Published in final edited form as:

Neuroimage. 2011 January 15; 54(2): 750–759. doi:10.1016/j.neuroimage.2010.09.068.

Differential effects of age and history of hypertension on regional brain volumes and iron

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Abstract

Aging affects various structural and metabolic properties of the brain. However, associations among various aspects of brain aging are unclear. Moreover, those properties and associations among them may be modified by age-associated increase in vascular risk. In this study, we measured volume of brain regions that vary in their vulnerability to aging and estimated local iron content via T2* relaxometry. In 113 healthy adults (19–83 years old), we examined prefrontal cortex (PFC), primary visual cortex (VC), hippocampus (HC), entorhinal cortex (EC), caudate nucleus (Cd), and putamen (Pt). In some regions (PFC, VC, Cd, Pt) age-related differences in iron and volume followed similar patterns. However, in the medial temporal structures, volume and iron content exhibited different age trajectories. Whereas age-related volume reduction was mild in HC and absent in EC, iron content evidenced significant age-related declines. In hypertensive participants significantly greater iron content was noted in all examined regions. Thus, iron content as measured by T2* may be a sensitive index of regional brain aging and may reveal declines that are more prominent than gross anatomical shrinkage.

Keywords

MRI; aging; brain; iron; vascular risk; susceptibility weighted imaging

1. Introduction

Aging, even in healthy individuals, is associated with significant brain changes. One of the best established changes in the aging brain is differential brain shrinkage that is prominent in the polymodal association cortices, striatum, and cerebellum and minimal in the primary sensory cortices (Fjell et al., 2009; Pfefferbaum et al., 1998; Raz et al., 2005; for a review see Raz & Rodrigue, 2006; Resnick et al., 2003). However, age effects on the brain are not limited to gross neuroanatomy but appear at multiple levels of analyses, including neurochemistry, vascular function, and neural connectivity (Bäckman et al., 2006; Hedden & Gabrieli, 2004; Madden et al., 2009; see Raz and Kennedy, 2009 for reviews). Furthermore, all aspects of brain aging are sensitive to numerous physiological,

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environmental, and genetic factors. The relationship among structural and metabolic aspects of brain aging and the role of physiological and neurochemical modifiers remains unclear.

One of the most prominent metabolic differences associated with brain aging is alteration of iron homeostasis (Andrews & Schmidt, 2007). Although indispensable in diverse physiological and neurochemical processes, iron is a potent agent of oxidative stress and neurodegeneration (Zecca et al., 2004). In the brain, iron is present in several forms. Ferric heme iron in the hemoglobin molecule makes deoxyhemoglobin paramagnetic (Pauling & Coryell, 1936) and creates local inhomogeneity detectable through magnetic resonance imaging (MRI) via change in $T2^*$ relaxation times (Ogawa et al., 1990). Thus, differences in $T2^*$ can indicate heme iron content. In addition to heme iron in blood that dynamically alters $T2^*$, the brain contains non-heme iron predominantly sequestered in ferritin (Schenk & Zimmerman, 2004). Brain distribution of non-heme iron is uneven and its highest concentration is in the motor nuclei (Hallgren & Sourander, 1958; Haacke et al., 2005). Regional non-heme iron content increases with age (Hallgren & Sourander, 1958; Schenk & Zimmerman, 2004), and in the aging brain there is another contributor of non-heme iron that can alter $T2^*$: iron-rich amyloid plaques (Collingwood et al., 2008; Quintana et al., 2006).

In a comparison of adults with Alzheimer's disease (AD) and mild cognitive impairment to normal controls, Rombouts, Scheltens, Kuijter, & Barkhof (2007) found significant reduction of $T2^*$ in the hippocampus, posterior cingulate gyrus and precuneus, parietal lobe, insula and putamen of AD patients. However, due to image filtering and smoothing, the validity of regional interpretation in this study is unclear. Recently, Sullivan and her colleagues used field dependent relaxation rate measures of $T2^*$ in a small sample of older adults, and observed significant associations between the $T2^*$ values in the basal ganglia and thalamus and cognitive and motor task performance (Sullivan et al., 2009). Notably, the regions examined in that study included neither neocortex nor hippocampus.

Few studies applied $T2^*$ relaxometry to investigation of the aging brain (Haacke et al., 2005). In early attempts, age-related reduction in $T2^*$ signal was observed in the entorhinal cortex (EC) of older adults (Small et al., 1999; 2002), and interpreted as reduction of resting metabolism. However, in those studies, signal intensity, not true $T2^*$ values, were measured from a single slice. Other studies found age-related declines in $T2^*$ in superior frontal gyrus, hippocampus and entorhinal cortex (Raz et al., 2007a), striatum (Siemonsen, et al. 2008; Cherubini, et al., 2009; Haacke et al., in press), globus pallidus (Aquino et al., 2009; Pfefferbaum et al., 2009), and the thalamus (Haacke et al., in press). Although $T2^*$ may not be the ideal *in vivo* index of brain iron (Haacke et al., 2005), it is nonetheless a sufficiently robust correlate of iron content (Ordidge et al., 1994; Martin et al., 1998; 2008, Péran et al., 2009; Duyn et al., 2007; Yao et al., 2009; Shmueli et al., 2009). Although $R2^*$ ($=1/T2^*$) is the sum of spin-spin relaxation rates, $R2$ ($=1/T2$), and the inhomogeneity-induced relaxation rate $R2'$ ($=1/T2'$), in regions with very little myelin the reciprocal of $R2^*$ but not $R2$, correlate highly with iron concentration across a wide range of field strengths (Péran et al., 2009; Yao et al., 2009). From these data, one can conclude that shortening of $T2^*$ is a good proxy for increased iron content. Despite this good correlation, others have suggested that the addition of phase information improves estimates of iron concentration in the basal ganglia (Pfefferbaum et al., 2009). Moreover, the correlations between age and $T2^*$ in the basal ganglia is driven almost entirely by iron-sensitive $T2'$ and not by $T2$ (Siemonsen et al., 2008).

