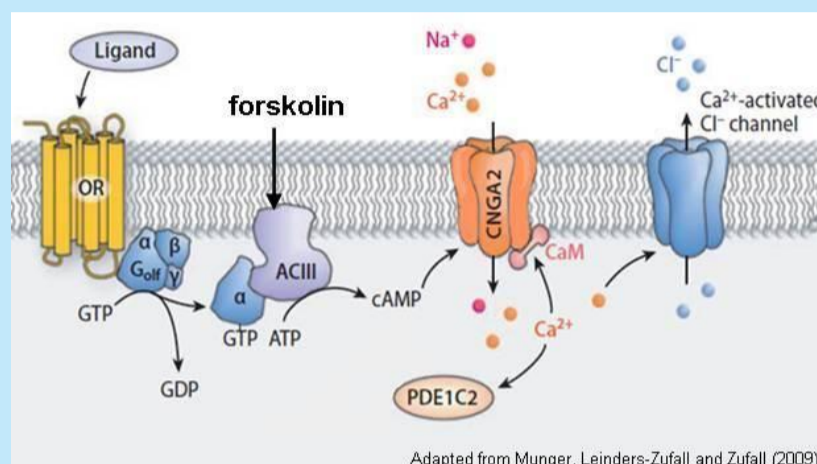


## Abstract

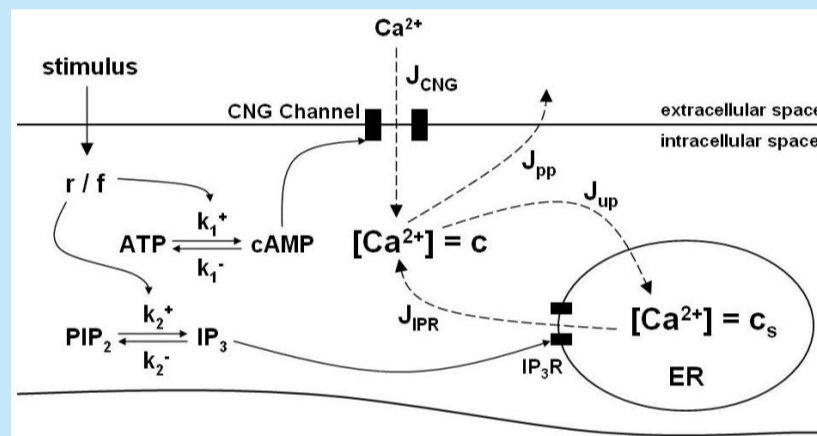
Signal transduction in mammalian olfactory sensory neurons (OSNs) is mediated by one or more signaling pathways where an increase in intracellular calcium level occurs in response to stimuli, which leads to excitation of the OSNs and smell sensation. The signal transduction process is initiated once an odorant binds to the G-protein coupled receptor (GPCR), resulting in dissociation of the alpha subunit ( $G_\alpha$ ) and the beta-gamma subunits ( $G_{\beta\gamma}$ ), thereby activating a major and a minor signaling pathway, respectively. The alpha subunit activates adenylyl cyclase (AC), which increases intracellular concentration of cyclic adenosine monophosphate (cAMP), leading to activation of cyclic nucleotide-gated (CNG) channels and subsequently an influx of calcium into the OSNs. The beta-gamma subunits trigger phospholipase C (PLC), which breaks down phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into diacylglycerol (DAG) and inositol trisphosphate (IP<sub>3</sub>), and the latter binds to IP<sub>3</sub> receptor on endoplasmic reticulum (ER) to release calcium from the internal store to intracellular space.

We applied chemicals to stimulate the two pathways and analyzed changes in intracellular calcium levels using techniques of calcium imaging. Based on literature and our results, we created a mathematical model between the two pathways and intracellular calcium level, and utilized MATLAB for simulation. By implementing data from calcium imaging, we were able to produce preliminary results that resembled the observed changes of intracellular calcium level.

## Graphics



The major olfactory signal transduction pathway and forskolin activation.



The diagram of Ca<sup>2+</sup> considered in modeling.

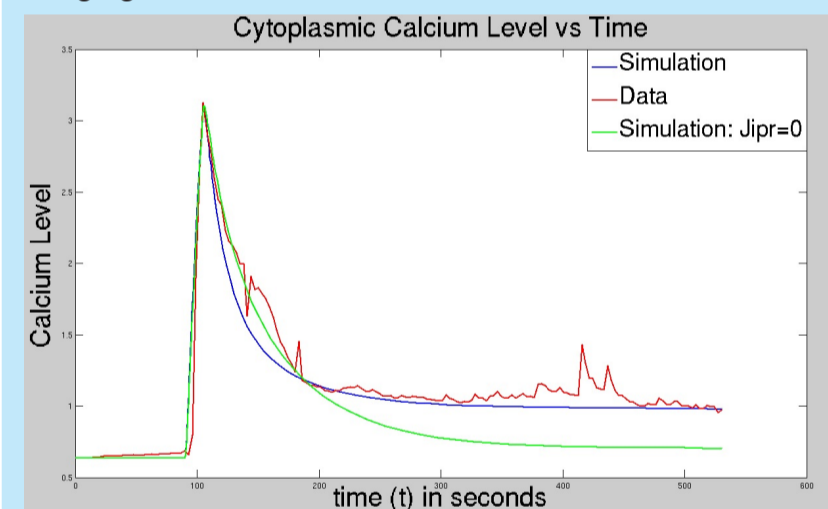
## Variables

- $c, c_s$  variation from rest values of calcium levels in the cytoplasm and endoplasmic reticulum, respectively
- $u, p$  relative concentrations to base level of cAMP and IP<sub>3</sub> in the cytoplasm, respectively
- $v$  ciliary membrane potential, scaled so that  $v = 0$  is the rest potential
- $y$  proportion of IP<sub>3</sub> receptors activated by Ca<sup>2+</sup>

In the model, an initial value of zero is used for all variables. The concentration of forskolin,  $f(t)$ , is a piecewise function of time. It is zero before application, constant at 2.5 μM during application, and decays exponentially when the application is done.

