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# Parsimonious modelling allows generation of the dendrograms of primate striatal medium spiny and pallidal type II neurons using a stochastic algorithm.

Patrick Mouchet[a], Jérôme Yelnik[b].
[a]Grenoble Institut des Neurosciences, Université Joseph Fourier Grenoble, France
[b]INSERM U679, Hôpital de la Salpêtrière, Paris, France

26 pages
7 figures
3 tables

Corresponding author.
Patrick Mouchet,
Grenoble Institut des Neurosciences,
Bâtiment Edmond J. Safra,
Chemin Fortuné Ferrini,
Université Joseph Fourier Site Santé,
BP170 38042 Grenoble Cedex 9
France
Tel: (33)0456520651
e-mail: Patrick.Mouchet@ujf-grenoble.fr

ABSTRACT

Data from quantitative three-dimensional analysis of primate striatal medium spiny neurons (MSNs) and pallidal type I and type II neurons were used to search for possible rules underlying the dendritic architecture of these cells. Branching and terminating probabilities per unit length of dendrite were computed from all available measurement points. In the three neuronal groups, terminating probabilities were found to be exponentially increasing functions of the path distance to soma. MSNs and type II branching probabilities could be accurately modelled with decreasing functions of both the metrical (exponential functions) and topological (power functions of the centrifugal branch order) distances to soma. Additionally, type II branching also slightly depended on the distance to the proximal tip of the supporting branches. Type I branching probabilities did not follow these rules accurately. Embedding the modelled probability functions in a stochastic algorithm allowed generation of dendrograms close to those of the real MSNs and pallidal type II neurons, while the algorithm failed to simulate type I dendrites. MSN and pallidal type II neuron branching and terminating probabilities are thus highly dependent on the position in the dendritic arbor. This relationship can be modelled with simple functions and has a strong incidence on the dendrogram structure of the cells concerned. The additional dependence of the branching probability on the within-branch position led us to propose an extension of a previous modelling study by Nowakowski and co-workers which could account for a large range of topological and metrical (length) dendritic tree structures.

Section 9: Computational and Theoretical Neurosciences

Key words: Basal Ganglia, Parsimonious modelling, Quantitative morphology, Dendrogram structure, Stochastic algorithm.

## 1.Introduction

Neuronal dendrites display an astonishing diversity in shape. This part of the nerve cells is important for several reasons. Firstly, it strongly influences the information processing performed by the cell, though how this influence is exercised is still debated. Secondly, the shape of dendritic arborisations can be used to classify neurons in a functionally meaningful way. Finally, some pathological states are well correlated with changes in dendritic morphology (Klapstein et al., 2001). For all these reasons, quantitative analysis of dendritic arbors could be useful and may additionally, through testable models, help to gain insight into the possible rules and mechanisms involved in the growth and maintenance of these structures. In this perspective, we present a model derived from the experimental data one of the authors obtained in a detailed quantitative study of the main primate striatal and pallidal neurons (Yelnik et al.,1984; Yelnik et al.1991).

The striatal cells were medium spiny neurons (MSNs), which represent the bulk of caudate and putamen nerve cells. Pallidal cells belonged to the large pallidal neurons, which constitute the main cell type in both the external (GPe) and internal (GPi) globus pallidus. They were grouped in either type I or type II neurons according to their average branch length (Ln). Indeed, it has been shown that this parameter is the main distinctive feature of these neurons, irrespective of the species (human versus monkey), anatomical location (GPe versus GPi) or tissue processing (Golgi staining versus biocytin labelling).

While several other statistical modelling studies of dendrites addressed the topological tree structure (Carriquiry et al.,1991; Devaud et al.,2000; Dityatev et al.,1995; Van Pelt et al.,1997), we have chosen an approach also dealing with metrical parameters of the three neuronal types. However, not all metrical aspects were taken into account because we did not consider diameter-linked parameters or those characterizing the dendritic spatial occupancy, which has been extensively described for these cells (François et al.,1984; Yelnik et al., 1984; Yelnik et al., 1991).

The exhaustive 3D quantitative analysis performed on the neurons of the three groups provided raw data from which we computed branching and terminating probabilities per unit length according to the method described by Burke et al. (1992). The dependence of these probabilities on the location in the dendritic arbor was then modelled with simple functions. To check whether these models could predict the corresponding dendrogram structure we used a stochastic algorithm also described by Burke et al. (1992). Trees generated with this method were compared to the real ones according to a large set of topological and metrical length parameters which served as emergent parameters (Ascoli and Krichmar, 2000; Ascoli et al., 2001).

