Graphical Modeling for Single-Cell Ca^{2+} Imaging

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1 Background and Data

In this project we set out to model the propagation dynamics of calcium throughout the dendritic arbors of neurons, the central cell type of the nervous system. Neurons are composed of three main components: (1) the dendrites, large tree-like structures that branch out to receive inputs to the cell, (2) the soma (or cell body), in which integration of inputs is classically considered to occur, and (3) the axon, which generates spikes and transfers this output to other cells.

Recent work suggests that input-processing in the dendrites may be more active than previously thought, and it is now believed that the efficacy of signal propagation into different dendritic branches may be a read out of the stabilization of plasticity processes that have increase the impact of that branch's inputs on global cellular computations. Unfortunately, due to experimental limitations, to date little is known about how these signals are propagated *in vivo*, or how factors such as branch identity or behavioral state affect this propagation.

On the experimental end, using calcium-sensitive fluorophores coupled with two-photon microscopy, we can generate movies of somatic and dendritic calcium activity (a proxy for intracellular electrical activity) in an awake mouse performing spatial navigation behaviors (see fig. 1). Using a piezo actuator to rapidly shift the depth of the microscope's focal plane, we cut multiple optical planes through the dendrites to sample large portions of the arbor. A final structural scan then yields a wire diagram of the cell, from which we can read out the connectivity of the sampled points. We therefore aimed to establish a modeling framework for analyzing data of this type.

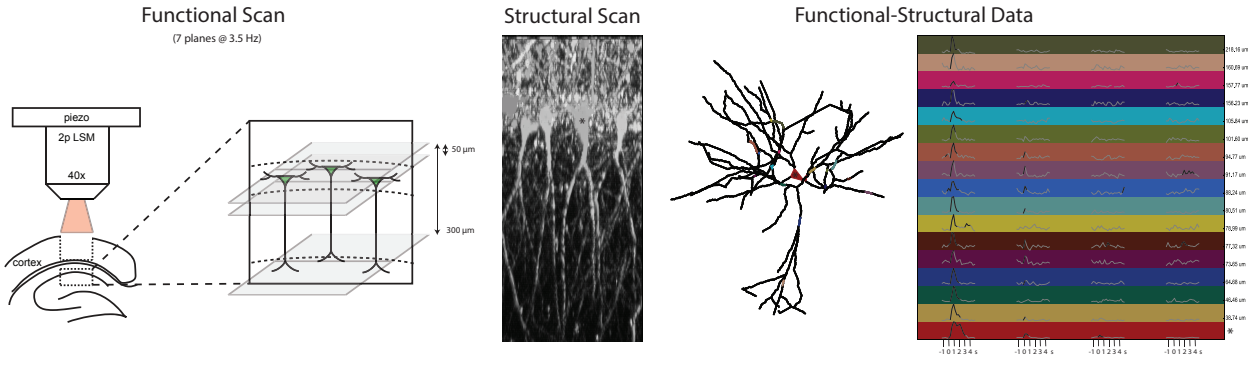

Figure 1: An example of the datasets simulated in this project. Cell activity is observed at discrete optical planes (left) and then structurally scanned to generate a wire diagram (center). The extracted signal can then be considered around transient events at all observed points which are now structurally labeled (color-coded at right) to allow connectivity to be read out.

2 Model

Because the calcium sensors we use in our experiments are quite slow relative to the signal itself (on the order of one second vs. one millisecond), and because it takes tens of milliseconds to jump between planes, it is impossible for us to determine the directionality of signal propagation in our prep. We therefore focus on modeling the total true underlying signal (i.e., the signal AUC) at all observed points in a given cell during small snapshots of time around interesting observed signals (i.e., detectable calcium transients) using an undirected graphical model. For each cell, the structure of the corresponding graphical model is exactly the structure of the cell that we get from the structural scan (see fig. 2).

