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Review

The functional changes of the circadian system organization in aging

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ABSTRACT

The circadian clock drives periodic oscillations at different levels of an organism from genes to behavior. This timing system is highly conserved across species from insects to mammals and human beings. The question of how the circadian clock is involved in the aging process continues to attract more attention. We aim to characterize the detrimental impact of aging on the circadian clock organization. We review studies on different components of the circadian clock at the central and periperal levels, and their changes in aged rodents and humans, and the fruit fly *Drosophila*. Intracellular signaling, cellular activity and intercellular coupling in the central pacemaker have been found to decline with advancing age. Evidence of degradation of the molecular clockwork reflected by clock gene expression in both central and peripheral oscillators due to aging is inadequate. The findings on age-associated molecular and functional changes of peripheral clocks are mixed. We conclude that aging can affect the circadian clock organization at various levels, and the impairment of the central network may be a fundamental mechanism of circadian disruption seen in aged species.

1. Introduction

The circadian clock is the endogenous pacemaker that generates the ˜24 h daily rhythms in behavior, physiology, metabolism and cellular processes, such as body temperature, endocrine secretion, sleep-wake cycles and locomotor activity (Panda et al., 2002). These circadian output rhythms and the intrinsic clock mechanism not only synchronize with environmental rhythms (entrainment) but also persist under constant conditions (free-running).

Aging is the inevitable process of functional decline and the circadian timing system is also susceptible, showing progressive deterioration with advancing age. As a prominent signature of aging, the decline of circadian output rhythms has been widely observed in fruit flies and mammals including humans (Kondratova and Kondratov, 2012). In humans, the circadian output changes associated with aging include the reduction of the amplitude and earlier timing of phase of daily rhythms, both evident in body temperature and sleep-wake cycle, as well as a disruption of quantity and quality of nocturnal sleep (Duffy et al., 2015; Hood and Amir, 2017). Mammalian studies on aging demonstrate change in the free-running period, shift in the phase and weakening of rhythmicity, which have been reported in body temperature, locomotor activity patterns and drinking behavior (Froy, 2011). Similarly, prolonged free-running period, decline in sleep consolidation and decrease of overall rest-activity rhythm strength, have been revealed in *Drosophila* (Koh et al., 2006; Umezaki et al., 2012).

It is widely accepted that the molecular mechanism of the circadian clock can be found in nearly all cells of the body (Brown and Azzi, 2013). Correspondingly, the circadian timing system is composed of a number of tissues and organs comprising cell-autonomous circadian oscillators. The well-organized circadian network is formed with interconnected cellular oscillators within individual tissues and organs, and these cooperate with each other within the system (Mohawk et al., 2012). Arguably the impact of aging on the circadian clock is likely to be complex, with the possibility that the clock can be arrested at the cellular, tissue/organ or systemic levels resulting in desynchrony. In the first part of this review, we introduce the architecture of the circadian clock system, and in the second, summarize how aging affects the function of different parties in the clock organization.

1.1. The functional organization of the circadian clock

The circadian clock consists of the central pacemaker and numerous peripheral oscillators, and each can be dissected into functional units showing circadian properties at the cellular and tissue/organ levels. In view of this, a circadian pattern of clock gene expression has been shown in individual cells and the tissue of the mammalian central clock (Honma, 2018). Likewise, evidence of the existence of clock mechanism has been found in peripheral cells and tissues/organs (Dibner et al., 2010). Ultimately, these circadian components collaboratively contribute to the timed functioning of the organism.

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1.1.1. Central clock at the tissue, intracellular and intercellular levels

The central clock in mammals is situated in the suprachiasmatic nucleus (SCN), which has been identified as both necessary and sufficient for controlling circadian physiology and behavior through a series of classic lesion and transplantation experiments (Lehman et al., 1987; Ralph et al., 1990; Stephan and Zucker, 1972). The SCN can be divided into regions exhibiting sequential spatiotemporal activation patterns of clock gene expression (Foley et al., 2011; Yamaguchi et al., 2003) and phase adjustment at different rates in response to light stimuli (Nakamura et al., 2005), as well as distinctive distribution of calcium rhythm (Enoki et al., 2012, 2017). The SCN is mainly entrained by the light, which is perceived by the retina and then transmitted to the SCN *via* the retinohypothalamic tract (RHT). The SCN receives direct photic input from intrinsically photoreceptive retinal ganglion cells (ipRGCs), in this case, the ipRGCs-expressing photo-pigment melanopsin are considered the principal circadian photoreceptors (Do and Yau, 2010).

