division. The distinctive subsidiary-cell divisions and the zonal organization of grass leaf development have been leveraged to reveal fundamental rules of plant cell division. These studies led to the identification of receptor-like proteins *PAN1* and *PAN2*, which localize to the membrane between guard cells and subsidiary cells and are required for correct subsidiary cell shapes, deepening our understanding of how the actin cytoskeleton and plant homologues of the WAVE/SCAR complex guide nuclear migration and asymmetric cell division.

Where can I find out more?

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Primer

The suprachiasmatic nucleus

Andrew P. Patton
and Michael H. Hastings*

Like it or not, your two suprachiasmatic nuclei (SCN) govern your life: from when you wake up and fall asleep, to when you feel hungry or can best concentrate. Each is composed of approximately 10,000 tightly interconnected neurons, and the pair sit astride the mid-line third ventricle of the hypothalamus, immediately dorsal to the optic chiasm (Figure 1A). Together, they constitute the master circadian clock of the mammalian brain. They generate an internal representation of solar time that is conveyed to every cell in our body and in this way they co-ordinate the daily cycles of physiology and behaviour that adapt us to the twenty-four hour world. The temporary discomfort associated with jetlag is a reminder of the importance of this daily programme, but there is growing recognition that its chronic disruption carries a cost for health of far greater scale. In this primer, we shall briefly review the historical identification of the SCN as the master circadian clock, and then discuss it on three different levels: the cell-autonomous SCN, the SCN as a cellular network and, finally, the SCN as circadian orchestrator. We shall focus on the intrinsic electrical and transcriptional properties of the SCN and how these properties are thought to form an input to, and an output from, its intrinsic cellular clockwork. Second, we shall describe the anatomical arrangement of the SCN, how its sub-regions are delineated by different neuropeptides, and how SCN neurons communicate with each other via these neuropeptides and the neurotransmitter γ-aminobutyric acid (GABA). Finally, we shall discuss how the SCN functions as a circadian oscillator that dictates behaviour, and how intersectional genetic approaches are being used to try to unravel the specific contributions to pacemaking of specific SCN cell populations.

Circadian clocks and rhythms

To maximise their success in the daily and seasonal world, living organisms display pronounced daily and seasonal rhythms of physiology and behaviour. For example, the pineal gland of all vertebrates secretes melatonin at night to promote nocturnal physiology, and in mammals, daylength-dependent changes in the duration of this nocturnal signal trigger photoperiodic rhythms that adapt them to the seasons. Daily and seasonal rhythms are not, however, a passive response to sunrise and sunset. Rather, they are cued by internal clock mechanisms that enable the organism to anticipate, and so prepare for, predictable environmental changes likely to occur several hours and sometimes many months in advance. The biochemical components of these circadian clocks vary across taxa, but their mechanistic basis invariably pivots around a cell-autonomous negative feedback oscillation. In turn, this oscillation directs daily patterns of gene expression that drive cellular metabolic rhythms and ultimately the circadian programme of the organism. The defining property of circadian rhythms and the clocks driving them, therefore, is that when deprived of environmental cues their intrinsic oscillation persists, 'free-running' with a period of approximately (*circa*-) one day (*diem*) (Figure 1B,C). To be fully adaptive, however, this biological timer needs to be synchronised with external solar time: circadian clocks must therefore receive input from photoreceptors to perform their biological functions.

Identification of the SCN as the mammalian circadian clock

Following the acceptance in the late 1950s that circadian rhythms are indeed driven by internal, self-sustaining biological clocks and are not an artefactual response to unidentified periodic environmental cues, the search for the clocks focused on photoreceptive structures. The eyes of invertebrates and the eyes and pineal gland of non-mammalian vertebrates were shown to express self-sustaining rhythms when isolated in culture and to be necessary for the expression of various overt circadian rhythms. In the case of mammals, ablation studies indicated that a

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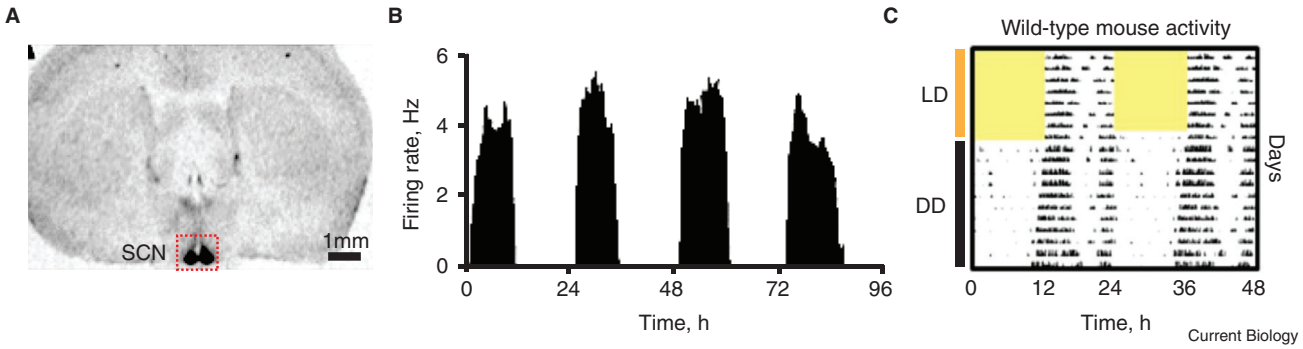

