SUPPORTING INFORMATION

Accompanying 'Cellular asymmetry and individuality in directional sensing' by Samadani *et al.*

Photoactivation of cAMP and gradient formation. A cAMP gradient was formed by photoactivation of a known concentration of NPE-caged cAMP. An inverted Nikon TE2000 microscope equipped with a 60× oil immersion objective and a 100 W mercury lamp was used. A UV-GFP (370-410 nm) filter was used to remove the visible component of the mercury lamp. The filtered light was focused through an objective into the observation chamber. To restrict the area of photoactivation, we place a 1 mm diameter pinhole in the light path before the objective. The light passing through the pinhole was focused by the objective to a 17 μm diameter spot on to the field of view. In all of our experiments the UV exposure time was 2 seconds. The setup was equipped with a CCD camera to permit time lapsed microscopy. Two types of short pulses were used in the experiments to stimulate cells: a directed pulse, which forms a spatio-temporal gradient of cAMP around the cell, and a pulse of spatially uniform cAMP concentration. A spatially uniform increase of cAMP concentration around the cell membrane was generated by illuminating the entire field of view with a low dose of UV exposure.

Quantifying the cAMP gradient. We have quantified the spatial and temporal variation of the cAMP concentration by numerically solving the two dimensional diffusion equation. The diffusion in the observation chamber which is a semi infinite reservoir is given by:

$$C(x,t) = \int_0^{\min(t_{pulse},t)} d\tau \tilde{C}(x,t-\tau), \quad \textbf{[S1]}$$

$$\tilde{C}(x,t) = \frac{C_0}{4\pi(Dt+\sigma_0)} \exp(-r^2 / 4(Dt+\sigma_0)), \quad \textbf{[S2]}$$

where r is the distance from the illumination region, D the diffusion coefficient, σ_0 the initial Gaussian width of the UV beam, C_0 the concentration of photoactivated molecules and t_{pulse} =2 s. $\tilde{C}$ is the concentration obtained from a very short, very bright pulse of light. To justify the validity of our calculations, we visualize a similar gradient forming by photoactivation of caged fluorescein and fit the solution of the diffusion equation to the fluorescein gradient. The inset of Fig. S1 shows the gradient formed by diffusion of photoactivated fluorescein from the illumination area, 6 seconds after the start of the pulse.

Estimate of the cAMP diffusion coefficient. cAMP diffusion was estimated based on the Stokes-Einstein equation for spherical molecules, $D=k_BT/6\pi\eta a$ where k_B is the Boltzmann constant, T the temperature, η the viscosity, and a the radius of the molecule. Based on the molecular weight of cAMP, the equivalent spherical radius is about 0.516 nm. Therefore the Stokes-Einstein equation predicts a diffusion coefficient of $D = 4.3\times10^{-6}$ cm^2/s at 24 °C, consistent with previously measured value for cAMP diffusion constant (1).

Estimate of the cAMP release. To measure the absolute value of cAMP concentration we have estimated the uncaging efficiency χ. This efficiency is the number of photoactivated molecules divided by the total number of molecules. The quantum yield of uncaging Φ is defined as the number of photoactivated molecules divided by the number of absorbed photons n_{abs}. Following the Lambert-Beer law, the number of absorbed photons n_{abs} equals $n_o(1-\exp(-Ecl))$, in which n_0 is the photon flux, E is the molar extinction coefficient, l the length of the light path and c the concentration of caged molecules. For small Ecl, one can approximate the above formula by $n_{abs} = n_0Ecl$. The total number of molecules in the irradiation volume is the product of Avogadro's number N_A, c and V the volume of the exposed region. Therefore χ is given by:

$$\chi = \frac{\Phi n_o Ecl}{N_A cV} \quad \textbf{[S3]}$$

When irradiated with full intensity the photon flux density n_0/At is 4×10^{18} photons $cm^{-2}s^{-1}$ as measured at the plane of focus, in which A is the area and t the time of exposure. Considering the extinction coefficient of $E = 1500$ $cm^{-1}M^{-1}$, at 400 nm and a quantum yield of 0.39 (2, 3). It can be estimated that a 2 second pulse of light with full intensity will cleave about 1% of the NPE-caged cAMP molecules. The photon flux density will be slightly higher for the maximum wavelength of 390 nm that we use in this set up. Therefore we estimate χ to be slightly above 1%. Small χ is an advantage in our experiment, because caged compounds do not deplete during the 2 second illumination.

Definition of *L*(*t*), *P*(*t*) and ϕ(*t*). The localization $L(t)$ is defined as the average value of $R(\theta,t)$, $L(t) = \langle R(\theta,t)\rangle_\theta$, and therefore reflects the average recruitment of CRAC-GFP molecules to the membrane a time t after cAMP was uncaged. The brackets $\langle ...\rangle_\theta$ denote that the response function is averaged over the coordinate θ (*Supplementary Information*). The polarization $P(t)$ is a measure of the amplitude of the response function and therefore reflects the difference between the maximum and minimum

value of $R_{fit}(\theta,t)$ of a single cell at a time t after cAMP uncaging. Finally, the polarization angle $\phi(t)$ is defined as the angle for which the fit function $R_{fit}(\theta,t)$ reaches its maximum value. Therefore, a value of $\phi = 0$ implies that the cell has recruited most of the CRAC-GFP molecules to the position at the membrane that is closest to the uncaging spot ($\phi = 0$, Fig. 1a). Conversely, a value of $\phi = 180^o$ means that the cell has polarized in the opposite direction and recruits most of the CRAC-GFP molecules to the position in the membrane that is furthest away from the uncaging spot.

