Altered neural activity in brain cough suppression networks in cigarette smokers

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Smokers are less sensitive to inhaled cough-evoking stimuli due to increased activity in brain circuits that inhibit coughing. Smoking history influences the nature of the inhibitory process engaged to reduce sensitivity to cough stimuli. http://bit.ly/2ZBTKlo

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ABSTRACT Cough is important for airway defence, and studies in healthy animals and humans have revealed multiple brain networks intimately involved in the perception of airway irritation, cough induction and cough suppression. Changes in cough sensitivity and/or the ability to suppress cough accompany pulmonary pathologies, suggesting a level of plasticity is possible in these central neural circuits. However, little is known about how persistent inputs from the lung might modify the brain processes regulating cough.

In the present study, we used human functional brain imaging to investigate the central neural responses that accompany an altered cough sensitivity in cigarette smokers.

In nonsmokers, inhalation of the airway irritant capsaicin induced a transient urge-to-cough associated with the activation of a distributed brain network that included sensory, prefrontal and motor cortical regions. Cigarette smokers demonstrated significantly higher thresholds for capsaicin-induced urge-to-cough, consistent with a reduced sensitivity to airway irritation. Intriguingly, this was accompanied by increased activation in brain regions known to be involved in both cough sensory processing (primary sensorimotor cortex) and cough suppression (dorsolateral prefrontal cortex and the midbrain nucleus cuneiformis). Activations in the prefrontal cortex were highest among participants with the least severe smoking behaviour, whereas those in the midbrain correlated with more severe smoking behaviour.

These outcomes suggest that smoking-induced sensitisation of central cough neural circuits is offset by concurrently enhanced central suppression. Furthermore, central suppression mechanisms may evolve with the severity of smoke exposure, changing from initial prefrontal inhibition to more primitive midbrain processes as exposure increases.

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Introduction

Cough is a neural process that plays an important role in airway defence and the maintenance of adequate ventilation. Functional brain imaging studies in humans and neuroanatomical investigations in animals provide insight into the central (brain and brainstem) sensory and motor networks involved in cough under nonpathological circumstances [1–5]. However, cough sensitivity can become altered, especially in disease states and from psychophysical (*e.g.* placebo or cognitive regulation of cough) or environmental (*e.g.* chronic exposure to airway irritants) factors [6–11]. Indeed, both enhanced and reduced cough sensitivity are possible, producing excessive or insufficient cough responses that prove challenging to control in the clinic [12].

The neural processes involved in altered cough sensitivity are poorly understood. Although altered airway peripheral sensory nerve activity and sensitivity is undoubtedly involved, aspects of the dynamic and chronic malleability of cough sensitivity is indicative that central mechanisms might also shape the sensorimotor processing of cough-related stimuli [7, 10, 13]. We recently reported alterations in the brain responses accompanying airway irritations in patients with chronic cough, demonstrating increased activity in cough sensory processing nuclei (evidence for sensitisation) accompanied by concomitant reduced activity in brain regions involved in cough suppression (indicative of reduced inhibitory control) [7]. Consistent with this, Cho *et al.* [14] recently reported that patients with chronic refractory cough display significantly reduced capacity to voluntarily suppress capsaicin-evoked coughing, while also being hypersensitive to cough-evoking stimuli. Thus, the relative balance between facilitatory and inhibitory brain processing may be central to the development or maintenance of altered cough sensitivity in different pathologies.

In the present study, we reasoned that the brain processes involved in altered cough sensitivity could be further investigated using functional brain imaging in cigarette smokers because they typically demonstrate reduced cough reflex sensitivity to inhaled challenge stimuli [13, 15, 16]. The previously reported central neural correlates of the urge-to-cough, cough induction and cough suppression mechanisms in healthy humans and in people who suffer from chronic cough provides a strong *a priori* prediction for how cigarette smoking could affect the central nervous system regions regulating cough [2–4, 7, 10]. Reductions in brain region responsivity associated with cough sensory processing, including in the sensorimotor or anterior insula cortices (important for urge-to-cough perception and stimulus intensity grading), posterior or parietal cortices (involved in spatial discrimination), or medullary and pontine neural circuitry (needed for integrating airway primary afferent inputs for reflex coughing) might be associated with reduced evoked cough sensitivity in smokers. Alternatively, changes in cough reflex thresholds observed in smokers could be due to enhanced cough suppression network activity, perhaps because exposure of the airways to smoke enlists tonic central adaptive processes to countervail the unwanted perceptions or responses to irritation. Brain regions involved in the voluntary suppression (cingulate cortex, inferior frontal gyrus, anterior insula and supplementary motor area) or involuntary suppression (dorsolateral prefrontal cortex and midbrain) of cough and the urge-to-cough could display enhanced activity in smokers [4, 10, 17].

Materials and methods

Full details are included in the supplementary material.

Participant recruitment and experimental protocol

16 active smokers without history of lung disease and 16 age- and sex-matched nonsmoking controls were recruited *via* advertisements displayed around the campus of The University of Melbourne (Melbourne, Australia) (10 males and six females, mean±SEM age 34.0±12.2 and 30.6±12.3 years, respectively). Smokers were defined as having smoked ⩾5 cigarettes per day for >1 year.

Exclusion criteria included a respiratory infection during the 8 weeks preceding the experimental session, claustrophobia, a history of respiratory or brain pathology, pregnancy, intellectual or mental impairment and use of psychotropic medication. Smoker participants abstained from cigarettes 3 h prior to the scanning session. Smokers completed the Wisconsin Smoking Withdrawal Scale to ensure that there were no significant withdrawal effects caused by the short period of smoking deprivation [18] and the Fagerström Test for Nicotine Dependence, which is a self-report measure of dependency on nicotine [19].

All participants consented to be involved in the study in compliance with procedures approved by the Melbourne Health Human Research Ethics Committee (approval 2013.262) and with the Declaration of Helsinki.

