Evidence for limbic system activation during CO_2-stimulated breathing in man

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1. The role of supra-brainstem structures in the ventilatory response to inhaled CO_2 is unknown. The present study uses positron emission tomography (PET), with infusion of $H_2{}^{15}O$, to measure changes in relative regional cerebral blood flow (rCBF) in order to identify sites of increased neuronal activation during CO_2-stimulated breathing (CO_2-SB) in awake man.

2. Five male volunteers were scanned during CO_2-SB (mean ± S.E.M.; end-tidal P_{CO_2}, 50·3 ± 1·7 mmHg; respiratory frequency, 16·4 ± 2·7 min^{-1}; tidal volume, 1·8 ± 0·2 l). As control, scans were performed during 'passive' isocapnic (elevated fraction of inspired CO_2) positive pressure ventilation (end-tidal P_{CO_2}, 38·4 ± 1·0 mmHg; respiratory frequency, 15·5 ± 2·2 min^{-1}; tidal volume, 1·6 ± 0·2 l). With CO_2-SB, all subjects reported dyspnoea.

3. The anatomical locations of the increases in relative rCBF (CO_2-SB *versus* control) were obtained using magnetic resonance imaging.

4. Group analysis identified neuronal activation within the upper brainstem, midbrain and hypothalamus, thalamus, hippocampus and parahippocampus, fusiform gyrus, cingulate area, insula, frontal cortex, temporo-occipital cortex and parietal cortex. No neuronal activation was seen within the primary motor cortex (at sites previously shown to be associated with volitional breathing).

5. These results suggest neuronal activation within the limbic system; this activation may be important in the sensory and/or motor respiratory responses to hypercapnia in awake man.

The increase in ventilation associated with inhaling carbon dioxide is thought to be mediated by the increased P_{CO_2} stimulating the peripheral and central chemoreceptors; this leads to increased efferent output from the brainstem respiratory centres to respiratory muscles. However, the ventilatory response to CO_2 in awake man is highly variable. In addition, breathing CO_2 is associated with sensations of dyspnoea, particularly of an 'urge to breathe'. Such observations suggest that, in man, supra-brainstem structures might modulate the ventilatory response to CO_2. This hypothesis is supported by observations in animals that the respiratory response to CO_2 is influenced by hypothalamic structures (Waldrop, 1991) and that respiratory-related activity is present during CO_2-stimulated breathing in neurones located in the thalamus (Chen, Eldridge & Wagner, 1992). It is also known that stimulation of forebrain structures can have marked influences on breathing (Hugelin, 1986).

We have previously used positron emission tomography (PET) to measure changes in regional cerebral blood flow (as an index of regional change in neuronal synaptic activity) that were associated with volitional breathing in man. Foci of neuronal activation were found bilaterally in the superolateral primary motor cortex and associated motor control areas (Colebatch *et al.* 1991; Ramsay *et al.* 1993*a*). Our most recent study suggests that these areas of the brain are also involved in exercise-induced hyperpnoea (Fink *et al.* 1993). The present study, using PET, was designed to test the hypothesis that supra-brainstem structures, in particular the primary motor cortex (Murphy, Mier, Adams & Guz, 1990), would also be involved in the ventilatory response to inhaled CO_2 in awake man. This work has already been presented in preliminary form (Corfield *et al.* 1994).

Table 1. Values of inspiratory time (T_I), expiratory time (T_E), respiratory rate (f_R), tidal volume (V_T), minute ventilation ($\dot{V}_I$) and end-tidal P_{CO_2} (P_{ET,CO_2}) for each subject during control (C) and CO_2-SB

