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REVIEW ARTICLE

Environmental Control of Biological Rhythms: Effects on Development, Fertility and Metabolism

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Internal temporal organisation properly synchronised to the environment is crucial for health maintenance. This organisation is provided at the cellular level by the molecular clock, a macromolecular transcription-based oscillator formed by the clock and the clock-controlled genes that is present in both central and peripheral tissues. In mammals, melanopsin in light-sensitive retinal ganglion cells plays a considerable role in the synchronisation of the circadian timing system to the daily light/dark cycle. Melatonin, a hormone synthesised in the pineal gland exclusively at night and an output of the central clock, has a fundamental role in regulating/timing several physiological functions, including glucose homeostasis, insulin secretion and energy metabolism. As such, metabolism is severely impaired after a reduction in melatonin production. Furthermore, light pollution during the night and shift work schedules can abrogate melatonin synthesis and impair homeostasis. Chronodisruption during pregnancy has deleterious effects on the health of progeny, including metabolic, cardiovascular and cognitive dysfunction. Developmental programming by steroids or steroid-mimetic compounds also produces internal circadian disorganisation that may be a significant factor in the aetiology of fertility disorders such as polycystic ovary syndrome. Thus, both early and late in life, pernicious alterations of the endogenous temporal order by environmental factors can disrupt the homeostatic function of the circadian timing system, leading to pathophysiology and/or disease.

Key words: melatonin, circadian, reproduction, clock gene, mouse, rat

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Time as a variable is frequently neglected when we consider the interaction between the organism and the environment. However, life on Earth means that we are under the geophysical cycles that impose approximately 12 h of light and approximately 12 h of dark, the oldest and major environmental time-cue on Earth. Organisms would have faced natural selection in intervals of opportunity and adversity that recur with precise and predictable frequencies, generating an innate temporal programme responsible for circadian oscillations. These are responsible for orchestrating physiology and behaviour and allow organisms to anticipate daily environmental changes (1). The presence of a circadian component in this temporal organisation is also implied in the way that abnormal entraining cycles often impair homeostasis. This can be observed from the simplest and most familiar stress imposed by rapid travel across time zones or, more dramatically, from the high frequency of cardiovascular disease, metabolic syndrome and insomnia observed in workers subjected to night (shift) work schedules (2–4). We begin our discourse with a discussion of the molecular basis for circadian oscillations and how robustly conserved temporal organisation is among organisms. This will lay the framework for a more comprehensive discussion of how chronodisruption impacts the aetiology of disease in our 24/7 society.

Molecular basis of endogenous biological clocks

Temporal organisation is codified at the genetic level because single gene mutations can lead to changes in circadian rhythm of activity

or even complete arrhythmia (5). This was first observed in *Drosophila melanogaster* with mutation of one gene located in chromosome X (6); and, later, in mammals such as the tau-mutant hamsters (7). Now, we have expanded our view of how evolution has worked to regulate temporal organisation. A molecular machinery is present in organisms as diverse as cyanobacteria (*Synecchus*), fungi (*Neurospora*), insects (*Drosophila*), fish (8) and mammals (9,10). This consists of a set of very-well conserved macromolecules, known as clock proteins, which function as transcription factors in a positive and a negative auto-regulatory loop that generates circadian rhythms of clock-controlled gene expression. In mammals, the proteins CLOCK and BMAL1 (brain and muscle Arnt-like protein 1) form heterodimers, and enhance the expression of *period [Per]* and *cryptochrome [Cry] genes* (11). PER and CRY dimerise and are phosphorylated by casein kinase (12). PER-CRY dimers are then transported to the cell nucleus where they inhibit their own transcription (13,14). In parallel, the expression of the transcription regulators REV-ERBα and RORa is driven by BMAL1:CLOCK and suppressed by PER and CRY proteins (15). When REV-ERBα is absent, *Bmal1* gene (and probably *Clock*) is released from its inhibition, starting a new circadian cycle (16). REVERBα and RORa are not core components of the transcriptional oscillator but act as a 'stabilising loop' to facilitate robustness within the core clock machinery.