In the *Drosophila* brain, the central circadian clock in each hemisphere is composed of approximate 150 pacemaker neurons, which are divided into multiple clusters based on their location, size, neuropeptide expression and function. These are dorsal lateral neurons (LN_d), pigment dispersing factor (PDF)-expressing large ventral lateral neurons (l-LN_v), lateral posterior neurons (LPN), PDF-positive small ventral lateral neurons (s-LN_v), PDF-negative s-LN_v, anterior dorsal neurons 1 (DN_{1a}), posterior dorsal neurons 1 (DN_{1p}), dorsal neurons 2 (DN_2) and dorsal neurons 3 (DN_3) (Helfrich-Förster, 2005).

First and foremost, the circadian clock is driven by the transcription-translation feedback loops of clock gene expression existing at a cellular level. Most clock genes are well conserved from insects to humans and, with few exceptions, play similar roles in the timekeeping mechanism. The details of transcription-translation feedback loops in animals are described elsewhere (*e.g.* Hardin and Panda, 2013). Briefly, in *Drosophila*, the negative feedback loop is mainly composed of gene *period* (*per*) and *timeless* (*tim*), whose translational products PERIOD (PER) and TIMELESS (TIM) in cytoplasm form a heterodimer. This enters the nucleus to inhibit the transcription of *per* and *tim* themselves. The positive feedback loop primarily consists of the gene *clock* (*clk*) and *cycle* (*cyc*), whose corresponding proteins CLOCK (CLK) and CYCLE (CYC) heterodimerize in the nucleus. They then facilitate transcription of other clock genes such as *per* and *tim* by recognizing their specialized promoter regions known as the E-box. These two feedback loops work closely together in an antiphase manner to generate circadian rhythms. The circadian molecular mechanism is mirrored in mammals, with a pair of transcriptional factors CLOCK and BMAL1 which form heterodimer to activate the transcription of genes encoding their repressors, PERIOD 1–3 (PERs) and CRYPTOCHROME 1–2 (CRYs).

Secondly, in order to produce functional cellular oscillations within the SCN, reciprocal interaction between molecular and cytosolic clocks is necessary (O'Neill and Reddy, 2012). The relationships among cellular membrane events, intracellular signaling cascades and transcriptional-translational regulation have been increasingly studied but not totally elucidated. The molecular clock machinery in SCN neurons drives neural activities such as a daily oscillation in spontaneous firing rate (namely membrane excitability or action potential) (Enoki et al., 2017). This in turn plays an essential role in the cellular expression of clock genes as well as the coupling between individual neurons within the SCN (Colwell, 2011; Yamaguchi et al., 2003). Recent data also suggest the essential contribution of intracellular signaling to the timekeeping mechanisms, and the calcium rhythmicity is one such example (O'Neill and Reddy, 2012). Found at a single-cell level in the SCN, robust circadian calcium rhythms play an important role to mediate the input signals to the core molecular loop for circadian rhythm generation (Enoki et al., 2012) and regulate the synchronization of rhythmicity across the population of SCN clock cells (Nahm et al., 2005). In *Drosophila* as in mammals, membrane depolarization and cellular signaling are similarly important for clock function at the intracellular level (Harrisingh et al., 2007; Nitabach et al., 2002) and at the intercellular level (Nitabach and Taghert, 2008).

Thirdly, individual cellular oscillators within the SCN are synchronized between each other through intercellular coupling, so that the entire SCN can perform as an efficient network system. In this case, the SCN population of neurons can be constrained to a narrow range of parameters such as 'free-running period' corresponding to the circadian output rhythms (Herzog et al., 2004; Honma et al., 2004). For instance, the circadian spontaneous firing rhythms are synchronized throughout the SCN slice (Enoki et al., 2017), though dispersed individual neurons display heterogeneity in periods, phases and strength of oscillation (Honma et al., 1998, 2004; Liu et al., 1997; Welsh et al., 1995). In addition, the essential role of intercellular coupling is also emphasized by the evidence that densely cultured neurons show not only higher synchrony but also stronger rhythmicity than dispersed ones (Aton et al., 2005; Webb et al., 2009).

The specific coupling mechanisms which maintain the SCN neurons synchronized as a functional unit include synaptic signaling and neuropeptides (Evans, 2016) and the latter one is so far believed to be unique to the SCN (Liu and Chang, 2017). Acting as the most important neurotransmitter in the SCN, vasoactive intestinal polypeptide (VIP) is necessary to synchronize the pace of timekeeping among individual neurons (Aton et al., 2005; Maywood et al., 2006). It is a given that inhibition of VIP-mediated coupling leads to loss of synchrony in clock gene expression throughout the SCN tissue (Abraham et al., 2010). Although the role of inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the SCN neuronal synchronization is still unclear, it is hypothesized that VIP works with GABA to promote synchrony of heterogeneous oscillators in the SCN. In this model, VIP produced by VIP-secreting cells in the SCN works as an essential driver for synchronization while GABA generated from both VIP-secreting and non-VIP-secreting cells serves as a negative feedback mechanism to balance cell coupling (Kingsbury et al., 2016).

