Phase-Locked Responses to Pure Tones in the Auditory Thalamus

Mark N. Wallace, Lucy A. Anderson, and Alan R. Palmer
Medical Research Council, Institute of Hearing Research, Nottingham, United Kingdom

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Wallace MN, Anderson LA, Palmer AR. Phase-locked responses to pure tones in the auditory thalamus. J Neurophysiol 98: 1941–1952, 2007. First published August 15, 2007; doi:10.1152/jn.00697.2007. Accurate temporal coding of low-frequency tones by spikes that are locked to a particular phase of the sine wave (phase-locking), occurs among certain groups of neurons at various processing levels in the brain. Phase-locked responses have previously been studied in the inferior colliculus and neocortex of the guinea pig and we now describe the responses in the auditory thalamus. Recordings were made from 241 single units, 32 (13%) of which showed phase-locked responses. Units with phase-locked responses were mainly (82%) located in the ventral division of the medial geniculate body (MGB), and also the medial division (18%), but were not found in the dorsal or shell divisions. The upper limiting frequency of phase-locking varied greatly between units (60–1,100 Hz) and between anatomical divisions. The upper limit in the ventral division was 520 Hz and in the medial was 1,100 Hz. The range of steady-state delays calculated from phase plots also varied: ventral division, 8.6–14 ms (mean 11.1 ms; SD 1.56); medial division, 7.5–11 ms (mean 9.3 ms; SD 1.5). Taken together, these measurements are consistent with the medial was 1,100 Hz. The range of steady-state delays calculated from phase plots also varied: ventral division, 8.6–14 ms (mean 11.1 ms; SD 1.56); medial division, 7.5–11 ms (mean 9.3 ms; SD 1.5). Taken together, these measurements are consistent with the medial was 1,100 Hz. The range of steady-state delays calculated from phase plots also varied: ventral division, 8.6–14 ms (mean 11.1 ms; SD 1.56); medial division, 7.5–11 ms (mean 9.3 ms; SD 1.5).

INTRODUCTION

There is considerable divergence and reconvergence of information in the ascending part of the central auditory system (Cant and Benson 2003; Oliver et al. 1997). In practice, it is difficult to anatomically identify the cellular components of a simple ascending pathway between the cochlear nerve and the cortex (Oliver et al. 1999), but one way of doing so physiologically is by measuring the steady-state delay and upper limit of phase-locking to pure tones (Liu et al. 2006). In the guinea pig auditory nerve, action potentials are produced at a particular phase relative to the stimulus waveform (up to a limit of about 3 kHz) and at a particular delay of 1–4 ms for cells with a characteristic frequency (CF) >200 Hz (Palmer and Russell 1986). Thereafter, as activity propagates along a multisynaptic pathway, the upper limit of phase-locking generally decreases and the delays increase (de Ribaupierre et al. 1980), even though the strength of phase-locking can increase as a result of convergence (Joris et al. 1994). We have previously shown that phase-locked activity is still present in the primary auditory cortex of the guinea pig, but by this level the upper limit is 250 Hz and the delays range from 11 to 18 ms (Wallace et al. 2002). If phase-locked information was propagated along parallel, linear pathways between the cochlear nucleus and the cortex then we would expect to record delays between 4 and 8 ms from the IC and between 8 and 12 ms from the MGB. A separate way of measuring the delays introduced by conduction times and synaptic processes is to record click latencies and these were also measured for most units irrespective of whether they showed a phase-locked response to tone pips.

In the present study, we measured the delay and upper limit of phase-locking in the main auditory area within the auditory thalamus, the medial geniculate body (MGB). Within the MGB we have recently reported (Anderson et al. 2006) that the latencies to a brief click vary between the different divisions, with the shortest latencies in the medial division and the longest in the dorsal division and shell. As a follow-up we sought to determine whether there were differences in the steady-state delays between different thalamic divisions and whether these correlated with the latencies of the click responses.

In a study of the cat MGB, Rouiller et al. (1979) found phase-locked responses in only about 2% of thalamic units. This may have been partly because they assumed that phase-locked responses would be recorded only from units with sustained responses and these formed about 10% of their responses. However, in a previous study of the guinea pig cortex (Wallace et al. 2002), we observed “onset” units that gave a transient response at CF, but were able to give a sustained, phase-locked response at 100 Hz. Here we wanted to determine whether phase-locked responses in the guinea pig MGB were restricted to sustained units, or whether some cells with onset properties at CF might show sustained phase-locked responses at lower frequencies.

Another factor that might affect the numbers of phase-locked responses is the presence of burst firing. Many recent studies of the thalamus have emphasized the importance of the tonic versus burst firing modes of the relay cells that carry information to the cortex. The prevalence of burst firing depends on the state of the animal and in the guinea pig MGB both sleep states and anesthesia increase the proportion of cells that show burst firing (Massaux et al. 2004). Thus we wanted to check whether cells showing burst responses could also phase-lock.

