Functionally Connected Brain Regions in the Network Activated During Capsaicin Inhalation

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Abstract: Coughing and the urge-to-cough are important mechanisms that protect the patency of the airways, and are coordinated by the brain. Inhaling a noxious substance leads to a widely distributed network of responses in the brain that are likely to reflect multiple functional processes requisite for perceiving, appraising, and behaviorally responding to airway challenge. The broader brain network responding to airway challenge likely contains subnetworks that are involved in the component functions required for coordinated protective behaviors. Functional connectivity analyses were used to determine whether brain responses to airway challenge could be differentiated regionally during inhalation of the tussive substance capsaicin. Seed regions were defined according to outcomes of previous activation studies that identified regional brain responses consistent with cough suppression, stimulus intensity coding, and perception of urge-to-cough. The subnetworks during continuous inhalation of capsaicin recapitulated the distributed regions previously implicated in discrete functional components of airway challenge. The outcomes of this study highlight the central representation of airways defence as a distributed network. *Hum Brain Mapp* 35:5341–5355, 2014. © 2014 Wiley Periodicals, Inc.

Key words: brain mapping; cough; sensation; magnetic resonance imaging

INTRODUCTION

The airways and lungs are endowed with specialized sensory receptors (nociceptors) for detecting potentially harmful substances that can reach the airway mucosa via way of inhalation or aspiration, or alternatively via local production in airways disease [Mazzone, 2005]. Noxious stimulation of these receptors evokes defensive responses including reflex or behavioral coughing which is dependent upon airway sensory nerve input to the CNS. This represents an essential process in protecting the airways from potentially damaging stimuli, thereby ensuring that normal respiratory function is preserved. Sensations also accompany stimulation of the airways in humans, which are thought to contribute to airway protection by promoting an awareness of the damaging insult, and these

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perceptual experiences have been dubbed the urge-to-cough. Typically, an urge-to-cough will involve an itching, burning or scratching sensation of variable intensity that is localized to the upper airways and is associated with a desire to cough, which may or may not be suppressible [Davenport, 2008; Lee et al., 2002]. The sensation is elicited by airway nociceptor stimulation and the intensity of the experience shows dose dependence [Davenport et al., 2002; Hegland et al., 2011]. Coughing usually leads to a resolution of urge-to-cough in healthy people, although this may not be true in patients with respiratory disease in which both cough and the urge-to-cough become sensitized and persistent [Chung and Pavord, 2008]. Thus describing the central neural mechanisms that regulate cough and the urge-to-cough is important for understanding the essential processes that sustain normal ventilation and the mechanisms that lead to cough dysfunction in disease.

Inhalation of capsaicin (from hot chilli peppers) evokes the sensation of urge-to-cough and this is associated with regional brain activations in humans that are thought to represent the broader cough neural network [Farrell et al., 2012; Mazzone et al., 2007, 2011]. The extent and distribution of regions activated during capsaicin inhalation are likely to represent multiple functional processes such as coding of afferent inputs, representation of perceptual experience, affective responses, and motor planning and execution. Indeed, results from recent functional brain imaging experiments have identified distributed modules of regional activation within the broader capsaicin-inhalation network that are associated with either stimulus-intensity coding or self-reported urge-to-cough or cough-suppression efforts. Intensity coding of inputs succeeding airway stimulation occurs in many of the regions activated during capsaicin inhalation including the thalamus, premotor cortices, cerebellum and insula, whereas the subjective urge-to-cough is represented in the primary and secondary somatosensory cortices and the posterior parietal cortex [Farrell et al., 2012]. Activations associated with efforts to suppress cough occur in regions that have been implicated in response suppression in other contexts, and include the right inferior frontal gyrus, adjacent anterior insula, ventromedial prefrontal cortex and supplementary motor area [Mazzone et al., 2011]. Additionally, the mid cingulate cortex shows activation related to stimulus intensity, urge-to-cough, and cough suppression, which would suggest that the mid cingulate cortex is particularly important for the integration of intensity coding with other functional consequences of capsaicin inhalation.

Collectively, the outcomes of activation studies would suggest that the distributed regions implicated in discrete functional processes are operating as subnetworks with the broader capsaicin-inhalation network. However, while activation studies can implicate distributed brain regions in a common functional process, it cannot be assumed that the commonly activated regions share additional variance in signal change that would be compatible with a mutual

pattern of ongoing responses. Further support for the notion that distributed regions are activating in a coordinated fashion can be achieved by the demonstration of functional connectivity between constituent components of a putative network. The rationale for functional connectivity is that regions involved in a mutual function are likely to show increased levels of inter-regional correlation in association with the appropriate physiological state, in this instance capsaicin inhalation, compared to a control state [Friston, 2011]. Additionally, the incultation of brain regions as a dissociable subnetwork is strengthened when these inter-regional correlations show common variance that is not shared with other components of the broader network.

The objective of this study was to test for levels of functional connectivity in three putative subnetworks that include regions that have previously shown activation responses compatible with roles in intensity coding, perception of urge-to-cough or cough suppression during capsaicin inhalation. We hypothesized that distributed brain regions previously implicated in functional components of capsaicin inhalation would fulfill two criteria compatible with coordinated inter-regional activity. First, analyses seeded from one region in a subnetwork would show levels of correlation with other regions in the same subnetwork during prolonged inhalation of capsaicin that was increased compared to levels of correlation during the same period of constant saline inhalation. Secondly, levels of correlation between a seed and other regions in the same subnetwork would exceed levels of correlation with alternative seeds from other subnetworks during capsaicin inhalation.

