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REVIEW

The suprachiasmatic nucleus: age-related decline in biological rhythms

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Abstract Aging is associated with changes in sleep duration and quality, as well as increased rates of pathologic/disordered sleep. While several factors contribute to these changes, emerging research suggests that age-related changes in the mammalian central circadian clock within the suprachiasmatic nucleus (SCN) may be a key factor. Prior work from our group suggests that circadian output from the SCN declines because of aging. Furthermore, we have previously observed age-related infertility in female mice, caused by a mismatch between environmental light–dark cycles and the intrinsic, internal biological clocks. In this review, we address regulatory mechanisms underlying circadian rhythms in mammals and summarize recent literature describing the effects of aging on the circadian system.

Abbreviations
SCN   Suprachiasmatic nucleus
SPZ   Subparaventricular zone
LD   Light–dark
DD   Constant dark
CT   Circadian time
MUA   Multi-unit neural activity

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Introduction

Sufficient duration of high-quality sleep is important to maintain an individual's physical and mental health. However, it has often been reported that age-related declines in alertness during active and deep sleep phases, as well as fragmented sleep, are observed in elderly adults. Disturbances in circadian rhythms are suspected as the primary cause of age-related changes to the biological clock. Circadian output from the suprachiasmatic nucleus (SCN) of the hypothalamus, the central biological clock, decreases with age [1]. In previous experiments using rodents, loss and reduced rhythmic expression of neuropeptides in the SCN [2], as well as decreased glucose metabolism [3], have been reported. Surprisingly, age-related declines in behavioral rhythmicity can be partially reversed by implanting fetal SCN tissue into the third ventricle of aged animals [4–7], indicating that the SCN plays an important role in age-related behavioral changes. Reproductive functions are also influenced by the aging and circadian process [8]. For instance, daily timing signals were shown to be essential for normal estrous cyclicity, even before researchers discovered the SCN [9]. SCN-lesioned animals do not demonstrate an estrous cycle, and transplantation of fetal SCN tissue cannot restore reproductive functions [10]. We have recently reported that in order to ameliorate early-onset estrous cycle irregularity, and resultant infertility in mice, it is important to synchronize the environmental day/night (light and dark) cycles with each individual's endogenous circadian rhythms [11]. However, disharmony in this cycle and circadian rhythms does not lead to reproductive dysfunction in young adults. Thus, the present review outlines regulatory circadian rhythm mechanisms in mammals. We also discuss aging mechanisms that influence SCN neural circuits and resultant decreases in physiological functions.

Circadian rhythm characteristics

In constant dark (DD) conditions, whereby mice lose their time cues, wheel-running activity starts approximately 15 min earlier each day [12, 13] (Fig. 1a). Activity onset regularly starts earlier thereafter, and the periodicity is extremely accurate. The 24-h behavioral rhythm can be regarded as an exogenous (reactive) rhythm, created by the light period in a day/night cycle. However, the approximately 24-h rhythm that continues at a steady state is an endogenous rhythm. This rhythm indicates the presence of an approximate 24-h cycle mechanism derived from inside the body, a phenomenon that is referred to as "free-running." The free-running period is not exactly 24 h, but the period has the ability to be synchronized into a 24-h cycle. This is referred to as "entrainment." Light responses of the circadian rhythm are time-dependent. Phase response curves for light pulses have been proposed as a model for enabling entrainment of the 24-h period due to the light environment. When a light pulse is applied to a free-running mouse in a constant dark environment early in the active phase (referred to as "subjective night"), activity onset the following day is greatly delayed. Moreover, when a light pulse is applied during late subjective night, or the beginning of a rest phase, the activity onset for the following rhythm is shifted toward an advanced direction. Even if a similar light pulse is applied during the rest phase (e.g., subjective daytime for mice), the phase for

subsequent rhythms does not change [14, 15] (Fig. 1b). This phase-dependent shift in circadian rhythms has revealed differences in factors such as responsiveness to light intensity and degrees of shift among species; however, this is also fundamentally conserved in humans [16, 17] (Fig. 1c).

