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STOCHASTIC RESONANCE IN THE SYNAPTIC TRANSMISSION BETWEEN HAIR CELLS AND VESTIBULAR PRIMARY AFFERENTS IN DEVELOPMENT

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Abstract—The stochastic resonance (SR) is a phenomenon of nonlinear systems in which the addition of an intermediate level of noise improves the response of such system. Although SR has been studied in isolated hair cells and in the bullfrog sacculus, the occurrence of this phenomenon in the vestibular system in development is unknown. The purpose of the present study was to explore for the existence of SR via natural mechanical-stimulation in the hair cell-vestibular primary afferent transmission. *In vitro* experiments were performed on the posterior semicircular canal of the chicken inner ear during development. Our experiments showed that the signal-to-noise ratio of the afferent multiunit activity from E15 to P5 stages of development exhibited the SR phenomenon, which was characterized by an inverted U-like response as a function of the input noise level. The inverted U-like graphs of SR acquired their higher amplitude after the post-hatching stage of development. Blockage of the synaptic transmission with selective antagonists of the NMDA and AMPA/Kainate receptors abolished the SR of the afferent multiunit activity. Furthermore, computer simulations on a model of the hair cell – primary afferent synapse qualitatively reproduced this SR behavior and provided a possible explanation of how and where the SR could occur. These results demonstrate that a particular level of mechanical noise on the semicircular canals can improve the performance of the vestibular system in their peripheral sensory processing even during embryonic stages of development.

Key words: noise, vestibular, inner ear, mechanoreceptor, primary afferents, development.

INTRODUCTION

The stochastic resonance (SR) is a counter-intuitive phenomenon of many physical and biological nonlinear systems that refers to the increase in the signal-to-noise ratio (SNR) on the output, obtained through an increase in the noise level on the input (Anishchenko et al., 1999; Gammaitoni et al., 1998, 2009; for reviews see Moss et al., 2004; McDonnell and Abbott, 2009; McDonnell and Ward, 2011; McDonnell et al., 2015). Typically, the plot of SNR vs. input noise is an inverted U-like function characterized by maximal enhancement of SNR at a specific noise amplitude value.

The isolated hair cells of the vestibular and auditory systems have been studied in the context of their response to a variety of physical stimuli, i.e., caloric, electrical, chemical or mechanical. In 1991, Zenner and Zimmermann, demonstrated that isolated vestibular hair cells from the guinea pig can produce motility of the cell body or sensory hairs by direct caloric, electrical or chemical stimuli applied on these cells. Such mechanical responses of the vestibular cells could contribute to micromechanical non-linearities of stereociliary displacements and gain control. In this context, in 1998, Jaramillo and Wiesenfeld published a pioneering study about SR elicited by mechanical stimuli in whole-cell recordings of isolated hair cells from the frog sacculus. These authors demonstrated that mechanical Brownian motion of the hair bundle provides an optimal noise level that increases the sensitivity of mechanoelectrical transduction to weak signals. Thus the SR can be elicited by intrinsic mechanisms of the hair cells when mechanical noise is applied to the hair bundle. A subsequent study by Indresano et al. (2003) reported that mechanical noise applied in the bullfrog sacculus produced a SR effect in the acoustic information conveyed by the 8th nerve. This result demonstrated for the first time that mechanical noise enhances the signal transmission in the bullfrog sacculus via the SR phenomenon. The studies by Jaramillo and Wiesenfeld (1998) and Indresano et al. (2003), show that the SR can be generated in both, the hair cells and in the second stage of the sensory transmission: the auditory primary afferents via the hair cell-primary afferents transmission.

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Abbreviations: IHC, inner hair cell; SNR, signal-to-noise ratio; SR, stochastic resonance.

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The above-mentioned studies show that the SR has been extensively studied in sensory and motor systems, even in the isolated hair cells and in the bullfrog sacculus (Jaramillo and Wiesenfeld, 1998 and Indresano et al., 2003); however, their physiological mechanisms in the synaptic transmission from the vestibular hair cells to the primary afferents during development are unknown. The study of SR in the synaptic transmission of the semicircular canals of the chicken inner ear during development is crucial to clarify the specific contribution of SR elicited by mechanical noise in the vestibular system, the organ of balance, which is a sensor of angular acceleration.