MATERIALS AND METHODS

Participants

Twenty-one healthy participants (14 males, 7 females, average age 24.6 ± 7.7 years), with no history of respiratory or neurological disease, were recruited for the experiment. Four of the recruits were unable to comply with the protocol, which required active suppression of cough during sustained inhalation of a tussive substance. Seventeen participants (12 males, 5 females, average age 25.1 ± 8.4 years) provided the data that is reported for this study. Data collected from the cohort of 21 participants has been reported in other studies [Farrell et al., 2012; Mazzone et al., 2011]. However, the specific group of seventeen participants described herein has not been previously reported, nor have there been any previous reports of the data specifically collected for this study of functional connectivity. The experiment was conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the guidelines of the Melbourne Health Human Research Ethics Committee and all participants provided informed consent prior to their participation (Approval #2008.217).

Psychophysical Session

Participants attended an initial psychophysical testing session, in which capsaicin (Sigma, Australia) doses requisite for scanning sessions were determined. A modified facemask was fitted to the participants and attached via tubing to a jet nebulizer (RapidFlo; Allersearch, Scoresby, Victoria, Australia) in series with medical air (flow rate = 5 L/min). The dose needed to evoke two coughs (C2 threshold) after a single vital capacity breath of capsaicin was determined using the method of limits and as previously described [Mazzone et al., 2007]. Doubling doses of capsaicin (starting at 0.49 μ M) were administered at 30-s intervals, until two coughs were elicited. Urge-to-cough ratings on a 10-point scale (0 is no urge; 10 is most intense urge-to-cough imaginable) were collected during four subsequent challenges at the C2 dose. The capsaicin concentration two dose increments lower than the C2 threshold (low dose) for each participant was used during a later imaging session to identify brain activation associated with repeated inhalations during 18 s blocks of stimulation. The low dose was used because it produced a relatively low level of urge-to-cough that would approximate the low levels that were needed to ensure tolerability and cough suppression during the prolonged periods of capsaicin inhalation required for functional connectivity image acquisition. The dose determined during the psychophysical session was inhaled by participants for 18 s before image acquisition commenced to assess if their level of sensitivity to capsaicin was the same at the time of scanning. In some cases an alternative dose to the low dose was used during functional brain imaging when participants showed either an increase or a decrease in their sensitivity to capsaicin compared to their responses during the psychophysical session.

The concentration for continuous inhalation was determined by administering capsaicin during ongoing inhalations and exhalations over the course of 1 min, at increasingly lower doses until the participant could successfully perform the task without coughing but with a perceivable urge-to-cough. To ensure that cough suppression was feasible for the planned duration of image acquisition, the dose that did not elicit any coughing during the 1-min trials was applied during 4 min of continuous challenge during which participants maintained tidal breathing. During these 4 min, the participants were asked to rate the urge-to-cough every twenty seconds. If suppression was not feasible during the 4-min inhalation then the dose was further reduced and the trial repeated.

Brain Imaging Session

Image acquisition

Brain images were obtained at the Murdoch Children's Research Institute, Melbourne, Australia using a 3-T MRI scanner (Trio, Siemens Medical Systems, Erlangen, Ger-

many) and 32-channel head coil. Anatomical images were acquired with a MPRAGE sequence (repetition time (TR) = 1,900 ms, echo time (TE) = 2.55 ms, flip angle = 9°, slice thickness = 0.9 mm, inplane resolution 0.94×0.94 mm). Echoplanar images were acquired to identify capsaicin activation (TR = 2,000 ms, TE = 32 ms, flip angle = 90°, slice thickness = 4.5 mm, inplane resolution 3.3×3.3 mm, 3 runs of 246 volumes per run). Echoplanar images were also obtained for functional connectivity analysis (TR = 1,600 ms, TE = 32 ms, flip angle = 90°, slice thickness = 5.5 mm, inplane resolution 3.3×3.3 mm, 2 runs of 150 volumes per run).

Imaging experimental protocol

Capsaicin and saline solutions were nebulized and delivered to participants using MR compatible equipment as previously described. Separate nebulizers connected by independent hoses to the facemask were used for saline and capsaicin doses. The capsaicin activation scans involved 18 s blocks of either saline or capsaicin in random order interleaved with 42 s no-stimulus periods. Projected cues visible through a mirror mounted on the head coil alerted participants to the onset of stimuli and prompted ratings after stimulus offset. They were instructed to breathe at a normal rate and depth throughout the stimulus periods. Participants were instructed to show the number of fingers corresponding to their urge-to-cough using the 0–10 scale when prompted to rate their experiences at the conclusion of each stimulus period. The functional connectivity component of the study involved two scanning runs each of 4 min duration. In one scanning run participants were continuously challenged with nebulized saline and in another scanning run they were continuously challenged with nebulized capsaicin. The order of saline and capsaicin runs was randomized between participants. Ratings of urge-to-cough were not collected during the acquisition of images for the functional connectivity component of the study.

Analysis

Behavior

Geometric means were calculated for the capsaicin doses required for the C2 threshold, the stimuli used during capsaicin activation scans, and the levels used during continuous inhalation of capsaicin. Descriptive statistics (means, standard deviations) were also calculated for urge-to-cough ratings collected during challenge with C2 doses, capsaicin activation scans, and continuous inhalation of capsaicin in the psychophysical session. Paired *t* tests were used to test for differences between urge-to-cough ratings and the logarithms of doses. Sequential measures of urge-to-cough ratings during continuous inhalation of capsaicin in the psychophysical session were tested for time effects with repeated measures ANOVA. Variance across time in

urge-to-cough ratings during continuous inhalation for individual participants was calculated with coefficients of variation using the means and standard deviations of the 12 sequential measures.

