Timing and Laminar Profile of Eye-Position Effects on Auditory Responses in Primate Auditory Cortex

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INTRODUCTION

Although spatial localization of auditory stimuli is important to survival, its mechanisms are only partially understood. Auditory spatial location is calculated using a combination of interaural intensity and delay cues as well as monaural spectral cues (Middlebrooks and Green 1991). Auditory cortex is essential for sound localization behavior in the macaque monkey (Heffner and Heffner 1990), but studies in cats suggest that spatial processing actually begins at the subcortical stage of the lemniscal auditory pathways and simply “feed-forward” into cortex. Rather, these effects may be conveyed to auditory cortex by feedback projections from parietal or frontal cortices, or alternatively, they may be conveyed by nonclassical feedforward projections through auditory koniocellular (calbindin positive) neurons.

The present study had two goals: to confirm the presence of eye-position influences in anatomically defined A1 as well as the belt regions of auditory cortex and to develop physiological evidence as to the source of the eye-position input. We assessed neuronal responses using laminar current-source density (CSD) and multieunit activity (MUA) profiles, sampled with linear array multielectrodes, positioned to straddle the layers of auditory cortex, while the subject performed a visual fixation task. In determining the presence of eye-position effects, CSD analysis is advantageous because it indexes the first-order response to synaptic input and transmembrane current flow and thus is extremely sensitive to “modulatory” influences the impact of which on local action potential rates is subtle or undetectable. Analysis of concomitant MUA addresses the relationship of the synaptic response pattern to any subsequent changes in action potentials that do occur and to relate our results to those of other studies the sole measure of which is the action potential (Schroeder et al. 1998). In investigating potential sources of eye-position signals, simultaneous recording across the cortical laminae is important as it allows us to distinguish feedforward (ascending) from feedback (descending) inputs (Mehta et al. 2000; Schroeder and Foxe 2002; Schroeder et al. 1998, 2001). The former tend to target Lamina 4, whereas the latter tend to exclude Lamina 4 (Fellman and Van Essen 1991; Rockland and Pandya 1979).

Our findings confirm the occurrence of eye-position effects in A1 as well as belt regions of auditory cortex. The laminar profile of eye-position effects indicates that they are projected to auditory cortex through either cortical feedback connections or ascending koniocellular afferents. The timing of eye-position effects relative to that of auditory sensory processing is consistent with either alternative.

METHODS

Two male rhesus macaques (Macaca mulatta), weighing 5–7 kg, were prepared for chronic-awake electrophysiological recording using standard methods (Schroeder et al. 1998). All procedures were approved in advance by the Institutional Animal Care and Use Committee of the Nathan Kline Institute. Prior to surgical preparation, each animal was adapted to a custom fitted primate chair and acclimated to the recording chamber. Surgical preparation was conducted under deep anesthesia (1–2% isoflurane). Using aseptic technique, the tissue overlying the calvarium was resected, and appropriate portions

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of the cranium were removed. The neocortex and overlying dura were left intact. To allow electrode access to the brain and to promote an orderly pattern of sampling across the surface of the auditory cortices, matrices of 18- and 20-gauge thin-wall stainless steel guide tubes were placed over auditory cortex. Not all of the guide tubes were used, but none were used more than once. These matrices were angled so that the electrode track would be normal to plane of auditory cortex, as determined through preoperative MRI, and placed within small, appropriately shaped craniotomies to rest against the intact dura. The matrices, along with socketed Plexiglas bars (permitting painless head restraint), were embedded in a pedestal of dental acrylic, secured to the skull with titanium orthopedic screws.

Subjects were permitted a minimum of 2 wk recovery before the commencement of visual fixation training. All training and recording took place in an electrically shielded, sound-attenuated chamber lined with sound-absorbing foam (Sonex ProSPEC Composite). During training and later recording, subjects were monitored continuously using electroencephalographic (EEG) and infrared video displays. Using a fruit juice reward, monkeys were trained to fixate on one of three light-emitting diodes (LEDs) positioned at 24.5, 0, and −24.5° eccentricity with 0° elevation. To initiate a “trial,” an LED was illuminated, and the monkey had to fixate on this LED and maintain fixation while one to five sounds were presented. Eye position was monitored using an ISCAN ETL-200 eye-tracking system, and sounds were presented only when the monkey’s gaze was held within a 3° window surrounding the fixation point. Stimulation paused whenever the monkey broke fixation. The fixation LED was kept the same over a block of trials lasting ~5 min and alternated randomly across trial blocks. Trial blocks were separated by brief breaks in which the monkey was checked and fed dried fruits and other preferred treats.