Psychophysical testing session

Sensitivity to capsaicin, the active ingredient of chilli peppers, was initially assessed using a modified functional magnetic resonance imaging (fMRI)-adapted protocol. Participants inhaled a single vital

capacity of capsaicin vapour delivered from a MRI-compatible jet nebuliser, prepared in doubling concentrations (0.06–125 µM), and rated their urge-to-cough sensation (0=no urge-to-cough to 10=maximum urge-to-cough). Cough frequency was noted after each challenge. Urge-to-cough threshold (C_U) was determined as the minimum concentration of capsaicin needed for the participant to perceive an urge-to-cough sensation. Cough threshold (C_2) was determined as the concentration of capsaicin needed to elicit two or more coughs. A further 10 stimuli (2×5 concentrations) were delivered in randomised order to generate a stimulus–response function: 1) capsaicin concentration at C_2, 2) one concentration increment below and above the C_2 ($C_{2\pm1}$) and 3) two concentration increments below and above the C_2 ($C_{2\pm2}$). Guided by these measures, the highest concentration of capsaicin that could be inhaled for 24 s (the challenge time used in fMRI) without coughing was defined as the maximum suppressible concentration (S_{max}). Participants were asked whether they experienced any other sensation during capsaicin inhalation. The $\log_{10}$ values of C_U, C_2 and S_{max} thresholds were tested for associations with pack-years in the group of smokers by calculating the Pearson r correlation coefficients between these continuous variables.

fMRI testing session

The imaging protocol included eight blocks of 24 s periods where the participants were administered saline, a low or a high concentration of capsaicin in a randomised order interspersed by 42 s periods of no stimulation (figure 1). Participants rated the level of urge-to-cough after each capsaicin challenge.

High and low concentrations of capsaicin were administered. The high concentration was the individual's S_{max} concentration, while the low concentration was tailored to be equal between matched pairs of smokers and controls. Thus, concentrations either 1) varied among the participants allowing for brain responses to be tested when participants were having a comparable behavioural experience (Like-Behaviour) or 2) allowed for comparisons of brain activity between participants during an identical stimulus intensity (Like-Stimulus).

Image acquisition parameters

Scanning was performed at the Murdoch Children's Research Institute (Melbourne, Australia) using a MAGNETOM Trio 3 T scanner (Siemens, Malvern, PA, USA) with a 32 channel head coil. Structural T1-weighted images were acquired in the sagittal plane (192 slices, 0.90 mm slice thickness, 0.84×0.84 mm^2 in-plane resolution, echo time (T_E) 2.59 ms, repetition time (T_R) 1900 ms, flip angle 9°). Blood oxygen level-dependent (BOLD) contrast echo-planar images (EPI) were acquired in the transaxial plane (36 slices, 4 mm slice thickness, 3.28×3.28 mm^2 in-plane resolution, T_E 32 ms, T_R 2000 ms, flip angle 0°), producing a total of 279 sequential volumes in 9 min 18 s of scanning time. Three EPI series were collected from all participants.

Imaging analysis

Statistical analyses of behavioural measures were performed with SPSS version 21.0 (IBM, Armonk, NY, USA). Image analysis was performed as previously described [2, 4, 7, 10] and is described in detail in the supplementary material.

Contrasts for high and low capsaicin concentrations against saline challenges were used in the analysis of group and between-group effects. Primary group effects were determined using a single voxel inclusion threshold of Z>3.09 and a cluster-wise family-wise error (FWE)-corrected threshold of $p_{corrected}<0.05$ based on the FMRIB Software Library Expert Analysis Tool (FEAT) implementation of the random field

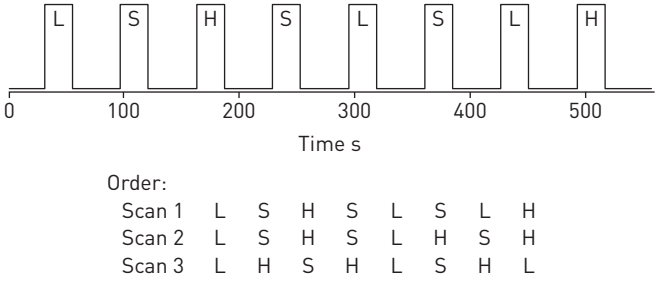

FIGURE 1 Experimental design schematic showing the timing and order of experimental challenges: each participant underwent three scanning runs (scans 1–3) with each run involving eight blocks of nebulised challenges consisting of either saline (S), a low personally relevant concentration of capsaicin (L) or a high personally relevant concentration of capsaicin (H). The order of the challenges was varied across runs, although each participant received in total eight challenges with each stimulus. Participants were informed visually immediately prior to the onset of a challenge, but were blinded as to the identity of the challenge.

theory [20, 21]. A group contrast was performed for paired concentrations between controls and smokers for the Like-Stimulus comparison. Only 15 pairs were included (not the original 16 pairs) as one smoker–control pair did not inhale matching capsaicin concentrations due to the smoker having a higher "low" concentration than the matched control's S_{max} concentration. An unpaired contrast of high concentrations for all participants was also performed to test the Like-Behaviour effect. Univariate correlation analyses were performed with demeaned measures of smoke exposure measured by pack-years (number of packs per day smoked multiplied by the number of years smoked) to identify brain region activations explained by the severity of smoking behaviour. Significant activations were determined using a single voxel inclusion threshold of Z>2.3 and a cluster-wise FWE-corrected threshold of $p_{corrected}<0.05$ [20].

Results

Behaviour

The mean±SEM score for smokers for the Fagerström Test for Nicotine Dependence was 3.2±1.6 (range 1–6), indicating a low to moderate level of dependence on nicotine. The mean±SEM total score for smokers for the Wisconsin Smoking Withdrawal Scale was 11.6±3.2 (range 6.7–16.9), indicating that smokers were neutral to all seven subscales (anger, anxiety, concentration, craving, hunger, sadness and sleep). The mean ±SEM pack-years for smokers was 6.6±6.0 (range 0.38–23).

