RESEARCH ARTICLE

Geometrical Differences Among Homologous Neurons in Mammals

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Abstract. *The dendritic arbors of sympathetic neurons in different species of mammals vary systematically: the superior cervical ganglion cells of smaller mammals have fewer and less extensive dendrites than the homologous neurons in larger animals. This difference in dendritic complexity according to body size is reflected in the convergence of ganglionic innervation; the ganglion cells of progressively larger mammals are innervated by progressively more axons. These relations have implications both for the function of homologous neural systems in animals of different sizes and for the regulation of neuronal geometry during development.*

A striking feature of the vertebrate nervous system is the wide variation in dendritic form (*1*). At one extreme are neurons that lack dendrites altogether; at the other are nerve cells with hundreds or even thousands of dendritic branches (Fig. 1). This diversity raises two related questions: why do animals harbor neurons with such different shapes, and how is this range generated during development?

One generally accepted rationale for diverse neuronal morphologies is that dendrites modulate synaptic strength by interposing a variable electrotonic distance between inputs and the region of the cell that initiates the action potential (usually the axon hillock) (*2*). Thus, by altering a cell's electrical properties, dendritic branches influence a neuron's integrative characteristics and, consequently, its output. Another idea is that geometrical diversity regulates the number of different inputs that a neuron receives (*3, 4*). The evidence for this view is that the number of axons that innervate neurons in some autonomic ganglia varies systematically according to the geometrical complexity of the target cells. Thus, ganglion cells that lack dendrites tend to be innervated by a single preganglionic axon, whereas neurons with dendritic branches are innervated by a number of different axons, that number increasing in proportion to the number of dendrites (*3, 4*).

We have explored this apparent link between neuronal geometry and convergence by examining the shape and innervation of homologous sympathetic neurons in a number of different mammals. Our initial purpose in this study was to assess the generality of the relation between convergence and geometry first described in a parasympathetic ganglion (*3*). Our results show a consistent proportionality between neuronal shape and convergence in the sympathetic ganglia of the several species we examined. During the course of the work, however, we found another unexpected relation: the length and complexity of dendritic arbors of homologous ganglion cells increase with the adult size of the animals studied. This systematic variation of dendritic complexity with body size has both functional and developmental implications that bear on the general questions of why neuronal shapes are so diverse and how such a wide range of geometries is generated.

Methods used to compare convergence and geometry in autonomic ganglia. We chose the superior cervical ganglion for this study because it is accessible and thoroughly described and because the number of axons innervating each neuron can be measured with reasonable accuracy by stimulating the relevant ventral roots (*5, 6*). Furthermore, sympathetic ganglion cells are considerably more complex in both shape and function than parasympathetic ganglion cells (*3, 4*) and are arguably better models for neuronal interactions in the central nervous system. To determine the number of axons innervating superior cervical ganglion cells in various species, young adult mice, hamsters, rats, guinea pigs, and rabbits were killed by an overdose of pentobarbital; the right ganglion was dissected in continuity with the sympathetic trunk, sympathetic chain ganglia, communicating rami, and ventral roots, down to the level of approximately the ninth thoracic segment (*5*). This isolated preparation was placed in a Lucite chamber and superfused with an oxygenated saline solution. Intracellular recordings from ganglion cells were made with glass microelectrodes filled with 0.5*M* potassium acetate. We determined the number of axons innervating each ganglion cell by counting the excitatory postsynaptic potentials that could be recruited by gradually increasing the stimulus strength to each of the ventral roots that supplies innervation to the ganglion (*5, 7*).

To assess the geometry of these neurons, we removed the right superior cervical ganglion in additional animals and labeled between six and ten neurons in each ganglion by intracellular injection of horseradish peroxidase (HRP) (*3*). After 1 to 4 hours the ganglia were fixed overnight and the HRP reaction product developed according to the method of Hanker *et al.* (*8*). The ganglia were dehydrated, cleared, and mounted whole; labeled neurons were drawn with a camera lucida (×300). Only cells whose axons were clearly visible for at least several hundred micrometers were included in the study. Very few labeled neurons failed to meet this criterion.

