A Parsimonious Description of Motoneuron Dendritic Morphology Using Computer Simulation

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Most quantitative descriptions of neuronal dendrite morphology involve tabulations of measurements and correlations among them. The present work is an attempt to extract from such data a parsimonious set of parameters that are sufficient to describe the quantitative features of individual and pooled dendrites, including their statistical variability. A relatively simple stochastic (Monte Carlo) model was devised to simulate branching dendritic trees. The necessary parameters were then derived directly from measurements of 64 completely reconstructed dendrites belonging to six gastrocnemius α -motoneurons, labeled by intracellular injection of HRP. Comparison of actual and simulated dendrites was used to guide the process of parameter extraction. The model included only two processes, one to generate individual branches given their starting diameters and the second to select starting diameters for the daughter branches produced at dichotomous branching points. The stochastic process for branch generation was controlled by probability functions for branching (P_{br}) and for terminating (P_{tm}), together with a constant rate of branch taper. All model parameters were fixed by motoneuron measurements except for branch taper rate, which was allowed to vary within limits consistent with observed taper rates in order to generate the appropriate total number of branches. The simplest model (model 1), in which P_{br} and P_{tm} depended only on local branch diameter, produced simulated dendrites that fit many, but not all, characteristics of actual motoneuron dendrites. Two additional properties produced significant improvements in the fit: (1) a small but significant dependence of daughter diameters on the normalized starting diameter of the parent branch, and (2) a dependence of P_{br} and P_{tm} on distance from the soma as well as on local branch diameter. The process of developing this model revealed unsuspected relations in the original data that suggest the existence of fundamental mechanisms for morphological control. The final model succinctly describes a large amount of data and will enable quantitative comparisons between the dendritic structures of different types of neurons, regardless of their relative sizes.

The advent of intracellular labeling methods, particularly the use of HRP (Snow et al., 1976), has produced a very large volume of qualitative and quantitative data about neuron morphology, including detailed studies of motoneurons (e.g., Cullheim and Kellerth, 1978; Ulfhake and Cullheim, 1981; Ulfhake and Kellerth, 1981, 1983; Cameron et al., 1985; Rose et al., 1985; Cullheim et al., 1987a,b; Kernell and Zwaagstra, 1989; Moschovakis et al., 1991). This literature contains a catalog of data that includes global indices of cell size (e.g., total membrane area, and total dendritic length and span) as well as a variety of specific relations, such as correlations between the diameter of the stem branch and the size and branching complexity of individual dendritic trees. Attempts have also been made to compare the morphologies of different species of neurons, for example, α - versus γ -motoneurons (Westbury, 1982; Moschovakis et al., 1991), and to define morphological changes that result from experimental manipulations such as axotomy (Cullheim et al., 1989), functional overload (Gollvik et al., 1990), or hormonal changes (Kurz et al., 1986; Sasaki and Arnold, 1991). In practice, it is often difficult to interpret such comparisons because of the large variance in the data and because of systematic interrelations between many measurements (e.g., Moschovakis et al., 1991). Such comparisons would, in principle, be facilitated if the raw data could be reduced to a relatively compact set of parameters and functions.

Hillman (1979) has suggested that it might be possible to extract from masses of morphological data those “fundamental parameters of form” (p 478) that can completely describe the data. The present work attempts to identify some of these parameters and to examine quantitatively the extent to which they can be said to “describe” the original data base. The strategy was simple: (1) devise a model system that can simulate dendritic trees, (2) derive the required model parameters directly from measurements of real dendrites, and (3) refine the parameter derivations or basic model assumptions, based on the degree of congruence between real and simulated dendrites. Morphological measurements of 64 dendrites from six completely reconstructed gastrocnemius α -motoneurons, described elsewhere (Cullheim et al., 1987a,b), served as the data base.

There are several factors that make this exercise biologically interesting. The *process* of developing such mathematical models requires examination of alternative designs and careful scrutiny of the original data to isolate those factors that appear to be important. The set of model rules and parameters can be said to describe the exemplar data set successfully only if the model output is congruent with the actual data in features that

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Figure 1. Scatter diagrams to show the distributions of the length (ordinate) of 1974 motoneuron dendritic branches in relation to their starting diameters (abscissa). The distribution for terminating branches indicates a direct dependence, while that for parents is more complex, with greater scatter but a basically inverse dependence. The inset illustrates histograms of the rate of branch taper for parent (solid bars) and terminating branches (shaded bars; overall mean, $-0.001 \pm 0.001 \mu\text{m}/\mu\text{m}$).

are not explicitly included in the model design (i.e., the model is nontrivial). Persistent discrepancies between model output and its real counterpart can reveal relations in the original data that are not obvious or even suspected. The present system successfully encapsulated a large amount of data into a relatively simple formulation, with a parsimonious set of parameters that provided clues to the nature of underlying biological factors that can control dendritic growth and maintenance.

Materials and Methods

The present model was developed using a relatively large data set obtained by completely reconstructing and measuring 64 dendrites from six gastrocnemius α -motoneurons in the cat spinal cord, each labeled by intracellular injection with HRP after physiological identification. With one exception, the measurements from these cells and the methods used to obtain them have been fully documented elsewhere (Cullheim et al., 1987a,b). The present data set included all dendrites from five gastrocnemius α -motoneurons described in those references (cells 36/4, 38/2, 41/2, 42/4, and 43/5), plus one gastrocnemius cell obtained at the Karolinska Institutet, using the same basic techniques. Raw data files were completely reanalyzed for the present work, using commercial statistical programs (DATADESK and STATVIEW II) and special-purpose programs written in PASCAL and implemented on Macintosh II computers. The model simulations were also implemented on Macintosh II computers in programs written in PASCAL, with certain features

checked using analytical functions developed using the symbolic manipulation package Mathematica®.

Rationale. A neuronal dendrite is composed of modules (individual branches) emanating from a stem branch and linked together at branch points that, in α -motoneurons, are almost exclusively dichotomous (Ulfhake and Kellerth, 1981; Cullheim et al., 1987a). Simulation of complete dendritic trees thus requires (1) a method to produce individual branches that have the correct distributions of diameters and lengths, as well as the correct proportions of branches that either bifurcate again ("parent" branches) or terminate (Fig. 1); and (2) a second method to generate the correct statistical properties for the diameters of the two daughter branches that arise at each branching point. A stochastic (Monte Carlo) simulation approach was used in order to mimic the variability observed in actual motoneuron dendrites.

