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REVIEW

Spontaneous activity in the developing auditory system

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Abstract Spontaneous electrical activity is a common feature of sensory systems during early development. This sensory-independent neuronal activity has been implicated in promoting their survival and maturation, as well as growth and refinement of their projections to yield circuits that can rapidly extract information about the external world. Periodic bursts of action potentials occur in auditory neurons of mammals before hearing onset. This activity is induced by inner hair cells (IHCs) within the developing cochlea, which establish functional connections with spiral ganglion neurons (SGNs) several weeks before they are capable of detecting external sounds. During this pre-hearing period, IHCs fire periodic bursts of Ca^{2+} action potentials that excite SGNs, triggering brief but intense periods of activity that pass through auditory centers of the brain. Although spontaneous activity requires input from IHCs, there is ongoing debate about whether IHCs are intrinsically active and their firing periodically interrupted by external inhibitory input (IHC-inhibition model), or are intrinsically silent and their firing periodically promoted by an external excitatory stimulus (IHC-excitation model). There is accumulating evidence that inner supporting cells in Kölliker's organ spontaneously release ATP during this time, which can induce bursts of Ca^{2+} spikes in IHCs that recapitulate many features of auditory neuron activity observed in vivo. Nevertheless, the role of supporting cells in this process remains to be established in vivo. A greater understanding of the molecular mechanisms responsible for generating IHC activity in the developing cochlea will help reveal how these events contribute to the maturation of nascent auditory circuits.

Keywords Cochlea · ATP · Glia · Spiral ganglion neuron · Inner hair cell

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Introduction

A major goal for developmental neuroscience is to elucidate how neurons assemble into a network to carry out its specific functions. While substantial progress has been made in understanding the genetic programs and guidance molecules required for proper connections, much less is known about another fundamental feature of developing neural circuits — the role of intrinsically generated or "spontaneous" activity. Action potentials that are not initiated by input from the external environment have been observed in the developing nervous system of many species and in many distinct regions of the nervous system (Blankenship and Feller 2010), including the retina (Galli and Maffei 1988; Meister et al. 1991), spinal cord (Landmesser and O'Donovan 1984), hippocampus (Ben-Ari et al. 1989; Garaschuk et al. 1998), cerebellum (Watt et al. 2009), and cochlea (Kros et al. 1998; Tritsch et al. 2007). The pervasiveness of this spontaneous activity suggests that it plays an important role in the maturation of neural circuits. Indeed, it has been implicated in regulating the proliferation, differentiation, and migration of neurons (Wong and Ghosh 2002; Moody and Bosma 2005; Spitzer 2006), and is thought to influence their structural maturation (Cohen-Cory 2002), axonal arborization, and ultimately their integration into neuronal circuits (Katz and Shatz 1996; Friauf and Lohmann 1999; Zhang and Poo 2001; Moody and Bosma 2005; Huberman et al. 2008; Blankenship and Feller 2010; Kirkby et al. 2013).

In the auditory system, spontaneous electrical activity that is present before the onset of hearing (defined as the age when auditory neurons reliably respond to airborne acoustic stimulation) has also been implicated in shaping the organization of nascent circuits. Most studies have been performed in either chicken (*in ovo*) or neonatal altricial animals, such as rodents and cats, which are born deaf and remain unresponsive to airborne sound until approximately the end of the 2nd postnatal week. In rodents, the inability to sense ambient sound is not due to the inability of hair cells to transform mechanical stimulation into electrical signals (Lelli et al. 2009) or the lack of functional connections between central cochlear neurons (Hoffpauir et al. 2009). Rather, deafness results from the combination of physical occlusion of the external ear canal, poor ossicular transduction in the middle ear, underdeveloped mechanics in the organ of Corti, immature ionic composition in the endolymph, and absence of the endocochlear potential (Bosher and Warren 1971; Anniko and Wroblewski 1986; Woolf and Ryan 1988; Rybak et al. 1992; Geal-Dor et al. 1993; Abe et al. 2007). The time of hearing onset for some common experimental animals is embryonic day 16 (E16) in chicken (Jones et al. 2006), postnatal day (P) 10–12 in mouse (Mikaelian and Ruben 1965; Ehret 1983), P11–13 in rat (Uziel et al. 1981; Ehret 1983; Geal-Dor et al. 1993), P10 in cat (Walsh and McGee 1987), and P12 in gerbil (Woolf and Ryan 1984), with the day of birth defined as P0.

