Research paper

Postnatal development of a large auditory nerve terminal: The endbulb of Held in cats

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Abstract

The endbulbs of Held are formed by the ascending branches of myelinated auditory nerve fibers and represent one of the largest synaptic endings in the brain. Most of the developmental changes in structure occur during the first 30 postnatal days of age. The neonatal endbulb begins as a flattened expansion with many filopodia, resembling a growth cone and characterized by numerous puncta adherentia and synapses associated with small postsynaptic densities; the most impressive feature of the ending at this age is its highly irregular plasma membrane that interdigitates with that of the postsynaptic spherical bushy cell. During these first 30 days, the number of puncta adherentia diminishes, postsynaptic densities nearly double in size, intermembraneous cisternae emerge, and plasma membranes flatten. These features endow the endbulb with an adult-like appearance. On the other hand, synaptic vesicle density increases progressively from approximately 50/μm² at birth to 100/μm² at adulthood. Mitochondria size remains constant over this developmental period but mitochondrial volume fraction increases until 60 days postnatal. Although many features of endbulb morphology stabilize by 30 days, other features suggest that endbulb development continues into the third month of age. Many of these observations correlate with the maturation of physiological response properties and suggest issues for further study.

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1. Introduction

Auditory nerve fibers give rise to as many as 100 different synaptic endings that vary widely in terms of size and postsynaptic targets (Held, 1893; Ramón y Cajal, 1909; Lorente de Nó, 1933; Brawer and Morest, 1975; Fekete et al., 1984). These endings range from small boutons (1–3 μm in diameter) to large, highly branched endbulbs of Held (15–30 μm in diameter). Light microscopic examination demonstrated that endbulbs in cats pass through a postnatal developmental process that commenced in the form of a solid, spoon-shaped growth cone and culminated in a highly branched, axosomatic arborization (Ryugo and Fekete, 1982); this sequence resembles what has been observed for chicks (Jhaveri and Morest, 1982a) and mice (Limb and Ryugo, 2000). A number of sequelae arise from this maturational process. One consequence is the generation of a structural substrate for synaptic transmission (Brugge et al., 1978, 1981; Kettenr et al., 1985; Walsh and McGee, 1987). This sequence resembles what has been observed for chicks (Jhaveri and Morest, 1982a) and mice (Limb and Ryugo, 2000). A number of sequelae arise from this maturational process. One consequence is the generation of a structural substrate for synaptic transmission (Brugge et al., 1978, 1981; Kettenr et al., 1985; Walsh and McGee, 1987). Another is enabling endings from other sources to admix with those of the auditory nerve along the surface of the postsynaptic cell body (Ibata and Pappas, 1976; Cant and Morest, 1979). A third is that the number of endbulbs contacting a spherical...
bushy cell decreases while the number of non-primary endings rises. This latter process implies that the endbulb must advance, split into multiple branches, and/or retract. Bidirectional communication must occur between the pre- and postsynaptic elements where molecular signals are presumably involved (Suter and Forscher, 2000). These dramatic changes prompted us to examine endbulb development with higher resolution. In the present study, we examined the ultrastructure of endbulbs in an age-graded series of kittens in an attempt to discover clues to structures that might foster these changes. We also sought to correlate specific anatomical features to the onset of physiological properties in order to better understand developmental dynamics.

2. Materials and methods

2.1. Subjects

Structural data were collected from an age-graded series of pigmented cats that ranged in postnatal age (PN) from newborn (PN-0) to adult. Twenty-seven cats were used for the present study, with 2 at each of the following postnatal (PN) ages: 0, 2, 5, 10, 20, 30, 60, 90, 120, 150, and 180 days. These subjects were selected for study because they exhibited the best ultrastructural appearance (e.g., no staining artifacts, no holes in the tissue, and good membrane preservation) when viewed with an electron microscope. Five cats older than 1.5 years were used because of the variability in quantitative values. All cats were pigmented with normal hearing, with the exception of one white kitten at PN-20. Standard auditory brainstem responses (ABRs) were collected from kittens at PN-20 and with normal hearing. All cats were pigmented with normal hearing status in cats, based on ABR thresholds and quantitative values. All cats were pigmented with normal hearing. The normality of kittens was inferred by the normal histologic appearance of their cochleae when viewed with a light microscope. We have previously presented data suggesting that hearing status in cats, based on ABR thresholds and cochlear histology, does not change appreciably with age (Ryugo et al., 1996, 1997).

