Responses in the Inferior Colliculus of the Guinea Pig to Concurrent Harmonic Series and the Effect of Inactivation of Descending Controls

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Nakamoto KT, Shackleton TM, Palmer AR. Responses in the inferior colliculus of the guinea pig to concurrent harmonic series and the effect of inactivation of descending controls. J Neurophysiol 103: 2050–2061, 2010. First published February 10, 2010; doi:10.1152/jn.00451.2009. One of the fundamental questions of auditory research is how sounds are segregated because, in natural environments, multiple sounds tend to occur at the same time. Concurrent sounds, such as two talkers, physically add together and arrive at the ear as a single input sound wave. The auditory system easily segregates this input into a coherent percept of each of the multiple sources. A common feature of speech and communication calls is their harmonic structure and in this report we used two harmonic complexes to study the role of the corticofugal pathway in the processing of concurrent sounds. We demonstrate that, in the inferior colliculus (IC) of the anesthetized guinea pig, deactivation of the auditory cortex altered the temporal and/or the spike response to the concurrent, monaural harmonic complexes. More specifically, deactivating the auditory cortex altered the representation of the relative level of the complexes. This suggests that the auditory cortex modulates the representation of the level of two harmonic complexes in the IC. Since sound level is a cue used in the segregation of auditory input, the corticofugal pathway may play a role in this segregation.

INTRODUCTION

In our normal busy lives we rarely hear isolated sounds. In most situations, such as the eponymous cocktail party (Cherry 1953; Cherry and Taylor 1954), many sound sources are present and their waveforms simply add together at the listener’s ears. Remarkably, listeners are able to segregate from this complex waveform the components from the different sources and group them together to form a coherent percept of each source. In such situations, despite the shared spectrotemporal characteristics of the sources, listeners can attend to a single talker despite multiple competing talkers. This segregation of a single input into multiple sound sources is part of a process commonly referred to as “auditory scene analysis” or “auditory image formation” (reviewed in Bregman 2001; Griffiths and Warren 2004; Kubovy and Van Valkenburg 2001; Nelken and Bar-Yosef 2008; Scott 2005; Shamma 2008; Yost 1991). A variety of cues have been identified that contribute to this process (Bregman 2001; de Cheveigné 2005; Moore and Gockel 2002), including the pitch and sound level. Previous studies at different levels of the auditory pathway have revealed much about the representation of concurrent sounds that differ in their pitch and level (cochlear nucleus: Keilson et al. 1997; Larsen et al. 2008; Palmer 1990; inferior colliculus: Sinex and Li 2007).

Sounds such as speech, music, and animal communication often contain harmonic complexes. A harmonic complex is composed of a tone (the fundamental frequency) and tones at integer multiples of the fundamental frequency (e.g., 125, 250, 375, 500 Hz, etc.). When harmonic complexes are played, a pitch is perceived with a frequency at the fundamental frequency of the harmonic complex. This perceived pitch can be created by interactions between the components of the harmonic complex within an auditory filter, e.g., 2,125 and 2,000 Hz. The individual components interact and create a modulation of the envelope at a rate equal to the difference in the components (e.g., 125 Hz = 2,125 Hz – 2,000 Hz). In the auditory nerve the fundamental frequencies of concurrent harmonic stimuli are well represented across the nerve fibers, although individual fibers tend to be dominated by individual components near the characteristic frequency (CF) of the fiber rather than by envelope modulations (Larsen et al. 2008; Palmer 1990). In the inferior colliculus (IC) the responses are greatly affected by the envelope of the harmonic complexes, as opposed to the individual components (Sinex and Li 2007); it is unknown whether these differences reflect processing subsequent to the auditory nerve, descending inputs to the IC, or a mixture of the two.

In the IC we have observed that descending projections from the auditory cortex may have radical effects on the representation of amplitude-modulated signals in terms of both firing rate (Nakamoto et al. 2008) and timing of the discharges (Palmer et al. 2007). Here we hypothesize that such inactivation of the descending modulation of afferent activity will also affect the representation of concurrently presented harmonic series and thus may contribute to the process of separating sound sources.

We used two harmonic series that were distinguishable on the basis of their pitch and level, even though the two sounds span the same frequency ranges and occur at the same time (125 and 145 Hz, Fig. 1, A and B; see Micheyl et al. 2006). The responses to the harmonic complexes were analyzed before, during, and after recovery from cortical inactivation by a cooling loop (Lomber et al. 1999). Inactivation of the auditory cortex changed the synchronization of IC units to the harmonic series and the magnitude of the responses.

METHODS

Anesthesia and surgical preparations

A total of 13 pigmented guinea pigs of both sexes and weighing 500–800 g contributed to this study. In all animals, anesthesia was induced with urethane (1.1 g/kg in 20% solution, administered intraperitoneally) supplemented as necessary by Hypnorm (fentanyl citrate 0.315 mg/ml; fluanisone 10 mg/ml, administered intramuscularly) to
Animals (Scientific Procedures) Act.

