A GENERAL THEORY TO EXPLAIN HEART RATE AND CARDIAC CONTRACTILITY CHANGES WITH ADVANCING AGE

Kenta Tsutsui[1], Oliver J. Monfredi[1, 2] and Edward G. Lakatta[1*]

[1]Laboratory of Cardiovascular Science, Biomedical Research Center, Intramural Research Program, National Institute on Aging, NIH, Baltimore, Maryland, USA.

[2] Institute of Cardiovascular Sciences, University of Manchester, United Kingdom

***Correspondence to:**
Edward G. Lakatta (LakattaE@grc.nia.nih.gov)

ABSTRACT

Sinus node dysfunction and chronic heart failure have been, and will continue to be, major health issues in humans for the foreseeable future. The heartbeat originates from spontaneously firing sinoatrial nodal (SAN) pacemaker cells. A coupled-clock system underlies the robust and flexible automaticity in these cells. The basal action potential (AP) firing rate of SAN cells is largely determined by the degree of phosphorylation of critical proteins in the coupled-clock system. Autonomic neuronal signaling from the brain effects changes in AP firing rate via modulation of cAMP and cAMP-mediated PKA-dependent phosphorylation. Age-associated alterations in intrinsic SAN cell behavior and associated changes in brain-heart communication play central roles in the development of SAN cell pacemaker failure. This mini-review provides integrated insights into the molecular mechanisms underlying the effects of aging on deterioration in beating rate and contractility of the heart in animal models and in apparently healthy humans.

Keywords: Aging, sinoatrial node, pacemaker cell, phosphorylation, coupled-clock system, general theory

Original received: May 23, 2016; Final revision received: May 27, 2016; Accepted: June 2, 2016

Background

Over 700,000 pacemakers are implanted worldwide annually [1], of which the total annual cost in the USA alone in 2010 was over 24 billion dollars [2]. Sick sinus syndrome is a major indication for pacemaker implantation [1]. More than 80% of all pacemaker recipients are over the age of 60, suggesting that aging is a major culprit in necessitating pacemaker implantation [1]. Pacemaker clock failure is therefore one of the major heartbreaks of getting old. So, what happens to heart rate (HR) regulation as we age?

Intrinsic heart rate covertly decreases with aging in humans

One crucial mechanism controlling how fast the heart beats is signaling from the brain via the autonomic nervous system. This modulates the so-called 'coupled-clock system' intrinsic to pacemaker cells within the SAN. Impulses via parasympathetic vagus nerves slow the heart, while those from sympathetic nerves increase HR. Circulating catecholamines from the adrenal medulla are another major sympathetic effector in addition to direct brain-derived neurotransmitter-mediated cardiac sympathetic innervation.
The observed HR at rest is determined by the overlying effects of autonomic neuronal efferent signaling on the intrinsic heart rate (IHR), which is defined as the underlying HR or intrinsic firing activity of the SAN remaining in the presence of dual blockade of the autonomic nervous system (usually performed with the combination of atropine and propranolol). While the IHR decreases at a constant rate with aging [3, 4] **(Figure 1A**), no overt appreciable change in observed *resting* HR accompanies advancing age in healthy humans (**Figure 1B**, green solid line) [5-8].

Resting HR appears to be stable over time, but maximum HR during exertion declines with aging in humans

The HR of healthy older humans when sitting upright from a supine posture and during graded exercise increases to a lesser extent than in younger persons (**Figure 1B**, red dotted line) [9]. It is not possible to ameliorate this blunted HR response with lifestyle measures - exercise training, for example, while being capable of increasing VO_{2max} and cardiac performance through various remodeling effects (including increased arterial compliance, stroke volume and ejection fraction) [9-13], **does not** slow the rate of the age-associated decline in maximum HR during exertion [8, 14].

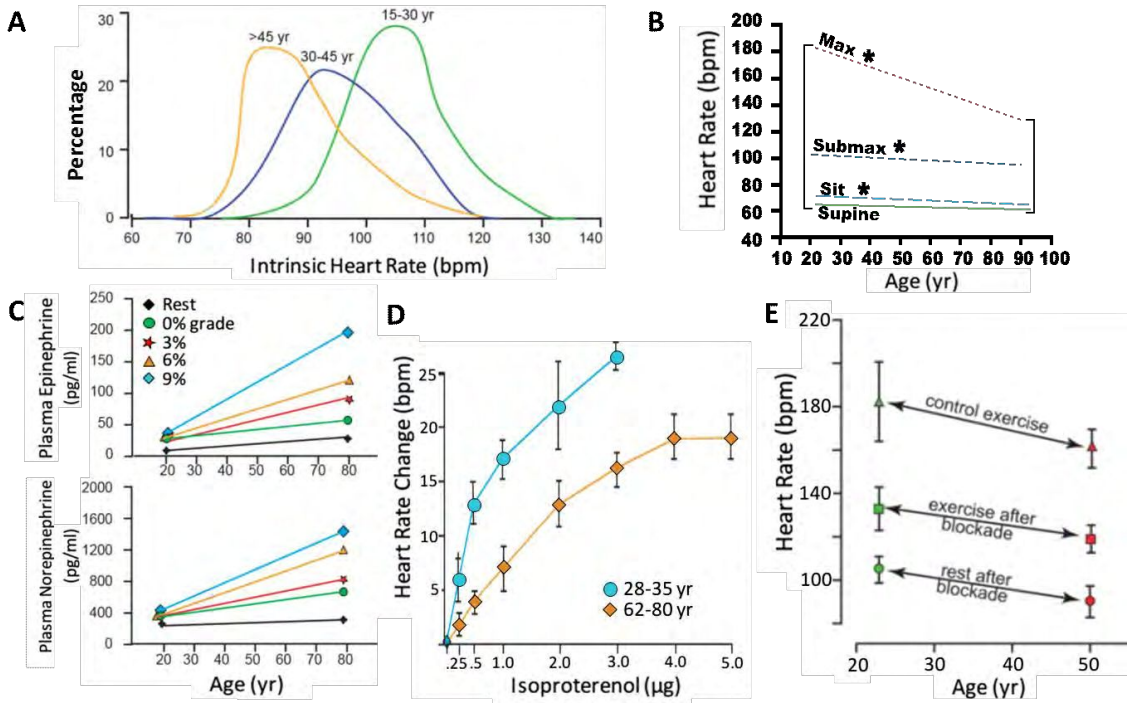


Figure 1. The human aging phenotype is characterized by (A) decreased IHR [3]; (B) apparantly stable resting HR (green solid line) but decreased HR in response to graded exercise (cyan, blue and red dot lines, * indicates statistical significance) [8]; and (C) increased plasma catecholamine secretion in response to graded exercise [15], which is confirmed by (D) reduced sensitivity to an infusion of synthetic agonist isoproterenol at rest [8]. (E) Peak HR both with and without autonomic blockade decreases with advancing age [16].

Brain-heart crosstalk withers with aging

In healthy individuals, both the HR and plasma catecholamine level increase simultaneously during graded exercise. One possibility why maximal HR in response to exercise is lower in older people could be that, with aging, there is a less marked increase in plasma catecholamines. However, this is not the case. **Figure 1C** shows that plasma catecholamine levels in response to exercise actually *increase* as a function of age. In participants of the Baltimore Longitudinal Study of Aging and other studies [8, 17], the heart does not respond to injection of the same dose of the synthetic catecholamine isoproterenol to the same degree as seen in younger individuals (**Figure 1D**). These findings suggest that cardiac catecholamine *responsiveness* is faulty, rather than the elaboration and secretion of catecholamines in older individuals. **Figure 1E** demonstrates that the IHR and maximal HR during exercise both decrease with aging, supporting the idea that mechanisms that reduce IHR with aging could be key contributors to the decreased maximal HR during exercise also seen with aging. Note that the rate at which peak HR declines in the absence of autonomic blockade is similar to that of IHR at rest and during peak exercise (**Figure 1E**) [18]. In other words, an age-associated decline in the acceleration of HR during exercise is, in part, due to age-associated decrease in intrinsic SAN cell (SANC) function, and not merely a function of deterioration of the autonomic signaling to the heart.

