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Functional Connectivity of the Cortical Swallowing Network in Humans

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Abstract

Introduction—Coherent fluctuations of blood oxygenation level dependent (BOLD) signal have been referred as “functional connectivity” (FC). Our aim was to systematically characterize FC of underlying neural network involved in swallowing, and to evaluate its reproducibility and modulation during rest or task performance.

Methods—Activated seed regions within known areas of the cortical swallowing network (CSN) were independently identified in 16 healthy volunteers. Subjects swallowed using a paradigm driven protocol, and the data analyzed using an event-related technique. Then, in the same 16 volunteers, resting and active state data were obtained for 540 seconds in three conditions: 1) swallowing task; 2) control visual task; and 3) resting state; all scans were performed twice. Data was preprocessed according to standard FC pipeline. We determined the correlation coefficient values of member regions of the CSN across the three aforementioned conditions and compared between two sessions using linear regression. Average FC matrices across conditions were then compared.

Results—Swallow activated twenty-two positive BOLD and eighteen negative BOLD regions distributed bilaterally within cingulate, insula, sensorimotor cortex, prefrontal and parietal cortices. We found that: 1) Positive BOLD regions were highly connected to each other during all test conditions while negative BOLD regions were tightly connected amongst themselves; 2) Positive and negative BOLD regions were anti-correlated at rest and during task performance; 3) Across all three test conditions, FC among the regions was reproducible ($r > 0.96$, $p < 10^{-5}$); and 4) The FC of sensorimotor region to other regions of the CSN increased during swallowing scan.

Conclusions—1) Swallow activated cortical substrates maintain a consistent pattern of functional connectivity; 2) FC of sensorimotor region is significantly higher during swallow scan than that observed during a non-swallow visual task or at rest.

Keywords

resting connectivity; reproducibility; seed based; deglutition

1. INTRODUCTION

Previous studies have identified distributed areas of brain activity associated with voluntary and spontaneous swallowing (Birn et al., 1998; Hamdy et al., 1996; Hamdy et al., 2001; Hamdy et al., 1999; Kern et al., 2001; Martin et al., 2001; Martin et al., 2004). The interest in identification of cortical areas associated with deglutition is driven by the high prevalence of dysphagia in many survivors of stroke (Barer, 1989; Daniels et al., 1996; Martino et al., 2005; Martino et al., 2001; Smithard et al., 1997; Smithard et al., 2007). A better understanding of cortical control of swallowing is essential for developing therapeutic and rehabilitative modalities for patients suffering from neurogenic dysphagia. Although the overall cortical regions activated during swallowing have been identified, it is not known whether these regions constitute a cohesive functional network; and how this connectivity compares during rest and swallowing.

One must identify the elements and interconnections of a network to understand its function. In that, previous investigations studied effective connectivity of cortical swallowing network using principal component analysis and structural equation modeling (Mosier and Bereznaya, 2001). These studies identified five independent BOLD positive functional clusters and proposed that these components were organized into two parallel cerebral loops rather than a hierarchical dual projection model put forward earlier (1997). Cerebral first and second loops were defined by connections to insula and cerebellum respectively and both were interconnected through the sensorimotor-cingulate cluster (Mosier and Bereznaya, 2001). More recently another study looked at laterality of components of the volitional swallowing network (Lowell et al., 2012). This study used the whole brain seed based cross correlation analysis in a block-designed swallowing task. Clusters of connected voxels were larger from insula than other swallow-related seed regions, and interactions of the insula with other brain regions were greater on the left side during volitional saliva swallowing. Authors concluded that insula especially on the left hemisphere played the primary integrative role for volitional swallowing (Lowell et al., 2012).

Biswal et al. (1995) first observed the specific and strongly correlated spontaneous blood oxygenation level dependent (BOLD) fMRI signal fluctuations in the right and left somatosensory cortex and introduced the term “functional connectivity” to describe this novel finding (Biswal et al., 1995). Since this original description, coherent and synchronized fluctuations of BOLD signal have emerged as an important technique to study underlying neuronal networks of the human brain (Fox and Raichle, 2007). These coherent BOLD fluctuations are even observed at rest when subjects are explicitly instructed not to focus on any stimulus or task. It is believed that the function of a particular coherent activity depends on the function of the brain system and it may play a role in input selection, consolidation of information or facilitation of synaptic plasticity (Buzsaki and Draguhn, 2004). Synchronous spontaneous fluctuations of the BOLD signal (or functional connectivity) are reported between multiple distant neuroanatomical brain systems across many behavioral, resting, sleep and anesthesia states (Arfanakis et al., 2000). Connectivity has been extensively studied and replicated in many brain systems including: somatomotor (Biswal et al., 1995; Van Dijk et al., 2010), visual and auditory (Cordes et al., 2000; Van

Dijk et al., 2010), attention (Fox et al., 2006a), language (Hampson et al., 2002) and memory (Stein et al., 2000; Vincent et al., 2006) systems but not swallowing network. The observed correlation patterns are strongly constrained by system-specific patterns of anatomical connectivity but probably do not simply reflect monosynaptic direct connections (Vincent et al., 2007). Functional connectivity may refer to any study examining inter-regional correlations in neuronal variability. This applies to both resting-state and task-state studies, and can refer to correlations across subjects, runs, blocks, trials or individual BOLD time points, an ambiguity that can become confusing (Horwitz, 2003). Our study examines *intrinsic baseline BOLD fluctuations* as an important manifestation of underlying spontaneous neuronal activity (Fox and Raichle, 2007). Henceforth, for simplicity throughout this manuscript, we will use the term functional connectivity (FC) for these spontaneous and synchronized low frequency fluctuations of the BOLD signal related to all cortical regions that exhibit either positive or negative BOLD signal change during swallow.

