Computational Investigation of Atrial Driving: how Sinoatrial Node Heterogeneity Affects the Heart Rate

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Abstract

Despite extensive experimental and computational investigation, the mechanism by which the sinoatrial node is able to drive the atrium is not completely understood. Current knowledge considers an insulating fibrous-fatty border, discrete sinoatrial exit pathways and gradients in cellular coupling as key elements in determining atrial excitation. However, it is not known if – and how – other aspects such as cellular heterogeneity affect this phenomenon. Here, a 2D model of rabbit atrium containing the SAN was developed and used to investigate the role of heterogeneity in pacing and driving mechanisms. Simulations with homogeneous tissue show simultaneous excitation of the atrium at the exit pathways (cycle length, CL = 355 ms). When n = 5 configurations of cellular heterogeneity are considered, the atrium is effectively stimulated by dominant exit pathways, resulting in a significantly shorter CL (339 $\pm$ 2.5 ms, $p < 0.001$). Interestingly, SAN cellular heterogeneity and the presence of fibroblasts increase the safety factor for conduction (2.73 vs 2.94 $\pm$ 0.13, $p < 0.05$ and 2.94 $\pm$ 0.06, $p < 0.01$). Additionally, the presence of heterogeneity protects from bradycardia in case of 50% I_f block (CL = 387 vs 410 ms). In conclusion, the model shows that cellular heterogeneity can play a role in determining atrial excitation and heart rate, enhancing SAN robustness.

1. Introduction

The mechanisms by which the sinoatrial node (SAN) excites the heart remain incompletely understood. Many SAN anatomical and electrophysiological aspects influence pacemaking and play a role in delivering the stimulus to the right atrium: insulating borders, exit pathways and gradients in cellular connectivity [1, 2]. Heterogeneity, in the form of different electrophysiological properties among SAN cells (inter-cellular heterogeneity) and presence of different cellular phenotypes inside the SAN (e.g., fibroblasts), represents another important and underappreciated factor that influences SAN function [3]. Recent computational evidence shows that inter-cellular heterogeneity enlarges the parametric space in which spontaneous depolarization is achieved in isolated SAN tissues in both physiological and diseased conditions [3]. Less explored remains the electrophysiological role of fibrosis inside the SAN, where – opposite to the working myocardium – high amounts of fibrosis can be found in physiological conditions, especially in humans [4]. Due to their depolarized resting potential, fibroblasts could have a strong effect on SAN pacemaking depending on their density and distribution. Consequently, in the present work we hypothesized that both inter-cellular heterogeneity and fibrosis can modulate the ability of the SAN to drive the atrium, and investigated this possibility by means of a bi-dimensional computational model.

2. Methods

The 2D tissue consists of a square matrix of 200×200 discrete cells. The SAN is represented by an ellipse surrounded by an insulating border and connected to the atrium via 5 exit pathways (SEPs) [2, 4], 10x10 cells in size (= 0.5x0.5 mm [4]). As single cell models, the rabbit SDiF [5], the rabbit Lindblad [6] and the human Morgan [7] models were used for the SAN, atrium and fibroblasts, respectively. Gap junctional coupling was achieved by ohmic 4-neighbours connectivity with values: R_{atrium} = 10 kΩ, R_{SAN} = R_{fibro}= [10 MΩ, 40 kΩ], R_{border} = $\infty\Omega$. Sigmoidal gradients from the SAN left side to the atrium with slope = [1, 0.1] and $R_{50\%}$= [115, 100] were set. The two different SAN gap junctional configuration were called "low coupling" (*lc*, with R_{SAN} = R_{fibro}= 10 MΩ, slope = 1, $R_{50\%}$= 115) and "high coupling" (*hc*, with R_{SAN} = R_{fibro}= 40 kΩ, slope = 0.1, $R_{50\%}$= 100). The rationale for this was investigating both a condition were the SAN is sufficiently isolated from the atrium and one where phase synchronization could be achieved when inter-cellular heterogeneity is present inside the SAN. Inter-cellular heterogeneity was achieved by SAN maximal conductances, currents and permeabilities (P_{CaL}, P_{CaT}, g_{Kr}, K_{NaCa}, i_{NaKmax}, g_{Na}, g_{Ks}, g_f) randomization (n = 5) obtained by

log-normal distribution (σ = 0.2) sampling, as previously done [3]. The presence of diffuse fibrosis was instead obtained by substituting SAN cells with active fibroblasts. A level of 40% fibrosis was imposed in the SAN excluding the SEPs [4], and n = 5 different random spatial distributions were tested.

