Mukherjee et al., http://www.jgp.org/cgi/content/full/jgp.201110706/DC1

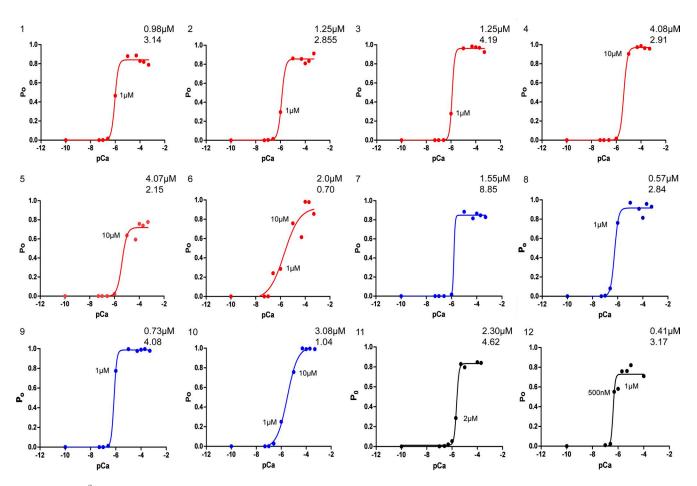


Figure S1. Ca²⁺ activation profiles for 12 hRyR2 channels (0–500 μM). These are color coded as follows: red, channels recorded at 0, 0.05, 0.1, 0.25, 1, 10, 50, 100, 200, and 500 μM [Ca²⁺]_{cyt}, which were all analyzed by MIL fitting; blue, channels recorded at the same concentrations as above, but data either showed periods of modal gating at $P_o \ge 0.8$ resulting in increased T_o values (channels 8, 9, and 10; see Fig. 2 C in main text) or did not exhibit any intermediate open probabilities (channel 7) and were therefore not included in any further analysis; black, channels recorded at additional intermediate concentrations (0, 0.1, 0.25, 0.5, 1, 2, 5, 10, and 100 μM [Ca²⁺]_{cyt}, and 200 μM for channel 11), which were also analyzed by MIL fitting. Individual EC₅₀ and Hill coefficient measurements are given in each top right-hand corner.

Λ.				1			В							
Α	Actual data		ual data	Model demonstrating ligand-dependent rates		Simulated data		3		tual data	Model demonstrating ligand-dependent rates		Simulated data	
	200µМ	Po	0.82	$ C_{NR} \rightleftharpoons C_R \rightleftharpoons O_1 \rightleftharpoons O_2 \rightleftharpoons O_3 \rightleftharpoons O_4 $	Po	0.8		200μΜ	Po	0.815	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$	Po	0.93	
		T _o	1.65		T _o	1.5			T _o	1.75		T _o	2.4	
		T _c	0.45	\ddot{C}_{F}	T _c	0.4			T _c	0.45	$\overset{\circ}{\mathbf{C}_{\mathbf{F}}}$	T _c	0.3	
	100μΜ	Po	0.82	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{f} \rightleftharpoons O_{2} \rightleftharpoons O_{5} \rightleftharpoons O_{4}$ $\downarrow \downarrow $	P _o	0.79		100μΜ	P _o	0.82	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ $\downarrow \downarrow $	P _o	0.90	
		T _o	1.8		T _o	1.2			т,	1.7		T.	1.7	
		T,	0.35		T _c	0.4			T _c	0.4		T _c	0.3	
	1μМ	Po	0.45	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{f} \rightleftharpoons O_{2} \rightleftharpoons O_{5} \rightleftharpoons O_{4}$ $\downarrow \downarrow $	P _o	0.42			P _o	0.339	$C_{NR} \rightleftharpoons C_R \rightleftharpoons O_1 \rightleftharpoons O_2 \rightleftharpoons O_3 \rightleftharpoons O_4$	Pa	0.399	
		T _o	1.8		T.	1.5		1μΜ	T _o	1.75		T _o	1.9	
		T,	2.35		T _c	0.7			T _c	3.35	C _E	T,	0.7	
	0.25μΜ	P _o	0.02	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ O_{UU}	P _o	0.026		0.25μΜ	P _o	0.0219	$C_{NR} \stackrel{\sim}{=} C_R \stackrel{\sim}{=} O_1 \stackrel{\sim}{=} O_2 \stackrel{\sim}{=} O_3 \stackrel{\sim}{=} O_4$	P _a	0.025	
		T _o	0.35		T _o	0.6			l r.	0.45		T,	1.05	
		T,	18.75		T _c	29			T T	34.1		T.	30.45	
	10 2014 ## 10 2014 ##													
C		Act	ual data	Model demonstrating ligand-dependent rates	Sim	ulated data	D		Ac	tual data	Model demonstrating ligand-dependent rates	Simu	ulated data	
С		Act	ual data 0.840		Sim P _a	ulated data 0.833	D		Ac P _o	tual data	Model demonstrating ligand-dependent rates	Simu	ulated data	