Entrainment of the circadian timing system

Among the environmental clues, light/dark cycles appear to be one of the most important daily synchronisers or 'Zeitgebers' (as used by Aschoff) of endogenous circadian oscillators (17). When one oscillator is coupled to and driven by another, it assumes its period; this coupling guarantees changes of the circadian period of an organism to precisely 24 h and establishes a functional phase-relationship between them (1). In insects, mollusks, reptiles, birds and mammals, there is a clear association between endogenous oscillators and photoreceptors, stressing the predominance of cycles of light in the entrainment of circadian rhythms (18). This relationship may indicate a co-evolution of these systems (perception of light and endogenous circadian clock) to the same selective pressure. This is the basis for the evolution of the 'escape from light' hypothesis: high temperatures found in the light phase of the day would have been hazardous for the stability of enzymes and physiological processes, as much as ultraviolet radiation would have been for DNA structure (19). This idea the of co-evolution of these systems has been confirmed by the recent study of Shen *et al.* (20), who demonstrated that rhodopsin, a photopigment found in visual photoreceptors, is required for temperature discrimination in *D. melanogaster*. Furthermore, the photopigment melanopsin, expressed in intrinsic light sensitive cells of the mammalian retina, also appears to participate in this response. Expression of melanopsin in rhodopsin-null mutants effectively rescues temperature discrimination (20).

By contrast to basal vertebrates, which possess extra-ocular photoreceptors (pineal complex and deep brain photoreceptors) (21), mammals rely only on their eyes for light perception (22). Surprisingly, classical photoreceptors for image forming visual function (i.e. the rods and cones) are not essential for light-entrainment (23). A subset of retinal ganglion cells, referred to as intrinsically-photosensitive retinal ganglion cells (ipRGC), are capable of capturing light radiation (24) and conveying this information to the suprachiasmatic nucleus (SCN) (25). IpRGCs express the photopigment melanopsin (24), which was first cloned in *Xenopus laevis* melanophores (26) and is now known to be present in the retina of all vertebrates, including humans (27). The melanopsin positive ipRGCs are fundamental for light entrainment of the circadian system (25,28). Visual photoreceptors, although not essential, have a permissive effect on this process (29).

Peripheral clocks

In the classic view of biological clock functioning, a light signal detected either by retinal photoreceptors or by extra-retinal photoreceptors is transmitted to a central oscillator (the SCN in mammals) and, subsequently, temporal information is disseminated to the remainder of the organism by the output of the central oscillator (30). However, Plautz *et al.* (31) elegantly demonstrated that *Per1* is rhythmically expressed in the legs, wings and antennas from a transgenic fly expressing the enzyme luciferase under the control of *Per1* gene promoter. This oscillatory profile is synchronised by light/dark cycles, suggesting that these tissues are able to independently detect light and translate this signal to the molecular clock. These results point to the existence of multiple oscillators, an idea suggested long ago by Aschoff from his study in humans, in which sleep–wake cycles can be dissociated from body temperature oscillations (32). In agreement with this decentralised view of the biological clock, the core molecular clock machinery is expressed in almost all cells and tissues (33,34). Robust cell-autonomous rhythms of clock gene and clock-controlled gene expression have been detected in isolated cell cultures from both mammals (35,36) and non-mammalian vertebrates (37–40). Together, these data strengthen the notion that, in addition to a central clock, there are multiple circadian oscillators throughout the mammalian body collectively referred to as peripheral clocks.

The identification of the central clock in mammals was based in large part on SCN transplant experiments. Transplants of this region reinstated rhythms in SCN lesioned animals (41,42) and *Clock or Cry1-2 double mutant*-mice (43,44). Furthermore, SCN lesions abolish rhythmic *Per2* gene expression in rat peripheral tissues (45). These results suggest that peripheral clocks are dependent or 'slave' to the SCN for the appearance of circadian oscillations (46). This notion was supported by the fact that the SCN generates its own rhythm and isolated SCN explants show sustained rhythms for more than 30 cycles, whereas peripheral clocks were attenuated after a few days in culture (33). This assertion was subsequently rejected based on the independence of the oscillator in many peripheral tissues (34,36,47,48). Furthermore, disruption of clock gene function in the liver abolishes the circadian regulation of clock-controlled genes (49,50), reinforcing the idea that peripheral tissues possess their own rhythm-generating machinery. It was thus determined that cultured organs from SCN lesioned mice retain their rhythmic expression of clock genes when isolated, although

with different phases from the intact animal, indicating the loss of synchronisation (34). It is now known that the rhythm attenuation of cultured cells (33,51) is a function of phase coherence among individual isolated cells. That is, when clock gene rhythms are analysed in populations of uncoupled cells, although *clock gene* oscillations persist in each cell, the rhythm appears damped because each cell oscillates in its own phase. This was demonstrated in elegant studies by Welsh *et al.* (36) in rat fibroblasts and by Carr and Whitmore (52) in a zebrafish embryonic cell line. These data indicate that circadian organisation begins at the cellular level and may be functionally dependent on cell-to-cell communication within a network of oscillators in each tissue.

