CORRECTIONS

EVOLUTION

The authors note that the incorrect URL for accessing the data in the Dryad database was published. The data discussed in this publication have been deposited in the Dryad Digital Repository database: http://hdl.handle.net/10255/dryad.908.

MEDICAL SCIENCES

The authors note that due to a printer’s error in adding an additional reference in the proof, beginning with the second citation for reference 12 on page 5130, right column, first paragraph, fifth line, all numerical references should have appeared as one number higher, e.g., reference 12 becomes reference 13.

This is with the exception of reference 1 on page 5133, left column, last paragraph, fifth line.

NEUROSCIENCE

The authors note that in the abstract of their paper, the sentence, “Phosphorylation of Rap1GAP is also associated with increased dendritic spine head size in cultured neurons” should instead appear as “Phosphorylation of Rap1GAP is also associated with decreased dendritic spine head size in cultured neurons.” This error does not affect the conclusions of the article.

RETRACTION

IMMUNOLOGY
Retraction for “B7-DC cross-linking restores antigen uptake and augments antigen-presenting cell function by matured dendritic cells” by Suresh Radhakrishnan, Esteban Celis, and Larry R. Pease, which appeared in issue 32, August 9, 2005, of Proc Natl Acad Sci USA (102:11438–11443; first published online August 1, 2005; doi: 10.1073/pnas.0501420102). The authors wish to note the following: “After a re-examination of key findings underlying the reported conclusions that B7-DCXAb is an immune modulatory reagent, we no longer believe this is the case. Using blinded protocols we re-examined experiments purported to demonstrate the activation of dendritic cells, activation of cytotoxic T cells, induction of tumor immunity, modulation of allergic responses, breaking tolerance in the RIP-OVA diabetes model, and the reprogramming of Th2 and T regulatory cells. Some of these repeated studies were direct attempts to reproduce key findings in the manuscript cited above. In no case did these repeat studies reveal any evidence that the B7-DCXAb reagent had the previously reported activity. In the course of this re-examination, we were able to study all the antibodies used in the various phases of our work spanning the last 10 years. None of these antibodies appears to be active in any of our repeat assays. We do not believe something has happened recently to the reagent changing its potency. Therefore, the authors seek to retract this work.”

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Sensitivity of MRI resonance frequency to the orientation of brain tissue microstructure

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Recent advances in high-field (>7 T) MRI have made it possible to study the fine structure of the human brain at the level of fiber bundles and cortical layers. In particular, techniques aimed at detecting MRI resonance frequency shifts originating from local variation in magnetic susceptibility and other sources have greatly improved the visualization of these structures. A recent theoretical study [He X, Yablonskiy DA (2009) Proc Natl Acad Sci USA 106:13558–13563] suggests that MRI resonance frequency may report not only on tissue composition, but also on microscopic compartmentalization of susceptibility inclusions and their relative orientation to the magnetic field. The proposed sensitivity to tissue structure may greatly expand the information available with conventional MRI techniques. To investigate this possibility, we studied postmortem tissue samples from human corpus callosum with an experimental design that allowed separation of microstructural effects from confounding macrostructural effects. The results show that MRI resonance frequency depends on microstructural orientation. Furthermore, the spatial distribution of the resonance frequency shift suggests an origin related to anisotropic susceptibility effects rather than microscopic compartmentalization. This anisotropy, which has been shown to depend on molecular ordering, may provide valuable information about tissue molecular structure.

MRI phase contrast | resonance frequency shift | anisotropic susceptibility | magnetic susceptibility tensor | white matter fiber tracking

Recent high-field (>7 T) human MRI studies have demonstrated that contrast based on resonance frequency shifts may substantially improve the visualization of fine-scale structural variation in the human brain. For example, many of the brain’s major white matter fiber bundles and distinct cortical laminations patterns have recently been visualized in vivo by observing the signal phase that is proportional to the resonance frequency in gradient-echo (GRE) MRI (1–4). Despite this achievement and the potential clinical and scientific significance, however, the mechanisms underlying magnetic resonance frequency shifts remain poorly understood.

