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Brain Aging in Strepsirhine Primates

Among primates, the small nocturnal mouse lemur appears to be a promising species for aging research. With a body size of 60 to 130 g, the mouse lemur lives up to about 13 years in captivity. This primate offers a good model system for aging chronobiology because its life span can be changed by manipulating photoperiodic cycles. Age-related behavioral alterations as well as amyloid deposits, neurofibrillary degeneration, and iron and lipofuscin deposits, the hallmarks of normal and pathological human aging, have been observed in the mouse lemur brain. Cerebral atrophy also occurred in some aged animals. Magnetic resonance imaging allowed the evaluation of the iron content-based indice of aging and the detection, *in vivo*, of iron levels in basal forebrain. Comparative studies of aging should help dissect species-specific factors contributing to aging and to the etiology of dementing illnesses in humans. © 2001 Academic Press.

I. Introduction

A small, rapidly maturing, and short-lived primate model would provide significant advantages for the study of cerebral aging. The small nocturnal primate *Microcebus murinus* (mouse lemur) appears to be a promising species for aging research. This strepsirhine primate belongs to the family Cheirogaleidae, which includes the genera *Microcebus* (mouse lemurs), *Cheirogaleus* (dwarf lemurs), *Allocebus*, and *Phaner*. Strepsirhine primates and tarsiers are usually called prosimian primates, but taxonomy distinguishes strepsirhine primates and their sister group, the haplorhine primates, which includes tarsiers, monkeys, apes, and humans.

Age-specific mortality rate data from mouse lemur (Fig. 27.1) indicate that the longevity of this small primate is far longer than what would be expected for a mammal of that body size (60 to 130 g). Despite a life expectancy of about 5 years in the wild, the mouse lemur lives up to about 15 years in captivity (Hakeem *et al.*, 1996). In comparison, the body size of *Rattus norvegicus* averages 265.5 g with a maximum longevity record of 37 months in captivity (Eisenberg, 1981). This situation is similar for the other cheirogaleid primates. The life span of the fat-tailed dwarf lemur, *Cheirogaleus medius* (body weight averaging at 179.0 g), in the wild is not known, but specimens up to 19 years old have been observed in captivity (Hakeem *et al.*, 1996). Cheirogaleid primates have the body size as well as the energetic and environmental constraints of small mammals, but their longevity is comparable to larger mammals. In this sense, they are both atypical primates (in terms of body size) and atypical small mammals (in terms of longevity). It should be noted that other mammals, as some bats or the naked mole rat (Sherman *et al.*, 1991), also combine small body size and a long life span.

In mouse lemur, puberty occurs by 1 year (Perret, 1992), and reproduction continues in females until about 6–8 years. Because of its small size, rapid maturity, fecundity, and relatively short life expectancy compared to other primates, the mouse lemur therefore constitutes a useful model system for the study of normal and pathological cerebral aging. This chapter summarizes studies on age-related changes in mouse lemur and explores some age-related features that could be studied *in vivo* in this primate.

II. Cognitive Function during Aging in Mouse Lemurs

The study of behavioral alterations is an important step for the evaluation of mouse lemurs as models for cerebral aging. This section focuses on age-related modifications of spontaneous social and sexual behavior, anxiety-related behaviors, and memory.

A. Spontaneous Social and Sexual Behavior

Mouse lemurs are tree-dwelling animals. The general strength and motor coordination of aged animals are fairly conserved. The only activities that become restricted are those requiring an extensive strength and/or good stretching capacities, such as jumps from the ground to an elevated platform placed 50 cm above the floor (Dhenain, 1998). Studies of locomotor behavior revealed heterogeneity in old animals. Some aged animals become more lethargic, e.g., walk instead of jumping and spend more time in their nests. This reduced activity is not the result of impaired physical shape. In contrast, some aged animals develop stereotyped hyperactive locomotor behaviors (Picq,

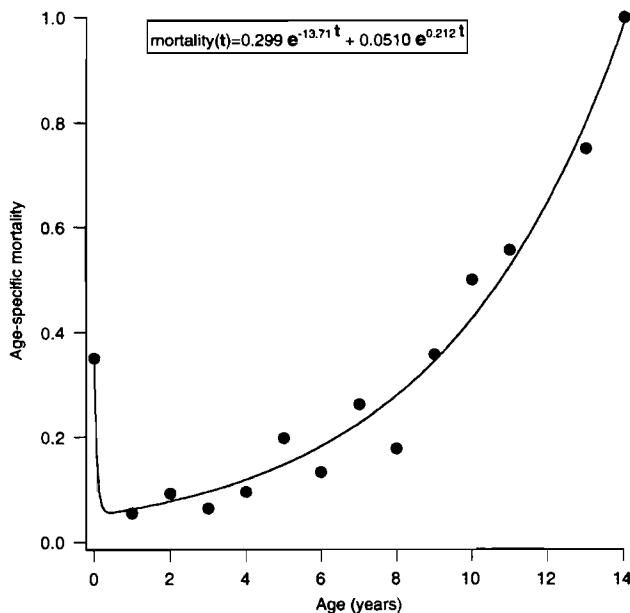


FIG. 27.1. Age-specific mortality data for *Microcebus murinus*. Data are fit by the equation shown in the box. The first term represents infant mortality and the second shows the effects of senescence. For this data set, $n = 140$. Data points represent rates for a population that had survived to a particular age. Unpublished data from Zachary Berger, John Allman, and Atiya Hakeem.

1993a; Dhenain *et al.*, 1997c). Curiously, the latter animals are often the ones with the worst physical shape (Picq, 1993a).

In the wild, active animals, which are observed only during the night, are relatively solitary. They move around in well delimited but partly superposed domains. During the day, they gather to sleep in shelters (hollow trees, nests made of leaves, unstuck barks or wood nests in captivity). Mouse lemur meetings, whether they occur by night or by day, are often associated with peaceful tactile contacts called allogrooming, sound emissions, and scent marking. Aggressive behavior is seldom displayed except occasionally during estrus periods (Martin, 1972; Petter *et al.*, 1977; Pages-Feuillade, 1988). Studies of social behavior from the observation of single-sex pairs of young and very old (9–12 years) captive animals showed that young animals seek positive social contacts and try to approach their partner to groom it. Older individuals may accept the contacts in a passive manner, but do not seek them. Sometimes, they even threaten or chase their partners in response to attempt of approach (Picq, 1992). This suggests that although they remain socially attractive partners, old mouse lemurs tend to withdraw from social interactions. Moreover, whether they are observed in social groups or alone, old mouse lemurs tend to increase the time spent in self-centered activities such as autogrooming (Picq, 1993a). This increase is at the expense of the time spent observing the environment. These modifications do not seem to be related to alteration of senses such as smell or sight (Picq, 1993a). The withdrawal of old animals from social interactions is similar to observations in other primates, such as macaques (Davis, 1978; Hauser and Tyrrel, 1984).

