YASUZO TSUKADA/BERNARD W. AGRANOFF

Neurobiological Basis of Learning and Memory

NEUROBIOLOGICAL BASIS OF LEARNING AND MEMORY

Edited by

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Preface

The search for a biological basis of behavioral change remains among the most challenging in science. The pursuit is in no small measure responsible for the recent rise of neurobiology as a discipline. New disciplines borrow heavily from existing ones, and in this case, biochemistry, pharmacology, psychology, physiology, genetics, and morphology, as well as the clinical sciences have served as the sources.

It is timely that the Taniguchi Foundation, having recently made a commitment to extend its goals into the brain sciences, has now turned to questions of learning and memory. This volume is itself a "memory" of such an effort, in the form of a symposium, held October 23–25, 1978 at Ohtsu-shi, Japan. The various aforementioned approaches have been applied to further our understanding of learning and memory and of brain plasticity in general. An important aim of this meeting was to exchange information and to strengthen lines of communication between young Japanese and visiting scientists. As might be predicted, cultural and linguistic barriers are minor compared with those between the scientific disciplines. Hopefully, the meeting served to help bridge the disciplinary gaps and to widen scientific horizons for the participants. To preserve the informality necessary for free exchange, the meeting was a small one. Consequently, this volume should be considered a sampling from a vast field rather than a comprehensive summation. The participants are grateful to Mr. Toyosaburo Taniguchi and the Taniguchi Foundation for sponsoring this meeting. The publication of this book marks the fiftieth year of the Foundation's efforts in the dissemination of knowledge.

Yasuzo Tsukada
Bernard W. Agranoff

Contents

1

Plasticity During Neuronal Differentiation: An Experimental Morphological Study of Developing Synapses and of Neuronal Networks

József Hámori

The genetically inherited capabilities of nerve cells and of neuronal networks do not automatically materialize during maturation. The proper expression of intrinsic potentialities is made possible by the remarkable developmental plasticity of the differentiating nerve elements. The main source of plasticity during brain development is—as known from the studies of Ramón y Cajal (43–45)—the excess neurons and, especially, neuronal processes. This results in a relative overdevelopment of synaptic connections during synaptogenetic processes. Through a selective process, the use or disuse of the neuronal elements and synapses will then determine which nerve cells, neuronal processes, and synaptic connections can survive. Only properly linked and functioning neurons and networks will be stabilized, whereas numerous "erroneous" or simply "unnecessary" nerve elements will be eliminated (3,6,9,41).

The aim of this chapter is to demonstrate that the same natural selection law, by "trial and error," also operates at the subneuronal level in the development of synaptic junctions.

QUANTITATIVE ELECTRON MICROSCOPY OF DEVELOPING SYNAPTIC JUNCTIONS

In the adult nervous system the chemical synaptic junction is characterized by the presence of an accumulation of synaptic vesicles and of dense projections opposite the

presynaptic membrane, as well as of a varying amount of osmiophilic material adhering to the postsynaptic membrane ("thickening") (Fig. 1-5). The synaptic cleft varies from 10 nm to 30 nm in interneuronal synapses.

Considerable differences of opinion exist about the nature of the earliest structural events associated with synaptic development. Glees and Sheppard (11), Hámori and Dyachkova (18), Larramendi (29), Alley (1), Spira (52), Bunge et al (4), Matthews and Faciane (34), and McArdle et al (35) have suggested that the first signs of impending synaptic development are desmosomoid membrane thickenings of presynaptic and postsynaptic membranes. Occhi (37) and Sheffield and Fischman (47), on the other hand, have observed an accumulation of vesicles in advance of any membrane thickening. In a more recent study of the synaptic differentiation of local interneurons in monkey lateral geniculate nucleus (20), it was found, however, that although synaptic vesicles in the newborn are present in the presynaptic dendritic processes, the profiles are exclusively postsynaptic until the end of the second postnatal week. Only at this time, after the appearance of postsynaptic thickenings, will the synaptic vesicles exhibit the characteristic presynaptic accumulation and will the neuronal process consequently become also presynaptic. Bodian (2) suggested that the two events, that is, the development of synaptic membrane thickenings and the accumulation of presynaptic vesicles, appear simultaneously. Indeed, from recent studies of the differentiation of cerebellar mossy fiber synapses (21), it is apparent that both mature and immature synapses (Figs. 1-13–1-15) can be found at a very early stage of synapse development. (Synaptic contacts exhibiting both presynaptic vesicle accumulation and an enhanced osmiophily of the synaptic membranes are considered "mature" [Fig. 1-5]; those having only postsynaptic membrane thickening [e.g., Figs. 1-1, 1-2], "immature.") Similarly, immature contacts on Purkinje and basket cell bodies (Figs. 1-1, 1-3, 1-4), as well as on Purkinje dendritic spines (Figs. 1-6, 1-7), could be also observed during early differentiation of these synaptic contacts, accompanied by the simultaneous appearance of more mature synaptic contacts (e.g., Fig. 1-4). It was also observed that during the synaptogenetic period, which occurs between one to five weeks postnatally, there was a gradual numerical diminution, and by the sixth week the immature contacts completely disappeared from rat cerebellar mossy terminals (21). This led to the logical assumption that the immature contacts have been transformed during synapse differentiation to mature synaptic junctions. Using quantitative electron microscopy, however, we have gained good evidence, at least for the two synaptic junctions studied, that this is not the case.