	T_I (s)		T_E (s)		f_R (min^{-1})		V_T (l)		$\dot{V}_I$ (l min^{-1})		P_{ET,CO_2} (mmHg)	
Subject	C	CO_2-SB	C	CO_2-SB	C	CO_2-SB	C	CO_2-SB	C	CO_2-SB	C	CO_2-SB
D.M.	2·73 (0·01)	2·48 (0·06)	2·66 (0·01)	2·86 (0·10)	11·2 (0·0)	11·3 (0·3)	2·13 (0·02)	2·26 (0·05)	23·7 (0·2)	25·4 (0·4)	34·4 (0·5)	44·0 (0·4)
G.M.	2·96 (0·15)	2·68 (0·13)	2·98 (0·14)	3·02 (0·17)	10·2 (0·5)	10·7 (0·5)	1·95 (0·04)	2·21 (0·10)	19·9 (0·9)	23·4 (0·9)	39·4 (0·6)	50·1 (0·5)
M.P.	1·35 (0·03)	1·19 (0·04)	1·38 (0·02)	1·19 (0·04)	22·0 (0·4)	25·6 (0·7)	1·13 (0·03)	1·30 (0·01)	24·8 (0·8)	33·0 (1·0)	39·7 (0·8)	52·5 (0·4)
N.D.	1·56 (0·12)	1·51 (0·03)	1·83 (0·11)	2·09 (0·07)	17·72 (0·04)	16·7 (0·33)	1·67 (0·04)	1·92 (0·03)	29·5 (0·7)	31·9 (0·3)	39·1 (0·4)	51·5 (0·3)
R.M.	1·22 (0·04)	1·38 (0·03)	2·35 (0·09)	1·91 (0·05)	16·90 (0·64)	18·3 (0·4)	1·22 (0·03)	1·29 (0·06)	20·7 (0·9)	23·5 (0·8)	39·2 (0·4)	53·3 (1·2)
Mean	1·96 (0·36)	1·85 (0·30)	2·24 (0·29)	2·22 (0·33)	15·5 (2·19)	16·4 (2·7)	1·62 (0·20)	1·79 (0·21)	23·7 (1·7)	27·4 (2·1)	38·4 (1·0)	50·3 (1·7)
P	0·211		0·855		0·237		0·009		0·032		0·0001	

Each value relates to the breaths during the final 2 min of the PET scan period and is the mean (S.E.M.) of all six runs. Statistically significant differences between control and CO_2-SB were noted if $P < 0·05$.

METHODS

Five healthy right-handed male subjects aged 23–49 years were studied; all had a normal ventilatory response to inhaled CO_2. Ethical approval (Hammersmith Hospital Medical Ethics Committee) and permission to administer radioactivity (Administration of Radioactive Substances Advisory Committee of the Department of Health, UK) were obtained. Subjects gave informed written consent but were unaware of the specific aims of the study.

Respiratory protocol and measurements

Each subject either breathed spontaneously or was ventilated, with the mouth closed, through a tightly fitting nasal mask whilst lying supine. Airflow was measured with an ultrasonic flow meter (Branta, UK); from this, tidal volume (V_T), inspiratory and expiratory time (T_I, T_E), respiratory frequency (f_R) and inspired minute ventilation ($\dot{V}_I$) were derived. Tidal P_{CO_2} was measured (47210A, Hewlett Packard, USA); from this, end-tidal P_{CO_2} (P_{ET,CO_2}) was derived. Pressure (P23B, Statham, USA) within the nasal mask (P_{nasal}) was used as an index of upper airway pressure. Arterial oxygen saturation (S_{a,O_2}) was monitored with a pulse oximeter (Biox 3700, Ohmeda, UK) using a finger probe. Data were recorded onto chart paper (2400, Gould SAF, France) and FM tape (R-71, Teac Corp., Japan).

CO_2-stimulated breathing (CO_2-SB). CO_2-SB was performed for periods of 6 min. Subjects breathed via a dead space (~1·5 l) attached to the breathing circuit. The fraction of inspired CO_2 (F_{I,CO_2}), and therefore P_{ET,CO_2}, could be controlled by trickling 100% CO_2 directly into the dead space. For CO_2-SB, P_{ET,CO_2} was maintained at around 50 mmHg. S_{a,O_2} was maintained above 97% by increasing F_{I,CO_2}, when necessary.

Passive ventilation (control). Passive eucapnic ventilation was performed, as control, for periods of 6 min. This was achieved by subjects relaxing respiratory muscles during intermittent positive pressure ventilation (Servo 900B, Siemens-Elma, UK). The adequacy of the relaxation was judged from the recordings of P_{nasal} (Colebatch *et al.* 1991). V_T and f_R were similar to those produced during CO_2-SB. This level of ventilation was substantially greater than that at rest and therefore P_{ET,CO_2} was maintained around eucapnia by increasing F_{I,CO_2}.