During recordings the surface of the brain was maintained at 38°C by a heating blanket and rectal probe. The animals were administered subcutaneously) was given to reduce bronchial secretions. All animals were tracheotomized and respired with oxygen to maintain normal end-tidal CO2 levels. 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 specula replacing the ear bars, inside a sound-attenuating room. The bullae on both sides were inserted into the IC through the intact cerebral cortex and into the auditory cortex. Over a 5-min period the surface of the right auditory cortex was cooled to 2–3°C using a 4-mm-diameter cryoloop (Lomber et al. 1999), which is large enough to cover primary and secondary auditory cortical areas (Wallace et al. 2000). Temperatures were recorded by a thermocouple that contacted the cortical surface below the cryoloop. Cortical surface temperatures of 2–3°C are sufficient to inactivate all six layers of the cortex (Lomber et al. 1999). This is important because the descending projections arise from the deeper layers (Winer and Prieto 2001). These temperatures do not affect the white matter below the auditory cortex and, due to the distance from the cooling loop, are unlikely to affect areas below the white matter (Lomber et al. 1999). Cortical inactivation was confirmed by the cessation of activity in the deeper cortical layers, as recorded by microelectrodes. Following the presentation of a battery of sounds (lasting ≤45 min), the cryoloop pump was turned off and the cortical surface returned to near normal temperature over a few minutes due to the normal cortical blood flow. Although normal temperature at the cortical surface was achieved in just a few minutes, cortical responses took longer to return to normal. We waited 20 min to ensure full recovery, which was confirmed by the reappearance of stimulus-locked activity on the cortical electrodes that was similar to the precouling condition. We then repeated the recordings. In this way, each neuron acted as its own control. We do not report any data here from neurons whose responses did not recover their spike count (to within 20% of the precouling maximum spike count) and temporal response (the dominant Fourier components were the same as in the precouling condition).

Analysis

Because the temporal structure of the two harmonic complexes is different, the degree of synchronization to the temporal structure of each can be independently determined by a Fourier transform. The
poststimulus time histogram (PSTH) in response to the concurrent harmonic series (bin width: 0.5 ms) was Fourier transformed to determine the frequencies that IC units synchronized to. To compare across units, the Fourier magnitude at each frequency was normalized to the total number of spikes in the histogram (DC component); this yields a measure equivalent to vector strength (Goldberg and Brown 1969). The vector strength value can vary between 0, indicating no synchronization, and 1, for perfect locking. Fourier magnitudes were considered to be significant if their Rayleigh values (2\pi n^2, where n is the number of spikes in the period histogram and r is the vector strength) were \( >13.8 \) (\( P < 0.0001 \)) (Rayleigh test of uniformity; Buunen and Rhode 1978). Only significant Fourier components were included in the analyses.

Unit selection

Responses to the concurrent harmonic series of 217 single units and multiunits were recorded. After exclusions (see following text) only those neurons (57) whose responses to the concurrent harmonic series fully recovered after cortical inactivation remained. Thirty-four units that responded only to stimulus onset were excluded because it was not possible to differentiate their responses to the two harmonic complexes. In all, 106 units were excluded due to lack of recovery in the postcooking condition; this is not surprising, considering that the precooking and cooled conditions took >1 h. In addition, both the spike count and the temporal pattern of the units had to recover. Twenty units were excluded because they were located outside of the central nucleus or the lateral (external) cortex of the IC. Only units with CFs \( >4.5 \text{ kHz} \) were examined; this was done to ensure that the synchronization of the cell was caused by interactions of multiple harmonic components within its response area and not synchronization to the component at 125 or 145 Hz.

Histology

During the withdrawal of the electrode, electrolytic lesions were made in some of the tracks by passing a current of \( 5 \mu \text{ A} \) for 10 s (electrode negative) through the electrode. At the end of the experiment, the animal was given an overdose of pentobarbital and perfused with 4% paraformaldehyde. The brain was removed and sectioned at \( 50–100 \mu \text{ m} \) on a vibratome. Sections were stained for cytochrome oxidase. The electrode tracks were located using the electrolytic lesions to provide confirmation of the recording locations within the IC. In the later experiments the electrodes were dipped in cresyl violet after each dip; Lim and Anderson (2007), consequently, the electrode tracks were clearly visible in the sections. Only cells in the central nucleus and lateral (external) cortex of the IC were analyzed.

