Acquisition and usage of circadian information by peripheral organs.

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Abstract
The SCN, or central pacemaker, is considered as the structure governing the circadian organization in our body and entraining other peripheral oscillators. Peripheral tissues, such as the liver, need an oscillator to economize energy expenditure, keep homeostasis and reduce harmful processes. The liver can be entrained by various factors and pathways including feeding rhythms and direct and indirect pathways originating from the SCN. Interestingly, the way the liver is entrained depends on the availability of food and the metabolic state of the body. This indicates that the circadian system can be flexible and is not entirely hierarchical organized.

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Introduction

The world we live in is rhythmically organized due to the rotation of the earth around its axis and around the sun. The eternal succession of days and nights and the continuous succession of the seasons cause everlasting modulations in the availability of food and predators. Hence, organisms need to adapt to these rhythms to enhance their survivability and their fitness. Therefore, at some point in evolution, organisms began to develop time-keeping mechanisms (clocks) in order to keep track of the rhythms in the environment, and eventually predict what will come next. These clocks, being daily or seasonal, need to be synchronized with the environment to ensure accurate time keeping. The most accurate signal on earth that reflects time is the light-dark cycle, which gives not only information about the time of the day, but also about time of the year (the length of the light phase). Consequently, most organisms have developed clocks which synchronize with the environment through light (Newman et al., 2003).

In mammals the central daily clock is situated in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus in the brain (Ralph et al., 1990). The SCN is synchronized with the light dark cycle by means of the light that is absorbed by the retina. The retina communicates this signal to the (SCN) via the retinohypothalamic tract. In the absence of light the SCN is not synchronized with the environment, but runs free. In humans, the free running rhythm has a period of approximately 24.2 hours (Czeisler et al., 1999). Each cell in the SCN generates electrical and hormonal output signals in order to regulate circadian rhythms. Underlying these output signals a molecular machinery of several interconnected feedback loops is present.

Through discovery of the clock genes, i.e., genes encoding for the proteins involved in de molecular clock mechanism, and the development of animals containing luciferase reporter constructs it became possible to localize tissues in which these clock genes are (rhythmically) expressed. Soon it became clear that cells in the entire body are showing circadian gene expression (Yamamoto et al., 2004). Cultured cells and tissue explants from the following organs are showing circadian gene expression: liver, lung, kidney, spleen, pancreas, heart, stomach, skeletal muscle, cornea, thyroid gland, and adrenal gland (Dibner et al., 2010; Yamamoto et al., 2004; Muhlbauer et al., 2004; Yamazaki et al., 2000; Yamazaki et al., 2009; Storch et al., 2002). Thus, besides the central clock in the SCN other oscillators exist in peripheral tissues. It seems that the molecular machineries in the cells of the central and the peripheral clocks are quite similar. However, the rhythms from liver, lung and skeletal muscle dampen after two cycles in vitro, in contrast to the SCN which damped after 32 days in vitro (Yamazaki et al, 2000). In order to explain this result Yamazaki et al. (2000) proposed the idea of an hierarchically organized circadian system, with the SCN entraining oscillators in peripheral tissues. Other articles support this result, currently this view is generally accepted (Stratmann and Schibler, 2006; Gachon et al., 2004; Dibner et al., 2010).

The pathways through which the SCN can entrain the peripheral tissues are not well understood. The SCN can entrain peripheral tissues by direct signals such as neural or non-neural (blood borne or hormonal) signals or by indirect signals (for instance by regulating crucial aspects of behaviour, like food intake; Stratmann and Schibler, 2006). These indirect cycles are governed by a rest activity cylce generated by the SCN. Most organisms need to be awake in order to be able to search for food, hence they will not eat during sleep. Further, the body requires more food in its active state. Therefore, the rest-activity cycle determines the time of food consumption. Thus, the SCN may entrain organs via an indirect feeding cycle. Apart from entrainment of peripheral tissues, by the SCN peripheral tissues might be entrained by their own specific Zeitgebers. It is known that peripheral tissues, such as the liver, can be entrained by food consumption (Hara et al., 2001). Normally the SCN entrains to light and via direct and indirect pathways it influences the circadian organization of the body. In some situations, such as under food restriction, the peripheral oscillators can be uncoupled from the central pacemaker or SCN (Damiola et al., 2000; Stokkan et al., 2001). In some experimental conditions, in which food is supplied at specific times, the SCN cannot influence the time of feeding and thus does not generate an indirect feeding pattern. In this case the liver entrains to an externally generated food rhythm. This demonstrates the impact of food intake as an external zeitgeber. Besides feeding other indirect entrainment signals may exist, such as temperature and/or metabolic state of the body.