An important factor not addressed in the reviewed studies of $T2^*$ in the aging brain, is the influence of vascular risk. Hypertension, one of the most common vascular risk factors, negatively affects brain structure (Burgmans et al., 2010; DeCarli et al., 1995; Giannaros et al., 2006; Raz, et al., 2003; Salerno et al., 1992). It is also linked to cerebral microbleeds

(CMB), which contain paramagnetic hemosiderin (Harrison & Arosio, 1996; Viswanathan & Chabriat, 2006). Thus, hypertension may have an additional impact on T2* beyond the effects of aging.

In summary, prior studies suggest that through T2* relaxometry, regional differences in heme and non-heme iron content can be detected in normal aging and age-related cognitive disorders. However, regional distribution of T2* values, their relation to age and to structural properties affected by aging, and the role of vascular risk in those associations remain unclear. The goals of the present study were threefold. First, we investigated age differences in iron content indexed by T2* values across several brain regions that differ in their sensitivity to aging. Second, we compared the pattern of age differences in local T2* values to the pattern of volume shrinkage in the corresponding regions and evaluated the relationship between two types of indices of brain aging. Third, we sought to determine whether a vascular risk factor such as hypertension could affect the strength of association between age and regional indices of iron content.

2. Materials and methods

2.1 Participants

The sample consisted of 113 participants recruited through media advertisements and paid for their participation. About 61% of the participants were from a previous preliminary study (Raz et al., 2007a). Persons with a history of cardiovascular, neurological or psychiatric disease, use of anti-seizure medication, anxiolytics, or antidepressants, head trauma with loss of consciousness for more than five minutes, thyroid problems, diabetes mellitus, drug and alcohol problems were excluded from the study. Persons with metal implants and dental prostheses that could affect image quality and T2* values were not included in the study. Participants had at least high school education, were native English speakers and consistent right-handers with Edinburgh Handedness Questionnaire score of at least 75 (Oldfield, 1971). To screen for dementia and depression we used the Mini-Mental State Examination (MMSE, Folstein, et al., 1975, a cut-off of 26), and Center for Epidemiology Studies Depression Scale (CES-D, Radloff, 1977; a cut-off of 15). See Table 1 for sample demographics.

Seventeen participants who reported a diagnosis of hypertension were taking antihypertensive medications: calcium channel blockers – one participant; angiotensin-converting enzyme inhibitors – two; angiotensin receptor II antagonist – one; beta-blockers – two; potassium-sparing diuretics – three; and eight participants took a combination of at least two of these medications. The hypertensive participants were significantly older than their normotensive peers (61.65 years vs. 52.59 years), $t = -3.11$, $p = .003$, but had similar education level ($t = -1.18$, *ns*) and MMSE ($t(24) = -.38$, *ns*). Mean systolic blood pressure for the hypertensive participants (137.85 mm Hg) exceeded that of the normotensive participants (126.71 mm Hg, $t = 2.99$, $p < .005$), whereas diastolic pressure did not (81.09 mm Hg vs. 77.88 mm Hg, $t = 1.91$, $p = .07$). All participants provided written informed consent at the beginning of the study and were debriefed after its completion.

2.2 Procedure

Blood Pressure Measurement—Blood pressure was measured by a mercury sphygmomanometer (Country Technology, Inc., Gays Mills, WI, model 12–525) with a brachial cuff on three separate occasions in participants seated in a comfortable chair in a climate-controlled room. The values were averaged across occasions.

MRI acquisition protocol—Images were acquired on a 1.5 Tesla Siemens Sonata scanner. Regional T2* measurements were obtained from a multi-echo 3D Gradient-Recalled Echo (GRE) sequence with 48 axial slices, echo time (TE) = 10, 20, 30, 40, 50, 60, 70, 80 ms; repetition time (TR) = 100 ms; field of view (FOV) = $256 \times 256 \text{ mm}^2$; bandwidth (BW) = 170 Hz/pixel, flip angle (FA) = 30° , and voxel size = $1 \times 1 \times 2 \text{ mm}^3$. Thus, the 8-echo data were collected in a single high-BW sequence, which further reduced problems associated with motion. Even-number echoes were flow-compensated in the read direction. Sequence duration was 15:22 min. For regional volumetry we acquired a magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence with 144 coronal slices, TE = 3.93 ms, TR = 800 ms, inversion time (TI) = 420 ms, FOV = $192 \text{ mm} \times 192 \text{ mm}$, BW = 130 Hz/pixel, acquisition matrix = 256×256 , FA = 20° , and voxel size = $0.75 \times 0.75 \times 1.5 \text{ mm}^3$. An 8-channel head coil was used for all sequences, thus considerably improving signal to noise ratio.