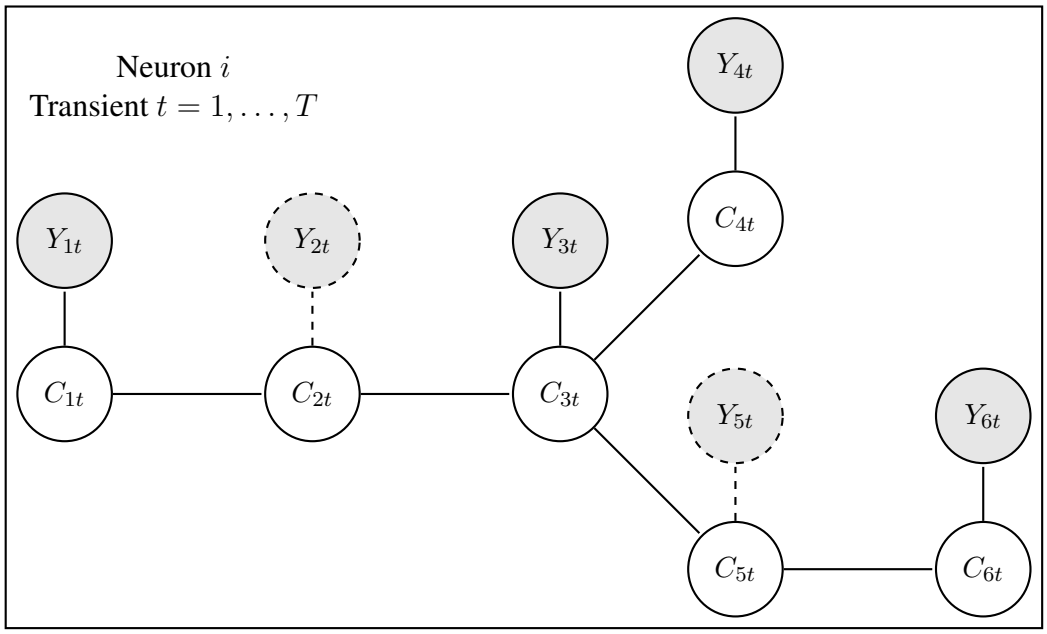

Figure 2: A small example cell with a single dendritic branch emerging from the soma C_{1t}. Note that some observations are missing, and different parameters may, for example, govern propagation of the primary ($C_{1t} - C_{3t}$) versus secondary ($C_{3t} - C_{4t}$ and $C_{3t} - C_{6t}$) dendrites (see text).

For a given cell and transient, the model is specified as follows:

We have noisy observations of the true underlying signal $C_i,\ i = 1, \ldots, N$

$$Y_i = aC_i + b + \epsilon,\ \epsilon \sim \mathcal{N}(0, \sigma_y^2) \tag{1}$$

Note that some nodes may not be observed during a given transient (due to slight differences in the configuration of the cell and the imaging angle from day to day), meaning that there may be missing observations.

We also assume some level of smoothness in the propagation of signal between adjacent latent nodes

$$C_i \sim \prod_{C_k \in \mathcal{N}_{C_i}} \mathcal{N}(C_k, \sigma_{c_{ik}}^2) \tag{2}$$

where $\mathcal{N}_{C_i}$ is the neighborhood of node C_i, and c_{ik} indexes into a list of possible variances which we assume to be constant within sections of the tree. E.g., in figure 2 edges $\{C_1, C_2\}$ and $\{C_2, C_3\}$ might be governed by $\sigma^2_{c_1}$ while all other edges are governed by $\sigma^2_{c_2}$. This choice allows us to model different propagation properties for different biologically relevant sections of the dendritic arbor.

We thus have a fully specified graphical model, with pairwise potentials

$$\psi^E(Y_i, C_i) = p(Y_i|C_i, \theta) = \mathcal{N}(Y_i|aC_i + b, \sigma^2_y) \tag{3}$$

$$\psi(C_i, C_j) = p(C_i, C_j|\theta) = \mathcal{N}(C_i - C_j|0, \sigma^2_{c_{ij}}) \tag{4}$$

and uninformative singleton potentials $\psi(C_i) = 1$, $\psi(Y_i) = 1$.

