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Cochlear Receptor Development in the Rat with Emphasis on Synaptogenesis*

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Summary. Maturation of the albino rat cochlea was studied using light and electron microscopy. Critical stages of receptor morphology were examined. At birth, cochlear structures are very immature, but even at this early stage synapes are recognizable. Under inner hair cells (IHCs) both afferent and efferent synapses are present. Under outer hair cells (OHCs) only afferent endings are seen. During the first postnatal week, synaptic development proceeds slowly. Between 6 to 12 days of age, substantial changes occur in the pattern of hair cell innervation. There are fewer efferent synapses at the IHC level and the first efferent junctions form on OHCs. In addition, a pattern of temporary innervation is seen under the OHC, with axo-dendritic synapses between efferent endings and afferent fibres. Between 12 and 16 days of age the main changes in hair cell innervation are at OHC level where afferent junctions regress and large efferent synapses form. By 16 days of age sensory-neural relationships seem adult-like. The results are discussed in relation to rat cochlear electrophysiological development and the period of supra-normal sensitivity to acoustic trauma.

Key words: Synaptogenesis – Rat cochlea – Development.

Introduction

The development of the rat cochlea has been studied by light microscopy (LM) (Wada 1923; Belanger 1956). We were interested in supplementing these findings with LM and also in extending them using the electron microscope (EM). The latter has been especially useful in helping to characterize organ of Corti synaptogenesis in the cat (Pujol et al. 1978, 1979). Apart from providing fundamental information on synaptic development in the rat organ of Corti, it was of interest to see if postnatal changes in synaptic organization were related

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to rapid ontogeny of auditory function in pre-weanling rats (Crowley and Hepp-Reymond 1966; Carlier et al. 1979; Pujol et al. 1980; Uziel et al. 1980) and to the peculiar susceptibility of such young rats to cochlear trauma (Lenoir et al. 1979; Lenoir and Pujol 1980).

Material and Methods

Fifteen Sprague Dawley albino rats ranging in age from a few hours after birth to 25 days were used. Under Pentothal anaesthesia, both cochleae were quickly removed and perfused with a 1% aqueous solution of osmium tetroxide. The cochleae remained in the fixative for $1^1/_2$ h, were then dehydrated, and subsequently slowly embedded in Spurr resin. Semi-thin transverse sections, made at various levels of the cochlea, were examined with a Reichert Zetopan microscope using Nomarski interference contrast optics. Thin sections from the basal, median and apical cochlear coils, mounted on formvar coated grids and counterstained with uranyl acetate and lead citrate, were examined with a Phillips 400 or a Jeol 100C EM.

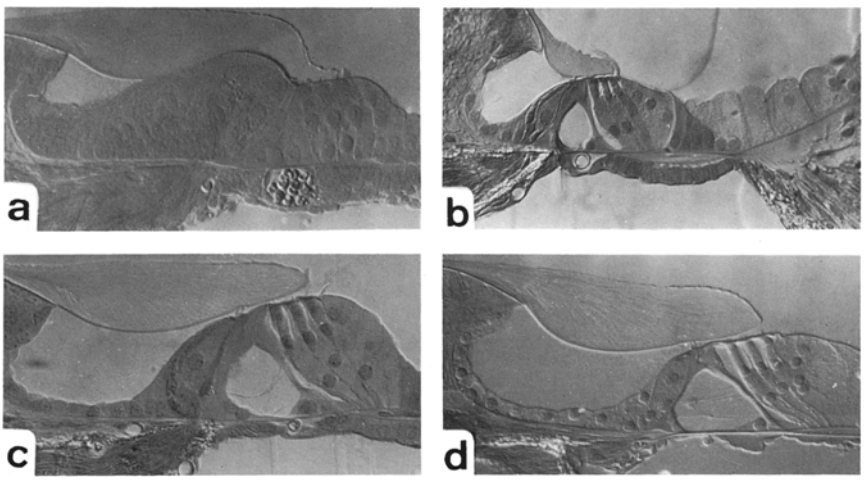


Fig. 1a–c. Four significant stages of the gross morphological development of rat organ of Corti. Nomarski optic. ×210 (a, b, d); ×320 (c). **a** Day 6 (median coil). Beneath the still fused pillar cells the large spiral vessel can be seen. **b** Day 12 (extreme basal coil). Note the internal spiral sulcus, the tunnel of Corti, the spaces of Nuel. **c** Day 12 (median coil). In contrast with (b), the tectorial membrane is no longer attached to the reticular lamina. **d** Day 16 (median coil). Mature organ of Corti. The spiral vessel is completely collapsed

