CHAPTER 12

Light entrainment of the mammalian biological clock

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Introduction

Mammalian circadian rhythms are controlled by a pacemaker which is located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Meijer and Rietveld, 1989). The SCN produces rhythms of roughly 24 h. In the external world, circadian rhythms are entrained to the environmental light/dark cycle and adopt the external period of 24 h. As a consequence, animals time their behaviour properly in view of the light/dark cycle which enhances their chance of survival. In this chapter, our current knowledge on photoentrainment will be reviewed.

Determinants of light-induced phase shifts

When animals are kept in constant darkness, their circadian period deviates from 24 h and is ‘free-running’. Such a constant condition is a good experimental situation to investigate the effects of various stimuli on the circadian pacemaker’s phase. After having established the animals’s freerunning period in constant conditions a stimulus can be applied at a selected phase of the circadian cycle. In the following days the freerunning period is re-established and it can be analyzed whether a phase advance or phase delay of the pacemaker’s rhythm has occurred as a result of the stimulus.

The hamster is an often-used animal model to investigate the pacemaker’s response to light because it exhibits a remarkable precise onset of running wheel activity. The onset of running wheel activity is taken as a marker of circadian phase and is defined as circadian time (CT) 12. The circadian cycle is subdivided in 24 circadian hours which are almost but not exactly equal to real hours. For instance when the circadian period is longer than 24 h, circadian hours are longer than 1 h by a fraction.

To understand the principle of photic entrainment, the effects of discrete light pulses on the circadian pacemaker have been analyzed as a function of the timing of the light pulse. A circadian cycle is subdivided in a subjective day and subjective night. When light pulses are presented at around the onset of subjective night, which corresponds with the onset of activity in nocturnal animals, a phase delay of the circadian activity pattern is observed. Steady state shifts in the onset of running wheel activity are an indication that the underlying rhythm of the circadian pacemaker has shifted in phase. When light pulses are presented towards the end of the animals subjective night, a phase advance of the activity cycle
occurs. During the animals subjective day light induces no phase shift. The effects of light on the pacemakers' phase are summarized in a phase response curve (PRC) in which the time of the pulse application is given on the x-axis and the magnitude of a phase advance or delay on the positive and negative y-axis, respectively (Daan and Pittendrigh, 1976; Takahashi et al., 1984).

What happens when a nocturnal animal is exposed to a normal light/dark cycle? When the animal leaves its burrow too early and before darkness, light will induce a phase delay and cause the animal to wake up later on the next day. When the animal returns to its burrow too late, the morning light will cause a phase advance and cause the animal to return earlier the next day. The consequence of the phase shifting effects at early morning or late afternoon is that the animal will confine its activity to the dark hours of the day.

In diurnal animals, the situation is in principle identical. The onset of activity is now defined as CT 0. During the subjective day, no phase shifts are induced by light. During the early subjective night phase delays are obtained and during late subjective night phase advances. In diurnal animals, circadian periods shorter than 24 h will result in light exposure at the delaying part of the circadian cycle. The resulting phase delays will compensate for the short freerunning period. A long circadian period, on the other hand, will result in light exposure during the phase advancing part of the PRC.

In conclusion, photic entrainment of the biological pacemaker is based on adjustments to the light/dark cycle made possible by a phase-dependent responsiveness of the pacemaker to light. Additional mechanisms increase the animal's ability to entrain. The magnitude of a phase shift that can be obtained at a given phase of the circadian cycle depends on: (a) light intensity; (b) duration of a light pulse; and (c) wavelength of light. The role of any of these three parameters is investigated by manipulating only one of them while keeping the other two constant.

**Light intensity**

When light intensity of light pulses is raised, an increase in the magnitude of a phase shift is obtained. At some point saturation occurs and further increases in intensity become ineffective. For low light intensities there appears to be a threshold intensity. Below this intensity no phase shifts are observed. The threshold for a phase shift is rather high as compared to the threshold for vision [for instance for the hamster: 0.1 lx (Meijer et al., 1992) or $10^{11}$ photons cm$^{-2}$ s$^{-1}$ or 0.1 cd m$^{-2}$ (Nelson and Takahashi, 1991)].

