TITLE

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

AUTHORS

Eilidh A MacDonald[1]

T Alexander Quinn[2,3]

AFFILIATIONS

[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, Scotland

[2]Department of Physiology and Biophysics, Dalhousie University, Halifax, Canada

[3]School of Biomedical Engineering, Dalhousie University, Halifax, Canada

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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+}-clocks'. The principle 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. 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.

KEY WORDS

Mechano-electric coupling; stretch; pacemaking; calcium-clock; membrane-clock; heart rate

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

1. 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 – which, incidentally, is one of the principal reasons that heart transplantation is possible. This is because the electrical excitation, which initiates the heartbeat, is produced within the organ itself. 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 (Keith and Flack, 1907). In the 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 remains incomplete, and fundamental questions unanswered.

Perhaps the most heavily studied – but still most highly contested – question regarding the SAN relates to the mechanism(s) responsible for its automaticity: what keeps us 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 relative 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 sub-systems 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, 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 circulating endocrine factors (MacDonald et al., 2020b). There are also *intracardiac* factors that critically 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).

2. The classical understanding of cardiac pacemaking

2.1 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 hyperpolarised (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 human is almost exclusively expressed in the SAN (Li et al., 2015). The vital importance of HCN channels for pacemaking has been corroborated in HCN knock-out 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 assert 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 (~ -40mV), 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, and in fact, prior to the identification of I_f their decay at the end of the SAN AP was thought 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 this occurs at the same time as a continuous reduction in their total current. Repolarisation of the cell 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 they 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 establish heart rate.

2.2 Ca^{2+}-clock

Intracellular Ca^{2+} cycling has also been shown to be a predominant 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 a 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 in part explained by the fact that while LCR were originally thought to be spontaneous, they are 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 then, 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).

2.3 Coupled-oscillator system

The combined action 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 found in the cell membrane and directly alters its potential. 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. So, 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 alone. 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 off cellular activity may occur is through cyclic stretch of the SAN as the right atrium fills and contracts, 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 'mechanics-oscillator'), as stretch effects are a key driver – and perhaps a master controller – of cardiac pacemaking.

3. The contribution of SAN stretch to cardiac pacemaking

3.1 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) (although interestingly not in mouse SAN, whose beating rate tends to decrease with stretch (Cooper and Kohl, 2005)) This evolutionary conservation 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 *via* 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 over-distension of the right atrium, while maintaining 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.

3.2 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, stretch of single SAN myocytes

results in activation of a current with a reversal potential of ~-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 assuming its importance (Quinn and Kohl, 2011), as there are several other mechano-sensitive components within SAN cells that may account for the response to stretch. In particular, mechano-sensitivity of membrane- and Ca^{2+}-clock components (Quinn and Kohl, 2012) may additionally 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 (but interestingly not T-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 response to SAN stretch (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 Ca^{2+} handling mechano-sensitivity for the SAN stretch response. 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 are 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.

3.3 Mechanical entrainment of SAN activity

In vivo, during atrial diastole the ventricles are contracting, pulling the atrio-ventricular valve-plane apically and causing stretch of the tissue containing the SAN (Hales et al., 2012). The SAN continues to be stretched 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 toward the threshold for AP generation, so any stretch-induced depolarising currents will act to mechanically 'prime' pacemaker cells for excitation. This allows for 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, 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 sub-threshold 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). This phase-resetting behaviour has been shown to also occur in SAN tissue (Fig. 3) (Sano et al., 1978; Jalife and Antzelevitch, 1979), has been corroborated by computational modelling (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), and is a phenomenon by which stretch-induced sub-threshold depolarisation may act to normalise heterogenous electrical activity across individual non-synchronous cells and help prevent irregularly fast or arrhythmic groups of cells from over-taking pacemaking by their mutual entrainment (Abramovich-Sivan and Akselrod, 1999). Since the SAN is constantly subjected to oscillating cyclic stretch *in vivo*, stretch may thus act to specifically enhance SDD and to 'smooth out' differences in automaticity between cells across the node to stabilise rhythm (Ushiyama and Brooks, 1977).