T2* Relaxometry—The raw data from the multi-echo GRE sequences were analyzed with locally designed software (SPIN; Signal Processing in NMR; Detroit). Images were sorted by echo time. T2* maps were created by fitting the logarithm of the data for each echo on a pixel-by-pixel basis. We used a linear fit on the logarithm of the data, with the number of echoes varying from pixel to pixel depending on the local signal. To reduce error, the signal from longer echoes that fell below two standard deviations of the noise was excluded from the fitting. The noise was calculated from 0.8 times the mean of the Rayleigh noise distribution.)

T2* values were sampled after interpolating the data by a factor of two in both in-plane dimensions of the transverse plane. The short-echo image (TE=10 ms) was used to anatomically identify the ROIs: hippocampal formation (HC), entorhinal cortex (EC), primary visual (calcarine) cortex (VC), superior frontal gyrus (SFG), caudate (Cd) and putamen (Pt). To ensure that roughly the same number of pixels was sampled from each region, an oval-shaped probe the size of 24–36 contiguous pixels (depending on ROI size) was placed within each ROI on the short TE image and copied onto the T2* map in the identical region to obtain the T2* mean and standard deviation values. To avoid partial volume artifacts, the operator was cautious not to place the sampling probe close to CSF. Test-retest reliability was assessed for one operator (KR) on 10 images (for each ROI) on two separate occasions, one week apart. All test-retest reliabilities (ICC3, Shrout & Fleiss, 1979) equaled or exceeded 0.89. The ROIs were determined with the aid of anatomical atlases (primarily Duvernoy, 1999) and were defined and measured as follows.

The T2* values in the head of the *hippocampus* (ICC3 = .90) were measured on four contiguous slices from the first slice on which it was apparent in the axial plane. This encompassed the extent of the anterior portion of the HC, which was chosen because of its reported sensitivity to aging (Hackert, et al., 2002). *Entorhinal cortex* T2* (ICC3 = .91) was measured on three contiguous slices beginning with the first slice of the HC measurement. T2* was measured in the gray matter lining of the *calcarine sulcus* (*primary visual cortex*, ICC3 = 0.89) on three contiguous slices, beginning on the last slice where the superior colliculi remained visible. The *superior frontal gyrus* T2* (ICC3 = 0.96) was measured on six consecutive axial slices beginning on the first slice where the AC-PC line is visible. The T2* values for the *caudate* nucleus (ICC3 = 0.94) and the *putamen* (ICC3 = 0.93) were measured on four consecutive slices from the first slices on which they appeared axially. Figure 1 illustrates ROI demarcation. All T2* measurements were obtained from the left and right hemispheres, separately and averaged.

Volumetry—The MP-RAGE images were reformatted to correct for head tilt, pitch, and rotation using the orthogonal tool in Analyze software (Biomedical Imaging Resource,

Mayo Clinic College of Medicine). All images were aligned along the anterior and posterior commissure line (see Raz, et al., 2004 for details). The hippocampal measurements were performed on images orthogonal to the long axis of the hippocampal formation. Aligned images were re-sliced into $0.5 \times 0.5 \times 0.5 \text{ mm}^3$ contiguous sections.

Images were displayed on a 21" monitor and magnified 2. Each ROI was traced manually with a stylus on a 21" LCD digitizing tablet (Wacom Cintiq 21UX), see details in Raz et al., 2004. Six regions of interest (ROI) were manually traced and measured on the coronal plane: HC, EC, Cd, Pt, dorsolateral prefrontal cortex (PFC), and VC. All ROI areas were measured with the ROI tool in the Analyze software, and volumes were computed by multiplying the sum of areas by the slice thickness. Intracranial volume (ICV) was used to correct for differences in head and body size via a linear equation: $Volume_{adj_i} = Volume_{raw_i} - b(ICV_i - Mean\ ICV)$ for each subject i . In this equation, $Volume_{adj_i}$ is adjusted ROI volume, $Volume_{raw_i}$ is the raw ROI volume, b is the slope of the ROI volume regression on ICV, and $Mean\ ICV$ is the sample mean of the intracranial volume.

Reliability of the regional volumetric measures was assessed by an intraclass correlation formula that presumes random selection of raters, ICC(2) (Shrout & Fleiss, 1979). Four trained and reliable raters obtained a minimum ICC(2) ≥ 0.90 on each region traced.

