# Part Sleep and normal aging



## Sleep and normal aging

## Aging and circadian rhythms: general trends

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## Introduction

Age-related changes in circadian rhythms occur in both animals and humans [1, 2, 3, 4]. It is also known that dysregulated circadian rhythms are a major cause of sleep pathology, and thus there tends to be a strong correlation between increasingly disrupted sleep patterns and advancing age. Circadian rhythms, which have a periodicity of approximately 24 hours (circa = about; dies = day), have been shown to have survival value [5, 6]. The progressive deterioration in functional vigor, the most pronounced hallmark of aging, is believed to be due to the loss of co-ordination between interdependent oscillatory processes. These processes become increasingly dissociated (disphasia) with age [7]. According to Samis, this disphasic condition is the consequence of aging because the aging organism has lost much of its adaptive resiliency, even though the diphasic episodes may occur only briefly or occasionally a complete re-entrainment of the aberrant rhythms does not occur. Small changes therefore have large consequences. Only a slight discrepancy between the diphasic rhythms and compensatory re-entrainment will, with time, produce increasing randomization in the affected processes.

## Aging and circadian rhythms

There are many age-related changes that affect circadian rhythms. It is generally believed that ageassociated circadian disruption occurs at various levels of biological organization. These age-related disruptions have been studied in laboratory animals and have been extensively reviewed in the literature [8, 9, 10, 11, 12, 13]. Such age-attenuated changes have been associated with several neural, endocrine, metabolic, and behavioral rhythms in animals [2, 3], and are closely linked to the period of the circadian pacemaker which controls these rhythms [14]. Indeed, anatomical and electrophysiological studies have shown that age-related changes occur within the suprachiasmatic nucleus (SCN, the mammalian "biological clock"), and that these changes occur in both humans and other mammals [15, 16, 17, 18, 19, 20, 21, 22].

Aging is often accompanied by reductions in the nocturnal melatonin and pineal *N*-acetyltransferase (NAT) rhythms [23, 24]. Additionally, aging is associated with a general decline in body temperature [25, 26], as well as reduction in the amplitude of light-induced phase response curve (PRC) [14]. Older animals take longer to re-entrain to phase-shifted light/dark (LD) cycles compared to their younger counterparts [27]. Further, there is a general tendency towards sleep loss during the day. The fact that older animals sleep less during the day (the sleep period of rats) suggests that it is a selective loss of sleep that accounts for the largest component of age-related reduction in total sleep time (TST).

Various systematic changes occur in rhythmic processes as organisms age. While these modifications may generally reflect the normal aging process, it is apparent that individual differences exist in the way that aging progresses. As a result, many attributes of normal development show a characteristic progression towards circadian disorganization, as manifested by increases in the standard deviations (SD) of their measured values.

Numerous changes in overt rhythmicity appear to be associated with aging [2, 3, 28]. Some of these have been attributed to deterioration in the functioning of circadian pacemakers, while others may result from a general decline in the capacity for entrainment ability and/or in the systemic processes that are clock controlled.

Observed changes in overt circadian patterns include:

 Reduction in the amplitude of rhythms [14], fragmentation of rhythms, and disorganization in their temporal order, vigor, and precision [7, 9, 10, 29, 30, 31];

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- (2) Loss of entrainment stability and responsiveness to zeitgebers [31]; and
- (3) Changes in clock period and stability.

In addition, alterations in the clock-regulated processes are seen. These include changes in the level of specific activities, in the temporal distribution of behaviors, in the levels of circulating hormones, and in the density of certain peptides, neurotransmitters, and receptors [32].

## Amplitude and organization

Decrease in the amplitude of rhythmic functions reflect a general loss of their "stability" or robustness [33]. The relationship between rhythm disturbances and aging has been discussed extensively in several reviews [2, 3, 9, 10, 12]. It has been reported in numerous studies that as aging progresses there is a general deterioration of wheel running activity of rodents [30, 34, 35, 36, 37, 38].