4. SAN mechano-sensitivity: the grandfather-clock of cardiac rhythm

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 for re-evaluating previous experimental evidence in mind, we propose that the strong emphasis put on the contributions of the membrane- and Ca^{2+}-clocks 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 tension 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 cells 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 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 application of physiological levels of baseline stretch restore 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 the other pacemaker 'clocks' to sustain SDD in the absence of a sufficient mechanical preload, which when applied restores function through a positive shift in MDP towards the AP threshold, resulting in regular, spontaneous AP generation. 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 build-up in the quiescent

cardiac tube appears to be a pre-requirement for the initiation of spontaneous cardiac excitation during ontogenesis (Rajala et al., 1976, 1977; Chiou et al., 2016).

A consequence of the apparent vital role of stretch for SAN automaticity is that it may be an important consideration in some forms of SAN dysfunction. The SAN stretch response is related to tissue structure and stiffness (MacDonald et al., 2020a), so as SAN dysfunction is associated with structural changes in advanced age and cardiac pathologies (in particular increased fibrosis), it is reasonable to hypothesise that there may be a causal link between changes in SAN structure and its function due to an altered stretch response, which may be further exasperated by changes in SAN mechano-sensitivity with electrical remodelling (Kistler et al., 2004; Morris and Kalman, 2014; Csepe et al., 2015). 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), so may be involved in SAN dysfunction in pathologies associated with atrial volume overload (Sparks et al., 1999; Morton et al., 2003; Sanders et al., 2003).

5. Conclusion

Clearly 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 other mechanisms contributing to SAN

automaticity, making SAN mechano-sensitivity the ‘grandfather-clock’ of cardiac rhythm (Fig. 1).

REFERENCES

Abramovich-Sivan, S., and Akselrod, S. (1999). Phase response curve based model of the SA node: Simulation by two-dimensional array of pacemaker cells with randomly distributed cycle lengths. Med. Biol. Eng. Comput. *37*: 482–491.

Anumonwo, J.M.B., Delmar, M., Vinet, A., Michaels, D.C., and Jalife, J. (1991). Phase resetting and entrainment of pacemaker activity in single sinus nodal cells. Circ. Res. *68*: 1138–1153.

Arai, A., Kodama, I., and Toyama, J. (1996). Roles of Cl- channels and Ca2+ mobilization in stretch-induced increase of SA node pacemaker activity. Am. J. Physiol. - Hear. Circ. Physiol. *270*: H1726-35.

Bainbridge, F.A. (1915). The influence of venous filling upon the rate of the heart. J. Physiol. *50*: 65–84.

Bartos, D.C., Grandi, E., and Ripplinger, C.M. (2015). Ion channels in the heart. Compr. Physiol. *5*: 1423–1464.

Bezold, A. von, and Hirt, L. (1867). Über die physiologischen wirkungen des essigsauren veratrins. Untersuchungen Aus Dem Physiol. Lab. Wurzbg. 75–156.

Blinks, J.R. (1956). Positive chronotropic effect of increasing right atrial pressure in the isolated mammalian heart. Am. J. Physiol. *186*: 299–303.

Bogdanov, K.Y., Maltsev, V.A., Vinogradova, T.M., Lyashkov, A.E., Spurgeon, H.A., Stern, M.D., et al. (2006). Membrane potential fluctuations resulting from submembrane Ca2+ releases in rabbit sinoatrial nodal cells impart an exponential phase to the late diastolic depolarization that controls their chronotropic state. Circ. Res. *99*: 979–987.

Brennan, J.A., Chen, Q., Gams, A., Dyavanapalli, J., Mendelowitz, D., Peng, W., et al. (2020). Evidence of superior and inferior sinoatrial nodes in the mammalian heart.

