Topographic Analysis of Epidural Pure-Tone–Evoked Potentials in Gerbil Auditory Cortex

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Ohl, Frank W., H. Scheich, and Walter J. Freeman. Topographic analysis of epidural pure-tone–evoked potentials in gerbil auditory cortex. J. Neurophysiol. 83: 3123–3132, 2000. This study investigated the tonotopic organization of pure-tone–evoked middle latency auditory evoked potentials (MAEPs) recorded at the auditory cortical surface in unanesthetized gerbils. Multielectrode array recording and multiple linear regression analysis of the MAEP demonstrated different degrees of tonotopic organization of early and late MAEP components. The early MAEP components P1 and N1 showed focal topography and clear dependence in location and size of cortical area covered on pure-tone frequency. The later components P2 and N2 showed a widespread topography which was largely unaffected in location and size of cortical area covered by pure-tone frequency. These results allow delimitation of the neural generators of the early and late MAEP components in terms of the spectral properties of functionally defined neural populations.

INTRODUCTION

Auditory events (e.g., acoustic clicks, pure-tones, or more complex and natural sounds) evoke characteristic temporal patterns of electrical potential recordable from various locations both within the brain and from its surface or from the scalp. Usually a series of short poststimulus latency (<10 ms), small amplitude (up to a few tens of μV) positive and negative deflections of electrical potential called the brain stem auditory evoked potential (BAEP), is distinguished from a series of longer latency (>10 ms), high-amplitude (tens to hundreds of μV) deflections, called the middle latency auditory evoked potential (MAEP). Whereas the BAEP is extensively studied and thought to reflect in its series of potential deflections a sequence of neuronal activations in separate nuclei of the auditory brain stem, the neurogenesis of the MAEP is only partly understood, despite the clinical relevance of this signal (Ichiki et al. 1998; Kraus and McGee 1992) and its possible relevance for our understanding of central auditory processing of sound (Bullock 1981; Kraus and McGee 1995).

Investigation of the MAEP has been impeded mostly by the circumstance that these potentials are unlikely to reflect a serial activation of separate auditory nuclei but may confound a number of different neuronal processes in separate but partially overlapping neuronal populations. As most approaches aimed at unraveling the different contributions of presumed generator populations evoked MAEPs by auditory clicks, which exhibit a spectrally broad energy distribution, a most salient feature of auditory neurons, viz., their tuning to characteristic confined frequency intervals of the auditory spectrum, has remained largely unexploited.

Within the last decade, understanding the neurogenesis of the MAEP has been advanced by spatial analysis of the MAEP (Barth and Di 1990, 1991; Kraus et al. 1985, 1988; Simpson and Knight 1993a,b; Steinschneider et al. 1992). This understanding was made possible by the combination of high-resolution recording using multielectrode arrays, depth recording through cortical laminae with microelectrodes, and multivariate analysis of the linear models of the MAEP and its component peaks (Barth and Di 1990, 1991; Di and Barth 1992, 1993). From these studies it was concluded that the peaks of the click-evoked MAEP recorded from the surface of the rat auditory cortex are generated by distinguishable but partially overlapping neuronal populations which are asynchronously activated by acoustic input. Specifically, the earlier MAEP components (called P1 and N1 to reflect their polarity and sequence of occurrence in the MAEP complex) were thought to be primarily caused by extracellular currents associated with thalamocortical activation of supragranular and, respectively, supra- and infragranular pyramidal cells in primary auditory cortex. The later components (P2 and N2) however, were presumed to involve contributions from corticocortical activity (Barth and Di 1991) but were shown to mainly reflect input from a second, more diffuse, thalamocortical projection system, probably originating in the medial part of the medial geniculate body (Barth and Di 1993).

