American Journal of Primatology 49:183–193 (1999)

BRIEF REPORTS

Topographical Localization of Lipofuscin Pigment in the Brain of the Aged Fat-Tailed Dwarf Lemur *(Cheirogaleus Medius)* and Grey Lesser Mouse Lemur *(Microcebus Murinus)*: Comparison to Iron Localization

EMMANUEL P. GILISSEN,* RUSSELL E. JACOBS, EVELYNN R. MCGUINNESS, AND JOHN M. ALLMAN
Division of Biology, California Institute of Technology, Pasadena

The present study was undertaken to explore the distribution of lipofuscin in the brain of cheirogaleids by autofluorescence and compare it to other studies of iron distribution. Aged dwarf *(Cheirogaleus medius)* and mouse *(Microcebus murinus)* lemurs provide a reliable model for the study of normal and pathological cerebral aging. Accumulation of lipofuscin, an age pigment derived by lipid peroxidation, constitutes the most reliable cytological change correlated with neuronal aging. Brain sections of four aged (8–15 year old) and 3 young (2–3 year old) animals were examined. Lipofuscin accumulation was observed in the aged animals but not in the young ones. Affected regions include the hippocampus (granular and pyramidal cells), where no iron accumulation was observed, the olfactory nucleus and the olfactory bulb (mitral cells), the basal forebrain, the hypothalamus, the cerebellum (Purkinje cells), the neocortex (essentially in the pyramidal cells), and the brainstem. Even though iron is known to catalyse lipid oxidation, our data indicate that iron deposits and lipofuscin accumulation are not coincident. Different biochemical and morphological cellular compartments might be involved in iron and lipofuscin deposition. The nonuniform distribution of lipofuscin indicates that brain structures are not equally sensitive to the factors causing lipofuscin accumulation. The small size, the rapid maturity, and the relatively short life expectancy of the cheirogaleids make them a good model system in which to investigate the mechanisms of lipofuscinogenesis in primates. Am. J. Primatol. 49:183–193, 1999.

Key words: cheirogaleids; iron; lipofuscin; neurodegenerative diseases

INTRODUCTION

The most reliable and widespread cytological change correlated with neuronal aging is the formation of fluorescent age pigment or lipofuscin, which con-

*Correspondence to: Emmanuel Gilissen, PhD, University of the Witwatersrand, Medical School, Department of Anatomical Sciences, 7 York Road, Parktown 2193, Johannesburg, South Africa. E-mail: 055ape@chiron.wits.ac.za

Received 13 July 1998; Accepted 20 February 1999

tains peroxidised lipids, proteins, and transition metals [Strehler, 1964; Whiteford & Getty, 1966; Sohal & Wolfe, 1986; Gutteridge, 1987; Kitani et al., 1995; Terman & Brunk, 1998]. Lipofuscin belongs to the lysosomal system and is a result of incomplete degradation of heterophagocytosed and/or autophagocytosed material [De Duve & Wattiaux, 1966]. The formation of lipofuscin is strongly related to oxidative enzymatic activity [Friede, 1962] as well as to oxidative stress and perturbation of protease activity [Ivy et al., 1984, 1990, 1996; Harman, 1990; Sohal & Brunk, 1990; Marzabadi et al., 1991; Ivy, 1992; Yu, 1993]. Lipofuscin accumulation can therefore be considered as an outcome of both physiological aging and abnormal oxidative metabolism [Sohal & Wolfe, 1986; Sheehy et al., 1995].

It has been observed that iron content increases with age in cheirogaleid brains and mainly accumulates in the extrapyramidal motor system [Gilissen et al., 1996; Dhenain et al., 1997, 1998]. Iron is an essential transition metal catalyst used by biological systems to facilitate aerobic metabolism. It can however also induce oxygen radical formation leading to oxidative stress and cell damage [Gutteridge, 1987]. Several lines of evidence support the hypothesis that oxidative stress is associated with the aging process [Harman, 1988, 1994; Sohal, 1988]. Iron-catalysed lipid peroxidations are common processes during senescence. As a catalyst for the interaction of superoxide anion radical (O_2^-), iron accelerates the formation of lipofuscin. The high rate of oxygen utilization of the brain, its high lipid content, and its relatively low level of endogenous antioxidants make it highly sensitive to iron-catalysed oxidative processes and lipofuscin formation.

The mouse lemur provides a useful model for the study of cerebral aging. Its life expectancy is about 4 years in the wild. In captivity however, it lives up to about 13 years. Some aged specimens (8–11 years old) undergo neuropathological and behavioural changes quite similar to those seen in normal and pathological human aging [Bons et al., 1995]. The life span of the fat-tailed dwarf lemur in the wild is not known, but specimens up to 19 year old have been observed in captivity [Hakeem et al., 1996]. The present study was undertaken to explore the distribution of lipofuscin in the brain of aged mouse and dwarf lemurs and compare it to previous work on iron distribution [Gilissen et al., 1998].

