Changes in the suprachiasmatic nucleus during aging: implications for biological rhythms

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Abstract

Animals have neural structures that allow them to anticipate environmental changes and then regulate physiological and behavioral functions in response to these alterations. The suprachiasmatic nucleus of the hypothalamus (SCN) is the main circadian pacemaker in many mammalian species. This structure synchronizes the biological rhythm based on photic information that is transmitted to the SCN through the retinohypothalamic tract. The aging process changes the structural complexity of the nervous system, from individual nerve cells to global changes, including the atrophy of total gray matter. Aged animals show internal time disruptions caused by morphological and neurochemical changes in SCN components. The effects of aging on circadian rhythm range from effects on simple physiological functions to effects on complex cognitive performance, including many psychiatric disorders that influence the well-being of the elderly. In this review, we summarize the effects of aging on morphological, neurochemical, and circadian rhythmic functions coordinated by the main circadian pacemaker, the SCN.
Keywords: suprachiasmatic nucleus, aging, morphological and neurochemical changes, circadian rhythms.

The suprachiasmatic nucleus

Environmental cues strongly influence living organisms, and the light/dark cycle is considered the most influential aspect of the regulation of rhythmic behavior (Dibner, Schibler & Albrecht, 2010). In mammals, periodic changes in the environment are recognized by an endogenous circadian clock. During evolution, organisms have developed numerous strategies to maintain an appropriate internal temporal order in response to alterations in the ecological niche (Hut & Beersma, 2011). Animals have neural structures that allow them to anticipate information about cyclical environmental changes and optimally adapt to new environmental conditions (Figure 1); thus, these characteristics have implications for the survival of the species (Ukai & Ueda, 2010; Menna-Barreto & Díez-Noguera, 2011). In mammals, these structures are part of the circadian timing system (CTS; Cavalcante, Nascimento Júnior & Costa, 2006), which includes a set of distinct structures that consist of a central pacemaker and afferent and efferent pathways. These structures are connected to behavioral effectors that are involved in generating and modulating biological rhythms (Dibner *et al.*, 2010; Golombek & Rosenstein, 2010; Figure 1).

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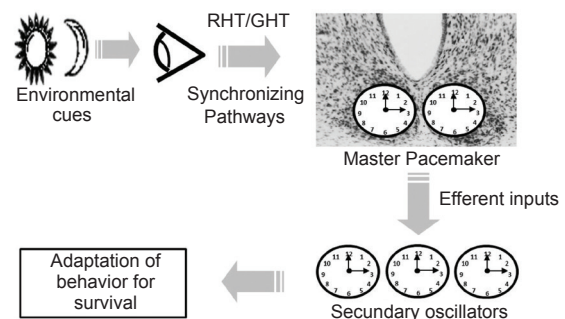

Figure 1. The circadian timing system. The control and adaptation of the biological mechanisms executed by the circadian timing system provide adequate adjustment to behavioral elaboration, thus enhancing the survival of animals.

Among the components of this system, since the 1970s, the suprachiasmatic nucleus (SCN) of the hypothalamus has been considered the mammalian central circadian pacemaker (Hendrickson, Wagoner & Cowan, 1972; Moore & Lenn, 1972). Damage to the SCN results in damage to the circadian rhythms of several behavioral and physiological parameters (Moore & Eicher, 1972). Fetal SCN transplants allow the recovery of circadian rhythms in animals that lost them as a consequence of bilateral injury of the SCN (Lehman *et al.*, 1987; Ralph, Foster, Davis & Menaker, 1990). Additionally, the rhythms that recovered in the animals that received the transplant had characteristics of the donor rather than the host (Ralph *et al.*, 1990).

Although the SCN displays variations with regard to tridimensional shape, volume, density, and cell size in all species studied (Figure 2), morphologically in mice, each unilateral SCN has approximately 10,000 neurons divided into two main neuronal populations: ventral or "core" (a producer of vasoactive intestinal polypeptide [VIP]) and dorsal or "shell" (which has vasopressin [VP] as its main neurotransmitter; Abrahamson & Moore, 2001; Morin, 2007; Mammen & Jagota, 2011). Approximately 1,100 neurons (~10%) in the unilateral SCN express VIP in the core subdivision. Approximately 2,100 neurons (~20%) in the shell express VP (Welsh, Takahashi & Kay, 2010). In rodents, the levels of VP and VIP show specific daily oscillations under light/dark conditions, suggesting that they act on the rhythm of behavioral expression (Dardente *et al.*, 2004).

