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# A Quantitative Investigation of Spine and Dendrite Development of Neurons in Visual Cortex (Area 17) of *Macaca nemestrina* Monkeys<sup>1</sup>

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**ABSTRACT** In a previous Golgi study (Lund et al., '77) which examined the development of the macaque monkey striate cortex (area 17) it was observed that the dendrites of neurons within the visual cortex show a marked increase in the number of spines on their surface during the first eight weeks of postnatal life. The qualitative observation was also made that all neurons then showed a marked decrease in spine numbers by the time the animal was adult. Since these spines are known to be sites of synaptic contact, changes in their numbers may reflect changes in synapse populations on these neurons.

This study examines *quantitatively* spine frequency and total dendritic development of Golgi impregnated neurons in monkeys ranging in age from 145 days gestation to adult. Four cell types were studied: spiny stellate neurons from laminae IVC $\alpha$  and IVC $\beta$  and pyramidal neurons with soma in either lamina IIIB or upper lamina VI. After consideration of possible sources of variation in spine numbers several conclusions are made: (1) Dendritic spine development appears to be a tightly controlled process both in terms of actual numbers of spines on a neuron at any one age and in the rate of change of spine frequency. (2) The neurons populations examined all show a gradual increase in spine numbers up to eight weeks of age. (3) At least two different trends are found in spine population maturation after the eight week point: (A)-the spine population may remain constant at the eight week level for same period of time or (B)-there may be a rapid decline in spine numbers following the eight week peak. (4) There is a suggestion that those neurons associated with direct input, or early stages in the relays, from the parvocellular geniculate laminae show trend B, while those associated with magnocellular input, or later order combined relays within the cortex, show trend A. (5) Different parts of a single pyramidal neuron dendrite may show either trend A or trend B, depending on the lamina location of the dendritic segment considered. (6) All neurons show spine population decreases between nine months of age and adult (5-7 years) suggesting continuing long term maturational changes.

Electrophysiological studies of feline and primate visual cortex development have indicated that maturation continues well into the postnatal period. In physiological studies of cat cortex for example, a proportion of area 17 units show some stimulus specificity at birth,

but both the number of stimulus-specific units and the degree of specificity for properties such as orientation tuning appear to increase

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for several weeks with postnatal experience in a normal visual environment (Hubel and Wiesel, '63; Pettigrew, '74; Blakemore and Van Slyters, '75; Sherk and Stryker, '76; Buisseret and Imbert, '76; Bonds, '78). Binocular deprivation of light or pattern during this period appears to cause deterioration in, or failure to develop, normal cortical receptive field organization in cats (Wiesel and Hubel, '65; Blakemore and Van Sluyters, '75; Buisseret and Imbert, '76) and in monkeys (Wiesel and Hubel, '74; Crawford et al., '75).

These physiological results raise the question as to whether the anatomical development of the visual cortex has been disturbed. Several anatomical studies comparing the deprived and normal visual system have been performed. Binocular deprivation has effects on cell size in the dLGN and number of spines and dendrites on neurons in the visual cortex in many species (Gyllensten et al., '65; Cragg, '67; Coleman and Riesen, '68; Valverde, '71; Hubel et al., '77). Monocular deprivation has more severe effects: cells in laminae of the dLGN to which the deprived eye projects are relatively smaller than cells in the non-deprived laminae (Wiesel and Hubel, '63; Headon and Powelle, '73; Von Noorden, '73; Guillery, '74; Von Noorden and Middleditch, '75) and afferent axon termination bands in layer IVC of monkey visual cortex associated with the projection of the experienced eye appear to widen at the expense of those associated with the deprived eye (Hubel et al., '77; Des Rosiers et al., '78). Thus, there is evidence that monocular and binocular deprivation cause alterations in geniculo-cortical connections and probably intracortical synaptic connectivity.

The normal developmental baseline to which the deprived cortex can be compared has not been fully investigated. Extensive formation, and perhaps loss, of connections appears to occur postnatally in the visual cortex of higher mammals. Cragg ('75) in a quantitative electron microscopic study of cat visual cortex described accelerated postnatal synaptic formation which reached a maximum population at roughly mid-critical period as shown in studies of monocular deprivation in the cat. The total number of synapses in visual cortex appeared to decline thereafter. Ruiz-Marcos and Valverde ('69) counted spine numbers from layer V pyramidal neurons in mouse cortex and concluded that for the distal portions of the apical dendrite, spine frequency

increased until 19 days of age and then decreased in older animals. Lund et al. ('77) in a descriptive study of Golgi-stained macaque monkey visual cortex, similarly reported that for most neurons spine frequency appeared to increase until approximately eight weeks postnatal and decreased at later ages. With electron microscopy, it has been demonstrated in rats, cats, and monkeys that each spine is usually associated with a single type I synapse (Gray, '59; Colonnier, '68; LeVay, '73). Hence estimates of the frequency of occurrence of spines along dendrites combined with estimates of the amount of dendrite per neuron may provide an estimate of the numbers of type I synapses of the neurons examined. These studies suggest that visual cortex development may involve loss as well as acquisition of synaptic contacts.

It would be useful to be able to compare this spine development pattern with other anatomical, physiological, and behavioral studies of development, and to determine more precisely the time course of spine frequency changes in macaque visual cortex. The present study is a quantitative analysis of the time course of development of dendrites and their spines in primary visual cortex (area 17) of normal macaque monkeys. The time course has been determined quantitatively from measurement of camera lucida drawings and counts of spines from more than 4,000 dendritic segments of more than 400 pyramidal and spiny stellate neurons from monkeys at 12 ages ranging from four weeks prenatal to adulthood.

#### METHODS

Tissue from *Macaca nemestrina* monkeys was used in these experiments. Normal gestation time for this species is 170 days (Sackett et al., '75). In table 1 are listed the ages of the animals and the number of cells counted for each cell type examined. The adults were of unknown age but estimated to be between five and seven years. The animals were anesthetized and perfused with phosphate buffered 4% paraformaldehyde. In the case of embryonic animals the fetus was removed from the anesthetized mother by caesarian section and immediately perfused. Blocks were removed from area 17 of the cortex, stained by the Golgi Rapid technique, and cut into 90  $\mu\text{m}$  sections (see Lund, '73, for further details).

The cells studied were all located in the same region of the outer occipital operculum

TABLE 1

Age	IVC $\alpha$ spiny stellate neurons		IVC $\beta$ spiny stellate neurons		IIIB pyramidal neurons		VI pyramidal neurons	
	No. animals	No. cells	No. animals	No. cells	No. animals	No. cells	No. animals	No. cells
145 days fetal	1	10	1	10	1	10		
160 days fetal	1	10	1	10	1	10	1	5
Birth	1	10	1	10	1	10	1	5
1 week					2	20	1	5
3 weeks	1	10	1	10	1	10	1	5
5 weeks	1	10	1	10	1	10	1	5
8 weeks	1	10	1	10	1	10	1	5
12 weeks	1	10	1	10	3	30	1	5
24 weeks	1	10	1	10	1	10	1	5
30 weeks					1	10		
36 weeks	1	10	1	10	2	20	1	5
Adult	1	10	1	10	2	20	1	5
Total	10	100	10	100	17	170	10	50

posterior to the lunate sulcus. This region receives input from the perimacular region of each retina and includes approximately the central ten degrees of the visual field (Talbot and Marshal, '41; Daniel and Whitteridge, '61).

#### Spine counting methods

All counts are expressed in terms of spine frequency per linear micrometer of dendrite. Spine frequency, as used in this paper, is not an absolute measure of spine frequency because spines directly in front of or behind a dendrite generally cannot be resolved. Every process protruding from the dendrite shaft was counted and therefore the term spine is being used to refer to a variety of morphological shapes. For a description of changes in spine morphology during development see Lund et al. ('77).

The tissue was first scanned with a 10  $\times$  objective; individual spines could not be resolved at this power. Well-stained cells which met specific selection criteria (see below) were chosen at this power. Each cell was then drawn at a magnification of 500  $\mu\text{m}$  using a camera lucida. Spine counts were made with an oil 90  $\times$  or 100  $\times$  objective (total magnification 1,125  $\times$  or 1,250  $\times$ ) from predetermined regions on each cell (see below), and each region that was counted was marked on the drawing. Branch order, length of sample region, distance from cell body, type of dendrite, and lamina were noted for each region counted.

