## Supplementary information

# Dynamic Impact of Temporal Context of $Ca^{2+}$ Signals on Inhibitory Synaptic Plasticity

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This file contains 5 figures, and supplementary information regarding systems biological simulation model.

Fig. S1 Context-dependent conversion of the positive effect of $[Ca^{2+}]_i$ increase to negative.

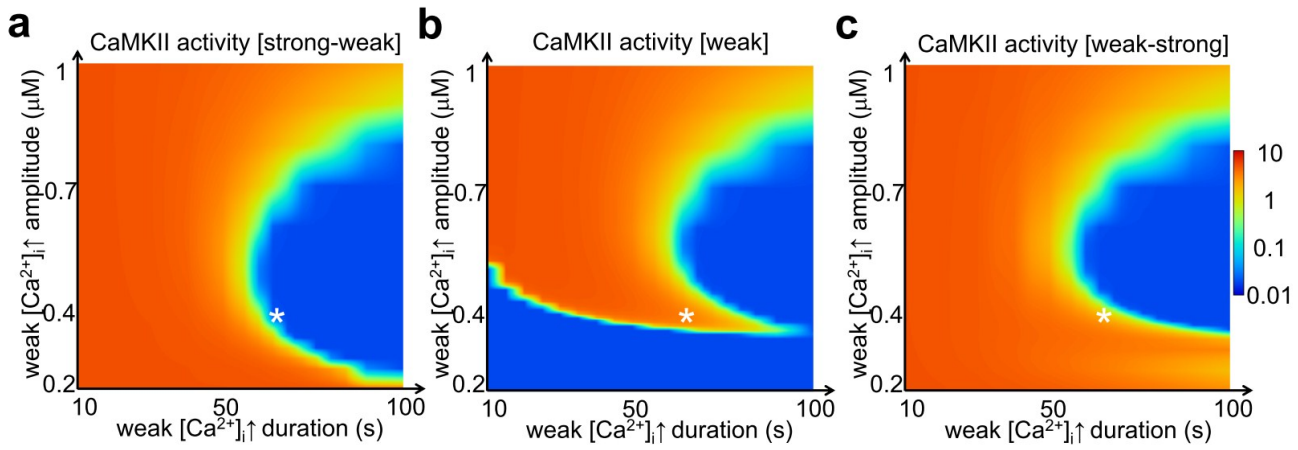
Fig. S2 Effect of inhibition of PDE1 or calcineurin on $Ca^{2+}$ increases.

Fig. S3 Effect of 10/15 mM $K^+$ or pharmacological agents on CaMKII activity.

Fig. S4 Distinct activities of signaling molecules in response to different temporal order of the strong and weak $[Ca^{2+}]_i$ increase.

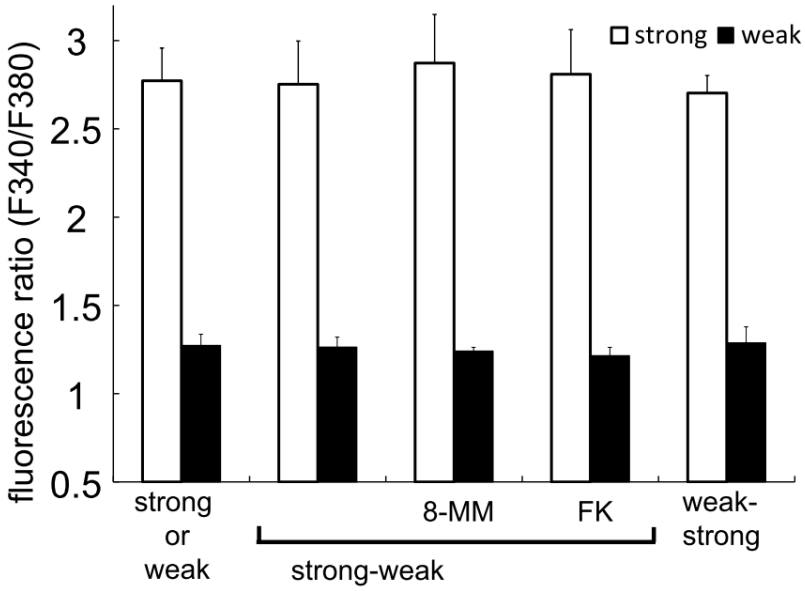
Fig. S5 Frequency-dependent dynamic regulation of CaMKII activity by trains of brief $[Ca^{2+}]_i$ increase (2 μM for 20 msec).

