Superficial white matter imaging: Contrast mechanisms and whole-brain in vivo mapping

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Superficial white matter (SWM) contains the most cortico-cortical white matter connections in the human brain encompassing the short U-shaped association fibers. Despite its importance for brain connectivity, very little is known about SWM in humans, mainly due to the lack of noninvasive imaging methods. Here, we lay the groundwork for systematic in vivo SWM mapping using ultrahigh resolution 7 T magnetic resonance imaging. Using biophysical modeling informed by quantitative ion beam microscopy on postmortem brain tissue, we demonstrate that MR contrast in SWM is driven by iron and can be linked to the microscopic iron distribution. Higher SWM iron concentrations were observed in U-fiber–rich frontal, temporal, and parietal areas, potentially reflecting high fiber density or late myelination in these areas. Our SWM mapping approach provides the foundation for systematic studies of interindividual differences, plasticity, and pathologies of this crucial structure for cortico-cortical connectivity in humans.

INTRODUCTION

Superficial white matter (SWM) is the thin layer of WM just underneath the cortical sheet. Its structure and function are substantially different from deep WM (DWM) and are strongly influenced by the proximity of the cortical gray matter (GM). SWM contains short association U-fibers that primarily connect adjacent gyri. These subcortical U-fibers represent most of the WM connections in the human brain (1) and are the last structures to be myelinated, maturing as late as the fourth or fifth decade of life. The important role of U-fibers in brain maturation, plasticity, and aging is reflected by the fact that reduced U-fiber density is observed in disorders such as autism (2), epilepsy (3), and Alzheimer’s disease (AD) (4).

The SWM also has a high density of interstitial WM neurons (5). With distinct inter-regional differences in density, the presence of these cells has challenged traditional views of SWM as a structure for passive information transfer. Neuronal circuits in SWM may modulate cortico-cortical connectivity by regulating the timing and signal transfer efficiency at the axonal connections (6).

Despite these proposals, surprisingly little is known about the structure, function, and metabolism of the SWM and the variation of U-fiber and interstitial neuron density across the human brain (7, 8). One major reason for that is the lack of reliable SWM and U-fiber imaging methods. Gold-standard molecular tracer studies are not feasible in humans due to their invasiveness, while noninvasive fiber tractography, based on diffusion-weighted magnetic resonance imaging (MRI) (DWI), does not provide satisfactory results in SWM. Low spatial resolution of current DWI approaches can neither resolve the thin SWM layer nor disentangle the multitude of crossing fibers within it (9).

One promising method for in vivo SWM mapping is ultrahigh-resolution structural MRI. Recent advances in ultrahigh field MRI, combined with biophysical modeling of MR contrast, have enabled the imaging and mapping of specific aspects of brain microstructure (10). Important progress has been made in mapping the laminar structure and myelination patterns of the cortex (11, 12) and fiber orientation-dependent WM contrast in DWM (13–16). However, no study has systematically applied microstructural imaging to SWM mapping yet.

One substantial obstacle is that the neurophysiological and biophysical mechanisms underlying MRI contrast in SWM are not well understood. It is now known that MR contrast in SWM (17) differs from that in DWM. In DWM, highly aligned myelinated axons, and their orientation, dominate MR contrast (13, 18). Drayer et al. (17) have demonstrated enhanced transverse relaxation rates (R2) in SWM compared to DWM, in patients across different ages. By a visual comparison with postmortem Perls staining, the authors qualitatively linked the observed contrast to increased iron concentration in subcortical U-fibers. This observation was supported by Bagnato et al. (19) at 7 T who demonstrated hyperintense SWM on effective transverse relaxation rate (R2*) and phase maps in postmortem brain samples of patients with multiple sclerosis and controls. Other studies have also observed elevated iron levels in the SWM (20–22).

Although the contribution of iron to the susceptibility, R2, and R2* MRI contrasts in SWM has been established, it remains unclear which morphological microscopic structures influence the contrast, such as cell bodies or myelinated fibers. It is also not clear what the relative contributions of other tissue components are, such as myelin,
to MRI contrast. Moreover, systematic whole-brain SWM mapping would require an understanding of the orientation dependence of MRI parameters within this structure with its complex geometry. A mechanistic understanding of the contrast mechanisms is therefore crucial for the interpretation of MRI parameters in SWM and the development of SWM mapping methods.

In this study, in order to lay the foundation for systematic SWM mapping, we aimed to establish a quantitative relationship between microstructure and MRI parameters. We studied several MR contrasts in SWM using high spatial resolution MRI in vivo and in human postmortem brain samples at 7 T. We show that \( R^2, R^2^*, \) and quantitative susceptibility maps (QSMs) exhibit a strong contrast between SWM, cortical GM, and DWM. By comparing in vivo and postmortem MRI with postmortem quantitative iron maps, we identified iron as the dominant source of contrast in SWM. The microscopic iron maps were obtained by proton-induced x-ray emission (PIXE) and laser ablation inductively coupled plasma mass spectroscopic imaging (LA-ICP-MSI) at different spatial resolutions ranging from 1 to 100 \( \mu \text{m} \). Furthermore, we developed a novel biophysical model that quantitatively links the iron-induced MR contrast to the microscopic iron distribution at the cellular level. Iron inside iron-rich oligodendrocyte bodies and other cellular tissue components was identified as the main source of susceptibility-related MR contrasts. In vivo imaging data show that the iron-induced contrast in SWM varies between different cortical brain areas. Increased iron deposits were observed in U-fiber–rich frontal, temporal, and parietal association areas, potentially reflecting higher fiber density or late myelination in these areas. This variability suggests a functional specificity of SWM and further supports the validity of susceptibility- and \( R^2^* \)-based markers.

The noninvasive mapping of \( R^2, R^2^*, \) and quantitative susceptibility thus opens the door for systematic studies of the SWM in humans. Interregional variation, interindividual differences, and developmental trajectories of this important brain structure can now be investigated in terms of health and disease.

**RESULTS**

The experiments described below followed a three-step approach. In a first step, we have empirically demonstrated that iron is the main contributor to MRI contrast in SWM. We have characterized the microscopic distribution of iron by combining in vivo and postmortem MRI with histological analyses and iron quantification methods. In a second step, we used the information about the meso- and microscopic iron distribution to develop models of MRI contrast in SWM. Two models were developed: an empirical linear model and a generative model. Both models combined iron and myelin as relaxation drivers, but the latter accounted for cellular iron distribution and orientation dependence of \( R^2^* \). In a third step, we applied the generative model to map the iron distribution in SWM throughout the entire human brain in vivo.

**SWM shows increased values of magnetic susceptibility, \( R^2, \) and \( R^2^* \) in line with elevated iron levels**

High-resolution whole-brain (400-\( \mu \text{m} \) isotropic resolution) quantitative multi-parameter maps, including the longitudinal relaxation rate (\( R_1 \)), effective transverse relaxation rate (\( R^2^* \)), proton density map (PD), and magnetic susceptibility (\( \chi \)), were obtained in vivo in four healthy human volunteers (11). Combined \( R^2 \) and \( R^2^* \) measurements (500-\( \mu \text{m} \) in-plane resolution) were performed in one slice on a fifth volunteer.

\( R^2, R^2^*, \) and \( \chi \) were strongly increased in a thin tissue strip underneath the cortex (Fig. 1). This hyperintense strip, 0.5 to 2 mm thick (as estimated at different locations in the brain), was identified as SWM due to its location below the GM–WM interface (Fig. 1A). The visibility of this strip varied across the brain areas (Fig. 1A).