The organisation of circadian timing, from interlocked loops of gene expression to coordinated timing of cell and tissue physiology, represents a sophisticated and precise system of temporal order. Understanding the basic mechanism and organisation of the circadian timing system is critical to advancing exploration of its complex role in metabolism, endocrine and neuroendocrine physiology. Disturbances to this program can often result in pathophysiology and disease.

Environment, biological rhythms, melatonin and energy metabolism

The circadian temporal structure of mammalian organism divides the 24-h day into one period of activity and another of rest, defining the daily rest/activity cycle. All physiological functions are accordingly distributed to be the effective support for the proper articulation of all the behavioural expressions typical of the rest/activity cycle. As an intrinsic part of this circadian temporal structure, energy metabolism is temporally distributed to maintain energy balance and to synchronise metabolism with the rest/activity cycle. In this way, the acquisition of energy through feeding is restricted to the activity phase, whereas fasting, or burning of the stored energy, is associated with the rest phase of the daily cycle. An exception to this distribution of the physiological functions synchronised to the rest/activity cycle is the synthesis and secretion of pineal melatonin. Pineal melatonin production is always allocated to the dark or night portion of the day, no matter what the behavioural distribution of the activity/rest cycle (i.e. in either diurnal or nocturnal species).

Melatonin is considered to be a potent chronobiotic because it is the interface between the light/dark environmental cycle and the organism so that its plasma profile is able to indicate whether it is light or day outside, by its absence or negligible blood concentration, or whether it is night, by its presence or high blood concentration (53). In addition, the neuroendocrine system that regulates the synthesis of pineal melatonin is such that the nocturnal plasma melatonin secretory episode reflects exactly the duration of the dark phase of the day or, in other words, the duration of the photoperiod or the season of the year (53). The conspicuousness of melatonin production and its strict association with the dark phase of the light/dark daily cycle is such that melatonin is an important element in the setting of the circadian temporal order. Thus, it is no surprise that melatonin is able to regulate most of the physiological systems in the mammalian organism such as the sleep/wake cycle, reproduction, the cardiovascular system and blood pressure, including energy metabolism and energy balance (54).

Melatonin regulates energy intake [feeding (55–57)] so that pinealectomised or aged animals (that have no or sharply reduced melatonin production, respectively) display increased food intake that is restored to normal when the animals are treated with melatonin on a daily basis [56,57]. In addition, melatonin promotes the maintenance of brown adipose tissue and the browning of white adipose tissue and also activates the thermogenic function in these tissues promoting energy expenditure (58). Thus, melatonin is able to regulate energy balance and body weight such that an absence or reduction in melatonin production induces obesity, whereas melatonin treatment is able to reduce body weight (56,57).

One of the main roles for melatonin in regulating energy metabolism is its synergism with insulin signalling (54). Melatonin, acting through its membrane receptors, is able to phosphorylate the insulin receptor and potentiate the insulin signalling cascade (59). In addition, melatonin promotes the synthesis of glucose transporter GLUT4 in muscle and adipose tissues (60,61). It should be stressed that the daily distribution of energy metabolism is dependent on the rhythmic production of melatonin because melatonin deficiency causes increased hepatic gluconeogenesis and hepatic insulin resistance during the active phase of the rest/activity cycle (62). This is largely detrimental because both metabolic processes should peak during the fasting period and not during the time of feeding. The absence or reduction of melatonin production leads to a metabolic scenario that involves insulin resistance, glucose intolerance, dyslipidaemia and obesity (60). This metabolic dysfunction is completely restored if the animal is treated with melatonin (61). As a corollary, metabolic diseases such as diabetes (either type 1 or type 2 diabetes) lead to a reduction in melatonin production aggravating the metabolic picture (Amaral *et al.*, submitted).

It is worth noting that light during the night and the nocturnal use of electronic devices with LED screens emitting light in the blue–green wavelength is one of the most powerful blockers of melatonin synthesis through the activation of the melanopsinergic retinal system projecting to the hypothalamus (28). The use of such LED screen technology, so predominant in our contemporary society, may reduce melatonin production and, as a consequence, contribute to the appearance of insulin resistance, glucose intolerance and obesity.

