown in Figure (3.6). Three-way ANOVA showed a significant effect of INTERVENTION*TIME*SITE interaction ($F(1, 15) = 4.639, P = 0.048$). Two-way ANOVA for dominant hemisphere showed a significant effect of interaction TIME*INTERVENTION on the percentage changes of PMEPs for carbonated solution swallowing compared to water and citric acid solutions ($F(1, 15) = 4.755, P = 0.046$) and a significant effect of INTERVENTION ($F(1, 15) = 9.440, p = 0.008$) was also observed. There was an increase in excitability of the dominant MEPS following the carbonated solution intervention for all the time-points with the highest values from the baseline at 45 minutes ($62.12 \pm 21.31\%$) and at 60 minutes ($61.31 \pm 26.31\%$).

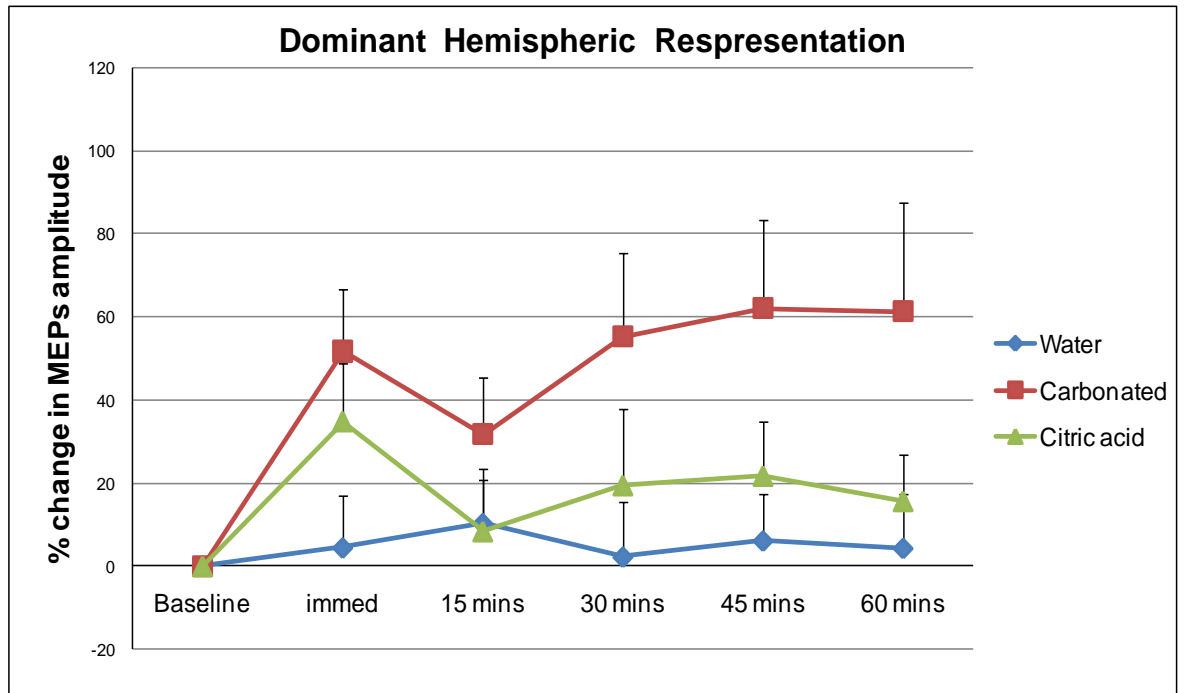


Figure 3.6: Mean percentage change in PMEPs amplitudes following three different solutions intervention. All interventions show variable increases in cortical excitability from their baselines and there was a significant difference for carbonated solution compared to water and citric acid solutions ($P=0.008$).

In contrast to dominant pharyngeal MEPs, there was not a significant TIME*INTERVENTION interaction for carbonated solution compared to water and citric acid solutions in the non-dominant pharyngeal projection. Also, there was no significant effect of INTERVENTION (carbonated solution) on this weaker projection compared to other solutions ($F(1, 15) = 2.273, P=0.152$). Across all time-points, the carbonated solutions visibly increased MEPs at 30 minutes ($48.73\% \pm 17.61$) and 60 minutes ($55.47\% \pm 25.54$) compared to water and citric acid solutions in non-dominant hemisphere.

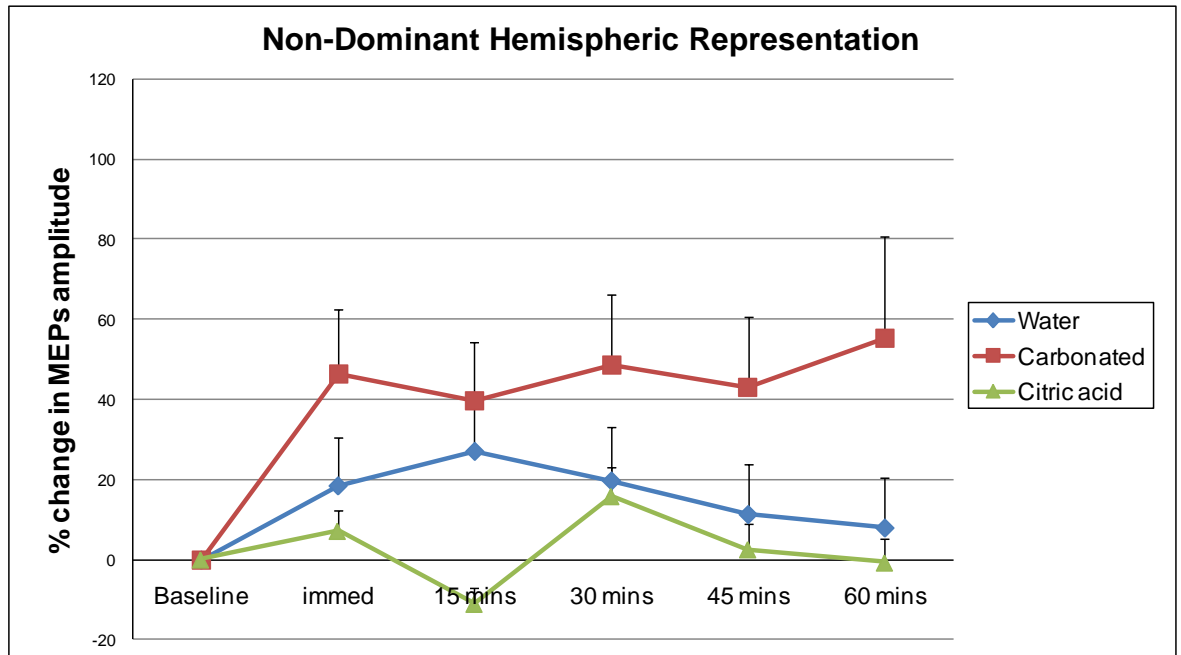


Figure 3.7: Mean percentage change in PMEPs amplitudes following three different solutions intervention. All interventions showed an increase in cortical excitability from their baselines except citric acid at 15 minutes. Carbonated solution showed higher increase than water and citric solutions but there were no significant changes ($P=0.152$).

3.3.3.2 Thenar

Also there was no significant INTERVENTION*TIME interaction for thenar representation, two-way ANOVA showed no effect for the different interventions on cortical excitability ($F(1, 15) = 0.279$, $P = 0.609$)

3.3.4 Latencies

The mean pharyngeal response latencies at the baseline and each time-point for the different swallowing intervention solutions for each hemispheric hotspot and for the thenar representation hotspot were shown in Table (A.1) in appendix A. There was no significant difference on two-way ANOVA for intervention, time, and different sites.

3.4 Discussion

This study investigated the effect of carbonated solutions on corticobulbar swallowing excitability compared to non-carbonated solutions. My hypothesis was that carbonation would influence the excitability of swallowing pathways, which would provide new data on how taste/sensory bolus stimuli interacts with human swallowing neurophysiology. The results from the current study in healthy volunteers showed that the swallowing of carbonated solutions can increase cortical excitability of pharyngeal projections compared to still water or weak acid solution (citric acid) swallowing although this appeared to be restricted to the dominant stronger projection. Such a finding merits further discussion.

As mentioned in previous chapters, swallowing is a complex process and to trigger a swallow response, it has been proposed that stimuli need to excite many peripheral sensory receptors in a sequential manner to activate the neurons in the NTS [218, 219]. To trigger taste perception, chemical stimuli are required to activate taste buds and stimulate neurons and interneurons in the NTS [95]; hence taste may play an important role in swallowing performance given the additional inputs arriving at the NTS [220]. According to Dessirier et al [112], activation of lingual noiceptors in the oral cavity is chemogenic in origin via the formation of carbonic acid and not solely mechanical in origin due to stimulation of mechanoreceptors by bursting CO₂ bubbles. This could imply that carbonated solutions can evoke more sensory input to the solitary tract nucleus than simple flavoured solutions.

Furthermore, carbonated solutions swallows led to reduction of aspiration/penetration into airways, reduced pharyngeal retention and the shortening in the pharyngeal transit time (PTT) compared to other liquids (thin and thick) [125]. This shortening in PTT is thought to be due to stimulation of faucial isthmus pillar receptors in the mouth by carbonic acid molecules which elicit more afferent impulses to NTS in midbrain leading to rapid pharyngeal swallowing [221]. However, there is another study which used cold tactile stimulation or topical anesthesia and concluded that normal pharyngeal swallowing was not effected by these stimulation. This may suggest that the pharyngeal swallowing response is not dependent on mechanical and cold stimulation of faucial pillar receptors [222].

Previous studies in the literature have shown a strong relationship between taste and swallowing cortical excitability. Afferent fibres of the cranial nerves involved in the swallowing process terminate in trigeminal spinal nuclei and the NTS. These afferent fibres are able to modulate motor neuron and interneuron circuitry as well as higher centres in the cerebral cortex. Importantly, different taste stimuli can excite or inhibit interneuron in the NTS [220]. For example, bitter and sweet tastes have detectable effects on corticobulbar excitability [204]. These stimuli showed reduction in PMEPs with TMS due to reduction of activity in the NTS which may cause a reduction in the activity of cortical swallowing centres [204]. Therefore, I would hypothesise that the carbonated solution causes an increase in sensory impulses to NTS that will lead to increased cortical excitability. This expectation was supported by the results in my study which showed an increase in cortical excitability for carbonated solutions compared to other solutions.

Despite the limited published data for the effects of carbonated solutions on swallowing compared to citric acid, my findings support the result of Bulow et al [125] who reported that the carbonated solution caused shortening of pharyngeal transit times, reduced pharyngeal retention, reduced penetration and aspiration to airway compared to thin liquids. In addition, Nixon [126] studied the effects of carbonated liquid and thin barium liquid on swallowing in four groups of patients/subjects under videofluoroscopy. Group one included patients who aspirated on thin liquids. Group two included patients who required modified and non-oral feeding. Group three included patients who required non-oral feeding. Group four included healthy volunteers. Nixon reported a shortening of oral transit time (OTT), pharyngeal transit times and reduction in aspiration in groups 1 and 2.

Furthermore, Krival et al [129] measured the difference between three types of liquid: water, carbonation and carbonation + gingerol (ginger beer) in peak lingual-palatal pressure, lingual-palatal pressure duration, and adjustments in the development of linguopalatal pressure in 20 healthy volunteers. They reported that carbonation and gingerol produced greater neuromotor activity compared to water during the oral stage of swallowing.

Moreover, Sdravou et al [128] reported a significant decrease in penetration and aspiration with carbonated thin liquid (5ml and 10 ml) compared to non-carbonated thin liquid in oropharyngeal swallowing in seventeen neurogenic dysphagic patients. Also, Miura et al

[124], studied the effects of taste, carbonation and cold stimulus on the power frequency content of submental sEMG in continuous swallowing of 60 ml solutions in healthy volunteers. They reported an increase in the high-frequency content of swallowing submental sEMG for sour taste, carbonated liquid and cold stimuli. This was speculated to generate more organized activation of submental muscle which provided more adequate and effective afferent inputs into the nucleus tractus solitarius (NTS) in brainstem.

These findings are contrary to the findings presented by Ding et al [123] who studied the effects of several tastants (sweet, salt, lemon, and water) on submental muscle contractions in healthy volunteers and found strong muscle contraction for the salt bolus and no effect of carbonated solution on the amplitude and duration of submental sEMG. These differences may be due to different experimental methodological conditions as I described earlier in Chapter two.

In addition, Cometto-Muniz et al and Yau and McDaniel [118, 119] investigated the taste intensity rating of tastants (sour and sweet) in aqueous solution before and after adding carbonation. They reported a significant increase of sourness of acid stimuli and decline in sweet taste in presence of carbonation. Moreover, Cometto-Muniz et al [119] examined the effect of carbonation on the tastes of salt and bitter solutions. They observed some salt enhancement with low concentration of NaCl (0.086 M). Also, Beverly [120] examined the total taste intensity of sweetness, saltiness, sourness, bitterness and their binary combinations with and without added carbonation. She found the rating of taste intensity slightly increased in the presence of carbonation whereas she observed a dramatic change in the perceived qualities of salt and sweet tastes. These taste intensity studies support my result regarding taste intensity of carbonation which I discussed in the previous chapter.

My findings also stand against many published data regarding the effect of citric acid solution. For example, Logemann et al [94] examined the effect of sour bolus on pharyngeal swallowing in two groups of dysphagic patients (19 who had dysphagia due to stroke and 8 who had dysphagia due to other neurological diseases) using videofluoroscopy. The results showed significant improvement in the onset of oral swallowing in all subjects, reducing oral transit time, pharyngeal transit time, pharyngeal delay time in the stroke group and reduction of oral transit time, pharyngeal transit time, aspiration in the other neurogenic cases. In addition, Chee et al [210] reported that sour,

sweet and salt tastes caused a decrease in swallowing speed compared to water swallows. Moreover, Pelletier and Lawless [95] compared the effects of two different concentrations of citric acid in water (2.7% citric acid in water, 1.11% citric acid in water) and 8% sucrose in water on swallowing in 11 neurogenic dysphagic patients. They found a significant reduction in aspiration and penetration with high citric acid concentration compared to low citric acid concentration and sucrose solution. Another study was done by Steele et al [209] examined movement of tongue during swallowing using electromagnetic articulography in 16 healthy volunteers. They used two different citric acid concentrations and moderate sweet and sweet-sour solutions. The results showed an increase in amplitude and peak velocity of tongue movements during swallowing with high citric acid concentration solution compared to other solutions.

On other hand, Hamdy et al [211] examined the effect of sour bolus versus water using a timed water test in both young and old healthy volunteers and in patients with dysphagia. They reported no effect of sour bolus alone in both groups except that when it was combined with cold, the speed of swallowing volume was reduced. This result corroborates the results of Palmer et al study [223] that compared sour bolus (50% citric acid) versus water at room temperature using intramuscular EMG. They found strong submental muscles contraction with sour bolus but they did not find any difference in muscle contraction duration, muscle activation time and swallow onset time. The effect of citric acid is therefore controversial between the different published papers. To try to overcome some of the problems, I used a equi-pH solution (citric acid) compared to carbonation to try to resolve the pH vs physicochemical properties of the bolus on swallowing neurophysiology. My results support the contention that physical and chemical bolus properties are additive to altering brain swallowing function.

With this in mind, given that Hamdy et al [203], showed that an increase in cortical excitability in an undamaged hemisphere is associated with recovery after stroke accompanied by an improvement in behaviour function, it places more importance on my findings. I would therefore contend that an increase in cortical excitability in my study corroborates the results from the pilot study and previous main study regarding the effect of carbonated liquid on swallowing behaviour compared to other solutions. Carbonated solutions may contribute to rehabilitation of dysphagia after stroke by increasing sensory stimulation of oral receptors, which may lead to improve swallowing performance.

CHAPTER 4

Carbonation reverses the inhibitory effects of a virtual lesion in the human pharyngeal motor cortex

4.1 Introduction

In chapter two, a significant increase in the percentage of successful challenged swallows was observed with carbonated solutions compared to still water and citric acid solution. Moreover, swallowing of carbonated solution increased the cortical excitability of the stronger pharyngeal representation compared to still water and citric acid solution (chapter three). Neurophysiological effects on the pharyngeal motor cortex coupled with behavioural changes were investigated in healthy volunteers but the brain effect of carbonated water in dysphagic stroke patients has not been previously investigated. My interesting findings in the previous two chapters may be different if the participants were dysphagic patients.