Similarly, postmortem brain tissue samples from the temporal lobe also exhibited substantially elevated \( R^2^* \), \( R^2, \) and \( \chi \) values in the SWM (Fig. 2, A to C). The averaged cortical profiles of \( R^2, R^2^* \), and susceptibility peaked within the SWM (Fig. 2, G to I), distinguishing it clearly from GM and DWM. The maximum \( \chi \) value in the averaged cortical profiles was found in SWM. A positive shift difference, between SWM and DWM susceptibility values was \( \chi_{\text{SWM}} - \chi_{\text{DWM}} = (21 \pm 3) \) parts per billion (ppb) (means \( \pm SD \)) (Fig. 2, C and I). The averaged \( R^2^* \) was significantly higher in SWM compared to DWM, whereas \( R^2 \) was only slightly increased in SWM: \( \Delta R^2^* = (10 \pm 0.8) \) s\(^{-1}\) (28% of the DWM \( R^2^* \) value) and \( \Delta R^2 = (3 \pm 0.25) \) s\(^{-1}\) (12.5% of the DWM \( R^2 \) value), respectively (Fig. 2, G to H).

In postmortem tissue, the orientation dependence of the SWM contrast was investigated by comparing \( R^2^* \) values recorded at two different orientations of the sample with respect to the main magnetic field (Fig. S1). Although some orientation-dependent contributions to the SWM \( R^2^* \) were identified, the contrast between SWM and DWM did not depend on the orientation with respect to the magnetic field. The effects may be tentatively attributed to orientation-dependent contributions of myelin to the relaxation rates (14).

The positive susceptibility shift and increased \( R^2^* \) and \( R^2 \) relaxation rates in SWM, both in vivo and in postmortem brain samples, supports earlier reports suggesting paramagnetic iron as the underlying contrast driver (17). The higher values of \( \Delta R^2^* \) compared to \( \Delta R^2 \) are in line with a substantial contribution of static intravoxel dephasing to \( R^2^* \) and thus an increase of \( R^2^* = (R^2^* - R^2) \), which can be explained by mesoscopic or microscopic inhomogeneous iron distributions within the MRI voxel (23).

**MR contrast in SWM disappears after iron extraction**

A tissue metal extraction experiment was performed to corroborate the role of iron in generating the SWM contrast and to quantify the relative impact of the local iron and myelin distributions on the MRI parameters. Parameter maps were obtained on postmortem brain samples before and after iron extraction with a deferoxamine mesylate salt solution (Fig. 2). The 0.5- to 2-mm-thin SWM strip with enhanced \( R^2, R^2^*, \) and \( \chi \) values, apparent before iron extraction (Fig. 2, A to C), vanished after iron extraction (Fig. 2, D to F). Furthermore, the maxima of the averaged profiles of \( R^2, R^2^*, \) and \( \chi \), located within SWM, disappeared after iron extraction (Fig. 2, G to I). \( R^2 \) and \( R^2^* \) in SWM were reduced by \( R^2_{\text{before}} - R^2_{\text{after}} = (12 \pm 2) \) s\(^{-1}\) and \( R^2_{\text{before}} - R^2_{\text{after}} = (6 \pm 0.6) \) s\(^{-1}\), respectively (means \( \pm SD \)). The vanishing contrast after iron extraction provided a direct indication that the difference in \( R^2, R^2^*, \) and \( \chi \) between SWM and DWM originated primarily from the elevated level of paramagnetic iron in SWM. While iron’s contribution dominates the \( R^2^* \) contrast between SWM and DWM, it represents a substantial, but not the dominant, part in transverse and effective transverse relaxation rates, explaining about \( (22 \pm 2) \% \) of the total \( R^2 \) and \( (32 \pm 7) \% \) of the total \( R^2^* \) in SWM.

Note that after iron removal, no differences between the susceptibility of SWM and DWM \( [\chi_{\text{SWM}} - \chi_{\text{DWM}} = (\pm 2 \pm 5) \text{ ppb}] \) was measured within experimental error, indicating that the myelin density in SWM is comparable to that in DWM. The WM appeared...
patchy in the $R2^*$ and susceptibility maps before iron extraction but homogenous after iron extraction (fig. S2, top right). The patches of alternating hypo- and hyperintensity may be caused by patchy iron distributions in the WM (fig. S2, bottom right), as has also been observed previously (19–21).

**Quantitative histology at mesoscopic and microscopic resolutions confirms dominating contribution of iron**

To link $\Delta R2^*$ and $\Delta \chi$ to tissue composition in SWM, the iron and myelin distributions were quantitatively mapped at the mesoscopic and microscopic scale with advanced histology methods and compared to postmortem MRI of the same tissue block. Quantitative iron distribution maps obtained by LA-ICP-MS revealed a 0.5- to 2-mm-thin strip with elevated iron levels below the WM to GM interface (Fig. 3A and fig. S3C). The iron concentration in the SWM was $(55 \pm 11) \mu g/g$ wet tissue weight (wtw). This value was significantly higher than in the upper cortical layers (spanning from pial surface to 25% of cortical depth) $(15.6 \pm 4) \mu g/g$ wtw, middle cortical layers (spanning from 30 to 65% of cortical depth) $(29.2 \pm 7) \mu g/g$ wtw, and in DWM $(33 \pm 10) \mu g/g$ wtw.

In contrast to the iron maps, the myelin volume fraction maps estimated from the measured quantitative phosphorus and sulfur concentrations (Fig. 3B) and myelin basic protein stain (fig. S4B) did not show any significant enhancement in the SWM myelin density compared to DWM. There is a notable similarity between the maps and cortical profiles of iron concentration (Fig. 3A) and $R2^*$ (Fig. 3C), emphasizing that iron strongly contributes to $R2^*$ in both GM and WM.

**High iron concentration observed in SWM oligodendrocytes**

The iron concentration in SWM was mapped to specific cell types and subcellular compartments using PIXE with 1-µm resolution within a field of view of 200 µm by 200 µm (Fig. 4B) (similar to the voxel sizes of the postmortem MRI experiment). Hotspots of iron concentration with an extent of approximately 5 µm were localized in SWM. Comparison with immunohistochemistry revealed that these hotspots were colocalized with the somata of oligodendrocytes and some astrocytes in the SWM (Fig. 4A). We found that not all oligodendrocyte somata had the same iron content.

The oligodendrocyte bodies contained 12% of the overall iron mass in the sub-volume scanned with PIXE. The remaining 88% of iron detected outside the SWM oligodendrocyte somata might be attributed to iron in oligodendrocyte processes or myelinated fibers, since a high similarity between phosphorus (as coarse cell membrane and myelin marker), immunohistochemically marked oligodendrocytes, and iron distributions in the PIXE maps (Fig. 4A) was found. The distribution of neurons and microglia differed prominently from the observed iron distribution (fig. S4), excluding them as relevant iron-containing microstructure. However, a precise assignment of this iron to a particular cellular compartment was not possible with the available PIXE resolution.

**Empirical linear model of $R2^*$ in SWM requires separate iron and myelin contributions**

To quantify the contribution of iron and myelin to $R2^*$, we first used the empirical linear model proposed in reference (21). The averaged cortical profile of $R2^*$ was modeled as a linear combination...
of the iron and myelin concentration profiles. The best fit was provided by the following parameter set

$$R_2^* = (0.35 \pm 0.25 \text{ s}^{-1}) c_F + (47 \pm 7 \text{ s}^{-1}) v_m + 13.7 \text{ s}^{-1} \quad (1)$$

where $c_F$ is the iron concentration in $\mu$g/g wtw, $v_m$ the myelin volume fraction, and the constant term ($13.7 \text{ s}^{-1}$) is an offset describing any relaxation processes unrelated to iron and myelin variations (Fig. 3D). This model explained 91% of the variance in cortical profiles, whereas an alternative model including only the myelin contribution and a constant term explained only 66% of the variance. The strongly improved fit and high relaxivity of iron estimated by the linear model provides additional support for the important role of iron in $R_2^*$ contrast generation in SWM.

**Generative model of $R_2^*$ relaxation in SWM needs to account for cellular iron distribution and orientation dependence of $R_2^*$**

The empirical linear model described by Eq. 1 has several limitations. First, it does not provide understanding of the microstructural underpinnings of iron-induced $R_2^*$, which is indispensable for the interpretation of $R_2^*$ data. Second, it does not account for the potential dependence of $R_2^*$ parameters on tissue orientation in the magnetic field (fig. S1) (24), which is important for whole-brain SWM mapping.

