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Synapses develop concurrently and at identical rates in different layers of the visual, somatosensory, motor, and prefrontal areas of the primate cerebral cortex. This isochronic course of synaptogenesis in anatomically and functionally diverse regions indicates that the entire cerebral cortex develops as a whole and that the establishment of cell-to-cell communication in this structure may be orchestrated by a single genetic or humoral signal. This is in contrast to the traditional view of hierarchical development of the cortical regions and provides new insight into the maturation of cortical functions.

THE CEREBRAL CORTEX IS DIVIDED into numerous cytoarchitectonic areas that are specialized structural and functional units (*1*). Cortical differentiation is most fully expressed in the human brain and underlies the subdivision of the cortex into sensory, motor, and associative systems. Although this cortical diversity is of major conceptual and biomedical importance, the mechanisms of its development are unknown (*2, 3*). Studies based on histological and histochemical parameters such as the density and distribution of myelin (*4*), levels of various enzymes (*5*), and metabolic activity (*6*) tend to support a hierarchical model of cortical development in which primary sensory and motor areas mature before adjacent secondary areas, and the association regions differentiate last. Although this model has had a major influence on physiological and psychological studies (*7*), a number of recent findings are not entirely consistent with it. For example, neurons in the primary visual cortex begin and complete their genesis later than neurons in the adjacent secondary visual areas (*8*), and the columnar organization of connections in the prefrontal association cortex (*9*) emerges prior to that of ocular dominance columns in the primary visual cortex (*10*).

We examined the pre- and postnatal course of synaptogenesis in five areas of the monkey cerebral cortex that mediate, respectively, visual, somatosensory, motor, associative, and limbic functions. On the basis of the available literature (*3–6*), we expected that synaptogenesis would proceed in clearly segregated waves in different cortical regions, with the sensory areas achieving maturation earlier than association areas. We also predicted that synaptogenesis would exhibit laminar specificity, and perhaps follow the inside to outside pattern of cortical neurogenesis (*11*) or the sequence of ingrowth of various afferents. Contrary to these expectations, however, our results revealed a simultaneous synaptogenesis in all areas and layers examined.

Rhesus monkeys (*Macaca mulatta*) of various pre- and postnatal ages were perfused with mixed aldehydes (*12*), and 1 by 2 by 3 mm blocks were dissected from the visual, somatosensory, motor, and prefrontal cortices, and the dentate gyrus of the hippocampus, and processed for electron microscopic analysis (Fig. 1). More than 500,000 synapses were identified from 22 monkeys in a total of 25,000 electron micrographs. Twenty percent of these synapses were selected randomly for further classification on the basis of their termination (on spines, dendritic shafts, or somas) or their morphology (symmetrical or asymmetrical) in each layer (*13*). The data are expressed as density of synapses per unit area of neuropil to provide a measure that is unaffected by age-related changes in the extracellular space, by the growth of neuronal perikarya, or by the addition of glial cells, myelin sheaths, or blood vessels.

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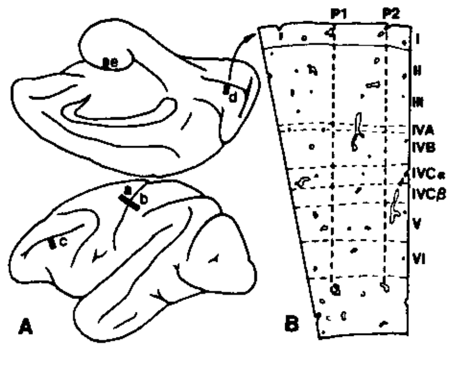

Fig. 1. (A) The lateral surface of the left cerebral hemisphere (bottom) and the medial surface, inverted (top), show the five cortical areas examined: a, motor cortex (Brodmann's area 4) in precentral gyrus; b, somatosensory cortex (area 1) in the postcentral gyrus; c, prefrontal cortex (area 9) in the upper bank of the principal sulcus; d, visual cortex (area 17) in the upper bank of the calcarine fissure; and e, molecular layer in the dentate gyrus (area 34). The blocks were postfixed in osmium, embedded in Epon-Araldite, and 600-angstrom sections were cut across the entire width of the cortex. (B) An outline of an ultrathin section across the visual cortex (d), as an example. The two vertical lines (P1 and P2) indicate the localization of two probes each yielding about 100 electron micrographs that were printed at a final magnification of ×14,000. Similar probes were prepared for other cortical areas except the dentate gyrus, where probes were taken only across the width of the molecular layer of the suprapyramidal and infrapyramidal limbs.