As shortest latency thalamocortical input is mediated by a projection from the ventral part of the medial geniculate body to the primary auditory cortex, the above interpretation allows formulation of two hypotheses amenable to experimental testing. The first hypothesis is that direct electrical stimulation of the ventral part of the medial geniculate body should evoke epicortical potentials similar in field-specific localization, topography, and timing to the P1 and N1 components evoked by acoustic stimulation. This hypothesis was experimentally verified by Di and Barth (1992) in the rat. As the projection from the ventral medial geniculate to the primary auditory cortex is tonotopically organized in the rat (Brett et al. 1994; Winer et al. 1999), as well as in other species (Winer 1992), a second hypothesis can be formulated on the topography of epicortical MAEPs: if the early, but not the later, components of the epicortical MAEP predominately reflect thalamocortical input from the ventral medial geniculate, then given a suitable spectrally defined acoustic input, the functional architecture of this projection into the primary auditory cortex should be visible in the topography of early MAEP components and should be less obvious or absent in later MAEP components.
The main objective of this study is to test this hypothesis by studying the surface topography of separate components of the MAEP evoked by pure-tones. Pure-tones, in contrast to acoustic clicks, activate the thalamocortical input into the auditory cortex in a frequency-dependent and systematic (i.e., tonotopic) manner. The frequency dependence of the surface topography of the pure-tone-evoked MAEP should allow evaluation of the above hypothesis. We have chosen the lissencephalic rodent species Mongolian gerbil (Meriones unguiculatus) as the experimental animal because its auditory cortex has been extensively investigated (Ryan et al. 1982; Scheich 1991; Scheich et al. 1993b, 1997) and the tonotopy of its primary field AI and of the anterior auditory field AAF are thoroughly described (Hess and Scheich 1996; Scheich et al. 1993a; Sugimoto et al. 1997; Thomas et al. 1993).

METHODS

Multielectrode arrays

For high resolution multichannel recordings two types of multielectrode arrays were configured from epoxy-insulated stainless steel microwires (100 μm diam) (Barrie et al. 1996). A rectangular 3 × 6 electrode array (interelectrode distance: 600 μm) and a linear 16 electrode array (interelectrode distance: 300 μm) were designed after optimizing interelectrode distances by spatial spectral analysis of recorded potentials (Freeman and Baird 1987) (Fig. 1A). Just before implantation (see Surgical preparation) the surface of the arrays were filed to concave shape matching the convexity of the cortical surface.

Surgical preparation

Six adult (aged 6 mo) male Mongolian gerbils weighing 85–100 g were chronically implanted with the multielectrode arrays (4 animals with rectangular arrays and 2 animals with linear arrays) under deep ketamine anesthesia (xylazine, 2 mg/100 g body wt ip; ketamine, 20 mg/100 g body wt ip). An array was positioned onto the dura exposed by craniotomy over the region of primary auditory cortical field AI (rectangular arrays) or covering both cortical core fields AI and AAF (linear arrays; see Fig. 1A). The positioning of the arrays was guided by visual inspection of the vascularization pattern (Ohl and Scheich 1997a,b; Sugimoto et al. 1997) and established by stereotactic coordinates relative to stereotactic landmarks (Ohl and Scheich 1996; Thomas et al. 1993). Both types of arrays were positioned with their length axes rostrally inclined into the ventral direction ~10° from the horizontal plane (Fig. 1A) to achieve orientation parallel to the tonotopic gradient in AI (Thomas et al. 1993) and fixed with dental acrylic. After the operation, animals were allowed to recover for 3 days before recording sessions began.

Acoustic stimulation

Pure-tone bursts (duration: 250 ms, including 5 ms linearly ramped rise/fall times) of frequencies varying from 0.25 to 8.0 kHz in 5 octave steps were free-field delivered over a calibrated loudspeaker at a rate of 0.2 Hz. This frequency range was selected because of its high spatial resolution in the tonotopic maps of fields AI and AAF in the gerbil (Scheich et al. 1993a; Thomas et al. 1993) to facilitate detection of the hypothesized tonotopic organization of MAEP components. Calibration was to equal a sound pressure level of 70 dB as measured by a condenser microphone at a distance of 22 cm from the speaker.

Recording

Single presentation MAEPs were recorded (ISO 4/8 differential amplifiers, World Precision Instruments; amplification: 10K; bandpass filtering between 3dB cutoff frequencies of 0.1 and 100 Hz) from all electrodes of the array monopolarly against the reference electrode. Data from 2 s prestimulus and 2 s poststimulus were sampled at 1 kHz sampling frequency and stored to disk for offline analysis. All data were obtained from fully awake unanesthetized animals that were sitting in a small compartment (18 × 16 × 22 cm) during acoustic stimulation through the calibrated loudspeaker mounted 22 cm over the compartment floor.

Postmortem examination

After the experiments, animals were killed by pentobarbital sodium overdose (12 mg/100 g body wt). The recording array was surgically removed and the underlying tissue was examined for any signs of damage, necrosis or regrowth of connective tissue.

