Astrocyte networks and intercellular calcium propagation

Derivation of the shell model

Supplementary Text

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1 Shell decomposition

In an astrocyte network, we define the r-th shell by the set of cells that are at topological distance r from a reference astrocyte where, by 'topological distance,' we mean the shortest path in terms of number of cells that one has to go through to reach the reference astrocyte from any cell in the r-th shell. Then, connections between cells can be either within this shell or between shell r and other shells. Figure S1A shows a schematic representation of three consecutive shells: $r-1$, r, and $r+1$. Each shell can be characterized by a triplet (N_i, W_i, E_i) where N_i is the number of astrocytes in the shell; W_i is the number of connections between astrocytes in the shell (*blue links*); and E_i denotes the number of connections between cells in shell i and cells in shell $i+1$ (*orange links*). In this fashion, a network formed by n shells can be described by $3n-1$ values. This description neglects "long distance" connections between cells that are in non-consecutive shells and may thus be expected to reflect effective topology only if the network under consideration has some sort of spatial constraints. In other words, if we assume that all connections are identical in strength, i.e. $F_{ij} = F$ in equation 5, our description effectively approximates the topology of any network where the number of connections from shell i and shells beyond $i+1$ is $\ll W_i$, E_i. If this is not the case, we should expect our shell decomposition to fail to properly describe ICW propagation.

An important corollary that follows from our description is that ICWs that originate from a stimulated astrocyte (i.e. shell '0'), can only radially propagate from it, activating outward consecutive shells in a sequential fashion. In this way, intracellular IP_3 balance in the $r+1$-th shell results from the combination of three IP_3 fluxes: (i) a diffusion flux mediated by GJCs from the activated astrocytes of shell r to astrocytes in shell $r+1$; (ii) an IP_3 flux directly ensuing from the stimulated astrocyte(s) in shell '0'; and (iii) the redistribution of IP_3 among cells in the shell $r+1$ by their intra- and inter-shell connections to unactivated astrocytes.

2 IP_3 flux between consecutive shells

In our description, when an ICW propagates from shell $r-1$ to shell r, it leaves behind all astrocytes in $r-1$ unactivated, while it activates only a fraction ρ_r of astrocytes in r. The IP_3 generated from these cells will then split among their unactivated neighbors. To estimate how much IP_3 shell r supplies to shell $r+1$, we assume that astrocytes in shell $r-1$, r and $r+1$ can be classified into three groups: activated astrocytes ('a') in shell r; unactivated astrocytes (or sinks 's') in shell $r-1$ (i.e. all cells there) and in shell r; and unactivated 'target'

astrocytes ('t') in shell $r+1$. We think of these three groups as homogeneous compartments with respective IP_3 concentrations I_a, I_t and I_s, and corresponding sizes of $N_a^* = \rho_r N_r$, $N_t^* = N_{r+1}$ and $N_s^* = N_{r-1} + \hat{N}_r$ where $\hat{N}_r$ is the number of unactivated astrocytes in shell r. Moreover, the activated cells (group 'a') are diffusively coupled with unactivated astrocytes (or sinks) in groups 't' and 's', with respective diffusion strengths $D_t = FE_r$ and $D_s = F(E_{r-1} + \hat{W}_r)$, where F denotes the rate of IP_3 diffusion (in s^{-1}) of single cell-to-cell connections (assumed identical), and $\hat{W}_r$ is the number of connections between activated and unactivated astrocytes of shell r.

As exemplified in Figure S1B, the relative size of each astrocyte group along with the magnitude of diffusion between different groups, ultimately dictate how much IP_3 flows to the 'target' astrocytes. However we also need to take into account IP_3 degradation. For simplicity, here we do not take into account the fact that this degradation may be dependent on Ca^{2+} and we assume it to just occur at constant rate Ω_I. In this way, the IP_3 balance for the three aforementioned astrocyte groups is regulated by the system of equations

$$N_s^* \frac{\mathrm{d}I_s}{\mathrm{d}t} = D_s\,(I_a - I_s) - \Omega_I N_s^* I_s \tag{S1}$$

$$N_a^* \frac{\mathrm{d}I_a}{\mathrm{d}t} = -D_s\,(I_a - I_s) - D_t\,(I_a - I_t) - \Omega_I N_a^* I_a \tag{S2}$$

$$N_t^* \frac{\mathrm{d}I_t}{\mathrm{d}t} = D_t\,(I_a - I_t) - \Omega_I N_t^* I_t \tag{S3}$$

