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Review

# The grey mouse lemur: A non-human primate model for ageing studies

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ABSTRACT

The use of non-human primate models is required to understand the ageing process and evaluate new therapies against age-associated pathologies. The present article summarizes all the contributions of the grey mouse lemur *Microcebus murinus*, a small nocturnal prosimian primate, to the understanding of the mechanisms of ageing. Results from studies of both healthy and pathological ageing research on the grey mouse lemur demonstrated that this animal is a unique model to study age-dependent changes in endocrine systems, biological rhythms, thermoregulation, sensorial, cerebral and cognitive functions.



Ageing seems to be the only available way to live a long life.
Auber, Daniel Francois Esprit

## 1. Introduction

Over the last century, human life expectancy has dramatically increased and the number of aged individuals is still rising. This trend results in the development of great public and scientific concern in ageing, as well as in a demand for strengthening the research effort on the mechanisms responsible for the ageing processes, and on the methods that could prevent age-related diseases.

Along with age, a series of physiological and morphological changes progressively transform young healthy adults into older adults exhibiting increased risks for expressing a wide range of potentially lethal diseases, but also for several kinds of disabilities, which are limiting facets of daily living. The ageing process, observed in virtually all organisms (including unicellular organisms, as yeast), is a complex biological phenomenon which needs to be understood notably for the possible benefits for humans.

Non-human primates are at the crossroad between genetic models (such as *Drosophila melanogaster* and inbred mouse strains), non-transgenic rodent models, and human beings, and constitute indispensable models for physiological and biochemical research on ageing. For ageing research, non-human primate models are more relevant to human ageing than classical biological models, such as rodents (Lavery, 2000), for two reasons: (i) they share several genetic, physiological, and anatomical similarities (a complex nervous system in particular) with humans, (ii) they mimic the heterogeneity observed in the human population. Moreover, they can be studied under controlled experimental conditions more easily than humans.

This review focuses on ageing of a non-human primate model: the grey mouse lemur, *Microcebus murinus* that will be referred to as "mouse lemurs" throughout the review. Using mouse lemurs has several advantages in ageing research with regard to using other non-human primates: (1) relatively shorter lifespan (8–12 years),

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implying that ageing in mouse lemurs can be observed faster than in other primates (e.g., rhesus macaques, which live approximately 35 years); (2) small size (body length ~12 cm, ~60–120 g), rapid maturity (first year) and relative high fecundity for a non-human primate (gestation: two month and weaning: two months; 1–3 offspring per litter) (Perret, 2005); (3) housing and dietary knowledge of mouse lemurs afford optimal living conditions and ease of maintenance in the laboratory; (4) environmental manipulations can alter lifespan (Perret, 1997); (5) information already available on ageing (present review). Moreover, mouse lemurs are exceptionally long-lived relatively to mammals of similar size; they live up to two-three times longer than mammals of equivalent body mass (Stuart and Page, 2010), suggesting that mouse lemurs could be used to address key questions about basic ageing processes. Finally, mouse lemurs are photoperiod-dependent animals, expressing marked seasonal rhythms in response to seasonal alternation of long day lengths (>12 h/day) and short day lengths (<12 h/day); this last feature allows for the study of adaptive responses under different photoperiodic conditions.

The present review aims at regrouping the major results of all studies on mouse lemurs ageing, and to update and complete earlier longevity data on this primate as previously described by Perret (1997). In humans, ageing is associated with changes in appearance, sensory and motor impairment, declines in sexual activity, endocrine modifications, declines in metabolism and thermoregulation, declines in cardiovascular, respiratory and immune functions, and impairment of certain memory functions (Lata and Alia, 2007). We will focus on several biological systems which are impaired in lemurs, as in humans: the sensori-motor system, the endocrine systems, the biological clock, thermoregulative system, and cerebral and cognitive functions.

## 2. Longevity

Per year, the Brunoy breeding colony averages about 500 mouse lemurs of various ages, originating from wild animals from south Madagascar 40 years ago. All studied mouse lemurs were laboratory born, and maintained under constant conditions of ambient temperature (24–26 °C) and relative humidity (55%). To control seasonal and daily rhythms of mouse lemurs, an artificial photoperiodic regimen was used consisting in alternating 26 week-period of short day lengths (10 h light/day, Malagasy winter-like photoperiod) and 26 week-period of long day lengths (14 h light/day, Malagasy summer-like photoperiod). The shift from one photoperiod to another was given without transition. Light was provided by cool fluorescent lamps (250–350 lux) and a dim red or blue light (about 0.002 lux) was provided during the dark phase. Animals were fed with fresh fruit, milky mixture (eggs, cereals, milk cheese and honey bread) and meal worms (~23–29 calories/day animal in short and long photoperiod respectively). Mouse lemurs were housed in a controlled social environment. Cages of different sizes (from ~1 to 6 $m^3$), provided with many supports (wooden branches, flexible cords) and wooden nests were used: the largest cages for groups (6–8 animals) either during sexual competition or during winter rest, the smaller for isolated animals (pregnant or lactating females, scientific experiments, injury). In a general way, except obviously during period of required sexual competition and breeding, sexes were maintained separated to avoid intra-sexual competition. To ensure breeding, heterosexual groups (2–3 females with 3 males) were constituted only for a short period (3–5 weeks) during the long day lengths. Body mass and health status were controlled at least once a month, and necropsies were performed in all animals that died spontaneously.

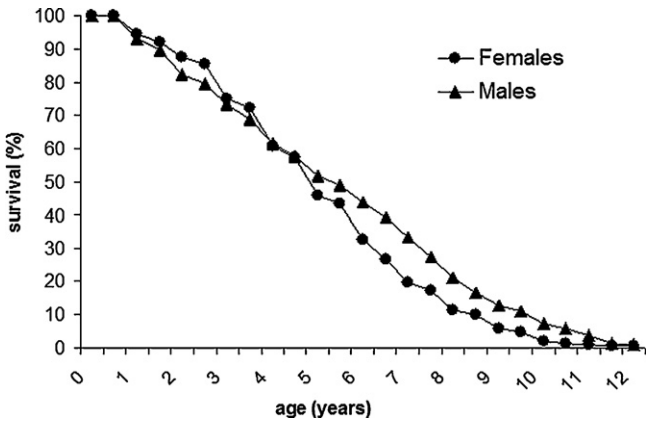


**Fig. 1.** Survival curve from 643 mouse lemurs (361 females and 282 males) of the Brunoy colony.

We have analyzed longevity of 643 mouse lemurs (361 females and 282 males) which were born and died in the laboratory (Fig. 1). The median survival time, time at which half of the population has died, is generally used to delineate the adult and aged portions of a population. In this colony, the median survival time of mouse lemurs is 4.9 years for females and 5.7 years for males. At 5/6-year-old, morphological modifications suggest ageing: bleaching of the fur of the face, belly and back, shortening of the snout and thickening of the border of the ear auricle (Fig. 2). Thus, for the sake of simplicity, we consider mouse lemurs younger than 5 years as adult animals, and older than 6 years as aged animals.