FIG. 1. A: lateral view of brain of a Mongolian gerbil (Meriones unguiculatus). Typical positioning of a rectangular 3 × 6 electrode array (●) and of a linear 16 electrode array (○) is indicated in relation to the rostrocaudal position of the anatomic landmark λ and the characteristic vascularization pattern formed by the ascending branches of the inferior cerebral vein (icv) and the descending branches of the middle cerebral artery (mca). The border between primary cortical field AI and anterior auditory field AAF is typically located at a characteristic ascending branch (br) of the icv and indicated by a dotted line. Note that the rectangular arrays (interelectrode distance 600 μm) were centrally positioned over cortical field AI whereas the linear arrays (interelectrode distance 300 μm) covered both the largest part of the rostrocaudal extent of AI and anterior auditory field AAF. The length axes of both array types were rotated ~10° from the horizontal plane to be parallel to the tonotopic gradient in AI grossly perpendicular to the AI/AAF border line (●●●). Other abbreviations: c; caudal, d; dorsal, r; rostral, and v; ventral. B: typical form of an averaged (n = 100) auditory evoked potential as recorded from the auditory cortical surface of an alert animal showing positive and negative deflections, P1, N1, P2, N2, P3, and N3 at their characteristic latencies.
Data analysis

For each pure-tone stimulus of given frequency single presentation MAEPs were averaged (n = 100). The average MAEP was DC corrected for interelectrode variability by subtracting the mean of the 2 s prestimulus average for each electrode channel. Analysis of spatiotemporal patterns of the MAEP and their linear modeling from spatial patterns obtained at the latencies of the four identified peaks P1, N1, P2, and N2 was performed by multivariate linear regression analysis (Dillon and Goldstein 1984) as introduced for this purpose by Barth and Di (1990). The multivariate linear regression yielded for each spatial pattern associated with one of the four peaks a standard partial regression coefficient (often referred to as “beta weights” or “regression weights”) as a function of time. These coefficients are a measure of the contribution of the spatial pattern of epicortical potential at the latency of a given peak to the total spatial contribution under the assumption of a linear model.

On the basis of the high-resolution data obtained with the linear arrays in two animals, tonotopic gradients for MAEP components could be determined by log-linear regression of the pure-tone frequency shifts to spatial coordinates of the local component maxima: a model of pure-tone octave shifts as a linear function of spatial coordinate was fitted to the data by minimizing the residual sum of squares between data and model. The fit was quantified by calculating the coefficient of determination R² and its goodness was evaluated statistically by comparing with a fit of a simpler model involving only a constant term. Analysis of variance (ANOVA), F ratios, and attainable significance levels are presented in Table 1. Note, that the determined P value corresponds to the significance level of t-testing the hypothesis of a tonotopic gradient unequal to zero. Only when this test yielded significant results was a tonotopic gradient determined.

All procedures were conducted according to protocol approved by the University of California at Berkeley Animal Care and Use committee with veterinary supervision by the Office of Laboratory Animal Care.

RESULTS

Figure 1A shows a lateral view of the brain of a Mongolian gerbil with the positioning of both a rectangular 3 × 6 epidural electrode array (interelectrode distances: 600 μm) and a linear 16 electrode array (interelectrode distances: 300 μm) schematically indicated over the right temporal cortex. The rectangular arrays covered an area of 3.0 × 1.2 mm² of cortical surface and were used to record two-dimensional potential distributions over the primary cortical field AI in four animals; the linear arrays covered a length of 4.5 mm and were positioned to record a one-dimensional spatial potential profile over a cortical region spanning both the field AI and the anterior auditory cortical field AAF in two other animals. Both fields show a clear topographic organization with tonotopic gradients from low- to high-frequency running from caudal to rostral in field AI and from rostral to caudal in field AAF so that both fields share a common border of high-frequency representation (Scheich et al. 1993a; Thomas et al. 1993). Figure 1B shows a typical recording of the MAEP from the epidural surface over field AI. The basic wave forms of the MAEP evoked by pure-tones of frequencies from 0.5 to 8.0 kHz corresponded to those of click-evoked responses recorded from other species. They invariably consisted of two subsequent positive/negative deflections, termed P1/N1 and P2/N2, and often a third positive/negative complex P3/N3 (see example in Fig. 1B). The regularly occurring components P1, N1, P2, and N2 peaked at characteristic latencies of 16.4 ± 3.1 (SD), 29.7 ± 2.9, 58.0 ± 5.1, and 115 ± 8.9 ms, respectively.