ROI Demarcation and Tracing Rules

Prefrontal Cortex (PFC)—The PFC was measured on 24 to 36 coronal slices located within 40% of the distance between the genu of the corpus callosum and the frontal pole. After the first slice, every 3rd slice (1.5mm) is measured resulting in a sampling of 8 to 12 total slices for this ROI. The PFC ROI includes superior, middle, and inferior frontal gyri and covers mainly Brodmann areas 9 and 46, and parts of areas 8, 10 and 45. The superior and inferior boundaries of the PFC are the most dorsomedial point of the cortex and the lateral orbital sulcus, respectively. The reliability of this measure is ICC(2) = 0.97.

Hippocampus (HC)—HC volume was measured on a series of 19 to 25 slices aligned perpendicular to the long axis of the right hippocampus. The mammillary bodies define the rostral boundary of the HC, and the slice showing the fornices rising from the fimbria mark the caudal boundary. Care was taken not to include the amygdala in this ROI. The reliability of this measure is ICC(2) = 0.94.

Entorhinal Cortex (EC)—In tracing this region, the rules developed by Insausti et al. (1998) were followed with minor modifications. The EC was traced on every third slice after the first slice spanning a total of 15–18 0.5mm thick slices (beginning with the first slice on which the anterior commissure is visible and continuing to one slice caudal to the posterior limit of the gyrus intralimbicus. The medial bank of the collateral sulcus defined the ventrolateral boundary. The reliability of this measure is ICC(2) = .93.

Visual (Calcarine) Cortex (VC)—The volume of the VC was estimated as the volume of the cortical ribbon lining the calcarine sulcus. This sulcus appears as the most ventromedial sulcus in the temporal-occipital cortex on the coronal slice that is mid-vermis or immediately caudal to mid-vermis. It is measured on the anterior 50% of the coronal slices between the mid-vermal slice and the occipital pole. The inferior and superior boundaries of this ROI are defined as the point at which the opening of the sulcus occurs. At this point, a line is drawn horizontally so that no cortex (dorsal or ventral) outside of the calcarine sulcus is included, confining this ROI to BA 17. VC is measured on a total of 15–18 0.5mm slices, sampling every 3rd slice after the initial slice. The reliability of this measure is ICC(2) = 0.94.

Caudate Nucleus (Cd)—The volume of the head and the body of the caudate were estimated from 15–20 coronal slices. The most rostral slice was the one on which the caudate first appeared; usually lateral to the lateral ventricles. On the one to three sections on which the nucleus accumbens was visible, a diagonal line was drawn from the most inferior tip of the internal capsule to the lateral ventricle. The caudate was traced on every other slice (interslice distance 3 mm) until no longer visible. The reliability of this measure was $ICC(2) = 0.95$.

Putamen (Pt)—The volume of the putamen was measured from 15 to 20 coronal slices (every other slice at the inter-slice distance of 3 mm). The operator began tracing on the most rostral slice on which the putamen was visible and continued to the most caudal slice on which it could be detected. The external capsule demarcated the putamen laterally throughout the ROI. The dorsal boundary of the putamen was the white matter. The internal capsule delineated the medial border until reaching the anterior commissure. Past that point, the globus pallidus became the medial border of the putamen. The limen insulae, optic radiations, the amygdala, the temporal horn, and the anterior commissure served as the ventral boundary of the putamen on various slices. The reliability of this measure was $ICC(2) = 0.93$.

Intracranial vault (ICV)—The auto-trace function in the Analyze program was used to measure ICV. Measurements were taken from 9 slices across the axial plane, 6 mm apart, which encompasses the majority of the cranium excluding only the base of the brain where the cranium is not yet fully apparent and vertex of the brain. A seed point was selected on the edge of the cranium and the auto trace scale was manually adjusted until the entire cranium is outlined by the Analyze ROI tool. The reliability of this measure is $ICC(2) = 0.99$. Illustrations of all ROIs are displayed in Figure 1.

Statistical analyses—The data were examined for the undue influence of outliers. The general linear model approach was used to test all hypotheses in this study. Age (centered at the sample mean) was a continuous independent variable, whereas sex and hypertension diagnosis were categorical factors. Region of interest (ROI) was a categorical six-level repeated measures (within-subjects) factor. Volume and $T2^*$ were used as the dependent variables in separate analyses. All interactions among the predictors were tested but discarded if found non-significant (using a conservative $p > 0.15$ criterion), after which reduced models were tested. Significant age effects for ROIs were examined with linear and polynomial regressions. Strength of associations between age and brain variables were compared by testing correlations with Steiger's test for related correlations (Steiger, 1980).

3. Results

3.1 Age-related regional differences in $T2^*$

The analysis revealed a significant main effect of age on $T2^*$: $F(1, 110) = 93.50, p < 0.001$. Advanced age was associated with shorter $T2^*$ across the sampled ROIs, with the correlation between age and mean $T2^*$ $r = 0.68, p < 0.001$. No effect of sex or its interaction with other variables was found. The within-subjects main effect of ROI was significant and reflected significant regional differences in $T2^*$ values: $F(5, 550) = 122.55, p < 0.001$.

