1

STRUCTURAL ASPECTS

Connectivity in local cortical circuits exhibits a salient feature: Examining the occurrence of connections in neuron pairs, studies have repeatedly found that bidirectionally connected neuron pairs appear much more frequently than expected from the network's overall connection probability. In layer 5 of the somatsosensory cortex studies from Markram (1997) and Perin et al. (2011) have found an overrepresentation of reciprocally connected pairs of thick tufted pyramidal cells, an observation that has also been reported in layer 2/3 (Holmgren et al. 2003) and layer 5 (Song et al. 2005) of the visual cortex. The overrepresentation of bidirectionally connected pairs is significant, Song et al. for example found such pairs represented four times the expected amount.

The underlying connection principle imposing this overrepresentation on the network however remains unclear. Song et al. discuss the possibility of known learning rules to explain their findings, leaving a definitive answer open to further investigation. More recent studies find overrepresentation of reciprocally connected pairs *in vitro* resulting from functional specificity (Ko et al. 2011) and *in silico* from dense neuron clustering rules (Klinshov et al. 2014), identifying specific network characteristics that may contribute to the reported overrepresentation *in vivo*.

Here we examine anisotropy in connectivity as a possible candidate for an underlying principle explaining the characteristic two neuron connection distribution. In random networks, the chance to encounter a specific mode of connection in a random pair of neurons be can easily be computed from the overall connection probability p. For this let X be the random variable of the number of edges between two different vertices in a Gilbert graph $G(n, p)$ with $n \geq 2$. As the edges are independently realized, resulting in a simple directed graph, we have

$$\begin{aligned} \mathbf{P}(X=0) &= (1-p)^2 && \text{unconnected pair,} \\ \mathbf{P}(X=1) &= 2p(1-p) && \text{single connection,} \\ \mathbf{P}(X=2) &= p^2 && \text{reciprocal connection;} \end{aligned} \tag{1.1}$$

in short $\mathbf{P}(X=k) = \mathcal{B}_{2,p}(k)$ for $k \in \{0,1,2\}$ and $\mathbf{P}(X=k) = 0$ otherwise. Using this probability distribution as the expectation for connectivity of neuron pairs in the various network types, a numeric analysis of the anisotropic sample graphs reveals that bidirectionally connected pairs appear almost twice as often as expected from the overall connection probability ($p = 0.116$) and equations 1.1, similarly as reported by Song et al. (Figure 1.1 A). However, comparing the pair probabilities in anisotropic networks with the probabilities in their rewired counterparts, we find that anisotropy does not influence the occurrence of two-neuron motifs (Figure 1.1 B) In fact, expected connections in neuron pairs are identical in distance-dependent and rewired anisotropic networks (**??**).

