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INVITED REVIEW

Complexities in cardiovascular rhythmicity: perspectives on circadian normality, ageing and disease

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Abstract Biological rhythms exist in organisms at all levels of complexity, in most organs and at myriad time scales. Our own biological rhythms are driven by energy emitted by the sun, interacting via our retinas with brain stem centres, which then send out complex messages designed to synchronize the behaviour of peripheral non-light sensing organs, to ensure optimal physiological responsiveness and performance of the organism based on the time of day. Peripheral organs themselves have autonomous rhythmic behaviours that can act independently from central nervous system control but is entrainable. Dysregulation of biological rhythms either through environment or disease has far-reaching consequences on health that we are only now beginning to appreciate. In this review, we focus on cardiovascular rhythms in health, with ageing and under disease conditions.

Keywords Circadian rhythm • Cardiovascular • Heart rate variability • Sinus node

And the rhythm of life is a powerful beat
Puts a tingle in your fingers and a tingle in your feet
Rhythm in your bedroom, rhythm in the street
Yes, the rhythm of life is a powerful beat
—Sammy Davis Jr, Rhythm of Life 1969

1. Introduction

Physiological rhythms are intrinsic to all forms of life, from microscopic bacteria to large mammals. These persist even when the organism is withdrawn from environmental cues that might otherwise be held responsible for their existence. Such rhythms occur across broad time scales, from once daily (light-dark, or 'circadian', from the Latin *circa diem*, meaning 'for about a day'),[1] to less frequent than once daily ('infradian'),[2] to more frequent than once daily ('ultradian').[3] Life on our planet is subject to a day–night cycle lasting 24 h, and the circadian period of organisms on earth matches this. Rhythmicity is likely to have persisted through evolution because it allows the body to adapt to optimal and appropriate functioning during the day and at night, conferring the 'selective advantage of anticipation',[4] allowing organisms to tailor their physiology to light/dark, activity/rest, and sleeping/wakefulness. Circadian rhythms are believed to have originally evolved in aerobic organisms due to solar-cycle driven fluctuations in environmental oxygen from photosynthetic bacteria.[5]

It was once thought that an organism's biochemistry and physiology were continuous, compartmentalized processes. It is now known that circadian, infradian, and ultradian rhythms exist in all organs of the body, and at all levels of biological organization, from organ systems, to tissues, to single cells and even molecules.[6–9] These rhythms are not unique to animals, indeed the origins of circadian biology were based upon observations made in plants.[10] The heart and cardiovascular system as a whole exhibit many co-ordinated rhythmical behaviours, and modification or loss of rhythmicity may be both a predictor and detector of morbidity and mortality across a wide gamut of both cardiac and non-cardiac conditions. In this article, we will review the rhythms that govern a wide variety of cardiovascular parameters. We will discuss what is considered normal, and how this changes with both disease and with ageing.

2. Physiological rhythms in cardiovascular biology

Rhythmicity in the heart exists because of the complex output from a number of intrinsic and extrinsic oscillating factors. Intrinsic rhythmicity begins at the subcellular level, with rhythmical output of proteins from

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genes, and the readout of this occurs in the form of measurable cardiovascular parameters; each of these is considered in detail below:

2.1 Cardiac genes

Before considering rhythms within the heart itself, one must recognize that there is a 'central clock' or 'master pacemaker' that generates circadian rhythms in mammals, located in the suprachiasmatic nuclei (SCN) of the hypothalamus. Light sensed in the eye is transmitted via the retinohypothalamic tract to the SCN, where a molecular clock exists, consisting of multiple sets of transcription factors engaged in autoregulatory transcription-translation feedback loops.[5] This conversion of photic energy into neuronal and hormonal signals is the essence of the master or central circadian clock. The current dogma (see *Figure 1A*) is that production of protein from the core clock genes *BMAL1* (also known as *ARNTL*) and *CLOCK*, or *BMAL1* and *NPAS2*, leads to their heterodimerization in the cytoplasm. Translocation of this complex to the nucleus is followed by binding to canonical enhancer-box (E-box) sequences of clock-controlled genes (CCGs), and further expression of the genes *CLOCK*, *BMAL1*, *PER*, *CRY*, *NR1D1*, *NR1D2*, *ROR*, and *CCG*. Expression of BMAL1 and CLOCK proteins simultaneously induces their own repression through expression of PER and CRY, which also heterodimerize, translocate to the nucleus and inhibit transcription. The cycle completes when levels of PER and CRY fall because of the falling levels of BMAL1/CLOCK that they themselves have caused. Multiple other modulators of this process exist, for example casein kinase that phosphorylates PER and CRY, leading to inhibition of their transcription. Comprehensive reviews of this process are given elsewhere (see Ref.11). Critically, one complete transcription-translation loop of this cycle takes 24 h. The central clock of the body interacts with and synchronizes multiple peripheral light-*insensitive* clocks that themselves are capable of oscillating autonomously.

The heart's specific peripheral clock uses essentially the same molecular components as the central clock described above (see *Figure 1*). In general, there are tissue specific differences in the actual clock proteins produced, which are necessary for each peripheral circadian oscillator to have effects tailored to the individual organ/tissue. The first description of the peripheral clock in the human heart came in 2009.[12] Like in the other central and peripheral clocks, cardiac clock genes are 'autonomous', i.e. intrinsically maintained and self-sustained, as proven by the presence of rhythmical changes in single cardiac cells, and cultured cardiomyocytes. In the heart, the molecular clock plays a critical role in all physiological processes, for example in the diurnal variation in myocardial oxidative and non-oxidative metabolism of carbohydrates and lipids (described in more detail below),[13] and the dysregulation of clock gene-controlled diurnal rhythms has been shown to lead to dysfunctional cardiac metabolism and function.[12] Core clock genes in the heart include *BMAL1*, *CLOCK*, *CRY1*, *CRY2*, *PER1*, *PER2*, *PER3*, *DBP*, *HLF*, and *TEF*. The current dogma of a transcription-translation loop of 24 h duration in cardiac cells is the same as that already described in the central oscillator of the SCN.[14]

Components of the core clock in the heart also regulate genes outside of the clock mechanism, designated as CCGs, which encode other transcription factors or proteins controlling rate-limiting steps in cardiac cell physiology.[15] Studies in mice using high-density oligonucleotide microarrays to study the 3-hourly myocardial expression of virtually all of the genes of the heart (almost 12 500) found statistically significant variation in 13% of these (1634/12 488) over a 24 h period, with some showing a smoothly rhythmic temporal profile change, with others demonstrating abrupt day–night/night–day transitions (see *Figure 1B*).[16] Those that did demonstrate significant cyclical change were mapped to key biological pathways, including growth, remodelling, transcription, translation, mitochondrial respiration, and signalling pathways.[16] Undoubtedly, 'the heart is transcriptionally a different organ in the day vs the night'.[17] These findings were confirmed in a later study comparing rhythmical gene expression in heart and liver—here >8–10% of 12 488 genes exhibited circadian regulation, but only 37 genes were regulated similarly in the two different organs, confirming the organ-specific heterogenous nature of circadian gene expression.[18]

In terms of location, expression of CCGs has been specifically shown to exhibit significant circadian variation in the atria of mice, in the hearts of rats,[19,20] and in human hearts.[12] The expression of clock genes in humans is in antiphase to that seen in rodents, reflecting the nocturnal waking life of the rodent vs. the daytime waking life of humans.[12] Destruction of the suprachiasmatic nucleus eliminates, while autonomic blockade only dampens, this circadian variation in expression of clock genes in mice,[19] suggesting that central control of clock genes in the heart is exerted in additional ways to autonomic nervous system (ANS) signalling.

Targeted disruption of clock genes disrupts circadian rhythms, and has far-reaching implications for the organism involved, including a shortened life span.[21] Specific disruption of core clocks genes, for example by overexpression of mutant CLOCK protein (the so-called 'CCM mouse'),[22] or through targeted cardiac-specific deletion of *Bmal1* (the so-called 'CBK mouse'),[23] has profound yet distinct effects on the molecular clock in cardiomyocytes, leading to significant reductions in heart rate (HR) through the day, altered substrate metabolism, and deficient contractile function. The importance of these genes is emphasized by the fact that the cardiomyocyte specific Bmal1 KO (CBK) mouse begins to exhibit echocardiographic features of heart failure by around 30 weeks of age, and uniformly dies by the age of 1 year (see *Figure 2*);[4] subsequent studies on the same mouse demonstrated evidence of progressive abnormal diastolic septal annular wall motion and reduced pulmonary venous flow at 28 weeks of age, progressive worsening of fibrosis in the interstitial and endocardial regions from 8 to 28 weeks, increased expression of collagen and matrix metalloproteinases at 28 weeks, increased transcript levels of neutrophil chemotaxis and leucocyte migration genes and decreased levels of enzymes indicating impaired resolution of inflammation.[24]

2.2 Protein production within cardiomyocytes

Although instructive to study gene expression levels in terms of mRNA, proteins underlie most biological processes, and levels of the two do not always correspond. Studying rhythmical or circadian variation in proteins themselves is necessary to ensure that mRNA data are not misleading.[25] The diurnal proteome has been studied in the mouse heart,[17] demonstrating considerable daily variation (7.8%, 90/1147 proteins studied), similar to that seen in mRNAs. The cardiomyocyte-specific clock mutant (CCM) mouse introduced above has a significantly altered proteome, with myofilaments exhibiting a loss of time-of-day-dependent maximal calcium-dependent adenosine triphosphate (ATP) consumption and altered phosphorylation rhythms, with effects on enzymes regulating vital metabolic pathways, with highly detrimental effects on cardiac metabolism.[17]

2.3 Cardiac contractility and cardiac output

Cardiac output is known to show diurnal variation, in humans decreasing by 24–29% at night compared with daytime averages, while stroke

