Figures and figure supplements

The Ca\textsuperscript{2+} transient as a feedback sensor controlling cardiomyocyte ionic conductances in mouse populations

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Figure 1. Schematic representation of the sarcolemmal currents and intracellular Ca\textsuperscript{2+} cycling proteins of the mouse ventricular myocyte model. DOI: https://doi.org/10.7554/eLife.36717.002
Figure 2. Effects of individual conductances on the Ca\(^{2+}\) transient (CaT). (A) CaT amplitude defined as the difference \(\Delta[Ca_i]\) between the peak and diastolic values of the cytosolic Ca\(^{2+}\) concentration \([Ca_i]\) versus \(G/G_{ref}\) where \(G\) is the individual conductance value and \(G_{ref}\) some fixed reference value. (B) Time-averaged \([Ca_i]\) over one pacing period \(\langle [Ca_i] \rangle\) versus \(G/G_{ref}\). Illustration of the effect of varying \(I_{Ca,L}\) conductance (C) and \(I_{lo,f}\) conductance (D) on AP and CaT profiles, where 50%, 100%, and 150% correspond to \(G_{ref} = 0.5, 1.0, \text{ and } 1.5\), respectively. (E) Effect of varying RyR conductance on SR Ca\(^{2+}\) concentration \([Ca_{SR}]\) and CaT. Different time windows are plotted for the CaT and SR load (0 to 150 ms) and AP waveforms (0 to 50 ms) in (C–E).

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Figure 3. Computationally determined good enough solutions (GES) with calcium sensing. (A) Examples of GES representing combinations of 6 conductances that produce a normal CaT and intracellular Na⁺ concentration. Each color represents a different GES and the corresponding AP and
CaT profiles are shown in B) and C), respectively. (D) Histograms of individual normalized conductances $G/G_{\text{ref}}$ for a collection of 7263 GES showing that some conductances are highly variable while others are highly constrained. (E) Three-dimensional (3D) plot revealing a three-way compensation between conductances of $I_{\text{Ca,L}}, I_{\text{Kur}}$, and $I_{\text{f}}$. Each GES is represented by a red dot. All GES lie close to a 2D surface in this 3D plot. Pairwise projections (grey shadows) do not show evidence of two-way compensation between pairs of conductances. (F) Alternate representation of three-way compensation obtained by plotting $I_{\text{Ca,L}}$ versus the sum of $I_{\text{f}}$ and $I_{\text{Kur}}$. Peak values of those currents after a voltage step from $-50$ to 0 mV are used to make this plot that can be readily compared to experiment. Different time windows are plotted for the AP waveforms and CaT in B and C, respectively.

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Figure 3—figure supplement 1. Histograms of individual ion channel conductances in 8320 GESs found by a GES search constrained only by Ca\(^{2+}\) transient amplitude and average, but not constrained by intracellular sodium concentration [Na\(_i\)].

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Figure 3—figure supplement 2. Correlation between $I_{Ca,L}$ and the sum of $I_{to,f}$ and $I_{Kur}$ is weaker but still significant when intracellular sodium concentration is not constrained.
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Figure 3—figure supplement 3. Computationally determined GES with voltage sensing. (A) Examples of GES representing combinations of 6 conductances that produce a normal AP with predominantly voltage sensing. Sensors are \( S_1 = \text{APD}_{90} \), \( S_2 = \text{APD}_{30} \) and \( S_3 = [\text{Na}]_i \) with \( \epsilon = 0.05 \). Each

Figure 3—figure supplement 3 continued on next page
Figure 3—figure supplement 3 continued

Color represents a different GES and the corresponding AP and CaT profiles are shown in (B) and (C), respectively. Correlation between $I_{\text{Ca,L}}$ and the sum of $I_{\text{to,L}}$ and $I_{\text{cur}}$ is not present among models constrained by $\text{APD}_{90}$ (D) or by $\text{APD}_{90}$, $\text{APD}_{30}$ and $[\text{Na}]_i$ (E).

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Figure 4. Good enough solutions in the Hybrid Mouse Diversity Panel (HMDP). (A) Central result of this paper showing quantitative agreement between theoretically predicted and experimentally measured compensation of inward Ca$^{2+}$ and outward K$^+$ currents. Equivalent plot of Figure 3F showing the sum of $I_{\text{tot}}$ and $I_{\text{Kur}}$ versus $I_{\text{Ca,L}}$ for nine different mouse strains using peak values of those currents (proportional to conductances) after a voltage step from $-50$ to $0$ mV. Mean current values (green filled squares) are shown together with standard errors of the mean (thin bars) for each strain. The number of cells used for each strain is given in Table 1 of the Materials and methods section. Computationally determined GES are superimposed and shown as faded red points using all three sensors (CaT amplitude, average $[\text{Ca}^2]_i$, and diastolic $[\text{Na}]_i$) and faded blue points for two sensors (CaT amplitude and average $[\text{Ca}^2]_i$). Lines represent linear regression fits using the method of Chi-squared minimization with errors in both coordinates including (solid line, $p=0.0144$) and excluding (dashed line, $p=0.0007$) the outlier strain BXA12/PgnJ marked by a red box. The small $p$ values of those fit validate the computationally predicted three-way compensation of Ca$^{2+}$ and K$^+$ currents. The three strains selected for the organ scale study (C57BL/6J, CXB1/ByJ, and BXA25/PgnJ) with low, medium, and high $I_{\text{Ca,L}}$ conductance, respectively, are highlighted by blue circles. (B) Cell shortening, measured as the fraction of resting cell length at 4 Hz pacing frequency in different HMDP strains where thick and thin bars correspond to standard error of the mean and standard deviation, respectively. A standard ANOVA test shows no significant differences in cell shortening between strains ($p=0.4136$).
Figure 4 continued

