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Visualizing the Entire Cortical Myelination Pattern in Marmosets with Magnetic Resonance Imaging

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Abstract

Myeloarchitecture, the pattern of myelin density across the cerebral cortex, has long been visualized in histological sections to identify distinct anatomical areas of the cortex. In humans, two-dimensional (2D) magnetic resonance imaging (MRI) has been used to visualize myeloarchitecture in select areas of the cortex, such as the stripe of Gennari in the primary visual cortex and Heschl's gyrus in the primary auditory cortex. Here, we investigated the use of MRI contrast based on longitudinal relaxation time (T_1) to visualize myeloarchitecture *in vivo* over the entire cortex of the common marmoset (*Callithrix jacchus*), a small non-human primate that is becoming increasingly important in neuroscience and neurobiology research. Using quantitative T_1 mapping, we found that T_1 at 7 Tesla in a cortical region with a high myelin content was 15% shorter than T_1 in a region with a low myelin content. To maximize this T_1 contrast for imaging cortical myelination patterns, we optimized a magnetization-prepared rapidly-acquired gradient echo (MP-RAGE) sequence. In whole-brain, 3D T_1 -weighted images made *in vivo* with the sequence, we identified six major cortical areas with high myelination and confirmed the results with histological sections stained for myelin. We also identified several subtle features of myeloarchitecture, showing the sensitivity of our technique. The ability to image myeloarchitecture over the entire cortex may prove useful in studies of longitudinal changes of the topography of the cortex associated with development and neuronal plasticity, as well as for guiding and confirming the location of functional measurements.

Keywords

brain anatomy; non-human primate; myeloarchitecture; magnetic resonance imaging; magnetization-prepared rapidly-acquired gradient echo sequence; Gallyas silver staining

Introduction

A major quest in neuroscience is to understand the anatomical and functional organization of the brain. A classic study of anatomical organization is Brodmann's division of the human

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cortex into distinct areas based on structural features including cellular density, cellular type, and the composition of the cortical layers (Brodman, 1909). Other classical studies (Campbell, 1905)(Elliott Smith, 1907)(Vogt & Vogt, 1919) organized the cortex based on myeloarchitecture, the density and arrangement of myelinated fibers across the depth of the cortex. While most myelin in the brain is located in discrete white matter tracts that connect various gray matter structures to each other, a significant amount of myelin resides as small bundles of fibers that run either vertically (radially) or horizontally (tangentially) within the layers of the cortical gray matter. Presumably this cortical myelination speeds the conduction of signals to the input layer of areas that require fast responses, such as the primary sensory areas.

Typically, myeloarchitecture is visualized *ex vivo* in myelin-stained sections of the cortex at low power magnifications (Annese et al., 2004). It has been shown, however, that magnetic resonance imaging (MRI) can differentiate specific human cortical areas *in vivo* based on myelin content. The presence of myelin in cortical gray matter produces MRI contrast based on proton density (Clark et al., 1992), longitudinal relaxation time (T_1) (Barbier et al., 2002), and transverse relaxation time (T_2) (Yoshiura et al., 2000). The idea of using MRI to visualize cortical myeloarchitecture has been demonstrated in specific primary sensory areas of the human cortex. For example, dense myelin bundles run horizontally throughout layer IV of the primary visual cortex in a feature called the stripe of Gennari. This stripe can be visualized with high resolution MRI to identify the extent of V1 in humans (Barbier et al., 2002). Shorter T_1 values have also been reported in Heschl's gyrus in the temporal lobe in humans and ascribed to the high myelin content of the primary auditory cortex (Sigalovsky et al., 2006).

The common marmoset (*Callithrix jacchus*) is an ideal non-human primate for visualizing cortical myelination patterns. It has a gray-to white-matter ratio close to that of humans, while presenting the lowest degree of cortical folding of all the primates (Zilles et al., 1989). Furthermore, the pattern of cortical myelination in the marmoset brain has been extensively characterized with conventional histological tools (Krubitzer & Kaas, 1990)(Burman et al., 2008) (Pessoa et al., 1992)(Rosa & Schmid, 1995)(Jeffs et al., 2009). Recently, transgenic marmosets have been successfully generated (Sasaki et al., 2009), adding to the importance of characterization of the marmoset neuroanatomy with non-invasive tools.