Auditory stimuli for the main experiment consisted of 60 dB SPL 100 ms (5-ms ON-OFF ramp) bursts of Gaussian noise produced using Tucker Davis Technology’s System III coupled with ES-1 free-field speakers positioned with 0° elevation at −90 and 90° azimuth relative to the head. We limited our analysis to two speaker locations to maximize the power in our statistical analysis (following text). That is, we wanted to get as many single trials as possible in each condition during the ~2 h period during which the monkeys typically performed the task and thus maximize the number of single-trial auditory responses in each experimental condition (2 speakers × 3 fixation locations = 6 conditions). Analyzing two speaker positions was justified because auditory cortical neuron spatial receptive fields are broad and nonbounded in both A1 and CM, although 5–6% of A1 neurons can be described as “spatially tuned,” meaning that their receptive fields are bounded if a criterion of >75% of the maximum rate is adopted (Recanzone et al. 2000b). It should be noted that neither A1 nor CM displays a clear spatial topography with methods used thus far (Merzenich and Brugge 1973; Recanzone et al. 2000b).

For a functional assignment of each recording site to A1 versus belt auditory cortex, and the location within the region’s characteristic frequency map, we evaluated the response to a series of seven pure tones ranging from 0.5 to 20 kHz. We used a suprathreshold method, entailing binural presentation of each tone (intensity of 60 dB SPL, duration of 100 ms, 5-ms ON-OFF ramp) in blocks of 100 with blocks in random order (Schroeder et al. 2001; Steinschneider et al. 1995). Although this method approximates a site’s characteristic frequency and tuning bandwidth on a coarser scale than the more common “threshold” methods (Merzenich 1983), it does so in <10 min, which is a critical time saving when experimenting with awake-behaving subjects, and both methods agree on the two key bits of information we require for the present experiments (A1 vs. belt and location within the characteristic frequency map).

Laminar activity profiles consisting of concomitant field potentials, CSD measures and MUA were obtained by recording with linear array multielectrodes constructed with an inter-electrode spacing of 150 μm (Schroeder et al. 2001). On each experimental day, a multielectrode was inserted through an electrode guide tube and lowered into auditory cortex. The guide tube matrices were positioned on the dorsal brain surface so that they would constrain the electrode array to an angle orthogonal to the laminae of auditory cortex. The laminar activity profile in response to bilateral Gaussian noise burst was used to position the electrode so that the array of contacts was distributed across the entire laminar expanses (i.e., layers 1–6) of auditory cortex. Once the position was refined, it was left stable for the duration of recording. The “grid” arrangement of the guide tubes in each matrix ensured that successive penetrations would sample different locations in auditory cortex with a relatively constant density across its surface. For all recordings, the reference was an epidural electrode located over occipital cortex. Signals were impedance matched in a preamplifier (10× gain) located near the electrode, and further amplified 1000 times with a band-pass of 1 Hz to 3 kHz by Model 8-16D Grass amplifiers. Field potential profiles were obtained by averaging single-trial responses over 100 stimulus presentations. Prior to averaging, amplifier outputs were integrated down to 1 kHz in and then digitized at 2 kHz. MUA was obtained from the same signal at each contact by high-pass filtering the raw amplifier output at 500 Hz, full-wave rectifying the high-frequency activity, integrating the resultant signal down to 1 kHz, digitizing (at 2 kHz), and averaging the single sweep responses (n = 100). With the rectification step, upward deflection represents an increase in action potentials and downward deflection represents decrease in action potentials relative to the prestimulus baseline.

One-dimensional CSD profiles were calculated from the field potential profiles using a three-point formula for estimation of the second spatial derivative of voltage (Nicholson and Freeman 1975)

\[
D = \frac{df}{dx^2} = \frac{[f(x-h) - 2f(x) + f(x+h)]}{h^2}
\]

in which \( f \) is the voltage, \( x \) is the point at which \( D \) is calculated, and \( h \) is the spacing between electrodes (150 μm). Electrode penetrations were made orthogonal to the local lamination pattern in keeping with the requirements of one-dimensional CSD analysis (Mitzdorf 1985). CSD analysis provides an index of the location, direction, and density of transmembrane current flow. Because transmembrane current flow is the first-order response to synaptic input, it provides the ability to assess synaptic responses either or not they produce net changes in local action potential rates. Quantification of the CSD waveforms in determining eye-position preference was performed by full-wave rectifying then averaging across appropriate channels (AVREC).