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[1] Abbreviations

As : tree asymmetry index ; D : tree degre ; GPe : external globus pallidus ; GPi: internal globus pallidus ; Hm : maximal branch order ; L : total dendritic length ; Lm : average parent branch length ; Ln : average branch length ; Lp : average terminal branch length ; MSN : medium spiny neuron ; P : average path distance to soma of all tree tips ; Q : average branch order; q:centrifugal branch order; x: path distance to soma; z: path distance between a point within a branch and the proximal tip of the branch.

## 2.Results.

### 2.1. Estimation and modelling of the branching and terminating probabilities per unit length.

Since neurons of the three groups exhibited a striking decrease of branching as path distances to soma increased (Fig.1), we computed the branching probabilities per unit length at increasing distances from the cell body. Being interested in real, finite trees, we also computed the terminating probabilities per unit length with respect to the same variable, which served as a basic parameter (Ascoli et al., 2001). As expected from the data, the dendrites of the three neuronal groups indeed showed a sharp decrease in branching probability at increasing distances from the soma (Fig. 2), but in pallidal type I neurons the decrease was less regular, and the branching probability displayed a reincrease starting about 400 μm from the soma. In the three neuronal groups the terminating probability per unit length showed a regular increase with the path distance to soma. However, MSNs differed from the neurons of the two pallidal types in that there was almost no overlap between the two probabilities, while for pallidal type II neurons there was a large range (between 200 and 500 μm) of path distances to soma where branch termination occurred as well as branching; for type I neurons the overlap was even greater due to the branching of distal parts of dendrites.
Searching for simple relationships between the topological probabilities per unit length and the path distance to soma, we found good to very good fits with exponential functions with, as expected, an exception concerning the branching probability of pallidal type I neurons for which the fit was poor. Denoting x the path distance to soma, the branching probability per unit length could be satisfactorily modelled with

$$p_{br}(x)=k_b\exp(-\alpha x),\ (k_b,\alpha>0)$$

for MSNs and pallidal type II neurons.
For the terminating probability, $p_{tm}$ , there was a good fit with

$$p_{tm}(x)=k_t(\exp(ax)-1),\ (k_t,a>0)$$

for the three groups.These equations were the basis of our modelling and constitute what we refer to as model 1.

### 2.2 Algorithmic generation of dendrites according to model 1.

Using coefficient estimations from the plots in Fig. 2, we applied the generating algorithm mentioned above to see whether such simple relationships could account for the main features of the corresponding dendritic trees. In spite of the poor fit of the branching probability of pallidal type I neurons, we also simulated the generation of their trees.
Real and simulated dendrites were compared according to two different sets of parameters. The first set was made of parameters averaged from the whole population of trees. These were scalar parameters (Ascoli et al.,2001). Most of them had been previously reported (Yelnik et al.,1984; Yelnik et al.,1991) for whole neurons, but not for isolated trees as presented here. We computed four topological parameters: (i) the average tree degree, which is the average number of tips per tree ; (ii) the average stature Hm (Hm is the maximal branch order) ; (iii) the average branch order Q, (ii) and (iii) being estimated using the centrifugal ordering (Uylings and Van Pelt, 2002); (iv) the average tree asymmetry index As (Van Pelt et al.,

1992). Metrical parameters concerning lengths were also included: as in the previous studies we considered the total dendritic length (L), the average branch length (Ln) and the average length of terminal branches Lp. We also considered the average length of parent branches Lm (the branches which terminate with a bifurcation) instead of separating primary trunks and internode branches. We further computed the average path distance to soma (P) of all tips in a tree.
The second set was made of distribution parameters estimated from all branches of a neuronal group. They describe the relationships between the branches and their topological (the order) and metrical distances to soma. They were the numbers of parent and terminal branches per order, the branching frequency per order, the average length per order of parent and terminal branches respectively, the average branch order and the total number of branches present at increasing path distances to soma.
For scalar values, the results provided by the simulations are shown in tables 1, 2 and 3.
For MSNs, scalar values issued from the simulations based on model 1 were in good agreement with the real values, with only one exception, the average branch length, Ln. Simulated trees displayed two remarkable features of MSNs: a weak asymmetry index and an extremely high terminal branch length. Indeed, terminal branches provided about 90% of the total dendritic length. Differences between simulated and observed features were greater when considering distribution parameters, for which the only good fit came from the plot of the total number of surviving branches versus the path distance to soma (Fig.5 C). The discordance was greatest for the variations in the numbers of branches with the branch order (Fig.3, A and C), the evolution of the frequency of branching with the order (Fig.3E) and the dependence of the average parent branch length on the branch order (Fig.4A) Concerning pallidal neurons, as expected, differences were very important for type I neurons, even for scalar parameters (table 2). For instance, the high Ln values of the simulated trees were not compatible with the definition of type I neurons. Though real and simulated dendrites did not significantly differ for D, As, L and P, we decided to discard this cell group from further analysis because our model clearly could not generate the morphology of these cells. Results were better for pallidal type II neurons. However, there were still marked differences between the modelled and real dendrites. For this cell group, the degree and the asymmetry index were the only topological parameters for which there was no statistically significant difference between real and simulated dendrites. Scalar metrical parameters also differed significantly, with the exception of the total dendritic length and the average tip path distance to soma (table 3). The distribution parameters were also very different. Apart from the relationship between the average terminal branch length and the branch order (Fig.4D) the discordances were the same as those concerning MSNs. Pallidal type II neurons were nevertheless investigated further.

