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Research paper

Development of hyperactivity after acoustic trauma in the guinea pig inferior colliculus

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ABSTRACT

The time of onset of hyperactivity (increased spontaneous firing rates) was investigated by single neuron recording in the inferior colliculus (IC) of guinea pigs subjected to unilateral acoustic trauma (exposure to a loud 10 kHz tone). Hyperactivity was present by 12 h post acoustic trauma whereas data obtained within approximately 4 h of the cessation of acoustic trauma found no evidence of hyperactivity. These data suggest that hyperactivity in the IC begins at some time between 4 and 12 h post trauma and is a relatively rapid plastic event beginning within hours rather than days post cochlear trauma. This is consistent with results reported in the cat auditory cortex (Norena and Eggermont, 2003). Hyperactivity did not show any further systematic increase between 12 h and up to 2 weeks post acoustic trauma. At recovery times of 12 and 24 h hyperactivity was widespread across most regions of the IC but at longer recovery times, it became progressively more restricted to ventral regions corresponding to the regions of the cochlea where there was persistent damage.

1. Introduction

Acoustic trauma is known in animal models to result in increased spontaneous activity (so-called hyperactivity), in a sub-population of neurons in central auditory structures such as the cochlear nucleus (CN), inferior colliculus (IC) and auditory cortex (AC) (Brozoski et al., 2002; Dong et al., 2010; Kaltenbach et al., 2000; Komiya and Eggermont, 2000; Mulders and Robertson, 2009, 2011; Norena and Eggermont, 2003; Seki and Eggermont, 2003). This hyperactivity has been suggested as a possible neural substrate for tinnitus, a phantom hearing sensation frequently associated with cochlear trauma (Bauer et al., 2008; Brozoski et al., 2002; Kaltenbach et al., 2004).

Surprisingly, the detailed time course of the onset of hyperactivity after cochlear trauma has received little systematic attention. In the AC of cats, Seki and Eggermont (2003) reported increased single unit spontaneous rates 1 week after loud sound exposure. In a similar preparation, Norena and Eggermont (2003) used spike sorting of cortical multiunit clusters and reported that spontaneous firing rates were not immediately altered but increased above pre-exposure levels “a few hours” post trauma. Single unit recordings in guinea pig IC and ventral cochlear nucleus (VCN), found hyperactivity 1 and 2 weeks post trauma respectively (Mulders and Robertson, 2009, 2011; Robertson et al., 2013). Mulders and Robertson (2009) reported no significant hyperactivity in guinea pig IC immediately after acoustic trauma (0–4 h post-trauma) but other recovery times shorter than 1 week were not investigated. In the dorsal cochlear nucleus (DCN) of hamsters, multiunit activity was reported to be reduced 2 days post-exposure and elevated between 2 and 5 days post trauma (Kaltenbach et al., 2000). In the IC of the same species, multiunit activity was elevated 7 days post-exposure (Manzoor et al., 2013), but shorter recovery times were not investigated.

In addition to differences in species, anaesthetic regimes, site of recording and recovery times, any comparison between these studies is further confounded by the fact that a range of acoustic trauma parameters was employed.

Hence the present study was undertaken to provide more complete data on the time of onset of hyperactivity in IC, in the one species, using a fixed acoustic trauma regime.

2. Materials and methods

2.1. Animals

Twenty four adult pigmented guinea pigs of either sex, weighing between 260 and 385 g at the time of initial anaesthesia were

Abbreviations: AC, auditory cortex; CN, cochlear nucleus; DCN, dorsal cochlear nucleus; VCN, ventral cochlear nucleus; IC, inferior colliculus; CNIC, central nucleus of inferior colliculus; CF, characteristic frequency; CAP, compound action potential of cochlea.

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included in this study. The experimental protocols conformed to the Code of Practice of the National Health and Medical Research Council of Australia, and were approved by the Animal Ethics Committee of The University of Western Australia.

2.2. Surgical procedures

Details of all procedures have been presented in previous publications (Mulders and Robertson, 2009, 2011; Robertson et al., 2013; Vogler et al., 2011). Briefly, animals were initially anaesthetized by intraperitoneal injection of Diazepam (5 mg/kg), followed 20 min later by an intramuscular injection of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; 1 ml/kg) and were allowed to breathe unassisted. Cochlear neural thresholds in the left ear were assessed using the threshold of the compound action potential (CAP) of the auditory nerve (Johnstone et al., 1979) for tone bursts ranging from 4 to 24 kHz. Only animals with CAP thresholds in the normal range were used.

One group of animals ($n = 4$) served as sham controls and these were allowed to recover from anaesthesia for 2 weeks without further treatment. Five acoustic trauma groups ($n = 4$ per group) underwent exposure to a loud unilateral tone (10 kHz, 2 h, 124 dB SPL) using a closed sound delivery system. CAP audiograms were again measured in the traumatized cochlea immediately after exposure. Four of the five acoustic trauma groups were allowed to recover for survival times of 12 h ($n = 4$), 24 h ($n = 4$), 48 h ($n = 4$) and 2 weeks ($n = 4$). After the recovery period, animals were prepared for single neuron recording by being re-anaesthetized by an intraperitoneal injection of pentobarbitone sodium (30 mg/kg) and a 0.15 ml intramuscular injection of Hypnorm. The maintenance anaesthesia regime consisted of full Hypnorm doses every hour and half doses of Nembutal every 2 h. Animals were placed on a heating blanket in a sound proof room and artificially ventilated on carbogen (95% O_2 and 5% CO_2). Paralysis was induced with 0.1 ml pancuronium bromide (2 mg/ml intramuscularly). Heart rate was continuously monitored using the electrocardiogram (EEG). CAP audiograms were recorded from the cochlea on both sides and only animals with normal thresholds in the cochlea that did not receive the prior acoustic trauma were used for assessment of hyperactivity in the IC.