Impact of gestational chronodisruption on foetal and postnatal physiology

Epidemiological and experimental evidence suggests that there are deleterious effects for human health with respect to working under 'light-at-night' conditions. Given that, in our 24/7 society, shift work affects approximately 20–25% of the workforce, even a modest detrimental impact upon health may have important public health implications (63). Data from the International Labor Organization indicate that 160 million workers are becoming ill because of workplace hazards; with approximately 36 million of them being subjected to chronodisruption. The term chronodisruption was

coined by Reiter and colleagues in 2003, to define 'a relevant disturbance of the circadian organization of physiology, endocrinology, metabolism and behavior' (64); which is characterised by plasma melatonin suppression under 'lights on' conditions (65). In 2007, the International Agency for Research on Cancer reclassified working at night from a possible to a probable human carcinogen, with the proof of principle being an increased prevalence of breast cancer in nurses and flight attendants (66). Recently, a link between shift work and type 2 diabetes has been inferred from statistical analyses of a large nurses cohort; whereas the poor diet frequently observed in shift workers was suggested as one weight gain factor predisposing to diabetes (63,67). Further epidemiological evidence together with studies of clock gene mutants also indicates the adverse effects of shift work on adult physiology: an increased risk of chronic diseases such as cardiovascular, metabolic and gastrointestinal disorders, some types of cancer and mental disorders including depression (68–70).

Regarding the effects of chronodisruption on pregnancy outcome, an increased risk of miscarriage, preterm delivery and low birth weight have been consistently reported in female shift workers (71–73); it is notable that both factors are strong predictors of chronic disease later in life (74). Against this background, a number of studies have established a relationship between antenatal deleterious environments (e.g. foetal undernutrition and/or hypoxia) and the onset of adult diseases including hypertension, coronary heart disease, stroke, as well as metabolic and neurological disorders (74).

It is conceivable that, in the physiological context of pregnancy, both the maternal and developing foetal circadian system may become targets of chronodisruption. Indeed, several organs appear to operate as peripheral oscillators in foetuses from different species, despite their SCN not yet being a functional master clock (75). Indeed, we have recently identified day/night changes in clock gene expression in the foetal rat heart, hippocampus, pineal and adrenal already entrained to the light/dark (LD) cycle at 18 days of gestation (75). The possibility of foetal rhythms being entrained by transplacental and/or biophysical cues is in line with our recent study in primates on the effects of constant light (LL) exposure during late gestation on rhythms of body temperature in newborns (76). This study showed that gestational chronodisruption affected newborn body temperature rhythms. This rhythm was entrained in control LD newborns, whereas LL newborns showed a random distribution of temperature acrophase over 24 h (i.e. a free-running circadian period). In mice, the perinatal photoperiod was shown to have a long-lasting effect on the molecular clockwork in the SCN and on neurobehavioural disorders (77). In rats, Varcoe and colleagues exposed dams to chronic phase shifts (CPS) in their photoperiod every 3–4 days throughout gestation and the first week after birth. The offspring of CPS rats displayed poor glucose tolerance and increased insulin secretion in response to an i.p. glucose tolerance test (78). Interestingly, this was also associated with increased anxiety in the CPS rat, supporting an influence of circadian disruption on deep brain structures involved in depression, including the hippocampus (78). Further experiments in murine models include a recent report using mice subjected to repeated shifting of the LD cycle, which showed both increased non-productive mating and decreased term pregnancies (79).

We recently characterised the foetal rat adrenal gland as a strong peripheral clock entrained by maternal melatonin (80). In further experiments, exposure of pregnant rats to LL (along the second half of gestation) induced intrauterine growth retardation, as well as changes in the relative expression of clockwork and steroidogenic genes and a reduced content of corticosterone (which in turn did not have a circadian rhythm) in the foetal adrenal, with no change in maternal plasma corticosterone (81). On the other hand, in the LD foetal rat hippocampus, we found oscillatory expression of different clock and clock-controlled genes, which was suppressed under LL conditions. Strikingly, we also found a significant spatial memory deficit in adult offspring that had been gestated under LL conditions (82). Of note, all these changes were reversed when the LL mother received melatonin during the subjective night, supporting the idea that the maternal plasma melatonin rhythm may drive the foetal circadian system (81,82).

