Study Title:

A functional magnetic resonance imaging study to investigate ATP-sensitive cough neural pathways in patients with chronic cough hypersensitivity.

Clinicaltrials.gov registration number:

NCT03722849

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Objectives & Hypotheses

To identify the neural circuits involved in ATP-dependent cough sensitization in humans by determining whether ATP-sensitive pathways in the brainstem and brain are altered in patients with chronic cough.

Clinical hypothesis.

ATP contributes to the development of cough hypersensitivity in patients by enhancing the neural activations mediated by vagal pathways involving jugular ganglia sensory neurons.

BACKGROUND TO PROBLEM

Chronic cough is one of the commonest clinical problems encountered by doctors both in general and hospital practice (1), that can persist for months and years. It remains a difficult problem to manage because of our poor understanding of the mechanisms underlying its initiation and persistence and the current lack of effective antitussive therapies (2). People living with chronic cough report that chronic cough impacted considerably on their daily-life activities with a significant deterioration in health-related quality of life, leading to feeling fed-up and depressed (3) Chronic cough is also a very common co-morbid problem accompanying many pulmonary (e.g. asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, idiopathic pulmonary fibrosis) and non-pulmonary (e.g. gastro-esophageal reflux disease, cardiac failure) conditions; in some patients, it exists as a distinct clinical entity termed 'refractory' or 'idiopathic' cough.

Patients with a chronic cough report that changes in ambient temperature, laughing, talking on the phone for more than a few minutes, aerosol sprays, or smoky atmospheres characteristically trigger bouts of coughing (4, 5). In a demographic profile of chronic cough in 4,500 patients attending UK specialist cough clinics (and over 10,000 patients worldwide) (6),we found a state of hypersensitivity as a key common pathophysiological characteristic (7). This has led to the Cough Hypersensitivity Syndrome (CHS) hypothesis as an underlying cause of chronic cough whereby nerves (in the airway and/ or the brain) become sensitized,

activated and perhaps damaged (neuropathic cough) by irritant factors including viral infection, chemicals and inflammation (8).

Sensory inputs from the upper and lower airways to the brainstem are relayed to higher brain regions where they are integrated in subcortical nuclei and then mapped to the cingulate, insula, orbito-frontal and somatosensory cortices in the cerebrum (9). Studies using fMRI of the human brain during challenges with inhaled airway irritants have shown these areas to be important for the different sensory and motor responses that accompany coughing. Importantly, these central neural pathways function differently in chronic cough patients (10). Thus, responses to sensory inputs are exaggerated while those needed to suppress (control) coughing are depressed. This exciting discovery has shed an entirely new perspective on problematic cough, suggesting that pulmonary conditions that lead to coughing may do so by changing the way in which the central nervous system responds to sensory inputs. The striking parallels regarding peripheral and central sensitization which link cough and pain syndromes have prompted recent collective effort to more completely understand the processes leading to these neural dysfunction (11).

Despite this important understanding of the process of CHS, the most important unmet need relates to the lack of effective antitussives. Clinically, amitriptyline and gabapentin are used as cough suppressants with central effects potentially on CHS pathways that remains to be determined, but their effect is not uniform in all patients with chronic cough (12). However, more recently, a blocker of the ATP receptor, P2X3, Gefapixant (600 mg twice a day for 14 days), has been shown to be extremely effective in suppressing the chronic cough of patients with an idiopathic cough, with a reduction in cough frequency by 75% when compared to placebo with daytime cough frequency reduced from a mean 37 coughs per hour to 11 coughs per hour (13). These results were confirmed in another randomized, double-blind, placebo-controlled, Phase 2b crossover study using lower doses of Gefapixant (50 mg, 100 mg, 150 mg and 200 mg twice daily for four days) demonstrated significant reduction in cough frequency, including at the lowest dose of 50 mg twice daily (14). However, contrasting with this positive effect of Gefapixant, studies using TRP receptor blockers such as TRPV1 and TRPA1 antagonists have failed to show any efficacy in chronic

cough patients (15-17). Therefore, the singular potent effect of Gefapixant as an antagonist of P2X3 ATP receptor in suppressing cough opens a new focus of investigation in the role of ATP and its receptor P2X3 in CHS.

