Chapter 11
Modifying Dendritic Structure After Function

Yuan Wang and Edwin W. Rubel

Abstract Neurons develop highly specialized dendritic architecture for certain operations of information processing in the brain. In this chapter, we review the development and regulation of characterized patterns of dendritic morphology and arrangement of bipolar neurons in the auditory brainstem, an excellent example of highly specialized dendritic architecture for their function in temporal coding and coincidence detection. We describe dramatic dynamics of the dendrites in both developing and mature systems and discuss the role of afferent synaptic input in influencing both the size of dendritic tree and the pattern of dendritic arborizations. The unique dendritic structure of these neurons provides an advantageous model for further understanding of the specific roles of neurotransmission, calcium signaling, protein synthesis, and cytoskeletal regulation in this important form of brain dynamics. Importantly, these neurons are highly conserved structurally and functionally across vertebrates including humans, emphasizing stereotyped dendritic regulation that is evolutionarily conserved for fundamental information processing operations in the vertebrate brain.

Keywords Neuronal morphology • Dendritic regulation • Afferent influence • Auditory brainstem • Sound localization • Bipolar neurons • Development • Nucleus laminaris • Medial superior olive

Functional neural circuits require that neurons develop their dendritic architecture to maximize information processing specific to that circuit. For a distinct neuronal cell type, dendritic architecture is determined not only by dendritic morphology of individual neurons but also by the way in which dendrites of all neurons within a neural circuit are arranged as a population. The former underlies how information is

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received and integrated within individual neurons, while the latter enables effective communication between neurons of the same or different types within a local network. Specialized dendritic architectures usually form gradually during development and may remain flexible after they mature. Such structural dynamics is presumed to be important for changes in perception, cognition, learning, and memory, but may also result in neurological dysfunction and degeneration at any stage of the life cycle.

An excellent example of highly specialized dendritic architecture with dramatic dynamics in both developing and mature systems is observed in bipolar neurons of the auditory brainstem. These neurons are located in the nucleus laminaris (NL) of reptiles and birds and in the medial superior olive (MSO) of mammals (Fig. 11.1). Across vertebrate species, these bipolar neurons are highly comparable in their dendritic morphology and arrangement, afferent and efferent connectivity, biochemistry particularly specialized levels of expression of a number of functionally specialized proteins, as well as physiology. They act as coincidence detectors for processing temporal properties of the incoming signals from the two ears and are exquisitely sensitive to interaural time differences (ITDs), the time disparities in the arrival of low-frequency information between the two ears. This ITD sensitivity is believed to be essential for normal sound localization in low-frequency hearing species including reptiles, birds, some rodents, primates, and humans (reviewed in Grothe 2000; Grothe and Pecka 2014). In addition, bipolar neurons in NL and MSO respond in a comparable manner to chronic changes in their environment, particularly to altered afferent input through manipulations of their excitatory input from the ears. These similarities emphasize stereotyped dendritic regulation that is evolutionarily conserved for fundamental information processing operations in the vertebrate brain.

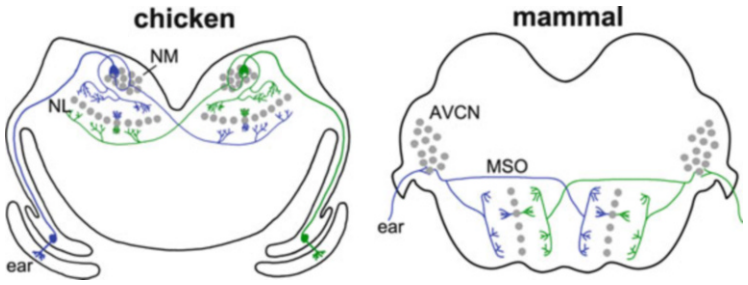

Fig. 11.1 Comparable patterns of cellular organization and major afferent excitatory projections in the chicken NL and mammalian MSO. Note the bipolar morphology and laminar organization of both NL and MSO. Afferent inputs from the left and right ears are colored in *blue* and *green*, respectively. Dendritic domains of NL and MSO neurons are colored corresponding to their afferent input. Individual neurons in NL and MSO receive information about acoustic signals at the same specific sound frequencies from both ears. The drawings are meant to represent an iso-frequency dimension. Abbreviations: *NM* nucleus magnocellularis, *NL* nucleus laminaris, *AVCN* anterior ventral cochlear nucleus, *MSO* medial superior olive

This chapter focuses on the development and regulation of dendritic architecture in the chicken NL, which has been most extensively studied owing to its simple anatomy and well-defined organization. We first describe a number of structural and organizational specializations of NL dendrites that are exquisitely tailored for its function as coincidence detectors and precise temporal processing. This is followed by a discussion of how these specializations are established during development and how they are regulated after onset of function, with a focus on the role of excitatory afferent input in these structural dynamics.

11.1 Structural and Organizational Specializations of NL Dendrites

11.1.1 Bipolar Configuration and Differential Innervation

The following description is of the chicken NL, and it should be recognized that variations of this basic plan are seen in other avian species (Carr and Code 2000). The somata of NL neurons are aligned into a single sheet, resulting in separate dorsal and ventral dendritic neuropil layers (Figs. 11.2a and 11.3a, b; Rubel et al. 1976; Seidl et al. 2010). These dendritic layers are largely free of glial cells but surrounded by a high density of glial cells just beyond the border of the dendritic arbors (Fig. 11.2c). At the single-cell level, the most characteristic structural property of the chicken NL neurons is their bipolar morphology with two segregated dendritic domains (Fig. 11.2b; Ramón y Cajal 1909; Smith and Rubel 1979; Wang and Rubel 2012). The two domains are largely comparable in their size and shape, as well as in their fine structure such as synapse distribution and organelle organization (Deitch and Rubel 1989a, b). Dendritic arbors are generally spineless but sometimes roughly surfaced. Distal dendritic endings are often characterized with an enlarged bulge with or without narrow filopodial-like extensions (Fig. 11.2d).

This bipolar configuration and the single-cell body layer provide the architecture for the organization of afferent inputs, which is critical for coincidence detection. NL receives glutamatergic excitatory inputs solely from the nucleus magnocellularis (NM) on both sides of the brain (Fig. 11.1). NM is a primary target of the cochlear ganglion cells and homologous to the mammalian anterior ventral cochlear nucleus (AVCN). This projection is arranged in a strict topographic manner resulting in a precise map of sound frequencies (tonotopic) in NL (Rubel and Parks 1975). NL neurons responsible for encoding high-frequency information are located in the rostromedial pole, and neurons that are optimally activated by lower-frequency sound are positioned in progressively caudolateral regions of the nucleus. Ipsilateral and contralateral terminals from the same parent neuron in NM target identical tonotopic positions in NL on the two sides of the brain, but segregate onto dorsal and ventral dendritic domains, respectively (Parks and