Aging effects on activity rhythms

Aging is commonly associated with changes to the quantity and quality of sleep, as well as rates of acquired sleep disorders (including sleep episode fragmentation), in humans and other mammals [18–20]. In rodents, aging induces changes to locomotor activity rhythms, including decreased amplitudes, increased fragmentation, shortened or lengthened free-running periods, slower re-entrainment following LD cycle shifts, and altered light sensitivity [21–24] (Fig. 2). For aged mice, a distinct "variation" between active and rest is lacking during both the night (active phase) and day (rest phase), respectively. Furthermore, early morning awakening in humans can be regarded as reflecting a change in the entrainment phase angle due to age-related changes to the endogenous period and photic response. Based on the similarities described above, examinations of neural mechanisms using rodents as an aging model are currently underway.

Fig. 1 Circadian rhythm characteristics. **a** A representative double-plotted actogram of wheel-running activity in a C57BL/6J mouse showing entrainment to the LD cycle, free-running under DD, and phase responses to 6-h light pulses. **b** Phase response curves detailing wheel-running activity rhythms for 6-h light pulses in male mice. The activity onset time was defined as CT; circadian time, 12. The free-running period is set to 24 circadian-hours. **c** Phase response curves of human sleep rhythms for 3 h of bright light. *Open circles* by Honma and Honma [16] and *closed circles* by Minors et al. [17]
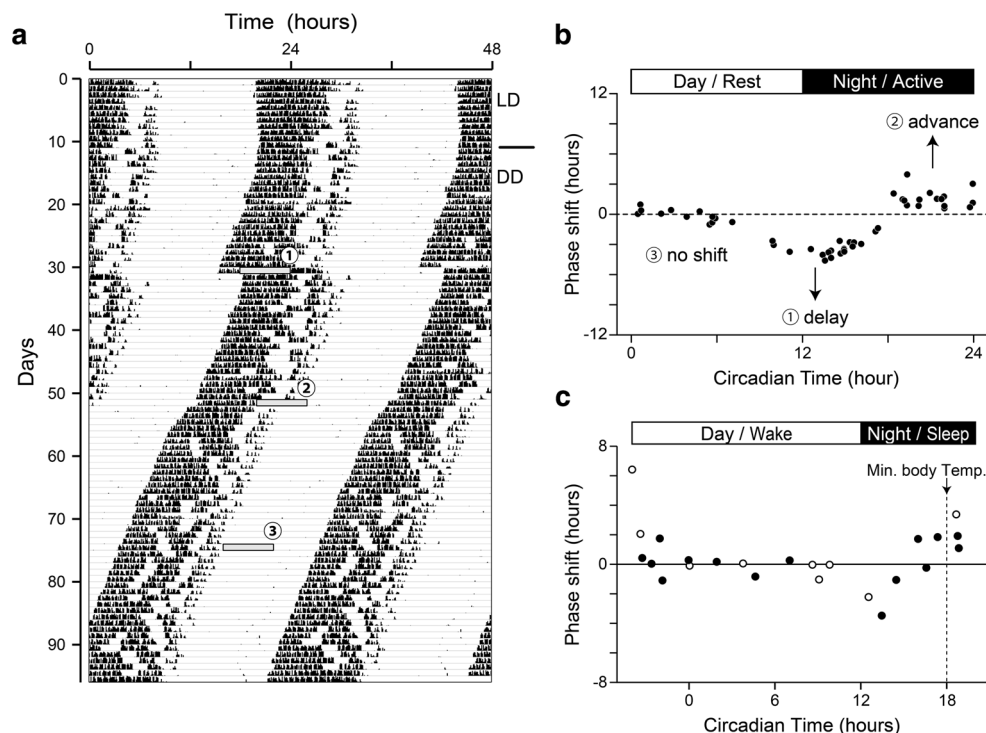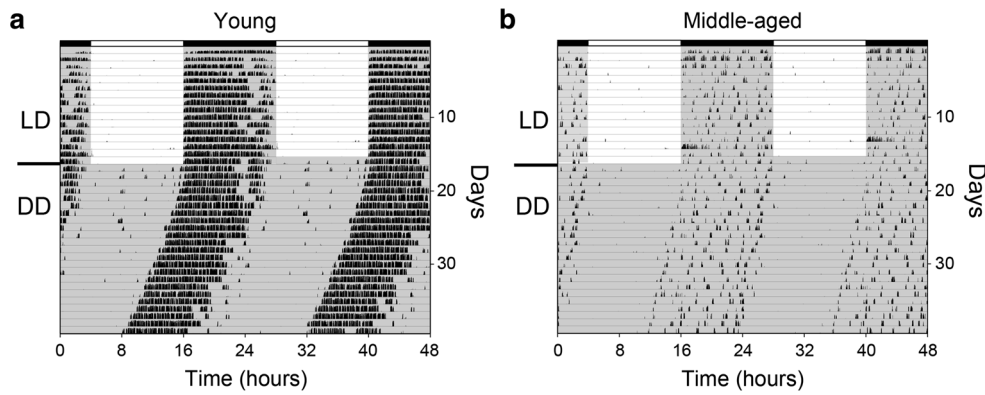
Fig. 2 Reduced behavioral circadian rhythms in middle-aged mice. Representative double-plotted actograms of wheel-running activity in C57BL/6J mice. These *plots* show the effects of aging on circadian rhythms influencing locomotor activity for both young (**a**) and middle-aged (**b**) mice. The mice were maintained in an LD cycle for 2 weeks and then transferred into DD. Light conditions are indicated at the *top* of the figure; *open bars* are light phases, and *closed bars* are *dark*

