

Chapter 12

The Mammalian Neural Circadian System: From Molecules to Behaviour

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Abstract Circadian rhythms pervade all aspects of mammalian physiology and behaviour, providing a near 24 h temporal architecture for all major brain and body systems. Mammalian chronobiology research has focused on nocturnal laboratory rodent models, and this has yielded valuable insight into the neural basis of circadian timekeeping. This has identified the suprachiasmatic nuclei (SCN) as the dominant circadian pacemaker and characterised its neurophysiology, neurochemistry, and potential mechanisms of behavioural control. However, nocturnality is only one of four possible temporal niches, and understanding of the neural circadian system in diurnal, crepuscular, and cathemeral mammalian species is very much in its infancy. In this chapter, we review the fundamental properties of the neural circadian system in nocturnal rodents and then compare this with what is known about neural timekeeping in diurnal species. Through this, we identify gaps in our knowledge and key problems to investigate in order to gain a more complete understanding of circadian control of behaviour and physiology, particularly with regard to temporal niche preference.

12.1 Introduction to the Suprachiasmatic Nucleus as the Central Brain Clock

Since the early twentieth century, behavioural research has shown that many wild-caught as well as laboratory-bred rodent species have a propensity for voluntarily exercising in running wheels with the onset of this activity tightly linked with the time of lights off. Further, when assessed in the absence of a light-dark cycle, rhythms in locomotor activity persisted, with a period that deviated from the 24 h pattern seen under light-dark conditions. Based on this and a multitude of other findings, researchers in the 1970s surmised that the daily brain clock controlling these rhythms should receive visual information. Through mapping visual pathways and making discrete lesions in brain nuclei receiving retinal innervation, it

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was discovered in 1972 that the suprachiasmatic nuclei (SCN) of the hypothalamus contained the circadian pacemaker controlling daily rhythms in the physiology and behaviour of nocturnal rats [1, 2]. Subsequent demonstration that behavioural rhythms are rescued in SCN-lesioned adult rodents by implants of fetal SCN tissue cemented the SCN as the master pacemaker in mammals and catalysed modern neuroscience investigation of the biological timekeeping mechanisms.

Neuroanatomists in the late nineteenth and early twentieth century had identified the SCN as distinct structures in the mammalian hypothalamus [3], but their function was unknown until the 1970s. The SCN are readily identifiable in histological preparations, in part, because the density of cell packing in this structure is relatively high compared to adjacent anterior hypothalamic structures. Consequently, the SCN are now well-characterised, particularly in nocturnal rodent species such as rat (*Rattus norvegicus*), mouse (*Mus musculus*), Syrian hamster (*Mesocricetus auratus*) and Siberian hamster (*Phodopus sungorus*). The SCN are bilobed, situated on either side of the ventral floor of the third ventricle in the periventricular zone of the anterior hypothalamus. In the adult laboratory rat, they are ~0.7 to 1 mm in length along its rostrocaudal axis, ~0.5–0.6 mm on the dorsoventral plane and ~0.3–0.5 mm wide on the mediolateral plane. The SCN vary in morphology along their rostrocaudal axis (described as ‘rugby ball shaped’ in the rat) as well as between species, and consequently, these parameters are general estimates.

Cytologically, the SCN contain both neurons and astroglia with an estimated ratio of 7–8:1 in the rat SCN (reviewed in [4]). In rodents, SCN neurons are among the smallest of the brain, with cell body diameter of ~8–10 μm . The size of these cells in the medial SCN is smaller (~8 μm in diameter) than neurons in more lateral areas (~10 μm). Similarly, the density of cell packing is heterogeneous in the rat SCN with more cells per volume in the dorsomedial compared to lateral areas. It has been speculated that this dense cell packing and reduced extracellular space between neurons in the dorsomedial SCN may enable ephaptic communication between these cells, an idea that has yet to be experimentally scrutinised.

Physiologically, four key features define circadian timekeeping in the nocturnal rodent SCN: (1) The SCN exhibits daily changes in the uptake of 2-deoxyglucose, a marker of metabolic activity [5]. Since this is higher during the day than at night, this indicates that the SCN is metabolically most active during the animal’s behavioural quiescent phase of the circadian cycle. (2) Consistent with the metabolic profile, *in vivo* and *in vitro* electrophysiological recordings show that SCN neurons of nocturnal rodents are spontaneously active and intrinsically generate ~24 h rhythms in the frequency of action potential (AP) discharge [6, 7]. This AP firing rate rhythm peaks during the behaviourally quiescent day, with the nadir during the active phase at night. (3) The 24 h variation in electrical activity does not depend on ‘network’ properties as dissociated SCN neurons isolated in culture also vary daily discharge of AP firing [8]. (4) SCN neuronal clocks are predisposed to synchronise their activity with another, and intercellular communication is necessary for this process. Thus, the SCN are composed of several thousand cell autonomous oscillators, and the ability of SCN neurons to maintain synchronous

activity in brain slices has enabled the development of ‘clock-in-a-dish’ preparations. With this *in vitro* model, the resetting actions of drugs on the firing rate rhythm can be readily assessed (see [9] for review).

