Phase-Locked Responses to Pure Tones in the Inferior Colliculus

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INTRODUCTION

The processing of acoustic information involves multisynaptic pathways that ascend from the cochlea to the neocortex. One simple way for following the sequence of steps involved in central processing is to study cells with the ability to transmit precise temporal information coding the low-frequency components from a complex sound (Spitzer and Semple 1995). Thus recording the phase-locked responses to pure tones provides a way of identifying one functionally separate stream of ascending information. At every step along a sequential pathway, the upper limiting frequency for phase-locking varied greatly between units (80–1,034 Hz) and between anatomical divisions. The upper limits in the three divisions were central nucleus, >1,000 Hz; dorsal cortex, 700 Hz; external nucleus, 320 Hz. The mean latencies also varied and were central nucleus, 8.2 ± 2.8 (SD) ms; dorsal cortex, 17.2 ms; external nucleus, 13.3 ms. We conclude that many cells in the central nucleus receive direct inputs from the brain stem, whereas cells in the external and dorsal divisions receive input from other structures that may include the forebrain.

Liu, Liang-Fa, Alan R. Palmer, and Mark N. Wallace. Phase-locked responses to pure tones in the inferior colliculus. J Neurophysiol 95: 1926–1935, 2006. First published December 7, 2005; doi:10.1152/jn.00497.2005. In the auditory system, some ascending pathways preserve the precise timing information present in a temporal code of frequency. This can be measured by studying responses that are phase-locked to the stimulus waveform. At each stage along a pathway, there is a reduction in the upper frequency limit of the phase-locking and an increase in the steady-state latency. In the guinea pig, phase-locked responses to pure tones have been described at various levels from auditory nerve to neocortex but not in the inferior colliculus (IC). Therefore we made recordings from 161 single units in guinea pig IC. Of these single units, 68% (110/161) showed phase-locked responses. Cells that phase-locked were mainly located in the central nucleus but also occurred in the dorsal cortex and external nucleus. The upper limiting frequency of phase-locking varied greatly between units (80–1,034 Hz) and between anatomical divisions. The upper limits in the three divisions were central nucleus, >1,000 Hz; dorsal cortex, 700 Hz; external nucleus, 320 Hz. The mean latencies also varied and were central nucleus, 8.2 ± 2.8 (SD) ms; dorsal cortex, 17.2 ms; external nucleus, 13.3 ms. We conclude that many cells in the central nucleus receive direct inputs from the brain stem, whereas cells in the external and dorsal divisions receive input from other structures that may include the forebrain.

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METHODS

Surgical preparation

A total of 17 pigmented guinea pigs of both sexes and weighing 147–736 g were used. Animals were anesthetized with urethan (1.1 g/kg for animals weighing <500 g, 0.9 g/kg for animals weighing >500 g; intraperitoneal, in 20% solution in 0.9% saline) and Hypnorm (0.2 ml im, comprising fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml). To prevent bronchial secretions, atropine sulfate (0.06 mg/kg sc) was administered at the start of the experiment. Anesthesia was supplemented with further doses of Hypnorm (0.2 ml im) on indication of forepaw withdrawal reflex.

An incision was made in the external ear flap to provide direct access to the auditory meatus and the meatus was cleared of wax. All animals were tracheotomized, and 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 sleeves replacing the ear bars, inside a sound-attenuating room. The bulla on each side was vented with a 10-cm long (0.5-mm OD) polyethylene tube. A small craniotomy was usually performed on the right side, and the dura reflected from the cortex overlying the midbrain. Heart rate and end tidal carbon dioxide levels were monitored and the animals were artificially respired with 100% oxygen. All experiments were performed in accordance with the UK Animal (Scientific Procedures) Act of 1986.