Imaging

Capsaicin activation. The purpose of the capsaicin activation study was to identify regions in the urge-to-cough network. FSL 4.1.4 (<http://www.fmrib.ox.uk/fsl>, Oxford, UK) [Jenkinson et al., 2012] was used for preprocessing including motion correction, removal of nonbrain voxels, high-pass filtering, spatial smoothing with a full-width half maximum kernel of 6 mm, and prewhitening [Woolrich et al., 2001]. FLIRT was used to calculate matrices for the transformation of mean functional images to the MNI standard template brain [Jenkinson and Smith, 2001; Jenkinson et al., 2002]. General linear models incorporated regressors for saline and capsaicin inhalation events, visual cues, and urge-to-cough rating events [Woolrich et al., 2009]. Contrasts of the parameter estimates for capsaicin and saline events were used to identify capsaicin-inhalation activations. These contrasts of parameter estimates were modeled as fixed effects to amalgamate scanning runs for each participant and the outcomes were then modeled as mixed effects to determine group activations. Voxels in the activation map were included when $z > 2.3$ and cluster corrected significance was $P < 0.05$ [Worsley et al., 1992].

Regions of Interest

Regions of interest (ROI) were used to acquire seeds for subsequent functional connectivity analyses. The objective was to identify one region each from three putative subnetworks activated during capsaicin inhalation. Identification of ROI was based on complementary information including outcomes of previous reports by our group of capsaicin-inhalation activations, the loci of capsaicin-inhalation activations for the cohort used in this study, and anatomical labels from the Harvard-Oxford Cortical and Subcortical Structural Atlas [Desikan et al., 2006] and the Talairach Daemon [Lancaster et al., 1997, 2000].

The first stage in the process of ROI definition was to examine the outcomes of previous studies to identify candidate regions that had empirical support for a functional role in either capsaicin-related stimulus-intensity coding, or urge-to-cough perceptual-intensity coding, or cough suppression. The left mid insula was chosen as a representative region for intensity-dependent activation. The insula in both hemispheres has previously shown high levels of graduated activation in response to two levels of stimulation [Farrell et al., 2012], but the left mid insula was chosen because it was distinct from right-sided anterior activation seen during cough suppression [Mazzone et al., 2011], and perception-related activation in the posterior insula [Farrell et al., 2012]. The region of interest chosen to seed the perception-related functional connectivity analysis

was the primary somatosensory cortex. Activations in the most ventral parts of SI in both hemispheres have shown very high levels of correlation with participants' ratings of urge-to-cough after excluding variance explained by stimulus attributes [Farrell et al., 2012]. The ventral portion of the opercula segment of the right inferior frontal gyrus was the region nominated to seed functional connectivity analysis of the cough-suppression network. This part of the inferior frontal gyrus has previously shown an interaction between cough behavior and capsaicin inhalation that was consistent with active suppression [Mazzone et al., 2011], and the same region is consistently activated by experimental paradigms involving other types of response inhibition [Steele et al., 2013] (see Fig. 1 for graphical representations of rationales for seed figures).

The second stage in the definition of seeds for functional connectivity involved examination of the capsaicin-inhalation statistical parametric map generated for the study cohort. Each of the three anatomical regions was searched for the voxel with the peak level in clusters of capsaicin-inhalation activation ($z > 2.3$, $P_{\text{corrected}} < 0.05$). The search of the ventral SI cortices involved both hemispheres and located the peak in the right hemisphere. The peak identified in each of the three target regions was used to localize the core of seeds for the functional connectivity analyses. The peak and neighboring voxels in the candidate regions constituted the seed in the space of the MNI standard brain (27 voxels, 1,617 mm³, see Fig. 1 for location of seeds). The inverse of transformation matrices generated from the registrations of the mean images of the motion corrected functional connectivity data sets to the standard brain were used to warp the seed regions to the native space of the participants' images. The mean time series from the seeds were subsequently extracted for all capsaicin and saline inhalation functional connectivity scanning runs.

Functional Connectivity

Functional connectivity data were preprocessed using the software and techniques previously described for the capsaicin-inhalation activation analysis. General linear modeling of the capsaicin and saline functional connectivity data included the time series of the three seeds as regressors. Nine additional regressors were added to take account of physiological and movement-related noise. The movement parameters for translations and rotations constituted six of the noise regressors. Standard deviation images of the time series of functional data sets were calculated and thresholded to identify the noisiest voxel for each run. Values extracted from the noisiest voxels were also included as noise regressors. The final two noise regressors consisted of time series of mean voxel intensities from regions in the white matter and ventricles.

Contrasts of parameter estimates were calculated for the correlates of each of the three seeds for capsaicin and

Figure 1.

A. Regional brain responses have been implicated in the suppression of cough. These regions include the (1) right prefrontal cortex, (2) right inferior frontal gyrus, (3) right anterior insula, (4) mid cingulate/ventromedial prefrontal cortices, and the (5) supplementary motor area. The right inferior frontal gyrus (arrowed) was chosen as the seed to investigate the functional connectivity of this network. **B.** Previously published work has shown that blood oxygen level-dependent (BOLD) signals from regions in the suppression network are increased during the combined contingencies of capsaicin inhalation and instructions to avoid coughing. **C.** The location of the seed for the right inferior frontal gyrus (IFG_R) was based on activation associated with the inhalation of a low dose of capsaicin. The peak voxel in the IFG_R was located and the signal from this voxel and the neighboring voxels were averaged to create the seed regressor. **D.** Some brain regions show activation levels that code stimulus intensity. Brain regions showing intensity-dependent activation include the (6) bilateral insula cortices, (7) bilateral premotor cortices, and the (8) mid cingulate cortex. The left insula cortex

was chosen as a seed to perform functional connectivity analyses of the intensity-dependent network. **E.** A previous experiment involving inhalation of saline and two doses of capsaicin (low and high) showed that intensity-dependent regions have graduated BOLD signal responses. **F.** The peak of low capsaicin activation and neighboring voxels in the left insula were used to generate a seed to investigate functional connectivity of intensity-dependent brain regions. **G.** Subjective ratings of the urge-to-cough correlate with regional brain responses in the (9) primary and (10) secondary somatosensory cortices as well as the (11) mid cingulate cortex. **H.** A previous analysis has shown that brain regions representing the urge-to-cough show correlated BOLD signal changes that are independent of any variance explained by dose levels of inhaled capsaicin. **I.** Inspection of low capsaicin activations showed that the peak voxel in the somatosensory cortices was in the right hemisphere. This peak and neighboring voxels were used as the seed to perform functional connectivity analyses.