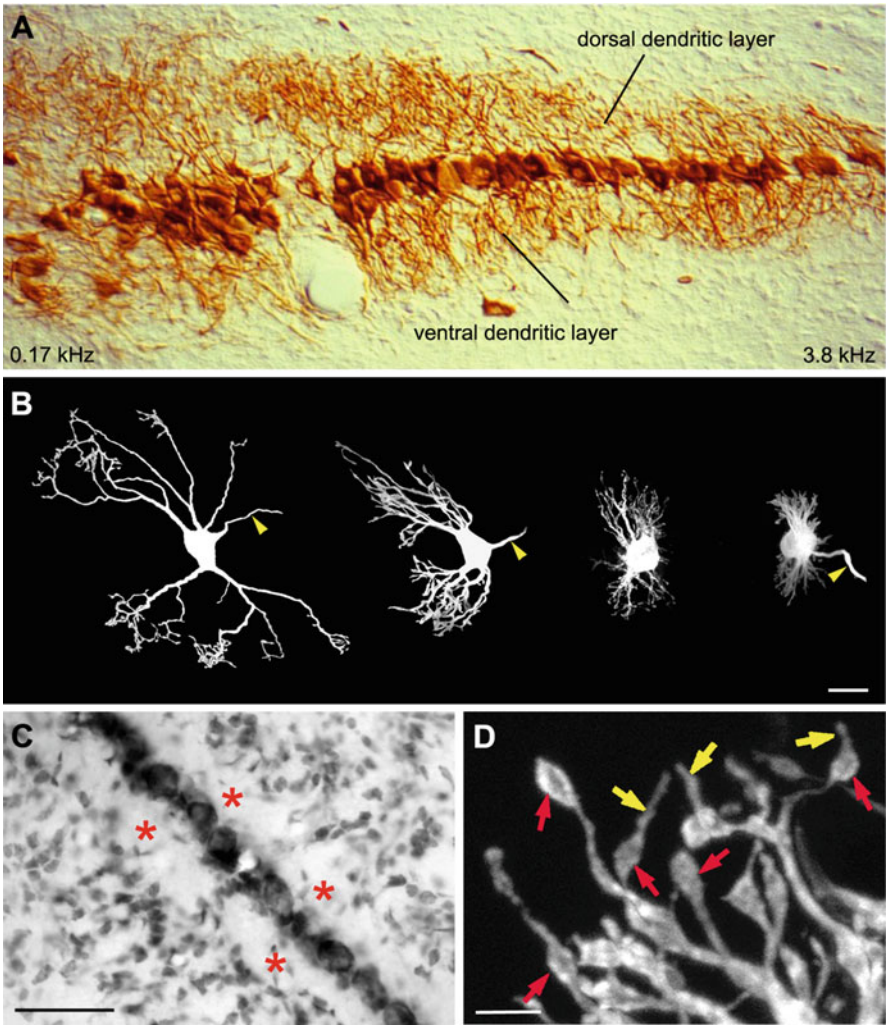

Fig. 11.2 Dendritic morphology and arrangement in the chicken NL. (**a**) MAP2 immunoreactivity (a somatodendritic marker) in NL. The plane of the section is approximately parallel to the caudolateral-rostromedial axis of the nucleus. Thus, this photo covers a large, although not the entire, range of the tonotopic axis. Note the spatial gradient of dendritic extension along this axis, as well as the three-layer configuration of the nucleus. The lowest and highest characterized frequencies recorded from the chicken NL are indicated (Rubel and Park 1975). (**b**) Examples of individual dye-filled NL neurons. These bipolar neurons vary their dendritic length and arborization pattern as a function of the location. A neuron responding optimally to low-frequency sounds is shown on the left, with neurons responding to progressively higher frequencies to the right. Axons are indicated by *yellow arrowheads*. (**c**) Nissl staining demonstrating that NL dendritic neuropil regions are largely glia-free (*red stars*). (**d**) Morphology of distal dendritic endings of NL neurons, characterized by an enlarged bulge (*red arrows*) with or without narrow extensions (*yellow arrows*). Scale bar: 10 μm in (**b**) (applies to **a** and **b**), 50 μm in (**c**), and 5 μm in (**d**)

Rubel 1975; Young and Rubel 1983). With this arrangement, individual NL neurons receive information about acoustic signals at the same specific sound frequencies from both ears.

To perform coincidence detection of action potential input from the two ears, ipsilateral and contralateral terminal arbors of each NM neuron form radically different and highly stereotyped morphologies. The ipsilateral axon bifurcates to provide equivalent axon length to dorsal dendrites of NL neurons along an iso-frequency dimension (Young and Rubel 1983). The contralateral axon, however, extends across the midline and bifurcates several times to create an orderly, serial set of axonal branches to ventral dendrites along a matching iso-frequency lamina of NL on the opposite side of the brain. This arrangement of innervation, predominately along the medial to lateral dimension, results in medial NL neurons receiving contralateral inputs from the shortest input axons and lateral NL neurons receiving from the longest axons. This systematic increase in axonal length across the ventral dendritic field effectively establishes a series of delay lines. The combination of these delay lines formed by the serial branching and differential conduction velocities of the ipsilateral and contralateral circuit compensates for time delays between the two ears as sound emanates from different positions in azimuth (Jeffress 1948; Young and Rubel 1983, 1986; Overholt et al. 1992; Seidl et al. 2010, 2014).

11.1.2 Spatial Gradient

In addition to their bipolar morphology, another dendritic specialization of NL neurons is its highly stereotyped dendritic gradient (Fig. 11.2a, b; Smith and Rubel 1979; Smith 1981; Deitch and Rubel 1984). This gradient is evident not only in the total dendritic branch length of individual neurons but also in the distance from the most distal dendritic branches to the soma, i.e., the "width" or dorsal-ventral extent of the dendritic field. The dendritic gradient conforms precisely to the tonotopic axis with an 11-fold increase in total dendritic branch length and approximately fivefold increase in the width of the dendritic band from high- to low-frequency neurons. Along with these parameters, the number of primary dendrites decreases about 13-fold from high-frequency to low-frequency coding regions of NL. While there is no evidence of spatial gradient in dendritic size and shape in the mammalian MSO, both dendritic extension and arbor density exhibit a dramatic gradient in the alligator NL (Wang et al. 2014).

This dendritic length gradient in NL appears to be an adaptation for ITD processing for particular sound frequencies. Low- and middle-frequency neurons, which have the longer dendrites, may exhibit dendritic filtering resulting from their large surface area (Kuba et al. 2005). A possible advantage of this filtering property is that it may enhance the electrical isolation of dorsal and ventral dendrites and, thus, the inputs from each ear. Indeed, basic biophysical modeling of NL neurons demonstrates that NL dendrites act as current sinks, improving coincidence

detection by allowing a nonlinear summation between the segregated inputs from the ipsilateral and contralateral NM (Agmon-Snir et al. 1998).

11.1.3 High Dendritic Activity

NL dendrites are highly active. In quite conditions, average multiunit spike rate is usually over 1000 per second in NM and NL (Born et al. 1991), while single units in NM spike around 100 times per second (Fukui et al. 2010). With acoustic stimulation, the maximal instantaneous spike rate of NM and NL neurons reaches up to several folds of their spontaneous activity. As required by this high spiking activity,

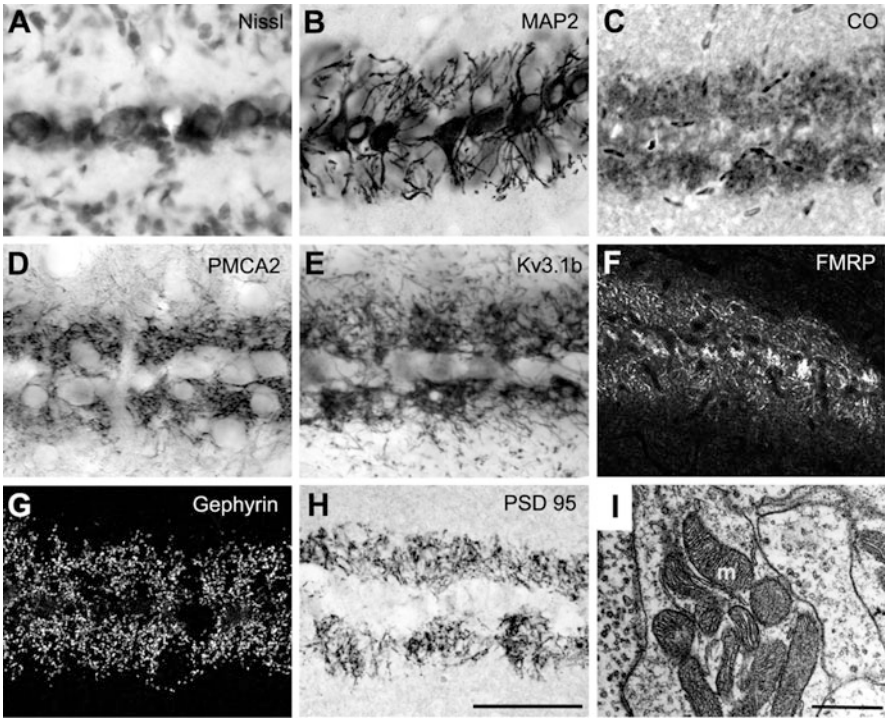

Fig. 11.3 Specialized proteins and cellular organelles in neuronal dendrites of the chicken NL. Images are taken from sections stained for Nissl (**a**), immunoreactivity for microtubule-associated protein 2 (MAP2, **b**), cytochrome oxidase (CO, **c**), and immunoreactivities for plasma membrane calcium ATPase type 2 (PMCA2, **d**), high voltage-gated potassium channel 3.1b (Kv3.1b, **e**), fragile X mental retardation protein (FMRP, **f**), gephyrin (**g**), and PSD 95 (**h**). NL dendrites contain high levels of CO, PMCA2, Kv3.1b, and FMRP and both excitatory and inhibitory synapses, indicated by PSD 95 and gephyrin staining. In **b**–**h** note that all of these highly expressed dendritic proteins are symmetrically distributed in the two dendritic domains. **i** An ultrastructural image illustrating a high density of mitochondria (m) in NL dendrites. Scale bar: 50 μm in (**h**) (applies to **a**–**h**) and 1 μm in (**i**)

