Bradycardia and Slowing of the Atrioventricular Conduction in Mice Lacking CaV3.1/α1G T-Type Calcium Channels

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Abstract—The generation of the mammalian heartbeat is a complex and vital function requiring multiple and coordinated ionic channel activities. The functional role of low-voltage activated (LVA) T-type calcium channels in the pacemaker activity of the sinoatrial node (SAN) is, to date, unresolved. Here we show that disruption of the gene coding for CaV3.1/α1G T-type calcium channels (caca1g) abolishes T-type calcium current (I_{Ca,T}) in isolated cells from the SAN and the atrioventricular node without affecting the L-type Ca^{2+} current (I_{Ca,L}). By using telemetric electrocardiograms on unrestrained mice and intracardiac recordings, we find that cacna1g inactivation causes bradycardia and delays atrioventricular conduction without affecting the excitability of the right atrium. Consistently, no I_{Ca,T} was detected in right atrium myocytes in both wild-type and CaV3.1/−/− mice. Furthermore, inactivation of caca1g significantly slowed the intrinsic in vivo heart rate, prolonged the SAN recovery time, and slowed pacemaker activity of individual SAN cells through a reduction of the slope of the diastolic depolarization. Our results demonstrate that CaV3.1/T-type Ca^{2+} channels contribute to SAN pacemaker activity and atrioventricular conduction. (Circ Res. 2006;98:1422-1430.)

Key Words: pacemaker activity ■ T-type calcium channel ■ sinoatrial node ■ conduction ■ knockout mice

The initiation of the heartbeat requires coordination between the automaticity of the sinoatrial node (SAN) and excitability of the atrioventricular (AV) conduction tissue: the AV node (AVN) and the His–Purkinje fiber network. SAN automaticity is caused by the presence of the diastolic depolarization.1 Multiple classes of ionic channels are expressed in the SAN,1,2 but the precise mechanism initiating the diastolic depolarization has not been entirely elucidated, and the relative contribution of different ionic channels in establishing the heart rate under specific physiological conditions is still a matter of debate.3–5 Strong functional, pharmacological, and genetic evidence show that the hyperpolarization-activated currents I_{Kd}6,7 and I_{Ca,T}8,10 play major roles in controlling the diastolic depolarization after decaying of the fast component of delayed-rectifier K+ currents (I_K). Diastolic release of Ca^{2+} mediated by ryanodine receptors (RyRs) has been indicated as an important mechanism for controlling SAN pacemaking under activation of the β-adrenergic receptor.11 The SAN also expresses neuronal tetrodotoxin (TTX)-sensitive and cardiac TTX-resistant voltage-dependent Na+ currents (I_{Na}).12 TTX-sensitive I_{Na} is involved in SAN pacemaking in the newborn rabbit13 and in the adult mouse.12,14 The cardiac TTX-resistant SCN5A-mediated I_{Na} is important for conduction from the SAN to the atrium and in intranodal conduction.15 I_{Na} is also expressed in the rabbit14 and guinea pig17 AVN and contributes to the fast AV conduction pathway.18 Consistently, heterozygous mice lacking SCN5A channels have major AV conduction dysfunction.19

I_{Ca,T} has also been proposed to contribute to pacemaking in primary SAN20 as well as in latent pacemaker cells of the right atrium (RA).21 I_{Ca,T} is expressed in pacemaker cells of mammals, including the rabbit20 and the mouse.22 I_{Ca,T} is also expressed in the cardiac conduction system, the rabbit AVN,18 and in canine Purkinje cells.23,24 The functional role of I_{Ca,T} in the cardiac primary pacemaker and conduction tissue remains to be elucidated.

Molecular cloning of 3 T-type Ca^{2+} channel pore-forming subunits, namely the CaV3.1/α1G, CaV3.2/α1H, and CaV3.3/α1I

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DOI: 10.1161/01.RES.0000225862.14314.49

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subunits, has fostered investigation of the role of native $I_{\text{Ca,T}}$.

Here, we describe that CaV3.1 knockout (CaV3.1−/−) mice lack $I_{\text{Ca,T}}$ in both SAN pacemaker and AVN cells and display slowed pacemaker activity and AV conduction. Our results constitute the first direct functional demonstration of the participation of Cav3.1 channels in pacemaking and cardiac conduction.

Materials and Methods

The generation of the CaV3.1−/− mouse line was originally described by Kim et al. The experimental procedure for electrophysiological recording of isolated cells from the SAN, the AVN, and the RA is described in the expanded Materials and Methods section in the online data supplement, available at http://circres.ahajournals.org. Detailed procedures for in vivo surface and intracardiac electrocardiograms (ECGs), measurement of blood pressure, RT-PCR, and quantitative RT-PCR experiments (supplemental Figure II) and numerical modeling of pacemaker activity are available in the expanded online Materials and Methods section. Results are presented as the mean±SEM. Statistical significance was assessed by the unpaired Student’s $t$ test. A value of $P<0.05$ was considered as statistically significant. $N$ indicates the number of mice used and $n$ the number of cells considered.

Results

CaV3.1−/− SAN Pacemaker Cells Lack $I_{\text{Ca,T}}$

Electrophysiological recordings obtained on SAN cells of wild-type (WT) and CaV3.1−/− animals revealed that $I_{\text{Ca,T}}$ related to CaV3.1 is a major component of the total Ca2+ current ($I_{\text{Ca}}$) in SAN cells (Figure 1). Figure 1A shows representative $I_{\text{Ca}}$ traces recorded from a holding potential (HP) of −90 mV in WT (Figure 1Aa) and CaV3.1−/− (Figure 1Ab) SAN cells. No difference in the cell capacitance was detected (see supplementary Figure I). Corresponding current–voltage (I-V) curves are presented in Figure 1B (filled symbols). $I_{\text{Ca,L}}$ was measured by applying depolarizing steps from a HP of −55 mV (Figure 1B, open symbols), revealing $I_{\text{Ca,T}}$ by subtraction of records from a HP of −90 mV from those obtained from a HP of −55 mV (Figure 1Ba, dotted line). Consistently, switching the HP from −90 to −55 mV significantly shifted $I_{\text{Ca}}$ voltage for half activation ($V_{0.5\text{act}}$) to more positive values (−45±1 mV; $k=5.7±0.6$ mV from a HP of −90 mV and −28±1 mV; $k=6.3±0.3$ mV from a HP of −55 mV, $N=9$, $n=17$, $P<0.05$) in all tested cells ($n=17$), revealing further a $V_{0.5\text{act}}$ of −47±1 mV for the net $I_{\text{Ca,T}}$ (Figure 1Ba, dotted line). In contrast to WT SAN cells, none

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**Figure 1.** $I_{\text{Ca,T}}$ in SAN cells from WT and CaV3.1−/− mice. A, Typical sample $I_{\text{Ca}}$ traces obtained using a HP at −90 mV in a SAN cell from WT (a) and CaV3.1−/− (b) mice. B, Average I-V curves of the Ca2+ current obtained on WT (a) and CaV3.1−/− (b) SAN cells using a HP at −90 mV (filled symbols) and −55 mV (open symbols). Traces obtained at a test potential of −35 mV, from a HP of −90 and −55 mV, are shown in each inset in a and b, as indicated. The net $I_{\text{Ca,T}}$ I-V curve recorded in WT SAN cells is indicated with a dotted line in a and b. C, Histogram showing the relative density of $I_{\text{Ca,T}}$ and $I_{\text{Ca,L}}$ at different test potentials as indicated. $I_{\text{Ca,T}}$ was measured in SAN cells from WT (filled bar), and $I_{\text{Ca,L}}$ was measured in SAN cells from CaV3.1−/− mice (open bar). HP was −90 mV for $I_{\text{Ca,T}}$ and $I_{\text{Ca,L}}$. D, Density of $I_{\text{Ca,L}}$ in SAN cells from WT (open bar) and CaV3.1−/− (filled bar) mice at different test potentials. ns indicates not significant.
of the tested Ca,3.1<sup>−/−</sup> cells displayed detectable I<sub>Ca,T</sub> (Figure 1Bb). Indeed, in Ca,3.1<sup>−/−</sup> SAN cells, switching to a HP of −55 mV did not alter significantly voltage dependence of I<sub>Ca</sub> activation (HP, −90 mV; V<sub>1/2act</sub> = −30±1 mV; k = 8.0±1.3 mV, n = 14; and HP −55 mV: −28±1 mV; k = 6.3±0.3 mV, N = 10, n = 15, P = NS). Subtraction between traces from HP −90 mV and from HP −55 mV identified no residual I<sub>Ca,T</sub>, because current waveforms displayed slow inactivation kinetics (Figure 1Bb, inset) and no criss-crossing of the current traces. A comparison of the current density values in WT and Ca,3.1<sup>−/−</sup> SAN cells at HP −90 mV is shown in Figure 1C (black and white bars, respectively) to estimate the I<sub>Ca,T</sub> component related to Ca,3.1. At −40 mV, I<sub>Ca,T</sub> density was 6.8±1.6 pA/pF (+/+, N = 9, n = 18) and I<sub>Ca,L</sub> density was 2.6±0.7 pA/pF, (−/−, N = 10, n = 14). I<sub>Ca,L</sub> density was not significantly affected by inactivation of Ca,3.1 channels (Figure 1D). Indeed, I<sub>Ca,L</sub> peak densities at −10 mV (HP, −55 mV) were 6.0±1.6 pA/pF, N = 9, n = 15; and 4.5±0.6 pA/pF, N = 10, n = 11 in Ca,3.1<sup>−/−</sup> and WT SAN cells, respectively. Taken together, these data showed that inactivation of Ca,3.1 channels abolished I<sub>Ca,T</sub> and had no significant effect on I<sub>Ca,L</sub> in SAN cells.