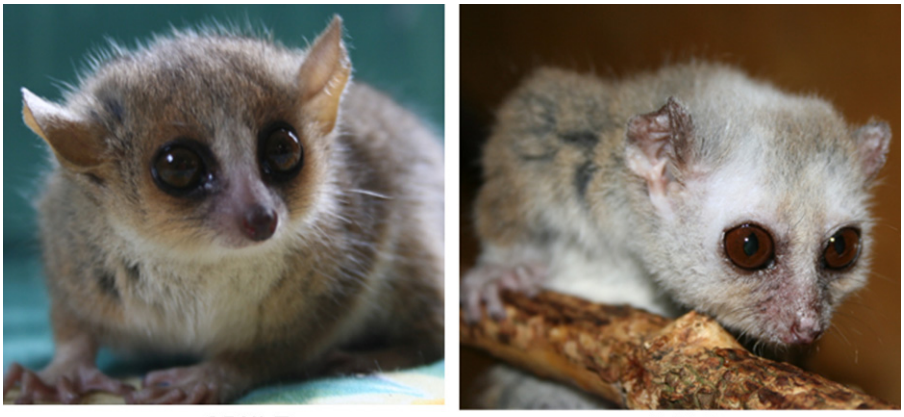


**Fig. 2.** Representative pictures of an adult and an aged mouse lemur.

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In the wild, animals rarely live to their maximum lifespan because they are exposed to several causes of death that do not exist in captivity (e.g., predation, food restriction, parasitism). In our laboratory, the maximum recorded lifespan is 12 years. The mean lifespan of the top 10% of the oldest individuals is shorter in females than in males ($9.9 \pm 0.2$ and $11.0 \pm 0.2$ years, respectively; $t(62) = 4.6$, $p < 0.0001$). Interestingly, whatever their age, mortality of mouse lemurs depends on both sex and period of the year (short versus long day length): 72% of females and 59% of males die during long day length (comparison long versus short day length; females: $\chi^2 > 20$, $p < 0.0001$; males: $\chi^2 = 10.8$, $p = 0.001$; comparison females versus males: $\chi^2 = 11.7$, $p < 0.001$). High mortality rate during the reproductive period (long day length) and sex-difference in lifespan may be linked to reproductive pressure. In adults, death was mainly due to complications resulting from wounds. In aged individuals, rare cases of cancers were observed and most of the deaths can be attributed to progressive renal insufficiency (chronic nephrosis with nephritis) leading to respiratory insufficiency.

## 3. Phenotypes of aged mouse lemurs

### 3.1. Age-associated alterations of the sensorial system

#### 3.1.1. Vision and ageing

The mouse lemur has an intriguing visual system which is interesting to explore for potential ageing effects. This nocturnal species shows a series of adaptations that improve photon capture under low levels of illumination: eye characteristics such as large globe and pear-shaped pupil are completed by an extensive tapetum which improves photon capture but likely at the expense of visual resolution (Dkhissi-Benyahya et al., 2001). Yet, no retinal abnormality has been observed in the eyes of adult and aged animals: the thickness and morphology of both the outer nuclear layer and the photoreceptor layer seemed well preserved suggesting no sign of retinal degeneration or atrophy, at least from an histological point of view (Beltran et al., 2007). A precise neurophysiological investigation would be required to examine whether age may affect the abundance and distribution of photoreceptors as well as their efficiency in light reception.

Cataract is the ocular lesion most frequently encountered in old mouse lemurs. An ophthalmological survey of 220 animals of all ages from two captive colonies has revealed the high incidence of this lesion, with nearly half of the old adults affected (Beltran et al., 2007). The youngest age at which lens opacities have been diagnosed is 3.5 years and the earliest age-group in which a majority of animals had cataracts is the 7–8-year group. Cataract is predominantly bilateral and progresses slowly with age. Other cataract-associated lesions are also observed such as hyphema, posterior synechiae, pupil seclusion, corneal degeneration or buphthalmia. Cataractogenesis can be caused by a diverse array of factors among which trauma, dietary and metabolic disorders (imbalance in amino-acids, sugars or vitamins), parasites, ocular inflammation, toxic substances, hereditary factors and ageing (Beltran et al., 2007). While proximate factors have not been yet identified in the mouse lemur, ageing is recognized as being a major risk factor for human cataract development (Rathbun and Holleschau, 1992). Because of potential help to understand the mechanisms of cataract formation in humans, the proximate role of oxidative stress has been studied in more detail in the mouse lemur. Age-related changes of the metabolic pathways of glutathione, a key component of the anti-oxidative system, have been demonstrated in mouse lemurs. As in humans, glutathione-synthetase activity decreases and glutathione-peroxidase activity increases with age in the lens of mouse lemurs. Nevertheless, the metabolic control of glutathione cycle in the mouse lemur differs from that of Old World simians including humans in some areas; for instance the glutamyl-cysteine synthetase activity increases with age in mouse lemurs, while activity of this enzyme decreases in Old World simians (Rathbun and Holleschau, 1992; Holleschau and Rathbun, 1994). The influence of glutathion cycle on cataract formation remains to be investigated in the mouse lemur.

#### 3.1.2. Olfaction and ageing

The mouse lemur has highly developed sensory modalities to ensure adaptive locomotor and feeding behaviors in dense forest biotope (e.g., jumping, climbing, capture of insects) but also social communication between solitary, nocturnal living individuals. In this species, the sense of smell is of high relevance for the modulation of both behavioral and physiological functions (Schilling et al., 1984; Aujard, 1997). Social communication mainly relies on chemical signals actively dispersed by mouse lemurs through typical marking behaviors such as urine-washing (Schilling and Perret, 1987). Chemosensory structures include large olfactory bulbs and a functional vomeronasal organ. These structures are especially developed reaching more than 2.6% of the cerebral mass, and represent the greatest olfactory surface in proportion compared to other primates (Smith et al., 2007). Moreover, this species provides the unique example within primates so far of the presence of true pheromones acting on sexual physiology (Schilling et al., 1984). Some elements from behavioral studies are already in favor of an altered chemosensory system in the aged mouse lemurs. First, males show with age a decrease in the frequency of sniffing and licking of the genitalia of receptive females and a decrease in the frequency of scent marking behaviors (Aujard and Perret, 1998). Second, similar behavioral and social communication patterns are observed in adult males deprived of either their vomeronasal organ (Aujard, 1997) or their main olfactory bulb (Araujo, 2003). A chemosensory discrimination test based on the discrimination between water and an odorant repellent surrounding food reveals a progressive decline in olfactory sensitivity with age (Aujard and Némoz-Bertholet, 2004). When exposed to the volatile phase of urine from proestrus females, aged mouse lemurs fail to exhibit the increase in testosterone level that is classically observed in adult males (Aujard and Némoz-Bertholet, 2004). Further investigations at the central level reveal that proestrus urine odor exposure induces Fos expression in the different cell layers of the main olfactory bulbs in adult mouse lemurs, whereas Fos expression is not increased by the odorant stimulation in aged individuals (Cayetanot et al., 2005b). This confirms that the lack of pheromonal effect on the sexual function of aged male mouse lemurs is clearly related to a lack of pheromonal input from the main olfactory system to the central nervous system. Taken together, these data constitute the first demonstration of a clear impairment of olfactory information processing in an aged non-human primate.

### 3.2. Motor function and ageing

In humans, motor capacities and balance decrease with increasing age. In mouse lemurs, aged animals move with greater difficulty (Némoz-Bertholet and Aujard, 2003). Motor coordination and endurance, measured by placing an animal on an accelerating rotating rod, is also impaired with age. From 4 year-old, balance performance decreases progressively (Némoz-Bertholet and Aujard, 2003). This age-related decrease is observed only during long day length, since both adult and aged performances are poor during short day length (Némoz-Bertholet et al., 2004).

### 3.3. Socio-sexual behavior and ageing

Decrease in social interactions with age has been frequently described in primates (Davis, 1978; Heydecke et al., 1986). In the mouse lemur, old animals remain attractive partners for mates, but

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show an active withdrawal from social interactions (Picq, 1992). During the breeding season, old mouse lemurs exhibit less sexual and aggressive behaviors than younger animals but they outrank younger males (except when they reach the oldest age, >9 years), in reaching dominant positions, increasing their reproductive success (Aujard and Perret, 1998).