Brain-heart crosstalk does indeed wither with aging, but the diminished IHR of the cardiac pacemaker and its responsiveness to these neurotransmitters [15] must be due to deficits within the SAN itself (referred to as the 'box' in the **Figure 2A**). Although it has been known

for many years that pacemaking originates from cells within the SAN, the exact details of the underlying mechanisms have remained the subject of intensive investigation and debate. So, what exactly is in 'the box'? In order to begin to understand why aging is associated with a reduction in HR, we need to understand the essence of what makes pacemaker cells 'tick'.

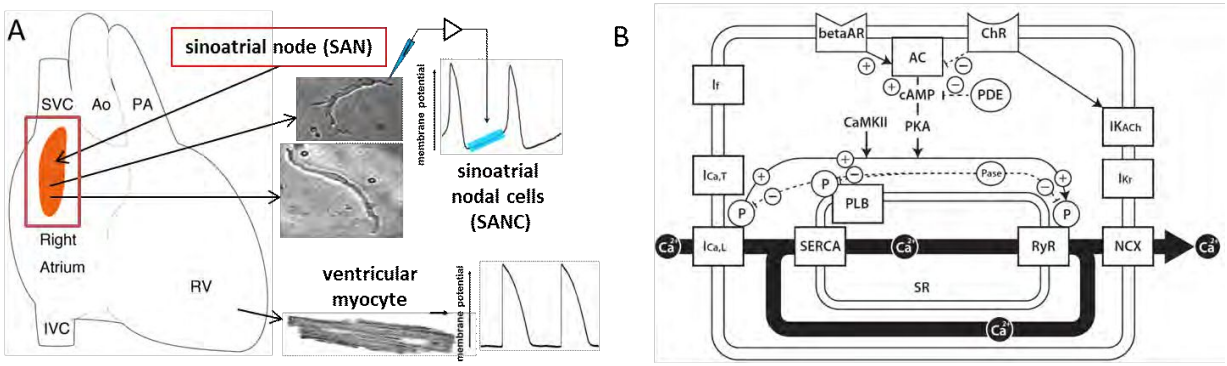


Figure 2. (A) The SAN (the 'box') discharges spontaneous electrical impulses that spread across the whole heart to execute a coordinated heartbeat. The SAN is located within the right atrium and, in the human, consists of thousands of SANC. SANC differ from working myocytes in both morphology and electrophysiology. (B) The 'coupled-clock system' in SANC comprises electrogenic membrane ion transporters (M clock, ensemble of membrane proteins on cell membrane) and intracellular Ca^{2+} cycling proteins operating between the sarcoplasmic reticulum (SR) and cytoplasm (Ca^{2+} clock). The frequency of spontaneous action potentials (AP) is determined by cAMP levels and the degree of phosphorylation of critical proteins within the coupled-clock system, and is kept in check by counteracting dephosphorylating mechanisms including phosphodiesterase activity (see text for details). Autonomic signals modify the degree of phosphorylation to manifest their accelerating/decelerating actions.

A coupled-clock in pacemaker cells keeps the time

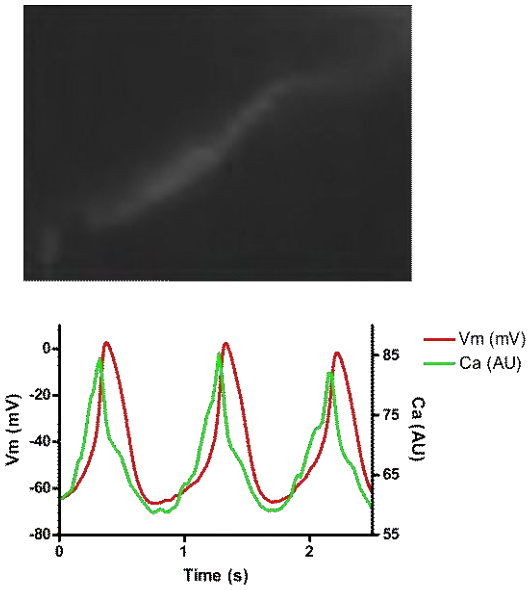
Cardiac automaticity emerges from spontaneously firing SANC, which can be enzymatically isolated from the SAN, allowing individual cellular, reductionist study [19]. Diastolic depolarization (DD) is the critical electrophysiological characteristic of pacemaker cells, which have a typically unstable, gradually depolarizing diastolic membrane potential. In contrast, much larger working atrial (AM) and ventricular myocytes (VM) have a stable diastolic resting membrane potential, and are electrically captured by signals originating in the SAN to execute the heartbeat by contracting and pumping blood (**Figure 2A**). Behind these differences are significant contrasts between SANC and AM/VM in several crucial electrophysiological aspects. For example, I_{K1}, an ionic current that maintains a stable resting membrane potential, is lacking in SANC but abundant in AM/VM [20], while Ca^{2+} cycling proteins are highly phosphorylated in the basal state SANC, but poorly phosphorylated in VM at rest [21].

Early investigations into the mechanisms underlying cardiac automaticity focused on the behavior of time- and voltage-dependent ion channels and other electrogenic molecules located in the surface membrane of SANC [22-25]. As our understanding of the presence of other mechanisms has evolved, we have come to call this particular ensemble the 'membrane clock' or 'M clock' [19].

A critical step in the development of M clock theory was the discovery of the funny current, I_f, carried by HCN channels in the early 1980s [26]. The funny current is so-called because of its peculiar nature: it is the only inward ion current to be activated upon membrane

hyperpolarization in SANC during the late phase of repolarization, close to the maximum diastolic potential [27]. It had initially been speculated that I_f protects SANC from the hyperpolarizing and suppressive effect of surrounding AM, thereafter it was proposed that I_f is '**the** pacemaker current' [25], considered by many to make the singularly substantial and critical contribution to the early stages of DD. Later, voltage-dependent Ca^{2+} channels were found to be important in DD [27]. In particular, recent evidence in murine SANC supports the view that $Ca_v1.3$, one of the subtypes of L-type voltage-dependent Ca^{2+} channel that has a more negative threshold for activation than those of $Ca_v1.2$ (which is involved in the action potential upstroke), substantially contributes to DD [27].

In parallel with the discovery of the components of the M clock in SANC, we and others had been studying the dynamics of intracellular Ca^{2+} release in VM with aging, in particular spontaneous Ca^{2+} releases, which, when dysregulated, can cause serious functional decline, including VM generated arrhythmias and loss of contractile function [28, 29]. The understanding that spontaneous Ca^{2+} releases in VM could generate spontaneous AP [21] led us to hypothesize that intracellular Ca^{2+} may similarly contribute to automaticity in SANC (suggesting the possibility of a 'Ca^{2+} clock'). We went on to show that in SANC there are indeed diastolic Ca^{2+} releases that are roughly periodic and persist even in the absence of M clock activity [30]. These Ca^{2+} releases in SANC emanate from the SR via ryanodine receptors (RyR), and Ca^{2+} is thereafter pumped back into the SR through the sarco-endoplasmic reticulum-Ca^{2+}-ATPase (SERCA). In SANC, Ca^{2+} release occurs in diastole spontaneously, sporadically, rhythmically and locally (in so-called 'local calcium releases (LCRs)'). Startling movies of 2D high-speed fluorescence imaging (**Supplemental movie 1** available online) demonstrate that LCRs 'dance' beneath the cell membrane during the diastolic phase between APs [31]. The electrical function of LCRs is executed via their interaction with the membrane bound electrogenic sodium-calcium exchanger (NCX), which mainly exchanges a single outward Ca^{2+} ion with three inward Na^+ ions, resulting in a net inward current that depolarizes the membrane potential, and contributes critically to DD [32]. Note that, similar to I_f, I_{NCX} is also voltage-dependent and is activated by hyperpolarization.



