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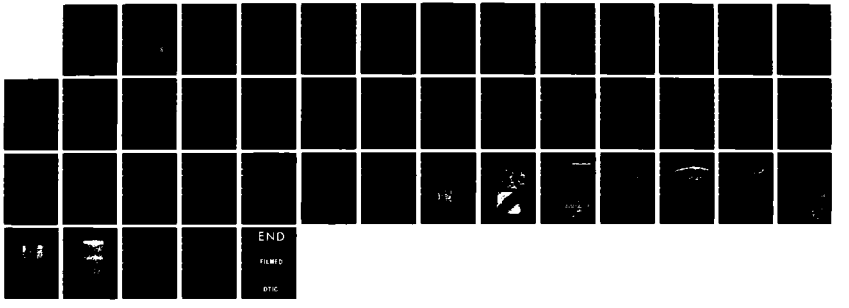

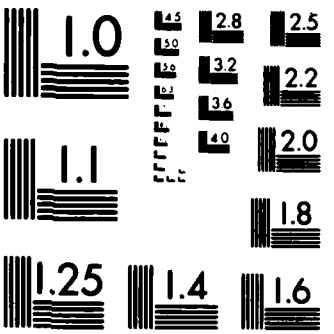

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The maturation of AChE-positive axons was not found to be fully complete until at least three months of age, and was characterized by several distinct developmental trends. AChE-positive fibers in layers IVc-VI proliferate

rapidly after birth until, by 4 weeks postnatal, they appear to exceed the adult density. They remain at this level as late as 8 weeks and then decrease to the adult density by 13 weeks. In contrast, the AChE-positive fibers in layer I do not show a substantial increase in density until 6 weeks of age and the adult level is not achieved before 3 months postnatal. Finally, the density of AChE-positive fibers in layers II-III appears to increase gradually from birth until the mature pattern is reached at about 6 weeks.

AChE could also be localized histochemically to cell bodies whose position and appearance depended on postnatal age. Stained cells first appeared in the white matter subjacent to layer VI shortly after birth. By two weeks of age, most cells in layer VI were also intensely AChE positive. The staining of these cells gradually disappears over the next two months until, at three months of age, there are no AChE-positive cells in cat striate cortex. However, a subpopulation of stained neurons appers in layer V by one year of age that persists into adulthood.

The possible contributions of acetylcholine and AChE to the postnatal development of kitten striate cortex are discussed.

POSTNATAL CHANGES IN THE DISTRIBUTION OF ACETYLCHOLINESTERASE IN KITTEN STRIATE CORTEX

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ABSTRACT

We have traced the postnatal development of axons and cells in kitten striate cortex that contain acetylcholinesterase (AChE) using a modification of Koelleś histochemical method.

The maturation of AChE-positive axons was not found to be fully complete until at least three months of age, and was characterized by several distinct developmental trends. AChE-positive fibers in layers IVc-VI proliferate rapidly after birth until, by 4 weeks postnatal, they appear to exceed the adult density. They remain at this level as late as 8 weeks and then decrease to the adult density by 13 weeks. In contrast, the AChE-positive fibers in layer I do not show a substantial increase in density until 6 weeks of age and the adult level is not achieved before 3 months postnatal. Finally, the density of AChE-positive fibers in layers II-III appears to increase gradually from birth until the mature pattern is reached at about 6 weeks.

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INTRODUCTION

One muscarinic action of acetylcholine (ACh) in cortex is a marked facilitation of transmission at excitatory synapses (Sillito and Kemp, 1983). Because pre- and post-synaptic coactivation appears to be essential for synaptic consolidation (Rauschecker and Singer, 1981), Singer (1979) has proposed that ACh contributes importantly to the stabilization of active synapses during the postnatal development of neocortex. Kitten striate cortex promises to be an ideal preparation for testing this idea. The functional connectivity of this cortical area may be readily altered by subtle manipulations of sensory input, such as monocular lid suture, during a critical period of development that extends from three weeks to about three months of age (Hubel and Wiesel, 1970). Indeed, a special role for acetylcholine during the critical period has been indicated recently by biochemical studies of several cholinergic markers. For example, muscarinic receptor binding is distinctly elevated in striate cortex at two months of age (Shaw et al, 1984) and the activity of acetylcholinesterase (AChE), the degradative enzyme for ACh, drops at the onset of the critical period and remains low until the third postnatal month (Potempska et al., 1979). These provocative findings have encouraged us to undertake a systematic investigation of cholinergic inputs to area 17 in the cat.

Cat visual cortex is known to receive a direct projection from large, AChE-rich neurons in the basal telencephalon that, collectively, are homologous to the Nucleus Basalis of Meynert in primates (Carnes et al., 1984; Tigges and Tigges, 1984). In a previous study, we demonstrated that kainate lesions of this region will deplete striate cortex of its normal complement of AChE-positive axons. In contrast, extensive lesions of the brainstem had no detectable effect on cortical AChE (Bear et al., 1984). These results, together with the fact that cortically projecting neurons in the Nucleus Basalis have been shown by immunocytochemistry to contain choline acetyltransferase (Mesulam et al., 1983; Stichel and Singer, 1984), suggest, first, that this input to area 17 employs acetylcholine as a transmitter; second, that the basal forebrain is the principal extrinsic source of cholinergic axons in cat visual cortex; and third, that AChE histochemistry accurately reveals the organization of this projection. These conclusions have been strengthened by the recent finding that axons in cat striate cortex with choline acetyltransferase immunoreactivity have a distribution and morphology very similar to that of AChE-positive fibers (Stichel and Singer, 1984).

In the present study we have extended this inquiry to the postnatal development of axons and cells in striate cortex that stain with AChE histochemistry. We find that cortical AChE does not achieve the adult pattern until 12 weeks of age. The postnatal changes in the distribution of AChE are consistent with the idea that the cholinergic projection to striate cortex contributes importantly to the shaping of connections during the critical period.

MATERIALS AND METHODS

Animals

Fourteen kittens were used in this study. Their ages at the time of sacrifice ranged from birth to one year (table 1). In addition, material from our previous work on adult cats (Bear et al., 1984) was used for comparison.

Tissue preparation

All animals were deeply anesthetized with sodium pentobarbital and perfused through the ascending aorta, first with 100-200 cc saline, then with 1-2 liters of fixative. The fixative was either 10% phosphate buffered formalin, pH 7.4 (11 kittens) or 1.25% glutaraldehyde and 1.0% formalin in phosphate buffer, pH 7.4 (3 kittens). In either case, half the fixative was perfused rapidly at a pressure of 120mm Hg; the second half was perfused slowly over 20-30 minutes. This was followed by a 30 minute vascular rinse with 2 liters of cold 10% sucrose in phosphate buffer, pH 7.4. The brains were removed from the skull, blocked in the appropriate plane of section, and frozen by immersion in 2-methylbutane at -50^{o}C for 5 minutes.

The brains were sectioned in a cyostat at 40 um, usually in the coronal plane. Adjacent sections, kept in serial order, were collected in 0.1 M phosphate buffer, pH 7.4, for Nissl staining and AChE histochemistry.

Histochemistry

The procedure used to localize AChE was a modification (Jacobowitz & Creed, 1983) of Koelleś method (Koelle, 1955). The tissue was first pre-incubated for 30 minutes at 38°C in 1.25 x 10^{-3} mM Iso-OMPA in 24% sodium sulfate. This was followed by incubation for 2-4 hours at 38°C in a solution containing 215.6 mg acetylthiocholine iodide, 2 mM copper glycine, 50 mM maleate, 2 mM copper sulfate, 80 mM magnesium chloride and 9.36 x 10^{-4} mM Iso-OMPA in a final volume of 187.5 ml of 24% sodium sulfate, pH 6.0. After washes in 20% and 10% sodium sulfate, the stain was developed at room temperature in 4% phosphate buffered ammonium sulfide, pH 6.0, for 1 minute. Next, the tissue was washed in distilled water, and the stain fixed in 10% phosphate buffered formalin, ph 7.4, for 20 minutes. The sections were again washed in water and the stain toned in 0.2% gold chloride for 5 minutes. After another water wash, the toner was developed in 5% sodium thiosulfate for 5 minutes. Finally, the tissue was washed in water, mounted onto glass slides, dehydrated through a series of alcohols, cleared in xylene and coverslipped. This material was

examined on a Leitz Orthoplan microscope using normal brightfield and interference contrast optics. With this technique, the cortical AChE reaction product is seen mainly in axons and cell bodies.