Smokers had significantly higher thresholds for C_u, C_2 and S_{max} measures ($t(30)=2.502$, $p<0.018$; $t(30)=2.820$, $p<0.008$ and $t(30)=2.096$, $p<0.045$, respectively) (figure 2a and b). Repeated-measures ANOVA revealed a main effect for capsaicin concentration ($F(4,120)=127.534$, $p<0.001$). The groups did not differ in their urge-to-cough ratings of the personally relevant concentrations ($F(1,30)=0.286$, $p=0.597$) nor was there an interaction between group and concentration ($F(4,120)=0.556$, $p=0.695$). The number of coughs recorded during the randomised capsaicin challenges did not differ between the groups ($t(30)=1.1$, nonsignificant) (figure 2b, inset). Capsaicin concentrations required to elicit a perceptible urge-to-cough $\log_{10}(C_u)$ were positively correlated with pack-years (r=0.687, 95% CI 0.291–0.883; p<0.003) in smokers, whereas thresholds to elicit coughing ($\log_{10}(C_2)$: r=0.224, 95% CI −0.31–0.65; p=0.403) and maximum suppressible concentrations ($\log_{10}(S_{max})$: r=−0.205, 95% CI −0.32–0.64; p=0.447) were not associated with smoking behaviour. Six out of 32 participants (18.75%) reported ancillary effects (three participants had urge-to-sneeze and three participants had a runny nose), unrelated to smoking. No patients reported eye irritation.

Brain imaging

Capsaicin-induced brain activation for nonsmoking controls and smokers

The group mean %BOLD signal increase, irrespective of matched capsaicin concentration or urge-to-cough sensation comparisons, was widely distributed across brain regions in the two participant groups including the cingulate cortex, supplementary motor area, primary somatosensory and motor

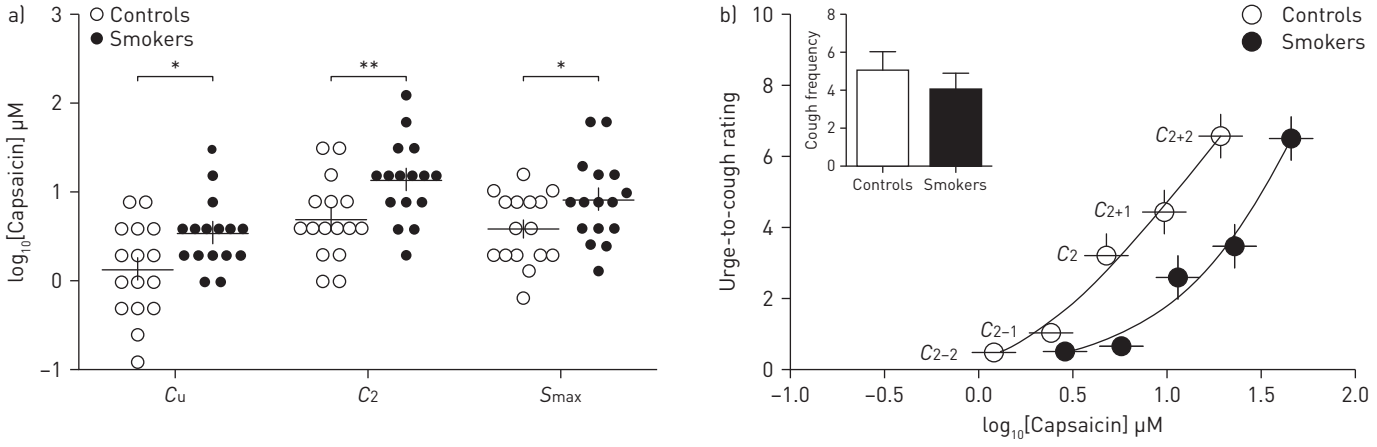

FIGURE 2 Behavioural data representing cough sensitivity measured during the psychophysical testing session. a) Participants inhaled doubling concentrations of nebulised capsaicin during single maximum inhalations. Urge-to-cough ratings (numerical rating scale of 0–10) and cough events were recorded. Thresholds were measured for detection of urge-to-cough (C_u), provocation of two coughs (C_2) and maximum suppressible concentration during 24 s of repeated inhalations (S_{max}). All three thresholds were significantly increased in smokers compared with controls. The "crosshairs" represent the geometrical mean of the threshold. *: p<0.05; **: p<0.01. b) Relationships between capsaicin concentrations and urge-to-cough ratings were assessed using repeated inhalations of five capsaicin concentrations at concentration increments less than, greater than and corresponding to participants' C_2 thresholds. Cough frequency during the challenge period was also recorded (inset). The smokers showed a rightward shift of the stimulus–response function and there were no differences in cough frequency during challenges. Lines through the symbols indicate SEM. Histograms represent the group mean±SEM.

TABLE 1 Capsaicin inhalation activation for matched capsaicin concentration in controls and smokers

Region	Controls				Smokers			
	Peak voxel coordinate			Z-score	Peak voxel coordinate			Z-score
	x	*y*	*z*		*x*	*y*	*z*	
Precuneous cortex	2	−76	48	5.54				
Anterior midcingulate cortex	−6	22	32	5.03	−2	36	22	4.60
Posterior midcingulate cortex					−4	−32	26	4.53
Supplementary motor area	10	10	56	5.01	−4	2	54	3.97
S1/M1	48	−10	34	4.20	58	2	40	5.09
Superior parietal cortex					−58	−38	36	3.88
Operculum	−56	−2	8	4.53	52	−6	8	4.90
Orbitofrontal cortex	50	22	−10	5.07	50	18	−10	3.97
Inferior frontal gyrus	48	16	−6	4.90	−56	8	0	5.29
Insula	40	22	−8	4.79	−38	−6	0	5.34
Putamen					−30	−8	6	5.43
Thalamus	2	18	8	3.84	12	−14	6	5.13
Pons					8	−32	−26	4.79
Medulla	12	−34	−50	3.74	−8	−40	−40	3.90
Cerebellum	4	−62	−8	4.92	12	−56	−30	5.14

S1/M1: primary somatosensory and motor regions.

regions (S1/M1), superior parietal cortex, frontal regions, insula, cerebellum, regions of the basal ganglia, and brainstem at a cluster-wise FWE-corrected threshold of $p_{corrected}<0.05$ (voxel inclusion of $Z>3.09$) (tables 1 and 2; activation maps in supplementary figure S1). This pattern of activation was consistent with previous capsaicin inhalation studies [2, 4, 5].

