Biological Rhythms and Aging

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Biological Rhythms

A regular and predictable pattern of any biological phenomenon which reoccurs with certain periodicity is considered as biological rhythm [106]. A biological rhythm can be endogenous, where it is controlled by the internal biological clock or exogenous where it is controlled by synchronizing internal cycles with external stimuli. Such stimuli are mostly with respect to the position of Earth to the Sun and to the Moon as well as on the immediate effects of such variations, for example, day alternating with night, high tide alternating with low tide, etc. [33]. Biological rhythms are genetically regulated, temperature independent, and resistant to pharmacological and chemical disruption. Based on the set of cues, the organism entrains and generates rhythms (Fig. 20.1) with varied periodicity such as, circannual rhythms – occurring in cycles of approximately 1 year; circalunar rhythms – occurring in cycles of approximately one lunar cycle; circatidal rhythms – occurring in cycles of approximately one ocean tide; infradian rhythms – occurring in cycles of frequency more than a day (>24 hours (h)); ultradian rhythms – occurring in cycles of frequency less than a day (<24 h) and circadian rhythms – occurring in cycles of approximately 24 h. In anticipation of these day–night phases, living organisms have evolved cellular and physiological rhythms having a periodicity of approximately 24 h known as circadian rhythms [124].

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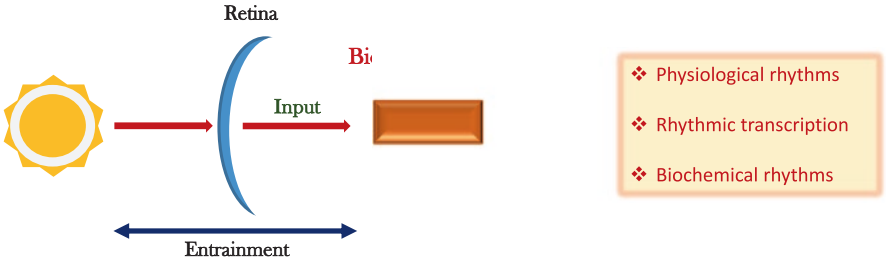

Fig. 20.1 Diagrammatic representation of mammalian circadian timing system. The biological localized in suprachiasmatic nucleus (SCN) gets entrained through photic input and then time information from SCN regulates physiological, biochemical, and transcriptional rhythms and hence synchronizing the entire body processes

Circadian Rhythms

Circadian rhythms regulate physiology and behavior with a period near 24 h. Circadian is derived from a Latin phrase meaning about (*circa*) a day (*dia*). There are several physiological, biochemical and behavioral aspects which follow circadian rhythms that include: sleep/wake cycle, body temperature, hormone secretion, blood pressure, digestive secretions, levels of alertness, etc. [69]. Exogenously they are set and entrained by zeitgebers, derived from German meaning time (*Zeit*) giver (*Geber*). The primary being Light–Dark cycles due to the rotation of earth around its axis though food, social cues, temperature, etc., can be considered as nonphotic zeitgebers. The circadian rhythms persist even in the absence of zeitgebers under constant conditions such as constant darkness or constant light. That means rhythms are endogenous. The persistence of rhythms in the absence of cues leads to free running situation, that is, rhythms continue to run, but with a slight deviation from 24 h. The rhythms are genetically determined [66] (Fig. 20.2). There are several other factors that act to the external stimuli and the result is the generation of the rhythms collectively known as the circadian timing system (CTS) [26]. These self-sustained, endogenous, and entrainable rhythms of sleep and wakefulness, foraging and feeding, body temperature, enzyme activity, hormonal release, energy metabolism, and several other molecular and behavioral parameters helped the organisms to effectively cope with ever-changing environment, thus improving their survival [56, 137, 150]. The importance of these oscillations on health and diseases has been rightly recognized with 2017 Nobel Prize in physiology and medicine jointly awarded to Jeffrey Hall, Michael Rosbash, and Michael Young *"for their discoveries of molecular mechanisms controlling the circadian rhythm"* [29].