The appearance of the stain depended on the quality and type of fixation. In agreement with our previous observations (Bear et al, 1984), 10% phosphate buffered formalin was the optimal fixative for the visualization of AChE-positive axons; 1.25% glutaraldehyde in 1.0% phosphate buffered formalin was superior for cell body staining. In either case, good perfusions were essential for the clear resolution of AChE-positive axons and cells. Nonetheless, in our experience, the results obtained with this method are highly reproducible when these variables are controlled adequately.

Specificity

To distinguish non-specific esterase activity from cholinesterase activity, we occasionally reacted tissue in the presence of 10^{-5}M eserine. This treatment abolished all stain in the tissue, indicating that the histochemistry specifically demonstrated cholinesterase in cortex. In addition, we routinely inhibited butyrylcholinesterase in the tissue with 1.25 x 10^{-3}mM Iso-OMPA. These precautions, together with our use of acetylthiocholine iodide as a substrate, make it very likely that the stain we describe is attributable solely to AChE. We are, of course, aware that the specificity of the enzyme may change during development (Silver, 1974). However, Krnjevic and Silver (1966) found, using specific inhibitors of AChE, that by birth virtually all the cholinesterase activity in cat cerebral cortex is due to AChE.

RESULTS

Birth

At the time of birth, AChE in striate cortex is histochemically localized to axons; stained cell bodies were not visualized. These fibers distribute largely within layers VI, V and lower IV (figure 1) and are characterized by terminal swellings that are reminiscent of synaptic boutons. Radially-oriented fibers are also commonly observed ascending through layers II and III. These axons branch just under the pial surface, and run tangentially in the upper third of layer I.

One source of AChE-positive axons in mature cat striate cortex is the large neurons that are embedded in the internal capsule (Bear et al., 1984). This region of the basal forebrain is well differentiated in newborn kittens (figure 2) and, together with the diagonal band of Broca, is presumably the source of many of the AChE-positive fibers in striate cortex at the time of birth.

One Week

With the usual formalin fixation, the main difference in cortical AChE at one week of age, as compared with that at birth, is an increased density of stained axons in layers I-III (figure 3A).

However, with glutaraldehyde fixation, a population of intensely stained cells is also observed (figure 3B & C). These cells lie in and just deep to layer VI, among the AChE-positive axons of the cortical white matter. In most cases, the proximal processes of these cells were also stained.

Two Weeks

The pattern of AChE staining changes radically during the second postnatal week. At low magnification, the stain can be seen to form bands in cortex that are similar to the adult pattern (figure 4). These AChE-rich bands lie in the outer half of layer I, in lower layer III, in layer IVc and in layer VI. However, at higher magnification, it becomes clear that these bands do not merely reflect the density of stained axons (figure 5). To the contrary, much of the stain observed in layer VI is associated with AChE-positive neurons. In one animal whose cortex was particularly well perfused with formalin on postnatal day 15 (K243, illustrated in figure 5) we found virtually all layer VI cells to be AChE-positive. Careful examination of these cells revealed, however, that only their proximal processes contain AChE; stained axons were not observed arising from layer VI neurons in striate cortex.

The transition from layer VI to white matter was not always sharp in AChE-stained material (figure 4). The white matter of the lateral gyrus was also filled with a reticulum of AChE-positive cells and

fibers, even in formalin-fixed tissue (figure 5). However, at the depths of sulci, this reticulum was easily distinguished from both layer VI and unstained white matter, and appeared to constitute a seventh cortical layer (figure 6A & B). The appearance of AChE-positive, "layer VII" cells depended on their location. Those in sulcal white matter appeared fusiform in shape with few stained processes (Figure 6C). Those in gyral white matter generally appeared multipolar (figure 6D).

In layers I-V, the stain was associated primarily with axons. Indeed, all layers contained numerous AChE-positive fibers. Plexuses of tangentially-oriented axons occured in outer layer I, lower layer III, and layer IVc as well as in layer VI.

Four Weeks

By 4 weeks of age, most of the AChE in striate cortex is again localized histochemically to axons. The density of stained axons appears to achieve or even exceed the adult level in all layers except layer I (figure 7). As at two weeks of age, plexuses of horizontally running axons occur in lower layer III, in layer IVc and in layer VI. However, these bands appear less distinct at 4 weeks because of the high density of fibers in upper layer III and in layer V.

In addition to the stained axons, AChE-positive cells are again observed in layer VI and in the subjacent white matter. However, the fraction of layer VI neurons that are AChE-positive is considerably smaller than that seen at 2 weeks. This is illustrated in figure 7. Figure 7C is a photomicrograph of layer VI stained by AChE histochemistry. Figure 7D shows this same photographic field after being counterstained for Nissl substance with cresyl violet acetate. It can be seen that by 4 weeks AChE-positive cells constitute only about half of all layer VI cells.

Six Weeks

The AChE staining pattern in striate cortex at 6 weeks of age is still characterized by a high density of stained axons (figure 8). The distribution of AChE-positive fibers resembles that at 4 weeks, with one notable exception: the entire thickness of layer I is now filled with a plexus of horizontally-running axons. In addition, the fiber plexuses in layers IVc and VI appear to be particularly dense at this age. These bands encroach on layer V so much in some places that they appear fused into one thick plexus of AChE-positive axons (figure 8).

Only occaisional AChE-positive cells are observed in layer VI at this age. However, numerous stained cells may still be found in the white matter of the lateral gyrus.

Subsequent Development

From 6 to 13 weeks, the AChE-positive axons in striate cortex gradually achieve the adult pattern (figure 9). The density of AChE-positive fibers in layer I increases during this period until, by 13 weeks, the molecular layer is black with stained axons. In contrast, the density of AChE-positive axons in the deep layers appears to decline markedly. For example, on postnatal day 93, the density of stained fibers in layer IVc is only slightly higher than in layer IVa+b which, together with layers II and V, has the fewest AChE-positive axons in cortex. Likewise, the density of stained fibers in layers V and VI is distinctly lower at 12 weeks than it is at 6 weeks.

The most striking change in striate cortex during the third postnatal month, however, is the disappearance of all the AChE-positive cells. By 93 days of age, all layers of striate cortex, including layer VI and the subjacent white matter, are devoid of stained cell bodies. This appears to be the case as late as 6 months of age. However, in cats aged beyond one year, we consistently observe AChE-positive neurons in layer V of striate cortex. These cells, described previously (Bear et al., 1984), constitute a subpopulation of pyramidal cells that are among the largest in this layer.

DISCUSSION

Development of AChE-positive fibers

The present study extends to postnatal development the classical description by Krnjevic and Silver (1966) of the embryonic ontogenesis of AChE in kitten forebrain. They showed that the AChE-positive innervation of fetal cortex arises from cells in the basal forebrain. This suggests that our previous conclusions, drawn from lesion experiments in adult cats (Bear et al., 1984), are also valid for infants; namely, that AChE-positive axons in striate cortex are cholinergic and derive largely from the basal telencephalon. It should be stressed, however, that experimental lesion and immunocytochemical studies must be performed before any firm conclusions can be reached about the source or cholinergic nature of the AChE-positive innervation of striate cortex in young kittens.

Figure 10 summarizes the postnatal changes in the AChE-staining of kitten striate cortex. At least three distinct developmental trends are evident. In layers IVc-VI, AChE-positive axons are present at birth and, by postnatal day 30, appear to exceed the adult density. This remains true as late as 8 weeks of age. However, by 13 weeks, the density of axons in layers IVc-VI has decreased to the adult level. In contrast, AChE-positive fibers in layer I do not achieve their greatest density until adulthood. These axons distribute

sparsely as late as postnatal day 30, and mostly to the outer half of layer I. Not until 6 weeks of age do AChE-positive fibers innervate the full thickness of layer I; the adult density is not achieved before 3 months postnatal. Finally, the development of AChE-positive axons in layers II-III appears to involve a smooth and gradual increase in fiber density over time. The mature pattern is apparent in these layers by six weeks of age. Thus, the development of AChE-positive axons in striate cortex is not fully complete until at least 3 months of age, and is characterized by a number of distinct events that are superimposed.

