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Laura O. Thomas,[1] Orest B. Boyko,[1,2] Douglas C. Anthony,[2] and Peter C. Burger[2]

PURPOSE: To provide further quantitative studies concerning the relationship with age between regional brain iron and T2 shortening. **METHODS:** *a*) Quantitative T2 calculations of eight anatomic regions (red nucleus, substantia nigra, dentate nucleus, corpus callosum, caudate, putamen, temporal lobe white matter, and frontal lobe white matter) from T2-weighted spin-echo images were performed in 60 patients aged newborn to 35 years. *b*) Quantitative brain iron concentrations were obtained in six of the eight anatomic regions (red nucleus, substantia nigra, dentate nucleus, corpus callosum, cauda, and putamen) using 13 autopsied brains (newborn to 78 years). Brain tissue from these six regions was digested with 0.6 N HCl–2.5% wt/vol $KMnO_4$ for 2 hours at 60°C. After centrifugation, 0.1 mL of an iron-chelating reagent (2 mol/L ascorbic acid, 5 mol/L ammonium acetate, 6.5 nmol/L ferrozine, 13.1 mmol/L neocuprine) was added and the absorbance was measured at 562 nm/L and compared with a standard curve with ferric chloride. *c*) The in vivo iron concentrations in tissue that were obtained were reproduced in four test tube phantom studies with ferric ammonium sulfate or ferrous ammonium sulfate dissolved in either deionized water or 5% agarose. T2 calculations of the phantoms were made with a single-section multiple repetition time, multiple echo time acquisition. **RESULTS:** *a*) *Clinical T2 calculations*—all eight anatomic regions showed a decrease with age in T2 value, beginning shortly after birth. During the first three decades, the T2 shortening was most significant in the region of substantia nigra. *b*) *Quantitative brain iron*—five anatomic regions but not the corpus callosum demonstrated an age-related increase in brain iron (1449.6 nmol/g for the red nucleus versus 261.8 nmol/g for the corpus callosum). *c*) *T2 effect of iron in vitro*—both the ferric and ferrous iron phantoms showed a decreased T2 value in the in vivo concentration range of iron obtained from the postmortem studies. The T2 shortening was most marked for the ferric phantoms. **CONCLUSION:** There is an age-related accumulation of iron in five regions of the brain, correlating with an associated decrease in T2 value that can be demonstrated in iron phantoms. Brain iron appears to contribute to the progressive decrease of T2 signal that occurs with aging.

Index terms: Iron, brain; Brain, magnetic resonance; Age and aging

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It is clear that the signal intensity on T2-weighted images of cerebral tissues shows considerable variability in regard to both regional anatomy and patient age (1). Studies correlating T2 signal shortening with the semiquantitative estimation of brain iron as inferred from whole-brain sections stained by the Perl method concluded that the deposition of this mineral was important for the mechanism of T2 shortening (2–4). Subsequent studies have challenged this conclusion (5, 6), one investigation suggesting that T2 signal intensity and the concentrations of iron are not closely related (6).

This study was undertaken in an attempt to investigate this issue by determining: *a*) the in vivo regional change in T2 value with age on MR scans of healthy patients; *b*) the iron concentration in these same anatomic regions as a function of age in postmortem studies; and *c*) the concentration of iron that was determined in the postmortem studies and whether it is of sufficient magnitude to alter the T2 signal in aqueous solution and agarose gels.

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Departments of Radiology[1] and Pathology[2], Duke University Medical Center, Durham, NC 27710. Address reprint requests to Peter C. Burger, MD.