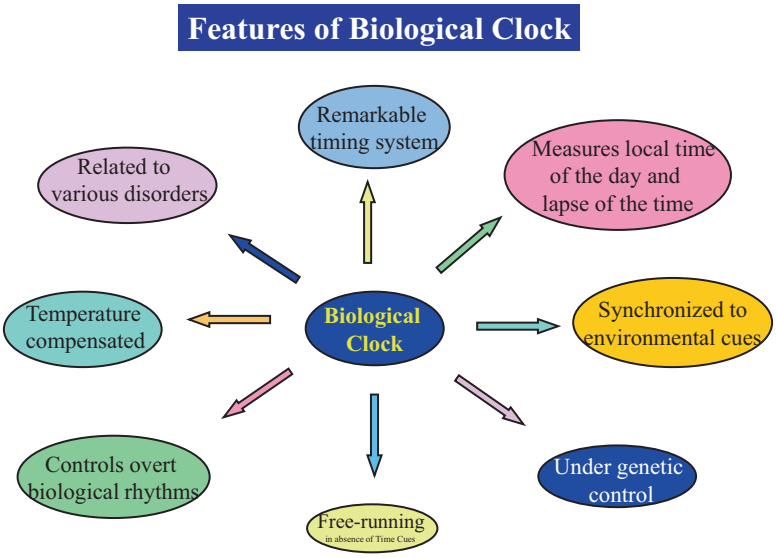

Fig. 20.2 Diagrammatic representation of various features of clock

Circadian Time-Keeping System (CTS)

The presence of temporally regulated rhythms in physiology and behavior hinted at the possible existence of a circadian clock which was confirmed by lesion experiments and was discovered to be located in the hypothalamic suprachiasmatic nucleus (SCN) in mammals [150]. SCN, the principal pacemaker, consisting of nearly 20,000 tightly packed neurons, acts as "master clock" by synchronizing the peripheral oscillators located in all other cells and tissues [1]. This hierarchical architecture of the CTS in mammals function robustly based on specialized input and output pathways.

Input Pathways to the SCN

Three major pathways convey information to the SCN resulting in entrainment of the master clock. Blue light activates photosensitive retinal ganglion cells in retina and will be communicated to SCN via *retinohypothalamic tract* (RHT) with the release of excitatory neurotransmitter glutamate and the neuropeptide pituitary adenylatecyclase-activating protein (PACAP). The release of these neurotransmitters leads to stimulation of signaling pathways involving Ca^{2+} and cAMP and leads to induction of clock genes [1, 22, 55].

RHT also indirectly communicates photic information to SCN via intergeniculate leaflets (IGL) *geniculohypothalamic tract* (GHT) pathway through gamma aminobutyric acid (GABA) and neuropeptide Y (NPY) [64]. However, the IGL also processes non-photic information such as arousal status received via pathway originating from the dorsal raphe nucleus (DRN), suggesting assimilation of photic and non-photic signals to entrain the SCN [30].

Another important input to the SCN comes directly from both the median raphe nucleus (MRN) and dorsal raphe nucleus (DRN) [96]. Here the serotonergic tract participates in entrainment of the circadian clock by non-photic cues such as physical activity and exercise [30].

Output Pathways from the SCN

Within hypothalamus, SCN axons project to the preoptic area, lateral septum, bed nucleus of the striaterminalis, the subparaventricular zone, and also to the arcuate nucleus and the dorsomedial hypothalamus. In thalamus, efferents from the SCN innervate the IGL and paraventricular nucleus (PVN). Glutamate, GABA, peptide neurotransmitters, AVP, VIP, prokineticin 2 (PK2), cardiolipin-like cytokine, and transforming growth factor α (TGFα) have been shown as SCN output signals [30, 54].

Melatonin, a multitasking molecule, also a messenger of darkness, is secreted from pineal gland and is considered to be an internal zeitgeber which communicates the time information from SCN to all other peripheral clocks through circulation. As the photic information reaches SCN via the RHT, the subsequent activation of signaling pathways lead to induction of clock genes which ultimately results in the regulation of biosynthesis and release of melatonin from pineal. The pathway governing melatonin synthesis is triggered during scotophase in the absence of light. In pineal, tryptophan is converted into serotonin (5-HT) by 5-hydroxytryptophan. Serotonin subsequently undergoes N-acetylation and methylation by arylalkylamine N-acetyltransferase (AANAT) and hydroxyindole-O-methyl-transferase (HIOMT) respectively, ultimately resulting in melatonin synthesis (reviewed in Jagota [55] and Reiter et al. [123]).