Figure 1. The SCN and circadian time-keeping.
(A) The SCN (boxed) is located at the base of the anterior hypothalamus immediately dorsal to the optic chiasm, here revealed in a coronal section of mouse brain showing autoradiographic binding of the SCN neuropeptide Prokineticin 2 to its receptor (Prokr2) (from Prosser *et al*., 2007). Scale bar = 1 mm. (B) When isolated in culture, individual SCN neurons exhibit persistent circadian cycles of electrical activity: 'a cellular clock in a dish'. (Redrawn from Hastings and Herzog, 2004.) (C) The SCN clock directs circadian physiology and behaviour, here represented by wheel-running activity cycles of a mouse initially entrained to a light–dark cycle (LD) then released into constant darkness (DD) when the rhythm persists with a period of approximately 24 h, in phase with anticipated lights-on and -off. Activity is shown by black bars, and is double-plotted for clarity. (Redrawn from Anand *et al*., 2013.)

centre in the hypothalamus was necessary for circadian behaviour, but its location was unclear. The SCN was first identified as a hypothalamic retinal target in rodents, and by implication a potential circadian control point following autoradiographic visualisation of its dedicated route of retinal innervation — the retinohypothalamic tract (RHT). It was initially thought to be a relay station, directing light–dark information to a clock located elsewhere. Loss of such a relay would cause animals to free run independently of the light dark–cycle, but in fact, ablation of the SCN caused animals to become arrhythmic and to lose photoperiodic seasonality. This was consistent with the SCN being the source of rhythmic behaviour, but did not prove it: the SCN were necessary but were they sufficient for circadian time-keeping? Confirmation that the SCN constitute the circadian clock driving behavioural rhythms came first with the demonstration that they can express sustained circadian rhythms when isolated from the brain and, second, that transplantation of SCN tissue can initiate circadian behaviour in an arrhythmic SCN-lesioned recipient. Critically, the period of the behavioural rhythm was set by the genetically specified period of the donor animal, not the period of the recipient. The SCN is therefore a circadian clock in receipt of retinal information and under natural conditions the SCN clockwork responds to retinal entraining cues in three ways. First, on each cycle its intrinsic period is set to exactly 24 hours by a small phase advance or phase delay. Second, in doing so internal time as defined by the SCN is locked into a strict phase relationship to solar time; i.e. biological (subjective) night matches the real night. Finally, changes in daylength are encoded into the ensemble circadian signal generated by the SCN cellular network to provide an internal calendar which tracks and predicts the seasons via changes in photoperiod.

The SCN as a vertebrate clock and calendar

The distinct role of the SCN as the central, retinally entrained pacemaker controlling behavioural rhythms in mammals is a significant refinement of a more distributed ground-plan for circadian time-keeping in lower vertebrates (Figure 2). For example, in birds, reptiles and teleosts both the retina and the pineal gland house light-responsive local oscillators and, depending on species, they can control circadian behavioural rhythms by the nocturnal secretion of melatonin. The avian equivalent of the mammalian SCN encompasses two interconnected hypothalamic nuclei, the melatonin-responsive medial SCN and the melatonin-insensitive visual SCN, which is innervated by the avian RHT. In some species of bird, ablation of the medial SCN causes arrhythmia similar to that seen after pinealectomy, suggesting that the avian SCN may direct behavioural rhythms as a downstream relay of the pineal clock. Equally, ablation of the SCN in some lizards also causes arrhythmia and loss of entrainment to melatonin. Entrainment of the network of light-sensitive clocks of lower vertebrates is also more complex than in mammals. A variety of opsin-based photoreceptors are expressed in the basal forebrain and in the hypothalamus (including preoptic and mediobasal areas), and contribute to circadian and/or seasonal timekeeping. Indeed, in the model teleost the zebrafish, many peripheral tissues (e.g. heart, liver) contain light-sensitive cellular clocks that can be entrained directly when isolated in culture. In mammals, local light-entrainable autonomous clocks are present in the retina but their role is limited to the control of retinal physiology, while circadian rhythms in the pineal are entirely dependent on signals from the SCN. Furthermore, the contribution of melatonin to the control of circadian functions in mammals is limited to the trans-placental circadian entrainment of the foetal SCN by the maternal SCN. In adult mammals, the role of the SCN-driven melatonin rhythm is as the critical seasonal transducer: acting via the anterior pituitary pars tuberalis and the mediobasal hypothalamus, long nocturnal melatonin patterns signal winter whereas short durations

