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REVIEW ARTICLE

Seeing the forest and trees: whole-body and whole-brain imaging for circadian biology

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Recent advances in methods for making mammalian organs translucent have made possible whole-body fluorescent imaging with single-cell resolution. Because organ-clearing methods can be used to image the heterogeneous nature of cell populations, they are powerful tools to investigate the hierarchical organization of the cellular circadian clock, and how the clock synchronizes a variety of physiological activities. In particular, methods compatible with genetically encoded fluorescent reporters have the potential to detect circadian activity in different brain regions and the circadian-phase distribution across the whole body. In this review, we summarize the current methods and strategy for making organs translucent (removal of lipids, decolourization of haemoglobin and adjusting the refractive index of the specimen). We then discuss possible applications to circadian biology. For example, the coupling of circadian rhythms among different brain regions, brain activity in sleep–wake cycles and the role of migrating cells such as immune cells and cancer cells in chronopharmacology.

Keywords: light-sheet microscope, single-cell resolution, tissue clearing

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The Circadian Clock as a Whole-body System

Many physiological processes, including the sleep–wake cycle, hormone secretion and body temperature, change according to a day–night 24 h cycle [1]. Most of these processes are under the control of a system called the circadian clock. The circadian clock works as an autonomous oscillator: the physiological activities of organisms with a clock can cycle every 24 h without the need for external stimuli for timing.

A series of seminal experiments established that the center of the mammalian circadian clock lies in the suprachiasmatic nucleus (SCN) located in the hypothalamus of the brain. The initial demonstrations of the role of the SCN were done by a brain-lesion experiment where ablation of the brain area including SCN disrupted the circadian cycle of behavioural activityand hormonal secretion in the absence of environmental light information (constant darkness condition) [2,3]. The critical role of the SCN for autonomous circadian behaviour was established by a SCN transplantation carried out by Ralph et al. [4]. They used mutant hamsters with a short circadian period in behavioural rhythmicity. When SCN from the mutant hamster was transplanted into the brain of a wild-type hamster, the transplanted hamster had a shorter period in behavioural rhythms. Similarly, a mutant hamster transplanted with a wild-type SCN had normal circadian rhythms. These results clearly indicated that behavioural rhythmicity is established by the circadian period encoded within the SCN.

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Because the SCN is a neuronal assembly, its activity can be monitored using electrophysiological recording. Intriguingly, explanted SCN cultured *in vitro* has rhythmic activity changes over a 24-h period [5,6]. The rhythmicity is preserved even in neurons dissociated from the SCN [7]. Thus, each SCN neuron works as an autonomous oscillator. Central components of this oscillator were revealed by genetic screening of mutant organisms; the *period* (*per*) gene was originally identified in fruit flies [8,9] and the *clock* gene was firstly identified in mice [10]. Currently ~20 genes are 'clock genes', which comprise the mammalian circadian oscillator [11]. Most clock genes encode transcriptional regulators that activate or repress the expression of other genes. The resultant transcriptional network is inherently complex, but its core architecture can be understood as a simple negative feedback loop (Figure 1). Clock and its binding partner Bmal1 bind to a promoter sequence called an E-box. The E-box regulates thousands of downstream genes including the clock genes *per* and *cry*. The expressed PER and CRY proteins form a heterodimer that binds to and inhibits the BMAL1–CLOCK complex. The total action therefore forms a negative feedback, where elevated CRY and PER inhibit their own transcription. The negative feedback loop can exhibit oscillatory dynamics, and in the mammalian circadian clock, this feedback loop of E-box regulation is thought to be the core of the cellular circadian oscillator.