The neural circuitry from SCN to pineal gland involves a multisynaptic pathway that includes PVN, intermediolateral cell column (ILCC), superior cervical ganglia (SCG), and finally terminate on pinealocytes and release norepinephrine (NE) which acts on both α1- and β-adrenergic receptors potentiating cAMP production to activate protein kinase A (PKA) and stimulating adenylatecyclase (AC) respectively. PKA phosphorylates cAMP response element-binding protein (CREB) which in turn activates N-acetyl transferase (*Nat*) gene that leads to melatonin synthesis and secretion. However, the cAMP also suppresses *Nat* expression by inducible cAMP early repressor (ICER) which competes with P-CREB [55, 138].

Melatonin exerts its effects and influences cellular physiology majorly by membrane bound G-protein-coupled receptors such as MT1 and MT2. These receptors

regulate cellular processes by inhibition of adenylatecyclase, followed by a decrease in cAMP levels and modulation of PKA activity [123, 160].

Molecular Mechanisms

The molecular mechanism governing the mammalian CTS involves two main processes such as the posttranslational modifications (PTMs) of proteins (e.g., phosphorylation) and the autoregulatory transcriptional–translational feedback loop (TTFL) that consist of tightly interconnected positive and negative limbs [137].The positive limb composed of transcriptional activators BMAL1 and CLOCK (or NPAS2 in brain), hetero-dimerize and bind to the E-box elements present in the promoter of several clock-controlled genes (CCGs) including the clock genes *Period* (*Per1* and *Per2*) and *Cryptochrome* (*Cry1* and *Cry2*). PER-CRY heterodimers enter the nucleus to repress BMAL-CLOCK activity by deacetylating histones 3 and 4 by recruiting PSF/Sin3-HDAC complex [34].The auxiliary feedback loops consist of nuclear receptors REV-ERBs and RORs which are transcriptionally controlled by the BMAL1-CLOCK. RORs activate the transcription of *Bmal1* while REV-ERBs inhibit the transcription of *Bmal1*, there by regulating their own activator [22, 137].

Moreover, the gene coding for adenine dinucleotide (NAD+) synthesis in the mammalian salvage pathway, nicotinamide phosphoribosyltransferase (*Nampt*), is a CCG.NAD+, a metabolic oscillator, modulates the transcriptional activity of clock through a histone deacetylase, SIRT1 [99]. This indicates that the cellular metabolism via NAD+ can feedback to the clock, suggesting an interplay between the elements of clock output and the clock itself [1].

Transcriptional regulation of circadian clock is also controlled by D-box elements [140]. The PAR-Zip transcription factors such as D-box-binding proteins (DBP) which are under the E-box-mediated transcriptional control bind to these elements, and hence, they indirectly regulate CCGs [72].

In addition to transcriptional regulation, PTMs regulate the subcellular localization and stability of PER–CRY complexes [45] allowing progressive and delayed (circa 24 h) maturation of PER/CRY as transcriptional repressors. CK1ϵ and mitogen-activated protein kinase play an important role in the activation or repression of BMAL1, while CK2α and GSK-3β help in cellular localization and proteasomal degradation respectively [75]. In case of PER, site-specific phosphorylation at a "priming site" (FASP site) delays phosphorylation at "sites" (PERs or βTrCP site) that would signal for nuclear entry and degradation by proteasomal pathways [141]. In addition, salt inducible kinase 3 (SIK3 kinase) modulates PER2 phosphorylation rhythms and abundance [47]. Similarly, microRNAs (miRNAs) and several RNA-binding protein complexes regulate RNA stabilization and degradation, circadian polyadenylation and splicing [112]. Overall, the molecular mechanisms underlying the regulation of circadian rhythms in a cell involves numerous complex processes.