Similar to the instability of isolated individual cellular oscillators in the SCN, single *Drosophila* central clock neurons are weak oscillators that need to be in an intact circadian circuit to generate robust circadian rhythms (Sabado et al., 2017). A sophisticated network is formed by the intercellular coupling among different clusters of clock neurons in *Drosophila*, and PDF strongly resembles those functions described for VIP in the SCN. Though only released by s-LN_v and l-LN_v, PDF also feeds back to non-PDF neurons, playing a key role in communication within the central clock neuronal network (Top and Young, 2017). Clock neurons from flies lacking PDF or PDF receptors are uncoupled and show impaired rhythmicity (Collins et al., 2014; Lin et al., 2004; Peng et al., 2003; Yoshii et al., 2009).

In summary, synchronization among the central pacemaker neurons requires neuropeptide signaling (VIP in the mammalian SCN and PDF in the *Drosophila* brain), regulated by a reciprocal interaction between the molecular clockwork and rhythmic neuronal activity, which in turn depends on intracellular signaling (Herzog et al., 2017).

1.1.2. Peripheral clocks and their connection with the central clock

The ubiquity of the circadian clock mechanism assures that aside from the central clock, endogenous oscillators exist in most cells throughout the body. These individual cells and the functional tissues/organs they comprise, are the origin of peripheral clocks. In many cases, peripheral clocks are specifically referred to peripheral tissues that show circadian clock property (Yamazaki et al., 2000; Yoo et al., 2004). Mammalian peripheral oscillators residing in cells/tissues/organs show circadian properties outside the SCN, including various non-SCN brain regions (such as the hypothalamus, forebrain, olfactory bulb and pineal gland) and non-neuronal tissues/organs (such as the liver, kidney, muscle, adipose tissue and blood cells (O'Neill and Reddy, 2011; Cermakian and Boivin, 2009). Peripheral clocks appear to have a similar molecular makeup to the oscillators in the SCN (Dibner et al., 2010; Yagita et al., 2001), despite the fact that there are large differences in the relative contributions of individual clock components as

well as the largely non-overlapping downstream genes under circadian control (Mohawk et al., 2012). Outside the central clock in the brain, peripheral clocks in *Drosophila* are found in almost all tissues which display rhythmic *per* expression (Liu et al., 1988; Plautz et al., 1997), showing the same core molecular program present in central pacemaker neurons with diverse tissue-specificity (Ceriani et al., 2002; Krishnan et al., 2001).

In normal circumstances, circadian oscillators in individual cells are synchronized within a tissue and individual tissues are kept in a stable phase-relationship with each other within the circadian system. In view of this, the SCN is not a generator of peripheral rhythms, instead being a coordinator and synchronizer at the head of a distributed organization of individual clocks, mainly through neural and humoral pathways (Balsalobre, 2002). Many fundamental properties of circadian oscillations in peripheral clocks *in vivo* (Tahara et al., 2012) and *in vitro* (Izumo et al., 2014) have been shown to persist even in the absence of SCN function. Meanwhile, it has been reported that clock gene expression dampens in mammalian peripheral tissues (such as the liver, spleen, kidney, heart and lung) *in vitro* within days in constant conditions, but sustains in the SCN explants for weeks (Yamazaki et al., 2000). Furthermore, in constant conditions, loss of the SCN function results in peripheral clocks that become desynchronized (Izumo et al., 2014; Yoo et al., 2004). Likewise, peripheral tissues in *Drosophila* can be clearly distinguished from the central oscillators due to their rapid dampening in constant conditions (Peng et al., 2003). These results arise from the fact that peripheral clocks are oscillators that require internal or external signals (including signals from the central clock) to synchronize and thus sustain their circadian rhythms, whereas the central clock is able to tick independently without entraining signals (self-sustaining). One possible explanation is that peripheral clocks lack the intercellular coupling mechanism that is unique to the central pacemaker (Brown and Azzi, 2013). Deficiency in coupling leads to a loss of synchrony among individual cells and thus dampening of the ensemble rhythm at the peripheral population level, from lacking internal or external cues (Welsh et al., 2004). Taking the argument further, this could also be the reason why peripheral tissues are more easily affected when facing challenges such as genetic perturbation in both mammals (Liu et al., 2007) and *Drosophila* (Weiss et al., 2014).

