Summer 2015

The effect of taste on swallowing function

Rachel Mulheren
James Madison University

The Effects of Taste on Swallowing Function

Rachel Mulheren

A dissertation submitted to the Graduate Faculty of

James Madison University

In

Partial Fulfillment of the Requirements

For the degree of

Doctor of Philosophy

Department of Communication Sciences and Disorders

August 2015

Table of Contents

List of Tables

Part I: Extended Literature Review

Part II: Article Manuscript

List of Figures

Part I: Extended Literature Review

Part II: Article Manuscript

Abstract

This study investigated the effects of taste on swallowing frequency and cortical activation in the swallowing network. The effects of salivary flow and taster status were also examined, along with genetic taster status. The effects of a 3ml bolus compared sour, sour with slow infusion, sweet, water, and water with infusion. Swallowing frequency was significantly higher 0-15 seconds after bolus delivery than 16-30 seconds. Swallowing frequency was higher in the sour conditions, whereas sweet and water did not differ. Functional near-infrared spectroscopy recordings measured changes in blood oxygenation (HbO) in the right and left hemispheres in the premotor, S1 and supplementary motor area in response to swallowing a bolus indicated a significant interaction of side and channel. Event-related analyses of HbO following bolus administration of taste solutions were significantly higher in the sensory than the motor area in the right hemisphere. A block average analysis of the response to taste between 17 and 22 seconds after bolus administration revealed significant differences between hemispheres and regions. Genetic taster status was not significant in any of the analyses. The highest activation in response to sour taste was in the motor regions of both hemispheres. The results indicated that sour taste effectively increased swallowing frequency and cortical activation while increasing salivary flow in comparison to water and sweet taste. In conclusion, sour taste may have peripheral effects on salivary flow while up-regulating the activation of the swallowing network at the cortical level.

Part I: Extended Literature Review

Chapter 1: Introduction

Background

Swallowing is a highly intricate process requiring neurological coordination of peripheral musculature and sensory feedback for successful deglutition. Swallowing is under voluntary and involuntary control. The healthy swallowing process may be disrupted by damage to the brain, to craniofacial and laryngeal musculature and structures, or to the nerves relaying motor and sensory information. Dysphagia, or disordered swallowing, manifests in a breakdown of the strength, timing and/or coordination of swallowing motor patterning or sensory triggers. Specific etiologies of dysphagia include stroke, traumatic brain injury, progressive neurological diseases, head and neck cancer, structural abnormalities, and tumors. The application of sensory stimulation to the oropharyngeal cavity is a potential method of rehabilitation for dysphagia, as research suggests that sensory input can modulate the coordination of swallowing at the level of the brainstem and cortex. The following chapters propose a course of study for establishing the effect of a sour bolus on swallowing frequency and cortical activation and examining the mechanisms responsible for this effect.

Swallowing Innervation

The coordination of swallowing musculature is controlled by motor efferents from the brainstem, and sensory afferents traveling to the brainstem from the larynx. The trigeminal nerve innervates the muscles of mastication through the maxillary and mandibular branches and provides sensory information from the anterior 2/3 of the tongue and the face, mouth and teeth. The facial nerve innervates the muscles of the face such as the lips and the buccinator and carries taste afferents from the anterior 2/3 of the

tongue. The glossopharyngeal nerve delivers efferents to the stylopharyngeus and carries afferents for taste and general sensation from the posterior 1/3 of the tongue and the soft palate and oropharynx. The vagus nerve supplies motor innervation to the pharynx, larynx, esophagus and soft palate; taste and sensation are delivered from the larynx, pharynx and trachea. The hypoglossal nerve innervates the intrinsic muscles of the tongue.

Swallowing Control in Animal Models

Early research in animals suggests that swallowing is both automatic and modifiable. Miller and Sherrington (1915) observed changes in swallowing latency of anesthetized cats as they altered bolus properties and stimulated the trigeminal and chorda tympani nerves and the floor of the fourth ventricle. However, Doty and Bosma (1956) found that the pattern of swallowing remained undisturbed by stimulation, transection, anesthesia, and manipulation to the pharynx and larynx in anesthetized cats, dogs and monkeys; they concluded that afferent and interneuron inputs were not required for a swallow to occur. Similarly, denervation of the inferior contrictor, posterior tongue, and thyrohyoid, anesthesia to the oropharynx, and motor paralysis did not change the motor sequencing of swallowing in anesthetized cats, suggesting an automatic patterning of swallowing (A. J. Miller, 1972b).

Peripheral stimulation and sectioning of the brainstem of the rostral reticular formation of the medulla and the rostral nucleus tractus solitarius (NTS) altered or eliminated the swallowing process in anesthetized cats, dogs and monkeys, indicating that these two areas may serve as swallowing central pattern generators (CPGs) (Doty, Richmond, & Storey, 1967). In support of this finding, electrical stimulation of the NTS

and the nucleus ambiguus (NA) induced swallowing in anesthetized cats (A. J. Miller, 1972a). The dorsal swallowing group (DSG) in the NTS is responsible for initiating and modulating the pattern of swallowing. The ventral swallow group (VSG) within the NA is driven by the DSG and communicates neural inputs to the motor neuron pools (A. Jean, 2001; Andre Jean & Dallaporta, 2006). Sumi (1963) found evidence for sensory input to the swallowing CPGs based on the selective response of sensory fibers of the superior laryngeal nerve (SLN) during deglutition in anesthetized cats, suggesting a direct pathway of sensory input to the brainstem.

The role of the cortex in modulating the brainstem swallowing reflex was elucidated by electrical stimulation of the anterolateral ("…lateral to the precentral area… or rostrolateral to the postcentral area…" (Sumi, 1969, p. 109)) cortex which resulted in changes to hypoglossal activity and swallowing rhythm in anesthetized rabbits (Sumi, 1970). Based on electrode stimulation to several brain structures of anesthetized sheep, Car, Jean & Roman (1975) posited a pathway of swallowing activation initiated by sensory stimulation to the SLN and traveling to either the NTS or to the pons and through the ventral posteromedial (VPM) thalamic nucleus to the orbitofrontal cortex. Miller and Bowman (1977) localized the intersection of the precentral gyrus and the Sylvian fissure as inducing a swallow in anesthetized monkeys. Evidence from animal studies suggests that swallowing activation follows both ipsilateral and contralateral pathways. Bilateral stimulation of the anterolateral cortices in anesthetized rabbits results in a higher frequency of repetitive swallows, although unilateral stimulation produced a swallow after severing the corpus callosum (Sumi, 1969), indicating ipsilateral input to the

brainstem CPG. Stimulation of the reticular formation induced a response in the contralateral frontal cortex (Sumi, 1972).

Neural Network of Swallowing in Humans

Neuroimaging of the activity of brain regions during swallowing has shown activation of many cortical and subcortical areas (M. L. Harris et al., 2005; Humbert & Robbins, 2007; Lowell et al., 2008; Malandraki, Sutton, Perlman, Karampinos, & Conway, 2009). However, several studies of healthy adults have highlighted the particular involvement of the primary motor (M1) and sensory (S1) cortices (Humbert & Robbins, 2007). During dry swallowing, positron emission tomography (PET) indicated the greatest increase in activation occurred bilaterally in the precentral gyrus and in portions of the postcentral gyrus (Zald & Pardo, 1999). Swallowing activated M1 and S1 as measured by magnetoencephalography (MEG), and anesthesia to the oropharynx decreased the activation of these areas during swallowing, supporting an impact of peripheral sensory input on cortical activity for swallowing (Teismann et al., 2007). The supplementary motor area (SMA) is also activated during swallowing (Lowell, et al., 2008; R. E. Martin et al., 2004; Satow et al., 2004) and may serve a "preparatory" role in motor planning.

To organize the various areas activated by swallowing into modules, Mosier and Bereznaya (2001) performed a principal components analysis of fMRI data during swallowing and found the following clusters of activation: sensorimotor and cingulate gyrus; inferior frontal gyrus, S2, corpus callosum, basal ganglia and thalamus; premotor cortex and posterior parietal lobe; cerebellum; insula. Path analysis of the data supported parallel connections between modules. Lowell et al. (2012) also explored the functional

connectivity of areas activated during swallowing; a cross correlation analysis of 5 specific areas with the rest of the brain indicated that the left insula had the most significant number of connections. Most recently, Babaei et al. (2013) found a bilateral increase in functional connectivity between the sensorimotor regions and other areas of the swallowing network. Based on the evidence of the role of S1, M1, and SMA in the swallowing network, these regions will be monitored for changes in cortical activation during swallowing.

Swallowing Frequency

Adults spontaneously swallow at a rate of approximately 1 swallow per minute when awake (Kapila, Dodds, Helm, & Hogan, 1984), with variation across individuals (range .19- 1.32/minute) (Lear, Flanagan, & Moorrees, 1965). Swallowing during sleep decreases significantly, with long periods during which no swallow occurs (Lear, et al., 1965). Spontaneous swallowing without a bolus is influenced by properties of saliva. Unstimulated saliva volume and flow rate significantly predicted the interval between swallows, with a high flow rate and low volume producing the highest frequency of swallowing ($r = .54$, $p < .05$) (Rudney, Ji, & Larson, 1995). Salivary flow does not change with age (Heft & Baum, 1984; Ship, Nolan, & Puckett, 1995; Tylenda, Ship, Fox, & Baum, 1988), thus the relationship with swallowing frequency is stable.

The frequency of swallowing is impacted by oral and oropharyngeal stimulation. In one study, swallowing frequency at baseline was reported as 4.31±.88 in a 5 minute period, or .862/minute, with an increase to 9.75 ±4.43, or 1.95/minute, with oropharyngeal air-pulse stimulation (Theurer, Bihari, Barr, & Martin, 2005). The frequency of swallowing and salivary flow each increased in response to intubation and a

peppermint lozenge (Kapila, et al., 1984). Swallowing frequency increased in patients with esophageal reflux from a baseline of .87/minute to 2.59/minute when reflux occurred (Bremner et al., 1993).

Swallowing frequency decreases with aging and medical compromise and is a sensitive measure of dysphagia. Young participants had a mean of 40.7±19.5/hour (.678/minute), whereas participants over 74 had a swallowing frequency of 9.4±4.9 (.157/minute); this value was even lower for bedridden elderly (6.8±3.3/hour, .113/minute) (Tanaka, Nohara, Kotani, Matsumura, & Sakai, 2013). Despite higher overall values in swallowing frequency (which may have been influenced by the method of instrumental examination), participants above the age of 60 had significantly fewer swallows (2.82/minute) than a group of young participants (2.96/minute); a third group of hospitalized patients over the age of 60 had the lowest swallowing frequency (.89/minute) (Murray, Langmore, Ginsberg, & Dostie, 1996).

Neuroanatomy of Taste

Taste is a critical component of deglutition as it signals nutritive (sweet, salty, sour, umami) vs. threatening (bitter) stimuli and impacts the pleasantness of eating. Taste preferences are shaped by food selection at birth, and can be modified throughout the lifespan by adaptation over time. Taste is detected by papillae in the mouth and throat and carried to the brainstem and cortex for processing. Taste buds in the fungiform papillae are located on the anterior two-thirds of the tongue and foliate and circumvallate papillae contain taste buds on the posterior tongue (Breslin & Huang, 2006). The majority of taste buds respond to more than one taste (Arvidson & Friberg, 1980).

The sensory process of taste begins peripherally and travels via neurochemical and electrochemical transmission to the brainstem, reticular formation, thalamus, and to the cortex and limbic structures. Taste transduction occurs through voltage-dependent ion channels and through direct permeation of ion channels in the epithelial cells of gustatory receptors (Breslin & Huang, 2006; Firestein, Margolskee, & Kinnamon, 1999). The neurotransmitters serotonin, glutamate, norepinephrine, GABA, and acetylcholine are present in mammalian taste buds (Herness et al., 2005). The chorda tympani branch of the facial nerve supplies the anterior 2/3 of the tongue including the fungiform papillae and the anterior foliate papillae of the tongue, and the greater superficial petrosal branch of the facial nerve supplies taste sensation to the soft palate ipsilaterally (Breslin & Huang, 2006; Lewis & Dandy, 1930). These two branches of the facial nerve then travel to the cell bodies of the geniculate ganglion. The lingual-tonsilar branch of the glossopharyngeal nerve carries taste information from the posterior 1/3 of the tongue including the posterior foliate and circumvallate papillae, to the cell bodies of the petrosal ganglion. Taste from the epiglottis, larynx, and pharynx and esophagus is carried by the SLN to the nodose ganglion.

The facial, glossopharyngeal and vagus nerves relay taste information to the NTS which projects to the VPM of the thalamus (Pritchard, Hamilton, & Norgren, 2000). Taste from the right and left sides of the tongue is under independent control and transmitted by a combination of neurochemical and electrochemical processes (Breslin & Huang, 2006). Each taste is projected by distinct pathways to the NTS, where the representation of tastes are spatially organized (Hashimoto, Doden, Ono, & Uematsu, 2012; Yokota, Eguchi, & Hiraba, 2014). Some of the projections to the thalamus and

cortex decussate at the level of pons, whereas others remain ipsilateral (Small, 2006). Taste is then carried to the primary gustatory cortex (PGC) located in the anterior/mid insula (Figure 1) (Iannilli, Noennig, Hummel, & Schoenfeld, 2014; Nakamura et al., 2013), which is also topographically organized in overlapping but unique patterns for each taste (Chen, Gabitto, Peng, Ryba, & Zuker, 2011). Projections to the superior insula are bilateral, whereas unilateral projections to the inferior insula are contralateral to the dominant hand (Faurion et al., 1999). Additional areas of activation during tasting include the orbitofrontal cortex (the "secondary gustatory cortex"), perisylvian region, supramarginal gyrus, frontal operculum, superior temporal gyrus, angular gyrus, cuneus, parahippocampal gyrus, anterior cingulate cortex, and prefrontal cortex (de Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003; Faurion, et al., 1999; Kringelbach, de Araujo, & Rolls, 2004; Okamoto, Dan, Clowney, Yamaguchi, & Dan, 2009a; Yamamoto et al., 2003). Figure 2 depicts the pathway for taste proposed in a review of taste disorders in humans and animal studies (Onoda, Ikeda, Sekine, & Ogawa, 2012).

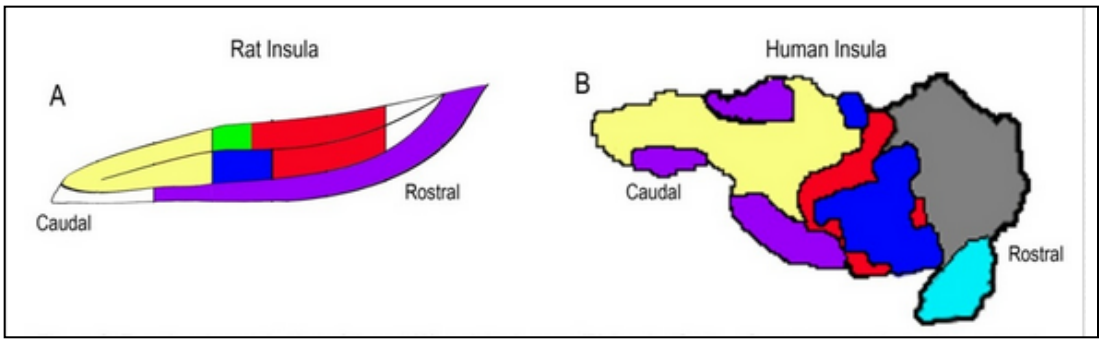


Figure 1. Functional organization of the rat and human insular cortex. Blue area represents taste-related functions. Adapted from Moraga-Amaro & Stehberg (2012).

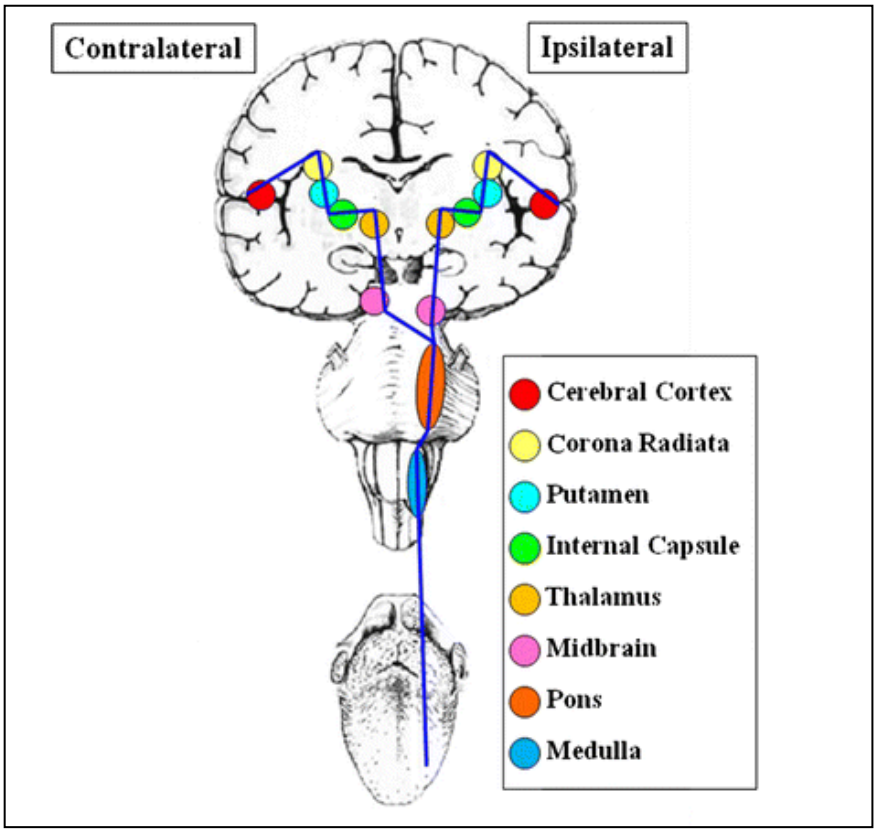


Figure 2. Central pathway for taste. Proposed by Onoda, Ikeda, Sekine, & Ogawa (2012)

Although several studies support the role of the PGC in taste sensation, a water bolus PET study of healthy participants found no difference in regional cerebral blood flow (rCBF) in this area during a taste recognition of sour or neutral; the authors suggest that this may be attributable to "the mechanical stimulation of [their] delivery method, because cells responsive to somatosensory stimulation of the mouth are extensively interspersed within the PGA…" (Small, Jones-Gotman, Zatorre, Petrides, & Evans, 1997b, pp. 5138-5139). Significant increases in rCBF were noted in the right and left caudolateral orbitofrontal cortex, the right anteromedial temporal lobe, and the right orbitofrontal cortex with sour as compared to water recognition. Based on these results and the finding of significantly increased sour recognition thresholds in patients with

unilateral anteromedial temporal lobe resection in comparison to patients with left resection of the same region and healthy controls, the authors propose that the right anteromedial temporal lobe is responsible for taste recognition (identification vs. sensation of taste) (Small, et al., 1997b).

Taste has a distinct temporal pattern in comparison to other sensory processes, and different tastes have unique temporal patterns of activation. The effect of taste on neural activity is slower (onset ~ 350 ms) and has a longer duration (up to 1 sec) than other somatosensory stimuli (Yamamoto, et al., 2003). An fMRI study found activation in the middle insula with "a sudden increase in activation induced by the salty taste… more rapidly but for a shorter duration than that for the sweet taste… activation due to the sweet taste increased more slowly and subsequently decreased more slowly than that for the salty taste" (Nakamura et al., 2012, p. 403). Similary, Kobayakawa et al. (1996) found shorter reaction times and shorter onset of gustatory magnetic fields (measured by magnetoencephalography) for salty tastants in comparison to sweet tastants.

In addition to differential temporal organization, tastes have unique patterns of spatial topography in the primary gustatory cortex. Two in vivo imaging studies of mice and rats found distinct spatial patterns of activation in the primary gustatory cortex in response to different tastes (Accolla, Bathellier, Petersen, & Carleton, 2007; Chen, et al., 2011). An fMRI study found similar resuls in the human primary gustatory cortex; despite individual variation in precise spatial patterns, overlapping but unique spatial patterns of activation were consistent within participants (Figure 3) (Schoenfeld et al., 2004).

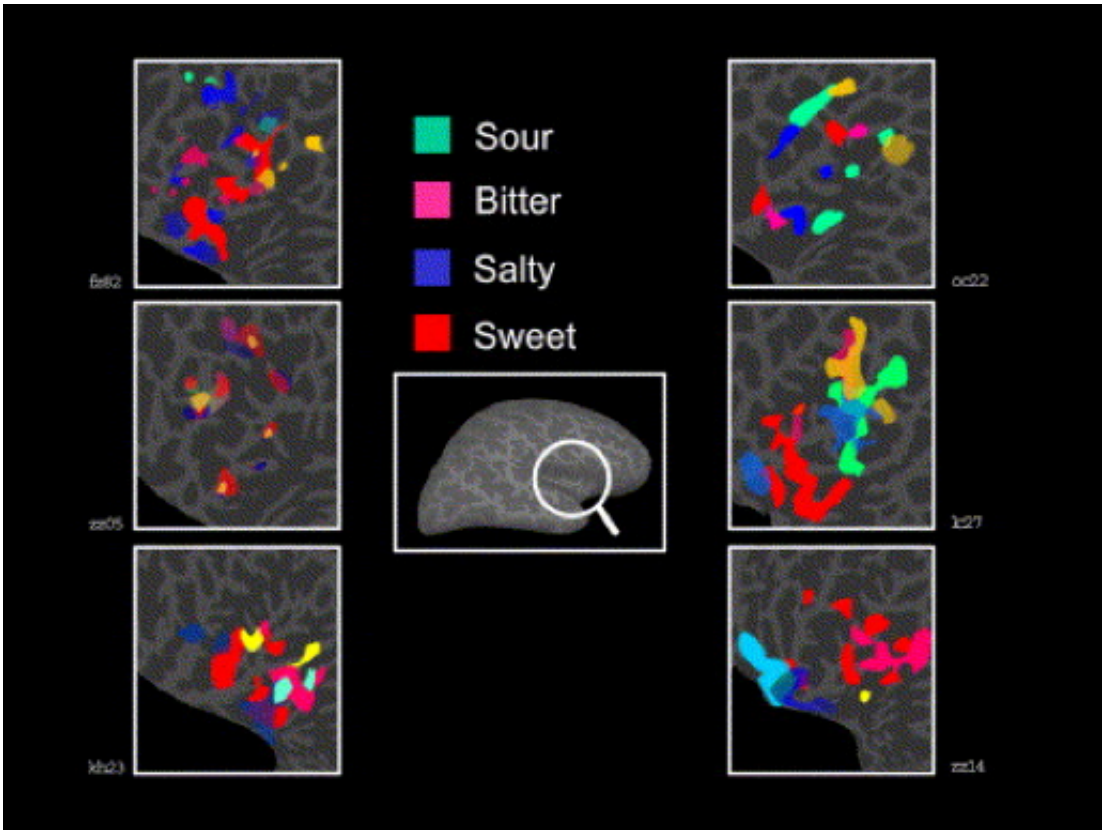


Figure 3. Activity in the insular/opercular cortex associated with individual tastes in 6 participants. Adapted from Schoenfeld et al. (2004).

Neural networks for taste are influenced by the nature of the tasting task and simultaneous cognitive processing. During passive tasting, connections between the left anterior insula and the bilateral amygdala are stronger. Assessment of taste presence (vs. tasteless stimulus) increases the connectivity between the orbitofrontal cortex and the striatum, cingulate and anterior insula. Further, assessment of pleasantness increases connections between the orbitofrontal cortex and the PGC (Bender, Veldhuizen, Meltzer, Gitelman, & Small, 2009). Connections between the insula and the hypothalamus, pallidum and striatum are greater for tasting nutritive stimuli (sweet and salty) than for bitter and astringent stimuli (Rudenga, Green, Nachtigal, & Small, 2010).

Measuring Taste Sensitivity

Absolute taste threshold detection is tested by gradually increasing the concentration of a tastant until a change in taste (compared to water) is reported by participants (H. Harris & Kalmus, 1949). The first increment above the absolute threshold that can be distinguished as a change is termed the just-noticeable difference. The psychophysical response to taste intensity is measured by the generalized Linear Magnitude Scale (gLMS), which matches intensity ratings across independent modalities (e.g. sound and taste) on an unmarked vertical line (Bartoshuk et al., 2004). Taster status is genetic and is determined by sensitivity to PTC/PROP, counting the number of fungiform papillae on the tongue surface, or a combination of these procedures.

A person with more fungiform papillae also has more taste buds and will perceive a higher taste intensity than someone with fewer fungiform papillae (I. J. Miller, Jr. & Reedy, 1990). Individuals with the highest perceptual intensity ratings are known as supertasters (Smith & Davis, 2000), whereas nontasters have very low intensity ratings of the same stimulus. The following percentages represent the approximate distribution of taster status in the general population: 25% supertasters, 50% medium tasters, 25% nontasters (Bartoshuk, et al., 2004). Females are more likely to be supertasters, but there is no effect for age or ethnicity (Bartoshuk, Duffy, & Miller, 1994). Though limited, recent research suggests that taster groups may differ in cortical activation for taste. A study of healthy participants found that taster status was significantly positively correlated with BOLD response in S1, S2, the middle and anterior insula, and the anterior cingulate when tasting fat emulsions (Eldeghaidy et al., 2011). Additionally, supertasters

had more activation in the bilateral ventrolateral prefrontal cortex than nontasters while tasting PROP-impregnated filter paper (Bembich et al., 2010).

Although taster status is not impacted by aging, the natural aging process does involve a decrease in sensitivity to taste (Breslin & Huang, 2006). The threshold for detection and identification of all tastes increases significantly and the perception of intensity differences decreases in individuals above 65 years of age (Methven, Allen, Withers, & Gosney, 2012). However, reduced sensitivity due to aging is "discrete" (localized to a specific area), and whole-mouth assessment of taste with high intensities of taste reveals no difference between young and old participants (Bartoshuk, 1989). Perception of taste intensity is also heightened by temporal and spatial summation (Breslin, 2000).

Overlap of Taste and Swallowing

A discussion of the functional neuroanatomy of the taste system indicates an overlap of taste and other sensations critical to healthy swallowing function. At the cranial nerve level, the glossopharyngeal nerve carries taste as well as all other sensory information from the posterior tongue; similarly, the SLN carries taste and sensory information from the larynx and pharynx. The convergence of taste fibers in the NTS may contribute to the activation of the swallowing CPGs at the level of the brainstem. At the cortical and limbic levels, taste may serve as a cue to initiate the swallowing pattern based on hedonics and nutritional value. Similarly, taste may modify the timing and muscle movement for swallowing. The concentration of taste may also alter the swallowing pattern by enhancing the level of awareness of a bolus in the mouth and pharynx/larynx. A stronger taste may be more likely to induce a swallow, and the

resulting swallows may be more numerous, of shorter latency, and of higher muscular amplitude than without taste. Based on the overlap of taste and swallowing neuroanatomy, it is proposed that a taste stimulus will alter swallowing function at both peripheral and central levels.

