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Location of cells giving phase-locked responses to pure tones in the primary auditory cortex

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ABSTRACT

Phase-locked responses to pure tones have previously been described in the primary auditory cortex (AI) of the guinea pig. They are interesting because they show that some cells may use a temporal code for representing sounds of 60–300 Hz rather than the rate or place mechanisms used over most of AI. Our previous study had shown that the phase-locked responses were grouped together, but it was not clear whether they were in separate minicolumns or a larger macrocolumn. We now show that the phase-locked cells are arranged in a macrocolumn within AI that forms a subdivision of the isofrequency bands. Phase-locked responses were recorded from 158 multiunits using silicon based multiprobes with four shanks. The phase-locked units gave the strongest response in layers III/IV but phase-locked units were also recorded in layers II, V and VI. The column included cells with characteristic frequencies of 80 Hz–1.3 kHz (0.5–0.8 mm long) and was about 0.5 mm wide. It was located at a constant position at the intersection of the coronal plane 1 mm caudal to bregma and the suture that forms the lateral edge of the parietal bone.

1. Introduction

Most or all of the auditory cortex is thought to use a rate or place code for representing rhythmic fluctuations in a stimulus of more than about 100 Hz (Elhilali et al., 2004; Wang et al., 2008). However it is not surprising that a specialised group of auditory cells show phase-locking at up to 300 Hz because the potential information content of a timing code is much higher than a rate code (Tiesinga et al., 2008). It can also be more robust as firing rate is usually more sensitive to changes in sound intensity and neural habituation than time based codes. In the primary somatosensory cortex phase-locked responses, by single units, to the input derived from rapidly adapting receptors in the glabrous skin, are thought to be involved in the conscious discrimination of low-frequency (up to 80 Hz) vibrations (Mountcastle et al., 1969). Local field potential studies in the monkey and human auditory cortex have shown evidence of temporal synchronization to low-frequency tones and speech sounds (Steinschneider et al., 1980; Ahissar et al., 2001) and these responses may also be involved in conscious perception. Conscious perception may involve the synchronized activity of the neurons in at least one column of cells. Cylindrical or slab-like columns are thought to represent a basic processing unit in the neocortex (Mountcastle, 1997). A column is normally defined as a vertically arranged group of cells that stretches from layer I to layer VI where the cells have a consistent and definitive response property. In this study we wished to test three hypotheses: 1) that a phase-locked response to low-frequency tones (60–300 Hz) can be reliably found in every animal examined, 2) cells with phase-locked responses are arranged in a substantial group of cells that take the form of a column, 3) that the phase-locked responses are consistently located at a particular point on the cortical surface of the guinea pig.

In the auditory thalamus neurons are able to give phase-locked responses up to frequencies of over 1000 Hz (Rouiller et al., 1979; Wallace et al., 2007). Phase-locked responses to pure tones of up to 300 Hz have been described in AI of the guinea pig, but only for a small proportion of low-frequency cells and it was not clear whether the phase-locking lasted for more than 100 ms (Wallace et al., 2002). Some units showed strong habituation over a period of 100 ms. Stimuli of a longer duration were not tested in our earlier study.

Area AI in the cat was originally defined as a short-latency area with the majority of ascending inputs from the ventral nucleus of the medial geniculate body and a smooth tonotopic gradient among cells that had best frequencies of 100 Hz–40 kHz (Rose and

Abbreviations: AI(LF), low-frequency (≤1.3 kHz) division of primary auditory cortex; CF, characteristic frequency; MCA, middle cerebral artery; MGB, medial geniculate body; PSTH, peristimulus time histogram.

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Woolsey, 1949). However subsequent work has shown that AI can be subdivided with a functionally separate dorsal zone (Middlebrooks and Zook, 1983; He and Hashikawa, 1998). In some species there appear to be subdivisions along the tonotopic axis as well. Some rodents such as the mouse have a separate ultrasonic area which forms a continuation of the tonotopic gradient of AI (Stiebler et al., 1997). In other species with good low-frequency hearing the low-frequency end of AI ($\leq$1.3 kHz) may also form a separate division that is particularly sensitive to timing information. We denote this as AI(LF). This division has many units that are sensitive to interaural phase differences (Fitzpatrick et al., 2000; Scott et al., 2009; Wallace and Palmer, 2009) and analyzing the time differences that these represent is an important element of localizing sounds in the azimuthal plane. We define AI(LF) as being the part of AI that contains cells that are sensitive to interaural timing differences. In the guinea pig inferior colliculus timing-sensitive neurons generally have a CF of $\leq$1.5 kHz (McAlpine et al., 1996) and a slightly lower cut-off point (1.3 kHz) is present in the cortex (Wallace and Palmer, 2009). Coding for interaural time differences requires a reasonable population of cells with CFs that are less than 1 kHz. Small rodents such as the mouse, with relatively poor low-frequency hearing, may not have a separate AI(LF) (Stiebler et al., 1997). We have previously shown that phase-locked responses to pure tones form about 13% of units in AI(LF) in the guinea pig (Wallace et al., 2002). In this study by using multi-electrodes to give simultaneous recordings, at fixed intervals across the length and depth of the cortex, we were able to sample a greater number of units than in our previous single electrode work.