The developmental trends we describe are not unique to striate cortex; similar changes were observed in all cortical areas examined. However, the timing of these events depended critically on location. For example, the maturation of AChE-positive axons in layer I occured first laterally and anteriorly in the hemisphere; as early as two weeks in entorhinal cortex. Striate cortex is the last neocortical area to mature apparently as a consequence its medial position in the postlateral gyrus.

The activity of choline acetyltransferase (ChAT), the synthetic enzyme for ACh, and muscarinic receptor binding have been measured recently as quantitative indices of the postnatal maturation of cholinergic synapses in striate cortex (Potempska et al., 1979; Shaw et al., 1984). Both of these markers are elevated over the adult level during the early postnatal period. Muscarinic binding, in

particular, exceeds the adult value from 3 weeks until at least 3 months of age. These developmental curves thus appear to correlate reasonably well with the density of AChE-positive axons in striate cortex during this same period, especially in the deep layers. However, the developmental curve for AChE activity is paradoxically different (Potempska et al., 1979). AChE enzyme activity apparently is almost 90% of the adult value at one week of age (per gram of cortex, wet weight), then drops to approximately 65% of the adult activity at 2 weeks. It remains at this level through the second postnatal month. Cortical AChE activity then increases until it is again 90% of the adult value by 3 months of age.

This apparent discrepancy, between the histochemistry and biochemistry, is possibly explained by developmental changes in the multiple isoenzymes of AChE. Two primary forms of this enzyme can be distinquished using sucrose gradient centrifugation (Rieger and Vigny, 1976; Wade and Timiras, 1980): a soluble 4S fraction and an insoluble, probably membrane bound 10S fraction. The relative amounts of these two forms of AChE have been shown to vary in rat cerebral cortex as a function of postnatal age (Wade and Timiras, 1980). The biochemical measurement of enzyme activity in homogenates of cortex pools the individual contributions of all the isoenzymes of AChE. On the other hand, the treatment of cortex with buffer and fixative for histochemical experiments has been shown to wash out the soluble fraction (Hüther and Luppa, 1979). Thus it is possible that the postnatal dip in the developmental curve for AChE specifically reflects a decrease in the soluble form of the enzyme.

Development of AChE-positive cells

Perhaps the most surprising histochemical change in cortical AChE during postnatal development is the transient appearance of stained cell bodies. We first observed these cells in the cortical white matter at one week of age. By two weeks, cells in layer VI and the adjacent white matter are intensely AChE positive. These cells gradually disappear over the next two months until, at three months of age, there are no AChE-positive cells in cat visual cortex. However, stained cells again appear in area 17 by 1 year, but at this age they are found in layer V.

The finding of AChE-positive cells in the cortical white matter is not a new discovery: Krnjevic and Silver (1966) originally described these cells in young kittens. The morphology and size of the cells characterizes them as neuronal, instead of glial elements. These cells are not, however, found scattered randomly throughout the white matter of the hemisphere, but rather are located in specific zones that are subjacent to layer VI. These zones are also characterized by a high density of AChE positive axons and therefore, at low magnifications, appear to form a seventh cortical layer. Krnjevic and Silver (1966) believed that such cells were late migrating neuroblasts, probably enroute to layer VI. Indeed, the fusiform appearance of the AChE-positive cells in sulcal white matter strongly resembles that of neuroblasts (Levi-Montalcini, 1964). On the other hand, in gyral white matter, these cells appear

to have differentiated into multipolar cell types deep to layer VI. Furthermore, AChE-positive neurons remain in the white matter as late in postnatal development as 6 weeks, over one month after the cellular differentiation of the cortical plate.

Marin-Padilla (1971, 1972, 1978) has shown, using the Golgi method, that polymorphic cells appear below the cortical plate very early in development. These cells are characterized by projections to layer I, and appear to persist during the subsequent prenatal maturation of cortex. It is thus conceivable that AChE-positive cells in the white matter are vestiges of these primordial neurons. Whatever their origin, these layer VII cells are in a good position to receive a synaptic input. Innocenti and Clarke (1984) have described a transient callosal projection in young kittens that appears to terminate specifically in the white matter of the lateral gyrus. Thus, both the AChE-positive cells in layer VII and the commissural connections within layer VII are present during the early postnatal period and are absent in striate cortex of adult cats.

The finding of stained layer VI neurons also agrees with the original descriptions by Krnjevic and Silver (1965, 1966) of cortical AChE in the cat. However, while they reported that these cells persist into adulthood, we find no AChE-positive layer VI neurons after about eight weeks of age, at least not in area 17. The disappearance of stained cells from layer VI cannot be explained by cell death since, at two weeks of age, almost all of the neurons in this layer can contain AChE. Rather, this appears to result from a loss of enzyme from these neurons.

The transient appearance of AChE in neurons during early postnatal development has been documented before in other systems (See Silver, 1974, for review). One frequently cited example is the Purkinje cells of the cerebellum (Joo, et al., 1963; Csillik, et al., 1964). In the rat, these neurons acquire AChE shortly after birth, but then lose the enzyme three weeks later. Purkinje cells are innervated by climbing fibers during this same period (Altman, 1972). Interestingly, removal of this climbing-fiber innervation in the adult cerebellum results in another transient surge in AChE activity in Purkinje cells (Kasa and Csillik, 1964; Phillis, 1968). In this light it may be important to note that the appearance of AChE-positive cells in layer VI coincides with the period of most rapid synaptogenesis in kitten visual cortex (Cragg, 1975).

It is not yet clear what mechanisms account for the changing levels of AChE in cortical neurons, or what purpose the enzyme serves in this location. On the one hand, synthesis of AChE by these neurons could signal the transient expression of a cholinergic phenotype. Such a process probably occurs in the developing sympathetic nervous system (Landis, 1983, for review). On the other hand, certain cortical neurons may synthesize AChE because they are prime targets of the cholinergic fiber innervation. For example, in the adult most "cholinoceptive" units are found in layer V (Krnjevic and Phillis, 1963) and many neurons in this layer are AChE-positive. A third possibility is that AChE-positive cortical neurons do not synthesize the enzyme at all, but rather sequester it from the extracellular space.

The role of acetylcholine in the postnatal development of striate cortex

It is well known that sensory experience plays a crucial role in the establishment and maintenance of synaptic patterns in the developing striate cortex. For example, temporary closure of one eye during a critical period that extends from three weeks to approximately three months of age results in a dramatic cortical rearrangement such that most neurons, originally binocularly activated, are dominated exclusively by the open eye (Hubel and Wiesel, 1970). However, an imbalance in the activity of geniculate afferents to cortex is not alone sufficient to shift ocular dominance; it is additionally required that the open eye is presented patterned visual stimuli that are effective in altering the firing rate of cortical cells (Rauschecker and Singer, 1981; See also Singer, 1979, for review). One factor that appears to modulate the effectiveness of excitatory synapses in striate cortex is acetylcholine (Singer, 1979; Sillito and Kemp, 1983). Thus, the cortical cholinergic projection is in a strategic position to determine when synapses may be modified by sensory experience. Consistent with this idea is recent evidence that suggests that both the availability and effectiveness of ACh are enhanced during the critical period. For example, the density of AChE-positive axons and the number of muscarinic binding sites (Shaw, et al., 1984) are elevated over the adult level during the second and third postnatal months. AChE activity, in contrast, is significantly reduced at these same ages (Potempska et al., 1979).

There are indications that, during the first half of the critical period, the modification of cortical circuitry by monocular deprivation involves primarily the thalamo-cortical connections in layer IV; subsequent changes are probably restricted to intrinsic connections in the supra- and infra-granular layers (LeVay et al., 1980; Presson and Gordon, 1982). Not only is the efficacy of ACh probably higher during the entire critical period, but the distribution of muscarinic receptors also reflects this laminar distinction: Shaw et al. (1984) have found that during the first six postnatal weeks, layer IV has the highest density of muscarinic binding sites. However, by 60 days, the receptors apparently redistribute above and below layer IV in a pattern that resembles the distribution of AChE. Thus, the levels of this cholinergic marker correlate with synaptic plasticity in both the spatial and temporal domains.