MATERIALS

The specimens used in this study are the same as those described by Gilissen et al. [1998]. They include aged and young animals (Table I). All animals died of natural causes. The diet of the dwarf lemurs is similar to the one described in Lee et al. [1996]. The mouse lemurs were fed cracked Purina Old World Monkey Chow (No. 5038), chopped fruits, vegetables, and crickets. The composition of the chow is as follows: 15.5% protein, 5.9% fat, and 4% fiber per daily serving. The chow contains 180 parts iron per million. It corresponds to the daily requirement in iron and is similar to the normal diet in the wild. There was no supplementation with vitamins or minerals. To prevent obesity in nocturnal lemurs during the winter (torpor period), a "winter diet" and a "summer diet" have been established. The winter diet is initiated in mid-August, with a gradual decrease of food amounts. The diet is then increased gradually starting in April, reaching the full summer diet amount in breeding season (David Haring & Janet Baer, personal communications).

METHODS

After removal from thawed cadavers (not perfused), the brains were refrigerated in 10% formalin solution with 30% sucrose overnight. Serial sections were

then cut in sagittal and coronal planes at 50 μm on a cryomicrotome. The sections were kept as free-floating sections in the same fixative. Some were kept unstained and some were treated for lipofuscin detection with the following histochemical procedures: Sudan III, oil red O, Schmorl, Nile blue sulfate, and Ziehl-Neelsen [Pearse, 1961]. The sections were then mounted on gelatin-coated slides.

Lipofuscin-like substances appear yellow to brown in unstained preparations of aging brain examined in the light microscope. In fact, the term *lipofuscin* has been classically used to refer to the material emitting green to yellow autofluorescence when irradiated with ultraviolet or blue light. These pigments can be easily defined and identified by their autofluorescence in situ [Dowson & Harris, 1981; Harman, 1990; Sohal & Brunk, 1990]. The sections that we kept unstained (see above) as well as histological material used for iron staining in the same animals [see Gilissen et al., 1998] were examined by fluorescence microscopy using epiillumination in a Zeiss Axiophot microscope. The semiquatitative assessment of Dhenain et al. [1998] was used for the degree of lipofuscin pigmentation: 0 = no lipofuscin detectable, 1+ = small, 2+ = large amount of lipofuscin (Table I).

RESULTS

External morphology of the brains showed no age-related changes. In our previous study [Gilissen et al., 1998], we observed no evidence of amyloid plaques or degenerated neurites in these specimens. We observed no changes at the cellular level between the young and the aged animals, but we did note the accumulation of lipofuscin granules in the aged animals. No lipofuscin was observed in the young animals (Fig. 1.1). The accumulation does not appear substantially different between the 8-year-old and the oldest animals and appears similar in the two species (Fig. 1.2–5). The presence of lipofuscin is not common to all brain regions (Table I, and see Figs. 2, 3). We observed no difference in regional lipofuscin distribution between the two species.

Lipofuscin is observed in the hippocampus [granular layer of the dentate gyrus, interneurons (large polymorphic neurons) of the hilus of the dentate gyrus, and pyramidal layer including its subicular part] (Figs. 1.2–5, 3.1). Pigments are also observed in the Purkinje cells of the cerebellum (Fig. 3.2) and in the cerebellar nuclei as well as in the mitral cells of the olfactory bulb (Fig. 3.3), the olfactory nucleus (Fig. 3.4), and the olfactory tubercle. The pigments are present in more anterior forebrain structures including the nucleus basalis of Meynert, and especially the diagonal band of Broca, the septum, and the nucleus accumbens (Fig. 3.6). They are also present throughout the amygdala and they show a clear lamination pattern throughout the whole cerebral cortex, where they are especially visible in the motor cortex pyramidal cells (Fig. 3.5). They are also visible in the subthalamic region (zona incerta), the hypothalamus, the interpeduncular nucleus, and the hindbrain structures (raphe, locus coeruleus, reticular formation).

A few, scattered pigments are also present in the pons, the inferior and superior colliculus, the thalamus, the globus pallidus, the substantia nigra (pars reticulata and pars compacta), the striatal fundus, and in the striatum (Fig. 3.7–8).

DISCUSSION

Iron promotes in vivo lipofuscin formation and is usually detectable in high concentrations in lipofuscin granules. Similarly, the amount of lipofuscin found in tissues or in cultured cells exposed to iron enriched environment is greater

