Respiratory Impairment in Stroke Patients
Lung Function, Respiratory Muscles, Voluntary and Reflex Cough

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Awarding institution:
King's College London

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Respiratory Impairment in Stroke Patients: Lung Function, Respiratory Muscles, Voluntary and Reflex Cough

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Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy
Date of Award: 1st May 2012
Author’s Declaration

The studies described in this thesis were performed between 2007 and 2010. Intellectual contribution for these studies is shared between my supervisors, Professor John Moxham, Professor Lalit Kalra, Professor Michael Polkey and Professor Kerry Mills and myself. I worked with Drs John Seymour, Caroline Jolley and Joerg Steier and Mr Charles Reilly to collect the data. Mr Prashant Rao, a medical student, assisted me with many of the studies described in Chapter 5 as part of his BSc in Physiology. I received assistance with respiratory physiology measurement techniques from Dr Gerrard Rafferty and Mr Alan Lunt. I take full responsibility for the collection, analysis and presentation of the data. This thesis was written entirely by me with guidance from my supervisors.

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I am indebted to my parents and my sister, who have always helped and encouraged my academic endeavours and to the patients and volunteers who
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Katie Ward
29th September 2011
Abstract

Stroke is a major public health problem and stroke patients suffer much mortality and morbidity due to chest infections, especially in the acute period. Chest infections are associated with respiratory muscle weakness and poor cough. We studied ischaemic hemispheric stroke patients within two weeks of their first-ever stroke to investigate their respiratory physiology, volitional and non-volitional respiratory muscle strength and voluntary and reflex cough function.

Patients were weak on voluntary but not involuntary tests of expiratory muscle function. They were also impaired on tests of both voluntary and reflex cough. The data we collected suggests that impairment may be due in part to ineffective coordination of the complex cough manoeuvre, following cerebral ischaemia.

To further investigate the underlying reasons for impaired cough flow we studied functional residual capacity (FRC) in a group of stroke patients with mild impairments. In the semi-recumbent position patients’ FRC was significantly lowered, compared with healthy controls even in these acute patients little residual disability. The low FRC was strongly associated with low cough inspired volume and low cough inspired volume was associated with poor cough flow.

Transcranial magnetic stimulation was used to investigate the corticomotor projection to the abdominal muscles. We also designed a cough training protocol to be tried initially in the lab, to see if there is an effect of cough training on corticomotor excitability. This was a feasibility study in two patients; we make recommendations to increase the training duration to ten minutes and suggest how TMS could be used to assess the effect of training on corticomotor excitability. If an effect is shown in the lab across a number of patients, the training regimen could then be tried over longer periods in a clinical trial.

The face of stroke has changed completely since this work began in 2006. However despite new hyperacute stroke units and the widespread use of thrombolysis treatment, eighty per cent of stroke patients still do not receive it. Of those who do receive alteplase, it is estimated that there is clinical benefit for only one in seven. Hence the burden of disability is still heavy and the work remains relevant, especially for those at risk of chest infections in the first few weeks after stroke.
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<th>Definition</th>
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<tr>
<td>95% CI</td>
<td>95% confidence intervals</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>Angiotensin-converting enzyme inhibitor drug</td>
</tr>
<tr>
<td>AP</td>
<td>Anterior-posterior. If a chest radiograph is taken AP, the X-rays travel from in front of the patient to a film placed behind the patient's back. See also PA.</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence intervals (used in statistical tests)</td>
</tr>
<tr>
<td>CMAP</td>
<td>Compound Muscle Action Potential</td>
</tr>
<tr>
<td>CMT</td>
<td>Corticometabolic threshold, used in transcranial magnetic stimulation</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CXR</td>
<td>Chest Radiograph</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion Weighted Imaging</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expired volume in one second</td>
</tr>
<tr>
<td>FEV₁/FVC ratio</td>
<td>(FEV₁/FVC) * 100</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>He</td>
<td>Helium</td>
</tr>
<tr>
<td>IQ range or IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>L</td>
<td>Litres</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor evoked potential</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
</tr>
<tr>
<td>ms</td>
<td>milliseconds</td>
</tr>
<tr>
<td>mV</td>
<td>millivolts</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximum Voluntary Contraction</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health (USA)</td>
</tr>
<tr>
<td>NIHSS</td>
<td>National Institutes of Health Stroke Scale</td>
</tr>
<tr>
<td>NINDS</td>
<td>National Institute of Neurological Disorders and Stroke (USA)</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PA</td>
<td>posterior-anterior</td>
</tr>
<tr>
<td>PCF</td>
<td>Peak Cough Flow</td>
</tr>
<tr>
<td>Pdi</td>
<td>Transdiaphragmatic pressure</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow rate</td>
</tr>
<tr>
<td>PEmax</td>
<td>Maximal static expiratory mouth pressure</td>
</tr>
<tr>
<td>Pgas</td>
<td>Gastric pressure</td>
</tr>
<tr>
<td>PImax</td>
<td>Maximal static inspiratory mouth pressure</td>
</tr>
<tr>
<td>Pnasal</td>
<td>Nasal pressure</td>
</tr>
<tr>
<td>Poes</td>
<td>Oesophageal pressure</td>
</tr>
<tr>
<td>R²</td>
<td>Coefficient of determination, used in regression; a measure of how well an outcome can be predicted by a model</td>
</tr>
<tr>
<td>RC</td>
<td>Reflex cough</td>
</tr>
<tr>
<td>S₅₀</td>
<td>Refers to a TMS stimulus response curve fitted with a Boltzmann curve; S₅₀ is the stimulus intensity required to obtain a response 50% of the size of the maximum response</td>
</tr>
<tr>
<td>Sats</td>
<td>Haemoglobin Saturation</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SR</td>
<td>Stimulus-response</td>
</tr>
<tr>
<td>Sniff Pdi</td>
<td>Transdiaphragmatic pressure during a sniff</td>
</tr>
<tr>
<td>Sniff Pnasal</td>
<td>Nasal pressure (in the occluded nostril) during a sniff</td>
</tr>
<tr>
<td>Sniff Poes</td>
<td>Oesophageal pressure during a sniff</td>
</tr>
<tr>
<td>TES</td>
<td>Transcranial Electrical Stimulation</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
</tr>
<tr>
<td>TwT₁₀Pgas</td>
<td>Twitch T₁₀ Pgas; the rise in gastric pressure after magnetic stimulation over the T₁₀ spinal nerve roots</td>
</tr>
<tr>
<td>VC</td>
<td>Voluntary cough</td>
</tr>
</tbody>
</table>
Chapter 1  Introduction

This thesis investigates respiratory problems and cough impairment encountered in patients who are in the first two weeks after their first ever ischaemic stroke in the vascular territory of the middle cerebral artery.

1.1 DEFINITION OF STROKE

Attempts to define stroke have been ongoing since the 1970s. The World Health Organisation (WHO) clinical definition of stroke, first published in 1978 is: “a neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours” (1). Since then the definition has been further refined, reflecting the increased use and expertise of neuroimaging in the diagnosis of stroke and associated conditions. The terms stroke and ischaemic stroke are used in this report in place of a new name, Acute Ischaemic Cerebrovascular Syndrome (AICS) coined by the US National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH / NINDS) in 2003 (2). The NIH / NINDS guidance uses a new, more precise description for AICS: “acute onset of neurologic dysfunction of any severity, consistent with focal brain ischaemia and imaging/laboratory confirmation of an acute vascular ischaemic pathology.” Imaging confirmation includes neuroimaging studies demonstrating a recent, appropriately located ischaemic lesion [for example diffusion weighted imaging (DWI) or computed tomography (CT)]; vascular imaging demonstrating an acute arterial occlusion appropriate to the clinical syndrome [for example Magnetic Resonance (MR) Angiography or CT angiography] (2).

1.1.1 The Burden of Stroke

Stroke accounts for more than five million deaths a year worldwide (3). One hundred and fifty thousand people a year have a stroke in the UK; 69% of these strokes are ischaemic. A quarter of a million people live with disabilities caused by stroke and it accounts for 11% of all deaths in England and Wales (4). Most deaths after stroke are caused by complications of which chest infections are most important (5). 16% of UK stroke patients develop pneumonia during their inpatient admission (6). Aspiration is common after stroke and is associated with an eleven-fold increase in risk of chest infection (7). There is inconclusive evidence for dysphagia treatments after stroke; a Cochrane review found only two trials of swallowing therapy suitable for inclusion in the review (total 85 patients) and their results suggested that formal swallowing therapy did not reduce end-of-trial dysphagia rates (8). However dysphagic patients may be protected from chest
infections if they still have an effective cough, which serves to clear the airways of inhaled material (9). This thesis investigates cough abnormalities in stroke patients and suggests ways of improving ineffective cough. If cough can be improved it could result in fewer deaths from chest infections amongst recovering stroke patients, although testing that hypothesis is beyond the scope of this thesis.

1.1.2 Stroke Severity
Stroke related impairment can be assessed using a number of different severity scales (10). The National Institutes of Health Stroke Scale (NIH stroke scale or NIHSS) is a fifteen item neurological examination. It is a simple, valid and reliable clinical stroke assessment tool to evaluate neurological status at the bedside in acute ischaemic stroke patients. The maximum score is 31, reflecting the most severe impairment (11). NIHSS score can also be assessed retrospectively from the medical notes using a published algorithm; this method has been shown to be reliable and unbiased (12).

NIHSS score was used to describe stroke severity in all studies in this thesis because all stroke patients have a score performed on admission to the hospital as part of usual clinical care. Stroke severity at onset influences many outcomes including mortality, length of hospital stay and functional recovery (12, 13). The baseline (admission) NIHSS score strongly predicts the likelihood of a patient’s recovery after stroke. A score of 16 or greater forecasts a high probability of death or severe disability whereas a score of 6 or less forecasts a good recovery (14).

1.2 RECOVERY FROM STROKE-RELATED IMPAIRMENT
Most recent research in stroke medicine concentrates on neuroprotection and neurovascular intervention in the acute stage. However as more patients survive the initial event, research into neurorehabilitation and the mechanisms underlying recovery from stroke becomes ever more important.

1.2.1 Measuring Recovery and its Mechanisms
Recovery from stroke is extremely variable; some patients with initial severe hemiparesis may recover completely whereas others may have little or no improvement (15). There is usually some spontaneous recovery of both motor and cognitive impairments.

1.2.1.1 Spontaneous Recovery
Spontaneous recovery of movement after stroke is thought to be complete in the weeks to months following the initial event although patterns of recovery are extremely variable. The most dramatic improvements in motor function usually
occur within 30 days but in patients with more severe disability significant recovery can occur up to three months after stroke. A study of arm function found 95% of recovery had occurred within 9 weeks of stroke onset (16). After the acute phase when oedema is resolved, possibly allowing the ischaemic brain to achieve reperfusion, it is thought that much of the spontaneous recovery involves plastic changes in the brain (17).

1.2.1.2 Cortical Plasticity
Cortical plasticity can be defined as the ability of the human brain, either healthy or damaged, to change and reorganise as a result of experience, for example due to learning, after amputation, brain insult or injury. This could be any enduring change in cortical properties such as unmasking of previously silent intracerebral connections, long-term potentiation or depression of existing synapses, altered representation or changed neuronal territories (either morphological or functional) (18-20). Evidence for plasticity has been given using brain stimulation techniques such as transcranial electrical or magnetic stimulation (TES or TMS); electroencephalography (EEG); or imaging techniques such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET) or single photon emission computerised tomography (SPECT) (17, 21). Transcranial magnetic stimulation (TMS) and other techniques used to measure plasticity are discussed further in section 1.5 and in the Methods section.

1.2.2 Promoting Functional Recovery After Stroke
1.2.2.1 Driving Plasticity
Although some spontaneous recovery of function is usual in stroke patients, a major aim for those working in rehabilitation is to find ways to enable and encourage plasticity so that it occurs more quickly and completely and so reducing the length of time for which patients are at risk of complications and death (15). Stroke patients with delayed recovery of function are at high risk of venous thrombosis and chest infection, for example. Investigators have studied healthy humans, healthy animals, animal models of stroke and stroke patients to look at functional recovery and its neurophysiology correlates. A few key studies are described here and further discussion is included in section 1.6.4 after the neurophysiology techniques have been explained more thoroughly.

Nudo et al, working with adult squirrel monkeys, caused small focal ischaemic infarcts affecting a portion of the representation of one hand (22). They showed that retraining of skilled hand use after the infarct prevented loss of the hand
territory adjacent to the infarct, as would be expected to happen without retraining. In some monkeys the hand representations expanded to include territories previously representing the arm and shoulder. They also found functional reorganisation in the unaffected hemisphere accompanying the recovery of skilled hand use. The monkeys’ cortical representations were mapped using intracortical microstimulation (precise but rarely used in human studies) and EMG recordings from the muscles of interest (22). This important study was one of the first to give insight into mechanisms of recovery after stroke. Further studies have shown that when healthy animals are trained to perform a new task, there is evidence of task specific cortical reorganisation but motor tasks that do not require new skill acquisition (and do not involve cognitive effort) are not associated with any significant motor cortical changes (23, 24).

Lotze and colleagues compared a 30 minute training period of active training with 30 minutes of passive wrist movements in healthy humans, using TMS to measure motor cortex excitability (25). Active training led to significant improvements in motor performance whilst passive training did not. Although passive movements do not seem to lead to cortical changes, sensory stimulation has been shown to play a part in cortical plasticity: Fraser, Hamdy et al used transcranial magnetic stimulation and functional MRI to demonstrate that cortical plasticity of the swallow regions could be promoted in humans using (passive) electrical stimulation of the pharynx (18). The work is described further in section 1.2.3.

Constraining the unaffected upper limb during intensive therapy for hemiparetic stroke has been shown to lead to considerable functional gains in the affected arm, even in chronic stroke patients. In some studies functional gains were accompanied by cortical plasticity demonstrated using TMS (26). Whitall and colleagues developed bilateral arm training using rhythmic cueing (BATRAC) in patients with chronic hemiparesis (stroke onset 2-15 years prior to the trial) (27). They used some of the principles of constraint-induced therapy: the training was task specific (reaching and retrieving) and forced use of the affected arm but did not involve constraint. The authors used a metronome set at the patients’ own preferred speed to give auditory cues to the patients. More studies in healthy people and stroke patients are described in section 1.6.4.
1.2.3 Recovery of Swallow after Stroke

The study of impaired swallow after stroke is particularly relevant to the work in this thesis, which describes respiratory muscle and cough impairments post-stroke. The respiratory muscles are also midline structures and skeletal muscles, like the pharynx. Cough and swallow are both complex manoeuvres with centres in the brainstem but higher control by the cortex. In patients with impaired swallow, an intact cough can clear foreign material from the airways.

A series of elegant studies from Hamdy et al at the University of Manchester investigated impairment of swallow and its subsequent recovery after cortical stroke (28). Swallow is commonly impaired after stroke, affecting up to fifty percent of patients but adequate function is usually restored within two to six weeks (8). The propensity for recovery of swallow may be due to its bilateral hemispheric representation; it has been postulated this is because the swallowing process involves midline muscular structures such as the pharynx. Hamdy's work on healthy subjects showed that swallowing has a bilateral but asymmetrical hemispheric representation; the hemisphere with a prominent swallow area was independent of the subject's handedness (see Figure 21) (28). The group also studied a stroke patient with dysphagia in the acute and recovery stages and showed that recovery of swallow was associated with increased pharyngeal representation in the unaffected hemisphere; another stroke patient without dysphagia was shown to have a dominant pharyngeal region in the unaffected hemisphere (28). The group then went on to show that cortical plasticity in dysphagic stroke patients could be enhanced by electrically stimulating the pharynx for short periods of time. After stimulation, patients had increased motor evoked potential (MEP) amplitude elicited by the application of TMS to the area of cortex controlling the pharynx and improved swallow function (18, 29).

Jayasekeran, Hamdy et al recently published a randomized controlled trial of pharyngeal electrical stimulation compared with sham stimulation in acute stroke patients. The intervention group demonstrated improved swallow function on videofluoroscopy, two weeks after completing three sessions of pharyngeal electrical stimulation (PES) (30). Patients who received PES also had a significantly reduced length of hospital stay compared with controls that did not receive the intervention. The stimuli (0.2ms pulses, 280V) were delivered at a set frequency of 5 Hz at 75% of maximum tolerated intensity for ten minutes, as reported by Fraser (18).
Hamdy’s studies describing passive electrical stimulation driving plasticity and improving pharyngeal function are in contrast to other authors’ failure to find functional improvements after passive electrical stimulation of limb muscles. For example in one study in healthy people the authors state that motor cortex excitability after repetitive common peroneal stimulation depends on voluntary drive (31). EMG triggered neuromuscular stimulation shows some promise; Kimberley et al using electrical stimulation applied to the median nerve on the hemiparetic side in stroke patients showed improved hand function and positive fMRI changes (32). In hemiparetic stroke patients with foot drop electrical stimulation of the peroneal nerve whilst walking leads to improved function and there are reports of patients whose foot drop remains improved after removal of the stimulator, suggesting motor relearning. Further studies are needed to confirm the suggested mechanisms (23, 33).

1.3 RESPIRATORY MUSCLES

The respiratory muscles and cough are described in this section, along with methods by which they may be studied and a review of previous research on respiratory muscles and cough in neuromuscular and stroke patients. The respiratory muscles drive ventilation by working together to achieve changes in the dimensions of the thorax and aid both inspiration and expiration; they are illustrated in Figure 1 (34). They are under the control of the respiratory centres in the brainstem, medulla and pons and the autonomic nervous system. The anatomy and peripheral nerve supply of the inspiratory and expiratory muscles will be discussed below. The cortical control of the respiratory muscles will be discussed after a description of the primary method used to study them, transcranial magnetic stimulation. The cortical control of these muscles is especially relevant to this thesis as the patients studied had all suffered strokes affecting the cerebral cortex.
1.3.1 Respiratory Muscles: Inspiratory

The diaphragm is a skeletal muscle, anatomically separating the thoracic and abdominal cavities and is the principal muscle required for inspiration. It is illustrated in Figure 2 (35). When the diaphragm contracts during normal breathing it descends 1 to 2cm towards the pelvis. In deep inspiration this can increase to 10cm. Together with the contraction of the external intercostal muscles the movement serves to increase the volume of the thoracic cavity. This increase in volume leads to a drop in intrathoracic pressure; the intrathoracic pressure falls below the external air pressure and this leads to air being drawn passively into the lungs (36).
Peripheral nerve supply to the diaphragm

The diaphragm receives its motor supply from the two phrenic nerves, one on each side. The phrenic nerves originate from the fourth cervical nerve but also receive contributions from the third and fifth cervical nerves (35). An illustration of the phrenic nerves in the neck is given in Figure 3 (37) and their course is shown in Figure 4 (35).

Figure 2 Inferior view of the diaphragm
Image from Gray 1918

Figure 3 The course of the phrenic nerves in the neck and upper thorax
Image adapted from Qvist 1977
1.3.3 Respiratory Muscles: Expiratory

The anterior abdominal wall consists of four muscles with an expiratory respiratory function. These are the internal and external obliques and the transverse and rectus abdomini (Figure 5 and Figure 6) (35, 38).Expiration during quiet breathing is mostly passive. From EMG studies with individual needle electrodes placed in each of the four muscles it appears that the transversus abdominis is active during both quiet breathing and forced manoeuvres. The external obliques and rectus abdomini appear inactive during resting breathing in some subjects but they are always recruited for forced manoeuvres such as cough (38).
1.3.4 Peripheral nerve supply to the abdominal muscles
The abdominal muscles receive their motor nerve supply mainly from tenth (T10), eleventh (T11) and twelfth thoracic nerves (T12). This is illustrated in Figure 7, adapted from Gray (35).

Figure 5 Muscles of the abdominal wall
The internal oblique is not shown but lies superficial to the transversus abdominis and deep to the external oblique, see Figure 6. Image adapted from Gray 1918.

Figure 6 Ultrasound Image of the Ventrolateral Wall of the Abdomen
Image adapted from De Troyer 1990
1.4 TESTING RESPIRATORY MUSCLE FUNCTION

Muscles contract to produce force. The force produced by the respiratory muscles can be measured by pressure catheters placed in the thoracic cavity (catheter usually in the oesophagus) and in the abdominal cavity (catheter placed in the stomach). The pressure across the diaphragm (Pdi), a specific measure of diaphragm contraction, can be calculated by subtracting the oesophageal pressure from the gastric pressure. Pressures are conventionally stated as a difference from barometric pressure (39).

Respiratory muscle strength can be tested using volitional and non-volitional methods. Volitional methods require participants to be awake, to understand instructions on how to perform the tests and to make maximum efforts. They are tests of overall respiratory muscle function and as such are integrated tests of the motor cortex, corticospinal tracts, motor neurons, peripheral nerves and respiratory muscles working together to produce a force. An abnormal result on testing could be due to a problem at any of these levels or due to lack of patient understanding or effort.

It is useful to look at groups of respiratory muscle tests rather than a single test (e.g. PI max and sniff tests for inspiratory muscles) when assessing respiratory...
muscle strength as some people may perform badly on some tests and well on others (40).

1.4.1 Volitional Tests

Volitional tests such as maximum inspiratory mouth pressure (PI max) or maximum expiratory mouth pressure (PE max) can provide an assessment of the strength of all the inspiratory (for PI max) or expiratory muscles (for PE max) working together. Mouth pressure tests are generally easy to perform but PI max and PE max tests require patient cooperation and a good mouth seal. If there is an unobstructed passage from the mouth to the lungs (e.g. no airway resistance), mouth pressures give a reasonable reflection of alveolar pressure and oesophageal pressure (39).

Results of lung function tests can also suggest respiratory muscle weakness. For example, a low vital capacity when lying flat is typically seen in patients with diaphragm weakness due to neuromuscular disease such as motor neurone disease or Duchenne muscular dystrophy (41). These are described further in section 1.9. It is usual for a number of different tests (volitional and non-volitional and lung function tests) to be used in the evaluation of a subject with suspected respiratory muscle dysfunction (40). Although normal ranges for results of respiratory muscle tests have been produced the cohorts used to produce them are small and it may be better to use cut-off values as shown in Figure 8 (40).

1.4.2 Non-volitional tests

1.4.2.1 Peripheral nerve stimulation

Non-volitional tests of muscle function do not require patient cooperation or effort so can be used in sedated patients, for example. They are also useful when it is unclear whether a subject is making a maximum effort. By stimulation of the peripheral nerve supplying a muscle or group of muscles e.g. phrenic nerves to test diaphragm, T10 nerves for abdominals using electrical or magnetic stimulation, the normal muscle contracts and produces force. Nerve stimulation is introduced in section 1.5. For the respiratory muscles the force produced by nerve stimulation is usually measured as a change in pressure. However these are tests of the function of the peripheral nerve and the supplied muscle together with the neuromuscular junction. Therefore an abnormal result could be due to peripheral neuropathy, neuromuscular junction disturbance or muscle weakness, or a combination of all three. By looking at the latency of compound muscle action
potentials (CMAPs) evoked when the nerve supplying that muscle is stimulated, the speed of nerve conduction can be estimated (see 1.5.3.1 for explanation of CMAPs). In a patient with respiratory muscle weakness, peripheral neuropathy is unlikely if nerve conduction speed is normal but disease at the neuromuscular junction e.g. myasthenia gravis should be excluded before diagnosis of a pure muscle disorder.

1.4.2.2 Cortical stimulation

If the cerebral motor cortex is stimulated, either directly during neurosurgery or transcranially using electrical or magnetic stimulation the function of the motor cortex, corticospinal tracts, motor neurons, peripheral nerves and respiratory muscles working together can be tested in a subject who is sedated or who may not be able to follow instructions.

Figure 8 Cut-off Values for Diagnosis of Weakness on Respiratory Muscle Testing
Table from Steier 2007

<table>
<thead>
<tr>
<th>Test</th>
<th>Sex</th>
<th>Calculation</th>
<th>Cut off (cm H₂O)</th>
<th>Rounded (cm H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmax</td>
<td>M</td>
<td>10.4–1.96 × 3.0 kPa</td>
<td>44.8</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>7.2–1.96 × 2.1 kPa</td>
<td>31.6</td>
<td>30</td>
</tr>
<tr>
<td>Sniff Poes</td>
<td>M</td>
<td>105–1.96 × 26 cm H₂O</td>
<td>54.0</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>92–1.96 × 22 cm H₂O</td>
<td>48.9</td>
<td>50</td>
</tr>
<tr>
<td>Sniff Pnasal</td>
<td>M</td>
<td>0.91 × 55 cm H₂O</td>
<td>50.1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.91 × 50 cm H₂O</td>
<td>45.5</td>
<td>45</td>
</tr>
<tr>
<td>Sniff Pdi</td>
<td>M</td>
<td>148–1.96 × 24 cm H₂O</td>
<td>101.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>121–1.96 × 25 cm H₂O</td>
<td>72.0</td>
<td>70</td>
</tr>
<tr>
<td>Twitch Pdi</td>
<td>M &amp; F</td>
<td>28–1.96 × 5 cm H₂O</td>
<td>18.2</td>
<td>18</td>
</tr>
<tr>
<td>Pmax</td>
<td>M</td>
<td>14.4–1.96 × 3.3 kPa</td>
<td>80.5</td>
<td>80</td>
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<tr>
<td></td>
<td>F</td>
<td>9.1–1.96 × 1.6 kPa</td>
<td>61.1</td>
<td>60</td>
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<tr>
<td>Cough Pgas</td>
<td>M</td>
<td>214.4–1.96 × 42.2 cm H₂O</td>
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<td></td>
<td>F</td>
<td>165.1–1.96 × 34.8 cm H₂O</td>
<td>96.9</td>
<td>95</td>
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<tr>
<td>Twitch T10</td>
<td>M &amp; F</td>
<td>x=1.6–1.96 × 0.20re-transformation: y=10^x</td>
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<td>16</td>
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Pmax, maximum inspiratory pressure; Poes, oesophageal pressure; Pnasal, nasal pressure; Pdi, transdiaphragmatic pressure; Pmax, maximum expiratory pressure; Pgas, gastric pressure.

Detailed methods of testing respiratory muscles are described in the Methods section (Chapter 2) and in the American Thoracic Society guidelines (39). An explanation of electrical and magnetic brain and nerve stimulation, used in non-volitional testing, is given below.
1.5 ELECTRICAL AND MAGNETIC STIMULATION OF NERVOUS TISSUE

Stimulation of peripheral nerves using electricity can be used to test the strength of the muscle that the nerve supplies, provided the nerve function is normal. The motor cortex can also be stimulated to evaluate the summed response of the brain, corticofugal pathways, peripheral nerves and muscles. This concept is introduced in 1.4.2. Electrical currents can be applied via needle electrodes, implanted electrodes or surface pads to stimulate nerves. Currents can also be applied to brain tissue during craniotomy (direct stimulation) or via scalp surface electrodes in awake subjects (transcranial electrical stimulation) (42). Merton and Morton showed that stimulation over the motor cortex could produce a twitch of contralateral limb muscles. However, the use of electrical currents for stimulation of the cerebral cortex has been limited because of considerable discomfort caused. The skull has 8-15 times the resistivity of soft tissues and so offers a significant barrier to applied electrical stimuli; as a result large currents are required for transcranial electrical stimulation to produce an effect (43). The large currents cause pain due to stimulation of nerve endings in the skin and superficial muscles.

In the early 1980s, the development of a magnetic stimulator by Polson et al made it possible to induce electrical currents in peripheral nerves with a minimum of discomfort (44). In 1985, Barker announced the development of a magnetic stimulator capable of non-invasively stimulating the human motor cortex through the skull in a letter to Nature, attracting a huge amount of interest (43). The magnetic field induced by such stimulators can pass through high resistance structures such as the skull without being diminished so the induction of current in other nearby structures is minimal and stimulation is virtually pain-free. Barker’s method of non-invasively stimulating the brain using electromagnetism was named transcranial magnetic stimulation (TMS). A large body of research literature about motor control using TMS has now accumulated and it is widely accepted as the principal method of non-invasive brain stimulation, especially for the study of motor control (45). A brief description of magnetic stimulation and its use for respiratory muscle testing follows.

1.5.1 Magnetic Stimulation: Mechanism of Action

Magnetic stimulation is based on the principle of electromagnetic induction, the production of voltage in a conductor moving through a magnetic field. In 1831
Michael Faraday showed that currents and voltages were induced in a conductor by a changing (time-varying) magnetic field (46). In the case of magnetic stimulation of the human nervous system the conductor is nervous tissue, the brain and peripheral nerves. The stimulator itself consists of a large capacitor, charged up from the mains supply of electricity, connected to a large wire coil. When the charge stored in the capacitor is discharged there is a brief surge of current through the coil. The current surge reaches a peak value of about five thousand amperes and lasts for up to 1 millisecond but 90% of the discharge occurs within the first 100 microseconds. This rapid discharge is crucial as it is the rate of change of the magnetic field which causes the electrical current to be generated in the tissue (47). The coil produces a magnetic pulse with field strength of up to 4 Tesla. The directions of forces produced by a circular coil are shown in Figure 9. As the lines of force pass through tissue a current is generated; if this is of sufficient amplitude and duration that the cell membrane is depolarized, neural tissue will be stimulated and an action potential produced in the same way as with electrical stimulation (47).

Figure 9 Circular Coil Showing Lines of Force Generated when Current Flows through the Winding

Image from the MagStim Company

1.5.2 Stimulating Coils

1.5.2.1 The Circular Coil

A 90mm circular coil is suitable for stimulation of the motor cortex and the spinal nerve roots. It produces a peak magnetic field strength of 2 Tesla; See Figure 9 and Figure 42. In expiratory muscle testing, the circular coil is used for peripheral magnetic stimulation over the tenth thoracic spinal nerve (T₁₀) roots. The rise in abdominal pressure after stimulation over the T₁₀ roots is a non-volitional measure
of abdominal (expiratory) muscle strength in subjects with normal peripheral nerves.

1.5.2.2 **Double Coils: Figure of Eight (Flat) and Double Cone (Shaped)**

Double coils have two copper windings placed side by side in a figure-of-eight or double cone configuration. Their advantage is that the induced current is at its maximum directly under the “waist” of the pair of coils so the point of greatest stimulation can be more accurately defined. Small figure-of-eight coils produce a relatively weak and less penetrating magnetic field; however the coils are well suited to stimulation of the phrenic nerve in the neck. Measurement of the pressure change across the diaphragm after magnetic stimulation over the phrenic nerve in the neck is a non-volitional test of diaphragm strength. The double cone coil (Figure 50) has a flat central section and angled sides, which are designed to fit the subject’s head near the vertex. The angled sides allow for better magnetic coupling and higher induced current in the central fissure. Figure 10 shows the magnetic field produced by the double cone coil; the lines of force are concentrated under the central section. It is used for bilaterally stimulating the motor cortex areas which control the lower trunk and limbs (47).

**Figure 10 Diagrams Showing the Concentration of the Lines of Force Produced by the Double Cone Coil**

Image from the MagStim Company

1.5.3 **Peripheral Magnetic Stimulation: Measures Made**

1.5.3.1 **Compound Muscle Action Potentials**

Motor nerve conduction is evaluated by recording and measuring the compound muscle action potential (CMAP) associated with contraction of a given muscle, in response to an electrical or magnetic stimulus of sufficient size to the motor nerves supplying the muscle. The evoked muscle contraction is known as a twitch. The
CMAP is the sum of all the action potentials from all the individually contracting fibres that make up the muscle and it is recorded from a pair of surface electrodes on the skin overlying the muscle, connected to electromyography (EMG) recording equipment. The method for this is described in the Methods section (2.9.6). A CMAP recorded from abdominal muscle is shown in Figure 44. The latency and amplitude of the CMAP are read from the computer.

1.5.3.2 Twitch pressures
The force of contraction of a muscle after stimulation of a peripheral nerve can give an assessment of muscle strength, independent of patient effort. The changes in intrathoracic and intra-abdominal pressure evoked by stimulating the motor nerves supplying the diaphragm and abdominal muscles are used to make a non-volitional assessment of diaphragm or abdominal muscle strength. Stimulation of the T₁₀ nerve roots over the spine and measurement of the evoked rise in gastric pressure (Pgas) is a well-described method of measuring expiratory or abdominal muscle strength. This test, sometimes known as TwitchT₁₀ Pgas, is described in section 2.9.5 (39). The results of Twitch T₁₀ Pgas are reproducible but often not supramaximal (48). Twitch potentiation, the augmentation of twitch tension following recent muscular contraction, (49, 50) should be avoided by resting the participant for twenty minutes prior to twitch tests.

1.5.4 Transcranial Magnetic Stimulation and Measures Made
When TMS is applied to the motor cortex, the coil produces a magnetic field in the motor cortex and this excites the corticospinal tract either pre- or trans-synaptically. When TMS is applied at stimulus intensities above the motor threshold so that sufficient pyramidal neurons are activated, alpha motor neurons in the spinal cord are depolarised and a response is elicited in the muscle of interest. This evoked electrical signal can be recorded using standard electromyography recording equipment and is known as the motor evoked potential or MEP. Signals recorded from muscle, after TMS is applied to the motor cortex are the result of electrical activity induced in the motor cortex being transmitted to the muscle via the corticospinal projection (i.e. axons arising from the cortex and terminating in the spinal cord), motor neurons in the spinal cord and peripheral nerves. Thus for the MEP to be a reflection of motor cortex or corticospinal excitability the motor neurons, peripheral nerves and muscle need to be intact and functioning normally. This is presumed to be the case for stroke patients with no other nerve or muscle disease. The area under the curve of the
rectified signal, the peak-to-peak amplitude and the latency of the signal can all be measured (39, 51) (Figure 55). These and other ways of using TMS to study the motor cortex and their possible meanings are described below.

### 1.5.4.1 Corticomotor Threshold

In physiological terms, the corticomotor threshold (CMT) is the lowest stimulus intensity that is required to produce a MEP of a defined size in the muscle of interest (52, 53). It represents the stimulus intensity required to activate the most excitable parts of the cortex, spinal cord and motoneurons (54). The resting corticomotor threshold (rCMT) reflects the excitability and the membrane characteristics of the cortico-cortical axons and their excitatory synapses with the motor cortical output neuron (55). A raised CMT could indicate reduced excitability of either of these components (51). Threshold can be measured on resting or active muscle and threshold for activated muscle is usually lower, see section 1.5.5.1. CMT is thought to be independent of age in adults (51). CMT has been shown experimentally to be a stable parameter, over periods of hours, days and up to five years for both individuals and groups (52). The method for finding the CMT is described in 2.12.9. Several studies have shown CMT to be raised after stroke, see section 1.6.

### 1.5.4.2 Cortical Topography: Mapping

Penfield showed that the motor cortex is somatotopically organised and there have been studies attempting to verify this using electrical and magnetic motor cortical stimulation (51). However, whilst trying to confirm Penfield’s findings Humphrey showed that the motor cortex is organised in terms of movements rather than muscles and individual motor cortex pyramidal cells connect to a number of different muscles (56).

To perform mapping studies, small figure-of-eight coils are required to achieve the focused and oriented stimulation required, but these coils have less depth of penetration than other coils. Using the figure of eight coil, it may be difficult to obtain any response at all from study participants with a high CMT, due to stroke for example (57). Many investigators complement their TMS mapping with fMRI studies which look at oxygen utilisation in brain regions of interest during specific tasks. Mapping is not further described in this thesis; Ridding and Rothwell propose that the information gained from mapping studies is equivalent to that obtained from motor cortical stimulus-response (SR) curves, described in section 1.5.4.5 below. SR curves may be less time consuming to elicit and more tolerable
for study participants as the coil stays in one place and the threshold does not need to be found before the study can start, as it does with mapping (20).

1.5.4.3 Twitch Pressures
The evoked force produced by the muscle twitch resulting from TMS over the motor cortex can be measured. For the abdominal muscles the rise in intra-abdominal pressure evoked by TMS can be measured using an intra-gastric pressure balloon, see section 2.8 (58).

1.5.4.4 Silent Period
In actively contracting muscles, a pulse of TMS induces an electrical silence that interrupts the ongoing background EMG activity. The duration of this inactivity is known as the silent period. Most of the studies described are of resting muscle (to enable inclusion of all stroke patients) so silent period was not measured. The silent period is an inhibitory phenomenon and we chose to study inhibition using paired-pulse TMS rather than silent period as both give similar information but with short interval paired pulse stimulation, inhibition can be seen with both active and resting muscles.

1.5.4.5 Motor-Cortex Stimulus-Response Relationships
Motor-cortical stimulus-response (SR) curves plot the size of the MEP response (e.g. area under the curve or amplitude) or muscle twitch response evoked by TMS of a fixed scalp site at a range of different stimulator intensities (20). They are also known as recruitment curves, dose-response curves or input-output curves. The relationship between stimulus intensity and evoked potential or force can be studied at rest and with voluntary background muscle contraction.

Boltzmann Sigmoidal Curve
SR relationships which take the form of a Boltzmann sigmoidal curve have been shown for the diaphragm (59) as well as for tibialis anterior (TA), the first dorsal interosseus (FDI) (60) and many other skeletal muscles; see Figure 11. The features of the curve can be described by the threshold, slope and plateau level, see Figure 11 and Equation 1 (54). The equation for the Boltzmann curve is given in Equation 1. In the equation, as written here, MEP amplitude is the response of interest, to be plotted on the y-axis and S is the stimulus intensity, to be plotted on the x-axis. It should be noted that to produce a sigmoidal curve, the size of the response has to reach a plateau and for individuals and muscles with a high threshold to TMS it may not be possible to give a large enough stimulus to get a maximal evoked response before reaching the maximum stimulator output. This
can be seen in one of the subjects in the TA panel of Figure 11. If the responses do not plateau, sigmoidal curve fitting may not be appropriate and a straight line may better describe the SR relationship.

SR curves are thought to provide a relatively sensitive overall measure of the excitability of the corticospinal projection. The slopes of SR curves are steeper in muscles with a large representation in the motor cortex (61). Ridding and Rothwell showed that increases in the cortical map area following ischaemic anaesthesia are accompanied by concomitant increases in the slope of the SR curve (62).

Some explanations of why the relationship takes a sigmoidal shape are given by Devanne (54). The plateau value is probably not the maximum response that could be obtained if the corticospinal volley was purely excitatory. Instead, it is likely to represent a balance between excitatory and inhibitory components of the corticospinal volley (54). The steepness of stimulus response curves can be increased by voluntary facilitation; facilitation may also lead to a higher plateau for a given individual and muscle (54). Devanne demonstrated stability of the stimulus response curve for the TA when the muscle was studied twice, 90 minutes apart (54).

**Equation 1 The Boltzmann Sigmoidal Non-Linear Regression**

The Boltzmann equation describing the relationship between MEP amplitude, plotted on the y-axis and magnetic stimulus intensity,(S) plotted on the x-axis is:

\[
\text{MEP amplitude (y)} = \frac{\text{MEP amplitude}_{(\text{max}-\text{min})}}{1 + e^{\frac{S_{50}-S}{\text{slope}}}}
\]

\(S_{50}\) is the stimulus at which \(y\) (the MEP amplitude) is halfway between \(y\) max and \(y\) min

The maximal steepness of the curve occurs at \(S_{50}\)
Area under the Curve

The area under the stimulus-response curve (AUC) is a useful way of summarising the information for a series of measurements in one individual, as would be required with our planned cough training study. The AUC outcome measure is frequently used in clinical pharmacology but has also been used in TMS studies (63, 64). The area under the curve is thought to be a robust overall measure of corticospinal output and projection strength. Calculation of the AUC is illustrated in Figure 53 (64).

1.5.5 Modifying the Motor Evoked Response by Voluntary and Involuntary Methods

1.5.5.1 Facilitation by Voluntary Muscle Activation

Voluntary contraction of the muscle being studied, whilst the TMS is being administered, has been shown to cause increased size (measured as area or amplitude) of the MEP for any given stimulus intensity. This phenomenon is known as facilitation. MEPs from contracted muscle are also more reproducible and occur at lower corticomotor thresholds (CMTs) (51). The relationship between the amplitude of the MEP and the level of muscle contraction (measured as background isometric force or background spontaneous EMG) has been described for several muscles. In the hand muscles facilitation rises sharply at low levels of force and reaches a maximum at about 10% of maximum voluntary contraction.
In contrast, for biceps the response amplitude continues to increase as background force rises to 25% of maximum voluntary contraction (MVC) and for the diaphragm the plateau is reached somewhere between 40 and 60% of MVC (51, 59). It is postulated that the increase in size of the MEPs is due to a greater proportion of the total motoneuron pool being accessed by the descending corticospinal volley. This could be because the volley itself is larger or because of increased accessibility of the motoneurons pool due to increased overall excitability (51).

The lowered CMT which occurs with activation of the muscle is thought to be due to the fact that the CMT is a function of the most excitable motoneurons and these are exactly the ones that become active first at low levels of motor activity (54).

