Aging of the Suprachiasmatic Clock

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Abstract

More than half of the elderly in today's society suffer from sleep disorders with detrimental effects on brain function, behavior, and social life. A major contribution to the regulation of sleep stems from the circadian system. The central circadian clock located in the suprachiasmatic nucleus of the hypothalamus is like other brain regions subject to age-associated changes. Age affects different levels of the clock machinery from molecular rhythms, intracellular messenger, and membrane properties to neuronal network synchronization. While some of the age-sensitive components of the circadian clock, like ion channels and neurotransmitters, have been described, little is known about the underlying mechanisms. In any case, the result is a reduction in the amplitude of the circadian timing signal produced by the suprachiasmatic nucleus, a weakening in the control of peripheral oscillators and a decrease in amplitude and precision of daily rhythms in physiology and behavior. The distortion in temporal organization is thought to be related to a number of serious health problems and promote neurodegeneration. Understanding the mechanisms underlying age-related deficits in circadian clock function will therefore not only benefit rhythm disorders but also alleviate age-associated diseases aggravated by clock dysfunction.

Keywords

amplitude, membrane properties, neuronal network, circadian clock, modeling, neurodegeneration, synchronization

Healthy aging is what most of us strive for once we realize we are eligible for a senior pass to the museum. This review will postulate that the challenges of today's 24/7 society with incidental disturbances in our biological clock are often hurdles in reaching this goal. Growing knowledge of the impact of aging on physiology and brain function offers new opportunities for finding countermeasures. One important impact of age on physiology affected by age is in the daily regulation of organ and brain functions. Virtually all organisms on this planet have developed a biological clock that controls the daily scheduling of most physiological processes within the body. In mammals, the central clock controlling daily rhythms is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Fig. 1; Saper 2013). The SCN generates a circadian rhythm in electrical activity, which is controlled by the action of molecular and cytosolic oscillators. Even in healthy humans aging weakens the signal of this endogenous clock resulting in symptoms like altered timing of sleep and less consolidation of sleep phases or diminished rhythms of body temperature and hormones (Dijk and Duffy 1999).

The age-associated attenuation of the central timing signal generated by the SCN may lead to increased variability in the phases of so-called peripheral oscillators controlling physiological and cognitive functions. It has been suggested that this loss of phase coherence can cause a number of health problems or aggravate them, like metabolic syndrome, neurodegenerative disorders, and cardiovascular diseases (Kondratova and Kondratov 2012). Chronotherapeutical interventions that could boost the circadian signal and restore the temporal order may not only alleviate the symptoms (e.g., sleep problems) but also prove beneficial for the prognosis of the disease.

One example for such a causal connection is Alzheimer's disease, the most prevalent neurodegenerative disorder. On the one hand, chronotherapy can reduce sleep disturbances of Alzheimer patients (Riemersma-van der Lek and others 2008), which would allow for an extended period of care at home. On the other hand, increasing the central clock output may also help a number of clock-controlled defense mechanisms against

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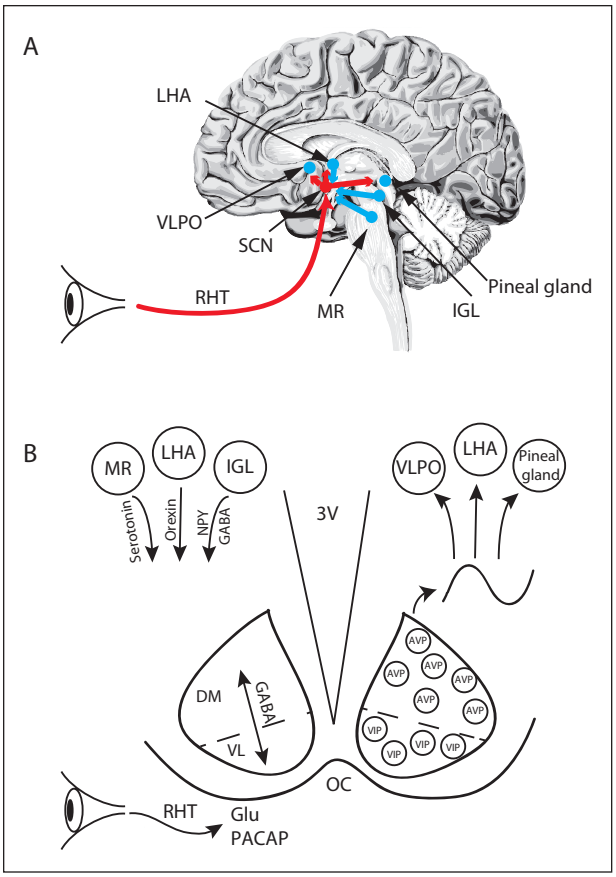


Figure 1. The location and organization of the suprachiasmatic nucleus (SCN). (A) The SCN is located in the anterior hypothalamus. Light information is transmitted to the SCN via the retinohypothalamic tract (RHT) which releases glutamate (Glu) and pituitary adenylate cyclase–activating polypeptide (PACAP). Several brain nuclei receive SCN output and/or project to the SCN, some involved in sleep/waking regulation (VLPO, LHA) and melatonin release (pineal gland). (B) The SCN is functionally organized in at least two distinct regions: dorsomedial (DM) and ventrolateral (VL) areas. Argenine vasopressin (AVP) producing cells mark the dorsomedial area and vasoactive intestinal peptide (VIP) producing cells are mainly found in the ventrolateral area. These neuropeptides, in concert with GABA, are involved in synchronization and neuronal interaction within the SCN. The SCN receives different inputs (left) and transmit its output to several brain nuclei and downstream targets. 3V = third ventricle, IGL = intergeniculate leaflet, LHA = lateral hypothalamus, MR = median raphe, OC = optic chiasm, VLPO = ventrolateral preoptic nucleus.

oxidative stress and DNA repair mechanisms that could slow the progression of the neurodegenerative disease (Kondratova and Kondratov 2012).

