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The Growth of Dendrites in the Mammalian Brain

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Summary. The histological features of developing dendrites are analyzed in age-graded series of cats, rats, rabbits, and opossums with the Golgi techniques. Growth cones and filopodia are consistent features of growing dendrites in the sensory nuclei, motor cranial nerve nuclei, brain stem reticular formation, cerebellar cortex, midbrain tectum, thalamus, hypothalamus, striatum, olfactory bulb, hippocampus, pyriform lobe, and neopallium. Terminal dendritic growth cones occur at the tips of dendrites; preterminal growth buds occur on the dendritic shafts. Filopodia are conspicuous on the growth cones and buds, but they occur on the dendritic shafts, also. Terminal growth cones, preterminal growth buds, and filopodia associate with incipient dendritic branches. Growth cones and filopodia generally disappear when the neurons are completely differentiated. But some dendritic growth cones persist after the rest of the brain is mature and consequently may be involved in learning and other plastic changes in neural function. The analysis illustrates the following general trends in neural ontogeny, none of which are expressed without modifications and qualifications. The dendrites of the specific types of neurons differentiate in typically circumscribed periods, which occur in a fixed sequence. Within a specific neuronal population there may be regional gradients in the degree of dendritic differentiation. Within the same region the dendrites of large cell bodies tend to differentiate before those of small cell bodies. The dendrites of Golgi Type II neurons differentiate later than neurons with long axons from the same thalamic nucleus. In the afferent sensory systems the neurons nearer the peripheral receptors usually begin their differentiation before those nearer the cerebral cortex. The dendrites of phylogenetically older neuronal groups tend to differentiate earlier than those of more advanced or more highly specialized groups. The dendrites and the other post-synaptic surfaces of the neurons differentiate in conjunction with the particular afferent axonal end-branches that are destined to synapse with the dendrites. Dendritic differentiation may be instigated by the afferent axons, controlled by local physico-chemical conditions, and guided by contact with the afferent axonal end-branches.

Key Words: Nervous system — Embryology — Neurogenesis.

Introduction

The growing axons and dendrites of the nervous system are distinguished by characteristic enlargements, the growth cones. Previous study (MOREST, 1968c, 1969) of the developing medial trapezoid nucleus demonstrated axonal and dendritic growth cones similar to those of regenerating peripheral nerves (HARRISON, 1910; SPEIDEL, 1964). Their activities establish the stereotyped branching patterns of the axonal and dendritic endings and participate in the formation of synapses in that nucleus. The present study is an attempt to identify dendritic growth cones and to trace similarities in the developmental patterns of dendrites in other

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parts of the brain. In order to indicate the generality of the analysis, the report gives a sample of the observations of the young neurons in neuronal groups from each major subdivision of the brain in several species. With the previous studies of the medial trapezoid nucleus as a guide, the approach has been to construct cytogenetic series, consisting of the transitional forms of differentiating neurons, to define the developmental stages of these series, and to trace the patterns of morphological change through succeeding stages in progressively older animals. The observations pertain to morphologically homogeneous populations of neurons, in regions where the necessary details of the mature neuronal architecture are available. The growth cones and sprouting filopodia are consistent features of dendritic growth in the different parts of the brain. Growth cones regularly accompany dendritic elongation and participate in the differentiation of the dendritic branching patterns and in the elaboration of the synaptic organization of the brain. The findings have some implications for the analysis of the factors involved in neurogenesis.

Materials and Methods

The observations apply to the brains of cats, rats, rabbits, and opossums, arranged in age-graded series from fetus to adult and prepared in serial sections with the rapid Golgi technique after perfusion-fixation, according to MOREST and MOREST (1966) and MOREST (1968b). A detailed account of the developmental series and of the analytical methods used appears in previous articles (MOREST, 1968c, 1969). Many of the findings are also verified in Golgi-Cox impregnations, available from previous investigations. In addition the present material includes rapid Golgi and Golgi-Cox preparations from four-, five-, six-, seven-, nine-, and twelve-month old cats. There are also brains from cats and rats, prepared by the Golgi-Kopsch technique (ROMEIS, 1948) after perfusion-fixation and by similar modifications of the Golgi techniques, based on primary formaldehyde, glutaraldehyde, and acrolein fixations. The illustrations are camera lucida drawings. Every detail has been checked with an oil-immersion, apochromatic or semi-apochromatic objective with a numerical aperture (N.A.) of 1.3 or 1.25. The kind of lens actually used to make each drawing is noted in the legends. Illustrations of many of the mature types of neurons under consideration are published elsewhere. Where the text does not refer to these, many useful illustrations may be found in the work of RAMÓN Y CAJAL (1909, 1911) or of RAMÓN-MOLINER (1962, 1967b, 1968).

Results

Specific types of neurons in the present material are distinguished on the basis of the number, size, and branching pattern of the dendrites, the shape of the dendritic field, and the appearance of the dendritic appendages of the mature neurons. Each neuronal population of a specific dendritic type appears to be homogeneous with respect to other morphological features as well, including the appearance of its cell bodies in Nissl-stained sections and the forms of the afferent axonal endings associated with it in rapid Golgi preparations. Only morphologically homogeneous populations are considered here. Within each brain stem nucleus and cortical area examined, the neurons of one population commonly intermingle with those of another. Fortunately, most types of neurons consistently mature at different ages. The dendritic morphology of many neuronal populations is sufficiently distinct that they may be identified at a stage when they are not yet fully differentiated. Each type of neuron differentiates during a predictable span of time, which may overlap with that of another type of neuron. For these reasons

the observations are limited to neurons that are unequivocally identified as to type. The observations of the gradual dendritic differentiation in progressively older neuronal populations indicate that they behave as developmental units. In the analysis they are treated as such.

The specific neuronal populations considered in the present report come from the thalamus, the cerebral cortex, the midbrain, and the hindbrain. The thalamus in typical Golgi impregnations contains discretely delineated groups of neurons, which consist of relatively few morphological types. In some of these groups only two types of neurons are recognized. These are the principal neurons, which project to the cerebral cortex, and the Golgi Type II neurons, the axons of which end in the same neuronal group in which they originate. Because of their relative morphological simplicity, compared to most other cerebral nuclei, some of the thalamic groups offer especially favorable opportunities to follow the maturation of their constituent neuronal types in the developmental series. The first example to be considered is the small Golgi Type II neuron of the lateral geniculate body. Its dendritic differentiation is treated in detail. The subsequent examples are described in less detail, since they conform more or less to the same general pattern. The consistent variations of the general pattern that have been recognized in each example are described. Finally some general features of the material are given in a separate section. Observations of the neuroblasts of some of the neuronal populations are given in a brief report (MOREST, 1968a), soon to be published in full.

In the lateral geniculate body the dorsal nucleus contains the principal neurons, the axons of which project to the striate cortex, and the Golgi Type II cells, or neurons with short axons, which ramify within the dorsal nucleus. A specific population of small Golgi Type II neurons occurs in the dorsal nucleus of the cat (Fig. 1). This population corresponds to the cell type illustrated by RAMÓN Y CAJAL (1911: Fig. 254) in the cat and by POLYAK (1957: Fig. 219) in the monkey. Its cell body is 10—15 μ in diameter in rapid Golgi impregnations from the six-week old cat. The cell body usually has a smooth surface, but an irregular shape. From the cell body extend four or five main dendrites. On the secondary branches of the dendrites are thin appendages or spicules. The spicules extend nearly perpendicular to the dendritic shaft. They are usually two or three microns long, but they may be shorter than one micron or longer than five microns. They may have enlargements at their tips, and sometimes they branch. The dendritic spicules of the Golgi Type II neuron impart a characteristic fringed appearance to the dendrites, which are clearly distinguished from those of all other types of cells. The tips of the dendrites end in a plumed array of delicate branches, which are longer than the spicules. The individual terminal branches taper to a point, or they end as tiny spheres, scarcely larger than the branches themselves. The main stem dendrites seem disproportionately thick, compared to those of the principal neurons. The axon forms numerous branches, which ramify mostly within a volume of tissue encompassed by the dendritic field of the parent neuron (Fig. 1, *E*, *G*). The short terminal axonal branches usually end in tiny knobs.