1. The *optic terminal* in the newborn monkey's lateral geniculate nucleus is in a relatively advanced developmental stage. Nevertheless, as was shown in an earlier study (40), it exhibits both mature and immature asymmetric (synaptic) as well as symmetric contacts with the dendritic processes of the principal neurons (Figs. 1-8,–1-10).

Figure 1-3. A transient form of immature synaptic contact between a basket terminal (ba) and Purkinje cell soma (P) from 14 dpn rat cerebellum. Early signs of differentiation of presynaptic dense projections (ringed arrows) are seen in addition to the presence of postsynaptic "thickening" (arrows). The synaptic vesicles in the axon terminals are ovoid or polymorphic (×78,300).

Figure 1-4. Mixture of mature (arrows) and immature (thick arrow) axosomatic synapses on basket neuron (*b*) from 14 dpn rat cerebellar cortex (×42,630).

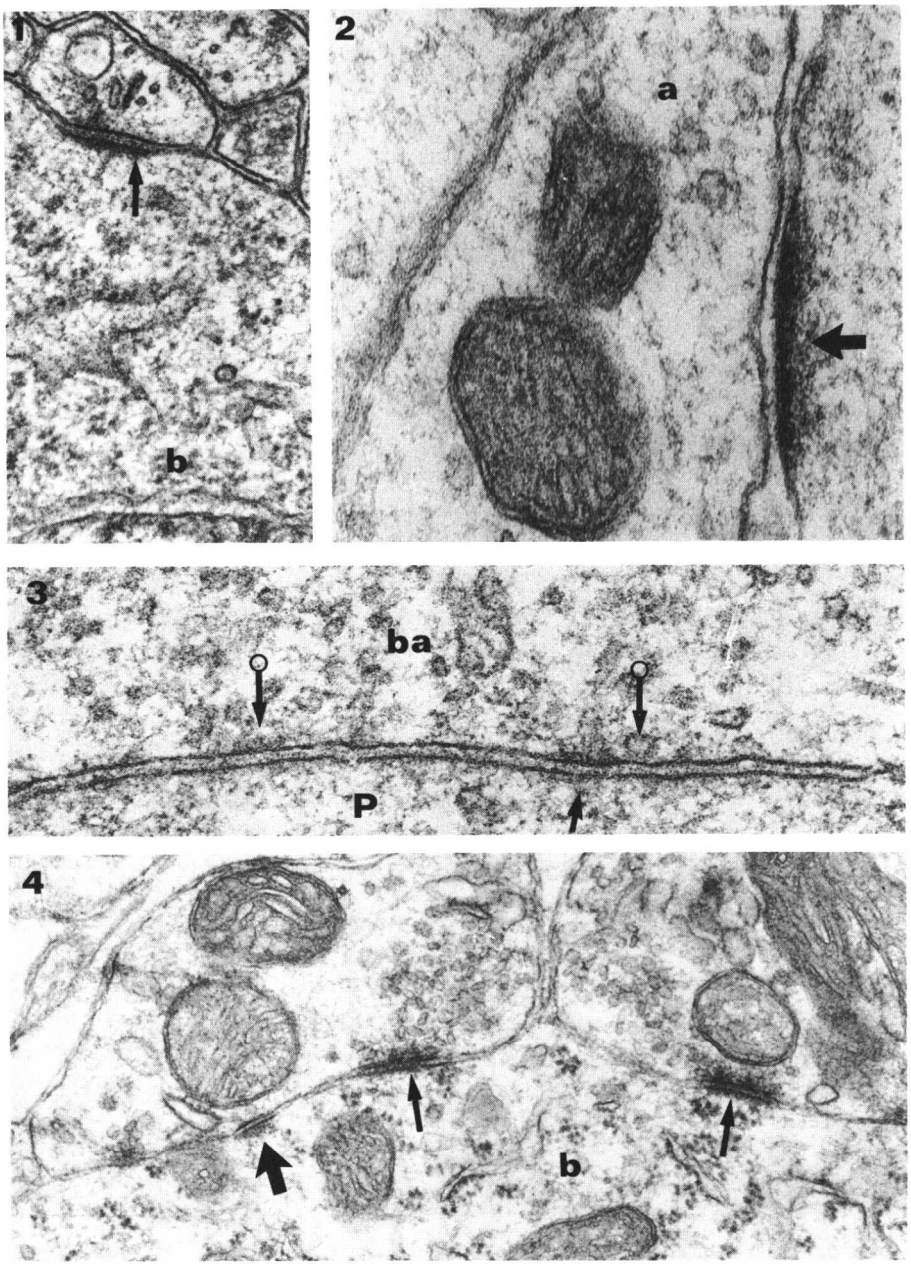


Figure 1-1. Immature axosomatic contact (arrow) on a differentiating basket neuron (b) in a 14 dpn rat cerebellar cortex (×4263).

Figure 1-2. Immature axondendritic synaptic contact from 6 dpn dog lateral geniculate nucleus. Note the presence of scattered synaptic vesicles in the axon terminal (a) but the absence of vesicle accumulation opposite to the well-developed postsynaptic membrane (arrow) (×97,440).

