ABSTRACT

This study describes the postnatal change in size of motoneurons in the hypoglossal nucleus that innervate the genioglossus muscle. Such anatomical information is essential for determining the cellular mechanisms responsible for the changes observed in the electrical properties of these motoneurons during postnatal development. The cells analyzed here are part of an earlier study (Nunez-Abades et al. [1994] J. Comp. Neurol. 339:401–420) where 40 genioglossal (GG) motoneurons from four age groups (1–2, 5–6, 13–15, and 19–30 postnatal days) were labeled by intracellular injection of neurobiotin in an in vitro slice preparation of the rat brainstem and their cellular morphology was reconstructed in three-dimensional space.

The sequence of postnatal dendritic growth can be described in two phases. The first phase, between birth (1–2 days) and 13–15 days, was characterized by no change in either dendritic diameter (any branch order) or dendritic surface area of GG motoneurons. However, maturation of the dendritic tree produced more surface area at greater distances from the soma by redistributing existing membrane (retracting some terminal branches). During the second phase, between 13–15 days and 19–30 days, the dendritic surface area doubled as a result of an increase in the dendritic diameter across all branch orders and a generation of new terminal branches. In contrast to the growth exhibited by the dendrites, there was little discernible postnatal growth of somata.

At all ages, dendrites of GG motoneurons show the largest amount of tapering in the first- and second-order dendrites. The calculated dendritic trunk parameter deviated from a value 1.0, indicating that the dendritic tree of developing GG motoneurons cannot be modeled accurately as an equivalent cylinder. However, the value of this parameter increased with age. Strong correlations were found between the diameter of the first-order dendrite and the dendritic surface area, dendritic volume, combined dendritic length, and, to a lesser extent, the number of terminal dendrites in GG motoneurons. Correlations were also found between somal and dendritic geometry but only when data were pooled across all age groups.

These data support earlier studies on kitten phrenic motoneurons, which concluded that postnatal growth of motoneurons was not a continuous process. Based on the fact that there was no growth in the first 2 weeks, the changes in the membrane properties described during this phase of postnatal development (e.g., decrease in input resistance) cannot be attributed to increases in the total membrane surface area of these motoneurons. It is proposed that these changes result from changes in the specific membrane resistance of these neurons.

Indexing terms: brainstem, hypoglossal nucleus, upper airways, respiration, three-dimensional reconstruction
sites on the tree will impact the integration at the axon hillock (Barrett, 1975). This paper is part of a larger study using in vitro brainstem slices to follow the postnatal changes in the morphological and electrophysiological properties of developing genioglossal (GG) motoneurons (Núñez-Abades et al., 1993, 1994). In a previous paper, we identified times during postnatal development when major changes occur in the active and passive membrane properties of GG motoneurons (Núñez-Abades et al., 1993). Some of these observed changes in the electrical properties of the motoneuron could be due solely to changes in cellular morphology. For example, there was a large decrease (50%) in mean input resistance of GG motoneurons between the first and the second weeks of postnatal life. One simple explanation for this decrease in resistance could be a doubling of membrane surface area. The present data demonstrate that this is not the case.

In the first paper examining the morphology of these developing motoneurons (Núñez-Abades et al., 1994), we described the postnatal changes in the dendritic branching of developing GG motoneurons. During the first 2 postnatal weeks, we found that the dendritic trees undergo a decrease in branching complexity (loss of terminal branches) that is simultaneous with an increase in the length of other branches. By the third postnatal week, the dendritic tree reevaluates by regenerating new branches, probably as a result of collateral outgrowths from terminal branches. Three-dimensional analysis revealed that this spatial redistribution of GG dendrites during postnatal life was nonrandom. In the present study, we have reexamined the temporal pattern of growth exhibited by GG motoneurons by focusing on the changes in the distribution of membrane area as a function of the distance from the soma. These data describing the changes in the dendritic geometry are important for generating an electrical model of the developing neuron (Rall et al., 1992).

On the basis of morphometric analyses of intracellularly labeled mammalian neurons, investigations have shown several common principles that govern the growth and maintenance of dendrites. A strong positive correlation has been found between the diameter of the first-order dendrites and dendrite length, volume, and combined length of the dendrite in several populations of adult cat spinal motoneurons (Ulfhake and Kellerhals, 1981, 1983, 1984; Cameron et al., 1985; Rose et al., 1985; Cullheim et al., 1987) and rat neocortical pyramidal cells (Larkman, 1991a,b). This relationship has also been described by workers from two laboratories in studies of developing spinal motoneurons of the kitten (Ulfhake and Cullheim, 1988; Ramirez and Ulfhake, 1991a; Cameron et al., 1991a). The present study extends these investigations to the development of brainstem motoneurons and to a different species, the rat. Preliminary reports of these data have been presented elsewhere (He et al., 1992; Núñez-Abades et al., 1992).