Mouse lemurs are strict seasonal breeders. During the breeding season, in the wild, males have to compete for the acquisi-

tion of large home ranges, which overlap several female home ranges. Laboratory studies suggested that the dominant status of males increases with age until the sixth to seventh breeding season. The dominant status only decreases in very old animals (10–13 breeding seasons) (Aujard and Perret, 1998). Young animals must display a high degree of aggressive behavior to reach a dominant rank and are contested routinely. Aged animals, however, do not need to display as much aggressive behavior to gain a dominant status and this status remains uncontested by other animals. Interestingly, when aged animals are paired with young animals, the aged animals must be more aggressive to obtain dominant status than when they are paired with other aged animals (Aujard and Perret, 1998). Observations of age-matched pairs of males after introduction of a preoestrous female in their cage revealed that old animals display less sexual behavior than young animals, but they are as efficient as young animals in term of reproduction. When old mouse lemurs are paired with young ones, the young animals become less active and display less sexual behavior than when they are paired with age-matched lemurs (Aujard and Perret, 1998). This might reflect the dominant status reached by old animals. This allows their priority access to estrous females and assures reproductive success.

B. Anxiety-Related Behaviors

Mouse lemurs are very sensitive to stress (Perret, 1982). Picq (1993a) described modifications of their behavior as a function of the surrounding stress. A low stress level provokes a rapid exploration of the environment, often characterized by oscillatory movements of the head and of the body. This exploration decreases rapidly in intensity and is replaced by behaviors such as resting, nutrition, or grooming. A moderate stress level brings very active exploratory behaviors. A stronger stress level produces long periods of visual explorations associated with alarm cries or aimless jumps. A very stressful situation gives rise to an absolute freezing where animals are literally petrified. The open-field test is a behavioral test, which consists of direct observation of animals when they are put in a new enclosure. It evaluates the spontaneous response to novelty and allows evaluation of anxiety levels (Halliday, 1966). Overall, there is a decrease of the sensitivity of animals to stress during aging. What slightly worries the young animal does not worry the old at all. What terrifies the young, just worries the old (Picq, 1993a; Dhenain, 1998). However, some aged animals are more stressed than young animals. The inter-individual heterogeneity is a common feature reported in many studies involving old mouse lemurs. It might reflect differences between normal and pathological aging. In a preliminary study, two out of two animals with abnormal sensitivity to stress had diffuse amyloid deposits in the brain (Dhenain, 1998) (Fig. 27.2). This suggests that the open-field test might have a predictive value to detect animals with abnormal aging.

C. Memory

For a long time, prosimian primates were not studied by specialists of animal intelligence because they were said to be timid and not cooperative. However, adaptation of experimental protocols to their sensory-motor capacities revealed very

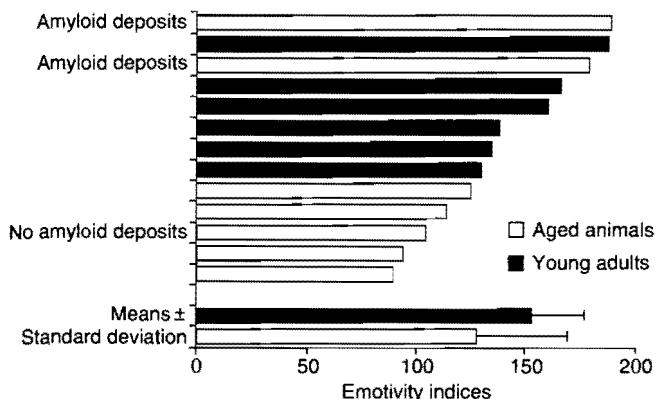


FIG. 27.2. Emotivity indices calculated for different animals. The higher the index, the higher the stress level. In this preliminary study, there was no statistical difference between young and aged animals (Mann-Whitney's $U = 11$, $p > 0.05$). The two aged animals with high stress levels had amyloid deposits in the brain. The brain of only one aged animal with a low stress level has been tested for amyloid deposits and had no amyloid.

good cognitive abilities (Ehrlich *et al.*, 1976). In mouse lemurs, it is possible to assess memory in tasks based on spontaneous behaviors where animals do not have to learn any rule to perform the task. As an example, the free object exploration task (Picq and Dhenain, 1998) consists of an enclosure containing various objects placed in permanent positions. First, animals are allowed to visit the enclosure for repeated periods of time to make the environment and its objects familiar and also to suppress anxiety or fear responses. After the habituation period, the object exploration task is done during six tests of 8 min during 6 consecutive days (one test per day). During each test, new objects are added in new locations or familiar objects are displaced to new locations and replaced by new objects. Aged mouse lemurs were more active than young animals in the task (Picq and Dhenain, 1998). This feature is often characteristic of aged animals in behavioral tasks and rules out the possibility that lower performances are related to physical alterations. Aged lemurs tended to spend more time exploring the objects (new or familiar) than young animals, which suggested a higher curiosity or motivation. Young and old animals spent more time exploring new objects than visiting familiar ones. However, only young animals explored new or displaced objects in priority. They explored the new objects for a longer time than old lemurs. In contrast to old animals, young animals' interest for new objects diminished greatly on second contact with the objects (after 1 day). The behavior of young animals indicated that, like other primates (Vauclair, 1987), mouse lemurs are able to mentally "map out" the experimental enclosure. They are able to construct internal models of their environment, including the nature and the location of each object. The internal map can be retained for at least 24 hr and be revised after a few minutes of exploration if changes occur in the environment. The behavior of aged animals suggests that they did not detect the new or displaced objects as quickly as young animals. Moreover, the new objects were not perfectly memorized or included in the internal map during the first contact. This suggests an age-related alteration of spatial memory (Picq and Dhenain, 1998).

