

Biophysical constraints on neuronal branching

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When examining the structure of the brain or even the structure of a simple neural network in the nervous system, one is faced with surprisingly complex wiring diagrams [1]. Even the neurons themselves, the individual entities that together construct neural networks, come in an extremely wide variety of shapes and forms. As in networks in general, there is a strong relation between the neural network's structure or wiring diagram, and its function (see [2] and references within). Neurite outgrowth, the branching pattern of single neurons leading to the formation of distinct synapses, is a dominant factor in determining the future output of neural circuits - behavior.

In this paper we investigate conjunct rules that govern the complex structures of neurons. We concentrate on neuronal arborization, specifically on the local geometry of the bifurcation of a neurite into its sub-branches (Figure 1). The relationship between the branch angles and the neurite diameters has been previously addressed by two theoretical approaches.

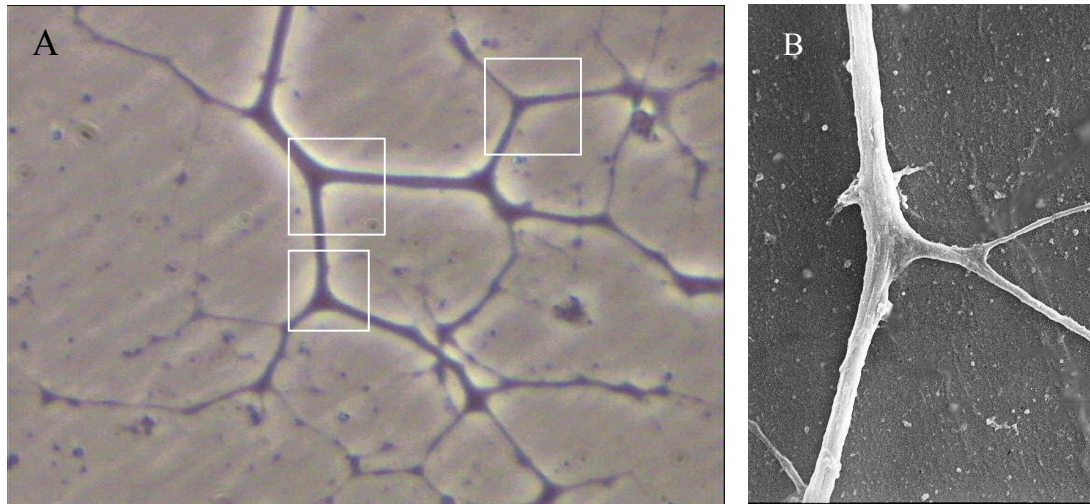


Figure 1: (A) Cultured neurons arborize into multiple branching neurites. Examples of measured branch points (bifurcations) are marked. (B) A scanning electron microscope image of the neurite branching area.

The first theoretical approach is based on the concept of optimization and has been previously applied to the blood vessels, living trees and more [3,4,5]. This approach is based

on postulating a cost function and then minimizing it under certain constraints. By postulating that the total volume of branches gives the cost function, Murray derived a relationship between branch diameters and angles of a single bifurcation for living trees and blood arteries [3,4]. Similar model can be applied to neurites [5]. Indirect comparison of the experimentally measured neurite diameters and branch angles with various minimization models (based on minimizing volume, length, signal propagation speed, or surface area) suggests that the volume minimization provides the best fit to the data [5,6,7,8,9].

The second theoretical approach is based on postulating the existence of the mechanical tension along the branches constructing the neuronal arbors [10]. One can compare these branches to ropes being pulled by their growth cones with forces increasing with their diameters. Then the equilibrium arrangement of the arbor segments is given by the mechanical equilibrium of tension forces. Although this approach seems different from the first one, it is mathematically equivalent. This is because the optimization problem can be formulated in the differential form, where derivatives of the cost function in respect to branch length are virtual forces. Then the condition for the minimum of the cost function is equivalent to the tension force equilibrium.