The latency of the response shortens with voluntary facilitation. This can be by as much as 5ms in the lower limb muscles (51).

Muscle responses can also be facilitated by contraction of muscles remote from the target muscle; for example Hess et al showed that MEPs from the right abductor digiti minimus (ADM) were facilitated by contraction of the left ADM (65). This method of facilitation may be useful in stroke patients with a hemiplegia. Thinking about moving the muscle of interest has also been shown to cause small but significant increases in MEP amplitude, although it is impossible to quantify or assess quality of the “thinking” (51). As stroke patients may not be able to contract their muscles on demand and thoughts of moving muscles are difficult to assess, most of the studies in this thesis are of resting muscle.

1.5.6 Paired-Pulse TMS

Pairs of stimuli can be applied to the motor cortex to study motor cortex excitability. The first stimulus, (the conditioning stimulus) of a pair has a modulatory effect on the second (test) stimulus. The intensity of both stimuli and the interstimulus interval affect the observed response. Intra-cortical inhibition, (decreased size of MEP amplitude) and intra-cortical facilitation (increase in MEP amplitude) with paired-pulse TMS are indicators of motor cortex excitability. These phenomena have been shown for many muscles including diaphragm (66) and adductor pollicis brevis (67).

With inter-stimulus intervals of 6-20 milliseconds, a conditioning stimulus of 70-90% of resting corticomotor threshold and a suprathreshold test stimulus facilitation usually occurs (61, 68, 69). The facilitation has been shown to occur in the motor cortex, although the exact mechanism is uncertain (70).
A short interval (1 to 5 milliseconds) between a sub-threshold conditioning stimulus and a supra-threshold test stimulus usually causes inhibition; the phenomenon is known as short-interval intracortical inhibition (SICI) (68, 71, 72). For maximum suppression a conditioning stimulus of 70-90% of resting corticomotor threshold is used. The inhibition was demonstrated to be cortical (rather than spinal) in origin by Di Lazzaro in 1998 and is thought to occur by activation of local cortico-cortical inhibitory circuits (72). GABA agonist drugs, such as benzodiazepines and alcohol can cause increased intracortical inhibition (73, 74).

1.5.7 Ipsilateral Responses to TMS
Contralateral MEP responses to TMS are expected and usual but ipsilateral responses are also well documented. It should be noted that it is very difficult to prove that cross-stimulation of the opposite hemisphere or volume conduction of the muscle response from the opposite side has not occurred and led to the production of the so-called ipsilateral response, especially in healthy people. Ipsilateral responses in healthy people are more likely to be recorded for midline muscles such as the abdominals and the pathways are thought to be more proximal than distal (75). Separate studies by Carr, Strutton and Harraf found ipsilateral responses to abdominal muscles in 80-100% of healthy people (58, 76, 77).

1.5.8 Effect of Gender, Age, Height and Handedness on TMS Parameters
Corticomotor threshold is independent of age in adults. Latency is not thought to correlate with age, after height is taken into account. Females have shorter latency to the lower but not the upper limbs (51). Conduction time from the motor cortex to a muscle depends on the distance between the two so there will be a relationship between MEP latency and height (78). Studies comparing left and right-handed subjects found that in right-handed people the corticomotor threshold was lower for activation of the muscles of the right arm and in left-handed people the threshold was lower for muscles of the left arm. However the threshold asymmetry was significantly influenced by how often the participants used their writing hand to perform other tasks (51).

1.6 TRANSCRANIAL MAGNETIC STIMULATION AND STROKE
Some of the changes observed in TMS-evoked potentials after stroke are discussed in section 1.2.1 and TMS studies of the respiratory muscles are described in sections 1.7 and 1.8. In summary, stroke patients often have absent
MEPs, or delayed and reduced amplitude MEPs when the affected hemisphere is stimulated using TMS. Corticomotor threshold is commonly raised (51, 79) and ipsilateral corticofugal pathways may be demonstrated.

1.6.1 Ipsilateral responses to TMS in Stroke Patients
In acute hemispheric stroke patients, TMS over the unaffected hemisphere may produce ipsilateral MEP responses in muscles on the hemiparetic side, in patients for whom there were no contralateral responses when the affected hemispheres were stimulated (58, 80). Ipsilateral MEP responses (from the hand) in Caramia’s study were only seen in acute stroke patients and not the control group. Ipsilateral responses may play an important role in motor recovery after stroke although it is unclear whether they are always beneficial (19).

1.6.2 Paired-Pulse Stimulation and Stroke
Paired-pulse stimulation has been used to facilitate MEPs from the affected side of the body but it can also be used to study the unaffected side. Paired-pulse stimulation was been used to demonstrate disinhibition of the unaffected hemisphere in acute stroke patients and in rats with MCA infarcts (67). In the rats, GABA receptors were downregulated and glutaminergic activity was enhanced (81). Liepert and colleagues studied the abductor pollicis brevis muscle (APB) and found evidence of motor cortex disinhibition (that is less inhibition with paired stimuli at short interstimulus intervals) in stroke patients who were compared with a healthy control group. The paired stimuli were at 95% and 130% of the active motor threshold. The active motor threshold was equivalent to the CMT, determined with the APB isometrically contracting at low force. Decreased intracortical inhibition may play a part in cortical plasticity and recovery after stroke (67, 82). A reduction of GABAergic transmission may contribute to the disinhibition in stroke patients; recovered stroke patients who are given the GABA\textsubscript{A} agonist midazolam suffer a transient recurrence of their original symptoms (83).

1.6.3 Stimulus-Response Curves in Stroke Patients
Liepert et al studied stroke patients with pure motor stroke and very mild impairments; the patients had MRC graded strength of at least 4 in both upper and lower limbs (79). They subdivided patients according to the region of infarct (shown on MRI scans) as follows: M1L patients (n=7) had an embolic lesion in the hand area of the primary motor cortex; SCL patients (n=13) had embolic lesions affecting the basal ganglia and internal capsule; ICL patients (n=13) had lacunar lesions in the internal capsule at the level of the basal ganglia; PL patients (n=10)
had paramedian pontine infarcts. Stimuli were given over the point of optimum excitation on both sides of the brain and MEP responses recorded from the first dorsal interosseous muscles. MEPs from the unaffected side, for stimuli at all stimulus levels (110 to 150% of CMT) were significantly larger in amplitude than those from the affected side in patients with internal capsule and pontine lesions. For those with striatocapsular lesions, there were no significant differences in MEP amplitude between the affected and unaffected sides. For the smaller group with motor cortex lesions, the MEPs from the unaffected side tended to be bigger but the difference was only significant for stimuli at 150% of corticomotor threshold. See Figure 12 for illustration of this (79).

**Figure 12 Stimulus Response Curves in 4 Different Stroke Groups**

The figure shows stimulus-response curves for TMS stimuli given over the point of optimum excitation on the affected and unaffected side of the brain and responses recorded from the first dorsal interosseus muscle on the contralateral side.

Key for Figure 12
Black symbols represent the affected side and open symbols represent the unaffected side.
* signifies a significant difference (P<0.05) between the affected and unaffected sides. From Liepert 2005

### 1.6.4 Use of TMS to Measure Motor Cortical Plasticity

Plasticity is introduced and defined in section 1.2.1.2. Evoked signals produced by TMS applied to the motor cortex can change after an insult or intervention because of changes in excitability in the cortex or corticomotor projection as well as because of changes in organisation, eg changes in cortex circuitry. It should be remembered that excitability effects can arise due to changes of the tonic input to
the motor cortex from other structures known to have influence, as well as because of changes in the cortex itself (20).

Evidence of an increase in excitability seen by TMS studies could be any or all of: decreased CMT, increased MEP amplitude for a given stimulus (where stimulus intensity is a set multiple of CMT), a shift of the SR curve to the right along the x axis, an increase in the slope of the SR curve and increased area under the SR curve.

fMRI, PET and SPECT (introduced in 1.2.1.2) are indirect methods of tracking plasticity as they look at cerebral blood flow rather than the motor cortical output, which can be directly studied with TMS. Problems with all the methods are the possible instability of the recorded signals over time in normal people without any intervention.

1.6.4.1 Healthy Humans and Training

Careful mapping at two separate time points (perhaps with an intervention in between) may indicate changes in the area of response to TMS for any given muscle. Maps can be modulated by motor learning as demonstrated by Pascual-Leone et al who gave subjects a one-handed, five fingered piano exercise to practice for five days (84). Fraser and colleagues showed an increase in the pharyngeal area of response to TMS one hour after a short period of electrical stimulation applied to the pharynx (18). They also showed increased amplitude of MEPs from the pharynx after electrical stimulation to that area and stated that this represented increased corticobulbar excitability (18). Whether the increased map area and larger MEPs are demonstrating one or two phenomena is not clear. Ridding and Rothwell showed that changes in the size of cortical maps are related to changes in cortical excitability (measured by a SR curve) and changes in cortical excitability can produce changes in the size of cortical maps. Indeed they argue that changes in topography may simply be a result of altered excitability because map changes are only apparent in resting and not in active muscle (20).

1.6.4.2 Stroke Patients: Spontaneous Recovery

It is thought that plastic changes in the damaged hemisphere are more likely to lead to better recovery from stroke than changes in the undamaged hemisphere (15) The presence of contralateral TMS-evoked potentials soon after stroke has been suggested as good indicator of recovery. In those who recover later, contralateral motor-evoked potentials have been shown to appear just before function returns (85).
In healthy people a fine balance of intracortical facilitation and inhibition helps with fine motor control. Cicinelli found decreased intracortical inhibition from the affected hemisphere of stroke patients and suggested this could represent a marker of cortical plasticity relevant to post-stroke recovery (82). This intracortical inhibition and facilitation can be studied with paired-pulse magnetic stimulation. Plasticity in stroke patients may also occur by recruitment of ipsilateral pathways from the unaffected cerebral hemisphere to the affected side of the body. These have been shown in animals and humans after surgical hemispherectomy; however the mechanism of ipsilateral plasticity leading to recovery is not well described in stroke patients, except perhaps for the recovery of midline muscles such as those involved in swallow (see 1.2.3) (18). Ipsilateral motor evoked potentials are seen in stroke patients but these are often associated with poor motor recovery (15, 86, 87). The presence of ipsilateral pathways alone does not mean that these pathways are performing any useful function (15). Gerloff and colleagues studied well-recovered patients after capsular stroke using several complementary techniques and concluded that recovery of hand movements was based on enhanced utilisation of both ipsilateral and contralateral hemispheres and corticofugal pathways (21).

1.6.4.3 Stroke Patients and Training with Functional and Neurophysiological Outcome Measures

Several authors have attempted to match functional outcomes in stroke rehabilitation to motor cortex changes. Unfortunately the studies all include very small heterogenous groups of patients with a wide variety of scales used to measure function and a variety of TMS (and other neurophysiological) parameters used as outcome measures. A meta-analysis of 13 upper-limb training studies concluded that neural changes in the sensorimotor cortex of the lesioned hemisphere (measured by TMS, fMRI, PET or SPECT) accompany functional paretic upper limb gains achieved with targeted rehabilitation interventions (88). Optimum duration and intensity of training, as well as the best outcome measure of function and plasticity, is unknown.

Motor skill training may have a greater effect on plasticity than repetitive strength training. For example, Jones' work on a rat model of stroke showed that animals given a stroke lesion and then skilled motor task training postoperatively recovered better function than those given a repetitive motor task to practice (89). Voluntary rather than passive exercise is thought to be more beneficial in terms of cortical
plasticity; one study of 25 healthy people showed more prominent increases in the SR curve and intracortical facilitation after 30 minutes of active wrist exercise compared with 30 minutes of passive movements in a crossover study by Lotze and colleagues (25). The success of the Hamdy group’s passive electrical stimulation of the pharynx seems an anomaly (30). Koski and colleagues studied five chronic stroke patients and suggested that changes in affected hemisphere neurophysiological response to a single 90 minute tailored upper limb occupational therapy session could predict response to longer term training (90). If it is possible to predict which long term rehabilitation strategies are likely to be successful by performing a one-off laboratory study first this would save much time and resources when planning future rehabilitation trials.

1.7 CORTICAL CONTROL OF RESPIRATORY MUSCLES

The development of transcranial magnetic stimulation led to greatly enhanced knowledge of the cortical control of the respiratory muscles and some of the key studies of healthy humans are described below.

1.7.1 Cortical Control of the Diaphragm

Breathing can be activated volitionally, via the corticospinal pathways and automatically via the brainstem-spinal pathways (91). Transcranial electrical stimulation (TES) was the first non-invasive method used to demonstrate that the diaphragm could be activated from the motor cortex but TES studies were limited by considerable discomfort for participants (92). Transcranial magnetic stimulation studies by Maskill and others added to knowledge gained from Gandevia’s work, showing that stimulation of an area 3cm lateral to the midline and 1-3cm anterior to the auricular plane led to diaphragm contraction, predominantly on the contralateral side but with some response on the ipsilateral side (76, 93-95). So there is evidence for both contralateral and ipsilateral corticofugal pathways to the diaphragm, although the contralateral pathways predominate.

Positron emission tomography (PET) scanning during volitional breathing showed activation of an area of the primary motor cortex, approximately 2.5cm lateral to the vertex, which corresponds with the point of optimum excitability found by Maskill et al using TMS (93, 96).

1.7.1.1 Facilitation of TMS evoked MEPs from the diaphragm

TMS studies by Sharshar et al demonstrated that MEPs from the diaphragm could be voluntarily facilitated with an inspiratory manoeuvre against a closed valve, similar to that performed when measuring maximum inspiratory pressure at the
mouth (see Methods section) (59). The facilitation reached a plateau with inspiratory efforts of between 40% and 60% of each individual’s maximum. Demoule and colleagues studied intracortical inhibition and facilitation of the diaphragm in healthy people using paired TMS (97). They used a conditioning stimulus at 80% of corticomotor threshold and a test stimulus of 125% of corticomotor threshold. If 125% of the threshold was greater than the maximum stimulator output, the test stimulus intensity was the maximum stimulator output. The authors found that interstimulus intervals shorter than 5ms were inhibitory; this was statistically significant for the 1ms and 3ms intervals. Interstimulus intervals longer than 6ms were facilitatory and the facilitation was maximal at 15ms. These results are very similar to those described for other muscles (see 1.5.6).

1.7.1.2 Diaphragm Motor Cortex Excitability
Sharshar and colleagues presented SR curves for the human diaphragm and showed a depression of diaphragm motor cortex excitability in healthy participants after application of isocapnic volume cycled non-invasive ventilation (98). In particular mechanical ventilation aiming for a tidal volume of the participant’s baseline tidal volume plus 5mls per kilogram, showed a shift of the SR curve to the right and a decrease in its slope. As explained in section 1.6, a shift of the entire curve to the right (i.e. an increase in the motor threshold) is indicative of reduced excitability of the cortico-cortical axons and their excitatory synapses with the motor cortical output neurons; and a decrease in the slope of the SR curve has been shown to accompany a decrease in the cortical map area (62).

1.7.2 Cortical control of abdominal muscles
The abdominal muscles are represented medially in the motor cortex, close to the interhemispheric fissure as first described by Penfield who directly stimulated the human brain during surgery (99). The area can be seen represented on the motor homunculus marked “trunk” in Figure 13.
Plassman & Gandevia used transcranial electrical stimulation (TES) of the motor cortex to non-invasively demonstrate corticofugal pathways to human abdominal muscles (100). TMS made further studies more tolerable for participants and using this technique it was found that the abdominal muscles are predominantly controlled by the contralateral motor cortex in the precentral gyrus of the frontal lobe. Plassman and Gandevia did record ipsilateral responses in their experiments but concluded that they could not be certain they were not due to spread of stimulus to the opposite cortex. A summary of TES and TMS studies of abdominal muscles in healthy people is given in Table 1.

Weak ipsilateral pathways from the motor cortex to the abdominal muscles have been demonstrated in several TMS studies (57, 76, 77, 101). Contralateral and ipsilateral pathways were seen by Carr in the activated rectus abdominis in 6/7 people studied (76); by Strutton in the active internal obliques in 85% of hemispheres studied (77) and by Harraf in the resting external obliques and rectus abdominis in all of 16 healthy subjects (58). Fujiwara et al studied the external obliques at rest and found that right hemisphere stimulation led to bilateral MEPs in eight of eleven healthy subjects and left hemisphere stimulation produced bilateral MEPs in five out of eleven (57). See Table 1 for details of the studies described in this section.
In all the studies describing ipsilateral and contralateral pathways it is difficult to know if the signals recorded are truly demonstrating ipsilateral pathways or whether they are merely a result of stimulus spread or volume conduction of EMG signals. Evidence to support ipsilateral pathways may be the increased latencies of the ipsilateral MEPs seen by Harraf and by Strutton (58, 77, 101). Strutton argued that the longer latencies of MEPs from the ipsilateral compared with the contralateral internal oblique in his study were too long to be attributed to current spread to the contralateral cortex and too short to be attributed to interhemispheric conduction via the corpus callosum. Harraf’s study participants had surface electrodes on the quadriceps muscles bilaterally but only contralateral MEPs were recorded, despite the cortical centres for the lower limb muscles being located close to those for the abdominal muscles and the midline (Figure 13). Harraf states that this implies that stimulus spread was unlikely to have been the reason for the presence of ipsilateral MEPs from the abdominal muscles (102). In addition, Harraf states that volume conduction was unlikely as the latency of MEPs recorded from the ipsilateral oblique were the same or shorter than those than MEPs from the contralateral rectus abdominis, despite the rectus being closer to the midline. Strutton et al compared areas of rectified contralateral and ipsilateral MEPs from the right and left internal obliques. They describe a bias of representation towards one cortex or another, independent of handedness. Hamdy reported similar asymmetry of cortical representation for the muscles of the pharynx (28, 77). Bilateral innervation and cortical bias for abdominal and upper airway muscles are extremely relevant for our cough studies in stroke patients. TMS studies of abdominal muscles in stroke patients are described in section 1.8.2 and Table 2.

1.7.2.1 Facilitation of TMS-Evoked MEPs from the Abdominal Muscles
Some authors describe difficulty in producing TMS-evoked MEPs from resting abdominal muscles for all participants so they employed various facilitation methods (101). Apparatus usually used for measurement of respiratory pressures (as seen in Figure 33) can be used to apply loads to the respiratory muscles to activate them and facilitate TMS, in trained individuals with good coordination (59, 101). Tsao et al used a forced expiratory manoeuvre (not described further) to activate the abdominals and facilitate MEPs. Participants first performed the manoeuvre three times with maximum effort, as spontaneous EMG from the transversus abdominis was recorded simultaneously (101). The mean maximum
amplitude of the rectified EMG signal, recorded over a 1s interval ($RMS_{\text{max}}$), was measured. For TMS facilitation participants performed the same manoeuvre with less effort. They watched the rectified EMG signal in real time and modified the effort level to aim for a target level on the computer screen set at 10% of $RMS_{\text{max}}$. The group’s paper does not quantify the increase in size of MEP achieved by this method of facilitation but the authors state that they were able to produce MEPs using a figure of 8 coil in some participants from whom no response could be recorded in resting muscle.

Tunstill and colleagues showed that bilateral trunk flexion in supine (fully flat) healthy people was shown to facilitate TMS to the rectus abdominis to a greater degree than forced expiration during breath holding, with peak-to-peak MEP amplitude 2.3 times greater during trunk flexion than forced expiration (103). They also found that by the time 30-40% of maximum voluntary EMG was attained the facilitation of the MEP tended to plateau. In some individuals the MEP amplitude fell again at very high levels of contraction (103).

Unpublished observations of our own have shown that when an individual is sitting in a chair (rather than lying down), the abdominal muscles are not completely inactive. These muscles are used to maintain trunk stability and sitting balance, as seen on spontaneous electromyography (EMG) traces of healthy humans sitting quietly at rest. Thus positioning participants in a chair (rather than having them lie flat) without any other manoeuvre will facilitate TMS to the abdominals to some extent.
# Table 1: Summary of Studies of Abdominal Muscles in Healthy People using TMS and TES

<table>
<thead>
<tr>
<th>Study author, year and reference</th>
<th>N</th>
<th>Muscle and activation</th>
<th>Stimulation details</th>
<th>Cortico motor threshold (% MSO)</th>
<th>MEP amplitude (mV)</th>
<th>Latency (ms)</th>
<th>Gastric pressure rise (cmH₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plassman and Gandevia 1989</td>
<td>5</td>
<td>Upper external oblique, activated by expiratory loading, trunk flexion and expulsive effort at 10-20% MVC</td>
<td>Electric stim at 750V in POE laterally; needle and surface electrodes</td>
<td>Mean 537V resting (electrical stim)</td>
<td>Not stated, around 1mV</td>
<td>13.90 active</td>
<td>Not given*</td>
</tr>
<tr>
<td>Carr 1994</td>
<td>7</td>
<td>Rectus abdominis, sitting upright with muscles activated</td>
<td>Magnetic stim with double cone coil over POE in dominant cortex at CMT+10% MSO</td>
<td>Mean 45 active</td>
<td>Not stated (MEP area measured not amplitude)</td>
<td>19.5 active</td>
<td>Not done</td>
</tr>
<tr>
<td>Fujiwara 2001</td>
<td>11</td>
<td>Upper external oblique at rest, semi-reclined in chair</td>
<td>Magnetic stim at 100% MSO</td>
<td>Mean Not done</td>
<td>Not stated (MEP area normalized to CMAP from T₁₀ root stim)</td>
<td>15.68 active</td>
<td>Not done</td>
</tr>
<tr>
<td>Tunstill 2001</td>
<td>6</td>
<td>Rectus abdominis resting and activated by forced exp breath hold, and trunk flexion from lying flat posn</td>
<td>Magnetic stimulation with figure of 8 coil</td>
<td>Mean 75 resting</td>
<td>0.19 resting</td>
<td>17.50 active</td>
<td>Not done</td>
</tr>
<tr>
<td>Tsao 2008</td>
<td>11</td>
<td>Transversus abdominis resting</td>
<td>Double cone coil; intramuscular EMG needle electrodes, inserted with USS</td>
<td>Mean 69 resting</td>
<td>Sig smaller than for active; figure not given</td>
<td>16-18 resting</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Continued overleaf with summary of abbreviations and extra notes
Table 1 Summary of Studies of Abdominal Muscles in Healthy People using TMS and TES

Continued from previous page

<table>
<thead>
<tr>
<th>Study author and ref</th>
<th>N</th>
<th>Muscle and activation</th>
<th>Stimulation details</th>
<th>Cortico motor threshold (% MSO)</th>
<th>MEP amp (mV)</th>
<th>Latency (ms)</th>
<th>Gastric pressure rise (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsao 2008</td>
<td>11</td>
<td>Transversus abdominis active 10% voluntary contraction from an expiratory manoeuvre</td>
<td>Double cone coil; intramuscular EMG needle electrodes, inserted with USS</td>
<td>Mean 53 active</td>
<td>Sig greater than for resting; figure not given</td>
<td>Not stated</td>
<td>Not done</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SD 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsao 2008</td>
<td>11</td>
<td>Transversus abdominis active 10% voluntary contraction from an expiratory manoeuvre</td>
<td>7cm figure of 8 coil; intramuscular EMG needle electrodes, inserted with USS</td>
<td>Mean 73 active</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Not done</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SD 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strutton 2004</td>
<td>10</td>
<td>Internal oblique, activated by abdominal hollowing</td>
<td>Magnetic stimulation with figure of 8 coil 2cm ant and 2cm lat to vertex; intensity enough to get bilat responses</td>
<td>Mean Not stated; MEP area measured but amplitude about 50 microvolts</td>
<td>16.10 active</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SD -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harraf 2008</td>
<td>16</td>
<td>Upper external oblique at rest (reclined)</td>
<td>Magnetic stim with double cone coil lateral over POE at 1.4xCMT; surface electrodes</td>
<td>Mean 72.40 resting</td>
<td>0.42 resting</td>
<td>21.42 resting</td>
<td>16.70 resting</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SD 16.70</td>
<td>0.30</td>
<td>2.12</td>
<td>11.50</td>
</tr>
</tbody>
</table>

Key for Table 1
TMS=transcranial magnetic stimulation; TES=transcranial electrical stimulation; ant=anterior; lat=lateral; CMT=corticomotor threshold; POE=point of optimum excitation; MSO=maximum stimulator output; MVC=maximum voluntary contraction
Dominant cortex=the cortex opposite to reported handedness as defined by the author. Latency, amplitude and threshold are for the MEPS from the contralateral muscle where both ipsilateral and contralateral MEPs were recorded
Threshold is for right side if both right and left were measured
*Plassman and Gandevia study used a pressure catheter to measure MVC for the abdominal manoeuvres and then measure the amount of gastric pressure (as % MVC) used to facilitate MEPs
1.8 RESPIRATORY MUSCLE ABNORMALITIES AFTER CORTICAL STROKE

The respiratory centres in the brainstem, medulla oblongata and the pons control ventilatory drive and automatic breathing. However the studies quoted above describe the involvement of the cortex in diaphragm and abdominal muscle control. Subjects with cortical damage, such as cortical stroke patients, may be expected to have abnormalities of respiratory muscle control and strength. Studies of cortical control of the respiratory muscles in stroke patients are described first followed by a review of volitional tests of respiratory muscle strength in similar patient groups.

1.8.1 The Inspiratory Muscles and Diaphragm after Stroke

Similowski and Urban made separate studies of hemiplegic stroke patients, measuring the diaphragm response to TMS over the motor cortex using surface EMG electrodes to measure diaphragm MEPs. Both authors found that diaphragm...
response on the paretic side of the body was absent (for more than half of subjects) or markedly delayed (104, 105).

Khedr studied the contralateral diaphragm MEPs in thirty-four hemiplegic stroke patients using TMS, comparing MEPs produced by stimulation over the affected hemisphere to those produced by stimulating the unaffected side (106). The group showed that the central conduction time of the phrenic nerve after TMS applied to the affected hemisphere was delayed compared with stimulation over the unaffected hemisphere; however the cervical MEP latency was unaffected. This indicates, as expected, that the effect of stroke on the diaphragm is due to a problem situated above the cervical cord and that the phrenic nerves are unaffected.

Radiological studies also suggest that cortical damage may affect diaphragm position and function. Hemiplegic stroke patients’ chest radiographs may show elevation of the diaphragm on the paretic side (107) but this is not universal and is contested by some authors (108). In addition, difference between the heights of the two hemidiaphragms as seen on chest X-ray is very variable in normal subjects.

Ultrasound studies of stroke patients show some (4 out of 8) have decreased diaphragm excursion on the paretic side during volitional breathing (108) Khedr used videofluoroscopy to demonstrate decreased diaphragm excursion during voluntary deep breathing in 40% of thirty-four stroke patients (106).

De Troyer and colleagues performed needle EMG studies of the parasternal intercostal muscles in 20 patients with acute hemiplegia and found reduced intercostal muscle activity on the hemiparetic side during deep voluntary breathing in most subjects. They suggest that decussated fibres innervate these respiratory muscles and respiratory muscle dysfunction may be implicated in chest infections in stroke patients (109). Lanini and colleagues studied eight hemiparetic stroke patients using optoelectronic plethysmography during quiet breathing, voluntary hyperventilation and ventilation driven by hypercapnia. They were particularly interested in ventilation symmetry between the paretic and normal sides of the chest and abdomen. During quiet breathing there was no difference between the weak and the normal sides. However during voluntary hyperventilation, the expansion of the chest wall on the paretic side was on average 582mls (SD 155mls) compared with 783mls (SD 317mls) on the unaffected side. (P for difference <0.05) In contrast, the affected side was more ventilated during
hypercapnic ventilation; mean expansion of the chest wall was 1114mls (SD 203mls) on the affected side compared with 805mls (SD 134mls) on the unaffected side. The authors concluded that the reduction in tidal volume on the paretic side during voluntary hyperventilation confirms that the involvement of voluntary control of the diaphragm in ischaemic hemiplegia is the same as that for other skeletal muscles and that the increased volume of the paretic side on hypercapnic ventilation was due to loss of cortical inhibition (110).

1.8.2 Abdominal Muscle Control after Stroke

Most studies of abdominal muscle control after stroke are in the context of postural control, which can be severely impaired in cortical stroke patients. Despite the contralateral and ipsilateral innervation of the abdominal muscles described in studies of healthy controls, the abdominal muscles on the hemiparetic side still seem to be significantly impaired by stroke in most cases of middle cerebral artery territory (MCA) stroke. One study of spontaneous abdominal EMG activity during trunk flexion in hemiplegic patients found activity to be reduced and delayed on the affected side (111). The patients studied had all had MCA strokes one to two months previously; they had hemiparesis but all patients were able to sit unsupported and they had a mean score on Carr's Motor Assessment Scale of 32 (SEM 16) (112). A maximum of 54 points is available on this scale (for those with normal function) (112).

A summary of TMS studies of the abdominal muscles in stroke patients is given in Table 2. Fujiwara et al studied the external obliques in twenty hemispheric stroke patients (3 months or more post-stroke) and eleven healthy controls, using TMS applied with a figure of eight coil. The participants were reclined in a chair with no facilitation except a presumed small amount of background activity in the abdominals. Results for healthy people were given in Table 1 above. Stroke patients’ impairments were scored on the Stroke Impairment Assessment Set (113). Patients’ upper limb impairments were scored 0-10 out of 30, and lower limb impairments ranged from 0-15 out of 30 (with 30 being the most severely affected possible). Stimulation over the affected motor cortex resulted in a contralateral MEP in only one of twenty patients; the others had no MEP response. Stimulation over the unaffected hemisphere produced contralateral and ipsilateral responses in nineteen out of twenty patients; ipsilateral MEP responses in stroke patients occurred in a higher proportion and were larger in amplitude than for controls (57). This disinhibition of the unaffected hemisphere has been described before in
studies of other muscles e.g. the hand (see section 1.2) and may be due to decreased inhibition of the unaffected cerebral hemisphere from the contralateral, damaged hemisphere (67).

Harraf and colleagues, interested in cough and expiratory muscle strength, studied the response of the external obliques and rectus abdomini to TMS over the affected and unaffected hemispheres of fifteen acute stroke patients, all within two weeks of stroke onset. The patients in the Harraf study were moderately severely impaired, with a mean NIH Stroke Scale score of 15 (SD 6) on admission and 9 (SD 7) on the day of testing. For the NIHSS score details, see Figure 22. The maximum NIHSS score is 31 for the most severely affected patients.

There were no MEPs recorded on either side from TMS over the affected hemisphere but both contralateral and ipsilateral MEPs were recorded from TMS over the unaffected motor cortex (Table 2). Contralateral MEPs were significantly bigger and of shorter latency than ipsilateral MEPs after TMS to the unaffected hemisphere (P<0.05 for both). Harraf’s stroke group had numerically bigger MEPs from the contralateral external oblique than controls, when stimulated over the affected hemisphere [stroke group mean 0.73mV (SD 0.86) vs. 0.42mV (SD 0.3) for controls]. Significance testing was not performed but the large SD for the stroke group makes it unlikely the difference was statistically significant. However the result fits with Fujiwara’s work described above and other studies describing motor cortical disinhibition in the unaffected hemisphere after cortical stroke (57, 114).

that it was often the case that no MEPs were recorded for some stroke patients. Neither Harraf nor Fujiwara used facilitation to see whether this would lead to MEPs being recorded for patients who had no response to unfacilitated TMS.
Table 2 TMS Studies of Abdominal Muscles in Stroke Patients

<table>
<thead>
<tr>
<th>Study author and reference</th>
<th>N</th>
<th>Muscle and activation</th>
<th>Stimulation details</th>
<th>Corticomotor threshold (% MSO)</th>
<th>MEP amplitude (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujiwara 2001</td>
<td>20</td>
<td>External oblique</td>
<td>TMS to unaffected hemisphere</td>
<td>Not done (All stims at 100%)</td>
<td>MEP area measured*</td>
</tr>
<tr>
<td>Fujiwara 2001</td>
<td>20</td>
<td>External oblique</td>
<td>TMS to affected hemisphere</td>
<td>Not done (All stims at 100%)</td>
<td>No contralateral or ipsilateral response in 19/20. Response on both sides in one patient with no stroke deficits</td>
</tr>
</tbody>
</table>

*All MEPs (contralateral and ipsilateral) from stimulation of the unaffected hemisphere were significantly increased in area compared with the control group

Table 2 continued overleaf with Key
1.8.3 Volitional Tests of Respiratory Muscle Strength After Cortical Stroke

Several studies have shown abnormalities on volitional respiratory muscle testing in cortical stroke patients; see Table 3 for details. Patients with cortical stroke in the middle cerebral artery vascular territory (MCA stroke) and a resultant hemiplegia should not have any damage to the brainstem respiratory centres. They should not have any peripheral neuropathy and the peripheral muscles are unaffected in the acute stage. The damage is purely cortical so abnormalities in muscle strength are due to damage to the motor cortex.

In a Brazilian study, respiratory rate, tidal volumes at rest, minute ventilation and percentage of inspiratory time related to total time of respiratory cycle (Ti/Ttot)
were not significantly different for a group of sixteen chronic stroke patients (stroke onset >9 months prior to study date) when compared with healthy controls of a similar age. The patients were relatively high functioning; all were living in the community and could walk. The investigators excluded patients with abnormal lung function and those with facial palsy (115). The investigators found that the patients’ respiratory muscle strength (measured by volitional mouth pressure tests) was reduced when compared with the controls. Mouth pressures can reflect respiratory pressures and therefore respiratory muscle strength if good technique is employed and there is no hyperinflation. Hyperinflation of the lungs flattens the diaphragm, placing it in a disadvantageous position for muscle strength tests (39, 116). The patient group had a mean (SD) PI max of 73.6 (20.6) cmH\textsubscript{2}O and a mean PE max of 89.4 (41.3) cmH\textsubscript{2}O. Both of these values are greater than the male cut-offs of 45 cmH\textsubscript{2}O for PI max and 80 cmH\textsubscript{2}O for PE max, (see Figure 8) below which respiratory muscle weakness may be diagnosed (40). Fugl-Meyer et al studied PI max and PE max in the supine position for hemiparetic or hemiplegic stroke patients (117). Patients’ results were expressed as percent of predicted using Ringvist’s Scandinavian values (117); 27 patients with severe impairment, judged on a scale thought to be similar to the Scandinavian Stroke Scale (118, 119) had PE max only 30% predicted (SD15%) and PI max 49% predicted (SD 23%). It should be noted that the Ringvist values are high when compared with other normal ranges for respiratory muscle tests (120). More recently Harraf studied 15 patients within two weeks of ischaemic stroke affecting the middle-cerebral artery vascular territory. Her moderately severely affected stroke patients had a mean (SD) PE max of 62.6 (28.0) cmH\textsubscript{2}O that would class them as weak compared to the usual cut-off value of 80 cmH\textsubscript{2}O and significantly weaker than the age matched control group studied, P=0.001 (40, 58). She also measured Pgas rise during voluntary cough, (cough Pgas). This is a test of expiratory muscle strength complementary to the PE max test and may be easier for some subjects to perform (121). The patients’ cough Pgas was also much reduced with a mean of 72.7 (64.5) cmH\textsubscript{2}O, lower than of the female cut-off of 95 cmH\textsubscript{2}O (Figure 8) and significantly lower than the controls mean value of 163.4 (55.8) cmH\textsubscript{2}O, p<0.001. The novel technique used in the Harraf study was the non-volitional assessment of respiratory muscle strength by magnetic stimulation of the supplying nerves. For non-volitional assessment of expiratory muscle strength the group performed magnetic stimulation over the tenth thoracic nerve roots; section 2.9.5 gives further
details of this test. By this measure, there was no significant difference in abdominal muscle strength between groups; the rise in Pgas after stimulation of the T₁₀ nerve roots (TwT₁₀Pgas) was mean (SD) 25.2 (7.8) cmH₂O for patients compared with 29.4 (12.4) cmH₂O for controls, P=0.153. Magnetic stimulation of the phrenic nerves in the neck was performed to obtain a non-volitional assessment of diaphragm strength. The pressures produced across the diaphragm as a result of the stimulation (twitchPdi) were not significantly different between groups; thus twitch Pdi for stroke group was 21.6 (7.2) cmH₂O and for the control group was 19.2 (3.4) cmH₂O; P=0.163. These results may be expected, as acute MCA stroke does not affect the muscles or the peripheral nerves (58). Patients’ poor results on volitional tests may be due to problems with the corticomotor control of the respiratory muscles, as discussed in Section 1.8.

**Table 3 Volitional Respiratory Muscle Tests in Stroke Patients: Summary of Previous Studies**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Patients</th>
<th>N</th>
<th>PEmax Mean (SD) cmH₂O</th>
<th>PImax Mean (SD) cmH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fugl-Meyer 1983</td>
<td>Chronic hemiplegic stroke patients from a rehab facility</td>
<td>17</td>
<td>30 (15)% predicted</td>
<td>49 (23)% predicted</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>15</td>
<td>46 (20)% predicted</td>
<td>64 (25)% predicted</td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>8</td>
<td>40 (24)% predicted</td>
<td>62 (24)% predicted</td>
</tr>
<tr>
<td>Teixeira-Salmela 2005</td>
<td>Community-dwelling chronic hemiplegic stroke patients (&gt;9 months since onset)</td>
<td>16</td>
<td>89 (41)</td>
<td>74 (21)</td>
</tr>
<tr>
<td>Harraf 2008</td>
<td>Acute hemispheric stroke patients (&lt;2 weeks since onset)</td>
<td>15</td>
<td>63 (28)</td>
<td>-</td>
</tr>
</tbody>
</table>

Key for Table 3 PEmax=maximum expiratory pressure at the mouth; PImax=maximum inspiratory pressure at the mouth; %=percent; SD=standard deviation

### 1.8.4 Expiratory Muscle and Cough Training in Patients with Neuromuscular Disease

Expiratory muscle weakness and poor cough are associated with respiratory failure and chest infections in patients with many acute and chronic neuromuscular diseases in addition to stroke. Therapist-applied cough interventions such as electrical stimulation of the abdominal muscles and use of the mechanical insufflator-exsufflator (CoughAssist®, Philips Respironics, Philips Healthcare, Guildford, Surrey, UK) are often used in patients with acute deteriorations of chronic progressive neuromuscular diseases and in those with irreversible neurological damage, such as spinal cord injury (122-124). These interventions can be used in stroke patients but they are thought unlikely to drive cortical...
plasticity due to the patient’s passive role (see 1.2.2). They will not be discussed further in this thesis. For groups of patients with a number of specific neurological diseases, expiratory muscle training has been trialled. Expiratory muscle training has been used and shown to improve maximum expiratory pressure measured at the mouth (PEmax) in Parkinson’s disease and multiple sclerosis as well as the sedentary elderly (125, 126) However it has not been shown to reduce chest infections or death or improve objective measures of voluntary cough. Training methods are briefly discussed below as they will be referred to later in this thesis when cough training in stroke patients is described.

1.8.4.1 Parkinson’s Disease

Pitts and colleagues enlisted ten male patients with Parkinson’s disease and aspiration problems into an expiratory muscle training programme with the aim of improving voluntary cough and swallow (127). The patients used an expiratory muscle strength training device daily, performing five sets of five repetitions at 75% of maximum effort each day 5 days a week for four weeks (127); see Figure 15 for the device used. A number of voluntary cough parameters were measured; significant improvements were seen in the expiratory phase rise time and in cough volume acceleration but no improvement in cough peak flow; see Figure 48 for explanation of these measures. It would seem unlikely that such a device would be useful for many stroke patients as the mouthpiece is very slim: a tight mouth seal is required to raise pressure across the device and this is often impossible to achieve after stroke.

Figure 15 Expiratory Muscle Training Device

The image shows the Threshold PEP® device (Respironics Ltd, Chichester, UK) a calibrated, spring loaded valve similar to the device used for expiratory muscle training in Parkinson’s patients. The mouthpiece, made of white plastic, is on the left of the picture.

1.8.4.2 Multiple Sclerosis

Chiara and colleagues studied 17 mild to moderately disabled patients with multiple sclerosis. The authors aimed to improve voluntary cough measures as a result of expiratory muscle training. Patients trained five days a week, performing
four sets of six repetitions a day for eight weeks (125). PEmax and peak expiratory flow were initially low and significantly increased following training. However no statistically significant improvements in voluntary cough measures were found after the expiratory muscle strength training. The authors acknowledge that to improve cough, future studies should use a paradigm that trains the muscles as they would be used during a cough (125).

1.8.4.3 Spinal Cord Injury

Invasive and non-invasive electrical stimulation has been tried to simulate cough, with some success, in spinal cord injured patients with lesions above T10 (122). Although the twitch pressures produced by electrical stimulation are slightly inferior to those produced by magnetic stimulation over the T10 nerve roots (Pgas rise approximately 30-40cm H2O with electrical stimulation compared with 35-50cm H2O for magnetic stimulation) the relatively small size and low cost of electrical stimulators makes them an attractive prospect for use in cough rehabilitation of stroke patients. If the electrical stimuli were EMG-triggered the process seems more likely to drive cortical plasticity and promote recovery than if the stimulation were passive, see 1.2.2 (122, 128).