413 JACC Clin. Electrophysiol. *6*: 1827–1840.
414 Brooks, C.M., Lu, H.H., Lange, G., Mangi, R., Shaw, R.B., and Geoly, K. (1966).
415 Effects of localized stretch of the sinoatrial node region of the dog heart. Am. J.
416 Physiol. *211*: 1197–1202.
417 Calabrese, B., Tabarean, I. V., Juranka, P., and Morris, C.E. (2002).
418 Mechanosensitivity of N-type calcium channel currents. Biophys. J. *83*: 2560–2574.
419 Calloe, K., Elmedyb, P., Olesen, S.P., Jorgensen, N.K., and Grunnet, M. (2005).
420 Hypoosmotic cell swelling as a novel mechanism for modulation of cloned HCN2
421 channels. Biophys. J. *89*: 2159–2169.
422 Chiou, K.K., Rocks, J.W., Chen, C.Y., Cho, S., Merkus, K.E., Rajaratnam, A., et al.
423 (2016). Mechanical signaling coordinates the embryonic heartbeat. Proc. Natl. Acad.
424 Sci. *113*: 8939–8944.
425 Coleridge, J.C., and Linden, R.J. (1955). The effect of intravenous infusions upon the
426 heart rate of the anaesthetized dog. J. Physiol. *127*: 31P.
427 Cooper, P.J., and Kohl, P. (2005). Species- and preparation-dependence of stretch
428 effects on sino-atrial node pacemaking. Ann. N. Y. Acad. Sci. *1047*: 324–335.
429 Cooper, P.J., Lei, M., Cheng, L.X., and Kohl, P. (2000). Selected contribution: axial
430 stretch increases spontaneous pacemaker activity in rabbit isolated sinoatrial node
431 cells. J. Appl. Physiol. *89*: 2099–104.
432 Coster, A.C.F., and Celler, B.G. (2003). Phase response of model sinoatrial node
433 cells. Ann. Biomed. Eng. *31*: 271–283.
434 Csepe, T.A., Kalyanasundaram, A., Hansen, B.J., Zhao, J., and Fedorov, V. V.
435 (2015). Fibrosis: A structural modulator of sinoatrial node physiology and
436 dysfunction. Front. Physiol. *6*: 1–8.
437 Deck, K.A. (1964). Dehnungseffekte am spontansehlagenden, isolierten

438 Sinusknoten. Pflügers Arch. *280*: 120–130.
439 Difrancesco, D. (2010). The role of the funny current in pacemaker activity. Circ.
440 Res. *106*: 434–446.
441 Difrancesco, D., and Noble, D. (2012). The funny current has a major pacemaking
442 role in the sinus node. Hear. Rhythm *9*: 299–301.
443 DiFrancesco, M.L., Mesirca, P., Bidaud, I., Isbrandt, D., and Mangoni, M.E. (2021).
444 The funny current in genetically modified mice. Prog. Biophys. Mol. Biol.
445 Donald, D.E., and Shepherd, J.T. (1978). Reflexes from the heart and lungs:
446 Physiological curiosities or important regulatory mechanisms. Cardiovasc. Res. *12*:
447 449–469.
448 Fenske, S., Krause, S.C., Hassan, S.I.H., Becirovic, E., Auer, F., Bernard, R., et al.
449 (2013). Sick sinus syndrome in HCN1-Deficient mice. Circulation *128*: 2585–2594.
450 Gaskell, W.H. (1882). Preliminary observations on the innervation of the heart of the
451 tortoise. J. Physiol. *3*: 369–379.
452 Guevara, M.R., and Jongsma, H.J. (1990). Phase resetting in a model of sinoatrial
453 nodal membrane: Ionic and topological aspects. Am. J. Physiol. - Hear. Circ. Physiol.
454 *258*: 734–747.
455 Guharay, F., and Sachs, F. (1984). Stretch-activated single ion channel currents in
456 tissue-cultured embryonic chick skeletal muscle. J. Physiol. *352*: 685–701.
457 Hales, P.W., Schneider, J.E., Burton, R.A.B., Wright, B.J., Bollensdorff, C., and Kohl,
458 P. (2012). Histo-anatomical structure of the living isolated rat heart in two contraction
459 states assessed by diffusion tensor MRI. Prog. Biophys. Mol. Biol. *110*: 319–330.
460 Hoffman, B., and Cranefield, P. (1960). Electrophysiology of the heart (New York:
461 McGraw-Hill).
462 Huang, X., Mi, Y., Qian, Y., and Hu, G. (2011). Phase-locking behaviors in an ionic