saline runs. In addition, contrasts were made between the parameter estimates of each seed with the other two seeds. Group effects were tested in a two-stage process. In the first stage the functional connectivity with each seed during capsaicin runs was contrasted with the paired functional connectivity for the same seed during saline runs using a mixed effects analysis. Activations for the first

stage had a voxel inclusion of $z > 2.3$ and a cluster corrected threshold of $P < 0.05$. These capsaicin-related maps of functional connectivity were subsequently used as masks in the second stage of the group analysis, which involved mixed effects analysis of the contrasts of parameter estimates of single seeds versus the remaining two seeds. The resulting statistical parametric maps were

thresholded using a voxel inclusion $z > 2.3$ and corrected clusters of $P < 0.05$. Thus, the final activation maps for any given seed represented brain regions that had levels of functional connectivity with the seed during capsaicin inhalation that was significantly greater than connectivity with the remaining seeds, and only included voxels that had significantly greater functional connectivity during capsaicin inhalation compared to saline inhalation. The analysis pathway for functional connectivity is summarized in Fig. 2.

RESULTS

Behavior

The geometric mean dose of participants' C2 thresholds was $7.5 \mu\text{M}$, which was significantly greater than the geometric mean of low doses [$1.62 \mu\text{M}$, $t(16) = 10.0$, $P < 0.001$] used during image acquisition for the activation study, which in turn was significantly greater than the geometric mean of doses delivered during constant inhalation ($0.68 \mu\text{M}$, $t(16) = 3.8$, $P < 0.001$) (Fig. 3A). Ratings of urge-to-cough during single breaths of C2 doses (4.3 ± 1.3) were significantly greater than the average ratings of the constant dose during prolonged inhalation for 4 min in the psychophysical session [2.6 ± 1.3 , $t(16) = 3.3$, $P < 0.005$]. Ratings of urge-to-cough in response to low doses during functional brain imaging (3.6 ± 2.2) did not differ significantly compared to the other two doses [constant $t(16)=1.8$, n.s., C2 $t(16) = 1.3$, n.s.] (Fig. 3B). The repeated measures of urge-to-cough during continuous inhalation in the psychophysical session did not demonstrate a significant pattern across time [$F(11,176) = 1.1$, n.s.] (see Fig. 3C). Regardless of the lack of systematic time effects, there was considerable variability in urge-to-cough ratings within participants during continuous inhalation, with average coefficients of variation of 74.9%, and average ranges of least to most intense urge-to-cough of 4.1 ± 2.0 .

Low Dose Activation and Seed Locations

Low doses of capsaicin inhaled during 18 s periods of tidal breathing was associated with activation in a widely distributed network including prefrontal, limbic, paralimbic, premotor, motor, thalamic, midbrain, and cerebellar regions (see Table I for details). The peaks of activation used for the definition of seeds were located at $x = 36$, $y = 24$, $z = 10$ for the right inferior frontal gyrus (Fig. 2C), at $x = -34$, $y = 10$, $z = 2$ for the left mid insula (Fig. 2F) and at $x = 62$, $y = -12$, $z = 20$ for the right primary somatosensory cortex (Fig. 2I).

Functional Connectivity

Right inferior frontal gyrus

The right inferior frontal gyrus (BA47) showed levels of functional connectivity greater than the other two seeds

during capsaicin inhalation in regions including neighboring subdivisions of the gyrus (BA44 and BA45), right anterior insula, bilateral ventromedial cortex (medial frontal gyri, BA8), bilateral superior frontal gyrus (BA10), premotor cortex (BA6), orbital areas of the middle frontal gyrus (BA11) in both hemispheres, and a more dorsal cluster in the later gyrus in the right hemisphere (BA9) (Table II and Fig. 4).

Left insula

The seed in the left mid insula showed levels of functional connectivity that was greater than connectivity with the other two seeds in the corresponding part of the insula in the contralateral hemisphere, bilateral posterior insula, bilateral precentral gyri (BA6), left inferior frontal gyrus (BA47), left superior frontal gyrus (BA9), left middle frontal gyrus (BA46), and bilateral putamen (Table III and Fig. 5).

Right primary somatosensory cortex

Functional connectivity with the seed in the right primary somatosensory cortex during capsaicin inhalation was greater than levels for the other two seeds in regions including a more dorsal location in the post central gyrus at the base on the central sulcus in both hemispheres, bilaterally in BA43, bilaterally in the operculum, bilaterally in the precentral gyrus adjacent to BA43 and more dorsally in the left hemisphere (BA4), in the right inferior frontal gyrus, and the left inferior parietal lobule (Table IV and Fig. 6).

DISCUSSION

Airways irritation and the urge-to-cough give rise to complex behaviors that encompass sensory discriminative processes, motor control of coughing, and the related cognitive and affective outcomes. The brain network responding to capsaicin-inhalation incorporates subnetworks that are involved in multiple functional processes. The results of functional connectivity analyses between previously reported regions identified three subnetworks with key loci in the mid insula, primary somatosensory cortex and inferior frontal gyrus. The subnetworks recapitulate the regions that have previously shown activation responses consistent with capsaicin inhalation intensity coding, perception of urge-to-cough and cough suppression [Farrell et al., 2012; Mazzone et al., 2011].

