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DIFFERENTIAL EFFECTS OF PRENATAL CHRONIC HIGH-DECIBEL NOISE AND MUSIC EXPOSURE ON THE EXCITATORY AND INHIBITORY SYNAPTIC COMPONENTS OF THE AUDITORY CORTEX ANALOG IN DEVELOPING CHICKS (*GALLUS GALLUS DOMESTICUS*)

V. KUMAR,[a] T. C. NAG,[a*] U. SHARMA,[b] N. R. JAGANNATHAN[b] AND S. WADHWA[a]

[a] *Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India*

[b] *Department of NMR, All India Institute of Medical Sciences, New Delhi 110029, India*

Abstract—Proper development of the auditory cortex depends on early acoustic experience that modulates the balance between excitatory and inhibitory (E/I) circuits. In the present social and occupational environment exposure to chronic loud sound in the form of occupational or recreational noise, is becoming inevitable. This could especially disrupt the functional auditory cortex development leading to altered processing of complex sound and hearing impairment. Here we report the effects of prenatal chronic loud sound (110-dB sound pressure level (SPL)) exposure (rhythmic [music] and arrhythmic [noise] forms) on the molecular components involved in regulation of the E/I balance in the developing auditory cortex analog/Field L (AuL) in domestic chicks. Noise exposure at 110-dB SPL significantly enhanced the E/I ratio (increased expression of AMPA receptor GluR2 subunit and glutamate with decreased expression of GABA(A) receptor gamma 2 subunit and GABA), whereas loud music exposure maintained the E/I ratio. Expressions of markers of synaptogenesis, synaptic stability and plasticity i.e., synaptophysin, PSD-95 and gephyrin were reduced with noise but increased with music exposure. Thus our results showed differential effects of prenatal chronic loud noise and music exposures on the E/I balance and synaptic function and stability in the developing auditory cortex. Loud music exposure showed an overall enrichment effect whereas loud noise-induced significant alterations in E/I balance could later impact the auditory function and associated cognitive behavior.

Key words: avian, field L, GABA, glutamate, PSD-95, gephyrin.

*Corresponding author. Address: Room Number-1026, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India. Tel: +91-1126594875; fax: +91-1126588663.

E-mail address: tapasnag@aiims.ac.in (T. C. Nag).

Abbreviations: ACx, auditory cortex; AuL, auditory cortex analog/Field L; DAB, 3,3′-diaminobenzidine; E/I, excitatory and inhibitory; NAA, *N*-acetyl aspartate; NMRS, NMR spectroscopy; SPL, sound pressure level; TSP, 3-trimethyl silyl propionic acid.

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INTRODUCTION

Prenatal auditory exposure influences the development of the auditory system as well as associated species typical perceptual preference in birds (Gottlieb, 1978) and mammals (Vince, 1979; DeCasper and Spence, 1986). Development of the functional (tonotopic) organization and signal-processing capabilities in the auditory cortex (ACx) critically depends on the early acoustic environments (Zhang et al., 2001; Mrsic-Flogel et al., 2003). Music at moderate sound pressure level (SPL) has been shown to enhance the ACx development in children (Shahin et al., 2004) and improve the response strength, sensitivity, and latency of the ACx neurons (Engineer et al., 2004) and auditory discrimination learning (Xu et al., 2009) in rats. On the other hand, noise exposure at moderate SPL in early postnatal rats retards the ACx maturation (Chang and Merzenich, 2003), disrupts the functional organization (Wang, 2004), processing of sound intensity (Grécová et al., 2009; Bures et al., 2010) and auditory spatial sensitivity (Xu et al., 2010).

With changing modern social and occupational culture, exposure to loud sound, whether loud music (recreational noise) or traffic/industrial sound (occupational noise) for longer duration is becoming inevitable (Rabinowitz, 2000; Goines and Hagler, 2007). Consistent occupational noise (85–95-dB SPL) during pregnancy caused high-frequency hearing loss in children when tested at 4–10 years of age (Lalande et al., 1986; Committee on Environmental Health, 1997). Altered electrophysiology in the inferior colliculus and median geniculate nucleus of mice (Basta and Ernest, 2004) and rabbits (Emmerich et al., 1990) were observed following acute noise exposure at 115-dB SPL. Noise-(115-dB SPL, 3-h duration) induced cell death has also been reported in the mouse inferior colliculus and ACx (Basta et al., 2005). Exposure to such loud sound in pregnant women and neonates (Zhang et al., 1992; Committee on Environmental Health, 1997) for prolonged period can disrupt the early development of the ACx. On the other hand, loud music-(ranging near 100 dB SPL) induced hearing loss or altered auditory behavior is still a controversial topic in auditory research as some studies on adult human have shown an increased risk of hearing loss (Gunderson et al., 1997; Häusler, 2004), while other studies found little to no correlation (Drake-Lee, 1992; Maia and Russo, 2008).