1.8.4.4 Cough Training in Stroke Patients

We found no previous studies of cough training in stroke patients. We wanted to develop a training protocol that would drive cortical plasticity and improve cough function. When planning the training we considered previous studies where cortical plasticity was demonstrated in healthy people or animals, (22, 25) and the expiratory muscle training studies described above, as well as Fraser’s work with electrical stimulation of the pharynx (18). It was decided that the training protocol should involve voluntary coughing, rather than expiratory muscle strength training, passive electrical or magnetic stimulation or use of the CoughAssist device (Philips Respironics). Functional electrical stimulation, that is stimulation triggered by patient EMG or movement would seem promising but is technically challenging to perform and measure outcomes. Expiratory muscle strength training seems less likely to improve cough than coughing practice itself, bearing in mind the results of previous training studies, see 1.6.4 (25, 89).

Koski and colleagues demonstrated that changes in affected hemisphere neurophysiological response to a single training session could predict response to long term training (90). The plan for our work was to find a cough training protocol
which caused changes in response to TMS after a single training session in a small number of patients and eventually take the successful training method onto the stroke ward to use in a larger randomised controlled trial of cough training in acute stroke. The training is further described in the Methods section and Chapter 8.

1.9 LUNG VOLUMES

Figure 16 Lung Volumes and Their Subdivisions

The image shows a spirometric trace of tidal breathing followed by a maximum inspiratory capacity manoeuvre (IC) and an expiratory vital capacity manoeuvre recorded from a rolling seal spirometer.

Lung volumes are commonly reduced in patients with neuromuscular disease and this will be described further in this section. Small lung volumes may be an indicator of respiratory muscle weakness. Lung volumes are simple and quick to measure and they provide useful information to supplement results of respiratory muscle testing. Lung volumes are the volumes of air the associated with different phases of the respiratory cycle. The physical size (i.e. height) of the subjects apart, lung volumes are all determined by a balance of forces.

1.9.1 Vital Capacity

Vital capacity (VC) is the maximum amount of air a subject can expel from the lungs after a maximum inspiration (41). It can be reduced by disease affecting the lungs, the chest wall or the respiratory muscles. As testing the VC requires a maximum effort, it will also be low with poorly motivated subjects or those who are unable to make maximum efforts due to tiredness from intercurrent illness, for example. Reduction in the VC must be due to a low total lung capacity, a high residual volume, or both (41).

1.9.1.1 Change in VC with posture

Allen and colleagues found a mean fall in forced vital capacity of 7.5% in 50 healthy controls between measurements in the standing and supine positions.
(standard deviation 5.7%, upper value of the 95% confidence interval for the mean 19%) (129).

1.9.1.2 Changes in VC with neuromuscular disease
For a patient with normal lungs and respiratory muscle weakness, (e.g. with motor neurone disease) vital capacity will only fall when the muscle weakness becomes moderately severe, as only small pressures are required to inflate the normal lung. Allen and colleagues concluded that a drop in FVC of more than 25% when moving from upright to supine, associated with normal or restrictive lung function should be an indication for further study of diaphragm function (129).

1.9.2 Changes in Vital Capacity with Stroke
Several authors have found reduced VC and restrictive spirometry in stroke patients and these are summarized in Table 4 (106, 108, 117, 130, 131). Cohen reported seven chronically hemiplegic stroke patients who had a mean seated VC of 79% predicted (SD 18) (108). An Egyptian study of a group of 34 acute stroke patients, in the first week after middle cerebral artery territory infarct showed the average sitting VC to be reduced to about half of that predicted (mean 52%) (106). Patients’ mean stroke severity, scored using the Scandinavian Stroke Scale, was 33. The maximum score on this scale is 70 (which would be achieved by someone with no impairments) so it is assumed these were patients with at least moderately severe strokes (119). Fugl-Meyer et al studied 54 chronic hemiplegic and hemiparetic stroke patients; the group with severe impairment had a mean supine VC 75% of predicted (sitting) values (SD 16%). 9 patients categorized as having mild impairment had a supine VC of 92% predicted (SD 14%). Increasing respiratory dysfunction was associated with increasing motor disability in both studies (106, 117).

1.9.3 Functional Residual Capacity
Functional residual capacity is the lung volume at the end of a quiet expiration at rest (41). In healthy subjects this may be considered the relaxation volume, where the inward elastic recoil of the lung is equal and opposite to the outward pull of the chest wall. At rest the FRC is equal to the end expiratory lung volume (EELV). On exercise, the EELV can go down (in healthy people) or up (in patients with obstructive airways disease) and in this situation the term EELV should not be used interchangeably with FRC (41).
1.9.3.1 Changes in FRC with posture

Stroke patients may spend a considerable amount of time in reclined or semi-supine positions in bed so the effect of posture on lung volumes is relevant. FRC is known to fall with supine posture in healthy people (132, 133). Yap et al (132) showed a mean fall in FRC of 20% (760ml) when healthy subjects were moved from the seated to supine position; they measured FRC using body plethysmography. Ibanez and colleagues measured FRC using helium dilution in 100 healthy males and females and found FRC to drop by an average of 29% (133). Fugl-Meyer’s group studied 12 healthy controls alongside stroke patients; their mean supine FRC measured using helium dilution was 83% (SD 9%) of predicted sitting values. On lying flat, the fall in FRC is thought to be due to the fall in relaxation volume, caused by gravity forcing the diaphragm upwards and an increase in intrapulmonary blood volume (132, 134).

1.9.4 Changes in FRC with anaesthesia

Hewlett and colleagues measured supine FRC in 26 males undergoing (non-cardiothoracic) minor surgery. Patients’ lung volumes were measured by gas dilution before and after being anaesthetised with thiopentone sodium, a barbiturate (134, 135). The mean reduction in patients’ FRC from pre-induction values was 16.1% (SD 13.4%); this was equivalent to a mean absolute reduction in FRC volume of 390ml, SD 403ml (corrected to BTPS).

1.9.5 Changes in FRC with increasing Body Mass Index

A considerable amount of work has been published on the effect of obesity on lung volumes. Obese people are more likely to suffer a stroke (136) and those stroke patients who are obese may be more likely to encounter respiratory complications. Jones and colleagues studied nearly 400 Canadians over a wide range of BMIs and found an exponential decay in FRC with increasing BMI (137). FRC is thought to reduce due to increased loading of the chest wall and abdomen, pushing the diaphragm up and the chest wall inwards (41).
1.9.5.1 Changes in FRC with neuromuscular and skeletal disease

Like neuromuscular patients, stroke patients perform poorly on volitional tests of respiratory muscle strength so a brief review of the effect of neuromuscular disease on lung volumes is relevant. In addition chronic stroke patients may develop spinal deformities. A low FRC occurs with low total lung capacity (TLC) in restrictive diseases such as those causing chest deformity (e.g. kyphosis, scoliosis) (138) or in cases of respiratory muscle weakness (139). For neuromuscular patients it has been suggested that the fall in FRC is partly due to microatelectasis (41), although this is disputed by a study of 14 patients with respiratory muscle weakness who had reduced static lung volumes but no evidence of microatelectasis on thoracic CT scans (140). It should be appreciated that CT scanning technology was then more primitive than it is now. Patients with inspiratory muscle weakness may have a large fall in FRC when supine (41, 141).

1.9.5.2 Changes in FRC in Stroke Patients

Three papers have described FRC measurements in chronic stroke but this was the main aim of the study in only one case. The studies are summarised in Table...
4. Fugl-Meyer et al measured FRC in 54 chronic hemiplegic or hemiparetic stroke patients and 12 healthy controls (117). FRC was measured by helium dilution with all participants lying flat and expressed as a percentage of predicted (142). The patients had been treated at a department of rehabilitation medicine but the time between onset of stroke and respiratory testing was not given. Patients were categorised as having severe, marked or moderate / slight impairments according to the Fugl-Meyer scale but it was not stated when these assessments had been made (118, 143). Patients with severe (n=27, FRC mean 69% predicted, SD 26) or marked impairments (n=18, FRC mean 72%, SD 21) were found to have significantly reduced FRC compared with controls (n=12, FRC 83%, SD 9). Patients with moderate / slight impairments (n=9, FRC mean 74%, SD 15) tended to have a lower FRC than controls but this was not statistically significant. However the moderate sample size and division of the stroke group into subgroups meant the study was not adequately powered to make any of the comparisons with controls (a post-hoc calculation of achieved power was calculated from their results, using G*Power software; see Chapter 2 for details). For t-test comparing controls and severe patients achieved power=0.52; for controls and marked severity patients power=0.42; for slightly impaired patients power=0.35 (144).

Lanini and colleagues measured FRC in 8 stroke patients, on average 26 days post stroke (range 14-55 days) (110). The patients were categorised as hemiplegic with moderate impairments. The investigators measured FRC by helium dilution in the seated position and quoted their results as a percentage of predicted, using the ECCS equations (145). Patients had a mean FRC of 99% predicted (SD 17) compared with controls’ 100% predicted (SD 16). However, FRC was not an outcome measure in the Lanini study, it was measured only to characterize the patient and control groups before a study of cortical control of ventilation. The sample size was not large enough to address the question of altered FRC.

Cohen and colleagues also used FRC to characterise seven chronic hemiplegic stroke patients, whose diaphragm movement they were studying with ultrasound (108). Patients all had a dense hemiplegia involving both arm and leg. Smoking history is not given but all were said to be without any chest disease on evidence from chest X-ray, medical history and physical examination. The patient group’s mean FRC, measured by helium dilution, (presumably in the sitting position) was 101% predicted (SD 22).
Figure 18 pictures B-F show possible reasons for reduced functional residual capacity in disease or with posture, as proposed by Hewlett et al (146). These will be briefly discussed below. Elevation of the diaphragm after stroke, (as illustrated in picture C) could lead to reduced FRC. The diaphragm has been seen to be elevated on the paretic side on chest radiographs of some hemiplegic stroke patients (107, 147) and movement of the diaphragm during volitional inspiration, measured by ultrasound, may be reduced on the paretic side in patients with chronic stroke and hemiparesis (108). Several authors also describe weakness of
the diaphragm on volitional tests of respiratory muscle function in stroke patients. Diaphragm weakness would lead to the diaphragm being pushed cranially by the abdominal contents in the supine posture. Impairment of corticofugal pathways to the diaphragm may also lead to decreased diaphragmatic tone and a raised hemidiaphragm on the weak or indeed both sides. Another cause of the reduced FRC could be collapse of the chest wall on the weak (or both) sides, as shown in picture D. Fluck and Korczyn both reported reduced movement of the whole hemithorax on the affected side during deep voluntary breathing. Respiratory drive, measured by parasternal EMG in 25 patients with flaccid hemiplegia, was abnormal on the hemiplegic side during voluntary hyperventilation. Lanini and colleagues used optoelectronic plethysmography to show that the chest wall on the paretic side moved less than on the healthy side during voluntary hyperventilation. Gas trapping would reduce FRC as measured by helium dilution, as was the case in two of the studies shown in Table 4. However there is no reason to believe the patients in any of the studies had any gas trapping as all were free of respiratory disease; also patients in a third study had normal FRC measured by the He dilution method. The scenarios shown in pictures F and B are thought unlikely to be a consequence of stroke so will not be discussed.

1.9.6 Total Lung Capacity
Total lung capacity (TLC) is the maximum volume to which the lungs can be expanded with a maximum inspiratory effort. At TLC, the inspiratory muscles have reached the limit of their ability to oppose the elastic recoil of the lungs as shown in Figure 16. TLC falls with weakness of the respiratory muscles, especially the diaphragm. It is also reduced by distortion of the chest wall and spine (reducing chest expansion), obesity and ascites (causing limited descent of the diaphragm) and pleural disease (e.g. pleural effusion occupying thoracic space).

1.9.6.1 TLC in Stroke
Fugl-Meyer et al also described total lung capacity in their group of 54 stroke patients. The twenty-seven patients categorized as severely impaired had a mean TLC of 79% predicted (SD 16) in the supine position, significantly worse than controls who had a mean (SD) TLC of 93 (6)% predicted. This comparison achieved power of 0.90 so the difference was highly unlikely to be due to chance, unlike the FRC comparisons that were all underpowered. Cohen showed a group of seven stroke patients had a mean TLC of 93% predicted (SD 19).
1.10 COUGH

Cough is a brainstem reflex that can be voluntarily initiated, inhibited or enhanced. Voluntarily initiated cough is referred to in this report as voluntary cough and cough that is initiated involuntarily is known as reflex cough but there is inevitably some crossover; reflex coughs can be cortically enhanced or suppressed. All coughs consist of an initial inspiratory phase followed by a compressive phase (against a closed glottis) and then a forced expulsion, associated with a characteristic sound (151). The changes in airflow and pressure during voluntary coughing can be seen in Figure 47. The inspiratory phase of voluntary cough is very variable in duration. The compressive phase usually lasts 0.2 seconds and the expulsive phase can last 50ms for a single cough, or longer for a single cough followed by a series of expirations with glottis closure in between, sometimes referred to as a cough bout.

1.10.1 Control of Voluntary Cough

Electromyography (EMG) studies of the inspiratory (chest) and abdominal muscles during voluntary cough in healthy people show a sequential activation of chest and then abdominal muscles (152). Mazzone and colleagues have identified what they believe to be a “cough region” of the cerebral motor cortex on functional MRI scans. See Figure 20 (153). This is sited laterally in the motor cortex and anatomically close to the swallow region described by Hamdy’s group (28).

1.10.2 Reflex Cough

The reflex cough pathway involves airway afferent nerves being activated by inhaled material (solids, liquids or gases). This results in modifications of the brainstem areas controlling breathing and the generation of a cough (154). Vagal afferent nerves are responsible for initiation of the cough reflex (155). The sensory terminations of these nerves are found throughout the airways; some of the better-described ones are known as Rapidly Adapting Receptors (RARs), slowly adapting stretch receptors (SARs) and C-fibres. Various subtypes have different roles. The majority of the airway afferents are C-fibres and they respond to mechanical and chemical stimuli including capsaicin and tartaric acid (156).

Absence of reflex cough leaves patients at high risk of chest infection (156). The expiratory pathways for reflex cough may be similar to those for voluntary cough; however there is evidence that the reflex cough response is quicker and perhaps better coordinated than voluntary cough. EMG studies show that the chest and abdominal muscles are activated early and simultaneously during reflex cough.
caused by nebulised tartaric acid (152). This may be expected for a reflex from the brainstem compared with the voluntary cough initiated from the cerebral cortex. It would be very difficult to measure the reflex caused for example by a peanut entering the airway. It would not be ethical to conduct these studies and instead investigators have tended to study reflex cough caused by inhalation of aqueous solutions, (157) L-tartaric acid (152, 158) or capsaicin (151).

Figure 19 Voluntary and Reflex Control of Cough

Cough is a brainstem reflex that can be voluntarily initiated, inhibited or enhanced. A provisional scheme is illustrated in the figure below. Diseases or conditions that can impair cough and the sites where each causes a problem are shown. Image adapted from Lee 2002.

Key for Figure 19 MCA=middle cerebral artery
**Figure 20 Voluntary Cough on Functional MRI scan**

Image from Mazzone 2009. The authors used blood oxygen level dependence (BOLD) functional MRI scanning to show signal changes in the motor cortex during voluntary cough. The magnetic resonance (MR) signal of haemoglobin changes with the level of haemoglobin oxygenation. BOLD fMRI imaging may indicate changes in cerebral blood flow and oxygen consumption using endogenous haemoglobin as the contrast medium. Note the asymmetry of the response in the motor cortex shown in image B (top right) and compare with that for the swallow pictures in Figure 21. This asymmetric representation of cough may be linked to the biased representation of the abdominal muscles found in TMS studies by Strutton described in 1.7.2.

![Graph showing respiratory movement and BOLD signal](image)

**Figure 21 Swallow fMRI Before and After Pharyngeal Stimulation**

BOLD fMRI picture from Fraser 2002. Fraser noted asymmetry of swallow representation, independent of handedness prior to stimulation. Pictures taken after pharyngeal electrical stimulation show greater bilateral functional activation in the sensorimotor cortex.

**NO STIMULATION**

![Brain images with no stimulation](image)

**STIMULATION**

![Brain images with stimulation](image)

Key for Figure 21: BOLD=Blood oxygen level dependence. fMRI=functional magnetic resonance imaging. For explanation of BOLD fMRI see Figure 20
1.10.3 Cough abnormalities after stroke

An effective cough requires powerful, coordinated contraction of expiratory (abdominal) muscles along with adequate static lung volumes, sufficient inspiration prior to cough, unobstructed airways and normal laryngeal function (159).

1.10.3.1 Voluntary Cough

Smith Hammond and speech therapy colleagues studied voluntary cough in a mixed group of 43 acute stroke patients with the aim of finding a predictor of aspiration (160). The stroke types included brainstem, subcortical and cortical, on either side and some patients had had more than one stroke. Several of the patients had pre-existing respiratory disease. They wanted to do the study because the gold-standard method of assessing dysphagia, the videofluoroscopy test is difficult to perform in stroke patients who cannot sit unsupported. They found that patients’ cough (expiratory) peak flows were only about 1/3 of those of controls (patients’ mean 78 l/min compared with controls 218 l/min, p=0.001). Patients cough sound (i.e. noise) levels were also significantly reduced compared with controls (160). The group performed a similar but larger study in 96 stroke patients that demonstrated that a voluntary cough flow of less than 174 l/min had a sensitivity of 82% and specificity of 83% for the identification of aspiration. The value of 174 l/min is close to the value of 160 l/min quoted by Bach as the minimum required for successful weaning from mechanical ventilation (161).

Harraf and colleagues also found reduced cough flows in 18 stroke patients of 203 l/min compared with 351 l/min for controls. However her group of patients had all had middle cerebral artery territory first-ever strokes with no brainstem involvement, affecting either side of the brain. None had a diagnosis of neurological or respiratory disease except stroke (58). This is good evidence for cortical control of voluntary cough, which is impaired after cortical stroke.

One study of right handed, middle cerebral artery territory stroke patients found that only patients with left brain strokes had impairment of voluntary cough (162). Unfortunately the assessment of cough as normal or abnormal was subjective, based on observer reporting. However patients with left hemisphere (usually the dominant hemisphere) strokes are often more severely impaired and more difficult to rehabilitate than those with right brain strokes.

1.10.3.2 Reflex Cough

Addington and colleagues performed a number of studies of the cough reflex induced by tartaric acid in stroke patients (162-164). In a study of 30 right-handed
patients with middle cerebral artery (MCA) infarcts all produced a cough in response to tartaric acid. They did not make any physiological measures of the cough, which was instead rated as normal or abnormal by an observer.

1.11 SUMMARY

Stroke patients often die of chest infections, which can be caused by a combination of aspiration and poor cough. Factors that may contribute to ineffective cough include weak respiratory muscles; poor coordination of respiratory muscles (inspiratory, expiratory and upper airway); altered resting lung volumes; poor cortical control of expiratory muscles and cough coordination.

We designed a series of studies to investigate the respiratory muscles (especially the expiratory muscles) and cough in middle cerebral artery territory acute ischaemic stroke patients and appropriate controls. The studies are listed below and described in subsequent chapters.

- Respiratory Function and Respiratory Muscle Tests in Stroke Patients and Controls (Chapter 3)
- Acute ischaemic hemispheric stroke is associated with impairment of reflex and voluntary cough (Chapter 4)
- Reduced Peak Cough Flow in Acute Stroke Patients is Associated with Reduced FRC and Low Cough Inspired Volume (Chapter 5)
- Motor Control of the Abdominal Muscles: Studies in Healthy Participants and Stroke Patients using Transcranial Magnetic Stimulation (Chapter 6)
- Motor-Cortical Stimulus-Response Curves in Controls: TMS and Motor-Evoked Potentials and Evoked Force (Chapter 7)
- TMS Studies of Abdominal Muscle Control in Stroke Patients and Attempts to Drive Plasticity Using Cough Training (Chapter 8)
Chapter 2 Materials and Methods

2.1 ETHICS

King’s College Hospital Local Research Ethics Committee (London, UK) granted permission for all studies in this report to be carried out under Ethics Approval number LREC 02-120. Participants gave written informed consent unless they were unable to write, for example after a hemiparetic stroke affecting the dominant hand. In this case they made a mark using their unaffected hand, witnessed by a relative or a member of the hospital ward staff not involved with the study.

2.2 PARTICIPANTS

2.2.1 Controls

Male and female healthy controls were recruited from a volunteer database and from amongst hospital and laboratory staff.

2.2.2 Acute Stroke Patients

Male and female patients were recruited from the Friends Stroke Unit of King’s College Hospital, London, UK. All patients were within two weeks of their first-ever ischaemic stroke, affecting the vascular territory of the middle cerebral artery (MCA) on either side of the brain. Patients with brainstem strokes were excluded because of likely involvement of the respiratory centres. We were interested in MCA strokes as respiratory problems in this group are poorly documented. Those with haemorrhagic strokes were excluded because of the risk of further haemorrhage with forced manoeuvres.

2.2.3 Exclusion Criteria

People with a previous diagnosis of respiratory or neurological disease were excluded from all studies. A history of (or current) heavy alcohol consumption and presence of diabetes also led to exclusion from the study. This was because of the possibility of peripheral neuropathy (that would affect the peripheral magnetic stimulation studies) and the association of both conditions with an increased risk of pneumonia. Those with any contraindications to magnetic stimulation e.g. metal implants, cardiac pacemakers, epilepsy were excluded from all studies incorporating peripheral nerve or brain stimulation (165). Potential participants taking cough suppressants were excluded from the cough studies.
2.3 BASELINE MEASURES

2.3.1 Height and Weight
The height and weight of every ambulant participant was measured using a stadiometer (Holtain Limited, Crymych, Dyfed, UK) and a seated automatic weighing scale (Marsden, Oxfordshire, Great Britain) respectively. Patients unable to get out of bed were weighed by nursing staff on a hoist scale. (Marsden MPHW-200, Oxfordshire, UK). For patients unable to stand, the most recent height measurement was obtained from the medical notes or the general practitioner (GP).

2.3.2 Smoking History
A smoking history was taken from the patient or a close relative. Smoking history was expressed in pack years. One pack year=20 cigarettes per day for 1 year.

2.3.3 Medication History
For patients, a history of medication taken prior to admission was obtained from the GP. Medications administered during the current admission including alteplase (for thrombolysis) and angiotensin-converting enzyme inhibitors were noted from the patient’s drug chart. For controls a medication history was taken from the participant by the investigator.

2.3.4 Past Medical History
Past medical history of participants was obtained from the participants themselves by an investigator. In cases where the participant (usually patient) had difficulty with communicating or had forgotten parts of the history, hospital and GP records were retrieved.

2.4 STROKE ASSESSMENTS

2.4.1 Stroke Diagnosis
A consultant stroke physician or a consultant neurologist made the diagnosis of ischaemic stroke based on clinical and radiological findings at the time of admission to hospital or after further clinical and radiological assessments the next day, if the initial diagnosis had been uncertain (2). Computerised tomography (CT) brain scans (without contrast) were performed at the time of admission to hospital and reported by a consultant neuroradiologist. If the stroke territory was unclear on the initial scan the results of further neuroimaging performed 24 hours later were used. Scans and reports were retrieved from the hospital imaging database, PACS.
2.4.2 Clinical assessments of stroke severity
A stroke physician scored stroke severity on the National Institutes of Health Stroke Scale on the day of admission. The NIHSS score was performed again for all patients by the investigator (KW) on the day of testing; see Figure 22 (11). The NIHSS score on admission was obtained from the patient medical records. If an admission NIHSS score was missing from the notes, the patient’s stroke severity was scored retrospectively using a validated algorithm (12).

2.4.3 Swallow Assessments
Patients had a bedside swallowing assessment performed by a stroke specialist speech and language therapist (SLT) within 24 hours of admission, using radio-opaque contrast to detect aspiration (166). The SLT reports were obtained from the medical notes. Patients with dysphagia were not excluded from the study, including those who were kept nil-by-mouth.

2.4.4 Handedness
All participants were asked which hand they would use for writing and in the patients’ case they were asked which hand they would have used for writing before the stroke. This simple assessment of handedness was used because tools to assess handedness such as the Edinburgh Inventory (167) are not valid for use on stroke patients.

2.5 PULSE OXIMETRY AND CHEST RADIOGRAPHS
All patients had a chest radiograph (CXR) taken in the accident and emergency department at the time of admission to hospital. They were usually anterior-posterior views as they were taken using mobile equipment in the resuscitation room. The radiographs were retrieved from the hospital’s computerised radiology archive. A specialist radiologist reported all radiographs. If a patient had been admitted to an outside hospital and transferred into King’s, the admission film and report was requested from the other hospital. If there was no admission CXR and no other CXRs available, a CXR was performed after the patient’s individual research study was completed. Oxygen saturations were measured prior to other measures, with participants at rest, breathing room air using a fingertip probe and a pulse oximeter (Ohmeda Biox 3740 Pulse Oximeter, BOC Healthcare).
# Figure 22 The National Institutes of Health Stroke Scale

<table>
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<tr>
<th>NIH Stroke Scale</th>
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<tbody>
<tr>
<td><strong>1a. Level of Consciousness</strong></td>
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<td>(Alert, drowsy, etc.)</td>
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<td><strong>1b. LOC Questions</strong></td>
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<td>(Month, age)</td>
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<td><strong>1c. LOC Commands</strong></td>
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<tr>
<td>(Open, close eyes; make fist, let go)</td>
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<td><strong>2. Best Gaze</strong></td>
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<td>(Eyes open-patient follows finger or face)</td>
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<td><strong>3. Visual</strong></td>
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<td>(Introduce visual stimulus to patient’s visual field quadrants)</td>
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<td><strong>4. Facial Palsy</strong></td>
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<td>(Show teeth, raise eyebrows and squeeze eyes shut)</td>
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<td><strong>5a. Motor Arm left</strong></td>
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<td>(Elevate extremity to 90° and score drift/movement)</td>
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<td><strong>5b. Motor Arm right</strong></td>
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<td>(Elevate extremity to 90° and score drift/movement)</td>
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<td><strong>6a. Motor Leg left</strong></td>
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<td>(Elevate extremity to 30° and score drift/movement)</td>
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<td><strong>6b. Motor Leg right</strong></td>
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<td>(Elevate extremity to 30° and score drift/movement)</td>
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<td><strong>7. Limb Ataxia</strong></td>
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<td>(Finger-nose, heel-shin)</td>
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<td><strong>8. Sensory</strong></td>
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<td>(Pin prick to face, arm, trunk, and leg- compare side to side)</td>
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<td><strong>9. Best Language</strong></td>
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<td>(Name items, describes a picture, reads sentence)</td>
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<td><strong>10. Dysarthria</strong></td>
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<td>(Evaluate speech clarity by patient repeating listed words)</td>
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<td><strong>11. Extinction and Inattention</strong></td>
</tr>
<tr>
<td>(Use information from prior testing to identify neglect or double simultaneous stimuli testing)</td>
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**Total Score**
2.6 SPIROMETRY

Spirometry is a physiological test that measures how an individual inhales or exhales air as a function of time (168). Forced vital capacity (FVC) is the maximum volume of air that can be expired during an exhalation made as forcefully and completely as possible starting from full inspiration (total lung capacity, TLC). Forced expiratory volume in one second (FEV₁) is the volume exhaled in the first second of an FVC manoeuvre.

2.6.1 Forced Expiratory Volume in One Second (FEV₁) and Forced Vital Capacity (FVC) and FEV₁/FVC ratio

Spirometry (FEV₁ and FVC) was performed according the European Respiratory Society standards with the exception of participants being on a bed instead of a chair (168). Spirometry was carried out with subjects on a bed, propped up with the backrest at 45 degrees. For the studies described in Chapter 3, a SpiroPro (Carefusion, Basingstoke, UK) handheld, battery-operated spirometer (Figure 23) was used in conjunction with a single patient use bacterial filter and an appropriately sized flanged mouthpiece (Hans-Rudolf, Kansas, USA). Patients found it easier to make a mouth seal using the flanged mouthpiece rather than the conventional cardboard flow tube. For the studies described in Chapter 5, the Medisoft SpiroAir System (Medisoft SA, Sorinnes, Belgium) was used to for spirometry and measurement of lung volumes. A 13-litre rolling seal spirometer was used for spirometry. Volume measurements for each spirometer were validated using a three-litre calibration syringe prior to each patient testing session. Volumes were expressed as if the air were saturated with water vapour at
body temperature and at ambient barometric pressure (BTPS correction). Ambient
temperature, pressure and humidity were measured using a thermometer / hygrometer / barometer (Fisher Scientific, B70247, Basingstoke, UK) and entered into the spirometer software to enable BTPS correction of volumes.

2.6.2 Expression of Lung Function Results as a Percentage of Predicted
FEV₁ and FVC were expressed as a percentage of predicted for age and height to enable comparison between groups of possibly different average age or sex make-up. The most recently published predictions were used; these employ data from nearly 4000 males and females aged 8 to 80 years (169). Percentage predicted values for each participant were derived using a computer look-up program, available as an add-in for Microsoft Excel software (170). FRC was expressed as a percentage of predicted using the European Community for Steel and Coal (ECSC) prediction equations, see Equation 2 and Equation 3 (145). This corrects for between-group differences in height, age and proportion of females.

Equation 2 Prediction of FRC in Males
FRC predicted in litres = 2.34Height in metres + 0.009Age (years) - 1.09

Equation 3 Prediction of FRC in Females
FRC predicted in litres = 2.24Height in metres + 0.001Age (years) – 1

No correction of results was applied for ethnicity as there is no consensus as to how this should be done (171). An example of adjustment factors is the finding that populations using standing height as the measure of size tend to over predict values measured in black African subjects by ~12% for total lung capacity (TLC), FEV₁ and FVC, and by ~7% for functional residual capacity (FRC) and residual volume (RV) (172, 173). Although lower values may be usual in some non-white populations, this may not be normal (174).

2.7 CLOSED CIRCUIT HELIUM DILUTION FOR MEASUREMENT OF FRC

Functional Residual Capacity (FRC), the volume of gas remaining in the lung after a normal expiration, was measured using the closed circuit helium (He) dilution technique (41). This equipment was also used to measure flows for some studies. Testing was performed using a Medi-soft SpiroAir System (Medisoft SA, Sorinnes, Belgium). The system consisted of a 13-litre rolling seal spirometer with a katharometer-based helium analyser and a chemical fuel cell oxygen analyser. A Lilly cone pneumotachograph was incorporated to enable measurement of flow
rates. The testing sequence was controlled by a computer running Exp’Air software, (Medisoft SA, Sorinnes, Belgium), which digitised and displayed measurements in real time and stored them at the end of testing. A soda lime cell, incorporated in the breathing circuit removed carbon dioxide (Medisorb Carbon Dioxide Absorbent, GE Healthcare, Helsinki, Finland).

2.7.1 Ambient air conditions
Ambient air conditions were measured using a certified combined barometer, hygrometer and thermometer (Fisher Scientific, Loughborough, Leics, UK) The SpiroAir incorporates its own temperature probe and the machine readings were verified with the Fisher Scientific thermometer.

2.7.2 Helium analyser
The katharometer based helium analyser works by measuring the difference in thermal conductivity of two gases or gas mixtures. The thermal conductivity of any gas is inversely proportional to the molecular weight of the gas. The katharometer contains two parallel tubes each containing gas and heating coils. One tube receives the He mixture to be analysed and the other tube contains a known concentration of helium. The reference gas is the gas mix in the spirometer before the participant is switched into the measuring circuit. The heating coils are arranged in a Wheatstone bridge circuit so resistance changes due to unequal gas cooling can be measured. The response time of the SpiroAir He analyser is stated to be 10 seconds and the concentration range it is capable of measuring is 0 to 15% helium. The calibration of the helium and oxygen analysers is automatic (Figure 24). The SpiroAir measures the zeros from ambient air and the gains with gases from separate certified cylinders containing 9.232% helium and 100.0% oxygen (BOC gases, Guildford, UK). The pneumotachograph was calibrated using a certified three-litre syringe (Medisoft SA). The SpiroAir measured the voltage offset automatically, and then the syringe was used to pump three litres into and out of the SpiroAir at different flow rates. The volume of air added to or removed from the SpiroAir was displayed on the calibration screen. The vertical bars were coloured green if the volume measurements were within 3% of 3 litres (Figure 25). This is the American Thoracic Society definition of an acceptable volume measurement.
2.7.3 Static Lung Volume Testing

Participants sat in a supportive, reclinable chair (with a headrest and armrests) for testing (Barton I-400, Barton Medical Corp, Austin, Texas, USA). They were tested in both the seated and reclined positions according to the study protocol. For reclined testing, the chair back was reclined to 45 degrees from upright, measured using a goniometer and the legs were raised to horizontal. Participants were connected to the SpiroAir via an appropriately sized flanged mouthpiece (Hans Rudolf Inc, Shawnee, Kansas, USA). They wore a nose clip to eliminate nasal air leaks. Initially the patient breathed room air whilst the tidal breathing trace was monitored on the computer screen. Once a stable breathing pattern had been achieved, the participant was switched into the rebreathing circuit, at end expiration. The rebreathing circuit contained 14% helium and 21% oxygen made.
up to 100% with nitrogen. The exact concentration of each gas was measured prior to connecting the participant to the circuit. Oxygen consumed by the participant during rebreathing was automatically replaced by the system and exhaled carbon dioxide removed from the circuit by the soda lime was replaced by oxygen. The participant performed tidal breathing whilst the helium concentration in the rebreathing circuit was monitored on screen along with the oxygen concentration and the breathing trace.

The static lung volume testing was performed at least twice. The mean of two functional residual capacity (FRC) values within 10% of each other was recorded. A minimum of ten minutes was allowed between repeat tests to allow for helium washout from the participant’s lungs.

2.7.4 Cough Flow Testing (using the rolling seal spirometer)

The rolling seal spirometer was set to measure a flow-volume loop. Participants were connected to the spirometer via a bacterial filter and an appropriately sized Hans-Rudolf facemask covering the nose, mouth and chin (Hans Rudolf Inc). To ensure a good air seal any gaps around the side of the mask were filled with silicone nosepieces and Silly Putty, a mouldable mixture of silicone oil and boric acid. (Binney & Smith Europe Ltd, Bedford, UK). The participant was asked to take a deep breath and cough as hard as possible into the mask. A minimum of three coughs was performed and the maximum flow value, out of three flows within 10% of each other, was recorded.

2.8 RESPIRATORY MUSCLE TESTS

2.8.1 Pressure Measurements

Respiratory pressures (gastric pressure, oesophageal pressure and mouth pressure) were measured using pressure balloons attached to individual pressure transducers (MP45, Validyne, USA). The manufacturer states that these transducers give a linear response between -150 and +350cmH₂O. For the studies described in Chapter 3 and Chapter 4, the pressure transducer signals passed through a sensor interface (CD-280, Validyne) and were digitised and acquired at 2KHz using an analogue-to-digital converter (Powerlab, ADinstruments) and a computer running Chart 5 software (ADinstruments). (For the studies described in Chapter 6 onwards the pressure transducer signals passed through the same sensor interface (CD-280, Validyne) but were instead digitised and acquired at 2KHz using a CED 1401 analogue-to-digital converter (Cambridge Electronic Design Ltd, Cambridge, UK) and a computer running Spike2 software version 6.13
(Cambridge Electronic Design Ltd). The gain of the CD-280 was adjusted so full-scale deflection (+/-5V) occurred at a pressure of approximately +/- 280 cmH20 to ensure that the pressure signals were of optimum resolution but not clipped. Calibration and linearity testing of the transducers was carried out using a Comark C9553 pressure meter (Comark Ltd, Hitchen, Herts) (Figure 27). The pressure meter is regularly calibrated by an external laboratory and is certified valid up to 350cmH20 with an uncertainty of +/-2mBar or 0.02cmH20 (JMW Calibration Lab, Harlow, England). Linearity of the pressure transducer responses was demonstrated; see Figure 28, Figure 29, Figure 30, Figure 31. Transdiaphragmatic pressure (Pdi) was obtained by online subtraction of oesophageal pressure (Poes) from gastric pressure (Pgas). A calculated Pdi channel can be seen on the sniff traces in Figure 40.

**Figure 26 Equipment for Recording EMG and Pressure Traces**
Figure 27 Equipment used to calibrate and test linearity of the pressure transducers

Figure 28 Relationship between Applied Pressure and Electrical Output of the Mouth Pressure Measurement System

The graph shows the response characteristics of the pressure transducer and amplifier used to measure mouth pressures.
Figure 29 Relationship between Applied Pressure and Electrical Output of the Oesophageal Pressure Measurement System

The graph shows the response characteristics of the pressure transducer and amplifier used to measure oesophageal pressures.

Key for Figure 29 V=volts; cmH\(_2\)O=centimetres of water

Figure 30 Relationship between Flow Rate and Electrical Output of the Flow Measurement System

The graph shows the response characteristics of the combined pneumotachograph, pressure transducer and amplifier used to measure airflow rates.

Key for Figure 30 V=volts; applied airflow is measured in litres per minute
Two-point calibrations of each of the pressure transducers were carried out before each patient testing session using the Comark pressure meter and the calibration function on the Chart software. Atmospheric pressure was used as the zero point and approximately +/-200 cm H2O pressure was introduced to the system shown in Figure 27. The Chart software saved the voltage-to-pressure conversion for each transducer, to be used throughout the patient testing session.

### 2.8.3 Rationale for measuring gastric and oesophageal pressures

Balloon catheters were used to record respiratory pressures, that is oesophageal pressure (Poes) and gastric pressure (Pgas). The convention of measuring oesophageal pressure as a reflection of pleural pressure and gastric pressure as a reflection of intra-abdominal pressure has been in use for many years (39, 175).

### 2.8.4 Balloon Catheters

Gastric and oesophageal pressures were both measured using separate 86 cm closed-end latex-free plastic catheters (CooperSurgical, Trumbull, CT, USA) incorporating a balloon of 9.5 cm in length, positioned over the catheter and 5 cm from the closed end. The catheters have an external diameter of 2 mm and a 1 mm lumen. The length from the tip is marked in centimetres on the outside of the catheters.
catheter to aid positioning. The part of the catheter covered by the balloon has nine holes evenly spaced in a spiral arrangement for smooth transmission of pressure from the balloon to the catheter lumen.

2.8.5 Frequency Response of the Balloon-Transducer System

When dynamic respiratory manoeuvres are performed (e.g. cough or sniff) the measuring system requires a high frequency response (>10Hz) to rapid changes in pressure. Catheters that have too narrow a lumen or which are very long could lead to damped responses. Previous studies suggest that catheters with an internal lumen of 1.4-1.7mm and 70-100cm provide an appropriate response (39). As the catheters we used had a narrower lumen than 1.4mm the frequency response of the balloon catheters was confirmed, using exactly the same equipment as would be used for patient testing. This is important as use of extension tubing and connectors can affect the response. The balloon catheter was inserted into an inflated latex toy balloon. The pressure inside the toy balloon was measured using the balloon catheter system. The balloon was then burst using a sharp scalpel. The trace from the computer is shown in Figure 32. The time for pressure to drop from 90% of initial pressure to 10% of initial pressure was taken. The frequency response was calculated using Equation 4 described by Jackson and Vinegar (176).

Equation 4 Frequency Response of Balloon Transducer System

\[
\frac{1}{[(10\% \text{time} - 90\% \text{time}) \times 3]}
\]

The \(10\% \text{time} - 90\% \text{time}\) in this case was 0.0155 seconds so the calculated frequency response for the balloon catheter (containing 2ml of air) was 21.5Hz.
**Figure 32 Dynamic Response of the Balloon-Catheter-Transducer System**

This trace is from the pressure transducer used to measure gastric pressure with a balloon catheter containing 2ml of air.

**Figure 33 Response to 0cmH₂O applied external pressure when balloon catheter filled with different amounts of air**

The graph shows the pressure response of the balloon, transducer, amplifier, analogue-to-digital converter and computer measurement system when the balloon catheter was filled with different amounts of air.

Key for Figure 32: volume of air measured in millilitres; pressure reading measured in centimetres of water
Figure 34 Response of Gastric Pressure Measurement System with 2ml air in Balloon Catheter

The graph shows the response of the gastric pressure measurement system (balloon catheter filled with 2ml of air, pressure transducer, amplifier, analogue to digital converter and computer) to applied pressures, confirmed with a manometer.

