Enhancing Effects of Flavored Nutritive Stimuli on Cortical Swallowing Network Activity

Running Title: Swallowing Network and Nutritive Stimulation

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Abbreviations: Functional magnetic resonance imaging (FMRI), Blood oxygenation level dependent (BOLD), Broadman area (BA)
ABSTRACT

Background & Aim: A better understanding of the central control physiology of deglutition is necessary for devising interventions aimed at correcting pathophysiologic conditions of swallow. Positive modulation of the cortical swallowing network can have clinical ramification in dysphagia due to central nervous system deficits. Our aim was to determine the effect of nutritive sensory input on cortical swallowing network.

Methods: We studied 14 healthy right handed volunteers. We quantified the number of activated voxels and their signal intensity within the left hemispheric cortical swallowing network by high resolution FMRI technique during five different swallow conditions utilizing a paradigm driven protocol. Swallow conditions included dry swallow (saliva), natural water, lemon, popcorn and chocolate flavored liquid swallows. Each flavored liquid was presented simultaneously by its image, scent, and taste in random order and tested x 3 runs. FMRI images were analyzed in a blinded fashion.

Results: Both average FMRI BOLD signal intensity and number of activated voxels during swallowing concurrent with nutritive gustatory, olfactory and visual stimulations were significantly increased compared to dry/natural water swallows throughout cortical swallow network (p<0.001 and p<0.05 respectively). Sub-region analysis showed the increased activity for flavored liquids in prefrontal, cingulate gyrus and sensorimotor cortex but not in precuneus and insula.

Conclusion: Concurrent gustatory, olfactory and visual nutritive stimulation enhances the activity of cortical swallowing network. This finding may have clinical implications in management of swallowing disorders due to cortical lesions.

Key Words: Flavor, Deglutition, Olfaction, Gustation
INTRODUCTION

The main physiological function of the digestive tract and ingestion related behavior in all multicellular life-forms in the animal kingdom is to acquire food and water to meet the nutritional needs of the organism(30, 39). Deglutition is the primary ingestive behavior that develops in utero in mammalian species(42) and contributes to several critical fetal developmental processes(39). The physical act of swallowing requires extensive sensory-motor coordination of oral, pharyngeal, laryngeal, esophageal, and diaphragmatic muscles(1, 2) that are primarily mediated by the “swallowing center” of the brainstem(19, 20). However, a large body of physiologic evidence indicates that the cerebral cortex plays a fundamental role not only in initiation(5, 36) but also in regulation and modulation of the deglutition(18, 29, 35). Furthermore, clinical observations have long documented the development of swallowing disorders due to cerebrovascular accident(13, 37) or traumatic brain injury(7, 12), in the absence of brainstem involvement(9, 14). Recent cortical mapping studies in humans using functional brain imaging(4, 10, 15, 16, 24, 28, 33, 45, 47) have shown that volitional swallowing bilaterally activates a number of cerebral cortical areas including the cingulate gyrus, prefrontal and sensory/motor cortices, the insula and the precuneus collectively considered to represent the “cortical swallowing network”.

However, the physiology of the cortical swallowing network is incompletely understood. Behavioral studies quantifying deglutitive biomechanical parameters following various gustatory and olfactory stimuli(27, 32, 48, 49), brain magnetic stimulation studies of the effects of gustatory stimulation on the swallow cortical motor pathways(31), as well as brain imaging studies evaluating the effect of acid-induced esophageal afferent signals on the swallowing network(21) indicate significant modulatory input to this distributed network from a variety of peripheral sensory fields.