Glossary

d_s	branch starting diameter (μm)
d_e	branch end diameter (μm)
d_0	diameter (μm) for first segment of stem branch of simulated dendrite
ΔL	length (μm) chosen for simulated segments
G	random variable with Gaussian distribution with mean μ and standard deviation σ
$l(d)$	length (μm) of a segment with diameter falling into diameter bin d
L_{br}	branch length (μm)
$N_{br}(d)$	observed number of segments with diameter in diameter bin d that branch
$N_{trm}(d)$	observed number of segments with diameter in diameter bin d that terminate
$P_{br}(d)$	observed branching probability for segments within a given diameter bin d
$P_{trm}(d)$	observed terminating probability for segments within a given diameter bin d
$P_{br}(d)$	calculated branching probability for segment with diameter d
$P_{trm}(d)$	calculated terminating probability for segment with diameter d
P	normalized parent branch start diameter (μm)
R	random variable ($0 \leq R \leq 1$) with flat distribution
S	coefficient of regression for sum of normalized daughter branch d , on normalized parent branch d

Results

Dendrite decomposition and model design

Figure 1 illustrates the basic features of 1974 dendritic branches belonging to 64 reconstructed dendrites from six gastrocnemius α -motoneurons (see Cullheim et al., 1987a). The main panels show the starting diameter (d_s , abscissae) and branch length (L_{br} , ordinates) for 1019 terminating and 955 parent branches. Both plots show considerable variance and are distinctly different in shape. In terminating branches, L_{br} values tended to increase linearly with d_s . The scatter pattern for parent branches is more complex, but both the average and maximum L_{br} values tended to decrease with d_s . Parent branches exhibited a wide range of d_s (0.8–18 μm), while the range of d_s for terminating branches was more limited (0.4–3.5 μm). A similar plot for pyramidal cell branches was presented by Hillman (1979, his Fig. 5). The shapes of these scatter plots suggest that the nature (i.e., parent or terminating) and length of dendritic branches are dependent on their starting diameters, with a direct dependence for terminations and more complex but basically inverse dependence for parent branches.

The inset in Figure 1 shows a histogram of the rate of taper for parent and terminating branches in the same sample, evaluated from a subset of branches for which at least two diameter measurements were obtained ($L_{br} \geq 200 \mu\text{m}$; total $n = 1036$).

The rate of branch taper was calculated as

$$\text{taper} = (d_e - d_s)/L_{br}, \quad (1)$$

where d_e is the final diameter at the point of branching or termination. The diameter of most branches tended to decrease [overall mean taper, -0.001 ± 0.001 ($\pm$ SD) $\mu\text{m}/\mu\text{m}$], although a substantial number showed no taper and a minority exhibited positive taper values (see also below).

In order to simulate the large variances in these distributions and the effect of branch taper, we simulated the data in Figure 1 using a stochastic growth process. An individual branch was envisioned to grow in discrete length increments, ΔL , with appropriate decrements in diameter depending on the selected taper rate. At each length increment, the fate of the branch (i.e., to end in either a branch point or a termination, or to continue growing) was determined stochastically using estimates of the probabilities of branching or terminating.

Estimation of branching and terminating probabilities

The probabilities of branching or terminating per unit length, P_{br} and P_{trm} , respectively, were estimated directly from observed morphological data. The original data were obtained by measuring motoneuronal dendrites as successions of cylindrical "segments," each with a constant diameter and a measured length (Cullheim et al., 1987a). Any given "branch" (delimited on either end by soma, branch points, or termination) in the original data set was represented as a sequence of one or (usually) more segments, most of them with lengths $\leq 200 \mu\text{m}$. The 1974 branches in the sample included more than 4000 such segments. The relations between d_i and L_{br} shown in Figure 1 suggested that P_{br} and P_{trm} might be functions of local branch diameter, d (i.e., the diameter of the i th segment). We initially assumed that these probabilities were independent of branch position within the growing dendritic tree (i.e., branch order, length, or distance from the somatic origin). Accordingly, we assembled all of the segment data in groups according to segment diameter bins, d , with bin widths that depended on the local density of data points (Fig. 1; bin widths: $0.25 \mu\text{m}$ for $d_i \leq 2.0 \mu\text{m}$, $0.5 \mu\text{m}$ for $2.0 > d_i \leq 4.0 \mu\text{m}$, and $1.0 \mu\text{m}$ for $d_i > 4.0 \mu\text{m}$).

Figure 2A shows a plot of the number of parent segments, $N_{br}(d)$, the number of terminating segments, $N_{trm}(d)$, and the total length of all segments in diameter bin d , $\Sigma l(d)$, on log-log coordinates (in order to display more clearly the important relations at small local diameters). Branches with d between 0.75 and $2.25 \mu\text{m}$ (denoted the "overlap region") either branched or terminated, and $N_{br}(d)$ and $N_{trm}(d)$ exhibited reciprocal changes while $\Sigma l(d)$ remained relatively constant.

The probabilities of branching or terminating per unit length for segments within a given bin d were estimated from the ratios of the number of branching or terminating segments in each bin divided by the total length of segments in the bins:

$$P_{br}(d) = N_{br}(d)/\Sigma l(d) \quad (2)$$

and

$$P_{trm}(d) = N_{trm}(d)/\Sigma l(d). \quad (3)$$

Figure 2B is a semilogarithmic plot of the probabilities derived from these data with $\Delta L = 25 \mu\text{m}$, which was used for most simulations, since it proved to be a good compromise between computational efficiency and length resolution. The log of the discrete values of $P_{trm}(d)$ varied inversely with d and were well fitted by a single exponential relation with negative slope. In

Figure 2. *A*, Observed data used for derivation of the probabilities of branching (P_{br}) and terminating (P_{trm}). The log-log graph shows the total length of dendritic segments (thick line; refer to right ordinate), and the numbers of parent (solid circles) and terminating segments (open circles; both refer to left ordinate) as functions of their diameters (abscissa; logarithmic scales used to clarify the details at small diameters). Note that segments with diameters between 0.8 and $2.2 \mu\text{m}$ (overlap region) could either branch or terminate. *B*, Semilogarithmic plot of the observed $P_{br}(d)\Delta L$ (open circles) and $P_{trm}(d)\Delta L$ (solid circles; $\Delta L = 25 \mu\text{m}$) derived from the data in *A* (see Eqs. 2, 3) as functions of local segment diameter (abscissa). The exponential functions $P_{br}(d)\Delta L$ and $P_{trm}(d)\Delta L$ fitted to these data are indicated by the lines drawn through the points. All had the form $P_x = k_1 \exp(k_2 d)$. Values for the parameters are given in Table 1. Note the discontinuity between the two exponentials fitted to P_{br} at $\approx 2.1 \mu\text{m}$.

Table 1. Summary of model 1 parameters for gastrocnemius α -motoneurons

Ending type	k1	k2
Parameters for branching or terminating probabilities		
Branching (overlap region)	2.58×10^{-5}	2.219
Branching (nonoverlap region)	2.34×10^{-3}	0.194
Terminating	2.62×10^{-2}	-2.955
Parameters for branch point R distribution		
μ_r		0.8255
σ_r		0.2125
a		-0.2087

The general form of probability equation was $P_x = k_1 \exp(k_2 d)$, where d is the local segment diameter. The units of k_1 and k_2 are μm^{-1} ; μ_r , σ_r , and a are dimensionless. P_{br} used for simulations was the minimum of the two exponential functions calculated using each set of branching parameters. In all models, the minimum allowable branch diameter was $0.25 \mu\text{m}$.

