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Research paper

Influence of the paraflocculus on normal and abnormal spontaneous firing rates in the inferior colliculus

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ABSTRACT

Spontaneous firing rates of neurons in the central auditory pathway, such as in the inferior colliculus, are known to be increased after cochlear trauma. This so-called hyperactivity is thought to be involved in the generation of tinnitus, a phantom auditory perception. Recent research in an animal model suggests behavioural signs of tinnitus can be significantly reduced by silencing or removal of the paraflocculus (PF) of the cerebellum. The current study investigated the effects of acute PF removal on spontaneous firing rates recorded from single neurons in the right inferior colliculus of guinea pigs with normal hearing (which did not receive acoustic trauma) or with hearing loss caused by acoustic trauma. Spontaneous firing rates were obtained at either 2 or 13 weeks after initial surgery on the left side. In half of the animals in each group the left PF was removed immediately prior to the spontaneous firing rates recordings. In the acoustic trauma groups, spontaneous firing rates in the inferior colliculus were higher when the PF was removed compared to animals with an intact PF. This effect of PF removal was not observed in animals that did not receive acoustic trauma. These results suggest that the PF has a tonic inhibitory effect on hyperactivity in the inferior colliculus in animals with hearing loss, but not on normal spontaneous firing rates in normal hearing animals.

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1. Introduction

Tinnitus, a common and potentially debilitating symptom, is frequently associated with hearing loss (Axelsson and Ringdahl, 1989). To date, the neural substrate for tinnitus is unconfirmed however one proposed mechanism is hyperactivity, i.e. increased spontaneous firing rates (SFRs) of neurons in the auditory pathway (Eggermont and Roberts, 2004). Hyperactivity is observed in multiple auditory brain regions following hearing loss as demonstrated in different animal models (Finlayson and Kaltenbach, 2009; Kalappa et al., 2014; Mulders and Robertson, 2009; Noreña et al., 2010; Vogler et al., 2011). Animals that show hearing loss and subsequent hyperactivity also demonstrate behavioural evidence of tinnitus (Brozoski et al., 2002; Kalappa et al., 2014; Mulders et al., 2014b). The co-occurrence of hyperactivity and tinnitus suggests hyperactivity may be involved in the generation of tinnitus.

Changes in central activity after cochlear trauma are not restricted to the auditory pathway. One of the non-auditory structures that shows hyperactivity after acoustic trauma and after development of tinnitus is the paraflocculus (PF) of the cerebellum (Brozoski et al., 2007). In addition, Mulders et al. (2014a) showed altered mRNA levels of inhibitory genes in PF after cochlear trauma. Finally, other studies revealed that PF removal or silencing by glutamatergic antagonists or lidocaine resulted in reduced behaviours associated with tinnitus (Bauer et al., 2013a, 2013b; Brozoski et al., 2013), whereas application of glutamatergic agonists induced tinnitus-like behaviour, which suggests a role for the PF as a modulator of tinnitus (Bauer et al., 2013b).

Traditionally, the PF has been associated with vision and movement (Azizi and Woodward, 1990; Burne et al., 1978; Lisberger et al., 1994; Rambold et al., 2002; Stone and Lisberger, 1990). However, the cerebellum has been shown to be activated when auditory input is processed (Petacchi et al., 2005) and the PF

Abbreviations: AC, Auditory Cortex; CAP, Compound Action Potential; CF, Characteristic Frequency; CN, Cochlear Nucleus; CNIC, Central Nucleus of the Inferior Colliculus; dB SPL, decibel Sound Pressure Level (re 20 μPa); DCN, Dorsal Cochlear Nucleus; IC, Inferior Colliculus; i.m., intramuscular; i.p., intraperitoneal; PF, Paraflocculus; s.c., subcutaneous; SFR, Spontaneous Firing Rate

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receives and integrates multi-modal sensory inputs (Azizi and Woodward, 1990). Anatomical studies have suggested inputs to PF from the cochlea, cochlear nucleus and indirectly from the auditory cortex (Azizi et al., 1985; Huang et al., 1982; Morest et al., 1997). In line with this connectivity, PF neurons have been shown to be responsive to auditory stimulation (Azizi and Woodward, 1990, 1985; Sun et al., 1990) as well as electrical stimulation of the auditory cortex (Azizi et al., 1985; Sun et al., 1990).

This study investigated the effect of acute PF removal on SFRs in the IC after acoustic trauma, because 1) removal of the PF can reduce behaviours associated with the presence of tinnitus (Bauer et al., 2013a; Brozoski et al., 2013), 2) acoustic trauma induced hyperactivity may be involved in the generation of tinnitus (Eggermont, 2005; Eggermont and Roberts, 2004; Kaltenbach and Afman, 2000) and 3) treatments that modulate hyperactivity also modulate tinnitus (Mulders et al., 2014b). In view of the above mentioned studies, it was hypothesized that acute PF removal would decrease the acoustic trauma induced hyperactivity in inferior colliculus. Single neuron recordings were made from guinea pig inferior colliculus (IC) either 2 or 13 weeks after acoustic trauma with or without acute unilateral removal of PF at the final time-point. In addition, the effects of PF removal were also investigated in separate group of animals that did not receive acoustic trauma.