Supplemental movie 1. (upper panel) A 2D Ca^{2+} fluorescent imaging of a spontaneously beating guinea pig SANC shows AP-induced whole cell Ca^{2+} transients and LCRs 'dancing' in the cytoplasm between AP-induced transients. (lower panel) Simultaneously recorded membrane potential (Vm) superimposed on whole-cell Ca^{2+} transients.

Through the discovery and characterization of this mechanism, we 'married' the Ca^{2+} clock to the M clock, and thereafter began to elucidate the intricate details of how they work together as a system in marriage (**Figure 2B**) [22, 33], as a COUPLED CLOCK SYSTEM. Spontaneous LCRs emanating from the intracellular SR activate NCX, causing membrane potential to reach the threshold for opening of L-type Ca^{2+} channels (-35 to -40 mV). The ensuing Ca^{2+} influx interacts with RyR to cause a larger, 'global' Ca^{2+} release across a cell, the so-called Ca^{2+} transient. This process, called 'Ca^{2+}-

induced Ca^{2+} release' in VM, depletes the SR. SERCA replenishes the SR with the available intracellular Ca^{2+} (derived from SR release itself and from extracellular influx via L- and T-type Ca^{2+} channels) to critically balance the oscillatory substrate, Ca^{2+}.

What drives the pacemaker cell coupled-clock system?

Constitutively-active, Ca^{2+}-activated adenylyl cyclases (AC), more specifically AC1 and AC8 in SANC [34, 35], play a central role in the coupled-clock system (**Figure 2B**). cAMP is the product of these enzymes, and is continuously produced, in turn activating important phosphorylating enzymes (including protein kinase A, PKA) in SANC, leading to phosphorylation of critical proteins that drive pacemaking. cAMP levels are constitutively higher in SANC than VM [36]. Ca^{2+} releases in SANC also activate calmodulin kinase II (CaMKII), which is also involved in regulation of the beating rate [37]. As a result, the ratio of phosphorylated to total (phosphorylated plus unphosphorylated) phospholamban and RyR molecules is higher in SANC than VM [36]. In addition to its downstream phosphorylating effect, cAMP also *directly* activates the M clock protein, I_f [38].
M clock and Ca^{2+} clock proteins interact with each other at several critical points; the intracellular Ca^{2+} released from the SR via RyR drives the M clock directly via flow of Ca^{2+} ions through NCX, and indirectly via regulatory effects to activate AC, contributing to the genesis of DD, while L-type Ca^{2+} channels and NCX contribute to the Ca^{2+} replenishment and depletion from the SR, respectively. This finding led us to consider that all of these molecules seen in the **Figure 2B** belong to **both** clocks, i.e. they are one SYSTEM. Indeed, both clocks are crucial for normal automaticity, and for the range and robustness of beating exhibited by SANC [19].
As noted above, Ca^{2+} being released from SR via RyR activates ACs, which generate cAMP, which indirectly phosphorylates and directly activates critical proteins of the coupled-clock system, including RyR themselves in a 'feed-forward' or positive feedback fashion. Left unchecked, the signal from this system would keep getting stronger and stronger until it plateaud at its maximum activity. To prevent this, 'brakes' exist in the system. One such brake is the phosphodiesterase (PDE) set of enzymes, which hydrolyze cAMP [39]. Another brake is a set of phosphatases that modulate activity of protein kinases, to keep phosphorylation levels of critical proteins in check. Even with these brakes in place, automaticity proceeds in SANC via maintaining cAMP-phosphorylation near its mid point; the same is not true in either AM or VM. Muscarinic receptor agonism (e.g. vagal stimulation leading to acetylcholine release, or application of the synthetic agonist carbachol) reduces phosphorylation levels with concomitant activation of IK_{Ach} at higher concentrations, acting as a brake on the same mechanisms of the pacemaker cell clock system that regulate intrinsic automaticity, leading to negative chronotropism (**Figure 2B**) [40]. When β-adrenergic receptors are stimulated (e.g. by the synthetic agonist isoproterenol, or by the natural ligands epinephrine and norepinephrine), the beating rate 'revs up' due to increased cAMP, phosphorylation levels and accelerated Ca^{2+} cycling in the coupled-clock system [36].

Do other mammals recapitulate human changes in heart rate with aging?

Do other animals experience the same age-related changes in HR, and if they do what can we learn from them? For example, do rodents mimic the human phenotype of the IHR deceleration with age? Because the life span of laboratory mouse is reported to be about 27

months [41] and the age at which 50% of animals are dead in our study is 24 months [42], 3 and 24 months old may be reasonably considered young and old, respectively for mice. **Figure 3A** demonstrates basal HR measured *in vivo* in mice, showing that (as in humans) it does not change during aging. However, if we uncover the IHR using dual autonomic blockade we see that IHR *in vivo* begins to drop off at around 18 months of age [42]. Beating rate in the isolated murine SAN, which consists of only about 500 cells [43], is similarly significantly reduced at 24 months, suggesting involvement of mechanisms intrinsic to the SAN rather than simply autonomic remodeling [44]. Therefore, the mouse does indeed replicate important human features in terms of a decrease in IHR with age.

To dissect out the mechanisms behind the underlying age-related changes in the coupled-clock system, the behavior of SAN preparations from 3 and 24 month old mice were compared. The sensitivity of the isolated SAN to β-adrenergic stimulation with isoproterenol is decreased at 24 months compared to 3 months. For cholinergic stimulation, the story is same, in that IC_{50} and maximal beating rate reduction are greater and less, respectively, in older mice [44] (**Figure 3B**).

So we know that aging leads to problems with translating autonomic signals at the receptor level into beating rate changes at the SAN level. However, are the actual *intracellular* signals downstream from the autonomic receptors that execute receptor-mediated signals also weaker with aging? To understand this, we can rev up the beating rate by blocking intracellular PDEs, which act distal to autonomic receptors, and in doing so bypass the autonomic receptors to ask more direct questions about what happens with aging to *intrinsic mechanisms inside* SANC. We demonstrated a significant decrease in sensitivity to PDE inhibition (IBMX) in 24 month old SAN (**Figure 3B**), indicating that the responsiveness of parts of the internal coupled-clock machinery is also decreased by aging [44].

