Sinoatrial Node Heterogeneity and Fibrosis Increase Robustness of Atrial Driving in a Computational Human Tissue Model

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Abstract

Cardiac pacemaking remains an unsolved matter under many points of view. Extensive experimental and computational research has been performed to describe sinoatrial physiology across different scales. Nevertheless, how the heartbeat arises inside the node and propagates to the working myocardium is still not fully understood. This work aims at providing quantitative information about this phenomenon by developing and studying a bi-dimensional computational model of human right atrial tissue including the sinoatrial node. The novelty of this study is the presence of cellular heterogeneity and fibroblast-myocyte coupling inside the sinoatrial node to investigate how they tune the robustness of stimulus formation and conduction under different conditions (baseline, ionic current blocks, autonomic modulation, external high frequency pacing). The simulations show that both heterogeneity and fibrosis significantly increase the safety factor for conduction by more than 10% in almost all the conditions tested and shorten the sinus node recovery time after overdrive suppression up to 60%. Fibroblasts help to capture the atrium especially in challenging conditions (e.g., with 25% L-type calcium current block). In conclusion, this work suggests a quantitative explanation to the astonishing overall heterogeneity shown by the sinoatrial node.

1. Introduction

The mechanisms by which the sinoatrial node (SAN) is able to excite the right atrium (RA), thus starting the cardiac cycle, are still incompletely understood. Several computational works have investigated driving mechanisms in animal models by exploring the role of gradient and mosaic configurations, the presence of transitional phenotypes in the SAN periphery and of specialized conduction pathways [1]. However, none of them considered randomized electrophysiological properties or fibroblast-myocyte interactions. Therefore, the behaviour of cellular heterogeneity and active fibroblasts is still unexplored with respect to atrial driving. Previous works in our and other labs [2, 3] highlighted a primary role for moderated levels of heterogeneity in SAN ionic properties in conferring robustness to cardiac pacemaking. At the same time, the experimental evidence of a much higher presence of fibrotic tissue in the mammalian SAN compared to the working myocardium [4] has still to be clearly explained.

In this work, bi-dimensional computational models of SAN-RA tissue featuring heterogeneity and fibrosis were developed with the aim of gaining quantitative information about atrial driving mechanisms by the SAN. The hypothesis expressed in this study is that both cellular heterogeneity and presence of fibroblasts in the SAN increase the robustness of driving capability in terms of safety factor for conduction, and of a larger parameter space for which atrial excitation is achieved in physiological and pathological conditions. Therefore, we hypothesize that heterogeneity and fibrosis specifically contribute to 1) overcome hyperpolarization from the atrium, 2) provide a current source in case of ionic current blocks in the SAN and 3) protect the SAN from overdrive suppression in the case of high-frequency stimulation.

2. Methods

The model consists of a square matrix of 200×200 cells with the sinoatrial node located at the center and surrounded by an insulating border which prevents the interaction with atrial tissue if not for 5 exit pathways (SEPs) of 20×11 cells in dimensions (Figure 1). We adopted the Koivumäki et al. 2011 model [5] for atrial cells (K model) and the Morgan 2016 [6] for fibroblasts. The density of the latter decreased from 45.7% in the central SAN to 27.7 in the SEPs [4] following a linear gradient. For the SAN, the maximal conductances of the main ionic currents and the maximal uptake rate of the SERCA pump of the Fabbri et al. model [7] were randomized as previously done [2]. No heterogeneity or fibrosis were considered in the atrium. Five different distributions of heterogeneity and fibrosis were investigated to improve the generalizability of the results (tissue #1-5). Coupling resistance values (R_{gap}) of $1\,\mathrm{G\Omega}$ were chosen for SAN cells and fibroblasts, according to experimental measures and previous computa-

tional works [1]. An R_{gap} of $1\,\mathrm{M\Omega}$ was set in the RA to achieve a conduction velocity of $100\,\mathrm{cm\,s^{-1}}$. This value is typical of the crista terminalis tissue and was obtained by doubling the maximal conductance of the fast sodium current g_{Na} in the K model [8]). A sigmoidal gradient in coupling conductivity from SAN to RA was implemented [8].