Notably, a significant Age $\times$ ROI interaction, $F(5, 550) = 6.28, p < .001$, indicated that the magnitude of age differences in $T2^*$ varied across the examined regions. Univariate post-hoc tests showed that all regions, except the primary visual cortex, evidenced a significant negative association with age. Table 3 summarizes regression analyses by ROI and scatter plots in Figures 2–4 depict the association of $T2^*$ with age by region. Examination of

overlap between confidence intervals of the regression slopes presented in Table 3 shows the steepest age-related decline was found in the hippocampal formation, which differed significantly from all other ROIs except the caudate nucleus. The caudate nucleus and the putamen showed similar age differences, exceeding the effects of age on T2* in prefrontal and entorhinal cortices, which did not differ from each other. Primary visual cortex presented significantly shallower (and nonsignificant) age slopes than all other regions examined in this study.

To test for nonlinear effects of age on T2*, a quadratic term was added to the regression for each ROI. Analyses revealed a significant quadratic effect only for the superior frontal gyrus T2* (see Table 4). As presented in Figure 3, T2* shortening in that region was apparent from young to middle-age, after which the estimated decline reached a plateau, although an overall increase in variability is apparent in the older adults. Examination of the scatter suggests, however, that the observed nonlinearity is likely to reflect inhomogeneity of variance across the age range with increased variability of T2* observed among the older participants.

3.2 Vascular risk and age-related T2* differences

Effects of Hypertension Diagnosis—To determine if diagnosis of hypertension affected the age-related differences in T2*, the analyses were first rerun with removal of the 17 diagnosed hypertensive participants. The pattern of results remained unchanged. However, due to the vastly unequal size of hypertensive and normotensive groups and the resulting small statistical power to detect differences between them, the hypertensive participants were matched on age, sex, race, and education to 17 normotensive participants in the study to determine if vascular risk played a role in age-related T2* differences, see Table 5 for details on matching.

Results of the GLM analyses indicated a significant main effect of Hypertension diagnosis, $F(1, 30) = 11.45, p < .002$, beyond the main effect of age, $F(1,30) = 6.36, p = .02$. There was no difference among ROIs in the effect of Hypertension: ROI $\times$ Hypertension interaction $F < 1$, ns. The differences in regional T2* means between normotensive and hypertensive groups are displayed below in Figure 5.

3.3 Regional brain volume and age

To examine the effect of age on regional brain volume, a mixed general linear model was fitted to the data, with Age, entered as a continuous predictor and Sex and Hypertension entered as categorical predictors. ROIs (HC, EC, PFC, VC, Cd, Pt) formed a six-level repeated-measures factor. Regional brain volume was a dependent variable.

The analyses revealed a significant main effect of Age on volume, $F(1, 108) = 28.15, p < .001$, i.e., advanced age was associated with smaller regional brain volumes across the ROIs. The main effect of Hypertension was not significant, $F < 1$. A within-subjects effect of ROI, $F(5, 540) = 1336.06, p < .001$, and the ROI $\times$ Age interaction, $F(5, 540) = 10.69, p < .001$, were significant, indicating that the association of regional volumes with age varied across the ROIs. There was also a significant main effect of Sex, $F(1, 108) = 4.23, p < .05$. However, these effects were qualified by a significant ROI $\times$ Age $\times$ Sex interaction, $F(5, 540) = 3.91, p < .002$, which indicated that the age differences in regional brain volumes varied across regions and between the sexes. Univariate post-hoc analyses revealed differential effects of age on regional brain volume for women and men, with women showing greater age-related differences in the hippocampal formation, prefrontal cortex and caudate nucleus than men. Men showed no significant age differences in those regions.

To assess the effect of hypertension diagnosis on regional brain volume, we repeated GLM analyses previously conducted on T2* values, in the group of 34 demographically matched hypertensive and normotensive controls. In contrast to the T2* analyses, we found neither group differences by Hypertension diagnosis nor ROI $\times$ Hypertension interaction (all $F < 1$).

3.4 Age-related differences in T2* versus age differences in volume

To test whether age differences in regional volumes and in T2* differed in magnitude, we compared the correlations between each region's volume and age with age-T2* correlations for the same or equivalent region using Steiger's Z* statistic (Steiger, 1980). The results show that with the exception of the medial-temporal regions, all ROIs showed equal strength of age differences for T2* and volume. For HC, the association between age and T2* ($r = -0.54$) was significantly stronger than that between HC volume and age ($r = -0.37$), whereas for the EC, the difference between a significant association of T2* and age ($r = -0.37$) and nonsignificant correlation between EC volume and age ($r = -0.15$) reached only a trend level ($p = 0.075$).