In the present work, we set out to visualize the pattern of myelination *in vivo over* the entire cortex of marmoset monkeys (*Callithrix jacchus*). We chose T_1 contrast because of the suitability of T_1 -weighted MRI sequences for high field anatomical imaging in the brain. (One could also investigate other contrast mechanisms widely used for imaging myelin in white matter tracts, such as transverse relaxation time (T_2) or magnetization transfer (Laule et al., 2007)). We use T_1 measurements in cortical gray matter with very high and very low myelin content to optimize the contrast in a T_1 -weighted magnetization-prepared rapidly acquired gradient echo (MP-RAGE) sequence for imaging cortical myelin at 7 Tesla. We show by comparison to histology that the resulting MRI contrast indeed reflects myeloarchitecture. Finally, we use this contrast to visualize the myelination pattern over the entire cortex in live marmosets.

Materials and Methods

Animal Methods

All experiments were approved by the NINDS Animal Care and Use Committee. Experiments were carried out in one male and four female common marmosets aged 8.4 ± 0.5 years (mean $\pm$ standard deviation) and weighing 410 ± 20 grams. Marmosets were housed two to a cage with a twelve-hour light/dark cycle on an ad libitum diet of Zupreem canned marmoset food, Purina 5040 biscuits, unfiltered water, P.R.A.N.G. rehydrator, and fruit and vegetable treats.

On the day of each animal's MRI experiment, an animal was fasted for 12 hours, then retrieved from its cage and transported to the MRI laboratory. The animal was sedated with an intramuscular injection of 10 mg/kg ketamine and placed in a prone sphinx-like position on an MRI-compatible cradle. The animal's head was fixed to a stereotaxic frame with non-piercing earpieces, placed in the ear canals after application of 2% lidocaine as a topical analgesic. The animal was allowed to breathe freely, and was anesthetized through a nose cone with isoflurane in a 2:2:1 mixture of medical air, nitrogen, and oxygen. Throughout the MRI session, the isoflurane concentration was varied around 2% to maintain anaesthesia, which was monitored by end tidal CO₂, heart rate, and SPO₂ using a capnograph and pulse oximeter (Surgivet, Waukesha, WI, USA). Rectal temperature was continuously monitored and the body temperature was maintained at 38.5 °C with a water heating pad.

MRI Methods

MRI was performed on a 7T/30 cm MRI scanner (Bruker Biospin Corp., Ettlingen, Germany) equipped with a 15 cm gradient set of 450 mT/m strength (Resonance Research Inc., Billerica, MA, USA). A custom-built 16-rung high pass birdcage radiofrequency coil with a 12 cm inner diameter was used for transmission, and a four-element phased-array receiver coil (Bruker Biospin) for reception. The elements of the receiver coil were arranged in a 2 by 2 array of 5 cm by 5 cm dimensions. The inner surface of the coil was slightly curved and was placed directly on top of the marmoset's head, approximately centered over the parietal cortex.

Tissue Measurements with MRI

T₁ mapping was performed in a single coronal slice through the middle temporal (MT) area in the occipital lobe using a 2D inversion-recovery echo-planar imaging (EPI) sequence with geometric inversion time sampling (TE = 30 ms, TR = 12500 ms, FOV = 34.8 mm × 17.4 mm, Matrix = 96 × 48, Slice thickness = 1.5 mm, Inversion times = 0, 14, 35, 67, 114, 185, 291, 451, 690, 1048, 1587, 2394, 3605, 5422, 8146, 12233 ms, Number of averages = 6), producing an in-plane resolution of 363 μm. The data were fit to a three parameter, single-exponential T₁ recovery function in Matlab (MathWorks, Natick, MI, USA) to produce a T₁ map. T₁s in specific regions were measured by region of interest (ROI) analysis in ImageJ (National Institutes of Health, Bethesda, MD, USA).

Proton density mapping was performed in the same slice location using a 2D multiple-echo spin echo sequence (TEs = 9, 18, 27, 36, 45, 54, 63, 72, 81, 90, 99, 108, 117, 126 ms, TR = 12000 ms, FOV = 34.8 × 34.8 mm, Matrix = 192 × 192, Slice = 0.75 mm, Number of Averages = 1) producing an in-plane resolution of 181 μm. The data were fit to a two parameter, single-exponential decay function in Matlab to produce proton density maps. Proton densities in specific regions were measured by region of interest (ROI) analysis in ImageJ. Relative proton density (ρ) was calculated by dividing the proton density values in specific regions by the proton density value in gray matter.