**Duration of a light pulse**

Longer durations of a light pulse increase the magnitude of a phase shift. The sensitivity to pulse duration has been investigated by manipulating the duration of a light pulse while the total number of photons was kept constant. The circadian system appears more sensitive to 300-s stimuli than to light pulses of either 30 or 3000 s (Nelson and Takahashi, 1991).

**Wavelength of light**

The spectral sensitivity curve of the hamster resembles the one of rhodopsin to some extent (Takahashi et al., 1984). At present, it is unclear whether rhodopsin mediates entrainment or whether other photopigments play an (additional) role.

In the hamster, the onset of running wheel activity is used as a marker of the phase of the pacemaker. All experimental results described above are based on steady state shifts in the onset of activity. Steady state shifts are usually not obtained at the first cycle after a light pulse. When a light pulse is presented at, for instance late subjective night, the pacemaker will respond to this with a phase advance. At the first few days after the light pulse the phase advance is not maximal but instead it grows in the course of several days. The period of the circadian activity rhythm
is not stable during these days. Such circadian cycles which occur shortly after a light pulse are called transient cycles. Whether or not such transient cycles reflect the behaviour of the mammalian pacemaker or secondary processes is unknown.

There have been a few studies in which the offset of running wheel activity was also used as a marker of phase and both of these studies indicated a difference between shifts in the onset and offset of activity (Elliott and Tamarkin, 1994; Honma et al., 1985; Meijer and De Vries, 1995). The difference between onset and offset of activity is especially clear when the transient cycles after a light pulse are observed but is also present in steady state shifts (Meijer and De Vries, 1995). Consider a phase advance that is induced by a light pulse at CT 19 (Fig. 1, second pulse). A number of transient cycles are observed at activity onset on the first few days after the light pulse. Activity offset, on the other hand, appears to have shifted immediately and no transients are visible.

Usually, activity offset is not very clearly expressed and the example above was carefully selected for its clear offset. A clear offset of running wheel activity can be introduced experimentally by lesions of the geniculate nucleus that include the intergeniculate leaflet (IGL; De Vries and De Vries, 1995). Syrian hamsters with a partial or complete lesion of the IGL and ventral lateral geniculate nucleus have a less variable circadian offset of running-wheel activity than sham and unoperated controls (Fig. 2). In the lesioned animals the mean deviation of the circadian offset from the fitted regression line through the offsets was 0.313 h whilst it was 0.678 h in control animals.

Hamsters with geniculate lesions have been used to investigate the effects of light on the onset and offset of activity in more detail and with an extensive quantitative analysis (Meijer and De Vries, 1995). Fig. 3 gives an example of a light-induced phase advance in a geniculate ablated animal. Large and immediate phase advance is observed in the offset of activity whereas

Fig. 1. Wheel-running activity record of a hamster in constant darkness (DD). At the days indicated by arrows, a light pulse is presented. The first light pulse is presented at around CT 14. It induces a clear delay in activity onset but not so clear in activity offset. The second pulse at around CT 19 induces an immediate advance in activity offset. The immediate advance in activity onset is much smaller.
the onset advance is generally characterized by transient cycles. Fig. 4 summarizes the mean values of onset and offset shifts for 22–24 light pulses, presented in ten animals. Immediate delays were larger in activity onset than in activity offset \((P < 0.0001)\). Immediate advances are larger in the offset than in the onset of activity \((P < 0.0001)\). The difference between onset and offset shifts is very clear on the first few days after a light pulse.

Steady state phase shifts in onset and offset of activity differ as well (Fig. 5). Steady state phase delays were larger in the onset than in the offset of activity \((P < 0.0001\), paired Student \(t\)-test) and steady state advances were larger in the offset than in onset \((P < 0.0005)\).

As a result of the difference in onset and offset shifts, the activity time \((\alpha)\) of the hamsters changed. We have plotted the difference in steady state shifts in the onset and offset of activity against the difference between the freerunning periods \((\tau)\) in activity onset and offset and found a significant positive correlation \((r = 0.486, P < 0.001)\). The difference in \(\tau\) onset and \(\tau\) offset compensates for this change in \(\alpha\) so that the initial magnitude of \(\alpha\) may be finally restored (Meijer and De Vries, 1995).