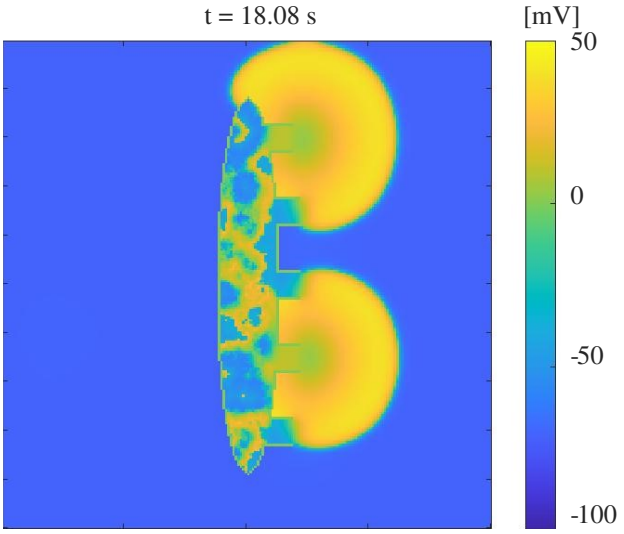

Figure 1. Successful atrial depolarization from two different SEPs in a heterogeneous SAN *lc* condition.

To gain further insights into the contribution of heterogeneity in SAN physiology, different pathological conditions were investigated. The first one consisted in the loss of fundamental ionic currents for SAN functioning – namely I_f and I_{CaL} – whose nominal maximal conductances were reduced by 50% and 25%, respectively. Secondly, a high-frequency (5 Hz) stimulus was delivered to a 5x5 cluster of atrial cells in the top-right corner of the tissue from second 6 to second 16, to assess how the SAN recovered from overdrive suppression due to arrhythmic conditions. In the last type of simulations the number of SEPs was varied from 1 to 7 in the uniform *lc* and *hc* configurations.

Simulations were started from single-cell steady-state conditions (500 s) and lasted 20 s to reach steady state in the tissue. The last 2 s of simulation were saved and analysed. In the case of arrhythmia simulation, the last 5 s of the action potential traces were analysed. Tissue cycle length (CL) was quantified as the time difference between two successive AP overshoots. The safety factor (SF) for conduction was computed as the ratio between the net charge exchanged by the cells with their neighbours (Q_{gap}) and the charge required to obtain a full upstroke in single-cell simulations (Q_{thresh}). In arrhythmia simulations, when a re-entrant circuit was established, we computed the time employed to re-obtain sinus rhythm after high-frequency stimulation is stopped (re-entry duration). CL and SF significative differences were assessed with one-sample or paired t-tests.

The simulation was initially developed as a C++ implementation of a fixed-step Euler integration algorithm (1 µs); however, the serial program proved to be too slow due to the large number of runs that are required. We modified the serial implementation to make use of GPU parallelism to speed up the critical part, i.e., the Euler integrator. The CUDA/C program running on a Linux workstation with a 12 GB Nvidia Titan V GPU is ∼120 times faster than the equivalent serial program running on a AMD Rhyzen Threadripper 2950x CPU@ running at 3.5 GHz. Data analysis was performed in Matlab R2019b.

3. Results

The simulations show that atrial driving can be achieved in different conditions: in the *lc* uniform and in the *hc* (both uniform and with heterogeneity) ones, the atrium is excited by all the 5 SEPs at the same time. In the *lc* configuration or in presence of fibrosis, specific SEPs become dominant depending on the leading pacemaker sites location (Fig 1).

Fig 3 reports the results of the CL and SF computation. As it can be seen, the presence of inter-cellular heterogeneity in the *lc* condition significantly affects the average CL (339 ± 2.5 vs 355 ms, p = 0.00013) and the robustness of atrial driving (SF = 2.94 ± 0.13 vs 2.77, p = 0.043). The latter effect is also the case in the *hc* condition (SF = 2.34 ± 0.02 vs 2.29, p = 0.0062). Interestingly, fibrosis on one hand prevents atrial propagation in the *hc* condition, but at the same time increases the SF in the *lc* one (SF = 2.94 ± 0.06 vs 2.77, p = 0.0037). However, the combination of inter-cellular heterogeneity and fibrosis did not result in a significant change of average CL or SF due to the large variability in the results.

