Available online at www.sciencedirect.com
ScienceDirect
Hearing Research 230 (2007) 53–63
Hearing Research
www.elsevier.com/locate/heares

Research paper

The role of spontaneous activity in development of the endbulb of Held synapse

Sarah M. McKay [a], Sharon Oleskevich [a,b,*]

[a] *Neuroscience Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW 2010, Australia*
[b] *University of New South Wales, Sydney, NSW 2052, Australia*

Received 25 February 2007; received in revised form 9 April 2007; accepted 14 May 2007
Available online 24 May 2007

Abstract

In the mouse brainstem cochlear nucleus, the auditory nerve to bushy cell synapse (endbulb of Held) is specialised for rapid, high-fidelity transmission. Development of this synapse is modulated by auditory nerve activity. Here we investigate the role of spontaneous auditory nerve activity in synaptic transmission using *deafness* (*dn*/*dn*) mutant mice that have abnormal hair cells and lack spontaneous auditory nerve activity. Evoked and miniature alpha amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor-mediated excitatory post-synaptic currents (eEPSCs, mEPSCs) were compared in *deafness* and normal mice before the age of hearing onset (post-natal day 7–11: P7–11) using variance-mean, miniature event and tetanic depression analyses. Amplitudes were significantly greater in *deafness* mice for eEPSCs (2.1-fold), mEPSCs (1.4-fold) and quantal amplitudes (1.5-fold). eEPSCs in *deafness* mice decayed more rapidly with increasing age, indicating an input-independent transition in post-synaptic AMPA receptor properties. A comparison of normal mice before and after the onset of hearing showed a change in synaptic parameters with an increase in eEPSC (1.7-fold), mEPSC (1.6-fold) and quantal amplitude (1.7-fold) after hearing onset while release probability remained constant (0.5). Overall, the results in *deafness* mice suggest that synaptic strength is altered in the absence of spontaneous auditory nerve activity.

Keywords: Endbulb of Held; Auditory; Development; AMPA; *dn*/*dn*; Spontaneous

1. Introduction

The endbulb of Held is an unusually large and powerful calyceal synapse between spiral ganglion neurons and spherical bushy cells in the auditory pathway. Spiral ganglion neurons transfer auditory input from the inner hair cells in the organ of Corti to bushy cells in the anteroventral cochlear nucleus (AVCN). Each spherical bushy cell receives input from up to four large endbulb terminals (Brawer and Morest, 1975; Ryugo and Sento, 1991). At maturity, the endbulb of Held is specialised for rapid, high-fidelity transmission via glutamatergic alpha amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor-mediated transmission. The AMPA-receptor mediated responses allow presynaptic action potentials to generate post-synaptic spikes with very few failures and at very high (up to 800 Hz) frequencies. Such rapid synaptic transmission is necessary for the precise processing of binaural cues required for sound localisation (Brenowitz and Trussell, 2001b; Gardner et al., 1999; Taschenberger et al., 2002).

Abbreviations: AMPA, alpha amino-3-hydroxy-5-methyl-4-isoxazole propionate; eEPSC, evoked excitatory postsynaptic currents; mEPSC, miniature excitatory postsynaptic currents; AVCN, anteroventral cochlear nucleus; aCSF, artificial cerebral spinal fluid; TTX, tetrodotoxin; Pr, the probability that a vesicle will be released from an active zone after the arrival of a single action potential; N, the number of release sites; Q_{av}, average quantal amplitude; RRP, ready releasable pool; P_{ves}, the probability of release for a single vesicle from the pool of readily releasable vesicles

* Corresponding author. Address: Neuroscience Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW 2010, Australia. Tel.: +61 2 9295 8290; fax: +61 2 9295 8281.
E-mail addresses: s.mckay@garvan.org.au (S.M. McKay), s.oleskevich@garvan.org.au (S. Oleskevich).

0378-5955/$ - see front matter
doi:10.1016/j.heares.2007.05.006

How does this synapse develop to produce rapid, high-fidelity properties of synaptic transmission? Is spontaneous auditory nerve activity important in development of the endbulb synapse? Previous reports show that hearing begins around postnatal day 12 (P12) (Kamiya et al., 2001; Romand, 1983, 2003) and sound-driven activity is required for development of frequency-selective tuning and organization in the auditory cortex (Rubel and Fritzsch, 2002; Zhang et al., 2001). Spontaneous nerve activity, which is present before auditory input (Jones et al., 2001; Lippe, 1994; Lu et al., 2007), regulates membrane properties and tonotopic maps (Leao et al., 2005, 2006; Walmsley et al., 2006). The *deafness* (*dn*/*dn*) mutant mouse offers an opportunity to examine the role of spontaneous auditory nerve activity in the development of synaptic transmission. The *deafness* mouse is profoundly deaf from birth and, unlike normal mice, lacks spontaneous auditory nerve activity (Durham et al., 1989; Leao et al., 2006). A mutation is present in the transmembrane cochlear-expressed gene 1 (Kurima et al., 2002) and inner hair cells fail to acquire mature potassium currents and exocytotic machinery (Marcotti et al., 2006). Hair cells with damaged stereocilia have been shown to result in a significant reduction in spontaneous nerve activity (Liberman and Dodds, 1984). The hair cells in the *deafness* mouse are initially intact with minor ultrastructural abnormalities (Pujol et al., 1983; Steel and Bock, 1980) and degenerate from postnatal days 15 to 40 (P15–40) (Faddis et al., 1998; Marcotti et al., 2006; Webster, 1992). Hence, this mouse provides an excellent model to study the absence of spontaneous activity in the auditory nerve without the complication of other abnormalities.