EXPERIMENTAL PROCEDURES

Animal preparation

Experiments were made using the isolated inner ear of the chicken (*Gallus domesticus*). We employed 17 embryos of 15, 17, 19 and 21 days (E15–E21) and three hatchlings of 5 days (P5). They were obtained from the ALPES poultry farm at Tehuacán, Puebla. The age of the embryos given in days was established by reference to the staging criteria of Hamburger and Hamilton (1951). The embryos were kept in incubation under strict temperature control, between 38.5 °C and 39.5 °C, and relative humidity of about 60% in a BG-E33 incubator (TPM Equipos, Instrumentos de Laboratorio, Ciudad de México, México).

Embryos were cooled at approx. −12 °C for 15 min. Then the embryos were extracted and immediately after the withdrawal reflex was abolished the animal was decapitated as described (Cortes et al., 2013; Galindo et al., 2013). Hatchling animals were first anesthetized intraperitoneally with sodium pentobarbital (6 mg/kg, Pfizer) and then decapitated as previously described (Cortes et al., 2013; Galicia et al., 2015). The otic capsule was immediately opened. The nerve of the posterior semicircular canal was dissected up to the brainstem. The cartilaginous otic capsule was cut and isolated from the cranium. All efforts were made to minimize animal suffering and to reduce the number of animals used for the experiments, as outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health. The isolated inner ear was transferred to a recording chamber and continuously perfused with Ringer solution of the following composition (in mM): NaCl 124, KCl 5, $CaCl_2$ 2, NaH_2PO_4 2.2, $NaHCO_3$ 26, $MgSO_4$ 2, glucose 10. The pH of this solution was 7.3–7.4 after saturation with 95% O_2 and 5% CO_2 (Peusner and Giaume, 1997). The saline was warmed by a Temperature Controller (TC-102, Medical System Corporation) and was circulated through the chamber at a rate of 3–5 ml/min. The temperature of the bathing medium was kept at 37–39 °C during the experiments. A small opening, approximately 1 mm^2, in the bony wall of the posterior semicircular canal was made, being careful not to disrupt the underlying membranous duct, through which the probe of the mechanical stimulating device was positioned as shown in Fig. 1A. The preparation was allowed to equilibrate for 30 min before recording.

Mechanical stimulation

A closed-loop mechanical stimulator-transducer (Chubbuck, 1966; Manjarrez et al., 2002a,b; Mendez-Balbuena et al., 2015) allowed measures of the displacement of the applied stimuli. The output of two independent function generators provided input to the stimulator-transducer (Fig. 1A). One of these (Tektronix CFG 253, Tektronix Inc., Beaverton, OR 97077, USA) generated a sinusoidal input waveform (test stimulus) while, the other (Wavetek Model 132, San Diego, CA, USA), supplied the superimposed noise. The inner ear was held in a fixed position to ensure that the exposed semicircular canal remained over the indenter arm of the mechanical stimulation device, which consisted of a 0.5-mm diameter probe. The probe tip was used to apply sinusoidal duct displacements of the posterior semicircular canal. Fig. 1E shows the input–output curve for the extracellular multiunit activity versus the sinusoidal stimulus strength in zero noise conditions (control).

Test sinusoidal-stimuli

We obtained input–output curves for the multiunit response of the semicircular canal for each animal to mechanical sinusoidal stimulation. We applied a single sine frequency of 1.1 Hz with a magnitude of the probe tip displacement from 0 to 50 μm. This method of natural stimulation has proven to be reliable and reproducible (Dickman et al., 1988; Boyle and Highstein, 1991). This natural stimulation produced endolymph movement and consequent cupula deflections as illustrated in Fig. 1A. The dashed lines in Fig. 1E indicate the stimulation intensity employed for the test stimulus (the control). This intensity produced a multiunit activity in the nerve of the semicircular canal of about 30% of their maximal response. We verified that this stimulation strength did not affect the membranous duct of the semicircular canal neither the functional response of the vestibular organ in the semicircular canal (i.e., we verified that the amplitude of the multiunit activity did not change along the experiment).

Noisy stimuli

Gaussian noise in the range from 0 to 250 Hz was continuously applied with the same indenter and it was superimposed to the test sinusoidal stimulation. Fig. 1D, F shows the power spectrum and the amplitude distribution of the noisy stimulus. We choose this broad bandwidth because it was more effective to produce SR effects in our preparation in our preliminary experiments. The range of the applied noise in our experiments was from 0 μm to 40 μm. It was adjusted to this amplitude range because within this range we found the optimal noise level which produced the maximal SR effect in our preliminary experiments. We also employed this range of noisy mechanical indentation with confidence because the maximal indentation was about 15% of the