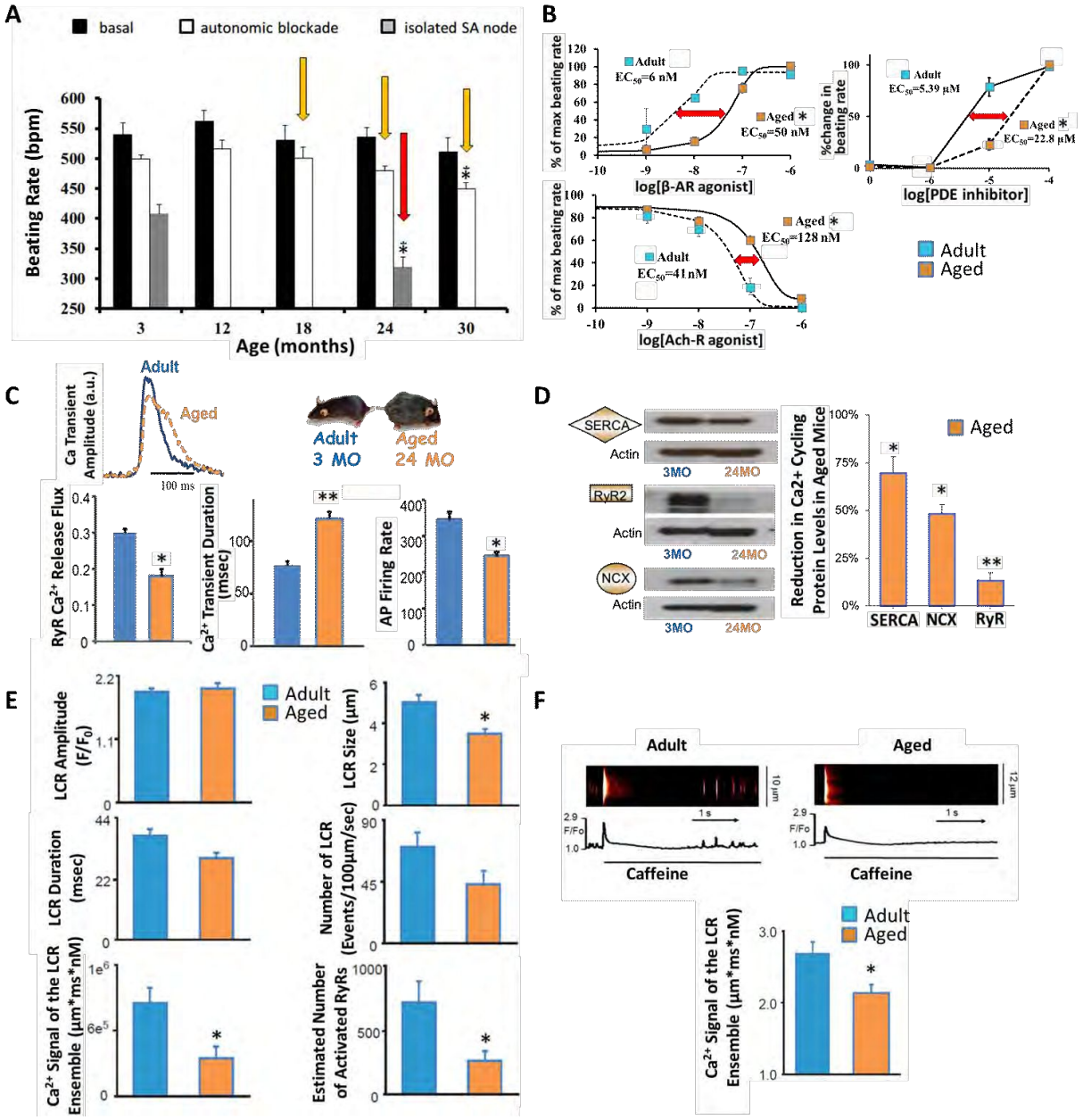


Figure 3. Observations from an in vivo rodent model of age-related SAN and SANC dysfunction. (A) As in humans, resting HR appears to remain stable across the ages; however, IHR covertly diminishes with aging [42]. (B) Shifts in dose-response curves suggest that it is not only autonomic receptor sensitivity but also mechanisms intrinsic to SANC that diminishes with aging [45]. Aging is associated with alterations in: (C) characteristics of intracellular Ca^{2+} transients, (D) mRNA and protein expression of SERCA, RyR2 and NCX, and (E) LCR characteristics and (F) SR Ca^{2+} load [44]. *P < 0.05 and ** P< 0.01 vs. adult.

What causes the malfunction in pacemaker cell clocks with advancing age?

Virtually everything crucial for SAN functioning changes with aging. It has been shown that gene expression of components of both the M- and Ca^{2+} clock in the SAN broadly changes with aging; there exist age-dependent decreases in RyR_2 mRNA and protein, increases in mRNA of $Na_v1.5$, $Na_v\beta1$ and $Ca_v1.2$ and decreases in mRNA of $K_v1.5$ and HCN1 [46]; expression of HCN proteins is down-regulated [47]; and the profile of HCN channel isoforms

shifts with aging, accompanied by a diminished sensitivity to the direct HCN blocker ivabradine in isolated rat SAN at 30 months [47, 48]. Besides this, the number of cells within the SAN decreases [49, 50], and there is greater inequality in the size and regularity of distribution of SANC [51], along with atrophy of SANC [52], fibrosis [53], and decreases in the gap junction protein Cx43 [54].

We have focused upon trying to understand how the Ca^{2+} clock 'breaks' in advancing age. Our studies comparing single SANC isolated from the young and old murine SAN demonstrate that spontaneous AP firing rate becomes slower with aging, while the maximum rate of the upstroke of the AP induced Ca^{2+} transient (an index of 'RyR Ca^{2+} release flux') decreases, and the duration of the Ca^{2+} transient increases [44] (**Figure 3C**). These age-related changes accompany reduced expression of the calcium cycling proteins SERCA, RyR2 and NCX (**Figure 3D**) [44].

In order to purely study the Ca^{2+} clock component of the coupled-clock system directly, membrane clock functions must be negated, otherwise interaction of the two clocks at multiple levels would hamper interpretation of results. One way to do this involves the use of permeabilized cells, i.e. those without a functioning cell membrane, in which the concentration of free Ca^{2+} in the bathing solution can be fixed, allowing ultimate control of the Ca^{2+} clock environ. Under these conditions, both aged and young SANC show 'roughly periodic' spontaneous LCRs generated by their intact SR. However, aging is associated with decreased size and lower numbers of LCRs and estimated number of activated RyRs (**Figure 3E**). Furthermore, the amplitude of all Ca^{2+} signals as well as SR Ca^{2+} load is reduced in older skinned cells (**Figure 3E, 3F**) [44].

These findings suggest that the Ca^{2+} clock aspect of the coupled-clock machinery deteriorates with aging, in addition to the other membrane clock changes already noted above.

General theory of HR and cardiac contractility and how these change with age

SANC and VM share have many common proteins (see **Figure 4**). VM indeed contain their own version of the SAN's coupled-clock, which can artificially be awakened (like a sleeping dragon) by increasing phosphorylation of critical proteins [21]. While SANC operate to initiate the heartbeat utilizing their coupled-clock system with its constitutively highly phosphorylation states, VM are on stand by to execute the heartbeat with a similar 'coupled-clock', which would normally stay dormant, in part due to low phosphorylation states of critical proteins in the resting state. But once activated by an AP from the SAN, the coupled-clock system in VM translates electrical excitation into mechanical contraction via Ca^{2+}-induced-Ca^{2+} release from SR RyR, which is triggered by Ca^{2+} influx via sarcolemmal L-type Ca^{2+} channels. Interaction of the Ca^{2+} released from SR with contractile proteins complete the duty cycle (excitation-contraction coupling, or EC-coupling) [9, 21, 55] (**Figure 4**). Therefore it is reasonable to assume that the slowed kinetics of Ca^{2+} cycling in SANC that occur with aging are also likely to occur in aging VM. So, what do we know about what happens to VM as they age?