463 model of sinoatrial node cell and tissue. Phys. Rev. E - Stat. Nonlinear, Soft Matter
464 Phys. *83*:.
465 Iribe, G., Ward, C.W., Camelliti, P., Bollensdorff, C., Mason, F., Burton, R.A.B., et al.
466 (2009). Axial stretch of rat single ventricular cardiomyocytes causes an acute and
467 transient increase in Ca2+ spark rate. Circ. Res. *104*: 787–795.
468 Jalife, J. (1984). Mutual entrainment and electrical coupling as mechanisms for
469 synchronous firing of rabbit sino-atrial pace-maker cells. J. Physiol. *356*: 221–243.
470 Jalife, J., and Antzelevitch, C. (1979). Phase resetting and annihilation of pacemaker
471 activity in cardiac tissue. Science (80-.). *206*: 695–697.
472 Jarisch, A., and Richter, H. (1939). Die afferenten bahnen des veratrineffektes in den
473 herznerven. Naunyn. Schmiedebergs. Arch. Exp. Pathol. Pharmakol. *193*: 355–371.
474 Keith, A., and Flack, M. (1907). The form and nature of the muscular connections
475 between the primary divisions of the vertebrate heart. J. Anat. Physiol. *41*: 172–89.
476 Kistler, P.M., Sanders, P., Fynn, S.P., Stevenson, I.H., Spence, S.J., Vohra, J.K., et
477 al. (2004). Electrophysiologic and electroanatomic changes in the human atrium
478 associated with age. J. Am. Coll. Cardiol. *44*: 109–116.
479 Krogh-Madsen, T., Glass, L., Doedel, E.J., and Guevara, M.R. (2004). Apparent
480 discontinuities in the phase-resetting response of cardiac pacemakers. J. Theor.
481 Biol. *230*: 499–519.
482 Lakatta, E.G., and DiFrancesco, D. (2009). What keeps us ticking: a funny current, a
483 calcium clock, or both? J. Mol. Cell. Cardiol. *47*: 157–170.
484 Lakatta, E.G., Maltsev, V.A., and Vinogradova, T.M. (2010). A coupled SYSTEM of
485 intracellular Ca2+ clocks and surface membrane voltage clocks controls the
486 timekeeping mechanism of the heart's pacemaker. Circ. Res. *106*: 659–73.
487 Lakatta, E.G., Vinogradova, T.M., and Maltsev, V.A. (2008). The missing link in the

mystery of normal automaticity of cardiac pacemaker cells. Ann. N. Y. Acad. Sci. *1123*: 41–57.

Lange, G., Lu, H.H., Chang, A., and Brooks, C.M. (1966). Effect of stretch on the isolated cat sinoatrial node. Am. J. Physiol. *211*: 1192–1196.

Lei, M., Goddard, C., Liu, J., Léoni, A.L., Royer, A., Fung, S.S.M., et al. (2005). Sinus node dysfunction following targeted disruption of the murine cardiac sodium channel gene Scn5a. J. Physiol. *567*: 387–400.

Li, N., Csepe, T.A., Hansen, B.J., Dobrzynski, H., Higgins, R.S.D., Kilic, A., et al. (2015). Molecular Mapping of Sinoatrial Node HCN Channel Expression in the Human Heart. Circ. Arrhythmia Electrophysiol. *8*: 1219–1227.

Lin, W., Laitko, U., Juranka, P.F., and Morris, C.E. (2007). Dual stretch responses of mHCN2 pacemaker channels: Accelerated activation, accelerated deactivation. Biophys. J. *92*: 1559–1572.

Lyford, G.L., Strege, P.R., Shepard, A., Ou, Y., Ermilov, L., Miller, S.M., et al. (2002). α1C (CaV1.2) L-type calcium channel mediates mechanosensitive calcium regulation. Am. J. Physiol. Physiol. *283*: C1001–C1008.

MacDonald, E.A., Madl, J., Greiner, J., Ramadan, A.F., Wells, S.M., Torrente, A.G., et al. (2020a). Sinoatrial node structure, mechanics, electrophysiology and the chronotropic response to stretch in rabbit and mouse. Front. Physiol. *11*: 1–15.

MacDonald, E.A., Rose, R.A., and Quinn, T.A. (2020b). Neurohumoral control of sinoatrial node activity and heart rate: insight from experimental models and findings from humans. Front. Physiol. *11*: 170.

MacDonald, E.A., Stoyek, M.R., Rose, R.A., and Quinn, T.A. (2017). Intrinsic regulation of sinoatrial node function and the zebrafish as a model of stretch effects on pacemaking. Prog. Biophys. Mol. Biol. *130*: 198–211.