More subtle memory alterations can be tested by artificial tasks in which animals have to reach a goal to get a reward. In many species the usual rewards are alimentary. In mouse lemurs, most of the studies used the "right to find refuge" for approximately 2 min in a nest box as a positive reinforcement. The reason for this choice is that mouse lemurs are very sensitive to stress, and during a test situation, nothing is more attractive than returning to the nest. In rodents, behavioral tasks are often based on spatial paradigms [Olton's radial maze (Olton, 1979), Morris' water maze (Hsiao *et al.*, 1996)]. Because of their small size, Olton's radial maze can be adapted readily to mouse lemurs (Picq, 1993b). The maze was made of a central platform from which radiated eight arms ending on a nest box. Each arm was obstructed by an opaque curtain. Four arms ("the blind arms") were blocked by a door placed just behind the curtain and thus not visible from the entrance. Four arms ("the free arms") with a free throughway were opened behind the curtain and might freely be explored right through the end where a transparent door blocked the access to the nest box. First, mouse lemurs were habituated to the enclosure. Thereafter, the test phase consisted of eight series of five trials (one series per day) during which the mouse lemur was placed in the central platform and then freely explored the radial maze. Animals had five trials to visit the four free arms. When they visited the fourth free arm, access to the nest box was allowed. Aged mouse lemurs did not visit the blind arms more often than the young. This suggested that their reference memory, which is the memory for data that are stable across trials in tests used in rodents (Olton and Papas, 1979), was not altered. However, during one set of five trials, old animals explored the same free arm several times, whereas young animals were able to visit a new free arm at every new trial. This suggests that old animals' memory for data, which varied from trial to trial (such as which free arms were already visited), was altered. This memory is called "rodent type" working memory (Olton and Papas, 1979) (working memory does not have the same meaning for human or other anthropoid primates and rodents).

The neuropsychological models used to assess memory involved in rodent and human tasks are different. This makes it difficult to compare alterations described in rodent tasks with alterations in humans. In anthropoid primates, several tasks, such as discrimination (Buerger *et al.*, 1974; Zola-Morgan and Squire, 1984), delayed response (Fletcher, 1965), delayed nonmatching-to-sample (DNMS) (Mishkin and Appenzeller, 1987) or delayed alternation (DA) tasks (Mishkin and Pribram, 1955; Winocur, 1991), have been developed to study animal models of amnesia. They are conceptually very similar to tasks that can be used in humans. These tasks have also been used to study aging primates. Generally, they are based on the visual recognition of objects and on manipulation of the recognized objects. Mouse lemurs have a limited ability to handle objects; for this reason, Picq developed a spatial version of these tasks. His apparatus (Fig. 27.3) is composed of a starting box in which the animal is placed at the beginning of the test. This starting box gives access to a work chamber from which there are six corridors leading to a reinforcement chamber housing the mouse lemur's nest box. The corridors can be shut off at each end by sliding doors. They can be illuminated, and their relevance is based on whether they are lit. An opaque screen

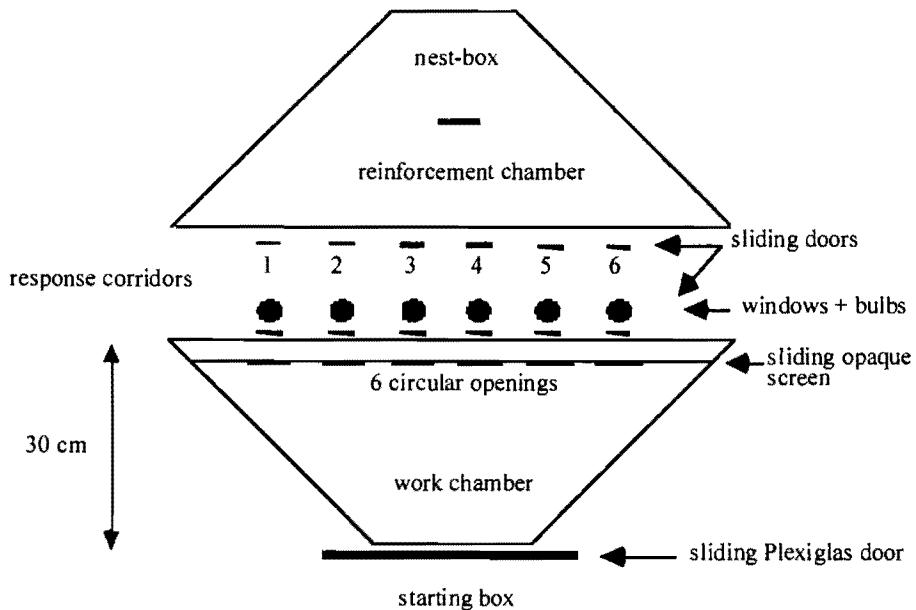


FIG. 27.3. Apparatus used in memory tasks in lemurs. The entire apparatus was made of plywood and was covered with a Plexiglas roof. It was lit by a 15 W red bulb. The mouse lemur's urge to find its nest box was the only motivation. No food reward was necessary.

can be slid between the corridors and the working chamber to impose delays between the visualization of illuminated corridors and the possibility to walk through them. A positively reinforced corridor gives access to the reinforcement chamber where the mouse lemur may rest in its nest box for 2 min. When a mouse lemur enters in a nonreinforced corridor, the exit door remains shut and the sliding entrance door is lowered behind it. The animal is immediately returned to the starting box for another trial.

Discrimination tasks have been performed with this apparatus. Mouse lemurs learned to go through corridors that were illuminated (which is against their natural instinct). This test required that animals associate the stimulus (illuminated corridor) to the response (walk through the corridor), which was associated to the reward (stay 2 min in the nest). This stimulus-response-reward test is a rule-based test (Ridley, 1991). Young and aged lemurs learned the task similarly (Picq, 1995). After 3 months without contact with the apparatus, they were both still able to perform the task (Picq, 1995). The apparatus can also be used to teach other rules to lemurs. For example, to teach a "nonmatching-to-sample" rule, one out of the six corridors was lit. The mouse lemur had to choose this corridor to access the reinforcement chamber. Five seconds later, the animal was put back in the starting box and two corridors were lit: the previous corridor and a new one. The animal had to go through the new corridor to get a reward. The task was performed similarly by young and old animals (Dhenain *et al.*, 1998b). In humans, general concepts and rules about the world are stocked in semantic memory. This memory also refers to language and knowledge of facts (Tulving, 1972). In the context of animal experiments, Ridley (1991) regarded the rules as equivalent to knowledge about the "meaning of things," e.g., associations among stimuli, responses, and re-

wards. They may be regarded as an equivalent of semantic memory (Ridley, 1991), especially if the behavioral response to the "rule-based test" requires flexibility (Eichenbaum *et al.*, 1992), as is the case for the DNMS test. In this context, rodent reference memory, which refers to data stable from one trial to another, is also comparable to semantic memory (Ridley, 1991). The performances of mouse lemurs during discrimination learning, nonmatching-to-sample learning, or reference memory tasks suggest that rule acquisition by semantic memory is not altered during aging and that this memory is stable for at least 3 months even in aged animals. In humans, semantic memory is not altered during normal aging (Nebes, 1992) but is altered during Alzheimer's disease (AD) (Nebes, 1989).