## 4. Age-associated alterations of biological rhythms

### 4.1. Daily rhythms and ageing

In humans, daily rhythms of sleep, thermoregulation and hormonal secretion are severely altered with ageing. Ageing is associated with changes in amplitude and temporal organization of many daily rhythms (Van Someren and Riemersma-VanDerLek, 2007). Although mouse lemurs are nocturnal, the rest-activity rhythm becomes fragmented in aged mouse lemurs (Aujard et al., 2006a), as in humans. Mouse lemurs exhibit very marked circadian rhythms with high levels of locomotor activity during the dark period and almost complete rest during the light period (Perret and Aujard, 2001). Aged animals show a significant increase in daily variability and an advance in activity onset compared to adult animals. Furthermore, in absence of timing cues (i.e., with constant dim red light), aged mouse lemurs exhibit a shortening of the free-running period compared to adult animals (Cayetanot et al., 2005a, 2009), suggesting that ageing affects the regulation of the central clock.

The daily locomotor activity patterns change between periods of short or long day lengths. Although some age-related differences in the locomotor activity rhythm can be observed under exposure to short day length, they are predominant under long day length (Aujard et al., 2007). Some mechanisms allowing adaptation to changing day length thus seem to be impaired with ageing. Conversely to adult animals, the pattern of urinary 6-sulfatoxymelatonin excretion (index of melatonin production) is significantly altered in aged mouse lemurs which do not show the classical nocturnal peak (Aujard et al., 2001). This strongly suggests that aged mouse lemurs lack efficient entrainment to annual photoperiod variations. This is corroborated by the age-related decreased amplitude in seasonal variations of most biological functions (e.g., body mass, resting metabolic rate, sexual hormones) studied so far in the mouse lemur (Aujard et al., 2001; Perret and Aujard, 2006).

### 4.2. Central clock and ageing

The main central clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus whose endogenous oscillations are mainly entrained by light. Light is transmitted primarily via the retino-hypothalamic tract, which terminates in the ventral part of the SCN, where vasoactive intestinal polypeptide (VIP)-containing neurons and calbindin-containing neurons are located (Antle and Silver, 2005). VIP cells are mainly intrinsic and project to the dorsal part of the SCN, where neurons containing arginine-vasopressin (AVP) reside. Calbindin cells are involved in the control of circadian rhythmicity (Kriegsfeld et al., 2004). In the SCN, cellular function and sensitivity to light show drastic changes with age in the mouse lemur. In adult animals, VIP-positive and AVP-positive SCN neurons exhibit daily rhythms of their secretion: AVP immunoreactivity peaks during the second half of the day, and VIP peaks during the night. In aged mouse lemurs, the peaks of AVP and VIP immunoreactivity are significantly shifted, so that AVP is most intense at the beginning of the night, whereas VIP peak at the beginning of daytime (Cayetanot et al., 2005a). In adult animals, calbindin-positive SCN neurons do not exhibit daily rhythms in their number or intensity, but exhibit significant daily variations in the percentage of cells with a calbindin-positive nucleus, characterized by high values during the daytime and low values during the night. Immunoreactive intensity peaks in the middle of the daytime. During ageing, calbindin expression in the nuclei of calbindin cells in the SCN tends to be modified. The amplitude of daily variation in calbindin expression is dampened, with a lower immunointensity during the daytime and a delayed decrease during the night. These changes seem to affect the ability of the SCN to transmit rhythmic information to other neural target sites, and thereby to modify the expression of some biological rhythms (Cayetanot et al., 2007).

The cellular response to photic inputs in the SCN of aged mouse lemurs also exhibits dramatic changes. SCN neuronal activations evaluated by Fos expression is reduced by 88% in the SCN of aged mouse lemurs following exposure to low levels of irradiance. Exposure to higher irradiance levels shows similar results, with a reduction of 66% in Fos expression in aged animals (Aujard et al., 2001). Taken together, these results highlight a deficit in transduction of the light signal to target areas with ageing.

### 4.3. Seasonal rhythms and ageing

Long-term acceleration of seasonal rhythms (i.e., experimental shortening of season and year duration) affects survival and longevity in mouse lemurs (Perret, 1997). In captivity, acceleration of seasonal rhythms is obtained by exposing the animals to alternating periods of long and short days over a periodicity shorter than a year (8 or 5 months). Independent of sex, the mean lifespan was shortened in mouse lemurs exposed during their whole life to an accelerated photoperiodic cycle of 8 months compared to animals that lived under an annual photoperiodic cycle (Perret, 1997). This reduction of about 30% of lifespan is not accompanied by a desynchronization of biological rhythms under photoperiodic control and is not related to an increase in reproduction or in duration of time spent in active conditions. However, when the number of seasonal cycles experienced by an individual is considered rather than chronological age, the mean lifespan is 5 seasonal cycles and maximum survival reaches 9–10 cycles, independent of sex or photoperiodic regimen (Perret, 1997).

In animals exposed to 3- to 5-years of accelerated seasonal photoperiodic rhythm ("annual" duration of 5 months), disturbances of the locomotor activity rhythm are observed, that resemble those of aged mouse lemurs, whereas animals are studied in entrained or in free-running conditions (Cayetanot et al., 2005a). Animals exposed to artificially accelerated ageing exhibit the same alterations in melatonin production and Fos response to light than animals that have been maintained in a routine photoperiodic cycle (Aujard et al., 2001). These results suggest that in mouse lemurs, as in other seasonal mammals, longevity depends on the expression of a fixed number of seasonal cycles rather than on a fixed biological age.

### 4.4. Biological rhythms, immune system and ageing

Human ageing can be associated with a reduction in acquired immune responses accompanied by elevated levels of pro-inflammatory cytokines (Nikolich-Zugich, 2005)). Recent findings suggest that interferon-γ (IFN-γ) can affect the function of the SCN, both *in vitro* and *in vivo* (Ohdo et al., 2001; Sadki et al., 2007; Palomba and Bentivoglio, 2008). The magnitude of age-related disturbances in biological rhythms is correlated with the plasma level of IFN-γ shown to increase with age in the mouse lemur. Most remarkably, increased levels of IFN-γ correlate positively with longevity in mouse lemurs (Cayetanot et al., 2009). These results in mouse lemurs demonstrate that the degree of circadian rhythm alterations in an individual is correlated with plasma IFN-γ levels during ageing, and that plasma IFN-γ levels may pre-

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dict ageing. Although only correlative, these results indicate that anti-inflammatory molecules may have the potential to regulate circadian rhythms in the elderly.

## 5. Age-associated alterations of the thermometabolic system

The mouse lemur offers a potentially unique model to investigate the thermometabolic system owing to several particularities. First, the mouse lemur shows a strong seasonal cycle of body mass and physical activity. Body mass and energy intake are maximal in winter when resting metabolic rate is down due to low thyroid and gonad activities. The orexigenic ghrelin hormone is involved in the seasonal body mass gain (Giroud et al., 2009). Second, one particularity of the mouse lemur is the use of daily torpor (daily decrease in body temperature during the first half of the diurnal phase) to further save energy during the period of limited resource availability of the winter season (Giroud et al., 2008; Canale et al., 2011). This confers a great plasticity in energy balance regulation in order to face both seasonal and non-seasonal unfavorable conditions, be they linked to food availability (energy challenge, Séguy and Perret, 2005; Giroud et al., 2008; Canale et al., 2011) or physical conditions (temperature challenge, Terrien et al., 2008, 2009a). Studies conducted on adult mouse lemurs converge to the idea of a great energy flexibility and demonstrate (1) the efficiency of torpor as an energy saving mechanism, (2) the strong influence of photoperiod on thermoregulation and energy balance regulation (Giroud et al., 2008) and (3) the capacity to use either protein or fat sparing strategies in response to the constraints of the environment of a given season (Giroud et al., 2010). Although the use of torpor and the strong seasonality of the mouse lemur confer specificities to study thermoregulation in this heterothermic primate, it can provide a powerful basis to study ageing of the thermometabolic system. Indeed, the maintenance of an equilibrated energy balance is critical but complex in the elderly (Morley, 1990; Meydani, 2001; Ritz, 2001).