NL neurons contain a high density of mitochondria throughout their dendrites (Fig. 11.3i; Deitch and Rubel 1989a). In addition, as in other central auditory neurons, NL is characterized by a high level of cytochrome oxidase (CO, Fig. 11.3c), a mitochondrial enzyme supplying ATP to the cell and thus an indicator for the amount of energy consumption (Dezsö et al. 1993). CO activity in NL is most intense in dendritic layers and adjacent glial regions containing NM axons. Similarly, NL exhibits strong glucose uptake in these regions as measured by 2-deoxyglucose method (Lippe et al. 1980; Heil and Scheich 1986). Studies with cochlea destruction reveal that this high metabolic level of NL is partially due to neuronal spiking which completely depends on cochlea integrity (Born et al. 1991) and is partially due to intrinsic cellular events as indicated by still higher CO level in NL (and other auditory nuclei) than most other regions in the brain even after bilateral cochlea removal (Dezsö et al. 1993). In addition, the brainstem region containing NM and NL is rich in blood vessels, and the amount of blood flow to this region is reduced following cochlea removal (Richardson and Durham 1990).

Consistently, these highly active NL dendrites exhibit strong localization of ion channels including high voltage-gated potassium channel 3.1b (Kv3.1b) (Fig. 11.3e), local protein translation regulators such as fragile X mental retardation protein (FMRP, Fig. 11.3f; see more details in 3.1—Molecular signals and pathways), as well as both excitatory and inhibitory synapses, indicated by PSD 95 and gephyrin staining (Fig. 11.3g, h). Importantly, NL dendrites adopt a number of cellular mechanisms to handle the challenges associated with this high activity. One such challenge is extensive calcium influx during action potentials (spikes), which could lead to calcium toxicity if overaccumulated. As one important mechanism for maintaining basal calcium homeostasis, NL dendrites are heavily loaded with the plasma membrane calcium ATPase type 2 (PMCA2, Fig. 11.3d), the fastest calcium efflux pump that expels calcium out of the cell and sets the resting calcium concentration (Wang et al. 2009).

11.2 Formation and Regulation of NL Dendrites During Development

11.2.1 Normal Development

Chickens can hear well before they hatch (about 21 days of incubation). Similar to other auditory neurons, the major developmental events in NL occur *in ovo* (Fig. 11.4). Detailed reviews of these developmental events are provided elsewhere (Rubel and Parks 1988; Wang et al. 2010). Below we highlight the main time course of dendritic and axonal development within NL.

NL neurons are formed at embryonic days 3–4 (E3–4) from the rhombomeres 5–6 of the neural tube (Rubel et al. 1976; Cramer et al. 2000). Following migration from the ventricular surface into the caudal brainstem, NL and NM are first

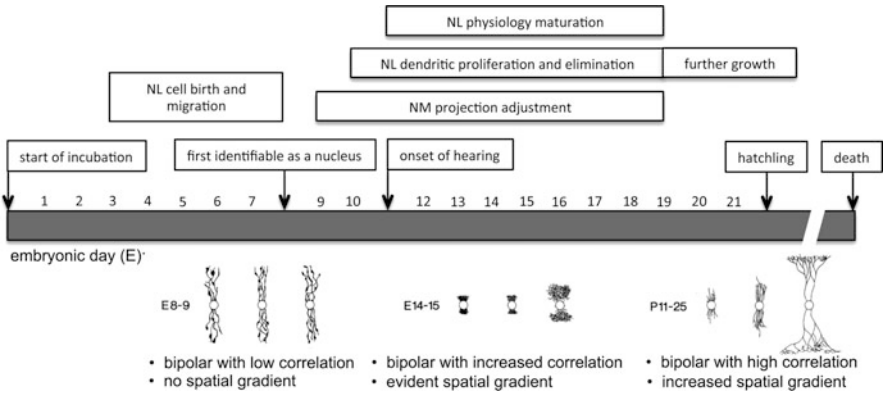

Fig. 11.4 Developmental time course of the chicken NL dendrites. Schematic drawings of dendritic morphology at three time points are illustrated below. For each time point, the three cells from left to right are examples from the caudal, middle, and rostral NL (These drawings are modified from Smith (1981))

identifiable as a nucleus at E8–9. At this time, the morphology and organization of NL and its input from NM have exhibited a number of principles of their mature architecture. Although the somata of NL neurons have not yet aligned into a single layer at this time, the glia-free margin surrounding it has already became apparent (Rubel et al. 1976). Individual NL neurons are recognizably bipolar, often with dorsally and ventrally oriented dendrites, characterized by dense branching of thin processes and wispy filopodia (Smith 1981). The connection between NM and NL is formed and organized topographically, although NM terminals appear immature in morphology and size of terminal field (Young and Rubel 1986). One important aspect of immaturity, though, is the apparent absence of spatial gradient of dendritic length and extension across the nucleus (Smith 1981).

Subsequently, NL neurons undergo dramatic changes in dendritic morphology (Smith 1981). Starting at E10, there is a spatiotemporal gradient of proliferation of numerous fine dendritic processes from rostromedial to caudolateral. This is followed by a spatiotemporal gradient of the elimination of dendritic processes along the same spatial direction. Such changes along with growth of dendritic trees as a function of the location result in the emergence of the distinct spatial gradient of remaining dendrites. This gradient is evident at E15 and becomes prominent by E19. During the same period of dendritic proliferation and elimination, the incoming NM axons gradually adjust their dorsal and ventral terminal fields to achieve the tonotopic specificity (Young and Rubel 1986). In addition, the maturation of intrinsic and synaptic properties of NL neurons also takes place primarily between E9–17 through extensive recomposition and relocalization of synaptic receptors and ion channels in dendrites (Kuba et al. 2002; Gao and Lu 2008; Sanchez et al. 2010, 2012). By E19, NL dendrites and their NM inputs are mature like structurally and physiologically, although the dendritic gradient continues to

develop after hatchling, as does the correlation of dendritic length between the two domains of individual neurons (Smith 1981).

11.2.2 Role of Afferent Input

In the chicken, onset of hearing and synaptogenesis in NL take place at E11–14 (Saunders, et al. 1973; Jackson et al. 1982; Rebillard and Rubel 1981). The development of these highly specialized structural features of NL dendrites appears temporally correlated with the establishment of synaptically driven neuronal activity and may be influenced by the level and pattern of this activity. Interestingly, spontaneous activity in the embryonic auditory brainstem emerges before the onset of sound-evoked activity (Lippe 1994, 1995). Possible roles of afferent activity, synaptically driven and/or spontaneous, in dendritic development of NL neurons are supported by significant effects on NL dendrites of otocyst (embryonic precursor of the inner ear) removal at E3. This manipulation prevents the formation of the inner ear and thus completely removes excitatory afferent input to the ipsilateral NM and then to the dorsal NL dendrites ipsilaterally and ventral NL dendrites contralaterally. As compared to normal development, unilateral otocyst removal produces 44 % reduction in total dendritic length of NL neurons at E17–18 (Parks 1981). Unexpectedly, bilateral otocyst removal produces a smaller (14 %) reduction (Parks et al. 1987). On the other hand, the spatial gradient of NL dendrites is unaffected following either unilateral or bilateral manipulation, indicating molecular factors inherent to the nucleus.