Different experiments contributed to these spine counts. Each observer cross checked with other observers on selected samples in

order to be sure that the same criteria were being used in making the counts. In addition, a subsample of cells was analyzed specifically for observer differences (RESULTS).

#### Analysis of dendrites

Camera lucida drawings of the cells were analyzed in two ways. First, a clear plastic sheet with concentric rings at the equivalent of 20  $\mu\text{m}$  intervals was centered on the soma and the intersections between the rings and the underlying dendrites were counted. These data are presented below as "ring intersections" per neuron. Second, the length of each dendritic branch as projected by camera lucida was measured for each cell using a map reader. Any branch which appeared to leave the section or was otherwise lost before either termination or branching was discarded; this analysis is therefore restricted to complete segments.

#### Selection criteria

Four cell types were studied: lamina IVC $\beta$  spiny stellate neurons, lamina IVC $\alpha$  spiny stellate neurons, lamina IIIB pyramidal neurons, and upper lamina VI pyramidal neurons. These four cell types are shown schematically in figure 1. Laminar boundaries and the laminar numbering system are as in Lund and Boothe ('75). (1) IVC $\alpha$  spiny stellate neurons: the dendrites of these neurons distribute primarily in lamina IVC $\alpha$ ; their axons project to laminae IVB and VA. These cells are likely to receive input from magnocellular layers of the dLGN (Hubel and Wiesel, '72; Lund, '78; Lund and Boothe, '75). Spines were counted from one or more dendrites on each neuron.

Whenever possible each dendrite was sampled by counting from 10  $\mu\text{m}$  segments every 20  $\mu\text{m}$  starting at the junction with the soma. However, for many of these neurons, segments which were convenient to count could not be found at these exact locations. Spines were therefore counted at intervals of opportunity and analyzed in terms of distance from the cell body.

(2) IVC $\beta$  spiny stellate neurons: The dendrites of these neurons largely avoid lamina VA and extend minimally into lamina IVC $\alpha$  while their axons project to laminae IIIB and VA. These cells are likely to receive input from parvocellular layers of the dLGN

(Hubel and Wiesel, '72; Lund, '73; Lund and Boothe, '75). One or more dendrites from each neuron were sampled for spine counts as described above for the lamina IVC $\alpha$  neurons.

(3) IIIB pyramidal cells: The somas of these neurons lie in lamina IIIB and their basal dendrites are distributed primarily within lamina IIIB. The apical dendrite often gives off collateral branches in lamina IIIB and then goes to lamina I with no or few branches in lamina IIIA or II. These neurons are likely to receive direct input from the lamina IVC $\beta$  spiny stellate cells (which in turn are likely to receive a parvocellular input) and therefore can be

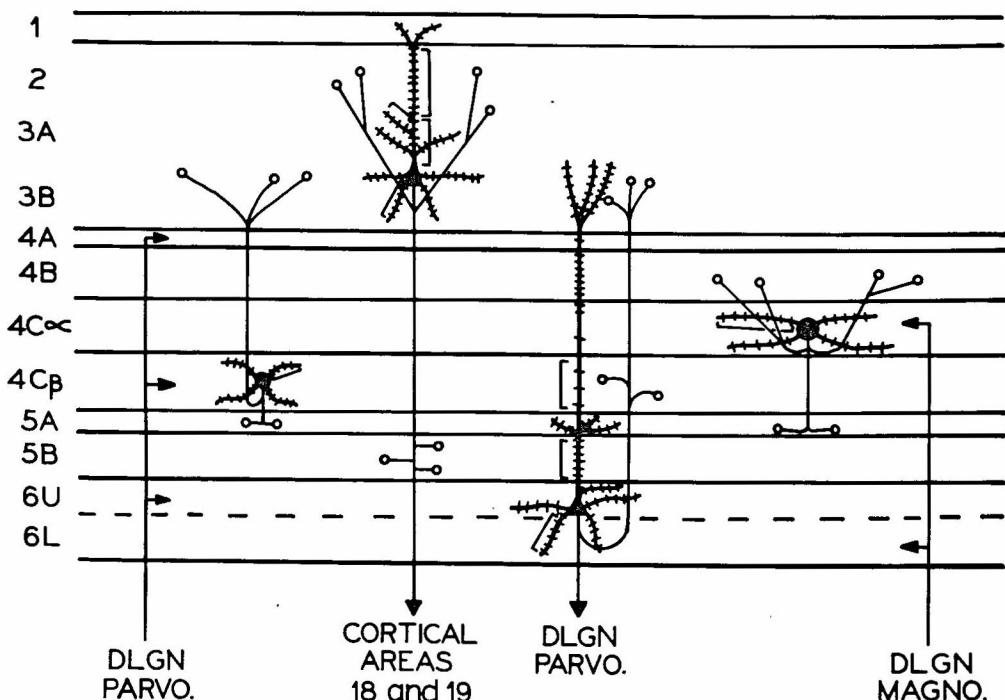


Fig. 1 The schematic shows the four cell types from which spine counts were obtained. The spiny stellate neuron with soma in lamina IVC $\beta$  is likely to receive a direct thalamic projection from parvocellular layers of the dorsal lateral geniculate nucleus (dLGN). It projects in turn to laminae VA and IIIB. Brackets indicate the dendritic regions sampled. Spine counts were made from the dendrites of these stellate neurons in lamina IVC $\beta$ . The pyramidal neuron with soma in lamina IIIB does not receive any direct input from the dLGN. However its basal dendrites are likely to receive second order parvocellular input via the axons from the IVC $\beta$  stellates. Spine counts were made from both the apical and basal dendrites in lamina IIIB, IIIA, and II. The spiny stellate neuron with soma in lamina IVC $\alpha$  is likely to receive a direct thalamic projection from the magnocellular layers of the dLGN. Its axons project to lamina VA and IVB. Spine counts were made from dendrites of this neuron in lamina IVC $\alpha$ . The pyramidal neuron with soma in lamina VIU may receive direct parvocellular input to its basal dendrites in lamina VIU and to the portion of its apical dendrite which passes through lamina IVC $\beta$ . It may receive second order parvocellular input via lamina IVC $\beta$  stellate neuron axons in laminae VA and IIIB. This pyramidal neuron may receive direct magnocellular input to its basal dendrites in lamina VIU and to the portion of its apical dendrite which passes through lamina IVC $\alpha$ . It may receive second order magnocellular input via the lamina IVC $\alpha$  stellate neuron axons in lamina VA and IVB. Spine counts were made from basal dendrites in lamina VIU and from the apical dendrite as it passes through laminae VB and IVC $\beta$ .

thought of as second order cortical neurons in the processing of parvocellular geniculate information (Lund and Boothe, '75). For each neuron the apical dendrite shaft and one basal dendrite were sampled for spine frequency by counting a 10  $\mu\text{m}$  segment every 50  $\mu\text{m}$  starting at the junction with the cell body. (4) Upper VI medium-sized pyramidal cells: The cell bodies of these neurons lie in upper lamina VI while their basal dendrites extend into deep lamina VI. The large Meynert cells which are also present in this region were not included in the sample. These upper lamina VI neurons project to parvocellular layers of the geniculate (Lund et al., '75) and in turn the upper half of lamina VI receives direct input from the parvocellular dLGN (Hendrickson et al., '78). The basal dendrites of these neurons often extend down into lower lamina VI which is associated more directly with magnocellular input (Hendrickson et al., '78). The apical dendrite passes through lamina V (which receives later order input probably combining both parvocellular and magnocellular pathways from laminae 2 and 3) and then into lamina IVC $\beta$  which receives parvocellular input. Not enough apical segments could be followed

to laminae superficial to lamina IVC $\beta$  to allow spine counts. For each neuron the apical dendrite shaft and one basal dendrite were sampled for spine frequency by counting a 10  $\mu\text{m}$  segment every 50  $\mu\text{m}$  starting at the junction with the cell body.