The above equations can be analytically solved for initial conditions $I_s(0) = I_t(0) = 0$ and $I_a(0) = I_0$ to estimate the fraction of IP_3 that flows from activated astrocytes to target cells (Appendix B). Denoting by Q_s and Q_t the amounts[1] of IP_3 that diffuse from activated astrocytes in shell r to unactivated cells in shells $r-1$ and r, and to target cells in shell $r+1$ respectively, this fraction equals to

$$\frac{Q_t}{Q_s + Q_t} = \frac{1}{1 + \dfrac{D_s N_s^* (D_t + \Omega_I N_t^*)}{D_t N_t^* (D_s + \Omega_I N_s^*)}} \tag{S4}$$

Accordingly, the average IP_3 supply to a target cell from shell r, i.e. Ψ_r^{out}, can be computed by

$$\begin{aligned} \Psi_r^{out} &= \frac{Q_t}{N_t^*} = \frac{Q_s + Q_t}{N_t^*} \frac{Q_t}{Q_s + Q_t} \\ &= \frac{\rho_r N_r}{N_{r+1}} \frac{1}{1 + \dfrac{(E_{r-1} + \hat{W}_r)(N_{r-1} + \hat{N}_r)(E_r + \frac{\Omega_I}{F} N_{r+1})}{E_r N_{r+1} \left(E_{r-1} + \hat{W}_r + \frac{\Omega_I}{F}(N_{r-1} + \hat{N}_r) \right)}} \end{aligned} \tag{S5}$$

where we used the fact that $Q_s + Q_t = \rho_r N_r$, along with the expressions for N_i^* and D_i ($i = \mathrm{a}, \mathrm{t}, \mathrm{s}$).

To evaluate equation S5 we ultimately need to estimate the number of unactivated astrocytes in shell r ($\hat{N}_r$), as well as the number of connections of these cells with activated astrocytes in shell r ($\hat{W}_r$). With this aim, if we assume intra-shell connections to be randomly distributed between activated and unactivated cells, given W_r effective intra-shell connections among $N_r(N_r - 1)/2$ possible ones, the probability of connection between two randomly chosen cells in shell r is $p_r^W = 2W_r / (N_r(N_r - 1))$. Accordingly, the probability of an unactivated astrocyte in shell r to be disconnected from *one* activated cell in this shell is $1 - p_r^W$. Because

[1] We normalize IP_3 quantities with respect to the average amount of IP_3 ($\bar{Q}_0$) produced by an astrocyte in the network. This amounts is estimated by simulation of the biophysical model presented in the main text, and is essentially constant as far as the cell's degree is ≥ 3 (Lallouette, 2014).

there are $\rho_r N_r$ activated astrocytes in shell r, the probability of an unactivated astrocyte in shell r to be disconnected from *any* activated cell in this shell thus reads

$$p_r^s = \left(1 - p_r^W\right)^{\rho_r N_r} \tag{S6}$$

In the realistic scenario that $W_r \ll N_r(N_r - 1)$, then $p_r^W \ll 1$, and the above equation can be expanded by Taylor series up to the first order, i.e.

$$p_r^s \approx 1 - \rho_r N_r p_r^W \tag{S7}$$

Accordingly, the probability p_r^a of an unactivated astrocyte in shell r to be connected with *at least one* activated astrocyte in that shell equals to

$$p_r^a = 1 - p_r^s = 2\rho_r \frac{W_r}{N_r - 1} \tag{S8}$$

While the total number of unactivated astrocytes in shell r is $(1-\rho_r)N_r$, those that are connected to an activated astrocyte are

$$\hat{N}_r = (1 - \rho_r) N_r p_r^a = 2\rho_r(1 - \rho_r) W_r \frac{N_r}{N_r - 1} \approx 2\rho_r(1 - \rho_r) W_r \tag{S9}$$

where the last approximation is motivated by the observation that $N_r/(N_r - 1) \to 1$ as r increases.