Statistical analysis of eye-position effects began with the processing of individual single-trial responses (each response or “sweep” includes all channels in the CSD profile). Individual sweeps were baseline corrected and screened using standard criteria, to eliminate movement, and electromyographic artifacts. Following these initial steps, a repeated-measures ANOVA, with trial as a repeated-measures factor, was used to evaluate the composite question: “is electrical activity associated with eye position (center, left, right) or time (0–150 ms post stimulus) for each channel of the CSD profile, stimulus (speaker 1 or 2), monkey, and experiment (electrode penetrations 1–29)?” Due to the processing limitations of our current server and workstation technology, it was not practical to analyze the entire time course of the response for the entire data set. To enable the analyses to incorporate all of the single trials in the data set, we limited our analysis to the first 150 ms poststimulus and ran the analysis program on every fifth data point. Differences were acknowledged only when alpha exceeded a criterion of \( P < .05 \) in ≥5 sequential time points, providing a Bonferroni correction level in excess of 0.05 or 3.125 in 10 million. Confirmation was provided by running serial analyses. Five different starting points were used, and the subsequent results were not different, illustrating that the location and time course of the effects was not dependent on the starting point of our analysis.

The rationale for the single-trial analysis we have used here is that it allows us to take advantage of the dynamic variability in signals across recording channels, time points, and single trials and to use this...
variability to increase the power of the analysis. The reason that this is important is that we have come to recognize that many of the effects we study vary dynamically from trial to trial in their latency, amplitude, and sometimes even in their presence or absence. More traditional analyses, such as averaging responses across trials, within condition and testing for significant differences between conditions are insensitive to this variability except insofar as it increases the noise term in the analysis.

Because the experimental analysis assumes that the position of the electrode array is stable throughout the experiment, it is important to rule out any contribution from an electrode shift, to the pattern of eye-position effects. The design of the experiment makes it unlikely that an electrode shift, could produce significant differences. There were two speakers and three eye positions for a total of six conditions. We typically cycled through these six condition blocks three or more times (minimally twice) in random order. An electrode shift between any two blocks would produce a significant difference if only those trial blocks were analyzed. However, the effect would have an "unphysiological" appearance as changes would be detected at response onset in nearly all channels. More importantly, because each condition is repeated over multiple trial blocks, the change (variance) between conditions in the two-trial block case would register as within condition variance. This would enlarge the error term in the statistical test, thereby decreasing the probability of any between condition differences reaching statistical significance. In short, electrode slippage would obliterate effects due to eye position. We also monitored for the possibility of electrode slippage by comparing the laminar profile of auditory-evoked response recorded at the beginning of the experiment (i.e., after initial positioning of the electrode), with one recorded using the same stimulus at the end of the experiment; this profile has characteristic qualities that identify specific cortical layers (Schroeder et al. 2001; Steinschneider et al. 1992, 1995). Based on these analyses, data from four experiments were eliminated from the analysis.

After serving as a subject in this experiment, monkey S went on to participate in additional experiments. However, this was the last of a series of experiments for monkey A, and his brain was therefore available for histological analysis and anatomical reconstruction of the recording sites. At the end of data collection the monkey was given an overdose of pentobarbital sodium (50 mg/kg iv), and once deeply anesthetized, was perfused through the heart with 4% paraformaldehyde. Following 3-day immersion in a 30% sucrose/phosphate buffer solution for cryoprotection, the brain was cut in 80-μm, whole-brain, coronal sections on a freezing microtome. The anterior-posterior angle of sectioning was set parallel to the angle of electrode penetrations, ~20° anterior of vertical (defined by the angle of the guide tube matrix). Alternate sections were stained for Nissl substance, acetylcholine esterase (AchE) and parvalbumin (PV) to help determine the borders between A1 and surrounding regions (e.g., Hackett et al. 1998a; Schroeder et al. 2001). Individual histological sections were digitally scanned, and a whole-brain three-dimensional reconstruction was made using MEDEX software (Abraham and Bear 1996; Schroeder et al. 2001). Because our monkeys are usually subjects in several experiments recording from auditory cortex, as well as other brain regions, the whole brain reconstructions are extremely valuable. With this technique, we can accurately reconstruct the complete pattern of electrode penetrations through the regions(s) of interest in each subject. In the present study, reconstruction of the brain of one subject (A) is presented.