**2.3. Modification of the model: model 2.**

The discrepancies between simulated and real MSNs, and above all type II dendrites, could not be ascribed to estimation errors because trying to obtain better fits by arbitrarily tuning the coefficients in the probability functions was unsuccessful, though simulation results exhibited a high sensitivity to the coefficient values. The main reason for the failure of the algorithm based on model 1 in generating satisfactory dendritic trees was a too low branching frequency for the lower branch orders (Fig.3). This suggested that the branch order also has an influence on the branching probability. Due to the prominent role of the path distance to soma, this order influence could have been blurred in the data we used to estimate the basic parameters. We therefore reexamined these data, this time selecting branch segments around some definite distances to soma, to which we applied the usual procedure by forming bins

according to the branch order of the selected segments. In spite of insufficient numbers of data points, which could generate considerable estimation errors, the branching probability per unit length was found to depend on the branch order. Here, power functions gave the best fits. Accordingly we modified our model, adding the branch order as a second basic parameter. The new model (model 2) was then defined by the branching probability function:

$$p_{br}(x,q)=k_b \exp(-\alpha x) q^{-\sigma}, (\sigma>0),$$

with q denoting the centrifugal branch order.
The $\sigma$ coefficient was estimated from the plot of the probability per unit length versus the branch order in the selected x ranges. Since the average path distance to soma of dendrite endings was already well established with model 1 for the three populations studied, we did not modify the terminating probability function which was left unchanged in all subsequent simulations.
Checking the new model with the stochastic algorithm necessitated reestimation of the coefficients of the dependence upon the path distance to soma. Estimating the $k_b$ and $\alpha$ coefficients independently of $\sigma$ required use of data points belonging to branches of the same order. In order to avoid the errors resulting from the estimation of the $\sigma$ coefficient, we performed the new estimation of $k_b$ and $\alpha$ with primary trunks only, applying the usual procedure.

**2.4. Algorithmic generation of dendrites according to model 2.**

Concerning MSNs, simulations done according to the modified model gave results much closer to the observed values and distributions. Indeed, the scalar parameters of simulated and real trees no longer showed any differences (table 1). There was also a great improvement in the simulated distribution parameters (Figs. 3, 4 and 5) and it was not considered meaningful to try to modify the model again in order to reduce the remaining slight discrepancies (see discussion). For pallidal type II neurons, however, model 2 based simulations again resulted in noticeable, new discordances (table 3). Indeed, introducing the dependence on the branch order definitely reduced the discrepancy observed with model 1 , as shown by the good fit of the model 2 derived branch frequency per order curve (Fig.3F), but simulated numbers of branches now exceeded the ones observed for almost every order (Fig.3, B, D). Accordingly, the total numbers of simulated branches present at increasing path distances to soma was higher than the real ones (Fig. 5, D).