2. Methods

2.1. Animals

Forty-one adult pigmented guinea pigs of either sex were used. At the time of initial surgery animals weighed between 265 and 532 g. All 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. A flow diagram showing the experimental design is shown in Fig. 1.

2.2. Recovery surgery

Surgery was similar to that which has been described previously (Mulders and Robertson, 2009; Robertson et al., 2013; Vogler et al., 2014). Prior to anaesthesia, animals were injected with 0.1 ml of atropine sulphate (0.6 mg/ml, subcutaneously (s.c.)). Anaesthesia consisted of an intraperitoneal (i.p.) injection of diazepam (5 mg/kg) followed 20 min later by an intramuscular (i.m.) injection of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; 1 ml/kg). Deep anaesthesia was indicated by the absence of the foot withdrawal reflex. Following this, animals were placed on a heating blanket in a soundproof room and mounted in hollow ear bars. A hole was made in the tympanic bulla and an insulated silver wire was placed on the round window of the left cochlea. Compound action potential (CAP) thresholds were recorded for frequencies between 2 and 24 kHz and were used to assess the animals' cochlear sensitivity as previously described (Johnstone et al., 1979). All sound stimuli were presented in a closed sound system using a 0.5 inch condenser microphone driven in reverse as a speaker (Bruel and Kjaer, type 4134). A computer, equipped with a DIGI96 soundcard, synthesized pure tone stimuli. This signal was then passed to an analog/digital interface (ADI-9 DS, RME Intelligent Audio Solution). Sample rate was 96 kHz. A custom-designed computer program (Neurosound, MI Lloyd) was used to regulate frequency and intensity and collect single neuron data. A different data acquisition system (Powerlab 4SP, AD Instruments) was used to amplify, filter (100 Hz–3 kHz bandpass) and record CAP signals. When initial cochlear sensitivity was within the normal range (Johnstone et al., 1979), animals were randomly assigned to one of two treatment groups, i.e. no acoustic trauma ($n = 21$) or acoustic trauma ($n = 20$). The acoustic trauma animals were exposed to a 2 h continuous 10 kHz pure tone at 124 dB SPL, while the contralateral ear was blocked using a plasticine plug. Post-acoustic trauma, CAP audiograms were recorded to assess the immediate loss in peripheral sensitivity. All wounds were sutured and animals were recovered for 2 or 13 weeks before the final single neuron recordings were made. All aspects of experimental design (e.g. anaesthesia, exposure surgery etc.) were identical between the no acoustic trauma animals and acoustic trauma animals, except that the no acoustic trauma group did not receive any acoustic trauma.

2.3. Non-recovery surgery for electrophysiological recordings

During the final non-recovery surgery at 2 or 13 weeks after the initial recovery surgery, half of the animals in each group (see flow diagram in Fig. 1) underwent an acute PF aspiration before single neuron recordings from the IC were obtained (PF- groups). In the other half of the animals in each group PF was kept intact before single neuron recordings from the IC were obtained (PF+ groups). PF aspiration was performed ipsilateral whereas IC recordings were done contralateral to the cochlea that underwent acoustic trauma.

Animals were administered 0.1 ml of atropine (s.c.) immediately followed by an injection of Nembutal (pentobarbital sodium, 30 mg/kg, i.p.) and 10 min thereafter a 0.15 ml i.m. injection of Hypnorm. Maintenance anaesthesia consisted of administering full Hypnorm doses every hour and half doses of Nembutal every 2 h. When deep anaesthesia was achieved (absence of the foot withdrawal) animals were placed on a heating blanket in a sound proof room and artificially ventilated on carbogen (95% O_2 and 5% CO_2). They were mounted in hollow ear bars and the right and left cochleae were exposed. CAP thresholds were measured on both sides, using methods identical to the recovery experiment. Prior to electrophysiological recordings in the central nucleus of the IC (CNIC), animals were paralysed with a 0.1 ml i.m. injection of pancuronium bromide (2 mg/ml i.m.). Anaesthesia level was monitored thereafter using a continuous electrocardiogram and heart rate never increased above pre-paralysis levels at any stage of

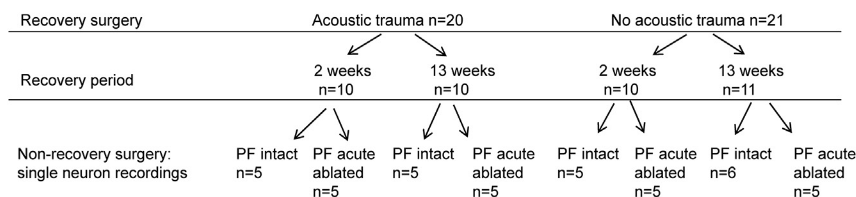

Fig. 1. Flow diagram showing the experimental design of this study. Animals were first exposed to surgery to measure CAP thresholds and half of the animals then received acoustic trauma and the other ones did not (no acoustic trauma). Animals were then allowed to recover for either 2 or 13 weeks at which time-point a final electrophysiological experiment was performed during which the SFRs of neurons in the IC were recorded. In each group PF was ablated in half of the animals (PF-) and not ablated (left intact) in the other half (PF+). This resulted in a total of 8 groups to be analysed.

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