Paired comparison: matched capsaicin concentrations (Like-Stimulus)

Nonsmoking participants were more often inhaling the S_{max} concentration for the comparison of equivalent concentrations in pairs of smokers and controls ($\chi^2=6.788$; $p<0.009$). The mean±SEM ratings of

TABLE 2 Capsaicin inhalation activation during maximum suppressible inhalation in controls and smokers

Region	Controls				Smokers			
	Peak voxel coordinate			Z-score	Peak voxel coordinate			Z-score
	x	*y*	*z*		*x*	*y*	*z*	
Precuneous cortex	10	−76	52	5.15	−12	−74	38	4.28
Anterior midcingulate cortex	−12	16	38	4.65	6	26	38	6.69
Posterior midcingulate cortex					4	−22	30	6.42
Supplementary motor area	−8	−10	58	4.74	−10	−2	64	4.21
S1/M1	−34	−12	46	4.73	−58	−24	24	6.72
Superior parietal cortex	54	−30	40	4.51	56	−40	42	5.51
Operculum	58	−10	6	4.04	−52	−2	10	9.93
Orbitofrontal cortex	48	18	−8	3.79	−46	14	−12	5.72
Inferior frontal gyrus	−58	4	2	4.86	−52	−2	12	10.38
Insula	34	14	6	4.31	38	10	8	5.67
Globus pallidus	18	2	0	3.12				
Putamen					−26	−8	6	7.01
Thalamus	−4	−14	4	4.81				
Pons	8	−38	−24	3.57	8	−36	−24	4.77
Medulla	10	−40	−48	6.01	16	−36	−42	3.56
Cerebellum	0	−84	−26	6.08	14	−56	−34	7.87

S1/M1: primary somatosensory and motor regions.

urge-to-cough during inhalation of equivalent concentrations were 4.3±0.7 for nonsmoking participants and 3.3±0.5 for smokers, but this difference was not significant ($t(14)=1.2$, nonsignificant) (figure 3a). Increased %BOLD signal during inhalation of equivalent concentrations of capsaicin between pairs was noted in the orbitofrontal cortex, bilateral ventral accumbens, cuneal cortex and cerebellum for controls greater than smokers at a cluster-wise FWE-corrected threshold of $p_{corrected}<0.05$ (voxel inclusion of $Z>3.09$) (table 3 and figure 3b). The substantial %BOLD signal change in the orbitofrontal cortex in controls was in contrast to that in smokers where the %BOLD signal change in this region was negative/approaching zero ($t(14)=3.657$, $p<0.003$) (figure 3c). Smokers showed increased %BOLD signal compared with the controls in the right sensorimotor ($t(14)=2.241$, $p<0.042$) and left insula cortices ($t(14)=5.277$, $p<0.001$) and regions of the basal ganglia including the bilateral putamen ($t(14)=3.653$, $p<0.003$) (table 3, and figure 3d and e).

Between-group comparison: maximum suppressible concentration (Like-Behaviour)

Smokers tolerated significantly higher capsaicin challenge concentrations compared with controls ($t(30)=2.131$, $p<0.041$), despite no significant differences in reported urge-to-cough between the two groups (figure 4a) and similar regional distributions of brain responses to S_{max} inhalation. However, the spatial distribution of sensorimotor activation in smokers appeared to be more extensive than in controls (figure 4b). A between-group difference was seen in the sensorimotor cortex as well as in the posterior parietal