Aging and Theories of Aging

Aging is an inevitable unidirectional process that eventually leads to the progressive decline of metabolism, physiology, and behavior and ultimately culminates in death. Aging is explained through a couple of theories. Programmed theory deems the timeline of different stages of life linked to change of metabolism, physiology, and behavior. First theory falls into three subcategories: (i) Programmed longevity – programmed switching of particular genes leading to senescence resulting in overt manifestations. (ii) Endocrine theory – evolutionarily conserved hormonal signaling such as insulin/IGF-1 signaling pathway regulates the process of aging. We have vividly discussed the endocrine regulation of aging elsewhere [59]. (iii) Immunological theory – preprogrammed deterioration of immune system.

Second, damage or error theory postulates that the accumulated damages or errors at several levels over the period of time would cause aging and is linked to metabolic disorders, epigenetic alterations, genomic instability, telomere attrition, loss of proteostasis, altered intercellular communications, cellular senescence, deregulated nutrient sensing, stem cell exhaustion and mitochondrial dysfunction, and DNA damage [82]. Of all the macromolecules that are being damaged as the age progresses, DNA is very important because it cannot be replaced like other macromolecules [38] and also slowing down of DNA repair mechanisms progresses aging process.

Age-Associated Circadian Dysfunction

Age has a marked effect on CTS, which impacts the temporal organization of circadian physiology and behavior (Fig. 20.3, Table 20.1). In humans, fragmented sleep and progressive advance in sleep phase has been recorded in elderly [35, 53]. Similarly, the amplitude of feeding rhythms, secretion of hormones, and body

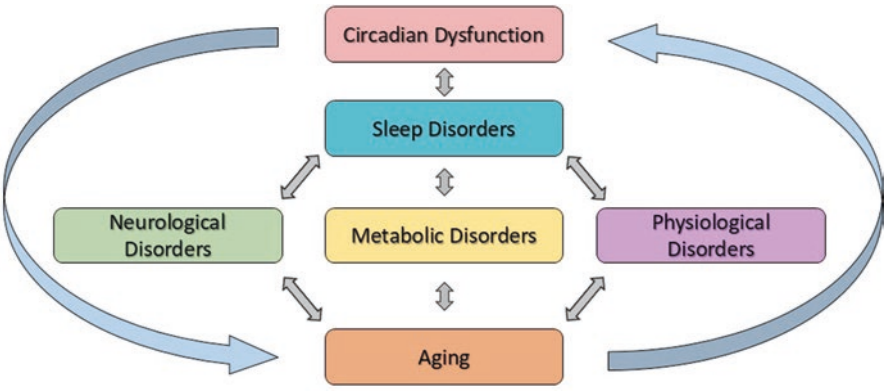

Fig. 20.3 Schematic diagram showing interaction between aging and clock dysfunction

Table 20.1 Effect of clock-associated genes on aging

Animal model	Clock-associated gene	Age-associated phenotypes	Author
Mice	*Clock* mutant	Age-related cataract development	[31]
		Age-related arthropathy	[161]
	Bmal1 mutant	Premature aging	[111]
		Premature aging of hippocampal neurogenic niche	[3]
		Induced severe age-dependent astrogliosis	[98]
		Progression of noninflammatory arthropathy	[15]
		Accelerated prothrombotic phenotype	[133]
		Accelerated age-dependent arthropathy	[158]
	Reduced Sirt1	Reduced amplitude of circadian rhythms in aging	[11]
	Sirt1 mutant	Premature aging	[147]
	Per1/2 mutant	Premature aging	[74]
	Per2 overexpression	Increased expression of aging markers	[147]
Irradiated mice	*Clock* mutant	Accelerated aging	[5]
Drosophila	*Per* mutant	Accelerated aging	[70]

temperature are known to decline with age [149]. In aged animals, decline in locomotor activity rhythms and disrupted sleep–wake cycles suggests age-associated circadian alterations [9]. Reports from mice have demonstrated that the aged animals are more vulnerable to negative effects of photoperiodic phase shifts as the adaptability of circadian system is compromised with aging [7]. In rodents, age-related changes in circadian rhythms have been reported for body temperature, activity–wakefulness, locomotor activity patterns, drinking behavior, and serotonin rhythms [57, 87, 121, 148, 152]. In addition, core clock in aged mice showed diminished response to the external stimuli suggesting CTS deterioration [83].