The goal of this thesis is to clarify how peripheral tissues obtain circadian information, considering direct and indirect pathways originating from the SCN and SCN independent pathways. Furthermore, the physiological significance of a peripheral oscillator will also be considered. Primarily because of the restricted availability of information in the literature not all peripheral tissues will be considered, instead this thesis will focus on the liver. The liver is an organ involved in various important metabolic and homeostatic processes in our body, including food intake, fat metabolism and glucose metabolism. Therefore, this organ is a proper example of a peripheral tissue. As in other organs, the SCN is a major factor governing entrainment of the liver. Consequently, it is necessary to address this brain structure first and to explain why it is so important.

The central pacemaker in the SCN

The original idea of the existence of a central pacemaker or clock in mammals originates from Pittendrigh (1960). Since then, researchers tried to locate such a structure in the brain. In the 1970s they succeeded. Lesion experiments identified the location of such a pacemaker in the suprachiasmatic nucleus of the hypothalamus (Moore and Eichler, 1972; Stephan and Zucker, 1972). Lesioned animals displayed a total loss of rhythmicity in their behaviour, a loss of circadian locomotor activity patterns and the disruption of the circadian based photoperiodic response. In 1987 Lehman transplanted fetal SCN tissue into the third ventricle of previously SCN-lesioned hamsters. To a large extent this restored the daily locomotor activity pattern the hamster exhibited before the lesion. Transplanting SCN tissue from *Tau* mutant hamsters,

displaying an rhythm with a short period (20 hrs for homozygous animals), into SCN-lesioned normal hamsters resulted in the deviant rhythm of the donor animal and *vice versa* (Ralph et al., 1990). These experiments show that the SCN governs the rest-activity cycles and that the period of the rest-activity rhythm is contained in the SCN. When isolating the entire SCN *in vitro*, the oscillations continue. This implicates that the SCN is oscillating independent of other brain structures. Furthermore, the electrical activity of individual SCN tissue cells, cultured *in vivo*, was shown to be circadian (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982) and persisted even after three weeks of culture (Bos and Mirmiran, 1990). The latter suggests that the 24-hour rhythm is not generated by means of cell-cell interaction.

Each cell is generating hormonal and electrical circadian output signals and has a molecular mechanism underlying that signal. The molecular cellular machinery of the clock consists of two main interconnected feedback loops. The feedback loops are interacting with each other and are influencing the RNA and protein levels of clock components. This machinery was first discovered in mice, DNA comparison showed that other animals and humans apply similar mechanisms. Two transcription factors CLOCK and BMAL1 are the basis of the system. In mice, they activate three period genes (mPer1–mPer3) and two cryptochrome genes (mCry1 and mCry2). Transcription of these genes results in an increase of mPER and mCRY protein levels. The protein mCRY acts as a negative regulator of CLOCK and BMAL1, thereby providing negative feedback. The second feedback loop arises when the BMAL1 protein activates the Rev-Erbα gene. The REV-ERBα protein represses the transcription of BMAL1, resulting in a fall of *bmal1* RNA levels and a rise in *mPer* and *mCry* RNA levels. The mCRY proteins enter the nucleus to inhibit transcription of mCRY, mPER and REV-ERBα. This results in a de-repression or activation of Bmal1 transcription (Reppert and Weaver, 2002), enclosing the second (positive) feedback loop. These two feedback loops result in a circadian cycle of gene expression and protein levels in a cell (for an overview see figure 1).