**2.5. Further modification: model 3**.

Apart from an inherent inability of our modelling to predict the dendrogram structure of pallidal type II neurons, the new discrepancies could have been due to errors in the coefficient estimations, which were performed with fewer data points. There was however another explanation, namely an inhibition of branching next to each bifurcation. Indeed such an inhibition has been reported for other types of neurons (Nowakowski et al.,1992; Van Pelt et al., 2001). Moreover, it played a pivotal part in a modelling work sharing imortant aspects with our own (Nowakowski et al., 1992). In spite of this similarity, we had not considered it before because the dendrites modelled in that study were very different from our striatal and pallidal cells (see discussion). This kind of inhibition induces a peak in the length histogram of the branches concerned. Drawing this histogram for our dendrites, we indeed found a peak around 35 micrometers for pallidal type II dendrites, which was clearly absent in model 2 simulated type II dendritic branches, since we had not modelled the underlying inhibition

(Fig. 6). We therefore tried to fit the branching probabilities of pallidal type II neurons with a function combining our current model with that of Nowakowski et al. (1992):

$$p_b(z,x,q)=k_b(1-\exp(-\beta z))\exp(-\alpha x)q^{-\sigma}, \quad (\beta>0)$$

with the new basic parameter z denoting the path distance to the previous bifurcation (or to the root of the tree for primary trunks).
Because MSN dendrites were satisfactorily simulated with model 2 and because the length histograms of simulated and real branches were not significantly different for this neuronal group, the new model (model 3) was applied to pallidal type II neurons only.
To avoid the incidence of the branch order, we used the data from primary trunks only, and found a very good fit (Fig.7). $k_b$, $\alpha$ and $\beta$ were reestimated accordingly.

**2.6 Algorithmic generation of pallidal type II dendrites according to model 3.**

Dendritic trees were generated with the new model.This resulted in a considerable increase in similarity between simulated and real pallidal type II dendrites. Indeed, none of the scalar parameters were significantly different (table 3). Emergent distribution parameters of simulated trees were also in good agreement with those of the real dendrites, with the exception of small residual discrepancies mainly concerning the distributions with respect to the path distance to soma (Fig. 5,D). The generated trees now exhibited the expected peak in the parent branch length histogram (Fig. 6).

**3.Discussion.**

**3.1.Summary.**

Three main conclusions may be drawn from our study. 1. In adult primate medium spiny and pallidal type II neurons the branching and terminating probabilities per unit length of dendrite are highly dependent on the metrical (for both probabilities) and on the topological (for the branching probability) distances to soma. Moreover, these dependencies may be modelled with simple exponential and power functions respectively. 2. These functions, used with the algorithmic procedure described by Burke et al.(1992), can generate dendritic trees looking like the real ones. Simulated MSN trees were very close to the observed dendrites while the fit was less satisfactory for pallidal type II neurons. 3. The branching probability function may be integrated with a modelling previously made by Nowakowski et al.(1992). This neatly improved the fit between simulated and real type II neurons and, above all, led us to propose an extension of Nowakowski et al.'s model, providing simple rules potentially able to account for a large range of dendritic arborisation shapes.

**3.2. Nature and limitations of our modelling.**

As other approaches (Burke et al.,1992; Devaud et al.,2000), our modelling is parsimonious because only a very few basic functions are used to algorithmically generate a large set of other parameters, the emergent parameters (Ascoli et al., 2001), close to the real ones. One of the main aims of such approaches is indeed to capture as much as possible of the dendritic morphological complexity with rules that are as simple as possible. Derivation of coefficients and generation of the simulated dendrites were done with the methods described by Burke et al.(1992), which were also used by Ascoli et al.(2001). We did not try to generate trees

exhibiting all the morphological aspects of the real ones because at this stage of our work we were more interested in structural rules possibly underlying the dendrogram structure.
The model of dendritic structure we present here is descriptive and statistical since it is derived from data obtained from fixed adult brains. Moreover, contrary to the real dendrite growth, the generating algorithm forms the dendritic branches one after the other. Accordingly, competitive or cooperative interactions between branches have not been considered here and no reference has been made to the underlying cellular mechanisms, as those pivotal in other neurite morphology models (Graham and Van Ooyen, 2004; Li et al., 1992; Van Veen and Van Pelt, 1992). Thus, although our model may possibly be interpreted in a developmental perspective in the future, it is not a biological growth model.
Another limitation, inherent to all modelling approaches of dendrite morphology (Ascoli, 2002; Burke et al., 1992) is that the possible adequacy of the models is relative only to the data set from which they are derived and to which they are applied. The extent to which this data set is representative of the real population under study depends on histological and measurement procedures which are independent of the modelling process. Here we used measures coming from a large set of neurons (21 MSNs and 39 pallidal neurons), the great majority of which had already served in previous reports (François et al.,1984, Yelnik et al., 1984,1991; Percheron et al.,1984) to describe the dendritic organisation of the primate pallidum and striatum. Many of these cells were labelled with the Golgi methods, and though they have been selected according to stringent conditions (see section 4.1), whether they were representative of the real populations is questionable. In the present work, good evidence in support of limited bias of this labelling method comes from a statistical analysis which showed that our biocytin labelled pallidal cells were not significantly different from their Golgi homologues.