Supplementary information about the modifications of the systems biological simulation model previously developed in Kitagawa et al., 2009.



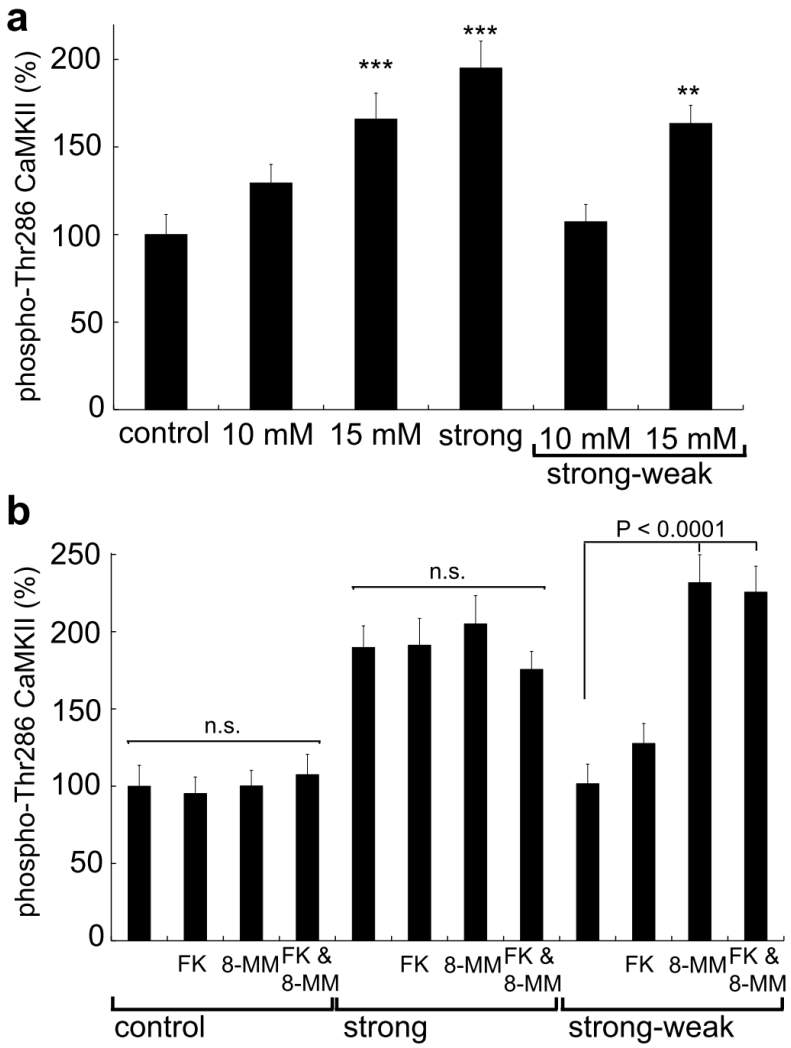

**Supplementary Figure S1**

Context-dependent conversion of the positive effect of $[Ca^{2+}]_i$ increase to negative. **a**-**c**, Simulated CaMKII activity at 30 minutes after the weak $[Ca^{2+}]_i$ increase without (b) or with coupling to the preceding strong $[Ca^{2+}]_i$ increase (a) or the following increase (c). The positive effect of weak and long duration of $[Ca^{2+}]_i$ increase (marked by *) was inverted by the preceding strong $[Ca^{2+}]_i$ increase, but not by the subsequent one.