However, in 1998 Davis and Viswanathan [38] did show that the free-running period of Syrian hamsters remained stable throughout their lifespan. Generally, aging is associated with increasingly disruptive changes in circadian rhythms. Age-related disturbances in the locomotor activity of humans, for instance, have been observed [39]. In addition, aging is associated with a reduction in the amplitude of other behavioral rhythms, including feeding, drinking [40], and sleep/wake cycles [41, 42].

In addition to behavioral changes, physiological rhythms are also affected during the aging process. This includes rhythms in body temperature (mice and rats), audiogenic convulsions (mouse), and oxygen consumption (mouse), as well as the excretion of potassium (humans), growth hormone (GH; human), testosterone, and leutenizing hormone (LH; humans) [6, 12, 25, 26, 43, 44, 45, 46, 47]. In 1982 Halberg reported that circadian rhythms of cortisol, aldosterone, prolactin, and GH in the blood plasma are altered in older human subjects [48]. Age-related changes have also been noted in the circadian hormonal rhythms of other mammals. For instance, epinephrine (E) and norepinephrine (NE) have been shown to undergo an age-related decline in circadian amplitude and mesor, without any apparent change in the acrophase [48].

It is difficult to assess to what extent a decrease in the amplitude of overt rhythms reflects changes in circadian pacemaker activity as opposed to parallel age-dependent losses of peripheral function. Satinoff *et al.* (1993) demonstrated that the SCN of older rats exhibits disturbed patterns and lower amplitude neuronal firing compared to younger animals (although their behavioral rhythms were not always disturbed) [19]. Wise *et al.* showed that there is a decrease in the SCN glucose utilization in older rats in response to LD transitions [18, 49]. There are also numerous reports of age-related changes in the structure and neurochemistry of the SCN, including alterations in the cells producing vasopressin (AVP) [15, 16, 21] and vasoactive intestinal polypeptide (VIP) [17, 50, 51]. While these alterations do not always correlate with overt changes in behavior and physiology [16, 52, 53], there is sufficient evidence for differences in the SCN of younger and older animals to suggest that a link exists between the functional impairment of the "master" circadian pacemaker (SCN) and observed changes in the overt circadian patterns. Moreover, the re-consolidation of the host-driven locomotor activity rhythm following SCN transplantation in older hamsters suggests that the SCN plays a primary role in maintaining temporal organization in metabolic functions in mammals [54].

A predictable consequence of the reduction in rhythm amplitude is a loss of synchrony or inappropriate phase-relationships among constituent circadian rhythms. It has been asserted that the primary function of biological clocks is to produce temporal organization among rhythmic processes, and to entrain them to appropriate environmental cycles. It is predictable, therefore, that if circadian clocks or their control mechanisms become impaired, this organization will be compromised. Disorganization generally appears as changes in the temporal structure of the organism's rhythmic physiology and behavior. Rhythms remain synchronized with each other but may assume inappropriate or variable phase relationships [7]. Such disorganization is well documented in humans [41, 55, 56].

Age-related changes in the amplitude of many physiological and behavioral rhythms have been noted in the rest/activity cycle, core body temperature (cBT), feeding, drinking, eating, and an organism's response to LD cycles. Such reductions in the amplitude of behavioral rhythms are quite similar to those observed in sleep/wake cycles.

# Entrainment and responsiveness to zeitgebers

In most studies, rhythms in aged organisms are measured in 24-hour LD cycles, so that inappropriate phase

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relationships with the environment can be detected. A common example of this is seen in elderly humans, in whom sleep/wake patterns become disorganized and variable compared to those of young adults [41]. In LD cycles, loss of temporal organization could result from either impairment of clock function, and/or due to a reduction in its sensitivity to zeitgebers. The primary zeitgeber for most organisms is the LD cycle; however, there is some evidence that other rhythmic factors in the environment can also assume this role [57], and it is almost certain, for instance, that non-photic stimuli can alter rhythms, and thus can act as zeitgebers [58, 59].

