Pergamon

PII: S0306-4522(01)00202-0

Neuroscience Vol. 105, No. 2, pp. 403–412, 2001

0306-4522/01 $20.00+0.00

www.elsevier.com/locate/neuroscience

ARTIFICIALLY ACCELERATED AGING BY SHORTENED PHOTOPERIOD ALTERS EARLY GENE EXPRESSION (FOS) IN THE SUPRACHIASMATIC NUCLEUS AND SULFATOXYMELATONIN EXCRETION IN A SMALL PRIMATE, *MICROCEBUS MURINUS*

F. AUJARD,[a] O. DKHISSI-BENYAHYA,[b] I. FOURNIER,[c] B. CLAUSTRAT,[c] A. SCHILLING,[a] H. M. COOPER[b] and M. PERRET[a]*

[a]Laboratoire d'Ecologie Générale, UMR CNRS 8571, 4 avenue du Petit Château, 91800 Brunoy, France

[b]Cerveau et Vision, INSERM U371, 18 avenue du Doyen Lépine, 69675 Bron, France

[c]Service de Radiopharmacie et Radioanalyse, Centre de Médecine Nucléaire, Hôpital de Neurocardiologie, 59 boulevard Pinel, 69334 Lyon Cedex, France

Abstract—In mammals, a number of anatomical and functional changes occur in the circadian timing system with aging. In certain species, aging can be modified by various factors which induce a number of pathological changes. In a small primate, the gray mouse lemur (*Microcebus murinus*), long-term acceleration of seasonal rhythms by exposing the animals to a shortened photoperiodic regime (up to 2.5 times the natural photoperiodic regime) alters longevity, based on survival curves and morphological changes. This provides a model for challenging the idea that modifications of the circadian pacemaker are related to chronological (years) versus biological (photoperiodic cycles) age.

To assess the effect of aging and accelerated aging on the circadian pacemaker of this primate, we measured body weight variations, the daily rhythm in urine 6-sulfatoxymelatonin and the light-induced expression of the immediate early gene (Fos) in the suprachiasmatic nucleus of mouse lemurs that had been exposed to different photoperiodic cycles. Urine samples were collected throughout the day and urine 6-sulfatoxymelatonin levels were measured by radioimmunoassay. Light-induced Fos expression in the suprachiasmatic nucleus was studied by exposing the animals to a 15-min monochromatic pulse of light (500 nm) at saturating or sub-saturating levels of irradiance (10^{11} or 10^{14} photons/cm^2/s) during the dark phase. The classical pattern of 6-sulfatoxymelatonin excretion was significantly altered in aged mouse lemurs which failed to show a nocturnal peak. Fos expression following exposure to low levels of irradiance was reduced by 88% in the suprachiasmatic nucleus of aged mouse lemurs. Exposure to higher irradiance levels showed similar results, with a reduction of 66% in Fos expression in the aged animals. Animals subjected to artificially accelerated aging demonstrated the same alterations in melatonin production and Fos response to light as animals that had been maintained in a routine photoperiodic cycle.

Our data indicate that there are dramatic changes in melatonin production and in the cellular response to photic input in the suprachiasmatic nucleus of aged mouse lemurs, and that these alterations depend on the number of expressed seasonal cycles rather than on a fixed chronological age. These results provide new insights into the mechanisms underlying artificial accelerated aging at the level of the molecular mechanisms of the biological clock.

Key words: aging process, circadian rhythms, biological clock, light response, melatonin, *Microcebus murinus*.

In mammals, the suprachiasmatic nucleus (SCN) contains a circadian pacemaker that regulates a variety of physiological and behavioral rhythms. This circadian organization allows an optimal adaptation of the organism to cyclic changes in the environment of which the daily light/dark cycle is the most reliable and predictable cue. With age, changes in several basic parameters of circadian rhythms have been observed in mammals (Brock, 1991), including decreased amplitude and increased fragmentation of the locomotor activity rhythm (Penev et al., 1997), changes in period length under free-running conditions (Pittendrigh and Daan, 1974; Davis and Menaker, 1980; Morin, 1988; Mayeda et al., 1997), and decreased rate of reentrainment following a shift in the light/dark cycle (Zee et al., 1992; Zhang et al., 1996).

Age-related changes in the circadian timing system have been correlated with anatomical and functional alterations within the SCN which is the target of a number of modifications. Indeed, age-related changes in circadian rhythmicity may be correlated with decreased sensitivity of the circadian system to light (Witting et al., 1993), demonstrated by the reduced Fos expression in response to light by the SCN (Sutin et al., 1993; Zhang et al., 1996; Benloucif et al., 1997). Altered melatonin production with age is also observed (Touitou and Haus, 1994) and melatonin-related compounds

*Corresponding author. Tel.: +33-1-60479238; fax: +33-1-60468118.

E-mail address: martine.perret@wanadoo.fr (M. Perret).

Abbreviations: aMT6s, 6-sulfatoxymelatonin; CT, circadian time; O.D., optical density; SCN, suprachiasmatic nucleus.

have been found to improve circadian disorders associated with aging (Weibel et al., 2000). The consequences of disruptions of temporal organization have been suggested to reduce individual fitness (Folkard et al., 1985; Sakellaris et al., 1975) and affect longevity. Indeed, a study on fruit flies has demonstrated that a disrupted circadian resonance can induce a decrease in longevity (Pittendrigh and Minis, 1972). However, the underlying causes of the observed changes in circadian rhythmicity remain unknown and the relationship between disrupted circadian function and longevity awaits clarification.

The gray mouse lemur (*Microcebus murinus*), a prosimian primate, exhibits seasonal and circadian rhythmic components in most of its biological functions studied so far. Seasonal variations have been observed in various biological parameters, such as reproductive function (Perret, 1992), thermoregulation (Aujard et al., 1998; Perret et al., 1998), body temperature and locomotor activity (Aujard et al., 1998). The strict control by photoperiod of these biological rhythms allows manipulation of survival and longevity by long-term acceleration of seasonal rhythms (Perret, 1997), providing a model for assessing various aspects of aging. In this previous study, it was shown that compared to the normal 12-month photoperiodic cycle, an 8-month photocycle did not modify physiological responses to photoperiodic variations. For example, no significant differences were found for cyclic changes in reproductive activity or for reproductive efforts within females, suggesting that an increase in metabolic expenditures cannot account for earlier mortality in animals exposed to the 8-month photocycle. These results were based on survival curves, morphological changes associated with aging and body weight variations.