With this feedback loop, the expression of several clock genes has robust 24 h rhythmicity. These genes are useful reporters to analyze circadian rhythmicity even in non-neuronal cells, because cell-autonomous circadian oscillation occurs in almost all cell types in mammals including lung and liver [12], and in cultured cell lines such Rat-1 [13], NIH3T3 [14] and U2OS cells [15]. The finding of circadian oscillation in non-SCN

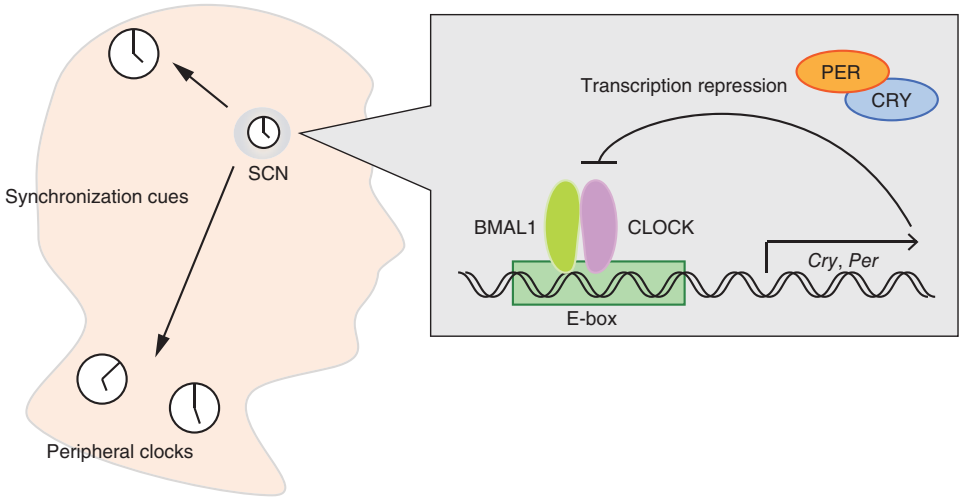

Figure 1. Regulation of E-box promoter activity by BMAL1-CLOCK and PER-CRY. Almost all cells in mammals have a cell-autonomous circadian clock. The core of the clock is thought to be a transcriptional/translational negative feedback loop, where the PER-CRY complex represses its own transcription. The clocks in an entire body are orchestrated by synchronization cues from the suprachiasmatic nucleus (SCN).

cells, namely 'peripheral clocks', leads to a hierarchical view of the circadian clock system; the SCN works as a master clock that generates a signal to synchronize peripheral clocks. This system is like adjusting a computer's current time – a time server delivers time information to all computers on the same network and each computer adjusts the time of its own clock accordingly. One fundamental difference between the clock in our computers and the circadian clock is the phase distribution; the phase of the circadian clock is not identical in all peripheral clocks [12,16] – each organ may be delayed relative to the central circadian clock. In other words, a body is an assembly of heterogeneous cells with different circadian phases.

Assuming the heterogeneous yet synchronized assembly of cellular circadian clocks in the whole body, the next challenge is to understand how the SCN synchronizes peripheral clocks throughout the body. How does the signal from SCN propagate and synchronize other peripheral clocks? What is the role of heterogeneity in peripheral clocks? To answer these questions, the first step is to observe the circadian clock in the entire body. The hierarchical organization of circadian system from cells to organs requires a technology to observe centimetre-scale 3D samples with cellular resolution. Recent advances in microscopic imaging and sample preparation techniques are opening up the possibility of whole-body imaging at single-cell resolution. In this review, we will revisit a few different state-of-the-art imaging methods and discuss what questions can be asked by using such whole-body imaging techniques.

Technology for Imaging Circadian Activities Throughout the Body

Method for Clearing Brain Tissue

The brain is the most complex organ in our body. The complexity comes from 'heterogeneity' in the brain – the nature of the brain as an information processing organ lies in the network architecture of wired neurons and glia cells. A homogeneous brain lysate loses this essential property.

> "The combination of tissue-clearing methods and light-sheet microscopy allows for whole-organ imaging with single-cell resolution."

