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Frequency Changes in a Continuous Tone: Auditory Cortical Potentials

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Abstract

Objective—We examined auditory cortical potentials in normal hearing subjects to spectral changes in continuous low and high frequency pure tones.

Methods—Cortical potentials were recorded to increments of frequency from continuous 250 Hz or 4000 Hz tones. The magnitude of change was random and varied from 0% to 50% above the base frequency.

Results—Potentials consisted of N100, P200 and a slow negative wave (SN). N100 amplitude, latency and dipole magnitude with frequency increments were significantly greater for low compared to high frequencies. Dipole amplitudes were greater in the right than left hemisphere for both base frequencies. The SN amplitude to frequency changes between 4 to 50% was not significantly related to the magnitude of spectral change.

Conclusions—Modulation of N100 amplitude and latency elicited by spectral change is more pronounced with low compared to high frequencies.

Significance—These data provide electrophysiological evidence that central processing of spectral changes in the cortex differs for low and high frequencies. Some of these differences may be related to both temporal- and spectral-based coding at the auditory periphery. Central representation of frequency change may be related to the different temporal windows of integration across frequencies.

Keywords

event related potentials; spectral temporal coding; dipole source analysis; N100

INTRODUCTION

Natural sounds in the environment such as speech and music are complex acoustic signals with changing pitch, intensity, and temporal features that provide cues for both speech comprehension and music perception (Moore, 2003). For instance, both frequency and

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temporal changes of formant transitions are essential for the identification of consonant-vowel and vowel-consonant transitions. Electrophysiological and neuromagnetic studies of auditory processing have traditionally employed short duration transient tone burst stimuli in contrast to everyday listening situations, where most of the auditory environment is in flux.

Neural mechanisms underlying the processing of stimulus change have been of interest for more than twenty years. There were several early studies of auditory cortical potentials to changes of pitch and/or intensity of an ongoing continuous tone (Arlinger et al., 1982; Jerger and Jerger, 1970; Kohn et al., 1978; Lavikainen et al., 1995; McCandless and Rose, 1970; Spoor et al., 1969; Yingling and Nethercut, 1983). The general findings were that N100/P200 cortical components occurred in response to changes of pitch and intensity and their amplitude increased with the magnitude of change. More recently the mismatch negativity (MMN), a potential related to the automatic processing of stimulus change, was shown to occur with frequency change of continuous tones and also to increase in amplitude with the magnitude of frequency change (Novitsky et al., 2004; Jacobson et al., 2003). In the past decade, continuous natural stimuli containing changes relevant for speech identification such as phoneme recognition, fricative to vowel transition (Ostroff et al., 1998), vowel to vowel transitions (Martin and Boothroyd, 2000) were shown to evoke N100/P200 components called “acoustic change complex”. Jones and colleagues (Jones et al., 1998; Jones and Perez, 2001; 2002) examined auditory cortical potentials accompanying changes in continuous stimuli using synthesized music in which one or more parameters (i.e., pitch or timbre) were varied and found “change N100 and change P200” components.

The ability to measure auditory potentials to frequency change can be used to examine central processes underlying differences in pitch perception as a function of base frequency. Neurophysiological studies in experimental animals have shown that neural codes for high and low frequencies differ. A “place code” appears to be utilized for discriminating pitch or frequency of a stimulus reflecting the motions of the basilar membrane that have a maximum displacement at the apex for low frequencies and the point of maximum displacement moves towards the base for higher frequencies (von Bekesey, 1960). This frequency order is preserved centrally by the differential orderly grouping of neural populations sensitive to high or low frequencies within auditory subcortical nuceli and auditory cortex. For auditory cortex in humans electrophysiological and neuromagnetic (Pantev et al., 1995, 1988), as well as fMRI studies (Wessinger et al., 1997) have shown that low frequencies are more medially and anteriorly represented than high frequencies.