Entrainment to LD cycles is a key feature of circadian clocks, and is known to be affected by agedependent changes in period and photic sensitivity. However, entrainment of circadian clocks is also affected by the organism's acute responses to light that may mask circadian gating. The simplest experiments for examining circadian responses to environmental stimulation have involved re-entrainment and phaseresetting paradigms. While there appear to be changes in these responses during aging, it is not consistent across species or even among experiments. For example, Rosenberg et al. reported that older rats take longer to respond to a phase-reversal in the LD cycles as compared to their younger counterparts [27]. On the other hand, Peng et al. reported that there is no difference between young and old rats in their rates of re-entrainment [40]. Zee et al. [60] found that it takes more time for younger hamsters to re-entrain to a phase-advanced LD cycle, but less time when the cycle is phase delayed, whereas Valentinuzzi et al. [37] reported that re-entrainment in old mice is accelerated when the LD cycles are phase advanced but remains unchanged when the LD cycles are phase delayed. The magnitude of light-induced phase delays increases with age in rats [61], but decreases with age in mice [62] and in hamsters [14]. Such changes, however, can be reversed by the use of bright light pulses [61, 63].

The reasons for these disparate findings are not known. Because different species have been used in separate experiments, and because experimental conditions change from laboratory to laboratory, such differences in findings may not be surprising. However, it is also possible that such discrepancies are related to the fact that there are fundamental interand intra-species specific differences in the way animals age. For example, some hamsters lose their highly consolidated pattern of wheel running behavior as they age, while others do not [64]. Because activity influences the circadian responses to light [65], and can also influence directly the phase and period of circadian rhythms, aging may have different effects on rhythmicity due to different changes in activity patterns.

Responses to non-photic signals are also affected by age. Phase-shifts induced by a serotonin (5-HT) agonist [66] or by the benzodiazepine (BZD) triazolam are reduced in aged hamsters [67]. The latter effect can be reversed by transplantation of a fetal SCN [68], and by a melatonin agonist [69]. Melatonin can also facilitate re-entrainment to a shifted LD cycle [70].

# Age-related changes in circadian organization

The most prominent age-related changes in circadian behavioral rhythms are those observed in free-running and entrained rhythms. Pittendrigh and Daan found that the free-running period decreases in rodents from puberty to old age [30]. With regard to entrained rhythms, Weitzman *et al.* proposed that changes in the relationship between endogenous rhythms and environmental rhythms accounted for the deficits in circadian organization that are known to occur in advancing age [41].

# Discrepancies in the age-related changes in period length

It should be noted that several discrepancies exist in the findings from studies on age-related changes in period length. There are many schools of thought regarding the interpretation of these changes. Several studies observed decreases in period length [10, 30, 41, 71], whereas other prominent studies observed increases [11, 37, 72, 73, 74]. Some researchers argued that, in fact, negligible changes in circadian period occur with age [75]. According to Sharma and Chandrashekaran, the differences in the reported findings on age-related period changes can be attributed to the fact that in several studies rhythms were not monitored for a long enough period and the observations were made on animals maintained under different LD conditions [75]. Czeisler et al. also concluded, based on their forced desynchrony protocol studies, that circadian period does not shorten reliably with age [76]. Because there is such a range of effects reported, it seems unwise at this point in time to make

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any definitive statements about systematic changes in circadian systems that occur with age. It may be possible that variability in circadian function increases in conjunction with variability in an individual's behavioral pattern. Thus, in contrast to elderly subjects who are healthy, and who often show greater regularity in the timing of their activities (possibly as an adaptive behavior to overcome a less robust circadian timing system [83]), demented elderly patients show a marked loss of stability.

## Modifications in circadian rhythms with aging

The amplitude of many intrinsic rhythms decreases with age, with an apparent decrease in the "maxima" for the rhythm. As summarized by Davis, these rhythms include those in body temperature (mouse and rat), audiogenic convulsions (mouse), oxygen consumption (mouse), potassium excretion (humans), growth hormone (human), testosterone (human), and leutenizing hormone concentration (humans) [6].

Under constant darkness, circadian rhythms "freerun" with an intrinsic (genetically determined) period ( $\tau$ ; tau) that is either slightly longer or slightly shorter than 24 hours. The average human  $\tau$  is believed to be in the range of 24.2 to 24.4 hours. In the elderly, however, the value of  $\tau$  decreases with age, though only by a small degree [2, 3, 14, 29, 30, 60, 61]. As the value of  $\tau$  increases or decreases, there is also an associated increase in its standard deviation. For example, some species exhibit an age-related lengthening of  $\tau$ , while other species show an age-related shortening of  $\tau$ [74, 77, 78, 79].

