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RESEARCH NOTE

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Long-term changes of GABAergic function in the sensorimotor cortex of amputees

A combined magnetic stimulation and ^{11}C-flumazenil PET study

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Abstract Primary sensory and motor areas of the cerebral cortex contain organised maps of the body. These maps appear to reorganise after damage to the peripheral parts of the sensory or motor systems, so that the cortical representation of undamaged structures expands at the expense of the damaged parts. Several studies in animals have suggested that decreased activity of the inhibitory GABAergic neurones is responsible for driving these changes. However, whether similar mechanisms sustain the effects in the longer term in humans is unknown. The present study addressed this question by examining reorganisation of sensorimotor areas of cortex in six unilateral upper limb amputees several years after the initial injury. We measured two independent indices of GABAergic function. Volumes of distribution of $GABA_A$ receptors were determined from ^{11}C-flumazenil binding measured with positron emission tomography (PET). The strength of inhibition in the motor cortex was measured with paired-pulse transcranial magnetic stimulation. In the six amputees taken as a whole and compared with 24 normal subjects, there was a highly significant increase in ^{11}C-flumazenil binding in the upper limb region of primary sensorimotor cortex bilaterally and in medial frontal cortex of the hemisphere contralateral to the amputation. Surprisingly, however, there was no change in the time course or strength of intra-cortical inhibition in the motor cortex of the amputees compared with matched control subjects. The increased ^{11}C-flumazenil binding may reflect up-regulation of $GABA_A$ receptors to compensate for a decrease in the GABA content or activity of inhibitory neurones. Up-regulation of $GABA_A$ receptors may also indicate that long-term changes require stabilisation of cortical organisation.

Key words Motor cortex · Cortical plasticity and inhibition · $GABA_A$ receptors · Supplementary motor area/Anterior cingulate cortex · Human amputees

Introduction

Over the past 20 years, it has become increasingly evident that cortical areas such as the primary sensory, visual and motor cortices undergo considerable reorganisation following damage to their inputs, outputs or to the area itself (Levy et al. 1990; Merzenich et al. 1986; Nudo and Milliken 1996; Ramachandran et al. 1998). Likewise, subcortical areas such as thalamic relay nuclei are known to undergo considerable reorganisation (Davis et al. 1998; Jones and Pons 1998). The acquisition of motor skills and learning difficult sensory discrimination tasks also involve reorganisation of the primary motor and sensory cortex, respectively (Nudo et al. 1996; Xerri et al. 1996).

One potential mechanism involved in plastic reorganisation was proposed by Hendry and Jones (Hendry and Jones 1986; see also Jones et al. 1994). They suggested that down-regulation of GABA and $GABA_A$ receptors lead to the unmasking of latent intracortical connections. In this way, latent connections within a cortical locus deprived of afferent input would be released from inhibition and allow adjacent cortical loci to drive the de-afferented cortical locus. This might account for the perceptual "filling-in" of scotomas (Gilbert 1990) following a localised retinal lesion – i.e. small blind spots are not perceived as such, but are perceptually filled-in. GABAergic neurones have also been suggested to underlie the maintenance of the motor map (Jacobs and Donoghue 1991) in the primary motor cortex (M1). In high cervical cord-injured patients and in ampu-

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tees, magnetic stimulation of the motor cortex has revealed an expansion of the representation of the neurologically intact body parts and a lower activation threshold (Hall et al. 1990; Levy et al. 1990). We reasoned that these physiological changes might be, at least in part, the result of down-regulation of GABAergic activity. This would account for the expansion of the representation of neurologically intact body parts and the lower activation thresholds. However, whether these mechanisms sustain the effects in the longer term in humans is unknown. To test this idea, we measured regional cerebral volumes of distribution (Vd) of $GABA_A$ receptors in six upper limb amputees, several years after their injury. The Vd is directly proportional to the number of available receptors (B_{max}), providing that the receptor affinity is not changed (Richardson et al. 1996; Koepp et al. 1998). The central benzodiazepine antagonist ^{11}C-flumazenil was used as a $GABA_A$ receptor marker (Richardson et al. 1996; Koepp et al. 1998). Additionally, we obtained a physiological measure of intra-cortical inhibition (ICI) from responses to paired magnetic stimuli applied to the motor cortex (Kujirai et al. 1993). This inhibition has been shown unequivocally to be of intracortical origin (Di Lazzaro et al. 1998) and there is evidence that it depends on GABAergic neurones (Ziemann et al. 1996).