A better understanding of the influence of physiological stimuli on cortical swallowing network activity may have ramification on the management of dysphagic patients. The aim of the present study therefore was to test the hypothesis that the activity of the cortical swallowing network can be augmented by food related sensory stimuli. We determined and compared the effect of several common flavors such as milk chocolate, lemonade and popcorn on a-priori defined components of the cortical swallowing network using functional magnetic resonance imaging (FMRI).
METHODS

Study Protocol: We studied 14 right handed, healthy subjects (28 yr ± 10, 7F). The Human Research Review Committee of the Medical College of Wisconsin approved the study protocol, and all volunteers signed a written informed consent. All participants completed a detailed health-related questionnaire. Following a detailed interview with a physician, subjects were confirmed to have no gastrointestinal disease and no gross deficit in their sense of taste or smell to common flavors. The subjects were asked to fast for 6 hours before the study except for drinking water. They were placed supine in a 3.0-tesla GE Signa System® scanner (General Electric Medical Systems, Waukesha, WI) equipped with a custom three-axes eight channel head coil designed for rapid gradient field switching and a shielded transmit/receive birdcage radiofrequency coil. A rear projection screen was placed at the head of the scanner bed to display visual cues and different visual stimuli. All anatomic scans were acquired using the high-resolution spoiled gradient recalled acquisition (SPGR) technique, consisting of 120 sagittal whole brain 1.2-mm-thick slices over a 240 mm field of view (FOV) and 256x256 within slice pixel resolution. These high-resolution anatomical images were used for subsequent superposition of cortical activity regions derived from the lower-resolution echo planar blood oxygenation level-dependent (BOLD) contrast image data in each subject.

Data Acquisition: Paradigm-driven functional MRI data were acquired during 120s runs while subjects performed 7 random single trial swallows. Each run was repeated three times. During each MRI scan, subjects had either no external sensory stimulant other than visual cues to swallow their saliva (dry swallow), or an additional sensory stimulant as one of the following flavors: water, lemonade, milk chocolate or popcorn. Dry swallow runs were always performed both at the beginning (x3) and at the end (x3) of each MRI scan session while four flavor runs were randomly performed in between. Each flavor was represented by an image (visual representation), standard scent (olfactory representation) and taste (gustatory representation) of the respective flavor. Each tastant was delivered by a Harvard Infusion pumping system through individual polyvinyl catheters to the mouth at a controlled flow rate of 2 ml/minute (~0.6 ml per swallow) during each sensory stimulant run. Popcorn taste was simulated by a mixture of commercial dry popcorn and powdered popcorn butter mixed in water. Bottled water, lemonade and chocolate milk were used for the other tastes. Nutritive contents for a total of 12ml of each flavor delivered to subjects during a study session based on information provided on their respective “nutritional fact” labels were as follows: popcorn (Calories 9, Fat 1000mg, Sodium 200mg, Carbohydrate 100mg, Protein 100mg), lemonade (Calories 6, Fat 0mg, Sodium 0mg,
Carbohydrate 1500mg, Protein 0mg), chocolate milk (Calories 9, Fat 100mg, Sodium 11mg, Potassium 20mg, Carbohydrate 1400mg, Protein 400mg). All tastants were prepared at room temperature to reduce the effect of temperature variability. Scent was delivered orthonasally by a custom made apparatus. Prior to each scan, a cotton tip applicator was immersed in the standard scented liquid extract of each flavor and then excess liquid was removed from the tip. This applicator was secured to a delivery system and guided through a fixed 6 feet long plastic tube to position the applicator tip 1 cm from the nares. To reduce cross contamination of different flavors after each run, we asked volunteers to have multiple wet swallows and waited a minute for aroma and taste to clear. Before initiation of the next flavor we verified with every subject that prior flavor has completely resolved.