**MATERIALS AND METHODS**

The morphological data were obtained in an earlier study of intracellularly labeled GG motoneurons with neurobiotin. The details of the surgery, recording, intracellular injection, and histochemical processing have been described previously (Núñez-Abades et al., 1994). In brief, rats were deeply anesthetized with halothane, tracheotomized, ventilated, transcardially perfused with a cold (1-4°C) sucrose-artificial cerebral spinal fluid (ACSF), and then quickly decapitated. The brainstem was removed and sectioned at 300 µm in the transverse plane using a Vibratome and then incubated at room temperature for at least 1 hour before transfer to the recording chamber. GG motoneurons had been retrogradely labeled by intramuscular injection of dextran rhodamine made several days prior to surgery. Intracellular impalements of GG motoneurons were made in the ventromedial region of the hypoglossal nucleus with the help of an upright microscope equipped with epilluminescence. A total of 40 GG motoneurons were selected for reconstruction and they represented each of the following groups: 1-2 days (n = 10), 6-8 days (n = 10), 13-15 days (n = 10), and 19-30 days (n = 10). Seven cells in each group had all their dendritic processes confined to the slice, and these processes could be traced to terminations measuring less than 1 µm in diameter. The remaining motoneurons could only be partially reconstructed because one or two dendritic branches left the plane of the section; these branches exiting the slice always had diameters less than 1 µm. The partially reconstructed cells were equally distributed among the age groups. The completely reconstructed dendrites from these partially reconstructed cells were included in the analysis involving individual dendrites, but data from these cells were excluded in analyses dealing with the morphometrics of the entire neuron.

All motoneurons were reconstructed using the Eutectic Neuron Tracing System running on an IBM-compatible computer (Capowski, 1989). With this system, the neurons were reconstructed by making multiple measurements (approximately every 5-10 µm) of each dendritic branch using a x60 dry objective (NA = 0.80). Each point was designated as either a branch point, a continuation point, or a natural (terminal) ending and was assigned X, Y, and Z and diameter values. This form of analysis provides a more accurate estimation of dendritic length, surface area, and volume than measurements derived from planar reconstructions (cells drawn in two dimensions with the aid of a drawing tube attachment; see Cameron et al., 1991a, for details). With the Eutectic system, the combined dendritic length was calculated as the summed lengths of all branches of a dendrite, and the total extent of the dendritic territory (area of influence) was quantified by calculating the minimum surface area required to enclose all the dendritic terminals of the neuron. Because of the difficulty of determining the somatodendritic boundary, the cell bodies and proximal dendrites were manually drawn using a camera lucida attachment. The geometry of the cell bodies was approximated by the largest ellipse that could be placed within the boundaries of the cell (Schade et al., 1981). The major and minor diameters (axes) of these ellipses and the diameters of the first-order dendrites were measured from the manual reconstruction using a digitizing tablet. Somal surface area and volume were calculated using the equation for a prolate spheroid (Wobber and Pleijchka, 1976).

The terminology used to describe the dendritic tree is the same as described previously in Núñez-Abades et al. (1994). In all reconstructed cells, each dendritic branch was assigned a branch order following a centrifugal ordering method (cell body to distal terminal), and each branch was identified as a preterminal or a terminal branch. The term preterminal branch was used to identify parts of a dendrite that connected the soma with the first branching point or two successive branching points. A terminal branch was defined as a branch that connected a branching point with a
termination. Also, each dendritic branching point was assigned a vertex index (Berrv and Flinn, 1984; Verweer and Van Pelt, 1985) indicating whether the parent branch gave rise to two terminal branches (V, node), one terminal branch and to another parent branch (Vh-node), or two new parent branches (no terminal branches; V,-node). The diameter of dendritic branches was calculated as the average of multiple measurements along the entire branch (every 5–10 μm), and this value was analyzed separately for terminal and preterminal branches. Further analysis of the preterminal branches was undertaken with respect to the branch order or type of branching node.

When modeling the neuron as an equivalent cylinder (Rall, 1959, 1977), one assumes that the dendritic tree can generate a constant surface area at varying distances from the soma. This assumption was tested by calculating the combined dendritic trunk parameter (Cameron et al., 1985, 1991a). The dendritic trunk parameter was defined as the sum of the daughter branch diameters, each raised to 3/2 power divided by the diameter of the first-order branch raised to the 3/2 power. If the dendritic tree is a perfect equivalent cylinder, then the value of this trunk parameter at various distances from the cell body would be 1.0. Data from all dendrites from each age group were pooled, and the dendritic trunk parameter was calculated at intervals of 100 μm.