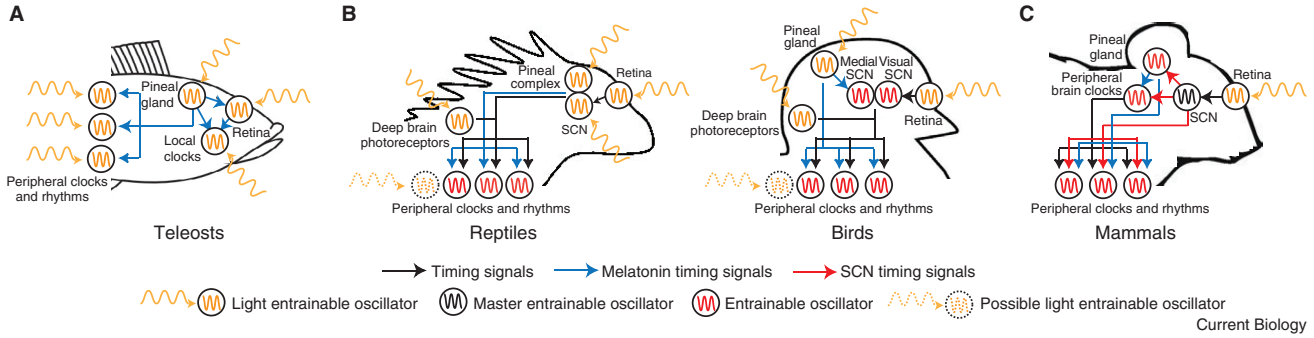

Figure 2. The ascent of the SCN: a comparison of circadian timing systems in vertebrates.
(A) Schematic view of the widely distributed system of photosensitive tissue clocks of zebrafish and other teleosts. (B) The SCN/ pineal-based system in reptiles and birds, in which melatonin is a critical mediator of clock control of circadian behaviour. (C) The SCN-based system in mammals, with a restricted access for photic entrainment via the retina and ultimate co-ordination of the entire system by the SCN. Note that the phylogenetic transition in lower vertebrates, whereby peripheral clocks cells lost intrinsic photoreception (if they did), is not clear.

signal summer. Intriguingly, there is emerging evidence that, beyond the SCN-dominated melatonin signal of mammals and the control by deep-brain photoreceptors in non-mammalian vertebrates, the neuroendocrine structures and signalling pathways that ultimately mediate seasonality have conserved elements; i.e. the neuroendocrine effectors of seasonality are shared but their upstream photoperiodic control systems diverge. Overall, therefore, the pattern of circadian organisation in mammals, with the SCN as the sole central pacemaker and the RHT as the single route for its entrainment by light, represents a much more focussed, stripped-down arrangement than that of non-mammalian vertebrates (Figure 2).

The cell-autonomous mammalian clock

So what is the cellular basis of circadian timekeeping in the SCN? Just as tissues across the zebrafish contain local clocks, then so does practically every nucleated cell in the mammalian body contain a cell-autonomous circadian clock. It is formed from interlocking transcriptional–translational feedback loops (TTFL) that drive spontaneous oscillations of gene and protein expression with a circadian period. At the core of this loop, expression of the Period (Per1 and Per2) and Cryptochrome (Cry1 and Cry2) genes is initiated by the transcription factors CLOCK and BMAL1 acting at E-box regulatory sequences. Subsequently, PER and CRY proteins accumulate over the course of circadian day, translocating in large multimeric complexes into the nucleus until they reach a level whereby they repress their own transcription (and that of other genes activated by CLOCK/BMAL1). This is followed over the course of circadian night by progressive degradation of the existing inhibitory complexes, and ultimately renewed transcription of Per and Cry approximately 24 hours after the previous initiation. Additional TTFL components, including the E-box-driven transcriptional regulators ROR and REV-ERB, feed into the core oscillation via RRE elements to control Bmal1 expression and thereby enhance stability, amplitude and precision. The representation of solar time generated by the effortless progression of these loops is conveyed to the rest of the cell in the form of circadian programmes of gene expression. These 'clock output genes' carry E-boxes and RRE sequences and so are subject to alternating circadian activation and suppression. Many are themselves transcription factors and so, by directing cascades of gene expression, they amplify the TTFL circadian signal and co-ordinate circadian programmes of gene expression that are cell-type- and tissue-specific. Co-ordinated across tissues by the SCN, these programmes are the origin of our adaptive circadian cycles of physiology, metabolism and behaviour: and it happens every day, for a lifetime. The biological elegance and sophistication of this organism-wide orchestration are astonishing.