We compared the LL and LD foetal heart using a microarray-based functional genomics approach (83). Differential expression was found for 383 transcripts in the LL foetal heart; with 42 displaying a $\geq$ 1.5-fold change in expression. Deregulation of several markers of cardiovascular disease may contribute to the observed alteration of diverse gene networks in the LL foetal heart, including local steroidogenesis, vascular calcification, cardiac hypertrophy, stenosis and necrosis/cell death. Indeed, gestational chronodisruption markedly inhibited the transcription not only of enzymes involved in cardiac steroid hormone synthesis (*Hsd3b1*, *Cyp21a1*, *Star, Cyp11a1* and *Cyp11b1*), but also of vascular calcification-related genes (*Gas6* and *Mgp*). Transcription of several additional markers of cardiovascular disease was either up-regulated (*Mdk*, *Cited1* and *Slc2a1*) or down-regulated (*Adra1a* and *Lrrc10*) in the foetal heart by gestational chronodisruption (83). Integrated analysis of significant pathway enrichment for pathological processes in the LL foetal heart is shown in Fig. 1. In addition, several gene ontology categories related to DNA integrity were over-represented (such as DNA metabolism, function and maintenance), including a 2.1-fold increase of *Hmga1* mRNA, which encodes for an abundant architectural transcription factor. At the epigenetic level, microRNA analysis revealed up-regulation of miRNAs 218-1 and 501 and concurrent down-regulation of their validated target genes (83). Among them, the nuclear factor, interleukin 3 regulated (*Nfil3*) gene appears to be under the negative control of both miRNA 218-1 and miRNA 501. *Nfil3* is a transcription factor that binds to the promoter region of interleukin-3. Regarding the remaining transcripts potentially targeted for degradation by increased miRNA 218-1 under gestational chronodisruption, Sarcalumenin (*Srl*) and Xin repeat-containing protein 2 (*Xirp2* or *Xinβ*) have been reported as being relevant for cardiac development and function (83).

The alteration in the transcription level of several disease markers in the foetal heart subjected to chronodisruption prompted us to examine any long-term effect on cardiomyocyte or whole heart morphology. In 90-day-old rats that had been gestated under constant light, we observed an altered left ventricle phenotype, as defined by an increased cardiomyocyte nuclear and cell diameter, as well as increased wall thickness, accompanied by a reduced cavity area (83). Next, we investigated whether the early onset of altered

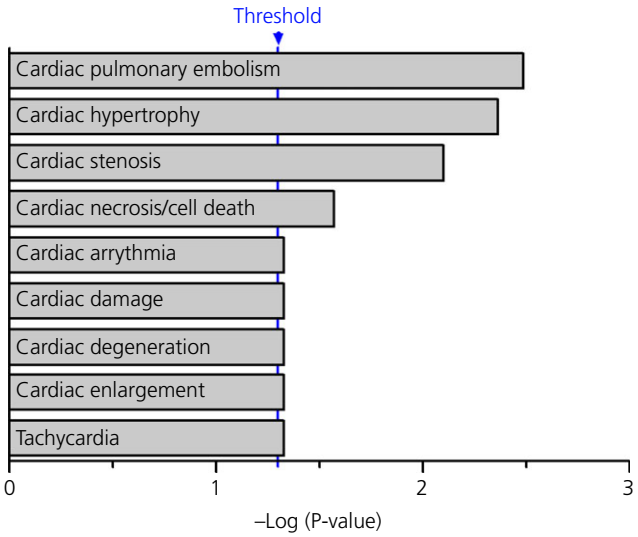

Fig. 1. Impact of gestational chronodisruption on foetal cardiac genomics. Identification of over-represented pathways by functional genomics analysis of foetal rat heart gestated under constant light (LL) relative to control photoperiod (LD). RNA isolated from foetal heart at 18 days of gestation was subjected to microarray analysis (Affymetrix platform for 28 000 genes; Affymetrix, Santa Clara, CA, USA). Significant differential expression was found for 383 transcripts in LL relative to LD foetal heart (for details, see text). Integrated transcriptional changes were assessed using INGENUITY PATHWAY ANALYSIS (IPA) software (Qiagen, Valencia, CA, USA). IPA was set to pinpoint pathological processes predictably affected by the interaction of the whole set of differentially expressed genes in the foetal rat heart subjected to the LL condition. The threshold (blue) line indicates P = 0.05. A similar set of results was reported by Galdames *et al.* (83).

transcription of cardiovascular disease markers may persist into adulthood for 10 selected transcripts in the LL versus LD heart of foetal and adult offspring. A lasting and significant alteration was found only for *Kcnip2* mRNA, which was decreased at both developmental stages. KCNIP2 is a calcium-binding protein acting as a subunit of the voltage gated potassium Kv4 channel complex, which is crucial for the conduction system to regulate calcium-dependent A-type currents. This result is particularly relevant given that studies in humans and mice have linked mutation of the *Kcnip2* gene with arrhythmogenesis and heart failure. Moreover, there is solid evidence accounting for a significant decrease of *Kcnip2* gene expression in hypertrophy, particularly in the left ventricle wall (83).