To mechanistically link the microscopic and mesoscopic iron distribution in SWM to $R_2^*$, we developed a generative biophysical model of iron-induced relaxation in SWM. This model accounts for the contribution of iron and myelin, as well as the magnetic field orientation dependence. Our complete theoretical considerations are provided in Materials and Methods. Here, we summarize the key findings relevant for SWM mapping of the entire brain. In the following, we assumed that $R_2^*$ can be considered as a sum of reversible ($R_2^*$) and irreversible contributions ($R_2$), i.e., $R_2^*/R_2 = R_2 + R_2^*$ for simplicity.

In the human brain, iron is mostly stored in paramagnetic form in the protein ferritin (25). Ferritin-bound iron contributes to $R_2^*$ via three distinct relaxation mechanisms operating at different temporal and spatial scales. At the nanoscale from tens of angstroms to hundreds of nanometers, fast fluctuating molecular interactions of water spins with ferritin-bound iron lead to irreversible transverse relaxation $R_2$ (23, 26). At the microscale spanning from micrometers to tens of micrometers, the heterogeneous cellular distribution of iron induces perturbation of local magnetic fields and therefore $R_2^*$ relaxation (23). Last, at the submillimeter mesoscale of the MRI voxel size, variation of the magnetic susceptibility within the SWM strip results in intravoxel signal dephasing and therefore contributes to reversible $R_2'$. Relaxation mechanisms resulting from these three mechanisms have different relative contributions to $R_2^*$ and $R_2$. Moreover, contributions of the three mechanisms depend on different aspects of the tissue iron distribution and reveal a different
dependence on the orientation of the SWM surface with respect to the static magnetic field. For example, nanoscale relaxation contributes to $R2$ only, is orientation-independent, and depends solely on the mean tissue concentration of iron (see Eq. M2). In contrast, microscopic relaxation contributes potentially to both $R2$ and $R2^*$ and is determined by the cellular iron distribution. Last, mesoscale iron-induced relaxation is orientation dependent and contributes only to reversible $R2^*$ (see Eq. M4).

Using theoretical considerations and quantitative iron maps obtained with LA-ICP-MSI and PIXE, we estimated the contributions of nanoscale, microscopic, and mesoscale relaxation processes to iron-induced $R2^*$ in SWM as described below. The nanoscale contribution of iron to $R2$ (and therefore to $R2^*$) was estimated using the averaged iron concentration in SWM $(55 \pm 11 \, \mu g/g \, wtw)$ obtained from the LA-ICP-MS experiment (Fig. 3) and Eq. M2. The resulting contribution of the nanoscale relaxation to $R2^*$ in SWM was $\Delta R2^*_{\text{nano}} = (1.2 \pm 0.2 \, s^{-1})$. This contribution is only a small fraction of the experimentally measured iron-induced relaxation rate of $(12 \pm 2) \, s^{-1}$ obtained in the tissue metal extraction experiment.

The mesoscale contribution to $R2^*$ was estimated using differences in the iron concentrations of SWM and DWM, measured with LA-ICP-MSI $(22 \pm 21 \, \mu g/g \, wtw)$ and by applying Eq. M4. Under the assumption of a perpendicular orientation of the SWM slab to

The microscopic contribution to $R2^*$ was estimated using microscopic maps of iron concentration obtained with PIXE. To estimate the line broadening, intravoxel distributions of proton resonance frequencies were predicted from cellular iron maps measured with PIXE (Fig. 4B). To investigate the influence of different cellular compartments, two simulations were performed. The first simulation estimated the effect of the entire iron content, while in the second simulation, only contributions from the iron-rich cell somata were taken into account. The resulting field maps and histograms for both cases of intravoxel Larmor frequency distributions are presented in Fig. 4, respectively.

Consideration of the total iron content led to a distribution of proton Larmor frequencies within the voxel that was well described by a Gaussian with full width at half maximum (FWHM) of $8 \, s^{-1}$ (Fig. 4E). Restricting the model to the contributions of the iron-rich cell somata resulted in a line shape close to a Lorentzian distribution with a FWHM of $1.2 \, s^{-1}$. Predictions from the first simulation model were in good agreement with experimentally obtained values from the iron extraction experiment, which revealed an impact of the tissue iron on the $R2^*$ contrast of $(12 \pm 3) \, s^{-1}$. By comparing the simulations obtained from the two tested models with the experimental results, the influence of the iron-rich fibers can be identified as the dominant contribution, which is bigger than the effect of the iron-rich cell somata. This result clearly shows that the applied approach is not only capable of assigning iron as the main source of MR contrast in SWM but also allows the prediction of MR relaxation rates based on the microscopic iron distributions. The microscopic contribution to $R2^*$ scales linearly with the total iron concentration. By dividing the obtained linewidth of $8 \, s^{-1}$ by the total iron concentration in an SWM voxel investigated with PIXE $(37 \, \mu g/g \, wtw)$, the effective microscopic relaxivity $(r2^*_{\text{micro}})$ was estimated $r2^*_{\text{micro}} = 0.215 \, s^{-1} / \mu g/g \, wtw$, which compares well with the relativity factor $(0.35 \pm 0.25 \, s^{-1} / \mu g/g \, wtw)$ found in the empirical model (Eq. 1). In summary, we found iron-induced contributions to $\Delta R2^*$ according to $\Delta R2^*_{\text{nano}} > \Delta R2^*_{\text{meso}} > \Delta R2^*_{\text{micro}}$ indicating that the dominating contribution originates from the microscopic scale.

$R2^*$ and QSM in the SWM vary across brain areas with sharp boundaries between cortical areas

The iron deposits in SWM were mapped over the entire human brain in vivo using $R2^*$ maps in combination with the generative model (Eq. M8) and QSM maps. Whole-brain multiparametric data were acquired in four participants with an isotropic resolution of 400 $\mu m$, which yielded intrinsically co-aligned maps of longitudinal relaxation rate $(R1)$, PD, $R2^*$, and QSM. The high contrast between GM and WM on $R1$ and PD maps was exploited to obtain the cortical GM-WM boundary. SWM was then determined as the surface $0.5 \, mm$ below the GM-WM boundary, and $R2^*$ and susceptibility values were mapped at this depth across the entire brain (Fig. 5, B and C). We defined the SWM as a surface running at a constant depth from the cortical GM-WM boundary to sample quantitative MRI parameters exclusively inside the SWM and to keep the partial volume effect with GM constant across the brain areas. The orientation-dependent influence of myelin and iron on $R2^*$ was estimated and removed using the general linear model (GLM) described in Eq. M8. Intracortical myelination and myelination of SWM were estimated from the $R1$ values in the middle of the cortex (Fig. 5D) and

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**Fig. 3. Elevated iron levels determine MR contrast in SWM.** (A) Quantitative iron map and (B) estimated myelin volume fraction map were obtained with LA-ICP-MSI and compared to (C) quantitative $R2^*$ map. Maps depicted in (A) to (C) were obtained from the same postmortem brain sample slice of the temporal lobe. Elevated iron levels in a thin (0.5 mm) stripe in the SWM dominate the $R2^*$ contrast. Note that the myelin volume fraction is not elevated in the SWM compared to DWM. (D) Averaged cortical and subcortical profiles of iron, myelin, and $R2^*$ obtained in the sulcus between the positions marked with the dotted line in (A). $R2^*$ fits, calculated based on the linear combination of myelin and iron contributions, are also shown in (D) (in yellow). Bars represent SEM across the profiles. The white square highlighted by the arrow in SWM in (A) indicates the position of the PIXE measurements shown in Fig. 4B.
in the SWM surface (fig. SSD), respectively [following Sereno et al. (27)] to relate $R2^*$- and QSM-based SWM maps to cortical myelination patterns.