Aim

Taking advantage of a number of available technological and analytical advancement, our aims were to: a) investigate the reproducibility of FC among swallow-associated regions/seeds; and b) compare the FC among these regions/seeds during swallow with a control task and resting state.

2. METHODS

Study Subjects

Sixteen asymptomatic right-handed adult subjects (ages 20-34, 9 female) were studied. Subjects were recruited by advertisement. The Human Research Review Committee of the Medical College of Wisconsin approved the study protocol, and subjects gave written informed consent before the study. None of the subjects had any history of dysphagia or other gastrointestinal-related diseases based on a completed detailed health-related questionnaire and interview with a physician. All subjects tolerated the procedure well and completed the protocol.

2.1. Data Acquisition

Subjects were placed supine in a 3.0T General Electric Signa LX scanner (General Electric Medical Systems, Waukesha, WI) equipped with an eight-channel receiver head coil and body quadrature transmit radiofrequency coil. Cardiorespiratory monitoring was performed at sampling rate of 40 Hz with a pulse oximeter and respiratory bellows equipment incorporated in the MRI system. A rear projection screen was placed at the head of the scanner bed to display visual cues to swallow, relaxation and fixation crosshair. Echo planar BOLD contrast image (EPI) data were acquired during six 540s whole brain scans, across two sessions on the same day. fMRI data were acquired in three different conditions: 1) swallow task condition cued by 21 random visual commands to *swallow* (SW) repeated twice (n=16 for first run and n=13 for second run); 2) control visual task with eyes open fixated on a crosshair, and similarly timed 21 random visual commands to *relax* (RX) replacing the fixation crosshair repeated twice (n=16); and 3) awake state with eyes closed at *rest* (RT) repeated twice (n=16). Subjects were instructed to avoid repetitive swallowing.

They were coached to perform a single pharyngeal swallow with their mouth closed without lower jaw or lip motion, yet swallow may have involved oral preparatory activity. Two anatomic scans were also acquired during each session using the high-resolution spoiled gradient recalled acquisition (SPGR) technique, consisting of 140 sagittal whole brain 1 mm-thick slices over a 240 mm field of view (FOV) and 256×224 within slice pixel resolution. These high-resolution anatomical images were used for subsequent superposition of the lower-resolution functional MRI data. Functional EPI scans were acquired over 34 contiguous 4-mm thick sagittal slices over the whole brain volume in an interleaved fashion without any gap or overlap, with a slice-wise pixel resolution of 64×64 pixels over a 240 mm field of view yielding a within-slice resolution of 3.75×3.75 mm, captured with an echo time (TE) of 23.4 ms and a repetition time (TR) of 2000 ms. At the end of the second scan session, a separate paradigm driven functional scan was performed, which was strictly used to independently identify cortical and subcortical areas activated with swallowing in order to use them as seed regions for FC analysis (*functionally guided connectivity analysis*). Details of the imaging parameters and analytical techniques of this “seed finder” scan have been previously described (Babaei et al., 2012).

2.2. Data Analysis

All fMRI signal analysis was carried out using Analysis of Functional Neuroimages (AFNI) software package (Cox, 1996). Functional EPI images were reconstructed into four dimensional time dependent volumes wherein each voxel was associated with 540s time series of fluctuating BOLD contrast data. We conditioned the data according to a rigorous series of signal pre-processing steps based on previously published fMRI connectivity analysis pipeline (Chen et al., 2011; Goveas et al., 2011). All participants maintained a peak-to-peak global (EPI volume) head motion of <1 mm. One subject showed >1 mm global head motion throughout the study that was related to swallow and her swallow run data was excluded from further analysis. Physiologic (cardiac and respiratory) related signal changes expressed as second order Fourier series expansion were retrospectively corrected (Glover et al., 2000). In anatomical data sets, brain was extracted from surrounding tissue (Smith, 2002). We then computed a mean anatomical dataset from the two scans acquired in each of the sessions to remove the bias of either session, and used it as the reference anatomical dataset for alignment of all EPI datasets. Reference anatomical scan was spatially normalized to match the standard Talairach-Tournoux stereotaxic template (Talairach and Tournoux, 1988). We performed T1 equilibration, slice timing correction and modeled head motion using 12 degrees of freedom (Ernst et al., 1999) (12 motion parameters from a general affine transformation matrix representing shift, rotational and shearing motion) indexed by time, which was used to interpolate the time series back to the original acquisition grid (Saad et al., 2009). We computed the alignment matrix between EPI datasets and reference anatomical scan, and applied that to register and align all datasets to the reference anatomic scan grid. The data were transformed and resampled into standard Talairach-Tounoux space with voxel size of $2 \times 2 \times 2$ mm in one interpolation using the AFNI package (Talairach and Tournoux, 1988). Voxel-wise extreme fluctuations of the signal (spike values) were replaced by a fitted smooth curve to the time series. fMRI BOLD signal trend components were removed over the course of time series voxel by voxel independently using linear least squares. White matter and cerebrospinal fluid containing