The light/dark cycle is considered the strongest synchronizer of circadian rhythms (Morin & Allen, 2006; Xu, Gu, Pumir, Garnier & Liu, 2012). Photic information is transmitted to the SCN through the retinohypothalamic tract (RHT), which originates from melanopsin-containing retinal ganglion cells. These cells project bilaterally to the SCN (Hendrickson *et al.*, 1972; Moore & Lenn, 1972) and geniculohypothalamic tract (GHT), which originates in the intergeniculate leaflet and, similar to RHT, terminate at the ventrolateral portion of the SCN. A third afferent pathway to the SCN is a serotonergic projection that comes from raphe mesencephalic nuclei, mainly from the median raphe nucleus (Hay-Schmidt, Vrang, Larsen & Mikkelsen, 2003; Pontes *et al.*, 2010). Interestingly, some findings suggest that serotonin (5-hydroxytryptamine [5-HT]) should modulate the photic entrance to the SCN by controlling the release of glutamate from the RHT (Morin, 1999; Figure 1).

Other neurotransmitters, in addition to VIP and VP, have been described in terminals and perikarya in the SCN, such as neuropeptide Y (NPY; Costa *et al.,* 1998; Abrahamson & Moore, 2001; Cavalcante, Alves, Costa, & Britto, 2002; Pinato, Frazão, Cruz-Rizollo, Cavalcante & Nogueira, 2009), 5-HT (Abrahamson & Moore, 2001; Cavalcante *et al.,* 2002; Pontes *et al.*, 2010), glutamate (GLU; Van den Pol, 1991; de Vries, Cardozo, van der Want, Wolf & Meijer, 1993), and γ-aminobutyric acid (GABA; Castel & Morris, 2000; Abrahamson & Moore, 2001).

The circadian pacemaker in the SCN is composed of multiple oscillator cells with individual electrical activity rhythms that become synchronized by synaptic coupling (Nakamura, Honma, Shirakawa & Honma, 2001). This was initially observed in dispersed cultures of SCN neurons (Welsh, Logothetis, Meister & Reppert, 1995) and later demonstrated in slice preparations (Nakamura *et al.*, 2001). In the SCN, groups of cells constitute several internally coupled oscillatory subsets that can differ in resetting mechanisms because of photic and non-photic time cues (Silver & Schwartz, 2005). Studies have shown a functional subdivision within the SCN. In fact, the left and right sides of the SCN may behave differently in different experimental protocols, and they can lead to the temporal splitting of rhythmic output functions, such as locomotor activity (de la Iglesia, Meyer, Carpino & Schwartz, 2000). In another forced desynchronization protocol, differences between the ventrolateral and dorsomedial SCN zones have been observed in rats (de la Iglesia, Cambras, Schwartz & Díez-Noguera, 2004), providing a novel approach to understanding the tissue organization of the SCN.
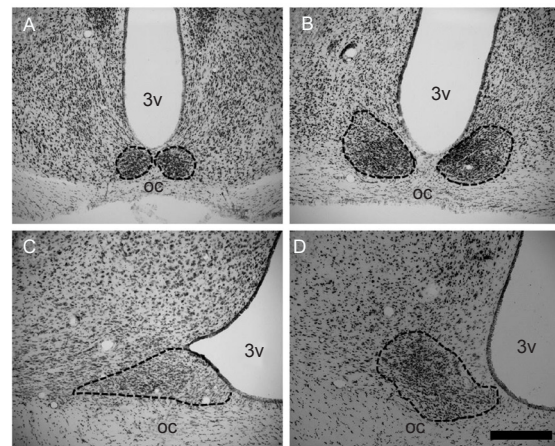

Figure 2. The suprachiasmatic nucleus. Digital images of coronal sections of Nissl staining of the suprachiasmatic nucleus that show morphological differences in four species: mouse (A), rat (B), marmoset (C), and cebus (D). 3v, third ventricle; oc, optic chiasm. Scale bar = 300 μm in A and B; 150 μm in C and D. The digital images were obtained in the Neuroanatomy Laboratory, Federal University of Rio Grande do Norte.

Aging

In human, aging is considered a multidimensional process, in which environmental factors may intensify or provide protection from natural degeneration (Schmidt, Peigneux, & Cajochen, 2012). The large variations during an organism's lifetime are mainly influenced by environmental factors, whereas the genetic contribution is approximately 25-30%. Over the years, the behavioral and biological manipulations have resulted in considerable increases in the human life expectancy (Deelen, Beekman, Capri, Franceschi & Slagboom, 2013).