### 5.1. Thermal challenges and ageing

In response to cold exposure, aged mouse lemurs exhibit increased frequency of deep torpor as compared to adult animals (Terrien et al., 2008). The greater occurrence of very low body temperature values is combined with decreased body mass without impairment of body composition. Furthermore, aged animals show a negative correlation between high rates of torpor use and low levels of insulin-like growth factor-1 (IGF-1) (Terrien et al., 2008), a growth factor that has been proposed to be involved in non-shivering process (Yamashita et al., 1994; Duchamp et al., 1997). Taken together, these results suggest that aged mouse lemurs exhibit great difficulty facing cold exposure, inducing very low levels of body temperature. Such impairment induces high rates of energy expenditure, ultimately leading to energy imbalance. It is noteworthy that these results were obtained with animals during the short day length period. In aged mouse lemurs in the long day length period, cold exposure does not induce the occurrence of deep torpor (Terrien et al., 2009a). These results highlight the strong effect of photoperiod on thermoregulatory capacity in the aged mouse lemur. Ability of the aged mouse lemur to maintain normothermia during long but not short day length could be due to indirect thermogenic effect of reproductive status (e.g., sexual steroids levels).

### 5.2. Energy metabolism and ageing

Exposure to low ambient temperatures ultimately induces increased energy expenditure (Gordon, 1990), reflecting the greater needs for body heat production. Consequently, animals increase their food intake to compensate high rates of energy metabolism and be able to fuel the thermogenesis processes (Balasko et al., 2006). In heterotherms, a trade-off between the energy saved during daily drop in body temperature and the energy cost of arousal ensures the maintenance of an equilibrated energy balance. In the mouse lemur, ageing significantly affects such a trade-off during cold-induced energy stress and alters the energetics of daily heterothermia.

Under short day length, ageing is associated with increased levels of energy expenditure during cold exposure, in concomitance with impaired energy balance. Interestingly, increased energy expenditure and depth of hypothermia phases are strongly correlated. Consequently, high energy needs are induced in the ageing mouse lemur exposed to cold, leading to great energy expenditure levels and making the torpor a counterproductive mechanism (Terrien et al., 2009b). Among heat-production mechanisms, non-shivering thermogenesis (NST) is of major importance in heterotherm species, including the mouse lemur (Genin et al., 2003). The enhancement of this process during cold exposure could be at the origin of energy imbalance in aged mouse lemurs. In reference conditions, the ability to activate NST is preserved during ageing as morphology of brown-adipose tissue and presence of uncoupling protein 1 (mitochondrial protein of brown-adipose tissue) do not differ between adult and aged mouse lemurs (Terrien et al., 2010a). Also, the pharmacological activation of NST reveals similar increased levels of $O_2$ consumption in adult and aged animals, confirming that no age effect can be evidenced on NST activation in reference conditions. However, the pharmacological activation of NST reveals an impaired evacuation of the excess body heat in aged animals, associated with increased energy expenditure. Thus, ageing seems to be related to decreased capacities in the maintenance of NST rather than to its activation. Energy mobilization could be impaired in the ageing mouse lemur but remains to be demonstrated.

### 5.3. Behavioral thermoregulation and ageing

In addition to autonomic thermoregulation, animals have the possibility to adjust their behavior to compensate for their physiological failures, and maintain normothermia (Gordon, 1985, 1987). Behavioral adjustments (such as nest sharing and huddling) are very useful to save energy and are widely used in mammal species (Gilbert et al., 2010; Terrien et al., 2011). Ageing affects behavioral thermoregulation in humans (Taylor et al., 1995). When animals are exposed to a choice among different ambient temperatures, aged mouse lemurs prefer warmer nests than adults. Thermal preference is dependent on photoperiod (Aujard et al., 2006a, 2006b; Terrien et al., 2010b) and sex (Terrien et al., 2010b). Actually, such behavior is particularly used in short day length, corresponding to the resting season, and could help animals to face low levels of available energy in the wild. Adults acclimated to long day length, and particularly males, choose colder nests, thus probably avoiding any possible hyperthermia occurrence. Indeed, steroid levels are very high in long day length and participate to maintain thermogenesis at a high level, thus decreasing the needs for behavioral adjustments. The seasonal effect is not maintained with age. With further experiments to accurately assess the occurrence of physiological changes, behavioral thermoregulation could be used as an early marker of ageing to predict the onset of disruption in energy homeostasis balance.

## 6. Age-associated alterations of the endocrine system

Maintenance of homeostasis involves both the central nervous system and the endocrine system. It is well known that age-related

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changes in the central nervous system include disorders particularly marked in the hypothalamus, hippocampus and the limbic system with modifications of neurotransmitters and neuro modulatory molecules (McEwen, 2002). Age-related changes in secretion and metabolism of various hormones have been already described. Their interpretation remains difficult considering the multiple hormonal deregulations occurring with age and the great inter-individual variability of the ageing processes (Epelbaum, 2009; Maggio et al., 2010; Barzilai and Gabrieli, 2010).

### 6.1. Hypothalamo–pituitary–somatotroph axis and ageing

The declining activity of the growth hormone (GH)-IGF-1-axis with ageing plays a role in the development of frailty and in several pathological conditions commonly observed during ageing, such as atherosclerosis, cardiovascular disease and cognitive decline (for review, see Smith et al., 2005). At the same time, a second body of evidence indicates that decreased insulin and IGF-1-like signaling is associated with increased lifespan in fruit flies and nematodes (Kenyon, 2010). More recently, rodent models with reduced GH and/or IGF-1 signaling have also been reported to have extended lifespan (Gontier and Holzenberger, 2010), though such findings are not yet available in the human species (e.g., Aguiar-Oliveira et al., 2010; Guevara-Aguirre et al., 2011). In the mouse lemur, IGF-1 levels have been assessed in order to determine if they were related to the rate of survival of this seasonal species. Cross-sectional blood samplings on 112 males of various ages indicated that IGF-1 levels remain high and constant during the long-day, breeding season, while a significant age-related decrease occurs from the 4th short-day, resting season onward. Interestingly, in 4 year-old lemurs, the ratio of IGF-1 to body mass in short day lengths appears as a good predictor of the animals' lifespan (Aujard et al., 2010).

### 6.2. Hypothalamo–pituitary–gonadal axis and ageing

Age-related changes in reproductive function have been extensively studied in primates, including humans. Classically, in both sexes, levels of sexual hormones decrease and daily rhythms of sexual hormones are of lower amplitude with age (Brock, 1991; Morley et al., 1997; Goncharova and Lapin, 2004). In mouse lemurs, the reproductive function is highly seasonal with a complete pause of gonadal and sexual hormones secretions in both sexes during the 6 month-period of non-breeding season. In both sexes, seasonal variations of the reproductive function are maintained even in aged individuals. However, levels of sex hormones, testosterone and estrogens, progressively decrease during the breeding season (Aujard and Perret, 1998; Perret, 2005). Except in the very old age (>9 years), these decreases have less impact on fecundity although the sexual motivation is highly reduced. Among reproductively active females, the oldest which produced young alive was 9 years old.

