function. Application of this measure can assist clinicians such as sports physicians, orthopaedic surgeons, and physiotherapists with clinical examination of injuries associated with hip muscle function, exercise prescription, and the monitoring of strength changes associated with intervention. Furthermore, this protocol offers a reliable means of measuring strength deficits and therefore injury risk as well as a reliable means of measuring performance at a strength-based level in sports where hip muscle function is important.

Future Directions

This study has established a reliable strength testing protocol for the assessment of strength of all six hip muscle groups. In contrast to previous methods, the protocol offers positions, which aim to maximise subject stability to allow the tester to counteract both the magnitude and direction of force produced by the hip musculature.

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Conflict of interest

None declared.

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In vivo anatomical and functional identification of V5/MT using high-resolution MRI: A technique for relating structure and function in human cerebral cortex

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Previous in vivo neuroimaging studies have clearly demonstrated the functional specialisation of the human cerebral cortex. However, precise anatomical localisation of functionally defined cortical areas is an ongoing challenge due to the poor spatial resolution of functional imaging techniques and significant inter-individual differences in the complex morphological structure of the human cortex. This study used high-resolution magnetic resonance imaging (MRI) to identify V5/MT in three subjects based on its distinctive magnetic resonance (MR)-visible myeloarchitectonic structure. Consistent with previous studies, V5/MT was localised to the junction of the ascending limb of the inferior temporal sulcus and the lateral occipital sulcus. This anatomically defined location of V5/MT was shown to correspond with its functionally defined location, identified using functional MRI in one subject. Structural MR images with high spatial resolution were acquired in this study by combining increased MR field strength, a multi-channel phased-array head coil for image acquisition and signal averaging across a series of T1-weighted images. This study confirmed that MR contrast can be used to resolve intracortical lamination known to be present on a histological level, enabling cortical substructure to be visualised in vivo. It provided proof of concept in a single human subject; therefore, further validation of this novel technique for identification of V5/MT and other functionally defined cortical areas is required. Application of this methodology in its own right, or integrated with other MR-based neuroanatomical mapping techniques, will facilitate structure-function correlation throughout the neocortex in living human subjects.

Functional specialisation of the human cerebral cortex has been demonstrated in vivo using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). [1] Current research is aimed at precise anatomical localisation of functionally defined areas. However, accurate localisation of active regions is an ongoing challenge given the poor spatial resolution of functional imaging and inter-individual variability in the complex morphological structure of the cerebral cortex. [2,3] Consequently, location of cortical areas must be examined within an individual to obtain precise structure-function information.

Various techniques have been developed to indirectly correlate structure and function in the cerebral cortex. Post-mortem human brain analysis, following functional imaging, would be ideal for precise anatomical localisation of functional regions within an individual; however, this is not readily available. Consequently, novel techniques were required to enable structure-function correlation in living humans.

Early research relied on presumed homology between human and non-human primate brains. Unfortunately, comparative variations in brain size, complexity, orientation and potential rearrangement of cortical areas between species makes comparison of functional anatomy difficult. [4]

Brain atlases developed using traditional histological techniques to provide standardised coordinates for neuroanatomical landmarks have also been widely used. However, significant inter-individual variation in brain topography limits their usefulness in precisely identifying functional regions. For example, Talairach and Tournoux’s [5] atlas is based on one brain’s structure, over which Brodmann’s [6] cytoarchitectonic map was projected. Given that functionally defined areas can vary in location by centimetres, such an atlas can provide only gross localisation. Additionally, Brodmann’s two-dimensional map contained no data about the intrasulcal surface, two-thirds of the cortical surface, so we can only estimate these borders. [7,8]

Humans who have had a stroke, tumour, or traumatic brain injury also provide localisation information. Correlation of lesion location and subsequent neurological deficits provides information about the damaged region’s role. However, this technique’s usefulness is limited because (1) lesions are often extensive, so accurately locating the area responsible for the missing function is difficult and (2) rarely is only a single function lost, because lesions tend to incorporate areas performing a range of roles. [9]

Recent studies have revealed a successful new method of achieving precise anatomical localisation of functionally identified areas. Co-registration of structural MR with functional images from the same individual enables functionally defined regions to be mapped onto the specific morphological structure of each subject. [10-13] This approach to structure-function correlation relies on distinct anatomical features, in particular MR-visible cortical myeloarchitecture, e.g. the densely myelinated stria of Gennari, which demarcates the primary visual cortex. [14,15] These techniques were used here to identify the visual motion area, V5/MT.
V5/MT is readily identifiable histologically because of its characteristic myelination. Clarke and Miklossy [16] first identified putative V5/MT in post-mortem brains at the occipito-temporal junction featuring distinctive myelination. These MR-visible myelin bands in layers I, IV and V (the latter two, the external and internal bands of Baillarger) and radial fibres crossing layers VI to IV help anatomically localise putative V5/MT at the junction of the ascending limb of the inferior temporal sulcus (ALITS) and lateral occipital sulcus (LOS) in the parieto-temporo-occipital cortex. [11,13] This is largely consistent with functional results, showing that this functionally defined area also has anatomical identifiers.