## RESULTS

### *Spine counts*

Spine counts for the stellate neurons are shown in figure 2. Spine frequency did not appear to vary systematically with distance along the dendrite from the soma and therefore the counts have been combined in figure 2 to give an average spine frequency over the entire neuron. Error bars in this figure indicate the standard errors across dendrite segments counted. It can be seen that the laminae IVC $\alpha$  and IVC $\beta$  neurons have overlapping standard errors at all ages except birth, eight weeks, and 36 weeks (fig. 2). Prenatally there appears to be a decline in spine frequency in the last four weeks prior to birth. However, this must be interpreted with caution because the morphology of spines is changing during this period and the counts may be reflecting different populations rather than a change in

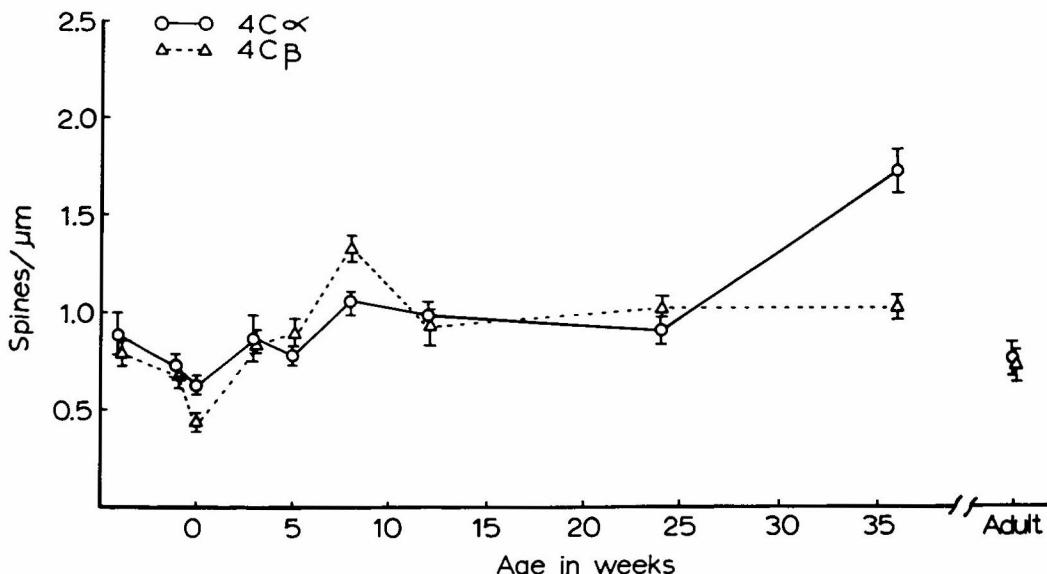


Fig. 2. Spiny stellate neurons. Spine frequency is plotted as a function of age for spiny stellate neurons in layers IVC $\alpha$  and IVC $\beta$ . Each data point is the mean spine frequency averaged across all dendrite segments counted at a particular age. Error bars indicate  $\pm 1$  standard error of the mean. Since only a single animal was counted at each age, the error bars indicate the variability between dendrite segments taken from the same neuronal type in the same lamina and from the same animal. These error bars allow statistical comparisons to be made between the two types of neurons at the same age, but not between ages.

frequency for a single population of spines (Lund et al., '77).

Postnatally the lamina IVC $\alpha$  neurons show a rather gradual rise in spine numbers from birth until about eight weeks and then level off until 24 weeks. Between 24 and 36 weeks there is a further increase in spine numbers. Comparison of the 36 week spine counts with those from the adult animals suggests that there must be a reduction in spine frequency on the lamina IVC $\alpha$  spiny stellate neurons which takes place sometime after 36 weeks. The lamina IVC $\beta$  spiny stellate neurons show a somewhat sharper rise in spine frequency between birth and eight weeks postnatal (fig. 2). Spine frequency appears to peak in this population at about eight weeks and all older ages have a reduction of at least 25% from the eight week level. These results in figure 2 suggest that laminae IVC $\alpha$  and IVC $\beta$  spiny stellate neurons follow a somewhat different time course. The lamina IVC $\beta$  neurons have a fairly dramatic increase in spine frequency from birth until a peak at about eight weeks. Spine frequency then drops off from this peak. The lamina IVC $\alpha$  neurons show a somewhat less dramatic increase in spine numbers from birth until eight weeks. Spine frequency then appears to plateau until after 24 weeks when there is a late postnatal increase. Spine frequency is reduced to the adult level sometime after 36 weeks postnatal. In order to see whether these differences in time course might be reflected in other neuronal populations, spine counts were made in two types of pyramidal neurons.

For pyramidal neurons spine frequency was found to vary as function of distance along the dendrite from the soma as well as a function of age. In lamina IIIB pyramidal neurons the apical dendrite at all ages retains the same profile of spine numbers, having at any age greater numbers of spines on the proximal 50–200  $\mu\text{m}$  of the apical dendrite than on the more distal regions. This is shown in figures 3a,b. However marked changes occur over time in the numbers of spines on any one region of the apical dendrite. In figure 3a it can be seen that spine frequency increases from the perinatal period to eight weeks postnatal. In figure 3b results are shown for ages from eight weeks postnatal (same curve as in fig. 3a) to adults. The number of spines decreases dramatically across these older ages. The 6- to 9-month-old monkey has approximately 30% fewer spines per micrometer than the 8-week

monkey. In fact, the adult monkeys are left with spine frequencies in the same range as the perinatal animals.

Spine counts at most ages were based on only one monkey so that standard errors across subjects are unknown. However, at the 1 week, 12 week, 36 week, and the adult ages (where more than 1 animal was counted) standard error bars are shown indicating the magnitude of variability between monkeys at the same age (figs. 3a,b). It seems clear that the perinatal ages have fewer spines than monkeys in the 5- to 12-week age range and that these ages in turn have more spines than the older postnatal ages. Counts were also made of spines on the basal dendrites of these lamina IIIB neurons and the results are shown in figure 3c. Within the limits of variability shown, the time course of spine development on the basal dendrites appears to be similar to that of the apical dendrite. Standard error bars in figure 3c indicate the magnitude of variability between counts made on different neurons of the same type and at equivalent distances from the soma.

The data in figures 3a,b and c are displayed in terms of the distance along the dendrites from the soma. However, equivalent distances along the dendrites may be falling in different laminae at different ages. The data have therefore been re-analyzed according to the lamina in which the spine count was made for this same population of lamina IIIB neurons and the results are shown in figure 3d. The same age trend can be observed in figure 3d as in 3a,b and c. Spine frequency increases steadily from the perinatal period until about eight weeks postnatal. This is followed by a decrease in spine frequency for the older ages. It appears that the same general age pattern of spine development is occurring over the entire neuron including the basal dendrites and the apical dendrite as it passes through layers IIIA and II. Standard error bars in figure 3d indicate the magnitude of variability between segments from the same neuronal type within the same lamina.

Results from the pyramids in lamina VI are shown in figure 4. Over the proximal portions of the apical dendrite (50  $\mu\text{m}$  to 200  $\mu\text{m}$ ) there appears to be an increase in spine frequency from the perinatal period to eight weeks postnatal which is similar to that seen for the lamina IIIB pyramidal neurons (fig. 4a). On more distal segments (from 250  $\mu\text{m}$  to 300  $\mu\text{m}$ ) the increase is less dramatic. For the

ages from 8 weeks through 36 weeks the results are much different for the lamina VI pyramidal neurons than for those in lamina IIIB (fig. 4b). Spine frequency appears to decrease gradually to adult levels on the more distal segments. However, the proximal segments appear to retain a high frequency of spines from 8 weeks through 36 weeks. Spine loss to the adult level does not take place on these portions of the dendrite until after 36 weeks postnatal.

The apical dendrite shafts of these lamina VI pyramidal neurons pass through lamina V and then into lamina IVC $\beta$ . Again, differences between proximal and distal portions of the dendrite might be due to laminar differences. This was examined in figure 4d by re-plotting the results in terms of the lamina in which the segments were located. The portions of the apical dendrite which pass through lamina IVC $\beta$  show a time course of spine frequency very similar to that of the lamina IVC $\beta$  spiny stellate neurons and lamina IIIB pyramidal neurons. There is a rise to a spine frequency peak at about eight weeks followed by a decrease at older postnatal ages. However, the spine frequency of the dendrite as it passes through lamina V follows a somewhat different time course. Spine frequency increases to eight weeks but then remains high until 36 weeks. The decrease to the adult level does not occur until some time after 36 weeks.

