The development of synapses in cat visual cortex

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A review of cortical development points to outstanding gaps in our knowledge. Counts of the distribution in depth of synapses are reported from two weeks before birth to adulthood. The rising density of synapses is compared with the developing physiological functions. The development of extrinsic connections is also considered.

Key words: cat visual cortex, development, synapses

The cerebral cortex is formed by an outward migration of neuronal cell bodies which are produced by mitosis of cells lying close to the ventricle. The migrating cells appear to be guided by contact with radial fibers of uncertain origin, and the ascent of a fiber by successive cells may be a factor in the formation of cell columns. The earliest cells to arrive in cortex are passed by later arrivals which come to occupy more superficial positions. In the rat, cells formed on day 16 of gestation migrate to form the deepest cortical layer, and cells produced on day 22 become the most superficial cortical layer. The first neuroglial cells are said to originate on day 21 in the rat, almost at the end of neuronal production. The glial cells are formed in a subventricular zone in which mitosis continues even in adult animals. The corresponding times in the cat are before birth and have not been determined by autoradiography.

The Golgi method has been used recently to study the formation of cat visual cortex and has yielded a number of interesting findings. The cortex into which the neurones migrate already contains a fiber plexus present on day 22 of gesta-
The exact provenance of these fibers has not been established. On day 25 neurons start to migrate into cortex and to form a compact cell layer. The cells that face the fiber plexus develop dendrites: apical dendrites from the uppermost cells, basal dendrites from the lowermost cells. Afferent axons may trigger the development of dendrites, but the mechanism is unknown. I find no synapses present at 37 days, and extremely few at 44 days of gestation. Desmosome-like junctions between fibers and cells are numerous before this time, but small gap junctions have not yet been described.

By day 45 of gestation a new group of afferent axons arrives on the deep side of the cell plate, and the dendrites here grow and separate the cell bodies into layers 5, 4, and lower 3. A third and more superficial group of afferent axons arriving by day 55 leads to the differentiation of the upper part of the cellular plate into layers 2 and upper 3. Birth follows at around day 60 of gestation. The development of spines upon the dendrites to receive the majority of synapses in the adult begins seven to 10 days after birth in cats. Myelin appears rather suddenly: it is present at 21 days as judged by light microscopy and in my material is seen by electron microscopy at 23 days after birth but is not present at 19 days. Brain weight goes on increasing and glial cells increase in numbers for several months after birth.

The desmosome-like contacts present in cortex before synapses are formed become infrequent by the time of birth. Since most synapses are formed after birth, these contacts are unlikely to be precursors of synapses. Numerous studies on cortex have shown that the structure of a synapse develops gradually toward the adult form. In brief, the earliest synapses have short regions of apposed membrane which is not yet fully thickened nor differentiated into dense projections. There is little difference between the pre- and postsynaptic membranes. Synaptic vesicles are scarce and may include a variety of sizes, while mitochondria are not yet accumulated in the axon terminal. An interesting question raised by gradual structural maturation is, when do synapses become functional? At the regenerating neuromuscular junction in the frog, transmission is not re-established until after other signs of nerve return can be detected (increase in frequency, and symmetric voltage distribution, of miniature end-plate potentials; retraction of muscle fiber sensitivity to acetylcholine back to the region of the end-plate). These are not features that could be detected in central nervous tissue. However, when rat spinal cord at 14 days of gestation was explanted and cultured in vitro electrical responses that seemed to indicate synaptic transmission appeared after three to four days. Electron microscopy showed that this was the time at which synapses could first be found frequently in the cultures. But no critical correlation can be obtained from these nonquantitative results. Little is known of precise times at which identifiable synapses begin functioning, and the visual cortex is probably too complex a system to make such a correlation. Single cortical units respond to flash stimulation at four to six days after birth in the cat, and responses in radiation fibers are detectable at three days. However, field potentials can be evoked from birth by electrical stimulation of the optic nerve so the functional maturation of some part of the retina is the limiting factor for the visual system of the cat, as had been found previously for the rabbit.