The coordination among peripheral clocks in *Drosophila*, which can respond directly to the same entrainment cues that set the phase of the central pacemaker neurons in the brain, is thought to occur mainly *via* light and temperature sensitive intracellular pathways (Agrawal et al., 2017; Dubruille and Emery, 2008; Krishnan et al., 2001). In this way, some peripheral clocks can maintain synchrony with external environmental cues independent of input from the central clock, such as the Malpighian tubules (Giebultowicz and Hege, 1997) and the antenna (Krishnan et al., 1999; Tanoue et al., 2004). Other peripheral oscillators such as the prothoracic gland are driven by the central clock, similar to those in mammals (Myers et al., 2003; Selcho et al., 2017). Meanwhile, an exceptional case has been reported in the fat body, where the cycling of some genes depends on the local clock whereas that of other genes occurs in response to central brain signals (Xu et al., 2008; Erion et al., 2016).

1.2. The circadian clock and aging

Some of the mammalian and *Drosophila* studies suggest that the function of the central and peripheral clocks is weakened by the aging process. Conversely, minimal or even no impact has been revealed in other studies. Clear evidence has been found to point at a damaged network at the central level which is related to aging process, whereas core molecular clock mechanism seems to stay intact based on current aging research findings. In this part of the review, we focus on how the function of the circadian clock changes with advancing age, including alterations of the circadian signals at the tissue and cellular levels (such as the neuronal electrical activity), the neuronal network interactions (such as neuropeptides in the SCN), the intracellular properties (such as membrane potential and ionic currents), as well as the expression of clock genes and their protein products.

1.2.1. The impact of aging on the central clock

The first clue that the central clock is involved in aging process comes from transplantation studies. Implantation of fetal SCN tissue in old animals rescues circadian output function such as daily behavioral rhythms in hamsters (Viswanathan and Davis, 1995) and diurnal rhythms of hormones in rats (Cai et al., 1997). Furthermore animals that have restored higher clock function end up with increased longevity (Hurd and Ralph, 1998). However, it is still unclear whether circadian decline observed in aged animals is paralleled with age-associated alterations in the structural organization of the SCN. The results of the changes of the SCN in both volume and total cell numbers under aging in both humans and rodents are mixed (Hood and Amir, 2017). For instance, despite that SCN neuronal cells have been found to reduce with increasing age in rats (Tsukahara et al., 2005), there are a number of studies showing no loss in the total number of cells (Madeira et al., 1995; Roozendaal et al., 1987) or the volume (Madeira et al., 1995; Tsukahara et al., 2005) of the SCN.

There is good evidence that with age comes impairment of light transmission from eye to the SCN, as well as the reduction of responsiveness of the SCN to light (Asai et al., 2001; Kolker et al., 2003; Lupi et al., 2012; Zhang et al., 1998). Despite this there is no reported age-related change of the RHT (Lupi et al., 2012; Zhang et al., 1998), but extensive evidence points to the decrease of short-wavelength light passage through the yellowing and thickened lens with increasing age in humans (Brainard et al., 1997; Kessel et al., 2010; Najjar et al., 2014) and rodents (Zhang et al., 1998). Aside from the decline of retinal function observed in humans (Freund et al., 2011; Gerth et al., 2002), the number of ipRGCs has been reported to decrease with advancing age in mice (Semo et al., 2003). Therefore a plausible mechanism of circadian clock decline is weaker entrainment by light leading to desynchrony among oscillators within the SCN. This in turn feeds back on the circadian clocks throughout the body worsening the systemic effect.

The results of age-associated decline in SCN neuronal activity at the tissue level are consistent, which may account for behavioral and physiological impairment. The electrical activity patterns of SCN neurons measured by multiunit activity (MUA) recordings indicate that the amplitude of SCN activity decreases with increasing age in brain slice preparations *in vitro* (Biello, 2009; Nygård et al., 2005; Satinoff et al., 1993; Watanabe et al., 1995) and *in vivo* (Nakamura et al., 2011). The decrease in neuronal electricity rhythms may reflect a loss of coherence in firing patterns across SCN cells in aged animals, ending up with a phase desynchronization. This hypothesis is supported by the findings of a wider phase distribution and an antiphase activation of cluster of cells during the night (Farajnia et al., 2012), as well as an elevated number of silent cells during the day (Nygård et al., 2005) with advancing age.

One of the possible explanations to the deterioration of the SCN neuronal signals at the tissue level is the age-related degradation of the central network system (Nakamura et al., 2016). Correspondingly, rhythms of clock gene expression recorded at single cell level rapidly become desynchronized and dissociated and gradually drift out of phase with each other in associated SCN neurons from aged animals, while those in young SCN cells stay synchronized (Nakamura et al., 2015), indicating a ‘network fault’ due to aging.