The auditory temporal neural code refers to the ability of groups of neurons to discharge at a particular phase of the fine structure of an incoming sound stimulus. This results in a periodicity of discharge with a temporal pattern reflecting the spectrum of the tone. The relevance for a temporal code for low tones was clearly demonstrated by Rose et al. (1967) by the finding that auditory nerve fibers in monkeys discharged at a particular phase of low but not high frequency tonal stimuli. In humans, the temporal encoding of low frequencies is reflected in the frequency following response or FFR (Moushegian et al., 1973) recorded with scalp electrodes as far field potentials of neurons of auditory nerve and auditory brainstem pathways. The FFR reproduces the waveform of low frequency acoustic stimuli to such a degree that when amplified and presented through a loudspeaker the resulting sound is that of the stimulus tone (Wever-Bray phenomenon). There are slight species’ differences in the upper range of phase-locking using measures of both single units and groups of fibers discharges that can be up to 1 kHz in guinea pigs and 2 kHz in cats (data from Palmer and Russell, 1986, Starr and Hellerstein, 1971). In humans the upper limit of the FFR is intermediate at approximately 1.5 kHz (Moushegian et al., 1973). At higher levels of the auditory system neural units have greater difficulty in preserving the one-to-one relationship with the stimulus and therefore a transformation of rate to place code has been suggested (Langner, 1992). At the cortical level, imaging studies suggest
that spectral and temporal codes appear to be separately processed at the cortex with left and right hemispheres being maximally sensitive to temporal and spectral encoding, respectively (Zattore and Belin, 2001). A recent MMN study also showed that right hemisphere differences were seen with frequency but not duration changes (Grimm et al., 2006).

This study examines auditory cortical responses to changes in frequency of an on-going tonal stimulus without changes in intensity. We hypothesized that cortical responses will differ as a function of the base stimulus frequency reflecting the participation of both spectral and temporal codes for low frequencies and only spectral codes for high frequencies.

**METHODS**

**Subjects**

Twenty-one (8 males) subjects (mean age: 21 years, all self-reported righthanded) with pure tone thresholds below 25 dB HL (500 to 6000 Hz) and no history of neurological deficits participated in the study after giving written informed consent.

**Stimuli**

Stimuli were continuous tones of 15 minutes duration containing brief upward frequency changes lasting approximately 100 ms occurring every 1.4 seconds. The base frequencies examined were 250 Hz and 4000 Hz and the magnitudes of frequency changes were 0%, 2%, 4%, 10%, 25%, and 50% for each base frequency. The order of the frequency changes in the stimulus sequence was randomly determined. The frequency change occurred for an integer number of cycles of the target frequency (lasting as close to 100 ms as possible). If the target frequency duration was set to exactly 100 ms, there would be non-integer cycles that would produce click-sounding transients. For example, the 250 Hz, 2% frequency change would have a base frequency of 250 Hz and a target frequency of 255 Hz (i.e. 2% above 250). In 100 ms, 255 Hz would have 25.5 cycles and thus produce a transient click. Therefore, in the case of 255 Hz, the number of cycles was rounded up to 26 cycles which lasted for 102 ms. Frequency changes occurred at 0 phase (zero crossing). For both 250 and 4000 Hz, the onset and offset of the frequency changes did not produce audible transients. However, spectral splatter at the transition of frequencies was present. We quantified the splatter as a function of the magnitude of frequency change using a 10 ms Gaussian window in the Praat phonetic software (www.praat.org). The splatter at 4000 Hz onset frequency change was 20 to 40 dB less than the splatter at 250 Hz.

These tonal stimuli were presented at 80 dB SPL using Etymotic-2A insert earphones. Long duration (30 s) tonal stimuli consisting of both base frequencies and corresponding percent changes were calibrated using a Brüel and Kjær (Investigator 2260) sound level meter set on linear frequency and slow time weighting with a 2 cc coupler. Given that insert earphones do not always have perfectly flat spectra, the stimuli were adjusted such that all the frequency changes were at 80 dB SPL. Although the continuous tones and frequency changes were presented at equal intensity (80 dB SPL) this does not necessarily mean that they are at equal loudness. Equal loudness contours describe the subjective loudness for a given frequency and intensity. We did not equate loudness because the contours are relatively flat at 80 dB SPL (Suziki and Takeshima, 2004). Moreover, the evoked potentials measured in this study saturate in amplitude and latency with increasing intensity (Näätänen and Picton, 1987). Therefore, although there is a potential confounding loudness effect we believe that in this study this contribution is minor.
Recordings

A 64-channel Neuroscan Synamps2 recording system was used to collect electrophysiological data. Electrode placements included the standard 10-20 placements and intermediate placements. Impedances were kept below 10 kΩ. Electro-oculograms were recorded using two bipolar electrodes above and below the right eye and two bipolar electrodes on left and right outer canthi. Signals were digitized at 1000 Hz, amplified and bandpass filtered (cutoffs at 0.05 and 200 Hz). Epochs were extracted using a -200 to 1900 ms window relative to the frequency increase. Offline analysis included re-referencing the recordings to an average reference (excluding the EOGs). Ocular artifact correction was performed in each subject using a singular value decomposition-based spatial filter based on principal component analysis of averaged eye blinks for each subject (Ille et al., 2002).

Averages for each of the frequency changes were based on 100 events for each frequency change.