Because the SCN harbors the major circadian pacemaker in the brain, it is strongly suggested that this circadian clock is involved in adaptation and survival. But its critical role in longevity remains unclear. Using a very accelerated photocycle (5 months), we were interested to determine whether artificially accelerated aging could induce modifications in the circadian pacemaker at physiological and cellular levels, similar to those observed in normal aging. Our hypothesis was that age-related changes in circadian physiology are based on biological age (number of seasonal cycles) rather than chronological age (number of years), and that clock pacemaker function can be a good predictor of longevity for a given organism in a given population. To test this hypothesis, we measured the light-induced expression of the immediate early gene c-*fos* in the SCN and the melatonin production of young and aged mouse lemurs that have been exposed to different photoperiodic cycles. Specifically, we tested the hypotheses that in mouse lemurs: (1) aging alters the generation and amplitude of the daily rhythm in melatonin, (2) aging is associated with a decreased photic induction of Fos in the SCN, and (3) these alterations depend on the number of expressed seasonal cycles rather than on a fixed chronological age.

EXPERIMENTAL PROCEDURES

Mouse lemurs (*Microcebus murinus*, Cheirogaleidae, Primates) are small (60–90 g) nocturnal prosimian primates restricted to Madagascar. Animals were kept in captivity in the Laboratory of General Ecology in Brunoy, France, where the breeding colony (Licence approval No. A91.114.1) has been established from wild animals originating from southwest Madagascar 30 years ago. All mouse lemurs used in this study were laboratory-born and maintained under constant conditions of ambient temperature (25°C) and humidity (55%), with food (fresh fruits, milk porridge and insects) available *ad libitum*.

Photoperiodic regimes

In this photoperiod-dependent seasonal breeder, daylengths longer than 12 h are stimulatory, leading to sustained activity of both behavioral and physiological functions including reproduction, whereas daylengths shorter than 12 h result in complete gonadal regression, weight gain and reduced behavioral activities (Perret, 1992). From birth to spontaneous death, animals were exposed to different photoperiodic regimes consisting of equal alternating periods of short days (winter-like) and long days (summer-like) of either a 12-month, an 8-month or a 5-month total 'annual' duration. These photoperiodic schedules trigger seasonal rhythms of similar amplitude but reduced period. Compared to natural photoperiod, where one chronological year corresponds to one seasonal cycle, the number of seasonal cycles progressively differs from the biological age in accelerated photoperiodic conditions (Fig. 1). For example, at an equivalent chronological age of 3 years, an animal exposed to the 8-month photocycle will have experienced 4.5 seasonal cycles, while an animal exposed to the 5-month photocycle will have experienced more than seven seasonal cycles. According to a previous study (Perret, 1997), survival and mean life span in mouse lemurs depend on the number of seasonal cycles experienced by the animals rather than actual chronological age. The fifth seasonal cycle corresponds to the 50% survival point in the captive population of mouse lemurs whatever the duration

Table 1. Group composition and age characteristics

	N	Photoperiodic cycle length (months)	Mean age ± S.E.M. (years)	Mean number of seasonal cycles ± S.E.M.
Young (< 5 years)	4	12	1.3 ± 0.3	1.3 ± 0.3
	5	8	2.4 ± 0.2	3.4 ± 0.2
Aged (> 5 years)	1	12	7.1	7.1
	5	8	7.3 ± 1.3	11.0 ± 1.5
Accelerated	4	5	3.5 ± 0.2	8.5 ± 0.5

In the case of exposure to the 12-month photocycle or to the 8-month photocycle, the animals belong to the 'young' or the 'aged' categories whether their age is expressed in years or in number of experienced seasonal cycles. In the case of exposure to the 5-month photocycle, the animals belong to the 'young' category if their age is expressed in years (chronological age) but they also belong to the 'aged' category if their age is expressed in seasonal cycles (biological age).

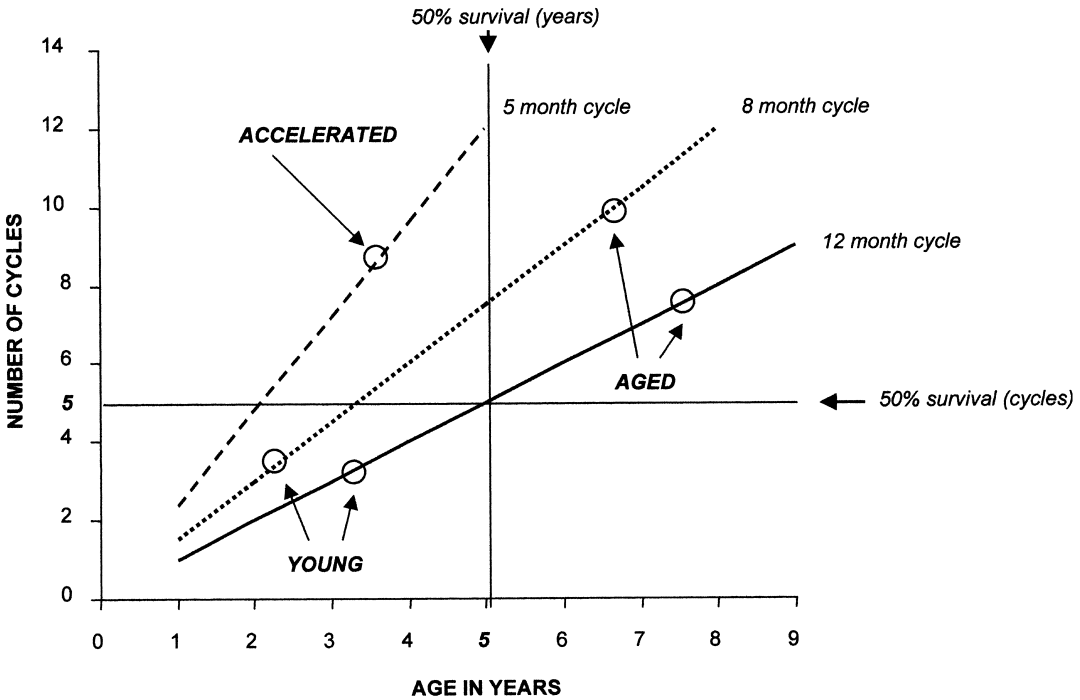

Fig. 1. Relationship between chronological age (in years) and number of experienced seasonal cycles according to the length of the photoperiodic cycle to which the animals were exposed. The 50% survival times (according to years or cycles) are represented on the graph and delineate the different age categories used in the present experiment as represented by circles on the curves. Young animals are less than 5 years in both age and cycles, aged animals are more than 5 years in age and cycles, whereas accelerated aged animals are less than 5 years in age but have experienced more than five cycles.

of the photocycle. This criterion was used in the present study to delineate young adults and aged adult animals (Fig. 1). Throughout their lives, animals were routinely examined for cyclic changes in body weight and reproductive state. Changes in body weight were used to ascertain the adequate response to photoperiod whatever the photocycle length. To do so, data obtained from the colony were plotted as a function of the number of expressed seasonal cycles.

