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PAPER

VERTEX ANALYSIS OF THE GROWTH OF PURKINJE CELL DENDRITIC FIELDS OF THE MOUSE

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ABSTRACT

The purpose of this study was to quantify the growth of Purkinje cell dendritic fields in mice, so that changes with time in segment frequency, length and topology are defined. Our results show that changes in the Va/Vb ratio are consistent with the hypothesis that growth is by random terminal branching with the intrusion of 5-8% trichotomy and with inhibitory constraints operating in the lateral domains. The changing frequency distribution of vertex orders with time indicates that initially growth predominates over high orders as a front of growth ascends a narrow corridor physically defined by the margins of juxtaposed trees. Subsequent branching occurs over low order vertices as the boundaries of the tree stabilise. Path lengths increment uniformly over established equivalent orders, because all segment lengths are similar, but growing segments have smaller, more variable lengths. All segments elongate by terminal and interstitial growth.

INTRODUCTION

This study attempts to describe the growth of the Purkinje cell (PC) dendritic field of the mouse quantitatively using the new technique of vertex analysis (Berry and Pymm, 1981). Within networks, vertices are points interconnected by arcs(lines). There are three types of arc, namely; pendant arcs, root arcs and arcs (fig.1). Pendant vertices (Vp) each connect 1 pendant arc, a root vertex (Vr) connects 1 root arc, dichotomous vertices (Vd) comprise primary vertices (Va) each connecting 2 pendant arcs and 1 arc, secondary vertices (Vb) each connecting 1 pendant arc and 2 arcs, and tertiary vertices (Vc) each connecting 3 arcs (fig.1). The four types of trichotomous vertex (Vt), i.e., Va',Vb',Vc' and Vd', are shown in figure 1. Quantitative relationships between vertices in a given network may be defined by simple mathematical formulae. Thus, in dichotomous trees, $Vp = Vr + Va + Vb + Vc$ (1). By definition $Vr = 1$ and, since all Va's drain 2 Vp's and all Vb's drain 1 Vp, $Vp = 2Va + Vb$ and thus $Vb = Vp - 2Va$.

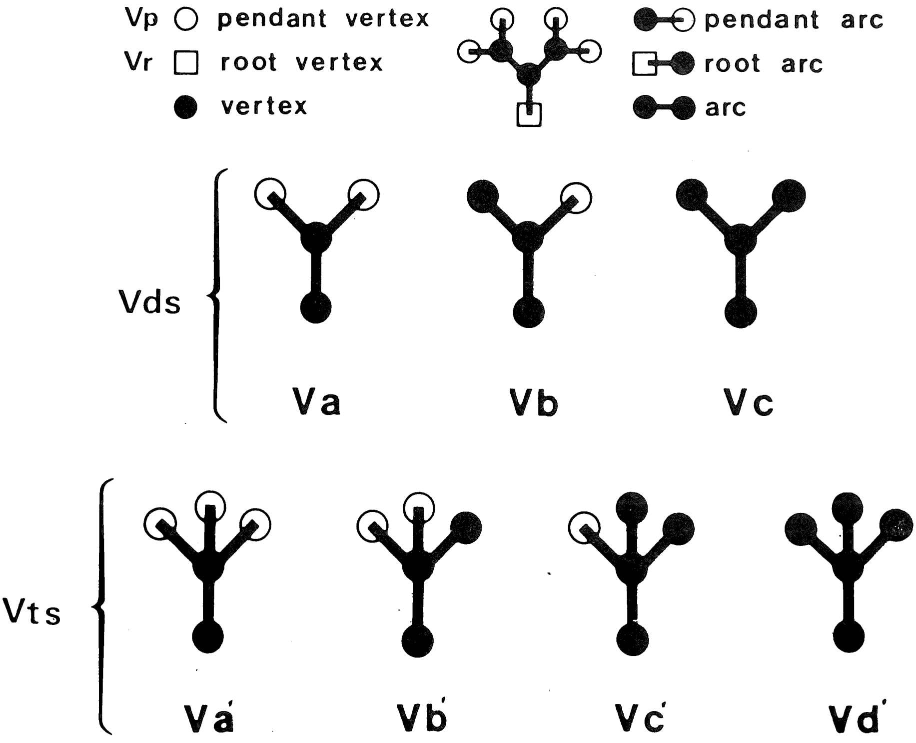

Figure 1. Definition of vertices and arcs and of the different forms of dichotomous and trichotomous vertex