To quantitate the symmetry of dendritic branching, the daughter-to-parent ratio (DBR) was calculated for each branch point as the ratio of the diameters of the larger daughter segment to that of the smaller daughter segment (Larkman, 1991a). A symmetrical branching would have a ratio of 1.0 with increasing values of the DBR indicating greater asymmetry. Tapering of dendrites was quantified by measuring the amount of taper per 100 μm of branch length:

\[
\text{Taper} = \frac{d_{\text{proximal}} - d_{\text{distal}}}{\text{length}} \times 100/\text{length of branch},
\]

where \(d_{\text{proximal}}\) and \(d_{\text{distal}}\) are diameters of the proximal and distal ends of the dendritic branch, respectively, and the value is expressed in micrometers of taper per 100 μm dendritic length. A positive value was derived when there was tapering and a negative value when the branch expanded.

For each geometric parameter, the statistical differences between two age groups' data were tested using the nonparametric Mann-Whitney U test, while comparisons among more than two groups were accomplished with the Kruskal-Wallis analysis of variance. In the text, statistical significance has been indicated as follows: *P < 0.05, **P < 0.01, and ***P < 0.001. The functions describing the relationships between the various quantitative measures of dendritic morphology were determined by the method of least squares fit. The data points were fitted by linear, power, exponential, or second-order polynomial functions, depending upon which yielded the largest correlation coefficient (r).

**RESULTS**

Original data consisting of light photomicrographs of intracellularly labeled neurons and their respective three-dimensional, computer-assisted reconstructions have been presented in an earlier paper (Núñez-Abades et al., 1994). In addition, representative reconstructions of cells from each of the four age groups were also presented in this earlier work.

**Growth of soma relative to dendrites**

In all cases, the shape of the cell body was best fit by an ellipse. Analysis of major, minor, and mean \([\text{major} + \text{minor}/2]\) diameters of the cell body revealed no changes in any of these dimensions during postnatal development and, as a result, the ratio of minor to major diameters was unchanged with age (Table 1). At all four age groups, the ratio of ~0.7 indicated a slight eccentricity of GG cell body; however, no preferential orientation of this eccentricity was noted. Motoneurons maintained their multipolar shape during development. Furthermore, the orientation of the major diameter was totally unrelated to the nonrandom spatial orientation of the dendrites (Núñez-Abades et al., 1994). Without significant changes in major or minor diameters, it follows that there was no significant increase in calculated somal surface area with age. Therefore, the adult size and shape of the GG somata were apparently established at birth.

Up to 13–15 days, there was no increase in total membrane surface area (see Table 1). Therefore, dendritic to somal surface area ratio was unchanged during the first 2 postnatal weeks. Between 13–15 and 19–30 days, the dendrites exhibited a significant increase in surface area at a time when somal dimensions increased by only 16%. The dendritic surface area nearly doubled between 13–15 days and 19–30 days and thereby increased the dendritic to somal surface area ratio by 46% (Table 1). Based on anatomical and physiological criteria (see Núñez-Abades et al., 1994), rat GG motoneurons appear to be mature by 19 days of postnatal life. If the 19–30-day-old group represents the adult state, then the cell bodies exhibit less growth during development than the dendrites exhibit.

Table 2 presents the number and diameter of the first-order dendrites in each age group. The increase in dendritic
TABLE 2. Quantitative Data of Developing Genioglossal Dendrites

<table>
<thead>
<tr>
<th>Postnatal age (days)</th>
<th>1-2</th>
<th>5-6</th>
<th>15-16</th>
<th>19-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. dendrites/.neuron</td>
<td>5.6 ± 1.1 (10)</td>
<td>6.1 ± 1.1 (10)</td>
<td>5.7 ± 1.1 (10)</td>
<td>6.3 ± 1.7 (10)</td>
</tr>
<tr>
<td>Diam. first-order dendrite (μm)</td>
<td>2.4 ± 1.3 (53)</td>
<td>2.9 ± 1.3 (59)</td>
<td>3.1 ± 1.4 (54)</td>
<td>3.2 ± 1.6 (68)**</td>
</tr>
<tr>
<td>Diam. all dendritic branches (μm)</td>
<td>0.61 ± 0.30 (166)</td>
<td>0.69 ± 0.31 (159)</td>
<td>0.74 ± 0.32 (155)</td>
<td>0.78 ± 0.33 (172)**</td>
</tr>
<tr>
<td>Total surface area/neuron (μm²)</td>
<td>15.62 ± 1.60 (7)</td>
<td>14.28 ± 1.26 (7)</td>
<td>14.177 ± 1.08 (7)</td>
<td>13.978 ± 0.99 (7)**</td>
</tr>
<tr>
<td>Dendritic volume/neuron (μm³)</td>
<td>5.93 ± 1.60 (7)</td>
<td>4.89 ± 1.19 (7)</td>
<td>4.97 ± 1.06 (7)</td>
<td>4.96 ± 0.98 (7)**</td>
</tr>
<tr>
<td>No. terminals/neuron</td>
<td>27.8 ± 6.9 (7)</td>
<td>21.8 ± 4.1 (7)</td>
<td>16.0 ± 4.1 (7)</td>
<td>21.6 ± 4.4 (7)**</td>
</tr>
<tr>
<td>Area of influence (μm²)</td>
<td>128.988 ± 50.148 (7)</td>
<td>181.058 ± 50.658 (7)</td>
<td>156.045 ± 55.738 (7)</td>
<td>128.548 ± 30.582 (7)**</td>
</tr>
</tbody>
</table>

Values and statistical representation as in Table 1.