Cellular time-keeping in the SCN

The adaptation of the SCN to its role as circadian pacemaker, orchestrating the innumerable circadian oscillations across the body, is exemplified by a remarkable property: when isolated *in vitro* and provided with the appropriate culture medium, it can sustain its intrinsic circadian cycle of TTFL activity indefinitely. In contrast, the circadian programmes of peripheral tissues slowly damp after several days in culture, as cell-autonomous clocks lose amplitude and phase coherence. These remarkably robust SCN oscillations have been comprehensively described by using organotypic slice cultures from genetically modified mice and rats carrying bioluminescent and fluorescent reporters of TTFL transcriptional (Per1, Cry1 and Bmal1) or translational (PER2::Luciferase, PER2::Venus) activity (Figure 3A). These cellular rhythms are tightly synchronised across the SCN network, and although they are generated cell-autonomously, they rely on interneuronal electrical communication for their maintenance. Pharmacological blockade of electrical firing with tetrodotoxin (TTX) desynchronises tissue-level rhythms and damps (but does not stop) the cell-autonomous rhythms. Put another way, in addition to it being the sole point of retinal access and possessing efferent connectivity to all of the subordinate clocks across the body, the secret of the SCN is the powerful intercellular coupling conferred by electrophysiological, synaptic activity. Thus, individual SCN

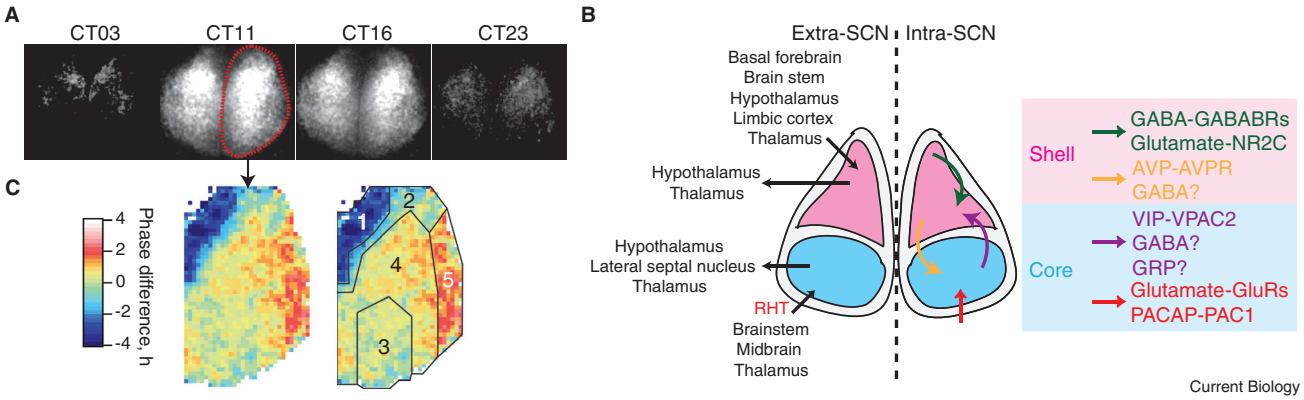

Figure 3. SCN networks.
(A) Time series of PER2::Luciferase bioluminescence images from SCN organotypic slice (CT, circadian time) reports cell-autonomous and circuit-level TTFL oscillations. (B) Schematic view of SCN afferent and efferent projections (left) alongside intra-SCN signals and neuroanatomical sub-divisions (right) (modified from Moore 2013). (C) False-coloured map of oscillator phases (expressed as phase difference from field mean) from right SCN in (A) reveals temporal heterogeneity of cellular TTFLs across the SCN that defines spatiotemporal clusters or sub-networks (1–5) across the circuit. (Modified from Brancaccio *et al*., 2013.)