Pure tones are not a natural stimulus for guinea pigs and so we also used a conspecific vocalization, termed the purr (Berryman 1976), that contained a low-frequency fundamental. We previously showed that a few cortical cells can phase-lock to the fundamental frequency (270 Hz) of the purr (Wallace et al. 2002). About 50% of cells in the guinea pig MGB respond to the purr, although the strength of response is generally better among cells with low CFs (Syka et al. 1997).

Address for reprint requests and other correspondence: M. Wallace, MRC Institute of Hearing Research, University Park, Nottingham, NG7 2RD, UK (E-mail: markw@ihr.mrc.ac.uk).

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We expected that some thalamic cells would be able to phase-lock to the fundamental of the call and possibly even to the first harmonic.

**METHODS**

**Surgical preparation**

Twenty pigmented guinea pigs of both sexes and weighing 388–870 g were used. In 10 animals anesthesia was induced with a single intraperitoneal (ip) injection of urethane (0.9 g/kg) and surgical anesthesia was obtained and maintained by giving supplementary doses of Hypnorn [0.2 ml administered intramuscularly (im), consisting of fentanyl citrate 0.315 mg/ml and fluamisone 10 mg/ml]. The other 10 animals were anesthetized by an injection of ketamine hydrochloride [60 mg/kg administered subcutaneously (sc)] followed by xylazine hydrochloride (Rompun) at 16 mg/kg xylazine (im) followed by supplementary doses of 15 mg ketamine hydrochloride (im) as required to achieve surgical anesthesia as judged by abolition of the corneal withdrawal reflex. Thereafter urethane was injected (ip) in doses of 0.1 g in a 20% solution until a stable level of anesthesia was reached (generally at around 0.9 g/kg). No noticeable difference was observed in the physiological responses obtained under the two anesthetic regimes. By using ketamine-xylazine followed by urethane we had greater control over the final level of anesthesia than when a single dose of urethane was given initially. Different guinea pigs had a different susceptibility to anesthetic agents even when they were from the same breeding colony. A single dose of atropine sulfate (0.06 mg/kg sc) was given to reduce bronchial secretions.

An incision was made in the external ear flap to provide direct access to the auditory meatus, which was cleared of wax. All animals were tracheotomized so that end-tidal carbon dioxide could be monitored and maintained within normal physiological limits by artificially respiring with oxygen. Core temperature was maintained at 38°C by a heating blanket and rectal probe. The animals were placed in a stereotaxic frame, with hollow plastic speculae replacing the ear bars, inside a sound-attenuating room. A standard stereotaxic position was achieved by leveling the skull between 5 and 13 mm rostral to ear bar zero, as described in the atlas of Rapisarda and Baccelli (1977). A cranioectomy was performed, 5 mm in diameter, usually on the right side alone, so that electrodes could be inserted vertically into the thalamus. The auditory thalamus was located at coordinates between 4.7 and 7.1 mm behind bregma and between 2.8 and 5.3 mm lateral to the midline (see Anderson et al. 2007). All experiments were performed in accordance with the UK Animal (Scientific Procedures) Act of 1986.

**Stimulation and recording**

Auditory stimuli were delivered to one or both ears through sealed acoustic systems, consisting of modified Radio Shack 40–1377 tweeters joined by a conical section to a damped, 2.5-mm-diameter probe tube that fitted into the speculum. The system was calibrated in each acoustic system, consisting of modified Radio Shack 40–1377 tweeters. Electrodes could be inserted vertically into the thalamus. These values are steady-state delays that should not be leveling the skull between 5 and 13 mm rostral to ear bar zero, as described in the atlas of Rapisarda and Baccelli (1977). A cranioectomy was performed, 5 mm in diameter, usually on the right side alone, so that electrodes could be inserted vertically into the thalamus. The auditory thalamus was located at coordinates between 4.7 and 7.1 mm behind bregma and between 2.8 and 5.3 mm lateral to the midline (see Anderson et al. 2007). All experiments were performed in accordance with the UK Animal (Scientific Procedures) Act of 1986.

**Identification of MGB divisions using cytochrome oxidase staining**

To reliably locate the position of the phase-locked units we had to establish the borders of the different parts of the MGB. **RESULTS**
The four main divisions of the MGB were identified by staining coronal sections for cytochrome oxidase (CYO) and using criteria we have defined previously (Anderson et al. 2007). The ventral division (V) of the MGB has relatively high CYO activity and forms an ovoid structure in the middle of the MGB (outlined by dashed white line in Fig. 1). The precision with which it is possible to define the border of the ventral division varies depending on its adjacent structures: the border with the medial division is defined by pale fiber bundles and can be determined to within 0.1 mm whereas the border with the dorsal division involves a more gradual change in the enzyme levels and is more difficult to discern. The medial division (outlined by the solid white line in Fig. 1) mainly has diffuse borders and contains a cluster of densely stained neurons at the caudal and middle levels of the MGB. The cells are shown at a higher magnification in Fig. 1, F and G, interspersed among pale-staining fiber bundles. Dorsal to the ventral and medial divisions lies the less densely stained dorsal division, which is subdivided into a dorsolateral (DL) and more medial suprageniculate (SG) zones (Fig. 1, B and D). At the caudal, ventral, and lateral margins of the ventral division there is a band of paler staining corresponding to the shell (S) division of the MGB (Fig. 1, A and B). At the caudal portion of the MGB the dorsal division is bounded by the lateral posterior nucleus (LP) as shown in Fig. 1E.