Materials and methods

Subjects and PET scanning methods

We studied six amputees who were recruited from a local rehabilitation and prosthetics clinic. The subjects were all men, ranging in age between 33 and 57 years (mean 43.8 years, SD 9.7 years). All had sustained traumatic amputation of at least one upper limb segment several years before this study. None were taking benzodiazepines or barbiturates. Three of these subjects were amputated above the elbow and three below. One of them used a cosmetic hand prosthesis only. A second one used a body-powered prosthesis at work and a myoelectric prosthesis during leisure times. Of the remaining four amputees, two used only a myoelectric prosthesis, whilst the two others used only a body-powered prosthesis. The amputees in our study had no history of neurological disorders. None complained to any significant extent about phantom limb sensations such as pain. Details on the side of the amputation, its level, prosthesis use and hand preference prior to the accident are given in Table 1 .

Twenty-four normal subjects, who had no evidence of neurological disorder and were on no medication, served as controls for the radioligand binding studies. Consumption of alcohol was not allowed for 48 h preceding the scan. Written informed consent was obtained in all cases and approval of local ethics committees and of the UK Administration of Radioactive Substances Advisory Committee (ARSAC) were obtained. Details and references on PET scanning methods, derivation of the plasma input function, production of parametric maps and statistical analysis can be found in several publications of the Hammersmith group (Richardson et al. 1996; Koepp et al. 1998). Briefly, data were acquired with an ECAT-953B PET scanner in three-dimensional (3D) mode. The derivation of the ^{11}C-flumazenil-tracer plasma input function was carried out as described previously (Richardson et al. 1996). Voxel-by-voxel parametric images of flumazenil volume of distribution were produced using the technique of spectral analysis (Cunningham and Jones 1993). Next the images were smoothed (10×10×6 mm full width at half max.) with a Gaussian kernel. Images from the left-hand-side amputees were reversed left to right so that in all cases the amputated side appears controlled by the left hemisphere. Statistical parametric maps (Friston et al. 1995) were used to characterise regionally specific effects in imaging data, global activity being included as a confounding covariate (Friston et al. 1990). A voxel-based approach allows the entire brain volume to be interrogated without making a priori anatomical hypotheses. The approach is thus data driven and objective.

Table 1 Subject characteristics (*RHS* right-hand side, *RHS* left-hand side, *AE* above elbow, *BE* below elbow, *BP* body powered, *MYO* myoelectric)

Subject	Amputated side	Level of amputation	Prosthesis	Hand use[a]
1	RHS	AE	BP	R
2	RHS	AE	MYO	R
3	LHS	BE	BP/MYO	R
4	RHS	BE	Cosmetic	R
5	RHS	AE	BP	R
6	LHS	BE	Cosmetic/MYO	R

[a]Hand use means preferred hand prior to accident

Magnetic stimulation

Cortico-cortical inhibition was investigated using the technique described by Kujirai et al. (1993). A subthreshold conditioning stimulus (90% of active threshold in the target muscle) was used to condition the response to a larger test stimulus capable of producing a response of about 0.5–1.0 mV peak-to-peak amplitude. Interstimulus intervals of 2, 3, 4, 5, 6 and 10 ms were studied. Because the level of amputation was different, we studied different target muscles in different patients: in two patients forearm muscles were studied, in three the deltoid and in one the pectoralis major. The same muscle was studied in the intact arm. We also investigated six control subjects. To make the data from this group similar to that of the amputees, we studied the forearm extensors in three subjects, whilst the deltoid was studied in the remainder.

Results

Statistical comparison of parametric images of ^{11}C-flumazenil Vds generated with statistical parametric mapping (SPM), on a voxel-by-voxel basis for the amputees and the normals, showed three distinct regions of significantly increased Vds in the amputees (Fig. 1). In the hemisphere controlling the amputated limb, a significant cluster of suprathreshold voxels with a peak *Z*-score of 4.78 (Table 2) was localised in the medial frontal cortex. A second significant cluster with a *Z*-score of 4.17 was localised in the upper limb representation of the sensorimotor cortex, spanning the central sulcus. In the opposite hemisphere, a near-symmetrical region was also found in the sensorimotor cortex with a peak *Z*-score of 4.37. However, no significant clusters were found in the supplementary motor area (SMA) or other premotor regions in this hemisphere. Additionally, taking the peak voxel value from each sensorimotor cluster (an objective measure of Vd in these empirically discovered regions), no significant difference was found between hemispheres. There were no regions of decreased ^{11}C-flumazenil binding in the amputees.