### 3.3. Residual inadequacies of the models.

The accuracy of the kind of models we have used is checked by the fits between observed and algorithmically generated dendrites. Significant differences between these sets of arborisations may result either from intrinsic flaws of the models (for instance the absence of dependence on the branch order in our model 1) or to errors in estimating the coefficients. Here, the intrinsic adequacy has been improved from model 1 to model 3. Concerning coefficient estimations, it is likely that errors decrease with increasing numbers of available measures. For this reason we used all the neurons in our data bases which satisfied the conditions of satisfactory impregnation (see section 4.1). We checked the effect of using smaller samples by random sampling a subset of MSNs (11 cells out of 21 available neurons, results not shown), and reestimating the coefficients of the functions of model 2 from this reduced data set. Whereas all the scalar parameters of the smaller sample were very close to those computed from the whole set, the dendrites generated according to the model 2 were significantly different from the real ones (for instance the average tree degree was now 5.21 instead of 6.67 for the observed value). This suggests that a reduced data set size would weaken the accuracy of the model.
In spite of the satisfactory results obtained with the terminal forms of our models (model 2 for MSNs and model 3 for pallidal type II neurons), there remain discrepancies between simulated and observed dendrites. The first is the reincrease in branching frequency at some high branch orders. Such a reincrease has already been reported for other neuronal types (Dityatev et al., 1995), for which it was hypothesised to arise from factors other than those providing the rules detected in those studies. Another slight, but clear, discrepancy concerns the evolution of the terminal branch length with branch order at the higher branch orders, in

pallidal type II dendrites more particularly than in MSN arbors. As stated for the high order branching frequencies, these two discrepancies may be due to rules or factors that our model does not capture. But since they only concern the high order branches, they may result from the very low number of these branches in our data samples. A third discrepancy concerns the total number of pallidal type II branches present at increasing path distances to soma (Fig. 5,D). In the 200-500µm range, the simulated branches are more numerous than the real ones, even with model 3. This may be due to the cumulative effect of coefficient estimation errors for both the branching and terminating probabilities. Indeed, this range of distances from the soma is precisely where the two probabilities overlap (Fig.2). Moreover, MSNs, for which the overlap is extremely small, show no mismatch between the simulated and real curves (Fig.5,C).

### 3.4. Basic contributions of the modelling.

The first contribution of our modelling is present in model 2, whose main feature is a high dependence of the branching and terminating probabilities per unit length of dendrite on the position in the dendritic tree. This global aspect distinguishes the present work from methodologically similar approaches mainly based on a purely local parameter, the dendrite diameter (Ascoli et al.2001; Burke et al.,1992; Donohue and Ascoli, 2005). However, it may be noticed that the distance to soma had to be added or suggested in some of these studies (Burke et al.,1992; Donohue and Ascoli, 2005) in order to obtain better simulated trees. Moreover, since diameters themselves may depend on the position in the arbor, they might be tree-dependent, too. The additional dependence on the path distance to soma mentioned in these works likely results from factors different from diameters per se, while the path distance to soma dependence of our models encompasses all these factors. Our simulations show that the incidence of this metrical parameter is especially strong. This was expected from simple examination of our raw data. However, the algorithm derived from model 1 failed to produce trees close to the real ones with respect to all parameters. Accordingly, it proved necessary to include a dependence of the branching probability on the branch order to reach a really good fit between simulated and real MSN dendrites.

### 3.5. Extension to model 3 and synthesis with Nowakowski et al.'s model.

The residual inadequacy of model 2 to satisfactorily predict the pallidal type II dendrograms pointed to an inhibition of branching next to each bifurcation, a feature we had not detected before. This illustrates how quantitative modelling may have unexpected outcomes and prompted us to consider the work of Nowakowski et al (1992), in which such branching inhibition played an essential part. The dorsal horn dendrites modelled by these authors have increasing, not decreasing, branching probabilities with more distal positions on the branch, and the location on the branch, irrespective of its place in the tree, was the basic parameter in their work. More precisely, the branching probability per unit length was an increasing exponential function of the distance from the proximal tip of the branch. These features were thus in sharp contrast with of our Basal Ganglia neuron data which display a clear decrease of the branching probability at increasing path distances to soma. This explains why we intitially missed Nowakowski et al.' model. In spite of these differences, adding a multiplicative factor which is exactly the probability function used by these authors (model 3) easily solved the discrepancy between model 2 generated and real type II dendrites. This amounted to replacing the asymptotic constant, towards which the probability function converged in the dorsal horn dendrite model, by the decreasing function of the metrical and topological distances to soma which so accurately describes the branching of MSN dendrites.