Taste is more resistant to complete peripheral damage than other sensations (e.g. smell) due to ipsilateral, independent projections to each side of the tongue. The frequency of hypogeusia (reduced taste sensitivity) is approximately 5%, whereas total ageusia is rare (Welge-Lussen, Dorig, Wolfensberger, Krone, & Hummel, 2011). Although a disorder of taste transduction or perception would lessen sensory input for swallowing, it would likely have little impact on overall swallowing function provided that sensory feedback such as bolus size and consistency remain intact. No known literature suggests that a disorder of taste would lead to dysphagia. Despite these strengths as a therapy method, however, taste is highly subjective and may be altered by smoking, neurological damage, eating disorders, and weight (Bartoshuk, 1989; Bertoli et al., 2014; Pavlidis, Gouveris, Kekes, & Maurer, 2014); therefore the threshold for excitation of motor swallowing structures by means of afferent taste projections may vary across individuals. For this reason, exclusionary criteria will be applied to participants in order to diminish the effect of mediating variables on taste sensation.

Taste and Salivation

Tasting a sour bolus affects the flow of salivation. Participants salivated and swallowed more when tasting lemon juice than when tasting water ($F(1,12)= 50.1$, $p< 0.001$) (Nederkoorn, Smulders, & Jansen, 1999). A sour taste induces a higher salivary flow rate than food or other taste stimuli (Lashley, 1916; Watanabe & Dawes, 1988).

Salivary flow increases as concentration of taste stimuli (NaCl, sucrose, citric acid) increase (Dawes & Watanabe, 1987; Watanabe & Dawes, 1988), and more intense perceptual ratings of taste are significantly correlated with a higher salivary flow (Bonnans & Noble, 1995) (boluses were tasted then expectorated). Maximum salivary flow occurs at approximately 9.4 sec after the onset of taste stimulus delivery (Dawes & Watanabe, 1987). Although adaptation to taste reduces its impact on salivary flow, oral movements counter adaptation by distributing the taste across several receptors in order to prolong the heightened salivary flow response to taste (Theunissen, Kroeze, & Schifferstein, 2000; Watanabe & Dawes, 1988). Similar to taste and swallowing, the neural control of salivation includes the NTS as part of the brainstem connections of efferent and afferent information to the peripheral salivary glands (Chatfield, 1941; Contreras, Gomez, & Norgren, 1980; Magoun & Beaton, 1942; Wang, 1943); therefore, taste modifies salivary flow at the level of the brainstem.

A portion of patients with xerostomia, or impaired salivation, also exhibits disturbances in taste perception. Heightened taste detection thresholds in patients with xerostomia (Gomez, Cassis-Nosthas, Morales-de-Leon, & Bourges, 2004; Henkin, Talal, Larson, & Mattern, 1972; Weiffenbach, Schwartz, Atkinson, & Fox, 1995) may be related to degeneration and abnormalities of the taste buds observed in Sjogren's syndrome (Henkin, et al., 1972) or to reduced availability of saliva to transmit stimuli to taste receptors (Hershkovich & Nagler, 2004; Negoro et al., 2004).

Due to the effects of taste on salivation, this study will attempt to control for these effects by comparing trials of liquid taste stimuli with trials of liquid taste stimuli coupled with a slow infusion of water. This slow infusion rate will provide adaptation to the flow

of a stimulus in the oral cavity and control variations in salivary flow that would otherwise occur due to the presence and taste of a bolus.

Chapter 2: Literature Review

Animal Studies of Taste and Swallowing

One study examined the effect of taste on swallowing functions in anesthetized rats. Infusion of acetic acid (taste, smell, and chemesthesis) and citric acid to the pharynx and larynx was found to elicit a higher swallowing frequency than water, whereas NaCl elicited a lower frequency; increasing concentrations of acetic acid produced more swallows (Kajii et al., 2002). Interestingly, the effect of taste was significant despite severed salivary glands, leading the authors to conclude that swallow frequency increased due to sensory input rather than due to increased salivary flow.

Human Studies of Sour Stimulus and Swallowing

Sour taste vs. Sour flavor

It is not possible to compare between studies with a pure taste stimulus and studies that use a sour flavor, which includes the additional sensory input of smell and somatosensation (Small & Prescott, 2005). In addition, the perceptions of taste, smell, and flavor have significantly distinct patterns of regional cerebral blood flow activation (Small, Jones-Gotman, Zatorre, Petrides, & Evans, 1997a). A number of studies have examined the effect of a sour stimulus on swallowing outcomes; however, the specific substance used to create a sour bolus differs between studies. Thirteen studies utilized lemon juice (Abdul Wahab, Jones, & Huckabee, 2010; Alves, Fabio, & Dantas, 2013; Cola et al., 2012; Cola et al., 2010; Ding, Logemann, Larson, & Rademaker, 2003; Gatto et al., 2013; Hamdy et al., 2003; Lee et al., 2012; Logemann et al., 1995; Palmer, McCulloch, Jaffe, & Neel, 2005; Pauloski et al., 2013; Sciortino, Liss, Case, Gerritsen, &

Katz, 2003; Wahab, Jones, & Huckabee, 2011), which provides a sour flavor (taste + smell + chemesthesis),whereas 11 studies used citric acid (a pure taste) (Chee, Arshad, Singh, Mistry, & Hamdy, 2005; Humbert, Lokhande, Christopherson, German, & Stone, 2012; Humbert & McLaren, 2014; Leow, Huckabee, Sharma, & Tooley, 2007; Miura, Morita, Koizumi, & Shingai, 2009; Nagy, Steele, & Pelletier, 2014b; Pelletier & Dhanaraj, 2006; Pelletier & Lawless, 2003; Pelletier & Steele, 2014; Pouderoux, Logemann, & Kahrilas, 1996; Steele, van Lieshout, & Pelletier, 2012). One study utilized lemonade (Babaei et al., 2010), which in addition to sour flavor also has added sugar and sweet flavor, and two studies presented HCl (pure taste) (Miyaoka et al., 2006).

Taste and Swallowing Function in Healthy Participants

Several studies have investigated the effect of a sour bolus on parameters of swallowing function such as EMG response, oropharyngeal pressure, frequency of swallowing, and timing of swallowing events. Significant increases in the amplitude of electromyographic (EMG) responses from suprahyoid muscles have been noted following a sour bolus diluted in water in comparison to water alone (Miura, et al., 2009; Palmer, et al., 2005; Pelletier & Steele, 2014) and other tastes (Leow, et al., 2007). One study also found that the power of EMG response increased with a sour bolus (Miura, et al., 2009). In contrast, two studies found no difference in EMG amplitude between tastes dissolved in a thickening agent (Miyaoka, et al., 2006) or between a combination of sour taste delivered by filter paper and smell and water (Wahab, et al., 2011).

Examination of the timing of swallowing as measured by EMG has yielded mixed results. As compared to a water bolus, a sour bolus has been found to reduce the latency of the onset of a pharyngeal swallow in isolation (Ding, et al., 2003) and in combination

with mechanical and cold stimulation (Sciortino, et al., 2003); however, one study found no effect of the 4 tastes on pharyngeal swallow onset in comparison to water (Pouderoux, et al., 1996). With the exception of one study, which reported prolonged EMG duration after sour, salty, and bitter in comparison to water and sweet stimulation (Leow, et al., 2007), no effect of sour bolus was found on swallow EMG duration in comparison to control stimulation (Miyaoka, et al., 2006; Palmer, et al., 2005; Sciortino, et al., 2003).

Lingual pressure increases immediately after stimulation with a sour bolus compared with water (Wahab, et al., 2011), particularly for high concentrations (Nagy, Steele, et al., 2014b; Pelletier & Dhanaraj, 2006; Pelletier & Steele, 2014), whereas pressure in the hypopharynx decreases (Wahab, et al., 2011). A sour bolus has been shown to increase the frequency of swallowing in comparison to water (Nederkoorn, et al., 1999). A sour bolus has also been associated with reduced duration of tongue movement during swallowing (Steele, et al., 2012), slower swallowing speed (Chee, et al., 2005; Hamdy, et al., 2003), and prolonged swallowing apnea duration in comparison to water (Plonk, Butler, Grace-Martin, & Pelletier, 2011). In contrast to these results, one study found no effect of taste on kinematics of oropharyngeal swallowing when compared to water (Humbert, et al., 2012).

Sour Stimuli and Swallowing Function in Dysphagia

The majority of research on the effect of sour boluses in dysphagia patients has focused on stroke and neurogenic etiologies. One study found that taste disorders are present in 30% of acute stroke patients, occurring most frequently in cases of frontal cerebrovascular accident (CVA) (Heckmann et al., 2005). In this study, significant predictors for taste disorder included sex (male), National Institutes of Health Stroke

Scale score, dysphagia, and partial anterior circular subtype CVA; resolution of taste disorders improved in a subset of patients at 3 months post-infarct. Neurodegenerative conditions, such as Alzheimer's Disease and Parkinson's Disease (27% prevalence (Shah et al., 2009)), and neoplasm may also affect taste (Heckman & Lang, 2006). Due to the evidence that dysphagia and hypogeusia may co-occur, a sour stimulus may be less effective for altering swallowing function in a subset of patients. Taste function should be assessed and factors such as localization and CVA type should be considered prior to implementing this form of therapeutic stimulation in these patient populations.

In a study of the effects of lemon juice combined with barium on swallowing measures during videofluoroscopy, stroke patient demonstrated decreases in oral transit time (OTT), swallow onset time, pharyngeal delay time, and pharyngeal transit time (PTT) and an increase in swallowing efficiency (% bolus swallowed through esophagus divided by total transit time) relative to swallows of barium with no taste added, whereas patient with other neurogenic etiologies of dysphagia had decreased swallow onset time and time of tongue base contact to pharyngeal wall and a later tongue base retraction onset (Logemann, et al., 1995). Patients with neurological etiologies of dysphagia had a significantly lower Pen-Asp score and a shorter OTT after a stimulus of lemon juice mixed with barium when compared to a water and barium mixture (Lee, et al., 2012). Significant reduction of overall penetration and aspiration and increase in the number of spontaneous swallows corresponded to boluses of citric acid compared to water boluses in neurogenic dysphagia patients (Pelletier & Lawless, 2003). A sour and cold bolus had the most pronounced reduction of OTT, oral phase duration, and PTT in ischemic stroke patients as compared to a water bolus (Cola, et al., 2012; Cola, et al., 2010; Gatto, et al.,

2013). Cold and sour boluses were also found to slow swallowing speed and capacity (volume per swallow) in stroke patients in comparison to water boluses (Hamdy, et al., 2003). A study focusing on the esophageal phase of stroke patients found that swallowing lemon juice resulted in significantly longer transit and clearance times in the distal esophagus in comparison to other tastes and water, whereas sucrose resulted in larger amounts of esophageal residue in patients than in controls (Alves, et al., 2013).

A study of head and neck cancer patients who had undergone chemoradiation and healthy controls found that a lemon juice barium mixture significantly reduced PTT immediately after and at 1 and 3 months following chemoradiation (or first experimental session) in patients and controls groups of participants in comparison to a neutral barium stimulus (Pauloski, et al., 2013). Although chemoradiation to the oral cavity may reduce sensitivity to taste (Vernia et al.), and the sample included patients with chemoradiation to this area, results were comparable between patients and controls in this study. In summary, a sour bolus has been shown to reduce latency and slow speed and capacity of oropharyngeal swallowing, to reduce penetration and aspiration, and to increase swallowing frequency and timing of the esophageal phase in dysphagia patients with neurological and chemoradiation etiologies.

Cortical Activation for Taste and Swallowing

A small number of recent fMRI studies have examined hemispheric activation of the swallowing network in response to taste. A greater change in blood flow occurred in response to flavored liquid and solid stimuli (lemonade, chocolate milk, popcorn) than to non-flavored stimuli (dry swallow or water) specific to the cingulate gyrus, prefrontal cortex, S1, and M1 (Babaei, et al., 2010). Swallowing a sour bolus yielded activity in S1,

anterior cingulate cortex, insula, supplementary motor area, inferior frontal gyrus, and inferior parietal gyrus; activation was higher for sour than water in the SMA, and a gradual sensitization (increase in BOLD response over 20 swallows) to the sour bolus was noted in S1 and the inferior parietal gyrus (Humbert & Joel, 2012). A subsequent study found significantly increased interconnectivity within the insula and between the insula and M1, S1, inferior frontal gyrus, supramarginal gyrus, and rolandic operculum when tasting a sour stimulus, with results lateralized to the left hemisphere (Humbert & McLaren, 2014). In summary, taste enhances cortical activation in areas known to be activated during swallowing.

Transcranial Magnetic Stimulation (TMS) has also been utilized to study changes in corticobulbar activity related to taste presentations. The amplitude of pharyngeal magnetic evoked potentials (MEPs) at rest decreased 30 minutes after swallowing an oral infusion of sweet and bitter tastes (Mistry, Rothwell, Thompson, & Hamdy, 2006). The amplitude of submental MEPs during swallowing was significantly greater at 30, 60 and 90 minutes following sour taste and smelling lemon odor in comparison to baseline (Abdul Wahab, et al., 2010). The discrepancy in the direction of amplitude change may be due to the use of different tastants. Additionally, the first study examined MEPs at rest whereas the second recorded MEPs during swallowing.

Limitations of Comparisons of the Literature on Taste and Swallowing

As previously noted, the sensory differences between a pure taste and flavor limit the validity of comparisons between studies. Parameters of swallowing function are measured by varying techniques across studies (e.g. eEMG, articulography, manometry, MBS). The concentration, viscosity, volume, and temperature of sour solutions varied

across studies of healthy and patient samples; each of these variables differentially impacts swallowing independently of taste (Clave et al., 2006; Dantas, Dodds, Massey, & Kern, 1989; Kendall, Leonard, & McKenzie, 2001; Nagy, Molfenter, Peladeau-Pigeon, Stokely, & Steele, 2014; Nagy, Steele, et al., 2014b; Triadafilopoulos, Tsang, & Segall, 1998), complicating the process of comparisons across studies. Delivery method (tubing to different locations in the oral & pharyngeal cavities, anterior faucial pillar stimulation, spoon, cup) and solvent (barium, water, filter paper, gelatin) varied across studies. Different etiologies of dysphagia in patient samples as well as different loci of injury hinders direct comparison, as different mechanisms may be responsible for disordered swallowing and the potential for recovery. Patient studies also reported differing times post-onset, indicating that some patients may have had more time for spontaneous recovery.

Taster Status and Swallowing Function

To date, 6 studies have examined the effect of taster status on swallowing function with mixed results. Higher linguapalatal pressure have been reported specific to supertasters when swallowing high concentrations of the 4 tastes (Nagy, Steele, et al., 2014b). Supertasters also had increases in submental EMG amplitudes at higher intensities of sour (Pelletier & Steele, 2014). However, no differences in taster status were observed on linguapalatal pressure and submental EMG when swallowing the 4 tastes, an ethanol mixture, and seltzer with and without barium (Nagy, Steele, & Pelletier, 2014a). Swallowing apnea duration did not differ by taster status when swallowing 4 tastes (Todd, Butler, Plonk, Grace-Martin, & Pelletier, 2012b) or barium mixtures (citric acid, carbonation, ethanol) (Todd, Butler, Plonk, Grace-Martin, & Pelletier, 2012a);

however, when barium was added to the tastes, nontasters had longer apnea duration than when swallowing the taste stimuli without barium (no effect for supertasters) (Todd, et al., 2012b). In contrast, supertasters were found to have longer swallowing apnea duration than nontasters when swallowing a strong citric acid solution and other chemesthetic stimuli (Plonk, et al., 2011). Despite mixed results, this limited body of research suggests that taster status may mediate the effect of taste stimuli on swallowing function, and thus will be included in this study.

Salivation and Swallowing

A study of healthy participants found no significant effect of salivary flow on the timing of swallowing (Sonies, Ship, & Baum, 1989); However, dysphagia is reported more frequently by patients with salivary dysfunction than by healthy controls (Kaplan, Zuk-Paz, & Wolff, 2008; Rhodus, Moller, Colby, & Bereuter, 1995). In comparison to healthy controls, patients with salivary gland dysfunction had significantly lower swallowing frequency and a longer PTT (Rhodus, et al., 1995), longer oral phase (Hughes et al., 1987), longer swallowing duration (Caruso, Sonies, Atkinson, & Fox, 1989).

Despite more frequent complaints of dysphagia and reduced salivary weight following chemoradiation for head and neck cancer, no relationship could be established between salivary weight and OTT, pharyngeal delay, and oropharyngeal residue (Logemann et al., 2003; Logemann et al., 2001). Similar results were found in a sample of patients with Sjogren's syndrome; these patients did have a significantly higher rate of oropharyngeal residue and penetration than healthy controls, but these values were within the normal range (Rogus-Pulia & Logemann, 2011). Patients with Sjogren's syndrome

and healthy controls have similar pharyngeal and esophageal manometric values, though abnormal peristalsis has been noted in approximately 30% of patients (Anselmino et al., 1997).

Chapter 3: Pilot Study and Methods

Rationale

The purpose of this study is to examine the effect of taste on swallowing function. Due to the influence of sensory stimulation at the level of the brainstem and cortex, taste may be a useful tool for rehabilitating patients with dysphagia secondary to neurological disorder. Before implementing taste in dysphagia therapy, it is important to determine if taste does have an effect on swallowing and the mechanism driving this potential effect. Although taste thresholds may be elevated in a subset of dysphagia patients due to natural aging processes, disease, or chemoradiation, these patients may still benefit from suprathreshold taste stimulation. When a taste disorder is suspected, patient should be tested to determine if they are candidates for this modality of sensory stimulation and at what intensity the stimulation be presented. The rarity of complete ageusia also supports targeting this sensory modality in dysphagia therapy.

The mechanisms involved in the effect of taste on swallowing have yet to be thoroughly elucidated. The only animal study on this topic suggests modulation of swallowing frequency at the level of the brainstem specific to sour tastes, particularly at high concentrations, in comparison to water, whereas salty taste had the reverse effect (Kajii, et al., 2002). Changes in activation of the cortical swallowing network in response a sour stimulus suggest that taste also mediates swallowing at the level of the cortex,

primarily in M1, S1, and the cingulate cortex (Abdul Wahab, et al., 2010; Babaei, et al., 2010; Humbert, et al., 2012; Humbert & McLaren, 2014).

Although the study by Kajii et al. (2002) suggested that the modulation of swallowing by sour taste at the level of the brainstem is not mediated by salivation, it is not known if this effect is similar in humans. The extent of current evidence indicates that a sour taste, in comparison to water and other tastes, maximally increases both swallowing frequency and salivary flow. Therefore it is necessary to consider the potential mediating effect of salivary flow on swallowing response and cortical activation of the swallowing network to taste. Sour is a preferable candidate for up-regulating the swallowing network as it is less susceptible to adaptation over time (Theunissen, Polet, Kroeze, & Schifferstein, 2000) and induces a higher salivary flow rate (Lashley, 1916; Watanabe & Dawes, 1988). In contrast, sweet taste has been shown to activate a large area of the primary gustatory cortex in humans (Schoenfeld, et al., 2004).

Based on limited literature examining the effect of taster status on cortical activation for taste, taster status likely mediates the effect. A small body of research suggests that supertasters have stronger motor swallowing responses (pressure, EMG amplitude, apnea duration) to strong concentrations of sour. It is not known how taster status may modulate the cortical activation for taste, or if differences in taster status and cortical activation would be correlated with differences in physiological swallowing characteristics. In the studies of taster status and swallowing function, the effect of taster status became evident with stronger concentrations. However, the literature concerning taste concentration and swallowing function is limited. One study found no concentration effect on timing of tongue movement in response to citric acid stimuli (Steele, et al.,

2012), whereas another found higher linguapalatal pressures in response to high concentrations of sour and salty stimuli (Nagy, Steele, et al., 2014b). If response to taste is in fact mediated by concentration, then patients may exhibit differential responses to a uniform concentration.

In conclusion, it remains to be determined how taster status mediates the effect of sour taste on swallowing, if the effect of sour taste on swallowing is mediated by changes to salivary flow, and if swallowing function and cortical activation differ in response to different tastes. Given the potential mediating effect of these factors, patients may respond differently to sour stimulation due to differences in genetic taster status, salivary flow, or in contrast to sweet. This study aims to answer these questions in order to determine if these factors need to be taken into consideration for implementation of taste stimulation in dysphagia management.

Unique Contributions

Only 2 published studies to date (available in English) have examined cortical activation for swallowing using functional near-infrared spectroscopy (fNIRS); one presented a comparison of motor imagery and motor execution of swallowing water (Kober & Wood, 2014) and the second study investigated the effect of pharyngoesophageal air and water stimulation on frontoparietal cortical responses in premature neonates (Jadcherla et al., 2014). Although many studies have utilized fMRI to measure cortical activation during swallowing, several aspects of fNIRS are more amenable to this line of research. Unlike fMRI, FNIRS allows participants to remain in a natural, upright position during swallowing tasks. FNIRS is better equipped to manage motion artifact, which is inevitable with movement during swallowing, in the signal that

fMRI. Although fNIRS is currently limited to examining activation patterns in the cortex, it has a higher temporal resolution than fMRI. For these reasons, this study will contribute to the existing literature on cortical activation when swallowing a sour bolus by introducing unique instrumentation that is more suitable to studies of swallowing function.

Although the effect of taste on swallowing function has been examined in the past, the potential mediating effect of salivary flow on swallowing function in response to taste stimuli has not been in explored. If, as the literature suggests, a sour taste increases salivary flow, an increase in swallowing frequency would follow in order to manage higher levels of saliva in the oral cavity; therefore, rather than directly influencing swallowing function via sensory input at the brainstem and cortical levels, taste may serve as the first step in a chain of events that result in higher swallowing frequency. Because of the impact of sour taste on salivation, which in turn requires an increase in swallowing frequency, manual delivery of sour and water boluses will include trial with and without a slow infusion of water. The purpose of the slow infusion is to provide habituation to the flow of a bolus at a rate that will not significantly influence swallowing frequency.

An additional problem that will be addressed in this study is the impact of taster status on the degree of response to sour stimulation in swallowing physiology and cortical activation for swallowing. Taster status of participants will be collected as the literature suggests that this variable impacts taste sensitivity; if certain individuals are more sensitive to the same level of taste concentration than others, the physiological response to gustatory stimulation may also differ in terms of swallowing. Five studies

have examined the impact of taster status on swallowing function with mixed results. One study found that supertasters had higher submental EMG amplitudes at higher intensities of citric acid (Pelletier & Steele, 2014). Two studies found that supertasters had longer swallowing apnea duration that nontasters when tasting citric acid and chemesthetic stimuli (Plonk, et al., 2011) and another reported shorter swallowing apnea duration in supertasters and a longer apnea duration for nontasters when barium was added to taste stimuli (Todd, et al., 2012b.). The remaining studies found no difference between supertasters and nontasters on swallowing apnea duration with barium (Todd, et al., 2012a) and different tastants and on submental EMG and palatal pressure (Nagy, Steele, et al., 2014b). This study will be the first to examine the effect of taster status on the outcome measure of swallowing frequency. In addition, only two studies have examined cortical activation patterns in participants of different taster status; these studies reported a significant difference between taster groups on hemodynamic response in response to fat emulsions (Eldeghaidy, et al., 2011) and in response to tasting a filter paper impregnated with PROP (Bembich, et al., 2010). No known studies have examined the effect of taster status on cortical activation in response to swallowing a tastant, therefore this study will introduce a new line of evidence.

Purpose and Hypotheses

The purpose of this study is to evaluate the effect of sour taste on spontaneous swallowing frequency and cortical activation in healthy participants from different taster groups. The response to sour stimuli will be compared to water and a sweet stimulus on the outcome measures (swallowing frequency and cortical activation), in different taster groups. As salivation often increases with taste of a sour bolus, it is possible that

swallowing increases are due to a combined effect of increases in salivation and taste. As the addition of water infusion in both conditions (sour and water bolus) would reduce the effect increases in salivation, it is expected that there will be less increase in swallowing frequency between sour with infusion and water with infusion in both taster groups. It is predicted that taste conditions including the slow infusion will result in less increase in swallowing frequency and less increase in cortical activation than in conditions without the slow infusion due to habituation to the flow of a bolus.

Hypotheses include:

1. Increases in swallowing in response to a sour stimulus will be greater in supertasters than nontasters, and that supertasters would have greater increases in cortical activation in response to a tastant.
2. Increases in swallowing frequency will be positively related to increases in oxygenated hemoglobin in M1.
3. Swallowing frequency and cortical activation will be greater for sour than for sweet stimuli.
4. The addition of continuous infusion will interfere with taste-induced increases in salivary flow and reduce responses to a tastant.

The results of this research will indicate how sour taste affects the frequency of swallowing and cortical activation for sensory and motor control of swallowing in different taster groups.

Pilot Study

A pilot study of 3 participants was conducted in order to assess the feasibility of the methodology presented below. Two of the 3 participants yielded complete datasets.

Participants 101 and 103 are supertasters. Table 1 and Figures 4-9 depict results from the study.

Table 1

Swallowing Frequency by Condition for Pilot Study Participants

	Sour	Sour + infusion	Water	Water + infusion	Control (no stimulus)
101	66	68	45	43	17
102	-	-	74	78	23
103	77	99	72	60	23

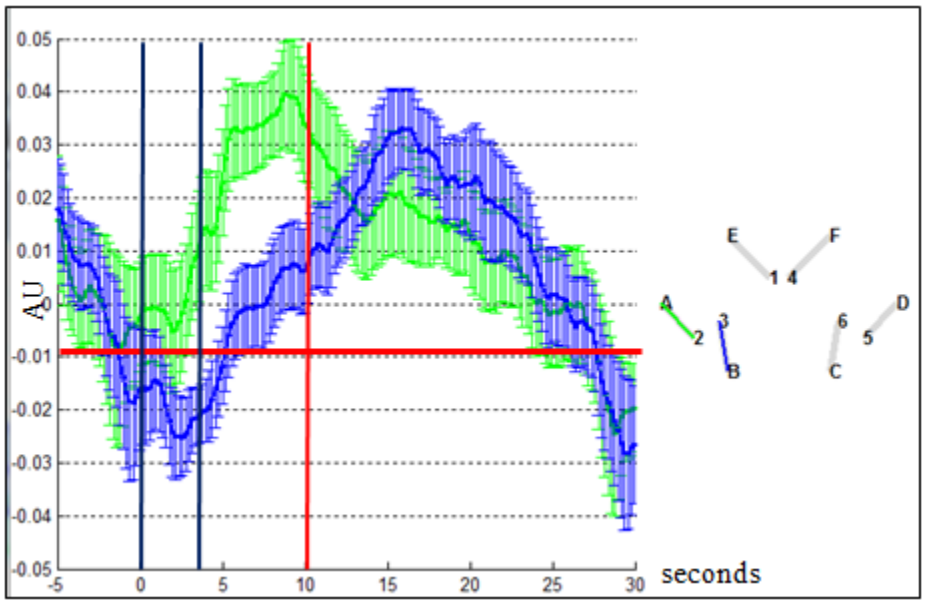


Figure 4. Response to sour stimulation in the right M1 (green) and S1 (blue), participant 101. Units on the y-axis are arbitrary. Units on the x-axis are seconds. Red horizontal line indicates level of baseline cortical activity. Vertical black line at 0 seconds indicates onset of bolus delivery. Vertical black line at 3 seconds indicates completion of bolus delivery. Vertical red line at 10 seconds indicates onset of response to taste. Epochs with swallows between 12 and 18 seconds after onset of bolus delivery removed.