Many hypotheses have attempted to explain the mechanism of aging. A possible way to explain the aging

process suggests an important role for mitochondrial dysfunction, which contributes to the increase in free radical species (Bishop, Lu & Yanker, 2010). In humans, a reduction of protein expression caused by the dysfunction of mitochondrial energy metabolism contributes to the cognitive decline that is typical of old age and Alzheimer's disease (Miller, Oldham & Geschwind, 2008). Molecular biology studies have suggested that this decrease in mitochondrial gene expression is a conserved characteristic in older organisms, from *Caenorhabditis elegans* to humans, in which the nervous system and muscles are particularly more susceptible to functional mitochondrial deficits (Bishop *et al.*, 2010). Together with mitochondrial dysfunction, oxidative stress also appears to be a strong candidate that promotes alterations in older organisms (Muller, Lustgarten, Jang, Richardson & Van Remmen, 2007). Park *et al.* (2009) reported that diets rich in antioxidants may decrease the expression of many genes related to the aging process in the mouse brain (Park *et al.*, 2009), and it may minimize cognitive decline in older rats (Liu *et al.*, 2002).

When focusing specifically on the effects of aging on the central nervous system (CNS), many morphological and neurochemical alterations during life result in several behavioral changes, such as cognitive dysfunction (Fjell & Walhovd, 2010). Aging alters the structural complexity of the CNS, resulting in decreased soma size, decreased dendrites and dendritic spines in individual neurons (Duan *et al.*, 2003), alterations in neurotransmitter receptors (Hof & Morrison, 2004), and changes in electrophysiological properties (Chang, Rosene, Killiany, Mangiamele & Luebke, 2005). Other general changes are also reported in the CNS, including substantial atrophy of total gray matter (Oh, Madison, Velleneuve, Markley & Jagust, 2013), and activation of the ipsilateral and contralateral prefrontal cortex appears to be activated in young individuals (Persson *et al.*, 2004), suggesting a compensatory mechanism of the CNS in response to the aging process (Figure 3).
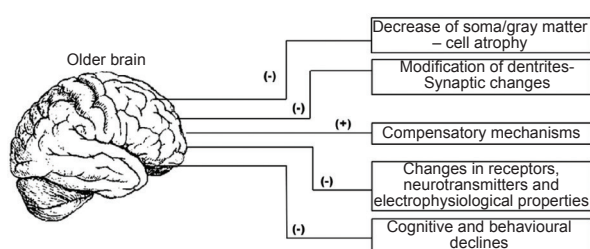
Many circuits can be interrupted by a decrease in synapses and cellular death during aging, but some areas of the CNS have differences in their vulnerability to neurodegeneration (Tomasy & Volkow, 2012). Once many CNS areas suffer from the effects of aging, some components of the CTS may change structurally and neurochemically, thus contributing to behavioral alterations. This idea is supported by data that show changes in synchronization parameters caused by photic and non-photic stimuli in elderly animals (Duncan, 2006).

The aging suprachiasmatic nucleus

The SCN allows the organism to have an internal temporal order, thus contributing to the adequate execution of physiological and behavioral mechanisms. However, when the circadian clock in young animals is not synchronized with the environment, homeostasis is disturbed and displays some functions similar to those observed in aged animals, such as a decrease in time precision (Zhdanova *et al.*, 2011; Davidson, Yamazaki, Arble, Menaker & Block, 2008).

Interestingly, longevity appears to be correlated with the adequate circadian rhythm pattern exhibited by the organism. The transplantation of tissue that contained neonatal SCN tissue in old hamsters increased life span, thus restoring normal rhythms in many physiological mechanisms (Hurd & Ralph, 1998). Additionally, the circadian rhythm in old mice was reported to be improved after they received a transplant of fetal tissues that contained the SCN in the third ventricle (Li & Satinoff, 1998).