**Supplementary Figure S2**

Fluorescence ratio of fura-2 (F340/F380) during the strong and weak conditioning depolarizations with or without temporal coupling. Effects of pharmacological agents 8-MM-IBMX (20 μM) and FK506 (400 nM) on the strong-weak sequence of conditioning depolarization are also shown. n = 9 for each. No significant difference was detected (strong, $p = 0.98$; weak, $p = 0.92$) by ANOVA.

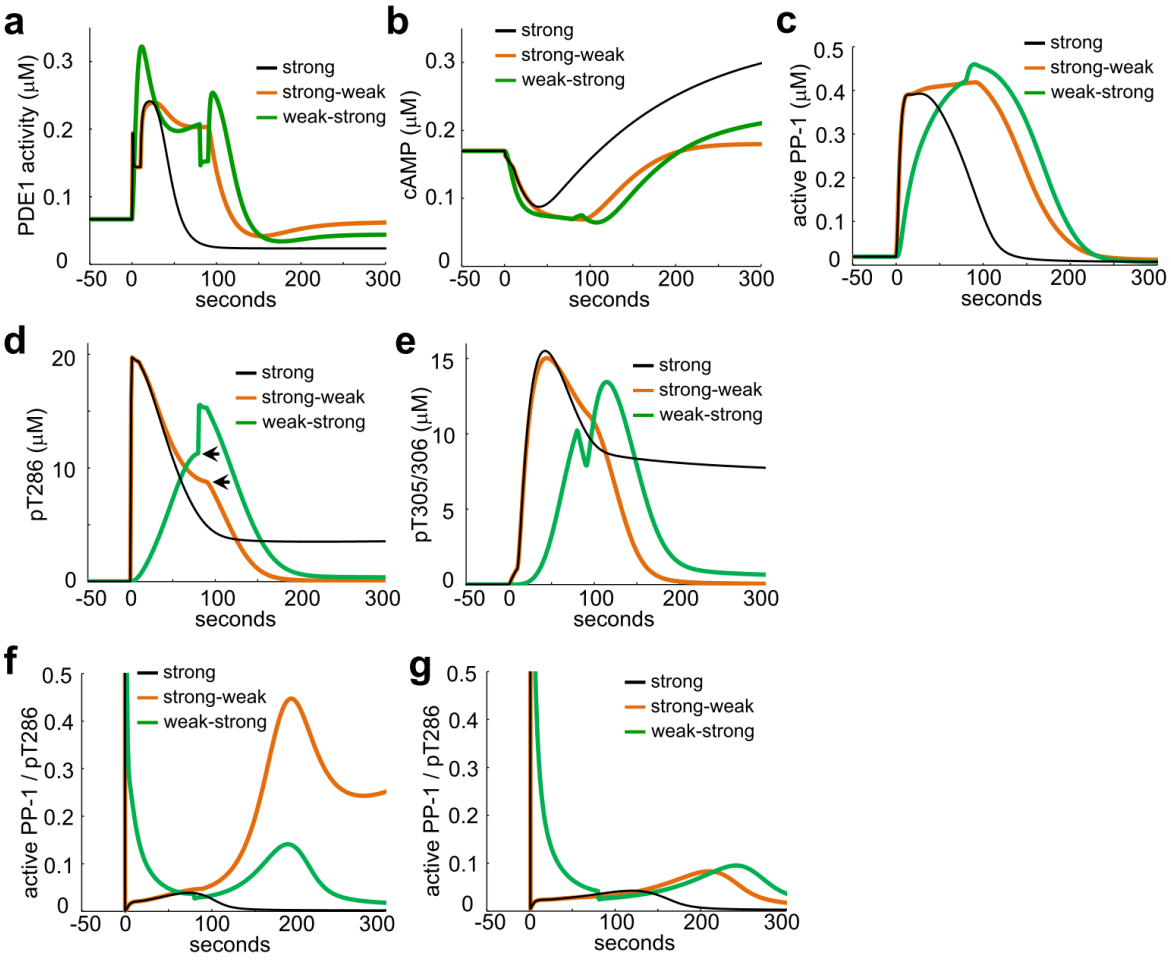



**Supplementary Figure S3**

Effect of 10/15 mM $K^+$ or pharmacological agents on CaMKII activity.