The more dramatic effect of unilateral otocyst removal on NL dendrites than that induced by the bilateral manipulation may suggest that symmetrical afferent input is more important to the regulation of NL dendritic length than the absolute level of this input during development. However, the underlying mechanism might be complicated if one considers potential involvement of newly formed neuronal connection induced by the manipulation. Early removal of one otocyst induces formation of a functional aberrant axonal projection to the ipsilateral NM from the contralateral NM (Jackson and Parks 1988). This projection is excitatory and maintains the tonotopic map in NM and NL on both sides of the brain (Lippe et al. 1992; Zhou and Parks 1993; Parks 1997) and thus may compensate, at least partially, the lost afferent input to the initially deprived NL dendrites. The formation of this aberrant projection is not unique to birds; a similar projection from the contralateral AVCN to the deprived ipsilateral AVCN is formed following early ablation of one cochlea in gerbils (Kitzes et al. 1995). Another new source of excitatory afferent input to the deprived NL dendrites may arise directly from axons of the contralateral NM, which are normally restricted to the opposite domain of NL dendrites (Rubel et al. 1981).

In support of the importance of afferent input to NL development, acoustic enrichment by overstimulation of species-specific and music sound during the prenatal period increases neuronal and glial cell numbers in the chicken NM and

NL, as well as the volume of NL neuropil regions (Wadhwa et al. 1999), although exact effect on NL dendritic architecture is not studied. In summary, it is clear that molecular markers, whose expression is genetically controlled, determine the principles of NL dendritic architecture. It is also evident that excitatory afferent input affects dendritic growth and/or maintenance during development.

11.3 Afferent Influence of NL Dendrites Following Maturation

After NL neurons become structurally and functionally mature, the dendritic arborizations maintain their ability to rapidly and dramatically change their architecture in response to changes in their environment. One such change is altered excitatory afferent input, evident by dramatic effects of afferent deprivation on the behavior, structure, and biochemistry of mature NL dendrites in young chickens.

11.3.1 Effects of Afferent Deprivation on Dendritic Structure

Direct Deafferentation The most direct way of depriving a postsynaptic target of afferent physiological input is to physically disconnect incoming axons to their postsynaptic targets. Of course deafferentation initiates a series of events beyond the elimination of activation of postsynaptic receptors. Nevertheless, it is a direct, repeatable, and productive manipulation and often reveals important information on the roles of a specific presynaptic input on postsynaptic cellular and subcellular elements. The location and organization of NL neurons and incoming NM axons allow complete elimination of excitatory to ventral NL dendrites in vivo by surgical transection of the crossed dorsal cochlear tract (XDCT) at the midline, which contains contralateral NM axons (Fig. 11.5a). Since dorsal and ventral NL dendrites receive excitatory input almost exclusively from ipsilateral or contralateral NM axons, respectively, XDCT transection does not directly alter the excitatory input to dorsal dendrites of the same NL neurons. In addition, there is no evidence of retargeting of damaged contralateral NM axons or sprouting of fibers from the ipsilateral NM to the ventral NL dendrites within 3 months (Rubel et al. 1981). Thus, this differential manipulation allows examination of the subcellular spatial resolution of the response to deafferentation.

XDCT transection produces rapid and progressive retraction of deafferented ventral dendrites in NL (Benes et al. 1977; Deitch and Rubel 1984; Wang and Rubel 2012). Within just 2 hours (h), total branch length of the ventral dendrites is 20 % shorter than those on the dorsal side of the same neurons or the ventral dendrites in control animals. Loss of ventral dendrites continues over the time, such that by 16 days

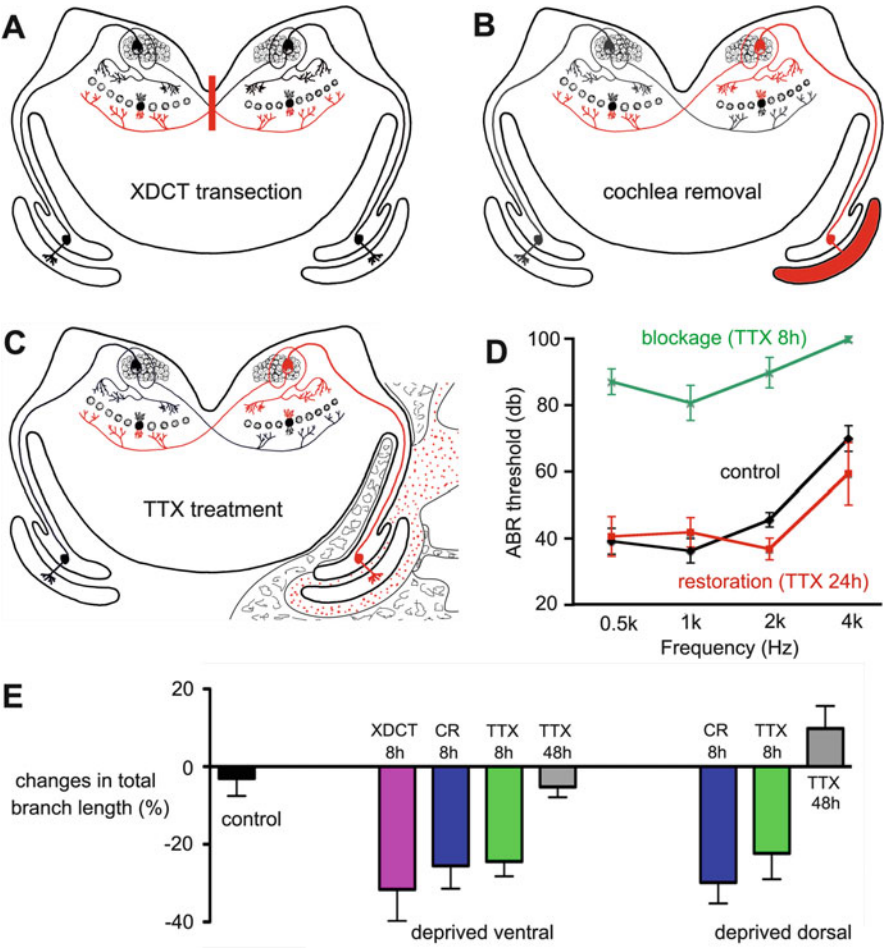

Fig. 11.5 Effects of afferent manipulations on dendritic length of NL neurons. (**a**–**c**) Schematic drawings of the chick brainstem illustrate the affected dendritic fields following XDCT transection (**a**), unilateral cochlea removal (**b**), and unilateral TTX treatment (**c**). *Red* color indicates the surgical site or drug application location for each manipulation, as well as deafferented axons and dendrites influenced by each manipulation. For TTX treatment, TTX is injected into the perilymph of the vestibular system to avoid disrupting the integrity of the middle ear or cochlear fluids (Born and Rubel 1988; Canady and Rubel 1992). (**d**) Effects and time course of TTX treatment on auditory brainstem response (ABR). ABR threshold is plotted as a function of the frequency of sound stimulation. ABR thresholds in response to sounds presented to the injected ear increase dramatically across chicken's entire hearing frequency range immediately after the TTX injection and remain so at 8 hours (h) (TTX 8 h, *green*) and then return to the control levels (*black*) by 24 h (TTX 24 h, *red*). (**e**) Change in total branch length of the deprived dendrites of individual NL neurons is plotted as a function of the manipulation. These changes are calculated as the differences of the deprived dendritic domain as compared to the intact domain of the same neurons. Error bar is standard deviation. At 8 h following XDCT transection (XDCT 8 h), unilateral cochlea removal (CR 8 h), or local TTX treatment (TTX 8 h), deprived dendrites exhibit 20–30 % reduction in total branch length (pink, blue, and green bars). Neurons at 48 h following TTX treatment (TTX 48 h) have more than 24 h with restored afferent activity, and the dorsal and ventral domains are no longer significantly different (*gray bars*), similar to control neurons (*black bar*)