Image Contrast Modeling

The measured tissue parameters (Table 1) were used to optimize a strongly T₁-weighted 3D magnetization-prepared, rapidly acquired gradient echo (MP-RAGE) sequence (Mugler & Brookeman, 1990). This sequence consisted of a 180 degree inversion pulse and an inversion delay (TI), followed by the acquisition of a single segment of a 3-D FLASH image. Next, there was a segment delay (TD) during which there was longitudinal relaxation, and then the process was repeated for the next segment. Diechmann et al. derived analytical equations to calculate the development of the longitudinal magnetization during the MP-RAGE sequence (Diechmann et al., 2000), which we used in our modeling to calculate the optimal TI and TD to maximize contrast between gray matter and high myelin content gray matter. Since

anaesthetized animals were to be imaged, the overall imaging efficiency was not optimized, as it would be in human imaging.

The first step in the modeling was to find the optimum TD which determined the amount of longitudinal magnetization available immediately prior to the inversion pulse. For each tissue type, the recovery of the longitudinal magnetization, M , was calculated following a FLASH segment that was long enough for the equilibrium magnetization, M_0 , to reach its saturation value, M_0^* using the following equation:

$$M = M_0 \cdot \left(1 - e^{-\frac{TD}{T_1}}\right) + e^{-\frac{TD}{T_1}} \cdot M_0^* \quad (1)$$

where T_1 was the longitudinal relaxation time. M_0^* was calculated based on the equations from Diechmann et al. using the FLASH parameters listed in Table 1 and ρ was used in place of M_0 , since they are directly proportional. The shortest TR possible on our scanner was used (12.5 ms) to maximize the efficiency of the FLASH acquisition, and a tip angle of 12 degrees was used to reduce the likelihood of signal oscillations during the first partition encoding steps due to variable contributions from stimulated echoes seen with higher flip angles. In our simulations, as TD was increased, the difference in longitudinal magnetization prior to the inversion pulse between the tissue types increased, which ultimately increased the contrast produced by the MP-RAGE sequence. Thus TD = 6000 ms was chosen as the segment delay time, where the longitudinal magnetization in gray matter had recovered to 97% of its maximum value. The calculated TD was much longer than that typically used in human imaging, but the calculated contrast was greatly improved at the cost of imaging efficiency.

Next, the optimum TI was calculated for TD = 6000 ms, using the equations from Diechmann et al.. The signal and the contrast for the first phase-encoding step in a centric phase-encoding method (where the central lines of k-space in the 3D phase encoding direction are acquired at the beginning of each FLASH segment) were calculated. In our simulations, the optimum contrast between gray matter and high myelin content gray matter was found to occur at a TI of 600 ms, and then decrease for longer TIs. However, the optimal TI of 600 ms was before the signal for any tissue type recovered past its null point. If one acquires data before the null point for a given tissue, then an artifact will occur when the signal reaches zero during the FLASH segment acquisition. Therefore, the optimal TI in practice was around 1200 ms, where the signal had just recovered past the null point for the slowest recovering tissue (gray matter) and the contrast was as high as possible.

T₁-Weighted Imaging

High resolution 3D T₁-weighted imaging was performed over the whole brain using the optimized MP-RAGE sequence with centric phase encoding (Inversion time = 1200 ms, TE = 4 ms, TR = 12.5 ms, Flip Angle = 12 degrees, FOV = 42.0 × 42.0 × 21.0 mm, Matrix = 256 × 256 × 128, Number of segments = 4, Segment delay = 6000 ms, Number of averages = 1) producing an isotropic resolution of 164 μm in about 51 minutes. A saturation pulse was applied to a slab positioned at the base of the marmoset's brain to saturate the signal from blood flowing into the brain and to prevent aliasing of signal outside the FOV, which covered just the animal's head. The saturation pulse was applied once per segment, before the inversion pulse. B₁ inhomogeneities were corrected using a reference image method (Wang et al., 2005). One marmoset was imaged with six averages of the MP-RAGE sequence in a single imaging session. The long imaging time of roughly six hours was permissible as the animal was to be sacrificed for histology immediately after imaging and not recovered from anaesthesia. Another marmoset was imaged with three averages in two imaging sessions on separate days

and recovered after each session. The resulting images were co-registered via an affine transformation and averaged in Amira (Visage Imaging Inc., Tewksbury, MA, USA).