2.2. Histological preparation

Animals were administered an anesthetic dose of sodium pentobarbital (45 mg/kg, IV) and when areflexic to corneal stimulation, were perfused through the heart with 25 cc of phosphate buffered isotonie saline with 0.5% NaNO₃ (pH 7.4) followed immediately by a phosphate buffered solution of 2% paraformaldehyde and 2% glutaraldehyde. Following perfusion, subjects were decapitated and the head immersed in the same fixative (5°C) with just enough bone removed to expose the brain stem and cochlear nuclei to fixative. The next day, the brains were dissected from the skull, and the cochlear nuclei dissected and embedded in a gelatin-albumin mixture hardened with glutaraldehyde. The tissue block was sectioned on the coronal plane (50–75 μm thickness) on a Vibratome. Sections were collected in serial order and separated into two series: one for light microscopy and one for electron microscopy. Those sections for electron microscopic analysis were placed in 1% OsO₄ for 15 min, rinsed in buffer, bloc stained in 1% uranyl acetate, rinsed, dehydrated in an ascending concentration of alcohols, and flat embedded in PolyBed 812 between two sheets of Aclar. After polymerization, pieces of the rostral anteroventral cochlear nucleus (AVCN) were cut out and re-embedded in BEEM capsules. Ultrathin sections were collected on Formvar-coated slotted grids and examined in an electron microscope.

2.3. Data analysis

Endbulbs of Held make contact with spherical bushy cells and are distributed in the rostral pole of the AVCN. Endbulb identification was based on previously published criteria showing their distinct properties at the ultrastructural level (Lenn and Reese, 1966; Gentschev and Sotelo, 1973; Cant and Morest, 1979; Ryugo et al., 1996, 1997). Because endbulbs are so large, only smaller pieces can ever be observed in any single ultrathin section; as a result, we refer to cut sections through an endbulb as a “profile”. Approximately 10 endbulbs from different cells were randomly selected from each animal for electron microscopic analysis, with more endings analyzed in adults, resulting in a total of 272 ending profiles used for morphometric analysis. The perimeter of each endbulb profile was traced; the perimeter of each mitochondrion within an endbulb profile was determined by outlining the outer edge of each organelle. Double membranous structures within an endbulb profile that exhibited no organelles were classified as inclusions. Areas were calculated within the perimeters for endbulbs (minus the values of inclusions), mitochondria, and inclusions using the Photoshop plugin.

Counts of subcellular structures were made for each endbulb profile. Postsynaptic density (PSD) and puncta adherentia length was traced along the outer membrane of endbulb profiles. Puncta adherentia were characterized by small, symmetric membrane thickenings. In contrast, PSDs were characterized by distinctly asymmetric membrane thickenings with the postsynaptic thickening more prominent, and the presence of at least one synaptic vesicle within a vesicle’s diameter of the PSD.

Synaptic vesicle density was determined by counting the number of vesicles in a 0.5-μm radius of the PSD. To determine this area within the endbulb, the PSD was traced using a 1-μm diameter pencil in Adobe Photoshop (Fig. 1) whose center was run along the PSD; mitochondria and inclusions were subtracted to yield cytoplasmic area. Synaptic vesicle density was calculated by dividing the vesicle count by the area of the 0.5-μm PSD radius. By restricting...
the vesicle counts to the area immediately surrounding the PSD, regions of axoplasm (which lack vesicles) were eliminated and counts focused on the readily releasable pools of vesicles. If PSDs were within 0.5 μm of each other, they were considered a single structure for the purpose of determining vesicle density so that no vesicle was counted twice.

Endbulb apposition was defined as the profile membrane adherent to the spherical bushy cell membrane; it was traced by drawing along the membrane’s outer leaflet. Apposition length complexity was determined by dividing the length of the endbulb-to-cell apposition by the straight-line length between the two ends of the endbulb-cell apposition. This ratio was called the form factor. A ratio with a low value indicated a complex membrane apposition, whereas a value approaching 1.0 signified a relatively simple apposition.