During the last 2 months of gestation, synaptic density increased at a rapid rate in the five cortical areas examined, reaching between 15 and 20 synapses per 100 μm^2 of neuropil by the time of birth (Fig. 2). Although this density is about the same as in sexually mature adults, it continued to increase during infancy and remained above adult levels for about the same length of time in all five areas (Fig. 2, A–E). The highest density ranged from 26 synapses per 100 μm^2 in the prefrontal cortex (Fig. 2C) to 34 synapses per 100 μm^2 in the visual cortex (Fig. 2D). Analysis of covariance revealed no significant differences among the slopes of increase in the four neocortical areas (Fig. 2, A–D), but the slope of the increase in the dentate gyrus (Fig. 2E) was lower than that of each other area ($P < 0.001$) (*14*). This lag in the rate of synaptic increase in the dentate gyrus might be because the dentate gyrus, unlike the neocortex which has a full complement of neurons before birth (*11*), acquires additional neurons during the first three postnatal months (*15*).

Synaptogenesis proceeded concurrently in all cortical layers; the density of synapses per unit area of neuropil in the relatively cell-poor layer I was not substantially different from that in neuron-rich layers II through VI in any of the five areas examined. Although the distribution of various classes of synapses differed from area to area as well as from layer to layer (*16*), the density per unit area of neuropil of all synaptic types combined was nevertheless similar in all areas and layers.

Synaptic density increased for several months after birth before beginning to decline in all layers and areas (Fig. 2). The decline occurred rapidly at first and then slowed during the second half of the first year; after this there was an even more gradual reduction throughout life (Fig. 2). The decrease in synaptic density cannot be attributed to dilution caused by an increase in cortical volume since extracellular space, neuronal somata, glial cells, and other tissue elements such as blood vessels and myelin sheaths were not included in our measurement. Furthermore, the percentage of neuropil in the cortex does not change appreciably during the period of synaptic decrease in the rhesus monkey (*17*). Finally, if the decrease in synaptic density were due to dilution, we would expect all classes of synapse to be affected similarly. However, synapses situated on dendritic spines, which make up 60 to 70 percent of the cortical synapses in the rhesus monkey, sustained the largest share of this loss. Synapses on dendritic shafts (30 to 40 percent) and cell somas (below 1 percent) contributed less to the age-related changes. In addition, the ratio between asymmetrical and symmetrical synapses changed in the prefrontal cortex from 4:1 at birth to 7:1 during the 4th month, and then again reached 4:1 at puberty. Changes in this ratio in the motor cortex were even larger—7:1 at birth, 24:1 during the 4th month, and again 7:1 in adult animals. We can conclude, therefore, that the decrease in synaptic density is achieved by elimination of synapses. Furthermore,