fMRI Analysis: Fifteen contiguous, sagittal gradient high resolution echo planar slices were obtained of the left hemisphere. MR images were acquired at a repetition time of 1.2 seconds and an echo time of 20 ms. We studied only the left hemisphere to facilitate using high-resolution echo planar imaging (EPI) techniques(21). The slice thickness for our functional image was 4.5 mm and a slice-wise pixel resolution of 96 x 96 pixels over a 240 mm field of view yielded a within-slice resolution of 2.5 x 2.5 mm. The reason for our unilateral brain imaging is that at the time of our study these high-resolution EPI scanning specifications and short repetition time (TR less than 1.5 s) needed for studying swallow, with scanner and computer processing limitations in our institution, made whole-brain scanning untenable. For example we were only able to accommodate 25 slices (not enough for both hemispheres) in TR=1200 ms used for current study. Furthermore, there has been no evidence showing fMRI activity associated with volitional swallowing to be restricted to one hemisphere(16, 24, 28, 34). Studies from our laboratory and others have shown bilateral activation of the cortical swallowing network with greater registration reported in the left hemisphere for all brain regions except the insula in which right hemispheric dominance has been reported in some studies and left hemispheric dominance has been reported in other studies(16, 24, 28, 33, 45). Since the limitation of the scanner technology constrains imaging of both hemispheres and all historical data points to either hemisphere as a viable target for measuring fMRI signal changes, the left hemisphere was arbitrarily chosen for our study scans(21).

All imaging data were mapped stereotaxically to the Talairach–Tournoux coordinate system for comparison and display purposes. The studied regions of interest (ROI) were all defined anatomically a priori and included the cingulate gyrus, the insula, the prefrontal region, the sensory-motor cortex and the precuneus. The cingulate region was defined as the portion
of the cortex confined by the cingulate sulcus and consisted of Brodmann’s areas (BA) 23, 24, 25, 29, 30, 31, 32 and 33. The prefrontal region was defined as predominantly the dorsolateral prefrontal cortex (BA 9 and 46), anterior prefrontal cortex (BA 10) as well as ventromedial prefrontal cortex (11 and 47). The insula was defined as the region deep within the lateral fissure consisting of several long gyri parallel to the lateral fissure as well as several short gyri more rostrally (BA13). The sensory region consisted of BA 1, 2 and 3. The motor region was defined as the precentral gyrus on the lateral surface of the cortex extending into the longitudinal cerebral fissure medially (BA 4), as well as the supplementary motor cortex and supplementary motor area (BA 6,8). The precuneus region was defined as the medial surface of the parietal cortex between the cuneus and the paracentral lobule (BA 7).

**Data Analysis:** All fMRI signal analysis was carried out using the Analysis of Functional Neuro-Imaging (AFNI)(8) software. Subtle changes in head position during MRI scanning sessions were corrected using 3-dimensional volume registration that realigns motions of a few millimeters and rotations of a few degrees using first-order Taylor series expansions at each point in the six motion parameters (3 shifts, 3 angles) and Fourier interpolation. All FMRI signal intensity data were normalized, voxel by voxel, to the mean signal intensity over the entire time series. General linear modeling (GLM) techniques that compute the voxel-wise hemodynamic response function from the magnetic signal time series were used to detect cortical regions that exhibit significant BOLD changes compared to random Gaussian variation of the signal. As a conservative estimate, we used an uncorrected p value of less than $10^{-5}$ as the cutoff threshold when identifying regional cortical activation using AFNI. We considered statistical output of the GLM to be significant if the probability of making a type I error was less than 0.05 after correction for multiple comparisons.

We identified the number of activated voxels based on the GLM test in each ROI and over the whole cortical swallowing network. The average and peak signal intensity change for all activated voxels in each ROI was also calculated. Average BOLD signal waveforms during the inter-stimulus period of each sensory stimulant were generated across all significantly activated voxels within each ROI of all subjects and during all trials (n=294). This was done by calculating mean value of percent signal intensity change for each of 12 TRs (14.4s) after a swallow among all activated voxels within a ROI of every subject. Group activity maps representative of each stimulant were also created. Additional statistical analysis further compared dry and water swallows to swallowing activity during flavor swallows and relevant modulation of the cortical swallow network.
We determined if a distinct area in each region of interest of cortical swallow network was activated by flavored sensory stimuli compared to either water or dry swallow. Signal intensity values were compared using two statistical techniques. All non-parametric statistical values are presented as median with range and compared using Kruskal–Wallis test and multiple comparisons. All parametric statistical values are reported as mean ± standard error of the mean and compared using analysis of variance with repeated measures and Tukey post-test for multiple pair-wise comparisons. Significance level for all hypothesis testing was set at p<0.05 corrected for multiple comparisons when appropriate.