V5/MT has been studied in non-human and human primates. Its location and role in non-human primates is similar, although not identical, to humans. [17,18] as expected given the limitations of such studies, discussed above. Research in brain-damaged humans also supports V5/MT’s role in visual motion detection [19-22]; however, lack of imaging or post-mortem analysis has prevented accurate lesion localisation. Even using transcranial magnetic stimulation to mimic deficits experimentally [23,24] is limited by the extensive area such lesions encompass, likely responsible for a range of functions. Thus, structure-function correlation based on MR imaging in healthy humans represents significant progress in the field.

Recent studies have produced a robust, non-invasive method of identifying functionally defined cortical areas in living humans. [e.g. 11] However, acquiring MR images with sufficient spatial resolution to precisely characterise underlying microarchitecture is an ongoing challenge. Our approach focused on improving resolution by applying advanced technology and analysis now available.

Precise anatomical localisation of functionally defined cortical areas relies on correlation of functional and structural images. Visualisation of cortical lamination requires a minimum MR resolution of 200-300μm, since the thickest myelin band is 250μm thick. [9] Standard T1-weighted images are generally acquired at 1.5-1.5mm resolution – it would take several hours to acquire a single T1-weighted image of 200-300μm with equivalent signal intensity and signal-to-noise ratio (SNR). It is clearly unfeasible to expect subjects to remain stationary within the scanner for this time.

Several ways of increasing spatial resolution of T1-weighted images without relying on long scan times were used here. Firstly, the scanner’s magnetic field strength was increased from 1.5 to 3 Tesla (T), nearly doubling available signal, producing a corresponding SNR improvement, [25] thus enhancing resolution. Secondly, a multi-channel phased-array head coil was used for image acquisition, increasing SNR using radiofrequency coils and combining multiple coils with individual receiver channels into an array covering the same volume as a larger coil with slight sensitive volume overlap. This produced a signal of equivalent amplitude but greatly reduced noise. [26] This improvement in SNR is particularly evident in surface regions, [27] such as V5/MT. Thirdly, averaging signal across a series of T1-weighted images significantly reduced noise, improving SNR and enhancing visibility of fine architectonic detail, [27] a technique validated by Walters et al. [11] Additionally, voxel oversampling during averaging due to jitter from small inter-scan head movements increased signal and reduced partial volume error of single scans, improving neuroanatomical detail. [28]

The current study thus applied a well-established methodology of non-invasive in vivo structural identification of functionally defined cortical areas developed by Walters et al. [11] to the precise anatomical localisation of V5/MT. We aimed to (1) validate this technique and (2) use technological advances to enhance MR images and to improve microarchitecture detection. It was hypothesised that this would produce improved spatial resolution of structural MR images, enhancing visualisation of cortical lamination within V5/MT.

Methods

Experiment 1: Identification of cortical microarchitecture using high-resolution MRI

Subject recruitment

Ethical approval was obtained. Three healthy male subjects (mean age 52) participated with informed consent.

Structural MRI

Twelve to sixteen high-resolution T1-weighted part brain images and three to four whole brain images were acquired over several sessions on a Siemens Trio 3T scanner using a 32-channel phased-array head coil (Siemens AG, Germany). Part brain parameters: three-dimensional magnetisation prepared rapid gradient echo (3D MP-RAGE) sequence: slices = 144; thickness = 0.75mm; field of view (FOV) = 220mm; in-plane resolution = 0.5x0.5mm;; echo time (TE) = 3.41ms; repeat time (TR) = 1800ms; inversion time (TI) = 900ms; flip angle (FA) = 99; number of excitations (NEX) = 1. Whole brain parameters: 3D MP-RAGE sequence: slices = 256; thickness = 0.60 mm; FOV = 265 mm; in-plane resolution = 0.6x0.6mm; TI = 2.81ms; TR = 1900ms; TI = 900ms; FA = 9º; NEX = 1. Raw images were transferred via a DICOM client program (Digital Jacket, Hewlett-Packard, CA), composed into contiguous volumes, and saved in Analyze (Radiological) format (Biomedical Imaging Resource, Mayo Foundation, MN).

Data analysis

Images were analysed using tools from Oxford Centre for fMRI of the Brain (FMRIB) Software library [29,30] and MRicro. [31] T1-weighted images were cropped at rostral spinal cord using MRicro and automatically segmented to remove non-brain tissue using the FMRIB Brain Extraction Tool (BET). [32] Each image was resampled at half the acquired voxel dimensions, producing volumes with voxel dimensions of 0.25x0.38x0.25mm³ for part and 0.30x0.30x0.30mm³ for whole brain images.