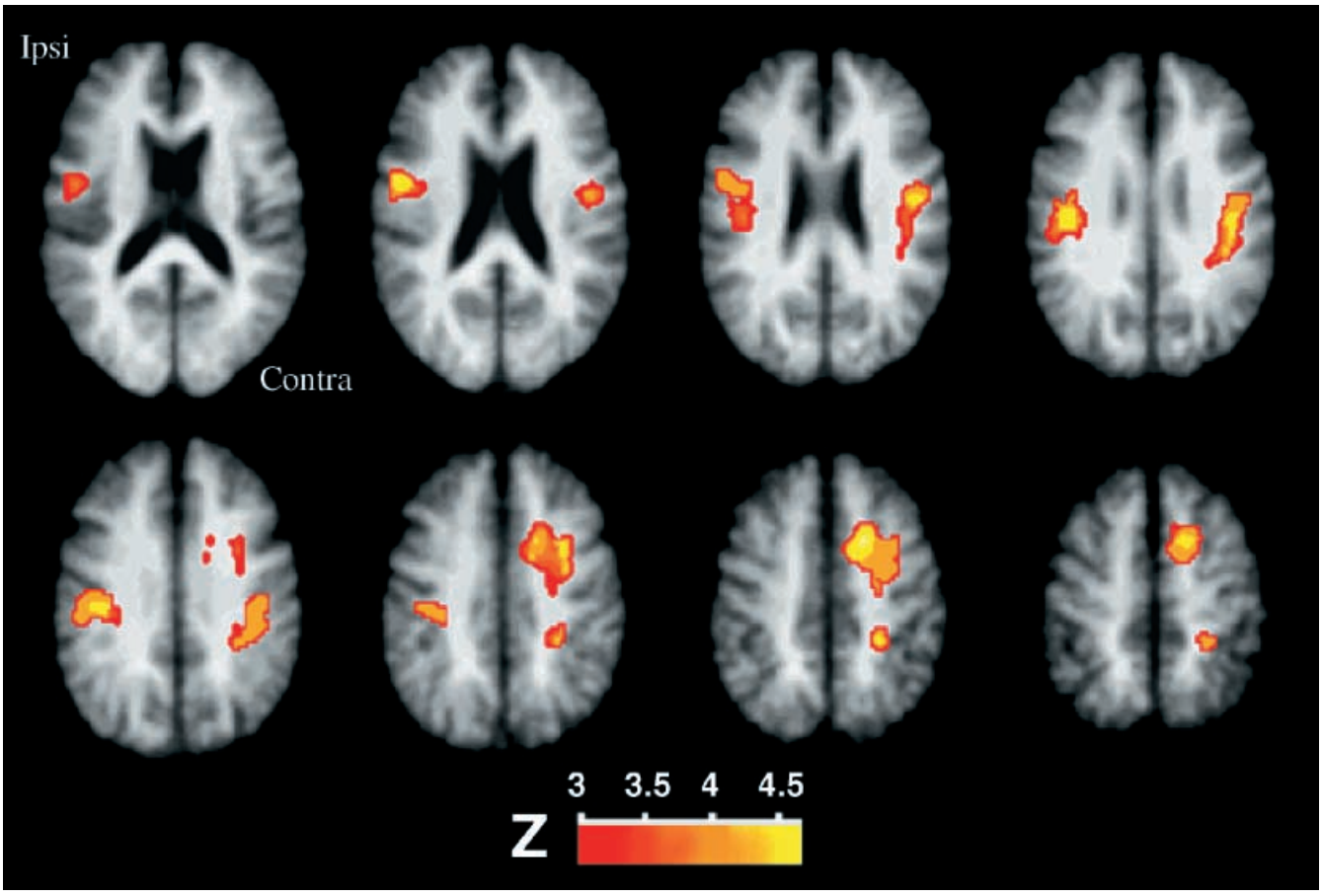

Fig. 1 Transverse sections through a mean magnetic resonance (MR) image, created from a voxel-by-voxel mean of the volumetrically normalised MR images of eight of the normal control subjects. Superimposed are the regions of significantly increased flumazenil binding found in the amputees. Three regions are seen: symmetrical increases in flumazenil binding in the sensorimotor cortices bilaterally; and a region of increased flumazenil binding encompassing the supplementary motor area and the lateral premotor area of the hemisphere contralateral to the amputation. The terms *Ipsi* and *Contra* refer to the hemisphere ipsilateral or contralateral to the amputation, respectively