There are other unusual structural aspects of synapses in young kittens: synapses formed en passage (Fig. 1, C) are much more common than in the adult. It may be that the region of axodendritic contact later buds off to form a spine and an axonal branch. Alternatively, it may simply be that neuronal processes are so much less tightly packed and convoluted in the kitten that long lengths of axons often appear in the plane of section (Fig. 1, C), and so the chance of seeing...
that they go on after making a synapse is much increased. Axodendritic synapses are said to appear before synapses on cell bodies, the latter being present at one week but not at one to four days after birth in cat cortex. However, in cat visual cortex I have found an occasional synapse on a cell body as early as 12 days before birth (Fig. 1, D), and they are easier to find at birth (Fig. 1, E). Since axo-
somatic synapses form a small proportion of the totality of synapses in the adult, while the frequency of synapses in the fetal cat is much less than in the adult, the expected number of fetal axosomatic synapses is very small. The fact that they can be found at all means that a quantitative investigation would be needed to re-establish the generalization that axodendritic synapses tend to precede axosomatic. This question is relevant to the means by which afferent axons may trigger the development of dendrites, and also to the observation that inhibitory processes are prominent in the electrophysiology of immature cortex. The latter has not yet been correlated with the appearance of flattened vesicles in either axosomatic or axodendritic synapses. The osmolarity of the buffer is important in demonstrating flattened vesicles in adults and Fig. 1, A, and B, shows that the distinction between round and flattened vesicles can be made before birth. More work is needed to determine how early the terminals with flattened vesicles appear, and what structures they end on.

Recently, the distribution in depth of cortical synapses has been counted for the first time in the somatic sensory area in the dog. Strata of high or low synaptic density related to the layering of cell bodies were found. The three animals examined were all new-born, but the finding raises the exciting possibility that at an earlier stage of development the distribution of cortical connections might be so limited that it would be possible to work out the initial neuronal circuitry. I have therefore counted synapses in depth in the visual cortex of 10 cats ranging from adult to two weeks before birth. In the earliest cat the number of synapses present in a unit volume is only one sixtieth of the adult number, but the distribution is already widespread (Fig. 2, A). A few synapses can be found at all depths, but they are least frequent in the compact plate of cell bodies. A similar result has been found qualitatively in rabbit visual cortex at day 20 of gestation.

The visual cortex of young cats shows peaks and troughs in the distribution of synapses, and these are related to the layering of cell bodies as found in the dog. In adult cats these peaks are flattened out (Fig. 2, B) and the density of synapses is remarkably even in depth. By looking at intermediate stages one can see whether there are centers of development, or whether synapses increase simultaneously at all depths. Between 15 and 10 days before birth (when a second group of afferent axons arrives at the deep side of the cortical plate and induces dendritic development there), the synaptic density shows a small gain that is greater on the deep side of the cortical plate than else-
where. But in the last 10 days before birth, when a third group of axons arrives on the superficial side of the cortical plate,\(^5\)\(^,\)\(^6\) the synaptic density shows a small gain superficial to the layer of cell bodies. Much larger increases in synaptic density follow in the same places and in the same order after a delay of three to four weeks. Thus there is a large gain in synaptic density in the deeper layers between one and eight days after birth, and a large gain in superficial density between eight and 27 days. Nevertheless, the development of synapses is not confined to any one depth at any epoch, for synapses increase at all depths to some extent during the period studied.