If this line of reasoning is correct, then it follows that an increase in cortical ACh might stabilize marginally effective synapses that otherwise would be eliminated. Indeed, cortical synapse density does exceed the adult value during the critical period (Cragg, 1975; Winfield, 1981). Moreover, the subsequent decline in synapse density coincides with an increase in AChE activity (Potempska et al., 1979) and a decrease in muscarinic receptor binding (Shaw et al., 1984). A correlation of cortical synapse density and cholinergic markers also exists under abnormal rearing conditions. For example, binocular deprivation apparently

increases the activity of AChE in kitten striate cortex (Potempska et al., 1979). Because there is not a parallel increase in ChAT activity, it is plausible that the half-life of released ACh is reduced in the visual cortex of animals reared under these conditions. This might partially explain why binocularly deprived kittens develop fewer synapses in striate cortex (Winfield, 1981).

It is interesting to note that the projection to striate cortex from the locus coeruleus, which employs norepinephrine (NE) as a neurotransmitter, might exert an influence on cortical synaptogenesis that is the opposite of what has been proposed for acetlycholine. Parnavelas and Blue (1983) found that neonatal depletion of cortical NE with 6-hydroxydopamine actually accelerates the rate of synapse formation in developing rat neocortex.

It is clear that specific experiments are required to test these ideas. Now that the baseline description is becoming more complete, striate cortex of the cat provides an excellent opportunity to establish the role of acetylcholine and AChE in cortical development.

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FIGURE LEGENDS

Figure 1: Kitten striate cortex on postnatal day 2 stained for Nissl substance (A) and for AChE (B). In this instance, the AChE-stained section was counterstained with cresyl violet after photography. Thus, the asterisks in A and B mark the same blood vessel. Note that the AChE stain at this age is restricted to axons that distribute mostly in layers IVc-VI. Scale bar: mm.

Figure 2: AChE in the basal forebrain on postnatal day 2. A: Photomicrograph of a coronal section through the internal capsule at the level of the anterior commissure. The boxed region is enlarged in B. B: Photomicrograph of the large AChE-positive neurons embedded in the internal capsule. In the adult, these cells give rise to a substantial fraction of AChE-positive axons in the striate cortex. Abbreviations: AC, anterior commissure; Ca, caudate; D, dorsal; GP, globus pallidus; IC, internal capsule; L, lateral.

Figure 3: AChE in striate cortex on postnatal day 8 in a case fixed with 10% buffered formalin (A) and in a case fixed with 1.25% glutaraldehyde in 1.0% buffered formalin (B). Note that the distribution of stained axons is the same regardless of the type of fixation. However, in the glutaraldehyde fixed material, stained cell bodies may also be observed deep to layer VI (C). Laminar borders were determined on adjacent sections stained for Nissl substance. Scale bars: A, mm; B, mm; C, mm.

Figure 4: Kitten striate cortex on postnatal day 15 stained for Nissl substance (A) and for AChE (B). Note the large increase in AChE at this age, especially in the deep layers. Scale bar: mm.

Figure 5: Photomontage of striate cortex on postnatal day 15 stained for AChE. Particularly note that much of the stain in the deep layers is attributable to labelled cell bodies, especially in layer VI and the subjacent white matter.

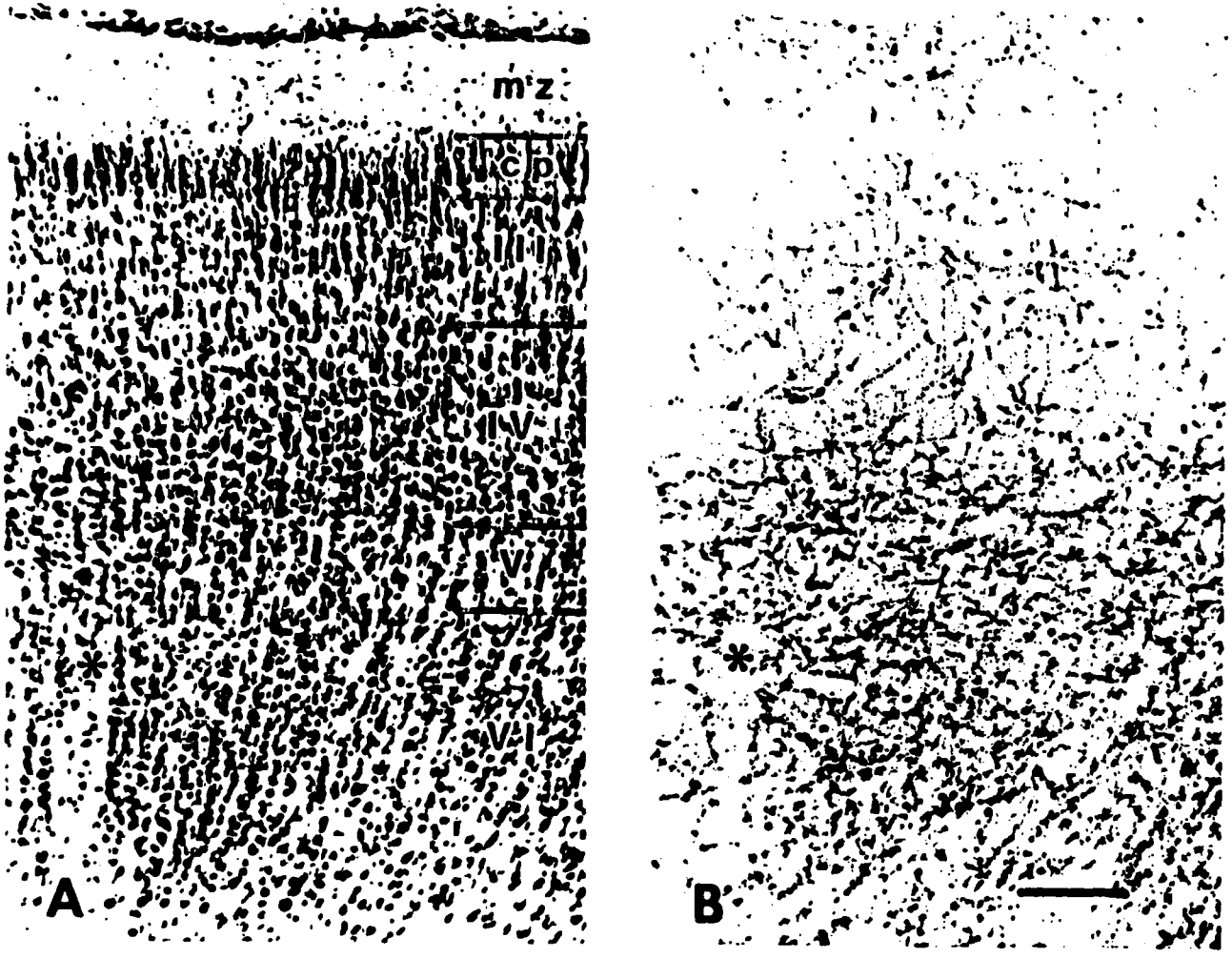
Figure 6: A: AChE in striate cortex on the superior lip of the splenial sulcus on postnatal day 15. The boxed region is enlarged in B. B: Photomicrograph to illustrate the AChE-positive cells and fibers that lie deep to layer VI. This reticulum forms a seventh cortical layer. Also note the AChE-positive neurons in layer VI. C: Layer VII cells, stained for AChE, as they appear in sulcal white matter. D: Layer VII cells, stained for AChE, as they appear in the white matter of the lateral gyrus.