Behaviourally, examination of nocturnal rodent wheel-running rhythms has allowed the description of several key properties of circadian rhythms. First, wheel running is synchronised or entrained to lights off such that the initiation of vigorous wheel running is coincident with lights off. Second, these rhythms are sustained in constant darkness, and their timing can be adjusted by exposure to short pulses of light. In nocturnal rodents, the onset of the active phase is defined as circadian time (CT12), and light pulses given in the early night (CT13-17) causes phase delays in the onset of the wheel-running rhythm, while the same stimulus given during the latter part of the active phase (CT18-22) causes phase advances. When given during the middle of the circadian day (CT4-8), light pulses are without phase-resetting effects. This phase response curve (PRC) to light is characteristic of many nocturnal rodent species (for review, see [10]).

Different types of stimuli that elicit increases in internal arousal, such as changing the cage or providing a novel running wheel, have very different phase-resetting actions [11]. During the subjective night, such stimuli have little phase-shifting effect, but, when given during the middle of the subjective day, they evoke phase advances in behavioural rhythms. As this PRC tends to be in anti-phase to that of light, such stimuli are called non-photic cues [12].

12.2 Connections of the SCN

12.2.1 Inputs

Neuroanatomically, antero- and retrograde tracer compounds as well as viral tools have revealed SCN efferents and afferents. Three main input pathways have been extensively characterised (Fig. 12.1):

1. The retinohypothalamic tract (RHT) is a monosynaptic pathway from melanopsin-containing retinal ganglion cells to the SCN. In some rodent species, such as the rat, the ventral aspect of the SCN is more densely innervated than the dorsal [3], whereas in the others, such as the mouse, retinal innervation is dense in both dorsal and ventral aspects [13]. The main neurotransmitter of the RHT is glutamate, and it primarily acts on ionotropic glutamate receptors expressed by SCN neurons. Glutamate depolarises and increases action potential firing of SCN neurons [14]. In addition, the neuropeptide, pituitary adenylatecyclase-activating polypeptide (PACAP), is co-localised with glutamate in RHT terminals in the SCN where it acts to modulate the actions of glutamate [15]. This is the direct route via which environmental light information is conveyed to the SCN.

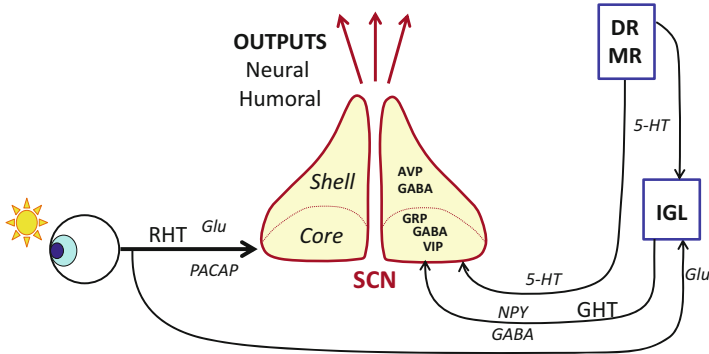


Fig. 12.1 Mammalian circadian system: main inputs and outputs to the master circadian clock, the suprachiasmatic nucleus (SCN). Photic cues are conveyed to the SCN through the retinohypothalamic tract (RHT) with glutamate (Glu) and pituitary adenylate cyclase-activating polypeptide (PACAP) as main neurotransmitters. The SCN also receives photic information indirectly from the intergeniculate leaflet (IGL) via the geniculohypothalamic tract (GHT). The neurotransmitters used in this pathway are γ -aminobutyric acid (GABA) and neuropeptide Y (NPY). GABAergic and peptidergic projections from the IGL and serotonergic (5-HT) projections from the median raphe (MR) convey non-photoc cues to the SCN. Master circadian clock is organised in two main subdivisions: the dorsomedial area or 'shell' characterised by the presence of neurons expressing mainly arginine vasopressin (AVP) and the ventrolateral part or 'core' where most neurons contain vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP). Virtually all neurons across the SCN express the neurotransmitter GABA. Timekeeping signals from the SCN are transmitted to the rest of the brain and body by neural outputs as well as by diffusible humoral signals