The difference between onset and offset shifts has important practical implications. In the common analysis of phase shifts of the pacemaker, only onset shifts are measured and offset shifts are not taken into account. When shifts in onset and offset appear to be different, the situation becomes complicated as there is no reason to attribute more value to the onset as a phase marker of the pacemaker than to the offset. What is the meaning of a difference in onset and offset shifts?

Partial lesions of the SCN as well as recordings from SCN slices or cultures have indicated that small parts of the SCN are able to generate a circadian rhythm (Green and Gillette, 1982; Groos

![Fig. 2. Double plotted wheel-running activity record of a hamster in DD before and after a lesion of the IGL and vLGN. (The lesion spared the most posterior IGL.) Day of the lesion is indicated by an arrow. Note the sharp activity offset after the lesion.](image)
slightly out of phase. Their mutual phase relation determines $\alpha$. The idea that $E$ and $M$ correspond to the onset and offset of activity is derived from tracing back split components of the hamster's activity rhythm to the time when splitting had not yet occurred, and is thus largely phenomenological. It is predicted from this model that a single light pulse will hit the two oscillators at a slightly different phase and cause, therefore, a different shift in onset and offset of activity. Our differential shifts in activity onset and activity offset can thus be viewed as support for the proposed model. However, the data may support other possible versions of a multi-oscillator model as well.

### Input pathways to the SCN

Although many vertebrate and invertebrate species of animals entrain by some form of extraretinal photoreception, mammals do not (Underwood and Groos, 1982). Photoentrainment in mammals is mediated exclusively via the retina. It is unknown which of the photopigments mediate entrainment but there is some indication for a role of rhodopsin (Takahashi et al., 1984), for a near-ultraviolet sensitive photopigment (Brainard et al., 1986; Podolin et al., 1987) and for a red sensitive pigment (Thiele and Meissl, 1987). From the photoreceptors in the eyes, light information is transmitted to the retinal ganglion cell layer from where several neuronal pathways project to the brain. One group of ganglion cells, with relatively large somata projects with fine unmyelinated fibres directly to the SCN (Pickard, 1982). This fibre bundle is called the retinohypothalamic tract (Moore, 1973). The retinohypothalamic tract is formed — maybe in part — by collaterals of a projection to the ventral lateral geniculate nucleus (vLGN, Pickard, 1985). The vLGN and especially the intergeniculate leaflet (IGL) of the LGN project in turn to the SCN and make up the geniculohypothalamic tract (GHT, Card and Moore, 1984; Harrington et al., 1987). The GHT...
contains neuropeptide Y and GABA (Harrington et al., 1985; Moore and Speh, 1993).

Increasing evidence exists that a third photic pathway enters the SCN. This pathway originates in the raphe nucleus and contains serotonin (Azmitia and Segal, 1978). The raphe itself is responsive to light (Kent and Sladek, 1978; Foote et al., 1978) but it is unknown whether the light-responsive cells in the raphe make a connection with neurons that project to the SCN. A fourth pathway that could be photic reaches the SCN from the pretectum (Mikkelsen and Vrang, 1994).

At present it seems that the RHT is sufficient for photic entrainment while the raphe and GHT seem to suppress the effects of light on the pacemaker. The RHT projects to the ventral and lateral parts of the SCN in many species of animals (Moore and Lenn, 1972; Moore, 1973; Pickard, 1982; Cassone et al., 1988). Some RHT terminals project to adjacent hypothalamic areas outside the SCN but with much lower density (Johnson et al., 1988a; Youngström, 1991; Levine et al., 1991; Speh and Moore, 1993). Also in humans the RHT has been demonstrated (Sadun et al., 1984). Lesions of the RHT by semicircular cuts in the hamster disrupt photic entrainment.

Fig. 4. Phase response curves for shifts in activity onset and activity offset in IGL/vLGN ablated hamsters induced by a 1-h light pulse of 140–180 lux. On the abscissa, the circadian time of light pulse application is given as a percentage of the active time of the animal (α) that has passed. On the ordinate, phase advances are indicated in positive direction and phase delays in negative direction. (A) Steady state shift in activity onset. (B) Immediate shift in activity onset. (C) Steady state shift in activity offset. (D) Immediate shift in activity offset. (Reproduced from Meijer and De Vries (1995) with permission.)
Fig. 5. (A) Mean (± SEM) immediate and steady state phase delays \( n = 22 \) in activity onset and offset. (B) Mean (± SEM) immediate and steady state phase advances in activity onset and offset \( n = 24 \) All phase shifts differed significantly from zero \( P \leq 0.001 \). (Reproduced from Meijer and De Vries (1995) with permission.)