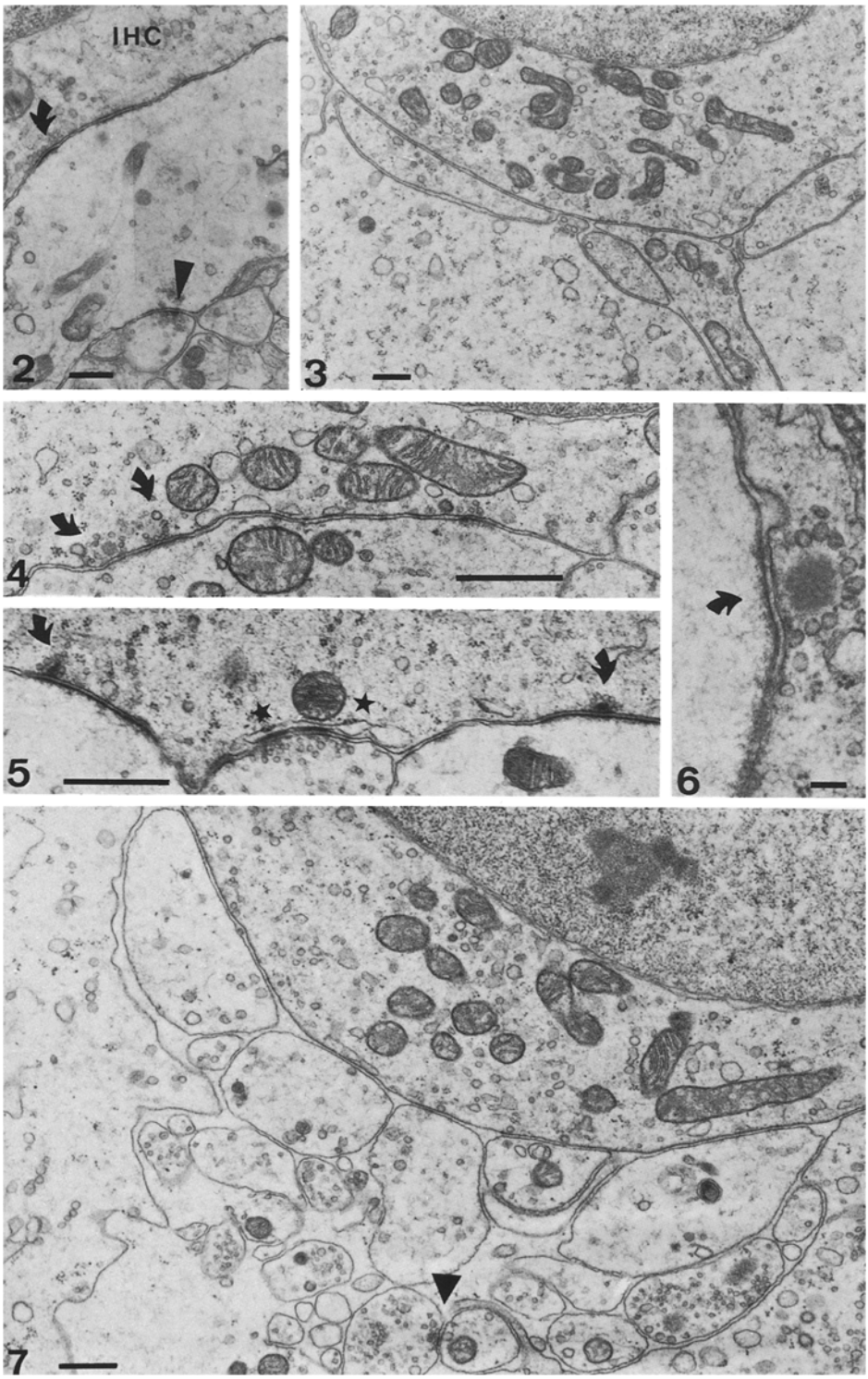
Figs. 2–7. Early stages of rat cochlear synaptogenesis. Scale: 0.5 μm except Fig. 6: 0.1 μm