2. The geniculohypothalamic tract (GHT) mostly innervates the ventral and central aspects of the rodent SCN and originates from neurons in the intergeniculate leaflet (IGL) of the visual thalamus [16]. The IGL is also retinally innervated and thus represents an indirect route through which environmental light cues are communicated to the SCN. The characteristic neurochemical of the GHT is neuropeptide Y (NPY). This neuropeptide predominantly inhibits SCN neurons (see [9]), while functionally, activation of the GHT, or microinjection of NPY into the SCN [17], resets rodent behavioural rhythms at different times of the circadian cycle to light (non-photoc PRC, phase advancing the SCN and circadian rhythms in wheel running during the subjective day, with much smaller effects during the subjective night). Stimuli that elevate internal arousal (such as cleaning a rodent's cage) activate the IGL, and the GHT signals this information to the SCN. In addition to NPY, GABA is also present in the GHT, reinforcing the inhibitory nature of this pathway.
3. The median raphe (MR) innervates the ventral and central SCN aspects, and the neurotransmitter serotonin (5-hydroxytryptophan or 5-HT) is the characteristic neurochemical of this pathway [18]. This neurochemical mostly suppresses SCN neuronal activity, although there is a range of pre- and postsynaptic 5-HT

receptors expressed in the SCN region, adding complexity to its actions. Similar to the GHT, input from the median raphe signals internal arousal state, although the magnitude of the resetting actions of 5-HT is less pronounced than those of the NPY. Nonetheless, current evidence indicates that both the GHT- and MR-SCN pathways are necessary for the resetting actions of non-photic stimuli to be fully realised [19].

In addition to these three pathways, there are also inputs arising from other hypothalamic structures. For example, the orexin neurons of the lateral hypothalamus provide a diffuse innervation to the SCN, with orexin-containing fibres in close proximity to clock gene-expressing neurons. Orexin alters SCN neuronal activity and acts to accentuate the resetting actions of NPY on the SCN, implicating this projection in non-photic entrainment of circadian rhythms [20].

In the SCN, the terminations of the RHT, GHT, and MR pathways overlap, particularly in the ventral aspects. Perhaps unsurprisingly, activation of non-photic pathways can limit the resetting effects of light pulses, while acute light exposure can reduce or eliminate shifts to non-photic stimuli [12]. Thus, SCN neurons actively integrate photic and non-photic cues to shape the phase of the molecular clock and the entrainment of the circadian system to the external world.

12.2.2 Major Projections

From mapping of rat SCN efferents, the idea emerged that the dorsal and ventral aspects of the SCN may differ in their outputs. A crescent-shaped dorsal area, labelled the ‘shell’ by Moore and colleagues [3], was found to project mainly to the hypothalamus including the dorsomedial hypothalamus (DMH), the preoptic area (POA), the medial subparaventricular zone (mSPVz) and the paraventricular hypothalamic nucleus (PVH). In addition, the ‘shell’ efferents also targeted the paraventricular nucleus in the thalamus (PVT) and the bed nucleus of the stria terminalis (BST). By contrast, the more ventral ‘core’ SCN projected mainly to peri-SCN as well as the lateral SPVz and basal forebrain including the lateral septal nuclei. The implication of these anatomical studies is that different compartments of the SCN share and communicate different types of information.

12.3 Molecular Basis of Timekeeping

Single SCN neurons function as cell autonomous clocks as they contain the molecular clockworks. Building on key findings on the molecular basis for circadian timekeeping in *Drosophila melanogaster*, from the mid to late 1990s, several labs identified and characterised the interactions of fundamental molecular components of the mammalian circadian clock. These include the *period* (*Per1-2*),

cryptochrome (*Cry1-2*), *Clock* and *Bmal1* genes and their associated proteins [21, 22]. There is now a generally accepted model of this molecular clockwork with PER1-2/CRY1-2 constituting the negative limb and CLOCK and BMAL1 the positive limb of the clock. This molecular clock is named the transcriptional-translational feedback loop (TTFL) and conceptually is similar to the molecular circadian clock of flies, yeast and plants. Additional components, such as *Rev-Erba* [23], *Chrono* [24], *dec1/dec2*, and others, function to fine-tune the molecular clock. Further, post-translational modulation of the clock function is provided by casein kinase 1 δ/ϵ enzymes which influence PER-CRY complex stability and translocation to the nucleus to influence circadian clock speed [25]. Similarly, F-box proteins that alter protein ubiquitination and degradation influence CRY protein stability and clock speed in the SCN and other circadian oscillators [26–28]. In addition to this TTFL clock, a second non-TTFL oscillator is present in red blood cells and may be present in neurons [29]. Intriguingly, current evidence indicates that the circadian variation in SCN neuronal activity acts to facilitate the internal molecular clockworks such that chronic interference with electrical activity damps molecular oscillations and desynchronises SCN neurons [9].