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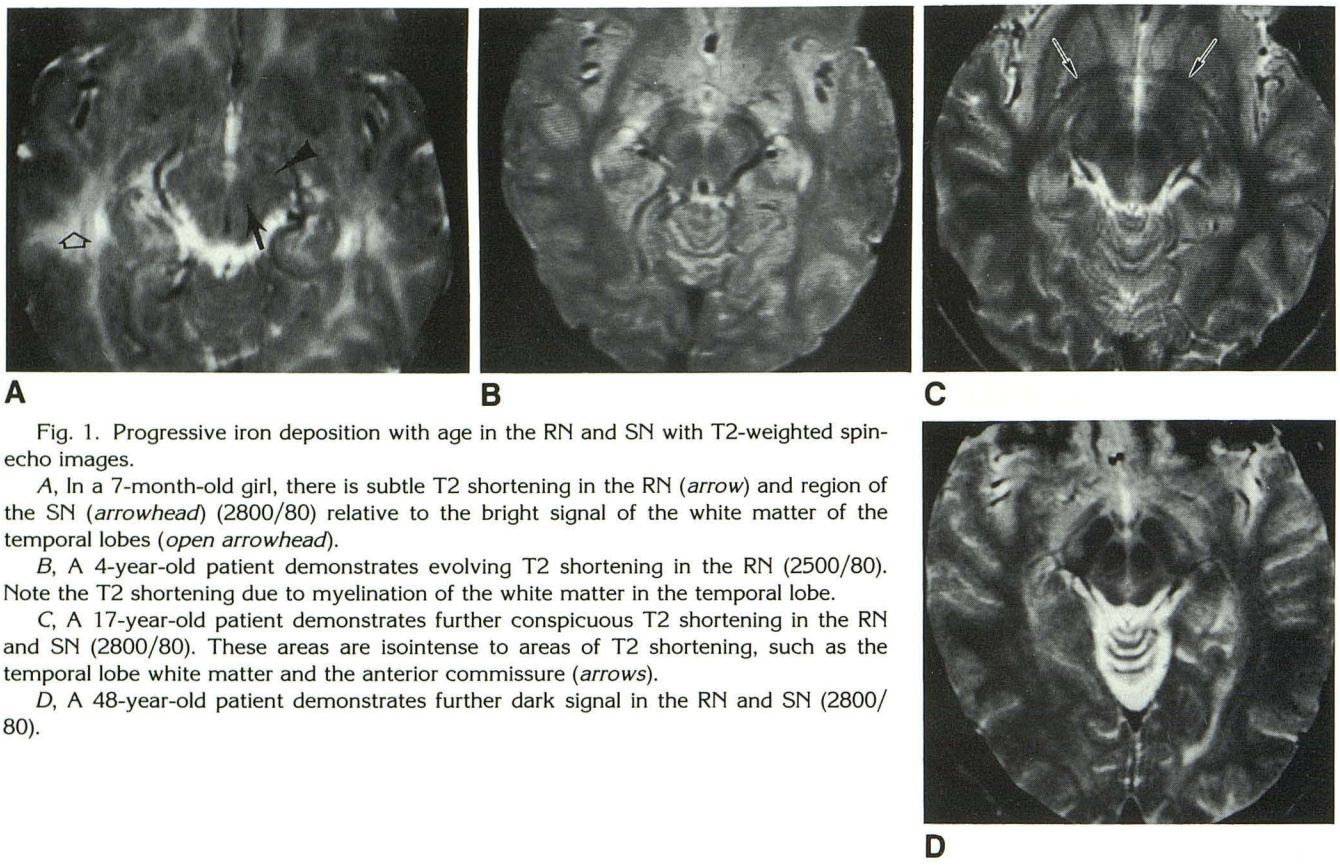

Fig. 1. Progressive iron deposition with age in the RN and SN with T2-weighted spin-echo images.

A, In a 7-month-old girl, there is subtle T2 shortening in the RN (*arrow*) and region of the SN (*arrowhead*) (2800/80) relative to the bright signal of the white matter of the temporal lobes (*open arrowhead*).

B, A 4-year-old patient demonstrates evolving T2 shortening in the RN (2500/80). Note the T2 shortening due to myelination of the white matter in the temporal lobe.

C, A 17-year-old patient demonstrates further conspicuous T2 shortening in the RN and SN (2800/80). These areas are isointense to areas of T2 shortening, such as the temporal lobe white matter and the anterior commissure (*arrows*).

D, A 48-year-old patient demonstrates further dark signal in the RN and SN (2800/80).