Several measures were used to evaluate the dendritic complexity of HRP-labeled superior cervical ganglion cells. (i) We counted the number of primary dendrites arising from each ganglion cell body. A primary dendrite was defined as a process other than the axon that extended radially from the cell body for a distance at least equivalent to diameter of the cell body. (ii) We measured the total length of a cell's dendrites from camera lucida drawings of the labeled neurons with a digitizing tablet and a computer. (iii) We determined the number of dendritic processes crossing a circle centered on the cell body with a radius of half the distance from the center to the farthest branches of the dendritic arborization. Each of these values provides a somewhat different index of the average complexity of the neurons in the species studied.

Correlation between the number of innervating axons and the dendritic ge-

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ometry of superior cervical ganglion cells in a single species. As a first step, we asked whether the correlation between inputs and target cell geometry observed in the rabbit ciliary ganglion (*3*) was also apparent in a sympathetic ganglion of a particular species.

Fifty-five neurons in the right superior cervical ganglia of 11 guinea pigs were filled with HRP after we had estimated the number of inputs to individual cells by successive stimulation of the relevant ventral roots. Individual neurons tended to receive a number of inputs proportional to the complexity of their dendritic arbors (Figs. 2 and 3). Thus, cells that had relatively simple geometries had fewer inputs, on average, than cells with more complex shapes. For example, the mean number of inputs recorded in the simplest cells in this series was only about half of that determined for the most complex neurons (Fig. 2). Figure 3 shows the relation of geometry and innervation for the full population of guinea-pig neurons studied; the same general result was obtained for each measure of dendritic complexity examined. These findings indicate that in sympathetic ganglia, as in simpler parasympathetic ganglia, the degree of convergent innervation is proportional to target cell geometry.

Correlation of superior cervical ganglion cell geometry and convergence in different species. We next examined neuronal geometry and innervation across a range of animals. A similar correlation between inputs and neuronal geometry was observed when, instead of comparing different neurons in a particular ganglion of one species, we compared the average innervation and dendritic morphology of superior cervical ganglion cells in different species.

Intracellular recordings were made from a large sample of superior cervical ganglion cells in mice, hamsters, rats, guinea pigs, and rabbits, and the mean number of inputs innervating each cell was determined. The cells received the following mean numbers of inputs: mouse, 4.5; hamster, 7.2; rat, 8.7; guinea pig, 12.3; and rabbit, 15.5 (Fig. 4A). We then evaluated the geometry of superior cervical ganglion cells in these animals. HRP labeling of at least 100 homologous neurons in each of the five species showed a systematic progression of dendritic complexity that followed the same order as increasing convergence (Figs. 4 and 5 and Table 1). The overall dendritic length of superior cervical ganglion cells also increased in the order mouse, hamster, rat, guinea pig, and rabbit (Fig. 4B and Table 1). Other measures of dendritic complexity (number of primary dendrites and degree of dendritic branching) gave the same result. For each of the species examined, the average number of axons innervating ganglion cells and average dendritic complexity varied together (Fig. 4).

Geometry and convergence as a function of body size. It was apparent that the order of both increasing convergence and increasing dendritic complexity corresponded to the order of increasing body size (Figs. 4 and 5 and Table 1). Thus the superior cervical ganglion cells in a small animal like the mouse are simpler and receive fewer inputs than the corresponding neurons in a larger animal like the rabbit; hamsters, rats, and guinea pigs have sympathetic neurons of intermediate dendritic complexity and convergence that also rank in order of animal size.

The superior cervical ganglion cells of a diminutive mammal like the mouse are not simply smaller copies of the homologous neurons in a larger animal like the

Table 1. The dendritic complexity of superior cervical ganglion cells in different species. These measurements are subject to some inaccuracy because camera lucida projections are two dimensional; however, since the arborization of the cells is largely in the plane of the ganglion surface, the error is probably small and, in any case, is similar for all the cells studied. Dendritic measures are given as means.