The effects of aging on circadian rhythm range from effects on simple physiological functions to effects on complex cognitive performance (Antoniadis, Ko, Ralph & McDonald, 2000). Considering that aging affects biological functions regulated by the SCN, the SCN could be a primary locus for changes related to the aging process (Yamazaki *et al.*, 2002; Figure 4).
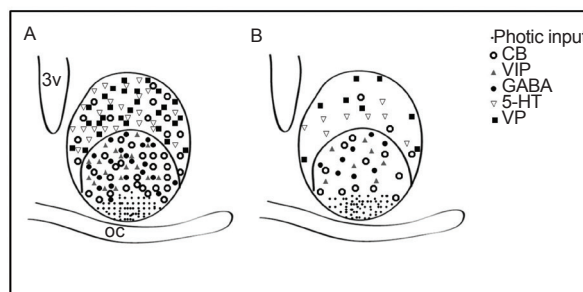

Figure 3. Alterations in the older brain. Several changes that are observed in the nervous system of elderly animals are deleterious (-) and result in physiological and behavioral alterations, thus impairing survival or welfare during aging. Mechanisms may have been developed by the CNS to mitigate these deleterious effects (e.g., bilateral activation in elderly animals of some areas during the execution of specific tasks, thus exhibiting compensatory mechanisms [+]).

Figure 4. The aging suprachiasmatic nucleus. Many morphological and neurochemical alterations may be seen in the SCN in aged animals (B) compared with young animals (A). These changes result in a decline of biological behaviors controlled by the central circadian pacemaker, such as fragmentation on the sleep-wake cycle in elderly humans. Some neurochemical compounds decrease in the SCN in aged animals compared with young animals, such as VIP, VP, GABA, 5-HT, and CB. 3v, third ventricle; oc, optic chiasm.

Morphological modifications in the aged suprachiasmatic nucleus

Zhang et al. (1998) reported that old animals are less sensitive to the synchronizing effects of the light/dark cycle than young animals. However, these authors observed that the RHT pathway was morphologically unaltered in old animals, thus suggesting that these synchronization deficits could be attributable to alterations in the retina or SCN (Figure 4). Little information is available about morphological alterations in the eyes as a possible site of the effects of aging that may result in circadian changes.

Some studies have reported an abrupt decrease in the absorption of blue light within the visible spectrum in the retina in humans who were over 60 years old (Dillon, Zheng, Merriam & Gaillard, 2004) and a small decrease in melanopsin-containing retinal ganglion cells (Kessel, Lundeman, Herbst, Andersen & Larsen, 2010). Some consequences of the changes in photoreception on the circadian rhythm have been reported. Healthy elderly individuals without cataracts exhibit a decrease in phase-delay responses to moderate light, but they did not differ from young individuals with regard to night exposure to a monochromatic and polychromatic white light (Duffy, Zeitzer & Czeisler, 2007; Münch et al., 2011). Molecular studies of aging in the SCN have found a significant decrease in c-*fos* expression induced by light in mice, rats, and hamsters (Zhang et al., 1998) and a decrease in *Per1* expression in mice (Kolker et al., 2003). Biello (2009) suggested that molecular changes in response to light stimuli may occur because of the decreased action of GLU on NMDA receptors during senescence.

Alterations related to aging cannot be purely explained by cellular death or atrophy of the SCN. Some studies have reported contradictory results with regard to the decrease in neuronal cells and neuroglia in the SCN in rats, particularly when comparing male and female animals (Madeira, Sousa, Santer, Paula-Barbosa & Gundersen, 1995; Tsukahara, Tanaka, Ishida, Hoshi & Kitagawa, 2005; Roberts, Killiany & Rosene, 2012). *Post mortem* studies showed that neurodegenerative processes occur in the SCN during senescence, suggesting progressive deterioration of the circadian pacemaker in the human brain during aging (Zhou & Swaab, 1999; Hofman & Swaab, 2006).

Madeira et al. (1996) performed a stereological study in male and female adult and aged rats and did not observe any effect of age or sex on the total number of neurons or astrocytes in the SCN. In a similar study, Tsukahara et al. (2005) reported no alterations in the total size of the SCN in rats, regardless of age and sex. However, a decrease in the number of neurons was observed in the SCN in elderly rats compared with young rats of both sexes. These authors also observed stability of the neuronal decline in adult male individuals until old age. In males, the neurodegeneration appears to occur during the adult phase. Recently, Roberts et al. (2012) confirmed the stability of neuronal decline in

the SCN in adult male rhesus monkeys compared with aged male animals. Roberts et al. (2012) also found a decrease in the number of neurons in the dorsomedial and ventrolateral portions of the SCN in adult male rhesus monkeys and adult and aged females compared with young individuals. In the same study, the authors analyzed the nuclear diameter of SCN cells, observing atrophy of the nuclear size of these neurons with increasing age in monkeys of both sexes (Roberts et al., 2012). Interestingly, the effects of aging on neuroglia differed from the effects observed in neurons. These same studies reported an increase in the amount of glial fibrillary acidic protein (GFAP) expression (i.e., a marker of astrocytes) in the SCN in rats and rhesus monkeys, especially in the dorsomedial part of this nucleus (Tsukahara et al., 2005; Roberts et al., 2012).