Familiarization. Prior to the study day, subjects experienced CO_2-SB and were trained to relax their breathing during positive pressure ventilation.

Statistical analysis. Mean values of V_T, T_I, T_E, f_R, $\dot{V}_I$ and P_{ET,CO_2} (determined over the final 2 min of each scan) were compared using a repeated measures analysis of variance (BMDP 2 V). Statistically significant differences between conditions were noted when $P < 0·05$.

PET scans. PET scans were performed, in 3-D mode (Townsend *et al.* 1991), to determine relative regional cerebral blood flow (rCBF) by measuring the regional distribution of cerebral radioactivity following the intravenous infusion of radiolabelled water ($H_2{}^{15}O$) (Mazziotta, Huang, Phelps, Carson, MacDonald & Mahoney, 1985; Fox & Mintun, 1989). Scans were performed alternately during six CO_2-SB and six control runs, 2 min after the commencement of each run and at intervals of 12 min to allow sufficient decay of radioactivity.

Scanning protocol. Each subject lay supine on an adjustable table attached to the PET camera (Siemens-CTI 953B, CTI Inc., USA); the head was supported by an individually fitted head mould. During all scans, subjects had their eyes closed; the room lights were switched off.

Data acquisition. A short transmission scan was first performed using an external $^{68}Ge/^{68}Ga$ ring source generating positrons. These data were used to determine the subject's head position; if necessary, the head position was corrected. Thereafter, a second 20 min transmission scan was undertaken to provide a correction factor for the effects of radiation attenuation by the skull and soft tissues of the head.

For each measurement of relative rCBF, emission scans were performed sequentially over 4 min, with a 1 min background scan (scan A) followed immediately by a 3 min scan (scan B). The $H_2{}^{15}O$ (15 mCi) was given as a slow bolus infusion (via the right antecubital vein) for 1 min at the start of scan B. Increased

Table 2. Sensations associated with CO_2-SB and control during the supplementary study

Subject	CO_2-SB	Control
N.D.	An increased urge to breathe Starved for air Air hunger [Breathing required more work] [Changed pattern of breathing]	Not in control of your breathing Changed pattern of breathing [Dry mouth]
D.M.*	An increased urge to breathe Breathing required more effort Air hunger [Flushed and warm]	Not in control of your breathing Breathing was rapid Changed pattern of breathing [Increased salivation]
M.P.	Changed pattern of breathing Breathing required more work [An increased urge to breathe] [Flushed and warm] [Slight sweating]	Changed pattern of breathing
R.M.	Breathing was rapid Changed pattern of breathing An increased urge to breathe [Flushed and warm]	Changed pattern of breathing
G.M.	Changed pattern of breathing Breathing required more effort Breathing required more work [An increased urge to breathe]	Breathing was shallow † Not breathing all the way in † Not breathing all the way out †

The replies, listed in order of preference, are the responses when asked to select up to three appropriate phrases from a list of possible respiratory-related sensations/descriptors. The bracketed responses are additional descriptors/sensations associated with the experience that were revealed by the questioning. * Subject does not like the experience of CO_2 stimulation. † Sensations were not associated with any discomfort or urge to breath.

radioactivity was first detected 45–50 s after the start of the infusion and during the final 2 min of the scan period. The integrated counts accumulated over scan B were corrected for background activity (derived from scan A); this gave a relative measure of cerebral blood flow (Mazziotta *et al.* 1985) during a period commencing approximately 4 min after the start of the breathing run. The emission scan data were then reconstructed as thirty-one axial image planes (each 3·4 mm thick); each plane was 128 × 128 pixels, with a pixel size of 2·0 × 2·0 mm.

Magnetic resonance scans

On a later occasion, each subject underwent a magnetic resonance (MR) scan (performed on a 1 tesla system; Picker HPQ Vista, UK) to produce high resolution 3-D images of brain morphology.