The fifth group of animals subjected to cochlear trauma was used to evaluate any acute changes in single neuron activity in the IC within the 4 h immediately post trauma period. These acute exposure animals ($n = 4$) were not allowed to recover from the initial anaesthesia. Instead, at the end of the 2 h exposure period, they were administered the same anaesthetic regime as that finally administered to the groups that were re-anaesthetized after recovery from the first anaesthesia. This procedure ensured that in both acute and recovery cochlear trauma groups, the trauma was administered under the same anaesthetic regime (Diazepam and Hynorm), and the subsequent collection of single neuron data post-cochlear trauma was performed in all groups under the second anaesthetic regime (pentobarbitone and Hypnorm). As for the recovery groups, in the acute group, the CAP audiogram was also measured in the unexposed cochlea and only animals with normal thresholds on that side were included in the study. In order to ensure as close as possible the same depth of anaesthesia during single neuron recordings in the acute and recovery groups, single neuron recordings in the acute group only commenced after switching to the barbiturate/Hypnorm regime when the heart rate measured by the EEG was the same as that observed in the other groups of animals under the same anaesthesia.

2.3. Electrophysiological recordings

Hyperactivity was assessed using extracellular single neuron recordings in the central nucleus of the inferior colliculus (CNIC) contralateral to the acoustically-traumatized cochlea, as previously described (Mulders and Robertson, 2009; Mulders et al., 2011). The location of the recording electrode in the CNIC was not routinely confirmed histologically. Presumed recording from CNIC was based on the presence of robust, short-latency and sharply-tuned cluster and single neuron responses of appropriate characteristic frequency (CF) in the regions of the IC unaffected by the acoustic trauma. For each single neuron encountered, the characteristic frequency (CF) and threshold at CF, were estimated audiovisually and spontaneous firing rate was measured over a 10 s sample. In order to reduce the possibility of sampling bias, an acoustic search stimulus (50 ms duration tone burst repeated 2 times per second) was continuously swept across the frequency and intensity range at each location in the CNIC and care was taken to record where possible from a similar number of neurons in each animal. For the sham control and all recovery exposure groups this ranged from 78 to 124 neurons per animal. However, for the acute exposure group, because the recording time was limited to a total of 4 h after the immediately preceding 2 h trauma, the number of neurons ranged from 37 to 132 per animal.

For statistical comparison of mean spontaneous firing rates between the different groups and between different regions in the IC, a Kruskall–Wallis test and a Dunn's multiple comparison post-test were used.

3. Results

3.1. Peripheral thresholds

As reported previously, the acoustic trauma resulted in immediate changes in CAP thresholds, followed by progressive but partial recovery of threshold. As shown in Fig. 1, immediately after exposure CAP thresholds were markedly elevated for sound frequencies from 8 to 24 kHz, but as recovery time increased, the range of frequencies over which the CAP thresholds remained elevated became mainly confined to frequencies above 10 kHz and the maximum CAP threshold loss also reduced in magnitude. This general pattern of threshold change immediately after exposure and after 2 weeks recovery agrees closely with previously reported results using identical methods (Mulders et al., 2011).

3.2. Spontaneous activity measurements

Fig. 2 shows the average spontaneous firing rate of all IC neurons in all groups regardless of each neuron's CF or location within the

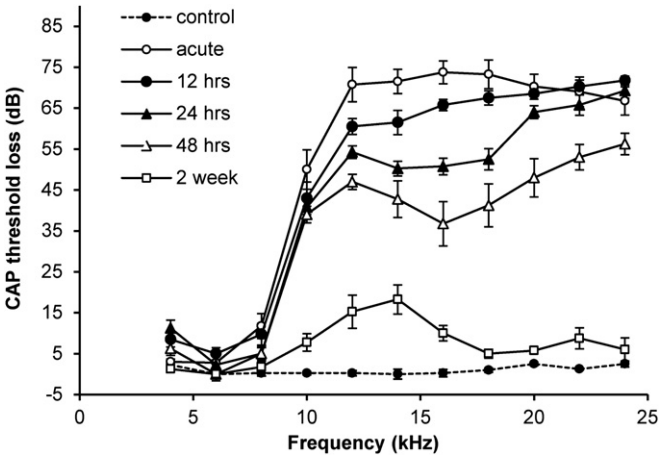

Fig. 1. CAP threshold changes relative to pre-exposure thresholds in the cochlea in guinea pigs exposed to acoustic trauma and allowed to recover for varying periods after trauma. Error bars are standard error of mean. $n = 4$ for all groups.