RESULTS

All subjects showed regional cerebrocortical FMRI BOLD response to swallowing with or without a flavor. There was FMRI activity in all known regions of cortical swallow network including the cingulate gyrus, insula, precuneus, prefrontal, sensory and motor regions. Table-1 illustrates the median number and range of activated voxels within each ROI. As seen the number of activated voxels in the insula and precuneus regions was smaller and more variable than other cortical regions. The average percent BOLD signal increase and number of activated voxels in cortical swallow network during dry swallow prior to the flavored swallows (PRE run) were slightly higher than dry swallow after flavored swallow runs (POST run), however the observed difference did not reach statistical significance.

**Cingulate Gyrus:** Swallow related average percent signal intensity change was significantly greater with flavored stimuli compared to dry swallow and water (figure 1A). Average swallow related BOLD signal waveform across all subjects and trials (n=294 for each flavor) in cingulate gyrus is presented in figure 1B. Average BOLD response peaked at 7.2s after swallow of flavored liquids compared to 6s with dry swallows (figure 1B). We analyzed anterior (BA 23, 24, 25, 32, 33) and posterior (BA 29, 30, 31,) cingulate separately and a similar increased activity with flavored stimuli compared to dry swallow and water was seen in both sub-regions. Talairach-Tornoux coordinates of peak FMRI signal intensity change in cingulate gyrus is located in mid-cingulate (or dorsal anterior cingulate) as presented in table 1C.

**Insula:** Average signal intensity increase associated with swallow paradigm in insula was highly variable and did not reach statistical significance when comparing flavored swallows to unflavored swallows (figure 2A). Average BOLD signal waveform related to swallow across all subjects and trials (n=294 for each flavor) in insula is shown in figure 2B. Insular average BOLD response peaked at 8.4s-10.8s after swallow with or without flavor (figure 2B). We analyzed
anterior and posterior insular swallow related BOLD activity separately and found no statistical
difference comparing signal intensity for dry or water swallows to other flavored stimuli swallows
due to high variability of response between subjects. Talairach-Tornoux coordinates of peak
FMRI signal intensity change within insula is located in dorsal anterior insula as presented in
table 2C.

Prefrontal Cortex: Prefrontal cortex is significantly activated during swallow. Swallow-
related average signal intensity increased significantly with flavored stimuli compared to dry
swallow and water (figure 3A). Average swallow related BOLD signal waveform across all
subjects and trials (n=294) is presented in figure 3B. Average BOLD response related to
swallow of flavored stimuli in prefrontal cortex peaked at 8.4-9.6s after swallow of flavored
liquids compared to 6-7.2s during dry and water swallow (figure 3B). Talairach-Tornoux
coordinates of peak FMRI signal intensity within prefrontal cortex is located dorsolaterally as
presented in table 3C.

Sensory-Motor cortex: Swallow related average percent signal intensity change was
significantly increased with flavored stimuli compared to dry swallow and water (figure 4A).
Average swallow related BOLD response across all subjects and trials (n=294) is presented in
figure 4B. Average BOLD signal in sensory-motor cortex peaked at 7.2s-8.4s after swallow of
flavored or non-flavored liquids (figure 4B). We analyzed motor and supplementary motor
cortices (precentral gyrus, BA 4 and 6) and sensory cortex (postcentral gyrus, BA 1, 2, 3, 43)
swallow related BOLD activity separately. Both sub-regions showed significantly increased
activity with flavored stimuli compared to dry swallow and water; flavored stimuli increased
average signal intensity within motor and supplementary motor cortex (figure 4D) more robustly
(two-way ANOVA with repeated measurements p<0.01) compared to the swallow related
sensory cortex (figure 4C). Talairach-Tornoux coordinates of peak FMRI signal intensity
increase is located in rolandic opercular region within sensory-motor cortex as presented in
table 5E.