Procedures

Subjects were seated in a comfortable reclining chair and watched a silent, closed-captioned movie of their choice. Stimuli were presented monaurally to either the left or right ear.

Psychoacoustic Measures

Psychoacoustic testing was carried out as a pilot experiment to determine if continuous-based stimuli had similar frequency detection thresholds as standard tone burst-based stimuli. Similar procedures were used as reported elsewhere (Zeng et al., 2005). Discrimination thresholds did not differ between continuous and tone burst stimuli. All subjects tested had frequency change thresholds below 1% for both 250 and 4000 Hz.

Waveform Analysis

After low-pass filtering (40 Hz) and baseline correcting (200 ms) peak analysis was carried out for all subjects in all conditions for the FCz electrode since it always had the largest N100. N100 peaks were defined as the most negative potential in the 70 to 150 ms post stimulus range. Peak measurements of P200 were not taken because not all subjects showed a clear peak. The slow negativity (SN) was evaluated by filtering (0.05 to 5 Hz) the averaged records and averaging the amplitude from 200 ms to 600 ms.

Dipole Source Analysis

Dipole source analysis was performed using Neuroscan’s Source software. Modeling was performed on the individual averaged waveforms (50 and 25% Δf) after band-pass filtering (1 to 30 Hz, 12 dB/octave). A symmetrical two dipole model was applied using a three-shell spherical head model. The source activity was allowed to vary in location, orientation and strength and was applied to a ±20 ms window around the N100 in global field power. A criterion of 90% goodness of fit (GOF) or better was used to determine if a fit was significant in each subject. A second grand average was performed using only those subjects who had GOFs above 90%. Based on these subjects (n=10 for 250 and 7 for 4000 Hz), separate averages were made for 50, 25, 10, and 4% frequency changes for 250 and 4000 Hz. Dipole source analysis was then repeated.

Dipole locations (NeuroScan Source native format) are given in (x,y,z) coordinates measured in millimeters where x extends left (negative) to right (positive) where 0 is in the middle. Y extends anterior (positive) to posterior (negative) where 0 is in the middle. Z extends superior (positive) to inferior (positive) where 0 is at the base of the bottom of the brain at the same plane as the bottom of the cerebellum. Figure 6 illustrates the coordinate system.
In order to improve signal to noise ratios, the 50 and 25 % frequency change data were collapsed across subjects for orientation and location comparisons.

Statistical Analysis

Repeated measures of analysis of variance (ANOVA) procedures were used to evaluate N100 amplitude and latency and SN amplitude differences associated with frequency change and to assess the effects of high- and low- base frequency. Post-hoc comparisons were conducted using the Tukey Honestly Significant Difference test.

Functions of frequency change versus amplitude and latency were assessed by means of Pearson correlations and slopes were evaluated using the form $y=mx+b$ in log-linear format. Differences between slopes were evaluated using an ANCOVA (Zar, 1999).

The detection of the slow negativity (SN) was quantified by calculating the standard deviation in each subject’s prestimulus baseline (200 ms). Next, the amplitude of the SN was quantified by taking the average amplitude from 300 to 600 ms. If the amplitude exceeded 2 prestimulus baseline standard deviations then the response was scored as significant. This was repeated for each subject and each frequency change.

Functions of frequency change versus amplitude and latency were assessed by means of Pearson correlations and slope functions were evaluated using the form $y=mx+b$ in log-linear format. Only subjects that had detectable N100’s and significant SN’s at 50, 25, 10, and 4 % Δf’s were used. Differences between slopes were evaluated using an ANCOVA (Zar, 1999).

Differences in dipole orientation were assessed using the Watson-Williams test (Zar, 1999, pp 625). This test is designed to evaluate differences between 2 or more groups of vectors and takes the form:

$$F=K \times (N - 2)(R_1 + R_2 - R) / (N - R_1 - R_2)$$

where $K$ is a correction factor related to the bias in the F calculation; $N$ is the combined sample size (groups 1 and 2); $R_1$ and $R_2$ are Rayleigh values for each group; and $R$ is a weighted average of the $R_1$ and $R_2$. Individual subjects (pooled across 50 and 25% Δf) were used in this statistic. Dipole orientations were compared between hemispheres (left versus right dipole in all 3 planes) and between 250 and 4000 Hz (each plane and hemisphere separately analyzed).

Differences in dipole location were assessed using a repeated measures ANOVA when comparing 50 and 25% differences for 250 and 4000 Hz and t-test was used to compare differences between 250 and 4000 Hz (pooled across 50 and 25% Δf).