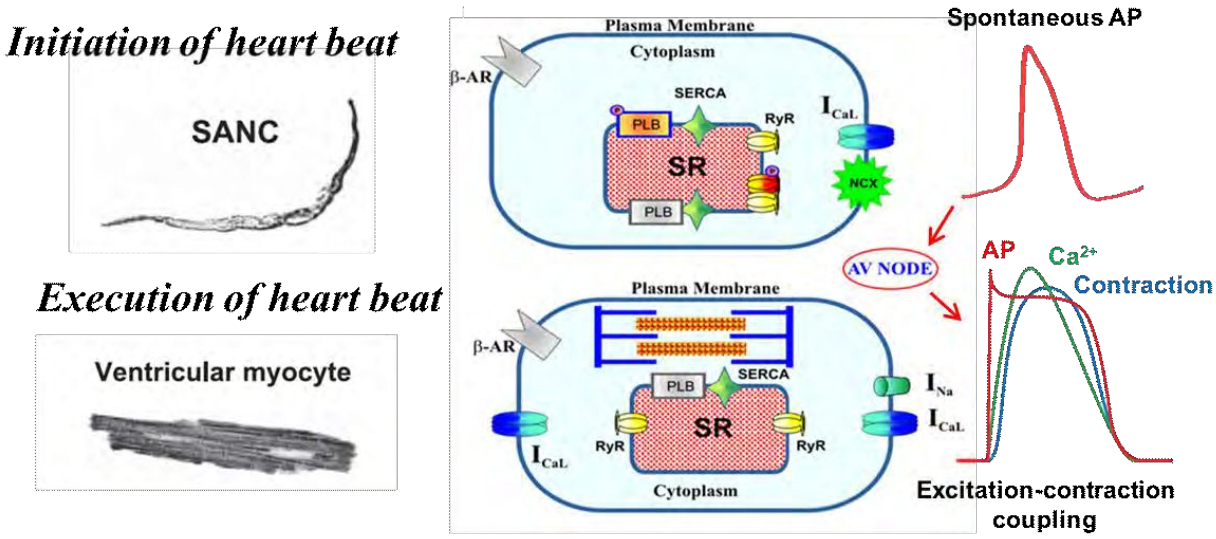


Figure 4. General theory of the heart: SANC and VM operate with shared crucial components. The coupled-clock system within SANC produces spontaneous AP, which subsequently spread to excite VM via the cardiac conduction system including the atrioventricular (AV) node, while VM execute the heart beat by converting the electrical exciation (AP) into an intracellular Ca^{2+} transient (Ca^{2+}-induced Ca^{2+} release from SR) and thereafter mechanical contraction (EC coupling) via their version of the coupled-clock, which critically involes many of the same molecules as involved in the duty cycle of the SANC. Image is from Ref [33] modified with permission.

Like the age-associated pacemaker clock dysfunction in SAN (**Figure 1B**), in apparently healthy aged humans, ventricular systolic dysfunction is not apparent at rest, when supine. Indeed, the end-systolic volume index (ESVI) measured by echocardiography is similar in resting young and old hearts (**Figure 5A**).

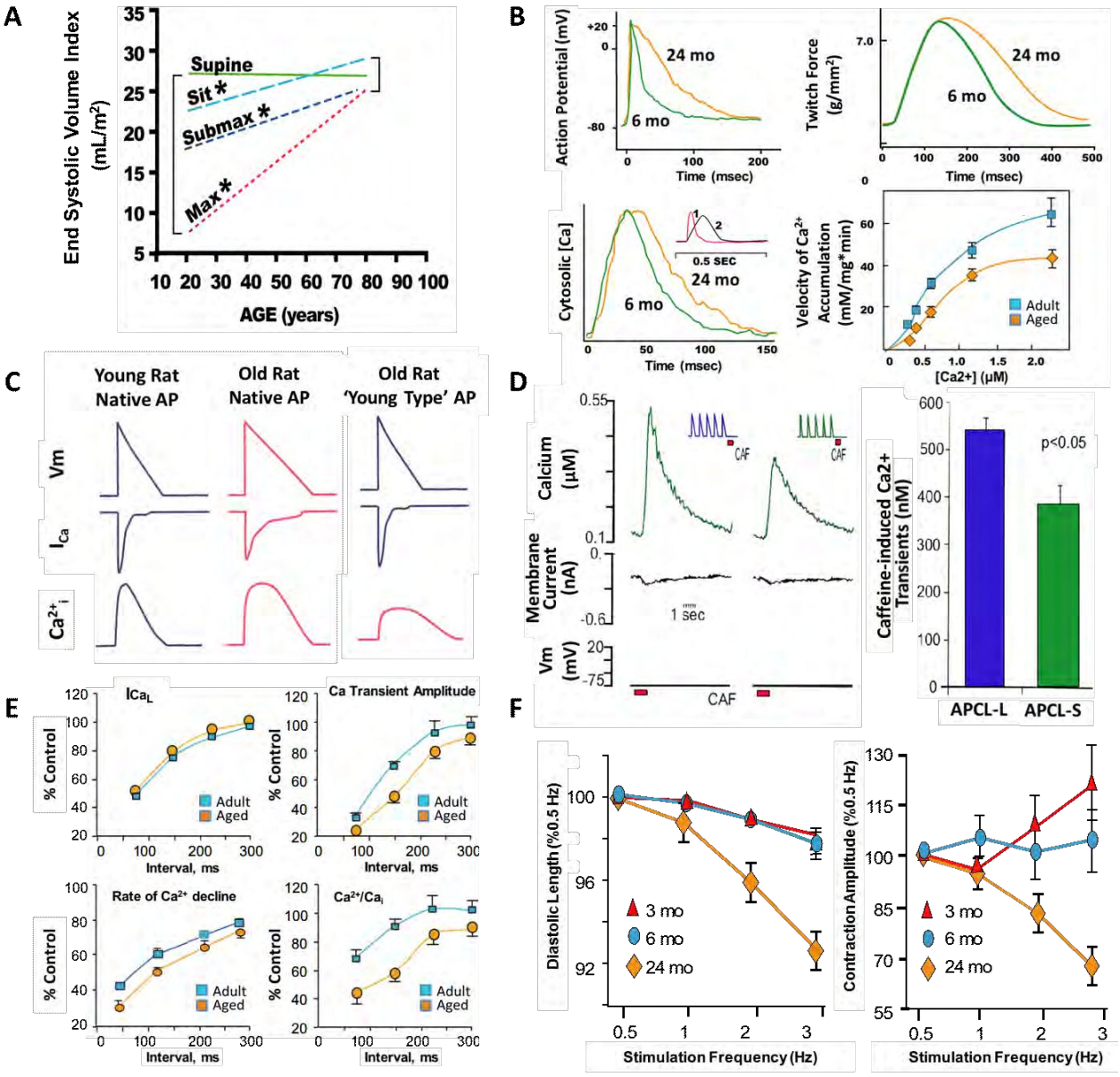


Figure 5. Dysfunction of the left ventricule (LV) in human BLSA participants (A) and rat (B-F). (A) ESVI of the human LV appears stable during aging at supine rest (green solid line); however, the functional reserve required with assumption of the upright posture or during graded exercise becomes substantially reduced with aging (cyan, blue and red dotted lines, * indicates statistical significance) [8]. (B) Studies using isolated rat LV trabecula show that LV muscle of advanced age (24 m, yellow) has prolonged repolarization time and longer contraction duration induced by an AP (upper left and upper right panel), and longer duration of intracellular Ca^{2+} release (6 mo, green line, lower left panel). Note that upstrokes of AP, twitch force and cytosolic Ca^{2+} are not substantially different between young and old, while the second half of the cycles i.e. repolarization of AP, relaxation from a contraction, decay of cytosolic Ca^{2+} are prolonged in old LV muscle. Finally, isolated SR from aged VM (orange) is less adaptive in terms of Ca^{2+} uptake than that isolated from younger VM (blue) in response to varying extracellular Ca^{2+} concentrations (lower right panel) [56]. (C) When an isolated single old rat VM (center panel) is stimulated with a 'young type AP' (so-called 'AP clamp'), old VM produce a Ca^{2+} transient that is diminished in amplitude and prolonged in duration (right, lower trace) [56]; (D) Caffeine-induced SR Ca^{2+} release following cessation of pacing with youthful APs demonstrates a significant reduction in SR Ca^{2+} load vs long AP clamp (APCL-L and APCL-S, respectively. Average data shown in right panel)[57]. (E) When 2 stimuli are given at decremental coupling intervals, 24 mo VM exhibit slower recovery kinetics of the Ca^{2+} transient