voxels were identified automatically based on signal intensity and manually verified. The average time courses in the white matter, cerebrospinal fluid and global brain were extracted for use as nuisance regressors (Van Dijk et al., 2010). We utilized functionally acquired nasopharyngeal signal and identified each swallow with its associated distinct susceptibility changes to extract timing of actual swallows. General linear modeling techniques with orthogonal least squares estimation were used to remove undesirable signal contamination correlated with swallow or null command (relax) related activity (where present), subject motion (12 motion parameters), white matter, cerebrospinal fluid and global noise temporal components out of the BOLD signal as covariates of no interest. Utilizing the residual signal of an event-related paradigm was investigated and deemed well suited for evaluation of underlying functional connectivity analysis (Fair et al., 2007b). Residual fMRI signal was spatially smoothed to full-width-at-half-maximum (FWHM) of 6 mm and band-pass filtered to a frequency target range of 0.015 – 0.1 Hz using Fourier transformation. Subsequently the first 60 seconds of each run was discarded (scanner and subject equilibration period).

We extracted positive (increased BOLD signal during swallow task) and negative BOLD (decreased BOLD signal during swallow task) activated clusters from independent “seed finder” scan and utilized them as seed regions for the functional connectivity analysis. Average time series over each seed region was then extracted (averaged time course across all voxels within a seed region) individually during each condition. We assessed functional connectivity (FC) between any two seed regions of the cortical swallow network (CSN) by calculating Pearson correlation coefficients (CC) between preprocessed 480s time series of the paired ROIs. Correlation coefficients can take values in the range of -1 to +1. Large positive values (closer to 1) are indicative of high FC, large negative values (closer to -1) are indicative of highly anti-correlated FC and small values (close to zero) are suggestive of low FC between corresponding pair of ROI. Large positive values are commonly depicted as warm colors (yellow – red), large negative values are demonstrated as cold colors (blue – navy) and small values are shown as neutral green colors. For each subject there were 780 $[(40 \times 39) / 2]$ pair-wise CC values among 40 ROIs of the CSN. These values were arranged into an individual FC matrix for each study condition. The matrix of correlation coefficients, hereafter called FC matrix, then will get averaged together to generate a group FC matrix for each study condition.

2.3. Statistical Analysis

Statistical analysis was performed using MATLAB® software (MathWorks, Natick, MA). FC values were z-transformed to achieve a normalized Gaussian distribution, and one-sample t-test was performed to identify statistically significant functional connectivity. We then used analysis of variance and paired t-test to compare FC matrices of repeated functional runs for three tested conditions in order to establish that CSN is reproducible. Bonferroni correction was used as the statistical method to correct for multiple comparisons and $p < 0.05$ was considered to be statistically significant. The corrected p-threshold was obtained by dividing the nominal alpha value by the number of multiple comparisons. Since there are 40 ROI's in the FC matrix, $(40 \times (40-1) / 2 = 780)$ hypotheses are being test simultaneously. Therefore, the Bonferroni-corrected p-value $(0.05 / 780 = 0.0000641)$.

We plotted cross correlation coefficients of FC matrices and ran linear regression analysis to confirm reproducibility of the data. We then performed 10000 random permutation resamplings of seed labels. Under the null hypothesis, randomly relabeled seeds allowed us to estimate the distribution of the correlations (r value, inset of Figure 1-3B) of the CC values. Then we compared the true observed CC value to the null distribution of CC values and determined if the null hypothesis was true *or* verified that cross correlation (CC) values were significant. The null distribution of actual cross correlation values was also assessed using a random block permutation approach to take into account temporal correlation of data points. Under standard normal theory, data points are assumed to be independent and normally distributed. To estimate the corresponding p-values without making these assumptions we implemented a random temporal block permutation-resampling scheme of data points in each time series. The ordinary (Block length = 1 point) permutations preserve the distribution of data but destroy its temporal correlation, whereas the (Block length > 1 point) block permutations preserve some of the temporal correlation structure of the data. More elaborate description of the block permutation is described in supplementary material. Also as a measurement of reproducibility, we calculated the difference in cross correlation of each pair of ROIs across session 1 and 2 as described previously (Fair et al., 2007a). Mean difference of CC values across all study subjects were then calculated for each experimental condition.

Subsequently the two FC matrices of each test condition were averaged for all subsequent analyses to decrease variance of the data and the number of comparisons. We defined the following FC scores across different study conditions in order to further investigate individual components of CSN (He et al., 2007). Since swallowing is primarily a motor function we defined the following FC scores specifically related to motor activity within BOLD positive activated component of the CSN: a) average FC score of rolandic operculum of either hemisphere and all cortical positive BOLD activated seeds; b) average FC score of sensory motor region of either hemisphere and all cortical positive BOLD activated seeds; c) average FC score of premotor area of either hemisphere and all cortical positive BOLD activated seeds, d) average FC score of supplementary motor area of either hemisphere and all cortical positive BOLD activated seeds. Furthermore, we calculated average FC score of all other positive BOLD cortical regions to the rest of positive BOLD activated seed regions respectively. We then used analysis of variance to compare pair-wise FC of all nodes within the network across three test conditions.