Behavioral Consequences of Capsaicin Inhalation

Discrete subnetworks in the brain are more likely to show correlated signals with patterns that distinguish one subnetwork from another if the functions they subserve vary independently. Measures made in the psychophysical

Figure 2.

A. Signals in seeds were extracted from sequential BOLD contrast images acquired during the continuous inhalation of capsaicin (Capsaicin 4D) and saline (Saline 4D). The seed represented by the red square (Capsaicin 4D) and the blue square (Saline 4D) is in the right inferior frontal gyrus in this example. A region of interest (ROI) in the middle frontal gyrus (BA10) is approximated with a black square. **B.** Voxel-wise analyses were undertaken to identify regions where signal changes were correlated with seeds. The upper panel shows time series of the right inferior frontal gyrus seed (IFG_R_Seed) and an ROI in the right middle frontal gyrus (ROI_Cap), for illustrative purposes, from a single participant inhaling capsaicin. The lower panel shows time series from the same participant for the seed (IFG_R_Seed) and ROI (ROI_Sal) during inhalation of saline. **C.** The outcomes of individual participants' voxel-wise analyses were tested for group effects for the inhalation of capsaicin (i), saline (ii), and the contrast of capsaicin greater than saline (iii). The group effects shown in this panel are from the analyses involving the right inferior frontal gyrus. Similar analyses were also performed for the other two seeds: the left insula and the right primary somatosensory cortex. The regions activated for the contrast of capsaicin greater than saline were used as masks in later stages of the analyses. **D.** A voxel-wise analysis for each participant was undertaken using BOLD images acquired during capsaicin inhalation (Capsaicin 4D) and regressors based on signals extracted from the three seeds: the right inferior frontal gyrus (approximate area—red oval), left insula (approximate area—red square) and right primary somatosensory cortex (approximate area—red cross). **E.** The inclusion of three seed regressors in the modeling of individual participants' BOLD signal

changes were used in voxel-wise analyses to test for differences in correlation levels between the seeds. Paired time points for an individual participant's BOLD signals from a ROI (right middle frontal gyrus, approximate area—black square in panel D) and each of the three seeds (IFG_R, Insula_L, SI_R) are plotted in a scattergram to illustrate differential levels of correlation between a region and the seed regressors. In this example the seed in the IFG_R explained a greater level of variance than the other two seeds. **F.** Correlates of a single regressor were contrasted with the variance predicted by the remaining two regressors. The graph in this panel shows the distribution of variances in an ROI (right middle frontal gyrus) explained by the seeds from the right inferior frontal gyrus (IFG_R), the left insula (Insula_L) and the right primary somatosensory cortex (SI_R). Each connected series of dots represents the data from a participant. The majority of participants had higher estimates of variance explained by the IFG_R than the other two seeds. **G.** Voxel-wise contrasts between single seeds versus the remaining two seeds were tested for group effects using masks derived from analyses that had involved a contrast of functional connectivity for capsaicin greater than saline. The voxels shaded in green in this panel showed activation for the contrasting levels of correlation with the right inferior frontal gyrus seed between capsaicin and saline scans. The contrast of functional connectivity of the right inferior frontal gyrus seed greater than the other two seeds was confined to those voxels that also showed greater correlation with the right inferior frontal gyrus seed during capsaicin inhalation compared to saline inhalation, and is rendered in red/yellow on top of the mask voxels. An arrow indicates the ROI in the right middle frontal gyrus used in previous panels.

Figure 3.

A. Participants inhaled doubling doses of capsaicin until two coughs were elicited; the C2 threshold. The column of filled circles on the right of the graph shows the distribution of participants' C2 doses. The middle column shows the doses that participants inhaled for 18 seconds at a time during the acquisition of functional brain images, which were subsequently used to show regional brain activation associated with a *Low* dose of capsaicin. The column of filled circles on the left of the graph shows doses of capsaicin inhaled continuously for four minutes (Constant) during the acquisition of functional brain images, which were subsequently used to calculate functional connectivity. The geometric means of each of the three distributions are indicated by open triangles. **B.** Participants rated their urge-to-

cough every 20 s during 4 min of continuous inhalation of capsaicin (Constant) in a psychophysical session before scanning, and the average of the repeated ratings for each participant are shown in the column on the left. The middle column represents the average urge-to-cough ratings of participants that were recorded after 18-s inhalations of low doses of capsaicin during functional brain imaging. Urge-to-cough ratings of C2 doses for each participant are shown in the column on the right. Averages of the three distributions are represented by open triangles. **C.** The average urge-to-cough ratings at 20-s intervals during continuous inhalation of capsaicin in the psychophysical session did not show significant time effects. $*p < 0.01$, note—paired *t* tests of differences in doses after conversion to logarithms.

session and evidence from additional sources suggest that stimulus intensity coding, cough suppression, and the urge-to-cough have dissociable relationships with airway

stimulation. Firstly, it should be noted that while the dose of capsaicin was held constant during image acquisition, the intensity of stimulation would be expected to vary