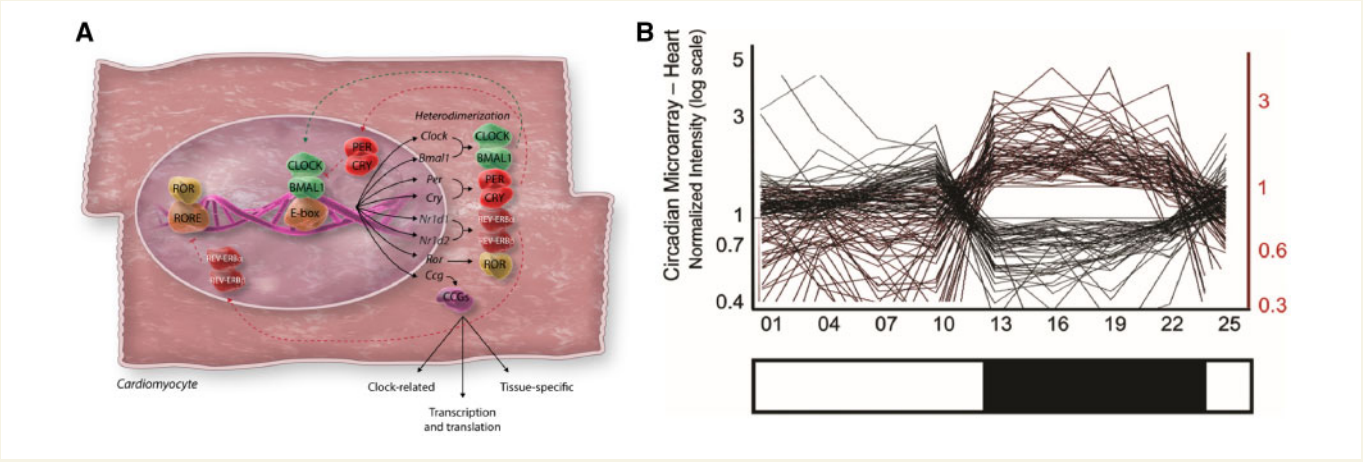

Figure 1 (*A*) Adapted from Scheiermann *et al.*[5] Transcription of the core clock genes CLOCK and BMAL1 results in their heterodimerization within the cytoplasm of the cell. This transcription factor heterodimer is then translocated to the nucleus, where it binds to E-box sequences, leading to further self-transcription, along with transcription of core clock genes (CCGs) that are largely tissue specific and drive circadian processes. In addition, the CLOCK/BMAL1 heterodimer leads to transcription of PER and CRY, which also form a heterodimer that acts as a negative regulator of the cycle by interfering with the binding of CLOCK/BMAL1 to the E-box sequences. Other positive regulators of the process are also produced, including ROR, which binds to ROR response elements (RORE) in the BMAL1 promoter zone to induce further expression of BMAL1. This is antagonized by another product of CLOCK/BMAL1 binding to E-box sequences that is the products of the Nr1d1 and Nr1d2 genes, REV-ERBα and REV-ERBβ. These form a complex that inhibit the expression of BMAL1 by interfering with ROR binding to RORE. The period of the ebbing and flowing of this transcription-translation loop is 24 h on earth. (*B*) (from Martino *et al.*[75]) illustration of the circadian variation of expression of a subset of cardiac genes in the mouse, demonstrating remarkably abrupt changes in expression, especially at the transition point between light and dark phases of the cycle. Black lines reflect light activated genes and red lines reflect dark activated genes.

volume decreases by 7%.[26,27] Atrial pressure in humans also exhibits a circadian pattern that is correlated with HR.[28] These observations are traditionally ascribed to fluctuations in neurohormonal influences in the intact organism.[29] However, such rhythmicity is also intrinsic to the heart. The *ex vivo* working rat heart, for example, has been shown to exhibit circadian rhythmicity in contractile function, with greatest contractile performance in the middle of the night,[30] a feature lost in hypertrophied hearts. Similarly, cardiac contractility in Langendorff-perfused murine hearts is greater when they are studied 3 h into the dark phase vs. 3 h into the light phase of a 12:12 h light:dark regime. Disruption of this circadian rhythm, by putting the mouse into a 10:10 h light:dark regime, causes normal diurnal variation in cardiac contractility to disappear.[17] Contractile reserve of *ex vivo* rodent hearts is greater in the dark phase of the circadian cycle, consistent with anticipation of workload demand during waking hours.[22] These changes in cardiac contractility may be related to recent observations suggesting molecular circadian control of the sarcomere, including circadian regulation of the titin-cap protein,[31] rhythmic mRNA expression of cAMP-dependent protein kinase A (PKA),[22] observations that disruption of diurnal pattern alters myofilament protein phosphorylation in a murine model of myocardial infarction (MI),[32] and observed daily oscillations of cardiac myofilament composition and function,[17,31] calcineurin activity, protein phosphorylation,[33] and myocardial excitation-contraction coupling.[34] Time-of-day-dependent oscillations in expression of the two isoforms of myosin heavy chain, a critical contractile protein in the heart, have also been demonstrated in rodent hearts.[20,35]

2.4 Cardiac metabolism

The circadian fluctuation in contractile function of the heart described in the prior section is inextricably linked to cardiac metabolism. Carbohydrate oxidation and oxygen consumption exhibit marked circadian variation.[36] In the working rat and mouse heart, this has been shown to be timed to coincide with periods of increased workload (e.g. exercise), with a peak in the middle of the night.[30,37,38] Glycogen content in the rat heart peaks in the dark-to-light phase transition, consistent with increased rates of glycogen synthesis during the awake-dark period.[37]

Unlike glucose utilization, fatty acid oxidation does not exhibit circadian variation in isolated rat or mouse heart preparations, suggesting that fatty acids form the consistent foundation for the energetic demands of the heart. However, triglyceride synthesis does oscillate in mice, peaking near the end of the dark/active phase,[30,39] while lipolysis is elevated during the light/sleep phase.[39]

Far less is known about circadian variation in protein and amino acid metabolism. Some evidence exists that net protein synthesis appears to be increased in the rat myocardium during the light/sleep phase *in vivo*.[40] Indirect evidence also points to diurnal amino acid metabolism, through the levels of certain amino acids fluctuating in a time-dependent manner,[40,41] and expression of genes for amino acid metabolism enzymes show diurnal variation.[16,18,22]

It has been similarly challenging to investigate the relationship between the circadian clock and cardiac mitochondrial metabolism. Cardiac-specific ablation of the *Bmal1* gene results in cardiomyocyte mitochondrial morphological and functional abnormalities, including reduced respiratory complex enzyme activity, and decreased expression of genes involved in fatty acid oxidation, the tricarboxylic acid cycle and the mitochondrial respiratory chain.[42] These mice also demonstrate impaired ketone body metabolism, impaired glucose utilization in the fed state, and abnormal metabolic responsiveness to acute fasting.[4] Furthermore, they develop severe progressive heart failure with age.[42] Similar features were present in normal (C57BL/6J) mice exposed to

chronic reversal of the normal light:dark cycle, confirming that the circadian clock is important for maintaining healthy mitochondrial dynamics and bioenergetics in the heart. The circadian component REV-ERBα regulates mitochondrial content and oxidative function in skeletal muscle, in part by repressing genes that trigger mitophagy.[43] A similar role in cardiac muscle might be predicted, and several papers have also pointed to the importance of effective mitochondrial autophagy for the prevention of contractile dysfunction and heart failure.[44–47]

The rhythms described above in cardiac metabolism may be affected by rhythmicity in sympathetic activity,[48] circulating insulin,[49] thyroid hormone,[50] and corticosteroid levels,[49] as well as circadian variation in circulating fuel availability such as glucose, fatty acids, and ketone bodies.[51,52]

Similar to changes seen in the CBK mouse noted above, CCM mice demonstrate abnormalities in metabolism, including increased rates of myocardial oxygen consumption and fatty acid oxidation, and decreased cardiac efficiency relative to wild-type animals.[22] CCM mice also exhibit lack of oscillatory lactate release and modest mitochondrial dysfunction, but no alterations in mitochondrial content or structure.[22]

The question of what links the cardiomyocyte's circadian clock genes with cellular metabolism is slowly being addressed, with circadian control of several key modulators of myocardial cell metabolism now established, including AMPK and NAMPT.[38,39,53] The latter of these contributes to NAD levels in the heart, and normal oscillation of NAD in wild-type hearts is lost in the hearts of CCM.[39,54]

2.5 Heart rate

HR in humans exhibits a clear circadian pattern,[55–60] with a morning 'acrophase' (a term used to denote a cycle's peak, occurring at 10 am to midday), a small afternoon nadir (at around 3 pm), an evening acrophase (at around 8 pm), and a profound nocturnal nadir (at around 3–5 am) (see *Figure 3A*).[55,61] CCM mice demonstrated decreased diurnal variation in HR.[22]

Increasing or decreasing HR *in vivo* occurs largely via the ANS's two opposing arms—the catecholaminergic, sympathetic nervous system, and the acetylcholinergic, parasympathetic nervous system. Circulating catecholamines epinephrine (indicator of adrenal medullary activity) and norepinephrine (indicator of sympathetic nervous system activity) exhibit clear circadian periodicity, with a rapid elevation on waking, and a peak around midday, with a gradual tailing-off thereafter (see *Figure 4*).[61,62] Plasma norepinephrine levels have been shown to be increased by upright posture and wakefulness, while levels of epinephrine are not so, suggesting that the latter is controlled by a central circadian oscillator, while the former is influenced by environmental conditions.[62] Detailed review of circadian epinephrine and norepinephrine rhythms is available elsewhere.[63]

Both arms of the ANS have direct effects on the cells of the sinoatrial node and their intrinsic clocks (described in detail later), leading to a so-called 'brain hierarchical clock system' (*Figure 5A*). The effects of the ANS are mediated by post-translational modification of critical intrinsic regulatory mechanisms of pacemaker cell automaticity via cAMP/PKA/CaMKII signalling. The nadirs in circadian HR have previously been ascribed to times when the parasympathetic nervous system becomes 'dominant' over the sympathetic.[64,65] Similarly, acrophases in circadian HR are ascribed to vice versa shifts in the parasympathetic-sympathetic balance. These conclusions rely on assumptions made *not* from direct measures of ANS functioning, but instead from heart rate variability (HRV) data, which is recognized to be limited by a dependence on HR, an often overlooked but critically important correction.[66–69] Although circadian variation in post-translational protein phosphorylating mechanisms has been demonstrated in other organs (e.g. CaMKII in chick retinal cells[70]), it is yet to be demonstrated in the cells of the heart, and further experiments are required to clarify whether circadian variation in phosphorylation is the 'end effector' of circadian variations in autonomic tone to the heart.

Despite their substantial contribution, the brain and its autonomic nervous signalling are not essential for the presence of circadian rhythms in HR, and a variety of rhythms (circadian, infradian, and ultradian) persist in so-called 'denervated' cardiac preparations, including transplanted hearts,[71] isolated Langendorff-perfused hearts,[22,30,66] monolayers of cultured cardiomyocytes,[72] and isolated sinoatrial nodal cells.[66] Though rhythms persist in these denervated preparations, they are distinct from the rhythms that exist in the intact organism with a functioning ANS, regardless of whether one focusses on time-, frequency-, or non-linear domain measures of HRV.[66] The best example of a 'denervated' SAN 'in vivo' is the transplanted heart. It has been shown that without intact autonomic innervation, such hearts beat faster and with less circadian variability than does the normally innervated healthy heart.[73] The reasons behind this are unclear, but will be related to the lack of an intricate interaction between SAN autonomic innervation and the rhythmic processes that are occurring at a tissue, cellular and subcellular level, explored in more detail in Section 3.