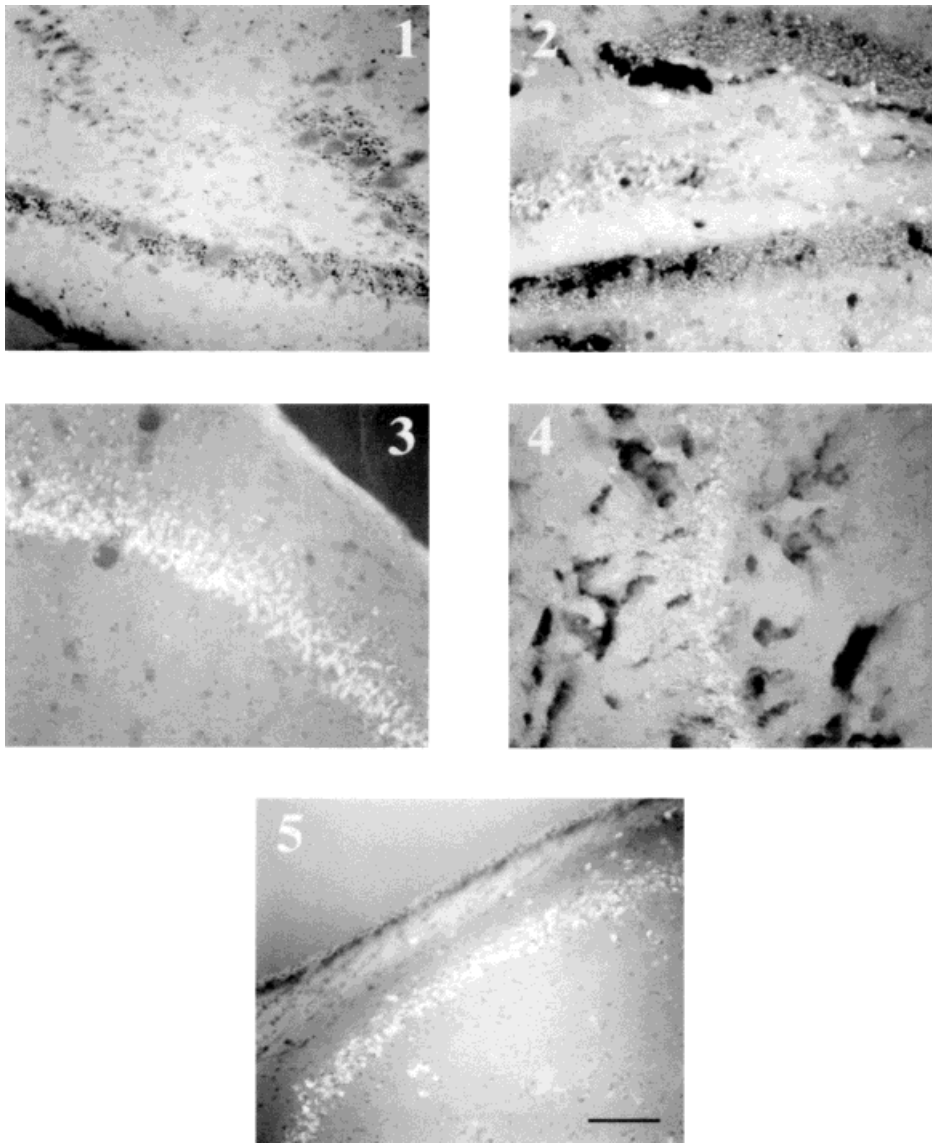

Fig. 1. Views of parasagittal sections of the hippocampus of several specimens examined by fluorescence microscopy using epiillumination. Views 1 and 2 show the dentate gyrus and a portion of the pyramidal layer. Views 3–5 show only a portion of the pyramidal layer. **1:** Three-year-old male dwarf lemur; **2:** 8-year-old male mouse lemur; **3:** same specimen as in 2; **4:** 12-year-old female mouse lemur; **5:** 15-year old male dwarf lemur. The autofluorescent pigments appear bright on the grey background. They are visible in all the specimens except in the 3-year-old one. Scale bar = 0.1 mm.

than in the controls [Sohal et al., 1977, 1985; Armstrong, 1984; Sohal & Wolfe, 1986; Marzabadi et al., 1988; Brunk, 1990; Sohal & Brunk, 1990; Katz et al., 1993; Yuan et al., 1995; Zs.-Nagy et al., 1995; Castelnau et al., 1998]. Because lipofuscin pigments occurring in cultured cells have similar characteristics to the material found in postmitotic cells in vivo [Brunk & Collins, 1981], one could expect a close correlation between the distribution of iron [Gilissen et al., 1998]