RATIONALE

Given that a blocker of the ATP receptor, P2X3, has been successful in inhibiting chronic cough, we wish to use this observation to understand the putative role of ATP sensitive vagal sensory neural pathways in the cough hypersensitivity syndrome. Vagal sensory neurons are located in the nodose and jugular vagal ganglia (Fig 1). Nodose neurons developmentally originate from cells within the embryological placodes (18). There are a series of placodes that form above the pharyngeal cleft during development, collectively referred to as the epibranchial placodes, and these comprise the geniculate, petrosal, and nodose placodes that eventually form the geniculate, petrosal and nodose ganglia, associated with the 7th, 9th and 10th cranial nerves. By contrast, most peripheral neurons, including the sensory neurons of the jugular and spinal dorsal root ganglia (DRG), are developmentally derived from neural crest cells (18). Jugular ganglia neurons contribute to the innervation of tissues of the head and neck and are phenotypically similar to DRG neurons, but quite distinct to nodose neurons. Because of these developmental differences, nodose and jugular afferent nerves have distinct activation profiles, central (brain) connectivity and likely serve distinct sensory functions (reviewed in (19)). Preclinical animal studies, for example, suggest that only nodose neurons are responsive to ATP even though P2X3 receptor subunits are ubiquitously expressed in neurons of both vagal ganglia. This is because only nodose neurons express P2X2 subunits needed for functional heteromeric channel assembly. Intriguingly, other stimuli of nodose neurons (such as serotonin and adenosine) do not produce much coughing. Thus, whilst stimuli (such as capsaicin) that activate both jugular and nodose vagal chemically sensitive afferents evoke cough in conscious animals and humans, nodose chemically sensitive afferent-selective stimuli (including ATP) do not reliably evoke cough, and some nodose stimuli may inhibit cough in certain circumstances (20-22). Indeed, there is a paucity of evidence that would support a role for most subtypes of nodose neurons in cough production. This, therefore, creates a

conundrum – nodose neurons don't evoke cough when activated but are responsive to ATP while jugular neurons readily evoke coughing but don't respond to ATP ordinarily. However, it is important to note that this work has only been conducted on neurons from healthy animals, and as such how ATP dependent responses at the neuronal level changes in pulmonary disease is not entirely clear.

Consequently, the role of nodose versus jugular afferent pathways in human chronic cough dependent upon purinergic mechanisms remains unclear.

The differential organization of nodose and jugular neurons within the central nervous system may allow insights into the relative contribution of nodose and jugular afferent pathways in ATP-dependent cough in health and disease. Thus, axonal guidance cues under the regulation of placodal transcription factor Phox2b attract nodose terminals to the medullary regions of the nucleus of the solitary tract (nTS) (23). By contrast, the Mazzone group have recently shown that the neural crest derived jugular ganglia neurons have their central terminations in the trigeminal nuclear complex, in a little-known region called the paratrigeminal nucleus (Pa5) (24, 25). Thus, in rats and guinea pigs injection of a retrograde tracing dye into the nTS labels only neurons in the nodose ganglia, while a comparable injection in the Pa5 labels only jugular neurons. Because of this differential brainstem wiring, the ascending pathways in receipt of nodose and jugular afferent inputs are also different (24). *Until now, this has been impossible to study in humans. Consequently, we know nothing about the neural mechanisms involved in ATP dependent cough in humans, nor do we know how these neural processes might behave in patients with chronic cough.* This issue is central to the current funding request because our team

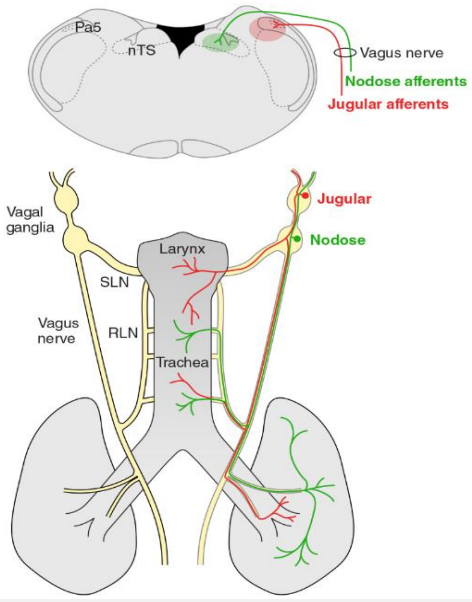


Fig 1. Pulmonary jugular (red) and nodose (green) vagal sensory pathways are anatomically and functionally distinct.

has optimized methods for assessing cough neural circuit activation driven by ATP in humans (see preliminary data) and are positioned to make major insights into these neural processes.