12.3.1 Compartments of the SCN

Visualisation of gene expression by *in situ* hybridisation indicates that not all regions of the SCN rhythmically express clock genes at the same phase or perhaps at all (for review, see [30]). In particular, a predominantly dorsal area that forms a crescent-like shape expresses detectable and robust rhythms in *Per1/Per2* which peak in the late day, while the ventromedial area that is largely enveloped by the dorsal area expresses rhythms that are weak or difficult to discern. These areas map well onto the ‘shell’ and ‘core’ regions previously described in relation to the differential regional pattern of SCN efferents. Similarly, imaging of luciferase reporters of *Per1* promoter activity or PER2 expression in living rodent SCN brain slices reveals ‘shell’ and ‘core’ regions that are strongly and weakly rhythmic, respectively. Through attempting to model these temporal patterns in bioluminescent signals, terms such as ‘waves’ and ‘tides’ have been used to capture the complex dynamic nature of spatio-temporal organisation of molecular activity in the SCN [30]. Subsequent studies have conceptualised that the dorsal SCN (roughly corresponding to the area designated the ‘shell’ on the basis of SCN extrinsic connections and intrinsic neurochemistry) as initiating the daily change in *Per1/PER2* expression such that the peak in these signals in some occurs several hours after the peak in the ‘shell’. Thus, while the SCN as a whole functions as the mammalian brain’s master circadian clock, intra-SCN timekeeping is heterogeneous with some areas appearing to lead daily changes in molecular clock activity, while others follow.

12.4 Intrinsic Neurochemistry

Neurochemically, all SCN neurons contain GABA, but they can, to an extent, be distinguished by the neuropeptides that they synthesise. The prominent neuropeptides contained in SCN neurons include vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), and arginine vasopressin (AVP) [31]. - VIP-expressing neurons appear anatomically partitioned from AVP-expressing neurons lending further credence to the idea of ‘core’ and ‘shell’ regions of the SCN (Fig. 12.1). As there are many studies of the organisation of the intrinsic neurochemistry of the SCN as well as the local neurophysiological actions of these substances, we direct the reader to recent comprehensive reviews on these topics.

12.4.1 VIP

VIP-containing neurons are present throughout the ventral aspect of the nocturnal rodent SCN. In rat, it is estimated that VIP neurons constitute around 24% of SCN neurons and these cells delineate the ‘core’ SCN. VIP neurons have extensive axonal arborisations throughout the SCN, and their axons form part of the SCN output to innervate other hypothalamic sites such as the dorsomedial hypothalamus. The cognate receptor for VIP, VPAC₂, is expressed throughout the dorsal and ventral aspects of mouse and rat SCN. Electron microscopy studies have indicated that some VIP neurons are directly retinorecipient and suggest that they form part of an interface with light input to the SCN. Consistent with this, the immediate early gene *c-fos* is induced by light in VIP neurons, while rat SCN firing rate rhythms are reset by VIP and VIP agonists in a pattern resembling the actions of light on behavioural rhythms (for review, see [32]). Further, microinjection of VIP into the SCN region evokes moderate phase shifts in wheel-running rhythms with a PRC resembling light [33].

The acute actions of VIP on SCN neurons are not well understood. Extracellular recordings from rat SCN neurons *in vitro* indicate that VIP evokes both activations and suppressions in the spontaneous firing rate of SCN neurons, with a larger portion of cells responding when tested during the night. The suppressive actions of VIP are mimicked by a VPAC₂ receptor agonist. Voltage clamp recordings indicate that VIP elicits the release of GABA, and, therefore, the mixed acute effects of VIP likely represent a combination of pre- and postsynaptic actions [33].

As an intrinsic signal, VIP-VPAC₂ communication plays a key role in synchronising SCN neurons; in transgenic mice lacking VIP or VPAC₂ expression (*Vip*^{-/-} and *Vipr2*^{-/-}, respectively), coordination among SCN cellular timekeepers is dysfunctional [34], and these mice express severely disrupted behavioural activity patterns, ranging from arrhythmic to rhythmic with an abnormally short tau (~22 h) [33, 35, 36]. Some of the alterations in cellular rhythmicity and synchrony arising from VIP-VPAC₂ signalling deficits can be circumvented by treatment with

other non-VIP agonists [37, 38], revealing functional redundancy in intercellular communication within the SCN.

12.4.2 GRP

Gastrin-releasing peptide (GRP) is synthesised by ~14 % of neurons in the central region of the SCN [3], and its cognate receptor, BB₂, is also expressed in the SCN. Similar to VIP neurons, some GRP neurons are retinorecipient, and GRP microinjected into the SCN causes light-like phase shifts in wheel-running behaviour [39]. Further, GRP resets rodent SCN firing rate rhythms *in vitro* with a temporal pattern of sensitivity resembling that of glutamate. GRP and associated peptides activate SCN neurons to elevate firing rate. A greater proportion of SCN cells respond to GRP when tested during the subjective night, and these actions are blocked by a BB₂ receptor antagonist. In mice, transgenic deletion of BB₂ expression does not affect ongoing rhythmicity but does reduce the phase-resetting actions of light [9].