Studies carried out by Davis and Viswanathan, Sharma and Chandrashekaran, and Czeisler *et al.* found that  $\tau$  remained relatively stable over the lifespan of the animals investigated [38, 75, 76]. Studies by Valentinuzzi *et al.* and Kendall *et al.* suggested that the clock period increases as the animals aged [37, 80]. However, these increases were attributed to the methodological differences such as a restriction in the range of ages studied, after-effects of previous entrainment, and/or activity feedback in the circadian pacemaker [80].

## Irregular circadian rhythms with age-related neurodegenerative disease

As is evident from this overview, age-related changes in circadian organization can be seen in many rhythm parameters, including their phase-relationship, amplitude, period, and entrainability. Van Someren and co-workers have pointed out the importance of investigating not only these parameters, but also their inherent variability over subsequent days. They provided methods for quantifying such variability, and found evidence for a specific loss of stability over days in the rest-activity rhythm of elderly patients suffering from Alzheimer's dementia [81, 82]. Thus, in contrast to elderly subjects who are healthy, and who often show greater regularity in the timing of their activities (possibly as an adaptive behavior to overcome a less robust circadian timing system [83]), demented elderly patients show a marked loss of stability in their temporal organization. It is likely that such poor stability in demented elderly patients contributes to both nocturnal sleep problems and diurnal sleepiness, as has been demonstrated experimentally in young adults [84, 85]. Moreover, poor stability may also contribute to the neuropathological changes in the medial temporal lobe area, and in the associated memory problems that are typical of Alzheimer's disease, a phenomenon that has been demonstrated in young adults who show irregular synchronization to the environmental LD cycle due to occupational demands [86, 87, 88]. For this reason, the application of whole-day bright light exposure has been effectively used as a practical intervention for improving the stability of rest-activity rhythms in demented elderly patients [89]. Moreover, preliminary data suggest that long-term application of such a therapeutic zeitgeber enhancement indeed attenuates cognitive decline [90].

### Summary

In this chapter, we have cited evidence showing that circadian rhythms have a tendency to become less robust with increasing age, i.e. they generally exhibit decreases in amplitude and less stability. Some obvious consequences of such reductions in rhythm amplitude are fragmentation of rhythms, complete loss of temporal order and structure, loss of stability of entrainment and responsiveness to zeitgebers, changes in clock period and its stability, and inappropriate phase relationships among behavioral and metabolic oscillations.

Age-related decreases in the amplitude of circadian rhythms in humans and other mammals have been linked to a deterioration of rhythmic behaviors such as those seen in locomotor activity, feeding, drinking,

and sleep and wakefulness. While some of these changes are due to molecular and physiological changes in the circadian pacemakers, others stem from a general decline in entrainment mechanisms or clockcontrolled processes. Anatomical and electrophysiological studies have shown that age-related changes occur within the biological clocks of mammals including humans. The SCN of aged animals have abnormal patterns and lower amplitude of neuronal firing compared to young animals, and there is a decrease in SCN glucose utilization in aged rats. There is increasing evidence for age-related changes in the structure and neurochemistry of the SCN, including alterations in cells producing vasopressin.

Many attributes of normal aging and development show a characteristic progression towards circadian disorganization, of which the most undesirable consequence, from the clock's perspective, is disturbances in the sleep/wake cycle. The translational aspect of such observations is that many of the findings related to animals show similarities to the findings in humans. In essence, disruption in rhythm, whether of shorter or longer duration of time, will have undesirable consequences for health and well-being. In general, circadian rhythms have a tendency to become less robust with advancing age, i.e. they show a decrease in amplitude and/or are phase advanced [10]. Moreover, an age-associated shortening or speeding up of the clock, which reflects phase advancement of the sleep/wake cycle, can produce changes in habitual bedtimes and awakening times in the elderly. Such phase advances may be related to changes in the core body temperature (cBT) rhythm that shows amplitude attenuation and phase advances [91]. Further, major age-related alterations in temporal organization result in a shortening of the circadian period of waking and of paradoxical sleep (PS) - a deep sleep with a brain wave pattern more like that of waking states than that of other states of sleep, which occurs during rapid eye movement (REM) sleep [92].