Finally, the model contains parameters

$\theta = a,$	observation gain
$b,$	observation offset
$\sigma^2_y,$	observation variance
$\{\sigma^2_c\}$	transition variances (constant within predefined regions)

To avoid potential issues with indeterminacy in the equation $Y_i = aC_i + b$, we also place a Gaussian prior on $a \sim \mathcal{N}(\mu_a, \sigma^2_a)$.

For the purposes of this proof-of-concept stage of the project, we arbitrarily choose $\mu_a = 1$ and $\sigma^2_a = 0.1$, but in actual application these values would be estimated from previous literature in this domain as well as the specific imaging parameters used to collect the data.

3 Expectation-Maximization

Given the observed data $\mathbf{Y}$, we wish to infer $\mathbf{C}$ and learn θ. To do this, we formulate the Expectation-Maximization (EM) algorithm for our model.

For a given cell with corresponding graph $G = (V, E)$ with observed transients $t = 1, \ldots, T$ we can write down the complete likelihood

$$\begin{aligned} p(\mathbf{Y}, \mathbf{C}|\theta) &= \prod_{t=1}^{T} \left[\prod_{i=1}^{N} \psi(C_{i,t}) \psi(Y_{i,t}) \psi^E(C_{i,t}, Y_{i,t}) \prod_{(i,j)\in E} \psi(C_{i,t}, C_{j,t}) \right] \\ &= \prod_{t=1}^{T} \left[\prod_{i=1}^{N} \mathbb{1}_{i,t} p(Y_{i,t}|C_{i,t}, \theta) \prod_{(i,j)\in E} p(C_{i,t}, C_{j,t}|\theta) \right] \end{aligned} \tag{5}$$

where $\mathbb{1}_{i,t}$ is an indicator for whether C_i yielded an observation Y_i on trial t.

3.1 E Step

To perform inference in the Expectation step, we must first specify a message passing protocol for each cell/transient. The messages passed are of two types: (1) from observations to latent nodes, simply

$$m_{Y_i \to C_i}(C_i) = \mathcal{N}\left(C_i | \frac{Y_i - b}{a}, \frac{\sigma_y^2}{a^2}\right) \tag{6}$$

and (2) between latent nodes.

$$\begin{aligned} m_{C_i \to C_j}(C_j) &= \int_{-\infty}^{\infty} \mathcal{N}(C_j | C_i, \sigma_{c_{ij}}^2) \prod_{k \in \mathcal{N}_{C_i} \setminus C_j} m_{k \to C_i}(C_i) dC_i \\ &= \mathcal{N}(C_j | \mu_M, \sigma_{c_{ij}}^2 + \sigma_M^2) \end{aligned} \tag{7}$$

Where for μ_{m_k}, σ_{m_k} from $m_{k \to C_i}$ calculated previously in the protocol we have

$$\mu_M = \left[\frac{a(Y_i - b)}{\sigma_y^2} + \sum_{k \in \mathcal{N}_{C_i} \setminus \{C_j, Y_i\}} \frac{\mu_{m_k}}{\sigma_{m_k}^2}\right] \sigma_M^2 \tag{8}$$

$$\sigma_M^2 = \left[\frac{a^2}{\sigma_y^2} + \sum_{k \in \mathcal{N}_{C_i} \setminus \{C_j, Y_i\}} \frac{1}{\sigma_{m_k}^2}\right]^{-1} \tag{9}$$

See appendix B for the full derivation.

Finally, the expression for the marginal $p(C_i | \mathbf{C}_{-i}, \mathbf{Y})$ is a Gaussian with mean and variance of the same form as (8) and (9), except the sums are over all latent neighbors $\mathcal{N}_{C_i} \setminus Y_i$.