### 6.3. Hypothalamo–pituitary–thyroid axis and ageing

For age-related changes in the thyroid function of humans, contradictory data have been obtained. Levels of thyroid hormones and TSH (thyroid stimulating hormone) are reduced, increased or unchanged (Kunikate et al., 1992; Magri et al., 2002). In healthy old subjects and in centenarians, general features of the thyroid function are maintained suggesting that ageing does not clearly affect the thyroid function (Ferrari et al., 2008; Mazzoccoli et al., 2010). Likewise, daily variations of the thyroxin secretion marked by a peak in the late afternoon seem to be spared by ageing. In old mouse lemurs, morphological changes of the thyroid suggest a reduced secretion of thyroid hormones during the breeding season (Perret, 1975).

### 6.4. Hypothalamo–pituitary–adrenals axis and ageing

Within the corticoadrenal steroids, the cortisol secretion appears relatively unchanged with ageing in humans despite a tendency for an advanced morning peak and a flattened daily amplitude (Mazzoccoli et al., in press). On the contrary, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) levels are significantly reduced during ageing in both sexes in primates including humans (Labrie et al., 1997; Morley et al., 1997; Beaulieu et al., 2000; Kemnitz et al., 2000; Tannenbaum et al., 2004) with the disappearance of the daily morning peak of secretion. The ratio cortisol/DHEA-S is considered as a relevant indicator of biological ageing in primates. The DHEA level could be a predictor of intra and inter-specific longevity (Labrie, 2010; Ohlsson et al., 2010; Muehlenbein et al., 2003). As in other primates, age-related changes in DHEA-S are characterized by a clear decline in old animals, but during the breeding season only. Longitudinal studies demonstrated that the earlier and faster is the decrease in DHEA-S, the shorter is the longevity (Perret and Aujard, 2005).

## 7. Age-associated alterations of the central nervous system

### 7.1. Cognitive functions and ageing

Human ageing is frequently accompanied by alterations of numerous aspects of behavior and cognition, namely intact procedural memory, progressive and widespread decline in executive functions with limited declarative memory dysfunction. In the case of age-associated pathologies such as Alzheimer's disease (Gabrieli, 1996) increased declarative memory dysfunction is also apparent. While age related cognitive decline is well described, the underlying mechanism remain poorly understood and available pre-clinical models provide low predictive capacity for the clinic. Due to their similarity in brain structure to humans, non-human primates are viewed as the most useful and valid models for understanding cognitive deficits and their neurobiological substrates in the elderly. Five general domains of cognitive function have been examined in young and aged mouse lemurs: recognition memory, stimulus reward associative learning, working memory, spatial navigation and set-shifting ability which indeed reflect changes similar to those present in human ageing.

#### 7.1.1. Recognition memory tasks

The delayed non-matching to sample task requires the animal to retain information about trial-specific stimuli over variable delay intervals and the general rule of the non-matching (Ridley and Baker, 1991). In a spatial version of this recognition memory task, the mouse lemur learns to enter the "novel" corridor to reach the reinforcement. Aged mouse lemurs display no deficit in acquisition of the rule, but about 40% of them express deficit in retention when latency between sample and choice increases (Dhenain et al., 1998a). In a free object exploration task (Picq and Dhenain, 1998), old animals exhibit difficulties in memorizing new objects in a complex and changing environment. Moreover, aged mouse lemurs do not seem to detect changes in location of familiar objects, maybe because of a deficit in memorizing the spatial position of these objects.

#### 7.1.2. Discrimination tasks (Stimulus-reward associative learning tasks)

Numerous versions of discrimination tasks were used to assess cognitive capacities in mouse lemurs with olfactory or

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visual/spatial cues (Joly et al., 2006; Picq, 2007). The go/no-go successive discrimination task requires an animal to emit a simple motor response to one cue while inhibiting the response in the presence of another cue (e.g., enter the lit corridor, and not the non-lit corridor). No deficits are observed in aged mouse lemurs, showing that the cognitive processes underlying simple stimulus-reward associations are preserved during ageing (Joly et al., 2006; Picq, 2007).

A generalization task (Picq, 2007), given after a simple simultaneous visual discrimination task, showed that a subpopulation of aged mouse lemurs (about 45%) was disturbed when it had to apply a previously acquired rule in a new context requiring new motor responses. Thus, some old animals have difficulties to make flexible use of acquired memories. A spatial rule-guided discrimination task was also performed: in each trial, the mouse lemurs were confronted with access to one pair of corridors, each individual corridor belonging to several pairs so that it had as well often positive as negative valence over a session. The valence of a given corridor was determined by its spatial position with respect to the other (Picq, 2007). A subgroup of aged mouse lemurs (about 30%) had severely impaired performances in this task which required comparison between items (the two corridors of a pair) and flexibility (the response to a given stimulus can be inverted between trials) and not only expression of biases toward single items, as in simple discrimination tasks.

#### 7.1.3. Working memory tasks

Old mouse lemurs had no difficulty learning and memorizing the stimulus-reward association rule (simple visual discrimination), whereas they displayed impaired performances when a delay was imposed between the stimulus presentation and the response (Picq, 1995). That suggested severe age-related alteration of working memory in the mouse lemur. Working memory was also evaluated in the three-panel runway task: animals had to pass through a series of gated panels, with two of the three gates locked and to memorize the sequence of the open gates that remained constant over a session but changed daily (Trouche et al., 2010). Adults displayed a higher level of perseverative errors compared with aged animals. This behavior is likely due to the high level of anxiety exhibited by younger animals when placed in a novel environment (Picq, 1993a; Némoz-Bertholet and Aujard, 2003). Conversely, aged lemurs showed a rapid habituation to this three-panel runway task but made more working (and reference) memory errors compared to young ones that improved quickly their performance.

#### 7.1.4. Spatial navigation task

Reference and working spatial memories were assessed in mouse lemurs in circular platform task and in radial maze task adapted to this species. The circular platform task is useful to evaluate the reference memory. The animal must use allocentric cues to reach the reinforcement, positioned under one randomly chosen hole among the 12 (Picq et al., in press). A large individual variability was observed: some of the older animals performing as well as younger ones whereas some other aged subjects were severely impaired. Thus a subset of aged mouse lemurs (about 60%) exhibited allocentric spatial memory impairments. In the eight-arm radial maze task, four of eight arms give a reinforcement (Picq, 1993b). Both aged and young groups memorized the position of the blind arms with an equal efficiency. However, the aged group displayed impaired performance for visiting alternatively the reinforced arms without repetition. That corroborates the theory that age would deteriorate working memory, but not reference memory.

#### 7.1.5. Set-shifting task

Executive functions were evaluated by testing the effects of intra- and extra-dimensional shifts on visual/spatial discrimination (Picq, 2007; Picq et al., in press). After a visual discrimination task, mouse lemurs had to perform a reversal task during which the reward contingencies were reversed; the dark corridor being now associated with the reward. Then mouse lemurs were tested in a task of shift of the discriminating stimulus (extra-dimensional shift). The positive stimulus became the right corridor, regardless of luminosity, thus requiring a shift of attentional set from visual characteristics to spatial location. Finally, animals were required to do a task of reversion of the spatial discrimination: a response to the corridor on the left was now correct and correlated with the rewarding. Performances at visual discrimination were independent of age, but on extra- and intra-dimensional shifts tasks, older mouse lemurs were significantly impaired. All the aged mouse lemurs were impaired in set-shifting tasks with no overlap between younger and old group scores (Picq, 2007; Picq et al., in press). This difficulty in reversing or shifting previous discriminations indicates a widespread age-related cognitive rigidity, widely described in both human and animal studies (Dempster, 1992; Barense et al., 2002; Moore et al., 2003). However aged animals differed in their pattern errors: some had difficulties in disengaging from the previous rule whereas some others displayed difficulties in maintaining the new rule. Results of reversion of visual/spatial discrimination are consistent with those found for reversion of olfactory discrimination which brought out a subgroup of aged mouse lemurs with strong cognitive impairment (Joly et al., 2006).