3. RESULTS

3.1. Seed identification

Studies of stage one from separately obtained and analyzed seed-finders can identified a total of forty regions exhibiting changes in BOLD signal during swallowing. Of these, 22 exhibited positive BOLD changes and 18 negative BOLD changes. Median volume for positive BOLD seeds was 136 mm^3 (range: $48\text{-}1288 \text{ mm}^3$) and for negative BOLD seeds was 1068 mm^3 (range: $96\text{-}5664 \text{ mm}^3$). The data from seed finder scan have been previously described and published in another study focusing on swallow-related BOLD activity (Babaei et al., 2012). All activated regions and coordinates of the centroid voxel of the

identified cluster along with their corresponding Brodmann area based on standard Talaraich-Tornoux atlas provided in AFNI are presented in Table 1.

3.2. Functional connectivity analysis, reproducibility and comparative studies

Group FC matrices of swallow condition (SW), control visual relax task (RX) and resting state (RT) in sessions one and two are shown in Figure 1-3A. Diagonal diameter of the FC matrix from upper left to lower right corner represents connectivity of each seed region to itself that is a value of 1 (fully correlated). Lower left and upper right triangles of FC matrix on either side of this diagonal diameter represent identical mirror image connectivity information.

FC matrices showed a uniform pattern of higher FC amongst BOLD positive activated seeds throughout all study conditions as demonstrated in upper left section, depicted in warm colors yellow-orange. FC matrices illustrated a consistent pattern of high FC amid BOLD negative activated seeds in lower right section, shown in warm colors yellow-orange. Interaction of BOLD negative activated seeds and BOLD positive activated seeds demonstrated mainly anti-correlation (or no significant correlation) in the lower left (or upper right) corner of FC matrices, demonstrated by cold colors green-blue. Sub-cortical seeds showed low but steady connectivity across both cortically positive and negative BOLD activated seeds as depicted in the middle yellow band of FC matrices (Panel A of Figure 1-3).

3.2.1. Reproducibility—We performed linear regression analysis on scatter plots of FC values amongst 780 distinct ROI pairs, across two sessions of an identical test conditions. Cross correlation coefficients of average group FC matrix for each condition were as follows: swallow task $r=0.965$ ($n=13$, $p<10^{-5}$), control visual task $r=0.975$ ($n=16$, $p<10^{-5}$) and resting state $r=0.962$ ($n=16$, $p<10^{-5}$). The representative linear regression analysis of FC matrices during swallow, rest and control task are demonstrated in panel B of Figures 1, 2 and 3 respectively. The confirmatory 10000 random permutation resamplings of ROI labels verified that cross correlation of connectivity matrices are significantly different from random occurrence in all study conditions ($p < 10^{-5}$). The representative histogram of random permutation and null distribution of r -values in each condition is demonstrated as the inset embedded in Figure 1-3B. Please note that the true group r values of each condition ($r > 0.95$) is well separated from the null distribution of permutation of r values ($r < 0.2$), and thus rejects the null hypothesis that observed r values are random occurrences. Individual observed r -values in each experimental condition were also distinctly apart from null distribution of permuted r -values. All individual scatter plots and their corresponding r -values along with their null distribution histograms are shown in supplementary Figure 1A-C. Results from temporal permutation resampling of the time series (Block length = 1 point) were consistent with the assumption of temporally independent data as shown in supplementary Figure 2A. However, utilizing a longer block length (Block length = 6 points) showed that p -values were skewed to the left and adopted a higher value that may represent a slight bias due to temporal correlation of consecutive fMRI measurements in a time series. This temporal correlation bias was consistent across experimental conditions (supplementary Figure 2B).

We measured CC difference as another marker of intra-subject reproducibility. Mean intra-subject CC differences ranged from 0.05 to 0.25 across 780 links of FC matrix of all experimental conditions. Mean CC difference of each ROI (39 links to all other ROIs) ranged from 0.11 to 0.17. Mean CC difference of right prefrontal anterior region (0.16) was significantly higher than other ROIs during swallow condition ($p < 0.001$) and no other difference among ROIs were observed across conditions. Mean CC difference was not significantly different between resting, control visual task and swallowing conditions. Mean CC difference in various components of the FC matrix (cortical BOLD positive, subcortical BOLD positive, BOLD negative and interaction of BOLD positive and negative) consistently ranged from 0.12 to 0.15 and was not significantly different (Panel C of Figure 1-3).

Furthermore, other statistical methods such as analysis of variance, paired t-test and principal component analysis (PCA), and also subdividing the connectome into positive and negative BOLD sub-networks did not show any statistically significant difference between FC matrices of two similar repeated sessions. PCA was performed on the entire individual FC matrices across all experimental conditions. The first principal component explained 13% of total variance of the data. The first principal component mainly identifies positive and negative BOLD components of the FC matrix and is shown in supplementary Figure 3A. Then we analyzed cumulative accounted percentage of total variance as a function of number of principal components. The first 30 principal components accounted for 90% of total variance of the 780 pairs of connections across 6 study sessions (supplementary Figure 3B). For example the first 9 principal components that explain more than 50% of total variance of data are shown in supplementary Figure 3C. None of the first 9 principal components showed a significant difference between first and second sessions of experimental conditions.