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 diam, 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. The main stimuli were pure tones (duration: 200 ms and repeated 100 times) gated on and off with cosine squared ramps lasting 2 ms and with a repetition period of 600 ms. In the first 14 experiments, times) gated on and off with cosine squared ramps lasting 2 ms and inserting a probe tube microphone close to the tympanic membrane. The system was calibrated in each experiment by inserting a probe tube microphone close to the tympanic membrane. The main stimuli were pure tones (duration: 200 ms and repeated 100 times) gated on and off with cosine squared ramps lasting 2 ms and with a repetition period of 600 ms. In the first 14 experiments, recordings, from 87 single units were made with single glass-insulated tungsten electrodes, advanced in the vertical direction, by a piezoelectric motor in steps of 2.5 μm. Extracellular action potentials were discriminated using a level-crossing detector (SD1, Tucker-Davies Technologies), and their time of occurrence was recorded with a resolution of 1 μs. In addition, samples of the response were captured and digitized so that the waveform of the units would be retained. The characteristic frequency (CF) and frequency response area were determined by making automated frequency-intensity plots using binaural stimulation at the same sound pressure level in each ear. In the last three experiments, custom-made multi-electrodes were used. These were composed of eight glass-insulated tungsten electrodes that had been glued to a circuit board in a line with their tips ~200 μm apart. Multichannel responses were gathered using a program (Brainware) that retained a digitized record of the responses and incorporated spike-sorting software that allowed 74 single spikes to be discriminated off-line. In these three experiments, in addition to frequency-intensity plots, we also stimulated the units with 50-μs clicks presented at an attenuation equivalent to 100 dB SPL for a 1-kHz tone giving ~60 dB SPL peak. This allowed us to calculate the mean first spike latency.

For all units, perstimulus time histograms (PSTHs) were constructed from responses to 100 presentations of tones, of at least eight frequencies, that were determined by the boundaries of the frequency response area. The tones were presented at either 80 or 90 dB SPL depending on the sensitivity of the unit at low frequencies. Period histograms were also plotted for the same sets of responses so that the degree of synchronization (vector strength) could be calculated (Goldberg and Brown 1969). Spikes were assigned to 100 bins/cycle, and each bin represented by a radius vector. The vectorial sum of the vectors normalized by the total spike count in the histogram gives a vector strength R and angle θ, where θ indicates the central tendency of the distribution of spikes during the period, or mean phase angle. 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). In all cases where there was a clear onset response, the analysis window for the period histograms started 10–30 ms after the stimulus onset because the onset response involved a burst of firing that masked the phase-locked response. Only the sustained portion of the response was analyzed.

Once the frequency with the largest vector strength had been determined, most units obtained with the single electrodes were stimulated contralaterally, ipsilaterally, and bilaterally at this frequency, and for some units, this frequency was presented at different sound levels depending on the dynamic range available. Phase plots were made by plotting the best mean phase angle against the stimulation frequency for units with Rayleigh values of >100. The slopes of these lines gave a measure of neural latency that represents the time between the sound waves leaving the earphone and an action potential being recorded in the inferior colliculus. These values are steady-state latencies that should not be affected by sensitivity to rise time or other problems associated with estimating onset latency.

Histological reconstruction of electrode tracks

At the termination of recording, two electrolytic lesions were made 1 mm apart by passing 5 μA of current for 10 s. A lesion was usually made at the most superficial position where auditory driving was recorded. At the end of the experiment, the animal was perfused transcardially with 0.5 l of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was postfixed in this solution overnight and then sectioned at 100 μm, approximately in the coronal plane. Sections were mounted on slides and stained to demonstrate cytochrome oxidase activity (Wallace et al. 2000a). The electrode tracks and recording positions of the units were reconstructed in three dimensions using a microscope with a motorized stage and computer software (Neurolucida, Microbrightfield, Colchester, VT).

RESULTS

Range and strength of phase-locking

Recordings were made from 161 single units. Of these units, 110 (68%) showed phase-locking to pure tones at one or more frequencies. Although the phase-locked units had spikes that were accurately timed with reference to the stimulus, these units only responded to a small proportion of the stimulus cycles. The stimulus cycles that produced a response varied with each repetition. This is illustrated in Fig. 1, which shows two original records of activity produced by two pulses of sound within a 1-s period. The first record (A) shows sustained activity throughout the period of the stimulus (pure tone at 1.8 kHz), but the unit is not phase-locked. The second record (B) shows a unit the firing of which is phase-locked to the stimulus (400 Hz) despite having a variable interspike interval and that continues to fire after the end of the stimulus. The response only consists of 10 or 12 spikes even although there were 80 complete waves within the stimulus.

The IC is also known to contain cells that show a regular pattern of spike timing that is not caused by the stimulus frequency but appears to be due to intrinsic membrane properties (Rees et al. 1997). We were careful not to confuse the two response types, and in all cases, we were able to check for true phase-locking by stimulating at two or more frequencies.
and showing that the period between the regular peaks in the PSTH was the same as the period of the stimulus waveform. An example of phase-locked responses of a single unit to different stimulus frequencies is shown in Fig. 2. The mean CF of the phase-locking units was 723 Hz [range: 115–4,500 Hz (after excluding 2 outliers)] and the median value was 500 Hz. Most single units (91%) showed phase-locking if their CF was <500 Hz. In units with a CF >500 Hz, there was a progressive decline in the proportion that were phase-locked as their CF increased. The CFs of most (81%, 89/110) phase-locked units were within the range of 100–1,000 Hz.

