

Topographical Localization of Iron in Brains of the Aged Fat-Tailed Dwarf Lemur (*Cheirogaleus medius*) and Gray Lesser Mouse Lemur (*Microcebus murinus*)

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Iron deposits in the human brain are characteristic of normal aging but have also been implicated in various neurodegenerative diseases. Among nonhuman primates, strepsirrhines are of particular interest because hemosiderosis has been consistently observed in captive aged animals. In particular, the cheirogaleids, because of their small size, rapid maturity, fecundity, and relatively short life expectancy, are a useful model system for the study of normal and pathological cerebral aging. This study was therefore undertaken to explore iron localization in the brain of aged cheirogaleids (mouse and dwarf lemurs) with histochemistry and magnetic resonance microscopy. Results obtained with both techniques were comparable. There was no difference between old animals in the two species. The young animals (3 years old) showed no iron deposits. In the old animals (8–15 years old), iron pigments were mainly localized in the globus pallidus, the substantia nigra, the neocortical and cerebellar white matter, and anterior forebrain structures, including the nucleus basalis of Meynert. This distribution agrees with previous findings in monkeys and humans. In addition, we observed iron in the thalamus of these aged nonhuman primates. Microscopic NMR images clearly reveal many features seen with the histochemical procedure, and magnetic resonance microscopy is a powerful method for visualizing age-related changes in brain iron. *Am. J. Primatol.* 45:291–299, 1998. © 1998 Wiley-Liss, Inc.

Key words: cheirogaleids; iron; histochemistry; MR microscopy; neurodegenerative diseases

INTRODUCTION

It has been observed in various mammals, including monkeys and humans, that iron content in the brain increases progressively with age and is unevenly distributed [for a review see Koeppen, 1995]. Studies revealed high concentrations in the globus pallidus, the substantia nigra, the dentate nucleus, the red

Contract grant sponsor: von Humboldt Foundation; Contract grant sponsor: Del Webb Foundation; Contract grant sponsor: Human Brain Project; Contract grant sponsor: National Institute on Drug Abuse; Contract grant sponsor: National Institute of Mental Health; Contract grant sponsor: National Science Foundation.

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Received for publication 23 June 1997; revision accepted 27 January 1998.

nucleus (only in humans), the subthalamic nucleus, and to a lesser extent in the putamen and the caudate nucleus. These brain regions are mainly involved in various aspects of motor behavior.

The question of iron absorption in captive strepsirrhine primates is of particular interest. Spelman et al. [1989] observed that all 49 lemurs necropsied since 1968 at the San Diego Zoo were hemosiderotic. Diet may have contributed to this situation. As in humans, the severity of the disorder was correlated to age, and the liver and kidney were especially affected. The work of Spelman et al. [1989] as well as previous studies by Benirschke et al. [1985] and Gonzales et al. [1984] described iron overload in various lemur body organs, including the duodenum, liver, kidney, and spleen. Brain iron content was not examined in these zoo autopsy studies. The brain is nevertheless probably more affected by iron overload than any other body organ. In healthy adult human brains, the total concentrations of iron in the globus pallidus, substantia nigra, and red nucleus (circa 20 mg/100 g) are higher than in healthy adult human liver (13 mg/100 g) [Hallgren & Sourander, 1958].

Of strepsirrhine primates, cheirogaleids are of special interest. The mouse lemur provides a good model for the study of cerebral aging. Some aged specimens (8–11 years old) undergo neuropathological and behavioral changes quite similar to those seen in normal and pathological human aging [Bons et al., 1995]. Iron homeostasis is disrupted in the aging brain, and increased iron-catalyzed peroxidative damage has been implicated in various neurodegenerative diseases in humans [Gelman, 1995; Imon et al., 1995].

The present study was therefore undertaken to explore iron in the brain of aged cheirogaleids. Their small size allows one to observe age-dependent processes with high-field magnetic resonance microscopy.

MATERIALS

Specimens included aged animals (one 15-year-old male dwarf lemur, one 12-year-old female mouse lemur, and two 8-year-old male and female mouse lemurs) and young animals (one 3-year-old male dwarf lemur and two 2-year-old male and female mouse lemurs). The mouse lemurs were obtained from the Duke University Primate Center. All animals died of natural causes. The housing conditions and the diet of the dwarf lemurs were similar to the ones described in Lee et al. [1996]. The mouse lemurs were fed cracked Purina Old World Monkey Chow (5038), chopped fruits, vegetables, and crickets. The chow contains 180 parts iron per million. There was no supplementation with vitamins or minerals [David Haring & Janet Baer, personal communications].