Since two fMRI runs were obtained in identical conditions and were similar, we averaged the two FC matrix of each subject in each condition for all further comparative analysis. Average group FC matrix during each condition is demonstrated in Figure 4A. As seen in these figures, amongst positive BOLD activated regions during all conditions high FC (shown encircled in 4A-I, swallow scan) exists between sensory-motor related seeds i.e. rolandic operculum, sensory motor and premotor seeds. Supplementary motor area showed less FC. Prefrontal operculum and insula (anterior and posterior) also demonstrated a very high FC (shown encircled in 4A-III, relax scan). Among negative BOLD seeds homologous regions show cross-hemispheric high functional connectivity (shown encircled in 4A-II, rest scan). Furthermore, Posterior cingulate was ipsilaterally connected with lateral parietal and precuneus regions. Premotor regions showed high connectivity to lateral parietal region. (Individual average FC matrices across each condition for all subjects are presented in supplementary Figure 4A-C).

We performed one sample t-test to identify statistically significant FC values between pairs of ROIs as shown in Figure 4B. Majority of significant FC pairs (distinctively marked by warm colors orange- red) reside in the BOLD positive and BOLD negative components of the network and only few in the interaction component of the matrix (Figure 4B). In the *cortical* BOLD positive component of the FC matrix (top left corner of all matrices)

amongst all possible 153 connections: 137, 136 and 133 of the connections displayed significant FC ($z > 1.96$) during swallow, control task, and resting condition respectively (87-90% functionally connected and none 0% anti-correlated). In the BOLD negative component of the FC matrix (bottom right corner of all matrices) amongst all possible 153 connections: 100, 108 and 101 of the connections displayed significant FC ($z > 1.96$) during swallow, control task, and resting condition respectively (65-70% functionally connected and minimally 5% anti-correlated). On the other hand, in the interaction of BOLD positive and negative component of FC matrix (bottom left corner of all matrices) amongst all possible 324 connections only 2, 3 and 8 of the connections displayed significant correlation FC ($z > 1.96$) during swallow, control task, and resting conditions respectively (Only 1-2% were functionally connected and mostly were anti-correlated). In the *subcortical* BOLD positive component of the FC matrix (middle band of all matrices) amongst all 150 connections: 44, 37 and 44 of the connections displayed significant correlation FC ($z > 1.96$) during swallow, control task, and rest condition respectively (25-29% functionally connected).(Figure 4B).

3.2.2 Comparison of functional connectivity (FC) among swallow and control tasks, and resting state—We compared FC of all 780 available ROI connections of the swallow network across study conditions using analysis of variance. Multiple pairs of ROIs involving the right parahippocampal gyrus were identified to be different during swallow task compared to control visual task and resting state (Figures 5A-B, $p < 0.001$ not corrected); the FC of this region (a *negative BOLD region*) to left sensory motor region (a *positive BOLD region*) diminished from FC_{RT} = 0.02 at rest, to FC_{RX} = -0.04 during control visual task, and finally to significantly anti-correlated value of FC_{SW} = -0.22 during swallow. This difference survived correction for multiple comparisons (Figure 5C).

Since deglutition is primarily a motor response, we focused on motor related cortical components of the connectome for further analysis and investigated FC score of sensory-motor related regions across different study conditions. Among sensorimotor related cortical regions, *right and left sensory motor* areas are significantly different across study conditions and that difference was due to higher FC of sensory motor regions during SW condition compared to both control visual task and resting state (Figure 6). We did not find higher functional connectivity of either sensorimotor cortex in this population and bilateral sensorimotor cortices showed a similar pattern of increased functional connectivity pattern during swallow. Scatter plot of functional connectivity of bilateral sensory motor regions to the remainder of deglutition network ROIs across study subjects are presented in supplementary Figure 5A and B. We performed FC score analysis of all the other cortical BOLD positive regions including insula, and did not find any significant functional connectivity score difference between the three experimental conditions. Therefore it appears that observed changes in functional connectivity was specific to bilateral sensorimotor regions and not others.

CONCLUSION

In this study, we determined the functional connectivity (FC) among regions of the brain involved in swallowing and characterized the differences in FC among these regions when

they were involved in swallowing and when they were at rest or engaged in a control task. Study findings indicate that FC among the swallow-related cerebral regions are reproducible and exhibit identifiable differences between rest and swallowing state. These differences involved sensorimotor cortices and represent their engagement in controlling the motor aspect of swallowing. The present study did not detect variances among regions involved in cognitive aspects of swallowing. This could simply reflect the insensitivity of the employed techniques for detecting potentially more subtle connectivity alterations.