(Johnson et al., 1988b). This may indicate that the RHT is required for entrainment but the possibility that fibres to or from the IGL have been disrupted by these cuts cannot easily be ruled out (Johnson et al., 1988b).

Lesions of the raphe nucleus, on the other hand, do not interfere with entrainment (Kam and Moberg, 1977; Levine et al., 1986). Lesions of the vLGN and IGL or disruption of their photic input do not interfere with entrainment either, although light-induced phase shifts can be smaller and the rate of re-entrainment to a shifted light/dark cycle can be reduced (Dark and Asdourian, 1975; Rusak, 1977; Zucker et al, 1976; Harrington and Rusak, 1986; Pickard et al., 1987; Meijer and De Vries, 1995).

With respect to the function of the RHT in photic entrainment, an even more extreme situation is obtained in slices when the SCN is deafferented from both the raphe and GHT. Electrical stimulation of the optic nerve in such a preparation induces a phase response curve with phase delays during early subjective night and phase advances during early subjective day (Shibata and Moore, 1993). The shape of this PRC is qualitatively similar to the phase response curve for optic nerve stimulation in intact animals (De Vries et al., 1993) and to the light pulse phase response curve (Takahashi et al., 1984).

The shape of the PRC obtained in the slice preparation is, theoretically, appropriate for a pacemaker to entrain to the external light/dark cycle. Hence, the RHT by itself can be sufficient for photic entrainment. We like to conclude as a working hypothesis that the RHT is responsible for the shape of the PRC and concentrate here on the neurophysiology and neuropharmacology of the RHT.

Electrical stimulation of the optic nerve in intact animals produces the light-type phase re-
sponse curve.* It indicates that light activates the ganglion cells of the eye that form a projection to the SCN. These ganglion cells are probably part of the W-type ganglion cells because they respond to light in a sustained way and with a rather long delay which is similar to the behaviour of light-responsive SCN cells (Groos and Meijer, 1985; Rowe and Palmer, 1994). The fine caliber fibres of this projection are unmyelinated, and have a slow conduction velocity (Moore, 1973). The terminals of the RHT can be found along the ventral and lateral borders of the SCN, and some differences are observed between different species of animals (Pickard, 1982; Cassone et al., 1988). For example in the rat, most fibres end in the ventral part of the SCN while in the hamster they end more laterally and dorsally as well.

Inside the SCN the RHT contacts SCN neurons and affects their activity. Three types of optic synapses have been observed in the SCN that is Gray type I and Gray type II and intermediate type synaptic contacts (Güldner, 1978). Gray type I may be excitatory and Gray type II inhibitory (Güldner, 1978). Identification of the RHT neurotransmitter has been the subject of much investigation in the last couple of years and at present, excitatory amino acids (EAAs) are the most likely transmitter candidates of the RHT. In vitro, an increase in glutamate and aspartate concentration can be recorded following optic nerve stimulation (Liou et al., 1986). Retinal terminals show glutamate-immunoreactivity (De Vries et al., 1993; Castell et al., 1993) and reactivity to N-acetylaspartylglutamate (NAAG) which decreases following optic nerve transection (Moffet et al., 1990). Moreover, retinal ganglion cells are labeled by \(^{3}H\)aspartate, a retrograde tracer of glutamatergic/aspartatergic pathways when it is injected into the SCN and adjacent hypothalamic nuclei of the Syrian hamster (De Vries and Lakke, 1995). Inside the SCN, glutamate receptors have been demonstrated (Gannon and Rea, 1993; Mikkelsen et al., 1993; Ishida et al., 1994).

Direct application of glutamate in vitro induces the light pulse type PRC (Ding et al., 1994) but in vivo a different PRC is obtained (Meijer et al., 1988). It is possible that indirect feedback pathways are over-stimulated by overall cannula application in vivo that have been cut off in vitro.