Location of units with phase-locked responses

Locations of all units were determined histologically by making lesions and relating them to the pattern of CYO staining in coronal sections. Most lesions were used to mark the beginning and end of tracks through the MGB (dorsal and ventral lesions in Fig. 1B), but 20 lesions were made at or near the location where phase-locked cells were recorded. The lesion in Fig. 1A and the middle lesion in Fig. 1B indicate phase-locked units at the medial tip of the ventral division. Four separate units within 300 μm of the middle lesion showed phase-locked responses. All four units had CFs of 0.14–0.35 kHz but, despite being located close to each other, they had different upper limits of phase-locking that ranged from 200 to 480 Hz. The beginning and end of auditory activity were also marked by lesions in the track shown in Fig. 1C, which is at the rostral pole of the MGB. Four phase-locked units were recorded between these two lesions. Another pale electrode track is shown in Fig. 1D and this time the two lesions straddle the position of three phase-locked cells in the medial division. The lesions are shown at higher power in Fig. 1F and they clearly straddle the darkly stained neurons at the center of the medial division. Another example of a pale track ending in a lesion marking a phase-locked unit in the medial division is shown in Fig. 1E and at higher power in Fig. 1G.

The three-dimensional positions of the lesions were reconstructed using NeuroLucida software and then they were superimposed on a single reconstruction of the MGB shown in a planar view from a dorsal perspective (Fig. 2). Each lesion, or pair of lesions, is shown by a white asterisk; positions of the lesions shown in Fig. 1, A–E are indicated by the letters A–E in Fig. 2. The lesions occur in three clusters: one at the caudal pole of the medial division (D and E), one along the medial edge of the ventral division (A and B), and a single pair at the rostral pole of the ventral division (C). These results show that in the ventral division the phase-locked cells are restricted to a discrete band of cells with low (<1.4 kHz)

![Image](https://via.placeholder.com/150)

FIG. 1. Coronal sections of the thalamus stained for cytochrome oxidase activity. Ventral (V) division is outlined by the dashed white line; the solid white line marks the border of the medial (m) division. Lesions are marked by black arrows with a white border and electrode tracks by small white arrows. A: lesion at the edge of the medial tip of the ventral division, close to the caudal pole of the medial geniculate body (MGB). Characteristic frequency (CF) of a phase-locked unit here was 180 Hz. B: total of 3 lesions have been made in 2 nearby tracks at locations where there was auditory driving. Dorsal lesion is in the suprageniculate nucleus (SG), the ventral is in the shell division (S), and the middle lesion is again at the medial tip of the ventral MGB. Four phase-locked units were located at the medial tip of the ventral division, close to the middle lesion. This section is at a more rostral part of the MGB than A and contains the caudal part of the lateral geniculate nucleus (LGN) immediately above the dorsolateral zone (DL) of the MGB. C: section at the rostral pole of the MGB showing 2 lesions at the edges of the ventral MGB. Pale line marked by arrows indicates the electrode track and 4 phase-locked units were recorded from this part of the track. All had CFs of between 250 and 500 Hz. D: section about 400 μm from the caudal pole of the MGB showing a pale electrode track passing through the SG. Toward the end of this track there are 2 lesions in the medial division that straddled 3 phase-locked units with CFs of 800–1,270 Hz. E: section about 600 μm from the caudal pole of the MGB showing a pale electrode track and a single lesion in the medial division. The lesion was close to a phase-locked unit with a CF of 600 Hz. At this level the relatively high enzyme activity of the anterior pretectal nucleus (APT) can be seen. F: higher-power view of section shown in D. G: higher-power view of section shown in E. Scale bars in A–E are 1 mm and in F and G are 250 μm.
CFs that are tightly arranged along the medial and rostral edges.

Of the 275 units recorded from the MGB, most (53%) were in the ventral division, with 22% in the medial division, 16% in the dorsal division, and 9% in the shell division (see Table 1). A total of 18% of units were phase-locked, but these were not evenly distributed between divisions: 40/146 (27%) of ventral units and 9/60 (15%) of medial units showed phase-locked responses. No phase-locked activity was recorded from the dorsal and shell divisions despite the fact that some of the units in these divisions had CFs of 1 kHz. The sample of units recorded was not random because we oversampled the low-frequency parts of the MGB where phase-locked responses were more common. Because most of the phase-locked units (98%) were recorded from parts of the MGB where the CFs were <1 kHz we could not obtain an accurate value for the total percentage of phase-locked units in the MGB. In our data 43% (40/94) of low-frequency (<1.4 kHz) units in the ventral division showed phase-locked (P-L) responses (Table 1). In the medial division, 28% (8/29) of the low-frequency units showed phase-locked responses and 3% (1/31) of high-frequency units.