**a**, Relative immunofluorescence intensity of phospho-Thr286 CaMKII in response to the conditioning treatment with high $K^+$-containing solution in various conditions, 10 or 15 mM $K^+$ treatment for 30 minutes; strong, treatment with 50 mM $K^+$ for 10 seconds followed by wash in the normal external solution until fixation at 30 minutes; strong-weak, 50 mM $K^+$ treatment for 10 seconds followed by 5 minutes treatment in 10 or 15 mM $K^+$–containing

solution and wash in the normal external solution. ***: $p < 0.001$, **: $p < 0.01$. n = 25 for each. Only mild depolarization by 10 mM $K^+$, which by itself did not significantly increase CaMKII activity, canceled the CaMKII activation by the strong treatment. **b**, Relative immunofluorescence intensity of phospho-Thr286 CaMKII in the basal condition or in response to strong (50 mM $K^+$ for 10 sec) or strong-weak (10 mM $K^+$ for 5 minutes after the strong one) conditioning treatments in the presence or absence of 8-MM-IBMX (20 μM) and/or FK506 (5 μM). PDE1 inhibition by 8-MM-IBMX affected the cancellation of CaMKII activation by the strong-weak treatment, but not the basal activity ($p = 0.91$) or the CaMKII activation triggered by strong alone treatment ($p = 0.61$). n = 22 for each.