Fig. 2 (Day 1). Base of an IHC connected with a large afferent dendrite: synaptic vesicles are seen in the hair cell (*arrow*). Note an axo-dendritic efferent synapse (*arrowhead*)

Fig. 3 (Day 1). Base of an OHC connected only with afferent dendrites

Figs. 4–6 (Day 6). Presynaptic bodies (*arrow*) surrounded by microvesicles are frequent at IHC-afferent synapses. In Fig. 5 a direct efferent-IHC synapse is seen (stars indicate the post-synaptic cistern)

Fig. 7 (Day 6). Base of an OHC still completely surrounded by afferents. The first efferent endings (filled with microvesicles) are seen to arrive at this level. An axo-dendritic efferent synapse can be seen (*arrowhead*)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Results

I. Gross Morphological Development

Birth. Cochlear structures were very immature. The hair cells were difficult to distinguish from surrounding epithelial cells, which were overlaid by a short tectorial membrane (TM).

Day 6 (Fig. 1a). Little morphological change had occurred. The hair cells were more readily distinguished and thick pillar cells could be seen. The internal spiral sulcus (ISS) began to take shape only under the proximal part of the TM. Several rows of epithelial cells, and a large spiral vessel, were seen under the basilar membrane (BM).

Day 9. The organ of Corti was now clearly recognizable in the basilar coil of the cochlea. Here the IHC had a mature pear-like shape and the space of Nuel began to appear. The tunnel of Corti was now open. Only in the basal coil of the cochlea was the ISS fully formed and bordered by a single layer of polygonal epithelial cells. At the apex many small Kölliker's cells were still seen in the ISS.

Day 12. Figure 1b and c show that the space of Nuel was now well formed, and that the TM had an adult length covering the three rows of OHC. Only at the extreme basal coil of the BM (Fig. 1b) was the TM still "hooked" to the top of the reticular lamina.

Day 16. Throughout the cochlea the organ of Corti had an adult-like gross morphology (Fig. 1d). The OHC and Hensen's cells were considerably longer, while the pillar cells and BM were thinner than at Day 12. Only in the basal

Figs. 8–15. Late stages of cochlear synaptogenesis. Scale: 0.5 μm

Fig. 8 (Day 6). Signs of synaptic competition below an OHC. An efferent (*e*) appears to be "growing" between afferents and the base of the hair cell. Fragments of subsynaptic cisterns (*stars*) are seen in the OHC