RESULTS

An example of the responses of a single unit in the central nucleus of the inferior colliculus to the two harmonic complexes is shown in Fig. 2. The stimulus conditions are illustrated in the first row; the level of the 125-Hz harmonic complex (H125) was held constant at 60 dB SPL and the level of the 145-Hz harmonic complex (H145) was varied from 40 to 60 dB SPL (Fig. 2, A1–A3). In the second row the PSTH in response to each stimulus is displayed (Fig. 2, B1–B3). It is not obvious from the PSTH which harmonic complex dominates this unit’s response. However, the synchronization to both harmonic series is observable in the Fourier transforms (Fig. 2, C1–C3). The vector strengths of the components at 125 and 145 Hz or 250 and 290 Hz represent the degree of synchronization to the fundamental frequencies (f0), which corresponds to the pitch of the harmonic complexes, or to their second harmonic (2f0). The two components of particular interest: the 125-Hz component and the 145-Hz component are marked with black and gray arrows, respectively, in Fig. 2, C1–C3. In the majority of conditions (79/3: three H145 levels at each of three H125 levels) either the f0 (125 or 145 Hz) or 2f0 (250 or 290 Hz) components had the largest vector strength. Since the f0s were well below the CF (6.4 kHz) of this IC unit, synchronization to these low-frequency components was most likely due to the interaction of harmonics falling within the unit’s response area (e.g., harmonic 48 at 6 kHz and harmonic 49 at 6.125 kHz). The vector strength of the 125-Hz component was greatest when the level of the 125-Hz complex was 20 dB above that of the 145-Hz harmonic complex (Fig. 2C1). The vector strength of the 125-Hz component declined as the level of H145 was increased (Fig. 2, C2 and C3). As the level of H145 was increased the vector strength of the 145-Hz Fourier component also increased and, in this example, was the largest when both harmonic complexes were equal in level.

The vector strengths of the 125- and 145-Hz Fourier components of the unit in Fig. 2 are plotted against the level of the H145 complex in Fig. 3 (A1: 125 Hz; A2: 145 Hz). It is clear that the synchronization to the constant level H125 (whether at 40, 50, or 60 dB SPL per component) decreased (Fig. 3A1) and the synchronization to the 145-Hz component increased (Fig. 3A2) when the level of H145 was increased. The synchronization to the 125-Hz component became progressively lower and the synchronization to the 145-Hz component became progressively higher with the reduction in the level of H125. If the data are replotted in terms of dB SPL (H125 – H145 dB SPL), a nearly linear relationship with vector strength becomes apparent; both the 125-Hz (Fig. 3B1) and the 145-Hz (Fig. 3B2) components were significantly correlated with dB SPL [ANOVA: \( F(1,7) = 79 \) (125 Hz), 12 (145 Hz); \( P < 0.01; R^2 = 0.97 \) (125 Hz), 0.98 (145 Hz)]. The vector strength of the 125-Hz component was positively correlated and the vector strength of the 145-Hz component was negatively correlated with dB SPL. This suggests a third correlation, the difference in vector strength of the 125- and 145-Hz components (125-Hz vector strength minus 145-Hz vector strength; \( \Delta VS \)), which is also significantly correlated with dB SPL [ANOVA: \( F(1,7) = 82, P < 0.01; R^2 = 0.99 \) (Fig. 3B3)]. In summary, the difference in synchronization to the f0s of the harmonic complex is approximately linearly related to the difference in the level of the harmonic complexes, suggesting that the relative level of the harmonic complexes is encoded temporally.

In Fig. 4 the 125- and 145-Hz response components of the unit in Fig. 2 are plotted for the different cooling conditions. As stated previously, both the 125- and the 145-Hz response components had a significant linear relationship with dB SPL, although across the population a cubic fit was better (similar \( F \) values, higher \( R^2 \)) and will be used for all subsequent analyses. As previously shown, the 125- and 145-Hz components (Fig. 4, A1 and A2, •, solid line) and \( \Delta VS \) (Fig. 4A3, •, solid line) were significantly related to dB SPL prior to cortical inactivation. When the auditory cortex was inactivated the synchronization to both the 125- and 145-Hz components was substantially reduced (Fig. 4, A1 and A2, *, dotted gray lines) and the 145-Hz component was no longer significantly related to dB SPL. The difference between the 125- and 145-Hz functions was also reduced. Consequently, \( \Delta VS \) was
no longer significantly related to ΔdB SPL and the function was substantially closer to 0 ΔVS (Fig. 4A3, ●, dotted gray line). After the auditory cortex had recovered to normal temperature, responses to the 125- and 145-Hz components were similar to the precooling conditions (Fig. 4, A1 and A2, ▲, dashed line). This is interesting on two levels. First, deactivation of the auditory cortex changed the synchronization of the unit in the IC (i.e., cortical deactivation affected the temporal response of the IC unit). Second, for this unit, when the cortex was inactivated the difference in the synchronization to the f0s of the harmonic complexes was no longer indicative of the relative level of the harmonic complexes (i.e., cortical deactivation altered the representation of the level of the concurrent stimuli).

The spike count (summed over the trials) evoked by the various stimulus and cooling conditions was tested against various parameters and it was found that the spike count correlated best with the summed power of the two harmonic complexes (power of the 125-Hz harmonic complex + power of the 145-Hz harmonic complex). There is very little frequency overlap between the individual components of the two harmonic complexes and, consequently, the combined level could be no more than 3 dB SPL greater than the more intense harmonic complex. In Fig. 4A4 the spike count of the unit in response to H125 and H145 is plotted against the summed level. Linear regressions were computed for the responses to the different level combinations of H125 and H145 for the precooling condition (Fig. 4A4, ●, solid line). For this unit, there were no significant differences in the spike counts due to inactivation of the cortex. In summary, for this unit there was no systematic change in the spike count during cortical deactivation (Fig. 4A4), even though there was a substantial change in the synchronization to the 125- and 145-Hz components (Fig. 4A3).