The SCN synchronizes with the environment by using the most accurate external time signal available: the light dark cycle. In mammals the SCN synchronizes with the environment by means of light which is absorbed by the retina. Axonal projections from retinal ganglion cells (RGCs) communicate this signal to the SCN. It is interesting to note that rod and cone photoreceptors are not required for retinal light absorption. Indeed, the same RGCs that project to the SCN can absorb light independently of rod and cone photoreceptors. These cells contain melanopsin which reacts to light. Newman et al. (2003) demonstrated that especially blue light reacts with melanopsin in the melanopsin-containing ganglion cells in the retina and is able to activate a G-protein (thus able to generate a signal). The melanopsin containing ganglion cells can also receive input from rod and cone photoreceptors (Perez-Leon et al., 2006). This implicates RGCs may respond to light via a pathway driven by rod and cone photoreceptors and via an intrinsic melanopsin-based pathway. Thus, via light absorbed by

the ensemble of photoreceptor cells in the retina the SCN is synchronized with the environment and is governing the circadian rhythms in physiology and behaviour.

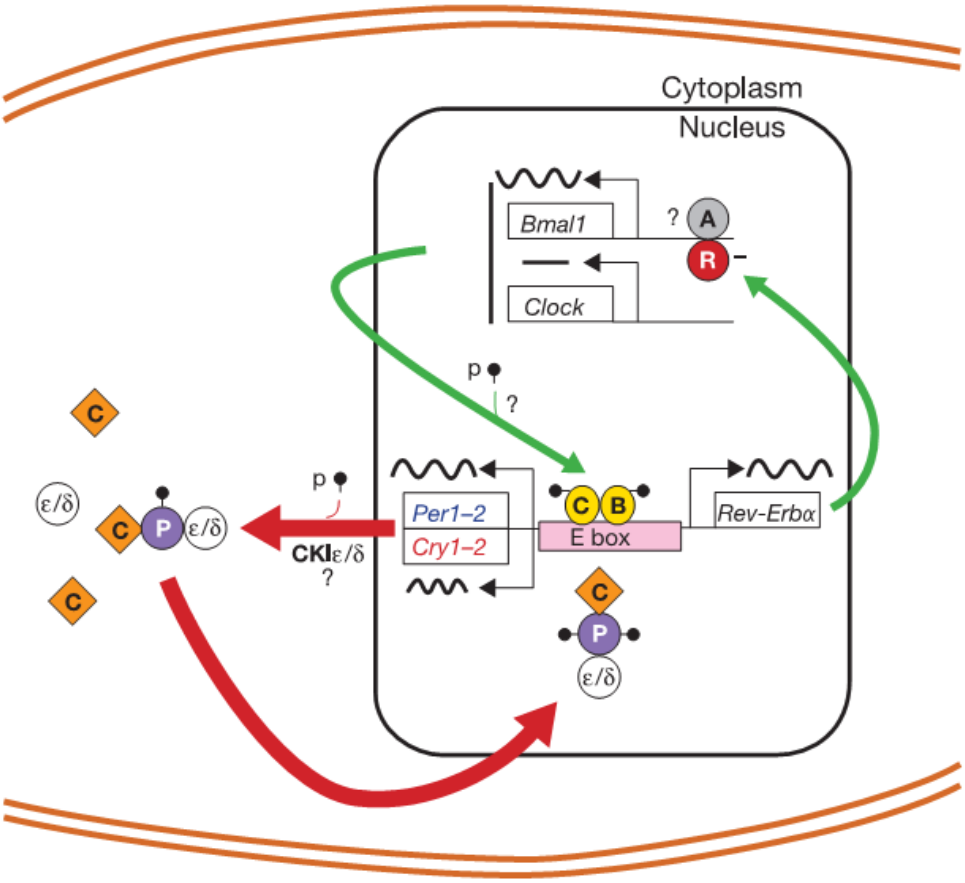

Figure 1; A model of the mammalian circadian clock proposed by Reppert and Weaver (2002). Two feedback loops are present, a positive (green) and a negative feedback loop (red). The transcription of Per, Cry and Rev-Erbα is activated by a protein complex of CLOCK (C, oval) and BMAL1 (B, oval) through E-box activation. PER (P, blue circle) and CRY (C, diamond) proteins form complexes and shut down the transcription of Clock and Bmal1. This comprises the negative feedback loop. In the positive feedback loop the REV-ERBα levels is increased and represses the transcription of Bmal1. Cry de represses Bmal1 transcription by inhibition of CLOCK-BMAL1 mediated transcription (thus inhibition of REV-ERBα mediated repression; Reppert and Weaver, 2002).

Within the brain the SCN can influence hormonal and neural pathways in order to rhythmically organize the body. The release of pituitary hormones may be influenced by the SCN. The SCN has projections to the Paraventricular nucleus of the hypothalamus (PVN) and hence may influence the sensitivity of the adrenal cortex to Adrenocorticotropic hormone ACTH (Kalsbeek and Buijs, 2002). Other hormone levels such as melatonin and corticosterone are also controlled by SCN efferents (Lehman et al., 1987; Kaneko et al., 1980). Furthermore, the SCN can regulate the circadian rhythms of the body by regulating sympathic and parasymphatic output. In the SCN a clear differentiation exists between pre-symphatic and pre-parasympathic neurons (Buijs et al., 2003). This indicates that, within the SCN, different specializations may exist with different functions. Kalsbeek et al. (2006) even

suggested that specific neurons in the SCN can target specific organs, such as the liver, the pineal, and the adrenal gland.

Synchronization of peripheral tissues by the SCN