Spines on basal dendrites of these lamina VI neurons were also examined and the results are shown in figure 4c. On segments from the basal dendrites very close to the soma (0-10  $\mu\text{m}$ ) the spine frequency shows a peak at eight weeks postnatal followed by a decline. However spine frequency on more distal parts of the basal dendrites follows a time course more similar to that of the apical dendrite in lamina V; spine frequency remains high through 36 weeks postnatal. More distal portions of the basal dendrite often extend deep into layer VI so that this proximal-distal difference may reflect a difference between upper and lower lamina VI.

#### *Mean length of dendrite segments*

An obvious question to be raised about the spine count data is whether changes in spine frequency reflect changes in spines per neuron or whether they merely reflect changes in the lengths of dendrites (i.e., spines per micrometer). For example, the decline in spine frequency seen in many neuronal populations

after eight weeks postnatal could reflect an actual decrease in spines or it could be that spine numbers per dendrite are remaining constant while the dendrites are growing longer. To check for this, measurements have been made of the mean length of dendrite segments for each of the four neuronal populations on which spines were counted. Results of some of these measurements are shown in figure 5. Examination of this figure clearly indicates that the mean length of dendrite segments is not increasing steadily over the postnatal period. In fact, for each cell population the mean length at eight weeks equals or exceeds the mean length at six or nine months. Therefore, any observed decreases in average spine frequency at the older postnatal ages can be interpreted as decreases in numbers of spines per dendrite segment and are not just due to segments growing longer. These measurements of mean dendrite segment lengths have also been analyzed separately for each branch order and for branches off the apical dendrite. Examination of all of these data reveals no obvious age changes in dendrite length (data not shown). These results suggest that if the dendrites are growing during the postnatal period, they do so by adding new branches rather than by increasing the length of existing segments.

#### *Concentric ring analysis*

If neurons are growing by adding new branch segments, this should be reflected in the concentric ring analysis which counts the number of dendrite intersections for a single neuron with a series of concentric rings. These intersection counts have been made for each of the four cell types and the results are presented in figure 6. Unfortunately, there is a relatively large amount of variability in these data, but a few general trends can be seen. First, it is apparent that lamina IVC spiny stellate neurons have considerably more dendritic material than the pyramidal neurons. The pyramidal neurons show an average of 30 to 50 intersections per neuron while the stellate cells are more often in the range of from 60 to 80 intersections and at some ages reach over 100 intersections per neuron. Another general trend is that the stellate neurons appear to be adding branches throughout the first nine months of the postnatal period (although there is too much variability to determine whether this is going on continually or in spurts). On the other

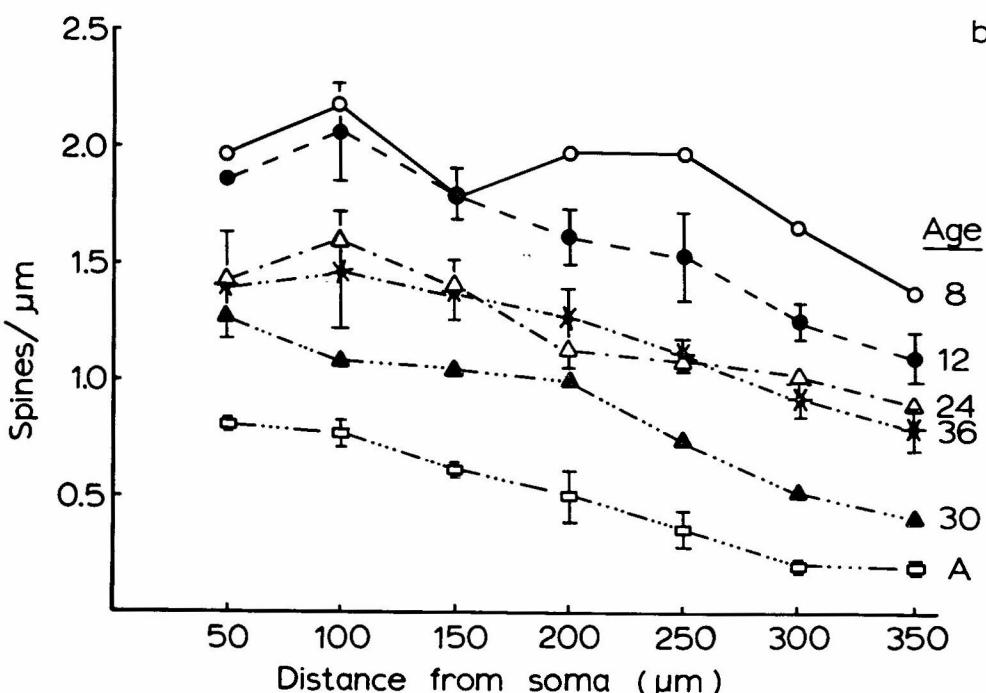
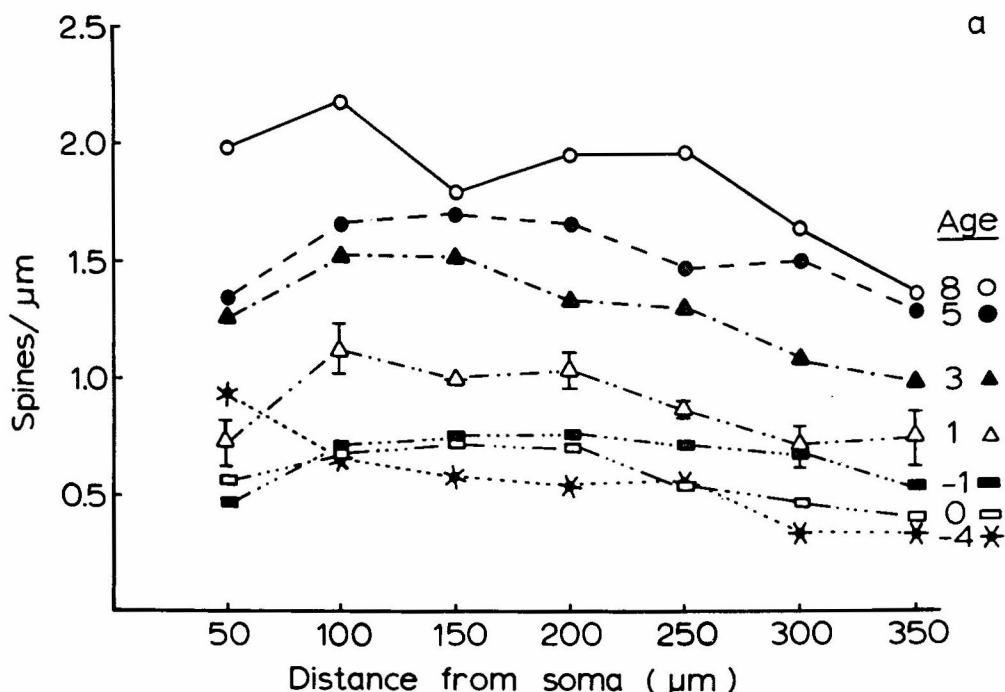