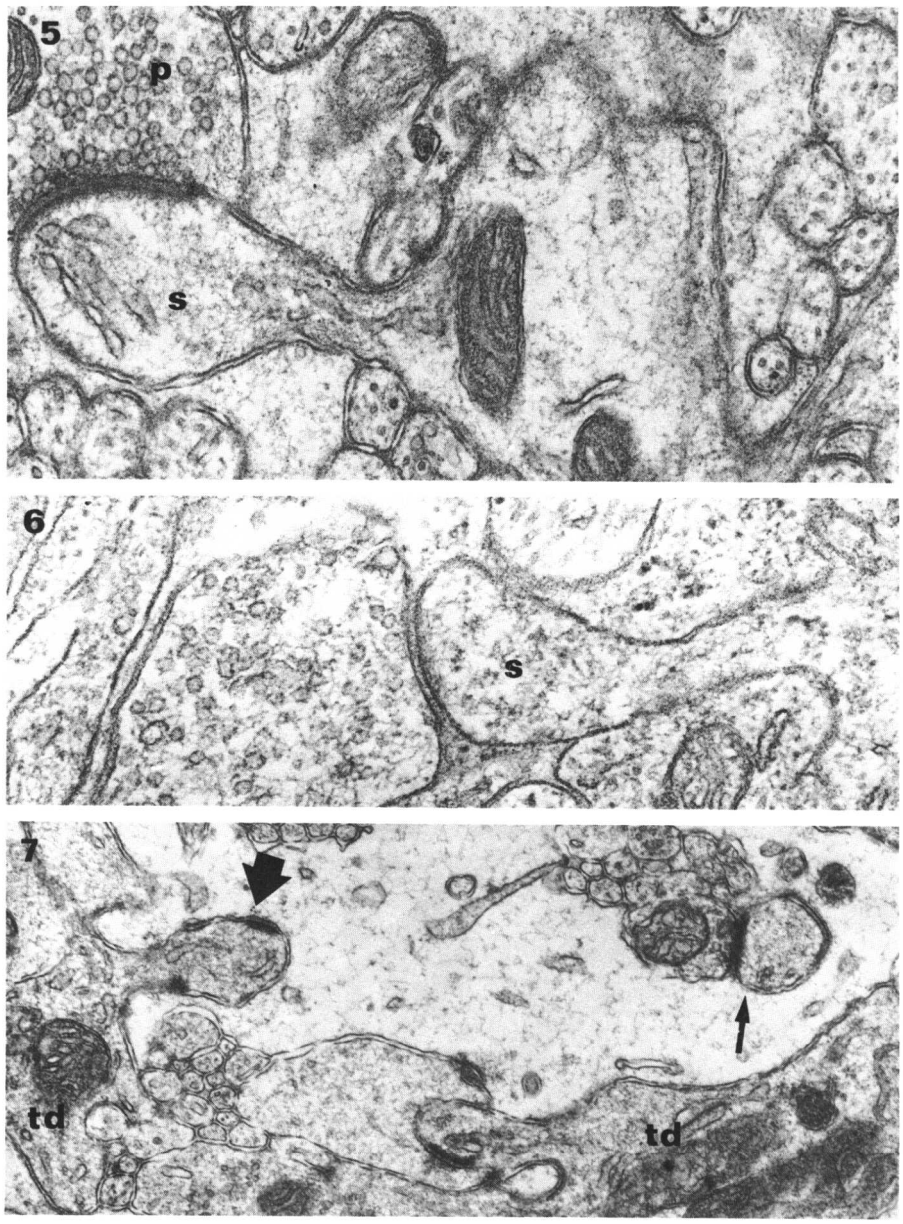


Figure 1-5. A typical axon-spine synapse between a parallel fiber (p) and a tertiary dendritic spine (s) of cerebellar Purkinje cell. Adult rat cerebellar cortex (×49,590).

Figure 1-6. A developing, not fully matured, axon-spine synapse from 15 dpn rat cerebellar cortex for comparison with Figure 1-5. s: dendritic spine (×56,550).

Figure 1-7. Synaptic (arrow) and nonsynaptic (thick arrow) tertiary dendritic spines of Purkinje cell from 11 dpn rat cerebellar cortex. Note the presence of postsynaptic membrane "thickening" on the nonsynaptic spine, which is contacted, however, only by glial process. td: tertiary dendrite (×27,840).