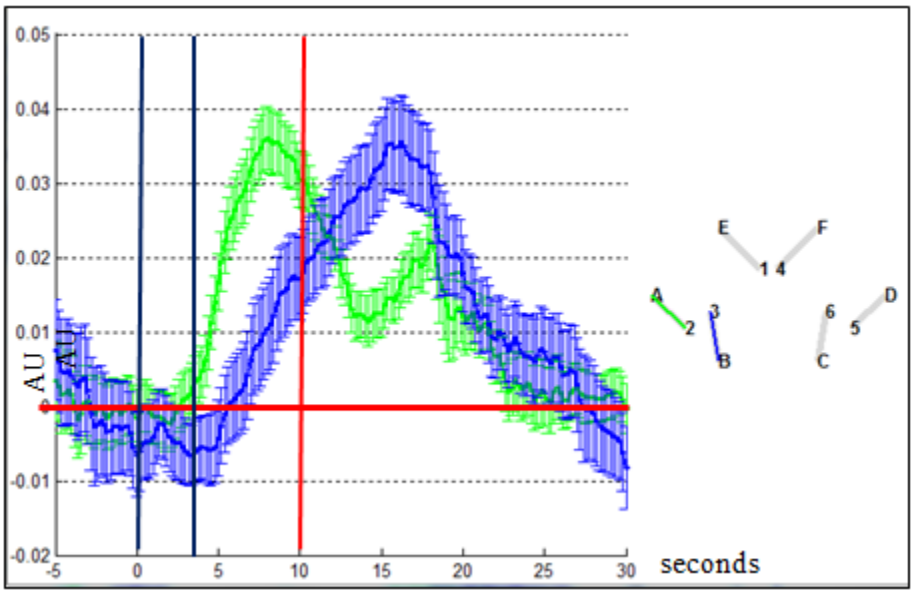


Figure 5. Response to water stimulation in the right M1 (green) and S1 (blue), participant 101.

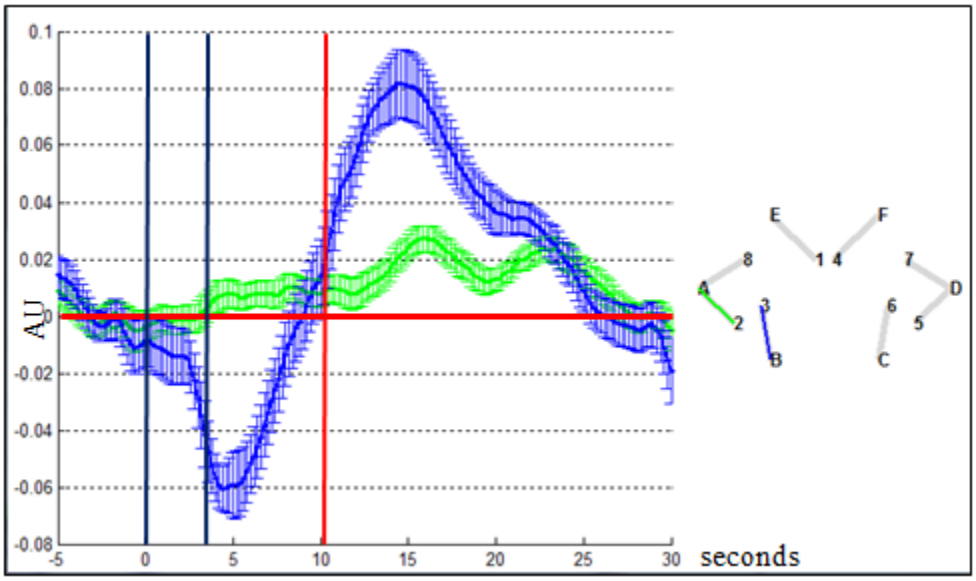


Figure 6. Response to sour stimulation in right M1 (green) and S1 (blue), participant 103.

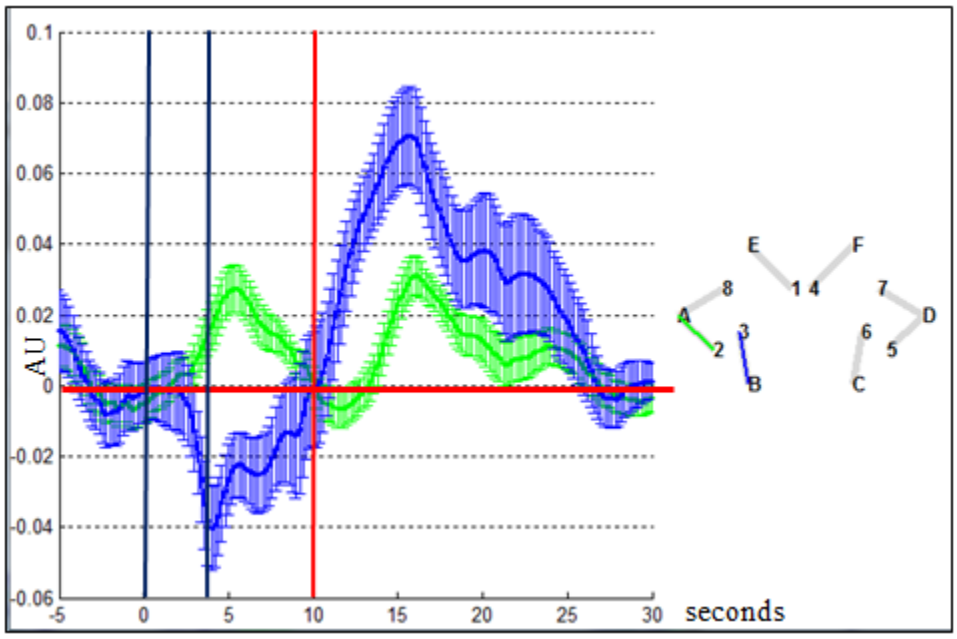


Figure 7. Response to water stimulation in right M1 (green) and S1 (blue), participant 103.

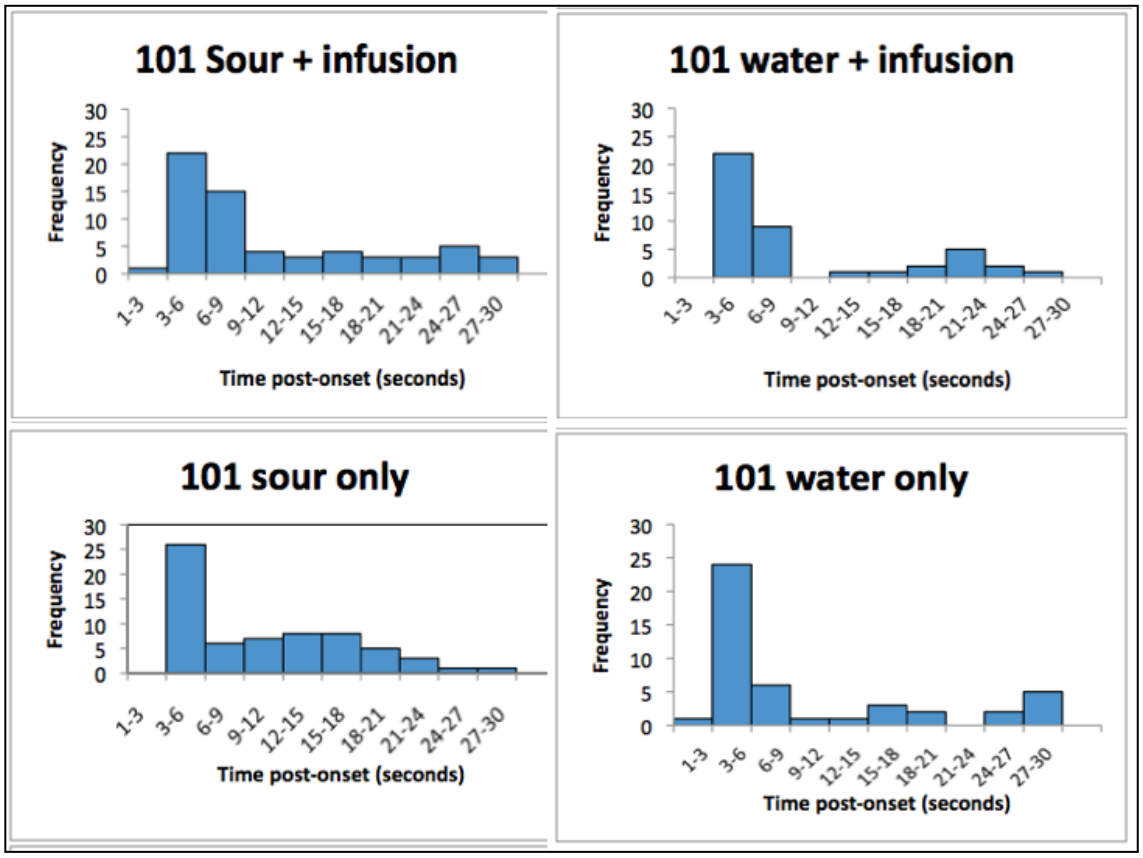


Figure 8. Histograms of swallowing frequency latency, participant 101.

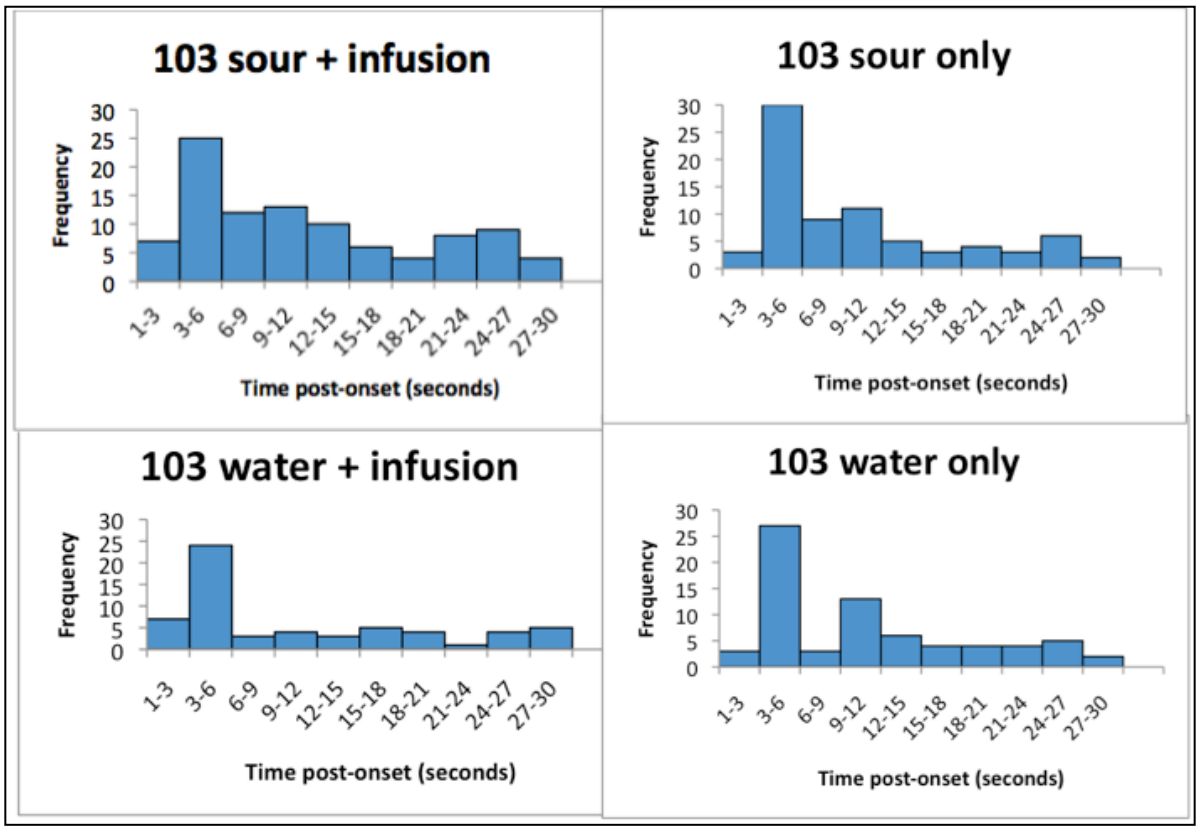


Figure 9. Histograms of swallowing frequency latency, participant 103.

Participants

Participants between the ages of 20 and 70 were recruited for the study. No compensation was provided for participation. The study was approved by the Institutional Review Boards of JMU and Rockingham Memorial Hospital.

Power Analysis

In order to determine the number of participants required to obtain meaningful results, an a priori power analysis for a repeated measures ANOVA with within- and between-subject factors was conducted with G*Power (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007). Desired power was set at 0.8. Results of the power analysis indicated a sample size of 10 was needed (Table 2).

Table 2

Statistics for Power Analysis

	Independent variable	Outcome variable	Effect size	Number of participants
(Kaatzke-McDonald, Post, & Davis, 1996)	Chemical stimulus (saline, glucose, water)	Swallowing frequency	3.5585	10
(South, Somers, & Jog, 2010)	Gum chewing	Swallowing frequency	2.632	20

Exclusionary Criteria

An initial telephone screen identified the following exclusions based on patient report:

- Left-hand dominance/preference
- History of swallowing complaints or problems
- Not able to hear and understand conversational level speech
- History of smoking
- Current diagnosis and/or management of reflux
- Current diagnosis of COPD
- Current diagnosis of epilepsy
- Diagnosis of a neurological disorder (including stroke)
- Current diagnosis of an eating disorder (e.g. anorexia, bulimia, or binge-eating disorder), which may alter taste perception
- Current psychiatric disorder
- Obesity (Figure 10), which may alter taste perception
- Subject-reported changes in diminished taste perception

- History of epileptic seizure
- Diagnosis of progressive neurodegenerative disorders, such as dementia, Parkinson's Disease, multiple sclerosis, peripheral neuropathy, or amyotrophic lateral sclerosis
- Speech disorder

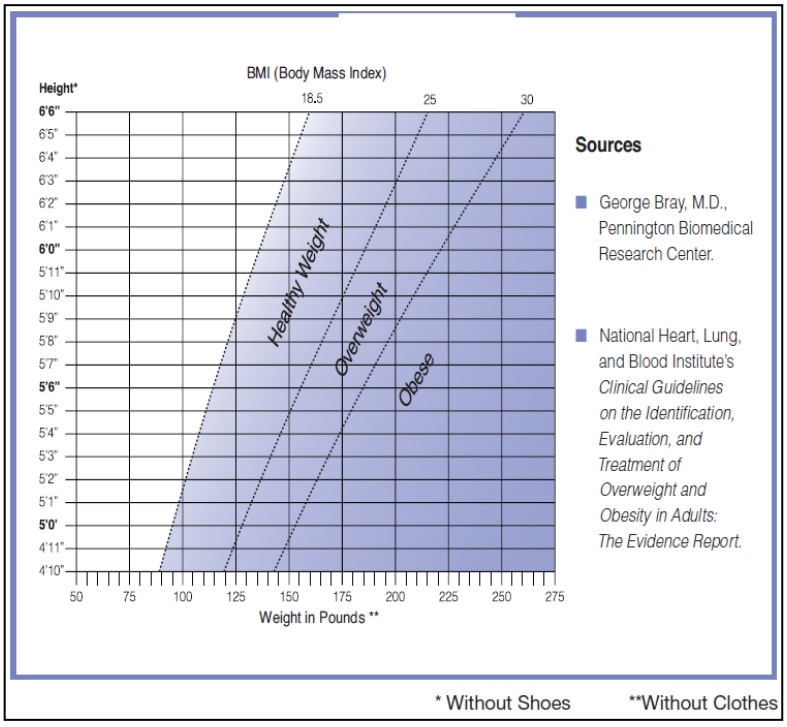


Figure 10. Obesity by height and weight

Initial Telephone Screen

Once potential participants contacted the researcher, a telephone screen was conducted to provide additional information and to determine qualification for the study. If candidates met all criteria for participation, an experimental session was scheduled at their preferred time and date.

Preliminary Paperwork and Consent Procedure

Upon their arrival at the Neural Bases of Communication and Swallowing Lab, participants completed a brief medical history form as well as the Edinburgh Handedness Inventory (Oldfield, 1971) in order to document any threats to exclusionary criteria. Participants then reviewed a written copy of the Informed Consent document (see appendix), which provides information concerning study purpose, risks and benefits. The researcher offered to answer any questions concerning the study and the consent. The participants, the researcher and a witness signed the consent form. All participants will be provided with a photocopy of the consent document.

Participants reviewed and signed forms concerning Release of Data for Educational Uses and Permission for Future Contact to notify participants of study results and conclusions (see appendix).

Confidentiality

Participation in this study and personally identifiable information (PII) was kept completely confidential. All data was stored in a locked and secure cabinet in the Neural Bases of Communication and Swallowing Laboratory that is only accessible to authorized researchers. Participant numbers were arbitrarily assigned to individuals in order to remove identifying information from the data. No identifying information was presented in the results of the study or in any subsequent presentations and publications. Video recordings of the sessions are stored on an encrypted drive with access limited to authorized researchers in the laboratory.

Time Course

Each participant completed the study in one session lasting approximately 2-3 hours (including paperwork and consent procedures).

Instrumentation

Stimuli

Experimental stimuli included distilled water, sour, and sweet. Oral stimuli were pre-loaded and delivered manually by syringes attached to a mouthpiece by plastic cannula; this delivery procedure aimed to minimize movement artifact of jaw opening and closing and overall head movement when drinking by spoon or cup. A slow, steady water infusion of .08 L/minute was delivered by a Masterflex motorized infusion pump (Cole-Parmer Instrument Co., Chicago, IL, model 7518-00); this infusion traveled to the mouthpiece via plastic cannula.

PTC Papers

Slips of paper impregnated with 3-5 micrograms of phenylthiocarbamide (PTC; Precision Laboratories, Waukegan, IL) were presented to participants as an additional method of determining taster status. PTC is a sensitive measure for dividing individuals by taste sensitivity (Lawless, 1980). Although evidence from animal studies suggests that PTC may be toxic at 1 mg (Wheatcroft & Thornburn, 1972), the levels utilized in this study were well below known toxic levels and there was only one administration of PTC per participant.

Piezoelectric Accelerometer

AC movement signals from a piezoelectric accelerometer (Kistler Instrument Corporation, Amherst, NY, Model 8778A599) was used to measure swallowing

frequency. For the purposes of this study, this device provided a voltage output reflecting change in direction along the y-axis as the larynx elevated at the initiation of a swallow and returned to a lower resting position once the swallow had been completed. The body of the accelerometer weighs less than a gram and measures 1 by 3 mm and it connects to a 16-channel voltage mode piezoelectric sensor power supply/coupler (Type 5148). The accelerometer was placed on the surface of the throat over the laryngeal area and secured with medical tape. The accelerometer signal is modulated by an AC pre-amplifier (Semiconductor Circuits Inc., Atkinson, NH) and recorded by PowerLab 8 software.

Inductotrace

The Inductotrace System (Ambulatory Monitoring, Inc., Ardsley, NY, model 10.9000) transduces change in chest wall circumference and can be used to identify periods of apnea to that typically occur during the adult swallow. Two flexible Inductotrace bands were fitted around a participant's rib cage and abdomen. The bands were connected to a transducer box, which connected to the amplifier (set at 1 for individual channels and 2 for the sum channel). The expansion and contraction of the bands during inhalation and exhalation was reflected in three LabChart channels: rib cage, abdomen, and their sum.

Functional Near-Infrared Spectroscopy

Changes in oxygenation of hemoglobin in selected cortical regions was measured with continuous-wave functional near-infrared spectroscopy (fNIRS; Techen Inc., Milford, MA, USA, model CW6). FNIRS consists of a series of light-emitting and –detecting optodes connected to an electronic control box, which interfaces with a desktop computer. fNIRS technology is non-invasive, less sensitive to motion artifact than fMRI,

and allows participants to remain upright during recordings. Light at wavelengths 690 and 830 nm is emitted from each optical emitter at approximately 3 and 6 mW. This laser light travels through the scalp and the first few centimeters of the cortical surface of the brain. An increase in cortical activation is accompanied by an increase in oxygenation and increase in bloodflow. The concentrations of oxygenated and deoxygenated hemoglobin fluctuate with changes in activation. Although fNIRS cannot calculate exact changes in concentration, the modified Beer-Lambert Law (MBLL) postulates that changes in the absorption of light emitted through and attenuated by cortical tissues are representative of changes in concentrations of HbO and HbR. (D. A. Boas et al., 2001; Villringer & Chance, 1997).

As the light passes through the cortex, a portion of it is absorbed by hemoglobin based on local cortical activity, and certain wavelengths of the original emitted signal is attenuated. A characteristic banana-shaped trajectory of light is formed between an emitter-detector pair. A detector or an array of detectors placed approximately 3 cm from the emitter receives this filtered signal and a plot of relative changes in hemoglobin oxygenation is recorded simultaneously. The CW6 system samples at a rate of 50Hz. The level of signals can be monitored in real time and manipulated by either the auto gain function or by manual adjustment of gain to individual detectors. Gain levels are adjusted at the onset of a session and are not altered throughout the recording period.

Based on the findings of previous neuroimaging research (Lowell, et al., 2008; R. Martin et al., 2007), cortical areas of interest for this study include precentral gyrus to M1, postcentral gyrus including S1, and supplementary motor area (SMA). An emitter and detector were spaced 3 cm away to form a channel overlapping each area in order to

detect changes in oxygenated and deoxygenated hemoglobin levels in the neural substrates between the emitter and detector. An additional channel (a detector placed 3 cm from an emitter) served as a "dummy" to ensure that cortical activity in the regions of interest was distinct from general neural activity. A short separation channel on each side formed by emitters H and G and detectors placed 1 cm away monitored changes in skin-level activity that was subtracted from the remaining channels to improve the signal-to-noise ratio (Gagnon et al., 2012; Takahashi et al., 2011). There were a total of 5 channels, 3 emitters and 5 detectors in the array for each hemisphere (Figure 11). The arrangement of optodes was plotted using Brainsight, and a template of this arrangement was created from sheets of thin foam and rubber for each hemisphere. The templates were sewn to strips of Velcro and elastic to form a self-adhesive headpiece. The templates were then wrapped with Coban during data collection to ensure close contact with the scalp.

SD GUI

The SD_gui executable file of HomER was used to create a template for arranging emitters and detectors. Optodes were plotted on a 2D graph and connections between optodes indicate channels of interest.

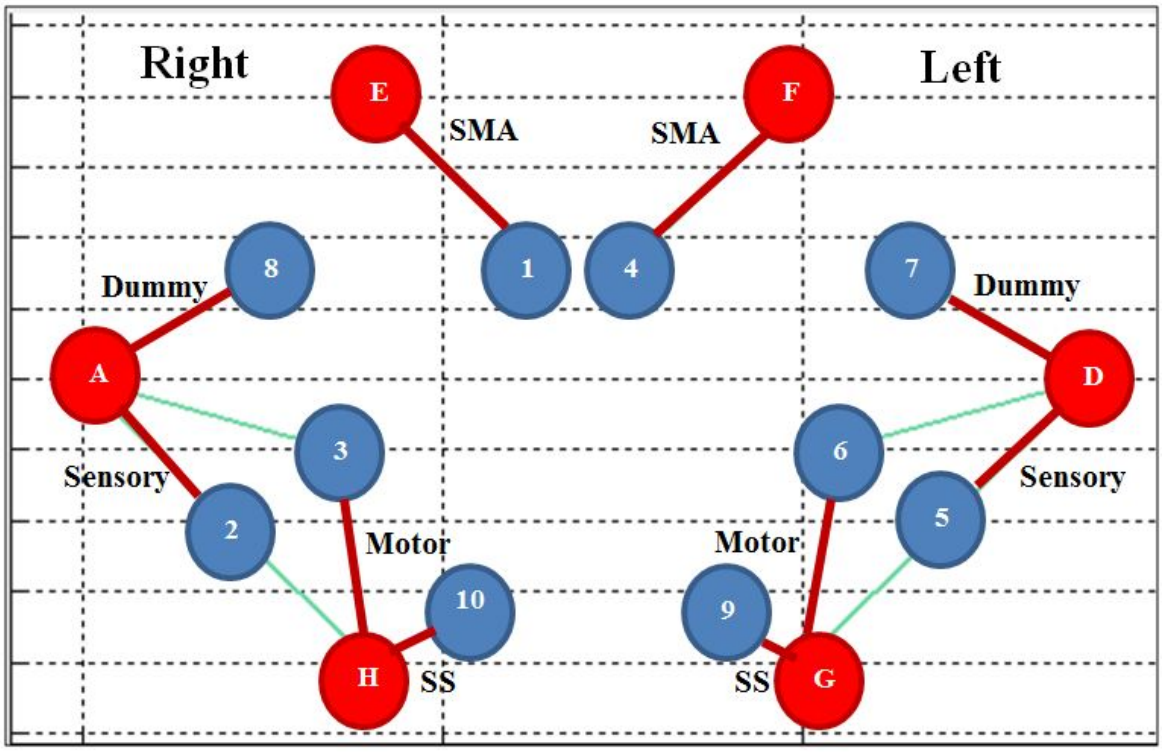


Figure 11. Array of emitters (red) and detectors (blue). Channels of interest marked in red.

Brainsight and Polaris

Brainsight 2.0 (Rogue Research, Montreal, Quebec) is a neuronavigation tool that was used to localize points on the head corresponding to the MNI or Talaraich neuroatlas (Table 3). A Vicon camera and pointer were used to mark and confirm the location of emitters on a participant's scalp based on a 3-D anatomical brain atlas.

Table 3

Coordinates for placement of fNIRS optodes (MNI)

	X	Y	Z
Right			
S1 emitter	54	-24	43
M1 emitter	53	8	8
SMA emitter	13	8	62
S1 detector	57	-9	25
M1 detector	53	8	8
SMA detector	13	38	62
Dummy detector	38	-2	58
Left			
S1 emitter	-54	-24	43
M1 emitter	-53	8	8
SMA emitter	-13	8	62
S1 detector	-57	-9	25
M1 detector	-53	6	38
SMA detector	-13	38	62
Dummy detector	-38	-2	58

Note. Based on Lowell (2008) and Soros (2008)

Pulse generators

Push buttons connected to pulse generator boxes were used to indicate: a). onset of manual bolus delivery via syringe and b). observation of hyolaryngeal elevation. Each

activation of the push buttons is represented by a square wave in Labchart 8 and the fNIRS file.

Powerlab and Labchart 8

A Powerlab 16/30 SP unit (16-bit analog-to-digital converter; AD Instruments, Colorado Springs, CO, model ML 880) was used to record and digitize data from the Inductotrace, accelerometer, and pulse generators and to display the data as synchronized waveforms in Labchart 8 software on a Dell laptop. Labchart 8 was used to analyze the data.

Hemodynamic Evoked Response (HomER) 2

The data from fNIRS was analyzed with HomER 2 software (D. Boas, Dubb, & Huppert, 2012) in Matlab 2013a (The MathWorks Inc., Natick, MA). HomER 2 is a Matlab-based program used to process raw fNIRS data to obtain relative changes in oxygenated, de-oxygenated and total hemodynamic responses in response to events defined by bolus delivery and swallow times. High- and low-pass filter settings removed physiological (e.g. Mayer's waves, respiratory and cardiac) signals from the signal. Portions of the signal containing motion artifact that is consistent across all fNIRS channels can be objectively extracted from analysis by setting thresholds for detection and omission in the processing stream.