The functional role of EAAs in transmitting light information to the SCN becomes especially clear when blockers are used in combination with light presentation or optic nerve stimulation. In vitro, non-selective antagonists of EAA receptors block the SCN's response to optic nerve stimulation (Cahill and Menaker, 1987, 1989). Intraperitoneal administration of the NMDA-receptor blocker MK 801 blocks light-induced phase advances and delays in vivo (Colwell et al., 1990, 1991). Intraperitoneal administration of MK 801 also strongly attenuates phase shifts induced by electrical stimulation of the optic nerve (De Vries et al., 1994) which excludes the possibility that MK 801 blocked light effects by acting on retinal processes (Fig. 6). CPP and DNQX, specific NMDA and non-NMDA receptor antagonists respectively, block light-induced phase shifts in vivo (Colwell and Menaker, 1992). Finally, light-induced Fos expression in the SCN is blocked with both NMDA and non-NMDA receptor antagonists (Abe et al., 1992) and in vitro both NMDA and non-NMDA receptors appear to mediate excitatory input from the RHT (Kim and Dudek, 1991, see chapter XX, van den Pol and Dudek).

The action of EAAs on SCN cells is nearly always excitatory (Nishino and Koizumi, 1977; Shibata et al., 1983; Meijer and Groos, 1988; Ito et al., 1991; Kim and Dudek, 1991; Mason and Rusak, 1991, Meijer et al., 1993; Bos and Mirmiran, 1993). Aspartate and NAAG appear somewhat less effective than glutamate which is also true in a number of other brain areas. Injections into the SCN (\(N = 4\)) at CT 19 \(\pm\) 0.4 with 0.75 \(\mu\)l NAAG (5 mM) produced a shift of \(-0.19\) h \(\pm\) 0.18 (de Vries, M.J., unpublished results). Three

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* Electrical stimulation of the optic nerve in vitro induces an increase of transmitter release which indicates that such a stimulation reflects an activation of the stimulated pathway (Liou et al., 1986)
classes of ionotropic postsynaptic receptors have been described quite extensively, and have been distinguished by use of selective antagonists (see for review: Nicoll et al., 1990). As light induced phase shifts or SCN responses to optic nerve stimulation were blocked by different types of antagonists (see above) there is probably more than one receptor type involved in entrainment. Glutamate, aspartate and NAAG generally activate all of the receptor types.

There is some indication that not only EAAs but also substance P is a transmitter of the RHT. As with EAAs, the main effect of substance P is excitation (Shirakawa and Moore, 1994). Substance P application in vitro induces a light-pulse type PRC (Shibata et al., 1992). Substance P containing ganglion cells project to the SCN of the rat and the density of substance P containing fibres in the SCN drops following enucleation (Takatsuji et al., 1991; Mikkelsen and Larsen, 1993).

Electrical activity of the SCN and its response to light

Light responses inside the SCN have been measured by electrophysiological recording techniques. With the aid of such techniques, changes
in electrical discharge of SCN neurones have been measured as a function of light duration and light intensity. Before the SCN's response to light will be discussed, we will first discuss the circadian changes in baseline activity of SCN neurones.

The SCN shows clear alterations in electrical activity in the course of the circadian cycle. This has been demonstrated for the first time in vivo in the rat by Inouye and Kawamura (1979) and was later confirmed in two other studies in the same species (Inouye and Kawamura, 1982; Inouye, 1984). In all of these studies, SCN activity appeared high during the (subjective) day when the animal was not active, and low during (subjective) night. In most recordings a knife cut was performed all around the SCN by which the SCN was neuronally disconnected from the surrounding hypothalamic areas. In some cases the connection with the incoming RHT was left intact (see for example: Inouye, 1984). As rhythmicity prevailed in the island preparations, these experiments formed a strong indication that the SCN contains an endogenous oscillator. This was later confirmed by in vitro studies (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982). In these studies, hypothalamic slices that contained the SCN were kept in totally constant conditions, and possible fluctuations in temperature and hormonal conditions could be ruled out. The in vitro experiments thereby proved unequivocally that the SCN contained an endogenous oscillator.