<table>
<thead>
<tr>
<th>Cortical Field</th>
<th>MAEP Component</th>
<th>Tonotopic Gradient (oct/mm)</th>
<th>R²</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>P1</td>
<td>3.3 ± 0.5</td>
<td>0.91</td>
<td>42.67</td>
<td>0.0028</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>3.3 ± 0.4</td>
<td>0.95</td>
<td>80.65</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>N1</td>
<td>3.3 ± 0.5</td>
<td>0.92</td>
<td>48.76</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>2.5 ± 0.3</td>
<td>0.94</td>
<td>66.00</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>2.5 ± 0.0</td>
<td>0.00</td>
<td>0.02</td>
<td>0.9043</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>0.7 ± 0.3</td>
<td>0.39</td>
<td>2.51</td>
<td>0.1882</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>0.7 ± 0.7</td>
<td>0.16</td>
<td>0.76</td>
<td>0.4327</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>0.0 ± 0.0</td>
<td>0.00</td>
<td>0.01</td>
<td>0.9236</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>0.0 ± 0.8</td>
<td>0.00</td>
<td>0.00</td>
<td>0.9587</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>0.0 ± 0.0</td>
<td>0.00</td>
<td>0.01</td>
<td>0.9198</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>0.0 ± 0.1</td>
<td>0.00</td>
<td>0.01</td>
<td>0.9265</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>0.0 ± 0.0</td>
<td>0.00</td>
<td>0.01</td>
<td>0.9265</td>
</tr>
</tbody>
</table>

Octave shifts in pure-tone frequency were regressed onto spatial coordinates of local potential profile maxima of middle latency auditory evoked potential (MAEP) components P1, N1, P2, N2, P3, and N3 in cortical fields AI and AAF by least squares fitting. For each combination of cortical field and middle latency auditory evoked potential (MAEP) component the coefficient of determination R², as well as F ratio and attainable significance level P of the fit statistics are given for gerbil 5 (top entries in each box) and gerbil 6 (bottom entries). If the model of a linear relationship between octave shift and spatial position reached significance (P ≤ 0.05) the regressed tonotopic gradient was specified ± SD.

The description of the spatial distribution of potential amplitudes of the four identified peaks was based on a multiple regression analysis of the MAEP as introduced for the analysis of the spatiotemporal organization of click-evoked potentials by Barth and Di (1990). MAEP components P3 and N3 were not included in this analysis because they were not reliably recordable as their occurrence seemed to be dependent on the behavioral state of the animal. Figure 2A shows typical isopotential distributions of a 2 kHz pure-tone–evoked MAEP of one animal (gerbil 1) at latencies corresponding to the four identified MAEP peaks. Components P1 and N1 showed similarly focal potential distributions with nearly identical location of the potential maxima in the rostral half of the recording area corresponding to central AI and similarly steep potential gradients. Components P2 and N2 had more widespread potential distributions and shallower potential gradients in comparison to P1 and N1. The potential maxima were located caudally with respect to the P1 and N1 foci near the border between caudal AI and rostral parts of the caudal auditory fields dorso-posterior field (DP) and ventro-posterior field (VP) (see Scheich et al. 1993a; Thomas et al. 1993).

The same general pattern was found for the components of MAEPs evoked by pure-tones of other frequencies from 0.25 to 8.0 kHz. Figure 2B shows standard partial regression coefficients of the MAEP regressed onto the spatial patterns of
potential distributions recorded at the four latencies corresponding to the MAEP peaks P1, N1, P2, and N2 as a function of time. The four regression weights are a measure of the contribution of a spatial pattern of potential distribution at these four specified latencies to the overall MAEP. The amplitudes were predictably maximal at the latencies of the respective peaks. However, the regression weights of all peaks showed substantial contribution to the overall MAEP at other latencies as well. This was especially apparent for the N1 regression weights which contributed to the overall MAEP at latencies corresponding to peak P1 as evident in a clearly discernible downward peak at the P1 latency. The P2 component showed a contribution to the overall MAEP at the N1 latency; similarly, but to a lesser degree, did component N2. Figure 2C shows spatiotemporal maps of potential reconstructed by multiplication of the isopotential distribution with the regression weight time series. The sum of these maps is a linear prediction of the spatiotemporal map of the overall MAEP and explained 98% of the variance across measurements. In the other three animals implanted with rectangular arrays multiple regression analysis yielded variance explanation of 94, 97, and 97%, respectively. The potentials showed a sharp fall-off in amplitude at the caudal border (Fig. 2C, left) of the array corresponding to locations near the caudal border of the primary auditory cortex (see Scheich et al. 1993a; Thomas et al. 1993).