This change introduces a new basic parameter into our branching probability function, but it leads us to propose that the present model 3 constitutes an extension of Nowakowski et al.'s model with interesting biological significance. Indeed, this generalisation extends the potential range of these models, which now seem able to account for a wider variety of dendritic shapes. This is the case if we assume that, in addition to MSNs and type II neurons, model 3 could satisfactorily describe important aspects of the dendritic morphology of the neurons studied by Nowakowski et al. in spite of the strong differences between their branching patterns. Such different morphologies can be simulated by the same model because of the interplay between the coefficients present in the branching probability function. When $\alpha$ and $\sigma$ tend towards 0, with $\beta$ not too great, the generated dendrites are likely to exhibit dendrograms close to those of the neurons analyzed by Nowakowski et al., with no incidence of the location in the tree. Conversely, a very large $\beta$ coefficient would lead to a probability function purely dependent on the position in the dendritic arbor, which corresponds to MSN dendrites. Pallidal type II neurons represent an intermediary situation in which branching depends on both the position within the branch and the position in the tree, but mainly on the latter. Other combinations of the coefficients would of course produce different branching patterns. So, an unexpected and important outcome of our work is the extension of the conceptual framework proposed by Nowakowski et al. The very simple mathematical form of this extended model indeed suggests that they may be relevant to other neuronal types. For instance it would be interesting to know whether the dendrograms of some reticular formation neurons, which look like the large pallidal cells (Yelnik et al.,1984), could be predicted by our model.

### 3.6. Contribution of the modelling to basal ganglia cell taxonomy.

The potential interest of our modelling may be illustrated by considering its application to the neuronal groups we have studied. The pallidal dendrites we have analyzed belonged to the large cells which constitute the vast majority of the neurons found in GPe and GPi. In primates, these cells apparently form a single population (Fox et al.,1974; Yelnik et al.,1984). However, a careful morphological quantitative analysis has shown they are actually made of two distinct groups according to the average dendrite branch length. These groups correspond to the type I and type II neurons of the present study. Our simulations provide an additional and methodologically different basis to the fact that the two pallidal cell types really differ in spite of their apparent morphological similarity. Indeed, type I dendrite morphology was clearly out of reach of our models while type II dendrograms could be accurately simulated with model 3. In our opinion these results support an early proposal (François et al.,1984;Yelnik et al. 1984) according to which all the main pallidal neurons, irrespective of their species (human versus monkey) or anatomical (GPe versus GPi) origin, have dendrites built on the same basic organisation characterised by long and poorly branched dendrites, which corresponds to the type II neurons. From this point of view, type I dendrites could result from the addition of other elements to the basic structural framework. These elements are the fine processes and complex endings specific of type I neurons (François et al.,1984). We propose that essential aspects of the basic framework are captured by our model but that the structural effects of the added elements are not.

While this result is mostly confirmatory, our modelling has also revealed the new and surprising suggestion that the rules underlying the basic pallidal dendrite organisation are shared with MSNs. This was not expected since pallidal dendrites exhibit low branching (tree degree less than 4 for pallidal type II neurons) and are very long, while MSNs have short dendrites with a greater number of branches (average tree degree near 7). Another conspicuous difference is the existence of densely packed spines on MSNs contrasting with

the absence of such dendritic protrusions in the large pallidal cells. Moreover, though not considered here, the dendritic spatial orientation of the two populations also clearly differs (Yelnik et al.,1984, 1991). On the contrary, pallidal type I and type II neurons have dendritic shapes and orientations much more alike. It is therefore an interesting result of our simulations that, in spite of their obviously different shapes, MSN and pallidal type II dendrograms are correctly predicted by the same model while pallidal type I dendritic trees are not. Thus, our models seem able to discriminate between fine morphological differences on the one hand, and yet can suggest similarities between apparently very different neuronal populations. They could thus serve as a classification tool, within and between neural cell groups.