METHODS

Magnetic Resonance Microscopy

Iron's paramagnetic characteristics allows for its visualization using magnetic resonance imaging (MRI). Brain regions with high ferric iron content have short relaxation times, yielding a hypointense (dark) signal in T2*-weighted images as compared to regions with low ferric iron content. This effect is more prominent at high-field strengths [Schenck, 1995]. MR microscopic images of the intact thawed cadaver head were made using a Bruker AMX500 11.7-Tesla MRI system (Ettlingen, Germany). Images were acquired using T2*-weighted 3-D Gradient Echo scheme [Haase et al., 1986; Chien & Edelman, 1991] with TR = 100 ms, TE = 9 ms, 30 micron isotropic voxel resolution, and ~4 h imaging time.

Histology

Within 24–72 h after MR imaging, the brains were removed and refrigerated in 10% formalin solution with 30% sucrose overnight. Serial sections were cut in sagittal and coronal planes at 50 microns on a cryomicrotome. The sections were kept as free-floating sections [Hill & Switzer, 1984] and divided into three sets of alternating sections. The first set of sections was immersed at room temperature for 30 min in Perl's (Prussian) solution (freshly prepared solution of equal parts 2% HCL and 2% potassium ferrocyanide). The Perl's blue reaction is a histochemical stain for non-heme iron [Pearse, 1961]. This reaction depends on the production of ferric ferrocyanide when ferric ions (Fe^{3+}) in the tissues react with ferrocyanide in acid solution. It generally reflects the quantity of Fe^{3+} stored in the brain parenchyma. We counterstained with pararosaniline for cell nuclei. The second set of sections was processed with Congo red stain and the third set treated with the Bielschowsky silver impregnation for amyloid plaques and degenerated neurites. The sections were then mounted on gelatin-coated slides.

RESULTS

External morphology of the brains showed no age-related changes. The 3-year-old animals showed no iron deposits. In the old animals, histochemistry demonstrated the presence of numerous Prussian blue-positive cells in tissue sections corresponding to the dark regions in NMR images (Figs. 3–5). The greatest concentration of iron was observed in the globus pallidus, the substantia nigra, and the neocortical white matter (corpus callosum) (Figs. 1–5). Iron deposits are also present in the striatal fibers, in more anterior forebrain structures including the nucleus basalis of Meynert, the diagonal band of Broca, the septum,

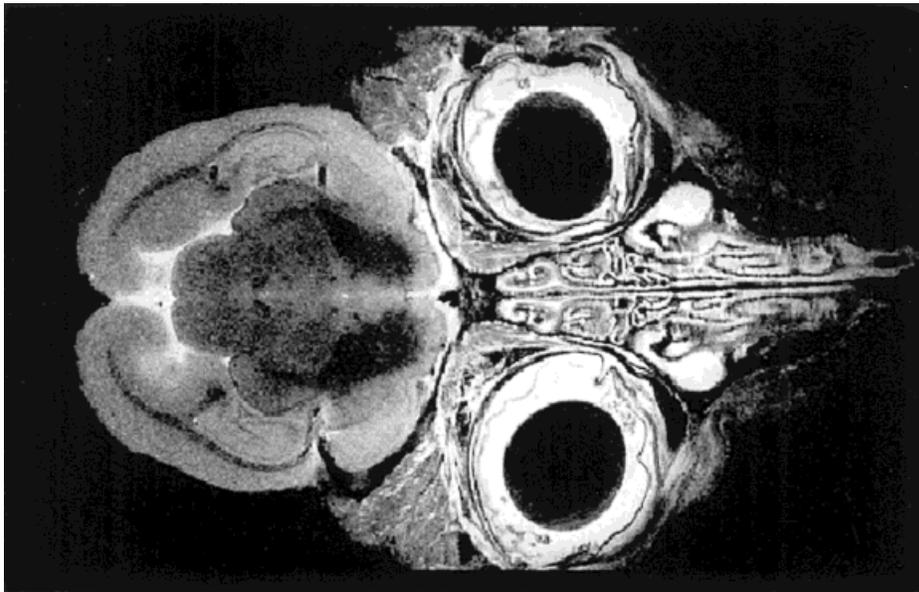


Fig. 1. Axial MR scan T2*-weighted image of the 12-year-old dwarf lemur brain (skull partially removed). The hypointense (dark) signal corresponds to iron accumulation in the globus pallidus, the striatal fundus, and the nucleus accumbens.