Optical microscopes use light. As long as we use light to observe the specimen, the transparency of the object greatly limits the depth of observation. This is one reason why transparent biological samples such as sea urchin embryos have contributed to our understanding of developmental processes. Adult mammals in general, however, are not transparent. There are at least two reasons for the opacity of biological samples. One reason is the presence of scattering media in organs. Such materials, typically lipid layers and red blood cells, scatter light and hide the image behind opaque materials. The second reason is different refractive indexes within the sample and between the sample and its environment (e.g. air or immersion oil filing the space between the sample and the objective lens). To overcome these limiting factors for the transmittance of light, several techniques have been developed to make fixed organs from mammals translucent.

Table 1 categorizes the tissue-clearing reagents/methods in three classes, each of which has advantages and limitations. The first category is methods that use organic solvent. The clearing chemical cocktail composed by benzyl alcohol and benzyl benzoate (BABB) [17], or the combination of tetrahydrofuran (THF) and dibenzyl ether (DBE) [18,19] are used in these methods. These chemicals effectively remove lipids and modify the refractive index, therefore that the whole brain becomes translucent within a few days. Because organic chemicals denaturize protein, reporters based on fluorescent proteins are, in general, incompatible with BABB. THF/DBE protocols are somewhat compatible with fluorescent proteins but fluorescent

Table 1. Summary of major tissue-clearing reagents/protocols.

Category	Reagent/protocol name	Key chemical components or procedures	Reference
I	BABB	Benzyl alcohol and benzyl benzoate	[17]
	THF-DBE	Tetrahydrofuran and dibenzyl ether	[18]
	3DISCO	Tetrahydrofuran and dibenzyl ether	[19]
	iDISCO	Tetrahydrofuran and dibenzyl ether	[20]
II	Sca*l*e	Urea	[21]
	SeeDB	Fructose	[22]
	Clear^{T}	Formamide, polyethylene glycol	[24]
	CUBIC	Aminoalcohol	[23,29]
	PACT-RIMS-PARS	Hydrogel-embedded samples and perfusion-mediated reagent circulation	[28]
	TDE	2,2′-thiodiethanol	[25]
III	CLARITY/Advanced CLARITY	Hydrogel-embedded samples and electrophoretic lipid removal	[26,27]

BABB, benzyl alcohol and benzyl benzoate; CUBIC, clear, unobstructed brain imaging cocktails and computational analysis; DBE, dibenzyl ether; TDE, 2,2′-thiodiethanol; THF, tetrahydrofuran.

photoquenching by organic solvent still occurs. Nonetheless, the immunostaining protocol of iDISCO allows the use of non-protein reporters such as Alexa Fluor dyes and q-dot to label specific proteins [20].

By contrast, methods in the second category use water-soluble chemicals, so that genetically encoded reporters are more functional. A major component in the Sca*l*e method – urea – is mostly responsible for lipid removal from the brain [21], whereas the fructose used in the SeeDB method aims to adjust the refractive index within the brain [22]. The clear, unobstructed brain imaging cocktails and computational analysis (CUBIC) method adopts both strategies with a slightly different set of chemicals [23]: a mixture of urea and aminoalcohol for the removal of lipids and sucrose to adjust the refractive index. *Clear^{T}* applies polyethylene glycol to stabilize proteins [24] and 2,2′-thiodiethanol (TDE) solutions for brain clearing [25]. The use of water-soluble chemicals is also advantageous for easy handling and safety for the user. The third category also uses water-soluble chemicals but the sample is embedded in an acrylamide gel [26,27]. The molecular mesh of acrylamide is thought to maintain the detailed structure of the neuronal network in the brain and enables an electrophoresis force to facilitate the removal of lipids.

> “The clearing method with water-soluble chemicals is compatible with genetically encoded reporters for neuronal activities, which depict integrated neuronal activities over the time range from minutes to hours.”