The SCN is the most important factor in keeping cells of the peripheral tissues synchronized within peripheral oscillators, the rest of the body and the environment. This is demonstrated by the fact that SCN-lesioned animals do not show a rest-activity cycle. Furthermore, experiments done in vitro support the notion that the SCN is the central clock. Cultured SCN tissue and cultured NIH/3T3 fibroblasts were separated by a semipermeable membrane. This implicated that humoral, but not neural, factors can pass the membrane to the other cultured tissue. The in vitro cultured SCN tissue was able to impose a rhythm in metabolic activity and *per* gene expression in the cultured NIH/3T3 fibroblasts. Fibroblasts that received a serum shock exhibited a rhythm in gene expression but not in metabolic activity. These fibroblasts could not impose a rhythm in untreated co-cultured fibroblasts (Allen et al., 2001; Li et al., 2008). The conclusion of these experiments is that SCN tissue, but not serum shocked fibroblasts, can give some signal that imposes a rhythm in untreated fibroblasts. Because the cultures were separated by a semi-permeable membrane it seems that this signal is non-neural and perhaps blood borne.

The only humoral output signal of the SCN that is demonstrated to be secreted *in vivo* is vasopressin. In the 1980s researchers discovered cycles of vasopressin in the cerebrospinal fluid (CSF) following day night cycles (Gillete and Reppert, 1987) and characterized this hormone as a humoral output signal of the SCN. However, it is not clear if this hormone acts as a circadian output signal that entrains the body. Fluctuations of vasopressin could be the result of a diffusion of vasopressin out of the SCN, released as neurotransmitters to function within the SCN, (a spillover; Kalsbeek et al., 2006). Other humoral factors have been proposed, but none have proven to be a circadian output signal *in vivo*. Besides humoral output the SCN exhibits electrical output. Thus, the SCN does have several output signals that rhythmically organize the body. This indicates that the mechanism of entraining the body is complex. This is supported by a parabiosis experiment between intact and SCN-lesioned animals performed by Guo et al. (2005). Parabiotic pairs consisted of a mouse with an intact SCN and an SCN lesioned mouse which were mutually connected by blood. These experiments are useful when investigating the influence of blood borne cues on physiology. This experiment showed that organs are entrained by different pathways. The circadian rhythm of SCN-lesioned animals, linked to animals that were not lesioned, was restored in the liver and kidney but not in heart, spleen or skeletal muscle. This suggests that non-neural signals are sufficient to maintain circadian rhythms in liver and kidney but not in heart, spleen or skeletal muscle (Guo et al., 2005). In addition, transplanting SCN tissue in hamsters did not restore rhythmic gene expression in heart, spleen and adrenal medulla (Guo et al., 2006). In conclusion, the SCN regulates the circadian gene expression of peripheral tissues via diverse factors and pathways.

