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REVIEW

What keeps us ticking? Sinoatrial node mechano-sensitivity: the grandfather clock of cardiac rhythm

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Abstract

The rhythmic and spontaneously generated electrical excitation that triggers the heartbeat originates in the sinoatrial node (SAN). SAN automaticity has been thoroughly investigated, which has uncovered fundamental mechanisms involved in cardiac pacemaking that are generally categorised into two interacting and overlapping systems: the 'membrane' and 'Ca^{2+} clock'. The principal focus of research has been on these two systems of oscillators, which have been studied primarily in single cells and isolated tissue, experimental preparations that do not consider mechanical factors present in the whole heart. SAN mechano-sensitivity has long been known to be a contributor to SAN pacemaking—both as a driver and regulator of automaticity—but its essential nature has been underappreciated. In this review, following a description of the traditional 'clocks' of SAN automaticity, we describe mechanisms of SAN mechano-sensitivity and its vital role for SAN function, making the argument that the 'mechanics oscillator' is, in fact, the 'grandfather clock' of cardiac rhythm.

Keywords Mechano-electric coupling · Stretch · Pacemaking · Calcium clock · Membrane clock · Heart rate

Introduction

Perhaps not surprising for such a critical element to life, the heart can independently maintain its function. Even when removed from the body, it continues to beat and intrinsically regulate its activity. This is possible as the electrical excitation that initiates the heartbeat is produced within the organ itself (which, incidentally, is one of the principal reasons that heart transplantation is also possible). The heart's intrinsic pacemaker, the sinoatrial node (SAN), rhythmically generates action potentials (AP) that propagate through the myocardium, causing the heart to contract. The myogenic origin of cardiac excitation was first identified nearly 140 years ago by Walter Gaskell (Gaskell 1882), and its anatomical location within the heart a few decades later by Keith and Flack (Keith and Flack 1907). In more than a century since, an entire field of research investigating SAN automaticity has emerged, which has taught us much about what drives pacemaker function, regulation, and dysfunction. Yet, our understanding is far from complete, and fundamental questions remain unanswered.

Perhaps the most heavily studied—but still most highly contested—questions regarding the SAN relate to the mechanism(s) responsible for its automaticity: what keeps it ticking? There is still no consensus as to the relative importance of the various subcellular mechanisms involved in spontaneously generating the SAN AP (Lakatta and DiFrancesco 2009; Noble et al. 2010; Rosen et al. 2012). Yet, despite contradictory perspectives regarding the importance of individual cellular components for SAN automaticity, there is now general agreement that SAN pacemaking consists of a robust, dynamic, and flexible system characterised by multiple integrated subsystems and contributors.

The SAN AP differs from the AP of working cardiomyocytes in multiple ways, the most important for automaticity being the period of spontaneous diastolic depolarisation (SDD, rather than diastolic rest), during which membrane potential gradually depolarises from its most negative value (maximum diastolic potential, MDP) towards the threshold for AP generation (Bartos et al. 2015). The slope of SDD is the key to determining the frequency of SAN firing,

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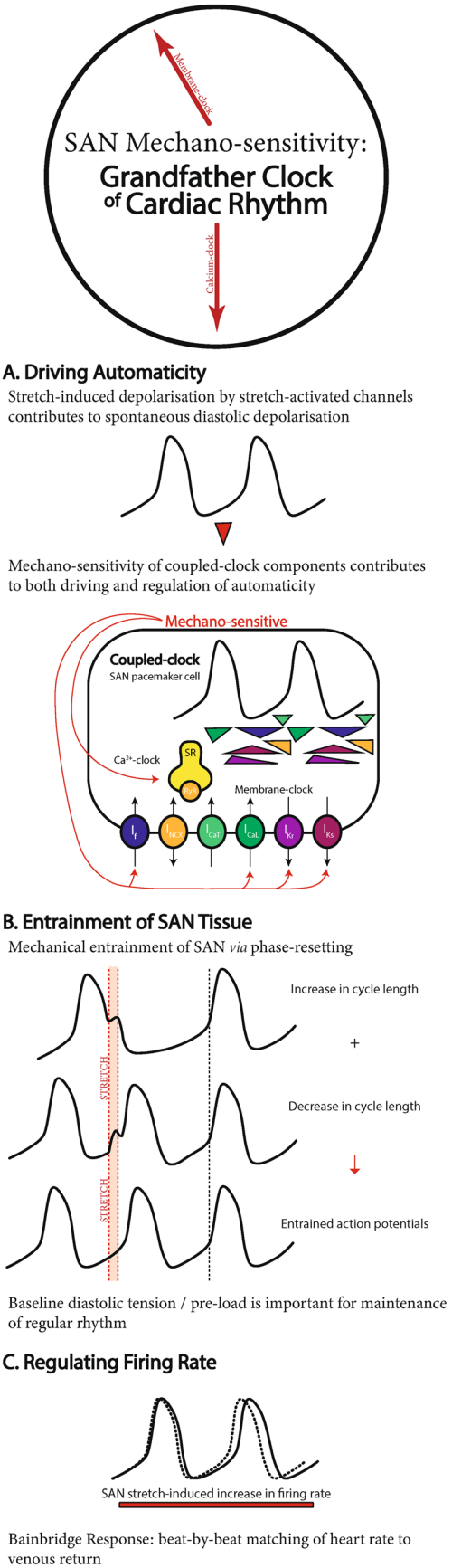

