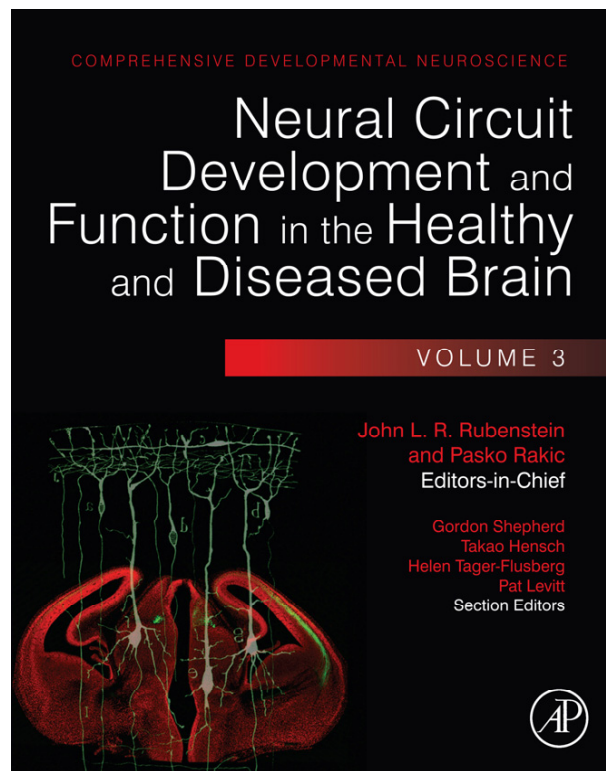


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Functional Circuit Development in the Auditory System

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2.1 INTRODUCTION TO AUDITORY SYSTEM DEVELOPMENT

2.1.1 A Neurobiological Approach to Studying Auditory System Development

The auditory system undergoes a series of profound changes from the time neural circuits begin forming in the fetal brain to the day, years later, when a child first comprehends a complete sentence. The processes unfolding during this period are a fascinating mixture of intrinsic molecular orchestration and activity-dependent refinement. In humans, auditory perceptual development is a protracted process that begins late in the second trimester when the fetus first shows discriminative changes in heart rate to variations in specific sound features. Progressive improvements in the ability to resolve variations in frequency, temporal patterns and spatial positions of sounds are observed throughout infancy and early childhood, and these changes often parallel an increasing capacity to discriminate phonological units of a child's native language (Werker, 2005). While a greater understanding of the processes at work in human auditory development is of paramount importance, these efforts are often complicated by an inability to isolate the contributions of sensory system development from other cognitive and physical factors.

The use of model systems such as birds and rodents has provided researchers with direct access to central and peripheral auditory circuits, and has elucidated many of the basic mechanisms that underlie their changes during ontogeny. Songbirds and chickens have long been popular model systems due to the greater ease of studying and manipulating the embryo in the egg (*in ovo*) rather than *in utero*. As a result, we can now draw information from an extensive corpus of work detailing the organization of brainstem and forebrain pathways, and the similarities by which songbirds acquire their song and humans acquire speech, particularly in their dependence on auditory feedback during sensitive periods of development. Interestingly, birds begin hearing approximately eight days before hatching, around embryonic (E) day 11, while altricial mammals such as mice, rats and gerbils do not respond to airborne sound until the second postnatal (P) week of life. The relatively late onset of hearing in rodents has aided research into the cellular and molecular changes that underlie abrupt changes in circuit development in the days before and after the onset of hearing.

Recent studies suggest that molecular cues, whose expression is genetically controlled, play an essential role in the formation of topographically ordered connections in the auditory system. It is also evident that factors linked to the flux of ions across the cell membrane during and following the action potential also support

neuron survival and regulate the growth and topographic specificity of axons and dendrites within auditory brain areas. In some brain areas, instructive electrical signals generated through the "closed-loop" spontaneous activity is sufficient to promote normal neural circuit development, whereas higher levels of the auditory pathway require structured activity patterns arising from acoustic signals to guide their final stages of assembly. The influence of each activity-dependent and activity-independent factor waxes and wanes within defined windows of development, and piecing together the chronology and mechanisms behind each epoch of auditory system development represents one of the fundamental challenges for researchers in this field. The chapter describes the current state of knowledge concerning the interplay of these factors in the establishment of functional circuits from the cochlea to the cerebral cortex.

2.1.2 Basic Concepts of Cochlear Transduction

The encoding of auditory information begins with a highly specialized receptor organ known as the cochlea in mammals and the basilar papilla in birds. In mammals, pressure waves are delivered into the fluid filled cochlea via the middle ear bones, setting the cochlear partition into motion. The cochlear partition consists of the basilar membrane (BM), the organ of Corti, and the tectorial membrane (Figure 2.1(a)). As a result of this motion and the mechanical properties of these structures, a relatively crude spectral analysis is performed by a vibratory pattern that occurs along the partition, a passive process referred to as the traveling wave (Von Békésy, 1960). This traveling wave results in a vibratory pattern such that high frequency sounds produce maximum displacement near the beginning (base) of the partition, while low frequency sounds vibrate maximally near the end (apex) of the partition.

The sensory receptors in mammals responsible for transducing these hydro-mechanical vibrations are located in the organ of Corti and are known as inner and outer hair cells (IHC and OHC, respectively). Both types of receptors are polarized epithelial cells that contain mechano-sensitive organelles located on their apical surface. These actin-filled hair-like processes are termed stereocilia and contain mechanotransducer channels near the tips of the ciliary bundles. The bundles are deflected as a result of partition movement, opening the channels and depolarizing the hair cell due to an influx of potassium (K^+). This unconventional form of depolarization activates voltage gated calcium (Ca^{2+}) channels, which triggers the release of the excitatory neurotransmitter glutamate. Glutamate binds to postsynaptic receptors located on first-order spiral

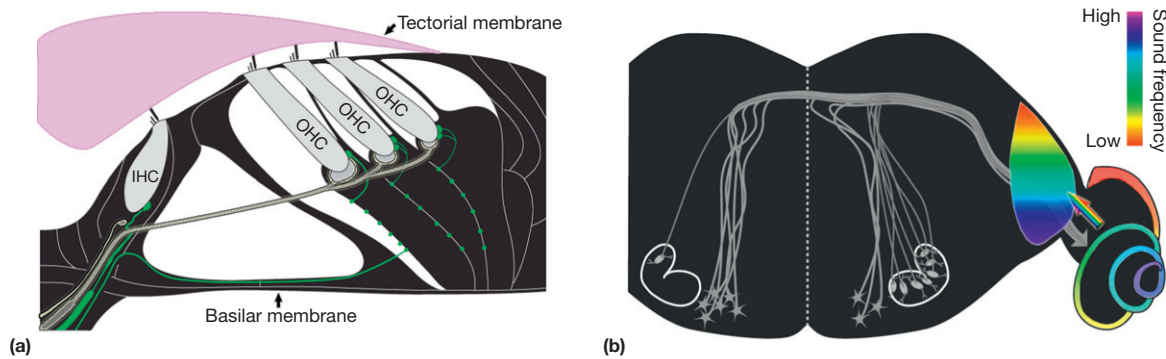


FIGURE 2.1 Connections within the adult auditory system. Schematics illustrate the position of cell bodies and their interconnections in mammalian Organ of Corti (a) and initial components of the auditory pathway (b). (a) Two classes of mechanical sensory receptors, inner hair cells (IHC) and outer hair cells (OHC) are innervated by dendrites that carry afferent signals to the brain (green) and efferent axons that convey signals to the brain (gray). (b) Sounds of varying frequencies maximally excite particular regions along the basilar membrane (BM). Maximal sensitiveness are mapped systematically across the length of the BM, such that high frequency vibrations excite the base of the BM and low frequencies excite the apex. This tonotopic organization of frequency preference is preserved through topographically ordered projections to the central auditory system. Distinct populations of cells on both sides of the brainstem send efferent projections to the cochlea.

ganglion neurons (green dendrites, Figure 2.1(a)), initiating saltatory conduction of action potentials to auditory nuclei in the brainstem.

The generic description above for sensory transduction holds true for all hair cells in both birds and mammals. However, the separation into IHCs and OHCs, and their differences, are remarkable and unique to mammals. The IHCs are the true sensory receptor, receiving approximately 95% of afferent innervation. In contrast, OHCs receive only around 5% of afferent contacts, suggesting a minor role in sensory transduction. Despite this dichotomy, OHCs are thought to have highly-specialized “motor” functions. One such active function is to enhance the bundle displacement caused by the vibration of the cochlear partition, acting as a positive feedback on the bundle and the consequent amplification of its displacement. A second active function involves an elongation and contraction of the OHC itself. As the OHC changes length, it feeds back mechanical force onto its surrounding environment, a process termed electromotility. This results in a highly significant and spatially segregated enhancement of the basilar membrane vibratory amplitude. Accordingly, the relatively crude sensitivity and frequency selectivity of the basilar membrane that arises through its passive biomechanical properties is substantially refined through hair cell active amplification. Not only do hair cells transmit signals to the central nervous system, they are also innervated by efferent axons from the brain, which communicate with OHCs to extend the dynamic range of hair cell signaling and protect the Organ of Corti from acoustic overexposure (Figure 2.1(b)). For detailed reviews of auditory transduction and the distinct mechanisms that create active cochlear amplification in mammals versus birds, readers are referred to (Hudspeth, 2008; Dallos, 2006).

2.1.3 Scope of this Chapter

A functional circuit can be defined as the connection between one or more cells or nuclei that transmit – and often transform – a signal. As such, topics relating to the proliferation, delamination and migration of cells and the development of their connecting projections in the auditory system are touched upon only briefly. Rather, this chapter is primarily concerned with describing the relative contributions of intrinsic molecular events, spontaneous action potentials and sound-evoked action potentials in the assembly of functional circuits within the peripheral and central auditory pathways.

This chapter is divided into three principal sections. The first section covers the ontogeny of local circuits within the cochlea and as well as the development of afferent and efferent circuits connecting the cochlea to the brain. The second section describes the establishment of circuits within the developing auditory brainstem and the influence of signaling from the auditory periphery. The final section addresses the formation of functional circuits in the auditory midbrain and cortex. When appropriate, we have cited notable seminal research papers, breakthrough findings and comprehensive review materials so that the interested reader may avail themselves of these more focused sources of information.