The highest frequency at which phase-locking was detected in isolated single units was 1,034 Hz. However, occasionally the multielectrodes picked up some very small potentials that we interpreted as fibers from the lateral lemniscus. Four of these multi-units/fibers had upper limits for phase-locking of 1,000–1,500 Hz. They, like other units which were not well isolated, were excluded from the study. For most units, the upper limit of phase-locking was much lower than 1,000 Hz. For some units, the upper phase-locking limit was determined by their tuning characteristics: those with a low CF (<300 Hz) often phase-locked up to the maximum frequency to which they gave a robust response. However, many units clearly stopped phase-locking well below 400 Hz even when they responded strongly at >1 kHz. This is illustrated in Fig. 3A where the upper phase-locking limit is plotted against CF. The upper limit of phase-locking varied between 80 and 1,034 Hz [mean: 386 ± 252 (SD) Hz]. The strength of phase-locking varied greatly for different units even at the stimulus frequency that gave the optimal response (Fig. 3B). Thus some units showed weak phase-locking where the vector strength never increased >0.3, whereas other units showed strong phase-locking and had vector strengths of >0.9. These responses were different from those seen at the level of the cochlear nerve where vector strengths seldom dropped below a value of 0.3 except for frequencies >2 kHz and were never above a value of 0.9 (Palmer and Russell 1986). The cells in the IC clearly stop phase-locking at much lower frequencies than the nerve
where significant phase-locking was still observed in some fibers at \( \leq 3 \) kHz. In the cochlear nerve, the vector strength does not fall \(<0.5\) until \( \sim 1,500\) Hz, whereas in the colliculus, there were only two units with a vector strength \(>0.5\) at frequencies \(>500\) Hz. However, a few cells in the IC have higher vector strengths than those normally seen in nerve fibers for stimulation frequencies between 200 and 400 Hz.

Another difference between cells in the IC and the nerve fibers was that all the nerve fibers showed low-pass phase-locking responses, whereas some of the IC cells showed band-pass responses, as measured by vector strength. There were 12 band-pass units in our IC sample (11%), whereas the rest of the sample of IC units were low-pass and had the best vector strengths at the lowest frequencies to which they gave a sustained response. Examples of five of the band-pass units that have their peak vector strengths at different frequencies are shown in Fig. 4. The changes in vector strength were largely independent of the spike number. For some units, the vector strength decreased even when the spike number was increasing.

Different cell types have been identified in the IC, and we wished to determine if there was any correlation between the quality or type of phase-locking and other response characteristics. Most of the phase-locking units in this study had CFs of \(<1\) kHz (Fig. 3A), and the frequency response areas did not show the variety of classes shown by cells with higher CFs. We were unable to identify clear differences between units based on their tuning curves as they all generally had "V"-shaped curves. An alternative way of classifying units is by comparing their responses over time as shown in their PSTH. We identified six types of units by following the definitions of Le Beau et al. (1996) for 102 phase-locking units where we had recorded PSTHs at close to the CF (see Table 1). The most common type of unit was the on-sustained unit (55%), and these had a wide range of CFs and included some units that showed band-pass phase-locking. The phase-locking strengths varied greatly between units, but some still showed good phase-locking (vector strength: \(>0.3\)) at \(>400\) Hz. Onset units (9%) had similar strengths and ranges of phase-locking and also showed a clear example of a band-pass unit. “Onset” units were defined as such by their response at their CF and often showed sustained activity at the low-frequencies where phase-locking was most commonly observed. This meant that although there was no sustained activity at CF there could still be strong phase-locking at lower frequencies. An example of one such single unit is shown in Fig. 5 where the unit had a CF of 1.19 kHz. This was one of only two units (both onset) that had a vector strength of \(<0.9\) when stimulated with a frequency close to 400 Hz. Both of the units had steady state latencies of 4.9 ms, and this was consistent with them being cells in the IC and not afferent fibers.

The strength of phase-locking for pauser units (23%) was generally weaker than for the on-sustained or onset units and for most the vector strength had fallen \(<0.3\) by 300 Hz. The vector strength had also fallen \(<0.3\) by 300 Hz for the sustained units, but our sample had a low mean CF (245 Hz), and so for most, the response rate was already declining by 300 Hz. Chopper units were uncommon (3%), among these low-CF units, but all showed their strongest phase-locking at \(\sim 400\) Hz. The single onset-chopper unit did show strong phase-locking but only \(\leq 120\) Hz. These results imply that the pauser and sustained units generally do not show as strong phase-locking at frequencies \(>300\) Hz as the on-sustained and onset units. However, there is considerable overlap in the strength of phase-locking between the four larger groups and it is not clear if there is a functional difference in their inputs.

