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Research paper

Evidence of activity-dependent plasticity in the dorsal cochlear nucleus, *in vivo*, induced by brief sound exposure

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ABSTRACT

The purpose of the present study was to investigate the immediate effects of acute exposure to intense sound on spontaneous and stimulus-driven activity in the dorsal cochlear nucleus (DCN). We examined the levels of multi- and single-unit spontaneous activity before and immediately following brief exposure (2 min) to tones at levels of either 109 or 85 dB SPL. Exposure frequency was selected to either correspond to the units' best frequency (BF) or fall within the borders of its inhibitory side band. The results demonstrate that these exposure conditions caused significant alterations in spontaneous activity and responses to BF tones. The induced changes have a fast onset (minutes) and are persistent for durations of at least 20 min. The directions of the change were found to depend on the frequency of exposure relative to BF. Transient decreases followed by more sustained increases in spontaneous activity were induced when the exposure frequency was at or near the units' BF, while sustained decreases of activity resulted when the exposure frequency fell inside the inhibitory side band. Follow-up studies at the single unit level revealed that the observed activity changes were found on unit types having properties which have previously been found to represent fusiform cells. The changes in spontaneous activity occurred despite only minor changes in response thresholds. Noteworthy changes also occurred in the strength of responses to BF tones, although these changes tended to be in the direction opposite those of the spontaneous rate changes. We discuss the possible role of activity-dependent plasticity as a mechanism underlying the rapid emergence of increased spontaneous activity after tone exposure and suggest that these changes may represent a neural correlate of acute noise-induced tinnitus.

1. Introduction

The DCN, one of the first central processing stations in the ascending auditory pathway, receives acoustic input from auditory nerve fibers and multimodal inputs from diverse brain areas through a population of granule cells and their parallel fiber axons. Both auditory nerve and parallel fibers have excitatory synapses onto the principal output neurons of the DCN, the fusiform cells. These cells have been shown to become hyperactive following intense noise exposure (Brozoski et al., 2002; Finlayson and Kaltenbach, 2009; Shore et al., 2008; Dehmel et al., 2012), which is manifest as an increase in spontaneous activity that develops gradually following induction of hearing loss and persists for up to at least 6 months (Kaltenbach et al., 1998, 2000). The induced hyperactivity is relayed to higher levels of the auditory system, and there is evidence suggesting that it may be an important factor contributing to tinnitus in noise-exposed animals (Brozoski et al., 2002; Kaltenbach et al., 2004; Ma et al., 2006; Shore et al., 2008).

Noise-induced hyperactivity is widely thought to result from a shift in the balance of excitation and inhibition in the central auditory system (see reviews of Roberts et al., 2010). There is evidence that noise exposure and related manipulations that impair peripheral function, cause decreases of inhibitory and increases in excitatory synaptic transmission in the DCN (Asako et al., 2005; Cransac et al., 1998; Muly et al., 2004; Potashner et al., 2000; Shore, 2011; Suneja et al., 1998; Wang et al., 2009; Zeng et al., 2009). Although little is known about the mechanism that triggers such a shift, one hypothesis is that the observed changes in synaptic connectivity are triggered by the loss of normal primary afferent input to neurons in the cochlear nucleus. Loss of hair cell

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integrity leads to degeneration or weakening of primary afferents, and the deafferented neurons may undergo transneuronal degeneration and/or various forms of plastic alterations, including sprouting of new synapses (Bilak et al., 1997; Kim et al., 1997, 2004; Morest et al., 1997), and/or up- and down-regulations of receptors on existing synapses (Wang et al., 2009; Milbrandt et al., 2000; Dong et al., 2010a; Zeng et al., 2009; Kaltenbach and Zhang, 2006). These changes are widely believed to underlie the chronic form of tinnitus, but they appear to emerge too slowly to underlie all forms of noise-induced tinnitus (NIT). The acute form of tinnitus, which is observed following moderate sound exposure conditions that are not permanently damaging to the auditory periphery, develops within seconds or minutes following exposure (Atherley et al., 1968; Loeb and Smith, 1967). This suggests a fast acting mechanism that is independent of deafferentation.

One mechanism that could underlie the acute form of tinnitus and could also set in motion changes that lead to the chronic form of tinnitus is activity-dependent plasticity (Tzounopoulos, 2008). Two forms of activity-dependent plasticity, LTP and LTD, have been found in DCN fusiform and cartwheel cells in brain slice preparations (Fujino and Oertel, 2003; Tzounopoulos et al., 2004). Intrinsic membrane properties can also be regulated in an activity dependent manner (Turrigiano et al., 1994; Aizenman and Linden, 1999; Desai et al., 1999; Egorov et al., 2002; Smith and Otis, 2003), leading to modulation of neuronal spontaneous rate. Thus far, studies demonstrating induction of hyperactivity in the DCN have not been comparable to those inducing LTP or LTD, since they have employed long exposure conditions (1–4 h) that cause injury to cochlear receptors and/or auditory nerve fibers, and changes in spontaneous activity have generally been observed in the range of days to months after exposure. In contrast, neither LTP nor LTD are dependent on injury to cochlear receptors, and both are observed within minutes following co-activation of their postsynaptic membranes and inputting parallel fibers (Fujino and Oertel, 2003; Tzounopoulos et al., 2004). The effect of intense tone exposure on spontaneous activity on the time scale of minutes and using conditions that do not damage the peripheral receptor epithelium has not been systematically tested and described.

