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Timing - Understanding Central and Peripheral Clocks
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Stepien JM,* Coates A, Banks S.*

a Sleep and Chronobiology Laboratory, Behaviour-Brain-Body Research Group, UniSA: Justice & Society, University of South Australia, Adelaide, South Australia

b Alliance for Research in Exercise, Nutrition, and Activity, UniSA: Allied Health & Human Performance, University of South Australia, Adelaide, South Australia

Abstract

From the discovery of the first clock genes outside of the ‘master clock’ – the suprachiasmatic nucleus (SCN) – to now, there has been extensive research into the location of these peripheral clocks and how they relate to the SCN and other timing signals. The purpose of this review is to provide an overview of the current knowledge in this area. Areas discussed will include: How the timing of sleep and wake in mammals is controlled by the central clock; how physiological processes during sleep and wake in mammals are coordinated by peripheral clocks; what changes in environmental signals affect the timing of SCN and peripheral clocks; how we measure central and peripheral clock timing; which environmental signals can entrain the SCN and peripheral clocks; and how disturbances in central and peripheral clock timing due to aspects of modern lifestyles including shiftwork and jet lag, as well as biological aspects such as blindness and chronotype, may have negative impacts on our health. By understanding how our biological timing systems work, we may be able to develop strategies to minimise disturbances in central and peripheral clock timing and therefore the associated negative health outcomes observed.

Keywords

Circadian rhythm; suprachiasmatic nucleus; peripheral clocks; clock genes; circadian disruption

*Corresponding author.
Jacqueline.Stepien-Hulleman@mymail.unisa.edu.au
1. Introduction

This review is designed to give an overview of the current understanding of how the body coordinates functions over the 24 hour period. It will look at why a timing system is advantageous, how timing is coordinated in mammals, what factors may influence timing in mammals, how we measure timing, and how disturbances in timing may be responsible for negative health outcomes in humans.

The day/night cycle offers a stable and dependable measure of time for a species to segment activity to regular periods. Different species have adapted to different sleep/wake patterns (such as being diurnal or nocturnal) due to a number of factors. Doing so favours survival by maximising activity efficiency – increasing activity when food and prey availability is maximal and predator risk is minimal, and decreasing energy expenditure when food and prey availability is minimal and predator risk is maximal (1). In turn, when an organism is active, metabolic processes are coordinated to harvest energy. During rest periods, metabolism switches to releasing energy stores to ensure adequate energy is available for various maintenance processes.

When mammals are not awake and seeking out food they use a large amount of their rest time to sleep. There are a number of theories that support the importance of sleep and the functions that occur during the sleep period. Sleep has been proposed to assist with a number of processes including brain plasticity (2), learning, and memory (3), thermoregulation providing brain and body cooling (4), tissue repair (5), immune functioning (6), energy conservation (7), and neuronal detoxification (8).

Adult humans, on average, spend 36% of time asleep and 64% awake (9). It has been demonstrated that when humans do not get enough sleep on a regular basis they are less able to maintain a healthy energy balance. Five days of inadequate sleep has been found to lead to an approximate 5% increase in total daily energy expenditure as well as an increase in energy intake above what is required to maintain energy balance (10). In relation to general health and wellbeing sleep quality, rather than sleep quantity, positively correlates with measures of physical and psychological health (11).

2. The timing of sleep and wake in mammals is controlled by the central clock

Although mammals have different sleep/wake patterns, mechanisms of circadian timing are highly conserved. The central clock is located in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus of the brain. The cells of the SCN contain a number of ‘clock’ genes that are able to keep time by transcriptional/translational feedback loops that operate on an approximate 24 hour cycle (12). The primary genes involved in this process are Clock (circadian locomotor output cycles kaput), Bmal1 (brain and muscle arnt-like protein-1, also known as Arntl), Cry (cryptochrome) 1 and 2, and Per (period) 1, 2, and 3 for the primary positive loop. Secondary loop genes include Rev-ERBA (reverse strand of ERBA alpha, also known as Nr1d1), Rev-ERBB (reverse strand of ERBA beta, also known as Nr1d2), RORα (retinoic acid receptor-related orphan receptor alpha, also known as Nr1F1), RORβ (retinoic acid receptor-related orphan receptor beta, also known as Nr1F2), and RORγ (retinoic acid receptor-related orphan receptor gamma, also known as Nr1F3). When cultured in the lab, SCN cells show robust rhythmicity for several weeks (13).