**Location of phase-locked units**

Different parts of the IC receive inputs from different structures. The belt areas that surround the central nucleus are considered to form part of an extralemnisical system and receive a different balance of inputs compared with the central nucleus. We identified the location of isolated units by making electrolytic lesions and staining the histological sections for cytochrome oxidase (Fig. 6). The edge of the IC has low levels of cytochrome oxidase activity and a pale staining, whereas the

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**TABLE 1. Range of phase-locking for different types of units defined by their response patterns at close to CF**

<table>
<thead>
<tr>
<th>Type of Unit</th>
<th>Proportion, %</th>
<th>Mean and Range of CFs</th>
<th>Mean Lower Limit of Phase-Locking</th>
<th>Mean Upper Limit of Phase-Locking</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-sustained</td>
<td>55 (56/102)</td>
<td>457 (114–1350)</td>
<td>99 (50–250)</td>
<td>407 (147–1034)</td>
</tr>
<tr>
<td>Pauser</td>
<td>23 (23/102)</td>
<td>702 (122–1207)</td>
<td>80 (50–260)</td>
<td>334 (94–1000)</td>
</tr>
<tr>
<td>Sustained</td>
<td>10 (10/102)</td>
<td>245 (171–395)</td>
<td>73 (50–100)</td>
<td>199 (115–311)</td>
</tr>
<tr>
<td>Onset</td>
<td>9 (9/102)</td>
<td>738 (230–1190)</td>
<td>167 (50–317)</td>
<td>396 (110–852)</td>
</tr>
<tr>
<td>Chopper</td>
<td>3 (3/102)</td>
<td>591 (578–605)</td>
<td>350 (300–400)</td>
<td>595 (587–874)</td>
</tr>
<tr>
<td>Onset-chopper</td>
<td>1 (1/102)</td>
<td>777</td>
<td>50</td>
<td>117</td>
</tr>
</tbody>
</table>

CF, characteristic frequency.
central nucleus has relatively high levels of enzyme activity. This difference in cytochrome oxidase levels is thought to be a useful method for defining the edge of the central nucleus (Syka et al. 2000). Although the change in enzyme activity may not coincide exactly with the border of the central nucleus, we felt confident that units in the pale areas were either in the dorsal cortex or external nucleus. All the other units were ascribed to the central nucleus as they were on tracks that were ≥2 mm lateral to the midline.

The locations of 77 single units were determined histologically and 11 were in the lateral part of the dorsal cortex, 8 in the external cortex, and 58 units were in the central nucleus. The locations of the other units were not determined. In Fig. 6A, the electrode track is seen as a thin pale line that joins two small lesions in the central nucleus (small arrows). In Fig. 6B, the thin arrows indicate lesions in the external cortex and the thick arrow indicates a lesion in the central nucleus. Of the 11 units in the dorsal cortex, 8 (73%) showed a phase-locked response to pure tones. All were low-pass units and only one showed significant phase-locking at over 320 Hz. Of the eight units recorded in the external cortex, only three (37%) showed a phase-locked response to pure tones. These were all low-pass units and none showed significant phase-locking at >320 Hz. Of the 58 units in the central nucleus, 44 (76%) showed phase-locked responses to pure tones. There was a wide range of different strengths of phase-locking with two units showing vector strengths of >0.9 at 400 Hz and four units that still showed significant phase-locking at 1,000 Hz. The relatively small number of units in the dorsal and external divisions made it difficult to assess how significant the differences were between them and the central nucleus, but the proportion of phase-locked cells in the external nucleus was significantly smaller than in the dorsal cortex or central nucleus ($\chi^2$ test, $P < 0.001$).

Linearity of phase plots used in calculating steady-state latency

One way of measuring pathway latency from the tympanic membrane to the inferior colliculus is by plotting the mean phase of the response at a range of frequencies (de Ribaupierre et al. 1980). An example of a series of period histograms for one unit stimulated at frequencies between 50 and 410 Hz is shown in Fig. 7. The mean phase is shown beside the frequency for each histogram and changes progressively as the frequency increases. Plots of mean best phase versus stimulus frequency were only made from units that had reasonably strong vector strength (Anderson et al. 1971) with Rayleigh values of >100 at a range of at least four frequencies. This was the case for 85 single units. The slope of these phase plots gives the overall delay from the sound source to the IC.