Distinct regions of the brain have been regularly observed to decrease their activity during attention demanding cognitive tasks suggesting an organized baseline default mode (Raichle et al., 2001), thought to mediate brain processes during resting state that gets suspended during goal directed behaviors (Corbetta and Shulman, 2002). Among forty distinct regions involved in swallowing nearly half comprised negative BOLD component and the other half involved cortical positive BOLD component of the CSN. Positive and negative BOLD activated clusters that were identified in the seed finder scan, and were subsequently used for functional connectivity analysis are shown in Figure 7, adapted from reference (Babaei et al., 2011) with permission. As illustrated, the negative BOLD regions were similar to constituents of the default mode network (Greicius et al., 2003), and positive BOLD clusters showed overlap with “task control” (Corbetta and Shulman, 2002; Dosenbach et al., 2006) networks, which have been previously described in literature. Previous studies have confirmed that functional connectivity maps of default mode (Van Dijk et al., 2010) and task control (Dosenbach et al., 2006) networks are highly reliable across different subjects, states and studies. However, deglutition is a unique primitive motor behavior that is different from other goal directed motor behaviors. It employs muscular structures that are only partially and during initial phase of swallowing under voluntary control. Observed FC matrix of CSN (at rest or during task) is in agreement with studies showing high FC amongst positively BOLD activated seeds, and amid negatively BOLD activated ROIs. Furthermore, the FC matrix in the current study exhibited anti-correlative pattern between positive and negative BOLD components of CSN consistent with previous studies of other cortical systems (Fox et al., 2005). Therefore as suggested previously, regions that were similarly modulated by swallow task paradigm (seed finder scan) were correlated at rest, and regions with opposing functionality were found to be anti-correlated in their spontaneous activity. Some of these studies have suggested that resting functional connectivity (FC) strongly reflects the direct structural connectivity (Greicius et al., 2009). However, others have shown that FC may rather reflect a polysynaptic link and not a direct axonal connection (Vincent et al., 2007). Current interpretation of functional connectivity (FC) demonstrated as synchronous spontaneous BOLD fluctuations is that FC is constrained by but is not equal to the anatomic structural connectivity (Fox and Raichle, 2007).

Strong intra- and inter hemispheric functional connectivity of sensorimotor regions involved in deglutition shown in current paper is consistent with previous studies of somatomotor network engaging in a broad range of motor behaviors (Biswal et al., 1995; Fox et al., 2006b; Morgan and Price, 2004; Van Dijk et al., 2010). The degree to which the correlation structure of spontaneous BOLD activity changes under task conditions remains an important topic of research interest (Fox and Raichle, 2007). Robust functional connectivity of insula and prefrontal operculum regions within CSN is in agreement with the recent FC study of

volitional swallowing network proposing a central integrative role for this region (Lowell et al., 2012). In our study, functional connectivity was equivalent in both sensorimotor cortices, and demonstrated increased FC bilaterally during swallow scan. Individual analysis of subjects in Lowell study also showed a mixed picture between different ROIs. While some subjects displayed left hemispheric dominance of insula, others showed much higher functional connectivity of right primary motor cortex. Similar inter-subject variance of functional connectivity could be inferred from results of principal component analysis in our study. First principal component explained only 13% of variance in our data and more than 30 components were needed to explain 90% of variance across subjects and conditions. Current publication did not address modular organization of CSN and parallel versus hierarchical projection model of deglutitive control reported previously by Mosier et al. due to limitations of space, and diversion of the main focus of the manuscript. Neuroanatomical model of cortical swallowing control is an area of intense research interest and warrants further dedicated comprehensive investigation.

Recent studies suggest that neural networks (visual and sensorimotor) consistently establish new patterns of synchrony at different stages of various tasks (Bressler et al., 1993; Rodriguez et al., 1999; Roelfsema et al., 1997) suggesting that networks reorganize into new systems with formation of dynamic links mediated by synchrony to accommodate a new state of brain (Varela et al., 2001). Therefore, connectivity could be modified under different physiological conditions. For example, connectivity is reported to increase within the language system when subjects continuously listened to a narrative text (Hampson et al., 2002), but remained unchanged within the motor system during a finger-tapping task (Morgan and Price, 2004). Current study shows that functional connectivity of sensory motor cortex to the remainder of cortical positive BOLD swallow network is amplified in the physiologic state of repetitive swallowing. On the other hand, in pathological conditions such as stroke it has been demonstrated that resting functional connectivity between structurally normal left and right posterior parietal regions correlate with the degree of spatial neglect as an objectively measured behavioral deficit (Corbetta et al., 2005). Loss of inter-hemispheric functional connectivity between homologous regions of the dorsal attention network correlated with objective functional deficits in the detection of contra-lesional targets (Carter et al., 2010; He et al., 2007). Moreover, longitudinal studies using functional connectivity have provided crucial information regarding neuroplasticity and cortical reorganization during recovery after stroke (Connor et al., 2006; Corbetta et al., 2005). These studies lend validity to the use of resting state functional connectivity for assessing the cortical swallowing network following neuronal injury. Current study interrogating the functional connectivity of cortical swallowing network in physiologic state lays the groundwork for future studies with implications for prognosis of dysphagia after stroke and during its course of recovery. Resting functional connectivity, which does not require an external input or performing a task has also a methodological advantage that it is applicable in patients who are not capable of proper performance of an overt task (Park et al., 2011). This advantage makes it an ideal paradigm for disease conditions where performance of task is either not feasible or challenging such as comatose or stroke patients (Park et al., 2011).