Animal	*n*	Weight (g)		Cells	Dendritic complexity		
		Range	Mean		Primary dendrites	Total dendritic length (μm)	Branches crossing 50 percent circle
Mouse	11	20 to 30	27	104	4.4	788.6	5.9
Hamster	12	70 to 140	99	104	6.5	1206.4	6.4
Rat	13	150 to 200	181	100	8.5	1701.4	9.5
Guinea pig	18	270 to 450	339	103	11.3	2640.0	12.6
Rabbit	10	900 to 1800	1262	100	11.9	4143.3	14.4

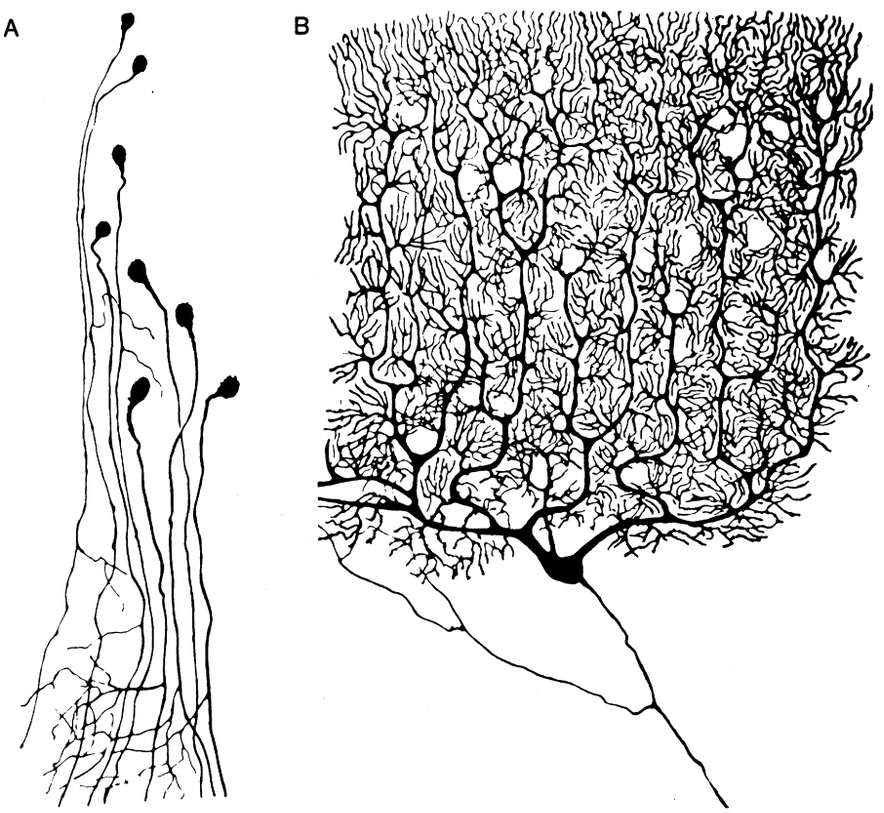


Fig. 1. Diversity of neuronal shapes in the mammalian nervous system. (A) Some classes of neurons—in this case motor neurons in the trigeminal nucleus—lack dendritic processes altogether. (B) Other neurons—in this example cerebellar Purkinje cells—have complex dendritic arbors. Virtually all intermediate forms between these two extremes can be found in various parts of the nervous system [adapted from (*1*)].