A decrease in electrical activity in the SCN cells was also reported (Nygård, Hill, Wikstrom & Kristensson, 2005). Nygård et al. (2005) used tissue slices from young and old mice to analyze the electrophysiological properties of the ventrolateral part of the SCN. Both young and old mice displayed significant variations in the mean firing rate between day and night. Old animals exhibited an elevated number of such silent cells during the day compared with young animals. The frequency of spontaneous inhibitory postsynaptic currents was also reduced in ventrolateral SCN neurons in old animals (Nygård et al., 2005; Farajnia et al., 2012). This reduction of the electrical activity of the cell membrane could be partially explained by age-dependent changes in the properties of the cell membrane (Farajnia et al., 2012).

Neurochemical implications of the aging suprachiasmatic nucleus

Together with the morphological alterations in the SCN induced by age, a decrease in the expression of many neurotransmitters is also observed, including VIP, VP, 5-HT, GABA, and other neurochemical compounds, such as calbindin (CB; Figure 4; Swaab, Flies & Fisser, 1985; Harper et al., 2008; Duncan, Hester, Hopper & Franklin, 2010).

Studies have reported different results with regard to the reduction of VIP immunoreactivity (Kawakami et al., 1997; Cayetanot, Bentivoglio & Aujard, 2005a) and VP (Cayetanot et al., 2005a) in the SCN in rodents and nonhuman primates. A nonsignificant decrease in the total number of VP-expressing cells was observed in elderly humans (Hofman, Fliers, Goudsmith & Swaab, 1988). However, nonhuman mammals, such as aged rodents and primates, exhibit consistent decreases in VIP expression (Kawakami et al., 1997; Cayetanot et al., 2005a) and VIP mRNA in the SCN (Duncan, Herron & Hill, 2001; Kalló, Kalamatianos, Piggins & Coen, 2004). Alterations in VP immunoreactivity were also observed in rats and nonhuman primates (Roozendaal, Van Gool, Swaab, Hoogendijk & Mirmiran, 1987; Cayetanot et al., 2005a), which showed decreased expression. Interestingly, the reduction of VIP and

VP expression in rats was restored in aged animals after treatment with the neurotrophin nerve growth factor (NGF; Pereira, Cardoso & Paula-Barbora, 2005). Importantly, the reinstatement of VIP- and VP-immunoreactive fibers in the SCN in aged rats did not clearly restore the circadian rhythmic impairments induced by aging (Pereira *et al.*, 2005).

Studies have reported substantial changes in temporal VIP and VP expression in the SCN in elderly nonhuman primates (*Microcebus murinus*) compared with adults (Cayetanot *et al.*, 2005a; Aujard, Cayetanot, Bentivoglio & Perret, 2006). Vasopressin exhibited a daily rhythm in neuron number and immunostaining in young animals, with higher peaks of VP occurring during the second part of the day. In elderly animals, VP expression was more accentuated in the early evening (Cayetanot *et al.*, 2005a; Aujard *et al.*, 2006). The peak VIP expression occurred during the night in adult individuals. In elderly animals, the highest VIP expression occurred at the beginning of the day (Cayetanot *et al.*, 2005a; Aujard *et al.*, 2006).

Vasopressin is described as a neurotransmitter involved in rhythm amplification, acting as a feedback regulator of electrical activity inside the SCN (Ingram, Terenzi, Howard & Windle, 1998). Animal experiments have demonstrated an important role for VP derived from the SCN in the control of neuroendocrine day/night rhythms, such as in the hypothalamic-pituitary-adrenal axis (Kalsbeek, Van Heerikhuize, Wortel & Buijs, 1996; Kalsbeek *et al.*, 2008). The remarkable correlation between the diminished presence of VP in the SCN and deterioration of sleep-wake rhythms during aging, in addition to neurodegenerative diseases and mood disorders, indicate that VP neurons significantly contribute to the rhythmic output of the SCN, even in humans (Liu *et al.*, 2000; Zhou *et al.*, 2001).