The properties of sound-independent activity present during auditory system development are distinct from those which occur after hearing onset. Previous studies have demonstrated that the majority of auditory nerve fibers (ANFs) in hearing cats and rodents are continuously active in the absence of acoustic stimulation. The spontaneous discharge rate of individual ANFs can vary from only a few Hz to more than 100 Hz (Kiang 1965; Liberman 1978; Walsh and McGee 1987; Taberner and Liberman 2005); the pattern of spiking over time is relatively continuous for a given fiber, which displays regular discharges without discernible periods of silence (Walsh and McGee 1987; Jones et al. 2007). Similar patterns of activity have also been recorded from brainstem auditory nuclei (Kopp-Scheinpflug et al. 2008; Sonntag et al. 2009; Crins et al. 2011), and in other species (guinea pig: Manley and Robertson 1976; Manley et al. 1991; chicken: Jones and Jones 2000). This regular firing behavior is distinct from the patterns of activity present in the developing auditory system. Before hearing onset, a larger proportion of ANFs and brainstem auditory neurons are silent and do not exhibit spontaneous discharges, a phenomenon that decreases with age (Romand and Marty 1975; Shnerson and Willott 1979; Romand 1984; Walsh and McGee 1987). Although the average discharge rate of active cells is only several Hz (Shnerson and Willott 1979; Brugge and O′Connor 1984; Romand 1984; Walsh and McGee 1987; Kotak and Sanes 1995; Jones et al. 2007; Sonntag et al. 2009; Crins et al. 2011), auditory neuron activity is concentrated into brief bursts that are followed by long periods of silence (Romand and Marty 1975; Shnerson and Willott 1979; Romand 1984; Walsh and McGee 1987; Kotak and Sanes 1995; Sonntag et al. 2009; Tritsch et al. 2010a; Crins et al. 2011). Similar firing behavior has been observed in the cochlear ganglion and in higher-order neurons in the embryonic chicken (Lippe 1994; Jones et al. 2001), in the auditory midbrain of pre-hearing horseshoe bats (Rubsamen and Schafer 1990), and in the cochlear nucleus from pouch young wallaby (Gummer and Mark 1994), suggesting that the mechanisms responsible for initiating this activity may be highly conserved.

In this review, we attempt to outline current knowledge about the generation, developmental changes, and function of spontaneous activity that occurs in the auditory system before hearing onset. For simplicity, we will focus on rodents and restrict our discussion of functional consequences to the auditory brainstem, where most experimental results have been obtained.

Spontaneous activity in the auditory system originates within the cochlea

Although spontaneous activity has been recorded within different central auditory nuclei, it is believed that this activity is initiated from the developing cochlea. Bursting activity in auditory brainstem neurons of embryonic chick was eliminated following removal of the cochlea or by application of tetrodotoxin (TTX) to the oval window to block firing of ANFs (Lippe 1994), and similar bursts of action potentials have been recorded directly from spiral ganglion neurons (SGNs) in vivo (Jones et al. 2001; 2007). Furthermore, it has been shown that the spontaneous action potentials recorded in vitro from SGNs exhibit a stereotyped pattern consisting of repeating mini-bursts, which is also seen in the spontaneous discharge of neurons in the medial nucleus of the trapezoid body (MNTB) and the central nucleus of inferior colliculus (IC) in vivo (Tritsch et al. 2010a). Although it is possible that this activity is intrinsically generated by SGNs, the spontaneous action potentials exhibited by SGNs are dependent on Ca^{2+}-mediated transmitter release from inner hair cells (IHCs) (Robertson and Paki 2002; Tritsch and Bergles 2010), suggesting that this distinct firing behavior reflects events happening within the developing organ of Corti before the onset of hearing.

IHCs in the pre-hearing cochlea are capable of firing Ca^{2+}-based action potentials (termed "Ca^{2+} spikes") (Kros et al. 1998), which have much slower kinetics than conventional Na^{+}-based action potentials. These spikes are mediated primarily by L-type Ca^{2+} channels containing the $Ca_v1.3$ subunit (Platzer et al. 2000; Brandt et al. 2003), but the kinetics of these events are also modified by other ion channels (Marcotti

et al. 2003a, 2003b, 2004; Kennedy 2012). Each Ca^{2+} spike allows a large bolus of Ca^{2+} to enter the cell, which is sufficient to trigger Ca^{2+}-mediated glutamate release from immature ribbon synapses (Beutner and Moser 2001; Glowatzki and Fuchs 2002; Johnson et al. 2005). This synaptic activity induces SGNs to fire action potentials that are carried to the auditory brainstem via the eighth nerve. IHCs exhibit small Ca^{2+} currents and exocytotic membrane capacitance changes in response to current injection as early as E16.5 (Marcotti et al. 2003a; Johnson et al. 2005), and by E17.5 depolarization elicits broad Ca^{2+} spikes in IHCs (Marcotti et al. 2003a). Thus, despite the immaturity of ribbon synapses and the lower Ca^{2+} efficiency of exocytosis (Sobkowicz et al. 1982; Beutner and Moser 2001; Johnson et al. 2005), IHCs are capable of releasing neurotransmitter several weeks before hearing onset.