Fig. 1 The grandfather clock of cardiac rhythm. Summary of the role of mechano-sensitivity in sinoatrial node (SAN) **A** automaticity, **B** entrainment, and **C** regulation. For expanded figures of the coupled-clock system, please refer to Lakatta et al. (2010), Quinn and Kohl (2012), and Bartos et al. (2015)

and thus heart rate (Mangoni and Nargeot 2008). Two predominant systems contributing to SDD have been identified and extensively studied: the so-called 'membrane clock' (consisting of sarcolemmal ionic currents) and the 'calcium (Ca^{2+}) clock' (comprising intracellular Ca^{2+} cycling) (Lakatta et al. 2008; Difrancesco 2010), which are mutually entrained to form a system of coupled oscillators capable of generating SAN automaticity (Lakatta et al. 2010).

This understanding of pacemaker function, however, has been developed based largely on investigations of mechanisms in isolated cells (and to a lesser degree, isolated tissue), which neglects factors acting in the whole heart. There are various important extracardiac neurohumoral factors that influence heart rate by acting directly on mechanisms of SAN automaticity, including those released locally by the autonomic nervous system and those released into the bloodstream by the endocrine system (MacDonald et al. 2020b). There are also intracardiac factors that acutely affect SAN function, perhaps the most well established being stretch, which is a major determinant of in vivo heart rate (Quinn and Kohl 2012).

In this review, after outlining the principal components of the two classical 'clocks' of SAN automaticity and their mutual entrainment, we briefly summarise the primary mechanisms of SAN mechano-sensitivity and the critical contribution of SAN stretch to pacemaking, making an argument for its role as the 'grandfather clock' of cardiac rhythm (Fig. 1).

The classical understanding of cardiac pacemaking

Membrane clock

During early SDD, the membrane clock is driven by the "funny" current (I_f), an inward cation current that becomes increasingly activated as membrane potential becomes more negative (Bartos et al. 2015). It is passed through hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels (Difrancesco 2010), of which there are four isoforms. HCN isoforms 1, 2, and 4 are expressed throughout the human heart and more prominently in the SAN than the atria, particularly HCN1, which in humans is almost exclusively expressed in the SAN (Li et al. 2015). The vital importance of HCN channels for pacemaking has been corroborated in HCN knockout mice, which display the hallmarks of SAN dysfunction,

including prolonged recovery, prolonged conduction time, bradycardia, sinus dysrhythmia, and recurrent sinus pauses (Fenske et al. 2013; DiFrancesco et al. 2021).

Although some have asserted that I_f is the predominant driver of SDD and the primary pacemaking mechanism (Difrancesco and Noble 2012), inward transmembrane Ca^{2+} currents also contribute to SDD. They are passed through both transient (T-type) and long-lasting (L-type) Ca^{2+} channels (Mangoni and Nargeot 2008). $Ca_v3.1$ T-type and $Ca_v1.3$ L-type Ca^{2+} channels contribute to the early portion of SDD, as they become activated at a relatively low membrane potential (Mangoni et al. 2003). SDD ends at the threshold for $Ca_v1.2$ L-type Ca^{2+} channels (approximately −40 mV), at which point their activation generates the upstroke of the SAN AP (Mesirca et al. 2015). Although fast sodium (Na^+) channels do not trigger the upstroke in SAN cells (as they do in working myocytes), they are heterogeneously expressed at low levels throughout the SAN and appear to make some contribution to automaticity (Lei et al. 2005).