Influence of Aging on Central and Peripheral Clocks

As the SCN communicates directly and indirectly to various peripheral clocks, circadian clock and aging may be interconnected by pathology at the level of the SCN and SCN output signals [91]. Though there appears no reduction in the total number of cells in aged SCN [50], the age-related loss of amplitude in SCN electrical activity [100] suggests alterations in cellular properties, neuronal circuitry, and clock genes [10]. At single cell level, the neurons of aged SCN showed diminished amplitude of potassium currents and resting membrane potential as a result of possible alterations in large conductance calcium-activated potassium channels (BK channels) [36]. Age-associated alterations in cellular communication in SCN has been evident with reports showing age-dependent loss of neuronal connectivity, marked by decline in synaptic spines and shortened dendrites, altered electrical activity, and altered signaling molecules [107]. Moreover, alterations in the expression of

neuropeptide vasoactive-intestinal polypeptide (VIP) and arginine-vasopressin (AVP) reported upon aging would hamper the SCN output as they are essential for intracellular coupling within the SCN [93]. Disrupted GABAergic signaling in aged SCN indicates clock deterioration [107]. Weakened melatoninergic feedback to the SCN is suggested by reports showing diminished MT1 receptor expression in aged human SCN [145]. Age-linked circadian disruption is significantly contributed by desynchrony between SCN and oscillators in peripheral tissues. Phase-shifting studies in elderly involving exposure to different LD regimens showed decline in the ability to re-entrain in several parameters such as rhythms of activity, rest/sleep and body temperature [51]. Similarly, phase advance study using PER2::LUC mouse demonstrated that oscillators in esophagus, thymus gland and lungs in older mice took longer time period to get entrained to specific light–dark schedule, in comparison with younger mice [128].

Aging is also known to be resulting in declined total melatonin secretion. Studies in humans, primates, and hamsters indicate that the normal nighttime peak in elderly is reduced and phase advanced compared to younger adults [51]. In addition, diminished pineal melatonin synthesis and SCN expression of melatonin receptors have been reported in individuals with Alzheimer's or Parkinson's disease [142]. Severe alterations in daily rhythms and levels of serotonin metabolism in SCN of aged rats and a rat model of PD have been reported from our lab [57, 89, 120]. Similarly, cortisol, a hormone under clock control, which also synchronizes peripheral clocks was observed to show age-related reduction in amplitude and advance in phase [51]. In a recent study analyzing hepatic transcriptome, it was observed that 2626 genes (44.8%) were exclusively oscillatory in young mice whereas in old mice only 1626 genes (28.4%) were rhythmic [126]. Further, age-dependent decline in cyclic global protein acetylation was observed in peripheral clock liver [126]. Recently, from our laboratory, we have reported the age-associated day–night variations of proteins in SCN, substantianigra (SN), and pineal gland of rats [61]. In SCN, the number of proteins showing day–night variations were found to have decreased from 32 (in young adults) to 9 (in old age). Similarly, SN also showed a decrease from 59 to 9. However, in pineal, the number of proteins showing oscillations increased from 51 to 62 [61]. Our earlier studies investigating daily rhythms of lipid peroxidation and antioxidant enzyme activities in rats showed age-dependent variations in liver [87]. Further, reports from our laboratory have demonstrated differential alterations in daily rhythms and levels of NO and *Socs1* expression in various peripheral clocks of aged rats [143, 144] suggesting desynchrony [61].

Influence of Aging on Clock Genes and Proteins

The canonical genes and proteins constituting the TTFL of the core clock machinery show drastic variations upon aging (Table 20.2). We have reported severe alterations in rhythms and levels of various clock genes in the SCN of mid- and old-aged rats [90]. Similarly, studies in aged mice SCN showed changes in *Rev-erb a*, *Dec1*, and *Dbp* expression [12]. Earlier studies in mice showed altered expression of the