Branch Model Flow Chart

Figure 3. Flow chart of the stochastic process of branch growth by adding segments with length = ΔL . Input at the initiation of the process is the branch start diameter (d_s).

contrast, the log of $P_{br}(d)$ values varied *directly* with d but showed an abrupt discontinuity at $d \approx 2 \mu\text{m}$. The initial model simulations used exponential equations ($P_x = k_1 e(k_2 d)$; see Table 1 for parameters) fitted to the data for $P_{trm}(d)$ and the two parts of $P_{br}(d)$ to give continuous functions of local diameter, $P_{trm}(d)$ and $P_{br}(d)$. The simulation program calculated $P_{br}(d)$ values with *both* sets of parameters and used the *smaller* of the two results in order to deal with the discontinuity. The exponential slopes of $P_{br}(d)$ and $P_{trm}(d)$ with $d < 2.1 \mu\text{m}$ were similar but of opposite sign.

Branch simulation with diameter-dependent probabilities

For each branch, the stochastic growth process (Fig. 3) began with a cylindrical segment, $i = 0$, of selected start diameter d_s and length ΔL . A uniformly distributed random variable ($0 \leq R \leq 1$) was generated and compared with the probability of branching, $P_{br}(d_s)\Delta L$, calculated for d_s . The branch ended in a branch point if $R \leq P_{br}(d_s)\Delta L$. If this test was false, another value of R was generated and compared with the $P_{trm}(d_s)\Delta L$, and the segment was terminated if $R \leq P_{trm}(d_s)\Delta L$. If both tests proved to be false, another segment, $i + 1$, was added, branch length was incremented by ΔL , diameter was decremented by the selected taper rate, and the process was repeated until the branch ended in a branch point or a termination. Since P_{br} was always tested first, there was a slight bias in favor of branching,

Figure 4. Histograms showing the distributions of observed lengths (thick lines) and the mean branch lengths produced by simulations (symbols; see text and Eqs. 4–7) for parent and terminating branches with $d_s = 1.37 \mu\text{m}$. The observed data are for branches binned for $\pm 0.125 \mu\text{m}$ of the indicated d_s values. Simulations were run for three values of taper rate, indicated by the model taper data. The initial total number of branches was 222. Note the marked effect of taper rate choice, particularly in terminating branches.

which peaked sharply (to about 2% error) when $P_{br} \approx P_{trm}$ (at $d \approx 1.3 \mu\text{m}$; see Fig. 2). The length of the particular branch under assembly and its nature (i.e., parent or termination) were controlled entirely by the model parameters.

The branch simulation process proved to be quite sensitive to the choice of taper rate, which was poorly constrained by the observed data (Fig. 1, inset). In order to test this sensitivity, the probability equations derived as in Figure 2 were used with different rates of branch taper to generate histograms of simulated branch length for comparison with the observed motoneuron data. The observed data were lumped into the same d_s bins used for the probability calculations (see above). The equations for P_{br} and P_{trm} derived from the data (Fig. 2, Table 1) were used to calculate the *mean* numbers of branches with a given d_s that would be expected to have lengths falling into successive length bins with repeated iterations. The number of surviving branches, N_{surv} , was initially set to equal the *experimentally observed* total number of branches for each d_s bin, and the simulation d_s was set to the midpoint value for that bin. For

Figure 5. Plot of E_{RMS} (ordinate; see Eq. 8) calculated for branch length histograms as in Figure 4, simulated using various constant rates of taper (abscissa). Note that error was minimal for terminating branches with taper about $-0.00135 \mu\text{m}/\mu\text{m}$ while the minimum for parent branches was at the smallest taper rate tested ($-0.00025 \mu\text{m}/\mu\text{m}$). The total error for parent and terminating branches taken together was minimal with taper rate of $-0.00125 \mu\text{m}/\mu\text{m}$.

each iteration, $i = 1 \dots n$, the mean numbers of parent and terminating branches in successive ΔL length bins were calculated as

$$\overline{N}_{\text{br}_i} = N_{\text{surv}_i} P_{\text{br}}(d_i) \Delta L \quad (4)$$

and

$$\overline{N}_{\text{trm}_i} = N_{\text{surv}_i} P_{\text{trm}}(d_i) \Delta L, \quad (5)$$

where d_i was the diameter of current segment (see Fig. 3). Before the next iteration, the number of survivors and the next segment diameter d_{i+1} , were decremented,

$$N_{\text{surv}_{i+1}} = N_{\text{surv}_i} - \overline{N}_{\text{br}_i} - \overline{N}_{\text{trm}_i}, \quad (6)$$

$$d_{i+1} = d_i + \text{taper } \Delta L, \quad (7)$$

and the process was repeated until $N_{\text{surv}_{i+1}} \leq 0$. The length bin for the i th iteration was simply $i \cdot \Delta L$. Although the total number of branches for each d_i bin was fixed by the motoneuron data, the proportions of parent versus terminating branches and their length distributions depended entirely on the model parameters. The calculations were repeated with different values of constant taper centered on the observed mean of $-0.001 \mu\text{m}/\mu\text{m}$ (inset, Fig. 1). The resulting histograms were combined into bins of $100 \mu\text{m}$ length for display. The averages produced by repeated Monte Carlo simulations (as in Fig. 3) were comparable.

The comparison between data and model output was most important for d_s values within the overlap region (Fig. 2; $d_s < 3.5 \mu\text{m}$), which contained over 80% of the total sample and included branches that could either branch again or terminate (Fig. 1). Figure 4 illustrates representative histograms that compare the observed L_{br} (thick line) with simulations using $d_s = 1.37 \mu\text{m}$ (symbols). These histograms illustrate the sensitivity of the distribution of L_{br} to the choice of constant taper rate, which was particularly marked for terminating branches. Some taper rates generated proportions of parent versus terminating branches similar to the observed data (see model taper data, Fig. 4), but no single value of taper gave reasonable fits for every

Figure 6. Data base for the algorithm used to choose daughter branch diameters at branch points. *A*, Scatter plot showing the negative correlation between the normalized diameters of daughter branches ($D1$ and $D2$; see text) at 955 motoneuron branch points ($D2 = 0.927 - 0.40D1$; $r = 0.39$; $p < 0.001$, F test). *B*, Histogram of the r distribution calculated from observed motoneuron data (shaded bars; see text) compared with a computed Gaussian distribution with the same mean and SD (solid line; $\mu_r = 0.8255$; $\sigma_r = 0.2125$; see Table 1). See text for full explanation.

value of d_s . The length histograms of simulated parent branches fitted the observed data poorly for $d_s \leq 2.0 \mu\text{m}$ but with larger starting diameters there was increasingly good agreement, because the sensitivity to taper rate became less with the relatively short parent branches that had large values of d_s (see Fig. 1). Low rates of taper (e.g., $-0.0005 \mu\text{m}/\mu\text{m}$) produced poor fits for terminating branches with all values of d_s .