The morphological attributes of the small Golgi Type II neuron are sufficiently distinct that it may be clearly distinguished from all other neuronal types when its dendrites are still immature. In the cat the principal neurons have nearly

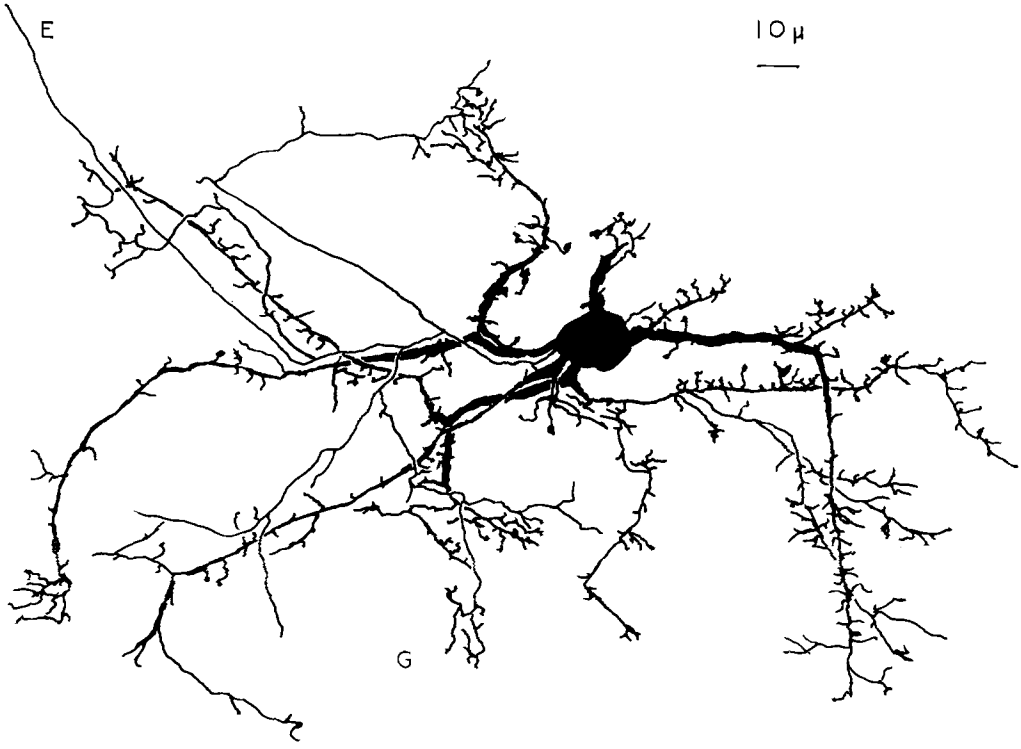

Fig. 1. A fully differentiated Golgi Type II neuron from layer *A 1* of the dorsal nucleus of the lateral geniculate body. The axon leaves the section at *E* after forming several collateral networks, e.g., at *G*. Parasagittal section from a 41-day old cat (littermate of specimen illustrated in Fig. 2 right). Camera lucida drawing of a Golgi-Cox preparation made with an apochromatic objective of numerical aperture (N. A.) 1.0

completed their differentiation by the end of the second postnatal week. During this time the differentiation of the small Golgi Type II neuron begins. In the second postnatal week one may find unequivocal examples of immature Golgi Type II neurons (Fig. 2, right). The cell body and the dendritic trunks seem disproportionately large, compared to the mature neuron, as if they contained accumulations of cytoplasm about to stream into the newly forming secondary branches. The cell body and the dendrites have fine, usually unbranched, filiform appendages, or filopodia, which are practically absent in the mature neuron. *The dendritic filopodia are particularly prominent at the ends of the immature dendrites, where they usually arise from enlargements, the terminal dendritic growth cones* (Fig. 2, *A*). The filopodia have the same appearance as the sprouts of the axonal growth cones in the medial trapezoid nucleus (Morest, 1968c). Some of the terminal dendritic growth cones contain tiny lacunae, possibly corresponding to vacuoles or to clumps of unimpregnated organelles (Fig. 2, *A*). Lumpy enlargements occur on the preterminal portions of the dendrites, particularly on the distal branches. They resemble the terminal dendritic growth cones. These preterminal growth cones or buds may also have thin, short filopodia. *Larger, thicker*

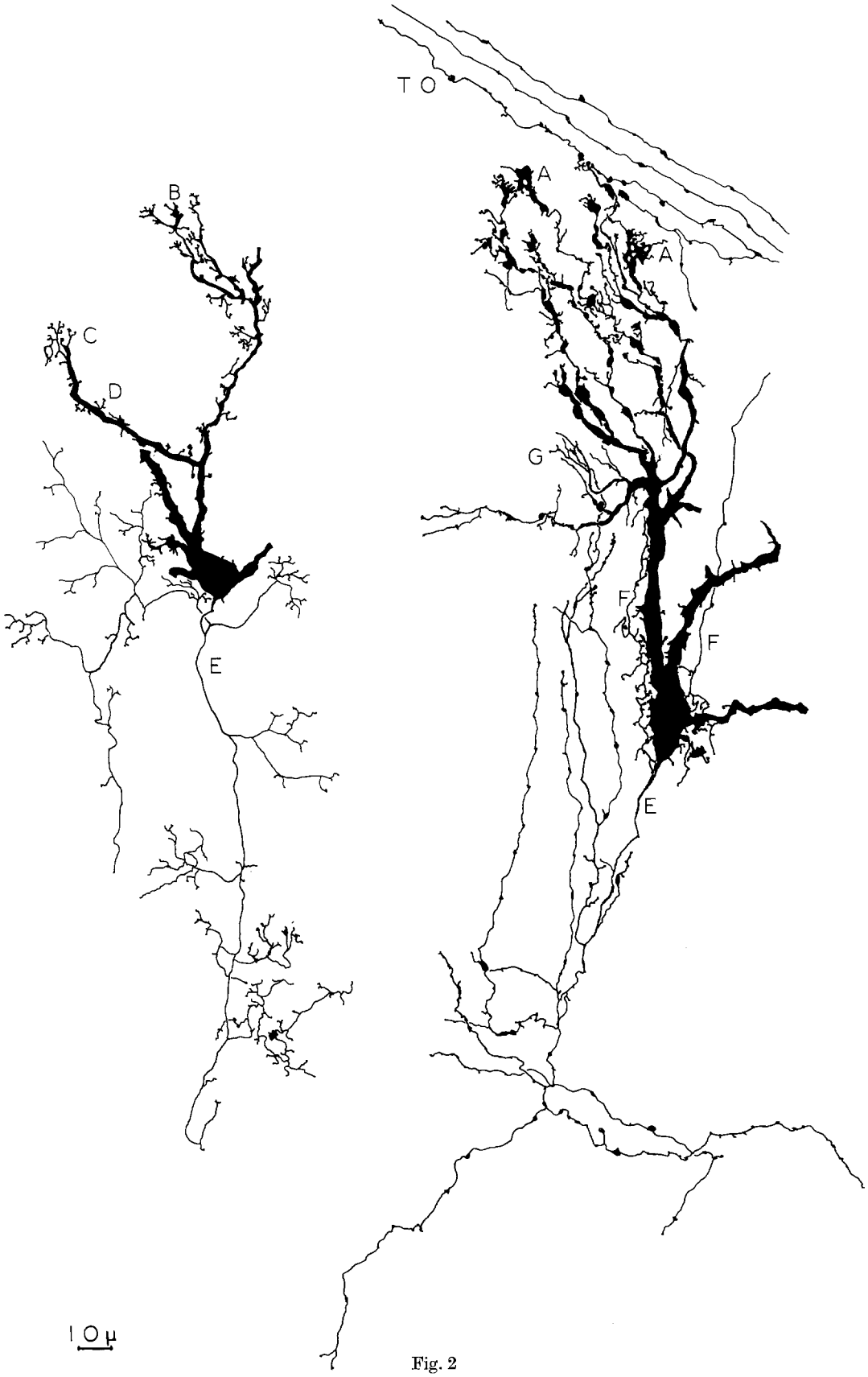

Fig. 2

processes, the incipient dendritic branches, consistently associate with the preterminal growth buds. The incipient branches themselves end in growth cones. The preterminal portions of some of the growing dendrites may be very thin, even when they end in relatively large growth cones, as if cytoplasm were accumulating at the ends of the dendrites. Beady enlargements, located along the thin dendritic branches, may represent packets of cytoplasm streaming through the dendritic process en route to the terminal growth cone. The beady enlargements are usually much smaller than the distal growth cones and are not associated with filopodia.

The axon of the Golgi Type II neuron begins to ramify in the immediate vicinity of the cell of origin very early in the neuron's development, during the first postnatal week (Fig. 2 right, *E*). Along the axis of the thin, delicate axon are some small beady enlargements, irregular in shape and arranged at uneven intervals. Many of these enlargements have sprouts and resemble preterminal axonal growth cones. They apparently are foci for the proliferation of the numerous short end-branches that characterize the entire length of the older Golgi Type II axon (Fig. 2 left, *E*). *Thus the axon grows and differentiates throughout its proximal extent as well as at its most distal ends.* The small, smooth, more regularly beaded enlargements along the course of the axon may be clumps of axoplasm streaming through the axon in one direction or the other. There is no indication that very much material collects at the tips of the axonal branches, as it does in the dendrites at this stage. Small enlargements, resembling axonal growth cones, do occur at the ends of some axonal branches. Many of the branches already have a tiny, rounded tip, which is indistinguishable from the mature axonal endings. In all of the examples of the young Golgi Type II neurons some of the axonal branches end in close relation, possibly in actual contact with the incipient dendritic branches of the parent neuron (Fig. 2, *G*). It is not clear whether this is a significant relationship or purely fortuitous (see Ramón-Moliner, 1967a).