### 3.7. Concluding remarks.

The probability functions which constitute the generalised quantitative framework of model 3, as well as the coefficients they contain, are all derived from neurons observed in fixed adult tissue. The dendritic shape of these neurons results from the many developmental and perhaps adult regulatory mechanisms (Scott and Luo, 2001; Woolley,2001) now known to shape dendrite morphology (Cline, 2001; Jan and Jan,2003; Scott and Luo, 2001). These processes include interstitial branching in addition to tip splitting, though the former remains controversial (Acebes and Ferus, 2000), branch elongation but also retraction and pruning (Cline, 2001). They involve the interplay of a wealth of factors of different kinds: regulators of intrinsic cellular origin, external molecular signals such as neurotrophins, synaptic stabilisation and synaptically driven electrical activity. It is surprising that such complex and dynamic phenomena can be translated into functions as simple as those we used to make an accurate prediction of the MSN and pallidal type II dendrograms. A useful task would then be to search for biological interpretations of these functions and the coefficients they contain. For instance, it may be asked whether they result predominantly from some particular factors (we think first of intrinsic factors, because MSNs and pallidal neurons evolve in different environments). Further models will have to rely on hypothetical and biologically plausible mechanisms in order to gain insight into such relationships.
They will also have to include diameters and diameter-linked parameters such as dendritic cross sectional areas, membrane areas and volumes, a task we are currently performing. These metrical parameters, not considered in the present study, must also be taken into account to improve our understanding of structure-function relationships at the dendrite level and to search for possible links with neuroanatomical modelling. Because of their size similarities, pallidal cells seem appropriate for such investigations, and we have already begun to explore these questions (Mouchet and Yelnik, 2004).

## 4.Material and methods.

### 4.1.Data sources and grouping of neurons.

The experimental data came from primate striatal (monkeys) and pallidal (humans and monkeys) neurons, most of which have been used for previous studies (François et al., 1984; Yelnik et al.,1984; Yelnik et al. 1991).
MSNs came from four Golgi stained monkey brains (two *Papio papio* and two macaques) selected from a 22 brain collection because their striata were especially well impregnated (Yelnik et al., 1991). This striatal sample was made of 21 neurons, providing 107 dendritic trees (totalling 1321 branches).

For the pallidal cells, 34 Golgi stained and 7 biocytin labelled neurons were considered. The Golgi stained neurons came from three human (20 neurons) and two macaque (14 neurons) brains which had been selected from a larger collection due to a satisfactory impregnation of the pallidal region (François et al.,1984). The biocytin labelled cells were obtained from the internal globus pallidus of two macaque brains used in other experiments (Arrechi-Bouchhioua et al., 1996). Previous statistical analysis showed there was no significant difference between these human and monkey pallidal cells, or between Golgi and biocytin labelled cells. All were large neurons, constituting the main neuronal component in the two pallidal segments (François et al.,1984; Yelnik et al.,1984). It has been shown previously (Yelnik at al.,1984) that they form two distinct groups according to their average dendritic length Ln. Indeed, this parameter displays a bimodal distribution from which large pallidal cells can be divided into types I (Ln lower than 250 µm) and II (Ln higher than 265 µm). These groups also differ with respect to the average tree degree (which is higher for type I cells) and the average terminal branch length (wich is higher in type II neurons). All other parameters describing the dendrograms of the members of these groups were identical (Yelnik et al., 1984). In the present work we again used Ln to separate our neurons into types I and II. This led us to discard two cells because, in spite of their being likely of type II, their Ln slightly failed to meet our classification criterion. The resulting samples used in our modelling were made of 18 type I( 15 Golgi stained and 3 biocytin labelled) neurons and 21 type II (17 Golgi impregnated and 4 biocytin labelled) neurons. Type I neurons provided 73 denditic arborisations (totalling 615 branches) and type II cells 90 trees (totalling 544 branches).
In the brains used, the Golgi stained neurons, which consitute the great majority of our samples (53 neurons out of 60) were selected on condition that their dendritic arborisations were complete. This means that the reconstruction was from serial sections without any silver precipitates or glial processes impairing the reconstruction and without any abrupt ending of a dendrite in the thickness of the section instead of a termination with a progressive tapering (Yelnik et al., 1984).
Dendritic trees of all neurons were quantitatively analyzed and the neurons reconstructed 3-dimensionally. They were all binary (branching occurs solely as bifurcations), which seems true for most dendrites described until now (Uylings and Van Pelt, 2002). Consequently, the models we have designed consider dendrites as binary trees.

### 4.2. Estimation of the branching and terminating probabilities per unit length of dendrite.

Branching and terminating probabilities per unit length of dendrite were derived from the original raw data, according to the method described by Burke et al.(1992). To do this, we used the quantitative analysis of the dendrites of the three groups of neurons (MSNs, pallidal types I and II) which directly provided the many short adjacent segments of known length needed. The segments were pooled into bins according to the features (the future basic parameters of the models) suspected of affecting the branching and terminating probabilities. In each bin, the branching and terminating probabilities per unit length were computed by dividing the numbers of segments ending with a bifurcation (respectively a termination) by the total length of all segments in the bin.