Sensorimotor lesions in hemispheric stroke patients result in swallowing difficulties for many reasons – such as inability to form a cohesive bolus and inability to perform voluntary movements inside the oral cavity due to paresis, swallowing apraxia and somatosensory impairment [17]. In addition, pharyngeal swallowing impairment increases the risk of aspiration and pharyngeal retention because of a decrease in pharyngeal motor function and poor sensory awareness in the laryngopharyngeal area [224, 225]. All of these complications can impair safe swallowing. Clearly, difficulty in swallowing (dysphagia) in stroke patients is a result of the impaired motor functions of the swallowing muscles as well as decreased sensory awareness in the oral cavity and laryngopharyngeal area, but this also raises challenges in terms of how to study swallowing safely in an experimental setting in this diseased population.

Based on the results from previous chapters, I hypothesised that swallowing carbonated water can increase the sensory input to the CNS, which translated in my experiments into an increase in both cortical excitability and percentage of successful challenged swallows. Therefore, it would be interesting to investigate whether the effects of swallowing carbonated solution, previously seen in healthy volunteers, could also be observed in dysphagic stroke patients. Prior to including stroke patients, which for the reasons given above are likely to be a challenging group to investigate, I would like to explore the effects of carbonation in a more controlled setting using a novel technique called “focal cortical suppression” or virtual lesion [189, 226, 227].

Focal cortical suppression or a virtual lesion is a technique that allows dysfunction to be evaluated in the healthy brain, which would give me the chance to study the effect of carbonated solution in a more controlled environment. Recently, it was reported that the application can produce inhibitory effects on the swallowing motor cortex. Using 1 Hz rTMS (600 single pulses in total) to the dominant swallowing motor cortex in healthy volunteers for 10 minutes, focal suppression (decrease in MEP amplitude) was observed immediately and continuously for up to 45 minutes after application [189]. This technique has been used previously to test the effects of neurorehabilitative techniques such as paired associative stimulation (PAS) [228], pharyngeal electrical stimulation (PES) [229] and high frequency rTMS [191].

Therefore, the aim of the current project was to investigate whether the swallowing of carbonated water could reverse the inhibitory effects of a virtual lesion to the pharyngeal motor cortex compared to still water and saliva swallowing, as a prelude to any studies in stroke patients.

4.2 Methods

4.2.1 Participants

Healthy volunteers were recruited through adverts placed around Salford Royal Hospital and through a departmental database of volunteers who have expressed an interest in participating in future research.

Sixteen healthy volunteers (seven female, mean age 33 years, age range from 21 to 61) participated in this study. All subjects were in good health and received the information sheet prior to obtaining written informed consent. They also had the opportunity to discuss any queries before and during the study. The volunteers were invited to attend the laboratory prior to starting the study in order to familiarise themselves with the equipment. All participants met the study inclusion criteria. Exclusion criteria included: a history of epilepsy, previous brain or throat surgery, cardiac pacemaker, prior history of swallowing difficulty, neurological disease, pregnancy, the presence of metal implants in eyes or head,

or intake of any medication which acts on the central nervous system or gastrointestinal tract. The subjects attended on three separate visits, with each study taking approximately 2–2.5 hours and with at least 3–4 days between each visit. The approval for this study was granted by Great Manchester (Central North West 7) Research Ethic Committee (10/H1008/61) and all studies were done in the clinical laboratory of the Gastrointestinal Physiology department at Salford Royal NHS Foundation.

4.2.2 Sample size

Power calculations performed by the Medical Statistics department at Salford Royal Foundation Trust using information obtained from Chapter 2 indicated that the number of participants required to allow appropriate statistical power would be 12, hence 16 participants were written into the protocol in order to obtain 12 full data sets with expected subject dropout. This sample would be sufficient for obtaining pilot data in order to illustrate any significant effects of an intervention according to results from previous work with TMS at the department of GI Sciences.

4.2.3 Procedures

The previous methodology, which utilised PES, TMS, pharyngeal EMG, thenar EMG and different boluses for mineral water and carbonated solution swallowing, remained identical for these studies {Chapter two, page (76,78), and chapter three, page (108,110,111) }.

4.2.3.1 Repetitive Transcranial Magnetic Stimulation (rTMS)

The virtual lesion (focal cortical suppression) was delivered over the dominant pharyngeal motor cortex (site of the lowest resting motor threshold over pharyngeal primary motor cortex) by giving 1 Hz rTMS at up to 120% of pharyngeal resting motor threshold (limited to a maximum of 100% of stimulator output) for 10 minutes [189]. A Magstim super rapid stimulator (Magstim Company, Wales) was used to deliver trains of stimulation via a

figure of eight coil, with a maximum output of 1.8 Tesla, run via computer software “Magstim Rapid Session” (Magstim Company, Whitland, Wales, UK).

4.2.4 Experimental procedures

4.2.4.1 Protocol

Subjects attended on three separate days at least 3–4 days apart. On each of the three occasions they received one of two different solutions to swallow in a single-blinded randomised manner – carbonated solution or non-carbonated solution (mineral water) – and on the other occasion swallowed their own saliva.

On each visit they sat in a comfortable chair and a pharyngeal EMG catheter was passed down according to their preference either orally (13-16 cm) or nasally (15-17 cm). The electrodes of the catheter were positioned in the pharynx and the correct placement was verified done dependent on the baseline pharyngeal EMG. A disposable surgical cap was put on the head and taped. Then the cranial vertex was identified and marked on the cap according to Jasper [217]. With this catheter, I was able to record sensory and tolerance thresholds with PES and baseline and follow-up MEPs were obtained using single pulse TMS as described in the previous chapter.

After that, the focal region of cortical suppression was created using 10 minutes of 1 Hz rTMS over the dominant pharyngeal motor cortex. They then received either the carbonated solution or non-carbonated solution (mineral water), or performed saliva swallows for 10 minutes as the intervention. The non-saliva swallowing tasks comprised delivering 3 ml boluses intra-orally via a plastic catheter connected to a hand-held syringe, every 15 seconds (40 swallows). A PowerPoint presentation was presented to them on a personal computer to direct them to swallow the liquid given. Following the interventions, the neurophysiological measurements were repeated, and they received a single pulse TMS immediately, then at 15, 30, 45 and 60 minutes to measure any change in MEP amplitude and latency.

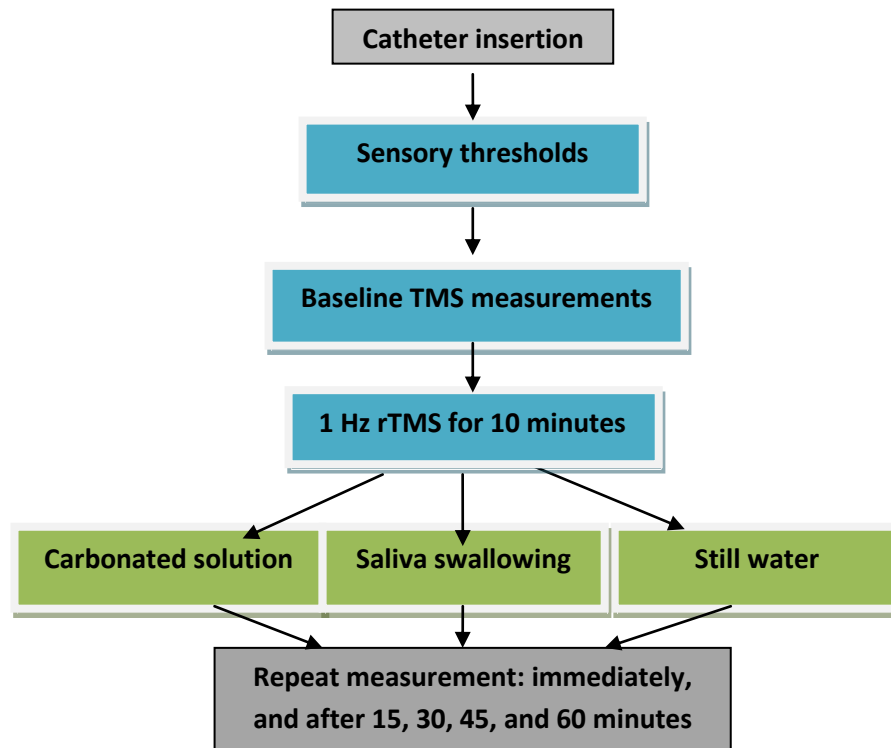


Figure 4.1: Diagram of the virtual lesion study protocol

4.2.5 Data analysis

The peak-to-peak amplitude of MEPs evoked by magnetic stimulation was used as a measure of motor cortex excitability. Signal software was used to review individual MEPs in microvolts (μV) and to identify the amplitude of each trace. Ten EMG traces for each hemispheric hotspot – dominant (stronger projection), non-dominant (weaker projection) and thenar muscle – were used for analysis. The average amplitude and latencies were calculated for each intensity and each time interval. The average of both intensities (MT+10% and MT+20%) for each time interval was calculated. After that, grand mean amplitudes for each time point were calculated for each individual; these were normalised to the baseline and were shown as a percentage change from baseline.

4.2.6 Statistical methods

SPSS 16 (SPSS Inc., Chicago, USA) was used for the statistical analysis of the normalised data. Comparisons were made between the different interventional groups for the cortical excitability changes for each hemispheric hotspot. The GLM repeated measures ANOVA was used, including each time point except the baseline. The data were expressed as mean (\pm SEM) unless stated otherwise and p values of less than 0.05 were taken as a measure of statistical significance. Across the different study days, the reproducibility of baseline raw data was investigated using non-parametric tests (Friedman's test).

4.3 Results

Studies were performed with no reported adverse incidents except for one subject suffering a possible provoked syncopal episode; this was fully investigated medically, but no cause was found. The subject remained well after investigation but, in view of the incident, this subject's data was removed from the full analysis. A second subject's data was also excluded because of incomplete participation and therefore non-completion of the full protocol. Therefore data are reported in fourteen completed subjects.

4.3.1 Pharyngeal sensory threshold

The mean sensory and tolerance pharyngeal thresholds are represented in table (4.1) below.

Subject No	Minimum (Sensory) Threshold (mA ± SEM)	Maximum (Tolerance) Threshold (mA ± SEM)
1	4.67 ± 0.48	13.38 ± 3.72
2	3.6 ± 0.49	6.62 ± 0.193
3	4.13 ± 1.15	15.79 ± 2.65
4	1.4 ± 0.52	3.97 ± 2.02
5	2.39 ± 0.48	4.11 ± 0.76
6	1.76 ± 0.39	5.75 ± 0.72
7	5.31 ± 0.82	11.21 ± 0.10
8	3.53 ± 0.56	31.14 ± 1.28
9	10.12 ± 2.37	15.21 ± 5.35
10	5.24 ± 1.73	6.99 ± 0.98
11	1.7 ± 0.42	8.19 ± 0.53
12	3.7 ± 0.93	5.34 ± 0.87
13	2.91 ± 0.50	6.26 ± 1.6
14	1.4 ± 0.51	3.99 ± 2.02

Table 4.1: Average of the pharyngeal sensory and maximum tolerated electrical thresholds for each subject (mA and SEM)

The baseline sensory and tolerance thresholds during the three studies were similar for each participant (Friedman test: sensory threshold: Chi squares: 0.298, p=0.862, tolerance threshold Chi squares: 0.298, p=0.862). The minimum (sensory) threshold ranged from 1.4 to 10.12 mA whereas the maximum (tolerance) threshold ranged from 4.0 to 31.14 mA.

4.3.2 Localisation of cortical representations

PMEPs were recorded in the fourteen subjects who reported no adverse incidents. Right hemispheric dominance was observed in eight of the fourteen participants. The hemispheric site evoking the greatest pharyngeal response at the lowest thresholds (hotspot) was between 5.7 ± 0.8 cm (mean \pm SD) anterior to the vertex and 3.8 ± 0.7 cm (mean \pm SD) lateral to the midline for the right hemisphere. For the subjects with left hemispheric dominance, the optimal site for stimulation was between 5.6 ± 1.0 cm (mean \pm SD) anterior to the vertex and 3.9 ± 0.6 cm lateral to the midline. The optimal sites for stimulation are shown in Figure (4.2).

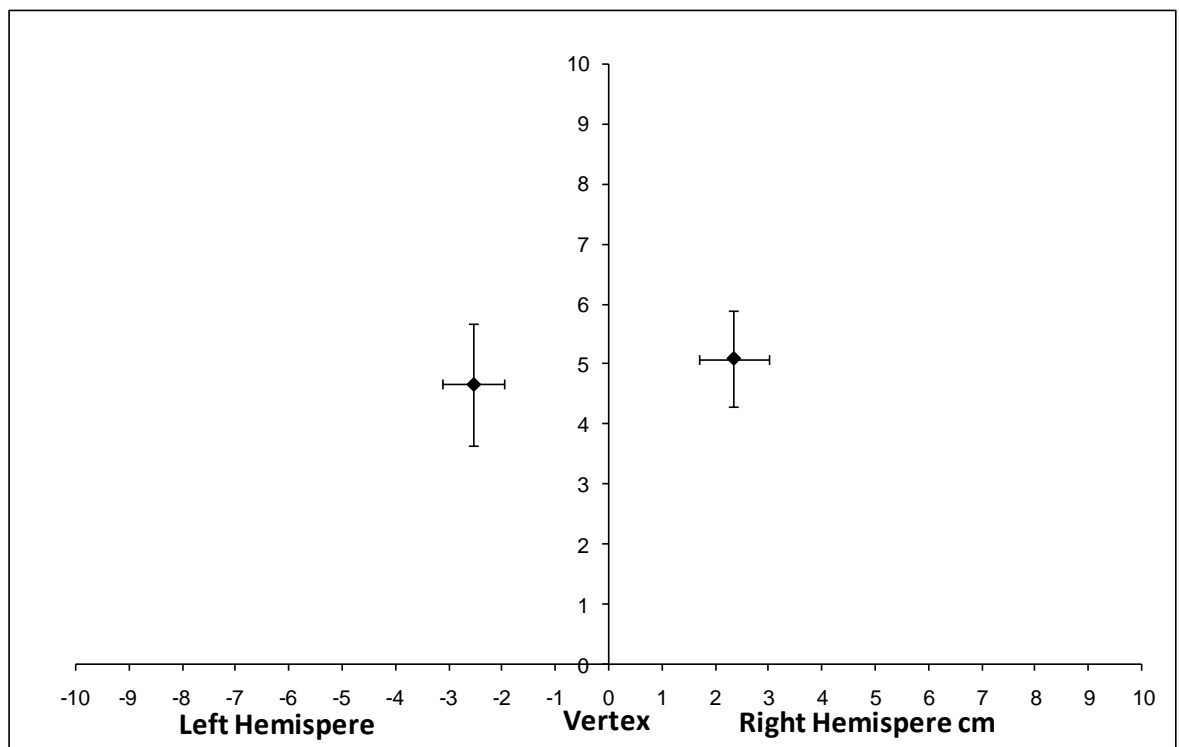


Figure 4.2: Plot illustrating the sites of maximum PMEPs in response to TMS. The right and left hemispheric dominant are represented in this figure by two points, with horizontal and vertical distance measured in centimetres from the vertex (mean \pm SD).

The average TMS intensity used for elicitation of PMEPs (pharyngeal EMGs after the application of single TMS pulses) from the dominant hemisphere was 63.45 ± 1.81 (mean \pm SEM) whereas from the non-dominant hemisphere was $69.62\% \pm 1.61$ (mean \pm SEM).

The average TMS intensity from the thenar representation was $35.79\% \pm 1.24$ (mean \pm SEM).

4.3.3 Amplitude

PMEP traces from one volunteer before (baseline) and after the virtual lesion and interventions with still water, carbonated solutions and saliva for the dominant hemisphere are shown in Figure (4.3).