Key for Figure 34 cmH₂O=centimetres of water
2.8.6 Balloon Catheter Position

Two catheters (for gastric and oesophageal pressure measurements) were lubricated and inserted at the same time via the nostril and nasopharynx with the aid of stylet introducers. Once the investigator felt the tip of the catheters had passed into the laryngopharynx, participants were asked to repeatedly swallow small sips of water to aid passage of the catheters into the stomach. Both catheters were passed up to the 70cm mark. The stylets were removed once the 70cm mark was reached. The catheters were each attached to an individual pressure transducer via a pressure extension tube and three-way tap. The balloon catheter used to measure oesophageal pressure was pulled back out from the stomach until the markings on the catheter read 40cm at the nose. 2ml of air was introduced into the gastric balloon and 0.5ml into the oesophageal balloon (after full inflation and deflation of the balloons to smooth out folds) using the three-way tap and a syringe (39). The participant was then asked to perform a sharp sniff. If the balloons were in the correct position the oesophageal pressure trace would show a sharp negative deflection and the Pgas trace would show a positive deflection. The balloon

Figure 35 Response of Oesophageal Pressure Measurement System with 0.5ml air in Balloon Catheter

The graph shows the response of the oesophageal pressure measurement system (balloon catheter filled with 0.5ml of air, pressure transducer, amplifier, analogue-to-digital converter and computer) to applied pressures, confirmed with a manometer.
positions were adjusted until this was the case after which the catheters were taped securely at the nostril.

Figure 36 Balloon Catheter Attached to Three Way Tap and Pressure Transducer, Showing Addition of Air to Balloon Using Syringe

2.9 VOLITIONAL RESPIRATORY MUSCLE TESTS

2.9.1 Maximum Inspiratory Mouth Pressure

Inspiratory muscle strength was assessed volitionally by measuring maximum static inspiratory mouth pressure (Plmax) and sniff pressures (39). For Pl max, participants wore a nose clip and made strong inspiratory efforts against a closed shutter, starting from functional residual capacity. The shutter was opened and closed by a tap and incorporated in a custom-made 30cm long, 2cm diameter hollow aluminium tube. The pressure measurement was taken from a small port close to the patient end of the tube (Figure 37). A flanged mouthpiece (Hans-Rudolf Inc, Shawnee, Kansas, USA) of appropriate size was used to try and achieve a good mouth seal. Well-fitting dentures were left in the participants’ mouths. The minimum mean pressure over one second was recorded (Figure 38). The Plmax manoeuvre was performed at least three times until consistency was achieved. If participants had problems making a mouth seal on the Plmax test an operator held the lips closed manually.
Measurement of oesophageal pressure during the PI max tests confirms correct positioning of the oesophageal balloon catheter. The fact that the PI max and the Poes measurements are within 10% of each other reassures the operator that the oesophageal pressure balloon is correctly placed and that there is no significant obstruction between the mouth and oesophageal balloon.

Key for Figure 38 PI max=maximum inspiratory mouth pressure; cmH\textsubscript{2}O=centimetres of water pressure
2.9.2 Sniff tests
Sniff tests are widely used as a global measure of inspiratory muscle function (39). Sniffing is a relatively natural manoeuvre which participants may find easier to perform than the PLmax. Sniff tests are complementary to the PLmax tests as a measure of inspiratory muscle strength (39, 40). A nasal probe (MicroMedical MicroRPM, Cardinal Health UK, Basingstoke, UK) was introduced into the nostril that did not contain the balloon catheters. The probe was attached to a pressure transducer via a pressure extension tube and a three-way tap (Figure 39). The participant was instructed to perform short sharp sniffs from FRC with the mouth closed (Figure 40). The sniffs were repeated until there were no improvement in the value of sniff nasal pressure (SNIP) and three SNIP values were within 10% of each other.

2.9.2.1 Sniff Nasal Pressure
Sniff nasal pressure (SNIP) was the most negative nasal pressure achieved during a sniff (see top row of pressure traces, Figure 40).

2.9.2.2 Sniff Oesophageal and Sniff Transdiaphragmatic Pressures
Sniff oesophageal pressure was the most negative oesophageal pressure achieved during a sniff. Sniff transdiaphragmatic pressure was the maximum pressure across the diaphragm achieved during a sniff (Figure 40).

Figure 39 Equipment for Measurement of Sniff Pressure
Volitional Expiratory Muscle Tests

Expiratory muscle strength was assessed volitionally by measuring maximum static expiratory pressure (PEmax). Participants made strong expiratory efforts against a closed shutter. The average (mean) maximum pressure over one second was recorded.

Volitional tests were performed at least three times until consistency was achieved. If participants had problems making a mouth seal an operator held the lips closed manually.
2.9.4 Cough Gastric pressure

The main expiratory muscles are used for cough, so the Pgas rise occurring during a cough can be used to assess expiratory muscle strength. As it is a natural manoeuvre, many people find it easier to perform than the PE max test. The method for this test is described in 2.10.3, page 102.

2.9.5 Non-Volitional Expiratory Muscle Test: Twitch Gastric Pressure

Non-volitional assessment of expiratory (abdominal) muscle strength was made, by measuring the rise in Pgas after magnetic stimulation over the T10 nerve roots (TwT10Pgas) supplying the abdominal muscles (40). A MagStim 200 stimulator (MagStim Company, Whitland, Wales, UK) was used with a 90mm high power, circular coil (HP 90mm 9784-00, MagStim Co, Whitland, UK). The stimulator was set to 100% of maximum output and the coil placed flat, with the centre over the intervertebral space between the T9 and T10 thoracic vertebrae. The participant was positioned on a bed, with the backrest at 45 degrees, leaning back onto the coil. The coil handle pointed caudally and marker A was on the outside (Figure 43 and Figure 43). The direction of current is not thought to make a significant difference as the coil produces a polyphasic rather than monophasic response but we wanted to ensure the method of stimulation was the same for all subjects (177). The participant was stimulated in the T9-T10 space and then 3cm above and below this to find the position where stimulation produced the biggest rise in Pgas.
At this position of maximum response five to ten stimulations were given to the limit of participant tolerance.

**Figure 42 Circular Coil for Magnetic Stimulation over the T<sub>10</sub> Nerve Roots**

The circular coil has faces labelled A and B. With Side A visible and Side B facing the area to be stimulated, the coil current flows in an anticlockwise direction and the induced tissue current flows in the clockwise direction. Image from The Magstim Company.

![Circular Coil](image1)

Causes clockwise current in tissue

**Figure 43 Position of Circular Coil for Stimulation over the T<sub>10</sub> nerve roots**

Image from Chokroverty (1995)

![Position of Circular Coil](image2)

![Key for Figure 43](image3)

Key for Figure 43 T<sub>10</sub>=tenth thoracic

**2.9.6 Peripheral Nerve Conduction and Compound Muscle Action Potentials**

Compound muscle action potential (CMAP) signals were acquired from the rectus abdominis muscles during and after magnetic stimulation over the tenth thoracic nerve roots. CMAPs are introduced in 1.5.3.1. Surface electrodes were placed over the rectus abdominis muscles. The skin surface was prepared using Nuprep Gel (Weaver and Co, Aurora, Colorado, USA) and alcohol rub. The electrodes were placed 5cm lateral to the umbilicus with the reference a further 5cm laterally.
The EMG electrodes were connected to a custom-made EMG amplifier (Pclab 3808, Guangzho, China). EMG signal between 10 and 1000 Hz was acquired at a sampling frequency of 10 000 Hz and amplified 1000 times. A 50 Hz band stop filter was applied to reduce mains noise contamination. The signals were then passed through an analogue to digital converter (Powerlab, AD instruments) to a computer running Chart 5 software. The Chart 5 files were converted into Spike 2 (Cambridge Electronic Design, Cambridge, UK) data to enable averaging using the Spike 2 software (as Chart 5 does not perform averaging). At least 5 (and up to 10) stimuli were averaged depending on the number of stimuli tolerated by the participant and the quality of the acquired data. Signals with magnetic stimulus noise superimposed over the evoked EMG were unusable. An averaged signal is shown in Figure 44 and Figure 45. The latency of the compound muscle action potential was measured (Figure 44); a normal result almost certainly excludes a diagnosis of peripheral neuropathy in the nerve being stimulated, which can lead to poor respiratory muscle function.

**Figure 44 Averaged CMAP and Measurements**

The latency of the CMAP is 7.4 ms and its peak-to-peak amplitude is 3.81 mV.
2.10 VOLUNTARY COUGH

For voluntary cough, participants were instructed to take a deep breath and cough "as hard as possible." The operator also demonstrated the cough, as some stroke patients performed better after a demonstration rather than a verbal order. The lung volume from which cough started was not set. Coughs were repeated at least five and up to twenty times, until there was no further improvement in cough Pgas and flow rates.

2.10.1 Cough Flow Rates

Airflow rates before, during and after cough were measured with participants wearing a well-fitting appropriately sized facemask (Hans-Rudolf, USA) connected to a pneumotachograph (Fleisch, Switzerland). Any air leaks around the side of
the mask were filled with Silly Putty, a mouldable mixture of silicone oil and boric acid (Binney & Smith Europe Ltd, Bedford, UK). The pneumotachograph was attached in series with a pressure transducer (MP45, Validyne, USA), a sensor interface / amplifier (Validyne CD-280), an analogue to digital converter (Powerlab) and a computer running Chart 5 software.

### 2.10.2 Correction of Cough Flow Rates for age, sex and height

Cough flow rates were expressed as percentage predicted peak flow rate. There are no published normal ranges for cough peak flow in adults; normal values are thought to range from 400 to 1200 litres per minute (178). Instead, cutoff values are used, for example to predict which patients are unlikely to wean from mechanical ventilation (those with cough peak flow less than 160 litres per minute) (161). It is argued that PEFR cannot be considered as a surrogate measure of cough efficiency as PEFR measures expiratory flow through an open glottis, whereas the forceful expiratory flow of a normal cough follows a glottis closure of about 0.2 to 0.5 seconds (178). As a consequence, cough peak flow measurements make some measure of assess bulbar-innervated muscle function.

For children and adolescents the strongest predictor of cough flow rate is height; this is also the strongest predictor of cough flow rate in adults (178, 179). So we argue that it is reasonable, though not ideal, to use predicted peak flow equations to correct for height differences between individuals.

Peak flow prediction equations used were: (179)

**Equation 5 Prediction of Peak Flow in Males**

\[ 6.14^* \text{ht (m)} - 0.043^* \text{Age (years)} + 0.15 \]

**Equation 6 Prediction of Peak Flow in Females**

\[ 5.50^* \text{ht (m)} - 0.030^* \text{Age (years)} - 1.11 \]

### 2.10.3 Cough Gastric Pressures

Intra abdominal pressures during cough were measured using a latex balloon catheter, placed as in the stomach described in 2.8.6. The gastric balloon contained 2ml of air. The pressure transducer signals were passed through a sensor interface / amplifier (CD-280, Validyne) and acquired at 2KHz using an analogue-to-digital converter (Powerlab, ADinstruments) and a computer running Chart 5 software (ADinstruments).
2.10.4 Cough Volumes

Cough inspired and expired volumes were calculated online by integration of the flow signal (Figure 47). This first required arithmetic conversion of the flow rate from litres/min to litres/second. The integration was reset to zero each breath cycle to prevent drift of the signal away from zero baselines.
Reflex cough can be induced by inhalation of aerosolised aqueous solutions of citric or L-tartaric acid. L-tartaric acid is thought to act on airway C-fibres to initiate coughing.

Voluntary cough was assessed before reflex cough, to avoid any effect of tartaric acid on voluntary cough. Participants started off with tidal breathing through the mask. They were then told to inhale maximally and produce the biggest voluntary cough possible, until five consistent readings of maximum cough gastric pressure were achieved.

**Figure 47 Traces From Three Voluntary Cough Bouts Showing Flow, Volume and Gastric Pressure**

Voluntary cough was assessed before reflex cough, to avoid any effect of tartaric acid on voluntary cough. Participants started off with tidal breathing through the mask. They were then told to inhale maximally and produce the biggest voluntary cough possible, until five consistent readings of maximum cough gastric pressure were achieved.

![Figure 47 Traces From Three Voluntary Cough Bouts Showing Flow, Volume and Gastric Pressure](image)

*Key for Figure 47 l/min=litres per minute; cmH₂O=centimetres of water pressure*

**Figure 48 Voluntary Cough Trace for a Stroke Patient with Guide to Measurements Made**

![Figure 48 Voluntary Cough Trace for a Stroke Patient with Guide to Measurements Made](image)

*Key for Figure 48 l/min=litres per minute; cmH₂O=centimetres of water pressure*

### 2.11 REFLEX COUGH

Reflex cough can be induced by inhalation of aerosolised aqueous solutions of citric or L-tartaric acid. L-tartaric acid is thought to act on airway C-fibres to
precipitate cough (156). Solutions for inducing cough were made by dissolving L-tartaric acid (Fisher Scientific, Loughborough, Leics, UK) in distilled water. The solutions were made up as shown in Table 5. Solutions were made immediately prior to each patient testing session and discarded at the end of the session.

**Table 5 Composition of Tartaric Acid Solutions For Reflex Cough Testing**

<table>
<thead>
<tr>
<th>L-tartaric Acid Solution Concentration (weight in volume)</th>
<th>Amount of L-tartaric Acid</th>
<th>Amount of Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>5g</td>
<td>100ml</td>
</tr>
<tr>
<td>10%</td>
<td>10g</td>
<td>100ml</td>
</tr>
<tr>
<td>15%</td>
<td>15g</td>
<td>100ml</td>
</tr>
<tr>
<td>20%</td>
<td>20g</td>
<td>100ml</td>
</tr>
</tbody>
</table>

Key for Table 5: % = percentage; g = grams; ml = millilitres

Reflex cough was induced by nebulising escalating concentrations [5%, 10%, 15% and 20% weight in volume (w/v)] of L-tartaric acid solutions through the facemask for 1 minute at a time during normal breathing (180). The L-tartaric acid was administered using a Porta-Neb® compressor and Sidestream® nebuliser (Philips-Respironics Ltd, Chichester, UK) attached to the pneumotachograph and facemask via a T-piece connector. Equipment used is shown in Figure 49 (151). The data sheet for the nebuliser states that 80% of the particles delivered will be 0.5µm diameter or smaller. Solutions were administered for 1 minute during normal breathing. Dose escalation was undertaken until 5 or more coughs were produced; if a participant failed to respond to 20% tartaric acid, no further solutions were administered. Corresponding cough spikes on the flow and Pgas traces were counted as coughs; all cough spikes within a cough bout were counted. Measures made were as in Figure 48. The concentration at which five or more coughs were elicited was noted (the suprathreshold concentration). A cough was counted if it produced simultaneous spikes on the flow and pressure traces. The actual numbers of coughs produced at this suprathreshold concentration were also counted.
2.12 TRANSCRANIAL MAGNETIC STIMULATION

Transcranial magnetic stimulation was used to study motor control of the abdominal (expiratory muscles). Before any magnetic stimulation took place participants completed a safety questionnaire (Figure 51). If there were affirmative answers to any of the questions, except question 11 (about medications) the participant was excluded from the study. For transcranial magnetic stimulation studies, participants sat in a supportive chair (Barton I-400, Barton Medical Corp, Austin, Texas, USA) in the upright position with the knees bent to 90 degrees from horizontal. The participant's head was kept firmly in position with the aid of a winged headrest and a small pillow. The cranial vertex (the highest point of the skull) was found using a tape measure and marked using indelible ink.

2.12.1 Magnetic Stimulator Device

The MagStim 200 magnetic stimulator (The MagStim Company, Whitland, UK) is based on the original Sheffield stimulator (43). The capacitor charges up to 2.8kV and produces a monophasic discharge current when triggered by the operator using a switch. The rise time of the discharged current is 100 microseconds and the total duration is less than 1 millisecond (47).

2.12.2 The BiStim Device

The BiStim device (MagStim Ltd, Whitland, Wales) enables a pair of pulses (from a pair of MagStim 200 stimulators) to be delivered through the same coil, with a
short, programmable interstimulus interval. The interval between stimuli is usually a number of milliseconds.

### 2.12.3 Stimulating Coil

Cortical magnetic stimulation was delivered using a 110mm double cone coil (MagStim 9902-00, The MagStim Company, Whitland, UK) shown in Figure 50. The coil was powered by a MagStim 200 magnetic stimulator (The MagStim Company). The peak magnetic field strength generated using the double cone coil is 1.4 Tesla (47). The coil was positioned so the current in the coil was anterior to posterior with respect to the patient, leading to a posterior to anterior current in the brain.

**Figure 50 Double Cone Coil**

![Double Cone Coil Image](image)

### 2.12.4 Stimulation Technique

Stimuli were delivered at end-expiration. Previous studies suggest that normal respiration can modulate the activity of the abdominal motoneurons and this could

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**Figure 51 TMS screening questionnaire**

1. Do you have epilepsy or have you ever had a convulsion or a seizure?
2. Have you ever had a fainting spell or syncope? If yes, please describe in which occasion(s)
3. Have you ever had severe (i.e., followed by loss of consciousness) head trauma?
4. Do you have any hearing problems or ringing in your ears?
5. Are you pregnant or is there any chance that you might be?
6. Do you have metal in the brain/skull (except titanium)? (e.g., splinters, fragments, clips, etc.)
7. Do you have cochlear implants?
8. Do you have an implanted neurostimulator? (E.g., DBS, epidural/subdural, VNS)
9. Do you have a cardiac pacemaker or intracardiac lines or metal in your body?
10. Do you have a medication infusion device? (e.g. insulin or chemo pump)
11. Are you taking any medications? (Please list)
12. Did you ever have a surgical procedure to your spinal cord?
13. Do you have spinal or ventricular derivations?
affect the MEP amplitude (181). To control for this subjects ceased breathing at the end of a normal expiration prior to the stimulus being delivered (101).

2.12.5 Motor Evoked Potentials
A motor evoked response or motor evoked potential (MEP) is an electrical action potential recorded from a muscle following stimulation of the motor cortex. MEPs were acquired from the resting lateral abdominal muscles during transcranial magnetic stimulation unless otherwise stated.

2.12.6 Surface Electrodes
Surface electrodes were placed bilaterally over the lateral abdominal muscles unless otherwise stated in individual study protocols. The skin surface was prepared using Nuprep Gel (Weaver and Co, Aurora, Colorado, USA) and alcohol rub. The positive electrodes were placed on an imaginary horizontal line drawn between the left and right anterior superior iliac spines, 2cm medial to each spine. The negative electrodes were placed 5cm medial to the positive electrodes on each side. The reference electrode was placed on the same horizontal line, directly caudal to the umbilicus. This position overlies the external oblique in most people but from this position surface electrodes may pick up EMG from underlying and adjacent muscles too. For patient 2 in the training study only (see Chapter 8) quadriceps EMG was recorded from electrodes placed over the belly of the rectus femoris muscle.

2.12.7 EMG Acquisition and Processing
The EMG electrodes were connected to an EMG amplifier (CED 1902, Cambridge Electronic Design, Cambridge, UK). EMG signal between 10 and 1000 Hz was acquired at a sampling frequency of 10 000Hz and amplified 1000 times. A 50Hz band stop filter was applied to reduce mains noise contamination. The amplified signals were passed through an analogue to digital converter (CED 1401 micro mark II) to a computer running Spike2 software version 6 (AD converter and software by Cambridge Electronic Design).

2.12.8 EMG Averaging and Measuring
Averaging of MEP responses was performed offline using Spike2 software. An explanation of measures made is given in Figure 52. A 150-millisecond sweep was averaged, starting from 50 milliseconds prior to the magnetic stimulus. Latency of the averaged responses was measured in milliseconds, with latency defined as the time up to the first deflection from baseline following TMS. Peak-to-peak amplitude of the averaged responses was also measured, in millivolts.
Note that area under the curve of the rectified MEP response and peak to peak amplitude of the unrectified signal are very strongly correlated so either may be used as a measure of MEP size (54). We chose to use peak-to-peak amplitude as our measure of muscle electrical activity as it was often difficult to pinpoint the exact termination of the rectified MEP signal, which is required for area to be determined.

2.12.9 Corticomotor Threshold
A definition and physiological meaning of the corticomotor threshold (CMT) is given in 1.5.4.1. Measurement of the resting CMT was performed with participants sitting quietly in a supportive chair with the backrest at 90 degrees to the horizontal and the hips flexed to 90 degrees, with the abdominal muscles in the relaxed state. Stimulations were given with the double cone coil at the cranial vertex, with the coil oriented as detailed in 2.12.3 (53). Starting at 30% of maximum stimulator output (MSO) and rising in 5% increments, ten stimulations were given at each output level. The CMT was defined as the minimum stimulus intensity required to produce a motor evoked potential (MEP) with peak-to-peak amplitude of 50 microvolts from the right-sided lateral abdominal muscles, in at least five out of ten consecutively recorded stimulations. If one level produced fewer than five suitable MEPs and the next level up produced ten MEPs, the CMT was said to be the average of those two output levels, rounded up to the next 1%. A separate threshold for the affected and unaffected sides was established for patients.
2.12.10  Studies to Try and Produce Larger and More Reproducible MEPs

2.12.10.1  Effect of Coil Position

First the resting CMT was established as in 2.12.9. A baseline MEP was established by delivering ten stimulations with the double cone coil at the cranial vertex with the stimulator set at 120% of CMT, with the participant resting as in 2.12.9. For patients, 120% of the CMT for the affected side was used. If 120% of the CMT for any participant was larger than 100%, the stimulator was set at MSO (100%). To establish the effect of different coil positions on MEPs, all stimulations were given at a stimulus intensity of 120% of the CMT (with exceptions as above). The double cone coil was then moved anteriorly in 1cm increments until the centre of the coil was 5cm anterior to the vertex. The coil was then moved posteriorly from the vertex in 1cm increments until the centre of the coil was 5cm posterior to the vertex. Five stimulations were given in each position, with approximately one minute rest for the participant whilst changing coil position. The MEPs were averaged using Spike2 software. The coil was only moved along the midline (saggital plane) as it was not possible to achieve good contact between the double-cone coil and skull in positions lateral to the midline. The averaged MEPs for each test position were compared with the baseline MEP using multiple comparison statistical tests; this is further explained in Chapter 6. The MEPs from

Figure 52 MEPS from the Right Lateral Abdominal Muscles

The figure shows an average of ten MEPs after transcranial magnetic stimulation at the cranial vertex with the magnetic stimulator intensity set to 100%. The mean signal and standard error of the mean are shown. Vertical cursor 1 is the time of the stimulus; cursor 2 marks the onset of the evoked potential; cursor 3 is at the trough and cursor 3 is at the peak of the evoked potential. The duration from 1 to 2 is the latency.
each test position were also normalised for clear illustration of the effect of coil position; see 2.12.11 and Chapter 6.

2.12.10.2 Paired Pulse Stimulation

The CMT was first established as detailed in 2.12.9. A baseline MEP was established by delivering ten stimulations with the double cone coil at the cranial vertex with the stimulator set at 120% of CMT, with the participant resting as in 2.12.9. For patients, 120% of the CMT for the affected side was used. If 120% of the CMT for any participant was larger than 100%, the stimulator was set at MSO (100%). Paired pulse TMS was delivered at the cranial vertex via the double cone coil, using two MagStim 200 stimulators triggered at set interstimulus intervals through the BiStim device (see 2.12.2). Participants sat as for establishment of the CMT. The first (conditioning) stimulus was set to 80% of the CMT and the second (activating) stimulus was set to 120% of the participant’s CMT (68). 5 pairs of stimuli were given for each interstimulus interval and the MEPs were averaged using Spike2 software. The intervals used were 1-5 milliseconds inclusive; 7, 9, 10, 12, 14, 15, 17, 19, 20, 22, 24 and 25 milliseconds.

2.12.10.3 Voluntary Muscle Activation

For the voluntary muscle activation sub studies participants sat unsupported on a height-adjustable stool with their hip flexors at 90 degrees to vertical and their feet on the floor, to achieve a low background level of trunk muscle activation. TMS was further facilitated using a modified Valsalva manoeuvre to activate the abdominal muscles. The manoeuvre comprised holding the breath at end-expiration and bearing down as if trying to pass a bowel movement.

Method of finding MVC for abdominal muscles

Each participant performed the modified Valsalva manoeuvre described above with maximum effort at least ten times, until the intra-abdominal pressure rise (measured with an intragastric balloon) had reached a plateau. The method of measuring intra-abdominal pressure is given in section 2.8. The average maximum pressure over one second was recorded. This was then called the abdominal muscle MVC.

Voluntary Facilitation

To establish a baseline MEP, ten stimuli were given at 100% of MSO at the vertex, using the double cone coil. An intra-abdominal pressure level target, representing of 20% of the previously found MVC, was then drawn onto the computer screen. We asked participants to raise their Pgas up to the target line and sustain it by
“bearing down” whilst TMS was delivered. TMS was then given at 100% of maximum stimulator output, whilst the participant was performing the manoeuvre. Participants did not find it easy to raise their Pgas to exactly 20% of MVC and sustain it there. We were pragmatic and told subjects to do the best they could and then retrospectively measured the Pgas on the averaged pressure trace for the 50ms prior to the stimuli, expressing it as a percentage of their own maximum. This was repeated for a target pressure level of 40%. Ten stimuli were given for each abdominal pressure level.

2.12.11 EMG normalisation

For the coil position, paired pulse stimulation studies and voluntary facilitation studies, MEP amplitudes were normalised by comparing to the baseline MEP. These were produced in by single-pulse TMS from the double cone coil at the vertex, see 2.12.10.1 and 2.12.10.2. For the baseline in the voluntary facilitation study only, the MEPs produced for each level of facilitation were compared with MEPs produced by stimulation at the vertex at 100% of maximum stimulator output with the participant sitting upright on a stool.

To normalise data we divided the peak-to-peak amplitude of the averaged MEP produced by the test condition of interest in an individual, with the peak to peak amplitude of the averaged baseline MEP for the same individual and multiplied by 100 to get a percentage of the baseline result. If the amplitudes of the two MEPs were the same, the normalised MEP amplitude would be 100%.

2.12.12 Stimulus-Response Relationships for Abdominal Muscle Response to TMS

These studies are described in Chapter 7 and Chapter 8. Stimuli were delivered at the cranial vertex using the double cone coil and the Magstim 200 as described above. Stimulus-response (SR) curves were constructed with the abdominal muscles in a relaxed state. Participants sat upright in a chair with the head, arms and legs supported with the backrest of the chair at 90 degrees to the horizontal. All participants rested for 20 minutes prior to commencement of the TMS protocol to avoid twitch potentiation. A ramp incremental technique was used and the healthy participants received ten stimulations at each level of stimulator output, increasing by 5% each time from 30% to 100% of maximum stimulator output. The responses, recorded simultaneously, were motor evoked potentials (MEP) from the lateral abdominal muscles (right oblique) and the rise in Pgas evoked by the TMS. The electrode positions are detailed in section 2.12.6. The method of
recording $P_{gas}$ is described in section 2.8. The signal acquisition equipment is described in 2.12.7. Responses (MEP amplitude and $P_{gas}$ rise) for each individual were plotted against the stimulus intensity on a scatter graph, using Prism software.

2.12.12.1 Area under the SR Curve

This is introduced in section 1.5.4.5. The data points on the scatter plot of stimulation intensity (on the x-axis) against MEP amplitude were joined with straight lines to get a “curve”. The area under the curve was calculated (in Prism) by adding the areas under the curve between each pair of consecutive observations; this method is known as the trapezium rule and is described by Altman (182). See Figure 53 for illustration of the method.

Figure 53 Stimulus-Response Graph for First Dorsal Interosseus Muscle Showing Calculation of Area Under the Curve

The image shown is taken from Talelli’s paper of 2008; see 1.5.4.5. The circles represent the collected data. The stimulation intensity, on the x-axis, is expressed as a percentage of the motor threshold of the active muscle (AMT). The y-axis MEP amplitude is expressed as a percentage of the CMAP amplitude. The line represents the relation predicted by the Boltzmann model. The area under the curve (grey area) was calculated with the method of trapezoid integration using the actual data collected during the construction of the curves.

Key for Figure 53 CMAP=compound motor action potential; MEP=motor evoked potential; AMT=active motor threshold; %=percentage

2.12.12.2 Boltzmann Sigmoidal Curve Fit

Prism software was used to fit the data points to a curve with the Boltzmann sigmoidal function (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). The extra sum-of-squares $F$ test was used (in GraphPad Prism) to compare the Boltzman sigmoidal fit with a straight line fit for each participant’s SR data. The test compares the
difference in sum-of-squares for the two models with the difference that would be expected by chance. The null hypothesis for this part of the study was that the straight line fit was best. The test used generates a P-value; if the P-value was <0.01 the null hypothesis was rejected and it was concluded that the sigmoidal model fitted the data significantly better than the straight line.

For the patient studies described in Chapter 8 the methods were as above except magnetic stimulus intensity increased by 10% each time from 30% to 100%. Patients had bilateral recording of abdominal muscle MEPs but Pgas measurements were not made. The results of these studies are described in Chapter 7 (healthy participants) and Chapter 8 (patients).

2.12.13 Cough Training and Its Effects

Stroke patients were studied. Patients were seated upright in the same supportive chair and position used for TMS and described in 2.12. The results of these studies are given in Chapter 8. MEPs and EMG (during training) were recorded from the lateral abdominals (both patients) and the quadriceps (patient 2) on both sides as detailed in 2.12.6.

2.12.13.1 Measurement of Maximum Cough Flow

Prior to cough training, patients’ maximum cough flow rates were measured. This was so that a proportion of the maximum flow rate could be set as a target for the cough training. Patients made five or more maximum cough efforts, continuing to cough until cough flow reached a plateau and three coughs with a cough flow rate within 10% of each other were produced. After measurement of the maximum cough flow rate, the patients rested for twenty minutes. Patients coughed into a tight-fitting Hans-Rudolf facemask connected in series with a calibrated Fleisch pneumotachograph, a pressure transducer (Validyne MP-45), a 1401 analogue-to-digital converter (CED, Cambridge, UK) and a computer running CED Spike 6 software. Further details regarding the measurement of cough flow rate is given in the Methods (Chapter 2), in particular 2.10.1.

2.12.13.2 Stimulus-Response Curves at Baseline and Post Training

TMS SR curves for the lateral abdominal muscles bilaterally were constructed immediately prior to the cough training and then again at ten and twenty minutes after the training had finished, to assess the effect of training on the motor cortex excitability. In addition, patient 2 had MEPs recorded from the quadriceps muscles bilaterally. The methods for the SR curves are given in 2.12.12. MEP outcome
measures, (MEP amplitude, MEP latency, corticomotor threshold, area under the SR curves), from before and at 10 and 20 after the training were tabulated.

2.12.13.3 Cough Training Protocol

Patients performed five coughs with a cough flow rate of 75% of their own maximum or greater, into the Hans-Rudolf mask and pneumotachograph equipment as described above. A visual target on the computer screen was set at 75% of the patients’ previously measured maximum voluntary cough flow rate. The coughs were cued using a metronome set at ten beats per minute. The five coughs were completed over 30 seconds and then patients rested for 30 seconds. In this way patients completed twenty-five coughs in a five-minute period. Cough flow and EMG from the abdominals and quadriceps (patient 2 only) were recorded during the training.

2.13 Statistical Analyses, Sample Size Calculations and Figure Construction

The statistical test methods used for individual studies are described in the chapters describing those studies. The software used and general principles of the statistical analyses are described here. Statistical analyses were performed using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California, USA); and SPSS 16.0.1 (SPSS Inc, USA). All datasets were tested for normality using the D'Agostino and Pearson omnibus method. P<0.05 was considered significant for all studies. Confidence Interval Analysis 2.2.0 (183) was used to calculate confidence intervals for the median difference between patient and control groups for non-parametric datasets. Univariate and multivariate linear regression modelling was used to investigate relationships between dependent and independent variables. All regression models included a constant. Sample size calculations throughout were performed using G*Power v3.0.8 software (Franz Faul, University of Kiel, Germany) (144). GraphPad, SPSS and PowerPoint 2008 for Mac were used for figure construction.
Chapter 3  Respiratory Function and Respiratory Muscle Tests in Stroke Patients and Controls

3.1  INTRODUCTION

The central theme of this work as a whole is the study of cough. An effective cough requires powerful, coordinated contraction of expiratory (abdominal) muscles along with adequate static lung volumes, sufficient inspiration prior to cough and unobstructed airways.

Previous studies of stroke patients have shown reduced arterial oxygenation, impaired spirometry, asymmetry of ventilation, reduced movement of the diaphragm and chest on the hemiparetic side and poor performance on volitional respiratory muscle tests (108, 110, 115, 184).

We sought to confirm our prior observations from a smaller study (58) that stroke patients who had no evidence of abdominal muscle weakness judged by peripheral nerve stimulation were classed as weak when assessed by voluntary tests of respiratory muscle strength. We thought that current patients might perform better than seen in the previous study due to the introduction and subsequent widespread use of therapeutic thrombolysis at our hospital and the consequent reduction in patient morbidity. The principal outcome measures were FEV$_1$, maximum expiratory mouth pressure (PEmax) and twitch gastric pressure (TwT$_{10}$Pg).

3.2  METHODS

3.2.1  Study Participants

Forty-five consecutive patients admitted to the stroke unit of King's College Hospital within two weeks of first-ever, middle cerebral artery territory ischaemic stroke were screened. Six patients were excluded as they did not wish to take part. Six patients with lacunar infarcts were excluded and fifteen were unsuitable due to diabetes, excess alcohol consumption, respiratory or neurological disease except stroke or inability to follow commands. Eighteen adults (seven women) were studied. Twenty healthy controls (five women) were recruited from a volunteer database and studied. The mean age and the proportion of female participants were not significantly different between groups. Institutional ethical approval was obtained (LREC 02-120) and the participants gave written informed consent.
3.2.2 **Baseline Assessments**
Smoking history, alcohol and ACE inhibitor use, height and weight were documented for all participants. For patients, stroke diagnosis and location were confirmed by brain CT scan. Stroke severity on admission was assessed using the National Institutes of Health Stroke Scale (NIHSS) score, See Methods section.

3.2.3 **Chest radiographs**
All patients had chest radiographs taken in the accident and emergency department at the time of admission to hospital. The radiographs were retrieved using the hospital online radiology archive, PACS. A specialist radiologist reported all radiographs.

3.2.4 **Pulse oximetry**
Oxygen saturations were measured with participants at rest, breathing room air using a fingertip probe and a pulse oximeter. See section 2.5 for details.

3.2.5 **Spirometry**
A handheld spirometer was used to measure forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC), which were expressed as percentage of predicted. See 2.6.1 for details of spirometry and 2.6.2 for % predicted calculations.

3.2.6 **Respiratory Muscle Tests**
Respiratory muscle strength measurements were performed under controlled laboratory conditions with participants on a bed, with the backrest at forty-five degrees and their legs raised but otherwise in accordance with the ATS/ERS Statement (185). This position ensured patients had the head end of the bed raised at an angle greater than the thirty degrees recommended to prevent aspiration and enabled inclusion of participants unable to sit upright. Section 2.8 details how the measurements were made. Respiratory muscle strength was assessed volitionally by measuring maximum static expiratory mouth pressure (PEmax), maximum static inspiratory mouth pressure (PImax) and sniff pressures. Further details are given in 2.9.3 (PEmax), 2.9.1 (PImax) and 2.9.2 (sniff tests).

Non-volitional assessment of expiratory (abdominal) muscle strength was made using magnetic stimulation over the spine at the level of the tenth thoracic nerve roots with the participant wearing a nose clip and at FRC (39). Section 2.9.5 details how this was done. Compound muscle action potentials (CMAPs) were recorded from surface electrodes over the rectus abdominis muscles after magnetic stimulation over the T₁₀ nerve roots; see 2.9.6 for details. Five to ten
reproducible signals were averaged and the latency of the averaged CMAPs was recorded.

3.2.7 Sample size and Data Analysis
The primary outcome measure was maximum expiratory mouth pressure (PEmax). In a previous study with similar methods, healthy participants had a mean (SD) PEmax of 102.7 (28.0) and the stroke group had a mean PEmax of 62.6 (30.0) cmH₂O (58). Using these data and G*Power v3.0.8 software (see Methods chapter) it was calculated that thirteen participants in each group were required for a 95% chance to detect a similar difference in PEmax between groups, at a significance level of 5%. We aimed to study twenty participants in each group, as we believed the patients in this study could be less severely affected by stroke than those in the previous study. Statistical analyses were performed using software described in 2.13. Datasets were tested for normality and t-tests or Mann-Whitney u-tests for two independent groups were used for comparisons (183). Univariate linear regressions were used to investigate the effect of stroke severity on PEmax and Plmax. Patients and controls were analysed together with controls being assigned a stroke severity score (NIHSS score) of zero for the purposes of these analyses. All regression models included a constant.

3.3 RESULTS
3.3.1 Participants
Acute infarction was present in the left hemisphere in nine and the right hemisphere in nine patients. Of the left infarcts, 3 were frontal, 1 frontoparietal, 1 temporofrontoparietal and 4 capsulostriate. Of the right infarcts, 3 were frontal, 1 frontoparietal, 4 temporofrontoparietal and 1 capsulostriate. Six of eighteen patients had been treated with thrombolysis.
### Table 6 Stroke Patient Characteristics

<table>
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<tr>
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<th>Ethnicity</th>
<th>Stroke Side</th>
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<th>No of days post stroke</th>
<th>Swallow judged safe</th>
<th>Handedness†</th>
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Key for Table 6
*NIHSS=National Institutes of Health Stroke Scale; m=male; f=female
†where patient or family were able to communicate this
Table 7 Baseline Characteristics

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<th>Controls</th>
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<td>Mean 56</td>
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<td>Males 15</td>
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<td></td>
<td>Proportion 0.61</td>
<td>Proportion 0.75</td>
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<td>-0.40 to 0.15</td>
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<td>Median 24</td>
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<tr>
<td>Days from stroke onset to test</td>
<td>Mean 6</td>
<td>Mean 3</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD 3</td>
<td>SD 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke severity (NIHSS score)</td>
<td>Mean 14</td>
<td>Mean 14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD 8</td>
<td>SD 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysphagic</td>
<td>Number 4</td>
<td>Number 4</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proportion 0.22</td>
<td>Proportion 0.22</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Taking ACE inhibitor</td>
<td>Number 9</td>
<td>Number 0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proportion 0.50</td>
<td>Proportion 0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Key for Table 7
*P-value for difference in proportions calculated using Fisher’s exact test. NIHSS=National Institutes for Health Stroke Scale; IQR=Interquartile range; SD=standard deviation; BMI=body mass index; CI=confidence interval; kg=kilograms; cm=centimetres

3.3.2 Pulmonary Function Tests

Results are given in Table 8. Patients’ oxygen saturations were lower than those of controls and their respiratory rate was higher. Reports of chest radiographs taken during the admission for stroke were available for fourteen out of eighteen patients. For ten patients, the radiologist reported the lung fields and pleura to be clear. For two patients, chest radiographs were reported as showing signs of chronic obstructive pulmonary disease (COPD) although these patients had not had a diagnosis of COPD made previously. One radiograph showed interstitial pulmonary oedema but as the relevant patient was unable to perform spirometry or cough, this did not affect group results for these tests. One radiograph showed a small left pleural effusion.
3.3.3 Respiratory Muscle Tests

Patients showed significant impairments on volitional respiratory muscle tests (Table 9 and Table 11). There was no difference between patient and control groups on the non-volitional expiratory muscle strength test, $T_{10}P_{\text{gas}}$. The patients' group's mean $T_{10}P_{\text{gas}}$ of 26.4cmH$_2$O (SD 6.6) was well above the published normal minimum value of 16cmH$_2$O, indicating that the expiratory muscles themselves were not weak but that the stroke patients could not fully recruit them volitionally (Table 9). The latency of the compound muscle action potential (CMAP) was similar for stroke patients and controls (Table 10) although many CMAPS were uninterpretable due to stimulus artefacts.

### Table 9 Expiratory Muscle Tests

<table>
<thead>
<tr>
<th>Normal value for male*</th>
<th>Stroke patients n=18†</th>
<th>Controls n=20</th>
<th>Mean or Median difference (95% CI)</th>
<th>P-value for diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{Emax}}$ (cmH$_2$O)</td>
<td>Median 50.5 IQR 39.5 to 69.5</td>
<td>106.0 82.9 to 140.0</td>
<td>-55.7 -74.3 to -31.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$T_{10}P_{\text{gas}}$ (cmH$_2$O)</td>
<td>Mean 26.4 SD 6.6</td>
<td>32.0 14.1</td>
<td>-5.6 -16 to 4.0</td>
<td>0.258</td>
</tr>
</tbody>
</table>

Key for Table 9: IQR=interquartile range; $P_{\text{Emax}}$=maximum expiratory mouth pressure; $T_{10}P_{\text{gas}}$=gastric pressure rise after $T_{10}$ nerve root stimulation; cmH$_2$O=centimetres of water pressure; *normal values from Steier 2007 †3 patients were unable to perform $P_{\text{Emax}}$ tests so n=15 for these tests in the stroke group.
Peripheral Nerve Conduction

Latency could not be measured for every subject because of non-biological artefact contaminating the EMG trace. Noise-free signals were obtained for nine patients and twelve controls and the means for each group are given in Table 10 below. An uncontaminated, averaged CMAP recording from a stroke patient is given in Figure 55. The results of the t-test should be interpreted with care due to the small sample size.