To determine whether the pharmacological sensitivity of the SAN I<sub>Ca,L</sub> is consistent with the expression of the Ca,3.1 subunit, we tested its sensitivity to Ni<sup>2+</sup> ions (Figure 2A and 2B). Application of Ni<sup>2+</sup> concentrations of 50 and 200 μmol/L inhibited 26±9% (N = 2, n = 4) and 61±10% (n = 9) of I<sub>Ca,T</sub>, respectively. Because the IC<sub>50</sub> values for Ni<sup>2+</sup> on recombinant Ca,3.1 and Ca,3.2 T-type channels are in the range of 100 to 200 μmol/L and 5 to 10 μmol/L, respectively,<sup>27</sup> these results indicate that I<sub>Ca,T</sub> in mouse adult SAN cells is generated by the Ca,3.1 subunit. Consistent with these findings, real-time RT-PCR experiments showed that the Ca,3.1 subunit is the predominant T-type Ca<sup>2+</sup> channel isoform in the SAN of the adult mouse (supplemental Figure II). Furthermore, application of 2 μmol/L of the 1,4-dihydropyridine I<sub>B,L</sub> blocker isradipine on Ca,3.1<sup>−/−</sup> SAN cells blocked I<sub>B,L</sub> by 93±4% (N = 5, n = 7, Figure 2C and 2E) from a HP of −90 mV and by 100% from a HP of −55 mV (N = 5, n = 8, Figure 2D and 2F). Such a sensitivity to isradipine further confirmed that the remaining I<sub>B,L</sub> in SAN cells from Ca,3.1<sup>−/−</sup> mice was I<sub>I,L</sub>.

**Sedated Ca,3.1<sup>−/−</sup> Mice Have Intrinsic Slower Heart Rate and Prolonged AV Conduction**

We next performed surface ECG recordings on sedated WT and Ca,3.1<sup>−/−</sup> mice (Figure 3). Because the heart rate is highly regulated by the balance between the sympathetic and parasympathetic outflow, which depends on the levels of stress and anesthesia, ECGs were also recorded after injection of propranolol and atropine to block the autonomic nervous system (ANS).<sup>28</sup> Recording examples collected from WT (+/+ ) and Ca,3.1<sup>−/−</sup> (−/−) mice before and after the ANS block are presented in Figure 3A. The loss of the Ca,3.1 subunit induced a significant (9%) prolongation of the atrioventricular conduction (PQ interval) under baseline conditions (34±1
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ms, N=11, and 37±1 ms, N=12 for WT and CaV3.1−/− mice, respectively; P<0.05). The heart beat (RR interval), P wave duration, and QRS interval (Figure 3B), as well as the QTc interval (data not shown; 62±1 ms and 61±1 ms for WT and CaV3.1−/− mice, respectively; P>0.05), were not significantly modified. In conditions of ANS block, the intrinsic heart rate was significantly slowed by ≈10%. Indeed, the RR interval was significantly longer in CaV3.1−/− mice than in WT animals (144±2 ms and 132±2 ms, respectively; P<0.05; Figure 3B). The PQ interval was also markedly prolonged (34±1 ms and 40±1 ms in WT and CaV3.1−/− animals, respectively; P<0.001; Figure 4B). The other ECG parameters were not significantly modified.

SAN Cells From CaV3.1−/− Mice Display Slowed Pacemaker Activity

The spontaneous activity of isolated SAN cells was studied to evaluate whether the slowing of the intrinsic heart rate observed in vivo in CaV3.1−/− mice was associated with dysfunction of SAN cell automaticity. Representative recordings obtained on WT and CaV3.1−/− SAN cells are presented in Figure 4A (upper and lower traces, respectively). Analysis of these recordings showed that inactivation of cacna1g gene resulted in a 37% slowing of the cellular beating rate (163±3 bpm, N=4, n=15 in CaV3.1−/−, and 234±19 bpm, N=5, n=14 in SAN cells from WT mice; P<0.01; Figure 4B). Slowing of cellular pacemaking was accompanied by a reduction of the diastolic depolarization slope of ≈44% (Figure 4C). No significant changes in the maximum diastolic potential (MDP) and the voltage threshold of the action potential upstroke (Em) were observed (MDP: −56.9±1.3 mV in WT, n=15, and −58.2±1.4 mV for CaV3.1−/− SAN cells, n=14; Em: −45±1.3 mV in WT and −43±1.4 mV in CaV3.1−/− SAN cells; Figure 4D). Also, the action potential duration (147±15 and 141±10 ms for CaV3.1−/− and WT SAN cells, respectively; Figure 4E) and the action potential amplitude (APA) (92±8 and 90±8 mV for CaV3.1−/− and WT SAN cells, respectively; not shown) were comparable in SAN cells from WT and CaV3.1−/− mice.

Intracardiac Electrophysiology Study of CaV3.1−/− Mice

The AV conduction delay in mice lacking CaV3.1 channels was measured under baseline intracardiac recording conditions. Eight of 10 WT and 7 of 9 CaV3.1−/− mice had a distinctly visible His-bundle electrogram (Figure 5A). In this group of animals, atrial-His (AH) conduction times were significantly longer in CaV3.1−/− mice (31±1 ms) than in WT animals (25±1 ms; P<0.001; Figure 5B). However, His-ventricle (HV) intervals were comparable (11±1 and 10±1 ms for WT and CaV3.1−/− mice, respectively; P>0.05). Effective refractory periods at the atrial (AERP), atrioventricular (AVERP), and ventricular (VERP) levels were investigated at a basic cycle length of 100 ms (Figure 5C). AERP and VERP were not significantly different between WT and CaV3.1−/− mice. In contrast, the AVERP was significantly longer in CaV3.1−/− mice (58±6 ms; N=8) than in WT animals (44±2 ms; N=10; P<0.05). Decremental atrial pacing revealed that Wenckebach cycle length was significantly longer in CaV3.1−/− mice than in WT mice (85±5 ms and 67±2 ms, respectively; P<0.01; Figure 5C). Atrial pacing confirmed SAN dysfunction. Indeed, the corrected SAN recovery time (cSNRT) was longer in CaV3.1−/− mice than in WT mice (31±5 in CaV3.1−/− and 17±2 ms in WT mice P<0.05; n=7 and 9, respectively).

AVN Cells From CaV3.1−/− Mice Lack Ica,L

We then tested whether dysfunction of AV conduction observed on inactivation of Ca3.1 channels was associated with downregulation of Ica,L in the AVN. Ica,L and Ica,T were recorded in isolated AVN cells using the same recording protocols as for SAN cells (Figure 6). Ica,L was found in all the WT AVN cells investigated (Figure 6A). Indeed, switching the HP from −90 to −60 mV significantly shifted the V0.5act of the total Ica from −34±0.7 mV (n=7, P<0.05). In contrast, no Ica,T could be recorded in CaV3.1−/− AVN cells (Figure 6Ab), and switching the HP from −90 to −60 mV did not shift the V0.5act value (−25±1.3 mV to

Figure 3. Surface ECGs obtained on sedated WT (+/+ ) and CaV3.1−/− (−/−) mice. A, Representative lead I ECG traces obtained from WT and CaV3.1−/− under baseline conditions (ANS+) (left panels) and after injection of atropine and propranolol to block the ANS (ANS−) (right panels). B, Histograms of the average RR interval, P wave duration, PQ interval, and QRS interval, respectively, obtained from WT (open bars) and CaV3.1−/− (filled bars) mice, before (ANS+) and after (ANS−) blockade of the ANS.
Bradycardia in Conscious Unrestrained Ca\textsubscript{v}3.1\textsuperscript{−/−} Mice

Both the heart rate and the AV conduction were altered in freely moving Ca\textsubscript{v}3.1\textsuperscript{−/−} mice (Figure 7A and 7B and the Table). The mean heart rate was significantly slowed in Ca\textsubscript{v}3.1\textsuperscript{−/−} mice, when considering a 24-hour period or during the day and night periods (Figure 7B and the Table). The maximum cardiac frequency during short periods of activity (40 seconds) was unchanged (Table), suggesting that activation of other ionic channels involved in SAN automaticity can compensate for the lack of \( I_{\text{Ca,T}} \) during strong activation of the sympathetic nervous system. Spectral analysis of RR signals in the frequency domain revealed no differences between the 2 groups of mice. Total spectral power, ultralow (ULF), very-low (VLF), low (LF), and high (HF) frequencies were not significantly modified between WT and Ca\textsubscript{v}3.1\textsuperscript{−/−} mice (Figure 7C, data not shown for ULF and VLF), indicating that the degree of autonomic regulation of heart rate was not altered in Ca\textsubscript{v}3.1\textsuperscript{−/−} mice.

Telemetric recordings also confirmed the prolongation of the PQ interval in Cav3.1\textsuperscript{−/−} mice (Table), demonstrating that the propagation of the heartbeat through the AV conduction system was delayed in Ca\textsubscript{v}3.1\textsuperscript{−/−} mice. This effect was independent from the heart rate because measurements were performed at comparable heart rates (600 bpm; cycle length of 100 ms). In contrast, the atrial conduction (as assessed by the P wave duration), the ventricular conduction (QRS interval), and ventricular repolarization (QT interval) were unchanged (Table). In addition, we did not find evidence for cardiac arrhythmias in Ca\textsubscript{v}3.1\textsuperscript{−/−} mice. Also, no significant difference in the systolic, diastolic, and mean arterial pressure (60±8 mm Hg, N=8 in WT and 72±5 mm Hg, N=8 in Ca\textsubscript{v}3.1\textsuperscript{−/−} mice; \( P=\text{NS} \)) was observed between the 2 mouse strains (Figure 7D).