Table 20.2 Age-induced circadian rhythm disorders

Sleep-associated disorders	
Advanced sleep phase	[24]
Irregular sleep–wake disorder	[162]
Circadian sleep–wake rhythm disorders (CSRD)	[65]
REM sleep behavior disorder (RBD)	[117]
Insomnia	[39]
Co-occurrence of obstructive sleep apnea and insomnia	[2]
Restless leg syndrome/Willis–Ekbom disease (RLS/WED)	[151]
Free running disorder	[156]
Sleep fragmentation	[77]
Reduced total sleep time	[16]
Reduced slow wave sleep time	[86]
Sleep disordered breathing	[62]
Advanced sleep–wake phase disorder (ASWPD)	[73]
Delayed sleep–wake phase disorder (DSWPD)	[73]
Neurological disorders	
Cognitive decline	[13]
Dementia	[91]
Alzheimer's disease	[78]
Parkinson's disease	[44]
Mood and behavior-related disorders	
Adult attention deficit hyperactivity disorder	[8]
Bipolar disorder	[125]
Depression	[97]
Major depressive disorder (MDD)	[92]
Metabolic disorders	
Cancer	[105]
Rheumatoid arthritis	[27]
Type II diabetes mellitus	[119]
Cardiovascular diseases	[13]

CLOCK and BMAL1 proteins in the SCN, hippocampus, amygdala, and several other brain regions by mid-age [153]. Additionally, altered expression of *Bmal1* and *Per2* transcripts in various non-SCN brain regions in aged hamsters has been reported [32]. Studies on Per1:luc rats showed slight age-related changes in *Per1* expression in SCN, whereas robust changes in peripheral oscillators [155]. Though, reports on rhythmic PER2, PER3, and BMAL1 expression in cortex of elderly humans suggest persistence of clock function in old age [76], altered PER1, 2, 3 rhythms in leukocytes indicated desynchrony [49]. A detailed account on age-linked alterations in the core clock gene expression in the SCN is discussed elsewhere [10].

The mechanisms underlying the modulation of clock gene expression in aging might be involving the cross-talk between circadian and metabolic regulatory systems [116]. The NAD+-dependent protein deacetylase SIRT1 is known to play a significant role in the age-related deterioration of the circadian clock [20]. The role of SIRT1 in the modulation of circadian clock is well known [99] and studies in the SCN of aged mice have demonstrated decline in SIRT1 levels concomitantly with levels of BMAL1 and PER2 [20]. Further, *Sirt1* knockout mice resulted in senescent-like phenotype and *Sirt1* overexpression resulted in antiaging phenotype with respect to alterations in clock [20]. In addition, role of SIRTs in age-associated epigenetic changes in the clock has also gained considerable importance [104].

Therapeutic Interventions

Understanding the molecular components and their feedback mechanisms that are involved in aging and circadian dysfunction helps the researchers to identify the target molecules and to develop the therapeutic drugs to delay the progress of aging and age-associated disorders. Here we discuss few of the potential therapeutic strategies that are showing promising results in combating the aging process and restoring the circadian clock.

Effect of Antioxidants on Aging and CTS

(i) **Melatonin**

The primary function of melatonin is to relay the circadian signals to the peripheral clocks and to synchronize them with the central clock [138]. It is also well established that melatonin relays seasonal temporal information [113]. Melatonin is shown to regulate several clock genes and considered as chronobiotic [59]. It is a multitasking molecule with several properties like anti-inflammatory and antioxidant, and can stimulate antioxidant enzymes like glutathione reductase, glutathione peroxidase, and catalase [87, 118]. Melatonin irregularities have been attributed to several circadian dysfunctions that are involved in cancers [122]. The secretary rhythm of melatonin has been linked with the immune changes and thyroid hormone in aging mice [114]. And also, the nocturnal secretion of melatonin is related to the cell-mediated immunity [84]. The peak expression of IL-1b, IL-2, IL-6, and TNF-α is observed shortly after the maximum melatonin serum levels [25].