Several candidate mechanisms have been suggested and investigated as sources for this frequency contrast (1, 5–8). A primary candidate is an altered bulk magnetic susceptibility due to such compounds as ferritin, myelin, and deoxyhemoglobin, all of which are found throughout brain tissue. Strong support for this mechanism comes from postmortem tissue analysis (9), and from the observation that the phase distribution within and between gray and white matter is consistent with calculations from susceptibility models.1

An additional contribution to MRI resonance frequency may come from the exchange processes between protons in water and in amide groups on proteins or other exchangeable sites (8). The frequency shifts arising from these mechanisms may provide important information about the chemical composition of brain tissues in vivo.

A recent theoretical study from He and Yablonskiy (10) suggests that resonance frequency shifts may also report on brain tissue microstructure and its orientation relative to the main magnetic field. The authors applied the Lorentzian cavity concept (11, 12) to estimate the magnetic field induced by microscopic variations in magnetic susceptibilities. Using a nonspherical Lorentzian cavity, proposed earlier in (12, 13), to model the compartmentalization of water protons in an anisotropic tissue structure, the authors suggested the presence of a net (voxel-averaged) frequency shift that depends on the tissue’s orientation in the externally applied magnetic field. This mechanism could explain some of the strong frequency shifts observed in the major fiber bundles (Fig. 1) and may have important implications for the application and interpretation of the resonance frequency in GRE MRI.

The extent to which the Lorentzian cavity concept, which is well established at the atomic (nanometer) scale for electric and magnetic fields (11, 14), should be used to account for the field distributions at larger (micrometer) scales that may be present in brain structures is not clear, however. Furthermore, the experimental evidence is sparse in the aforementioned study (9), and interpretation of the results is confounded by large-scale geometric effects inherent to the susceptibility-related contrast mechanism.

The purpose of the current study was to further investigate the potential effect of brain microstructure on MRI resonance frequency.

Results

The effect of tissue microstructure on MRI resonance frequency was investigated by manipulating microstructure while minimally affecting macrostructure. Experiments were performed on an elongated section of human corpus callosum, a major white matter fiber bundle of the human brain. A part of the corpus callosum was chosen that had relatively uniform (microstructural) fiber orientation, with fibers running mostly parallel to the long direction of the section (Fig. 2A). The section was cut into five subsections, which were aligned parallel to the MRI main magnetic field (B0) in a sample holder. The section was imaged under two conditions: one in which all subsections were placed in their original orientation (condition A; Fig. 2C), and the other in which two of the subsections (C2 and C4) were rotated by 90 degrees relative to B0 (condition B; Fig. 2D). Microscopic fiber orientation was confirmed by diffusion tensor imaging (DTI) (15).

Author contributions: J.L., K.S., M.F., and J.H.D. designed research; J.L. performed research; J.L., H.M., and A.C.S. contributed new reagents/analytic tools; J.L., K.S., M.F., P.v.G., and J.H.D. analyzed data; and J.L. and J.S., and J.H.D. wrote the paper.

The authors declare no conflict of interest.

1Shmueli K, et al. The dependence of tissue phase contrast on orientation can be overcome by quantitative susceptibility mapping. Proceedings of the Annual Meeting of the International Society of Magnetic Resonance Medicine, April 18–24, 2009, Honolulu, HI; p. 466.

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showing parallel orientation for condition A and alternating parallel and perpendicular orientations for condition B (Fig. 3A and B). Macroscopic tissue orientation was parallel to $B_0$, and the overall shape was similar for both conditions.

Images representing signal amplitude and resonance frequency from the two different microstructural orientations are shown in Figs. 3C–F. The average resonance frequency of the tissues in condition A was $-3.89 \pm 0.76$ Hz relative to the saline solution. Under condition B, the resonance frequency of the rotated segments C2 and C4 showed a positive shift of $0.56 \pm 0.67$ Hz (mean difference $\pm$ pooled SD over voxels) (Fig. 3F) relative to condition A (Fig. 3E). This effect is shown more clearly in Fig. 4, in which the average cross-sectional profile of the difference image is plotted. The large SD is attributed to the nonuniform phase shift within a tissue segment and the true susceptibility variation within the tissue.