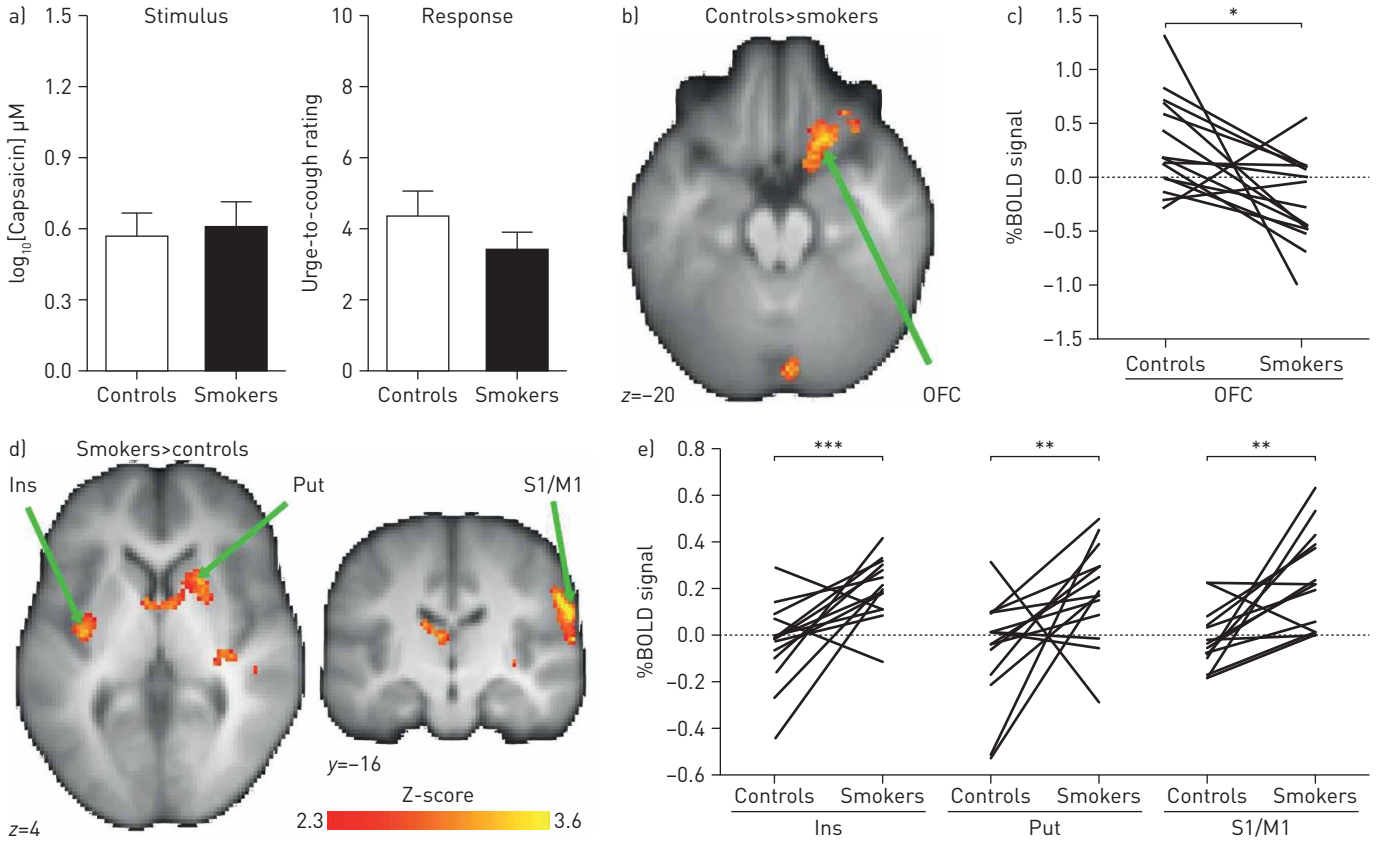

FIGURE 3 Brain responses during paired comparison of matched capsaicin concentrations. Participants were challenged with low and high concentrations of capsaicin during functional magnetic resonance imaging. BOLD: blood oxygen level-dependent; OFC: orbitofrontal cortex; S1/M1: primary somatosensory and motor regions; Ins: insula; Put: putamen. "High" corresponded to participants' maximum suppressible concentration during 24 s of repeated inhalations (S_{max}) and smokers' "low" concentration was matched to controls. Not all smokers had a higher capsaicin threshold than their matched controls, in which case at least one of the concentrations that the nonsmoking controls inhaled was matched to the capsaicin concentration of their paired smoker for the matched capsaicin concentration between-group comparison. a) The similar stimulus concentrations were associated with marginally, but not significantly, decreased urge-to-cough responses represented by their ratings in smokers (the difference in ratings did not reach significance; $p<0.2$). b) Differential regional activation was seen where controls had increased %BOLD signal in the OFC compared with smokers, also evident in c) the line graph showing individual %BOLD signal responses of the paired subjects. d) Regions of the sensorimotor (S1/M1) and Ins cortices, as well as regions of the basal ganglia (in particular, Put), showed increased %BOLD signal in smokers compared with controls, also evident in e) the line graphs showing individual %BOLD signal responses of the paired subjects. All axial brain images are presented in the neurological convention (left side of the image is the left hemisphere of the brain). Activations have a voxel inclusion of $Z>2.3$ and cluster-wise family-wise error-corrected threshold of $p_{corrected}<0.05$. Histograms represent the group mean±SEM. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

TABLE 3 Between-group differences during pair-matched capsaicin concentration inhalation

Region	Peak voxel coordinate			Z-score
	x	*y*	*z*	
Controls>smokers				
Cuneal cortex	2	−76	50	3.55
Orbitofrontal cortex	22	20	−20	3.46
Cerebellum	−18	−82	−26	4.62
Smokers>controls				
Postcentral gyrus	66	−18	26	3.91
	58	−16	34	3.74
Inferior parietal lobule	60	−22	32	4.28
Insula	−36	−8	10	3.95
Thalamus	−6	−16	14	3.37
Caudate	20	16	6	3.24
Putamen	−28	0	−6	3.17
	22	10	2	3.31

cortex in smokers compared with controls at a cluster-wise FWE-corrected threshold (Z>2.3; $p_{corrected}<0.05$) (figure 4c and table 4). Notably, %BOLD signals extracted from the left posterior parietal and extended region of the left sensorimotor cortices showed positive increases during inhalation of S_{max} concentrations in smokers, whereas controls showed negative or no mean signal change during the same stimulus (figure 4d).

Correlations between regional capsaicin activations and pack-years in smokers

Both negative and positive associations were identified between the severity of smoking behaviour and brain regional responses (figure 5). Negative correlations between S_{max} activation levels and pack-years were evident in the superior frontal gyri and middle frontal gyri bilaterally (table 5 and figure 5a) as well as in the left precentral gyrus. Estimates of %BOLD signal change in the middle frontal gyri were predicted by pack-years, with R^2=0.330 in the right hemisphere and R^2=0.568 in the left.

Positive correlations between pack-years and S_{max} activation were evident in bilateral peaks of activation in the lateral midbrain (figure 5c and table 5), two rostrocaudal levels of the pons (figure 5d and e, and table 5), a central location of the rostral, dorsal medulla (figure 5f and table 5) and in symmetrical cerebellar regions incorporating the dentate nuclei (table 5). Estimates of %BOLD signal change from clusters of activation in the brainstem were regressed against pack-years and showed levels of shared variance ranging from R^2=0.203 to R^2=0.583.