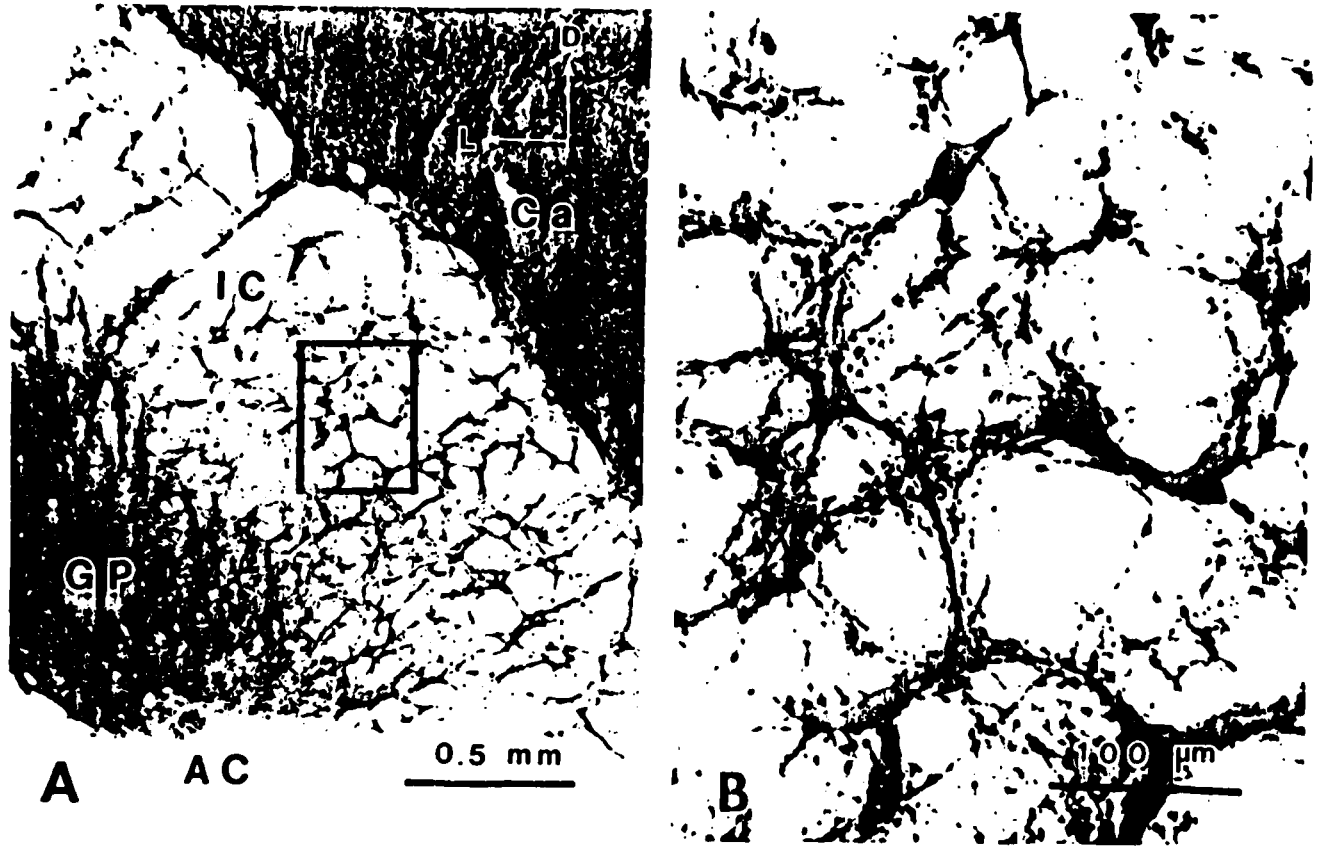
Figure 7: Kitten striate cortex on postnatal day 30 stained for AChE (A) and counterstained for Nissl substance (B). C: Layer VI as it appears in AChE-stained material. D: The same photographic field as C counterstained with cresyl violet. Note that only about half the cells in layer VI are AChE-positive at this age.

Figure 8: Kitten striate cortex on postnatal day 46 stained for Nissl substance (A) and for AChE (B). Note the high density of AChE-positive axons in layers IVc-VI and the increase in stained fibers in layer I. Scale bar: mm.

Figure 9: Photomontage of striate cortex on postnatal day 93 stained for AChE. Particularly note (1) the increase in layer I fiber density, (2) the decrease in layer VI fiber density and (3) the virtual absence of stained cell bodies. Laminar borders were confirmed on an adjacent section stained for Nissl substance.

Figure 10: Summary of the postnatal changes in the distribution of the AChE in kitten striate cortex. The prefix (P) stands for "postnatal day"; the day of birth is defined as P 0.