The fact that changes occur in the sleep/wake cycle (increased wakefulness at night and increased sleepiness during the day) suggests that aging is associated with desynchronization of circadian rhythms [93]. Arguably, some of the sleep dysfunctions experienced by certain aged individuals are due to insufficient exposure to zeitgebers (environmental time cues, such as the daily changes in luminance from sunlight), particularly among those who are house-bound or institutionalized [94].

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There is also evidence that the rest-activity rhythms of the elderly suffering from Alzheimer's dementia show a general loss of stability with age. It is likely that such poor stability in demented elderly patients contributes to both nocturnal sleeping problems and diurnal sleepiness, and to the neuropathological changes in the medial temporal lobe area and



Figure 1.1. Locomotor activity data illustrating activity/rest profile of (a) young (~4 month old) and (b) old (~18 months) field mouse Mus booduga. The locomotor activity of the animal was monitored using an activity running wheel (diameter  $\approx$  20 cm) attached to a transparent plexiglas cage of dimension 0.07 m  $\times$  0.11 m  $\times$  0.09 m, with a small opening of 0.02 m diameter. Reed-relays attached to the wheels activated the writing stylets of an Esterline Angus A620X Event Recorder when the running mice caused revolutions of the wheel. The activity patterns of as many as 18 mice in separate running wheels, placed on open shelves in the experimental room, could be assayed concurrently in constant darkness. The temperature inside the experimental rooms (3.05 m × 2.44 m × 4.01 m), which did not have windows but were gently ventilated, remained constant at  $25 \pm 1^{\circ}$ C and the relative humidity was  $75 \pm 5$  %. Food (millet and grain) and water were available ad libitum. Actograms were obtained by pasting 24 hr activity/ rest strips chronologically one below the other in the standard manner and double plotted.

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the associated memory problems that are typical of Alzheimer's disease. For this reason, the application of bright light exposure has been effectively used as a practical intervention for improving the stability of rest-activity rhythms in demented elderly patients [88, 95] and general well being [96].

Table 1.1. Summary of some of the age-related changes in circadian rhythm in animals: changes in general characteristics

Criteria	Comments	Reference(s)
Precision	The day-to-day stability of the different sleep states is reduced in old rats, whereas that of the drinking rhythm is enhanced	79
Impairment of rhythm	Age-related deficits in learning and memory, changes in emotional behavior, and abnormality of circadian rhythms in mice. In aged animals, endothelial NO synthase activity was markedly decreased during the daytime, along with impairment of clock gene expression and the circadian variation in blood pressure	97, 98, 99, 100, 101, 102
Internal desynchronization	Activity and core body temperature rhythms dissociate with aging	102, 103
Light/dark (LD) activity difference	Age-related changes include decrease of wheel-running activity, decrease in circadian rhythm amplitude, increase in proportion of light activity, and increase in split activity rhythms	102, 104, 105, 106, 107, 108, 109
Rate of resynchronization	Old animals take significantly longer to re-entrain compared to younger ones. Resynchronization is significantly slower in old mice. Middle-aged hamsters resynchronized more rapidly after a phase advance in LD cycle than young hamsters, whereas young hamsters phase delay more rapidly than middle-aged hamsters. Following an advance of the LD cycle, circadian rhythms in the pineal NAT activity and melatonin content reappeared in young rats, but was abolished in old rats	37, 60, 110, 111, 112
Circadian period (t)	The circadian period of active wakefulness, body temperature, and drinking behavior are significantly shortened in old rats	79
	Relationship between circadian period and wake time, circadian phase, and diurnal preference in older subjects are not different from those in young subjects. Period did not change with age in two inbred strain of mice	38, 75, 78, 111, 113
	Circadian period is significantly lengthened in two inbred strains of mice and in blind humans	37, 74, 80, 114
Phase of entrainment	Delay in evening activity peak and advance in morning activity peak. Age-related difference in the phase of entrainment of activity rhythm is greater under LD 6:18 than LD 14:10. Age-related alterations in the phase of entrainment to LD cycle	2, 3, 36, 37, 88, 96, 103, 115, 116, 117, 231
	Relationship between circadian period and wake time, circadian phase, and diurnal preference in older subjects are not different from those in young subjects	113
Attention pattern rhythm	Age modifies rhythm in attention and also the distribution of interindividual differences which occur in kindergarten children	117
Phase-resetting	Phase shifts do not differ among old and young groups. Compared to the young, older adults are significantly phase-advanced in sleep, cortisol, and aMT6s onset, but not in aMT6s or temperature rhythm. The effect of increase in daily light duration is attenuated in old animals compared to younger lemurs. Age-related increase in light-induced phase shifts. Loss of responsiveness to phase shifting/ entraining effect of stimuli. Aging is associated with attenuation of 8-OH-DPAT-induced phase shift and its ability to attenuate the photic phase. Decreased sensitivity to phase delaying effect of light. Magnitude of phase delays does not differ between old and young individuals, but phase advances are significantly attenuated in old	2, 14, 66, 69, 96, 116, 118, 119, 120, 121, 122, 123, 124
Feeding rhythm	Age-related shift from nocturnal to diurnal eating habits in mice. Rhythm in in gestive responses to SKF-10,047 is absent in old animals. Also, old mice failed to show any significant increase in ingestive response following opiate administration	125, 126
Phase-shifting effects of nocturnal exercise	The dim-light melatonin onset (DLMO) phase delays more after exercise. On average, the difference in phase shift between exercise and control conditions is similar for old and young subjects	127