Discussion

Although cough can be a troubling symptom associated with cigarette smoking, smokers often display reduced sensitivity in evoked cough reflex testing indicative of complex peripheral or central sensory nervous system adaptation to chronic airway stimulation [13, 15, 16]. We similarly demonstrated reduced sensitivity to airway challenge with capsaicin in smokers compared with nonsmokers and showed this to be reflected by differences in capsaicin-evoked brain responses. Although smokers showed increased activation in brain regions thought to encode airway sensations, there was a concomitant increase in putative central inhibitory network activity. Interestingly, brain responses to capsaicin inhalation among smokers were shown to vary according to individuals' smoking histories, with a transition from prefrontal (dorsolateral prefrontal cortex) to midbrain (nucleus cuneiformis) activations as the severity of smoking behaviour increased. Both the dorsolateral prefrontal cortex and nucleus cuneiformis have been implicated in the modulation of responses to tussive stimuli in other circumstances [7, 10], suggesting an evolving pattern of brain processing as smoking behaviour persists. Smoking was also associated with decreased capsaicin-evoked activation compared with controls in the orbitofrontal cortex and cerebellum, although these differences were only seen when participants inhaled comparable capsaicin concentrations and not when challenged with concentrations eliciting maximum levels of cough suppression. Collectively, these data further highlight the importance of central facilitatory and inhibitory neural network balance for shaping sensory and motor responses to airway irritation.

Evidence for altered airway sensory processing in the brain of smokers

Capsaicin inhalation challenge of both smokers and controls activated brain regions that are consistent with previous reports, and this adds further support to the conclusion that airways irritation and

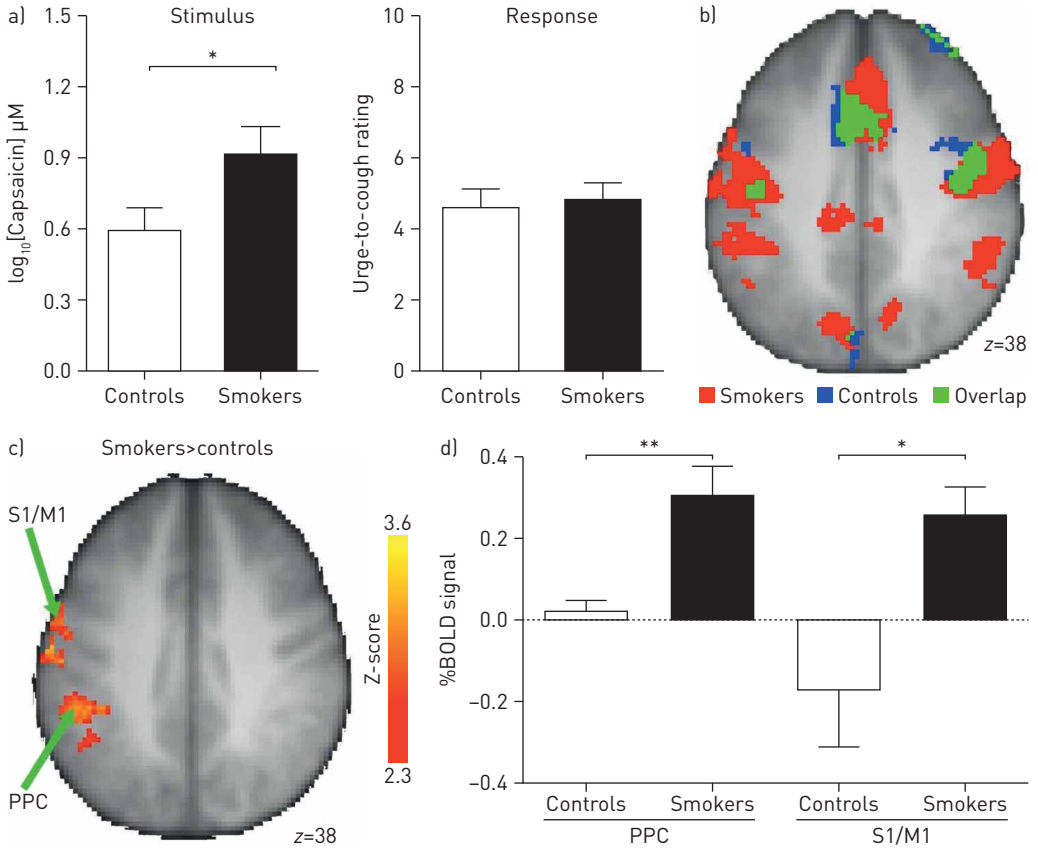

FIGURE 4 Brain responses during between-group comparisons of maximum suppressible concentration comparison. Participants were stimulated with low and high concentrations of capsaicin during functional magnetic resonance imaging. BOLD: blood oxygen level-dependent; PPC: posterior parietal cortex; S1/M1: primary somatosensory and motor regions. "High" corresponded to participants' maximum suppressible concentration during 24 s of repeated inhalations (S_{max}). During the matched urge-to-cough sensation comparison, all participants were inhaling their S_{max} concentration. a) Despite a significantly lower mean stimulus in controls compared with smokers, high concentrations were associated with similar mean urge-to-cough rating response between the two groups. b) Logical map of group activation shows brain regions activated during match urge-to-cough sensation in controls, smokers and overlapping regions of activation. Group activations have a voxel inclusion of Z>3.09 and cluster-wise family-wise error-corrected thresholds of $p_{corrected}$<0.05. c) Differential regional activation was seen where smokers had increased %BOLD signal in the PPC and S1/M1. Activations for group differences have a voxel inclusion of Z>2.3 and cluster-wise family-wise error-corrected thresholds of $p_{corrected}$<0.05. d) Between-group differences are evident in the mean %BOLD signals extracted from both the PPC and S1/M1. All axial brain images are presented in the neurological convention (left side of the image is the left hemisphere of the brain). Histograms represent the group mean±SEM. *: p<0.05; **: p<0.01.

associated processes are represented in a distributed brain network [1, 2, 7, 22–24]. The constituent regions of the network include the prefrontal, cingulate, sensorimotor, posterior parietal and insula cortices, as well as the thalamus, cerebellum, basal ganglia and brainstem. Differences between smokers and controls did not occur uniformly throughout the brain regions activated, which implies differential effects of smoking on functional modules within the broader network.