SIGNIFICANCE OF SELECTED TOPIC

This is an important area of research in CHS because this provides us for the first time with some clues as to the potential mechanisms for CHS. ATP is known to activate and sensitize signal transmission at sensory sites including primary afferent neurons such as airway vagal afferent nerves via its P2X3-containing trimers and P2X3 antagonists are active in many inflammatory and visceral pain models, by inhibiting inappropriate chronic signals and decreasing peripheral and central hypersensitivity (26). Discovering the mechanisms by which P2X3 contributes to CHS may not only lead to discovery of the important peripheral and central pathways where P2X3 is crucial, but also to other potential targets for therapy. This may also lead to an understanding of the role of the P2X2 component that has been implicated, and to understand now to dissociate the anti-tussive effects of Gefapixant from its hypo- or ageustic effects. Finally, our study could provide an empirical test that would predict whether an antitussive would have the capacity to work or represent a new approach to studying the efficacy of P2X3 targeting compounds, or any other anti-tussives being developed. Regardless of the future applications, the work represents cutting-edge neuroscience in the area of cough and pulmonary sensation, and this will have impact in the field.

PRELIMINARY DATA

Peripheral effects of ATP via P2X3 receptors

ATP has been shown to be a tussive agent particularly on chronic cough patients who were more sensitive than non-cough subjects to inhaled ATP (27). ATP has been shown to augment the cough response to capsaicin in patients with asthma (28). Gefapixant at a single oral dose of 50 mg did not modulate capsaicin cough responses in normal volunteers and chronic cough subjects while inhibiting ATP-induced cough particularly in chronic cough

subjects (22). These observations would suggest that ATP has a direct effect on the sensory neurons that evoke coughing through the activation of P2X3 receptors (detailed above).

The use of fMRI to provide insights into the peripheral and central sites of activation by ATP/P2X3 activation

We have generated functional brain imaging (fMRI) data to suggest that the different brain circuits in receipt of nodose and jugular ganglia neuron inputs (as identified in animal studies) are conserved in humans. When inhaled, the tussigenic compound capsaicin (from hot chili peppers) indiscriminately stimulates both nodose and jugular chemosensitive afferents and we have published that capsaicin inhalation produces brain activations in the primary sensory, anterior and mid-insula, cingulate, premotor, motor and orbitofrontal cortices (29, 30). These regions are presumed to encode perceptual awareness of airway irritation, and the associated emotional, cognitive and behavioral (motor) consequences. For example, activity in the human primary sensory cortex (which receives jugular ganglia inputs in animal studies) correlates with an individual's perception of airway irritation (their perceived need/ urge to cough) while activity in the insula (in receipt of nodose inputs) relates closely to the actual magnitude of the delivered stimulus independent of perception. We have now built upon these published findings by using high resolution brainstem fMRI during the inhalation of ATP (expected to only activate P2X2/3 expressing nodose-derived airway afferents) versus capsaicin (expected to activate both jugular and nodose chemosensitive afferents). Our results are striking and reveal that ATP inhalation evokes an in increased signal level in the brainstem regions corresponding to the nTS, while capsaicin inhalation produces activations in both the nTS and in an area of the dorsal spinal trigeminal nucleus on the lateral margins of the brainstem that contains the paratrigeminal nucleus (Fig 2). Indeed, our healthy participants did not cough as much to ATP compared to capsaicin, consistent with studies cough in animals and humans and the relatively poor cough-evoking properties of ATP in healthy humans (20, 21). However, the perception of airway irritation was identical between ATP and capsaicin stimuli (Fig 3). We believe that cough production will ultimately be dependent upon activation of the neural circuit that integrates in the paratrigeminal nucleus (i.e.' the jugular afferent pathway) and therefore

we hypothesize that there is an upregulation of the capacity of ATP to act via jugular ganglia pathways in chronic cough patients.

The fMRI studies described above provide an exciting opportunity to assess for the first time which primary airway afferent pathways are likely excited or sensitized by ATP and, in turn, what aspects of the central processing of airway sensory information is altered by ATP. We have reported previously that patients with chronic cough display functional brain responses consistent with a state of central sensitization (10) that closely resembles the central sensitization accompanying chronic pain (31).