Apart from direct pathways, such as the neural and non neural pathways described above, the SCN can entrain the peripheral tissues indirectly. The SCN drives feeding-fasting rhythms which indirectly can entrain the liver. When exposing animals to experimental conditions such as restricted feeding, in which animals can only feed during fixed times, the liver entrains to the feeding rhythm. In a normal situation the feeding rhythm is influenced by the SCN and thus the liver and SCN are in synchrony. This raises the possibility of organs driven by cycles, such as temperature or feeding, that are driven indirectly by the SCN (Stokkan et al., 2001; Hara et al., 2001; Strattmann and Schibler, 2006). These findings are raising questions about what circadian information the liver utilizes to entrain itself. There are several pathways that probably play a role: entrainment by a feeding cycle which can be indirect influenced by the SCN, direct neural signals, direct non-neural signals or a combination. This complex network of entraining signals will be discussed in the next chapter (see figure 2 for an overview).

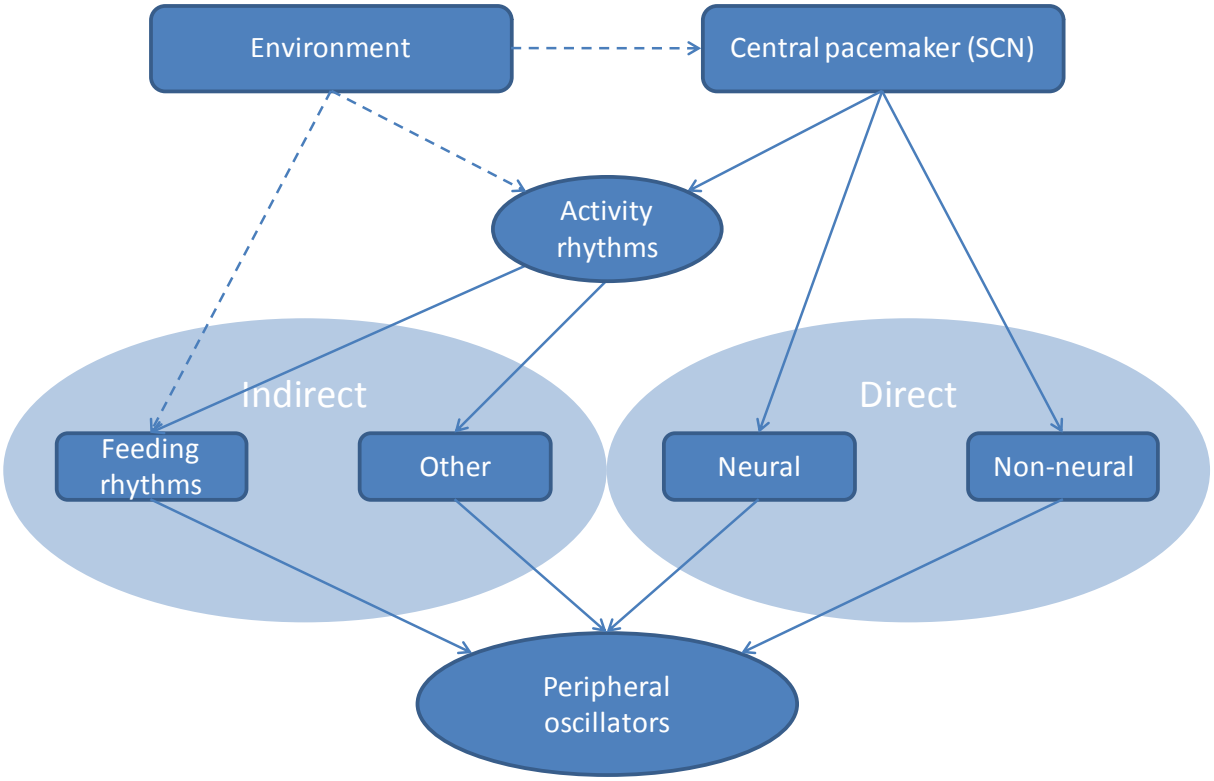

Figure 2; The pathways in which the SCN can entrain peripheral tissues. Under normal conditions the SCN is entrained by light from the environment which is absorbed by the retina. However, when light is absent the SCN is free-running and not synchronized with the environment (hence the interrupted arrow). The SCN can entrain oscillators in peripheral tissues, such as the liver, by indirect (feeding rhythms) and direct (neural and non-neural) signals. Under normal conditions, the direct influence of the environment on activity and feeding cycles is hard to distinguish from the regulating influence of the SCN. Experimental conditions such as restricted feeding schedules are needed to show the impact of the environment on the feeding and activity rhythms and consequently the entrainment of the peripheral oscillators (hence the interrupted arrows).

Entrainment of the liver

The liver is an organ with various functions in metabolism and homeostasis. A large number of genes in the liver are expressed in a circadian way (Kornmann et al., 2007) and important processes need to be synchronized with the environment. Hence, the liver needs circadian information from the SCN and/or from food to entrain itself. In this chapter the different signals that may play a role in the entrainment of the liver will be attended. Non-neural signals maintained the circadian rhythms of gene expression in the liver (Guo et al., 2005; Guo et al., 2006). So the SCN can entrain the liver by a direct signal, which is most likely blood borne. This does not exclude neural signals as a factor influencing the circadian organization and entrainment of the liver. Moreover, a feeding rhythm indirectly influenced by the SCN may also play a role (see figure 2).