To quantify the effect of cortical inactivation on the spike count, the spike count during cortical inactivation was normalized to the precooling spike count and the percentage difference was measured. A positive value indicates a higher spike count before cortical inactivation and a negative value indicates a higher spike count during cortical inactivation. Changes in synchrony was quantified as the sum of the squared differences between the precooling VS and the inactivated VS [VS DIF: Σ (precooling ΔVS − inactivated ΔVS)]. A large value on this measure indicates a large difference between the VS curves in the precooled and cooled conditions, which could be due to an overall shift in the curves, a flattening of
one, or a change in sign of the slope of one of them. However, the most common result was a flattening (i.e., reduction in slope) of one of the curves, which indicates that the responses to H125 and H145 were substantially more similar in one cooling condition compared with the other—i.e., the neural representations of the level differences ($\Delta$H9004) were substantially smaller in one condition. To distinguish which condition had a larger difference a positive value was assigned if there was a greater difference between the 125- and 145-Hz functions before cooling; a negative value was assigned if there was a greater difference during cortical inactivation. The unit displayed in Figs. 2–4 had a VS DIF of 0.25 and a maximum spike difference of $-7\%$.

Additional examples of the effect of cortical inactivation on the responses to the harmonic complexes are shown in Fig. 5. For the unit in the first row (CF: 12.8 kHz) there was little change in $\Delta$VS during cortical inactivation (Fig. 5A1). However, the spike count during cortical inactivation was substan-

FIG. 3. In the 1st row the vector strengths of the 125- and 145-Hz Fourier components of the unit shown in Fig. 2 are replotted against the level of the 145-Hz harmonic complex. The 3 different lines signify the 3 different levels of the 125-Hz harmonic complex. In B1 and B2 the Fourier components are plotted against $\Delta$dB SPL (125-Hz harmonic complex dB SPL minus 145-Hz harmonic complex dB SPL). In B3 the $\Delta$VS is plotted against $\Delta$dB SPL. $\Delta$VS is defined as the difference in vector strength between the 125- and 145-Hz Fourier components. If the 125-Hz component was greater, $\Delta$VS was positive; if the 145-Hz component was greater, $\Delta$VS was negative.

FIG. 4. The responses of the unit shown in Figs. 2 and 3 are shown for the 3 different cortical cooling conditions. The solid and dashed lines signify the precooling and recovered conditions, respectively. The dotted gray line signifies the response during cortical inactivation. In the 1st row the 125-Hz, 145-Hz, and $\Delta$VS functions are shown; the axes are the same as Fig. 2, B1–B3. The spike count in response to the stimuli is shown in A4. The x-axis is the summed level (in dB SPL) for the 125- and 145-Hz harmonic complexes.
tially reduced, compared with the precooling and recovered conditions (Fig. 5A2, dotted gray line compared with the solid and dashed lines). The VS DIF for this unit was 0.02 and the maximum spike count difference was 35%. For the unit in the second row (CF: 6.4 kHz) both the synchronization and the spike count were affected by cortical inactivation. The 125- and 145-Hz components of the unit responses shown in Fig. 5B1 (dotted gray line) became more similar during cortical inactivation as indicated by the VS function, which was closer to zero during cortical inactivation. The spike count was substantially reduced during cortical inactivation (Fig. 5B2, dotted gray line). The VS DIF for this unit was 0.74 and the maximum spike count difference was 84%. In contrast, the responses of the unit (CF: 9 kHz) in Fig. 5, C1 and C2 were less affected by cortical inactivation. The VS DIF for this unit was 0.21 and the maximum spike count difference was 12%.

In summary, in this section we have shown examples of the effect of cortical cooling on the responses to harmonic complexes. Inactivation was shown to reduce the synchronization (Fig. 5, B1 and C1) and to reduce the firing rate (Fig. 5, A2 and B2), but these effects were independent; compare Fig. 5B, where both synchronization and firing rate were reduced, with Fig. 5A, where only the firing rate was reduced, and Fig. 5C where only the synchronization was reduced. We discuss this further in the following text, where data for the entire population of recorded cells are compared (see Population synchronization).

Population analyses

Vector strength. The Fourier components with the largest vector strengths were generally at the fundamental frequency (f0) or 2f0 of the two harmonic complexes; this was true when the cortex was active or inactivated. The largest Fourier components were determined for each unit and the percentages of units responding best to each component were calculated for the various \( \Delta dB \) SPLs (Table 1). A \( \Delta dB \) SPL of 10 would indicate that H125 was 10 dB SPL higher in level than H145 and -10 \( \Delta dB \) SPL would indicate that H125 was 10 dB SPL.