2.2 DEVELOPMENT OF PERIPHERAL CIRCUITS

2.2.1 Developing Networks Within the Cochlea

Cochlear hair cells initiate the process of hearing by converting mechanical deflections of their stereocilia bundles into electrochemical signals that are distributed

throughout the rest of the auditory system. Before mature and normal transduction can occur, a number of critical developmental events take place between hair cells and non-sensory cells within the cochlea. The specialized function of IHCs and OHCs depends in part upon developing networks of non-sensory supporting cells within the organ of Corti and the lateral wall of the cochlea.

The precision of mechanoelectrical transduction can be attributed, in part, to the unusual electrical potential and ionic milieu in the endolymphatic space surrounding the apical surface of the hair cell. Prior to and during the first week of hearing, an endocochlear potential is established between the endolymph and surrounding perilymph, which increases from 0 mV to +80 mV. The ramping up of the electrical potential is complemented by the accumulation of high levels of K^+ in the endolymphatic space, which further exaggerates the electrical gradient across the negative resting potential of the hair cell membrane. The combination of high extracellular K^+ and the positive endocochlear potential work synergistically to effectively drive ionic currents through open mechanotransducer channels, creating the large and rapid receptor potential changes that mediate glutamate release at the synapse between the hair cell and the auditory nerve. The endocochlear potential is established through the development of tight cellular junctions between local networks of epithelial cells, connective tissue and supporting cells that completely partition the endolymph from the surrounding perilymph. These tightly bound networks also efficiently recycle K^+ from the hair cell back into the endolymphatic space where they can once again be used in sensory transduction.

The spontaneous generation of action potentials from sensory receptors is considered essential for normal neural circuit development throughout the brain. In the developing auditory system, the mechanisms responsible for spontaneous action potential activity are still unresolved but recent reports suggest that this spontaneous activity is generated by IHCs of the cochlea. The cartoon of the IHC region in the immature Organ of Corti represents one proposed set of developmental changes that occur in cochlear circuitry (Figure 2.1(a)). Compared to IHCs in mature animals, which are surrounded by one or two supporting cells (see Figure 2.1(a)), the pre-hearing Organ of Corti features a structure known as the greater epithelial ridge, or Kollikers organ (Ko). This structure consists of non-neuronal inner supporting cells (ISCs) that are present up to the onset of hearing. However, by the time of hearing onset, Ko undergoes programmed cell death and subsequent removal of the majority of ISCs. Despite this dramatic change in the structure of the organ of Corti, recent studies have identified a potential role for ISCs in the initiation of electrical signaling within the auditory nerve (Tritsch, 2007). One to two weeks prior to the onset of hearing, the elongated

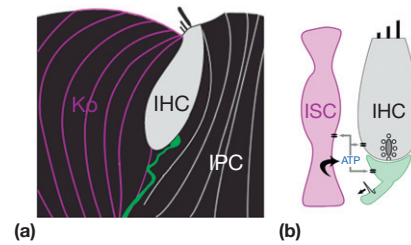


FIGURE 2.2 Transient microcircuits in the developing Organ of Corti. (a) Schematic of the region surrounding the inner hair cell in the pre-hearing rodent. Compared to the same region in the adult cochlea (Fig. 2.1(a)), the immature cochlea features a proliferation of elongated inner supporting cells called Kollikers' organ (Ko, in purple) and inner pillar cells (IPC). (b) Inner supporting cells in Ko (ISC, purple) release ATP prior to hearing onset. ATP binds to purinergic receptors (black ellipses) to promote Ca^{2+} -dependent glutamate release from ribbon synapses within the inner hair cell (IHC, gray) and action potentials in auditory nerve fibers (green).

ISCs within Ko begin to spontaneously release adenosine tri-phosphate (ATP) into the extracellular space (Figure 2.2(b)). ATP activates purinergic receptors on neighboring IHCs, peripheral processes of the auditory nerve and on the ISCs themselves. Binding of ATP on the IHC depolarizes the membrane potential, inducing Ca^{2+} -dependent glutamate release and bursts of action potentials in auditory nerve fibers. ATP release is local and desynchronized along the length of the cochlea. In this manner, spatially and temporally independent volleys of electrical signals initiated by non-sensory neurons entrain the firing patterns of SG and, ultimately, central auditory neurons. This process is thought to play a role in the strengthening of functional circuits prior to the onset of hearing.

Despite this role of ATP release from Ko, it remains uncertain how early action potential activity is patterned and whether ATP binding drives IHC membrane voltage or provides weaker modulatory control. More recent data suggests that during the first postnatal week of life, developing IHCs intrinsically generate the voltage changes that elicit action potentials in SG neurons. The frequency and pattern of this spontaneous action potential activity varies between regions of the cochlea (i.e., high-frequency versus low-frequency) and are modulated in multiple ways by the release of acetylcholine (ACh) and ATP near the IHCs (Johnson, 2011). It has been proposed that this pattern of action potential activity, along with ACh and ATP modulation, could be important for guiding tonotopic organization and the refinement of sensory information along the central auditory pathways before the occurrence of experience-drive information becomes relevant.

2.2.2 Development of the Place Code

The basilar membrane acts as a spectral analyzer that translates vibration frequencies within the cochlear fluid

pressure waves into positions of maximal displacement along its length. In mature animals, the BM is relatively narrow and taut at its base (violet region, [Figure 2.1\(b\)](#)) compared to the apex, which is wider and more mobile (red region, [Figure 2.1\(b\)](#)). As previously mentioned, this structural gradient confers a smooth shift of preferred vibration frequency along its length, with high frequencies maximally activating basal regions of the BM and low frequencies maximally activating apical areas. As with the visual and somatosensory pathways, the spatial organization of the receptor organ is maintained through topographic connections between the receptor epithelia and successive levels of the central sensory pathways. In the auditory system, the one-dimensional tonotopic arrangement of preferred frequency along the length of the BM is preserved in tonotopic maps of preferred frequency within the central auditory nuclei.

In nearly every respect, the development of basal (high frequency) regions of the cochlea occurs before apical (low frequency) regions; apical hair cells are the last to differentiate and the last to be innervated by afferent and efferent nerve fibers that convey signals to and from the brain. Therefore, one would predict that sensitivity to high frequency sounds would emerge before low frequencies in development. Interestingly, behavioral and neurophysiological hearing assessments in dozens of avian and mammalian species show just the opposite: high frequency sensitivity is the last to mature. This developmental mismatch implies that either tonotopic connections between the periphery and central auditory system are undergoing large-scale rewiring, or that developmental changes within the cochlea cause a given position along the BM to vibrate at progressively higher frequencies during the early period of hearing. Direct neurophysiological recordings from first-order auditory ganglion neurons that innervate a fixed point within the basal cochlea provide clear support for this latter scenario ([Echteler, 1989](#)). Basal cochlear regions were found to be maximally sensitive to low frequencies at the onset of the hearing and then become gradually responsive to higher frequencies. This developmental shift in the cochlear place code has been traced to a progressive maturation of OHC mechanics ([Norton, 1991](#)).

2.2.3 Development of Afferent and Efferent Circuits

The mature functional circuit linking the auditory periphery to the brain has four essential processing stations: 1) sensory hair cells in the auditory periphery, 2) first-order spiral ganglion cells (SG) that send a peripheral dendrite to the IHCs and OHCs and a central axon to the brain (i.e., the auditory nerve), 3) the cochlear nucleus (CN), a second-order auditory brain nucleus that is heavily innervated by auditory nerve fibers, and 4)

brainstem neurons whose efferent projections innervate IHCs, OHCs and neurons of the CN ([Figure 2.3\(a\)](#)). The two types of sensory receptors in the mammalian cochlea (IHCs and OHCs) are each innervated by two types of SG neurons: Type I and Type II. Type I afferents comprise approximately 95% of all SG neurons and each contact a single IHC. A single IHC, in turn, can be innervated by 10–20 Type I afferents, providing parallel and topographically specific information transfer from a single IHC to the CN. By contrast, a single Type II SG neuron contacts 30–60 OHCs, providing a weaker, spatially integrated signal to CN neurons. Although the OHCs contribute comparatively little afferent input to the brain, they are the predominant targets of efferent axons from the medial olivocochlear neurons (MOC) in the brainstem. In mature animals, the central processes of the auditory nerve terminate in the CN. In some cases, one or two auditory nerve fibers contact a single CN neuron via a massive axosomatic synapse called the endbulb of Held. The process through which this circuit achieves its mature form reflects an interplay of molecular processes and intrinsic activity-dependent processes that can be broken down into three phases.

Phase 1: Well Before Hearing Onset

In rodents, the peripheral processes of SG neurons innervate basal regions of the cochlea approximately five days before birth, or approximately 17 days prior to hearing onset ([Figure 2.3\(b\)](#)). Within a day of growing into the peripheral epithelia, afferents can be sorted into Type I or Type II morphologies. It is generally agreed that there are no gross errors or widely exuberant connections between SG dendrites and sensory hair cells. However, the exact precision of longitudinal (basal to apical) and radial (IHC to OHC) innervation patterns by Type I afferents is not entirely understood. Genetic fate mapping studies have described highly precise and rapid initial targeting ([Koundakjian, 2007](#)), while ultrastructural and histochemical studies find that Type I afferents initially innervate multiple IHCs and OHCs ([Echteler, 1992](#)). The weight of evidence favors this latter characterization. ACh-releasing efferent fibers grow into the sensory epithelia at the same time or slightly before afferent fibers. Compared to afferent innervation, however, efferent fibers show a clear developmental shift in their spatial targeting, as olivocochlear efferent fiber innervation is initially biased towards IHCs rather than OHCs ([Pujol, 1978](#)).