513 Mangoni, M.E., Couette, B., Bourinet, E., Platzer, J., Reimer, D., Striessnig, J., et al.
514 (2003). Functional role of L-type Cav1.3 Ca2+ channels in cardiac pacemaker
515 activity. Proc. Natl. Acad. Sci. U. S. A. *100*: 5543–8.
516 Mangoni, M.E., and Nargeot, J. (2008). Genesis and regulation of the heart
517 automaticity. Physiol. Rev. *88*: 919–982.
518 Mattick, P., Parrington, J., Odia, E., Simpson, A., Collins, T., and Terrar, D. (2007).
519 Ca2+-stimulated adenylyl cyclase isoform AC1 is preferentially expressed in guinea-
520 pig sino-atrial node cells and modulates the If pacemaker current. J. Physiol. *582*:
521 1195–1203.
522 Mesirca, P., Torrente, A.G., and Mangoni, M.E. (2015). Functional role of voltage
523 gated Ca2+ channels in heart automaticity. Front. Physiol. *6*: 19.
524 Morris, C.. (2011). Pacemaker, potassium, calcium, sodium: stretch modulation of
525 the voltage-gated channels. In Cardiac Mechano-Electric Coupling and Arrhythmias,
526 P. Kohl, F. Sachs, and M. Franz, eds. (Oxford: University Press, Oxford), pp 42–49.
527 Morris, G.M., and Kalman, J.M. (2014). Fibrosis, electrics and genetics -
528 Perspectives on sinoatrial node disease -. Circ. J. *78*: 1272–1282.
529 Morton, J.B., Sanders, P., Vohra, J.K., Sparks, P.B., Morgan, J.G., Spence, S.J., et
530 al. (2003). Effect of chronic right atrial stretch on atrial electrical remodeling in
531 patients with an atrial septal defect. Circulation *107*: 1775–1782.
532 Noble, D., Noble, P.J., and Fink, M. (2010). Competing oscillators in cardiac
533 pacemaking. Circ. Res. *106*: 1791–1797.
534 Pathak, C.L. (1958). Effects of changes in intraluminal pressure on inotropic and
535 chronotropic responses of isolated mammalian hearts. Am. J. Physiol. *194*: 197–9.
536 Pathak, C.L. (1973). Autoregulation of chronotropic response of the heart through
537 pacemaker stretch. Cardiology *58*: 45–64.

538 Peyronnet, R., Nerbonne, J.M., and Kohl, P. (2016). Cardiac mechano-gated ion
539 channels and arrhythmias. Circ. Res. *118*: 311–329.
540 Quinn, T.A. (2015). Cardiac mechano-electric coupling: A role in regulating normal
541 function of the heart? Cardiovasc. Res. *108*: 1–3.
542 Quinn, T.A., and Kohl, P. (2011). Systems biology of the heart: Hype or hope? Ann.
543 N. Y. Acad. Sci. *1245*: 40–43.
544 Quinn, T.A., and Kohl, P. (2012). Mechano-sensitivity of cardiac pacemaker function:
545 Pathophysiological relevance, experimental implications, and conceptual integration
546 with other mechanisms of rhythmicity. Prog. Biophys. Mol. Biol. *110*: 257–268.
547 Quinn, T.A., and Kohl, P. (2016). Rabbit models of cardiac mechano-electric and
548 mechano-mechanical coupling. Prog. Biophys. Mol. Biol. *121*: 110–122.
549 Quinn, T.A., and Kohl, P. (2021). Cardiac mechano-electric coupling: acute effects of
550 mechanical stimulation on heart rate and rhythm. Physiol. Rev. *101*: 37–92.
551 Quinn, T.A., Kohl, P., and Ravens, U. (2014). Cardiac mechano-electric coupling
552 research: Fifty years of progress and scientific innovation. Prog. Biophys. Mol. Biol.
553 *115*: 71–75.
554 Rajala, G.M., Kalbfleisch, J.H., and Kaplan, S. (1976). Evidence that blood pressure
555 controls heart rate in the chick embryo prior to neural control. J. Embryol. Exp.
556 Morphol. *36*: 685–95.
557 Rajala, G.M., Pinter, M.J., and Kaplan, S. (1977). Response of the quiescent heart
558 tube to mechanical stretch in the intact chick embryo. Dev. Biol. *61*: 330–337.
559 Reiner, V.S., and Antzelevitch, C. (1985). Phase resetting and annihilation in a
560 mathematical model of sinus node. Am. J. Physiol. - Hear. Circ. Physiol. *18*:.
561 Roddie, I.C., Shepherd, J.T., and Whelan, R.F. (1957). Reflex changes in
562 vasoconstrictor tone in human skeletal muscle in response to stimulation of