The influence of branch taper rate on the fit between observed and simulated branch length histograms was examined by calculating the root-mean-square (RMS) error of the deviations of the calculated length histograms from observed distributions for all d_s values from 0.375 to $8.5 \mu\text{m}$, which included more than 90% of the observed sample. The RMS error was calculated as

$$E_{\text{RMS}} = \sqrt{\frac{\sum (N_{\text{mdl}_{d,l}} - N_{\text{obs}_{d,l}})^2}{N_{\text{comp}}}}, \quad (8)$$

where $N_{\text{mdl}_{d,l}}$ and $N_{\text{obs}_{d,l}}$ are, respectively, the model and observed numbers in each diameter and length bin of the histograms, and N_{comp} is the number of entries in which $N_{\text{mdl}_{d,l}} > 0.1$ and $N_{\text{obs}_{d,l}} \geq 0$. Figure 5 shows that E_{RMS} was systematically larger for terminating branches (triangles) than for parents (open circles),

Figure 7. Log-log plots of characteristics of individual dendrites in the observed motoneuron data (dots) and the average values obtained for 20 runs of model 1 (open circles; see text). Motoneuron data are largely from Cullheim et al. (1987a; see text). **A**, Relation between stem dendrite diameter d_0 (abscissa) and the total membrane area A_d (ordinate) for 64 individual dendrites. Lines drawn through the points are least-square power function fits to the respective data sets (motoneurons: thinner line, $A_d = 3320 d_0^{1.32}$, $r = 0.59$; simulation: thicker line, $A_d = 986 d_0^{1.88}$, $r = 0.97$). **B**, Relation between d_0 (abscissa) and the number of dendritic terminations T_d (ordinate) for the same 64 real and simulated individual dendrites. Lines are power function fits to the respective data sets (motoneurons: thinner line, $T_d = 0.58 d_0^{1.56}$, $r = 0.67$; simulation: thicker line, $T_d = 0.44 d_0^{1.70}$, $r = 0.97$).

with total E_{RMS} (solid circles) intermediate between the two. Minimum total E_{RMS} for parent and terminating branches taken together occurred with taper rate around $-0.00125 \mu\text{m}/\mu\text{m}$.

The fact that minimum E_{RMS} was produced by different taper values for parent and terminating branches was consistent with the fact that the average taper for actual parent branches $\geq 200 \mu\text{m}$ in length was smaller ($-0.00087 \pm 0.0018 \mu\text{m}/\mu\text{m}$) than for terminating branches ($-0.00112 \pm 0.00076 \mu\text{m}/\mu\text{m}$; see inset in Fig. 1). Although this difference was statistically significant ($p < 0.05$, two-tailed t test), we elected for simplicity to retain a model design that used the same taper rate for all branches. In addition, the taper rates are particularly sensitive to the errors inherent in cell reconstruction with the light microscope.

Branch points: assigning simulated daughter branch diameters

In order to simulate complete dendritic trees, it was necessary to formulate a rule to generate d_s values for the daughter branches at branch points that match the statistical properties observed

in actual dendrites (e.g., a mean “ $D^{3/2}$ power ratio” $= \Sigma d_{\text{daughter}}^{3/2} / d_{\text{parent}}^{3/2} = 1.11 \pm 0.28$ SD for the six motoneurons; see Cullheim et al., 1987a). As noted above, we assumed that all branch points generate only two daughter branches (dichotomous branching). Analysis of the gastrocnemius motoneuron data set showed a significant negative correlation (regression coefficient $\beta[d1, d2] = -0.4$; $r = -0.38$; $p < 0.001$, F test) between the d_s of the two daughter branches, when normalized by d_e of the parent branch ($d1 = d_{s1}/d_{e\text{par}}$; $d2 = d_{s2}/d_{e\text{par}}$; Fig. 6A; see also Hillman, 1979). An algorithm was required that preserved this negative correlation and at the same time produced the observed distributions of $d1$ and $d2$. We should note here that $d1$ and $d2$ were not dependent on $d_{e\text{par}}$ or on location within the tree (i.e., branch order or distance from the soma).

Since the probability distributions of the two daughter diameters were the same, our approach to generating them was symmetrical. If two numbers, $r1$ and $r2$, are drawn independently from the same distribution, r , they can be linearly combined,

$$d1^* = r1 + a r2 \quad \text{and} \quad d2^* = r2 + a r1, \quad (9)$$

so that, when the constant a is negative, $d1^*$ and $d2^*$ exhibit a negative correlation coefficient with a value approximately twice that of a . As discussed in the Appendix, the empirical distribution, r , was obtained from the observed values of $d1$ and $d2$ as follows:

$$\begin{aligned} r1 &= (1/(1 - a^2))(d1 - a d2) \\ &\text{and} \\ r2 &= (1/(1 - a^2))(d2 - a d1). \end{aligned} \quad (10)$$

Because $d1$ and $d2$ have the same distributions, the values for $r1$ and $r2$ were combined into a single distribution, $r = r1 \cup r2$. The value of $a = -0.2087$ was obtained from Equation A9 (see Appendix) using the observed coefficient of correlation between $d1$ and $d2$ ($\beta[d1, d2] = -0.4$). As illustrated in Figure 6B, the empirical r distribution (bars) was simulated reasonably well by a Gaussian distribution, G , with the same mean, $\mu_r = 0.826$, and standard deviation, $\sigma_r = 0.213$ (solid curve), when constrained by the observed limits of the r distribution ($g_{\min} = r_{\min}$; $g_{\max} = r_{\max}$). At every simulated branch point, two numbers $r1^*$ and $r2^*$ were drawn at random from G and used as in Equation 9 to calculate normalized daughter diameters, $d1^*$ and $d2^*$, that had the same distributions as the observed branch point data. The required absolute values for the two daughter branch diameters, Diam1 and Diam2 , were then obtained by multiplying these normalized diameters by d_e of the simulated parent branch:

$$\text{Diam1} = d_{e\text{par}} d1^* \quad \text{and} \quad \text{Diam2} = d_{e\text{par}} d2^* \quad (11)$$

The Appendix contains a complete description of this approach.

Simulation of complete dendritic trees

The stochastic algorithms for generating dendritic branches given d_s (see Fig. 3) and selecting start diameters for daughter branches at branch points (Eqs. 9, 11) were combined into a Monte Carlo model that generated structural maps [essentially the dendrograms, including branching structure (encoded by a binary numbering system for each branch; see Cullheim et al., 1987a)] and the lengths and diameters of all branches of the complete dendritic tree. Simulation of each dendrite began by specifying a stem diameter, d_0 , equal to a initial stem branch diameter for a dendrite in the original motoneuron data. In the

Model 1

Figure 8. Further comparison of observed and simulated dendrite properties for model 1 simulations. *A* and *B* show, respectively, the aggregate branch order (*abscissae*) distributions of the numbers and average end diameters, d_e (*ordinates*), for all branches of 64 real (*lines*) and simulated (*symbols*) dendrites. The data for observed motoneuron parent and terminating branches are denoted by *solid* and *broken* lines, respectively. Results for the simulation run are indicated by symbols (*open circles*, parents; *solid diamonds*, terminations). *C* and *D* are similar, except that the *abscissae* denote path distance from the soma, with data collected in 100 μm bins. Note that the distributions of parent and termination path distances for simulated dendrites were broader and less peaked than those for actual motoneurons.

model, as in the original motoneuron data (see Cullheim et al., 1987a), each component branch was identified by integers that specified its branch order and identification number. Termination of the last growing branch signaled completion of the dendrite under construction, whereupon the program automatically proceeded to build the next, until all 64 were simulated. The program also calculated a variety of measurements for individual dendrites and of aggregate statistics for the entire set.