Video Recording

A webcam (Logitech Carl Zeiss Tessar, Newark, CA, model HD 1080p) recorded sessions through Labchart 8 Video Capture module, which synchronized the video input with other Labchart 8 data.

SPSS/SYSTAT

SPSS 17 (IBM, Armonk, NY) and SYSTAT 13 (San Jose, CA) were used for statistical analysis of swallow frequency and cortical activity data.

Experimental Design

In this within-subjects study, five conditions were administered to each participant: sour bolus only, sweet bolus only, water bolus only, sour bolus plus slow infusion, water bolus plus infusion (Table 4). The order of conditions was randomized by means of a random numbers generator. Each condition included 30 presentations of sour or water and lasted 15 minutes. The duration of bolus delivery was 3 seconds with a 30 second inter-stimulus interval (or 27 seconds between offset and onset). Independent variables included the liquid solutions and infusion. Primary outcome variables were swallowing frequency and changes in hemodynamic response.

Table 4

List of Conditions

A	B	C	D	E
Slow flow + water bolus	Slow flow + medium sour bolus	Water bolus	Sour bolus	Sweet bolus

Experimental Procedure

At least 12 hours prior to a study session, experimental stimuli were prepared and loaded into syringes. An anatomical MRI was processed with Brainsight software to include MNI coordinates. Additional set-up procedures were conducted at least an hour prior to participant arrival. Equipment was turned on and software opened. Batteries for the pulse generator motor boxes were checked for full charge. An adjustable table serving as a chin rest was covered with layers of paper towels and a liquid-resistant dental bib to

absorb any lost saliva. Two pieces of plastic tubing were attached to a mouthpiece with dental putty (3M, St. Paul, MN, ESPE Express STD); one cannula was connected to the infusion pump and the other was taped to the table. A new mouthpiece and fresh tubing were utilized for each participant.

When participants arrived at the laboratory, they completed preliminary and consent paperwork. They were then seated in a stationary chair and fitted with Brainsight plastic glasses, which contain probes that can be detected by the Polaris camera to interface with the Brainsight software. A Brainsight pointer registered facial landmarks (tip of noise, nasion, left and right tragi) to fit a 3D anatomical MRI to each participant's head. Once Brainsight had synced the anatomical MRI to the individual's head, the pointer located the placement of the 5 emitters on each side of the scalp corresponding to MNI coordinates of the regions of interest (corrections for scalp-brain difference automatically computed by Brainsight for each participant). These points were marked with an orange or yellow highlighter. The remaining optodes and reference points were marked on the scalp through a rubber copy of the template. Hair was parted and directed away from the marked points with styling paste and hairpins. The researcher presented a strip of PTC paper and asked participants to rate the intensity of the tastes on a visual analog scale using sound intensity as an anchor. At this point the participant were offered a restroom and water break.

Participants were fitted with Inductotrace bands and seated in an upright dental chair with cushions as needed. The Inductotrace bands were attached to the transducer. After palpation during a volitional dry swallow, the Kistler accelerometer was attached over the thyroid cartilage with medical tape. The fNIRS optodes were matched to their

corresponding marks on the participant's scalp and the optode array was secured with Velcro and wrapped with Coban self-adherent bandage (3M, St. Paul, MN). The fNIRS signals were checked for appropriate intensity and cardiac response, and gain was manually adjusted and maintained at a uniform level throughout the recording; if necessary, the optode array was re-fitted to promote close contact with the scalp and accurate placement. The adjustable table was raised to the level of the participant's chin and the mouthpiece inserted (and secured with Coban according to participant preference). Researchers verbally verified that the participant was comfortable and rearranged equipment accordingly. Equipment function was tested by recording a dry swallow on command. A silent video free from written language was played during all conditions to facilitate an awake and alert state in participants. At the conclusion of each condition, the Labchart and fNIRS files were saved by condition name and participant number. Figure 12 depicts the equipment set-up and connections to participant.

Liquid Stimuli

Two experimental conditions included manual delivery of 3 ml boluses of sour in two conditions (.08 M citric acid ((Nagy, Steele, et al., 2014b)), sweet in one condition (1 M sucrose), and de-ionized water in two conditions. All chemicals met USP standards and were pure tastes. They were kept at room temperature for at least 12 hours in order to ensure uniform temperature.

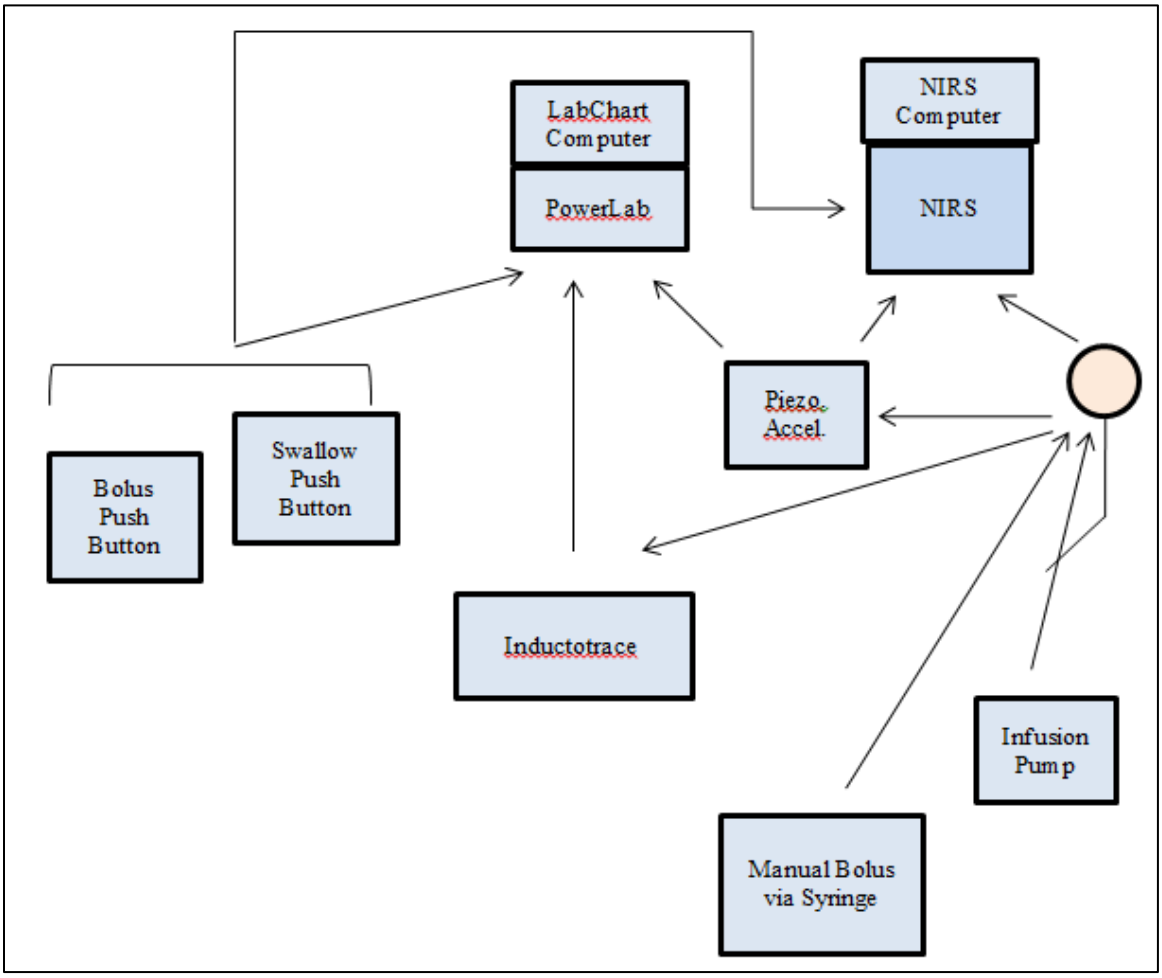


Figure 12. Flowchart of study instrumentation.

Presentation of Stimuli

The experimental conditions proceeded in randomized order for each participant. With the exception of one participant who could not tolerate one condition, every participant received each of the five stimulus conditions. Liquid boluses were delivered manually via pre-loaded syringe through a plastic cannula at 30-second intervals (total of 30 boluses per condition). The timing of bolus delivery onset was monitored by stopwatch and marked by simultaneous button press. A second plastic cannula delivered a continuous, slow flow of de-ionized water in the two infusion conditions. The

mouthpiece to which the 2 plastic cannulas were attached remained in the mouth through all five conditions.

Risks

Accelerometer and Inductotrace

Both instruments are non-invasive and do not pose any known risks. Brief discomfort and redness may result from removal of the medical tape used to attach the accelerometer to the neck. The Inductotrace bands are elastic and fit over the partcipants' clothing.

fNIRS

There is a risk of optode light being directed to the eyes, but the intensity of the emitters is similar to that of laser pointer. The emitters were turned on until they are secured to the head and none were positioned in particpants' line of vision.

Highlighter marks on the head to indicate optode location were easily removed with an alcohol wipe at the end of the session. Participants were frequently asked about discomfort from parting and pinning of the hair and optode array pressure, and adjustments were be made accordingly.

Taste Stimuli

All taste stimuli met USP standards and are safe for human consumption. There was a risk that stimuli may be perceived as unpleasant or unpalatable.

Outcome Measures

Swallowing frequency was calculated by counting the number of swallows in each 15-minute condition. Based on the results from the pilot study indicating a difference in swallowing frequency between early and late swallow, the overall

swallowing frequency was divided into swallow occurring 0-15 seconds and 16-30 seconds after bolus delivery. Swallowing frequency was compared across the five taste conditions.

Changes in cortical activation for swallowing are indicated by relative changes in oxygenated and de-oxygenated hemoglobin. A repeated measures analysis compared these changes in the five conditions and between taster status and location. An example of the time course of cortical response to taste using fNIRS is depicted in Figure 14.

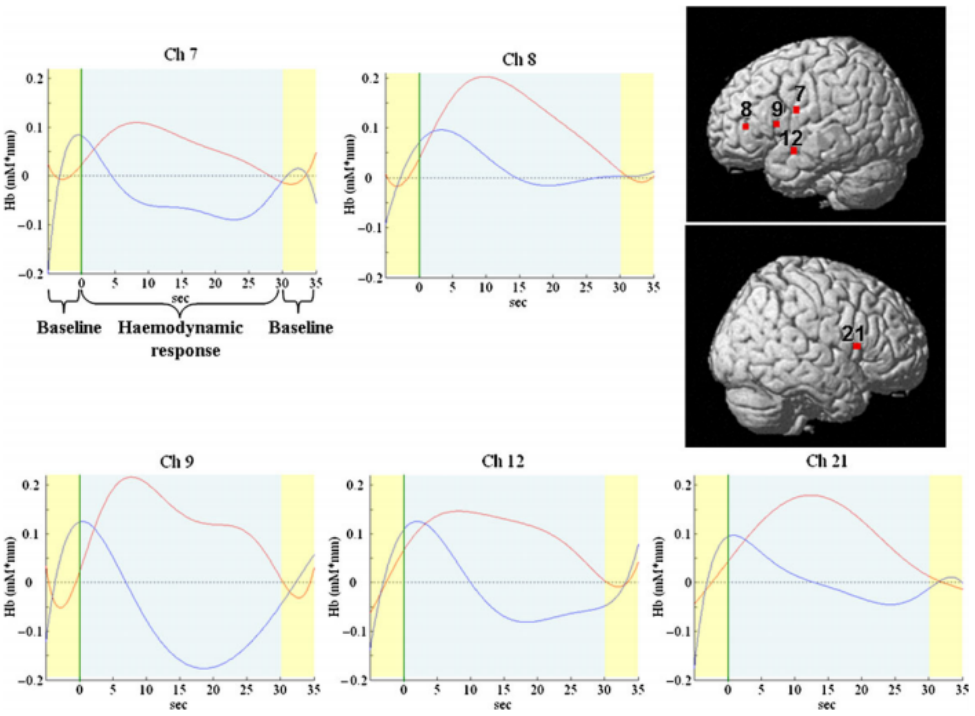


Figure 3 Time courses of variation in concentration of haemoglobin (Hb) in mM*mm during PROP administration in the taster group, HbO_2 significantly increased, showing cortical activation, in the left DLPFC (ch7–ch8), in the left VLPFC (ch9–ch12), and right VLPFC (ch21). (Curves indicate changes in HbO_2 and Hbb concentration. The vertical line shows when the filter paper disk touched the tip of the tongue). The right side of the figure shows the Chs cortical location on a rendered brain.

Figure 14. Time course of cortical response to taste using fNIRS, from Bembich et al. (2010)

Data Analysis

Swallowing Frequency

The frequency of swallows per 15-minute condition was determined by a combination of inductive plethysmography and piezoelectric accelerometry. The ribcage, abdomen and sum channels of the Inductotrace indicated respiratory cycles in three Labchart 8 channels. At the time of data analysis, an additional channel was added to Labchart 8 and programmed to calculate the first derivative of the sum channel. Periods of swallowing apnea on exhalation were indicated by a flat signal in the first derivative channel occurring around 0 V/s and lasting more than 350 ms (Klahn & Perlman, 1999; Martin-Harris et al., 2005).

A large, sharp peak in the accelerometer channel was typically observed when a swallow occurred. Although motion artifact also induces a sharp peak, swallows followed a stereotypical pattern in the shape of the signal and occurr simultaneously with a moment of apnea in the first derivative of the Inductotrace sum channel.

The Labchart 8 channel for the signal from push button (depressed whenever a swallow was observed and represented by a square waveform) was coupled with the apneic moment and peak of laryngeal elevation to confirm a swallow (Figure 13). If all parameters for a swallow were met, a comment was inserted at the onset of apnea. If the occurrence of a swallow remained unclear, the synchronous video recording was viewed for laryngeal elevation. Swallows that remained in doubt were excluded from the analysis. Once all swallows had been marked for a condition, comments and their times were exported to Excel. The swallowing frequency was calculated for each condition for each participant. Group means by condition were compared using a repeated-measures

ANOVA with taster status as a between-groups factor and swallow latency (early vs. late) and taste condition as within-subjects factors.

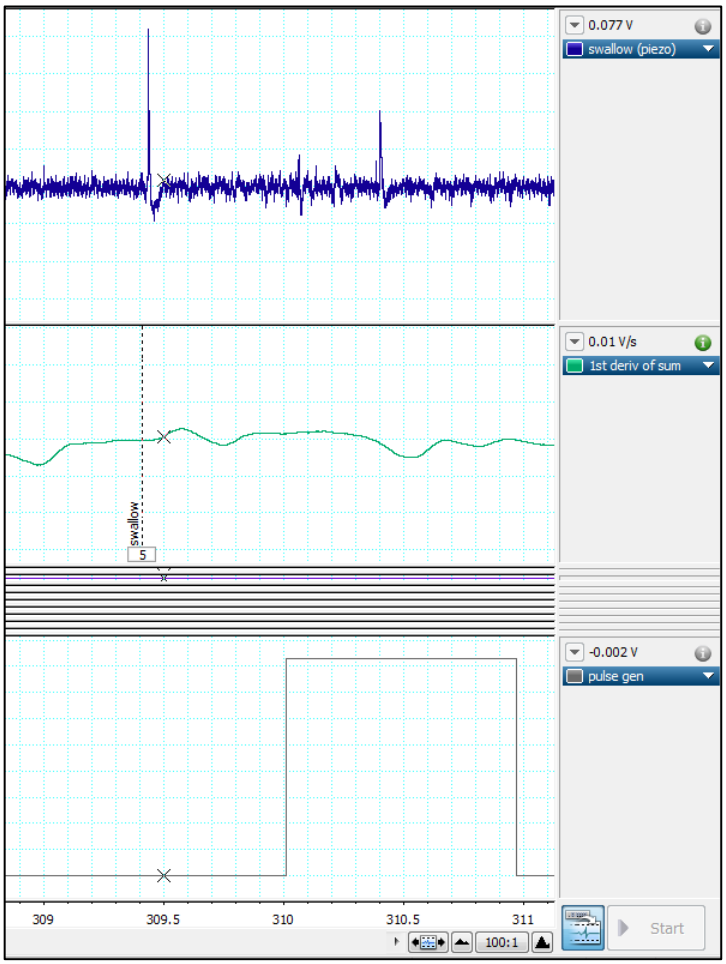


Figure 13. Marking a swallow with accelerometer, Inducotrace, and push button

Changes in Hemodynamic Response

Cortical activity was assessed using HomER software. The time of Labchart and fNIRS signals were synced by linear interpolation of bolus delivery onset in Excel. The onset of bolus delivery was indicated by the fNIRS auxiliary channel for the bolus push button; these stimuli were marked by condition (e.g. “sour + infusion”). The times of

bolus swallows from Labchart were converted by linear interpolation and added to HomER as stimulus markers ("swallow [name of condition] bolus".

Two sets of analyses were performed in HomER: an event-related analysis of the bolus swallows from 2 to 7 seconds and a block average analysis from 17 to 22 seconds. The event-related analysis was tied to the event of bolus onset, whereas the block average analysis calculated a mean of response across the selected time window. The raw was processed with a high pass filter of .01, a low pass filter of .5, and a threshold of 15 mm for detection and extraction of the response in the short separation channels from the channels over regions of interest.

This processing stream yielded images and values of the average hemodynamic response (HRF) for each channel by condition and participant. The HRF plots were examined for latency of responses and artifact as indicated by oxygenated and deoxygenated responses moving in the same direction. Processed data for HbO were exported to Excel and organized by participant, condition, hemisphere, location, taster status and HbO and HbR. Z-scores relative to baseline (-5 to 0 seconds of water condition) were computed. The data was then be exported to SYSTAT/SPSS and a repeated measures GLM analysis was run comparing across conditions, taster status, hemispheres, and locations. Hemodynamic response and swallowing frequency were also examined by correlation analysis in order to determine the relationship between the two primary outcome measures.

Part II: Manuscript

Title: The Effect of Taste on Swallowing Elicitation and Cortical Activity Using Functional Near-Infrared Spectroscopy

Authors: Rachel Mulheren: experiment design, running of study, data analysis, preparation of manuscript

Erin Kamarunas: running of study, preparation of manuscript

Christy Ludlow: experiment design, data analysis, preparation of manuscript

Affiliations: Department of Communication Sciences and Disorders, James Madison University, Harrisonburg, VA

Corresponding Author: Rachel Mulheren

rach.whit.mul@gmail.com

701 Carrier Drive, MSC 4304, Harrisonburg, VA 22807

Running Head: Effect of Taste on Swallowing and Cortical Activity

Abstract

This study investigated the effects of taste on swallowing frequency and cortical activation in the swallowing network. The effects of salivary flow and taster status were also examined, along with genetic taster status. The effects of a 3ml bolus compared sour, sour with slow infusion, sweet, water, and water with infusion. Swallowing frequency was significantly higher 0-15 seconds after bolus delivery than 16-30 seconds. Swallowing frequency was higher in the sour conditions, whereas sweet and water did not differ. Functional near-infrared spectroscopy recordings measured changes in blood oxygenation (HbO) in the right and left hemispheres in the premotor, S1 and supplementary motor area in response to swallowing a bolus indicated a significant interaction of side and channel. Event-related analyses of HbO following bolus administration of taste solutions were significantly higher in the sensory than the premotor area in the right hemisphere. A block average analysis of the response to taste between 17 and 22 seconds after bolus administration revealed significant differences between hemispheres and regions. Genetic taster status was not significant in any of the analyses. The highest activation in response to sour taste was in the premotor regions of both hemispheres. The results indicated that sour taste effectively increased swallowing frequency and cortical activation while increasing salivary flow in comparison to water and sweet taste. In conclusion, sour taste may have peripheral effects on salivary flow while up-regulating the activation of the swallowing network at the cortical level.

Keywords: Taste, Swallowing, Functional Near-Infrared Spectroscopy, Salivary Flow, Taster Status

Introduction

Swallowing is a highly intricate process that is both automatic and modifiable. Early research in animals suggests that the process of swallowing may be altered by manipulating bolus properties (F. R. Miller & Sherrington, 1915) and peripheral stimulation (Doty, et al., 1967) via direct sensory connections from the cranial nerves to the swallowing central pattern generators (CPGs) in the brainstem (Sumi, 1963). Research with human participants indicates that swallowing physiology may be altered by peripheral changes in sensation such as anesthesia (Jafari, Prince, Kim, & Paydarfar, 2003; Mansson & Sandberg, 1975) and by manipulation of bolus properties including temperature, taste, viscosity, texture and volume of ingested material (Bisch, Logemann, Rademaker, Kahrilas, & Lazarus, 1994; Bove, Mansson, & Eliasson, 1998; Butler et al., 2009; Hamdy, et al., 2003; Lazarus et al., 1993). Sensory stimulation from the CPGs is relayed from the nucleus of the solitary tract (NTS) via the thalamas bilaterally to each hemisphere (Car, et al., 1975), where the pattern of swallowing and swallowing initiation may be altered. One method of sensory stimulation for swallowing is the introduction of a sour bolus. Similar to general sensory input from structures involved in swallowing, taste is transmitted via the superior laryngeal, facial and glossopharyngeal nerves (Breslin & Huang, 2006; Lewis & Dandy, 1930) to the nucleus of the solitary tract (NTS) (Pritchard, et al., 2000). In addition to this convergence of sensory information, which favors taste as an effective stimulus for manipulating swallowing physiology, independent projections from the brainstem and cortex to each side of the tongue yield a low frequency of hypogeusia and rare complete ageusia (Welge-Lussen, et al., 2011). In recent years, several studies have suggested that a sour bolus affects submental muscle amplitude, oropharyngeal pressure, frequency of swallowing, and timing of swallowing

events in comparison to water and other tastes in healthy participants (Chee, et al., 2005; Ding, et al., 2003; Hamdy, et al., 2003; Leow, et al., 2007; Miura, et al., 2009; Nagy, Steele, et al., 2014b; Nederkoorn, et al., 1999; Palmer, et al., 2005; Pelletier & Dhanaraj, 2006; Pelletier & Steele, 2014; Plonk, et al., 2011; Sciortino, et al., 2003; Steele, et al., 2012; Wahab, et al., 2011). Additionally, a sour bolus has been shown to alter timing and frequency of swallowing and to improve scores on the Penetration Aspiration Scale in patients with a swallowing disorder (Alves, et al., 2013; Cola, et al., 2012; Cola, et al., 2010; Gatto, et al., 2013; Hamdy, et al., 2003; Lee, et al., 2012; Logemann, et al., 1995; Pauloski, et al., 2013; Pelletier & Lawless, 2003). Taste also enhances cortical activation in areas activated during swallowing, including S1, the supplementary motor area (SMA) (Humbert & Joel, 2012) and the pre- and postcentral gyri, with a long-lasting effect peaking at 22 seconds (Okamoto, Dan, Clowney, Yamaguchi, & Dan, 2009b).

The mechanisms involved in the effect of taste on swallowing have yet to be thoroughly elucidated. Changes in activation of the cortical swallowing network in response a sour stimulus suggest that taste also modulates swallowing at the level of the cortex, primarily in M1, S1, and the cingulate cortex (Abdul Wahab, et al., 2010; Babaei, et al., 2010; Humbert, et al., 2012; Humbert & McLaren, 2014). Current evidence indicates that a sour taste, in comparison to water and other tastes, maximally increases both swallowing frequency and salivary flow (Lashley, 1916; Nederkoorn, et al., 1999; Watanabe & Dawes, 1988), with a direct relationship between increases in salivary flow, taste concentration and perceptual ratings (Bonnans & Noble, 1995; Dawes & Watanabe, 1987; Watanabe & Dawes, 1988). The amount of salivation in response to taste stimuli is positively correlated with changes in hemodymanic signals supplying the parotid gland

(Sato et al., 2011). Similar to taste and swallowing, the neural control of salivation includes the NTS as part of the brainstem connections of efferent and afferent information to the peripheral salivary glands (Chatfield, 1941; Contreras, et al., 1980; Magoun & Beaton, 1942; Wang, 1943). Therefore, it is necessary to consider the potential mediating effect of salivary flow on swallowing response and cortical activation of the swallowing network in response to taste. Sour is a preferable candidate for up-regulating the swallowing network as it is less susceptible to adaptation over time (Theunissen, Polet, et al., 2000) and induces a higher rates of salivary flow (Lashley, 1916; Watanabe & Dawes, 1988). In contrast, sweet taste has been shown to activate a large area of the primary gustatory cortex in humans (Schoenfeld, et al., 2004).

Individual differences in perception of taste may mediate the relationship between taste and swallowing. Perception of taste varies across individuals due several factors, including smoking, neurological damage, medications, eating disorders, and weight (Bartoshuk, 1989; Bertoli, et al., 2014; Pavlidis, et al., 2014). Taste perception also varies as a result of genetic taste status. Individuals with the highest perceptual intensity ratings are known as supertasters (Smith & Davis, 2000), whereas nontasters have very low intensity ratings of the same stimulus. The distribution of taster status in the general population is approximately 25% supertasters, 50% medium tasters, 25% nontasters (Bartoshuk, et al., 2004). Though limited, recent research suggests that activation patterns in the ventrolateral prefrontal cortex, S1, S2, insula and anterior cingulate differ by genetic taster status (Bembich, et al., 2010; Eldeghaidy, et al., 2011) and that genetic taster status differentially affects swallowing function (Nagy, Steele, et al., 2014b; Pelletier & Steele, 2014; Plonk, et al., 2011; Todd, et al., 2012b).

It remains to be determined how taster status mediates the effect of sour taste on swallowing, if the effect of sour taste on swallowing is mediated by changes to salivary flow, and if swallowing function and cortical activation differ in response to different tastes. Given the potential mediating effect of these factors, humans may respond differently to sour stimulation due to differences in genetic taster status, salivary flow, and in contrast sweet. This study aims to answer these questions to determine the role of peripheral, genetic and cortical responses to tastants during swallowing. We hypothesize that:

1. Increases in swallowing in response to a sour stimulus will be greater in supertasters than nontasters, and that supertasters would have greater increases in cortical activation in response to a tastant.
2. Increases in swallowing frequency will be positively related to increases in oxygenated hemoglobin in M1.
3. Swallowing frequency and cortical activation will be greater for sour than for sweet stimuli.
4. The addition of continuous infusion will interfere with taste-induced increases in salivary flow and reduce responses to a tastant.

Materials and Methods

Participants

The protocol, consent and recruitment materials were approved by the James Madison University Internal Review Board. Participants between the ages of 20 and 70 were recruited through bulk email. No compensation was provided for participation. Once potential participants contacted the researcher, a telephone screen was conducted to

provide additional information and to determine qualification for the study. If candidates met all criteria for participation, an experimental session was scheduled at their preferred time and date. Exclusionary criteria included: left hand dominance/preference, history of swallowing complaints or problems, history of smoking, current diagnosis and /or management of reflux, chronic obstructive pulmonary disorder, epilepsy, neurological disorder, eating disorder, psychiatric disorder, obesity, reported changes in taste perception, and speech disorder.

Design

In this within-subjects study, five conditions were administered to each participant: sour bolus only, sweet bolus only, water bolus only, sour bolus plus slow infusion, water bolus plus infusion. The order of conditions was randomized across participants. Each condition included 30 presentations 3 ml of a taste solution or water and lasted 15 minutes. The duration of bolus delivery was 3 seconds with a 30 second inter-stimulus interval that yielded 27 seconds between offset and onset. Independent variables included the liquid solutions and infusion. Primary outcome variables were swallowing frequency and changes in blood oxygenation levels from 0 to 30 sec over time.

