In a recent article in the *Jpn J Physiol* Matsuoka *et al.* [1] present in their discussion section a comparison of their cardiac action potential model and the Luo-Rudy dynamic (LRd) model [2]. Figure 10 in their paper compares the action potential (AP), calcium transient (CaT) and selected ionic currents during the AP. Several results of their LRd simulations are conducted using parameters/protocols that influence the comparison and are not provided in their paper. We repeated all simulations presented in their paper and provide relevant results and discussion in this Letter to the Editor.

**Simulation results**

Matsuoka Fig. 10 shows simulations pacing their model at steady-state at a cycle length (CL) of 400 ms; they compare the results to simulations using LRd. Using the current version of the LRd model [3], our results for the same protocol (steady state, CL = 400 ms; Fig. 1) differ from their LRd simulations. Important differences include: a) CaT exceeds 1 µM in amplitude, while in their simulations it barely reaches half this value. b) The computed Ca²⁺ gain is 3.85; they report 2.33. c) In Fig. 1, the early spike of $I_{Ca(L)}$ is deeper than the dome; it is opposite in their Fig. 10. As shown by Faber and Rudy [3] such results can depend on the sodium concentrations used in the simulations. Unfortunately, Matsuoka *et al.* do not provide the sodium concentration in their simulation using the LRd model.

**Comparison to experiments—L-type calcium current $I_{Ca(L)}$**

In their discussion of the magnitude of peak $I_{Ca(L)}$, Matsuoka *et al.* do not consider an important experiment performed by Linz and Meyer [4]. In this experiment, shown in Fig. 3 of Linz and Meyer, AP clamps of decreasing peak potential are shown to elicit $I_{Ca(L)}$ tracings of increasing peak amplitude. For 40 mV peak potential, measured peak $I_{Ca(L)}$ amplitude is approximately 6 µA/µF. The LRd peak potential for a 400 ms cycle length stimulus at steady-state is 41.5 mV, and peak $I_{Ca(L)}$ is 6.00 µA/µF, in agreement with that measured by Linz and Meyer. On a more general note, when presented as whole-cell current in pA (as in Matsuoka *et al.* Fig. 10), current amplitude depends strongly on cell size used in the simulation. Instead, currents should be normalized to cell size and compared in units of µA/µF that correspond to current density.

Regarding $I_{Ca(L)}$ during the plateau of the AP, Matsuoka *et al.* state that the LRd $I_{Ca(L)}$ magnitude is too large by comparison with Linz and Meyer [4] AP clamp measurements. The AP used in the Linz and Meyer clamp protocol is exceedingly long (APD₉₀ is 377.8 ms), and is preceded by a 200 ms step from resting potential to −35 mV. We simulated the exact voltage protocol of Linz and Meyer with the LRd model (the AP-clamp waveform was traced using *Data Thief II*, http://www.nikhef.nl/~keeshu/datathief/). The result in Fig. 2 shows that $I_{Ca(L)}$ as formulated in the LRd model reproduces well the recording of Linz and Meyer during the AP plateau.

**CaT and gain**

When paced to steady-state, CaT amplitude in the LRd model exceeds an amplitude of 1 µM and its morphology during the AP (Fig. 3) is very similar to that measured recently by Choi and Salama [5]. (Another
example of close correspondence between LRd simulated AP and CaT and their measured counterparts [6] is provided on our web site: http://rudylab.wustl.edu/research/cell/methodology/cellmodels/LRd/schematic.htm#ap).

Matsuoka et al. report a gain of 15.2 for the their T. O’HARA et al. Japanese Journal of Physiology Vol. 54, No. 5, 2004

Fig. 1. Our reproduction of the simulation shown in Fig. 10b of Mastuoka et al. [1] for the LRd model. $I_{Ca(L)}$ is the L-type current carried by Ca$^{2+}$ which participates in triggering calcium release from the sarcoplasmic reticulum, included in computation of gain. Gain measurement is according to the protocol and definition of Matsuoka et al. The number of pacing pulses needed to reach steady-state depends on the frequency of pacing, initial conditions and other details of the protocol. We typically define steady-state when state variables vary by less than 1% with additional pacing.