TABLE I. Low capsaicin greater than saline

Region	BA	Side	MNI coordinates			z score
			x	y	z	
Medial frontal gyrus	6	Right	8	10	52	3.76
Middle frontal gyrus	10	Right	36	50	14	3.43
	46	Left	-40	48	22	3.46
Inferior frontal gyrus	47	Right	36	24	-10	^a 3.88
Inferior parietal lobule	40	Right	50	-34	42	3.75
Mid-cingulate cortex	24	Right	10	8	44	3.90
	24	Right	8	20	26	4.56
	24	Left	-10	16	38	4.24
Supplementary motor area	6	Left	-2	4	64	3.35
Precentral gyrus	6	Right	56	6	28	3.31
	6	Left	-60	4	26	3.54
Post central gyrus	43	Right	62	-12	20	^a 3.80
	43	Left	-56	-8	16	3.67
Pre/post central gyrus	3/4	Right	40	-14	30	4.15
	3/4	Left	-40	-18	30	3.46
Central operculum		Right	60	0	8	4.82
		Left	-56	-4	6	4.79
Anterior insula	13	Right	30	16	2	4.56
	13	Left	-32	18	2	4.41
Mid insula	13	Left	-34	10	2	^a 4.59
Globus pallidus		Right	14	2	0	3.93
		Left	-18	-4	4	3.84
Thalamus—ventral lateral		Right	14	-12	0	3.27
		Left	-10	-10	2	3.95
Midbrain—dorsal		Right	4	-26	-6	3.16
		Left	-2	-26	-6	3.11
Midbrain—ventral		Right	12	-18	-6	3.46
		Left	-12	-20	-4	3.62
Medulla			0	-38	-40	3.02
Cerebellum		Right	14	-62	-22	3.47
		Left	-22	-68	-26	3.59

^aCluster loci used to define seed regions for functional connectivity analyses.

subtly in accordance with changes in the flow rate and direction of nebulized air throughout the respiratory cycle. For instance, respiratory rate would naturally vary during the 4 min that participants were inhaling capsaicin leading to differences in the rate that nebulized particles would pass across the airways. Furthermore, the amount of capsaicin in contact with the airways was likely to differ between inhalation and exhalation within a single breath. This variation in the way the stimulus interacts with the airways would produce a dynamic pattern of intensity levels. It is likely that this input would contribute, in part, to variation in a number of functional processes. However, behavioral measures herein and reports elsewhere suggest that stimulus attributes are not solely responsible for variability in behavioral responses to capsaicin inhalation.

In this study and others, ratings of urge-to-cough show substantial variability that is not explained by stimulus intensity [Dicpinigaitis et al., 2012; Davenport et al., 2002]. In a previous report, we have shown that participants' ratings of urge-to-cough at a constant capsaicin dose are vari-

able, and that ratings at two different doses can be the inverse of what would be expected [Farrell et al., 2012]. In this study participants reported highly variable levels of urge-to-cough during continuous inhalation that occurred despite the delivery of a constant dose of capsaicin (i.e., average coefficients of variation of 74.9%). This level of variability is much greater than might be expected as a consequence of minor variations in respiratory parameters during inhalation and exhalation. Thus, the behavioral evidence would suggest that urge-to-cough and any associated regional brain responses are likely to show considerable variability during the course of constant inhalation of a single dose of capsaicin.

Suppression of cough can be remarkably variable depending on participants' conscious efforts to hold cough at bay [Hegland et al., 2012; Hutchings et al., 1993], and the likelihood of coughing cannot reliably be predicted from ratings of urge-to-cough [Dicpinigaitis et al., 2012; Farrell et al., 2012]. Data from this study provides additional evidence of a complex relationship between tussive

TABLE II. Functional connectivity right IFG > right SI and left insula and capsaicin > saline

Region	BA	Side	MNI coordinates			z score
			x	y	z	
Medial frontal gyrus	8	Right	2	26	42	5.25
	8	Left	-2	32	36	4.91
Superior frontal gyrus	6	Right	16	22	54	3.29
	10	Right	30	50	-4	4.09
	10	Left	-28	56	-4	3.98
Inferior frontal gyrus	45	Right	50	24	16	3.83
	44	Right	54	24	8	3.76
	47	Right	40	24	-10	7.63
Middle frontal gyrus	9	Right	44	28	30	3.09
	11	Right	38	48	-10	4.81
	11	Left	-38	50	-12	3.42
Anterior insula	13	Right	36	22	-4	5.15

stimulation and cough events, in that the doses used to ensure a cough-free period of 4 min during continuous inhalation of capsaicin were well below the C2 threshold, and below doses used to achieve a low level of urge-to-cough during 18 s of inhalation. The absence of systematic time effects on urge-to-cough ratings during continuous inhalation of capsaicin would argue against temporal summation effects in the subjective experience, and consequently this is unlikely to explain the lower doses required for successful suppression during prolonged inhalation. Instead, it would appear that the duration of capsaicin inhalation interacts with efforts to suppress cough in ways that remain latent, but are unlikely to be simply explained by stimulus attributes and concurrent feelings of urge-to-cough.

Collectively, the behavioral evidence points to dissociable temporal patterns in stimulus intensity coding, urge-to-cough, and cough suppression during continuous inhalation of capsaicin that facilitated the identification of subnetworks using functional connectivity analyses.

Multiple Discrete Functional Networks Activated by Capsaicin Inhalation

The conjunction of two tests of differential functional connectivity was used to characterize the correlates of seed regions: capsaicin inhalation greater than saline inhalation, and seed region greater than other seed regions during capsaicin inhalation. The demonstration of increased levels of correlation with a seed between capsaicin and saline inhalation constituted a preliminary test to implicate shared variance in signal change with responses to noxious airway stimulation. Subsequent contrasts of functional connectivity between the three seeds provided support for subnetworks within the broader capsaicin-inhalation network. This final test of between-region differences is predicated on, and supports the notion that the activation patterns of the seeds are

contingent on dissociable responses among the multiple behaviors occurring during sustained inhalation and exhalation of a single dose of capsaicin. However, it should be noted that the demonstration of unshared variance between the subnetworks does not preclude common variance among the constituent brain regions, which would likely reflect common drivers of responses, or other forms of integration.