The communication among SCN neurons undergoes significant impairment in aged animals due to lacking of effective messengers. It has been reported that the expression level and the amplitude of VIP mRNA and the expression level of its receptor mRNA in the SCN decrease in the course of mammalian aging (Kalló et al., 2004; Kawakami et al., 1997). Such attenuation is associated with both a decline in the level of mRNA within each cell as well as in the number of VIP-producing cells in the SCN (Krajnak et al., 1998). This is well supported by human

research, showing decreased number of VIP neurons in the SCN of middle-aged males (Zhou et al., 1995; Zhou and Swaab, 1999). The disruption of VIP expression has functional implications in the aging of the circadian clock. The extent of age-related changes of circadian amplitude of behavior rhythms are correlated with the number of VIP-expressing SCN neurons in humans (Wang et al., 2015). In addition, the presence of VIP-expressing transplanted cells successfully restores circadian output behavior and physiology in aged mice (Li and Satinoff, 1998).

Aside from neuropeptides, quantification of synaptic terminals in the SCN has demonstrated an age-related reduction in synaptic spines and a shortening of dendrites (Palomba et al., 2008). This marked reduction in the synaptic signaling of the aged SCN affects equally GABAergic terminals and the remaining subset. Furthermore, patch-clamp recordings in hypothalamic slices show that GABAergic postsynaptic currents decrease in frequency (Nygård et al., 2005) and amplitude (Farajnia et al., 2012) in SCN neurons of aged mice.

It is evident that an age-related loss of neuronal connectivity exists at the central level, but the molecular clock in the SCN as a whole is relatively resistant to aging (Mattis and Sehgal, 2016). Indeed, a number of studies have compared the expression of core clock genes in the aged SCN and have shown conflicting results with either normal or reduced expression of various clock genes. This is aside from a consistent age-related decline in expression of *Bmal1* and a consistent lack of age-related change in the expression of *Per1* (Banks et al., 2016). In a recent study reporting the gene profiling in the SCN of a primate model, core clock gene expression at both mRNA and protein level has been found to stay unchanged in aged animals (Eghlidi et al., 2018). In view of this, causal links between clock gene expression patterns in the central clock and age-associated disturbance in circadian rhythms cannot be conclusively demonstrated. For instance, with the same purpose to investigate the intrinsic age-related changes of the central clock mechanism, results of *Per2*/PER2 expression (same clock gene) measured in the SCN under constant conditions in aged mice (same species) when compared with young counterparts from different studies are diverse. Age-related reduction has been found in *Per2* mRNA measured by *in situ* hybridization *in vitro* in free-running aged male mice (approx. 15 months) when compared to young mice (approx. 4 months) (Weinert et al., 2001). Likewise, the amplitude of PER2::LUC rhythms *in vitro* drops markedly in aged male mice (13–15 months) when compared to young mice (3–5 months) (Nakamura et al., 2015). Nonetheless, only minor deficits of PER2 expression measured by both immunohistochemistry and bioluminescence *in vitro* have been shown in free-running aged male mice (13–16 months) when compared to young mice (3–6 months) (Nakamura et al., 2011). In a recent study where animals with more advancing age are used, *Per2* rhythms are measured in old male and female $Per2^{Luc}$ mice (24–26 months) *in vitro*, and the difference is not notable when compared to younger animals (8–13 months) (Polidarová et al., 2016).

The molecular clockwork is also believed to be intact at the single cellular level in the central clock (Nakamura et al., 2016). Previous attempts to measure the clock genes in individual neurons under aging are contradictory and have shown both preservation of the clock gene profiles (Asai et al., 2001) and lower amplitude oscillations (Weinert et al., 2001). A recent study using an ultra-sensitive camera system capable of recording single cell imaging in PER2::LUC reporter mice, has provided strong evidence on the preservation of the intracellular clock mechanism by showing clear circadian rhythms and similar amplitude in individual cells in both young and aged SCN (Nakamura et al., 2015).

Despite maintenance of normal clock gene expression, individual neurons in the SCN have shown functional decline resulting from aging. By using long-term single-cell recording *in vivo*, the effect of aging on firing rate patterns of individual SCN neurons has been revealed, displaying a decreased amplitude of circadian impulse activity at the cellular level (Aujard et al., 2001). Not only the neuronal activity, but also the intracellular signaling is modified by aging process. For instance, the reduction in the circadian amplitude of certain potassium currents has been observed in aged SCN neurons (Farajnia et al., 2012; Itri et al., 2005, 2010). Another study also reports the loss of the circadian modulation of large-conductance Ca^{2+}-activated K^+ (BK) channels (Farajnia et al., 2015). Cellular signaling is important in the regulation of the resting membrane potential and the action potential, thus their age-related changes may lead to diminished rhythm of neuronal activity in the SCN cells from aged animals (Farajnia et al., 2012).