We used global noise (average signal over entire brain) as a signal of non-interest in our regression model. This preprocessing step has been controversial and some studies have suggested that it may overall reduce cross correlation values and introduce spurious anti-correlation patterns into the functional connectivity matrix (Murphy et al., 2009; Saad et al., 2012). Therefore, we avoided emphasis on anti-correlation patterns of CSN matrix for this reason. However, it should be noted that other investigators have shown that anti-correlation patterns exist even without global signal regression that may suggest an underlying biological origin for anti-correlations (Chai et al., 2012). Since our main emphasis was to compare FC matrix across different conditions with exactly similar pre-processing steps, we do not think this step had an effect on the reported comparative results across conditions. We are aware of some other limitations of the technique used in the current study. Swallowing physiologically is always locked into expiratory phase of respiration, and is inherently associated with oropharyngeal motion. In spite of our rigorous analytical steps to reduce effects of non-physiologic artifacts such as motion or physiologic noise such as respiration, we cannot exclude the possibility that observed differences could originate from these non-neuronal confounders. However, we think that these confounders won't specifically target sensorimotor regions and not other areas of CSN.

In summary, swallowing is associated with BOLD positive and BOLD negative fMRI signal activity. Functional connectivity of these regions is robust and reproducible across various experimental conditions. Members of each BOLD activity group consistently exhibit strong connectivity amongst them, but show anti-correlative pattern in between. Functional connectivity of sensorimotor cortex among regions of the deglutition network strengthens significantly during task of swallowing compared to control visual task or rest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CSN	Cortical swallowing network
fMRI	functional magnetic resonance imaging
BOLD	Blood oxygenation level dependent
FC	functional connectivity
CC	cross correlation coefficient

Figure 1. Functional connectivity matrix during swallow task

Participants watched a screen with commands to swallow in random intervals. Swallow-related signal has been removed and fMRI signal residue has been used to measure functional connectivity (FC). Forty regions of interest that showed significant group BOLD activation (either positive or negative) during an independently obtained fMRI scan in the same population were used as seeds for subsequent connectivity analysis. A) Group functional connectivity matrices during session 1 and 2 of swallow task are shown. Forty seeds are enumerated on x- and y- axes, and functional connectivity values are between -0.40 to +1.00. Color scale in the middle shows the spectrum of FC values from lowest possible -1 to highest 1. First eighteen rows and columns (1-18) of matrix represent FC of cortical positive BOLD activated seeds (top left corner of FC matrix) and the last eighteen rows and columns (23-40) denote FC of negative BOLD activated seeds (bottom right corner). Middle four (19-22) rows and columns are FC of subcortical positive BOLD activated seeds (middle band). Cells corresponding to first eighteen columns (rows) and last eighteen rows (columns) represent interaction of FC between positively and negatively BOLD activated seed regions (bottom left or top right corner of FC matrix). B) Linear

regression analysis of 780 functional connectivity values among forty seed regions between session 1 and sessions 2 of swallow task. Y-axis in the plot represents session two and x-axis depicts session one FC values. Fitted line shows an intercept of 0.007 and slope of 0.952 confirming quite reproducible results across both sessions. This was highly statistically significant ($p < 10^{-5}$). Inset figure demonstrates histogram of cross correlation analysis of 10000 random permutation resamplings of forty seed labels to show null distribution of r-values. X-axis of inset figure corresponds to r-value of each permutation and Y-axis represents frequency occurrence of all the permutations having that r-value. Histogram confirms that random occurrence of cross correlation values with 99% confidence interval will still remain less than 0.2 far separate from observed r-value > 0.95 . C) Mean intra-subject cross correlation (CC) difference of various components of FC matrix of swallow network ($p > 0.1$). Data is shown as mean $\pm$ standard error of mean.

Functional Connectivity Matrix: Control Visual Task

Figure 2. Functional connectivity matrix at rest. Participants were awake but closed their eyes fMRI signal residue has been used to measure functional connectivity. A) Group functional connectivity matrices at rest in session 1 and 2 are shown (n=16). B) Linear regression analysis between session 1 and session 2 at rest. Fitted line shows an intercept of 0.004 and slope of 0.944 confirming quite reproducible results across both sessions ($p < 10^{-5}$). Inset figure demonstrates histogram of 10000 random permutation resamplings of forty seed labels and null distribution of r-values. C) Mean intra-subject cross correlation (CC) difference of various components of FC matrix of swallow network ($p > 0.1$). See Figure 1 legend for further details.

Figure 3. Functional connectivity matrix during control visual task

Participants watched a screen with commands to relax in random intervals. Command related signal has been removed and fMRI signal residue has been used to measure functional connectivity. A) Group functional connectivity matrices during control visual task in session 1 and 2 are shown ($n=16$). B) Linear regression analysis between session 1 and sessions 2 during visual task. Fitted line shows an intercept of 0.003 and slope of 1.007 confirming quite reproducible results across both sessions ($p < 10^{-5}$). Inset figure demonstrates histogram of 10000 random permutation resamplings of forty seed labels and null distribution of r -values. C) Mean intra-subject cross correlation (CC) difference of various components of FC matrix of swallow network ($p > 0.1$). See Figure 1 legend for further details.