The findings in the mouse lemur enforce the idea of a selective vulnerability of cognitive functions to ageing and, consequently, of a selective vulnerability of the neural systems subserving these cognitive functions. Thus, the following pattern of cognitive ageing in mouse lemurs can be established: (1) The ability to form simple stimulus-reward associations, as required on tasks of simultaneous or concurrent discriminations, is preserved. To the extent that such tasks encourage implicit acquisition of habit through repetition of the learning events, they involve procedural memory. Thus, procedural memory appears to be spared during ageing in mouse lemurs. (2) Working memory and ability to rapidly shift strategy when previous strategies become irrelevant are severely impaired in most aged mouse lemurs as demonstrated by radial maze, delayed response and set-shifting tasks. These cognitive capacities are part of executive functions. Consequently, executive functions are especially vulnerable to ageing in mouse lemurs. (3) Reference spatial memory, spatial and object recognition memory and capacity to use flexibly acquired information (generalization and spatial rule-guided discrimination tasks) are impaired in a subset of aged mouse lemurs. It can be argued that these cognitive abilities characterize declarative memory. Indeed, according to several authors (Sherry and Schacter, 1987; Squire, 1992; Eichenbaum, 1999), declarative memory refers to a memory system fundamentally designed to recollect specific events and which, consequently, possesses several key-features: (i) ability to form conjunctions between arbitrary items (because a unique event is a conjunction of arbitrary items); any task requiring comparison of items (such as the spatial rule guided discrimination test) or relational representation of items (such as circular platform test) taps into that ability; (ii) capacity to store rapidly information specific to a single episode as required on recognition tasks; and (iii) ability to use acquired information with flexibility as on delayed non-matching to sample, spatial rule-guided discrimination or generalization tasks. Thus, declarative memory seems to be impaired in a subpopulation of aged mouse lemurs.

These behavioral studies show that mouse lemurs mimic the pattern of cognitive ageing described in humans, namely intact procedural memory, progressive and widespread decline in executive functions, limited declarative memory dysfunction except in case of age-associated pathologies such as Alzheimer's disease (Gabrieli, 1996). Not all the aged mouse lemurs are equally

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affected by behavioral and cognitive ageing. Some are profoundly impaired while others perform as well as younger animals. That increasing individual variability accompanying ageing is consistently reported in animal and human studies (Valdois et al., 1990; Bachevalier et al., 1991; Rapp and Amaral, 1992; Ylikoski et al., 1999). It provides the opportunity to identify biological, physiological and neural correlates of successful versus impaired cognitive ageing. It also offers the possibility to distinguish between normal and pathological ageing.

### 7.2. Cerebral morphological alterations and ageing

In humans, cerebral atrophy is one of the most described cerebral alterations that occurs during normal and pathological ageing. During normal ageing, cerebral atrophy occurs mainly in frontal regions of the brain (Raz et al., 2005; Kalpouzos et al., 2009) as well as in the caudate (Raz et al., 2005). An alteration of white matter is also described in some studies (Resnick et al., 2003; but see Good et al., 2001). White matter alterations have been related to hypertensive alterations (Benisty et al., 2009). During pathological ageing, such as during Alzheimer's disease, changes are first detected in the hippocampus and cingulate cortex (Chetelat et al., 2002).

In mouse lemurs, histological studies have shown that some aged animals present atrophy of brain areas such as the white and grey matter of the cortex, the hippocampus, the basal ganglia, the brainstem and sometimes the cerebellum (Bons et al., 1992). The most marked atrophy being observed in the cortex, which is accompanied by a significant enlargement of the lateral ventricles compared to young animals where the cavities are limited (Bons et al., 1992).

In mouse lemurs, as in humans, magnetic resonance imaging (MRI) has been used to evaluate age-associated cerebral atrophy (Dhenain et al., 1997, 2000). An atrophy process starts between 5 and 8 years of age in some animals and it evolves rapidly once initiated (Dhenain et al., 2000). Atrophy thus appears to be an age-related pathological condition and not an inevitable effect of age. Brain atrophy in lemurs leads to accumulation of cerebrospinal fluid first in the frontal pericortical areas, then in parietal and temporal pericortical regions and finally in all the pericortical regions (Dhenain et al., 2003; Kraska et al., 2011). These patterns are consistent with a progression of the atrophy starting from sharply demarcated regions toward a more generalized process encompassing the whole brain. A more recent study evaluated the precise location of atrophied tissues. It reveals a severe atrophy in the caudate and white matter of all the aged animals (Picq et al., in press). Because atrophy of these regions occurs in all old animals and is well correlated with age, it is considered as part of the "normal" ageing process in lemurs. A cortical atrophy was also detected in the cingulate, occipital, and temporal (including entorhinal) cortices. On the other hand, in some regions, namely the hippocampus and the septum, the atrophy involves only a subcategory of animals, which suggests that it is related to pathological ageing. Even more interesting, in aged mouse lemurs, cognitive performances (in spatial reference memory and executive tasks) are correlated to atrophy levels in the hippocampus, entorhinal, and septum (Picq et al., in press). To our knowledge, such a relationship between alterations of a given cognitive function and macroscopic atrophy of specific cerebral regions has never been described in other non-human primates. This reinforces the values of biomarkers based on atrophy as indicators of functional alterations. The tissue lesions underlying atrophy processes in lemurs have been poorly evaluated so far. One study has however suggested a link between brain atrophy and intracellular amyloid depositions as well as astrogliosis (Kraska et al., 2011).

On the basis of cerebral atrophy, one can classify animals as having a physiological or pathological ageing. Biomarkers based on MRI can thus be used to select animals and further evaluate histological alterations associated to pathological ageing and treatments against age-related neuropathologies.

### 7.3. Neuropathological alterations and ageing

Brains from animals with cortical atrophy show striking signs of degenerating structures: neuritic debris including argyrophilic rings of degenerated axon terminals, pyramidal neurons with argyrophilic filaments in the perikarya and apical dendrite, and lesions characterized by clusters of argyrophilic neurites associated with dystrophic glial cells and surrounding an amyloid core (Bons et al., 1992). Immunohistochemical study confirms that β amyloid peptide (Aβ) and its precursor (Amyloid Precursor Protein, APP) are present in vascular and extracellular deposits (Bons et al., 1994; Silhol et al., 1996). Leptomeningeal and cortical vascular deposits are observed in 30% of old mouse lemurs (Mestre-Francés et al., 1996). For parenchymal deposits, different types are evidenced according the maturation: pre-amyloid stage detected only by silver impregnation, diffuse Aβ immunoreactive deposits and focal deposits characterized by a dense core of amyloid surrounded by a diffuse halo of amyloid. Most of the diffuse plaques are strongly positive for Aβ42 whereas only a subset of deposits are positive for Aβ40, particularly in the amygdalar nuclei (Mestre-Francés et al., 2000). Aβ42 in mouse lemurs is associated with early stages of plaque maturation as also shown in humans (Iwatsubo et al., 1994). Plaques are generally distributed first into the neocortex and finally in the hippocampus of some animals. In young lemurs, astrocytes are observed only in cortical white matter (corpus callosum). In old lemurs, some reactive astrocytes characterized by a thickening of filaments into the perikarya and astrocytic processes are detected in some cortical areas. When old lemurs present amyloid plaques, the level of glial fibrillary acidic protein (GFAP) is two fold increased compared to old animals (Bons et al., 2000).