Volume Rendering of 3D MRI Data

The cortex in each marmoset MP-RAGE image was masked in Amira using a rough manual segmentation followed by an automatic refinement based on image intensity thresholding. The image data for the cortex alone was then displayed using the “Vortex” rendering function in Amira. For this, each voxel in the cortical volume was assumed to emit and absorb light. The amount of emitted light and the amount of absorption was determined from the MRI magnitude data and displayed using a grayscale lookup table to intuitively show regions of T_1 -enhancement over the extent of the cortex.

Histology

One marmoset was sacrificed under deep anesthesia (sodium pentobarbital, 100 mg/kg, intravascular) immediately following imaging via perfusion fixation through the ascending aorta with 500 ml of ice cold 4.0% paraformaldehyde in 0.1 M phosphate buffer solution (PBS) (pH 7.4). The brain was removed and further fixed by immersion in the paraformaldehyde solution for 2 hours at 4° C and then cryoprotected in 30% sucrose in 0.1M PBS. Coronal 40 μ m thick sections were cut in a microtome and collected in 10% formalin and processed to visualize myelin using a modified Gallyas silver staining method (Pistorio et al., 2006). Briefly, sections were placed in 2:1 pyridine: acetic anhydride for 30 minutes then re-hydrated through washes in 80, 60, 40, and 20% pyridine followed by two water rinses. Sections were then immersed in fresh ammoniacal silver nitrate solution for 45 minutes and stored in 10% formalin solution following washing in 0.5% acetic acid. Sections were again washed in 0.5% acetic acid and incubated for 10 – 15 minutes in a freshly prepared developer solution containing 2.5% sodium carbonate, 0.1% ammonium nitrate, 0.1% silver nitrate, 0.5% tungstosilicic acid and 73 μ l of 4% paraformaldehyde per 20 ml of total volume. The reaction was monitored under a dissecting microscope and stopped by immersion in two washes of 1.0% acetic acid. Sections were mounted onto subbed slides, dehydrated and coverslipped. Sections were analyzed under a light microscope and digital pictures were taken and calibrated with Adobe Photoshop 8 (Adobe Systems, San Jose, CA, USA).

Statistics

Significant differences in tissue T_1 s, p s, and T_2 s were tested using a one-way ANOVA performed in Matlab (Mathwork, Natick, MI, USA) followed by a Tukey multiple-comparison test. $P \leq 0.05$ was used to establish statistical significance.

Results

Tissue Measurements with MRI

To investigate T_1 shortening in high myelin content gray matter, we measured a region-of-interest (ROI) in the middle temporal area (MT) in a coronal T_1 map of the occipital cortex. MT is a distinctive area in marmosets that is identified by dense myelin bands running vertically through layers III – VI (Bourne et al., 2007) (Figure 1 – Left). For cortical gray matter with a low myelin content (herein referred to simply as gray matter), we chose an ROI in an adjacent and more medial region of the cortex with little myelination. Finally, we also chose an ROI in white matter. Figure 1 – Right shows a representative T_1 map with the ROIs identified. We measured T_1 to be 1830 ± 30 ms (mean $\pm$ standard deviation, $n = 4$) in gray matter, 1560 ± 20 ms in high myelin content gray matter in MT, and 1220 ± 30 ms in white matter. There were significant differences in T_1 for all pair-wise comparisons between the three tissue types ($p < 0.05$). Specifically, the difference in T_1 between low and high myelin content gray matter was

270 ms, meaning that T_1 was 15% shorter in high myelin content gray matter versus gray matter.

We also measured the relative proton density (ρ) of each tissue type using a multiple-echo spin echo pulse sequence in the same slice as the T_1 maps, since ρ is an important parameter for optimizing T_1 -weighted sequences. We defined ρ to be 1.00 for gray matter, and measured ρ to be 0.97 ± 0.01 (mean $\pm$ standard deviation, $n = 4$) for high myelin content gray matter and 0.86 ± 0.03 for white matter. There were significant differences in ρ for pair-wise comparisons between gray matter and white matter and high myelin content gray matter and white matter ($p < 0.05$). The difference in ρ between low and high myelin content gray matter was 0.03, meaning that ρ was 3% smaller in high myelin content gray matter versus gray matter.