Discussion

The major finding of this study is that genetic inactivation of the Ca\textsubscript{v}3.1/\( \alpha \text{Ca}_1 \) T-type Ca\textsuperscript{2+} channels in mice results in a significant slowing of the heart rate and AV conduction. Downregulation of \( I_{\text{Ca,T}} \) in both SAN and AVN cells of Ca\textsubscript{v}3.1\textsuperscript{−/−} animals is documented, accounting for this in vivo phenotype. The lack of Ca\textsubscript{3.1} channels in isolated SAN cells induces slowing of pacemaker activity through a reduction of the slope of the diastolic depolarization. Our study provides novel and compelling genetic evidence for a direct contribution of Ca\textsubscript{3.1} channels in the setting of the mammalian cardiac impulse generation and propagation by contributing to both the diastolic depolarization in the SAN and impulse conduction through the AVN.
Cav3.1 channels did not significantly modify the expression of Cav3.1 Channel subunits do not compensate for the lack of Cav3.1 channels in observed on recombinant Cav3.1-mediated Cav3.1.

First, bradycardia was evident in sedated mice under pharmacological blockade of the ANS input for heart rates that attributable to slowing of the pacemaker activity in the SAN. Several lines of evidence indicate that bradycardia is and slowing of the atrioventricular conduction in Cav3.1. ECGs and intracardiac recordings document both bradycardia and AV Wenckebach cycle length (Wenck.) in WT (open bars; N=8) and Cav3.1 (filled bars; N=7) mice. RR intervals were 111±3 and 122±4 ms for WT and Cav3.1 mice, respectively (P<0.05). C, Histograms of the average atrial effective refractory period (AERP), atrioventricular ERP (AVERP), ventricular ERP (VERP), and AV Wenckebach cycle length (Wenck.) in WT (open bars; N=9 to 10) and Cav3.1 (filled bars; N=6 to 8) mice.

I\textsubscript{Ca,T} in SAN and AVN Cells Following Inactivation of Ca\textsubscript{3.1} Channels
A striking observation of this study is that I\textsubscript{Ca,T} in the mouse SAN is predominantly generated by the Ca\textsubscript{3.1} subunit because we found no evidence for a residual I\textsubscript{Ca,T} in Cav3.1 mice in both SAN (Figure 1) and AVN cells (Figure 6). The low sensitivity of SAN I\textsubscript{Ca,T} to Ni\textsuperscript{2+} is consistent with that observed on recombinant Ca\textsubscript{3.1}-mediated I\textsubscript{Ca,T}.\footnote{7} Also consistent with our general findings is the recent observation that Cav3.2 mice showed no ECG alterations.\footnote{29} Inactivation of Ca\textsubscript{3.1} channels did not significantly modify the expression of I\textsubscript{Ca,L} in Cav3.1 mice in both SAN and AVN cells. Our data show, therefore, that expression of Ca\textsubscript{3.2}, Ca\textsubscript{1.3}, and Ca\textsubscript{1.2} subunits do not compensate for the lack of Ca\textsubscript{3.1} channels in Cav3.1 mice.

Bradycardia and Atrioventricular Dysfunction in Ca\textsubscript{3.1}\textsuperscript{−/−} Mice
EGCs and intracardiac recordings document both bradycardia and slowing of the atrioventricular conduction in Ca\textsubscript{3.1}\textsuperscript{−/−} mice. Several lines of evidence indicate that bradycardia is attributable to slowing of the pacemaker activity in the SAN. First, bradycardia was evident in sedated mice under pharmacological blockade of the ANS input for heart rates that were comparable with the mean heart rate observed in freely moving mice. Second, slowing of the heart rate was observed in freely moving mice in the absence of a significant change both in the heart rate variability profile and in the arterial blood pressure, thus arguing against the hypothesis that bradycardia would be caused by dysfunction in the autonomic regulation of the heart rate or could be secondary to a change in the control of the vascular tone. The prolongation of the SAN recovery time also indicates dysfunction in SAN automaticity. Finally, the slowing of pacemaker activity observed in isolated SAN pacemaker cells of Ca\textsubscript{3.1}\textsuperscript{−/−} mice is consistent with bradycardia being attributable to the lack of I\textsubscript{Ca,T} in the SAN. The reduction of the mean heart rate measured on freely moving Ca\textsubscript{3.1}\textsuperscript{−/−} mice is moderate, compared with the slowing of the cycle length in isolated pacemaker cells (10% in freely moving mice and 37% in isolated cells). This difference can be caused by the compensatory adrenergic tone in vivo, which stimulates ionic mechanisms involved in pacemaking, such as Ca\textsubscript{1.3}-mediated I\textsubscript{Ca,L}\textsuperscript{9,10} and I\textsubscript{T}\textsuperscript{9} and RyR-dependent diastolic release of Ca\textsuperscript{2+}\textsuperscript{11}. The observation that the maximal heart rate is comparable in WT and
mice is consistent with this hypothesis. Comparison of mice lacking Cav1.3 and Cav3.1 channels (this study) indicate that I_{Ca,L} and I_{Ca,T} play distinct roles in pacemaker activity in vivo. Indeed, knockout of Cav1.3 channels reduces the basal and intrinsic heart rate in sedated conditions by approximately 20% and 60%, respectively, compared with the 10% reduction observed in mice lacking Cav3.1 channels in both freely moving and block of ANS conditions. These observations suggest that Cav3.1 channels contribute to the setting of the basal heart rate but have less impact on the dynamic regulation of pacemaker activity by the ANS. The lack of a significant change in the variability of the RR intervals in Cav3.1 mice is consistent with this hypothesis.

We report that the slowing of the AV conduction in Cav3.1 mice is caused by dysfunction in the excitability of the AVN. Indeed, intracardiac recordings show a delay in the AH conduction time and AVERP, in the absence of a prolongation of the P wave, which indicates similar conduction velocities in atria from WT and Cav3.1 mice. Consistently with ECGs and intracardiac recordings, we found no I_{Ca,T} related to CaV3.1 channels because it was no longer detectable in Cav3.1 mice. We did not find significant changes in the HV, QRS, and QT intervals (see Figures 3 and 5 and supplemental Figure III), indicating that the lack of Cav3.1 channels does not affect conduction through the His–Purkinje fiber network or the ventricular muscle. This phenotype differs from that of SCN5A mice, which shows prominent alteration of the P wave and the QRS complex duration, stressing the importance of the cardiac

### Telemetric ECG Parameters in Freely Moving WT and CaV3.1 Mice

<table>
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<tr>
<th>Heart Rate (bpm)</th>
<th>P Wave Duration (ms)</th>
<th>PQ Interval (ms)</th>
<th>QRS Interval (ms)</th>
<th>QT Interval (ms)</th>
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<td>Daytime Mean HR</td>
<td>Nighttime Mean HR</td>
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<td>578±14</td>
<td>526±11</td>
<td>630±18</td>
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<td>CaV3.1</td>
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<td>0.015</td>
<td>0.022</td>
<td>0.039</td>
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ECG parameters were set at a cycle length of 100 ms. WT, N=6; CaV3.1, N=6. HR indicates heart rate.
I_{Ca,T} in intraatrial and His–Purkinje conduction. Also in contrast with SCNS5A \textsuperscript{−/−}, \textsuperscript{19} CaV3.1 \textsuperscript{−/−} mice do not present ventricular tachiarhythmias. This SCNS5A-mediated \textit{I_{Ca,T}} can possibly compensate for the lack of \textit{I_{Ca,T}} in the His–Purkinje system.

**Pacemaking in CaV3.1 \textsuperscript{−/−} Mice**

We describe that inactivation of CaV3.1 channels significantly prolongs the pacemaker cycle length in isolated SAN cells by reducing the slope of the diastolic depolarization, demonstrating the involvement of CaV3.1-mediated \textit{I_{Ca,T}} in the setting of the diastolic depolarization in mouse SAN cells. In an attempt to compare the physiological roles of CaV3.1-mediated \textit{I_{Ca,T}} with that of CaV1.3-mediated \textit{I_{Ca,L}} and \textit{I_{Na}}, we have developed a numerical model of mouse SAN electrophysiology based on previous studies on mouse SAN pacemaking in normal and genetically modified mouse strains (see supplemental Figures IV and V). Our numerical simulations indicate that CaV3.1 channels contribute to pacemaking by activating during the diastolic depolarization for more negative voltages than TTX-sensitive \textit{I_{Na}} and CaV1.3-mediated \textit{I_{Ca,L}} (see supplemental Figure VB and VE). As a consequence, CaV3.1 channels can accelerate the diastolic depolarization rate in a voltage range in which \textit{I_{Ca,T}} is still not activated. This property can explain, at least in part, how CaV3.1 channels can contribute to pacemaking in spite of their relatively low availability at SAN diastolic potentials. An expanded discussion about the development, the features and limitations of our numerical model, and the physiological significance of CaV3.1-mediated \textit{I_{Ca,T}} compared with that of \textit{I_{Ca,L}} and \textit{I_{Na}} is available in the online data supplement.