FIG. 5. The \( \Delta VS \) and the spike-count functions are shown for 3 units. The axes are the same as those in Fig. 4, A3 and A4. The VS DIF for each cell is shown in the 1st column and in the 2nd column the difference between the precooling maximum spike count and the inactivated maximum spike count is shown. VS DIF is the sum of the square difference between the precooling \( \Delta VS \) function (1st column, solid black lines) and the inactivated \( \Delta VS \) function (1st column, gray lines).
lower in level than H145. The majority of units synchronized best to the \( f_0 \) (125 and 145 Hz) and \( 2f_0 \) (250 and 290 Hz) of the harmonic complex with the higher level. Again, since the \( f_0 \)s were well below the CFs of these units the synchronization to \( f_0 \) and \( 2f_0 \) is likely to be due to the interactions of higher-frequency components within the response area of these cells.

One thing to note is that whereas the largest Fourier components were the \( f_0 \) or \( 2f_0 \) of the louder harmonic complex, the majority of cells also synchronized to the \( f_0 \) or \( 2f_0 \) of the other harmonic complex. Even with a large (20 dB SPL) level difference between the harmonic complexes both harmonic complexes were represented in the temporal response; i.e., the different harmonic complexes did not appear to be represented by different neural populations.

The largest Fourier components during cortical inactivation were determined for each unit at every \( \Delta dB \) SPL (Table 2). In general, most units synchronized best to the \( f_0 \) or \( 2f_0 \), although the population responding to those components was reduced compared with the precooling condition.

**TABLE 1. Precooling percentage of population synchronizing**

<table>
<thead>
<tr>
<th>Fourier Component</th>
<th>( 20 )</th>
<th>( 10 )</th>
<th>( 0 )</th>
<th>( -10 )</th>
<th>( -20 )</th>
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</thead>
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<tr>
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<tr>
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<tr>
<td>290</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>26</td>
<td>24</td>
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<td>13</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of units, for the precooling condition, showing the greatest synchronization to different Fourier components for the different \( \Delta dB \) SPLs.

**TABLE 2. Percentage of population synchronizing during cortical inactivation**

<table>
<thead>
<tr>
<th>Fourier Component</th>
<th>( 20 )</th>
<th>( 10 )</th>
<th>( 0 )</th>
<th>( -10 )</th>
<th>( -20 )</th>
</tr>
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<tbody>
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<td>38</td>
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<td>39</td>
<td>28</td>
</tr>
</tbody>
</table>

Percentage of units, when the cortex was inactivated, showing the greatest synchronization to different Fourier components for the different \( \Delta dB \) SPLs.

**FIG. 6.** The mean vector strength of the population is plotted against the difference in level \( \Delta dB \) SPL for H125 (solid line) and H145 (dashed line). The mean vector strength was higher for the stimulus that was higher in level (positive \( \Delta dB \) SPL for H125 and negative \( \Delta dB \) SPL for H145). Vertical bars represent the SD.

\[ SD = 0.22 VS; \text{H145: } \mu = 0.44 VS, SD = 0.27. \]

The synchronization to each harmonic complex appeared to depend on the level difference between the two harmonic complexes, which is in agreement with the conclusion reached by Sinex and Li (2007).

During cortical inactivation the synchronization of the population to H125 and H145 was reduced, although the synchronization to H125 and H145 was not affected equally across all levels. The synchronization to H125 was reduced during cortical inactivation, although it was only significantly reduced when its level was higher than or equal to the level of H145 [Fig. 7A1, \( 0 \) to \( 20 \Delta dB \) SPL; respectively, \( t(319.741) = 5.052, P < 0.001, t(200.789) = 5.912, P < 0.001 \) and \( t(95.885) = 4.016, P < 0.001 \)]. The synchronization to H145 was also significantly reduced during cortical inactivation, but only when its level was higher than or equal to the level of H125 [Fig. 7A2, \( -20 \) to \( 0 \Delta dB \) SPL; respectively, \( t(100.631) = 4.497, P < 0.001, t(211.776) = 6.456, P < 0.001 \) and \( t(333.912) = 5.727, P < 0.001 \)]. In summary, the synchronization of the
population to the more intense harmonic complex was significantly reduced during cortical inactivation.

Cubic fits. Across the population the synchronization versus $\Delta$dB SPL plots were fit well with a cubic function. When the 125- and 145-Hz Fourier components were plotted against $\Delta$dB SPL 75% (43/57) of the unit’s responses were fit well by a cubic function (ANOVA, $P > 0.05$) when the cortex was active. For the units with significant cubic fits the slope of the 125-Hz Fourier component was generally positive (38/43) and the slope of the 145-Hz Fourier component was generally negative (35/43). The point at which the two functions intersected was on average $-0.0219$ (SD 0.876) $\Delta$dB SPL; i.e., the point at which the synchronizations at 125 and 145 Hz were equal was when they were similar in level.

During cortical inactivation only 22% (19/57) of the units had a 125- and a 145-Hz synchronization versus $\Delta$dB SPL plot that had a significant cubic fit, a substantial reduction from the 75% when the cortex was active. In 25% of the units (11/43) there was a substantial reduction in the spike count (>80% reduction), which in turn reduced the vector strength to below the significance level (Rayleigh $<13.8$, $P > 0.001$) and no cubic fit was possible. Thirty percent of the units (13/43) had significant (Rayleigh $>13.8$, $P < 0.001$) vector strengths but were no longer significantly related to $\Delta$dB SPL [ANOVA: $F(1,7)$, $P > 0.05$].

