Supplemental Figure Legend:

Fig. S1

Cells continuously exposed to low concentrations of carbachol exhibit calcium oscillations. As the stimulant concentration is increased, the oscillation period decreases. The period for individual cells varies greatly however, highlighting cell-to-cell variability in a population exposed to the same stimulus. Bars represent the standard deviation.

Fig. S2

Caveats for controlling the timing of calcium signals with continuous (A and B) and periodic stimulation (C). A) Upon continuous exposure to 25 nM Carbachol, cells exhibited calcium oscillations (as measured by FRET) with large variability. B) A mathematical model of calcium signaling was able to reproduce oscillations and variability to some extent. Due to cell-to-cell variability, the timing of calcium responses cannot be precisely controlled. For the top graph, the *in silico* cell had a receptor number of ~$8.0 \cdot 10^4$ and for the bottom graph, the receptor number was $8.0 \cdot 10^5$. C) Periodic stimulation is predicted to overcome the timing issues observed with continuous stimulation: calcium responses only result when stimulation is applied. However, not every stimulation event elicits a calcium response, indicative of a loss of fidelity. The top and bottom graphs had receptor numbers corresponding to those from B).

Fig. S3

Schematic of the mathematical model of calcium signaling used in this study. Solid lines represent translocation or binding events, while dotted lines represent enzymatic events. Symbols: R = Receptor, G-GDP = inactive G-protein, G-GTP = active G-protein, PLC = inactive phospholipase C, PLC* = active PLC, PIP3 = Phosphatidylinositol (4,5)-bisphosphate, IP3 = inositol 3-phosphate, deg = degradation, IP3R = IP3 receptor, IP3R(i) = inactive IP3R, Ca2+ = calcium, Ca2+(ER) = Endoplasmic Reticulum Ca2+.

Fig. S4

Distribution plots of intracellular calcium oscillation periods from LHS-generated *in silico* cells stimulated continuously. The red bars represent the fraction of cells that exhibited oscillations less than 60 s, while the blue bar represents the fraction of cells that exhibited oscillations equal to or greater than 60 s. As was observed experimentally, the *in silico* cells exhibited variability as well as larger average oscillation periods upon exposure to lower stimulant levels.

Fig. S5

Periodic stimulation parameters are predicted to modulate calcium response fidelity in cell populations with cell-to-cell variability. The *in silico* cell populations with cell-to-cell variability exhibited higher calcium response fidelity as a population with increases in stimulant concentration (C), stimulation duration (D), and rest period (R). A) Effect

of C on response fidelity; D and R were kept fixed at 24 s, respectively. B) Effect of D on response fidelity; C and R were kept fixed at 10 nM and 24 s, respectively. C) Effect of R on response fidelity; C and D were kept fixed at 10 nM and 24 s, respectively.

Fig. S6

Western Blot Results. Cell lysates were prepared, run on a 12% SDS-PAGE gel, transferred to an Immobilon-P transfer membrane and probed with an anti-HA antibody to detect RGS4 expression and an anti-actin antibody for normalization of protein. Transiently transfected (lane 1) or mock-transfected (lane 4) HEK-293T cells are compared for RGS4 expression with Flp-In RGS 4 cells incubated with (lane 3) or without (lane 2) 1 μg/ml doxycycline for 45 hours.

Fig. S7

Average calcium oscillation period elicited by 25 nM Carbachol decreases with application of doxycycline (1 ug/mL for 45 hrs) or atropine (1 nM). Bars represent standard error. All differences were statistically significant based upon the Student T-test, with $p < 0.05$.

Fig. S8

Small changes in protein levels are predicted to have drastic effects on the phase-locking ratio. Within the dashed box area, less than a 1% change in RGS proteins results in a precipitous change between a phase-locking ratio of 0.5 (bottom) and 1 (top). The periodic stimulation conditions for this simulation were C = 30 nM, D = 10 s, and R = 70 s.

Fig. S9

In an experiment, a periodically stimulated cell initially exhibits a phase-locking ratio of 0.5 then abruptly switches to a phase-locking ratio of 1, which was observed on several occasions across many experimental conditions. Cells were stimulated with the following parameters: C = 10 nM, D = 24 s, and R = 24 s.

Fig. S10

Potential consequences of low fidelity calcium signaling on cAMP signaling. Intracellular calcium is intimately linked with several signaling pathways, including cAMP. Using a model of calcium-cAMP signaling [41], we predicted the effects of low and high fidelity calcium signaling upon the result cAMP signals; the differences in fidelity were a result of a only a 10% difference in stimulus strength. Low fidelity calcium signaling (bottom panel) is predicted to exhibit lower frequency and lower average amplitude cAMP responses (middle panel- red trace) compared to high fidelity calcium signaling (middle panel- blue trace).