Animals

For the purpose of the present experiment, 19 male gray mouse lemurs were selected according to their photoperiodic history (Fig. 1, Table 1). Among the groups of animals exposed to the 12-month or the 8-month photocycle, nine animals belonged to the 'young' category (less than 5 years) and six belonged to the 'aged' category (more than 5 years) whether their age was expressed in number of seasonal cycles (biological age) or in years (chronological age). By contrast, the animals exposed to the 5-month photocycle ($n = 4$) were young adults when their age was expressed in years and were aged (more than five seasonal cycles) when their age was expressed in number of seasonal cycles. These animals will be designated as 'accelerated aged' for this reason. During the experiments, animals were maintained in individual cages (1 m^3 volume) provided with nests and branches and isolated from any visual or olfactive stimulus from conspecifics. Daily variations of light according to photoperiodic state were maintained by dual cool fluorescent tubes (300 lux) during the day and red light (0.002 lux) during the night, controlled by a timer.

All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC). All efforts were made to minimize animal suffering throughout the experiments.

Sulfatoxymelatonin measurement

Animals were studied during their long-day photoperiod (14:10). Urine was collected every 2 h during the light phase and every hour during the dark phase. Urine samples were immediately stored at −20°C prior to assay. Urine 6-sulfatoxymelatonin (aMT6s) concentrations were measured by radioimmunoassay according to the method previously described (Harthé et al., 1991).

To control for variations in the volume and concentrations of the voided urine, the creatinine content of each sample was determined by a creatinine colorimetric test (Sigma #555A; Saint Quentin, France) using 1:10 diluted urine. Urinary hormone concentrations are expressed as mass/mg creatinine. In order to eliminate a possible alteration of liver conjugation with age, we previously determined the melatonin/aMT6s ratio in young and aged mouse lemurs. The ratio did not differ significantly between the two age categories ($t = 1.24$, $df = 10$, $P = 0.240$). We also examined the relation between age, creatinine levels and aMT6s levels. There was no relationship between creatinine concentrations and aMT6s excretion ($r = 0.437$, $df = 18$, $P = 0.070$), and no effect of either age ($P = 0.953$) or time of the day ($P = 0.257$) could be found.

Photic stimulation

Because animals exposed to the 12-month photocycle and those exposed to the 8-month photocycle belonged to the same age categories whether their age was considered in years or in number of seasonal cycles, and in order to minimize the number of animals used, we decided to kill only animals exposed to the 8-month photocycle [referred as young ($n = 5$) and aged ($n = 5$)] and compare them to the animals exposed to the 5-month photocycle [referred as accelerated ($n = 3$)]. All ani-

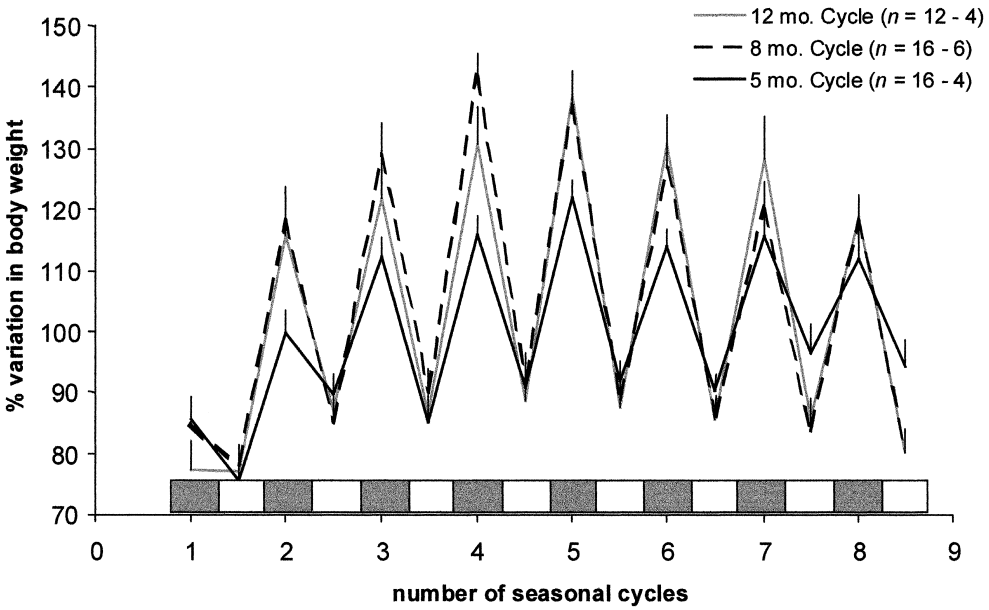

Fig. 2. Cyclic body weight variations in male mouse lemurs exposed to either 12-month (mo.), 8-month or 5-month photocycles. Variations in body weight between short-day (gray areas) and long-day periods are expressed as the percentage of individual mean life body weight (±S.E.M.). When the age of the animals is expressed according to equivalent numbers of seasonal cycles (biological age), similar relative cyclic variations in body weight are maintained throughout life in all photoperiodic regimes.

mals were maintained at least 10 days under a 24-h light/dark cycle. On the day of testing, the 24-h light/dark cycle was discontinued and the animals were maintained in constant darkness. Thus, the time at which light onset would normally have occurred (zeitgeber time, ZT0) was used as the beginning of the subjective day (circadian time, CT0; Schwartz et al., 1994; Dkhissi-Benyahya et al., 2000). Animals were stimulated with a light pulse at CT14, because this time corresponded to the period of the circadian cycle of maximum amplitude of phase delay in locomotor activity (Schilling et al., 1999). Animals were exposed to a 15-min monochromatic pulse of light ($\lambda = 500$ nm) at two different irradiance levels: 10^{14} or 10^{11} photons/cm^2/s. In other species, these irradiance values correspond approximately to saturation and half-saturation levels (Dkhissi-Benyahya et al., 2000). Following the light pulse, animals were returned to complete darkness and perfused 60 min after the beginning of the exposure. Control animals were handled identically but did not receive the light pulse.

The stimulus apparatus used a tungsten–halogen bulb (24 V, 150 W). Monochromatic light was obtained using a monochromatic interference filter with a maximum transmission at 500 nm (Corion; Franklin, MA, USA; half-peak bandwidth 10 nm). Irradiance was controlled using neutral density filters (Kodak). Peak irradiance and spectral output ($\lambda = 500$ nm, half bandwidth 9.34 nm) were measured in the stimulation chamber using an International Light model IL 700 photometer and a spectrophotometer (Ocean Optics S2000; Eerbeek, The Netherlands).