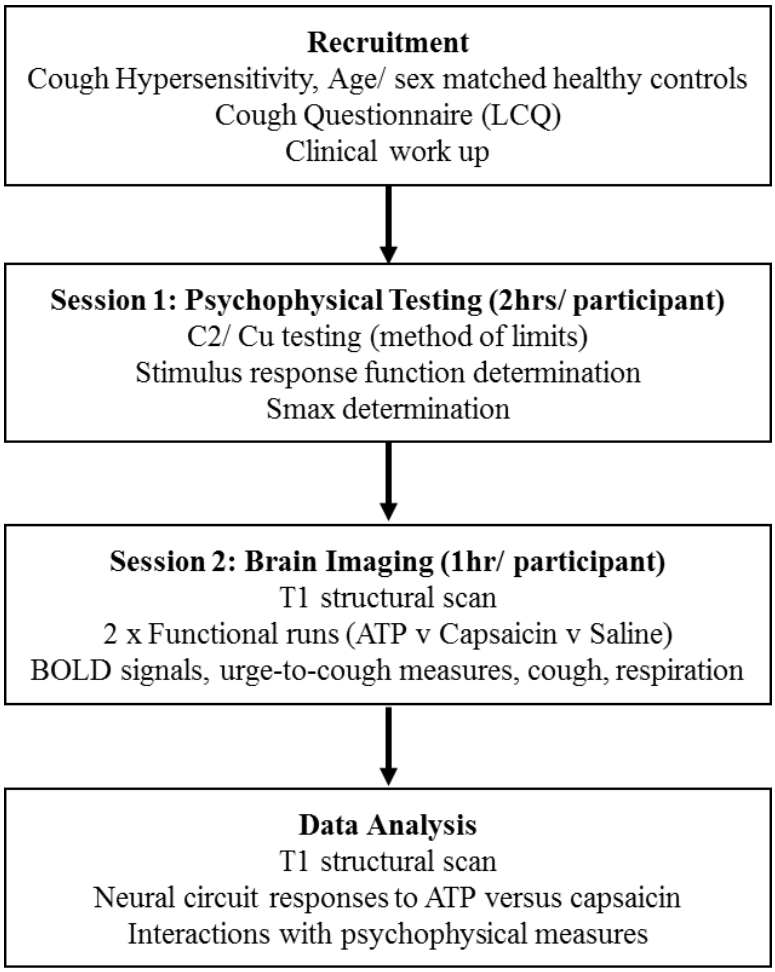
We will extend upon these findings by determining whether ATP-sensitive pathways in the brainstem and brain are altered in patients with chronic cough, and in doing so provide insight into whether ATP effects vagal afferent processing through an interaction with nodose and/ or jugular neural pathways.

Study Design

The study will involve 25 participants with cough hypersensitivity and 25 age and sex matched healthy controls. All participants will provide written informed consent to participate in the study, which will be approved by the Melbourne Health Human Research Ethics Committee (Australia). Before inclusion, all participants undergo screening interviews to ascertain their eligibility for the study. All participants will be non-smokers with no history of neurological disease or any recent history (over 8 weeks) of acute respiratory infections. Cough hypersensitive participants will be recruited from respiratory specialist clinics and included if they have experienced chronic cough (coughing for over 8 weeks) and are considered idiopathic/ refractory coughers (to avoid the confounds of heterogeneity of precipitating diseases). Healthy control participants will have no history of chronic respiratory disease and will be individually age and sex matched to each cough hypersensitive participant.

Participants will attend two experimental sessions. The first is used to ascertain their individual sensitivities and tolerances to inhaled challenges with capsaicin and ATP

(psychophysical testing session) and the second to determine their brain responses to challenge stimuli (brain imaging session). This is not an interventional study but rather a comparison between responses in healthy control participants versus patients with chronic cough syndrome.



All participants will first undergo a psychophysical testing session where their level of sensitivity to capsaicin and ATP is measured using a method of limits approach. The following measures of sensitivity are determined: stimulus dose evoking 2 coughs (C2), the stimulus dose required for the initial detection of an urge to cough (Cu) and the maximal suppressive stimulus dose where a participant can resist coughing (Smax). Patients will provide subjective measures of their urge-to-cough using an eleven point numerical rating scale (0, no urge-to-cough; 10, maximum urge-to-cough) evoked by single breath challenges with tussive stimuli. The capsaicin and ATP solutions are nebulized to participants via a

facemask fabricated to deliver stimuli through oral breathing (and to be compatible for hands free use in an MRI scanner). Doubling doses of stimuli are used throughout the study ranging from 0.06µM to 125µM for capsaicin and 0.45 – 232.5mM for ATP. Once the C2 dose is identified, participants will receive randomized challenges with doses two increments above and below the C2 (C2-2, C2-1, C2, C2+1 and C2+2) to accurately measure the stimulus response function for each individual. Finally, in preparation for fMRI scanning, participants will be challenged for 24 seconds with stimuli above and below the C2 dose to determine challenge stimuli doses that are tolerable for brain imaging (defined as the Smax). This session is used to ascertain each participant's sensitivities and tolerances to inhaled stimuli and to inform the participants about the design of the subsequent fMRI session.