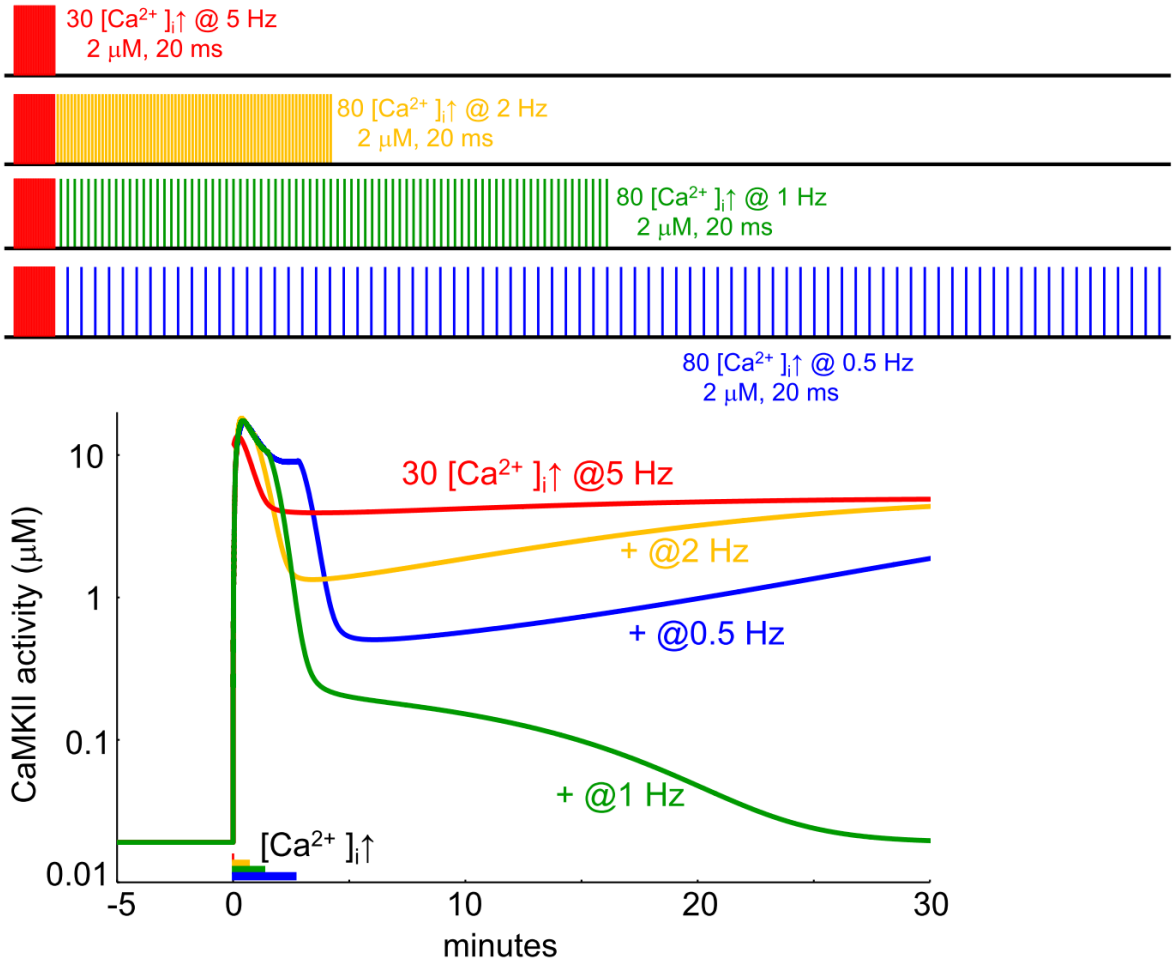

**Supplementary Figure S4**

Distinct activities of signaling molecules in response to different temporal order of the strong and weak $[Ca^{2+}]_i$ increase.

**a**-**e**, Simulated time courses of PDE1 activity (**a**), cAMP amount (**b**), PP-1 activity (**c**), phospho-Thr286 CaMKII amount (**d**), and phospho-Thr305/306 CaMKII amount (**e**), in response to the strong alone, the strong-weak, or the weak-strong sequence of $[Ca^{2+}]_i$ increase.

**f**, **g**, Simulated time courses of ratio of the amount of active PP-1 and that of phospho-Thr286

in the original model (f) and the modified model lacking negative feedback regulation by Thr305/306 phosphorylation (g). Upon the strong-weak $[Ca^{2+}]_i$ increase, the phospho-Thr286 peaked rapidly and decayed to less than a half at the end of stimulation (at 90 seconds, see d), around which the PP-1 activity peaked (see c). On the other hand, both phospho-Thr286 and PP-1 activity showed similar timing of peaks (around 100 sec) in response to the weak-strong $[Ca^{2+}]_i$ increase (see c, d). As a result, the ratio of active PP-1 and phospho-Thr286 CaMKII reflecting the strength of suppressive effect on CaMKII activity was about four times larger after the strong-weak $[Ca^{2+}]_i$ increase (f). In the modified model which lacks the negative feedback regulation through Thr305/306 autophosphorylation, the ratio of PP-1 activity and phospho-Thr286 showed similar time courses irrespective of the temporal sequence of $[Ca^{2+}]_i$ increase (g).