Model 1: diameter-dependent probabilities

The initial model (model 1) used the exponential probability curves illustrated in Figure 2*B* together with the branch point algorithm discussed above (fixed parameters are given in Table 1). The only free parameter was rate of taper, which was constant for any given model run but was adjusted to produce the same *total* number of parent and terminating branches (within 1%) as observed in the 64 actual dendrites ($n = 1974$), when averaged for 20 repetitions. Averaging of multiple model runs was done in order to clarify the central tendencies for a given set of input

parameters. Individual model runs produced variability equivalent to that seen in the experimental data.

When the observed mean taper rate of $-0.001 \mu\text{m}/\mu\text{m}$ was used (Fig. 1, inset), 20 simulation runs consistently produced >2000 total branches. The taper rate had to be increased to $-0.0015 \mu\text{m}/\mu\text{m}$ to get reasonably consistent numbers near the target total of 1974, but the average number of branches produced by the 20 model runs continued to fluctuate between about 1935 and 2075. The plots in Figures 7 and 8 illustrate comparisons between actual and simulated dendrites from 20 model repetitions in which the average branch total was 1978. Observed and simulated individual dendrites exhibited clear correlations between the stem diameter, d_0 , and the summed area of individual dendrites (A_d ; Fig. 7*A*), as well as with the number of terminations for each dendrite (T_d ; Fig. 7*B*; see Cullheim et al., 1987a, their Fig. 2). Both correlations were best fitted by power functions ($y = ad_0^b$) that were somewhat different (see Fig. 7 caption) and individual simulation runs exhibited greater scatter than the observed data (the average residual vari-

Figure 9. Data base for the “grandparent correction” for simulated daughter diameters at branch points (see text for full explanation). *A*, Scatter plot showing a weak but statistically significant ($p < 0.01$, F test) negative correlation between the sum of normalized daughter diameters, $d1$ and $d2$ (ordinate), and the normalized starting diameter P of the parent branch (abscissa; see inset diagram). *B*, The thick line shows the regression line from *A*, replotted using the “grandparent correction factor,” $1 + (P - \mu_P)$, as the ordinate scale. The correction to be applied to $d1$ and $d2$ at simulated branch points is determined by P for the parent branch and is denoted by the shaded areas (see text).

ance for the relation between d_0 and A_d from 23 runs of model 1 was about 38% larger than that of the observed data).

Plots of the numbers of parent and terminating branches as functions of branch order (Fig. 8*A*) showed reasonable agreement but, in both cases, the simulated data were more skewed, with larger than expected numbers with orders ≥ 8 . The average d_e values of simulated parent branches were 10–15% larger than expected at branch orders ≥ 3 , but the d_e values of terminating branches were quite close to the expected values for all orders (Fig. 8*B*). The greatest discrepancy from observed data for model 1 simulations was found in the spatial distributions of branch points and terminations as functions of path distance from the soma (Fig. 8*C*). The path distance distribution of simulated branch points was broader than in actual dendrites and had a peak closer to the soma. In contrast, the spatial distribution of terminations peaked at about the same point as in the observed data but was broader in both proximal and distal directions (note that the total numbers of branches making up the curves were nearly identical). The spatial distributions of simulated d_e values (Fig. 8*D*) showed reasonable agreement with observed values for both parent and terminating branches.

Modifications to the model

Given the complexity of motoneuron dendrite morphology and the relative simplicity of model 1 (notably, the fact that P_{br} and P_{trm} depended only on local branch diameter; see Table 1), the degree of fit between the simulated and observed data shown in Figures 7 and 8 was, in many respects, surprisingly good. Model 1 can be viewed as a concise description of gastrocnemius motoneuron dendrites that, while not satisfactory in all details, nevertheless contains considerable information about dendritic morphology. However, the discrepancies clearly indicated that model 1 lacked features that were important to constrain the spatial distributions of branch points and terminations along the paths from the soma.

An additional problem, not evident in the averaged data shown in Figures 7 and 8, was a tendency for individual dendrites produced by some model runs to be either much larger or much smaller than expected on the basis of their d_0 values. Upon detailed examination of the dendritic paths in such dendrites, we noted a tendency, not found in real dendrites, for relatively large or small daughter diameters to occur at successive branch points. This occurred because model 1 had no “memory” of past choices for Diam1 and Diam2, which depended only on randomized draws from the G distribution (Fig. 6) and d_e of the parent branch (Eq. 11). When the actual motoneuron data was reexamined for possible serial dependence of daughter branch diameters at successive branch points, we found a weak but significant dependence between the sum of the normalized daughter diameters at a given branch point (Σd_{dau}) and the normalized starting diameter, P , of the parent branch ($P = d_{spar}/d_{egpar}$) that gave rise to that branch point (i.e., the “grandparent” branch point; Fig. 9*A*). This dependence on the history of branch diameters in dendritic paths presumably reflects some global (i.e., metabolic or cytoskeletal) constraint on the amount of material that can be sustained in actual dendritic trees (see Discussion).

We implemented a correction to deal with this problem by adjusting Diam1 and Diam2 (Eq. 11) by comparing the value of P (defined above) for the branch point under consideration to the mean value, μ_P , of P for all actual branch points:

$$\text{Diam1}^* = \text{Diam1}(1 + S(P - \mu_P)) \quad \text{and} \quad (12)$$

$$\text{Diam2}^* = \text{Diam2}(1 + S(P - \mu_P)),$$

where S is the regression coefficient (slope) of the relation in Figure 9*A* (Table 2). Because S was negative, values of $P > \mu_P$ produced decrements in Diam1*, and vice versa. We will refer to this as the “grandparent correction.” The graph in Figure 9*B* illustrates the magnitude and polarity of the correction factor (shaded areas), which was less than $\pm 10\%$ for most branch points. When introduced into the basic model, this relatively small correction considerably moderated the model’s tendency to produce “runaway” large or small dendrites and reduced the variability in total branch numbers between model runs. For the relation between d_0 and A_d (e.g., Fig. 11*A*), the average residual variance for 23 runs of model 2 without the “grandparent correction” was about 12% larger than in the observed data, while with the correction, it was about 9% less.