Fig. 1. Dendritic branch diameter of developing genioglossal (GG) motoneurons as a function of dendritic branch order and age. In the oldest age group, there was an increase in mean diameter at all dendritic branch orders. Dendritic diameter was calculated as the average of all dendrites of a given branch order. The cellular dimensions presented in this and the next six figures represent the means from seven totally reconstructed cells in each age group.

Morphometry of individual dendrites

To understand how growth of surface area occurs in different parts of the dendritic tree, we analyzed the changes occurring in different generations of branching (terminal and preterminal) and nodal types. The mean diameter of all dendritic branches showed no significant growth up to 13-15 days. However, there was an increase in the diameter of first-order dendrites between 13-15 days and 19-30 days that also produced an increase in the combined diameter of first-order dendrites.

The surface area observed was not due to an increase in the number of dendrites per cell; their number remained unchanged postnatally. However, there was an increase in the diameter of first-order dendrites between 13-15 days and 19-30 days that also produced an increase in the combined diameter of first-order dendrites.

Morphometry of individual dendrites

To understand how growth of surface area occurs in different parts of the dendritic tree, we analyzed the changes occurring in different generations of branching (terminal and preterminal) and nodal types. The mean diameter of all dendritic branches showed no significant growth up to 13-15 days. Then, it increased significantly ($P < 0.01$) from $1.04 ± 1.05$ μm at 13-15 days to $1.36 ± 1.13$ μm at 19-30 days. The mean branch diameter in the different branch orders is shown in Figure 1. There was a rapid decline in diameter between the first and the second branch orders in all age groups. For all four age groups, the diameter of the second branch order was approximately one-half that of the first branch order. In higher order branches of the dendritic tree, the decline in mean branch diameter was less pronounced. Between birth and 13-15 days, there was no significant increase in diameter of any branch order. However, the growth between 13-15 days and 19-30 days was characterized by a significant increase ($P < 0.05$) in the diameter of all dendritic branch orders. This increase ($P < 0.01$) was most evident in the first and second branch orders. A similar pattern emerged when the caliber of terminal and pretermi-
nal branches was analyzed separately (Fig. 2A,B), where there was an increase in mean diameter in all dendritic branch orders (either preterminal or terminal branches) at 19–30 days. In relative terms, the decrease in diameter between subsequent branch orders was larger for preterminal than for terminal branches. There were also growth patterns in preterminal branches that were specific to nodal vertex type (Fig. 2C). In the four age groups, the diameter of preterminal branches at Vb-nodes was statistically smaller (P < 0.001) than that at Vc- or Vh-nodes. At 1–2 days and 19–30 days, the diameter of the different nodal types was Va < Vh < Vb, while at 5–6 days and 13–15 days, the pattern was Vb < Vh < Va. Figure 3A shows the relationship between dendritic branch diameter and dendritic branch length for preterminal and terminal branches (inset) at 19–30 days. Although only data from the 19–30 day group is presented in Figure 3, the average diameter of the terminal branch was positively correlated (P < 0.001) with the branch length for all four age groups. In contrast, this relationship of diameter to length for the preterminal branches was best fit by a second-order polynomial function (P < 0.05). When this analysis of preterminal branches was extended to specify the nodal type (Fig. 3B), a negative linear correlation (P < 0.001) was found for only Vh-nodes while no correlations were found for either Vc- and Vb-nodes.

Geometry of dendritic trees

In order to predict how synaptic current generated at various locations on the dendritic tree will passively spread to the cell body and initial segment, it is important to describe the geometry of the motoneuron membrane. In Figure 4, the distribution of dendritic surface area is plotted as a function of distance along the dendrite for each of the four age groups. There was a rapid decline in dendritic surface area within the first 300 μm from the soma at all ages. In general, maturation of the GG dendritic tree produces more surface area at greater dendritic distances from the soma, as evidenced by the fact that there were no dendrites (and, therefore, no surface area) beyond 700 μm from the soma found prior to 13–15 days. Total neuronal surface area, volume, and combined dendritic length remained unchanged in the first 2 weeks of postnatal life (Table 2). At 13–15 days, growth beyond 700 μm was counterbalanced by a small decrease in dendritic surface area within the first 200 μm. In a previous paper (Núñez-Abades et al., 1994), we described that the elongation in terminal branches at 13–15 days was accomplished by redireciting membrane of resorbed branches. Later (beyond 13–15 days), the dendritic surface area doubled as a result of increases in both diameter and branch length. This growth was achieved by increasing the dendritic diameter along the whole dendritic tree (see Fig. 1) and increasing the combined dendritic length of the neuron, in part by reelaborating new terminal branches (see Table 2 and Núñez-Abades et al., 1994). Coincident with the increase in the dendritic surface area between 13–15 days and 19–30 days, there was also roughly a doubling in the dendritic volume and the area of influence.