3.5 Associations between regional T2* and volume

We assessed a possible contribution of regional T2* to explaining age differences in regional brain volumes in a series of univariate analyses. In those analyses, Age, Sex, Hypertension diagnosis and regional T2* served as predictors of the corresponding brain volume for each ROI. The analysis for the entorhinal cortex revealed a significant Age $\times$ T2* interaction, $F(1, 107) = 3.80, p = 0.05$, indicating that only older adults (65 years or older, $N = 43$) showed a positive association between entorhinal T2* and volume, with smaller volumes corresponding to shorter T2*, $t(42) = 2.38, p = 0.02$. For other regions, the effect of regional T2* on brain volume depended on sex. In the HC, a trend for Sex $\times$ T2* interaction was noted: $F(1, 107) = 3.53, p = 0.06$. A significant positive association between the hippocampal volume and HC T2*, was observed only for women: $F(1, 72) = 4.35, p = .04$. In the basal ganglia, Age $\times$ Sex $\times$ T2* interactions were found for both Cd, $F(1, 104) = 4.10, p = .05$ and Pt, $F(1, 104) = 6.20, p = .01$. For Pt, the sex effect was due to an Age $\times$ T2* interaction for men only, $F(1, 32) = 3.87, p = .06$, with older men only showing a positive association between T2* and volume. For the Cd, the interaction was also due to the presence of an Age $\times$ T2* effect among men, but not among women. However, when one outlier (a 66 year old male with the largest Cd volume in the sample, 11.09 cm^3 , Studentized residual = 3.98) was removed the higher order interaction effect became nonsignificant. In the neocortical regions, primary visual and prefrontal, there were no significant contributions of T2* to local volume variance.

4. Discussion

To the best of our knowledge, this is the first study to examine *in vivo* the relationship between regional brain iron content and age-related differences in regional brain volumes in healthy adults. An excellent correspondence between the T2* values and regional concentrations of non-heme iron (from Table 3, Haacke et al., 2005), supports the validity of T2* as an index of iron content (see Figure 6). Although we observed age-related increase in iron content, the regional pattern of T2* shortening differed from that of age-related shrinkage.

In the neostriatum and the prefrontal cortex, age-related differences in volume and iron content were of similar magnitude, and no age differences were found in the primary visual cortex by either method. In contrast, within medial temporal lobe, age-related differences in T2* exceeded the differences in volume. It is unclear, whether this reflects increased

sensitivity of T2* to aging, a response to age-related changes, or an early indication of incipient pathology.

T2* is affected by local content of two types of iron: heme, mainly blood-born, and non-heme, mainly sequestered, in ferritin within glia and endothelium and in transferrin within the neurons (Andrews & Schmidt, 2007; Benarroch, 2009; Haacke, et al., 2005). Thus, the differences in T2* may stem from alterations of cerebral microvascular dynamics and tissue oxygenation and/or from the changes in non-heme iron homeostasis.

Non-heme iron is distributed unevenly throughout the brain (Haacke et al., 2005; Hallgren & Sourander, 1958; Morris et al., 1992), and its regional contents increase with age (Hallgren & Sourander, 1958). Concentration of iron-binding proteins increases with age in the frontal white matter, basal ganglia, and substantia nigra (Bartzokis et al., 2007; Connor et al., 1995; Zecca, et al., 2001). Although, in contrast to the basal ganglia, non-heme iron content in the neocortex is relatively low (Hardy et al., 2005; Ogg, 1999), direct experimental manipulations of iron content show that the influence of deoxyhemoglobin and its heme iron on neocortical T2* is negligible in comparison to that of ferritin, i.e. non-heme iron (Fukunaga et al., 2010; Lee et al., 2010). It is possible, however, that the role of heme iron in cortical T2* increases with age because of changes in cortical vasculature. The findings by several research groups (Asslani et al., 2009; Heo et al., 2010; Small et al., 2004) suggest that for the medial temporal lobe this may be the case. However, because that work was limited to the hippocampal formation and the entorhinal cortex, it is still unclear whether their findings generalize across neocortical regions.

In comparison to the neocortex and the striatum, the medial temporal regions present additional difficulty in interpretation of T2* differences, because they may contain iron trapped in the amyloid plaques (Bartzokis, et al., 2007; Collingwood et al., 2008; Quintana et al., 2006). Indeed, hippocampal ferritin content is elevated with age (Bartzokis, et al., 2007). Postmortem (Price et al., 2009) and *in vivo* (Bourgeat et al., 2010; Rowe et al., 2007) studies show significant amyloid load in 22–34% of cognitively normal persons (for review see Rodrigue, et al., 2009). Amyloid burden in the inferior temporal cortex correlates inversely with hippocampal volume (Bourgeat et al., 2010); limbic and neocortical plaque counts obtained postmortem are associated with suppressed memory scores measured before death (Price et al., 2009). It is possible therefore, that differential age effects on T2* of the medial temporal structures reflect elevation in the amyloid plaque load therein. Early accumulation of amyloid plaques involves only the entorhinal and perirhinal cortices, with later proliferation into the hippocampus and subsequent involvement of the neocortical sites (Braak & Braak, 1991, 1997). It is noteworthy in that respect that we observed a significant association between EC volume and T2* only in the older participants, and no such association was found in the neocortical regions that are not expected to contain a significant number of plaques. This pattern suggests the possibility that amyloid plaques in the EC significantly contribute to T2* shortening in that structure. However, only concurrent assessment of brain iron content and amyloid burden *in vivo* can test that proposition.