563 receptors in a low-pressure area of the intrathoracic vascular bed. J. Physiol. *139*:
564 369–76.
565 Rosen, M.R., Nargeot, J., and Salama, G. (2012). The case for the funny current and
566 the calcium clock. Hear. Rhythm *9*: 616–618.
567 Sanders, P., Morton, J.B., Davidson, N.C., Spence, S.J., Vohra, J.K., Sparks, P.B.,
568 et al. (2003). Electrical remodeling of the atria in congestive heart failure. Circulation
569 *108*: 1461–1468.
570 Sano, T., Sawanobori, T., and Adaniya, H. (1978). Mechanism of rhythm
571 determination among pacemaker cells of the mammalian sinus node. Am. J. Physiol.
572 - Hear. Circ. Physiol. *235*: H379-84.
573 Sénatore, S., Reddy, V.R., Sémériva, M., Perrin, L., and Lalevée, N. (2010).
574 Response to mechanical stress is mediated by the TRPA channel painless in the
575 Drosophila heart. PLoS Genet. *6*: e1001088.
576 Sirenko, S., Yang, D., Li, Y., Lyashkov, A.E., Lukyanenko, Y.O., Lakatta, E.G., et al.
577 (2013). Ca2+-dependent phosphorylation of Ca2+ cycling proteins generates robust
578 rhythmic local Ca2+ releases in cardiac pacemaker cells. Sci. Signal. *6*: ra6.
579 Sparks, P.B., Mond, H.G., Vohra, J.K., Jayaprakash, S., and Kalman, J.M. (1999).
580 Electrical remodeling of the atria following loss of atrioventricular synchrony.
581 Circulation *100*: 1894–1900.
582 Starzinsky, and Bezold, A. von (1867). Von dem einflusse des intracardialen
583 blutdruckes auf die hauflgkeit der herzschlage. Untersuch Phys Lab 195–214.
584 Torrente, A.G., Mesirca, P., Neco, P., Rizzetto, R., Dubel, S., Barrere, C., et al.
585 (2016). L-type Cav1.3 channels regulate ryanodine receptor-dependent Ca2+
586 release during sino-atrial node pacemaker activity. Cardiovasc. Res. *109*: 451–461.
587 Tsalikakis, D.G., Zhang, H.G., Fotiadis, D.I., Kremmydas, G.P., and Michalis, K.

588 (2007). Phase response characteristics of sinoatrial node cells. Comput. Biol. Med.
589 *37*: 8–20.
590 Ushiyama, J., and Brooks, C.M.C. (1977). Interaction of oscillators: Effect of
591 sinusoidal stretching of the sinoatrial node on nodal rhythm. J. Electrocardiol. *10*:
592 39–44.
593 Verheijck, E.E., Wilders, R., Joyner, R.W., Golod, D.A., Kumar, R., Jongsma, H.J., et
594 al. (1998). Pacemaker synchronization of electrically coupled rabbit sinoatrial node
595 cells. J. Gen. Physiol. *111*: 95–112.
596 Vinogradova, T.M., and Lakatta, E.G. (2009). Regulation of basal and reserve
597 cardiac pacemaker function by interactions of cAMP-mediated PKA-dependent Ca2+
598 cycling with surface membrane channels. J. Mol. Cell. Cardiol. *47*: 456–474.
599 Vinogradova, T.M., Lyashkov, A.E., Zhu, W., Ruknudin, A.M., Sirenko, S., Yang, D.,
600 et al. (2006). High basal protein kinase A-dependent phosphorylation drives rhythmic
601 internal Ca2+ store oscillations and spontaneous beating of cardiac pacemaker cells.
602 Circ. Res. *98*: 505–514.
603 Vinogradova, T.M., Zhou, Y., Bogdanov, K.Y., Yang, D., Kuschel, M., Cheng, H., et
604 al. (2000). Sinoatrial node pacemaker activity requires Ca 2+ /Calmodulin-dependent
605 protein kinase II activation. Circ. Res. *87*: 760–767.
606 Yaniv, Y., Maltsev, V.A., Escobar, A.L., Spurgeon, H.A., Ziman, B.D., Stern, M.D., et
607 al. (2011). Beat-to-beat Ca2+-dependent regulation of sinoatrial nodal pacemaker
608 cell rate and rhythm. J. Mol. Cell. Cardiol. *51*: 902–905.
609 Ypey, D.L., Meerwijk, W.P.M. Van, and Bruin, G. de (1982). Suppression of
610 pacemaker activity by rapid repetitive phase delay. Biol. Cybern. *45*: 187–194.
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FIGURES