The SCN regulates behavioral circadian rhythms

The SCN is located just above the medial portion of the optic chiasm on the left and right side of the third ventricle (Fig. 3a). The SCN's size in mice is approximately that of a poppy seed; however, because of its characteristic structure of nearly 8000, tightly packaged neurons, identification is relatively easy. When the SCN is experimentally lesioned, behavioral circadian rhythms and photic entrainment are completely lost [25] (Fig. 3c). However, behavioral circadian rhythms recover when an SCN obtained from fetuses or newborns is transplanted into a lesioned animal [26–28].

Fig. 3 The SCN regulates behavioral circadian rhythms. **a** The SCN in a coronal section (50-μm thickness) of the hypothalamus stained by neutral red. The *scale bar* indicates 200 μm. A pair of nuclei with densely packed neurons is apparent in the middle base of the brain. **b** A male mouse on a running wheel. **c** A representative actogram of wheel-running activity in an SCN-lesioned mouse under an LD cycle. The nocturnal (night-active) rhythm has disappeared except for an ultradian rhythm, with a period of 2–4 h. Light condition is indicated at the *top* of the figure; the *open bar* is a light phase, and the *closed bar* is *dark*

Results of an experiment using mutant hamsters with a naturally occurring "tau"; circadian period, mutation (which creates genetically shortened circadian free-running periods; [29]) indicates the SCN's importance as the circadian pacemaker. When a wild-type SCN that possessed an approximate 24-h cycle was transplanted into hamsters with tau-mutant SCN lesions, behavioral rhythm cycles returned to 24-h cycles. Conversely, when a tau-mutant SCN is transplanted into hosts with wild-type SCN lesions (causing a lack of periodicity), short-period behavioral circadian rhythms are recovered [30]. Results of SCN transplants with different genetic free-running periods provide conclusive evidence that the SCN is the circadian pacemaker regulating behavioral circadian rhythms. Moreover, an experiment assessing a transplanted SCN within a semipermeable polymeric capsule before transplantation (which prevents neural outgrowth but allows diffusion of humoral signals) indicates that behavioral circadian rhythms are not controlled by neural projections and synapses. Rather, circadian rhythms function through the regulation of secreted substances from the transplanted SCN [31]. Moreover, it has become apparent that both behavioral activation and inhibiting factors related to the SCN functions in a coordinated manner [32].
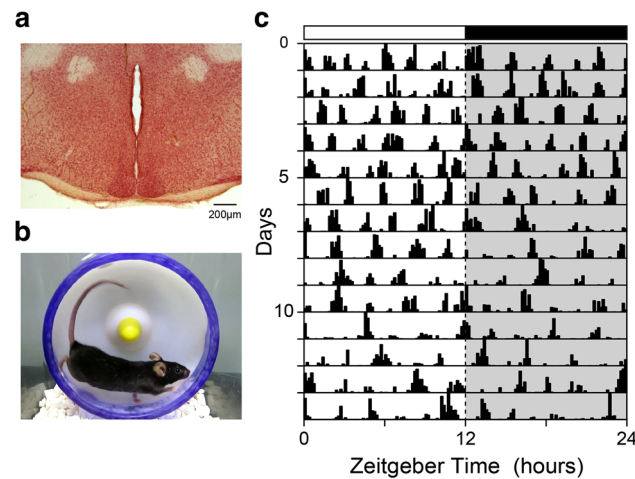
Multiple circadian oscillators in the SCN

Since the identification of the mammalian clock genes—*period1* and *clock*—in 1997 [33, 34], mechanisms underlying circadian rhythms generated by the molecular clock have been considered to consist transcriptional-translational feedback loops. These loops involve clock genes that exist within single cells. Two years before our initial understanding of this molecular mechanism, Welsh et al.