A strong variation of iron-induced $R2^*$ was observed in the SWM across different brain regions (Fig. 5A). Higher $R2^*$ values were found in frontal, temporal, and parietal association areas, whereas lower $R2^*$ values were observed in the primary visual and the auditory cortices (Fig. 5A). Similar patterns were observed on the QSM maps (Fig. 5B). These patterns of variation across brain regions were distinct from the variation of SWM curvature (fig. S5C), SWM myelination (fig. SSD), and the orientation-dependent term in Eq. M8 (fig. S5B) and were also visible before the correction for orientation-dependent contribution (fig. S5A). The orientation-dependent terms in the model explained only $8 \pm 3\%$ (means $\pm$ SD across four participants).
of the variance in whole-brain R2* maps (fig. S5B). Correction of orientation-dependent effects reduced their impact even further (fig. S1). Thus, the patterns are most likely driven by the variation of SWM iron content.

It is known that the cortical primary motor, somatosensory, and auditory areas are heavily myelinated and myelinate early during development (27), which is reflected in higher intracortical R1 values (Fig. 5C). In contrast, low R2* values were observed in the SWM underneath these brain regions (Fig. 5A). This pattern reversed for the frontal and temporal association areas: Low intracortical R1 values were colocalized with high R2* values in the SWM. This is consistent with a low density of myelinated intracortical and ascending long range fibers but a high density of short association fibers, which myelinate late during the life span (28). Sharp boundaries between the primary and secondary visual areas were visible in the SWM. The primary motor and somatosensory areas showed a third type of pattern with increased values of both R1 in the cortex and R2* in SWM.

**DISCUSSION**

We have demonstrated that SWM can be reliably identified and differentiated from both cortical GM and DWM, in vivo, with high-resolution quantitative R2, R2*, and susceptibility mapping at 7 T. The high iron concentration in the somata of oligodendrocytes and other microstructural components in SWM were identified as the main source of MR contrast by using advanced histology and postmortem MRI. A unified biophysical model was developed to quantitatively correlate MR parameters with iron distribution at the micro- and mesoscopic level. With this model, the SWM iron deposits were mapped over the entire human brain in vivo. Iron deposits were increased in the U-fiber rich frontal, temporal, and parietal association areas, while they were reduced in areas with high intracortical myelination (including primary visual and auditory areas, but not primary sensorimotor areas). The sharp boundary between the primary and secondary visual cortex, in terms of iron accumulation identified in vivo, is in line with previous studies in which histology and postmortem quantitative iron mapping were used (20, 29). These findings confirm area-dependent iron concentration in the SWM and may also reflect U-fiber density or myelination patterns of the underlying cortical areas.

Our results have several important implications. First, they demonstrate that SWM iron deposits can be mapped by quantitative MRI in vivo, which holds great promise for the study of various pathologies with impaired iron homeostasis (30). For example, the regional distribution of plaques in AD could be partially explained by the interregional distribution of SWM iron. It has recently been shown that both plaques and tangles in AD are more prominent in sulcal fundi (where the U-fiber density is increased) than in gyral crowns (31). Higher vulnerability of sulcal fundi to AD could be related to enhanced SWM iron levels in these regions.

Second, structural SWM contrasts may potentially be used for in vivo mapping of cortico-cortical U-fiber densities, thus significantly advancing future studies in local brain connectivity and studies of the human connectome. Our supposition that iron-induced contrast in the SWM is related to U-fiber density is supported by the colocalization of iron with somata of oligodendrocytes, as demonstrated here by cellularly resolved iron mapping. This colocalization may reflect the specific myelination processes in SWM, since iron is recognized as an important cofactor for myelin synthesis and oligodendrocyte proliferation (32). Additional support for our assumption comes from the inhomogeneous SWM contrast distribution in the brain, as this excludes nonspecific global effects and supports area-specific processes or structures. The increased SWM iron content in the frontal and temporal association areas may reflect distinct myelination processes of U-fibers in these late myelinating areas, as late myelinating oligodendrocytes have an increased metabolic demand and iron is a basic requirement for oxidative metabolism, which is crucial for lipid synthesis and normal myelination (32).

Detailed knowledge of the U-fiber topography in the human brain, which could be used to validate our hypothesis, is very limited. Early dissection studies reported U-fiber systems in frontal, occipital, and temporal areas (33), which is in excellent agreement with the high SWM contrasts observed in our study. While several studies have focused on short cortico-cortical connections in the occipital lobe, precentral and postcentral gyri, and frontal (8) and temporal areas in humans and primates, there has been no systematic mapping of U-fibers across the entire human brain. Recent in vivo MRI studies, based on the magnetization transfer contrast (4), have revealed higher U-fiber densities in the frontal and temporal lobes compared to the occipital pole. The regional SWM density distributions reported for healthy control groups in the mentioned study correspond well to the iron-induced SWM contrast observed for the four participants in our study. Although the in vivo and postmortem experiments strongly suggest that R2* maps reflect U-fiber density, additional studies are needed to corroborate this claim. For example, comparisons should be made to tracer studies and to U-fiber density measurements from emerging submillimeter resolution DWI techniques that can substantially improve the detectability of subcortical U-fibers.

An alternative explanation for the observed differences in the iron content of SWM over the brain may be regional differences in its cellular organization (5, 6). It has been demonstrated that the density of interstitial neurons, their type, and morphology vary across brain regions, with the lowest density of WM neurons in the visual cortex (5). This may explain the sharp boundary we observed between the primary and secondary visual cortex.

Generally, our findings demonstrate that iron strongly influences the MR contrast in WM. The role of iron in laminar-specific contrast in the cortex (20, 21) and subcortical nuclei (34) has been widely recognized. However, most MR contrast models in WM neglect the iron contribution (13, 18). In addition, the relationship between the microscopic iron distribution and R2* is an important step in the quantitative understanding of iron-induced contrast in the human brain and, in particular, the understanding of the differential contributions of the different cell populations to iron-induced contrast. Quantitative microscopic iron maps as obtained in our study (Fig. 4), in combination with theoretical concepts developed for R2* contributions of microscopic magnetic perturbations (23), pave the way for in vivo MR iron histology. Our biophysical model predicts R2* within 30% error without any free fitting parameters, which is an advancement over previous work that included free parameters or lacked quantitative predictions. The residual difference may be addressed by including iron-induced effects on R2 resulting from water diffusion in inhomogeneous fields induced by iron-rich cells (23).

Our findings may be helpful in studies of the human cortex, especially of its parcellation. The SWM strip appears to be a more prominent imaging feature on R2* and susceptibility maps than the variation of myelination in cortical layer IV, which is often directly or indirectly used for parcellation (11, 27). Because SWM contrast
is region-dependent, cortical parcellation methods based on intra-
cortical myelin-sensitive R1 contrast may benefit from adding R2*
and QSM information from the SWM. In addition, MRI-based seg-
mentation algorithms could benefit from SWM contrast to better
define cortical boundaries.

A limitation of our work is that only the total iron content and
not the spin state was assessed. In addition, the limited resolution of PIXE (approximately 1 μm) makes it difficult to assign iron to
specific subcellular compartments. It has been shown that iron is
localized in outer and inner layers of the myelin sheaths (35). However, the precise localization of elevated iron concentrations
in SWM to a specific compartment, such as myelin sheaths, oligo-
dendrocyte processes, or the extracellular space, requires further in-
vestigations with methods capable of iron quantification at the
nanometer scale.

Here, R2 mapping was based on the acquisition of a single slice,
which limits the extractable information on the distribution of R2
across the cortical areas. Future development of the described method
toward three-dimensional (3D) intrinsically co-aligned R2/R2*
correlation may add additional information.