Although the precise timing of synapse formation between SGNs and IHCs has not been clearly defined, the peripheral neurites of developing SGNs extend into the sensory epithelium even before hair-cell differentiation, with nascent contacts between these cells formed as early as E18 in both rat (Pujol et al. 1998) and mouse (Huang et al. 2007). Glutamate receptors are expressed by SGNs before birth (Luo et al. 1995; Puyal et al. 2002), and morphologically-defined synapses are present at P0, in which intracellular ribbon-like structures in IHCs are apposed to electron-dense regions of SGN dendritic membranes (Sobkowicz et al. 1982). Consistent with the presence of these synaptic structures, depolarization-evoked glutamate exocytosis from IHCs can trigger action potentials in SGNs at P0 (Tritsch and Bergles 2010). This activity is unlikely to be restricted to the cochlea, as the central projections of SGNs grow into the hindbrain quite early (Appler and Goodrich 2011) and reach the cochlear nucleus by E15.5 (Koundakjian et al. 2007). Within the brainstem, functional synapses between auditory relay neurons are also present at birth (Friauf and Kandler 1990; Kandler and Friauf 1995b; Hoffpauir et al. 2006, 2009; Rodriguez-Contreras et al. 2008). Together, these anatomical and functional studies indicate that the transduction pathway from IHCs to developing circuits of the brain is established well before hearing onset, providing a substrate that spontaneous activity can act upon to influence its developmental trajectory.

Despite the capacity of IHCs to generate Ca^{2+} spikes and release glutamate before hearing onset, it is still under debate whether the spontaneous activity exhibited by IHCs arises from cell intrinsic processes or is induced (or modified) by an external stimulus. Previous studies indicate that IHCs in the pre-hearing cochlea of rodents continuously fire Ca^{2+} spikes in vitro without apparent external stimulation (Kros et al. 1998; Marcotti et al. 2003a, 2003b, 2004; Brandt et al. 2007). To reconcile this pattern of activity with the discontinuous, bursting pattern recorded from auditory neurons in vivo, it has been proposed that phasic inhibitory cholinergic input from medial olivocochlear (MOC) efferents periodically interrupts the tonic firing of IHCs (Kros 2007) (Fig. 1). Indeed, cholinergic efferent discharge can hyperpolarize IHCs and disrupt the initiation of Ca^{2+} spikes (Glowatzki and Fuchs 2000); if these efferents fire prolonged, high-frequency bursts in vivo, they could suppress the generation of Ca^{2+} spikes (Goutman et al. 2005). In support of this model, inhibition of acetylcholine receptors in acutely isolated cochleae changed the firing pattern of IHCs from bursting to sustained activity (Johnson et al. 2011), suggesting that cholinergic efferents can sustain high rates of release even when removed from their cell bodies in the brainstem. The involvement of these cholinergic efferents could explain the discrepancy between in vitro and in vivo activity patterns, if the extent of release from these axons varies after cochlea isolation. This model satisfies the cochlear origin of spontaneous activity, and provides an explanation for the abrupt decline in burst firing at hearing onset, which coincides with the cessation of both MOC efferent innervation of IHCs (Katz et al. 2004; Roux et al. 2011) and Ca^{2+} spike generation by IHCs (Kros et al. 1998). Nevertheless, several observations suggest that cholinergic input is not essential for generating bursts of activity in IHCs and auditory neurons during this period.

At present, there is a lack of consensus about whether IHCs fire continuously in vitro at this age. While some studies have shown that IHCs sustain tonic firing, as noted above (Kros et al. 1998; Marcotti et al. 2003a, 2003b, 2004; Brandt et al. 2007), others reported that IHCs fire bursts of Ca^{2+} spikes in the apex (Tritsch and Bergles 2010; Johnson et al. 2011), or all along the length of the cochlea (Sendin et al. 2014). Moreover, it has been shown that IHCs and SGNs exhibit burst activity in cochlear explants that have been maintained in vitro for several days (Tritsch et al. 2010a), despite the absence of cholinergic axons. Furthermore, to accommodate the long periods of silence between bursts observed in vivo, the cholinergic model requires that MOC neurons fire sustained discharges for up to several seconds; unfortunately, there have been no in vivo recordings from these neurons to determine if they exhibit such activity during this developmental stage. Finally, this model predicts that removal of MOC cholinergic input would result in a conversion of auditory neuron activity from bursting to continuous firing. However, a recent study showed that MNTB neurons in vivo continue to fire in discrete bursts in mice that lack the $\alpha 9$ acetylcholine receptor subunit, which is required for MOC-mediated inhibition of IHCs (Vetter et al. 1999; Clause et al. 2014). Together, these results indicate that inhibitory input from MOC efferents is not essential to induce burst firing in auditory neurons, and suggest that there may be other extrinsic mechanisms to trigger periodic excitation of IHCs.