RESULTS

Figure 1 presents the stimulus (top) consisting of a continuous tone that changes in frequency for 100 ms beginning at the vertical arrow and the associated grand averaged evoked responses at the FCz electrode (below) in response to a 50% frequency change from 250 Hz and from 4000 Hz. Labeled are N100, P200, and a late SN extending from 150 to 800 ms. The N100 and SN accompanying frequency changes of 250 and 4000 Hz have a similar appearance whereas P200 is a single component with 250 Hz but a double-peaked component, noted as “a” and “b”, with 4000 Hz. The proportion of subjects showing a detectable N100 and SN are shown in Table 1.
N100 amplitude and latency to frequency change from low (250 Hz) and high (4000Hz) tones

Increases in frequency of both high and low frequency tones were accompanied by N100 components whose amplitude and latency changed with the magnitude of frequency change (Figure 2, Table 2). A comparison of the regression slopes shown in Figure 3 between 250 and 4000 Hz shows that the extent of the N100 changes with magnitude of the frequency change for 250 Hz was significantly greater than those of 4000 Hz for both latency \([F(1,60)=38.7; P<0.001]\) and amplitude \([F(1,60)=5.0; P=0.029]\). Pearson correlations between frequency change and N100 latency were significant for both 250 Hz and 4000 Hz while N100 amplitude correlation with frequency change was only significant with 250 Hz base frequency.

An overall main effect of frequency change on N100 amplitude was observed with 250 Hz where smaller frequency change elicited a smaller N100 \([F(3,18)=15.8; P<0.001]\). No significant frequency change effects on amplitude were seen with 4000 Hz \([F(3,18)=2.0; P=0.134]\). In contrast to the amplitude data, both the 250 and 4000 Hz latencies showed significant effects of frequency change on N100 \([F(3,18)=32.6; P<0.001]\) and \([F(3,27)=3.1; P=0.046]\) respectively. The results of the post-hoc test are shown in Table 3.

In 7 subjects, both 250 and 4000 Hz frequency change recordings were obtained that were directly compared and showed significant interactions between base frequency and frequency change for amplitude \([F(2,12)=1.73; P=0.037]\) and latency \([F(2,12)=5.6; P=0.02]\). Post-hoc analysis revealed that the N100 to the 50% frequency change, was larger for 250 compared to 4000 Hz \((P=0.028)\). The N100 to the 10% frequency change was delayed for 250 versus 4000 Hz \((P=0.005)\).

Slow Negativity amplitude to frequency change for low (250Hz) and high (4000Hz) tones

Both the occurrence (Table 1) and amplitude (Table 4) of the slow negative potential were not affected by the extent of frequency change for low and high frequencies (Figures 2 and 4).

Dipole Source Analysis

Grand average dipole fits are shown in Figure 5. The left column shows the 250 Hz data and 4000 Hz is shown on the right. The mean 250 Hz data show an orderly posterior to anterior shift with increasing frequency change (i.e. z/y and y/z). No such organization was seen with 4000 Hz. Table 5 summarizes the results of the dipole source analysis.

Orientation—x/z (coronal) plane (Figure 5; top row): The 250 Hz data showed a non-significant trend \([F(1,34)=3.6; P=0.065]\) toward differences between left and right dipole orientations. On the other hand, 4000 Hz did not show any trend for orientation differences \([F(1,22)=0.02; P=0.901]\). y/z (sagittal) plane (Figure 5; second row): Differences between left and right dipole orientations are seen in the figure, the 250 Hz left dipole was always more vertically oriented than the right dipole \([F(1,34)=11.6; P=0.002]\). y/x (axial) plane (Figure 5; third row): The 250 Hz orientations were oriented more radially on the left side \([F(1,34)=10.8; P=0.002]\). No such hemispheric differences were seen with 4000 Hz.

Direct comparisons between 250 and 4000 Hz dipole orientations did not show significant differences. However, the 250 Hz left dipole did show a trend towards being more vertically oriented in the sagital plane \((y/z) [F(1,28)=3.9; P=0.058]\) and more radial in the axial \((y/x)\) plane \([F(1,28)=3.0; P=0.095]\) compared to its 4000 Hz counterpart.
**Location**—Significant location differences were seen between 250 and 4000 Hz in the medio-lateral direction (x; \( P < 0.001 \)) and superior-inferior direction (z; \( P = 0.004 \)). Figure 6 illustrates the location differences.

**Magnitude**—Right dipoles were always larger than the left ones for both 250 and 4000 Hz \([F(1,19) = 18.2; \ P = 0.024]\) and \([F(1,12) = 12.1; \ P < 0.001]\). In contrast to the N100 peak amplitude data, the dipole magnitudes did not differ between 250 and 4000 Hz.