Image processing and transformation

Image manipulations and calculations were performed using ANALYZE (Version 5; BRU, Mayo Foundation, Rochester, MN, USA), PROMATLAB (MathWorks Inc., Sherbon, MA, USA) and SPM (MRC Cyclotron Unit, London) software. For the group analysis, individuals' PET images were transformed into standard stereotactic space (Talairach & Tournoux, 1988) using information from the MR image data (e.g. level of line between anterior and posterior commisures, brain height).

The results presented, for the relative rCBF measurements, are based on a simple categorical (subtraction) analysis between the activation (CO_2-SB) and the control task (passive ventilation). A pixel-based analysis of covariance was performed using 'global' activity (reflecting blood flow) as the covariate to control for differences in global cerebral blood flow (gCBF) associated with the different conditions. The condition-specific (activation or control) mean values of relative rCBF and the associated error variances were then calculated for each pixel across all subjects. Comparisons of these means were made using Student's t distribution. The resulting images constitute statistical parametric maps (SPM{t}) of the areas of significant relative rCBF changes. The values of the relative rCBF changes underwent Z transformation to produce Z values (population distribution with $\bar{x} = 0$ and S.D. = 1). The pixels of the local maxima of significant relative rCBF changes were derived in terms of x, y and z stereotactic co-ordinates (defined in Table 3). The anatomical location of these co-ordinates was established using both the stereotactic atlas of Talairach & Tournoux (1988) and the group mean image of all individual MR scans after stereotactic normalization.

Supplementary study

On a separate day and following the PET scans, subjects underwent three CO_2-SB runs alternating with three control runs. After the final run of each condition, subjects described their symptoms using a structured interview and questionnaire.

RESULTS

Respiratory measurements

Table 1 shows the average values of the respiratory variables for each subject. The group mean (± S.E.M.) P_{ET,CO_2} was significantly higher during CO_2-SB than during control (50·3 ± 1·7 *versus* 38·4 ± 1·0 mmHg, respectively). During

Table 3. Anatomical locations and co-ordinates of maximally activated relative rCBF foci during CO_2-SB

	Left				Right			
Region	x	y	z	Z value	x	y	z	Z value
Upper brainstem	−8	−28	−12	4·2	—	—	—	—
Midbrain/hypothalamus	−6	−26	−8	4·2	—	—	—	—
Thalamus	−4	−8	4	5·8	8	−32	4	4·4
Hippocampus/parahippocampus	−24	−26	−12	3·8	—	—	—	—
Fusiform gyrus	−44	−44	−16	4·6	28	−38	−16	5·6
Cingulate area								
Frontal	−24	30	20	5·2	12	32	16	6·4
Intermediate	−20	6	44	5·6	10	−12	40	6·2
Posterior	−2	−44	24	5·3	14	−40	32	5·4
Insula	—	—	—	—	40	14	−4	5·1
Frontal cortex								
Area 9	−14	46	36	4·9	6	48	28	5·3
Area 8	−12	20	52	4·9	2	34	52	4·5
Temporo-occipital cortex	−24	−72	16	4·4	26	−62	20	5·2
Parietal cortex	−10	−56	52	4·8	12	−66	52	4·9
Cerebellar vermis	0	−54	−16	5·0	—	—	—	—

Co-ordinates (in standard stereotactic space; Talairach & Tournoux, 1988) refer to maximally activated foci during CO_2-SB as indicated by the highest Z value within an area of activation associated with the differences between CO_2-SB and control. x, distance (mm) to right (+) or left (−) of the mid-sagittal line; y, distance anterior (+) or posterior (−) to vertical plane through the anterior commissure; z, distance above (+) or below (−) the intercommissural (AC–PC) line.

CO_2-SB, V_T, and consequently $\dot{V}_I$, was significantly greater than during control. There were no statistically significant differences in the group mean values for T_I, T_E and f_R. Examination of the P_{nasal} trace, during control, indicated that all subjects were able to relax their respiratory muscles and that breathing was passive during this condition.

Symptoms associated with CO_2-SB

Subjects reported no undue difficulty in completing the studies. Four of the five subjects (D.M., G.M., N.D. and R.M.) spontaneously volunteered that they had felt short of breath or had noticed their breathing increase during CO_2-SB. Few further comments were reported. The sensations associated with the two conditions were addressed more fully in the supplementary study (Table 2). Descriptors associated with a changed pattern of breathing were associated with both CO_2-SB ($n = 4$) and control ($n = 5$). All subjects reported symptoms of discomfort associated with their breathing during CO_2-SB although these were not always chosen as one of the principal descriptors.