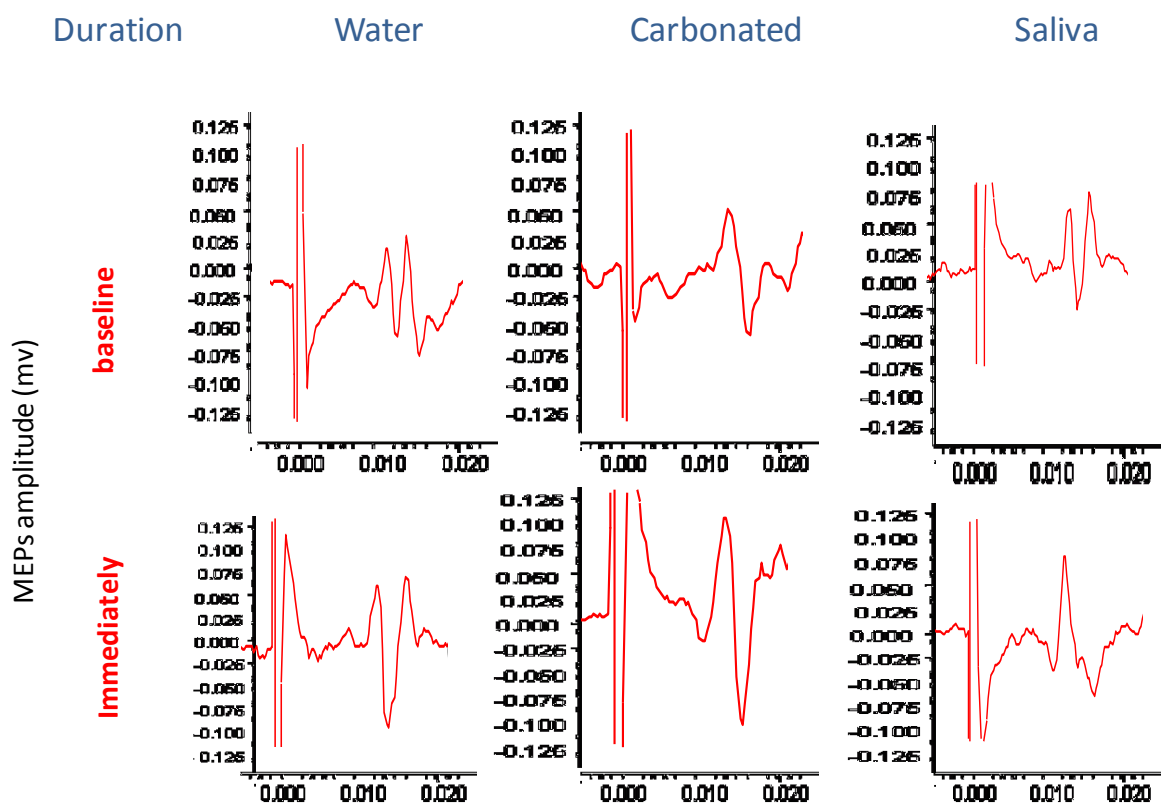


Figure 4.3: Amplitudes of PMEPs of the dominant hemisphere projection at baseline and immediately after still water, carbonated solution and saliva swallowing intervention

The baseline of the cortical excitability prior to the application of the different solution swallowing intervention was similar across all six arms for each site: the dominant and

non-dominant pharyngeal representation and thenar cortical representation (Friedman's test, dominant cortical representation: Chi squares: 3.85, $p=0.145$, non-dominant cortical representation: Chi squares: 1.286, $p=0.52$, thenar cortical representation: Chi squares: 1.857, $p=0.395$).

A three-way ANOVA was used to analyse data with factors of INTERVENTION (carbonated, water and saliva), TIME (immediately, 15 minutes, 30 minutes, 45 minutes and 60 minutes) and SITE (dominant hemisphere, non-dominant hemisphere and thenar), then separate two-way ANOVAs were used to analyse data with factors of INTERVENTION (carbonated, water and saliva), TIME (immediately, 15 minutes, 30 minutes, 45 minutes and 60 minutes) and SITE (dominant hemisphere, non-dominant hemisphere and thenar).

4.3.3.1 Pharynx

The percentage change in the PMEPs amplitude of dominant pharyngeal motor projection from the baseline for the fourteen subjects after the focal cortical suppression 1 Hz rTMS for different types of swallowing intervention are shown in Figure (4.4) The three-way ANOVA shows a significant effect of INTERVENTION*TIMEPOINTS* SITE interaction ($F(1, 13) = 5.287$, $p=0.039$). In addition, the two-way ANOVA for the dominant hemisphere showed a significant effect of INTERVENTION on the percentage change of PMEPs for carbonated solution swallowing compared to still water and saliva swallowing ($F(1, 13) = 7.519$, $p=0.017$) but no significant effect of interaction TIME*INTERVENTION was observed. There was thus an increase in the percentage change in the dominant hemisphere following swallowing of carbonated solution for all the time points with the highest values from the baseline at 30 minutes ($80.31\% \pm 38.9$) and 60 minutes ($74.61\% \pm 26.4$).

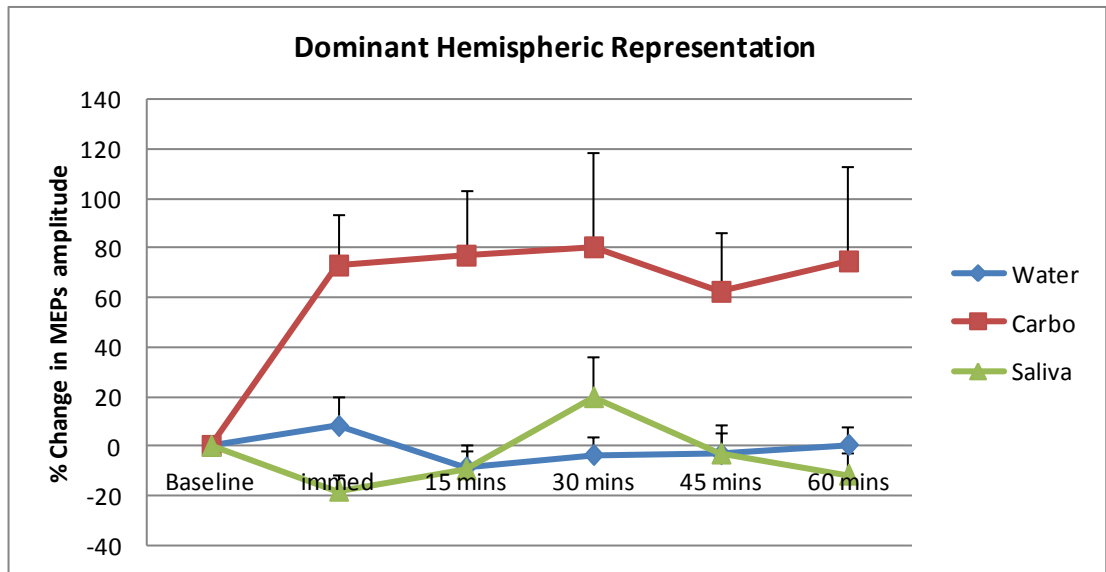


Figure 4.4: Mean percentage change in PMEP amplitudes following two different solutions and saliva intervention. PMEPs are plotted as mean data across TMS intensities (MT+10% + MT+20%) \pm SEM. Carbonated solution intervention showed a dramatic increase in cortical excitability and reversal of virtual lesion over all time points with significant changes compared to water and saliva. Both water and saliva swallowing intervention showed mild fluctuation up and down from their baseline in cortical excitability for all time points ($p=0.017$).

In addition, there was a significant effect of TIME*INTERVENTION interaction for carbonated solution compared to saliva swallowing ($F(1, 13) = 5.394, p=0.037$) and a significant effect of INTERVENTION (carbonated solution) on the non-dominant hemisphere compared to still water and saliva swallowing ($F(1, 13) = 4.731, p=0.049$). During follow-up for the non-dominant hemisphere, the carbonated solution resulted in an increase in percentage change compared to water and saliva (the highest at immediately (45.94% \pm 17.3) and 15 minutes (45.1% \pm 17.3)). Also, it is noticeable that the effect of water and saliva are lower than the baseline for most of the time points (the lowest at immediately for saliva swallows (-18.93 \pm 9.06) and at 15 minutes for water swallows (-6.09 \pm 4.99)).

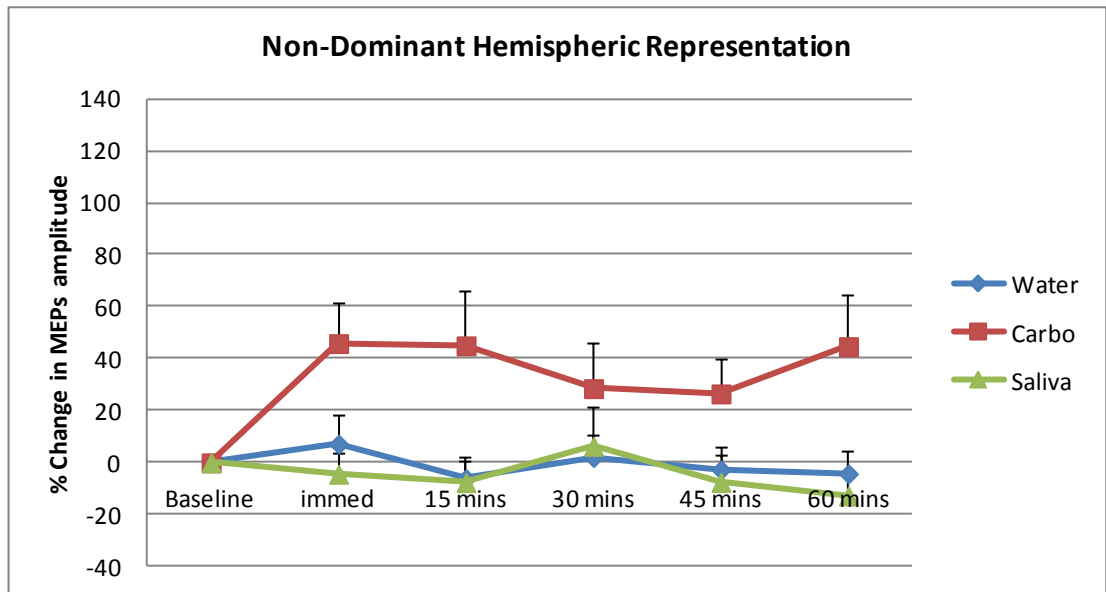


Figure 4.5: Mean percentage change in PMEP amplitudes following two different solutions and saliva swallowing intervention. Carbonated solution intervention showed an increase in cortical excitability from its baseline at all time points with significant changes compared to water and saliva, whereas water and saliva decreased cortical excitability from their baseline mostly at all time points ($p=0.049$).

4.3.3.2 Thenar

For the thenar representation, there was no significant INTERVENTION*TIME interaction ($F(1, 13) = 0.291, p=0.599$). Also, a two-way ANOVA showed no effect for the different interventions on cortical excitability ($F(1, 13) = 0.374, p=0.551$).

4.3.4 Latencies

The mean pharyngeal response latencies at the baseline and at each time point for the different swallowing intervention solutions delivered after the focal lesion suppression 1 Hz rTMS for each hemispheric hotspot and for the thenar representation hotspot are shown in Table (A.2) in the appendix. There was no significant difference in the two-way ANOVA for interventions, times and different sites.

4.4 Discussion

The results from my virtual lesion study in healthy volunteers showed that swallowing carbonated solution reversed the inhibitory effects of a unilateral virtual lesion with significant increases in cortical excitability of pharyngeal representation bilaterally (dominant and non-dominant hemisphere) compared to still water and saliva swallowing. This study corroborates the results of the previous two studies for the effect of swallowing carbonated liquids. Moreover, it provides neurophysiological evidence that the physical properties of carbonated solution are enough to cause greater neuronal activity compared to still water swallowing.

Following the development of the virtual lesion model to the pharyngeal motor cortex, many studies have used this technique to test the efficacy of neurostimulation and gain further information regarding the underlying mechanisms for beneficial plasticity in healthy volunteers. For example, Jefferson et al. [191] investigated the effect of high frequency rTMS (5 Hz rTMS) on the contralesion hemisphere following an induced unilateral virtual lesion in the pharyngeal motor cortex in healthy volunteers. They found the active rTMS (5 Hz rTMS) reversed the cortical suppression induced by a virtual lesion and led to an increase in pharyngeal cortical excitability and improvement in swallowing behaviour compared to sham treatment measured with SRT. Jayasekeran et al. [229] examined whether pharyngeal electrical stimulation (PES) could reverse the effects of a virtual lesion to the dominant of thirteen healthy volunteers. The results showed that the PES was equally effective at restoring brain function after experimental lesioning. Furthermore, Michou et al. [228] examined the behavioural and neurophysiological effect of the paired associative stimulation (PAS) to the both ipsilesional and contralesional pharyngeal motor cortex in twelve healthy volunteers after creating focal cortical suppression on the dominant hemisphere. The results showed that PAS to the contralesional cortex was most powerful at reversing the cortical suppression induced by 1 Hz rTMS. Both PES and PAS were then applied to stroke patients with acute and chronic lesions respectively with positive behavioural results [228, 229].

By comparison, Verin et al. [230] studied the swallowing behavioural in nine healthy volunteers using focal cortical suppression in the oropharyngeal motor cortex in both hemispheres (dominant and non-dominant) in different sessions. Videofluoroscopy was used

to assess swallowing behaviour after each rTMS session. They reported an increase in swallowing reaction time in VFS and decrease in the oral transit time when performed rTMS over the dominant lesioned hemisphere, and only a decrease in oral transit time when applied over the non-dominant hemisphere. However, there was no effect in aspiration/penetration scores. These results indicate that focal cortical suppression of the oropharyngeal motor cortex causes subtle changes in swallowing, which are partially similar to post-stroke oropharyngeal dysphagia. Therefore, the virtual lesion in the pharyngeal motor cortex might be seen as a legitimate method to examine different treatments for stroke dysphagia.

According to Mistry et al. [189], 1 Hz rTMS applied over the dominant pharyngeal motor cortex is able to decrease the output of motor-evoked potentials for up to 45 minutes and a similar effect on the non-dominant pharyngeal motor cortex was observed to lesser extent. The inhibitory effects are due to the suppression or change of the synaptic efficacy of neurons in the motor cortex. Stroke lesions are clearly different from the virtual lesion method because the former produce permanent pathological damage to neurons in the cerebral cortex. In this study, an increase in cortical excitability after the inhibitory effect of the virtual lesion was observed with swallowing intervention with carbonated solution compared to still water and saliva swallowing. It could therefore be speculated that carbonation may produce this effect because the conversion of CO₂ to carbonic acid in carbonated water can lead to the activation of lingual nociceptors, which excites trigeminal neurons involved in signalling oral irritation [112]. This could lead to an increase of sensory input to NTS in the midbrain, which in turn could lead to an increase in cortical excitability. It would therefore seem reasonable to infer that such a mechanism would also be active in stroke patients.

The effects of sensory oral receptors were supported by Fraser et al.'s study [6], which compared the effects of volitional (water) swallowing, pharyngeal electrical stimulation and anaesthesia on human pharyngoesophageal motor excitability in healthy volunteers. They reported early increases in pharyngoesophageal corticobulbar and craniobulbar excitability after water swallowing, whereas pharyngeal electrical stimulation causes a late increase in the corticobulbar excitability. In addition, both corticobulbar and craniobulbar projections showed an inhibited effect with anaesthesia. These results show that peripheral sensory information plays an important role in the cortical swallowing process.

Although the number of published papers regarding the effects of carbonation are limited and as far as I know there are no data available describing the effect of carbonation on the cortical excitability, the findings of the current study support the results of Bulow et al. [125] and Nixon et al. [126], who reported that carbonated solution in adults with dysphagia reduced penetration and aspiration and improved swallowing physiology. Furthermore, Krival et al [129] measured differences between three types of liquid – water, carbonation and carbonation+gingerol – in peak linguapalatal pressure, linguapalatal pressure duration, and adjustments in the development of linguapalatal pressure in twenty health volunteers. They speculated that carbonation and gingerol might produce greater neuromotor activity compared to water during the oral stage of swallowing. Moreover, Sdravou et al. [128] investigated the effect of carbonated thin liquid (CTL) solution compared to effect of non-carbonated thin liquid (NCTL) on oropharyngeal swallowing in seventeen neurogenic dysphagic patients using videofluoroscopy (VFS). They reported that a significant decreased in penetration and aspiration with carbonated solution (5 ml and 10 ml) compared to non-carbonated solutions.

Also, Miura et al. [124] studied the effects of taste, carbonation and cold stimulus on the power frequency content of submental sEMG in continuous swallowing of 60 ml solutions in health volunteers. They reported an increase in the high-frequency content of swallowing submental sEMG for sour taste, carbonated liquid and cold stimuli. This was suggested to reflect more organised activation of submental muscles, which provided more adequate and effective afferent inputs into the nucleus tractus solitarius (NTS) in brainstem.

In contrast, according to Ding et al. [123], carbonated water did not increase the submental muscle contractions in healthy adults. These differences may be due to different experimental methodologies, as I described earlier in chapter two.