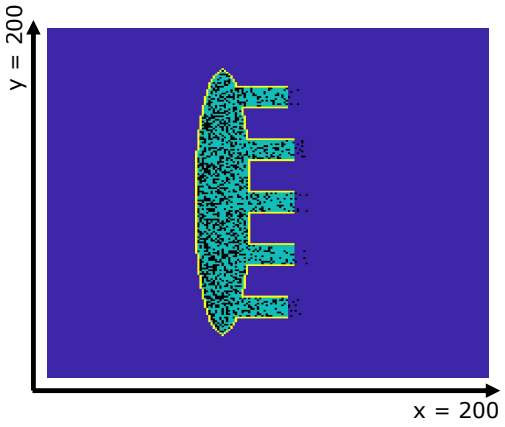

Figure 1. Geometry of the 2D human model. The yellow contour line shows the insulating border, while dark blue indicates atrial tissue. SAN tissue is reported in green while fibroblasts are represented in black. The SEPs have been numbered #1-5 from top to bottom.

Simulations were run starting from $500\,\mathrm{s}$ single cell steady-state conditions and lasted for $50\,\mathrm{s}$. The last $5\,\mathrm{s}$ were saved and analyzed to compute the safety factor for conduction (SF, computed as in [9] and averaged on the atrial cells at the interface of the leading SEP). To investigate the response to overdrive suppression of the SAN, a high-frequency ($2\,\mathrm{Hz}$) pacing protocol was performed [10] from second 32 to second 42, then the pacing was stopped. In this case, the last $10\,\mathrm{s}$ of the simulations were exported to compute the sinus node recovery time $SNRT$ (the time difference between the first atrial action potential occurring after the last stimulus and the time instant of the last stimulus [10]). Additional simulations with 50% and 25% blocks in I_f and I_{CaL} were performed and the effects of $25\,\mathrm{nM}$ acetylcholine (ACh) and $1\,\mu\mathrm{M}$ isoproterenol (ISO) continuous infusion were assessed in both baseline and stimulated conditions. For clarity, we define "U" as the uniform condition (no heterogeneity or fibrosis), "H" as the condition with SAN heterogeneity, "UF" as the condition with fibrosis but no heterogeneity and "HF" as the condition in which both SAN heterogeneity and fibrosis are included. Statistical differences were assessed via one-sample or paired t-tests. P-values of 0.05 or lower were deemed significant. All data analyses were performed in Matlab R2019b.

The simulations took advantage of High Performance Computing approaches. An efficient CUDA/C program exploiting GPU parallelization was developed to update the cells' state variables. The program was run on a Linux server equipped with a Nvidia Titan V GPU with 5120 cores and $12\,\mathrm{GB}$ of device RAM, and an AMD Rhyzen Threadripper 2950x CPU running at $3.5\,\mathrm{GHz}$. A time step of $5\,\mu\mathrm{s}$ was used and data were undersampled at $200\,\mu\mathrm{s}$.

3. Results

The simulation results show that both fibroblasts and heterogeneity act on the pace and drive ability of the SAN by increasing the SF with respect to a uniform tissue condition (1.62 ± 0.02 and 1.58 ± 0.06 vs. 1.45, $p = 2 \times 10^{-5}$ and $p = 0.009$ respectively, Figure 2). The combination of the two provides a raise in the SF slightly higher than heterogeneity alone (1.60 ± 0.05, $p = 0.002$ vs. uniform), even if lower than fibroblasts alone (both not significant).

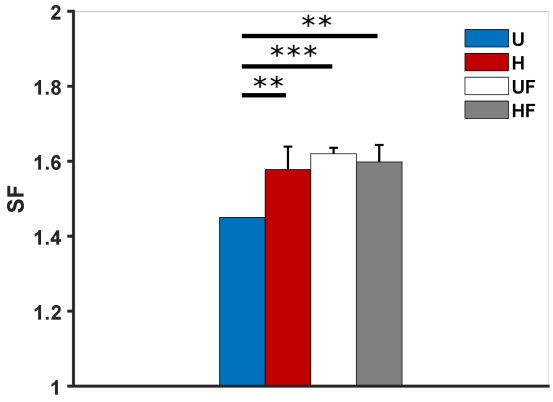

Figure 2. Safety factor results in different conditions: U (uniform tissue), H (SAN heterogeneity), UF (uniform tissue with fibrosis) and HF (SAN heterogeneity and fibrosis). *p<0.05, **p<0.01, ***p<0.001.