Figure 1. The grandfather-clock of cardiac rhythm. Summary of the role of mechano-sensitivity in sinoatrial node (SAN) automaticity, entrainment, and regulation.

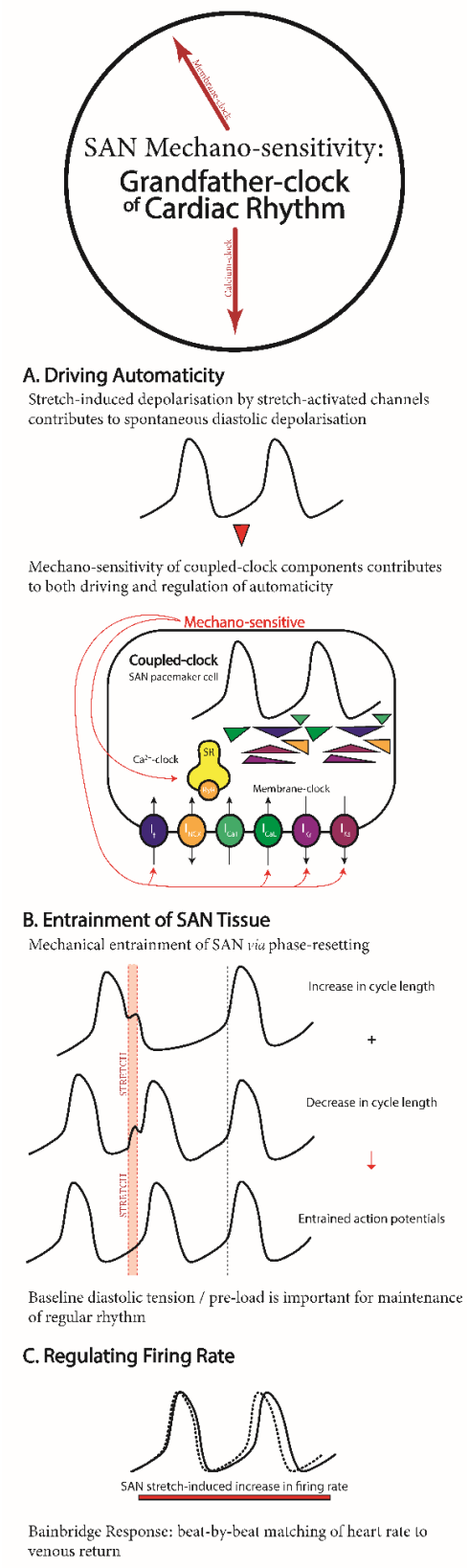

Figure 2. Stretch-induced increase in the beating rate of isolated sinoatrial node preparations. (**A**) Intracellular sharp electrode recording of transmembrane potential (top) and applied and generated force (bottom; passive stretch and active contraction pointing upwards) in spontaneously beating 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.