An experiment performed by Kornmann et al. (2007) shows that circadian gene expression in the liver can be influenced by both local hepatocyte oscillators and systemic cues possibly originating from the SCN. They used a mouse model in which they could discriminate between local hepatocyte oscillators and direct or indirect signals controlled by the SCN. The model is based on the tetracycline dependent overexpression of REV-Erbα which represses Bmal1. Without Bmal1 the clock does not function. Thus by controlling the expression of Bmal1, using tetracycline, they could switch the liver oscillator on or off. 31 genes in the mouse, including mPer2, oscillated while the liver clock was turned off and thus they oscillated independently of the circadian oscillator in the liver. When the liver clock was turned on 350 genes (including the 31 genes mentioned above) were rhythmically expressed. Therefore, the circadian gene expression of clock genes, including mPer2, can be governed by systemic cues and hepatocyte oscillators. Moreover, this result suggests that the rhythmical expression of most genes is controlled by local hepatocyte oscillators (Kornmann et al., 2007). However, there may be clocks that are independent from Bmal1, hence it is not sure that those 31 genes that oscillate in the liver when the circadian oscillator is turned off are activated by the SCN. The SCN and local hepatocyte oscillators together are governing circadian gene expression in the liver. The SCN may also entrain these local hepatocyte oscillators and may thus entrain the liver via different pathways.

Besides the above described direct pathways originating from the SCN, the liver can be entrained by food cycles. Stokkan et al., (2001) demonstrated that the liver rapidly entrains to a feeding rhythm when the animal is subjected to restricted feeding in daytime. While the SCN was entrained to the light dark cycle, the liver shifted its rhythm by 10 hours in 2 days. An experiment in which the SCN was lesioned was done by Hara et al. (2001). They showed that the entrainment of the liver to restricted feeding is SCN-independent: it also occurs in SCN-lesioned animals. Other studies revealed that both rhythmic feeding and autonomic input participate in a steady rhythm in plasma glucose concentration. The biological clock thus uses multiple pathways to drive glucose rhythms (Caliotto et al., 2009).