In the present study we set out to investigate whether neuronal arbors are adequately described by the above models and determine the relationship between branch diameter and the cost function, or, alternatively, the dependence of the tension force on the diameter. To

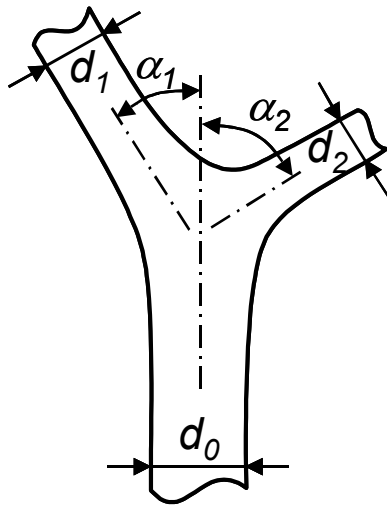


Figure 2: Geometry of a neurite branch point is characterized by the diameters of parent segment (d_0), daughter segments (d_1 , d_2) and branch angles (α_1 , α_2). We measured these parameters in cultured neural networks and compared them with the theoretical predictions.

this end, we employed two dimensional neuronal networks growing in cultures of neurons dissociated from insect ganglia [11, 12]. This system is simple (relative to any *in-vivo* network), and allows control over many of its variables. It also enables easy access for optical observations and mechanical manipulation of the cells.

We analyzed simple branch bifurcations in which a parent segment splits into two daughter segments (see Figure 1). Branching points with obvious abnormalities such as extreme changes in segment thickness, irregular angle changes and other disruptions to growth were excluded. We measured the diameters of the parent and daughter segments and the angles formed between the orientation of the parent segment and that of each

of the daughter segments (Figure 2). These experimental parameters were tested against the theoretical predictions of the optimization and tension models.

Assuming that the branch points are in equilibrium (or, equivalently, the cost function is minimized) there is a relationship between the tension forces and the branch angles. This relationship follows from the Law of Sines for the force triangle and is given by the following three equations:

$$\text{I. } \frac{T_1}{T_0} = \frac{\sin(\alpha_2)}{\sin(\alpha_1 + \alpha_2)} \quad \text{II. } \frac{T_2}{T_0} = \frac{\sin(\alpha_1)}{\sin(\alpha_1 + \alpha_2)} \quad \text{III. } \frac{T_2}{T_1} = \frac{\sin(\alpha_1)}{\sin(\alpha_2)}$$

where T_0 is the tension along the parent neurite and T_1 and T_2 are the tensions along the daughter neurites. If tension is proportional to branch diameter to some power ν then we have:

$$\text{I. } \frac{d_1^\nu}{d_0^\nu} = \frac{\sin(\alpha_2)}{\sin(\alpha_1 + \alpha_2)} \quad \text{II. } \frac{d_2^\nu}{d_0^\nu} = \frac{\sin(\alpha_1)}{\sin(\alpha_1 + \alpha_2)} \quad \text{III. } \frac{d_2^\nu}{d_1^\nu} = \frac{\sin(\alpha_1)}{\sin(\alpha_2)}$$

Because the third equation follows from the first two, we plot data for the ratios of daughter and mother branch diameters only (Figure 3)

The results presented in figure 3 demonstrate correlation between diameters and angles measured in our cultured neurons following the predictions in equations I-III. However, the