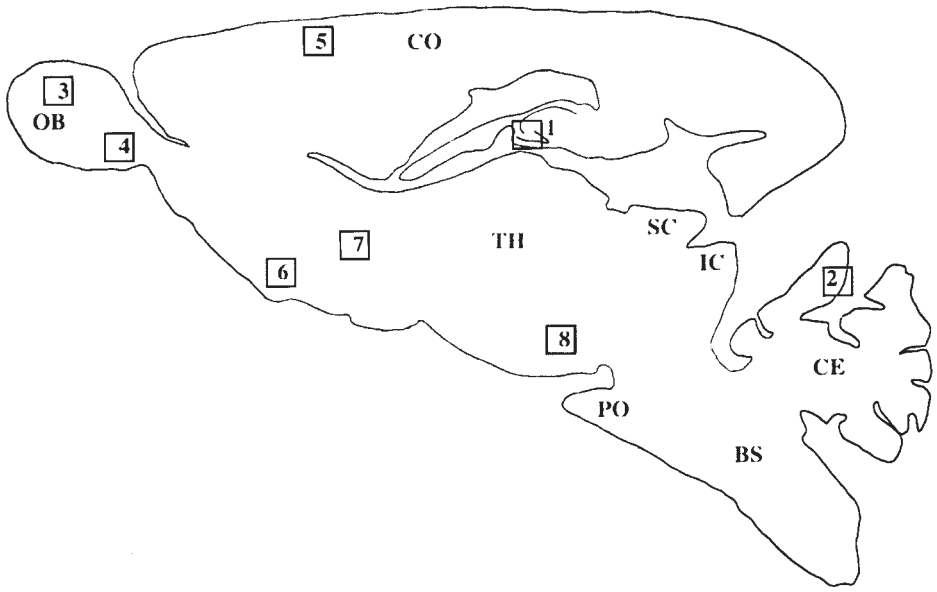

Fig. 2. Schematic representation of a parasagittal cryostat section of the aged dwarf lemur brain. The insets correspond to the views in Figure 3. CO, cortex; OB, olfactory bulb; TH, thalamus; PO, pons; SC, superior colliculus; IC, inferior colliculus; CE, cerebellum; BS, brainstem (hindbrain structures).

and the distribution of lipofuscin in aged cheirogaleid brains. Our data rather suggest that the in vivo distributions of iron and lipofuscin are to a large extent segregated (compare for instance Gilissen et al.'s [1998] Figs. 3, 4 and Fig. 3.7–8 in this study).

Lipofuscin accumulation in cheirogaleids shows similarities with rodents and macaques [Brizzee et al., 1974], but we observed very little lipofuscin in thalamus, pons and midbrain (inferior and superior colliculus) compared to the reported accumulations in these mammals. In aging rodents, the hippocampus shows the most abundant and the highest rate of lipofuscin accumulation [Samorajski et al., 1968; Nandy et al., 1988; Naguro & Iwashita, 1992; Oenzil et al., 1994]. Pigment bodies are also present in the Purkinje cells of aging carnivores [Riis et al., 1992]. In contrast with other mammals, including cheirogaleid primates, the

TABLE I. Species, Age (Years), Gender, and Regional Lipofuscin Rates of Specimens Examined*

Species	Age	Gender	H	C	OB	ON	N	BF	GP	SN
Dwarf lemur	15	Male	xx	xx	xx	xx	xx	xx	x	x
Mouse lemur	12	Female	xx	xx	xx	xx	xx	xx	0	x
Mouse lemur	8	Male	xx	xx	xx	xx	xx	xx	x	0
Mouse lemur	8	Female	xx	xx	xx	xx	xx	xx	0	0
Dwarf lemur	3	Male	0	0	0	0	0	0	0	0
Mouse lemur	2	Male	0	0	0	0	0	0	0	0
Mouse lemur	2	Female	0	0	0	0	0	0	0	0

*Abbreviations: H, hippocampus; C, cerebellum; OB, olfactory bulb; ON, olfactory nucleus; N, neocortex; BF, basal forebrain; GP, globus pallidus; SN, substantia nigra. Semiquantitative assessment made according to Dhenain et al. (1998): 0 = no lipofuscin detectable, 1+ = small, 2+ = large amount of lipofuscin.