**Conclusion**

In conclusion, our study demonstrates that CaV3.1 channels contribute to the heartbeat by influencing pacemaking and the AV conduction. To date, the presence of T-type channels in human heart is poorly documented. Molecular analyses, such as dot blots and Northern blots of human heart mRNAs, have identified transcripts for CaV3.1 and CaV3.2 \textsuperscript{31,32} T-type channels may play a role in the human SAN and the conduction system. For instance, the causality between congenital heart block induced by maternal autoantibodies against T-type channels and children showing SAN bradycardia and atrioventricular block has recently been documented.\textsuperscript{33} There is growing evidence that T-type channels may constitute a promising pharmacological target for the treatment of human diseases, such as epilepsy and chronic pain.\textsuperscript{34} From our observations, T-type channel inhibition would have no deleterious consequences in cardiac physiology. It is, therefore, tempting to speculate that selective blockers of CaV3.1 channels may hold promise for the therapeutic management of the cardiac diseases that require moderate heart rate reduction, such as cardiac ischemia and coronary heart disease.

**Acknowledgments**

This work was supported by the Association Francaise Contre les Myopathies, the Action Concertee Initiatice (Developmental Biology and Integrative Physiology) of the French Ministry for Education, the Fondation de France, and the Chemoinformatics Program of Korean Institute of Science and Technology, Korea. A.T. is supported by a fellowship from the CNRS Lebanon. We are grateful to Patrick Ager for excellent technical assistance.

**References**


Bradydardia and Slowing of the Atrioventricular Conduction in Mice Lacking Ca\(\gamma\)3.1/\(\alpha\)1G T-Type Calcium Channels
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*Circ Res.* 2006;98:1422-1430; originally published online May 11, 2006;
doi: 10.1161/01.RES.0000225862.14314.49
*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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http://circres.ahajournals.org/content/98/11/1422

Data Supplement (unedited) at:
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Expanded Material and Methods

Mice handling was done in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1996), and European directives (86/609/CEE) and every effort was made to minimize animal suffering and the number of animals used.

Results are presented as the mean ± the standard error of the mean (SEM); n indicates the number of observations. Statistical significance was assessed by the unpaired Student’s t test. A value of $P<0.05$ was considered as statistically significant and n.s. stands for not significant. $N$ indicates the number of mice used in indicated experiments.

Electrophysiology on isolated SAN, AVN and RA and cells

SAN tissue samples and pacemaker cells were isolated from age- and sex-matched 129 sv WT or Ca$_{V3.1}^{-/-}$ adult mice as previously described. Myocytes from the right atrium were isolated using a similar procedure as described for SAN cells. The AVN was identified by using landmarks as in. Individual AVN cells were isolated by applying the same digestion/dissociation procedure as for SAN cells. Ca$^{2+}$ currents were recorded at 26°C with the conventional whole-cell patch-clamp technique using extracellular 2 mM Ca$^{2+}$ and analyzed as described previously. The cell access resistance in these recording conditions was around 10 M$\Omega$, series resistance was evaluated at the peak of $I_{Ca}$ and could be corrected up to 75%. Capacitative transients were not compensated electronically. Ca$^{2+}$ currents were recorded using an extracellular solution containing (in mmol/L): TEA-Cl, 130; CaCl$_2$, 2; MgCl$_2$, 1; 4-amino-pyridine, 10; HEPES, 25; (adjusted to pH 7.4 with TEAOH). Whole-cell pipettes were filled with a solution containing (mM) CsCl, 130; EGTA, 10; HEPES, 25; Mg-ATP 3 and Na-GTP 0.4 (pH at 7.2 with CsOH). Pacemaker activity of SAN cells was
recorded at 35°C under perforated-patch conditions using β-escin 4 into the pipette solution at a final concentration of 30 µM. The pipette solution was (in mmol/L): KCl, 130; NaCl, 10; ATP-Na\(^+\) salt, 2; creatine phosphate, 6.6; GTP-Mg\(^2+\), 0.1; CaCl\(_2\), 0.04 (pCa 5.7); Hepes-KOH, 10; (adjusted to pH 7.2 with KOH). The extracellular solution contained (in mmol/L): NaCl, 140; KCl, 5.4; CaCl\(_2\), 1.8; MgCl\(_2\), 1; Hepes-NaOH, 5; and D-glucose, 5.5; (adjusted to pH 7.4 with NaOH). Myocytes from the RA were isolated using a similar procedure as described above and according to a previous study 5. All voltage values were corrected for the appropriate liquid junction potential. All electrophysiological data were recorded and analyzed using the pCLAMP suite ver 9.0 (Axon Instruments).

**Electrocardiograms (ECGs) on anesthetized mice**

Adult mice were anesthetized with etomidate (30 mg/kg i.p.). Body temperature was maintained at 36°C-37°C with a warming pad controlled by a rectal temperature probe. Surface ECGs were recorded with 25-gauge subcutaneous electrodes on a computer through an analog-digital converter (IOX 1.585, EMKA Technologies) for monitoring and later analysis (ECG Auto 1.5.7, EMKA Technologies). ECG channels were filtered between 0.5 and 250 Hz. Reported measurements were averaged from 3 consecutive PQRST complexes in lead I. Criteria used for interval measurements have been described elsewhere 6. The QT interval was corrected for heart rate using the formula, QTc=QT/(RR/100)\(^{1/2}\) established for mice with QT and RR expressed in ms 7. ECGs were first recorded under baseline conditions and then 10 min after atropine sulfate (0.5 mg/kg i.p.) and propranolol (1 mg/kg i.p.) injection to block the autonomic nervous system (ANS).
**Intracardiac recording and pacing.**

*Mouse preparation.* Animals were anesthetized with an intra-peritoneal injection of etomidate (8 mg/kg) and pentobarbital (30 mg/kg). Body temperature was maintained at 36°C-37°C with a warming pad controlled by a rectal temperature probe. Under sterile conditions, an octapolar 2F catheter with an electrode spacing of 0.5 mm (Cordis Webster®) was introduced into the right atrium and ventricle through the right internal jugular vein. Using this catheter, simultaneous atrial and ventricular pacing and recording were performed. His bundle activity was also recorded when detected. Intracardiac electrograms were filtered between 30 and 500 Hz. Surface ECG (lead I) and intracardiac electrograms were recorded to a computer through an analog to digital converter (IOX 1.585, EMKA Technologies) for monitoring and off-line analysis and measurement. Intracardiac pacing was performed with a Biotronik® UHS20 stimulator, modified by the manufacturer to pace at short coupling intervals.

**Electrophysiological study protocols.** Standard pacing protocols were used to determine the electrophysiologic parameters. Sinus node function was evaluated by measuring the rest sinus cycle length and the sinus node recovery time. After a period of normal sinus rhythm (RR interval values of 109± 5 ms for WT and 120 ± 9 ms for Ca,3.1−/− mice; NS; n=9 and 7 respectively), atrial pacing was applied for a period of 30 s at cycle lengths of about 80% of the intrinsic sinus cycle length (78% ± 2% in WT mice, n = 9; 77% ± 1% in Ca,3.1−/− mice, n = 7; NS). For each pacing cycle length, sinus node recovery time (SNRT) was determined as the longest pause from the last paced atrial depolarization to the first sinus return cycle. Here we report cSNRT corrected by subtracting the intrinsic sinus cycle length from the recovery interval). Anterograde AV nodal conduction properties were assessed by pacing the atrium at rapid rates and determining the maximum cycle length resulting in Wenckebach phenomenon.
Refractory periods were determined by delivering an 8-stimulus drive train (S1) at a cycle length of 100 ms followed by a premature stimulus (S2) progressively decremented in 2 ms intervals. Atrial, atrioventricular nodal and ventricular effective refractory periods (AERP, AVERP and VERP, respectively) were defined as the longest S1-S2 coupling interval for each region that failed to generate a propagated beat with S2.

**ECG recording in conscious mice**

For long-term ECG recording, adult male mice were anesthetized with etomidate (35 mg/kg i.p.). A midline incision was made on the back along the spine to insert a telemetric transmitter (TA10EA-F20, Data Sciences International) into a subcutaneous pocket with paired wire electrodes placed over the thorax (chest bipolar ECG lead). Local anesthesia was obtained with lidocaine (1%) injected subcutaneously at the sites of electrodes and transmitter implantation. Experiments were initiated at least 8 days after recovery from surgical implantation. Mice were housed in individual cages with free access to food and water and were exposed to 12-hour light/dark cycles (light, 8:30 AM to 8:30 PM) in a thermostatically controlled room. ECG signals were computer-recorded with the use of a telemetry receiver and an analog-to-digital conversion data acquisition system for display and analysis by Dataquest™ A.R.T.™ software (Data Sciences International). Heart rate values were determined from RR intervals averaged every 10 seconds. Mean heart rate values were obtained in each mouse for an overall 24-hour period from 8:30 AM to 8:30 PM and for the corresponding 12-hour light and dark periods. Maximal and minimal heart rate values were determined on 40-second periods during dark and light periods respectively. ECG parameters were measured at a fixed RR interval of 100 ms with ECG Auto 1.5.7 software. Spectral analysis using a fast Fourier transformation algorithm on sequences of 512 points was performed using the ECG Auto 1.5.12.10 software (EMKA Technologies) on RR data series.
recorded between 11 AM and 2 PM. The area under the curve was calculated for the ultra-
low-frequency (ULF: 0 to 0.026 Hz), very-low-frequency (VLF: 0.026 to 0.32 Hz), low-
frequency (LF: 0.32 to 1.2 Hz), and high-frequency (HF: 1.2 to 3.2 Hz) bands, as previously
defined in the mouse species 8.