## Results

Only the forskolin activated cAMP pathway has been modeled as of now. Scaling is done so that the plot of  $c(t)$  can be compared to the unitless ratio given by calcium imaging data.



Simulation vs Data from calcium imaging vs Simulation where  $J_{IPR} = 0$ . The intracellular Ca<sup>2+</sup> level is represented by the ratio (F ex340nm/F ex380nm).

This shows the recovery level's dependence on Ca<sup>2+</sup> from the endoplasmic reticulum.

## Methods

**Tissue Preparation** - Adult TRPM5 mice were euthanized, and OSNs from the main olfactory epithelium (MOE) were enzymatically dissociated. The cells were then kept healthy in Tyrodes solution which resembles the extracellular environment.

**Calcium Imaging** - We used the ratiometric calcium dye Fura-2AM as our calcium level indicator. The dye's peak excitation shifts from 380 nm to 340 nm when bound to calcium ions, and the ratio of the emission intensities indicates intracellular calcium level, which are recorded at 3 Hz. We used Olympus X71 light microscope and Imaging Workbench software for the imaging.

**Chemical Stimuli** - 2.5 μM of forskolin and 25 μM of m-3MFBS were used to activate AC and PLC, respectively. Application durations varied, ranging from 15 to 100 seconds.

**Imaging Analysis** - Based on the imaging data we were able to determine quantitative changes of intracellular calcium level in the dendritic knobs in response to the stimuli. Using Microsoft Excel we calculated amplitudes and rates of change in calcium level.

**Simulations** - MATLAB was used to carry out the simulations using built in ODE solver ode23t. Simulations were scaled to match the amplitude of the data and shifted so that the base levels and times of excitation are consistent with the data.

## Model

1.  $\frac{dc}{dt} = J_{CNG} + J_{IPR} - J_{up} - J_{pp}$
2.  $C_m \frac{dv}{dt} = -I_{CNG} - I_{Cl(Ca)} - I_\ell$
3.  $\frac{du}{dt} = k_1 f(t) - k_{-1} u$
4.  $\frac{dc_s}{dt} = \gamma(-J_{IPR} + J_{up})$
5.  $\tau_y(c, p) \frac{dy}{dt} = y_\infty(c, p) - y$
6.  $\tau_p \frac{dp}{dt} = k_2 r(t) - k_{-2} p$

where

$$\begin{aligned}
 I_{CNG} &= g_{CNG} \frac{u^n}{K_u^n + u^n} (v - E_{CNG}) \\
 I_{Cl(Ca)} &= g_{Cl(Ca)} \frac{c^m}{K_c^m + c^m} (v - E_{Cl(Ca)}) \\
 I_\ell &= g_\ell (v - E_\ell) \\
 J_{CNG} &= -\alpha I_{CNG} \\
 J_{IPR} &= \left\{ r_2 + r_1 \left[ \frac{pc(1-y)}{(p+K_1)(c+K_5)} \right]^3 \right\} (c_s - c) \\
 J_{up} &= Q_1 c - Q_2 c_s \\
 J_{pp} &= \frac{v_{pp} c^2}{K_{pp}^2 + c^2} \\
 \tau_y &= \left[ \frac{(A_1 + A_2 p)c}{p + A_3} + \frac{B_1 + B_2 p}{B_3 + p} \right]^{-1} \\
 y_\infty &= \tau_y \frac{(A_1 + A_2 p)c}{p + A_3}
 \end{aligned}$$

and we have parameters:

$$C_m, g_{CNG}, g_{Cl(Ca)}, g_\ell, E_{CNG}, E_{Cl(Ca)}, E_\ell, K_u, K_c, n, m, v(0), k_1, k_{-1}, u(0), \alpha, r_1, r_2, K_1, K_5, Q_1, Q_2, v_{pp}, K_{pp}, c(0), \gamma, c_s(0), A_1, A_2, A_3, B_1, B_2, B_3, y(0), \tau_p, k_2, k_{-2}, p(0), f, r.$$

Parameters were either obtained from research papers or adjusted to better fit data by eye.

## Conclusion

Though accuracy has yet to be tested, the simulation does show promising results for the forskolin activated cAMP pathway. We will soon begin analyzing the PLC pathway. Eventually, we will compare our model to activation by odorants, rather than chemicals.

## Acknowledgements

Our model is based on "Modeling IP<sub>3</sub>-Dependent Calcium Dynamics in Non-Excitable Cells" [Sneyd, MBI Tutorials, 2005].

This work was supported by a NSF grant for the UBM program, NIH/NICDC grant DC009269, and ARRA administrative supplement to Weihong Lin.