Substituting for Vb in equation (1), $Vc = Va - 1$. Thus, as the magnitude of Vp increases Va and Vc approximate to equality. Therefore, the connectivity of a given large arborisation may be defined by the Va/Vb ratio. For example, in trees grown by random terminal branching Va/Vb = 1; in those generated by random segmental growth the ratio is 0.5; and in those that grow collaterally it approaches 0. Symmetrical dichotomous networks produce Va/Vb ratios which are very large.

The frequency of Vt's may be used as an estimate of the rate of interaction of axons with dendritic growth cone filopodia during development (Berry et al,1978). Their frequency and distribution within the tree may also be an important pointer to local factors influencing plasticity and development. Equation (1) is modified by the intrusion of trichotomy as follows; Vp = Vr + Va + Vb + Vc + 2(Va' + Vb' + Vc' + Vd') (fig.1), i.e., Vp = Vr + Vd + 2Vt. However, in order to facilitate analysis, all trichotomous or higher order vertices are transformed into equivalent Vd's thus Va' = Va + Vb; Vb' = Va/3 + 4Vb/3 + Vc/3; Vc' = Vb + Vc and Vd' = 2Vc. In trees grown by random terminal branching in which all Vt's are represented as equivalent Vd's, the Va/Vb ratio deviates predictably from 1 as the probability of trichotomous branching increases. Thus, the Va/Vb ratio will detect the frequency of trichotomous vertices in a transformed dichotomous tree. This is an important advantage of the method because Vt's may be naturally transformed into Vd's by nodal growth (Berry and Bradley, 1976c).

Using the Coleman and Riesen (1968) ordering system, the spatial positions of all vertices can be defined. Vertices of the same order (z) are equivalent since they are removed from the root by (z-1) vertices. All daughter arcs may be given the same order magnitude as that of the parent vertex from which they originate. This method of ordering allows Vt and all other higher order nodes to be included in the analysis.

MATERIALS AND METHODS

At least 10 C57 mice were used at each of the 7,10, 12,15,20 and 30 days-post-natal (dpn) age-groups. Beyond this, at 50 and 100 dpn, 5 mice were used. Each litter was reduced to 8 animals at birth and 1 animal of either sex was removed at random from each litter at each age. Brains were removed and treated by Scholl's (1953) modification of the Golgi-Cox technique. Fully impregnated PCs were selected from along the primary fissure of 80 μm thick parasagittal vermal sections. At least one cell was drawn from each animal using a camera lucida method. Subsequent analysis was performed using a computer and bit-pad to reduce each tree to a numerical computer-readable form for vertex analysis.

RESULTS

Total segment length gives a measure of the total expansion of the tree. Figure 2 shows that initially the tree expanded rapidly from 7 to 20 dpn, accounting for 65% of the total growth, after which the remaining 35% was added between 20 and 100 dpn. Terminal path length is the mean distance from the tip of any terminal segment through the tree to Vr and is a measure of the rate of migration of the boundaries of the arbor. This parameter showed an initial rapid increase from 7 to 15 dpn (72% of total) followed by a slower rise to a ceiling value around 150μm maintained between 50 and 100 dpn (fig.3). Vertex path lengths are the mean distances from vertices of a given order to Vr. At each age there was a period of regular increases in vertex path lengths through the orders, which spanned more orders as age advanced. At the highest