neurons exhibit spontaneous rhythms in membrane potential and firing rate that track solar time: regardless of whether an animal is behaviourally diurnal or nocturnal, the peak of SCN electrical activity occurs in the middle of the subjective day. It is therefore reasonable to believe that electrical activity in the SCN does not exist to merely facilitate communication of molecular programmes between SCN neurons: it encodes important contextual information. In the intact circuit, SCN neurons undergo pronounced oscillations in membrane potential of between 6 and 10 mV, being depolarised during the subjective day with a daytime resting membrane potential of between –55 and –50 mV and generating spontaneous action potentials at frequencies of around 10 Hz. What is remarkable about SCN neurons, however, is that they can sustain these firing rates for long intervals (up to 6 hours at a time) without spike-frequency adaptation. These electrophysiological rhythms are truly cell-autonomous, intrinsic oscillations as evidenced by their persistence when SCN neurons are dissociated in culture, although as with the molecular TTFL rhythms, dissociation renders the electrical circadian signal less robust. A fundamental question, therefore, is where and how do these rhythmic electrical properties in SCN neurons arise? Possible cell-autonomous means for this can be loosely categorised into 3 mechanisms: daily depolarisation, facilitation of firing, and nightly hyperpolarisation.

Molecular control of electrical rhythms in the SCN

In order to determine how rhythmic electrical activity arises in SCN neurons, studies have utilised transcriptomics, proteomics and electrophysiology over the circadian cycle to identify potential regulatory targets of the TTFL clock. In the case of daily depolarisation, voltage-gated sodium and calcium channels, along with hyperpolarisation-activated, cyclic nucleotide gated (HCN) channels, and the NALCN sodium leak channel are thought to contribute to circadian excitatory drive, whilst high-frequency firing during circadian day is sustained by the fast delayed rectifiers (FDRs) and A-type potassium (I_A) channels. Two potassium conductances have been implicated in nocturnal circadian hyperpolarisation of SCN neurons: the two-pore potassium (K2P) channels and calcium-activated large conductance potassium (BK) channels. Members of both are rhythmically transcribed in the SCN, peaking during the night, and genetic deletion of the pore-forming subunit of BK channels reduces the amplitude of neuronal firing and behavioural rhythms. Overall, therefore, it seems that the main electrical rhythm is generated by cyclic expression of hyperpolarising K^+ conductances during the night and daytime depolarising Na^+ conductances. This daily programme of balancing ionic conductances in concert with molecular oscillations means that the SCN is potentially a very complex and unique electrophysiological brain region, and the exact mechanism generating this electrophysiology and linking it to the core molecular clock is still unknown. Second-messenger signals are prime candidates for such linkage, and SCN cells indeed display circadian oscillations of cyclic AMP (cAMP) and calcium ($[Ca^{2+}]_i$). Given that the Per genes contain Ca^{2+}/cAMP-response elements (CREs) in their promoters, these cytosolic oscillations can influence core clock gene expression. Indeed the TTFL can be accelerated or suspended by pharmacological manipulations of adenylyl cyclase, the enzyme that synthesises cAMP. The levels of cAMP in the SCN peak in the middle of circadian day, alongside those of $[Ca^{2+}]_i$, which in neurons correlates with the daily peak of neuronal firing rate and membrane depolarisation. The precise mechanisms governing this rhythmicity of $[Ca^{2+}]_i$ are unknown: electrical activity and ryanodine-sensitive intracellular stores likely contribute. Interestingly, neurons are not the only SCN cell type to exhibit a circadian $[Ca^{2+}]_i$ oscillation: astroglial cells also show strong oscillations that are antiphasic to neuronal oscillations, peaking during the circadian night

when neurons are inactive. Thus, the interplay between cell-autonomous core molecular, electrical and cytosolic oscillations is complex and it is unclear where circadian TTFL input ends and output begins.

The SCN as a network: inputs

Neurons of the SCN are predominantly GABAergic, but this homogeneity is striking considering that SCN neurons also express a wide range of neuropeptides and the neuropeptide receptors that mediate input from extra-SCN afferent pathways as well as intra-SCN networks (Figure 3B). The most important afferent connection is the RHT, which terminates in the ventral, retinorecipient core of the SCN. This tract brings non-image-forming visual information from the intrinsically photosensitive retinal ganglion cells (iPRGCs) of the inner retina directly into the SCN and these projections are sufficient to entrain the SCN to the light–dark cycle in the absence of conventional rod and cone (image-forming) photoreceptors. The iPRGCs are defined by expression of the invertebrate-like photopigment melanopsin, a photopigment first discovered in amphibian skin that provides an echo, perhaps, of the early vertebrate ground-plan for circadian organisation. Melanopsin causes depolarisation in response to light and results in the release of glutamate and the neuroactive peptide pituitary adenylyl-cyclase activating peptide (PACAP) from RHT terminals in the SCN. In turn, this results in the electrical activation of retinorecipient SCN cells, downstream activation of their intracellular kinase signalling cascades and consequent induction of Per and other immediate-early genes. These events constitute the first steps in maintaining or slightly re-adjusting the phase of the SCN TTFL oscillation in response to light. A second, less well characterised form of entrainment involves non-photic cues associated with behavioural arousal. Afferents to the SCN from the non-visual thalamus, the mid-brain and the brainstem Raphe employing, respectively, NPY, dopamine and serotonin as neurotransmitters are the principal mediators of non-photic entrainment. Intriguingly, this resetting can involve suppression of Per gene expression rather than the induction associated with photic entrainment.