In conclusion, recent findings in murine models of gestational chronodisruption indicate persistent metabolic, cognitive and cardiovascular alterations in the adult offspring. These results support epidemiological data obtained in humans, suggesting that circadian misalignment during gestation may have detrimental consequences for pregnancy outcome. Collectively, these data raise challenging questions about the consequences of shift work during pregnancy.

Chronodisruption and fertility: a role for the timing system in reproductive pathophysiology

In mammals, reproductive physiology and metabolism are both directly and indirectly regulated by the circadian timing system (2,3,84). Circadian disruption as a result of chronic sleep disturbances, shift-work or persistent transmeridian travel (jet-lag) can produce irregular menstrual cycles in women (85). Furthermore, genetic or environmental manipulations that alter or abolish clock function have significant negative impacts on both fertility and metabolic homeostasis (2,78,86,87). Central circadian pacemakers, including the SCN, play established and well-described roles in female reproductive physiology (88–90). Recently, cellular clocks outside the brain, including those in the pituitary gland, oviduct, uterine and ovarian cells, have been implicated in reproductive function (91). It has been suggested that the clock in the ovary may play a significant role in the timing of ovulation, steroid hormone synthesis and follicular maturation (92–95). Furthermore, clocks in the female reproductive tract have been implicated in the processes of embryo maturation, implantation and parturition (96–101). Together, these data suggest that circadian clock function at the cellular level appears to be critical for normal reproductive physiology.

Fertility disorders represent a considerable health issue worldwide, with fertility rates dropping by almost 45% in the USA alone in the past 40 years (102,103). Although some of this decline in fertility can be related to socioeconomic factors, the impacts of environmental contaminants and metabolic disease are considerable (102–106). Early developmental exposure to steroid-mimetic endocrine disrupting compounds (e.g. bisphenol A), maternal obesity and endocrinopathies such as polycystic ovary syndrome (PCOS) have been linked to infertility and adverse metabolic function (107–109). PCOS is particularly common and devastating, affecting approximately 10% of unselected women during their childbearing years (110). Despite the frequency of diagnosis and the intense focus of scientific research, the aetiology of the disease remains largely unknown (111). The primary symptoms of the disease include abnormal ovarian physiology, specifically anovulation and a polycystic ovarian morphology (112,113). The disease is often co-morbid with a metabolic syndrome characterised by hyperinsulinaemia, obesity, and an increased risk of type-2 diabetes and cardiovascular disease (112–114). Developmental programming as a result of excess androgen exposure *in utero* is considered to be a significant factor (114–116). In several animal models, prenatal androgenisation produces a PCOS phenotype in adult female offspring (106,117). Much of the reproductive dysfunction of PCOS, from altered gonadotrophin secretion to anovulation, has been directly or indirectly linked to excess foetal or pubertal androgen (118–120).

It is widely considered that synchronisation among central and peripheral oscillators (e.g. SCN, ovary, liver, etc.) is a fundamental property of physiological homeostasis (11). Thus, internal circadian organisation is broadly defined as the coordinated and synchronised timing of central and peripheral clocks, as well as the timing of each tissue clock relative to the environment (Fig. 2A, top). A decline in internal circadian organisation, coincident with, and potentially caused by chronodisruption (as seen during chronic jet-lag, rotating shift-work and non-24-h entrainment), can lead to health issues, including pre-diabetes, cancer and cardiovascular disease (121–124). Although chronodisruption is most commonly associated with nonstandard lighting conditions and altered sleep–wake