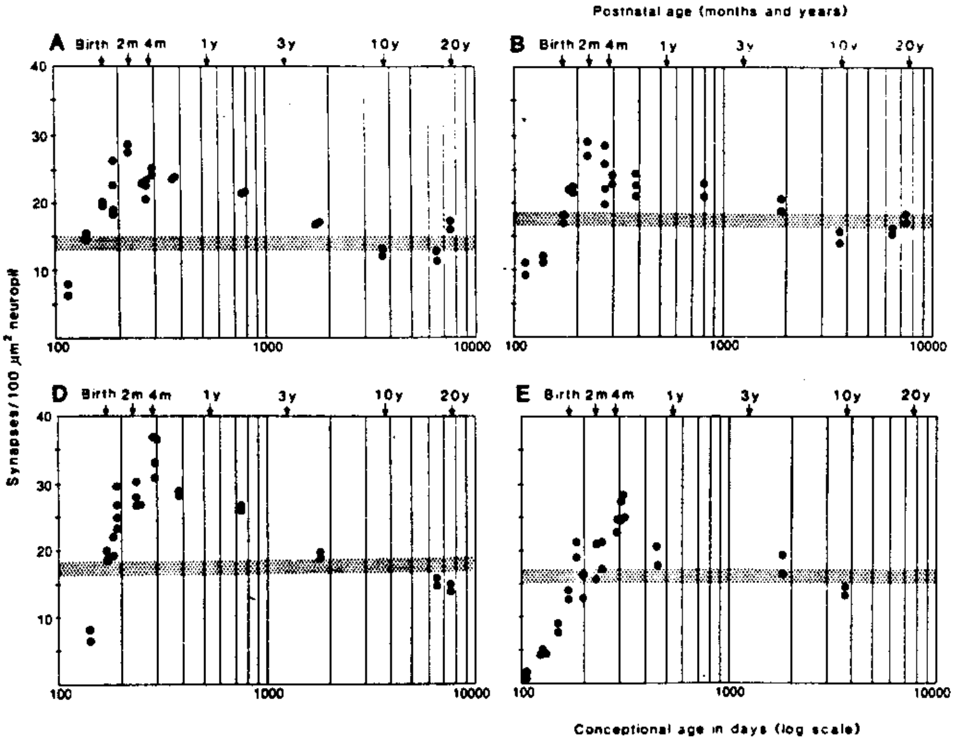

Fig. 2. Histograms of the density of synapses per 100 μm^2 of neuropil in (A) motor, (B) somatosensory, (C) prefrontal, (D) visual, and (E) limbic cortices at various ages. Each black circle represents the value obtained from a single electron microscopic probe (Fig. 1). Dotted horizontal stripe denotes average synaptic density in the adult monkey for each area. Age is presented in conceptional days on a logarithmic scale in order to fit the entire life span of the monkey onto a single graph.

synaptic density finally stabilizes at the same value of 15 to 20 synapses per 100 μm^2 of neuropil in all five regions examined (dotted stripes in Fig. 2). This value is similar to that found previously for structures of the primate brain as different as the retina and neostriatum (*18*). The value of 15 to 20 synapses per 100 μm^2 of neuropil may be structurally, metabolically, or physiologically optimal. Whether this value is species-specific remains to be determined.

Although transient overproduction of synapses could be predicted from previous observations made in various species (*19*), as well as in human cortex (*20*), our study compares the timing and magnitude of these events in different cortical layers and brain regions from the same specimens. The isochronic course of synaptogenesis in the primate cerebral cortex during infancy was unexpected because, since the time of Flechsig (*4*), the areas examined have been thought to mature anatomically, biochemically, and functionally at different rates (*3, 5–7*). It was also unexpected that synaptic density increased at identical rates in all cortical layers, since neurons of each layer are generated at different times (*8, 11*) and receive different ratios of monoaminergic, thalamic, cortico-cortical, and local synaptic connections (*21*). The simultaneous "overshoot" phase in diverse areas and layers of the cortex and the final common density achieved suggests that the cortex develops as a whole rather than regionally, and that formation of synapses throughout the entire cortical mantle may be regulated by common genetic or humoral signals. Simultaneous overproduction of synapses may be essential for competitive interactions between extrinsic afferents such as the competition that has been postulated between the projections of the two eyes during the formation of visual centers (*10, 22*). Our results, as well as other recent studies on the visual (*22*) and peripheral (*23*) nervous systems, suggest that if experience alters synaptic number during development it does so by causing selective survival of certain synapses, and not by regulating their initial formation.

Our findings contrast with the classical view of a hierarchical sequence of functional development from the sensory to motor, and finally to associative functions (*7*). However, they may help to explain certain behavioral findings that were heretofore puzzling. For example, rhesus infants as young as 2½ months of age have the capacity to tactually discriminate texture and size differences at the level of an adult monkey (*24*). Likewise, visual tracking of small objects, visually guided reaching, and discrimination of facial features, skills indicative of visual cortical function, appear between 1½ and 2 months (*25*); visual object discrimination performance first becomes possible at about 2 months of age (*26, 27*). Although fully independent use of the digits does not mature until between 7 and 8 months after birth, some independent finger usage begins at 2 months and is quite efficient by 4 months (*28*). Numerous other indices of adult posture and progression, as well as regression of infantile motor reflexes commonly attributed to the development of "descending" control, occur around 2 months of age (*29*). Performance on a memory task sensitive to hippocampal damage in adult rhesus monkeys is possible at 2 months and reaches mature levels at approximately 4 months of age (*27*). Delayed-response performance follows a similar ontogenetic sequence (*30*). The latter task measures cognitive functions that are mediated by the principal sulcus from which the prefrontal sample was taken in our study (*31*).

Thus, various indices of sensory, motor, limbic, and associative cortical function are all expressed between 2 and 4 months of age, a time period which coincides with excess synapse production. The attainment of these behavioral milestones within the first few postnatal months indicates that the synchronous production of a critical mass of synapses in each cortical area may be essential for their parallel emergence. However, behavioral competence continues to increase beyond the stage of excess synapses. This suggests that full functional maturation may be related to synapse elimination and acquisition of synaptic efficiency at the molecular level. Increasingly complex cortical capacities might also evolve from the accretion and storage of information and subsequent interactions among cortical areas rather than from further changes in the number of synaptic contacts.