In the second session, participants return for fMRI scanning. Structural and functional MRI data are collected on a Siemens Magnetom Trio 3 Tesla scanner (Siemens AG, Erlangen, Germany) with a 32-channel head coil located at the Monash Biomedical Imaging Facility, Melbourne, Australia. Anatomical T1 ‐ weighted images are acquired in the sagittal plane (192 slices, 0.90 mm slice thickness, 0.84 × 0.84 mm2 in ‐ plane resolution, echo time (TE) = 2.59 ms, repetition time (TR) = 1900 ms, flip angle = 9). Two functional MRI (fMRI) scans of 491.4 seconds duration are performed using the BOLD contrast. Functional runs are collected using a partial field of view (FOV) with a gradient-echo echo-planar parallel imaging sequence (GRAPPA, PAT factor=3, reference lines=36, TR=1790ms, TE=30ms, 27 slices, 2.5mm thickness, 1.88mm in-plane resolution, flip angle=70 degrees). This facilitates a restricted focus on the brainstem necessary to define regional activations in this area (see Figure 2).

During image acquisition, participants lay comfortably on the scanner bed with their head stabilised with foam padding and hearing protection. Participants are fitted with the facemask and apparatus used in the psychophysical testing session. Online respiratory monitors are fitted around their chest throughout the experiment (AD Instruments). A periscope mirror attached to the scanner head coil enables participants to view a projector

screen upon which visual instructional cues are presented throughout the experimental session. Within each functional scanning run, three stimulus blocks of capsaicin, three of ATP and two of saline are administered in a pseudorandom order. Each stimulus block lasts 24 seconds in duration, and is separated from the next by 36.8 seconds. Visual cues are used to time the participant's inhalation with the onset of stimulus delivery in order to standardise the respiratory cycle between participants. At the end of each challenge block, participants give a numerical rating of their urge-to-cough.

Study Duration

18 months will be required to recruit and scan 25 cough patients and 25 age/ sex matched healthy controls, this includes 6 months for fMRI data analysis which is time consuming. Total study duration will be 18 months.

Statistical analysis plan

Our team has previously published in detail our methods for analyzing functional brain imaging data with associated physchophysical metrics, many of which have been rigorously optimized by our group to control for confounding respiratory noise. We have extensive experience in these types of studies and all the required expertise to analyze and interpret brain imaging data exists within A/Prof Mazzone's research team in Melbourne. This includes investigating relationships between behavioral measures and brain responses (e.g., see ref 10). Professor Chung and Dr McGarvey will contribute to the interpretation of clinical metrics related to cough phenotype and cough questionnaire survey results.

Variables/Time Points of Interest

Two types of data will be collected, (1) Blood Oxygen Level Dependent signal data collected during fMRI scans and used to determine regional brain activity, and (2) psychophysical data during preliminary testing sessions and during fMRI scanning. The latter includes the stimulus dose evoking 2 coughs (C2), the stimulus dose required for the initial detection of an urge to cough (Cu) and the maximal suppressive stimulus dose where a participant can resist coughing (Smax). As part of the clinical work up of the patients we will also collect

subjective patient scores of their cough severity and quality of life, using validated tools (the Leicester Cough Questionnaire (LCQ)) which can be also used to help interpret fMRI data. The primary variable of the study is the stimulus delivered (ATP versus capsaicin) compared between cough patients and healthy controls, assessing the differential outcomes on BOLD signal changes and associated psychometrics.