3.2 M Step

Given our updated estimates $\hat{C}_i$ we can now update our estimate of θ.

$$a^{new} = \frac{\frac{\mu_a}{\sigma_a^2} + \frac{1}{\sigma_y^2} \sum_{t=1}^{T} \sum_{i=1}^{N} \mathbb{1}_{i,t} \left[(\hat{C_{i,t}} - \bar{C}_t)(Y_{i,t} - \bar{Y}_t)\right]}{\frac{1}{\sigma_a^2} + \frac{1}{\sigma_y^2} \sum_{t=1}^{T} \sum_{i=1}^{N} \mathbb{1}_{i,t} \left[(\hat{C_{i,t}} - \bar{C}_t)^2 + \hat{V}_{i,t}\right]} \tag{10}$$

where $\bar{C}_t$ and $\bar{Y}_t$ are the means over all estimates and observations, respectively, within transient t, and $\hat{V}_{i,t}$ is the marginal variance for $\hat{C_{i,t}}$

Note the similarity between this expression and the expression for a Least-Squares Estimator with a Gaussian prior on the weights.

$$b^{new} = \frac{1}{\mathcal{E}} \sum_{t=1}^{T} \sum_{i=1}^{N} \mathbb{1}_{i,t} \left[Y_{i,t} - a^{new} C_{i,t} \right] \tag{11}$$

where $\mathcal{E} = \sum_{t=1}^{T} \sum_{i=1}^{N} \mathbb{1}_{i,t}$ is the total number of observations.

Note that this is simply the mean residual between the observations and our gain-transformed latent estimates.

$$\sigma_y^{2\ new} = \frac{1}{\mathcal{E}} \sum_{t=1}^{T} \sum_{i=1}^{N} \mathbb{1}_{i,t} \left[(Y_{i,t} - (a^{new} \hat{C}_{i,t} + b^{new}))^2 + (a^{new})^2 \hat{V}_{i,t} \right] \tag{12}$$

For each of the transition variance parameters governing propagation in a region, denoted by all edges in the set $\mathcal{N}_c$ we have

$$\sigma_c^{2\ new} = \frac{1}{T} \sum_{t=1}^{T} \frac{1}{|\mathcal{N}_c|} \sum_{i,j \in \mathcal{N}_c} \left[(\hat{V}_{i,t} + \hat{C}_{i,t}^2) - 2 \left[\frac{\hat{V}_{i,t\setminus j} \hat{V}_{j,t}}{\hat{V}_{i,t\setminus j} + \sigma_c^{2\ old}} + \hat{C}_{i,t} \hat{C}_{j,t} \right] + (\hat{V}_{j,t} + \hat{C}_{j,t}^2) \right] \tag{13}$$

where $\hat{V}_{i,t\setminus j}$ is the variance of $\prod_{k \in \mathcal{N}_{C_{i,t}} \setminus C_{j,t}} m_{k \to C_{i,t}}$

3.3 Missing Observations

Once the EM algorithm has converged, we can straightforwardly use θ^{final} to estimate the values of missing observations

$$\hat{Y}_{i,t} = a^{final} \hat{C}_{i,t}^{final} + b^{final} \tag{14}$$

4 Simulation Results

While real experimental data is not yet quite ready for use (and in any case will require extensions beyond the scope of the current model, see below), we tested a software implementation[1] of the model described above on simulated data. To do so, we procedurally generated neurons with varying numbers of nodes, and pseudo-randomly picked gold-standard parameter values. We then picked an initial calcium value for C_1 and used our generative model to propagate the calcium throughout the cell (to get simulated true calcium) as well as to generate noisy observations of those calcium values (the "data").

[1] All code is available for use at github.com/t-rutten/dendrites_prop_model

We then ran the EM algorithm on the neurons and observed the convergence of the log likelihood, the parameters θ, and the sum of squared errors (SSE). The results of a representative run are shown in the figures below. Typically the results converged fairly quickly, though the number of iterations appears to scale with the size of the cell. Unfortunately, a small but significant subset of runs resulted in highly aberrant, diverging estimates of the latent calcium (though the parameters tended to stay within a reasonable range). This suggests there was either a bug in the code, or perhaps a "bug" in one of our update derivations - unfortunately we could not solve this issue in the time allotted.