Table 2 Summary of statistical parametric mapping analysis and anatomical locations showing increased binding

Region	Voxels (n)[a]	$P(n_{max}>k)$	Z-score[b]	Talairach (x, y, z)	Anatomical location
1	168	0.005	4.78	–18, 14, 36	Contralateral medial frontal
			3.83	–28, 6, 36	Contralateral medial frontal
			3.55	–12, 16, 44	Contralateral medial frontal
2	155	0.011	4.37	36, –18, 28	Ipsilateral sensorimotor
			3.86	48, 4, 20	Ipsilateral sensorimotor
			3.77	46, –22, 28	Ipsilateral sensorimotor
3	141	0.015	4.17	–42, –4, 20	Contralateral sensorimotor
			3.97	–32, –32, 28	Contralateral sensorimotor
			3.73	–36, –22, 28	Contralateral sensorimotor

[a] Region size
[b] Uncorrected

The level of intracortical inhibition (Fig. 2) was not statistically different between the cerebral hemispheres of the amputees, concordant with the ^{11}C-flumazenil PET findings (mean level and standard error of inhibition contralateral to the intact arm, 78±8%; contralateral to the amputated arm, 64±5%; paired *t*-test, $P>0.05$). However, there was also no difference in the level of intracortical inhibition measured in the motor cortices of the amputees compared with that measured in six matched control subjects (mean 65±10%; unpaired *t*-test between groups, $P>0.05$). At long conditioning-testing (C-T) intervals (circa 10 ms), the conditioning stimulus produces a facilitation of the test MEP, referred to as intracortical facilitation (ICF). This variable may be a measure of cortical excitatory interneuron excitability (Ziemann et al. 1996). On this measure as well, no differences between the amputees and the normal subjects were found. Additionally, we found no difference in the

Table 3 Magnetic stimulation thresholds, expressed as a percentage (±SD) of the maximum current discharged through the stimulating coil

Normals		Amputees			
		Normal side		Amputated side	
Relaxed (%)	Active (%)	Relaxed (%)	Active (%)	Relaxed (%)	Active (%)
52±11.3	35±9.4	60±21.7	40±14.7	52±17.7	40±15.5

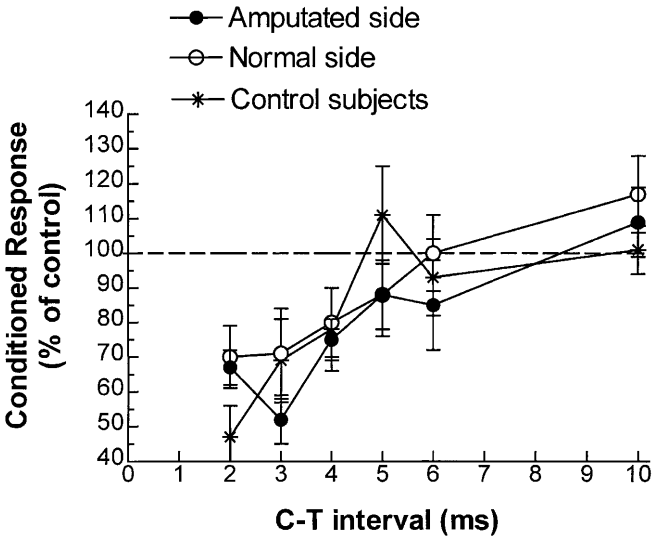

Fig. 2 Summary of the inhibition of a test motor-evoked potential (MEP) by a preceding sub-threshold conditioning stimulus to the motor cortex. Note the strong inhibition at short (2–3 ms) conditioning-testing (*C-T*) intervals. At longer intervals (7–10 ms), the test response is facilitated. Each point is the mean (±1 SE) obtained from six amputees and six matched normal subjects. The *dashed horizontal line* is the reference control (unconditioned) response

activation thresholds between hemispheres in the amputees, either at rest or during small tonic contractions (Table 3). More importantly, we found no difference in the activation thresholds between the amputees and the normal subjects in either condition.