Method for Clearing the Whole Organism

While the primarily application of these clearing methods is the brain, it should be possible to apply these techniques to other organs. Indeed, methods in each category were extended to other organs and even whole animals [28,29]. In the case of several non-brain organs (e.g. liver), however, red blood cells cannot be completely removed by the perfusion of buffers in the fixation process. The leftover colouring limits transparency of the organs. Amino-alcohols used in the CUBIC method can overcome this problem because the chemical effectively elutes heme from haemoglobin and clears the colour of blood [29]. The CUBIC method can clear the whole body of adult mice with a simple protocol; soaking the whole body in clearing mixtures for several weeks. The cleared whole mice sample is sufficiently translucent for fluorescent light-sheet microscopy (see next section) with resolution to distinguish nuclei of individual cells.

Light-sheet Microscopy for Efficient Image Acquisition of Large Samples

In order to obtain 3D images of the cleared organs, optical sectioning with high-resolution on the *z*-axis is also required. Conventional confocal and two-photon microscopes can focus excitation light at a specific voxel within the 3D sample. To reconstruct the whole 3D image, the voxel is scanned along every *x*-, *y*- and *z*-axis, limiting the throughput of image acquisition. By contrast, light-sheet microscopy generates a thin laser plane to excite an optical section within a specimen [30]. The image is then captured by a wide-field camera placed at the orthogonal direction of illumination. This optical setting makes it possible to acquire a whole image section in a single acquisition. Scanning is required only in the *z*-axis direction. This optical configuration, however, requires the specimen to be highly translucent, otherwise the sheet laser cannot illuminate the center of optical section and the fluorescence from the excited surface cannot reach the detector. Highly cleared organs are one of the best applications for this state-of-the-art microscopy; and several clearing methods outlined above can be used with light-sheet microscopy. Figures 2 and 3 are examples of whole-brain and whole-mice imaging obtained by the combination of clearing treatment (CUBIC) and light-sheet microscopy. The brain image has enough resolution to distinguish axon projections and the heart image reveals the detailed mesh structure of blood vessels.

Image Processing and its Computational Analysis

Comparison of different samples is an issue for data processing, especially when the images are complex three-dimensional ones. For this purpose, brain imaging using magnetic resonance