Table 10 Compound Muscle Action Potential Latency

<table>
<thead>
<tr>
<th></th>
<th>Stroke (n=9)</th>
<th>Controls (n=12)</th>
<th>Mean difference (95% CI)</th>
<th>P-value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency of CMAP of rectus muscle, after magnetic stimulation of the T₁₀ nerve roots (ms)</td>
<td>9.56 (1.80)</td>
<td>9.10 (1.57)</td>
<td>0.46 (-1.089 to 2.00)</td>
<td>0.544</td>
</tr>
</tbody>
</table>

Key for Table 10 CMAP=compound muscle action potential; CI=confidence interval

Figure 54 Maximum Expiratory Mouth Pressure

Scatter dot plot to show PEmax for stroke patients and controls. The lines represent median and interquartile range. P value for the difference between medians = <0.001

Key for Figure 54 PEmax=maximum expiratory pressure at the mouth; cmH₂0=centimetres of water pressure; P value calculated with Mann-Whitney u-test
Figure 55 Close Up of Averaged CMAP from Right Rectus Abdominis in Stroke Patient

Compound muscle action potential from surface electrodes over the right rectus muscle recorded during magnetic stimulation of the T10 nerve roots. The traces show the averaged signal, the mean (and standard error of mean) results of 10 stimulations in a 50 year old male patient with a left cortical infarct and resultant right hemiparesis. The clinical severity of the stroke was moderate with the patient scoring 15 on the NIH stroke scale. The magnetic stimulus is given at time 0 on the x-axis. The latency of the CMAP (from cursor 1 to cursor 2) is 7.4ms and its peak to peak amplitude is 3.81mV (shown below at 1000x amplification).

Key for Figure 55 CMAP=compound muscle action potential; EMG=electromyograph; SEM=standard error of the mean

Table 11 Inspiratory Muscle Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal value for male*</th>
<th>Stroke patients n=18†</th>
<th>Controls n=20</th>
<th>Mean difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PImax (cmH2O) †</td>
<td>&gt;45</td>
<td>Mean 38.9</td>
<td>95.1</td>
<td>-56.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 25.1</td>
<td>33.0</td>
<td>-77.7 to 34.7</td>
<td></td>
</tr>
<tr>
<td>Sniff nasal pressure (cmH2O)†</td>
<td>&gt;50</td>
<td>Mean 40.7</td>
<td>92.7</td>
<td>-52.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 25.8</td>
<td>25.9</td>
<td>-70.2 to -33.8</td>
<td></td>
</tr>
<tr>
<td>Sniff Poes (cmH2O) †</td>
<td>&gt;55</td>
<td>Mean 57.7</td>
<td>109.3</td>
<td>-51.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 36.7</td>
<td>28.7</td>
<td>-76.1 to -27.3</td>
<td></td>
</tr>
<tr>
<td>Sniff Pdi (cmH2O) †</td>
<td>&gt;100</td>
<td>Mean 63.2</td>
<td>121.1</td>
<td>-58.0</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 40.6</td>
<td>38.7</td>
<td>-88.4 to -27.4</td>
<td></td>
</tr>
</tbody>
</table>

Key for Table 11 PImax=maximum inspiratory pressure at the mouth; Poes=oesophageal pressure; Pdi=pressure across the diaphragm; cmH2O=centimetres of water pressure; *normal values from Steier 2007
3 patients were unable to perform PImax and sniff tests so n=15 for these tests in the stroke group.
Figure 56 Comparison of Maximum Inspiratory Mouth Pressure for Patients and Controls

Scatter dot plot to show PImax for stroke patients and controls. The lines represent mean and standard deviation from the mean. P value for the difference between means = <0.001

Key for Figure 56
PImax=maximum inspiratory mouth pressure; cmH2O=centimetres of water pressure

3.3.5 Predictors of Respiratory Muscle Strength

The results of univariate linear regression with PEmax as the dependent variable and stroke severity as the predictor are shown in Figure 57.

Figure 57 Stroke Severity is a Significant Predictor of PE max

Scatter diagram to show the relationship between stroke severity (NIHSS score) and maximum expiratory pressure at the mouth (PE max). The equation for the regression line is given on the figure. 95% confidence interval for the constant 92.1 to 120.2; 95% confidence interval for the slope -5.0 to -2.0. P<0.001.

Key for Figure 57 NIHSS=National Institutes of Health Stroke Scale; PEmax=maximum expiratory pressure at the mouth
3.4 DISCUSSION

These studies show that acute ischaemic hemispheric stroke patients perform poorly on volitional tests of respiratory muscle strength but have similar strength to control subjects on non-volitional testing. They also show that patients have impaired spirometry in line with that seen in patients with neuromuscular disease. Our prediction that this new group of study patients would perform better than a previous group (58) because of an increase in use of thrombolysis in acute stroke care, was proved wrong. The previous group had a mean PE max of 62.6 (SD 28.0) cmH₂O whereas this stroke patient group had a median PE max of 50.5cmH₂O (IQ range 39.5 to 69.5). There was no significant difference in stroke severity between both groups, NIHSS 14(8) for this study and 15(6) in the Harraf study. The stroke group described in this new study was weaker than the cutoff for diagnosis for respiratory muscle weakness for males (80cmH₂0) and females (60cmH₂0) (40).

As may be expected our group of acute, moderately severe patients performed worse on the PE max than the chronic stroke patients studied in Brazil (115). Their patients could all walk and had had strokes more than 9 months previous to testing. Their PE max was mean 89.4cmH₂0 (SD 41.3) which although lower than that of the control group would not be considered weak in our laboratory, (40) and Figure 8.

Our patient also performed poorly on spirometry with a mean FEV₁ of 60 % predicted (SD 22) and mean FVC of 73% predicted (SD 22). A normal subject would be expected to achieve greater than 80% predicted. An FEV₁ of 60% predicted could possibly be explained by chronic obstructive pulmonary disease but the FEV₁/FVC ratio of our patient group at 0.71 could not be classed as obstructive. No patients had previously been diagnosed with any respiratory disease although two showed signs of chronic obstructive pulmonary disease (COPD) on chest radiographs (CXR). A previous study of 34 moderately severe Egyptian stroke patients with an average age of 57 also reported reduced FEV₁ (mean 2.66 +/- 0.47 litres) and FVC (mean 3.6 +/- 0.57 litres) (106). It is unclear what the percent predicted values are as there seems to be an error in their paper; they quote the FVC of 3.6 litres is 52% of predicted but this is very unlikely. The oxygen saturations of their patients were similar to ours, mean 95.7% (SD 2.9) compared with our median 96% (IQ range 92-98).
This reduction in FEV\textsubscript{1}, FVC, oxygen saturations and volitional respiratory muscle strength is typical of patients with neuromuscular disease, for example amyotrophic lateral sclerosis (ALS) (186). However most patients with neuromuscular disease are also weak on non-volitional respiratory muscle testing. This was not the case with our patients who had intact peripheral nerves and normal muscles.

### 3.4.1 Critique of the Methods

We did not hold the cheeks of subjects during the PEmax manoeuvre. The ballooning of cheeks during the forced expiration may lead to reduced maximum pressure achieved. Stroke patients could have reduced facial tone due to the stroke and this could lead to more ballooning of cheeks than occurred in controls. It is difficult or perhaps impossible to achieve supramaximal stimulation of the abdominal muscles using the equipment as described above (187). However all participants received the same, maximal magnetic stimulus. We did not perform phrenic nerve stimulation to make a non-volitional assessment of diaphragm strength (39). The diaphragm was not our main focus and phrenic nerve stimulation would have added another twenty minutes to a study that was already very tiring for this group of acute patients.

### 3.4.2 Conclusions

Stroke patients are weak on volitional tests of respiratory muscle function but their results on a non-volitional test (the rise in Pgas after stimulation of the T\textsubscript{10} nerve roots) are no different to those of controls and well above the cutoff for diagnosis of weakness. The weakness on volitional testing is likely to be due to the stroke effect on the cortex. Any attempts to improve patients' respiratory muscle strength should focus on improving this cortical control.
Chapter 4  Acute ischaemic hemispheric stroke is associated with impairment of reflex and voluntary cough flow

4.1 INTRODUCTION

Stroke accounts for more than five million deaths a year worldwide (3). Most stroke deaths are caused by complications, of which chest infections are most important. One large study showed that 30% of acute stroke patients diagnosed with pneumonia had died before hospital discharge. Aspiration is common after stroke and is associated with a eleven-fold increase in risk of chest infections (7). Cough is important for clearing the lungs of aspirated material. This is demonstrated by studies showing a higher incidence of aspiration and chest infections in stroke patients with a weak voluntary cough (160, 164) and a significant association between absent cough reflex in acute stroke patients and subsequent development of pneumonia (188). A strong cough, whether voluntary or reflex, requires powerful, coordinated contraction of expiratory (abdominal) muscles along with adequate inspiration prior to cough, low upper airways resistance, adequate duration of glottis closure, fast and complete glottis opening and the ability to keep small airways patent during sudden rises in intra-thoracic pressure (152). That some of these abilities are impaired after stroke is suggested by studies of stroke patients showing asymmetry of ventilation, reduced movement of the diaphragm and chest on the hemiparetic side, poor performance on volitional respiratory muscle tests and reduced voluntary cough flow rates and sound (108, 110, 115). More recent studies suggest, as expected, cortical involvement in cough production. Cortical activation during voluntary cough has been demonstrated in healthy volunteers in functional MRI studies (153, 189). We have recently shown, using transcranial magnetic stimulation, increased latency and decreased amplitude of the motor evoked potentials from the abdominal (expiratory) muscles and reduction of the evoked rise in Pgas in acute stroke patients compared with controls, suggesting impaired cortical control of the abdominal muscles after stroke (58). However, reflex cough may be more important than voluntary cough in ensuring adequate airway protection and clearance after acute stroke (156, 188). Reflex cough is thought to be primarily brainstem in origin but previous studies have noted cortical stroke patients with absence of reflex cough in response to food swallowing (188) or an inhaled
noxious substance (162). These studies did not describe intensity measures (flow, pressure, sound) for any reflex cough produced. Therefore we considered further evaluation of reflex cough in hemispheric stroke to be worthwhile. The null hypotheses were that patients with first ever cortical hemisphere stroke would have the same results as a group of non-stroke, age-matched controls. The primary outcome measure of cough intensity was cough flow rate for both voluntary and reflex cough.

4.2 MATERIALS AND METHODS

4.2.1 Study Participants
Forty-five consecutive patients admitted to the stroke unit of King's College Hospital within two weeks of first-ever, middle cerebral artery territory ischaemic stroke were screened. Six patients were excluded as they did not wish to take part. Six patients with lacunar infarcts were excluded and fifteen were unsuitable due to diabetes, excess alcohol consumption, respiratory or neurological disease except stroke or inability to follow commands. Eighteen adults (seven women) were studied. Twenty healthy controls (five women) were recruited from a volunteer database and studied. The mean age and the proportion of female participants were not significantly different between groups. Institutional ethical approval was obtained (LREC 02-120) and the participants gave written informed consent.

4.2.2 Baseline Assessments
Smoking history, alcohol and ACE inhibitor use, height and weight were documented for all participants. For patients, stroke diagnosis and location were confirmed by brain CT scan. Stroke severity on admission was assessed using the National Institutes of Health Stroke Scale, shown in Figure 22. Patients had a bedside swallowing assessment within 24 hours of admission, using radio-opaque contrast to detect aspiration (166). Oxygen saturations were measured with participants at rest, breathing room air; see section 2.5. Radiologist's reports of chest X-ray examinations (for patients only) were acquired from the hospital records.

4.3 VOLUNTARY AND REFLEX COUGH TESTS
Full details of cough testing are given in 2.10 (voluntary cough) and 2.11 (reflex cough). Airflow rates before, during and after cough were measured with participants wearing a face-mask (Hans-Rudolf, USA) connected to a pneumotachograph (Fleisch, Switzerland). Cough inspired and expired volumes
were calculated online by integration of the flow signal (Figure 47). Voluntary cough was assessed before reflex cough, to avoid any effect of tartaric acid on voluntary cough. Participants were told to inhale maximally and produce the biggest voluntary cough possible, until five consistent readings of maximum cough Pgas were achieved. Reflex cough was induced by nebulising escalating concentrations of L-tartaric acid solutions.

4.3.1 Cough measures made
These are all illustrated in Figure 48. Peak cough flow (PCF) was the maximum expiratory flow achieved during cough. PCF was recorded, and also expressed as a percentage of peak expiratory flow (PEF), to correct for a difference in height between groups. Cough inspired volume and cough expired volume were expressed as a percentage of predicted FVC (190). Cough Pgas was the maximum rise in Pgas during cough. For each participant, the five coughs (voluntary and reflex) with the biggest peak cough flows were averaged and the following derived measures made:

(a) Compression time: duration of zero airflow from the time cough Pgas started to rise to the onset of expiratory flow; this is likely to represent the glottis closure period; (b) Cough pressure acceleration: the maximum cough Pgas divided by the time taken to reach maximum, starting from the onset of expiratory flow; and (c) Cough volume acceleration: peak cough flow divided by time taken to reach maximum flow.

4.3.2 Number of Reflex Coughs Produced at Suprathreshold Stimulus
To ensure a suprathreshold tartaric-acid stimulus of similar intensity was delivered to all participants, the numbers of reflex coughs (simultaneous spikes on the flow and Pgas traces) produced by each participant during a suprathreshold stimulus were counted.

4.3.3 Sample size and Data Analysis
The primary outcome measure was cough flow rate, for both voluntary and reflex cough. In a previous study with similar methods, healthy participants had a mean voluntary cough peak flow of 351 (SD 112) l/min, which was 200 l/min greater than that of the stroke group (58). Using these data and G*Power software (see 2.13) it was calculated that thirteen participants in each group were required for an 85% chance to detect a 150 l/min difference in peak cough flow between groups, at a significance level of 5%. Statistical analyses were performed using software described in 2.13. P<0.05 was considered significant. Data were tested for
normality and t-tests or Mann-Whitney u-tests for two independent groups were used for comparisons (183).

Univariate and multiple linear regressions were used to investigate possible causes for impaired voluntary and reflex cough flow rates. Patients and controls were analysed together with controls being assigned a stroke severity score (NIHSS score) of zero for the purposes of these analyses. Cough flow rate, for both voluntary and reflex cough was the dependent variable and stroke severity (NIHSS score), height and FEV₁/FVC ratios were entered as independent predictors. All models included a constant.

4.4 RESULTS

4.4.1 Participants

Baseline characteristics of participants are given in Table 6 and Table 7. Acute hemispheric infarction was present in the left hemisphere in nine and the right hemisphere in nine patients. Of the left infarcts, 3 were frontal, 1 frontoparietal, 1 temporofrontoparietal and 4 capsulostriate. Of the right infarcts, 3 were frontal, 1 frontoparietal, 4 temporofrontoparietal and 1 capsulostriate. Six of eighteen patients had been treated with thrombolysis. Five of eighteen were judged to have poor swallow.

4.4.2 Voluntary Cough

Voluntary cough was significantly impaired in patients (Table 12 and Figure 58). Two patients were unable to produce any voluntary coughs and so were excluded from the cough intensity analysis. Patients’ mean cough Pgas of 98.5cmH₂O is well below the cutoff value of 130cmH₂O used to aid diagnosis of expiratory muscle weakness (40).
Figure 58 Voluntary Cough in Normal Participant Compared to Stroke Patient

A five second sweep of flow and gastric pressure traces during maximum voluntary cough. The traces for a moderately impaired stroke patient (in black) are superimposed on those of control participant (in grey). Note the patient’s cough flow does not appear so severely impaired as the gastric pressure.

Key for Figure 58 l/s=litres per second; cmH\textsubscript{2}O=centimetres of water pressure
Results for reflex cough are given in Table 13. The median concentration of tartaric acid solution required to produce 5 coughs was 10% for both patients and controls. One patient and two controls found tartaric acid inhalation intolerable so the reflex cough test was not performed. Three of the remaining 17 patients (17.6%) had no reflex cough response to 20% tartaric acid; all 18 normal participants produced a cough response (0% non-responders). In our early studies we continued to give participants higher concentrations of TA after they had reached 5 coughs to see if cough intensity would increase further. All four images in Figure 61 show tartaric acid stimuli causing five or more cough spikes on the flow and Pgas traces. The cough flow rate does not increase when the concentration of L-tartaric acid increases from 15% to 20% for the control or from 10% to 20% for the patient.

### Table 12 Maximum Voluntary Cough

<table>
<thead>
<tr>
<th></th>
<th>Stroke (n=16)</th>
<th>Controls (n=20)</th>
<th>Mean difference (95% CI)</th>
<th>P-value for diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak cough flow (l/min)</td>
<td>287 (171)</td>
<td>497 (122)</td>
<td>-210 (-314 to -106)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak cough flow as % predicted PEFR</td>
<td>70 (43)</td>
<td>102 (19)</td>
<td>-32 (-55 to -10)</td>
<td>0.005</td>
</tr>
<tr>
<td>Cough Expired Volume(ml)</td>
<td>1170 (755)</td>
<td>2100 (864)</td>
<td>-930 (-1541 to -319)</td>
<td>0.004</td>
</tr>
<tr>
<td>Cough Expired Volume as % predicted FVC</td>
<td>33 (21)</td>
<td>47 (16)</td>
<td>-14 (-28 to -1)</td>
<td>0.041</td>
</tr>
<tr>
<td>Peak Inspiratory Flow Rate (l/min)*</td>
<td>112 (80 to 164)</td>
<td>213 (157 to 256)</td>
<td>-88 (-136 to -42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cough Inspired Volume (ml)</td>
<td>1519 (546)</td>
<td>2710 (818)</td>
<td>-1191 (-1773 to -609)</td>
<td>0.003</td>
</tr>
<tr>
<td>Cough Inspired Volume as % predicted FVC</td>
<td>43 (21)</td>
<td>62 (18)</td>
<td>-19 (-34 to -3)</td>
<td>0.020</td>
</tr>
<tr>
<td>Cough Gastric Pressure (cmH2O)</td>
<td>98.5 (61.6)</td>
<td>208.5 (61.3)</td>
<td>-110.0 (-152.4 to -67.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cough Pressure Acceleration (cmH2O/s)</td>
<td>583 (512)</td>
<td>927 (303)</td>
<td>-344 (-644 to -45)</td>
<td>0.026</td>
</tr>
<tr>
<td>Compression Time (ms)</td>
<td>212 (105)</td>
<td>261 (127)</td>
<td>-49 (-138 to 40)</td>
<td>0.265</td>
</tr>
<tr>
<td>Cough Vol Acceleration (l/s²)</td>
<td>83 (57)</td>
<td>200 (70)</td>
<td>118 (-165 to 70)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Key for Table 12**  
*PEFR*=peak expiratory flow rate; *FVC*=forced vital capacity; *l=litres; %=percentage; *ml=millilitres; *ms=milliseconds; *min=minute; *s=second; *CI=confidence intervals.

### 4.4.3 Reflex cough

Results for reflex cough are given in Table 13. The median concentration of tartaric acid solution required to produce 5 coughs was 10% for both patients and controls. One patient and two controls found tartaric acid inhalation intolerable so the reflex cough test was not performed. Three of the remaining 17 patients (17.6%) had no reflex cough response to 20% tartaric acid; all 18 normal participants produced a cough response (0% non-responders). In our early studies we continued to give participants higher concentrations of TA after they had reached 5 coughs to see if cough intensity would increase further. All four images in Figure 61 show tartaric acid stimuli causing five or more cough spikes on the flow and Pgas traces. The cough flow rate does not increase when the concentration of L-tartaric acid increases from 15% to 20% for the control or from 10% to 20% for the patient.
The participants who did not cough were not included in the cough intensity analysis. Patients’ mean reflex cough P_{gas} of 179.0 cm H_{2}O was well above the normal cutoff value of 130 cm H_{2}O for voluntary cough P_{gas} (40).

**Table 13 Maximum Reflex Cough Response to L-Tartaric Acid**

<table>
<thead>
<tr>
<th>Metric</th>
<th>Stroke (n=14) Mean (SD)</th>
<th>Control (n=18) Mean (SD)</th>
<th>Mean difference (95% CI)</th>
<th>P-value for difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tartaric Acid Concentration Required to Produce Five (Or More) Coughs in One Minute</strong>*</td>
<td>10 (5-10)</td>
<td>10 (10-20)</td>
<td>0 (-5 to 0)</td>
<td>0.610</td>
</tr>
<tr>
<td><strong>Number of Coughs Elicited in One Minute by Suprathreshold Tartaric Acid Stimulus</strong></td>
<td>11.4 (5.0)</td>
<td>12.3 (7.6)</td>
<td>0.9 (-3.9 to 5.7)</td>
<td>0.705</td>
</tr>
<tr>
<td><strong>Peak cough flow (l/min)</strong></td>
<td>204 (111)</td>
<td>379 (110)</td>
<td>-175 (-253 to -96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Peak cough flow as % predicted PEFR</strong></td>
<td>43 (34)</td>
<td>77 (20)</td>
<td>34 (-54 to -15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Cough Expired Volume (ml)</strong></td>
<td>478 (203)</td>
<td>1269 (1119)</td>
<td>791 (-1412 to -169)</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>Cough Expired Volume as %predicted FVC</strong></td>
<td>13 (5)</td>
<td>27 (25)</td>
<td>-14 (-29 to -0.3)</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Cough Inspired Volume (ml)</strong></td>
<td>763 (406)</td>
<td>1172 (699)</td>
<td>-410 (-893 to -74)</td>
<td>0.093</td>
</tr>
<tr>
<td><strong>Cough Inspired Volume as % predicted FVC</strong></td>
<td>22 (13)</td>
<td>26 (15)</td>
<td>-4 (-15 to 7)</td>
<td>0.479</td>
</tr>
<tr>
<td><strong>Cough Gastric Pressure (cmH_{2}O)</strong></td>
<td>179.0 (78.0)</td>
<td>208.2 (77.4)</td>
<td>-29.3 (-81.9 to 23.4)</td>
<td>0.266</td>
</tr>
<tr>
<td><strong>Cough Pressure Acceleration (cmH_{2}O/s)</strong></td>
<td>788 (297)</td>
<td>947 (216)</td>
<td>-159 (-393 to 75)</td>
<td>0.171</td>
</tr>
<tr>
<td><strong>Compression Time (ms)</strong></td>
<td>280 (236 to 383)</td>
<td>270 (197 to 325)</td>
<td>23 (-52 to 99)</td>
<td>0.661</td>
</tr>
<tr>
<td><strong>Cough Volume Acceleration (l/s^2)</strong></td>
<td>99 (58)</td>
<td>179 (64)</td>
<td>-80 (-129 to 31)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Key for Table 13

PEFR=peak expiratory flow rate; FVC=forced vital capacity; l=litres; %=percentage; ml=millilitres; ms=milliseconds; min=minute; s=second; CI=confidence intervals.

*Tartaric acid strength data are ordinal and compression time data are skewed: values given are medians (IQ range) and median difference (95% CI). P-value is calculated using the Mann-Whitney u-test.
Figure 59 Raw Data Traces Showing Reflex Coughs For a Control Participant and a Severely Affected Stroke Patient

Key for Figure 60 l/min=litres per minute; cmH₂0=centimetres of water pressure
4.4.4 Suprathreshold Reflex Coughs

The average number of coughs (simultaneous spikes on the flow and Pgas traces) produced by each participant during a suprathreshold tartaric acid stimulus was similar for both patient and control groups. Results for each group are given in Table 14 and a comparison between groups is shown in Figure 60.

Table 14 Number of Reflex Coughs Produced at Suprathreshold Tartaric Acid Concentration

<table>
<thead>
<tr>
<th></th>
<th>Stroke patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of values</td>
<td>14.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>25% Percentile</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Median</td>
<td>11.5</td>
<td>9.5</td>
</tr>
<tr>
<td>75% Percentile</td>
<td>15.8</td>
<td>18.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>21.0</td>
<td>29.0</td>
</tr>
<tr>
<td>Mean</td>
<td>11.4</td>
<td>12.3</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>5.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Std. Error</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>D'Agostino &amp; Pearson omnibus normality test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K2</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td>P value</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Passed normality test (alpha=0.05)?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>P value summary</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Key for Table 14 Suprathreshold tartaric acid stimulus=concentration of tartaric acid required to produce five or more coughs.

Figure 60 Numbers of Coughs Elicited by Suprathreshold Tartaric Acid

Scatter dot plot to show the number of coughs elicited by the maximum strength of tartaric acid administered to each participant. Horizontal lines show mean and the 95% confidence interval for the mean.

Key for Figure 60
P value calculated using unpaired t-test

4.4.5 Cough Intensity and Increasing Stimulus Intensity

In our early studies we continued to give participants higher concentrations of TA after they had reached 5 coughs. All four images in Figure 61 show tartaric acid
stimuli causing five or more cough spikes on the flow and Pgas traces. The cough flow rate does not increase when the concentration of L-tartaric acid increases from 15% to 20% for the control participant or from 10% to 20% for the stroke patient.

**Figure 61 Suprathreshold Reflex Coughs**

**Healthy control**

![Graph showing flow and Pgas traces for healthy control with 15% and 20% L-tartaric acid]

**Stroke patient**

![Graph showing flow and Pgas traces for stroke patient with 10% and 20% L-tartaric acid]

Key for Figure 61 Pgas=gastric pressure; % stands for % weight in volume, i.e., number of grams of L-tartaric acid per 100ml of water

**4.4.6 Predictors of Voluntary and Reflex Cough Flow Rate**

The results of univariate linear regression with peak cough flow as the dependent variable and stroke severity as the predictor are shown in Figure 62 and Figure 63.
Figure 62 Scatter Diagram showing the Relationship Between Stroke Severity and Voluntary Cough Flow

Controls were assigned an NIHSS score of 0. The regression line and its 95% confidence intervals are shown with the equation for the regression line. 95% confidence interval for the constant 437.1 to 549.8; 95% confidence interval for the slope -20.3 to -9.1. P<0.001.

Figure 63 Scatter Diagram showing the Relationship Between Stroke Severity and Reflex Cough Flow

The regression line and its 95% confidence intervals are shown. Controls have an NIHSS score of 0. Controls were assigned an NIHSS score of 0. The regression line and its 95% confidence intervals are shown with the equation for the regression line. 95% confidence interval for the constant 315.3 to 415.8; 95% confidence interval for the slope -15.8 to -5.7. P<0.001.
4.4.7 Multivariate Linear Regression

Results of multivariate linear regression analysis performed to predict voluntary cough flow rate from NIHSS score (stroke severity score), height and FEV1/FVC ratio.

The only significant predictor of reflex cough flow rate was NIHSS score; details are given in Figure 63. ACE inhibitor use (or not) was tried as a predictor for both voluntary and reflex cough but did not exert a statistically significant effect.

Table 15 Multivariate Linear Regression to Predict Voluntary Cough Flow Rate

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.830*</td>
<td>.689</td>
<td>.653</td>
<td>109.246</td>
</tr>
</tbody>
</table>

* a. Predictors: (Constant), FEV1/FVC ratio, Height(cm), NIHSS score

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>95.0% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>t</td>
<td>Sig.</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>-873.0</td>
<td>444.4</td>
<td>-2.0</td>
<td>.060</td>
</tr>
<tr>
<td></td>
<td>NIHSS score</td>
<td>-12.6</td>
<td>3.2</td>
<td>-4.0</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>5.7</td>
<td>2.3</td>
<td>2.4</td>
<td>.022</td>
</tr>
<tr>
<td></td>
<td>FEV1 /FVC ratio</td>
<td>5.1</td>
<td>2.3</td>
<td>2.2</td>
<td>.037</td>
</tr>
</tbody>
</table>

* a. Dependent Variable: Voluntary cough flow rate (l/min)

Key for Table 15 NIHSS=National Institutes of Health Stroke Scale Score; FEV1=forced expiratory volume in 1 second; FVC=forced vital capacity

4.5 DISCUSSION

This study shows that voluntary and reflex cough are both impaired after acute hemispheric infarction. Reductions in voluntary cough flows and Pgas in stroke patients have been described previously (160, 164, 58, 191) but reflex cough may be considered more important for airway protection and clearance (156). The novel and important finding of this study is that despite patients achieving normal reflex cough Pgas, flow rates and expired volumes for reflex cough are both decreased.

4.5.1 Critique of the Methods

The differences in reflex cough flow rate, reflex cough expired air volume and reflex cough volume acceleration between patients and controls could not be attributed to differences in air volume inspired prior to cough, concentration of tartaric acid solution required to produce five or more coughs, duration of glottis closure, peripheral nerve conduction or expiratory muscle strength. Higher stroke
severity score predicted impairment of both voluntary and reflex cough flow rates, suggesting a physiological basis for impaired cough in acute stroke.

Spirometry, respiratory muscle strength and voluntary cough are measured by volitional tests in the sense that they require a patient to make a maximal effort and their interpretation depends on the vigour of that effort being maximal. Stroke patients may theoretically perform badly because the tests depend upon participant understanding and effort (185), although stroke’s more obvious manifestations concern motor skills. Even so these factors would not affect reflex cough where any impairment observed is likely to be due to non-volitional factors. Reflex cough Pgas and glottis closure times in patients were no different to controls. Reflex cough inspired air volume tended to be smaller for patients but this did not reach statistical significance.

It is possible that reduced functional residual capacity (FRC) in patients may have contributed to reduced cough flows, as a lower starting lung volume will result in higher airways resistance and reduced flow rates. Little is known of FRC in stroke patients at rest (and none at all during cough) but one small study showed normal FRC in moderately severe patients at 2-4 weeks post-onset (110). Patients had more airways obstruction than controls (significantly reduced FEV₁ and FEV₁/FVC ratio). Airways obstruction would be expected to lead to reduced voluntary cough flow rate although from the regression models, the influence of FEV₁/FVC ratio on voluntary cough flow rates was smaller and less significant than the effect of stroke severity. Neither FEV₁ nor FEV₁/FVC ratio were significant predictors of reflex cough flow rate.

Previous studies have separately described intra-abdominal pressure changes during reflex and voluntary cough in normals (158) but this is the first study to describe peak cough flows, volumes and pressures during reflex and voluntary cough in an homogeneous sample of stroke patients and controls. A merit of our study is that participants with diabetes or previous heavy alcohol use were excluded because these may affect cough (192, 193). Similarly application of lidocaine to the pharynx, (to pass pressure catheters) can alter cough (163) so cough tests were performed at least 90 minutes after administration. Lastly, although ACE inhibitor use was recorded because of the previously described effect on cough (194), it was not found to be a significant independent predictor of cough flow rate in our study.
4.5.2 Significance of the findings
The rapid rise of Pgas but not expiratory flow during reflex cough suggests that the sensory pathways are intact since abdominal muscles must be recruited to generate a positive Pgas. However the slower rise of expiratory flow suggests an additional flow limitation as a manifestation of ischaemic cortical injury. We suspect that this injury may affect the coordinated activation of the upper airway muscles with the abdominal and thoracic muscles used for cough production (152). Cortical involvement in reflex and voluntary cough is supported by studies showing voluntary suppression of capsaicin-induced reflex cough in healthy volunteers, (195) absent or delayed conduction in cortico-respiratory tracts on stimulating the affected hemisphere of stroke patients (58) and cortical modulation of pharyngeal coordination in stroke (18).

This study is of modest size but the sample appears representative and shows baseline characteristics similar to those in other studies. The patients’ mean voluntary cough peak cough flow found here is similar to that found 6 days post stroke onset in a recent study of 96 patients (261±188 l/min) (191). As the definition of effective cough remains elusive, (156) it is impossible to know whether the reduction in patients’ cough flows are clinically meaningful. One method would be to correlate cough impairments with incidence of chest infections but the number of events in this study (n=2) precludes such analysis. Reflex cough produced in the laboratory does not accurately replicate the response to aspirated fluid or food, which cannot easily be studied for safety reasons. Although desirable, measurements and imaging of the upper airways during cough could not be performed because of logistic and patient discomfort considerations.

This study shows that acute stroke patients have impaired voluntary and reflex cough; this may result in impaired lung clearance. The data suggests that impairment may be in part due to ineffective coordination of different muscle groups following cerebral injury. Further studies are required of the mechanisms that may underlie reflex and voluntary cough impairment and to test interventions that may improve cough function, in order to try and reduce the incidence and consequences of aspiration after stroke.
Chapter 5 Reduced Peak Cough Flow in Acute Stroke Patients is Associated with Reduced FRC and Low Cough Inspired Volume

5.1 INTRODUCTION

Acute hemiparetic stroke patients have impaired voluntary and reflex cough flows (58, 160, 191, 196) and the more severe the patient’s stroke, the worse the cough flow (196). Reduced FRC could cause impaired cough flow due to impaired flow volume characteristics of the deflated lungs and because reduced lung volume causes shortening of the expiratory muscles, so reducing pressure generating capacity.

In a published study of acute stroke patients we showed that three predictors (stroke severity score, height and FEV$_1$/FVC ratio) accounted for two-thirds of the variability in voluntary cough flow (196) this is detailed in Chapter 4. We concluded that there must be additional factors implicated in variability of cough flow after stroke and one could be reduced functional residual capacity (FRC). It is known that the volume of air in the lungs, immediately prior to an expulsive manoeuvre, has a linear relationship with the flow (197).

Probably due to the difficulty of making the measurements in stroke patients there are few published data on FRC in these patients, and none in those with acute stroke. While FRC *per se* simply represents the equilibrium point between the lung and chest wall, it is usual to inspire before coughing and the volume inspired could be reduced in stroke if there was impaired inspiratory muscle activation.

FRC could become reduced as a consequence of previously described pulmonary abnormalities in stroke patients such as elevation of the diaphragm on the paretic side (107, 147); reduced movement of the diaphragm during inspiration (108); weak respiratory muscles (58, 115, 117); impaired corticofugal pathways to the diaphragm (105, 184) or flattening or reduced movement of the chest wall on the weak side (149, 150, 198).

Stroke patients can spend a lot of time in reclined positions so the effect of posture on lung volumes is also relevant; see Chapter 1. We believed evaluation of FRC in acute hemiparetic stroke was necessary in order to better understand cough impairment in stroke patients. The reclined position was used for measurement as this replicates most stroke patients’ position in a hospital bed and enabled us to
include subjects who were unable to sit. A healthy control group was studied for comparison, as normal values of FRC in the reclined position are not known. The null hypothesis was that FRC, measured by helium dilution in the reclined position, would be the same for patients with hemiparetic stroke as for a group of non-stroke controls. FRC would be expressed as percent predicted (%predicted) to allow for differences in age, height or gender balance between the two groups. As a secondary hypothesis we sought to confirm that stroke patients have impaired voluntary cough flow and reduced air volume inspired prior to cough (cough inspired volume) when compared with controls (196). These secondary measurements would enable exploration of the relationship between FRC, cough flow and cough inspired volume.

5.2 MATERIALS AND METHODS

Patients were recruited from the Acute Stroke Unit of King’s College Hospital. Seventy-seven patients admitted to the stroke unit of King’s College Hospital within two weeks of middle cerebral artery territory infarcts were screened. Patients unable to follow verbal commands were excluded (n=13), as were those unable to use a flanged mouthpiece correctly whilst wearing a nose-clip (n=12). Others were excluded because of previous stroke (n=8) or respiratory disease (n=15). A total of twenty-seven participants were suitable and consented. A group of thirty healthy controls were recruited from amongst hospital volunteers and laboratory staff. Institutional ethics approval was obtained (LREC 02-120) and participants gave written informed consent.

5.2.1 Baseline Assessments

Smoking history, self-reported ethnicity, height and weight were recorded for all participants. For patients, stroke diagnosis was made by a consultant physician and confirmed radiologically. Stroke location was obtained from CT or MRI scan reports made by a consultant neuroradiologist. Stroke severity on admission and on the day of testing was assessed using the National Institutes of Health Stroke Scale (NIHSS) see Figure 22. Stroke patients who met the thrombolysis eligibility criteria had received alteplase treatment at the time of admission (127). Pulse oximetry was performed at rest in the reclined position, breathing room air; see section 2.5. Radiologists’ reports of patients’ chest radiographs (taken at the time of admission) were acquired from hospital records; we were interested in the presence of pleural effusions or atelectasis, which could cause reduced FRC.
Lengths of stay data were acquired from hospital records and were not determined by the investigators.

5.2.2 FRC and Cough Measurements

FRC was measured using the closed circuit helium dilution technique on a Medisoft SpiroAir system controlled using a computer running Exp’Air software (Medisoft SA, Sorinnes, Belgium). The methods are fully described in 2.7. Participants sat in a supportive chair (with a headrest and armrests) with chair back reclined to 45 degrees from upright and the legs raised to horizontal. FRC testing was performed at least twice and the mean of two FRC values within 10% of each other was recorded. Total lung capacity (TLC), inspiratory capacity (IC) and slow vital capacity (VC) values were acquired by the participant making inspiratory and expiratory vital capacity manoeuvres at the end of each FRC test cycle. FRC, VC, TLC and IC measurements were recorded and also expressed as % predicted using the European Community for Steel and Coal prediction equations (145).

For measurement of voluntary cough flow on the SpiroAir see 2.7.4. At least three separate cough attempts were made and the maximum cough flow, out of three within 10% of each other, was recorded. The volume of air inspired prior to cough (equivalent to the inspiratory capacity on the flow-volume loop) was taken from the cough with the maximum flow. Cough flows were expressed as % predicted peak expiratory flow (PEF) using equations from Cotes (179). Cough inspired volumes were expressed as % predicted vital capacity (VC) (190). All equations used for % predicted are for seated subjects (145, 179, 190).

FRC testing was repeated in the seated upright position for participants able to do so, after the reclined testing had been completed. This was to enable comparison of our data with those of previous authors (110) and also to look what effect sitting the patients upright would have on lung volume. Patients rested in the upright sitting position for twenty minutes before upright testing commenced.

5.2.3 Statistical Analyses including Sample Size Calculation

The primary outcome measure was % predicted FRC, with FRC measured in the reclined position (145). An analysis of achieved power was performed after reclined FRC data had been obtained on 30 controls and 21 patients. It was calculated that the study had achieved 82% power at the 5% significance level so recruitment to the study was halted.
Datasets were tested for normality using the D'Agostino and Pearson omnibus method. Comparisons between patients and control groups were made using unpaired t-tests or Mann-Whitney u-tests. Difference in proportions between groups was calculated using Fisher’s exact test. Where participants underwent testing in both the reclined and seated positions the results (absolute values) from the two positions were compared using paired t-tests or Wilcoxon tests.

5.2.3.1 Correlation and Regression

Correlations were described using Pearson coefficients or Spearman’s rho (for non-parametric data) to 2 decimal places with 2-tailed P-values. As we are ultimately interested in cough flow, univariate linear regression analyses were performed to investigate prediction of cough flow by cough inspired volume and the prediction of cough inspired volume by FRC (all in the reclined position); analyses used absolute values of volume and flow and included a constant. Patients and controls were analysed together. Univariate and multivariate linear regression was also used on patient data to investigate the effect of FRC, cough flow and NIHSS score on patient outcome (length of stay in hospital, dependent variable). All regression models included a constant. GraphPad Prism version 5.00, (GraphPad Software, San Diego California USA, www.graphpad.com) and SPSS Statistics 17.0 (SPSS Inc, Chicago, USA) computer software were used for statistical analysis and construction of figures. CIA software (University of Southampton, UK) was used to calculate confidence intervals for non-parametric data.

5.3 RESULTS

The baseline characteristics of participants are given in Table 16. The patients’ mean age of 68 years (SD 11) was 10 years older than that of controls. Further details of the stroke patients are given in Table 17. FRC and other static lung volumes measured in the reclined position are given in Table 18. 27 patients had baseline assessments (Table 16) but for six of these it was not possible to obtain two reclined FRC readings due to intolerance of the position for the required length of time. One of these six was also unable to perform any coughs. The twenty-one patients with FRC measurements in the upright position (Table 21) include 15 who had both reclined and upright readings and the six who did not tolerate reclined readings.
Six patients were unable to complete the minimum of two satisfactory FRC tests. The results of cough flow tests and spirometry are given in Table 19 and Figure 65. One patient was unable to complete any cough tests.