amplitude (upper right) and prolonged recovery time (lower left panel) of Ca^{2+} transient relaxation, while L-type Ca^{2+} current recovery kinetics are similar in old and young myocytes (upper left panel), resulting in a net decrease in the ratio of SR Ca^{2+} release to a given quantity of L-type Ca^{2+} current i.e. a reduced recovery of the gain of EC coupling (lower right panel) [56]. (F) at faster pacing rates, old VM (24 mo, orange) demonstrate failure to relax completely, while relaxation of younger VM is less affected by pacing rate (left panel); similarly, old VM fail to maintain contraction amplitude at faster stimulation frequencies, which again is not replicated by young VM at higher pacing rates (right panel), demonstrating both diastolic and systolic dysfunction at faster rates in old vs young VM [56].

However, incremental exercise reveals reduced systolic reserve in aged hearts (**Figure 5B**). Experiments on isolated LV trabeculae and isolated single VM provide further insights into age-related exercise-induced pump dysfunction. The absolute amplitude of the AP and its upstroke velocity, twitching force, and the cytosolic Ca^{2+} concentration of aged LV muscle are comparable to values from young animals; however, the older LV muscle exhibits a prolonged duty cycle in all of these parameters (**Figure 5B**, upper two panels and lower left panel). The substantially prolonged AP duration in old LV muscle (**Figure 5B**, upper left) may be explained by the delayed inactivation of L-type Ca^{2+} channels and reduced transient outward K current (I_{to}) [8] and consequently prolonged Ca^{2+} transient (**Figure 5B**, lower left), leading to increased inward NCX current. Note, however, that the L-type Ca^{2+} current density does not change with aging [8]. The SR of aged VM also shows a diminished relative rate of Ca^{2+} uptake in response to increasing extracellular Ca^{2+} concentrations (**Figure 5B**, lower right panel). This reduced rate of cytosolic Ca^{2+} uptake seems to be consistent with other research showing reduced mRNA and protein level of SERCA, the major Ca^{2+} pump responsible for refilling the SR [9]. It is noteworthy that as discussed above, the mRNA and protein levels of SERCA, RyR and PLB in old isolated murine SANC are also reduced, as shown in **Figure 3D**. When isolated single VM from old rat hearts are stimulated in a way that simulates the AP of young VM (so-called 'AP clamp'), the ensuing Ca^{2+} transient shows a substantially smaller amplitude, and has a longer duration compared to AP clamp in myocytes from young rats (**Figure 5C, right panel**). These observations raise the possibility that the prolonged AP duration of aged VM is an attempt to compensate for the age-related decline in intracellular Ca^{2+} handling [56, 57].

The function of old VM deteriorates further when they are forced to contract at faster rates, when time to react to increased sarcolemmal Ca^{2+} influx reduces. **Figure 5D** illustrates the effects of varying cycle length in AP-clamp experiments on old VM. SR Ca^{2+} load (measured by caffeine-induced whole SR emptying) significantly reduces when the same cells in **Figure 5C** were AP-clamped at shorter cycle lengths ('APCL-S' in **Figure 5D**) compared to longer cycle lengths ('APCL-L' in **Figure 5D**, left panel) [57]. When a so-called 'double-pulse' protocol is applied to VM, to systematically test phase-dependent alterations in recovery of the components of the coupled-clock system, old and young VM have similar revocery kinetics of the L-type Ca^{2+} current following a prior pulse (**Figure 5E**, upper left panel), but the recovery kinetics of the Ca^{2+} transient (**Figure 5E**, upper right and lower left panels) are reduced in older VM, thus the relative response of the SR to release Ca^{2+} in response to a given amount of L-type Ca^{2+} current is reduced; thus the recovery kinetics of the gain of EC-coupling (the ratio Ca^{2+} transient amplitude to that of the L-type Ca^{2+} current) are reduced with aging (**Figure 5E**, lower right panel) [56]. Similarly, the velocities of relaxation and contraction in old VM decrease at higher steady-state pacing rates. In contrast, in VM from young rats, relaxation and contraction kinetics are less affected by higher pacing rates (**Figure 5F**) [56]. Thus the age-associated reduction in ventricular EC coupling parallels the age-related reduction in SANC coupled-clock system kinetics. This concomitant age-associated decline in

SAN firing rate function and slower kinetics of the VM duty cycle may be an adaptation to avoid incomplete relaxation, reduced contraction and the tendency to develop VM generated arrhythmia.

Summary

The coupled-clock system within SANC comprises a membrane localized M clock and an intracellular Ca^{2+} clock that mutually interact to ensure life-sustaining robust and flexible cardiac automaticity. The SAN responses to autonomic receptor stimulation become reduced with aging. Sympathetic compensation maintains SAN function at rest so that it appears stable with aging. However, in reality, it is not, and IHR tails off with age. Exercise also reveals age-associated deficiency of SAN function i.e. a reduction in maximum achievable HR with age. Recent experimental findings suggest that the pacemaker clock fails because of complex flaws in the coupled-clock system, and that this underlies the observed age-related deterioration in SAN automaticity. Similarly, ventricular function in humans and rodents deteriorates with aging along similar lines, giving rise to a GENERAL THEORY of age-related decline across cardiac cells. Deterioration in VM resembles the age-related deterioration in SANC with regard to multiple similarities in reduced functional reserve in respective coupled-clock systems. Although there have been several novel discoveries regarding the mechanisms contributing to age-related decline in SANC and VM function, more research needs to be undertaken so that we are able to ideally avoid or if necessary treat the consequences of these **heartbreaks of getting old**.

Abbreviations

AC	adenylyl cyclase
AP	action potential(s)
AM	atrial myocyte
CaMKII	Calcium/calmodulin dependent kinase II
EC coupling	excitation-contraction coupling
DD	diastolic depolarization
ESVI	end systolic volume index
HR	heart rate
IHR	intrinsic heart rate
LCR(s)	local Ca^{2+} release(s)
LV	left ventricle, or ventricular
PKA	Protein kinase A
RyR	ryanodine receptor
SAN	sinoatrial node
SANC	sinoatrial nodal cell(s)
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
VM	ventricular myocyte

