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Research Paper

Medial geniculate neurons show diverse effects in response to electrical stimulation of prefrontal cortex

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ABSTRACT

Phantom perceptions have been proposed to arise due to dysfunctional sensory gating at the level of the thalamus. Recently, it has been suggested that tinnitus, a phantom perception of sound, may arise from altered cortico-limbic circuitry and its connection with the auditory thalamus, the medial geniculate nucleus (MGN). Indeed, some elements of this cortico-limbic circuitry, such as the prefrontal cortex (PFC), as well as elements of the auditory pathway, have been shown to be altered in humans with tinnitus. However, the functional connectivity between PFC and MGN has not yet been explored. We therefore investigated the effects of activation of the PFC on neuronal activity in MGN in normal anaesthetized Wistar rats. Bipolar electrical stimulation was delivered to the PFC while recording single neuron activity in the MGN. The majority (81%) of MGN neurons sampled showed a change in their spontaneous firing rate in response to electrical stimulation of the PFC. The effects observed varied greatly between neurons and included combinations of inhibitory and excitatory effects with a wide range of latencies. The effects were not dependent on acoustic response type or MGN subdivision. These data demonstrate that PFC activation can modulate MGN neuronal activity and this connection could potentially play a role in sensory gating of auditory signals.

1. Introduction

Sensory gating in the central nervous system plays an important role in filtering salient from irrelevant neural information (Barbas et al., 2011; McCormick and von Krosigk, 1992; Mease et al., 2014; Xiao et al., 2009; Zliang and Deschênes, 1998). Dysfunctional sensory gating has been suggested to be involved in the generation of tinnitus, a phantom auditory perception (De Ridder et al., 2014; Leaver et al., 2011; Rauschecker et al., 2010; Vanneste et al., 2010). The functionality of sensory gating has been shown to be influenced by behavioural states such as stress and anxiety, implicating the involvement of the limbic system (Halassa et al., 2014; White and Yee, 1997). Indeed, it has been suggested that cortico-limbic circuitry contributes to conscious perception of auditory information by inhibiting negative or unnecessary signals (McCormick and von Krosigk, 1992; Zliang and Deschênes, 1998) and that a break-down of this circuitry could therefore lead to abnormal activity reaching perception, leading to tinnitus. In line with this proposed mechanism, tinnitus patients show structural changes and abnormal neural activity in both auditory and cortico-limbic circuitry, such as in the auditory cortex and in the prefrontal cortex (PFC) (Landgrebe et al., 2009; Leaver et al., 2012, 2011; Maudoux et al., 2012; Mühlau et al., 2006; Seydell-Greenwald et al., 2012).

The auditory thalamus, or medial geniculate nucleus (MGN) has been shown to be a target for sensory gating via predominantly inhibitory inputs from the thalamic reticular nucleus (TRN) (Yu et al., 2009). The TRN in turn receives many corticofugal and non-auditory inputs (O'Donnell et al., 1997). This local circuitry at the level of the MGN allows for the modulation of auditory information before it reaches the auditory cortex, at which point a perception may arise. In tinnitus, a failure of this circuitry at the level of the thalamus may therefore result in abnormal neural activity being brought to conscious perception (Rauschecker et al., 2010; Vanneste et al., 2010; De Ridder et al., 2013).

Animal studies have shown that the PFC projects to TRN (O'Donnell et al., 1997; Yu et al., 2009) and may therefore be able to

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Abbreviations

CF	characteristic frequency
MGN	medial geniculate nucleus
PFC	prefrontal cortex
PB	phosphate buffer
PSTH	peristimulus time histogram
SFR	spontaneous firing rate
TRN	thalamic reticular nucleus
dB	decibel
SPL	sound pressure level

affect indirectly the auditory processing in MGN and subsequently in auditory cortex. In agreement, clinical studies have used transcranial magnetic stimulation of the PFC to treat tinnitus and have shown some success reducing tinnitus intensity and distress, supporting a role for PFC in auditory processing and tinnitus, possibly via an effect on MGN (De Ridder et al., 2013, 2011). However, direct evidence that PFC can modulate activity in MGN is lacking. Therefore, in the present study we investigated in normal Wistar rats the effects of PFC electrical stimulation on the spontaneous firing rates (SFRs) of single neurons in MGN.