Studies with regard to the effects of prenatal chronic high SPL spectrally complex sound (music) or noise exposure on the developing ACx are limited. Molecular or biochemical changes including major regulators of excitatory/inhibitory (E/I) circuits in the ACx have been sparingly studied together. Most of the earlier studies have focused either on acute exposure of noise (Sun et al., 2008) or used pure pulse tone for chronic exposure (Komiya and Eggermont, 2000; Seki and Eggermont, 2003). Accordingly, the present study investigated the effects of prenatal chronic 110-dB SPL music and noise exposure on the development of the auditory cortex analog/Field L (AuL) of domestic chick. The latter is a well-established acoustic model (Sanyal et al., 2013a) with remarkable similarity of its AuL in the functional organization (Scheich, 1983) and processing ability of spectral and temporal composition of species-specific vocalizations (Heil et al., 1992; Nagel and Doupe, 2008) to the ACx of mammals. Following exposure with 110-dB SPL noise or music during the last 10 days of embryonic development, expression of various molecular components, which maintain the excitatory/inhibitory (E/I) balance and synaptic functions in the developing AuL, was analyzed.

EXPERIMENTAL PROCEDURES

Fertilized eggs and incubation conditions

The Institution Animal Ethics Committee (546/IAEC/10), AIIMS, New Delhi approved the protocol and number of animals used in the experiments, adhering to the guidelines laid down by the NIH (USA) regarding the care and use of animals for experimental procedures. Fertilized eggs (day 0) of healthy White Leghorn domestic chicks (*Gallus gallus domesticus*) weighing 50–60 g, were obtained from a registered poultry farm (Venkys (India) Ltd., Haryana, India). Eggs were incubated under controlled conditions (Sanyal et al., 2013a) in a double-insulated egg incubator (Widson Scientific Works Ltd., New Delhi, India).

Chick as an acoustic model

Chicks show prenatal acquisition of auditory cues (precocious development similar to humans) and have comparable organization and function of the auditory system (including the ACx) to that of humans (Scheich, 1983; Heil et al., 1992; Rose, 2000; Boumans et al., 2008; Nagel and Doupe, 2008; Wang et al., 2010). Bird's ability to perceive musical contents is quite similar to humans and their ability to distinguish different styles and rhythms is not elementary rather well refined and sophisticated, (Porter and Neuringer, 1984; Watanabe and Sato, 1999; Kaplan, 2009) which suggests that there is great similarity in the way the birds and humans hear music (Toukhsati et al., 2005). Similarities in the acoustic features of avian complex learned sounds to those of human speech (Singh and Theunissen, 2003) including the overlapping avian hearing frequency range (40 Hz–40 kHz) (Beason, 2004) and its dependency on precocious development, provide an excellent system to study the effects of prenatal acoustic exposure. Furthermore our earlier studies have established the validity of chick as an acoustic model (Wadhwa et al., 1999; Chaudhury et al., 2010; Sanyal et al., 2013a) including the report showing that chicks exposed to prenatal species-specific calls or music develop a preference toward maternal calls (Jain et al., 2004).

Sound exposure protocol

In order to replicate the present scenario of increased occupational or recreational exposure time (6–8 h per day, ranging near 100–120-dB SPL) (National Institute for Occupational Safety and Health, USA) to loud sound developing chick embryos were exposed to noise or music at 110-dB SPL for 15 min per hour throughout the day (total 6 h per day). Considering the ethical point, the exposure was divided into several time periods instead of a continuous 6-h exposure per day. Behavioral response to external stimuli/hearing is evident around E15-17 (Jackson and Rubel, 1978; Jones et al., 2006). Therefore in the present study, the sound exposure started from E10 until hatching so that the chronic effects of sound exposure over the developmental period could be studied. Details of the audio system settings inside the incubator and acoustic profile of sounds used have been explained in earlier publications (Sanyal et al., 2013a,b). Briefly, the frequency of the music stimulus ranged between 100 and 4000 Hz and that of the noise stimulus within 30 to 3000 Hz with a peak at 2.7 kHz. The noise sound consisted of simple waves with a constant wave length whereas the sitar music (a string instrument) had a complex wave form. Variations in the energy with frequency and time imparted a rhythmic pattern to the music stimulus whereas for noise stimuli, the energy did not vary over time for a particular frequency giving it a relatively continuous and arrhythmic pattern. Noise sound having peak and dominant energy distribution near 2.7 kHz has been used based on the fact that most hearing loss occurs at higher frequencies and which forms the basis for including 2-, 3- and 4-kHz frequencies in damage-risk formulae in most studies (WHO and EHC 12, 1980). A-weighted output of 110 ± 3-dB SPL inside the incubator for both of these exposures was matched and controlled which was confirmed though a Class-I sound level meter (8928 AZ-Instruments, Taichung City, Taiwan).

Experimental groups

The eggs ($n = 69$ each group) were divided into following three groups for three separate incubations:

Group I: Control. Incubated under normal conditions with no additional sound exposure.

Group II: Music. Embryos exposed to rhythmic sitar music within a frequency range of 100–4000 Hz at 110-dB SPL from E10 until hatching.

Group III: Noise. Embryos exposed to arrhythmic vehicle horn noise of frequency range 30–3000 Hz at 110-dB SPL from E10 until hatching.