Vasoactive intestinal polypeptide cells innervate the SCN itself, suggesting the presence of a self-feedback loop within the SCN (Piggins & Cutler, 2003). These cells receive a direct retinal input (Tanaka, Ichitani, Okamura, Tanaka & Ibata, 1993; Glass, Guinn, Kaur & Francl, 2010), and photic stimulation activates these neurons, reflected by the activation of Fos proteins (Romijn, Sluiter, Pool, Wortel & Buijs, 1996), thus adjusting the phase of the SCN in response to zeitgebers. Consequently, a decrease or change in the time or expression of VIP may cause irregular electrical activity of SCN neurons and alterations in behavioral execution (Ingram *et al.*, 1998; Cayetanot *et al.*, 2005a). Vasoactive intestinal polypeptide cells receive from the SCN (Piggins & Cutler, 2003). The decrease in the expression of VIP mRNA or product may be partially responsible for the decrease in the ability to respond to photic and non-photic stimuli reported in aged animals (Zhang *et al.*, 1996; Duncan, 2006).

Deficits have been described in reproductive mechanisms controlled by the SCN. In old female rats, the ability of the SCN to stimulate the production of hormone waves weakens. Vasoactive intestinal polypeptide mRNA becomes arrhythmic in the SCN in middle-aged female hamsters (Krajnak, Kashon, Rosewell & Wise, 1998; Gerhold, Katherine & Wise, 2005), and VIP deprivation in the SCN in young female hamsters induced deficits in the release of gonadotropin releasing hormone and luteinizing hormone, thus simulating what occurs in aged female hamsters (Gerhold *et al.*, 2005).

Alterations in the density of GABAergic terminal axons and the GABA$_A$ α3 receptor subunit have been reported in the SCN in elderly mice (Palomba *et al.*, 2008). GABA plays an important role in the regulation and synchronization of the amplitude of electrical activity in SCN neurons (Aton, Huettner, Straume & Herzog, 2006), and it appears to act together with VIP, in which 70% of the synaptic terminals may contribute to a decrease in or inadequate signaling of VIP neurons in the SCN (Palomba *et al.*, 2008).

In mammals, age-related changes in the serotonergic system are involved in distinct disorders, such as changes in sleep patterns, mood disorders (Meltzer *et al.*, 1998), and neurodegenerative diseases (Benninghoff *et al.*, 2012; Fidalgo, Ivanov & Wood, 2013). 5-HT regulates some of the behavioral parameters of the CTS that undergo aging-related alterations, such as the circadian rhythm of wheel-running activity or phase shifts (Penev, Zee, Wallen & Turek, 1995). 5-HT is a melatonin precursor. It has been shown that the synthesis and metabolism of 5-HT during the day is reduced in older pigeons (Garau, Aparicio, Rial, Nicolau & Esteban, 2006), which could be related to a decrease in melatonin during aging (Hardeland, 2013). Many studies have reported changes in 5-HT receptors in the SCN in aged animals (Duncan & Franklin, 2007; Duncan *et al.*, 2010), and these alterations may influence behaviors that depend on neurotransmitter release (Garau *et al.*, 2006). Additionally, a previous study showed that systemic administration of the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) mimicked non-photic phase shifts in young adult hamsters, whereas it was ineffective in old hamsters (Penev et al., 1995). These findings suggest a possible decrease in the sensitivity of the serotonergic system. Similar to what is seen in the GABA system, high 5-HT$_7$ receptor expression was found in the dorsal subdivision of the SCN, a brain area characterized by CB expression (Duncan & Franklin, 2007).

Synaptic plasticity is known to critically depend on the influx of neuronal Ca^{2+} and the signaling pathways mediated by this ion. The decline in the expression of calcium-binding proteins, such as CB, renders the neurons more vulnerable to excitotoxic insults mediated by Ca^{2+}, thus contributing to neurodegeneration (Geula *et al.*, 2003). A decrease in the daily variation of CB immunoreactivity, the most prominent chelating protein for Ca^{2+}, has been reported in the primate SCN (Cayetanot, Deprez & Aujard, 2007). Calbindin-immunoreactive cells within the SCN constitute a small cluster of cells that express Fos protein in

response to light (Silver *et al.*, 1996). Injury of the SCN leads to the disruption of the circadian rhythm of locomotor activity (Kriegdfeld, LeSauter & Silver, 2004). These behavioral alterations are quite similar to the locomotor activity changes observed in elderly animals (Valentinuzzi, Scarbrough, Takahashi & Turek, 1997). Altogether, these data suggest a possible relationship between the disruption in the circadian rhythm of locomotor activity and a decrease in CB-immunoreactive cells in the SCN.