Procedures

Participants scaled the intensities of the lowest and highest sounds imaginable on an unmarked 100 mm vertical line. Then they marked the perceived intensity of 3-5 micrograms of phenylthiocarbamide (PTC; Precision Laboratories, Waukegan, IL), a sensitive measure for dividing individuals by taste sensitivity (Lawless, 1980).

Participants were seated in a stationary chair for marking emitter locations on the scalp corresponding to Montreal Neurological Institute (MNI) coordinates of the regions of interest. Based on the findings of previous neuroimaging research (Lowell, et al., 2008; R. Martin, et al., 2007), cortical areas of interest for this study included precentral gyrus to M1, postcentral gyrus including S1, and SMA (Table 1). An emitter and detector were spaced 3 cm apart to record blood oxyngenation changes over each area and to detect changes in oxygenated and deoxygenated hemoglobin levels in the neural substrates between the emitter and detector. An additional channel (a detector placed 3 cm from an emitter) served as a "dummy" to ensure that cortical activity in the regions of interest was distinct from general neural activity. A short separation channel on each side formed by emitters and detectors placed 1 cm away monitored changes in skin-level activity that was subtracted from the remaining channels to improve the signal-to-noise ratio (Gagnon, et al., 2012; Takahashi, et al., 2011). There were a total of 5 channels, 3 emitters and 5 detectors in the array for each hemisphere (Figure 1). Templates were constructed with strips of Velcro and elastic to form a self-adhesive headpiece. The arrangement of optodes was placed on a participant's scalp based on an anatomical MRI fitted to the pariticpant's head. Optodes were placed by MNI coordinates using the Brainsight 2.0 neuronavigation system (Rogue Research, Montreal, Quebec), which computed and corrected for scalp-brain difference automatically computed for each participant. After the emitters were located on the scalp and marked by yellow or orange highlighter, the detectors and reference points were located with a rubber copy of the templates. Hair was parted and directed away from the marked points with styling paste and hairpins so that the optode locations on the scalp were free from obstruction.

An Inductotrace System (Ambulatory Monitoring, Inc., Ardsley, NY, model 10.9000) was used to identify periods of apnea to that occurred during a swallow. Two flexible Inductotrace bands were fitted around a participant's rib cage and abdomen and connected to a transducer box, which connected to the amplifier (set at 1 for individual channels and 2 for the sum channel). The expansion and contraction of the bands during inhalation and exhalation was reflected in three LabChart 8 (ADInstruments, Colorado Springs, CO) channels: rib cage, abdomen, and their sum. After participants were fitted with Inductotrace bands, they were seated in an upright dental chair.

A piezoelectric accelerometer (Kistler Instrument Corporation, Amherst, NY, Model 8778A599) was placed on the skin over the thyroid notch to sense laryngeal elevation during swallowing. The signal was pre-amplified (Semiconductor Circuits Inc., Atkinson, NH) and used to measure swallowing frequency. The accelerometer was secured with medical tape. Pulse generators were used to indicate: a). onset of manual bolus delivery via syringe, which was initiated simultaneously with depression of a push button and lasted 3 seconds, and b). observation of hyolaryngeal elevation by a trained observer. Each activation of the push buttons was represented by a square wave in Labchart 8 and the fNIRS file. A Powerlab 16/30 SP unit (16-bit analog-to-digital converter; AD Instruments, Colorado Springs, CO, model ML 880) was used to record, digitize, and display data from the Inductotrace, accelerometer, and pulse generators. Each session was videorecorded and synchronized by Labchart 8 Video Capture.

Changes in oxygenation of hemoglobin in selected cortical regions were measured with continuous-wave functional near-infrared spectroscopy (fNIRS; Techen Inc., Milford, MA, USA, model CW6). FNIRS technology is non-invasive and allows

participants to remain in a natural upright during recordings. The modified Beer-Lambert Law (MBLL) postulates that changes in the absorption of light emitted through and attenuated by cortical tissues are representative of changes in concentrations of oxygenated (HbO) and deoxygenated (HbR) hemoglobin. (D. A. Boas, et al., 2001; Villringer & Chance, 1997). Light at wavelengths 690 and 830 nm is emitted from each optical emitter at approximately 3 and 6 mW. The CW6 system samples at a rate of 50Hz. The fNIRS optodes were matched to their corresponding marks on the participant's scalp and secured to the head to ensure close contact with the scalp. The level of signals were monitored in real time and manipulated by auto gain function and by manual adjustment of gain to individual detectors at the onset of a session only. If necessary, the optode array was re-fitted to promote close contact with the scalp and accurate placement. One end of the adjustable table was raised to the level of the participant's chin to reduce movement artifact from head movement and the mouthpiece was inserted. A silent scenic video free from written language was played in front of the participants during all conditions to facilitate an awake and alert state in the participants.

Experimental conditions included boluses of medium sour (.08 M citric acid (Nagy, Steele, et al., 2014b), strong sweet (1 M sucrose), and de-ionized water. Oral stimuli were pre-loaded and delivered manually by syringes inserted into a stopcock attached to a plastic cannula; this delivery procedure aimed to minimize movement artifact of jaw opening and closing and overall head movement. During the two conditions with infusion, a slow, steady water infusion of .08 L/minute was delivered continuously through a second plastic cannula by a Masterflex motorized infusion pump (Cole-Parmer Instrument Co., Chicago, IL, model 7518-00). The two plastic cannulas

were inserted and secured in a mouthpiece with dental putty (3M, St. Paul, MN, ESPE Express STD). Both cannulas were attached to the surface of a side table with tape. A new mouthpiece and fresh tubing were utilized for each participant.

Analysis

The frequency of swallows per 15-minute condition was determined by a combination of inductive plethysmography and piezoelectric accelerometry. The ribcage, abdomen and sum channels of the Inductotrace indicated respiratory cycles in three Labchart 8 channels. At the time of data analysis, an additional channel was added to Labchart 8 and programmed to calculate the first derivative of the sum channel. Periods of swallowing apnea on exhalation were indicated by a flat signal in the first derivative channel occurring around 0 V/s and lasting more than 350 ms (Klahn & Perlman, 1999; Martin-Harris, et al., 2005). A large, sharp peak in the accelerometer channel was typically observed when a swallow occurred. Although motion artifact also induces a sharp peak, swallows followed a stereotypical pattern in the shape of the signal and occur simultaneously with a moment of apnea in the first derivative of the Inductotrace sum channel. The Labchart 8 channel for the signal from push button (depressed whenever a swallow was observed and represented by a square waveform) was coupled with the apneic moment and peak of laryngeal elevation to confirm a swallow. If all parameters for a swallow were met, a comment was inserted at the onset of apnea to indicate that a swallow had occurred. If the occurrence of a swallow remained unclear, the synchronous video recording was viewed for laryngeal elevation. Swallows that remained in doubt were excluded from the analysis. Once all swallows had been marked for a condition, times were exported to Excel. Swallows were then divided into those occurring 0-15

seconds after bolus delivery and those occurring 16-30 seconds after the onset of bolus delivery.

The data from fNIRS was analyzed with HomER 2 software (D. Boas, et al., 2012) in Matlab 2013 (The MathWorks Inc., Natick, MA). The time of Labchart and fNIRS signals were synced by linear interpolation of bolus delivery onset in Excel. The onset of bolus delivery was indicated by the fNIRS auxiliary channel for the bolus push button; these stimuli were marked by condition (e.g. "sour + infusion"). High- and low-pass filter settings set at .01 and .5 removed physiological (e.g. Mayer's waves, respiratory and cardiac) signals from the signal. Short separation channels were identified by a threshold of 15 mm channel length. Based on the latency of cortical responses across participants, two sets of analyses were performed in HomER: an event-related analysis of the response to bolus delivery from 2 to 7 seconds and a block average analysis from 17 to 22 seconds. The response noted in the latter time window was likely related to taste effects, as previous research using fNIRS reported peak response to taste around 22 seconds after initial introduction (Okamoto, et al., 2009b).

The processing stream yielded images and values of the average hemodynamic response (HRF) for each channel by condition and participant. The HRF plots were examined for artifact as indicated by oxygenated and deoxygenated responses moving in the same direction. Processed data from HoMER 2 was exported and organized by participant, condition, channel, taster status and HbO and HbR. In each of these analyses, data were converted to z-scores using the mean and standard deviation from baseline activity of -5 to 0 seconds before the onset of water bolus delivery.

Statistical Methods

An a priori power analysis for repeated measures ANOVA with within- and between-subject factors indicated a sample size of 10 was needed. SPSS 22 (IBM, Armonk, NY) and SYSTAT 13 (San Jose, CA) were used for statistical analysis of swallow frequency and cortical activity data. Group means for swallowing frequency were compared using a 2 way repeated measures ANOVA with taste condition and swallowing latency (early vs. late) as repeated measures and taster status as a between-subjects factor. Post hoc comparisons were computed between levels of significant main effects and interactions. A 3 way repeated measures GLM analysis was run comparing within condition, location and side and between participant taster effects. Post hoc tests were used to examine paired contrasts between levels. Finally, Pearson correlation coefficients and p-values were computed to determine the relationship between the number of swallows and mean z-scores of cortical activation from 2 to 7 seconds and from 17 to 22 seconds after bolus delivery. Alpha levels were set at .05 for ANOVAs and post hoc testing.

Results

Participants

A pilot study was run to test feasibility of instrumentation and design; this arm included 2 participants, both female. The full study included 15 healthy volunteers ranging in age from 20 to 55 (mean = 29), 4 of whom were male. Once a taste group had reached its maximum of 5, volunteers were screened by taste status to achieve three taster groups with five members per group. Sixteen individuals volunteered and gave consent to

participate. Each participant completed consent procedures, paperwork and participation in research in one session of 2-3 hours.

With the exclusion of one participant (supertaster) who could not tolerate the sour + infusion condition, participants completed each of the five conditions. One participant (supertaster) could not tolerate more than 10 minutes of the sour condition; the remaining conditions for this participant were normalized to a 10-minute cut-off in the analysis of frequency of swallowing.

Swallowing Frequency

The results indicated that swallows were most frequent 0-6 seconds after onset of bolus delivery (Figure 2); after 6 seconds, swallowing frequency tapered until 15 seconds, then stabilized. Swallows occurring in the early 0-15 sec window were thus considered to be associated with the necessity of disposing of the bolus, whereas swallows after this period were related to the residual effects of taste. Swallowing frequency for early swallows associated with the bolus was significantly higher than swallowing frequency for later swallows associated with the effect of taste ($F(1,11)=93.267$, $p<.001$) (Figures 3 and 4). A significant main effect of taste condition was found ($F(4, 44) = 21.643$, $p < .001$). There was no effect of taster status ($F(1,11)=.261$, $p=.775$). There was no significant interaction between swallowing time (early vs. late) and taste condition ($F(4, 44)=1.609$, $p=.189$).

Post hoc comparisons indicated a significant difference between sour + infusion and water ($p<.001$) as well as between sour + infusion and water + infusion ($p=.001$). Sour and water demonstrated a significant difference ($p<.001$). There was a significant difference between sour + infusion and sweet ($p<.001$) and sour and sweet ($p<.001$).

Water + infusion had a higher swallowing frequency than water (p=.003) and sweet (p=.034). There was no difference between sweet and water (p=.149). Sour and sour + infusion did not differ (p=.288).

Event-related Cortical Response to Bolus Delivery

Using a GLM 3 way repeated measures ANOVA, for taste condition, hemisphere and location, there was no significant main effect of condition ($F(4,40)= 1.735$, $p=.161$), side ($F(1,10)= 59.775$, $p=.441$) or location ($F(3,30)=2.687$, $p=.064$). There was no effect of taster status ($F(2,10)= 2.64$, $p=.12$). The only significant finding was an interaction between location and side ($F (3, 30)= 3.12$, $p=.041$) (Figures 5-7). There was no significant interaction between condition and location ($F(12,120)= .408$, $p=.958$) or condition and hemisphere ($F(4,40)= .964$, $p=.438$). The only post hoc comparison that showed a difference was between the right premotor and right sensory z-scores (p=.011).

Block Average Cortical Response

For the block average analysis, there was a significant main effect of taste condition ($F (4, 40)= 4.427$, $p=.005$). There was also a significant main effect of location ($F(3,30= 16.686$, $p<.001$) (Figure 8). There was no effect of side ($F(1,10)= .659$, $p=.436$) or taster status ($F(1,10)= .483$, $p=.63$). No significant interactions were found between condition and channel ($F(12, 120)=1.751$, $p=.064$), condition and side ($F(4, 40)=1.345$, $p=.27$), or channel and side ($F(3,30)= .114$, $p=.951$).

Post hoc contrasts for the main effect of taste condition indicated a significant difference between sour and water (p=.012) and sour + infusion and water (p= .002) (Figure 9). There was a significant difference between the sour + infusion and sweet conditions (p=.018) (Figure 10). There was no difference between the sour and sour +

infusion conditions (p=.124) (Figure 9) or between sour and water + infusion (p=.12). There was no significant differences between sweet and water conditions (p=.315) or water and water + infusion (p= .088).

The mean z-scores were greater in the premotor cortical location than in the sensory cortical location (p=.004), the SMA (p=.001) and the dummy channel (p=.002). The mean z-scores for the sensory cortical location were greater than in the dummy (p=.008) and SMA channels (p=.002). There was no significant difference between the SMA and dummy channels (p=.323).

Correlations between Swallowing Frequency and Cortical Activation

Based on these results, correlations were calculated between z-scores for the event-related cortical response to bolus delivery and early bolus swallowing frequency (2-7 seconds after bolus delivery), and z-scores for the block average cortical response and late swallowing frequency (17-22 seconds after bolus delivery).

A significant correlation between the z-scores for event-related cortical response and early swallows was found during the water condition in the right premotor region (r=.513, p=.035) (Figure 11). Additionally, there were significant correlations between these measures during the sweet condition in the left SMA region (r=.459, p=.049) and during the water + infusion condition in the left sensory region (r=.624, p=.006).

There was a significant correlation between block average cortical response and late swallowing frequency during the sour condition in the left premotor (r= -.551, p=.017) (Figure 12) and right premotor regions (r= -.574, p=.013) (Figure 13). There was also a significant correlation between these measures during water + infusion in the left premotor region (r= -.533, p=.02) (Figure 14). There were also significant correlations

between cortical response and swallowing frequency during the sour + infusion condition in the right sensory region (r=.464, p=.047) and during the water + infusion condition in the left SMA region (r= -.615, p=.007).

Discussion

This study examined variations in swallowing frequency and cortical activation in different genetic taster groups while swallowing different taste solutions. The results indicate that a sour bolus elicits a higher swallowing frequency than water and sweet taste. Although swallowing frequency was higher for the water +infusion condition than the water condition, there was no difference between the sour and sour + infusion conditions. This suggests that the response to sour taste was not dependent upon salivary flow as it was similar with and without infusion. In addition, taster status did not have an effect on swallowing frequency. A similar pattern was found for cortical activation of the swallowing network in response to taste, with a similar increase in cortical activation in response to sour and sour + infusion, and a greater cortical response to sour boluses in comparison to other tastes. A similar cortical response to was found to water and sweet. There was no effect of taster status, nor of infusion. In both hemispheres, the premotor areas had the greatest response to taste, followed by the sensory channels, while the dummy and SMA channels did not differ. There was significantly greater cortical response to the bolus delivery in the sensory region than in the premotor region on the right side only. Swallowing frequency and cortical response to water 2-7 seconds after bolus delivery were positively correlated in the right premotor area. At 17-22 seconds after onset of bolus delivery, negative correlations were found between swallowing

frequency and left and right premotor areas during the sour condition and between swallowing frequency and the left premotor region during water + infusion.

Taste Condition

The present finding that sour maximally enhances swallowing frequency in comparison to water is supported by the literature (Nederkoorn, et al., 1999). The effect of modulates swallowing in the CPGs by introducing sensory input that increases firing rates of neurons that instigate the onset of a swallow. In previous studies, sour taste trials resulted in higher submental EMG amplitudes than all other tastes, including sweet, which had the lowest EMG amplitude (Leow, et al., 2007). Although it was predicted that sour taste would result in a higher swallowing frequency, the unexpected finding that sweet taste did not differ from the water condition indicates that sweet taste does not up-regulate the swallowing network to the same degree as sour.

The similar cortical activation results indicate that a sour taste affects not only the brainstem level programming of swallowing, but also the cortical activation of the swallowing network. The premotor cortices in each hemisphere demonstrated the highest response 17-22 seconds after bolus delivery; the introduction of a sour taste likely altered cortical activation for swallow initiation after a bolus. These results are further substantiated by a small body of literature. A greater change in blood flow occurred in response to flavored stimuli, including lemonade, than to non-flavored stimuli (dry swallow or water) specific to the cingulate gyrus, prefrontal cortex, S1, and M1 (Babaei, et al., 2010). Swallowing a sour bolus yielded activity in S1, anterior cingulate cortex, insula, supplementary motor area, inferior frontal gyrus, and inferior parietal gyrus; activation was higher for sour than water in the SMA, and a gradual sensitization

(increase in BOLD response over 20 swallows) to the sour bolus was noted in S1 and the inferior parietal gyrus (Humbert & Joel, 2012). A subsequent study found significantly increased interconnectivity within the insula and between the insula and M1, S1, inferior frontal gyrus, supramarginal gyrus, and rolandic operculum when tasting a sour stimulus, with results lateralized to the left hemisphere (Humbert & McLaren, 2014). Due to this supporting evidence, sour taste can play an effective role in up-regulating the swallowing network.

Taster Status

Although the lack of difference between genetic taster status groups on all outcome measures was not expected, the existing literature on this topic is limited and includes mixed results. For example, supertasters reportedly demonstrated higher linguapalatal pressure, larger submental EMG amplitudes, and longer swallowing apnea duration than nontasters when swallowing high concentrations of tastes (Nagy, Steele, et al., 2014b; Pelletier & Steele, 2014) (Plonk, et al., 2011). In contrast, other studies have indicated no differences between taster status groups on the same measures of swallowing function (Nagy, Steele, et al., 2014a; Todd, et al., 2012a, 2012b). The small body of neuroimaging research reports a significant effect of taster status group when tasting fat emulsions and (Eldeghaidy, et al., 2011) and PROP-impregnated filter paper (Bembich, et al., 2010). Genetic taster status may lead to different responses in certain aspects of swallowing physiology not captured by swallowing frequency. Additionally, differences in cortical activation patterns may be limited to certain types of stimuli such the previously mentioned fat emulsions and PROP. However, taster status was not a

significant factor in this study, with not effect on swallowing frequency, cortical response to a bolus, or in late cortical response 17-22 seconds after the bolus.

Salivary flow

It was expected that the increase in swallowing frequency with a slow infusion to control the effect of salivary flow due to sour taste would be the same as a sour bolus alone as salivary flow would increase as a result of a sour bolus. Further, it was expected that there would be an increase in activation in S1 without slow infusion. Research has indicated that taste stimuli alter salivary flow, with the highest salivary flow being induced by a sour bolus (Lashley, 1916; Nederkoorn, et al., 1999; Watanabe & Dawes, 1988). The results of this study aligned with previous findings, as swallowing frequency did not significantly differ between the sour condition with infusion and the sour condition without infusion. The effect of sour taste heightened salivary flow to the extent that the sour condition had the same swallowing frequency with and without the introduction of additional liquid via infusion pump. The finding of a higher swallowing frequency associated with water + infusion in comparison to water without infusion resulted from a larger amount of liquid to be swallowed in the former, with no effect of taste to impact salivary flow. As indicated by Humbert et al. (2009), "…water represents a primarily tactile stimulus to the oropharynx because it is tastless…" (p. 990), and thus was not seen to effect salivary flow to the same extent as sour taste. The similarity between the sour conditions with and without slow infusion indicates that the effect of taste on salivary flow and swallowing frequency occurs at the level of the brainstem, where salivary gland secretion is initiated in structures including the NTS (Matsuo,

1999), and that the effect of taste on salivary flow is distinct from the sensation of a bolus in the oral cavity.

Hemisphere

There was no effect of hemisphere in response to taste, indicating similar degrees of bilateral activation. The significant interaction between hemisphere and cortical location in response to the bolus was specific to a higher response in S1 of the right hemisphere. Previous research reported bilateral activation of the sensory and motor regions in response to air pulse stimulation (Lowell, et al., 2008; Soros, et al., 2008) and swallowing of water boluses (Hamdy et al., 1999; R. E. Martin, Goodyear, Gati, & Menon, 2001) using fMRI. In contrast, swallowing water boluses yielded lateralization in the left primary motor and sensory regions, which subsequently shifted to a right lateralization (Mihai, Otto, Platz, Eickhoff, & Lotze, 2014), whereas another study found alternating patterns of lateralization, with great lateralization in the right hemisphere in response dry and bolus swallows (K. M. Mosier, Liu, Maldjian, Shah, & Modi, 1999). A consensus has yet to be developed as to the lateralization of swallowing response, and it is likely that there is no systemic hemispheric dominance across participants.

Correlation between swallowing frequency and cortical response

During bolus delivery, there was a significant correlation between early swallowing frequency and amplitude of activation in M1 in the water condition. This result suggests that the amplitude in M1 increases in response to the presence of a bolus, and that the sensory effects of the presence of a bolus up-regulates swallowing frequency at the level of the cortex. The negative correlations between later swallowing frequency

after the bolus and amplitude of response in M1 indicate that amplitude increases may have been related to taste rather than swallowing. The different degrees of cortical activation in relation to taste occurred despite a decrease in swallowing frequency.

Limitations

The results of this study may have limited generalizability to different age populations. Although taster status is not impacted by aging, the natural aging process does involve a decrease in sensitivity to taste (Breslin & Huang, 2006). The threshold for detection and identification of all tastes increases significantly and the perception of intensity differences decreases in individuals above 65 years of age (Methven, et al., 2012). However, reduced sensitivity due to aging is "discrete" (localized to a specific area), and whole-mouth assessment of taste with high intensities of taste reveals no difference between young and old participants (Bartoshuk, 1989).

Due to the evidence that dysphagia and hypogeusia may co-occur, a sour stimulus may not be effective for altering swallowing function in some persons with swallowing disorders. Research indicates that stroke patients may have a transient disorder of taste (Heckmann, et al., 2005). Neurodegenerative and neosplastic etiologies of dysphagia may also involve a taste disorder (Heckman & Lang, 2006; Shah, et al., 2009). Taste function should be assessed in patients with different types of neurological disorders.

Despite the effect of sour taste on swallowing function, the palatability of sour may limit its application to persons with different genetic taster status. Although the concentration of the sour stimulus was set at a medium intensity level, one supertaster could not tolerate one of the sour conditions, and a second supertaster could only complete 10 minutes of a 15-minute sour condition.

Conclusions

The results of this study indicate that a sour bolus increases the frequency of swallowing to a greater degree than other taste stimuli through both brainstem and cortical connections. Salivary flow increases in response to taste, and is brainstem-mediated. The response to the presence of a bolus was greatest in the right sensory area in the cortex. Cortical changes in activation long after the bolus was swallowed continued between 17 and 22 seconds after initial presentation, showing a prolonged effect of taste at the cortex after presentation.

Table 1

Coordinates for cortical regions of interest (MNI)

		X	Y	Z
Right				
	S1	59	-17	35
	M1	57	8	23
	SMA	13	8	62
Left				
	S1	-56	-16	35
	M1	-55	8	23
	SMA	-13	8	62

Figure Captions

Figure 1. Array of emitters (red) and detectors (blue). Regions of interest marked in green.

Figure 2. Histogram of latency of swallows after sour and water onset of bolus delivery.

Figure 3. Swallowing frequency by taste condition 0-15 sec. after bolus delivery

Figure 4. Swallowing frequency by taste condition 16-30 sec. after bolus delivery

Figure 5. Event-related response to bolus by side and channel for nontaster group.