Finally, we need to estimate $\hat{W}_r$. We note however that for the networks considered in this chapter, the average number of intra-shell connections per astrocyte is ≤ 1, and thus we also assume that $\hat{W}_r = \hat{N}_r$. In this fashion, we can rewrite equation S5 in the form of equation 14, where

$$g(r, \rho_r) = \frac{1}{1 + \dfrac{(E_{r-1} + 2\rho_r(1-\rho_r)W_r)(N_{r-1} + 2\rho_r(1-\rho_r)W_r)(E_r + \frac{\Omega_I}{F} N_{r+1})}{E_r N_{r+1}\left(E_{r-1} + 2\rho_r(1-\rho_r)W_r(1 + \frac{\Omega_I}{F}) + \frac{\Omega_I}{F} N_{r-1}\right)}} \tag{S10}$$

3 IP_3 flux by stimulation

We estimate the IP_3 supply to shell r directly due to stimulation (Ψ_r^{stim}) by computing the difference $\Psi_r^{tot} - \Psi_{r-1}^{out}$ for different r values in the biophysical network models. With this aim, we make the simplifying assumption that IP_3 supplied to the network by stimulation is minimally affected by degradation, and fast redistributes in the unactivated cells in the network. In this fashion, we directly measure Ψ_r^{tot} from simulations of the biophysical network models, while estimating Ψ_{r-1}^{out} for different r values and for $\Omega_I = 0$ by equation S5. Estimation of Ψ_r^{tot} in a biophysical network model is as follows. We consider the IP_3 supplied to any astrocyte i in a given shell r, i.e. Q_r^i. This quantity is computed integrating the total IP_3 diffusion flux only when incoming to cell i. Then $\Psi_r^{tot} = \sum_i \max Q_r^i(t)/N_r$.

Figure S1C shows the results of our estimation of Ψ_r^{stim}, conveniently plotted as a function of the number of astrocytes in the sphere of radius r centered on the stimulated cell, i.e. $V_r = \sum_{i=0}^{r} N_i$. It may indeed be seen that the data points can be well fit by a power law (*dashed line*), i.e.

$$\Psi_r^{stim} = S \cdot V_r^{-\eta} \tag{S11}$$

where S and η are left as free parameters.

4 IP_3 redistribution and shell activation

We start by defining the IP_3 threshold for activation of a shell r (i.e. ψ_r^θ) as the IP_3 amount needed to activate on average half of the astrocytes in the shell. This threshold can be directly estimated by numerical simulations of the biophysical network model, and can be approximated by a linear function of the mean degree $\langle k_r \rangle$ of cells in shell r, i.e.

$$\psi_r^\theta = A\langle k_r \rangle + B = A\frac{E_{r-1} + E_r + 2W_r}{N_r} + B \tag{S12}$$

In general, $\langle k_r \rangle$ is preserved across the network, so that the dependence on r in the above formula may be neglected. In this fashion we consider $\psi_r^\theta = \psi_\theta(\langle k \rangle)$ where $\langle k \rangle$ is the network's mean degree.

To quantify the fraction of activated cells per shell, i.e. ρ_r, we simulate ICW propagation in biophysical network models, and quantify the number of activated cells as a function of the difference $\Psi_r^{tot} - \psi_\theta(\langle k \rangle)$. The results of these simulations are shown in Figure S1D where the existence of a sigmoid relationship may be evinced for ρ_r as a function of $\Psi_r^{tot} - \psi_\theta(\langle k \rangle)$. We choose to fit this relationship by

$$\rho_r = \frac{1}{2}\left(1 + \tanh\left(\frac{\Psi_r^{tot} - \psi_\theta(\langle k \rangle)}{\delta}\right)\right) \tag{S13}$$

with δ being a parameter that controls the slope of the sigmoid. The latter equation coincides with equation 15, with the exception that in the simulation of the shell model, Ψ_r^{tot} is replaced by equation 13, which is computed according to equations S5 and S11.

5 Shell model simulations

For each realization i of a biophysical network model, we compute N_r^i, E_r^i, and W_r^i for all shells in the network centered on the stimulated astrocyte. Equations 13, S5 and S11 are then used to iteratively compute ρ_r until $\rho_r N_r < 1$ or the outmost shell R is reached. In doing so, we estimate the number of activated astrocytes in the shell model by $N_{sim}^i = \sum_{r=0}^{R} \rho_r N_r$.