The proposed model of R2* contrast mechanisms relies on the
following assumptions. The mass susceptibility of iron was estimated
on the basis of a value for ferritin-bound iron found in the litera-
ture. This value, however, may slightly differ from the mass suscep-
tibility of iron in brain tissue due to presence of different iron forms,
contributing to systematic error of our model. Any systematic error
of the assumed brain tissue density and experimentally estimated
tissue shrinkage factor would influence the mass iron concentration
used in the modeling. In addition, the orientation-dependent myelin
contribution was estimated assuming no preferential orientation of the U-fibers within the SWM plane. In the future, more realistic fiber
orientation distributions obtained from DWI may be used to im-
prove the model. Last, the in vivo measurements are prone to partial
volume effects and image processing inaccuracies, since the strip of
SWM is only approximately 0.5 mm thick. In particular, the thick-
ness and exact location of SWM with respect to the GM-WM boundary
may slightly vary.

Our study was based on four in vivo datasets and one postmor-
tem brain only. However, since our results relate mainly to the funda-
camental physical mechanisms of SWM contrast, we do not expect
that the model and the contrast mechanism will differ in the popu-
lation and influence its generalization to the healthy population.
Different neuropathologies can lead to different iron distributions,
which the model may not adequately explain. These situations re-
quire further study. However, the presented biophysical model,
multi-parameter acquisitions, and the corresponding image pro-
cessing offer the opportunity to further study the SWM contrast in
pathological and/or interindividual variations.

In summary, we have presented a novel biophysical generative
model and performed the first systematic investigations of SWM
iron distribution throughout the brain in vivo. Our postmortem
experiments provide a mechanistic explanation of the observed
contrast, linking iron, late-myelinating axons, and oligodendro-
cytes. Our results suggest that the maps may reflect U-fiber density
throughout the brain, providing a unique window into short corti-
co-cortical connections. The newly developed method can be used to
assess inter-regional, interindividual, and developmental differ-
ences in SWM and U-fibers in healthy and pathologically altered
brains.

**MATERIALS AND METHODS**

**In vivo MRI measurements**

**Whole-brain R2*, R1, PD, and QSM maps**

Four healthy volunteers (28 ± 1 years) were scanned over three ses-
sions on a 7 T MR system (Magnetom 7 T, Siemens Healthineers,
Erlangen, Germany) using a 32-channel radio frequency (RF) head
coil (Nova Medical Inc., Wilmington, MA, USA). The study was
approved by the local ethics committee. Quantitative parameter
maps, including R2*, R1, and PD maps, were obtained using a gradient- and RF-spoiled multi-echo 3D gradient-echo sequence with
400-μm isotropic resolution [repetition time (TR) of 31.8 ms, eight equidistant echoes acquired with alternating readout gradient pola-
ry, first echo time (TE1) of 3.4 ms, and distance between echoes
(ΔTE) of 2.6 ms], readout bandwidth (BW) of 434 Hz per pixel, and
matrix size of 560/640/416 (phase/read/slice) (11). In each session,
both PD-weighted (flip angle α = 5°) and T1-weighted (α = 28°) im-
ages were acquired in addition to calibration data to correct for RF
transmit field nonuniformity. Parallel imaging with acceleration
factor of 2 was applied in both phase-encoding (PE) directions, en-
abling acquisition of each volume in 32 min. Prospective motion
mismatch (Kineticor, HI) was used to correct for both intra- and
interscan motion. Due to the large size of the acquired datasets, raw
data were streamed online, and images were subsequently recon-
structed offline using a SENSE-based parallel imaging algorithm.

Sensitivity maps were estimated from integrated k-space reference
lines (N = 84, N = 88 lines in each PE direction). Transmit RF field
mapping was performed using echo planar imaging acquisition of
spin and stimulated echoes with 15 different refocusing flip angles.

Quantitative R2*, R1, and PD maps were created from the weighted
datasets using the hMRI toolbox (http://hmri.info) and SPM12
(www.fil.ion.ucl.ac.uk/spm/) within MATLAB (MathWorks, MA).
Maps created from each of the three sessions were skull-stripped,
coregistered to the maps of the last session using the Optimized Au-
tomated Registration as implemented in CBS Tools in MIPAV (www.
nitrc.org/projects/cbs-tools/), and then averaged across the three ses-
sions to increase signal-to-noise ratio (SNR).

For each of the three multi-echo PD-weighted acquisitions, QSM
maps were reconstructed, registered to the third scan session, and
averaged across scan sessions. The QSM maps were computed from
the phase information of all eight echoes of the PD-weighted acquis-
tions. Phase discrepancies between odd and even echoes were com-
penated (36), and echoes were averaged in an SNR-efficient manner
(37). Unwanted background phase contributions were removed us-
ing sophisticated harmonic artifact reduction for phase data with
varying spherical kernels (V-SHARP; radii range, 0.4 to 4 mm) (38).

The background-free phase data were then scaled to yield the local
magnetic field distribution, and homogeneity enabled incremental
dipole inversion was carried out for field-to-susceptibility inversion.

(37) We referenced all in vivo susceptibility maps to the average sus-
ceptibility of the brain tissue within the field of view.

**Comparison of R2 and R2**

For one participant, quantitative R2 and R2* maps were acquired
in a separate, single session. Quantitative R2* maps were obtained
using a gradient- and RF-spoiled multi-echo 3D gradient-echo se-
quence (four echoes, TE1 = 9.18, ΔTE = 8.15 ms, TR = 44 ms, flip
angle α = 14°, and isotropic resolution 500 μm) by mono-exponential
fitting of the TE-dependent signal in each voxel.

A single-slice quantitative R2 map was acquired using a 2D spin-echo (SE) sequence [TR = 2 s, flip angle = 90°, partial Fourier
R2* relaxation values, we modeled these contributions to SWM kernel of 6 mm) and resampled to an average surface template (“fsaverage” approach) to assess the pattern of intracortical sampled at middle cortical surface (50% cortical depth using FreeSurfer’s “white” surface). Postmortem sample was imaged at two orientations with respect to the main magnetic field \( B_0 \). Between the two acquisitions, the sample was rotated by 73° about an axis perpendicular to the field. QSM maps were reconstructed using the same approach as for the in vivo scans. The postmortem QSMs were referenced to the average susceptibility of the tissue sample and its embedding medium. To improve visual conspicuity, we manually segmented the brain tissue from the embedding medium and presented masked images. As the experimental setup was identical for all postmortem tissue samples, the susceptibility profiles between them could be compared. However, special care should be taken when transferring the postmortem results to the in vivo maps.

Averaged cortical profiles of quantitative MRI parameters and iron and myelin concentrations were extracted for postmortem tissue blocks. To this end, manual segmentation into GM and WM was performed on several consecutive slices. Surface normals with respect to the GM-WM boundary were generated at 20 equidistant points along the WM boundary in sulcal walls and the fundus located between two gyri (see dashed lines in Figs. 2 and 3). The surface normals were extrapolated from the cortex into the WM for the length of the cortical depth at each cortical location. Quantitative \( R2^* \), R2, and QSM values were sampled at 40 equidistant positions along the surface normals. Obtained profiles were averaged across the 20 profiles covering the entire cortical region between the two gyri. Correspondingly, the SD at each cortical depth was calculated across the 20 sampled profiles. The averaged SVM values were determined by averaging the cortical profile values over the band spanning from the GM-WM boundary to 20% of cortical depth into the WM (corresponding to a band about 0.5 mm thickness). DWM values were calculated by averaging values between 50 and 100% of cortical depth into the WM. Cortical layer IV was identified in immunohistochemical stains for myelin as a band of dense intracortical fibers. Location of layer IV in MRI images at each position along the sulcus was determined by manual coregistration between histology and MRI images. Averaged depth location of cortical layer IV in the cortical profiles is indicated on Figs. 2 and 3.
The same procedure of extracting a cortical profile was applied to the quantitative iron and myelin concentration maps obtained with LA-ICP-MSI. The cortical profiles of iron and myelin concentrations were used as regressors in the GLM describing the cortical profile of $R_2^*$ (Eq. 1 and Fig. 3D). Two models were used: one containing both myelin and iron profiles as predictors and a reduced model containing only myelin. For both models, the variance of the residuals after regression was compared with the total variance of the original data, and the percentage of the explained variance was calculated.