Figure 6. Event-related response to bolus by side and channel for medium taster group

Figure 7. Event-related response to bolus by side and channel for supertaster group

Figure 8. Block average response to taste by condition and channel. Boxes within each plot are in the following order: left motor, left sensory, left SMA, right motor, right sensory, right SMA, left dummy, right dummy

Figure 9. Within participant block average response to sour + infusion vs. sweet in the left and right premotor cortical areas

Figure 10. Within participant block average response to sour conditions for sour vs. water (A) and sour + infusion vs. water (B) in the right and left premotor cortical areas

Figure 11. Correlation between right motor cortical response z-scores and swallowing frequency 2-7 sec after bolus delivery during water condition

Figure 12. Correlation between left motor cortical response z-scores and swallowing frequency 17-22 sec after bolus delivery during sour condition

Figure 13. Correlation between right motor cortical response z-scores and swallowing frequency 17-22 seconds after bolus delivery during sour condition

Figure 14. Correlation between left motor cortical response z-scores and swallowing frequency 17-22 seconds after bolus delivery during water + infusion condition

Figure 1

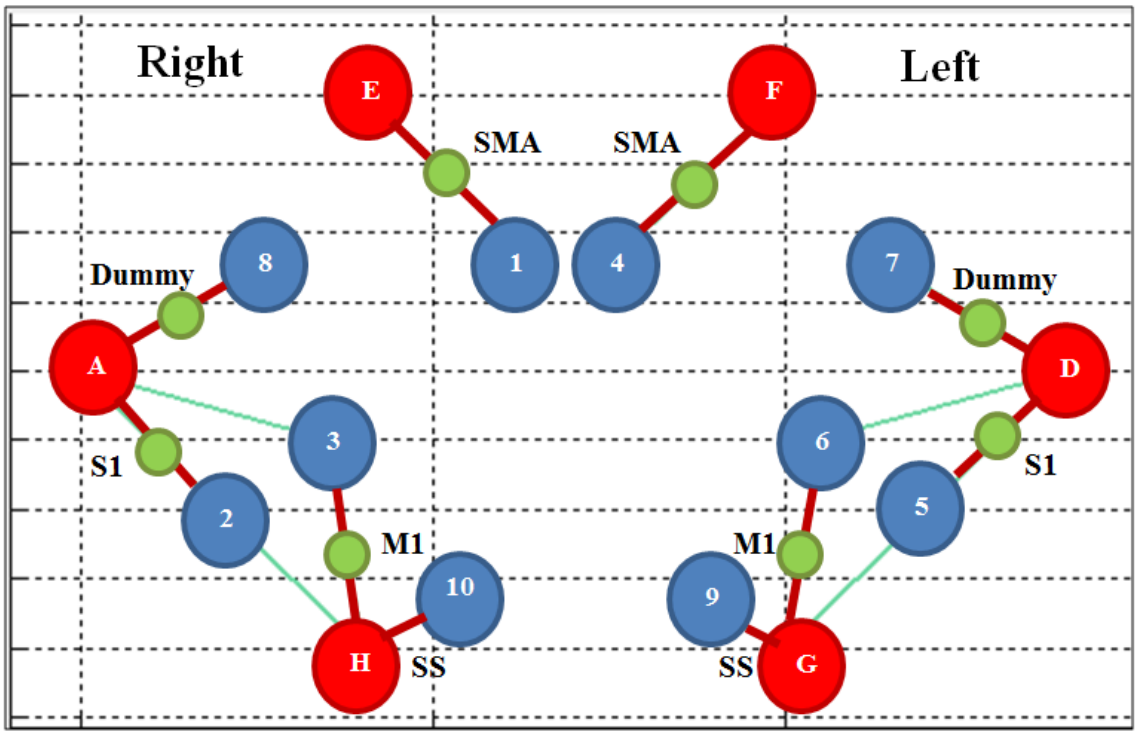

Right
Left
E
F
SMA
SMA
1
4
Dummy
8
7
Dummy
A
D
S1
S1
3
6
2
5
M1
M1
10
9
H
G
SS
SS

Figure 2

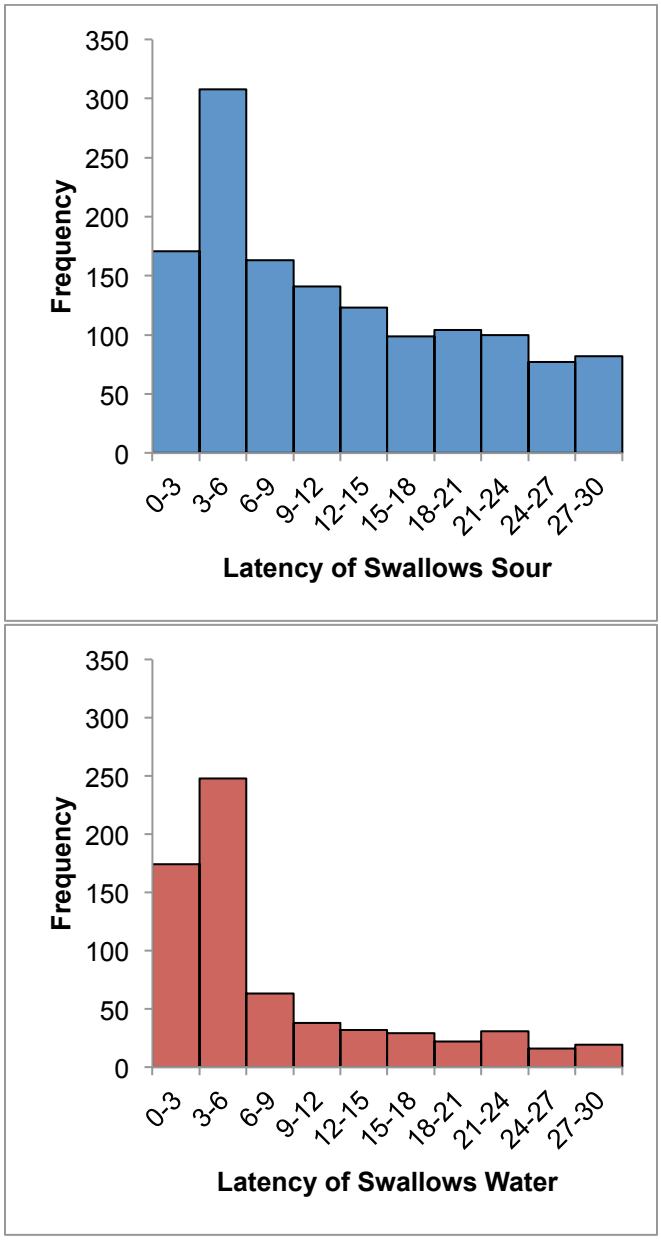

350
300
250
200
150
100
50
0
Frequency
0-3
3-6
6-9
9-12
12-15
15-18
18-21
21-24
24-27
27-30
Latency of Swallows Sour
350
300
250
200
150
100
50
0
Frequency
0-3
3-6
6-9
9-12
12-15
15-18
18-21
21-24
24-27
27-30
Latency of Swallows Water

Figure 3

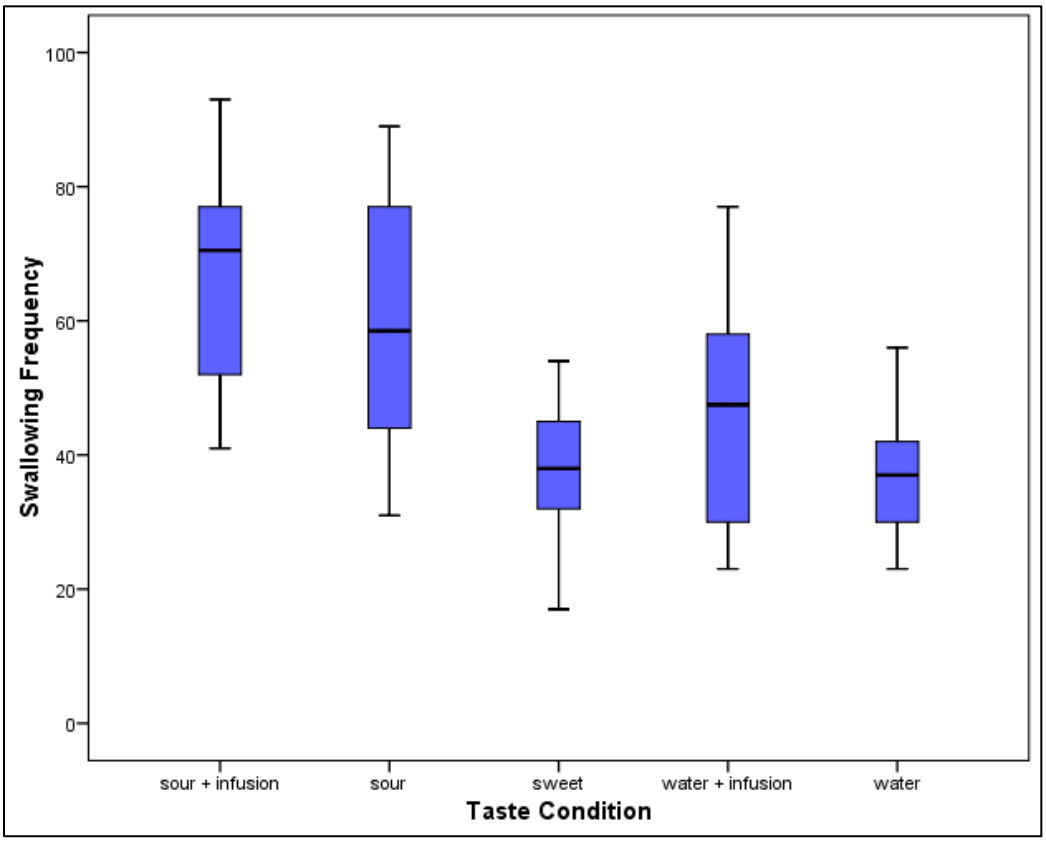

100
80
60
40
20
0
Swallowing Frequency
sour + infusion
sour
sweet
water + infusion
water
Taste Condition

Figure 4

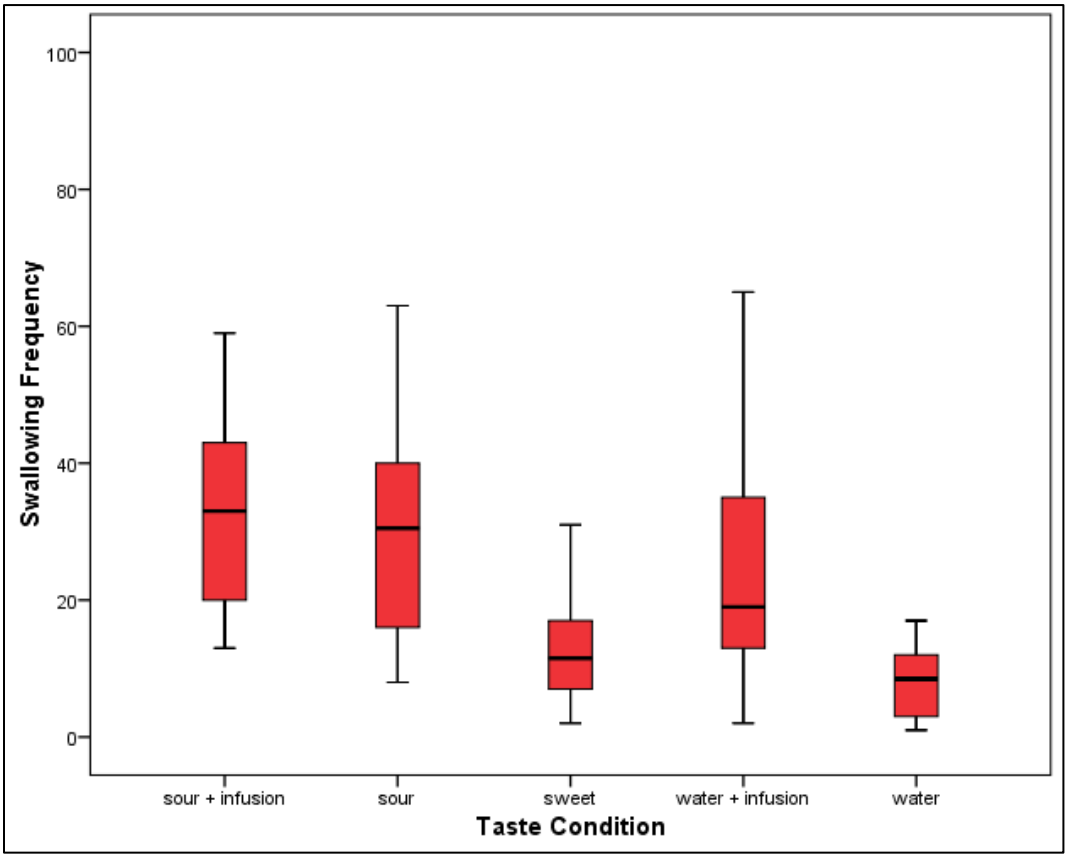

100
80
60
40
20
0
Swallowing Frequency
sour + infusion
sour
sweet
water + infusion
water
Taste Condition

Figure 5

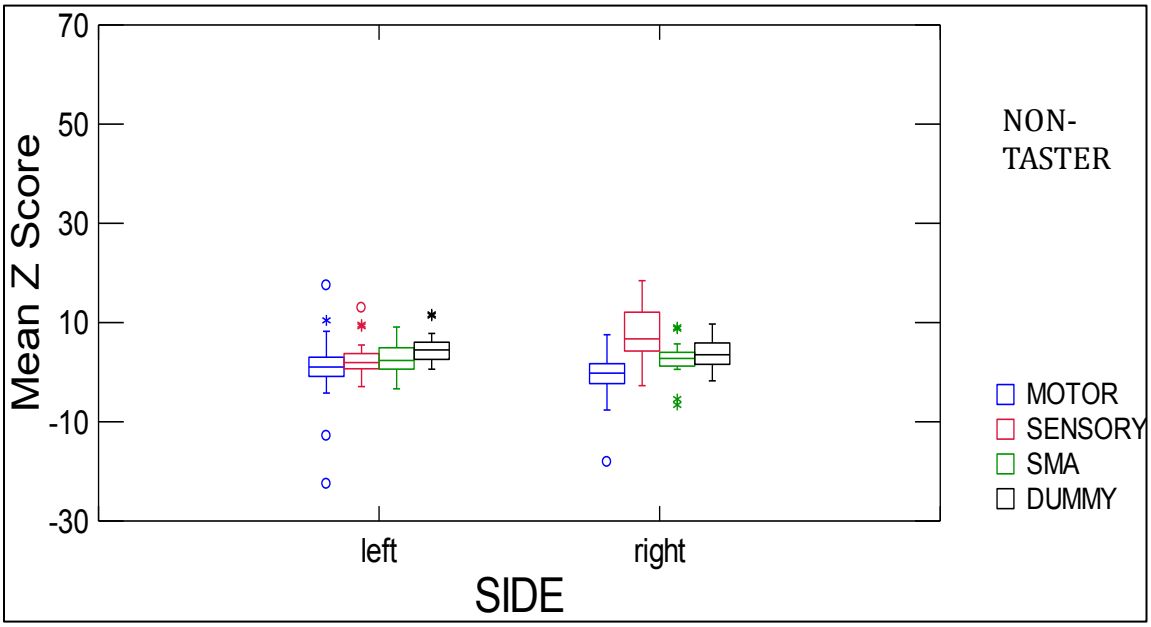

70
50
30
10
-10
-30
Mean Z Score
left
right
SIDE
NON-
TASTER
MOTOR
SENSORY
SMA
DUMMY

Figure 6

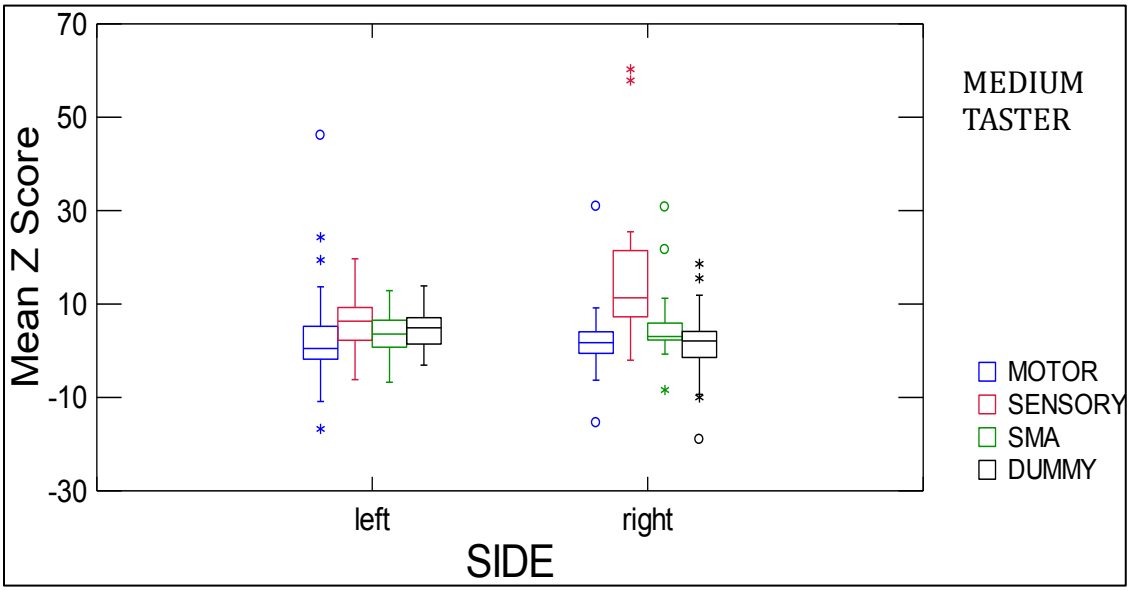

70
50
30
10
-10
-30
Mean Z Score
left
right
SIDE
MEDIUM
TASTER
MOTOR
SENSORY
SMA
DUMMY

Figure 7

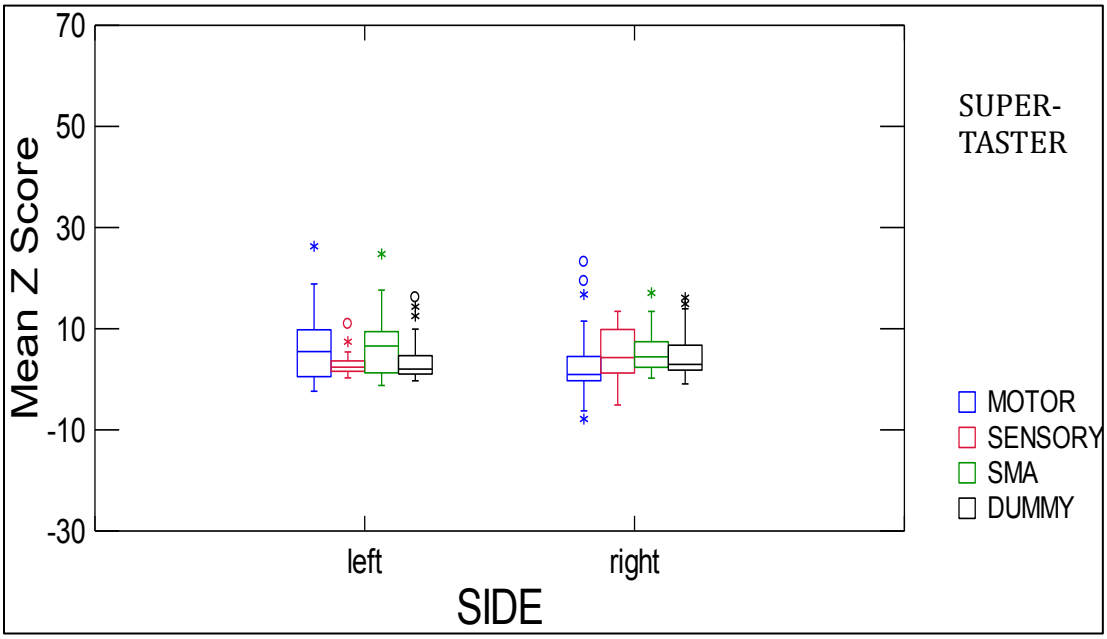

70
50
30
10
-10
-30
Mean Z Score
left
right
SIDE
SUPER-
TASTER
MOTOR
SENSORY
SMA
DUMMY

Figure 8

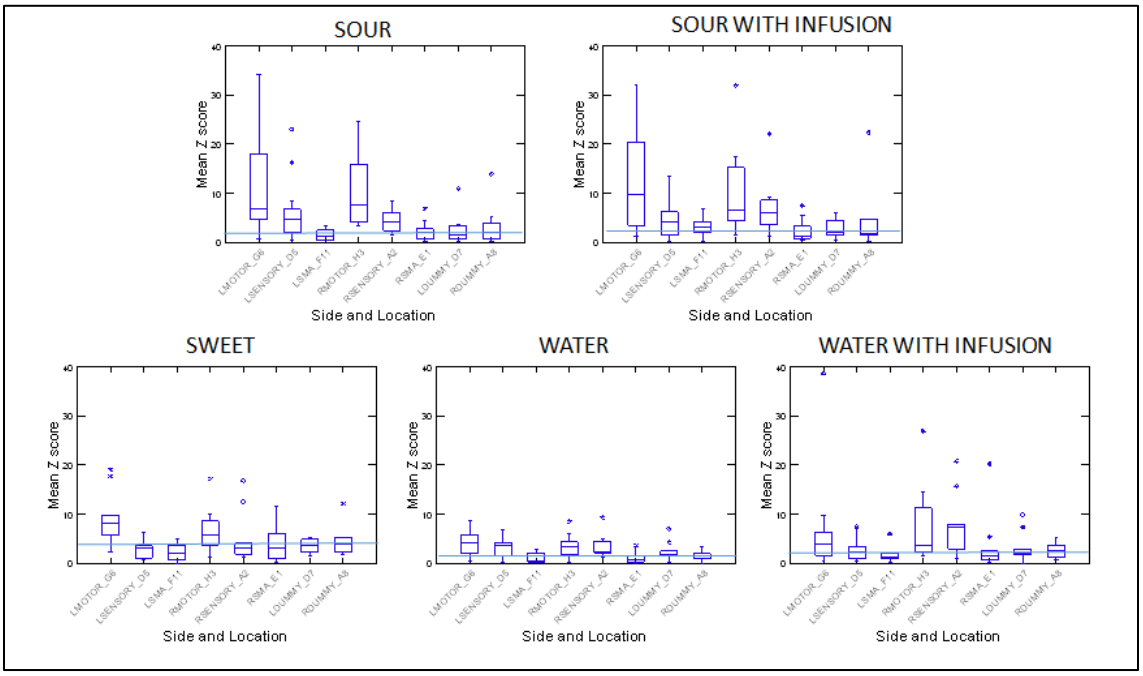

SOUR
SOUR WITH INFUSION
SWEET
WATER
WATER WITH INFUSION
Mean Z score
Side and Location
LMOTOR_G6
LSENSORY_D5
LSMA_F11
RMOTOR_H3
RSENSORY_A2
RSMA_E1
LOUMMY_D7
ROUMMY_A8

Figure 9

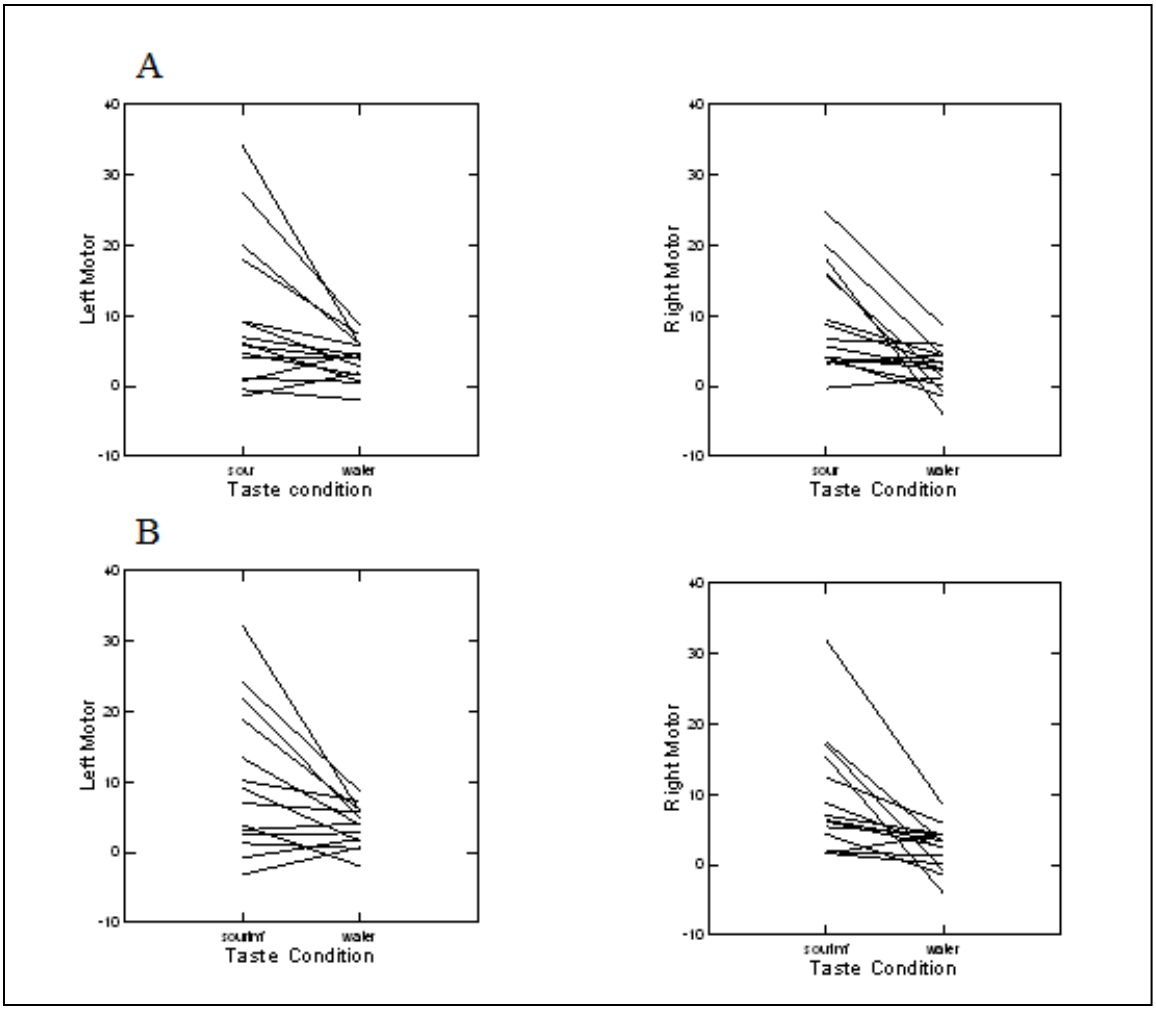

A
Left Motor
Taste condition
sour
water
Right Motor
Taste Condition
B
Left Motor
Taste Condition
sourlmf
water
Right Motor
Taste Condition

Figure 10

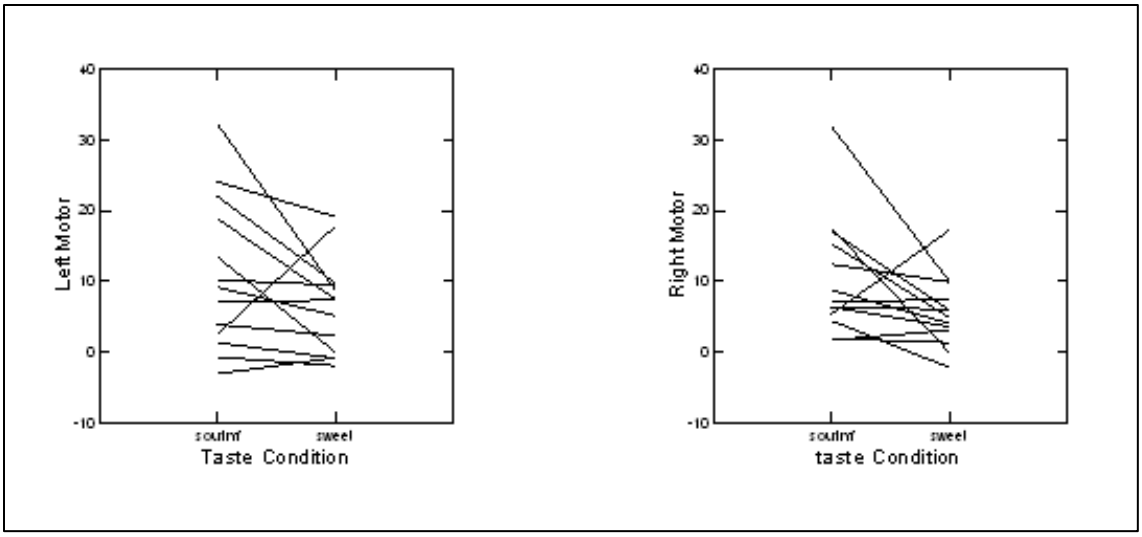

Left Motor
40
30
20
10
0
-10
soutnf
sweel
Taste Condition
Right Motor
40
30
20
10
0
-10
soutnf
sweel
taste Condition

Figure 11

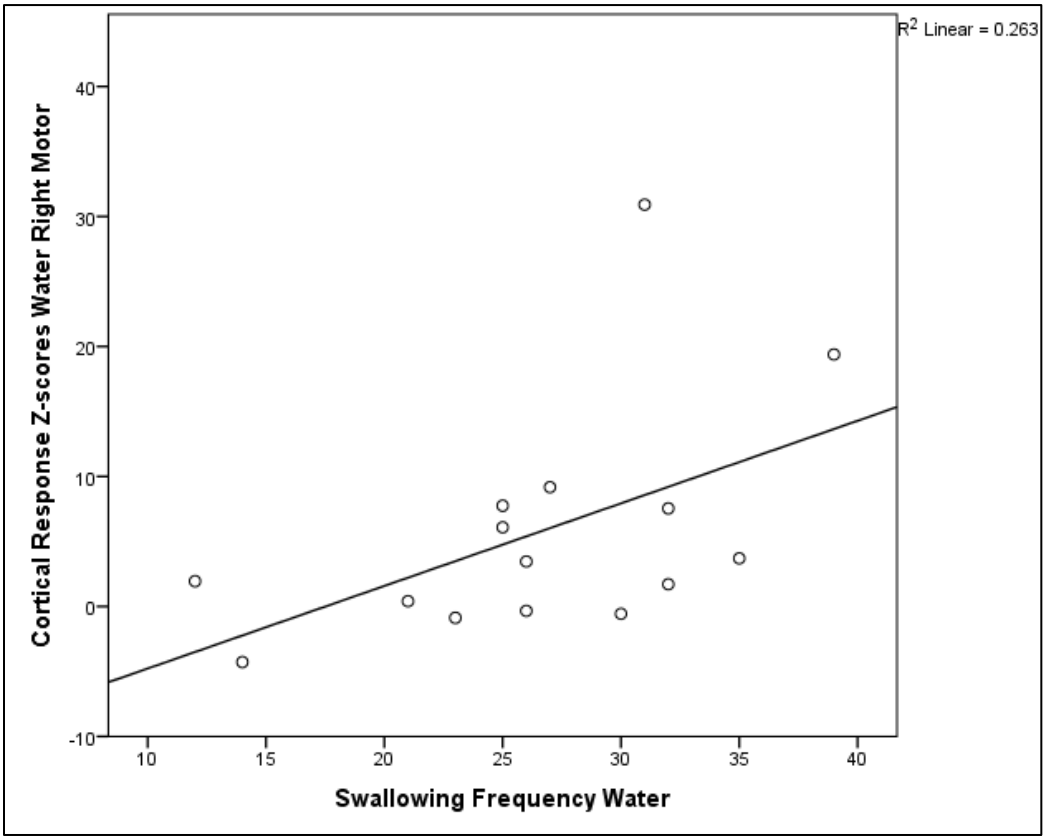

R2 Linear = 0.263
Cortical Response Z-scores Water Right Motor
40
30
20
10
0
-10
10
15
20
25
30
35
40
Swallowing Frequency Water