Fig. S11

Our mathematical model predicts that skipping (left column) likely arises from the non-linear nature of IP3 signaling, which propagates to calcium signaling. Elements upstream of IP3, activated G-protein (G-GTP) and activated PLC, do not exhibit skipping, suggesting that these components are not directly responsible for the skipping phenomenon. The right column represents a 'non-skipping' case for comparison.

Fig. S1

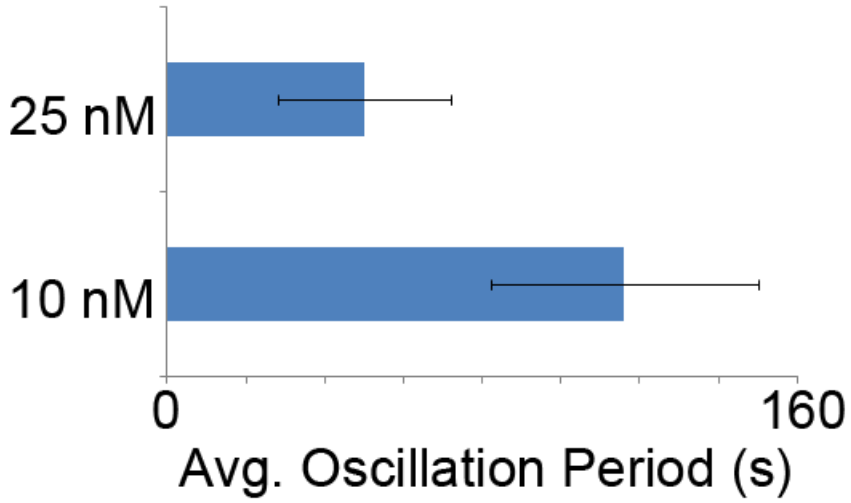


Fig. S2

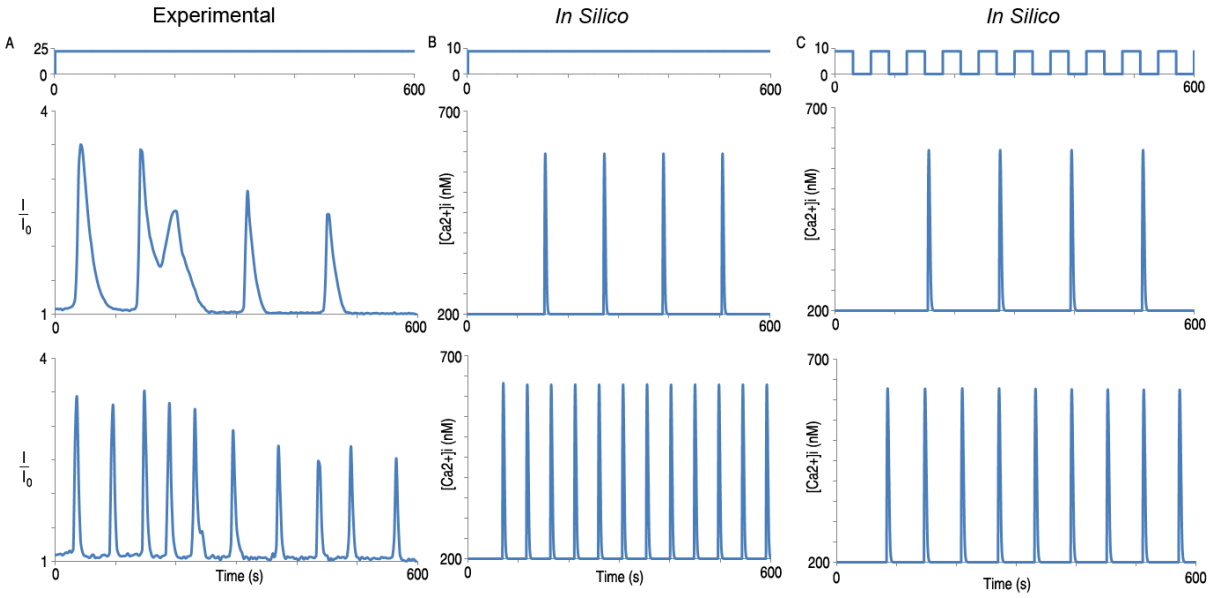


Fig. S3

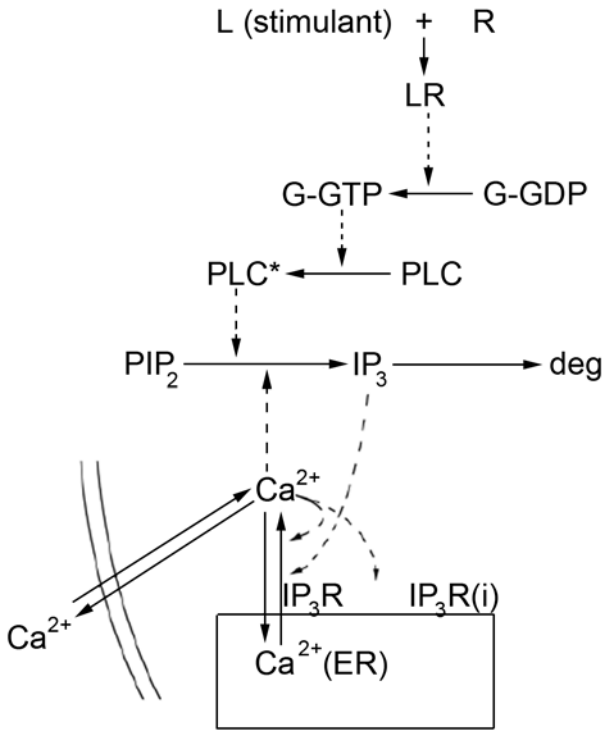

L (stimulant) + R
LR
G-GTP
G-GDP
PLC*
PLC
PIP2
IP3
deg
Ca2+
IP3R
IP3R(i)
Ca2+
Ca2+(ER)