**Tissue iron extraction**

One of the two smaller subsamples was subjected to an iron extraction procedure (20, 21) to quantify iron-induced contributions to $R_2^*$, $R_2$, and $\chi$. After MR scanning, this sample was cut into two equally sized pieces. One half of the sample was incubated in a solution of 2% deferoxamine mesylate salt (Desferal) and 2% sodium dithionite dissolved in PBS at 37°C for a period of 15 days. The deferoxamine solution was exchanged every 3 days. The other half of the sample was incubated in pure PBS under the same conditions and served as a control. Subsequently, MR scans were obtained from both samples using the same acquisition parameters as before iron extraction.

**Histology and tissue preparation**

After completing MRI, the same tissue blocks were processed for histology and quantitative iron mapping. After dehydration in increasing ethanol concentrations the samples were embedded in paraffin (Histowax). Frontal sections of 12-μm thickness were cut with a sliding microtome (SM2000R, Leica). The sections were transferred to Superfrost Plus glass slides, deparaffinized with xylene, and embedded in a mounting medium (DePeX, Merck) and subsequently removed from the object slides. The 16-μm-thick DePeX foils containing the brain sections were placed into aluminum frames for LA-ICP-MSI analysis or left unstained for LA-ICP-MSI.

Staining was performed to analyze the distribution of potentially iron rich cell types and the distribution of myelin, iron, and iron-proteins (transferrin and ferritin) in SWM. Neurons, oligodendrocytes, astroglia, microglia, and myelin basic protein, ferritin, and transferrin were stained using the antibodies listed in Table 1.

Before staining, the slices were treated for 1 hour with 60% methanol and 2% H$_2$O$_2$, followed by 1-hour incubation in a blocking solution (2% bovine serum albumin, 0.3% milk powder, and 0.5% donkey serum) to reduce unspecific staining. Primary antibodies were incubated in blocking solution overnight at 4°C. After incubation, brain slices were washed in PBS-Tween (0.02% Tween 20, pH 7.4) three times and then incubated with secondary biotinylated antibodies (1:1000; Dianova) for 1 hour at room temperature. All brain slices were three times washed in PBS-Tween followed by 1-hour incubation with peroxidase-conjugated avidin (ExtrAvidin, Sigma-Aldrich; 1:2000) at room temperature and rinsing in tris-HCl (pH 8.0). The staining was enhanced by 3,3′-diaminobenzidine (Sigma-Aldrich) and nickel (nickel ammonium sulfate, purity grade of 99.999%; Sigma-Aldrich) in tris-HCl (pH 8). Brain slices were lastly rinsed in tris-HCl and PBS-Tween again. In addition, Perls’ (Fe$^{3+}$) and Turnbull’s (Fe$^{2+}$) staining for the two chemical forms of iron were performed.

For quantitative elemental imaging with LA-ICP-MS, the unstained brain sections were dehydrated in increasing ethanol concentrations and air-dried. For quantitative PIXE, the immunohistochemically stained brain sections, still on Superfrost Plus object slides, were embedded in a mounting medium (DePeX, Merck) and subsequently removed from the object slides. The 16-μm-thick DePeX foils containing the brain sections were placed into aluminum frames for PIXE analysis.

### Table 1. Primary and secondary antibodies used for staining of specific compartments.

| Stained compartment | Antibodies/binding proteins | Source | Dilution | Treatment for antigen retrieval |
|---------------------|-----------------------------|--------|----------|--------------------------------|
| **Cell stains**      |                             |        |          |                                |
| Neurons (HuCD)       | Mouse anti-HuCD             | Molecular Probes | 1:400 | tris buffer, 20 min, pH 8, 90°C |
| Oligodendrocytes (Olig2) | Rabbit anti-Olig2 | Immuno-Biological Laboratories | 1:100 | Citrate buffer, 20 min, pH 6, 90°C |
| Microglia [Iba 1] ionized calcium binding adapter molecule 1 | Rabbit anti-Iba1 | Wako | 1:800 | / |
| Astroglia [GFAP] glial fibrillary acidic protein | Rabbit anti-GFAP | Dako | 1:500 | / |
| Astroglia [GLT-1] glial glutamate transporter 1 | Guinea pig anti-GLT-1 | Millipore | 1:500 | / |
| **Myelin stains**    |                             |        |          |                                |
| Myelin basic protein (MBP) | Rat anti-MBP | Abcam | 1:400 |                                |
| Myelin oligodendrocytes (CNPase) | Mouse anti-CNPase | BioLegend | 1:300 | Citrate buffer 20 min, pH 6, 90°C |
| **Iron-proteins**    |                             |        |          |                                |
| Ferritin             | Goat anti-Ferritin          | Santa Cruz Biotechnology | 1:200 | / |
| Transferrin          | Rabbit anti-Transferrin     | Abcam | 1:5000 | / |
Quantitative iron microscopy with PIXE
Quantitative elemental maps with microscopic resolution were obtained by PIXE using the high-energy ion nanoprobe LIPSION at the Leipzig University. LIPSION provides a 1-μm proton beam with an energy of 2.25 MeV. The proton beam was scanned over multiple 200-μm by 200-μm sized brain regions, and the induced x-rays emitted from the sample were recorded. A total charge of about 70 μC was accumulated for each sample. For iron quantification, Rutherford backscattering (RBS) spectra were used to calibrate for particle exposure. RBS is used to measure the energy of proton backscatter from the sample, thereby allowing to determine the organic composition (carbon, nitrogen, and oxygen and that of hydrogen indirectly) of the sample, and thus permitting the simultaneous detection of both low and high atomic number (Z) elements when combined with PIXE. From the recorded x-rays, tagged with the position, quantitative element maps were created using dynamic analysis, which is part of the GeoPIXE II software (http://nmp.csiro.au/GeoPIXE.html). The maps were smoothed with a Gaussian filter with 2-μm kernel. Quantitative iron, phosphorus, sulfur, and nickel maps were obtained as described in (21). Quantitative volume iron concentrations obtained with PIXE and LA-ICP-MS on tissue sections were converted into mass iron concentrations using a density of the brain tissue of 1.05 g/ml and an experimentally determined tissue volume shrinkage factor of 0.7. This value was obtained by a comparison of the distances between landmarks identified in optical microscopy and MRI images of the studied samples. The total iron concentration in the SWM tissue was calculated by integrating the iron content over the investigated regions. In addition, the iron fraction contained in the oligodendrocyte bodies within the studied regions was calculated using oligodendrocyte body masks. The latter were manually segmented on the PIXE nickel maps. On the maps, they were made visible by nickel enhanced immunohistochemical staining.

Quantitative iron mapping with LA-ICP-MS
For LA-ICP-MS iron mapping, tissue sections were ablated continuously in line-by-line scans using a commercial laser ablation (LA) system (NWR213, ESI, Portland, USA), operating at a wavelength of 213 nm with a laser spot diameter of 150 μm, energy fluence of 0.06 J/cm², scan speed of 120 μm/s, and a repetition rate of 20 Hz. To ensure full sample removal, overlapping laser spot scans (30 μm overlap) were applied during line scanning.

The ablated tissue was transported with a helium gas flow of 1 liter/min to an ICP sector field mass spectrometer (Element XR, Thermo Fisher Scientific, Germany). Ablated tissue was ionized by an RF plasma source with a power of 1350 W using argon as plasma gas and auxiliary and transport gas, with flows of 15, 1, and 0.6 liter/min, respectively. Mass spectra were continuously recorded with mass resolution of 300 m/Δm and time averaged to a sampling time of 0.52 s. This setup provided elemental maps with a resolution of 120 μm by 61 μm.