At an intermediate stage of development, in the four-week old cat, the cell body assumes its normal size in relation to the dendritic branches (Fig. 2, left). The cell body and the dendrites have fewer thready appendages. They apparently lose their filopodia with maturation. After the bulk of the filopodia are lost, there is a new outgrowth of dendritic appendages. These gradually increase in frequency in the later stages of development. They take on the typical appearance of the mature dendritic spicules and terminal dendritic plumes (Figs. 1; 2, *C*). The dendrites generally assume a less lumpy appearance. Terminal growth cones and preterminal growth buds are considerably smaller and less frequent. On the same neuron some dendrites may be much more differentiated than others. Moreover,

Fig. 2. Two stages in the differentiation of Golgi Type II neurons in the dorsal nucleus of the lateral geniculate body. In each example only one dendrite is completely shown. *Right* an immature neuron in a transverse section from layer *A1* of a 13-day old cat (littermate of specimen illustrated in Fig. 1). *Left* a nearly mature neuron in a parasagittal section from layer *A* of a 28-day old cat. *A* terminal dendritic growth cones with filopodia and lacunae; *B* nearly mature dendritic tips with an advanced growth cone; *C* fully differentiated terminal dendritic plume; *D* branched dendritic appendage; *E* axons of the Golgi Type II neurons; *F* afferent axonal end-branches of undetermined origin; *G* region where collaterals of the Golgi Type II axon end next to the dendrites of the same cell; *TO* axons of the optic tract. Rapid Golgi technique. Camera lucida, N.A. 1.3 (apochromatic)

different portions of the same dendrite may exhibit various degrees of maturity. Branches sometimes appear to be forming on a proximal portion of a dendrite that already displays a fully mature terminal plume of branches. Sometimes the reverse situation occurs. *There is no clear sign of a simple developmental gradient, in which the more proximal dendritic branches would differentiate ahead of the more distal ones, or vice versa.* The degree of dendritic development does progress in parallel with that of the afferent axonal plexus associated with the dendrites. The axon of the Golgi Type II neuron in an intermediate stage of development has a considerably more elaborate network of the short end-branches (Fig. 2 left, *E*). Beady enlargements on the axons are now rare, while the terminal axonal branches end uniformly in tiny knobs. The plexus formed by the Golgi Type II axon acquires its mature pattern before the dendritic branches are fully developed, about the same time as the dendritic pattern of the principal neurons matures (the second to third postnatal week).

The differentiating branches of afferent axons are recognized at the same time as the cell body and dendrites of the small Golgi Type II neuron are growing (Fig. 2, *F*). Some of the afferents come from the optic tract as well as from Golgi Type II axons. The afferent axons exhibit growth cones and sprouts (e.g., the branches of *TO* in Fig. 2 near *A*). Their terminal ramifications develop in a consistent relationship with the growth cones, filopodia, and incipient branches of the growing cell bodies and dendrites. *The terminal branches of the afferent axons and the receptive parts of the neurons differentiate at the same time and in parallel. There is an intimate association, possibly actual physical contact, between the afferent axonal endings and the potential post-synaptic surfaces of the neurons, as they differentiate.*

The principal neuron of the dorsal nucleus of the lateral geniculate body sends its axon to the striate cortex. Its cell body is 25—30 μ in diameter in rapid Golgi impregnations from the six-week old cat. In its dendritic differentiation a similar sequence to that already described for the Golgi Type II neuron has been traced through the age-graded series of cats, rabbits, and opossums. Two examples at different developmental stages are illustrated here (Figs. 3, 4 A).

In the first example, from a 13-day old cat, the dendrites have very nearly completed their differentiation (Fig. 3). They have not quite attained their adult lengths. Some dendrites have acquired the mature, tufted branching pattern and numbers of dendritic spines (Fig. 3, *C*). Although their tufted branching pattern can be recognized, some dendrites still have small terminal growth cones, considerably smaller than at an earlier stage of development (Fig. 3, *A*). There are a few, very short somatic and dendritic filopodia. The terminal growth cones of most of the dendrites have practically disappeared. They can still be recognized by their sprouts (Fig. 3, *B*) and, in many instances, by minute Y-shaped forks (Fig. 3, *BC*). In the fully mature state the dendrites typically taper to a point (Fig. 3, *C*). Some dendrites have formed spines proximally, while their tips still show signs of growth (Fig. 3, *A*). In other cases the distal segments of the dendrites are relatively well differentiated (Fig. 3, *B*, *C*, *BC*), while proximally some major branches are just starting to bud (Fig. 3, *D*). This illustrates a common finding. *The degree of dendritic differentiation may not be uniform in all parts of a dendritic field.*

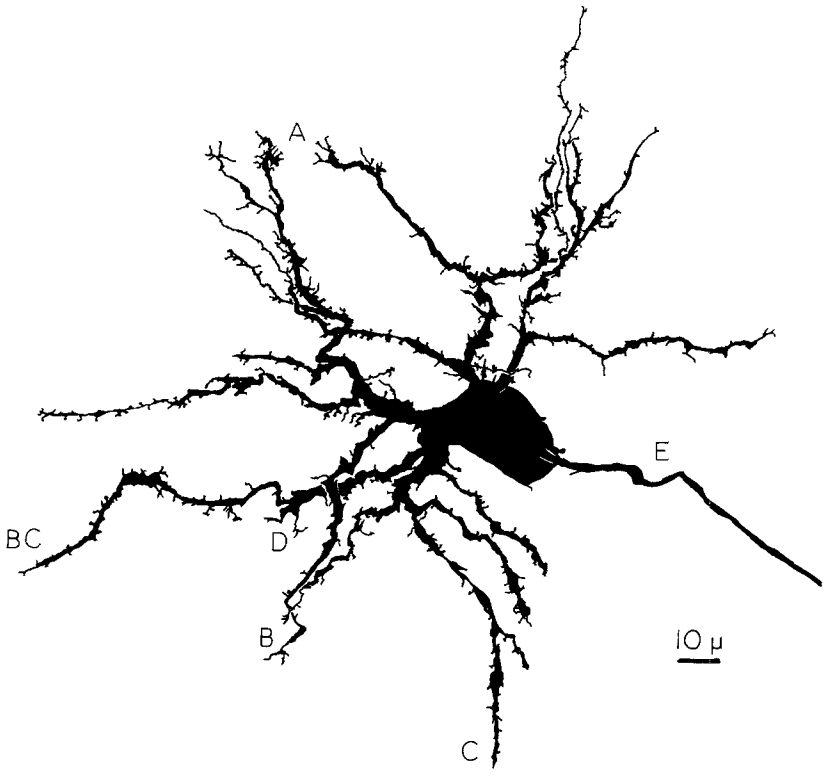

Fig. 3. A nearly mature principal neuron from layer *A1* of the dorsal nucleus of the lateral geniculate body of a 13-day old cat (from the same animal as Fig. 2 right). *A* terminal dendritic growth cones with filopodia; *B*, *BC* progressively more differentiated tips of growing dendrites; *D* preterminal dendritic growth bud and two incipient branches; *E* initial portion of the axon, which enters the visual radiation. Rapid Golgi technique, transverse section. Camera lucida, N.A. 1.3 (apochromatic)

The other example of a principal neuron of the lateral geniculate body comes from a one-week old rabbit. The dendrites have just begun to differentiate (Fig. 4A). It represents a much earlier stage of development than the previous example. The incipient dendrites and disproportionately large cell bodies are covered with filopodia. The tips of many of the dendrites end in very large growth cones (Fig. 4A, *A*). Although the dendrites and cell bodies at this age appear to be in a very primitive state, the efferent axons have already grown out of the dorsal nucleus into the visual radiation and the subcortical white matter. Collaterals of the efferent axons appear in the dorsal nucleus, where they also occur in the adult. *As a rule, the axons grow out and differentiate sooner than the dendrites.*

In the medial geniculate body of the cat and the opossum the dendritic patterns of the specific neuronal populations are sufficiently well known to permit the unambiguous construction of series of transitional forms (Morest, 1964a, 1965). Growing dendrites of principal neurons in Golgi-Cox impregnations of the dorsal nucleus of the medial geniculate body of the cat have already been illustrated and described (Morest, 1964a: Fig. 10 and p. 622). The tips of the immature