The T_1 and ρ values of high myelin content gray matter lay between the values for gray and white matter, confirming that the contrast needed to visualize cortical myelination patterns is higher than the contrast typically needed for imaging anatomy based on gray and white matter structure.

Image Contrast Modeling

Table 1 summarizes the general tissue and imaging parameters we used to model the optimum parameters for imaging cortical myelin with a 3D T_1 -weighted MP-RAGE sequence (see Methods for details). From our modeling, we determined an optimal segment delay (TD) of 6000 ms and an optimal inversion delay (TI) of 1200ms for the MP-RAGE. During imaging, we confirmed the best contrast for visualizing cortical myelination patterns occurred at TI = 1200 ms.

T_1 -Weighted Imaging

Figure 2 shows slices from an *in vivo* marmoset brain image made with the 3D MP-RAGE sequence with TD = 6000 ms and TI = 1200 ms. A coronal myelin-stained histology section is shown in each of the major myelinated cortical areas beside a corresponding slice from the MRI. The histology and MRI are from the same marmoset. Areas are identified based on the myelin-stained histology sections with reference to literature on myelination of the marmoset cortex. Three primary sensory areas are identified in the figure: the primary auditory (A1) (Pistorio et al., 2006), somatosensory (S1) (Krubitzer & Kaas, 1990), and visual (V1) cortices (Lyon & Kaas, 2001)(Rosa & Tweedale, 2000). Myelin bands in A1 and S1 run vertically between the white matter and the middle layers of the cortex, while in V1, the bands run horizontally in layer IV (the stripe of Gennari).

The other densely myelinated areas are all associated with extrastriate visual areas: the middle temporal (MT) (Pessoa et al., 1992) and dorsomedial (DM) (Rosa & Schmid, 1995) areas, and the frontal eye fields (FEF) (Krubitzer & Kaas, 1990). As in A1 and S1, the myelin bands in these areas run vertically between the white matter and the middle layers of the cortex. Generally, areas of high cortical image intensity in the MRIs correspond well with areas of high myelination in the histological images.

To visualize the pattern of cortical myelination over the entire brain, we show volume rendered 3D MRIs from a dorsal anterior lateral viewpoint (Figure 3). All areas identified in Figure 2 are visible on the surface of the brains shown in Figure 3. From this volume-rendered representation, we can see the size and relation of areas to each other.

A better presentation of the data is shown in Figure 4 – Figure 7. In these figures, the volume-rendered data for relatively flat regions of the cortex is shown from a view tangential to the surface. This approximates what one would see in flat-mounted histological sections of the cortex, except that the image reflects the average image intensity through the entire depth of

the cortex, and not the optical density at a specific depth, as would be the case for a histological section.

Figure 4 shows the occipital cortex. The key feature that is visible in the figure is a clear boundary between the primary visual cortex (V1) and the secondary visual cortex (V2). This contrast arises from the strong T_1 -weighted enhancement in the Stripe of Gennari that was also visible in the cortical sections in Figure 2. The dorsal portion of area DM is also visible in this figure. Interestingly, there is a region of high intensity within V1 that may indicate the foveal representation (denoted by * in Figure 4).

Figure 5 shows the occipital and parietal cortex. There is a medial area of enhancement that extends into the cerebral fissure and corresponds to the location of DM described on histology. We have outlined the approximate shape of DM as described by others; however, the exact shape is difficult to define, as there is a continuous gradient of enhancement in the region. A feature that can be readily identified in the figure is the border between the dorsal and ventral subdivisions of the posterior parietal cortex (PPd and PPv). This feature is also seen in histological sections based on differences in myelination between the two subdivisions (Rosa et al., 2009); our ability to visualize it shows our technique can be used to study detailed myeloarchitecture in the cortex beyond the major myelinated areas.