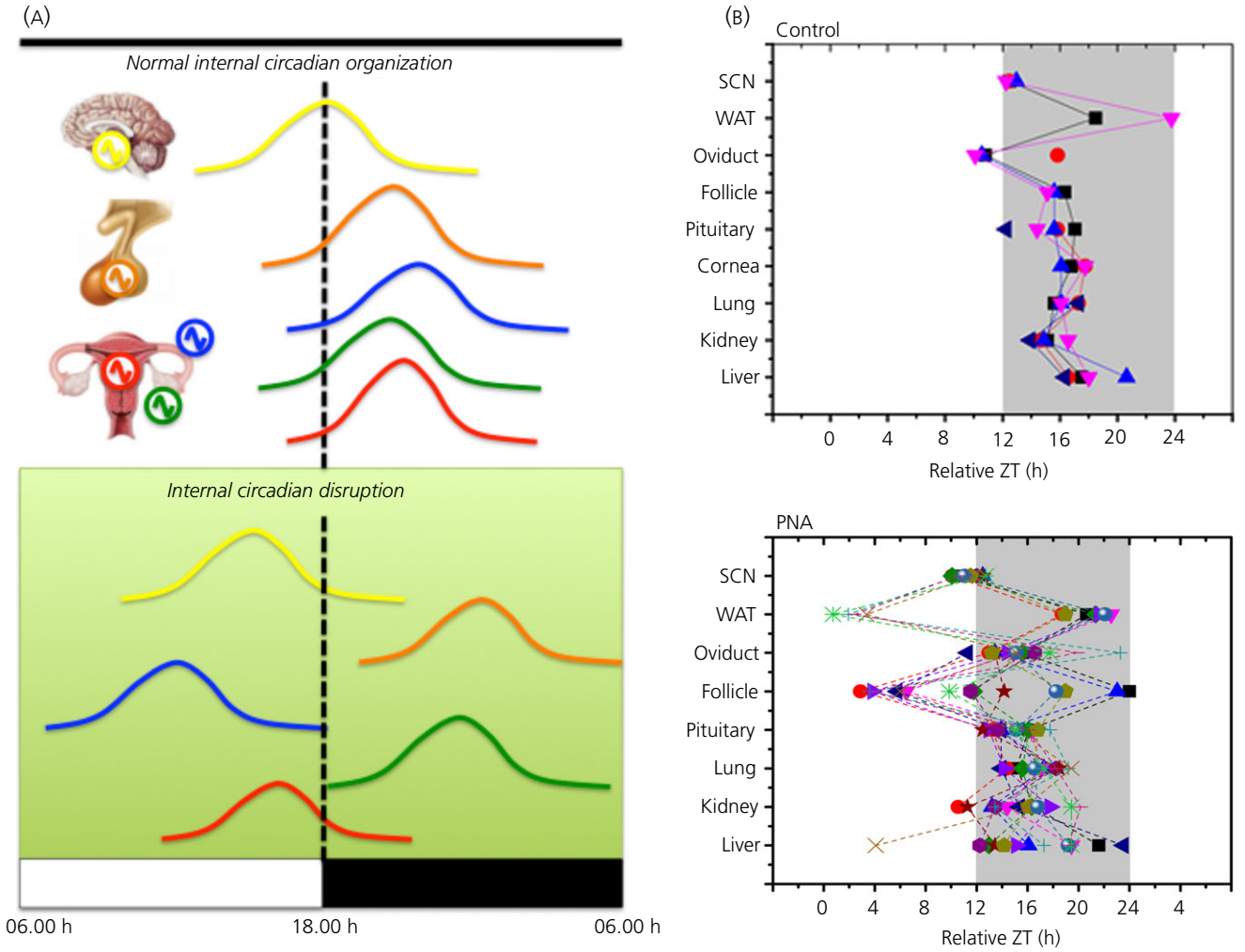

Fig. 2. Chronodisruption and fertility: impact of androgen-dependent developmental programming on the circadian timing system. (A) Synchronisation among central and peripheral oscillators [e.g. suprachiasmatic nucleus (SCN), ovary] is a fundamental property of physiological homeostasis. Coordination of gene expression and cellular physiology among oscillators and phase synchrony between oscillators and the environment (e.g. entrainment) are critical features of internal circadian organisation. (B) In control animals, phase synchrony among central and peripheral oscillators of both the hypothalamic-pituitary-ovarian and metabolic axes is robust and cohesive (n = 2–5 explants/tissue). After prenatal androgenisation, phase distribution within several tissues, including follicles and liver, increases dramatically, resulting in considerable internal circadian disruption (n = 8–12 explants/tissue). This decline in circadian organisation is coincident with abnormal reproductive cycles, a hallmark of polycystic ovary syndrome. (B) Each symbol represents a tissue from a different animal and tissues from the same animal are connected by a solid line. The peak phase of PER2::LUC expression is plotted as a function of 'relative Zeitgeber Time (ZT)' where ZT0 = the time of lights on for the animal before euthanasia. The 12-h dark phase is shaded in grey. PNA, prenatal androgenisation; WAT, white adipose tissue.