In the absence of time cues, the central clock has its own genetically determined ‘free run’. This means that without any indication of what the time of day is, the SCN is able to generate a regular rhythm to maintain the timing of physiological processes within an organism to maintain homeostasis. In rats this ‘free run’ has been reported to be around 24.4 hours (14) and in mice around 23.6 hours (15) while in humans the average free run period is 24.2 hours (16-18). The primary zeitgeber (timegiver) in mammals is sunlight that synchronises, or entrains, the central clock every 24 hours to ensure physiological processes align with the environmental light/dark cycle (Figure 1). Light entrains the SCN utilising non-visual pathways via the retina to regulate the transcriptional/translational feedback loop by activating the transcription of Per genes.

In both animal models and humans, changes in light exposure have been shown to re-entrain, or phase shift, SCN activity in a dose dependent manner. Phase-response curves of locomotor activity in rodents (20), melatonin in rats (21) and humans (22), and core body temperature in humans (23)
Figure 1. The non-visual pathway of light from the environment to the suprachiasmatic nucleus (SCN) and how it regulates the timing of the transcriptional/translational feedback loop (19). Once the light signal reaches the cells of the SCN, it is the induction of *Per1* and *Per2* gene expression that synchronises SCN timing with the environment.

in response to light show that when kept under constant lighting conditions, only light presented during subjective darkness (the time that the SCN is signalling to the body that it is night time) can phase shift these rhythms. Light presented during the subjective day (the time that the SCN is signalling to the body that it is daytime) has no phase shifting effect. When light is presented during the first half of the subjective night, the onset of the phase marker measured will delay (occur later) the following day. Conversely, when light is presented during the second half of the subjective night, the onset of the phase marker being measured will advance (occur earlier) the following day. It has also been shown that the degree of phase shift light exposure can elicit depends on the time of exposure (22), length of exposure (24), and intensity of exposure (25). It has also been shown that the wavelength of light presented can affect the degree of phase shift with shorter wavelengths (blue/green) more effective than longer wavelengths (26), and that intensities as low as 50 lux can both acutely suppress SCN activity and phase shift SCN activity on the following night (25).

While the evidence above demonstrates that the SCN is the master clock that signals to the rest of the body environmental light information, there is also evidence that clock gene expression is not exclusive to the SCN.

3. Physiological processes during sleep and wake in mammals are coordinated by peripheral clocks

Along with the SCN, there are a number peripheral clocks that have been identified in almost all organs. These clocks also use the transcriptional/translational feedback loop system to coordinate physiological processes at the cellular, organ, and system level. Unlike the SCN, peripheral clock cells grown in the lab lose their rhythmicity after a few days (13, 27). Furthermore, studies in rats (28) and mice (29, 30) where the SCN has been removed have demonstrated that in the absence of the SCN, peripheral clock gene expression is no longer rhythmic. As a result, peripheral clocks need to receive signals from the SCN to maintain rhythmicity and this is achieved via a number of different pathways, evidence of which is detailed below.
3.1. The Autonomic Nervous System

The autonomic nervous system provides a direct pathway between the SCN and target organs. Identified targets include the submandibular salivary glands (31, 32), liver (33-35), pancreas (31), lung (36) and adrenal glands (31, 37, 38). The regulation of these tissues, both sympathetic and parasympathetic, have been directly traced from the SCN to target peripheral tissues (39).

3.2. Melatonin

Melatonin is a hormone produced and released into the circulation by the pineal gland. This release is controlled by the SCN via the sympathetic nervous system with minimal levels circulating during the day and maximal levels reached during the night. Melatonin synchronises peripheral clocks with the SCN, acting as a global zeitgeber that controls the transcription/translation cycle of peripheral clock genes. This has been demonstrated in both cell culture (40) and in animal models (41). The mechanism by which melatonin synchronises peripheral clocks has not yet been elucidated but it has been suggested that melatonin may mediate its effects on peripheral clocks via post-transcriptional processes (42) and this in turn, may explain why phase shifts occur more slowly in peripheral clocks than in the SCN.