Precuneus: Average percent signal intensity change associated with swallow
paradigm in precuneus region with flavored swallows was variable and didn’t reach statistical
significance compared to dry swallow (figure 5A). Average BOLD signal waveform related to
swallow in precuneus across all subjects and trials (n=294) is shown in figure 5B. Average
BOLD response peaked at 8.4s-9.6s after swallow of non-flavored and flavored liquids.
Talairach-Tornoux coordinates of peak FMRI signal intensity change in precuneus presented in table 5C.

**Total Cortical Swallow Network:** Activated cortical regions of the swallowing network are shown from grouped analysis in the rendered anatomical display in figure 6A. Swallow related average percent signal intensity change throughout entire cortical swallow network sub-regions significantly increased with flavored stimuli compared to dry swallow and water (figure 6B). Average BOLD signal waveform related to swallow in cortical swallow network across all subjects and trials and regions (n=294) is shown in figure 6C. Average BOLD signal in cortical swallow network peaked at 8.4s-9.6s after swallow of flavored and non-flavored liquids (figure 6C).

**DISCUSSION**

In this study we have demonstrated the enhancing effect of concurrent olfactory, gustatory and visual stimulations by the ingested material on the cortical swallowing network. Study findings indicate that, although the fMRI signal intensity changes of the total swallowing network shows significant increase during swallowing liquids that stimulates all three ingestion-related senses compared to inert materials such as pure water or saliva, this effect is not distinguishable in all components of the swallowing network. Notably, the insula and precuneus activity, contrary to cingulate gyrus, sensory motor and prefrontal cortices, exhibit significant variability and overall do not show a statistically significant augmentation due to flavor of the ingested materials.

Flavor of prepared food, one of the most complex and powerful human sensations(11), engages almost all human senses, particularly the retronasal olfactory system as well as the gustatory system(41). The sense of taste influences evaluating the nutritious content of food(6) like sweet for energy-rich carbohydrates, salty for proper dietary electrolyte balance or umami (savory) for amino acids(50), and prevents the ingestion of possible toxic or noxious substances that may taste bitter and sour respectively(6). Peripheral taste signals are modulated and relayed through the nucleus tractus solitarius (NTS) and the taste nucleus of the thalamus(43) projecting bilaterally onto the anterior insula(40, 43, 45) and adjacent fronto-parietal opercula(17, 40, 45) as primary, and onto orbitofrontal cortex (OFC)(3) as secondary gustatory cortex. Olfactory signals pass through the olfactory bulb to project bilaterally onto the fronto-temporal pyriform cortex(51, 52) as primary and to OFC(46, 52) as secondary olfactory cortex. In fact, the orbitofrontal cortex receives convergent somatosensory, visual, olfactory and
gustatory afferents(38, 43, 44), and combines these inputs with hedonic experience to form a
sensory integration area; the “flavor center”(26, 41). The brain flavor perceptual system is
directly linked to other brain systems like memory, language, motivation and emotion circuitry,
that are closely involved in the complex control of eating behavior(41). Various cortical drives
like appetite, thirst and hunger are known to stimulate complex eating behaviors consummating
in swallowing(39). It is known that the human sensory systems generate flavor as the main
internal representation of food, and swallowing is the essential step in ingestion of the food.
Therefore, investigation of the interaction between the respective cortical networks of sensory
systems and swallowing is essential to our understanding of the brain-gut interaction in context
of eating behavior.

Activation of insula, precuneus, cingulate, sensorimotor and pre-frontal cortices have
been reported in relation to swallowing and various other gastrointestinal events such as
subliminal esophageal acid exposure(25), heartburn(22), and external anal sphincter
contraction(23). Activation of these same areas has also been reported by gustatory and
olfactory stimuli. Despite the common areas of processing, the observed augmentation of these
regions during swallowing flavored fluid compared to inert water does not represent the
summation of the deglutition related activity and those induced by sensory stimulation of the
flavor. This assertion is supported by the fact that the nutritive stimuli, i.e. flavored water, was
delivered continuously into the mouth as described in the methods section, therefore, any
activity induced by them would have formed the baseline upon which the intermittent swallowing
activity was registered and processed. Since the measurement of percent fMRI signal intensity
change is determined by signal increase from the baseline, the comparison would only take into
account signal change due to swallowing.