Statistical Methods

Statistical analysis of psychophysical parameters will be performed with SPSS 22.0. Independent t- tests will be used to test the effects of group on C2, Cu and Smax thresholds. A repeated-measures ANOVA is used to test the effects of group, dose and their interaction on urge-to-cough ratings in response to random stimulus challenges. Independent t-tests will be used to assess group effects on capsaicin/ ATP doses and urge-to-cough ratings associated with the fMRI scanning.

fMRI image analysis will be performed with FEAT, version 5.98. Regressors for each dose of capsaicin/ ATP and rating events will be included in a general linear model that also includes motion parameters and confound variables to take account of physiological noise. Contrasts for capsaicin/ ATP doses will be averaged across the three scans for each participant and used in the analysis of group and between-group effects. Group contrasts include high doses for all participants (Like-Behaviour), and activation levels will be tested for correlations with Cu and frequency of coughing during repeated stimulus challenges. Higher level analyses will be carried out for inter-group averaging and between-group comparisons. Z (Gaussianised T/F) statistic images are thresholded to define clusters of contiguous voxels activated at a significance level of $Z > 2.3$. To correct for multiple comparisons, a corrected cluster probability threshold (based on Gaussian Random Field Theory of $p < 0.05$ is applied. Between-group contrasts are made using two different comparisons: i) during the inhalation of Smax for all individuals in both groups (matched urge-to-cough sensation). Activation levels associated with Smax doses are tested for relationships with the Cu threshold and the frequency of coughing during the psychophysical sessions.

Multiplicity

N/A

Power/Sample Size

Sample size estimates have been made using BOLD signal changes extracted from regions of interest in the data set shown in Figure 2 and analyzed with fMRIpower (32). Inclusion of 25 participants will provide at least 80% power to detect a between-group difference of 0.48 to 0.72 standard deviation units (analogous to Cohen's d) in the regions of interest. This is sufficient power to resolve significant effects of disease (chronic cough) on ATP and capsaicin evoked brain activations in brainstem nuclei (as shown in Figure 2, data collected from n=24 with one additional participant excluded because of excessive head movement during scanning). Males and females will be included in the study.

Adverse Incident Reporting

We do not expect that there will be any serious adverse events during the experimental procedures. Should a serious event occur, the experiment will be stopped immediately and the Institutional health and safety guidelines will be followed. We will call a trained first aid officer and contact our local emergency services if required.

Risks

Inhaling capsaicin causes irritation of the throat. The irritation quickly resolves after exhaling. Capsaicin does not harm the tissues of the throat. Throat irritation and urge-to-cough are integral components of the experimental protocol. Levels of these sensations will be assessed using psychophysical procedures whereby the individual participant's sensitivity to the substances dictate the doses used in the experiment, thus avoiding excessive discomfort. Adverse responses to capsaicin inhalation, other than transient throat irritation, are unlikely. A systematic review of the literature and personal communications with investigators established the absence of any adverse outcomes of capsaicin inhalation among 4,833 participants that included healthy adults, children, and patients with respiratory disease (33) Our own reports involving capsaicin inhalation are similarly without

incident (e.g., refs 6, 9, 10, 29, 30). The amount of capsaicin inhaled is very low, equivalent to 0.0038%. These low levels are sufficient to evoke transient feelings of airway irritation, but much lower than concentrations typically encountered for other uses of capsaicin such as medicinal topical applications (0.025-0.25%), experimental protocols involving topical application to produce cutaneous hyperalgesia (i.e. 5%), or as a spray in law enforcement (1-2%).

Experience with ATP is less extensive than capsaicin, but reported effects on throat irritation are similar for the two stimuli (34). ATP can also cause bronchoconstriction and associated increases in reported dyspnea in asthma patients (35). Asthmatics will not be recruited to this study.

MRI scanning is safe providing appropriate prescreening is performed. Participants will be screened by the MRI radiographer prior to scanning to exclude any risk factors (namely implanted or embedded metals) before going in proximity to the high magnetic field of the scanners. Due to the unknown risks of MRI scanning on fetal development, women who are pregnant, or suspect they may be pregnant, will be excluded from participating in the study. On rare occasions, MRI scanning identifies unknown pre-existing pathologies in participants. Under such circumstances A/Prof Mazzone will contact the individual and advise them to consult a medical practitioner to further discuss any implications of the findings. Copies of the Radiology report will be forwarded to the participant and to a nominated medical practitioner if the participant wishes.

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EXPLANATORY STATEMENT

Project Title:

Behavioural and brain haemodynamic responses to inhalation of capsaicin and adenosine tri-phosphate (ATP) in patients with idiopathic chronic cough.

Chief Investigator: Prof Stuart Mazzone
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Human Ethical Approval number: 1852642

You are invited to take part in this study. Please read this Explanatory Statement in full before deciding whether or not to participate in this research. If you would like further information regarding any aspect of this project, you are encouraged to contact the researchers via the phone numbers or email addresses listed above.