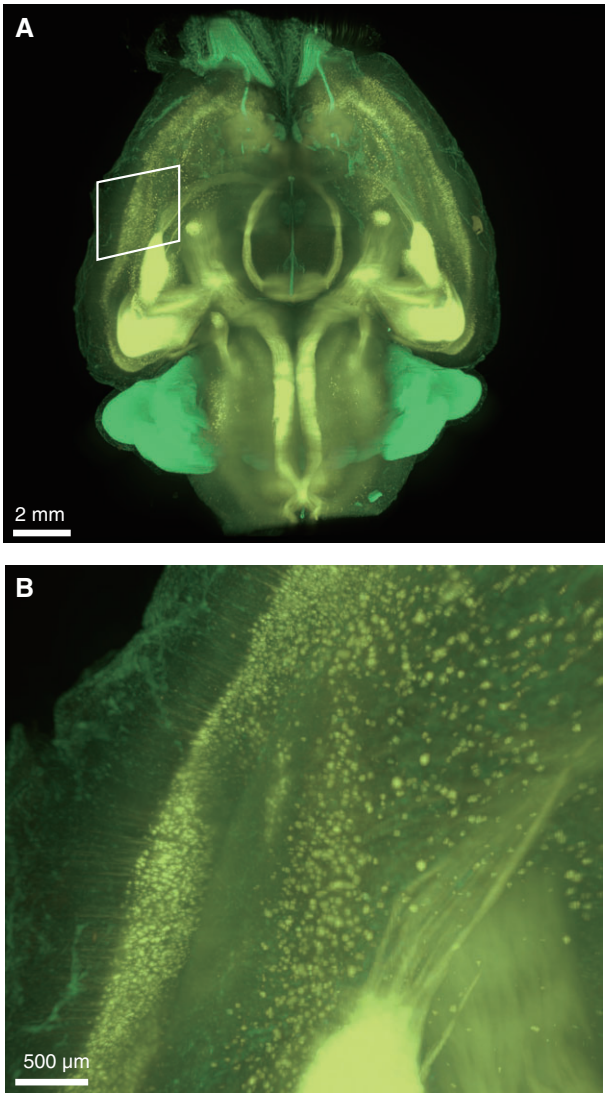

Figure 2. Whole-brain imaging with the CUBIC method. (A) 3D-reconstituted whole-brain image of an adult mouse acquired with a light-sheet fluorescent microscope. The mouse expressed YFP (shown in yellow) under a Thy1 promoter and was stained with propidium iodide (PI) (shown in green). (B) Magnified view of the white-box region in A. See reference [23] for the detailed process.

imaging (MRI) or magnetoencephalography (MEG) use the standard brain atlas. By fitting obtained images to the standard brain, we can compare and annotate each image based on the same format. A similar approach was used in the CUBIC protocol [23]. Each brain image is transformed to fit the shared standard brain by a method based on affine transformation. This fitting works well in the case of the brain, an organ with relatively simple structure, but it will be highly difficult to fit the other organs, especially whole body images because the position of the arms and legs are variable for individual specimens and digestive organs (e.g. a stomach or intestines) change shape upon food intake. An algorithm to standardize such complex 3D images will be required for future image processing.

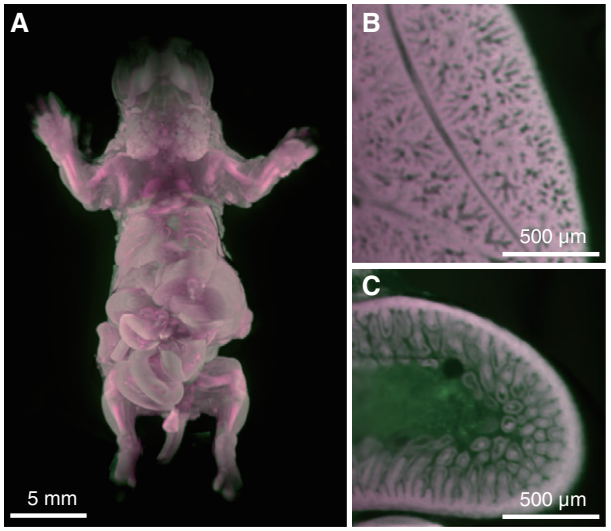

Figure 3. Whole-body imaging with the CUBIC method. (A) 3D-reconstituted whole-body image of P1 mouse acquired with a light-sheet fluorescent microscope. The mouse expressed EGFP (shown in green) under a CAG promoter and was stained with propidium iodide (PI) (shown in purple). (B and C) Coronal plane images of the mouse lung (B) and intestine (C). See reference [29] for the detailed process.

Whole Brain Imaging of Neuronal Network and its Activity

Tracing Neuronal Connections

In the following section, we will discuss the potential application of whole-brain and whole-organism imaging to circadian biology. A typical application of whole-brain imaging may be to illustrate the network architecture of neuronal connections between SCN and other brain areas. Cleared brains with water-soluble chemicals keep the detailed structure of axon projections and synapses, and are compatible with genetically coded fluorescent reporters. Thus, it is possible to track neuronal connections. For example, tracing of axon projections from the SCN will be possible by inducing fluorescent proteins under the control of SCN-specific promoters (see below) or by introducing viral neuronal tracers to identify functional synaptic connections.