Figure 12

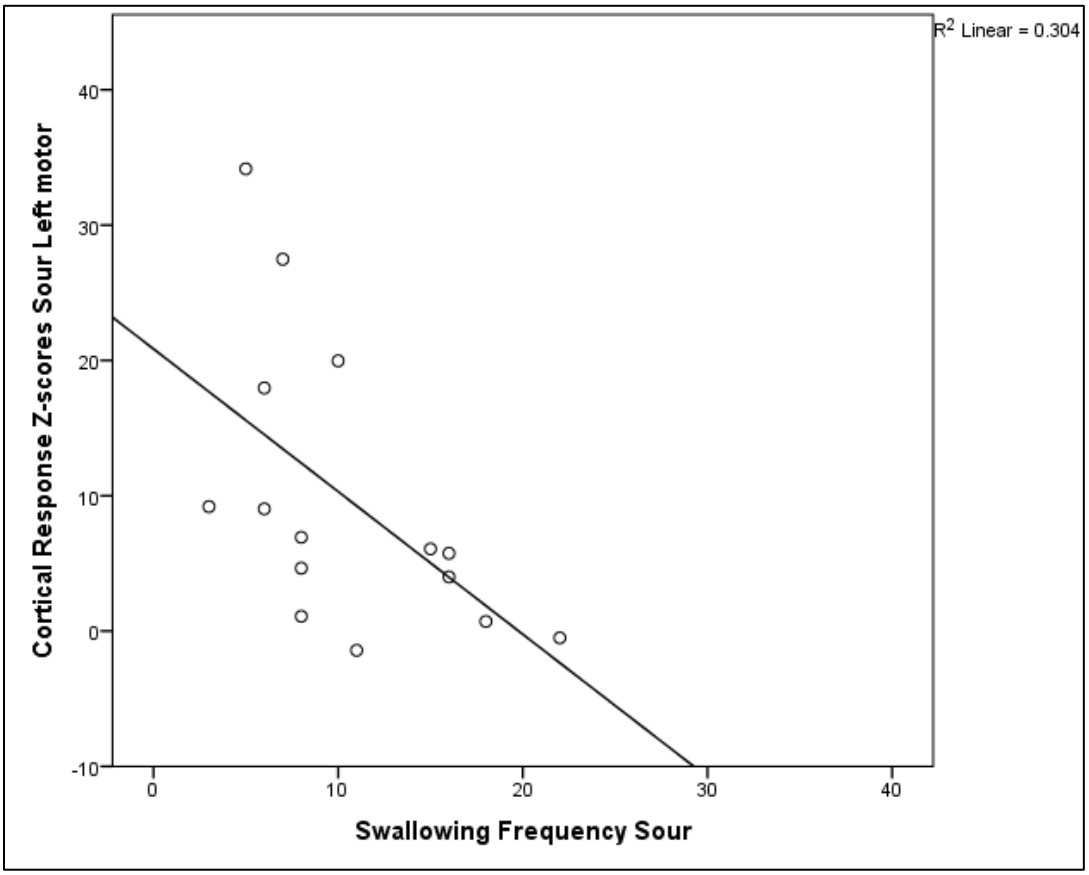

R2 Linear = 0.304
Cortical Response Z-scores Sour Left motor
40
30
20
10
0
-10
0
10
20
30
40
Swallowing Frequency Sour

Figure 13

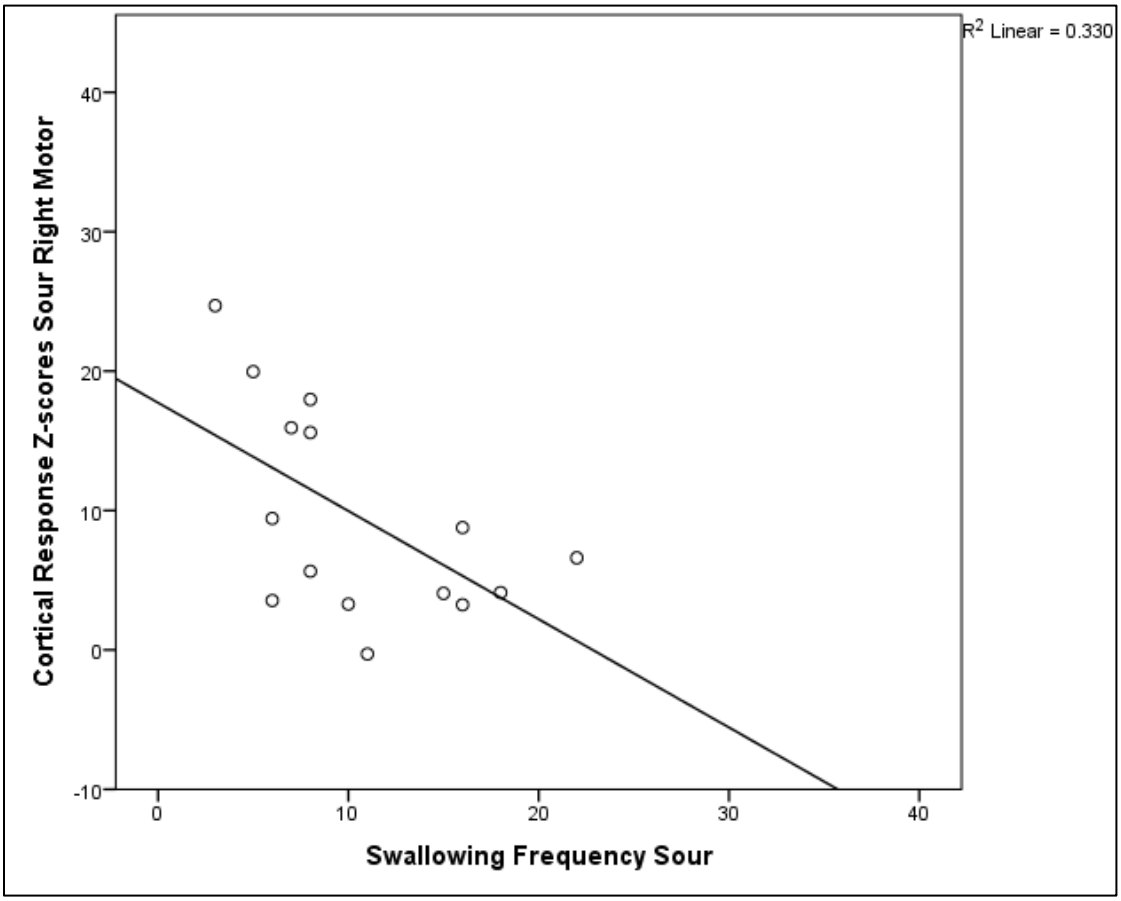
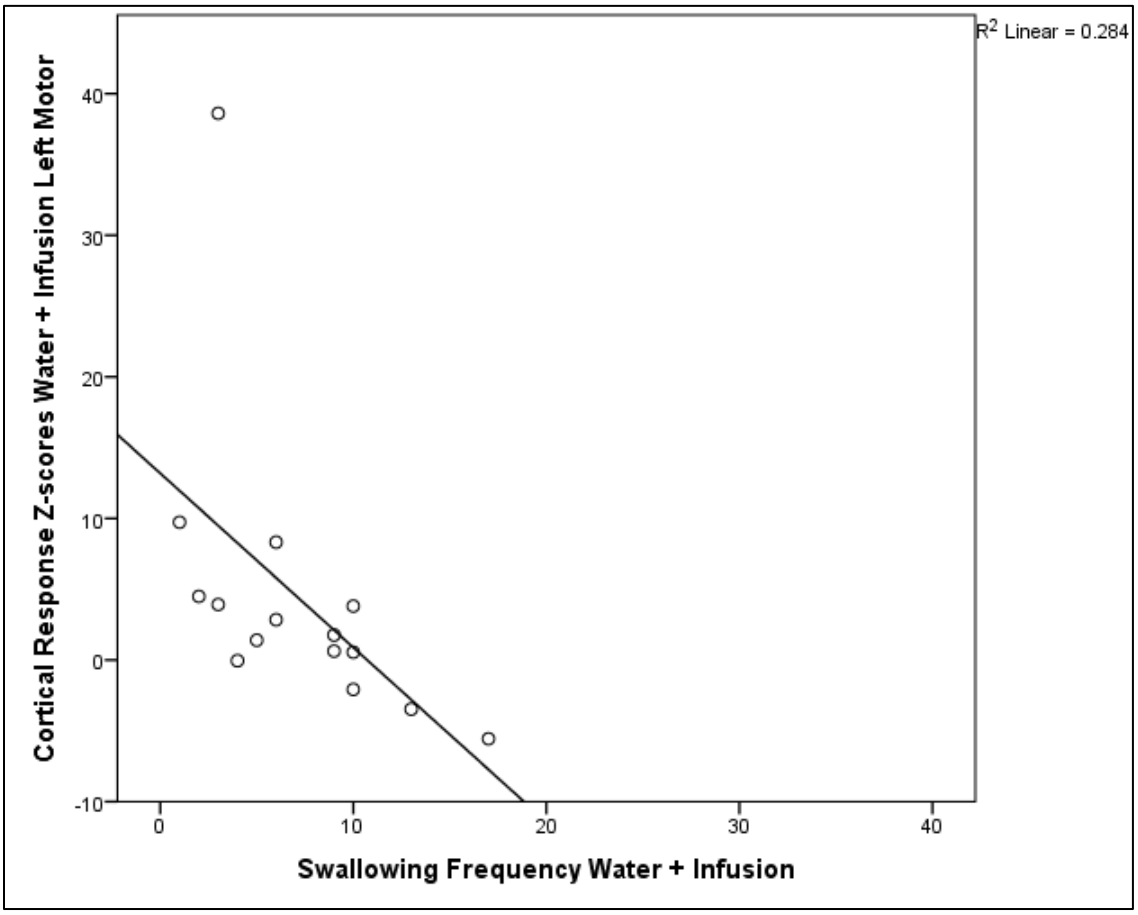

R2 Linear = 0.330
Cortical Response Z-scores Sour Right Motor
40
30
20
10
0
-10
0
10
20
30
40
Swallowing Frequency Sour

Figure 14


R2 Linear = 0.284
Cortical Response Z-scores Water + Infusion Left Motor
40
30
20
10
0
-10
0
10
20
30
40
Swallowing Frequency Water + Infusion

References

Abdul Wahab, N., Jones, R. D., & Huckabee, M. L. (2010). Effects of olfactory and gustatory stimuli on neural excitability for swallowing. *Physiology & behavior, 101*(5), 568-575. doi: 10.1016/j.physbeh.2010.09.008

Accolla, R., Bathellier, B., Petersen, C. C., & Carleton, A. (2007). Differential spatial representation of taste modalities in the rat gustatory cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 27*(6), 1396-1404. doi: 10.1523/JNEUROSCI.5188-06.2007

Alves, L. M., Fabio, S. R., & Dantas, R. O. (2013). Effect of bolus taste on the esophageal transit of patients with stroke. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / I.S.D.E, 26*(3), 305-310. doi: 10.1111/j.1442-2050.2012.01366.x

Anselmino, M., Zaninotto, G., Costantini, M., Ostuni, P., Ianniello, A., Boccu, C., . . . Ancona, E. (1997). Esophageal motor function in primary Sjogren's syndrome: correlation with dysphagia and xerostomia. *Digestive diseases and sciences, 42*(1), 113-118.

Arvidson, K., & Friberg, U. (1980). Human taste: response and taste bud number in fungiform papillae. *Science, 209*(4458), 807-808.

Babaei, A., Kern, M., Antonik, S., Mepani, R., Ward, B. D., Li, S. J., . . . Shaker, R. (2010). Enhancing effects of flavored nutritive stimuli on cortical swallowing network activity. *American journal of physiology. Gastrointestinal and liver physiology, 299*(2), G422-429. doi: 10.1152/ajpgi.00161.2010

Babaei, A., Ward, B. D., Siwiec, R. M., Ahmad, S., Kern, M., Nencka, A., . . . Shaker, R. (2013). Functional connectivity of the cortical swallowing network in humans. *NeuroImage, 76*, 33-44. doi: 10.1016/j.neuroimage.2013.01.037

Bartoshuk, L. M. (1989). Taste. Robust across the age span? *Annals of the New York Academy of Sciences, 561*, 65-75.

Bartoshuk, L. M., Duffy, V. B., Green, B. G., Hoffman, H. J., Ko, C. W., Lucchina, L. A., . . . Weiffenbach, J. M. (2004). Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching. *Physiology & behavior, 82*(1), 109-114. doi: 10.1016/j.physbeh.2004.02.033

Bartoshuk, L. M., Duffy, V. B., & Miller, I. J. (1994). PTC/PROP tasting: anatomy, psychophysics, and sex effects. *Physiology & behavior, 56*(6), 1165-1171.

Bembich, S., Lanzara, C., Clarici, A., Demarini, S., Tepper, B. J., Gasparini, P., & Grasso, D. L. (2010). Individual differences in prefrontal cortex activity during perception of bitter taste using fNIRS methodology. *Chemical senses, 35*(9), 801-812. doi: 10.1093/chemse/bjq080

Bender, G., Veldhuizen, M. G., Meltzer, J. A., Gitelman, D. R., & Small, D. M. (2009). Neural correlates of evaluative compared with passive tasting. *The European journal of neuroscience, 30*(2), 327-338. doi: 10.1111/j.1460-9568.2009.06819.x

Bertoli, S., Laureati, M., Battezzati, A., Bergamaschi, V., Cereda, E., Spadafranca, A., . . . Pagliarini, E. (2014). Taste sensitivity, nutritional status and metabolic syndrome: Implication in weight loss dietary interventions. *World journal of diabetes, 5*(5), 717-723. doi: 10.4239/wjd.v5.i5.717

Bisch, E. M., Logemann, J. A., Rademaker, A. W., Kahrilas, P. J., & Lazarus, C. L. (1994). Pharyngeal effects of bolus volume, viscosity, and temperature in patients with dysphagia resulting from neurologic impairment and in normal subjects. *J Speech Hear Res, 37*(5), 1041-1059.

Boas, D., Dubb, J., & Huppert, T. (2012). Hemodynamic Evoked Response (HomER 2) [Software], from Available from http://www.nmr.mgh.harvard.edu/PMI/resources/homer2/home.htm

Boas, D. A., Gaudette, T., Strangman, G., Cheng, X., Marota, J. J., & Mandeville, J. B. (2001). The accuracy of near infrared spectroscopy and imaging during focal changes in cerebral hemodynamics. *NeuroImage, 13*(1), 76-90. doi: 10.1006/nimg.2000.0674

Bonnans, S. R., & Noble, A. C. (1995). Interaction of salivary flow with temporal perception of sweetness, sourness, and fruitiness. *Physiology & behavior, 57*(3), 569-574.

Bove, M., Mansson, I., & Eliasson, I. (1998). Thermal oral-pharyngeal stimulation and elicitation of swallowing. *Acta Otolaryngol, 118*(5), 728-731.

Bremner, R. M., Hoeft, S. F., Costantini, M., Crookes, P. F., Bremner, C. G., & DeMeester, T. R. (1993). Pharyngeal swallowing. The major factor in clearance of esophageal reflux episodes. *Annals of surgery, 218*(3), 364-369; discussion 369-370.

Breslin, P. A. (2000). Human Gustation. In T. Finger, W. Silver & D. Restrepo (Eds.), *The neurobiology of taste and smell* (2 ed., pp. 423-461). New York, NY: Wiley-Liss.

Breslin, P. A., & Huang, L. (2006). Human taste: peripheral anatomy, taste transduction, and coding. *Adv Otorhinolaryngol, 63*, 152-190. doi: 93760 [pii]
10.1159/000093760

Butler, S. G., Stuart, A., Castell, D., Russell, G. B., Koch, K., & Kemp, S. (2009). Effects of age, gender, bolus condition, viscosity, and volume on pharyngeal and upper esophageal sphincter pressure and temporal measurements during swallowing. *J Speech Lang Hear Res, 52*(1), 240-253. doi: 10.1044/1092-4388(2008/07-0092)

Car, A., Jean, A., & Roman, C. (1975). A pontine primary relay for ascending projections of the superior laryngeal nerve. *Experimental brain research, 22*(2), 197-210.

Caruso, A. J., Sonies, B. C., Atkinson, J. C., & Fox, P. C. (1989). Objective measures of swallowing in patients with primary Sjogren's syndrome. *Dysphagia, 4*(2), 101-105.

Chatfield, P. (1941). Salivation in response to localized stimulation of the medulla. *Am J Physiol, 133*, 637-641.

Chee, C., Arshad, S., Singh, S., Mistry, S., & Hamdy, S. (2005). The influence of chemical gustatory stimuli and oral anaesthesia on healthy human pharyngeal swallowing. *Chemical senses, 30*(5), 393-400. doi: 10.1093/chemse/bji034

Chen, X., Gabitto, M., Peng, Y., Ryba, N. J., & Zuker, C. S. (2011). A gustotopic map of taste qualities in the mammalian brain. *Science, 333*(6047), 1262-1266. doi: 10.1126/science.1204076

Clave, P., de Kraa, M., Arreola, V., Girvent, M., Farre, R., Palomera, E., & Serra-Prat, M. (2006). The effect of bolus viscosity on swallowing function in neurogenic

dysphagia. *Alimentary pharmacology & therapeutics, 24*(9), 1385-1394. doi: 10.1111/j.1365-2036.2006.03118.x

Cola, P. C., Gatto, A. R., da Silva, R. G., Spadotto, A. A., Ribeiro, P. W., Schelp, A. O., . . . Henry, M. A. (2012). Taste and temperature in swallowing transit time after stroke. *Cerebrovascular diseases extra, 2*(1), 45-51. doi: 10.1159/000339888

Cola, P. C., Gatto, A. R., Silva, R. G., Spadotto, A. A., Schelp, A. O., & Henry, M. A. (2010). The influence of sour taste and cold temperature in pharyngeal transit duration in patients with stroke. *Arquivos de gastroenterologia, 47*(1), 18-21.

Contreras, R. J., Gomez, M. M., & Norgren, R. (1980). Central origins of cranial nerve parasympathetic neurons in the rat. *J Comp Neurol, 190*(2), 373-394. doi: 10.1002/cne.901900211

Dantas, R. O., Dodds, W. J., Massey, B. T., & Kern, M. K. (1989). The effect of high- vs low-density barium preparations on the quantitative features of swallowing. *AJR. American journal of roentgenology, 153*(6), 1191-1195. doi: 10.2214/ajr.153.6.1191

Dawes, C., & Watanabe, S. (1987). The effect of taste adaptation on salivary flow rate and salivary sugar clearance. *Journal of dental research, 66*(3), 740-744.

de Araujo, I. E., Rolls, E. T., Kringelbach, M. L., McGlone, F., & Phillips, N. (2003). Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain. *The European journal of neuroscience, 18*(7), 2059-2068.

Ding, R., Logemann, J. A., Larson, C. R., & Rademaker, A. W. (2003). The effects of taste and consistency on swallow physiology in younger and older healthy individuals: a surface electromyographic study. *Journal of speech, language, and hearing research : JSLHR, 46*(4), 977-989.

Doty, R. W., & Bosma, J. F. (1956). An electromyographic analysis of reflex deglutition. *Journal of neurophysiology, 19*(1), 44-60.

Doty, R. W., Richmond, W. H., & Storey, A. T. (1967). Effect of medullary lesions on coordination of deglutition. *Experimental neurology, 17*(1), 91-106.

Eldeghaidy, S., Marciani, L., McGlone, F., Hollowood, T., Hort, J., Head, K., . . . Francis, S. T. (2011). The cortical response to the oral perception of fat emulsions and the effect of taster status. *Journal of neurophysiology, 105*(5), 2572-2581. doi: 10.1152/jn.00927.2010

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behavior research methods, 41*(4), 1149-1160. doi: 10.3758/BRM.41.4.1149

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods, 39*(2), 175-191.

Faurion, A., Cerf, B., Van De Moortele, P. F., Lobel, E., Mac Leod, P., & Le Bihan, D. (1999). Human taste cortical areas studied with functional magnetic resonance imaging: evidence of functional lateralization related to handedness. *Neuroscience letters, 277*(3), 189-192.

Firestein, S. J., Margolskee, R. F., & Kinnamon, S. (1999). Taste. In G. J. Siegel, B. W. Agranoff, R. W. Albers, S. K. Fisher & M. D. Ulher (Eds.), *Basic*

Neurochemistry: Molecular, Cellular and Medical Aspects (6 ed.). Philadelphia: Lippincott-Raven.

Gagnon, L., Cooper, R. J., Yucel, M. A., Perdue, K. L., Greve, D. N., & Boas, D. A. (2012). Short separation channel location impacts the performance of short channel regression in NIRS. *NeuroImage, 59*(3), 2518-2528. doi: 10.1016/j.neuroimage.2011.08.095

Gatto, A. R., Cola, P. C., Silva, R. G., Spadotto, A. A., Ribeiro, P. W., Schelp, A. O., . . . Henry, M. A. (2013). Sour taste and cold temperature in the oral phase of swallowing in patients after stroke. *CoDAS, 25*(2), 164-168.

Gomez, F. E., Cassis-Nosthas, L., Morales-de-Leon, J. C., & Bourges, H. (2004). Detection and recognition thresholds to the 4 basic tastes in Mexican patients with primary Sjogren's syndrome. *European journal of clinical nutrition, 58*(4), 629-636. doi: 10.1038/sj.ejcn.1601858

Hamdy, S., Jilani, S., Price, V., Parker, C., Hall, N., & Power, M. (2003). Modulation of human swallowing behaviour by thermal and chemical stimulation in health and after brain injury. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society, 15*(1), 69-77.

Hamdy, S., Mikulis, D. J., Crawley, A., Xue, S., Lau, H., Henry, S., & Diamant, N. E. (1999). Cortical activation during human volitional swallowing: an event-related fMRI study. *Am J Physiol, 277*(1 Pt 1), G219-225.

Harris, H., & Kalmus, H. (1949). The measurement of taste sensitivity to phenylthiourea. *Annals of eugenics, 15*(1), 24-31.

Harris, M. L., Julyan, P., Kulkarni, B., Gow, D., Hobson, A., Hastings, D., . . . Hamdy, S. (2005). Mapping metabolic brain activation during human volitional swallowing: a positron emission tomography study using [18F]fluorodeoxyglucose. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 25*(4), 520-526. doi: 10.1038/sj.jcbfm.9600042

Hashimoto, T., Doden, T., Ono, Y., & Uematsu, T. (2012). Bilateral hypogeusia caused by a small lesion in the lower midbrain tegmentum. *BMJ case reports, 2012*. doi: 10.1136/bcr-2012-006837

Heckman, J. G., & Lang, C. J. G. (2006). Neurological causes of taste disorders. In T. Hummel & A. Welge-Lussen (Eds.), *Taste and smell. An update.* (Vol. 63, pp. 255-264). Basel: Karger.

Heckmann, J. G., Stossel, C., Lang, C. J., Neundorfer, B., Tomandl, B., & Hummel, T. (2005). Taste disorders in acute stroke: a prospective observational study on taste disorders in 102 stroke patients. *Stroke; a journal of cerebral circulation, 36*(8), 1690-1694. doi: 10.1161/01.STR.0000173174.79773.d3

Heft, M. W., & Baum, B. J. (1984). Unstimulated and stimulated parotid salivary flow rate in individuals of different ages. *Journal of dental research, 63*(10), 1182-1185.

Henkin, R. I., Talal, N., Larson, A. L., & Mattern, C. F. (1972). Abnormalities of taste and smell in Sjogren's syndrome. *Annals of internal medicine, 76*(3), 375-383.

Herness, S., Zhao, F. L., Kaya, N., Shen, T., Lu, S. G., & Cao, Y. (2005). Communication routes within the taste bud by neurotransmitters and neuropeptides. *Chemical senses, 30 Suppl 1*, i37-38. doi: 10.1093/chemse/bjh101

Hershkovich, O., & Nagler, R. M. (2004). Biochemical analysis of saliva and taste acuity evaluation in patients with burning mouth syndrome, xerostomia and/or gustatory disturbances. *Archives of oral biology, 49*(7), 515-522. doi: 10.1016/j.archoralbio.2004.01.012

Hughes, C. V., Baum, B. J., Fox, P. C., Marmary, Y., Yeh, C.-K., & Sonies, B. (1987). Oral-pharyngeal dysphagia: a common sequela of salivary gland dysfunction. *Dysphagia, 1*(4), 173-177.

Humbert, I. A., Fitzgerald, M. E., McLaren, D. G., Johnson, S., Porcaro, E., Kosmatka, K., . . . Robbins, J. (2009). Neurophysiology of swallowing: effects of age and bolus type. *NeuroImage, 44*(3), 982-991. doi: 10.1016/j.neuroimage.2008.10.012

Humbert, I. A., & Joel, S. (2012). Tactile, gustatory, and visual biofeedback stimuli modulate neural substrates of deglutition. *NeuroImage, 59*(2), 1485-1490. doi: 10.1016/j.neuroimage.2011.08.022

Humbert, I. A., Lokhande, A., Christopherson, H., German, R., & Stone, A. (2012). Adaptation of swallowing hyo-laryngeal kinematics is distinct in oral vs. pharyngeal sensory processing. *Journal of applied physiology, 112*(10), 1698-1705. doi: 10.1152/japplphysiol.01534.2011

Humbert, I. A., & McLaren, D. G. (2014). Differential psychophysiological interactions of insular subdivisions during varied oropharyngeal swallowing tasks. *Physiological reports, 2*(3), e00239. doi: 10.1002/phy2.239

Humbert, I. A., & Robbins, J. (2007). Normal swallowing and functional magnetic resonance imaging: a systematic review. *Dysphagia, 22*(3), 266-275. doi: 10.1007/s00455-007-9080-9

Iannilli, E., Noennig, N., Hummel, T., & Schoenfeld, A. M. (2014). Spatio-temporal correlates of taste processing in the human primary gustatory cortex. *Neuroscience, 273*, 92-99. doi: 10.1016/j.neuroscience.2014.05.017

Jadcherla, S. R., Pakiraih, J. F., Hasenstab, K. A., Dar, I., Gao, X., Bates, D. G., & Kashou, N. H. (2014). Esophageal reflexes modulate frontoparietal response in neonates: Novel application of concurrent NIRS and provocative esophageal manometry. *American journal of physiology. Gastrointestinal and liver physiology, 307*(1), G41-49. doi: 10.1152/ajpgi.00350.2013

Jafari, S., Prince, R. A., Kim, D. Y., & Paydarfar, D. (2003). Sensory regulation of swallowing and airway protection: a role for the internal superior laryngeal nerve in humans. *J Physiol, 550*(Pt 1), 287-304. doi: 10.1113/jphysiol.2003.039966

Jean, A. (2001). Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiological reviews, 81*(2), 929-969.