Neuropeptide Y is another neuropeptide that has important functions in the SCN. It is implicated in the photic (Yannielli & Harrington, 2004) and non-photic (Biello, Janik & Mrosovsky, 1994) regulation of this brain area. Studies have revealed changes in NPY expression and some of its receptors in various areas of the CNS in aged animals (Kuruba, Hattiangady, Parihar, Shuai & Shetty, 2011; Veyrat-Durebex, Quirion, Ferland, Dumont & Gaudreau, 2013), such as in cortical areas (Huh, Kim, Lee, Kim & Ahn, 1997). However, no data have shown any changes in other components of the CTS beyond the SCN. Therefore, a wide field exists for studies on other alterations that may interfere with the precise function of the circadian clock in elaborating and regulating biological mechanisms that optimize the execution of behavior.

Implications of the aging suprachiasmatic nucleus on circadian rhythm

Changes in many basic parameters of circadian rhythm in mammals are associated with aging. These changes include alterations in the length of the circadian period and a reduction of the amplitude and duration of the phase of rhythm activity regulated by the clock (Satinoff *et al.*, 1993; Watanabe, Shibata, & Watanabe, 1995; Figure 5), resulting in altered responses to photic and non-photic stimuli (Brock, 1991; Duncan, 2006).
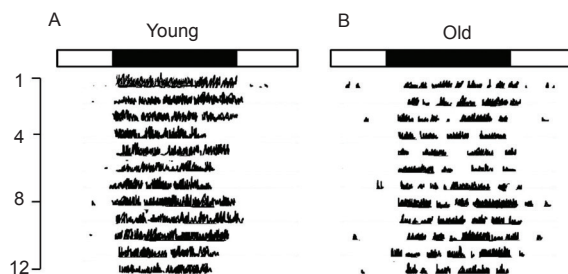

Figure 5. Hypothetical representation of activity records from a young (A) and old (B) nocturnal animal entrained to a 12 h/12 h light/dark cycle. Successive days are plotted from top to bottom. One to 24 h periods of activity are represented on the x-axis. The black bar indicates the dark phase of the light/dark cycle. The old animals exhibit a fragmented rhythm with a decrease in amplitude.

A decrease in the amplitude of circadian rhythm is followed by an increase in the percentage of the ultradian component during the last week of life in mice

(Weinert & Weinert, 1998). This decrease enhances the splitting of circadian rhythm (Morin, 1988). This loss of amplitude, together with a broadening of the period, may be attributable to weaker coupling between two or more oscillators that control circadian rhythm (Pittendrigh, 1981a). Consistent with the theory of coupled oscillators (Pittendrigh, 1981b), age-dependent differences in the phase angle between circadian rhythms and the exogenous zeitgeber might be caused by changes in period lengths or may be modified by coupling strength. Studies of the coupling strength between the circadian oscillator and zeitgeber have investigated the response of the oscillator to external stimuli. During aging, this response declines (Zhang *et al.*, 1998). Witting, Mirmiran, Bos & Swaab (1993) found that the amplitude of the activity rhythm during synchronization to the light/dark cycle could be increased simultaneously with the intensity of light. Therefore, the amplitude of the rhythm in older animals that are exposed to very intense light may resemble the amplitude exhibited by young animals, demonstrating a decrease in the sensitivity of cells. Aged rats and mice exhibit deficits in their photic synchronization abilities (Benloucif, Masana & Bubocovich, 1997), with a loss in response to light pulses (Zhang *et al.*, 1998; Benloucif *et al.*, 1997) and non-photic stimuli (Van Reeth, Zhang, Reddy, Zee & Turek, 1993; Duncan, 2006). Furthermore, the locomotor activity patterns of these animals show increased fragmentation, period lengthening, and resynchronization changes (Nakamura *et al.*, 2011; Valentinuzzi *et al.*, 1997).

A recent study reported a decline in the number of dopaminergic neurons in aged rats, which might be related to the changes seen in the circadian period of locomotor activity (Tanaka *et al.,* 2012), circadian drinking behavior (Burwell, Whealin & Gallagher, 1992), locomotor activity (Valentinuzzi *et al.*, 1997), and body temperature rhythm (Weinert, 2010), all of which have a reduced amplitude in older rats.