### 4.3.Modelling and simulations.

The relationships of the branching and termination probabilities per unit length to the variables used to form the bins were modelled with functions derived from the plots of the

corresponding probabilities (Fig.2). These functions were obtained using either logarithmic linearisations or the Levenberg-Marqhart algorithm (Press et al.,1989). They were then used to generate dendritic trees according to the algorithm described by Burke et al. (1992), with a slight modification concerning the decision process.
Briefly, the procedure started at the soma. At each step a random number s was generated from a uniform distribution between 0 and 1. It was compared to the value $p_{br}$ of the branching probability function which was strictly defined according to x (model 1), x and q (model 2) or x, q and z (model 3), where x was the path distance to soma (in micrometers), q the centrifugal branch order and z the distance in micrometers to the previous bifurcation (or to the soma for primary trunks). If $s<=p_{br}$, the branch ended with a bifurcation. If not, s was compared to the conditional terminating probability $p_{tc}=p_{tm}/(1-p_{br})$. If $s<=p_{tc}$ the branch was terminal. If these comparisons did not result in either bifurcation or termination the branch was further elongated by 1µm. The procedure was continued to the end of all branches. In order to gain confidence in the significance of possible fits between observed and simulated dendrites, we generated a higher number of arborisations than the real ones, for each model. The algorithm was programmed in C language and run on a PC workstation.

**Aknowledgements**.

We thank Prof. C.Feuerstein for fostering this work, Dr. A. Dicky for help in modifiying the decision process of the stochastic algorithm, Dr. F.Hemings for revising the English and L.Davoust for help in preparing the illustrations.

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LEGENDS OF FIGURES.

Fig. 1.
Planar views (upper row) and the corresponding dendrograms (lower row) of neurons representative of the three cell groups studied. The neurons were labelled with the Golgi method. The medium spiny neuron comes from monkey putamen. The pallidal type I and type II neurons come from monkey internal globus pallidus.

Fig. 2.
Branching and terminating probabilities per unit length of dendrite versus the path distance to soma for the three neuronal groups studied. The probabilites were computed as described in the text from 14947 segments ( for 21 MSNs), 8131 segments (for 18 pallidal type I neurons) and 12499 segments (for 21 pallidal type II neurons). The MSNs, totalling 107 dendritic trees, had been selected from 4 monkey brains. Pallidal types I (totalling 73 trees) and II (totalling 90 trees) had been selected from 2 human and 5 macaque brains.
The solid lines indicate the best fits to the branching (empty circles) and terminating (empty squares) probabilities according to the Levenberg-Marqhart algorithm.
A ($R^2$=0.995) and B ($R^2$=0.956) :MSNs; C ($R^2$=0.695) and D ($R^2$=0.909) : pallidal type I neurons; E ($R^2$=0.956) and F ($R^2$=0.946): pallidal type II neurons.
The equations were those of model 1 (see text).

Fig. 3.
Distribution of parent (A,B), terminal (C,D) average numbers of branches and branching frequencies (E,F) according to the centrifugal branch order for the real (solid lines) and simulated MSNs (observed:n=107, simulated: n=1070) and pallidal type II neurons (observed:n=90, simulated:n=900)..

Fig. 4.
Distribution of parent (A,B) and terminal (C,D) average branch lengths according to the centrifugal branch order for the real (solid lines) and simulated MSN (observed:n=107, simulated: n=1070) and pallidal type II dendrites (observed:n=90, simulated:n=900).

Fig. 5.
Evolution of the average branch order (A,B) and the total number of surviving branches (C,D) at increasing path distances to soma for real (solid lines) and simulated MSN and pallidal type II dendrites. For the simulated trees, the number of branches has been divided by ten to normalise the result to the real populations.

Fig. 6.
Primary trunk length distribution of real and simulated type II neurons. See table 3 for the parameters used for model 2 and model 3 simulations.

Fig. 7.
Branching probabilitiy per unit length of dendrite versus the path distance to soma for the pallidal type II primary trunks. The solid line indicates the fit to the function

$k(1-\exp(-\beta x))\exp(-\alpha x)$ (with $k,\beta,\alpha > 0$), according to the Levenberg-Marquart algorithm ($R^2=0.9775$).