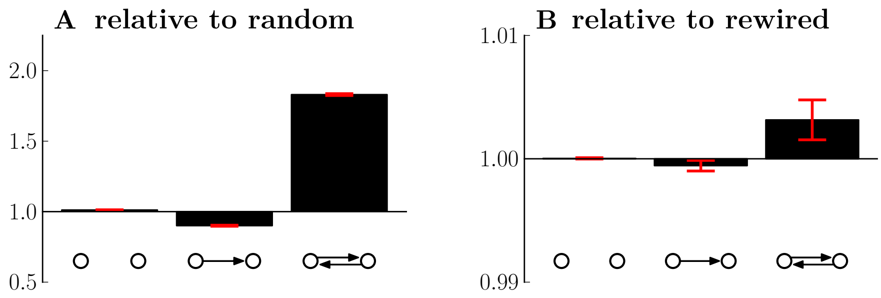

Figure 1.1: Overrepresentation of reciprocal connections in anisotropic networks due to distance-dependent connectivity Extracting the counts of unconnected, one-directionally and bidirectionally connected neuron pairs in the anisotropic sample graphs, overrepresentation of reciprocally connected pairs is identified as a feature of the network's distance dependency as opposed to anisotropy in connectivity. **A)** Showing the quotient of the counts for the three pair types, extracted from the set of sample graphs, with the number of expected pairs in Gilbert random graphs $G(n, p)$, where $n = 1000$ and $p = 0.116$ were matched to the sample graph parameters. While single connections appear less often than in Gilbert random graphs, reciprocal connections are significantly overrepresented. Errorbars SEM. **B)** Comparing appearance of connection pairs in the anisotropic sample graphs with their respective appearance in the rewired sample graphs, we find that eliminating anisotropy does not significantly change the counts for the connection types, indicating that anisotropy does not influence two neuron connection probabilities. Errorbars SEM. (`c5f1462b`)

We further support this observation by computing the probability distribution for the expected number of edges between to random vertices in the anisotropic graph model. For this we assume that only the distance-dependent connection probability $C(x)$ determines the occurrence of edges in vertex pairs in the anisotropic graph model. Then, using the probability distribution $f(x)$ for the a random neuron pair to be at distance x, we calculate

$$\begin{aligned}
\mathbf{P}(X=0) &= \int_0^{\sqrt{2}} (1-C(x))^2 f(x)\,dx, \\
\mathbf{P}(X=1) &= \int_0^{\sqrt{2}} 2C(x)(1-C(x)) f(x)\,dx \quad \text{and} \\
\mathbf{P}(X=2) &= \int_0^{\sqrt{2}} C(x)^2 f(x)\,dx.
\end{aligned}$$

Inserting the distance-dependent connection probabilities $C(x)$ in the anisotropic graph model as computed in Theorem **??** and the probability distribution $f(x)$ from Theorem **??** we obtain

$$\begin{aligned}
\mathbf{P}(X=0) &= 0.791336 & 0.7907 \pm 0.0008 \\
\mathbf{P}(X=1) &= 0.184151 & 0.1846 \pm 0.0007 \\
\mathbf{P}(X=2) &= 0.024513 & 0.02462 \pm 0.00009,
\end{aligned}$$

perfectly matching the probabilities extracted from anisotropic sample graphs in the right column (error SEM, `c5f1462b`). Noting that distance-dependency alone is sufficient to accurately predict edge probabilities in neuron pairs in the anisotropic network model and combined with the observations in Figure 1.1, we conclude that varying degrees of anisotropy do not affect the occurrence of neuron pair motifs.

The discussion in the last section focused on the effect of anisotropy in connectivity on the occurrence of neuron pair motifs. Could distance-dependency itself, as imposed by the specific geometry, be a decisive factor in the distribution of edge counts in neuron pairs? Song et al. (2005), as well as Perin et al. (2011), report an overrepresentation of reciprocal connections independent from distance-dependent connectivity, opposing the observations made in the last section (Figure 1.1 A). Furthermore, the connectivity profile in the anisotropic graph model, as identified in Section **??**, follows purely from abstract geometry rather than being motivated by connectivity found in cortical circuits. In an attempt to rectify this and to allow for a more differentiated examination of two neuron connections, in this section we step away from simplistic geometry and "tune" the anisotropic networks to display a distance-dependent connectivity as reported by Perin et al. by adjusting the width $w(x)$ at any point x along the axon's projection.

For this we introduce anisotropic networks tuned to reflect a given distance-dependent connection profile $C(x)$. We are facing the following problem: Given $C(x) : [0, \sqrt{2}) \to [0, 1]$, find $w : [0, \sqrt{2}) \to [0, \infty)$ such that the probability to have a connection from v_1 to v_2 for arbitrary vertices $v_1 \neq v_2$ in an anisotropic graph $G(n, w)$ with distance $\mathrm{d}(v_1, v_2) = x$ is $C(x)$. The problem is in general highly complex when nothing can be assumed about $C(x)$. We find an approximate solution to the problem considering the following geometric relation:

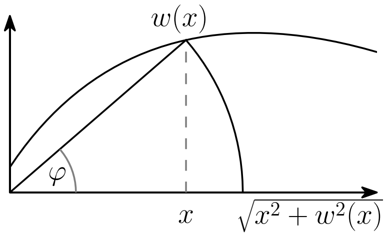

Figure 1.2: Computing connection probability $C(x)$ from non-constant $w(x)$

From Figure 1.2 we have the relation

$$C\left(\sqrt{x^2 + w^2(x)}\right) = \frac{1}{\pi} \arctan \frac{w(x)}{x}. \tag{1.2}$$

In order to solve for $w(x)$ we first consider a linear approximation, expanding

$$C\left(\sqrt{x^2 + w^2(x)}\right) \approx C(x) + \left(\sqrt{x^2 + w^2(x)} - x\right) C'(x).$$

The resulting transcendental equation

$$C(x) + \left(\sqrt{x^2 + w^2(x)} - x\right) C'(x) = \frac{1}{\pi} \arctan \frac{w(x)}{x}$$

is however still too complex in the context of this work. Instead we propose the approximation $\sqrt{x^2 + w^2(x)} \approx x$, which inserting into 1.2 yiels

$$C(x) \approx \frac{1}{\pi} \arctan \frac{w(x)}{x}. \tag{1.3}$$

Under the assumption that $C(x) < \frac{1}{2}$ for all x we obtain the identity

$$w(x) = x \tan\left(\pi\, C(x)\right), \tag{1.4}$$

being aware that it only holds as well as approximation 1.3 does.

Here we use relation 1.4 to generate anisotropic networks reflecting the distance-dependent connectivity profile as found by Perin et al. (2011). For this we finally need to adjust the before arbitrarily determined side length of the network's surface. Perin et al. mapped connectivity in layer 5 of the rat's somatosensory cortex up to a distance of 300 µm. Using this reported distance connectivity to generate anisotropic networks via 1.4, the chosen side length s determines the networks overall connectivity (Figure 1.3 A). We determine $s = 296$ µm to match the overall connection probability of $p = 0.116$ as used before and reported by Song et al. (Figure 1.3 B). The obtained value for s is consistent with the slice thickness of 300 µm used in Perin et al.'s experiment.

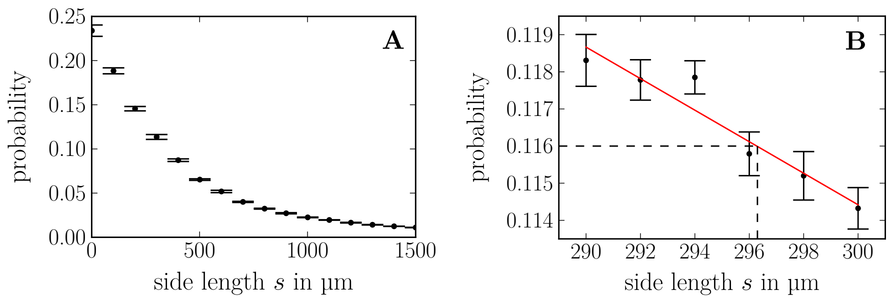

Figure 1.3: Network side length adjusted to match overall connection probability Side length of the network's surface determines the overall connection probability in the network when axon width function $w(x)$ is fixed. **A)** Connection probability declines with rising side length **B)** Determining side length as $s = 296$ µm to match $p = 0.116$ as reported by Song et al. (2005). (`6154302f`, `ef0e785d`)

Having determined the neotwork's side length s, we're extending the quiver of generated sample networks for the numerical analysis once more by the "tuned anisotropic graphs" , in which the axon width

$w(x)$ was determined such that the networks reflect Perin's connectivity profile. Analyzing the obtained axon width function we note that $x \gg w(x)$ holds for most x, justifying the approximation

$$\sqrt{x^2 + w^2(x)} \approx x$$

a posteriori (Figure 1.4). From the 25 generated networks overall connection probability is extracted as $p = 0.1160 \pm 0.0006$ (SEM), as expected from the choice of s (`f11dca65`).