Changes in relative rCBF (CO_2-SB *versus* control)

The brain volume successfully imaged in all subjects extended from the vertex to the upper cerebellum. Figure 1 illustrates the areas of significantly increased relative rCBF ($P < 0{\cdot}05$; corrected for multiple comparisons; $Z > 3{\cdot}68$) associated with CO_2-SB in the data averaged across all individuals and all runs. Areas of significantly increased relative rCBF were found within the upper brainstem (left side), midbrain and hypothalamus (left), thalamus, hippocampus and parahippocampus (bilaterally), fusiform gyrus (bilaterally), throughout the cingulate area (bilaterally), within the insula (right side), frontal cortex (bilaterally), temporo-occipital cortex (right), the parietal cortex (bilaterally) and cerebellar vermis (left). Table 3 gives the stereotactic co-ordinates and anatomical locations of the local maxima within these areas. The localization of these activation sites was independently confirmed on the group mean MR image. No changes in relative rCBF were seen within the primary sensorimotor cortex.

DISCUSSION

The principal observations of the present study are that, during CO_2-stimulated breathing at a level sufficient to induce dyspnoea, there is no increase in relative rCBF in the primary sensorimotor cortex but there are widespread increases within the upper brainstem and midbrain and in structures that are part of, or closely associated with, the limbic system. The brain volume imaged in the study did not contain the lower portion of the brainstem including the medulla.

Use of passive ventilation as control

To determine increases in neuronal activation that are associated with CO_2 stimulation, cerebral blood flow measured during a control condition must be subtracted from that determined during CO_2 stimulation. In the present study, and in common with previous studies from our group using PET (Colebatch *et al.* 1991; Ramsay *et al.* 1993*a*; Fink *et al.* 1993), we have used passive mechanical positive pressure ventilation at an elevated level of minute ventilation that matches that produced during the

'activation' condition. The intention of using this control, rather than the alternative of spontaneous breathing at rest, is that sensory differences between the two conditions related to differences in the level of ventilation are minimized and therefore increases in relative rCBF should reflect neuronal activation associated specifically with CO_2 stimulation. Passive ventilation has further advantages over spontaneous breathing as a control, for respiratory-