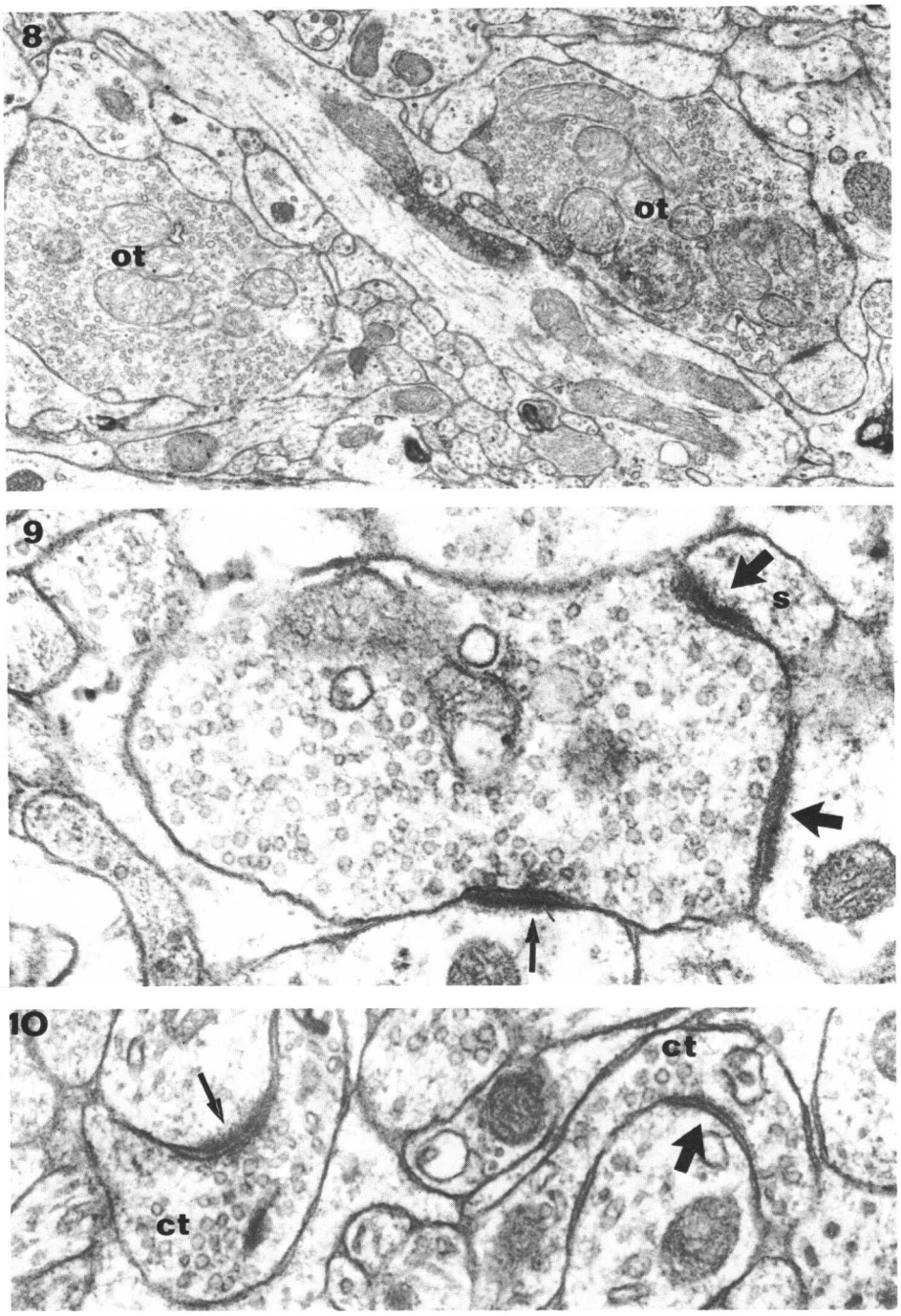


Figure 1-8. Two optic terminals (ot) from lateral geniculate nucleus of newborn monkey. The axon terminals are surrounded by vesicle-bearing presynaptic dendrites and by principal cell dendritic processes (×24,360).

Figure 1-9. Mature (arrow) and immature (thick arrows) synaptic contacts between optic terminal and postsynaptic dendrites and dendritic spines (s). Lateral geniculate nucleus (LGN), newborn monkey (×56,550).

Figure 1-10. Mature (arrow) and immature (thick arrow) axodendritic synaptic contacts between cortical terminals (ct) and postsynaptic dendritic processes in newborn monkey LGN (×56,550).

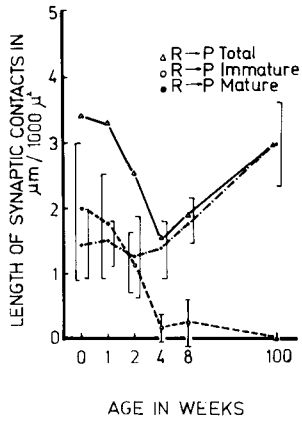


Figure 1-11. Diagram showing the results of quantitative measurements on electron micrographs from developing LGN of monkey. Note that increase in the number of mature retinoprincipal synapses begins only after the apparent disappearance of immature contacts between the two synapsing elements. R: retinal terminal, P: projective neuronal dendrites.

Between 0 and 28 postnatal days (dpn) there is a definite decrease in asymmetric contacts, which is caused by the gradual disappearance of immature contacts. Contrary to our expectations, however, the decrease in the number of immature contacts is not accompanied by a simultaneous increase in the number of mature synaptic junctions, which will occur only after the almost complete disappearance of the immature contacts (Fig. 1-11).

2. The same trend characteristizes the developing mossy fiber synapse in the cerebellar cortex of the rat. At the beginning of synapse differentiation, that is, 6 dpn, mature and immature synaptic contacts occur simultaneously (Figs. 1-13–1-15). The number of both contact types increases until about the twelfth postnatal day. From the seventeenth postnatal day onward, simultaneously with the pronounced development of the characteristic labyrinthine structure of the synaptic glomerulus (Fig. 1-12), a definite diminution in the number of immature contacts can be observed (Figs. 1-16,1-17). At the same time, however, the number of mature contacts exhibits the same decline and, after a steady decrease, will reach the adult level, that is, 5.5% of the whole mossy fiber membrane surface, at about 8 weeks after birth.

These observations clearly show that during early synaptogenesis there is an excess offer of synaptic contacts, both mature and immature. It is also obvious from both examples (optic and mossy terminals) that most of the immature contacts are not transformed to mature, permanent synaptic contacts but will simply be absorbed, together with many already mature contacts, with progression of the differentiation of the whole synaptic complex. This indicates that elementary synaptogenesis consists of a secondary selection accomplished by the disappearance—possibly as a consequence of disuse—of the excess of immature or otherwise nonfunctioning synapses.