Findings in aged *Drosophila* are similar to those in mammals, including a controversial result on changes of molecular oscillators at the central level and a convincing pattern of decline in intercellular synchronization among central clock neurons. On one hand, it has been reported that PER and TIM oscillations in almost all central clock neuron groups (s-LN_v, l-LN_v, DN_1, DN_2 and LN_d) deteriorate with advancing age (Umezaki et al., 2012). However, this is challenged by the finding that PER is robustly expressed in synchrony among different groups of clock neurons (s-LN_v, DN_1 and LN_d (Luo et al., 2012) and DN_{1-3} and LN_d (Zhao et al., 2018) in aged flies. On the other hand, the evidence showing reduction of the levels of PDF in the central clock network is consistent (Luo et al., 2012; Umezaki et al., 2012). Moreover, overexpression of PDF in PDF-positive neurons increases certain clock gene expression in specific central clock neurons, which leads to the partial rescue of rhythmicity and period changes of output behavior in old flies (Umezaki et al., 2012).

These results in both mammals and *Drosophila* suggest the important role of age-associated attenuation of intercellular communication within the central neuronal network. With advancing age, it becomes increasingly difficult for the rhythms of individual neurons to remain synchronized with each other and thus the rhythmic output of the network is fragmented. Here we predict a hypothesis that the network is serving as a protective mechanism rather than a pure victim under the process of aging. Age-induced dampening of circadian rhythms of neuronal activity is greater at the cellular level when compared to the whole SCN *in vitro* (Farajnia et al., 2012) and *in vivo* (Nakamura et al., 2011), suggesting a compensatory role of the SCN network capable of covering for functional deficiency of individual cells under aging (Farajnia et al., 2014).

1.2.2. The impact of aging on the peripheral clocks

One possible mechanism underlying circadian disruption with increasing age is the attenuation of the SCN's ability to drive local oscillators, leading to relative desynchrony between the SCN and oscillators in the peripheral tissues (Hood and Amir, 2017). There is evidence suggesting that age-related changes in the SCN can affect the function of peripheral oscillators, given that the SCN signals such as neuronal activity decline under aging as discussed above (1.2.1). The SCN regulates peripheral clocks *via* pathways including neural control through the autonomic nervous system and humoral control through circulation (Mohawk et al., 2012). It has been reported that deficiency in sympathetic input from the central clock is in part responsible for age-related disturbance of the peripheral oscillators. For instance, a weaker response to noradrenergic stimulation as well as decreased adrenergic receptor mRNA expression in target peripheral tissues have been found in aged animals (Tahara et al., 2017).

Other findings also support the idea that an age-related dampening of signal from the master clock may be responsible for functional decline in the periphery. It has been revealed that the entrainment of peripheral clocks (the esophagus, lung and thymus gland) in aged mice is slower under a shift of the light-dark (LD) schedule *in vitro* than the response in young counterpart, meanwhile a more rapid reaction has been found in the aged SCN (Sellix et al., 2012). Likewise, phase-resetting in the SCN appears nearly normal in aged rats, yet re-synchronization of peripheral clocks is disrupted in some (such as the liver) but speeds up in others (such as the arcuate nucleus and pineal gland) (Davidson et al., 2008). This study raises the concern that tissue-

specific changes of entraining speed cannot be simply explained by the weakening signal from the SCN in aged animals, rather, peripheral oscillators themselves may be affected and directly involved in the aging process.

The age-related deterioration of circadian signal from peripheral clocks has been shown in some tissues. For example, the neuronal activity rhythms are clearly degraded in one of the peripheral tissues (the sub-paraventricular zone) in the brain (Nakamura et al., 2011). Accordingly, studies focusing on the molecular changes in aged peripheral clocks have indicated disruption in clock gene expression in certain cases. In a study using *Per1* bioluminescence reporter *in vitro*, circadian rhythmicity of the lung in aged rats (24–26 months) has been found to be disturbed (Yamazaki et al., 2002). By using the same technique, *Per2* expression in aged mice (24–26 months) has also been shown to decline in the lung *in vitro* (Novosadová et al., 2018). Moreover, a human study has found consistent effects of aging on the circadian pattern of PER1 and PER2 expression in post-mortem brain samples (the prefrontal cortex) collected from old (> 60 years) and young (< 40 years) subjects, showing a phase advance and an amplitude reduction (Chen et al., 2016).