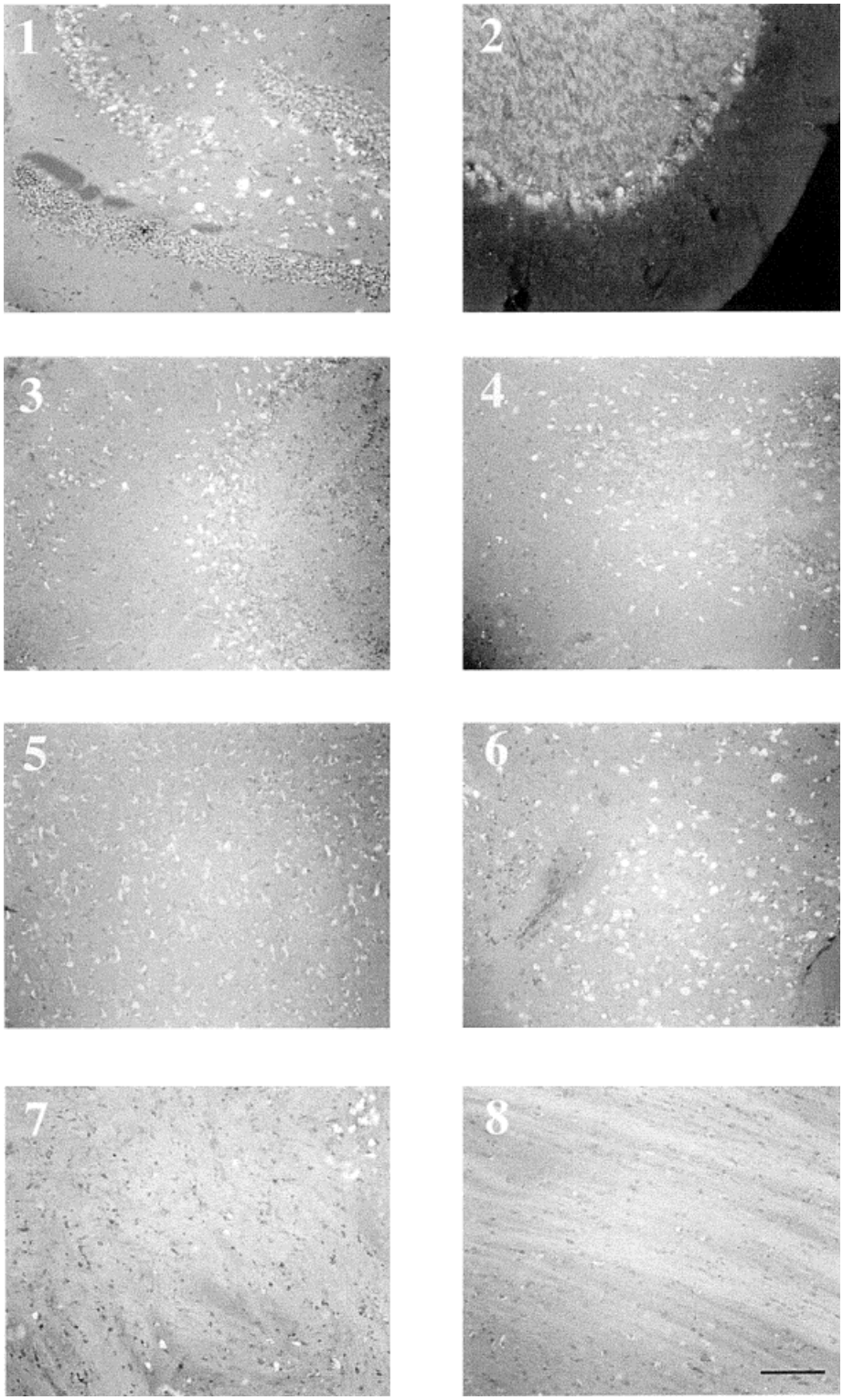

Figure 3.

human cerebellar Purkinje cells appear to remain relatively free of lipofuscin granules [Shima & Tomonaga, 1988; Mrak et al., 1997].

Experimentally induced lipofuscin accumulation in young rats reveals that the most affected brain cells are some of the most active in the brain [Friede, 1962; Ivy et al., 1984; comments of G.O. Ivy in Sohal, 1988, p 144; Ivy et al., 1990; Ivy, 1992]. They include most of the large neurons, the granule cells and interneurons of the hippocampal dentate gyrus, and the cerebellar Purkinje cells. It is therefore possible that a high rate of neural activity causes an increased turnover of cellular products such as lipofuscin, providing a way to measure the relative neuronal activity of different brain regions. In this view, the apparently high lipofuscin content of mitral cells and olfactory nuclei emphasizes the importance of olfaction in cheirogaleids.

The high iron levels of the extrapyramidal system led Morris et al. [1992] to suggest that potentially toxic iron needs to be removed from sites of high neuronal density and high metabolic activity. These regions (cortex, hindbrain structures, hippocampus) are vulnerable to free radical damage. The iron would therefore preferentially be stored in areas of low neuronal density and metabolic activity such as the globus pallidus. In cheirogaleids, iron is totally absent from the hippocampus, where lipofuscin is clearly visible (Figs. 1.2–5, 3.1). In basal forebrain and hindbrain structures, there is some overlap but the regions where lipofuscin is present do not always correspond to intense iron staining. Lipofuscin is visible in Purkinje cells which do not stain for the Perl's blue reaction. The situation is somewhat similar in the olfactory bulb; lipofuscin is visible in the mitral cells, but iron appears to be restricted to the internal granular and glomerular layers. There was no or very little autofluorescence in basal ganglia (striatum, globus pallidus) and substantia nigra (Fig. 3.7–8), which show high iron content [Gilissen et al., 1998]. The substantia nigra nevertheless is neither a low cell density nor low metabolic rate region. Other influences as well as quantitative aspects of lipofuscin distribution need to be considered; differences in sensitivity to perturbations of the lysosomal system have been noted between cells of different brain regions [Cataldo et al., 1996] and are beyond the goal of this work. Our qualitative analysis suggests that while iron is involved in the biochemical mechanisms that lead to lipofuscin formation, the age-related deposition of iron in the brain seems to be anatomically independent from that of lipofuscin. This suggests that different biochemical and morphological cellular compartments might be involved by iron and lipofuscin deposition.

A more radical approach involving regional biopsies and adequate quantitative analysis [Dowson & Harris, 1981; Shima & Tomonaga, 1988; Dowson et al., 1992] that could be repeated longitudinally is necessary for a better understanding of the relationships between metabolic activity, catalyzing role of iron in free radical reactions, resulting hydroxyl radical formation, lipid peroxidation, lipofuscin accumulation, and their regional differences. Such a study could elucidate how much iron is necessary to catalyse lipofuscin formation in vivo. More specific methods for iron detection are nevertheless required. The standart Perl's and Turnbull's methods allow the demonstration