ROLE OF PRESYNAPTIC ELEMENTS IN THE DEVELOPMENT OF POSTSYNAPTIC STRUCTURES

The next logical question to be raised is whether the development (and overdevelopment) of postsynaptic junctional complexes is an autonomous process, independent of the connectivity of the particular neurons, or if this plastic process of synapse differentia-

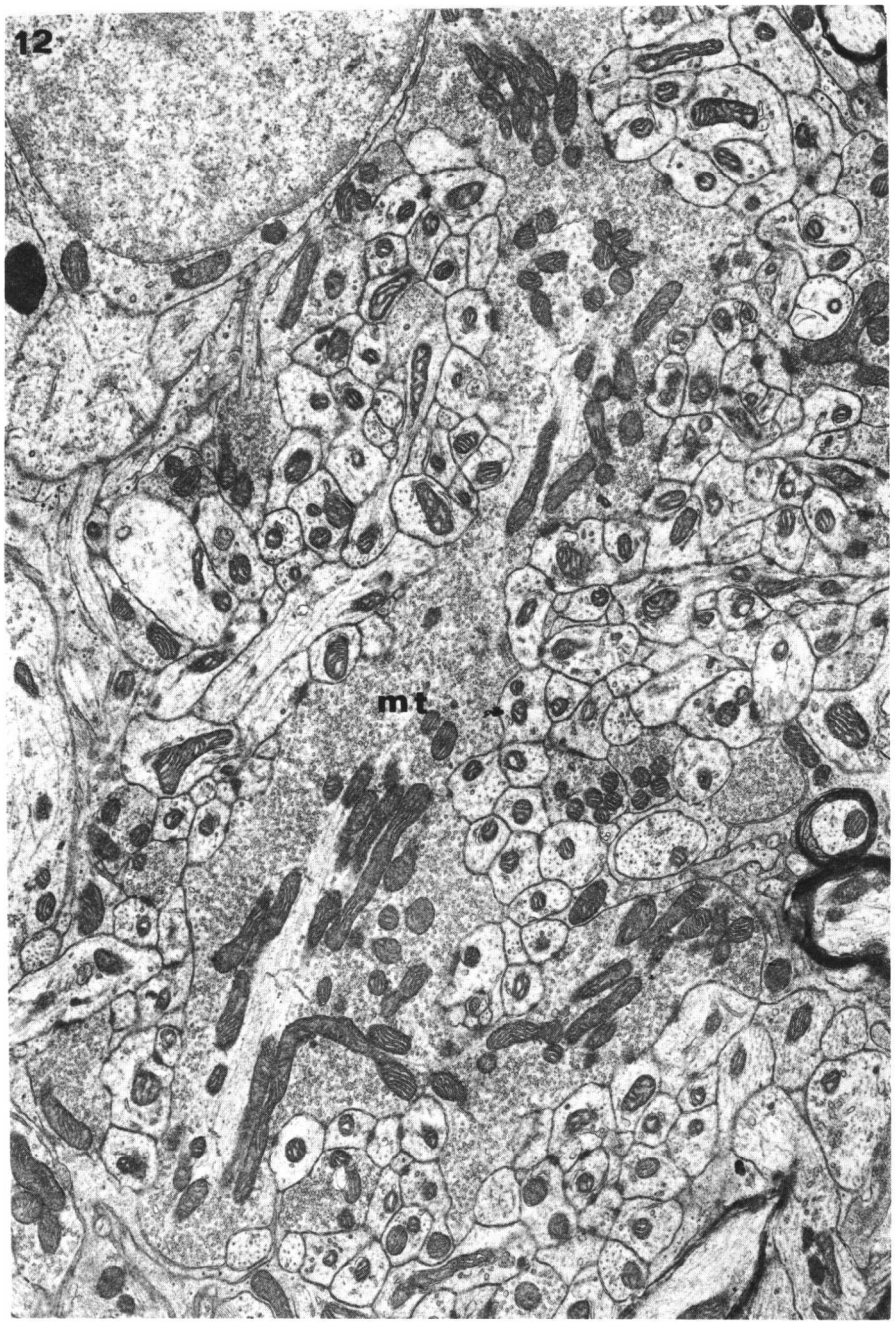


Figure 1-12. Cerebellar glomerulus from adult rat cerebellar cortex. The central big mossy terminal (mt) is surrounded by numerous postsynaptic dendrites and dendritic digits of granule neurons. Synaptic contacts are all mature by morphological criteria (×13,050).