2. General methods

2.1. Animals

10 male Wistar rats, weighing between 390 and 500 g, were used. Experimental protocols complied with 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. Electrophysiological recordings

Anaesthesia was induced by intraperitoneal injection of urethane (1.3 g/kg). Ten minutes later, animals received a subcutaneous injection of 0.05 ml atropine sulfate (0.05 mg/ml) and an intramuscular injection of 0.1 ml Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone). Some animals required an additional dose of 0.1 ml Hypnorm to maintain deep anaesthesia. When full depth of anaesthesia was reached, as assessed by foot withdrawal in response to foot pinch, animals were placed on a heating blanket in a soundproof room. Rectal temperature was checked every hour and maintained at 37.5° C. Animals then received a tracheostomy and were then mounted in a stereotaxic frame using hollow ear bars. The head was levelled and a partial craniotomy was performed using a small dental drill at identified coordinates (Paxinos and Watson, 2006) to allow access to prefrontal cortex (PFC) and medial geniculate nucleus (MGN). In order to eliminate the possibility of muscle activity contributing to changes in neural firing animals received an intramuscular injection of 0.1 ml pancuronium bromide (2 mg/ml) 15 min before data collection. Paralysed animals were artificially ventilated on carbogen (95% O_2 and 5% CO_2) and depth of anaesthesia was confirmed by an absence of increase in heart rate (measured by ECG) in response to foot pinch every half hour. Animals required a further intramuscular injection of 0.1 ml pancuronium bromide every 2 h to maintain full paralysis. This was indicated during the experiment by an increase of overall noise in the recording which was accompanied by the observation that the animals were no longer dependent on the ventilator, suggesting paralysis was wearing off.

Sound stimuli were delivered to the left ear while electrophysiological recordings were made in the right MGN. The right ear was blocked with plasticine. All sound stimuli were presented in a calibrated sound system through a ½″ condenser microphone driven in reverse as a speaker (Brüel & Kjær, type 4134). Noise and pure tone stimuli (50-ms duration, 1 ms rise/fall times) were synthesized by a computer using custom software (Neurosound MI Lloyd) and a DIGI 96 soundcard connected to an analog/digital interface (ADI-9 DS, RME Intelligent Audio Solution). Sample rate was 96 kHz. The sound system was calibrated using an $^1/_8$″ microphone (Brüel & Kjær, type 2670) in place of the animal's eardrum and a calibrated sound source (Brüel & Kjær, type 4231) to measure the output of the sound system (dB SPL re 20 μPa).

Noise stimuli were used as a search stimulus for single neurons in MGN. Single neuron recordings were obtained using tungsten in a glass microelectrode (Merrill and Ainsworth, 1972) or platinum iridium electrode (Frederick Haer & Co). Analysed data were recorded from neurons that showed spikes which were clearly distinguishable from background electrical activity. When a single neuron was isolated, its characteristic frequency (CF) and acoustic threshold at CF were determined audio-visually as described previously when recording from guinea pig inferior colliculus neurons and rat MGN neurons (Barry et al., 2015; Mulders and Robertson, 2011). The spontaneous firing rate (SFR) was then measured for a period of 10 s while input to the speaker was turned off to eliminate the possibility of a low-level background noise emanating from the sound system. Peristimulus time histograms (PSTH) were then constructed from 75 to 100 stimulus presentations (0.1 ms bin size) at CF 20 dB above threshold (50 ms tone; repetition rate of 2/s, rise/fall time 1 ms) to identify the neurons' acoustic response type. This was followed by construction of histograms of 500 ms samples of SFRs, with and without electrical stimulation of PFC using the same histogram program (50–100 sweeps) to assess acute effect of electrical stimulation on SFR. In order to assess whether a change in firing rate occurred, the total spike counts (from 50 to 500 ms in order to exclude stimulus artefacts in first 50 ms) in histograms obtained without and with electrical stimulation of PFC were compared. Changes of 10% or larger were considered to constitute a response whereas changes smaller than 10% were not.

Electrical stimuli were delivered through a custom-made bipolar tungsten electrode (Barry et al., 2015; Mulders and Robertson, 2000) connected to an A-M Systems Isolated Pulse Stimulator (Model 2100). The timing of electrical stimuli was controlled by the Neurosound software. Electrical stimuli were delivered as shock trains (pulse duration 0.5 ms, train duration 50 ms, rate 125–300 Hz). Current intensity ranged from 100 μA to 1 mA. Maximum current (1 mA) was initially applied to increase the likelihood of seeing an effect of stimulation in the MGN. Then, when time allowed, current was reduced until the threshold for any observable effect was reached. In two animals, the effect of altering the position of the simulating electrode in dorsal-ventral direction was assessed using a starting position of the tips of the stimulating electrode 0.5 mm dorsal to the usual PFC location used for all other animals and moving ventrally in 0.3 mm increments for a total penetration length of 2 mm. Histograms (75–100 stimulus presentations; 0.1-ms bin size; experimental period of 500 ms) of MGN SFR were obtained at each stimulating electrode location.

2.3. Histological analysis

At the end of the experiment, animals were euthanized (0.3 mL Lethabarb) and transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were collected and stored in fixative overnight before they were

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