Once animals have learned the discrimination or the non-matching-to-sample rules, delays can be added between the presentation of the stimulus (illuminated corridor) and the response (go in a corridor). Delayed response (DR) tasks have been performed with mouse lemurs by adding delays up to 60 sec between the presentation of one lit corridor out of four and access to go through the corridors. To get a reward, animals had to choose the corridor illuminated during the presentation phase (application of discrimination rule). Performances of young animals were decreasing slightly from zero to 15-sec delays and after remained stable. Old animals displayed severe time-related memory alterations. After 30 sec, they were performing at chance level (Picq, 1995). The time-related deficiency for delays inferior to 30 sec suggested a severe alteration of short-term memory in old mouse lemurs (Wright *et al.*, 1985; Baddeley, 1998). Such an alteration is very similar to the short-term memory alteration described in other primates (Bartus, 1979; Rapp and Amaral, 1992) or reported by some authors in humans during normal aging (Craik, 1977) or AD (Nebes, 1989).

Delayed nonmatching-to-sample tasks have been performed with mouse lemurs by imposing 40 sec delays between the presentation and the choice phases of the nonmatching-to-sample task. Tests involving delays from 30 to 60 sec with intervening distracting events are known to assess long-term memory in animals (Ridley, 1991). The distracting event used in lemurs consisted of moving the animal from the reinforcement chamber to the work chamber. Two kinds of DNMS-related tasks exist (Ridley, 1991). If different pairs of objects are used for each trial, then the DNMS is a trial unique task because there is no interference between the current trial and former trials. However, if a very limited number of stimuli is used, the DNMS is a trial-dependent task; earlier trials provide interfering information and the task requires the memory of the event presentation (Rapp, 1993; Pertrides, 1994). The small number of corridors that mouse lemurs had to discriminate during the DNMS (six corridors) would make the task a trial-dependent task (see Dhenain *et al.*, 1998b, for discussion). Good performance in the task depends on the integrity of temporomedian and prefrontal areas (Otto and Eichenbaum, 1992). The memory involved is similar to the human episodic memory, which is a memory for precise events, specific of every subject life and occurring in particular temporospatial contexts (Squire *et al.*, 1993). Performances of aged mouse lemurs in the DNMS task were significantly poorer than those of young adults (Dhenain *et al.*, 1998b). Interestingly, as described previously, aged mouse lemurs' memory is also impaired in the "rodent" working memory task of the radial maze, which is also a trial-dependent task. This suggests an alteration of the long-term episodic memory in aged mouse lemurs. This alteration is similar to episodic memory alteration described during human normal aging (Nebes, 1989) and also in AD (Baddeley *et al.*, 1991).

Age-related behavioral alterations in mouse lemurs suggest that prefrontal areas are altered during aging. This is supported by the observed DNMS memory impairment and also by poor performances of aged mouse lemurs in other tasks known to involve this area, such as the DR task (Fletcher, 1965; Goldman and Rosvold, 1970). The perseverance tendency of aged animals in several tasks (Picq, 1993b) also favors this hypothesis. Alteration of the prefrontal area in mouse lemurs is similar to the one described in other primates (Rapp, 1993) and also in human during normal aging (Schacter *et al.*, 1996; Petit-Taboue *et al.*, 1998). The temporal lobe also seems altered during aging in mouse lemurs, as suggested by the poor performances in the DNMS and in spatial memory tasks such as the free object exploration task or the radial maze. The hippocampus in particular is the temporal region involved in spatial tasks (Olton and Papas, 1979). A medial temporal alteration has been described in a subpopulation of primates (although in a task more dependent on entorhinal cortex) (Rapp and Amaral, 1992; Rapp, 1993). Temporal alteration has been shown to be particularly severe in subjects with AD (Desgranges *et al.*, 1998).

As a conclusion, mouse lemurs present behavioral alteration during aging. Actual studies described average behavioral changes in populations of aged animals. In these studies, individual heterogeneity has often been reported in aged animals. Future work must characterize the normal pattern of age-related behavioral changes so that pathological aging can be identified accurately by comparison.

III. Age-Related Cerebral Atrophy and Neuronal Alterations in Mouse Lemurs

Neuromorphological studies revealed ventricular enlargement associated to a severe cortical and hippocampal atrophy in a few aged specimens. Other regions, such as the corpus callosum, the fornix, the basal ganglia, the brain stem, and the cerebellum, are atrophied in some aged animals (Bons *et al.*, 1991a,b). More recently, Dhenain *et al.* (2000) showed in a longitudinal magnetic resonance imaging (MRI) study that cerebral atrophy only occurs in aged mouse lemurs but that the aged animals are not all atrophied. Atrophy thus appears to be an age-related pathological condition and not an inevitable effect of age. The atrophy process evolves within a few months and starts between 5 and 8 years old. Several subgroups of animals can be distinguished on the base of regional atrophy. For example, some animals showed a severe atrophy of the temporal lobe, whereas others displayed diffuse cortical atrophy in addition to temporal atrophy. Further studies are necessary to determine the relationships between these different types as well as the origin of cerebral atrophy, which might be associated with the presence of neurites and neuronal degeneration (Bons *et al.*, 1991b). The detection and follow-up of the atrophic process *in vivo* with MRI offer the possibility to search for the biological correlates of ongoing neurodegeneration.

IV. Amyloid Deposits, Amyloid Angiopathy, and Cytoskeletal Alterations

Senile plaques are extracellular β -amyloid deposits associated to reactive astrocytes, microglial cells, dystrophic neurites, and neurofibrillary tangles (NFT). Amyloid angiopathy is amyloid deposits surrounding blood vessels. NFT are neuronal accumulations of hyperphosphorylated tau proteins, and with senile plaques, and amyloid angiopathy, are the neuropathological hallmarks of AD (Wisniewski *et al.*, 1989; Ball *et al.*, 1997). They can also be present in nondemented aged subjects, although in lower density and in more restricted areas (Hyman *et al.*, 1993).