The fact that in this study we only interrogated the effect of flavored nutritive stimuli
on left hemispheric swallow related cortical activity might have underestimated the enhancing
effect. This may explain why the upward trend in insular activity in response to flavored stimuli
did not reach statistical significance. Furthermore, considering the small volume of swallowed
testants in the present study which potentially could have resulted in their reduced contact with
the entire surface of the tongue it is possible that we may have underestimated the magnitude
of gustatory effects on enhancing cortical swallowing network. Despite the shortcoming imposed
by technical limitations the findings of present study indicates an accentuating effect of flavored
nutritive stimuli on cortical swallowing network. We did not compare taste prototypes like
dextrose water, NaCl, quinine or hydrochloric acid as they were less representative of common
nutritive and flavored liquids, and lack the widely recognizable associated image or odor of flavor. Furthermore, it was not the aim of the present study to differentiate individual contribution of each sensory stimulus to the observed enhancement of swallow related cortical activity. Future studies delineating degree of influence of flavor prototypes (sweet, sour, savory, salty and bitter) and each of basic senses (gustation, olfaction and vision) in accentuating swallow related cortical activity are awaited to achieve optimal modulatory outcome.

In the present study, we did not evaluate the functional consequences of the observed enhancement of the swallowing network activity. However, a number of previous studies have evaluated the effect of various tastants and odorants on swallowing function. Measuring swallowing intervals by recording sub-mental electromyography have shown facilitation of voluntary swallow induced by chemical stimulation of the posterior tongue and pharyngeal region in humans(49). Studies of deglutition using concurrent gustatory & olfactory stimulation have shown an increase in frequency and velocity of swallowing after retronasal olfactory stimulation compared to orthonasal(48). Sweet and bitter tastes have been shown to modulate the human cortical swallowing motor pathway excitability(31). Furthermore, earlier studies have shown different influences of various tastes, temperature and carbonation of ingested substances on UES opening and deglutitive submental muscle contractions(32). Previous studies have also demonstrated a differing modulatory effect of various tastes on the swallowing biomechanical parameters such as the duration of oral bolus preparation, deglutitive submental muscle EMG amplitude and duration(27). The flavor induced stimulation of the cortical swallowing network as documented in the present study provides a mechanistic explanation for those previously reported modulatory effects of gustatory and olfactory stimulation on the deglutitive biomechanical parameters. However, the central control mechanisms of swallowing associated with the above mentioned modulations in response to gustatory and olfactory stimulation were not investigated in these studies. We recognize these modulations can occur either at the brainstem or the cerebral cortical level or both, therefore observed cortical enhancement does not exclude the possibility of contribution of brain stem swallowing center to the observed enhancement of cortical swallowing network activity. The findings of the present study suggest the potential for a new approach to dysphagic patients utilizing more robust gustatory, olfactory and visual stimuli to enhance their deglutitive function. This assertion is also supported by earlier studies using flavored substances in healthy individuals as stated above.
In summary, concurrent olfactory, gustatory and visual stimulation of the ingested substances enhances the activity of the cerebral cortical swallowing network. This increased activity may have implications in the management of dysphagic conditions.
<table>
<thead>
<tr>
<th>Voxel Count</th>
<th>Dry Swallow (Pre)</th>
<th>Chocolate</th>
<th>Lemon</th>
<th>Popcorn</th>
<th>Water</th>
<th>Dry Swallow (Post)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cingulate</td>
<td>16 (4-35)</td>
<td>31 (25-82)</td>
<td>27 (4-41)</td>
<td>26 (6-49)</td>
<td>13 (5-39)</td>
<td>10 (3-24)</td>
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<td>Insula</td>
<td>0 (0-11)</td>
<td>6 (0-73)</td>
<td>1 (0-31)</td>
<td>0 (0-71)</td>
<td>4 (0-22)</td>
<td>0 (0-12)</td>
</tr>
<tr>
<td>Prefrontal</td>
<td>17 (7-36)</td>
<td>107 (71-150)*</td>
<td>79 (43-115)*</td>
<td>68 (38-177)*</td>
<td>35 (14-56)</td>
<td>17 (5-31)</td>
</tr>
<tr>
<td>Precuneus</td>
<td>4 (0-32)</td>
<td>2 (0-100)</td>
<td>0 (0-96)</td>
<td>9 (0-87)</td>
<td>7 (0-43)</td>
<td>0 (0-33)</td>
</tr>
<tr>
<td>Sensory Motor</td>
<td>16 (9-23)</td>
<td>41 (30-115)*</td>
<td>57 (27-95)*</td>
<td>43 (26-101)*</td>
<td>32 (17-40)</td>
<td>15 (9-30)</td>
</tr>
<tr>
<td>Swallow Network</td>
<td>58 (46-128)</td>
<td>238 (151-455)*</td>
<td>190 (127-296)*</td>
<td>169 (102-290)*</td>
<td>117 (57-167)</td>
<td>50 (41-84)</td>
</tr>
</tbody>
</table>