Delacourte et al. (1995) previously showed in several old animals an increase in the molecular weight of Tau proteins (microtubule-associated protein). This increase is due to a change of conformation and a stabilization in the hyperphosphorylated state. This change was also investigated by immunohistochemistry with an antibody labeling both normal and abnormally phosphorylated Tau. In cortical pyramidal neurons, Tau protein is aggregated and localized close to the cytoplasmic membrane of the cell bodies and neurites instead of a regular cytoplasmic distribution (Bons et al., 1995). The prevalence and the density of Tau protein-immunoreactive accumulations in the neocortex increases steadily with age (Giannakopoulos et al., 1997). Neurons of neocortical areas are frequently Tau-immunoreactive even in young animals whereas the subiculum and entorhinal cortex are Tau-positive only in animals older than 8 years. No correlation is observed between Aβ deposits and Tau-protein accumulation in the neocortex.

The population of old mouse lemurs can be classified into 4 groups: animals presenting amyloid plaques without (5–10%) or with a tauopathy (1%), animals presenting tauopathy in the absence of amyloid plaques (1%), and animals without any lesion (90%). These data suggest that most of the mouse lemurs undergo a normal ageing, whereas some others show age-associated pathologies.

### 7.4. Iron accumulation and ageing

In humans, during normal ageing, a strong iron accumulation is described in the globus pallidus, the substantia nigra, the red nucleus, the putamen, the caudate nucleus, the dentate nucleus as well as the subthalamic body (Hallgren and Sourander, 1958). Because of its good sensitivity to iron, MRI was used to detect iron

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in mouse lemurs. As in humans, a strong accumulation of iron was found in the globus pallidus, the substantia nigra, and the thalamus in old lemurs. Other structures such as the neocortical and cerebellar white matter, anterior forebrain structures, including the nucleus basalis of Meynert were also shown to accumulate iron while the animals aged (Dhenain et al., 1998b; Gilissen et al., 1999). An interesting point is the overlap of the iron accumulation with the distribution of cholineacetyltransferase-immunoreactive neurons (Gilissen et al., 1999), which are precisely some of the neurons that are involved in age-associated pathologies in lemurs but also in humans.

### 7.5. Synaptic function and ageing

Functional imaging studies of the ageing human brain have revealed that different brain regions that interact to subserve higher-order cognitive functions show less coordinated activation with ageing, suggesting a global loss of integrative function (Andrews-Hanna et al., 2007). Changes in the synaptic physiology of ageing neurons may contribute to altered connectivity and higher order integration.

Acetylcholine is widely distributed in the nervous system and it plays a significant role in developing cerebral cortex in a number of mammalian species (Hohmann and Berger-Sweeney, 1998). A substantial number of studies suggest that it helps in establishing synaptic contacts in networks of cells in developing brain that will subserve complex cognitive functions in adulthood (Berger-Sweeney and Hohmann, 1997; Berger-Sweeney, 1998). With ageing, these complex networks of cholinergic system have been described to undergo moderate neurodegenerative changes resulting in age-related memory deficits (Bigl et al., 1990; Härtig et al., 2002). Changes in the cholinergic system during ageing have been determined by assessing the acetylcholine synthesizing and degrading enzyme, cholineacetyltransferase (ChAT) and acetylcholinesterase. The first semiquantitive measurement of forebrain ChAT-containing neuron in mouse lemurs ranging from 3 months to 12 years of age demonstrated variable cholinergic alterations without any direct correlation with age (Mestre and Bons, 1993). A year later, Dournaud et al. (1994) reported an age-related increase in ChAT activity in the cortex of middle-aged to aged mouse lemurs. Their exclusion of a young age group was influenced by a study performed by Wenk et al. (1989) on 23 rhesus monkeys that demonstrated a cortical age-associated decline in ChAT activity in the frontal cortex but a relative increase between middle-aged and aged monkeys. Also, another study in lemurs reported that the atrophy of basal ganglia compromises functionality of the forebrain cholinergic system (Mestre and Bons, 1993). A neuronal loss of cholinergic neurons is detected in some aged mouse lemurs. This loss reaches 40% into the accumbens nucleus, 70% into the caudate nucleus and 80% into the globus pallidus. It is also accompanied by an alteration of the cytology of the remaining cholinergic neurons: neuritic processes are shortened, devoid of ramifications, and the cytoplasm of the perikarya is characterized by the presence of large vacuoles. By contrast, adult animals show numerous cholinergic neurons with large and ramified neuritic processes (Mestre and Bons, 1993).

The cholinergic system is not the only neurotransmitter system that is modified along with ageing: the serotoninergic system also shows a loss of neurons reaching 76% in the medial raphe and 63% in the raphe pallidus. A neuronal loss reaching 63% is also detected in the reticular formation (Jallageas et al., 1998) whereas the cortical level of somatostatin remains unchanged with ageing (Dournaud et al., 1994).

Endocannabinoid signaling is another critical system for brain function. It plays an important role in synaptic function by presynaptic $CB_1$ receptors on neurons and modulates neurotransmitter release from axon terminals (Lutz, 2004). A study performed by Harkany et al. (2005) identified the distribution of receptors in the forebrain of mouse lemurs with particular reference to CB1 receptor distribution in basal forebrain cholinergic areas and their output pathways. They did not find any age-related differences in $CB_1$ receptors distribution patterns based on the analysis of the neocortex, hippocampus and cholinergic basal forebrain nuclei.

### 7.6. Transcriptomic approaches

Furthermore, these observations have been recently corroborated by transcriptomic data of the temporal cortex of *M. murinus* using human DNA chips (Abdel Rassoul et al., 2010). This was made possible by the fact that numerous genes had shown between 90% and 100% of identity with their human counterparts as it was previously observed for APP (100%, Silhol et al., 1996), presenilin 1 (95.3%, Calenda et al., 1996), presenilin 2 (95.6%, Calenda et al., 1998) or ApoE4 allele (92.7%, Calenda et al., 1995). Gene expression profiles were assessed in the temporal cortex of young adults, healthy old animals and "Alzheimer's Disease-like" ("AD-like") animals that presented β-amyloid plaques and cortical atrophy. The temporal cortex was chosen because this region is connected to the hippocampus and to the frontal cortex, two critical structures for learning and memory which are altered in Alzheimer's disease. By SAM (significance analysis of microarrays), Abdel Rassoul et al. identified 47 genes that discriminated young from healthy old and "AD-like" animals. In addition, ANOVA of the expression data from the three groups identified 695 genes (including the 47 genes) with significant changes of expression in old and "AD-like" in comparison to young animals. Very interestingly, hierarchical clustering analysis indicates that each group has distinct and characteristic expression profiles. Functional categorization shows that most of the genes that were up-regulated in healthy old and down-regulated in "AD-like" animals belong to metabolic pathways, particularly protein synthesis. These data suggest the existence of compensatory mechanisms during physiological brain ageing that disappear in "AD-like" animals (Abdel Rassoul et al., 2010).