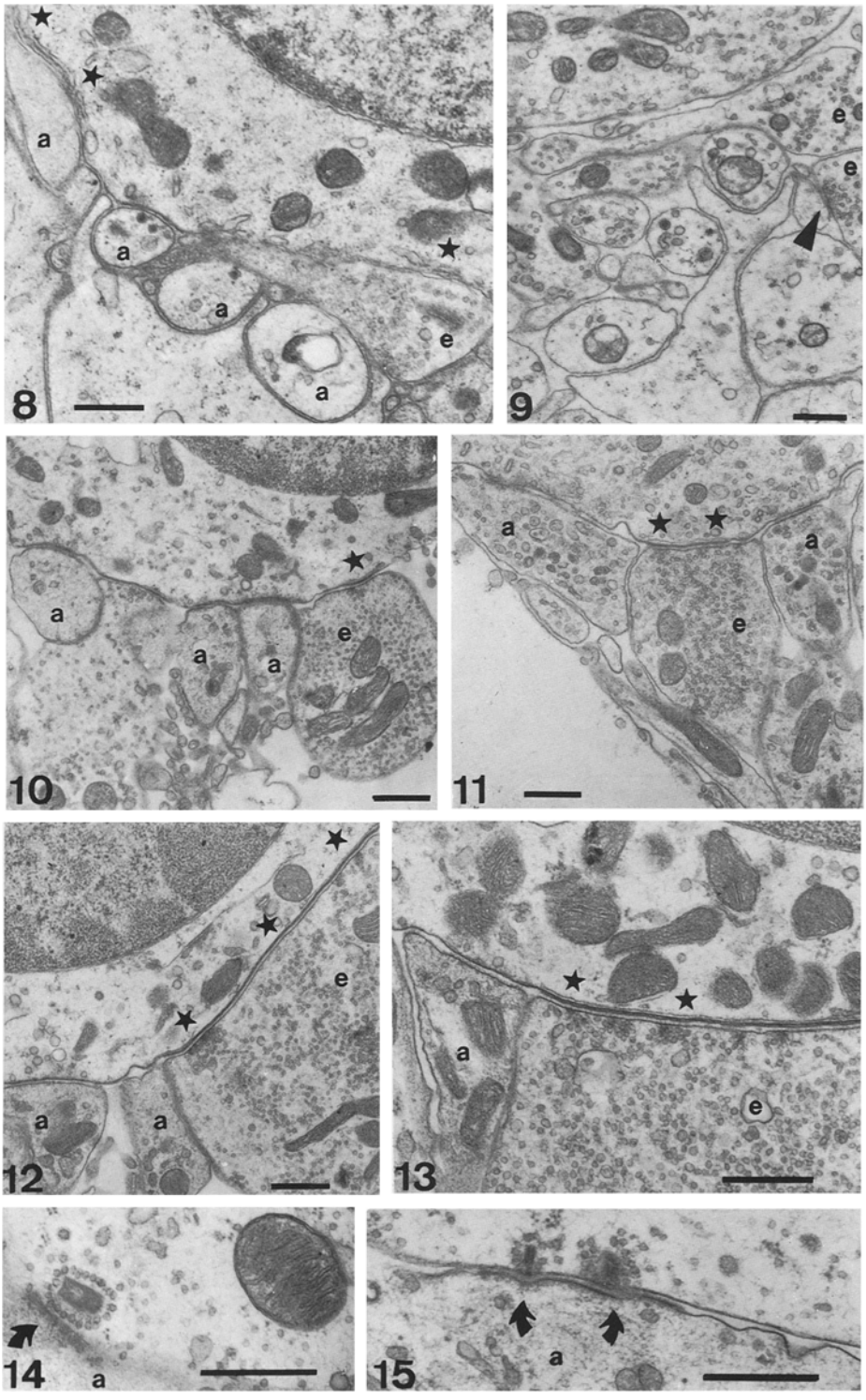
Fig. 9 (Day 9). Efferents (*e*) beneath an OHC become more numerous. An axo-dendritic efferent synapse is still noted (*arrowhead*)

Fig. 10 (Day 11). Base of an OHC connected by 3 afferents (*a*) and one efferent, the latter (*e*) forming a short neuroepithelial synapse (*star*)

Fig. 11 (Day 12). Base of an OHC. Note the long postsynaptic cistern (*stars*) opposite the efferent ending (*e*)

Figs. 12 and 13 (Day 16). Base of OHC with large efferent synapses. Note the length of the post-synaptic cistern (*stars*) and the arrangement (packets) of microvesicles within the efferent ending. No presynaptic specializations are seen opposite the few remaining afferent dendrites (*a*)

Figs. 14 and 15 (Day 25). The IHC-afferent synapses are still characterized by pre-synaptic bodies (*arrows*)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coil of the cochlea was it still possible to see a remnant of the fibrous process that once attached the TM to the top of the reticular lamina. The spiral vessel of the BM, underneath the tunnel of Corti, had collapsed.

After 16 days of age the only changes noted were small increases in the length of OHC and Hensen's cells.

II. Synaptogenesis

The following presents a summary of representative ultrastructural findings based on 30 cochleae. At each age synaptic development in the basal coil was approximately 2 days in advance of that seen in the apical coil. No substantial differences in the pattern of development were noted along the cochlear spiral. Unless otherwise noted, the subsequent description refers to the basal coil.

Birth. Even at the apical part of the cochlea, nerve fibres were seen under the immature IHCs and OHCs. At the level of the IHC, numerous afferent and efferent nerve terminals were present. Afferent synapses appeared to be well developed since pre-synaptic vesicles and post-synaptic thickenings were seen (Fig. 2). While vesicles in some terminals attested to the presence of efferent fibres, only axodendritic efferent-afferent synapses were well developed (Fig. 2). At the level of the OHC there were only afferent dendrites (Fig. 3). Pre-synaptic specializations were noted only in a few OHC of the apical coil. Elsewhere, the afferent nature of the fibers was established by their spiral course between Deiter's cells.