**Measurements of the blood pressure**

Systolic and diastolic blood pressures were measured on conscious mice using a computerized
tail-cuff method (BP-2000, Visitech Systems, Apex, NC). Following a 7-day acclimation
period, mice were trained for 6 days on the equipment prior performing measurements.
Animals were maintained restrained at 37°C in the dark and tail cuffs were placed on the tail
of each of the mice. In order to acclimate the mice, 10 preliminary measurements were
performed. Blood pressure was then determined and recorded. Mice were tested over 3 days
and 10 measurements per day were collected. The final systolic blood pressure value was
calculated as the average of 30 measurements.

**RT-PCR Analysis**

For RT-PCR analysis, total RNAs from sino-atrial node (SAN), the right atrium (RA), the left
ventricle (LV) and the atrio-ventricular node (AVN) adult mice were extracted using the
SNAP™ Kit (Invitrogen). Reverse transcription (RT) was performed with total RNA using
random primers and Superscript II RNase H reverse transcriptase (Invitrogen). Following RT,
the cDNAs for Ca3.1, Ca3.2 and Ca3.3 were amplified using the following set of primers :
Ca3.1-F 5’-GTCTCCGACGCTCTGTAAC-3’ (NM_009783, nucleotide (nt) 329), Ca3.1-
R, 5’-CCACAGCAAAGAA GGCAAAG-3’ (nt 499, expected size 170bp), Ca3.2-F 5’-
TGGGAACGTGCTTTCTCTCT-3’ (NM_021415, nt 690), Ca3.2R,
GGGATGTGTGTGACATTTCT (nt 918, expected size 228bp), CaV3.3-F 5’-GACACCACCTCCCCACACACT-3’(XM_139476, nt 3250), CaV3.3-R, CCTCCTGTTCCTCCTCCTTC-3’ (nt 3487, expected size 237 bp). PCR was performed in a final volume of 25 µl containing 2 µl of reverse transcription reaction, 10 pmol of each primer, 2.5 mM each dNTP (Pharmacia), 1.5 mM MgCl₂ and 1 unit of Taq polymerase (Sigma) in a MJ Research Inc Thermal Cycler with the following cycling protocol: after 3 min at 94°C, 35 cycles (94°C, 45s; annealing temperature, 45s; 72°C, 35s) of PCR were performed followed by a final elongation period of 10 min at 72 °C. Annealing temperatures were as follows: 57°C for CaV3.1, 55°C for CaV3.2 and 59°C for CaV3.3. To visualize the presence and size of the amplified fragments, 10 µl of the PCR products were electrophoretically separated on an ethidium bromide-stained 2% agarose gel.

Quantitative RT–PCR

CaV3 mRNA expression was determined by quantitative RT-PCR using a real time TaqMan PCR technology (ABI Prism 7000, Applied Biosystems) using the following sets of specific primers for CaV3.1, CaV3.2 and CaV3.3 and hypoxanthine guanine phosphoribosyl transferase (HPRT) : CaV3.1 : Q-CaV3.1-F 5’-CCAAGCAACGAGAGTCA-3’ (NM_009783, nt 1310) and Q-CaV3.1-R 5’-GCTAGCATTGGACAGGAATCG-3’ (nt 1371, expected size 61bp) ; CaV3.2 : Q- CaV3.2-F 5’-CAGCGGCTACGCCTATGC-3’(NM_021415, nt 3230) and Q- CaV3.2-R 5’-CAAAAGAGTGTGGGCCATGTC-3’(nt 3294, expected size 65bp) ; CaV3.3 : CaV3.3-F 5’-CCTTTTTTGCTCTTGTGTTTTCTG-3’ (XM_139476 nt 1106), CaV3.3-R 3’-ACTTTAGGGAGGCATATATTCC-3’ (nt 1219, expected size 113bp) and HPRT : Q-HPRT-F 5’- GCAGTACAGCCCCAAAATGG-3’ (NM_013556, nt 540), and Q-HPRT-R 5’-GGTCCTTTTCCACCAGCAAGC-3’ (nt 591, expected size 51bp). Experiments were performed using 1X SYBR Green PCR Master Mix TaqMan PCR buffer from Applied
Biosystems and 300 nM of each primer. The cycling conditions included a hot start for 10 min at 95°C, followed by 40 cycles at 95°C for 15s, and 60°C for 1 min. All primers sets were tested under the Taqman PCR conditions using mouse genomic DNA as template. In all cases, the presence of a single product of the appropriate size was controlled by agarose gel electrophoresis (Figure 2B). Each amplification was normalized to HPRT, the reference mRNA. mRNA samples that were incubated without reverse transcriptase during cDNA synthesis showed no amplification (negative controls).

**Numerical modelling of mouse SAN cell pacemaker activity**

Numerical modelling of pacemaker activity was performed according to the central SAN model by Zhang et al. 9. We have chosen this model since it fairly reproduces basal pacemaking in spite of the absence of experimental data on intracellular ionic concentration and homeostasis, which is in fact the case for mouse SAN pacemaker activity. Also, this model has been developed to account for pacemaking in small cells likely from the center of the node and having similar capacitance than that used in our study (21 pF). The original SAN model has been appropriately modified in accordance to experimental data obtained by different groups in mouse pacemaker cells. To this aim, we have added equations to account for the presence of Ca\(_{v1.3}\)- and Ca\(_{v1.2}\)- mediated \(I_{\text{Ca,L}}\), according to previous studies on Ca\(_{v1.3}^{-/-}\) mice 3,10. These current components have been separated with respect to their differential steady state parameters for activation and inactivation. Similarly, TTX- sensitive and TTX- insensitive \(I_{\text{Na}}\) currents have been included in the model according to experimental results on isolated mouse SAN cells by Lei et al. 11. Ca\(_{v3.1}\)- mediated \(I_{\text{Ca,T}}\) was modelled to match voltage clamp results obtained in Fig. 1 and in ref. 3. Also, equations for the \(I_{K1}\) and \(I_{st}\) were added according to a previous report on mouse SAN cells 12. Numerical simulations were performed by employing the XPPAUT software, freely available at [http://www.pitt.edu/~phase/](http://www.pitt.edu/~phase/). Due to the absence of experimental data from mouse SAN
cells, all equations and parameter values for background voltage-independent currents, ionic pumps and exchangers were kept as in the original model of rabbit central SAN cells\(^9\) (see also the online supplementary table 1). The complete list of parameter values and experimental current densities used for simulations are shown in the online supplementary table 1 and 2, respectively. The list of initial values is shown in the online supplementary table 3.

**Modelling of \(I_{Ca,T}\)**

A Hodgkin-Huxley (HH) model of \(Ca_{v3.1}\)-mediated \(I_{Ca,T}\) was constructed according to the equations described in Destexhe & Huguenard\(^\text{13}\). Steady state parameters for activation were taken from our \(I_{Ca,T}\) records in SAN cells from WT mice. Steady-state inactivation parameters of \(I_{Ca,T}\) were from our previous study on SAN cells from \(Ca_{v1.3^{-/-}}\) mice\(^3\) which allow precise measurement of \(I_{Ca,T}\) at negative test potentials (see also ref.\(^14\)). Activation and inactivation time constants were measured in sample records from WT mice at 36 °C (data not shown) and compared with records of \(I_{Ca,T}\) from \(Ca_{v1.3^{-/-}}\) mice\(^3\). For \(I_{Ca,T}\) reactivation kinetics, we used experimental records obtained from recombinant \(Ca_{v3.1b}\) channels\(^15\) which are expressed in the heart\(^16,17\) and show the greatest similarity in steady state properties with the native mouse SAN \(I_{Ca,T}\).

**Modelling of \(I_{Ca,L}, I_{Na}\) and \(I_{sp}\)**

For modelling of \(I_{Ca,L}\), we have used the same equations as in Zhang et al\(^9\). According to experimental evidence from SAN cells of \(Ca_{v1.3^{-/-}}\) mice, we have assumed that the total whole-cell \(I_{Ca,L}\) is composed by both \(Ca_{v1.3}\) and \(Ca_{v1.2}\) channels. Two independent groups of equations were used for \(Ca_{v1.3}\)- and \(Ca_{v1.2}\)-mediated \(I_{Ca,L}\). Activation and inactivation steady-state parameters used in the model, as well as current densities of \(Ca_{v1.3}\)- and \(Ca_{v1.2}\)-
mediated $I_{Ca,L}$ are from our previous study on Ca$_{v1.3}^{-/-}$ mice. For modelling $I_{Na}$, we have used equations as in the atrial cell model by Nygren and co-workers. We have assumed that the total whole-cell $I_{Na}$ is composed by TTX-sensitive ($I_{Na_s}$) and TTX-resistant ($I_{Na_r}$) $I_{Na}$ according to Lei et al. Steady-state activation/inactivation parameters as well as the relative current densities of $I_{Na_s}$ and $I_{Na_r}$ for a SAN cell of 21 pF are from Fig. 3F and 2K in Lei et al, respectively. The $I_{st}$ current was also added in our model. To this aim we have employed the HH empirical model of $I_{st}$ from Shinagawa et al. $I_{st}$ densities and activation are from mouse SAN cells according to ref. 

**Modelling of $K^+$ currents and $I_f$**

All voltage-dependent $K^+$ currents included in the original model by Zhang et al. have been included in our calculations, except for $I_{K1}$, that we have added to account for the observed $I_{K1}$ in mouse SAN cells. $I_{K1}$ was calculated according to equations in Nygren et al. $I_{K1}$ density was taken from Cho et al. For modelling of $I_{Kr}$, we used the equations from Kurata et al. These equations, fairly reproduced experimental results on mouse SAN pacemaker cells published in Clark et al. The conductance of all other voltage-dependent $K^+$ currents included by Zhang et al., have been kept as in the original model of rabbit central SAN cells (see online supplementary table 1). For $I_f$, we used the same equations as in Zhang et al. To account for mouse SAN cells, $I_f$ density and activation were adjusted according to experimental results by Mangoni & Nargeot.