Fig. 3. Different views in parasagittal sections examined by fluorescence microscopy using epiillumination and corresponding to the insets in Fig. 2. The age pigments appear bright on the grey background. **1:** Hippocampus; **2:** cerebellar folium; the age pigments are localized in the Purkinje cell layer; **3:** olfactory bulb; the age pigments are mainly localized in the mitral cell layer; **4:** olfactory nucleus; **5:** neocortex; **6:** basal forebrain; **7:** globus pallidus; **8:** substantia nigra. The age pigments are less visible in the globus pallidus and the substantia nigra, where iron deposits are abundant [Gilissen et al., 1998]. Scale bar = 0.1 mm.

of nonhaem Fe(III) and Fe(II). Iron in tissues is present largely in ferric rather than ferrous form [Koeppen, 1995], but no protocol has yet been designed for the specific measurement of the free Fe(II) levels. Most types of nerve cells retain a characteristic pigmentation throughout an animal's life [Braak, 1984]. However, selective changes of the lipofuscin architecture might be associated with age-related dysfunctions [Hilbig et al., 1997]. Congestion of the cytoplasm by lipofuscin may also reduce the degradative efficiency of the lysosomal system, resulting in a slowing of intra- and intercellular interactions [Sohal & Wolfe, 1986]. For instance, age-related neuronal atrophy in basal forebrain cholinergic neurons in the mouse lemur is correlated with the accumulation of large cytoplasmic vacuoles [Mestre & Bons, 1993]. The cells may suffer by having to supply large amounts of enzymes to a system containing material which cannot be lysed. The amount of lysosomal enzymes available for endocytosis and autophagocytosed material may therefore be insufficient. In this view, the accumulation of lipofuscin in aging cells may be considered as a kind of physiological "storage disease" [Brunk & Collins, 1981]. Moreover, although there is no evidence of toxicity of the lipofuscin, the process underlying their formation might be detrimental to cellular functions. Mecocci et al. [1993] demonstrated that there is a progressive age-related accumulation in oxidative damage to DNA in human brain and that mitochondrial DNA is preferentially affected. Lipid peroxidations during the aging process occur simultaneously with large-scale deletions of the mitochondrial DNA [Wei et al., 1996].

CONCLUSIONS

1. Abnormalities of the lysosomal system lie at the crossroad of various etiological pathways [Cataldo et al., 1996]. Examination of the topology of the accumulation of lipofuscin in experimental animal models of aging will provide important information in this crucial area [Bernardis & Davis, 1996]. Lipofuscin granules were present in all the aged specimens studied here. The hippocampus, the olfactory structures, the basal forebrain, the hypothalamus, the motor cortex pyramidal cells, the cerebellar Purkinje cells, and the brainstem show lipofuscin accumulation.

2. Iron is an important catalyst in free radical formation and lipofuscin accumulation is strongly related to oxidative stress. The distributions of lipofuscin and iron deposits do not strictly overlap. Different biochemical and morphological cellular compartments might be involved in iron and lipofuscin deposition.

ACKNOWLEDGMENTS

We thank Dr. Kenneth Glander, Duke Primate Center, for providing the mouse lemur brains. We thank Drs. Ludmilla Staneva-Dobrovski, University of Düsseldorf; John Shih, Mary Dickinson, Janet Baer, California Institute of Technology; and David Haring, Duke University Primate Center; for helpful assistance in the preparation of this work. We also thank the four anonymous reviewers for their very thoughtful comments. Funding was provided by the von Humboldt and the Del Webb Foundations to EG. Additional funding was provided by the Beckman Institute at Caltech and the Human Brain Project with contributions from the National Institute on Drug Abuse, the National Institute of Mental Health, and the National Science Foundation.

REFERENCES

Armstrong D. 1984. Free radical involvement in the formation of lipopigments. In: Armstrong D, Sohal RS, Cutler RG, Slater TF, editors. Free radicals in molecular biology, aging, and disease. New York: Raven Press, pp. 129–141.

Bernardis LL, Davis PJ. 1996. Aging and the hypothalamus—Research perspectives. Physiol Behav 59:523–536.

Bons N, Jallageas V, Mestre-Francés N, Silhol S, Petter A, Delacourte A. 1995. *Microcebus murinus*, a convenient laboratory animal model for the study of Alzheimer's disease. Alzheimer Res 83–87.

Braak H. 1984. Architectonics as seen by lipofuscin stains. In: Peters A, Jones EG, editors. Cerebral cortex. Vol. 1. New York: Plenum Press, pp. 59–104.

Brizzee KR, Ordy JM, Kaack B. 1974. Early appearance and regional differences in intraneuronal and extraneuronal lipofuscin accumulation with age in the brain of a nonhuman primate (*Macaca mulatta*). J Gerontol 29:366–381.

Brunk UT. 1990. On the origin of lipofuscin; the iron content of residual bodies, and the relation of these organelles to the lysosomal vacuome. A study on cultured human glial cells. In: Porta EA, editor. Lipofuscin and ceroid pigments. New York: Plenum Press, pp. 313–322.

Brunk UT, Collins VP. 1981. Lysosomes and age pigments in cultured cells. In: Sohal RH, editor. Age pigments. Amsterdam: Elsevier.

Castelnau PA, Garrett RS, Palinski W, Witztum JL, Campbell IL, Powell HC. 1998. Abnormal iron deposition associated with lipid-peroxidation in transgenic mice expressing interleukin-6 in the brain. J Neuropathol Exp Neurol 57:268–282.

Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA, Nixon RA. 1996. Properties of the endosomal-lysosomal system in the human central nervous system: Disturbances mark most neurons in populations at risk to degenerate in Alzheimer's disease. J Neurosci 16:186–199.

De Duve C, Wattiaux R. 1966. Functions of lysosomes. Annu Rev Physiol 28:435–492.

Dhenain M, Volk A, Picq JL, Perret M, Boller F, Michot JL. 1997. Age-dependence of the T2-weighted MRI signal in brain structures of a prosimian primate (*Microcebus-murinus*). Neurosci Lett 237:85–88.

Dhenain M, Duyckaerts C, Michot JL, Volk A, Picq JL, Boller F. 1998. Cerebral T2-weighted signal decrease during aging in the mouse lemur primate reflects iron accumulation. Neurobiol Aging 19: 65–69.

Dowson JH, Harris SJ. 1981. Quantitative studies of the autofluorescence derived from neuronal lipofuscin. J Microsc 123: 249–258.

Dowson JH, Mountjoy CQ, Cairns MR, Wilton-Cox H. 1992. Changes in intraneuronal lipopigment in Alzheimer's disease. Neurobiol Aging 13:493–500.

Friede RL. 1962. The relation of the formation of lipofuscin to the distribution of oxidative enzymes in the human brain. Acta Neuropathol 2:113–125.

Gilissen E, Ghosh P, Ahrens ET, Jacobs RE, Allman JM. 1996. MRI microscopy of a non-human primate model of Alzheimer's disease: Iron deposits in the brain of the aged mouse lemur. Soc Neurosci Abstr 22:2139.

Gilissen E, Ghosh P, Jacobs RE, Allman JM. 1998. Topographical localization of iron in brains of the aged fat-tailed dwarf lemur *(Cheirogaleus medius)* and grey lesser mouse lemur *(Microcebus murinus)*. Am J Primatol 45:291–299.

Gutteridge JMC. 1987. Oxygen radicals, transition metals and aging. In: Totaro EA, Glees P, Pisanti FA, editors. Advances in age pigments research. Oxford: Pergamon Press, pp 1–22.

Hakeem A, Sandoval GR, Jones M, Allman JM. 1996. Brain and life span in primates. In: Birren JE, Schaie KW, editors. Handbook of the psychology of aging. New York: Academic Press, 1996.

Harman D. 1988. Free radical theory of aging: Current status. In: Zs.-Nagy I, editor. Lipofuscin—1987: State of the art. Amsterdam: Elsevier, pp. 3–21.

Harman D. 1990. Lipofuscin and ceroid formation: The cellular recycling system. In: Porta EA, editor. Lipofuscin and ceroid pigments. New York: Plenum Press, pp. 3–15.

Harman D. Aging: prospects for further increases in the functional lifespan. Age 17: 119–146, 1994.

Hilbig H, Jürgens M, Dinse HR. 1997. Pattern of lipofuscin accumulation in the fore- and hindpaw representation of somatosensory cortex in aged rats parallel functional age-related differences of cortical organization. Soc Neurosci Abstr 23:1800.

Ivy GO. 1992. Protease inhibition causes some manifestations of aging and Alzheimer's disease in rodent and primate brain. Ann NY Acad Sci 674:89–102.

Ivy GO, Kanai S, Ohta M, Smith G, Sato Y, Kobayashi M, Kitani K. 1990. Lipofuscin-like substances accumulate rapidly in brain, retina and internal organs with cysteine protease inhibition. In: Porta EA, editor. Lipofuscin and ceroid pigments. New York: Plenum Press, pp. 31–47.

Ivy GO, Roopsingh R, Kanai S, Ohta M,

Sato Y, Kitani K. 1996. Leupeptin causes an accumulation of lipofuscin-like substances and other signs of aging in kidneys of young rats: Further evidence for the protease inhibitor model of aging. Ann NY Acad Sci 786:12–23.

Ivy GO, Schottler F, Wenzel J, Baudry M, Lynch G. 1984. Inhibitors of lysosomal enzymes: accumulation of lipofuscin-like dense bodies in the brain. Science 226: 985–987.

Katz ML, Stientjes HJ, Gao CL, Christianson JS. 1993. Iron-induced accumulation of lipofuscin-like fluorescent pigment in the retinal-pigment epithelium. Invest Ophthalmol Vis Sci 34:3161–3171.

Kitani K, Ivy GO, Shimasaki H. 1995. Lipofuscin and ceroid pigments. State of the art 1995. Gerontology 41(Suppl 2):1–330.

Koeppen AH. 1995. The history of iron in the brain. J Neurol Sci 134(Suppl):1–9.

Lee JT, Miller CA, McDonald CT, Allman JM. 1996. Xanthogranuloma of the choroid plexus in the fat-tailed dwarf lemur (*Cheirogaleus medius*). Am J Primatol 38:349–355.

Marzabadi MR, Sohal RS, Brunk UT. 1988. Effect of ferric iron and desferrioxamine on lipofuscin accumulation in cultured rat heart myocytes. Mech Ageing Dev 46:145–157.