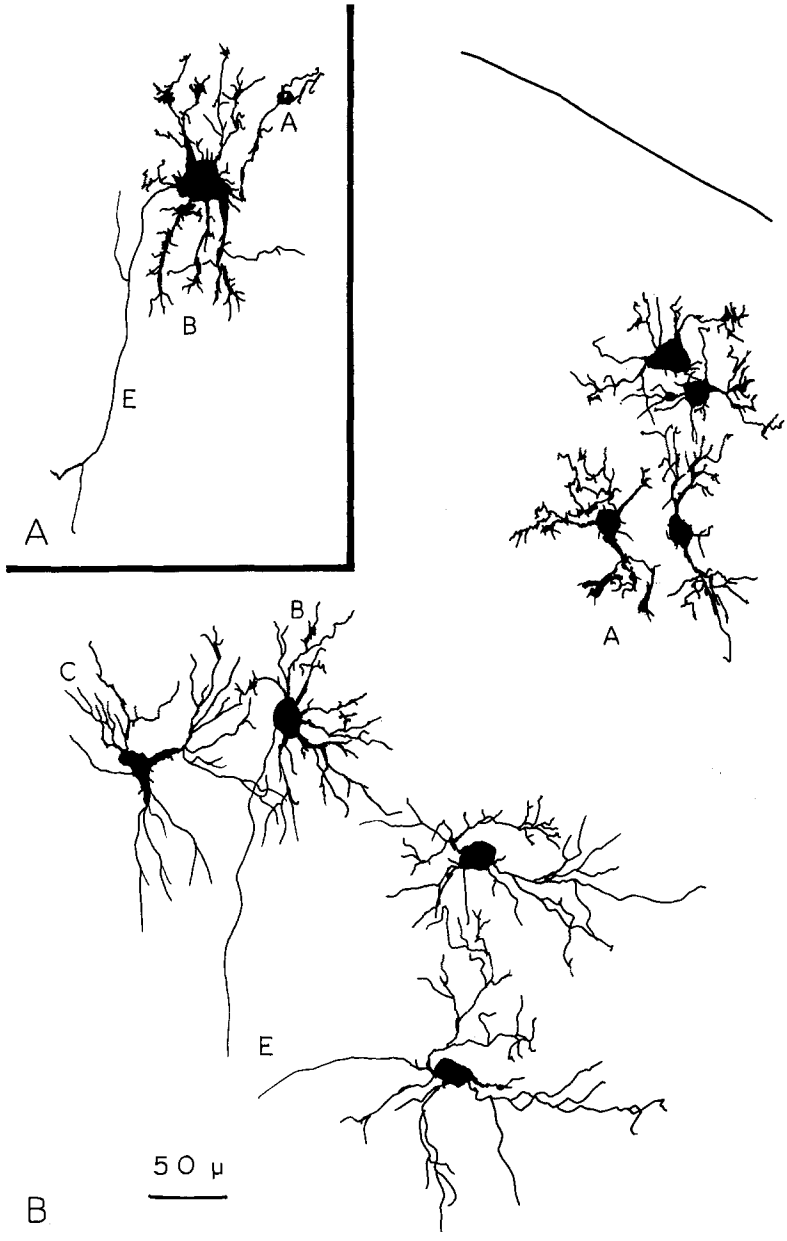

Fig. 4. A (inset) Immature principal neuron from the dorsal nucleus of the lateral geniculate body of a seven-day old rabbit. Golgi-Cox technique, parasagittal section. B Principal neurons in a transverse section from the medial geniculate body of an opossum fetus (50 mm crown-rump length). *Upper right* (*A*) immature principal neurons from the dorsal nucleus near the surface of the geniculate body. *Lower left* (*B, C, E*) nearly mature principal neurons from the ventral nucleus. Golgi-Cox technique. *A* primitive terminal dendritic growth cones; *B* growth cones of partially differentiated dendritic tips; *C* fully differentiated dendritic branches; *E* corticopetal axons. Camera lucida, N.A. 0.65 (apochromatic)

dendrites were shown to have forked sprouts similar to those mentioned above in the lateral geniculate body (Fig. 3, *B*, *BC*). The developmental series of the present study confirms this interpretation and extends it to earlier stages in perfusion-fixed rapid Golgi preparations from the cat and the opossum (Fig. 4B). The dendritic development of the principal neurons of the dorsal and ventral nuclei follows very closely the pattern just described for the principal neuron of the dorsal nucleus of the lateral geniculate body. The present analysis also confirms the previous impression that *the dendrites of the principal neurons differentiate in the ventral nucleus before they do in the dorsal nucleus of the medial geniculate body* (Fig. 4B, *A*, *B*; MOREST, 1964a). As in the lateral geniculate body, *the typical dendrites of the small Golgi Type II neurons differentiate later than the principal neurons, in their respective nuclei.*

In the cerebral cortex the growth and differentiation of the dendrites and axons also closely follow the appearance and progressive elaboration of growth cones and filopodia. Using these features as indicators of growth, one may confirm that the basal dendrites of pyramidal cells often differentiate later than the apical dendrites (RAMÓN Y CAJAL, 1911: p. 852). The example shown in the present study is a young, large stellate neuron from an intermediate layer of the area insularis of an opossum fetus (Fig. 5). Although the dendrites have scarcely begun to form, the axon has already grown into a deeper layer. *The dendritic growth cones can be enormous, relative to the size of the cell body.* They frequently enclose lacunae similar to those of the Golgi Type II neurons of the lateral geniculate body (Fig. 5, *A*). *The large stellate neurons at this stage of development often have a long process extending to the external limiting layer.* The process may eventually be incorporated in the dendritic tree of the neuron. Its attachment to the external limiting layer disappears in later developmental stages in older animals. The differentiation of the secondary dendritic branches seems to proceed at the same time as afferent axonal branches grow into the immediate vicinity (Fig. 5,*F*). These afferent axons still exhibit growth cones and sprouts during the time that the stellate dendrites are maturing.

In the posterior colliculus of the opossum the central nucleus clearly differs from the cortical zone on the basis of its neuronal morphology, just as in the cat (RAMÓN Y CAJAL, 1911; MOREST, 1964b, 1966). The disc-shaped neurons of the central nucleus are oriented in nearly vertical layers, arranged in parallel with afferent axons ascending from the lateral lemniscus (Fig. 6A, *F*). The typically tufted dendritic branching pattern of the disc-shaped neurons may be recognized in the opossum fetus. Their growth and differentiation are accompanied by dendritic growth cones and filopodia in a manner reminiscent of the tufted dendrites of the lateral geniculate body. *The dendrites of the cortex of the posterior colliculus begin to grow and to differentiate later in development than those of the central nucleus.* Within the cortex the morphologically distinct layers of the neuropil contain different types of neurons (Fig. 6A, *1—4*). *The dendrites of the neurons of the deeper layers tend to differentiate in advance of those of the more superficial layers. The larger types of neurons tend to differentiate in advance of the smaller neurons.* For example, in the intermediate stages of the cortical differentiation the large neurons of the fourth layer (Fig. 6A, *4*) exhibit fewer and relatively smaller dendritic growth cones than the somewhat smaller neurons in the second and

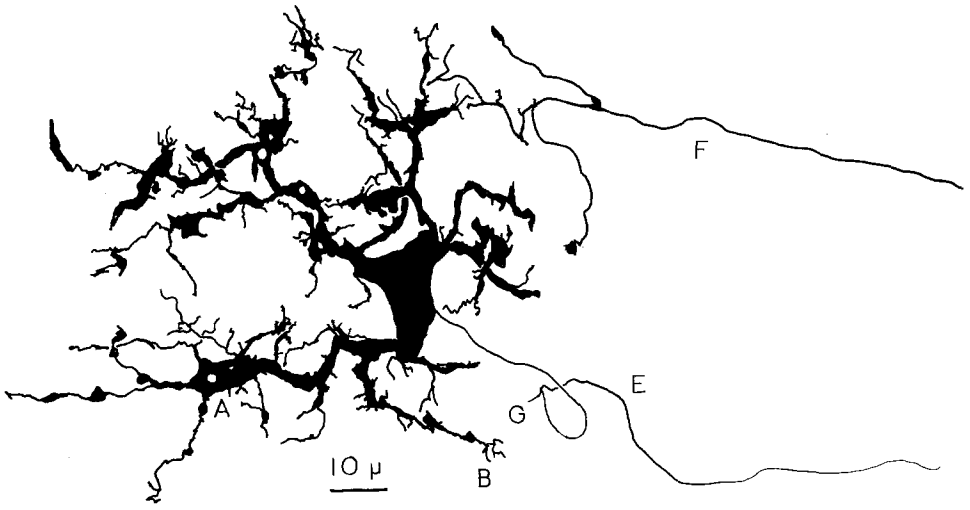

Fig. 5. Immature stellate neuron from an intermediate layer of the cortical plate in the area insularis of an opossum fetus (33 mm crown-rump length). *A* primitive dendritic growth cone with lacunae, short filopodia, and larger, incipient branches; *B* tip of a partially differentiated dendritic branch; *E* stellate axon extending as far as the deepest layer of the cortical plate; *F* afferent axon with growth cones, traced from the underlying presumptive white matter of the intermediate cortical zone; *G* collateral of the stellate axon. Rapid Golgi technique, horizontal section. Camera lucida, N.A. 1.25 (semi-apochromatic)

third layers. Exceptions to these trends are the neurons of the most superficial layer, which begin to differentiate earlier than expected, and neurons in the third layer, which continue their differentiation longer than expected. It is not yet clear when the small neurons of the fourth layer differentiate.

Some dendritic growth cones and filopodia persist after the rest of the brain has matured. In the most superficial, capsular layer of the posterior colliculus small enlargements with short sprouts appear at the tips of the indigenous dendrites (Fig. 6B). These resemble the terminal dendritic growth cones in late stages of dendritic development. At progressively more advanced ages these dendritic growth cones become progressively smaller and more strictly limited to the distalmost dendritic branches. Although the dendritic branching pattern of these neurons appears to be qualitatively complete in the 100-day old pouch young, the dendritic growth cones persist in a small, but readily demonstrable fraction of the cells in the older specimens, including young, reproductively competent adults. The incidence of appearance of these persisting growth cones could change in older adult or in senile opossums, but such specimens have not been studied.

The anteroventral cochlear nucleus contains principal neurons that have two or three thin dendrites with numerous short delicate branches, arranged in the form of terminal tufts (see Ramón y Cajal, 1909; Ramón-Moliner, 1968). The dendritic branching pattern of the principal neurons is so characteristic and striking that it may be recognized in the different mammalian species at early stages in morphogenesis. The principal neurons receive axosomatic endings in the