Types of units that phase-locked

Recordings were made from 241 single units and 34 multiunits within the MGB that responded to pure tones. For all units, PSTHs were measured in response to tones 20 dB above threshold at their characteristic frequency (CF). Different workers tend to classify temporal responses in slightly different ways. We used the same criteria as those used by Edeline et al. (1999) except that we subdivided the sustained units into three groups (Fig. 3, right column). Out of all the units, 51% (140/275) showed a transient type of response that included onset, offset, or on-off responses (see examples in Fig. 3). The distinction between offset and late responses was established by varying the duration of the stimulus. True offset units showed a consistent latency after the end of the stimulus. Some of the onset units (including the one illustrated) showed a regular firing pattern involving two or more spikes at consistent times.

**TABLE 1. Range of CFs and click latencies in the four divisions of the MGB**

<table>
<thead>
<tr>
<th>Division</th>
<th>Ventral</th>
<th>Medial</th>
<th>Dorsal</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.4 kHz</td>
<td>&gt;1.3 kHz</td>
<td>&lt;1.4 kHz</td>
<td>&gt;1.3 kHz</td>
</tr>
<tr>
<td>Total number of units</td>
<td>94</td>
<td>52</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Number of P-L units</td>
<td>40</td>
<td>0</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Range of phase delays, ms</td>
<td>8.6–14</td>
<td>7.5–11</td>
<td>9.26, 1.5</td>
<td></td>
</tr>
<tr>
<td>Mean and SD of phase delay</td>
<td>11.14, 1.6</td>
<td>9.26, 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range of click latencies in P-L units, ms</td>
<td>7–12.8</td>
<td>3.1–9.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Mean and SD of click latencies</td>
<td>9.02, 1.6</td>
<td>5, 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range of click latencies in non-P-L units, ms</td>
<td>6.3–18.5</td>
<td>4.3–15.1</td>
<td>7.7–32.3</td>
<td>9.3–40.9</td>
</tr>
<tr>
<td>Mean and SD of click latencies</td>
<td>9.74, 2.3</td>
<td>8.67, 3.2</td>
<td>13.1, 5.1</td>
<td>24, 10.8</td>
</tr>
</tbody>
</table>

P-L, phase-locking.
intervals (chopping). For all onset units the firing rate was at or below the background rate by the end of the 50-ms stimulus.

The remaining units (49%, 135/275) had a sustained response where the firing rate was still above background at the end of the stimulus. Most of these cells had a sharp onset and continued to fire at a lower rate throughout the duration of the stimulus (on-sustained units). There were two other types of sustained unit: buildup responses where there was no onset response, but a gradual increase in firing rate during most or all of the stimulus, and pauser responses where the firing rate fell to background after the onset response before rising again. The temporal response pattern of some units was observed to change with frequency: some units with a transient response at CF showed a sustained response at frequencies around 100–200 Hz (where phase-locking was most likely to occur). Out of the 275 units recorded, 49 (18%) showed evidence of phase-locking to at least one stimulus frequency (Fig. 4). Of these phase-locked units, 25 (51%) had transient responses at their CF, whereas 24 had sustained responses at their CF. Thus units that showed a transient response at their CF were as likely to show phase-locking to low frequencies as units with sustained responses at CF.

The number of units that phase-lock to pure tones may have been underestimated. All units were stimulated at CF, but many of the high-frequency units were not stimulated at the low frequencies where phase-locking was found to occur. Indeed only 13/49 units that showed phase-locked responses did so at their CF, and none of these had a CF of >800 Hz. Three of the units illustrated in Fig. 3 showed a phase-locked response at 100 Hz: only the offset, on-off, and buildup units did not. Only two of the six units (on-sustained and pauser) showed a phase-locked response at CF. Many of the high-frequency units were tested at CF only because they did not give a significant response at 100 Hz (as indicated by the frequency response area), which was the other main frequency used in searching for phase-locking. Only one unit with a CF of >1.3 kHz was identified as showing evidence of phase-locking; this was a broadly tuned unit in the medial division that also responded at 100 Hz.

Some cells clearly responded at 100–300 Hz but gave no evidence of a phase-locked response. This may result from the lack of an effective phase-locked input from the inferior colliculus (IC), but could also be the result of intrinsic membrane properties. We could not study the membrane dynamics directly but were able to look at the pattern of action potential generation. Some of the phase-locked units showed bursts of two to four spikes separated by 2- to 4-ms intervals in their spontaneous activity (Fig. 5, C–F). Bursts of spikes were also often observed during the onset response (Fig. 5, A and B) of phase-locked units, but these units showed tonic firing during the sustained part of the phase-locked response (Fig. 5, G–I). Thus some of the phase-locked units were able to switch from burst firing to tonic firing as they became entrained to the stimulus frequency. Burst firing was common among MGB units, especially when they were stimulated at CF, but some bursting units did not show evidence of bursting activity when stimulated at low frequencies.

Monaural versus binaural responses to tones and clicks

The responses to binaural tones and a click are illustrated in Figs. 6 and 7, which show the same, representative phase-locked unit in the ventral MGB. When stimulated with a tone of 140 Hz the onset response is followed by a sustained phase-locked response that continues for the duration of the 200-ms stimulus. Weaker phase-locking is still observed at 180 Hz but, by 220 Hz, while there is still some sustained activity, it is not phase-locked. In this particular unit, there is a late response occurring at about 140 ms after the stimulus onset whether it is a tone or a click. The main response to the click is a prominent onset response.