Fitting of data from biophysical network models and estimation of parameters such as Ω_I/F (equation S5), S and η (equation S11), A and B (equation S12), and δ (equation S13) were performed by Covariance Matrix Evolution Strategy (CMA-ES) (Hansen, 2006), minimizing the difference between the number of activated astrocytes in the simulated shell model with respect to the corresponding biophysical network model, averaged over $n = 20$ realizations of the same network, for all network topologies. With this regard, we considered the fitness function:

$$\mathcal{C} = \sum_{\text{networks}} \left(\log(\langle N_{act}^i \rangle) - \log(\langle N_{sim}^i \rangle)\right)^2 \tag{S14}$$

taking into account all networks in Figure 6E. Parameters used in the simulation of the shell model are reported in Table C4.

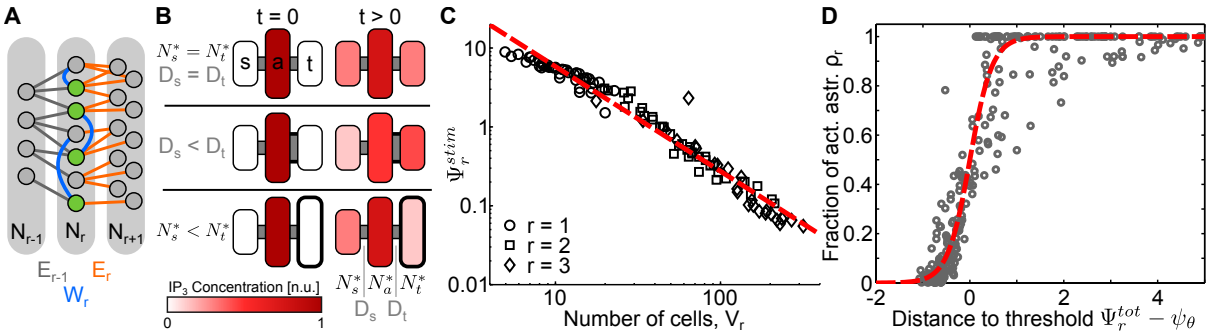

Figure S1: Shell model derivation. **A** Schematics of network decomposition by three consecutive shells: $r-1$, r, $r+1$ (*grey rounded boxes*). Among these shells, the r-th shell contains N_r astrocytes, interconnected by W_r intra-shell links (*blue links*) and sending out E_r connections to cells in the next shell (*orange links*). **B** Illustration of IP_3 redistribution by GJC-mediated diffusion from a group of activated astrocytes in a given shell ('a') to unactivated cells within the same shell or the inner one ('s') as well as to target cells in the outer shell ('t'). In total we consider N_a activated astrocytes connected with N_s and N_t cells by diffusion pathways with strength D_s and D_t respectively. The set of activated cells functions as IP_3 source, while unactivated 's' and 't' cells may be regarded as IP_3 sinks. At $t = 0$ all IP_3 is in the activated cells (*left*, dark red boxes), while it diffuses to unactivated cells for $t > 0$ (*right*). Only when the number of connections from activated cells to 's' and 't' cells is the same and so is the size of these two sets, IP_3 is evenly distributed between these cells (*top row*), otherwise it is not (*middle* and *bottom rows*). **C** IP_3 supply to shell r by stimulation (Ψ_r^{stim}) as a function of the number of astrocyte contained within this shell (V_r) estimated by simulations of biophysical network models. Data points can be conveniently fitted by a power law (*red dashed line*). Each data point represents the mean Ψ_r^{stim} for a shell $r \leq 3$ for $n = 20$ realizations of a particular biophysical network model. **D** Per-shell activation ratio may be regarded as a sigmoid function centered on $\Psi_r^{tot} = \psi_\theta$ (equation S13, *red dashed curve*). Each marker corresponds to mean values across 20 realizations for a given shell on a given network class with a given parameter set. In this example: $A = 0.07$ and $B = 0.35$. All networks and all shells are displayed on this figure. Biophysical network model parameters as in Figure 3. Shell model parameters as in Table C4.

References

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