The relationship between the circadian clock and brain aging was recently discussed with a focus on neurodegenerative diseases (Kondratova and Kondratov 2012). In the present review, we will first summarize the age-related changes in sleep and circadian rhythms in humans and different animal models to demonstrate the usefulness and limitations of the use of animal models. We will then focus for the reminder of the review on age-related physiological changes in the SCN of mammals and discuss potential mechanisms.

Aging and Sleep

Sleep is controlled by an interaction between homeostatic and circadian processes (Achermann and Borbely 2011). The homeostatic processes keep track of the prior waking duration and are reflected in the activity of the slow-waves (slow-wave activity [SWA]) of the non–rapid eye movement (NREM) sleep electroencephalography (EEG). In all mammalian species investigated, SWA increases as a function of prior waking duration and in several species a dose–response relationship has been established between waking duration and subsequent SWA in NREM sleep (reviewed in Deboer 2007). The circadian process is controlled by the SCN and provides the homeostatic process with a circadian framework (Fig. 1B).

Several researchers have found an age-associated decrease in the circadian amplitude of overall rhythms and this includes the amplitude of the sleep–wake behavior. Nighttime sleep is often interrupted by awakenings in the elderly and daytime naps occur during the main waking period (Bliwise 2011). In most humans, sleep latency at sleep onset is not severely affected by age, but the amount of REM sleep in the early night increases and more waking episodes occur in the later part of the night (Bliwise 2011; Dijk and Duffy 1999; Landolt and others 1996). In addition, all these differences between young and elderly are differentially influenced by the circadian phase (Dijk and Duffy 1999). Within NREM sleep, the deeper stage of NREM sleep (slow-wave sleep [SWS]) is reduced (Bliwise 2011; Dijk and Duffy 1999; Landolt and others 1996; Munch and others 2004). This may in part be caused by a decrease in the amplitude of the EEG slow-waves as the definition of SWS depends on the amplitude of the slow-waves in the NREM sleep EEG (Bliwise 2011; Dijk and Duffy 1999; Landolt and Borbely 2001; Landolt and others 1996). Next to a decreases in slow-wave amplitude the amplitude of sleep spindles and K-complexes in the EEG, which are mainly found in the lighter phases of sleep, were also reduced (Bliwise 2011). Remarkably, in humans, many of the changes observed in sleep and the sleep EEG already start before the age of 60 years.

In nocturnal rodents, a decrease in the daily amplitude of sleep and waking is also observed, but this is mainly due to an increase in the amount of sleep during the active period (Colas and others 2005; Farajnia and others 2012;

Hasan and others 2012; Welsh and others 1986), whereas the amount of sleep during the rest period only showed minor changes. Remarkably, REM sleep decreased during the rest phase in the course of aging in some mouse strains, but not all (Colas and others 2005; Farajnia and others 2012; Hasan and others 2012; Welsh others 1986). The latter suggests that this also depends on the genetic background. Compared with humans, EEG changes in aging rodents are less pronounced (Farajnia and others 2012; Hasan and othrrs 2012).

In contrast to clear changes in the circadian timing and overall amount of sleep, the age-dependent changes in the homeostatic response to sleep deprivation are less pronounced. Despite a clear decrease in the amount of high-amplitude slow-waves in NREM sleep in the course of aging in humans (Bliwise 2011; Dijk and Duffy 1999; Landolt and others 1996; Munch and others 2004), the increase in the amount of sleep and SWA during NREM sleep is intact in healthy older humans (Bliwise 2011; Munch and others 2004), and similar findings were obtained in aged rodents (Farajnia and others 2012; Hasan and others 2012).

Several changes in sleep in the course of aging are accompanied by increases in sleep disorder breathing and restless legs (Bliwise 2011). Both result in increased sleep fragmentation and a reduction in deeper NREM sleep stages. Restless legs syndrome is exceedingly common in the elderly population and is characterized by an urge to move the legs. Sleep disorder breathing exists in many forms and in aging is related to age-dependent risk factors such as decreased lung capacity, ventilatory control, muscular endurance, and thyroid function (Bliwise 2011).

Local circadian modulations are present in sleep controlling centers in the brainstem, thalamus, and cortex, and circadian control of these centers is important for daily rhythms in sleep and waking (Saper 2013). The importance of the circadian clock for the timing of sleep has been demonstrated, among others, by the finding that changes in core clock genes *Per1* and *Per2* changes timing and distribution of sleep in mice (Kopp and others 2002; Shiromani and others 2004) and that a mutation in the *PER2* genes in humans results in advance sleep phase disorder (Toh and others 2001). Many clock gene mutant models also develop a sleep phenotype (Deboer 2007). Changes in SCN functioning in the course of aging are therefore likely to influence sleep.