3.3. Glucocorticoids

Glucocorticoids (corticosterone in rodents, cortisol in humans) are hormones produced in the adrenal gland and this release is controlled by the SCN via the sympathetic nervous system. Glucocorticoid response elements have been found to be located in the regulatory regions of mouse core clock genes Bmal1, Cry1, Per1 (43, 44), and Per2 (45) suggesting that glucocorticoid binding may lead to the transcriptional activation of these and other clock genes (46). While glucocorticoid receptors are expressed throughout the brain and periphery, the SCN does not express these receptors (47). On their own, circulating glucocorticoids have been shown in the rat liver to maintain peripheral clock gene rhythmicity in the absence of autonomic nervous system input from the SCN or feeding signals, albeit with lower amplitude of expression (48). Exogenous administration of the glucocorticoid analogue dexamethasone has been shown to phase shift clock gene rhythms in rat peripheral tissues grown in the laboratory (49).

3.4. Temperature

The SCN controls the core body temperature rhythm in mammals (50). It has been shown that rat fibroblasts cells grown in the laboratory can maintain previously induced rhythms when exposed to the natural temperature rhythms measured in mice for a number of days compared to fibroblasts maintained at a constant temperature, but not establish a new rhythm in these cells (51). Temperature effects are most likely mediated by heat shock factor 1 (HSF1) which has been shown to oscillate in liver cells and modify its rhythm in line with temperature changes (52). The use of HSF1 inhibitors have been demonstrated to stop the shift in transcription of clock genes due to changes in temperature rhythms (53).

3.5. Behaviour

Behavioural processes such as locomotor activity and feeding are also influenced by the SCN in mammals (54, 55). These in turn can influence endocrine activity and body temperature. Under unrestricted feeding conditions, activity and feeding follow a regular rhythm in line with the SCN (and light/dark cycle).

As shown above, the SCN employs a number of ways to communicate environmental light information to the peripheral clocks to ensure physiological processes are appropriately coordinated during sleep and wake.
4. The measurement of central and peripheral clock timing

By measuring the timing of the acrophase (or peak) of clock gene expression researchers can compare the timing of different clocks within an organism. It has been demonstrated in mice that the *Per1* expression acrophase occurs at different times in different tissues (56) with the acrophase occurring in the SCN at zeitgeber time (ZT)6.5, the lung at ZT11.8, the liver at ZT15.1, and skeletal muscle at ZT15.8 (Figure 2).

Similar phenomena occur in humans. It has been demonstrated that different peripheral tissues have different acrophases for the same clock genes. Bjarnason and colleagues measured *Per1* expression in oral mucosa and skin and found that expression peaked at ZT0.5 and ZT6.7 respectively (57). Differences have also been demonstrated between leukocytes and beard hair follicles (58). In human cultured epidermal cells, it has also been demonstrated that different clock genes have different acrophases within the same cell types and that different cell functions can be mapped to these differing peaks (59). The cycling of gene expression may allow the separation of chemically incompatible reactions, for example, ensuring glycogen synthase and glycogen phosphorylase expression does not occur at the same time so that glycogen stores are built up when energy is being absorbed and broken down when energy is no longer being taken up (60). Peripheral clocks have been measured in a range of human tissues obtained from live individuals (Table 1).

Figure 2. The differences in the timing of peak *Per1* expression in central and peripheral clocks as measured in mice over the 24 hour period drawn from data in Yamanaka et al. (56). This demonstrates that under normal circumstances the timing of *Per1* expression in each tissue is not precisely synchronised. Zeitgeber time (ZT) 0 is the time of exposure to the timegiver (in this case time of lights on). SCN=suprachiasmatic nucleus.
Table 1. Human tissues where primary and secondary loop clock gene expression has been measured and the clock genes that were examined.

