A Comparison of Neuron Response Properties in Areas A1 and CM of the Marmoset Monkey Auditory Cortex: Tones and Broadband Noise

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Kajikawa, Yoshinao, Lisa de La Mothe, Suzanne Blumell, and Troy A. Hackett. A comparison of neuron response properties in areas A1 and CM of the marmoset monkey auditory cortex: tones and broadband noise. J Neurophysiol 93: 22–34, 2005. First published September 1, 2004; doi:10.1152/jn.00248.2004. The purpose of this study was to compare response properties of two adjacent areas of the marmoset monkey auditory cortex. Multiunit responses to 50 ms tones and broadband noise bursts (BBN) were recorded in the core area, A1, and the caudomedial belt area, CM, of ketamine-anesthetized animals. Neurons in A1 and CM exhibited robust low-threshold short-latency responses to BBN and tones, whereas neurons in adjoining lateral belt areas were poorly responsive or unresponsive to tones and noise. Except for a population of broadly tuned units in CM, the characteristic frequency (CF) could be determined for all recording sites in A1 and CM. Both areas were tonotopically organized and shared a high CF border. Whereas the tonotopic gradient in A1 was smooth and continuous across the field, the gradient in CM was discontinuous, and the intermediate CF range was underrepresented. For BBN stimuli, rate level functions were largely monotonic in A1 and CM. Response profiles were also similar in both areas. As a population, neurons in CM were distinguished from A1 by significantly shorter response latencies, lower thresholds, and broader tuning bandwidth at higher intensities. The results indicated that, while A1 and CM represent anatomically and physiologically distinct areas, their response profiles under anesthesia overlapped considerably compared with the lateral belt areas. Therefore refinements of current models of the primate auditory cortex may be needed to account for differences in organization among the auditory belt areas.

INTRODUCTION

In recent years, we have adopted a model of auditory cortical organization in primates based on the collective findings of the field (Hackett 2002; Hackett and Kaas 2004; Kaas and Hackett 1998, 2000). The model defines auditory cortex as the corpus of cortical areas that are the preferential targets of neurons in either the ventral (MGv) or dorsal (MGd) divisions of the medial geniculate complex (MGC). By this definition, three regions of the superior temporal cortex comprise the auditory cortex in Old World and New World primates: core, belt, and parabelt. The core region includes two or three tonotopically organized subdivisions that receive thalamic inputs from MGv. In the belt region, surrounding the core, as many as seven distinct areas have been proposed. These fields receive inputs from the core and MGd. At least some of the lateral belt areas are tonotopically organized in a manner that parallels the adjacent core area (Kosaki et al. 1997; Merzenich and Brugge 1973; Rauschecker and Tian 2004; Rauschecker et al. 1995). The belt areas project topographically to the caudal and rostral divisions of the parabelt region, which receive inputs from the MGd, but not the MGv (Hackett et al. 1998a,b). Therefore, it is assumed that there is a regional processing hierarchy in auditory cortex in which information proceeds from the core through the belt to the parabelt (Kaas and Hackett 1998). Within regions, however, it seems that individual areas are processing inputs in parallel from the thalamus and cortical areas within adjoining regions.

With the exception of auditory area 1 (A1), the anatomical and physiological features of the primate auditory cortex remain poorly characterized (for reviews, see Hackett 2002; Hackett and Kaas 2004). One of these fields, the caudomedial belt area (CM), has been explored sporadically over the last 25 years. Generally it has been reported that either a reversal or disruption in the tonotopic gradient occurs at the A1/CM border and that neurons in CM are more broadly tuned than those in A1 (Imig et al. 1977; Kosaki et al. 1997; Merzenich and Brugge 1973; Rauschecker et al. 1997; Recanzone et al. 2000a; Selezneva et al. 2003). Rauschecker et al. (1997) extended these basic findings in an influential study in which neuron responses were recorded in CM and the rostral core area (R) before and after ablation of A1 in macaque monkeys. The main finding was that responses to tones were abolished in CM, but remained responsive to complex sounds. Responses in R were unaffected by the A1 lesion. These data suggested that responses to tones in CM were dependent on intact feedforward inputs from A1. The findings were also consistent with a model of auditory cortical processing in which parallel inputs from the MGv target the core, while the belt areas depended on serial inputs from the core region and nonlemniscal subcortical pathways.

These conclusions received support from two papers in which responses of neurons in A1 and CM of awake-behaving macaque monkeys were compared (Recanzone et al. 2000a,b). The major findings were that neurons in CM had higher thresholds, longer latencies, and broader tuning than A1, consistent with the model. They also found that, compared with A1, a greater proportion of neurons in CM were sensitive to spatial location. This is consistent with the observation that CM is the only auditory belt area with projections to the ventral intraparietal area (VIP) in posterior parietal cortex (Lewis and Van Essen 2000; see also Pandya and Seltzer 1982). These

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results may also relate to the discovery of multisensory interactions in CM (Fu et al. 2003; Schroeder et al. 2001, 2003). Some neurons in CM are responsive to auditory and somatic sensory stimuli. Accordingly, CM shares properties of the neighboring temporo-parietotemporal area (Tpt), in which multisensory interactions have been clearly documented (Falachier et al. 2002; Leinonen et al. 1980).