Fos immunohistochemistry

Animals were rapidly anesthetized in complete darkness with halothane followed by an intraperitoneal injection of nembutal (sodium pentobarbital; 100 mg/kg i.p.) and then perfused intracardially (in dim light with the head covered) with warm (37°C) heparinized saline followed by Zamboni's fixative at 4°C. Perfusion was initiated within 2–3 min after anesthesia. Brains were post-fixed overnight in the same fixative at 4°C, removed from the skull, and subsequently stored in phosphate buffer with 0.1% sodium azide. Prior to sectioning, brains were cryoprotected in 30% phosphate-buffered sucrose for 24 h. Serial coronal sections were made at 40 μm on a freezing microtome. Endogenous peroxidase was first suppressed using a solution of 50% ethanol in saline with 0.03% H_2O_2. Free-floating sections were briefly rinsed in phosphate-buffered saline (0.01 M, pH 7.2) containing 0.3% Triton and 0.1% sodium azide and blocked with 1.5% normal goat serum. Sections were incubated in the anti-Fos primary antibody (Ab-5 rabbit antiserum, Oncogene Research Products; Calbiochem, Cambridge, MA, USA; dilution 1/10 000) for 3 days at 4°C. Immunoreactivity was visualized using a Vectastain ABC (avidin–biotin–peroxidase) Elite kit (PK-6100; Vector Laboratories, Burlingame, CA, USA), followed by incubation in 0.2% 3,3′-diaminobenzidine with 0.5% ammonium nickel sulfate and 0.003% H_2O_2 in Tris buffer (0.05 M, pH 7.6). Control sections were made by pre-incubation of the primary antibody with Fos peptide (Oncogene Research, #PP10; Calbiochem), by omitting the primary antiserum or by replacement with normal serum at the same concentration as the antibody. No labeling was observed in control sections.

Quantitative analysis

The total optical density of Fos-like immunopositive product in the SCN was determined using computer assisted image analysis (Visiolab 2000; Biocom, Les Ulis, France). Optical density of label was measured bilaterally from digitized images of the SCN. The method is based on quantification of the total optical density of Fos labeling, defined as the integral sum of the surface area of all pixels in the SCN multiplied by their corresponding optical density value. The optical density (O.D.) was obtained by first subtracting the background density value determined from an adjacent area of hypothalamus that did not contain Fos-positive cells. The background for each animal was systematically measured and in general did not vary more than ±3%. A threshold value was determined for detection of label significantly above the background level. The identity of the individual animals was unknown to the observer during the

analysis, and every section of the SCN of each series was analyzed (for technical details see Dkhissi-Benyahya et al., 2000).

Statistical analyses

All values are expressed in mean ± S.E.M. Data were statistically analyzed using an analysis of variance (ANOVA), followed by a Tukey *post-hoc* test on least square-adjusted means. Differences between age categories were determined by Student's unpaired *t*-test and correlations were performed using a Spearman correlation test. The probability level for statistical significance was $P < 0.05$.

RESULTS

Body weight variations

In mouse lemurs, exposure to short days leads to a high gain in body weight whereas exposure to long days has the reverse effect. When considering maximal body weight under short days and minimal body weight under long days, expressed as a percentage of the individual mean life body weight, cyclic variations in body weight were maintained throughout life in all photoperiodic regimes (Fig. 2). No significant difference was observed in the mean body weight of accelerated-reared animals when compared to animals reared under a normal 12-month annual photoperiod ($F = 1.06$, df = 2/42, $P = 0.354$). Owing to the shorter periods of long and short days in the case of accelerated photocycles, animals from the three groups expressed significantly different mean amplitudes of body variations between short and long days ($F = 6.31$, df = 2/42, $P < 0.01$): the amplitude of body weight variations decreased with shorter photocycle. The general feature of the cyclic variations was highly similar and regardless of the photoperiodic condition, the amplitude of body weight variations expressed a maximum around the fifth seasonal cycle and thereafter regularly decreased ($r_{10} = 0.669$, $P < 0.05$).

Levels of urinary sulfatoxymelatonin

Within each chronological age category there was no significant difference in aMT6s levels between animals exposed to the 8-month and the 12-month photocycles (young: $P = 0.176$; aged: $P = 0.080$). Consequently, animals were grouped according to their chronological age and referred to as young ($n = 9$) and aged ($n = 6$). Young mouse lemurs exposed to a 24-h light/dark cycle (14/10) exhibited a marked rise in urine aMT6s that started immediately after the onset of darkness and reached a three-fold increase after 2 h of darkness (Fig. 3). Daily mean value was 39.8 ± 5.0 ng/mg creatinine. Values significantly varied according to the time of the day ($F = 4.45$, df = 17/83, $P < 0.001$), with a significant peak at 114.2 ± 22.7 ng/mg creatinine occurring 8 h after lights off ($P = 0.01$). During the entire light phase values remained low. By comparison, aged males showed a clear alteration of the daily rhythm in urine aMT6s. Old males did not exhibit any significant variation in urine aMT6s levels throughout the entire day/night period ($F = 1.3$, df = 17/68, $P = 0.205$), and mean daily levels were significantly lower (mean value: 16.8 ± 1.5 ng/mg creatinine; $F = 24.7$, df = 1/176, $P < 0.001$) when compared to the levels measured in young males.

To assess the effect of accelerated aging on aMT6s production, mean day and night levels of urine aMT6s were compared in young, aged and accelerated aged males (Fig. 4). Accelerated aged animals exhibited significantly lower levels of urine aMT6s compared to young animals, both during the day ($P < 0.01$) and during the

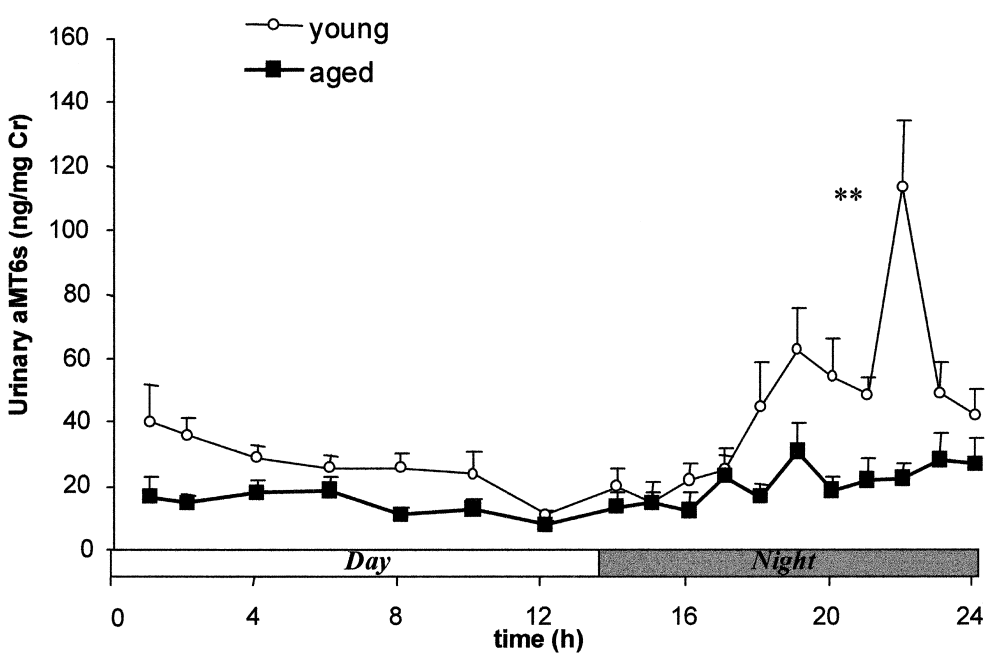

Fig. 3. Urinary aMT6s levels (mean ± S.E.M.) in young adult ($n = 9$) and aged ($n = 6$) mouse lemurs exposed to a 24-h light/dark cycle (14/10). Gray bar indicates dark phase. $^{**}P < 0.01$. The marked rise in urine aMT6s observed during the night in young mouse lemurs is dramatically altered in aged animals which fail to express the normal night-time peak. Cr, creatinine.