The third goal of the current study was to investigate the modifying effect of hypertension on T2*. We found that groups of hypertensive individuals carefully matched to their normotensive peers evidenced comparatively shortened T2* (i.e., greater iron presence) in all examined regions. Thus, hypertension may be associated with increased content of heme and non-heme iron contents beyond the normal effects of age. The addition of a vascular risk factor on top of the normal effects of aging resulted in two distinct findings. First, the hypertensive group displayed shorter T2* than the control group. Second, the differentiated pattern of brain aging (as measured by iron content inferred from T2* measures) was lost in the hypertensive group. Thus, this study replicates and extends previous findings which

suggest that vascular risk exacerbates brain aging and is associated with an anterior-to-posterior gradient of regional decline (Raz et al., 2007b), and extends this pattern from the structural level to the level of brain iron homeostasis. Notably, all hypertensive participants were treated well with anti-hypertensive agents. It is unclear, however, what if any effect different antihypertensive medication might have had on brain iron content. This important question should be addressed in future studies with larger number of participants undergoing various types of anti-hypertensive therapy.

Although the presence of non-heme iron may partly explain the effect of hypertension, T2* variations across the regions may also reflect differences in deoxyhemoglobin content in the local vasculature. Hypertension is associated with increased vascular resistance (Seals et al., 2006), and a particular vulnerability to hypoperfusion (Bang et al., 2008; Dai et al., 2008; Nobili et al., 1993) and ischemia (Ay et al., 2005; Baumbach & Heistad, 1988; Yao et al., 1991; Schwartz et al., 2007), which may account for a significant share of the T2* shortening. Although most of the hypertensive participants were taking anti-hypertensive medications that could improve cerebral perfusion (Bertel et al., 1987; Efimova et al., 2008), such improvement was likely to be only partial (Nobili et al., 1993). Notably, the negative effect of hypertension on age-related differences was observed in regional T2* values but not in regional volumes. Thus, it is possible that T2* is a more sensitive index of brain aging than regional volumes and that changes in T2* due to iron accumulation precede local shrinkage.

Finally, age-related shortening of T2* may reflect hemosiderin in CMB. Although CMB are relatively common in the general population of elderly (Greenberg et al., 2009), it is unlikely that many are present in this selective sample. In a sample with less stringent exclusion criteria, only 11% of healthy participants had CMB and none had more than three (Ayaz et al., 2010). Nonetheless, as CMB are more likely in persons with hypertension (Ochi et al., 2009; Sun et al., 2009; Viswanathan & Chabriat, 2006) they could have contributed to the observed T2* differences between hypertensive and normotensive participants. Even in that case, however, CMB would be observed in the regions that, with the exception of neostriatum, were not sampled in this study: deep white matter, thalamus, cerebellum, and brainstem (Kato et al., 2002).

T2* maps used in this study included no phase information and thus cannot distinguish between iron and other inorganic materials, such as calcium (Chavhan et al., 2009). In normal adults, calcification is common in the pineal, choroid plexus, and leptomeninges (Vigh et al., 1998) as well as in the basal ganglia. In this study, only in the latter could calcification confound the effect attributed to iron. In light of the excellent correspondence between regional iron concentration values and the observed T2*, this confound unlikely.

Some previous studies (Siemonsen et al. 2008) indicate that because T2* is a combination of T2 (spin-spin relaxation) and T2' (inhomogeneity effect), the latter may be a better index of iron content. Because T2 values were not available in this study, we could not compute T2'. However, age differences in T2* and T2' in gray matter (e.g., striatum) are equivalent (Siemonsen et al., 2008) and it is unlikely that use of T2' would alter the results.

This study has additional limitations. First, we excluded the participants with many common age-related pathologies and risk factors (e.g., heart disease and diabetes). Thus, our sample is not representative of aging in the general population. Second, the relatively low field strength employed in this study could have been insufficient for detection of more subtle differences in T2* that are now possible with higher-field magnets, which in spite of the added susceptibility artifacts, afford a substantially greater contrast-to-noise ratio (Duyn et al., 2007; Shmueli et al., 2009).

4.1. Conclusions

In conclusion, the results of this study show that regional iron concentration is sensitive index of brain aging. In longitudinal study, *in vivo* assessment of regional content of brain iron may shed light on the origins of age-related regional brain shrinkage. Moreover, altered iron metabolism may mediate exacerbation of brain aging by vascular risk factors. The mechanisms underpinning the relative contribution of various types of iron to age-related T2* shortening remain to be determined. Developing more sensitive and specific MRI methods of estimating brain iron will allow a better understanding of processes that link structural and metabolic aging of the brain. The results reported here underscore the importance of closely monitoring age-related changes in vascular health, as they can be modified by behavioral and pharmacological means (Nilsson, 2008).

Acknowledgments

The work was supported by the National Institutes of Health (grants R37 AG-011230 to NR, training grant T32 HS-013819 to KR via the Institute of Gerontology), and a Dissertation Award from the American Psychological Association (to KR). We thank Hanzhang Lu for valuable comments.

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Figure 1.

Definition of the regions of interest (ROI), indicated in red. Each ROI was marked on the T1-weighted image (first echo, TE = 10 ms, the a. panel throughout the figure), and copied to the T2* map (indexed as b. in the figure). T2* values that were computed from eight echoes were read from the T2* map. The figure presents two neocortical ROIs (1), two medial-temporal ROIs (2), and two neostriatal ROIs (3). In T2* maps, the values above 100ms were set to zero to make GM/WM more easily visible.