What does the research involve?

The aim of this study is to identify regions in the brain that respond to inhalation of stimuli that evoke coughing.

You will be invited to attend two sessions if you choose to participate in this study. Both sessions may occur on the same day or be up to a week apart depending on equipment access and your availability.

The first session is at either the University of Melbourne on Grattan St in Parkville or Monash Biomedical Imaging in Blackburn Road, Clayton, depending on what is convenient for you. In this session you will complete some questionnaires about your daily experience with cough, and then take single breaths of vapour containing chemicals that briefly irritate the airways. These chemicals are capsaicin and adenosine tri-phosphate (ATP). Capsaicin is the 'hot' ingredient of chilli peppers. ATP is a substance inside the body that plays a role in how cells use energy. You will place a ventilated mask over your nose and mouth for each inhalation of the chemical vapour. You are likely to feel a need to cough and sometimes you will cough after inhaling the vapour. The sensation and coughing resolve quickly in the first or second breath that you take after you exhale the vapour. You will be asked to give ratings of your need to cough for each inhalation and the researcher will also confirm with you if the sensation is disappearing with a breath of fresh air. This information will be used to determine a suitable level of stimulus to be used during the second session when brain imaging occurs. We will first check that the stimulus level is tolerable for you by having you breathe in and out the chemical repeatedly during 24 seconds and adjust the stimulus level to ensure that you only feel mild irritation. The duration of these trials is the same as the timing of repeated inhalations used during brain scanning. These trials will show if you are able to resist coughing during repeated inhalation, or if a reduction of stimulus level will be required to avoid any coughing. This session will take around 1 hour to complete.

Your second session will involve Magnetic Resonance Imaging (MRI) scanning of your brain. These scans will happen at Monash Biomedical Imaging, on Blackburn road in Clayton as this is the location of the MRI machine. The radiographer at the scanner will interview you to make sure it is safe for you to be scanned. You will lie on

your back on a moveable bed with a circular cage around your head. The ventilated mask will be placed in position over your nose and mouth. You will also wear hearing protection because the scanner is noisy. The bed will be moved until you are in the middle of the scanner tunnel. A mirror on top of the head cage will provide a view along the scanner tunnel, and a TV screen outside the scanner will be in your field of view. The scanning will take about one hour. There will be three scans that last nine minutes and two scans that last four minutes, with breaks in between. During the nine-minute scans you will see visual messages on the TV that tell you when the chemical vapour will be pumped into the mask. The chemical vapour will be turned off after 24 seconds on each of nine occasions during the scan. After each period of chemical vapour, visual information on the TV will appear asking you to rate your need to cough. During the four-minute scans you will constantly inhale chemical vapour.

Why were you invited to participate in this research?

The objective of this research is to localise the brain regions that respond to information coming from the airways. You have been approached to be involved because you are either a healthy person without any diseases involving your airways or because you have troublesome cough that is proving difficult to treat.

Source of funding

The pharmaceutical company Merck Sharp and Dohme Australia has provided funding for this research because they are interested in mechanisms that lead to excessive coughing in disease.

Consenting to participate in the project and withdrawing from the research

Participation in this research is voluntary. If you consent to be involved in this research, you will be asked to sign and return a Consent Form. By signing the Consent Form, you indicate that you:

- understand the information;
- give your consent to participate in the research project;
- consent to participate in the research processes that are described;
- consent to the use of your information as described

You have the right to withdraw from further participation in the research at any stage.

You will be given a copy of the Explanatory Statement and Consent Form to keep as a record.

Possible benefits and risks to participants

There will not be any direct benefits to you as a consequence of participating in this research. Information obtained during this research may eventually be used to develop new methods to control unwanted coughing.

Inhaling capsaicin and ATP causes irritation of the throat. The irritation quickly resolves after exhaling. The chemicals do not harm the tissues of the throat. The irritation is not painful.

There are no known risks from the MRI scanning except for dangers associated with metal implants such as cardiac pacemakers, prosthetic heart valves, implanted cardiac defibrillators, and vascular clips. The scanner generates a very strong magnetic field, so if you have metal implants you will not be able to participate. Bringing metal into the scanner can be dangerous because of the very strong magnetic field. You must not bring any metal into the MRI scanner. Before you go into the scanner, you will be thoroughly screened by an MRI

radiographer to ensure you are not at risk. There is no harmful radiation with MRI scanning and injections are not required.