**Table 16 Baseline Characteristics of Participants**

<table>
<thead>
<tr>
<th></th>
<th>Stroke</th>
<th>Control</th>
<th>Difference (95% CI)</th>
<th>P value for diff</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number assessed</strong></td>
<td>27</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>58</td>
<td>10</td>
<td>4 to 16</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>White Europ / Other</td>
<td>21 / 6</td>
<td>28 / 2</td>
<td>-0.16</td>
</tr>
<tr>
<td></td>
<td>Proportion White Europ</td>
<td>0.78</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males / Females</td>
<td>17 / 10</td>
<td>15 / 15</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Proportion male</td>
<td>0.63</td>
<td>0.50</td>
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</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>169.6</td>
<td>169.7</td>
<td>-0.1</td>
<td>-5.6 to 5.6</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>7.9</td>
<td>0.997A</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>78.0</td>
<td>72.1</td>
<td>4.2</td>
<td>3.0 to 11.0</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>7.9</td>
<td>0.182B</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.5</td>
<td>25.5</td>
<td>1.0</td>
<td>4.0 to -1.0</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>7.9</td>
<td>0.193B</td>
</tr>
<tr>
<td><strong>O₂ sats breathing air (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>97</td>
<td>0</td>
<td>-1 to 1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>7.9</td>
<td>0.660B</td>
</tr>
<tr>
<td><strong>Ever smoked</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ever / Never smoked</td>
<td>13 / 14</td>
<td>12 / 18</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Proportion ever smoked</td>
<td>0.48</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking (pack years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 to 5</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td>7.9</td>
<td>0.485B</td>
</tr>
</tbody>
</table>

Key for Table 16
SD=standard deviation; IQ range=inter-quartile range; CI=confidence interval; %=percentage; Europ=European; O₂=oxygen; sats=saturations; BMI=body mass index. kg=kilograms; m=metres; ^P-value calculated using unpaired t-test; ^P-value calculated using Mann-Whitney u-test; ^P-value calculated using Wilcoxon test
## Table 17 Stroke patient group details

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Inter-Quartile Range</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stroke severity on admission</strong> (NIHSS score)</td>
<td><strong>9</strong></td>
<td></td>
<td><strong>4 to 24</strong></td>
</tr>
<tr>
<td><strong>Stroke severity on day of testing</strong> (NIHSS score)</td>
<td><strong>4</strong></td>
<td></td>
<td><strong>2 to 6</strong></td>
</tr>
<tr>
<td><strong>Stroke affecting left or right brain?</strong></td>
<td><strong>18 / 9</strong></td>
<td></td>
<td><strong>0.67</strong></td>
</tr>
<tr>
<td><strong>Thrombolysed with alteplase</strong></td>
<td><strong>17 / 10</strong></td>
<td></td>
<td><strong>0.63</strong></td>
</tr>
<tr>
<td><strong>Days post stroke when tested</strong></td>
<td><strong>3</strong></td>
<td></td>
<td><strong>1 to 14</strong></td>
</tr>
<tr>
<td><strong>Total length of stay in hospital (days)</strong></td>
<td><strong>9</strong></td>
<td></td>
<td><strong>2 to 78</strong></td>
</tr>
</tbody>
</table>

Key for Table 17
NIHSS=National Institutes of Health Stroke Scale. Worst impairment=NIHSS 31

### Figure 64 Comparison of Reclined FRC in Stroke Patients and Controls

The figure shows FRC %predicted for 21 patients and 30 controls. Medians and inter-quartile ranges are marked with horizontal lines. See Table 18 for further information.

![Figure 64](image)

Key for Figure 64
FRC=functional residual capacity; %=percentage; P-value calculated using a Mann-Whitney u-test.
Table 18 Functional Residual Capacity and Total Lung Capacity in the Reclined Position

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>Difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>21</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>FRC (%predicted)</td>
<td>Median</td>
<td>76.0</td>
<td>90.0</td>
<td>-14.0</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>66.5 to 89.5</td>
<td>79.8 to 105.0</td>
<td>-22.0 to -5.0</td>
</tr>
<tr>
<td>FRC (litres)</td>
<td>Median</td>
<td>2.50</td>
<td>2.78</td>
<td>-0.27</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>2.32 to 3.60</td>
<td>2.26 to 2.90</td>
<td>-0.71 to 0.12</td>
</tr>
<tr>
<td>TLC (%predicted)</td>
<td>Mean</td>
<td>75.3</td>
<td>103.7</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>16.0</td>
<td>12.4</td>
<td>-37.3 to -19.6</td>
</tr>
<tr>
<td>TLC (litres)</td>
<td>Mean</td>
<td>4.40</td>
<td>6.22</td>
<td>-1.83</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.37</td>
<td>0.28</td>
<td>-2.75 to -0.92</td>
</tr>
</tbody>
</table>

Key to Table 18
FRC=functional residual capacity; TLC=total lung capacity; %=percentage; SD=standard deviation; CI=confidence interval; IQR=interquartile range; <sup>a</sup>P-value calculated using Student’s t-test

Table 19 Peak Cough Flow and Spirometry in the Reclined Position

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>Difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>26</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Peak Cough Flow (litres.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Mean</td>
<td>297</td>
<td>380</td>
<td>-83</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>133</td>
<td>121</td>
<td>-153 to -14</td>
</tr>
<tr>
<td>Peak Cough Flow (%predicted PEF)</td>
<td>Mean</td>
<td>61.2</td>
<td>86.3</td>
<td>-25.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>32.6</td>
<td>17.3</td>
<td>-38.8 to -11.4</td>
</tr>
<tr>
<td>Volume Inspired Before Cough (litres)</td>
<td>Mean</td>
<td>2.22</td>
<td>3.41</td>
<td>-1.19</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.83</td>
<td>0.72</td>
<td>-1.72 to 0.67</td>
</tr>
<tr>
<td>Volume Inspired Before Cough (%predicted VC)</td>
<td>Mean</td>
<td>64.3</td>
<td>94.6</td>
<td>-30.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>19.5</td>
<td>15.6</td>
<td>-42.2 to -18.5</td>
</tr>
<tr>
<td>Vital Capacity (litres)</td>
<td>Mean</td>
<td>2.75</td>
<td>4.058</td>
<td>-1.31</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.89</td>
<td>1.24</td>
<td>-1.92 to -0.71</td>
</tr>
<tr>
<td>Vital Capacity (%predicted)</td>
<td>Mean</td>
<td>79.1</td>
<td>111.9</td>
<td>-32.7</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>24.3</td>
<td>17.3</td>
<td>-44.1 to -21.4</td>
</tr>
<tr>
<td>Inspiratory Capacity (litres)</td>
<td>Mean</td>
<td>2.47</td>
<td>3.56</td>
<td>-1.09</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.79</td>
<td>1.01</td>
<td>-1.76 to -0.43</td>
</tr>
<tr>
<td>Inspiratory Capacity (%predicted)</td>
<td>Mean</td>
<td>80.1</td>
<td>101.0</td>
<td>-20.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>19.5</td>
<td>13.7</td>
<td>-33.3 to -8.4</td>
</tr>
</tbody>
</table>

Key to Table 19
min=minute; %=percentage; PEF=peak expiratory flow; SD=standard deviation; CI=confidence interval; IQR=interquartile range; VC=slow vital capacity; all P-values calculated using Student’s t-test
5.3.1 Chest Radiology

The reports of chest radiographs were obtained for fourteen patients, eleven of whom had an FRC measurement. For eight of the eleven the lung fields were reported to be clear. One radiograph showed a small pleural effusion (patient’s FRC was 80% predicted); another showed linear atelectasis in the lower zones (patient FRC was 87% predicted). One further radiograph had patchy consolidation bilaterally (patient FRC 95% predicted). Complete details of the chest radiographs and the corresponding FRC results are shown in Table 20.

### Table 20 Reclined FRC values and Chest Radiograph Reports for Stroke Patients

<table>
<thead>
<tr>
<th>FRC %predicted reclined</th>
<th>Chest radiograph report</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>Lungs clear</td>
</tr>
<tr>
<td>68</td>
<td>Normal</td>
</tr>
<tr>
<td>71</td>
<td>Clear</td>
</tr>
<tr>
<td>73</td>
<td>Clear</td>
</tr>
<tr>
<td>76</td>
<td>Lungs clear</td>
</tr>
<tr>
<td>80</td>
<td>Small R pleural effusion</td>
</tr>
<tr>
<td>86</td>
<td>Clear lung fields</td>
</tr>
<tr>
<td>87</td>
<td>Linear atelectasis left lower zone</td>
</tr>
<tr>
<td>92</td>
<td>Normal</td>
</tr>
<tr>
<td>95</td>
<td>Patchy consol L and R</td>
</tr>
<tr>
<td>118</td>
<td>Hyperinflated</td>
</tr>
</tbody>
</table>

Key to Table 20

FRC=functional residual capacity; %=percentage
5.3.2 Upright FRC values and Effect of Moving to Seated Position

Table 21: Sitting FRC Values and FRC Change from Reclined to Upright

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>P value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>21</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>FRC sitting (litres)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.84</td>
<td>2.92</td>
<td>0.31</td>
</tr>
<tr>
<td>IQ range</td>
<td>2.30 to 3.15</td>
<td>2.54 to 3.84</td>
<td></td>
</tr>
<tr>
<td><strong>FRC sitting % predicted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>89.6</td>
<td>99.9</td>
<td>0.04</td>
</tr>
<tr>
<td>SD</td>
<td>19.7</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td><strong>FRC increase from lying to sitting (litres)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.49</td>
<td>0.20</td>
<td>0.06</td>
</tr>
<tr>
<td>SD</td>
<td>0.75</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

Key for Table 21
P value calculated using A Mann-Whitney u-test or B unpaired t-test
6 of the 21 who did lying measures did not do sitting measures
6 stroke participants only had sitting measurements as they did not tolerate lying down with the mask on;
15 had lying and sitting measurements

5.3.3 Correlations

There were significant relationships between FRC and height, volume of air inspired before cough and cough flow (P<0.001 for all). There was no significant relationship between stroke severity (NIHSS score) and FRC in the reclined position. Correlation coefficients and individual P-values are given in Table 22. There was also a significant relationship between cough flow and length of hospital stay (for patients) but not between FRC and length of hospital stay (Table 23).

Table 22 Significant Correlations for FRC in the Reclined Position

<table>
<thead>
<tr>
<th></th>
<th>Height</th>
<th>Peak cough flow (L.min⁻¹)</th>
<th>cough inspired volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC</td>
<td>Spearman's rho</td>
<td>0.68</td>
<td>0.42</td>
</tr>
<tr>
<td>Significance (2 tailed)</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Key for Table 22 FRC=functional residual capacity

Table 23 Correlations for Length of Hospital Stay (Stroke Patients)

<table>
<thead>
<tr>
<th></th>
<th>Length of hospital stay Spearman's rho</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC %predicted (reclined)</td>
<td>Correlation Coefficient</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Peak cough flow %predicted (reclined)</td>
<td>Correlation Coefficient</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>
5.3.4 Regression

5.3.4.1 Prediction of Cough Flow

Univariate regression analyses were performed to investigate predictors of the dependent variable, cough flow in the reclined position; all analyses used absolute values of volume and flow and included a constant. Patients and controls able to complete FRC and cough flow tests in the reclined position were analysed together. Volume inspired before cough (reclined) was the strongest predictor of reclined cough flow rate (adjusted $R^2 = 0.56, P<0.001$). FRC (reclined) was a strong and significant predictor of volume inspired before cough (adjusted $R^2 = 0.45, P<0.01$). See Figure 66 for illustration.

The resultant prediction equations were:

- Cough inspired volume (L) = $-1.21 + 0.70 \times \text{FRC (L)}$; adjusted $R^2 = 0.45$ and $P<0.01$. 95% CI for the constant = -0.01 to 1.71 and 95% CI for the slope = 0.42 to 0.98
- Peak cough flow (L.min$^{-1}$) = $73 + 100 \times \text{cough inspired volume (L)}$; adjusted $R^2 = 0.56$ and $P<0.01$. 95% CI for the constant = -19 to 165 and 95% CI for the slope = 70 to 131
Figure 66 Reclined FRC Predicts Cough inspired Volume, which in turn predicts Cough Flow

All measures shown on the scatter plots were taken in the reclined position. Panel A shows a scatter graph of FRC in 34 patients and controls plotted against cough inspired volume. Panel B shows a scatter graph of cough inspired volume plotted against cough flow in 36 patients and controls. The regression line (solid) and its 95% CI (dotted lines) are marked on both panels.

Key for Figure 66
vol=volume; l.min⁻¹=litres per minute
5.3.4.2 Prediction of Length of Hospital Stay

A univariate linear regression analysis showed reclined FRC (%predicted) was not a significant predictor of length of hospital stay (P=0.057). However peak cough flow (%predicted PEF) was a significant predictor of length of hospital stay ($R^2=0.70$, $P<0.001$). See Figure 67.

**Figure 67 Scatter Diagram to Show the Relationship Between Peak Cough Flow and Length of Hospital Stay**

The data for twenty-six stroke patients is illustrated below. The prediction equation was: \( \text{Length of stay (days)} = 49 + 0.55 \times \text{(peak cough flow \%predicted)} \) adjusted \( R^2 = 0.70 \) and $P<0.001$. 95% confidence intervals for the constant = 40 to 59 days and 95% confidence intervals for the slope = -0.70 to -0.40.

5.3.4.3 Multiple Linear Regression

For the stroke patients a multiple regression model using both peak cough flow and NIHSS score on the day of respiratory testing as predictors and length of hospital stay as the dependent variable better predicted length of stay than either variable alone. See Table 24.
5.4 DISCUSSION

This study is the first to measure FRC in acute stroke patients within two weeks of stroke. Patients’ mean FRC (%predicted), measured in the reclined position was significantly reduced compared with that of similar controls despite most patients having only mild overall stroke-related impairment. While FRC was significantly related to peak cough flow, the most significant predictor of peak cough flow was the volume of air inspired prior to cough. The results show that despite good performance on a standard stroke scale patients can have significant respiratory impairment. Strategies that enhance cough inspired volume in stroke patients would likely improve cough efficacy in acute cough.

5.4.1 Critique of the Study

The study includes a moderate number of patients and their stroke-related impairments were mostly mild. The median NIHSS score of 4 on the day of respiratory testing means an excellent outcome is likely (14) although this also makes the finding of reduced peak cough flow more striking. The high usage of thrombolysis in our cohort likely resulted in patients with less severe disability than was considered probable when the study was conceived. There was a narrow range of stroke severity scores and this may be the reason that stroke severity did not emerge as a significant predictor of FRC or of peak cough flow, as it did in our previous study (196). Deep inspiration and coughing could lead to expansion of areas of pulmonary atelectasis and an increase in FRC. However, this was the case for both patients and controls. Sitting FRC was measured for 16 patients and 30 controls. This was a secondary measure performed after the end of reclined

Table 24 Multiple Linear Regression to Predict Length of Hospital Stay

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Interval for B</th>
<th>Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>t</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>32.90</td>
<td>7.87</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>Peak cough flow reclined (%predicted)</td>
<td>-0.41</td>
<td>0.09</td>
<td>-0.62</td>
</tr>
<tr>
<td></td>
<td>NIHSS on respiratory test day</td>
<td>1.60</td>
<td>0.64</td>
<td>0.33</td>
</tr>
</tbody>
</table>

a. Dependent Variable: length of hospital stay (stroke patients)

Key for Table 24: NIHSS=National Institutes of Health Stroke Scale Score; %=percentage
testing. The increases shown in patients’ FRC on sitting only reflects what happens for the least affected patients, as the 5 worst affected were unable to complete sitting tests.

Chapter 1 describes the three previous studies in chronic stroke only; they include small numbers of participants and have conflicting findings (108, 110, 117). The FRC we found for 21 acute stroke patients (median reclined FRC 76% predicted, IQ range 67 to 90%) was very similar to that found by Fugl-Meyer for a smaller group of chronic stroke patients with mild to moderate impairments (n=9, mean supine FRC 74% predicted, standard deviation 15%)(117). Peak cough flow for the patients [mean 297 litres per minute, (l.min\(^{-1}\)) SD 133] was in line with that found in recently work published by our group (mean 287 l.min\(^{-1}\), SD 171) (196) and others (mean 262, SD 188) (191).

It is proposed that the reduced FRC as well as TLC, VC and IC after cortical stroke are due to several factors. These include weak respiratory muscles, at least when acting under voluntary control, and consequent displacement of the diaphragm cranially in addition to decreased expansion and convexity of the chest wall on the hemiparetic side. Reduced FRC in the patients compared with controls is unlikely to be due to patients’ slightly higher body mass index (BMI); since the median difference between the two groups was only 1kg/m\(^2\) (95% CI 4 to -1 kg/m\(^2\)) and the difference was not statistically significant. The patients’ low FRC is unlikely to be due to atelectasis. Of the chest radiographs retrieved for eleven stroke patients, seven were reported as normal and the median reclined FRC of this subgroup was similar to that of the whole group. Increased elastic recoil of the lungs is very unlikely to be the reason for decreased FRC as all patients were free of lung disease and stroke is not known to affect lung parenchyma directly.

### 5.4.1.1 Significance of the Findings

Low peak cough flow rate is associated with an increased risk of chest infections and possibly death in stroke patients (160, 164, 188). FRC was reduced in patients at an early stage despite only mild stroke related impairments. The volume of air inspired prior to cough was the modifiable predictor accounting for more than half (56%) of the variability in peak cough flow; FRC accounted for almost half (45%) of the variability in cough inspired volume. FRC was not a significant predictor of length of hospital stay but stroke severity (NIHSS score) on
test day and peak cough flow used together better-predicted length of stay than either predictor used alone.

Moving stroke patients from supine to upright posture is likely to increase their FRC; the mean increase in FRC for our patients was 255ml (95% CI -0.387 to -0.123ml) and this repositioning was only from 45 degrees reclined to upright. Intermittent application of continuous positive airway pressure (CPAP) may also have a positive effect on end expiratory lung volume. Both strategies are suitable for patients unable to cooperate due to lack of understanding and motor control secondary to stroke.

Increasing inspired volume prior to cough would seem a logical strategy to try and increase peak cough flow in stroke patients. Asking patients to try and increase their own inspired volume by verbal instruction and demonstration is simple and more likely to promote recovery of the cough motor control in the damaged or contralateral cerebral hemisphere than use of passive therapies such as cough assist devices.

5.4.2 Conclusion

Functional residual capacity (%predicted) measured in the reclined position is significantly reduced in acute hemiparetic stroke patients compared with controls; this is associated with poor cough flow. Strategies to try to increase functional residual capacity include sitting the patients upright and application of continuous positive airways pressure. Early intervention may prevent chronically reduced FRC, low cough volumes and flow and chest infections.
Chapter 6  Motor Control of the Abdominal Muscles: Studies in Healthy Participants and Stroke Patients using Transcranial Magnetic Stimulation

6.1 INTRODUCTION

Stroke patients have impaired cough and it is thought this may be due to impaired motor cortical control of the abdominal muscles. Motor cortical inputs to the abdominal muscles can be studied using transcranial magnetic stimulation (TMS). TMS studies of the respiratory muscles are introduced in section 1.5. Previously published studies in hemiplegic stroke patients have often failed to produce any motor evoked potentials (MEPs) from the diaphragm and abdominal muscles on the hemiparetic side of the body after TMS at the vertex or over the damaged cerebral hemisphere (57, 58). We planned to use TMS to study cortical plasticity for a future cough training study. The aim of these studies of healthy controls and stroke patients was to develop a stimulation protocol suitable for use in patients and likely to lead to large, reproducible MEPS from abdominal muscles bilaterally. The TMS protocol could then be used to study motor evoked potentials in the abdominal muscles before and after cough training.

6.1.1 Methods of enhancing TMS evoked MEPS

The failure of previous studies to produce TMS evoked MEPS in some patients could be due to patient factors such as the stroke type or severity and it is possible that no method of stimulation would produce a signal in those patients. However, if the patient’s corticomotor threshold (CMT) to TMS was raised due to stroke, failure to produce a MEP could have been due to the patient’s CMT being higher than the maximum output of the stimulator-coil combination. Unfortunately investigators are always limited by the maximum output of the stimulator and coil combination. With this in mind, optimisation of coil position, use of paired stimuli and having the participant contract the muscle of interest voluntarily may reduce the CMT to TMS and increase the size of the MEP for any given stimulus. See section 1.5.5 for further details.

6.1.1.1 Magnetic stimulus intensity and corticomotor threshold

The magnetic stimulus intensity produced by any combination of magnetic stimulator and coil is determined by the power output of the coil and the maximum stimulator output. The maximum output of the MagStim 200 stimulator used could
not in itself be modified but choice of coil influences the intensity of the applied stimulus. Early TMS studies of respiratory muscles used small flat figure-of-eight coils. These produce focused stimulation (useful in mapping studies) but they have less depth of penetration than larger coils (57, 199). The coil geometry of double cone coils means that when they are applied in the midline they fit closely to the shape of the scalp and this leads to better magnetic coupling (see 2.12.3 and Figure 50). The MagStim double cone coil predominantly stimulates over the central fissure, which is especially good for studies of the trunk and lower limbs (199). Tsao and colleagues (101) showed that the improved coupling of the double cone coil led to a decreased CMT for activated transversus abdominis muscle compared with a 7cm figure of eight coil [mean 53% of maximum stimulator output (SD 6) compared with 73% (SD 10) for the figure of 8 coil], see Table 1. For this reason we decided to use the double cone coil for the studies described here.

6.1.1.2 Coil position on the head
The abdomen is represented medially on the motor homunculus as shown in Figure 13. Some TMS studies of abdominal muscles have placed the stimulator coil in a lateral position on the skull to try and achieve specific stimulation of only one hemisphere, although it is arguable whether this is possible due to the size of the magnetic field and the fast spread of induced current through nervous tissue (51, 103). Harraf delivered TMS at the vertex and over each hemisphere to produce MEPs in the recti and oblique muscles on both sides (58). For our planned stroke patient studies, we wanted to deliver TMS in the skull midline for two reasons. Firstly, the double cone coil does not closely fit the skull when used in lateral positions (so coupling is poor and CMT increased) and secondly we wanted to study signals from both affected and unaffected sides of the body in stroke patients, with the smallest possible number of stimulations. Although it may be unclear whether the MEPs recorded on each side of the body are produced from the contralateral or ipsilateral hemisphere, or more likely a combination of both, this would be the case both before and after the training intervention. If the relative contribution of either side to the MEP changed after the training, the form of the MEP and the latency would change and this would be evident on the recording. Ideally we wanted to use the same coil position for all participants as moving the coil around to find the point of optimum excitation can be very time consuming and involve lots of stimulations. We decided to compare the signals
produced by stimulation at the vertex with stimulations in more anterior and posterior positions, to find the optimum point of stimulation to produce MEPs from the lateral abdominal muscles bilaterally. We do not refer to individual muscles by name, as it is difficult to know that the electrical signal is coming from one muscle when there are surface electrodes over several layers of muscles.

6.1.1.3 Paired Pulse TMS: Intensity of Stimuli and Interstimulus interval
See section 1.5.6 for an introduction to paired-pulse TMS. An initial, “conditioning” magnetic stimulus is closely followed by a second stimulus known as the test stimulus. Paired-pulse stimulation, with interstimulus intervals of 10-20ms, usually facilitates TMS, leading to bigger MEPs in healthy people. However at short interstimulus intervals (1-5ms) it can be inhibitory. In stroke patients, disinhibition has been demonstrated by paired-pulse stimulation, see section 1.6.2. An online search found no previously published paired-pulse TMS studies of abdominal muscles; however Demoule and colleagues published paired pulse TMS studies of the diaphragm describing maximal facilitation with a conditioning stimulus at 80% of CMT, a test stimulus of 125% of CMT (or 100% of maximum stimulator output) and an interstimulus interval of 15ms It was decided to use a conditioning stimulus of 80% of CMT and a test stimulus of 120% of CMT in our studies. We chose 120% of CMT as the test stimulus is required to be suprathreshold; this meant that participants with CMTs up to 80% were able to receive the full 120% of CMT, ie 96% of maximum stimulator output. Multiples of CMT greater than 120% are impossible to achieve in participants with thresholds of more than 80%, as the maximum stimulator output only reaches 100%.

6.1.2 Voluntary Muscle Activation to Facilitate TMS
Maintenance of a voluntary contraction in the muscle being studied with TMS leads to more reproducible MEPs, larger amplitudes and shorter latencies (See Introduction, 1.5.5.1). Muscle activation at just 1.5% of maximum voluntary contraction (MVC) caused a three fold increase on MEP amplitude from small hand muscles when a suprathreshold magnetic stimulus is applied; MEP amplitude reaches a plateau at about 10% of MVC (200). For the diaphragm, the plateau is reached somewhere between 40 and 60% of MVC (51, 59). Previously published TMS studies of abdominal muscles in stroke patients have been of resting muscles, perhaps due to the difficulty of activating the abdominals to a set level (58).
6.1.2.1 Activation Methods
We had the participants sit unsupported on a stool to see what level of background abdominal muscle activation was achieved by this alone. Sitting unsupported is a simple method of activating the trunk (including abdominal) muscles at a low level for patients who can sit safely. We considered abdominal muscle activation methods previously described for healthy people, such as bilateral trunk flexion and expiration against resistance (see section 1.7.2.1) but rejected these as too challenging for many stroke patients. Tunstill used a forced breath hold at end-expiration to facilitate TMS to rectus abdominis in healthy people. We decided to use a modified Valsalva manoeuvre, similar to the forced expiration during breath holding described by Tunstill, to try and voluntarily facilitate TMS (103). The maximum intra-abdominal pressure rise recorded during a maximum effort modified Valsalva manoeuvre would be described as the MVC.

6.1.2.2 Quantifying Abdominal Muscle Activation
We wanted to quantify the amount of muscle activation achieved, as it was likely that with a suprathreshold magnetic stimulus, the facilitation effect would plateau before the muscle activation level reached maximum. Muscle activation level is usually expressed as a proportion of MVC in similar studies and activating to a preset level is technically simple to achieve for limb muscles. Measuring the muscle activation level for abdominal muscles is more difficult as assessment of the force of contraction involves invasive measurement of intra-abdominal pressure and a number of muscle groups are involved in raising the Pgas. Although some authors describe muscle activation level for these kinds of studies in terms of the rectified surface EMG trace (101), in our pilot studies we found that the baseline EMG trace was very variable and the signal noisy when participants were sitting up. Also the relationship between the size of the MEP recorded from the skin surface over the abdominal muscles and the intra-abdominal pressure rise has not been described.

Preliminary studies showed that for healthy people the background level of activation was about 10% of MVC even when sitting on a stool so we decided to set an intra-abdominal pressure level target at 20% and 40% of their own MVC for participants performing the modified Valsalva.

If we were to be able to demonstrate that the facilitation effect levelled off at a low proportion of MVC we could be reassured that if we went on to ask participants in
future studies to make a maximum manoeuvre, even if these were not very reproducible and the intra-abdominal pressure not measured, the activation was very likely to be above the plateau activation level and thus maximum voluntary facilitation would be achieved.

6.1.3 Hypotheses
The null hypotheses were that for TMS using the double cone coil and MEPs measured from lateral abdominal muscles:

1) MEPs from single pulse stimulations at the cranial vertex would be the same size as for stimulation anterior or posterior to the vertex.

2) MEPs from paired pulse stimulation at the cranial vertex would be the same size as MEPs produced by single stimuli.

3) MEPs from activated muscles would be the same size as those from resting muscles, for single pulse stimulations at the vertex where size is taken to mean peak-to-peak amplitude

A series of studies was designed to test these hypotheses.

6.2 METHODS
6.2.1 Participants
Healthy participants aged over 18 years were recruited from laboratory staff and a hospital volunteer database. Patients were recruited from the Stroke Unit of King’s College Hospital and were within three weeks of first-ever ischaemic stroke in the territory of the middle cerebral artery. Patients only participated in the coil position and paired-pulse stimulation studies. The research protocol was approved by King’s College Hospital Local Research Ethics Committee, reference LREC 02-120. All participants gave written informed consent. All participants were screened for suitability using a TMS safety questionnaire and those unsuitable for TMS (e.g. pregnancy, metallic implants, pacemakers) were excluded (165).

6.2.2 Baseline Characteristics
Demographic data (age, height, weight, self-reported handedness) for patients and controls were collected using the methods described in Chapter 2. Patient data (e.g. about the type of stroke) was collected from hospital records. A trained investigator performed NIHSS scoring on patients, on the same day that patient testing occurred (see 2.4.2 and Figure 22 for details).
6.2.3 Transcranial Magnetic Stimulation
Stimulation was using a MagStim 200 stimulator and a 110mm double-cone coil, with participants sitting upright in a supportive chair for all studies except the voluntary facilitation study where they sat on a stool. See the Methods section 2.12 for further details. The coil was oriented as described in 2.12.3.

6.2.4 EMG recording
55mm diameter surface electrodes were used to record motor evoked potentials from the lateral abdominal muscles on the right side for controls and both sides for patients. The positive electrode was positioned 2cm medial to the anterior superior iliac spine (ASIS), on a horizontal line drawn between the right and left ASIS. The negative electrode was positioned 5cm medial to this with the reference electrode on the ASIS. Full details of electrodes, skin preparation, EMG acquisition, amplification and processing are given in the Methods section 2.12.

6.2.5 Gastric pressure measurements
Gastric pressures (Pgas) were measured for the facilitation by voluntary muscle activation study only. Pgas was measured using an 86cm closed-end latex free plastic catheter passed into the stomach through the nose. The pressure catheter was attached in series to a pressure transducer (Validyne MP45), a sensor interface (Validyne CD-280), an analogue to digital converter (CED 1401) and a computer running Spike2 software version 6.13. Full details are given in section 2.8.

6.2.6 Corticomotor Threshold to TMS and Establishing a Baseline MEP for Comparisons
The first part of the study was to establish the resting corticomotor threshold (CMT) for the abdominal muscles in each participant. See 2.12.9 for details of the method. For the patients, a separate corticomotor threshold was established for the affected and unaffected sides. Baseline MEPs were then obtained with stimuli at 120% of CMT delivered at the vertex.

6.2.7 Coil Position Study
The Methods chapter, section 2.12.10.1 gives details of the study protocol.

6.2.8 Effect of Paired Stimuli
See Methods section 2.12.10.2. for full details. A conditioning stimulus at 80% of CMT and a test stimulus of 120% of CMT were applied at the cranial vertex; 5 pairs of stimuli were given for each interval. The interstimulus intervals used were
1.5 milliseconds inclusive; 7, 9, 10, 12, 14, 15, 17, 19, 20, 22, 24 and 25 milliseconds.

6.2.9 Voluntary Muscle Activation and Effect on MEPs
Healthy participants performed an abdominal muscle manoeuvre to find their abdominal muscle MVC (expressed in terms of intra abdominal pressure rise) and then had facilitated TMS by 1) sitting unsupported on a stool 2) performing a modified Valsalva to reach 20% then 40% of MVC. Ten stimuli, at 100% of maximum stimulator output, were given at each level of facilitation. See Methods section 2.12.10.3 for details. Averaged facilitated MEPs were compared with baseline MEPs produced by the stimulation at the vertex at 100% of MSO (see 2.12.10.3 and 2.12.11).

6.2.10 Data Processing and Statistical Methods
150 millisecond long sweeps containing the MEP responses recorded for each test condition, (with the sweep starting 50ms prior to the stimulus) were averaged offline using Spike2 software. All statistical calculations were performed using Prism 5.0 software for Windows. Datasets were tested for normality using the Kolmogorov-Smirnov test in Prism 5.0.

6.2.10.1 Baseline MEP Latency
MEP latency was measured offline, on averaged MEPs produced by baseline stimulations at the vertex with the stimulator set to 120% of the participant's CMT.

6.2.10.2 Comparison of Different Coil Positions
An averaged MEP was produced for each participant in every coil position and also for the baseline position, the vertex. The peak-to-peak amplitude was measured for each averaged MEP. The amplitude for each participant in each test position was normalised (see 2.12.11) and normalised data for each coil position across all participants were recorded in a table and tested for skewness (see 6.2.10).

6.2.10.3 Paired Stimulation: Comparison of Different Interstimulus Intervals
An average MEP was produced for each participant for each interstimulus interval and the peak-to-peak amplitude was measured for each averaged MEP. These were normalised as described in 2.12.11. The data for each healthy individual at each interstimulus interval was entered into a table and tested for skewness. The
average normalised amplitude for each interstimulus interval was compared with the amplitude of the baseline MEPs.

6.2.10.4 Voluntary Muscle Activation

Participants were generally unable to maintain a constant contraction at 20% and 40% of MVC. We averaged the ten responses and a 50ms period before the stimulus was given. We measured the mean Pgas over 50 milliseconds before the stimulus and then expressed this as a percentage of the maximum voluntary contraction figure obtained from the maximum modified Valsalva. The exact percentage of MVC at the time of stimulus was different for different participants. These data are shown as graphics and data tables for each individual.

6.3 RESULTS

6.3.1 Demographics and Corticomotor Threshold

Healthy control and stroke patient participants are described in Table 25 and Table 26. Exact numbers of those participating in each sub study are given with the results of the sub studies below. Table 39 gives further details of the stroke patients’ lesions and impairments.

Table 25 Demography and Corticomotor Threshold for Nine Healthy Participants in the Coil Position Study

<table>
<thead>
<tr>
<th>Initials</th>
<th>PR</th>
<th>TC</th>
<th>GR</th>
<th>KW</th>
<th>CR</th>
<th>LAF</th>
<th>SB</th>
<th>GM</th>
<th>CJ</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>38 (19)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21</td>
<td>23</td>
<td>41</td>
<td>36</td>
<td>27</td>
<td>23</td>
<td>67</td>
<td>71</td>
<td>36</td>
<td>38 (19)</td>
</tr>
<tr>
<td>Sex (male or female)</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>56% males</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178</td>
<td>169</td>
<td>188</td>
<td>172</td>
<td>175</td>
<td>168</td>
<td>161</td>
<td>168</td>
<td>158</td>
<td>171 (9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66</td>
<td>75</td>
<td>107</td>
<td>60</td>
<td>85</td>
<td>50</td>
<td>63</td>
<td>72</td>
<td>53</td>
<td>70 (18)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.8</td>
<td>26.3</td>
<td>30.3</td>
<td>20.3</td>
<td>27.8</td>
<td>17.3</td>
<td>24.3</td>
<td>25.5</td>
<td>21.2</td>
<td>23.8 (4.2)</td>
</tr>
<tr>
<td>Handedness (right or left)</td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>R</td>
<td>78% right handed</td>
</tr>
<tr>
<td>Threshold to TMS (% MSO)</td>
<td>50</td>
<td>75</td>
<td>60</td>
<td>65</td>
<td>75</td>
<td>45</td>
<td>60</td>
<td>80</td>
<td>70</td>
<td>64 (12)</td>
</tr>
<tr>
<td>Latency* (ms)</td>
<td>21.0</td>
<td>18.6</td>
<td>21.6</td>
<td>22.9</td>
<td>23.0</td>
<td>17.0</td>
<td>18.6</td>
<td>21.2</td>
<td>20.1</td>
<td>20.5 (2.1)</td>
</tr>
</tbody>
</table>

Key for Table 25. Sex: M=male; F=female. Handedness: R=right; L=left. BMI=body mass index. kg=kilograms; ms=milliseconds; cm=centimetres; MSO=maximum stimulator output.

*Latency is for stimulation at 120% of corticomotor threshold.
6.3.2 Corticomotor Threshold: Stroke Patients and Controls

There was no significant difference in resting corticomotor threshold between the affected side in stroke patients (median 70% of maximum stimulator output, range 60-85%) and the right side in healthy controls (median=65%, range 45-80%). It was not deemed appropriate to perform statistical tests comparing affected and unaffected sides in stroke patients, due to the small number of them.

6.3.3 Effect of Changing Coil Position

Nine healthy controls participated in the coil position study (Table 25). The study was also performed with one stroke patient, GJ. Data were skew so the averaged MEP amplitude for each coil position was compared with the averaged baseline amplitude using Dunn’s multiple comparison tests in the Prism 5.0 software package.

For healthy people the only significant difference between positions was found when the coil was placed 5cm anterior or 5cm posterior to the vertex (Figure 68 and Table 28). The patient’s data is shown graphically in Figure 69; no MEPs were produced with stimulation positions posterior to the vertex.
Figure 68 Normalised MEP Amplitude from Right Lateral Abdominal Muscle for Different Coil Positions Compared to the Vertex in Healthy Controls

The figure shows the median (horizontal line), interquartile range (box) and range (whiskers) of normalised MEP amplitudes for each coil position, obtained from nine healthy participants. Normalisation was achieved by dividing a participant’s MEP amplitude by the MEP amplitude obtained from a single stimulus.

Table 28 Comparisons of Different Coil Positions in Healthy Controls

<table>
<thead>
<tr>
<th>Dunn's Multiple Comparison Test</th>
<th>Difference in rank sum</th>
<th>Significant?</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (vertex) vs. 1cm</td>
<td>13.50</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. 2cm</td>
<td>25.25</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. 3cm</td>
<td>26.90</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. 4cm</td>
<td>39.90</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. 5cm</td>
<td>51.60</td>
<td>Yes</td>
<td>**</td>
</tr>
<tr>
<td>0 vs. -1cm</td>
<td>23.30</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. -2cm</td>
<td>28.25</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. -3cm</td>
<td>32.40</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. -4cm</td>
<td>25.30</td>
<td>No</td>
<td>ns</td>
</tr>
<tr>
<td>0 vs. -5cm</td>
<td>41.60</td>
<td>Yes</td>
<td>*</td>
</tr>
</tbody>
</table>

Key for Table 28
In column 1, 1cm to 5cm inclusive are positions anterior to the vertex; -1cm to -5cm inclusive are positions posterior to the vertex.
ns=non significant
*=significant at the 0.05 level
**=significant at the 0.01 level

Key for Figure 68
MEP=motor evoked potential; cm=centimetres
The horizontal line at 100% represents the amplitude of the normalised baseline MEP
6.3.4 Paired Pulse TMS in Healthy Controls and Stroke Patients

Eight healthy participants and three stroke patients took part. All healthy participants were the same as those studied for the coil position study, detailed in Table 25, with the exception of Participant 4, GR. Stroke patients who participated are described in Table 26. As data were not normally distributed, the Wilcoxon Signed Rank Test was used to compare the median normalised MEP amplitude for each stimulus interval with the theoretical median of 100%. For stroke patients the normalised data for each participant is shown graphically.

6.3.4.1 Paired Pulse Stimulation: Healthy Participants

Paired stimuli at short interstimulus intervals (1-5ms) were inhibitory. Median MEP amplitudes produced by paired stimuli with interstimulus intervals of 1-5ms were significantly smaller than those produced by single stimuli, P<0.020 for all. An interstimulus interval of 15ms was facilitatory, leading to a statistically significant increase in the size of the MEPs produced, compared with single stimuli (P=0.031). See Figure 70, Figure 71 and Table 29.
Figure 70 Normalised MEP responses from the right lateral abdominals to paired TMS for healthy participant 2

Conditioning stimuli were at 80% and the test stimuli were at 120% of threshold. Stimuli were given at the vertex using a double cone coil. At each interstimulus interval, the size of the conditioned response is given as a percentage of the size of the control response, a stimulus of intensity 120% of threshold given at the vertex. Each point gives the peak-to-peak amplitude of the averaged MEP resulting from five pairs of stimuli.

Key for Figure 70 MEP=motor evoked potential; ms=milliseconds; TMS=transcranial magnetic stimulation
6.3.4.2 Paired Stimulation for Stroke Patients

Data for paired TMS in individual patients is presented in Figure 71, Figure 72 and Figure 74. The effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable. For two stroke patients, the effect of paired stimulation was very variable.
patients (patients 1 and 2) paired stimuli produced no increase in size of the MEPs from the affected side (Figure 72 and Figure 73). For the unaffected side MEP amplitude was multiplied by a factor of 2-7 at interstimulus intervals of 15ms and above. One patient, VL, demonstrated a large increase in the size of MEPs from both the affected and unaffected sides. MEP amplitudes for VL were increased by a factor of 10 to 15 times for both the affected and unaffected sides, see Figure 74.

6.3.4.3 Paired Stimulation: Comparisons between Stroke Patients and Controls

Participant numbers were small so statistical comparisons were not made. However on visual inspection of the graphs, patients appeared to show less intracortical inhibition for short interstimulus intervals and greater intracortical facilitation at longer interstimulus intervals than healthy controls.