Functional correlates of the right inferior frontal gyrus during capsaicin inhalation incorporated regions that have previously been implicated in cough suppression [Mazzone et al., 2011] including the anterior insula, medial frontal gyrus, superior frontal gyrus and additional parts of the inferior frontal gyrus extending beyond the location of the seed. The network seeded from the inferior frontal gyrus in this study is also very similar to the regions implicated in response suppression more generally, notably operationalized in go/no-go experimental paradigms [Steele et al., 2013]. The initiation of coughing subsequent to airway irritation occurs as a reflex mediated by brainstem regions in the absence of countervailing inputs from the forebrain, which are likely to be recruited as a discretionary response to a perceptible urge-to-cough. As such, the urge-to-cough constitutes an interoceptive trigger for an action that is subsequently suppressed, as opposed to the exteroceptive cues that typically characterize go/no-go paradigms. The regions showing functional connectivity with the inferior frontal gyrus could represent shared functional roles in the suppression of cough throughout the prolonged period of capsaicin inhalation. Importantly, the shared variance among the components of the inferior frontal gyrus network is distinct from the variance of other regions that typically activate during capsaicin inhalation, suggesting a dissociable functional role for the network in the context of a multidimensional response to airway irritation.

Widely distributed brain regions show stimulus intensity-dependent activation during inhalation of varied doses of capsaicin [Farrell et al., 2012]. The bulk of these regions have graduated levels of activation to low and

Figure 4.

Regions showing greater levels of functional connectivity with the right inferior frontal gyrus during capsaicin inhalation compared to saline inhalation are shown in green. Analyses of functional connectivity with the right inferior frontal gyrus greater than the other two seeds was confined to voxels showing capsaicin > saline functional connectivity, and are rendered on top of the green voxels using red/yellow. **A.** Functional connectivity in the region neighboring the seed in the inferior frontal gyrus (IFG) (BA47) showed levels of functional connectivity that were greater than levels during saline inhalation and with the other seeds during capsaicin inhalation. Capsaicin-related functional connectivity was also increased in the middle frontal gyrus (MidFG) bilaterally (BA11). Other regions showing increased

functional connectivity with the right inferior frontal gyrus during capsaicin inhalation included, **B.** the right anterior insula (AI), **C.** the right inferior frontal gyrus (IFG)(BA44), **D.** the medial frontal gyrus (MedFG) bilaterally, the right middle frontal gyrus (MidFG)(BA9), and **E.** the right superior frontal gyrus (SFG). **F.** Increased capsaicin-related functional connectivity between the right inferior frontal gyrus and the insula was confined to the anterior (AI) and inferior parts of the insula in the right hemisphere. **G.** Functional connectivity with the right inferior frontal gyrus during capsaicin inhalation was seen in the medial frontal gyrus (MedFG) but was not present in the supplementary motor area.

TABLE III. Functional connectivity left insula > right SI and right IFG & capsaicin > saline

Region	BA	Side	MNI coordinates			z score
			x	y	z	
Superior frontal gyrus	9	Left	-32	42	30	4.10
Middle frontal gyrus	46	Left	-38	44	22	3.82
Premotor cortex	6	Left	-56	6	14	4.18
Central (rolandic) operculum		Left	-56	-2	6	4.13
Inferior frontal gyrus	47	Left	-36	32	0	4.30
	47	Left	-42	26	-12	3.43
Mid insula	13	Right	38	12	4	4.72
Posterior insula	13	Right	38	-16	6	3.32
	13	Left	-34	-24	4	3.37
Putamen		Right	24	8	4	4.16
		Left	-26	-10	4	4.94

Figure 5.

Functional connectivity with a seed in the left mid insula is shown in regions that were activated for the contrast capsaicin > saline (green) and insula seed > other seeds during capsaicin inhalation (red/yellow). Functional connectivity with the left insula that was greater than levels of connectivity with the other seeds included. **A.** The left inferior frontal gyrus (IFG), **B.** bilateral posterior insula (Pins), mid insula (MIns), and putamen

(PUT), **C.** The left middle frontal gyrus (MFG) and left precentral gyrus (PreCG), **D.** and the superior frontal gyrus (SFG). **E.** Functional connectivity activation in the right insula was in the superior part of the cortex and extended from the mid (MIns) to posterior regions. **F.** The left insula showed increased functional connectivity in proximity to the seed and extended to both the anterior (AIns) and poster regions of the cortex.

TABLE IV. Functional connectivity right SI > left insula and right IFG and capsaicin > saline

Region	BA	Side	MNI coordinates			z score
			x	y	z	
Post central gyrus	3	Right	42	-18	40	5.18
	3	Left	-36	-22	36	3.58
	3	Right	56	-8	32	5.61
	43	Right	54	-10	20	6.47
	43	Left	-58	-12	20	4.79
Central operculum		Right	46	-14	22	7.67
		Left	46	-10	22	4.31
Precentral gyrus	4	Left	-38	-18	40	3.90
	4	Left	-62	-4	24	4.93
Premotor cortex	6	Right	58	0	22	4.95
	6	Right	56	10	22	3.48
Inferior parietal lobule	40	Left	-38	-30	38	3.19

high doses of capsaicin, whereas a smaller number of regions such as the supplementary motor area, adjacent superior frontal gyrus and cerebellum activate exclusively at high doses of capsaicin. We have speculated that these differential regional responses encode unique aspects of sensory discrimination [Farrell et al., 2012]. This study

showed a network of regions functionally connected with the left anterior insula during capsaicin inhalation that largely replicates those regions that have previously demonstrated graduated responses to different doses of capsaicin, and includes the right anterior/mid insula, operculum, premotor cortex, putamen, and prefrontal regions including the superior and inferior frontal gyri. Additionally, the posterior insulae bilaterally showed functional connectivity with the left anterior insula, whereas our previous study had implicated this region in the perception of urge-to-cough. The close match between the regions in the network seeded from the insula and those regions previously identified as capsaicin intensity-dependent suggests that a coordinated, distributed brain response codes inputs from more caudal regions in receipt of primary afferent inputs from the airways during continuous inhalation of capsaicin.