It is not known if MRI scanning is harmful to the unborn child. Therefore, females should not participate in this project if you know or suspect that you are pregnant.

Some participants may experience minor discomfort during MRI scanning. Because the scanner operation generates a loud hammering noise, you will wear special earmuffs to reduce the noise level. In addition to the noise, some participants may find the tunnel shape of the scanner confining. In very rare cases, the experience may be so claustrophobic that it is not possible to continue. When you are in the scanner you have visual and verbal contact with the researchers and you also have a button to squeeze to stop the scanning immediately should you find it necessary.

What happens if something abnormal is found on my scans?

As part of your study we will obtain a limited number of pictures of your brain. Our research studies are designed to improve our knowledge of the brain. They are not designed for diagnostic or clinical purposes. After your scan, a specialist will examine these pictures (this will not be done on the day of your study). Minor changes are sometimes found in completely healthy people. You should be aware that because our pictures are taken for a specific purpose, not all abnormalities that might be detected by other MR scans are necessarily seen. On extremely rare occasions, we might find an abnormality that is significant. Prior to scanning we will ask you to complete a form to indicate what you would like us to do in the event that an abnormality is identified. If we find such an abnormality in your brain, and if you have indicated in advance that you wish to know, Prof Stuart Mazzone will call you at the contact number you supply and discuss the findings with you. Usually, such contact will be made within 2 weeks of your scan. Although a significant abnormality is extremely unlikely, you should be aware that if such an abnormality is detected and you are informed, then this knowledge might have consequences for you. Please take the time to consider carefully what it would mean to you if we told you of an abnormality in your brain which might, or might not, affect you later in life. Knowledge of an abnormality may affect your ability to work in certain professions, obtain life or health insurance and other facets of daily living.

Payment

We will offer you payment of $50 in the form of gift vouchers to reimburse you for your time involved in the study. You will receive this at the conclusion of the second session.

Confidentiality

Any information obtained in connection with this research that can identify you will remain confidential. It will only be disclosed with your permission, except as required by law. We plan to publish the results of this research in peer reviewed scientific journals and at specialised research conferences. In any publication, information will be provided in such a way that you cannot be identified. Information collected from individuals will be combined into group data for our analyses and reporting so your personal data will not be reported.

Storage of data

All data for this research will be stored in locked filing cabinets and/or secure electronic databases that can only be accessed by the researchers involved in the study or administrators of the electronic resources. These records will be maintained for seven years after the publication of study results, to allow sufficient time to resolve scientific queries and challenges.

Use of data for other purposes

It is possible that data collected for the purposes of this study may provide useful information for future research projects related to cough and brain structure and function. You can decide whether to approve your data being included in future research related to this project. Your approval will be considered in future applications to the relevant Human Research and Ethics Committee to undertake future research. If you agree to your data being used to answer related research questions in the future, all identifying information will be removed to protect your privacy.

Results

A report summarising the outcomes of this research will be made available to participants after the conclusion of the project. Only de-identified information will be included in the report.

Complaints

Should you have any concerns or complaints about the conduct of the project, you are welcome to contact the Manager, University of Melbourne Office of Research Ethics and Integrity:

Manager
University of Melbourne Office of Research Ethics and Integrity (OREI)
Research Ethics & Integrity
Level 4, 161 Barry Street
The University of Melbourne
VIC 3010

Tel: +61 3 8344 2073 Email: HumanEthics-Enquiries@unimelb.edu.au

Thank you,

Prof Stuart Mazzone

CONSENT FORM

Project Title:

Behavioural and brain haemodynamic responses to inhalation of capsaicin and adenosine tri - phosphate (ATP) in patients with idiopathic chronic cough.

Chief Investigator: Prof Stuart Mazzone
Department of Anatomy and Neuroscience
Phone: 8344 6457
email: stuart.mazzone@unimelb.edu.au
Human Ethical Approval number: 1852642

I have been asked to take part in the University of Melbourne research project specified above. I have read and understood the Explanatory Statement and I hereby consent to participate in this project.

I consent to the following:	Yes	No
Completing questionnaires about my daily experiences with coughing	☐	☐
Providing ratings of sensations associated with inhalation of vapour containing chemicals that cause throat irritation	☐	☐
Collection of images of my brain that can be used to show regions that respond to throat irritation	☐	☐
The data that I provide during this study may be used for future research projects	☐	☐

Name of Participant _____

Participant Signature _____ Date _____