These distributions were found by counting about 1,500 synapses in each cat, and if the density is averaged in depth a smooth curve of increasing average synaptic density is obtained as shown in Fig. 3. A similar S-shaped curve has been obtained previously for the development of synapses in the lateral geniculate nucleus (LCN) of the rat, where there is a sharp rise seven to 13 days after birth.\(^2^4\) The LCN of the cat has not yet been counted. In the cerebral cortex of the rat, there is some disagreement between two groups of authors. Counts of axon terminals showed a sharp rise between four and 14 days after birth in superficial motor cortex.\(^2^5\) An earlier count of synaptic bars stained with phosphotungstic acid in parietal cortex found the main rise in density after 12 days from birth.\(^1^0\) The disparity in counts at 12 to 14 days could be due to either of two factors: it would be easy to miss immature synaptic bars in the material stained only with phosphotungstic acid, and on the other hand the vesicle-filled profiles counted as axon terminals may possibly not all have formed synaptic appositions. This could be tested in serial sections. Fortunately there is excellent agreement\(^2^5\) between both groups of authors and myself on the density in adult

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**Fig. 3.** Growth of brain weight (W), density of synapses (S), density of cell nuclei (N), and number of synapses per neurone (S/N) in 8 kittens and 2 adult cats.
rat cerebral cortex \((1.2 \times 10^{12}\) synapses per cubic centimeter). The counts that I am reporting in cats were of synaptic appositions in conventionally stained material. The steepest increase in synapses in cat visual cortex is shown in Fig. 3 to be in the four weeks that follow eye opening at seven days after birth. The cell density falls as the synaptic density rises, but one can estimate the average number of synapses associated with each cell by dividing the synaptic density by the cell density. This is also plotted in Fig. 3, and rises from about 14 synapses per cell two weeks before birth, to 7,000 or 8,000 synapses per cell in adult cats.

There are three surprising features in these results. First, the density of synapses at 36 to 39 days exceeds that of two adults. To be sure that there really is a trimming down in this period more counts are needed in litter-mate cats. Secondly, at eight days after birth there is an average of only 112 synapses per cell (1.5 per cent of the adult number) yet the specificity of some cortical units for orientation and direction of stimulus movement has developed by this age. More recently it has been shown that one of the mechanisms for stereopsis has not yet developed at this age. The fact that 98 per cent of the synapses have still to come suggests that there are other immaturities still to be found unless many of the later formed synapses never do become operative.

The third surprising feature is that the development of synapses is nearly complete at 27 to 36 days, at which time a few days of closure of one eye can lead to a lasting deficit in response of cortical units to the eye that has been closed. It may be that the synapses present at 27 days are functionally immature and would become operative during the critical period for eye closure. But if so the receptive field specificity at eight days would be produced by even fewer than 1.5 per cent of synapses that has developed structurally at that time. A possibility that is easy to test is that eye closure may produce its effect by causing axon terminal degeneration in the cortex consequent to the cell shrinkage that has been detected in the LGN. The visual cortex can be searched by electron microscopy for blackened or otherwise abnormal terminals (especially in layer 4) a few days after a sufficient period of eye closure. After closure of one eye from 28 to 36 days after birth, Mrs. R. Anker has found no degenerating terminals in visual cortex, and I have found none after eye closure from 21 to 34 days after birth. Unfortunately there is at present no known sign by which one could recognize a retraction of axon terminals that did not involve actual degeneration. Spontaneously degenerating blackened processes (but not axon terminals) are found until soon after birth in normal cortex especially in the superficial visual cortex, and may be related to the unexplained disappearance of Retzius-Cajal cells from layer 1 at about the time of birth.

In thinking about critical periods, one of the large gaps in our knowledge concerns the time of development of extrinsic cortical connections. These contribute relatively few synapses, and most of the synapses counted in Figs. 2 and 3 are of intrinsic origin. Thus in layer 4 of the visual cortex of an adult cat the proportion of axon terminals that are derived from the cells of the LGN has been estimated at 2 to 4 per cent, 5 to 10 per cent, or less than 10 per cent. So extrinsic connections could develop while there is little further increase in total synaptic count. It was once fashionable to try to correlate the development of myelin with emerging behavior. Anatomical methods for studying the development of extrinsic connections have existed for more than 10 years. The only relevant work I have been able to find is a recent abstract reporting a superficial distribution of optic radiation fibers in visual cortex of kittens up to two weeks after birth. This differs from
the adult distribution and has yet to be confirmed. The time of first appearance of the terminals derived from the LGN has not been established, and the precise origin of the three groups of afferent axons that appear in Golgi preparations is unknown.