Cayetanot, Van Someren, Perret & Aujard (2005b) and Aujard *et al.* (2006) showed that 5-month-old lemurs (*Microcebus murinus*) that were subjected to an acceleration of seasonal cycles exhibited the same changes in locomotor pattern that are observed in chronobiologically aged animals (6-9 years of age). Additionally, aged individuals and animals with seasonal acceleration exhibited significant differences in locomotor activity compared with adults and adolescents (2-4.5 years of age). Chronobiologically elderly animals and artificially accelerated animals (2-4.5 years old; 5-9 seasonal cycles) showed a decrease in night activity and an increase in diurnal activity. This disruption of the sleep-wake cycle and activity fragmentation pattern are similar to observations in senescent humans.

In humans, healthy aging is associated with normal changes that occur in sleep architecture and patterns, such as a decrease in sleep during the night period, a decline of the deep sleep stage, and an increased frequency and duration of naps during the day, thus

presenting a loss of amplitude and fragmentation of the activity-rest rhythm (Huang *et al*., 2002; Tractenberg, Singer & Kaye, 2005). Perhaps the most characteristic sign is a phase advance of the normal circadian cycle. Therefore, elderly people have a propensity toward earlier sleep onset, accompanied by an earlier morning wake signal (Wolkove, Elkholy, Baltazan & Palayew, 2007). There is a reduction of delta sleep (stages 3 and 4; the deepest and most refreshing form of sleep) that begins in middle-age, with some reports that these deeper stages are completely absent after the age of 90 (Ohayon, Carskadon, Guilleminault & Vitiello, 2004). Similar sleep pathologies are found in the elderly, including insomnia (defined as difficulty falling or staying asleep; Monane, 1992; Reid *et al*., 2006) and rapid-eye-movement sleep behavior disorder (characterized by a loss in muscle atonia; Wolkove *et al*., 2007), that usually affects people older than 60 and is frequently associated with other neurodegenerative conditions such as Parkinson´s disease, multiple sclerosis or Alzheimer dementia (Olso, Boeve & Silber, 2000). An interesting study reported that healthy elderly appeared to need less sleep time physiologically (Duffy, Willson, Wang & Czeisler, 2009). A longitudinal sleep pattern study of rhesus monkeys observed similar modifications as in humans, such as a decline in the daily activity period associated with many modifications of sleep quality and quantity (Zhdanova *et al*., 2011).

In the elderly, several factors possibly contribute to changes in sleep and circadian rhythm desynchronization (Cooke & Ancoli-Israel, 2011). As discussed above, the SCN deteriorates with age (Zhou & Swaab, 1999; Hofman & Swaab, 2006), which may result in weaker or more disrupted circadian rhythms. Furthermore, other disturbances involved in the entrainment of the circadian rhythm of sleep may appear (Cooke & Ancoli-Israel, 2011). For example, the nocturnal secretion of endogenous melatonin gradually decreases with age (Hardeland, 2012). The reduction of melatonin in aged animals could possibly occur through insufficient environmental illumination (Mishima, Okawa, Shimizu & Hishikawa, 2001) or a decrease in photic absorption in older animals (Zhang *et al*., 1996, Zhang *et al*., 1998). In mammals, pineal melatonin biosynthesis and release are, in turn, under the control of the SCN and largely confined to the night (Tricoire, Locatelli, Chemineau & Malpaux, 2003). Melatonin secretion plays an important role in the sleep-wake cycle, and this decline may result in reduced sleep efficiency and an increased incidence of disturbances of the circadian rhythm of sleep (Hardeland, 2012; Hardeland, 2013).

Elderly people may have exogenous cues that are too weak to entrain to the light-dark circadian rhythm (Zhang *et al*., 1996; Aujard *et al*., 2001), thus influencing the sleep-wake rhythm. Light is one of the most powerful zeitgebers. Studies have shown that elderly patients, especially those who are institutionalized, spend too little time in daylight. This reduced level of bright light is associated with

nighttime sleep fragmentation and disorders of the circadian rhythm of sleep (Shochat, Martin, Marler & Ancoli-Israel 2000).

Changes in the circadian rhythm of activity, such as sleep, become irregular with age, leading to a decline in quality of life by inducing, among others, cognitive deficiency and emotional stress (Singletary & Naidoo, 2011). The severe interruption of circadian rhythm is associated with an increased susceptibility to disease (Gibson, Williams & Kriesgsfeld, 2009), which raises interest about circadian changes caused by the aging process.

Acknowledgements

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