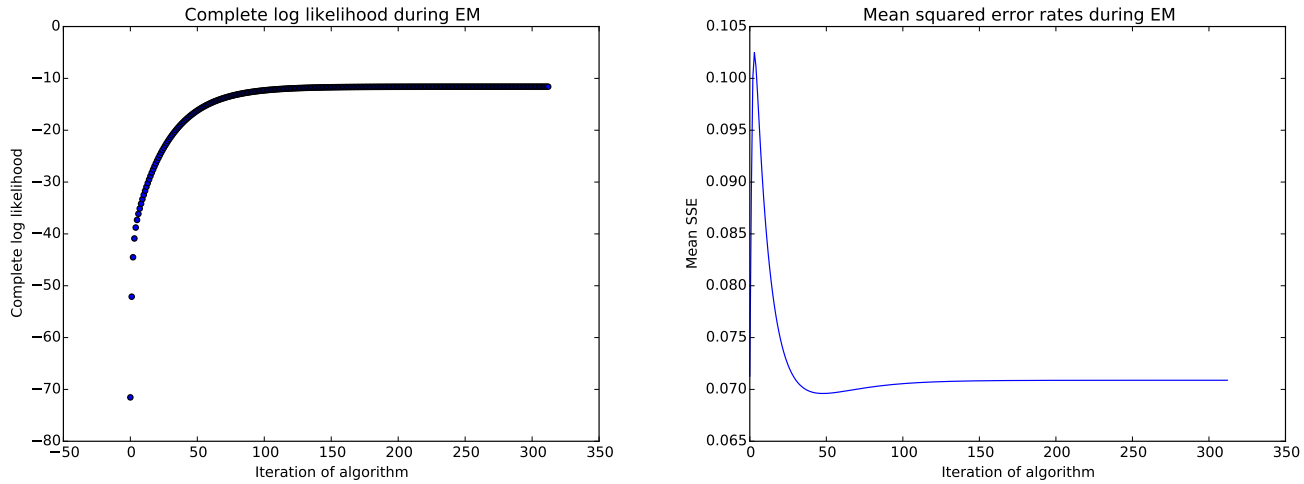

Figure 3: Complete log likelihood and mean squared error of model parameters during EM.

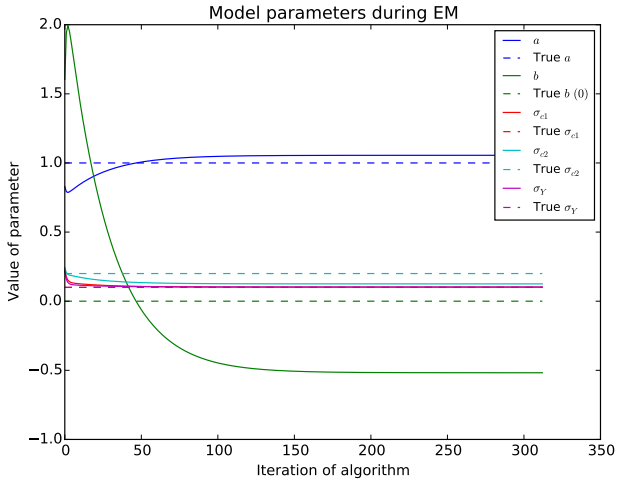

Figure 4: Convergence of the model parameters during EM.

5 Conclusions and Extensions

The model proposed here appears to be a useful tool for the single-neuron imaging datasets of the type described. Based on our preliminary results from simulated datasets, it appears that we may be able to (1) generate smoothed estimates of the latent signal, (2) learn potentially different parameters governing propagation in distinct regions of the dendritic arbor, and (3) estimate the values of missing observations.

The obvious next step for this project will be to apply this model to actual data (once they are done being collected). There also exist a number of clear extensions for the model. We may wish to learn a distinct a_i, b_i, σ^2_{yi} for each node C_i if the number of transients recorded in a cell makes such estimates statistically feasible. Another important extension will be to include some type of distance-dependent scaling for the transition potentials $\psi(C_i, C_j)$, as the observed nodes will not be equidistant as is assumed here, and the decay of intracellular signal with distance is a well-studied phenomenon. Finally, by playing games with the way in which we define the neighborhoods $\mathcal{N}_c$ governed by each σ^2_c, we can ask questions not just about regional differences in these parameters, but also differences with respect to any other behavioral or cognitive covariates along which we can define partitions of the transients or nodes. For example, by learning a different σ^2_c for transients that occur during running versus stationary periods, we can ask whether this behavioral dimension affects propagation properties across cells.