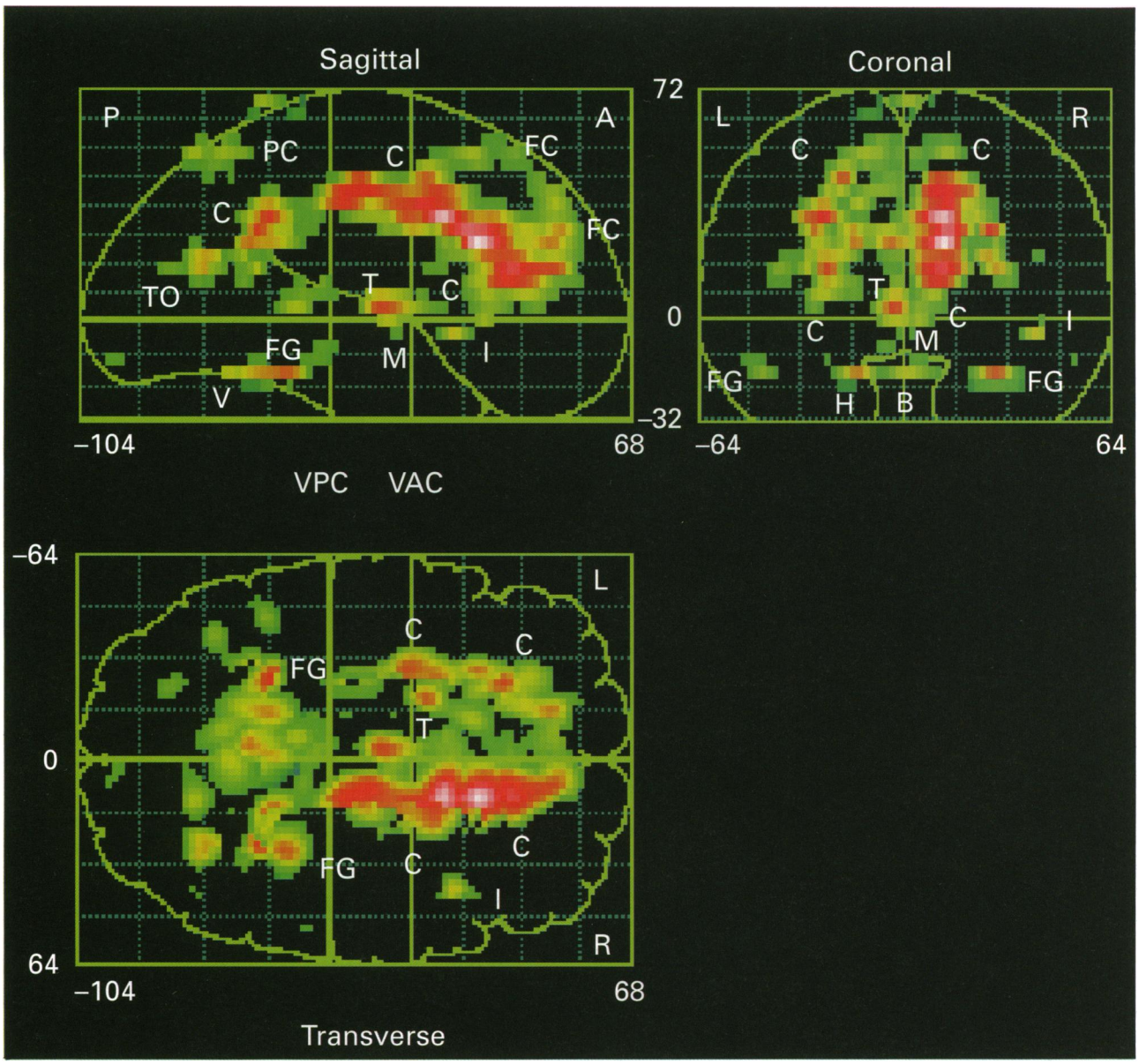

Figure 1. Group results of significant relative rCBF increases during CO_2-SB

Areas of significant relative rCBF increases during CO_2-SB, averaged from the group of 5 subjects, are shown as projections onto representations of sterotactic space (Talairach & Tournoux, 1988). The upper left (sagittal) image views the brain from the side, the upper right (coronal) image from the back and the lower (transverse) image from the top. A, anterior; P, posterior; L, left; R, right; VAC, vertical plane through the anterior commissure; VPC, vertical plane through the posterior commissure. Numbers by axes refer to co-ordinates of stereotactic space (see Table 3). Areas of activation are shown with increasing significance by an arbitrary colour scale ranging from green, through yellow and red, to white. The anatomical locations of the areas of activation are indicated by lettering placed next to the corresponding region of interest (B, upper brainstem; M, midbrain/hypothalamus; T, thalamus; H, hippocampus/parahippocampus; FG, fusiform gyrus; C, cingulate area; I, insula; FC, frontal cortex; TO, temporo-occipital cortex; PC, parietal cortex; V, cerebellar vermis).

related motor activity during mechanical ventilation is absent or minimal and fluctuations both in the level of ventilation and in P_{ET,CO_2} are prevented. The control condition is therefore more closely defined and controlled than if spontaneous breathing were used.