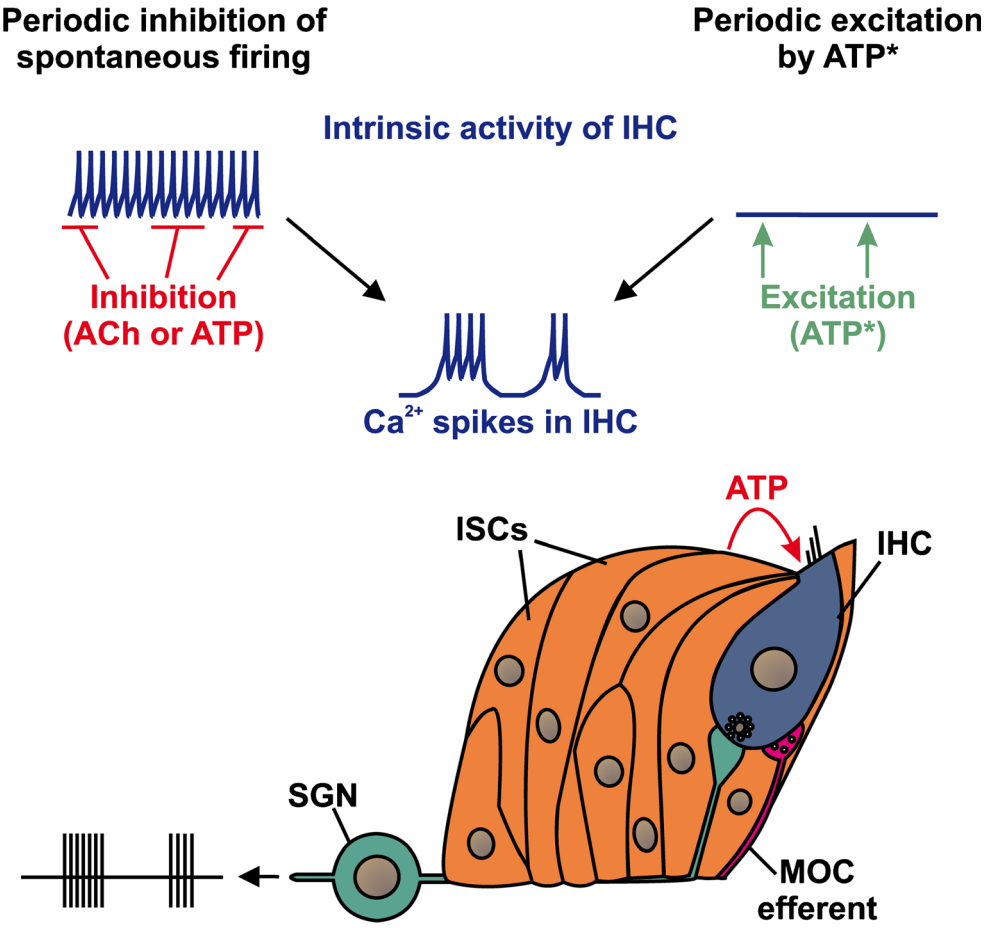

Fig. 1 Two models to explain how spontaneous bursts of activity could be induced in IHCs before hearing onset. The "IHC-inhibition" model (at *left*) proposes that IHCs are depolarized and therefore tend to fire Ca^{2+} spikes continuously. Periodic inhibition of IHCs by an external modulator such as acetylcholine (ACh) or adenosine triphosphate (ATP) would interrupt this firing, transforming continuous activity into burst firing. The "IHC-excitation" model (at *right*) proposes that IHCs are hyperpolarized and thus predominantly silent in the absence of an external stimulation. When ATP (* or another excitatory modulator) is spontaneously released from ISCs, IHCs are slowly depolarized, triggering a brief train of Ca^{2+} spikes. In both scenarios, the bursts of IHC Ca^{2+} spikes induce glutamate release and bursts of action potentials in SGNs that are subsequently carried to the auditory brainstem via the eighth nerve

An alternative model proposes that IHCs are excited by the periodic release of adenosine triphosphate (ATP) from neighboring supporting cells. Previous studies have shown that a group of pseudostratified inner supporting cells (ISCs) that together form Kölliker's organ (greater epithelial ridge), which lies medial to IHCs, exhibit spontaneous inward currents in the pre-hearing cochlea that are mediated by ATP. Similar events can be elicited by exogenous ATP or UTP, an agonist of P2Y receptors, and inhibited by purinergic receptor antagonists (suramin, PPADS). Strikingly, these cells shrink or crenate when stimulated with ATP or UTP, an effect that is triggered by a rise in intracellular Ca^{2+} (Tritsch et al. 2010b). Although the significance of these crenations is not known, it provides a mean to pinpoint the source of ATP — since all of the cells crenate when exposed to ATP, the site of crenation indicates where ATP was released. These studies indicate that the supporting cells themselves release ATP, which induces crenation by activation of P2 autoreceptors. Recordings from IHCs revealed that they exhibited similar slow inward currents, many of which were of sufficient magnitude and duration to induce bursts of Ca^{2+} spikes, and simultaneous recordings from IHCs and ISCs, while crenations were imaged, revealed that these three phenomena were coincident and required activation of purinergic receptors (Tritsch et al. 2007). Moreover, recordings from SGNs in isolated cochleae revealed that this IHC activity was sufficient to induce bursts of action potentials, providing a plausible mechanism to explain the periodic activity of auditory neurons observed in vivo. Indeed, subsequent studies revealed that SGNs exhibit a stereotyped firing pattern during this prehearing period, in which each burst is comprised of a series of mini-bursts, a reflection of the ability of each IHC Ca^{2+} spike to induce repetitive firing of SGNs (Tritsch et al. 2010a). This stereotyped firing pattern within bursts is also exhibited by neurons in the cochlear nucleus and the MNTB (Tritsch et al. 2010a; Clause et al. 2014), providing further support for the cochlear origin of this activity. Together, these experiments indicate that ATP is spontaneously released from ISCs in the pre-hearing cochlea, which activates purinergic receptors on IHCs (and ISCs), ultimately leading to bursts of action potentials in SGNs and auditory neurons of the brain (Fig. 1). Random variations in the location and amount of ATP released from ISCs provide an explanation for observed variations in the duration and frequency of action potential bursts in auditory neurons. This model also provides an explanation for the decline in phasic spontaneous activity around hearing onset, as Kölliker's organ progressively regresses after birth, ultimately forming the inner sulcus around the time of hearing