**Supplementary Figure S5**

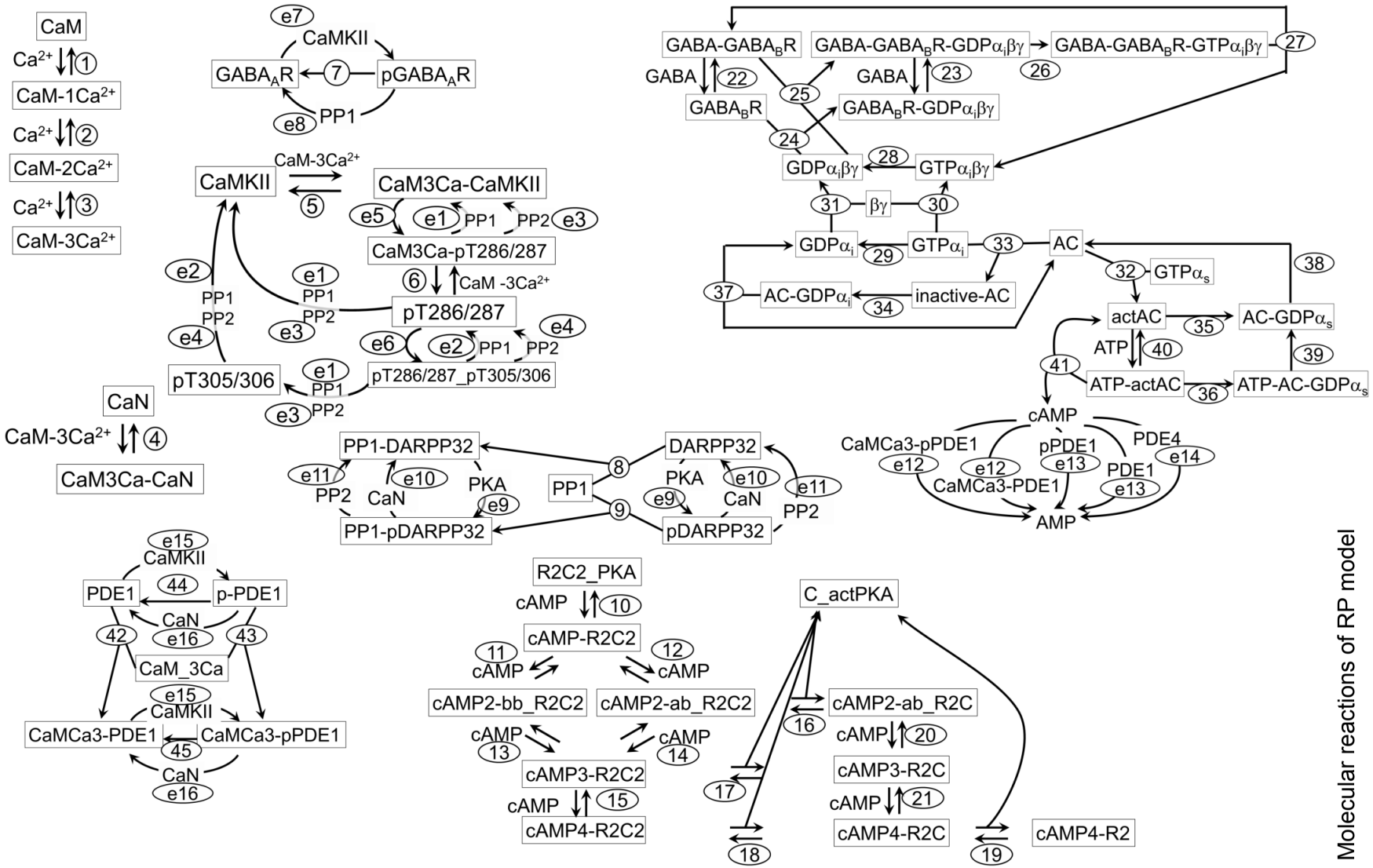
Frequency-dependent dynamic regulation of CaMKII activity by trains of brief $[Ca^{2+}]_i$ increase (2 μM for 20 msec).

Simulated time courses of CaMKII activity before and after the 30 pulses of $[Ca^{2+}]_i$ increase at 5 Hz with or without 80 subsequent pulses at 0.5, 1, or 2 Hz. Only moderate frequency (1 Hz) of large $[Ca^{2+}]_i$ increases cancelled the CaMKII activation triggered by the preceding high frequency $[Ca^{2+}]_i$ increases.