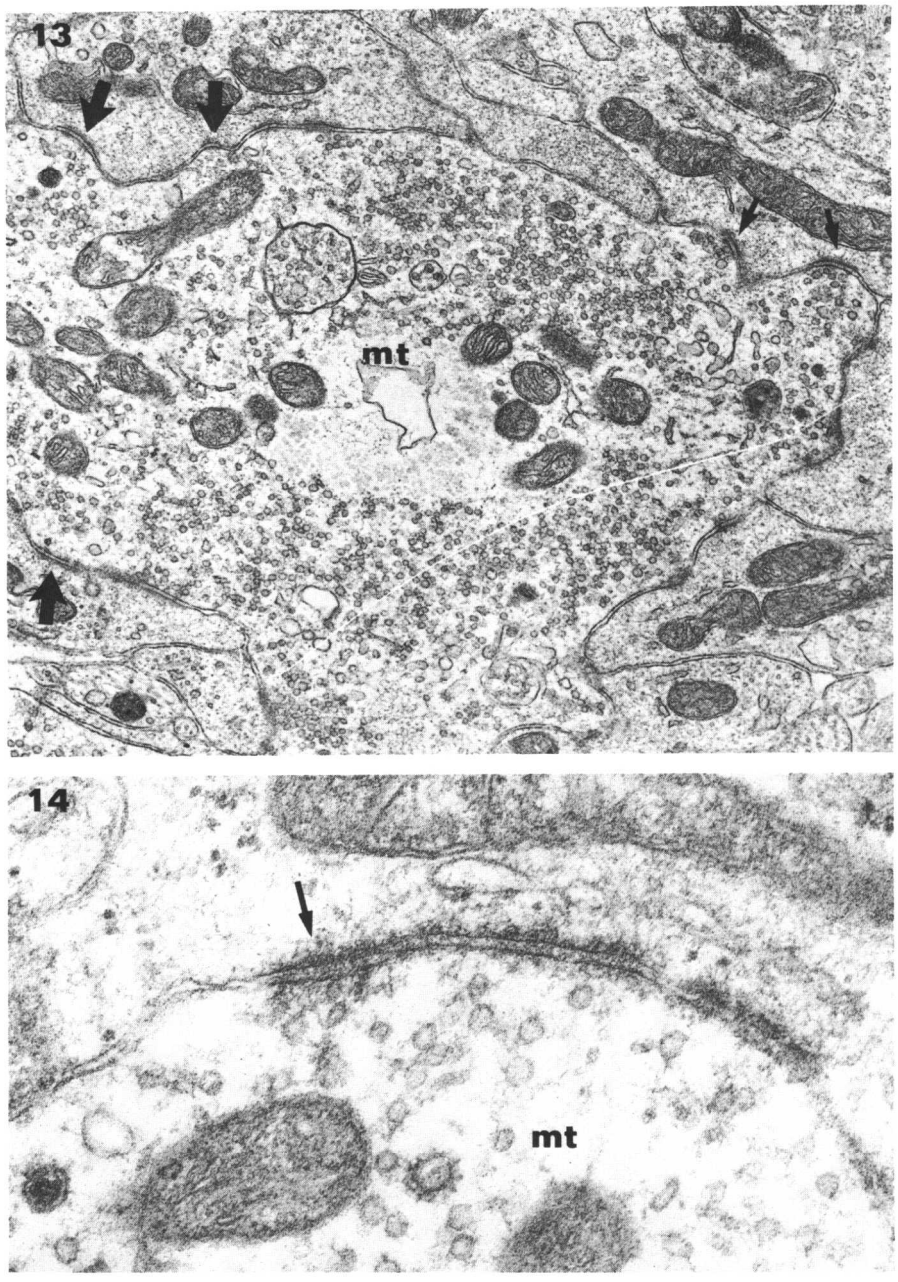
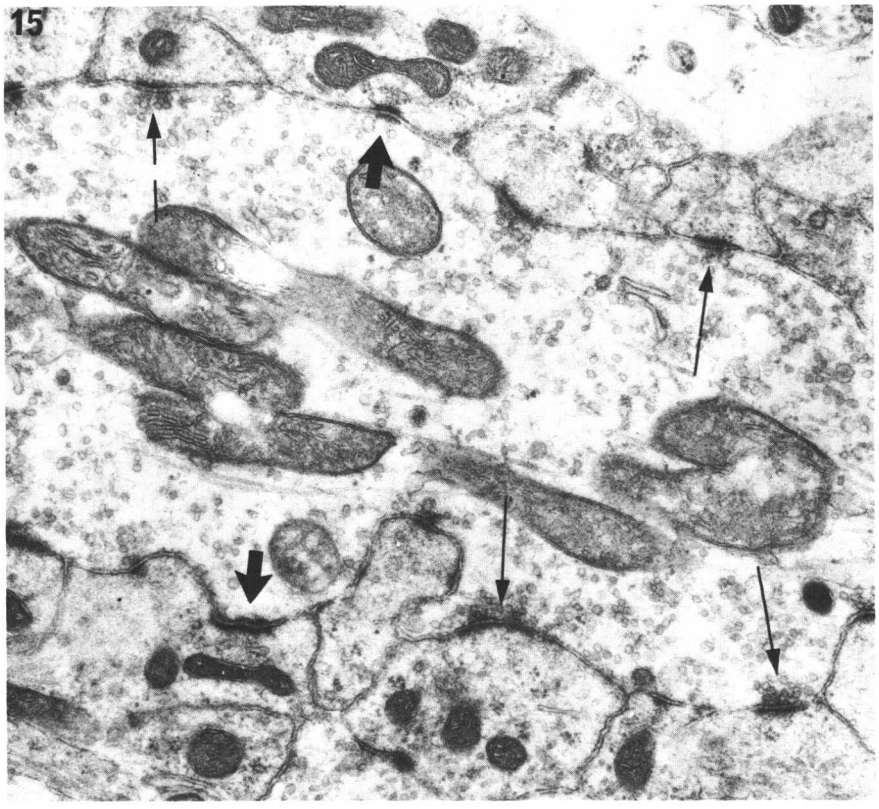