Simulations with high-frequency stimulation in the atrium show suppression of spontaneous activity in the SAN in all conditions. However, when the stimulation is stopped, the *lc* and *hc* conditions show different behaviours: the latter is affected by re-entrant activity through the SEPs which tends to last less when heterogeneity is considered (Fig 3, 1.45 ± 0.61 vs 1.89 s, p = 0.18), whereas the *lc* condition does not show re-entry onset at all.

The results of simulations with ionic current blocks show that, while the *hc* condition is more robust with respect to 25% I_{CaL} block (since atrial driving is not achieved with the *lc* one), in the *lc* configuration the presence of heterogeneity seems to be protecting the SAN from bradycardia when a 50% I_f block is tested (Tab 1).

Finally, the number of SEPs (1-7) in the uniform *lc* configuration does not seem to affect the average CL or the SF at all, while it slightly modulates both in the *hc* one

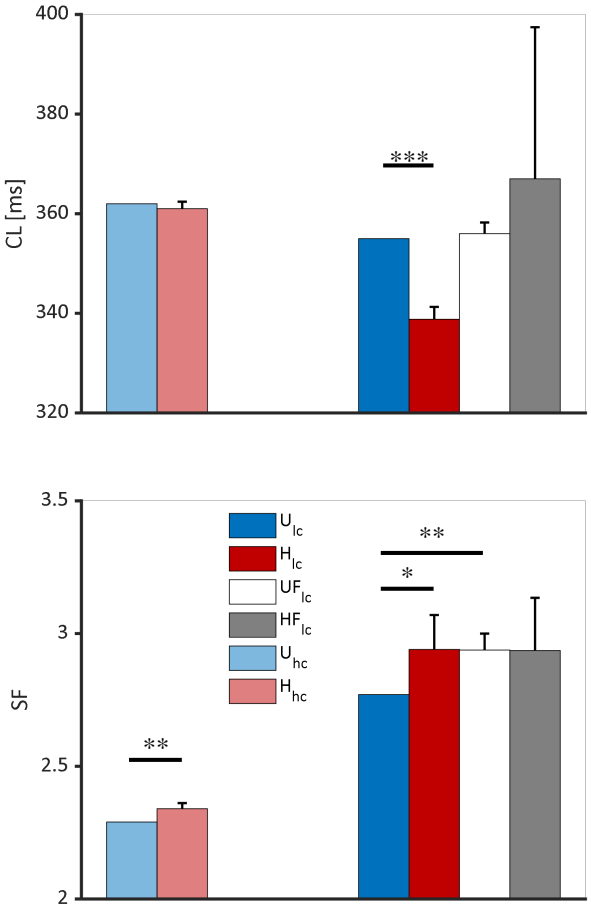

Figure 2. Cycle lengths and safety factor results in different conditions: U (uniform tissue), H (SAN heterogeneity), UF (uniform tissue with 40% fibrosis) and HF (SAN heterogeneity with 40% fibrosis) in the low coupling (*lc*) and high coupling (*hc*) SAN configurations. * p<0.05, ** p<0.01, *** p<0.001

(CL = 356 ms and SF = 2.19 with 1 SEPs vs. CL = 366 ms and SF = 2.31 with 7 SEPs).

4. Discussion and Conclusion

The results reported above show that atrial driving in the model tissue can be achieved in different ways. However, some are more robust (and thus, more likely) than others: the SF computation suggests that the *lc* configuration is safer since, by isolating the SAN, it avoids its hyperpolarization by the atrium, consequently providing a stronger propagation. About this, and specific to the aims of this work, heterogeneity significantly increases driving robustness by allowing the SAN to provide more current to atrial cells at the SEPs interface. Fibrosis, at the level tested (40% of SAN area) has a similar effect but only if the SAN is sufficiently decoupled from atrial tissue (otherwise in the *hc* condition it prevents propagation). Interestingly, the combination of inter-cellular heterogeneity and fibrosis leads to variable results in terms of average CL and SF. This is perhaps due to the fact that depending on the distribution of fibroblasts, leading pacemaker sites with different intrinsic properties (mainly the beating rate) form inside the tissue and become dominant. This could point to limitations of the present model, namely to how fibroblasts (but also SAN cell properties) are distributed in the tissue and to the absence of autonomic control over the SAN. Indeed, from the experimental evidence that fibroblasts form a cellular network encircling different SAN cell clusters [8], one could speculate that the autonomic nervous system selects one of these clusters to become the leading pacemaker site according to systemic needs. Otherwise, randomly-distributed fibrosis may represent a pathological