Age-Associated Changes in Circadian Rhythms

Aging is an innate process occurring in almost all multicellular organisms. The impact of age on circadian clock function seems to be comparable between a number of invertebrate (Koh and others 2006) and vertebrate models (Yeoman and others 2012; Zhdanova and others 2011). The most obvious signs of clock aging are found in behavior with reduced amount of activity, which is more often interrupted by rest phases. This low amplitude, fragmented locomotor activity has been reported in humans (Czeisler and others 1992; Oosterman and others 2009), rodents (Weinert and Waterhouse 2007) and nonhuman primates (Zhdanova and others 2011). Our longitudinal study showed a gradual increase in fragmentation from 100 to 900 days of age in mice (Fig. 2A; Farajnia and others 2012). Other changes in behavioral rhythms occur at different lifetimes. Whereas the decrease in activity, the increase in fragmentation, and the lengthening in endogenous period already occurred in adulthood (100 days of age), the increase in duration of rest phase only manifested late in life (700 days of age). These findings are of interest since they indicate that different mechanisms may underlie different aspects of age-related rhythm deterioration. Since locomotor activity can influence SCN function via neuronal feedback loops (Hughes and Piggins 2012) using different neurotransmitters (NPY, serotonin, orexin), the reduction and fragmentation in activity in the course of aging can worsen the age-associated defects in the central clock function, leading to a downward spiral.

As we age, the daily timing of physiological process in our body changes. For instance, the daily decrease in body temperature and the onset of sleep occurs almost 2 hours earlier compared with younger subjects (Czeisler and others 1992). This age-induced phase advance has also been described in a number of model systems such as nonhuman primates (Aujard and others 2006) and rodents (Zee and others 1992.). The gradual increase with age of early chronotypes found in an epidemiological study in humans between 20 and 80 years of age may also be an indicator for phase advanced circadian rhythms in the elderly (Roenneberg and others 2007), since a correlation of chronotype and phase of circadian rhythms has been recently demonstrated (Novakova and others 2013).

The effect of age on the intrinsic period length of the circadian clock seems to depend on species and genetic background. The endogenous circadian period of locomotor activity shortens in old hamsters (Pittendrigh and Daan 1974; Watanabe and others 1995), primates (Aujard and others 2006) and rats (van Gool and others 1987), whereas the period lengthens with age in inbred mice (Farajnia and others 2012; McAuley and others 2002). The best controlled study performed in humans to date did not find a significant change in intrinsic period of the circadian rhythms with average values of 24.18 hours in young and old subjects (Czeisler and others 1999). Recent evidence supports the old idea of resonance between intrinsic period and 24-hour rhythm in the environment