RESULTS

Auditory responses were sampled during 29 electrode penetrations distributed across A1 and the posterior auditory belt regions in two monkeys. Based on histological analysis, illustrated in Fig. 1, four of the relevant electrode penetrations (red dots) in subject A were in A1 and the remainder (6) were in belt cortex. The more rostral fields of primary auditory cortex (R and RT) were not systematically explored in this study, although, because we did not record enough of the more anterior sites to define the functional sign of the A1/R border (i.e., the reversal of the tonotopic progression), it is possible that some of the low-frequency (anterior) sites are in R. The black dots represent penetrations made for earlier, unrelated experiments (see METHODS). These dots are shown because they provide information on the position of our recording sites relative to the local characteristic frequency map at the posterior border of A1. Analysis of pure-tone responses in A1 and belt penetrations revealed narrow and broad frequency tuning profiles, respectively. This point is illustrated in Fig. 2A by using individual frequency-tuning profiles to contrast the tuning characteristics of representative A1 sites (thick black line) with representative belt cortex (gray dotted line) sites with low characteristic frequencies (1 kHz) and in Fig. 2B by depicting the same comparison for representative A1 and belt sites with higher characteristic frequencies (16 kHz). To promote ready comparison, response amplitude is expressed as percentage of the maximum response amplitude at the characteristic frequency; however, this does obscure another clear difference between A1 and belt recordings. That is, A1 pure-tone responses at the characteristic frequency were generally two to three times larger in absolute scale than those noted in belt regions. A1 sites often display off-frequency inhibition, evident in points at which the tuning curve assumes a “negative” amplitude (Fig. 2A). This is rarely observed in belt regions. To express the differences in tuning characteristics more quantitatively, Fig. 2C presents mean half-amplitude bandwidth for the entire sample of 29 penetrations in this study. Half-amplitude bandwidth differs significantly between A1 and belt region recordings (Wilcoxon Matched-Pairs Signed-Ranks Test, \( P < 0.01 \)). The contrast of narrow characteristic frequency tuning in A1 with much broader tuning in belt regions is typical of findings with these methods (Schroeder et al. 2001; Steinschneider et al. 1992, 1995) and in agreement with threshold-based evaluation of single-unit responses (Kosaki et al. 1997; Merzenich and Brugge 1973). Because histological confirmation in the second subject is not yet available, assignment to A1 (10 penetrations) versus belt cortex (9 penetrations) is based on functional criteria alone.

To quantify the effects of eye position, modulation indices (MIs) were calculated for each experiment (electrode penetration). The MI expresses modulation due to eye position as a function of the auditory evoked response amplitude. Figure 3 illustrates several points relevant to a firm appreciation of this analysis. On the far left of Fig. 3 is a depiction of the multielectrode positioned with respect to the laminae of auditory cortex. To the right is an overlay of two averaged laminar CSD profiles (red and black traces) generated in response to Gaussian noise burst stimulation of the contralateral ear. Based on our earlier work, (Schroeder et al. 2001; Steinschneider et al. 1992, 1995), these are in all respects typical auditory cortical response profiles with initial responses (current sinks) in and just above Layer 4, corresponding to the ascending activation of local stellate cells with subsequent current sources and sinks above and below corresponding to the postsynaptic responses of supra- and infragranular pyramidal cell ensembles. The relevant difference between these profiles is that in one case (red) the animal was fixating at the center location.
throughout the trial block, whereas in the second (black), the animal was fixating at the location to his left. The areas of significant difference between the “left” and “center” responses (i.e., modulation due to eye position), as determined by the statistical tests (see METHODS), are shaded gray. The subsequent columns in the figure illustrate the methods for calculating the MI using the quantified significant differences as a function of the total response amplitude. Separate MIs were calculated for the supragranular, granular, and infragranular layers (for simplicity, only that for the supragranular layers is shown in Fig. 3). First, significant eye-position-related differences in response amplitudes are determined for each electrode channel (as described in METHODS). These are full-wave rectified and summed over the channels comprising the laminar groupings. Figure 3 illustrates the computation of the MI for the supragranular laminae (CSD channels 1–6). Second, the sensory responses from the eye-position conditions used to compute the significant differences, for each of the same channels, are rectified and then summed across channels. These operations yield the total significant difference due to eye position, and total sensory response, respectively. For each laminar grouping, the first quantity is divided by the second quantity, thus scaling the effects of eye position by the total response amplitude. At each recording site, 6 comparisons were made (left vs. center, center vs. right, right vs. left for 2 speakers). The MI for a recording site is the sum of the MIs from all six comparisons. This provides a numerical value allowing us to compare effects across laminae, sites in auditory cortex, and across subjects.