Apart from the frequency shift observed inside the rotated tissue segments, subtle effects also were seen outside the rotated tissue segments. For example, Fig. 3F shows a slightly positive frequency shift in the fluid immediately lateral to the rotated tissue segments. The frequency profile in Fig. 4 suggests the presence of a shift in the unrotated segments (C1, C3, and C5) as well, with the strongest effect seen in C3. Inside of C1 and C5, the frequency gradually decreases close to the C1 side of the C1–C2 boundary and the C5 side of the C4–C5 boundary. This can be interpreted as the effect of C2 and C4 extending outside of those tissue sections, with an amplitude that decreases with distance. In summary, the results suggest that the orientation of the microscopic structure affects MRI resonance frequency both locally and remotely.

**Discussion**

Our findings represent direct experimental evidence for a dependence of MRI resonance frequency on the orientation of brain microstructure relative to the main magnetic field. Our experimental design allowed the separation of microscopic and macroscopic orientation effects that often coexist under general in-vivo imaging conditions. Such effects have complicated the
interpretation of earlier studies (9, 16). The observed sensitivity to orientation may result from different mechanisms (see below) that could provide important clues about the cellular organization of the tissue.

The Nonspherical Lorentzian Cavity. Although the dependence on microstructural orientation found in this study might appear consistent with the theoretical predictions of He and Yablonskiy (9), there is an obvious discrepancy that suggests inadequacy of their theory. The nonspherical Lorentzian approach does not explain the resonance frequency changes observed at the boundaries and outside of the tissues (e.g., the positive frequency near the lateral edges of C2 and C4, and the decreased frequency in C3 and at the C1–C2 and C4–C5 boundaries); rather, it predicts a frequency shift that is purely local to the area of anisotropic microstructure. We propose that the experimental observations are better explained by a model of anisotropic susceptibility rather than one of anisotropic compartmentalization.

Anisotropic Magnetic Susceptibility. Anisotropic magnetic susceptibility is a phenomenon commonly seen in crystals of highly anisotropic atomic structure. It also has been noted in constituents of biological tissues, including muscle fibers (17), retinal rods (18), nucleic acids (19), proteins (20–23), and lipid bilayers (24–27). Because the proteins and lipid bilayers associated with white matter fibers are highly ordered, anisotropic susceptibility may have contributed to our present findings.

Anisotropic white matter susceptibility could lead to a shift in susceptibility on rotation of the microscopic fiber orientation, as occurred in tissue segments C2 and C4 in the experiment described above. Computer simulations suggest that anisotropic susceptibility would lead to a pattern of resonance frequency shifts closely resembling the experimental observation, including the effects outside the rotated segments (Fig. 5). Assuming that the frequency shifts were caused entirely by anisotropy of the magnetic susceptibility, the magnitude of this effect was calculated using simulations. When the primary fiber orientation in all of the tissue sections was parallel to B0, the susceptibility value (χk) that gave the best match between the experiment and the simulation was −9.093 ppm. The susceptibility value χk that gave the minimum residual error between simulations and measurements made of the difference between condition A and condition B was −9.105 ppm. This value is more diamagnetic than χ⊥ (both relative to saline solution). These results suggest that the effect of microstructure orientation on the resonance frequency in white matter can be explained by a model based on anisotropic magnetic susceptibility with a microstructural orientation–dependent susceptibility difference (χ∥ − χ⊥) of 0.012 ppm. Note that this model does not include any contribution of the molecular exchange effect to the frequency shift; if that were taken into account, the absolute susceptibility values could change. The anisotropic susceptibility difference (χ∥ − χ⊥) would not change, however, because it is based on the exchange-averaged frequency difference images.

Fig. 6 shows the susceptibility difference profile calculated directly from the frequency difference image. The measured susceptibility difference (χ∥ − χ⊥) between the tissue segments varies from 0.006 ± 0.007 ppm (mean difference ± pooled SD over voxels between C4 and C5) to 0.010 ± 0.007 ppm (between C2 and C3). We attribute the large SD to the true susceptibility variation within the tissue and/or the noise amplification occurring during the inversion process that is required to convert frequency distributions into susceptibility distributions (16). Despite the large SDs, the susceptibility difference was highly statistically significant between all χ∥ and χ⊥ tissue combinations with P values <10−16 when averaged over the voxels.