Jean, A., & Dallaporta, M. (2006). Electrophysiologic characterization of the swallowing pattern generator in the brain. *GI Motility online*. doi: 10.1038/gimo9

Kaatzke-McDonald, M. N., Post, E., & Davis, P. J. (1996). The effects of cold, touch, and chemical stimulation of the anterior faucial pillar on human swallowing. *Dysphagia, 11*(3), 198-206.

Kajii, Y., Shingai, T., Kitagawa, J., Takahashi, Y., Taguchi, Y., Noda, T., & Yamada, Y. (2002). Sour taste stimulation facilitates reflex swallowing from the pharynx and larynx in the rat. *Physiology & behavior, 77*(2-3), 321-325.

Kapila, Y. V., Dodds, W. J., Helm, J. F., & Hogan, W. J. (1984). Relationship between swallow rate and salivary flow. *Digestive diseases and sciences, 29*(6), 528-533.

Kaplan, I., Zuk-Paz, L., & Wolff, A. (2008). Association between salivary flow rates, oral symptoms, and oral mucosal status. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics, 106*(2), 235-241. doi: 10.1016/j.tripleo.2007.11.029

Kendall, K. A., Leonard, R. J., & McKenzie, S. W. (2001). Accommodation to changes in bolus viscosity in normal deglutition: a videofluoroscopic study. *The Annals of otology, rhinology, and laryngology, 110*(11), 1059-1065.

Klahn, M. S., & Perlman, A. L. (1999). Temporal and durational patterns associating respiration and swallowing. *Dysphagia, 14*(3), 131-138.

Kobayakawa, T., Endo, H., Ayabe-Kanamura, S., Kumagai, T., Yamaguchi, Y., Kikuchi, Y., . . . Ogawa, H. (1996). The primary gustatory area in human cerebral cortex studied by magnetoencephalography. *Neuroscience letters, 212*(3), 155-158.

Kober, S. E., & Wood, G. (2014). Changes in hemodynamic signals accompanying motor imagery and motor execution of swallowing: a near-infrared spectroscopy study. *NeuroImage, 93 Pt 1*, 1-10. doi: 10.1016/j.neuroimage.2014.02.019

Kringelbach, M. L., de Araujo, I. E., & Rolls, E. T. (2004). Taste-related activity in the human dorsolateral prefrontal cortex. *NeuroImage, 21*(2), 781-788. doi: 10.1016/j.neuroimage.2003.09.063

Lashley, K. S. (1916). Reflex secretion of the human parotid gland. *Journal of experimental psychology, 1*(6), 461-493. doi: 10.1037/h0073282

Lawless, H. T. (1980). A comparison of different methods used to assess sensitivity to the taste of phenylthiocarbamide (PTC). *Chemical senses, 5*(3), 247-256

Lazarus, C. L., Logemann, J. A., Rademaker, A. W., Kahrilas, P. J., Pajak, T., Lazar, R., & Halper, A. (1993). Effects of bolus volume, viscosity, and repeated swallows in nonstroke subjects and stroke patients. *Arch Phys Med Rehabil, 74*(10), 1066-1070.

Lear, C. S., Flanagan, J. B., Jr., & Moorrees, C. F. (1965). The Frequency of Deglutition in Man. *Archives of oral biology, 10*, 83-100.

Lee, K. L., Kim, D. Y., Kim, W. H., Kim, E. J., Lee, W. S., Hahn, S. J., . . . Ahn, S. Y. (2012). The influence of sour taste on Dysphagia in brain injury: blind study. *Annals of rehabilitation medicine, 36*(3), 365-370. doi: 10.5535/arm.2012.36.3.365

Leow, L. P., Huckabee, M. L., Sharma, S., & Tooley, T. P. (2007). The influence of taste on swallowing apnea, oral preparation time, and duration and amplitude of submental muscle contraction. *Chemical senses, 32*(2), 119-128. doi: 10.1093/chemse/bjl037

Lewis, D., & Dandy, W. E. (1930). The course of the nerve fibers transmitting sensation of taste. *Arch Surg, 21*, 249-288.

Logemann, J. A., Pauloski, B. R., Colangelo, L., Lazarus, C., Fujiu, M., & Kahrilas, P. J. (1995). Effects of a sour bolus on oropharyngeal swallowing measures in patients with neurogenic dysphagia. *Journal of speech and hearing research, 38*(3), 556-563.

Logemann, J. A., Pauloski, B. R., Rademaker, A. W., Lazarus, C. L., Mittal, B., Gaziano, J., . . . Newman, L. A. (2003). Xerostomia: 12-month changes in saliva production and its relationship to perception and performance of swallow function, oral

intake, and diet after chemoradiation. *Head & neck, 25*(6), 432-437. doi: 10.1002/hed.10255

Logemann, J. A., Smith, C. H., Pauloski, B. R., Rademaker, A. W., Lazarus, C. L., Colangelo, L. A., . . . Newman, L. A. (2001). Effects of xerostomia on perception and performance of swallow function. *Head & neck, 23*(4), 317-321.

Lowell, S. Y., Poletto, C. J., Knorr-Chung, B. R., Reynolds, R. C., Simonyan, K., & Ludlow, C. L. (2008). Sensory stimulation activates both motor and sensory components of the swallowing system. *NeuroImage, 42*(1), 285-295. doi: 10.1016/j.neuroimage.2008.04.234

Lowell, S. Y., Reynolds, R. C., Chen, G., Horwitz, B., & Ludlow, C. L. (2012). Functional connectivity and laterality of the motor and sensory components in the volitional swallowing network. *Experimental brain research, 219*(1), 85-96. doi: 10.1007/s00221-012-3069-9

Magoun, H., & Beaton, L. (1942). The salivatory motor nuclei in the monkey. *Am J Physiol, 136*, 720-725.

Malandraki, G. A., Sutton, B. P., Perlman, A. L., Karampinos, D. C., & Conway, C. (2009). Neural activation of swallowing and swallowing-related tasks in healthy young adults: an attempt to separate the components of deglutition. *Human brain mapping, 30*(10), 3209-3226. doi: 10.1002/hbm.20743

Mansson, I., & Sandberg, N. (1975). Oro-pharyngeal sensitivity and elicitation of swallowing in man. *Acta Otolaryngol, 79*(1-2), 140-145.

Martin-Harris, B., Brodsky, M. B., Michel, Y., Ford, C. L., Walters, B., & Heffner, J. (2005). Breathing and swallowing dynamics across the adult lifespan. *Archives of otolaryngology--head & neck surgery, 131*(9), 762-770. doi: 10.1001/archotol.131.9.762

Martin, R., Barr, A., MacIntosh, B., Smith, R., Stevens, T., Taves, D., . . . Hachinski, V. (2007). Cerebral cortical processing of swallowing in older adults. *Experimental brain research, 176*(1), 12-22. doi: 10.1007/s00221-006-0592-6

Martin, R. E., Goodyear, B. G., Gati, J. S., & Menon, R. S. (2001). Cerebral cortical representation of automatic and volitional swallowing in humans. *J Neurophysiol, 85*(2), 938-950.

Martin, R. E., MacIntosh, B. J., Smith, R. C., Barr, A. M., Stevens, T. K., Gati, J. S., & Menon, R. S. (2004). Cerebral areas processing swallowing and tongue movement are overlapping but distinct: a functional magnetic resonance imaging study. *Journal of neurophysiology, 92*(4), 2428-2443. doi: 10.1152/jn.01144.2003

Matsuo, R. (1999) Central connections for salivary innervations and efferent impulse formation. *Vol. 11. Neural mechanisms of salivary gland secretion* (pp. 26-43). Basel: Karger.

Methven, L., Allen, V. J., Withers, C. A., & Gosney, M. A. (2012). Ageing and taste. *Proceedings of the Nutrition Society, 71*(04), 556-565. doi: doi:10.1017/S0029665112000742

Mihai, P. G., Otto, M., Platz, T., Eickhoff, S. B., & Lotze, M. (2014). Sequential evolution of cortical activity and effective connectivity of swallowing using fMRI. *Hum Brain Mapp, 35*(12), 5962-5973. doi: 10.1002/hbm.22597

Miller, A. J. (1972a). Characteristics of the swallowing reflex induced by peripheral nerve and brain stem stimulation. *Experimental neurology, 34*(2), 210-222.

Miller, A. J. (1972b). Significance of sensory inflow to the swallowing reflex. *Brain research, 43*(1), 147-159.

Miller, A. J., & Bowman, J. P. (1977). Precentral cortical modulation of mastication and swallowing. *Journal of dental research, 56*(10), 1154.

Miller, F. R., & Sherrington, C. S. (1915). Some observations on the bucco-pharyngeal state of reflex deglutition in the cat. *Experimental Physiology, 9*(2), 147-186.

Miller, I. J., Jr., & Reedy, F. E., Jr. (1990). Variations in human taste bud density and taste intensity perception. *Physiology & behavior, 47*(6), 1213-1219.

Mistry, S., Rothwell, J. C., Thompson, D. G., & Hamdy, S. (2006). Modulation of human cortical swallowing motor pathways after pleasant and aversive taste stimuli. *Am J Physiol Gastrointest Liver Physiol, 291*(4), G666-671. doi: 10.1152/ajpgi.00573.2005

Miura, Y., Morita, Y., Koizumi, H., & Shingai, T. (2009). Effects of taste solutions, carbonation, and cold stimulus on the power frequency content of swallowing submental surface electromyography. *Chemical senses, 34*(4), 325-331. doi: 10.1093/chemse/bjp005

Miyaoka, Y., Haishima, K., Takagi, M., Haishima, H., Asari, J., & Yamada, Y. (2006). Influences of thermal and gustatory characteristics on sensory and motor aspects of swallowing. *Dysphagia, 21*(1), 38-48. doi: 10.1007/s00455-005-9003-6

Moraga-Amaro, R., & Stehberg, J. (2012). The insular cortex and the amygdala: shared functions and interactions. B. Ferry (Ed.) *The amygdala- a discrete multitasking manager* Retrieved from http://www.intechopen.com/books/the-amygdala-a-discrete-multitasking-manager/the-insular-cortex-and-the-amygdala-shared-functions-and-interactions. doi:10.5772/48495

Mosier, K., & Bereznaya, I. (2001). Parallel cortical networks for volitional control of swallowing in humans. *Experimental brain research, 140*(3), 280-289.

Mosier, K. M., Liu, W. C., Maldjian, J. A., Shah, R., & Modi, B. (1999). Lateralization of cortical function in swallowing: a functional MR imaging study. *AJNR Am J Neuroradiol, 20*(8), 1520-1526.

Murray, J., Langmore, S. E., Ginsberg, S., & Dostie, A. (1996). The significance of accumulated oropharyngeal secretions and swallowing frequency in predicting aspiration. *Dysphagia, 11*(2), 99-103.

Nagy, A., Molfenter, S. M., Peladeau-Pigeon, M., Stokely, S., & Steele, C. M. (2014). The effect of bolus volume on hyoid kinematics in healthy swallowing. *BioMed research international, 2014*, 738971. doi: 10.1155/2014/738971

Nagy, A., Steele, C. M., & Pelletier, C. A. (2014a). Barium versus nonbarium stimuli: differences in taste intensity, chemesthesis, and swallowing behavior in healthy adult women. *Journal of speech, language, and hearing research : JSLHR, 57*(3), 758-767. doi: 10.1044/2013_JSLHR-S-13-0136

Nagy, A., Steele, C. M., & Pelletier, C. A. (2014b). Differences in swallowing between high and low concentration taste stimuli. *BioMed research international, 2014*, 813084. doi: 10.1155/2014/813084

Nakamura, Y., Goto, T. K., Tokumori, K., Yoshiura, T., Kobayashi, K., Honda, H., . . . Yoshiura, K. (2012). The temporal change in the cortical activations due to salty and sweet tastes in humans: fMRI and time-intensity sensory evaluation. *Neuroreport, 23*(6), 400-404. doi: 10.1097/WNR.0b013e32835271b7

Nakamura, Y., Tokumori, K., Tanabe, H. C., Yoshiura, T., Kobayashi, K., Honda, H., . . . Goto, T. K. (2013). Localization of the primary taste cortex by contrasting passive and attentive conditions. *Experimental brain research, 227*(2), 185-197. doi: 10.1007/s00221-013-3499-z

Nederkoorn, C., Smulders, F. T., & Jansen, A. (1999). Recording of swallowing events using electromyography as a non-invasive measurement of salivation. *Appetite, 33*(3), 361-369. doi: 10.1006/appe.1999.0268

Negoro, A., Umemoto, M., Fujii, M., Kakibuchi, M., Terada, T., Hashimoto, N., & Sakagami, M. (2004). Taste function in Sjogren's syndrome patients with special reference to clinical tests. *Auris, nasus, larynx, 31*(2), 141-147. doi: 10.1016/j.anl.2004.01.005

Okamoto, M., Dan, H., Clowney, L., Yamaguchi, Y., & Dan, I. (2009a). Activation in ventro-lateral prefrontal cortex during the act of tasting: an fNIRS study. *Neuroscience letters, 451*(2), 129-133. doi: 10.1016/j.neulet.2008.12.016

Okamoto, M., Dan, H., Clowney, L., Yamaguchi, Y., & Dan, I. (2009b). Activation in ventro-lateral prefrontal cortex during the act of tasting: an fNIRS study. *Neurosci Lett, 451*(2), 129-133. doi: 10.1016/j.neulet.2008.12.016

S0304-3940(08)01704-7 [pii]

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia, 9*(1), 97-113.

Onoda, K., Ikeda, M., Sekine, H., & Ogawa, H. (2012). Clinical study of central taste disorders and discussion of the central gustatory pathway. *Journal of neurology, 259*(2), 261-266. doi: 10.1007/s00415-011-6165-z

Palmer, P. M., McCulloch, T. M., Jaffe, D., & Neel, A. T. (2005). Effects of a sour bolus on the intramuscular electromyographic (EMG) activity of muscles in the submental region. *Dysphagia, 20*(3), 210-217. doi: 10.1007/s00455-005-0017-x

Pauloski, B. R., Logemann, J. A., Rademaker, A. W., Lundy, D., Sullivan, P. A., Newman, L. A., . . . Bacon, M. (2013). Effects of enhanced bolus flavors on oropharyngeal swallow in patients treated for head and neck cancer. *Head & neck, 35*(8), 1124-1131. doi: 10.1002/hed.23086

Pavlidis, P., Gouveris, C., Kekes, G., & Maurer, J. (2014). Changes in electrogustometry thresholds, tongue tip vascularization, density and form of the fungiform papillae in smokers. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies, 271*(8), 2325-2331. doi: 10.1007/s00405-014-3003-9

Pelletier, C. A., & Dhanaraj, G. E. (2006). The effect of taste and palatability on lingual swallowing pressure. *Dysphagia, 21*(2), 121-128. doi: 10.1007/s00455-006-9020-0

Pelletier, C. A., & Lawless, H. T. (2003). Effect of citric acid and citric acid-sucrose mixtures on swallowing in neurogenic oropharyngeal dysphagia. *Dysphagia, 18*(4), 231-241. doi: 10.1007/s00455-003-0013-y

Pelletier, C. A., & Steele, C. M. (2014). Influence of the perceived taste intensity of chemesthetic stimuli on swallowing parameters given age and genetic taste differences in healthy adult women. *Journal of speech, language, and hearing research : JSLHR, 57*(1), 46-56. doi: 10.1044/1092-4388(2013/13-0005)

Plonk, D. P., Butler, S. G., Grace-Martin, K., & Pelletier, C. A. (2011). Effects of chemesthetic stimuli, age, and genetic taste groups on swallowing apnea duration. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 145*(4), 618-622. doi: 10.1177/0194599811407280

Pouderoux, P., Logemann, J. A., & Kahrilas, P. J. (1996). Pharyngeal swallowing elicited by fluid infusion: role of volition and vallecular containment. *The American journal of physiology, 270*(2 Pt 1), G347-354.

Pritchard, T. C., Hamilton, R. B., & Norgren, R. (2000). Projections of the parabrachial nucleus in the old world monkey. *Experimental neurology, 165*(1), 101-117. doi: 10.1006/exnr.2000.7450

Rhodus, N. L., Moller, K., Colby, S., & Bereuter, J. (1995). Dysphagia in patients with three different etiologies of salivary gland dysfunction. *Ear, nose, & throat journal, 74*(1), 39-42, 45-38.

Rogus-Pulia, N. M., & Logemann, J. A. (2011). Effects of reduced saliva production on swallowing in patients with Sjogren's syndrome. *Dysphagia, 26*(3), 295-303. doi: 10.1007/s00455-010-9311-3

Rudenga, K., Green, B., Nachtigal, D., & Small, D. M. (2010). Evidence for an integrated oral sensory module in the human anterior ventral insula. *Chemical senses, 35*(8), 693-703. doi: 10.1093/chemse/bjq068

Rudney, J. D., Ji, Z., & Larson, C. J. (1995). The prediction of saliva swallowing frequency in humans from estimates of salivary flow rate and the volume of saliva swallowed. *Archives of oral biology, 40*(6), 507-512.

Sato, H., Obata, A. N., Moda, I., Ozaki, K., Yasuhara, T., Yamamoto, Y., . . . Koizumi, H. (2011). Application of near-infrared spectroscopy to measurement of hemodynamic signals accompanying stimulated saliva secretion. *J Biomed Opt, 16*(4), 047002. doi: 10.1117/1.3565048

Satow, T., Ikeda, A., Yamamoto, J., Begum, T., Thuy, D. H., Matsuhashi, M., . . . Shibasaki, H. (2004). Role of primary sensorimotor cortex and supplementary motor area in volitional swallowing: a movement-related cortical potential study. *American journal of physiology. Gastrointestinal and liver physiology, 287*(2), G459-470. doi: 10.1152/ajpgi.00323.2003

Schoenfeld, M. A., Neuer, G., Tempelmann, C., Schussler, K., Noesselt, T., Hopf, J. M., & Heinze, H. J. (2004). Functional magnetic resonance tomography correlates of taste perception in the human primary taste cortex. *Neuroscience, 127*(2), 347-353. doi: 10.1016/j.neuroscience.2004.05.024

Sciortino, K., Liss, J. M., Case, J. L., Gerritsen, K. G., & Katz, R. C. (2003). Effects of mechanical, cold, gustatory, and combined stimulation to the human anterior faucial pillars. *Dysphagia, 18*(1), 16-26. doi: 10.1007/s00455-002-0076-1

Shah, M., Deeb, J., Fernando, M., Noyce, A., Visentin, E., Findley, L. J., & Hawkes, C. H. (2009). Abnormality of taste and smell in Parkinson's disease. *Parkinsonism & related disorders, 15*(3), 232-237. doi: 10.1016/j.parkreldis.2008.05.008

Ship, J. A., Nolan, N. E., & Puckett, S. A. (1995). Longitudinal analysis of parotid and submandibular salivary flow rates in healthy, different-aged adults. *The journals of gerontology. Series A, Biological sciences and medical sciences, 50*(5), M285-289.

Small, D. M. (2006). Central gustatory processing in humans. *Adv Otorhinolaryngol, 63*, 191-220. doi: 93761 [pii]
10.1159/000093761
Small, D. M., Jones-Gotman, M., Zatorre, R. J., Petrides, M., & Evans, A. C. (1997a). Flavor processing: more than the sum of its parts. *Neuroreport, 8*(18), 3913-3917.
Small, D. M., Jones-Gotman, M., Zatorre, R. J., Petrides, M., & Evans, A. C. (1997b). A role for the right anterior temporal lobe in taste quality recognition. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 17*(13), 5136-5142.
Small, D. M., & Prescott, J. (2005). Odor/taste integration and the perception of flavor. *Experimental brain research, 166*(3-4), 345-357. doi: 10.1007/s00221-005-2376-9
Smith, D. V., & Davis, B. J. (2000). Neural representation of taste. In T. Finger, W. Silver & D. Restrepo (Eds.), *The Neurobiology of Taste and Smell* (2 ed., pp. 353-392). New York: Wiley-Liss.
Sonies, B. C., Ship, J. A., & Baum, B. J. (1989). Relationship between saliva production and oropharyngeal swallow in healthy, different-aged adults. *Dysphagia, 4*(2), 85-89.
Soros, P., Lalone, E., Smith, R., Stevens, T., Theurer, J., Menon, R. S., & Martin, R. E. (2008). Functional MRI of oropharyngeal air-pulse stimulation. *Neuroscience, 153*(4), 1300-1308. doi: 10.1016/j.neuroscience.2008.02.079
South, A. R., Somers, S. M., & Jog, M. S. (2010). Gum chewing improves swallow frequency and latency in Parkinson patients: a preliminary study. *Neurology, 74*(15), 1198-1202. doi: 10.1212/WNL.0b013e3181d9002b
Steele, C. M., van Lieshout, P. H., & Pelletier, C. A. (2012). The influence of stimulus taste and chemesthesis on tongue movement timing in swallowing. *Journal of speech, language, and hearing research : JSLHR, 55*(1), 262-275. doi: 10.1044/1092-4388(2011/11-0012)
Sumi, T. (1963). The activity of brain-stem respiratory neurons and spinal respiratory motoneurons during swallowing. *Journal of neurophysiology, 26*, 466-477.
Sumi, T. (1969). Some properties of cortically-evoked swallowing and chewing in rabbits. *Brain research, 15*(1), 107-120.
Sumi, T. (1970). Activity in single hypoglossal fibers during cortically induced swallowing and chewing in rabbits. *Pflugers Archiv : European journal of physiology, 314*(4), 329-346.
Sumi, T. (1972). Role of the pontine reticular formation in the neural organization of deglutition. *The Japanese journal of physiology, 22*(3), 295-314.
Takahashi, T., Takikawa, Y., Kawagoe, R., Shibuya, S., Iwano, T., & Kitazawa, S. (2011). Influence of skin blood flow on near-infrared spectroscopy signals measured on the forehead during a verbal fluency task. *NeuroImage, 57*(3), 991-1002. doi: 10.1016/j.neuroimage.2011.05.012
Tanaka, N., Nohara, K., Kotani, Y., Matsumura, M., & Sakai, T. (2013). Swallowing frequency in elderly people during daily life. *Journal of oral rehabilitation, 40*(10), 744-750. doi: 10.1111/joor.12085
Teismann, I. K., Steinstraeter, O., Stoeckigt, K., Suntrup, S., Wollbrink, A., Pantev, C., & Dziewas, R. (2007). Functional oropharyngeal sensory disruption interferes with

the cortical control of swallowing. *BMC neuroscience, 8*, 62. doi: 10.1186/1471-2202-8-62

Theunissen, M. J., Kroeze, J. H., & Schifferstein, H. N. (2000). Method of stimulation, mouth movements, concentration, and viscosity: effects on the degree of taste adaptation. *Perception & psychophysics, 62*(3), 607-614.

Theunissen, M. J., Polet, I. A., Kroeze, J. H., & Schifferstein, H. N. (2000). Taste adaptation during the eating of sweetened yogurt. *Appetite, 34*(1), 21-27. doi: 10.1006/appe.1999.0275

Theurer, J. A., Bihari, F., Barr, A. M., & Martin, R. E. (2005). Oropharyngeal stimulation with air-pulse trains increases swallowing frequency in healthy adults. *Dysphagia, 20*(4), 254-260. doi: 10.1007/s00455-005-0021-1

Todd, J. T., Butler, S. G., Plonk, D. P., Grace-Martin, K., & Pelletier, C. A. (2012a). Effects of chemesthetic stimuli mixtures with barium on swallowing apnea duration. *The Laryngoscope, 122*(10), 2248-2251. doi: 10.1002/lary.23511

Todd, J. T., Butler, S. G., Plonk, D. P., Grace-Martin, K., & Pelletier, C. A. (2012b). Main taste effects on swallowing apnea duration in healthy adults. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 147*(4), 678-683. doi: 10.1177/0194599812450839

Triadafilopoulos, G., Tsang, H. P., & Segall, G. M. (1998). Hot water swallows improve symptoms and accelerate esophageal clearance in esophageal motility disorders. *Journal of clinical gastroenterology, 26*(4), 239-244.

Tylenda, C. A., Ship, J. A., Fox, P. C., & Baum, B. J. (1988). Evaluation of submandibular salivary flow rate in different age groups. *Journal of dental research, 67*(9), 1225-1228.

Vernia, N., Feng, F. Y., Braun, T., Vineberg, K., Chepeha, D., Murdoch-Kinch, C., & Eisbruch, A. Dosimetric Predictors of Taste Impairment in Patients Treated with Chemoradiation for Head and Neck Cancer. *International Journal of Radiation Oncology ,Ä¢ Biology ,Ä¢ Physics, 75*(3), S179. doi: 10.1016/j.ijrobp.2009.07.416

Villringer, A., & Chance, B. (1997). Non-invasive optical spectroscopy and imaging of human brain function. *Trends in neurosciences, 20*(10), 435-442.

Wahab, N. A., Jones, R. D., & Huckabee, M. L. (2011). Effects of olfactory and gustatory stimuli on the biomechanics of swallowing. *Physiology & behavior, 102*(5), 485-490. doi: 10.1016/j.physbeh.2010.11.030

Wang, S. (1943). Localization of the salivatory center in the medulla of the cat. *J Neurophysiol, 6*, 195-202.

Watanabe, S., & Dawes, C. (1988). The effects of different foods and concentrations of citric acid on the flow rate of whole saliva in man. *Archives of oral biology, 33*(1), 1-5.

Weiffenbach, J. M., Schwartz, L. K., Atkinson, J. C., & Fox, P. C. (1995). Taste performance in Sjogren's syndrome. *Physiology & behavior, 57*(1), 89-96.

Welge-Lussen, A., Dorig, P., Wolfensberger, M., Krone, F., & Hummel, T. (2011). A study about the frequency of taste disorders. *Journal of neurology, 258*(3), 386-392. doi: 10.1007/s00415-010-5763-5

Wheatcroft, P. E., & Thornburn, C. C. (1972). Toxicity of the taste testing compound phenylthiocarbamide. *Nature: New biology, 235*(55), 93-94.

Yamamoto, C., Takehara, S., Morikawa, K., Nakagawa, S., Yamaguchi, M., Iwaki, S., . . . Yamamoto, T. (2003). Magnetoencephalographic study of cortical activity evoked by electrogustatory stimuli. *Chemical senses, 28*(3), 245-251.

Yokota, T., Eguchi, K., & Hiraba, K. (2014). Topographical representations of taste response characteristics in the rostral nucleus of the solitary tract in the rat. *Journal of neurophysiology, 111*(1), 182-196. doi: 10.1152/jn.01031.2012

Zald, D. H., & Pardo, J. V. (1999). The functional neuroanatomy of voluntary swallowing. *Annals of neurology, 46*(3), 281-286.