Figure 72 Stroke Patient 1 BL (Mild Right Hemiparesis) Effect of Paired Stimuli on MEP amplitude

Key for Figure 72 MEP=Motor evoked potential; ms=milliseconds
Figure 73 Stroke Patient 2 GJ (Dense Left Hemiparesis) Effect of Paired Stimuli on MEP amplitude

Key for Figure 73 MEP=motor evoked potential; ms=milliseconds

Figure 74 Stroke Patient 3 VL (Mild Right Hemiparesis) Effect of Paired Stimuli on MEP amplitude

Key for Figure 74 MEP=motor evoked potential; ms=milliseconds
6.3.5 Effect of Voluntary Muscle Activation on Motor Evoked Potentials in Healthy Controls

Three participants had TMS whilst sitting unsupported and also whilst they performed two different levels of Valsalva. The three proportions of MVC achieved were approximately 10%, 20% and 40% of MVC; exact numbers for each participant are given in the tables. The data for each participant are presented on the following pages (see Figure 75, Figure 76 and Figure 77 and Table 30, Table 31 and Table 32). Facilitation by the modified Valsalva manoeuvre led to an increase in size of the peak-to-peak MEP amplitude and a reduction in MEP latency for all participants. It was not possible for participants to maintain this activation at a set, constant level for a full stimulus response curve to be produced at different levels of abdominal muscle activation.
Figure 75 Healthy Participant Miss AC: Voluntary Facilitation of MEPs and Pgas rise

Overdrawn motor evoked potentials (MEPs) and gastric pressures recorded simultaneously when stimuli (100% maximum stimulator output) were delivered with abdominal muscles at rest and with background voluntary activation, using a modified Valsalva manoeuvre. Stimuli delivered at time 0 on x-axis. See Table 30 for numerical data.

Key for Table 30
*Background muscle activation measured in cmH₂O then expressed as % of the maximum gastric pressure achieved by this participant with a modified Valsalva manoeuvre.
†MEP amplitude and Pgas rise normalised by expression as a % of that achieved with 100% stimulation and low level abdominal muscle activation from sitting on a stool.
Figure 76 Healthy Participant VM: Voluntary Facilitation of MEPs
Overdrawn motor evoked potentials (MEPs) produced when stimuli (100% maximum stimulator output) were delivered with abdominal muscles at rest and with background voluntary activation using a modified Valsalva manoeuvre. Stimuli delivered at time 0 on x-axis.

<table>
<thead>
<tr>
<th>Background muscle activation* (%) MVC</th>
<th>MEP amplitude (mV)</th>
<th>Normalised MEP amplitude† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3.52</td>
<td>100</td>
</tr>
<tr>
<td>17</td>
<td>3.98</td>
<td>113</td>
</tr>
<tr>
<td>29</td>
<td>4.38</td>
<td>124</td>
</tr>
</tbody>
</table>

Key for Table 31 MVC=maximum voluntary contraction; mV=millivolts; MEP=motor evoked potential
†MEP amplitude normalised by expression as a % of that achieved with 100% stimulation of resting muscle.
DISCUSSION

6.4.1 Principal Findings

Our studies, performed in healthy controls and a small number of stroke patients, show that MEPs recorded from surface electrodes over the lateral abdominals have similar characteristics as those found for limb muscles and diaphragm when various TMS protocols are applied to the motor cortex (see section 1.5.4).

Voluntary muscle activation leads to an increase in the size of the MEP and this effect may level off when voluntary activation reaches about 40% of MVC, however the manoeuvres were difficult even for healthy people to perform reliably.

Paired pulse stimulation is inhibitory at short interstimulus intervals (1-5ms) and facilitatory with an interstimulus interval of 15ms. As previously described for other muscles, patients appear to have reduced intracortical inhibition, demonstrated by

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**Table 32 Healthy Participant KW Voluntary Facilitation of MEPs**

See Figure 77 for illustration.

<table>
<thead>
<tr>
<th>Background muscle activation* (% MVC)</th>
<th>MEP amplitude (mV)</th>
<th>Normalised MEP amplitude† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (rest)</td>
<td>1.73</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>2.42</td>
<td>140</td>
</tr>
<tr>
<td>44</td>
<td>2.25</td>
<td>130</td>
</tr>
</tbody>
</table>

Key for Table 32 *Background muscle activation measured in cmH₂O then expressed as % of the maximum gastric pressure (MVC) achieved by this participant with a modified Valsalva manoeuvre †MEP amplitude normalised by expression as a % of the maximum MEP amplitude achieved with patient sitting on a stool but no other voluntary abdominal muscle activation.
much enhanced MEPs from the unaffected side with paired pulse stimulation. Small changes in position of the double cone coil make little difference to the size of MEPs from the lateral abdominals so stimulations with the double cone coil should be delivered at the vertex. This is easy to find and the double cone coil fits the shape of the skull best in this position, leading to better coupling.

6.4.2 Strengths and Weaknesses of the Study

Only three stroke patients were studied. It is unlikely that all the methods of increasing the size of MEPs found for healthy people would be successful in stroke patients. Studies need to be repeated in more stroke patients with different kinds of brain lesions. Controls were younger than the patients but this is unlikely to be the reason for discrepancies in corticomotor threshold or latency, see sections 1.5.4.1 and 1.5.7. We placed the surface electrodes in a lower (more caudal) position over the external oblique than was done in previous studies (57, 100) as we had problems getting any EMG responses in overweight participants and we saw much more ECG contamination of ECG signals from the higher (more cranial) position. This is likely to have contributed to the longer MEP latency we found compared to previous authors. In addition, Plassman and Gandevia used electrical stimulation, which always produces shorter latency responses than magnetic stimulation.

MEP responses were normalised by comparison with single stimuli at the vertex with the muscle at rest and the stimulator set to 120% of threshold. Ideally we would have normalised the MEP area with the CMAP area produced by stimulation over the T10 nerve roots, as was done by Fujiwara and colleagues (57). However with trial stimuli over the T10 nerve roots it was difficult to determine the start of CMAPs as the EMG traces were obscured by magnet noise, with the magnetic coil being positioned very close to the recording electrodes and the CMAP latency being very short. The end of both the CMAP and MEP responses was also very difficult to determine; it was not possible to calculate CMAP or MEP areas without a clear start and end to the signals.

6.4.3 Comparison with Previous Studies

6.4.3.1 Healthy Participants: Threshold, Latency

See Table 1 in the Introduction for a summary of previous TMS studies of abdominals in healthy participants and section 1.8.2 for a summary of studies in stroke patients. The corticomotor threshold (CMT) of the lateral abdominals to
TMS we found for the healthy controls (mean 64% of maximum stimulator output, SD 12) was slightly lower than that found by Harraf in her description of the external obliques in 16 healthy controls (mean 72%, SD 17) (58). It is unlikely the difference in threshold is statistically significant but it should be noted that her participants were reclined from the waist to 60 degrees from the upright. The participants in our study were upright and it is likely the abdominal muscles had a higher background level of activation in this position, leading to a lower threshold. Carr studied the rectus abdominis in 7 healthy subjects and found the median activated threshold to be 45% (range 35-55) but the background level of activation was not measured (76). The latency of MEP response from the lateral abdominals with the stimulator set to 120% of CMT (mean 20.5ms, SD 2.1) was similar to that described by Harraf (mean 21.4ms, SD 2.1) although she stimulated lateral to the vertex with a stimulus intensity of 140% of CMT (58).

6.4.3.2 Stroke Patients: Threshold, Latency
As only three patients, with heterogenous lesions, were studied, statistical testing to compare signals from affected and unaffected sides was not appropriate but our findings are in line with those from previous authors. The median resting corticomotor threshold for lateral abdominals for the affected side (70%) was higher than that for the unaffected side (60%). Liepert studied patients with pure motor strokes and very mild impairments; patients had strength of 4 or greater on the MRC scale in both limbs. He found significant differences in resting motor threshold between affected and unaffected sides in TMS studies of the first dorsal interosseus in patients with lacunar lesions at the level of the basal ganglia (n=13) or with paramedian pontine infarcts (n=10) (79). The thresholds for the affected hemispheres were significantly greater than for the unaffected side. There was also a tendency for the thresholds to be larger on the affected side in a smaller group of seven patients with stroke in the region of the motor cortex but the difference was not significant (P=0.06). Other authors did not evoke signals from stimulation of the affected side so thresholds could not be measured. Harraf’s patients were much more severely impaired than those described in Liepert’s studies or our own (58).

6.4.3.3 Paired stimulation
The pattern of intracortical inhibition at interstimulus intervals of 1-5ms and facilitation at interstimulus intervals of 10-20ms, demonstrated previously for other
muscles such as the first dorsal interosseus, abductor pollicis brevis and the diaphragm, was also found for the lateral abdominals in healthy subjects and stroke patients (67, 69, 201).

On the unaffected side, patients exhibited significantly less intracortical inhibition than controls at the 5ms interstimulus interval. Patients also tended to have more intracortical facilitation at the 15ms interval. Motor cortex disinhibition of the unaffected hemisphere has been previously been shown in a study of stroke patients with large infarcts and a dense paresis of the upper limb (67). Liepert’s study also showed that acute stroke patients with pure motor cortex lesions had significantly decreased intracortical inhibition demonstrated when paired stimuli were applied to the affected hemisphere and responses were measured from the first dorsal interosseus muscle, see 1.6.2 and (79).

When the effects of paired stimulation are compared with single stimuli this gives the investigator more effects to study than if only single pulse stimuli are given. However paired stimulation as described here still requires the CMT to be found which is time consuming and may involve delivering lots of stimuli; and it is not always possible to deliver 120 or 130% of the corticomotor threshold as the magnet can only be turned up to 100%.

6.4.3.4 Voluntary activation

Voluntary activation of the abdominal muscles led to increased size of MEPs in healthy participants but it was difficult for the participants to maintain a set level of Pgas using the modified Valsalva manoeuvre, even when given visual feedback. The level of background muscle activation at which the effect on abdominal muscle response is supramaximal was not confirmed by this study but from our data it may occur at around 40% of MVC. This is in line with previous studies of the diaphragm and abdominals. In studies of the diaphragm, Sharshar concluded that the maximal effect of voluntary contraction was at 40% of MVC (59). Tunstill’s study did not measure force but showed the effect of facilitation by voluntary activation of the abdominals to level off when the average EMG level reached 30-40% of the maximum (spontaneous) EMG level, see 1.7.2.1 and Table 1 (103).

If the plateau had occurred at around 10% of MVC as it does for the small muscles of the hand, sitting on a stool alone would have been enough to near-maximally facilitate the abdominal response. Sitting unsupported would have been possible
for some patients but performing manoeuvres reliably was difficult for healthy people and unlikely to be possible for patients.

6.4.4 Conclusion

For assessment of MEPs from the lateral abdominals before and after cough training, TMS delivered at the cranial vertex is likely to produce MEP responses in the muscles on both sides but it is not possible to know exactly which cortex the responses are generated from. The advantage of vertex TMS rather than giving stimulation from the POE on both sides is that it can be done in half the time. If no signals can be produced from resting muscle, voluntary activation of the muscles may lead to an MEP because of facilitation. Unless a set level of contraction can be maintained though, or the level of contraction always exceeds that required for supramaximal facilitation, this will lead to variable responses.

Paired stimulation at an interstimulus interval of 15ms can lead to an increase in the size of MEP produced compared with single stimulation. Study of the effects of paired stimuli with short (1-3ms) and long (15-20ms) interstimulus intervals may provide additional information on intracortical inhibition and intracortical facilitation after stroke. These results should be interpreted with caution and the experiments need to be repeated in more stroke patients. They should also be repeated on separate occasions in the same individuals to check the stability of the responses over time.

Study of the stimulus-response curve to TMS is an alternative approach which may provide further insight into the cortical control of the abdominal muscles and will be described in the next two chapters.
Chapter 7  Motor-Cortical Stimulus-Response Curves in Controls: TMS and Motor-Evoked Potentials and Evoked Force from Abdominal Muscles

7.1 INTRODUCTION

The previous section (Chapter 6) evaluated various methods of producing large MEPs from abdominal muscles in response to TMS. Facilitation by voluntary activation of abdominal muscles to a set level of contraction (proportion of maximum voluntary contraction) was difficult even for healthy participants and thus thought not to be feasible for patients. Paired stimulation requires the participant’s corticomotor threshold (CMT) to be found prior to starting the paired stimulation protocol, which can take a long time and involves many stimuli being applied; it is not possible find a threshold for patients with no MEP response. The stimulus-response (SR) curve is an alternative method of describing motor cortex excitability, see 1.5.4.5 and (62).

Motor-cortical SR curves plot the size of the MEP response (e.g. area under the curve or amplitude) and / or the muscle twitch response evoked by magnetic stimulation of a fixed scalp site at a range of different stimulator intensities, see 1.5.4.5 (20) CMT does not need to be found first. The CMT can be established to within 5% offline, after the SR data has been acquired; this is helpful for patients who cannot tolerate lengthy studies due to tiredness. SR relationships often take the shape of a Boltzmann sigmoidal curve and changes in the curves are seen during voluntary muscle activation and after administration of some drugs (59). Curve parameters, for example slope and plateau, as well as MEP amplitudes at set stimulus intensities, may change after a training intervention (202). Some training studies simply describe MEPs elicited by a single set stimulus given before and after the intervention but these ignore the fact that training may change the CMT. Devanne et al used SR curves extensively to study the tibialis anterior muscle in experiments on the motor control of walking (54, 60, 203).

We found no previously published TMS SR curves for the abdominal muscles where MEP and twitch response (Pgas) rise were recorded simultaneously. Although there are studies describing TMS-evoked responses from the abdominal muscles in healthy people (98, 101, 103) and stroke patients (58) at set levels of stimulator output, we only found one describing the SR relationship for MEP response (from the rectus abdominis; not a primary outcome measure of the
paper) (98) and none describing the SR relationship for Pgas response. Sharshar showed SR plots for MEP from the rectus abdominis in healthy people undergoing mechanical ventilation. The primary aim of that study was to look at the effects of non-invasive ventilation on the diaphragm (not the abdominals). Curve fitting was not performed for the abdominal responses but on visual inspection of the plots the relationships appear to be relatively linear between 60 and 100% of stimulator output (98).

Harraf et al studied stroke patients and healthy controls (58). Both the external oblique MEP and the evoked rise in Pgas after TMS was recorded but SR relationships were not described and the relationship between the size of the MEP and the Pgas rise was not reported. One diaphragm TMS study measured changes in Pgas (59) and another changes in abdominal circumference (201) evoked by TMS but these measures were in pursuit of finding the pressure across the diaphragm, Pdi (Pdi=Pgas-Poes where Poes is the intraoesophageal pressure). The Pgas changes themselves are not reported in the papers. The studies described above are explained further in the Introduction and Table 1.

Pgas rise in response to TMS is of interest as it is the rise in Pgas that drives out airflow during cough and there is evidence for cortical control or modulation for both voluntary and reflex cough. Quantification of the rise in Pgas achieved during cough is one way of describing cough intensity (along with cough flow and cough sound). A strong relationship between abdominal EMG amplitude, Pgas rise and cough sound intensity for voluntary cough was demonstrated in work undertaken in collaboration with colleagues from the University of Manchester and presented in abstract form in 2009 (204).

Experiments were designed to study the relationship between stimulus intensity (of TMS) and response of the lateral abdominal (oblique) muscles, measured by 1) MEP amplitude and 2) evoked rise in Pgas. This was done in order to find which parameters could be used to study corticomotor changes in training studies designed to improve cough. Cough flow rate would be the primary outcome measure for the planned training study but demonstration of changed response to TMS (both Pgas rise and MEP response) would suggest any improvements in cough were due to cortical rather than peripheral changes.
7.2 METHODS

TMS was applied at the cranial vertex using the 110mm double cone coil and a MagStim 200 stimulator, with the participants sitting upright in a supportive chair with the backrest at 90 degrees to the horizontal. Further description of the equipment and methods for TMS and MEP recording are given in the Methods section, 2.12.

7.2.1 SR Curves: MEP and Force

Motor cortical SR curves were constructed in the relaxed state as described in 2.12.12. The responses, recorded simultaneously, were motor evoked potentials (MEPs) from the lateral abdominal muscles (right side) and the rise in Pgas evoked by the TMS. The electrode positions are detailed in section 2.12.6. The methods for recording Pgas are given in 6.2.5 and 2.8.

7.2.2 Resting Corticomotor Threshold

MEPs were reviewed offline, after the SR protocol had been completed. Resting corticomotor threshold (CMT) was found for the right lateral abdominals in these healthy controls. CMT was defined as the stimulus intensity required to produce a MEP of greater than 50µV in the lateral abdominals, for at least five out of ten consecutive trial stimuli. If one stimulus level produced less than five adequate MEPs and the next stimulus level up produced more than five MEPs, the average of the two levels was said to be the corticomotor threshold. See 2.12.9 for further details of the method.

7.2.3 Data Analysis and Curve Fitting

For every participant at each level of magnetic stimulus intensity, ten unrectified MEP responses were averaged over a time period of 150 milliseconds, starting 50 milliseconds prior to the magnetic stimulus. Averaging was performed offline using Spike2 software. Peak-to-peak amplitude of the averaged responses was measured (MEP amplitude and Pgas rise) and plotted against magnetic stimulus intensity on a scatter graph. Area under the curve (AUC) of the MEP SR scatter plot, with data points joined with straight lines, was calculated in Prism. Boltzmann curves and their parameters ($S_{50}$, MEP max, MEP min) described in 1.5.4.5 were generated for each individual by Prism and entered into a data table for further analyses. Curve fitting and comparison with a straight-line fit is described in 2.12.12.
7.2.4 Group Data Analysis
Each participant's MEP and Pgas responses were normalised, by dividing the absolute MEP amplitude or Pgas recorded for a given stimulus intensity by the maximum MEP amplitude or Pgas achieved by that individual for any stimulus intensity. This meant that the relationship between magnetic stimulus intensity and average response (MEP amplitude and Pgas rise) could be plotted for the group as a whole. SR curves for both normalised responses were constructed as for individuals, see 2.12.12. The relationship between MEP amplitude and Pgas rise was described by means of a scatter plot.

7.3 RESULTS
Participants' details are given in Table 33 and SR relationships for each participant are shown individually below.

Table 33 Healthy Participants for Stimulus-Response Curve Study

<table>
<thead>
<tr>
<th>Participant number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37</td>
<td>27</td>
<td>23</td>
<td>22</td>
<td>22</td>
<td>21</td>
<td>24</td>
<td>25.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>29% males</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171</td>
<td>172</td>
<td>175</td>
<td>162</td>
<td>180</td>
<td>163</td>
<td>183</td>
<td>172.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60</td>
<td>61</td>
<td>58</td>
<td>76</td>
<td>44</td>
<td>81</td>
<td>52.0</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.5</td>
<td>20.7</td>
<td>18.9</td>
<td>20.6</td>
<td>23.5</td>
<td>16.5</td>
<td>24.7</td>
<td>20.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Handedness</td>
<td>R</td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>R</td>
<td>L</td>
<td>29% left handed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key for Table 33: F=female; M=male; ms=milliseconds; TMS=transcranial magnetic stimulation; %=percentage; kg=kilograms; cm=centimetres; m=metres; SD=standard deviation

Table 34 Healthy Participants TMS parameters

<table>
<thead>
<tr>
<th>Participant number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold to TMS (% max stimulator output)</td>
<td>50</td>
<td>65</td>
<td>60</td>
<td>70</td>
<td>70</td>
<td>65</td>
<td>80</td>
<td>65.7</td>
<td>9.3</td>
</tr>
<tr>
<td>Latency at 100% (ms)</td>
<td>18.2</td>
<td>20.3</td>
<td>23.2</td>
<td>23.6</td>
<td>22.4</td>
<td>19.9</td>
<td>21.4</td>
<td>21.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Max MEP amplitude (mV)</td>
<td>1.72</td>
<td>1.68</td>
<td>2.73</td>
<td>0.83</td>
<td>1.55</td>
<td>4.99</td>
<td>0.43</td>
<td>1.99</td>
<td>1.52</td>
</tr>
<tr>
<td>Area under the SR curve (mV.%MSO)</td>
<td>36.2</td>
<td>26.9</td>
<td>91.0</td>
<td>13.3</td>
<td>29.7</td>
<td>102.7</td>
<td>6.1</td>
<td>43.7</td>
<td>37.8</td>
</tr>
</tbody>
</table>

Key for Table 34: ms=milliseconds; TMS=transcranial magnetic stimulation; %=percentage; cm=centimetres; m=metres; SD=standard deviation; H₂O=water; Pgas=gastric pressure

7.3.1.1 Form of the Motor Evoked Potentials
Figure 78 and Figure 79 show the typical form of the motor-evoked potentials from the right lateral abdominals for two different individuals. Figure 80 illustrates the reproducibility of responses for stimuli just above CMT in participant 6. As the stimulus intensity was increased, the MEPs became more reproducible.
Figure 78 Motor Evoked Potentials for Participant 4, Overlaid

Ten overlaid MEPs from the resting right oblique muscle for participant 4. The stimulator was set to 100% of maximum stimulator output.

Key for Figure 78: X axis shows time in seconds. The stimulus artefact is seen at time 0. Y axis shows right oblique EMG, measured in millivolts.

Figure 79 Reproducibility of Motor Evoked Potentials for Participant 1: Waterfall Plot

Ten MEPs from the resting right oblique muscle for participant 1 with the stimulator set to 100% of maximum stimulator output. In this waterfall plot, traces from consecutive stimuli are slightly offset along the X and Y axes to enable clear viewing. There is a large stimulus artefact at time 0.

Key for Figure 79: X axis shows time in seconds. Y axis shows right oblique EMG, measured in millivolts.
7.3.1.2 Individual SR Relationships

For all participants, the amplitude of the MEPs increased with increasing stimulus intensity from threshold to saturation with little change in the shape of the MEP. Absolute values of MEP and Pgas varied greatly between participants. However the nature of the SR relationship was very similar for all. Figure 81 shows the increase in peak-to-peak amplitude of the MEP with increasing stimulus intensity for a single individual. Figure 82 shows a scatter of stimulus intensity plotted against MEP response for a single individual, with a curve fitted using the Boltzmann sigmoidal equation.
7.3.2 Individual Stimulus Response Relationships

Figure 83 to Figure 89 inclusive show SR plots for each participant. Separate plots are shown for the MEP and Pgas responses. The plots on the right side of the pages have been fitted with non-linear regression curves using the Boltzmann equation, see Equation 1 for further details. The Boltzmann curve fit accounted for greater than or equal to 95% of the total variance of the data points for all participants, for both MEP and Pgas responses (ie $R^2 \geq 0.95$). The Boltzmann model was always a significantly better fit than the straight line: $P<0.01$ for all individuals, for both MEP and Pgas responses. See Table 35 and Table 36 for details of curve parameters for each participant.
Figure 82 Scatter Plot of Relationship Between Stimulus Intensity and Peak-to-Peak MEP Amplitude with Curve Fitted Using the Boltzmann Equation

The plot is for a single participant (no 4) and is marked with $S_{50}$ and $\text{MEP}_{\text{max}}$ and their 95% confidence intervals. The steepness of the curve is maximal at $S_{50}$.

For this curve the parameters are as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Best-fit values</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>0.00</td>
<td>-0.04 to 0.05</td>
</tr>
<tr>
<td>Top</td>
<td>1.95</td>
<td>1.73 to 2.17</td>
</tr>
<tr>
<td>$S_{50}$</td>
<td>82.82</td>
<td>80.29 to 85.35</td>
</tr>
<tr>
<td>Slope</td>
<td>8.70</td>
<td>7.00 to 10.39</td>
</tr>
</tbody>
</table>

Goodness of fit

<table>
<thead>
<tr>
<th>Degrees of Freedom†</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2$</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Absolute Sum of Squares (SS)

| $S_y, x^*$          | 0.02 |

$S_{y, x}^* = \sqrt{\frac{SS}{df}}$

Key for Figure 82

MEP=motor evoked potential; mV=millivolts

† Degrees of freedom for a non-linear fit= number of data points minus the number of parameters fit

* $S_y, x$ quantifies the scatter of the data points around the best fit curve

Equation 7 Scatter of Data Points around the Best Fit Curve

Where SS is the sum of squares and df is the degrees of freedom; a low value of $S_y, x$ indicates a good fit
Figure 83 Stimulus-Response Relationships for Participant 1

Figure 84 Stimulus-Response Relationships Participant 2
Figure 85 Stimulus-Response Relationships Participant 3

Figure 86 Stimulus-Response Relationships Participant 4
Figure 87 Stimulus-Response Relationships Participant 5

![Graphs showing MEP amplitude and Pgas rise as a function of stimulus intensity for Participant 5.]

Figure 88 Stimulus-Response Relationships Participant 6

![Graphs showing MEP amplitude and Pgas rise as a function of stimulus intensity for Participant 6.]

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Key for Figure 83 to Figure 89 inclusive
MEP=motor evoked potential; mV=millivolts; Pgas=gastric pressure; cmH₂O=centimetres of water pressure
Table 35 Individual Parameters for Stimulus-Response Curves (MEP amplitude)

<table>
<thead>
<tr>
<th>Boltzmann sigmoidal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best-fit values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>0.00</td>
<td>-0.02</td>
<td>-0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.04 to 0.04</td>
<td>-0.05 to 0.02</td>
<td>-0.30 to 0.10</td>
<td>-0.02 to 0.01</td>
<td>-0.03 to 0.04</td>
<td>-0.40 to 0.22</td>
<td>-0.02 to 0.01</td>
</tr>
<tr>
<td>Top</td>
<td>1.95</td>
<td>1.93</td>
<td>2.68</td>
<td>0.83</td>
<td>1.70</td>
<td>6.78</td>
<td>0.48</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.73 to 2.17</td>
<td>1.69 to 2.16</td>
<td>2.41 to 2.95</td>
<td>0.72 to 0.93</td>
<td>1.56 to 1.85</td>
<td>3.80 to 9.75</td>
<td>0.30 to 0.68</td>
</tr>
<tr>
<td>Slope</td>
<td>8.69</td>
<td>7.02</td>
<td>9.40</td>
<td>5.88</td>
<td>5.23</td>
<td>11.83</td>
<td>8.08</td>
</tr>
<tr>
<td>95% CI</td>
<td>7.00 to 10.39</td>
<td>5.62 to 8.43</td>
<td>6.17 to 12.64</td>
<td>4.01 to 7.75</td>
<td>4.04 to 6.41</td>
<td>5.94 to 17.71</td>
<td>4.11 to 12.05</td>
</tr>
<tr>
<td>S_{50}</td>
<td>82.82</td>
<td>86.59</td>
<td>64.32</td>
<td>83.42</td>
<td>83.08</td>
<td>87.46</td>
<td>88.16</td>
</tr>
<tr>
<td>95% CI</td>
<td>80.29 to 85.35</td>
<td>84.37 to 88.81</td>
<td>61.05 to 67.59</td>
<td>81.02 to 85.83</td>
<td>81.58 to 84.58</td>
<td>75.91 to 99.01</td>
<td>80.66 to 95.66</td>
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<tr>
<td>Goodness of fit</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Degrees of freedom</td>
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<td>12</td>
<td>13</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>R square</td>
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<td>0.98</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.97</td>
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<td>0.01</td>
<td>0.04</td>
<td>0.15</td>
<td>0.00</td>
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<tr>
<td>Sy.x</td>
<td>0.04</td>
<td>0.04</td>
<td>0.14</td>
<td>0.03</td>
<td>0.05</td>
<td>0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>No of points analysed</td>
<td>16</td>
<td>15</td>
<td>17</td>
<td>16</td>
<td>17</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Is Boltzmann better fit than straight line?*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>P value</td>
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<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Key for Table 35:
- 95% CI=95% confidence intervals; S_{50}=stimulus intensity required to obtain a response 50% of the maximum; Sy.x=standard deviation of the residuals; R square=coefficient of determination
- P value obtained from extra sum-of-squares F test comparing straight line fit with Boltzmann sigmoidal fit
Table 36 Individual Parameters for Stimulus-Response Curves (Pgas rise)

<table>
<thead>
<tr>
<th>Boltzmann sigmoidal</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>0.07</td>
<td>-0.02</td>
<td>-0.23</td>
<td>-0.03</td>
<td>0.01</td>
<td>0.18</td>
<td>-0.10</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.62 to 0.75</td>
<td>-0.12 to 0.07</td>
<td>-1.66 to 1.19</td>
<td>-0.16 to 0.08</td>
<td>-0.03 to 0.06</td>
<td>-0.51 to 0.88</td>
<td>-0.61 to 0.42</td>
</tr>
<tr>
<td>Top</td>
<td>17.32</td>
<td>4.32</td>
<td>43.54</td>
<td>2.48</td>
<td>2.67</td>
<td>35.10</td>
<td>7.65</td>
</tr>
<tr>
<td>95% CI</td>
<td>11.25 to 23.40</td>
<td>3.36 to 5.27</td>
<td>37.33 to 49.80</td>
<td>0.40 to 4.56</td>
<td>2.47 to 2.86</td>
<td>29.56 to 40.59</td>
<td>5.70 to 9.59</td>
</tr>
<tr>
<td>Slope</td>
<td>5.72</td>
<td>7.04</td>
<td>9.55</td>
<td>9.78</td>
<td>4.08</td>
<td>6.45</td>
<td>5.86</td>
</tr>
<tr>
<td>95% CI</td>
<td>2.64 to 8.80</td>
<td>5.06 to 9.01</td>
<td>7.05 to 12.06</td>
<td>2.49 to 17.06</td>
<td>3.23 to 4.93</td>
<td>4.75 to 8.15</td>
<td>2.01 to 9.61</td>
</tr>
<tr>
<td>S50</td>
<td>90.03</td>
<td>90.16</td>
<td>79.75</td>
<td>91.40</td>
<td>86.48</td>
<td>89.47</td>
<td>82.22</td>
</tr>
<tr>
<td>95% CI</td>
<td>84.72 to 95.35</td>
<td>86.37 to 93.94</td>
<td>76.23 to 83.27</td>
<td>73.00 to 109.80</td>
<td>85.40 to 87.55</td>
<td>86.72 to 92.22</td>
<td>77.40 to 87.03</td>
</tr>
<tr>
<td>Goodness of fit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>12</td>
<td>11</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>R square</td>
<td>0.97</td>
<td>0.99</td>
<td>0.99</td>
<td>0.96</td>
<td>0.99</td>
<td>0.99</td>
<td>0.95</td>
</tr>
<tr>
<td>Sum of squares</td>
<td>10.16</td>
<td>0.13</td>
<td>25.18</td>
<td>0.20</td>
<td>0.07</td>
<td>1.68</td>
<td>6.13</td>
</tr>
<tr>
<td>Sy.x</td>
<td>0.92</td>
<td>0.11</td>
<td>1.39</td>
<td>0.13</td>
<td>0.07</td>
<td>0.58</td>
<td>0.69</td>
</tr>
<tr>
<td>No of points analysed</td>
<td>16</td>
<td>15</td>
<td>17</td>
<td>16</td>
<td>17</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Is Boltzmann better fit than straight line?*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>P value</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Key for Table 36: Pgas=gastric pressure; 95% CI=95% confidence intervals; S50=stimulus intensity required to obtain a response 50% of the maximum; Sy.x=standard deviation of the residuals; R square=coefficient of determination; * P value obtained from extra sum-of-squares F test comparing straight line fit with Boltzmann sigmoidal fit.

7.3.3 Group Results

Stimulus response curves for the group as a whole are shown in Figure 90 and Figure 91.

7.3.3.1 Averaged and Normalised Responses

Table 37 Average Responses for Seven Healthy Participants

For latency, MEP amplitude and gastric pressure rise, the results are for TMS at 100% of maximum stimulator output at the vertex.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric pressure rise (cmH₂O)</td>
<td>13.8</td>
<td>14.0</td>
</tr>
<tr>
<td>MEP amplitude (mV)</td>
<td>1.99</td>
<td>1.52</td>
</tr>
<tr>
<td>Corticomotor threshold (% MSO)</td>
<td>65.7</td>
<td>9.3</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>21.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Area under the SR curve</td>
<td>43.7</td>
<td>37.8</td>
</tr>
</tbody>
</table>

Key for Table 37: MSO=maximum stimulator output; SD=standard deviation; mV=millivolts; cmH₂O=centimetres of water pressure; ms=milliseconds
Figure 90 Stimulus Intensity and Mean MEP Amplitude from Right Oblique Muscle

The graph shows the mean response to magnetic stimuli at the cranial vertex for seven healthy participants. The MEP peak-to-peak amplitude has been normalised by dividing by each participant’s own maximum response. The error bars represent one standard error of the mean (1 SEM). The superimposed curve has been fitted using the Boltzmann sigmoidal equation. $S_{50}$ and MEP max (solid lines) and their 95% confidence intervals (dotted lines) are shown on the plot.

For this curve the parameters are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Best-fit values</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>0.00</td>
<td>-0.03 to 0.03</td>
</tr>
<tr>
<td>Top</td>
<td>1.18</td>
<td>0.92 to 1.43</td>
</tr>
<tr>
<td>$S_{50}$</td>
<td>84.73</td>
<td>79.70 to 89.77</td>
</tr>
<tr>
<td>Slope</td>
<td>9.42</td>
<td>6.52 to 12.32</td>
</tr>
</tbody>
</table>

**Goodness of Fit**

| Degrees of Freedom | 112            |
| Absolute Sum of Squares (SS) | 1.33     |
| $S_{y,x}$          | 0.11           |

Is Boltzmann curve better fit than straight line? *Yes*  

*P value obtained from extra sum-of-squares F test comparing straight line fit with Boltzmann sigmoidal fit

Key for Figure 90 MEP=motor evoked potential; $S_{50}$= stimulus intensity required to obtain a response 50% of the maximum; $S_{y,x}$ = standard deviation of the residuals; $R^2$=coefficient of determination
7.3.4 Relationship Between Peak to Peak MEP Amplitude and \( P_{gas} \) rise

There was a strong and significant relationship between the MEP amplitude and \( P_{gas} \) rise evoked by TMS. An extra sum of squares F test was performed to compare the straight line and Boltzmann fits; this showed the Boltzmann curve to be a significantly better fit than the straight line, \( P<0.001 \). \( R^2 \) for the Boltzmann curve fit=0.99.
7.4 DISCUSSION

7.4.1 Principal Findings

For transcranial magnetic stimuli of varying intensity given at the vertex and responses measured as MEP amplitude from the lateral abdominals (for the electrical response) and Pgas rise (for the twitch response), the size of the responses is strongly and significantly related to the intensity of the stimuli and both the TMS-MEP and TMS-Pgas stimulus-response relationships are better described by a Boltzmann sigmoidal fit that a straight line. There is also a strong and significant relationship between the two responses measured simultaneously, i.e. the size of the MEP recorded from the lateral abdominals and twitch force (Pgas rise).

Rather than just measuring the size of the MEP for a single stimulus intensity level before and after an intervention, a sigmoidal (Boltzmann) SR curve gives an increased number of parameters to study. Participants do not need to wait for the operator to calculate the CMT before moving onto the next part of the study (as would need to be done for paired stimulation study for example). The size of the Pgas twitch response to TMS is strongly related to peak-to-peak amplitude of the

Figure 92 Relationship Between Normalised MEP Amplitude and Gastric Pressure Rise For Seven Healthy Participants at Rest

The scatter graph shows 7 participants’ averaged and normalised MEP responses to TMS stimuli (intensities ranging from 20% to 100% of maximum stimulator output), plotted against the averaged normalised gastric pressure rise for the same stimuli. A straight line fit (dashed line) and Boltzmann curve fit are superimposed on the scatter plot.
motor evoked potentials from the lateral abdominal muscles in this group of healthy people, so invasive measurement of intra-abdominal pressures may be avoidable in further studies.

7.4.2 Critique of the Methods and Comparison with Other Studies

MEP response was only measured in one place on the abdomen although there are several abdominal muscles in overlapping layers (Figure 5 and Figure 6). Although the surface electrodes were placed in a position which overlies the external oblique in most people it is not possible to tell which muscle layer or muscle the EMG is coming from. MEPs were recorded from the right side only. This side was chosen because EMG on the right side is less likely to be contaminated by cardiac artefact than signals recorded on the left. An alternative method of normalisation would have been to compare the MEP response area to that of the CMAP produced after stimulation of the T_{10} nerve roots, as was done by Fujiwara and colleagues (57); this was discussed in 6.4.2.

The results for our group for corticomotor threshold, MEP latency and maximum responses were compared to 16 healthy controls described in an earlier TMS study of the abdominals by Harraf et al (58), described in the Introduction section 1.7.2 and Table 1. Their paper did not describe SR relationships for TMS and it should be noted that their controls were much older than our group. Despite the difference in ages, results of corticomotor threshold, MEP latency and P_{gas} rise for TMS to the external obliques are similar for the two studies. Difference in maximum MEP amplitude may be explained by different positioning of the participants in the two studies and this is discussed below. Maximum MEP amplitudes for participants in our study (mean 1.99mV, SD 1.52) were much larger than those in the Harraf study (mean 0.42mV, SD 0.30). A higher background level of activation could produce larger responses due to facilitation (see section 1.5.5.1). Harraf’s participants were reclined to 60 degrees from vertical and ours were sitting upright; the background level of abdominal muscle activation was likely to have been higher for participants in our study, who were required to make some use of their trunk muscles to maintain sitting balance.

Sharshar and colleagues described the SR relationship between TMS and MEPs from the resting rectus abdominis in six healthy people (98); as for our participants their SR relationships did not appear to plateau either. TMS was delivered from the point of optimum excitation (POE) for the diaphragm rather than the vertex or
the POE for the abdominals. Their participants were semi-supine and probably had less activated abdominal muscles than our group who were sitting upright. CMT, MEP latency or absolute MEP amplitudes were not given and curve fitting was not described (98).

As previous authors have found for the abdominals (98) and other muscles such as the tibialis anterior (60), it is difficult to achieve a supramaximal response from resting / relatively inactive muscle using TMS. This is likely to be due to limitations of the output of the stimulator and coil combination. Although authors (54) describe the SR relationship as sigmoidal we do not know that the response necessarily plateaus at very high stimulus intensities and instead we may find a different response were it possible to stimulate at higher intensity.

7.4.3 Unanswered Questions and Further Work

SR curves produced for healthy young people with normal body mass index may not be representative of the results that could be achieved from stroke patients, healthy elders or overweight subjects, although age by itself is not thought to change the response to TMS (see 1.5.8). Repeatability studies are necessary to find out whether the SR relationship and the relationship between the MEP and twitch force responses are stable over minutes, hours, days and weeks. Studies in patients will be described in the next chapter.

7.4.4 Conclusion

TMS stimulus response relationships, where the responses are the MEP amplitude from the lateral abdominals (for the electrical response) and Pgas rise (for the twitch response) have similar characteristics to the relationships previously described for limb muscles such as the tibialis anterior and can be described by a Boltzmann sigmoidal fit. There is also a strong and significant relationship between the two responses measured simultaneously, i.e. the size of the MEP recorded from the lateral abdominals and twitch force (Pgas rise). If they are found to be stable over time SR curves may be a useful method of studying changes in corticomotor excitability arising as a result of training. SR curves in stroke patients are described in Chapter 8.
Chapter 8  TMS Studies of Abdominal Muscle Control in Stroke Patients and Attempts to Drive Plasticity Using Cough Training

8.1 INTRODUCTION

The ultimate aim of this programme of work is to improve cough in acute stroke patients, in order to increase lung clearance of foreign material and reduce the incidence of chest infections. In addition, we seek to find out whether any improvement in cough is accompanied by changes in corticomotor excitability, which would suggest neuroplasticity. The inspiration for this project was work by Hamdy and colleagues introduced in section 1.2.3 (18). However, the functional and neurophysiological changes for swallow shown to occur after electrical stimulation of the pharynx have not as yet been translated to other skilled tasks i.e. passive electrical stimulation has only been shown to improve function and drive plasticity for swallow. We wanted to have patients’ voluntary input into our planned cough training intervention as it was thought this was more likely to be effective. The aims of these preliminary experiments in stroke patients were:

1) To study the evoked responses from the abdominal muscles on the affected and unaffected sides after TMS at the vertex; and to study stimulus-response (SR) relationships
2) To compare TMS evoked responses from patients with those of healthy controls
3) To develop a cough training protocol for use in stroke patients
4) To develop a protocol for evaluation of the effect of cough training on corticomotor excitability

8.1.1 TMS in stroke patients

In general stroke patients have a raised corticomotor threshold (CMT) to TMS when compared with healthy controls; and motor evoked potentials (MEPs) from muscles on the affected side may be absent, delayed or decreased in amplitude (see section 1.6). Ipsilateral responses may be elicited, (see 1.6.1) and there may be disinhibition of the unaffected hemisphere (see 1.6.2).

The two previously published TMS studies of the abdominals in stroke patients are described in Table 2 and section 1.8.2. Both describe moderate to severely affected patients and there were no MEPs recorded from abdominal muscles on either side in most or all cases (57, 58) after TMS over the affected hemisphere.
Since the use of thrombolysis treatment for stroke has become widespread the number of patients with severe impairment has reduced and we believed the study of a new group of patients with a different TMS protocol was worthwhile. It was thought that the CMT of patients with less ischaemic damage than those studied previously could be low enough for TMS evoked responses to be seen on the affected side of the body.