Appendix

A References

1) Bickson, Danny. "Gaussian Belief Propagation: Theory and Application." (2008).
2) Bishop, Christopher M. *Pattern Recognition and Machine Learning* (2006).
3) Jordan, Michael I. *An Introduction to Probabilistic Graphical Models.* (2003).
4) Blei, David. Lecture Notes. (2016).

B Message Passing Derivation

For Gaussian belief propagation, messages are passed using the integral-product rule. Suppose we have a node C_i, and a message is being sent to a node C_j. The message is composed of three parts: (1) the pairwise potential, (2) message from the evidence node Y_i, and (3) messages from the neighboring nodes, denoted by the set $\mathcal{N}_{C_i} \backslash C_j, Y_i$. The message is thus:

$$m_{C_i \to C_j} = \int_{-\infty}^{\infty} \mathcal{N}(C_j | C_i, \sigma_C^2) \mathcal{N}\left(C_i | \frac{\bar{Y_i} - b_i}{a_i}, \frac{\sigma_{Y_i}^2}{a_i^2}\right) \prod_{k \in \mathcal{N}(C_i) \backslash C_j, Y_i} \mathcal{N}(C_i | \mu_{m_k}, \sigma_{m_k}^2) dC_i \tag{15}$$

It can be seen that the product of n Normal distributions, $\prod_{i=1}^{n} \mathcal{N}(\mu_i, \sigma_i^2)$ is

$$\prod_{i=1}^{n} \mathcal{N}(\mu_i, \sigma_i^2) = \mathcal{N}\left(\frac{\sum_{i=1}^{n} \frac{\mu_i}{\sigma_i^2}}{\sum_{i=1}^{n} \frac{1}{\sigma_i^2}}, \frac{1}{\sum_{i=1}^{n} \frac{1}{\sigma_i^2}}\right) \tag{16}$$

Combining the second and third term of equation (15):

$$\mathcal{N}\left(C_i | \frac{\bar{Y_i} - b_i}{a_i}, \frac{\sigma_{Y_i}^2}{a_i^2}\right) \prod_{k \in \mathcal{N}(C_i) \backslash C_j, Y_i} \mathcal{N}(C_i | \mu_{m_k}, \sigma_{m_k}^2) = \mathcal{N}(C_i | \mu_M, \sigma_M^2) \tag{17}$$

where

$$\sigma_M^2 = [\frac{a_i^2}{\sigma_{Y_i}^2} + \sum_{k \in \mathcal{N}(C_i) \backslash C_j, Y_i} \frac{1}{\sigma_{m_k}^2}]^{-1} \tag{18}$$

$$\mu_M = [\frac{a_i(\bar{Y_i} - b_i)}{\sigma_{Y_i}^2} + \sum_{k \in \mathcal{N}(C_i) \backslash C_j, Y_i} \frac{\mu_{m_k}}{\sigma_{m_k}^2}][\frac{a_i^2}{\sigma_{Y_i}^2} + \sum_{k \in \mathcal{N}(C_i) \backslash C_j, Y_i} \frac{1}{\sigma_{m_k}^2}]^{-1} \tag{19}$$

It can also be shown that the integral of the product of two Gaussian distributions, $C_x \sim \mathcal{N}(C_y, \sigma_1^2) \propto e^{-\frac{(C_x - C_y)^2}{2\sigma_1^2}}$ and $C_x \sim \mathcal{N}(C_z, \sigma_2^2) \propto e^{-\frac{(C_x - C_z)^2}{2\sigma_2^2}}$ is a Gaussian distribution, $C_y \sim \mathcal{N}(C_z, \sigma_1^2 + \sigma_2^2)$.

In other words,

$$\int_{-\infty}^{\infty} \mathcal{N}(C_y, \sigma_1^2)\mathcal{N}(C_z, \sigma_2^2) dC_x = \mathcal{N}(C_z, \sigma_1^2 + \sigma_2^2) \tag{20}$$

The proof is below.