m
z
I
c
p
II
III
IV
V
VI
A
B


D
L
Ca
IC
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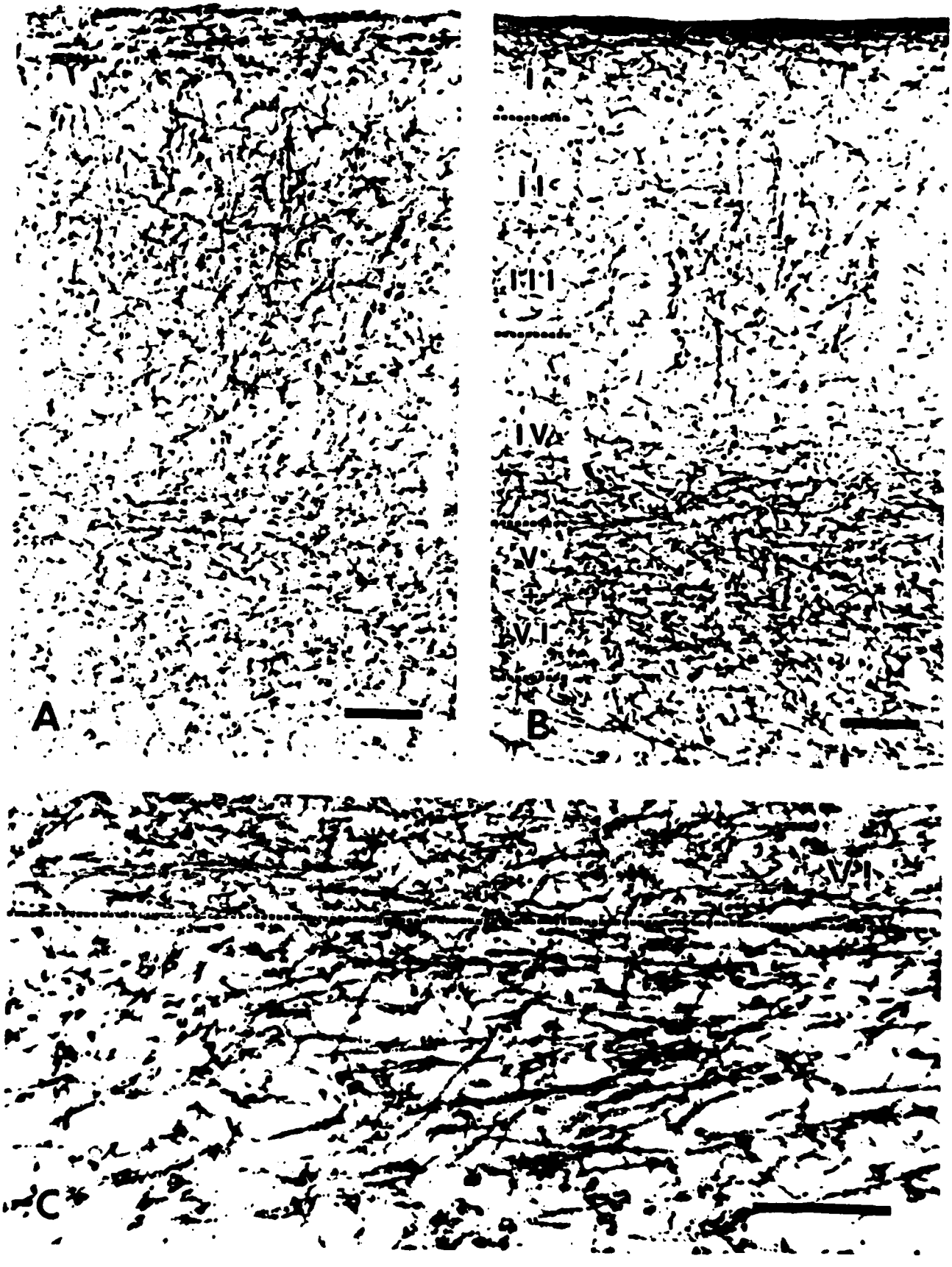
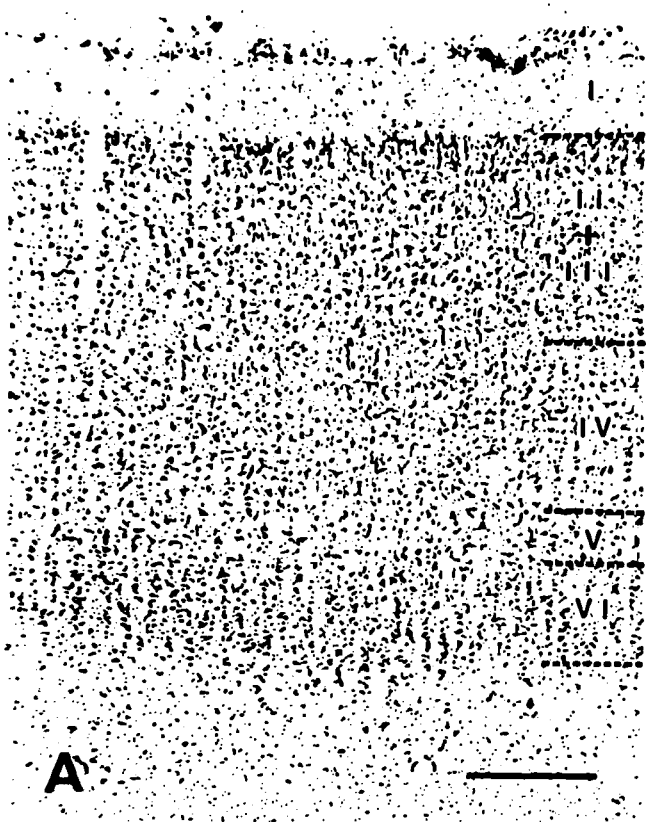