The unit in Fig. 6 was phase-locked at frequencies from 60 to 180 Hz and showed its strongest phase-locked responses to an 80-Hz binaural stimulus (Fig. 7A). The stimulus frequency giving the strongest phase-locking response was chosen to compare the monaural responses with the binaural. For this unit stimulating the left (contralateral) ear produced an onset response that was as strong as the binaural response but there was much less sustained activity and it was not phase-locked (Fig. 7C). Stimulating the right ear produced a small onset response and some sustained activity that was weakly phase-locked to the stimulus (Fig. 7E). Thus the phase-locked component of the response showed binaural facilitation where the binaural response was much greater than the sum of the monaural responses.

All units were stimulated with binaural tones because this was the most reliable stimulus but 17 of the best phase-locking units were also stimulated monaurally to assess the relative

![Figure 4](image-url)
Most units were also stimulated binaurally with a click; this was an effective stimulus for nearly all the units and it allowed us to measure the response latency to one stimulus at a constant sound level. The response for the unit shown in Fig. 6 was typical in that it had a brief onset response that lasted about 20 ms. This onset was composed of five peaks of increased firing probability that were spaced about 1.5 ms apart (Fig. 7B). A similar regular response occurred when the left ear was stimulated (Fig. 7D) but the response to the right ear, although multi-peaked, had peaks that were less regular and more widely spaced (Fig. 7F). A regular firing pattern was observed in the click responses of all eight medial MGB units that gave phase-locked responses to tones, and in 18/26 phase-locked units in the ventral MGB. We wanted to determine whether this regular firing might be directly related to the ringing of the basilar membrane and the regularity of the afferent input (Pfeiffer and Kim 1972) or to a regular chopping activity linked to the membrane properties of the thalamic cell. Consequently the period between the multiple peaks of the click response was converted to a frequency value and this was plotted against the CF of the unit at 60 dB SPL (the sound level of the click). There was only a weak correlation ($R^2 = 0.003$) between the click response frequency and the CF. Among the units, as the CF varied, there was no consistent change in the frequency of the regular response to click.

Variation of phase-locking strength with stimulus frequency and duration

The effective range of frequencies that could produce significant phase-locked responses in the ventral MGB ranged between 60 and 520 Hz, whereas in the medial division it was 60–1,100 Hz. The vector strengths for individual units varied over this range in either a low-pass or a band-pass fashion. Examples of these two types of response are shown in Fig. 8 by plotting vector strength against stimulus frequency.

Units were classified as band-pass if their vector strength increased by $>20\%$ from the lowest frequency at which they gave a robust response to a frequency that was $\approx 40$ Hz higher. Among the single units in the ventral division, 10 were classified as low-pass and 15 as band-pass. Within the medial division, five single units were classified as low-pass and two as band-pass. Other units did not give phase-locked responses over a wide enough range of frequencies to classify them. The peaks of the band-pass units varied in frequency between 100 and 300 Hz. In a study of phase-locking in the cat MGB most responses were low-pass, but band-pass responses were also described (Rouiller et al. 1979).

We mainly used tones of 200-ms duration in this study, but in some units responses to tones of 1 or 5 s were recorded. Six units in the ventral division and three in the medial division were stimulated for 1 s. Two of the ventral units were also stimulated for 5 s. All continued to show phase-locked responses throughout the period of stimulation. For all units there was a dip in firing rate after the onset response but, after the first 200 ms, the firing rate and vector strength remained fairly constant for the remainder of the stimulus. Only two of the units [one from the ventral division (CF 220 Hz) and one from the medial division (CF 610 Hz)] had vector strengths $>0.9$; these values were found only at frequencies of 100 and 150 Hz.

![Figure 5](image_url)

**FIG. 5.** Examples of the waveform (A) and a raster plot (B) of a unit in the medial MGB that showed a burst response with 2 or 3 spikes at the onset of a tone at its CF (610 Hz). Examples of waveforms and raster plots of burst firing recorded in the spontaneous activity of 2 phase-locked units from the ventral (C, E) and medial (D, F) divisions of the MGB. In the raster plots groups of 2, 3, or 4 spikes are indicated by the small arrows. Example of a raster plot (H) and PSTH (I) from a single unit in the ventral MGB. Response is phase-locked to a stimulus of 220 Hz, 78 dB SPL, and 200-ms duration. Waveform of the stimulus is shown in G.

contribution of each ear individually. Out of these 17 units, 8 showed a clear dominance for the contralateral (left) ear with the ipsilateral ear either not producing any response by itself (3 units) or producing an onset response with only weak evidence of phase-locking (vector strength $<0.2$). Five of the units showed a clear preference for the ipsilateral ear, with the contralateral ear not producing any strong (vector strength $>0.2$) phase-locking. All the ipsilaterally dominant units were located in the ventral MGB. Three of the units gave a strong (vector strength $>0.2$) phase-locked response only to a binaural stimulus, and either did not respond or gave only weak phase-locking to either ear alone. Only one unit showed good phase-locking to stimuli presented to each ear individually and binaurally.
The strongest phase-locking was always found at frequencies below CF for units in the medial division and for most units in the ventral division. Only 3/30 units in the ventral division had their highest vector strength at CF (CFs of 100–250 Hz) and one unit had its highest vector strength at a frequency above CF (CF was 180 Hz).