One possible source of this susceptibility anisotropy is the phospholipid bilayers in myelin. The susceptibility anisotropy (χ∥ − χ⊥) measured in the corpus callosum is ∼3–5 times smaller than the anisotropy measured in the lipid bilayers of lecithin membranes (24, 25). This range is reasonable considering the smaller lipid fraction (−16%) in white matter (28) and potential confounds, such as the differences in the molecular composition of the bilayers and differences in cellular structure.

Fig. 5. Simulation results for anisotropic susceptibility. (A) simulation result for condition B. (B) Cross-sectional profile of the best-fit susceptibility model (solid line) and the simulated frequency (dashed line). The simulated frequency image in A shows a strong similarity to the experimental results shown in Fig. 3F. The average cross-sectional profile (dashed in B) also agrees well with the experimental results (Fig. 4).
Although the qualitative agreement between the frequency distribution determined in the experiments and that predicted by the anisotropic susceptibility model is striking, the possible contribution of other sources to the observed dependence of MRI resonance frequency on microstructure cannot be ruled out. Further research is needed to confirm the effect size of the anisotropic susceptibility.

**Experimental Considerations.** One limitation of the current experiment is that the resonance frequency images (Fig. 3E and F) and the cross-sectional profiles (Fig. 4 and Fig. S1) could have been somewhat affected by the cut surfaces and the gaps between the tissue segments. However, these effects are limited in space and fail to explain the overall negative frequency shift in unrotated C3 (Fig. 4 and Fig. S1) and the positive frequency shifts observed in the fluid lateral to C2 and C4 (Fig. 3F). In addition, a gap should create a mirrored profile outside the gap and centered on it; however, the profiles around the C1–C2 and C4–C5 gaps in Fig. S1B are not mirrored, indicating that the effect of the gaps does not dominate the profile or explain the profile change between conditions.

Because the current study was performed on a fixed tissue sample that had been preserved in formalin, some of the biological parameters may not accurately reflect values in vivo. A recent study suggested that washing fixed tissues for 12 h helps restore MRI parameters (T1, T2, and diffusivity) to close to their values in vivo (29). We followed a similar procedure to restore the MRI parameters. The DTI results indicate that the microstructure was preserved even after fixation, as was suggested by Sun et al. (30). Moreover, frequency contrasts quantitatively similar to those observed in vivo have been observed in fixed tissues (31). Nevertheless, further research is needed to investigate the effects of fixation on resonance frequency contrast.

**Implications.** The finding of microstructure orientation–dependent resonance frequency shifts may have significant implications for the study of brain structures with MRI. First, it should improve the understanding of previous observations of frequency shifts in high-resolution brain images (1), which may lead to improved methodology for their detection. Second, the microstructural information available through this mechanism could complement data available with MRI measures of water diffusion (32). Interpreting resonance frequency data directly remains challenging, given the presence of multiple contributing mechanisms. Nonetheless, when combined with other information such as T2* (33) and magnetization transfer contrast (34), these resonance frequency data could be used to extract microstructural information for use in various clinical and scientific applications (e.g., high-resolution fiber tracking).

**Materials and Methods**

**Experimental Setup.** Because the aim of the present study was to investigate the effect of tissue microstructure and its orientation on MRI resonance frequency, we selected a section of white matter known to have a relatively uniform fiber orientation. The experiment was carefully designed to separate out any effects of tissue microstructure from well-known larger-scale geometric shape effects. A square cylindrical piece of corpus callosum was sectioned from a coronal slice of a fixed human brain from a 70-year-old female with no history of neurologic disorders, preserved in 10% formalin (Fig. 2A). The piece of corpus callosum was cut such that the main white matter fiber orientation was parallel to the piece’s long axis. The square cylindrical piece was then laid flat and cut into five square cylindrical subsections with a surgical knife: two long pieces (C1 and C5) with a short side of ~5.6 mm and a long side of ~16–18 mm) and three cubic pieces (C2–C4, with sides of ~5.6 mm) (Fig. 2B). Before MRI scanning, the tissue was soaked in saline solution for >48 h to restore T1 and T2 values and water diffusivity to values closer to those found in vivo (29). A cylindrical PVC container (62 mm diameter, 54 mm deep) was designed with a bottom plate with a central groove (59 mm long, 5.5 mm wide, 1.5 mm deep) (Fig. 2B). When the container was placed inside of the scanner, the cylindrical axis of the container was perpendicular to B0 and the orientation of central groove was parallel to B0. The container was filled with saline solution. The tissue pieces fitted tightly into the groove while leaving a large portion of the tissue above the groove. The two long pieces (C1 and C5) were placed at the ends, holding the three cubes (C2–C4) in place, as shown in Fig. 2B.