Cisternae were defined as separations between the endbulb-cell apposition that measured at least 50 nm in diameter in width and at least 100 nm in length. The endbulb–cisternae interface (cisternae length) was not included in the length measurement of apposition. Cisternal area and count per profile ending were also recorded. All measurements were made with the Image Processing Toolkit (Reindeer Graphics) for Photoshop.

3. Results

The most salient feature of the cellular architecture in the neonatal AVCN was that the facing membranes of every endbulb and spherical bushy cell were thrown into irregular ruffles and pleats whenever they touched (Fig. 2). In contrast, the membrane away from such contact was relatively smooth. Endbulbs of Held at this age exhibited a spoon-shaped appearance with filopodia as viewed with a light microscope (see inset drawings in Fig. 3). When viewed in section with the electron microscope, the endbulbs had a “caterpillar-like” appearance where the interdigitations resembled feet with furry soles (Figs. 3 and 4). Aside from these endbulbs, the spherical bushy cell had virtually no other inputs on its cell body.

3.1. Ages 0–10 days

The finger-like projections thrown up by the cell body were characteristically devoid of organelles (Figs. 3 and 4). This irregularity of the membranes was quantified by measuring the length of the undulating membranes and making a ratio with the straight-line segment connecting across the apposing membranes (Fig. 1B). A small ratio value indicated significant membrane irregularities; as the membrane ruffles diminished, the ratio approached unity. During the first 10 postnatal days, this ratio and the fine structure of the endbulb remained relatively constant. The endbulb profile was large (Fig. 5A), the apposition length relatively long (Fig. 5B), and the form factor value was small (Fig. 5C). In three dimensions, these irregularities materialized as interlocking digits, approximately 1–2 μm in length and less than a micrometer in thickness. They were irregular in...
shape and tended to branch. Pieces of these somatic evaginations were isolated in cuts through the endbulb and appeared as islands of membrane-bound cytoplasm (Figs. 3 and 4). Counts of these inclusions correlated with the irregularity of the facing surfaces (Fig. 5D).

There were numerous membrane thickenings in these young endbulbs (Figs. 3 and 4). Puncta adherentia (also known as attachment plaques) were characterized as short, symmetrical membrane thickenings that were not associated with synaptic vesicles. Filamentous strands project into the cytoplasm of the endbulb, connecting the dense membrane material to mitochondria or tubular structures, or fading in the cytoplasm. They were commonly found in close proximity to PSDs, and sometimes these structures seemed to merge together. There were on average 3–5 puncta adherentia per endbulb profile (Fig. 6A) and an additional 10 asymmetric membrane thickenings (Fig. 6B) that were marked by the presence of round synaptic vesicles in the endbulb compartment.

There was a pronounced membrane thickening on the somatic side that we referred to as the PSD, averaging 0.20–0.23 μm in length (Fig. 6C). The bushy appearance of these membrane thickenings along the endbulb “feet” was highly characteristic of the developing endbulb. The membrane thickenings tend to form along the tips of the endbulb protrusions into the cell body, but rarely form on the tips of the somatic appendages that penetrate the endbulb (Figs. 3 and 4). During this postnatal epoch, mitochondria size (0.064 ± 0.04 μm³, Fig. 7A), mitochondria volume fraction (7–10%, Fig. 7B), and synaptic vesicle density (roughly 50 synaptic vesicles per μm³, Fig. 7C) remained relatively constant. The intermembranous cisternae, appearing as small bubbles between the pre- and postsynaptic membranes in single sections, characterize adult endbulbs but have not yet emerged in the younger animals (Fig. 7D).