<table>
<thead>
<tr>
<th>System</th>
<th>Tissue/Cell</th>
<th>Clock Gene Measured</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integumentary</td>
<td>hair follicle</td>
<td>Bmal1</td>
<td>Per1</td>
</tr>
<tr>
<td></td>
<td>oral mucosa</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td></td>
<td>skin</td>
<td>Bmal1</td>
<td>Per1</td>
</tr>
<tr>
<td></td>
<td>skin fibroblasts</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td></td>
<td>skin keratinocytes</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td></td>
<td>skin melanocytes</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td></td>
<td>epidermal stem cells</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td>Circulatory</td>
<td>whole blood</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>monocytes</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>mononuclear blood cells</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>leukocytes</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>lymphocytes</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>mast cells</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>eosinophils</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>CD4+ T-cells</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>polymorphonuclear cells</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td></td>
<td>papillary muscle cells</td>
<td>Bmal1</td>
<td>Cry1</td>
</tr>
<tr>
<td>Endocrine</td>
<td>pancreatic islet cells</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>visceral adipose tissue</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>subcutaneous adipose tissue</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td>Bone</td>
<td>CD4+ (haematopoietic) bone marrow cells</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
<tr>
<td></td>
<td>whole bone marrow cell samples</td>
<td>Clock</td>
<td>Bmal1</td>
</tr>
</tbody>
</table>

Note: Clock (circadian locomotor output cycles kaput); Bmal1 (brain and muscle arnt-like protein-1); Cry1 (cryptochrome 1); Cry2 (cryptochrome 2); Per1 (period 1); Per2 (period 2); Per3 (period 3); Rev-ERBα (reverse strand of ERBA alpha); Rev-ERBβ (reverse strand of ERBA beta); RORA (retinoic acid receptor-related orphan receptor alpha).
Table 2. Evidence for the effect of identified entrainers on central and peripheral clock timing in humans and animal models.

<table>
<thead>
<tr>
<th>Tissue where the clock is found</th>
<th>Model</th>
<th>Light</th>
<th>Activity</th>
<th>Temperature</th>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN</td>
<td>Animal</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No (timing) yes (caloric restriction 66%)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Yes</td>
<td>Yes (partial)</td>
<td>-</td>
<td>No (timing) yes (caffeine)</td>
</tr>
<tr>
<td>Liver</td>
<td>Animal</td>
<td>Yes - rat and mouse</td>
<td>Yes - mouse</td>
<td>Yes/no - mouse</td>
<td>Yes (timing) - rat and mouse yes (caffeine) - mouse</td>
</tr>
<tr>
<td>Pineal Gland</td>
<td>Animal</td>
<td>Yes - rat</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adrenal Gland</td>
<td>Animal</td>
<td>Yes - rat and mouse</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Assumed (salivary cortisol)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heart</td>
<td>Animal</td>
<td>Yes - rat</td>
<td>-</td>
<td>-</td>
<td>Yes (timing) - rat and mouse</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>Animal</td>
<td>Yes - rat and mouse</td>
<td>Yes - mouse</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>-</td>
<td>Possible</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>Animal</td>
<td>Yes - mouse</td>
<td>Yes - mouse</td>
<td>-</td>
<td>Yes (timing) - rat yes (caffeine) - mouse</td>
</tr>
<tr>
<td>Hair Follicle</td>
<td>Human</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>Animal</td>
<td>-</td>
<td>-</td>
<td>No - mouse</td>
<td>No (timing) - mouse yes (caffeine) - mouse</td>
</tr>
<tr>
<td>Submandibular Gland</td>
<td>Animal</td>
<td>-</td>
<td>-</td>
<td>No - mouse</td>
<td>-</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Animal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unsure (timing) - mouse</td>
</tr>
</tbody>
</table>

Note: - indicates that no experiments have been done for this entrainer in this tissue. For food, (timing) refers to timed restricted feeding.
5. Is light the only signal that can entrain the SCN and peripheral clocks?

As shown above, light synchronises SCN activity. Recent work in animal models has looked at whether a change in light exposure can produce phase shifts in peripheral clocks similar to those in the SCN. When a one-hour light pulse is presented to rats during the subjective night, immediate changes to clock gene expression are seen in the pineal gland, adrenal gland, liver, heart, and muscle though the changes are not uniform in each tissue and are dependent on the time the light pulse is delivered (35). In mice, it has been shown that light exposure during the subjective night caused an immediate increase in Per1 expression in the adrenal gland but had no immediate effect on Per1 expression in the liver or kidney (38). A light-induced increase in salivary cortisol is also seen in humans in a phase dependent manner (100, 101) with morning light causing an increase but not evening exposure. This may be caused by an autonomic mediated increase in adrenal sensitivity to adrenocorticotropic hormone controlled by the SCN in a circadian manner (100). It should be noted that a corresponding change in peripheral clock timing can only be assumed as direct sampling of the human adrenal gland was not possible.