REFERENCES AND NOTES

1. K. Brodmann, *Vergleichende Lokalisations lehre der Grosshirnrinde*. (J. A. Barth, Leipzig, 1909); F. O. Schmitt, F. G. Worden, G. Adelman, S. G. Dennis, Eds. *The Organization of the Cerebral Cortex* (MIT Press, Cambridge, MA, 1981); M. A. B. Brazier and H. Petsche, Eds. *Architectronics of the Cerebral Cortex* (Raven, New York, 1978); A. Peters and E. G. Jones, Eds., *Cerebral Cortex* (Plenum, New York, 1984).
2. V. B. Mountcastle, in *The Neurosciences, Fourth Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, MA, 1979), pp. 21–42; P. Rakic and P. S. Goldman-Rakic, Eds., *Neurosci. Res. Program. Bull.* **20**, 429 (1982).
3. J. L. Conel, *Postnatal Development of the Human Cerebral Cortex* (Harvard Univ. Press, Cambridge, MA, 1939–67), vols. 1–8; S. A. Sarkisov, Ed., *Development of a Child's Brain* (Medicina, Leningrad, 1965).
4. P. Flechsig, *Anatomie des menschlichen Gehirns und Ruckenmarks auf myelogenetischer Grundlage* (Thieme, Leipzig, 1920); P. I. Yakovlev and A. R. Lecours, in *Regional Development of the Brain in Early Life*, A. Minkowski, Ed. (Blackwell, Oxford, 1967), pp. 3–70.
5. E. Farkas-Bargeton and M. F. Diebler, in *Architectonics of the Cerebral Cortex*, M. A. B. Brazier and H. Petsche, Eds. (Raven, New York, 1978), pp. 175–190; R. L. Friede, Ed. *Topographic Brain Chemistry* (Academic Press, New York, 1966).
6. C. Kennedy *et al.*, *Ann. Neurol.* **12**, 333 (1982).
7. R. J. Ellingson and R. C. Wollcott, *J. Neurophysiol.* **23**, 363 (1960); S. L. Friedman and M. Sigman, *Preterm Birth and Psychological Development* (Academic Press, New York, 1981).
8. P. Rakic, *Exp. Brain Res. Suppl.* **1**, 244 (1976).
9. P. S. Goldman and W. J. H. Nauta, *Brain Res.* **122**, 369 (1977).
10. P. Rakic, *Nature (London)* **261**, 467 (1976); *Philos. Trans. R. Soc. London Ser. B* **278**, 245 (1977).
11. ——, *Science* **183**, 425 (1974).
12. ——, *J. Comp. Neurol.* **145**, 61 (1972).
13. The criteria for identifying synapses was the presence of paired membrane thickenings, two or more synaptic vesicles located at the presynaptic site, and a synaptic cleft. The density of synapses was expressed per unit area of neuropil as described [M. L. Cooper and P. Rakic, *J. Comp. Neurol.* **215**, 165 (1983)]. On each photomicrograph, we calculated the percentage of tissue that was occupied by neuropil (neurite profiles) and the percentage occupied by blood vessels, extracellular spaces, and glial and neuronal perikarya.
14. Each data set presented in Fig. 2, A–E, was divided into ascending and descending components at the time of the highest synaptic density. The data from animals older than 10 years were not included in this analysis. Analysis of covariance [G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State University Press, Ames, IA, 1967), pp. 419–443] revealed that the linear components of the ascending portion of the synaptogenesis functions in Fig. 2, A–E, are statistically significant ($P < 0.001$). Further comparison of individual slopes showed a significant difference among the slopes [($F(4, 67) = 5.86$, $P < 0.001$)] that was due to a difference between group E (dentate gyrus) and each other group; groups A, B, C, and D are not significantly different from one another either in terms of slope or shift along ordinate [($F(3, 46) = 0.76$, $P < 0.2$, for slopes and $F(1, 46) = 0.31$, $P < 0.2$, for shift along ordinate].
15. P. Rakic and R. S. Nowakowski, *J. Comp. Neurol.* **196**, 94 (1981).
16. A detailed description of region-specific and layer-specific differences in composition of synaptic classes is not yet available.
17. N. Zecevic and P. Rakic, *Abstr. Soc. Neurosci.* **11**, 1288 (1985).
18. S. Brand and P. Rakic, *Anat. Embryol.* **169**, 21 (1983); Y. Nishimura and P. Rakic, *J. Comp. Neurol.* **241**, 420 (1985).
19. J. S. Lund, R. G. Booth, R. D. Lund, *J. Comp. Neurol.* **176**, 149 (1977); J. O'Kusky and M. Colonnier, *ibid.* **210**, 278 (1982); C. J. Duffy and P. Rakic, *ibid.* **214**, 225 (1983).
20. P. R. Huttenlocher, *Brain Res.* **163**, 195 (1979); P. R. Huttenlocher, C. deCourten, L. J. Garey, H. Van der Loos, *Neurosci. Lett.* **33**, 247 (1982).
21. J. H. Morrison *et al.*, *Brain Res. Bull.* **9**, 309 (1982); P. Levitt, P. Rakic, P. S. Goldman-Rakic, *J. Comp. Neurol.* **227**, 23 (1984); J. S. Lund, in *The Organization of Cerebral Cortex*, F. O. Schmitt, Ed. (MIT Press, Cambridge, MA, 1981), pp. 106–124; J. H. R. Maunsell and D. C. Van Essen, *J. Neurosci.* **3**, 2563 (1983); K. S. Rockland and D. N. Pandya, *Brain Res.* **212**, 249 (1981); E. G. Jones, in *Dynamic Aspects of Neocortical Function*, E. M. Edelman *et al.*, Eds. (Wiley, New York, 1984), pp. 7–40; P. Rakic, *Neurosci. Res. Bull.* **13**, 295 (1975).
22. D. H. Hubel, T. N. Wiesel, S. LeVay, *Philos. Trans. R. Soc. London Ser. B* **278**, 377 (1977); P. Rakic, *Science* **214**, 928 (1981); R. L. Mayer, *ibid.* **218**, 589 (1982); T. N. Wiesel, *Nature (London)* **299**, 583 (1982); J. T. Schmidt and D. L. Edwards, *Brain Res.* **269**, 29 (1983); M. P. Stryker and W. A. Harris, *J. Neurosci. J. Neurosci.*, in press.
23. P. Benoit and J.-P. Changeux, *Brain Res.* **99**, 354 (1975); J.-P. Changeux and A. Danchin, *Nature (London)* **264**, 705 (1976); D. A. Riley, *Brain Res.* **143**, 162 (1978); W. D. Thompson, D. P. Kuffler, J. K. S. Jansen, *Neuroscience* **2**, 271 (1979); M. C. Brown, W. G. Hopkins, R. J. Keynes, *J. Physiol. (London)* **329**, 439 (1982).
24. M. Carlson, *Dev. Brain Res.* **16**, 69 (1984).
25. R. G. Booth, V. Dobson, D. Y. Teller, *Annu. Rev.*