**Simulation of mouse pacemaker activity**

We have developed a model of mouse SAN electrophysiology to gain insights into the behaviour of $I_{Ca,T}$ during pacemaking (supplementary Figure 4, 5). To this aim, we have adapted the model for central SAN cells by Zhang and co-workers to account for published
data on isolated mouse SAN cells ionic conductances (see the Methods section). The model takes into account the expression of Cav1.3- and Cav1.2-mediated $I_{\text{Ca,L}}$, as well as TTX-sensitive and TTX-resistant $I_{\text{Na}}$. The model also includes $I_s$ current according to a recent study on mouse SAN cells by Cho and coworkers. $I_{K_F}$ was also modelled to account for experimental data in Clark et al. $I_{K_I}$ was also included in the model, since this current has been consistently found in mouse SAN cells (M. Mangoni, unpublished observations. As shown in supplementary Figure 4A, our model fairly reproduces current densities and waveforms experimentally observed in voltage-clamp conditions. $I_{\text{Ca,T}}$ density and voltage dependence was set according to experimental parameters (Figure 1B). The density of Cav1.3-mediated $I_{\text{Ca,L}}$ was set to 10 pA/pF (see supplementary table 2). This value is higher than that found experimentally in this study (5-6 pA/pF, see Figure 1). We have preferred to assume similar densities for Cav3.1 and Cav1.3-mediated currents since Cav1.3 channels are already partially inactivated at a $H_p$ of -55 mV (see supplementary Figure 4B), leading to a possible underestimation of the absolute $I_{\text{Ca,L}}$ density compared to $I_{\text{Ca,T}}$ in voltage-clamp conditions. Furthermore, $I_{\text{Ca,L}}$ densities higher than 6 pA/pF can be found on isolated SAN cells and activation of the $\beta$-adrenergic receptor strongly stimulates Cav1.3- and Cav1.2-mediated $I_{\text{Ca,L}}$. As to TTX-sensitive and TTX-resistant $I_{\text{Na}}$, our modelling work reproduced the data by Lei and co-workers. Steady-state voltage dependencies for activation and inactivation of both $I_{\text{Na}}$ current components are shown in the supplementary Figure 4C. The density of TTX-sensitive $I_{\text{Na}}$ was chosen to match that in reported action potential clamp experiments, thereby yielding a current amplitude between 10 and 20 pA (20 pA in our simulations) during the diastolic depolarisation (see Figure 3E of ref. 11). The density of TTX-resistant $I_{\text{Na}}$ was set as for cells having capacitance of 30 pF in Lei et al. This density has been measured in low-$Na^+$ conditions. This can lead to some underestimation of TTX-resistant $I_{\text{Na}}$ in our calculations even if the presence of this current in SAN cells of less than 30 pF has not been
directly demonstrated. However, it was necessary to include TTX-resistant $I_{Na}$, since it has been demonstrated that $SCN5A^{+/−}$ mice show moderate bradycardia and that TTX-resistant $I_{Na}$ participate to the setting of the normal SAN function.

When parameters in supplementary table 1 are applied, the model generates a basal rate of 278 bpm (cycle length is 216 ms, Supplementary Figure 5A), with a MDP of -65 mV and a takeoff potential of about -45 mV. Abolition of $Ca_{v}3.1$-mediated $I_{Ca,T}$ from the model induced 8% rate reduction, by slowing the diastolic depolarisation. Upon deactivation of large $I_{Kr}$, $I_f$ is the first voltage-dependent inward current to activate in the diastolic depolarisation (supplementary Figure 5C), $I_f$ is also present in the same range of voltages and drives a residual fraction of current which has not completely deactivated after repolarization (supplementary Figure 5D). $Ca_{v}3.1$-mediated $I_{Ca,T}$ starts to activate at about -60 mV and peaks at -40 mV (supplementary Figure 5D, E). As a comparison, TTX-sensitive $I_{Na}$ activates during the late phase of the diastolic depolarisation at about -50 mV and peaks around -30 mV (supplementary Figure 5E). This observation is consistent with the behaviour of TTX-sensitive $I_{Na}$ in action potential clamp experiments (see Figure 3 E, F in Lei et al. and the related discussion). Our simulations predict that $Ca_{v}3.1$-mediated $I_{Ca,T}$ can contribute to the diastolic depolarisation by activating earlier than the fast TTX-sensitive and TTX-resistant $I_{Na}$. (supplementary Figure 5B). The negative activation range of $Ca_{v}3.1$-related $I_{Ca,T}$ indicate that TTX-sensitive $I_{Na}$ cannot compensate for the lack of $I_{Ca,T}$ in $Ca_{v}3.1^{+/−}$ mice, at least under basal conditions. $Ca_{v}1.3$-mediated $I_{Ca,L}$ is predicted to be present throughout the diastolic depolarisation phase (supplementary Figure 5B, D). This persistent component is likely to be due to both slow inactivation kinetics of $Ca_{v}1.3$ channels and/or to the predicted window $Ca_{v}1.3$-mediated $I_{Ca,L}$ (supplementary Figure 4B). Incomplete inactivation of $Ca_{v}1.3$-mediated $I_{Ca,L}$ has also been observed upon application of pacemaker action potential waveforms to HEK cells expressing recombinant human $Ca_{v}1.3$ channels. This persistent $I_{Ca,L}$ component
contribute to the diastolic depolarisation by summing to $I_{Ca,T}$, $I_{st}$ as well as to both TTX-sensitive and –resistant components of $I_{Na}$ close to the cell takeoff potential. For this reason, $Ca_{1.3}$-related $I_{Ca,L}$ is the major voltage-dependent Ca$^{2+}$ current contributing to mouse SAN pacemaking. In our model, $I_{st}$ is activated in the same voltage range as $I_{Ca,T}$. Together with $Ca_{1.3}$-related $I_{Ca,L}$, $I_{st}$ is strongly sensitive to $\beta$-adrenergic agonists. These currents can thus constitute two major mechanisms of compensation of the lack of $Ca_{3.1}$ channels at maximal heart rates which is presumably driven by strong $\beta$-adrenergic input on the SAN (see table1). Compared to $Ca_{1.3}$ and TTX-sensitive channels, $Ca_{3.1}$ channels are strongly inactivated at positive MDPs. We can expect that the relative contribution of $Ca_{3.1}$-mediated $I_{Ca,T}$ to pacemaking will depend upon the cell MDP as well as the degree of stimulation of currents by the $\beta$-adrenergic/cAMP-dependent signalling pathway. Accordingly, switching the model MDP to more positive voltages (supplementary Figure 5F), reduces the effect of $I_{Ca,T}$ abolition on the calculated pacing rate.

We have also directly measured in SAN cells the level of $Ca_{3.1}$-mediated $I_{Ca,T}$ at a test potential of -50 mV from different HP ranging from -70 to -55 mV. To this aim, we have employed WT SAN cells in which $I_{Ca,L}$ did not show detectable activation at -50 mV. In these conditions, averaged $Ca_{3.1}$-mediated $I_{Ca,T}$ peak (isochronal) densities were $0.66\pm0.3$ pA/pF from a HP of -65 mV, $0.18\pm0.065$ pA/pF from a HP of -60 mV and $0.044\pm0.026$ from a HP of -55 mV ($N=2, n=5$, peak $I_{Ca,T}=7.5\pm2$ pA/pF at -30 mV, $Hp=-90$ mV). As a comparison, our model predicts in voltage-clamp conditions $1.9$ pF peak $I_{Ca,T}$ from a HP of -65 mV, $0.47$ pA/pF from a HP=-60 mV and $0.11$ pA/pF from $Hp=-55$ mV ($I_{Ca,T}$ peak density 10 pA/pF).

**Interest of the model study**

The development of a numerical model of mouse SAN electrophysiology based on experimental data coming from normal and genetically-modified mouse strains constitute an
important step toward understanding the physiological role and significance of Ca\textsubscript{v3.1}-mediated $I_{Ca,T}$ compared to other ionic channels. The goal of our numerical simulations is to define the voltage range of activation as well as the relative level of $I_{Ca,T}$ during pacemaker activity with respect to other ionic currents involved in the genesis and regulation of automaticity. Calculations indicate that Ca\textsubscript{v3.1}-mediated $I_{Ca,T}$ can contribute to the diastolic depolarisation by activating early in the diastolic depolarisation phase together with $I_{st}$. The contribution of Ca\textsubscript{v3.1}-mediated $I_{Ca,T}$ to overall pacemaking is predicted to be moderate, an observation which is experimentally verified. Our calculations are thus also important for indicating how Ca\textsubscript{v3.1} channels can contribute to the diastolic depolarisation in spite being partly inactivated at positive MDPs. The physiological significance of Ca\textsubscript{v3.1}-related $I_{Ca,T}$ can also be linked to the presence of large deactivating $I_{Kr}$ at the end of repolarization. Indeed, the presence of $I_{Ca,T}$ can favour the development of the diastolic depolarisation in a voltage range in which large outward $K^+$ currents are still present. In our numerical model, we did not observe a change in the upstroke phase upon abolition of Ca\textsubscript{v3.1}-related $I_{Ca,T}$ (supplementary Figure 5A). Consistently with the work of Lei et al.\textsuperscript{11}, abolition of TTX-sensitive and resistant $I_{Na}$ affected the SAN rate, as well as the takeoff potential and the upstroke phase in our simulations (not shown). These observation indicate that Ca\textsubscript{v3.1}-related $I_{Ca,T}$ and $I_{Na}$ play complementary and distinct roles in mouse SAN pacemaking.