**Upper limits and delays of phase-locked responses to pure tones**

The upper limit of significantly phase-locked responses varied between units and between divisions as shown in Fig. 9A. The upper limits in the ventral division varied between 80 and 520 Hz, whereas in the medial division they varied between 100 and 1,100 Hz. These upper limits were significantly different (using Scheffé ANOVA, $P < 0.001$). We also measured the steady-state delays of single units in both structures. The mean phase angle varied linearly with the stimulation frequency as shown by the phase plots in Fig. 9B. The slope of these plots corresponds to the steady-state delay. When the steady-state delays were plotted against the upper phase-locked limit, (Fig. 9C) there was a trend ($R^2 = 0.43$) for shortest delays to be found with the highest upper limits. We have previously collected similar data for cortical area AI (Wallace et al. 2002) and the IC (Liu et al. 2006) and these are plotted for comparison in Fig. 9D.

The units in the MGB and IC show almost the same range of upper limits for phase-locking, but the range of MGB delays is narrower. Not surprisingly some units in the IC have delays that are ≤4 ms shorter than any in the MGB, but a few units in the rim of the IC (dorsal cortex and external nucleus) also have delays that are ≤6 ms longer than any in the thalamus. There is also some overlap between MGB and AI units, with a few thalamic units having lower upper limits and longer latencies.
FIG. 9. A: plot of upper limit of phase-locking to pure tones against the CF of units in the ventral and medial divisions of MGB. Four of the medial units (open triangle) have limits higher than those of any in the ventral division (solid circle). B: phase plots derived by plotting the mean phase angle against the stimulus frequency for single units from the medial and ventral divisions. Slope of these lines corresponds to the steady-state delays of these units as indicated at the bottom right. C: plotting the upper limit of phase-locking against the steady-state delay derived from phase plots from both divisions of the MGB showed that there was a correlation between these 2 parameters. A linear regression line had an $R^2 = 0.43$. D: a similar correlation between the upper limit of phase-locking and the steady-state delay was also seen for units recorded previously in the central nucleus of the IC (ICC), primary auditory cortex (AI), and the dorsal cortex and external nucleus of the IC (ICCx) and these are plotted together with the results from MGB. E: plot of click latencies vs. steady-state delay for phase-locked units in the medial and ventral divisions. Dotted line indicates where the points would lie if both values were the same. F: plot of click latency vs. CF for the non-phase-locked units in the ventral and medial divisions of the MGB. Units in both divisions have a wide range of CFs and the shortest latencies are found in the medial division at all frequencies.
than the best units in AI. Altogether, there is much more overlap in the latencies and upper limits than would be expected for a simple linear hierarchy.

In some units different pathways may mediate the onset and the tonic phase-locked response. If the same pathways were involved in both responses the two parameters should covary. However, when click latency was plotted against steady-state delay (Fig. 9E), there was no correlation (R² = 0.06) between the two parameters (for the 26 units where these measurements were available).

The ranges of steady-state (phase) delays are 4–6 ms for both the ventral and medial divisions (Table 1) and their means are significantly different (t-test, P = 0.016). The ranges of click latencies for the cells that phase-lock to tones in both the ventral and medial divisions are about 6 ms and their means are significantly different (t-test, P = 0.003). In the medial division the mean of the click latencies for the cells that phase-lock is also significantly different from those that do not phase-lock (t-test, P = 0.001). The range of click latencies observed among the non-phase-locked cells of the ventral and medial divisions are shown in Fig. 9F. The shortest click latencies are clearly in the medial division over a wide range of frequencies. The shorter latencies and delays in the phase-locked cells of the medial division than those among any other group in the MGB (Table 1) imply they have a unique afferent input.

**Phase-locking to the fundamental frequency of a vocalization**

A short guinea pig purr was used to stimulate 30 of the MGB units that showed phase-locked responses to tones. We used the same example of purr as had previously been used in studying the cortex (Wallace et al. 2005). This example contains nine rhythmic pulses of sound as shown by its waveform in Fig. 10A. Nearly all (27/30) of the units tested gave a response to multiple pulses of the purr call. An example of a response from a unit in the medial division is shown in Fig. 10B and the mean response to the purr in the 21 phase-locked units recorded from the ventral MGB is shown in Fig. 10C. Most of the units responded to all of the pulses of sound, with the largest response usually to the first pulse and the smallest responses to pulses 2, 3, and 9, which were the least intense pulses. Pulses 6, 7, and 8 were the most intense and produced the most consistent responses. The waveform of pulse 6 is shown for a 50-ms-long segment of the call in Fig. 10D; Fourier analysis of this segment in Fig. 10E shows that it has a fundamental frequency at 267 Hz, and harmonics at 534, 801, and 1,068 Hz. Pulse 6 produced the most consistent response across units in both divisions of the MGB and responses to this pulse have been analyzed in detail in the cortex (Wallace et al. 2002).