## Supplementary Information

### Detailed information for systems biological model of RP

A computational model of signaling cascades regulating RP we previously developed (Kitagawa et al., 2009) was used with some modifications. First, CaMKII autophosphorylation at Thr286 and at Thr305/306 was assumed to take place in an intra-holoenzyme manner alone, although we previously assumed both intra- and inter-holoenzyme reactions. Second, the basal activity of “PP2s”, which was separately assumed to balance the basal phosphorylation level of CaMKII and that of DARPP-32 in the previous model, was integrated into one PP2. Third, calcineurin-mediated dephosphorylation of PDE1 was explicitly included in the present version. Fourth, some parameters were changed so that the temporal profile of context-dependent negative regulation of CaMKII in the model simulation better matches that obtained by experiments shown in Fig. 5. Details of the molecular interactions and modification are listed below.




Molecular reactions of RP model

Table S1 Molecular concentration

| molecule | concentration (μM) | previous version | notes and references |
|---|---|---|---|
| $Ca^{2+}$ | 0.1 | same | See Kitagawa et al., 2009. |
| CaM | 60 | same | |
| CaN | 10 | 5 | For better fitting to the experimental results shown in Figure 4, CaN amount was doubled. |
| CaMKII | 20 | same | See Kitagawa et al., 2009. |
| PP1 | 0.54 | 0.6 | Assumed to balance the level of CaMKII phosphorylation. |
| PP2 | 0.2 | 0.014 & 0.3 | In the previous version of model, the basal activities of PP2 which dephosphorylates CaMKII and DARPP-32 were separately assumed. In this version, they were integrated into one PP2. |
| $GABA_AR$ | 1 | same | See Kitagawa et al., 2009. |
| DARPP32 | 1.1 | 1.8 | Assumed to be double of the PP-1 amount. |
| R2C2_ PKA | 0.25 | 0.07 | To fit the time course of model behavior to the experimental result shown in Figure 4, rapid regulation of DARPP-32 was needed. Therefore, the amount of PKA was increased. |
| GABA | 0.01 | same | See Kitagawa et al., 2009. |
| $GABA_BR$ | 0.5 | same | |
| $GDP\alpha_i\beta\gamma$ | 1.5 | same | |
| AC | 0.02 | 0.01 | To enable rapid regulation of cAMP concentration, the amount of AC was increased. |
| cAMP | 0.1 | same | See Kitagawa et al., 2009. |
| ATP | 2000 (constant) | same | |
| $GTP\alpha_s$ | 0.00156 (constant) | 0.0037 (constant) | Because of the doubled amount of AC, the amount of $GTP\alpha_s$ to produce the basal cAMP (~ 0.1 μM) was set about a half. |
| AMP | 1000 (constant) | same | See Kitagawa et al., 2009. |
| PDE1 | 0.6 | 0.33 | To enable rapid regulation of cAMP concentration, the |

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|---|---|---|---|
| | | | amount of PDE1 was increased. |
| PDE4 | 0.4 | 0.2 | To enable rapid regulation of cAMP concentration, the amount of PDE4 was increased. |

Table S2 Kinetics of molecular interactions

| ID | $k_f$ ($\mu M^{-1}s^{-1}$) | $k_b$ ($s^{-1}$) | previous version | | notes and references |
|---|---|---|---|---|---|
| 1 | 4 | 80 | 40 | 960 | Modified to slightly increase the amount of $Ca^{2+}$/calmodulin complex in response to $Ca^{2+}$ increase. |
| 2 | 40 | 640 | 40 | 720 | |
| 3 | 40 | 700 | 40 | 880 | |
| 4 | 400 | 3 | 1000 | 10 | For better match to Meyer et al., 1992. |
| 5 | 400 | 8 | 1000 | 15 | The time constant of the binding of $Ca^{2+}$/CaM and CaMKII was increased. |
| 6 | 400 | 0.24 | 1000 | 0.6 | |
| 7 | 0.04 | 0 | 0.2 | 0 | Basal $GABA_AR$ dephosphorylation rate was assumed to be lower. |
| 8 | 0.5 | 0.5 | same | | See Kitagawa et al., 2009. |
| 9 | 500 | 0.5 | same | | |
| 10 | 2 | 0.75 | same | | |
| 11 | 1 | 1.5 | same | | |
| 12 | 10 | 7.5 | same | | |
| 13 | 20 | 7.5 | same | | |
| 14, 20 | 1 | 0.75 | same | | |
| 15 | 10 | 15 | same | | |
| 16, 17 | 0.005 ($s^{-1}$) | 5 ($\mu M^{-1}s^{-1}$) | same | | |
| 18 | 6 ($s^{-1}$) | 5 ($\mu M^{-1}s^{-1}$) | same | | |
| 19 | 3 ($s^{-1}$) | 10 ($\mu M^{-1}s^{-1}$) | same | | |
| 21 | 10 | 7.5 | same | | |
| 22, 23 | 1 | 2 | same | | |
| 24 | 0.2 | 0.1 | same | | |