TABLE 4 Between-group differences during maximum suppressible sensation

Region	Peak voxel coordinate			Z-score
	x	*y*	*z*	
Smokers>controls				
Posterior parietal cortex	−40	−38	36	3.30
Precentral gyrus	−60	−2	34	2.96
Postcentral gyrus	−60	−12	38	3.29

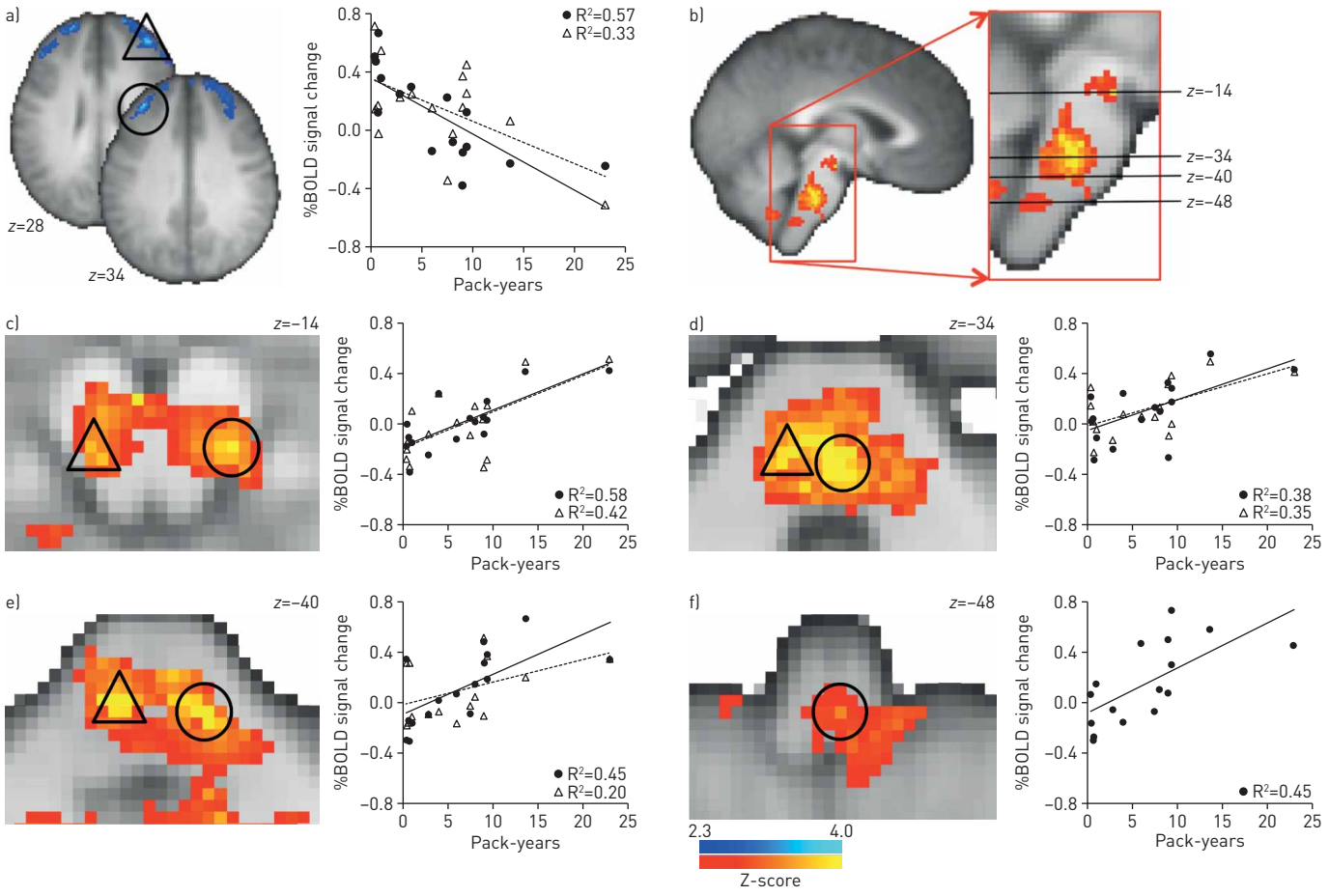

FIGURE 5 Behavioural measure correlation analyses. Controls: triangles and dotted lines; smokers: circles and solid lines. a) Levels of capsaicin inhalation activation in the dorsolateral prefrontal cortices in both hemispheres were negatively correlated with pack-years in smokers. b) Positive correlations between pack-years and levels of capsaicin inhalation activation were seen in clusters distributed throughout the brainstem. Black lines through the expanded midline section indicate the position of axial slices in the remaining panels of the figure. c) Symmetrical clusters in the lateral midbrain showed activation associated with pack-years. d) Capsaicin inhalation activation in bilateral dorsal pons was positively correlated with pack-years. e) The rostral, ventral medulla contained symmetrical clusters of capsaicin inhalation activation that correlated with pack-years. f) Pack-years was positively correlated with capsaicin inhalation activation in the dorsal medulla at a level ~10 mm rostral of the obex. Left side of axial images is left side of the brain and ventral is at the top of the images. Activations have a voxel inclusion of $Z>2.3$ and a cluster-wise family-wise error-corrected threshold of $p_{corrected}<0.05$.