[35] dissected the SCN from a rat that was treated with enzymes, dispersed, and cultured. The spontaneous firing rates of individual SCN neurons revealed circadian patterns, and these patterns revealed that each cell had its own period (Fig. 4a, c). These results indicated that the smallest circadian unit is inherent within a single cell. Although it is possible to reveal a circadian rhythm in a single cell, without the underlying SCN mechanism available for synchronizing individual rhythms, the SCN is unable to produce a stable, holistic rhythm. Even if 8000 SCN neurons oscillating within their own rhythm gathered (without coupling) in the SCN region, misalignment would eventually occur in regards to neuronal firing. This would lead to constant firing throughout the day. In fact, SCN neurons dispersed in a culture oscillate the circadian rhythm with their individual cycles. However, individual cells in an SCN slice culture exhibit the same coordinated circadian periods between neurons [36–39] (Fig. 4b, d).

Importance of neural output from the SCN

How does a timing signal from the SCN regulate physiological functions, including behavioral circadian rhythms? In an experiment where tetrodotoxin, a voltage-gated Na^+

channel blocker, was perfused near the SCN, it was shown that behavioral circadian rhythms in a rat disappeared [40]. This result indicates that neural firing (action potentials) is important for the SCN rhythm output. In another study, we inserted two stainless wires (100 μm in diameter) into the SCN and constructed an experimental system (in vivo multi-unit neural activity recording: MUA) to record neuronal activity in freely moving mice with a running wheel [41]. Neuronal groups in the SCN actively fired during the daytime (light period), the rest period, but firing frequency gradually decreased from several dozen minutes before the light was turned off and remained at a low level during the night. Conversely, SCN neuronal firing frequency gradually increased from approximately 1 h before the light was turned on, and a high firing level was maintained during the daytime. Additionally, we observed that even when the action rhythm was obtained during free-running under DD conditions, the neuronal firing rhythm that was active during the subjective day (and decreased during the subjective night) was maintained. In nocturnal rodents, with reference to the day-active neural activity in the SCN, behavioral inhibiting factors have been looked for. To date, three SCN-secreted factors have been proposed and all of them show an expression peak during the day, the time of behavioral resting phase and in parallel to the neural
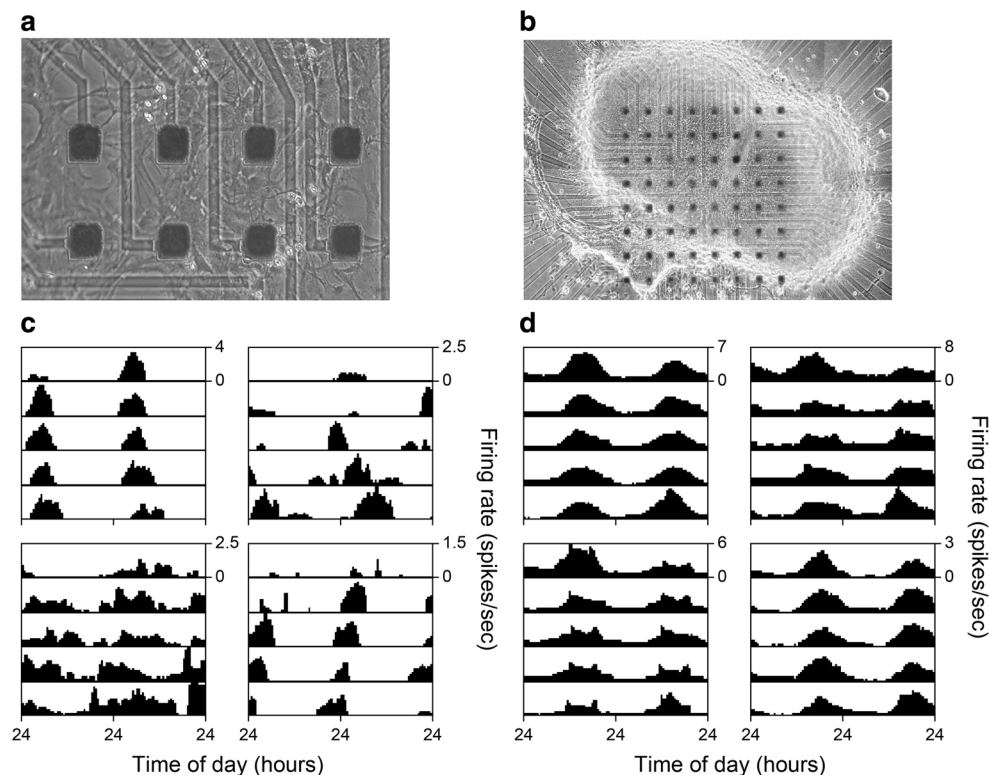