There are several assumptions regarding the geometry of the motoneuron that permit us to approximate the dendritic tree as an equivalent cylinder (Rall, 1959, 1977). One such assumption is that the dendritic trunk parameter (see Materials and Methods) should be approximately equal to 1.0 at varying distances from the soma. In Figure 5, the value of the dendritic trunk parameter has been plotted for four age groups as a function of distance from the soma along the dendrite. Similar to the distributions found for surface area, the values of the dendritic trunk parameter...
Fig. 3. Relationship between branch diameter and branch length in 19-30 days old GG motoneurons. The diameter represents the mean of all measurements (5-10 μm increments) along the segment. A: Preterminal branches. Inset: Terminal branches. Preterminal branches in A are best fit by a second-order polynomial function (r = -0.44) and terminal branches by a linear function (r = 0.66). B: Preterminal branches vs. diameter replotted specifying the nodal vertex type. A significant linear correlation was found only for preterminal branches of Vb-nodal type (r = -0.52, P < 0.001).

were characterized by a rapid decline in this parameter over the first 300 μm. This plot demonstrates that the dendritic trunk parameter decreases with distance at all ages studied. The large deviations of this value from 1.0 indicate that developing GG dendrites are not accurately modeled as an equivalent cylinder.

The observed deviations of the dendritic tree from an equivalent cylinder could result from asymmetrical branching and/or tapering of branches (Barrett and Crill, 1974). Branch symmetry was evaluated by calculating the daughter branch diameter ratio (DBR). When DBRs were calculated for the GG motoneurons in the four age groups, all ratios exceeded 1.0 to varying degrees. Initially, similar values were obtained for 1-2 days and 5-6 days (1.31 ± 0.43 and 1.29 ± 0.43, respectively) while significantly larger (P < 0.05) values were found at 13-15 days (1.54 ± 0.96) and 19-30 days (1.52 ± 0.51). Therefore, the asymmetry at branching points in GG motoneurons increased with age. Further analyses were done taking into account the nodal vertex type (Table 3). Vc-nodes had a value closer to 1.0 than those found in Vb- and Vc-nodes at four age groups. While the values of DBR remained unchanged in Vc-nodes,
Fig. 4. Distribution of dendritic surface area per cell at 100 µm intervals from soma along the dendrite as function of age. In this figure and the next two figures plotting distance along the dendrite, the X axis starts at 100 µm, not at zero, which would be equivalent to the boundary with the soma. Note that the data are calculated at regular increments of distance from soma (e.g., 100 µm, 200 µm, etc.). In order to extend greater distances, the dendrite GG motoneurons at 13–15 days generated less surface area within the first 600 µm than that found for the dendrites at 1–2 days.

DBR in V_r and V_b nodes increased significantly ($P < 0.001$ and $P < 0.05$, respectively) postnatally.

Another parameter indicates that dendritic asymmetry is an unequal length from the soma to dendritic terminations among dendritic branches of the same dendrite. Figure 6 shows the mean number of terminal branches per cell as a function of distance along the dendrite at 100 µm intervals from the soma. The distribution of terminal endings was similar between 1–2 and 5–6 days, with a peak at 300 µm from the soma. However, dendrites at 13–15 and 19–30 days produced fewer numbers of terminal endings within the first 200 µm from the soma and a larger number of them at a distance at 700 µm and beyond. The lower number of terminal branches found in the first 400 µm at 13–15 days was due to the less complex branching of the dendrites at this age (Núñez-Abades et al., 1994). In general, terminal branches end at nonuniform lengths from the soma at all four age groups. Although our analysis has been restricted to anatomical distance, a close relationship between electrotonic and anatomical distance has been described in cortical neurons (Larkman et al., 1992). Therefore, tapering may account for the rapid decline in dendritic trunk parameter found within the first 200–300 µm of the dendritic tree at birth and in the first 200 µm of the dendritic tree of older GG motoneurons.