References

[1] **Mond HG, and Proclemer A**. The 11th World Survey of Cardiac Pacing and Implantable Cardioverter-Defibrillators: Calendar Year 2009 − A World Society of Arrhythmia's Project. *Pacing and Clinical Electrophysiology.* 2011.
[2] **Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, et al.** Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation.* 2014; 129: e28-e292.
[3] **Jose AD, and Collison D**. The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res.* 1970; 4: 160-167.
[4] **Opthof T**. The normal range and determinants of the intrinsic heart rate in man. *Cardiovascular research.* 2000; 45: 173-176.
[5] **Erikssen J, and Rodahl K**. Resting heart rate in apparently healthy middle-aged men. *Eur J Appl Physiol Occup Physiol.* 1979; 42: 61-69.
[6] **Kostis JB, Moreyra AE, Amendo MT, Pietro JD, Cosgrove N, and Kuo PT**. The effect of age on heart rate in subjects free of heart disease. Studies by ambulatory electrocardiography and maximal exercise stress test. *Circulation.* 1981; 65: 141-145.
[7] **Fleg JL, O'Connor F, Gerstenblith G, Becker LC, Clulow J, Schulman SP, and Lakatta EG**. Impact of age on the cardiovascular response to dynamic upright exercise in healthy men and women. *J Appl Physiol (1985).* 1995; 78: 890-900.
[8] **Lakatta EG**. Cardiovascular regulatory mechanisms in advanced age. *Physiol Rev.* 1993; 73: 413-467.
[9] **Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, Yin FC, and Lakatta EG**. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation.* 1993; 88: 1456-1462.
[10] **Arbab-Zadeh A, Perhonen M, Howden E, Peshock RM, Zhang R, Adams-Huet B, Haykowsky MJ, and Levine BD**. Cardiac remodeling in response to 1 year of intensive endurance training. *Circulation.* 2014; 130: 2152-2161.
[11] **De Luca A, Stefani L, Pedrizzetti G, Pedri S, and Galanti G**. The effect of exercise training on left ventricular function in young elite athletes. *Cardiovasc Ultrasound.* 2011; 9: 27.
[12] **Stickland MK, Welsh RC, Petersen SR, Tyberg JV, Anderson WD, Jones RL, Taylor DA, Bouffard M, and Haykowsky MJ**. Does fitness level modulate the cardiovascular hemodynamic response to exercise? *J Appl Phys.* 2006; 100: 1895-1891.
[13] **Rodeheffer RJ, Gerstenblith G, Becker LC, Fleg JL, Weisfeldt ML, and Lakatta EG**. Exercise cardiac output is maintained with advancing age in healthy human subjects: cardiac dilatation and increased stroke volume compensate for a diminished heart rate. *Circulation.* 1984; 69: 203-213.
[14] **Katzel LI, Sorkin JD, and Fleg JL**. A comparison of longitudinal changes in aerobic fitness in older endurance athletes and sedentary men. *J Am Geriatr Soc.* 2001; 49: 1657-1664.
[15] **Fleg JL, Tzankoff SP, and Lakatta EG**. Age-related augmentation of plasma catecholamines during dynamic exercise in healthy males. *J Appl Physiol (1985).* 1985; 59: 1033-1039.
[16] **Jose AD, Stitt F, and Collison D**. The effects of exercise and changes in body temperature on the intrinsic heart rate in man. *Am Heart J.* 1970; 79: 488-498.
[17] **Vestal RE, Wood AJ, and Shand DG**. Reduced beta-adrenoceptor sensitivity in the elderly. *Clin Pharmacol Ther.* 1979; 26: 181-186.
[18] **Christou DD, and Seals DR**. Decreased maximal heart rate with aging is related to reduced {beta}-adrenergic responsiveness but is largely explained by a reduction in intrinsic heart rate. *J Appl Physiol (1985).* 2008; 105: 24-29.
[19] **Lakatta EG, Maltsev VA, and Vinogradova TM**. A coupled SYSTEM of intracellular Ca2+ clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ Res.* 2010; 106: 659-673.
[20] **Satoh H**. Sino-atrial nodal cells of mammalian hearts: ionic currents and gene expression of pacemaker ionic channels. *J Smooth Muscle Res.* 2003; 39: 175-193.
[21] **Sirenko S, Maltsev VA, Maltseva LA, Yang D, Lukyanenko Y, Vinogradova TM, Jones LR, and Lakatta EG**. Sarcoplasmic reticulum Ca2+ cycling protein phosphorylation in a physiologic Ca2+ milieu unleashes a high-power, rhythmic Ca2+ clock in ventricular myocytes: relevance to arrhythmias and bio-pacemaker design. *J Mol Cell Cardiol.* 2014; 66: 106-115.

[22] **Maltsev VA, Yaniv Y, Maltsev AV, Stern MD, and Lakatta EG**. Modern Perspectives on Numerical Modeling of Cardiac Pacemaker Cell. *Journal of Pharmacological Sciences.* 2014; 125: 6-38.
[23] **Noma A**. Ionic mechanisms of the cardiac pacemaker potential. *Japanese heart journal.* 1996; 37: 673-682.
[24] **Noble D**. Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations. *Nature.* 1960; 188: 495-497.
[25] **Noble D**. The surprising heart: a review of recent progress in cardiac electrophysiology. *J Physiol.* 1984; 353: 1-50.
[26] **DiFrancesco D**. A new interpretation of the pace-maker current in calf Purkinje fibres. *J Physiol.* 1981; 314: 359-376.
[27] **Mangoni ME, and Nargeot J**. Genesis and regulation of the heart automaticity. *Physiol Rev.* 2008; 88: 919-982.
[28] **Strait JB, and Lakatta EG**. Aging-associated cardiovascular changes and their relationship to heart failure. *Heart Fail Clin.* 2012; 8: 143-164.
[29] **Lakatta EG**. Functional implications of spontaneous sarcoplasmic reticulum Ca2+ release in the heart. *Cardiovasc Res.* 1992; 26: 193-214.
[30] **Vinogradova TM, Zhou Y-YY, Maltsev V, Lyashkov A, Stern M, and Lakatta EG**. Rhythmic ryanodine receptor Ca2+ releases during diastolic depolarization of sinoatrial pacemaker cells do not require membrane depolarization. *Circulation research.* 2004; 94: 802-809.
[31] **Monfredi O, Maltseva LA, Spurgeon HA, Boyett MR, Lakatta EG, and Maltsev VA**. Beat-to-Beat Variation in Periodicity of Local Calcium Releases Contributes to Intrinsic Variations of Spontaneous Cycle Length in Isolated Single Sinoatrial Node Cells. *PLoS One.* 2013; 8: e67247.
[32] **Bogdanov KY, Vinogradova TM, and Lakatta EG**. Sinoatrial nodal cell ryanodine receptor and Na+-Ca2+ exchanger - Molecular partners in pacemaker regulation. *Circulation Research.* 2001; 88: 1254-1258.
[33] **Monfredi O, Maltsev VA, and Lakatta EG**. Modern concepts concerning the origin of the heartbeat. *Physiology (Bethesda).* 2013; 28: 74-92.
[34] **Younes A, Lyashkov AE, Graham D, Sheydina A, Volkova MV, Mitsak M, Vinogradova TM, Lukyanenko YO, Li Y, Ruknudin AM, et al.** Ca(2+) -stimulated basal adenylyl cyclase activity localization in membrane lipid microdomains of cardiac sinoatrial nodal pacemaker cells. *J Biol Chem.* 2008; 283: 14461-14468.
[35] **Mattick P, Parrington J, Odia E, Simpson A, Collins T, and Terrar D**. Ca2+-stimulated adenylyl cyclase isoform AC1 is preferentially expressed in guinea-pig sino-atrial node cells and modulates the I(f) pacemaker current. *J Physiol.* 2007; 582: 1195-1203.
[36] **Vinogradova TM, Sirenko S, Lyashkov AE, Younes A, Li Y, Zhu W, Yang D, Ruknudin AM, Spurgeon H, and Lakatta EG**. Constitutive phosphodiesterase activity restricts spontaneous beating rate of cardiac pacemaker cells by suppressing local Ca2+ releases. *Circ Res.* 2008; 102: 761-769.
[37] **Wu YJ, Gao Z, Chen BY, Koval OM, Singh MV, Guan XQ, Hund TJ, Kutschke W, Sarma S, Grumbach IM, et al.** Calmodulin kinase II is required for fight or flight sinoatrial node physiology. *Proceedings of the National Academy of Sciences of the United States of America.* 2009; 106: 5972-5977.
[38] **DiFrancesco D, and Tortora P**. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature.* 1991; 351: 145-147.
[39] **Younes A, Lukyanenko Y, Lyashkov A, Tarasov K, Sirenko S, Ziman B, Juhaszova M, Graham D, and Lakatta EG**. Phosphodiesterase Expression Pattern and Activity Partitioning in Lipid Raft and in Non-Lipid-Raft Microenvironment in Cardiac Sinoatrial Nodal Cells. *Biophys J.* 2014; 106: 111A.
[40] **Lyashkov AE, Vinogradova TM, Zahanich I, Li Y, Younes A, Nuss HB, Spurgeon HA, Maltsev VA, and Lakatta EG**. Cholinergic receptor signaling modulates spontaneous firing of sinoatrial nodal cells via integrated effects on PKA-dependent Ca(2+) cycling and I(KACh). *Am J Physiol Heart Circ Physiol.* 2009; 297: H949-959.
[41] **Miller RA, Harper JM, Dysko RC, Durkee SJ, and Austad SN**. Longer life spans and delayed maturation in wild-derived mice. *Exp Biol Med (Maywood).* 2002; 227: 500-508.
[42] **Yaniv Y, Ahmet I, Tsutsui K, Behar J, Moen JM, Okamoto Y, Guiriba TR, Liu J, Bychkov R, and Lakatta EG**. Deterioration of autonomic neuronal receptor signaling and mechanisms intrinsic to heart pacemaker cells contribute to age-associated alterations in heart rate variability in vivo. *Aging Cell.* 2016; in press.
[43] **Verheijck EE, van Kempen MJ, Veereschild M, Lurvink J, Jongsma HJ, and Bouman LN**. Electrophysiological features of the mouse sinoatrial node in relation to connexin distribution. *Cardiovasc Res.* 2001; 52: 40-50.
[44] **Liu J, Sirenko S, Juhaszova M, Sollott SJ, Shukla S, Yaniv Y, and Lakatta EG**. Age-associated abnormalities of intrinsic automaticity of sinoatrial nodal cells are linked to deficient cAMP-PKA-Ca(2+) signaling. *Am J Physiol Heart Circ Physiol.* 2014; 306: H1385-1397.