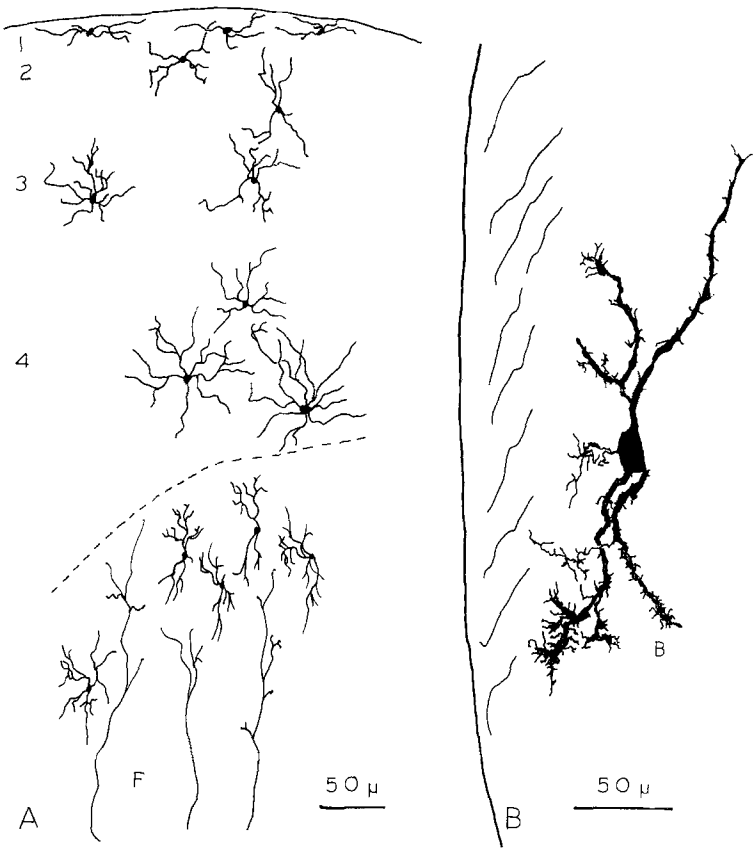

Fig. 6. A The posterior colliculus in a transverse section from an opossum fetus of 50 mm crown-rump length (80 mm snout-rump length), the same animal as Fig. 4 B *1—4* superficial layers of the cortex of the posterior colliculus; *F* afferent axons ending in vertical layers, parallel to the disc-shaped neurons of the central nucleus. The free, dorsal surface is at the top. Golgi-Cox technique. Camera lucida, N.A. 0.65 (apochromatic). B Mature elongated neuron, in a horizontal section from layer one of the cortex of the posterior colliculus, among the superficial fibers of the capsule (at the left). Note the terminal dendritic growth cones (e.g., *B*). Opossum pouch young (115 mm snout-rump length), rapid Golgi technique. Camera lucida, N.A. 1.0 (apochromatic)

form of the end-bulbs of Held and their collaterals from the cochlear nerve (Ramón y Cajal, 1909; Lorente de Nó, 1933). Other projections come from the descending auditory pathways (Rasmussen, 1960). The axons of the principal neurons project to parts of the superior olivary complex, the lateral lemniscus, and the posterior colliculus (Warr, 1966). Subdivisions of the anteroventral cochlear nucleus may be defined on the basis of the varieties of the specific neuronal populations. In the opossum, neurons in the anterolateral third of the nucleus can be identified by their branching pattern, which resembles that of the principal neuron of the medial trapezoid nucleus (Morest, 1968 b: Fig. 5). Within the anteroventral cochlear nucleus of the opossum fetus (30 mm crown-rump length) are newly differentiated end-bulbs of Held and the growth cones of the other afferent

axons. The characteristic tufted dendritic branching pattern can already be recognized in the young neurons (Fig. 7). However, many of the tufts do not appear to have all of their definitive branches; at least all of them are not yet in their definitive arrangement. The stems and main bifurcations of the dendrites have a lumpy, varicose appearance. From the varicosities and from the perikarya extend a number of filopodia (Fig. 7). *The filopodia must be transient, since they are rarely recognized in the mature brain.* Nor are there so many or such long processes on the perikaryon or the main dendrites, as there clearly are in the fetus. As the dendrites gradually assume their mature appearance in successively older littermates (42 and 58 mm crown-rump length), the number and length of the filopodia decrease. During the same period there is a decrease in the incidence and size of the sprouting dendritic varicosities, which assume increasingly more peripheral locations in the dendritic field. They have the same morphological appearance and follow the same pattern of change, as the dendritic growth cones previously demonstrated in the medial trapezoid nucleus (Morest, 1969). In the anteroventral cochlear nucleus the axons of the immature neurons also have varicosities, irregularly spaced, both in the cochlear nucleus and in the trapezoid body (Fig. 7). When the axons are first growing into the medial superior olivary nucleus (25 mm crown-rump), some of their varicosities display filopodial sprouts. Varicosities, with or without filopodia, rarely appear on these axons in the mature specimens.

In the lateral vestibular nucleus of the cat, large, medium, and small neurons have been illustrated (Ramón y Cajal, 1909: Fig. 320; Mannen, 1965; Ramón-Moliner, 1968: Fig. 23; Hauglie-Hanssen, 1968). The nucleus receives projections from the vestibular labyrinth and the cerebellum and has connections with several parts of the brain stem and spinal cord (Brodal, 1964). The connections of the medium-sized neurons in particular are uncertain. These neurons have perikarya about 30 μ in diameter. They have long, straight, radiating dendrites with very few branches and few spines (Fig. 8, *3*). In kittens the medium-sized neuron may be distinguished from the other neuronal types in the nucleus at an intermediate stage of development. The perikaryon has its mature shape, and the dendritic branching pattern is practically complete, although the dendrites have not reached their mature lengths (Fig. 8, *1*, *2*). Such dendrites typically have terminal enlargements, or growth cones, at their growing tips. The growth cones have a number of thin, short filopodia and occasionally some lacunae (Fig. 8, *1*). Their form resembles axonal and other dendritic, terminal growth cones. They seem surprisingly small, compared to those of other types of dendrites of comparable size and degree of development. There is a tendency for the distal shaft of the dendrite to have lumpy growth buds with short sprouts, but, again, these buds seem surprisingly small. *The young dendrites of the medium-sized vestibular neurons are often enlarged distally for considerable distances*, sometimes more than a hundred microns. The distal dendritic shafts are covered by numerous fine, short sprouts, or filopodia. These are not to be confused with the spines and appendages of the mature dendrites, which are relatively thick, tapered, and sparse. The dendritic filopodia decline in length and incidence, as the dendrites approach their mature lengths in successively older kittens (compare Fig. 8, *1*, *2*, *3*). Finally in the typically mature condition the dendrites of the medium-sized neurons taper gradually to a point or to a fork-shaped array of minute sprouts (Fig. 8, *3*).

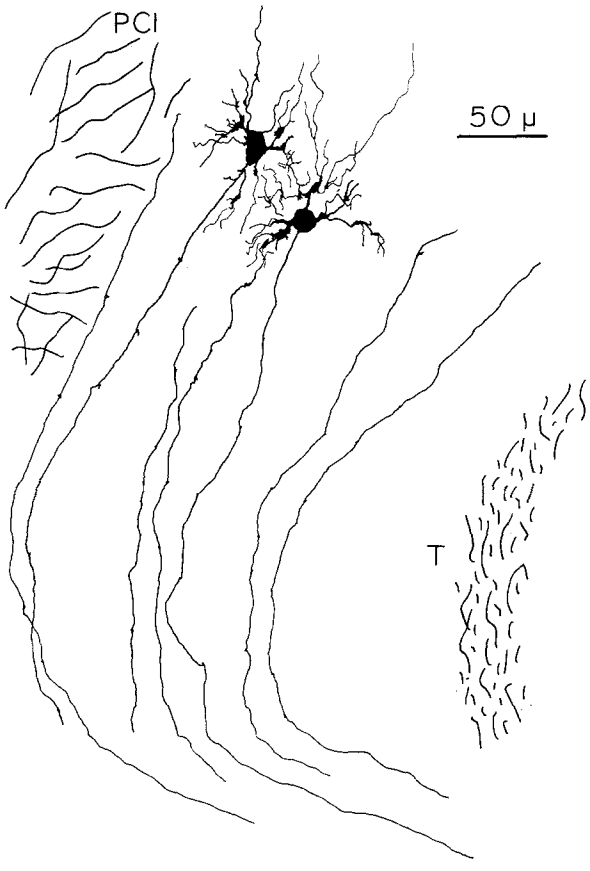

Fig. 7. Immature neurons from the anteroventral cochlear nucleus of an opossum fetus (35 mm crown-rump length). The neurons are located at the level of the vestibular nerve rootlets along the anteroventral edge of the inferior cerebellar peduncle (*PCI*), as it enters the cerebellum. The axons of these neurons pass ventrally through the cochlear nucleus and lateral to the descending trigeminal tract (*T*) to enter the trapezoid body. Rapid Golgi technique, transverse section. Camera lucida, N.A. 1.0 (apochromatic)

In the lateral vestibular nucleus of the growing cat and rat the dendrites of the large neurons, the so-called cells of Deiters, exhibit transitional stages with growth cones like those of the medium-sized cells observed in the cat. Like the latter, many of the dendrites of the fully differentiated large neurons in the older kittens and adult rats taper to a point or to a forked formation (Fig. 8, *4C*). *Many mature dendrites seem to retain terminal growth cones with filopodia, resembling those of the young growing cells* (Fig. 8, *4A*, *4B*). Some of the dendritic enlargements may reach exceptionally large proportions in the rat (Fig. 8, *5*). These may occupy either terminal or preterminal positions on the dendrite, especially its distal portion. They resemble terminal growth cones or preterminal growth buds (Fig. 8, *5D*). They vary considerably in size and shape. Sometimes they appear as tubular swellings of the main dendritic shaft, sometimes as mace-like formations or as complicated branches and convolutions or as clavate, corallike excrescences.

But, whatever their sizes and shapes, these clavate excrescences commonly bear filopodia and small growth cones. In fact there is not a clear separation between the largest growth cones and the clavate excrescences, either in their shape, size, time of appearance in the developmental series, or in their positions on the dendrites. The largest and most complicated clavate excrescences are more commonly seen in the adult rats. Comparable formations have not been observed in the other cell groups. Nor have they been identified as yet in the cat. Occasionally something similar occurs on the stellate neurons in the medial trapezoid nucleus of the cat (Morest, 1968b: Fig. 7).