3.2. Ages 20 days and older

Noteworthy changes in endbulb structure were apparent starting at 20 days postnatal (Fig. 4). Endbulbs stained by the Golgi method were no longer club-like in appearance but were transforming into a tree-like structure (Ryugo and Fekete, 1982). Fissures and fenestrations appeared in the broad swelling and accounted for the obvious quantitative changes. The transformation was reflected in an average reduction in profile size (Fig. 5A) and apposition length (Fig. 5B). There was an associated reduction in the number of puncta adherentia and PSDs per profile (Fig. 6A and B). The membrane apposition between the endbulb and spherical bushy cell was smoother (Figs. 4 and 5C) and marked by a near absence of somatic inclusions within the endbulb itself (Figs. 4 and 5D).

Although the branching of the endbulb continued to grow more complex as revealed by light microscopic examination (Figs. 8 and 9, insets), many of the fine structural details tended to stabilize (Figs. 8 and 9). Endbulb profile size (4.76 ± 2.5 μm²), PSD number (3.55 ± 1.9), and PSD diameter (0.34 ± 0.12 μm) have remained constant, and mitochondria size (0.058 ± 0.03 μm³) has not changed since birth. The postsynaptic density has assumed its usual place on the inner surface of the somatic convexity (Fig. 9). Inter-
Membraneous cisternae emerged between postnatal days 20–30 and became a regular feature of the endbulb profiles (Figs. 7D, 8 and 9). In three dimensions, reconstructed cisternae form a system of canals that lie between the pre- and postsynaptic membranes. Mitochondria volume fraction showed a steady growth until the kittens reach 60 days of age (Fig. 7B). The density of synaptic vesicles continued its slow and steady increase to reach approximately 100 vesicles per μm² in adults (Fig. 7C).

3.3. Myelin formation

There was no myelin surrounding axons in the cochlear nucleus of the newborn cat (Figs. 2 and 3). We examined 63 axons from newborn kittens and 61 axons had no myelin. Two axons exhibited loose lamellae that numbered 6 and 7, respectively. At two days postnatal, 77 axons were examined for which 75 had no myelin. Two axons had 3 and 5 lamellae. At five days postnatal, there was beginning signs.
of myelination. Of 48 axons examined, nine had 2–11 lamellae (mean 7.8 ± 4.2). Twenty-seven axons were examined in the 10-day-old kitten, and 19 exhibited some degree of myelination. The number of lamellae averaged 6.7 ± 3.4. The percentage of fibers exhibiting myelination increased with age as did the number of lamellae. The number of lamellae was as follows: PN-20, 12.5 ± 3.2; PN-30, 17 ± 4.7; PN-60, 15.7 ± 7.8. In older animals, the vast majority of axons were myelinated but the myelin was so compact that reliable counts could not be made.

Astrocytic lamellae were also developing around the endbulb. During the first two weeks, endbulbs of postnatal kittens exhibited intermittent covering by glial processes. Sometimes they were organized into thin sheets (0.1 μm in thickness, Fig. 3, bottom). Other times, there was incomplete coverage and the back of the endbulb was exposed to extracellular space (Fig. 4, right panel). This pattern continued for several months until the glial processes became thinner and layered themselves over the endbulbs (Figs. 8 and 9).
3.4. Synapse growth

During the first postnatal week PSDs were plentiful with much variability in length (Figs. 3, 4 and 6C). Some release sites were evident as short PSDs with one or several associated synaptic vesicles, whereas others were considerably longer (Fig. 3, inset). Puncta adherentia were often found in association with a PSD and synaptic vesicles. As the animal aged to 30 days postnatal, there was a progressive restriction in the range of lengths (Fig. 6C). As the large club-like endbulb differentiated into thinner branches and smaller swellings, ending profiles were naturally reduced in size and the number of PSDs per profile also diminished (Fig. 6B and C). The PSDs appeared as punctate membrane thickenings marking the somatic convexities (Fig. 10). Synaptic vesicle density in the immediate vicinity (0.5 μm radius) of the PSD grew from approximately 55 per μm² in newborn kittens to just above 75 per μm² in 180-day-old cats. There was a continued increase in synaptic vesicle density to more than 100 per μm² in cats greater than a year in age (Fig. 7C).
4. Discussion