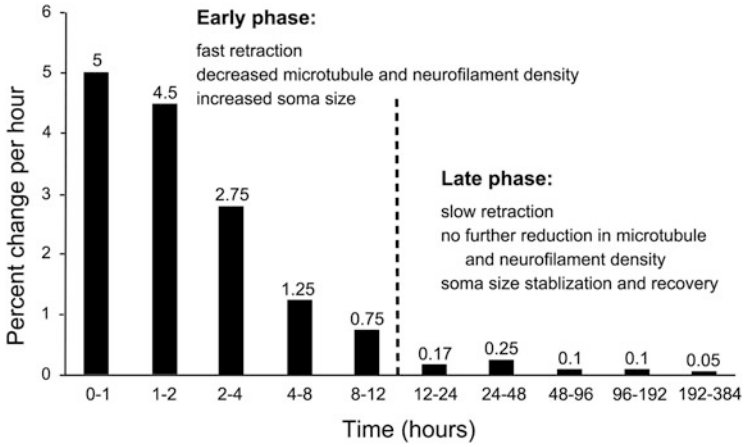

Fig. 11.6 Time course of dendritic retraction following XDCT transection in the chicken NL. The y-axis is the percent change per hour of total dendritic branch length of the deprived ventral dendrites of individual NL neurons as compared to the intact dorsal dendrites of the same neurons. The time course of dendritic change appears to have two phases, which differ in the retraction rate of dendritic branches, in cytoskeletal reorganization and in somatic alternations. The retraction rates are recalculated from Deitch and Rubel (1984). Changes in soma and cytoskeleton are described by Deitch and Rubel (1989a, b)

following XDCT transection, 60 % of the ventral dendrites have disappeared. Quantitative analyses reveal that the changes in dendritic branches are very fast during the first 12 h (on average 2 % reduction per hour) and slow down later (less than 0.25 % reduction per hour, Fig. 11.6). The short-term changes appear characterized by a rapid decrease in the density of dendritic cytoskeleton (microtubule and neurofilament), while the late phase exhibits no further changes in cytoskeletal density but shows a reduced density of cellular organelles (Deitch and Rubel 1989a, b). Importantly, at any given time point within 16 days following the manipulation, the percentage, not the absolute amount, of dendritic lost is the same across the tonotopic axis of the nucleus. Hence, the tonotopic gradient of ventral dendrites remains, but this gradient is "flattened" because the large (low-frequency coding) neurons loose more total dendrite than the smaller (high-frequency coding) dendrites. This observation is consistent with the notion that both the formation and maintenance of dendritic gradient in NL are largely independent of excitatory afferent input, but the overall size of any specific dendritic arbor is dramatically regulated by presynaptic activity.

Careful ultrastructural examination of synapses and axonal structure was unable to reveal degeneration during the early stage of ventral dendritic reorganization following XDCT transection (Deitch and Rubel 1989a). This result is consistent with the interpretation that the early changes in dendritic structure are due to the loss of excitatory synaptic input onto the ventral dendrite and not the result of degeneration of the afferent elements, per se. Surprisingly, no evidence of

degeneration of dendritic plasma membrane is evident until at least 2 days following XDCT transection. Examination of the soma, however, reveals an increase in volume within hours, which is maintained up to 8 days following the lesion (Deitch and Rubel 1989b). This result suggests that resorption of dendritic plasma membrane is apart of the process of dendritic remodeling, at least the early decrease in ventral dendritic length.

The most important observation from these experiments is that the changes in dendrites are confined to the ventral (deafferented) domain (Benes et al. 1977; Deitch and Rubel 1984, 1989a). There is no evidence of altered morphology or structure in the dorsal dendrites of the same NL neurons, indicating that the cellular response is confined to the dendritic surface which is directly deafferented. This specificity provides a clear example of the fact that afferent excitatory inputs can independently regulate different parts of the same neuron, an observation that has now been confirmed in a variety of other neuronal systems. Detailed quantitative studies in NL have demonstrated that structural difference between the two domains of the same neuron reliably predicts the actual change in the deprived domain following differential afferent deprivation. Thus NL provides a convenient model to study local dynamics of dendrites with the benefit of a matched control for structural properties within the same neuron.

Transneuronal Afferent Deprivation The rapidity and domain specificity of dendritic retraction following XDCT transection raise the possibility that these postsynaptic changes, at least the short-term changes, are exclusively or primarily due to the cessation of presynaptic action potentials. This possibility is confirmed by comparable changes in NL dendrites following transneuronal deprivation of afferent activity. As the cochlea provides the only excitatory input to the ipsilateral NM, unilateral cochlea removal eliminates presynaptic action potentials to the ipsilateral NM and then to the dorsal dendrites of the ipsilateral NL and ventral dendrites of the contralateral NL, without directly damaging NM axons (Fig. 11.5b; Born et al. 1991). By 8 h following unilateral cochlea removal, the total dendritic length of deprived NL dendrites decreases by ~30 % as compared to the intact dendritic domain of the same neurons, comparable to that following XDCT transection (Wang and Rubel 2012). The similar retraction rate following the two manipulations strongly suggests that presynaptic activity, instead of lesion-related events, is responsible for maintaining dendritic arbors at the early stage.

The short-term modifications of deprived NL dendrites are fully reversible, as evidenced by complete recovery of normal dendritic length and dorsal-ventral symmetry following restoration of normal afferent activity (Wang and Rubel 2012). Local exposure of one ear with tetrodotoxin (TTX) completely blocks action potentials in the auditory nerve, totally silences action potential generation of NM neurons, and is completely reversible (Fig. 11.5c; Born and Rubel 1988; Canady and Rubel 1992). TTX treatment with carefully adjusted amount of the drug allows complete blockage of activity for at least 8 h, followed by rapid recovery; the level of activity and hearing thresholds return to control levels by 24 h and are maintained thereafter (Fig. 11.5d; Wang and Rubel 2012). NL dendrites deprived

of excitatory input for 8 h by this manipulation show comparable retraction to that seen following cochlea removal or XDCT transection (Fig. 11.5e), further confirming presynaptic action potentials as a key afferent component in regulating postsynaptic dendrites. These dendrites then regrow to their normal size by 48 h following the onset of TTX treatment, by which time NM neurons have had at least 24 h with restored normal activity. This rapid recovery of dendritic structure demonstrates that retracting NL dendrites, following afferent deprivation, maintain a high degree of plasticity and actively respond to changes in afferent input in either direction.

In the paragraphs above, we only consider the early phase of dendritic changes following afferent deprivation and argue that these changes are mostly or entirely due to the deprivation of action potentials, presumably glutamate release and activation of postsynaptic receptors on the local postsynaptic elements (dendrites). On the other hand, integrity of presynaptic axons, in addition to their activity, may contribute to dendritic regulation during the later phases of dendritic remodeling following afferent axon elimination. To assess this hypothesis, we further studied the effects of unilateral cochlea removal. It is well known that unilateral cochlea removal in young chickens leads to extensive, but not complete, neuronal death in the ipsilateral NM (Born and Rubel 1985). Therefore, this manipulation produces partial deafferentation of specific dendritic domains in both ipsilateral and contralateral NL. The variation between animals in the amount of neuronal loss allowed us to compare the amount of dendritic retraction in the affected dendritic domains of NL neurons with the amount of NM cell loss in each animal. Across animals, the degree of dendritic retraction in NL exhibits a highly significant correlation ($r = 0.76$, $p < 0.01$) with the percentage of cell death in NM at 4 days following unilateral cochlea removal (Fig. 11.7). One interesting possibility is that the maintenance of remaining dendrites is affected by the integrity of NM neurons that continue to innervate these dendrites. Alternatively, cell survival in NM and long-term dendritic maintenance in NL may be influenced by similar mechanisms that depend on action potential-mediated signals.