Fig. 4 Multiple circadian oscillators in the SCN. a, b Phase-contrast photomicrograph of SCN neurons in a dispersed cell culture (a) and slice culture (b) on a multi-electrode dish. The *black squares* indicate the 64 electrodes with a size of 50 μm square, with 150 μm separation (a), and 20 μm square, with 100 μm separation (b). c, d Circadian firing rhythms of four representative SCN neurons from a dispersed cell culture (c) and a slice culture (d). *Numbers* on the *right* margin of the first *lane* indicate the firing rate (mean spikes/s in 15 min)

rhythm [42–44]. The main nerve projection path from the SCN goes through the subparaventricular zone (SPZ), dorsally adjacent to the SCN, and reaches the dorsomedial hypothalamic nucleus (DMH) [45]. This is where time information is sent to the central nervous system, which is responsible for various biological functions related to the DMH [46]. A cell-specific lesion study indicated that the dorsal part of the SPZ is relaying circadian signals from the SCN for body temperature rhythms, on the other hand the ventral part is responsible for rhythms of sleep and loco-motor activity [47]. In other words, the SCN-SPZ output system reflects the major SCN pathway for circadian rhythms of sleep, locomotor activity, body temperature, and furthermore reproductive functions.

Age-related declines in SCN output

We measured in vivo MUA in aged mice [1]. SCN neu-ronal firing rhythms in aged mice are generally maintained at a high level during the day and at a low level at night.

However, the neural firing frequency variance during measurement time bouts (1 min) is large, and day–night variations are diminished. These results demonstrated that circadian rhythm amplitudes significantly decreased in aged relative to young mice (Fig. 5). This decrease in amplitude is similar in the SPZ; these results suggest that the behavioral decrease in circadian rhythms in aged mice could be due to decreased functioning in the output system from the SCN [1]. Furthermore, single cell imaging for PER2::LUCIFERASE reporter mice using an ultra-sensi-tive CCD camera system revealed that each SCN cell's rhythm was approximately normal, but each rhythm was dissociated [48]. These results demonstrate that SCN cells can normally produce circadian rhythms, but due to decreased functioning in the neural network inside the SCN, the SCN output system is weakened. Given that significant decreases in activity rhythms associated with aging were not observed for degenerated dopamine neurons in the substantia nigra [49], it appears that the circadian aging mechanism is not due to the molecular clocks in the individual SCN cells. Rather, this mechanism is due to a