Under $25\,\mathrm{nM}$ ACh administration, heterogeneity and fibrosis still provide strength to atrial driving (SF = 1.60 ± 0.05 and 1.54 ± 0.09 for the H and HF conditions vs. 1.44 of the uniform one, $p = 0.002$ and $p > 0.05$) even if the HF set-up did not provide significantly higher values. When $1\,\mu\mathrm{M}$ ISO is applied, the highest SFs are obtained, with a remarkable increase (>20%) in the H and HF model compared to the uniform one (1.88 ± 0.08 and 1.86 ± 0.08 vs. 1.53, $p = 5 \times 10^{-4}$ and $p = 7 \times 10^{-4}$).

Stimulation of atrial tissue at $2\,\mathrm{Hz}$ resulted in overdrive suppression of the SAN, with all paced stimuli entering the SAN from all of the 5 SEPs. The H condition shows a slightly lower – but significant – value than the uniform set-up ($1047\pm18\,\mathrm{ms}$ vs. 1102, $p = 0.002$). However, in these conditions the first recovered beat originates from

the SEPs and then macro re-entry circuits with complex patterns (e.g., 2:1 exit block in the SEPs) are stabilized in most of the tissues. Exit blocks do not happen in the F (1124±9 ms, p = 0.005 vs U and p = 0.0009 vs H) and HF conditions (1009±164 ms, p>0.05), but all 5 tissues show re-entrant activity in both cases.

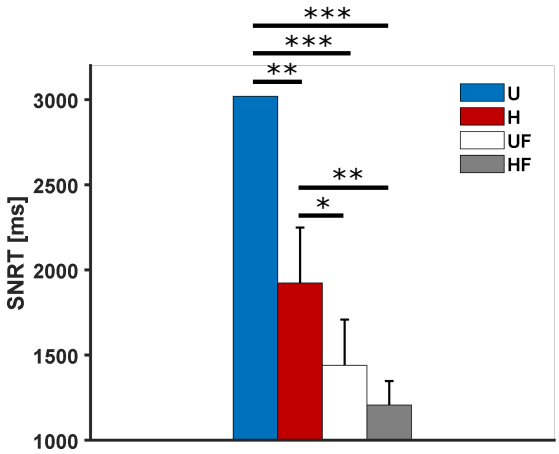

Figure 3. Sinus node recovery time results with 25 nM acetylcholine administration after 2 Hz pacing in different conditions: U (uniform tissue), H (SAN heterogeneity) and HF (SAN heterogeneity and fibrosis). *p<0.05, **p<0.01, ***p<0.001.

Figure 3 shows that the H, UF and HF set-ups determine significantly lower $SNRT$ values: 1923±325 ms for the heterogeneous tissue, 1440±269 ms with fibroblasts only and 1206±141 ms in presence of both heterogeneity and fibroblasts vs. 3020 ms of the uniform model (p = 0.002, p = 2×10^{-4} and p = 9×10^{-6}, respectively). The decrease in $SNRT$ given by the addition of fibroblasts resulted significant also with respect to the presence of cellular heterogeneity alone (UF vs. H: p = 0.03, HF vs. H: p = 0.007). Compared to results without ACh administration, the uniform condition sees a $SNRT$ prolongation of $+174\%$. In this condition, SAN depolarization originates in the middle of the SEPs as before but no re-entry is established even though 2:1 exit blocks occur for the first two beats. In presence of heterogeneity, re-entrant circuits are formed in all tissues, but the administration of ACh increases the number of exit blocks after the cessation of pacing. With fibroblasts, only tissue #3 of the HF condition shows exit blocks from the SEPs.

Compared to control, 1 µM ISO shortens the $SNRT$ in the U condition (841 ms vs. 1102 ms, -24%). The H and HF setups provide an even faster recovery ($SNRT$ = 775±18 ms and 790±31 ms, respectively p = 0.001 and p = 0.02 vs. U) also compared to their control values (p = 4×10^{-6} and p = 0.047).

When a 50% block in I_f was tested, a 12% CL prolongation was obtained in the U condition (912 ms vs. 814 ms). Similarly, the UF and HF conditions showed CL prolongations of 10.7% (745±27 ms vs. 665±55 ms) and 11% (907±2 ms vs. 817±1 ms, only 3 tissues showing beating). However, the presence of heterogeneity (H and HF conditions: 741±25 ms and 745±27 ms) determined a significantly shorter CL compared to the U and UF set-ups.