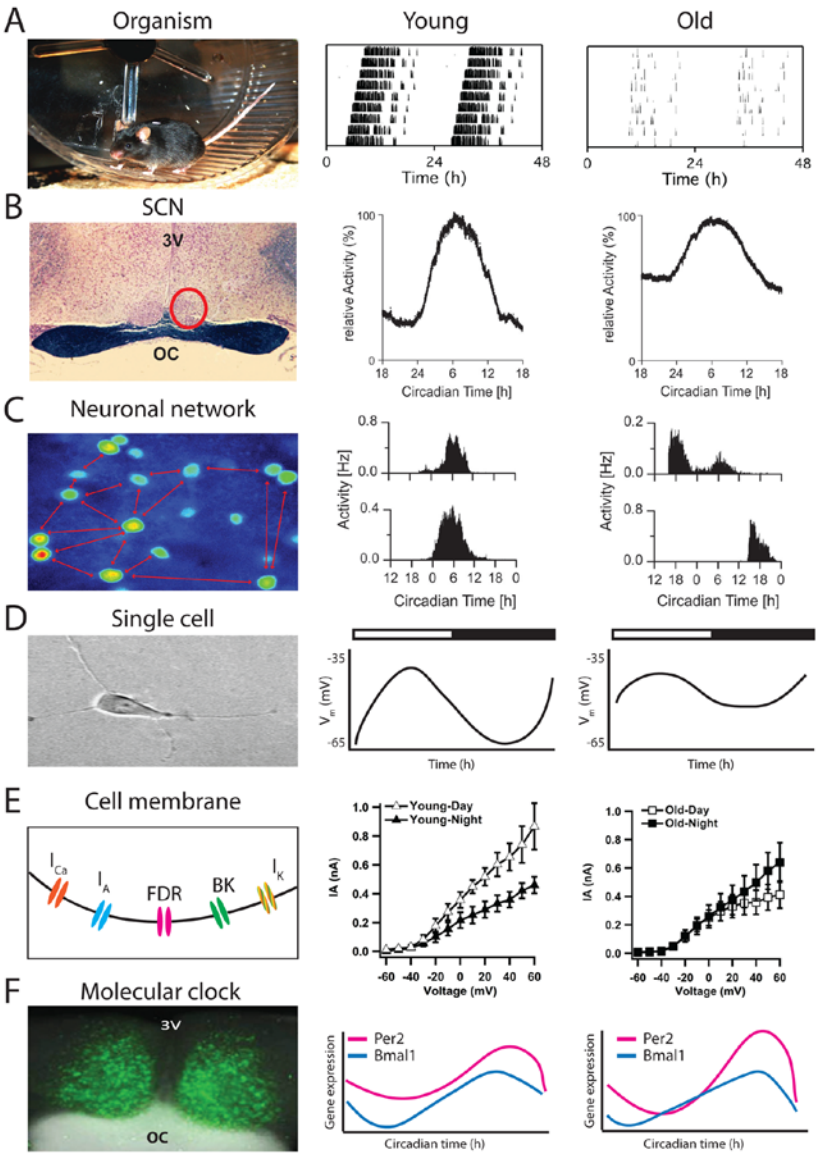


Figure 2. Impact of aging on different levels of organization. (A) The amount of activity is reduced as aging progresses. Double plotted wheel-running activity of a young (middle) and old animal (right) are shown. C57/bl6 mouse in running wheel is shown on the left. (B) The amplitude of the electrical activity rhythm in the aged suprachiasmatic nucleus (SCN) (right) is reduced by about 50% compared with young SCN (middle). Nissl-stained hypothalamic slice containing the SCN (circle) is shown on the left. (C) Aging changes neuronal network properties in the SCN. The distribution of the neuronal activity of subpopulations is more dispersed in old SCN (right) compared with recordings form young SCN slices (middle). The left panel shows an image of SCN neurons in a slice loaded with the Ca^{2+} indicator dye Fura-2 as one method to test network interactions (red lines symbolize putative connections). (D) Single cell properties are modified in aging. For instance, the resting membrane potential (V_m) recorded from neurons of aged SCN (right) does not reach the hyperpolarized night values as seen in young animals (middle). The white and black bars on top of the graphs represent the day and night, respectively, of the light regime prior to the recording. A photograph of a single SCN neuron in culture is shown on the left. (E) Several circadian controlled ionic conductances are modified in aging. A-type transient K^+ currents (I_A) that lose their rhythmicity in aging have been shown as an example (right). A sketch of some of the known clock-controlled channels is depicted on the left. (F) Clock genes and their related proteins are down regulated (*Bmal1*) or up regulated (*Per2*) by aging. Image of bioluminescence recording of *Per2* expression in organotypic SCN slices (left). Sketch of circadian rhythms in *Per2* and *Bmal1* demonstrate age-associated changes in gene expression. 3V = third ventricle, OC = optic chiasm. Data in A, B, C, and E adapted from Farajnia and others (2012).

(Wyse and others 2010). Hybrid mice (a cross between two inbred mouse strains) with intrinsic period close to 24 hours had a significantly increased longevity compared with littermates with longer or shorter periods (Libert and others 2012). A transgenic mouse model with increased life span compared to the wild-type littermates, showed an intrinsic period closer to 24 hours compared with wild-type. This period did not show alterations in the course of aging (Gutman and others 2011). Decreased caloric uptake in this model complicates the interpretation of the results, but the combined evidence of the studies support the idea that a stable period close to 24 hours throughout life will increase longevity. For humans this implies that we are fairly close to an optimum with an age-independent period of close to 24 hours (Czeisler and others 1999). The lesson to be learned from animal models, however, is that any clock disturbances that will alter our circadian period (e.g., medications) could have detrimental influence on our health and life span.

For the SCN to function as a reliable chronometer predicting time for the organism, it needs to be synchronized (or entrained) to the environmental cycles. The strongest time cue or zeitgeber in the environment is the daily changes of light and darkness. This light information is processed and relayed to the SCN by photosensitive retinal ganglion cells using glutamate and pituitary adenylate cyclase–activating peptide (PACAP) as neurotransmitters (Hannibal and others 2002). The retinorecipient SCN neurons are activated by light depolarizing membrane potential and increasing action potential frequency. This activation leads to an increase of intracellular calcium concentration that can influence the expression of genes involved in the molecular machinery of rhythm generation causing a phase shift of the rhythm (Antle and others 2009). The phase-shifting capacity of light is restricted to the night time and corrects deviations of the phase of the internal clock from the phase of the external light–dark cycle. The ability to synchronize and reset phase is hampered in aged humans (Klerman and others 2001) and rodents (Benloucif and others 1997; Farajnia and others 2012; Valentinuzzi and others 1997; Zhang and others 1996) as shown for behavioral, electrical, and molecular rhythms in the SCN. Aging can influence the resetting capacity of the clock at many levels from the light perception in the eye to the molecular level in SCN neurons. The light intensity required to achieve comparable entrainment can be 10 times (1 log unit) higher in the elderly compared with young subjects (Turner and Mainster 2008), which is similar in nonhuman primates (Gomez and others 2012). Age-dependent reduction of light transmission of lens and pupil alone can amount to 90% in the elderly (Turner and Mainster 2008). In rodents, an aging induced decline in photosensitive ganglion cells seems to account for most of the reduction of circadian photoreception in the eye (Lupi and others 2012). Evidence of age-associated changes in SCN glutamate receptor function (Biello 2009) and modulation (Duncan and others 2000) points to a major contribution of the SCN to age-related deficit in entrainment. Recently, it was suggested that the observed reduction in phase shifting capacity in the old clock is in part due to a modified neuronal network with a wider distribution of phases of single neurons activity pattern (Meijer and others 2012). The latter seems to contribute to low amplitude rhythms and reduction in light-induced phase response as will be discussed below.

Impact of Age on Suprachiasmatic Nucleus Function

The bilaterally organized nuclei of the SCN consist of a neuronal network of about 20,000 cells generating a circadian rhythm in electrical activity which peaks in the middle of the day. Molecular (Dibner and others 2010) and cytosolic clocks (O'Neill and Reddy 2012) supposedly interact to accomplish a circadian modulation in cell physiology and neuronal excitability. These intracellular clocks control a variety of ionic conductances, which then affect neuronal activity (Fig. 3; Brown and Piggins 2007). The result is a depolarization of the membrane potential and an increase in action potential frequency during the day, which lasts about 4 hours in most SCN neurons (Vanderleest and others 2007). One major tasks of the neuronal network of the SCN is the arrangement of phases of these single unit electrical patterns to construct an ensemble waveform that unambiguously encodes dawn, dusk, and day-length (Vanderleest and others 2007). The mechanisms of phase (re-)setting and synchronization within the SCN network are not fully understood yet, but the neurotransmitters vasoactive intestinal peptide (VIP; Colwell and others 2003) and GABA (Albus and others 2005) play an important role.