The fiber orientation in the two long pieces (C1 and C5) and the middle cube (C3) was parallel to B0 whereas the other two cubes (C2 and C4) initially had a primary fiber orientation perpendicular to B0 (condition B). To demonstrate the effects of microstructure and allow separation of these effects from large-scale shape effects, these two cubes of tissue were later rotated by 90 degrees such that their primary fiber orientation was parallel to B0 (condition A). The entire container was placed in a custom-designed passive PVC shim set, similar to that described previously, to reduce the large-scale field effects from the shape of the container (in air). The long axis of the entire square cylindrical tissue assembly was placed parallel to B0 to minimize the contribution of the geometry of the tissue assembly to the measured phase.

**Data Acquisition.** Fig. 1 was acquired using a 7-T ($f_1 = 298.095$ MHz)/94-cm human whole-body system (GE), whereas all other MRI images were acquired in a 7-T ($f_0 = 300.390$ MHz)/30-cm MRI system (Bruker BioSpin) equipped with a 15-cm gradient set (Resonance Research). A 12-cm linear hombuilt birdcage coil was used for excitation, and a four-element homebuilt phased array coil (each of 28 mm diameter) was used for signal reception. First, the tissue was localized using a three-plane localizer sequence, and region-of-interest–based shimming (MAPSHIM; Bruker BioSpin) was performed to increase the magnetic field homogeneity in and around the tissues. Then a DTI was performed to confirm the fiber orientation in the corpus callosum tissue sections. The sequence parameters for the DTI scan were as follows: field of view (FOV), 60 × 60 mm²; in-plane resolution, 0.5 × 0.5 mm²; slice thickness, 0.5 mm; slice gap, 0.05 mm; acquisition bandwidth, 10 kHz; flip angle, 90°; repetition time (TR), 15 s; echo time (TE), 56.4 ms. Four axial slices were acquired over 3 h using a spin-echo sequence with 20 diffusion gradient directions based on the downhill simplex method (35), with a b value of 3,000 s/mm². Three additional baseline images were acquired with no diffusion gradients.

After the DTI scan, a 2D GRE sequence (FOV, 60 × 60 mm²; slice thickness, 0.5 mm; slice gap, 0.05 mm; acquisition bandwidth, 26 kHz; flip angle, 90°; TR, 3 s; TE, 10 ms) was used to acquire phase images over four axial slices over 15 min. After the first GRE scan, the container was removed from the magnet, and the two cubes of tissue (C2 and C4) that originally had a main fiber orientation perpendicular to B0 (condition B) were rotated by 90 degrees such that their primary fiber orientation was parallel to B0 (condition A). When the cubic tissue sections were rotated, great care was taken to avoid moving the other tissue sections. In this way, only the microstructural orientation was altered, leaving the overall geometry and shape of the tissues undisturbed. The tissue phantom was carefully placed back into the scanner, and the three-plane localization was repeated to ensure that the tissue sections were located-

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sufficiently close to their previous positions to obviate the need for any image coregistration in later processing of the two sets of images. Another set of 2D GRE images was acquired with identical parameters, and the DTI scan was repeated as well.

Data Analysis. The DTI data were reconstructed by taking the root sum of squares of the individual coil magnitude images. The reconstructed images were processed further using DTIFIT (36) to generate eigenvectors, eigenvalues, and fractional anisotropy (FA) maps. The FA maps, multiplied by the primary eigenvector maps, were displayed as red-green-blue color-coded images in which each voxel was assigned a color based on the direction of its primary eigenvector and an intensity proportional to its FA.