Figure 4. Average functional connectivity matrix of swallow task, visual control task and resting state and corresponding normalized one-sample t-test

Forty seeds are identified and shown on x- and y- axes numerically. Similar to figures 1-3 first eighteen rows and columns (1-18) of matrix represent FC of cortical positive BOLD activated seeds (top left corner of FC matrix) and the last eighteen rows (23-40) denote FC of negative BOLD activated seeds (bottom right corner). Middle four (19-22) rows are FC of subcortical positive BOLD activated seeds (middle band). Cells corresponding to first eighteen columns and last eighteen rows represent interaction of FC between positively and negatively BOLD activated seed regions (bottom left or top right corner of FC matrix). Color scales analogous to FC and Z values are depicted. A) Functional connectivity values multiplied by 100 have been inserted in each cell for connectivity enthusiasts. Components of deglutition network with strong functional connectivity such as motor and opercular regions or cross-hemispheric homologous connections are encircled. B) Functional connectivity values were z-transformed and a one-sample t-test was performed. Absolute z-values higher than 1.96 are considered statistically significant and all insignificant

connections with z-values below the threshold are demonstrated in white. Absolute z-values above 3.99 are considered significant, corrected for multiple comparisons.

Figure 5.

Analysis of variance of functional connectivity across three study conditions (Swallow, relax and rest) and two scans (session one and two). A) The corresponding p values of comparing 780 functional connectivity values among forty seed regions in three experimental conditions are demonstrated as a color scale. Forty seed regions are listed and shown on x- and y- axes numerically. B) Several functional connectivity values show a difference among study conditions with an uncorrected $p < 0.001$. All of these differences are located in the interaction section of BOLD positive and negative within the FC matrix. C) The connectivity of left sensory motor and right parahippocampus is significantly reduced during swallowing compared to control visual relax task and resting state. This difference survives bonferroni correction for multiple comparisons.

Functional Connectivity Score

Figure 6. Functional connectivity score of motor-related cortical regions

We measured average functional connectivity of bilateral rolandic operculum, sensory-motor, premotor and supplementary motor regions to the remainder of positively BOLD activated regions of the deglutition connectome. Analysis of variance and post-hoc analysis showed that FC score of bilateral sensory motor regions during swallow task is significantly higher than the control task and the resting state (*uncorrected* $p < 0.05$). Rest: resting state while eyes are closed. Relax: control visual task cued to relax. Swallow: performing swallow task.

Swallowing Network Seeds

Figure 7. BOLD positive and BOLD negative swallow-related activity in “seed finder” scan Sagittal, axial, and coronal images are displayed to demonstrate the extent and location of activity throughout the whole brain. Positive BOLD clusters are shown in orange-yellow and negative BOLD clusters are depicted in blue-cyan color, modified and adapted from (Babaei et al., 2012) with permission.

Table 1

Independently identified swallows related BOLD activity

Participants swallowed in random intervals twenty one times triggered by visual cue. Swallow induced BOLD signal change was measured using event-related fMRI analysis. Clusters in group-analysis with significant activity, corrected for multiple comparisons, were used as seeds for subsequent functional connectivity analysis.

Positive BOLD Activation			Negative BOLD Activation		
Region of Interest	BA	Centroid of Activity (xyz)	Region of Interest	BA	Centroid of Activity (xyz)
1. L Prefrontal Operculum	44,45	-38, -9, 17	23.L Anterior Prefrontal	10	-23,49,17
2. L Middle Cingulate	24	-2, 0, 40	24.L Dorsolateral Prefrontal	9,46	-47,18,20
3. L Anterior Insula	13	-36, -4, 13			
4. L Posterior Insula	13	-32, -15,18	25.L Premotor	6, 8	-39,4,45
5. L Supplementary Motor	6,8	-4, -10, 53	26.L Anterior Cingulate	24,32	-4,39,22
6. L Premotor	6,8	-50, 1, 15	27.L Posterior Cingulate	23,31	-5,-47,28
7. L Sensory Motor	1-4	-48,-15,37	28.L Parahippocampus	24	-17,-36,-6
8. L Rolandic Operculum	43, 4	-53, -9, 20	29.L Precuneus	7	-4,-60,36
9. L Inferior Parietal	40	-54,-30,24	30.L Lateral Parietal	39,40	-45,-58,35
10.R Prefrontal Operculum	44, 45	43, 4, 8	31.L Cerebellum		-33,-64,-32
11.R Middle Cingulate	24	5, 0, 40	32.R Anterior Prefrontal	10	31,46,12
12.R Anterior Insula	13	39, 1, 8	33.R Dorsolateral Prefrontal	9,46	45,17,25
13.R Posterior Insula	13	37, -17, 5			
14.R Supplementary Motor	6	19, -8, 63	34.R Premotor	6, 8	35,9,45
15.R Premotor	6	52, -2, 23	35.R Anterior Cingulate	24,32	5,43,16
16.R Sensory Motor	4, 3	49, -14, 34	36.R Posterior Cingulate	23,31	5,-54,25
17.R Rolandic Operculum	43, 4	54, -8, 15	37.R Parahippocampus	24	18,-37,-10
18.R Inferior Parietal	40	57, -36, 24	38.R Precuneus	7	4,-62,35
19.R Dorsal Striatum		26, -4, 2	39.R Lateral Parietal	39,40	42,-60,36
20.R Thalamus		9, -20, 12	40.R Cerebellum		33,-61,-29
21.R Red Nucleus		5, -23, -4			
22.L Cerebellum		-9,-55,-11			