Units phase-locking to tones of 300 to 600 Hz should also phase-lock to the fundamental and possibly the first harmonic frequency of the purr. To test this, we performed an autocorrelation on the PSTH of the purr response between 515 and 565 ms after stimulus onset.

An example of a response from a ventral MGB unit that phase-locked to tones of ≤480 Hz is shown in Fig. 10F. The response shows eight peaks of activity at regular intervals and the autocorrelogram (Fig. 10G) shows three prominent peaks at 3.75, 7.55, and 11.45 ms. The major peak is at a lag time of 3.75 ms, corresponding to a frequency of 267 Hz. The other peaks correspond to 133 and 87 Hz and represent spikes occurring locked to the purr but not on adjacent cycles. Of the 11 units in the ventral MGB that phase-locked at ≥250 Hz and were stimulated with the purr, eight gave responses with autocorrelation peaks corresponding to 260–267 Hz. The other three gave strong responses where the spikes were not locked to any of the stimulus frequencies and there was no sign of regular firing within the response to a single pulse. None of the units gave a prominent peak in the autocorrelogram corre-
sponding to the first harmonic frequency of 534 Hz. In three of the units the responses to pulses 5–9 also gave autocorrelation peaks at 260–267 Hz.

In the medial MGB, four of the units phase-locked to tones of ≥600 Hz and two showed phase-locking at ≤400 Hz. All six of these units gave multiple peaked responses to the purr and showed autocorrelation peaks corresponding to frequencies of 260 to 274 Hz in response to pulse 6. However, none was phase-locked to the first harmonic of 534 Hz. Non-phase-locked cells also responded to all nine pulses of the purr but with no sign of regular firing within the response to a single pulse.

Overall, we found that most (16/20, 80%) units that phase-locked to 250-Hz tones also phase-locked to the fundamental frequency of the purr.

**DISCUSSION**

**Types and numbers of phase-locked cells in the MGB**

Spike timing is an important way of coding spectral information at the level of the cochlear nucleus (Lavine 1971) and the lower brain stem (Oertel 1999), but by the level of the neocortex temporal mechanisms are thought to have largely been replaced by rate or place mechanisms (Phillips et al. 1991). One factor in this change could be burst firing in cells of the MGB. In the rat auditory thalamus there is in vitro evidence that burst firing induces a slow after-hyperpolarization (AHP) that can inhibit neuronal firing over many seconds (Hu and Mooney 2005). If this slow AHP were present in vivo it would prevent bursting cells from showing a sustained, phase-locked response. This mechanism did not appear to be operating in our anesthetized guinea pigs because many of the cells with phase-locked responses also showed evidence of bursting in their onset response and during spontaneous activity (using the criteria for bursts of Massaux et al. 2004). According to the different burst criteria of He and Hu (2002) some cells recorded from both medial and ventral divisions were bursting during the sustained, phase-locked activity.

The current study provides further evidence that for low-frequency (<1 kHz) stimuli, temporal mechanisms may still have a role in spectral analysis. Indeed, there may be a higher proportion of phase-locked cells in the MGB than was previously thought (Rouiller et al. 1979). Uncertainty about the proportion of MGB units that can give a phase-locked response to a tone remains; an accurate assessment of the proportion would require random sampling and an unanesthetized preparation to avoid the effects of anesthetics. Anesthetics may reduce the accuracy with which central neurons can fire action potentials: in a study of IC cells in the awake rabbit, Kuwada et al. (1989) showed that the addition of barbiturate anesthesia reduced the sensitivity to interaural time differences.

None of the studies of phase-locked responses in the MGB (cat: Rouiller et al. 1979; rabbit: Stanford et al. 1992) used random sampling methods. In the cat study, only 10% of high-frequency units (those with a sustained response) were stimulated with the low frequencies to which a phase-locked response was most likely to occur. Other neurons were stimulated at CF. We have shown that many cells that have an onset response at CF can give a sustained, phase-locked response at low frequencies. Thus the estimate of 2–3% of cells that gave a phase-locked response in the MGB of the anesthetized cat (Rouiller et al. 1979) may be a significant under representation at least for high-level stimuli. A much higher proportion of phase-locked cells was found in the awake restrained rabbit (15.6%) (Stanford et al. 1992). However, although the electrode tracks were widely distributed and apparently randomly positioned, only cells sensitive to interaural time differences were tested (mainly low-frequency cells), and so this study may also have overestimated the number of phase-locked cells in the MGB. Because there is no evidence of phase-locked responses in the dorsal or shell divisions, the proportion of phase-locked responses in the ventral division must be higher than that for the MGB as a whole. Among the low-frequency cells (CF ≤1.3 kHz) of the ventral division the proportion of cells showing phase-locking may be >40% in the guinea pig.