Fig. S4

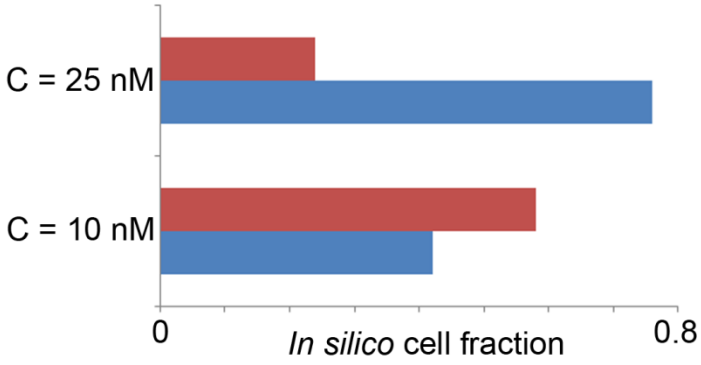

C = 25 nM
C = 10 nM
0
0.8
In silico cell fraction

Fig. S5

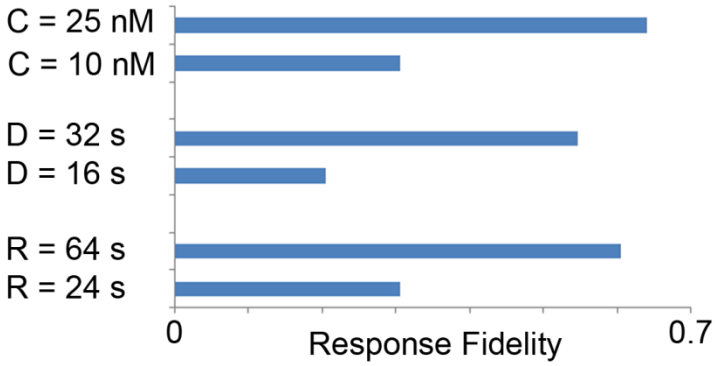


Fig. S6

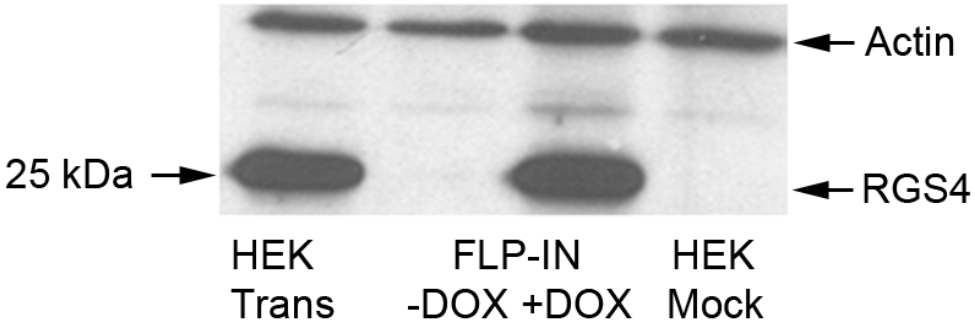


Fig. S7