Fig. 2. Coronal MR scan T2*-weighted image of the 15-year-old dwarf lemur brain. The hypointense (dark) signal corresponds to the globus pallidus.

and the nucleus accumbens, in the olfactory bulb, the thalamus, the subthalamic nucleus, the cerebellar nuclei (especially the dentate nucleus), and the cerebellar white matter. The anterior commissure (entorhinal and inferotemporal interhemispheric connections), the optic chiasm, and the fornix are densely stained. Lower levels of the ferrocyanide reaction product are also found in the hypothalamic nuclei, including the zona incerta (fields of Forel) and in mid- and hindbrain structures, including the inferior and superior colliculi and the interpeduncular nucleus. In white matter, iron is mainly present in a diffuse form. Elsewhere, the iron-positive structures contain numerous fine granular deposits localized around the cell nuclei and in the cell processes (Fig. 6).

We observed no evidence of amyloid plaques or degenerated neurites in our specimens, and we observed no significant difference in histochemical and MR characteristics between the two species.

DISCUSSION

Although the function of the uneven distribution of iron is unknown, iron metabolism plays an essential role in normal brain function, and its homeostasis is tightly controlled [Gelman, 1995; Connor & Menzies, 1995]. Iron is preferentially localized around the nuclei and in the processes of glial cells (astrocytes, oligodendrocytes) and in the macrophages. It can also be found in association with the perikarya and neuronal processes of nerve cells, especially in the globus



Fig. 3. Parasagittal MR scan T2*-weighted image of the 15-year-old dwarf lemur brain. Arrowheads, main iron deposits.

pallidus and substantia nigra [Hill & Switzer, 1984; Morris et al., 1992]. A comparable cellular distribution is observed in aged cheirogaleids (Fig. 6). Iron deposits can be classified as diffuse and finely granular [Spatz, 1922; Connor & Menzies, 1995]. The diffuse iron content of white matter defines the blue matrix and corresponds to the staining of myelinated axonal systems [François et al., 1981]. Relationships exist between iron acquisition and myelin production [Connor & Menzies, 1996].

Most of the finely granular brain iron is present as ferritin, a major iron-regulatory protein. The contribution of ferritin is very high in iron-rich gray matter

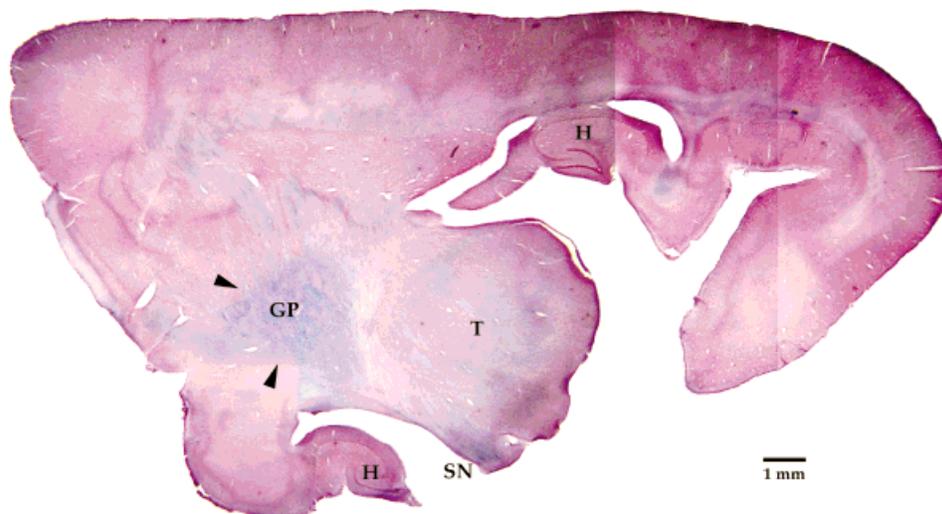


Fig. 4. Parasagittal cryostat section of the aged dwarf lemur brain corresponding to the parasagittal MR view of Fig. 3 (Prussian blue reaction and pararosaniline stain). Arrowheads, main iron deposits; GP: globus pallidus; H, hippocampus; SN, substantia nigra; T, thalamus. Iron reaction product is intense in the globus pallidus and the substantia nigra and can also be seen in the thalamus.