When examined together, previous work has demonstrated that a change in light schedule does shift both central and peripheral clock timing, but not to the same degree or direction, nor in the same timeframe (56, 102, 103). It has also been demonstrated in mice that different clock genes re-entrain to a change in light schedule at different rates within the same clock and there is no consistent pattern to this re-entrainment in different clocks (103). Why this is the case remains to be elucidated.

Researchers have examined whether there are other environmental factors that can influence the timing of central and peripheral clocks (Table 2). The following section considers factors other than light that can influence central and peripheral clock timing.

5.1. Activity

Activity, in the form of exercise, has been examined as a possible entrainer. In the SCN, previous experiments have shown that scheduled exercise can enhance the shift of behavioural rhythms to a change in light schedule in mice (a nocturnal animal). Adding three hours of wheel running at the start of the dark period (their normal active time) for four days had no additional phase shifting effect on SCN Per1 expression when exposed to either an eight hour phase advance or delay in light schedule (56). When the exercise is administered at the end of the dark period the results are the same (104). When the light schedule remains constant but exercise is scheduled eight hours before lights off (either voluntary or forced) Per2 expression in the SCN remains entrained to the light schedule (105).

Human studies looking at central clock timing have shown that depending on the time of day, exercise can have an effect on the following melatonin onset. Phase shifts have been seen in both directions with evening exercise (106-108) but this did not carry through to the following night where the melatonin onset was no longer different to the no exercise group (106). When combined with a change in light schedule, exercise may assist with the re-entrainment of the SCN. When placing subjects onto a 23.67 hour day, twice daily two hour bouts of exercise scheduled three and seven hours after waking were able to significantly advance the melatonin peak when compared to subjects who did not exercise (107). Another set of experiments in which subjects had a nine hours phase delay in their sleep/wake cycle under dim light conditions (< 5 lux), three 45 minute bouts of exercise performed in the evening over seven days produced a significant phase delay of melatonin onset of 3.17 hours when compared to a no exercise group (1.67h phase delay) (109). These findings suggest that while exercise could be used to help the SCN to entrain to a new light schedule, the amount required to make a significant impact is most likely impractical for individuals moving to a different light schedule, such as travellers moving to a different time zone.
emerges, with variation in responses between tissues. When the light schedule remains unchanged but exercise is scheduled during the normally inactive period for mice, eight hours before lights off (either voluntary or forced) for four weeks, mouse Per2 expression in skeletal muscle is phase advanced up to 3 hours but a non-significant phase advance is seen in the lung (105). These results demonstrate that altering the timing of muscle activity has a direct effect on the muscle clocks and the associated increase in oxygen demand may have an influence in lung clocks though the results from this study were not conclusive. As expected, no change in Per2 expression was seen in the SCN. In humans, changes in gene expression in skeletal muscle after a single bout of resistance exercise showed that at six hours post exercise, there was a modest yet statistically significant upregulation of expression of Cry1, Per2, and Bmal1. As only two time points were examined, it was not possible to determine if gene expression had phase shifted for these clock genes.

When exercise accompanies a change in light schedule, the changes in the rhythms of clock gene expression depend on whether the light schedule is advanced or delayed from baseline. After four days of an eight hours advance in light schedule Per1 expression in the mouse liver did not entrain to the new light schedule in either the control group or the group that exercised at the start of the dark period (56). However, in the same set of experiments Per1 expression did entrain to the new light schedule in the lung and skeletal muscle in the exercise group but not in the control group. When light exposure was phase delayed by eight hours both groups showed phase delays in Per1 expression in all tissues examined but exercise did not significantly affect the rate of phase shift over control. If the exercise is given at the end of the dark period, phase advanced mice no longer show re-entrainment in lung and skeletal muscle after 4 days (104). To date there have been no studies that have studied the effect of activity on peripheral clock timing in humans.

It has been shown that exercise can effect circulating levels of melatonin but results have been mixed with some studies showing an increase (106, 110), a decrease (111), or no change (106, 108, 110) and this appears to be dependent on exercise duration, intensity, and time of day. Therefore, it appears that any possible effects of exercise on peripheral gene expression are unlikely due to melatonin. If exercise can directly affect peripheral clock gene expression a possible candidate to mediate this effect are glucocorticoids. It has been demonstrated in mice and rats that bouts of increased activity increases corticosterone levels (112).