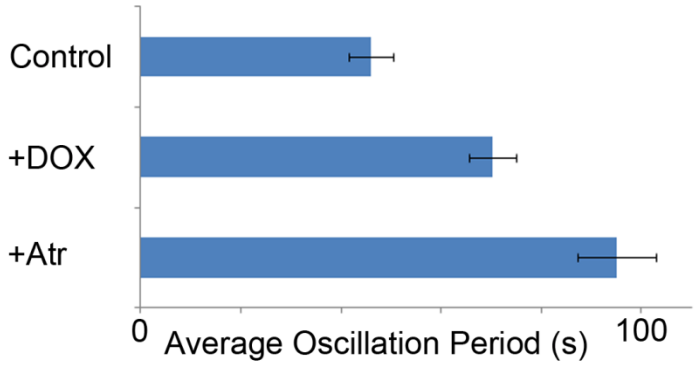


Fig. S8

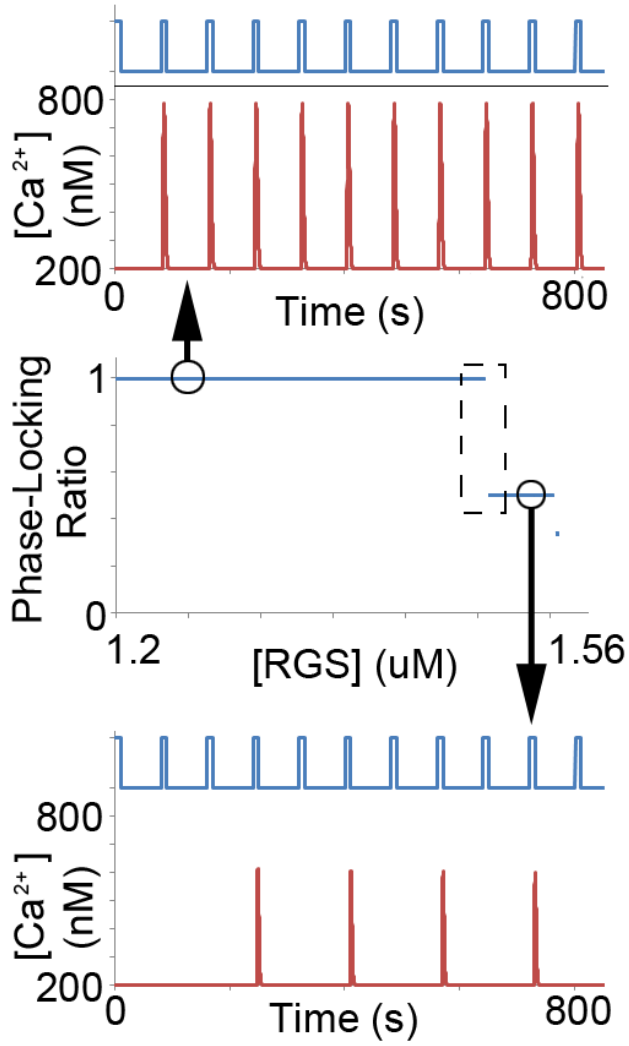

800
[Ca2+] (nM)
200
0
Time (s)
800
1
Phase-Locking Ratio
0
1.2
[RGS] (uM)
1.56
800
[Ca2+] (nM)
200
0
Time (s)
800