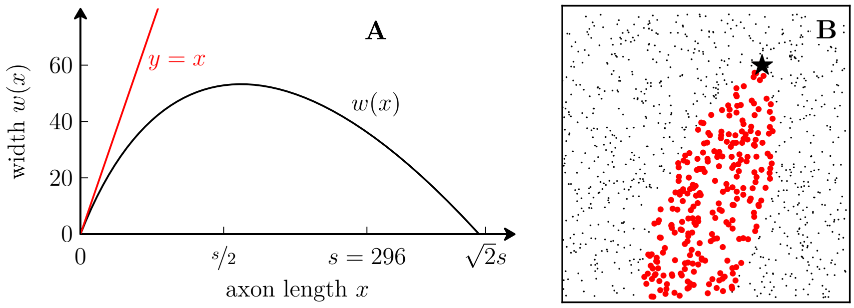

Figure 1.4: Anisotropic network model with tuned axon width w(x) **A)** Resulting axon width function $w(x)$ from tuning to distance-dependent connection profile as reported by Perin et al. (2011), see also Figure 1.5. Note that $x \gg w(x)$ for most x, supporting approximation 1.3. **B)** Showing for a single neuron (star) connected (red) and unconnected (gray) neurons in the tuned anisotropic network, revealing the characteristic axon shape. (`d45c02e4`, `8f0d65e4`)

Overall distance-dependent connection probabilities in the anisotropic graphs clearly match the profile of Perin et al., presenting strongest the argument for the approximations made earlier (Figure 1.5 A).

revisiting two neuron connections

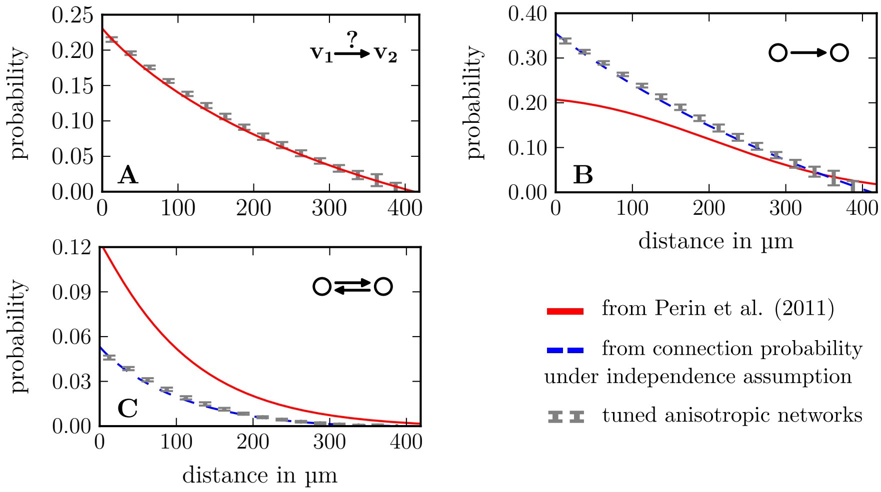

Figure 1.5: Distance-independent overrepresentation of reciprocal connections Comparison of occurrences of one- and bidirectionally connected neuron pairs in the tuned anisotropic networks (gray) with profiles found by Perin et al. (red), shows that overrepresentation of bidirectional pairs is distance-independent and not connected to anisotropy. **A)** Overall connection probability in the tuned anisotropic networks was successfully adjusted to reflect connection probability found by Perin et al. **B)-C)** Showing in blue the probabilities to obtain a neuron pair motif (single edge in B, two edges in C) calculated under independence assumption from the overall probability from A), we find that counts in the tuned anisotropic networks (gray) match the independence assumption and do *not* show the overrepresentation present in Perin et al.'s experiment. (`875505b0`)