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Research Paper

Effects of noise-induced hearing loss on parvalbumin and perineuronal net expression in the mouse primary auditory cortex

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ABSTRACT

Noise induced hearing loss is associated with increased excitability in the central auditory system but the cellular correlates of such changes remain to be characterized. Here we tested the hypothesis that noise-induced hearing loss causes deterioration of perineuronal nets (PNNs) in the auditory cortex of mice. PNNs are specialized extracellular matrix components that commonly enwrap cortical parvalbumin (PV) containing GABAergic interneurons. Compared to somatosensory and visual cortex, relatively less is known about PV/PNN expression patterns in the primary auditory cortex (A1). Whether changes to cortical PNNs follow acoustic trauma remains unclear. The first aim of this study was to characterize PV/PNN expression in A1 of adult mice. PNNs increase excitability of PV+ inhibitory neurons and confer protection to these neurons against oxidative stress. Decreased PV/PNN expression may therefore lead to a reduction in cortical inhibition. The second aim of this study was to examine PV/PNN expression in superficial (I-IV) and deep cortical layers (V-VI) following noise trauma. Exposing mice to loud noise caused an increase in hearing threshold that lasted at least 30 days. PV and PNN expression in A1 was analyzed at 1, 10 and 30 days following the exposure. No significant changes were observed in the density of PV+, PNN+, or PV/PNN co-localized cells following hearing loss. However, a significant layer- and cell type-specific decrease in PNN intensity was seen following hearing loss. Some changes were present even at 1 day following noise exposure. Attenuation of PNN may contribute to changes in excitability in cortex following noise trauma. The regulation of PNN may open up a temporal window for altered excitability in the adult brain that is then stabilized at a new and potentially pathological level such as in tinnitus.

1. Introduction

Even relatively brief exposure to loud noise can cause hearing loss or threshold shifts. Such noise-induced threshold shifts remain a common, but preventable, hearing disorder. Noise exposure may also lead to the development of tinnitus and hyperacusis (Roberts et al., 2010). Several lines of evidence suggest that noise exposure increases excitability in the central auditory system perhaps as a consequence of damage to cochlear hair cells and the resulting reduction in afferent input. This compensatory increase in gain manifests across the auditory neuraxis and occurs over multiple and overlapping temporal trajectories suggesting complex underlying mechanisms (Syka et al., 1994; Syka and Rybalko, 2000; Yang et al., 2011, 2012; Pilati et al., 2012; Berger and Coomber, 2015; Luo et al., 2016; reviewed in Wang et al., 2011; Eggermont, 2015). The cellular correlates of these changes in excitability are not well characterized.

One prominent hypothesis for noise-induced increase in excitability in the primary auditory cortex (A1) is reduced inhibition (Syka and Rybalko, 2000; Yang et al., 2011; Llano et al., 2012). While physiological studies have characterized synaptic inhibition and how inhibition changes following noise exposure, the cellular substrates that are altered are only beginning to be understood (Scholl and Wehr, 2008; Novák et al., 2016). Inhibitory interneurons in sensory cortices can be classified based on co-expression of various markers and physiological response properties. Novák et al. (2016) showed that cortical somatostatin and parvalbumin-expressing (PV+) interneurons show relatively fast and layer-

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specific changes in activity following noise trauma potentially leading to increased gain. Whether changes in responses of these cells are associated with circuit level or intrinsic factors remain unclear.

The present study focused on perineuronal nets (PNN), a cellular structure commonly found around GABAergic cells (reviewed in Takesian and Hensch, 2013). PNNs are specialized extracellular matrix components that consist of chondroitin sulfate proteoglycans (CSPG). These CSPGs are found throughout the extracellular matrix, but are highly dense around cortical PV+ inhibitory interneurons (McRae et al., 2007). While PV/PNN expression has been well studied in somatosensory and visual cortex of rodents, focus on A1 is relatively recent and sparse (Happel et al., 2014; Fader et al., 2016; Brewton et al., 2016; reviewed in Sonntag et al., 2015). PNNs are involved with developmental and adult plasticity (Happel et al., 2014; Nakamura et al., 2009; Pizzorusso et al., 2002) and provide protection against oxidative stress for PV+ cells (Cabungcal et al., 2013). These data suggest that changes in PNN expression following acoustic trauma may contribute to cortical plasticity leading to increased excitability. A loss of PNNs may decrease excitability of PV+ interneurons and thus reduce inhibition in the cortical circuit (Balmer, 2016). Therefore, the main aim of this study was to quantify cortical PNN expression following acoustic trauma that induces persistent threshold shifts. We report here that noise exposure does not change the density of PV+, PNN + or PV/PNN co-localized cells. However, PNN intensity is reduced in a cortical layer-and cell-type specific manner. The effect of trauma on PNN intensity appears to be relatively more severe on PNN cells that do not express PV. Some changes are seen even at the earliest examined time point (1 day post-exposure). These data suggest that altered PNN properties may be at least one of the cellular mechanisms involved in enhanced excitability of cortical neurons following acoustic trauma.