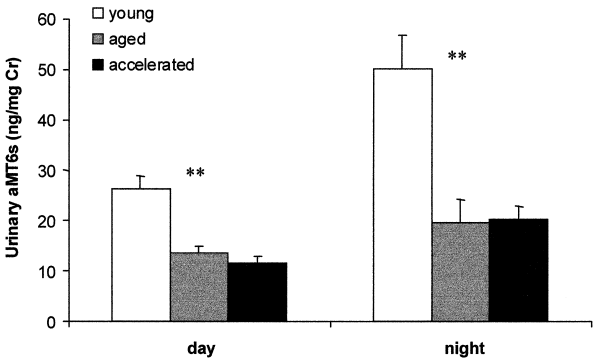

Fig. 4. Mean (±S.E.M.) day and night levels in urinary aMT6s concentration in young ($n=9$), aged ($n=6$) and accelerated aged ($n=4$) male mouse lemurs. $^{**}P<0.01$. When compared to young animals, day and night levels of urine aMT6s are similarly reduced in both accelerated and aged animals. Cr, creatinine.

night ($P<0.01$). Mean values were reduced by half, dropping from 50.3 ± 6.6 ng/mg creatinine in young to 20.3 ± 2.5 ng/mg creatinine in accelerated aged animals during the night, and from 26.4 ± 2.6 ng/mg creatinine to 11.5 ± 1.5 ng/mg creatinine during the day. Interestingly, the day and night aMT6s levels of accelerated aged animals did not differ from the levels measured in normal aged animals (day values: $P=0.571$; night values: $P=0.894$), although their chronological age was different. Moreover, the clear daily rhythm in urinary aMT6s, illustrated by the significant difference between mean day and night values in adult mouse lemurs ($P<0.01$), was absent both in aged ($P=0.266$) and in accelerated aged animals ($P=0.060$).

Fos expression in the SCN

In dark control animals, little or no nuclear Fos staining was observed in the SCN. Regardless of the age of the animal, the density of Fos-like immunopositive staining increased with increasing irradiance. The Fos-positive cells were concentrated in the SCN, with scattered cells extending dorsally to the periventricular region of the hypothalamus (Fig. 5).

In young animals, the lower irradiance level delivered in our study (10^{11} photons/cm^2/s) induced an increase in Fos expression at CT14 compared with dark control animals (mean O.D. = 2731 ± 1345; Fig. 6). The density of Fos induction in the SCN increased proportionately with increasing irradiance, with a mean optical density value of 5611 (±1108) at the high irradiance level (10^{14}

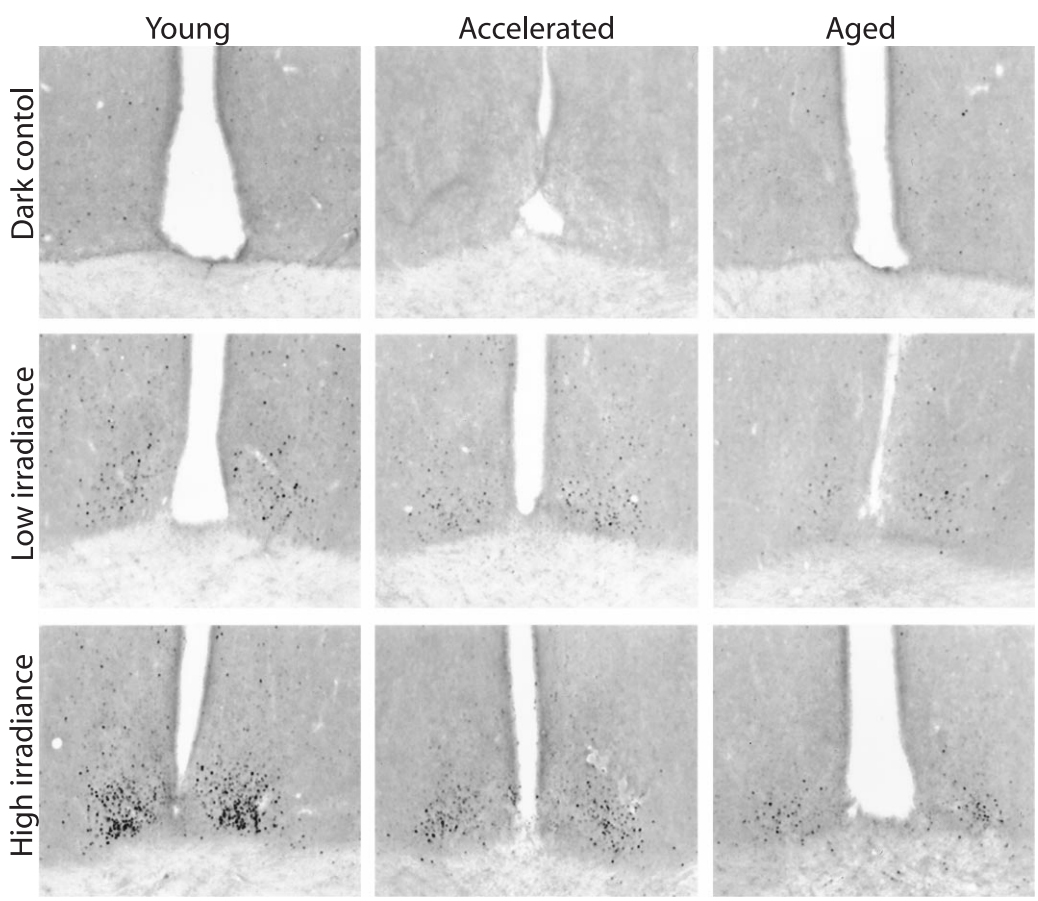

Fig. 5. Fos expression in representative SCN sections from young, aged and accelerated aged mouse lemurs, exposed to a 15-min monochromatic pulse of light (irradiance of 10^{11} or 10^{14} photons/cm^2/s) at CT14. Control animals remained in darkness. Fos-positive labelled cell nuclei appear in black. The differences in Fos expression are related to both age category and irradiance level.