Figure 1-13. Maturing cerebellar glomerulus from 14 dpn rat cerebellar cortex. A few "mature" synaptic contacts (arrows) can be detected with simultaneous appearance of immature or transient type of synaptic contacts (thick arrow) (×24,360.)

Figure 1-14. Mossy fiber (mt) to granule cell dendrite synapse from 14 dpn rat cerebellum: the larger portion of the contact is of immature type lacking synaptic vesicle accumulation at the presynaptic membrane. Only small area (arrow) shows indication of mature synaptic contact (×84,390).



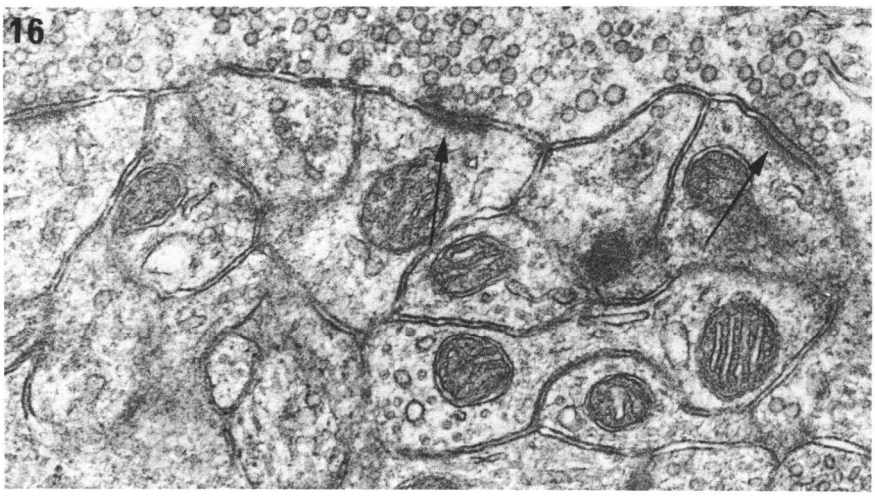


Figure 1-15. Part of a cerebellar glomerulus from 18 dpn rat cerebellum. Arrows point to mature synaptic junctions; thick arrows, to immature and desmosome-like contacts (×28,710).

Figure 1-16. Synaptic contacts (arrows) from 30 dpn rat cerebellum are exclusively "mature" by morphological criteria (×49,590).