One part brain with minimal motion artefact was made the template for each scanning session. All images obtained in that session were registered to the template using a rigid body model with six degrees of freedom with the FMRIB Linear Image Registration Tool (FLIRT). [33] Template images for each session were then registered to the template for session one. Transformation matrices were concatenated and applied to each image. Transformed images were averaged using fslmaths, [29] producing a mean high-resolution image. A single whole brain T1-weighted image was acquired in each scanning session. Each of these images was registered to that acquired in session one. Transformation matrices were applied to each and transformed images averaged using fslmaths, [29] producing a mean high-resolution image. SNR was calculated prior to and following image co-registration, with regional intensity measured using ImageJ version 1.45. [34]

V5/MT’s site was estimated based on its postulated location at the ALITS and LOS intersection. Slices through this region were identified and two-dimensional cortical lamination analysis conducted. Intensity line profiles were manually generated using ImageJ version 1.45. [34] The number, intensity and relative location of each stationary point or point of inflection between cortical surface and grey-white matter boundary were calculated. These measurements were used to generate a cortical lamination map and enabled comparison of lamination between subjects.

Experiment 2: In vivo structural identification of V5/MT using high-resolution MRI

Subject recruitment

Subject 2 was previously involved in Walters et al. [11]

fMRI

fMRI data for subject 2 was obtained from Walters et al. [11] Images were acquired on a 1.5T scanner (Signa Echospeed, General Electric). Subjects observed a moving checkerboard stimulus. [13] Further details available in Walters et al. [11]
Data analysis

Functional analysis was carried out by Walters et al. [11] using FLIRT [33] and SPM99. [35,36] High-resolution T1-weighted anatomical image obtained from subject 2 was aligned with the average greyscale-normalised surface coil T1-weighted image acquired by Walters et al. [11] The functional activation map was overlaid and used to identify functionally defined V5/MT for comparison with the location of anatomically defined putative V5/MT from Experiment 1.

Results

Experiment 1: Identification of cortical microarchitecture using high-resolution MRI

Figure 1 shows three T1-weighted MR slices from subject 2. Figure 1(i) shows the raw T1-weighted MR image, prior to de-skulling. The second panel (Fig 1(ii)) is from a single T1-weighted MR image; the third panel (Figure 1(iii)) shows the effect of co-registering multiple T1-weighted MR images within and across scanning sessions. The

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Figure 1. The effect of de-skulling then signal averaging across multiple T1-weighted MR images for subject 2. (i) A coronally-oriented slice from a single T1-weighted image prior to de-skulling. (ii) The same coronally-oriented slice from a single T1-weighted image after de-skulling using BET. (iii) An equivalent coronally-oriented slice from subject 2’s average T1-weighted part brain image, derived by co-registering multiple T1-weighted MR images using a linear algorithm using FLIRT.

Figure 2. In vivo structural MR results from subjects 1 (left hemisphere), 2 (left hemisphere) and 3 (left hemisphere). The left column shows coronally-oriented slices through putative anatomically defined V5/MT in subjects 1 (i), 2 (iv) and 3 (vii). The region of interest has been highlighted (white boxes). The middle column shows an enlarged view of the highlighted area in (i), (iv) and (vii) for subjects 1 (ii), 2 (v) and 3 (viii) with a red line (AB) through the one of the banks of the sulcus, indicating the site of cortical lamination analysis. The right column shows intensity line profiles along the red line AB of (ii), (v) and (viii), showing two intensity maxima for subjects 1 (iii), 2 (vi) and 3 (ix), with an additional point of inflection at 90% cortical depth for subject 2. Cortical depth is normalised to 0-100% from the outer cortical boundary to the grey/white matter junction.
significant increase in spatial resolution produced by averaging is clearly evident. Quantitatively, this reflects a 34% improvement in SNR due to co-registration.

Slices through putative anatomically defined V5/MT in the co-registered high-resolution structural T1-weighted MR images for all subjects are shown in Figure 2 (left). Visual examination of the areas of interest (Figure 2, middle) showed two horizontally-oriented bands within the cortical ribbon. Intensity line profile analysis through putative V5/MT at this point (AB) is also shown in Figure 2 (right). This enabled quantification of the location of these bands. The first was close to the cortical surface, at 30% grey matter depth, while the second was at 65% cortical thickness. The intensity line profile for subject 2 (Figure 2 (vii)) also suggested a third band near the grey-white matter junction (90% depth), consistently identified as either a local maximum or point of inflection.

Intensity line profiles of putative V5/MT are clearly different to the surrounding cortex. The opposite sulcal bank is characterised by a single wide peak at 45% cortical thickness (Figure 3).