Afferent sensory information during CO_2-SB will, however, differ from that during control due to the use of mechanical ventilation. Inspiration arising from the contraction of respiratory muscles will produce negative pressures within the lung and airways; conversely, mechanical ventilation will produce positive pressures in the nose mask, airways and lung during inspiration. Use of a nose mask ensured that the positive pressure was localized over a small area on the face immediately around the nose, and the principal facial sensation associated with the respiratory apparatus was the pressure of the nose mask strapped onto the face; this stimulation was the same during both CO_2-SB and control. Overall, movement of the lungs and intrathoracic airways, and therefore of lung stretch receptor-mediated sensory information, will be similar with both forms of ventilation since the evidence available suggests that such receptors fire in response to lung stretch, due to changes in transpulmonary pressure, and not to absolute pressure (G. Sant'Ambrogio, personal communication). Sensory information may vary if the relative movements of the chest wall and abdomen differ with positive pressure ventilation; however, differences in the discharge of lung receptor afferents are only apparent with marked conformational changes (Davenport & Sant'Ambrogio, 1981) that did not occur in the present study. The principal differences in sensory information will therefore relate to differences in the movement of the extra-thoracic airways and, also, to changes in discharge from respiratory muscle spindles. It is not possible to quantify directly these influences in the present study but the importance of any effects on the present observations can be inferred by comparing the present work with earlier studies which have investigated the volitional control of ventilation with PET when positive pressure ventilation was also used as a control (Colebatch *et al.* 1991; Ramsay *et al.* 1993*a*). Were positive pressure ventilation to produce a systematic effect on the observations it would be expected that some similar areas of increased rCBF would be present in all three studies. The present study, with CO_2 stimulation, has no areas of increased rCBF that are common to those found with the studies of volitional ventilation. We therefore feel that the use of passive positive pressure hyperventilation is the most appropriate control that is feasible for the present study.

Non-specific effects of CO_2 on cerebral blood flow

It is generally assumed that an increase in rCBF is due to an increase in local metabolic demand that is produced by a change in neuronal activity. Our principal concern in this study is that CO_2 itself has a direct action on cerebral blood flow, due to vasodilatation, that is independent of metabolism; this action may influence our observations in two ways. Firstly, the analysis assumes that increases in rCBF are independent of gCBF; however, it is possible that increased neuronal activation may be associated with a change in the response of rCBF when gCBF is elevated. This concern was addressed in a previous study using similar methodology (Ramsay *et al.* 1993*b*); steady-state changes in P_{ET,CO_2} (24, 40 and 55 mmHg) at near-constant ventilation were used to alter gCBF (24, 37 and 67 ml min^{-1} dl^{-1}, respectively). rCBF was determined with and without visual activation (eyes open with bright lighting *versus* eyes closed with dim lighting). This stimulation was associated with increased rCBF in the visual cortex that was independent of the changes in gCBF produced by the changes in P_{ET,CO_2}. Assuming that other cortical areas behave in a similar manner to the visual cortex, the results indicate that activation-dependent increases in rCBF will occur and will be quantitatively independent of gCBF, even when gCBF is elevated by CO_2. The study of Ramsay *et al.* (1993*b*) also supports the use of the analysis of covariance (with gCBF as the covariate) to normalize for changes in gCBF (Friston, Frith, Liddle, Dolan, Lammertsma & Frackowiak, 1990).

The second concern is that changes in relative rCBF may not be related to increased neuronal activation but may reflect a differential vascular reactivity of some cerebral areas to changes in CO_2. However, the sites of increased relative rCBF were many and various, the tissues having differing cyto-architectures and multiple blood supplies. For example, the cingulate area is supplied from the anterior, middle and posterior cerebral arteries; these vessels also supply many other cortical structures. To our knowledge there is no evidence that the vascular reactivity of the observed areas to CO_2 is different to that of other closely related cerebral structures. This conjecture is supported by the observation that, in anaesthetized baboons, elevated P_{ET,CO_2} produces uniform increases in cortical blood flow (Pinard, Mazoyer, Verrey, Pappata & Crouzel, 1993); this would further suggest that the increases in relative rCBF described in the present study are specifically related to CO_2 stimulation during wakefulness.

Motor cortical activation

It is clear that breathing in man can be controlled by supra-brainstem structures that are likely to include the motor cortex. Foerster (1936) first demonstrated, during neurosurgery, that the human diaphragm could be excited by electrical stimulation of the motor cortex. More recently, the existence of short latency pathways from the motor cortex to the diaphragm has been demonstrated using both anodal electrical stimulation of the scalp (Gandevia & Rothwell, 1987) and transcranial magnetic stimulation (Maskill, Murphy, Mier, Owen & Guz, 1991). Studies using PET have demonstrated activation associated with volitional breathing in the primary motor cortex, the

supplementary motor areas and in other associated motor areas (Colebatch *et al.* 1991; Ramsay *et al.* 1993*a*).