The SCN as a network: intrinsic organisation and neuropeptides

The neuropeptidergic identity of cells can be used to subdivide the SCN into two regions: the ventral core and the dorsal shell. The retino-recipient core is delineated by vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP). The shell, delineated by cells that express arginine vasopressin (AVP), receives input from the core region (as evidenced by the high density of the VIP receptor, VPAC2), and directs SCN outputs to other brain areas, as well as feeding back to the core (Figure 3B). Although SCN topology is normally divided into these two regions, it is evident from CCD recordings of the TTFL oscillation in SCN organotypic slices that the circuit organisation is more complex with differentially phased, spatially discrete clusters (Figure 3C). Recently, advanced transcriptomic approaches have identified markers that can be used to assign different cell groups to phase-specific and functional modules based on neurochemical combinations and transcriptional responses to light. Of the numerous neurochemicals expressed within the SCN, VIP and GABA have attracted the most study. VIP is particularly important for the correct function of the SCN, as it is expressed in the retinorecipient cells and therefore likely dictates responses of the rest of the network to light. Indeed, loss of VIP, or its receptor VPAC2, results in arrhythmic behaviour in mice held in constant darkness, the loss of circadian responses to light and impaired synchrony and amplitude of cellular TTFL rhythms. Thus, VIP signalling is required for correct SCN function, and restoring VIP signalling *in vitro* by grafting wild-type SCN on top of arrhythmic VIP-null SCN immediately restores synchronous rhythmicity to the host slice with a period determined by the graft SCN (Figure 4A). This synchronisation is dependent on neuropeptidergic paracrine signalling from the graft to host, and can be eliminated by placing a membrane with a 2 kDa cut-off between the graft and host SCN, and reinstated by inverting the membrane to allow contact between graft and host SCN. This paracrine synchronisation is not solely dependent on graft-derived VIP signalling, however, as it can also be observed in host SCN lacking VPAC2, albeit taking longer to achieve. Indeed, pharmacological tests indicate a contribution from graft-derived neuropeptides AVP and GRP, revealing that a hierarchy of neuropeptides (VIP>AVP>GRP) maintains circuit-level circadian coherence in the SCN. Consistent with this hierarchical view, loss of AVP signalling does not preclude circadian oscillations, but in the absence of AVPR1a and AVPR1b receptors, mice and SCN slices phase-shift more rapidly to resetting cues, which is indicative of weaker coupling across the network. In addition to coupling SCN neurons, neuropeptides also likely act as an output from the oscillator. For example AVP signals circadian time to pre-autonomic pathways and loss of signalling by prokineticin causes striking deficits in circadian outputs, most notably loss of the activity bout associated with early circadian night. Thus, SCN neuropeptidergic axes may specify certain circadian-timed behaviours by directly controlling particular outputs from the SCN.

The SCN as a network: intrinsic organisation and GABA

In contrast to VIP, the specific role for GABAergic signalling in the SCN is less well understood. As GABA is the dominant neurotransmitter in the SCN, synthesised by all neurons, it might be expected that disrupting GABAergic transmission pharmacologically would disrupt ongoing SCN function, but this is not the case: the cell-autonomous and circuit-level TTFL oscillations continue when SCN slices are treated with the broad-spectrum blocker Gabazine. GABAergic signalling comes in two forms: ionotropic $GABA_A$ signalling, and (oft-neglected) metabotropic $GABA_B$ signalling. $GABA_A$ signalling is suggested to oppose VIPergic signalling and thereby maintain the differential phasing of sub-populations of SCN neurons that encodes photoperiod at the network level. Moreover, intracellular chloride concentration ($[Cl^-]_i$) and excitatory GABAergic tone in the SCN have been observed to regionally track photoperiod with high $[Cl^-]_i$ expressed