A variety of other approaches were examined to deal with the discrepancies noted in connection with Figures 7 and 8. These included various manipulations of taper rate and the use

of calculated P_{br} and P_{trm} functions that depended not only on local diameter but also on order of branching or on the length of individual dendritic branches. Both of these additional dependencies were in fact found in the original motoneuron data. Probabilities dependent on both diameter and branch length improved the fits for branch length histograms, as in Figure 4, but *none* of these modifications significantly improved the fits illustrated in Figures 7 and 8. We concluded that, although these dependencies exist in actual motoneuron dendrites, they are epiphenomena produced by another factor not yet included in the model.

Model 2: probabilities that depend on both local diameter and path distance

Experience with variations of model 1 suggested that the problem evident in Figure 8C could be resolved only if both P_{br} and P_{trm} varied systematically with path distance from the soma. The original data were reexamined from this point of view, and significant differences were indeed found in the probability curves obtained when the numbers of branch points and terminations, and the total length of dendritic material (see Eqs. 2, 3) were binned by path distance, p , as well as local diameter, d , into a two-dimensional matrix. The resulting matrices gave the probabilities of branching or terminating as functions of both local diameter and path distance:

$$P_{br}(d, p) = N_{br,d,p}(\Sigma l_{d,p})^{-1} \quad (13)$$

and

$$P_{trm}(d, p) = N_{trm,d,p}(\Sigma l_{d,p})^{-1}. \quad (14)$$

When plotted against local diameter, d , for a given path distance bin, p , the probability values $P_x(d, p)$ exhibited exponential dependence on local diameter, as in Figure 3B. That is,

$$P_x(d, p) = A(p)\exp[B(p) d]. \quad (15)$$

In addition, some of the coefficients, A , and slopes, B , of the fitted equations also varied systematically with path distance, p . Over the observed range of path distance bins p , $A(p)$ and $B(p)$ were well fitted by linear functions of path distance: $A(p) = a1 + a2 p$ and $B(p) = b1 + b2 p$. Given linear dependence of A and B on path distance and the observed parameters (Table 2), P_x was calculated as a continuous function of local path distance, p , and diameter, d :

$$P_x(d, p) = (a1 + a2 p)\exp[(b1 + b2 p)d] \quad (16)$$

The parameters A and B for P_{trm} both showed a linear dependence on path distance. Therefore, the best-fitted equation to the observed $P_{trm}(d, p)$ exhibited a complex curvature with increasing path distance on a three-dimensional semilog graph (Fig. 10A). For a given local diameter, $P_{trm}(d, p)$ increased with increasing path distance up to about 1600 μm from the soma, beyond which there was some decline.

The situation with $P_{br}(d, p)$ was more complex because, as with the simple diameter-dependent probabilities of branching (Fig. 2B), two intersecting surfaces (Fig. 10B) were required to fit $P_{br}(d, p)$. The $b2$ coefficients of Equation 16 did not exhibit any significant dependence on path distance in either the small- or large-diameter regions of the data (i.e., $b2 \cong 0$ in both regions; see Table 2). However, the other coefficients ($a1$, $a2$, and $b1$) did vary significantly with path distance, and these account for the curvature of the $P_{br}(d, p)$ surfaces shown in Figure 10B. As in model 1, both $P_{br}(d, p)$ equations were calculated for each

Table 2. Summary of model 2 parameters

Constant	Value	r	p
Branching probabilities			
Overlap region—small diameters			
a1	1.28×10^{-4}	0.74	<0.01
a2	-9.24×10^{-8}		
b1	1.8175	0.18	NS
b2	0		
Nonoverlap region—large diameters			
a1	2.41×10^{-4}	0.90	<0.01
a2	2.65×10^{-6}		
b1	0.341	0.13	NS
b2	0		
Terminating probabilities			
a1	9.90×10^{-2}	0.81	<0.01
a2	-4.76×10^{-5}		
b1	-6.102	0.90	<0.01
b2	2.83×10^{-3}		
Parameters for branch point $\mathbf{r}$ distribution			
μ_r	0.8255		
σ_r	0.2125		
a	-0.2087		
Parameters for grandparent correction			
S	-0.198		
μ_p	0.73		

The general form of probability equation was $P_x = (a1 + a2 p) \exp[(b1 + b2 p) d]$, where p = path distance from the soma and d = local segment diameter. The units were, for **a1** and **b1**, μm^{-1} , and **a2** and **b2**, μm^{-2} ; μ_r , σ_r , **a**, **S**, and μ_p are dimensionless. The values under r are the linear coefficients of correlation for the equations **a1** + **a2** p and **b1** + **b2** p ; values under p are the probabilities that the slopes of the correlations are zero. The minimum allowable branch diameter was 0.25 μm .

growing segment and the *smaller* value of the two was used for comparison with random numbers.

The shading on the surfaces shown in Figure 10 denotes regions that were defined by the observed motoneuron data. As would be expected, the simulations involved probability calculations in the same regions of the probability spaces that were defined by the observed data. The introduction of distance dependence into the model doubled the number of parameters needed to define the required probabilities from 6 to 12 (see Table 2). For comparison, the thick lines in Figure 10 denote the loci of the diameter-only-dependent probabilities (cf. Fig. 2B).

Figures 11 and 12 illustrate the averaged results of 20 repetitions of what will be referred to as model 2, which includes the grandparent correction factor in the calculation of daughter branch diameters at branching points (Fig. 9, Eq. 12) and the path distance- and diameter-dependent probabilities illustrated in Figure 10. The relations between d_0 and A_d (Fig. 11A), and T_d (Fig. 11B) for individual dendrites were quite similar to the results with model 1 (cf. Fig. 7).

However, the fits between model and observed branch-order distributions were improved in the region of the large-order tails (Fig. 12A). Even more striking, however, was the improvement in fit for the path distance distributions of both branch points and terminations (Fig. 12C), which were very close to those observed in the actual motoneuron data. The average d_c for parent and terminating branches, as functions of branch order (Fig. 12B) and of path distance (Fig. 12D), were also well matched with observed data. The illustrated simulation used a constant

Figure 10. Three-dimensional graphs illustrating surfaces that reflect the dependence of P_{br} and P_{irm} on both local diameter and path distance from the soma. The equations that were fitted to observed motoneuron data to estimate $P_{irm}(d, p)25$ (A) and $P_{br}(d, p)25$ (B; $\Delta L = 25 \mu\text{m}$) are given in the text (Eqs. 13–16), and the parameters are in Table 2. Shading indicates regions where motoneuron data existed (density of shading denotes relative concentrations). The thick lines denote the loci of the diameter-dependent probabilities $P_{irm}(d)25$ and $P_{br}(d)25$, as in Figure 2.

taper rate of $-0.00125 \mu\text{m}/\mu\text{m}$, which was closer to the observed overall mean value of -0.001 (Fig. 1, inset) than the rate ($-0.0015 \mu\text{m}/\mu\text{m}$) that had to be used in model 1 in order to obtain total branch numbers equal to those observed experimentally. The possible significance of the remaining small discrepancies with model 2 will be taken up in the Discussion.