For 74% (63/85) of the units, the phase plots were linear and examples of linear phase plots are shown in Fig. 8A. However, many of the phase plots were slightly nonlinear and some had distinct nonlinearities. We did not collect enough data to develop meaningful statistical tests to determine if the slight nonlinearities were due to sampling errors or reflected a shift in some underlying process. Examples of nonlinear phase plots from single units are shown in Fig. 8B.

When latencies were calculated from the slopes of the phase plots there was a tendency toward shorter latencies among units with higher CFs ($R^2 = 0.36$ for logarithmic regression line) as shown in the plot of 57 single units from the central nucleus in Fig. 9A. These units either had a linear phase plot over the entire range of frequencies tested or a plot that was linear for values obtained in the range of 100–250 Hz. Although the trend is toward longer latencies among units with low CFs, there is a broad scatter and clear examples where the latencies for units with low CFs is shorter than for units with higher CFs.

FIG. 5. Example of an “onset” unit that shows vector strengths of >0.9 in the frequency range of 180–425 Hz. This unit did not show any sustained activity at frequencies close to its CF (1.19 kHz). A representation of the stimulus frequency is shown as a sine wave at the top of each panel above the numerical value. To the right of each response is the period histogram and the numbers in the box beside it correspond to the mean phase (in cycles), the vector strength ($r$), the number of spikes ($n$) in the period from 15 to 200 ms after stimulus onset, and the Rayleigh value.

FIG. 6. Histological sections were cut in the coronal plane and stained for cytochrome oxidase activity to reconstruct the position of the recording sites. A: electrode track appears as a pale line running through the caudal pole of the darkly stained central nucleus. Two small lesions along the track are indicated by arrows. The dark area that we defined as the central nucleus is outlined by a line. B: further rostral, the border of dark staining that marks the lateral border of the central nucleus is rather ragged. However, there is always a paler rim of low enzyme activity at the edge of the colliculus, that we identified as the external nucleus and dorsal cortex. There are 2 lesions in the external nucleus (marked by the thin arrows) indicating that the corresponding electrode track was restricted to this division. The lesion marked by the thick arrow came from a separate electrode track that passed through the central nucleus. CN, central nucleus; DC, dorsal cortex; EN, external nucleus. Scale bar = 1 mm.
higher CFs. The latencies of units in the dorsal cortex and external nucleus were generally longer than those of units in the central nucleus so that the central latencies lie below the other divisions of the IC in Fig. 9A. The range of latencies in the different divisions were: central nucleus, 4.6–15.4 ms (mean: 8.2 ± 2.8 ms); dorsal cortex, 13.2–19.6 ms (mean: 17.2 ms); external nucleus, 12.1–14 ms (mean: 13.3 ms). The differences between the means of the central nucleus and the combined dorsal and external divisions are significant (Student’s t-test, \( P = 0.002 \)). Neural latency values as low as 4.6 ms were slightly surprising but studies of the guinea pig IC using pure tone stimuli at a level of 10 dB above threshold have also shown latencies as low as 5 ms (Astl et al. 1996; Syka et al. 2000) while latencies in the mustached bat IC can be as low as 4 ms (Fuzessery et al. 2003).

The range of latencies among units with any one CF is much greater in the IC than the cochlear nerve (Palmer and Russell 1986), presumably reflecting the different pathways bringing phase-locked information to the IC. Short-latency, monosynaptic pathways would be expected to preserve timing information better than longer latency, multisynaptic pathways where the presence of synaptic jitter would be expected to reduce the high-frequency cut-off for phase-locking. To test this idea, we plotted cut-off frequency against latency for all the single units in the IC, and this is shown in Fig. 9B. The logarithmic regression line for the units from all three divisions does fit the data fairly well (\( R^2 = 0.57 \)), but there is still a wide range of latencies at any particular cut-off frequency.