Figure 2.
Volume and T2* as a function of age in the hippocampus and the entorhinal cortex.

Figure 3.

Regression of volume and T2* on age in the neocortical regions: prefrontal and occipital. The correlation coefficient for the superior frontal gyrus (a part of the lateral prefrontal cortex) T2* is for the combination of linear and quadratic components. For the linear component only, $r = -.001$.

Figure 4.
Regressions of volume and T2* on age in the caudate nucleus and putamen.

Figure 5. Regional T2* differences between hypertensive and normotensive participants: Group means and standard errors of the means.

Figure 6.

Calculated regional T2* correspond well to concentrations of iron (C) found in postmortem material (medians from multiple studies summarized in Haacke et al., 2005, Table 3). Note, that because there are no reports on the entorhinal cortex iron content, we used the reported hippocampal values. The values of T2* and C, though age-sensitive, are averaged across the respective life-span samples. Vertical bars are standard errors of T2* means. Abbreviations: Pt –putamen, Cd –caudate nucleus, VC –(primary) visual cortex, SFG –superior frontal gyrus, EC –entorhinal cortex, HC –hippocampus.

Table 1

Demographic characteristics of the sample and comparison of men and women.

	Age	Education	MMSE	CES-D
Total (N = 113)	53.96 ±15.39	15.63 ±2.44	28.62 ±1.12	4.59 ±4.14
Women (N =76)	51.76 ±15.71	15.35 ±2.40	28.78 ±.98	4.57 ±4.14
Men (N = 37)	58.46 ±13.82	16.19 ±2.46	28.27 ±1.31	4.65 ±4.18
<i>t</i> -test for difference	-2.31	-1.70	2.14	-0.10
<i>p</i> value	0.02	0.09	0.04	<i>p</i> = <i>ns</i>

Note. MMSE – Mini Mental State Examination; CES-D – Center for Epidemiologic Studies Depression Scale. The descriptive statistics are means ± standard deviations; the comparison of men and women is conducted via a two-sample t-test.

Table 2
Pearson correlations among age and regional T2* and the variables' means and standard deviations

	Age	HC	Cd	Pt	EC	SFG	VC
HC	-.54***						
Cd	-.50***	.37***					
Pt	-.50***	.23*	.49***				
EC	-.37***	.36***	.15	-.07			
SFG	-.38***	.44***	.30***	.25**	.26**		
VC	-.08	.20*	.12	.09	.11	.17	
Mean	53.96	68.72	57.81	54.18	69.10	66.33	66.65
Std dev	15.39	8.22	7.40	6.04	6.74	6.40	4.67

Note. HC – hippocampus; CD – caudate nucleus; PT – putamen; EC – entorhinal cortex; SFG – superior frontal gyrus; VC – primary visual cortex.

* p < .05,
** p < .01,
*** p < .001.

Table 3

Univariate regressions of regional T2* on age

ROI	Slope (ms/year)	SE	95% Confidence Limits		t	p
			lower	Upper		
HC	-.286	.0429	-.3329	-.2471	-6.68	.001
CD	-.242	.0395	-.2795	-.2005	-6.13	.001
PT	-.196	.0323	-.2323	-.1677	-6.05	.001
EC	-.164	.0386	-.1986	-.1214	-4.25	.001
SFG	-.160	.0365	-.1965	-.1235	-4.37	.001
VC	-.023	.0288	-.0488	.0088	-.81	Ns

Note. HC – hippocampus; CD – caudate nucleus; PT – putamen; EC – entorhinal cortex; SFG – superior frontal gyrus; VC – primary visual cortex; SE – standard error; 95% CI – upper and lower confidence intervals. Slope is estimate of T2* ms slowing per calendar year.

Table 4

Linear and quadratic effects of age on T2*

ROI	Regression Coefficient		R ²		F (1, 110)	p
	linear	quadratic	linear	quadratic		
HC	-.29	-.003	.285	.294	1.40	ns
EC	-.16	-.002	.140	.149	1.16	ns
VC	-.02	-.002	.005	.015	1.12	ns
SFG	-.16	-.005	.146	.186	5.40	.05
Cd	-.241	-.001	.253	.253	< 1	ns
Pt	-.20	-.001	.248	.250	< 1	ns

Note. HC – hippocampus; Cd – caudate nucleus; Pt – putamen; EC – entorhinal cortex; SFG – superior frontal gyrus; VC – primary visual cortex. Linear slope is estimate of T2* ms slowing per calendar year; quadratic slope is estimate of T2* ms slowing per year squared; R² is proportion of variance explained by age.

Table 5

Sample demographics for matched hypertension analyses

	Age	Education	MMSE	Systolic	Diastolic
Hypertensive Group 4 men, 13 women	61.65 ±10.01	16.35 ±2.80	28.71 ±.99	137.85 ±10.37	81.09 ±5.12
Control Group 4 men, 13 women	61.53 ±10.06	16.00 ±2.31	28.88 ±.93	126.71 ±11.33	77.88 ±4.59