5.2. Temperature

Mammals display an internally generated circadian temperature cycle controlled by the SCN, both directly by melatonin (50) and indirectly by sleep/wake and activity cycles. The effects of environmental temperature cycles have been examined to see if they also influence circadian rhythmicity. Mice that were exposed to an inverted environmental temperature profile with normal 12L:12D cycle for six days (37°C at night, 24°C during the day) showed no change in SCN rhythmicity, but expression rhythms in the liver were shifted by 9.5 hours. The core body temperature rhythm was also shifted with the normal temperature peak shifted from late afternoon to late evening and activity peak shifted from early evening to early morning. Observations showed that feeding rhythms in these mice also shifted by 6.5 hours as they consumed the majority of their food during the day (51). In addition to the studies that invert the environmental temperature cycle, the effect of a two hour
temperature increase during the light period on peripheral clock timing has been examined in mice (113). *Per2* peak expression immediately after the heat increase was phase advanced in the kidney and submandibular gland but not in the liver. Exposing mice to the two-hour temperature increase on two consecutive days saw *Per2* peak expression advance in the liver as well as the kidney and submandibular gland immediately after the exposure to the second temperature increase. When measured 24 hours later, *Per2* peak expression had returned to the same phase as seen before the exposure to the two-hour temperature increase. To our knowledge, research on the effects of environmental temperature on central and peripheral clocks has not been conducted in humans.

### 5.3. Food Timing

It has been shown in mice and rat models that changes in feeding schedule does not have an effect on SCN rhythmicity in light:dark conditions (114-117) or constant darkness (114). There is, however, a suggestion that timed caloric restriction may affect SCN rhythmicity. Mice that had received 66% of average daily caloric intake during their normal sleep period at ZT6 (six hours after lights on) demonstrated a decrease in amplitude of *Per1* expression in the SCN and a phase advance of 1.4 hours when compared to ad libitum fed mice or mice that were given a normocaloric intake at ZT6 (118). To date there is a lack of human studies looking at the effect of meal timing on SCN rhythmicity.

Animal models examining the effect of food timing on peripheral clocks has been shown to be dependent on the tissue examined. In the rat lung, timed restricted feeding to 4 hours during lights on (when they would normally be asleep) was shown to result in a six-hour phase advance in *Per1* expression after seven days with no further shift seen after 19 days (115). When the time food was available was extended to eight hours there was no longer any difference in *Per1* expression when compared to ad libitum fed rats. Restricting feeding in mice to when the lights were on did not alter gene expression in the kidney after four days while in the pancreas the results were not clear (114). In the heart, one study in mice was unable to demonstrate a clear result (114), but others have shown phase advances in a number of clock genes in both mice and rats (117, 119). The most commonly examined tissue in animal studies is the liver. When feeding is restricted to lights on, mouse (114, 120) and rat (115, 120) models show an almost complete inversion of expression in a number of genes after seven to nine days, while others found this inversion occurs even faster (119). Further restricting feeding to just four hours during lights on gave similar results (115, 116).

When kept under constant light conditions, *Per2* expression in mouse liver, kidney, and submandibular gland showed a decrease in amplitude and a broadening in the distribution of peak phases. While remaining in constant light, timed restricting feeding to what would have been lights off (20:00 - 08:00) was unable to increase the amplitude of expression of *Per2* in these tissues but it did reverse the distribution of peak phases to what is seen in animals kept in light/dark conditions (121).