Here we investigate the effect of spontaneous auditory nerve input on synaptic transmission by comparing *deafness* mice to normal mice before the age of hearing onset (P7–11). We also test the effect of sound-driven activity by comparing normal mice before and after the onset of hearing (P7–11 versus P13–16). This study represents a novel comparison compared to previous reports in mice ($P < 25$ days versus $P > 40$) (Wang and Manis, 2005) and provides additional information using variance-mean, miniature event and tetanic depression analyses. Results from the *deafness* mutant mice confirm previous reports of an increase in synaptic strength in *deafness* compared to normal mice (Oleskevich et al., 2004; Oleskevich and Walmsley, 2002) and present new evidence regarding the role of spontaneous auditory activity in development of synaptic transmission at the endbulb synapse.

2. Methods

Brainstem slices were obtained from normal CBA mice before (P7–11, $n = 14$) and after ear canal opening (P12–16, $n = 19$) and from *deafness* (*dn*/*dn*) mutant mice (P7–11; $n = 9$; P12–16; $n = 13$). *Deafness* (*dn*/*dn*) mutant mice were obtained from the MRC Institute of Hearing Research, Nottingham, UK. These mice were recently crossed with CBA mice (to improve breeding), and subsequently bred to obtain homozygous *dn*/*dn* mice with a CBA background. Auditory brainstem responses cannot be recorded in mice until P12 and response onset correlates closely with opening of the ear canal (Kamiya et al., 2001). Because P12 represents a transition day in hearing onset, data from P12 mice ($n = 2$) were not included in comparisons of age-grouped data but were included in correlation analysis of postnatal day. All normal mice that were P13 or older had their ears and eyes open at the time of the experiment. Tissue was collected in accordance with the Garvan Institute of Medical Research/St. Vincent's Animal Experimentation Ethics Committee. Saggital slices of AVCN (200 μm for P7–10, 150 μm for P11–16) were cut in low calcium/high magnesium ice-cold artificial cerebral spinal fluid (aCSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 5 Mg_2SO_4, 1 $CaCl_2$, 25 glucose, bubbled with carbogen (95% O_2, 5% CO_2). Slices were transferred to normal aCSF (mM): 125 NaCl, 25 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 1 Mg_2SO_4, 2 $CaCl_2$, 25 glucose, bubbled with carbogen and maintained at 35 °C for 30–60 min. Thereafter, slices were kept in normal aCSF at room temperature.

Whole-cell recordings were made at room temperature from AVCN neurons visualised using differential interference contrast optics. Patch electrodes (4–7 MΩ) were filled with internal solution containing (mM): 120 CsCl, 4 NaCl, 4 $MgCl_2$, 0.001 $CaCl_2$, 10 Hepes, 2 Mg-ATP, 0.2 GTP-tris, 10 EGTA, adjusted to pH 7.3 and 295–300 mOsm with sorbitol. Series resistance, which was <10 MΩ, was compensated by 80%. A caesium chloride-based internal solution blocked potassium conductances and intracellular addition of lidocaine *N*-ethyl bromide (QX-314, 2 mM) blocked sodium channels thus minimising variance in the amplitude of the synaptic currents. All recordings were filtered at 10 kHz with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA) and sampled at 20 kHz. Data acquisition and analysis were performed with AxoGraphX (AxoGraph Scientific, Australia).

Evoked excitatory post-synaptic currents (eEPSCs) were recorded in voltage clamp (−60 mV) by stimulation of auditory nerve afferents. The stimulating electrode was filled with normal aCSF and stimulation intensity was set at 1.5 times the response threshold for each cell (0.1 ms; 0.2 Hz). Thresholds ranged from 15 to 70 V for both *deafness* and normal mice. Since spherical bushy cells have one or two inputs and globular bushy cells and stellate cells can have up to 40 inputs, the evoked currents were identified as single-fibre synaptic currents by their all-or-none response to graded stimulation intensities, minimal eEPSC amplitude of greater than 1 nA and fast kinetics at a membrane potential of −60 mV (Isaacson and Walmsley, 1995; Oleskevich et al., 2000). AVCN neurons did not exhibit slow mEPSCs (at −60 mV), as recorded in some types of neurons in other regions of the cochlear nucleus (Gardner et al., 2001). The electrode was placed between 50 and 100 μm from the cell at a tissue depth of approximately 50 μm. Paired stimuli