Population synchronization

In Fig. 8 the change to the spike count caused by cortical inactivation is compared with the difference in 125- and 145-Hz vector strength (VS DIF). The majority of units had a larger spike count precooling (Fig. 8, positive y value) and a larger difference between the 125- and 145-Hz vector strength functions precooling (Fig. 8, positive y values). The two variables were significantly correlated [Pearson’s correlation = 0.51, $P$(two-tailed) $< 0.01$]; e.g., a relationship was found between the effect of cooling on the maximum spike count and the similarity of the 125- and 145-Hz functions. This is caused by the cells with large reductions in spike count during cortical inactivation: if cells with reductions >80% are removed then the variables are no longer correlated. Also apparent in the graph are a few cells (9%, 5/57) in which there was a large change in the maximum spike count (>50%) and little change in the VS DIF (<0.2) and few examples (9%, 5/57) of cells with smaller changes in the maximum spike count (<50%) and a change in the VS DIF (>0.2, as in Fig. 5C1). Histograms of the population for VS DIF and the difference in the maximum spike count are shown outside of the scatterplot, orientated to their respective axes, in Fig. 8. VS DIF is distributed around a mean of 0.39 VS DIF; on average, cortical inactivation reduced the difference in vector strength by 0.39 across the five $\Delta$dB SPL values.

Potentially the effect of cortical inactivation could be selective to the 125- or 145-Hz Fourier components. To eliminate this possibility the synchronization to the 125- and 145-Hz Fourier components was separately assessed to see whether the effect of cortical inactivation was similar for both components summed across $\Delta$dB SPL, which will remove the level-dependent effects. The vector strengths of the 125- and 145-Hz components were summed across conditions before cooling and compared with the summed vector strength during cortical inactivation (Fig. 9, A and B). In general, cortical inactivation reduced the synchronization to both the 125- (Fig. 9A) and the 145-Hz (Fig. 9B) components. The difference between the precooling vector strength and the vector strength during cortical inactivation was calculated for the 125- and 145-Hz components and plotted in Fig. 9C against each other. The 125- and the 145-Hz differences were significantly linearly related [ANOVA: $F(1,57) = 202.290; P < 0.001$]. Cortical inactivation affects both the 145- and the 125-Hz functions to a similar degree across the $\Delta$dB SPL values.

Multitunits versus single units

Of the 57 units studied 37 were multitunits and 20 units were well-isolated single units. No significant difference was found between the multitunits and the singles units in terms of the effect of cooling on the maximum spike count [ANOVA: $F(1,55) = 0.287; P > 0.05$]. No significant difference was found between the multitunits and the single units in terms of VS DIF [ANOVA: $F(1,55) = 3.924, P > 0.05$], although the mean VS DIF of the multitunits (0.28) was lower than the mean of the single units (0.622).

Cortical inactivation alters IC sound level processing

In 7% of the units, cortical inactivation had a radical effect on the spike count in response to the harmonic complexes. Before cooling and after recovery from cooling the spike count was significantly linearly related to the summed level [Fig. 10A1, black lines; ANOVA: $F(1,7) = 149.223, P < 0.001$]; however, during cooling the spike count was not significantly related to the summed level [Fig. 10A1, gray lines; ANOVA: $F(1,7) = 13.8$, $P > 0.05$].
2.879, \( P = 0.134 \). The spike count before and during cooling is replotted against H145 level in Fig. 10A2. When H125 was at 50 and 60 dB SPL (Fig. 10A2, gray solid and dashed lines) increasing the level of H145 reduced the spike count. These responses are substantially different from the response during the precooling condition (Fig. 10A2, black lines). During cortical inactivation the manner in which the levels of the two harmonic complexes affected the spike count changed compared with the precooling and recovered conditions.

Comparison of IC nuclei

Responses were recorded in both the central nucleus (CNIC: 39 units) and the lateral cortex of the inferior colliculus (LCIC: 18 units). No significant difference was found between units in the CNIC and LCIC in terms of the effect of cooling on the maximum spike count [ANOVA: \( F(1,55) = 0.380; P > 0.05 \)] or on VS DIF [ANOVA: \( F(1,55) = 0.107, P > 0.05 \)].

DISCUSSION

The purpose of this study was to analyze the manner in which the descending pathway from the auditory cortex to the inferior colliculus affects the neural representation of concurrent stimuli. We used two harmonic series as stimuli that are easily perceptually segregated when all components are available. This can be conceptualized as a highly simplified version of two people speaking, two musical notes, or two animals vocalizing at the same time. For the fundamental frequencies we used here and when only higher-frequency components are present (i.e., in the region of the characteristic frequencies that we have analyzed in this study) two equal-level harmonic complexes are perceived as a unitary noise-like sound (Carlyon 1996a,b). However, when there is large level difference between the two harmonic complexes the more intense harmonic complex is likely to dominate the percept (Micheyl et al. 2006). It might reasonably be assumed that this latter effect is due to energetic dominance of one component over the other. In this study, though, we show that inactivation of the auditory cortex decreased the difference in synchronization to concurrent stimuli and, consequently, the state of cortical activation alters the representation of the relative level of these stimuli.