12.4.3 AVP

Arginine vasopressin (AVP) is arguably the most abundant of SCN neuropeptides (~37 % of rat SCN neurons [3]). AVP is expressed in neurons along the medial border of the SCN as well as in a small cluster of neurons near the ventrolateral pole of the SCN. This neuropeptide is often used as the neurochemical marker of the 'shell' compartment. AVP expression overtly varies over the 24 h light-dark (as well as constant dark) cycle, with high levels of mRNA/protein during the middle of the day and lower levels at night. AVP is a clock-controlled gene and its expression is predictably altered by clock gene mutations. Electrophysiological investigation reveals that AVP activates rodent SCN neurons [40]. These effects are mediated by AVP V1a and V1b receptors that are expressed in the SCN [41].

The role of AVP in the circadian rhythms is suggested to be noncritical since Brattleboro rats that do not express AVP continue to show rhythmic behaviour. However, recently, a study has indicated that AVP-V1a/1b receptor signals are necessary for effectively controlling the magnitude of resetting to changes in the external light-dark cycle [41]. In mice lacking both V1a and V1b expression, the SCN resets to large changes in the phase of the LD cycle much more quickly than in wild-type animals.

12.5 Outputs

The peptide prokineticin-2 (PK2) is synthesised in the mouse SCN and is implicated in conveying circadian information to the rest of the brain. Levels of PK2 mRNA in the SCN vary across the light-dark and circadian cycles. The main receptor for PK2 in the brain is PK2r. PK2r is expressed by distinct populations of the SCN including AVP- and GRP-containing neurons, and PK2 acting via the PK2r alters SCN electrical activity [42]. PK2 is predominantly excitatory in the SCN and appears to act by reducing GABA release. Further, injections of PK2 into the lateral ventricle of rats at night, when the level of PK2 in the SCN is very low, reduce motor activity. Since PK2 mRNA peaks during the day when rats are behaviourally quiescent, then this suggests that PK2 functions as an inhibitory output signal. It is unclear whether this represents a direct inhibition of motor centres or arises through indirect pathways. PK2 expressing cells in the SCN potentially innervate a number of extra-SCN sites including the dorsomedial hypothalamus and the habenula (Hb), and PK2r mRNA is also expressed in these structures. There has been very little examination of the influence of PK2 at the target sites of SCN efferents, but, in the lateral Hb, PK2 acts presynaptically to enhance GABA release and reduce neuronal activity [43]. The model that has emerged is that PK2 released from SCN efferents during the day acts to suppress motor centres of the brain and that as PK2 release declines during the late day, this disinhibits these motor centres and is permissive of the expression of locomotor activity. Unfortunately, there has been limited follow-up on PK2's role, and while this model remains an intriguing possibility, more insightful experiments are required to be fully confident that PK2 does indeed function as an SCN output signal.

There is limited evidence that other factors such as transforming growth factor- α (TGF α) [44] and cardiotrophin-like cytokine [45] are involved, but, aside from these early initial investigations, there is little additional evidence to support such a role for these chemicals.

12.6 Circadian Organisation in Diurnal Species

Most of our current knowledge of the biological timekeeping mechanisms in mammals arises from laboratory investigations focused on nocturnal rodent models, but studies in diurnal species are much more limited. One layer of complexity in understanding the neural mechanism underpinning diurnal temporal niche preference in mammals is that diurnalism has re-emerged in several independent evolutionary lineages [46]. Thus, comparative analysis of diurnal species from different taxonomic groups is necessary to identify convergent adaptations that are common to a diurnal niche and therefore more likely to be shared by most diurnal species, including humans [47].