Endbulbs of Held have been the subject of extensive study because their large size identifies them as a unique entity with specializations that could contribute to our understanding of synaptic transmission and stimulus coding (Lorente de Nó, 1933; Gentschev and Sotelo, 1973; Brauer and Morest, 1975; Ibata and Pappas, 1976; Ryugo and Fekete, 1982; Sento and Ryugo, 1989; Ryugo and Sento, 1991; Wang et al., 1998; Limb and Ryugo, 2000; Nicol and Walmsley, 2002; Trussell, 2002; Lee et al., 2003; Wang and Manis, 2005). The endbulb also is hypothesized to have a role in the circuitry that mediates timing information inherent to auditory stimuli (Pfeiffer, 1966a,b; Molnar and Pfeiffer, 1968; Carr and Konishi, 1990; Smith et al., 1993; Köppl, 1994; Köppl and Carr, 1997). As an outgrowth of these issues, the structure of the endbulb has been studied to seek answers related to synapse formation, target specificity, and the effects of deafness (Gulley et al., 1977; Neises et al., 1982; Ryugo and Fekete, 1982; Carr and Boudreau, 1996; Limb and Ryugo, 2000; Ryugo et al., 1997, 1998; Oleskevich et al., 2004). The developmental studies reported

Fig. 6. Plots that quantify structural features of endbulb membranes related to age. The number of (A) puncta adherentia and (B) PSDs per endbulb profile diminishes as animals age. A prominent change occurs between 10 and 20 days postnatal. (C) The average length of PSDs grows during the first 40 postnatal days, when length reaches adult values. (D) The relative amount of surface area occupied by PSDs is illustrated. The PSDs show continuous growth to about 60 days postnatal and this growth is complemented by the progressive shrinkage of endbulb profile area as the endbulb branches into smaller elements. Consequently, the relative PSD surface contact increases until 60 days and then levels off.
a transformation of the flat, club-shaped ending with many filopodia into a highly branched, interconnected network of fibers and swellings that encapsulated up to half a postsynaptic cell body (Jhaveri and Morest, 1982a; Ryugo and Fekete, 1982; Carr and Boudreau, 1996; Limb and Ryugo, 2000). Our data describe the fine details of endbulb development in the cat and furnishes a possible model for studying mechanisms of developmental differentiation (Fig. 11). Moreover, our observations are consistent with the ultrastructural progression of postnatal synaptogenesis reported for the vertebrate central nervous system (Vaughn, 1989).

4.1. Structure–function correlations

The dramatic age-related transformation of the endbulb provided structural details that we thought might be correlated with the maturation of spike discharge properties between the auditory nerve and spherical bushy cells in the
anteroventral cochlear nucleus. The hypothesis was that developmental stages of the endbulb synapse could provide clues as to substrates for physiological benchmarks observed in the postsynaptic neuron. During the first postnatal week, physiological properties in the auditory nerve and cochlear nucleus were immature. Neural thresholds to acoustic stimuli were uniformly high (>100 dB SPL), tuning curves were shallow, spontaneous and sound-evoked spike discharges had low rates, and synchronous responses to low frequency tones (phase-locking) were limited to frequencies below 600 Hz (Brugge et al., 1978, 1981; Kettner et al., 1985; Walsh and McGee, 1987). These response properties corresponded to when the endbulb was club-shaped, its membranes were highly infolded, PSDs were small, synaptic vesicle density was low, and intermembrane cisternae were absent. The relatively longer latency to sound-evoked first spikes is undoubtedly due, at least in large part, to the lack of myelin on auditory nerve fibers.

During postnatal days 10–20, the spoon-shaped endbulb began to fenestrate and divide (Ryugo and Fekete, 1982), and the branching was reflected in the smaller profiles seen with electron microscopy. Membrane smoothing under the endbulb was almost adult-like and there was a 10% growth in PSD length. There was no change in synaptic vesicle density or cisternal counts but auditory nerve fibers continued myelination. The reported physiological properties of AVCN neurons were in step with the morphology. There was a continued growth in the maximal spike rate (an increase from roughly 100 s/s to 200 s/s), lowering of threshold at best frequency to 40–60 dB SPL, shortening of first spike latency to 6–7 ms, and synchronous responses to tone frequencies up to 2 kHz (Brugge et al., 1978, 1981; Kettner et al., 1985).