Murphy *et al.* (1990) demonstrated that facilitation of diaphragmatic excitation produced with transcranial magnetic stimulation occurred during both voluntary and CO_2-stimulated ventilation. Assuming facilitation was a cortical phenomenon, the authors proposed that the results were consistent with a role for the motor cortex in mediating the ventilatory response to inhaled CO_2. More recently it has become clear that the facilitation associated with transcranial magnetic stimulation may occur both at the level of the motor cortex and at the level of the phrenic motor nucleus within the spinal cord (Davey, Murphy, Maskill, Guz & Ellaway, 1993). Therefore the facilitation observed by Murphy *et al.* (1990) during CO_2 stimulation cannot be attributed with certainty to the motor cortex. Nevertheless the possibility remains that the increased breathing associated with CO_2 stimulation may be mediated in some part by the motor cortex. Gozal *et al.* (1994) have used functional magnetic resonance (MR) imaging to determine brain activation during CO_2 breathing in man and reported decreased signal intensity (an index of neuronal activation) within the motor cortex, medulla, pons and cerebellum. This report, of putative activation within the motor cortex, contrasts with the observations of the present study and is not explained by differences in the stimulus intensity (i.e. P_{ET,CO_2}). In the study of Gozal *et al.* the authors state that decreases in signal intensity may be related not only to increased neuronal activation but also to increased cerebral blood flow and/or blood volume. In the present study we were able to allow for the increases in global cerebral blood flow associated with the increased P_{a,CO_2} (see above); such an allowance was not made in the study of Gozal *et al.* (1994). In addition, MR signal changes during stimulation were compared with measurements during resting breathing and therefore might reflect changes in afferent activity to the cortex that were related to the increase in ventilation. In the present study, differences in sensory stimulation were minimized by using mechanical hyperventilation as the control state. The areas of signal change within the motor cortex, observed by Gozal *et al.* (1994), cannot be directly related to the foci of increased rCBF in the motor cortex associated with volitional breathing (Colebatch *et al.* 1991; Ramsay *et al.* 1993*a*) for these foci are centred at least 14 mm from the midline and are outside the brain region imaged in the study of Gozal *et al.* (1994).

In conclusion, the present study cannot exclude a role for the motor cortex in mediating the ventilatory response to CO_2 at higher levels of stimulation or when behavioural conditions are different. It is notable, however, that breathing can be increased by CO_2 stimulation to a level almost 4 times that at rest without apparent motor cortical involvement.

Supra-brainstem and limbic system activation

The results suggest that a region of neuronal activation extends from the upper brainstem, up through the midbrain and hypothalamus, to the thalamus during CO_2 stimulation. This pattern of activation is consistent with the observation that, in the cat, the posterior hypothalamus modulates the ventilatory response to CO_2 (Waldrop, 1991). In addition, CO_2-induced respiratory-related activity in the cat is present within the midbrain (Chen, Eldridge & Wagner, 1991) and thalamus (Chen *et al.* 1992) when connections with the medulla are intact.

Activation of the limbic system (Maclean, 1992) is consistent with the changes in relative rCBF seen here within the cingulate area, parahippocampus, hippocampus, fusiform gyrus and insula. Such activation would also be consistent with associated activation occurring within the parietal and frontal cortices. Activation of the limbic system might be explained by the uncomfortable sensations associated with CO_2 breathing that were reported by all subjects in the study. This might be a primary effect, directly related to CO_2, suggesting that sensations of dyspnoea may be mediated via the limbic system. Alternatively, it may be a secondary effect, related to the general unpleasantness of the sensation. It is notable that the extent of the activation seen in the present study is much wider than that activated during somatic pain (Jones, Brown, Friston, Qi & Frackowiak, 1991).

In addition to the possible sensory aspects of limbic system (and related) activation, the observations may also reflect motor-related influences on breathing. Support for this is provided from studies in animals, where electrical stimulation of the forebrain has many respiratory effects (Hugelin, 1986), and in man, where stimulation of the anterior cingulate area modifies breathing (Devinsky & Luciano, 1993).

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