The central projections of SG neurons reach their targets in the CN approximately one day before their peripheral dendrites innervate the sensory epithelia. Despite the fact that the cochlear nucleus is still forming at this stage, projections from the SG are remarkably precise and demonstrate a clear spatial organization long before intrinsic or sensory-evoked action potential signaling begins. Although little is known about the

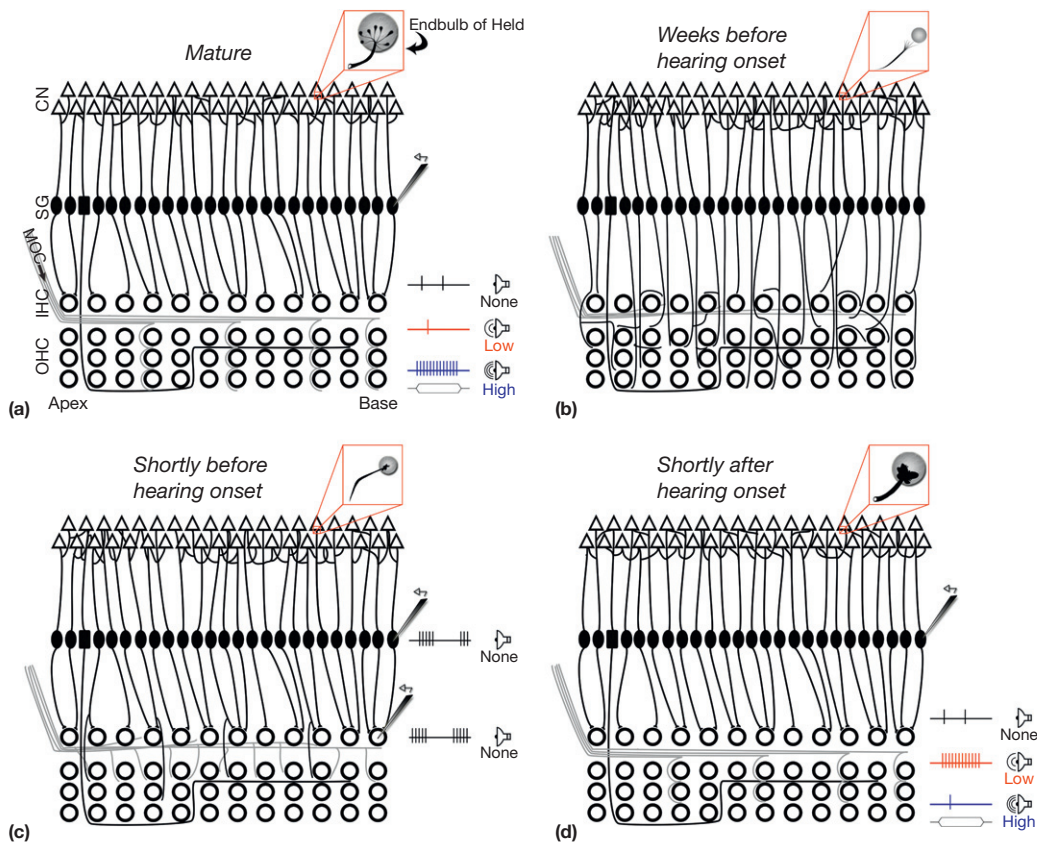


FIGURE 2.3 Development of afferent and efferent circuits linking the periphery and the brain. (a) In mature mammals, Type I (ellipse) and Type II (rectangle) spiral ganglion neurons (SG) extend a peripheral processes to a single row of inner hair cells (IHC) or three rows of outer hair cells (OHC), respectively. The central projection of SG neurons form elaborate axosomatic synapses called endbulbs of Held on specific types of neurons within the cochlear nucleus (CN, triangle). Efferent axons from medial olivocochlear (MOC) neurons innervate OHCs. Recording from a SG neuron innervating to the base of the cochlea would reveal occasional action potentials in the absence of sound or in response to low frequency sounds but elevated firing rates in response to high frequency sound (sound onset and offset represented by the trapezoidal stimulus). (b) Weeks before hearing onset, patterns of peripheral, central and efferent connections have been established, but lack the precise organization seen in mature animals. (c) In the days leading up to hearing onset, Type I/ II and MOC connections are sorted into their correct hair cell targets and rhythmic trains of Ca^{2+} spikes initiated in IHCs entrain the firing patterns of Type I SG neurons. Endbulb synapses are beginning to form. (d) In the days following hearing onset, peripheral innervation patterns are largely mature and central projection are approaching the topographic specificity and synaptic structure observed in mature animals. SG neurons innervating basal regions of the cochlea are responsive to low frequency tones, rather than high, due to immature basilar membrane and stereocilia mechanics.

molecules or cellular interactions participating in the establishment of auditory nerve fiber topography, several growth factors and receptors are expressed at approximately the time that connections are being established. In particular, differential distribution of the Eph/ephrin family of receptor tyrosine kinases in the auditory nerve and CN suggests an axon guidance mechanisms that shapes the formation of topographic connectivity within the auditory brainstem (Cramer, 2005).

Phase 2: Shortly Before Hearing Onset

Following the initial period of exuberant connectivity, the dendrites from Type I ganglion cells begin to coalesce around individual IHCs (Figure 2.3(c)). Conversely, efferent MOC fibers are in a transitional state during which immature connections to IHCs are maintained alongside newly formed connections with OHCs. IHC

mediated spontaneous spiking is robust during this period and electrophysiological recordings reveal temporally patterned IHC Ca^{2+} spikes that are mimicked by the firing patterns of SG neurons (Tritsch, 2010). In addition, functional synapses between SG and CN neurons are established, but the shape and overall size of endbulbs in the CN are still quite immature.

Phase 3: Shortly After Hearing Onset

At this stage, peripheral innervation of the receptor epithelia is essentially mature with Type I and Type II afferent endings making appropriate contacts with IHCs and OHCs, respectively. MOC efferents drop their IHC connection and make nearly exclusive contact with OHCs. Rhythmic spontaneous bursting gives way to stochastic spontaneous action potentials intermingled with temporal and topographically structured sound-evoked

responses. However, recordings from SG neurons innervating basal IHCs reveal an immature preference for low frequency sounds, as described in the section on place code development above. The topography from the SG neurons to the CN is initially very precise. However, more recent findings in mammals show that subtle topographic refinement continues around the onset of hearing and continues for several months, suggesting a combination of intrinsic and extrinsic activity-dependent mechanisms (Leake, 2002). Additionally, endbulb synapses continue to undergo clear structural modifications. Though they do not yet reach the state of a fully developed calyx that engulfs most of the CN cell body in mature animals, the axon terminals increase in diameter by an order of magnitude relative to the pre-hearing period (Jhaveri, 1982; Ryugo, 1982).

2.2.4 Conclusions

In a matter of weeks, what began as an outpocketing of epithelial cells near the embryonic hindbrain becomes an exquisite functional circuit, capable of encoding mechanical vibrations spanning three orders of magnitude in frequency, a 120 dB dynamic range for amplitude encoding (a million-million-fold change in signal energy), and sensitivity to sub-atomic stereocilia displacements with microsecond mechanical response times. The physical attributes of sound are captured by a tonotopically-organized array of sensory hair cells, which form topographic connections with neurons in the CN to initiate the psychological experience of hearing. This complex circuitry arises through the interaction of sensory and non-sensory supporting cells within the developing cochlea in addition to afferent and efferent connections that link the cochlea to the brain. Other than a modest topographic refinement in the spatial distribution of central projections to the CN and a fairly dramatic transformation of the endbulb synaptic terminal shape and overall size, these circuits reach a mature form independent of sensory input. Instead, maturation appears to reflect the dominant influence of genetic programming and molecular guidance cues with a subordinate role for cell-cell interactions that may include - but are not limited to - internally generated action potential patterns.

In humans, the development of peripheral circuitry is paralleled by enormous changes in infant phonological perception. The earliest stages of perceptual refinement can be attributed, at least in part, to the physical maturation of the outer ear, middle ear and cochlea. However, the scope of perceptual processes that continue to come online after peripheral circuits have matured as well as their dependence upon normally patterned sensory input both point toward the essential role of central auditory circuits in the development of hearing.

2.3 DEVELOPMENT OF BRAINSTEM CIRCUITS

2.3.1 Functional Circuit Assembly in the Brainstem

The assembly of functional circuits within the auditory system has been the subject of intense study over the past 30 years. Auditory brainstem nuclei are derived from progenitor cells within the hindbrain that migrate to their appropriate positions shortly before hair cells in basal regions of the cochlea are born. Like the central projection of SG neurons to the CN, guidance of embryonic brainstem neurons and assembly of their interconnections are thought to be mediated by the Eph/ephrin signaling, although the detailed signaling pathways have yet to be defined (Cramer, 2005). Axonal connections into second- and third-order nuclei are fully formed several days before hearing onset in birds and rodents. Direct electrical stimulation of the afferent axons in pre-hearing animals reveal that these connections form functional synapses shortly after they innervate their target nuclei (Jackson, 1982). The tonotopically organized connection between the auditory spiral ganglion neurons and the CN is preserved in projections to higher-order nuclei. That this topography is initially present from the time connections are formed further supports the influence of activity-dependent mechanisms on circuit formation in the auditory system.

Functional circuits in the auditory brainstem of mammals (Figure 2.4(a)) and birds (Figure 2.4(b)) can be separated into three functional divisions: 1) an excitatory projection from the second-order auditory nucleus (green), 2) inhibitory inputs (red), and 3) third-order nuclei that establish binaural sensitivity by integrating these excitatory and inhibitory inputs (white).

In mammals, CN neurons receive afferent input from the SG and project ipsilaterally to the lateral superior olive (LSO) and contralaterally to the medial nucleus of the trapezoid body (MNTB). The medial superior olive (MSO) gets binaural excitatory inputs from both the ipsilateral and contralateral CN. The MNTB extends a glycinergic (inhibitory) projection ipsilaterally to the MSO and LSO.

In birds, cochlear ganglion neurons extend a central process into two second-order brainstem nuclei, nucleus magnocellularis (NM) and nucleus angularis (NA). NM neurons make bilateral projections to nucleus laminaris (NL), a third-order nucleus analogous to the MSO in mammals. Whereas the mammalian MSO receives a powerful direct inhibitory input from MNTB, the inhibitory brainstem nucleus in birds, SON, modulates the convergent excitatory strengths of bilateral inputs to NA, NM, and NL.