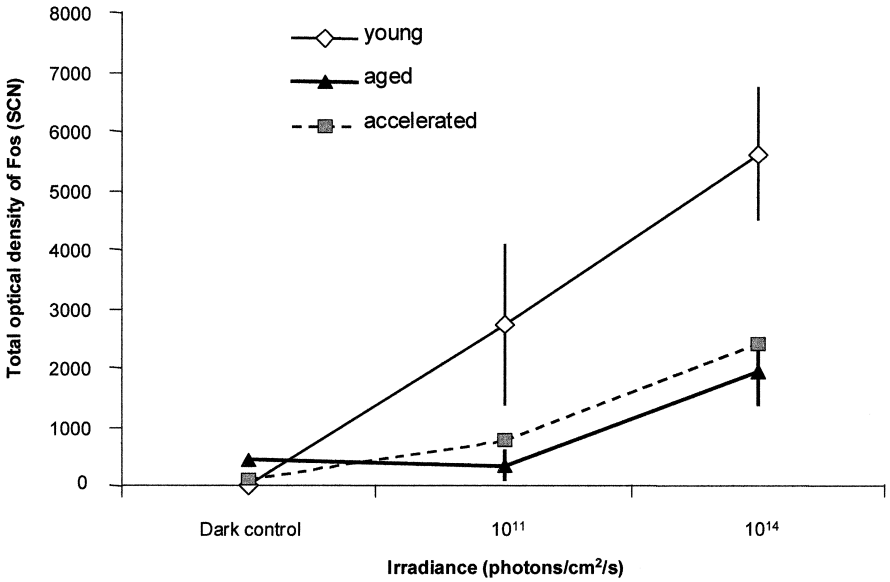

Fig. 6. Amplitude of total optical density of Fos-like immunoreactivity (±S.E.M.) in labelled cells of the SCN in young ($n = 5$), aged ($n = 5$) and accelerated aged ($n = 3$) animals in relation to irradiance. Both accelerated and aged mouse lemurs exhibit a clear reduction in Fos expression following exposure to low and high levels of irradiance when compared to young controls.

photons/cm^2/s). This result suggests that the irradiance threshold for induction of Fos in the SCN is below 10^{11} photons/cm^2/s and that the saturation of Fos induction requires at least 10^{14} photons/cm^2/s.

Although Fos expression in the dark control aged animal (O.D. = 436) was slightly higher than in the young dark control, exposure to the low level of irradiance failed to increase Fos induction in the SCN of aged animals (mean O.D. = 335 ± 244). Furthermore, the optical density of Fos-like immunopositive cells in the SCN was 88% less than that of young subjects exposed to the same low irradiance level (10^{11} photons/cm^2/s). A significant increase in Fos induction in aged animals was only observed at the highest irradiance, with a mean optical density of 1923 (±558). This value was similar to the values obtained in young animals at an irradiance three log units lower (Fig. 6).

Animals subjected to an artificially accelerated aging demonstrated a similar decreased sensitivity as compared to the young animals. Fos induction at low irradiance was similar to that of the chronologically aged animals (O.D. = 775), but was reduced by 72% compared to young animals (Fig. 6). When compared to younger animals, exposure to the high irradiance level led to a 66% reduction in Fos expression (O.D. = 2388) in the SCN in the case of normal aging and 57% in the case of accelerated aging.

DISCUSSION

These results show that, with aging, the amplitude of melatonin production and the responsiveness to light by the circadian pacemaker are strongly altered in mouse lemurs, and that these age-related deficits depend on biological age rather than chronological age. Taken together with previous data on the biological rhythms in this species, these findings demonstrate the uniqueness of this primate for the study of rhythmic entrainment, aging processes, and artificially accelerated aging.

Although numerous age-related alterations of activity and body temperature circadian rhythms have been demonstrated in rodents (Welsh et al., 1986; Rosenberg et al., 1991; Zee et al., 1992; Witting et al., 1994; Turek et al., 1995; Scarbrough et al., 1997; Valentinuzzi et al., 1997; Satinoff, 1998), and humans (Weitzman et al., 1982; Reilly et al., 1997), the decrease in melatonin production with aging is more controversial. Indeed, recent studies have shown a decrease of 10% in plasma melatonin levels (Waldhauser et al., 1998) and of 36% in urine aMT6s levels (Kennaway et al., 1999) during senescence, whereas Zeitzer et al. (1999) demonstrate that reduction of plasma melatonin concentration is not a general characteristic of aging in healthy subjects. The differences in these age-related effects could be explained by modified kinetics of melatonin degradation in aged people, but aMT6s excretion has been demonstrated to be a good surrogate measurement of plasma melatonin secretion even in older people (Baskett et al., 1998). In the present study, aged mouse lemurs demonstrate a decrease of 58% in urine aMT6s levels measured throughout the day. Owing to the particularities of the circadian clock of mouse lemurs, characterized by a short free-running period of locomotor activity of 22.5 h and an almost immediate resynchronization of the locomotor activity rhythm to both phase advances and phase delays in the light/dark cycle (Schilling et al., 1999), we suspect that the circadian rhythm of melatonin would play a major role in photoperiod transduction in this species and that the decrease in melatonin with aging would be involved

in the alteration of photoperiod response in aged mouse lemurs (Aujard and Perret, 1998).

In adult mouse lemurs, the amplitude of Fos expression induced by a 15-min pulse of light increases with increasing irradiance level. The levels of irradiances were chosen with regard to values similar to those required for saturation and half-saturation in rodents (Kornhauser et al., 1990; Zhang et al., 1996; Dkhissi-Benyahya et al., 2000), and light induction of Fos in the SCN of the mouse lemur follows a similar pattern. As is the case in old hamsters (Zhang et al., 1996), the threshold of Fos induction is shifted towards higher irradiance in aged mouse lemurs, since an irradiance of 10^{11} photons/cm^2/s does not induce significant induction of Fos in the aged group. Moreover, at the highest irradiance, saturation does not appear to be achieved in comparison to young animals, since Fos expression is reduced by 66%. This effect is more pronounced than that observed in rodents with a relative reduction of only 42% in mice (Benloucif et al., 1997) and 43% in hamsters (Zhang et al., 1996).

There are several hypotheses to explain the observed decrease in sensitivity to light in the SCN with aging: (1) a modification in the kinetics of the activation of signaling pathways in the SCN; (2) a reduction in the amplitude of photic information transmitted by the retina to the clock; (3) age-related changes within the clock mechanism of the SCN itself. It is difficult to determine whether activation of signaling pathways in the SCN is altered with aging, but an age-related decrease in light transmission from the retina seems unlikely, since no significant decrease in lens transmittance and in the amount of light transmitted by the retina to the clock was observed in aged hamsters (Zhang et al., 1998). Although it is clear that the age-related alteration of Fos induction could involve one or several steps in the transduction pathway of photic information to the clock, there is compelling evidence that the SCN itself is greatly affected by aging. Previous studies have demonstrated a disruption of circadian (Hofman and Swaab, 1994) and seasonal rhythms in vasopressin (Hofman and Swaab, 1995) and a progressive loss of vasopressinergic cells with aging (Roozendall et al., 1987; Lolova et al., 1996). The functional activity of the SCN is also altered with a loss of day/night differences in vasoactive polypeptide mRNA levels of aged rats (Kawakami et al., 1997), alteration in glucose utilization (Wise et al., 1988), and in cAMP-response element-binding protein phosphorylation (Zhang et al., 1996).