Convergence of phase-locked inputs

Some indication of the variety of pathways involved in providing a phase-locked input to cells in the inferior colliculus was gained by measuring the steady-state latency of the phase-locked responses (derived from phase plots) after stimulating each ear individually and comparing them with the latency of the phase-locked responses to binaural stimuli. Twelve single units gave good phase-locked responses to binaural stimulation as well as stimulation with contralateral and/or ipsilateral ears alone. For seven of these units the binaural phase plots were nonlinear, whereas the phase plots measured after monaural stimulation were linear. The other five units had linear binaural phase plots, but the steady-state latencies, measured from their slopes, were different from the latencies derived from their phase plots to unilateral stimulation, for at least one ear. The differences between latencies for binaural and unilateral stimulation ranged between 1.7 and 3.9 ms. Thus none of these cells appeared to be acting as a simple binaural comparator where there was a simple summation of the inputs from the two ears. Three units were recorded which did appear to act as

![FIG. 7](http://jn.physiology.org/)

When period histograms were plotted for different stimulus frequencies, there was a progressive shift in the mean phase of the phase-locked spikes. This is shown for a single unit stimulated at 13 frequencies between 50 and 410 Hz. For this unit the number of spikes in the sustained response remained fairly constant over the range 80–410 Hz. The frequency and mean phase are given beside each period histogram.

![FIG. 8](http://jn.physiology.org/)

The cumulative mean phase at successive stimulus frequencies was plotted for 85 single units so that the slope could be measured and an estimate of neural latency derived. A: examples of 6 units from the central nucleus with straight slopes and latencies of between 4.6 and 15.4 ms. (The number beside each line indicates the slope in ms.) B: not all of the phase plots had single straight slopes—some units appeared to have 2 or 3 different slopes over different frequency ranges.
Kuwada et al. (1984) studied 82 units in the cat IC, of which properties such as sensitivity to interaural time differences IC has been studied previously as part of investigations into properties (Rees et al. 1997). In general, phase-locking in the locking would not interfere with an analysis of chopping randomly relative to the tone burst envelope so that phase-concerned with phase-locking, and in one study of temporal comparison with other studies of phase-locking in the IC presumably provide an input to the IC.

**DISCUSSION**

**Comparison with other studies of phase-locking in the IC**

Most studies of response properties in the IC have not been concerned with phase-locking, and in one study of temporal properties, the phase of the tone was deliberately set to vary randomly relative to the tone burst envelope so that phase-locking would not interfere with an analysis of chopping properties (Rees et al. 1997). In general, phase-locking in the IC has been studied previously as part of investigations into properties such as sensitivity to interaural time differences (Kuwada et al. 1984; Rose et al. 1966; Stanford et al. 1992). Kuwada et al. (1984) studied 82 units in the cat IC, of which 15 (18%) were phase-locked; phase-locked responses were rarely seen >600 Hz and never >1,200 Hz. Similar results were found in the unanaesthetized rabbit (Stanford et al. 1992), where only ≈24% of IC cells showed phase-locking to pure tones, and this was rarely observed at frequencies >700 Hz (the upper phase-locking limit to contralateral tones was 593 ± 298 Hz). Even at lower frequencies the vector strengths were seldom >0.5. In a detailed study of response properties in the mouse inferior colliculus, only 2 of 414 units showed any sign of phase-locking (Willott and Urban 1978) despite many of the cells responding at frequencies <300 Hz.

Kuwada et al. (1984) showed that the proportion of phase-locked cells in the cat IC increased to 48% among cells with CFs of <600 Hz. The results from rabbit and mouse are also consistent with the proposal that in mammalian species the proportion of phase-locked cells increases among cells with the lowest CFs. In our study of the guinea pig, 68% of cells showed phase-locking, apparently because 26% of our recorded units had CFs of ≤300 Hz and 55% had CFs ≤600 Hz. Cats and rabbits have lower proportions of cells with these very low CFs, whereas the mouse probably has none. The gerbil, like the guinea pig, has a high proportion of cells with very low CFs and a higher proportion of phase-locked cells was apparently found in the gerbil IC than in the cat. The exact proportion of phase-locked cells was not stated (Harris et al. 1997), but like the guinea pig, the strength of phase-locking fell off between 500 and 1,000 Hz and no phase-locking was observed >1,000 Hz. Another reason for the high proportion of phase-locked cells in the present study is that 100 stimulus repetitions were used allowing some units to be classified as phase-locking even when the effect was very weak. Phase-locking among units with high CFs was less common partly because some of the units were narrowly tuned and did not respond at frequencies <500 Hz where most of the phase-locking occurred.

The vector strengths of phase-locking in the IC occasionally reached values of 0.99, and this was higher than was found in the auditory nerve at corresponding frequencies (Palmer and Russell 1986). Vector strengths that are higher than those in the auditory nerve have also been described at other central synapses where the strength of phase-locking actually increases as a result of synaptic convergence and specialized nerve endings. Thus most low-frequency (=700 Hz) globular bushy cells show stronger phase-locking than their auditory nerve inputs (Joris et al. 1994) and can have vector strengths almost as high as 0.99. Similarly some cells in the medial nucleus of the trapezoid body also have vector strengths that are higher than those found in the auditory nerve of the rat and gerbil (Kopp-Scheinpflug et al. 2003; Paolini et al. 2001). Convergence of different phase-locked inputs in the IC, along with short membrane time constants in certain onset cells may also lead to the high vector strengths of >0.8 recorded in this study.