Figure 3

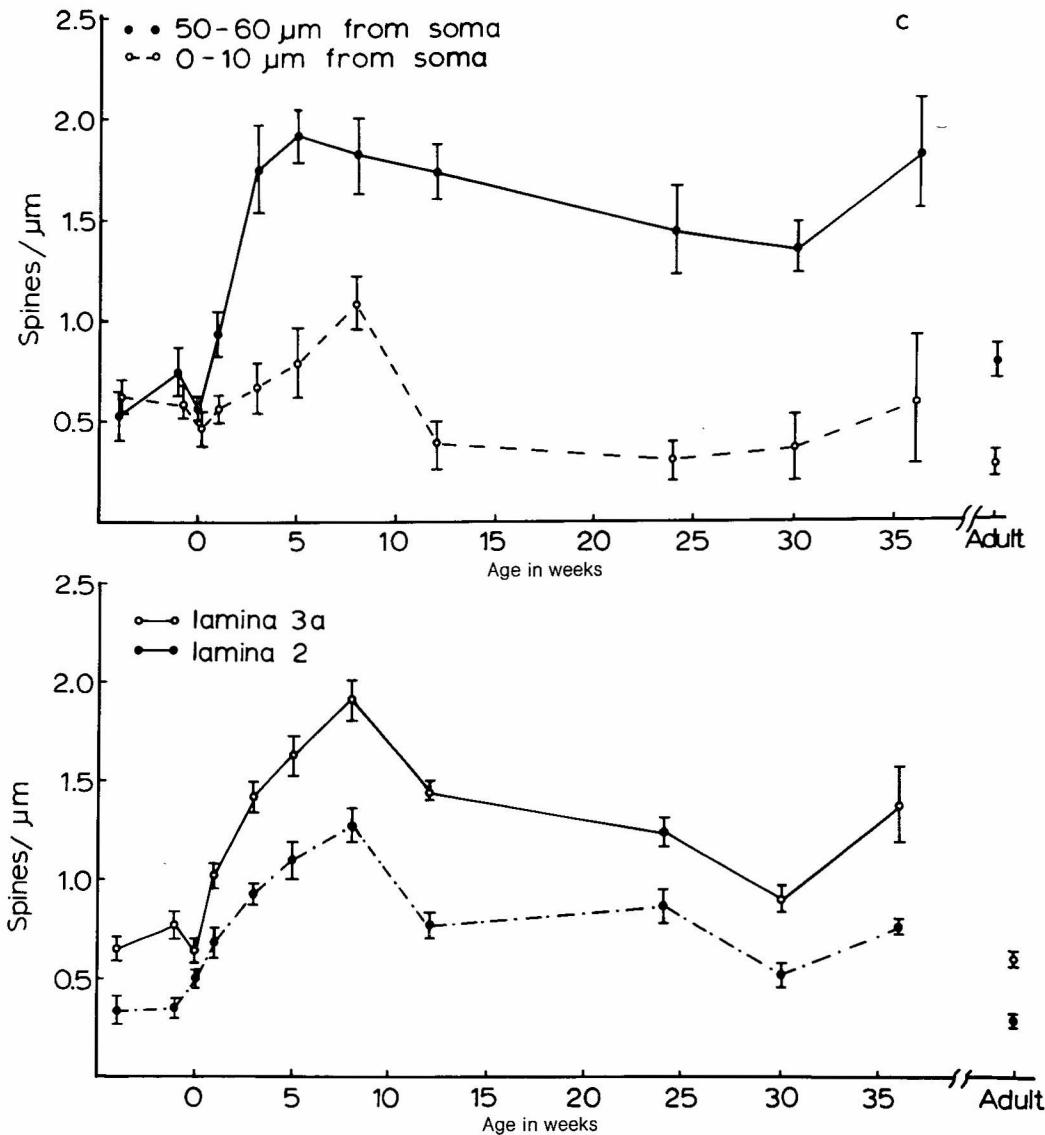


Fig. 3 Pyramidal neurons with soma in lamina IIIIB.

Figure 3a shows spine frequency as a function of distance along the apical dendrite from the soma. Separate functions are shown for seven ages ranging from four weeks prenatal to eight weeks postnatal. The age in weeks is indicated to the right of each function. Each data point is the mean spine frequency averaged over all dendrite segments at a given age and at a given distance from the soma. At one week postnatal two animals were included in the counts. The error bars for the one week data are  $\pm 1$  standard error of the mean computed by comparing the means in the individual animals. These error bars indicate the variability between animals at the same age. At 150  $\mu\text{m}$  the standard error was smaller than the plotting symbol.

Figure 3b shows spine frequency as a function of distance along the apical dendrite from the soma as in figure 3a. Separate functions are shown for six ages ranging from eight weeks postnatal (same curve as in fig. 3a) to adulthood. The age in weeks is indicated to the right of each function. The adults are indicated by A. More than one animal was included in the counts at 12 weeks, 36 weeks and the adult ages. For these ages standard errors between individual animal means are indicated as described for figure 3a.

Figure 3c shows spine frequency on the basal dendrites as a function of age. Two separate curves are shown: one for counts on segments 0-10  $\mu\text{m}$  from the soma and the other for counts on segments 50-60  $\mu\text{m}$  from the soma. Each data point is the mean spine frequency averaged over all dendrite segments at a given age and at a given distance from the cell body. Each neuron provides only a single dendrite segment at each data point and therefore variability between dendrite segments is equivalent to variability between neurons. Error bars in this figure are  $\pm 1$  standard error of the mean computed across individual neurons.

Figure 3d shows spine frequency as a function of the lamina in which the dendrite segment is found. Each data point is the mean spine frequency averaged over all dendrite segments at a given age and within a given lamina. Error bars in this figure are  $\pm 1$  standard error of the mean computed across dendrite segments within the same lamina.

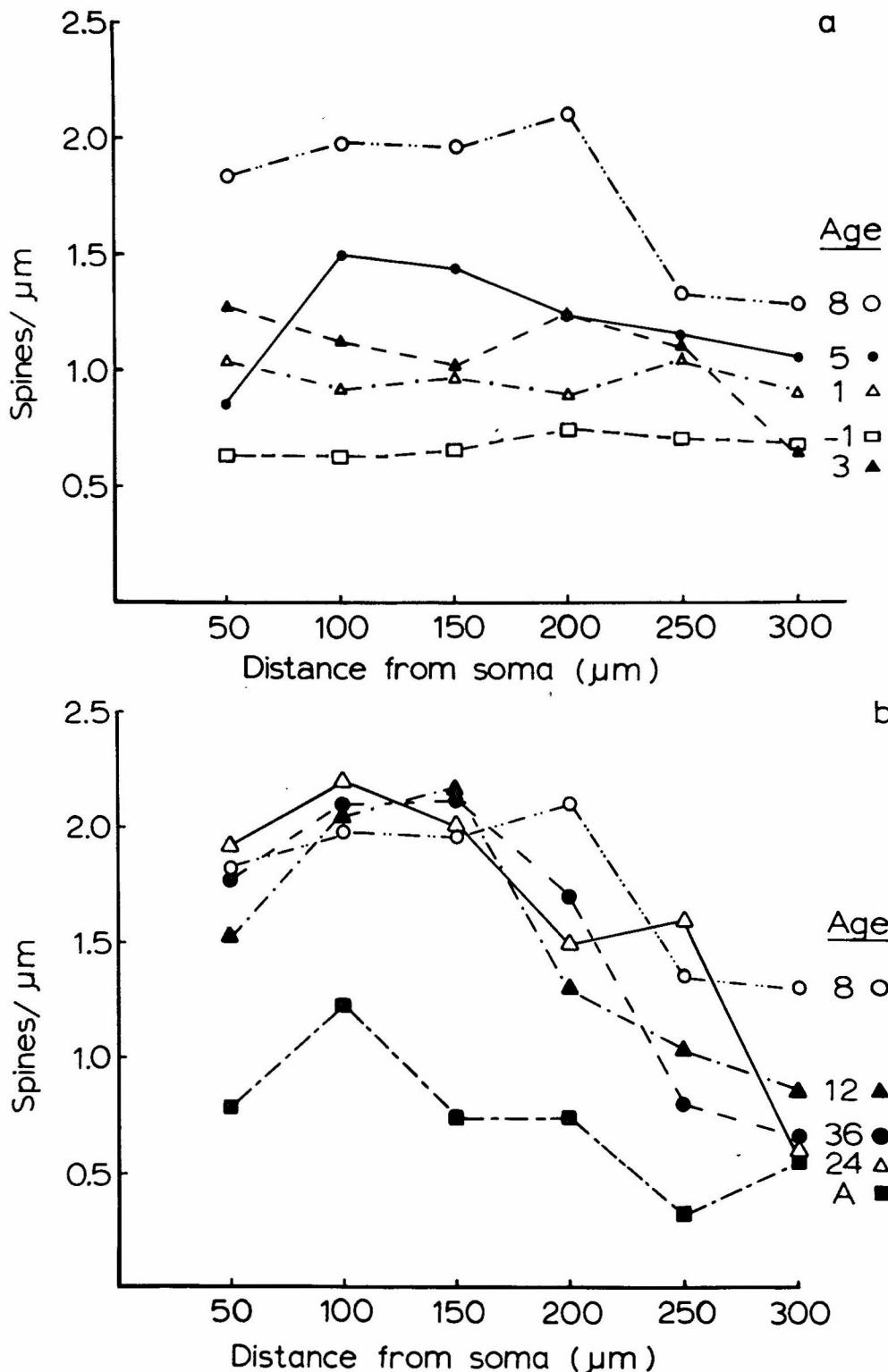
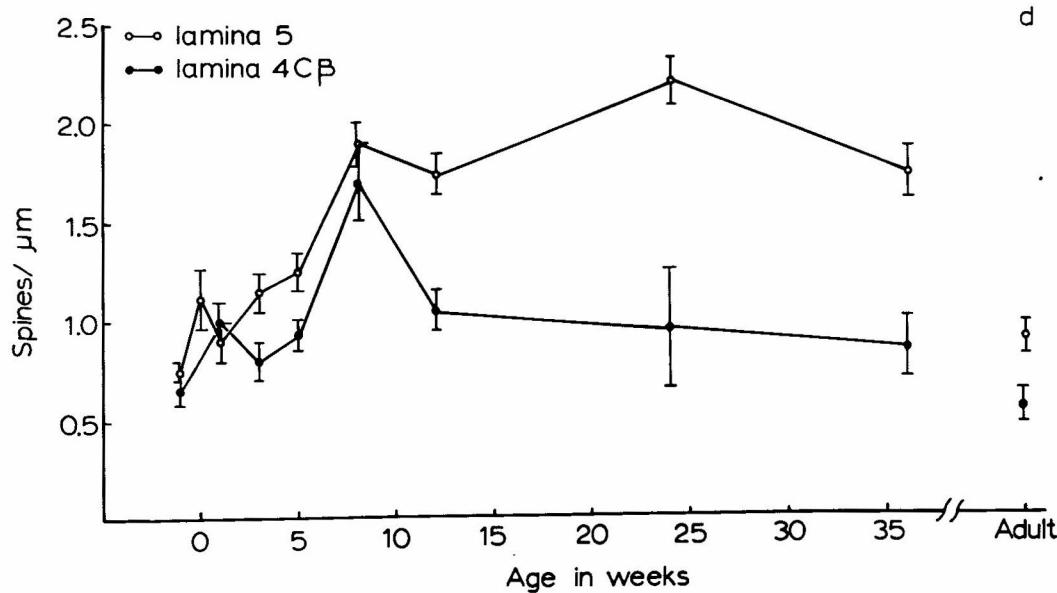
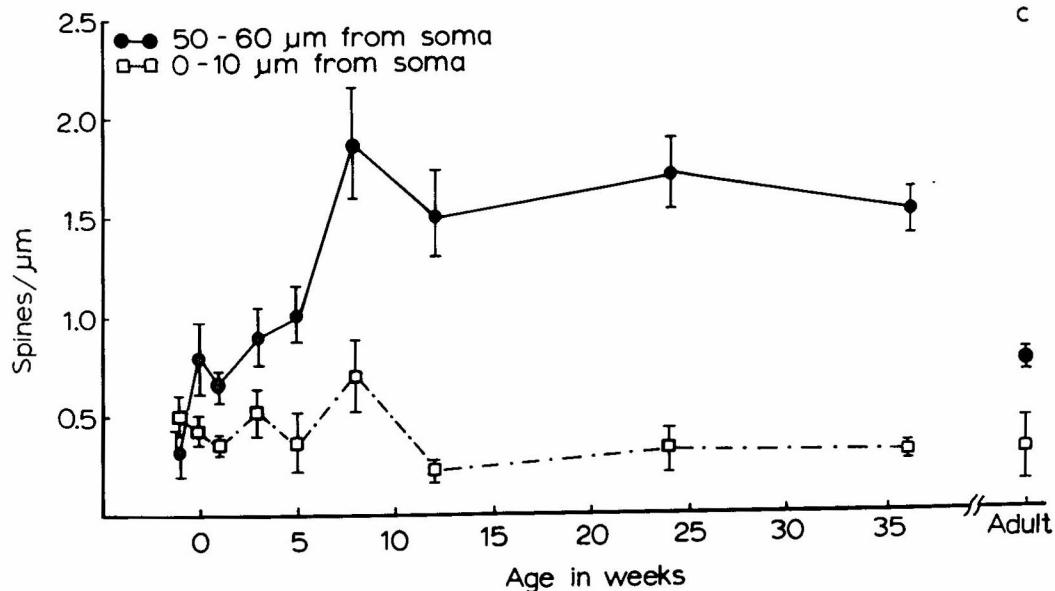