Proof. The product of two Gaussian distributions:

$$\mathcal{N}(C_y, \sigma_1^2)\mathcal{N}(C_z, \sigma_1^2) \propto e^{-\frac{1}{2\sigma_1^2}(C_x^2 - 2C_xC_y + C_y^2) - \frac{1}{2\sigma_2^2}(C_x^2 - 2C_xC_z + C_z^2)}$$

The integral over the product of the two Gaussian distributions:

$$\int_{-\infty}^{\infty} e^{-\frac{1}{2}\left(\frac{1}{\sigma_1^2} + \frac{1}{\sigma_2^2}\right)C_x^2 + \left(\frac{C_y}{\sigma_1^2} + \frac{C_z}{\sigma_2^2}\right)C_x - \frac{1}{2}\left(\frac{C_y^2}{\sigma_1^2} + \frac{C_z^2}{\sigma_2^2}\right)} dC_x$$

We use the Gaussian identity:

$$\int_{-\infty}^{\infty} e^{-ax^2 + bx} dx = \sqrt{\frac{\pi}{a}} e^{\frac{b^2}{4a}}$$

Where $a = \frac{1}{2}\left(\frac{1}{\sigma_1^2} + \frac{1}{\sigma_2^2}\right)$ and $b = \frac{C_y}{\sigma_1^2} + \frac{C_z}{\sigma_2^2}$

The integral reduces to $\propto e^{-\frac{(C_y - C_z)^2}{2(\sigma_1^2 + \sigma_2^2)}}$, which is just $C_y \sim \mathcal{N}(C_z, \sigma_1^2 + \sigma_2^2)$ □

Now we compute the full message from (15). Substituting equation (17):

$$m_{C_i \to C_j} = \int_{-\infty}^{\infty} \mathcal{N}(C_j | C_i, \sigma_C^2)\mathcal{N}(C_i | \mu_M, \sigma_M^2) dC_i \tag{21}$$

Now using equation (20), our message is:

$$m_{C_i \to C_j} = \mathcal{N}(C_j | \mu_M, \sigma_C^2 + \sigma_M^2) \tag{22}$$

C Maximization

C.1 a^{new} Update

To write an expression for a, we first write down the regression equation for a, for a single time step.

$$\hat{a}^{new} = \frac{Cov[C_i, Y_i]}{Var[C_i]} = \frac{\sum_{i=1}^{N} \mathbb{E}\left[(\hat{C_{i,t}} - \mathbb{E}\left[\bar{C}_{i,t}\right])(Y_{i,t} - \mathbb{E}\left[\bar{Y}_{i,t}\right])\right]}{\sum_{i=1}^{N} \mathbb{E}\left[\hat{C_{i,t}^2}\right]} \tag{23}$$

We do this for all time steps of a. Further, we only consider calcium nodes which have observations. For this, we use $\mathbb{1}_{i,t}$, the indicator function which is 1 if the calcium node $\hat{C}_i$ has an observation $\hat{Y}_i$ at time t, and 0 if not.

$$\hat{a}^{new} = \frac{\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[(\hat{C_{i,t}} - \bar{C}_t)(Y_{i,t} - \bar{Y}_t)\right]}{\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[(\hat{C_{i,t}} - \bar{C}_t)^2 + \hat{V_{i,t}}\right]} \tag{24}$$

We also want to put a prior distribution on a. To achieve this, we add a Gaussian prior $\mathcal{N}(\mu_a, \sigma_a^2)$

$$\hat{a}^{new} = \frac{\frac{\mu_a}{\sigma_a^2} + \frac{1}{\sigma_y^2}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[(\hat{C_{i,t}} - \bar{C}_t)(Y_{i,t} - \bar{Y}_t)\right]}{\frac{1}{\sigma_a^2} + \frac{1}{\sigma_y^2}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[(\hat{C_{i,t}} - \bar{C}_t)^2 + \hat{V_{i,t}}\right]} \tag{25}$$

C.2 σ_y^{2new} Update

Variance equation, noting only the calcium nodes with observations, over all time steps:

$$\sigma_y^{2\ new} = \frac{1}{\mathcal{E}}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[\mathbb{E}[(Y_{i,t} - (a^{new}\hat{C}_{i,t} + b^{new}))^2]\right] \tag{26}$$