Repolarising currents are also fundamental to the membrane clock's contribution to pacemaking; in fact, prior to the identification of I_f, their decay at the end of the SAN AP was thought by many to be the main driver of SAN automaticity. The rapid and slow delayed rectifier potassium (K^+) currents (I_{Kr} and I_{Ks}) repolarise SAN myocytes to their MDP, but at the same time, their total current is continuously reduced. This repolarisation allows for a simultaneous increase in the activation of I_f, driving depolarisation (Mangoni and Nargeot 2008). Importantly, this depolarisation is not prevented by inwardly rectifying K^+ channels (which stabilise and maintain the negative resting membrane potential of working cardiomyocytes), as those channels are minimally expressed or absent in SAN myocytes (Bartos et al. 2015).

So, overall, the balance of depolarising inward and repolarising outward membrane clock currents is one of the main determinants of SDD slope and largely responsible for the oscillations that drive SAN AP firing, which ultimately establishes heart rate.

Ca^{2+} clock

Intracellular Ca^{2+} cycling has also been shown to be a major contributor to SDD and SAN automaticity (Bartos et al. 2015). Local Ca^{2+} releases (LCR) from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyR) result in an increase in cytosolic Ca^{2+} concentration. Some of this Ca^{2+} is returned to the SR by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), while the remainder is extruded from the cell by the Na^+-Ca^{2+} exchanger (NCX)—with 1 Ca^{2+} ion exiting for 3 Na^+ ions entering—which generates an electrogenic, depolarising current (Lakatta et al. 2008, 2010). Unlike Ca^{2+} sparks released from the SR at rest in working cardiomyocytes, LCR during SDD in the SAN are rhythmic, larger in amplitude, and longer in duration (Sirenko et al. 2013). This may be partly explained by the fact that while LCR were originally thought to be spontaneous, they are actually, at least in part, triggered by Ca^{2+} influx via $Ca_v1.3$ L-type Ca^{2+} channels (Torrente et al. 2016). The importance of diastolic intracellular Ca^{2+} cycling in SAN myocytes is further enhanced by the fact that they have higher basal cyclic adenosine monophosphate (cAMP) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) levels than working cardiomyocytes, which results in greater phosphorylation of Ca^{2+} handling proteins (L-type Ca^{2+} channels, RyR, and phospholamban) and an increase in their activity (Vinogradova et al. 2000, 2006).

Overall, the rate and amplitude of intracellular LCR, and the balance between Ca^{2+} reuptake into the SR by SERCA and extrusion via NCX, are important determinants of SDD slope, and thus of heart rate (Vinogradova and Lakatta 2009).

Coupled-oscillator system

The combined actions of the membrane and Ca^{2+} clocks form a robust and redundant system for SAN automaticity. These individually defined 'clocks' are tightly coupled, as the action of one influences the other (Bogdanov et al. 2006; Mattick et al. 2007; Lakatta et al. 2008). Changes in membrane potential driven by the membrane clock influence intracellular Ca^{2+} balance, while LCR, as part of the Ca^{2+} clock, activate NCX, which is located in the cell membrane and directly alters its potential. The activity of the two clocks is further coordinated through entrainment by mutual intracellular regulatory mechanisms (MacDonald et al. 2020b). In fact, the clocks are so tightly coupled and interdependent one must question whether it is even productive or beneficial to distinguish between them; the oscillatory nature of the SAN is the result of the combined activity of the various components of the membrane and Ca^{2+} clocks, even though none alone are independently oscillatory. None of I_f, trans-sarcolemmal Ca^{2+} or Na^+ flux, activation of NCX by LCR, or the decay of I_K can independently produce the rhythmic membrane potential oscillations that result in SAN automaticity. Also, the SAN continues to fire even with the loss of individual clock components, indicating a protective redundancy. Therefore, the system driving SAN automaticity is best thought of as a system of coupled oscillators (rather than individual 'clocks').

It is important to recognise, however, that the activity of an individual pacemaker cell in well coupled SAN tissue will not be able to excite the entire node on its own. Thus, not only are the cellular contributors to automaticity within SAN cells mutually entrained but so must be the activity of individual cells within SAN tissue, resulting in their synchronous excitation (Jalife 1984). One mechanism by which this tissue-level entrainment of cellular activity may occur is through a cyclic stretch of the SAN, as the right atrium fills with blood during each heartbeat, which directly influences cell-level

automaticity due to the inherent mechano-sensitivity of SAN myocytes (Quinn and Kohl 2012, 2021). In fact, as described below, SAN mechano-sensitivity might itself be considered a 'mechanics clock' (or better a 'mechanics oscillator'), as stretch effects are a key driver—and perhaps a master controller—of cardiac pacemaking.