Fig. 8. Electron micrographs of endbulbs from 30-, 60-, 90- and 120-day-old kittens. As illustrated by the Golgi-stained endbulb of a 45-day-old kitten (middle left), the ending is branched and exhibits a mature look. The smaller endbulb components (yellow) are evident in the micrographs. There is a decrease of synapses and puncta adherentia in these older animals and the myelin sheaths have doubled in thickness. Intercellular cisternae (red asterisks) may serve as canals for transmitter diffusion. Scale bar equals 1 μm.
By 30 days postnatal, cisternal counts, PSD lengths and numbers, and membrane smoothing were nearly adult-like. The maturation of these features could represent the basic infrastructure that enables the rest of the system to progress. The membrane smoothing with the mature PSDs become paired with cisternae whose canal-like structure may act as “gutters” to facilitate transmitter inactivation. After 30 days postnatal and older, many structural and physiological features have stabilized. In contrast, there is a progressive growth in mitochondria volume fraction until 60 days and in synaptic vesicle density that continues into adulthood (beyond 12 months of age). These features accompany the progressive increase in average spontaneous and maximum driven discharge rates of auditory nerve.
fibers (Walsh and McGee, 1987; Romand, 1984), and may reflect the increased metabolic and transmission demands by these endings.

These developmental analyses provided a rough sketch for a timeline of structure–function correlations yet at the same time were unfulfilling. Developmental changes within the inner ear must influence the physiological properties in the central nervous system but it is not clear which ones. Furthermore, the time intervals between examining structural and physiological properties were too great. From a physiological perspective, the number of units sampled at any particular age was relatively small, and since the cochlear nucleus is not a homogeneous structure, it cannot be certain that data were collected from neurons of a single class. Our anatomical observations were pertinent primarily to those studies of AVCN units, specifically to those with prepotentials. Key structural changes in the cat seemed to occur between 10 and 20 days of age, and this epoch was consistent with some of the changes in physiological properties. Consequently, this age would be an

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Fig. 10. Electron micrographs illustrating the development of endbulb synapses. Membrane specializations become more regular in size and shape. Initially, the PSDs are often in close proximity to puncta adherentia (arrowheads) and the association of synaptic vesicles with membrane thickenings is variable. Over time, the somatic appendages withdraw and convex PSDs (*) form on what appear to be their remnants. Scale bar equals 0.5 μm.
appropriate focus for further anatomical and electrophysiological examination.

4.2. Membrane–membrane interactions

One of the most striking morphologic features observed during endbulb development was the interdigitation of endbulb and spherical bushy cell membranes. This relationship has been noted in previous reports of the developing endbulb (Jhaveri and Morest, 1982b; Neises et al., 1982; Carr and Boudreau, 1996). Clearly this specialization enhances surface contact between the two neural elements. What, then, does this specialization signify? Highly infolded membranes are found in the marginal cells of the stria vascularis, epithelial cells of the distal convoluted tubule in the kidneys, and epithelial cells of striated ducts associated with glands of the oral cavity. These infoldings occur in epithelial cells in which there is demand for efficient transmembrane transport of water and ions. For the endbulb, it seems that the dynamic process of synapse formation and stabilization must require bidirectional communication between the pre- and postsynaptic partners. It would be reasonable to hypothesize that the postsynaptic spherical bushy cell (SBC) implements localized release of some signaling molecule and that the endbulb possesses the complementary receptors. There are many potential candidate receptor molecules that could be investigated (Rubel and Fritzsch, 2002; Fritzsch et al., 2004; Lu, 2004). The other mystery is to determine the nature of the presynaptic signal to the SBC. The transmembrane exchanges could activate transcription factors, initiate second messenger cascades, or open channels, leading to the attraction, stabilization, and/or elimination of a particular synaptic ending.

The somatic appendages have been postulated to have a role in the guidance of the axon to a specific location on the surface of the neuron (Neises et al., 1982). Indeed, the filopodia of the endbulb and SBC resemble the contact sites of advancing axon growth cones and dendrites.
(Jontes et al., 2000; Lendvai et al., 2000). Along these membrane interdigitations, there is a conspicuous presence of membrane “adhesions” marked by accumulations of electron-dense material that represent puncta adherentia or immature synapses. These puncta adherentia exhibit polarization with respect to their appearance with the endbulb. They form within the somatic cytoplasm opposite the endbulb protrusions into the cell body and not in the endbulb cytoplasm opposite the somatic protrusions. It would seem that the endbulb is unable to manufacture its part of the junction.

