

Mini review

Extrinsic sound stimulations and development of periphery auditory synapses

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Abstract

The development of auditory synapses is a key process for the maturation of hearing function. However, it is still on debate regarding whether the development of auditory synapses is dominated by acquired sound stimulations. In this review, we summarize relevant publications in recent decades to address this issue. Most reported data suggest that extrinsic sound stimulations do affect, but not govern the development of periphery auditory synapses. Overall, periphery auditory synapses develop and mature according to its intrinsic mechanism to build up the synaptic connections between sensory neurons and/or interneurons.

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In mammals, the neuronal network is an integrated system in which neurotransmitters are well organized and transmitted through nanometer structures connecting different types of neurons. These nanometer structures are defined as synapses and believed to work as a fundamental component of the nervous system. Current data suggest that synapses are primarily responsible for sensation, emotions, learning, memory and behaviors (Mayford et al., 2012; Lee et al., 2012; Kandel et al., 2014). Most synapses are chemical synapses, whose functional status is largely dependent upon neurotransmitters release. In contrast, only a few synapses are electrical synapses, in which the neuronal signals are delivered much quicker via gap junctions compared with chemical synapses (Fukuda, 2007). It is known that synapses are the structural basis of most neurological activities controlling general sensations and behaviors. Undoubtedly, hearing is one of major sensations in mammals, and auditory synapses have been found to play a

key role in sound encoding and transduction. It has been reported that a number of genes are involved in the development of periphery auditory synapses, including OTOF, MYO7a, SLC17A8, and DIAPH3 (Varga et al., 2003; Roux et al., 2006; Ruel et al., 2008; Schoen et al., 2010) that have been identified to associate with cochlear ribbon synapses — the first afferent periphery synapses in the auditory pathway. Also, some neuronal factors, such as neurotrophins, Brain-derived neurotrophic factor (BDNF), Synapsins, and NT3, are required for peripheral auditory synapses development and maturation (McCutchen et al., 2002; Ferreira et al., 1996; Wang and Steven, 2011). For those mature synapses in peripheral auditory pathway, three types of ionotropic glutamate receptors can be identified: excitatory neurotransmitter receptors: AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid), kainate and NMDA (N-methyl-D-aspartate) receptors. Activation of any of these ionotropic glutamate receptors can stimulate an excitatory synaptic releasing and subsequent signal conduction to the brain. A normal level of NMDA glutamate receptors is necessary for appropriate neuronal and synaptic development (Constantine-Paton et al., 1990).

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Development of peripheral auditory synapses is a key element to build up normal hearing function. On the other hand, sound stimulation is believed to work as an important factor for auditory synaptogenesis. A number of studies show that stimulation deprivation of vision or hearing can delay significantly the development of related synapses (Rittenhouse et al., 1999; Bures et al., 2010). Deprivation of auditory stimulation can be achieved by cochlear damage via surgical treatment or drug toxicity. Auditory deprivation can also be acquired by congenital or genetic dependent hearing impairment (Zhang et al., 2015). The function of neuronal circuits of hearing or sensory experiences will be disrupted when the transduction of sound or sensory signals along the circuits is blocked. Under such conditions, maturation of auditory synapses is delayed significantly and cannot achieve average levels of normal controls. It has been proposed that deprivation of sound stimuli affects auditory synaptogenesis. Furthermore, the neuronal circuit of hearing is unable to fulfill proper sound coding and transduction under the condition of lacking sound stimulation (Rittenhouse et al., 1999; DiCristo et al., 2001).