[45] **Liu J, Sirenko S, Juhaszova M, Ziman B, Shetty V, Rain S, Shukla S, Spurgeon HA, Vinogradova TM, Maltsev VA, et al.** A full range of mouse sinoatrial node AP firing rates requires protein kinase A-dependent calcium signaling. *Journal of molecular and cellular cardiology.* 2011; 51: 730-739.
[46] **Tellez JO, McZewski M, Yanni J, Sutyagin P, Mackiewicz U, Atkinson A, Inada S, Beresewicz A, Billeter R, Dobrzynski H, et al.** Ageing-dependent remodelling of ion channel and Ca2+ clock genes underlying sino-atrial node pacemaking. *Exp Physiol.* 2011; 96: 1163-1178.
[47] **Huang X, Yang P, Du Y, Zhang J, and Ma A**. Age-related down-regulation of HCN channels in rat sinoatrial node. *Basic Res Cardiol.* 2007; 102: 429-435.
[48] **Huang X, Yang P, Yang Z, Zhang H, and Ma A**. Age-associated expression of HCN channel isoforms in rat sinoatrial node. *Exp Biol Med (Maywood).* 2015.
[49] **Fujino M, Okada R, and Arakawa K**. The relationship of aging to histological changes in the conduction system of the normal human heart. *Jpn Heart J.* 1983; 24: 13-20.
[50] **Davies MJ, and Pomerance A**. Quantitative study of ageing changes in the human sinoatrial node and internodal tracts. *Br Heart J.* 1972; 34: 150-152.
[51] **Lev M**. Aging changes in the human sinoatrial node. *J Gerontol.* 1954; 9: 1-9.
[52] **Shiraishi I, Takamatsu T, Minamikawa T, Onouchi Z, and Fujita S**. Quantitative histological analysis of the human sinoatrial node during growth and aging. *Circulation.* 1992; 85: 2176-2184.
[53] **Csepe TA, Kalyanasundaram A, Hansen BJ, Zhao J, and Fedorov VV**. Fibrosis: a structural modulator of sinoatrial node physiology and dysfunction. *Front Physiol.* 2015; 6: 37.
[54] **Jones SA, Lancaster MK, and Boyett MR**. Ageing-related changes of connexins and conduction within the sinoatrial node. *J Physiol.* 2004; 560: 429-437.
[55] **Maltsev VA, Vinogradova TM, and Lakatta EG**. The emergence of a general theory of the initiation and strength of the heartbeat. *J Pharmacol Sci.* 2006; 100: 338-369.
[56] **Janczewski AM, and Lakatta EG**. Modulation of sarcoplasmic reticulum Ca(2+) cycling in systolic and diastolic heart failure associated with aging. *Heart Fail Rev.* 2010; 15: 431-445.
[57] **Janczewski AM, Spurgeon HA, and Lakatta EG**. Action potential prolongation in cardiac myocytes of old rats is an adaptation to sustain youthful intracellular Ca2+ regulation. *J Mol Cell Cardiol.* 2002; 34: 641-648.

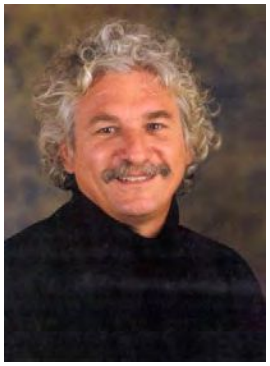
Acknowledgement

The work was entirely supported by the Intramural Research Program, National Institute on Aging, National Institutes of Health.

About the Authors

Dr. Kenta Tsutsui is MD, PhD and practice a clinical cardiology in Teikyo University Mizonokuchi hospital for 9 years treating wide range of cardiovascular diseases. When he read Dr. Lakatta's papers on the coupled-clock pacemaker system, he was inspired to join the Laboratory of Cardiovascular Science as a visiting fellow.

Dr. Oliver Monfredi is an NIHR Clinical Lecturer in Cardiac Electrophysiology in the Institute of Cardiovascular Sciences at the University of Manchester, UK. He is also a visiting scientist at the Laboratory of Cardiovascular Sciences at the NIH in Baltimore, where he has been working since 2009. He completed his PhD with Professor Mark Boyett in Manchester, looking at the fundamental mechanisms underlying heart rate variability, and has written several highly cited original science and review papers in the field. Clinically, he has an interest in the treatment of supraventricular arrhythmias and AF, and in implantable device therapy. His ongoing work includes the characterization of heterogeneity within the sinoatrial node, and the effect of obesity and athletic training on arrhythmias.



Dr. Edward Lakatta is the chief of the Laboratory of Cardiovascular Science, National Institute on Aging, NIH, USA. He also holds adjunct appointments as Professor, Department of Physiology, University of Maryland School of Medicine, and Professor, Cardiology Division, Johns Hopkins School of Medicine. Dr. Lakatta is recognized as an expert in cardiovascular research. He has authored over 450 original publications in top peer reviewed cardiovascular journals, written over 250 invited reviews/book chapters and delivered over 320 invited lectures. His studies over the last decade on pacemaker cell function, one of the research topics of his laboratory, shed light on the mechanisms of pacemaker automaticity, providing new and strong evidence that rhythmic, spontaneous calcium releases from SR within cardiac pacemaker cells prompt the surface membrane to generate rhythmic APs.