onset (Hinojosa 1977; Kelley 2007); ISCs and IHCs remain responsive to ATP after hearing onset (Tritsch and Bergles 2010), suggesting that a key event in the cessation of spontaneous activity is the decline in ATP release from ISCs. In this model, burst firing does not require sustained firing of MOC efferents, consistent with the preservation of bursting activity in MNTB neurons in α9 knockout animals (Clause et al. 2014), although this cholinergic input could provide feedback inhibition to influence burst firing (e.g., the duration and magnitude of bursts, as well as the number of IHCs that are activated). ATP appears to be released focally along the length of the cochlea, and is degraded by ectonucleotidases, which are expressed in the pre-hearing cochlea (O′Keeffe et al. 2010). As a result, each ATP release event is accompanied by the activation of discrete groups of IHCs proximal to the site of ATP release; such synchronous activity among IHCs, and thus SGNs, could help establish tonotopic wiring in the auditory pathway through Hebbian-like plasticity mechanisms (Tritsch et al. 2007).

Despite this evidence that supporting cell dependent excitation of IHCs is a primary driver for spontaneous activity in the developing auditory system, there are a number of unresolved issues, as well as experimental observations that appear to contradict this model. For example, it has been shown that exogenous ATP can have excitatory (Tritsch et al. 2007), inhibitory (Sendin et al. 2014), or dual effects (Johnson et al. 2011) on IHC firing, depending on the concentration and method of application used. In addition, one study reported that purinergic receptor *inhibition* increases the firing rate of IHCs (Johnson et al. 2011). Moreover, the activity of IHCs recorded in whole mount preparations from the early postnatal cochlea varies from infrequent Ca^{2+} spikes and bursting behavior (Brandt et al. 2007; Tritsch and Bergles 2010) to steady firing (Marcotti et al. 2003a; Sendin et al. 2014).

Although the reasons for these different observations have not yet been determined, it is possible that they reflect differences in experimental conditions between different laboratories. The organ of Corti is contained in a specialized environment in vivo, with one side exposed to endolymph containing a high K^+ concentration (157 mM) and the other side surrounded by perilymph (6.0 mM K^+ in perilymph of scala vestibule and 4.2 mM K^+ in scala tympani) (Wangemann and Schacht 1996). Unfortunately, it is not possible to mimic such a polarized environment in vitro. Moreover, the distinct ionic compositions of endolymph and perilymph are not fully established until the end of first postnatal week (Bosher and Warren 1971; Anniko and Wroblewski 1986), a time when there are many physiological changes occurring in the organ of Corti. Although many laboratories perform experiments in 6 mM extracellular K^+; it is unclear whether this K^+ concentration mimics the in vivo ionic environment. In addition, the kinetics of Ca^{2+} spikes are affected by temperature and the degree of intracellular Ca^{2+} buffering (Johnson et al. 2011). While these conditions can be reasonably replicated by different laboratories, it is more difficult to control for differences in the state of the tissue after isolation. IHCs exhibit high resting Ca^{2+} levels following tissue isolation (Wang and Bergles, unpublished results) and reduced responsiveness to ATP. Although resting IHC Ca^{2+} levels are lowered in preparations cultured for as little as one day (Wang and Bergles, unpublished results), exposure to culture media and isolation from the CNS may induce other changes that cause it to diverge from the in vivo state. Finally, MOC efferent axons are isolated from their cell bodies upon cochlear isolation, which prevents an analysis of their physiological activity patterns and extent of feedback from central auditory neurons. Thus, although acutely isolated cochleae and cochlear slices preserve complex intercellular relationships and offer greater experimental access, they have significant limitations that prevent ready extrapolation to in vivo conditions, necessitating confirmation of key findings in the intact nervous system.