Restricted feeding changes the metabolic state of the body and thereby changes the circadian organization of the liver. In a normal situation the liver is synchronized with the rest of the body and with the light dark cycle. In this situation entrainment can occur by the SCN via different neural or non-neural pathways. In the normal situation feeding can also entrain the liver and this pathway is indirectly influenced by the SCN. Thus, depending on the metabolic state and the availability of food, the liver can entrain to different factors (see figure 2).

The physiological significance of a circadian oscillator in the liver

This chapter will focus on the role of circadian rhythmicity on the various metabolic and homeostatic processes governed by the liver. Furthermore, the question will be addressed why the liver needs circadian information. The liver is an organ with multiple functions in metabolism and homeostasis. First off all, the liver is a storage place for glycogen and the primary source for glucose. It has a central role in the lipoprotein and fatty acid synthesis using lipids derived from food and has an important role in lipid metabolism (Nguyen et al., 2008). Furthermore, this organ is an important place for synthesis and degradation of proteins, such as albumin and fibrinogen (De Feo and Lucidi, 2002). Last but not least the liver secretes bile, is storing a variety of substances (glycogen, iron, zinc, copper and fat soluble vitamins) and has a role in the detoxification of the body (Silverthorn, 2007). These processes require to be tuned to the day-night cycle in order to adjust to energy need.

The liver can store glucose as glycogen and can free glucose if needed. The storage and release of glucose is important for homeostasis, especially during exercise (Silverthorn, 2007). The SCN can entrain the daily rhythm in glucose concentrations via multiple pathways, including the discussed direct and indirect pathways (see figure 2; Caliotto et al., 2007). Lamia et al. (2008) reported that Bmal1 is required for proper function and rhythm in glucose concentrations. This rhythm is thus probably governed by the local hepatocyte clock. Further, Lamia et al. (2008) suggested that the liver counterbalances the feeding fasting cycle generated by the SCN with a rhythm of hepatic glucose export. In addition, mice that have a mutation in a key clock gene named *Clock* have a tremendously disturbed energy balance and develop disorders associated with metabolic syndrome (Turek et al., 2005). Thus the liver is trying to keep homeostasis while the brain is generating a rhythm that is based on the rhythmic changes in the environment, and hence causes a loss of homeostasis.

The liver is important in the digestion of food by secreting bile to the gastro intestinal tract. This bile facilitates transport of lipids into gastrointestinal cells. In these cells chylomicrons are formed in order to transport these lipids to the tissues. Remnants of chylomicrons, the parts that are not used as energy or stored in adipose tissue, are taken up and metabolized by the liver. This organ than adds these remnants to its lipid pool. The liver can synthesise new cholesterol and fatty acids if needed. Access cholesterol is converted into bile salts and secreted in the bile. The liver has a major part in cholesterol metabolism, it monitors and regulates the cholesterol available in the blood and tissues (Silverthorn, 2007).

The liver is thus important in the storage and release of energy (glucose), has a role in digestion of lipids and is important in maintaining homeostasis by regulating the amount of cholesterol and glucose in the blood and tissues. The latter is especially important after a meal or during exercise. Therefore, through using circadian information the liver may prepare itself and the rest of the body for a risk of loss of homeostasis and/or for an increased need for energy.

There are multiple reasons why a peripheral organ such as the liver has an oscillator. Many processes that are governed in a circadian manner have some role in metabolism or homeostasis. It would by logical to assume, based on the function of processes which are governed by circadian rhythmicity, that the body is rhythmically organized to economize energy expenditure and to prepare for a possible loss of homeostasis. Dibner et al. (2010) suggest that the energy savings obtained by the cyclic activation of genes are negligible. Consequently, this may not be the main purpose of circadian gene expression. Due to a rhythm in food intake the liver needs to adjust the glucose level in the blood to maintain homeostasis. Thus, the liver needs to rhythmically compensate the behaviourally induced rhythmicity in order to maintain homeostasis (Lamia et al., 2008). Apart from that, the circadian activation may be evolved due to incompatible processes that need to be assigned to different time windows. Harmful processes can be assigned to the time window in which they are absolutely required and thus, for example, generate smaller amounts of reactive oxygen species (Dibner et al., 2010; Schibler, 2007).