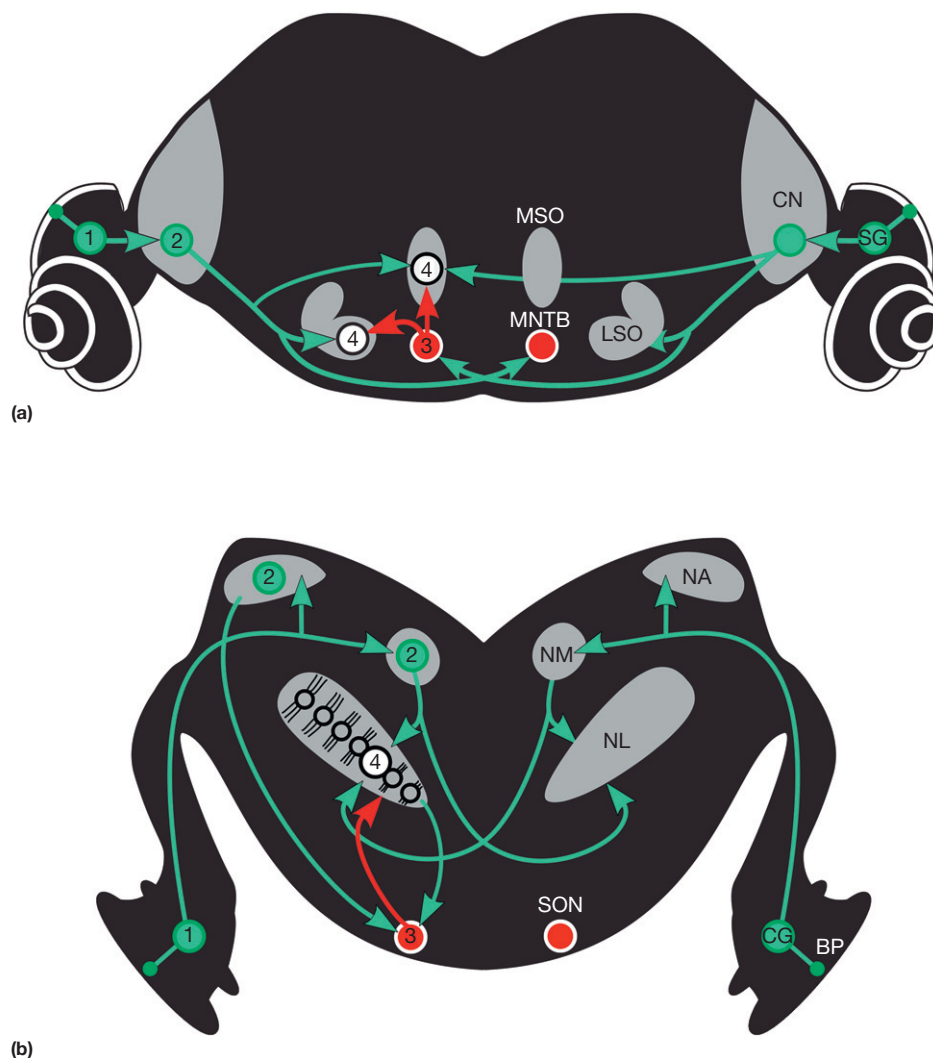


FIGURE 2.4 Organization of mammalian and avian brainstem circuitry. Schematic coronal sections of the auditory brainstem nuclei in rodents (A) and chicken (B). Brainstem circuits feature central projections from the primary sensory ganglion neurons (1) to 2nd order auditory nuclei in the brainstem (2). 3rd order brainstem nuclei (4) integrate inputs from 2nd order nuclei and local inhibitory nuclei (3). Excitatory projections are shown in green, inhibitory in red, nuclei in grey. For mammals, SG=spiral ganglion, CN=cochlear nucleus, LSO=lateral superior olive, MSO=medial superior olive, MNTB=medial nucleus of the trapezoid body. For birds, BP=basilar papilla, CG=cochlear ganglion, NA=nucleus angularis, NM=nucleus magnocellularis, NL=nucleus laminaris, SON=superior olivary nucleus. For both schematics, the dorsal surface of the brainstem is facing up.

2.3.2 Development of Fine-Scale Connectivity in the MSO

The spatial position of a visual or tactile stimulus can be encoded according to where, along the two-dimensional layout of the retina or skin, activity is greatest. In the auditory system, the functional layout of the cochlea is reduced to a single dimension mapped to sound frequency. The horizontal position of a sound source in space must be computed centrally, by neurons sensitive to differences in the loudness or timing of sounds arriving to each ear. Neurons in the MSO of mammals (NL in birds) are specialized to extract

microsecond differences in the timing of excitatory inputs from the CN (or NM) associated with each ear. In mammals with low frequency hearing, such as gerbils, this precise tuning to interaural time differences (ITDs) appears to be enhanced by developmental changes in the subcellular positioning of inhibitory glycinergic inputs from the MNTB (Werthat, 2008). Glycinergic terminals from MNTB are evenly distributed across the dendrites and cell bodies of MSO neurons round the time of hearing onset. In the days and weeks that follow, the less effective inhibitory synapses on the distal ends of the dendritic tree are eliminated, sparing the

proximal axosomatic inhibitory synapses (Figure 2.5). Alterations at the subcellular level are paralleled by a progressive refinement in the overall topographic breadth of presynaptic axon innervation across the topographic map in MSO. These anatomical changes are associated with increased temporal precision and efficacy of synaptic inhibition onto MSO neurons as well as sharpening of neural ITD tuning functions in the auditory brainstem, suggesting a direct link between physiological maturation and its structural underpinnings. Taken together, the developmental shifts in the topographic and subcellular distribution of inhibitory inputs to the MSO work synergistically to sharpen the spatial and temporal sensitivity of MSO neurons to the excitatory CN inputs arriving from each side of the brain.

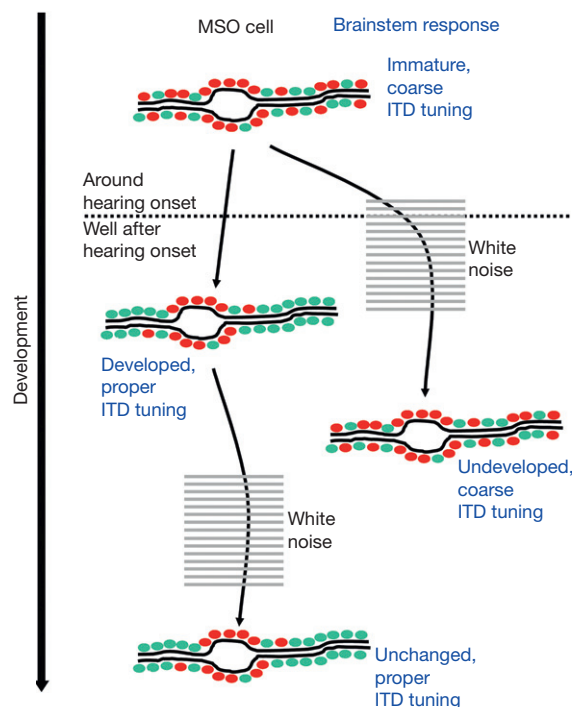


FIGURE 2.5 Correlation between development of inhibitory inputs and ITD tuning. Before hearing onset, inhibitory glycinergic synapses (red dots) and excitatory glutamatergic synapse (green dots) on MSO neurons are distributed over soma and dendrites. After hearing onset, the glycinergic synaptic inputs get refined to the cell body. This refinement depends on meaningful acoustic experience and can be interrupted by the exposure of omnidirectional white noise during hearing onset. White noise exposure at an adult stage has no effect on glycine receptor distribution. ITD tuning responses in the auditory brainstem correlate with the development of inhibitory inputs to MSO. When animals were exposed to white noise between P10 and P25, ITD tuning responses recorded were found to have the same characteristics as right after hearing onset, when neuronal responses to ITDs are immature and coarse. Under control conditions, i.e. with normal exposure to sound, ITD sensitivity developed normally. White noise exposure in adult animals has no effect on ITD tuning.

2.3.3 Development of Fine-Scale Connectivity in the LSO

LSO neurons are sensitive to interaural sound level differences via tuning to the relative strength of excitatory inputs from the ipsilateral ear and inhibitory inputs from the contralateral ear. Unlike the MSO, contralateral inputs are only expressed indirectly, via a local inhibitory connection from the MNTB. The sharp binaural tuning of LSO neurons is thought to arise from the integration of tonotopically matched excitatory inputs from the ipsilateral CN and MNTB. By contrast to MSO synapses, which are predominantly remodeled after hearing onset, topographic plasticity in the LSO proceeds in two phases: a functional silencing of MNTB inhibitory inputs prior to hearing onset followed by a structural pruning of MNTB axon terminals after the onset of hearing.

The pre-hearing functional refinement phase takes place contemporaneously with the period of ATP-induced Ca^{2+} spikes in IHCs. Accordingly, recordings from MNTB neurons at this age reveal spontaneous action potential bursts with the same rhythmic patterns observed in SG neurons (Tritsch, 2010). These activity patterns also depend upon an intact connection with IHCs, which may indicate that intrinsic spontaneous “test patterns” generated in the Organ of Corti prior to hearing onset may play a role in both peripheral and central circuit formation. During this 3–4 day period, the spatial spread of MNTB-derived inhibition shrinks by approximately 75%, considerably sharpening the breadth of inhibition along the tonotopic axis (Kim, 2003).

The onset of hearing ushers in a phase of structural refinement. In the week following hearing onset, dendritic arbors of postsynaptic LSO neurons increase in branching complexity yet are culled to more confined space within the topographic map. These postsynaptic modifications are accompanied by the physical elimination of tonotopically misaligned presynaptic axon terminals from the MNTB. For a more detailed description of inhibitory circuit development the reader is referred to Chapter 131 of this volume by K. Kandler et al.

2.3.4 Afferent Regulation of Cochlear Nucleus Development

These functional and structural changes in the brainstem synaptic networks during the weeks surrounding hearing onset provide correlational evidence that spontaneous and sensory-evoked events are needed to achieve the elegant organization of neurons and connectivity observed in mature animals. The strong test of this hypothesis has been carried out by dozens of studies over the last sixty years that examine age-dependent deviations from normative development following

disruption or complete elimination of afferent input from the cochlea. The seminal study by Rita Levi-Montalcini (Levi-Montalcini, 1949) removed the otocyst (the precursor of hair cells and ganglion neurons) of chicks at an early stage of embryonic development, thereby depriving auditory brainstem nuclei of cochlear input. She noted that neurons in NA and NM developed normally until E11 (the approximate time of hearing onset in the chick), at which point the size and overall number of surviving neurons rapidly declined. This observation suggested a dependence of CN neuron survival upon an intact connection with the periphery and has inspired a number of researchers to delve deeper into the role of afferent signaling in the formation and maintenance of brainstem circuitry.