A. Amyloid Deposits

The $\text{A}\beta$ peptide sequence in mouse lemurs is homologous to the human $\text{A}\beta$ (Silhol *et al.*, 1996). In mouse lemurs older than 7 years old, histological studies (amyloid-specific silver method, histochemistry for $\text{A}\beta$ 1–40 and APP, binding of the amyloid-indicating dyes, thioflavin and Congo red, and immunocytochemistry against $\text{A}\beta$) revealed the presence of $\text{A}\beta$ protein aggregates in the cerebral cortex, the meninges, and the cerebral vasculature (amyloid angiopathy), of neuritic plaque-like deposits composed of degenerated neurites surrounding an amyloid deposit, and of neurofibrillary changes such as bundles of argyrophilic filaments in pyramidal neurons (Bons *et al.*, 1991a,b, 1994, 1995b; Mestre-Francés *et al.*, 1996) (Fig. 27.4). The temporal and parietal lobes are especially affected by $\text{A}\beta$ deposition. In animals older than 7 years, $\text{A}\beta$ protein deposits were observed in four forms, from small cloudy deposits to a dense core of $\text{A}\beta$ surrounded by a halo



FIG. 27.4. Amyloid deposits in a 11.4 year old female mouse lemur cortex ($\times 20$, top) and amyloid angiopathy in leptomeningeal vessels ($\times 20$, arrows, bottom). Tissues were stained with antibody E50 against A β .

of amyloid fibrils (Mestre-Francés *et al.*, 1996). These different forms may characterize different stages of maturation. Only a few animals over 7 years presented very numerous amyloid deposits, which seemed to represent a characterized pathology (Mestre-Francés *et al.*, 1996). Diffuse amyloid deposits were also found in very young adults (1 year old).

B. Cytoskeletal Alterations

Tau is a cytoskeletal microtubule-associated protein. In humans, NFT are neuronal accumulations of pathological hyperphosphorylated tau proteins that form the basic matrix of paired helical filaments (PHF) within degenerating neurons. The biochemical profile of tau proteins during cerebral aging has been characterized in the mouse lemur using immunoblotting and several probes directed against human tau proteins and PHF by Delacourte *et al.* (1995). The molecular weight of tau proteins increased during aging in mouse lemurs, reflecting age-related states of hyperphosphorylation (Bons *et al.*, 1995b; Delacourte *et al.*, 1995). The localization of abnormally phosphorylated and PHF-immunoreactive tau proteins has been studied in lemurs by immunohistochemistry (Bons *et al.*, 1995a,b; Giannakopoulos *et al.*, 1997). Antibodies directed against "normal" human tau proteins (Flament *et al.*, 1989) revealed staining in almost all of the tested animals. Antibodies

directed against both normal and abnormally phosphorylated tau proteins (961-S28T) (Delacourte *et al.*, 1990) revealed tau proteins aggregated in thick granules in almost all of the tested animals (independent of their age). This particular form of tau proteins seems to be specific of lemurs and has not been described in humans (Bons *et al.*, 1995b). Numerous aggregated tau proteins were always found in lemurs with numerous amyloid deposits. Lemurs without amyloid deposits either displayed numerous neurons with aggregated tau proteins or few labeled neurons (Bons *et al.*, 1995a). Neocortical areas were frequently affected, even in young mouse lemurs, whereas the subiculum and entorhinal cortex were only involved occasionally in animals older than 8 years (Giannakopoulos *et al.*, 1997). The fact that the hippocampus was relatively nonlabeled in lemurs, whereas it is one of the first structure to be involved by NFT formation in AD (Braak and Braak, 1991), could be explained by different characteristics of neuroprotection in nonhuman primate and human hippocampus. It could also be related to the nonspecificity of the antibodies used in the lemurs to detect abnormal tau proteins characteristic of AD. Finally, the use of antibodies directed against abnormally phosphorylated tau proteins revealed either no staining (Dhenain *et al.*, 2000) or a weak immunolabeling in comparison to the one obtained in AD patients (adsorbed anti-PHF antibody; Bons *et al.*, 1995a). Animals that displayed a weak immunolabeling with anti-PHF antibody labeling displayed numerous aggregated tau proteins with 961-S28T antibodies. This suggests that PHF-immunoreactive tau proteins are related to the presence of high number neurons with aggregated tau proteins. However, some animals showing numerous aggregated tau proteins with 961-S28T antibodies did not display labeling with anti-PHF antibodies.

In conclusion, alterations in tau metabolism occur during aging in lemurs and pathological tau metabolism might be related to amyloid deposits. However, the tau immunoreactivity that is displayed by plaque neurites in mouse lemurs can hardly be compared to the tau immunoreactivity seen in AD (it is weaker or absent) and PHF have never been described in lemurs. From these results, it appears that the production of highly specific immunological probes directed against specific tau protein isoforms is needed to evaluate better the tau pathology displayed by lemurs.

C. Genetic Origin of "Alzheimer-like" Lesions in Lemurs

In humans, mutations of different genes, such as the β -amyloid precursor protein (APP) gene (which codes for large glycoproteins, which can be metabolized to form the A β peptide) (Kang *et al.*, 1987), presenillin 1 (PS1), and presenillin 2 (PS2), have been associated with familial forms of AD. These genes have been sequenced in lemurs. APP and PS1 distributions in mouse lemur brain are similar to that observed in humans and, similar to humans, are localized in the same brain structures (Calenda *et al.*, 1996; Silhol *et al.*, 1996). In contrast, few neurons can be marked with combined PS2 protein and APP immunoreactivity (Calenda *et al.*, 1998). The presence of genes related to AD in lemurs suggests that they are genetically susceptible to display mutations in genes involved in familial forms of AD. However, so far, neither

the presence of mutations involved in familial cases of AD nor the presence of mutational founder effects has been described in animals with amyloid deposits.

Variations in the age of onset and risk of AD are associated with the apolipoprotein E locus in humans (Strittmatter and Roses, 1996). Three isoforms (E2, E3, and E4) exist and are distinguished by cysteine-to-arginine substitutions at residues 158 and 112 (Mahley, 1988). A strong association has been found between the presence of the E4 isoform and the occurrence of AD (Schmeichel *et al.*, 1993; Gómez-Isla *et al.*, 1996). The human apoE allele system has unique characteristics when compared to other primates (Finch and Sapolsky, 1999), and only the E4 isoform of apolipoprotein E was evidenced in the mouse lemur. ApoE genotyping revealed that nine amino acids differ between human and mouse lemur ApoE isoforms. However, the two amino acids characteristic of the human ApoE4 isoform are conserved, suggesting that the ApoE in mouse lemurs is phenotypically analogous to human ApoE4 (Calenda *et al.*, 1995). This result is similar to results in macaque monkey, baboon, cow, pig, mouse, and rat, but not rabbit (Poduri *et al.*, 1994).