Developmental changes in spontaneous activity

As described above, anatomical and physiological studies indicate that the transduction pathway from IHCs to the brain is established in early postnatal life in rodents; however, there have been only a few in vivo studies to assess the developmental time course of spontaneous activity in the cochlea or auditory centers of the brain. Juxtacellular recordings from rat MNTB neurons revealed that they fire isolated spikes at P0, but the proportion of burst-firing neurons rapidly increases with age, and constitutes over 91 % of all units by P4 (Tritsch et al. 2010a). The principal neurons of MNTB are readily excited by glutamatergic inputs at P0-P1 as a result of their high input resistance (Rusu and Borst 2011), suggesting that the lack of burst-firing at P0 is not because principal neurons cannot respond to synaptic inputs, but because there is little peripheral input at this age. Another in vivo study in rat also showed that MNTB neurons exhibit bursting activity at P4, which transitions to continuous firing after the middle of the 2nd postnatal week, reaching the adult pattern after hearing onset (Crins et al. 2011). Similar observations have been reported in mice, where MNTB neurons shift rapidly from bursting activity at P8–P10 to a regular, non-bursting discharge pattern at P11–12, just before the onset of hearing (Sonntag et al. 2009). These results indicate that spontaneous activity in pre-hearing rodents emerges postnatally, with a distinct bursting pattern emerging before P4 that is maintained until hearing onset.

A more extensive in vitro assessment of developmental changes in the spontaneous firing of IHCs has been performed primarily using cochlear tissue isolated from rodents. Nevertheless, there remains a lack of consensus about the changes in IHC activity patterns before hearing onset. Some

studies have reported that IHCs spontaneously fire Ca^{2+} spikes only during the early postnatal period in mouse (E17.5 to P6: Marcotti et al. 2003a; until P7: Brandt et al. 2007), but spontaneous IHC Ca^{2+} spikes have also been observed in P7-8 mice (Seal et al. 2008). In contrast, spontaneous generation of Ca^{2+} spikes has been documented in the rat cochlea from P3 until hearing onset (Brandt et al. 2007; Tritsch and Bergles 2010), while other studies showed that current injection is necessary to elicit Ca^{2+} spikes in IHCs of P7-P11 rats (Glowatzki and Fuchs 2000; Goutman et al. 2005). It has also been reported that IHCs in the apical and basal regions of cochlea exhibit different activities, with apical IHCs firing Ca^{2+} spikes in bursts and basal IHCs firing continuously (P2–P5) (Johnson et al. 2011). In contrast, a recent study found that both apical and basal IHCs exhibited burst-firing behavior (P1–P9) (Sendin et al. 2014).

The stereotyped firing behavior produced in SGNs by IHC Ca^{2+} spikes provides a template with which to evaluate in vivo activity patterns in auditory centers of the brain. These data suggest that from the end of the 1st postnatal week until hearing onset, IHCs fire brief trains of Ca^{2+} spikes that lead to bursts of action potentials in SGNs, which then propagate through neurons in various auditory centers of the brain. The patterns of activity that precede (developmentally) this burst firing has been more difficult to establish — in vivo recordings from the MNTB in anesthetized rats showed limited activity (Tritsch et al. 2010a), consistent with some in vitro studies (Brandt et al. 2007; Tritsch and Bergles 2010), but counter to other reports of robust spontaneous firing of IHCs at this early developmental stage (Marcotti et al. 2003a; Brandt et al. 2007; Johnson et al. 2011; Sendin et al. 2014). In-vivo recordings from central auditory neurons in pre-hearing rodents have consistently failed to detect high rates of continuous firing such as that reported by these latter studies, suggesting that such activity may be confined to the cochlea at this age or that cochlear isolation in some cases enhances the excitability of IHCs.

Functional roles of spontaneous activity in the auditory system

Spontaneous activity in the developing nervous system is thought to play an important role in the maturation of neural circuits (Feller 1999; O'Donovan 1999); however, the particular contributions of this activity to different aspects of circuit refinement, and the mechanisms through which it influences these changes, are not well-understood. The most intensively studied example of developmental spontaneous activity involves coordinated activity of retinal ganglion cells (retinal waves) that occurs before eye opening and its involvement in retinotopic refinement in the lateral geniculate nucleus (LGN) (Huberman et al. 2008). Cholinergic retinal waves are initially induced by the spontaneous release of acetylcholine from a subset of starburst amacrine cells (Feller et al. 1996). Mice lacking the β2-subunit of the neuronal nicotinic acetylcholine receptor (nAChR) do not exhibit cholinergic retinal waves (Bansal et al. 2000; Muir-Robinson et al. 2002), while retinal waves at other developmental stages are unaffected. Application of the high-affinity cholinergic agonist epibatidine also blocks cholinergic waves by desensitizing nAChRs (Penn et al. 1998), and elimination of spontaneous activity after intraocular injection of epibatidine has been demonstrated in vivo (Ackman et al. 2012). Using both transgenic mouse models and pharmacological manipulations, it has been shown that axons of retinal ganglion cells project to correct retinotopic positions in the superior colliculus and lateral geniculate nuclei when retinal waves have been blocked or reduced, but form abnormally diffuse arborizations (Grubb et al. 2003; McLaughlin et al. 2003; Pfeiffenberger et al. 2006). Thus, sensory-independent activity within the developing retina is necessary for establishing proper retinotopic maps (Kirkby et al. 2013).