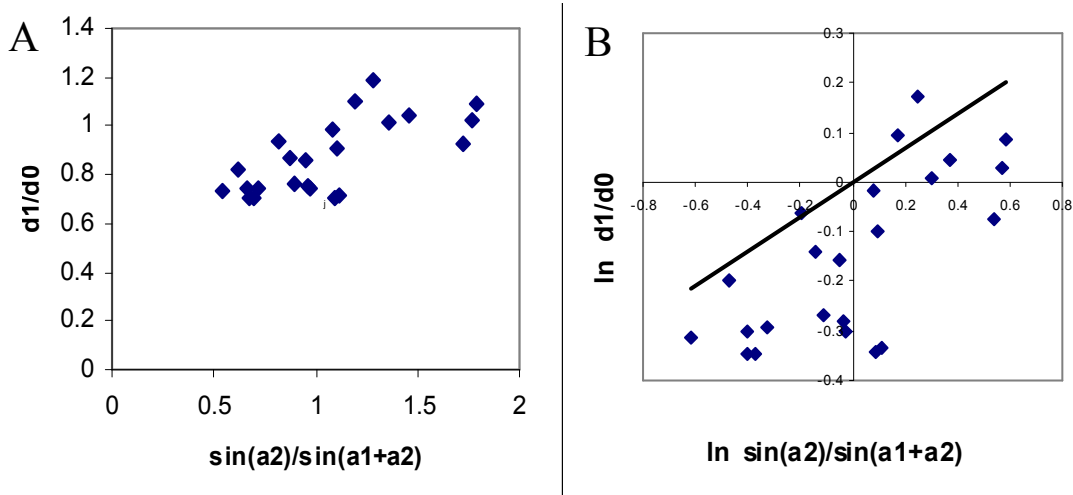


Figure 3: Mechanical tension equations tested for 22 measured neuron bifurcations. (A) The graph shows the results for equation I (dots). (B) The same data plotted on the log log graph. The data is fitted by a straight line passing through the origin. The slope of the line gives the exponent ν in the dependence of the tension force on the diameter (or cost function on the diameter).

data shows significant spread. This spread may arise because either the relationship between the tension force and diameters is more complicated than postulated or tension is not the only force determining branch angles. We investigated the second possibility.

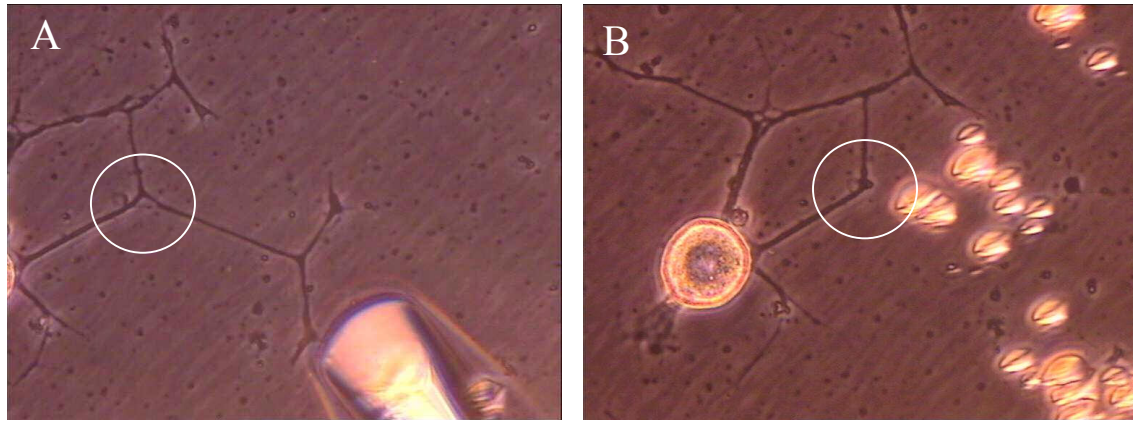


Figure 4: A cultured neuron two days after dissociation, before and after mechanical manipulation. (A) the developing neurites before the glass rod released the right hand side branch. The circle marks the tested junction. (B) the same neuron after manipulation. The whole neurite was loosened. The junction, marked with a circle, stayed almost stable during the entire process of neurite detachment and absorption. The bright spots on the right are scratches on the substrate as a result of the manipulation process.

The junction point itself may be attached to the substrate and acts as an anchor to the branching point [11,12,13] thus adding an additional force to the local equilibrium of forces. To test this assumption and study the nature of the junctions, we mechanically manipulated a daughter neurite by means of a thin glass rod until its growth cone was detached from the substrate and tension along the segment was released. We followed the position of the junction during the process of neural detachment and absorption in several cultured neurons. Figure 4 presents an example of a junction before and after such manipulation. It is clear that although the neurite is entirely absent, the junction point itself has only slightly moved.

To conclude, our results show that the tension theory and the volume optimization principles provide a reasonable approximation to the data. The deviation from pure tension forces equilibrium is explained by an additional force exerted by the anchoring of the junction to the substrate. This force confers plasticity upon the structure of the branch junction, which enables optimization of junction geometry without contradicting physical constraints. Plasticity is achieved by an additional degree of freedom to the relation between diameter and angle and is limited by the magnitude of the force produced by the junction as an anchor. We suggest that elements of both theories tested here participate in ensuring the construction of neurite junctions that will satisfy functional requirements. We further believe the principles demonstrated in our two-dimensional experimental system are also applicable *in-vivo* [14],

where the neuronal growth and branching process take place within a three-dimensional substrate.

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