Twenty-four of 29 penetrations displayed significant effects of eye position on auditory responsiveness. Eye-position effects were quantified using the MI described in the preceding text (see Fig. 3). The MI is the ratio of the response modulation due to eye position scaled by the amplitude of the raw sensory response. Collapsing modulation across time (from 0 to 150 ms post stimulus), space (combined from relevant channels within the same lamina grouping, i.e., supragranular, granular, or infragranular), and condition (the sum of 6 individual MIs), the MI provides an overall index of modulatory activity in a specific laminar grouping at a given recording site. Figure 4 presents the distribution of MIs for all recording sites, organized by laminar and anatomical location, along with the average supragranular MIs for both A1 and belt cortex. Because signifi-
To examine the physiology of eye-position modulations, we compared the CSD profiles with their corresponding MUA profiles. However, significant MUA modulations were only found in 4 of the 24 recording sites exhibiting CSD modulations, limiting the number of direct comparisons. Figure 5 illustrates results from one site in which a significant CSD effect (Fig. 5A) was accompanied by a significant MUA modulation (Fig. 5B). These measures were both taken from the same electrode channel in supragranular Layer 3. Significant differences between center fixation (red) and left fixation (black) are outlined in gray. A relative increase in current sink amplitude is found when the subject fixates toward the center as opposed to the left position. An increase in MUA, congruent with the CSD effect, albeit of shorter duration, is seen in the same condition. The lower Lamina 3 current sinks generally indicate net inward flowing transmembrane currents (depolarization) in the local pyramidal neuron ensemble (Schroeder et al. 2001) and, with the coincident MUA burst, indicate that the ensemble response is a net local excitation. The eye-position-related difference, therefore appears to be one of increased excitatory response to the auditory stimulus when the monkey fixates to the center versus to the left position.

To test for directionality in eye-position effects, for each electrode penetration (recording site) that showed a significant eye-position effect, eye-position preference was determined using statistical comparison of auditory responses across the three fixation conditions. Initially, responses to ipsilateral and contralateral speakers were evaluated separately. For each speaker, we compared the auditory response under central fixation versus ipsilateral (to the side of stimulation), central versus contralateral, and ipsilateral versus contralateral fixation conditions. If the response was larger under center fixation than under ipsilateral or contralateral fixation, then it was assigned to the “center preference” category. Using the same rules, the response was assigned to “ipsilateral preference” or “contralateral preference,” or “not center” in cases where responses under ipsilateral and contralateral fixation were equivalent, and both greater than the response under center fixation. The results for auditory responses combined across speakers, are summarized in Fig. 6A.

The smallest category of preference is “not center” (n = 3), a category that would predict a preference map with more than one mode. The largest category is preference for center, followed by lesser but comparable proportions of preference for ipsilateral and contralateral eye positions. Each of these categories (center, ipsilateral, and contralateral) predicts a preference with one mode. When responses to stimuli presented contralateral to the recording site were analyzed separately, (Fig. 6B) there was even stronger preference for the center eye position. Cases exhibiting center preference were nearly twice as frequent as the next strongest preference (the fixation position contralateral to the side of stimulation), in turn nearly twice as frequent as preference for the fixation position ipsilateral to stimulation. “Not center” was the least-preferred category. When we separated out the responses to sounds presented ipsilateral to the recording site (Fig. 6C), auditory responses appeared to prefer the ipsilateral fixation position (i.e., the position nearest the origin of the auditory stimuli). The effect is slight, however, (9 cases favoring ipsilateral vs. 7 cases favoring center), and in other respects, the pattern of preference is like that for responses to the contralateral speaker.
Using the integrated amplitude of the AVREC (see METHODS) waveform as the basis for comparison, 22 of the 24 recording sites with eye-position modulation had auditory responses with greater amplitude when stimuli were presented from the contralateral speaker. Regardless of any eye-position preferences, this finding is in keeping with earlier estimates of the proportions of auditory cortical neurons dominated by contralateral, as opposed to ipsilateral, ear inputs (Brugge and Merzenich 1973). Our sample of sites dominated by ipsilateral ear inputs is not large enough to make a statement about any differences from the contralaterally dominated response sites.