Cortices at the ventral extent of the post central gyrus and contiguous regions of the central operculum showed capsaicin inhalation-related functional connectivity with the seed in the right primary somatosensory cortex. Observations made during brain surgery have implicated the ventral part of the central gyrus, including Brodmann Area 43 in sensations of the mouth and throat [Penfield and Boldrey, 1937], which suggests that this region of the somatosensory cortex is involved in the processing of afferent inputs from airway regions from which the urge-to-cough can be generated (i.e., the pharynx and larynx). Lesion studies and fMRI activation studies would suggest that the rolandic extensions of the primary cortices into the operculum are also involved in sensorimotor processing in the mouth and throat region [Soros et al., 2009]. Other regions to show functional connectivity with the

Figure 6.

Figure 6.

Regions showing functional connectivity with a seed in the right primary somatosensory cortex during capsaicin greater saline inhalation are represented in green in this figure. Levels of functional connectivity with the primary somatosensory cortex that were greater than levels with the other two seeds are represent in red/yellow. **A.** The seed was located near the ventral end of the right post central gyrus in BA43 (SI), and functional connectivity with the seed during capsaicin inhalation in this region exceed levels during saline inhalation as well as exceeding levels of connectivity with the other seeds during capsaicin inhalation. **B.** The corresponding region of BA43 (SI) in the left hemisphere also showed functional connectivity with the seed. **C.** Functional connectivity in the primary somatosensory cortex (SI) extended bilaterally into a region deep in the sulcus that corresponds with the loci of activation during intermittent inhalation of capsaicin. The cluster in the left hemisphere extended in the posterior parietal cortex to include a region of the inferior parietal lobule (IPL)(BA40). **D.** Opercular regions (CO) in both hemispheres also showed increased levels of functional connectivity with the seed.

seed in the right primary somatosensory cortex were the left posterior parietal cortex, left primary motor cortex, and right premotor cortex. Collectively, the regions showing functional connectivity with the seed in the right primary somatosensory cortex are compatible with a role in the perception of an urge-to-cough. Stimulation of the airways with capsaicin evokes feelings of itch or scratch or burning that is localized to the pharyngeal and laryngeal airways and is accompanied by a sense that cough is imminent or needed [Davenport et al., 2002; Farrell et al., 2012]. It is very likely that these sensations and impulses are represented in somatosensory and motor regions in the locations that show correlated activation during continuous capsaicin inhalation.

Capsaicin Regional Responses not Implicated in Functional Subnetworks

It is notable that the mid cingulate cortex did not show seed-specific capsaicin-related functional connectivity in any of the three networks identified in this study, yet outcomes from our present and previous activation studies have implicated the mid cingulate cortex in all three of the functional processes that motivated the choice of seeds [Farrell et al., 2012; Mazzone et al., 2008, 2011]. We have previously speculated that the mid cingulate cortex is likely to play an integrative role in the processing of inputs from noxious stimulation of the airways [Farrell et al., 2012], and this putative role may have impacted on the functional connectivity of the region. If the mid cingulate cortex activates in coordination with each of the networks described in this study then it is unlikely that levels of correlation with any single seed would exceed levels for the other seeds, a requirement of the analytical approach employed to identify dissociable subnetworks. Further analyses would be required to assess the extent of regions that correlate with the mid cingulate cortex during the inhalation of capsaicin.

Several other regions in the broader capsaicin inhalation network that were seen for the contrast of low capsaicin greater than saline were not identified in the three seeded networks. The majority of regions seen in the capsaicin contrast and absent in the functional connectivity networks were in subcortical structures including the globus pallidus, ventrolateral thalamus, midbrain, medulla, and cerebellum. The absence of these structures in the subnetworks is not unexpected, given that the regions have not been implicated in the functional processes that motivated seed choices. Nevertheless, it does raise the possibility that regional differences in hemodynamic responses might have reduced shared variance between cortical and subcortical structures. However, in the one instance where a subcortical region had previously shown a functional profile compatible with a seed, namely graduated responses to capsaicin doses in the putamen [Farrell et al., 2012], the structure did indeed show functional con-

nectivity with the salient seed in the left anterior insula. An alternative explanation for the relative lack of subcortical regions in the functional networks is that capsaicin activations in these regions are involved in process unrelated to the functions that motivated seed choices for the functional connectivity analyses. The location of many of these regions corresponds with reports of activations in association with breathing control [McKay et al., 2010], and it could be that capsaicin inhalation leads to adjustments in breathing pattern that were reflected in the subcortical activations seen for the contrast of low capsaicin greater than saline. Measures of respiration were not recorded during image acquisition in this study and consequently it was not possible to test the proposition that breathing patterns were systematically influenced by capsaicin inhalation. However, previously reported observations from our group showed that the respiratory rate did not differ significantly between saline and capsaicin challenges during stimulus periods of 18 s, although the reported measures did not assess the depth of breathing [Farrell et al., 2012]. Future studies will be required to assess how inhalation of a tussive substance interacts with breathing control and if these putative effects are associated with regional brain responses.

CONCLUSIONS

The distributed network of regional brain activations responding to inhalation of capsaicin incorporates at least three dissociable subnetworks. These subnetworks are likely to be engaged in mutual processes related to functional components of capsaicin inhalation including sensory discrimination, motor control of coughing and related cognitive and affective outcomes. One subnetwork, seeded from the inferior frontal gyrus includes regions implicated in cough control and response suppression more generally. A second subnetwork is constituted of paralimbic, premotor, prefrontal, and subcortical regions that have previously been implicated in intensity coding of inputs from the airways. The third subnetwork so far identified includes somatosensory and posterior parietal cortices that are likely to represent the perception of urge-to-cough. Collectively, these networks represent coordinated brain responses that govern many of the behavioral responses to challenge of the airways.

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