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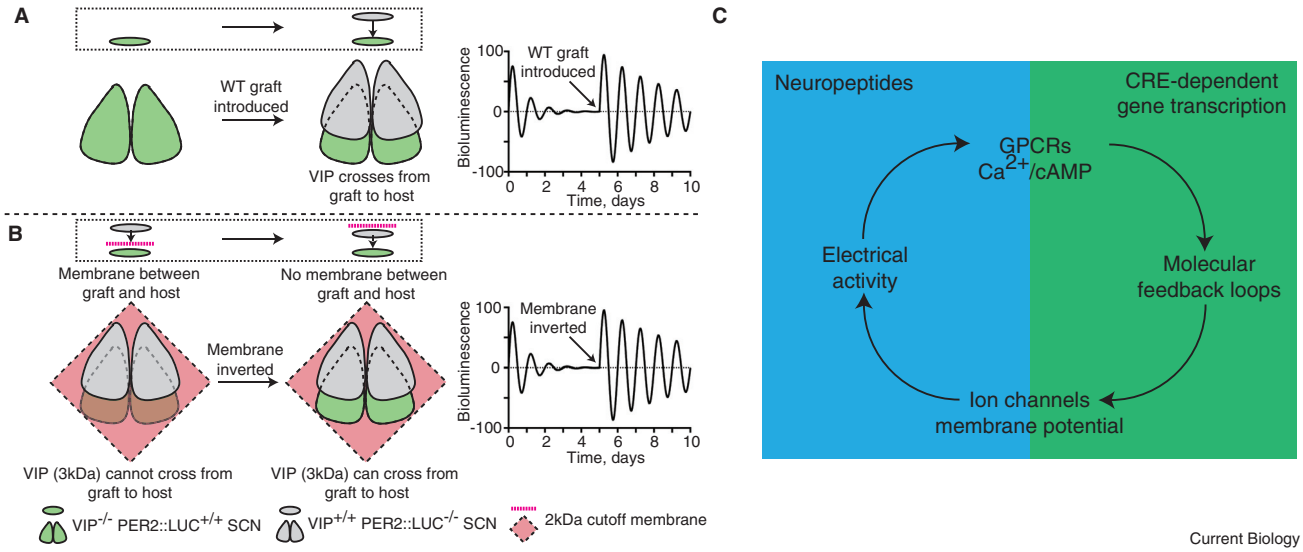

Figure 4. Paracrine signalling in the SCN.
(A) Schematic representation of co-cultures in which placement of a wild-type (WT; graft) SCN rescues TTFL function in a VIP-null (host) SCN, as reported by PER2::Luciferase bioluminescence. (B) When a 2 kDa cut-off membrane is placed between graft and host to preclude peptidergic signalling, the graft can not drive host TTFL rhythms. When the membrane is inverted, however, paracrine signalling from graft to host becomes possible and initiates TTFL function in the VIP-null host. (C) Schematic view of how neuropeptidergic signalling in the SCN acts via CRE-dependent gene transcription to regulate the TTFL and thereby control circadian electrical activity and neuropeptide release: output of the TTFL clock becomes its input.

preferentially in the dorsal region during long photoperiods. $GABA_B$ signalling in the SCN is less well studied, even though $GABA_B$ receptors are expressed in the SCN, especially dorsally. Pharmacological activation of $GABA_B$ receptors in the SCN can phase-shift electrical rhythms *in vitro*, attenuate photic circadian responses *in vivo* and influence presynaptic GABA release via auto-regulation of voltage gated calcium channels. Overall, the circadian role of GABA signalling remains an enigma. But there is a further aspect to this. In addition to neurons, the SCN is also densely populated by glia in an estimated ratio of approximately 3:1 (neurons to glia), but only recently has the development and widespread application of new genetic tools allowed glial function in the SCN to be dissected. Astrocytes potentially influence the SCN in at least two ways. First, by circadian changes in their morphology altering the access between neurons, they may regulate the activity of RHT synapses in the core SCN and thereby entrainment by lighting cycles. Second, the nocturnal increase of activity in astrocytes in circadian night, as evidenced by a sustained increase of $[Ca^{2+}]_i$, may be a prime controller of nocturnal elevations of extracellular glutamate levels. Acting via the NR2C subunit of NMDA receptors expressed pre-synaptically by neurons of the dorsal shell, the rhythm of glutamate may in turn drive circadian cycles of GABAergic tone that facilitate nocturnal hyperpolarisation of the neuronal circuit. In this model, control of the neuronal network is devolved to the astrocytic network via its nocturnal pre-synaptic facilitation of GABA release. But the quandary remains: why does complete pharmacological blockade of GABAergic signalling not compromise the ongoing oscillation?