Figure 4



Figs. 4 Pyramidal neurons with soma in lamina VIU.

Figure 4a shows spine frequency as a function of distance along the apical dendrite from the soma. Separate functions are shown for five ages ranging from one week prenatal to eight weeks postnatal. The age in weeks is indicated to the right of each function.

Figure 4b is the same as figure 4a for five ages ranging from eight weeks postnatal (same curve as in fig. 4a) to adulthood (adult age is indicated by A).

Figure 4c shows spine frequency on the basal dendrites as a function of age. Two separate curves are shown: one for counts on segments 0 to 10  $\mu\text{m}$  from the soma and the other for counts on segments 50-60  $\mu\text{m}$  from the soma. Error bars are as in figure 3c.

Figure 4d shows spine frequency as a function of the lamina in which the dendrite segment is found. Error bars are as in figure 3d.

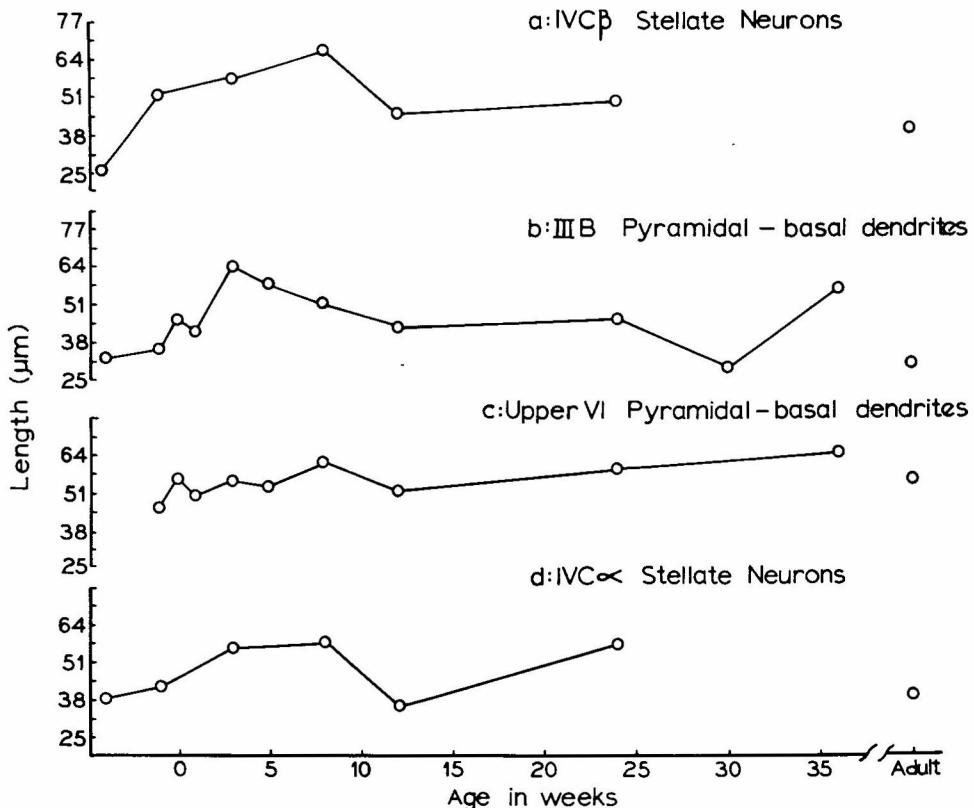


Fig. 5 Mean length of dendrite segments is plotted as a function of age for each of the four neuron types examined. Each data point is the average overall length of segments measured for a given neuron type at a given age. Branches which were not complete within the section were not measured. These length measures are projected lengths as measured with camera lucida and have not been corrected for tilt within section.

hand, the pyramidal neuron data is much more flat with no obvious trend toward steadily adding new branches during these first postnatal nine months.

The pyramidal neuron data often has local peaks. For example, the lamina IIIB pyramidal neuron apical and basal dendrites both show peaks at five weeks. The upper VI pyramidal neurons show a possible local peak at six months for their basal dendrites and at five weeks for their apical dendrite branches. These results suggest that pyramidal neurons are not only forming and then losing spines during the postnatal development period, but might also be forming and then losing dendrite branches. In addition it should be noted that for all neuron types, the nine month data consistently show more intersections/neuron than the adult data. However, these data are too variable to be more than suggestive.