The contribution of SAN stretch to cardiac pacemaking

The physiological importance of stretch effects on heart rate

The direct effects of stretch on SAN activity were first established by Starzinsky and von Bezold, who showed in rabbits with severed connections between the heart and the autonomic nervous system that an increase in venous return caused sinus tachycardia (Starzinsky and von Bezold 1867). More generally known, however, is the work of Francis Bainbridge, who demonstrated that right atrial distention by intravenous fluid injection caused an acute increase in heart rate in anaesthetised dogs (Bainbridge 1915). This response was later corroborated in humans by passively lifting the legs of healthy subjects to increase venous return to the heart—without a simultaneous change in arterial pressure—which similarly increased heart rate (Roddie et al. 1957; Donald and Shepherd 1978). This effect is now commonly known as the 'Bainbridge response'. An acute increase in heart rate or SAN beating rate in response to sustained atrial or SAN stretch has been shown to also occur in a multitude of experimental animals across the invertebrate (Sénatore et al. 2010) and vertebrate (Pathak 1973) phyla, and most recently in zebrafish (MacDonald et al. 2017). Interestingly, this is not the case in the mouse SAN, however, where beating rate tends to decrease with sustained stretch (Cooper and Kohl 2005). This species difference in the heart rate response to stretch can be explained by the relation of the reversal potential of the stretch-activated channels involved (discussed further below) to the species-specific action potential morphology (Cooper and Kohl 2005; MacDonald et al. 2020a); however, that explanation is yet to be experimentally verified. Regardless, the evolutionary conservation of the heart rate response to stretch demonstrates the fundamental nature of stretch effects on SAN automaticity. While originally considered to be an extracardiac, neurohumorally mediated effect, an increase in beating rate with stretch is observed not only in intact animals but also in isolated hearts and right atrial tissue (Blinks 1956), the SAN (Deck 1964), and single SAN cells (Cooper et al. 2000) (Fig. 2), indicating that intracardiac mechanisms are key contributors. For a comprehensive summary of the clinically and experimentally observed effects of stretch on SAN function, please see (Quinn and Kohl 2012, 2021).

The acute increase in SAN automaticity that occurs with distension of the right atrium (the Bainbridge response) is a critical regulator of heart rate, which along with stretch-induced changes in ventricular stroke volume via the Frank–Starling mechanism allows the heart to match cardiac output (= heart rate × stroke volume) to changes in venous return on a beat-by-beat basis (Quinn and Kohl 2016). The Bainbridge response also opposes the baroreceptor response (the Bezold–Jarisch or depressor reflex, which reduces heart rate when arterial blood pressure is increased, von Bezold and Hirt 1867; Jarisch and Richter 1939), thus preventing excessive bradycardia or overdistension of the right atrium, while helping to maintain cardiac output and adequate circulation during hemodynamic changes that increase both venous return and arterial pressure. Thus, the increase in heart rate with SAN stretch is vital for maintaining balanced cardiovascular system performance, while also matching the throughput of the left and right sides of the heart over any period of time (Quinn and Kohl 2012; Quinn 2015). Being a fundamental autoregulatory mechanism of cardiac function, it is perhaps not surprising that the mechanisms of the SAN stretch response are not only intrinsic to the heart but in fact to SAN myocytes themselves (Cooper et al. 2000), reflecting their inherent mechano-sensitivity.

Mechanisms of SAN mechano-sensitivity

Although the cellular mechanisms of the SAN stretch response remain incompletely understood (Quinn and Kohl 2012, 2021), it is clear that they relate to acute feedback of the heart's mechanical status to its electrical activity, a process known as "mechano-electric feedback" or "mechano-electric coupling" (Quinn et al. 2014; Quinn and Kohl 2021). Clinical and experimental observations of the acute effects of SAN stretch can generally be explained by evoking a mechano-sensitive trans-sarcolemmal current with a reversal potential between the MDP and maximum systolic potential (MSP) of SAN myocytes. Cation non-selective stretch-activated channels (SAC_{NS}), with a reversal potential between −20 and 0 mV (Guharay and Sachs 1984) would pass such a current. In fact, a stretch of single SAN myocytes results in the activation of a current with a reversal potential of approximately −11 mV (Cooper et al. 2000) and its pharmacological block with *Grammostola spatulata* mechanotoxin-4 (GsMTx-4) reduces the increase in SAN beating rate seen with stretch (Cooper and Kohl 2005).