Neurosci. **8**, 495 (1985); M. J. Mendelson *et al.*, *Dev. Psychol.* **19**, 387 (1983).

26. R. Boothe and G. Sackett, in *Rhesus Monkey. I. Anatomy and Physiology*, G. H. Bourne, Ed. (Academic Press, New York, 1975), pp. 343–363; P. S. Goldman, H. T. Crawford, L. P. Stokes, T. W. Galkin, H. E. Rosvold, *Science* **186**, 540 (1974); H. Harlow, *Am. Sci.* **47**, 459 (1959).
27. H. Mahut and S. Zola, *Abstr. Soc. Neurosci.* **3**, 428 (1977); H. Mahut and M. Moss, in *The Hippocampus*, K. Pribram and R. Isaacson, Eds. (Plenum, New York, in press); J. Bachevalier and M. Mishkin, *Behav. Neurosci.* 98, 770 (1984).
28. D. G. Lawrence and D. A. Hopkins, *Brain* **99**, 235 (1976).
29. M. Hines, *Contrib. Embryol. Carnegie Inst.* **30**, 153 (1942).
30. A. Diamond and P. S. Goldman-Rakic, *Soc. Res. Child Dev. Abstr.* **5**, 85 (1985).
31. P. S. Goldman and H. E. Rosvold, *Exp. Neurol.* **27**, 291 (1970); N. Butters, D. Pandya, D. Stein, J. A. Rosen, *Acta Neurobiol. Exp.* **32**, 305 (1972).

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