Since then, in vitro recordings have appeared as a very strong technique for research on the SCN pacemaker for a great number of reasons. As the SCN is deafferented from the rest of the brain, a lot of variables can be excluded which provides an ideal experimental situation for many research questions. On the other hand, in vitro studies do not allow one to investigate the effects of light on the pacemaker, and a rough indication of its light response can only be obtained by electrical stimulation of the optic nerves or by transmitter infusions in the SCN. Moreover, the awareness is growing that not only the RHT but also other incoming pathways affect the functioning of the circadian pacemaker and its response to light. These were often cut off in vivo and always in in vitro experiments. We have recently obtained data on electrical baseline activity as well as on light responses of the SCN of freely moving intact animals.

Rats were implanted with stainless steel electrodes, that were placed either inside or just outside the SCN in a nearby hypothalamic area (maximum distance from the SCN: 1.5 mm). The animal's drinking rhythm and its activity rhythm was recorded throughout the experiment to estimate the circadian phase. When the electrode was placed inside the SCN, electrical activity was high during subjective day and low during subjective night (Fig. 7A). This pattern is consistent with previous recordings in freely moving animals in which island preparations were made as well as with recordings in vitro. Apparently, incoming feedback pathways from the raphe or IGL do not result in a qualitatively different pattern of activity. The present recordings in intact animals therefore demonstrate that the general finding of high activity at subjective day and low activity during subjective night holds for the SCN of intact animals as well.

In several marine molluscs such as Bulla gouldiana and Aplysia californica, the eyes contain a circadian pacemaker. These eyes express a circadian rhythm in frequency of optic nerve compound action potentials (CAPs). CAP rhythms are present in the isolated eyes. In vivo, the waveform is more irregular (Block et al., 1993). The irregularity may be caused by efferent optic nerve signals (Aplysia) or may be correlated with the animal's behaviour which appears to affect the pattern of the optic nerve impulse activity (Bulla). Is there any indication that the amplitude or waveform of the recorded rhythm in intact rats is different from that in the neuronally isolated SCN in vivo? We find that indeed our amplitudes are smaller and more irregular than those observed in SCN islands in previous experiments. However, we have not performed measurements in hypothalamic islands and therefore a differ-
Fig. 7. Electrical multi-unit activity inside (A) and outside (B) the SCN of a freely moving rat in constant darkness. On the abscissa the circadian time is indicated. The dotted line at the bottom represents the animal’s movements.
ence between techniques (such as the previous use of differential recording techniques) may account for the observed differences in amplitude as well. This matter needs further study.

Outside the SCN, the reversed neuronal activity pattern was observed, that is, high activity during the night and low activity during the day (Fig. 7B). A similar reversal of activity has previously been recorded when electrodes were placed outside the SCN but within the islands (Inouye and Kawamura, 1979). The way in which the SCN imposes its rhythm on the activity levels in the

![A diagram](image)

**Fig. 8.** Response of light-activated (A) and light-suppressed cells (B) to a 6-min light pulse. The timing of the light pulse is indicated above the record.
brain is still a matter of dispute. However, a general tendency appears to be that pacemaker activity is high during the subjective day and low during the subjective night, both in nocturnal and in diurnal vertebrate and invertebrate species of animals. A phase reversal between SCN activity and cellular activity around the pacemaker is present in nocturnal animals only.

We have presented light pulses of different durations during the animals' subjective day and observed sustained responses to light for exposure periods up to 360 s. In most cases, the responses were of the light-activated type and in a few cases they were light-suppressed (Fig. 8). Light-activated responses were often preceded by a transient overshoot during the first 10–30 s of light exposure. Similar transients have been observed in anaesthetized species of mammals (Meijer et al., 1986). The sustained response pattern has previously been described in anaesthetized hamsters, cats, rats and squirrels (Groos and Mason, 1978; Groos and Mason, 1980; Meijer et al., 1986; Meijer et al., 1989). In the nocturnal species, light-activated responses dominated the light suppressed responses whereas in the diurnal squirrel there was no significant difference in the number of activated and suppressed cells. A distinct topographic organisation has thus far not been observed with respect to the occurrence of light-activated and light-suppressed responses. In the multiunit measurement set-up many cells are simultaneously recorded. When indeed activated and suppressed cells would intermingle completely, the recording of a light-suppressed response would be highly unlikely because of the large dominance of activated over suppressed cells. Our recordings of light-suppressed cells in the multiunit configuration are suggestive for an anatomical organisation or grouping of similarly responding cells within the SCN.

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