Nevertheless, the molecular identity of SAC_{NS} in the SAN has not yet been determined (Peyronnet et al. 2016) and one must be cautious not to fall into a 'plausibility trap' by

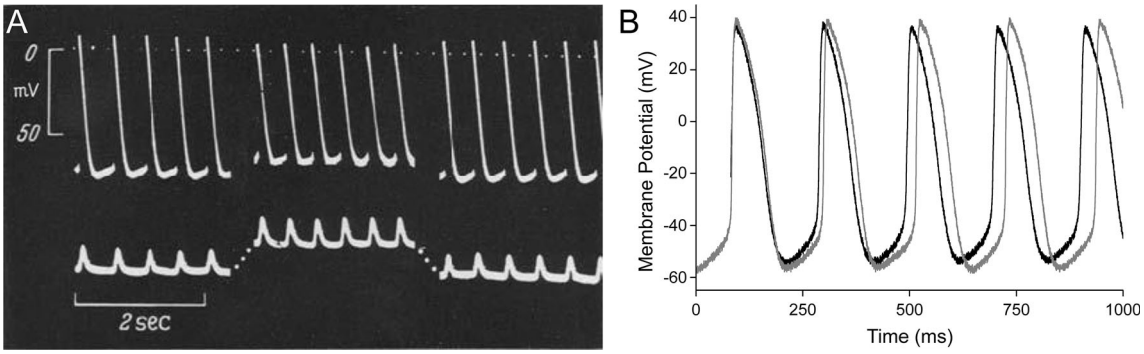

Fig. 2 Stretch-induced increase in the beating rate of isolated sinoatrial node preparations. **A** Intracellular sharp electrode recording of transmembrane potential (top) and applied and generated force (bottom; passive stretch and active contraction pointing upwards) in spontaneously beating cat isolated sinoatrial node (SAN) tissue (from Deck 1964) and **B** patch-clamp recording of transmembrane potential in a spontaneously beating rabbit isolated SAN cell (light curve, before stretch; dark curve, during stretch) (from Cooper et al. 2000). Both show an increase in beating rate during stretch, accompanied by a reduction in the absolute value of maximum diastolic and maximum systolic potential

assuming its critical importance (Quinn and Kohl 2011), as there are several other mechano-sensitive components within SAN cells that may also contribute to the stretch response. In particular, mechano-sensitivity of membrane and Ca^{2+} clock components (Quinn and Kohl 2012) may partly mediate the effects of SAN stretch on automaticity.

To start, I_f has been shown to be mechano-sensitive. In cell expression systems, the activation, deactivation, and current amplitude of HCN channels are altered by mechanical stimuli (Calloe et al. 2005; Lin et al. 2007), which results in a frequency-dependent alteration in the rate of cell excitation (Lin et al. 2007). Other components of the membrane clock have also been shown to be mechano-sensitive, including L-type Ca^{2+} channels (Calabrese et al. 2002; Lyford et al. 2002), fast Na^+ channels, and delayed rectifier K^+ channels (Morris 2011). Components of the Ca^{2+} clock have likewise been shown to be mechano-sensitive in other cardiac cell types, as axial stretch of ventricular myocytes results in an increase of Ca^{2+} sparks (Iribe et al. 2009). Lowered extracellular Ca^{2+} and pharmacological inhibition of SERCA (which prevents the reuptake of Ca^{2+} into the SR) or of RyR (which prevents Ca^{2+} release from the SR) results in a reduction in the SAN stretch response (Arai et al. 1996). These findings, along with the immediate change in SAN cell beating rate that occurs with acute changes in intracellular Ca^{2+} concentration (Yaniv et al. 2011), support the potential importance of the mechano-sensitivity of Ca^{2+} handling in the response of the SAN to stretch. Ultimately, if any of the above mechanically induced changes seen in other cell types occur in SAN myocytes, they could make significant contributions to SAN mechano-sensitivity, and while the specific mechanism(s) leading to the acute response of the SAN to stretch remain unclear, it seems reasonable to assume that like the coupled-oscillator system driving automaticity, multiple mechanisms may be involved. What is clear is that stretch generally leads to an increase in SAN beating rate, which may be important for in vivo SAN function.

Mechanical entrainment of SAN activity

In vivo, during atrial diastole, the ventricles are contracting, pulling the atrioventricular valve plane apically and causing a stretch of the atrial tissue containing the SAN (Hales et al. 2012). The SAN is then stretched further as blood returns to the heart and fills the right atrium. Peak SAN stretch levels coincide with the period of SDD, as membrane potential is moving towards the threshold for AP generation, so any stretch-induced depolarising currents will act to mechanically 'prime' pacemaker cells for excitation. This allows for a beat-by-beat adaptation of heart rate to changes in venous return, such as occur with exercise, alterations in posture, or modulation of thorax-abdomen pressure gradients caused by respiratory activity (Quinn and Kohl 2012).