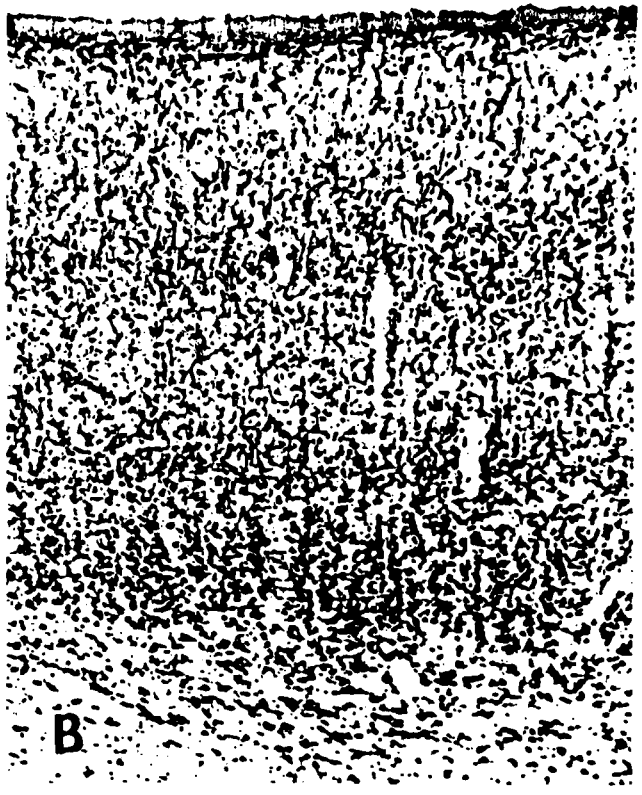
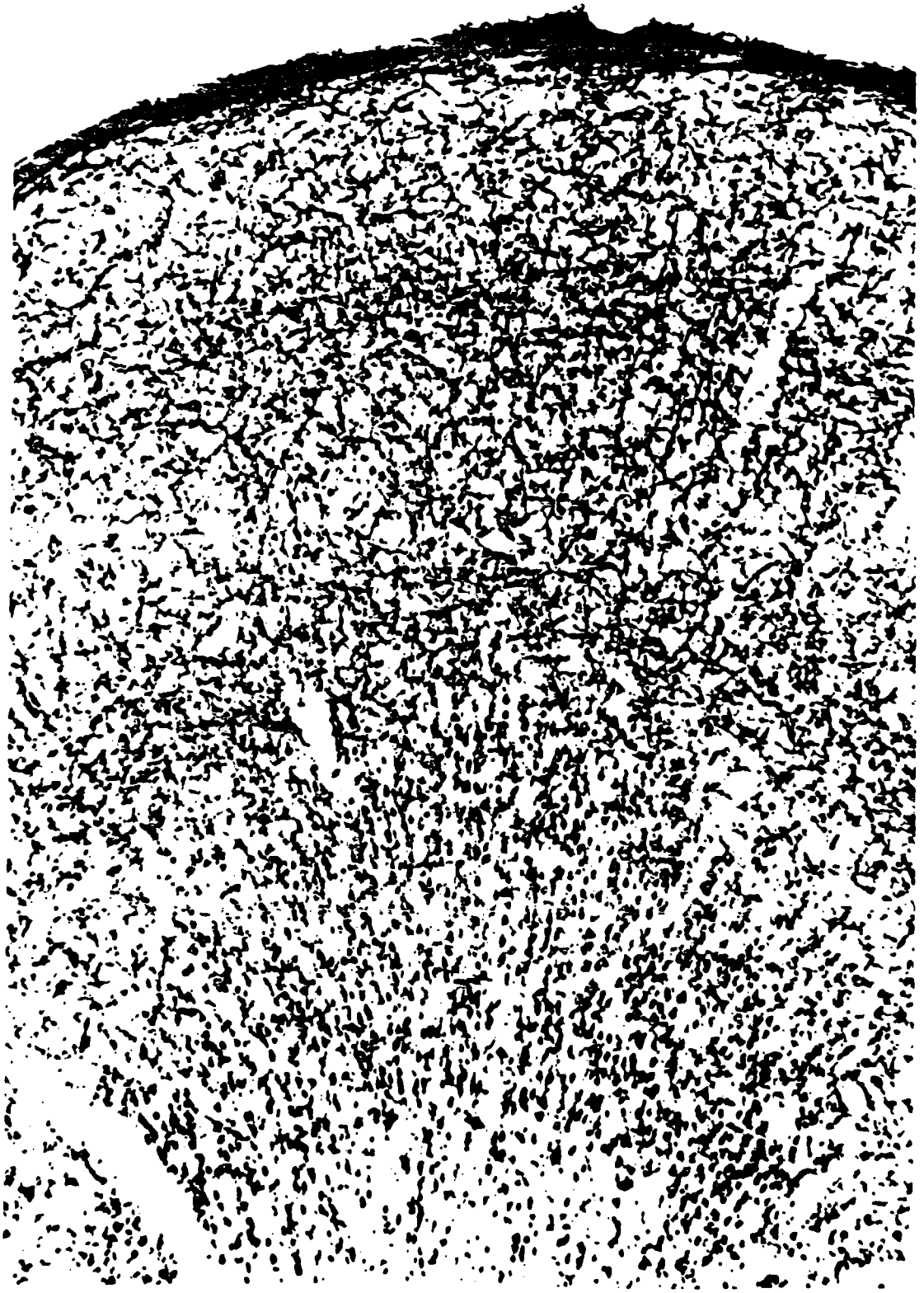
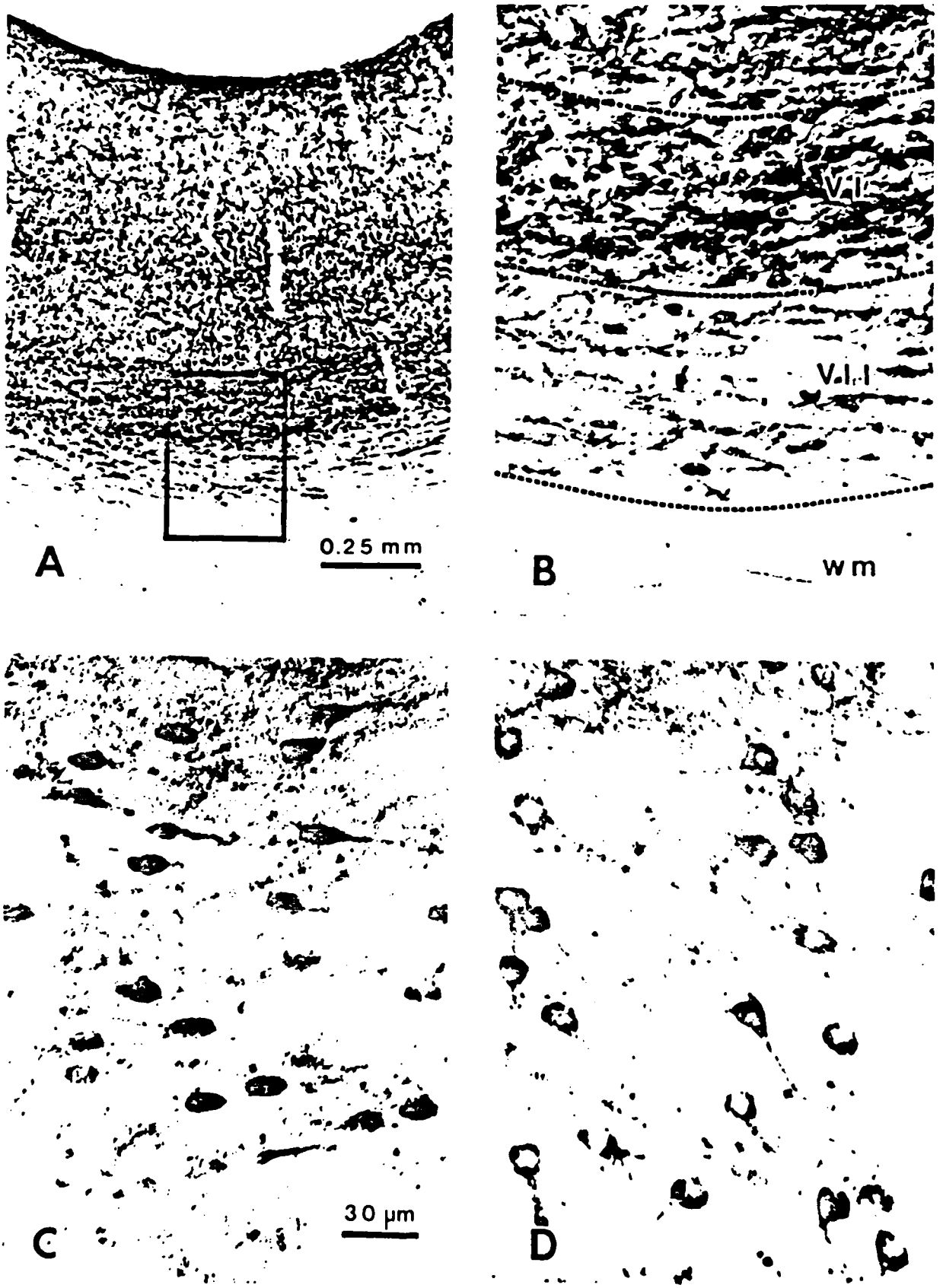
A
AC
0.5 mm
B
100 µm