As seen in Fig. 3, eye-position modulation occurs over an extended time frame and generally lags the onset of the sensory response. To visualize the overall temporal pattern of eye-position effects, Fig. 7 presents a grand mean of the significant CSD modulations (in the supragranular laminae) due to eye position, averaged across penetrations, in contrast with the grand mean of the total sensory response averaged across the same recordings. Data from each experiment (penetration) include all of the significant eye-position effects from that experiment. To compute the grand means, we condensed the data using the same procedure used to compute the MI for each individual penetration (described in the preceding text) with the exception of the last step of dividing by the total sensory response. That is, significant eye-position effects were summed across conditions and averaged across electrode channels to yield a single waveform. Then the resultant quantities were averaged together across experiments. To avoid giving undue weight to single cases, data from each individual experiment (penetration) were normalized before averaging. Onset latency of significant eye-position modulation was determined for each experiment by testing the single condensed representation of the eye-position effects. The first time point of sustained deviation (>4 ms) by ≥2 SD units from the baseline, was defined as the onset latency of the eye-position effect for that penetration. Based on these measurements across the set of 24 experiments showing significant eye-position effects, the onset latency of the effect ranges from 12.5 to 127.5 with an average

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Analysis of eye-position effects on the laminar current-source density (CSD) profile. Far left: a depiction of the recording electrode positioned with respect to the laminae of auditory cortex. In the next column are CSD profiles (D) calculated from the averaged auditory-evoked field potential profile, in different trial blocks, with the monkey fixating center (black traces) or to the left (red traces); in this case, the recordings were from auditory belt cortex of the right hemisphere. Because the CSD profile is calculated using a 2nd-derivative approximation, the signals from the 2 outer-most channels are lost. In this case, the uppermost of the remaining signals was a null response (flat line) sampled from Layer 1 and thus is not shown. Current sources are signified by upward deflections from baseline, and sinks are signified by downward deflections. Significant differences (see METHODS) are shaded light gray. The 3rd column presents the full-wave rectified CSD waveforms for these conditions with the superimposed significant differences. Further to the right are shown the remaining steps taken to compute the modulation index for the supragranular laminae, both for the single comparison shown (i.e., \( MI_{comp} \)) and combined across the 6 conditions for this experiment (\( MI_{tot} \)). The \( MI_{tot} \) represents the overall effect of eye position, scaled by amplitude of the underlying sensory response for any laminar grouping. In the case shown here, \( MI_{tot} \) calculation is shown for the supragranular layers alone. In this experiment, an MI was also calculated for the significant eye-position effect in Layer 4 (calculation not shown). Note that in stage 1 of this analysis, no significant eye-position effects were found for the infragranular laminae in this case, and thus the infragranular responses did not enter the later stages of analysis.

![Figure 4](http://jn.physiology.org/)

**FIG. 4.** Distribution of modulation indices (MIs). MIs for individual penetrations grouped by laminae (supra-, granular, infra-) and region (A1, belt) of auditory cortex, considering only the 24/29 penetrations that yielded significant effects. The overwhelming majority of sites displayed 0 modulation indices for the granular and infragranular (not displayed) layers. The average indices for the supragranular modulations in A1 and the posterior belt are also plotted with SE bars.
of 51.8. The average onset of the eye-position modulation lags the average onset of the sensory response by about 40 ms, however there is no systematic relationship between the two latency values on a case-by-case basis ($r = -0.13; P < .42$). The average onset latency of eye-position modulation in A1 (53.7 ms) and Belt regions (49.9 ms) do not differ significantly ($P < 0.69$), nor do the average auditory onset latencies in A1 (9.6 ms) differ from those in the belt region (11.6 ms) ($P > 0.14$). Finally, there is no significant difference ($P > .05$) between the latencies of eye-position modulation when responses to contralateral versus ipsilateral speakers are compared.