8.1.1.1 Stimulus (TMS) Response (MEP) Relationships in Stroke Patients

Previous SR studies performed in acute stroke patients include those of Liepert described in section 1.6.3 (79); for all stimulus intensities MEP amplitudes from the unaffected side tended to be larger than those from the affected side but the difference between sides was only significant for the subgroup of patients with internal capsule or pontine lesions.

More information, such as area under the curve, could be elicited using a stimulus response protocol rather than applying TMS with single stimulus intensity as was done in the previous studies. Fujiwara stimulated all patients at 100% of magnetic stimulator output and Harraf stimulated at 130% of CMT, or 100% of MSO.

With our training protocol in mind it would be incorrect to use the same set stimulus (level of stimulator output or proportion of pre-training threshold) before and after a training session. The intervention could change the CMT, so if the MEP responses were altered it would not be possible to know if this was because the magnitude of the stimulus had changed in relation to the CMT. SR plots from before and after training would demonstrate any change in CMT as a shift of the curve along the x-axis; the area under the curve measure would take into account the slope and maximum MEP amplitude.

8.1.2 Cough Training

Training which is goal-orientated, active, repetitive and task-specific, with auditory cues and which requires cognitive effort on the part of the participant has been shown to promote cortical reorganisation (22-24, 27); see section 1.2.2. We aimed to design a cough training protocol for stroke patients to fit as many of these criteria as possible. Passive methods of inducing cough such as electrical stimulation of the abdominal muscles, which have been used in spinal injury patients (122) or mechanical cough assist devices, which have been used in neuromuscular weakness patients (124) are thought less likely to promote cortical reorganisation than active movements, so were not used in this study.
We were unsure of the optimum effort level and duration of cough training but thought that short periods would be preferable for use in patients. Fraser and colleagues found a significant increase in cortical excitability after 5 minutes of pharyngeal electrical stimulation (at 75% of maximum tolerated intensity) and a maximum effect with 10 minutes of stimulation. Small but significant effects on cortical excitability were seen immediately for both five (MEP amplitude increased by about 20%) and ten minute (MEP amplitude increased by about 25%) stimulation periods. The largest effects were seen one hour after the ten-minute stimulation periods, with MEPs increased by 75% from baseline. Twenty minutes of stimulation had no significant effect on the MEP. The studies of Fraser, Hamdy and colleagues are described further in the Introduction, section 1.2.3.

From the Fraser study findings, it would seem appropriate to train patients for ten minutes in the first instance. However when ten minutes of high effort coughing was tried some healthy subjects complained of light-headedness, headaches and sore throats. As five minutes stimulation had shown small but significant effects for Fraser et al we designed a five minute training protocol in the first instance (18). We decided to use a metronome set at 10 beats per minute for an auditory cue to cough, as auditory cues were previously used with some success in stroke in the BATRAC and other stroke rehabilitation studies (27). For details of the BATRAC study see the Introduction, Section 1.2.2.

We decided upon a training session where patients performed twenty-five coughs over a five-minute period. Each training cough had to have a flow greater than 75% of the individual’s own maximum cough flow. This protocol of five sets of five repetitions is similar to an expiratory muscle training programme used in Parkinson’s patients (127). See section 1.8.4.1 for further details of that study. TMS assessments would be used before and after the training to look for any change in corticomotor excitability.

8.2 METHODS
8.2.1 Participants and Exclusion Criteria
Patients were recruited from the Acute Stroke Unit of King’s College Hospital and written consent obtained, in accordance with local ethics committee approval LREC 02-120. Further details about ethical approval for the study, consent and patient recruitment are given in section 2.1 and 2.2. Exclusion criteria are listed in
section 2.2.3. The TMS safety questionnaire was administered (Figure 51) and potential participants excluded if there was doubt about the safety of TMS.

8.2.2 Stroke Diagnosis and Assessments; Baseline Assessments
Senior medical staff specialising in stroke made clinical and radiological diagnoses of new stroke in the territory of the middle cerebral artery. The investigator made an assessment of stroke severity using the NIHSS score on the day of TMS testing. Details of stroke diagnosis and severity assessment are given in section 2.4. Height and weight were measured as described in section 2.3.1.

8.2.3 Transcranial Magnetic Stimulation and Motor Evoked Potentials
Equipment and methods for TMS and EMG recording are given in section, 2.12. MEPs were recorded from surface electrodes placed on the lateral abdominal muscles on both sides of the trunk. The electrode positions are detailed in section 2.12.6.

8.2.4 Stimulus-Response Curves
2.12.12 gives the methods for construction of SR curves. To try and decrease the length of the protocol, patients received only five stimuli for each level of stimulus intensity, rather than the ten stimuli given previously to healthy controls. If patients were very tired or intolerant of TMS the stimulus intensity was increased by 10% each time, instead of the 5% increments used for healthy controls. A subset of patients also participated in the cough training. The methods are given in 2.12.13. For every level of magnetic stimulus intensity, the five unrectified MEP responses were averaged over a time period of 150 milliseconds, starting 50 milliseconds prior to the magnetic stimulus. Averaging was performed offline using the Spike2 software; see 2.12.8 for details.

8.2.4.1 Resting Corticomotor Threshold
Resting corticomotor threshold (CMT) was found for the affected and the unaffected sides of the body for each patient. See 2.12.9 for further details. CMT was defined as the stimulus intensity required to elicit a MEP of greater than 50µV in the lateral abdominals, for at least three out of five consecutive trial stimuli. MEPs were reviewed offline, after the SR protocol had been completed. If one level of stimulus intensity produced less than three adequate MEPs and the next stimulus level up produced more than three MEPs, the average of the two levels was said to be the corticomotor threshold.
8.2.4.2 Latency and MEP Amplitude

Peak-to-peak amplitudes of the averaged MEP responses were measured for each individual and all stimulus intensities. Latency of the averaged responses was also measured, with latency defined as the time up to the first deflection from baseline following TMS. Latency was measured at 100% of MSO or the highest stimulus intensity tolerated by the patient.

8.2.4.3 Stimulus-Response Relationships: Line of Best Fit and Area Under the Curve

Peak-to-peak amplitudes of the averaged MEP responses were plotted against magnetic stimulus intensity on scatter plots. Prism software was used to display the data on the scatter plots. They were presented with the data points joined with straight lines and fitted to a straight line or a curve obeying the Boltzmann sigmoidal function. The extra sum-of-squares F test was used to compare the Boltzmann sigmoidal fit with a straight line fit for each participant’s SR data. Details of the methods are given in 2.12.12. Area under the SR curve was measured as per the method described in 2.12.12.1.

8.2.5 Cough Training Study

See 2.12.13 for details of the training study and the pre and post training cough flow and TMS assessments. PH had TMS at baseline and 20 minutes post training. FC had TMS at baseline and again at ten and twenty minutes post training. FC also had MEPs recorded from the quadriceps bilaterally as a control muscle. SR curves were constructed for each individual for each muscle at each time point. TMS outcome measures were made before and after training. These were corticomotor threshold, maximum MEP amplitude, MEP latency at 100% MSO and area under the SR curve (AUC).

8.2.6 Statistical testing

Statistical testing and curve fitting were performed using Prism software; see section 2.13. Unpaired t-tests were used to compare demographics between patients and controls (from Chapter 7). Paired tests were performed to examine within-patient differences between MEP responses and SR relationships from the affected and unaffected sides. Responses for patients were also compared to responses from the right side in the healthy controls described in Chapter 7, using unpaired t-tests. As four comparisons were being made, $P \leq 0.01$ was considered
significant. Comparisons between patients and controls for the Boltzmann parameters were not made due to insufficient patient data.

8.2.6.1 Training Study

MEP max, CMT, latency at 100% MSO and area under the SR curve were measured for each patient and each muscle, for every time point and entered into data tables. Stimulus-response plots were constructed for each muscle in each patient at each time-point. Statistical testing of results obtained before and after training was not appropriate in this feasibility study. Data for Patient 1 fitted well to a Boltzmann curve, so comparison of Boltzmann parameters pre and post training were tabulated in addition to the other outcome measures.

8.3 RESULTS

8.3.1 Stimulus-Response Relationships

Seven stroke patients were studied. Three patients did not receive stimuli across the full range of intensities due to intolerance of the high dose TMS. Patient demographics and the maximum stimulus intensity tolerated are given in Table 38. Stroke details are given in Table 39. Resting corticomotor threshold, MEP latencies and AUCs are given in Table 40.

Patients’ SR relationships are shown individually on the following pages (Figure 93 to Figure 99 inclusive) and each patient’s data should be interpreted with reference to the stroke type and severity. Four images are shown for each patient; there is a pair of images for the MEPs from the abdominal muscles of the affected side and a pair of images for the unaffected side. Data is joined point-to-point on the left hand images and the lines of best fit (straight or curve fit) are drawn on the right hand images. Details of the lines of best fit are given in Table 41, Table 42, Table 43 and Table 44.

Table 38 Stroke Patient Demographics

<table>
<thead>
<tr>
<th>Patient number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29</td>
<td>62</td>
<td>70</td>
<td>44</td>
<td>62</td>
<td>88</td>
<td>70</td>
<td>60.7</td>
<td>19.2</td>
</tr>
<tr>
<td>Sex (male or female)</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>28% female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178</td>
<td>175</td>
<td>162</td>
<td>162</td>
<td>178</td>
<td>173</td>
<td>165</td>
<td>170.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>95</td>
<td>80</td>
<td>87</td>
<td>96</td>
<td>82</td>
<td>75</td>
<td>84</td>
<td>85.6</td>
<td>7.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.0</td>
<td>26.1</td>
<td>33.4</td>
<td>36.6</td>
<td>25.9</td>
<td>25.0</td>
<td>30.9</td>
<td>29.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Handedness (right or left)</td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>14% left handed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side of weakness (right or left)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>43% right hemi</td>
<td>57% left hemi</td>
<td></td>
</tr>
</tbody>
</table>

Key for Table 38 SD=standard deviation; kg=kilograms; cm=centimetres; BMI=body mass index; cm=centimetres; m=metres; hemi=hemiparesis; R=right; L=left
Table 39 Details of Stroke Patients: Lesions and Functional Impairments

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Stroke territory</th>
<th>NIH stroke scale score</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Large left middle and anterior cerebral artery territory infarct (terminal ICA thrombus)</td>
<td>cortical</td>
<td>Dense paralysis right upper limb, mild weakness right lower limb, right facial palsy, dysarthria. Unable to walk.</td>
</tr>
<tr>
<td>2</td>
<td>Left frontoparietal infarct (MCA occlusion)</td>
<td>cortical</td>
<td>Right face and arm weakness with dysphasia and dysarthria. Walking unaided.</td>
</tr>
<tr>
<td>3</td>
<td>Small left posterotemporal infarct</td>
<td>cortical</td>
<td>Walking unaided</td>
</tr>
<tr>
<td>4</td>
<td>Right internal capsule posterior limb infarct</td>
<td>subcortical</td>
<td>Walking unaided</td>
</tr>
<tr>
<td>5</td>
<td>Right posterotemporal and right thalamus</td>
<td>cortical</td>
<td>Mild left hemiparesis (face/arm/leg) with partial neglect.</td>
</tr>
<tr>
<td>6</td>
<td>Right internal capsule infarct</td>
<td>subcortical</td>
<td>Mild left hemiparesis (face/arm/leg) with dysarthria. Walking with stick.</td>
</tr>
<tr>
<td>7</td>
<td>Right parietal lobe infarct</td>
<td>cortical</td>
<td>Walking with stick, left hand weakness and mild left foot drop</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Key for Table 39: MCA=middle cerebral artery; ICA=internal carotid artery

Table 40 MEP Amplitudes, Areas under the Curve, Corticomotor Thresholds and Latencies for Individual Stroke Patients

Latencies given are for stimulations at 100% of maximum stimulator output (MSO) or the maximum tolerated by the patient

<table>
<thead>
<tr>
<th>Patient number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold to TMS unaffected side (% MSO)</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>60</td>
<td>55</td>
<td>60</td>
<td>-</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Threshold to TMS affected side (% MSO)</td>
<td>65</td>
<td>60</td>
<td>85</td>
<td>70</td>
<td>70</td>
<td>75</td>
<td>65</td>
<td>70</td>
<td>8</td>
</tr>
<tr>
<td>Latency unaffected (ms)</td>
<td>19.1</td>
<td>19.4</td>
<td>13.7</td>
<td>22.4</td>
<td>19.0</td>
<td>25.4</td>
<td>-</td>
<td>19.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Latency affected (ms)</td>
<td>20.4</td>
<td>20.2</td>
<td>15.1</td>
<td>25.4</td>
<td>21.0</td>
<td>23.8</td>
<td>22.1</td>
<td>21.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Max MEP amplitude unaffected (mV)</td>
<td>1.79</td>
<td>0.28</td>
<td>0.24</td>
<td>0.79</td>
<td>0.09</td>
<td>0.21</td>
<td>-</td>
<td>1.03</td>
<td>1.35</td>
</tr>
<tr>
<td>Max MEP amplitude affected (mV)</td>
<td>0.63</td>
<td>0.87</td>
<td>0.25</td>
<td>0.46</td>
<td>0.08</td>
<td>0.16</td>
<td>0.53</td>
<td>0.43</td>
<td>0.28</td>
</tr>
<tr>
<td>Area under SR curve unaffected (%MSO.mV)</td>
<td>52.88</td>
<td>3.48</td>
<td>7.88</td>
<td>23.01</td>
<td>1.41</td>
<td>4.52</td>
<td>-</td>
<td>15.53</td>
<td>18.14</td>
</tr>
<tr>
<td>Area under SR curve affected (%MSO.mV)</td>
<td>14.38</td>
<td>8.42</td>
<td>3.70</td>
<td>10.88</td>
<td>1.01</td>
<td>3.51</td>
<td>10.4</td>
<td>7.41</td>
<td>4.48</td>
</tr>
</tbody>
</table>

Key for Table 40: ms=milliseconds; MSO=maximum stimulator output; MEP=motor evoked potential; AUC=area under the SR curve, joined point to point with straight lines. PH had no interpretable signals from the unaffected side.
Figure 93 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 1 with a Dense Right Hemiparesis

Patient 1 did not tolerate stimulus intensities above 80%.

Key for Figure 93 to Figure 99 mV=millivolts; MEP=motor evoked potential
Figure 94 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 2 with a Mild Right Hemiparesis

Patient did not tolerate stimulus intensities above 65%.

Figure 95 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 3 with Mild Right Hemiparesis
Figure 96 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 4 with a Dense Left Hemiparesis

Figure 97 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 5 with a Mild Left Hemiparesis
Figure 98 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 6 Mild Left Hemiparesis

Figure 99 Stimulus-Response Curves for the Lateral Abdominal Muscles for Patient 7 Left Hemiparesis

Key for Figure 99: PH had no interpretable responses from the unaffected side
Table 41 Individual Parameters for Unaffected Side where SR relationship best fits a Boltzmann curve

The stimulus is TMS at the cranial vertex and the response is MEP from the lateral abdominal muscles (obliques) on the unaffected side.

<table>
<thead>
<tr>
<th>Boltzmann parameters</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.33 to 0.32</td>
<td>-0.06 to 0.04</td>
<td>-0.01 to 0.01</td>
</tr>
<tr>
<td>Top</td>
<td>1.73</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.52 to 1.94</td>
<td>0.18 to 0.26</td>
<td>0.07 to 0.11</td>
</tr>
<tr>
<td>Slope in mV per unit change in % MSO</td>
<td>0.95</td>
<td>1.61</td>
<td>5.34</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.60 to 7.59</td>
<td>0.60 to 10.87</td>
<td>1.89 to 8.79</td>
</tr>
<tr>
<td>S_50</td>
<td>49.1</td>
<td>63.6</td>
<td>63.7</td>
</tr>
<tr>
<td>95% CI</td>
<td>45.9 to 52.3</td>
<td>57.4 to 69.7</td>
<td>59.5 to 67.9</td>
</tr>
</tbody>
</table>

Goodness of fit

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>2</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>R square</td>
<td>0.89</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Sum of squares</td>
<td>0.36</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sy.x</td>
<td>0.30</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>No of points analysed</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Key for Table 41
CI=confidence intervals; S_50= stimulus intensity required to obtain a response 50% of the maximum; Sy.x = standard deviation of the residuals; R square=coefficient of determination

Table 42 Individual Parameters for Unaffected Side where SR relationship best fits a straight line

The stimulus is TMS at the cranial vertex and the response is MEP from the lateral abdominal muscles (obliques) on the unaffected side.

<table>
<thead>
<tr>
<th>Straight line fit parameters</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>x intercept when y=0 (%MSO)</td>
<td>38.1</td>
<td>47.9</td>
<td>38.5</td>
</tr>
<tr>
<td>95% confidence interval for x intercept</td>
<td>27.2 to 43.3</td>
<td>34.1 to 55.7</td>
<td>12.2 to 51.4</td>
</tr>
<tr>
<td>Slope in microvolts per unit change in % MSO</td>
<td>36.8</td>
<td>8.5</td>
<td>25.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>22.7 to 51.0</td>
<td>5.7 to 11.4</td>
<td>15.0 to 36.9</td>
</tr>
</tbody>
</table>

Goodness of fit

| R square              | 0.96 | 0.94 | 0.85 |
| Sy.x                  | 0.09 | 0.07 | 0.03 |
| No of points analysed | 5    | 8    | 8    |

Key for Table 42
Sy.x = standard deviation of the residuals; R square=coefficient of determination; MSO=maximum stimulator output; CI=confidence intervals; %=percentage
Comparisons of Responses from Affected and Unaffected Sides for Patients and Comparisons with Healthy Controls

8.3.2 Demography

The control group, with a mean (sd) age of 25 (6) years were significantly younger than the patients who had a mean age of 61 (19) years, P for difference <0.01. There was no significant difference between groups for height and proportion of females. Raw data can be seen in Table 38 (patients) and Table 33 (controls). Age
is not known to affect corticomotor threshold, MEP amplitude or latency, see section 1.5.8.

**8.3.2.2 Threshold, Latency, MEP Amplitude and AUC**

See Table 45 for a summary of the data and Table 46 for comparisons and their statistical significance. There was a significant difference (P<0.01) in CMT between the affected and unaffected side in stroke patients; the small differences in CMT between controls and the affected side in patients did not reach significance at the required level of 0.01. None of the other comparisons were statistically significant.

**Table 45 Corticomotor Threshold, Latency and MEP amplitude to TMS for Stroke Group and Control Group**

<table>
<thead>
<tr>
<th></th>
<th>Stroke patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unaffected side</td>
<td>Affected side</td>
</tr>
<tr>
<td>Number of values</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Corticomotor threshold (% MSO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>54</td>
<td>70</td>
</tr>
<tr>
<td>95% CI for the mean</td>
<td>46-63</td>
<td>62-78</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>19.9</td>
<td>21.1</td>
</tr>
<tr>
<td>95% CI for the mean</td>
<td>15.7 to 23.9</td>
<td>18.1 to 24.1</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>3.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Max MEP amplitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.68</td>
<td>0.43</td>
</tr>
<tr>
<td>95% CI for the mean</td>
<td>0.00 to 1.35</td>
<td>0.17 to 0.68</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.64</td>
<td>0.28</td>
</tr>
<tr>
<td>Area under the SR Curve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17.22</td>
<td>7.62</td>
</tr>
<tr>
<td>95% CI for the Mean</td>
<td>0.00 to 37.23</td>
<td>3.10 to 12.14</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>19.06</td>
<td>4.89</td>
</tr>
</tbody>
</table>

Key for Table 45 CI=confidence intervals; std=standard; ms=milliseconds; MEP=motor evoked potential; MSO=maximum stimulator output; TMS=transcranial magnetic stimulation.
8.3.3 Training Study

Two patients participated; they are patients 6 and 7 from Table 38 and Table 39. This was a feasibility study; the results for the two patients will be described separately. It took up to one hour to explain the study to the patients, consent them and set up the equipment ready for the cough flow and surface EMG measurements to be made. The set-up time was not uncomfortable for either patient but both became tired before the patient recordings had started. Patient PH was poorly tolerant of the TMS as she was very tired and she slept in the chair in the twenty-minute period between training and the repeat TMS protocol.

8.3.3.1 Patient Number 7 PH With Left Hemiparesis

PH had a cough flow rate of 350 l/min at baseline. A baseline TMS SR protocol was performed which the patient found somewhat uncomfortable. As she was very keen to complete the study, PH completed the training protocol and one further TMS SR protocol, twenty minutes after the training. It was not possible to record any interpretable biological signals from the abdominals on the right, unaffected side. The cough training did not have an effect on either corticomotor excitability as measured by TMS or cough flow rate. The SR relationships from the left, affected side were very similar pre and post-training and MEP max and
corticomo
tor threshold were almost identical. These data are shown in Table 48 and illustrated in Figure 100. F tests showed the Boltzmann fit to be better than the straight line fit for both pre and post-training TMS datasets for the affected side. The cough flow measured 20 minutes after the end of testing was 340 l/min.

**Table 47 Patient 7: Effect of Training on Affected (Left) Sided Abdominal Responses and Cough Flow**

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Pre-training value</th>
<th>20 minutes post-training value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEP max</td>
<td>mV</td>
<td>0.53</td>
</tr>
<tr>
<td>CMT</td>
<td>% MSO</td>
<td>65</td>
</tr>
<tr>
<td>Latency to TMS</td>
<td>ms</td>
<td>22.1</td>
</tr>
<tr>
<td>Area under SR curve</td>
<td>mv.%MSO</td>
<td>10.40</td>
</tr>
<tr>
<td>Cough flow</td>
<td>l.min⁻¹</td>
<td>350</td>
</tr>
</tbody>
</table>

Key for Table 47
% MSO=percentage of maximum stimulator output; CI=confidence intervals; TMS=transcranial magnetic stimulation; MEP max=motor evoked potential maximum peak to peak amplitude; mV=millivolts; ms=milliseconds; l=litres; min=minute

**Figure 100 Patient PH: Effect of Training on Affected (Left) Sided Abdominal Responses**

An F test showed the Boltzmann curve fit was better than a straight line fit for both datasets; P<0.01 for both. Characteristics of both fits are given in Table 48.
Table 48 Patient 7 Effect of Training on Affected (Left) Side

<table>
<thead>
<tr>
<th>Boltzmann curve parameters</th>
<th>Pre training</th>
<th>20 mins post training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.05 to 0.05</td>
<td>-0.03 to 0.01</td>
</tr>
<tr>
<td>Top</td>
<td>0.77</td>
<td>0.93</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.07 to 1.46</td>
<td>0.33 to 1.51</td>
</tr>
<tr>
<td>Slope in mV per unit change in % MSO</td>
<td>11.0</td>
<td>14.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.0 to 21.0</td>
<td>9.0 to 20.6</td>
</tr>
<tr>
<td>S_{50}</td>
<td>90.7</td>
<td>97.2</td>
</tr>
<tr>
<td>95% CI</td>
<td>69.3 to 112.0</td>
<td>79.4 to 115.0</td>
</tr>
</tbody>
</table>

Goodness of fit

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>R square</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>Sum of squares</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>S_{y,x}</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>No of points analysed</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Key for Table 48
MEP=motor evoked potential; mV=millivolts; MSO=maximum stimulator output

8.3.3.2 Patient Number 6 FC, Male With Mild Left Hemiparesis

FC’s baseline cough flow rate was 280 l/min. He managed to complete TMS at baseline, cough training and repeat TMS and ten and twenty minutes post-training. A recording taken whilst FC performed the cough training protocol is shown in Figure 101. Although the training protocol was designed to work the abdominal muscles it can be seen in the recording that this participant is activating the quadriceps during coughs. Figure 103 shows the averaged MEPs, with the largest peak to peak MEP amplitude, recorded before training (left image) and after (right image), demonstrating slightly increased peak-to-peak amplitude post training. Table 50 gives a summary of the SR characteristics found before and after training in the abdominals (test muscle) and the quadriceps (control muscle). Figure 103 shows the SR relationships for each test muscle. The largest MEP amplitudes for all muscles were seen at 10 minutes after the end of training (Figure 102 and Figure 103). The cough flow measured after training was 278 l/min i.e. no change from baseline.
Figure 101 Cough Training Protocol Patient 6

Recordings taken during the five minute cough training protocol from patient FC, showing five sets of five coughs with 30 seconds rest between each set. Note activity in the quadriceps as well as abdominals during the cough training exercise.

Key for Figure 101
V=volts; l/min=litres per minute; L=left; R=right; abdo=lateral abdominals; quads=quadriceps.
Flow is calibrated with expiratory flow leading to a positive deflection on the y axis. EMG traces marked in volts have been amplified by 1000.

Figure 102 Averaged Maximum MEPs From Lateral Abdominals Pre-and 10 Minutes Post-Training, Patient Number 6

The black lines represent the mean of 5 responses and the red lines the standard error of the mean.

Key for Figure 102 Right abdominal signals are shown in the top trace of each panel and left abdominal signals on the bottom. MEP=motor evoked potential; x-axis is marked in seconds, y axis in volts (amplified x 1000).
Figure 103 Pre and Post Training Stimulus-Response Relationships for Stroke Patient 6 with Very Mild Left Hemiparesis

TMS was performed immediately before training and again at ten minutes and twenty minutes after the start of cough training. MEPs recorded after TMS at the cranial vertex. The largest responses in all muscles were recorded 10 minutes after the end of cough training. Responses from the abdominals and quadriceps taken from the affected side 10 minutes post training are shifted to the left. Both of these effects may indicate increased corticomotor excitability post training.

Key for Figure 103 MEP=motor evoked potential; mV=millivolts; %=percentage
8.4 DISCUSSION

8.4.1 Principal findings

Stimulus-response relationships for MEPs from the lateral abdominal muscles were demonstrated for seven stroke patients. MEP amplitude increased with increasing stimulus intensity but only in a few cases did the data points fit a Boltzmann curve; neither straight line nor a Boltzmann curve fits could reasonably be used to describe the SR relationships for all patients. For assessment of corticomotor excitability in stroke patients, area under the stimulus response curve may be the best overall measure as it is affected by the threshold, slope of the “curve” and the maximum MEP amplitude (see 1.5.4.5). Patients’ responses varied considerably and should be taken in the context of the type and severity of their stroke.

Cough training and assessment by TMS SR protocols is feasible. For future studies, a 10-minute cough training session would be recommended. If patients are able to tolerate it, TMS assessment of corticomotor excitability should be made twice before training (to assess the effect of time alone on the MEP responses).

<table>
<thead>
<tr>
<th>Contracted Muscles</th>
<th>Abdominals</th>
<th>Quadriceps (Control Muscle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>units</td>
<td>Side of body</td>
<td>Pre</td>
</tr>
<tr>
<td>MEP max mV</td>
<td>Unaffected (right)</td>
<td>0.2087</td>
</tr>
<tr>
<td>MEP max % of baseline MEP</td>
<td>Unaffected (right)</td>
<td>100</td>
</tr>
<tr>
<td>CMT % MSO</td>
<td>Unaffected (right)</td>
<td>60</td>
</tr>
<tr>
<td>Latency at 100% stimulus intensity</td>
<td>Unaffected (right)</td>
<td>25.1</td>
</tr>
<tr>
<td>Area under the SR curve mV</td>
<td>Unaffected (right)</td>
<td>4.52</td>
</tr>
<tr>
<td>Area under the SR curve % of baseline AUC</td>
<td>Unaffected (right)</td>
<td>100</td>
</tr>
<tr>
<td>Cough flow l.min⁻¹</td>
<td>280</td>
<td>278</td>
</tr>
</tbody>
</table>

Key for Table 49: MEP=motor evoked potential; %=percentage; MSO=maximum stimulator output; mV=millivolts; CMT=corticomotor threshold; AUC=area under the curve; ms=milliseconds; SR=stimulus response
and then at ten-minute intervals post training for up to one hour unless it can be shown that the responses are stable over time at rest.

8.4.2 Critique of the Study

As with many studies of stroke patients, using TMS and involving training, the patient numbers are small. It was only possible to include patients who could follow commands and sit in a supportive chair. The most severely affected patients who are perhaps most likely to benefit from training are the ones who are least able to tolerate a prolonged assessment, training session and subsequent evaluation of the effect and its possible mechanisms. The patients’ strokes were all within the middle cerebral artery territory but they varied from small lacunar infarcts to large cortical infarcts secondary to carotid occlusion. Abnormalities of the MEP could be due to a problem at any level from the corticomotor projection to the muscle of interest. We do not give results of peripheral nerve stimulation but stimulation over the T10 nerve roots and measurement of the compound muscle action potential (CMAP) amplitude and latency would have allowed us to assess peripheral nerve conduction and muscle electrical activity. Some authors also normalise their MEP amplitudes by expressing them as a proportion of the CMAP amplitude. Peripheral nerve studies would have further lengthened the protocol, which was already very tiring for patients. We attempted to record CMAPs from the lateral abdominals in healthy controls but the magnetic stimulus artefact obscured the short-latency (6-7ms) CMAP in about half of cases. However, by excluding patients with no known neuromuscular diseases except stroke it was unlikely any had peripheral neuropathies or muscle disease; any abnormalities of MEPs were likely to originate in the cortex or the corticospinal projection.

We describe the muscles we recorded from as the lateral abdominals; because we made surface recordings it is difficult to say from which muscle the potentials arose. Plassman’s work with surface and needle electrodes recording simultaneously from the lateral abdomen tells us it is likely that the potentials we recorded arose from the external obliques, with a contribution from the internal obliques (Table 1).
8.4.2.1 Comparison between affected and unaffected sides and between patients and controls

TMS was applied at the skull vertex in our study and responses were recorded from both unaffected and affected sides, except in one case where responses were not obtained on the unaffected side due to technical difficulties. The two previous abdominal muscle studies in stroke have applied TMS over each hemisphere in turn and recorded responses from the contralateral and ipsilateral sides (57, 58). In both studies the authors failed to record any responses from the muscles on either side in most (57, 58) or all cases (57, 58), after TMS was applied to the affected hemisphere. Harraf’s patients were on average more severely impaired than ours, with a mean (sd) NIHSS score of 9 (7) on the day of the TMS study compared with our groups mean (sd) score of 5 (2).

It is difficult to tell whether the responses recorded from the muscles on the affected side in our patients were contralateral to TMS acting on the damaged hemisphere or a result of the vertex TMS acting on the unaffected motor cortex and signals travelling in ipsilateral pathways. The MEPs we recorded are likely to be made up of a combination of contralateral and ipsilateral potentials. Ipsilateral responses have been shown for abdominal muscles in healthy controls (Table 1) and stroke patients (Table 2) and disinhibition of the unaffected hemisphere after cortical stroke can unmask latent ipsilateral corticomotor projections.

We believe that if we are making the same measurements before and after an intervention TMS at the vertex will give an idea of change in corticomotor excitability whether this occurs in the contralateral or ipsilateral corticofugal projection, or both. Use of this position for stimulation shortens the protocol by more than half and enables inclusion of patients who could not tolerate the length of time required for finding the point of optimum excitation on each side before the SR protocol begins.

With this in mind it may not be completely correct to compare responses from the affected and unaffected sides. However, paired tests were used and in line with other studies where the hemispheres were stimulated separately, the corticomotor threshold was significantly higher for the affected side than the unaffected (P<0.01).

As described for other muscles the corticomotor threshold tended to be higher for patients (either side) than controls; the MEP amplitude and the area under the
curve for the affected side for patients tended to be lower than for controls. The small numbers mean results should be interpreted with caution.

Although the exact source of our MEPs is unclear we can compare them to those found by others. For our patient group, the average MEP latencies to abdominals on the unaffected side, mean 19.9 (3.9) ms and the affected side, mean 21.1 (3.3) ms were slightly shorter than those found by Harraf for the contralateral (mean 20.6ms, sd 2.5) and ipsilateral external oblique (mean 22.6ms, sd 2.6) after TMS over the unaffected hemisphere of 15 patients, see Table 2. Our patients’ CMT for the unaffected side 54% (sd 8) was lower than her patients’ 74% (20). The shorter latency and the lower threshold could be due to our patient groups lower stroke severity.

8.4.2.2 SR relationships
The Boltzmann sigmoidal curve did not describe the stimulus response relationship well for most patients, in contrast to the controls described in Chapter 7. Patients often did not have enough data points for any curve to be a better fit than a straight line. Also, the Boltzmann curve is characterised by a plateau and the patients’ response amplitudes usually failed to reach a plateau. As discussed in the introduction (section 1.5.4.5) the plateau on the healthy human’s SR curve is probably due to a balance of excitatory and inhibitory components of the corticospinal volley (54). Stroke can reduce cortico-cortical inhibition arising from the affected hemisphere and acting on the unaffected hemisphere; this imbalance of excitation and inhibition may mean the responses on the unaffected side never reach a plateau and instead continue to increase. Increased CMT of the affected hemisphere also meant that it was not usually possible to achieve a maximum response (if such a maximum existed) before the magnetic stimulator had reached its maximum output level. Boltzmann or straight-line fit parameters could not be compared between sides or between patients and controls due to small numbers and a lack of a uniform best fit for patients.

8.4.2.3 Cough Training
The aim was to find a cough training that could feasibly be used in patients. Only after this is done can the training be tried in a study with a suitable sample size to test the training’s effectiveness. That said, the five-minute training regime made no difference to the cough flow and MEP amplitudes for the two patients studied here. Using the same protocol but continuing for ten minutes may be more effective.
Fraser et al saw changes (approx 10-30% increases) in MEP amplitude after just 5 minutes of pharyngeal electrical stimulation but they found the maximum effect was with ten minutes’ stimulation where they saw 20-80% increases in MEP amplitude (18).

8.4.2.4 TMS assessments of cough training
TMS may be used before a training protocol is rolled out to discover if it has an effect on corticomotor excitability. If a training regime is shown to increase excitability in the short term it may be more likely to have an effect on function in the long term, even if the improvement in function lags behind the neurophysiological changes. The quadriceps is unsuitable for use as a control muscle. We used the quadriceps as a control muscle for our second patient in the training study but as illustrated in Figure 101, the patient activates his quads during each cough. A hand muscle such as the adductor pollicis brevis may be a more appropriate control but EMG recordings should be made during training to see whether it becomes activated.

Two baseline assessments should be made, repeating the TMS SR protocol and cough flow after twenty minutes of sitting quietly, to look at what happens to TMS evoked responses and cough flow without any intervention. The patient would then perform the training and TMS could be repeated at 5, 10, 20, 30 minutes and one hour after the training. Unfortunately this would require the patient to be in the lab, awake and sitting up for at least two hours and many would not or could not do it. It would be possible for the TMS to be performed on patients in bed but many tend to fall asleep in that position so they would need to be woken for each set of TMS. In addition, if the patients were lying in bed, the small background activation of abdominal muscles (which helps us achieve signals by facilitation when the participants are sitting upright) is unlikely to be present.

8.4.3 Conclusion
TMS stimulus-response relationships were described for the abdominal muscles in stroke patients. SR curves provide information about corticomotor excitability and the area under the curve measures could be useful to assess corticomotor excitability before and after a training intervention. For further training studies ten minutes of cough training is recommended and a hand muscle should be tried as a control.
Chapter 9  Summary of Findings, Related and Future Work

This thesis describes abnormalities of respiratory physiology including reduced non-volitional respiratory muscle strength, impaired voluntary and reflex cough and reduced static lung volumes in acute hemispheric stroke patients compared with healthy controls. Testing respiratory and stroke physiology in stroke patients is very challenging but more than 50 patients, some with severe impairments, took part in this series of studies. We believe the work is novel and important, adding to scant knowledge of this field.

We suggest patients should be sat upright, out of bed, whenever possible to help their diaphragms move downwards with gravity and increase their functional residual capacity which in turn would increase their cough volumes and flows. We also suggest that patients be prompted to practice deep breaths and coughing as part of their physiotherapy regimes in order to promote cortical plasticity and cough recovery. These interventions have great potential to reduce respiratory complications of stroke.

Chapters 3 and 4, a summary of which was published in the European Respiratory Journal in 2010 (196), describe volitional weakness of expiratory muscles and impairment of both voluntary and reflex cough in patients with cortical strokes. Chapter 4 describes the first detailed study of reflex cough physiology in stroke patients. This is important because if patients aspirate, it is the reflex cough that will remove the inhaled material and reduce the chance of pneumonia. We made the important observation that the driving force for cough, the cough gastric pressure, was much reduced for voluntary but not reflex cough. Patients’ reflex cough flows were reduced however; since the driving force was relatively normal in patients we suggest that the reduced cough flow could be due to poor synchronisation of the chest and abdominal muscles with the muscles of the upper airways. We believe our data gives evidence for cortical modulation of the brainstem-originating cough reflex in healthy people, and impairment of this after cortical stroke. Imaging and measurement of the upper airways during cough in stroke patients would be of great interest.

The studies in this PhD were developed from earlier work on cough conducted in the laboratory at King’s and published most recently by Harraf and Lasserson (58, 152). As a result of these latest experiments we went on to work with colleagues
from Manchester University on voluntary cough physiology in healthy controls. This related cough effort to cough EMG, pressures and sound. The results have been presented orally and in abstract form and await submission to a physiology journal (204). The results may be used for improvements to the VitaloJAK cough monitor (207). The cough physiology work continues at King’s with studies in chronic cough and interstitial lung disease patients, after data we acquired for stroke patients was used as part of a local research grant application.

A study of respiratory muscle training in stroke patients, funded by the National Institute for Health Research, commenced in 2011 (208). Outcome measures will include assessment of aspiration, cough flow and respiratory function as well as chest infections.

Chapter 5 is a study of stroke patients’ static lung volumes, cough inspired volumes and cough flows. The patients described in this chapter had very mild stroke related impairments; they were less severely impaired by stroke than the patients described in Chapter 3 and Chapter 4, yet their cough flow rates and inspired volumes were still significantly reduced compared with controls. It was demonstrated that FRC accounted for nearly half of the variability in cough inspired volume and cough inspired volume accounted for more than half the variability in cough flow. The work from Chapter 5 has been presented in oral and abstract form and awaits submission to a stroke journal (209).

These findings of Chapter 5 suggest a couple of very simple clinical interventions may have a positive effect: sitting patients upright, preferably out of bed and getting patients to take deep breaths. Both of these are likely to improve FRC and thus cough flow and perhaps reduce chest infections. If successful, there is potential for a massive impact on stroke related morbidity from chest infections. Therapist applied treatments such as CPAP could be used in patients unable to sit up or follow commands. These interventions would be suitable for a clinical trial and a grant application under Research for Patient Benefit or similar schemes, like the ongoing expiratory muscle training trial described above.

Chapter 6 and Chapter 7 both describe investigations into the best way to use TMS to study the corticomotor projection to the abdominal muscles. It was decided that a stimulus-response protocol was best and this was then used in subsequent experiments. In Chapter 8 TMS stimulus-response relationships were described for the abdominal muscles in stroke patients; this has not been described
previously in any published studies. SR curves provide information about
corticomotor excitability and the area under the curve (AUC) measure could be
useful to assess corticomotor excitability before and after a training intervention.
For further training studies, ten minutes of cough training are recommended and a
hand muscle should be tried as a control.
The face of stroke has changed dramatically since this programme of work
commenced at the end of 2006. There has been national prioritisation of improving
stroke care in the UK, set out in the National Stroke Strategy 2007 (205) and the
re-organisation of stroke services for hyperacute care, to increase rates of
thrombolysis treatment. Seven hyperacute stroke units opened in London in
February 2010. Initial reports suggest that this has significantly increased the
number of patients receiving acute stroke care and those being thrombolysed,
from less than 1% to about 20% (206). However, the impact of these changes on
mortality or disability is unknown and remains to be quantified. It is important to
remember that 80% of stroke patients do not receive thrombolysis and clinical
opinion is that thrombolysis benefits only one in seven patients receiving the
treatment. Hence, the burden of disability remains significant in stroke despite the
progress made and the work described in this thesis remains highly relevant to
patients recovering from stroke.
Chapter 10 Resulting Publications and Awards

10.1 ORIGINAL RESEARCH PUBLICATIONS

10.2 POSTER PRESENTATIONS WITH ABSTRACT PUBLICATIONS

10.3 POSTER PRESENTATIONS
Medical Research Society Clinical Scientists in Training Meeting 2009
Reflex and Voluntary Coughs are Both Impaired after Acute Hemiplegic Stroke

10.4 SPOKEN PRESENTATIONS
Respiratory muscle activation during voluntary coughing in healthy volunteers

10.5 AWARDS
Medical Research Council Clinical Research Training Fellowship, (Funded by the Royal College of Physicians) 2008-2011
British Lung Foundation Research Fellowship Award for the American Thoracic Society Conference, Toronto, May 2008
British Lung Foundation Research Fellowship Award for European Respiratory Society Conference, Vienna, September 2009
King’s College London School of Medicine Travel Bursary 2008 and 2009
References