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Criteria	Comments	Reference(s)
Rest/activity rhythm	Amplitude of core body temperature (cBT) rhythm is reduced in elderly subjects	96, 102, 103, 107, 111
Respiratory rhythm	Aging decreases the amplitude of circadian respiratory rhythm and modifies the phases of ultradian respiratory rhythms	127
Body temperature rhythm (cBT)	Rest/activity rhythm becomes fragmented in aged primates, and shows increased activity during resting period. Aging induces a decrease in amplitude of cBT rhythm and an increase in energy consumption. Various hormonal secretions decrease with age. Activity and CBT rhythms do not change simultaneously with age. In comparison to adults, CBT rhythm in the elderly is poorly developed. Daily activity episodes, total activity, and body temperature are significantly lower in old mice	25, 26, 47, 88, 106, 109, 128, 129, 130, 131
Melatonin rhythm	Plasma melatonin concentration during sleep is considerably decreased with aging in men	132
Myelopoietic progenitor cell rhythm	The d-8 CFU-S cell numbers decline in aging mice. The amplitudes and 24 h mean values decline in aged mice. With aging a significant advance of peak is observed	133, 134
Mitotic activity of endocrine cells	Old mice show higher mitotic indices during the darkness. The average mitotic activity over the entire cycle is lower in old mice	135
Rectal temperature, organ weights, blood pressure, and Ca and Mg	17 parameters could be approximated in young rats, 1 parameter in old animals. In some cases large age-dependent alterations in amplitude could be observed	136
Blood pressure and heart rate during sleep	Sensitivity of baroreflex control of heart rate is significantly depressed; spontaneous increase in mean arterial pressure and body temperature during REM sleep and drop at the end of REM sleep are significantly enhanced in aged rats	137
Food anticipatory activity rhythm to restricted feeding	Under restricted feeding, aged rats take longer to show food anticipation pattern and show a lower amplitude food anticipation rhythm compared to young rats. Despite the absence of entrainment to LD cycles, both SCN-lesioned and aged groups show entrainment to restricted feeding	138, 139, 232
Activity rhythm	Age-related disruption of circadian timing. Older mice showed decrease in amplitude and high levels of activity during the light phase of LD cycle. Activity rhythms of older animals "split"	140, 141
Shift work	Ability to do shift work decreases	142, 143, 144, 145, 146, 147, 148, 149
Clock genes		
Clock genes	Impairment of <i>mPer</i> expression	150
BMAL1 and PER	Molecular clock mechanism in the SCN, PVN, and pineal body is preserved against aging, whereas impairment of light-induced <i>Per1</i> induction in SCN results in impaired behavioral photic entrainment in aged rats	150
CLOCK	CLOCK mutant mice respond to low-dose irradiation by accelerating their aging program	150, 151, 233, 234
Per1	Old rats display age-related period shortening in mPer1 rhythmicity	
Per2	Age-dependent difference in mice is found in the case of <i>mPer2</i> (but not <i>mPerI</i> ) mRNA expression	153
Per3	Strength of association of 4-repeat allele of PER3 with evening types, and 5-repeat allele with in morning types attenuates with age	
Per1–2, CLOCK and Bmal1	Age alters the 24 h expression profile of <i>Clock</i> and its binding partner <i>Bmal1</i> in the hamster SCN. Light pulses induce smaller phase shifts in old animals than in young, leads to decreased induction of <i>mPer1</i> , but not of <i>mPer2</i> in the SCN of old hamsters	155
Per1–3, Bmal1	The evening <i>mPer</i> expression in the liver of old rats show significant decrease. The heart showed similar profiles with only a tendency towards a decrease of <i>mPer</i> expression and an increased <i>Bmal1</i> expression in the evening in old rats	156