Description of the polar plots. Since the cell-to-cell variability is most pronounced for the polarization P and polarization angle ϕ it is convenient to present the data in a polar plot as shown in Fig. 3c and Fig. 3d. In these figures one data point represents data from a single cell at T_{max}. The distance from a data point to the origin of the polar plot equals the polarization $P(T_{max})$. The angle between the x-axis and the line which connects the data point to the origin of the polar plot is the polarization angle $\phi(T_{max})$. The x-axis is along the line which connects the center of each cell to the center of the uncaging spot ($\theta = 0$, Fig. 1a) and the y-axis is perpendicular to that line ($\theta = 90^o$). In this representation the polarization component along the x-axis P_x equals $P(T_{\max})\cos[\phi(T_{\max})]$. The polarization component along the y-axis, P_y equals $P(T_{\max})\sin[\phi(T_{\max})]$. For example, cells responding along the 'right' direction ($\phi = 0$) are characterized by $P_x > 0$ and $P_y = 0$, whereas cells that respond in the 'wrong' direction ($\phi = 180^o$) are characterized by $P_x < 0$ and $P_y = 0$.

The noise at the level of cAMP-receptor binding does not significantly contribute to the observed noise in the polarization *P* and the polarization angle ϕ. In order to verify whether the noise at the level cAMP-receptor binding is significant, we varied the extracellular concentration of cAMP over two orders of magnitude (4-6). $P\sin(\phi)$ versus $P\cos(\phi)$ at T_{max} for a population of 100 cells, which are stimulated with cAMP concentrations of 1 nM to 0.1 μM are plotted in Fig. S2a. Probability distributions of $|\phi(T_{max})|$, when the extracellular concentration of cAMP is varied from 1 nM to 0.1 μM are plotted in Fig. S2b. The standard deviations for the probability distributions are approximately 100° for all cAMP concentrations used. We observed that by increasing the extracellular concentration over two orders of magnitude, the probability of finding a cell with ϕ in the direction of the extracellular gradient ($\theta = 0$) does not significantly increase. This indicates that an intracellular mechanism is responsible for the observed variability in P and ϕ.

Description of the curve fittings in Fig. 5. The plots of ϕ as a function of θ_s in Fig 5c, 5d and 5e were fitted to the geometric model described by the following function:

$$\phi = \arctan\left[\frac{\alpha\sin(\phi_\varepsilon)+\sin(\theta_s)}{\alpha\cos(\phi_\varepsilon)+\cos(\theta_s)}\right], \textit{ where} \quad \textbf{[S5]}$$

$$\alpha = \frac{\varepsilon S_0}{S_1}, \quad \textbf{[S6]}$$

with α and ϕ_ε as the fitting parameters. The ratio of S_1/S_0 was independently calculated to be 0.38 by integrating the extracellular gradient from $t = 0$ to $T_{\max}$ using Eq. [S1] and Eq. [S2]. Our calculation for S_1/S_0, in addition was experimentally verified by using a caged fluorescent marker.

The prediction of the geometric model for curves in Figs. 3e and 3f. Equations 4–8 (*Methods*) can be simplified to demonstrate that the only relevant parameters in calculating the fraction of cells as a function of $|\phi(T_{\max})|$ (Fig. 3e) are α and ϕ_ε:

$$\phi = \arctan\left[P_y / P_x\right] = \arctan\left[\frac{\sin\phi_\varepsilon}{(1/\alpha + \cos\phi_\varepsilon)}\right] \quad \textbf{[S7]}$$

In order to obtain a mean value for α (and therefore ε), we fitted the experimental curves of ϕ as a function of θ_s obtained from 20 individual cells to Eq. [S5] (as described in previous section). From these fits, the mean value of α was measured to be 3.6 (giving a mean value of 1.3 for ε). The distribution of ϕ_ε was also experimentally measured to be uniformly distributed between [0, 2π] (Fig. 4e, Fig. S3a). Therefore both α and ϕ_ε (also ε) are experimentally determined and therefore the red line in Fig. 3e is the prediction of the geometric model with no fitting parameters.

Similarly, we demonstrate that the correlation between $L(T_{max})$ and $P(T_{max})$, and $|\phi(T_{max})|$, illustrated in Fig. 3f, depend on ϕ_ε,α and ε (Eq. [S8] and [S9]). However these also depend on an undetermined constant, which is the ratio between the cAMP concentration and the experimentally measured response function $R(\theta)$. The two red lines in Fig. 3f, are the geometric model fit to the curves $L,P(T_{max})$ versus $|\phi(T_{max})|$ using only one fit parameter.

$$L = S_0\left(1+\frac{\varepsilon^2}{2\alpha}\cos\phi_\varepsilon\right) \qquad \textbf{[S8]}$$

$$P = \varepsilon S_0\left(\left(\tfrac{1}{\alpha}+\cos\phi_\varepsilon\right)^2+\sin^2\phi_\varepsilon\right)^{1/2} \qquad \textbf{[S9]}$$

$$P/L = \varepsilon\frac{\left[\left(\tfrac{1}{\alpha}+\cos\phi_\varepsilon\right)^2+\sin^2\phi_\varepsilon\right]^{1/2}}{\left(1+\frac{\varepsilon^2}{2\alpha}\cos\phi_\varepsilon\right)} \qquad \textbf{[S10]}$$

The ratio of P and L at T_{max} as a function of $|\phi(T_{max})|$ illustrated in Fig. 3f is a function of ϕ_ε, α and ε given by Eq. [S10]. All these three parameters are experimentally determined and therefore the blue line in Fig. 3f is the prediction of the geometric model with no fitting parameters.

Supplementary references:

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