Methods

Clinical scans

MR proton images in 60 patients without any mass lesions by spin-echo pulse sequence were randomly selected. Ages ranged from newborn to 85 years. Patients were excluded from the study if there was a gross radiographic abnormality such as tumor, hemorrhage, or post-surgical changes. Quantitative T2 calculations were made from operator-selected regions of interest with standard software (GE Signa Image Analysis functions) with an iterative χ^2 minimization program. T2 values were obtained by the use of the first and second echoes from a dual-echo acquisition. On three scans, images through the basal ganglia were not archived and T2 values for the caudate (CA) or putamen were not available. In pediatric brains, using the standard adult 20-cm field-of-view generated images where the cursor size was occasionally larger than the region of interest (for example, the red nucleus [RN] and substantia nigra [SN]), we centered the cursor over the region of interest to minimize overlap. Eight anatomical regions (RN, SN, dentate nucleus [DN], corpus callosum, CA, PM, and bilaterally, temporal lobe white matter) and frontal lobe white matter were studied. Initially, the globus pallidus was studied also, but because of the heterogenous anatomic organization with gray matter, the presence of white matter, and blood vessels with frequently enlarged perivascular spaces, we could not obtain intrapatient T2 values that were as reproducible as were those for other regions. The globus pallidus was, therefore, not included in the regions studied. Images were obtained with a 1.5-T GE Signa system, T2-weighted spin-echo pulse sequence (2700–3200/30, 80/1 (repetition time/echo time/excitations) acquisition matrix 256 × 256) to produce 5-mm sections with a 2.5-mm intersection gap in the axial plane. The data was plotted with graphics software (Cricket Graph; Cricket Software).

Measurement of brain iron

Samples from 13 formalin-fixed brains were obtained from the Autopsy Service of the Duke University Medical Center. Brains had been fixed in 20% formalin for 2 to 4 weeks. Brains were excluded from selection if there was any intracranial abnormality at the time of autopsy or if a neurologic disease had been noted during life. Tissue samples, 0.1 to 1.0 g, from six anatomic regions (RN, SN, DN, corpus callosum, CA, putamen) were homogenized in 10 mL of distilled water, and 1.0-mL aliquots were digested (7) in triplicate with 0.6 N HCl–2.25% wt/vol $KMnO_4$ for 2 hours at 60°C in a water bath. The samples were stabilized at room temperature for 30 minutes and then centrifuged at 12,000 × *g* for 5 minutes. After the addition of 0.1 mL of an iron-chelating reagent (2 mol/L ascorbic acid, 5 mol/L ammonium acetate, 6.5 mmol/L ferrozine, 13.1 mmol/