The SCN as a circadian pacemaker: outputs

For all its neurochemical complexity, the SCN seems to have a simple role: it links behaviour and physiology to the daily world by creating and broadcasting an internal representation of solar time. This broadcasting, however, involves a considerable amount of coordination and one would expect the outputs of the SCN to be as numerous and complex as its neurochemical architecture. This does not appear to be the case, however, as the SCN projects sparsely, principally to interneurons in other hypothalamic areas and with some direct connectivity to neuroendocrine neurons (Figure 3B). Despite these limited direct connections, the control of daily physiology by the SCN goes beyond the simple scheduling of behavioural activity patterns: the SCN controls physiological responses as diverse as water intake, sleep quality, aggression and body temperature. It is likely, therefore, that the SCN provides the contextual timing information to which it is specialised, and delegates direct control of behavioural and physiological states to more appropriate brain regions. The identity of the SCN outputs and the 'temporal code' they convey via GABAergic and neuropeptidergic cues are currently unknown. As functional and anatomical circuit-tracing tools improve and opportunities for genetically targeted manipulations expand, the means by which information is passed from the SCN to other brain regions and, in turn, peripheral clocks will be decoded. For example, recent advances in mouse intersectional genetics have been used to interrogate the contribution of different SCN neuronal populations in controlling circadian behaviour. In particular, Cre recombinase-expressing driver lines have been

used to conditionally alter properties of the core clock by changing cell-autonomous period, deleting TTFL function, and/ or compromising synaptic signalling. Such studies have revealed a hierarchy of neurochemically defined cell types that contribute to the correct functioning of the SCN as a network and thereby circadian behaviour. Of note are Neuromedin S-positive (NMS) cells, which constitute ca. 40% of SCN neurons. Synaptic signalling in these cells is essential for the SCN to control circadian behaviour, and changes in the properties or competence of the cell-autonomous TTFL in NMS cells are echoed at the level of mouse behavioural rhythms. In contrast, manipulations of AVP- or VIP-expressing cells (which are sub-groups of the NMS population) have a more modest effect. Paradoxically, however, loss of NMS itself has no obvious effect on the clockwork. Furthermore, recent developments of the intersectional approach have shown that, astonishingly, neurons are not the only active cellular participants in SCN timekeeping. Through conditional manipulations of the astrocyte TTFL, it is clear that the astrocytic clock can also control the ensemble SCN molecular clockwork and overt circadian behaviour of the mouse.

Prospect

The SCN is a unique brain area: two clusters of 10,000 cells exert global, hour-by-hour control over every vital function of the organism. It generates an ensemble circadian signal that has extreme robustness, persistence and precision (varying by ca. ±5 minutes over the 24 h cycle, i.e. 0.35% error). These emergent properties are established by the linkage between cell-autonomous and circuit-level mechanisms, such that outputs of the cellular clock become inputs to it (Figure 4B). Significant questions remain as to how individual SCN neurons generate day-to-day electrophysiological rhythms, what these rhythms mean for the SCN and how they are linked to the molecular clock. At the network level, the neurochemistry that 'glues' the SCN together is poorly understood: we have a fair grasp of the distribution and release of some individual neuropeptides, but no deep understanding of how peptides and other neurotransmitters come together to create a robustly functional network. Finally, we do not know the full hierarchy of signals within the SCN at the level of neurochemicals or cell-type and their topology, nor how these signals are directed outside of the SCN to determine organismal physiology. Time to get to work.

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Extremely rapid maturation of a wild African annual fish

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Ephemeral habitats can impose challenging conditions for population persistence. Survival strategies in these environments can range from high dispersal capacity to the evolution of dormant stages able to tolerate a harsh environment outside the temporal window of favourable conditions [1]. Annual killifish have evolved to live in seasonal pools on the African savannah and display a range of adaptations to cope with an unpredictable environment [2,3]. For most of the year, killifish populations survive as diapausing embryos buried in dry sediment. When savannah depressions fill with rainwater, the fish hatch, grow rapidly and, after attaining sexual maturity, reproduce daily [2,4]. *Nothobranchius furzeri*, a model species in ageing research [2,3], is distributed in a region where the climate is particularly dry and rains are unpredictable [5]. Here, we demonstrate that the fast juvenile growth and rapid sexual maturation shown by *N. furzeri* in captivity is actually an underestimate of their natural developmental rate. We estimated the age of *N. furzeri* in natural populations by counting daily-deposited increments in the otoliths and performing histological analysis of gonads. We found that *N. furzeri* are capable of reaching sexual maturity within 14 days after hatching, which to our knowledge is the fastest rate of sexual maturation recorded for a vertebrate. We also demonstrate that *N. furzeri* can grow from an initial length of 5 mm up to 54 mm over the course of a two-week period. Such rapid juvenile development is likely to be adaptive since some pools were entirely desiccated 3–5 weeks after filling, but retained a viable killifish population that reproduced before the adults succumbed to the disappearance of their pool.

We surveyed natural populations of *N. furzeri* across its range in southern