Discussion

Mathematical models of tree structures have been used in biology to examine the structure of botanical trees and modular organisms like bryozoans (see Waller and Steingraeber, 1985, for review) and in geology to describe river systems (Leopold, 1971; Wittmann et al., 1991). Quantitative models of botanical trees have progressed beyond simple description (e.g., Honda, 1971) to formulations that attempt to interrelate structural growth rules with costs versus benefits of photosynthetic biomass production (Ford et al., 1990), in order to investigate the ways in which observed tree structures are optimized for the tasks that they perform (see Wittman et al., 1991). Such studies show the advantages to be gained by describing a complex entity, represented in large amounts of raw data, using a “machine” (algorithms and associated parameters) that will reproduce the essential features of the original data. The complexity of the original entity can be formally measured by the length of the machine’s description (see Rissanen, 1990).

There has been some interest among neurobiologists in analytical models of neuronal dendrite morphology. These have dealt primarily with the topology of branching (Smit and Uylings, 1975; Percheron, 1982; Berry and Flinn, 1984; Verwer and van Pelt, 1986) or analysis of dendritic trees as fractal objects (Smith et al., 1989). The increasing abundance of quantitative data about neuronal dendrites offers the opportunity to go beyond simple description to pose quantitative questions about the factors that control neuronal shapes (Hillman, 1979, 1988)

and to investigate whether the observed morphologies represent optimal compromises between, for example, synaptic connectivity and electrotonic current flow versus cytoskeletal and metabolic maintenance constraints.

The present morphological model defines a parsimonious set of data-derived parameters and a simple stochastic growth process that results in computed structures with statistical properties that closely resemble those of actual α -motoneuron dendrites. All of the relevant factors are assumed to be intrinsic to the motoneurons themselves. It should be noted that this model was designed *not* to describe the way in which actual motoneuron dendrites grow but rather as a tool to describe their characteristics in fully formed, adult neurons. The defining feature of model 1 is that the parameters depend only on *local* branch diameter. Despite its simplicity, model 1 was successful in producing simulated dendritic trees with many of the properties of actual motoneuron dendrites (Figs. 7, 8).

Nevertheless, the discrepancies noted in Figure 8 forced us to include additional factors that reflect more global dependencies: (1) the “grandparent correction” (Fig. 9) that modifies daughter branch diameters at successive branching points, and (2) the dependence of the P_{br} and P_{irm} on distance from the soma, as well as local diameter (Fig. 10). The spatial distributions of branch points and terminations produced by model 2 were very close to those observed in actual motoneuron dendrites (Fig. 12C), but the global characteristics of simulated dendrites were almost the same as produced by model 1 (compare Figs. 7, 11). Both models strongly suggest that the dependence of P_{br} and P_{irm} on local branch diameter (Fig. 3) is a critical factor in describing dendritic morphology.

Neurons, measurements and models

Before discussing the present results in detail, it is important to recognize that our approach began with measurements with

many inherent technical problems. These include tissue shrinkage during histological preparation, the limited resolution of light microscopy, the need to fractionate a continuous object into discrete pieces, and the possibility that HRP labeling induces morphological artifacts. These problems necessarily influence the conclusions that can be drawn about living neurons from cell reconstructions made using light microscopy. However, they do *not* negate the fact that the present model system successfully encapsulates the original measurements *per se*; rather, they affect the next step, which is to relate the measurements to the actual morphology of α -motoneurons. The magnitudes of the individual sources of error are relatively small, although perhaps not negligible in aggregate. The following discussion should be read with these caveats in mind. Nevertheless, our approach appears to be sufficiently robust that it would be equally applicable when "better" data become available.

Possible significance of parameter dependencies

The striking dependence of P_{br} and P_{tm} on local branch diameter, which reflects cytoplasmic cross-sectional area of the branch, suggests that the local cytoskeleton within a given branch probably plays an important role in constraining whether it can give rise to a branch point or only terminate. Hillman (1979, 1988) has discussed evidence that the number of microtubules in branches of different diameters controls their eventual fate. The steep reciprocal changes in the two probabilities in the region of diameter overlap (Figs. 3, 10) are consistent with this idea but suggest that there is no single transitional diameter that eliminates further branching. Rather, there seems to be a range of diameters with a lower limit (about 0.8 μm) below which α -motoneuron dendrites never branch and above which (about 2.5 μm) they always branch again. Ultrastructural studies of α -motoneuron dendrites within this diameter range may provide clues to the factors that produce this observation.

In α -motoneurons, the observed rates of branch tapering contribute relatively little to loss of branch diameter with distance from the soma (on average, about 0.1 $\mu\text{m}/100 \mu\text{m}$ length; Fig. 1, inset). Dendritic branching is the major factor that produces the precipitous decline in average branch diameter with path distance or branching order in actual and simulated dendrites (Figs. 8, 12). In the present sample of real dendrites, the average diameter of each daughter branch was about 0.67 that of the parent end diameter, giving a summated cross-sectional area for both daughter branches that is about 10% less than that of the parent. Ultrastructural studies of the fate of cytoskeletal components at branch points will be important to elucidating this loss in cross-sectional area at branch points (see Hillman, 1988), particularly if a method can be devised to relate the findings to distance from the soma. It should also be noted that the size and location of organelles (e.g., mitochondria) and other cytoplasmic elements are also important in determining dendritic diameter and volume (Stevens et al., 1989), and these may also vary with distance from the soma.

The "grandparent correction" and the dependence of probabilities on path distance needed in model 2 suggest that local factors related to branch diameter cannot alone explain dendritic morphology. It seems reasonable to postulate the existence of *global* cytoskeletal and perhaps metabolic constraints that also act to limit the total size to which any dendrite can grow and be maintained (Figs. 7, 12; see also Ulfhake and Kellerth, 1981, 1983; Cullheim et al., 1987a). We should note that the parameter dependencies in the present simulation are probably not in

Figure 11. Comparison as in Figure 7 of the correlations between d_0 and A_d (A) and T_d (B) for individual dendrites produced by model 2 simulations (large open circles; average of 20 simulation runs) and observed motoneuron dendrites (dots). Power function regressions denoted by thicker and thinner lines, respectively. The fitted equations for model 2 simulations were similar to those for model 1 (cf. Fig. 7 caption; $A_d = 1244 d_0^{1.81}$, $r = 0.98$; $T_d = 0.39 d_0^{1.76}$, $r = 0.98$).

themselves "causal" in any biological sense but rather result from, and therefore reflect, the operation of underlying biological factors that remain to be defined. One utility of morphological modeling is to identify the existence of such factors and to suggest how they might be studied.

Residual discrepancies and alternative models

The existence of constraining factors not included in the model probably also explains the residual discrepancies in model 2 simulations. For example, some of the actual motoneuron dendrites with relatively large stem diameters ($d_0 > 8 \mu\text{m}$) had considerably less membrane area than predicted by the model. These largely account for the disparities between power function fits between d_0 and dendritic area that are evident in Figures 7A and 11A. The operation of additional global constraints, unaccounted for in *either* model 1 or 2, on total dendritic size in actual motoneurons is an obvious candidate to explain this discrepancy in both models.