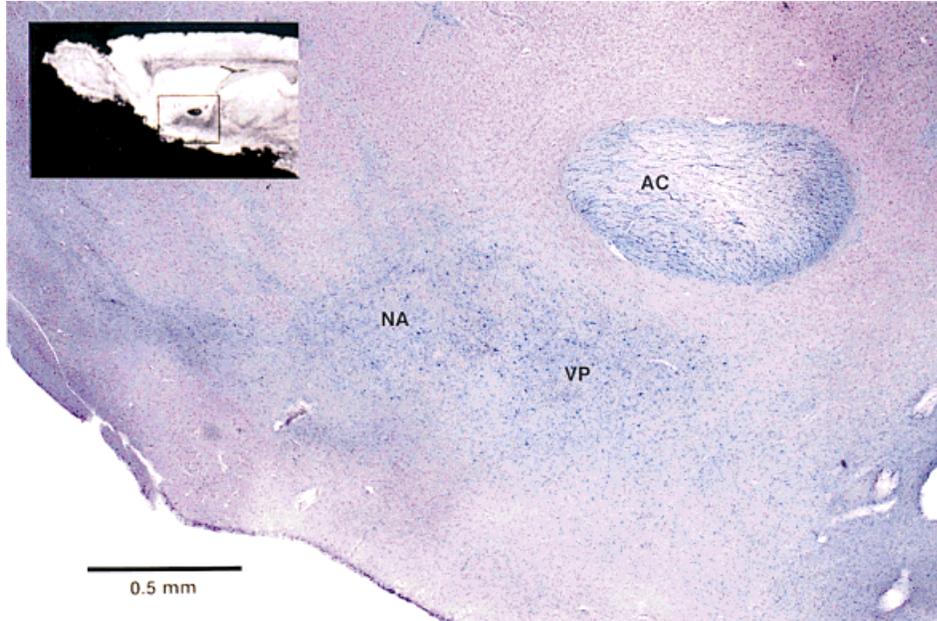


Fig. 5. Parasagittal cryostat section of the aged dwarf lemur brain. The Prussian blue reaction is intense in the anterior commissure (AC) and the region of the nucleus accumbens (NA) and ventral pallidum (VP). The corresponding parasagittal MR view is shown in the **inset**.

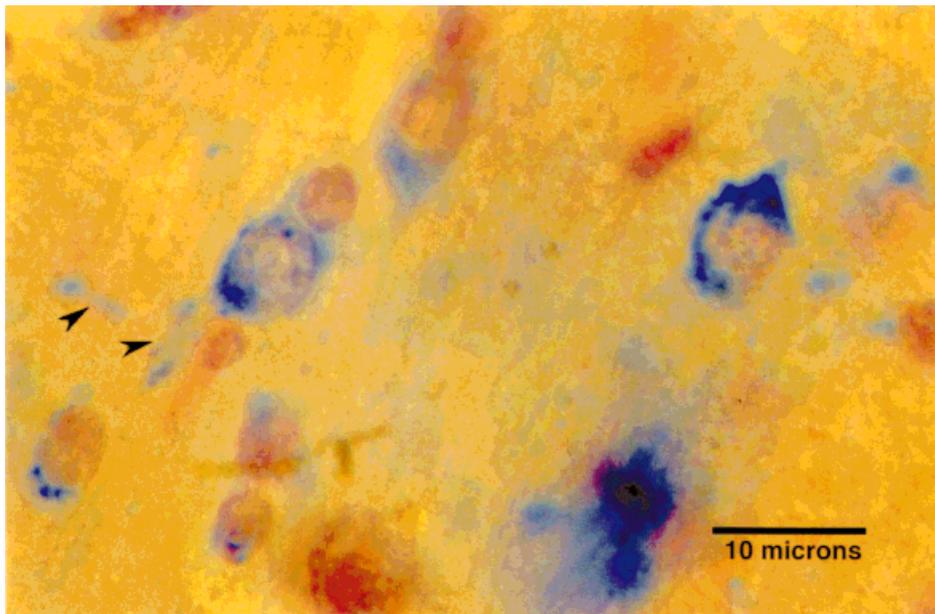


Fig. 6. High magnification of a small portion of the globus pallidus in the 15-year-old dwarf lemur. Iron pigments (blue) are intracytoplasmic and surround the cell nuclei (pink). Iron can also be found in association with the cell processes (arrowheads).

areas such as the globus pallidus [Vymazal et al., 1995]. Findings support the theory that ferritin iron is the primary determinant of MRI contrast in normal gray matter [Vymazal et al., 1996]. Magnetic resonance imaging studies reveal age-correlated increasing iron content in the globus pallidus, substantia nigra, putamen, and thalamus of both humans [Schenck, 1995] and cheirogaleids [Dhenain et al., 1996, personal communication; Gilissen et al., 1996]. Hypointense regions of MR images are consistent with histochemical staining for non-heme iron [Zhou et al., 1993; Schenck, 1995]. Microscopic NMR images clearly reveal many features seen with the histochemical procedure (Figs. 3–5) [Gilissen et al., 1996].