Discussion

The increased ^{11}C-flumazenil Vds may be due to increased affinity of the $GABA_A$ receptors or to an increased number of receptors. No evidence for increased receptor affinity has been found in previous studies, including those done in vitro (Richardson et al. 1996; Koepp et al. 1998). We suggest, therefore, that the increased ^{11}C-flumazenil binding is probably due to an upregulation of $GABA_A$ receptor availability, possibly in response to decreased levels of GABA. Thus, reorganisation of the sensorimotor cortex may initially require decreases in the GABA levels in neurones (Hendry and Jones 1986). However, in order to maintain stability of the cortical networks in the long term, we suggest that a compensatory increase in postsynaptic $GABA_A$receptors may be required. For example, in adult monkeys, large-scale sprouting of long-range intracortical connections is evident in S1 several years after forelimb trauma (Florence et al. 1998). It is clear that such sprouting upsets the balance between intracortical excitation and inhibition. It is possible, therefore, that the increased number of $GABA_A$ receptors reported in the present study is a natural counterpart of this increased collateral sprouting. This also suggests an explanation for why ICI was no different in the amputees compared with the normal subjects; inhibition remains balanced with excitation.

The lack of difference of ICI between hemispheres in our upper limb amputees is consistent with the recent study of Chen et al. (1998) on lower limb amputees. However, they reported that there was less ICI in the hemisphere controlling the amputated limb compared with normal subjects. The reason for our failure to find this is not clear. Methodological differences such as their use of a round, non-focal coil, which is known to activate a larger cortical territory, may be relevant. Interactions between widespread areas of a more fractionated motor cortical map in normal subjects are difficult to predict (Capaday et al. 1999). Genuine differences in the mechanisms involved in reorganisation of leg versus arm area of the motor cortex may also be involved.

There are two noteworthy findings concerning the anatomical sites at which neurochemical changes were found. First, the changes were confined to the upper limb region of the sensorimotor cortex; but they were bilateral, involving the hemisphere controlling the amputated side as well as the one controlling the intact limb. The focus of experimental attention thus far has been on the hemisphere contralateral to the damaged limb – or the trained limb in studies of learning mechanisms. The present findings strongly suggest that adaptive reorganisations also occur in the opposite hemisphere, consistent with results obtained in the rat barrel field cortex (Micheva and Beaulieu 1995). This is easily understandable in the case of amputees, since use of the intact limb is likely to have been in part adapted to compensate for the incurred deficit. By this very fact, such an adaptation involves new movement coordination patterns between the forelimbs. The increased ^{11}C-flumazenil binding in the medial frontal cortex contralateral to the amputated limb is thus particularly interesting. According to the Talairach coordinates (Table 2) this region corresponds to the anterior cingulate cortex. However, given the size of the region, the spatial smoothing of the images and the uncertainties of the atlas, this region may include the SMA. This structure has been identified as a higher level motor cortical area involved in bimanual coordination, associated postural responses and movement sequencing (Roland et al. 1980; Hugon et al. 1982; Brinkman 1984; Goerres et al. 1998). Likewise the

anterior cingulate cortex is a higher-level motor area whose activity has been associated with task difficulty.

$GABA_A$ receptors are involved in experience-dependent plasticity during development of the visual system (Hensch et al. 1998). Specifically, intrinsic GABAergic cortical circuits appear to detect an imbalance between inputs from the two eyes during the critical period (Hubel and Wiesel 1970). Plastic changes in the adult cortex may perhaps involve reactivation of these mechanisms to allow for reorganisation of the circuits. Such reorganisations, at least in the sensory cortex, can be maladaptive (Flor et al. 1995; Ramachandran et al. 1998). In the motor cortex there is hope that such reorganisations are, on the contrary, potentially beneficial (Nudo et al. 1996). Indeed, the rules of reorganisation may be different in M1 versus S1 (Schieber and Deuel 1997). The detailed understanding of the mechanisms of plasticity in the motor cortex and their potential exploitation for the rehabilitation of patients is thus of great clinical relevance. Here we have discovered one potential path to follow in this quest; defining the functional role that up-regulation of $GABA_A$ receptors has in reshaping sensorimotor cortical circuits.

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