The other three animals implanted with rectangular electrode arrays showed very similar patterns of isopotential maps, regression weights, and reconstructed spatiotemporal maps. Figure 3 depicts the 50% isopotential contours of the 2-kHz pure-tone–evoked MAEP at the latencies of the four identified peaks for all four animals plotted superimposed on each other (Fig. 3A) and the corresponding partial regression coefficients (Fig. 3B). Despite considerable variance across individuals the following features were invariant. The isopotential distributions of the early components P1 and N1 were similar in location and shape to each other and much more focal than those of the later components P2 and N2. The locations of the earlier peaks were more consistent across animals than those of the later peaks. The isopotential distributions of P2 and N2 tended to overlap those of the earlier peaks whereas their foci were shifted toward the caudal boundary of primary field AI; this was especially manifest for peak N2. The variance in the regression weights across individuals was higher for the later peaks. For all four peaks, regression weights were maximal at their corresponding latencies but also indicated contribution of their spatial potential patterns to the overall MAEP at other latencies: component N1 contributed to the overall MAEP at latencies of P1 and component P2 contributed to the MAEP at N1 latencies. In those cases where the component N3 was measurable, a contribution of the N2 component to the MAEP at N3 latencies was found (Fig. 3B).

Figure 4 shows the dependence of the location of the pure-tone–evoked MAEP on pure-tone frequency (color-coded).
separately for the four components P1, N1, P2, and N3. The spatial location of the P1 and N1 components showed a clear tonotopic shift from caudal to rostral across field AI with increasing pure-tone frequency from 250 Hz to 8 kHz in 5 octave steps (Fig. 4, top two rows). The 50% isopotential contours of components P1 and N1 of the MAEP evoked by a tone of a given frequency were similar in size, shape and localization of covered surface area within an individual. The change in size of the 50% isopotential contour with varying frequency presented at identical absolute sound pressure levels in a given individual was similar in the other individuals. Sizes were maximal for the frequencies between 1 and 4 kHz. The later components P2 and N2 did not show a clear tonotopic organization. P2 and N2 component isopotentials in a given individual showed lesser similarity compared with P1 and N1, especially with respect to localization (see gerbils 2 and 3). The fact of more widespread potential distributions in later MAEP components compared with earlier components was evident for middle range and high pure-tone frequencies but less evident for the 0.25 kHz stimuli. Across individuals a greater variation of isopotential contours for the later components was also detectable in comparison to the early components, especially with respect to size and shape.

To obtain a more quantitative determination of the tonotopic shift of MAEP component localization, two additional gerbils were implanted with linear electrode arrays of doubled spatial resolution (inter electrode distance: 300 μm) oriented along the tonotopic gradients in fields AI and AAF. Both fields have a clear topographic organization with tonotopic gradients running approximately in the rostrocaudal direction and isofrequency dorsoventrally oriented isofrequency contours running approximately parallel to their common border (see Fig. 1A) where high frequencies are represented in both fields. Figure 5 shows the spatial profiles of MAEP component amplitudes in one animal across a linear 16-electrode array spanning both cortical fields AI and AAF. In each panel, numbers at the nearly horizontal abscissae index the electrodes from caudal positions over AI to rostral positions over AAF; the other

![Fig. 3](image-url)

**FIG. 3.** Isopotential maps (A) and regression weights (B) obtained from multivariate regression analysis of the 2 kHz pure-tone evoked MAEP in all 4 animals implanted with rectangular arrays. A: for each of the 4 MAEP peaks P1, N1, P2, N2, the 50% isopotential contours of all 4 animals are plotted together. B: regression weight time series (200 ms) for each of the 4 animals plotted together. Bold curves represent the means of all 4 regression weight time series. Symbols as in Fig. 2.