Subsequent studies in the chick and rodents have further characterized the nature and timing of CN degeneration following deafferentation. Unilateral otocyst removal at E3 or cochlea removal prior to sexual maturity in chicks or cochlear destruction shortly after birth in rodents produces a variety of changes, including: 1) the CN on the ablated side has significantly fewer neurons than the intact side and the surviving neurons have smaller soma and neuropil area; 2) an ectopic – yet tonotopically aligned – projection from the normally innervated CN grows into the CN on the deafferented side; and 3) in mammals, the endbulb of Held development described in Figure 2.3 never reaches the fully mature state (reviewed in Harris, 2006).

In most animals, these plasticity effects are strictly limited to a developmental critical period. As noted above, the effects of otocyst removal in the E3 chick are not apparent for another eight days, when active connections with the periphery are first established. The critical period for CN neuron survival ends as abruptly as it begins, as cochlear destruction in gerbils during the first postnatal week causes 45-90% of CN cells to die, yet has no effect on the number of surviving neurons when the same manipulation is performed at P9, just prior to the onset of hearing (Tierney, 1997). The remarkably rapid changes in susceptibility of CN neurons to deprivation appears to reflect a differential weighting of factors that promote versus inhibit cell death.

Collectively, studies of CN development demonstrate that the proliferation, migration and formation of appropriate topographic connections are complete before action potentials begin to appear in auditory nerve fibers. The arrival of normal spontaneous and sound-evoked afferent action potentials has little effect on cellular morphology or topographic specificity. However, pathological deviations from the normal developmental trajectory (e.g., cochlear removal) that occur within a defined critical period window radically alter CN organization, leading to pronounced cell death and atrophy of surviving cells. The cellular mechanisms that close

the critical period of CN vulnerability are not yet fully established, but gene array analyses suggest that glial proliferation and up-regulation of immunity-related genes may play an important role.

In addition to deafferentation-induced cell death and atrophy, hearing loss also induces a long-term enhancement of neuronal excitability. *In vitro* measurements of neurons in acute slices of the CN demonstrate a shift towards enhanced excitation and diminished inhibition. The loss of balanced excitation and inhibition arises from an abnormal sorting of membrane-bound ligand-gated neurotransmitter receptors and voltage-gated ion channels. Ongoing research is exploring the hypothesis that this pathological over-excitability in the brainstem and other stations of the central auditory pathways may be the source of tinnitus, the perception of phantom sounds that can accompany hearing loss.

2.3.5 Afferent Regulation of 3rd-Order Brainstem Nuclei

All auditory-evoked signals in the brain are initially routed through the CN. Therefore, alterations in CN morphology resulting from cochlear ablation could also impact the organization of downstream nuclei. Indeed, the post-hearing structural refinement of axon terminals from the MNTB to the MSO and LSO described previously is substantially diminished without a connection to an intact cochlea. Moreover, unilateral cochlea removal before hearing onset enhanced an otherwise weak physical connection between the normally innervated CN and the opposing side of the MSO.

Unlike the inhibitory projections from the MNTB to the LSO and MSO, excitatory projections from the CN undergo considerably less developmental refinement and are largely insensitive to cochlear removal. The CN, for example, forms a giant excitatory synapse onto MNTB neurons called the calyx of Held. This calyx is the largest synapse in the mammalian brain and features glutamatergic CN terminals that almost completely envelop the MNTB neuron, ensuring high-fidelity transmission necessary for sharp interaural time- and level-dependent tuning observed in the MSO and LSO, respectively. In mature brains, one calyx innervates a single MNTB neuron. Although the calyx undergoes substantial changes in shape during the first weeks of postnatal development, the one-to-one connectivity is present from the time CN projections initially arrive in the MNTB and is established with or without spontaneous or evoked action potential from the auditory nerve (Hoffpauir, 2006).

The effects of deafferentation have been extensively studied in NL of the chicken. The intrinsic organization and connectivity patterns within NL lend themselves to

studies of afferent regulation of individual dendrites. First, NL exhibits a clear gradient of dendritic geometry that varies from small, short and stubby to large, long and elaborate across the high-to-low tonotopic map. This gradient appears to begin to form around the time of hearing onset. Second, NL neurons have two sets of symmetrical dendrites, one set oriented dorsally to contact glutamatergic inputs from the ipsilateral NM, the other ventrally to contact glutamatergic inputs from the contralateral NM. This organization permits researchers to directly compare the effects of unilateral manipulations (which would affect the dorsal dendrites on the ipsilateral NL and the ventral dendrites on the contralateral NL) to transection of the NM axons at midline (which would affect the ventral dendrites on both sides of the brain).

Using both of these approaches, researchers have discovered an interesting dichotomy in the afferent regulation of intrinsic features versus functional circuit properties. Removal of synaptic input to one side of NL induces a progressive retraction of dendrites on the corresponding side on a surprisingly short timescale (Figure 2.6). Tracking these changes over time reveals that retraction begins within 1 hour, can last over 2 weeks, and can amount to as much as a 60% reduction in length, demonstrating that NL neurons can rapidly regulate significant amounts of membrane surface devoted to specific excitatory inputs (Deitch, 1984). By contrast to the rapid calibration of dendritic length allocated to the dorsal versus ventral NM inputs, the short-to-long gradient of relative dendrite length across the tonotopic axis of NL is largely unaffected by cochlear removal.

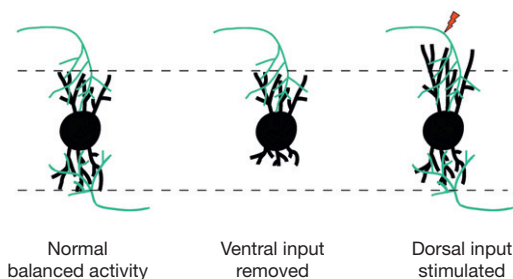


FIGURE 2.6 Compartment-specific regulation of afferent activity on dendritic structure in the chick nucleus laminaris (NL). NL neurons have bipolar dorsal and ventral dendrites (black), receiving highly segregated excitatory inputs by an axon from either the ipsilateral or contralateral ear via the nucleus magnocellularis (NM). (Left), Under normal physiological conditions, the two dendritic domains receive balanced inputs (green), each elicited by one ear, and their dendrites are of similar length. (Middle), Following the deafferentation of the inputs to one domain, e.g. when axons from one side of the brain get cut, deprived dendritic branches rapidly retract, while the length of the other dendrite remains unchanged. (Right) Physiological stimulation of the axon inputs to one set of dendrites (red lightning bolt), but not the other, leads to a growth of the stimulated dendritic branches and a retraction of unstimulated dendritic domain.

2.3.6 Influence of the Source and Pattern of Afferent Activity on Brainstem Circuits

The effects of cochlear removal on brainstem circuits point towards a panoply of developmental events that depend upon afferent signals from the periphery. Cochlear removal eliminates both spontaneous and sound-evoked action potentials in addition to the physical degeneration of SG neurons. Because the dependence on afferent activity is most commonly observed after hearing onset, one might assume that sound-evoked activity provides important signals for the fine-tuning of brainstem circuits. An alternative explanation holds that any afferent action potential signaling, be it spontaneous or evoked, could be sufficient for normal assembly of brainstem circuits.

To isolate the relative contributions of spontaneous action potentials versus sound-evoked activity, researchers have compared the effect of pharmacologically silencing all afferent action potentials versus simply blocking the transduction of acoustic signals. The effects of tetrodotoxin infusion into the inner ear at the time electrical signaling first begins were indistinguishable from the effects of cochlear removal: approximately 40% of the neurons died with widespread neuronal atrophy in the survivors (Born, 1988). On the other hand, simply blocking sound-evoked activity (by disrupting the sound transmission mechanisms of the outer or middle ear) without eliminating spontaneous activity in auditory nerve did not cause atrophy or cell death in the CN (Tucci, 1985). These results suggest that action potentials, or more probably the voltage-gated changes in glutamate and calcium signaling, provide a necessary source of trophic support during the critical period of CN development. Although the particular patterning of sound-evoked action potentials was not necessary for the normal cellular maturation of this second-order nucleus, it had a significant impact on stimulus selectivity and circuit formation in downstream nuclei. For instance, rearing animals in omnidirectional noise interferes with low frequency signals necessary to calculate interaural time differences. Absent these instructive environmental signals, the MSO fails to develop at a normal pace and features widely branching axodendritic MNTB synapses rather than the topographically focused axosomatic synapses found in normally reared animals (Seidl, 2005; Figure 2.5). Similarly, interaural level difference tuning in brainstem neurons is significantly altered when owls are reared with an earplug that deprives them of normally calibrated binaural cues (Mogdans, 1994).

2.3.7 Conclusions

In summary, functional circuit development and refinement in the auditory brainstem is thought to reflect

an interplay between molecular cues coupled with spontaneous and evoked action potential activity. Factors such as connectional topography in the LSO and spatial gradients of dendritic length in NL appear to be governed by intrinsic, activity-independent mechanisms and form prior to the onset of hearing. Cochlear action potentials before and after the onset of hearing predominantly regulate the size and subcellular positioning of synaptic contacts. The development of precise connectivity is less categorical: the proper development of axonal projections from the MNTB to MSO and LSO is dependent upon afferent signaling after hearing onset, the topographic specificity of SG connections into the CN are subtly modified by afferent signaling, while excitatory projections from the CN to their ipsilateral and contralateral brainstem targets develop independently of cochlear signaling (although they make aberrant connections should one cochlea be removed).

Compared to peripheral circuits, which have matured in many respects by hearing onset, brainstem circuits undergo additional refinement after sound-evoked activity is introduced to the system. These phenomena are likely to contribute to the progressive improvement in infant auditory perceptual acuity in humans and animals. For example, human infants become increasingly capable of resolving the fine positioning of sounds along a horizontal plane, which may reflect the developmental calibration of synaptic properties within the MSO and LSO. Other aspects of phonological development, including the neural specializations that underlie the acquisition of language, can only be understood by examining the development of functional circuits in higher levels of the central auditory system.