Given the central role of the SCN in the circadian control of behavior and physiology, it can be expected that many age-related deficits in the circadian system are based on altered physiology and function within this central clock. Indeed, implantation of fetal SCN tissue in old animals partially restored circadian function in hamsters (Van Reeth and others 1994) and rats (Cai and others 1997) and can even lead to an increase in longevity in hamsters (Hurd and Ralph 1998).

Amplitude of the Suprachiasmatic Nucleus Rhythms

One of the hallmark features of the aged clock is the reduction in amplitude of the circadian rhythms in physiology and behavior, as mentioned above. Recordings of the electrical activity rhythm in the aged SCN of mice found a significant reduction of amplitude of this important output

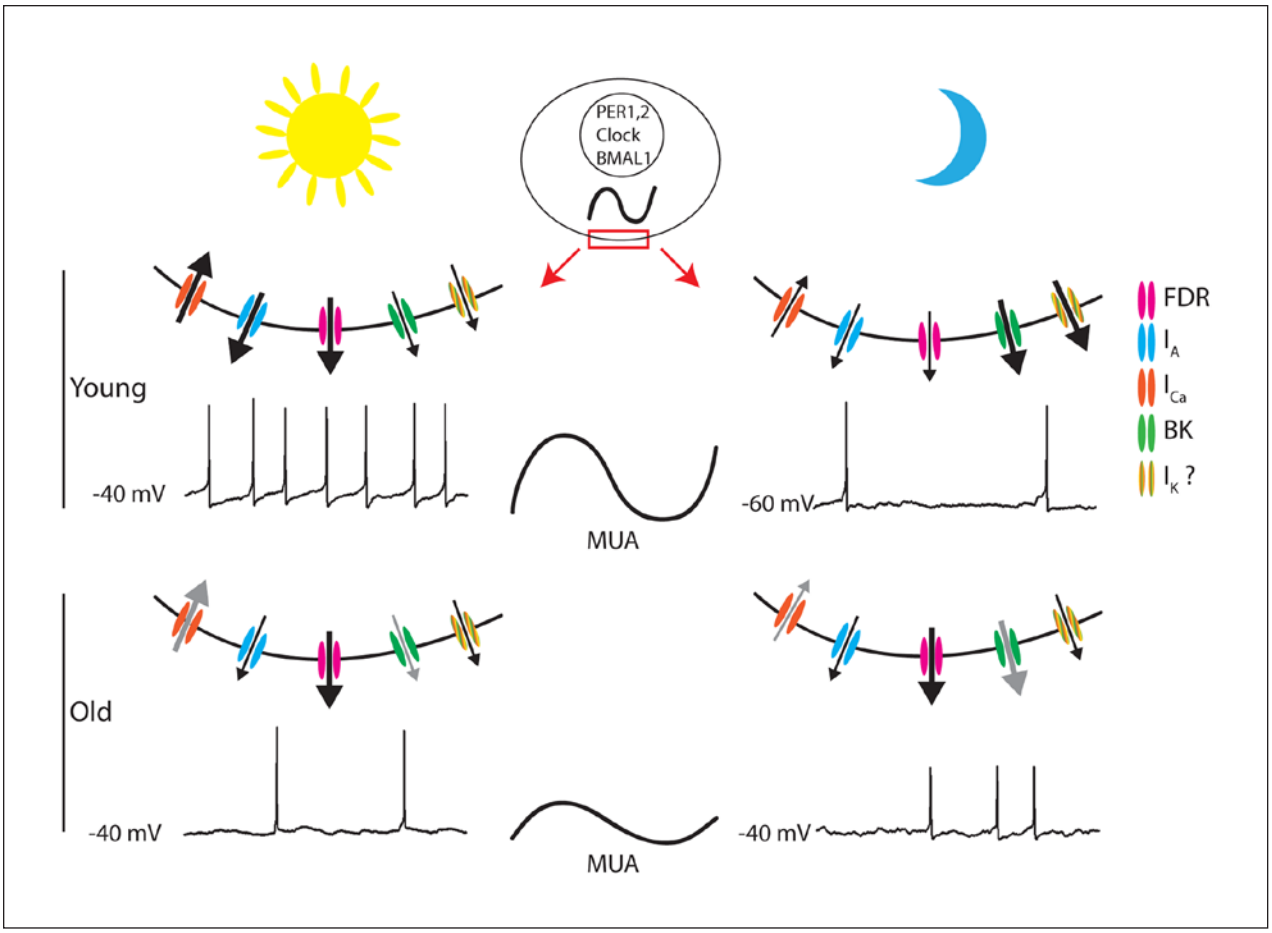


Figure 3. Age-related changes in circadian controlled ionic currents in the suprachiasmatic nucleus (SCN). The clock-controlled fast delayed rectifier (FDR), A-type K^+ currents (I_A), large conductance Ca^{2+} activated K^+ currents (BK) and voltage-dependent Ca^{2+} currents (I_{Ca}) modulate membrane excitability in a circadian manner. In young mice, this results in a higher frequency of action potential during the day compared to the night (upper panels). A yet to be identified K^+ current (I_K) contributes to regulation of membrane potential, which is more depolarized during the day as compared with the night. In the course of aging (lower panels), membrane potential does not reach the hyperpolarized night values found in young animal. In addition, the circadian modulation of I_A and FDR currents is diminished, leading to a blunted rhythm in electrical activity. Consequently the amplitude of the multiunit activity (MUA) rhythm of the SCN is reduced in aged animals. The thickness of the arrows illustrates the magnitude of the current that passes through channels (gray arrows mark conductances yet to be studied in aged SCN).

signal by about 50% (Fig. 2B; Farajnia and others 2012; Nakamura and others 2011). Obviously, one potential explanation for the reduction in amplitude of the output signal could be a loss of SCN neurons due to age-related neurodegeneration. However, most studies found no significant difference in the overall cell count within the SCN of aged mice (Miller and others 1989) or rats (Roozendaal and others 1987), compared with young animals. Aging does lead to a decrease in subpopulations of SCN neurons expressing VIP in rats (Chee and others 1988) and humans (Zhou and others 1995) and arginine vasopressin in rats (Roozendaal and others 1987). The loss of arginine vasopressin neurons in the SCN of humans was also described but only occurs from an age of 80 years or older (Swaab and others 1985). These findings indicate that the reduction in SCN output may be caused by age-dependent alterations in specific neurotransmitter signaling.