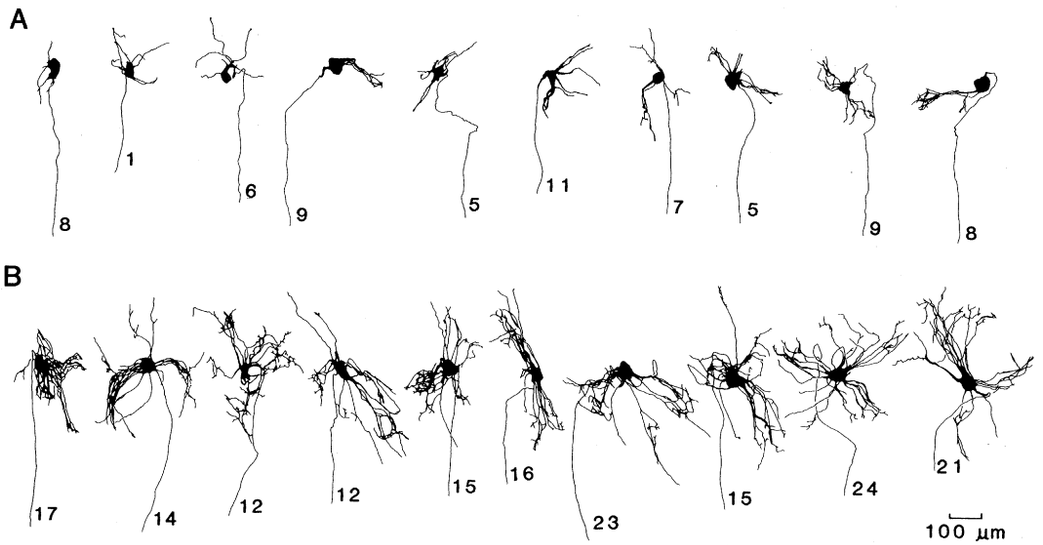


Fig. 2. Camera lucida drawings of (A) the ten least complex superior cervical cells and (B) the ten most complex cells from a series of 55 HRP-labeled neurons in 11 guinea pigs. The cells are arranged from left to right according to the criterion of total dendritic length. The electrophysiological estimate of the number of inputs received by each cell is indicated beside the axon (which in each case extends much farther than shown). On average, geometrically simpler cells received fewer inputs than more complex neurons. There were, however, individual deviations from the general rule that increasingly complex neurons receive proportionately more innervation: some cells were innervated by either more or fewer axons than expected from their shape. This observation indicates that the relation of inputs and geometry is probably not rigidly determined according to the number of primary dendrites. More than one axon must often impinge on a primary dendrite and its secondary and tertiary branches, whereas in other instances one axon must innervate more than one primary dendrite (*4*).

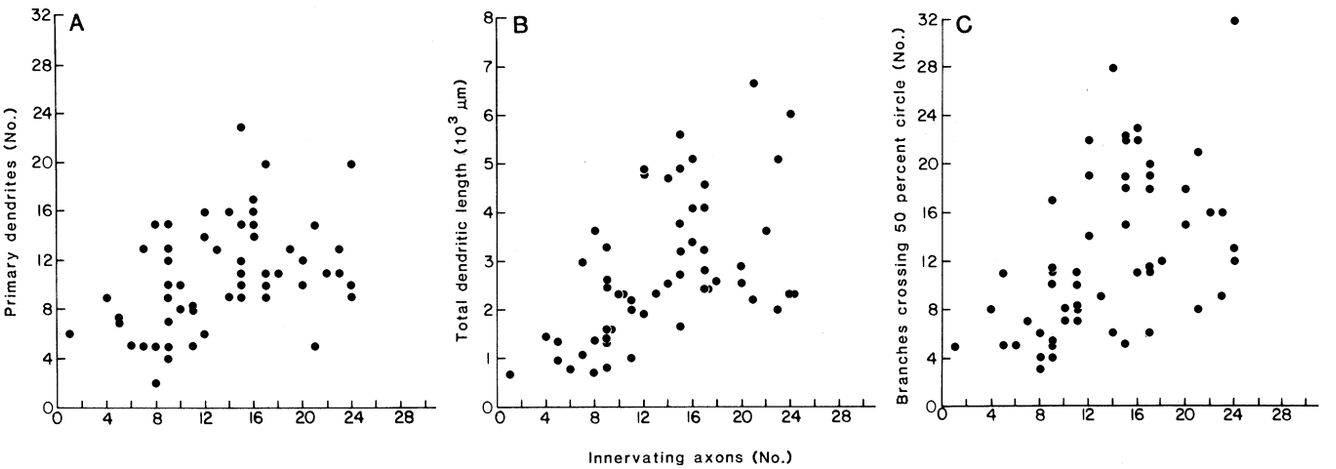
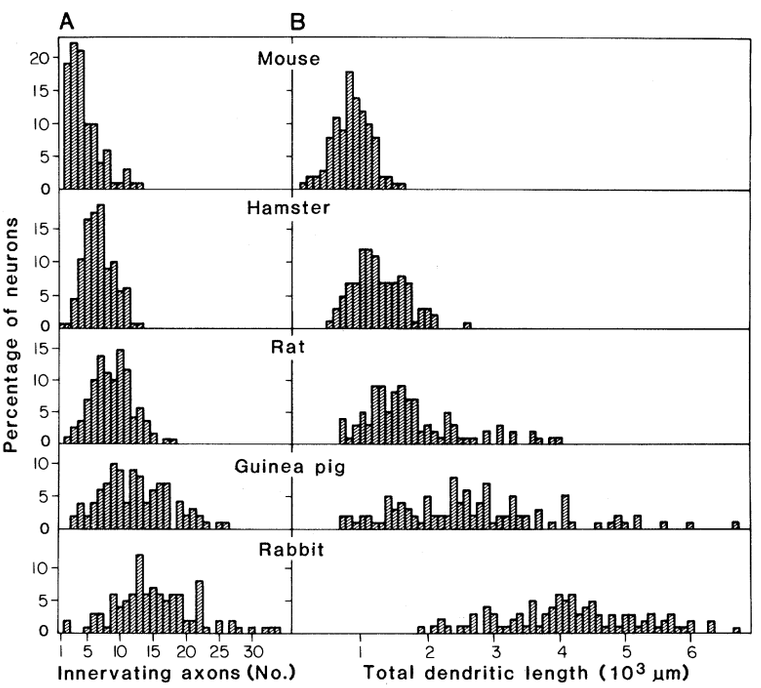