Somatic and dendritic appendages, called filopodia and protospines, exhibit structural evidence of synapse formation with incoming axons (Vaughn, 1989; Dunn et al., 1998). Because these appendages represent transitory developmental structures, they are implicated in an interactive process of afferent targeting during synaptogenesis (Moreira et al., 2001). For example, the differentiation of cerebellar Purkinje cells coincides with the establishment of climbing fiber synapses on dendrites and basket cell synapses on cell bodies (Larramendi, 1969; Mugnaini, 1969). Likewise, differentiation of the spherical bushy cell coincides in time with the maturation of its afferent source, the endbulb of Held (Ryugo and Fekete, 1982).

Synaptic vesicles also accumulate on the endbulb side but apparently only in association with future synapses. Synapses (associated with vesicles) and puncta adherentia can be found lying side-by-side, forming a large area of membrane thickening. In the opposing membranes of spherical bushy cells, there is a marked incidence of “omega figures” that flank the membrane thickenings and represent the endocytotic phase of vesicle recycling. It seems that vesicle recycling is sparked by activity and is part of the sequence of events involved in synaptogenesis (Ahmari et al., 2000; Friedman et al., 2000). The prevalence of puncta adherentia disappears between the 10th and 20th postnatal day. Given the apparent dynamic interactions between endbulb and SBC during early development, the puncta adherentia are assumed to play a role in keeping them attached, as they are well known for their role in mechanical attachments between adjacent cells (Peters et al., 1991).

4.3. Endbulb pruning

Since endbulbs at the early ages (younger than PN-5) were broad, pan-shaped and lacking lobes, each large endbulb profile seen in the electron microscope was inferred to represent a separate ending. The basis for this proposal is that the shape of the club prevents it from being cut in such a way to yield two large pieces when examined under an electron microscope. Thus every endbulb profile is assumed to originate from a separate ending. This logic leads to the conclusion that a single SBC in the neonatal kitten may be contacted by as many as 6–10 endbulbs (Fig. 2). Since it has been inferred that 1–3 endbulbs contact a SBC in the adult cat (Lorente de Nó, 1981; Ryugo and Sento, 1991), there must be pruning of “extra” endbulbs. How individual endbulbs are directed to their final destination remains a mystery, but because each ganglion cell contacts a single inner hair cell (Spoendlin, 1973; Liberman, 1980, 1982b), there is a one-to-one relationship between receptor cell and SBC. The precision of this connection is remarkable and further study of this relationship could provide novel insights into pathfinding and targeting.

4.4. Nature versus nurture

Ingrowth of auditory nerve fibers to their near-proper place in the cochlear nucleus apparently occurs without the benefit of hearing (Snyder and Leake, 1997). There is, however, a refinement of these projections during postnatal development because frequency laminae are more compressed in adults (Leake et al., 2002). With respect to endbulb morphology, it also seems to proceed on its own without the benefit of fully developed spike activity. The basic form of the endbulb is complete by around the end of the first postnatal month, and this time corresponds with the emergence of near-adult patterns of spontaneous spike discharges and patterned activity to tonal frequency (Brugge et al., 1978, 1981; Kettner et al., 1985; Walsh et al., 1986; Walsh and McGee, 1987, 1988; Walsh and Romand, 1992). Both morphological and physiological properties continue to undergo subtle maturational changes as sound begins to help shape the final product. The necessity of auditory nerve activity for normal endbulb development gets support from studies that have shown significant pathologic manifestations of endbulb morphology in congenitally deaf animals where there is no auditory nerve activity (Ryugo et al., 1997, 1998; Limb and Ryugo, 2000; Lee et al., 2003), and “synaptic rescue” in congenitally deaf animals where auditory nerve activity is returned through electrical stimulation by cochlear implants (Ryugo et al., 2005).

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References