The age-related decrease in amplitude of the circadian clock output may to a great extent be the result of reduced synchronization between SCN neurons as a result of changes in neurotransmitter signaling (Box 1). The electrical activity patterns of SCN neurons usually cluster around midday in young mice. SCN multiunit activity recording in old mice revealed a wider phase distribution and also an additional cluster of cells active during the night, in antiphase (180°) to the main group during the day (Farajnia and others 2012). This results in a reduction in the amplitude of the 24-hour rhythm and an increase in neuronal activity during the night, the phase of the circadian cycle where electrical activity should be low.

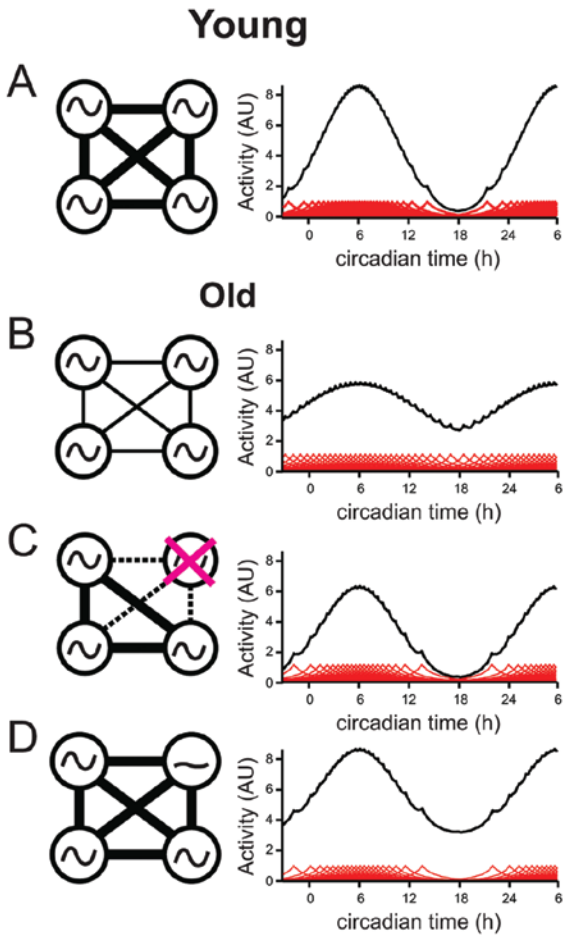
Box 1. Modeling the suprachiasmatic nucleus neuronal network in aging.

Most, if not all the 20 000 neurons in the SCN exhibit an endogenously rhythmic firing pattern with a period of approximately 24 hours (Honma and others 1998). Recordings from SCN cells in culture have shown the autonomous nature of rhythm generation (Welsh and others 1995). When dispersed in low density the largely uncoupled individual cells show high variability in their endogenous periods, whereas communication among neurons within the SCN neuronal network reduces this variability (Herzog and others 2004). This indicates that phase synchrony is enhanced by intercellular coupling, leading to a reinforcement of rhythm amplitude at the population level (Welsh and others 2010).

The SCN has the ability to synchronize or entrain its endogenous rhythm to the external 24-hour light–dark cycle. When entrained, the SCN produces a rhythmic pattern in electrical activity with a peak at midday and a trough at midnight (Brown and Piggins 2007). This sinusoidal-shaped activity pattern of the ensemble differs from the much shorter activity pattern of the single cells and is explainable by a specific phase distribution of the individual neuronal activity patterns. Mathematical modeling studies have indicated the significance of the phase distribution (Rohling and others 2006). If the firing patterns of all cells would be evenly distributed in phase over the 24-hour period, no overall SCN rhythm would be present. Only when more cells are active during midday than during midnight and when the cells are not fully synchronized, a rhythmic sinusoidal-like pattern can emerge at the population level (Rohling and others 2006; Vanderleest and others 2007). These conditions reflect the situation in young mammals that show robust, high-amplitude rhythms (panel A). In the figure, each circle represents a neuron and the symbol inside the circle denotes whether the cell is rhythmic or not. Each line between two circles represents a connection between the neurons. The thickness of the lines represents the strength of the connection. In the right panel, the corresponding activity patterns of the individual cells (in red) and of the population (in black) is shown. The phases of the single neuron activity patterns follow a Gaussian distribution. For young mammals, the network is intact and all cells express a circadian rhythm.

The degree of synchronization of the cellular oscillators is not static, but flexible. A change of environmental lighting conditions, such as a change in photoperiod, causes the network to reorganize its phase synchronization (Johnston 2005; Rohling and others 2006; Schaap and others 2003; Vanderleest and others 2007). Also, in aged animals, changes in synchronization occur. At the population level, the aged SCN shows a broad, low-amplitude peak in electrical activity (Farajnia and others 2012; Nakamura and others 2011). As compared with the higher amplitude rhythms for young animals, the network of aged animals is less synchronized.