Our results show a dramatic effect of aging at both upstream and downstream levels of the circadian clock. At the present time, it is difficult to impute a causal relationship between these two effects. The physiological mechanism underlying the decrease in melatonin with age is still undetermined, and whether this decrease results from an alteration of properties of the SCN or of pathways further downstream remains to be elucidated. However, a recent study demonstrating the usefulness of melatonin-related compounds in the treatment of circadian disorders associated with aging (Weibel et al., 2000) and the present exacerbated effects of aging on melatonin and light responsiveness in mouse lemurs indicate the relevance of this primate as a model for testing the predictive value of these parameters with regard to the individual longevity.

Animals exposed to an accelerated photocycle show the same alteration in aMT6s excretion and in the Fos response to light in the SCN as that of animals exposed to a longer photocycle when the age corresponds to more than five seasonal cycles. This argues in favor of the idea that the age-related alteration of the circadian pacemaker in mouse lemurs depends on the number of expressed seasonal cycles rather than on a fixed chronological age. Present models of accelerated aging in mammals are limited to the mouse, on which extensive studies are currently being carried out (Takeda, 1999). Accelerated senescent mice manifest various pathobiological phenotypes such as deficits in learning and memory, brain atrophy, and abnormal circadian rhythms. Even though melatonin seems to reduce brain damage in some strains of senescent accelerated mice (Morioka et al., 1999), the functional differences of the circadian clock observed between strains are still difficult to ascertain, owing to the genetic background of these mice (Sanchez-Barcelo et al., 1997; Takeda, 1999).

Our results provide the first primate model showing phenotypical accelerated aging and the interpretation of the present findings leads to several conclusions. First, in addition to the demographic and morphological demonstration of accelerated aging (Perret, 1997), these data demonstrate that the accelerated aging paradigm can affect the organism at the physiological and cellular levels of the circadian clock. These studies need to be extended to investigations including other pathobiological parameters such as brain atrophy occurring during normal aging in mouse lemurs (Dhenain et al., 2000). Second, the data emphasize the high predictive value of circadian clock function with regard to longevity in mouse lemurs. Finally, taken together, these results provide new insights into the mechanisms underlying artificially accelerated aging at the level of cellular and possibly molecular mechanisms of the biological clock, and the use of the circadian clock function as a marker of life span will help clarify the mechanisms in age-associated pathologies.

Acknowledgements—We thank Naura Choulamountri for technical assistance. This research was supported by grants from Biomed2 (BMH 4-CT97-2327) and the Ministry of Research and Technology.

REFERENCES

Aujard, F., Perret, M., 1998. Age-related effects on reproductive function and sexual competition in the male prosimian primate, *Microcebus murinus*. Physiol. Behav. 64, 513–519.

Aujard, F., Perret, M., Vannier, G., 1998. Thermoregulatory responses to variations of photoperiod and ambient temperature in the lesser mouse lemur: a primitive or an advanced adaptive character? J. Comp. Physiol. B 168, 540–548.

Baskett, J.J., Cockrem, J.F., Antunovich, T.A., 1998. Sulphatoxymelatonin excretion in older people: relationship to plasma melatonin and renal function. J. Pineal Res. 24, 58–61.

Benloucif, S., Masana, M.I., Dubocovich, M.L., 1997. Light-induced phase shifts of circadian activity rhythms and immediate early gene expression in the suprachiasmatic nucleus are attenuated in old C3H/HeN mice. Brain Res. 747, 34–42.

Brock, M.A., 1991. Chronobiology and aging. J. Am. Geriatr. Soc. 39, 74–91.

Davis, F.C., Menaker, M., 1980. Hamsters through time's window: temporal structure of hamster locomotor rhythmicity. Am. J. Physiol. 239, R149–R155.

Dhenain, M., Michot, J.-L., Privat, N., Picq, J.-L., Boller, F., Duyckaerts, C., Volk, A., 2000. MRI description of cerebral atrophy in mouse lemur primates. Neurobiol. Aging 21, 81–88.

Dkhissi-Benyahya, O., Sicard, B., Cooper, H.M., 2000. Effects of irradiance and stimulus duration on early gene expression (Fos) in the suprachiasmatic nucleus: temporal summation and reciprocity. J. Neurosci. 20, 7790–7797.

Folkard, S., Minors, D.S., Waterhouse, J.M., 1985. Chronobiology and shift-work: current issues and trends. Chronobiologia 12, 31–54.

Harthé, C., Claustrat, B., Brun, J., Chazot, G., 1991. Direct radioimmunoassay of 6-sulfatoxymelatonin in plasma with use of an iodinated tracer. Clin. Chem. 37, 536–539.

Hofman, M.A., Swaab, D.F., 1994. Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res. 651, 134–142.

Hofman, M.A., Swaab, D.F., 1995. Influence of aging on the seasonal rhythm of the vasopressin-expressing neurons in the human suprachiasmatic nucleus. Neurobiol. Aging 16, 965–971.

Kawakami, F., Okamura, H., Tamada, Y., Maebayashi, Y., Fukui, K., Ibata, Y., 1997. Loss of day-night differences in VIP mRNA levels in the suprachiasmatic nucleus of aged rats. Neurosci. Lett. 222, 99–102.

Kennaway, D.J., Lushington, K., Dawson, D., Lack, L., van den Heuvel, C., Rogers, N., 1999. Urinary 6-sulfatoxymelatonin excretion and aging: new results and a critical review of the literature. J. Pineal Res. 27, 210–220.

Kornhauser, J.M., Nelson, D.E., Mayo, K.E., Takahashi, J.S., 1990. Photic and circadian regulation of *c-fos* gene expression in the hamster suprachiasmatic nucleus. Neuron 5, 127–134.

Lolova, I.S., Davidoff, M.S., Yakimoff, N.A., 1996. Vasopressin- and oxytocin-immunoreactive nerve cells in the aging rat hypothalamus. Acta Physiol. Pharmacol. Bulg. 22, 7–16.

Mayeda, A.R., Hofstetter, J.R., Possidente, B., 1997. Aging lengthens TauDD in C57BL/6J, DBA/2J, and outbred SWR male mice (*Mus musculus*). Chronobiol. Int. 14, 19–23.

Morin, L.P., 1988. Age-related changes in hamster circadian period, entrainment and rhythm splitting. J. Biol. Rhythms 3, 237–248.