**DISCUSSION**

The present findings demonstrate that eye position affects the neuronal responses to sound in primary auditory cortex, A1, as well as secondary belt regions. Multisensory/sensorimotor interactions in auditory association cortex are not unexpected as these regions display convergent visual inputs (Butler et al. 2001; Calvert et al. 1997; Doniger et al. 2000, 2002; Foxe et al. 2002) and somatosensory inputs (Abraham and Bear 1996; Foxe et al. 2002; Levane et al. 1998; Schroeder et al. 2001). The presence of such an effect in A1 was unexpected because the visual multisensory convergence regions generally do not seem to include A1 (Schroeder et al. 2003). However, effects of eye position on neuronal responses in A1 was reported recently by another laboratory (Werner-Reiss et al. 2003), and our report confirms this finding anatomically as well as physiologically. There are several differences between the two studies. The most prominent methodological difference is that Werner-Reiss and colleagues sampled extracellular single-unit recording with a single electrode, whereas the present study sampled laminar multunit activity and CSD profiles. These approaches have offsetting strengths and limitations. CSD analysis has the strength of being an index of the first-order synaptic response, transmembrane current flow and can represent this response whether or not the activation is sufficient to cross the threshold for action potential generation in postsynaptic neurons. In addition, the present methods have the advantage that recording of activity across all cortical layers simultaneously allows us to define the laminar activation sequence, and thus to distinguish Layer 4-initiated (feedforward) from non-Layer 4-initiated (feedback and lateral) responses (Walker et al. 1995, 1998, 2001). Despite these strengths in defining the net response in a population of neurons, our approach has the limitation that it cannot isolate and separately analyze the activity of single neurons. In isolating single neuron responses, Werner-Reiss et al. (2003) reported that ~1/3 of the neurons in auditory cortex show eye-position effects, and many of these display modulation of baseline activity, in the absence of auditory input. Perhaps because the multunit recording method does not easily distinguish individual neurons’ action potentials trains, it fails to detect prestimulus effects of any sort and appears to underestimate the incidence of effects of eye position on action potentials. CSD analysis, as currently implemented, appears insensitive to effects that manifest in the baseline activity level.

**Laminar profile of eye-position effects**

The finding of eye-position effects in auditory cortex is predictable from similar findings in the inferior colliculus (Groh et al. 2001). However, the laminar pattern of the responses at most recording sites in A1 and the posterior belt is not the one predicted by the anatomy of a feedforward projection through the primary auditory pathways. The main (frequency specific) input to auditory cortex comes from the ventral nucleus of the medial geniculate (MGv), which projects to Lamina 4 and lower Lamina 3 of A1 (Hackett et al. 1998). If effects of eye position were combined with the auditory input at an earlier stage of the lemniscal auditory pathways and fed-forward into A1, the effects should manifest first in Lamina 4 of A1. The same prediction would hold for belt cortices because the feedforward projection of the specific input from A1 also targets in and near Layer 4 (Hackett et al. 1998). Although significant eye-position effects in the supragranular layers of auditory cortex were detected in 24/29 penetrations, corresponding effects in the granular layers were resolved in only three cases, and only one penetration revealed an effect in the infragranular laminae. The small numbers of granular and infragranular effects preclude any meaningful analysis of laminar differences in magnitude or quality of effects.

**Mediation by cortical feedback?**

An alternative possibility is that eye-position effects are conveyed by feedback from a higher cortical area. Neural activity in several parietal cortical areas involved in spatial processing is affected by the position of the eyes (Bremmer et
al. 1999; Russo and Bruce 1993, 1994; Stricanne et al. 1996), and several of these areas project to posterior auditory cortex (Lewis and Van Essen 2000; Romanski et al. 1999). Feedback projections (Felleman and Van Essen 1991) usually manifest in the supragranular or supra- and infragranular layers (Foxe et al. 2002; Mehta and Schroeder 2000), and this corresponds with our results. Additionally, though the onset latency of eye-position effects in auditory cortex is highly variable, on average, it is much later that that of the ascending auditory response. This is consistent with the view that eye-position effects are mediated by phasic cortical feedback inputs triggered by auditory input. Unlike the study of Werner-Reiss et al. (2003) our findings do not suggest that eye-position effects entail a tonic bias in excitability in auditory cortical neurons as we do not detect eye-position effects in the baseline prestimulus period. As discussed in the preceding text, this may stem from methodological factors. If eye-position effects in auditory cortices are driven by feedback from higher cortical regions, it follows that effects in the tectum (Groh et al. 2001) may be driven by feedback from cortex.

Mediation by koniocellular input?

Another possible explanation for our findings is that eye-position effects are conveyed to cortex by the koniocellular system. As reviewed earlier (Jones 1998), there are two thalamic sensory systems: a core system that projects topographically (or tonotopically) organized sensory information to well-defined primary sensory cortical areas and a matrix system, composed of koniocellular neurons that project less specific sensory information to a wider array of areas, including non-