![Fig. 4](image-url)

**FIG. 4.** Topography of pure-tone-evoked MAEPs for all 4 animals (columns) implanted with a rectangular array and for all latencies of the 4 identified peaks P1, N1, P2 and N3 (rows). In each panel the 50% isopotential contours of the MAEP evoked by pure-tones of variable frequency (color code) are plotted together. Other symbols as in Figs. 2 and 3.
abscissae specify the frequency of the pure-tones (0.25–8.0 kHz in 5 octave steps) used to evoke the MAEPs. The vertical ordinates specify the component amplitudes. For better comparison all amplitudes were plotted upward as absolute values. Across MAEP components amplitudes were of comparable absolute value (0.27 ± 0.18 mV) with higher values (>0.4 mV) found for the P1 and N1 component evoked by higher range frequencies, 2.0, 4.0, and 8.0 kHz. Components P1 and N1 showed more peaked spatial profiles of potential amplitude than components P2, N2, P3, and N3 did. The amplitudes were minimal at the edges of the recording line and in the center around electrode index 9 corresponding to the border between AI and AAF. The local maxima of the spatial profiles over fields AI (electrode indices 1–9) and AAF (electrode indices 10–15) are marked by a black top of the corresponding column for each pure tone frequency. Note the sharp amplitude decay for MAEP components P1 and N1 between electrode indices 9 and 10 corresponding to localization of AI/AAF boundary (cf. Fig. 1A).
profiles of components P3 and N3 were basically flat and remained unchanged with pure-tone frequency.

The other animal implanted with the linear 16-electrode array showed very similar results. We used the data from both animals to determine the tonotopic gradients of the MAEP components P1 and N1 by log-linear regression of the frequency shifts produced by varying pure-tone frequency onto the spatial rostrocaudal coordinates of the local maxima in the MAEP profiles. Figure 6 shows the spatial coordinates of the local profile maxima of components P1 (top) and N1 (bottom) measured in the two animals plotted against the octave shift from 0 octaves (corresponding to 250 Hz) to 5 octaves (8.0 kHz). The regression lines are also depicted for both animals and both cortical fields AI and AAF. Table 1 gives the numerical results of the regression. Only MAEP components P1 and N1 showed a clear tonotopic organization in fields AI and AAF (Fig. 6) which can be fitted to the model of a linear relationship between octave shift and spatial position (Table 1). The steeper tonotopic gradients in field AAF compared with field AI are a result of the smaller spatial extent of AAF (see Fig. 5 and Scheich et al. 1993a; Thomas et al. 1993). MAEP components P2 and N2 show a focal organization over field AI and over field AAF, but no systematic relationship of octave shift with spatial position (Table 1). MAEP components P3 and N2 showed smaller amplitude variation over the recording array for a given pure-tone frequency than did P1, N1, P2, and N2 (Fig. 5) and no systematic relationship of the local amplitude maximum to the octave shift (Table 1).

DISCUSSION

The purpose of this study was to test the hypothesis that successive components of the MAEPs as recorded epidurally over the auditory cortex mainly result from activity in differing but partially overlapping neuronal populations in auditory cortex. Specifically our objective was to exploit the different degree of tonotopic organization in separate thalamocortical projection systems to delineate their different contributions to the MAEP. Based on earlier work on click-evoked MAEPs from the rat auditory cortex (Barth and Di 1990, 1991; Di and Barth 1992), we hypothesized that the early but not the later components of the MAEP should exhibit tonotopic organization of their surface topography. Thus we evoked the middle latency responses by pure-tones rather than clicks and studied their surface topography with multiple regression analysis. The discussion first addresses methodological issues relevant for the interpretation of our present experimental approach and then places the present results into the functional context from earlier work.

Methodological considerations

We evoked MAEPs in unanaesthetized animals by pure-tones from a loudspeaker in the free field. A general problem with free-field stimulation is a reduced control of stimulus levels compared with closed-field stimulation (speakers inserted into the meatus). However, such a preparation is incompatible with recording from fully awake unsedated animals which was mandatory for this study because of known differential drug effects on the tonotopic and nontonotopic thalamocortical projection systems (Herz et al. 1967). A further problem with acoustic stimulation of the freely moving animals is the uncontrollable variance in binaural input which affects precise timing of MAEP components (Di and Barth 1993) thereby possibly disguising shoulder structures in MAEP peaks (Simpson and Knight 1993a). However, given the random orientations of the constantly moving animal relative to the loudspeaker during the recording periods and the averaging over large number of repetitions (n = 100) these differences cannot have systematically biased our results, although they excluded the possibility to further investigate possible subcomponents of P1 in the gerbil. In summary, the free field stimulation imposed no constraints on our data analysis as the latter was based not on single condition observations but on comparisons between experiments (different pure-tone stimuli) the intertrial variance each of which was similarly affected by the chosen experimental situation.