**Discussion**

This study employed a well-established methodology of non-invasive *in vivo* identification of functionally defined cortical areas to determine V5/MT’s precise location. Efficacy of the technique pioneered by Watson et al. [13] and extended to fMRI by Walters et al. [11] was confirmed. High-resolution structural MR images were successfully acquired and intensity line profiles drawn through putative V5/MT. Coregistration of functional data from Walters et al. [11] with new high-resolution structural data confirmed the putative anatomical location of V5/MT at the junction of ALITS and LOS in subject 2, consistent with previous studies [10,11,13].

Intensity line profile analysis has been used previously [11,37] to quantify cortical lamination. Current results are consistent with previous findings, demonstrating light-coloured bands at 30% and 65% cortical depth. Subject 2’s data suggest a third band near the grey-white matter junction. The first two likely correspond to the heavily myelinated internal and external bands of Baillarger identified in putative V5/MT in post-mortem brains. [11] The origin of the third band may correspond with radial fibres traversing lower cortical layers, also described in post-mortem specimens. These results thus confirm V5/MT’s characteristic T1-weighted MR appearance. Intensity line profiles distinguish putative V5/MT from the surrounding cortex. Profiles of the opposite sulcal bank are characterised by a single, wide peak at 45% depth (Figure 3), consistent with lamination described by Walters et al. [11]

There is a strong correlation between putative V5/MT’s spatial...
location and functional results for subject 2. This confirms that the characteristic intensity line profiles from this region are distinctive anatomical identifiers for human V5/MT, verifying Walters et al. [11] Quantitative comparison of the intensity of line profile maxima through V5/MT here and in Walters et al. [11] was not possible due to different techniques used to derive cortical lamination maps. Qualitatively, however, the peaks of local maxima (Figure 2) are less well defined than those previously obtained. This reflects reduced SNR with the present paradigm.

On a whole brain level, high-resolution T1-weighted MR images acquired in this study clearly have superior spatial resolution compared to those in Walters et al. [11] However, this overall improvement in resolution is at the expense of reduced SNR at the site of interest, i.e. putative V5/MT. This is reflected qualitatively as less well-defined maxima on intensity line profiles. In comparison, images obtained previously have high resolution at V5/MT, but lower resolution across the remaining cortex. This represents a significant benefit of using surface coils placed directly over the cortex compared to the current paradigm. The potential for concurrent use of these techniques warrants exploration.

This novel approach to improving MR spatial resolution combined technological advances with innovative image analysis. MR scanning hardware is improving rapidly with improved access to high magnetic field strength scanners and multi-channel phased-array head coils. The technique of signal averaging across images from successive sessions is also relatively recent. We demonstrated that these new tools can be successfully applied to produce T1-weighted MR images with high SNR, enabling detection of cortical lamination.

The application of this new approach to in vivo structure-function correlation in the human cortex requires further validation. This study provides proof of concept in a single subject. High-resolution structural MR images obtained for two other subjects clearly demonstrate the efficacy of this approach in enabling visualisation of cortical microarchitecture; unfortunately, functional identification of V5/MT in these subjects was not possible. Future extension of this study will help resolve this issue.

Imaging techniques used here significantly reduced analysis complexity. Twin surface coil arrangements used by Walters et al. [11] with narrow fields of view resulted in complicated signal attenuation requiring correction prior to registration. This processing step was not required here. Furthermore, use of a multi-channel phased-array head coil also enabled examination of subcortical microarchitecture since high SNR is not restricted to cortical surface. Acquisition of images with high SNR over a larger field of view represents significant progress.

As suggested by Walters et al. [11], this technique can be broadly applied to identification of cortical architecture in living humans. Its usefulness has not yet been fully explored with studies restricted to V1 and V5/ MT [11,12,14]. Given the strong contribution of myeloarchitecture to MR signal, further studies investigating non-visual cortex should focus on Flechsig's [37] fields of increased myelination to maximise initial success.

Development of imaging and analysis techniques enabling visualisation of cortical lamination opens up new research areas. For example, if functionally active areas are well-characterised microanatomically in vivo using high-resolution MR, major input and output cortical layers can be identified. This would require new task paradigms with multiple conditions activating a functional area in different ways, thus involving distinct pathways. [39] Additionally, anatomical localisation of functionally defined areas could guide medical therapy, like that achieved with deep brain stimulation in Parkinson's disease. [40] Further research in these areas is required.

Conclusions
This study confirmed that MR contrast can resolve intracortical lamination present histologically, enabling visualisation of cortical substructure in vivo. It employed improved MR hardware and analysis to validate Walters et al. [11], including V5/MT’s characteristic MR profile, and identified further microarchitectonic detail. Further optimisation of techniques to improve lamination detection is required to maximise results. Application of this methodology alone, or integrated with other MR-based mapping, will facilitate structure-function correlation throughout the neocortex in living humans.

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Conflict of interest
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