Effect on synchronization of the descending control from the auditory cortex

It has been long known that the auditory cortex, via corticofugal connections, can affect the temporal response of neurons in the medial geniculate body (MGB) and the IC. Ryugo and Weinberger (1976) demonstrated that an active auditory cortex supports long-latency rhythmic discharges in response to pure tones in the MGB, but does not affect the onset response (<20 ms). Changes in sustained responses were also found in 35% of the neurons in the rat IC after auditory cortical inactivation by tetrodotoxin (Popelar et al. 2003).

The dependence of the temporal pattern of IC neurons on the relative level of the two harmonic complexes, as opposed to the

![Fig. 9. Scatterplots of the summed vector strength for the precooling 125-Hz (A, x-axis) and 145-Hz (B, x-axis) Fourier components plotted against the summed vector strength of these Fourier component during cortical inactivation (125 Hz: A, y-axis; 145 Hz: B, y-axis). The vector strength was summed over the 9 harmonic complex level combinations. The differences between the precooling and cortical inactivation summed vector strength (x and y axes of A and B) are plotted for the 125 Hz (C, x-axis) and 145 Hz (C, y-axis) in C.](http://jn.physiology.org/)

![Fig. 10. A1: the spike count for a single unit; the axes are the same as those in Fig. 4, A3 and A4. A2: the spike count is plotted against the level of the 145-Hz harmonic complex. The 3 different lines indicate the different levels of the 125-Hz harmonic complex and the colors indicate the cortical status.](http://jn.physiology.org/)
absolute level of the stimuli, has been previously demonstrated in the chinchilla (Sinex and Li 2007). In the present study we have shown that, for many IC units, an active auditory cortex is necessary for the relative level of two simultaneously presented harmonic complexes to be reflected in a difference in the synchronization. This difference in synchronization (ΔVS) was related to the difference in the level (ΔdB SPL) of the two harmonic complexes. A change in ΔVS is thus likely to represent a change in the neural representation of the relative level of the two harmonic complexes. This suggests that an active auditory cortex is necessary to maintain the neural representation of the level difference between the two harmonic complexes.

It has been demonstrated that an active auditory cortex is involved in synchronizing responses across neurons in the MGB (Villa et al. 1999) and in the IC (Popelar et al. 2003). It has been hypothesized that the descending system acts as a gain or gating function (Crick 1984; Villa et al. 1999). With an active cortex the vector strength of the response to the more intense stimulus is much greater than that to the less intense (opposite slope of curves in Figs. 4, A1 and A2 and 7, A1 and A2; large slope on Figs. 4A3 and 5). When the cortex is cooled, these differences decrease and the slope of ΔVS as a function of ΔdB decreases. Thus deactivating the auditory cortex can have a differential effect on the temporal responses of two simultaneously presented harmonic complexes. The decrease in the difference between the temporal responses during cortical deactivation suggests that an active cortex increases the difference in the temporal response. One interpretation of this is that an active auditory cortex amplifies the representation of one concurrent stimulus and reduces the representation of the second concurrent stimulus. This would be supportive of the idea of the descending system adjusting the gain of one stimulus relative to another.

It was surprising to find that inactivation of the auditory cortex could separately affect the spike count and the synchronization to the harmonic complexes. For some cells the spike count and the synchronization were separately affected during cortical inactivation (Figs. 5 and 8). Selective recovery of the spontaneous activity, but not the temporal response pattern, has been previously reported in the auditory thalamus (Villa et al. 1991). Such data raise the possibility that there are two mechanisms of descending control on different neurons, one affecting the synchronization of IC units and one affecting the firing rate of IC units.

Other considerations

Urethane anesthesia has an effect on response in both the auditory cortex and the inferior colliculus and these effects need to be taken into consideration. Urethane is a widely used anesthetic for animal studies because it has comparatively weak effects on cardiovascular and respiratory systems (Maggi and Melli 1986; Soma 1983). Urethane enhances the function of a subset of γ-aminobutyric acid (GABA) and glycine receptors (23 and 33% increase, respectively) and also inhibits the function of a subset of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (10 and 18% decrease, respectively) (Hara and Harris 2002). This is in contrast to pentobarbital, which has a primary effect on GABA receptor function (>100% increase) (Sanna et al. 1995; Ueno et al. 1999), and ketamine, which has a primary effect on NMDA receptor function (80% reduction) (Yamakura et al. 1993, 2000, 2001). The weaker effect of urethane on multiple receptors, as opposed to the primary effects of pentobarbital and ketamine, could have less of an effect on timing since GABA, NMDA, and AMPA receptors play a crucial role in spike timing in the IC (Wu et al. 2004). In fact urethane has been shown to have a weaker effect on spike timing in the IC (Astl et al. 1996), compared with that of pentobarbital. This suggests that the descending effects noted in this study may differ in magnitude from the awake preparation, but are probably not qualitatively different.