supporting the hypothesis that different combinations of conductances produce a similar CaT and contractile 
activity.
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Figure 4—figure supplement 1. Patch clamp measurements of mean $I_{Ca,L}$ (A), $I_{i,f}$ (B), and $I_{Kur}$ (C) functional current density averaged over multiple cells for nine HMDP mouse strains with standard errors (thick bars) and standard deviations (thin bars).

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Figure 5. Organ scale compensation. (A) Mean $I_{Ca,L}$ conductance in three different HMDP strains where thick and thin bars denote standard error and standard deviation, respectively. (B) Sets of conductances generated to be representative of individual cells within ventricular tissue of the three strains.

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Figure 5 continued on next page.
by assigning normally distributed random values to the $I_{Ca,L}$, $I_{tof}$, and $I_{Kur}$ conductances using experimentally determined means and standard deviations. The blue, green, and red points correspond to the three HMDP strains with low (C57BL/6J), medium (CXB1/ByJ), and high (BXA25/PgnJ) $I_{Ca,L}$ conductance, respectively, and the grey points are the results of the three-sensor GES search (same as Figure 3F). (C) Variable AP waveforms in uncoupled myocytes with conductances randomly chosen from the distribution shown in B for C57BL/6J and D) AP waveforms for coupled myocytes in tissue for C57BL/6J and the two other strains. AP waveforms of uncoupled cells vary significantly from cell to cell as observed experimentally (Fig. Figure 5—figure supplement 1) but are uniform in electrotonically coupled cells, as expected. (E) Histograms of Ca$^{2+}$ transient (CaT) amplitude ($\Delta$Ca) and action potential duration (APD) for C57BL/6J in electrotonically uncoupled and coupled cells. Importantly, in coupled cells, the more uniform APD translates into a much more uniform CaT amplitude, reflecting the strong effect of the cell’s APD on its CaT amplitude. (F) Distribution of CaT amplitudes within electrotonically coupled cells in tissue scale simulations using the parameter distributions from B. The three strains have the same mean CaT amplitude averaged over all cells marked by a thick vertical gray line, thereby demonstrating that compensation of Ca$^{2+}$ and K$^+$ currents remains operative at a tissue scale. (G) Distribution of CaT amplitudes obtained by varying only $I_{Ca,L}$ conductance and with $I_{tof}$ and $I_{Kur}$ conductances fixed to their reference values. Lack of compensation between Ca$^{2+}$ and K$^+$ currents in this case yields different mean CaT amplitude.

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Figure 5—figure supplement 1. Action potential recordings from isolated myocytes for mouse strain C57BL/6J paced at 4 Hz under current clamp. The recordings illustrate the typical degree of cell-to-cell variability of AP morphology observed in all strains.
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Figure 5—figure supplement 2. Histogram of average Ca$^{2+}$ concentration corresponding to Figure 5E for C57BL/6J in electrotonically uncoupled and coupled cells.
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Figure 6. Correlation between L-type Ca$^{2+}$ current conductance and cardiac hypertrophic response to a stressor for different HMDP strains. The Pearson correlation is $r = 0.86$ ($p=3e-4$).

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Figure 7. Compensation and gene expression. Plot showing the existence of a statistically very significant correlation (Pearson correlation coefficient $r = 0.47$ and p-value, $p = 8.1 \times 10^{-11}$) between the expression level of Kcnip2, encoding the KChIP2 accessory $\beta$ subunits that interact with Kv4.2 channels ($I_{\text{h,t}}$) and of Cacna1c, a gene encoding the $\alpha_{1C}$ subunit of the Cav1.2 L-type calcium channels ($I_{\text{Ca,L}}$) across 206 mice. Cardiac gene expression was measured in 106 control (Pre-ISO) strains and 21 days after injection of isoproterenol (post-ISO) in 100 HMDP strains (a smaller number due to higher mortality of certain strains). Note that the significant correlation holds when considering separately pre-ISO (blue points, $r = 0.59$, $p = 2 \times 10^{-11}$) and post-ISO (red points, $r = 0.42$, $p = 1.5 \times 10^{-3}$) data. Lines show best fits of a linear model for pre-ISO (blue), post-ISO (red), and pre- and post-ISO combined (black). Expression data is taken from Santolini et al. (2018) and is averaged over all microarray probes for each gene.

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