Fig. 3 (above). Preganglionic inputs to guinea-pig superior cervical ganglion cells (n = 55) as a function of (A) the number of primary dendrites, (B) total dendritic length, and (C) the number of dendritic branches crossing a circle at half the radius of a circle encompassing all of the dendrites. In each case, convergence increases with dendritic complexity. Fig. 4 (right). Comparison of convergence and geometry in the superior cervical ganglion of different species. (A) Distribution of the number of inputs received by superior cervical ganglion cells measured electrophysiologically during ventral root stimulation. The number of neurons studied in each species ranged from 100 to 205. [Results for the hamster are adapted from figure 4 in (*6*)]. (B) Distribution of dendritic complexity according to the criterion of total dendritic length. Each histogram represents measurements of 100 to 104 cells labeled with HRP.



rabbit, however; the neurons in progressively larger animals not only have more total dendritic length but are also more complex, having more primary dendritic branches, more higher order branches, and dendritic branches that extend farther from the cell soma (Fig. 5 and Table 1). This presumably means that the rate or duration of dendritic growth increases in progressively larger animals.

Implications. One implication of these results is that the relation between the number of innervating axons and the shape of autonomic ganglion cells is general. In the several instances in which both the number of inputs and target cell geometry are known for different classes of autonomic neurons, these two measures vary concordantly. Thus, parasympathetic ganglion cells that lack dendrites are generally innervated by a single axon (*3, 7, 9*), whereas sympathetic neurons with dendrites are generally innervated by a number of different axons (*5, 10*). The significance of this relation is underscored by the demonstration that, even within a single parasympathetic ganglion, target cell shape and convergence are correlated (*3, 4*). Here we show a similar relation in a sympathetic ganglion (Figs. 2 and 3) and among homologous sympathetic ganglion cells in different animals (Figs. 4 and 5). It is unlikely that these differences arise from qualitative differences among the targets of superior cervical ganglion cells since these neurons innervate the same classes of end organs in each species examined. Taken together, these observations indicate that the relation between geometry and convergence probably holds throughout the autonomic nervous system and raise the possibility that a similar relation may obtain throughout mammalian nervous systems.