Figure 6 shows the parietal and temporal cortex. The main enhancing feature in the figure is the kidney-shaped area MT, which was a location we chose for our tissue relaxation properties measurements because of dense myelination in its lower layers. Ventral and anterior to MT is an enhancing region corresponding to the fundus of the superior temporal area (FST) (Rosa & Elston, 1998)(Krubitzer & Kaas, 1990). The middle temporal crescent (MTc) can also be identified in the figure as an area of intermediate intensity bordered by PPv and MT (Rosa & Elston, 1998). Finally, A1 can be seen in the dorsal temporal cortex and in the lateral bank of the lateral sulcus.

Figure 7 shows the parietal, temporal, and frontal cortex. A key feature visible in the figure is the strongly enhancing area S1. Within S1, a lightly enhancing septum can be seen at its bend. This feature has been described on histology as a lightly myelinated area separating the representations of the face and the hand (Krubitzer & Kaas, 1990). Within the medial wall of the lateral sulcus, the secondary somatosensory area (S2) can be seen (Krubitzer & Kaas, 1990). The posterior border of the lightly enhancing primary motor cortex (M1) is also visible in the figure, as it is on histology (Burman et al., 2008). Finally, the most anterior enhancing feature on the dorsal frontal cortical surface, FEF, is visible, as are two enhancing areas on the ventral surface corresponding to the densely myelinated areas 12 and 13 (Burman et al., 2008).

In Figure 4–Figure 7, we have qualitatively identified the boundaries of major myelinated cortical regions based on their high T_1 -weighted signal intensity. This is similar to identifying highly myelinated areas in flat mounted sections based on the optical density of the myelin stain (Karlen & Krubitzer, 2006). It can be seen from our images, however, that the myelin exists in a continuum throughout the cortex and the boundaries of areas can be diffuse in appearance. Therefore, care must be taken in morphological studies that report the sizes of cortical areas to make sure the criteria for identifying heavily myelinated regions is robustly defined (Pessoa et al., 1992).

Discussion

We have shown, with 3D T_1 -weighted MRI of the marmoset brain, that differences in T_1 associated with the presence of myelin in cortical gray matter allow the visualization of the pattern of myelination over the entire cerebral cortex. We did not investigate if or how the

presence of myelin in cortical gray matter shortens T_1 , but we did not verify with histology that decreases in T_1 in cortical gray matter are correlated with increases in myelination. To study the actual mechanism may require an extensive analysis of T_1 in the different tissues. For instance, we treated each tissue as though it had a single T_1 , yet other researchers have shown that there are actually two components to T_1 in white matter: a short component associated with water trapped in the myelin bilayers, and a longer component associated with intra- and extracellular water (Labadie et al., 2008). It would be interesting to see if these components are also present in high myelin content gray matter.

Overall, we observed in the MRI images and confirmed with histology six major cortical areas: the primary sensory areas A1, S1, and V1, and areas in the extrastriate visual pathway MT, DM and FEF. The ability to detect these areas in the intact brain *in vivo* opens up new avenues in morphological and functional studies that require longitudinal follow up of the changes in size and location of these areas during development or in response to plasticity-induced reorganization due to learning or injury. The location and geometry of these regions agreed well with previous studies of myeloarchitecture in the marmoset brain. The detection of these areas requires images with a high resolution and contrast, as the myelin is only distributed within some layers of the cortex and the tissue properties of high myelin content gray matter are not as distinct as those of gray or white matter, which are typically used to produce contrast in structural MRI.

The primary auditory cortex, A1, in marmosets is a prominent myelinated feature of the temporal lobe (Pistorio et al., 2006), which is unsurprising as the marmoset has a well-developed repertoire of tonal vocalizations, a similar hearing range as humans (Aitkin & Park, 1993), and can even discriminate the pitch of complex tones (Bendor & Wang, 2005). We readily identified A1 in our images by its strong T_1 enhancement relative to surrounding gray matter. In humans, short T_1 s have been noted in this area and related to a high myelin content (Sigalovsky et al., 2006). Interestingly, that study measured lower T_1 s in Heschl's gyrus on the left side of the brain than on the right, suggesting that there are asymmetries in cortical myelination that may reflect specialized functional processing. It would be interesting in a future study to see if the same asymmetries are present in marmosets, either in the T_1 values, or in the size of A1.