Similar to the N100 amplitude and latency data, the dipole fits as a function of frequency showed significant differences between 250 and 4000 Hz. Like the N100 data, 4000 Hz dipole amplitude did not show a significant fit with log frequency change. Figure 7 shows the dipole magnitude and latency functions with frequency change demonstrating steeper slopes with the 250 Hz base frequency.

**Discussion**

The results of this study showed that cortical potentials to frequency change differ between low and high frequencies. These differences include N100 latency, amplitude and hemispheric asymmetries. Three particular processes will be discussed: (1) the temporal window of integration; (2) the responsiveness of neural populations and (3) the N100 dipole generators. We will discuss physiological mechanisms underlying these differences and their relation to psychoacoustic measures of frequency discrimination for high and low frequency tones.

**Temporal window of integration**

We found that the peak latency of the cortical N100 to frequency change can be up to 50 ms longer for low frequencies compared to high frequencies. This observation suggests that cortical processes to low frequency change require more time for processing compared to high frequencies. This may be related to the concept of a temporal window of integration. However, “temporal windows of integration” have different operational definitions for electrophysiologists and psychoacousticians. In the human evoked potential literature, temporal integration time refers to the minimal stimulus duration that produces maximal amplitude. Using click trains in an MEG study, Fross et al. (1993) have suggested that integration times are on the order of 20 to 25 ms. Early work by Onishi and Davis (1968) has shown that beyond 30 ms the N100 is essentially unchanged for a 1000 Hz tone burst. More recent work examining the frequency dependence of the temporal integration was examined by Alain et al. (1997). In that study it was shown that N100 amplitude increases with stimulus duration and was greater for low than high frequencies suggesting a frequency dependence on the temporal window of integration. In animal models, Geisler and Sinex (1982) examined cat auditory nerve fiber frequency resolution as a function of characteristic frequency and duration. Resolution was measured by examining the peak of the total number of discharges in the post stimulus histogram. Stimulus durations required 10 and 2 ms for equal resolution between low and high frequencies. These data suggest that at both central and auditory nerve stages, low frequencies require more time for accurate resolution compared to high frequencies.

Temporal windows of integration for tones in human psychoacoustic studies are quantified differently. The most common approach is to measure thresholds as a function of tone duration. Audibility improves when subjects are presented long versus short duration tones. The relationship between threshold and duration is an exponential function with a time constant that decreases with increasing frequency. For example, the time constant for low frequencies (125 to 250 Hz) range from 150 to 175 ms whereas high frequencies (3000 to 4000 Hz) range from 40 to 60 ms (Watson and Gengel, 1969). Moore (1973) examined frequency discrimination thresholds as a function of tonal duration ranging from 125 to 8000 Hz. A tenfold increase in threshold for a 6 ms duration tone versus 200 ms duration tone was observed for...
250 Hz whereas 4000 Hz showed only a twofold increase in threshold. Certainly temporal window of integration is a common sense concept for both electrophysiology and psychoacoustics but the relationship between their operationally defined measures still needs work. However, all of these studies support the idea that at low frequencies, more time is needed for frequency discrimination compared to high frequencies. It is consistent with the present observation of N100 latency differences for low and high frequencies.

The overall longer N100 latencies we observed for high versus low frequencies are in general agreement with other studies (Jacobson, 1992; Stufflebeam et al., 1998; Woods et al., 1993). Woods et al. (1993) examined frequency related (250 vs. 4000 Hz) latency differences by measuring the auditory brainstem response (ABR), the middle latency response (MLR) and cortical N100. Evoked responses to high frequencies were earlier than low frequencies. These differences became progressively larger at successive stages of processing (i.e., brainstem to cortex). The authors explained the ABR differences by longer traveling wave times along the basilar membrane for the low frequencies. However, the larger N100 latency differences between 250 and 4000 Hz were on the order of 20 ms and were harder to account for. Latency differences can, in part, be attributed to different thalamocortical neural conduction times to high frequency (deep source) and low frequency (superficial source) regions of the brain. One problem however, is that this explanation may not fully account for such a large latency difference because a 2 cm difference in location (high versus low) would only be compatible with a slow (1 m/s) conduction time.