While the MSO of low-frequency hearing mammals presents many of the same advantages for dendritic analyses as NL, detailed structural and temporal analyses are not yet available. Nonetheless, similar dendritic changes have been observed in MSO of young rats (Feng and Rogowski 1980) and adult gerbils (Russell and Moore 1999). Unilateral cochlea ablation also produces domain-specific changes in dendritic structure days and months later; whether these relatively long-term changes correlate with neuronal survival in AVCN and whether there are earlier changes in MSO dendrites have not been examined.

Dendritic Properties Regulated by Afferent Input To understand functional significance or consequence of afferent-regulated dendritic dynamics in NL, we need to identify the major dendritic properties that are both sensitive to changes in afferent input and essential to the function of the neuron. Two such properties have been identified, the size of dendritic surface which helps define the number of synapses

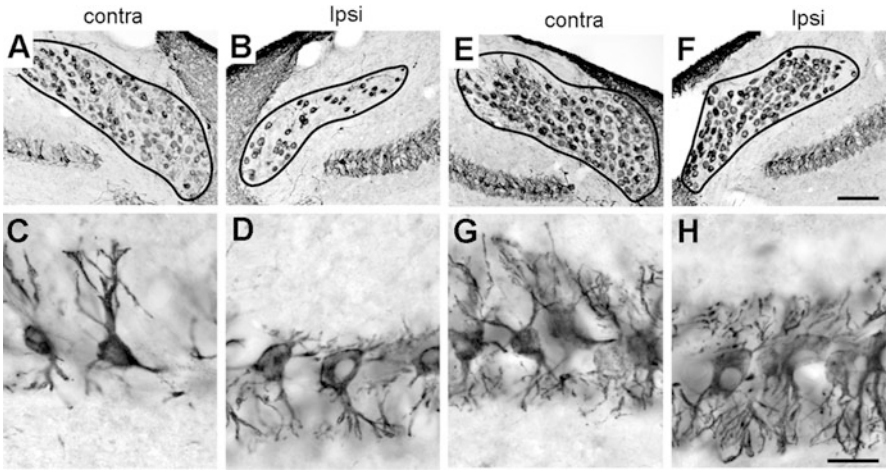

Fig. 11.7 Correlation of cell survival in NM with dendritic maintenance in NL. All images were taken at 4 days following unilateral cochlea removal. (**a**–**h**) Photomicrographs of coronal sections labeled with MAP2 immunoreactivity through NM (**a**, **b**, **e**, **f**) and NL (**c**, **d**, **g**, **h**). (**a**–**d**) Were taken from one animal, and **e**–**h** from the second animal. Left (**a**, **c**, **e**, **g**) and right (**b**, **d**, **f**, **h**) columns are from the contralateral and ipsilateral side, respectively. In both cases, the ipsilateral NM has a notable reduction in cell number as compared to the contralateral NM of the same case. Within NL, the ventral dendrites of the contralateral side and the dorsal dendrites of the ipsilateral side show clear retraction as compared to the other domain of the same side. The difference between the two cases is that the first case (**a**–**d**) exhibits a much larger degree of cell death in the ipsilateral NM as compared to the second case (**e**–**h**). Correlated to this difference in transneuronal NM cell death, dendritic retractions in NL are more dramatic in the first case than in the second case. *Solid lines* in **a**, **b** and **e**, **f** outline the borders of NM (Images are modified from Wang and Rubel (2012)). Scale bar: 100 μm in (**f**) (applies to **a**, **b** and **e**, **f**) and 20 μm in (**h**) (applies to **c**, **d** and **g**, **h**)

that can be formed and the dendritic arborization pattern which contributes to the way synapses are distributed and how inputs are integrated.

Quantitative analyses at the single-cell level following XDCT transection reveal no changes in the average diameter of dendritic branches (Deitch and Rubel 1984; Wang and Rubel 2012). Consistently, the overall size of dendritic surface correlates with total dendritic length in both control and deprived dendrites (Smith and Rubel 1979; Deitch and Rubel 1984). As a result, afferent activity influences dendritic surface area by dynamically regulating dendritic length.

Afferent activity also influences the arborization pattern of NL dendrites by reorganizing the dendritic tree in a branch-specific manner. First, the number of primary dendrites is unaffected by afferent deprivation (Deitch and Rubel 1984; Wang and Rubel 2012), suggesting that retraction mostly takes place in higher-order dendritic arbors. This suggestion is also supported by in vitro time-lapse imaging studies showing that deafferentation-induced dendritic retraction is observed primarily at the distal portion of the NL dendritic arborizations (Sorensen and Rubel 2006, 2011). Second, the number of dendritic endings or segments is

regulated by afferent activity in such a way that it reliably predicts changes in total branch length of both retracing and regrowing dendrites (Wang and Rubel 2012). This finding indicates that selective elimination of a number of distal arbors and addition of new branches contribute substantially to the overall changes in total branch length. In vitro time-lapse imaging further demonstrates that individual dendritic arbors of a particular neuron are always dynamic (Sorensen and Rubel 2006). At any time over any 30-min period, about 50 % of the dendritic branches remain stable (less than 10 % change) in length. This statement holds true under both normal (balanced) and imbalanced conditions of afferent activity. The other 50 % of the dendritic branches either grow or retract by over 10 %. Over a 7-h imaging period, most or all terminal branches could be found growing or retracting. Changes in total branch length result from changes in the *balance* of arbors that are growing, retracting, or remaining stable at any time, not a sudden surge of growth or retraction. Thus, afferent-regulated dendritic reorganization does not take place uniformly along all affected dendritic branches and may occur in a branch-specific manner by which the fate (growing, retracting, or remaining stable) of individual branches is influenced by a combination of afferent inputs and intrinsic properties of each branch. This suggestion is consistent with the compartmentalization of electrical, calcium, and other biochemical signaling with single branch specificity in neuronal dendrites (Rinzel and Rall 1974; Rinzel 1975; Sjöström et al. 2008; Branco and Häusser 2010; Siegel and Lohmann 2013). Similar regulatory patterns have been reported in visual neurons for dendritic growth induced by sensory stimulation and for dendritic retraction following neurotransmission inactivation (Rajan and Cline 1998; Rajan et al. 1999; Sin et al. 2002).

Responsible Components of Afferent Activity Both XDCT transection and unilateral transneuronal manipulations deprive presynaptic activity to one, but not both, dendritic domain of NL neurons. Thus, changes in either the balance or the overall level of activity, or both, could be responsible for induced changes in postsynaptic NL dendrites.

In vitro studies using acute slice preparations of the chicken NL support the importance of the balance of afferent activity to the two dendritic domains of the same neurons (Sorensen and Rubel 2006, 2011). Slices of the brainstem were maintained in artificial CSF for several hours with bipolar stimulating electrodes on both auditory nerves, while individual fluorescently labeled NL neurons were images with a multiphoton microscope (Fig. 11.8). Two control conditions were included: (1) both auditory nerves were stimulated at a rate of 10 Hz for 7 h and at a level that reliably evoked action potentials in NL neurons or (2) neither auditory nerve was stimulated for the same period of time. In both of these conditions, NL neurons exhibited very little changes in the total dendritic length over the 7 h, although the arbors were continuously growing and retracting. This observation indicates that NL neurons maintain their dendrites in the absence of presynaptic action potentials to both dendritic domains *in vitro*. In contrast, unilateral electrical stimulation, activating NM axons to only one set of NL dendrites (either dorsal or ventral), results in the unstimulated dendrites losing total dendritic branch length