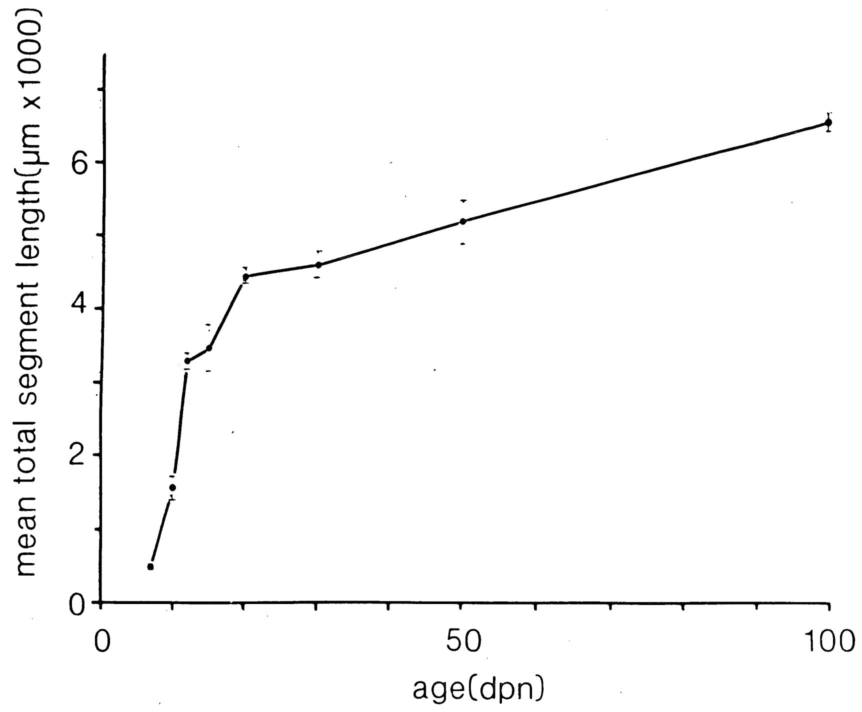

Figure 2. Changes in mean total segment length with age - see text

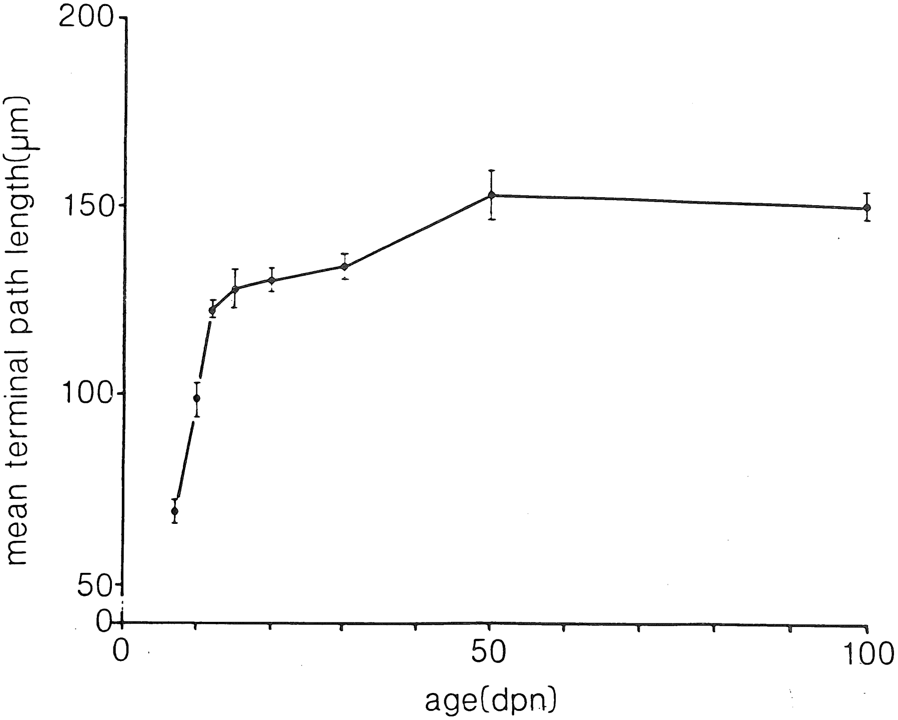

Figure 3. Changes in mean terminal path length with age - see text

orders of each age the regularity of vertex path length increments was lost as segment lengths became more varied at the active growth region of the tree (fig.4). Segment and terminal number also exhibited biphasic growth; a rapid rise from 7 to 20 dpn resulted in the elaboration of 85% of the total followed by a slow increase up to the values achieved at 100 dpn.