Another point of controversy raised by Fraser et al. [169] is their finding that multiple repeated swallows led to excitation of cortical excitability in healthy volunteers. Repetitive swallowing could have worked as behavioural-motor stimulation and could reverse the 1 Hz rTMS inhibitory effect on cortical excitability. However, still water and saliva swallowing interventions had a similar number of repeated swallows as the swallowing intervention with carbonated water (40 swallows during 10 minutes) and they did not show

any significant change in cortical excitability. The implication is that, while there may be sensorimotor changes in the brain during these tasks, they are not by themselves powerful enough to induce any long-lasting plasticity in the swallowing network that can overcome a virtual lesion.

Evidence for the role of the lesioned and contralesioned hemisphere in swallowing recovery comes from earlier work by Hamdy et al. [203]. There was a marked increase in the cortical excitability of pharyngeal representation in the undamaged hemisphere in the stroke patients who recovered, whereas there was no change in the cortical excitability of pharyngeal representation in stroke patients with persistent dysphagia or non-dysphagic stroke patients. Therefore, an increase in cortical excitability was associated with improvement in swallowing behaviour function. The results from the previous chapters showed that, the use of carbonated solution can lead to an increase in cortical excitability of pharyngeal representation and improved behavioural function compared to swallowing still water or weak acid solution (citric acid) in healthy volunteers. The increase in cortical excitability after carbonated swallows may have occurred due to the stimulation of the oral peripheral receptors. This effect appears similar to the PES effect but potentially different from the effects of PAS and rTMS in the mechanisms that cause an increase in cortical excitability.

In conclusion, I found that carbonated solution is able to reverse the inhibitory effects of 1 Hz rTMS in my study, which provides reasonable evidence for the benefit of using carbonated solutions in the rehabilitation of dysphagia after stroke. But, prior to the use of carbonated solution in stroke patients, it would be interesting to investigate the effect of carbonated solutions on the behavioural performance following 1 Hz rTMS in healthy volunteers.

CHAPTER 5

DISCUSSION

5.1 Overview of chapters

In Chapter 1, a brief introduction on the anatomy and physiology of human swallowing in health and its neurological control by peripheral, brainstem, subcortical and cortical regions was presented. The effects of taste, smell and texture on the swallowing process were also discussed. In addition, an overview of the current and established imaging methods used in the assessment of swallowing was included. The effects of stroke on swallowing and the role of cortical plasticity in the functional recovery after stroke were discussed. Lastly, a review of transcranial magnetic stimulation technique which can be used in the treatment of dysphagia after stroke, allowing the manipulation of neuroplasticity properties was explained in detail.

Chapter 2 described the pilot and the main experiments investigating the effect of carbonated solutions on swallowing performance using my validated swallowing reaction time tasks. I was able to demonstrate that carbonated solutions promoted an increase in the number of correct challenged swallows compared to water and citric acid solutions.

Chapter 3 examined the effect of carbonated solutions on the excitability of pharyngeal motor representation. Results indicate that increased cortical excitability of the strong pharyngeal projection after 10 minutes of the carbonated solution intervention, compared to still water and citric acid solutions.

Chapter 4 explored the response of healthy swallowing motor cortex to carbonated solutions, following the application of a virtual lesion. The control arms for this study were saliva and still water swallowing. The carbonated solution paradigm was able to reverse the inhibitory effects of the virtual lesion in the stronger human pharyngeal motor projection.

In this final chapter I attempt to consolidate the different discussions from the previous chapters and add further discussion to this analysis which provides further evidence about the beneficial effects of carbonated solution on swallowing performance to a virtual lesion in pharyngeal motor cortex from the preliminary study in health volunteers. My results have increased knowledge regarding the use of carbonated solutions on which could ultimately add further credence to using carbonated boluses in stroke patients.

5.2 Novel findings

Despite the many published data regarding the effect of sour boluses on aspiration [94, 95], my studies provide the first neurophysiologic evidence that the carbonated liquids, compared to the weak acid solution with an equal pH level, can alter beneficially swallowing performance (chapters 2 and 3). Moreover, I have shown that the carbonation has the ability to reverse the inhibitory effects of a virtual lesion in the human pharyngeal cortex (chapter 4). These novel findings provide new insights into how a sensory bolus can interact with swallowing and add new knowledge into the mechanisms by which physico-chemical properties of liquids can favourably enhance the swallowing neural network. These findings are of importance and so merit a final summation of their relevance highlighted below.

5.3 Summary discussion

5.3.1 Swallowing performance

Both studies (the pilot and the main) described in chapter 2 were designed to investigate the effect of carbonation on swallowing performance using a swallowing reaction times paradigm. Correlation between the repeated trials with the same solutions was strong in both experiments. This finding supports the sensitivity of swallowing reaction task as a valid method for demonstrating differences in performance. In the challenged swallowing tasks, participants performed better with carbonated water compared to still water. Nevertheless, no significant difference was observed concerning swallowing latencies of normal and fast swallowing between different solutions (still water, carbonated solution in the pilot study; and still water, carbonated and citric acid solutions in the main study). In addition, carbonation was perceived as having strong taste intensity, compared to still water and citric acid solutions. Taking into consideration that the citric acid solutions and carbonated solution have the same pH level, the effects of carbonation are more likely to be due to the chemical characteristics of the taste solution.

There are some published papers on the beneficial effects of carbonation in dysphagic patients that used videofluoroscopy to measure the transition time of different swallowing stages. However, the studies performed in healthy volunteers investigating the effect of carbonated solutions have not previously shown any significant effects of carbonated water on the amplitude or the duration of the submental EMG [123] or any change in swallowing apnoea duration (SAD) with chemesthetic perception in 80 healthy subjects [231]. Therefore, my finding of little or no difference between still water, carbonated and citric acid solutions in the latencies of normal and fast swallows is not surprising, and implies that more sensitive measures of swallowing performance (such as the challenge task) are required to delineate subtle but important effects of bolus inputs on the swallow system.

There are multiple sensory systems that respond to carbon dioxide such as olfaction [232], nociception [111, 113] and chemoreception for respiratory regulation [233]. Interestingly, a recent study conducted by Chandrashekar et al. [114] reported that taste receptors cells detected and respond to carbonation. They also demonstrated that sour cells housed the taste receptors responding to carbonation and carbonic anhydrase 4 (Car4), a glycosylphosphatidylinositol-anchored as a specific cellular (taste) sensor for carbonation. We also know that the conversion of CO₂ in carbonated water leads to activation of lingual nociceptors which excite trigeminal neurons involved in signalling oral irritation [112]. Regarding my findings, carbonation may act via cortical pathways rather than, or in addition to, the taste system alone [110, 111]. Therefore, the observed changes with carbonation water on the challenged swallows could be regarded as changes in higher centres that play a role in the control of the swallowing process and taste centre.

Furthermore, the same swallowing reaction time paradigm has been used in the study of peripheral electrical stimulation and the same change in challenged swallows was observed [229]. Moreover, another study conducted by Michou et al [228], which used a combination of the peripheral and cortical stimulation (PAS) observed the same changes in challenged swallows only, without any changes in fast or normal swallowing. Hence, my finding supports the idea that physical and chemical bolus properties of carbonation are additive to altering brain swallowing function as well as pharyngeal stimulation and cortical stimulation.

5.3.2 Neurorehabilitation

Previous clinical research has shown that carbonated liquids can reduce aspiration in patient with neurogenic dysphagia [125, 126, 128]. Until recently, however, the underlying mechanisms inducing the effects of carbonated liquids remain unclear. It was interesting to find (as described in chapter 3 of my thesis) that swallowing of carbonated solutions promoted a significant increase in the cortical excitability of pharyngeal projections compared to still water or weak acid solution (citric acid) swallows. Moreover, pH level of both solutions (carbonated and citric acid) was equal to 4.1. Therefore, the effect of carbonated liquids referred to its “fizz” sensation was likely providing a strong stimulation to oropharyngeal receptors, resulting in increased neuronal activity compared to the citric acid solution, which also led to improved swallowing performance, as demonstrated in chapter 2.

Focal cortical suppression 1 Hz rTMS has been previously described to inhibit swallowing motor function [189, 226, 227]. In chapter 4, I demonstrated the effects of carbonation on healthy volunteers after application of 1 Hz rTMS (a virtual lesion) on the stronger representation hemisphere. The carbonated solution reversed the inhibitory effects of a unilateral virtual lesion, highlighting the role of peripheral sensory stimuli in swallowing recovery following neurological injury. A significant bilateral (dominant and non-dominant) increase in cortical excitability was observed with swallowing carbonated solutions, compared to still water and saliva swallowing. The findings in my thesis support the notion that chemesthetic properties of carbonation may provide the required peripheral sensory information that can influence greater neuronal activity compared to still water swallowing. These results provide the platform for considering the use of carbonation as facilitating stimuli in dysphagic patients who aspirate on thin liquids.

5.3.3 Preliminary study of carbonated solution on swallowing behaviour to a virtual lesion in pharyngeal motor cortex

As aforementioned, the application of 1 Hz rTMS to the strong pharyngeal hemisphere decreased cortical excitability and altered swallowing behaviour, as measured by the

swallowing reaction times task [189]. Also, the inhibition of the human oropharyngeal motor cortex resulted in transient change in swallowing behaviour, as assessed by videofluoroscopy that is similar to the functional changes seen in stroke patients [230]. With reference to these studies, this novel model (virtual lesion) is useful for assessing various treatments for stroke dysphagia. Therefore, I wanted as a provide proof of principle study to assess the behavioural effect of carbonated solutions after the inhibitory effect of rTMS. As this was a pilot study, only 4 healthy volunteers have participated in this study. Methods, experimental procedures, data analysis and the full result are shown in appendix (A.3). A summary diagram of virtual lesion study protocol is shown below.

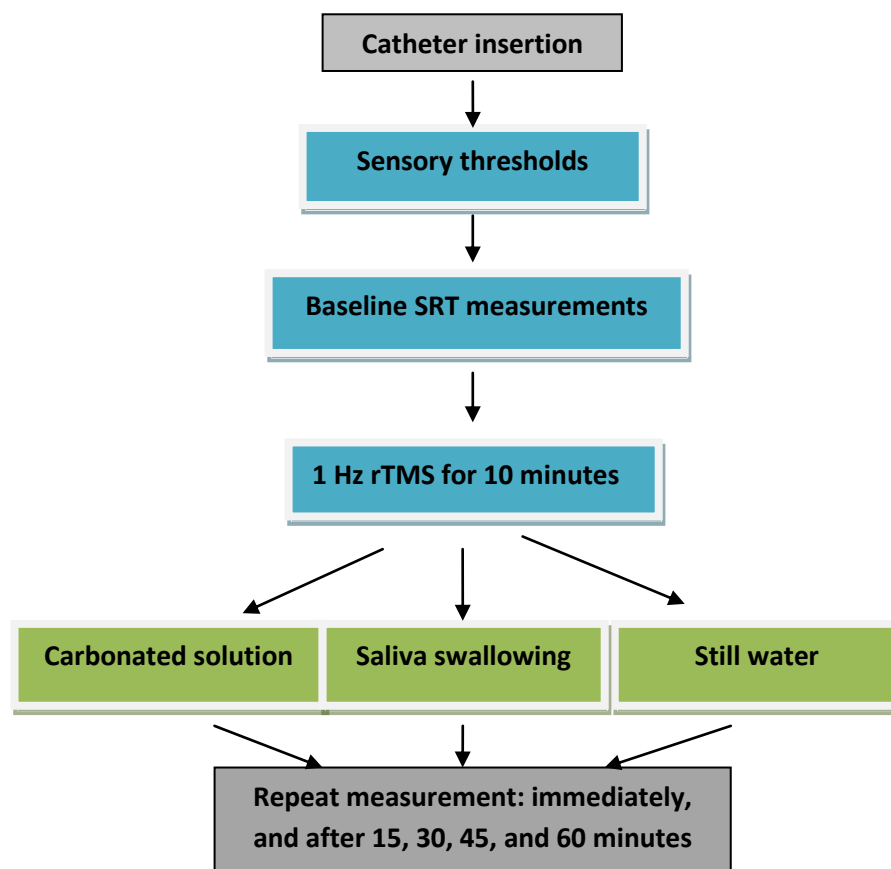


Figure 5.1: Diagram of the virtual lesion study protocol

SRT: Swallowing Reaction Tasks

The results of this study are described below.

5.3.3.1 Normal Swallowing Reaction Time

ICC between the five post intervention time points for two solutions (still water, carbonated solution) and saliva swallows showed a good agreement (0.821, 0.821 and 0.921 respectively). There was no significant difference for the swallowing latencies for the normal swallows between still water and carbonated solution {Chi squares: 17.554, $p=0.093$ }. This result is consistent with the previous result reported in Chapter 2. But there was a significant difference between saliva swallowing and other two solutions {carbonated solution versus saliva: Chi squares: 74.260, $p=0.000$, still water versus saliva: Chi squares: 68.702, $p=0.000$ }.

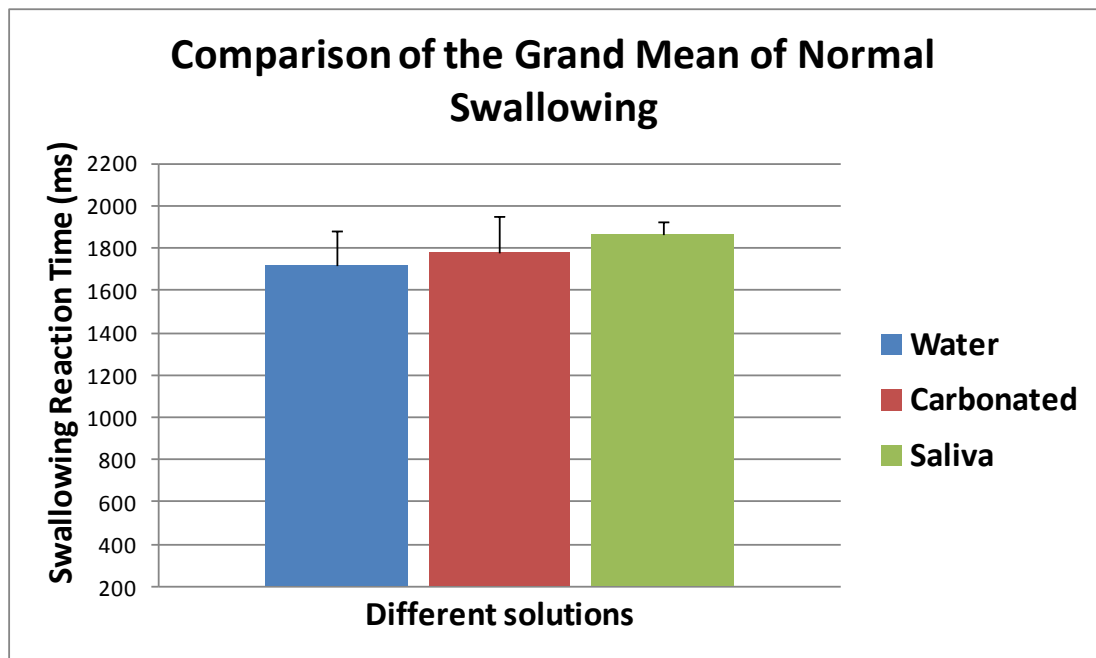


Figure 5.2.: The grand means of normal swallows (\pm SEM) for still water, carbonated solution and saliva swallows. This figure shows the grand means of response latencies of normal swallows in milliseconds for two different solutions (still water, carbonated) and saliva swallows. Each grand mean was calculated from the mean of each of 4 subjects at each time-point, and each mean calculated from response latency of the five time points for each volunteer.

This increase in response latency to evoke a cued swallow with saliva when applied for 10 minutes of 1 Hz rTMS to the dominant hemisphere (that is, the hemisphere evoking stronger pharyngeal responses) could be explained as follows: Firstly, the number of swallows was 10 times over a 50 second period. The participants found it challenging to produce an adequate volume per swallow of saliva quickly, compared to water and carbonation, when they received 3ml each swallow. Secondly, in previous brain imaging studies, saliva swallows had the ability to activate more cortical regions and had a higher Blood-Oxygen-Level-Dependent (BOLD) response bilaterally in the ventrolateral postcentral gyrus compared to water [234]. However, According to Martin et al [97] water swallows activated four times more volume than saliva swallows in healthy older adults. In addition, trying to prevent dehydration in patients with dysphagia is dependent on their oral intake of fluids, so saliva swallowing does not have role in that.

5.3.3.2 Fast Swallowing Reaction Time

ICC between the five time points for two solutions (still water, carbonated solution) and saliva swallows showed a perfect agreement (0.933; 0.969; and 0.999 respectively). There were significant differences between swallowing latencies for the fast swallows between the three different solutions {carbonated solution versus saliva: Chi squares: 102.192, $p=0.000$; still water versus saliva: Chi squares: 99.147, $p=0.000$; still water versus carbonated solution: Chi squares: 101.339, $p=0.000$ }.