More recent MEG data (Roberts and Poeppel, 1996; Stufflebeam et al., 1998, Crottaz-Herbette and Ragot, 2000) have described differences in N100m latencies as a function of frequency. Using tone bursts ranging from 100 to 5000 Hz they found that low frequency tones elicited N100m responses that were 35 ms later compared to high frequency tones. The authors interpreted these results by suggesting a different mode of frequency processing they termed “tonochrony” where latency of the evoked response reflects a code for pitch perception. In a subsequent review (Roberts et al., 2000) the authors suggest that the N100m latency for different frequencies represents a “fixed” 100 ms plus 3 periods of the stimulus tone (i.e., 30 ms for a 100 Hz and 3 ms for 1000 Hz). Their observations relate well to the psychophysical studies suggesting that several cycles of a stimulus are needed for pitch perception (Kay, 1982; Mark and Rattay, 1990; Doughty and Garner, 1947, 1948). We are cautious about using N100 latency as a code for pitch and frequency because N100 latency will vary with stimulus parameters (e.g., intensity) and subject states (e.g., attention). Phillips (1998) also failed to find a correlation between latency and stimulus frequency in single unit responses from cat cortex.

Longer N100 latencies with low frequencies may have nothing to do with the tonal spectral content but rather the perception of low frequency pitch. Krumholz et al. (2003) examined a novel evoked response they termed the pitch onset response (POR). The POR is elicited when using a stimulus that initially sounds like filtered noise. The temporal microstructure of the stimulus changes such that an interval is repeated and successively added. The pitch perception that is elicited relates to the duration of the repeated interval. For example, an 8 ms interval repeat elicits a pitch that is 125 Hz (1/8 ms). Additionally, the greater the number of interval iterations the more salient the pitch. An important feature of such a stimulus is that the energy and auditory nerve activity remains the same before and after the transition to pitch. Therefore the evoked response is not confounded by an energy onset-related N100 but rather the emergence of pitch. The POR was found to be larger with more interval iterations (strong versus weak pitch) and higher pitch (250 versus 16 Hz). The latency of the POR decreased with higher pitch and did not change with salience. The POR latency, like our N100 latency, shows that higher frequencies (and associated pitch perception) are associated with earlier N100s.
In summary, at initial, peripheral stages of processing, low frequency stimuli require longer stimulus durations to be processed compared to high frequencies. The magnitude of such differences progressively increases at more central levels of processing. The disproportionately longer times for cortical processing of low frequencies seen in this study may reflect the time required for transforming the subcortical temporal codes into central rate codes.

**Neural populations activated**

A larger N100 response to the low base frequencies suggests that a larger population of neurons is activated for their processing compared to higher frequencies. Our N100 amplitude versus frequency change shows a similar relationship to the latency data albeit with more variability. Our 4000 Hz N100 responses were smaller than the 250 Hz responses. This is in agreement with Jacobson et al. (1992) who found larger N100 responses for low versus high frequencies. They observed that the N100 evoked by a 4000 Hz tone burst was 40% smaller than to 250 Hz. They reasoned that the amplitude differences were in part related to 2 factors: (1) low frequencies cause a basal spread of activation along the basilar membrane and therefore a larger pool of neurons; (2) the generator of the high frequency response was located deeper below the surface of the cortex and therefore through volume conduction resulted in smaller amplitudes.

Auditory nerve recordings in the squirrel monkey to low frequency stimuli have shown that the activation of fibers can be defined over a wide range of frequencies reflecting the use of a temporal and not absolute rate code (Rose et al., 1967). For example, the authors describe a fiber whose best frequency was 1000 Hz. When stimulated with lower tonal frequencies, the fiber showed robust periodic discharges ranging from 400 to 900 Hz. These data suggest that temporal coding at the auditory nerve level will stimulate many low frequency coding neurons. This will result in more neurons becoming activated at low frequencies compared to high frequencies with the overall net effect of larger cortical responses. More recent data from the marmoset monkey using tones of varying frequency revealed a disproportionately larger number of units responding to low frequency than to high frequency stimuli in the caudal medial belt area compared to A1 (Kajikawa et al., 2005). This observation relates well to our data where large changes in N100 amplitude are seen with 250 Hz and minimal changes with 4000 Hz.

**Dipole generators**

We found hemispheric differences for the N100 during the processing of low and high frequencies. The right hemisphere sources were larger than the left for both high and low frequencies. This effect cannot be attributed to differences in ear of stimulation as seen with tone bursts (Pantev et al., 1988) since left and right ears were equally balanced across subjects. This finding relates well to other studies using PET (Zatorre and Belin, 1996) and MMNs (Grimm et al., 2007) suggesting a right hemisphere preference for spectral processing. Additionally, the 250 Hz data showed a hemispheric orientation difference whereas 4000 Hz did not. The orientation difference may be related to different cortical structures for spectral and temporal processing of high and low frequencies.