Fig. 5 Reduced MUA rhythms in the SCN of middle-aged mice in vivo. Representative serial-plotted actograms of neural and locomotor activity showing diurnal and circadian rhythms of MUA in the SCN of young (**a**) and middle-aged (**b**) mice. Light conditions are indicated at the *top* of the figure; *open bars* are light phases, and *closed bars* are *dark*. The *bottom* trace represents simultaneously recorded locomotor activity. The number of spikes for MUA or locomotor activity was counted every minute
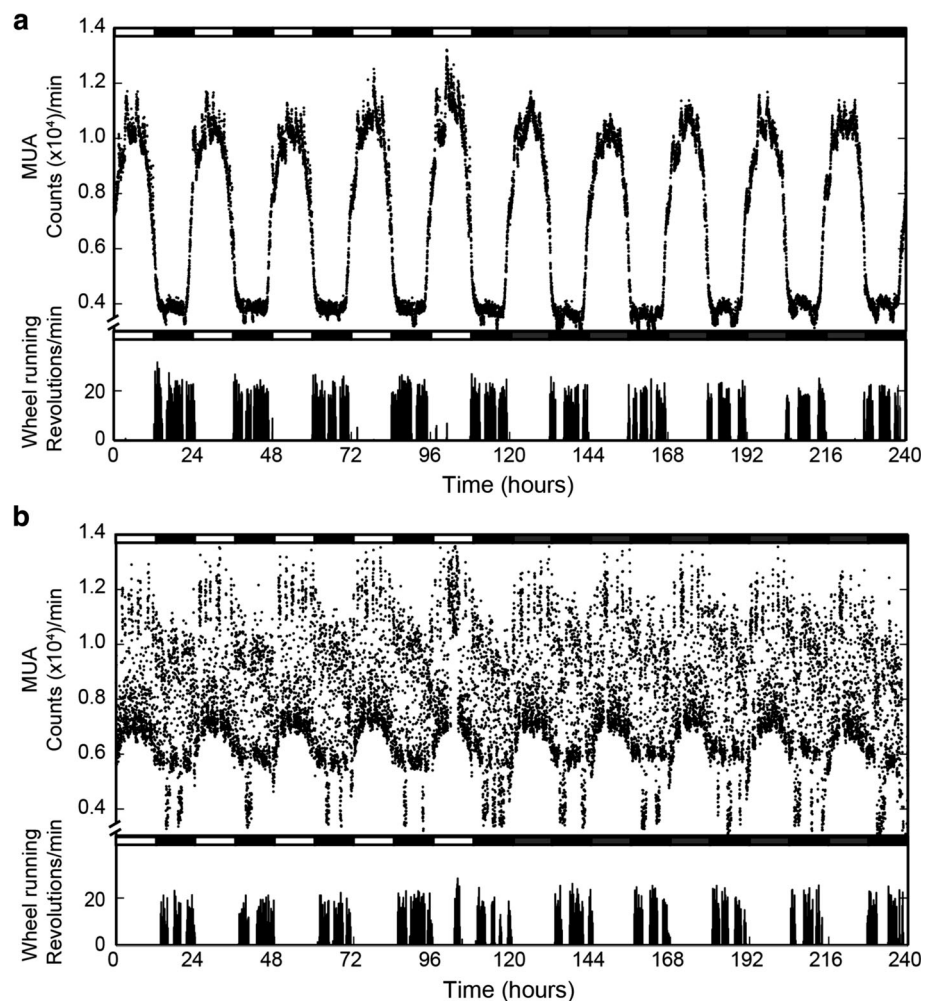
decrease in the SCN output rhythm associated with diminished neural networks in the SCN.

## Circadian fluctuations during the estrous cycle

Humans have a menstrual cycle of approximately 28 days, however, rodents (such as rats, mice, and hamsters) have estrous cycles that range from 4 to 5 days and are divided into four phases: proestrus, estrus, metestrus, and diestrus. Estrogen levels in the circulating blood increase rapidly from the beginning of the proestrus light period, a subsequent mass secretion of gonadotropin (LH; luteinizing hormone, surge) induces ovulation, and progesterone concentration also rises [50]. However, behavioral rhythms increase at night, from the proestrus to estrus phases, and progress toward the active period. This phenomenon is referred to as "scalloping". Accelerations in activity and phase advances are only observed during a transition from the proestrus to the estrus and are not seen during other stages [51]. Scalloping was observed in wheel-running activity rhythms from normal, young, female, experimental mice. Molecular machinery of the circadian rhythm, which has been observed in almost every organ of the body, can autonomously exhibit a circadian rhythm in each organ [52, 53]. Molecular clocks are also present in organs that comprise the hypothalamus-pituitary-gonadal axis (HPG-axis) responsible for estrous cycle regulation [54, 55]. We compared the expression rhythm of clock genes (*Per1*, *Per2*, and *Bmal1*) in the SCN and peripheral tissues using in situ hybridization and quantitative real-time PCR. Results revealed that the SCN is largely unaffected by the estrous cycle, but estrous cycle effects were observed in peripheral tissues (liver, uterus, and ovaries). Although the types of genes and tissues were not constant, when we focused on the expression of *Per2* mRNA in the uterus and ovaries, the rhythm amplitude during the proestrus stage

was large, and the phase had advanced in comparison with that in the other stages [56]. Furthermore, we confirmed through in vivo and in vitro experiments that these changes in clock gene expression rhythms during the estrous cycle are due to the effects of estrogen and progesterone [57, 58]. In other words, quantitative changes in ovarian steroid hormones associated with the estrous cycle are effective in influencing timing. Thus, it appears that desynchronization between the SCN and other tissues affects the female reproductive system.