Although, it is well-known that the SCN regulates various physiological activities, which are also regulated by different areas in the brain, the molecular and cellular mechanisms of the co-ordination among the SCN and other brain regions is not completely understood. A pioneering experiment of SCN transplantation demonstrates indirect (not through synaptic connection) regulation of behavioural rhythmicity by the SCN; transplanted SCN encapsulated in semi-permeable material sets the behavioural rhythm in host hamsters [31]. It is also known that SCN neurons directly project to several other regions, primarily the hypothalamus [32]. Retrograde tracing revealed direct or multi-synaptic projections from several brain areas to the SCN. Through this network, the SCN delivers circadian signals and SCN afferent pathways may contribute to reset the circadian clock in the SCN. Because the current afferent/efferent projection models of the SCN

neuronal network are largely based on the ensemble of many studies with different species and techniques, and there are several inconsistent observations between studies, it should be worthwhile to carry out a systematic investigation of projection patterns to and from the SCN on a whole-brain scale.

Tracing the Circadian-phase Variation in the Brain

The direct/indirect signals from SCN will also be important to reset and synchronize the peripheral clocks in non-SCN neurons. Direct projection may be important for regulating specific brain areas, while indirect spreading of neurotransmitters will affect a broad area next to the SCN. Heterogeneous distribution of circadian phase is also true in the brain. Interestingly, the phase of circadian clock may vary depending on the brain area [33], and food restriction may selectively affect the clock phase of the cerebral cortex and hippocampus [34]. The role of clocks in the brain outside the SCN may be more actively important for the development of neuronal network, not just for keeping the robust circadian rhythms. A recent study revealed that selective deletion of *Clock* or *Bmal1* in the parvalbumin-expressing inhibitory neuron, which is not involved in the SCN, delays the critical period of visual cortex plasticity upon monocular deprivation [35].

A detailed illustration of circadian phase in a cleared brain would be done by using fluorescent-protein-based circadian reporters. Although phase determination by real-time monitoring of circadian reporters (e.g. Per2-luciferase monitoring) is impossible for a fixed brain, relative values of two or three circadian-expressing reporters at a few time points can determine the phase of oscillation [36–38]. With such a strategy, the heterogeneous phase distribution of circadian phase in the brain (or ultimately in the entire body) may be quantified.

In order to trace circadian synchronization originating from the SCN, it is important to perturb the circadian phase specifically at the SCN, and trace how the altered phase at the SCN affects the phase of other brain regions. The phase adjustment of the SCN may be accelerated by knockdown of the arginine vasopressin (AVP) receptor or elevated by vasoactive intestinal peptide (VIP) action [39,40]. These conditions disrupt the neuronal coupling within the SCN and make the circadian phase more flexible to the environmental light phase (i.e. reducing the 'jet lag' effect). In these cases, non-SCN brain regions will experience unusually drastic phase shifts. Do other brain regions faithfully trace the altered time? Alternatively, possible circadian coupling between brain regions may resist drastic phase shifts. Neuromedin S promoter is another promising tool to observe the response of other brain regions to the selective inhibition of circadian output from the SCN. Recently, Lee et al. succeeded in disrupting circadian rhythmicity by inducing abnormal expression of clock genes at the subpopulation of SCN neurons by using promoter of neuromedin S [41], a neuropeptide that selectively expresses in the SCN [42]. Those studies have mainly focused on the circadian time-keeping mechanism within the SCN, but with whole-brain imaging, the observation can be extended to the response to and coupling of circadian signals within the whole brain, and ultimately within the whole body.

Illustration of the Neuronal Activity in the Whole Brain

The firing activity of a neuron is the physical nature of information processing in the brain. Current brain-clearing protocols require fixation of samples, which make it impossible to obtain the millisecond dynamics of electrophysiological neuronal actions. Fortunately, by using makers for the expression of immediate-early genes (IEGs), such as *c-fos* and *Arc*/*Arg3.1* the imaging of a fixed whole brain can be an excellent method to investigate the integrated change in neuronal activity over a minutes-to-hours scale [43]. This time scale also matches with the time resolution necessary for tracking circadian dynamics. Genetically encoded fluorescent protein under the control of the promoter of IEGs has been a reliable tool to monitor neuronal activity, and such tools work well with the water-soluble brain clearing methods. Thus whole-brain imaging of IEG activity provides complementary information that one may not expect from *in vivo* electrophysiological recording or real-time imaging of voltage/ion sensitive reporters, which are generally limited to a local area of the brain.