Although some nascent synaptic connections between auditory nuclei are established embryonically (Hoffpauir et al. 2009), the auditory brainstem circuit experiences remarkable structural and functional modifications during the first two postnatal weeks, during which auditory neurons obtain their adult morphology (Sanes et al. 1992; Sanes and Takacs 1993) and electrical properties (Sanes 1993; Kandler and Friauf 1995a; Youssoufian et al. 2005; Lu et al. 2007). During this time, synapses undergo morphological changes (Hoffpauir et al. 2006; Youssoufian et al. 2008; Ford et al. 2009), they are strengthened or eliminated (Sanes 1993; Kotak and Sanes 1995; Taschenberger and von Gersdorff 2000; Brenowitz and Trussell 2001; Iwasaki and Takahashi 2001; Kim and Kandler 2003; Awatramani et al. 2005; Youssoufian et al. 2005; Hoffpauir et al. 2006; Lu et al. 2007), and undergo spatial refinement to achieve precise tonotopic connections (Sanes et al. 1992; Sanes and Takacs 1993; Gabriele et al. 2000a; Leake et al. 2002; Kim and Kandler 2003). Throughout this time of synaptic refinement, spontaneous bursts of activity propagate through auditory brainstem circuits, raising the possibility that correlated activity among inputs could help achieve tonotopic segregation (Friauf and Lohmann 1999; Rubel and Fritzsch 2002; Kandler et al. 2009). Unfortunately, little is known about the roles of this activity, in part because of our limited understanding of the cellular and molecular mechanisms that initiate this activity in the cochlea — knowledge that is required to perform targeted disruption of activity patterns in vivo, similarly to what has been achieved in the visual system. Previous studies addressed the role of spontaneous activity by neonatally deafening animals through cochlear ablation, by injecting ototoxic drugs, or by using transgenic or naturally-occurring animal models of deafness. Unfortunately, these approaches do more than

simply block burst firing in the cochlea. For example, cochlear ablation and delivery of ototoxic drugs induce degeneration of afferent fibers, depriving neurons in cochlear nuclei of not only electrical input but also crucial trophic support. Also, the level of spontaneous activity during the pre-hearing period has not been examined in vivo in many deafness models, which may not be completely absent (Youssoufian et al. 2008). Furthermore, many studies have delayed their analysis of the consequences of deafferentation until after hearing onset, making it difficult to discriminate changes resulting from the lack of spontaneous activity from those that occur from the loss of sound-evoked activity. Moreover, the mutations that cause deafness in these models may also affect cells in the brainstem (Schug et al. 2006; Noh et al. 2010; Hirtz et al. 2011), and the consequences of deafness can vary between mutations and mouse strains (Kandler et al. 2009). Despite these limitations, these pioneering studies have revealed some possible functions of pre-hearing spontaneous activity.

The most consistent observation is that depriving SGNs of excitatory input, through either cochlear removal, IHC degeneration, or through use of genetic models that exhibit impaired Ca^{2+}-dependent glutamate release from IHCs, leads to apoptotic degeneration of SGNs and neurons in the ventral cochlear nuclei (Hashisaki and Rubel 1989; Mostafapour et al. 2000; Glueckert et al. 2003; Harris and Rubel 2006; Seal et al. 2008; Hirtz et al. 2011). In the CNS, more severe neuronal loss was observed when cochlear input was eliminated during the pre-hearing period, while no degeneration was observed when the cochlea was ablated after the onset of hearing (Hashisaki and Rubel 1989; Tierney et al. 1997; Mostafapour et al. 2000), suggesting that there is a critical period of development, prior to sensory experience, when afferent activity from IHCs supports neuronal survival.

Spontaneous activity that occurs during this time may also influence the physiological properties of central auditory neurons. Cochlear removal before hearing onset impairs the developmental decrease of intracellular Cl^- in auditory neurons, possibly through changes in the expression of K^+-Cl^- co-transporter KCC2 (Shibata et al. 2004). This aberrant Cl^- homeostasis prevents the shift of GABA/glycinergic responses from depolarizing to hyperpolarizing, and disrupts inhibition of neurons in lateral superior olive (LSO) and IC (Kotak and Sanes 1996; Vale and Sanes 2000, 2002; Vale et al. 2003). Moreover, principal neurons of the MNTB in congenitally deaf *dn/dn* mouse show enhanced excitability and non-synchronized firing in response to synaptic inputs (Leao et al. 2004a, 2005, 2006a), and lose their gradient in expression of ion channels along the tonotopic axis (Leao et al. 2006b). The firing properties and channel expression of LSO neurons also are altered in congenitally deaf mouse lines (Couchman et al. 2011; Hirtz et al. 2011), providing further indication that lack of peripheral excitatory drive disrupts the physiological maturation of central auditory neurons.