It has been shown that the auditory cortex is necessary for fine temporal sensitivity in the rat (Cooke et al. 2007) and the ferret (Kelly et al. 1996). In the rat, lesioning of secondary auditory cortical areas had a greater effect on the ability to process sinusoidally amplitude modulated (>100 Hz) noise. Although effects in the current study have been largely attributed to inactivation of the primary auditory cortex it is possible that there is also a contribution from inactivation of secondary auditory cortical areas.

Descending connections from the auditory cortex either directly or indirectly affect all subcortical auditory nuclei (Coomes et al. 2005; Schofield 2009; Winer 2006); thus it is possible that the effects measured in this study were caused by removing the descending control to nuclei other than the inferior colliculus. However, there is strong evidence that temporal responses to sounds are altered at the level of the IC (Caspar et al. 2002; Zhang and Kelly 2003), suggesting that the effects noted herein could be due to changes in the response at the level of the IC.

Coding of harmonic components

There are differences in the perception of harmonic complexes that consist of resolved (relatively high f0, low spectral region) or unresolved (relatively low f0, high spectral region) components. In the present study we concentrate on the representation of unresolved components of harmonic series by choice of high characteristic frequency neurons and low f0 stimuli. Earlier studies concentrated mainly on resolved harmonics, comparing the responses in the auditory nerve, cochlear nucleus, and the inferior colliculus to mistuned harmonic complexes. The response of the auditory nerve to mistuned harmonic complexes or concurrent vowels is dominated by individual components, with frequencies near the characteristic frequencies of the fibers (Larsen et al. 2008; Palmer 1990; Sinex et al. 2003). Mistuning a single component did not greatly affect the temporal pattern of the auditory nerve fiber responses. In the IC, mistuning one component in a harmonic complex greatly altered the temporal discharge pattern. These changes appeared to be produced by the envelope modulations created by the interaction between components (e.g., a 250-Hz component interacting with a 270-Hz component and creating a 20-Hz modulation, which is the difference between the components), rather than response to the individual components or the f0s of the harmonic complexes (Sinex et al. 2002, 2005). The difference between the responses at the auditory nerve and the IC were attributed to processing at or below the IC.
In contrast to the studies by Sinex and colleagues, in the present report the largest synchronization was to the \( f_0 \)s and \( 2f_0 \)s of the harmonic complexes. Two key differences between the study by Sinex and ours are the difference in the \( f_0 \)s used and the selection of characteristic frequencies of the cells studied. In the study by Sinex the \( f_0 \)s were generally \( >200 \) Hz and the characteristic frequencies between 0.24 and 3.1 kHz; thus he primarily studied the processing of resolved harmonics. In our study, we used \( f_0 \)s of 125 and 145 Hz and characteristic frequencies \( >4.5 \) kHz and thus we primarily studied unresolved harmonics. It has been demonstrated psychophysically in humans that different mechanisms are likely used to analyze harmonic components in these different frequency regions (Shackleton and Carlyon 1994). Thus the two sets of studies are complementary.

In our preliminary studies we used \( f_0 \)s of 250 \( + 270 \) Hz and 500 \( + 520 \) Hz. Synchronization to these higher fundamentals occurred less often than synchronization to the \( f_0 \)s at 125 and 145 Hz. This suggests an upper limit for synchronization of IC units to concurrent harmonic complexes, somewhere between 145 and 250 Hz. The limit for synchronization between 145 and 250 Hz may be partially due to the methods used in the current report and in the studies by Sinex and colleagues. In the current report, the first 50 ms were not analyzed and in Sinex’s studies the first 100 ms were not analyzed. In a report by Shackleton et al. (2009) using a 100-ms stimulus and analyzing the complete response, including onset, it was shown that 40\% of the units phase locked at 400 Hz. The difference in the phase-locking limit between the studies is possibly due to the difference in the window of analysis.

A third difference between Sinex’s studies and the current report is the first eight components of two concurrent harmonic complexes were presented in Sinex’s study, whereas all components \( \leq 10 \) kHz were presented in our study. The increased number of components allows for a greater degree of interaction, at the \( f_0 \), between components at high frequencies.

**Conclusion**

This report demonstrates that the descending system can alter the neural representation of concurrent harmonic stimuli in the IC. The descending system from the auditory cortex has a precise effect on the synchronization of cells in the inferior colliculus. This was demonstrated with a global inactivation of the auditory cortex.

Using global cortical cooling can demonstrate only that a pathway exists that affects the representation of concurrent stimuli. The present methods cannot determine whether the effect occurs on a stimulus by stimulus basis, whether it is topographically organized or whether it is activated or inactivated under specific stimulus or brain states. Although the results here are suggestive, further research would be necessary to demonstrate whether these cortical descending controls are involved in grouping or separating components of an auditory object (stimulus by stimulus effect) or whether it is involved only in longer-term phenomena, such as auditory plasticity or auditory learning.

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