Conclusions and perspectives

The body needs to be rhythmically organized to adapt to the everlasting rhythm of the environment. The SCN is considered to be the structure that facilities this rhythmical organization and can influence the circadian organization in various ways. This brain structure synchronizes with the light dark cycle by means of light absorbed by the retina, thereupon a signal reaches the SCN via the retinohypothalamic tract. The SCN can influence pituitary hormone expression (Kalsbeek and Buijs, 2002) and control melatonin and corticosterone levels (Lehman et al., 1987; Kaneko et al., 1980). Furthermore, it can regulate the parasympathic and sympathic output (Buijs et al., 2003). Thus by regulating and influencing neural and hormonal processes, the SCN can influence downstream circadian rhythms.

After the molecular machinery of the clock and the genes behind it were discovered, it was much more easy to investigate if more oscillators exist outside the SCN or even outside the brain. The conclusion of these investigations was that most cells in mammals exhibit circadian gene expression. This led to the view of peripheral oscillators in our body and the SCN governing these oscillators. This is supported by lesion experiments and cell culture experiments in fibroblasts and SCN tissue (Allen et al., 2001; Li et al., 2008). These experiments also suggest that the signals entraining peripheral oscillators are non-neural. However, other experiments suggested that the signals that facilitate entrainment differ

between organs (Guo et al., 2005; Guo et al., 2006). Kalsbeek et al. (2006) even suggested that a single neuron in the SCN may target specific organs. This is raising questions about the acquisition and usage of circadian information in the peripheral tissues.

Two main pathways that are influencing the circadian organization of the periphery are considered: the direct and the indirect pathway. Direct pathways influence the peripheral organs via neural and non-neural (blood borne or hormonal) signals. Lesion experiments showed the liver can be entrained by non-neural signals, this however does not exclude neural signals as an entrainment signal. Kornmann et al., 2007 showed that both local hepatocyte oscillators and systemic cues can influence and entrain circadian gene expression in the liver. Indirect pathways are cycles in temperature or feeding that can indirectly be governed by the SCN and can entrain peripheral organs such as the liver (Stokkan et al., 2001; Hara et al., 2001). When animals experience restricted feeding the oscillator in the liver entrains to an enforced feeding rhythm which is not synchronized by the light dark cycle. In this case the SCN entrains to the light dark cycle while the liver is entrained to the food cycle, this implicates the SCN and the liver are uncoupled. Thus, the liver can entrain to different factors dependent on the metabolic state of the body and the availability of food. This indicates that the circadian system can be flexible and is not entirely hierarchically organized.

The liver is important in regulating lipid and glucose metabolism. Due to the daily activity rhythms the need for nutrients changes during the day. As a consequence the body needs to free nutrients, glucose and free fatty acids to support activation of the body. Furthermore, the body needs to absorb nutrients, this means the activation cycle generates a feeding fasting cycle. The circadian oscillator in the liver prepares the body for systematic changes in the need of energy and systematic changes in the level of nutrients in the blood by regulating the glucose and lipid metabolism. Thus, the liver needs a circadian oscillator to counter the feeding fasting cycle indirectly generated by the SCN and to prepare for a changed need of energy. In case of the feeding fasting cycle the liver responds with a storage or release of glucose (Lamia et al., 2008). Besides keeping homeostasis a circadian oscillator is needed to assign incompatible processes to different time windows (Dibner et al., 2010; Schibler, 2007). In conclusion, a circadian system may not only exist to economize energy expenditure but also to keep homeostasis and to limit harmful processes.

Since the discovery of oscillators in peripheral tissues, the knowledge of the acquisition and usage of circadian information by peripheral tissues has rapidly increased. However, each new discovery raises more questions about the components involved. It seems that these different components are bound together and cannot be separated easily. Furthermore, the circadian organization can be flexible and is not entirely hierarchical, adding another factor that should be taken in to account. This makes research in this area very interesting but also difficult and challenging. Future research should focus on separating the different components involved in entrainment and investigating the effect of environment and metabolism on the circadian organization of the body.

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