С	200μΜ			Model demonstrating ligand-dependent rates $C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$	_	-	D	200μM	T	tual data	Model demonstrating ligand-dependent rates		ulated data No data	
С	200μΜ	Po	0.840		Po	0.833	D	200μΜ	Po		Model demonstrating ligand-dependent rates	Po		
С	200μΜ	P _o	0.840 1.95	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow$	P _o T _o	0.833	D	200μΜ	P _o			Po		
С	200μΜ	P _o T _o T _c	0.840 1.95 0.3		P _o T _o T _c	0.833 1.0 0.25	D	200μM	P _o T _o T _c	No data	Model demonstrating ligand-dependent rates $C_{NR} \rightleftharpoons C_R \rightleftharpoons O_1 \rightleftharpoons O_2 \rightleftharpoons O_3 \rightleftharpoons O_4$	P _o T _o T _c	No data	
C		P _o T _o T _c P _o T _o	0.840 1.95 0.3 0.846	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow$	P _o T _o T _c P _o T _o	0.833 1.0 0.25 0.814	D		P _o T _o T _c P _o	No data 0.82 1.7		P _o T _o T _c P _o T _o	No data 0.83 0.9	
C		P _o T _o T _c P _o T _c T _c	0.840 1.95 0.3 0.846 2.0	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow$	P _o T _o T _c P _o T _o T _c	0.833 1.0 0.25 0.814 0.75	D		P _o T _o T _c P _o T _c	0.82 1.7 0.45	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{I} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ $\downarrow C_{F}$	P _o T _o T _c P _o T _c T _c	No data 0.83 0.9 0.35	
C	100μΜ	P _o T _o T _c P _o T _c P _o T _o	0.840 1.95 0.3 0.846 2.0 0.5	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow$	P _o T _o T _c P _o T _c P _o	0.833 1.0 0.25 0.814 0.75 0.3	D	100μΜ	P _o T _o T _c P _o T _c P _o	No data 0.82 1.7 0.45 0.588		Po To To To To To	0.83 0.9 0.35	
C		P _o T _o T _c P _o T _c Contact P _o T _c Contact P _o C	0.840 1.95 0.3 0.846 2.0 0.5 0.054	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \qquad \downarrow \downarrow \qquad \qquad \downarrow \qquad \qquad \downarrow \qquad \qquad \downarrow \qquad \qquad \downarrow \downarrow \qquad \qquad$	P _o T _c P _o T _c P _o T _c T _c	0.833 1.0 0.25 0.814 0.75 0.3 0.12	D		P _o T _o T _c P _o T _c T _c T _o	No data 0.82 1.7 0.45 0.588 1.25	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{I} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ $\downarrow C_{F}$	P _o T _c P _o T _c P _o T _c T _c	0.83 0.9 0.35 0.36 1.45	
C	100μΜ	P _o T _o T _c P _o T _c T _c T _c T _c T _c	0.840 1.95 0.3 0.846 2.0 0.5 0.054 0.5	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \qquad \downarrow \qquad \downarrow \downarrow$	P _o T _c P _o T _c T _c T _c T _c T _c	0.833 1.0 0.25 0.814 0.75 0.3 0.12 1.5	D	100μΜ	P _o T _o T _c P _o T _c T _c T _c T _c	No data 0.82 1.7 0.45 0.588 1.25 1.65	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ C_{F} $C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ \emptyset C_{F}	P _o T _c P _o T _c P _o T _c T _c T _c	0.83 0.9 0.35 0.36 1.45	