Although we have known that deprivation of sound stimuli may cause inappropriate maturation of auditory peripheral synapses, however, the studies mentioned above have not answered the question regarding whether the development or maturation of peripheral auditory synapses is largely dependent on sound stimulations. Wang et al. once demonstrated development of cochlear ribbon synapses in an *in vitro* study (Wang and Steven, 2011). Cochlear ribbon synapses form between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). Numerous studies have proposed that cochlear ribbon synapses serve as the first afferent neuronal connection in the hearing pathway (Meyer et al., 2009; Glowatzki and Fuchs, 2002; Merchan-Perez and Liberman, 1996; Liberman, 1980, 1982). In current experimental studies, cochlear ribbon synapses are well identified by labeling the specific pre-synaptic scaffold protein RIBEYE/CtBP2, which normally is located beneath cochlear IHCs. In Wang's experiment, the samples were cultured and contained three rows of outer hair cells (OHCs), one row of inner hair cells (IHCs) and spiral ganglion cells (SGCs) (Wang and Steven, 2011). The samples were obtained on postnatal day 3–5 (P3–P5) in mice and the cultured basilar membrane contained intact hair cells (both OHCs and IHCs) that had not been exposed to any sound or other auditory stimulations. As it has been proposed that the time point of onset of hearing in mice is postnatal day 12 (P12) (Webster, 1983; Sobkowicz et al., 1982), no synaptic puncta or other synaptic structures were captured by immunostaining or TEM in the cultured basilar membrane collected on P3 in these mice, indicating that cochlear ribbon synapses had not developed on P3 in mice. Thus, Wang's study may suggest that cochlear ribbon synapses may develop *in vitro* in lack of sound stimulations (see Fig. 1). Excitotoxic damage to synaptic terminals, as induced by excess glutamate release from IHCs, can be used to imitate sound exposure to hair cells *in vitro*. A brief application of glutamate agonists can cause loss of IHC-SGN synapses in IHCs, mimicking excitotoxicity. However, the IHC-SGN synapse grows well spontaneously when glutamate

agonists are not involved (Zhang et al., 2015). Further, synaptic signals in Wang's study could carry out synaptic functions, as hair cells in the cultured samples displayed endocytosis, taking up the styryl dye FM1-43 and its analog AM1-43 (Zhang et al., 2015). Consistent with these observations, other experiments also show that cochlear ribbon synapses formed in *in vitro* studies can be functional, as shown in IHC endocytosis using FM1-43 dye labeling, suggesting that vesicles cycling and synaptic releasing can be active in the cultured system without sound or other auditory stimulations (Geleoc and Holt, 2003; Meyers et al., 2003; Griesinger et al., 2002; Meyer et al., 2001).

Similar models of *in vitro* synapse development systems have shown synaptic connections established in isolated sensory neurons in *Aplysia*. In the past several decades, the sensory synapse culture system of *Aplysia* has been used as an ideal culture model to investigate mechanisms underlying learning and memory (see Fig. 2). In this system, sensory synapses develop well between sensory neurons and motor neurons or interneurons (Castellucci and Kandel, 1976; Kandel and Schwartz, 1982; Hawkins et al., 1983). The developed sensory synapse displays a wide range of synaptic plasticity (Castellucci and Kandel, 1976; Kandel and Schwartz, 1982; Hawkins et al., 1983; Walters and Byrne, 1985; Frost et al., 1985; Montarolo et al., 1986, 1988). Moreover, some studies also report that the developed sensory synapse formed between isolated sensory and motor neurons can present long term facilitation (LTF) (Schacher and Wu, 2002; Liu et al., 2003), providing solid data showing that sensory synapses developed *in vitro* are also functional. Moreover, these synapses are protein-synthesis dependent, because anisomycin application (one type of protein synthesis inhibitor) can significantly reduce the quantity of synaptic connections (the number of varicosity) and amplitude of EPSP. Thus, external stimulation is not necessarily needed during synapse development and formation in this system. A number of genes were found to play a key role in the formation of isolated sensory synapses of *Aplysia* (Schacher and Wu, 2002; Martin et al., 1997; Casadio et al., 1999), showing that synapse development and formation are largely determined by its inheritance characteristics. Additional supporting data show that local protein synthesis at or near the sensory varicosities may be associated with LTF expression (Liu et al., 2003; Casadio et al., 1999; Bailey and Chen, 1988; Wainwright et al., 2002). Although exciting stimulations (mimicked via 5-HT application in the culture system of *Aplysia*) or stimulation suppression (such as anisomycin application) can boost or reduce synaptic formation significantly, overall, sensory synapse development is substantially spontaneous (Liu et al., 2003; Santarelli et al., 1996; Schacher et al., 1999; Hu et al., 2002).