2.6 Blood pressure

Circadian changes in blood pressure (BP) have been shown in humans,[26,74] with a pattern mirroring HR in both systolic and diastolic pressures (see *Figure 3B*), for example, night-time BP is decreased by around 10% compared with daytime values,[75] with the daily increase in BP starting between 4 and 7 am.[26] This is true of both normotensive and hypertensive patients.[76] Such a morning 'surge' and the associated increase in cardiac output have implications for the risk of atherosclerotic plaque rupture and likely plays a role in diurnal variation in incidence of MI, stroke, sudden death, and ventricular arrhythmia discussed elsewhere in this review. Hypertensive patients can be split into those who exhibit nocturnal dipping in BP and those who do not. Nocturnal dipping is believed to be a healthier state, and clinical studies have shown that loss of this behaviour (in patients referred to as 'non-dippers') is associated with left ventricular hypertrophy, increased risk of MI, renal failure, and an increase in cerebrovascular disease.[77–79]

The occurrence of vasovagal syncope in human patients also exhibits a circadian pattern, with more events occurring in the morning (0600 h to noon), thought to be related to differences in vagal tone or responsiveness to vagal outflow that occur through the course of the day.[80]

Excessive dietary Na^+ intake is recognized to adversely affect circadian rhythms in BP and nocturnal BP dipping in humans, while Na^+ restriction and treatment with diuretic medications can restore circadian dipping behaviour to non-dippers.[81] Counter-intuitively, total peripheral resistance of the systemic arterial circulation behaves in almost the complete opposite manner to BP (and HR and cardiac output), with a nadir at around 10 am, being highest through the night.[26] One might therefore predict that BP would be lowest around 10 am, but clearly this is not the case, and other factors than total peripheral resistance must contribute to circadian BP variation. *In vitro*, pressure induced hypertrophy in rats replicating that seen in response to long-standing hypertension attenuates circadian BP rhythms through attenuation of transcription of clock genes.[20]

Behavioural circadian clock disruption can have deleterious effects on the progression of hypertensive heart disease: in normal C57BL/6 mice exposed to pressure overload causing left ventricular hypertrophy,

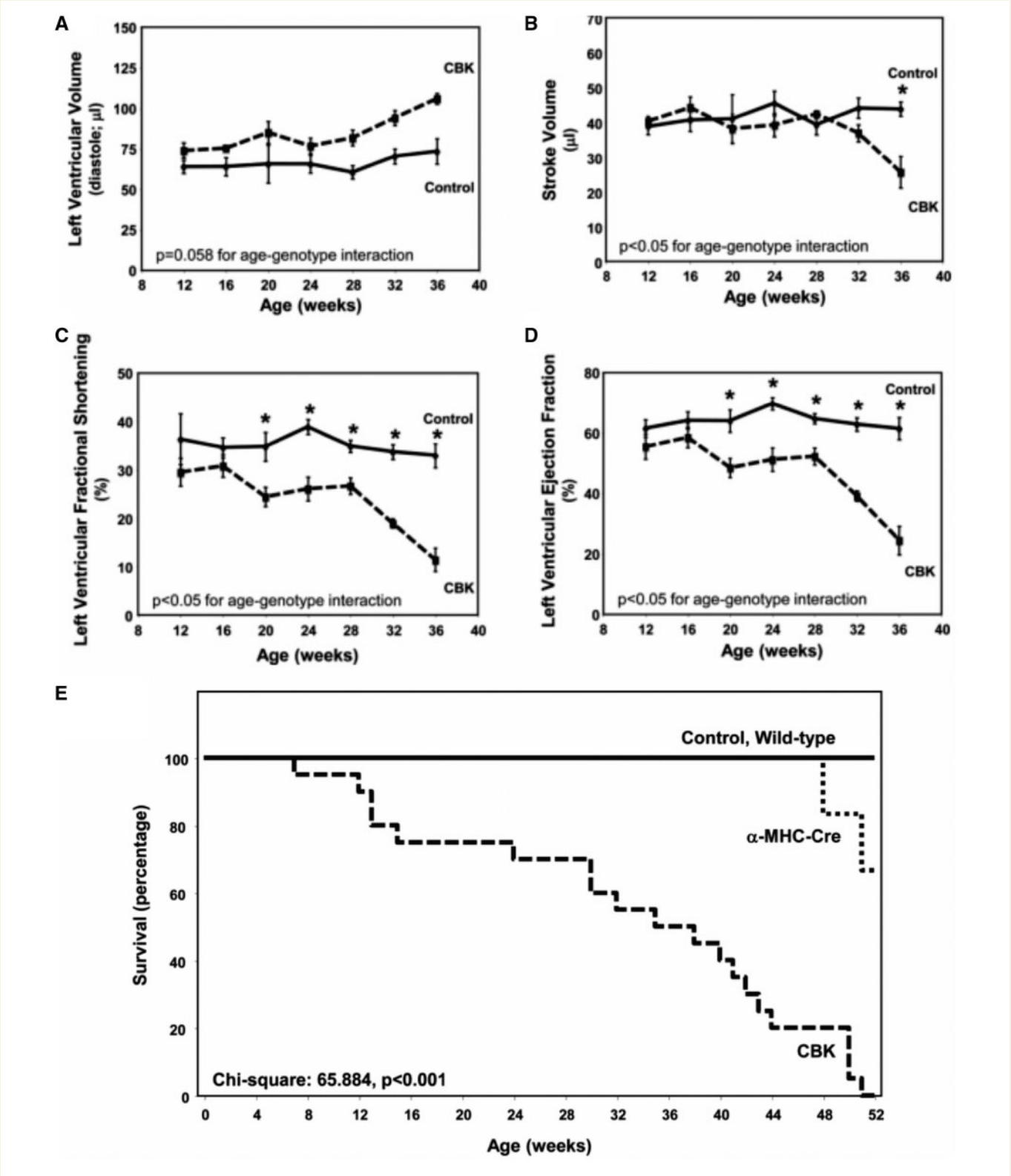

Figure 2 Adapted from Young *et al.*'s[4] seminal paper studying CBK mice. Age-dependent depression of cardiac function in CBK, but not littermate controls, including left ventricular volume during diastole (*A*), stroke volume (*B*), left ventricular fractional shortening (*C*), and left ventricular ejection fraction (*D*). Serial echocardiography was performed at 4 week intervals at the same time in the light:dark cycle. (*E*) It shows markedly decreased survival in CBK mice compared with littermate controls.

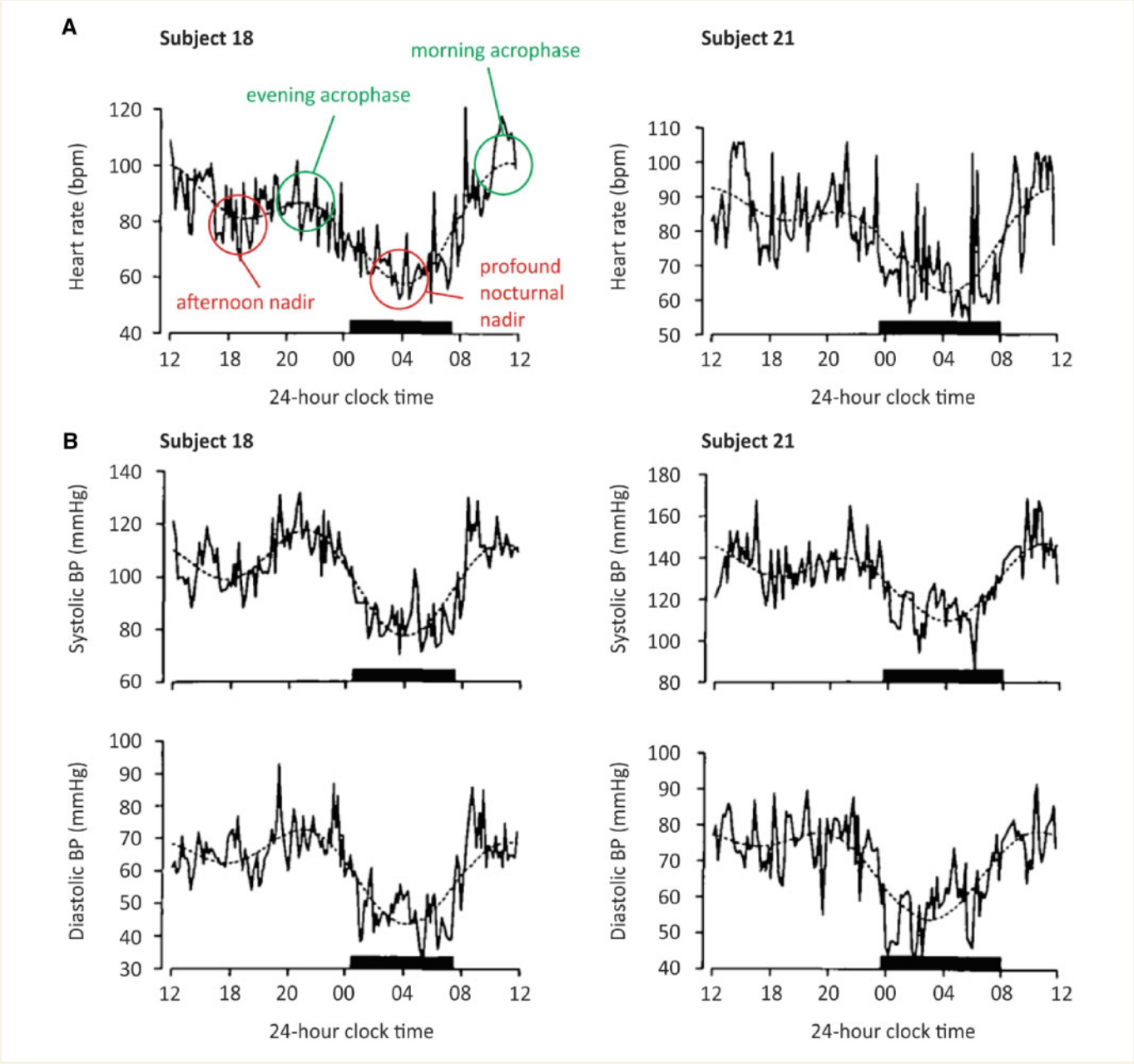

Figure 3 Typical nadirs and acrophases of both HR (*A*) and BP (*B*) in two young and healthy subjects. Reproduced and modified with permission from Ref.[55]

shifting them from a 24 h light:dark cycle to a 22 h light:dark cycle made cardiac remodelling more marked and the progression to heart failure more rapid.[82] Disrupting the circadian clock genetically by knocking out BMAL1,[83,84] or by mutating CLOCK[83] or PER2[85,86] leads to aortic endothelial dysfunction that is likely to contribute to hypertension, confirming the importance of the symbiotic relationship of circadian rhythms, cardiac contractility with systemic vascular resistance in the genesis of hypertension.