**Significance of nonlinear phase plots**

Some previous studies of phase-locking in the cochlear nerve have shown that there is a linear relationship between the cumulative mean phase angle and the stimulus frequency (Anderson et al. 1971; Palmer and Russell 1986). This linear relationship has also been shown at the level of the thalamus (de Ribaupierre et al. 1980). However Pfeiffer and Molnar (1970) showed that for most cochlear nerve fibers in the cat,
the slope of the relationship between stimulus frequency and phase changed at a distinct point close to the CF. It is not clear why the three cochlear nerve studies obtained such different results, but they are consistent with our recordings in the IC that showed that although many cells have a linear frequency/phase relationship, some others are nonlinear. This nonlinear relationship that we observed in some IC units could be explained by a number of mechanisms. The simplest is that their input from the cochlear nerve was nonlinear. Alternatively there might have been convergence of linear inputs arising from nerve fibers that had different phase slopes over different frequency ranges. A third possibility is that there was convergence of input that came via pathways with different numbers of synaptic delays or where the fibers had different conduction velocities. Evidence for convergence of inputs from the lateral and medial superior olive onto the same cell has already been shown in the guinea pig and rabbit by studying the mean cumulative phase of the responses to interaural phase differences produced by bimalar beats of different frequencies (Fitzpatrick et al. 2002; McAlpine et al. 1998). Some low-frequency cells appeared to receive inputs from the medial nucleus and some from the lateral nucleus of the superior olive while others had inputs from both.

Potential origins of phase-locked input from the brain stem

There are a number of parallel pathways bringing phase-locked information directly into the IC. The first of these arises in the cochlear nucleus. There are three cell types in the cochlear nucleus which have a direct projection to the IC. One of these is the type I multipolar (Cant and Benson 2003) cell, which corresponds to the chopper units that send out axons in the trapezoid body (Adams 1979; Osen 1972; Palmer et al. 2003). These cells phase-lock up to ~1.5 kHz and have a vector strength of 0.8 at 500 Hz in the guinea pig (Winter and Palmer 1990). The other two cell types are in the dorsal cochlear nucleus and are the fusiform and giant cells (Alibardi 1999; Oliver 1984). Some of these may have phase-locked responses as cells in the cat DCN show phase-locking at frequencies as high as 1.5–2 kHz (Goldberg and Brownell 1973; Lavine 1971). Input from the cochlear nucleus would be primarily monaural but is likely to be combined with another input such as that from the lateral superior olive by the recipient cells of the IC (Oliver et al. 1997).

The medial and lateral divisions of the superior olive both contain phase-locking cells (Finlayson and Caspary 1991; Mousshegian et al. 1967; Yin and Chan 1990) and are thought to be major sources of phase-locked input to the IC (Oliver et al. 2003). The medial division is largely composed of coincidence detectors that fire optimally to simultaneous, phase-locked input from the large spherical bushy cells of the cochlear nuclei on each side (Batra and Yin 2004; Goldberg and Brown 1969; Spitzer and Semple 1995; Tollin et al. 2000). Most of these cells also show a phase-locked response to unilateral inputs, but a few only give a phase-locked response when both ears are stimulated (Yin and Chan 1990). The lateral superior olive also receives excitatory phase-locked input from spherical bushy cells as well as a phase-locked inhibitory input from the medial nucleus of the trapezoid body (Boudreau and Tsuchitani 1968; Finlayson and Caspary 1991; Fitzpatrick et al. 2002; Joris and Yin 1995). Much of the output from the lateral division is from high-frequency cells, but there is also a distinct low-frequency output to the IC (Shneiderman and Henkel 1987). In addition to the two main divisions of the superior olive, there are a number of perilisory nuclei, some of which project to the IC. However, at present there doesn’t seem to be any evidence that they provide a phase-locked output. The superior paralirvatory nucleus in guinea pigs is large and receives an input from the octopus cells of the cochlear nucleus. The octopus cells form calyceal endings that are specialized for high temporal fidelity (Schofield and Cant 1997) in the ventral nucleus of the lateral lemniscus, but they do not appear to form calyceal endings in the superior paralirvatory nucleus. A recent study of the superior paralirvatory nucleus of the gerbil did not find any phase-locked cells (Behrend et al. 2002).