Each numerical model of pacemaking has its internal limitations. In our model, limitations are linked to the assumption that internal $Ca^{2+}$ concentration remains constant and to the available data on mouse SAN automaticity. Indeed, even if detailed modelling of diastolic release of $Ca^{2+}$ have been developed for rabbit SAN cells\textsuperscript{23}, no quantitative experimental data are available on this newly described pacemaker mechanism in mouse SAN cells. Consequently, inclusion of RyR-dependent $Ca^{2+}$ release in our model would be based only on assumptions about parameters scaling. To this respect, the basal calculated rate is about half than that
observed in freely-moving mice (table1). This discrepancy can be in part ascribed to $I_{Kr}$ density that has been set to account for data coming from larger cells and to the inclusion of $I_{Ks}$ and $I_{sus}$ in the model. Furthermore, our model does not include diastolic release of Ca$^{2+}$ which has been shown to strongly accelerate the diastolic depolarisation rate in rabbit SAN cells$^{24,25}$ and in numerical models of pacemaking$^{23,26}$. Finally, the basal adrenergic tone in vivo which stimulates $I_{Ca,L}$, $I_f$, $I_{st}$ and diastolic Ca$^{2+}$ release beyond their basal levels can also explain the difference between the basal model rate and that observed in freely-moving mice.

The lack of experimental data on diastolic Ca$^{2+}$ release in mouse SAN cells also prevented us to get insights into pacemaking during activation of the $\beta$-adrenergic receptor. Nevertheless, our model can be updated as new experimental data on mouse SAN will be available.

The observation that the reduction of pacing rate is higher in SAN cells from Ca$_v$3.1$^{-/-}$ mice (see Figure 4 A, B) than that predicted in our simulations suggests that the contribution of Ca$_v$3.1-mediated $I_{Ca,T}$ to the diastolic depolarisation may also be linked to intracellular Ca$^{2+}$ signalling. Indeed, Huser et al.$^{27}$ have reported that in latent pacemaker cells, T-type channels could trigger Ca$^{2+}$ release, since diastolic Ca$^{2+}$ sparks were generated in a voltage range compatible with T-type channel activation and were blocked by 50 µM Ni$^{2+}$. This view has been challenged in a recent work on diastolic Ca$^{2+}$ release in primary rabbit SAN pacemaker cells$^{25}$ in which no change in Ca$^{2+}$ sparks were reported in the presence of 30 µM Ni$^{2+}$. The fact that Ca$_v$3.1 channels are only partly inhibited at 50 µM Ni$^{2+}$ indicate the necessity to investigate if SAN Ca$_v$3.1 channels are coupled to Ca$^{2+}$ release during diastolic depolarization in the mouse SAN. On the other hand, a species-dependent influence on the functional expression of Ca$_v$3.2 in the adult heart cannot be excluded. For instance it has been reported that $I_{Ca,T}$ was strongly inhibited by 50 µM Ni$^{2+}$ in cat latent pacemaker cells$^{27}$, thus rising the possibility of functional Ca$_v$3.2 channels in these cells.
References cited in the supplementary data:


Online Supplementary Figure Legends

Online supplementary Figure 1:

No difference in capacitance and cell morphology between WT and Ca,3.1⁻⁰ cardiac cells. Electrophysiological recordings of SAN cells from WT and Ca,3.1⁻⁰ mice animals showed no change in the cell capacitance (19±2 pF N=13, n=17 and 20±1 pF N=12, n=18, respectively). Consistently, the averaged upstroke velocities in action potentials recorded (see Fig. 4 in the main text) were 20±5 V/S (N=5, n=14) and 21±5 V/S, (N=4, n=15) in SAN cells from WT and Ca,3.1⁻⁰ mice. These values are consistent with the leading mouse SAN pacemaker cells presumably from the center of the SAN. In addition, no difference in their morphology could be identified (supplementary Figure 1A). Similarly, inactivation of Ca₃.1 induce no change in capacitance for AVN cells (17±1 pF N=5, n=7 and 18±4 pF, N=4, n=7; supplementary Figure 1B) and for RA cells (33.3±1.4 pF and 32.7±2.3 pF N=3, n=8; supplementary Figure 1C).

Online supplementary Figure 2.

Expression of T-type Ca²⁺channel isotype mRNAs in wild-type (+/+) and Ca₃.1⁻⁻ (−/−) mouse heart. Transcripts for the Ca₃.1 and Ca₃.2 subunits, but not for the Ca₃.3 subunit, were detected in the SAN, as compared to that obtained with hippocampus mRNA used as a positive control (Supplementary Figure 2A). The disruption of the Cav3.1 gene was assessed by using a set of primers overlapping from the first to the third exon. Expectedly, no Ca₃.1 transcripts were detectable in Ca₃.1⁻⁻ mice since the forward primer used to identify Ca₃.1 transcripts was comprised within exon 1 that was deleted to generate a null allele for cacna1g. Because RT-PCR experiments indicated that Ca₃.1 transcripts were present in
various cardiac tissues, real-time TaqMan PCR was developed to quantitatively analyze T-type channel mRNA expression levels in various cardiac areas: SAN, sino-atrial node; AVN, atroventricular node; RA, right atrium; LV, left ventricle (Supplementary Figure 2B). mRNA contents were normalized according to HPRT mRNA contents and the results are expressed as $2^{-\Delta Ct} \times 100$. Each value represents the mean of at least 6 experiments in triplicate out of 3 independent RT samples. The upper panel shows the corresponding gels (Mw: molecular weight). The highest amounts of Ca$_v$3.1 mRNA were detected both in the SAN and in the AVN, while the amount of Ca$_v$3.1 mRNA in the RA and in the LV was significantly lower. Real-time PCR also confirmed the absence of Ca$_v$3.3 mRNA in all cardiac tissues while Ca$_v$3.2 mRNA was detected in mouse cardiac tissues, especially in the RA. We found mRNAs for both Ca$_v$3.1 and Ca$_v$3.2 channels in the adult mouse SAN, AVN and the RA (see supplementary Figure 2). Our RT-PCR results are consistent with in situ hybridisation data describing lower Ca$_v$3.2 mRNA levels as compared to Ca$_v$3.1 $^{29}$. Interestingly, several recent studies have revealed that after birth only $I_{Ca,T}$ related to Ca$_v$3.1 channels is detectable, while both Ca$_v$3.1 and Ca$_v$3.2 channels are expressed in the foetal heart $^{17,30-32}$. These data suggest a developmental isotype switch in the mouse heart with embryonic $I_{Ca,T}$ corresponding to a mix of Ca$_v$3.2 and Ca$_v$3.1 channels, while after birth only Ca$_v$3.1 channels would be functionally expressed.

Online supplementary Figure 3.

Relation between the QT and RR ECG intervals recorded in sedated WT (open circles) and Ca$_v$3.1$^{-/-}$ mice (filled circles). Each data point represents one mouse.
Online supplementary Figure 4.

Voltage-dependent properties of ionic currents calculated using the mouse SAN electrophysiology model. (A). Voltage clamp traces of voltage-dependent ionic currents (B). Activation and inactivation curves for Ca\textsubscript{v}3.1 ($d_T, f_T$), Ca\textsubscript{v}1.3 ($d_{LD}, f_{LD}$) and Ca\textsubscript{v}1.2 ($d_L, f_L$) channels used in the model, as indicated in the panel legend. (C). Activation and inactivation curves for TTX-sensitive and TTX-resistant $I_{Na}$, as indicated in panel legend. Abbreviations used in panels B, C for gating variables are referred in supplementary table 3.

Online supplementary Figure 5.

Simulated mouse SAN action potentials and underlying ionic currents. (A). Effect of abolishing Ca\textsubscript{v}3.1-mediated $I_{Ca,T}$ on a simulated SAN action potential obtained with parameters indicated in supplementary tables 1-3. (B). Activation of Ca\textsubscript{v}3.1-mediated $I_{Ca,T}$ compared with Ca\textsubscript{v}1.3, Ca\textsubscript{v}1.2 and TTX-sensitive and resistant $I_{Na}$ as indicated in the panel legend. (C). Activation during the pacemaker cycle of voltage-dependent $K^+$ currents, together with $I_{K1}$ and $I_f$. (D). Current-to-voltage relationship of Ca\textsubscript{v}3.1, Ca\textsubscript{v}1.3 and Ca\textsubscript{v}1.2 channels during the pacemaker cycle shown in (A). The predicted $I_{st}$ current is also shown here. (E). Current to voltage relationships comparing Ca\textsubscript{v}3.1 channels to TTX-sensitive and TTX-resistant $I_{Na}$ as indicated in the panel legend, for the pacemaker cycle in (A). (F). Effects of Ca\textsubscript{v}3.1 abolition in a simulated SAN action potential obtained by setting $I_{K1}$ density to zero and by lowering the conductance of $I_{Kr}$ to 0.0018 $\mu$S.
Online Supplementary table 1.

*Parameter values of the mouse SAN cell model.* Generally, abbreviations are the same as in the original model by Zhang et al. Other abbreviations define the conductance of $I_{st}$ ($g_{st}$) and that of $I_{KI}$ ($g_{KI}$).