2.7 Endocrine rhythmicity relevant to the heart

Circadian oscillation is present in the release characteristics of nearly every hormone. *Cortisol* secretion from the adrenal cortex, co-ordinated by the hypothalamic-pituitary-adrenal axis, exhibits clear diurnal variation, the peak of which occurs just after awakening, at a time coincidental with the maximal observed increase in plasma catecholamine levels and HRV parameters suggesting sympathetic dominance (see *Figure 6*).[87–89] Some have proposed that cortisol is utilized by the central clock as a critical intermediary in the entrainment of peripheral clocks.[90] Cortisol appears likely to contribute to the circadian rhythm of HRV, although exogenous administration of hydrocortisone only affects chronic but not acute markers of HRV.[91] It is possible to completely reverse the daily cortisol rhythm by deliberate circadian misalignment in humans, wherein study subjects are made to eat and sleep 12 h out of phase from their habitual times.[89]

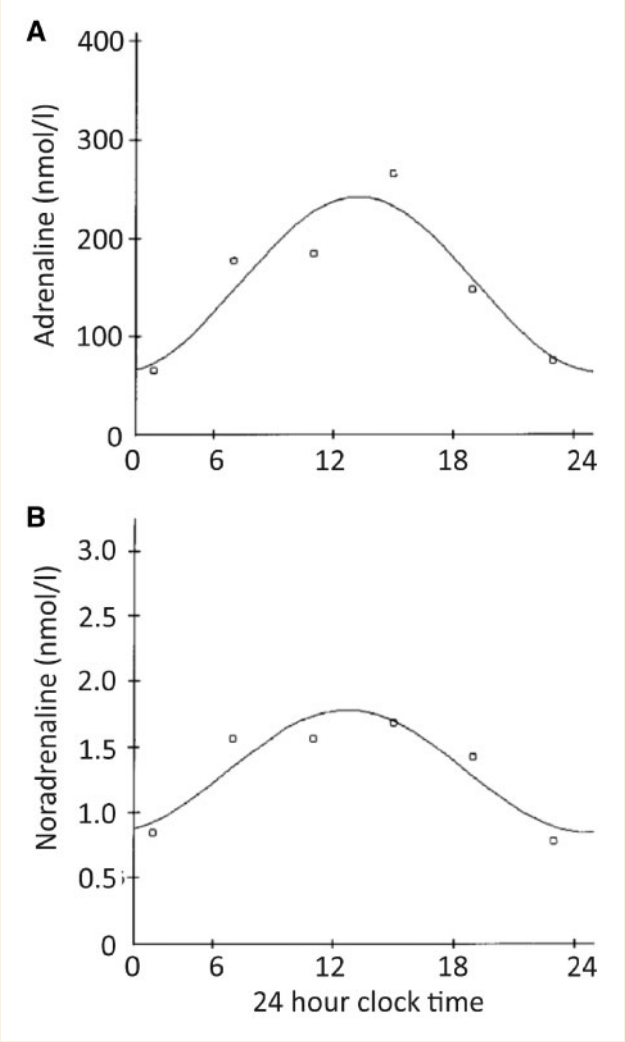

Figure 4 Adrenaline (*A*) and noradrenaline (*B*) plasma levels (in nmol/L) during a 24-h observation period in control subjects. Curves are periodic regression curves for circadian variation. Reproduced and modified with permission from Kondo *et al.*[223]

Melatonin is 'the most pronounced hormonal rhythm driven by the circadian system'.[92] It is a highly lipophilic molecule synthesized and secreted by the pineal gland during darkness—in humans its secretion starts soon after sundown and peaks in the middle of the night around 2–4 am. During the day, serum concentrations are very low. It acutely inhibits neuronal firing in the mammalian SCN. Despite this, acute effects of melatonin on clock gene expression in the hypothalamus are not seen immediately following its administration. Rather, it takes around 48 h to see changes in clock gene mRNA in response to melatonin injection, suggesting that its effect is post-translational.[93] It is considered to be an endogenous synchronizer of peripheral clocks to central 'time', with some viewing it as 'the master clock output'.[94] Melatonin administration in rats has been shown to effect the levels of cardiac clock gene expression,[95] and lead to favourable effects on BP in humans.[96] It also has marked antioxidant properties[97] and has been shown to protect against ischaemia-reperfusion myocardial damage and normalize lipid profiles through its ability to directly scavenge free radicals and indirectly through its antioxidant and anti-inflammatory properties.[98] This effect of melatonin is relevant since it is known that loss of function of BMAL1 leads to expression of genes related to oxidative stress, cardiac remodelling and inflammation,[42] and some studies have demonstrated circadian rhythms in the expression of pro-oxidation enzymes like glutathione peroxidase.[99]

The *renin-angiotensin-aldosterone* system has circadian periodicity that coincides with that of the HR and BP.[61,100,101] This particular circadian rhythm is likely to have profound effects on diurnal BP rhythms because of the powerful BP regulating effects of angiotensin II and aldosterone.

Brain natriuretic peptide (BNP) mRNA levels demonstrate circadian variability in the ventricles of mice, with a nadir at between 1200 and 1600 h (circadian time) and an acrophase at around midnight.[102] Since natriuretic peptides are important in the regulation of fluid homeostasis and vascular tone, and protecting against accelerated local fibrosis in the myocardium, this study suggests that in the ventricles, circadian patterns in BNP may partially account for variation in outcome from MI based on the time of day the infarct occurs.[103–106]

2.8 Coagulation

The observed excess of thrombotic cardiovascular events, including MI and stroke, at certain times of day[107–109] has generated interest in circadian coagulation rhythms. Circadian rhythms exist in both human *vascular endothelial function*[110,111] and in *platelet function*.[88,112] Regarding the former, flow-mediated vasodilatation of systemic vessels as an index of endothelial function is poorest in the morning,[110] and coronary segments with dysfunctional endothelium exhibit exaggeration in early morning vasomotor activity,[111] making coronary occlusion more likely at that time of day. Platelet aggregability demonstrates complex circadian rhythmicity, with some evidence that it peaks bimodally through the course of the day, at noon and 9 pm.[88] Other evidence demonstrates circadian rhythms in platelet surface activated glycoprotein IIb-IIIa, Ib, and P-selectin,[112] peaking at around 8–9 am, right when the excess of thrombotic cardiovascular events occurs. This article also demonstrated circadian peaks in other indices of platelet behaviour including count, aggregability and ATP release, peaking later in the day, between 3 and 8 pm,[112] and further that circadian influences on these platelet behaviours was significantly greater than any environmental stressors. It is interesting to consider what might be the cause of circadian rhythms observed in platelet biology, since they are anucleate. It has been suggested[112] that humoural factors control rhythms in platelet biology, including the previously discussed circadian rhythms in endocrine hormones (epinephrine, norepinephrine, melatonin, and cortisol), endothelial derived factors (nitric oxide and prostaglandin), and platelet-derived factors (ATP). Alternatively, circadian variation in organ sequestration and release of platelets by spleen/liver/lungs/bone marrow may be the critical factor. Without a nucleus, the clock genes and oscillating transcription-translation feedback described in the initial section cannot be the cause of rhythmical variation in platelet biology. Some insight has been given into the possibility of innate rhythmicity in platelets by studies of circadian rhythmicity in zero-DNA containing, anucleate human red blood cells.[113] These studies have shown that non-transcriptional mechanisms involving peroxiredoxins (highly conserved antioxidant proteins) undergo reduction-oxidation cycles with a period of around 24 h, which persist for many days under constant conditions in the absence of external cues.[113] These rhythms in red blood cells were entrainable to environmental cues and were also temperature compensated (did not change in response to fluctuation in ambient temperature),[113] both of which represent key features of circadian rhythms. This work suggested for the first time that having a nucleus was not a fundamental requirement for the presence of circadian rhythms in mammalian cells.

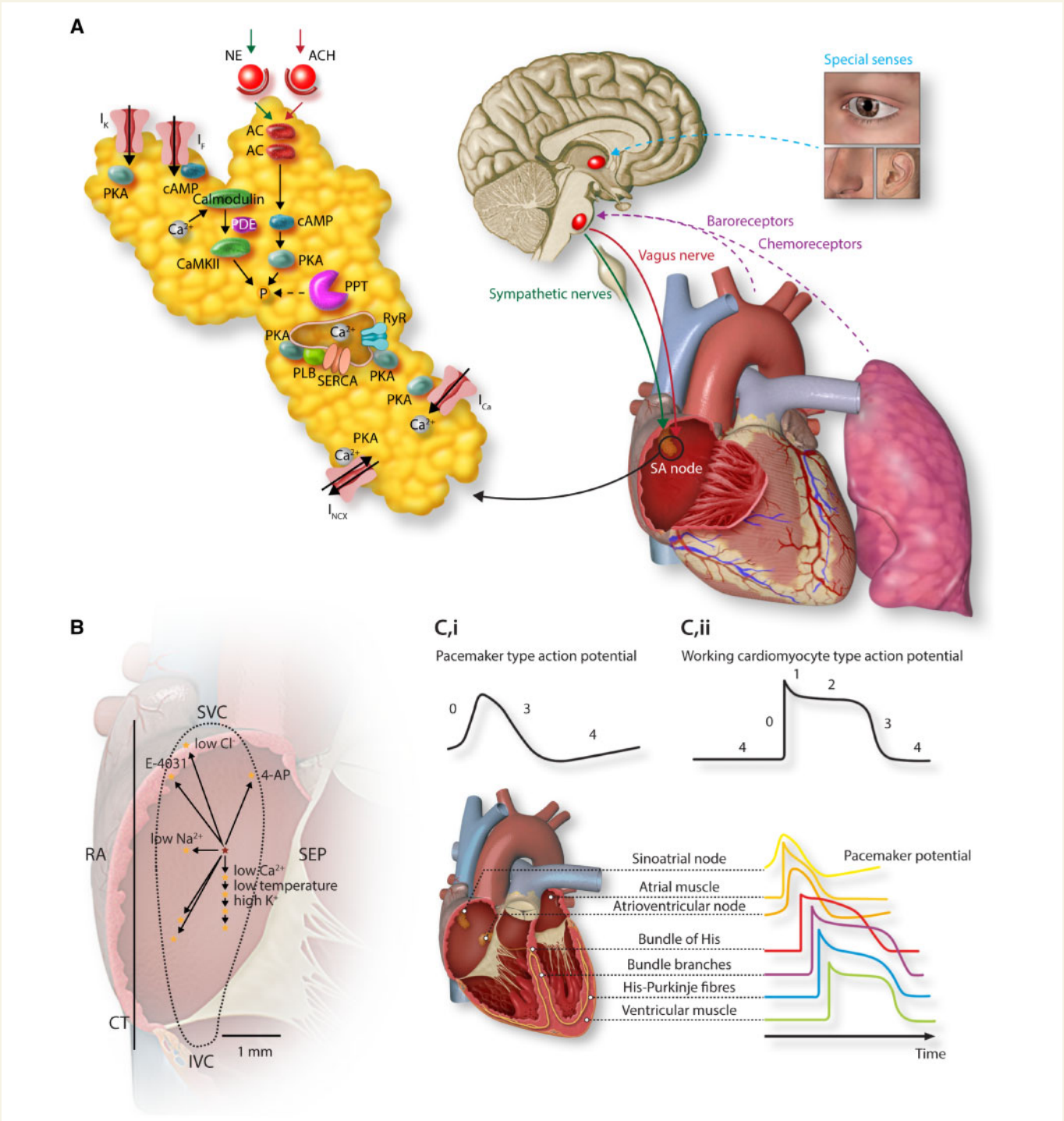

Figure 5 (*A*) Schematic figure of the brain-hierarchical clock system controlling SAN function. Special senses (vision, smell, and hearing) and chemical and baroreceptors feed information from the environment into the brainstem centres responsible for the central control of HR. Via largely opposing actions, the sympathetic and parasympathetic arms of the autonomic nervous system emerge from these brainstem centres, innervating the sinoatrial node directly, and having direct effects on levels of cAMP and phosphorylation of coupled clock proteins via their interaction with adrenoceptors and muscarinic receptors, respectively. The SAN is enlarged in yellow, and within is shown a schematic of the coupled clock system dictating automaticity. (*B*) Shift in leading PM site occurs in response to a variety of changing environmental conditions and drugs. Non-standard abbreviations: SVC, superior vena cava; SEP, septum; RA, right atrium; CT, crista terminalis; IVC, inferior vena cava; Ach, acetylcholine; Nif, nifedipine. Reproduced with permission from Ref.[121]. (*C*) Phases of the action potential in pacemaker type cells (i), which typically display an unstable resting membrane potential also known as phase 4 diastolic depolarization, and in working cardiomyocytes of the atria and ventricles (ii), which have a stable phase 4 resting membrane potential. The rapid upstroke of the action potential is referred to as phase 0, while repolarization is referred to as phase 3. In working cardiomyocytes, there are two further phases of the action potential—phase 1, which is early repolarization, and phase 2, which is referred to as the plateau potential. (*D*) the actual morphology of the action potential changes discretely but definitively as one moves down the cardiac conduction system through the working myocytes of the atria and ventricles.