Three different mechanisms for aging can be explored with computational approaches (methods described in Rohling and others 2006). The first mechanism that may play a role in aging is a weakening of the connections between the neurons (panel B). This leads to a more desynchronized phase distribution of neurons, resulting in a wide SCN activity pattern with low amplitude. The second mechanism is cell loss (panel C), which will result in a small decrease in rhythm amplitude, even if the connections between the remaining cells remain intact. Finally, it is possible that single cells may lose their rhythms. These cells will consequently have a constant activity level throughout the 24-hour cycle, resulting in a higher trough of the SCN rhythm (panel D). Possibly, all three mechanisms occur with aging, resulting in a wider activity pattern with lower amplitude and higher trough compared with the pattern in young animals.



The neurotransmitters VIP and GABA, which contribute to the synchronization within the SCN neuronal network, are both affected by aging. The expression level and the amplitude of circadian modulation of VIP mRNA (Kawakami and others 1997) and VPAC2 receptor mRNA (Kallo and others 2004) were reduced in the course of aging. The number of GABAergic synaptic terminals in the SCN are diminished by 26% in old mice compared with values found in young adults (Palomba and others 2008). Patch-clamp recordings in hypothalamic slices showed that GABAergic postsynaptic currents are reduced in frequency (Nygard and others 2005) and amplitude (Fig. 2C; Farajnia and others 2012) in SCN neurons of old mice. The age-associated decline in functionality of both VIP and GABAergic signaling could account for a loss in phase coherence within the old SCN neuronal network with the subsequent reduction in amplitude of the output signal.

Phase Resetting

The effect of aging on the state of the SCN network may also explain part of the age-associated deficits in resetting and phase-shifting capacity of the circadian clock. One analogue example is the photoperiod-induced phase dispersion described in the SCN of animals exposed to a long-day photoperiod. Because of the phase dispersal, a light stimulus will reach the cells at different phases of their cycle. It is expected therefore that the phase shifting effect of light on the different cells will be diverse, and will lead to reduction in phase response of the population as a whole when compared to a more synchronized network. Indeed, phase shifting response is diminished in animals exposed to long summer days and simulations confirmed that the degree of desynchronization among the neurons correlates with the phase shifting capacity of the ensemble (Vanderleest and others 2009).

In addition, neurotransmitters and neuromodulators more directly involved in photic information processing are also affected by age. The expression of PAC-1 mRNA, a receptor mediating the PACAP-induced enhancement of glutamatergic signaling in retinohypothalamic terminals of the SCN, is reduced in aged rats (Kallo and others 2004). Transporters and receptors of serotonin, which suppress the photic transmission in the SCN, are enhanced in the course of aging (Duncan and others 2000). And finally, mRNA expression of gastrin releasing peptide, which also modulates light-induced phase shifts of the circadian clock, is reduced in SCN of aged hamsters (Duncan and others 2010). Thus, age-associated deficits in phase resetting and entrainment of the circadian clock seem to involve SCN network modifications as well as alterations in photic information processing.

Cellular Physiology

Cell death is seemingly not the primary reason for the reduction in the amplitude of the SCN output signal and changes in neurotransmitter signaling effecting network properties are more likely contributors. The physiology of cells in the SCN on the other hand is also modified by aging and may play a role in age-related deficits in clock function (Aujard and others 2001; Satinoff and others 1993). Electrophysiological recordings in brain slice of old mice revealed an increased fraction of silent cells (Nygard and others 2005) and a decrease in excitability of SCN neurons (Farajnia and others 2012) during the day. The regulation of action potential frequency in SCN neurons is controlled by a number of ionic channels, among them two circadian controlled K^+ conductances, which we recently studied in old mice (Fig. 3; Farajnia and others 2012). The fast delayed rectifier K^+ current (Itri and others 2005) and also the transient K^+ current (Itri and others 2010) lost their circadian modulation in old SCN neurons, which is consistent with the diminished rhythm in neuronal activity (Fig. 2E). Importantly, not all ionic currents were affected by age. The slow delayed rectifier current was not altered in activation or current amplitude in old SCN neurons. The circadian rhythm in membrane potential, normally being depolarized during the day and hyperpolarized during the night, was also compromised in aged SCN neurons exhibiting depolarized night values (Fig. 2D; Farajnia and others 2012). The underlying conductance, regulating membrane potential in the SCN neuron, is not identified yet.

The decrease of the daily peak of the electrical rhythm observed in the single cell recordings and the increase of the trough caused by neurons active at the "wrong" phase at night, as found in subpopulation recordings, result in the dampened amplitude observed in the whole SCN ensemble. In general, the age-induced dampening of circadian rhythms of neuronal activity was greater on the cellular level compared to the whole SCN in vitro (Farajnia and others 2012) or in vivo (Nakamura and others 2011) suggesting a compensatory role of the SCN network.