Regional capsaicin activation levels in smokers showed both increases and decreases relative to nonsmoking controls. Most notably, the primary somatosensory and posterior parietal cortices were more activated in smokers. However, the regions of the somatosensory cortex commonly activated in both groups did not show differences in %BOLD signal levels. Instead, the differences occurred in neighbouring lateral cortical regions where activation occurred exclusively in smokers. These patterns of %BOLD signal changes suggest an expanding representation of the airways in the somatosensory cortices of smokers. Changes in the somatosensory homunculus have been reported in response to alterations of sensory input, and representations have shown both expansions and contractions depending on whether afferent inputs from the periphery increased [25–28] or decreased [29–32].

The increased spatial extent of somatosensory responses in smokers occurred in the context of decreased acuity for detecting capsaicin and a rightward shift of urge-to-cough ratings. It has been speculated that a loss of sensitivity to tussive stimuli in smokers could be due to functional loss of airway afferents [15]. However, other conditions involving deafferentation are usually associated with shrinking somatosensory representations [30, 32] and consequently the expansion seen in the cortices of smokers is not consistent with a loss of airway afferents. Studies demonstrating rapidly reversible changes in sensory sensitivity when smoking is ceased and resumed again also argue against any substantive loss of afferent nerve terminals themselves as a consequence of smoking [16]. It may be that other factors, including the influence of nicotine [33, 34], discrepant impacts of embryologically different sensory neural inputs [23, 24] or the recruitment of a countervailing central neural process, could (present study) explain the

TABLE 5 Activation during maximum suppressible inhalation correlated with pack-years of smoking

Region	Peak voxel coordinate			Z-score
	x	*y*	*z*	
Positive correlation				
Midbrain	12	−24	−12	3.69
	−8	−26	−16	3.62
Pons	4	−30	−36	4.06
	−4	−28	−34	4.25
	8	−32	−40	4.35
	−6	−30	−40	4.37
Medulla	2	−38	−48	3.07
Cerebellum	18	−64	−38	3.80
	−12	−62	−36	4.27
Negative correlation				
Superior frontal gyrus	38	44	28	3.96
	30	56	24	4.70
Middle frontal gyrus	−34	40	34	4.24
	44	46	18	4.10

concurrence of increased somatosensory activation with decreased subjective sensitivity to capsaicin inhalation in smokers.

Capsaicin inhalation activation in the controls exceeded that of smokers in the orbitofrontal cortex and cerebellum, although this was confined to the contrast involving paired concentrations and not when concentrations were adjusted to produce similar levels of urge-to-cough ratings. The activations in the orbitofrontal cortex and cerebellum may therefore have reflected a more challenging situation for the control participants, a greater proportion of whom were inhaling the maximum suppressible concentration. The unpleasantness of the experience when inhaling a more challenging concentration could have been a factor in shaping the activation levels, most notably in the orbitofrontal cortex that has a role in emotional responses to sensory experiences [35, 36].

Evidence for altered central inhibitory network activity in smokers

Previous studies by our group have implicated prefrontal and midbrain regions in the modification of urge-to-cough. For instance, bilateral activation in the dorsolateral prefrontal cortex accompanies placebo-related decreases in urge-to-cough [10] and the loci of these activations are similar to the regions that were increased to a greater degree among participants with the least severe smoking behaviour. Additionally, capsaicin inhalation responses in midbrain regions encompassing the nucleus cuneiformis distinguish patients with chronic cough and the activation levels in these regions correlate with airway sensitivity in hypersensitive cough patients [7]. The severity of smoking behaviour bore relationships with regional levels of capsaicin inhalation activation. Relatively increased capsaicin inhalation activations in the dorsolateral prefrontal cortex were seen among participants with the lowest pack-years, whereas more severe smoking behaviour was associated with increased activation in the midbrain and hindbrain.

The midbrain regions showing a positive association with smoking behaviour are very similar to those identified in chronic cough patients [7]. A parsimonious explanation for the respective positive and negative correlations between pack-years and regional capsaicin inhalation activation levels is that modulation of airway afferent input undergoes development as the severity of smoking behaviour increases. It could be that activity in prefrontal regions represents recruitment of cognitive processes that downregulate responses to airway afferent inputs when smoking behaviour is less severe, possibly due to the motivational drive to continue smoking despite the frequent irritation of the airways. As smoking behaviour increases, prefrontal responses may be replaced by activity in midbrain and brainstem regions that directly regulates incoming airway afferent processing. It is salient to note that increasing pack-years was associated with increases in thresholds to detect an urge-to-cough (*i.e.* less sensitivity), which supports speculation that midbrain and brainstem responses could contribute to decreased sensitivity to capsaicin challenge among smokers and may be an adaptive response in chronic coughers [7, 17, 37] in attempts to suppress persistent airway inputs.

Conclusion

This study demonstrates that cigarette smoking may impact on the central processing of airway sensations. Adaptation to regular airway irritation in smokers may involve the development of responses with the

potential to modulate airway sensory processing. One caveat of the study is that we assess pulmonary function in smokers and although they universally reported no history of lung disease, we cannot exclude the possibility that the brain imaging results are impacted by an underlying pathology. Additionally, we can only speculate about the molecular mechanisms underlying the observed functional effects. Chronic exposure to nicotine is known to upregulate the expression of many nicotinic receptor subunits, both peripherally and in the central nervous system, and this is thought to contribute to the addictive properties of smoking [38]. Airway sensory neurons are directly activated by nicotine to induce coughing, notably *via* α3β4 nicotinic receptors [39]. Upregulation of these would be expected to enhance sensitivity to airway delivery of nicotine, perhaps consistent with the increased neural activation seen in the brain regions directly encoding these sensory inputs. Conceivably, the enhanced activity of central suppressive networks may also be nicotinic receptor dependent. Consistent with this, a single inhaled exposure to nicotine in nonsmokers is sufficient to reduce cough and urge-to-cough evoked by inhaled capsaicin [33, 34]. Smoking may further enhance inhibitory processing by upregulating nicotinic receptor expression in these central suppressive pathways. In this regard, the outcomes of current early-phase trials with centrally acting α7 nicotinic receptor agonists in chronic refractory cough will be interesting.

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