Here, we investigated the immediate effect of acute sound exposure on spontaneous and stimulus-driven activity in the DCN of anesthetized hamsters. The level and duration of exposure were selected so as to constitute a condition of acoustic overexposure without inducing significant injury to the primary afferents. This allowed us to test the effects of over-activation of primary afferents without the confounding effects of deafferentation and hearing loss. The exposures were also designed to activate inputs to fusiform cells from auditory nerve fibers and parallel fibers and to resemble stimuli that have previously been shown to induce acute tinnitus in human subjects (80–109 dB) (Loeb and Smith, 1967; Atherley et al., 1968). We also examined the effects of these exposure conditions on stimulus-driven activity over a wide range of sound levels. We report that limited exposure to moderate level tones induces an immediate change of spontaneous activity and evoked responses in neurons with the properties of fusiform cells.

2. Materials and methods

2.1. Animal subjects

The animals used in all experiments were adult male Syrian golden hamsters (LVG strain) obtained from Charles River Laboratories. Animals were housed in the animal vivarium of the Cleveland Clinic and were cared for in accordance with NIH Guidelines for the care and use of animals in research. Animals were between 2 and 3 months of age at the time experiments were conducted.

2.2. Surgical preparations

Animals were anesthetized by i. m. injections of ketamine (117 mg/kg) and xylazine (18 mg/kg). Areflexia was maintained with supplementary injections of anesthetic. Core body temperature was maintained at 37 °C using a rectal probe and heating pad. A tracheotomy was performed to maintain normal breathing during anesthesia. The animal's head was held firmly in a brace, and the left DCN was exposed by parieto-occipital craniotomy followed by aspiration of the overlying portion of the cerebellum.

2.3. Electrophysiological recordings

Electrodes were micropipettes filled with 0.3 M NaCl and had tip impedances of either 0.4–0.5 MΩ for multiunit recordings or 2–10 MΩ for single unit recordings. The output of the microelectrode was fed through an amplifier (1000X) and bandpass-filtered (0.3–10 kHz). The electrode was lowered until contact was made with the DCN surface as signaled by the disappearance of 60 cycle noise and the emergence of neural activity. Recordings were conducted before, during and after presentation of an exposure tone (see below). For the multiunit recordings, only a single attempt at tone-induced plasticity was made in each animal to avoid the potential for contamination from previously induced plasticity. For single unit recordings, due to the lower abundance and long term stability of units, two attempts of exposure-induced plasticity were made in each animal with a recovery period of at least 60 min between exposures. At the beginning of the pre-exposure period, frequency tuning properties were tested with pure tones (50 ms including 5 ms rise-fall ramps) over a range of frequencies (3–32 kHz) and intensities (6–96 dB SPL). Generally, frequency tuning curves from multiunit clusters were similar to those of single units, except that the multiunit tuning was sometimes slightly broader. However, we found no evidence to suggest that the BFs based on multiunit recordings differed from those of single units taken from the same recording sites. Spontaneous rates were quantified and monitored over an extended duration spanning the periods before and following the exposure tone. The time axis was divided into 5 s time bins (epochs). For multiunits recordings, spontaneous rates were measured by counting in each epoch the number of voltage events exceeding (more negative than) −100 μV. These counts were converted to rates, expressed in events/s, by dividing the counts by 5 (the duration of each epoch). Single unit spontaneous rates were quantified and monitored over a similar time frame, but in each 5 s epoch, rates were measured by counting the number of spikes of similar amplitude and waveform in each bin and dividing by 5. Both multi- and single-unit spontaneous rates and tuning properties were acquired using customized Matlab software. Thresholds and tuning properties were based on frequency-intensity response maps as described previously (Finlayson and Kaltenbach, 2009). These properties were determined for both single- and multi-unit recordings after a pre-exposure baseline was established just before the exposure tone, then again after a 25–30 min post-exposure recording of spontaneous activity was completed. Any recordings that showed large shifts of tuning curve tip thresholds ($\geq$18 dB) after tone exposure were discarded. PST histograms (PSTHs) with bin resolutions of 1 ms were obtained from responses to 100 presentations of 40 ms tones set at the units' best frequency (BF) at 70 dB SPL presented at 1/s using Datawave (Sciworks) software. For both tuning curves and PST histograms, sound stimuli were presented monaurally at a rate of 10/s to the left ear through a Beyer Dynamic DT-48 headphone coupled to the external auditory meatus using a plastic conical insert (Finlayson and Kaltenbach, 2009).