In the descending trigeminal nucleus of the cat one may trace the transitional forms of the small neurons (Ramón-Moliner, 1968: Fig. 51, *L*) during the period of their differentiation in the anterolateral region of the nucleus. Dendritic growth cones and buds are readily identified. They follow a developmental pattern similar to that of other sensory neurons, e.g., the medium-sized neurons of the lateral vestibular nucleus. *Many of the dendritic growth cones in the descending trigeminal nucleus are very tiny.* They have long, thin, branching filopodia. *The long terminal filopodia also commonly occur at ages when the neurons appear to be fully differentiated* (Fig. 8, *6*).

In the cerebellar cortex of the opossum, rat, and cat it has been possible to confirm many of the observations of Ramón y Cajal (1911) concerning neurogenesis in this structure. He has provided adequate illustrations of the immature cells (see also Ramón y Cajal, 1960). Suffice it to say that the post-migratory neuroblasts of the granule and Purkinje cells exhibit numerous long filopodia on their perikarya and dendrites. The progress of the growing dendrites and of the growing mossy and parallel fibers has been followed in the developmental series by reference to the growth cones that distinguish these structures.

Some General Observations. It will be convenient to present here some observations generally applicable to the present material but not readily treated in the preceding topographical format.

The present findings can be verified in age-graded series of brains, impregnated with the rapid Golgi technique, the Golgi-Cox technique, or the varieties of the Golgi-Kopsch technique. The rapid Golgi preparations display the most delicate processes of the neuropil and the fine filopodia more consistently, perhaps because they are more uniformly preserved to begin with. In these preparations the filopodia of growing neurons are so thin that they are scarcely visible with the best lenses. They are more consistently demonstrable in material prepared by perfusion-fixation than by immersion-fixation, except possibly in the early opossum fetus. In the Golgi-Cox preparations of the thalamus and the midbrain of the newborn there

Fig. 8. Dendritic segments from the lateral vestibular nucleus (*1—5*) and the spinal trigeminal nucleus (*6*). *1, 2, 3* show the ends of progressively more differentiated dendrites of medium-sized neurons in newborn, one-day old, and 14-day old cats, respectively. *4* portion of a large neuron from a 23-day old cat: *A* terminal dendritic growth cone; *B* dendritic growth cone, smaller and perhaps more advanced than the preceding; *C* extremely fine dendritic growth tip. *5* clavate excrescences on dendritic tips and preterminal segments from an adult rat: *D* incipient dendritic branches in a preterminal position. *6* end of a dendrite of a small neuron in the anterolateral region of the descending trigeminal nucleus from a 23-day old cat. *E* axon

Rapid Golgi technique, transverse sections. Camera lucida, N.A. 1.3 (apochromatic)

Fig. 8

is a tendency for the impregnated cells or their cell bodies to be grouped in clusters. This is not a consistent feature of the rapid Golgi preparations. The basis for this difference is uncertain. Some of the observations could also be repeated in brains prepared by the Golgi-Kopsch technique or other variants of chrome-silver impregnation following fixation with aldehydes. However, these preparations have been less informative than the rapid Golgi impregnations, especially in very young animals, but also in the adult.

Within each specific neuronal population most of the neighboring neurons at intermediate stages of development usually display much the same degree of development. There is variation, at least to the extent that individual dendrites of the same or of nearby neurons may have more or less differentiated branches. Also an occasional neuron that has scarcely begun to differentiate can occur amidst neurons that are advanced in their dendritic development. The tendency for dendrites of the same type in a specific neuronal population to differentiate at a particular juncture in the developmental sequence is widespread in the brain, although it may not be completely general. This is not to deny the existence of developmental gradients within the neuronal populations. *Dendritic differentiation is often most advanced in one region of a neuronal population and continuously more retarded in successively more distant regions.* For this reason the critical comparisons of different developmental stages are restricted to corresponding regions of the same nucleus. *Within corresponding regions there seems to be a tendency for the dendrites of the larger types of neurons, or those with larger cell bodies, to differentiate before those of the smaller types* (see also Ramón y Cajal, 1909: p. 617). This cannot be promulgated as a law of neurological development, because it is not clear how the sizes of neurons should be judged.

Although the most critical observations presented here apply to sensory neurons in the brain stem, the growing dendrites of motor neurons also exhibit dendritic growth cones and filopodia and conform to the general pattern of dendritic development outlined above. Included also are neurons of the brain stem reticular formation, the ventral nucleus of the lateral lemniscus, the anterior colliculus, the hypothalamus, the striatum, the hippocampus, the olfactory bulb and cortex, and the neocortex. *There is a tendency for the dendrites of neurons in the motor field of the neuropil to begin to differentiate before those in the sensory field at the same anteroposterior level of the brain stem.* For example, among the cranial nerve nuclei some of the earliest dendrites to differentiate belong to the branchial motor column. The large neurons of the facial nucleus begin to differentiate before those of the adjacent auditory neurons of the superior olivary complex. The large neurons of the nucleus pontis centralis caudalis in the adjacent part of the reticular formation and the large neurons of the lateral vestibular nucleus form their dendrites very early in development, even before some of the nearby motor neurons. However, in some respects these neurons resemble motor neurons rather more than sensory neurons. The branching pattern of their dendrites resembles that of motor neurons (Ramón-Moliner, 1962); their axons probably contribute to the reticulo-spinal and vestibulo-spinal tracts of the extra-pyramidal motor pathways.

Within the sensory systems the constituent neurons seem to begin their development in a rigid sequence. In general, there is a tendency for the dendrites of the

neurons nearest the peripheral receptor to differentiate first. For example, in the auditory system dendrites in the cochlear nucleus begin to differentiate before those of the superior olivary complex and the posterior colliculus. The latter begins its dendritic differentiation before the medial geniculate body. The auditory cortex is the last major sector of the auditory system to begin to differentiate. This generalization does not necessarily apply to all of the constituents of the auditory system, especially in regard to the descending auditory pathways. Similar developmental progressions could be traced in the somesthetic, visual, and olfactory systems. Motor cranial nerve nuclei begin to develop before motor centers in the forebrain, but no attempt was made with the motor systems to trace developmental sequences analogous to those of the sensory systems.

Discussion

Particular morphological features of the neurons in the present study can be regarded as immature, because they typify the neurons of the fetus but not those of the mature animal. *Immature features of neurons are the filiform appendages of the cell bodies, the cell bodies and dendritic trunks that are disproportionately large compared to those of young adults, and the enlargements and their sprouts at the ends of dendrites and along their stems, namely, the growth cones.* The types of neurons differ considerably in their morphology from region to region, but within each region are populations of neurons that are homogeneous with respect to the morphological features that typify their dendrites. Wherever such a population is unambiguously defined, its constituent neurons follow the same course in their morphological differentiation. This is not to imply that all of the neurons and dendrites of a population necessarily evolve strictly in unison or even in lock step. For one thing, in each population all of the neuroblasts that migrate to the same region may not complete their migrations at the same time. It is rather a matter of the relative proportions of immature, transitional, and mature forms. With increasing age there is a regular succession of transitional stages, gradually leading to the mature, characteristic dendritic patterns. Thanks to these circumstances, it has been possible to reconstruct the sequence of morphological changes undergone by the individual developing neurons. This sequence may also be demonstrated in those populations with a spatial gradient of differentiation, at an intermediate stage of development, such that the dendrites are nearly mature in one region but less so in successively farther regions.

The neuroembryological literature scarcely deals with the growing dendrite, much less the dendritic growth cone and its properties. Ramón y Cajal (1909: p. 614), in his discussion of the development of dendrites, refers to their "large varicosities and the short triangular appendages in the form of 'crêtes d'impression', which characterize them in their first developmental stage". Otherwise that author's drawings of immature dendrites in the spinal cord, the retina, and in the cerebral and the cerebellar cortices show several examples of structures that we may now interpret as dendritic growth cones (Ramón y Cajal, 1909, 1911, 1960). Others have had occasion to observe immature dendrites in various histogenetic contexts (e.g., Held, 1909; Coghill, 1924; Conel, 1939—1967; Barron, 1946; Eayrs and Goodhead, 1959; Poliakov, 1961; Godina and Barasa, 1964;

Himwich and Himwich, 1964; Purpura and Schadé, 1964; Bernhard and Schadé, 1967; Minkowski, 1967; Hassler and Stephan, 1967). Some of these authors, e.g., Conel (1939), considered varicosities to be immature features of dendrites and axons. Dendritic growth cones and some other cytological features of dendritic growth have been reported in the medial geniculate body (Morest, 1964a) and in the medial trapezoid nucleus (Morest, 1969). In electron micrographs from the fetal spinal cord and cerebellum, Bodian (1966) and Del Cerro and Snider (1968) reported vesicular elements in dendritic varicosities, said to be growth cones. These authors believe that the vesicular elements correspond to cytological components in both axonal and dendritic growth cones, rather than to the varicosities and vesicular artifacts commonly found in poorly fixed neurons. In the lateral vestibular nucleus of the adult rat Sotelo and Palay (1968) observed varicosities filled with mitochondria and glycogen granules on the distal dendrites of Deiter's cells. These authors suggested that such varicosities may be growth cones. The students of tissue culture are strangely silent about the growth of dendrites. It is disappointing that so little information is available about this. The dendrites surely provide most of the synaptic loci in the central nervous system.

Some Limitations. One problem encountered in the present approach concerns the critical demonstration of the truly mature neurons. Failure to demonstrate them would not invalidate the present analysis, which rather depends on the changes in progressively older animals. However, it is a proper concern of neuroembryology to determine when the maturation of the brain is complete. Moreover, recognition of the mature state helps to establish the relevance of observations in very young animals.