The isotopes $^{31}\text{P}$, $^{34}\text{S}$, and $^{57}\text{Fe}$ were selected for analysis. The less abundant isotopes of $^{57}\text{Fe}$ and $^{34}\text{S}$ with natural abundances of 2.2 and 4.21%, respectively, were selected for iron and sulfur concentration mapping due to strong interference from $^{16}\text{O}_2$ and $^{40}\text{Ar}^{18}\text{O}$ with the most abundant isotopes $^{32}\text{S}$ and $^{56}\text{Fe}$, respectively. For matrix-matched calibration of $\text{P}$, $\text{S}$, and $\text{Fe}$, solution drops of $\text{KH}_2\text{PO}_4$, $\text{CuSO}_4$, and $\text{Fe}$-standard [1000 parts per million (ppm) in diluted $\text{HNO}_3$] were dropped in multiple replicates onto de-ironed brain tissue sections and air-dried for calibration with a matrix-matched sample as described in (39).

Measured element intensity time profiles of the ICP-MS were converted to 2D maps and further processed with MATLAB. For quantification, element concentrations were integrated over calibrating spot areas of the standard drops, and calibration coefficients between ICP-MS signal intensity and element concentrations were obtained using linear regression. These coefficients were used to convert ICP-MS signal intensities of the measured isotopes into quantitative elemental maps.

Myelin volume fraction was estimated from quantitative maps of sulfur and phosphorus concentrations using the method reported by Stüber et al. in (21) (see “Myelin” maps section) and described in detail in section S5.

Generative model of iron-induced $R_2^*$ relaxation in SWM
We developed a generative biophysical model of iron-induced relaxation in SWM. On the basis of an empirical linear model (Eq. 1) and following previous work (21), we partitioned $R_2^*$ in SWM into myelin and iron contributions

$$R_2^* = R_{2\text{myelin}} + R_{2\text{iron}} + R_{2\text{other}}$$

Subscripts indicate the contributions of myelin, iron, and other tissue components. We assume that the last term in Eq. M1 originates from tissue components other than myelin and iron and that it is constant across brain regions and orientation independent. In the following, we treat this contribution as a constant offset.

Paramagnetic iron in the brain, which we assumed to be stored mostly in the protein ferritin, contributes to $R_{2\text{Fe}}^*$ via three distinct relaxation mechanisms operating at different temporal and spatial scales: (i) nanoscale, (ii) microscale, and (iii) mesoscale. We also assume that $R_2^*$ can be considered as a sum of reversible ($R_2'$) and irreversible contributions ($R_2$), i.e., $R_2^* = R_2 + R_2'$. This simplification is valid for fast and slow processes (23). In the following, we first provide theoretical considerations for each of the relaxation mechanisms separately. We then reformulate Eq. M1 according to these considerations and formulate a GLM capable of mapping iron-induced $R_2^*$ in SWM across the cortical areas.

Nanoscale mechanism
At the nanoscale, water molecules engage in rapidly fluctuating interactions with iron-storage molecules, mainly the iron-rich protein ferritin (23, 26). These interactions contribute to $R_2^*$ via changes in $R_2$ only, since fast diffusion averaging occurs at these short time scales and distances (23). The nanoscale contribution to the $R_2^*$ and $R_2$ relaxation rate constants can therefore be approximated by

$$\Delta R_{2\text{nano}} = r_{2\text{nano}} c_{\text{Fe}}$$

where $r_{2\text{nano}}$ is the relaxivity of ferritin-bound iron and $c_{\text{Fe}}$ the tissue iron concentration. The relaxivity of ferritin-bound iron was measured at 7 T in ferritin solutions with physiological pH and temperature and reported as $r_{2\text{nano}} = 0.0225$ s⁻¹/ppm wtw (26).

Microscale mechanism
At the microscale with a typical range between one to tens of micrometers, the paramagnetic iron distribution in cells (Fig. 4) induces local magnetic field inhomogeneities within an MRI voxel. These inhomogeneities result in static line broadening and, therefore, in a $R_2^*$ decay (23).

PIXE iron maps were used to calculate intravoxel distributions of proton Larmor frequencies. The 3D maps of iron concentration

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were obtained by concatenating quantitative PIXE iron measurements performed on three consecutive slices, which were repeated three times to provide circular boundary conditions. The 3D iron maps were converted to magnetic susceptibility distributions by multiplying them with the mass magnetic susceptibility of ferritin-bound iron $\chi = 1.37 \times 10^{-9} \times c_{Fe}$ (where $c_{Fe}$ is the tissue iron concentration in $\mu g$/g wet wt) (25). The mass susceptibility of ferritin-bound iron is $1.37 \times 10^{-9}$ per $\mu g$/g wet wt (25). This value constitutes the upper bound of the reported mass susceptibilities of iron in the brain, (see section S1 for more details)

contribution $R_{2}'$ then the frequency offsets within the slab will result in dephasing, (Fig. 2C). Water diffusion was neglected in the theoretical considerations and a static dephasing limit was assumed to estimate the microscopic $R_{2}^*$ contributions (23). Water diffusion in locally inhomogeneous magnetic fields partially averages the static line broadening. This effect reduces the overall $R_{2}^*$ from microscopic mechanisms but enhances the contribution to $R_{2}^*$. Therefore, our estimation provides an upper limit for the iron-induced microscopic relaxation rates.

Mesoscale mechanism

At the mesoscale, ranging from hundreds of microns to the voxel size, SWM can be considered as a tissue slab with enhanced iron concentration and therefore high magnetic susceptibility (Fig. 2C). Water protons within the continuous endless slab experience a frequency offset, which depends on the orientation of the slab with respect to the magnetic field

$$\delta \Omega = \gamma B_0 \Delta \chi c_{Fe} \left( \sin^2 \theta - \frac{2}{3} \right)$$

(M3)

where $\theta$ is the angle between the slab surface normal and the external magnetic field $B_0$, $\Delta \chi c_{Fe}$ is the difference in iron concentrations between the SWM slab and the surrounding tissue, and $\chi$ is the volume susceptibility of ferritin-bound iron.

If the voxel size is comparable or larger than the slab thickness, then the frequency offsets within the slab will result in dephasing, which manifests itself as an orientation-dependent $R_{2}^*$ contribution (see section S1 for more details)

$$\Delta R_{2,nano}^* \approx \frac{p(1-p)}{2} \delta \Omega^2 T_E = \frac{p(1-p)}{2} T_E (\gamma B_0 \Delta \chi c_{Fe})^2 \left( \sin^2 \theta - \frac{2}{3} \right)^2$$

(M4)

where $p$ describes the partial volume within the voxel and $T_E$ is an echo time.

Myelin

The myelin contribution to $R_{2}^*$ depends on the fiber orientation with respect to the static magnetic field. Empirically, the myelin contribution, $R_{2,myelin}^*$ to $\Delta R_{2}^*$, can be described as a sum of orientation-dependent and orientation-independent terms (14, 24, 44)

$$\Delta R_{2,myelin}^* = C_{R_{2}^*} + a_1 \sin^2 \theta^* + a_2 \sin^4 \theta^*$$

(M5)

where $C_{R_{2}^*}$ is the orientation independent myelin contribution to $R_{2}^*$, $\theta^*$ is an angle between fibers and the magnetic field, and $a_1$ and $a_2$ are empirical coefficients that scale orientation-dependent terms. The hollow cylinder model (13, 14) provided theoretical justification of Eq. M5, demonstrating that parameters $a_1$ and $a_2$ are determined by the properties of the fibers, including fiber volume fraction, the $g$-ratio, and the fiber orientation dispersion [see equation 7 and Appendix A in (14)].