This study focused on the anatomic gerbil auditory cortical field AI and the rostrally adjacent anterior auditory field AAF. Both fields can be considered “core cortex” and “primary” (Patterson 1977) on the basis of their shared cyto- and myeloarchitecture, characterized by increased cellular density within layer IV and characteristic high-density of myelinated fibers in granular and infragranular layers (Budinger and Scheich 2000a), the dominating input from the ventral part of the medial geniculate nucleus (Budinger and Scheich 2000b), their precise tonotopic organization (Scheich et al. 1993a; Thomas et al. 1993), and their indistinguishable electrophysiological characteristics of single units to pure-tone stimuli (Schulze et al. 1997; Thomas et al. 1993) and linear frequency-modulated tones (Schulze et al. 1997). Our method of localizing auditory
cortical fields AI and AAF has proved to be useful in previous studies (Ohl and Scheich 1997; Ohl et al. 1999) and could be posthoc verified by these results.

**Pure-tone–evoked versus click-evoked MAEPs**

We found pure-tone–evoked MAEPs to share the basic wave form and overall topography with click-evoked MAEPs characterized by steep fall-off in amplitude beyond the region of auditory cortex. We have focused on the first four major deflections recorded over the region of the primary auditory cortex, termed P1, N1, P2, and N2, adopting the nomenclature from Shaw (1988) to facilitate comparison with these studies and with those of Barth and coworkers (Barth and Di 1990, 1991; Di and Barth 1992). Simpson and Knight (1993a) have described multiple subcomponents of P1 in click-evoked MAEPs occurring at latencies earlier and later than the main component. In this study it was not our aim to resolve any subcomponents of the classical ones. Kraus and coworkers had earlier described a similar MAEP recorded from the auditory cortical surface of the guinea pig (Kraus and McGee 1995; Kraus et al. 1985, 1987, 1988). The comparable latencies and corresponding polarities suggest that their components A, B, and C correspond to P1, N1, and P2, respectively. Later components like P3 and N3 were not regularly observed in this study and seemed to be dependent on nonstimulus-related parameters like the vigilance and state of attention of the experimental animals. Whereas these later components tend to be reduced or absent in anesthetized animals (Borbély and Hall 1970; Kiang et al. 1961; Pradhan and Galambos 1963; Schwender et al. 1996; Simpson and Knight 1993b), their dependence on behavioral state has been described already in the early studies of both awake animals with chronic electrodes (e.g., Teas and Kiang 1964) and humans (Picton and Hillyard 1974).

Our results on the general spatial localization of the four major components of the pure-tone evoked MAEP are in basic agreement with comparable studies on click-evoked potentials in the rat auditory cortex (Barth and Di 1990, 1991; Brett et al. 1996; Di and Barth 1992; Sukov and Barth 1998). All studies reported a more focal and spatially confined localization of the components P1 and N1 compared with P2 and N2. Given that AAF must be considered a primary field in the gerbil the present data support the general notion of confinement of these peaks over primary auditory cortex. Similar, but in their latencies distinguishable MAEP components were measured over the surface of the secondary auditory cortex in the rat (Barth and Di 1991; Di and Barth 1992). A difference in the results using click-evoked potentials in rat auditory cortex is the very similar size of areas encircled by corresponding isopotential contours of the P1 and N1 components. In the rat, N1 consistently covered an area approximately twice as large as P1. As the current source/sink pattern giving rise to the surface N1 component was hypothesized (Barth and Di 1990) to be mediated by ascending collateral fibers from the supragranular cells (Jones 1984, 1985) this difference could be a result of slightly different collateral circuitry patterns being less divergent in the gerbil compared with the rat. The closer similarity of the N1 spatial activity pattern to the P1 pattern in the gerbil compared with the rat is also evident in the very marked peak of the N1 regression weight at the P1 latency in the gerbil (Figs. 2 and 3) not regularly reported in the rat (e.g., Barth and Di 1990).