The GRE magnitude images were reconstructed using the root sum of squares method. Phase images were calculated from the complex sum of the individual coil data after correcting for each coil’s phase offset (37). A circular mask (32 mm diameter) around the tissue region was chosen in which to perform all further phase processing and analysis (Fig. 3, dashed circles). The phase was unwrapped (38), and large-scale background phase variations were removed by subtracting an eighth-order polynomial fitted to the data (in 2D) within the mask. During the polynomial fitting procedure, the tissue sections and nearby areas were not included in the fitting mask, to prevent the removal of phase contrast caused by the tissue structures. The difference image was calculated to illustrate the effect of changing the orientation of the microstructure. This difference image was obtained by subtracting the unwrapped phase images, not the data fitted with an eighth-order polynomial. The background phase variation was removed from the difference image using the procedure described earlier, but with a second-order polynomial fit. This procedure was necessary due to the significant drift in the scanner gradient and/or shim systems between the two acquisitions. The resulting difference image was more reliable than the original phase images because any orientation independent molecular exchange contrast would be canceled out in the difference image. Also, the large-scale background phase variations and polynomial fitting procedure had less effect on the difference images. A cross-sectional profile of the difference image—the average within the central 4 mm of each tissue section—was plotted (excluding air bubbles). In the profile, the values in C1 and C5 were affected by the order of background polynomial fitting and the choice of mask (including the tissues or not) and were less reliable than the values in C2, C3, and C4. All phase images and profiles were scaled (by dividing by 2π × TE) to give frequency images or profiles in Hz. To calculate the frequency shift in each tissue, the central 20 (width) × 23 (length) × 4 (slices) voxels were averaged, excluding air bubble areas. The resulting frequency shift measurements are given as mean ± SD or mean difference ± pooled SD.

Simulation for Anisotropic Susceptibility and Susceptibility Calculation. A computer simulation was performed to test the hypothesis that the observed resonance frequency changes could be explained by anisotropic susceptibility. Square cylindrical structures were designed to match the tissue pieces used in the experiment. The matrix size was 300 × 300 × 300, with 28 × 28 × 28 voxels in C2, C3, and C4. 28 × 28 × 93 voxels for C1; and 28 × 28 × 81 for C5. The susceptibility value for the saline was assumed to be that of water (−9.05 ppm). Frequency maps were calculated by assigning a susceptibility value to each tissue section and performing a Fourier-based field calculation (39). An initial susceptibility model was set up for condition A in which all of the tissues had their primary fiber orientation parallel to B0 and all of the tissue sections were assigned the same susceptibility value (χ0). The simulation was repeated, varying χ0 from −9.085 ppm to −9.105 ppm in steps of 10−5 ppm to see which value gave a minimum root mean squared error between the calculated and measured frequency images (the central 4 mm of the tissues excluding air bubbles and tissue boundaries). Once χ0 was chosen, another computer model was designed to simulate condition B. In this second model, C1, C3, and C5 were assigned χ0, whereas the susceptibility value for C2 and C4 (χ⊥) was varied between −9.090 ppm and −9.110 ppm in steps of 10−5 ppm. Both C2 and C4 were assumed to have the same susceptibility value. A frequency difference image was calculated by subtracting the results of the two simulations. The calculated and measured frequency difference images were then compared within the central 4 mm of the tissue section over all four slices, excluding air bubbles and the tissue boundaries. The susceptibility value that gave a minimum root mean squared error between the measured and calculated frequency difference images was chosen as χ⊥: the susceptibility of tissue sections with a microstructure perpendicular to B0.

In addition to the simulations, in which each tissue section was assumed to have a single susceptibility value, the susceptibility difference between the tissue sections was calculated from the measured frequency difference image. The inverse-Fourier method (16) was applied to the frequency difference image, and the susceptibility was calculated using a truncation value of 5 for the k-space deconvolution filter (16). Note that in this method and in the previous Fourier-based frequency map calculation, the susceptibility value in a voxel is constrained to be single-valued and, therefore, is a partial volume-averaged quantity. For the susceptibility calculation, a slightly larger circular mask (40 mm diameter) was used to avoid effects due to excluding the edges of the tissue sections. Air bubbles were excluded from the calculation to reduce streaking artifacts. A cross-sectional profile through the calculated susceptibility difference map in the original 32 mm mask was plotted as the average within the central 4 mm of the tissue over all four slices. The susceptibility of each tissue segment was calculated as the mean within this central 4 mm by 4.6 mm over all slices (20 × 23 × 4 voxels in each segment), excluding air bubble areas.

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