A second factor contributing to the decrease in dendritic trunk parameter is dendritic tapering (Barrett and Crill, 1974; Rose et al., 1985). Figure 7 shows the taper (measured in µm/100 µm length) as a function of dendritic branch order. The largest taper was found in the first branch order in the older animals and, to a lesser extent, in the first- and second-order dendrites of the newborn. Therefore, tapering may account for the rapid decline in dendritic trunk parameter found within the first 200–300 µm of the dendritic tree at birth and in the first 200 µm of the dendritic tree of older GG motoneurons. Further analysis of tapering was conducted by subdividing branches into terminal and preterminal branches. Tapering in terminal or preterminal branches of adult GG motoneurons (1.24 ± 0.96 and 0.40 ± 0.29, respectively) had values similar to those at birth (0.92 ± 0.79 and 0.35 ± 0.29, respectively). For all four age groups, preterminal branches exhibit greater tapering (all significant, at least $P < 0.05$) than that found in terminal branches. The quantitation of tapering in the finer terminal dendrites may be restricted by our ability to accurately resolve diameters less than 0.5 µm. Given this limited resolution, there might be real differences between ages in tapering in higher order branches that cannot be resolved at the level of light microscopy. Because of the importance of the surface area measurement to the conclusions of this paper, we calculated that surface area would decrease by less than 5% if all terminal branches tapered to endings of 0.1–0.2 µm instead of 0.4–0.5 µm.
Fig. 5. Distribution of the dendritic trunk parameter as a function of distance from soma and age. The dendritic trunk parameter was calculated as the sum of all dendritic branch diameters raised to $3^{1/2}$ power, measured at 100 μm intervals, then divided by the sum of the first-order dendritic diameters raised to $3^{1/2}$ power. Values less than 1.0 indicate that the developing dendritic tree deviates from an ideal equivalent cylinder.

TABLE 3. Daughter Branch Ratios

<table>
<thead>
<tr>
<th>Postnatal age (days)</th>
<th>1-2</th>
<th>5-6</th>
<th>13-15</th>
<th>19-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_a$ nodes</td>
<td>1.00 ± 0.15 (62)</td>
<td>1.12 ± 0.17 (57)</td>
<td>1.19 ± 0.18 (57)</td>
<td>1.22 ± 0.39 (50)**</td>
</tr>
<tr>
<td>$V_b$ nodes</td>
<td>1.41 ± 0.49 (62)</td>
<td>1.44 ± 0.60 (63)</td>
<td>1.22 ± 1.29 (28)**</td>
<td>1.71 ± 0.02 (59)**</td>
</tr>
<tr>
<td>$V_a$ nodes</td>
<td>1.58 ± 0.55 (25)</td>
<td>1.44 ± 0.42 (9)</td>
<td>1.65 ± 0.65 (10)</td>
<td>1.45 ± 0.32 (13)</td>
</tr>
</tbody>
</table>

Values and statistical representation as in Table 1.

Relationship between somal and dendritic geometry

Several dimensions of the soma and dendrites that are relatively easy to measure have been shown to predict the extent and complexity of the dendritic tree in cat spinal motoneurons (Zwaagstra and Kernell, 1980; Ulfhake and Kellerm, 1981, 1983). These relationships are also found throughout postnatal development of cat spinal motoneurons (Ulfhake and Cullheim, 1988; Cameron et al., 1991a). The plots of dendritic dimensions as a function of somal size are presented in Figure 8 for developing brainstem (GG) motoneurons of the rat. Correlations were found between the somal diameter and the combined dendritic diameter of first-order dendrites ($r = 0.42, P < 0.01$; Fig. 8A), and the dendritic surface area ($r = 0.50, P < 0.001$; Fig. 8B). Similarly, correlations were found between the somal surface area and the combined dendritic diameter ($r = 0.49, P < 0.01$; Fig. 8C), and the dendritic surface area ($r = 0.60, P < 0.001$; Fig. 8D) for data pooled across age. However, no correlations between these parameters were found when analyzed within any individual age group. Furthermore, no correlations were found between either the somal dimensions and the number of first-order dendrites, or the
It is important to know if there are any dimensions of the dendrites that could serve to predict the size and complexity of a dendritic tree without performing laborious, detailed reconstructions. Strong correlations were found within the individual age groups for geometry intrinsic to the dendrites. Figure 9 illustrates the relationship between the diameter of the first-order dendrite and the dendritic surface area for each age group. Linear correlation coefficients ($r$) values ranged between 0.94 and 0.97 (all significant, $P < 0.001$). Similar results were found in Figure 10A for the relationship between the diameter of the first-order dendrite and the dendritic volume at all ages ($r = 0.92-0.95, P < 0.001$). In general, the slope of the regression lines varied during development with the oldest age group tending to have a steeper slope than that of the newborn group.

The size of the dendritic tree is also determined by the diameter and the combined dendritic length of the dendrite in GG motoneurons. Linear correlations were also found between combined dendritic length and dendritic surface area ($r = 0.93-0.96$; not shown) and dendritic volume ($r = 0.76-0.84$; not shown). Because both dendritic surface area and volume are correlated positively to the diameter of the first-order dendrites as well as the dendritic combined length, it follows that the two latter parameters should be interrelated. The correlation coefficients for the combined dendritic length as a function of the diameter of the first-order dendrite ranged from $r = 0.85-0.93$ across the four age groups (Fig. 10B). During postnatal development, there was a transient increase in the slope of the regression line between 1–2 and 5–6 days that subsided at the older ages. This transient implies a different temporal pattern for the elongation in GG motoneurons with age, as previously described by Núñez-Abades et al. (1994).