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#### Table 1.1. (cont.)

Criteria	Comments	Reference(s)
Per2 and Bmal1	A significant age-related difference in mPer2 expression is detected	
Jun-B and Jun-D and CRH	In young rats, light induces a robust increase in the number of Jun-B positive cells in SCN. In middle-aged rats, the light-induced increase in the number of Jun-B positive cells was significantly attenuated. Transplantation of fetal SCN tissue into middle-aged rats successfully restored light-induced Jun-B expression to the levels of young rats. Unlike young rats, no rhythm in CRH mRNA expression is detected in the PVN of old rats	157, 158, 159
TAT	Age-related shift in the peak TAT enzyme activity rhythm	160
Role of Clock	Age-related changes in circadian rhythmicity occur equally in wild-type and heterozygous CLOCK mutant mice	161
Central and periphera	oscillators	
Glucose utilization	Unlike young animals, old rats show a more gradual increase in LCGU after lights-on, with no further increase prior to the LH surge, and a premature decrease during the afternoon and evening	18
Synaptic number	The population of the major SCN synapses formed with dendrite and total number of synapses reduce with advancing age	162
Morphology	The neurons and neuroglial cells in SCN of the old rats display more lipofuscin accumulation in comparison to younger animals. More neuroglial cells with broader somatic membrane appositional to that of neuron participate in satellitosis in the old age group	163
Electrophysiological properties	Aging leads to decrease in amplitude of impulse activity in dispersed SCN neurons in cultures. The frequency of spontaneous inhibitory post-synaptic currents is reduced in SCN of older animals	19, 163, 164, 165, 166
Responsiveness to melatonin	In SCN slices from aged mice, PACAP alone induced comparable levels of phospho-CREB	167
VIP mRNA expression	Aging selectively decreased the VIP mRNA expression in SCN without affecting AVP mRNA or SS mRNA	51
V1a and V1b receptors	The amplitude of V(1a) receptor mRNA rhythm is reduced and of V(1b) mRNA elevated in aged group	168
VIP	Loss of day-night difference in VIP mRNA levels in the SCN of aged rats	50
VIP, VPAC2, and PAC1 receptors	Aging reduces VIP and VPAC2 receptor mRNA and eliminates diurnal expression of VIP mRNA within the SCN of aged male rats	169
Cytokine receptors	Marked changes of several functional, cellular, and molecular parameters are observed in the aged SCN	170
Presynaptic network including GABAergic terminals	The number of and the area covered by presynaptic terminals and by their GABAergic subset are significantly decreased in old mice. Marked reduction in synaptic network of aging SCN, which also affects GABAergic terminals. Alterations of the GABAergic network during senescence	171, 172
AVP	Circadian organization in rats is progressively disturbed in senescence. An increase in SCN volume and nucleus diameter and an overall decrease in cell density are observed. Staining with antivasopressin and morphometry revealed a decrease in the number of SCN neurons, while the vasopressin cells became larger	16
Fos expression	No effect of age on the pattern of <i>c-fos</i> induction by light in SCN	173
Light-induced gene expression	A decrease in the response of <i>c-fos</i> and NGFI-A but not NGFI-B is noticed in the SCN of old animals after photic stimulation	20, 155
Adrenoceptors	Alpha(1)ARs and betaARs density in heart and brain and betaARs in submandibular glands are significantly lower in old mice	174
Neurotransmitter binding	In the aged animals, time of the binding maxima is no longer locked to the same phase of the LD cycle. Cycle amplitudes are smallest in young animals, increases significantly in old rats	175