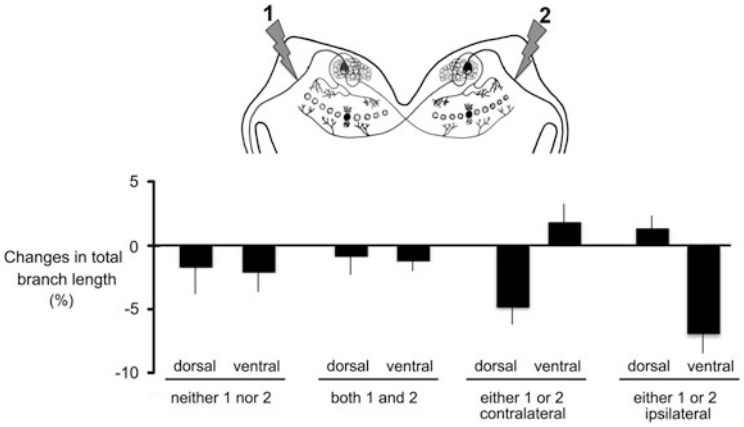

Fig. 11.8 Differential effects of balanced and imbalanced afferent inputs on dendritic length of the chicken NL. The y-axis is the changes in total branch length of NL dendrites. Control conditions on the left show that there is no significant change in the dorsal or ventral dendrites in response to no stimulation (neither 1 nor 2) or bilateral stimulation (both 1 and 2) at 10 Hz for 7 h. In contrast the data on the right show that unilateral (either 1 or 2) stimulation using the same parameters of afferent input from NM causes dramatic and opposite changes in branch lengths of the dorsal and ventral dendrites (Data are derived from Sorensen and Rubel (2006, 2011))

and the stimulated dendrites of the same NL neurons exhibiting a net growth. This result suggests that imbalanced input results in differential changes in the two sets of dendrites in a way that dendritic real estate can rapidly be shifted from inactive inputs to active inputs.

On one hand, these studies are consistent with the importance of the balance of afferent activity. On the other hand, dendritic growth or retraction during changes in afferent activity may depend on differences in the exact level and pattern of presynaptic activity elicited by electrodes as compared to that derived from the intact ears *in vivo*. That is, it is possible that both the balance between the two afferent inputs and the specific properties (level and/or pattern) of each input influence the maintenance of NL dendrites.

Molecular Signals and Pathways Examining effects of afferent alternations on dendritic biochemistry helps identify intracellular signals and pathways involved in afferent-regulated dendritic reorganization, especially if such effects show similar domain specificity as exhibited by changes in structure following differential afferent manipulations.

One such pathway is calcium-mediated microtubule regulation (Fig. 11.9). Following XDCT transection, one of the first changes in the fine structure of the deafferented ventral dendrites is dramatic reduction in the density of microtubules (Deitch and Rubel 1989b), a major cytoskeletal component of neuronal dendrites. This reduction may be associated with rapid decrease in the microtubule-associated

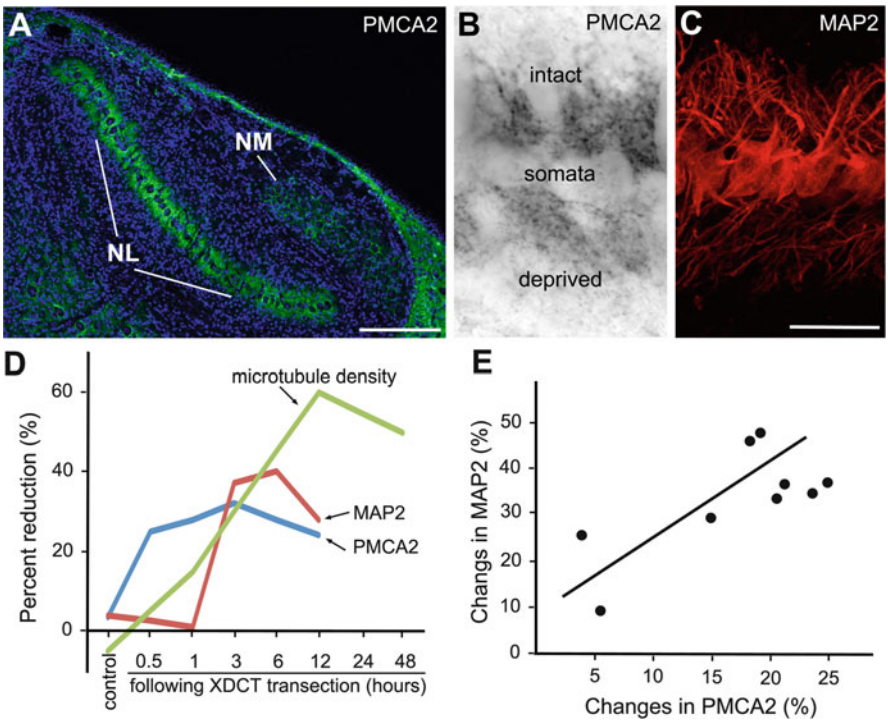

Fig. 11.9 Effects of afferent deprivation on dendritic levels of PMCA2 and MAP2 in the chicken NL. (**a**) A low-magnification photomicrograph showing the overall pattern of PMCA2 immunoreactivity in NM and NL. Note high PMCA2 level in NL dendritic layers. Also shown in Fig. 11.3d. (**b**, **c**) Immunoreactivities for PMCA2 (**b**) and MAP2 (**c**) in NL neurons at 6 h following XDCT transection. The levels of PMCA2 and MAP2 are reduced dramatically in deprived ventral dendrites as compared to the intact dorsal dendrites of the same NL neurons. (**d**) Time courses of percent reductions in dendritic levels of PMCA2 (*blue line*), MAP2 (*red line*), and microtubule density (*green line*) following XDCT transection (Recalculated from Wang and Rubel (2008), Wang et al. (2009), and Deitch and Rubel (1989a)). (**e**) Scatter plot showing relationship between changes in PMCA2 and MAP2 in deprived NL dendrites following unilateral cochlea removal. Each data point represents an individual case. Animals with a larger change in PMCA2 immunoreactivity tend to have a larger change in MAP2 immunoreactivity. Abbreviations: *PMCA2* plasma membrane calcium ATPase type 2, *MAP2* microtubule-associated protein 2. Scale bar: 200 μm in (**a**) and 25 μm in (**c**) (applies to **b** and **c**)

protein 2 (MAP2) that is confined to afferent-deprived NL dendrites (Wang and Rubel 2008). MAP2 is essential for microtubule stabilization and is believed to play a fundamental role in regulating dendritic morphology in developing and adult brains (reviewed in Dehmelt and Halpain 2005). Importantly, changes in MAP2 are correlated with changes in the plasma membrane calcium ATPase type 2 (PMCA2), a high-affinity calcium efflux protein, in deprived NL dendrites (Wang et al. 2009). Neurons with a larger change in PMCA2 immunoreactivity tend to have a larger

change in MAP2 immunoreactivity. Changes in PMCA2 start as early as 30 min following afferent deprivation, prior to detectable changes in MAP2 and the structure of these dendrites. This temporal property provides a potential mechanism by which deprivation can change calcium transport that, in turn, may be important for rapid, domain-specific dendritic remodeling. This possibility is strongly supported by the well-known regulation of MAP2 and microtubule by calcium signaling (Vaillant et al. 2002), as well as with the pivotal role of intradendritic calcium signals in activity-dependent dendritic regulation (Lohmann and Wong 2005; Redmond and Ghosh 2005). Further support for an important role of this pathway is provided by the observations that afferent or sensory deprivation leads to changes in the expression of a number of calcium-binding proteins (parvalbumin, calretinin, and calbindin) in the mammalian MSO in adults (Caicedo et al. 1997; Alvarado et al. 2004). These data naturally lead to the speculation that deprivation of afferent excitatory activity leads to chronic changes in dendritic Ca^{++} regulation in the affected dendrite, a hypothesis supported by the observation that $[Ca^{++}]_i$ appears to be independently regulated in the dorsal and ventral dendritic domains (Blackmer et al. 2009). This is an important area for further investigation using modern imaging methods.