An unexpected finding in the comparison of homologous ganglion cells in different species was the systematic variation of dendritic geometry and neural convergence with animal size (Fig. 6). The apparent interdependence of these three variables raises several general issues about neural function.

The different sizes of animals must require different quantitative relationships between pre- and postsynaptic cells in the autonomic and other parts of the nervous system. The mass of the peripheral neural targets in an animal like the rabbit is much greater than the mass of the homologous targets in a smaller mammal, such as the mouse. Thompson and others (*11*) pointed out many years ago that one way of compensating for this disparity would be to increase the number of nerve cells in the neural systems of larger animals. Although to our knowledge this issue has not been examined in detail, the number of neurons in the superior cervical ganglion of a relatively large animal like the rabbit (and presumably the number of preganglionic neurons that supply the ganglion) is greater than the number of homologous neurons in the mouse. Modulation of neuronal number by the normal death of redundant neurons (a developmental phenomenon that is regulated by the mass of the target tissue) seems to be one strategy by which the presynaptic population is matched to the size of the target (*12*). Our results show that the quantitative relation between pre- and postsynaptic populations also varies with the geometry of target neurons; the more complex the target cells, the greater the convergent innervation they receive. Altered convergence is likely to have a number of functional consequences; the tonic rate of neuronal activity, for instance, increases as a function of convergence in the rabbit ciliary ganglion (*13*). The number of neurons innervated by each axon (the neural unit size)