At 7 and 10 dpn there was a symmetrical distribution of terminals throughout the orders of the tree (fig.5). Beyond 10 dpn the symmetry was lost as terminals were produced in increasing frequency over higher equivalent orders. The peak terminal frequency shifted from order 10,

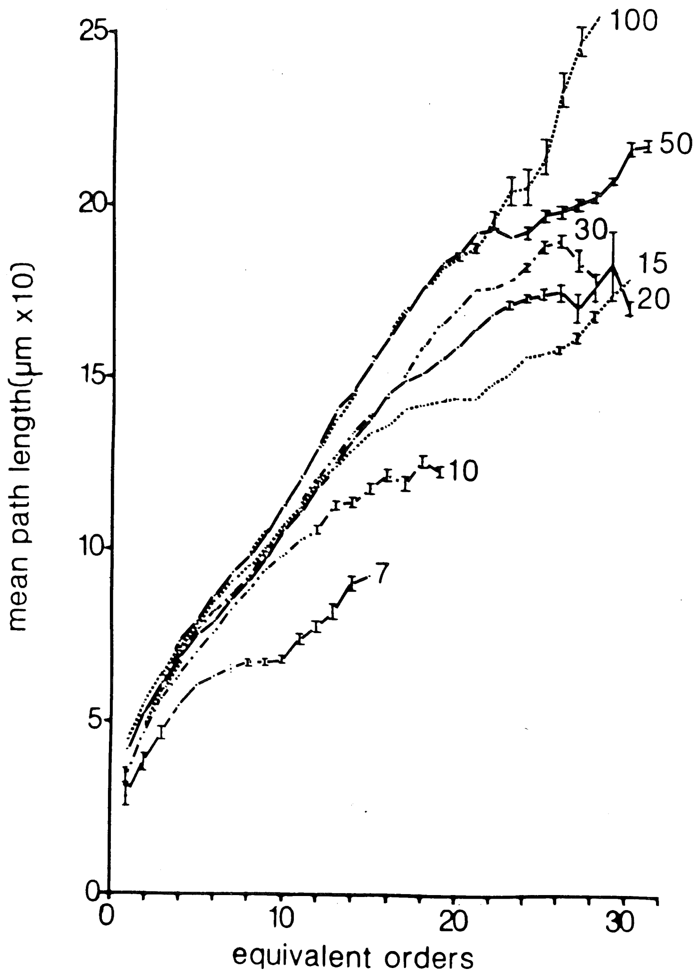

Figure 4. Changes in vertex path length with age for each age group - see text

at 10 dpn, to order 16, at 50 dpn. By 100 dpn there had been a large increase in the number of terminals between orders 8 and 18, largely restoring the symmetry of distribution. At 7 and 10 dpn the Va/Vb ratio was greater than 1, falling to a lowest value at 20 dpn of 0.84, before rising again to stabilise at approximately 0.9. The trends exhibited in the distribution of Va, Vb and Vc were similar to those shown by the terminal frequencies (fig.5). This is to be expected, since a Va of order n contributes 2 Vp's and a Vb contributes 1 Vp at order n + 1. The shift in maximum vertex distribution over ascending orders was obvious especially at 30 and 50 dpn. Symmetry was re-established at 100 dpn. The changes in the frequency of Vt's with age showed that the proportion of trichotomous nodes varied between 8 and 5% throughout development.