2.4 DEVELOPMENT OF AUDITORY MIDBRAIN AND FOREBRAIN CIRCUITS

Higher auditory circuits are assembled from a diverse set of excitatory and inhibitory connections arising from three critical sources: 1) the inferior colliculus (IC), a midbrain auditory brain structure, 2) the medial geniculate (MG) and reticular (Rt) divisions of the thalamus, and 3) the auditory cortex (Actx) (Figure 2.7). These nuclei are interconnected through a complex array of feedforward, feedback and intrinsic connections. Inputs from the two ears are heavily intermixed, given the feedforward binaural inputs from the MSO and LSO as well as interhemispheric connections between each IC and Actx. Both the IC and MG are obligatory relays for auditory signals reaching the Actx and, consistent with brainstem circuits, a tonotopic organization within these connections is evident 5-7 days before birth in rodents, long before the initiation of afferent signaling from the periphery.

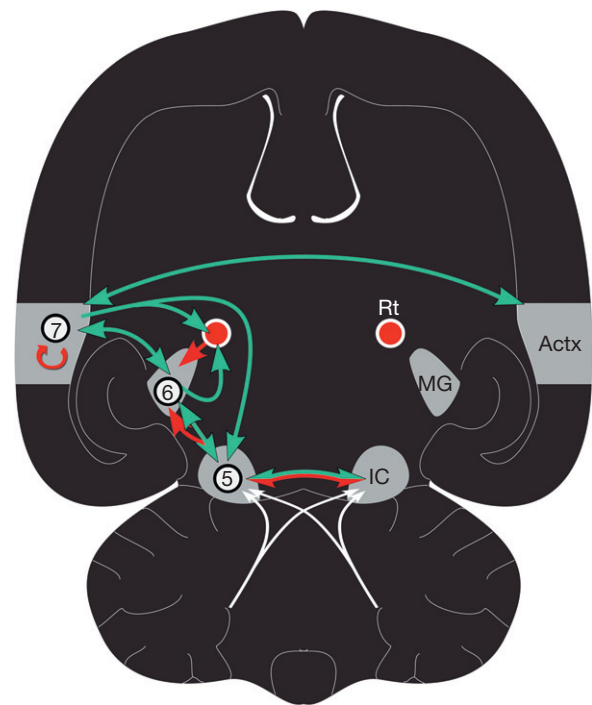


FIGURE 2.7 Organization of higher auditory circuits. Schematic of a horizontal section through a rodent brain. Convergent bilateral input from the brainstem (white arrows) projects to the inferior colliculus (IC), an auditory midbrain structure (5). The IC connects to the auditory thalamus (6) and cortex (7) through a complex chain of feedforward, feedback and intrinsic excitatory (green) and inhibitory (red) connections. MG=medial geniculate body, Rt=reticular nucleus of the thalamus, Actx=auditory cortex. Top of the figure is rostral (i.e., anterior), bottom is caudal (i.e., posterior).

2.4.1 Development of Thalamocortical Subplate Circuitry

Compared to the wealth of studies in NL, LSO and MSO, functional circuit developments in higher auditory brain areas have yet to be characterized in detail at the cellular level. One emerging exception is the observation of a transient microcircuit linking the neonatal thalamus and cortex. Cortical neurons originate from the ventricular zone and reach their final positions within the six-layered cortical plate via local radial migration or, in the case of several classes of inhibitory interneurons, via long-distance horizontal migration along the rostral stream (additional information on patterning and neural migration within the cerebral cortex can be found in [Rubenstein and Rakic, 2013](#)). Subplate neurons (SPN) reside in the white matter beneath the cortical plate, physically interposed between the ascending MG axons and the incipient circuits forming in the more superficial layers of the cerebral cortex (for a comprehensive review of subplate neurons, see [Kanold, 2010](#)).

SPN development occurs at an accelerated pace compared to the other cortical neurons; SPNs are among the

first neurons to appear in the cerebral cortex, the first cortical neurons to fire action potentials, and are almost completely eliminated around the time of hearing onset. SPNs have elaborate dendritic trees that integrate excitatory inputs from the MG and local inhibitory inputs. SPN axon terminals ramify extensively in the same layers of the cerebral cortex that will receive the bulk of direct axonal input from the MG in subsequent stages of development. SPNs have high input resistances, relatively depolarized resting membrane potentials and are electrically coupled to one another via gap junctions, making them a sensitive and potent source of excitatory input to Actx prior to hearing onset.

Their precocious morphological and biophysical development combined with their physical position between MG and Actx make SPNs ideal interlocutors in the postnatal assembly of thalamocortical circuits. Indeed, as represented in Figure 2.8, MG axons innervate the subplate days before birth and wait there for days or weeks (depending on the species) before innervating the middle cortical layers. During this waiting period, SPNs make excitatory glutamatergic projections to excitatory and inhibitory neuron subtypes within Actx, potentially providing a source of activity-dependent synaptic refinement before connections are established with the MG.

2.4.2 Postnatal Development of Local Cortical Circuits

Cortical circuits undergo a subsequent wave of refinement during the second postnatal week, after the

majority of SPNs have been eliminated. At the onset of hearing, sound-evoked responses in Actx are restricted to frequencies at the center of the hearing range presented at high sound levels. Over the following 2–3 days, response thresholds decrease and sensitivity to a broader range of sound frequencies begins to emerge. Following this brief period of change, which almost certainly reflects peripheral maturation, recordings from Actx neurons reveal a gradual improvement in their ability to synchronize action potential timing to rapid modulations of an incoming sound source. This improvement in cortical temporal processing is a hallmark feature of central auditory development and thought to be essential for the accurate encoding of important environmental sounds such as speech.

Through targeted recordings of excitatory and inhibitory neuron subtypes in Actx using an *in vitro* brain slice preparation, researchers have discovered that the progressive elimination of temporal “sluggishness” towards the end of the second postnatal week is associated with a confluence of synaptic and intrinsic changes in Actx neurons. In addition to afferent input from MG, acetylcholine (ACh)-positive axon terminals from neurons located in the basal forebrain begin innervating the Actx in postnatal week 1 and reach adult levels by postnatal week 2. The maturation of cholinergic terminals coincides with significant changes in the levels or composition of nicotinic ACh receptors and NMDA receptors (nAChRs and NMDARs, respectively) in Actx (Metherate, 2003). Although ACh does not excite Actx neurons directly, it can enhance excitatory transmission indirectly by binding to nAChRs, which in turn modulate glutamatergic NMDARs. The decline of nAChRs

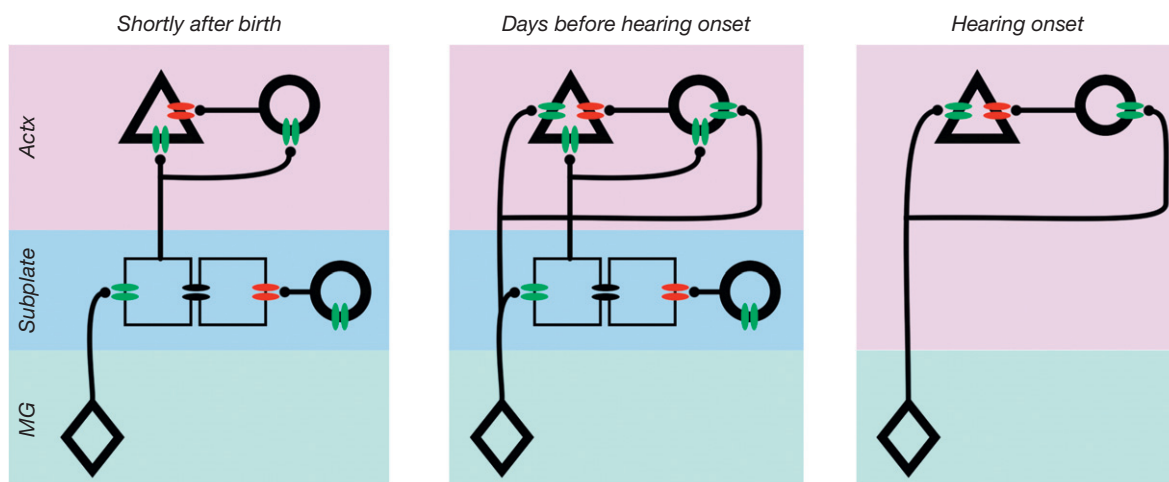


FIGURE 2.8 Subplate neurons form a transient microcircuit linking the auditory thalamus and cortex. Subplate neurons (SPNs, squares) mediate excitatory signaling from the medial geniculate nucleus of the thalamus (MG, diamond) to excitatory (triangle) and inhibitory (circle) cells in the auditory cortex (Actx, triangle). Shortly after birth (left), SPNs are believed to be the exclusive source of subcortical input to Actx. In the days before hearing onset (middle), SPNs mediate direct excitatory projections to Actx before they are eliminated, shortly thereafter (right). Green ellipses = glutamate receptor, red ellipses = GABA receptor, black ellipses = electrical synapse.

in Actx by postnatal week 3 works synergistically with changes in AMPA and NMDA glutamate receptor subunit composition to reduce the duration and increase the amplitude of excitatory postsynaptic currents in Actx (Figure 2.7(a)). Thus, the transient appearance of nAChRs during postnatal week 2 represents a critical period during which ACh can prolong the time course of NMDA-dependent synaptic excitation around the period of hearing onset.

The sharpening of synaptic excitation in Actx around the time of hearing onset is mirrored by a progressive enhancement of synaptic inhibition. Presynaptically, the voltage-gated K^+ channel subtypes present in GABAergic interneurons change significantly over the first three weeks of postnatal development to adjust the resting membrane potential and shorten the refractory period following an action potential. These biophysical changes are complemented by the progressive loss of GABA_B receptors on presynaptic interneurons, enabling higher sustained firing rates without fatiguing. Postsynaptically, the GABA_A receptor subunit composition in excitatory Actx neurons changes over the first weeks of development to eliminate the $\alpha 3$ subunit in favor of the $\alpha 1$ and $\beta 2/3$, both of which are associated with faster inhibitory synaptic current rise times (Figure 2.9(a)). Collectively, these intrinsic and synaptic changes in the time course of excitatory and inhibitory synaptic

transmission endows Actx neurons with an improved ability to track rapid temporal fluctuations in sound signals with high fidelity (for review see Sanes, 2009).