The fact that sound stimulation affects, but does not govern peripheral auditory synapse development may provide us with an optimistic prospect in future clinical applications. For example, in infants with profound hearing loss at birth, the peripheral auditory synapse may still develop along the hearing pathway, despite the lack of sound stimulation. Logically,

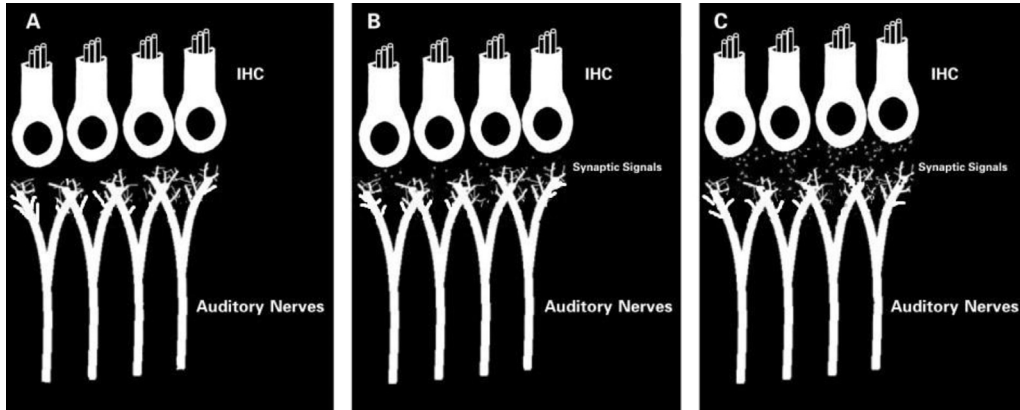


Fig. 1. Signals of cochlear ribbon synapses identified during *in vitro* development. A: No positive synaptic signals between IHCs and terminals of auditory nerves around postnatal day 2–3 (P2-3) in cultured samples of basal membranes harvested from the inner ear of mice. B: Some cochlear ribbon synapse (RIBEYE/CtBP2) signals are seen beneath IHCs around postnatal day 5–6 (P5-6). C: Abundant ribbon synaptic signals are identified between IHCs and terminals of auditory nerves around postnatal day 7–8 (P7-8).

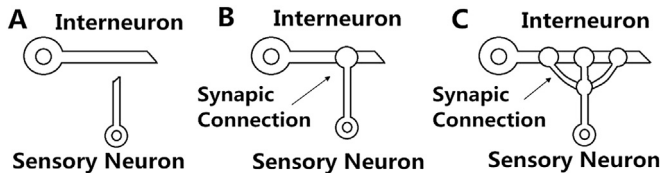


Fig. 2. *In vitro* development of isolated sensory synapses in *Aplysia*. A: One sensory neuron and one interneuron isolated and cultured together in the same dish, with no synaptic connections identified at the initial stage. B: Gradually, a synaptic connection appears in the axon of the interneuron. C: Numerous synapse connections are seen along the axon of the interneuron during continued co-culturing.

cochlear implant may still be an effective choice to restore auditory neuronal circuits via bypassing the broken sites in the peripheral auditory pathway, to enhance neural circuit integrity and promote auditory signal transmission. Alternative choices to repair damaged auditory neural circuits include substance that can promote synaptic synchronization or neural excitability in the auditory pathway, so auditory signals can be conducted faithfully from the ear to the brain (Starr et al., 1991, 1996). However, the ideal substances have yet to be found and need to be explored in future studies.

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