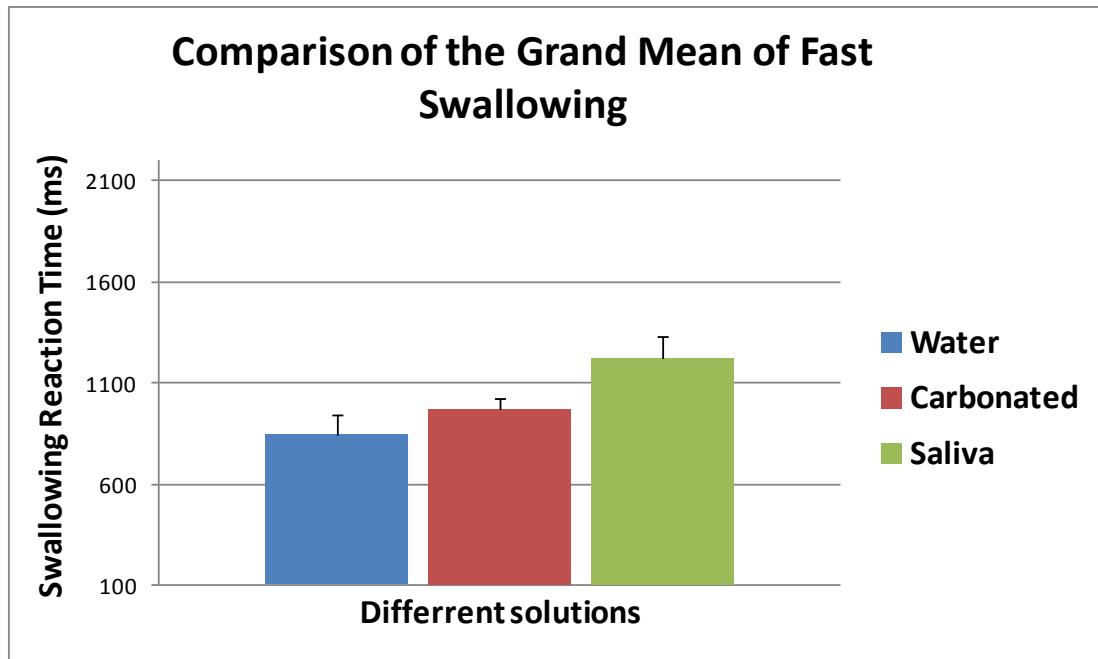


Figure 5.3: The grand means of fast swallows (\pm SEM) for still water, carbonated solution and saliva swallows. This figure shows the grand means of response latencies of fast swallows in milliseconds for the two different solutions and saliva swallows. Each grand mean was calculated from the mean for 4 subjects, and each mean was calculated from the response latency of the five time points for each volunteer.

The mean swallowing reaction time for saliva swallows was longer compared to still water and carbonated solution swallows. This increase in response latency to evoke a cued swallow with saliva may be explained due to the same reasons mentioned above. The mean swallow reaction time with carbonated solution was longer compared to still water swallows. This finding is contrary to those of Bulow et al. [125] and Nixon et al. [126] that showed that carbonated liquids decrease pharyngeal transit time; but their studies were carried out with normal rather than fast swallows. In contrast, the present finding is supported by Parker et al [208], who reported that slow water drinking with small volumes resulted in a good control of the bolus in the dysphagic patients. Also, Mistry et al. [189] reported that there was a significant change in swallowing behaviour (that is, a reduction in the response time to evoke a cued response) after application of 10 minutes of 1Hz rTMS to the dominant hemisphere.

5.3.3.3 Challenged Swallows

ICC between the five time points for two solutions (still water and the carbonated solution) and saliva swallows showed low agreement (0.59; 0.628; and 0.647 respectively). The grand mean of the percentage of successful swallowing of the carbonated solution was higher than the still water and saliva swallows. In addition, Friedman's test showed significant differences between the carbonation solution and the other two interventions {carbonated solution versus saliva: Chi square: 37.510, $p=0.000$; carbonation solution versus water: Chi square: 30.499, $p=0.000$ } but no significant difference between still water and saliva {still water versus saliva: Chi square: 16.383, $p=0.127$ }. The following figure represents the grand mean of the percentage of correct swallowing time in the two different solutions and saliva swallows.

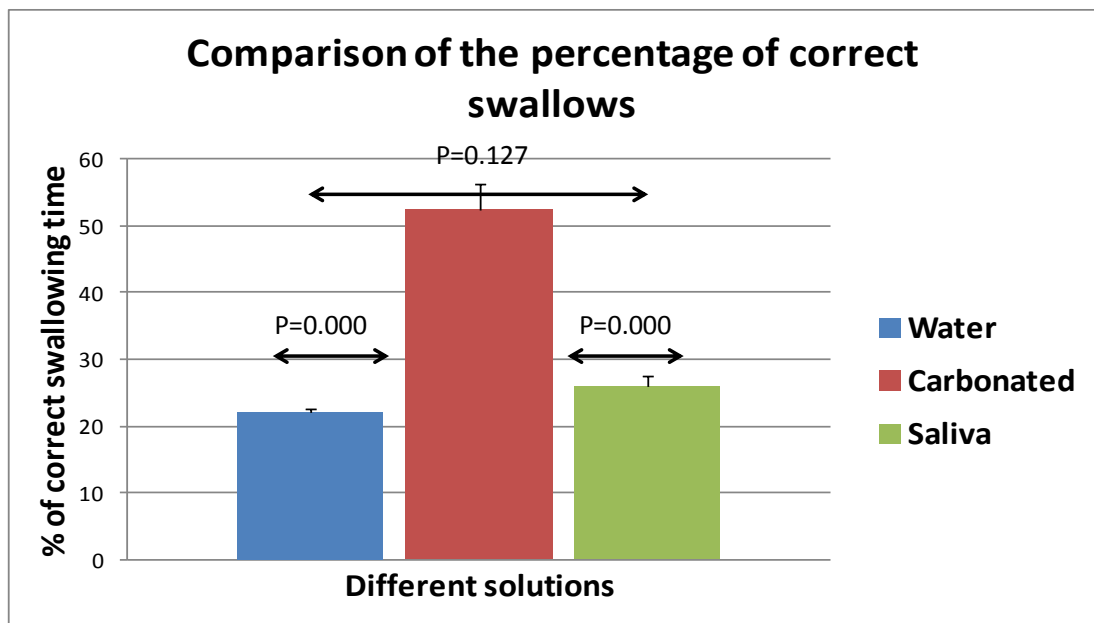


Figure 5.4: shows the grand means of challenged swallows (\pm SEM) for still water, carbonated solution and saliva swallows. This figure shows the grand means of the percentage of correct swallows for two different solutions and saliva swallows, each grand mean calculated from the mean of 4 subjects, and each mean calculated from the number of correct swallows of the five time points for each volunteer.

The findings of the current pilot study support the results of Bulow et al [125] and Nixon et al [126], who reported that the use of a carbonated solution in adults with dysphagia reduced penetration and aspiration and improved swallowing physiology. Furthermore,

according to Newman et al [127], carbonated thin liquids caused a significant reduction in the incidence of spillover, delayed pharyngeal response, and laryngeal penetration, compared to non-carbonated liquids, in 24 infants and children with dysphagia. Also, Sdravou et al [128] demonstrated that carbonated thin liquid showed a significant reduction in penetration and aspiration in patients with neurogenic dysphagia compared to non-carbonated thin liquid. Moreover, Krival et al [129] reported that carbonation and ginger influence greater neuromotor activity compared to water during the oral stage of swallowing in 20 healthy volunteers.

The results provide evidence for the benefit of carbonated solution on behavioural measurement and the basis for future studies looking into the effects of carbonation solution in dysphagic stroke patients. However, it will be important to apply the required sample size to validate these findings.

5.4 Limitations of the experimental protocols

The calculation of the sample size of TMS studies was based on previous work with TMS at the department of GI Sciences. There are, however, some differences in the methodology of my studies compared to previous TMS studies in which I tested the effects of different solutions on cortical excitability, and other studies examining the effects of different neurostimulation techniques such as PES, PAS, and high intensity of rTMS on cortical excitability.

5.5 Direction for future research

This thesis has examined the physiological and behavioural effects of carbonated liquids in healthy volunteers. Therefore, there are several ways to use these findings in future projects for the study of carbonation effects.

5.5.1 Reproducibility studies

In my current studies with healthy volunteers, the subjects attended on six separate days, at least 3-4 days apart, to reduce any chance of false positive results. Moreover, the baseline data across arms for each site (dominant and non-dominant pharyngeal representation and thenar cortical representation) of the study were similar and stable. This finding indicates that baseline values were reproducible and reduced the possibility of methodological effects on my results. I measured the EMG recordings immediately and thereafter each 15 minutes until one hour after the intervention which allowed me to record any post-intervention changes and decreased the possibility of missing possible changes. The ICC between the five timepoints for the three solutions ranged from good to perfect agreement. This finding supports the likelihood that the swallowing reaction time task is a sensitive measure of performance.

Carbonated water was prepared in the Soda Siphon maker which kept water under constant pressure and temperature; and iSi soda charger contains a fixed amount of CO₂ (8 grams). Moreover, carbonated water does not contain any additive such as sugar, salt or flavour. Therefore, my liquid is pure carbonated water which is very easy to reproduce.

5.5.2 Outcome measures

In my studies, I presented the pilot results concerning the beneficial effects of carbonated liquid on swallowing performance to a virtual lesion in the pharyngeal motor cortex which adds further credence to using carbonation in stroke patients. This study needs to be extended to allow the participation of the required number of subjects (12 participants) which will enable appropriate statistical power. Moreover, having found reasonable evidence for the beneficial effects of carbonated solution on cortical excitability and swallowing performance in healthy volunteers, it would be interesting to investigate the effects of carbonated solution in dysphagic patients. These patients, who aspirate thin liquid, should be carefully selected however, to prevent aspiration. For example, temporally aspiration with thin liquid included and avoids who permanently aspirated.

In addition, in my study I used single pulse TMS and SRT to measure physiological changes and swallowing performances for each solution. It should be noted, however, that functional magnetic imaging (fMRI), positron emission tomography (PET) and Magnetoencephalography imaging tests have been used in many studies to assess the cortical processes involved in swallowing function, or to measure the physiological changes in response to cortical stimulation of the hand motor area. These techniques have the ability to give information regarding the whole brain related to the multiple cortical areas involved in the control of swallowing and its modification by TMS. These techniques may provide an idea of the temporal sequencing of these events and therefore should be used in future work to investigate the effects of carbonation.

Furthermore, there are many studies that have used videofluoroscopy for the evaluation of swallowing in healthy volunteers; however, the correlation between videofluoroscopy and swallowing reaction times outcome has not been yet investigated. Therefore, a comparison between these two measurements should be performed in future.

5.6 Conclusion

Data presented in this thesis have demonstrated that carbonated liquids have beneficial neurophysiological and swallowing performance effects. These data support the notion that the chemical properties of carbonated liquids may provide the required peripheral sensory information that increases cortical excitability, which leads to improved swallowing performance. These data provide reasonable evidence for the benefit of using carbonated liquids in swallowing rehabilitation after stroke.

References

1. Fasano, A., *Cellular microbiology: can we learn cell physiology from microorganisms?* American Journal of Physiology-Cell Physiology, 1999. **276**(4): p. C765-C776.
2. Hamdy, S., *Central nervous system injury*, in *Pathophysiology of the enteric nervous system: a basis for understanding functional diseases*, S. Robin, and Grundy, D, Editor. 2004, Blackwell publishing. p. 176-182.
3. Ellis, H., *The head and neck*, in *Clinical Anatomy*. 2002, Blackwell publishing p. 293-298
4. Gosling, J. A., Harris. P. F., Whitmore, L., & Willan, P. L. T., *Head and neck*, in *Human anatomy,color atlas and text*. 2002, Mosby. p. 306-327
5. Snell, R. S., *The head and neck* in *Clinical anatomy for medical students*. 2000, Lippincott Williams and Wilkins. p. 736-742.
6. Fraser, C., et al., *Differential changes in human pharyngoesophageal motor excitability induced by swallowing, pharyngeal stimulation, and anesthesia*. Am J Physiol Gastrointest Liver Physiol, 2003. **285**(1): p. G137-44.
7. Dent, J., et al., *Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects*. J Clin Invest, 1980. **65**(2): p. 256-67.
8. Lear, C.S., et al. , *The Frequency of Deglutition in Man*. Arch Oral Biol, 1965. **10**: p. 83-100.
9. Dodds, W.J., et al., *A comparison between primary esophageal peristalsis following wet and dry swallows*. J Appl Physiol, 1973. **35**(6): p. 851-7.
10. Helm, J.F., et al., *Acid neutralizing capacity of human saliva*. Gastroenterology, 1982. **83**(1 Pt 1): p. 69-74.
11. Cichero, J., & Murdoch, B., *Dysphagia, foundation, theory and practice* 2006: John Wiley & Sons, Ltd.
12. Hiemae, K.M., & Palmer, J.B, *Food transport and bolus formation during complete feeding sequences on foods of different initial consistency*. Dysphagia, 1999. **14**(1): p. 31-42.
13. Guinard, J.X., Zoumas-Morse, C., & Walchak, C., *Relation between Parotid saliva flow and composition and perception of gustatory and trigeminal stimuli in food*. physiology and Behaviour, 1998. **63**(1).
14. Dua, K.S., et al., *Coordination of deglutitive glottal function and pharyngeal bolus transit during normal eating*. Gastroenterology, 1997. **112**(1): p. 73-83.
15. Guyton, A.C., & Hall, J.E., *Propulsion and mixing of food in the alimentary tract*, in *Text book of medical physiology*. 2000, Saunder Company. p. 728-731.

16. Crary, M. A & Groher, M.E., *Introduction to adult swallowing disorders*. Elsevier Science:Butterworth Heinemann., 2003.
17. Logemann, J.A., *Evaluation and treatment of swallowing disorders*. second edition ed. 1998: Austin Texas:Pro-ed.
18. Bisch, E.M., et al., *Pharyngeal effects of bolus volume, viscosity, and temperature in patients with dysphagia resulting from neurologic impairment and in normal subjects*. J Speech Hear Res, 1994. **37**(5): p. 1041-59.
19. Dantas, R.O., et al., *Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing*. Am J Physiol, 1990. **258**(5 Pt 1): p. G675-81.
20. Kahrilas, P.J. & Logemann, J.A., *Volume accommodation during swallowing*. Dysphagia, 1993. **8**(3): p. 259-65.
21. Lazarus, C.L., et al., *Effects of bolus volume, viscosity, and repeated swallows in nonstroke subjects and stroke patients*. Arch Phys Med Rehabil, 1993. **74**(10): p. 1066-70.
22. Tasko, S.M., et al., *Variability in tongue movement kinematics during normal liquid swallowing*. Dysphagia, 2002. **17**(2): p. 126-38.
23. Dodds, W.J., et al., *Influence of bolus volume on swallow-induced hyoid movement in normal subjects*. AJR Am J Roentgenol, 1988. **150**(6): p. 1307-9.
24. Logemann, J.A., *Efficacy, outcome, and cost Effectiveness in Dysphagia*, in *Measuring outcome in speech-language pathology*, C. Frattali, Editor. 1998, New York Thieme. p. 321-33.
25. Ekberg, O., et al. , *Relation of bolus size and pharyngeal swallow*. Dysphagia, 1988. **3**(2): p. 69-72.
26. Jacob, P., et al., *Upper esophageal sphincter opening and modulation during swallowing*. Gastroenterology, 1989. **97**(6): p. 1469-78.
27. Kahrilas, P.J., et al., *Oropharyngeal accommodation to swallow volume*. Gastroenterology, 1996. **111**(2): p. 297-306.
28. Logemann, J.A., et al., *Temporal and biomechanical characteristics of oropharyngeal swallow in younger and older men*. J Speech Lang Hear Res, 2000. **43**(5): p. 1264-74.
29. Hiss, S.G., et al. , *Effects of age, gender, bolus volume, and trial on swallowing apnea duration and swallow/respiratory phase relationships of normal adults*. Dysphagia, 2001. **16**(2): p. 128-35.
30. Preiksaitis, H.G., Mayrand, S., et al., *Coordination of respiration and swallowing: Effect of bolus volume in normal adults*. Am J Physiol, 1992. **263**(3 Pt 2): p. R624-30.