Day 6. The number of endings on IHC had increased. Many pre-synaptic bodies indicated afferent synapses (Figs. 4, 5, 6). IHC-efferent synapses were well differentiated, with post-synaptic cisterns and presynaptic vesicles (Fig. 5). Figure 7 shows that the arrival of efferent fibers underneath OHC was accompanied by the formation of axodendritic synapses. Synaptic competition seemed to occur between afferents and efferents, since a portion of the efferent ending was often seen between the afferent dendrite and the OHC membrane (Fig. 8). Formation of neuro-epithelial efferent synapses was signalled by sub-synaptic cistern fragments in OHC (Fig. 8). In contrast, presynaptic OHC specializations were no longer seen.

Day 9. The number of efferent endings directly connected with IHC had diminished so that most of the innervation was afferent. The IHC still contained synaptic bodies. At the OHC level the synaptic competition, described above, and axodendritic junctions were still clearly seen (Fig. 9).

Days 10 to 12. No significant change was noted in the characteristics of IHC innervation. The first neuro-epithelial synapses between efferent fibers and OHC were seen (Figs. 10 and 11). By 12 days of age, the density of synaptic vesicles had increased, the junction and the post-synaptic cistern had lengthened (Fig. 11).

Day 16. Throughout the cochlea both types of hair cells appeared to have acquired an adult innervation pattern. The majority of efferent synapses at the IHC level were axodendritic. The base of each OHC was almost completely surrounded by one or two large efferent terminals. Packets of synaptic vesicles were arranged near the pre-synaptic membrane and the post-synaptic cistern now extended along the entire length of the OHC-efferent fiber interface (Figs. 12, 13). Very few afferent, contacts persisted on each OHC, and these made poorly-defined synapses, without pre-synaptic specialization.

Day 25. At this time it was still possible to see a few efferent endings on IHC. Synaptic bodies continued to be seen within IHC opposite afferents (Figs. 14, 15). At the OHC level, modification of vesicular arrangements suggests minor development of efferent synapses.

Discussion

The light microscopic findings reported here are in accord with the timing of rat cochlear maturation described by Belanger (1956) and Wada (1923). At birth, the rat cochlea is very immature and it develops rather slowly during the first postnatal week. After a phase of quick development, between 9 and 12 days of age, the gross morphology of the cochlea is adult-like at about day 16.

Also in agreement with previous data on the rat (Belanger 1956; Wada 1923) and with findings in other mammals (see Pujol and Hilding 1973) is the observation that cochlear maturation generally proceeds from base to apex. For example, at 9 days of age, the following structures appear more mature at the base than at the apex of the cochlea: the size of the ISS, tunnel of Corti, space of Nuel, and TM. However, with regard to TM "detachment", development in the most basilar part of the cochlea seems delayed. At 12 days of age, the TM is free from the cuticular plate in the whole cochlea except for the extreme base, where it remains hooked until about day 16. The precise functional significance of this observation is unclear at present. It may be worthwhile noting that the well-known role of TM in cochlear excitation may also include frequency selectivity ("tuning") (Steele 1973; Manley 1978; Zwislocki and Kletsky 1979). Thus, the maturational changes in TM attachment reported here may be related to the parallel ontogenetic increase in cochlear tuning (Carlier et al. 1979).

Our electron microscopy data agree with developmental findings in other mammals (Kikuchi and Hilding 1965; Pujol and Hilding 1973; Pujol and Abonnenc 1977; Pujol et al. 1978, 1979) that an adult-like configuration of synapses appears sooner at IHC than at OHC. Indeed, IHC synaptogenesis appears to be, at least qualitatively, almost completed at birth. In contrast, the postnatal development of OHC synapses is quite complex (see below) and is not mature until about 16 days of age. These data may also be related to the development of rat cochlear function. In adult mammals a number of experiments suggest that OHC are involved in the discriminative properties of the cochlea (see

Harrison and Evans 1977). Indeed, the late maturation at the OHC level reported here correlates well with the increase between 12 and 16 days of age of rat cochlear sensitivity (Crowley and Hepp-Reymond 1966; Carlier et al. 1979).