Table 1 - Number of activated voxels in cortical swallowing network (Presented as median-range). Note that in prefrontal, sensory-motor cortices and cortical swallowing network as a whole there are significantly more activated voxels with flavored stimuli compared to dry swallow (p<0.05).
**Figure Legends**

**Figure - 1** Swallow related cortical activity in cingulate gyrus  
A) Average percent signal increase in cingulate gyrus. Flavored swallows showed significant more FMRI activity than dry/water swallow. We performed ANOVA, and Tukey test for multiple pair-wise comparisons. † p<0.01 compared to dry swallow (pre), * p<0.05 compared to water, ‡ p<0.01 compared to dry swallow (post). Data are presented as mean ± SEM;  
B) Average swallow-related BOLD response waveforms across all trials in cingulate gyrus (n=294). As seen there were considerable differences in magnitude of FMRI activity waveforms for the flavor stimulated swallows compared to dry swallow and water;  
C) Table represents coordinates of peak signal intensity with each flavor within cingulate gyrus based on Talairach-Tournoux system (x, y and z in milimiters represent left, posterior and superior directions respectively). Corresponding z-score of peak activity voxel with each flavor is also presented.

**Figure - 2** Swallow related cortical activity in insula  
A) Average percent signal increase in insula. Difference between flavored stimuli and dry swallow failed to reach statistical significance. We performed non-parametric Kruskal-Wallis test due to unequal variances among groups. Data are presented as mean ± SEM;  
B) Average swallow related BOLD response waveforms across all trials in insula are shown (n=294). As seen there were differences in the magnitude of FMRI waveforms for the flavor stimulated swallows, however these differences did not reach statistical significance;  
C) Table represents coordinates of peak signal intensity with each flavor within insula based on Talairach-Tournoux system (x, y and z in milimiters represent left, posterior and superior directions respectively). Corresponding z-score of peak activity voxel with each flavor is also presented.