**Tonotopic organization of pure-tone–evoked MAEPs**

Few studies so far have addressed the question of MAEP tonotopy (e.g., Kraus and McGee 1988; Scheel 1988; Woods et al. 1995). In a recent study on MAEPs recorded from the human scalp no tonotopic effects on MAEP topography were found after probing with loudness-matched 250 Hz and 4 kHz pure-tones mixed with continuous broadband masking noise (Woods et al. 1995). From these results the authors were careful not to conclude that there was a lack of tonotopic organization in the MAEP generators but also considered the possibility of an unfavorable orientation of neural generators impeding detection of tonotopic organization (Bertrand et al. 1991).

The input into the primary auditory cortex (fields AI and AAF in the gerbil) is predominantly from the ventral division of the medial geniculate body (Budinger and Scheel 2000b), similar to conditions in other species (for review see Winer 1992; for a discussion of species differences in certain anatomic details see Winer et al. 1999). At least one other thalamocortical projection system into primary auditory cortex originates from the medial division of the medial geniculate body. Whereas the first projection system is generally thought to be tonotopically organized, the latter is assumed to be more unspecified with respect to frequency (Masterson and Glendenning 1978) because the ventral part of the medial geniculate nucleus itself is precisely tonotopically organized (cat: Imig and Morel 1985; rat: Winer et al. 1999) and the medial part is not (cat: Rouiller et al. 1989; rat: Winer et al. 1999).

Our results have shown that the early components P1 and N1 of the pure-tone–evoked MAEP have a clear tonotopic organization. In the two cases of the animals implanted with the narrowly spaced linear electrode configuration the spatial resolution was high enough to determine the tonotopic gradients to 3.3 and −4.2 octaves/mm in AI and AAF, respectively, for component P1 and to 3.0 and −5.5 octaves/mm in AI and AAF, respectively, for component N1. These values agree with the tonotopic gradients estimated from multiunit mapping (Thomas et al. 1993) and 2-deoxyglucose mapping (Scheich et al. 1993a) of pure-tone representations in these two fields. These results provide strong support for the hypothesis that these components result from depolarization of primary cortical pyramidal cells by thalamocortical input from the tonotopically organized medial geniculate body as was concluded on the basis of a current source density analysis of laminar recordings of click-evoked potentials (Barth and Di 1990) and analysis of cortical activity patterns after electrical stimulation of the ventral medial geniculate body (Di and Barth 1992).

Corresponding isopotential contours of P1 and N1 obtained for the different pure-tone frequencies differed not only in tonotopic location but also in size. P1 and N1 isopotential contours obtained for frequencies between 1.0 and 4.0 kHz consistently covered larger areas than those evoked by lower and higher frequencies. Because the sound delivery system was calibrated to equal absolute intensities for all stimuli this result most likely reflects the representational geometry of isointensity input intrinsic to field AI which is characterized by a spatial “over-representation” of these frequencies reflecting the...
frequency dependence of pure-tone hearing threshold (Ryan 1976) as was demonstrated with electrophysiological mapping (Thomas et al. 1993), 2-deoxyglucose mapping (Scheich et al. 1993a), and mapping of intrinsic optical signals (Hess et al. 1996). Because 2-deoxyglucose accumulation is predominately a measure of dendritic input activity (Scheich 1990), which in AI results primarily from the ventral medial geniculate (Budinger and Scheich, 2000b), this is another independent argument in favor of the above hypothesis of the neural generators of the MAEP components P1 and N1.

Our approach, taking advantage of spectrally confined stimuli rather than clicks, sheds some light onto the question whether the later MAEP components are of a thalamocortical or rather corticocortical origin. Based on their finding in the rat, that P2 can be elicited even in the absence of the P1/N1 complex, Di and Barth (1993) have already concluded that P2 cannot solely reflect corticocortical activity induced by the P1/N1 response but has to be caused by activity of a second, albeit more diffuse, thalamocortical projection. Although a corticocortical contribution cannot be ruled out its possible relevance is further deemphasized by the finding that the P2/N2 shows almost no signs of tonotopy. Given that intracortical connections in gerbil auditory cortex are highly tonotopic (Budinger and Scheich, 2000a), a higher level of tonotopic organization of the P2/N2 response would have been expected if the organization was to a large degree the result of the P1/N1 complex.

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