V. Neurochemical Alterations

Immunocytochemical studies reveal that the structure of the cholinergic basal forebrain, essentially the nucleus basalis of Meynert, is very similar in both strepsirrhine and haplorhine primates, including humans. Cholinergic neurons have been observed in the mouse lemur brain with choline acetyltransferase (ChAT) immunocytochemistry in the septum, the diagonal band of Broca, the nucleus accumbens, the nucleus basalis of Meynert, the caudate, the putamen, the globus pallidus, and the olfactory tubercle. In the aged mouse lemur, cytological changes and neuronal loss have been observed in these structures, suggesting an alteration of the cholinergic function during aging (Mestre and Bons, 1993). However, the ChAT activity increases during aging in lemurs (Dournaud *et al.*, 1994). The apparent contradiction between immunocytochemical and biochemical studies might be related to different age ranges of the animals included in both studies. Studies of other neurotransmitter systems revealed a loss of serotonergic and catecholaminergic neurons during aging (Jallageas *et al.*, 1998). Biochemical studies revealed that cortical somatostatin levels do not change with aging (Dournaud *et al.*, 1994).

VI. Iron Accumulation

A. Captive Lemurs and Iron Overload

In various mammals, including human and nonhuman primates, a progressive increase of iron deposits in the brain, especially the basal ganglia, is characteristic of normal aging (see Koeppen, 1995, for a review). Strepsirrhine primates are of particular interest in the study of iron absorption and deposition because hemosiderosis has been observed consistently in captive aged animals. Spelman *et al.* (1989) observed that all 49 lemurs necropsied since 1968 at the San Diego Zoo were hemosiderotic. Their high protein chow diet may have contributed to excessive iron absorption. The absorbed iron is first

apparent in the macrophages of the intestinal lamina propria, in Kupffer cells, in splenic cells, and in other nonparenchymal cells. With increasing iron overload, liver parenchymal cells are also affected, and hepatic cell necrosis, as well as periportal fibrosis, is observed. Iron overload in lemurs to some extent resembles human transfusional siderosis (Iancu and Shiloh, 1994). As in humans, the severity of the disorder is correlated to age. Indeed, iron homeostasis is disrupted during the aging process (Gelman, 1995).

Histochemistry of brain iron deposits, using Perl's staining, in old animals (8–15 years old) revealed that iron pigments are localized mainly in the globus pallidus, the substantia nigra, the neocortical and cerebellar white matter, the thalamus, and in anterior forebrain structures, including the nucleus basalis of Meynert (Fig. 27.6, see color insert) (Dhenain *et al.*, 1998a; Gilissen *et al.*, 1998, 1999b). This distribution agrees with previous findings in monkeys and humans, but among primates, only cheirogaleids and humans show iron deposits in the thalamus. Nonheme iron in the brain is encapsulated by ferritin, the primary tissue iron storage protein.

B. In Vivo Detection of Iron with MRI during Brain Aging

MRI can detect *in vivo* naturally occurring brain depositions. Because of iron's paramagnetic characteristic, brain regions with a high iron content have short T2 relaxation times, yielding a hypointense (dark) signal in T2 or T2*-weighted images as compared to regions with a low iron content (Drayer, 1989). This effect is more prominent at a high magnetic field because susceptibility effects, such as iron-related T2 relaxation time decrease, are at least linearly dependent on magnetic field strength (Fisel *et al.*, 1991; Lee, 1991; Bartzokis *et al.*, 1993; Lee *et al.*, 1995; Schenck, 1995; Vymazal *et al.*, 1995; Gati *et al.*, 1997). In addition, an iron-dependent contrast is enhanced with increasing field strength, whereas the other T2 effects are not changed. The effects of other processes that can affect T2 relaxation times (tissue characteristics) (Bartzokis *et al.*, 1993) would therefore be proportionately less important at a high magnetic field.

Brain iron content was examined in young and aged cheirogaleid primates by Dhenain *et al.* (1997a,b, 1998a) with 4.7 T magnetic resonance (Bruker Biospec 47/30 system) and by Gilissen *et al.* (1998, 1999b) with high field magnetic resonance microscopy (Bruker AMX500 11.7 T MRI system) (Narasimhan and Jacobs, 1996). Results obtained from MR images were corroborated to histochemistry studies. The age-dependent MRI signal decrease in dwarf and mouse lemur brains appeared to be related to iron accumulation.

1. Iron Content of the Globus Pallidus: An *In Vivo* Indice of Brain Aging

Dhenain *et al.* (1997a,b) observed very high correlations between the T2-weighted MRI signal decrease (T2ws) and the natural logarithm of mouse lemur age in the globus pallidus ($r = 0.95$), in the substantia nigra ($r = 0.81$), and in the thalamus ($r = 0.80$). The T2ws decreased rapidly until the age of 4 years. After this age, in middle-aged and older animals the signal decrease became less important. A rapid

T2ws decrease in young individuals, less rapid in middle-aged and slower in aged individuals, has also been observed in humans (Pujol *et al.*, 1992; Schenker *et al.*, 1993; Bartzokis *et al.*, 1997). Further studies are necessary to assess if the kinetics of signal intensity decrease during aging is similar in mouse lemurs and humans.

The high correlation coefficient for the pallidum ($p < 0.0001$) suggests that it should be an excellent structure to study the T2 signal decrease as a marker of age. The T2 signal measure might be useful in assessing the efficiency of pharmaceutical agents aimed at reducing iron deposition in the aging process as well as standardizing the brain iron content during pharmaceutical tests.

2. Iron in the Basal Forebrain

The nucleus basalis of Meynert, the largest source of cholinergic fibers in the brain, is affected during pathological aging in humans (Samuel *et al.*, 1991; Cullen *et al.*, 1997 see Chapter 19). Bartzokis *et al.* (1994, 1997) suggested that future directions of *in vivo* evaluation of tissue iron with MR should focus on structures that are highly relevant for pathological aging, such as the basal forebrain. In particular, the nucleus basalis of Meynert is structurally and functionally closely associated with the ventral extension of the globus pallidus. Iron levels in the ventral globus pallidus are among the highest in the brain and are well visualized in humans with standard clinical magnets (1.5 T). Increased iron levels in the globus pallidus may be a marker of increased iron in the basal forebrain (Bartzokis, 1997). In humans, iron deposition in basal forebrain cholinergic structures is difficult to visualize *in vivo* because of their small size. High resolution and high magnetic field (11.7 T) MRI images of mouse lemurs allowed the detection of iron in the basal forebrain (Gilissen *et al.*, 1999b). The areas of iron accumulation largely overlap the distribution of ChAT-

immunoreactive neurons. Moreover, the MR contrast resulting from the iron content is comparable in the basal forebrain structures and in the globus pallidus (Figs. 27.5 and 27.6). This suggests that iron deposits affect basal forebrain cholinergic structures because of their close relationship with the ventral globus pallidus. Further longitudinal studies are necessary to assess how correlated are the iron depositions in the basal forebrain and the globus pallidus.