Interactions between circadian rhythms and the estrous cycle

We recently reported a relationship between circadian rhythms and reproductive functioning in female animals. During the early aging stage of clock gene knockout mice (*Cry* KO mice), in which the shortening (22.5 h, for *Cry1* KO) or lengthening (24.5 h, for *Cry2* KO) of genotype-specific circadian periods are known, estrous cycle irregularities and resultant infertility were observed [10]. When the light–dark environmental cycles were adjusted to intrinsic periods of the biological clock, the estrous cycle irregularities were ameliorated, and successful pregnancy rates dramatically increased. Moreover, when wild-type mice with no genetic defects were placed in light–dark environmental conditions that caused "weekly jet lag" during "middle age", in which normal pregnancies and birth are possible (8–12 months of age), the regular estrous cycle diminished and became irregular (Fig. 6). These results indicate that age-related changes in early onset estrous cycle irregularities and resultant infertility are strongly dependent on biological clock functioning. Desynchronization between the environment and the biological clock (i.e., "circadian timing shift") has a major impact on female reproductive functions.

Fig. 6 Interactions between circadian rhythms and the estrous cycle. A scheme for age-related decline in biological rhythms. Stable circadian rhythms are essential for regular estrous cycles (**a**). Age-related declines in circadian output of the SCN and environmental perturbations of LD cycles may be potential risk factors for irregular estrous cycles and resultant infertilities (**b**)
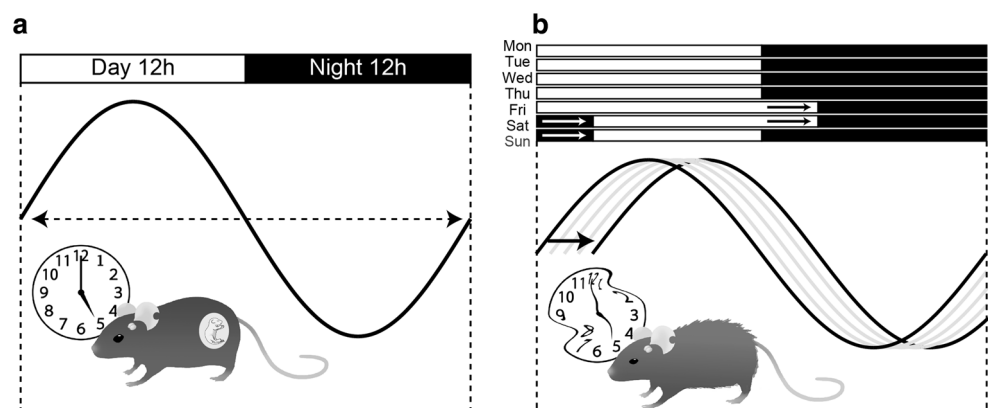
Conclusions

The SCN autonomously produces circadian timing in mammals and adjusts the biological clock on the basis of this timing information from environmental cycles. The SCN then acts like a control tower by outputting timing signals to the peripheral clocks responsible for various physiological functions. In other words, the SCN's clock mechanism is important for predicting changes in the environment and taking appropriate actions at proper times. Currently, the effects of aging on the SCN are unavoidable. However, our data shows the importance of circadian output from the SCN, and an appropriate light environment, for ameliorating age-related changes to certain physiological functions [59, 60]. Although aging in mice may well differ from aging in humans, our analysis reveals a hidden vulnerability in the SCN, suggesting that the aging circadian system is more sensitive to environmental challenges than previously suspected. We speculate that this vulnerability plays a role in age-related circadian dysfunction, including reproductive system issues. If our speculations are correct, these findings re-enforce the importance of living in a temporally structured environment.

Compliance with ethical standards

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