**Sources of phase-locked input to the MGB**

The main source of phase-locked input to the MGB is the inferior colliculus (IC). The central nucleus of the IC provides the main ascending input to the ventral division of the MGB (Malmierca et al. 1997; Wenstrup 2005; Winer 1992). Although the current study is consistent with the IC providing a phase-locked input to the ventral division only a small proportion of this input will be producing phase-locked excitation. Somewhere between 10 and 30% of fibers from the IC are inhibitory (Peruzzi et al. 1997; Winer et al. 1996) and will not contribute to phase-locking directly. Among the low-frequency cells in the IC 68% showed some evidence of phase-locking (Liu et al. 2006), although it is not known how many of these provide excitatory input to the ventral MGB. None of the units in the ventral MGB phase-locked at ≥520 Hz, whereas cells in the IC phase-locked at ≤1,000 Hz (Liu et al. 2006). The presence of synaptic jitter and longer membrane time constants could account for the reduction in the upper phase-locking limit in going from the IC to the ventral MGB (de Ribaupierre et al. 1980). Vector strengths as high as 0.96 were found in one cell of the ventral MGB, but vector strengths of ≤0.99 have been observed in the guinea pig IC (Liu et al. 2006) and the presence of convergent inputs may actually increase vector strengths of output cells (Joris et al. 1994). Overall, the presence of such high vector strengths in the ventral MGB is consistent with an input from the central nucleus of the IC. The other major source of afferent input to the MGB is the auditory cortex (Winer 1992, 2005), but there is no evidence to suggest that the cortex provides any significant phase-locked input to the MGB in any species. Similarly there is no evidence that the external nucleus or dorsal cortex of the IC provides a phase-locked input.

The presence of binaural facilitation and the weak or non-existent phase-locking produced by monaural stimulation, in many cells, is consistent with some form of coincidence detectors of the type shown in the superior olive (Goldberg and Brown 1969). The complexity of the inputs was further illustrated by the lack of binaural facilitation in either the onset response or the click response observed in most units. The click response was usually multipaked and in a few cases the interval between the peaks coincided with the period of the cell’s CF. Thus for a few cells the click response may have represented the ringing in the basilar membrane that causes the firing in cochlear nerve fibers to be locked to their CF (Pfieffer...
and Kim 1972). However, in most cells tested, the interval between the peaks did not coincide with the CF period, presumably because the CF was higher than the upper limit at which the cell could phase-lock. The multiple peaks appear to represent a chopping response.

This study has shown that the phase-locked cells in the medial division of the MGB have properties significantly different from those in the ventral division. Thus the medial cells have higher upper limits of phase-locking, shorter steady-state delays, and shorter click latencies than those of cells in the ventral division. We have previously suggested (Anderson et al. 2006) that the short click latencies in the medial division are due to a direct input from the dorsal cochlear nucleus. Even if this were true it need not imply that the phase-locked input to the medial division is also coming directly from the cochlear nucleus. The absence of any correlation between the click latencies and the phase delays, in the same units, implies that the click responses may involve a separate pathway from the phase-locked responses. The upper limit of phase-locking recorded for two units in the medial division of the MGB was as high as the best units in the IC and the range of upper limits was the same for both structures (Table 2). This hierarchical equivalence implies that some cells in the medial division of the MGB may have a direct phase-locked input from a pathway that is not relayed through the IC. There are a number of other nuclei that project directly to the medial division apart from the dorsal cochlear nucleus. These include the ventral nucleus of the lateral lemniscus, which receives specialized calyceal endings (Adams 1997; Kudo 1981; Schofield and Cant 1997; Whitley and Henkel 1984), the sagulum, and the intermediate nucleus of the lateral lemniscus (Hutson et al. 1991). Little is known about these pathways and it is not known whether they are excitatory or whether they carry phase-locked information. The phase-locked output from the medial division may not go to the cortex because the medial division also projects to subcortical structures such as the amygdala and putamen (Winer 1992). It would be useful to know the target of the phase-locked outputs because this may give an indication of their function.

### Table 2. Steady-state delay and upper phase-locking limit in six auditory structures

<table>
<thead>
<tr>
<th>Source</th>
<th>Central IC</th>
<th>Medial MGB</th>
<th>Ventral MGB</th>
<th>Cortical Belt (VRB)</th>
<th>Cortical AI</th>
<th>Rim of IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of units</td>
<td>57</td>
<td>7</td>
<td>19</td>
<td>3</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Range of delays, ms</td>
<td>4.6–15.4</td>
<td>7.5–11</td>
<td>8.6–14</td>
<td>10.5–14.6</td>
<td>11.1–18.3</td>
<td>12.1–21.3</td>
</tr>
<tr>
<td>Mean delay ± SD</td>
<td>8.2 ± 2.8</td>
<td>9.3 ± 1.5</td>
<td>11.1 ± 1.6</td>
<td>12.5 ± 2.1</td>
<td>14.9 ± 2.4</td>
<td>15.4 ± 3.1</td>
</tr>
</tbody>
</table>

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Present address of L. A. Anderson: UCL Centre of Auditory Research, 332 Gray’s Inn Road, London, WC1X 8EE, UK.

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