Spontaneous activity during development has also been shown to promote the functional elimination of synapses in brainstem auditory neurons, as well as their maturation. In particular, synaptic strength is enhanced at synapses between auditory nerves and bushy cells, the endbulbs of Held, in the congenitally deaf *dn/dn* mouse (Oleskevich and Walmsley 2002; Oleskevich et al. 2004; McKay and Oleskevich 2007). Moreover, the fenestration of calyceal synapses between bushy cells and MNTB principal cells (the calyx of Held) is disrupted in neonatal deafened gerbils and *dn/dn* mice (Youssoufian et al. 2008; Ford et al. 2009), but effects on synaptic strength have not been consistently observed (Oleskevich et al. 2004; Youssoufian et al. 2005; Erazo-Fischer et al. 2007). Abnormalities have also been documented in other synapses within the anteroventral cochlear nucleus, MNTB and LSO (Leao et al. 2004b; Lu et al. 2007; Cao et al. 2008; Clause et al. 2014), indicating that removal of afferent input results in widespread changes in the innervation of auditory neurons.

There is accumulating evidence that spontaneous activity during the pre-hearing period also helps to establish appropriate connections in the auditory pathway. Cochlear ablation in neonatal gerbils leads to ectopic projections from the cochlear nucleus to superior olive and IC (Moore and Kitzes 1985; Kitzes et al. 1995; Russell and Moore 1995). De-afferentation in older animals did not cause abnormal projections, indicating that this effect is similarly specific for pre-hearing spontaneous activity (Russell and Moore 1995). Moreover, the formation and maintenance of the tonotopically arranged afferent innervation patterns from the dorsal nucleus of lateral lemniscus to the IC is disrupted after deafening rats at an early postnatal age (Gabriele et al. 2000b; Franklin et al. 2006, 2008). In addition to these gross disruptions in neuronal projections, several studies have focused on the role of spontaneous activity in the formation and refinement of precise tonotopic maps in the auditory brainstem. Unexpectedly, the tonotopic organizations of brainstem auditory pathways in several congenitally deaf mouse lines appear normal (jerker: Cao et al. 2008; dn/dn: Youssoufian et al. 2008; Vglut3−/−: Noh et al. 2010). However, in neonatally deafened gerbil (Sanes and Takacs 1993) and cat (Leake et al. 2006), the axons of brainstem auditory neurons have more branches and broader arbors, resulting in less precise tonotopic organization. Afferent deprivation also causes degeneration of auditory neurons, so it is possible that these aberrant or broader projections might result from axonal sprouting secondary to neuronal loss, rather than the absence of spontaneous activity. Most recently, mice lacking cholinergic inhibition of IHCs exhibited axonal pruning and tonotopic refinement deficits (Clause et al. 2014); these animals exhibited different burst firing patterns, raising the

possibility that the precise temporal structure of activity within bursts, or the timing and duration of bursts, influence the segregation of inputs in auditory nuclei according to their ultimate frequency response.

While most studies have focused on the central auditory pathway, spontaneous activity might also participate in the development of cochlea itself. In particular, it has been shown that the frequency and duration of Ca^{2+} spikes in IHCs is important for the maturation of Ca^{2+} efficiency of vesicle fusion at ribbon synapses (Johnson et al. 2013). And while this review has focused on spontaneous electrical activity, there may be many other forms of spontaneous activity, for example, driven by growth factor release, that affect the maturation of different cell types in the cochlea, which has downstream consequences for development of auditory circuits in the brain.

Conclusions

The auditory system establishes initial connections during early development, and is capable of conveying signals from the periphery to central neurons several weeks before normal hearing thresholds are established. During this postnatal, pre-hearing period in rodents, IHCs undergo periodic depolarizations, triggering bursts of Ca^{2+} spikes that reliably induce glutamate release onto SGNs. These events induce highly stereotyped bursts of action potentials in SGNs that are transmitted to auditory circuits of the brain, where it may promote neuronal survival, induce their physiological maturation, and refine their connections to establish tonotopically segregated pathways. However, in spite of extensive in vivo and in vitro studies over the past several decades, the roles of spontaneous activity in auditory development remain largely speculative. This lack of understanding is mainly due to our limited knowledge about the molecular mechanisms that initiate this activity in the cochlea and the lack of genetic tools to specifically manipulate this activity in vivo. The cochlear explant preparation has proven to be a powerful in vitro system to reveal the potential molecular targets involved in generating spontaneous activity, but a consensus about the precise sequence of events responsible for IHC activation during the pre-hearing period has not yet emerged. Two models have been proposed to explain the periodic excitation of IHCs necessary to trigger burst activity in SGNs — one that requires periodic inhibition of spontaneously active IHCs, and another which requires periodic excitation of otherwise silent IHCs. Genetic manipulation of distinct pathways accompanied by in vivo assessment of auditory neuron activity patterns will be required to establish which model is correct. Previous studies have revealed that removing activity entirely during this period leads to degeneration of afferent fibers, loss of trophic support, and apoptotic death of peripheral and central auditory neurons. Thus, an ideal manipulation to assess the role of spontaneous activity in the development of brain auditory circuits should selectively disrupt spontaneous activity, but preserve sound-evoked activity after hearing onset. A greater understanding of the mechanisms responsible for generating this activity and its effects on nervous system development may have broad implications for the development of other sensory systems, provide new strategies to restore auditory function after acoustic trauma, and facilitate integration of cochlear implants in hearing impaired patients.

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