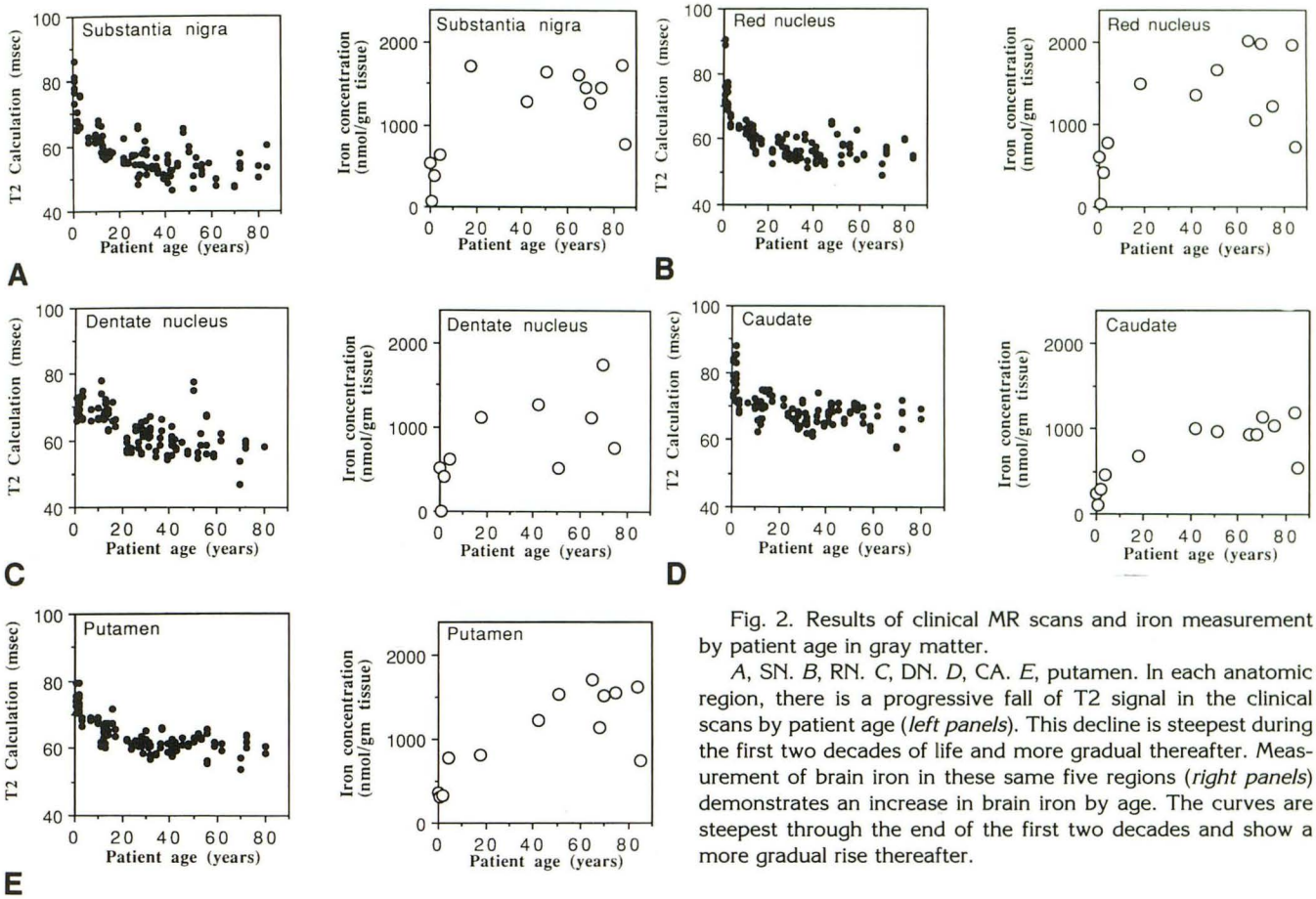

Fig. 2. Results of clinical MR scans and iron measurement by patient age in gray matter.

A, SN. *B*, RN. *C*, DN. *D*, CA. *E*, putamen. In each anatomic region, there is a progressive fall of T2 signal in the clinical scans by patient age (*left panels*). This decline is steepest during the first two decades of life and more gradual thereafter. Measurement of brain iron in these same five regions (*right panels*) demonstrates an increase in brain iron by age. The curves are steepest through the end of the first two decades and show a more gradual rise thereafter.

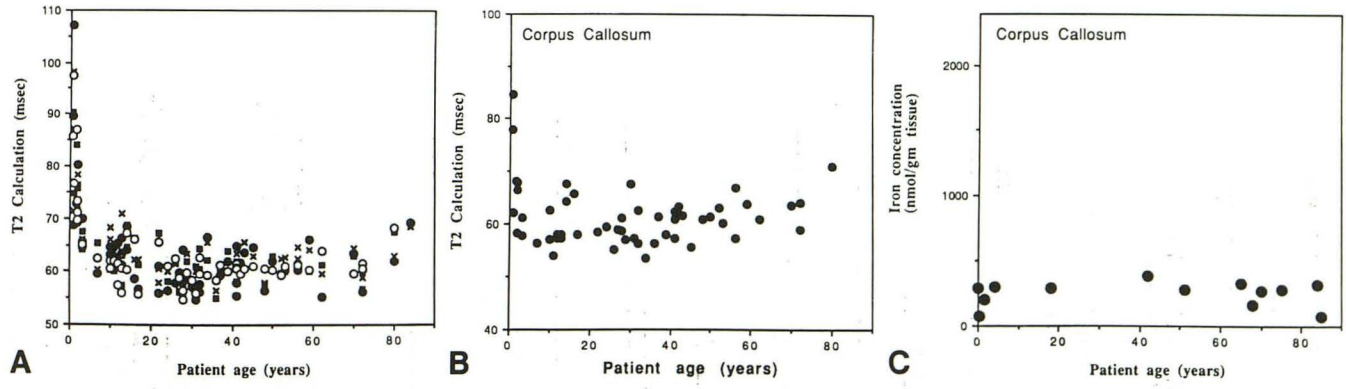

Fig. 3. Results of clinical MR scans and iron measurement by patient age in white matter.

A, T2 values in the temporal and frontal white matter of the right and left sides (x, right temporal lobe white matter; ●, left temporal lobe white matter; ■, right frontal lobe white matter; ○, left frontal lobe white matter).

B, T2 values in the corpus callosum. *C*, Iron concentration measured in the corpus callosum. Early decrease in T2 values is seen coinciding with myelination of the central nervous system, occurring predominantly before the age of two. There is no detectable change in T2 beyond this age, and there is no detectable change in iron levels over the ages studied.