VII. Lipofuscin: Another Marker of Aging Unrelated to Iron Deposits

Accumulation of lipofuscin, an age pigment derived by lipid peroxidation, constitutes another reliable but invasive marker of aging. Iron promotes *in vivo* lipofuscin formation and is

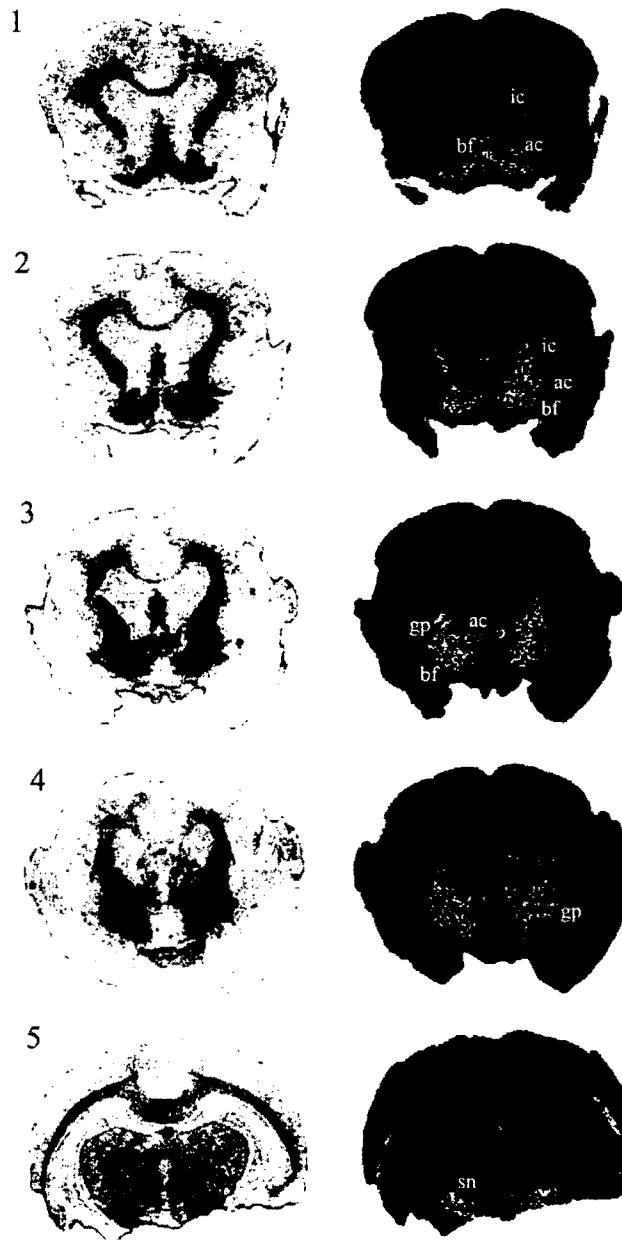


FIG. 27.5. Coronal MR scan T2*-weighted images of a 12-year-old mouse lemur brain. The hypointense (dark) signal on the left images corresponds to iron accumulation in basal forebrain structures (bf) such as the septum, the diagonal band of Broca, and the substantia innominata (1–3), in globus pallidus (gp) (3 and 4), and in substantia nigra (sn) (5). Iron is also present in the anterior commissure (ac) and internal capsule (ic). Signal intensity was measured using a gray scale ranging from 0 (black) to 255 (white). Gilissen *et al.* (1999b) assessed the maximum intensity value of the globus pallidus, substantia nigra, and basal forebrain structures. Upper thresholds were finally applied in order to set defined ranges of voxel values to be displayed. These thresholds correspond to the maximum intensity values of globus pallidus, basal forebrain structures, and substantia nigra. The lower voxel threshold was set at 0. In the right images, the voxel values beyond the upper thresholds are eliminated. In the case of the specimen illustrated here, upper thresholds were set at 35.2 (1–4) and 41.0 (5). To permit the remaining voxels to be visualized, the intensity histogram was adjusted to enhance contrast. In the right images, the resulting views display the basal forebrain structures (1–3), the globus pallidus (3 and 4), and the substantia nigra (5), as well as some adjacent structures with similar maximum intensity values. On a series of four aged animals (8–15 years old), the mean value of the upper threshold is 34.7 for the globus pallidus, 35.1 for the basal forebrain structures, and 40.8 for the substantia nigra (Gilissen *et al.*, 1999b). The right images indicate that the contrast resulting from the iron content is similar in the basal forebrain structures and in the globus pallidus. (From Gilissen *et al.*, *J. Neurol. Sci.*, vol. 168, 1999. Reprinted with the permission of Elsevier Publishers.)