Another highly myelinated primary sensory area in the marmoset is the primary somatosensory cortex S1 (also referred to as area 3b). This area features a characteristic somatotopic organization, with sub-areas corresponding to representations of different parts of the body within S1 being distinguished by patchy regions of high myelination surrounded by poorly myelinated regions (Krubitzer & Kaas, 1990). S1 is strongly enhanced in our T_1 -weighted images (Figure 2 and 3), and the patchiness is also apparent.

V1 was one of the first cortical areas to be segregated based on its high myelin content (Gennari, 1782). Since, the stripe of Gennari has been the major structural feature used to distinguish V1 from other cortical areas both in monkeys (Pessoa et al., 1992), as well in humans (Clarke & Miklossy, 1990). V1 is the area that has been most extensively visualized with MRI in humans *in vivo* and *ex vivo* (Clark et al., 1992a)(Barbier et al., 2002)(Bridge et al., 2005) (Eickhoff et al., 2005)(Bridge & Clare, 2006b)(Hinds et al., 2008c)(Walters et al., 2003). From a technical standpoint, it is actually one of the most difficult myelinated areas to image because of the high spatial resolution needed to visualize the stripe of Gennari (Peters & Sethares, 1996). All the other highly myelinated areas we identified contained myelinated fibers spanning multiple lower layers of the cortex which make them easier to image. In V1, the myelinated fibers are confined to just layer IV. In a recent publication, we were able to better visualize the stripe of Gennari in the marmoset visual cortex using manganese as an MRI contrast agent (Bock et al., 2009). In that study, we acquired images with the same spatial resolution as the present work,

but our ability to identify the extent of V1 was improved by the strongly preferential accumulation of the contrast agent in V1. This suggests that there may be other contrast mechanisms unique to V1 besides myeloarchitecture that aid its identification in images.

Another visual area that has been extensively studied in monkeys based on myeloarchitecture is the middle temporal area, MT (Bourne et al., 2007)(Van Essen et al., 1981), which has been implicated in processing visual motion (Ungerleider & Desimone, 1986). In the marmoset, MT is a heavily myelinated region situated dorsal and posterior to the temporal sulcus. Since the myelinated fibers span from layer III to VI (Bourne et al., 2007), we were able to readily identify MT in both our T₁ maps and in our T₁-weighted images. The corresponding homologous area in humans, V5/MT, has been identified using both anatomical MRI with myelin-based contrast, and with functional MRI using a stimulation protocol selective for moving stimuli (Walters et al., 2003). This shows the potential of correlating anatomical and functional areas in the cortex using *in vivo* MRI methods like ours and fMRI.

The final two major areas we identified are also associated with the visual pathway in New World monkeys – the dorsomedial area (DM) and the frontal eye field (FEF). DM is similar in size to MT and receives projections from V1 (Lyon & Kaas, 2001)(Rosa & Schmid, 1995). This region has not been defined in humans, but like MT, is strongly enhancing in our T₁-weighted images because of dense myelinated fibers spanning layers III to VI. The border of DM is less well-defined using myeloarchitecture, however, and our labeling may also contain some of the dorsal area of V3 (Lyon & Kaas, 2001). The frontal eye field (FEF), is analogous to the oculomotor field in humans. It has substantial connections with DM and MT, and can be identified based on its myelination (Burman et al., 2006). In our T₁-weighted images, it was less enhancing than other areas and had less clearly defined boundaries, but it is interesting as the only myelinated area on the dorsal surface of the frontal cortex.

The marmoset is an ideal primate for visualizing cortical myelination patterns, because it has the lowest degree of cortical folding of all the primates (Zilles et al., 1989). Thus, we were able to visualize the myelination pattern over the cortex in volume-rendered 3D images. In other primates with more extensive gyrification (including humans), it would be better to display the 3D MRI data in the cortex as a virtually flattened map.

The ability to visualize myeloarchitecture *in vivo* and non-invasively opens new avenues for studies that require longitudinal follow up of the changes in size and location of cortical areas. For example, the technique could be used to monitor post-natal development non-invasively with each animal serving as its own control. This would be interesting since other studies (Burman et al., 2007)(Bourne & Rosa, 2006) have shown that the primary sensory areas we detected (A1, S1, and V1), as well as MT are the first to mature histologically in the marmoset. Our technique could also be used to study plasticity-induced reorganization caused by learning or injury. Finally, it may prove useful for investigating the morphology of cortical areas non-invasively in a variety of species and for co-localizing structural organization in the cortex with functional organization.