Weaker correlations were found between the number of terminal branches and the diameter of the first-order dendrite ($r = 0.75-0.84$), dendritic surface area ($r = 0.75-0.87$), and dendritic volume ($r = 0.63-0.76$) than those correlations mentioned above. The strongest correlation for the number of terminal branches ($r = 0.82-0.91$) was found with dendritic combined length (Fig. 11). There was a trend for the regression to decrease its slope with age. This change in slope reflects the elimination of terminal branches observed between 5–6 days and 13–15 days, and the reelaboration of the original dendritic complexity at 19–30 days (Núñez-Abades et al., 1994).

DISCUSSION

The goal of this study was to describe the growth of rat GG motoneurons. During postnatal development, the sequence of changes in size, as previously found for changes in dendritic branch structure (Núñez-Abades et al., 1994), can be described in two phases for GG motoneurons. The first phase (from birth to 13–15 days) was characterized by no growth in either dendritic diameter or dendritic surface area. However, the cell morphology is not static during this period; rather, maturation produces more surface area at greater distances from the soma by redistributing preexistent membrane. During the second phase (beyond 13–15 days), the dendritic surface area doubled due to both an increase in dendritic diameter at all branch orders and the...
A relationship was observed between mean somal diameter and combined dendritic diameter of first-order dendrites (A) and total dendritic surface area (B). Relationship between somal surface area (SA) and combined dendritic diameter (C) and total dendritic surface area (D). Data from seven totally reconstructed cells and three partially reconstructed cells in each age group were used for A and C, while only data from seven totally reconstructed cells in each age group were used for B and D (see Materials and Methods). Significant correlations (P < 0.01) were found only for data pooled across ages but not for any individual age group.

Postnatal changes in the receptive domains

To date, a variety of studies have described the rate of growth of the soma in a number of motor nuclei (Mellstrom and Skoglund, 1969; Soto et al., 1978; Rose et al., 1984; Arvidsson et al., 1987; Tatton and Theriault, 1988; Cameron and He, 1989). One of these studies examined the growth in cat GG motoneurons (Brozanski et al., 1989). Using a similar method to measure somal dimensions, these authors reported that the shape and size of the somata changed during postnatal development. Specifically, the mean somal diameter value at birth was only two-thirds of the adult value and the soma became more elongated with age. In contrast, we found that rat GG motoneurons had essentially achieved their adult size and shape at birth. This discrepancy between the species could be due to differences in methodology (intracellular vs. retrograde labelling), or a more accurate estimate of mean diameter from the cat study based on a larger sample (100–150 cells per age group), or simply that rat GG motoneurons mature earlier in postnatal life than those in the cat.

The dendritic tree of GG motoneurons represents 90% of the total surface area at birth. This percentage is smaller than values reported in adult spinal motoneuronal pools. In cat spinal motoneurons, the dendritic tree accounts for greater than 95% of the total membrane surface area in adults (Ulfhake and Kellerth, 1981, 1983; Cameron et al., 1985; Rose et al., 1985; Cullheim et al., 1987). Therefore, the somata of these rat brainstem motoneurons represents a larger relative area available for potential synaptic contacts than the somata of cat spinal motoneurons. In addition, we found that the dendritic to somal surface area ratio increased postnatally in GG motoneurons, as previously reported for developing lumbar and phrenic motoneurons (Ulfhake and Cullheim, 1988; Cameron et al., 1991a). This change in the ratio represents a different time...
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Fig. 9. Relationship between the diameter of the first-order dendrite and the dendritic surface area for the four age groups. The points were fit by a least squares analysis yielding the following equations: 

- 1-2 days (n=55): \( y = 999x - 78, r = 0.94 \) (1-2 days); 
- 5-6 days (n=58): \( y = 1212x - 396, r = 0.97 \) (5-6 days); 
- 13-15 days (n=54): \( y = 1139x - 288, r = 0.95 \) (13-15 days); 
- 19-30 days (n=62): \( y = 1371x - 776, r = 0.95 \) (19-30 days).

All correlation coefficients significant \((P < 0.001)\). Data of totally reconstructed dendrites (number in parentheses) from ten cells (seven total and three partial) in each age group were used in this and the next figure.

Course of growth for the soma as compared to the dendrites. A similar progression was found in developing triceps surae motoneurons of the cat where the soma reached its adult size at 44-46 postnatal days while dendrites were only one-half of their adult surface area (Ulfhake and Cullheim, 1988). In general, it would appear that the somal surface area constitutes a larger target for afferent synapses in developing motoneurons than it does in the adult motoneurons.