Morioka, N., Okatani, Y., Wakatsuki, A., 1999. Melatonin protects against age-related DNA damage in the brains of female senescence-accelerated mice. J. Pineal Res. 27, 202–209.

Penev, P.D., Zee, P.C., Turek, F.W., 1997. Quantitative analysis of the age-related fragmentation of hamster 24-h activity rhythms. Am. J. Physiol. 273, R2132–R2137.

Perret, M., 1992. Environmental and social determinants of sexual function in the male lesser mouse lemur (*Microcebus murinus*). Folia Primatol. 59, 1–25.

Perret, M., 1997. Change in photoperiodic cycle affects life span in a prosimian primate (*Microcebus murinus*). J. Biol. Rhythms 12, 136–145.

Perret, M., Aujard, F., Vannier, G., 1998. Influence of daylength on metabolic rate and daily water loss in the male prosimian primate *Microcebus murinus*. Comp. Biochem. Physiol. A 119, 981–989.

Pittendrigh, C.S., Daan, S., 1974. Circadian oscillators in rodents: a systematic increase in their frequency with age. Science 186, 548–550.

Pittendrigh, C.S., Minis, D.H., 1972. Circadian systems: longevity as a function of circadian resonance in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 69, 1537–1539.

Reilly, T., Waterhouse, J., Atkinson, G., 1997. Aging, rhythms of physical performance, and adjustment to changes in the sleep-activity cycle. Occup. Environ. Med. 54, 812–816.

Roozendall, B., Van Gool, W.A., Swaab, D.F., Hoogendijk, J.E., Mirmiran, M., 1987. Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. Brain Res. 409, 259–264.

Rosenberg, R.S., Zee, P.C., Turek, F.W., 1991. Phase response curves to light in young and old hamsters. Am. J. Physiol. 261, R491–R495.

Sakellaris, P.C., Peterson, A., Goodwin, A., Winget, C.M., Vernikos-Danellis, J., 1975. Response of mice to repeated photoperiod shifts: susceptibility to stress and barbiturates. Proc. Soc. Exp. Biol. Med. 49, 677–680.

Sanchez-Barcelo, E.J., Megias, M., Verduga, R., Crespo, D., 1997. Differences between the circadian system of two strains of senescence-accelerated mice (SAM). Physiol. Behav. 62, 1225–1229.

Satinoff, E., 1998. Patterns of circadian body temperature rhythms in aged rats. Clin. Exp. Pharmacol. Physiol. 25, 135–140.

Scarbrough, K., Losee-Olson, S., Wallen, E.P., Turek, F.W., 1997. Aging and photoperiod affect entrainment and quantitative aspects of locomotor behavior in Syrian hamsters. Am. J. Physiol. 272, R1219–R1225.

Schilling, A., Richard, J.-P., Servière, J., 1999. Duration of activity and period of circadian activity-rest rhythm in a photoperiod-dependent primate, *Microcebus murinus*. C.R. Acad. Sci. Paris Life Sci. 322, 759–770.

Schwartz, W.J., Takeuchi, J., Shannon, W., Davis, E.M., Aronin, N., 1994. Temporal regulation of light-induced Fos and Fos-like protein expression in the ventrolateral subdivision of the rat suprachiasmatic nucleus. Neuroscience 58, 573–583.

Sutin, E.L., Dement, W.C., Heller, H.C., Kilduff, T.S., 1993. Light-induced gene expression in the suprachiasmatic nucleus of young and aging rats. Neurobiol. Aging 14, 441–446.

Takeda, T., 1999. Senescence-accelerated mouse (SAM): a biogerontological resource in aging research. Neurobiol. Aging 20, 105–110.

Touitou, Y., Haus, E., 1994. Biological rhythms and aging. In: Touitou, Y., Haus, E. (Eds.), Biologic Rhythms in Clinical and Laboratory Medicine. Springer, Berlin, pp. 188–207.

Turek, F.W., Penev, P., Zhang, Y., VanReeth, O., Takahashi, J.S., Zee, P.C., 1995. Alterations in the circadian system in advanced age. Ciba Found. Symp. 183, 212–226.

Valentinuzzi, V.S., Scarbrough, K., Takahashi, J.S., Turek, F.W., 1997. Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. Am. J. Physiol. 273, R1957–R1964.

Waldhauser, F., Kovacs, J., Reiter, E., 1998. Age-related changes in melatonin levels in humans and its potential consequences for sleep disorders. Exp. Gerontol. 33, 759–772.

Weibel, L., Turek, F.W., Mocaer, E., Van Reeth, O., 2000. A melatonin agonist facilitates circadian resynchronization in old hamsters after abrupt shifts in the light-dark cycle. Brain Res. 880, 207–211.
Weitzman, E.D., Moline, M.L., Czeisler, C.A., Zimmerman, J.C., 1982. Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. Neurobiol. Aging 3, 299–309.
Welsh, D.K., Richardson, G.S., Dement, W.C., 1986. Effect of age on the circadian pattern of sleep and wakefulness in the mouse. J. Gerontol. 41, 579–586.
Wise, P.M., Cohen, I.R., Weiland, N.G., London, E.D., 1988. Aging alters the circadian rhythm of glucose utilization in the suprachiasmatic nucleus. Proc. Natl. Acad. Sci. USA 85, 5305–5309.
Witting, W., Mirmiran, M., Bos, N.P., Swaab, D.F., 1993. Effect of light intensity on diurnal sleep-wake distribution in young and old rats. Brain Res. Bull. 30, 157–162.
Witting, W., Mirmiran, M., Bos, N.P., Swaab, D.F., 1994. The effect of old age on the free-running period of circadian rhythms in rat. Chronobiol. Int. 11, 103–112.
Zee, P.C., Rosenberg, R.S., Turek, F.W., 1992. Effects of aging on entrainment and rate of resynchronization of circadian locomotor activity. Am. J. Physiol. 263, R1099–R1103.
Zeitzer, J.M., Daniels, J.E., Duffy, J.F., Klerman, E.B., Shanahan, T.L., Dijk, D.J., Czeisler, C.A., 1999. Do plasma melatonin concentrations decline with age? Am. J. Med. 107, 432–436.
Zhang, Y., Brainard, G.C., Zee, P.C., Pinto, L.H., Takahashi, J.S., Turek, F.W., 1998. Effects of aging on lens transmittance and retinal input to the suprachiasmatic nucleus in golden hamsters. Neurosci. Lett. 258, 167–170.
Zhang, Y., Kornhauser, J.M., Zee, P.C., Mayo, K.E., Takahashi, J.S., Turek, F.W., 1996. Effects of aging on light-induced phase-shifting of circadian behavioral rhythms, Fos expression and CREB phosphorylation in the hamster suprachiasmatic nucleus. Neuroscience 70, 951–961.

(*Accepted* 24 *April* 2001)