2. Material and methods

2.1. Animals

All animal procedures were approved by the University of California, Riverside Institution Animal Use and Care Committee. Female CBA/CaJ mice at 4 weeks old were received from Jackson Laboratory and housed at a 12:12 light/dark cycle. Standard lab chow and water were given *ad libitum*. All animals were housed in the same room except for the noise exposure and auditory brainstem response (ABR) measurements. Each of the four groups (control and 1, 10, and 30 days post-exposure) consisted of n = 5 mice.

2.2. Noise-induced hearing loss paradigm

Noise exposure was done in a sound-attenuating booth (Gretch-Ken, OR). Mice were placed in a standard cage and were able to freely move during the duration of the exposure to noise. A Fostex 96TX speaker was placed facing down on top of the cage's lid. The sound stimulus used was a 102–104 dB SPL, narrowband noise (6–12 kHz) for 8 h. No food or water was provided during the duration of the exposure to noise. The control mice spent the same amount of time in the sound-attenuating booth, but did not receive noise exposure.

2.3. Auditory brainstem response (ABR)

Animals were anesthetized with isoflurane inhalation for the duration of the ABR procedure at a concentration of 0.5–0.75% in air. Three platinum coated electrodes were placed under the dermis of the head: the recording electrode was on the vertex, the ground electrode was in the left cheek and the reference electrode was in the right cheek. The sound stimuli were delivered via a free field speaker (MR1 Multi-Field Magnetic Speakers, Tucker-Davis Technologies) that was placed 10 cm away from the left ear at 45°. Clicks of alternating ± 1.4 V (duration 0.1 ms) were generated and delivered using RZ6 hardware (Tucker-Davis Technologies, FL). Intensity of the clicks ranged from 10 to 90 dB in 10 dB steps. The goal of the ABR measurement was to determine if threshold shifts occurred following noise exposure and to ensure that such shifts lasted at least 30 days. The goal was not to identify precise frequency-specific hearing levels over the course of the experiments. Therefore, clicks with a sound level resolution of 10 dB steps were used for threshold measures. The ABRs were filtered and amplified (Grass Technologies) and averaged (BioSigRZ, Tucker-Davis Technologies) before analysis. The ABR measurements were made on all mice before exposure to noise and after the noise exposure at 1 day, 10 days and 30 days post-exposure (PE). ABRs from control mice were also measured at the same four time points referenced to when they were placed in the sound booth without noise exposure.

2.4. Immunohistochemistry and image analysis

Mice were overdosed with sodium pentobarbital (i.p. 125 mg/kg) and perfused transcardially with cold solutions of 0.1 M phosphate buffered saline (PBS) (pH = 7.4) followed by 4% paraformaldehyde (PFA) (pH = 7.4). Mice were perfused for each time point (1, 10, and 30 days) post-exposure (PE) to noise. The control mice were perfused along with the 30-day PE mice. The brains were extracted from the skull and post-fixed at 20 °C in 4% PFA for 2 h before storage in 0.1 M PBS with sodium azide. Brain tissues were sunk in 30% sucrose for 24–48 h and coronal sections of 40 μm thickness were cut with a cryostat (CM 1860, Leica Biosystems). Three to six sections containing A1 were stained and analyzed per mouse. The distance between the sections was between 40 and 480 μm. It is possible that there is differential penetration of PV and WFA antibody in the 40 μm thick sections. However, because our main aim was to determine how noise exposure alters PV/PNN expression, the comparison across experimental groups is unlikely to be influenced by differential antibody penetration. All immunohistochemistry was done on a shaker at room temperature unless stated otherwise. Free floating sections were washed at room temperature with 0.1 M PBS 2× for 15 min then quenched with 50 mM of NH_4Cl for 15 min and then washed with 0.1 M PBS 3× for 10 min. Next, the sections were permeablized with 0.1% triton-x for 10 min. Sections incubated in blocking solution consisting of 5% normal goat serum (NGS) and 1% bovine serum albumin (BSA; Fisher BioReagents Bovine Serum Albumin, Fraction V, Cold-ethanol Precipitated; BP1605-100) in 0.1 M PBS for 1 h. The sections were then incubated overnight at 20 °C in 1% NGS, 0.5% BSA 0.1% Tween-20, 1:500 agglutinin *Wisteria floribunda* (fluorescein conjugated, FL-1351, Vector Laboratories) and 1:5000 rabbit anti-parvalbumin (PV-25, Swant). Sections were washed with 0.5% Tween-20 3× for 10 min and incubated in secondary antibody solution consisted of 1:500 donkey anti-rabbit 647 (A-31573, Life Technologies) in 0.1 M PBS. The sections were then washed with 0.5% Tween-20 2× for 10 min and with 0.1 M PBS for 10 min, mounted on a glass slide and allowed to air dry. The slides were cover-slipped with the mounting medium, Vectashield containing DAPI (Vector Laboratories), and the edges of the coverslip were sealed (Cytoseal 60, Richard-Allan Scientific).

The location of A1 was identified as previously described by Martin del Campo et al. (2012). In this previous study, electrophysiological mapping was used to identify tonotopy in both A1 and anterior auditory field (AAF). The boundary between A1 and