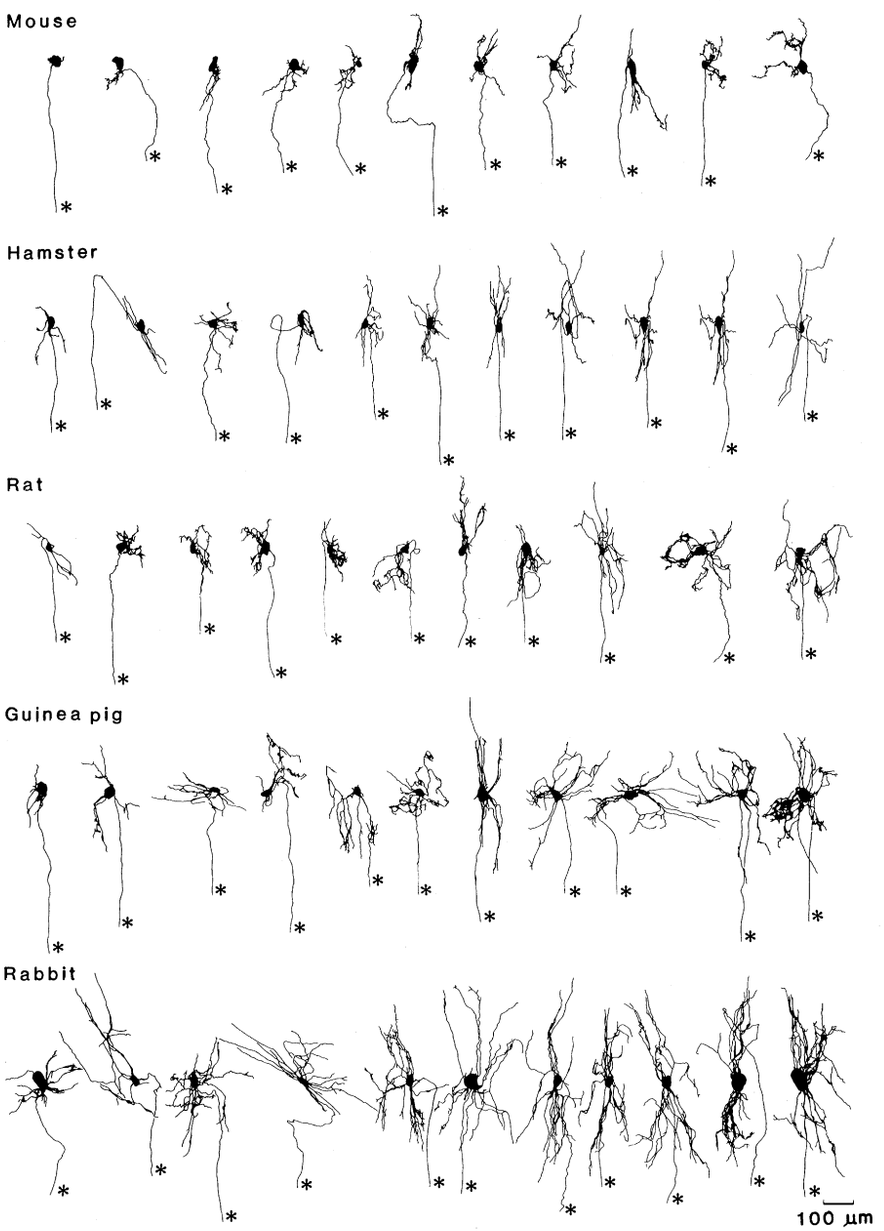


Fig. 5. Camera lucida drawings of every tenth superior cervical ganglion cell from samples of 100 to 104 neurons studied in each species. The neurons have been arranged in order of increasing total dendritic length to show the range of dendritic complexity in each animal; the asterisk denotes the axon (which in all cases extends farther than shown). Although the range of dendritic complexity overlaps between species to some degree, the intricacy of the dendritic arborizations of these homologous neurons increases in parallel with increasing size of the animal examined (Table 1).

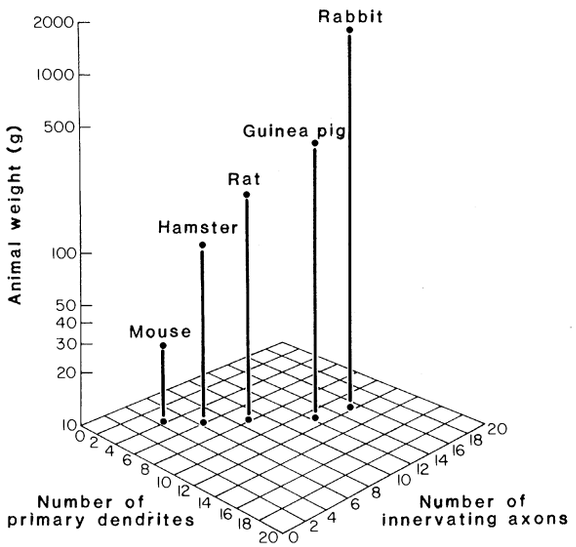


Fig. 6. The association of dendritic complexity, convergence, and animal size. The index of dendritic complexity here is the number of primary dendrites. Size is given as weight on a logarithmic scale. Values are taken from Fig. 4A and Table 1.

is also likely to be affected. Perhaps modulation of (i) the overall number of cells in a neural system and (ii) the degree of convergent innervation are related strategies for varying the efficacy of neural pathways in different sized animals.

Another question raised by the relation of animal size and dendritic complexity concerns the developmental mechanisms underlying the generation of neuronal form. One way that animals generate neurons with diverse shapes is presumably by a progressive change in genetic instructions during early development (that is, neuronal differentiation). Another way of generating neuronal diversity, however, may involve altering only quantitative aspects of development, such as the rate or length of time that dendrites proliferate. The differences in dendritic complexity among the homologous neurons that we have described here may arise primarily in this manner. Dendritic complexity in the five animals we examined is roughly correlated with the duration of their development. Differences in both animal size and the dendritic complexity of homologous neurons may arise in part from the different developmental timetables of these animals.

Conclusion. The relation between adult neuronal geometry and the number of axons impinging on target neurons seems to be a general one in the peripheral nervous system of small mammals. The further relation of the dendritic complexity of superior cervical ganglion cells to animal size suggests that different animals may regulate the degree of convergence in homologous neural pathways by varying the geometry of the target neurons. The manner in which regulation of dendritic complexity occurs and the functional significance of the ever-greater preganglionic convergence in the sympathetic system of progressively larger animals are issues that should be explored in these and other neural systems.

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