A 25% I_{CaL} block leads to loss of driving in the U and in all H models. Intriguingly, 4 out of 5 HF models show a pace and drive behaviour. Tissue #3 and #4 show however 3:1 and 2:1 exit blocks with consequent extreme atrial bradycardia (CL = 2583 ms or 23 bpm, showing only 2 APs in 5 s and CL = 1797±0 ms or 33 bpm). Tissue #5 has a complex electrical activity as well, with re-entrant excitation showing 1 exit block in the last 5 second of the simulation, with a resultant CL of 1142±463 ms. Only tissue #2 shows a regular and physiological rate (CL = 806±0 ms), but the activation starts from the center of SEP #2. For this 4 models, the SF appears to be slightly reduced (1.48±0.14), even if not significantly.

A final simulation including heterogeneity, fibroblasts (HF tissue #3) and simultaneous administration of 25 nM ACh and 1 µM ISO (named "full" model), was run to model a condition as close as possible to SAN physiology. In this setting, a CL of 961 ms and a SF of 1.75 were achieved. Atrial excitation occurred after 120 ms through SEP #5 and the slow CV inside the SAN allowed the depolarization to enter from the other SEPs. In the absence of fibroblasts, pacemaking activity was interestingly completely suppressed. If R_{gap} was lowered to 10 and 100 MΩ for SAN cells and fibroblasts, respectively, more physiological activation times and sequences were achieved (80 ms) but at the cost of a bradycardic rate (CL = 1192 ms).

4. Discussion

The simulations reported above suggest that heterogeneity and fibrosis increase SAN driving capability and improve its recovery from overdrive suppression.

In all conditions, the H configuration shows significantly higher SF values ($> +10\%$). This is likely due to the presence of "stronger" cells (i.e. expressing for example more I_{CaL}) that deliver more current at the SEP interface. UF and HF conditions have a similar behaviour, since fibroblasts act as current sources and allow SAN cells to provide more charge to atrial tissue. Indeed, heterogeneity and fibrosis determine depolarized MDPs (U: -58.5±1.6, H: -56±2.5, F: -55.8±2.6, HF -54.4±6.4 mV) and higher maximal upstroke velocities (U: 7.4±1.3, H: 8.5±1.9, F: 7.9±2.2, HF: 8.4±2.6 $\mathrm{mV\,ms^{-1}}$) in the leading SEP, compared to when they are absent. This same mechanism explains why the presence of fibroblasts determines atrial

driving despite the reduction in I_{CaL} (U: -58.5±2.1, H: -55.7±2.5, HF: -53.6±3 mV and U: 5.4±0.6, H: 6.1±1.2, HF: 6.5±2.4 $\mathrm{mV\,ms^{-1}}$).

In this work the indirect $SNRT$, measuring the time interval between the first atrial AP and the last paced beat [10] was implemented to quantify the ability of the SAN to recover the driving capability after overdrive suppression. All conditions show $SNRT$ values after pacing at 2 Hz similar to those obtained in two explanted human hearts: U = 1102 ms, H = 1047±18 ms, UF = 1124±9 ms and HF = 1063±163 ms vs. 1020 and 1459 ms [10]. When corrected for the basal CL, the $SNRT$ value of the uniform condition (288 ms) is in agreement with computational reports (167 ms with pacing at 950 ms [10]). However, after external pacing no exit blocks were observed in the experiments [10], but were obtained only with reduced sodium current and ACh administration or in heart failure remodelling conditions leading to re-entry.

However, heterogeneity and especially its synergism with fibroblasts determine faster recoveries, particularly under challenging conditions such as during ACh administration. In the latter case indeed, fibroblasts greatly reduce the $SNRT$ compared to heterogeneous tissues (UF: -25% and HF: -37%), which in turn recovered much earlier than uniform ones (-36%). In addition, heterogeneity and fibroblasts protect SAN pacemaking from bradycardia and failure in case of I_f and I_{CaL} block, respectively.

The limitations of the model include 1) the absence of a human SAN-specific fibroblast model; 2) the random distribution of fibroblasts, instead of a more physiological network/scaffold architecture; 3) the simplified 2D geometry and 4) the slow conduction velocity inside the SEPs, which favours re-entrant activity. Despite this, our model suggests a novel and intriguing role for nodal heterogeneity and fibrosis in atrial driving.

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