L neocuprine), the absorbance was measured at 562 nm and compared with a standard curve of ferric chloride.

T2 effect of iron in vitro

To evaluate the relationship between T2 shortening and iron concentrations in vitro, ferric ammonium sulfate and ferrous ammonium sulfate solutions were serially diluted to produce concentrations of 0, 0.2, 0.5, 0.75, 1.5, and 2.0 mmol/L in deionized water or in 0.5% agarose. Samples were then scanned in 10-mL plastic tubes placed in a styrofoam container in the same MR scanner with the same quadrature head coil used clinically. T2 images of the

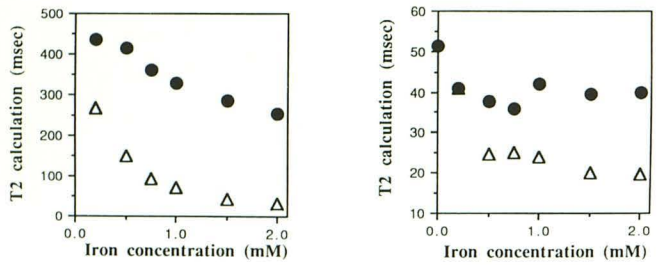

Fig. 4. Relationship between iron concentration (●, ferrous ion; △, ferric ion) and T2 signal in iron-containing solutions (*a*) and in iron-containing agarose phantoms (*b*). An increase of ferrous and ferric ion concentration within the physiologic range depresses the T2 signal. (In water, 1 mmol/L corresponds to 1000 nmol/g.)

phantoms were acquired with a 15-mm single section, multi-TR (3200, 1600, 800, 400, 200 milliseconds) and multi-TE (20, 40, 60, 80 milliseconds) spin-echo acquisition at 256 × 192, 1 signal averaged, 22-cm field of view. Quantitative T2 values were obtained at three different image levels along the tubes, again with the GE software image analysis package, and all measurements were done in duplicate. The final plotted T2 calculation was the average of the six determinations.

Results

Clinical scans

All eight anatomic regions showed a decrease with age in T2 value on clinical MR scans (Figs. 1 through 3). This decrease began shortly after birth and continued through the first three decades in iron-containing gray-matter structures or over the first 2 years in white matter. There was a suggestion of a gradual decrease in T2 throughout the subsequent decades in iron-containing gray-matter structures only. There was considerable overlap in some regions between patients. In all ages over 6 months, the T2 signal was shortest in the region of the SN, followed in turn by the RN, DN, putamen, and CA nucleus.

Measurement of brain iron

All six anatomic regions, with the exception of the corpus callosum, showed an increase in brain iron with age (Fig. 2). This increase was detected as early as 6 months and rose rapidly with a flattening of the curves thereafter. The mean amount of iron in patients over 40 (mean age, 67.5 years), was in nmol/g of tissue, 1449.6 for the RN, 1404.0 for the SN, 1257.1 for the putamen, 1085.0 for the DN, 972.1 for the CA nucleus and 261.8 for the corpus callosum. The level of iron was lowest in the corpus callosum and was independent of age (Fig. 3c).

T2 effect of iron in vitro

The in vitro effect on T2 values of iron in phantoms in the concentration range obtained from the postmortem work was determined (Fig. 4). The effect of increasing the concentration of ferric and ferrous ions on the T2 value in aqueous solutions is shown in Figure 4a. The presence of ferric and ferrous ions in solution decreased the T2 number, with the T2 shortened by 50% at 2 mmol/L ferrous ammonium sulfate and by more than 90% at the same concentration of ferric ions. The effect was especially pronounced for the ferric solution.

The relationship between iron concentration and T2 value in agarose phantoms is shown in Figure 4b. Although the magnitude of T2 shortening is less than in the aqueous solution, the presence of either ferric or ferrous ions clearly demonstrated shortened T2 relaxation.

Discussion

The in vivo experiments presented here recorded a fall with age in the T2 value in all five gray matter and three white matter structures studied, as has been noted daily in clinical practice and as has been documented in previous systematic studies (1). By studying scans of living patients, we found it possible to accumulate a large number of values for T2 in nondiseased tissue. The T2 shortening began shortly after birth and continued during life, although the majority of the change had occurred by the end of the third decade. Because these same areas are those that are deeply and progressively stained by the Perl method (3, 8–14), it seems reasonable to conclude that the amount of iron and the T2 change are related. This relationship is supported by the well-recognized ability of MR to detect hemosiderin around chronic hemorrhagic foci (15–17).