In humans, melatonin metabolite 6-hydroxymelatonin sulfate showed similar pattern in both young and healthy centenary subjects; this is evident to claim that melatonin can be a proper aging marker [37]. Melatonin reduces the microglial activity in brain which is considered as anti-inflammaging property [46]. It also has shown to alleviate age-induced memory impairments and neuronal degeneration [130]. Cognitive deficits and neurodegeneration were shown to be alleviated with melatonin administration [103]. Melatonin has also shown the beneficiary effects on

the sleep deprivation-induced memory deficits [4] and also improved sleep efficacy [135]. Further, melatonin supplementation has slowed down the progress of cognitive deficits and also ameliorated the sundowning syndrome in Alzheimer's disease patients [18]. In correspondence to Alzheimer's disease, melatonin has reduced β-amyloid, anomalous nitration of proteins, β-fibrillogenesis, tau phosphorylation, and also increased survival rate of AD transgenic mice [79, 80, 88]. We have recently demonstrated the restoratory effects of melatonin on age-induced alterations in NO daily rhythms and *Socs1* expression in peripheral oscillators [143, 144]. We further demonstrated that melatonin administration could differentially restore the circadian phase, amplitude and the expression levels of clock genes such as *Bmal1, Per1, Per2, Cry1,* and *Cry2* [89, 90] in aged and a rat model of PD. Additionally, we demonstrated age and PD-related changes in the number of oscillatory proteins which shows day–night variations, and the melatonin administration has resulted in differential restoration of these proteins in both aging and PD [61]. In concordance with it, several other researchers have shown the beneficial effects of melatonin in age-associated disorder like Parkinson's disease [19].

The beneficiary effects of melatonin can be attributed to its amphiphilic nature that can easily cross the blood brain barrier [123]. The effect of melatonin on several clock-associated genes has been extensively discussed elsewhere [59].

(ii) **Resveratrol**

Resveratrol (3,5,4′-trihydroxy-trans-stilbene), a polyphenol purified from natural sources is known to modulate CTS [102] and also rescues from various age-related impairments [41]. Studies in nocturnal primate gray lemur further revealed the influence of resveratrol on circadian clock. Resveratrol improved the synchronization of old animals to light–dark cycles and restored the rhythms of locomotor activity and body temperature [115]. Interestingly, few researchers have demonstrated its role in clock-mediated rescue from disorder of lipid metabolism in a rodent model [134]. Corroborative evidence on beneficial effects of resveratrol came from a report where it attenuated the insulin resistance in liver by modulating core clock elements as well as SIRT1 [163]. Considering the importance of clock in healthy aging [28], an earlier study emphasized SIRT1 and its activator resveratrol's role in BMAL1-CLOCK-mediated transcription of clock genes, highlighting its influence on CTS [109].

(iii) **Curcumin**

Curcumin (diferuloylmethane), a potent antioxidant, is a polyphenol derived from rhizomes of *Curcuma longa* and known for its multiple beneficiary effects [129]. It has been reported that curcumin can cross blood brain barrier [139]. Its role as antioxidative, anti-inflammatory, anticancerous, neuro-protective, and clock restoratory agent is widely explored in various animal models [17]. Several studies have also suggested curcumin as a potential SIRT1 activator [157], it could mediate antiaging effects. Reports showing *Bmal1* and SIRT1 activation by curcumin

suggest a modulatory role for curcumin in age and associated circadian disorders [43]. We have shown the influence of curcumin on circadian clock by studying serotonin (5-HT) and its metabolite 5-hydroxy indole acetic acid (5-HIAA) rhythms in the SCN and pineal of rats [58]. Interestingly, a recent study demonstrated a synergistic function of melatonin and curcumin in tumor suppression [131]. Overall, these reports suggest curcumin can be a potential drug candidate in reversing age-linked clock dysfunction.

(iv) ***Withania somnifera***

Withania somnifera (WS), known as Ashwagandha, is known to promote health, enhance longevity, and create a sense of well-being [85]. With various biologically active constituents, the leaf, root, and fruit extracts of the plant have potential regenerative properties and is used for the treatment of various disorders [146]. Several studies have explored the free radical scavenging activity, regulation of lipid peroxidation, glutathione-S-transferase activity, and anti-inflammatory property of WS [63, 108]. Similarly, clinical and preclinical experiments have revealed the potential of WS against cancer, insomnia, anxiety, stress, cognitive, and age-associated neurodegenerative disorders such as AD and PD [68, 85, 127]. Evidence for antiaging effects of WS has also come from studies reporting downregulation of senescence in human fibroblasts and lifespan extension in *C. elegance* [71]. Moreover, we have recently reported the differential restoratory effect of Ashwagandha leaf extract on age-induced alterations in SCN core clock transcript expression rhythms [60]. Our results showed an age-specific action of WS, as we observed restorations in the phase of *Per1*, *Cry1,* and *Bmal1* in the SCN of mid-aged (12 m) rats and only *Per1* in old-aged (24 m) rats [60].