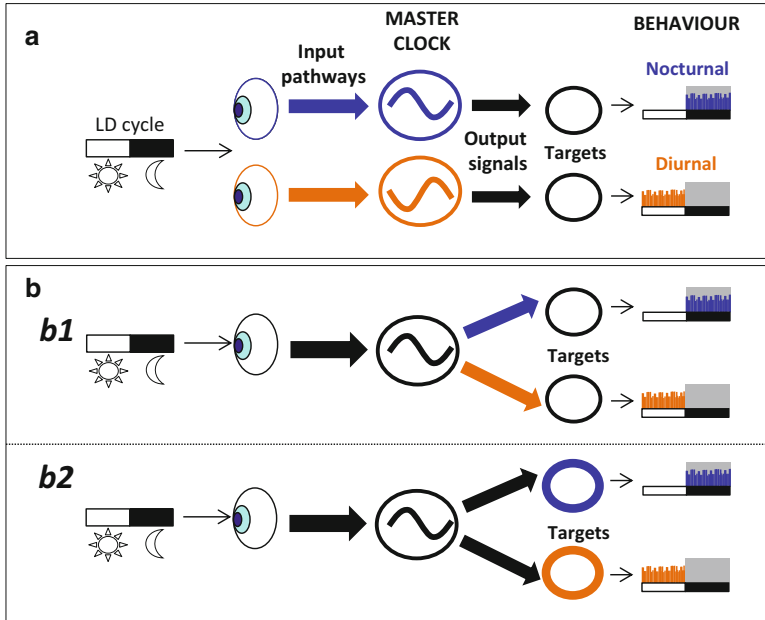


Fig. 12.2 Schematic diagram of the possible neural mechanisms underlying temporal niche preference. Different behavioural outputs in diurnal and nocturnal animals could arise from (a) a reversed phase relationship between the SCN and the external light-dark cycle (LD) and, (b) alternatively, changes in the coupling between the SCN and the functions that it controls, including reversed expression and release of SCN timekeeping signals (*b1*) or altered responsiveness in target areas downstream from the SCN (*b2*) (Adapted from [81])

From a theoretical point of view, the different patterns of behaviour and physiology in diurnal and nocturnal species could be the result of (1) changes in the central master oscillator and in the mechanisms involved in its entrainment to the LD cycle, but similar coupling of the clock to the functions it controls, and (2), alternatively, a circadian master oscillator with similar fundamental features and entrainment to the LD cycle but with different coupling between the clock and the functions that it regulates (Fig. 12.2) [48].

12.6.1 Inputs to the Central Pacemaker

In the natural environment, nocturnal and diurnal species differ considerably in the lighting conditions which they routinely experience. Indeed, diurnal species are exposed to light for longer durations and at higher intensities than nocturnal ones. It is therefore not surprising that evolutionary adaptation to a diurnal temporal niche has resulted in a visual system that is distinctly different from nocturnal animals. This includes, for example, lens pigmentation which confers strong filtering

properties and high cone density in the retina for colour vision processing [49]. In addition, anatomical studies have revealed that some diurnal species possess thicker optic nerves and more prominent optic chiasm and tracts than nocturnal animals [50, 51]. However, the significance of such differences, from a circadian point of view, is though unknown.

Adaptation to light-saturated environments by diurnal species could also account for the finding that the circadian systems of such species appear to be less sensitive to light than those of nocturnal animals. This is reflected in higher thresholds for inducing sustained photic responses in SCN neurons in diurnal rodents [52]. Intriguingly, differences between diurnal and nocturnal species are reported in the polarity of electrophysiological responses of their SCN neurons to photic stimulation. For example, the majority of photically responsive SCN neurons are suppressed by light in diurnal species, including the degu (*Octodon degus* [52]) and 13-lined ground squirrel (*Spermophilus tridecemlineatus* [53]), in contrast to predominantly excitatory responses observed in SCN neurons of nocturnal animals. This finding was also replicated *in vitro* by recording SCN responses to optic nerve stimulation [54]. Additional studies using other diurnal species are needed to confirm if these differences in the physiological effects of light inputs are a common feature of diurnalism.

A fundamental property of the circadian system is the PRC which describes the resetting effects of light on the SCN clock. As stated earlier, the shifting effects of light on the SCN clock depend on the time of day when light is applied. With pulses of light given during the night, the pattern of PRC appears to be quite similar across a wide range of diurnal and nocturnal species. Thus, a light pulse early and late in the subjective night causes phase delays and phase advances, respectively, regardless of the timing of activity. However, with light pulses given during the subjective day, differences between diurnal and nocturnal species emerge. For several hours during the subjective day, nocturnal animals are generally unresponsive to the phase-shifting actions of light pulses (the so-called dead zone), whereas in diurnal species, including degus and humans, the duration of photic insensitivity during daytime is much shorter or even absent [47, 55]. Indeed, this daytime sensitivity of the diurnal SCN to light is also mirrored when the activity of the immediate early gene, *c-fos*, is examined. Light pulses during the subjective day cause changes in *c-fos* expression in the diurnal SCN, but have no effect in nocturnal rodents [56]. However, these subtle variations in the shape of the PRC do not seem sufficient to explain the 180° phase reversal in the entrained activity pattern between nocturnal and diurnal species [47].

Anatomical pathways involved in conveying non-photic internal arousal to the SCN in nocturnal animals are conserved in diurnal species. However, it is unclear if their neurophysiological characteristics and the processing of information conveyed to the SCN are also conserved. As stated previously, arousal-promoting cues are conveyed to the SCN by the GHT, which co-releases NPY and GABA in the SCN, as well as via a brainstem pathway originating in the median raphe, which uses serotonin as its neurochemical signal. To date, studies comparing the role of GABA or serotonin in the SCN of diurnal and nocturnal animals are few, and the

limited evidence available suggests that these two neurochemicals elicit distinct behavioural effects in diurnal and nocturnal animals [57].