cycles (e.g. shift-work), other factors may have an equally dramatic impact upon internal circadian organisation (125). We have determined that exposure to excess androgen (hyperandrogenaemia) during foetal development [prenatal androgenisation (PNA)] disrupts reproductive function and causes a considerable decline in internal circadian organisation (Fig. 2B). Developmental programming by excess androgen alters reproductive function and metabolism in adult female offspring, producing a rodent model of the PCOS (117). Measurement of peak PERIOD2::LUCIFERASE expression in the SCN and peripheral tissues from PNA transgenic mice reveals a significantly reduced phase synchrony among peripheral oscillators, with the most pronounced effects apparent in tissues of the hypothalamic-pituitary-ovarian (HPO) axis and the liver. The reduction in phase-coherence among oscillators is striking given that these mice remained under a standard 12 : 12 h light/dark cycle and appear to have completely normal SCN phase (Fig. 2B). The observed decline in circadian organisation is quite similar to the established effect of SCN lesions on internal circadian organisation (126). This is notable because it was recently shown that lesions of the SCN produce insulin resistance and metabolic disease in mice, a metabolic phenotype similar to that of PCOS animals (127). Although the number of explants in the control group is smaller, relative to the PNA mice, we failed to detect a single tissue from control mice peaking more than 4–5 h away from the mean phase. This is in dramatic contrast to PNA mice, in which we see several tissues peaking during the relative day, in some cases 8–12 h out of phase

with other tissues from the same animal. This result is parallel to our finding in rats exposed to excess androgen during late adolescence and puberty (125). It is also worth noting that the impact of PNA on internal circadian organisation is not ubiquitous and may correlate with the degree of hyperandrogaemia, metabolic disease and/or reproductive dysfunction in each animal. It will be critical to establish the strength of correlation between the severity of disease progression and the degree of internal circadian disorganisation.

Nonetheless, these data suggest a direct influence of androgen on target oscillators in the HPO axis and/or disruption of the systemic cues needed to mediate adequate synchronisation among peripheral oscillators (30). How might androgens influence the timing of the circadian clock? There is considerable evidence linking androgen signalling to circadian clock function in mammals. Androgens affect circadian rhythms of locomotor activity (128–132) and can directly affect clock gene expression in prostate cancer cells (133). These data reveal that PER1 interacts with the androgen receptor (AR), PER1 expression is altered by androgen exposure and PER1 directly suppresses the transactivation of AR gene expression (133). Our preliminary data suggest that androgen may alter the period of the clock in target tissues, such as the ovarian granulosa cell, in a tissue-dependent manner. Although the site of the effects of androgen is unclear, the fact that androgens can alter the timing of the clock in multiple cell types and under various conditions suggests that developmental programming by androgen may mediate its affects in part by its impact upon the circadian timing system. A more comprehensive and focused examination of the interaction between androgen signalling and the clock, particularly as it relates to diseases of androgen excess, will provide additional insights and perhaps lead to new therapeutic targets for the treatment of common fertility disorders such as PCOS.

Summary and conclusions

The circadian timing system is a complex and integral regulator of physiological homeostasis, particularly in the endocrine and neuroendocrine systems. The timing system is evolutionarily conserved, with distinct mechanisms of parallel function across phylum. In mammals, the central pacemaker in the SCN receives direct retinal input and synchronises or entrains central and peripheral clocks to the external environment. The means of this synchronisation are multifaceted and integrative; incorporating both hormonal (e.g. glucocorticoids, melatonin) and neural cues (e.g. sympathetic nerves). Disruption of temporal order within the circadian system, or chronodisruption, as a result of abrupt and/or chronic changes in lighting condition (i.e. shiftwork, jet-lag) can have devastating impacts on physiology. Chronodisruption is associated with an increased risk of cancer, metabolic, cardiovascular and mental disease. As an output of the central clock, melatonin is a potent chronobiotic that modulates a number of physiological functions, including energy metabolism. A decline or even absence of normal melatonin secretion as a result of 'light at night' and/or shift work schedules is a salient feature of chronodisruption. Chronodisruption during pregnancy has considerable effect on gene expression (both clock and clock-controlled genes) with negative consequences for the life of the progeny, including persistent metabolic, cognitive and cardiovascular dysfunction. Although aberrant light exposure (e.g. light at night) is the most established and well-characterised form of chronodisruption, other environmental influences can also produce circadian disruption. As an example, steroid hormone-dependent developmental programming produces a polycystic ovary and reduces fertility with a parallel decline in internal circadian organisation. Thus, chronodisruption may be a common feature of fertility and metabolic diseases arising from foetal exposure to disruptive lighting conditions or an irregular hormonal milieu. Life in a 24/7 society together with the evidence discussed in the present review emphasises that there is the need for a continued and profound discussion regarding chronodisruption and its consequences for human health.

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