Effect of Calorie Restriction (CR) on Aging and CTS

CR is demonstrated to be a potential strategy in lifespan extension and improved health in various organisms [14]. A recent report elucidating the role of CR in rescuing age-dependent circadian alterations by involving SIRT1 activation in peripheral clock liver [126] corroborated the previous knowledge on antiaging effects of CR [81]. CR, a strong metabolic cue and known to function as a zeitgeber for peripheral clocks has been shown to modulate peripheral gene expression [6, 111]. Studies investigating the SCN VIP expression, pineal melatonin, blood glucose, and locomotor activity rhythms suggested the influence of CR on circadian clock. In addition to it, CR could also synchronize the peripheral clocks and influence clock-controlled output systems, such as the food anticipatory activity (FAA) and body temperature. Further, studies exploring the influence of CR on photic responses suggested a role for CR in entrainment of circadian clock [94]. Transcriptome analysis in various peripheral clocks under CR demonstrated *Per2* as the most upregulated gene in majority of the clocks [136]. Emerging studies have also linked CR with elevated expression of several core clock genes including *Per1*, *Per2*, *Cry2*,

and *Bmal1*. Similarly, some of the key CCGs which code for transcription factors such as *Dbp, Dec1*, *Dec2, Hlf*, *Tef*, *and E4bp4* were differentially affected in under CR [110]. These observations indicate that CR could directly synchronize central clock as well as peripheral clocks and rescue from the age-associated circadian ailments [126]. Interestingly, a recent study using *Bmal1* knockout mice has highlighted the role of BMAL1 in CR-mediated longevity effects [110].

Effect of Small Molecules as Modulators in Aging and CTS

In the recent years, there is an upsurge in the studies related to usage of small molecules as drugs. Through chemical screening approaches more than 2,00,000 small molecules have been identified as circadian regulators that may act as modifiers of period length, phase delay, phase advance, phase attenuation, and amplitude (Table 20.3) [22]. Small molecules such as CKI inhibitors and synthetic ligands for the nuclear receptors CRY1, REV-ERBs, and RORs have been proposed as therapeutic alternatives for several CTS dysfunction [22]. Studies on small molecules would open a new avenue in modulating age-related circadian dysfunctions toward healthy and slowly progressive aging.

Table 20.3 Small molecules modulating circadian clock

Small molecule	Circadian effect	References
KN-62	Targets CaMKII and attenuates phase shifts	[42]
Lithium	Stabilizes REV-ERBα and lengthens circadian period	[159]
PF-670462	Inhibits CK1δ resulting in period lengthening	[95]
L-methyl selenocysteine	Enhances transcriptional activation of *Bmal1*	[52]
SR9009 and SR9011	REV-ERB agonists Altersgene expression and circadian behavior in obese mice	[132]
Compound 5 and compound 6	Induction of cAMP leading to phase delays	[21]
KL001	Stabilizes CRY resulting in period lengthening	[101]
SSR 149415 and OPC-21268	AVP receptors antagonists Accelerate re-entrainment after shift work and jet lag	[154]
Resveratrol	SIRT1 activator Modulates circadian clock	[20]
2-ethoxypropanoic acid	Targets CRY Activates E-box transcription	[23]
Neoruscogenin	ROR agonist Induces *Bmal1* expression	[48]
SR8278	Targets REV-ERB Reduces anxiety and induces maniac-like behavior	[67]
Nobiletin	Targets ROR receptors Increases the amplitude of target gene expression	[40]

Conclusion

Aging is associated with disruption of the chronobiological cycle. CTS undergoes reduced sensitivity to external cues with aging in various physiological, biochemical, and molecular parameters. Numerous clinical studies have established a direct correlation between abnormal circadian clock functions and the severity of neurodegenerative and sleep disorders. Therapeutic interventions using various pharmacological agents such as antioxidants, CR, and small molecules may help to restore CTS dysfunction in elderly.

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