Only a few studies have provided information to date on the postnatal elaboration of the dendritic tree. One study found that the diameter of the dendrites of cat lumbar motoneurons increase during postnatal development (Tatton et al., 1983). Growth in diameter has been reported to occur between two months and the adult in phrenic motoneurons (Cameron et al., 1991a), while 50% of the diameter increase in lumbosacral motoneurons takes place after three weeks of age (Ramirez and Ulfhake, 1991). Growth in membrane surface area in developing triceps surae motoneurons of the cat has been reported as a continuous process (Ulfhake and Cullheim, 1988). However, investigations of phrenic motoneurons in the cat (Cameron et al., 1991a), and in the rat (Spielmann et al., 1992), as well as the present study of GG motoneurons in the rat show that the process of motoneuronal growth is not continuous. Instead, there are periods when there is no increase in total surface area, and these periods coincide with a remodeling of the dendritic tree that produces a substantial change in branching complexity and dendritic length. These dramatic changes in morphology are accompanied by important changes in the electrophysiological properties of these cells (Cameron et al., 1991a; Mazza et al., 1992; Núñez-Abades et al., 1993).

Functional significance

One goal of the present study was to determine whether the growth of total membrane surface area could explain the rapid decrease in mean input resistance of GG motoneurons measured between 5-6 days (30.4 ± 25.9 MR) and 13-15 days (10.5 ± 18.6 MR; Núñez-Abades et al., 1993). In the present study, we conclude that there was no increase in surface area between 5-6 days and 13-15 days and, therefore, the increase in membrane surface area cannot account for the decrease found in input resistance. A similar conclusion was derived from work in cat phrenic motoneurons where the mean input resistance of those neurons decreased from 4.1 ± 0.7 MR at two weeks to 1.9 ± 1.0 MR at one month (Cameron et al., 1991b). During that period, there was a similar lack of increase in total membrane surface area (Cameron et al., 1991a). Therefore, we propose that the mechanisms to explain the decrease in input resistance involves a change at the level of the unit membrane. One possibility to explain the decrease in input resistance is a decrease in specific membrane resistance of...
the diameter and the dendritic volume for the four postnatal age groups. The equations for the linear regression are: $y = 506x + 245$, $r = 0.93$ (1-2 days); $y = 473x - 232$, $r = 0.95$ (5-6 days); $y = 533x - 318$, $r = 0.92$ (13-15 days); $y = 763x - 659$, $r = 0.90$ (19-20 days). B: Relationship between the diameter of the first-order dendrite and the combined length dendritic for the four postnatal age groups. The equations of the linear regression are: $y = 294x + 47$, $r = 0.85$ (1-2 days); $y = 41x - 102$, $r = 0.93$ (5-6 days); $y = 356x - 21$, $r = 0.87$ (13-15 days); $y = 318x - 56$, $r = 0.85$ (19-20 days). All significance levels exceeded $P < 0.001$.

The growth of the dendritic membrane area will also alter the dendritic cable properties of the cell and, thus, the postsynaptic effects of its synapses (Rail, 1977; Horwitz,
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Fig. 11. Relationship between combined dendritic length and number of terminal branches for the four age groups. The equations of the linear regression are: \( y = 0.0047x + 0.62, r = 0.91 \); \( y = 0.0032x + 0.92, r = 0.82 \); \( y = 0.0033x + 0.66, r = 0.67 \) for 1-2, 5-6, 13-15, and 19-30 days, respectively. All correlations significant \((P < 0.001)\).

1981; Wolf et al., 1992). The detailed morphology generated in the present study has been used to test some anatomical assumptions underlying the equivalent cylinder model of the motoneuron (Rall, 1959, 1977). We found that the dendritic trunk parameter deviated from a value of 3.0, indicating that developing GG dendrites are not well suited to be modeled as an equivalent cylinder at any age. We concluded that the rapid decline in dendritic trunk parameter in the first 300 \( \mu \)m of the dendritic tree was due to the large amount of tapering found in the first- and second-order dendrites at four age groups. Because tapering is small in terminal branches and in the higher branch order dendrites, nonuniform lengths of branches may be more responsible for the observed decrease in dendritic trunk parameter beyond 400 \( \mu \)m. However, tapering in the terminal branches could be underestimated because of the accuracy to which we could resolve diameters less than 0.5 \( \mu \)m. Furthermore, tapering could be influenced by the way of estimation itself. The absolute amount of taper, as used here, is influenced by the decrease in average branch diameter that occurs with increasing distance (branch order); therefore, tapering may be underestimated (Rose et al., 1985). To avoid this problem, different formulas have been used to normalize taper values (i.e., by the branch start diameter) that in some cases overestimate the degree of expansion, or overestimate the degree of taper as well (see Rose et al., 1985).

In the present study, using measurements of absolute taper, we found that dendritic tapering was most marked on first-order dendritic branches, as previously demonstrated for cervical motoneurons of the cat using normalized tapering (Rose et al., 1986) or absolute tapering (Cameron et al., 1990). In addition, values of absolute tapering allow data to be compared between different motor pools. Further investigations are now required to explore the electrotonic properties of developing mammalian motoneurons, and to determine whether dendritic growth is regulated to maintain a constant electrotonic length, or whether other mechanisms are employed to maintain synaptic efficacy of even the most remote synapses.

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LITERATURE CITED