Whether food timing has an effect on a particular peripheral clock may depend on the types of signals it receives from the SCN. The regular timing of meals in a rhythmic pattern has been demonstrated to maintain clock gene expression rhythmicity and amplitude in the liver of rats when autonomic nervous system and corticosterone inputs from the SCN are abolished (48). Glucose production in the rat liver has been shown to be controlled by sympathetic nerves by the SCN regardless of feeding schedule (33, 34). It has also been shown that when rats are restricted to daytime feeding, *Per1* expression in rat submandibular salivary glands remains in sync with the SCN or becomes arrhythmic (32). The submandibular salivary glands receive direct signalling from sympathetic nerves from the SCN, destruction of this neural pathway in rats causes *Per1* expression shift 180 degrees and entrain to the daytime feeding schedule. In the same rats, liver *Per1* expression was shifted by feeding schedule (32), and this shift has also been shown by others (114, 115, 119, 122-124). Feeding induced increases in temperature could also have an entraining effect on peripheral clocks (46). Further evidence of differential signalling to peripheral clocks has been shown using parabiosis – two mice joined together in order to share their blood supply. Parabiotic pairs of mice consisting of one intact and one with the SCN removed were entrained to a 12L:12D cycle before being released into constant darkness for three days. On examination it was found that the mice with the SCN removed were able to maintain circadian rhythms of clock gene expression in the liver and kidney (suggesting a blood borne signal was responsible) but not in heart, spleen, and skeletal muscle (suggesting possible alternate signalling) (125).
Again, at this point in time there are few data available on the effect of meal timing on peripheral clocks in humans. This, in part, has to do with the ability of researchers to directly access suitable tissues over time to determine if time peripheral clocks are likely to be influenced by the timing of eating. The identification of surrogate markers of peripheral clock activity would assist in observing the possible effects of meal timing in humans.

If feeding patterns are out of sync with central clock timing, these become dominant zeitgeber over SCN in certain peripheral clocks resulting in a slow shift of peripheral clock gene expression to match feeding rhythms, and this has been demonstrated in mouse (114) and rat (115) liver. This slow shift may be SCN-influenced as SCN-lesioned mice have been shown to rapidly invert clock gene expression (one to two days) in response to restricted feeding during the subjective day (120) and may exist in order to prevent a transient shift in feeding from making immediate, lasting changes to the timing of physiological processes (60). In addition, destruction of the SCN or isolating cells from the SCN in mice results in a dampening in amplitude of clock gene expression in the liver (29, 120, 126). This suggests that even though the timing of peripheral clocks in the liver is determined by the timing of food intake, the SCN still has an effect by influencing the amplitude of gene expression in peripheral tissues.

5.4. Diet Composition

There has also been some interest into the effect of macronutrient composition on central and peripheral clock timing. Mice subjected to a high fat diet showed no change in liver and adipose tissue timing but the amplitude of clock gene expression rhythms was attenuated (15). In rats, parenteral nutrition, specifically glucose, amino acids, and saline administration, has been demonstrated to affect both SCN and liver Per2 expression (127). Human studies showed that switching from a low fat/high carbohydrate diet to a high fat/low carbohydrate diet had no effect on Bmal1, Nr1d1, Per1, Per2, and Per3 rhythmicity but had a significant effect on amplitude of expression for Bmal1, Per1, Per2, and Per3 in monocytes (75). This change in expression may be an uncoupling of central and peripheral clock timing that in turn...
alters metabolic processes (75).

The effect of caffeine on clock gene expression has been investigated in both animal models and humans. Caffeine is able to elevate cytosolic calcium and therefore likely to influence the timing of clock gene expression through the activation of early transcription factor CREB (Figure 1). In the SCN of mice that have been removed and observed in the lab, short term administration of caffeine given early during the day did not induce phase shifts in Per2 expression but it did induce phase shifts in Per2 expression in mouse liver, lung, and kidney. These shifts were dependent on the time of administration, with caffeine given early in the day producing advances and administration late at night producing phase delays (128). In mice, long-term caffeine administration has been shown to cause phase delays in Per2 expression in livers that have been removed and observed in the lab (128) but phase advances in liver Per1 and Per2 and delays in Bmal1 expression when they remain in the animal (129). Human studies have shown that caffeine can acutely inhibit salivary melatonin levels (130) and when administered three hours prior to habitual bedtime causes a delay in melatonin onset on the following night (131). This shift in SCN timing would have the effect of delaying sleep onset, which in turn could contribute to shortened sleep times. It has yet to be determined what the effect of caffeine administration later into the night might be, whether it might cause a more significant delay or, as is the case with light, a phase advance in SCN timing.