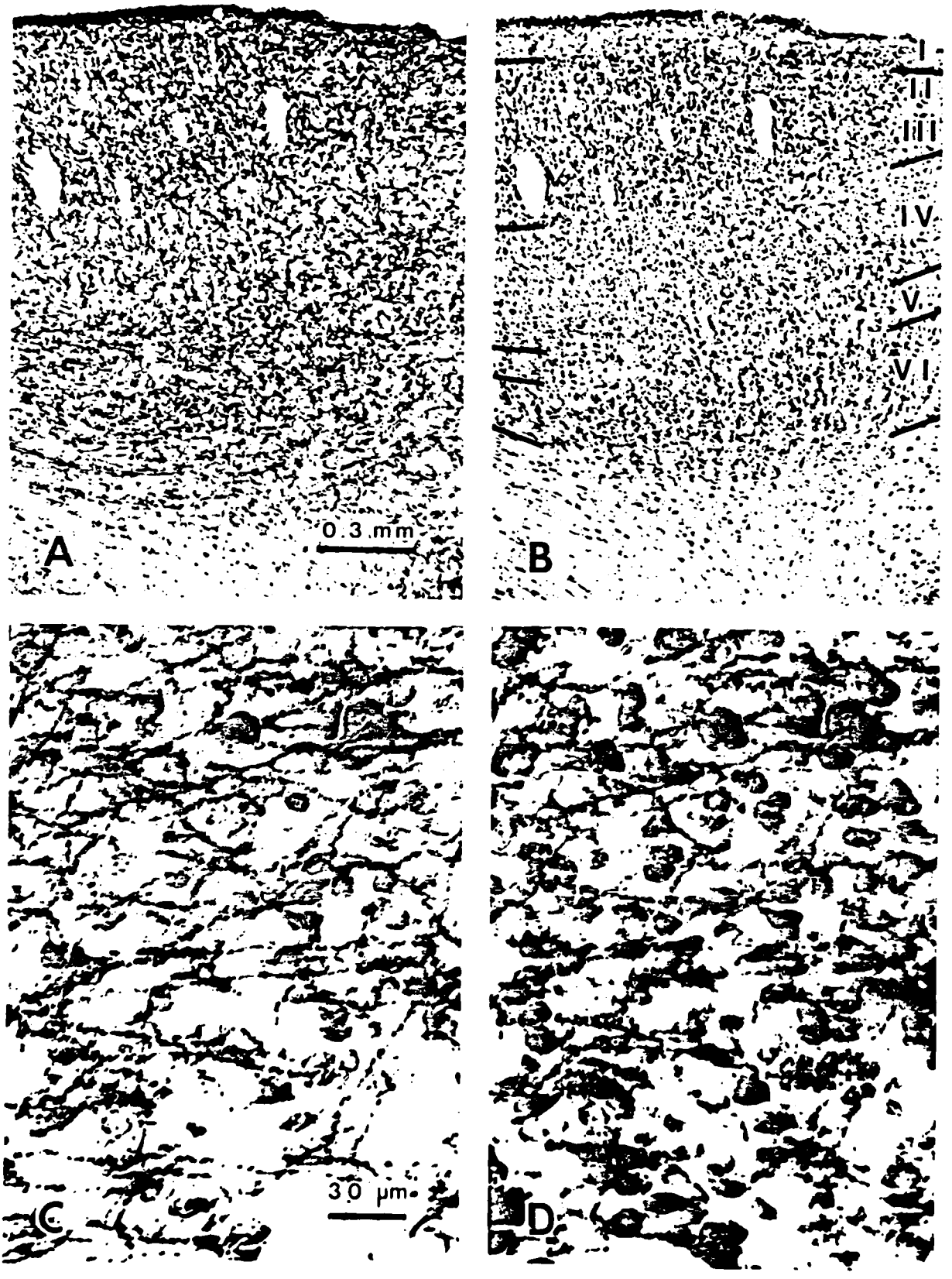

A
I
II
III
IV
V
VI
B
VI
C

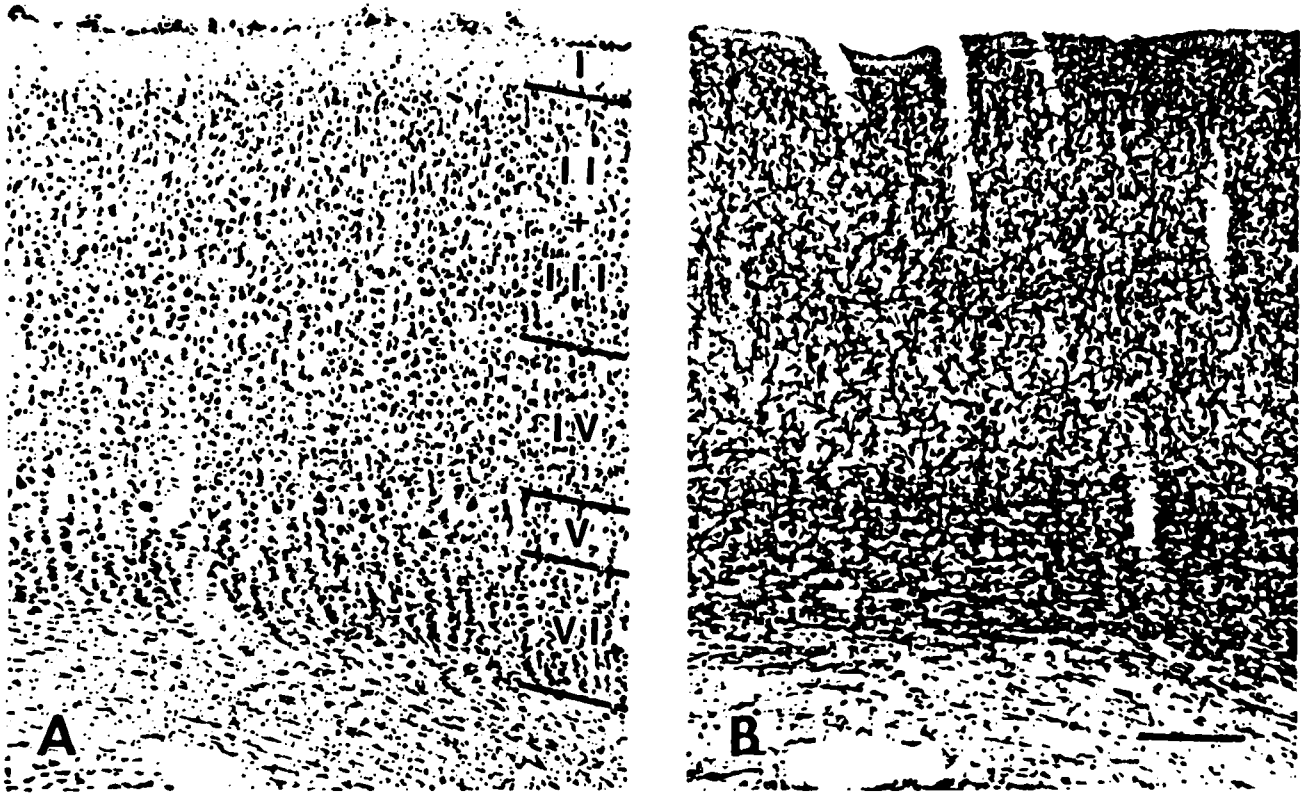

I
II
III
IV
V
VI
A

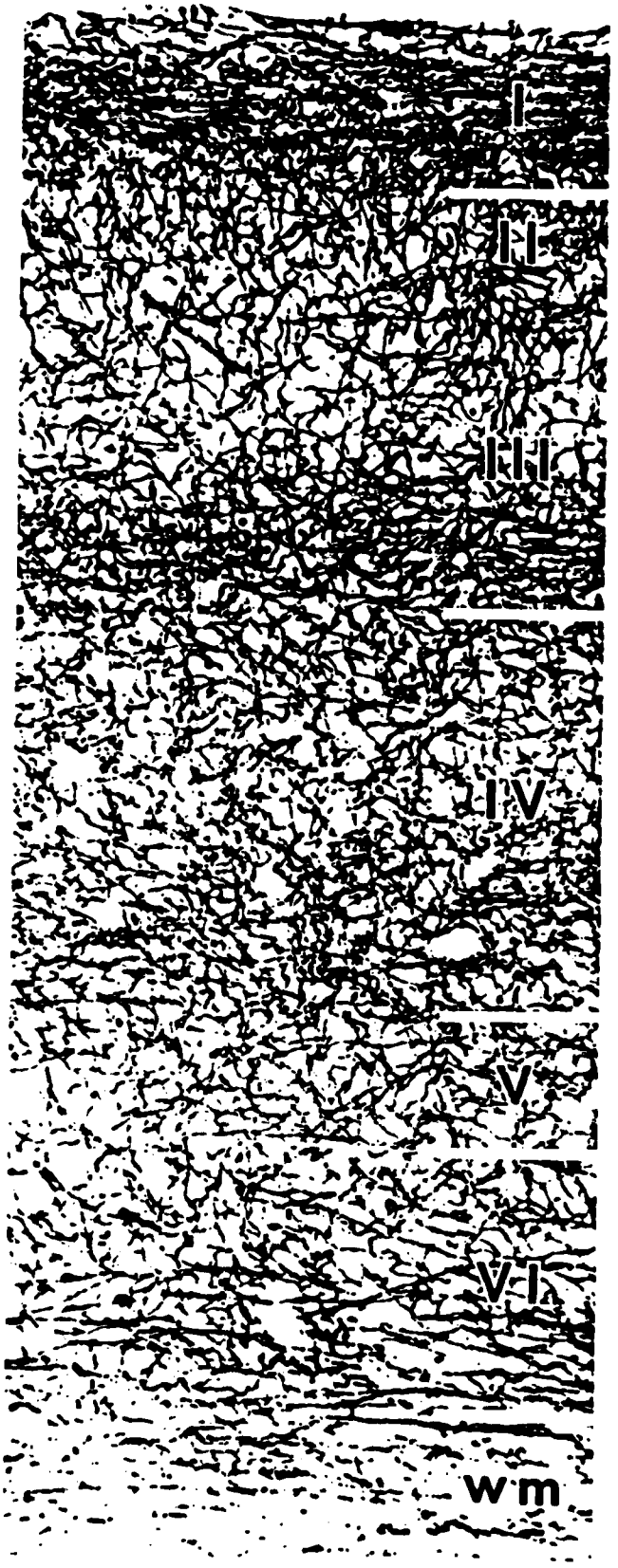

B




A
0.25 mm
B
V.l
V.l.l
wm
C
30 µm
D


A
0.3 mm
B
I
II
III
IV
V
VI
C
30 µm
D


I
II
III
IV
V
VI
A
B


I
II
III
IV
V
VI
wm

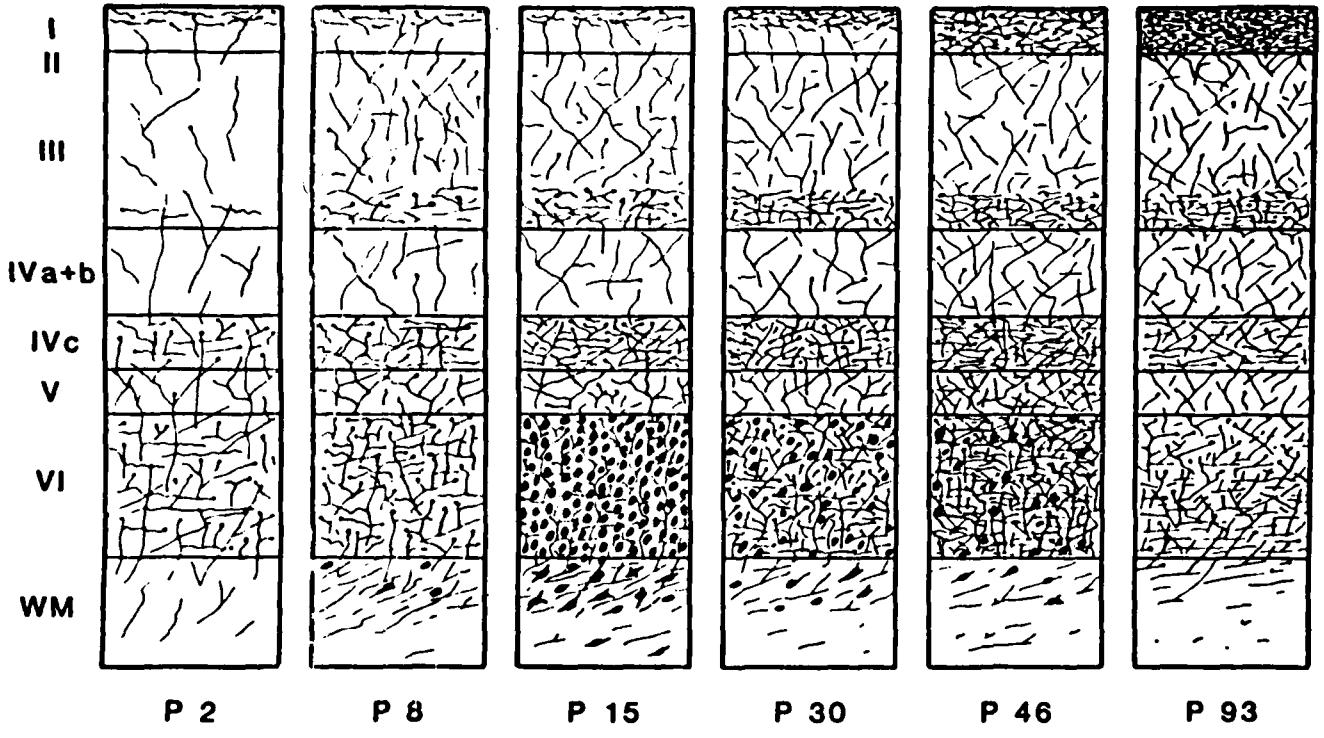

I
II
III
IVa+b
IVc
V
VI
WM
P 2
P 8
P 15
P 30
P 46
P 93

TABLE 1

ANIMAL HISTORY

ANIMAL	AGE (days)
K252	0
K238	2
K239	8
K244	8
K245*	8
K243	15
K254	15
K247*	21
K248	30
K240	46
K242	61
K210	93
K232*	224
K241	348

Asterisks denote animals perfused with 1.25% glutaraldehyde and 1.0% formalin. All others were perfused with 10% phosphate buffered formalin. Day of birth is defined as postnatal day 0.

END

FILMED

3-85

DTIC