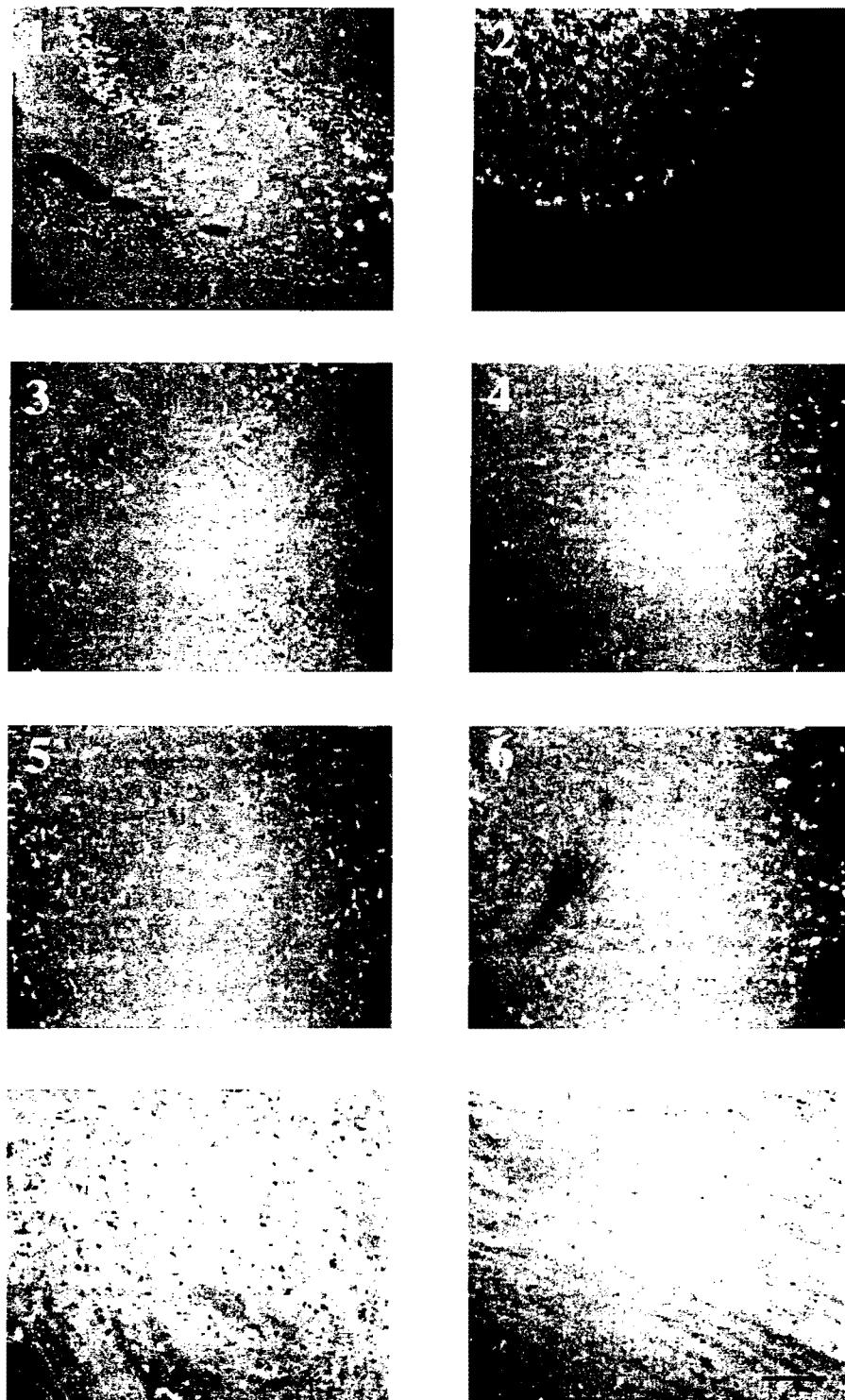


FIG. 27.7. Different fluorescence microscopy views of parasagittal cryostat sections of a 15-year-old dwarf lemur brain. The age pigments appear bright on the gray background. 1, hippocampus; 2, cerebellar folium; the age pigments are localized in the Purkinje cell layer; 3, olfactory bulb; the age pigments are mainly localized in the mitral cell layer; 4, olfactory nucleus; 5, neocortex; 6, basal forebrain; 7, globus pallidus; and 8, substantia nigra. The age pigments are less visible in the globus pallidus and the substantia nigra, where iron deposits are abundant. Scale bar: 0.1 mm. (From Gilissen *et al.*, *Am. J. Primatol.*, vol. 49, 1999. Reprinted with the permission of Wiley-Liss Publishers.)

usually detectable in high concentrations in lipofuscin granules. Nevertheless, even though iron is known to catalyze lipid oxidation (see Gilissen *et al.*, 1999a, for a review), Gilissen *et al.*, (1999a) observed that iron deposits and lipofuscin accumulation are not always coincident. Brain sections of aged (8–15 years old) and young (2–3 years old) dwarf and mouse lemurs were examined by autofluorescence and processed for iron histochemistry. Similar to iron, 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 brain stem. Nevertheless, only a few scattered pigments were present in the thalamus, the globus pallidus, the substantia nigra, the striatal fundus, and the striatum, where dense iron deposits were observed (Fig. 27.7). Different biochemical and morphological cellular compartments might be involved in iron and lipofuscin deposition. Finally, the nonuniform distribution of lipofuscin indicates that brain structures are not equally sensitive to the factors causing their accumulation.

VIII. Manipulation of Aging: Changes in Photoperiodic Cycle

The mouse lemur is a good model system for the study of aging chronobiology. This primate exhibits behavioral and physiological seasonal cycles that are strictly controlled by variations in day length (photoperiodic variation). Perret (1997) revealed that, in the mouse lemur, seasonal rhythms can be accelerated by exposing captive animals to accelerated photoperiodic conditions consisting of 5 months of a long photoperiod followed by 3 months of a short photoperiod instead of 6 months of a long period followed by 6 months of a short period (as is the case in the natural environment). Long-term acceleration of seasonal rhythms obtained with accelerating photoperiodic cycles reduced the mean life span from 63.2 ± 2.5 to 45.5 ± 2.1 months and the maximal life span from 98 ± 3.9 to 79.3 ± 3.3 months. This corresponds to about a 30% reduction of life span and is neither accompanied by a desynchronization of biological rhythms nor related to an increase in reproduction or in duration of time spent in active conditions (Perret, 1997). A striking conclusion emerges from this study. When the number of seasonal cycles experienced by one individual is considered rather than chronological age, the mean life span was 5 seasonal cycles and maximum survival reached 9–10 cycles, independent of sex or of photoperiodic conditions. It is therefore possible that in mouse lemurs, and probably in other seasonal mammals, longevity may depend on the expression of a fixed number of seasonal cycles rather than on a fixed biological age (Perret, 1997).

IX. Summary and Conclusions

When compared to other primates, the life history parameters of the mouse and dwarf lemurs make them an excellent

model system for the study of normal and pathological cerebral aging. Age-related behavioral alterations, as well as cerebral atrophy, have been observed in some aged animals. Amyloid deposits and neuritic degeneration associated with the presence of abnormally phosphorylated tau proteins (probably different than those observed in AD) have also been observed in the cerebral cortex of mouse lemurs. Other markers of aging, such as lipofuscin and iron deposits, have also been described in the brain of these primates. Iron distribution constitutes a reliable marker of cerebral aging. Brain regions with a high iron content have short relaxation times, yielding a hypointense (dark) signal in T2- and T2*-weighted MR images. T2 measures in the pallidum might be a useful *in vivo* marker for assessing the efficiency of pharmaceutical agents aimed at reducing iron deposition during the aging process. Because iron homeostasis is disturbed in pathological aging, the estimation of iron content in basal forebrain is of importance because of the implication of this structure in aging or AD. In humans, the basal forebrain is, however, very small when compared to the rest of the brain, and its iron content is difficult to study *in vivo*. Because of their specific brain proportions, mouse and dwarf lemurs appear to be an ideal model system for studying *in vivo* iron changes in the basal forebrain in relation to aging and neurodegeneration. Finally, this primate offers a good model system for aging chronobiology because its life span can be changed by manipulating photoperiodic cycles.

Aspects of age-related neurodegeneration differs between primate species or even between strepsirrhine primates and related species (Schmeichel *et al.*, 1996). Different species-specific mechanisms can induce species-specific hallmarks of normal and pathological aging. If the combination of factors contributing to the onset of AD is specific to the human species, comparative studies are certainly of prime interest for dissecting the relationships between these factors and for elaborating therapeutic strategies.

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