As demonstrated above, there are a number of factors that can alter the timing of central and peripheral clocks, and these factors do not affect timing in all clocks in a uniform manner. As a result, central and peripheral clock timing can become uncoupled and this may lead to undesirable consequences. Observational studies have demonstrated that changes to activity that affect timing such as shiftwork have negative consequences on health such as obesity (132, 133), diabetes (134), metabolic dysfunction (132, 135-137), cardiovascular disease (134), and cancer (134, 138). Metabolic disturbances, such as lowered glucose tolerance, have been observed humans when individuals eat at night (139). This disturbance is presumed to occur because of the misalignment of peripheral clocks as a result of the change in meal timing out of alignment with the timing of the SCN but, as yet, this has not been elucidated in humans. As a result of the change in meal timing, homeostasis is disrupted and results in changes in leptin release, glucose and energy metabolism, and insulin sensitivity (60). A possible way of offsetting this change in metabolic functioning is by altering meal patterns in shiftworkers so they better fit in with circadian rhythms of nutrient metabolism and glucose tolerance (140). By gaining a better understanding of how these different clocks work, we may be able to develop ways of minimising clock desynchrony and the associated negative outcomes that would otherwise occur.

6. Melatonin – more than just a peripheral clock entrainer

While the SCN controls endogenous levels of melatonin, there is evidence that exogenous melatonin can phase shift the SCN (141), although the mechanism by which melatonin achieves this has not been elucidated. One theory that has been presented is that melatonin does not affect the transcriptional/translational feedback loop directly but instead has an effect on post-translational factors involved with protein breakdown (42). This has been supported by observations in the rat SCN. While melatonin injections did not immediately influence the levels of clock gene expression, significant and differential changes were seen 24 hours later (142). Endogenous melatonin phase-response curves to exogenous melatonin have been established (143) and are 12 hours out of phase with melatonin phase-response curves to light (141, 144) and appear similar to a dark pulse phase-response curve (145).

It is possible that exogenous administration of melatonin could be used to resynchronise central and peripheral clocks when they become out of sync due to factors such as shiftwork and travel, but more research is required.

7. Studies in the blind

While there are suggestions that there may be other non-photic entrainers of the SCN, it is generally not possible to determine whether light exposure is a confounder in these studies (143). An exception to this is with studies in blind human subjects who have lost the ability to perceive light as well as lost their vision. These studies show that even when social activity coincides with the light/dark cycle, the SCN cycles to its own non-light entrained rhythm (free run), ranging from 23.9 - 25.0 hours (146-148) and as a
result these individuals suffer from non-24-hour sleep wake disorder (149). These free running individuals move in and out of sync periodically with environmental light/dark cycle despite scheduling their lives (work, meals, social interactions, sleep) in line with the environment. This demonstrates that the SCN is not entrained by non-photic cues in these individuals (149). In these blind subjects that lack light perception, it has been demonstrated that each individual’s free running period is not consistent. In this population the free run period slows and accelerates over time (150, 151). Period shortens as the melatonin onset occurs during the early evening and night while it lengthens as the melatonin onset occurs during the morning and afternoon. This variability in free run period suggests that there are non-photic cues that can influence but not entrain the SCN depending on the time of phase they occur, but current studies have not elucidated what these non-photic cues may be. The long term health implications of non-24-hour sleep wake disorder include increased incidence of severe sleep disturbance, increased fatigue, impaired performance, and decline in mood when SCN timing is not in sync with the environment (148, 149).

In this population, the use of exogenous melatonin has been studied to try and realign the SCN with the environmental light/dark cycle (152-155). As yet, dosage of melatonin, method of delivery, and timing of administration still needs to be refined.

8. Conclusion

There is a growing interest in the different ways our bodies keep time to coordinate processes. Living in a world that is increasingly operating on a 24/7 basis means that individuals are active, eating, and exposed to light at times when our bodies are expecting it to be dark and to be sleeping. Observational studies have highlighted that this change in timing of activities have a number of negative consequences on the health of shiftworkers and strategies are required to lessen these health burdens, both on the individual as well as society. Possible interventions may include controlling the timing of meals of shiftworkers, the use of molecules that can alter circadian timing, such as caffeine or exogenous melatonin, or other behavioural modifications around shift rostering or scheduling exercise. By gaining a clearer understanding of how the central and peripheral clocks are affected by mistimed activity, we may be able to come up with novel ways to minimise the misalignment and improve the health of those affected.

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10. References


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