31. Miller, J.L. & Watkin, K.L., *The influence of bolus volume and viscosity on anterior lingual force during the oral stage of swallowing*. *Dysphagia*, 1996. **11**(2): p. 117-24.
32. Shaker, R., et al., *Pressure-flow dynamics of the oral phase of swallowing*. *Dysphagia*, 1988. **3**(2): p. 79-84.
33. Shaker, R., et al., *Effect of aging, position, and temperature on the threshold volume triggering pharyngeal swallows*. *Gastroenterology*, 1994. **107**(2): p. 396-402.
34. Robbins, J., et al., *Oropharyngeal swallowing in normal adults of different ages*. *Gastroenterology*, 1992. **103**(3): p. 823-9.
35. Reimers-Neils, L., et al., *Viscosity effects on EMG activity in normal swallow*. *Dysphagia*, 1994. **9**(2): p. 101-6.
36. Freson, M. & Stokmans, R., *The radiologic investigation of dysphagia*. *Acta Otorhinolaryngol Belg*, 1994. **48**(2): p. 127-37.
37. Rosenbek, J.C., et al., *A penetration-aspiration scale*. *Dysphagia*, 1996. **11**(2): p. 93-8.
38. Pracy, J.P., et al., *Acute pneumonitis caused by low density barium sulphate aspiration*. *J Laryngol Otol*, 1993. **107**(4): p. 347-8.
39. Teasell, R.W., et al., *Frequency of videofluoroscopic modified barium swallow studies and pneumonia in stroke rehabilitation patients: a comparative study*. *Arch Phys Med Rehabil*, 1999. **80**(3): p. 294-8.
40. Aviv, J.E., *The bedside swallowing evaluation when endoscopy is an option: what would you choose?* *Dysphagia*, 2002. **17**(3): p. 219.
41. Sellars, C., et al., *Swallowing abnormalities after acute stroke: A case control study*. *Dysphagia*, 1999. **14**(4): p. 212-8.
42. Dziewas, R., et al., *Towards a basic endoscopic assessment of swallowing in acute stroke - development and evaluation of a simple dysphagia score*. *Cerebrovasc Dis*, 2008. **26**(1): p. 41-7.
43. Warnecke, T., et al., *Fiberoptic endoscopic Dysphagia severity scale predicts outcome after acute stroke*. *Cerebrovasc Dis*, 2009. **28**(3): p. 283-9.
44. Martin, R.E. & Sessle, B.J., *The role of the cerebral cortex in swallowing*. *Dysphagia*, 1993. **8**(3): p. 195-202.
45. Buchholz, D., *Neurologic causes of dysphagia*. *Dysphagia*, 1987. **1**: p. 152-6.
46. Anderson, D.J., et al., *Sensory mechanisms in mammalian teeth and their supporting structures*. *Physiol Rev*, 1970. **50**(2): p. 171-95.
47. Luschei, E.S. & Goodwin, G.M., *Patterns of mandibular movement and jaw muscle activity during mastication in the monkey*. *J Neurophysiol*, 1974. **37**(5): p. 954-66.

48. Ertekin, C. & Aydogdu, I., *Neurophysiology of swallowing*. Clinical Neurophysiology, 2003. **114**(12): p. 2226-44.
49. Jean, A., *Brain stem control of swallowing: neuronal network and cellular mechanisms*. Physiol Rev, 2001. **81**(2): p. 929-69.
50. Mansson, I. & Sandberg, N., *Effects of surface anesthesia on deglutition in man*. Laryngoscope, 1974. **84**(3): p. 427-37.
51. Green, B., G., *Psychophysical measurement of oral chemesthesis*, in *Methods in chemosensory research*, S.A.S. M.A.L.Nicolelis, Editor. 2002, CRC Press. p. 3-22.
52. Green, B.G., *Oral chemesthesis: an integral component of flavor*, in *Flavor Perception*, T.A. D.Roberts, Editor. 2004, Blackwell Publishing: Oxford,UK. p. 151-171.
53. Darian-Smith, I., et al., *Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli*. J Neurophysiol, 1979. **42**(5): p. 1297-315.
54. Bradley, R.M., *Sensory receptors of the larynx*. Am J Med, 2000. **108 Suppl 4a**: p. 47S-50S.
55. Yoshida, Y., Tanaka, Y., et al., *Sensory innervation of the pharynx and larynx*. The American Journal of Medicine, 2000. **108**(4): p. 51-61.
56. Green, B., G., *Sensory interactions between capsaicin and temperature*. Chem.Senses, 1986. **11**: p. 371-382.
57. Green, B.G., *The Effects of Temperature and Concentration on the Perceived Intensity and Quality of Carbonation*. Chemical Senses, 1992. **17**(4): p. 435-450.
58. Green, B.G., et al., *Chemesthesis and taste: evidence of independent processing of sensation intensity*. Physiol Behav, 2005. **86**(4): p. 526-37.
59. Liu, L. & Simon, S.A., *Similarities and differences in the currents activated by capsaicin, piperine, and zingerone in rat trigeminal ganglion cells*. J Neurophysiol, 1996. **76**(3): p. 1858-69.
60. Yuanyuan, Y.W., Rui, B.C., & Emily, R.L., *TRPA1 is a component of the nociceptive response to CO₂ (CO₂ sensing by TRPA1)*. J Neurosci, 2010. **30**(39): p. 12958-12963.
61. Dhaka, A., et al., *TRPV1 is activated by both acidic and basic pH*. J Neurosci, 2009. **29**(1): p. 153-8.
62. Duffy, V.B., *Variation in oral sensation: implications for diet and health*. Curr Opin Gastroenterol, 2007. **23**(2): p. 171-7.
63. Miller, A.J., *The neuroscientific principles of swallowing and dysphagia (dysphagia series)*. First ed. 1999: San Diego:Singular Publishing.
64. Witt, M., Reutter, K., & Miller, I.J, *Morphology of the peripheral taste system*, in *Handbook of olfaction and gustation*, R.L.Doty, Editor. 2003, Marcel Decker,Inc: New York.

65. Dantas, R.O., et al., *Effect of swallowed bolus variables on oral and pharyngeal phases of swallowing*. Am J Physiol, 1990. **258**(5 Pt 1): p. G675-81.
66. Accolla, R., et al., *Differential spatial representation of taste modalities in the rat gustatory cortex*. J Neurosci, 2007. **27**(6): p. 1396-404.
67. Mu, L. & Sanders, I., *Sensory nerve supply of the human oro- and laryngopharynx: a preliminary study*. Anat Rec, 2000. **258**(4): p. 406-20.
68. Kingsley, R.E., *Concise text of neuroscience*. second ed. 2000: Baltimore:Lippincott Williams & Wilkins.
69. Margolskee, R.F., *Molecular mechanisms of bitter and sweet taste transduction*. J Biol Chem, 2002. **277**(1): p. 1-4.
70. Dulac, C., *The physiology of taste, vintage 2000*. Cell, 2000. **100**(6): p. 607-10.
71. Mistry, S. & Hamdy, S., *Neural control of feeding and swallowing*. Phys Med Rehabil Clin N Am, 2008. **19**(4): p. 709-28, vii-viii.
72. Zemlin, W.R., *Speech and hearing science;Anatomy and physiology*. 1997: Boston:Allyn&Bacon.
73. Jean, A., *Brainstem control of swallowing:Localization and organization of the central pattern generator for swallowing*, in *Neurophysiology of the jaws and teeth*, A. Talyor, Editor. 1990, MacMillan Press: London.
74. Jean, A., *Brainstem organization of the swallowing network*. Brain Behav Evol, 1984. **25**(2-3): p. 109-16.
75. Miller, A.J., *The search for the central swallowing pathway: the quest for clarity*. Dysphagia, 1993. **8**(3): p. 185-94.
76. Bieger, D. & Hockman, C.H., *Suprabulbar modulation of reflex swallowing*. Exp Neurol, 1976. **52**(2): p. 311-24.
77. Bernheimer, H., et al., *Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations*. J Neurol Sci, 1973. **20**(4): p. 415-55.
78. Hamdy, S., et al., *Identification of the cerebral loci processing human swallowing with H2(15)O PET activation*. J Neurophysiol, 1999. **81**(4): p. 1917-26.
79. Bastian, H.C.A., *Atreatise on aphasia and other speech defects*. 1898, London: Lewis.
80. Hamdy, S., et al., *Induction of cortical swallowing activity by transcranial magnetic stimulation in the anaesthetized cat*. Neurogastroenterology and Motility, 2001. **13**(1): p. 65-72.
81. Penfield, W. & Boldrey, E., *Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation*. Brain, 1937. **60**: p. 389-443.

82. Robbins, J., et al., *Swallowing after unilateral stroke of the cerebral cortex*. Arch Phys Med Rehabil, 1993. **74**(12): p. 1295-300.
83. Hamdy, S., et al., *Cortical activation during human volitional swallowing: an event-related fMRI study*. Am J Physiol, 1999. **277**(1 Pt 1): p. G219-25.
84. Hamdy, S., et al., *Cranial nerve modulation of human cortical swallowing motor pathways*. Am J Physiol, 1997. **272**(4 Pt 1): p. G802-8.
85. Hamdy, S., et al., *The cortical topography of human swallowing musculature in health and disease*. Nat Med, 1996. **2**(11): p. 1217-24.
86. Hamdy, S. & Rothwell, J.C., *Gut feelings about recovery after stroke: the organization and reorganization of human swallowing motor cortex*. Trends Neurosci, 1998. **21**(7): p. 278-82.
87. Zald, D.H. & Pardo, J.V., *The functional neuroanatomy of voluntary swallowing*. Ann Neurol, 1999. **46**(3): p. 281-6.
88. Mosier, K. & Bereznaya I., *Parallel cortical networks for volitional control of swallowing in humans*. Exp Brain Res, 2001. **140**(3): p. 280-9.
89. Martin, R.E., et al., *Cerebral cortical representation of automatic and volitional swallowing in humans*. J Neurophysiol, 2001. **85**(2): p. 938-50.
90. Kurihara, K. & Kashiwayanagi, M., *Introductory remarks on umami taste*. Ann N Y Acad Sci, 1998. **855**: p. 393-7.
91. Barr, M., L., & Kiernan, J, A, *The human nervous system*, in *An anatomical viewpoint*. 1988, Philadelphia: JB Lippincott Co.
92. Rolls, E.T., *Taste, olfactory, and food texture processing in the brain, and the control of food intake*. Physiol Behav, 2005. **85**(1): p. 45-56.
93. Kaattzke-McDonal, M.N., Post, E., & David, P.J., *The effects of cold, touch, and chemical stimulation of anterior faucial pillar on human swallowing*. Dysphagia, 1996. **11**.
94. Logemann, J.A., et al., *Effects of a sour bolus on oropharyngeal swallowing measures in patients with neurogenic dysphagia*. J Speech Hear Res, 1995. **38**(3): p. 556-63.
95. Pelletier, C.A. & Lawless, H.T., *Effect of citric acid and citric acid-sucrose mixtures on swallowing in neurogenic oropharyngeal dysphagia*. Dysphagia, 2003. **18**(4): p. 231-41.
96. Lanessa, A.H., Michelle, E.F., Donald, G.M., et al., *Neurophysiology of swallowing: effects of age bolus type*. Neuroimage, 2009. **44**.
97. Martin, R., et al., *Cerebral cortical processing of swallowing in older adults*. Exp Brain Res, 2007. **176**(1): p. 12-22.

98. Rika, Y., Kazuhisa, O, A., et al., *Facilitation of voluntary swallowing by chemical stimulation of the posterior tongue and pharyngeal region in humans*. Neuroscience Letter, 2008. **448**.
99. Cerf-Ducastel, B. & Murphy, C., *fMRI brain activation in response to odors is reduced in primary olfactory areas of elderly subjects*. Brain Res, 2003. **986**(1-2): p. 39-53.
100. Poellinger, A., et al., *Activation and habituation in olfaction--an fMRI study*. Neuroimage, 2001. **13**(4): p. 547-60.
101. Sobel, N., et al., *Time course of odorant-induced activation in the human primary olfactory cortex*. J Neurophysiol, 2000. **83**(1): p. 537-51.
102. Kovacs, T., *Mechanisms of olfactory dysfunction in aging and neurodegenerative disorders*. Ageing Res Rev, 2004. **3**(2): p. 215-32.
103. Vroom, P., Van Amerongen, A., & De Vries, H., *Smell: The secret seducer*. 1997, New York: Farrar, Stratus & Giroux.
104. Rozin, P., *"Taste-smell confusions" and the duality of the olfactory sense*. Percept Psychophys, 1982. **31**(4): p. 397-401.
105. Shepherd, G.M., *Smell images and the flavour system in the human brain*. Nature, 2006. **444**(7117): p. 316-21.
106. Small, D.M., et al., *Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans*. Neuron, 2005. **47**(4): p. 593-605.
107. Moore, K.L., & Dalley, A.F, *Clinically oriented anatomy*. fourth ed. 1999: Philadelphia. Lippincott Williams & Wilkins.
108. Clave, P., et al., *The effect of bolus viscosity on swallowing function in neurogenic dysphagia*. Aliment Pharmacol Ther, 2006. **24**(9): p. 1385-94.
109. Lambert, H.C., et al., *McGill Ingestive Skills Assessment (MISA): development and first field test of an evaluation of functional ingestive skills of elderly persons*. Dysphagia, 2003. **18**(2): p. 101-13.
110. Komai, M. & Bryant, B.P., *Acetazolamide specifically inhibits lingual trigeminal nerve responses to carbon dioxide*. Brain Res, 1993. **612**(1-2): p. 122-9.
111. Simons, C.T., et al., *Neurobiological and psychophysical mechanisms underlying the oral sensation produced by carbonated water*. J Neurosci, 1999. **19**(18): p. 8134-44.
112. Dessirier, J.M., et al., *Psychophysical and neurobiological evidence that the oral sensation elicited by carbonated water is of chemogenic origin*. Chem Senses, 2000. **25**(3): p. 277-84.
113. Dessirier, J.M., et al., *The oral sensation of carbonated water: cross-desensitization by capsaicin and potentiation by amiloride*. Chem Senses, 2001. **26**(6): p. 639-43.

114. Chandrashekar, J., et al., *The taste of carbonation*. Science, 2009. **326**(5951): p. 443-5.
115. Yau, N.J.N. & Mcdaniel, M.R., *The Effect of Temperature on Carbonation Perception*. Chemical Senses, 1991. **16**(4): p. 337-348.
116. Harper, S.J. & Mcdaniel, M.R., *Carbonated Water Lexicon - Temperature and Co2 Level Influence on Descriptive Ratings*. Journal of Food Science, 1993. **58**(4): p. 893-898.
117. Mcllellan, M.R., et al., *Sensory Analysis of Carbonated Apple Juice Using Response-Surface Methodology*. Journal of Food Science, 1984. **49**(6): p. 1595-1597.
118. Yau, N., et al., *Carbonated interactions with sweetness and sourness*. J Food Sci, 1992. **57**: p. 1412-1416.
119. Cometto-Muniz, J.E., et al., *Interactions between CO2 oral pungency and taste*. Perception, 1987. **16**(5): p. 629-40.
120. Beverly, J., C., *The addition of CO₂ to traditional taste solutions alters taste quality*. Chem. Senses, 1998. **23**: p. 397-402.
121. Green, B.G., *Chemesthesis: Pungency as a component of flavor*. Trends in Food Science & Technology, 1996. **7**(12): p. 415-423.
122. Lederer, C., et al., *The Effect of Carbonation Level on the Sensory Properties of Flavored Milk Beverages*. Journal of Dairy Science, 1991. **74**(7): p. 2100-2108.
123. Ding, R., et al., *The effects of taste and consistency on swallow physiology in younger and older healthy individuals: a surface electromyographic study*. J Speech Lang Hear Res, 2003. **46**(4): p. 977-89.
124. Miura, Y., et al., *Effects of taste solutions, carbonation, and cold stimulus on the power frequency content of swallowing submental surface electromyography*. Chem Senses, 2009. **34**(4): p. 325-31.
125. Bulow, M.R., et al., *Videoradiographic analysis of how carbonated thin liquids and thickened liquids affect the physiology of swallowing in subjects with aspiration on thin liquids*. Acta Radiol, 2003. **44**(4): p. 366-72.
126. Nixon, T.S., *Use of carbonated liquids in the treatment of dysphagia*. . Network: A Newsletter of Dietetics in Physical Medicine and Rehabilitation, 1997.
127. Newman, L., Armstrong, R., Rogers, T., et al, *The effect of carbonation on sensory dysphagia in the pediatric population*. Dysphagia, 2001. **16**: p. 146-150.
128. Sdravou, K.M., et al., *Effects of carbonated liquids on oropharyngeal swallowing measures in people with neurogenic dysphagia*. Dysphagia, 2012. **27**(2): p. 240-50.
129. Krival, K. & Bates, C., *Effects of club soda and ginger brew on linguapalatal pressures in healthy swallowing*. Dysphagia, 2012. **27**(2): p. 228-39.