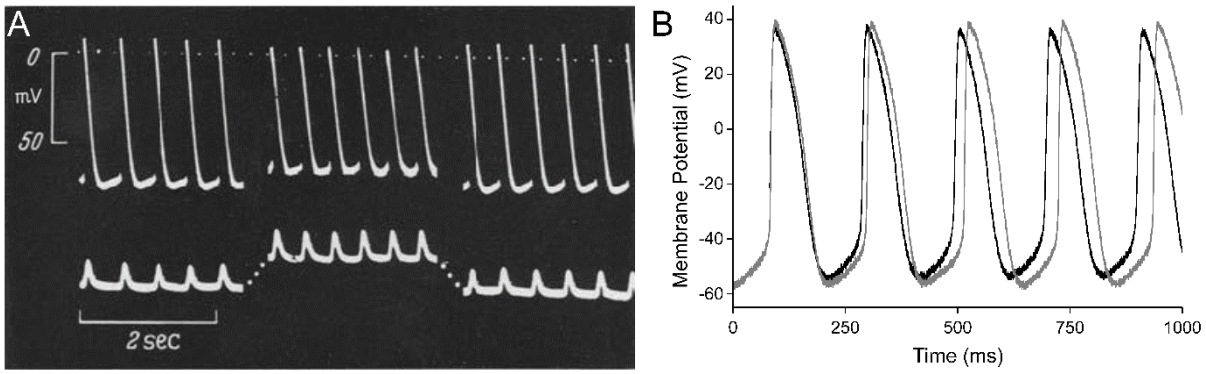

Figure 3. Phase-resetting in the sinoatrial node (SAN). (**A**) Application of subthreshold square-wave pulse in the early (1), middle (2), and late (3) phase of the cardiac cycle in the rabbit isolated SAN (lower tracings in each section are action potentials from the SAN region close to the atrium to show time of stimulus artifacts) and (**B**) the relationship between cycle length and time of stimulation in the cardiac cycle, showing that sub-threshold 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).

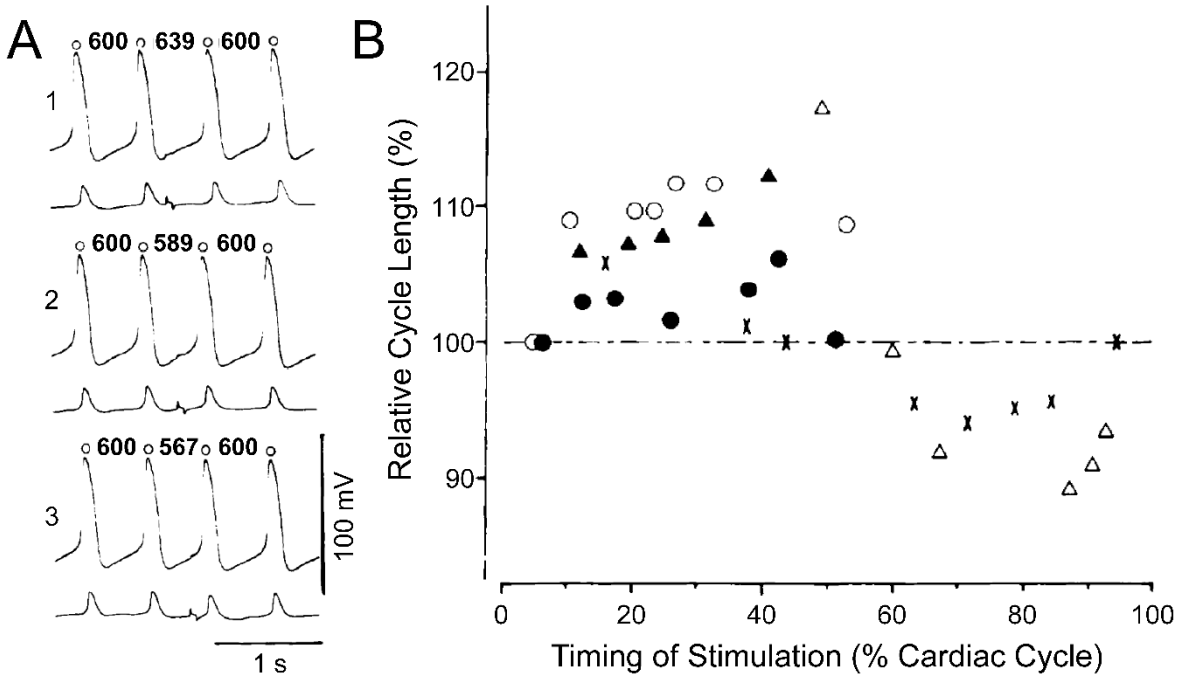

Figure 4. Effects of physiological levels of baseline stretch on isolated sinoatrial node (SAN) beating rate. Floating microelectrode recordings of transmembrane potential in cat isolated SAN, showing a stretch-induced shift of the maximum diastolic potential towards less negative values, resulting in: (**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).