The other persistent discrepancy in our simulations was the larger-than-expected average diameters for simulated parent branches with branch orders ≥ 3 (Figs. 7B, 12B). This result was sensitive to taper rate and disappeared as the value of branch taper increased, although the simulations were in other respects unsatisfactory. As noted in the Results, the average rates of

Model 2

Figure 12. Distributions as in Figure 8 of aggregate numbers (*A* and *C*) and average d_e (*B* and *D*) for parent and terminating branches as functions of branch order (*A* and *B*) and path distance (*C* and *D*). Simulation results were averaged for 20 runs of model 2 (unconnected symbols), compared with observed motoneuron data (lines). These fits were considerably better than those for model 1 (cf. Fig. 8).

branch taper in the motoneuron data were higher for terminating than for parent branches (Fig. 1, inset) and taper also depends to some extent on the initial branch diameter (see Moschovakis et al., 1991, their Fig. 12). We have not reported on models constructed with these additional complexities because we felt that restricting the simulation to a single adjustable parameter (i.e., constant taper rate) was the greatest strength of the present formulation.

It is possible to envision alternative model designs that would take global constraints more directly into account. For example, analysis of the gastrocnemius motoneuron data showed that the membrane area distal to any given parent branch was related to the approximate square of the parent end diameter (distal area = $904 d_{e,par}^{1.97}$; $r = 0.86$; not illustrated, but see Moschovakis et al., 1991, their Fig. 13). Examination of this relation in model 2 simulations also showed exponents near 2. It might be possible to incorporate this factor in future simulations, perhaps to limit the range of daughter branch diameters at branch points in a manner analogous to the "grandparent correction" in the present model. Given the relative success of the present approach,

we have not implemented such an alternative, but it could, at least in principle, be used to evaluate the possible influence of dendritic transport rates of proteins and other metabolites, if such data can be developed for morphologically characterized cell types. There has been considerable attention to such global factors in quantitative models of botanical trees (e.g., Leopold, 1971; Ford et al., 1990), but their nature in neurons remains speculative. Suffice it to say that variations of the present model system can be used to explore the existence of features of neuronal growth and maintenance that are not at all obvious in an examination of raw data on the morphology of neurons.

Conclusions

To a large extent, the system described in this article represents an exercise in data analysis that is directed by model design and validated by model output. The present model formulation is not an end product but rather a developmental stage that raises new questions and suggests how they may be answered. One potentially fruitful avenue of further exploration would be to compare the morphologies of different neuron types using the

present system, in order to isolate the factors that appear to explain different dendritic morphologies (see Moschovakis et al., 1991). The present model design allows quantitative comparisons between dendrites with very different starting diameters and thus appears to be useful in making structural comparisons between neurons that are independent of their absolute size.

Appendix

Simulation of complete dendritic trees requires choosing two normalized starting diameters, $\delta 1_j$ and $\delta 2_j$, for the daughter branches at each branch point j . Let $\delta 1$ and $\delta 2$ represent the set $\delta 1 = \{\delta 1_j\}$ and $\delta 2 = \{\delta 2_j\}$. We required that the mean and variance of $\delta 1$ and $\delta 2$ equal the mean and variance of the combined values for the observed normalized diameters, $d1$ and $d2$:

$$\mu(\delta 1) = \mu(\delta 2) = \mu(d1 \cup d2) \quad \text{and} \quad (A1)$$

$$\text{VAR}(\delta 1) = \text{VAR}(\delta 2) = \text{VAR}(d1 \cup d2).$$

The coefficients of regression for the two sets of ordered pairs must also be the same:

$$\beta'(\delta 1, \delta 2) = \beta(d1, d2). \quad (A2)$$

In order to satisfy Equation A2, we note that, if one draws two numbers, $r1_j$ and $r2_j$, independently from some distribution, r , a simple pair of linear combinations will produce a new pair of numbers with a regression coefficient that is controlled by a constant, a :

$$\delta 1_j = r1_j + a r2_j \quad \text{and} \quad \delta 2_j = a r1_j + r2_j. \quad (A3)$$

Rewriting these equations in matrix form gives

$$\delta = [\delta 1, \delta 2] = [r1, r2] \begin{bmatrix} 1 & a \\ a & 1 \end{bmatrix} = rA. \quad (A4)$$

The constant a is derived from the regression coefficient, β' , of the observed $d1$, $d2$ distribution as follows. By definition,

$$\beta' = \frac{D1 \cdot D2}{D1 \cdot D1}, \quad (A5)$$

where $D1 = \delta 1 - \mu(\delta 1)$, $D2 = \delta 2 - \mu(\delta 2)$, and the operator " $\cdot$ " denotes the dot product of the vectors (i.e., $x \cdot y = \sum_{j=1}^n x_j y_j$). With the identities in Equation A3, Equation A5 can be rewritten as

$$\beta' = \frac{(R1 + aR2) \cdot (R2 + aR1)}{(R1 + aR2) \cdot (R1 + aR2)}, \quad (A6)$$

where $R1 = r1 - \mu(r1)$ and $R2 = r2 - \mu(r2)$. Because the individual values of $r1_j$ and $r2_j$ are drawn independently from the same distribution, we have

$$R1 \cdot R2 = 0 \quad \text{and} \quad R1 \cdot R1 = R2 \cdot R2 = R \cdot R, \quad (A7)$$

which reduces Equation A6 to

$$\beta' = \frac{2aR \cdot R}{(1 + a^2)(R \cdot R)} = \frac{2a}{1 + a^2}. \quad (A8)$$

The value of a is obtained by setting β' to the observed $\beta = -0.4$ to satisfy Equation A2, and rearranging Equation A8 using the well-known quadratic formula to give

$$a = \frac{1}{\beta} + \sqrt{\frac{1}{\beta^2} - 1} = -0.2087. \quad (A9)$$

In order to satisfy Equation A1, we obtain the distribution r with Equation A4, using the observed distribution, d , instead of δ , where d is the set of ordered pairs, $d1_j$ and $d2_j$, with values randomly shuffled in pairwise manner to remove any bias introduced during cell reconstruction. Multiplication of d by the inverse of matrix A produces the required two vectors, $r1_j$ and $r2_j$ (denoted r):

$$r = [r1, r2] = \frac{1}{(1 - a^2)} \begin{bmatrix} 1 & -a \\ -a & 1 \end{bmatrix} [d1, d2] = A^{-1}d \quad (A10)$$

Combining Equations A4 and A10 gives, in simplified form,

$$\delta = rA = dA^{-1}A = d, \quad (A11)$$

where δ and d represent the ordered pairs of vectors for simulated and observed normalized daughter diameters, respectively. In the simulation program, $\{r1_j, r2_j\}$ were actually drawn from a smooth Gaussian distribution with the same mean and variance as the empirical distribution of $A^{-1}d$ (see Fig. 6B). Equations A10 and A11 imply that the distributions of $\delta 1$ and $\delta 2$ will have the same statistical properties as those of the original $d1$ and $d2$, thus satisfying Equation A1. This fact was verified in the simulation runs.

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