Fig. S9

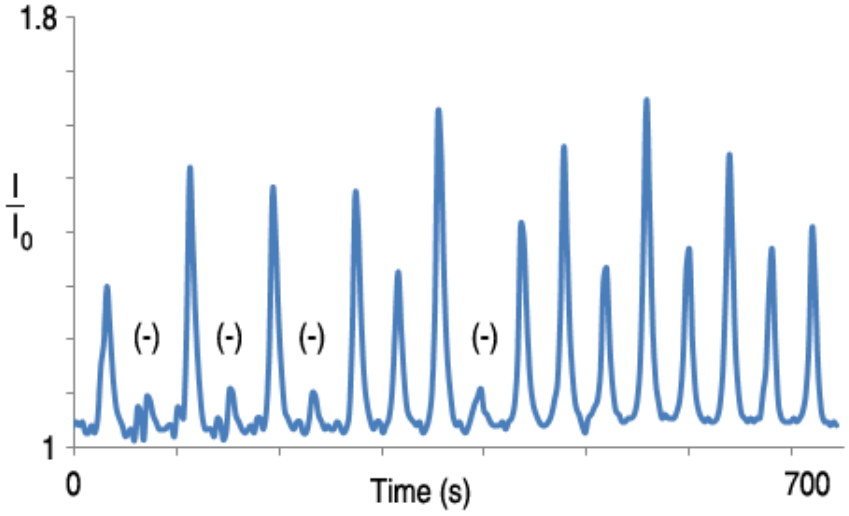


Fig. S10

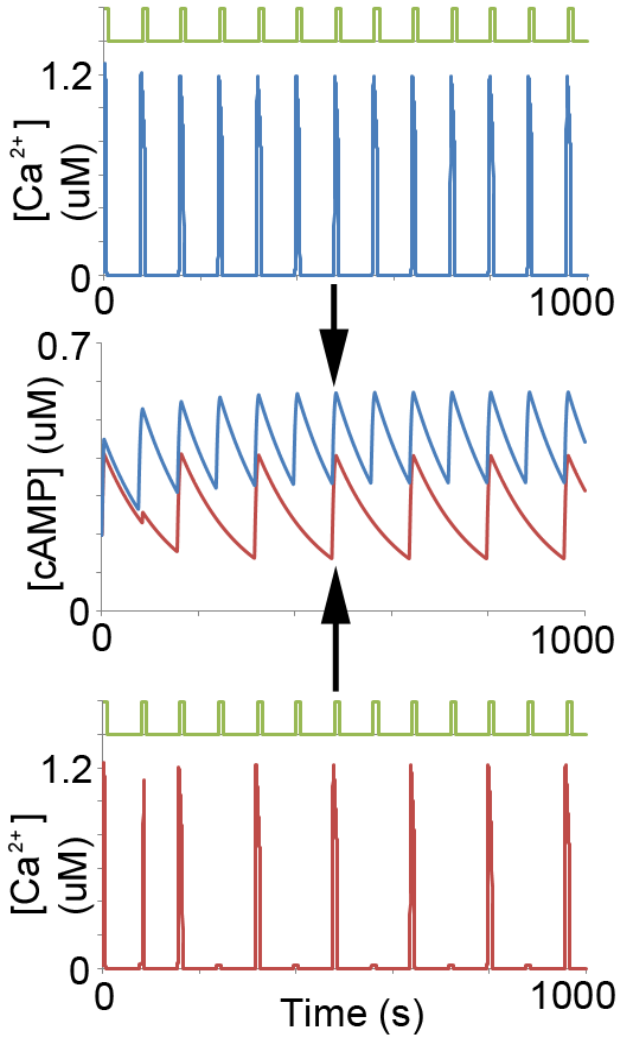
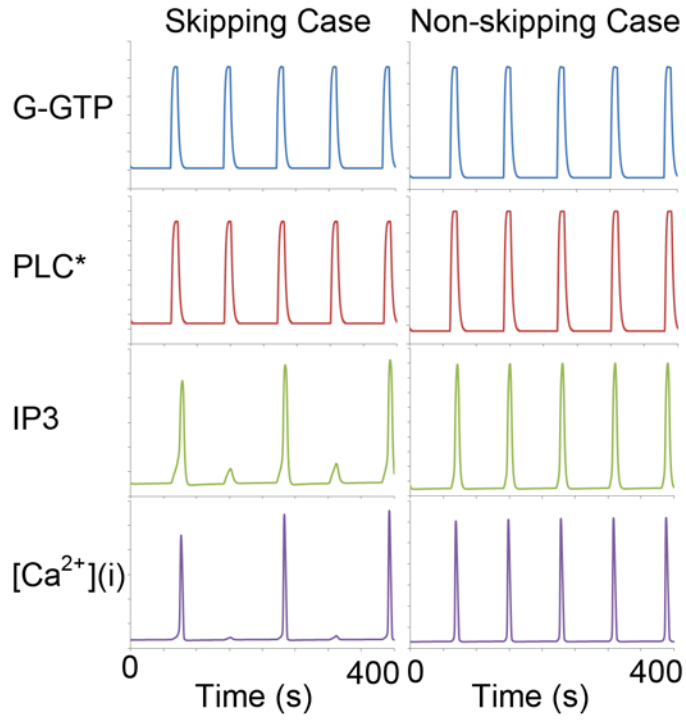


Fig. S11


Skipping Case
Non-skipping Case
G-GTP
PLC*
IP3
[Ca2+](i)
0
400
Time (s)
0
400
Time (s)