## 8. Conclusion

In summary, longitudinal assessment of endocrinology and cognitive performance has demonstrated that mouse lemurs display many features in common with human ageing (Table 1). Together this data suggests that the mouse lemur may provide an ideal system in which to:

(i) Understand the mechanisms and the dynamic evolution of healthy and pathological ageing. Numerous investigations in mouse lemurs highlight the biological manifestations of ageing in various research fields (e.g., endocrinology, neurosciences) by comparing aged animals to adults. Some physiological and behavioral modifications seem to appear in the same age range and evolve in parallel with healthy ageing (e.g., IFN-γ level and fragmentation of the activity rhythms (Cayetanot et al., 2009)) and pathological ageing (e.g., cognitive performance and cerebral atrophy (Picq et al., in press)). In mouse lemurs, longitudinal studies will be useful to determine the evolution of each criterion of ageing.
(ii) Offer predictive biomarkers of longevity and neuropathological ageing. To date, DHEA-S (Perret and Aujard, 2005), IGF-1 (Aujard et al., 2010) and IFN-γ levels (Cayetanot et al., 2009) have shown properties to be validated as good predictors of longevity in mouse lemurs.

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**Table 1**
Age-related changes in mouse lemurs.

| Age-related changes | Mouse lemur | | | Human references |
|---|---|---|---|---|
| | References | Age criteria (years): Adult versus old | | |
| Seasonal rhythms | | | | |
| ↓amplitude of seasonal variations of body mass, resting metabolic rate, sex hormones and behavioral thermoregulation | Perret and Aujard (2006) | 1 –4 | 6–11 | |
| | Terrien et al. (2011) | 1–4 | 6–11 | |
| Daily rhythms | | | | |
| ↑Fragmentation of rest-activity rhythm | Aujard et al. (2006a, 2006b) | 1–4 | 5–8 | Van Someren and Riemersma-VanDerLek (2007) |
| ↓Activity amplitude | Aujard et al. (2006a, 2006b) | 1–4 | 5–8 | |
| | Aujard et al. (2007) | 2.5 ± 0.5 | 7.3 ± 0.8 | |
| ↓Free-running period | Cayetanot et al. (2005a, 2005b) | 2.2 ± 0.2 | 6.3 ± 0.5 | |
| | Aujard et al. (2007) | 2.5 ± 0.5 | 7.3 ± 0.8 | |
| ↓Nocturnal peak of melatonin | Aujard et al. (2001) | 2.4 ± 0.2 | 7.3 ± 1.3 | Waldhauser et al. (1998) |
| Modification of cellular function in the suprachiasmatic nuclei of the hypothalamus | Aujard et al. (2001) | 2.4 ± 0.2 | 7.3 ± 1.3 | |
| | Cayetanot et al. (2005a, 2005b, 2007) | 2.2 ± 0.2 | 6.2 ± 0.2 | |
| Thermo-regulation | | | | |
| ↓Body temperature and increasing of torpor frequency during cold challenge | Terrien et al. (2008) | 2.3 ± 0.3 | 7.1 ± 0.2 | |
| | Terrien et al. (2009b) | | | |
| Modification of behavioral thermoregulation | Terrien et al. (2011) | 1–4 | 6–11 | |
| Physiological parameters | | | | |
| ↓IGF-1 level | Aujard et al. (2010) | 0.5–11 | | Morley et al. (1997) |
| ↓Testosterone and oestrogen levels | Perret (1997, 2005) | 1–3 | 6–11 | |
| IDHEA-S level | Perret and Aujard (2005) | 1–3 | 6–11 | |
| ↓IFN-y (cytokine) level | Cayetanot et al. (2009) | 4–7.8 | | Nikolich-Zugich (2005) |
| Behaviors | | | | |
| ↓Sexual and aggressive behaviors | Aujard and Perret (1998) | 3.2 ± 0.4 | 8.4 ± 0.7 | Staudinger (2005) |
| ↓Social interaction | Picq (1992) | 1–3 | 9–12 | |
| ↓Anxiety | Picq (1993a) | 1–3 | 8–12 | |
| | Némoz-Bertholet and Aujard (2003) | 2.9 ± 0.5 | 8.0 ± 0.8 | |
| Sensori-motor functions | | | | |
| ↓Motor coordination and balance | Némoz-Bertholet and Aujard (2003) | 2.9 ± 0.5 | 8.0 ± 0.8 | Kenny (2005) |
| ↓Odor perception | Némoz-Bertholet et al. (2004) | 2.2 ± 0.2 | 6.3 ± 0.4 | Margrain and Boulton (2005) |
| | Cayetanot et al. (2005a, 2005b) | 2.2 ± 0.2 | 6.3 ± 0.4 | |
| Ocular pathology: Cataract | Beltran et al. (2007) | 1–4 | 7–11 | |
| Cognition | | | | |
| No deficit of simple associative memory | Joly et al. (2006) | 3–4 | 6–14 | |
| | Picq (2007) | 2–4 | 7–11 | |
| ↓Working memory | Picq (1993b) | 1–4 | 9–10 | Grady and Craik (2000) |
| | Picq (1995) | 2–4 | 9–10 | |
| | Trouche et al. (2010) | 2–3 | 6–12 | |
| ↓Executive function | Joly et al. (2006) | 3–4 | 6–14 | |
| | Picq (2007) | 2–4 | 7–11 | |
| | Picq et al. (in press) | 2.4 ± 0.4 | 8.0 ± 1.4 | |
| Cognitive pathology: ↓declarative memory (spatial/object recognition, behavioral flexibility, spatial reference memory) | Dhenain et al. (1998a, 1998b) | 1–4 | 7–10 | Gabrieli (1996) |
| | Picq and Dhenain, 1998 | 1–4 | 8–9 | |
| | Picq (2007) | 2–4 | 7–11 | |
| | Picq et al. (in press) | 2.4 ± 0.4 | 8.0 ± 1.4 | |
| Brain | | | | |
| Cerebral atrophy in the caudate and the white matter | Picq et al. (in press) | 2.4 ± 0.4 | 8.0 ± 1.4 | Raz et al. (2005) |
| ↑ChAT activity | Dournaud et al. (1994) | 5–8 | 10–11 | Gottfries (1990) |
| Iron accumulation | Dhenain et al. (1998b) | 0.5–2.0 | 7–9 | Hallgren and Sourander (1958) |
| Brain pathology: Abrupt and localized atrophy in the hippocampus, septum, and cortex (temporal, occipital, cingulate) | Bons et al. (1992) | 2–3 | 8–11 | Chetelat et al. (2002) |
| | Dhenain et al. (2000) | 1.0–2.5 | 3.5–10.3 | |
| | Kraska et al. (2011) | 1.9–2.5 | 6.4–11.3 | |
| | Picq et al. (in press) | 2.4 ± 0.4 | 8.0 ± 1.4 | |
| Amyloid deposits | Bons et al. (1994) | 2 | 8–13 | Castellani et al. (2010) |
| | Silhol et al., 1996 | 1–5 | 8–11 | |
| | Mestre-Francés et al. (1996, 2000) | 2–4 | 8–13 | |
| Gliosis | Kraska et al. (2011) | 1.9–2.5 | 6.4–11.3 | |
| Structural modification and aggregation of Tau proteins | Delacourte et al. (1995) | 2 | 6–9 | |
| | Bons et al., 1995 | 2 | 7–10 | |
| | Giannakopoulos et al., 1997 | 1–4 | 8–13 | |
| Loss of cholinergic neurons | Mestre and Bons (1993) | 1 | 7–10 | Whitehouse et al. (1982) |
| | Jallageas et al. (1998) | 2–5 | 8–13 | |

(iii) Bring new insights into the biology of ageing and to offer potential targets for therapeutic interventions.

In the mouse lemur, age-related physiological changes resemble those observed in humans: hormonal deregulations (DHEA-S, IGF-1, testosterone, oestrogen), modifications of biological rhythms (fragmentation, desynchronization), alteration of thermoregulation, decrease in sensorimotor and cognitive capacities, structural and functional modifications of brain. Given these recent advances in the understanding of the age-related changes in this primate (Table 1) and given the recent development of the knowledge of its genome, the mouse lemur is a useful model of human ageing.

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