While we attempted to standardize counting methods between experimenters by occasionally comparing our counts of single typical segments, at least some difference between experimenters due to biases in selection of neurons and dendritic segments, as well as criteria for spines, is inevitable. Figure 7 presents one comparison of experimenter differences together with individual animal differences. The spine frequency along the apical dendrites of lamina IIIB pyramidal neurons was counted in three separate animals at 12 weeks postnatal. Animal 1281 was counted independently by experimenters RB and JL. Animal 1211 was counted only by JL. Animal 73201 was counted independently by RB and KW. Curves for each of these five independent counts are shown in figure 7. By examining this figure it is possible to determine the amount of variability encountered between individual animals and between

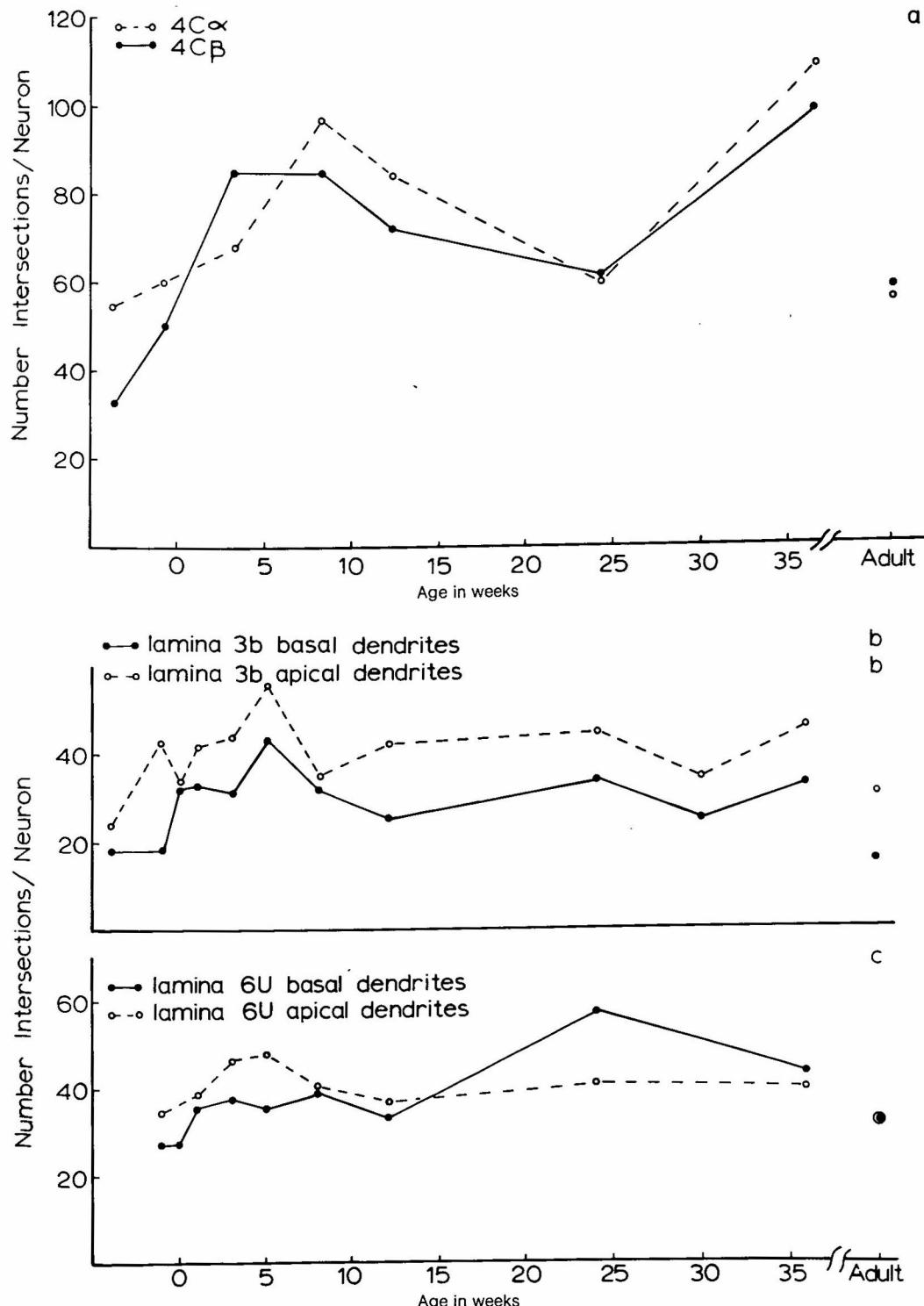


Fig. 6 The number of dendritic intersections per neuron in the concentric ring analysis is plotted as a function of age for three neuronal populations. Figure 6a shows results for spiny stellate neurons in laminae IVC $\alpha$  and IVC $\beta$ . Figure 6b shows results for lamina IIIB pyramids and figure 6c for lamina VIU pyramids.

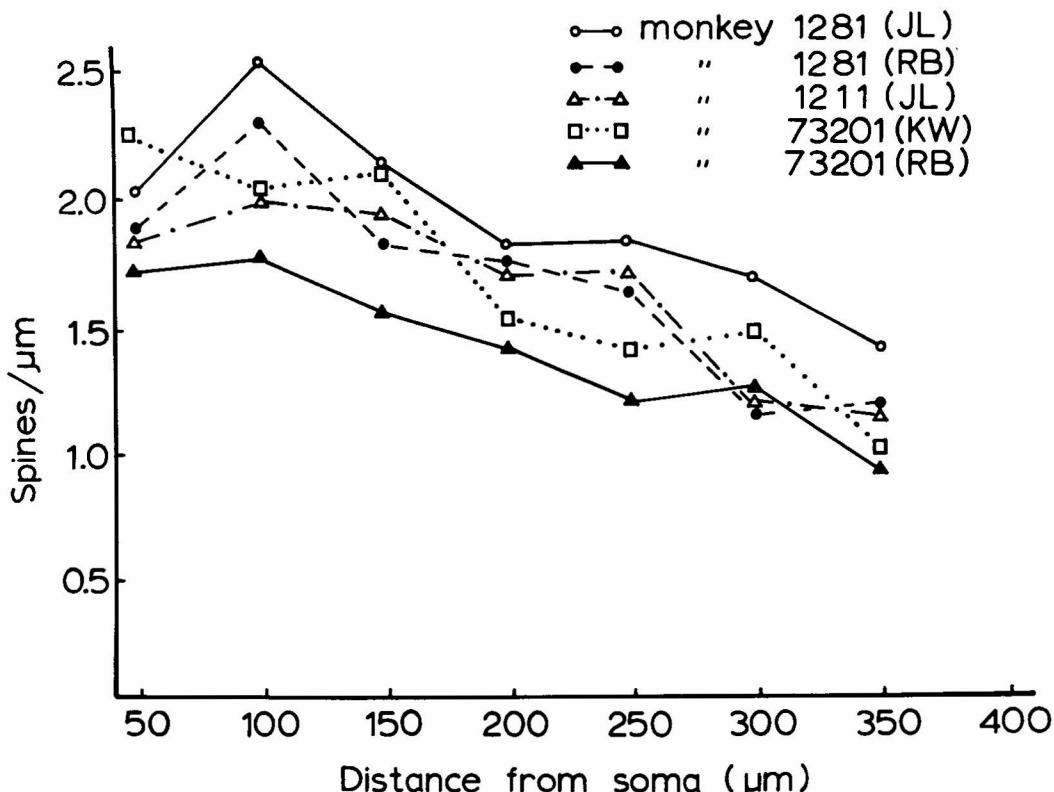


Fig. 7 Spine frequency along the apical dendrite of lamina IIIB pyramidal neurons for three different animals at 12 weeks of age. The difference between individual animals at 12 weeks postnatal is shown, as well as the difference between the counts of different experimenters. Each curve shows results from a single animal made independently by a single experimenter.

different experimenters. While absolute values vary somewhat, all five curves run roughly parallel indicating that the major conclusions, are not likely to be affected by experimenter differences (DISCUSSION section).