Another intracellular signaling pathway that may be involved in afferent regulation of dendritic structure is fragile X mental retardation protein (FMRP, Fig. 11.10). FMRP is an mRNA-binding protein, associated with a highly selected group of mRNAs (Darnell et al. 2011). A major function of FMRP is thought to be regulating local protein synthesis of its mRNA targets, particularly in neuronal dendrites (reviewed in Santoro et al. 2012). Loss of FMRP protein leads to abnormal dendritic structure and reduced dendritic dynamics in response to neuronal activity (Galvez et al. 2003, 2005; Restivo et al. 2005; Zarnescu et al. 2005; Thomas et al. 2008; Santoro et al. 2012). We have recently shown that *dendritic* FMRP is expressed at unusually high levels in NL and MSO across a wide range of low-frequency hearing vertebrates (alligator, chicken, gerbil, and human; Wang et al. 2014). Remarkably, dendritic FMRP in NL and MSO neurons accumulates at branch points and enlarged distal tips, loci known to be critical for branch-specific dendritic arbor dynamics. This localization pattern raises the possibility that FMRP may play an important role in determining the fate of individual branches either branching out, retracting, or staying unchanged and/or provides quick supplies of required proteins for such cellular events on an individual branch basis. In support of this possibility, in neurotrophin-stimulated neurites of PC12 cells, FMRP granules are predominantly localized to swellings along developing neurite, often locations where new branches are generated (De Diego Otero et al. 2002). Finally, it is interesting to note that FMRP mechanisms may be linked with calcium and microtubule regulation in NL and MSO dendrites as both mRNAs encoding PMCA2 and MAP2 show a very high binding affinity with FMRP (Darnell et al. 2011). In addition, Kv3.1b, a functionally specialized protein in NL dendrites (Fig. 11.3e), is also an identified target of FMRP (Darnell et al. 2011).

Given the activity dependency of afferent-regulated dendritic structure, it is reasonable to argue for a critical role of neurotransmission in this regulation.

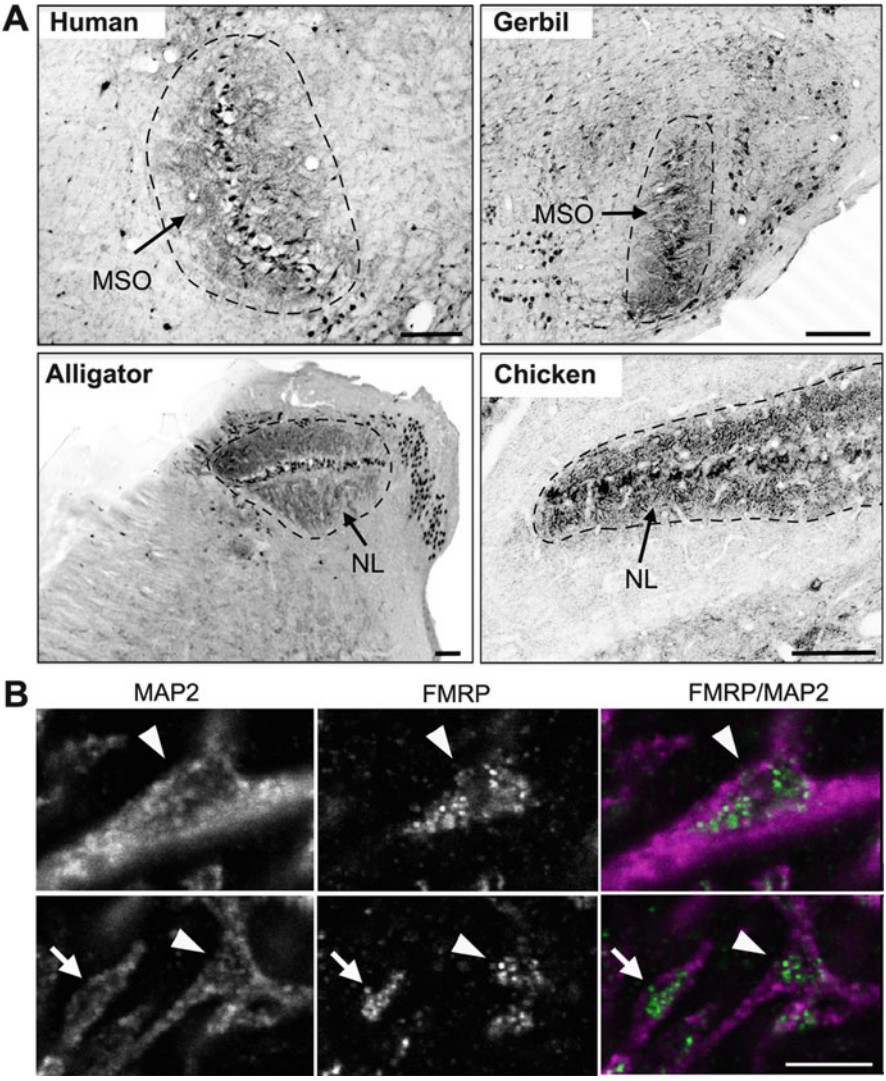

Fig. 11.10 Intense and specialized dendritic localization of FMRP in NL and MSO neurons. (**a**) High expression levels of dendritic FMRP immunoreactivity in human and gerbil MSO, as well as in alligator and chicken NL, as compared to the surrounding regions. (**b**) Complimentary pattern of MAP2 and FMRP in NL dendrites of the chicken. Note the strong accumulation of FMRP immunoreactivity in branch points (*arrowheads*) and terminal endings (*arrows*) and the relatively low level of MAP2 in these locations. Abbreviations: *FMRP* fragile X mental retardation protein. Scale bar: 100 μm in (**a**) (all panels) and 5 μm in (**b**)

Indeed, in the chicken NL, blocking excitatory neurotransmission using glutamate receptor inhibitors produces dendritic retraction (Sorensen and Rubel 2006), and unilateral cochlea removal also affects GABAa receptor expression (Code and

Churchill 1991). In the mammalian MSO, unilateral cochlea removal leads to significant changes in GABA and glycine release and uptake, as well as in the regulatory signaling of neurotransmitter release (Suneja et al. 1998a, b, 2000; Mo et al. 2006; Yan et al. 2007).

11.3.2 Other Regulatory Factors

NL and MSO neurons are among neuronal cell types with a high sensitivity to cues in the environment, including but not limited to changes in afferent input from the ear. For example, alcohol exposure affects dendritic length and structure in the chicken NL (Pettigrew and Hutchinson 1984) and neuronal number of the monkey MSO (Mooney and Miller 2001). Interestingly, this change in neuronal number may be exclusive to MSO, as compared to relatively smaller changes in other auditory regions in the brainstem. As another example, both the number and extension of MSO dendrites are reduced in underfed rats as compared to rats normally nourished (Salas et al. 1994). Similarly, aging-associated neurochemical changes are observed in the MSO but not other auditory groups in the monkey brainstem (Gray et al. 2014), and in the brainstem of humans with autism spectrum disorder (ASD), neuronal loss and disorganization appear most dramatic in MSO as compared to other auditory neurons (Kulesza 2007). This high vulnerability of MSO neuronal morphology and neuronal survival is consistent with reduced sound localization ability as a hallmark of age-related hearing deficits and as a common phenotype in a number of neurological disorders.

11.4 Conclusion

Afferent synaptic input is one important factor regulating neuronal structure including dendritic architecture in developing and mature brains. Bipolar neurons in NL and MSO present an example of dendritic architecture highly specialized for their function in temporal coding and coincidence detection. These neurons are also highly sensitive to cues in their environment, particularly variations in afferent input during both development and in the mature brain. Relatively brief changes in presynaptic glutamate-driven synaptic activity dramatically influences both the size of dendritic surface and the pattern of dendritic arborizations, and these changes appear largely localized to the precise regions of the altered input, providing unparalleled access to the membrane compartments undergoing plasticity with appropriate control compartments in the same neurons. The unique dendritic structure of NL and MSO neurons provides an advantageous model for further understanding of the specific roles of neurotransmission, calcium signal, protein synthesis, and cytoskeletal regulation in this important form of brain dynamics.

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