Tracking Sleep–Wake Activity Throughout the Brain

Sleep–wake cycle is one of the most significant phenomena regulated by the circadian system. Nevertheless, the brain center responsible for controlling sleep is still under discussion. Sleep–wake cycle is believed to contain homeostatic regulation, namely sleep homeostasis independent of the circadian regulation. With this homeostasis, an animal's sleep-time to wake-time ratio is kept constant so that an animal that could not sleep enough would take extended sleep time in the next sleep phase (recovery sleep). Given this hypothesis, brain regions activated under the condition of sleep loss would be regions responsible for sleep homeostasis, and thereby regulate the normal sleep-to-wake activity ratio. The ventrolateral preoptic nucleus (VLPO) is one of the best understood brain regions matching these criteria. Recent findings suggest differences between natural sleep cycles and sleepiness elicited by the forced loss of sleep time. Inhibition of gliotransmission by the induction of a dominant-negative form of SNARE protein (dnSNARE) in glia cells caused a defect in sleep homeostasis. Mice expressing dnSNARE had normal sleep time/quality even after significant loss of sleep [44]. The mice were also insensitive to the induction of increased sleep pressure by lipopolysaccharide (LPS) injection [45]. Strikingly, the mice exhibit almost a normal time-course of sleep–wake cycle in the absence of sleep deprivation or LPS injection. These results suggest decoupling between the mechanisms responsible to generate a normal sleep–wake pattern and those responsible for homeostatic recovery sleep. A study focusing on the effect of dexmedetomidine, an $\alpha 2$ adrenergic agonist clinically used for sedatives, may be consistent with this decoupling [46]. Dexmedetomidine induced elevated activity of VLPO and several other regions judged by *c-fos* promoter activation. A similar activation pattern was obtained by forced sleep deprivation, but surprisingly, natural sleep did not induce the activation of VLPO and the other regions, at least not to the same extent of dexmedetomidine application or sleep deprivation. Of course, the uncoupling may be due to a quantitative difference: natural sleep may only

require weak gliotransmission or activation of VLPO. Still it will be important to re-evaluate the role of VLPO and other brain regions on the normal sleep–wake cycle. A whole-brain activity atlas during the sleep–wake cycle will be a good reference for that evaluation. For example, when we carried out time-course *c-fos* imaging of the whole brain, there are several brain regions that show elevated activity at specific times of the day. Although more detailed analysis is required to investigate neuronal control of sleep, imaging allows us to quantitatively compare the cumulative activity of neurons across different brain areas. We also would like to point out the potential importance for having the brain atlas of sleep state in the view of pharmacological treatment of mental disorders. Because sleep disorders are associated with many mental disorders and *vice versa*, medicines targeting the central nerve system often affect sleep–wake dynamics. Thus, a detailed sleep–wake brain atlas will contribute to understanding potential effects/side-effects of medicines by comparing activity maps between brains under different medicines.

Tracking Non-circadian Autonomous Oscillators

Time-course comparison of brain activity also has the potential to identify brain centers for non-circadian oscillators. A methamphetamine-induced oscillator (MPO) was proposed based on the observation that chronic intake of methamphetamine alters the period of behavioural rhythms [47]. The elicited rhythmicity is SCN independent – methamphetamine induces behavioural oscillations even in circadian-deficient animals [48]. Similarly, the restricted-feeding induced oscillator (FEO) was also shown to operate independently from the SCN [49,50]. If there is a brain center responsible for these non-circadian oscillators, the center should show cyclic activity changes with a period matched to the non-circadian oscillation.