When a specific neuronal population is followed serially in progressively older brains, morphological changes may be detected after short intervals, e.g., from day to day, in the early stages of its differentiation. In later stages changes may be appreciated after longer intervals, perhaps weeks. Eventually a period ensues when changes are no longer detected, even after intervals of several months, usually by the time the animal is sexually mature. The qualitative features of the neurons observed in this stable period are presumably mature traits. It is possible that quantitative changes in this period could be detected with suitable statistical analyses. A prolonged period of morphological stability in the young adult may not necessarily preclude a resumption of growth. For that matter it is possible that a limited degree of neuronal growth may continue in adulthood and even in old age. Here the problem of distinguishing histological evidence of growth from signs of degenerative or regenerative activity remains a real one. Within limits, then, the present approach provides a basis for characterizing the mature neuron. This characterization does not differ from that currently accepted by most neurocytologists and previously derived from Golgi impregnations of very young brains by Ramón y Cajal and others. Ramón y Cajal (1909: p. 615) had already anticipated the need for the present approach.

So far, most reported findings with the Golgi techniques on very young animals are still deemed valid for the functionally mature brain. This is not to suggest that the student of the mature brain need not establish the relevance of observations made on growing brains. This need becomes increasingly critical, as more interest attaches to detailed observations and to the minute features of the

neuropil. For this purpose histologically inferior variants of the Golgi techniques cannot be justified solely on the grounds that they have been used on adult animals. However, the rapid Golgi technique, applied to young brains after perfusion-fixation, has been a most fruitful method.

One may question the present analysis on the grounds that the growth cone and other immature features may be artifacts of technique or pathological phenomena. Against the notion that the observations represent artifacts of the rapid Golgi technique is the fact that they have been verified with the Golgi-Cox technique. Although both of these techniques provide for the impregnation of cells and their processes, they have entirely different bases. The rapid Golgi technique involves the precipitation of silver dichromate within the cells, after fixation in a chrome-osmium solution. The Golgi-Cox technique relies upon the reduction of mercury in the cells, after fixation with a mercurial salt. The notion that the Golgi techniques do not reliably demonstrate normal histological structure was refuted a long time ago by RAMÓN Y CAJAL (1909) and cannot be regarded seriously by contemporary neurocytologists.

It is unlikely that the terminal dendritic growth cones and preterminal growth buds represent artifacts of fixation. A previous study did indeed show that beady enlargements on dendrites could result from inadequate histological preservation (MOREST and MOREST, 1966c: Fig. 10). Characteristic of such beaded dendrites is a lack of the delicate dendritic appendages found in adequately fixed specimens. The dendritic appendages in the present material are very well preserved, particularly those associated with the dendritic growth cones and growth buds. The same observation rules out the likelihood that the growth cones and buds are examples of *l'état moniliforme*, supposedly a pathological beading of dendrites, which has been observed under conditions highly conducive to inferior fixation (see VAN GEHUCHTEN, 1900; RAMÓN Y CAJAL, 1909: pp. 70—73). More difficult to interpret in the present material are the beady dendritic enlargements of growing dendrites that are not directly associated with filiform appendages. These enlargements could correspond to clumps of cytoplasm that may normally stream through the growing dendrites. The possibility that occasional examples may be the result of faulty fixation cannot be ruled out. It is unlikely that many examples in the present material can be explained on this basis. In the first place, the dendrites that exhibit the beady enlargements appear to be well preserved. They retain their delicate appendages. In the second place, the beady enlargements are readily demonstrated in the present material, prepared by the technique of perfusion-fixation. This satisfies light microscopic criteria of well preserved Golgi preparations (MOREST and MOREST, 1966). In addition, it can now be stated that such material, when stained in epoxy sections with toluidine blue (MOREST, unpublished), satisfies the light microscopic criteria for good cellular preservation elaborated by CAMMERMEYER (1960) with cytoplasmic and nuclear stains.

Do the morphological features ascribed to immaturity actually represent pathological phenomena? No. The immature morphological features, e.g., the dendritic growth cones, consistently follow a regular sequence of changes with increasing age. The pattern of this developmental sequence applies to all of the individual animals observed and to the different species. The sequence is continuous; it ends with the normally differentiated neuron. In short, the morphological

features in question define a state of immaturity. This benign state is obligatory for all normally developing individuals. It is possible that similar morphological features may occur in certain pathological states. For example, there are the structures resembling dendritic growth cones with sprouts among the hippocampal pyramidal cells in the senile dog, which Lafora (1914) interpreted as new growths in response to a regenerative or progressive irritative process. Ramón y Cajal (1928) has illustrated several examples of neurons, undergoing traumatic degeneration, with structures resembling dendritic sprouts or sprouting axonal growth cones (so-called "turtles") in reduced silver preparations. These could be interpreted as transitory regenerative phenomena. The same author cited studies of similar neoformations in cases of tuberous sclerosis and cerebral ganglioneuroma (Ramón y Cajal, 1928: II, p. 571). It is not certain to what extent degenerative and regenerative phenomena may occur normally in adult or aged brains.

Some Generalizations. The growth cone is a manifestation of dendritic growth and differentiation in the brain. In every case of the proliferation, enlargement, and morphological maturation of dendrites, dendritic growth cones and dendritic filopodia are in evidence. This condition applies not only to the sensory nuclei of the brain stem but also to many other cell groups in the motor and intermediate fields of the neuropil. The sample seems representative enough of the mammalian brain that the following general propositions now deserve serious consideration. First, *the presence of structures descriptive of dendritic growth cones and filopodia is presumptive evidence of dendritic growth.* Second, *the growth cones and filopodia actively participate in the emergence, the enlargement, and the differentiation of the dendrites and in the elaboration of their branching patterns. The same rules would apply to growing axons and cell bodies.* Related to these hypotheses is a third one, previously advanced (Morest, 1969), that *neuronal growth occurs at the very sites of the growth cones and the filopodia*, wherever else it may occur. The fourth proposition is that *the differentiation of the dendritic branches coincides in time and place with the differentiation of the afferent axonal end-branches that form synaptic contacts with the dendrites.*

The validity of the first proposition, as an induction, depends on the number of observations supporting it. The present study provides a large number of supporting observations in four species. Additional observations of more cell types and of other species should extend and modify it. This generalization applies to the form of the growth cones and the pattern of their behavior. While conforming to the general pattern, the dendritic growth cones and filopodia in different neuronal populations may vary in their size, shape, complexity, and in the time and duration of their occurrence. The young stellate neurons of the insular cortex are remarkable for their enormous, loculated dendritic growth cones with many branching filopodia. This constellation foreshadows the wide-spreading dendritic trees of the mature large stellate cells. At the other extreme in size are the minute terminal growth cones with few and very long filopodia, belonging to the slender dendrites of the small neurons in the descending trigeminal nucleus. These dendrites have few branches, but they retain their terminal growth cones much longer than the other types of neurons in the vicinity. In the case of the medium-sized neurons of the lateral vestibular nucleus the entire distal segment of the growing dendrite could be regarded as an exceptionally long equivalent of a growth cone.

Perhaps this variety of dendritic growth is related to the paucity of dendritic branches, especially distally, and to their great length and slenderness. Very complicated shapes are displayed by the large, clavate dendritic excrescences of the large neurons of the lateral vestibular nucleus. These excrescences may represent aggregations of many simple growth cones or conglomerations of multiple growth foci. Their appearance suggests a kind of dendritic overgrowth, like an exostosis, induced by an unusually strong, prolonged, and localized growth stimulus. Local obstacles in the surrounding neuropil, such as dendritic or axonal processes, may constrain the unusually large and active growth cones, giving them a sculptured appearance. Such a situation would be most pronounced in a mature neuropil. In fact the clavate excrescences are more commonly seen in the mature than in the immature brain. Possibly these excrescences, as well as the postmature growth cones of the descending trigeminal nucleus and of the posterior colliculus, are the morphological expression of a continual regenerative activity, perhaps as part of a normal cycle of degeneration and regeneration of the mature dendrites of these cells. SOTELO and PALAY (1968) have discussed this possibility in connection with the large dendritic expansions, full of mitochondria, which they observed in electron micrographs of the lateral vestibular nucleus of the rat. Perhaps these expansions correspond to the clavate excrescences. Now open for exploration is the possibility that morphological alterations involving the axonal and dendritic growth cones in the mature brain could be correlated with behavioral conditioning, trophic modifications, and other plastic changes in neural function.

The second proposition is that the growth cones and filopodia are involved in the emergence, the enlargement, and the differentiation of the dendrites and in the elaboration of their branching patterns. The third proposition is that growth occurs precisely at the sites occupied by growth cones and filopodia; this is a corollary of the first two propositions. The growth cones and filopodia are reliable indicators of the degree of dendritic differentiation and are useful in reconstructing the developmental stages of the dendritic trees that evolve in specific neuronal populations of progressively older animals. This implies that the development of the dendritic branching patterns of each specific neuronal type is a function of the strategic locations of the dendritic growth cones and the distribution of dendritic growth activity, as evidenced by the dendritic filopodia. To a certain extent, the dendritic growth cones and the filopodia may guide the development of the patterns of synaptic arrangements that endow each neuronal population with its particular functional capabilities. For the number and size of the dendrites and their branching patterns must limit the integrative capacities of neurons and the patterns of signal activity that they can mediate by way of axodendritic synapses.

The fourth proposition is that the elaboration of the dendritic branches proceeds *pari passu* with the development of the afferent axonal plexus associated with the dendrites. This proposition is more fully treated in a previous article, which suggests that the afferent axons could induce or influence the formation of dendritic branches in the medial trapezoid nucleus (MOREST, 1969). The present report does not contain enough data to tell if afferent axons in all parts of the brain regularly contact the neuroblast before it begins to form dendrites. It does generally

appear that dendritic branches ordinarily differentiate in conjunction with afferent axonal branches that are destined to form synapses with the dendrites. After the corresponding dendrites have formed, afferent axons must continue to make axodendritic synapses for a time, e.g., with dendritic appendages.