Expanding the square:

$$\sigma_y^{2\ new} = \frac{1}{\mathcal{E}}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[\mathbb{E}[Y_{i,t}^2] - 2\mathbb{E}[Y_{i,t}(a^{new}\hat{C}_{i,t} + b^{new})] + \mathbb{E}[(a^{new}\hat{C}_{i,t} + b^{new})^2]\right] \tag{27}$$

Expanding the square again:

$$\sigma_y^{2\ new} = \frac{1}{\mathcal{E}}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[\mathbb{E}[Y_{i,t}^2] - 2\mathbb{E}[Y_{i,t}(a^{new}\hat{C}_{i,t} + b^{new})] + \mathbb{E}[a^{new2}\hat{C}_{i,t}^2] + \mathbb{E}[2a^{new}b^{new}\hat{C}_{i,t}] + \mathbb{E}[b^{new2}]\right] \tag{28}$$

Note that $\mathbb{E}[a^{new2}\hat{C}_{i,t}] = a^{new2}\hat{C}_{i,t} + a^{new2}\hat{V}_{i,t}$

$$\sigma_y^{2\ new} = \frac{1}{\mathcal{E}}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[Y_{i,t}^2 - 2Y_{i,t}(a^{new}\hat{C}_{i,t} + b^{new}) + a^{new2}\hat{C}_{i,t}^2 + a^{new2}\hat{V}_{i,t} + 2a^{new}b^{new}\hat{C}_{i,t} + b^{new2}\right] \tag{29}$$

$$\sigma_y^{2\ new} = \frac{1}{\mathcal{E}}\sum_{t=1}^{T}\sum_{i=1}^{N}\mathbb{1}_{i,t}\left[(Y_{i,t} - (a^{new}\hat{C}_{i,t} + b^{new}))^2 + (a^{new})^2\hat{V}_{i,t}\right] \tag{30}$$

C.3 σ_c^{2new} Update

We want to compute the variance for the pairwise potentials between calcium nodes. We first write the variance equation over all pairs of calcium nodes of the same region N_c, and over all time steps:

$$\sigma_c^{2new} = \frac{1}{T}\sum_{t=1}^{T}\frac{1}{|N_c|}\sum_{i,j\in N_c}\mathbb{E}\left[(\hat{C}_{i,t}-\hat{C}_{j,t})^2\right] \tag{31}$$

Expand the square:

$$\sigma_c^{2new} = \frac{1}{T}\sum_{t=1}^{T}\frac{1}{|N_c|}\sum_{i,j\in N_c}\mathbb{E}[\hat{C}_{i,t}^2]-2\mathbb{E}[\hat{C}_{i,t}\hat{C}_{j,t}]+\mathbb{E}[\hat{C}_{j,t}^2] \tag{32}$$

Note that $\mathbb{E}[\hat{C}_{i,t}\hat{C}_{j,t}] = Cov[\hat{C}_{i,t},\hat{C}_{j,t}] - \mathbb{E}[\hat{C}_{i,t}]\mathbb{E}[\hat{C}_{j,t}]$.

$$\sigma_c^{2new} = \frac{1}{T}\sum_{t=1}^{T}\frac{1}{|N_c|}\sum_{i,j\in N_c}\left[(\hat{V}_{i,t}+\hat{C}_{i,t}^2)-2Cov[\hat{C}_{i,t},\hat{C}_{j,t}]-2\mathbb{E}[\hat{C}_{i,t}]\mathbb{E}[\hat{C}_{j,t}]+(\hat{V}_{j,t}+\hat{C}_{j,t}^2)\right] \tag{33}$$

$$\sigma_c^{2new} = \frac{1}{T}\sum_{t=1}^{T}\frac{1}{|N_c|}\sum_{i,j\in N_c}\left[(\hat{V}_{i,t}+\hat{C}_{i,t}^2)-\frac{2\hat{V}_{i,t\backslash j}\hat{V}_{j,t}}{\hat{V}_{i,t\backslash j}+\sigma_c^{2\ old}}-2\hat{C}_{i,t}\hat{C}_{j,t}+(\hat{V}_{j,t}+\hat{C}_{j,t}^2)\right] \tag{34}$$