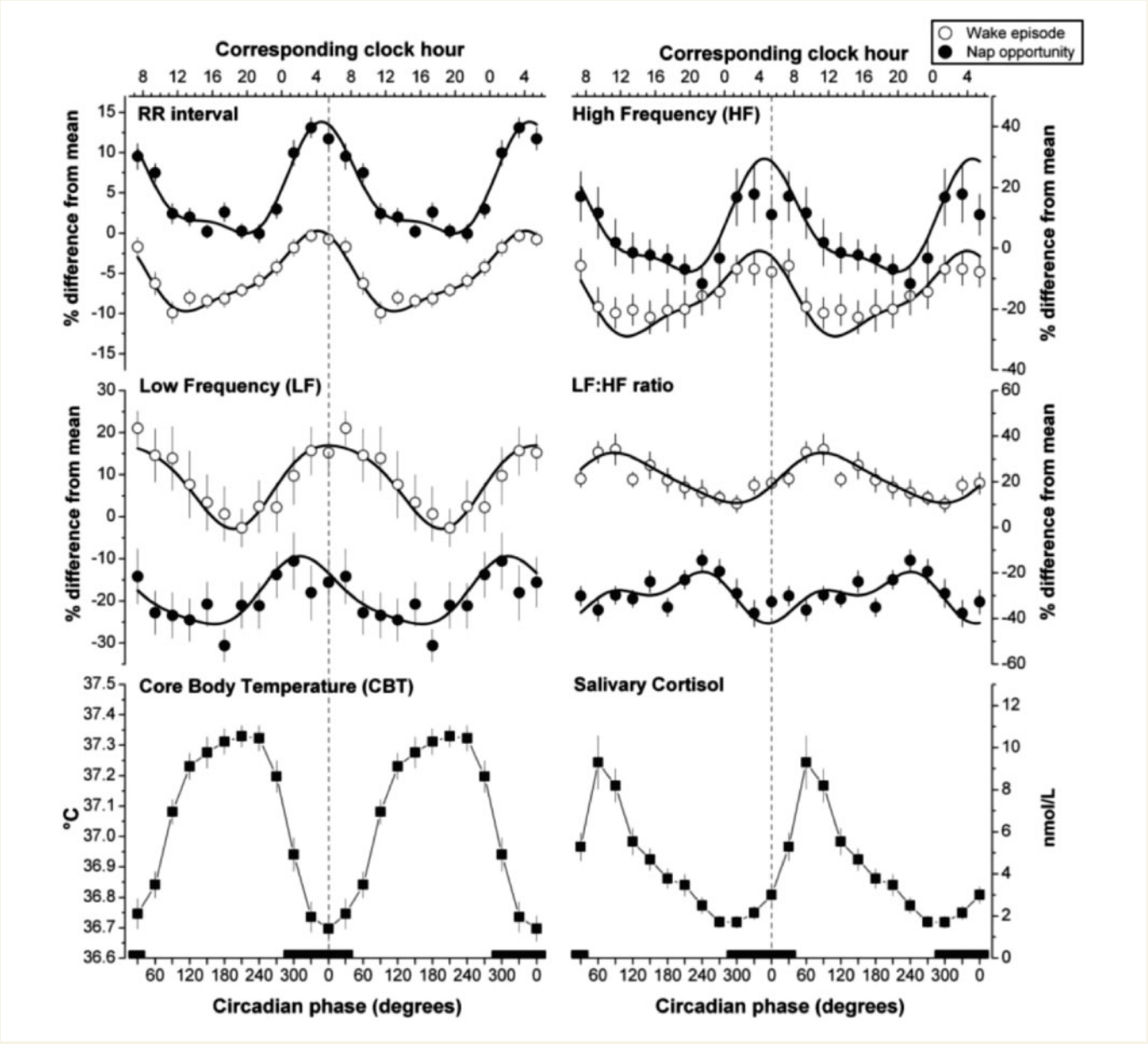

Figure 6 Marked circadian variability in frequency domain HRV parameters, core body temperature and salivary cortisol. Reproduced with permission from Ref.[87]

This backed several other observations that suggested transcription-translation independent circadian rhythmicity, including in the simplest organism known to exhibit circadian rhythmicity (the cyanobacterium *Synechococcus elongatus*), which demonstrates biochemical oscillations in the absence of transcription and translation.[114,115]

Indices of coagulation also show diurnal variation, with morning hypercoagulability and hypofibrinolysis being related to a broad variety of circadian changes in plasma levels of activators and inhibitors of coagulation and fibrinolysis.[116] For example, plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis and is a key circulating prothrombotic factor that is known to rise in the morning in humans, peaking around 06:30 h.[117] Data exist that show a direct effect of core clock genes (including *BMAL1*, *CLOCK*, and *PER2*) on the activity of haemostatic factors such as von Willebrand factor and megakaryocytes.[118–120] Further, disruption of the so-called 'positive' limb of the transcription-translation cycle (*CLOCK*[118] and *BMAL1*[119]) favours a thrombotic phenotype, while disruption of the so-called 'negative' limb (*PER2*[120]) favours a haemorrhagic phenotype.

3. The heart beat itself as a system of ultradian rhythms

The *heart beat* is comprised of discrete yet intricately woven 'clocks' that have been unpicked over the last 15–20 years. A small number of cells within the SAN dictate the heart's beating rate—the 'leading pacemaker site'—the location of which can vary depending on environmental conditions (see *Figure 5A, B*)[121] due to marked electrophysiological heterogeneity in SAN cells.[122] The cells leading pacemaking exhibit the fastest

rate of phase IV diastolic depolarization (see *Figure 5C-i, ii*), and are the first to reach the threshold for the firing of an 'all-or-nothing' action potential. As these cells fire an action potential, they entrain and electrically capture the cells around them, causing almost simultaneous firing of action potentials in a ripple effect, to the rest of the heart (see *Figure 5D*). At the cellular level, SAN automaticity is governed by complex and dynamic interaction between two oscillatory clocks (*Figures 5A and 7*).[123]

3.1 The membrane clock

This rhythmic 'clock', distinct from the circadian clock, was the first described mechanism that could plausibly account for automaticity in sinoatrial node cells (*Figure 7*, top panel). Charged species ('ions') pass through membrane-bound ion channels, taking advantage of the differences in ionic concentrations between the cytoplasm and extracellular milieu of sinoatrial node cells, in a time- and voltage-dependent manner. The opening of ion channels is a fleeting event producing electrical currents that contribute to both diastolic depolarization and the action potential itself. Because of their different characteristics, different ion channels are important at different times in the action potential (see *Figure 8*). The fact that these ion channels exhibit time-dependence means that they have *fundamental rhythmicity* that contributes to ultradian rhythms in the heart. Circadian variation exists in important members of the membrane clock, as follows:

3.1.1 Sodium channels

Knockout of *Bmal1* has been shown to cause loss of circadian expression of the *Scn5a* gene, normally encoding the principle cardiac voltage gated Na^+ channel, $Na_v1.5$,[14] with a decrease in $Na_v1.5$ protein expression, and in the levels of I_{Na} in ventricular myocytes.[14] It is likely that this contributes to HR slowing, loss of circadian HR variation, QRS prolongation and episodes of arrhythmia seen in these animals.

3.1.2 Repolarizing potassium channels

In rat heart, mRNA from two potassium channel genes, *Kcna5* and *Kcnd2* [the pore-forming subunit for the fast component of the transient outward K^+ current ($I_{to,\,f}$)], exhibits significant circadian variation, which can be modulated by pharmacological blockade of the ANS.[124] Similarly, in mice, there is also significant circadian variation in *Kchip2* and *Kcnk3* in both the atria and the ventricles.[19] Other studies in mice have confirmed the circadian variation in KChIP2 (the regulatory beta-subunit for the transient outward K^+ current, I_{to}), and also in the expression of the alpha subunit for I_{to}, $K_v4.2$, brought about through the clock genes *Clock* and *Bmal1*, via the transcriptional activator *Klf15*.[125] Abnormalities in *Klf15* led to loss of diurnal QT variation, abnormal repolarization and a higher rate of potentially life threatening ventricular arrhythmias. Inducible cardiac-specific knockout of *Bmal1* in murine hearts disrupted the previously robust circadian pattern of expression of *Kcnh2*, responsible for the hERG channel that underlies the rapidly activating delayed-rectifier K^+ current,[126] which is 50% smaller in ventricular myocytes from knockout animals, leading to prolonged QTc on the electrocardiogram (ECG).[126] Studies in humans have shown that high levels of circadian variation in ECG parameters of repolarization (so-called QT diurnality) are associated with ventricular arrhythmias in patients with a history of MI and a reduced ejection fraction, and that this seemed to be associated with hERG channel dysfunction.[127]

3.1.3 Funny current (I_f)

There is some early experimental evidence from our laboratory that circadian rhythms exist in the expression of I_f in murine sinus node cells, with corresponding circadianicity in HCN4 mRNA and protein expression (Lakatta, unpublished data).

3.2 The calcium clock

Rhythmical cycling of Ca^{2+} in and out of its major intracellular store, the sarcoplasmic reticulum (SR), occurs in two phases via its release channel, the ryanodine receptor (RyR) (*Figure 7*, bottom panel). The first involves rhythmical spontaneous, stochastic, locally propagating releases that occur in diastole known as 'local Ca^{2+} releases', 'LCRs'. These emerge as the SR refills, courtesy of the sarco-endoplasmic reticulum ATPase (SERCA). Following the production of an LCR, the RyR exhibits a delay before a subsequent LCR can be produced, giving them an inherent rhythmicity. LCRs grow as diastole proceeds, in number, size, and signal mass.[128] The Ca^{2+} that is produced in the form of these LCRs interacts critically with the membrane-bound electrogenic Na^+-Ca^{2+} exchanger (NCX, *Figure 7* middle panel), to generate inward current that increases diastolic depolarization rate, length of diastole, and therefore beating rate of the heart. The second phase of SR-derived Ca^{2+} release, involving *en masse* release of Ca^{2+} from the SR, known as the 'Ca^{2+} transient', is triggered by the action potential occurring when the diastolic membrane potential reaches a critical threshold. This resets the Ca^{2+} clock—the SR is completely depleted, and the Ca^{2+} clock starts to tick once more as the SR begins to refill.