130. Horner, J. & Massey, E.W., *Silent aspiration following stroke*. Neurology, 1988. **38**(2): p. 317-9.
131. Linden, P. & Siebens A.A., *Dysphagia: predicting laryngeal penetration*. Arch Phys Med Rehabil, 1983. **64**(6): p. 281-4.
132. McQuaid, K., R., *Symptoms and signs of gastrointestinal disease: Alimentary tract*, in *Current Medical Diagnosis and Treatment*. M.T.J.Lawrence., Stephen, J.M., Maxine, A. P, Editor. 2003, McGraw-Hill. p. 550.
133. Horner, J. & Massey, E.W., *Silent aspiration following stroke*. Neurology, 1988. **38**(2): p. 317-9.
134. Daniels, S.K., et al, *Clinical assessment of swallowing and prediction of dysphagia severity*. Am J Speech Lang Path, 1997. **6**: p. 17-24.
135. Leder, S.B. & Espinosa, J.F. *Aspiration risk after acute stroke: comparison of clinical examination and fiberoptic endoscopic evaluation of swallowing*. Dysphagia, 2002. **17**(3): p. 214-8.
136. McCullough, G.H., et al., *Inter- and intrajudge reliability for videofluoroscopic swallowing evaluation measures*. Dysphagia, 2001. **16**(2): p. 110-8.
137. Paik, J., *Dysphagia*. Rehabilitation Medicine, 2008.
138. Simth, H.A., & Connolly, M.J, *Evaluation and treatment of dysphagia following stroke*. Geriatric Rehabilitation, 2003. **19**(1): p. 43-59.
139. Gordon, C., et al., *Dysphagia in acute stroke*. Br Med J (Clin Res Ed), 1987. **295**(6595): p. 411-4.
140. Schmidt, J., et al., *Videofluoroscopic evidence of aspiration predicts pneumonia and death but not dehydration following stroke*. Dysphagia, 1994. **9**(1): p. 7-11.
141. Elliot, J.L, *Swallowing disorders in the elderly: A guide to diagnosis and treatment*. Geriatrics, 1988. **43**: p. 95-100, 104, 113.
142. Mrazek, S.A., *Bronchopneumonia in terminally ill patients*. J Am Geriatr Soc, 1969. **17**(10): p. 969-73.
143. Feinberg, M.J., et al., *Aspiration and the elderly*. Dysphagia, 1990. **5**(2): p. 61-71.
144. Finegold, S.M., *Aspiration pneumonia*. Rev Infect Dis, 1991. **13 Suppl 9**: p. S737-42.
145. Cameron, J.L., et al., *Aspiration pneumonia. Clinical outcome following documented aspiration*. Arch Surg, 1973. **106**(1): p. 49-52.
146. Davalos, A., et al., *Effect of malnutrition after acute stroke on clinical outcome*. Stroke, 1996. **27**(6): p. 1028-32.
147. Touho, H., et al., *Hypermetabolism in the acute stage of hemorrhagic cerebrovascular disease*. J Neurosurg, 1990. **72**(5): p. 710-4.

148. O'Neill, P.A., Davies, I., et al., *Stress hormone and blood glucose response following acute stroke in elderly*. Stroke, 1991. **22**.
149. Steffee, W.P., *Malnutrition in hospitalized patients*. JAMA, 1980. **244**(23): p. 2630-5.
150. Detroyer, A., et al., *Function of the Respiratory Muscles in Acute Hemiplegia*. American Review of Respiratory Disease, 1981. **123**(6): p. 631-632.
151. Counihan, T.J., *Cerebrovascular disease*, in *Cecil Essentials of Medicine*, c. T.E Andreoli, C. C.J., Griggs, R, C., and Loscalzo, J., Editor. 2004, Philadelphia: WB Saunders.
152. Warlow, C., et al., *Stroke*. Lancet, 2003. **362**(9391): p. 1211-1224.
153. Wolfe, C.D.A., *The impact of stroke*. British Medical Bulletin, 2000. **56**(2): p. 275-286.
154. Macleod, M., & Mumford, C.J., *The nervous system*, in *Medicine*, C.E. John. Munro., Editor. 2004, Boston:Blackwell science. p. 203-215.
155. Fatahzadeh, M. & Glick, M., *Stroke: epidemiology, classification, risk factors, complications, diagnosis, prevention, and medical and dental management*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2006. **102**(2): p. 180-91.
156. Smithard, D.G., et al., *Authors' reply to "A comment on 'The natural history of dysphagia following a Stroke' (Dysphagia 13:230-231, 1998)"*. Dysphagia, 1999. **14**(1): p. 54.
157. Aviv, J.E., et al., *Supraglottic and pharyngeal sensory abnormalities in stroke patients with dysphagia*. Ann Otol Rhinol Laryngol, 1996. **105**(2): p. 92-7.
158. Paciaroni, M., et al., *Dysphagia following Stroke*. Eur Neurol, 2004. **51**(3): p. 162-7.
159. Daniels, S.K. & Foundas, A.L., *Lesion localization in acute stroke patients with risk of aspiration*. J Neuroimaging, 1999. **9**(2): p. 91-8.
160. Smithard, D.G., et al., *The natural history of dysphagia following a stroke*. Dysphagia, 1997. **12**(4): p. 188-93.
161. Logemann, J.A., & Murdoch, B., *Non-invasive approaches to deglutitive aspiration*. Dysphagia, 1991. **8**.
162. Garon, B.R., Engle, M., & Ormiston, C, A., *Randomized control study to determine the effects of unlimited oral intake of water in patients with identified aspiration*. J Neuro Rehabil, 1997. **3**(11).
163. Perez, I., et al., *Pharmacological treatment of dysphagia in stroke*. Dysphagia, 1998. **13**(1): p. 12-6.
164. Rosenbek, J.C., et al., *Thermal application reduces the duration of stage transition in dysphagia after stroke*. Dysphagia, 1996. **11**(4): p. 225-33.

165. Lazzara, G.D.L., Lazarus, C., & Logemann, J.A., *Impact of thermal stimulation on the triggering of the swallowing reflex*. Dysphagia, 1986. **1**.
166. Rosenbek, J.C., et al., *Comparing treatment intensities of tactile-thermal application*. Dysphagia, 1998. **13**(1): p. 1-9.
167. Power, M., Hamdy, S., et al., *Electrical stimulation of the anterior faucial pillar suppresses pharyngeal excitability*. Dysphagia, 2000. **15**(2).
168. Freed, M.L., et al., *Electrical stimulation for swallowing disorders caused by stroke*. Respir Care, 2001. **46**(5): p. 466-74.
169. Fraser, C., et al., *Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury*. Neuron, 2002. **34**(5): p. 831-40.
170. Tihomir, V.I., & UIF, Z., *Exploring motor cortical plasticity using transcranial magnetic stimulation in human*. New York Academy of Sciences, 2006. **1048**(1).
171. Donoghue, J.P., *Plasticity of adult sensorimotor representations*. Curr Opin Neurobiol, 1995. **5**(6): p. 749-54.
172. Topka, H., et al., *Reorganization of corticospinal pathways following spinal cord injury*. Neurology, 1991. **41**(8): p. 1276-83.
173. Buonomano, D.V. & Merzenich, M.M., *Cortical plasticity: from synapses to maps*. Annu Rev Neurosci, 1998. **21**: p. 149-86.
174. Selzer, M.E., *Textbook of neural repair and rehabilitation*. 2006: Cambridge;Newyork:Cambridge University Press.
175. Hallett, M., *Transcranial magnetic stimulation and the human brain*. Nature, 2000. **406**(6792): p. 147-50.
176. Cohen, L.G., et al., *Studies of neuroplasticity with transcranial magnetic stimulation*. J Clin Neurophysiol, 1998. **15**(4): p. 305-24.
177. Maeda, F. & Pascual-Leone, A., *Transcranial magnetic stimulation: studying motor neurophysiology of psychiatric disorders*. Psychopharmacology (Berl), 2003. **168**(4): p. 359-76.
178. Barker, A.T.R., et al., *Non-invasive magnetic stimulation of human motor cortex*. Lancet, 1985. **1**(8437): p. 1106-7.
179. Simone, R., et al., *Safety,ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research*. Clinical Neurophysiology, 2009. **120**(12): p. 2008-2039.
180. Jalinous, R., *Technical and Practical Aspects of Magnetic Nerve-Stimulation*. Journal of Clinical Neurophysiology, 1991. **8**(1): p. 10-25.
181. Roth, B.J., et al., *The heating of metal electrodes during rapid-rate magnetic stimulation: a possible safety hazard*. Electroencephalogr Clin Neurophysiol, 1992. **85**(2): p. 116-23.

182. Rothwell, J.C., *Techniques and mechanisms of action of transcranial stimulation of the human motor cortex*. J Neurosci Methods, 1997. **74**(2): p. 113-22.
183. Terao, Y. & Ugawa, Y., *Basic mechanisms of TMS*. J Clin Neurophysiol, 2002. **19**(4): p. 322-43.
184. Kobayashi, M. & Pascual-Leone, A., *Transcranial magnetic stimulation in neurology*. Lancet Neurol, 2003. **2**(3): p. 145-56.
185. Liepert, J., *TMS in stroke*. Suppl Clin Neurophysiol, 2003. **56**: p. 368-80.
186. Nollet, H., et al., *Transcranial magnetic stimulation: review of the technique, basic principles and applications*. Vet J, 2003. **166**(1): p. 28-42.
187. Chen, R., et al., *Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation*. Neurology, 1997. **48**(5): p. 1398-403.
188. Lefaucheur, J.P., *Stroke recovery can be enhanced by using repetitive transcranial magnetic stimulation (rTMS)*. Neurophysiol Clin, 2006. **36**(3): p. 105-15.
189. Mistry, S., et al., *Unilateral suppression of pharyngeal motor cortex to repetitive transcranial magnetic stimulation reveals functional asymmetry in the hemispheric projections to human swallowing*. J Physiol, 2007. **585**(Pt 2): p. 525-38.
190. Gow, D., et al., *Induction of long-term plasticity in human swallowing motor cortex following repetitive cortical stimulation*. Clinical Neurophysiology, 2004. **115**(5): p. 1044-51.
191. Jefferson, S., et al., *Reversal of a virtual lesion in human pharyngeal motor cortex by high frequency contralesional brain stimulation*. Gastroenterology, 2009. **137**(3): p. 841-9, 849 e1.
192. Verin, E. & Leroi, A.M., *Poststroke dysphagia rehabilitation by repetitive transcranial magnetic stimulation: a noncontrolled pilot study*. Dysphagia, 2009. **24**(2): p. 204-10.
193. Wassermann, E.M., *Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the international workshop on the safety of repetitive transcranial magnetic stimulation, June 5-7, 1996*. Evoked Potentials-Electroencephalography and Clinical Neurophysiology, 1998. **108**(1): p. 1-16.
194. Bridgers, S.L., *The Safety of Transcranial Magnetic Stimulation Reconsidered - Evidence Regarding Cognitive and Other Cerebral Effects*. Electroencephalography and Clinical Neurophysiology, 1991: p. 170-179.
195. Loo, C.K., T.F., McFarquhar, and P.B. Mitchell, *A review of the safety of repetitive transcranial magnetic stimulation as a clinical treatment for depression*. Int J Neuropsychopharmacol, 2008. **11**(1): p. 131-47.
196. Belmaker, B., et al., *Managing the risks of repetitive transcranial stimulation*. CNS Spectr, 2003. **8**(7): p. 489.

197. Aziz, Q., et al., *The topographic representation of esophageal motor function on the human cerebral cortex*. Gastroenterology, 1996. **111**(4): p. 855-62.
198. Paine, P.A., et al., *Assessing the temporal reproducibility of human esophageal motor-evoked potentials to transcranial magnetic stimulation*. J Clin Neurophysiol, 2006. **23**(4): p. 374-80.
199. Penfield, W. & Boldrey, E., *Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation*. Brain, 1937. **60**: p. 389-443.
200. Hamdy, S., et al., *Sensorimotor modulation of human cortical swallowing pathways*. Journal of Physiology-London, 1998. **506**(3): p. 857-866.
201. Siebner, H.R. & Rothwell, J., *Transcranial magnetic stimulation: new insights into representational cortical plasticity*. Exp Brain Res, 2003. **148**(1): p. 1-16.
202. Hamdy, S., et al., *Explaining oropharyngeal dysphagia after unilateral hemispheric stroke*. Lancet, 1997. **350**(9079): p. 686-92.
203. Hamdy, S., et al., *Recovery of swallowing after dysphagic stroke relates to functional reorganization in the intact motor cortex*. Gastroenterology, 1998. **115**(5): p. 1104-12.
204. Mistary, S., et al., *Modulation of human cortical swallowing motor pathways after pleasant and aversive taste stimuli*. Am J Physiol Gastrointest Liver Physiol, 2006. **291**: p. 666-71.
205. Martino, R., et al., *Dysphagia after stroke: incidence, diagnosis, and pulmonary complications*. Stroke, 2005. **36**(12): p. 2756-63.
206. Terre, R. & Mearin, F., *Resolution of tracheal aspiration after the acute phase of stroke-related oropharyngeal Dysphagia*. Am J Gastroenterol, 2009. **104**(4): p. 923-32.
207. Paine, P.A., et al., *Modulation of activity in swallowing motor cortex following esophageal acidification: a functional magnetic resonance imaging study*. Dysphagia, 2008. **23**(2): p. 146-54.
208. Parker, C., et al., *Awareness of dysphagia by patients following stroke predicts swallowing performance*. Dysphagia, 2004. **19**(1): p. 28-35.
209. Steele, C.M., et al., *Variations in swallowing-related tongue movements across 5 different tastant stimuli*. Dysphagia, 2007. **22**(4): p. 367-367.
210. Chee, C., et al., *The influence of chemical gustatory stimuli and oral anaesthesia on healthy human pharyngeal swallowing*. Chem Senses, 2005. **30**(5): p. 393-400.
211. Hamdy, S., et al., *Modulation of human swallowing behaviour by thermal and chemical stimulation in health and after brain injury*. Neurogastroenterol Motil, 2003. **15**(1): p. 69-77.

212. Palmer, P.M., et al., *Effects of a sour bolus on the intramuscular electromyographic (EMG) activity of muscles in the submental region*. *Dysphagia*, 2005. **20**(3): p. 210-7.
213. Lund, J.P. & Kolta, A., *Generation of the central masticatory pattern and its modification by sensory feedback*. *Dysphagia*, 2006. **21**(3): p. 167-74.
214. Ertekin, C., et al., *Effect of mucosal anaesthesia on oropharyngeal swallowing*. *Neurogastroenterol Motil*, 2000. **12**(6): p. 567-72.
215. Miller, A.J., *Deglutition*. *Physiol Rev*, 1982. **62**(1): p. 129-84.
216. Power, M., et al., *Changes in pharyngeal corticobulbar excitability and swallowing behavior after oral stimulation*. *Am J Physiol Gastrointest Liver Physiol*, 2004. **286**(1): p. G45-50.
217. Jasper, H.H., *The ten twenty electrode system of the international federation*. *Electroencephalography and Clinical Neurophysiology*, 1958. **10**: p. 371-375.
218. Miller, A.J., Bieger, D., & Conklin, J.L., *Functional controls of deglutition*, in *Deglutition and its disorders: anatomy, physiology, clinical diagnosis and management*, A.L.a.S.-D. Perlman, K. S, Editor. 1997, San Diego, CA:Singular.
219. Shingai, T. & Shimada, K., *Reflex swallowing elicited by water and chemical substances applied in the oral cavity, pharynx, and larynx of the rabbit*. *Jpn J Physiol*, 1976. **26**(5): p. 455-69.
220. Smith, D.V. & St John , S.J., *Neural coding of gustatory information*. *Curr Opin Neurobiol*, 1999. **9**(4): p. 427-35.
221. Nilsson, H., *Quantitative aspects of swallowing- with particular reference to disturbances of swallowing in neurological disorders*. 1998, Lund University.
222. Ali, G.N., et al., *Influence of cold stimulation on the normal pharyngeal swallow response*. *Dysphagia*, 1996. **11**(1): p. 2-8.
223. Palmer, P.M., et al., *Effects of a sour bolus on the intramuscular electromyographic (EMG) activity of muscles in the submental region*. *Dysphagia*, 2005. **20**(3): p. 210-7.
224. Perlman, P.W., et al., *The risk of aspiration of pureed food as determined by flexible endoscopic evaluation of swallowing with sensory testing*. *Otolaryngol Head Neck Surg*, 2004. **130**(1): p. 80-3.
225. Setzen, M., et al., *Laryngopharyngeal sensory deficits as a predictor of aspiration*. *Otolaryngol Head Neck Surg*, 2001. **124**(6): p. 622-4.
226. Chen, R., et al., *Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation*. *Neurology*, 1997. **48**(5): p. 1398-403.
227. Muellbacher, W., et al., *Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior*. *Clinical Neurophysiology*, 2000. **111**(6): p. 1002-7.