At the tissue level, a stretch of the SAN may play another important role. While the majority of SAN cells will experience stretch-induced depolarisation during a similar period of SDD, cells that are not firing synchronously will experience it at some other point in the cardiac cycle. The response to this 'out-of-phase' stretch may act to entrain (or reset) the electrical activity of those cells via a phenomenon known as 'phase-resetting', so that excitation is more uniformly timed across the entire SAN. It has been shown that injection of a subthreshold (i.e. non-excitatory) depolarising current pulse into spontaneously beating SAN cells (as would occur with SAN stretch) can result in an increase or a decrease in their beating rate, depending on the timing of the stimulation within the cardiac cycle (Anumonwo et al. 1991), which can entrain SAN cell activity (Verheijck et al. 1998). Phase-resetting behaviour, in response to an externally applied, subthreshold electrical stimulus, has been shown to also occur in SAN tissue (Fig. 3) (Sano et al. 1978; Jalife and Antzelevitch 1979) and has been corroborated by computational modelling

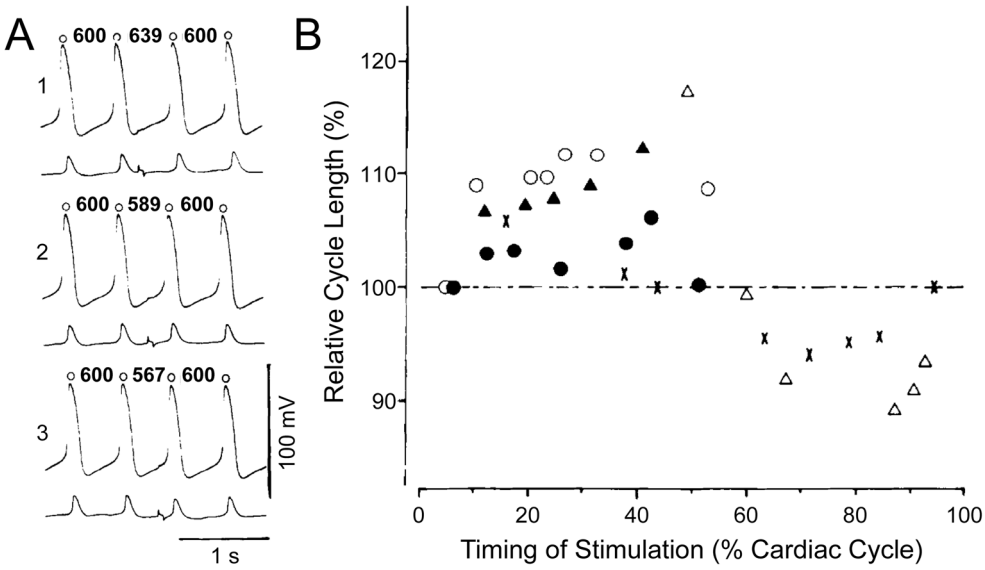

Fig. 3 Phase resetting in the sinoatrial node (SAN). **A** Application of subthreshold square-wave pulse in the early (1), middle (2), and late (3) phase of the cardiac cycle in the rabbit isolated SAN (lower tracings in each section are action potentials from the SAN region close to the atrium to show time of stimulus artefacts) and **B** the relationship between cycle length and time of stimulation in the cardiac cycle, showing that subthreshold depolarising current pulses result in an increase or a decrease in cycle length, depending on the timing of the stimulation within the cardiac cycle. From Sano et al. (1978)

(Ypey et al. 1982; Reiner and Antzelevitch 1985; Guevara and Jongsma 1990; Anumonwo et al. 1991; Coster and Celler 2003; Krogh-Madsen et al. 2004; Tsalikakis et al. 2007; Huang et al. 2011). Through this phenomenon, subthreshold depolarisation of SAN cells generated intrinsically by stretch may act to normalise heterogeneous electrical activity across non-synchronous cells and help prevent abnormally fast or arrhythmic groups of cells from overtaking pacemaking by their entrainment (Abramovich-Sivan and Akselrod 1999). Since the SAN is constantly subjected to oscillating cyclic stretch in vivo, the stretch may thus act to specifically enhance SDD and to 'smooth out' differences in automaticity between cells across the node, thus stabilising rhythm (Ushiyama and Brooks 1977).