Microinjections of GABA_A agonists, such as muscimol, directly into the SCN during the mid-subjective day cause phase shifts of behavioural activity in both diurnal and nocturnal animals. Interestingly, these shifts are in opposite directions, phase advance in nocturnal hamsters and large phase delay in diurnal Nile grass rat, *Arvicanthis niloticus*. These effects of muscimol are not prevented by blocking voltage-gated sodium channels with tetrodotoxin, indicating that they most likely represent direct actions of GABA_A activation in the SCN region (for review, see [51]). These phase alterations have been associated with downregulation of both *Per1* and *Per2* expressions in nocturnal animals and only decrease of *Per2* in the SCN of *A. niloticus*. GABAergic stimulation reduces the shifting effects of light at night in both diurnal and nocturnal animals [58].

The circadian window of sensitivity to serotonin-resetting actions also appears to differ between the nocturnal and diurnal species studied to date [57]. In nocturnal species, such as hamster, *in vivo* administration of the 5-HT agonist (8-OH-DPAT) causes phases advances only during the mid-subjective day. By contrast, in the diurnal *Arvicanthis ansorgei*, serotonergic activation induces phase advances during the subjective night. Intriguingly, serotonergic stimulation potentiates photic-resetting effect at night in diurnal *A. ansorgei* [59], instead of reducing it as it occurs in nocturnal animals.

Similarly to nocturnal animals, orexin fibres are found in the SCN of diurnal species [60]. Unfortunately, how orexin influences SCN activity and its potential role in conveying/modulating non-photic cues to the SCN in diurnal species is unknown. Importantly, the temporal pattern of activation of orexin cells is phase reversed in nocturnal and diurnal animals, that is, higher activation at night in nocturnal animals and during the day in diurnal rodents [48]. Thus, it will be interesting to study if orexinergic cues would induce phase shifts of the SCN only at night and if such cues can potentiate light-induced phase shifts as is seen for serotonergic stimulation.

12.6.2 *The Central Pacemaker*

Interspecific differences in the morphological organisation of the SCN at both the structural and neurochemical levels have been found; however, none seem clearly associated with diurnality. For example, the SCN in some diurnal species, including *O. degus* [61] and *Funambulus palmarum* [50], have an elliptical shape in the coronal plane, in contrast with the typical oval shape described for nocturnal rats and hamsters. However, this feature cannot be generalised to all diurnal species as diurnal Nile grass rats, for example, also have oval-shaped SCN [62]. The typical organisation of the SCN into 'core' and 'shell' described in nocturnal species seems to be present in some but not all diurnal species. For example, cell density in degus SCN does not appear greater in any part of the nucleus. Interestingly, AVP cells in

this species are found not only in the dorsomedial part but also in the central region of the SCN [61]. Recently, a study in capuchin monkeys has also reported the presence of AVP in both ventral and dorsal SCN regions [63]. Such pattern contrasts with the typical distribution of the AVP cell bodies limited to the dorsomedial SCN found in nocturnal species. Future studies are needed to explore the functional impact of these differences.

Despite the varied activity patterns across animal species, some features of the SCN are fundamentally conserved. For example, global SCN metabolic activity (assessed by 2-deoxyglucose) peaks during the day in both nocturnal and diurnal rodent species [5]. However, whether more subtle regional differences in SCN metabolism are related to temporal niche remains to be examined. Further, the possibility that the SCN output signal generated differs between nocturnal and diurnal species needs to be interrogated. For example, such output signals may vary in terms of their ratio of synthesis and release of inhibitory vs excitatory neurotransmitters [46].

Studies of electrical activity rhythms in diurnal species are scarce and the available data variable. *In vivo* multiunit activity SCN recordings made from the diurnal chipmunk (*Eutamias sibericus*) mirror the population-level metabolic rhythm. Thus, chipmunk SCN neurons generate daily rhythms in electrical outputs, being significantly more active during the day than at night [64]. However, a lack of significant daily variation in spontaneous firing was found in the degu SCN (in both light-responsive and light-unresponsive cells) [52]. At present, there is insufficient data to conclude on the nature of SCN neuronal rhythms in diurnal species, and many more such assessments from other diurnal species are necessary. Further, the possibility that temporal niche preference may be captured by one cell population but not another needs examining. Therefore, more detailed investigations are required to determine the SCN neuronal properties that contribute to the emergence of nocturnal and diurnal behavioural patterns.

At the molecular level, there do not appear to be any major alterations in the daily pattern of the SCN TTFL that can account for differences in temporal niche. Indeed, the phasing of core-clock gene expression, and their corresponding protein rhythms in the SCN, is remarkably similar in diurnal and nocturnal mammals despite major differences in their activity patterns (e.g. [65, 66]). Patterns of *Per1* and *Per2* expression, with high levels during the light phase and low levels at night, have been found in all diurnal rodent species studied so far including degus, barbary-striped grass mouse, ground squirrel, *A. ansorgei* and *A. niloticus* [58, 65, 67–70] and in typical nocturnal species, such as rats, mice and hamsters. In addition, recent studies comparing individuals with different activity patterns (e.g. day active vs night active) within the same species have found no differences in the phases of *Per1* and *Per2* clock gene expression or in their protein rhythms [66, 67].