3.2.1 Circadian rhythmicity, Ca^{2+} cycling, and the Ca^{2+} clock

There is little evidence linking the Ca^{2+} clock to circadian variation in cardiovascular parameters, with inferences being made from neuronal research. For example, cytosolic Ca^{2+},[129] and L-type Ca^{2+} current density[130] exhibit diurnal fluctuations in mammalian SCN neurons in the hypothalamus that, in the case of cytosolic Ca^{2+} fluctuations at least, are not sensitive to the presence or absence of an action potential.[131] As far as the heart is concerned, circadian type fluctuations have been demonstrated in the hearts of chicks[132] with respect to Ca^{2+} channel subunit and Ca^{2+} current densities. Further, time of day of isolation in adult rat left ventricular cardiomyocytes plays a role in their Ca^{2+} transient characteristics, related to differences in intracellular Ca^{2+} and SR Ca^{2+} load during the so-called 'resting' and 'active' periods (higher diastolic and systolic intracellular calcium, elevated peak Ca^{2+} release and more rapid relaxation in cells isolated during the resting period).[34] There was also a significant difference in L-type Ca^{2+} current measured in response to isoprenaline depending on the time of day of isolation (higher L-type Ca^{2+} current during the active period), despite evidence from elsewhere documenting higher gene and protein expression of the voltage gated Ca^{2+} channel subunit α1D.[133] Ventricular myocytes isolated during the active period were less sensitive to isoprenaline induced 'arrhythmia'.[34] This article was also the first to demonstrate diurnal variation in excitation: contraction coupling in ventricular myocytes.

Left ventricular myocytes from guinea pigs demonstrate circadian rhythmicity in expression of the alpha subunit of L-type Ca^{2+} channels, CACNA1C, associated with increases in action potential duration during periods of highest expression.[134] Overexpression of CLOCK:BMAL1 in these cells significantly reduced the levels of CACNA1C, suggesting that the molecular clock has a fundamental role to play in the expression of

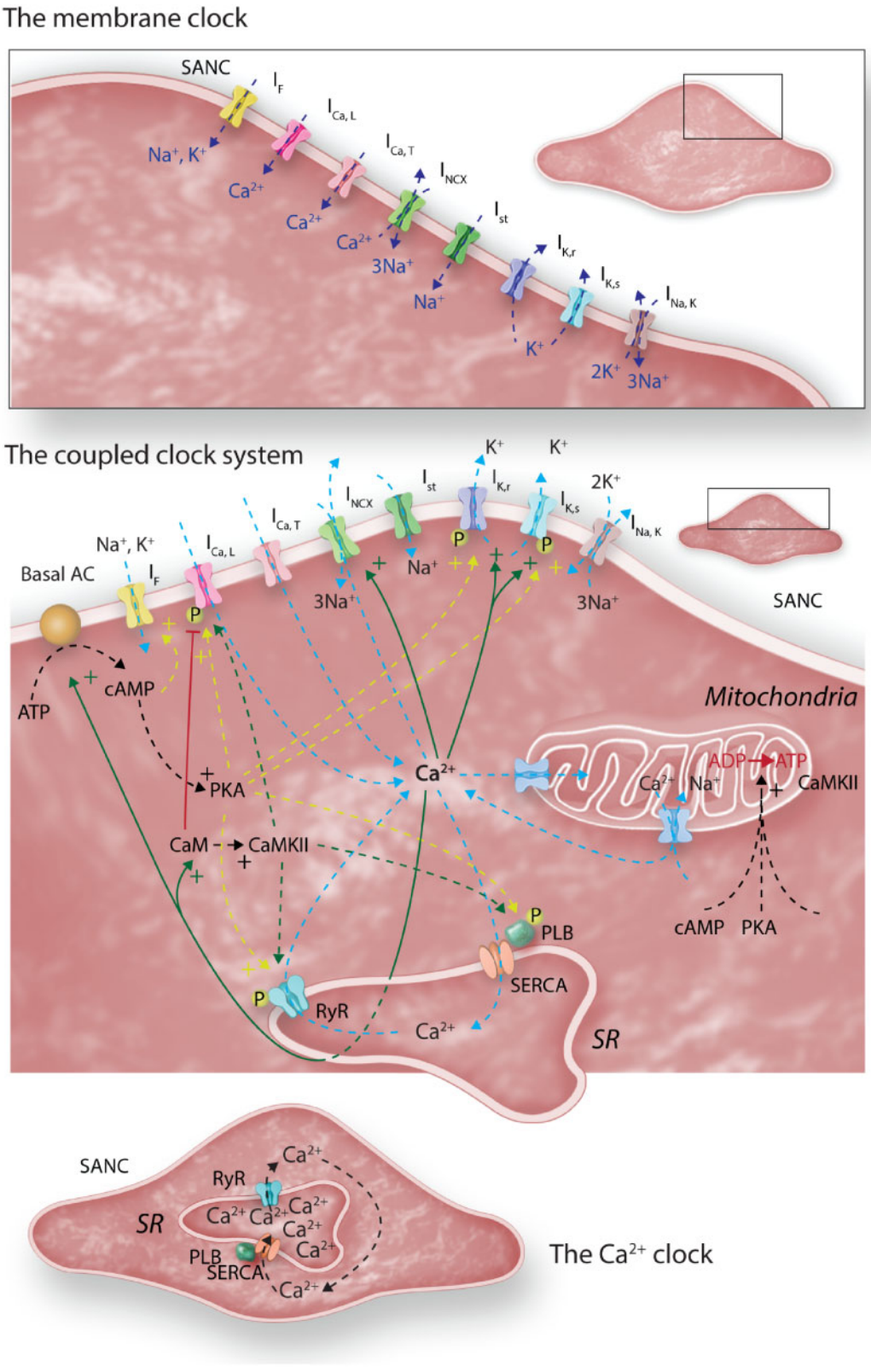

Figure 7 Adapted from Ref.[222]; schematic figure of the coupled clock system. The upper inset shows the membrane clock of sarcolemmal ion channels in isolation. The lower inset shows the intracellular Ca^{2+} clock in isolation. When put together, as in life, and as shown in the main central panel of the figure, it is clear that there are multiple areas of crossover between the two clocks, which are facilitated by the so-called 'nodes' of Ca^{2+} and phosphorylation. A full description of the coupled clock system is given in the text. The role played by mitochondria in the couple clock system remains poorly understood, though it is known that they are involved in intracellular Na^+ and Ca^{2+} homeostasis. Whether fluctuations in mitochondrial uptake and release of these crucial ions influence the coupled clock system is presently unclear.

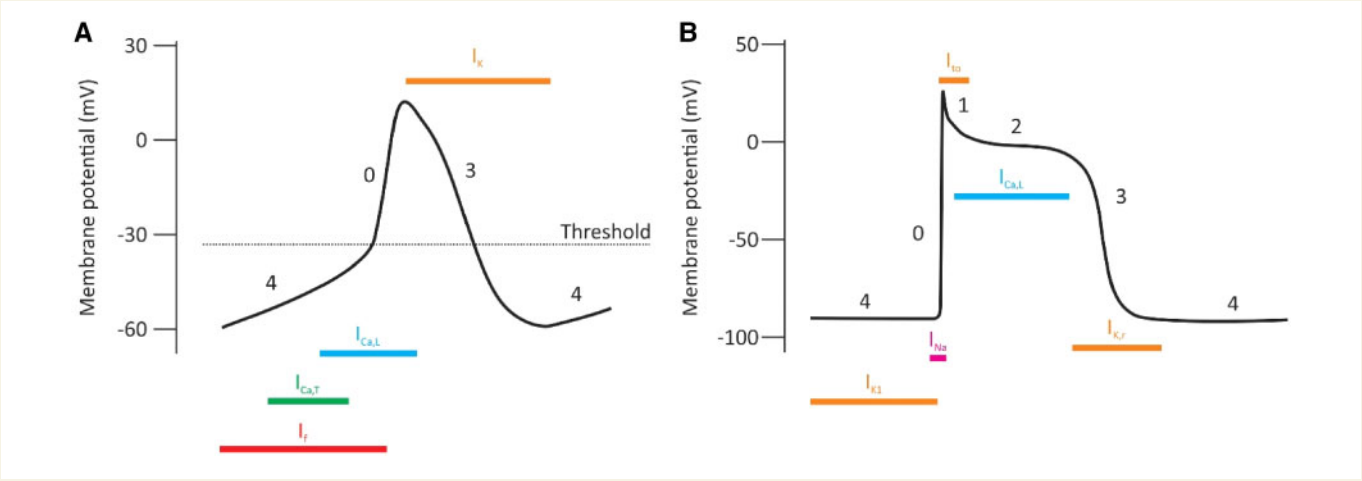

Figure 8 Ionic current density from ion channels varies at different times during the action potential in both the sinoatrial node (*A*) and the working ventricular myocyte (*B*) due to voltage- and time-dependence.

the L-type Ca^{2+} current, and disrupting it may affect action potential duration in a manner that is likely to be pro-arrhythmic.

3.3 HRV reflects rhythmical complexity in the heart beat

It is possible to dissect out rhythms in the beating of the heart to reveal complex variability existing on a beat-to-beat basis—'HRV' (see *Figure 9*). Historically, HRV is almost entirely ascribed to behaviour of the ANS, while the modern view is that it is likely significantly contributed to by the ultradian rhythms in the behaviour of components of the membrane- and Ca^{2+}-clocks described above.

Many parameters for objectively quantifying HRV exist (see *Table 1*).[65] The need for so many descriptors emphasizes the complexity of variability contained within the HR signal as a whole, and also the fact that no single parameter is capable of comprehensively quantifying HRV. Certain parameters of HRV selectively reflect sympathetic and parasympathetic components of innervation to the heart, and sympathovagal balance.[65] Although this is debated,[69] phosphorylation changes effected by sympathetic and parasympathetic signalling contribute substantially to ultradian rhythmical variation in HR. For example, sympathetic signalling not only quickens HR, but also decreases indices of ultradian variability.[135,136] Contrastingly, parasympathetic stimulation slows HR and increases ultradian measures of HRV.[137,138] Parameters of HRV are capable of predicting and detecting morbidity and mortality in a wide variety of cardiovascular and non-cardiovascular conditions, and even in healthy populations.[65] HRV initially increases from birth to early adulthood,[139,140] but from early adulthood it decreases,[140–150] as does the complexity of cardiovascular dynamics,[150–152] possibly related to the recognized age-associated decline in autonomic agonist sensitivity.[153–156] HRV parameters vary in a circadian fashion (*Figure 6*).[87,157] Across most parameters, HRV increases during the night and decreases during the day.[61,157] Indirect HRV-based measures of sympathovagal balance have been legitimized by direct measures of plasma catecholamine levels (see *Figure 4*).[158] The effect of vigilance state transitions on HRV parameters also varies dependent on the time of day,[87] with sleep-to-wake transitions that occur in the morning being most associated with increased sympathetic nervous activity compared with similar transitions at other times of the day. This suggests that both behaviour and circadian processes interact to affect the autonomic modulation of the heart in a complex way to produce optimal cardiovascular responses to vigilance state transitions. The presence and severity of cardiovascular disease also has an effect on the circadian periodicity of HRV—for example, the presence and severity of angina destabilizes circadian rhythms in HRV,[159] causing phase shifts in the nadirs and acrophases of several HRV parameters.[160] Similarly, ischaemic stroke reversibly abolishes circadian fluctuations in HRV, with loss of the vagal nocturnal dominance of HRV, reverting to normal after around 6 months of recovery.[161]