2.4.3 Afferent Regulation of Higher Auditory Circuit Development

Detailed characterizations of auditory brainstem circuit development in the absence of cochlear signaling have revealed a combination of activity-dependent and activity-independent processes. To assess the generality of these principles throughout the central auditory pathways, researchers have also examined the role of an intact periphery on the maturation of IC and Actx circuits. The potentially confounding influence of CN degeneration has been avoided in this type of experiment by bilaterally removing the cochleae after the critical period for CN cell death, but before the onset of hearing, such that higher auditory circuits have the potential to be shaped by spontaneous – but not sound-evoked – action potentials.

Recordings made in the acute brain slice preparation weeks after cochlear ablation reveal dramatic alterations in the strength and time course of excitatory and inhibitory synaptic currents in the IC (Figure 2.9(b)) and Actx (Figure 2.9(c)). Compared to control slices taken

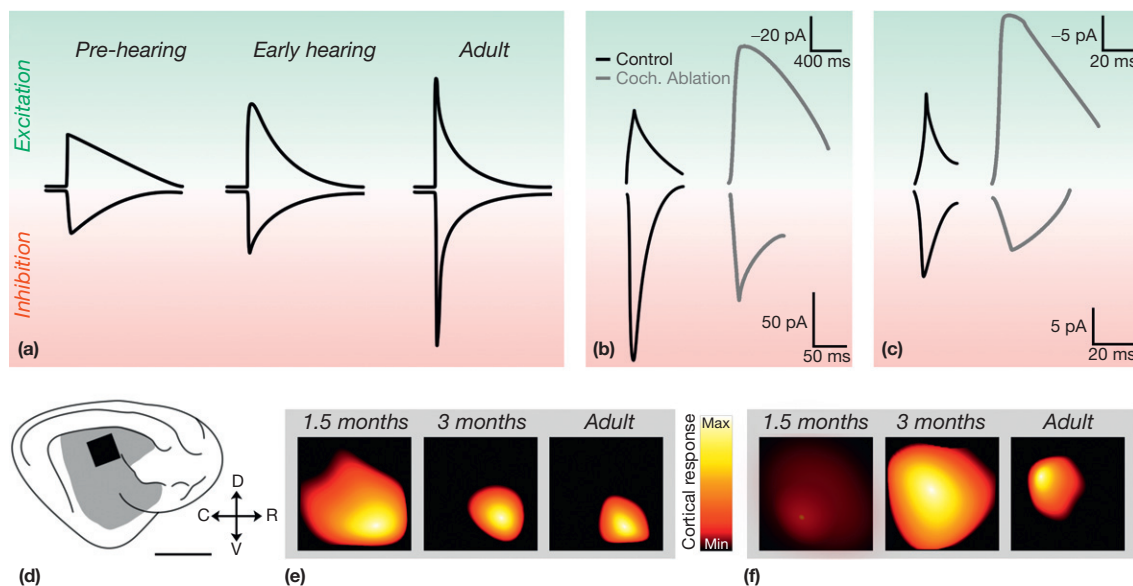


FIGURE 2.9 Afferent regulation of higher auditory circuits. (a–c) Black lines represent excitatory (upward) and inhibitory (downward) synaptic currents recorded from brain slices containing auditory cortex (a, c) or inferior colliculus (b) neurons. Auditory cortex neurons display faster and stronger synaptic currents over development based on changes in intrinsic and synaptic properties. This process is arrested following bilateral cochlear ablation, wherein neurons are hyperexcitable and display immature temporal dynamics. Current amplitude is plotted on the vertical axis. (d) Lateral surface of the cat brain. Gray denotes total area of auditory cortex in normal cats. Black square denotes area from which neurophysiological signals are measured in panels e and f. D = dorsal, R = rostral, V = ventral, C = caudal. Horizontal bar = 1 cm. Inward negative EPSCs are plotted upwards, and outward positive IPSCs are plotted downwards, by convention. (e–f) Areal extent of neural responses in auditory cortex evoked from activation of a stimulating electrode implanted proximal to the auditory nerve fibers in acutely deafened (e) or congenitally deaf (f) cats. Current traces in (b) and (c) are adapted from data presented in Sanes and Bao, 2009.

from normally hearing animals, neurons in cochlear-ablated slices areas show weaker, prolonged inhibition that is qualitatively similar to activity found in normal animal prior to hearing onset. Synaptic excitation is also prolonged in a similar fashion to pre-hearing animals, yet the amplitudes are far greater than those observed in the course of normal development, suggesting that deafferentation tips the homeostatic balance between excitation and inhibition towards greatly enhanced excitability, in a similar fashion to the CN (Kotak, 2005).

Recent studies have identified a combination of intrinsic and synaptic factors that may explain the failure of synaptic inhibition to mature normally. In terms of intrinsic biophysical mechanisms, the first postnatal weeks are marked by a substitution of voltage-gated K^+ channels that mediate faster membrane kinetics as well as the appearance of a K^+ -dependent intracellular chloride transporter, KCC2. During the first week of postnatal development, when KCC2 expression levels are low, intracellular chloride concentrations are higher than the electrochemical equilibrium potential and GABA release from inhibitory neurons induces a depolarization of the membrane potential in postsynaptic neurons, rather than the expected hyperpolarization. As levels of membrane-bound KCC2 increase in the second week of postnatal development, greater amounts of intracellular chloride are extruded into the extracellular space, thereby lowering the equilibrium potential and establishing the normal hyperpolarizing influence of GABA. This developmental process is arrested in the IC of cochlea-ablated animals, where the inhibitory reversal potential can be elevated by as much as 24 mV above normally hearing age-matched controls, reducing the hyperpolarizing effect of GABA binding. Although overall expression levels of KCC2 are not affected, pharmacological experiments reveal that cochlear ablation arrests the normal age-dependent maturation of KCC2 function, contributing to reduced levels of inhibitory signaling (Vale, 2003).

A synaptic piece of the puzzle was discovered through a comparison of GABA_A receptor subunit composition in the Actx of normal and deafferented animals. Recall from the description above that GABA_A receptors composed of the $\alpha 1$ and $\beta 2/3$ subunits appear during the second postnatal week and are partially responsible for the transition to shorter, larger-amplitude inhibitory synaptic currents. Following bilateral cochlear ablation, juvenile Actx neurons fail to express the mature form of the membrane-bound GABA_A receptor, thereby preventing the sharpening of the inhibitory postsynaptic currents observed in age-matched controls (Kotak, 2008). Taken together, these results demonstrate a broad spectrum of intrinsic and synaptic events that fail to develop in the absence of sound evoked-activity. The sum total of these events render IC and Actx neurons

incapable of tracking rapid fluctuations in sound properties, an essential characteristic of normal hearing.

2.4.4 Developmental Regulation over Reinstating Hearing in the Deaf

Synaptic transmission studies in the brain slice preparation shed some light on the molecular targets of afferent signaling and help to identify the complications and possibilities associated with reinstating hearing in deaf individuals. Unlike birds, and other non-mammalian vertebrates, which can regrow hair cells throughout life, mammals are born with all the cochlear hair cells they will ever have. Nevertheless, hearing is a possibility for profoundly deaf individuals through the use of the cochlear implant, a neural prosthetic device that bypasses the dysfunctional transduction machinery within the cochlea and reinstates afferent signals through direct electrical stimulation of auditory nerve fibers. Approximately 200,000 individuals have been fitted with cochlear implants over the past 40 years and it was discovered early on that the age of surgical implantation plays a crucial role in the quality of hearing experienced by cochlear implant users. While post-lingually deaf individuals often recover acceptable hearing and speech recognition whether they are implanted as children or adults, congenitally deaf individuals stand the best chance of experiencing the full benefit of the cochlear implant if they undergo the implantation procedure at an early age, typically by the time they are 7 years old (Dorman, 2007).

Through careful study of cochlear implants in a special breed of congenitally deaf cats, researchers have begun to understand how auditory brain areas represent signals delivered through the cochlear implant and the manner by which these representations are shaped through development and experience. As an experimental control, normally hearing cats are acutely deafened with an ototoxic drug and immediately fit with a cochlear implant. Neural recordings are made from the Actx of acutely or congenitally deaf cats at various ages in response to brief electrical pulses delivered the auditory nerve (Figure 2.9(d)). A comparison of activation patterns across development in acutely deafened cats reveals an exuberant spatial spread of neural activity across the Actx in young kittens that is culled to a topographically restricted activation area by 3 months of age (Figure 2.9(e)). By contrast, activating the implant in congenitally deaf kittens at 1.5 months evokes a weak cortical response (Figure 2.9(f)). At 3 months postnatal, deaf cats show the exuberant activation patterns comparable to normally hearing kittens at 1.5 months and these activation areas are not consolidated until early adulthood (Kral, 2005). The hyper-excitability of the Actx in congenitally deaf cats at three months may stem from

the diminished synaptic inhibition and augmented synaptic excitation observed in brain slices of rodents that undergo cochlear ablation in infancy (Figure 2.9(c)).

Although the mechanisms governing the recovery of hearing in cochlear implant users remain unclear, one possible clue has been found through analyzing the endbulb of Held synapse in deaf cats that began hearing through chronic use of the cochlear implant at a young age. As described previously, the endbulb synapse fails to develop normally in the absence of cochlear signaling. Strikingly, the endbulb synapse from cats using the cochlear implant for 3 months beginning at an early age was largely indistinguishable from normally hearing cats (Ryugo, 2005). Therefore, reinstating afferent activity to the central auditory system in early life can rescue the progressive synaptic degradation observed at several levels of the central auditory system.

2.4.5 Experience-Dependent Influences on Functional Circuit Development

For the most part, the influence of afferent signaling on neural circuit development has been described in the context of all-or-nothing manipulations; comparisons to normally hearing animals are made through cochlea removal, genetic deafness, or pharmacological silencing of the auditory nerve. However, many facets of auditory perceptual development depend upon the specific patterns of auditory experience rather than its presence or absence. For example, before a child utters a first word in its native language, it will have been exposed to hundreds of thousands of words that bear the phonemic structure specific to that language. One school of developmental cognitive psychology has argued that this repeated auditory exposure to speech sounds, when appropriately timed during childhood development, “primes” the auditory and vocal motor areas of the brain to specialize in the nuances of a given language. A half century of inspired neurobiology and neuroethology research has shown that the processes through which songbirds learn their vocal repertoire offers a striking parallel to human language acquisition. Song and speech learning both involve a complex interplay between innate predispositions and experience, both forms of learning are shaped by developmental critical periods, both require skilled control over the motor vocal apparatus, and both depend upon precisely calibrated auditory feedback (for a review of song learning, the reader is referred to (Brainard, 2002).