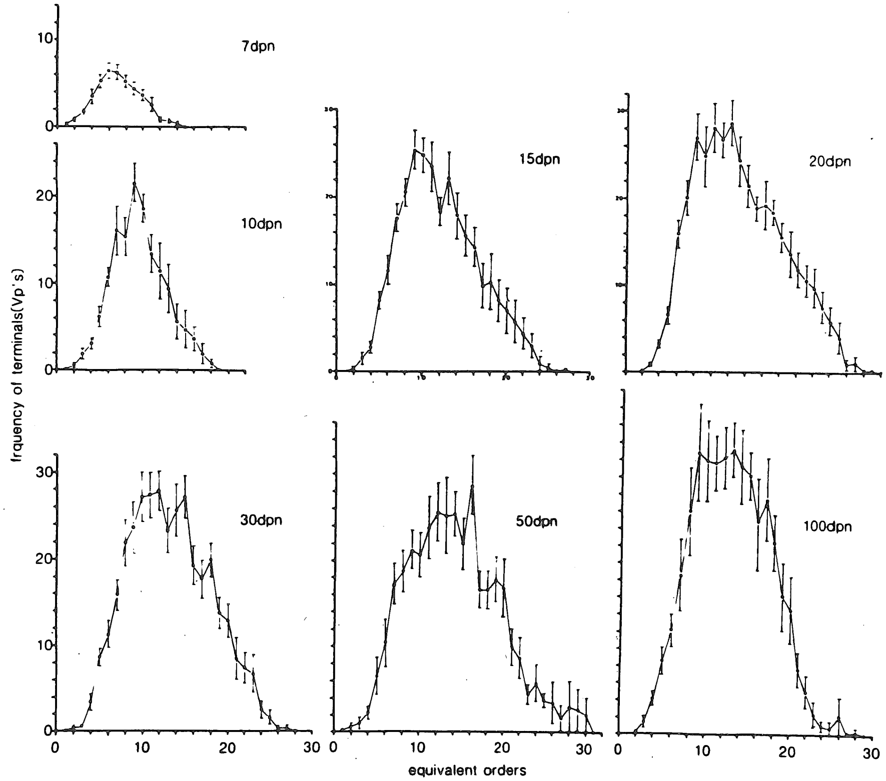

Figure 5. Changes in the frequency of terminals at each order at different ages - see text

DISCUSSION

The metrical parameters show two distinct phases in the development of the PC dendritic tree. The first growth spurt occurs between 7 and 20 dpn and is associated with a rapid elaboration of segments producing expansion in total dendritic length and terminal path length. This period correlates with similar growth seen by other workers (Weiss and Pysh, 1978 ; Berry and Bradley, 1976a,b). It is the time of deposition of parallel fibres (Fujita 1967,1969),the axonal system possibly responsible for the direction of growth and branching frequency (Berry et al, 1978). Over this period the lateral domains of the tree are established (Berry and Bradley, 1976a). Thereafter the growth of segments is slower and is not allied with changes in the boundaries of the tree. Most trees have established their lateral and vertical domains of growth by 20-30 dpn so that any further growth occurs within the tree rather than at its periphery.

The terminal and vertex frequencies per order show an asymmetric distribution beyond 7 and 10 dpn produced by a preponderance of terminals in the higher orders. This supports the suggestion that growth occurs by random terminal branching along a front which advances up a corridor physically defined by the margins of juxtaposed trees. Behind this front of activity and at the margins of the dendritic field inhibitory factors may initially prevent further growth (Berry and Bradley 1976a). Beyond 30 dpn, however, the limits of the growing zone have been reached and both the vertex and terminal distributions show a shift in peak frequency resulting from a massive elaboration from within the tree, especially marked between 50 and 100 dpn.

The Va/Vb ratio has a value of above 1 at 7 and 10 dpn indicating that the initial branching of the tree deviates significantly from random terminal growth. Between 10 and 20 dpn changes in the Va/Vb ratio suggest that growth is becoming more segmental in nature leading to the formation of an excess of Vb's as the tree reorientates its growth from a lateral to a vertical direction. Beyond 30 dpn a stable Va/Vb ratio with a 6-7% trichotomy is compatible with growth by random terminal branching but which is confined to terminals initially at the growth front and later within the tree.

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