Some Implications for the Analysis of Neurogenetic Mechanisms. The preceding considerations have particular relevance to the mechanisms controlling neurogenesis. Because of the functional importance of the dendritic and axonal branching patterns and their rigid formal invariance in the different neuronal populations, it is not difficult to imagine that their assembly must come under genetic control, either directly or indirectly. However, it would be impossible for the genetic code to specify directly each synaptic contact in the vertebrate brain, since there are far too many synapses. In this context the following questions arise. What are the events leading directly to the assembly of the synaptic organization ? At what points in this process is genetic control exerted ? The present study approaches the first question. Directly relevant to this question are the relative positions and morphological status of the afferent axons and the dendrites destined to engage in synaptic contact. This is the focus of the present series of papers (MOREST, 1968a, 1968c, 1969, in preparation).

The formation of dendrites and their synapses has not been explained. The theories of stimulogenous fibrillation (BOK, 1915) and neurobiotaxis (ARIËNS KAPPERS, 1921) have been contradicted too thoroughly to have more than historical interest. RAMÓN Y CAJAL (1909: p. 615) advanced a hypothesis regarding the early outgrowth of dendritic processes of the ventral horn cells of the spinal cord and of the granule and Purkinje cells of the cerebellum. Observing that many more dendrites seem to grow out initially than actually appear on the mature neurons, he proposed that the early dendrites grow out in random directions (see RAMÓN Y CAJAL, 1911: Fig. 63). Only those that successfully made functional contacts with afferent axons would survive to maturity, while the rest would degenerate. In the present material the growing cerebellar granule cells and Purkinje cells do not exhibit a premature outgrowth of numerous dendrites, but rather an initial proliferation of somatic filopodia that are identical to the filopodia of other growing neurons. The filopodia are evidently a general manifestation of neuronal growth. Perhaps they correspond to Ramón y Cajal's premature dendrites. The filopodia could also provide surfaces to anchor the proper afferent axons. Once the axons had been located or contacted, dendritic branches, led by growth cones, could grow into the positions most favorable for making synaptic contacts with the afferent axons. The growth cones and filopodia of the ingrowing afferent axons could play a similar role with respect to the differentiation of the axonal end-branches contacting the dendrites. This hypothesis takes into account typical structural features of growing neurons, their growth cones and filopodia. It does not require that many dendrites begin to differentiate, only to degenerate, while their neighbors continue to evolve. It does not contradict this notion, which certainly deserves a more thorough analysis.

The dendritic differentiation of a neuroblast may not begin until its axon has grown out to reach its destination, or nearly so, RAMÓN Y CAJAL (1909: p. 611) once suggested. BARRON (1943) has endorsed this speculation in the case of the spinal motor neurons. However, the fact that the axon differentiates before the

dendrites does not prove that the axonal ending must reach its destination before dendritic differentiation may proceed. Nor is there any evidence that the arrival of the axonal ending at its destination triggers the ensuing dendritic differentiation. This would be a difficult rule to apply to the brain. Most cerebral axons send collaterals to more than one destination, often to the same region in which the axons originate. HELD (1909: p. 34) reported instances of dendrites that grew out from the neuroblasts before the axons. These interesting matters deserve more attention.

After the initial dendrites form in the ventral horn, BARRON (1943) suggests, the subsequent differentiation of the dendrites of neighboring motor neurons may be stimulated by the earlier forming dendrites ("assimilative induction"). This hypothesis has the complication that the mechanisms governing the dendritic outgrowths of the first neurons would differ from those governing subsequent neurons. The validity of this hypothesis for cerebral dendrites is not supported by the present observations, which fail to show the postulated contacts of dendrites and neuroblasts or the appropriate sequences of their development. However, interneuronal contacts by the filopodia may occur in the primordial medial trapezoid nucleus (MOREST, 1969: Fig. 1) and in the anterior colliculus (MOREST, 1968a). BARRON (1944) also proposed that the dendritic branching patterns form by contact guidance. Since the texture of the neuropil changes as the spinal cord matures, dendrites forming at different times would have different patterns. In the brain this scheme would not account for the fact that the neurons of a specific population invariably develop the same type of branching pattern, although they may differentiate at different times in the same region. Nor would it explain how different branching patterns may overlap in their development in time and space. To be sure, mechanical factors may influence the shapes of neurons secondarily. In the present studies the correlations between the differentiation of the dendritic branching patterns and the elaboration of those elements of the afferent axonal plexus specifically related to the dendrites argue for other explanations. These could involve the induction of dendritic differentiation by the growth cones and filopodia of afferent axons and the mechanical guidance and chemical interactions of the growing parts of the dendritic and axonal branches destined for synapsis. It is interesting that TERRAZAS (1897) and RAMÓN Y CAJAL (1909: p. 658) speculated along similar lines a long time ago.

Suppose that the form of the axonal end-branches were specified directly by the nucleus or controlled by the perikaryon of the parent neuroblast. How would the same axon form one type of ending in one place, and other types of endings in other places by way of its collaterals? A dozen types of end-branches occur on the collaterals of the auditory nerve fibers in the different parts of the cochlear nucleus (LORENTE DE NÓ, 1933). Would a signal from the genetic material of the nucleus or a protein or other substance streaming from the perikaryon through the axon penetrate some collaterals and not others? It seems likely that the form of the afferent end-branches, as well as the dendritic branches contacting them, would be partially determined by local conditions. How would different patterns of axonal end-branches develop in the same locality? Since many axonal endings form at separate times in development, the pertinent local conditions may change with time. Where the development of different branching patterns over-

laps, the several types of axons must be so constituted as to react differently to the same environment. This would not be surprising, for such axons regularly derive from neurons that have different cytological features and different developmental histories, if not different anatomical locations. The same arguments can be applied to the dendritic branching patterns of the various neuronal types. These considerations argue for a chemotactic role as well as mechanical guidance in neuronal differentiation and synaptogenesis.

The temporal sequence in which the morphological features of the synaptic organization appear is as typical for each region as the spatial patterns of its mature neuronal architecture. The temporal sequence may be related to cell size or number, time of last cell division, and time of migration of the neuroblasts (e.g., see Angevine, 1965; Pierce, 1967). It may also depend on spatial gradients of the local physico-chemical factors controlling differentiation. Sometimes there seems to be a certain logic to these temporal features, with respect to the mature architectural and functional relationships that the different structures are to assume. In the lateral geniculate body the principal neurons and the primary retino-geniculate axonal endings differentiate before the small Golgi Type II neurons. This may be related to the secondary relationships that the latter structures must establish with the former. The cortex of the posterior colliculus derives a large part of its functional significance from its synaptic relationships with the axonal collaterals of the earlier developing central nucleus and of the later developing cerebral cortex (Morest, 1966). As one more example, some of the components in the ascending auditory system generally seem to differentiate in the ascending order, in the same direction as their axons grow and ultimately conduct impulses. The same rule may not apply to other systems, particularly the descending motor systems to the spinal cord, where seemingly the opposite tendency holds. These sequences may be incidental to the "logistics" of cellular proliferation, migration, and differentiation. They may also be related to the changing systemic needs of the growing organism, which may require some parts of the nervous system to function before others.

Some of the temporal features of dendritic maturation seem to respect their phylogenetic status. The small Golgi Type II neurons of the thalamus are thought to become prominent late in vertebrate phylogeny (Ramón y Cajal, 1911, 1952). They develop later than the principal neurons. In the medial geniculate body the principal neurons of the ventral nucleus differentiate earlier than those of the dorsal nucleus. The latter appears to be more advanced phylogenetically than the former (Morest, 1965). The late developing cortex of the posterior colliculus is better developed in the phylogenetically more advanced cat than in the opossum. In the medial trapezoid nucleus the dendrites of the stellate neurons mature earlier than those of the principal neurons. The stellate dendrites belong to the least highly specialized category of the brain stem, which Ramón-Moliner and Nauta (1966) regard as phylogenetically less advanced. The more peripherally located parts of the auditory system start to differentiate ahead of those parts nearer the cerebral cortex. The latter are functionally more prominent in the more advanced auditory adaptations.

The notion that the ontogenetic sequence of the dendrites follows the order of their evolutionary development is limited. The motor nuclei of the brain stem

and spinal cord generally begin to differentiate before the sensory nuclei. Yet the organization of the motor systems is surely no less evolutionarily advanced in these regions than the sensory systems. The morphological history of the dendritic ontogenesis of particular cell types does not commonly recapitulate the evolutionary history of these cell types. Growing dendrites in the cat brain, with their growth cones and filopodia, do not resemble their mature homologues in the opossum any more than they do in the cat. Nor do they resemble generally the neurons in more remote and less advanced vertebrate classes, such as reptiles and fish. Situations can be found in which the ontogenetic sequence of certain neuronal features does respect their phylogenetic precedence, as, e.g., the formation of the apical before the basal dendrites of cerebral cortical pyramidal cells (RAMÓN Y CAJAL, 1911: p. 861), the primacy of the axon over the dendrite, and the late embryonic appearance and advanced phylogenetic status of the dendritic spines in some regions. Nevertheless, the notion that ontogeny recapitulates phylogeny, as the expression of a general biological tendency, is hardly intended to support a very detailed application. That would require a considerably more rigorous formulation.

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