Block	Uniform	SAN heterogeneity
	lc	
25% I_{CaL}	No driving	No driving
50% I_f	401 ms	387 ms
	hc	
25% I_{CaL}	381 ms	376 ms
50% I_f	424 ms	420 ms

Table 1. Simulation results in diseased *lc* and *hc* conditions: 25% I_{CaL} and 50% I_f block in SAN tissue.

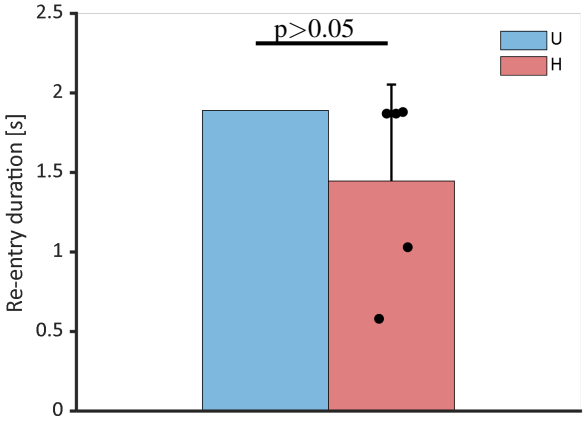

Figure 3. Results of arrhythmia simulation in the *hc* condition. No driving was obtained with 40% fibrosis inside the tissue while no re-entrant activity occurred with the baseline *lc* condition.

condition in which the fibroblasts network is lost, with a consequent heart rate dysregulation. The present model is well suited to investigate both leading pacemaker site shifts in physiological conditions and diseased condition such as the one now hypothesized. While future developments are represented by the investigation of other anatomical and electrophysiological configurations suggested by experimental data, at the present stage the model provided a preliminary confirmation [3] of the role of heterogeneity in protecting the SAN from loss of key ionic currents (Tab 1). Additionally, in certain conditions heterogeneity shows a tendency (Fig 3) in limiting re-entry onset after high-frequency stimulation (mimicking ectopic activity in the atrium). This is again probably due to the presence of cells with different intrinsic electrophysiological properties, with distinct sensitivities to overdrive suppression. However, the too high inter-cellular coupling blunts the effect of heterogeneity by homogenizing the cells' intrinsic properties, thus limiting the protection from re-entry onset in most random distributions (Fig 3) and from bradycardia with 50% I_f block (Tab 1). The fact that no re-entry was elicited in the *lc* condition suggests indeed that the most important parameter in order to achieve a robust atrial driving is inter-cellular coupling. While in this model a gradient in gap junctional conductivity in the SEPs was found to be necessary for the SAN to pace the atrium, both *lc* and *hc* conditions allowed the stimulus to propagate in the atrium, showing advantages and disadvantages (e.g. opposite to the *lc* configuration, the *hc* one allowed driving with 25% I_{CaL} block). The *hc* condition is rather unlikely if compared to gap junctional resistance experimental values ($\sim$1 GΩ [9]) and to the evidence of SAN electrical isolation established in literature. Also, no atrial depolarization is obtained when fibrosis is added to the tissue. In the *lc* condition in turn, the coupling gradient parameters might have to be tuned for the SAN to provide enough depolarizing current in order to make atrial driving (and not only SAN pacemaking [3]) robust to I_{CaL} current blocks. Also, atrial gap junctional resistance values set to obtain physiological conduction velocities in atrium are lower than experimentally reported ($\sim$5 MΩ in atrium [10]). Finally, the fact that the number of SEPs does not influence the average CL or the SF might suggest that SAN cells at the openings and inside the SEPs provide a strong enough source to depolarize the atrium at every exit, with SEPs length and width – rather than their number – being more critical parameters [2] in terms of successful propagation and average beating rate.

While further work is needed to overcome the model's limitations, this study suggests that – in particular coupling conditions – inter-cellular heterogeneity and fibrosis can be beneficial, even if not necessary, for the SAN to excite the atrium.

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