Fig. 2. Simulated LRd $I_{Ca(L)}$ subject to the 40 mV Linz and Meyer AP-clamp waveform. $I_{Ca(L)}$ has an amplitude that is less than 3 µA/µF during the entire plateau. Compare with Fig. 3 from Linz and Meyer [4].

model, computed as the total (integrated over time) calcium flux through the ryanodine receptor (RyR) divided by the total calcium influx carried by $I_{Ca(L)}$. Our calculation using the same gain definition in LRd (steady-state, CL = 400 ms) gives a gain of 3.85. Matsuoka et al. refer to a gain measurement by Wier et al. [7] for validation. The definition of gain in this measurement differs from that of Matsuoka et al.; it computes the ratio of the instantaneous maximum calcium flux through RyR to that through $I_{Ca(L)}$ during steps from −40 mV to various test potentials for 200 ms. Approximating the AP as a step to +50 mV for 200 ms, Wier et al. results suggest a gain close to 5 (see their Fig. 3C). In this context of gain definition, it is important to note that gain is strongly species dependent (Wier et al. [7] report gain in the rat which tends to be high). Also, it is important to decide on a consistent formulation of gain computation among all laboratories. In particular, the definition should include the $I_{Ca(L)}$ component carried by Ca$^{2+}$ and Ca$^{2+}$ influx through the Na$^{+}$–Ca$^{2+}$ exchanger. If integra-

Fig. 3. CaT from Choi and Salama [5] (their Fig. 3) and simulated by the LRd model. In both experiment and LRd simulation, CaT decays slowly, extending beyond the AP.
We have read with interest the letter to the JJP edition submitted by O’Hara et al. It raises the issues surrounding our simulation results of the Luo-Rudy dynamic (LRd) model [2] published recently in the *Japanese Journal of Physiology* [1].

O’Hara et al. show simulation results by using the current version of the LRd model in their Fig. 1, which is different from Fig. 10 in Matsuoka et al. [1], and point out that the intracellular Na⁺ concentration is a key factor that explains the difference between our simulation and theirs. We obtained results similar to theirs in Fig. 1 by using the source code of the LRd model from their Web site (http://www.cwru.edu/med/CBRTC/LRdOnline/). However, the current version of the LRd model, which they used, is different from the one in our simulation [1], which was based on their original paper [2] and partly on the source code that was available on their Web site a few years ago. We found several subtle differences between the two versions, such as the Na⁺-activated K⁺ channel, the Hill coefficient of fCa gate in the L-type Ca²⁺ channel, the Na⁺ pump, and the Na⁺ channel. Besides these differences, in our simulation the extracellular K⁺ concentration was set at 5.4 mM instead of the 4.5 mM of the original LRd model. These differences might affect the simulation results.

We agree that the intracellular Na⁺ concentration has a significant effect on simulation results. With the LRd model we used, however, we found it hard to reach a steady state. This was probably because ion (K⁺) carried by the stimulus current, as described in another article by their group [9], was not taken into account. This problem has been overcome in the latest version. We presented the simulation results obtained when the intracellular Na⁺ concentration was 9.5–10 mM (initial value was 9 mM) in our Fig. 10. However, we found that the intracellular Na⁺ concentration seems to rise more than 15 mM during the long pacing [9]. The main cause of the difference between our simulation and theirs may be the intracellular Na⁺ concentration.

O’Hara et al. claimed that current amplitude depends strongly on cell size (cell capacitance) and that currents should be normalized according to cell capacitance. However, in our paper we defined that our cell size was 132 pF in the ventricular cell [1]. Since experimental data are not always expressed as the normalized form, we think this is not a critical matter. But we agree that for programming purposes, normalization by cell size is important.

We also agree with their argument about a consistent formulation of the calcium gain both in the experiment and the computation. In our Fig. 10 we showed the total current via the L-type Ca²⁺ channel because it is difficult to obtain pure Ca²⁺ current in the experimental study, and we integrated Ca²⁺ influx through the L-type Ca²⁺ current. The Ca²⁺ influx through the Na⁺/Ca²⁺ exchange was not considered for the calculation of “gain.” Gain is an important parameter in the cardiac E-C coupling, though experimental measurements of it have several limitations, which O’Hara et al. pointed out. Further study in both the experiment and the computer simulation should be undertaken.

The importance of simulation study has been widely recognized. However, it is unfortunately sometimes difficult to repeat and reconfirm a previous simulation study because of typographical errors and inaccurate information about modeling as a result of limited space in journals. We believe that free access to the source codes of the mathematical models, as both the LRd and Kyoto models (http://www.card.med.kyoto-u.ac.jp/Simulation/index.html) are available on the Web site, leads to profound discussions.

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**Response**

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