| | | | | | |
|---|---|---|---|---|---|
| 25 | 10 | 0.1 | same | | |
| 26 | 0.25 | 0 | same | | |
| 27 | 1 | 0 | same | | |
| 28, 29 | 0.066667 | 0 | same | | |
| 30 | 0.01 | 1 | same | | |
| 31 | 0.7 | 0.0013 | same | | |
| 32, 33 | 10 | 0.02 | same | | |
| 34, 35, 36 | 0.33333 | 0 | same | | |
| 37, 38 | 0.1 | 0 | same | | |
| 39 | 10 | 0 | same | | |
| 40 | 0.2 | 48 | same | | |
| 41 | 12 | 0 | same | | |
| 42 | 600 | 0.06 | 2400 | 0.1 | $K_d$ and time constant for association of PDE1 and $Ca^{2+}$/CaM was increased based on Meyer et al., 1992. |
| 43 | 600 | 0.36 | 2400 | 0.6 | |
| 44, 45 | 0.2 | 0 | same | | See Kitagawa et al., 2009. |

Table S3 Kinetics of enzymatic reactions

| ID | $K_m$ (μM) | $k_{cat}$ ($s^{-1}$) | previous version | | notes and references |
|---|---|---|---|---|---|
| e1, e2 | 3 | 2 | same | | See Kitagawa et al., 2009. |
| e3, e4 | 10 | 0.2 | 5 | 1 | The basal PP2 activity regulating the phosphorylation level of CaMKII and that of DARPP-32 was integrated in this study. Therefore, the basal activity level was again assumed here. |
| e5 | - | 11 | 4 | | In this study, phosphorylation of CaMKII at Thr286 or Thr305/306 was assumed to take place between neighboring subunits in a holoenzyme. Thus, the velocity of phosphorylation was simply defined as multiplication of the $k_{cat}$ and the ratio of the active CaMKII and the total amount. |
| e6 | - | 0.5 | 0.27 | | |
| e7 | 11 | 1 | same | | See Kitagawa et al., 2009. |
| e8 | 3 | 2 | same | | |

| | | | | | |
|---|---|---|---|---|---|
| e9 | 2.4 | 2.7 | same | | |
| e10 | 1.6 | 0.5 | 1.6 | 0.2 | For rapid DARPP-32 regulation, $k_{cat}$ was increased. |
| e11 | 2 | 0.4 | 25 | 2 | The basal PP2 activity regulating the phosphorylation level of CaMKII and that of DARPP-32 was integrated in this study. Therefore, the basal activity level was again assumed here with lower efficiency than the dephosphorylation by CaN. |
| e12 | 12 | 5 | 12 | 10 | For better fitting to the experimental results shown in Figure 4, the velocity of cAMP hydrolysis by PDE1 was decreased. |
| e13 | 12 | 0.1 | same | | See Kitagawa et al., 2009. |
| e14 | 2 | 0.05 | same | | |
| e15 | 11 | 2 | 11 | 1 | For better fitting to the experimental results shown in Figure 4, the velocity of PDE1 phosphorylation by CaMKII was increased. |
| e16 | 2 | 0.5 | newly defined | | PDE1 dephosphorylation by CaN was explicitly defined in the present version based on Sharma and Wang, 1986. |