Although the circuitry underlying song acquisition is more closely linked to sensorimotor integration than auditory processing *per se*, auditory experience has also been found to exert a profound influence over the development of dedicated auditory circuitry within IC and

Actx. In rodents, a tonotopic map of preferred sound frequency tuning can be readily delineated from the primary field of the auditory cortex (AI), which is visible along the lateral surface of the brain (Figure 2.10(a)). In mature animals, the tonotopic map features an orderly low-to-high gradient of frequency tuning across the posterior-to-anterior extent of AI, with similar tuning organized into orthogonal iso-frequency contours (Figure 2.10(b)). Most recording sites feature closely matched frequency tuning for tones presented to each ear, albeit tuning evoked from contralateral stimuli is more robust than the weaker, high-threshold tuning for tones presented to the ipsilateral ear. By contrast to the adult map, AI recordings made shortly after the onset of hearing (P11-14) reveal narrowly tuned, high threshold responses restricted to the middle frequency regions of the tonotopic map (Figure 2.10(c)).

A series of studies over the past decade demonstrate that experimentally modifying the ambient acoustic environment experienced by young animals during critical periods of higher auditory circuit development can dramatically alter the normal developmental sequence described in Figures 2.10(b) and 2.10(c). For example, rearing litters of rodents in a sound environment dominated by the repeated presentation of a single tone at a fixed frequency more than doubles the area of the tonotopic map tuned to that exposure frequency (Figure 2.10(d)). As illustrated in Figure 2.10(d), exposure to a repetitive middle frequency tone for just 96 hours beginning at the onset of hearing is associated with an expansion of the corresponding area within the tonotopic map at the expense of flanking frequency representations. The tonotopic map retains this distorted organization into adulthood despite the fact that animals hear normal acoustic stimuli from P16 onwards. Conversely, rearing animals older than P16 to the repeated single frequency has no effect on the map regardless of the length of exposure time (de Villers-Sidani, 2007). Similar effects of tone rearing have been reported in the IC.

Whereas rearing animals in fixed frequency tone environments induces a region-specific reallocation of territory within the cortical map, rearing animals in broad-spectrum noise is associated with a degeneration of Actx tonotopy and an abundance of recording sites with abnormally broad tuning (Figure 2.10(e)). Similar to the extended visual cortex critical periods observed in animals reared in total darkness, the critical period for the effects of single tone exposure is abnormally prolonged in animals reared in white noise. Thus, deprivation of patterned acoustic input postpones the flip of a molecular switch that normally limits the critical period, and the effects of single tone exposure can distort the map many weeks after the critical period would normally have closed (Chang, 2003). That a critical period normally observed in infancy can be delayed

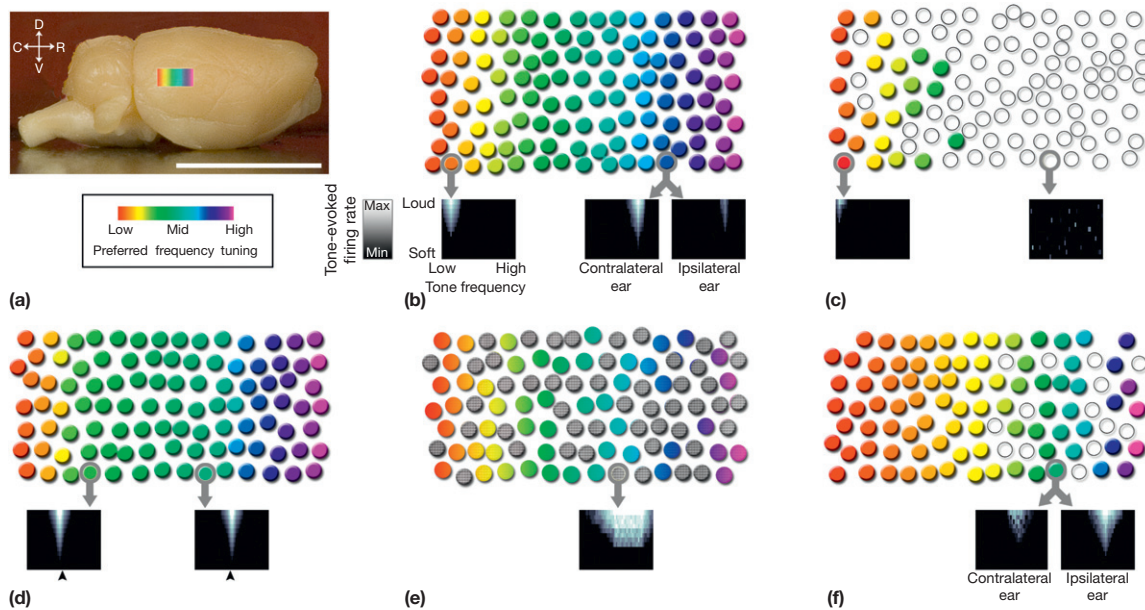


FIGURE 2.10 Experience-dependent map reorganization in the developing auditory cortex. (a) Photograph of the lateral surface of the rat brain. Color gradient denotes the position and orientation of the tonotopic map within the primary auditory cortex (AI). Horizontal line = 1 cm. (b–f) Schematics of tonotopic maps reconstructed from microelectrode recordings of tone-evoked action potentials at approximately 100 different points within AI. At each recording site, the frequency response area (grayscale surface plots) is determined by presenting tones at various frequencies and levels and identifying the frequency to which the neuron is tuned at its threshold intensity (indicated by the color of each dot). (b) Normal adult animals display a smooth and orderly tonotopic gradient in which neurons at the caudal and rostral boundaries of AI prefer low and high frequency tones, respectively. Neurons are tuned to the same frequency for sounds presented to each ear, yet responses to the contralateral ear are more robust than the ipsilateral ear. (c) Tonotopy at the onset of hearing is narrow, high-threshold and restricted to low frequency areas of the map. Open circles denote recordings sites with no evoked response. (d) Rearing rodents in acoustic environments dominated by presentation of a single middle frequency tone (represented by green colors and vertical arrows on surface plots) show an over-representation of that tone frequency within the tonotopic map such that recording sites at distant points within the map have similar tuning. (e) Rearing rodents in continuous white noise is associated with a disrupted tonotopic map featuring numerous recording sites with abnormally broad frequency tuning (represented by gray dots). (f) Reversibly closing the contralateral ear during critical periods of development is associated with an over-representation of low frequency tones, the loss of contralateral tuning at several points (open circles) and an enhancement of tuning strength to tones delivered to the developmentally unobstructed ipsilateral ear.

into adulthood supports the possibility that progressive development of auditory feature representation in Actx may be more closely linked to an experiential timeline, rather than a strict chronological timeline.

A parallel can also be drawn between the reversible lid suture method used extensively in studies of visual cortex development and the effects of reversible ear canal ligation on Actx and IC development. Ligating the ear canal temporarily interferes with the transmission of acoustic signals to the middle ear, and ultimately the brain, particularly at high frequencies. By reopening the ear canal prior to recording from the contralateral AI, researchers have observed a degraded tonotopic map populated by weak high-threshold responses to contralateral tones and the occasional absence of contralateral tuning in higher frequency regions of the map (Popescu, 2010). By contrast to the contralateral bias observed in normal animals (Figure 2.10b), the quality of tuning for stimuli delivered to the ipsilateral ear is often superior to the normally dominant contralateral ear (Figure 2.10f). Interestingly, ipsilateral inputs were only enhanced

when the ear canal was ligated in infant and juvenile animals, but not in adulthood. Collectively, these results indicate that the allocation of representational resources within the Actx and IC is quite dynamic and can be adaptively reassigned to the inputs that are most prominent during critical periods of early postnatal development.

2.4.6 Conclusions and Directions for Future Research

Compared to the periphery and brainstem, the assembly of circuits in higher auditory brain areas appears to be particularly sensitive to sound-evoked patterns of afferent action potentials. Eliminating hearing or even perturbing the normal balance of signals between the ears or across various frequency channels can dramatically alter auditory signal processing. This plasticity has been documented at multiple levels of analysis, ranging from the synaptic transmission between two neurons up to the coordinated arrangement of frequency tuning across

hundreds of thousands of neurons. Although circuit reorganization at higher levels of the auditory system is striking, additional information on the normative course of development in the IC, MG and Actx as well as a deeper mechanistic understanding about the contributions of transient microcircuits such as the SPN to circuit assembly prior to hearing onset will greatly aid efforts to understand how the stable networks that underlie normal hearing are constructed. Absent data that bridge the gap between synapses and neural networks in normally developing animals, it is difficult to know whether activity- or experience-dependent modifications derail normal development or introduce an abnormal outcome following the conclusion of normal maturation.

The past fifty years of auditory developmental research have revealed a great deal about the interplay between intrinsic molecular events and dynamic electrical signaling in functional circuit maturation. The scope of research in the coming years may widen to include other biological factors that help to shape functional circuits. Although neurons are the central players in functional circuits, they do not operate in isolation. Networks of developing non-sensory glial cells, cells that form the developing vasculature and the molecules that form the extracellular matrix play may key roles in modulating neural signaling and providing a physical substrate for growth. As we have seen in the cochlea, the non-sensory cells that make up Kölliker's organ may act as the catalyst for electrical signaling throughout the pre-hearing auditory system and it is probable that exciting new connections between neural development and the non-neural cells and molecules that support them remain to be discovered. On the other end of the spectrum, it will be important to link the observations made at a neurobiological level to their potential behavioral consequences measured at the level of the whole organism. Demonstrations of abnormal circuit connectivity or map organization are worthwhile and interesting in their own right, but take on additional importance when they can be associated with changes in perceptual abilities. Studies that bridge the gaps between these various levels of analysis and stations of processing along the auditory pathway promise to reveal more about the etiology and therapeutic possibilities for treating hearing impairment, and may teach us a great deal about the fundamental principles of neural development.

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