**Figure - 3** Swallow related cortical activity in prefrontal cortex  
A) Average percent signal increase in prefrontal cortex. Significant differences were found comparing average FMRI signal intensity for all flavored swallows compared to dry/water swallow. We performed ANOVA, and Tukey post-test for multiple pair-wise comparisons. † p<0.001 compared to dry swallow (pre), * p<0.01 compared to water, ‡ p<0.001 compared to dry swallow (post). Data are presented as mean ± SEM;  
B) Average swallow-related BOLD response waveform across all trials in prefrontal cortex (n=294). As shown there were considerable differences in magnitude of FMRI activity waveforms for the flavor stimulated swallows compared to dry swallow and water;  
C) Table represents coordinates of peak signal intensity with each flavor within prefrontal cortex based on Talairach-Tournoux system (x, y and z in milimiters represent left, posterior and superior directions respectively). Corresponding z-score of peak activity voxel with each flavor is also presented.
Figure – 4 Swallow related cortical activity in sensory-motor cortex A) Average percent signal increase in sensory-motor cortices. Significant differences were found comparing average FMRI signal intensity change for all flavored swallows compared to dry/water swallow. We performed ANOVA, and Tukey test for multiple pair-wise comparisons. † p<0.001 compared to dry swallow (pre), * p<0.001 compared to water, † p<0.001 compared to dry swallow (post). Data are presented as mean ± SEM; B) Average swallow related BOLD response waveform across all trials in sensory-motor cortices (n=294). As shown there were considerable differences in magnitude of FMRI activity waveforms for the flavor stimulated swallows compared to dry swallow and water; C) Average percent signal increase in sensory cortex. Significant differences were found comparing average FMRI signal intensity change for all flavored swallows compared to dry/water swallow. We performed ANOVA, and Tukey test for multiple pair-wise comparisons. † p<0.001 compared to dry swallow (pre), * p<0.001 compared to water, † p<0.001 compared to dry swallow (post), Data are presented as mean ± SEM; D) Average percent signal increase in motor cortex. Significant differences were found comparing average FMRI signal intensity change for all flavored swallows compared to dry/water swallow. We performed ANOVA, and Tukey test for multiple pair-wise comparisons. † p<0.001 compared to dry swallow (pre), * p<0.001 compared to water, † p<0.001 compared to dry swallow (post); Data are presented as mean ± SEM. Note that swallow related BOLD response to flavored liquids in motor cortex is more robust than sensory cortex (p<0.01); E) Table represents coordinates of peak signal intensity with each flavor within sensory-motor cortex based on Talairach-Tournoux system (x, y and z in milimiters represent left, posterior and superior directions respectively). Corresponding z-score of peak activity voxel with each flavor is also presented.

Figure – 5 Swallow related cortical activity in precuneus region A) Average percent signal increase in Precuneus. Difference between flavored stimuli and dry swallow failed to reach any statistical significance. We performed non-parametric Kruskal-Wallis test due to unequal variances among groups. Data are presented as mean ± SEM; B) Average swallow-related BOLD response waveforms across all trials in precuneus are shown (n=294). As shown there were no significant differences in the magnitude of FMRI waveforms for the flavor stimulated swallows compared to dry swallow; C) Table represents coordinates of peak signal intensity with each flavor within precuneus region based on Talairach-Tournoux system (x, y and z in milimiters represent left, posterior and superior directions respectively). Corresponding z-score of peak activity voxel with each flavor is also presented.
• Group analysis of water swallows did not show any significant activity within precuneus. Z-score within the region is presented only for comparative purposes.

**Figure – 6** Total cortical swallow network activity

A) Activated cortical regions during swallow showing the total cortical swallowing network. Significant activity associated with each stimulus throughout cortical swallowing network from medial anterior oblique view of a glass brain (All of the activity is seen through the tissue). Note that flavored associated swallows demonstrate more robust activity compared to saliva or water swallows. B) Average percent signal increase in cortical swallow network. Significant differences were found comparing average FMRI signal intensity change for all flavored swallows compared to dry/water swallow. We performed ANOVA, and Tukey test for multiple pair-wise comparisons. † p<0.001 compared to dry swallow (pre), * p<0.001 compared to water, ‡ p<0.001 compared to dry swallow (post). Data are presented as mean ± SEM; C) Average swallow related BOLD response waveform across all trials in cortical swallow network (n=294). As shown there were considerable differences in magnitude of FMRI activity waveforms for the flavor stimulated swallows compared to dry swallow and water.
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<td>2.</td>
<td>Asoh R and Goyal RK.</td>
<td>Manometry and electromyography of the upper esophageal sphincter in the opossum.</td>
<td>Gastroenterology</td>
<td>74</td>
<td>514-520</td>
<td>1978</td>
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<td>19.</td>
<td>Jean A.</td>
<td>Control of the central swallowing program by inputs from the peripheral receptors.</td>
<td>J Auton Nerv Syst</td>
<td>10</td>
<td>225-233</td>
<td>1984</td>
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