One important point to note is that a number of other studies has found no apparent change of peripheral clocks resulting from the course of aging. A recent study has revealed that neuronal firing is not affected by aging at either the single cell level or as the overall pattern in the mice motor cortex (McKillop et al., 2018). Besides, the circadian expression profiles of clock gene *Per1*, *Per2*, or *Cry1* mRNA measured by reverse transcription polymerase chain reaction (RT-PCR) *in vitro* are quite similar between young and aged rats (22–26 months) in the peripheral tissues (the paraventricular nucleus (PVN) and pineal gland) (Asai et al., 2001). This finding is supported by subsequent studies showing almost identical clock genes *Per2* and *Bmal1* expression by using similar method between young and aged mice (15 months) in the brain, heart and liver (Oishi et al., 2011), and normal *Per1* and *Per2* mRNA in aged mice (> 18 months) in the kidney, liver and submandibular gland (Tahara et al., 2017). Further, these three peripheral clocks measured in PER2::LUC aged mice (> 18 months) *in vivo* exhibit normal oscillation amplitudes (Tahara et al., 2017). Similarly, with bioluminescence reporters *in vitro*, *Per1* expression in some peripheral tissues (the PVN, pineal gland, arcuate nucleus, cornea, pituitary, liver and kidney) in aged rats (24–26 months) (Yamazaki et al., 2002) and *Per2* expression of the pancreas in aged mice (24–26 months) (Novosadová et al., 2018) have been found to be unchanged. In a recent human study, the phase of *Per3* expression in peripheral hair tissues from very old subjects (83–94 years old) has been shown to be similar with young controls (Yamaguchi et al., 2018).

It is not difficult to tell from these results that there is no clear evidence pointing at an age-associated peripheral disturbance in mammals. Under aging, changes of neuronal signal as well as rhythmic clock gene expression have been observed in some but not other peripheral oscillators. The challenge lies in the fact that a strong tissue-dependent manner of molecular mechanism within individual oscillators would complicate the general interpretation when it comes to the discussion on peripheral clocks and aging. For instance, in addition to studies mentioned above (Novosadová et al., 2018; Yamazaki et al., 2002), tissue-dependent changes of CLOCK and BMAL1 expression has been reported in a number of extra-SCN regions in aged mice (Wyse and Coogan, 2010).

One potential solution is to look at age-related peripheral alternations at the cellular level, although current results are inadequate to draw a confirmative conclusion. It has been reported that circadian expression of clock genes PER2 and BMAL1 in serum-stimulated senescent cells is significantly impaired compared with that in young cells both *in vitro* and *in vivo* (Kunieda et al., 2006). Nevertheless, it is also suggested that basic clock properties of peripheral cells do not change during aging, by showing the identical period length, amplitude, and phase of *Bmal1* expression in aged fibroblast *in vitro* (Pagani et al., 2011).

In recent years, instead of only focusing on damage of the core molecular mechanism, a novel theory putting emphasis on the age-associated change of the clock-controlled effector genes is rising. Through analyzing the whole transcriptome in aged mice (> 18 months), it has been revealed that epidermal and muscle stem cells retain a robust core circadian machinery, with comparable amplitude and phase both in LD conditions and the following constant darkness. However, although still active and rhythmic, the downstream oscillating transcriptome is extensively reprogrammed, switching from genes involved in homeostasis to those involved in tissue-specific stresses, such as inflammation, DNA damage and inefficient autophagy (Solanas et al., 2017).

Concerning the peripheral clocks in *Drosophila*, many studies indicate age-related molecular decline. First, reduction in amplitude of circadian clock gene expression has been found throughout the body in aged animals (Driver, 2000). Likewise, *per* and *tim* luciferase reporters has shown decline in expression of peripheral clocks throughout the fly body with advancing age (Luo et al., 2012; Zhao et al., 2018). Further, *per* mRNA oscillations (Krishnan et al., 2009) as well as *per* and *tim* at both mRNA and protein levels (Luo et al., 2012; Rakshit and Giebultowicz, 2013) have been reported to decrease in heads of old flies. It has also been shown in a recent study by using both techniques of RNA sequencing and qPCR that *tim* expression declines in aged flies' heads (Kuintzle et al., 2017).

Even though many results in *Drosophila* studies show molecular decline at the gross peripheral level, different tissues or body parts seem to respond differently. For example, transcriptional oscillations of *per* and *tim* have been proved to decrease in heads but not bodies of aged flies (Rakshit et al., 2012). As one of the peripheral clocks, the retinal photoreceptor cells in the compound eyes of old flies show decline in expression of PER, yet that is not the case for another peripheral oscillator, the Malpighian tubules, where strong PER oscillations have been observed in aged animals (Giebultowicz and Long, 2015).