4. The clinical importance of rhythmicity

Evidence of the importance of maintaining healthy ultra-, infra-, and circadian rhythms is apparent throughout cardiovascular research. Starting with the most easily measurable ultradian cardiovascular rhythm, the beating rate of the heart, it is known across a variety of organisms that low resting HR equates to longer life span.[162] Disruption of normal circadian rhythms increases cardiovascular disease risk. Such disruption is a modern day epidemic—night shifts, travelling across time zones, ubiquitous electrical lighting, and sleep disorders[163] are all increasingly prevalent with time as our lifestyle changes, and all uncouple our lives from our circadian clock, at a substantial cost. For example, humans working night shifts are more prone to heart disease, having a 40% increase in coronary heart disease risk and risk of cardiac events compared with non-night shift workers.[164–167] This has led to the suggestion of prophylactic anti-platelet medication use in night shift workers.[112] This increase in risk has significant public health ramifications, given that around 28% of the Western workforce operates outside of the conventional working day.[168] Deliberate circadian misalignment, such as that which occurs when study subjects are made to eat and sleep 12 h out of phase, leads not only to complete inversion of the cortisol profile described above, but also increases postprandial glucose (to pre-diabetic levels in some previously healthy individuals) despite increasing insulin levels, increases mean arterial pressure, and decreases plasma leptin and sleep efficiency.[89]

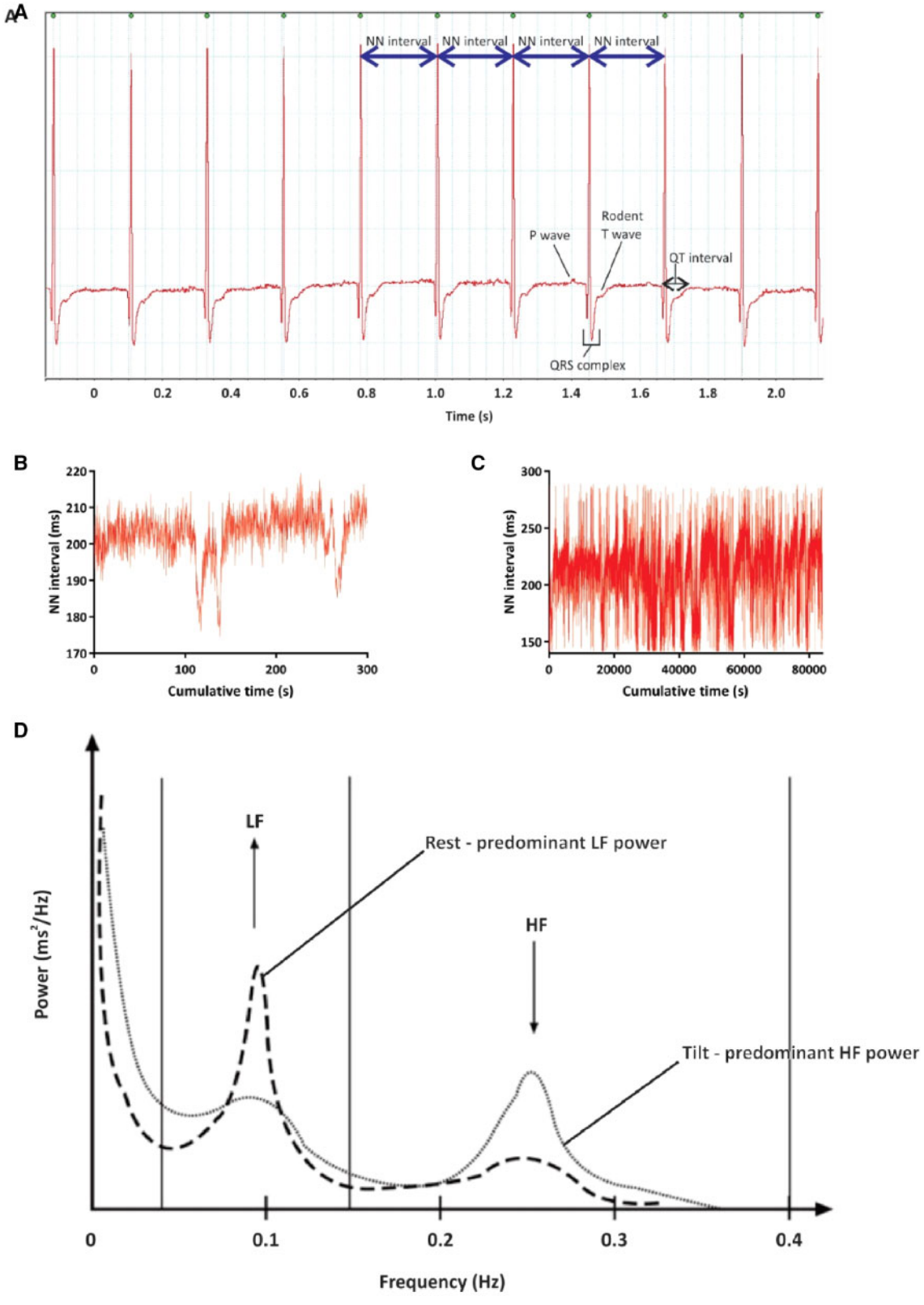

Figure 9 HRV in recordings of spontaneous ECG data from *in vivo* rats. (*A*) HRV data are calculated from consecutive normal-to-normal (NN) intervals, usually being measured at the peak of the R-wave of the ECG. Although not obvious to the naked eye, when consecutive NN intervals are plotted on a graph of cumulative time (a so-called tachogram, shown in *B* and *C*) there is marked variation over both the short and long term. (*B*) Tachogram of spontaneous NN intervals over 5 min in a rat. (*C*) Tachogram of spontaneous NN intervals over 24 h in the same rat as shown in panel (*B*). (*D*) Reproduced and modified with permission from Gunther *et al.*[224]: typical power spectrum from frequency domain analysis of human short-term HRV. Note especially the peaks localized to the low and high frequency bands, and how they vary between rest and in this case tilt testing. The frequency bands are dependent on the mean HR of the organism being investigated and should be altered based on the mean HR of the organism being studied.

Table 1 Different parameters used to objectively assess HRV across multiple domains

Time domain	Frequency domain	Non-linear domain
Standard deviation (SDNN)	Total power (TP)	Entropy (approximate, multi-scale, etc.)
Standard deviation of segment averages (SDANN)	High frequency (HF) domain power	$\alpha 1$, $\alpha 2$ (short and long-term fractal exponents)
Root mean square of successive differences (RMSSD)	Low frequency (LF) domain power	Beta slope
Coefficient of variation (CoV)	Very low frequency (VLF) domain power	SD1 and SD2 of Poincare plot
Standard error (STENN)	Ultra low frequency (ULF) domain power	Detrended fluctuation analysis (DFA)
SDNN:SDSD	LF:HF ratio	Hurst exponent
Range NN		
Number of intervals differing by a given number (NNx)		
Proportion of all intervals differing by a given number (pNNx)		

This list is not exhaustive, and, for example, the frequency domain parameters can be expressed in a variety of ways, including raw units, or normalized to the total power, or normalized to the total power minus the VLF/ULF band power.

Alterations of the circadian rhythm in HR also predict cardiovascular morbidity and mortality. For example, with respect to peripheral and coronary atherosclerosis, higher levels of diurnal HR variation predict greater atherosclerosis.[169] Significant circadian variations in the incidence of cardiac arrhythmias and sudden cardiac death exist in almost all acquired and hereditary forms of heart disease.[109,170] This is likely to be at least in part due to circadian variation in conduction and refractoriness throughout the heart. In general, circadian rhythms in conduction are attenuated at night (manifest by significant prolongation of effective refractoriness), while conduction is enhanced during daylight hours (with associated shortening of the effective refractory period).[61,171–173] Sinus node recovery time (the length of time taken for the sinus node to recommence spontaneous beating following a period of fast pacing) in patients with sick sinus syndrome also exhibits a circadian rhythm, with an acrophase between midnight and 7 am.[174] ECG manifestations of repolarization, specifically the QT interval (shown in *Figure 9*), demonstrate diurnal variation in a variety of species, including mice.[19,175] Destruction of the hypothalamic SCN, or use of ANS blockers leads to a loss of this circadian QT variation in mice. Such loss of circadian QT rhythm leads to an enhanced susceptibility to ventricular arrhythmias.[125] Similarly, QT dispersion, an infrequently used ECG parameter that describes beat-to-beat heterogeneity in ventricular repolarization (high QT dispersion being associated with greater risk of sudden cardiac death)[176] shows circadian variation, being greatest in the morning hours, both in normal humans and those with coronary heart disease. Ventricular late potentials are considered to be indicators of risk for malignant arrhythmias of the heart,[177] and in patients post-MI with a history of ventricular fibrillation, late potentials exhibit circadian rhythmicity,[178] being localized to the morning hours between 6 and 12 am. Late potentials do not exhibit such rhythmicity in people post-MI with a history of less severe arrhythmia or with no history of arrhythmia at all. Such a phenomenon may plausibly be used to risk stratify post-MI patients. Like QT dispersion and ventricular late potentials, T-wave alternans is an ECG marker of malignant arrhythmia risk, describing beat-to-beat variability in amplitude/morphology/polarity of the T wave. It too exhibits circadian periodicity, with a higher density during morning hours.[179] Circadian and ultradian rhythms also exist in the occurrence of premature atrial and ventricular ectopic beats, which can be associated with the development of arrhythmia and cardiomyopathy.[60] It is possible that underlying this are circadian variations in the expression of ion channels (described above), or in the physiological responsiveness of heart cells to autonomic signalling via the multi-synaptic pathways leading from the brain (cells in the SCN of the hypothalamus) to the end effectors (the cells of the SAN and working cardiomyocytes of the atria and ventricles).

Circadian rhythms also exist in the incidence of other cardiovascular events, not only arrhythmias, including the onset of heart failure and MI,[180–184] with morning hours being the time of greatest risk.[87] For example, the hours between 6 am and midday impart a 40% increased risk of MI and a 29% increased risk of sudden cardiac death in diurnally active individuals.[184] The reasons underlying this remain unclear, yet time-of-day variation in the incidence and severity of MI, sudden cardiac death, ventricular arrhythmia and stroke have been shown repeatedly, and are concisely summarized in Ref.[185] Basic science reinforces this message, with the molecular pathways that are activated in mice in response to coronary artery ligation differing depending on the time of day that this occurs,[186] and such differences are likely to affect the organ response to this insult, including favourable or unfavourable remodelling, and survivorship. Furthermore, disturbing circadian rhythms and sleep in the days following MI in a mouse interferes with the orderly cascade of humoural and cellular healing that is necessary, and leads to a worse long-term outcome.[32] This is especially worrying given the known detrimental effect on sleep and circadian rhythm that is known to occur in human patients when they are hospitalized.[187,188]

While the above evidence suggests that the disease incidence/prevalence can be affected by the hearts clock, vice versa the hearts clock can be affected by a variety of disease states, including pressure overload,[20,23] hypertension,[23,189] diabetes mellitus,[190,191] obesity,[190] coronary artery ligation,[192] simulated shift work,[193] and ageing.