228. Michou, E., et al., *Targeting unlesioned pharyngeal motor cortex improves swallowing in healthy individuals and after dysphagic stroke*. *Gastroenterology*, 2012. **142**(1): p. 29-38.
229. Jayasekeran, V., et al., *Adjunctive functional pharyngeal electrical stimulation reverses swallowing disability after brain lesions*. *Gastroenterology*, 2010. **138**(5): p. 1737-46.
230. Verin, E., et al., *"Virtual" lesioning of the human oropharyngeal motor cortex: a videofluoroscopic study*. *Arch Phys Med Rehabil*, 2012. **93**(11): p. 1987-90.
231. Plonk, D.P., et al., *Effects of chemesthetic stimuli, age, and genetic taste groups on swallowing apnea duration*. *Otolaryngol Head Neck Surg*, 2011. **145**(4): p. 618-22.
232. Hu, J., et al., *Detection of near-atmospheric concentrations of CO₂ by an olfactory subsystem in the mouse*. *Science*, 2007. **317**(5840): p. 953-7.
233. Lahiri, S., et al., *Regulation of oxygen sensing in peripheral arterial chemoreceptors*. *Int J Biochem Cell Biol*, 2001. **33**(8): p. 755-74.
234. Humbert, I.A., et al., *Neurophysiology of swallowing: effects of age and bolus type*. *Neuroimage*, 2009. **44**(3): p. 982-91.

Appendix

Appendix A.1: Cortico-pharyngeal latencies - Chapter 3

Site	Baseline	0 min	15 mins	30 mins	45 mins	60 mins
Dominant	0.00821	0.00842	0.00965	0.00819	0.00953	0.00934
Non-Dominant	0.00915	0.00964	0.00929	0.01023	0.01017	0.00935
Hand	0.0235	0.02349	0.02356	0.02357	0.02351	0.02358

Table A.1.1: Averages of response latencies for water

Site	Baseline	0 min	15 mins	30 mins	45 mins	60 mins
Dominant	0.00822	0.00922	0.00823	0.01222	0.00827	0.0093
Non-Dominant	0.00919	0.00823	0.00922	0.01224	0.00823	0.00923
Hand	0.02334	0.02319	0.02336	0.02326	0.0233	0.02329

Table A.1.2: Averages of response latencies for carbonated solution

Site	Baseline	0 min	15 mins	30 mins	45 mins	60 mins
Dominant	0.00822	0.00922	0.00822	0.00921	0.00822	0.00921
Non-Dominant	0.00822	0.00921	0.00821	0.01212	0.00922	0.01207
Hand	0.02305	0.0225	0.02337	0.02334	0.0235	0.02371

Table A.1.3: Averages of response latencies for citric acid solution

Appendix A.: Cortico-pharyngeal latencies - Chapter 4

Site	Baseline	0 min	15 mins	30 mins	45 mins	60 mins
Dominant	0.008468	0.008573	0.008323	0.008677	0.008339	0.008637
non-Dominant	0.008512	0.008863	0.008258	0.009161	0.009024	0.009218
Hand	0.021633	0.021976	0.021927	0.022065	0.022048	0.022016

Table A.2.1: Averages of response latencies for water

Site	Baseline	0 min	15 mins	30 mins	45 mins	60 mins
Dominant	0.008651	0.008871	0.008584	0.008898	0.008889	0.008808
non-Dominant	0.008862	0.008495	0.008611	0.008754	0.008557	0.008817
Hand	0.021788	0.021828	0.021846	0.022204	0.022267	0.022419

Table A.2.2: Averages of response latencies for carbonated

Site	Baseline	0 min	15 mins	30 mins	45 mins	60 mins
Dominant	0.009012	0.00871	0.009032	0.008863	0.009315	0.009113
non-Dominant	0.008835	0.008734	0.008839	0.009194	0.009048	0.009435
Hand	0.021919	0.021548	0.022065	0.021903	0.022153	0.021992

Table A.2.3: Averages of response latencies for saliva swallows

Appendix A.3: Pilot study

A.3.1 Methods

A.3.1.1 Participants

Healthy volunteers were recruited through advertisements placed around Salford Royal Hospital and through contacting those on the departmental database of volunteers who have expressed an interest in participating in future research.

As this was a pilot study, only 4 healthy volunteers (two female, mean age 37.25 ± 11.18 (\pm SEM) years, age range from 22 to 70) have participated in this study. All subjects were in good health and received the information sheet prior to obtaining written informed consent. Also they had the opportunity to discuss any queries before and during the study. The volunteers were invited to attend the laboratory prior to starting the study in order to familiarise themselves with the equipment. All participants met the study inclusion criteria. Exclusion criteria included: a history of epilepsy, previous brain or throat surgery, cardiac pacemaker, prior history of swallowing difficulty, neurological disease, pregnancy, the presence of metal implants in eyes or head, or intake of any medication which acts on the central nervous system or gastrointestinal tract. The subjects attended on three separate visits, with each study taking approximately 2-2.5 hours and with at least 3-4 days between each visit. The approval for this study was granted by Great Manchester (central North West 7) Research Ethic Committee (10/H1008/61) and all studies were done in the clinical laboratory of the Gastrointestinal Physiology department at Salford Royal NHS Foundation.

A.3.1.2 Procedures

The previous methodology which utilized of PES, SRT, rTMS and different boluses for mineral water and carbonated solution swallowing remained identical for these studies. {Chapter two, page (76, 78), chapter four, page (128)}

A.3.1.3 Experimental Procedures

A.3.1.3.1 Protocol

Subjects attended on three separate days at least 3-4days apart. On each of the 3 occasions they received one of 2 different solutions to swallow in a single-blinded randomised manner: (carbonated solutions, non-carbonated solutions (mineral water)) and on the other occasion swallowed their own saliva. On each visit they were asked to swallow the pharyngeal EMG catheter, then sensory threshold with PES were recorded before swallowing, as described in chapter 2. A pair of electrodes was placed on the back of the subject's hand. This was used to deliver an electrical pulse which was cued the subject to swallow. Subjects were asked to perform baseline measurements of swallowing behaviour (30 swallows, 10 normal, 10 fast and 10 within a challenging time window). After that, the focal region of cortical suppression was created using 10 minutes of 1 Hz r TMS over the dominant swallowing motor cortex. Following the inhibitory 1 Hz stimulation swallowing reaction times and challenged swallows were performed at 15, 30, 45 and 60 minutes after baseline with each solution (still water, carbonated and citric acid) on each assigned randomised visit.

A.3.1.2 Data analysis

Four subjects were involved in this study and data was used for analysis. Variability of pharyngeal manometry within the three different conditions was assessed on SPSS 16.0 by calculating the intra-class correlation coefficients (ICC) for five runs of still water, five runs of carbonated solution and for five runs of saliva solution. The data were presented as mean (\pm SEM) and non-parametric Friedman's test was applied to three-sample designs to see the difference between still water, carbonated solution and saliva for each of three types of swallows (normal, fast and challenged). Then Wilcoxon's signed ranks test was used to see the difference between each run and its baseline for three types of swallows (normal, fast and challenged) in each solution. P value of less than 0.05 was taken as measure of statistical significance.

A.3.1.3 Result

A.3.1.3.1 Pharyngeal sensory threshold

The averages of both sensory and maximum tolerance pharyngeal thresholds are represented in table (A.3.1) below. All participants completed the studies with no adverse events.

Subject NO	Minimum (Sensory) Threshold (mA± SEM)	Maximum (tolerance) Threshold (mA± SEM)
1	3.22±1.50	4.39±1.6
2	4.63±0.44	10.50±1.91
3	0.65±0.12	4.53±0.77
4	2.87±0.22	5.23±0.62

Table A.3.1: Average of the pharyngeal sensory and maximum tolerated electrical thresholds for each subject (mA and SEM).

The baseline sensory and tolerance thresholds during the three studies were similar for each participant (Friedman test: sensory threshold: Chi squares: 3.500, $p=0.174$, tolerance threshold Chi squares: 0.500, $p=0.779$). The minimum (sensory) threshold ranged from 0.65 to 4.63 mA, whereas the maximum threshold ranged from 4.39 to 10.50 mA

A.3.1.3.2 Normal Swallowing Reaction Time

All raw data was analysed and the grand mean for each normal swallow solution was calculated. The following table (A.3.2) shows the grand mean and standard error of means for each solution of normal swallows. Also it shows the mean for each volunteer for the different three solutions.

Swallowing type	Still water	Carbonated solution	Saliva
Subject			
1	1724.2	1488.27	1560.47
2	1069.32	1293.63	1984.92
3	2624.04	2790.74	2107.53
4	1456.76	1549.22	1818.9
Mean(ms)	1718.58	1780.46	1867.95
SEM	165.2	170.57	59.17

Table A.3.2: The response latencies for normal swallowing reaction time. The table includes mean response latencies in milliseconds (ms) of the baseline and follow up four time points for each volunteer in the different two solutions and saliva swallows. The grand mean and standard error of the normal swallows for each solution also was included.

Intra-class correlation (ICC) was calculated to check the variability of the five time points for two solutions (still water, carbonated solution) and saliva swallows during the normal swallowing using SPSS16.0. The result for still water was 0.821, for carbonated solution was 0.821 and for saliva swallows was 0.921. This means a high level of correlation for the two solutions and saliva swallows. However, the variability of the carbonated liquid and still water looks more compared to saliva swallows, the saliva swallows had significant difference between baseline and different time points.

In addition, Friedman's test showed a significant difference between saliva swallows and other two solutions {carbonated solution versus saliva: Chi squares: 74.260, $p=0.000$, still water versus saliva: Chi squares: 68.702, $p=0.000$ }, but there was no significant difference between still water versus carbonated solution: Chi squares: 17.554, $p=0.093$.

According to result from the Wilcoxon's test which compared each time point to baseline, there was no significant change of the response latencies in two solutions (still water, carbonated solution) for the normal swallowing for all time-points compared to baseline, still water: baseline versus immediately swallows (Z value=-0.255 $p=0.799$), baseline versus 15 minutes (Z value=-1.070, $p=0.285$), baseline versus 30 minutes (Z value=-0.459, $p=0.646$), baseline versus 45 minutes (Z value=-0.255, $p=0.799$), baseline versus 60 minutes (Z value=-1.478, $p=0.139$), and for carbonated solution: baseline versus

immediately swallows (Z value=-1.070, p=0.285), baseline versus 15 minutes (Z value=-1.682, p=0.093), baseline versus 30 minutes (Z value=-1.478, p=0.139), baseline versus 45 minutes (Z value=-0.459, p=0.646), baseline versus 60 minutes (Z value=-0.153, p=0.878). However, there was a significant difference between the four time points(15, 30, 45, and 60 minutes) response latencies in saliva swallows (Z value=-2.803, p=0.005) and no significant difference between immediately swallows and baseline (Z value=-0.255, p=0.799).

A.3.1.3.3 Fast Swallowing Reaction Time

The grand mean for each fast swallow solution was calculated after the analysis of all raw data. The following table (A.3.3) shows the mean for each volunteer for the different three solutions, and the grand mean and standard error of means for each solution of fast swallows.

Swallowing type	Still water	Carbonated solution	Saliva
Subject NO			
1	1166.38	1050.71	1152.94
2	536.13	790.53	1734.37
3	495.71	770.98	677.05
4	1195.87	1255.51	1321.26
Mean(ms)	848.52	966.93	1221.4
SEM	96.15	57.7	109.37

Table A.3.3: The response latencies for fast swallowing reaction time. The table includes mean response latencies in milliseconds (ms) of the baseline and follow up four time points for each volunteer in two solutions and saliva swallows, the grand mean and standard error of the fast swallows for each solution and saliva.

Intra-class correlation (ICC) was calculated to check the variability of the five time points for each solution and saliva swallows during the fast swallowing using SPSS16.0. The result for still water was 0.933, for carbonated solution was 0.969 and for saliva swallows was 0.999. These results indicate that a high level of correlation for the three different

interventions, however, the great agreement between the values of saliva swallows compared to the other two solutions.

Friedman`s test showed a significant difference between three different interventions {carbonated solution versus saliva: Chi squares: 102.192, p=0.000, still water versus saliva: Chi squares: 99.147, p=0.000, still water versus carbonated solution: Chi squares: 101.339, p=0.000}.

Wilcoxon`s test showed significant difference between the five time points response latencies compared to baseline in the two solutions and saliva swallows except at 60 minutes for still water and carbonated solution. For the still water and carbonation solution, the first four time points (immediately, 15, 30, and 45 minutes) response latencies versus baseline are (Z value=-2.803, p=0.005), and baseline versus 60 minutes is (Z, value=-1.478, P= 0.139 and (Z, value=-0.663, P= 0.508) respectively. For saliva swallows, five time points (immediately, 15, 30, 45 and 60 minutes) response latencies versus baseline are (Z value=-2.803, p=0.005).

A.3.1.3.4 Challenged Swallows

The number of successful swallows from each run (10 swallows) was recorded and the percentage of the grand mean (average of five repeated runs) of correct swallows for each volunteer and for each solution was then calculated.

The following table (A.3.4) shows the grand mean percentage of correct swallows and standard error of means for each solution of challenged swallows. Also it shows the mean percentage of correct swallows for each volunteer for two solutions and saliva swallows.

Swallowing type	Still water	Carbonated solution	Saliva
Subject NO			
1	23.33	60	28.33
2	21.67	68.33	28.33
3	23.33	33.33	15
4	20	48.33	21.67
Mean (%)	22.08	52.5	26
SEM	0.4	3.8	1.6

Table A.3.4: The percentage of correct swallows for challenged swallowing reaction time. The table shows the means of percentage of correct swallows (baseline and five repeated runs) for each volunteer in two solutions and saliva swallows and the grand mean percentage of correct swallows and standard error of percentage of correct swallows for each solution and saliva swallows.

The mean swallowing time for the carbonated solution was higher than still water and saliva swallows. In addition, the Friedman's test showed a significant difference between carbonation solution and other two interventions {carbonated solution versus saliva: Chi squares: 37.510, $p=0.000$, carbonation solution versus water: Chi squares: 30.499, $p=0.001$ } and no significant difference between still water and saliva {still water versus saliva: Chi squares: 16.383, $p=0.127$ }

Intra-class correlation (ICC) was calculated to check the variability of the five time points for each solution and saliva swallows during the challenged swallowing using SPSS16.0. The result for still water was 0.59, for carbonated solution was 0.628 and for saliva swallows was 0.647. These results indicate that a lower level of correlation for the three different interventions, however, the Wilcoxon's test showed no significant difference between baseline and five time-point for carbonated solution and saliva swallows. For carbonated solution: baseline versus immediately swallows (Z value=-0.061, $p=0.951$), baseline versus 15 minutes (Z value=-0.175, $p=0.861$), baseline versus 30 minutes (Z value=-0.577, $p=0.564$), baseline versus 45 minutes (Z value=-0.730, $p=0.465$), baseline versus 60 minutes (Z value=-0.289, $p=0.773$). For saliva swallows, baseline versus immediately swallows (Z value=-0.707, $p=0.480$), baseline versus 15 minutes (Z value=-0.816, $p=0.414$), baseline versus 30 minutes (Z value=-1.100, $p=0.271$), baseline versus 45 minutes (Z value=-1.406, $p=0.160$), baseline versus 60 minutes (Z value=-0.707, $p=0.480$).

For still water: The result was mixed, there are no significant differences between the baseline versus immediate swallows (Z value=-0.707, p=0.480) and baseline versus 15 minutes (Z value=-0.307, p=0.763) whereas there are a significant differences between baseline versus 30 minutes (Z value=-1.994, p=0.046), baseline versus 45 minutes (Z value=-0.000, p=1.000), and baseline versus 60 minutes (Z value=-0.000, p=1.000).