In brief, it is known that tissues/organs age at different rates and have tissue-specific signatures in both mammals and *Drosophila* (Girardot et al., 2006), adding the complexity of age-related properties within individual oscillators. Besides, various peripheral tissues/organs may have different functional implication to the entire circadian system. Therefore, there is no unifying theory to summarize the comprehensive changes of the peripheral clocks due to aging at this stage.

2. Conclusions

In this review we compare the changes of various components of the circadian clock system at different levels during the aging process. The main impairment lies in the age-related breakdown of the central network, the maintenance of which could serve as a protective role against aging. The intercellular coupling as well as the intracellular signaling have been proved to be disrupted at the central pacemaker. Although age-associated impairment of the molecular clockwork in central oscillators is currently unconfirmed, it is evident that the neuronal activity measured from both single-cell and tissue levels declines with advancing age. The deterioration of the peripheral oscillators, including molecular machinery, cellular activity and downstream clock-controlled genes, has been proposed in some aging studies. However, the study on the peripheral field is still largely unclear and one possible reason is that diverse tissues/organs change differently under the circumstance of aging. Presumably, the combination of the damage from various functional units is reflected by the layout at the body level, resulting in disrupted behavioral or physiological output rhythms seen in aged animals.

One main limitation of existing studies investigating how the circadian clock changes with aging is that they have failed to confirm the causal relationship between the two. On the one hand, it is difficult to fully ascertain that the effects on the circadian clock seen in the aged animals are simply due to aging. One cannot exclude that other factors,

such as disease status, may also actively affect the circadian clock. On the other hand, it also raises the question of whether the dampening of the circadian clock rhythms contributes to the aging process. System-wide knockouts of clock genes creating “accelerated aging” phenotypes in both *Drosophila* and mice suggest a causal role of the central and peripheral molecular clockwork on aging (Hood and Amir, 2017). For instance, null mutation of the *per* gene (Krishnan et al., 2009) or *tim* gene (Vaccaro et al., 2017) in *Drosophila* is associated with significantly shortened lifespan, as well as impaired locomotor rhythm. Likewise, deficiency of the BMAL1 or CLOCK protein significantly affects longevity and results in the development of age-specific pathologies, such as sarcopenia, osteoporosis and cataracts (Kondratov et al., 2006).

Given that not a particular part but instead the diverse components of the clock can be affected by aging, rescue strategies should aim at preservation of the circadian system organization. Caloric restriction (CR) or dietary restriction (DR) seems to be a desirable candidate to reset different levels of the clock system to slow down the deterioration caused by aging. To date, CR/DR is the only known intervention capable of consistently and reproducibly prolonging lifespan in several animal models from yeast to mammals (Cornélissen and Otsuka, 2017). The effects of CR/DR are a coordinated systemic response, through regulation of both central and peripheral oscillators (Guarente, 2013). At the cellular level, CR/DR has the capacity to prevent the age-associated gene reprogramming and to restore homeostasis (Sato et al., 2017; Solanas et al., 2017). At the central level, CR/DR is implicated in the regulation of the central pacemaker to maintain robust circadian control against aging (Chang and Guarente, 2013). At the peripheral level, CR/DR preserves the cycling of most circadian clock genes which mediates the lifespan-extending effects (Katewa et al., 2016).

Research on human beings also indicates the protective effect of CR/DR against age-associated metabolic disarrangement, inflammation and oxidative stress, sharing similar mechanisms that have been shown in mammalian studies (Fontana et al., 2010). Although there is no direct evidence showing that CR/DR prolongs human lifespan, a promising prospect comes from a more-than two-decade longitudinal study conducted in non-human primate rhesus monkeys (Colman et al., 2009, 2014). It suggests that long-term CR/DR significantly delays the onset of age-related disorders such as diabetes, cardiovascular diseases and cancer, and what’s more, reduces the incidence of age-related and all-cause deaths.

While the link between aging and the circadian clock has attracted growing interest there is still much to learn. Future studies are warranted to find out answers to questions such as what is the causal relationship between the clock and aging, whether individual clock cells/tissues/organs contribute to the systemic decline of the circadian organization, and how the alterations of circadian mechanism contribute to the behavioral and physiological changes seen in aged species. With the development of advancing techniques, such as next-generation sequencing approaches (Takahashi, 2017) and single-cell circadian reporter technology (Hong et al., 2018; Nakamura et al., 2015), the knowledge will continue to expand towards the broader and deeper fields. If more is known about the circadian system and its interaction with aging, we will be able to investigate potential strategies with which to approach age-associated problems.

Declarations of interest

None.

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