In a study of New and Old World monkeys, Bronson and Schoene [1980] suggested that brain iron may occur only in Old World monkeys, apes, and man. It is now clear that brain iron deposits occur in strepsirhine primates, at least in cheirogaleids. The regions that are most intensively stained with the Perl's blue reaction correspond closely with the findings of previous studies (see Introduction). The association of iron with the pallidonigral system could provide insights into the pathogenesis of neurodegenerative movement disorders; a hypointense signal in T2*-weighted MR images of the globus pallidus is commonly related to degenerative diseases of the extrapyramidal nuclei in humans [Sethi et al., 1988]. In particular, it is interesting to observe the MR images presented by Sethi et al. [1988] in parallel with our Figure 2. The two first figures presented by these authors show a decreased signal localized to the globus pallidus of patients with the Hallervorden-Spatz disease (a degenerative disease of the extrapyramidal nuclei). Similar findings in monkeys have been compared with those of neurodegenerative diseases in humans. They are reported to remain clinically silent in monkeys [Bronson & Schoene, 1980]. This indicates the need for a thorough longitudinal study correlating iron deposition (monitored in vivo with MRI) and motor behavior.

In this study, we observed the presence of iron in the thalamus of aged nonhuman primates (Figs. 3, 4). Previous studies emphasized the pallidonigral accumulation of iron [Bronson & Schoene, 1980]. Dhenain et al. [personal communication] observed no histochemical evidence of iron in the thalamus of the aged mouse lemur despite some indication in magnetic resonance [Dhenain et al., 1996]. François et al. [1981] undertook a description of the exact topographical and cytological localization of iron in rat and monkey brains. They reported reaction products of iron in the globus pallidus, the substantia nigra, the dentate nucleus, and, to a lesser extent, the striatum and its efferent fibers in two adult *Macaca speciosa* and one *Macaca mulatta*. In contrast with our study, they observed no iron trace in the thalamus, in spite of the use of an intensified Perl's reaction mixture and an intense staining of the striato-pallido-nigral system. François et al. [1981] reported no differences between monkeys and rats, but low reaction products of iron have been observed in some parts of the rat thalamus by Hill and Switzer [1984].

The young specimens studied here showed no iron deposits, but all the aged specimens that we examined as well as those studied by Dhenain et al. [1996] showed significant iron deposits. The predilection of non-heme iron for the pallidonigral system is not easily explained in humans [Morris et al., 1992]. The size of many brain structures is positively correlated with maximum recorded life span in haplorhine primates. In strepsirhine primates, however, only two brain structures are significantly related to life span: the globus pallidus and the subthalamic nucleus [Allman et al., 1993; Hakeem et al., 1996]. These structures are among those containing the greatest iron accumulation. These relations need to be further explored to understand the functional correlates of extrapyramidal iron accumulation.

The small size, the rapid maturity, the fecundity, and the relatively short life expectancy of the cheirogaleids provide considerable advantages for experimental investigations on the question of brain iron accumulation. Imon et al. [1995] observed that iron deposits are common in old patients with various neurological diseases and may be related to their etiology as well as to changes in the normal aging brain. It is therefore important to take into account the brain iron content and its possible relations to neuropathological changes when using the cheirogaleids as model of normal and pathological brain aging [Bons et al., 1995]. In particular, it needs to be demonstrated that iron increase is related to cytological changes and neuronal loss observed in the basal forebrain cholinergic neurons of aged cheirogaleids [Mestre & Bons, 1993].

CONCLUSIONS

Brain iron was present in all the aged lemurs studied here. Iron accumulation occurs in normal aging, but it must also be considered in animal models of neurodegeneration. Indeed, the breakdown of the homeostatic process of intracellular iron and the iron accumulation is related to the development of various neurodegenerative diseases in humans [Connor & Menzies, 1995]. The iron-containing regions in cheirogaleids correspond closely to the findings of previous studies, but among primates only cheirogaleids and humans show iron deposits in the thalamus. Cheirogaleids are a good model system for the study of non-heme iron accumulation in aging, and MRI offers a noninvasive means of identifying patterns and extents of accumulation over time. The specific life-history parameters of the cheirogaleids offer many advantages as compared with other primates.

ACKNOWLEDGMENTS

We thank Kenneth Glander, Duke Primate Center, for providing the mouse lemur brains, Ludmilla Staneva-Dobrovski, University of Duesseldorf, David Haring, Duke University Primate Center, and Janet Baer, California Institute of Technology, for helpful assistance. Funding was provided by the von Humboldt and the Del Webb Foundations to E.G. Additional funding was provided by the Human Brain Project with contributions from the National Institute on Drug Abuse, the National Institute of Mental Health, and the National Science Foundation.

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