2. Materials and methods

2.1. Surgical preparation

Eight pigmented, adult guinea pigs of both sexes and weighing 322–702 g were used for physiological recording. Animals were anaesthetized with urethane (0.9 g/kg i.p., in 20% solution in 0.9% saline) supplemented as necessary by 0.1 ml Hypnorm (fentanyl citrate 0.315 mg/ml; fluanisone 10 mg/ml i.m.) to maintain forepaw areflexia. A single dose of atropine sulphate (0.06 mg/kg s.c.) was given to reduce bronchial secretions. All animals were tracheotomised and core temperature was maintained at 38 °C by a heating blanket and rectal probe. Heart rate and end-tidal carbon dioxide levels were maintained within normal limits by artificial respiration with 100% oxygen. An incision was made in the external ear flap to provide direct access to the auditory meatus and the meatus was cleared of wax. The animals were placed in a stereotaxic frame, with hollow plastic speculae replacing the ear bars, inside a sound-attenuating room. A craniotomy was performed over the right auditory cortex and the dura removed. During recordings the cortex was covered with agar. All experiments were performed in accordance with UK Home Office regulations.

2.2. Stimulation and recording

Auditory stimuli were delivered diotically through sealed acoustic systems, comprising modified Radio Shack 40–1377 tweeters joined via a conical section to a damped, 2.5 mm diameter, probe tube that fitted into the speculum. The system was calibrated in each experiment by inserting a probe tube microphone close to the tympanic membrane. Stimuli were digitally synthesized (Tucker-Davis Technologies, System 3) at 50 kHz sampling rate and output through 24-bit sigma-delta digital-to-analog converters. Tonal stimuli were of 100 or 200 ms duration, switched on and off simultaneously in the two ears with cosine-squared gates with 2 ms rise/fall times (10–90%). The maximum output was within 3 dB of 100 dB SPL up to 1 kHz.

Recordings were made using a silicon multiprobe manufactured at the University of Michigan. This probe had 4 shanks spaced at 200 μm and each shank had 4 recording sites spaced at 200 μm intervals. The square recording sites had sides of 35 μm and an impedance of 0.4 MΩ. All tracks were oriented approximately orthogonal to the cortical surface. Extracellular action potentials were amplified and filtered between 300 Hz and 3 kHz (Tucker-Davis Technologies, Alachua, FL). Responses were collected using Brainware (v7.43, Jan Schnupp, Oxford University) and spike sorted based on waveform shape and amplitude. Even after spike-sorting we preferred to retain multiunits as these contained more spikes and gave a more reliable measure of whether or not phase-locking was present. We had isolated single units with a tungsten electrode in our previous study of phase-locked responses (Wallace et al., 2002) but this had not led to measurements of higher vector strengths or higher limits of phase-locking frequency. A frequency response area and characteristic frequency (CF) were first obtained using an array of randomly presented, diotic, tonal stimuli (0–80 dB [for lowest-frequency units] or 10–100 dB in 5 dB steps, 50 Hz–4.5 kHz in 0.2 octave steps). This was followed by a randomly interleaved array of pure tone stimuli at 100 Hz presented at 50, 60, 70 and 80 dB SPL to both ears and repeated 10 times. If there was any evidence of phase-locking then another set of pure tone stimuli was presented diotically in a pseudorandom fashion (60–300 Hz in 20 Hz steps) at 60 and 80 dB SPL for 100 or 150 repeats. For some units tones were also presented at 320, 350 and 400 Hz.

Peristimulus time histograms (PSTHs) were constructed of 100 or 150 responses at each of a variety of frequencies and intensities. Period histograms were also plotted so that the degree of synchronization (vector strength) could be calculated (Goldberg and Brown, 1969). Units were only considered to be phase-locked to the stimulus frequency when their vector strength was above the 0.1% significance level (Rayleigh test of uniformity $>$13.8) (Mardia, 1972). For responses to the 200 ms long stimuli, the period histograms started 50 ms after the stimulus onset to avoid dynamic changes associated with the onset.

2.3. Histological reconstruction of tracks

In three of the later experiments lesions were made at some of the recording sites by passing 10 μA of current (electrode negative) for 10 s. Animals were then perfused transcardially with 400 ml of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Their heads were repositioned in the stereotaxic headholder. The electrode was replaced with a scalpel blade oriented in the same plane and a cut was made in the brain about 3 mm more medial than the electrode track. This cut surface was used as the base of the block to ensure that the sections were in the same orientation as the electrode track. The block was postfixed in 2% paraformaldehyde (pH 7.4) overnight and then washed for 24 h in 30% sucrose before being sectioned on a vibratome at a thickness of 100 μm. Sections were mainly stained for cytochrome oxidase as this is a useful stain for identifying cortical layers in AI (Wallace and Palmer, 2008), but some sections were also stained for Nissl substance with cresyl violet.

3. Results

3.1. Characteristics of phase-locked responses

We made a total of 26 placements of the four-pronged recording array in the AI(LF) of eight animals and identified phase-locked responses to tones in the range of 60–350 Hz for a total of