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Table 1.1. (cont.)

Criteria	Comments	Reference(s)
Lipogenic enzymes, serum triglyceride and insulin levels	The activities of malic enzyme and alpha-glycerophosphate dehydrogenase are lower in the liver of old rats in contrast to young animals. Unlike young animals, the activity of glucose-6-phosphate dehydrogenase in old animals does not show any circadian rhythm	176
Norepinephrine level and serotonin	Old rats have lower amplitude and lower mesor values of 24 h variations in pineal NE content and 5-HT turnover	177
Melatonin and its receptor expression	Circadian rhythm of 2-[1251]iodo-melatonin binding showed age-related decrease in the SCN of old mice. Mel1a receptor mRNA expression in SCN decreased during day, but not during night	62a
Central and peripheral oscillators	While phase-resetting in the SCN following an advance or a delay of light cycle appears nearly normal in young rats, resynchronization of the liver is seriously disrupted. Arcuate nucleus and pineal gland exhibited faster resetting in aged rats relative to young controls. SCN rhythmicity in old rats displays age-related shortening in circadian period. Circadian rhythmicity in some peripheral tissues is unaffected by aging, while in other tissues is either phase advanced relative to the light cycle or absent	122, 153
Stress hormones and leptin	Aging augmented circulating leptin. Corticosterone levels correlated significantly with plasma adrenocorticotropin releasing hormone (ACTH) and leptin only in young rats	178
Lutenizing hormone (LH) and follicle stimulating hormone (FSH)	Baseline LH levels show a significant 24 h periodicity in adult males. FSH concentrations exhibit significant diurnal variations in juvenile and adult males. There is a significant influence of age on the temporal pattern and 24 h plasma hormone levels	179
Liver or brain enzymes	15 enzymes from young and 8 from aged mice showed a significant regression to a 24 h cosine curve. Old mice showed larger variance in 9 enzymes. 12 enzymes from aged mice had decreased mesors, 2 had increased mesors, and 9 were unchanged	180
Immune response, number of cells in lymphoid organs	With age, the peaks in amount of cells in thymus and its mass in the night and in autumn and peaks in the antibody titers in autumn diminish and intrasystemic relations undergo changes	181
HCRT-1 levels and HCRT receptors	Old rats had significantly less HCRT-1 in the CSF and continued to have low levels of HCRT-1 after 8 h of prolonged waking	182
Sensitivity of AD receptors	Old rats have more extracellular levels of AD and sleep compared to young rats across the 24 h diurnal cycle	183
5-HT7 receptors	Aging significantly inhibited the phase advances in running-wheel rhythms induced by 8-OH-DPAT	184
D2 receptor binding	Younger human subjects show reduced evening D2 receptor binding while older subjects show increased D2 receptor binding	185
2-[125l]iodo- melatonin binding	Specific 2-[1251]iodomelatonin binding sites are seen in the SCN of prenatal hamsters but not in older hamsters	186
Calbindin D28K protein cells	Calbindin expression in the SCN is modified by aging. Amplitude of oscillation in calbindin expression is damped, with a lower immunointensity during daytime and delayed decrease during night	187
Effect of lithium	The basal levels of phosphorylated GSK-3 (pGSK-3) protein expression in old hamsters are much lower, and lithium did not affect the period of the activity rhythm or pGSK-3 expression	188
Bcl-2 and Bax expression	In old rats CORT exposure decreases the bcl-2 to bax mRNA ratio in the morning but not in the evening	189