The cells clustered around the lateral lemniscus form a complex group of nuclei that vary between species in their details but collectively may contain ~50% of the cells in the brain stem that project to the IC (Brunso-Bechtold et al. 1981; Moore 1988). In the guinea pig, the ventral division of the ventral nucleus receives afferents from thick axons with specialized calyceal endings that appear to originate in the octopus cells of the ventral cochlear nucleus. (Adams 1997; Schofield and Cant 1997). These recipient cells apparently correspond to those in the lateral division of the ventral nucleus of the rabbit (Batra and Fitzpatrick 2002), which phase-locked to low-frequency pure tones with a vector strength that was roughly equal to that of cochlear nerve fibers (0.8) (Batra and Fitzpatrick 1999). The ventral nucleus of the lateral lemniscus also receives input from the type I multipolar cells and spherical bushy cells of the ventral cochlear nucleus (Schofield and Cant 1997) and is a major source of input to the central and external nuclei of the IC (Kudo 1981). Glycinergic neurons in the ventral nucleus, which receive specialized end bulbs of Held, appear to send large-diameter axons to the IC (Schwartz 1992). These would be expected to provide a phase-locked inhibitory input. Evidence of a separate group of phase-locking cells in the dorsal nucleus of the lateral lemniscus has been provided in the cat (Aitkin et al. 1970). It is not clear if these correspond to the inputs to the IC, from the dorsal nucleus, that are responsible for an inhibitory input to low-frequency cells of the guinea pig IC (Faingold et al. 1993; McAlpine and Palmer 2002).

Potential origins of phase-locked input from the forebrain

Phase-locked responses to pure tones have been recorded in the thalamus of the cat (Rouiller et al. 1979), rabbit (Stanford et al. 1992), and guinea pig (Wallace et al. 2004), and the cells responsible are located in the ventral and medial divisions of the MGB. Both the external nucleus and the central nucleus of the IC are known to receive inputs from the medial division of the MGB (Kuwabara and Zook 2000; Winer et al. 2002). Some cells in the guinea pig medial division phase-lock to frequencies of ~1,100 Hz and their steady-state latency calculated from their phase plots vary between 7.5 and 11 ms (Wallace et al. 2004). For any cells in the IC to be receiving a phase-locked input from the thalamus, the latency would need to be ~1 ms longer than this to account for an extra synapse. Units with latencies of 12–21 ms, based on their phase plots, were observed in all three divisions of the IC, and none of the latencies...
in the dorsal and external nuclei was <12 ms. Thus the reason for the long latencies of some phase-locked cells in the IC may be that they are receiving their input from the thalamus. The measured latencies seem to indicate that neither the dorsal nor the external divisions of the IC provide a phase-locked input to the medial division of MGB.

The other forebrain structure that projects to the IC is the auditory cortex, and this provides a larger input to the dorsal cortex and external nucleus than any other part of the brain (Winer 2005). In the cat, 11 of 12 auditory cortical areas project to the IC. Phase-locking cells have never been studied in the cat cortex because they are difficult to find, and the evidence for them has not been published. In the guinea pig cortex, phase-locked responses to pure tones have now been described in the ventrorostral belt (Wallace et al. 2000b) and the primary auditory area (Wallace et al. 2002). The guinea pig auditory cortex provides a dense input to the dorsal cortex and external nucleus and a much smaller input to the central nucleus that is thought to be glutamatergic (Feliciano and Potashner 1995). This projection is capable of exciting units in the IC after electrical stimulation of the cortex (Torterolo 1998). The latency of units in the primary auditory area, derived from phase plots, ranged from 11 to 18.5 ms, and so these are shorter latencies than some found in the three divisions of IC (Wallace et al. 2002). The origin of the corticocollicular pathway is layer V pyramidal cells (Feliciano and Potashner 1995; Winer 2005) and recordings of phase-locked cells were made from depths of >1,000 μm; this corresponds to layer V. Cells phase locked at frequencies of ≤250 Hz and so could potentially provide a phase-locked input to cells in the external nucleus and dorsal cortex where phase-locking was weak and some cells did not phase-lock at >200 Hz.

In conclusion, by studying the phase-locked responses to pure tones at successive levels of the auditory brain within one species, it should be possible to make objective deductions about the sequences of steps involved in processing an acoustic stimulus. In future it should be possible to follow information even when it passes along parallel pathways. Nevertheless to make progress more data will be required from the guinea pig cortex and brain stem nuclei such as those in the superior olive and located around the lateral lemniscus.

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