SAN mechano-sensitivity: the grandfather clock of cardiac rhythm

Maintenance of baseline heart rate and rhythm via SAN mechano-sensitivity

Even though it was discovered over 110 years ago (Keith and Flack 1907), our understanding of the SAN continues to develop. For instance, it has just recently been revealed that there are two distinct and competing pacemaker regions within the SAN, localised near the superior and inferior vena cava, which preferentially drive fast and slow heart rates, helping explain previous observations of pacemaker shifts in response to various physiological inputs (Brennan et al. 2020). With the importance of re-evaluating previous experimental evidence in mind, we propose that the strong emphasis put on the contributions of the membrane and Ca^{2+} oscillators to SAN automaticity have meant that a critical contributor—the mechanics oscillator—has been largely overlooked. While the importance of the Bainbridge response as a beat-by-beat regulator of heart rate in response to changes in venous return is well established, the fundamental importance of diastolic stretch and mechanical oscillations to SDD and SAN automaticity are underappreciated (yet critical), as SAN mechano-sensitivity may be involved in maintaining the regularity of baseline rhythm and entraining myocytes across the node. The contribution of mechanical load to SDD was first established in 1964 by Klaus Deck, using microelectrode recordings of membrane potential during an equi-biaxial stretch of cat and rabbit isolated SAN tissue (Deck 1964). The critical nature of a 'minimal' diastolic tension for the generation and stabilisation of rhythmic SAN excitation was confirmed soon after, as slack isolated SAN tissue often shows irregular or no rhythm, and the application of physiological levels of baseline stretch restores regular activity (Fig. 4) (Brooks et al. 1966; Lange et al. 1966; Ushiyama and Brooks 1977). In such cases, it is apparent that missed beats or SAN quiescence are due to the failure of other pacemaker mechanisms to sustain SDD in the absence of a sufficient mechanical preload, which when applied restores function through a positive shift in MDP towards AP threshold, resulting in regular, spontaneous excitation of the SAN. The importance of an adequate preload for SAN pacemaking may in fact be present from the very first heartbeat during embryonic development, as fluid pressure buildup in the quiescent cardiac tube appears to be a prerequirement for the initiation of spontaneous cardiac excitation during ontogenesis (Rajala et al. 1976, 1977; Chiou et al. 2016).

Implications of SAN mechano-sensitivity in cardiac disease and in ageing

A consequence of the apparent vital role of stretch for SAN automaticity is that it may be an important consideration in

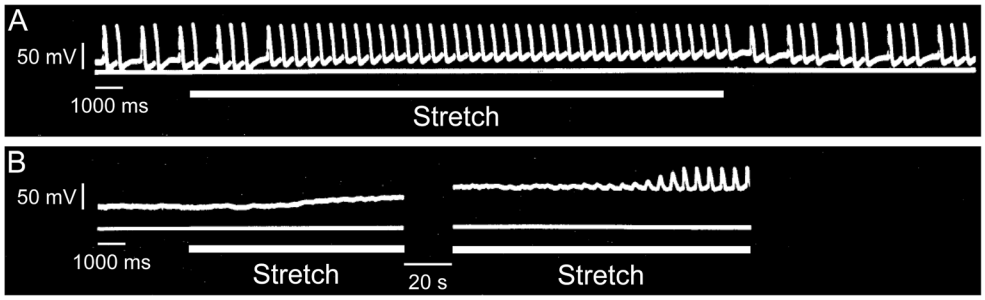

Fig. 4 Effects of physiological levels of baseline stretch on isolated sinoatrial node (SAN) beating rate. Floating microelectrode recordings of transmembrane potential in cat isolated SAN, showing a stretch-induced shift of the maximum diastolic potential towards less negative values, resulting in **A** restoration of regular rhythm in a SAN with irregular activity at slack length or **B** initiation of spontaneous excitation in a previously quiescent SAN. In both examples, tissue length was increased by ~40% from slack, with periods of stretch indicated by the lower horizontal lines. From Lange et al. (1966)

some forms of SAN dysfunction. The SAN stretch response has been shown to be influenced by tissue structure and stiffness (MacDonald et al. 2020a), so there may be a stretch-dependent link between SAN dysfunction and structural or mechanical changes that occur with advanced age and in many cardiac pathologies. Stretch-induced SAN dysfunction with ageing and in disease may be further exasperated by changes in SAN mechano-sensitivity secondary to electrical remodelling (Kistler et al. 2004; Morris and Kalman 2014; Csepe et al. 2015) or by an increase in electrically-coupled, mechano-sensitive fibroblasts (Kohl et al. 1994; Kohl and Noble 1996; Quinn et al. 2016) with increased fibrosis, which will also affect the normal patterns of stretch during the cardiac cycle, leading to an altered stretch response. Furthermore, the stabilisation of heart rate by stretch appears to be functional only within a certain range, as excessive stretch results in irregular rhythms (Lange et al. 1966) and multifocal activity (Hoffman and Cranefield 1960), which may in part account for SAN dysfunction in pathologies associated with atrial volume overload (Sparks et al. 1999; Morton et al. 2003; Sanders et al. 2003).