#### DISCUSSION

Two kinds of issues require discussion. First, procedural or technical issues and, second the implications of these results. The major procedural question concerns variability within the spine count data. Several sources of variability contributed to the counts; these include differences between experimenters' counting methods, individual differences between animals, neuron to neuron variability within the same animal, and laminar differences within the cortex. Standard errors have been calculated in different ways in order to show representative magnitudes for these various sources of

variability. For example, error bars are presented showing spine number variability between dendrite segments on a neuron type in the same lamina (figs. 2, 3d, 4d), between neurons of the same type in the same animal (figs. 3c, 4c) and between animals of the same age (figs. 3a,b). Differences between experimenters can be seen in figure 7. The variability in all cases is surprisingly small. These factors are important because we wish to determine whether differences in spine numbers between ages are really developmental or whether they are due to other sources of variability.

Three arguments can be made that the observed differences in spine frequency obtained in this study are really developmental. First, when separate monkeys of the same age were examined the results were generally similar. Figure 7 presents spine frequency along the apical dendrite of lamina IIIB pyramidal neurons for 3 different animals at

12 weeks of age. Both the absolute values and the pattern according to distance from the cell body are reasonably consistent between animals. Moreover, across three different experimenters and three different animals in this figure, only two points fall outside the boundaries established by the means for 8- and 24-week animals in figures 3a, b. Second, most of the important conclusions can be seen as trends in the data and do not depend on data from single animals. Third, trends in the data are not consistently associated with the same individual. For example, the 8-week monkey consistently exhibits the highest spine frequency for those neurons and laminae associated with parvocellular input. However, this is not just due to the fact that this 8-week monkey is a particularly "spiny" individual; lamina IVC $\alpha$  neurons of the same animal do not show a peak at this age compared to older or younger ages. Differences between lamina VI neuron segments in lamina IVC $\beta$  versus lamina V also show up consistently over a number of ages.

In discussing individual differences some mention should be made of the adult animals examined. In all of the neuron types examined, the adult animals consistently have fewer spines than the 6- to 9-month-old monkeys. This may indicate that spine frequency continues to decrease over a long postnatal period. However, one must be cautious in making such comparisons because the adults were wild born while all of the other monkeys were born and reared in the laboratory.

While we would like to assume a consistent relationship between spine frequency and synapse frequency, there is no way of knowing whether the spines observed in this study were associated with synapses at all ages. For example, it might be that many spines are initially formed, but those which do not make a functional synaptic contact are then lost. Studies are underway to try and resolve this issue by examining Golgi impregnated neurons from these monkeys with the electron microscope. However, other evidence also suggests that the loss of spines observed in this study may reflect an actual loss of synaptic contacts following a peak in their numbers. Indications of similar developmental peaks in synapse numbers followed by loss of contacts are present in electron microscopic analysis of cat visual cortex (Cragg, '75).

Turning next to a discussion of some implications of these results, it is clear that

dramatic anatomical changes occur in postnatal development of the primate visual cortex. The development of spine populations on neurons in the primate visual cortex appears to be highly ordered both in time course and numbers of spines. The smooth progression of spine frequency changes from one age to the next is apparent in all of the counts made in this study. It appears therefore, that spine frequency must be tightly controlled during the developmental period. The similarity in spine numbers for the two adult animals suggests that there may be a final adult level which is also under rigid control. The predictability of spine development has been previously noted in the mouse (Ruiz-Marcos and Valverde, '69). Although the time course of spine development is predictable, it is not the same for all neuron types or for dendritic segments falling in different laminae. The results for spine frequency have demonstrated that all neuronal populations undergo acquisition of spines during the first eight postnatal weeks. This may be rapid (e.g., lamina IVC $\beta$  spiny stellate neurons), or slower (e.g., lamina IVC $\alpha$  spiny stellate neurons). From the eight week point, some populations undergo a dramatic loss, while others plateau. The lamina IVC $\beta$  stellate neurons and lamina IIIB pyramidal neurons undergo a rapid spine loss of some 25% to 30% from the eight week peak. Selected parts of the dendritic surface of the upper lamina VI pyramidal neurons show a similar progression, while other parts plateau as do the lamina IVC $\alpha$  spiny stellate neurons.

It is interesting to ask whether those sets of neurons (or parts of neurons) which show a peak and those which plateau after eight weeks have features in common. One obvious difference concerns the processing of information derived from parvocellular versus magnocellular layers of the dLGN. Terminals from parvocellular layers end in lamina IVC $\beta$  (Hubel and Wiesel, '72). The dendrites of lamina IVC $\beta$  spiny stellate neurons arborize heavily in this region and these neurons show a spine peak at eight weeks postnatal. The upper lamina VI pyramids also show a strong spine peak over those portions of their apical dendrite shafts which are passing through lamina IVC $\beta$ . Axons from the lamina IVC $\beta$  stellates terminate in lamina IIIB (Lund, '73) and the lamina IIIB pyramidal neurons also exhibit an obvious eight week peak on both apical and basal dendrites. Therefore neuronal populations or laminae examined

which are likely to be processing first or second order relays derived from the parvocellular layers show a peak population of spines at eight weeks followed by a spine loss. On the other hand, those neuronal populations or laminae which receive primarily a magnocellular input (lamina IVC $\alpha$  spiny stellate cells), or which receive later order relays with a combination of parvo- and magnocellular derived information (lamina VB) do not show this same peak; rather they plateau, falling to adult levels sometime after 36 weeks. Basal dendrites of upper lamina VI pyramidal neurons which extend down into lower lamina VI also do not show an obvious peak; the extension of their basal dendrites throughout lamina VI may tend to pool parvocellular and magnocellular influence. (The magnocellular layers relate reciprocally to the deeper half of lamina VI while the parvocellular layers project to and receive input from upper VI [Hendrickson et al., '78]). Lamina VB does not deal predominantly with either parvo- or magnocellular derived information and contains output neurons sending axons to the superior colliculus. This lamina also does not appear to show an obvious spine peak, at least for upper lamina VI pyramidal cell apical dendrites passing through it.

There is a suggestion of a late rise in spine numbers at 36 weeks in the lamina IVC $\alpha$  spiny stellates. This may indicate that further marked changes in spine numbers can occur in the later postnatal period. The spine loss between 36 weeks and adult in all neuron varieties provides additional evidence for late postnatal changes. It will be necessary to conduct spine counts on several additional neuronal populations in order to determine whether only parvocellular related neurons or regions exhibit an obvious eight week peak. For example, it would be predicted that the basal dendrites of both lower lamina VI pyramidal neurons and lamina IVB pyramids, would not show an eight week peak since they are primarily associated with magnocellular relays.

Regardless of the details of the exact time course, all neuronal populations which were examined go through an initial formation of spines followed by a subsequent reduction sometime during the postnatal period. Hence peaks or plateaus and subsequent reduction in the number of spines and synapses appear to be a feature of visual cortex development which must be taken into account by any

theoretical model of visual development. It has been proposed (Changeaux and Danchin, '77; Lund et al., '77; Greenough, '78) that selective preservation of synaptic connections may be one mechanism whereby the developing central nervous system achieves its mature pattern of functional organization, as has been proposed in the periphery (e.g., Brown et al., '76). The neuronal activity, generated by visual input in the young animal, which can affect binocular interactions, orientation selectivity, and movement sensitivity (e.g., Blakemore and Van Sluyters, '75; Buisseret and Imbert, '76; Cyander and Chernenko, '76; Packwood and Gordon, '75) may be a major determinant in various selection processes. For example, Hubel et al. ('77) and Rakic ('76), have proposed that geniculostriate afferents to area 17 from the two eyes in the macaque overlap extensively at birth and that the adult pattern into which these inputs are separated may arise through retraction of these overlapping inputs into discrete columns or bands by six weeks of age. In the monocularly deprived monkey, the bands associated with the deprived eye appear to regress excessively while those associated with the experienced eye retain and perhaps add connections within their broader neonatal width. The loss of spines seen in the present study may reflect a similar process of retraction, the disappearing spines representing those which have lost out in a competitive interaction. Since the geniculostriate afferents appear to be separated by six weeks of age, these spine count results indicate that refinement of connection patterns associated with other aspects of development may continue for longer periods. The differently timed process of spine maturation for the spiny stellate neurons of laminae IVC $\alpha$  and IVC $\beta$  and for neurons receiving their later relays may indicate important differences in the nature of their dLGN inputs. Perhaps the parvocellular input and relays are consolidated and refined at an earlier age than the magnocellular input. The magnocellular relays may remain modifiable for considerably longer periods of time, perhaps beyond the 36 week study period.

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