Within the context of cochlear structure-function relationships, a final comment can be made. The maximum effect of noise trauma on cochlear function in seen at about day 20 (Lenoir et al. 1979), a time when there are only very minor changes at the synaptic level. Thus, it seems unlikely that some aspect of rat organ of Corti synaptogenesis is related to the period of heightened cochlear susceptibility to acoustic trauma (Lenoir and Pujol 1980). Perhaps the basis for the "critical period" lies in a metabolic lability of the cochlea coincident with the drastic change in its blood supply, due to the collapse of the basilar membrane spiral vessel, during the third postnatal week (see also Wada 1923; Belanger 1956).

The data prompt some fundamental remarks regarding synaptogenesis at the OHC level. The immature OHC is initially connected only with afferents, some of which are temporarily characterized by pre-synaptic hair cell specializations. This confirms findings in the cat foetus (Pujol et al. 1979). The evolution of OHC innervation is then marked by the disappearance of pre-synaptic specializations and the formation of axo-dendritic synapses between the arriving efferent fibres and the afferent dendrites. As in the cat (Pujol et al. 1979), these synapses are temproary. The efferent fibres then appear to compete with the afferent processes, already well in place, for synaptic sites on OHC membrane. Subsequently, efferent fibres synapse directly on OHC. Finall one or two large efferent endings synapse with almost the entire base of the OHC. It is of interest that post-synaptic cisterns first begin to be seen within OHCs while these are still

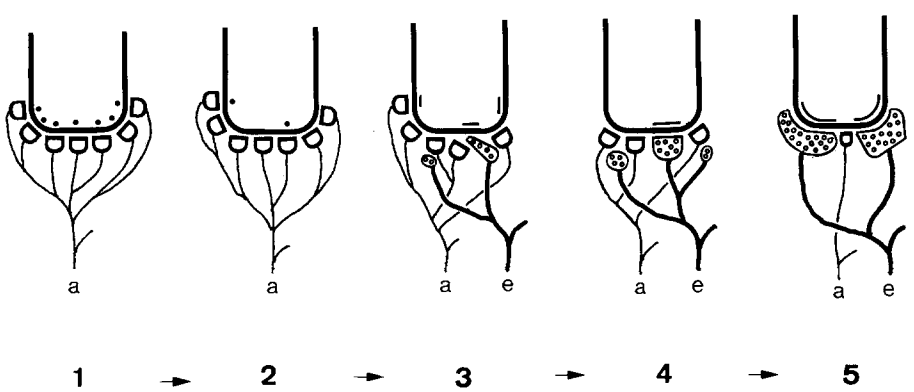

Fig. 16. Schematic representation of synaptogenesis at the OHC level in the rat. *Stage 1:* Before birth (predicted on the basis of data from the cat foetus; see Pujol et al. 1979). Innervation is exclusively afferent (*a*), the hair cell contains numerous presynaptic bodies (●). *Stage 2:* At birth, innervation is still entirely afferent, but presynaptic specializations are now rare. *Stage 3:* By day 6, efferent endings (*e*) make their first contact with the cell and with afferent processes. Cisterns (—) are sometimes seen within the hair cell before contact is achieved. *Stage 4:* By 12 days of age, the number of afferent endings has greatly diminished. Efferents make well-defined synapses on OHC. *Stage 5:* An adult-like configuration is seen by about 16 days post-partum. One or two efferent endings filled with microvesicles (○) make large, cup-shaped, contact with the OHC. Within the hair cell the cistern extends the full length of the contact zone

primarily innervated by afferents. This suggests that the OHC itselfs may play a role in the diminution of afferent synapses and facilitate their replacement by the burgeoning efferents. The disappearing afferent endings to a given OHC may be branches of a single fiber or a number of spiral fibers (see Spoendlin 1975). A Golgi study on young rats (Perkins and Morest 1975) suggests a diminution in the number of dendritic collaterals from a single ganglion cell. Figure 16 schematically summarizes the evolution of synapses on the rat OHC.

The last point relates to the remaining afferent synapses on each OHC in the mature rat organ of Corti. Such synapses are distinctly different from afferent synapses on IHC. First, OHC-afferent synapses are very few in number. Second, no synaptic body is seen within the OHC opposite the afferent dendrite. The presence of these particular synapses support the possibility of two afferent systems within the cochlea in addition to the two distinct efferent systems to OHC and IHC documented above and already described in the cat (Warr 1975; Pujol et al. 1978; Warr and Guinan 1979).

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