Interestingly, however, the expression patterns of these core-clock genes outside the SCN appear to peak in phase with the animal's activity pattern. Indeed, in extra-SCN brain regions (e.g. the hippocampus and cortex), *Per1/Per2* rhythms in nocturnal animals peak at night, which is nearly 180° out of phase with the peaks

seen in diurnal species [65, 67, 69]. Together, this suggests that the different temporal niche preferences in most diurnal or nocturnal species should result from different coupling between the clock and functions that it regulates.

Two cases are noteworthy to mention where changes in the pattern of coupling between the SCN and the LD cycle potentially influence an animal's activity phase preference: (i) Mice lacking the inner-retinal photopigment melanopsin (OPN4) and RPE65, a key protein used in retinal chromophore recycling, exhibit a switch from nocturnal to diurnal activity. The phase of clock gene expression rhythms is also reversed in the SCN of these animals suggesting that neural responses to light upstream of the SCN can produce acute temporal-niche switching. In the same way, a switch from nocturnal to diurnal phenotype also occurs when wild-type mice are transferred from an LD cycle with standard light intensity to an LD cycle with scotopic levels of light during the 12 h light phase [71]. (ii) In the case of the subterranean mole rat *Spalax ehrenbergi*, most individuals are active during the light phase, but a subset of them can switch from being active during daytime to becoming active at night. Interestingly, the molecular clock gene, *Per2*, also shifts to exhibit a peak during the dark phase in the SCN, while *Per1* oscillates in a biphasic manner [72].

12.6.3 Circadian Outputs

The SCN convey temporal information to other brain regions and to peripheral organs through rhythmic release of output signals (potentially including AVP, VIP, TGF α , PK2, GABA). Oppositely phased activity rhythms in nocturnal and diurnal species may result from reversed expression and release of these output signals (Fig. 12.2(b1)). However, to date, comparative analyses of PK2 and TGF α expression in diurnal and nocturnal species have revealed similar rhythmic profiles regardless of their pattern of activity [73, 74]. In the same way, the daily pattern of expression of VIP mRNA under LD conditions in the SCN of diurnal and nocturnal animals seems overtly similar [75]. AVP expression is under clock control. Measurement of its concentration in cerebrospinal fluid, as well as AVP mRNA expression in the SCN, reveals circadian rhythms in AVP peaking during daytime. Hence, both transcription and release of AVP appear to peak during daytime regardless on the animal's behavioural pattern. Collectively, these studies suggest that the fundamental mechanisms for diurnality should be located downstream the SCN. Thus, target areas would show reversed responses to SCN output signals (Fig. 12.2(b2)). An interesting example supporting this idea is the control of corticosterone rhythms by AVP-containing projections from the SCN to the paraventricular nucleus of the hypothalamus (PVN). Plasma corticosteroids (corticosterone in rats and mice; cortisol in degus, humans, and non-human primates) show daily variations with maximal values at dusk in nocturnal species and at dawn in diurnal mammals. This prepares the organisms for the forthcoming period of increased activity (night or day, respectively). Intriguingly, PVN neurons of diurnal

and nocturnal rodents show different responsiveness to AVP administration. Indeed, AVP infused in the PVN of the diurnal grass rat (*A. ansorgei*) stimulates release of plasma corticosterone. By contrast, the same treatment has a strong inhibitory effect on corticosterone levels in nocturnal rats [76]. The authors propose that changes in the phenotype of the neurons targeted by the SCN efferents (i.e. glutamatergic instead of GABAergic) could underlie these reversed responses to AVP in animals with different activity patterns.

12.7 Summary and Questions of Interests

- SCN neurons exhibit intrinsic circadian variation in molecular, metabolic, and electrophysiological characteristics.
 - Regional differences in neurochemical and timekeeping characteristics in the SCN are pronounced in some species.
 - SCN molecular clock does not appear to differ between nocturnal and diurnal species.
1. What processes and mechanisms make an animal diurnal?
 2. How do SCN output signals influence activity in specific target areas?
 3. Why are ‘core’ and ‘shell’ compartments more discernable in some species and not others?
 4. What are the mechanisms underlying temporal niche switching within the same species?

Suggested Further Reading

Very recent studies indicate new roles for known neuropeptides in the SCN [77, 78] and identify a new G-protein-coupled receptor and its function in the SCN [79]. Further, interactions between the SCN and other hypothalamic regions shape diurnal temperature preference [80].

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