FIG. 7. Temporal pattern of eye-position modulation. Grand mean of the significant CSD modulations in the supragranular laminae due to eye position averaged across all conditions and penetrations (■) in contrast with the grand mean of the total sensory response averaged across the same recordings (□); the total sensory response includes all of the individual fixation conditions used to test for significant differences. In all cases, data from each individual experiment (penetration) were normalized before averaging (see text for details).
primary cortices (Jones 1998). The koniocellular neurons project to the superficial layers across broad expanses of cortex unrestricted by architectonic boundaries. This projection pattern reconciles with our findings of little difference in the effect between A1 and the posterior belt regions, and it dovetails with our finding that the most prominent effects are detected in the supragranular layers. On the other hand, the timing of eye-position effects in auditory cortex is not informative as we have no information on the relative latencies of “core” and “matrix” inputs to auditory cortex. Several nuclei of the inferior colliculus project to divisions of the MGN that are involved in the matrix system. The pericentral, dorsal, and external nuclei of the inferior colliculus project to the dorsal (MGd) and the magnocellular (MGm) nuclei (Catania et al. 2000). These nuclei in turn project to the core, belt, and parabelt areas of auditory cortex (Hackett et al. 1998; Molinari et al. 1995). In the initial report of eye-position effects in the inferior colliculus (Groh et al. 2001), there was some uncertainty in localizing the effects to a specific nucleus. It is thus possible that the Groh et al. (2001) findings did not reflect responses in the central nucleus of the inferior colliculus, the one that projects to MGv, but rather, in one or more of the inferior colliculus nuclei that project in parallel through MGd and MGm.

Do eye-position effects correspond to selective attention?

The complexity of eye-position effects in auditory cortex (Werner-Reiss et al. 2003) argues that these effects are not simply a result of visuospatial attention. Although visuospatial attention can be moved independently of eye position (Harter et al. 1982; Hillyard et al. 1985; Moran and Desimone 1985; Treue and Maunsell 1996), the direction of visual attention generally correlates with the position of the eyes (Emery et al. 1997; Jellema et al. 2000), and it is clear that the cortical mechanisms in control of spatial attention overlap extensively with those that control eye position (Bisley and Goldberg 2003; Goldberg et al. 2002). Although one study (Robinson et al. 1995) reported attentional suppression of stimulus-evoked activity in parietal cortex, the nature of this effect is likely related to the mechanistic orienting of attentional resources rather than a general reflection of attention’s effects on sensory processing. Because attention generally enhances the processing of effective stimuli (Harter et al. 1982; Hillyard et al. 1985; Moran and Desimone 1985; Treue and Maunsell 1996), we would expect that if the eye-position effect is due to a shift in visuospatial attention, then auditory responsiveness would increase when the subject looked toward the sound source and decrease when he looked away. This pattern was not observed by the present study. The most common effect we encountered across recording sites was enhancement of the auditory response when the subject fixated centrally regardless of actual sound source. Beyond this, enhancement of the auditory response was just as common when the monkey fixated in the field opposite to the sound source as it was for fixation in the direction of the sound source. Although the foregoing makes a circumstantial argument against an attentional interpretation of eye-position effects, the question remains open. In the present and earlier experiments (Groh et al. 2001; Werner-Reis et al. 2003), monkeys were required to fixate at points varying in direction and distance from the sound source, but they were not required to discriminate the location of the sound itself. Additional experimentation will be necessary to determine if eye position interacts with auditory spatial selective attention.

Relationship to hypothesized auditory spatial functions?

Posterior auditory cortex, including the core area A1 and the caudal belt and parabelt regions, are hypothesized to compose a “where” pathway for auditory localization (Rauschecker 1998; Rauschecker and Tian 2000). Neurons in the caudomedial belt area (CM) demonstrate better spatial tuning than neurons in the anterior belt regions (Cannestra et al. 2001; Rauschecker et al. 1995), and their responses are correlated with sound-localization behavior (Recanzone et al. 2000a). Further, posterior auditory cortex projects to the frontal eye fields and caudal principle sulcus (Hackett et al. 1999; Romanski et al. 1999) as well as to the posterior parietal cortex (Lewis and Van Essen 2000) areas involved in spatial processing (Tune et al. 1993). In addition to the present results, eye-position effects in auditory cortex have been reported in a published study by an independent collaborator (Werner-Reiss et al. 2003) and in a preliminary report by a third laboratory (Wu and Andersen 2000). In the first two cases, effects were noted in posterior A1 and posterior auditory association areas, whereas in the preliminary report, the findings were attributed to Area Tpt (temporoparietal cortex), though it is not clear which part of Tpt (Wu and Andersen 2000). The findings in the posterior auditory cortices are consistent with the proposed role of these regions in auditory spatial processing; however, it should be noted that no one has yet determined whether or not eye-position effects are present in the more rostral auditory cortices. Thus the linkage of posterior auditory cortex with spatial processing functions remains speculative.

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