Conclusions and future research

The SAN is a vital piece of tissue for sustaining life, and thus its electrical activity is driven by a system of integrated and redundant mechanisms to ensure it continues to operate under a wide variety of (patho-)physiological conditions. SAN stretch is one fundamental contributor to pacemaking, as it: (A) drives SAN automaticity by contributing to SDD through SAC_{NS} and/or mechano-sensitivity of coupled-clock components; (B) entrains pacemaker cells across the SAN via phase-resetting caused by their mechanically induced subthreshold depolarisation, with the level of baseline stretch being important for the stability of rhythm generation; and (C) regulates SAN firing rate through the Bainbridge response, by which stretch results in the beat-by-beat matching of heart rate to venous return (Fig. 1).

One important consequence of a critical role for SAN mechano-sensitivity in pacemaking is the need for its consideration in future experimental investigations of SAN function. For instance, in isolated, Langendorff perfused hearts that are not in working mode (so have no atrial filling), the Bainbridge response is not engaged, meaning the effect of stretch on diastolic depolarisation is not present (which could partly account for the generally slower heart rate seen in isolated compared to in vivo hearts). Often, mechanical uncouplers (e.g. blebbistatin) are used in these preparations, which affect mechanics by preventing contraction (while preserving electrical activity). The loss of contraction should have no effect on heart rate, as it will not change the amount of stretch experienced by the SAN and in fact, might cause an increase in SAN stretch if there is a buildup of fluid in the atria that is no longer ejected. But in all cases, it is important to recognise that cells in tissue are always under some level of baseline mechanical load, which in isolated hearts (with or without mechanical uncouplers) may contribute to the regularity of SAN firing. Targeted manipulation of mechanical activity and baseline load in healthy and diseased whole hearts (e.g. working vs. non-working Langendorff, with or without blebbistatin) may be a means to further explore the importance of the various contributions of stretch to SAN (dys-)function in the whole heart.

Another potential means of exploring the influences of stretch on SAN function is computational modelling (Quinn and Kohl 2013). Highly complex, three-dimensional, electromechanically coupled whole heart models now exist (Travanova 2011; Niederer et al. 2019), which could be modified to include the hypothesised subcellular mechanisms of SAN mechano-sensitivity (i.e. SAC_{NS} or mechano-sensitivity of coupled-clock components) to gain further experimentally inaccessible insight into the relative importance of the different effects of stretch on SAN function, as well as which mechano-sensitive mechanisms can account for

experimentally observed effects, to help identify the most likely mechano-sensitive candidates for experimental follow-up.

SAN mechano-sensitivity may also represent an unappreciated therapeutic target for the treatment of SAN dysfunction. If subcellular mechanisms responsible for stretch effects can be identified, then they may be pharmacologically manipulated as an anti-arrhythmic therapy for the restoration of normal cardiac rhythm. There is also the potential to directly target SAN mechanics in diseases where it has been disrupted, using novel devices or biomaterials to normalise stretch and restore normal function.

In summary, SAN automaticity is driven by the combined actions of multiple oscillators that drive SDD and ultimately cause membrane potential to cross the threshold for AP generation. The importance of diastolic load and cyclic stretch for SAN function has been previously underappreciated. They may in fact be crucial for the stabilisation of pacemaking through the mechanical priming and entrainment of SAN cells and through their effect on the activity of other mechanisms contributing to SAN automaticity, suggesting SAN mechano-sensitivity is the 'grandfather clock' of cardiac rhythm (Fig. 1).

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Abbreviations *AP,* Action potential; Ca^{2+}, Calcium; *CaMKII,* Calcium/calmodulin-dependent protein kinase II; *cAMP,* Cyclic adenosine monophosphate; *HCN,* Hyperpolarisation-activated cyclic nucleotide-gated channels; I_f, "Funny" current; I_{Kr}, Rapid delayed rectifier potassium current; I_{Ks}, Slow delayed rectifier potassium current; K^+, Potassium; *MDP,* Maximum diastolic potential; *MSP,* Maximum systolic potential; *NCX,* Sodium-calcium exchanger; *LCR,* Local calcium releases; Na^+, Sodium; *RyR,* Ryanodine receptors; SAC_{NS}, Cation non-selective stretch-activated channels; *SAN,* Sinoatrial node; *SDD,* Spontaneous diastolic depolarisation; *SERCA,* Sarco/endoplasmic reticulum calcium-ATPase; *SR,* Sarcoplasmic reticulum

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