Some cortical connections have been studied histochemically. Axons containing acetylcholine esterase were found to appear in cat cortex around the time of birth, while esterase-containing cells were still migrating into cortex during the first two postnatal weeks. These late-developing cells are an exception to the migration scheme, for they occupy a deep position in adult cortex. Sensitivity of cortical units to iontophoretically applied acetylcholine matures during the third postnatal week. An adrenergic projection to cortex has also been described and shown to arise in the locus ceruleus. The time of development of this projection has not been studied; it has not yet been identified in the cortex by electron microscopy, and it is difficult to judge how dense or sparse it may be. I have started to look at the maturation of callosal and descending connections of visual cortex by removing the visual areas on one side in kittens, and using electron microscopy and the Fink-Heimer method to identify degenerated terminals in ipsilateral thalamus and contralateral visual cortex. It seems that the descending connections to the thalamus are present at 17 days after birth, but the callosal projection is still little developed 33 days after birth. It is, however, technically difficult to establish such negative results.

An interesting new approach to cortical organization is offered by the recent finding that peroxidase injected into muscle is taken up into motor nerve terminals and transported back to the nerve cell body. If such a method would work in the central nervous system it could be applied to many problems, for example, the identification of the output cells of the visual cortex which project to the LGN, superior colliculus, pretectal area, peristriate cortex, and corpus callosum. I have not yet been able to detect any transport on injecting peroxidase into rat visual cortex, but it is possible that a successful modification of the method may be found.

In conclusion, there is a great deal of structural work that can be done and has not yet been done on developing cortex. But the goal of unraveling the neuronal circuitry is remote, mainly because of the luxuriant connectivity of the neuropil and the difficulty of identifying the origin of the neuronal processes found in it. At eight days after birth, when some of the cortical stimulus specificities have developed, there are already over 100 synapses associated with an average neurone. It seems unlikely that anything more than a statistical description of neuronal circuitry will be possible. All structural studies reveal a mass of detail whose significance cannot be understood. There is a great need for functionally inspired questions to extract meaning from the structural appearances.

REFERENCES
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Discussion

LUND: We've been working on the effects of visual deprivation on the superior colliculus (R. D. and J. S. Lund: Vis. Res. Suppl. 3: 281, 1971; J. S. and R. D. Lund, Anat. Rec. 172: 358, 1972), and I would like to compare our results with yours. In the rat colliculus, synaptic development is largely postnatal. In the early days of synaptic development there are many structures which one calls synapses, i.e., asymmetric contacts, etc., but they have no vesicles in the presynaptic process. Do you find this? A second point regards dendrites which we agree grow long before synapses form. However, in the five days after initial synapse formation starts one finds an enormous number of dendritic growth cones in the region, and these disappear later on as if the initial synapse formation elicits a secondary growth of dendrites. Have you observed this? Have you considered the ratio of synapses with spherical and flattened vesicles? We've found essentially what Bodian found in the spinal cord (Bodian: J. Comp. Neurol. 133: 113, 1968) that there are proportionally more spherical vesicle terminals initially and then the flattened ones increase later. In regard to visual deprivation, we've found that in the rat superior colliculus there are three periods of development: a slow one before the eyes open like yours, then a rapid one with proliferation particularly of optic terminals just after eye opening, again like yours, and then there appears to be a final period of filling in of intrinsic flat vesicle synapses. If you keep the eyes closed between postnatal Days 20 to 35 this third period doesn't occur. I would suggest that maybe there is a real intrinsic population that doesn't develop rather than a degeneration of formed elements at this critical stage in development. This would be a slightly different interpretation. One final thing is that Dr. Jenny Lund has been looking at monkey visual cortex and found that prenatally or in newborn animals the pyramidal cells often have somatic spines just like Purkinje cells in the cerebellum, and by electron microscopy these spines have flat vesicles, terminals normally characteristic of the dendritic trunk. Have you seen evidence of this?

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