Marzabadi MR, Sohal RS, Brunk UT. 1991. Mechanisms of lipofuscinogenesis—Effect of the inhibition of lysosomal proteinases and lipases under varying concentrations of ambient oxygen in cultured rat neonatal myocardial-cells. APMIS 99:416–426.

Mecocci P, MacGarvey U, Kaufman AE, Koontz D, Shoffner JM, Wallace DC, Beal MF. 1993. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. Ann Neurol 34:609–616.

Mestre N, Bons N. 1993. Age-related cytological changes and neuronal loss in basal forebrain cholinergic neurons in *Microcebus murinus* (lemurian, primate). Neurodegeneration 2:25–32.

Morris CM, Candy JM, Oakley AE, Bloxam CA, Edwardson JA. 1992. Iron histochemical distribution of non-haem iron in the human brain. Acta Anat 144:235–257.

Mrak RE, Griffin WST, Graham DI. 1997. Aging-associated changes in human brain. J Neuropathol Exp Neurol 56:1269–1275.

Naguro T, Iwashita K. 1992. Olfactory epithelium in young-adult and aging rats as seen with high-resolution scanning electron-microscopy. Microsc Res Techn 23:62–75.

Nandy K, Mostofsky DI, Idrobo F, Blatt L, Nandy S. 1988. Experimental manipulations of lipofuscin formation in aging mammals. In: Zs.-Nagy I, editor. Lipofuscin—1987: State of the art. Amsterdam: Elsevier, pp. 289–304.

Oenzil F, Kishikawa M, Mizuno T, Nakano M. 1994. Age-related accumulation of lipofuscin in 3 different regions of rat brain. Mech Ageing Dev 76:157–163.

Pearse AGE. 1961. Histochemistry. Theoretical and applied. London: J. & A. Churchill.

Riis RC, Cummings JF, Loew ER, Delahunta A. 1992. Tibetan terrier model of canine ceroid lipofuscinosis. Am J Med Genet 42:615–621.

Samorajski T, Ordy JM, Rady-Reimer P. 1968. Lipofuscin pigment accumulation in the nervous system of aging mice. Anat Rec 160:555–574.

Sheehy MRJ, Greenwood JG, Fielder DR. 1995. Lipofuscin as a record of rate of living in an aquatic poikilotherm. J Gerontol Ser A Biol Sci Med Sci 50:B327–B336.

Shima A, Tomonaga M. 1988. Microfluorimetric characterization of in situ autofluorescence of lipofuscin granules in the aged human brains. In: Zs.-Nagy I, editor. Lipofuscin—1987: State of the art. Amsterdam: Elsevier, pp. 147–157.

Sohal RS. 1988. Oxidative stress and cellular aging. In: Zs.-Nagy I, editor. Lipofuscin—1987: State of the art. Amsterdam: Elsevier, pp. 135–144.

Sohal RS, Allen RG, Farmer KJ, Newton RK. 1985. Iron induces oxidative stress and may alter the rate of aging in the housefly, *Musca domestica*. Mech Ageing Dev 32:33–38.

Sohal RS, Brunk UT. 1990. Lipofuscin as an indicator of oxidative stress and aging. In: Porta EA, editor. Lipofuscin and ceroid pigments. New York: Plenum Press, pp. 17–29.

Sohal RS, Peters PD, Hall TA. 1977. Structure, origin, composition and age-dependence of mineralized dense bodies (concretions) in the midgut of the adult housefly, *Musca domestica*. Tissue Cell 9:87–102.

Sohal RS, Wolfe LS. 1986. Lipofuscin: Characteristics and significance. In: DF Swaab DF, Fliers E, Mirmiran M, Van Gool WA, Van Haaren F, editors. Progress in brain research, Vol. 70. Amsterdam: Elsevier, pp. 171–183.

Strehler BL. 1964. On the histochemistry and ultrastructure of age pigment. Adv Gerontol Res 1:343–384.

Terman A, Brunk UT. 1998. Lipofuscin: Mechanisms of formation and increase with age. APMIS 106:265–276.

Wei YH, Kao SH, Lee HC. 1996. Simultaneous increase of mitochondrial DNA deletions and lipid peroxidation in human aging. Ann NY Acad Sci 786:24–43.

Whiteford R, Getty R. 1966. Distribution of

lipofuscin in the canine and porcine brain as related to aging. J Gerontol 21:31–44.
Yu BP. 1993. Oxidative damage by free radicals and lipid peroxidation in aging. In: Yu BP, editor. Free radicals in aging. Boca Raton, FL: CRC Press, pp. 57–88.
Yuan XM, Brunk UT, Olsson AG. 1995. Effects of iron-loaded and hemoglobin-loaded human monocyte-derived macrophages on oxidation and uptake of LDL. Arterioscler Thromb Vasc Biol 15:1345–1351.
Zs.-Nagy I, Steiber J, Jeney F. 1995. Induction of age pigment accumulation in the brain cells of young male rats through iron-injection into the cerebrospinal fluid. Gerontology 41(Suppl 2):145–158.