To apply Eq. M5 to SWM, we assume for simplicity that there is no preferential orientation of the fibers within the SWM slab. We therefore averaged the orientation-dependent terms $\sin^2 \theta^*$ and $\sin^4 \theta^*$ in Eq. M5, over all possible orientations of the fibers within the SWM plane. As shown in section S2, the averaged $\sin^2 \theta^*$ and $\sin^4 \theta^*$ terms can be expressed as a linear combination of $\sin \theta$ and $\sin^4 \theta$ terms, where $\theta$ is the angle between the SWM surface normal and the static magnetic field. Therefore, Eq. M5 can be rewritten as

$$\Delta R_{2,myelin}^* = C_{R_{2}^*}^* + a_1^* \sin^2 \theta + a_2^* \sin^4 \theta$$

(M6)

Generative linear model for whole-brain SWM mapping

By inserting the total contributions of iron and myelin (Eqs. M2, M4, and M6) to the relaxation rate $R_{2}^*$ into Eq. M1, it follows

$$R_{2,SWM}^* = (r_{2,nano}^* + r_{2,myelin}^*) c_{Fe} + \frac{p(1-p)}{2} T_E (\gamma B_0 \Delta \chi c_{Fe})^2 \left( \sin^2 \theta - \frac{2}{3} \right)^2 + ...$$

$$+ C_{R_{2}^*} + a_1^* \sin^2 \theta + a_2^* \sin^4 \theta + R_{2,others}^*$$

(M7)

The terms in Eq. M7 can be divided into three different types. The first type includes orientation-independent terms that are linearly dependent on the iron concentration in SWM and can vary between different brain regions. The second type of terms contains orientation-dependent contributions of iron and myelin. The third type represents the orientation independent contributions of myelin and other tissue components. The latter contributions can be considered constant, assuming that there is no systematic variation of the myelin density in the SWM over the brain. Based on this approach, we formulated a GLM, which we used to map iron deposits in the SWM across the brain

$$R_{2,SWM}^* = \beta_0 + \beta_1 \sin^2 \theta + \beta_2 \sin^4 \theta + e(\chi c_{Fe})$$

(M8)

In Eq. M8, the terms $\beta_0$ and $e(\chi c_{Fe}) = (r_{2,nano}^* + r_{2,myelin}^*) c_{Fe} + \frac{2p(1-p)}{9} T_E (\gamma B_0 \Delta \chi c_{Fe})^2 + C_{R_{2}^*} + R_{2,others}^*$ is the contribution of myelin, iron, and other tissue components, which are independent of the orientation to the static magnetic field. $\beta_0$ term represents the averaged value across brain areas, and $e(\chi c_{Fe})$ explains variation between brain areas.

Assuming the contributions of myelin $C_{R_{2}^*}$ and other tissue
components, $R^2$ others is constant over the brain. Then, the variation of iron content $c_{Fe}$ across the brain is the main source of variance for the $R^2$ between brain areas after regressing out orientation dependent part. Note that since terms $\sin \theta$ and $\sin \phi$ are strongly correlated these two terms could not be reliably separated from each other in GLM analysis. Since they describe effects of no interest in the SWM mapping, the colinearity did not affect the SWM mapping approach.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/SUPPLEMENTARY_MATERIALS

**REFERENCES AND NOTES**

1. A. Schue, V. Braitenberg, *Cortical Areas: Unity and Diversity* (Taylor & Francis, 2002), pp. 377–386.
2. B. Zikopoulos, H. Barbas, Changes in prefrontal axons may disrupt the network in autism. *J. Neurosci. 30*, 14595–14609 (2010).
3. M. Liu, B. C. Bernhardt, S.-J. Hong, B. Caldairou, A. Bernasconi, N. Bernasconi, The superficial white matter in temporal lobe epilepsy: A key link between structural and functional network disruptions. *Brain 139*, 2431–2440 (2016).
4. C. Scharlach, L. Müller, W. Wagner, Y. Kobayashi, H. Kratz, M. Ebert, N. Jakubowski, E. Schellenberger, LA-ICP-MS allows quantitative microscopy of europium-doped iron oxide nanoparticles and is a possible alternative to ambiguous Prussian blue iron sensing.
5. M. L. Suárez-Solá, F. J. González-Delgado, M. Pueyo-Morlans, O. C. Medina-Bolívar, N. C. Hernández-Acosta, M. González-Gómez, G. Meyer, Neurons in the white matter and the macaque brain with diffusion tensor imaging.
6. K. Oishi, H. Huang, T. Yoshioka, S. H. Ying, D. S. Zee, K. Zilles, K. Amunts, R. Woods, D. H. Herman, L. S. Clasen, A. W. Toga, J. L. Rapoport, P. M. Thompson, Dynamic mapping of human cortical development during childhood through early adulthood. *Proc. Natl. Acad. Sci. U.S.A. 101*, 8174–8179 (2004).
7. S. Hametner, V. Endemary, A. Deistung, P. Palmirch, M. Prihoda, E. Haimburger, C. Menard, X. Feng, T. Haider, M. Leisser, U. Köck, A. Kaider, R. Höftberger, S. Robinson, J. R. Reichenbach, H. Lassmann, H. Traxler, S. Trattnig, G. Grabner, The influence of brain iron and myelin on magnetic susceptibility and effective transverse relaxation - A biochemical and histological validation study. *Neuroimage 179*, 117–133 (2018).
8. K. Oishi, H. Huang, T. Yoshioka, S. H. Ying, D. S. Zee, K. Zilles, K. Amunts, R. Woods, D. H. Herman, L. S. Clasen, A. W. Toga, J. L. Rapoport, P. M. Thompson, Dynamic mapping of human cortical development during childhood through early adulthood. *Proc. Natl. Acad. Sci. U.S.A. 101*, 8174–8179 (2004).
9. S. Hametner, V. Endemary, A. Deistung, P. Palmirch, M. Prihoda, E. Haimburger, C. Menard, X. Feng, T. Haider, M. Leisser, U. Köck, A. Kaider, R. Höftberger, S. Robinson, J. R. Reichenbach, H. Lassmann, H. Traxler, S. Trattnig, G. Grabner, The influence of brain iron and myelin on magnetic susceptibility and effective transverse relaxation - A biochemical and histological validation study. *Neuroimage 179*, 117–133 (2018).
10. T. A. Rouault, Iron metabolism in the CNS: Implications for neurodegenerative diseases. *Nat. Rev. Neurosci. 14*, 551–564 (2013).
11. T. Arendt, M. Morawski, U. Göttinger, N. Fröhlich, F. Schulze, N. Wohmann, C. Jäger, C. Eisenloeffel, H.-J. Gertz, W. Mueller, K. Brauer, Inhomogeneous distribution of Alzheimer pathology along the isocortical relief. Are cortical convulsions an Achilles heel of evolution? *Brain Pathol. 27*, 603–611 (2016).
12. J. R. Connor, S. L. Menzies, Relationship of iron to oligodendrocytes and myelination. *Glia 17*, 83–93 (1996).
13. Z. D. Théodoridou, L. C. Triarhou, Challenging the supremacy of the frontal lobe: Early views (1900–1909) of Christoff Jakob on the human cerebral cortex. *Brain Cereb. Cortex 48*, 15–25 (2012).
14. A. deistung, A. Schüfer, A. Fröhner, K. Amunts, J. Schellenberger, LA-ICP-MS allows quantitative microscopy of europium-doped iron oxide nanoparticles and is a possible alternative to ambiguous Prussian blue iron staining. *J. Biomed. Nanotechnol. 12*, 1001–1010 (2016).
15. F. Schweser, A. Deistung, B. W. Lehr, J. R. Reichenbach, Quantitative imaging of intrinsic magnetic tissue properties using MRI signal phase: An approach to in vivo brain iron metabolism? *Neuroimage 54*, 2789–2807 (2011).
16. C. Langkammer, F. Schweser, N. Krebs, A. Deistung, W. Goessler, E. Scheurer, K. Sommer, G. Reichenbach, Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *Neuroimage 62*, 1593–1599 (2012).
17. W. Zheng, H. Nicol, S. Liu, Y.-C. Cheng, E. Maacke, Measuring iron in the brain using quantitative susceptibility mapping and X-ray fluorescence imaging. *Neuroimage 78*, 68–74 (2013).
18. J. P. Marques, R. Bowtell, Application of a Fourier-based method for rapid calculation of field inhomogeneity due to spatial variation of magnetic susceptibility. *Concepts Magn. Reson. 35B*, 65–78 (2009).
19. S.-H. Oh, Y.-B. Kim, Z.-H. Cho, J. Lee, Origin of B0 orientation dependent R2* (=1/T2*) in white matter. *Neuroimage 73*, 71–79 (2013).

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