The dipole source analysis also revealed location differences between high and low frequencies where high frequencies were located more medially and superiorly. This relates well to other studies using MEG (Pantev et al., 1998) and fMRI (Wessinger et al., 1997) that suggested a tonotopic organization of auditory cortex. The dipoles based on the grand mean data (Figure 5) suggest that area, or the spatial spread of the dipoles, of cortex for frequency change is larger for low compared to high frequencies. This relates well to data in the marmoset monkey showing more units responding to low frequencies compared to high frequencies in the auditory caudal medial area (Kajikawa et al., 2005).
Although we did not find a significant difference between high and low frequency in dipole orientations in the axial plane \((p=0.056)\) the grand average data suggested that low frequencies are more radially oriented than high frequencies. This is in direct contrast to previous studies using tone bursts (Verkindt et al., 1995) where high frequency tones were oriented more radially compared to low frequencies. One possible reason for this discrepancy may be related to the different stimuli we used. Tone burst stimuli will evoke activity in primary auditory cortex and may be dominated by an onset response. Our stimuli on the other hand may involve other auditory cortical areas relating to frequency change. For example, the POR, which is free of an onset-related N100 has been shown to have a dipole source anterior and inferior to that of N100 (Krumbholz et al., 2003). Animal data suggest that complex stimuli will activate regions outside the core (Tian et al., 2001). We did not employ tone burst stimuli in this experiment and it is therefore difficult to relate the N100 arising from frequency changes to the N100 arising from tone bursts. The opposite dipole orientations in our study compared to Verkindt et al. (1995) suggest that the N100 generator for frequency change may be different to that of tone bursts. Different evoked responses can different tonotopic representations. For example, Pantev et al. (1996) found that both N100 and 40 Hz auditory steady-state responses show medial (high frequency) to lateral arrangement (low frequency). On the other hand the middle latency response had the opposite arrangement, namely lateral (high frequency) to medial (low frequency).

Relations to psychoacoustic auditory filters

The slope differences between high and low frequencies we observed are not due to auditory filter widths. The auditory filter at a particular frequency is often quantified using the equivalent rectangular band (ERB). It can be estimated using Moore’s formula: \[ \text{ERB} = 24.7(4.37F + 1) \] [Moore, 2003]; where ERB is the auditory filter width and \(F\) is the frequency in kHz. The base frequencies used in this study, 250 and 4000 Hz, have corresponding auditory filter widths of 52 and 456 Hz respectively. The 50% change for 250 Hz is 375 Hz which is outside the bandwidth for 250 Hz (2.4 ERBs away). Similarly for 4000 Hz, the 50% change is 4.4 ERBs away from the 4000 Hz auditory filter. In fact, our stimuli are biased towards larger slope differences for 4000 Hz Therefore the differences in slope (low versus high frequency) cannot be attributed to differences in frequency separations.

Waveform morphology differences

The most apparent waveform morphology difference between high and low frequency evoked responses was that the high frequencies elicited a double peaked P200 (labeled “a” and “b” in Figure 1). The double peaked P200 to 4000 Hz may derive from overlapping waveforms to the frequency increase (“onset”) and the subsequent frequency decrease (“offset”) 100 ms later. The N100 to the frequency decrease occurs at the same time as the original P200 to the frequency increase. Given the current paradigm it is difficult to disentangle these two waveforms. The different waveform morphologies of P200 to low and high frequencies strengthen the idea that the auditory processes involved in low and frequencies are different.

Slow negativity

The evoked potentials to frequency change that we recorded resembled a typical N100/P200 complex that is also recorded using transient tone bursts, with some important differences. The appearance of a late slow negativity (SN) is not seen in passive listening conditions with tone bursts. The generators of the SN are unresolved. We propose that its time of occurrence would be consistent with N200 and MMN. The N200 is a complex waveform that can have many overlapping components (Naatanen and Picton, 1986) and is related to stimulus discrimination (Simson et al., 1977) when the subject is engaged in a task. Because the SN did not systematically vary with magnitude of change we do not think it is likely a MMN. This SN...
may be related to a sustained DC shift as shown by Picton et al. (1978). This sustained potential is characterized by a negative shift persisting for the duration of the stimulus with a source that is located anterior to the N100. The SN we recorded far outlasts the 100 ms duration of the frequency change and initial examination of the source analysis of the SN revealed sources more anterior to N100 (data not shown). The SN may represent an activated neuronal state accompanying the continuous tone prior to the frequency change in addition to an offset response. The absence of a P200 may be related to the SN causing a baseline shift resulting in the P200 not reaching above baseline levels. A separation of the components may help to resolve these issues.