Putative Mechanisms of Age-Associated Suprachiasmatic Nucleus Dysfunction

Aging affects SCN function mainly by altering synaptic transmission and network properties as well as modifying cellular physiology (Fig. 4). At the level of rhythm generation, elements of the molecular feedback loop seem to be sensitive to aging although the few reports on this issue have conflicting results. Some studies found robust rhythms in the expression of *Period* genes in old SCN

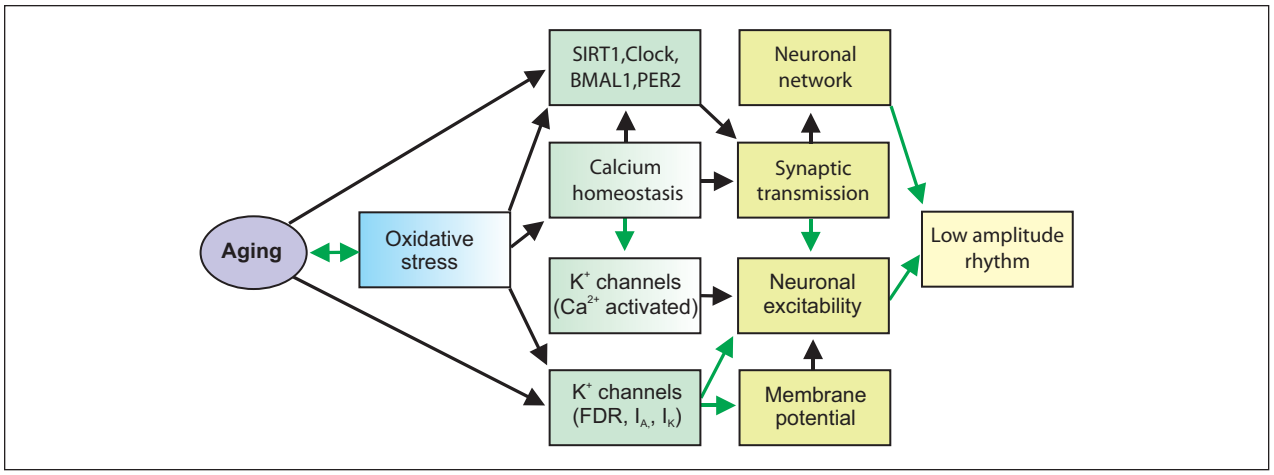


Figure 4. Hypothetical model for mechanism involved in aging-related decline in central circadian signal and function. Aging can lead to an increase in oxidative stress and cause modifications of K^+ channel activity, Ca^{2+}-homeostasis and gene expression in the suprachiasmatic nucleus (SCN). Other, more direct influence of aging on cellular components is also possible. The change in molecular expression and membrane properties lead to changes in excitability, synaptic activity and network modifications. The weaker network interactions and the reduced excitability result in decline of the circadian timing signal issued by the SCN. Light-colored boxes indicate weak experimental evidence. Arrows show possible interactions (green = based on experimental results).

neurons (Asai and others 2001), while other studies report age-related disruption in *Per2* mRNA (Weinert and others 2001) as well as *Clock* and *Bmal1* mRNA (Fig. 2F; Kolker and others 2003; Wyse and Coogan 2010). A recent study identified a reduction of sirtuins (SIRT1) expression together with a reduction in BMAL1 and CLOCK proteins in the SCN of old mice (Chang and Guarente 2013). SIRT1 activates the transcription of *Clock* and *Bmal1* and SIRT1 deficient young mice showed age-typical changes in circadian behavior such as period lengthening, fragmented activity, and reduced entrainment capacity.

Among the intensely discussed potential causes of aging-related neuronal dysfunction is the oxidative stress and the increase of endogenous reactive oxygen/nitrogen species (ROS) in the aged brain. ROS is still considered as one of the factors that may inflict structural damage on various macromolecules and consequently on neuronal interactions and cellular properties (Dirksen 2002; but see Yeoman and others 2012). Among the targets for ROS are plasma membrane ionic channels and transporters, which are critical to preserve normal physiological cellular functions (Annunziato and others 2002). Oxidation of K^+ channels by ROS, for instance, has been shown as a major cause for loss of neuronal function (Sesti and others 2010) could also underlie the age-associated change in K^+ channel activity observed in SCN neurons. Another interesting influence of increased oxidative stress in aging involves the modification of intracellular Ca^{2+} homeostasis in neurons. In hippocampal cells, aging leads to increased Ca^{2+} entry through voltage-dependent channels and changes in ryanodin receptor–regulated Ca^{2+} stores lead to a chronic increase in baseline cytosolic Ca^{2+} concentration (Foster 2007). One of the consequences is a decrease in excitability by increased activity of Ca^{2+}-activated K^+ currents. SCN neurons have similar mechanisms of Ca^{2+} regulation and the role of Ca^{2+}-activated K^+ currents for excitability and circadian rhythms have been well documented. Whether these components are also affected by aging in the SCN is an intriguing question for future research.

Conclusions and Future Directions

Healthy aging is a natural but complex phenomenon that involves different levels within the organism. The age-related changes in the circadian system lead to reduction of the amplitude, phase changes, as well as fragmentation in rhythms of behavior and physiology. The decay of the daily control of processes represents a risk factor for a number of diseases and can also aggravate symptoms of age-associated neurodegenerative diseases. The decline in amplitude of circadian rhythms can be traced back to decline in output signal of the central clock and further to age-induced alterations in neuronal network function, membrane properties and molecular components of circadian pacemaker cells in the SCN. The understanding of the causal connections between different parts of the circadian system affected by age and the knowledge of cellular and molecular targets may eventually help design new treatments aimed to alleviate age- and clock-related diseases. The challenges for future

research are to identify mechanisms of neuronal aging that go beyond today's hypothesis of cell death and oxidative stress. Neuronal network remodeling, changes in Ca^{2+} homeostasis, the role of senescent cells (Naylor and others 2013) and alterations in metabolic pathways (e.g., insulin/insulin-like growth factor signaling) are just a few directions worth following. Finally, the search for better animal models for healthy aging and age-associated neurodegenerative diseases needs to continue to facilitate research on the aged brain.

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