C	100μM 1μM	P _o T _o T _c P _o T _c P _o T _c P _o T _c P _o T _o	0.840 1.95 0.3 0.846 2.0 0.5 0.054 0.5 12.4	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \qquad \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \downarrow \downarrow \qquad \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \qquad \downarrow \qquad \downarrow \downarrow$	P _o T _o T _c P _o T _c P _o T _c P _o T _c	0.833 1.0 0.25 0.814 0.75 0.3 0.12 1.5 11.8	D	100μM 1μM	P _o T _o P _o T _o T _o	No data 0.82 1.7 0.45 0.588 1.25 1.65 0.024	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ C_{F} $C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ \emptyset C_{F}	P _o T _o P _o T _o T _o	0.83 0.9 0.35 0.36 1.45 1.1	
C	100μΜ	P _o T _o T _c P _o T _c T _c T _c T _c T _c	0.840 1.95 0.3 0.846 2.0 0.5 0.054 0.5	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{1} \rightleftharpoons O_{2} \rightleftharpoons O_{3}$ $\downarrow \downarrow \qquad \qquad \qquad \downarrow \downarrow \qquad \qquad \downarrow \qquad \qquad \downarrow \qquad \qquad \downarrow \qquad \qquad \downarrow \downarrow \qquad \qquad$	P _o T _c P _o T _c T _c T _c T _c T _c	0.833 1.0 0.25 0.814 0.75 0.3 0.12 1.5	D	100μΜ	P _o T _o T _c P _o T _c T _c T _c T _c	No data 0.82 1.7 0.45 0.588 1.25 1.65	$C_{NR} \rightleftharpoons C_{R} \rightleftharpoons O_{I} \rightleftharpoons O_{2} \rightleftharpoons O_{3} \rightleftharpoons O_{4}$ $\downarrow C_{F}$	P _o T _c P _o T _c P _o T _c T _c T _c	0.83 0.9 0.35 0.36 1.45	

Figure 52. Dependence of backward as well as forward rates on ligand concentration denotes possible ligand-dependent inhibition at high $[Ca^{2+}]_{cyt}$. Panels show P_o , T_o , and T_c from actual and simulated data. Simulations were performed using the 10- μ M model for four channels from Fig. S1: A, channel 1; B, channel 2; C, channel 11; D, channel 12 (where data at 200 μ M $[Ca^{2+}]_{cyt}$ was not available). All models apart from that in C achieve four ligand-bound open states. Rates shown in bold were constrained to be linearly dependent on ligand concentration, whereas the others remained fixed. At high $[Ca^{2+}]_{cyt}$ ($100-200~\mu$ M), making both forward and backward rates from C_R to O_3 or O_4 ligand dependent simulates data comparable to that acquired, suggesting inhibition at these concentrations. At intermediate $[Ca^{2+}]_{cyt}$ ($1~\mu$ M), ligand dependency of forward (from C_R to O_3 or O_4) and backward rates between open states only (i.e., from O_1 to O_3 or O_4) simulates data similar to that obtained in practice. At low ($0.25~\mu$ M) $[Ca^{2+}]_{cyt}$ ligand dependence of forward rates (from C_R to O_3 or O_4) only simulates data comparable to that recorded. At this $[Ca^{2+}]_{cyt}$ the addition of O_{UL} (using fixed-rate constants from the individual channel, if this state was resolved, as in A, C, and D), and the removal of C_F further improved the match between actual and simulated data.