Deliberate circadian misalignment and its consequences is an active field of study—for example, it is possible to mutate one allele of the protein tau (casein kinase 1ϵ) in hamsters to cause their circadian clock to shorten from 24 to 22 h.[194] When these hamsters are housed in a 12:12 h light:dark environment (dyssynchronization between circadian clock and environment) they demonstrate an excess of age-dependent cardiovascular disease, developing a marked cardiomyopathy, extensive myocardial fibrosis, and severely impaired contractility. When the environment is switched to an 11:11 h light:dark environment (resynchronization of circadian clock and environment) the excess cardiovascular disease disappears.[36]

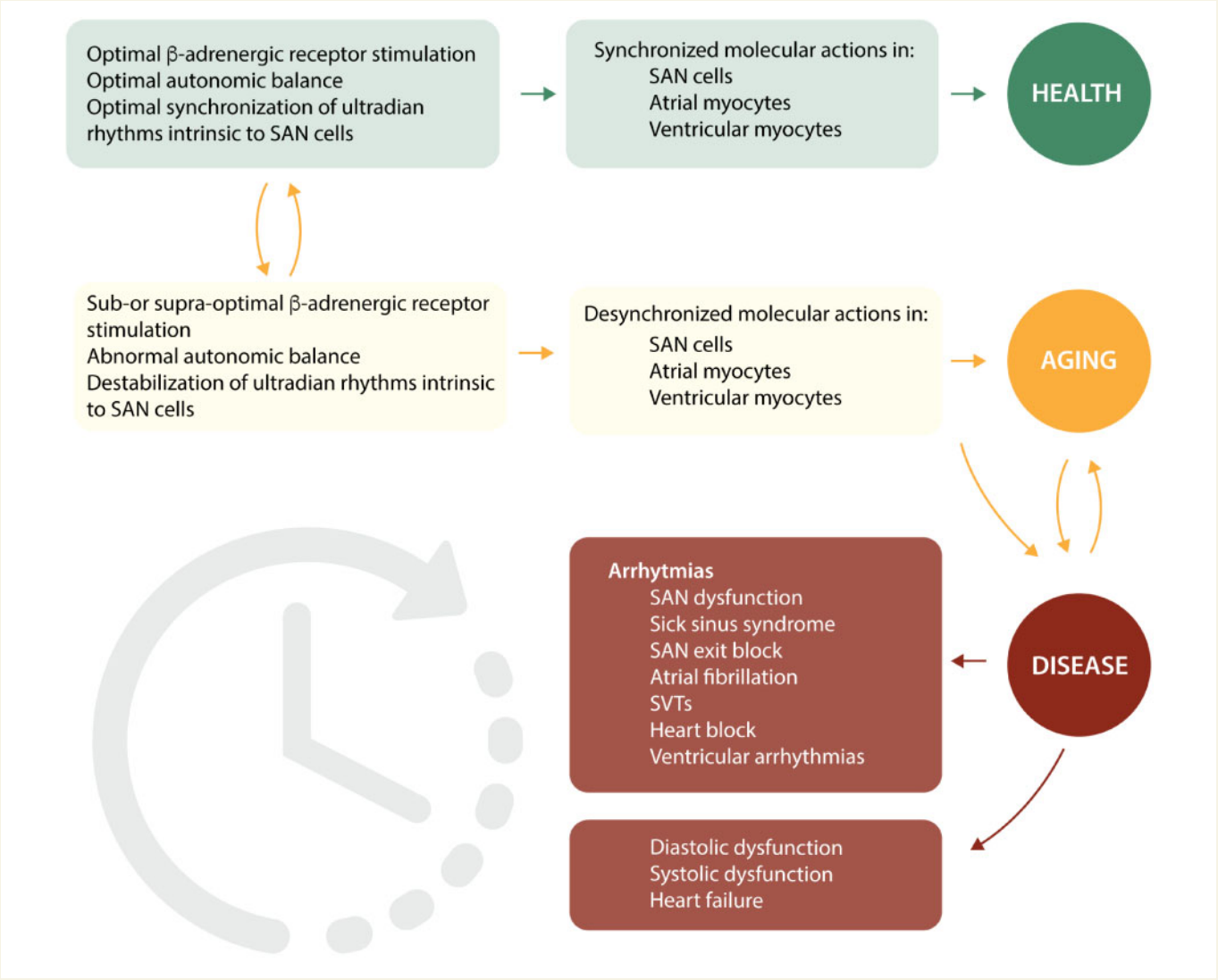

Figure 10 A schema depicting potential links between the extent of synchronization of molecular actions. RyR activation and Ca^{2+} release, to cardiac function and structure in health and disease'. Synchronization within and between cells of the heart is crucial for optimal chronotropism and inotropism of the heart. If this becomes suboptimal or disordered, then the clinical outcome is of arrhythmias and heart failure. ECG panel shows the irregularly irregular rhythm typical of atrial fibrillation, while the echocardiogram snapshot shows an enlarged dilated left ventricle with thinned walls typical of severe heart failure. Reproduced and modified with permission from Ref.[205]

5. Ageing and cardiovascular circadian rhythms

Ageing influences cardiovascular circadian rhythms. For example, it decreases the body's ability to respond to perturbation in the circadian clock, decreasing the speed of adaptation to night working,[195,196] which even in the young only occurs at a rate of 1 h per day.[197] Ageing significantly reduces the amplitude and phase of circadian rhythms in both HR and BP,[198,199] possibly via age-dependent changes in responsiveness to cardiac autonomic signalling, although circadian variations in plasma catecholamine levels do continue to occur, irrespective of age.[158] While epinephrine levels are not different with ageing, norepinephrine levels are, being 28% higher during the day and 75% higher at night in older individuals, possibly contributing to the insomnia of ageing.[158] It may be that age-associated changes in intrinsic clock mechanisms within SAN cells are responsible for the age-blunted circadian rhythms in HR—evidence to support this includes age-associated decrease of beating interval variability in mice, suggesting both imbalance in autonomic neural input to the heart and altered SAN cell responses to neural input.[200]

It would appear that, unlike the situation with HR, circadian variation of HRV is maintained in older people, although the extremes of day–night differences are smaller than in young individuals,[144] and the effect of ageing on circadian changes in HRV is different in the two sexes.[201,202] Elsewhere, however, it has been shown that there is a decrease in the total power of the power spectrum of 24 h circadian rhythm in very elderly patients, with a shift in the circadian acrophase to a later time point.[203]

Ageing is one of the major risk factors for cardiovascular disease, and it is recognized to decrease the amplitude of many circadian rhythms, and increase the tendency towards 'internal desynchronization',[165]

which can manifest clinically as heart failure. Perturbing the circadian system, for example by mutating the *Clock* gene in mice (specifically *Clock*$^{\Delta 19/\Delta 19}$ that results in the production of a protein incapable of transcriptional activation), leads to the age-dependent development of a heart failure phenotype, with cardiac dilatation, cardiomyocyte hypertrophy, interstitial fibrosis, and maladaptive remodelling, with eventual development of systolic dysfunction (decreased fractional shortening and decreased ejection fraction).[185] This suggests that maintaining circadian 'health' is critical in the avoidance of cardiovascular morbidity as we age—in the case of *Clock*, this occurs through an effect on the PTEN-AKT signalling pathway, known to be crucial for cardiac growth and renewal,[185] and such work raises the intriguing possibility of specifically targeting the circadian mechanism as a novel therapeutic approach to the management of cardiovascular disease. Notably, female mice with the same *Clock*$^{\Delta 19/\Delta 19}$ mutation did not develop the heart failure phenotype unless they were ovariectomized, suggesting that female hormones protect the heart in this model of accelerated cardiac ageing due to clock gene mutation.[204]

6. Conclusions and future directions

Rhythmical variations in HR and other cardiovascular parameters represent a basic quality of human physiology. The molecular mechanisms that control these rhythms exist at multiple levels of both chronicity and location (from single molecules, all the way up to cells, tissues, and organs). Variation in gene transcription for critical molecules involved in the coupled clock system is rhythmical at the level of hours and days, whereas endocrine modulation of HR, especially that involving the renin-angiotensin-aldosterone system, occurs over the order of minutes to hours. Similarly, autonomic sympathetic rhythmical control of HR occurs over minutes, whereas autonomic parasympathetic control of HR occurs over seconds. Finally, rhythms involving the behaviour of molecules involved in SR Ca^{2+} cycling, or in ion channel activation and inactivation, or in mitochondrial ATP production occur over the order of milliseconds or faster. The rhythms are caused by a variety of factors that are both extrinsic and intrinsic to the heart/SAN cell itself. Extrinsic autonomic rhythmicity has traditionally been believed to be the major player responsible for this rhythmicity, but an increasing body of evidence suggests that this extrinsic rhythmicity has to interact with highly complex intrinsic rhythmicity detailed painstakingly above exhibited by the cells of the different areas of the heart on multiple levels, which is further complicated because it changes in the presence of ageing and disease.

Synchronization of both extrinsic and intrinsic rhythms is crucial for optimal functioning of the heart,[205] and represents one important way in which the full chronotropic repertoire of the heart is achieved. Desynchronization of rhythms leads to pathological phenotypes, including cardiac arrhythmias, and also because a similar set of molecules are involved in atrial and ventricular myocytes as in SAN cells, desynchronization here can lead to the heart failure phenotype (see *Figure 10*).[205]

Future progress is required to turn what we have learned about circadian rhythms affecting the heart into therapeutic applications, focusing on restoring or maintaining these rhythms, and the effect of doing so on restoring and maintaining health. Most interventions, predominantly pharmacological, are presently given to have an effect *throughout* any given 24 h period, effectively neglecting the evolutionary significance of circadian rhythmicity. There is however some reasonable suggestion that targeting interventions to certain times of the day would maximize their benefit,[206–208] while leaving other periods in the day 'free' from substantial intervention, where the likely beneficial effect of the intervention is less substantial.[185] Examples include preferential timing of angiotensin-converting enzyme (ACE) inhibitor therapy (sleep-time dosing of ACE inhibitors in mice with pressure overload hypertrophy preferentially mitigated against cardiac remodelling, whereas dosing this medicine during wake time had no discernibly different effect than placebo, a difference reflecting the diurnal rhythmicity of the renin-angiotensin-aldosterone system),[209] evening administration of anti-hypertensives for non-dippers,[210] nocturnal aspirin to prevent morning thrombo-occlusive events in the cardio- and cerebrovascular systems,[211] nocturnal haemodialysis to cause regression of left ventricular hypertrophy,[212] and nocturnal continuous positive airways pressure for patients with obstructive sleep apnoea.[213] The design of true 'chronotherapeutic oral drug absorption systems' has been reported (e.g. Ref.[214]) and their use has shown some promise in preferentially targeting high risk times of day, although uptake into clinical practice has been limited, and the field of 'chronomics' is underexplored.[215,216] Working to simply maintain normal circadian rhythms by preventing sleep disturbance and excessive exposure to electric lighting during admission to hospital would seem to be one relatively easy way to prevent harm from circadian dysregulation, and work is ongoing in this field.[217–219]

Certainly, more detailed studies are required into chronomic therapy, and into the exciting field of cardiovascular rhythms in general, including the important differences that are beginning to be uncovered between males and females,[220] so that novel approaches to therapy may be developed to tackle the epidemic of cardiovascular diseases worldwide.[221]

Conflict of interest: none declared.

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