If the progressive shortening of the T2 signal is related to local iron concentration, then one should be able to document a relationship between the magnitude of T2 shortening and the regional concentration of iron. This study records such a relationship with the age-dependent accumulation of iron coinciding with the rate of change of T2 signal. Similar results were reported by Hallgren and Sourander (1, 10, 12, 18).

Despite these concurrent changes in iron concentration and T2 signal, Chen et al (6) have suggested that, although iron in pathologic states such as around hemorrhages can affect T2 relax-

ation time and shorten T2, the normal concentration of iron, even in the aged, is below the threshold of detection by MR. We therefore imaged iron-containing solutions and agarose phantoms to determine whether the presence of iron in the physiologic concentration range is associated with a change in T2 signal intensity and value. In aqueous solutions, increasing the concentration of both ferrous and ferric ions showed a profound effect on T2, an effect much greater than the change in T2 signal with age in normal patients. The curves of quantified brain iron by patient age were thus largely the inverse of the curves tracing the progressive decrease in in vivo T2 shortening with age. Because T2 is so much longer in aqueous solution (500 milliseconds) than in the brain (70 milliseconds), we also studied the effect of iron in agarose phantoms. These revealed much lower T2 values and a less dramatic decline in T2 with increasing iron concentration, but one more comparable to the values seen in patients. Nevertheless, both ferrous and ferric irons led to a progressive decline in T2. Wismer et al (19) have demonstrated a similar effect of Fe_3O_4 on T2 in agar.

Given the concurrent changes in iron and in T2 values that occur with age and the similar effects of equivalent concentrations of iron in vitro, it seems likely that the incremental accumulation of iron in certain gray matter structures accounts for the progressive decrease in T2 relaxation time. We do not, however, suggest that all structures with a low T2 number are rich in iron. Clearly, compact white matter pathways such as the corpus callosum, fornix, and anterior commissure have a short T2 signal that becomes progressively lower with the deposition and maturation of the myelin (20, 21), but not with the accumulation of iron. However, myelination occurs predominantly before the age of two and our data (Fig. 3) support that T2 shortening in white matter does occur in a time frame similar to that for regions containing iron. After this age, however, T2 values are relatively constant in white matter. Thus, it appears that the completion of myelination leads to constant T2 values in white matter. In contrast, T2 values continue to decrease in gray matter structures, even after myelination has been completed, and in the same time frame as iron accumulation.

Both our present quantitative study and our past investigations using the Perl method for iron have shown a very low iron concentration in these heavily myelinated areas (21). This effect of myelin on T2 is independent of iron and may help to explain why Chen et al (6) failed to find a relationship between T2 shortening and iron concentrations when both white and gray matter structures are considered together. When only gray matter structures are analyzed, their data (6) do demonstrate a dramatic relationship between iron and T2 values. This also has been concluded by another author (4). It has been suggested that if only gray matter structures are considered, the relationship between iron and T2 signal can be identified but is less dramatic (4). Our results lead us to concur with the latter conclusion. We extend the work by Chen et al (6) by having done quantitative assessment in iron-containing anatomic regions beyond the basal ganglia and especially in ones that contain no significant amount of myelin (ie, SN).

In light of the low T2 numbers of compact white matter fiber pathways, it is appropriate to ask whether myelin could contribute to the low T2 signal in certain gray matter structures. Myelinated bundles are prominent, for example, in the basal ganglia and RN. It is of interest, therefore, that although our quantitative data show that both the SN and RN have similar amounts of iron, the latter structure is darker on the T2-weighted image. Thus, myelin may contribute to the T2 shortening of certain gray matter structures but cannot account for the progressive T2 shortening with age in the SN, which contains little myelin. One limitation in our study is the difficulty in anatomically positioning the cursor in measuring the T2 of the SN and eliminating contamination of the nearby cerebral peduncle, which is myelinated and has T2 shortening.

Our work leads us to conclude that there is an age-related accumulation of iron in five regions of the brain (RN, SN, DN, CA, putamen), correlating with an associated decrease in T2 value from clinical scans, which can be also demonstrated in iron-containing phantoms. Brain iron appears to contribute to the progressive decrease of T2 signal that occurs with aging in these iron-containing regions of the brain.

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