Uncover the Role of Peripheral Clocks in the Whole Body

Heterogeneous Phase Distribution in Body Clocks

As discussed in the previous sections, whole-body imaging with single-cell resolution would allow us to illustrate the heterogeneous distribution of circadian phases. Synchrony of the circadian clock in the entire body is often schematically described, but the genuine distribution of circadian phase is unclear. The phase distribution and its transition upon various environmental signals will elucidate how the signal from SCN propagates to peripheral clocks and *vice versa*.

> “Whole-organ imaging will be an ideal technology to investigate the heterogeneous nature of cell assembly, such as circadian-phase variance of peripheral clocks and their synchronization process.”

Circadian phase and period of peripheral clocks are determined by the combination of cell-intrinsic peripheral clocks and synchronization cues from the SCN. Pando et al. succeeded in showing this relationship by an elegant clock-defective mouse embryonic fibroblast (MEF) transplantation experiment [51]. When *Per1*−/− MEF with a short circadian period is implanted into wild-type mice, the period of mutant MEF is returns to a wild-type period. This result indicates the SCN's dominance over peripheral clocks. At the same time, the peripheral clock is not just a slave to the central clock – the intrinsic clock mechanism plays a significant role. The synchronization signal from the SCN is not sufficient to induce *de novo* circadian oscillation in non-oscillatory $Clock^{c/c}$ mutant MEFs. The role of peripheral clocks were also demonstrated by brain-specific rescue of clock function that can rescue the behavioural rhythmicity but cannot rescue organ-specific gene expression [52].

Potential Application to Chronopharmacology and Chronotherapeutics

Given that peripheral circadian clocks are driven by the combination of an intrinsic molecular oscillator and environmental synchronization signals, what if a cell migrates to different organs with different environmental conditions? Because these mobile cells are a minority in the destination organ, a conventional bioluminescent assay that monitors average circadian activity among cell populations is not suitable to analyze the rhythms in these minority populations.

Whole-organ imaging is particularly important for focusing on smaller numbers of certain cells – one may easily imagine its potential application to cancer biology, where a small number of transformed cells are critical for tumour invasion. Numerous experiments have shown that circadian timing is a critical factor for the effectiveness of anti-cancer drugs [53]. Most anti-cancer drugs (e.g. chemicals that induce DNA damage or inhibit cell division) target transformed cells using the fast-replicating feature of the cell cycle in transformed cells. Because the circadian clock regulates cell cycle progression and the efficacy of DNA repair mechanisms, the clock affects the tolerance of transformed cells to anti-cancer drugs. The circadian clock also largely affects the pharmacokinetics of drugs, as clock timing affects the activity of metabolic pathways to uptake, deliver and metabolize drugs. Therefore, a precise chronotherapeutic model requires understanding of the circadian phase in the whole body at single-cell resolution.

The circadian clock also controls immune responses, where the migration of cells is essential to defend the organism from the threat of infection. It is well known that the level of hormones, cytokines and the number of circulating immune cells shows global circadian variation in the body [54]. Notably, several studies have shown that the peripheral circadian clock in local cells is also critical for gating the induction of inflammatory responses. Keller et al. showed that circadian clock acting in macrophage fulfils the circadian cytokine response [55], and Gibbs et al. showed that the intrinsic clock in epithelial bronchiolar cells is responsible for rhythmic secretion of chemokine, thereby controlling the circadian anti-inflammatory response in the lung [56]. Given the emerging role of circadian clocks in immune responses, the interplay between clocks in migrating cells and clocks in local tissue

cells will be of great interest. Such circadian interplay might be involved in local, tissue-specific crosstalk among the local environment and tissue-resident macrophage and memory T cells [57,58]. Whole-organ imaging has the potential to survey these heterogeneous minor populations.

Conflict of interest

The authors declare that there is no conflict of interest regarding this manuscript.

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