It is unfortunate that we could not reliably measure the dipole sources of the SN. A study by Gutschalk et al. (2002) used a long click train stimulus to elicit a sustained field. Dipole sources of the sustained fields suggested that pitch and loudness were separable. In that study, two types of click trains were used: regular interval clicks (giving rise to pitch) and irregular clicks (no discernible pitch). The authors found that dipole sources from a regular click train were more anterior to the sources of an irregular click train. Moreover, the anterior source magnitude did not vary with increases in intensity with irregular clicks but an increase in amplitude was seen with the regular click train. How a tonotopic gradient of the SN for pitch frequency differs from that of the N100 remains to be explored.

Spectral splatter

Spectral spread of acoustic energy occurs whenever there is an abrupt change in a sound. Our stimuli consisted of a pure tone that instantaneously changed in frequency and contained energy at other frequencies during the transition. A concern is that the splatter contributed to the different N100 amplitude/latency slopes between 250 and 4000 Hz. We do not think this was the case because the 4000 Hz N100 amplitude/latency slopes were relatively flat whereas its spectral splatter was of smaller amplitude than for the 250 Hz stimulus.

In summary these data provide evidence that high and low frequencies are processed differently by the auditory system and the differences are reflected by latency and amplitude changes at the level of cortex. Differences in place and temporal coding occurring at the subcortical levels are reflected at higher levels. The different slopes provide evidence of processing differences for spectral and temporal coding. This observation suggests that using a continuous tone paradigm allows the investigation of feature-specific processing strategies used by the cortex not previously possible using traditional tone burst stimuli. Additionally, using these types of low- and high frequency stimuli may give researchers and clinicians additional clues to temporal and spectral processing in abnormal auditory function such as auditory neuropathy and central processing disorders.

ACKNOWLEDGEMENTS

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REFERENCES


Figure 1.
Relationship between stimulus and evoked potentials. The stimulus was a continuous pure tone with periodic changes in frequency lasting 100 ms (top). A 50% frequency change is shown by the arrow. Below are grand averaged potentials to the frequency change for base frequencies of 250 and 4000 Hz stimuli. The potentials include an N100, P200 and slow negativity. Note the differing waveform between 250 and 4000 Hz.
Figure 2.
Evoked potentials to all the frequency changes from base frequencies of 250 and 4000 Hz. The shaded regions illustrate the extent of the slow negativity.
Figure 3.
Mean and standard errors of N100 amplitude and latency as functions of frequency change magnitude. The frequency change functions are steeper with 250 compared to 4000 Hz.
Figure 4.
Mean and standard errors of Slow Negativity amplitude as a function of frequency change magnitude. No significant effect of change magnitude was seen on SN amplitude.
Figure 5.
N100 equivalent dipoles and frequency changes. 250 and 4000 Hz responses are shown on the left and right columns respectively. The dipole sources and orientations are shown on a standard MRI image. Note that 250 Hz dipoles are oriented more vertically for the left hemisphere (first column, second row) compared to the right. Also note that the area covered by the cluster of 250 Hz dipoles (spatial variability) is greater than 4000 Hz (second and third rows).
Figure 6.
Mean and standard errors of N100 dipole locations for 250 and 4000 Hz. The three orthogonal planes are shown in the 3 rows. Data are given on the left column. The box overlaying the brain shows the location and extent of the axis of the data on the left. Significant differences are marked by asterisks. The 250 and 4000 Hz data are shown in black and white circles respectively.
Figure 7.
Mean and standard errors of N100 dipole amplitude and latency as functions of frequency change magnitude. The left and right columns show the dipole functions for 250 and 4000 Hz, respectively. Note that the right dipole is always larger than the left dipole.
Table 1
Proportion of subjects (out 15 showing an N100 or SN)

<table>
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<tr>
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<th>Δf(%)</th>
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<tr>
<td>4000 Hz</td>
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<tr>
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<tr>
<td>4000 Hz</td>
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Δf(%) = Proportion of subjects (out 15 showing an N100 or SN)
### Table 2

Mean amplitude and latencies.

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<tr>
<th>Δf (%)</th>
<th>250 Hz N100 amplitude (μV±SD)</th>
<th>4000 Hz N100 amplitude (μV±SD)</th>
<th>250 Hz N100 latency (ms±SD)</th>
<th>4000 Hz N100 latency (ms±SD)</th>
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<td>Amplitude significance</td>
<td>Latency significance</td>
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* P<.05
** P<.01
*** P<.001
### Table 4

SN amplitudes

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<td>Strength (nAm)</td>
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