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Figure 1.

(Left) A 40 μ m thick coronal histological section through the occipital lobe of a marmoset monkey stained for myelin showing the highly myelinated middle temporal area (MT).

Figure 1 (Right) A representative *in vivo* coronal T₁ map in a marmoset showing the regions of interest (ROIs) used to measure tissue T₁s. The map is masked to only show the brain. (GM = gray matter, GM (high) = high myelin content gray matter in the middle temporal area, WM = white matter).

Figure 2.

40 μm thick coronal myelin-stained histology sections through the marmoset brain and corresponding 167 μm slices from an *in vivo* T₁-weighted MRI. The MRIs are masked to only show the brain and presented on a gray background to make the edges of the cortex visible. The number in the upper right hand corner of each MRI indicates the order of the slices from rostral to caudal. (FEF = frontal eye fields, A1 = primary auditory cortex, S1 = primary somatosensory cortex, MT = middle temporal area, DM = dorsomedial area, V1 = primary visual cortex). Note that the contrast is reversed in the two types of images, with myelinated areas appearing dark in the histological sections and bright in the MRI.

Figure 3.

Volume renderings of 3D T_1 -weighted MP-RAGE images in two marmosets. **Marmoset 1)** A nine-year-old female marmoset imaged in one session with six averages of the MP-RAGE sequence. The marmoset was sacrificed for histology following the imaging session.

Marmoset 2) Another nine-year-old female marmoset imaged in two sessions of three averages each. The marmoset was recovered following each session. The images from both sessions were co-registered prior to volume rendering. Major myelinated areas of the cortex are shown by the labels. (D = dorsal, V = ventral, A = anterior, P = posterior)

Figure 4.

Tangential views of volume-rendered 3D MRI data in the occipital cortex in Marmoset 1. The cerebral fissure surface has been rotated into the same plane as the dorsal cortex surface for viewing. The border between the primary visual cortex (V1) and the secondary visual cortex (V2) can be seen. As well, an area of enhancement (*) can be seen within V1 which may correspond to the foveal representation. (DM = dorsomedial area, D = dorsal, V = ventral, A = anterior, P = posterior, M = medial, L=lateral)

Figure 5.

Tangential views of volume-rendered 3D MRI data in the occipital and parietal cortex in Marmoset 1. The cerebral fissure wall has been rotated into the same plane as the dorsal cortex for viewing. The border between the ventral (PPv) and dorsal (PPd) parietal posterior cortex can be seen. (DM = dorsomedial area, D = dorsal, V = ventral, A = anterior, P = posterior, M = medial, L=lateral)

Figure 6.

Tangential views of volume-rendered 3D MRI data in the parietal and temporal cortex in Marmoset 1. The lateral wall of the lateral sulcus has been rotated into the same plane as the dorsal cortex for viewing. The border between the ventral posterior parietal area (PPv) and the middle temporal crescent (MTc) can be seen. (MT = middle temporal area, FST = fundus of the superior temporal area, A1 = primary auditory cortex posterior, D = dorsal, V = ventral, A = anterior, P = posterior, M = medial, L=lateral)

Figure 7.

Tangential views of volume-rendered 3D MRI data in the parietal, temporal, and frontal cortex in Marmoset 2. The medial wall of the lateral sulcus and the ventral frontal cortex have been rotated into the same plane as the dorsal cortex for viewing. The posterior border of the lightly enhancing primary motor cortex (M1) is visible. The arrow denotes a lightly enhancing septum separating the hand and face representations in the primary somatosensory cortex (S1). (S2 = secondary somatosensory cortex, FEF = frontal eye fields, 12 = area 12, 13 = area 13, D = dorsal, V = ventral, A = anterior, P = posterior, M = medial, L=lateral)

Table 1

Parameters used for image contrast modeling.

Tissue Type	T₁ (ms)	ρ
Gray Matter	1830	1.0
High Myelin Content Gray Matter	1560	0.97
White Matter	1220	0.86
FLASH Parameters		
Repetition Time, TR (ms)	12.5	
Echo Time, TE (ms)	4	
Flip Angle (degrees)	12	
MP-RAGE Parameters		
Number of FLASH Segments	4	
Segment Duration (ms)	800	
K-Space Ordering	Centric	