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Absolute value</th>
<th>Abbreviation</th>
<th>Absolute value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_m$</td>
<td>21 pF</td>
<td>$Pinac$</td>
<td>-15</td>
</tr>
<tr>
<td>$d_{NaCa}$</td>
<td>0.0001</td>
<td>$F_{K,r}$</td>
<td>0.4</td>
</tr>
<tr>
<td>$E_{Ca,L}$</td>
<td>46.4 mV</td>
<td>$g_{to}$</td>
<td>0.00049 µS</td>
</tr>
<tr>
<td>$E_{Ca,T}$</td>
<td>45 mV</td>
<td>$g_{sus}$</td>
<td>0.000065 µS</td>
</tr>
<tr>
<td>$G_{Na}$ (TTX resistant)</td>
<td>7.5x10^{-7} µS</td>
<td>$g_{K,r}$</td>
<td>0.000518 µS</td>
</tr>
<tr>
<td>$I_{Na} V_{0.5act}$</td>
<td>-44 mV</td>
<td>$g_{I,Na}$</td>
<td>0.002 µS</td>
</tr>
<tr>
<td>$I_{Na} V_{0.5inact}$</td>
<td>-65 mV</td>
<td>$g_{I,K}$</td>
<td>0.002 µS</td>
</tr>
<tr>
<td>$G_{Na}$ (TTX sensitive)</td>
<td>7.5x10^{-6} µS</td>
<td>$g_{b,Na}$</td>
<td>0.000058 µS</td>
</tr>
<tr>
<td>$I_{Na} V_{0.5act}$</td>
<td>-29 mV</td>
<td>$g_{b,Ca}$</td>
<td>0.0000152 µS</td>
</tr>
<tr>
<td>$I_{Na} V_{0.5inact}$</td>
<td>-56 mV</td>
<td>$g_{b,K}$</td>
<td>0.0000252 µS</td>
</tr>
<tr>
<td>$g_{Ca,L}$</td>
<td>0.0022 µS</td>
<td>$I_{pmax}$</td>
<td>0.0478 nA</td>
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<tr>
<td>$I_{Ca,L} V_{0.5act}$ (Ca_{1.2})</td>
<td>-3 mV</td>
<td>$K_{m,K}$</td>
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<tr>
<td>$I_{Ca,L} V_{0.5inact}$ (Ca_{1.2})</td>
<td>-28 mV</td>
<td>$K_{m,Na}$</td>
<td>5.64</td>
</tr>
<tr>
<td>$g_{Ca,D}$</td>
<td>0.0064 µS</td>
<td>$\gamma_{NaCa}$</td>
<td>0.5</td>
</tr>
<tr>
<td>$I_{Ca,D} V_{0.5act}$ (Ca_{1.3})</td>
<td>-28 mV</td>
<td>$k_{NaCa}$</td>
<td>0.000027 nA</td>
</tr>
<tr>
<td>$I_{Ca,D} V_{0.5inact}$ (Ca_{1.3})</td>
<td>-48 mV</td>
<td>$[Na^+]_o$</td>
<td>140 mM</td>
</tr>
<tr>
<td>$g_{Ca,T}$</td>
<td>0.01 µS</td>
<td>$[Na^+]_i$</td>
<td>8 mM</td>
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<tr>
<td>$I_{Ca,T} V_{0.5act}$ (Ca_{3.1})</td>
<td>-45 mV</td>
<td>$[Ca^{2+}]_o$</td>
<td>2 mM</td>
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<tr>
<td>$I_{Ca,T} V_{0.5inact}$ (Ca_{3.1})</td>
<td>-71 mV</td>
<td>$[Ca^{2+}]_i$</td>
<td>0.0001 mM</td>
</tr>
<tr>
<td>$g_{st}$</td>
<td>0.017 µS</td>
<td>$[K^+]_o$</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>$g_{KI}$</td>
<td>0.0009 µS</td>
<td>$[K^+]_i$</td>
<td>140 mM</td>
</tr>
<tr>
<td>$g_{K,r}$</td>
<td>0.0022 µS</td>
<td></td>
<td></td>
</tr>
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Online supplementary table 2.

Current densities. The table show the experimental ionic current densities used in modelling mouse SAN cell pacemaking. The test potential and the reference from which each value has been deduced are also shown.

<table>
<thead>
<tr>
<th>Current</th>
<th>Density (pA/pF)</th>
<th>Test potential (mV)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na}$ (TTX resistant)</td>
<td>10</td>
<td>-20</td>
<td>11</td>
</tr>
<tr>
<td>$I_{Nas}$ (TTX sensitive)</td>
<td>135</td>
<td>-20</td>
<td>11</td>
</tr>
<tr>
<td>$I_{Ca,L}$ (Ca$_{1,2}$)</td>
<td>3.1</td>
<td>+10</td>
<td>3</td>
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<tr>
<td>$I_{Ca,LD}$ (Ca$_{1,3}$)</td>
<td>10.4</td>
<td>-20</td>
<td>This study, see also 3</td>
</tr>
<tr>
<td>$I_{Ca,T}$</td>
<td>10.5</td>
<td>-30</td>
<td>This study</td>
</tr>
<tr>
<td>$I_{st}$</td>
<td>7.4</td>
<td>-30</td>
<td>12</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>2.1</td>
<td>-10</td>
<td>21</td>
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<tr>
<td>$I_{K1}$</td>
<td>6.2</td>
<td>-120</td>
<td>12</td>
</tr>
<tr>
<td>$I_{f}$</td>
<td>21</td>
<td>-120</td>
<td>1</td>
</tr>
<tr>
<td>$I_{to}$</td>
<td>15.45</td>
<td>+50</td>
<td>9</td>
</tr>
<tr>
<td>$I_{sus}$</td>
<td>0.54</td>
<td>+50</td>
<td>9</td>
</tr>
<tr>
<td>$I_{Ks}$</td>
<td>0.66</td>
<td>+40</td>
<td>9</td>
</tr>
<tr>
<td>$I_{b,Na}$</td>
<td>0.43</td>
<td>-100</td>
<td>9</td>
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<tr>
<td>$I_{b,K}$</td>
<td>0.002</td>
<td>-100</td>
<td>9</td>
</tr>
<tr>
<td>$I_{b,Ca}$</td>
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<td>-100</td>
<td>9</td>
</tr>
<tr>
<td>$I_{NaCa}$</td>
<td>0.013</td>
<td>-100</td>
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<tr>
<td>$I_{p}$</td>
<td>0.098</td>
<td>-100</td>
<td>9</td>
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Supplementary table 3.

Model initial values. Abbreviations are defined as in the original model by Zhang et al$^9$. $d_{LD}$, $f_{LD}$, $d_{st}$, $f_{st}$ are the activation and inactivation gating variables for the Ca$_{1,3}$ and $I_{st}$ current, respectively.

<table>
<thead>
<tr>
<th>$V_M$ (mV)</th>
<th>$d_T$</th>
<th>$f_T$</th>
<th>$d_{st}$</th>
<th>$f_{st}$</th>
<th>$y$</th>
<th>$n$</th>
<th>$p_{a,f}$</th>
<th>$p_{a,s}$</th>
<th>$p_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-60.1</td>
<td>0.02217</td>
<td>0.06274</td>
<td>0.017</td>
<td>0.283</td>
<td>0.0528</td>
<td>0.0767</td>
<td>0.400</td>
<td>0.327</td>
<td>0.991</td>
</tr>
</tbody>
</table>
Mangoni et al. Supplementary Figure 1

A  SAN :

\[
\begin{align*}
\text{Cm (pF)} & \quad \text{ns} \\
+/- & \quad \text{ns} \\
\end{align*}
\]

B  AVN :

\[
\begin{align*}
\text{Cm (pF)} & \quad \text{ns} \\
+/- & \quad \text{ns} \\
\end{align*}
\]

C  RA :

\[
\begin{align*}
\text{Cm (pF)} & \quad \text{ns} \\
+/- & \quad \text{ns} \\
\end{align*}
\]

+/- 5 µM
A

\[
\begin{array}{ccc}
\text{Ca}_{\gamma}3.1 & \text{Ca}_{\gamma}3.2 & \text{Ca}_{\gamma}3.3 \\
\text{SAN} & \text{Hipp.} & \text{SAN} & \text{Hipp.} & \text{SAN} & \text{Hipp.} \\
+/- & -/- & +/- & -/- & +/- & -/-
\end{array}
\]

B

\[
\begin{array}{cccccc}
\text{Ca}_{\gamma}3.1 & \text{Ca}_{\gamma}3.2 & \text{Ca}_{\gamma}3.3 \\
\text{Mw} & \text{SAN} & \text{AVN} & \text{RA} & \text{LV} & \text{SAN} & \text{AVN} & \text{RA} & \text{LV} & \text{SAN} & \text{AVN} & \text{RA} & \text{LV}
\end{array}
\]

\[
\begin{array}{c}
\text{mRNA Ca}_{\gamma}3/\text{HPRT (\%)} \\
\text{Ca}_{\gamma}3.1 \\
\text{Ca}_{\gamma}3.2 \\
\text{Ca}_{\gamma}3.3
\end{array}
\]

\[\begin{array}{c}
\text{mRNA Ca}_{\gamma}3/\text{HPRT (\%)} \\
\text{Ca}_{\gamma}3.1 \\
\text{Ca}_{\gamma}3.2 \\
\text{Ca}_{\gamma}3.3
\end{array}\]

\text{Mangoni et al. Supplementary Figure 2}
Mangoni et al. Supplementary Figure 3
ICaT (Cav3.1)  
Hp = -90 mV

ICaL (Cav1.3)  
Hp = -60 mV

II  
Hp = -80 mV

INaTTx  
Hp = -120 mV

IKr  
Hp = -60 mV

IKr + If  
Hp = -35 mV

Mangoni et al. Supplementary Figure 4