In an effort to more fully characterize the medial belt areas and refine our working model of the primate auditory cortex, we have initiated anatomical and physiological studies to compare the core and medial belt areas of the primate auditory cortex. The goal of this study was to document similarities and differences between these regions and test the specific hypothesis that neurons in areas A1 (core) and CM (medial belt) could be distinguished on the basis of responses to simple acoustic stimuli (e.g., tones, Gaussian noise). To address this hypothesis, neuroanatomical tracing and architectonic analyses were combined with physiological recordings to derive anatomical and physiological profiles of the lateral and medial belt areas in the common marmoset (Callithrix jacchus jacchus). Here we present the results from recordings in the core area, A1, and the caudomedial belt area, CM. The results revealed clear differences between neurons in A1 and CM, but also indicated that neurons in the two areas shared many features, suggesting that CM has characteristics of both core and belt auditory cortex (e.g., Eggermont 1998). In that sense, CM is distinct from the lateral belt areas that were poorly responsive to the stimuli used in this study. A preliminary report of these findings was briefly presented elsewhere (see Kajikawa et al. 2003).

METHODS

Animal subjects

All experimental procedures were conducted in marmoset monkeys (C. jacchus jacchus) in accordance with National Institutes of Health Guidelines for the Use of Laboratory Animals under a protocol approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Five to 10 days prior to neurophysiological recording experiments, neuroanatomical tracer injections were made into the left auditory cortex of each animal subject (de la Mothe et al. 2002). Areas targeted for injections included several areas of the auditory cortex [CM, rostromedial (RM), AI, R, anterolateral (AL), middle lateral (ML), caudolateral (CL), caudal parabelt (CPB)], temporal parietotemporal (Tpt), and somatosensory areas (SS) in the lateral fissure (see Table 1). The neurophysiological recordings were obtained from the right hemispheres of six adult marmoset monkeys, ages 2–5 yr, during a terminal experiment that commenced after the survival period (Table 1). Limiting recordings to the hemisphere opposite the injection site minimized potential effects of tracer injections on neuron response properties. Most of the recordings were obtained from neuron clusters in areas A1 and CM, but also included a few clusters in adjacent areas. Only one verifiable recording site was identified in A1 of case 1. It was included in all analyses but not shown in Fig. 3. The experimental history of each animal subject is summarized in Table 1.

General surgical procedures

Animals were premedicated with cefazolin (25 mg/kg), dexamethasone (2 mg/kg), cimetidine HCl (5 mg/kg), and robenil (0.015 mg/kg). Anesthesia was induced by intramuscular injection of ketamine hydrochloride (10 mg/kg) and maintained by intravenous administration of ketamine hydrochloride (10 mg/kg) supplemented by intramuscular injections of xylazine (0.4 mg/kg). Body temperature was kept at 37°C with a water circulating heating pad. Heart rate, expiratory CO2, and O2 saturation were continuously monitored throughout the surgery and used to adjust anesthetic depth. Oxygen was delivered passively through an endotracheal tube at a rate of 1 l/min.

The head was held by hollow ear bars affixed to a stereotaxic frame (David Kopf Instruments, Tujunga, CA). A midline incision was made exposing the skull, followed by retraction of the temporal muscle. A craniotomy was made exposing the right dorsal superior temporal gyrus, lateral fissure, and overlying parietal cortex. After retraction of the dura mater, warm silicone was applied to the brain to prevent desiccation of the cortex. Photographs of the exposed cortical surface were taken for recording the locations of electrode penetrations in relation to blood vessels and the lateral sulcus (LS).

Auditory stimulation

Experiments were conducted in a sound-proof chamber (Industrial Acoustics). Acoustic stimuli were generated by Tucker-Davis Technologies (TDT, Gainesville, FL) System II hardware and software (SigGen). Auditory stimulation was accomplished by coupling STAX MK3 electrostatic earphones (Stax, Miyoshi, Japan) to a hollow ear bar inserted into the contralateral ear. Stimuli were calibrated using a one-quarter-inch microphone (Model 7017, ACO Pacific) and custom technologies (TDT, Acoustics). Acoustic stimuli were generated by Tucker-Davis Technologies (TDT, Gainesville, FL) System II hardware and software (SigGen). Auditory stimulation was accomplished by coupling STAX MK3 electrostatic earphones (Stax, Miyoshi, Japan) to a hollow ear bar inserted into the contralateral ear. Stimuli were calibrated using a one-quarter-inch microphone (Model 7017, ACO Pacific) and custom software (SigCal, TDT). Amplitude corrections were saved in a data file and applied to each stimulus to flatten the response of each earphone independently. Stimulus presentation was controlled by the data acquisition software.

For each recording site, the frequency response area (FRA) of the neuron or neuron cluster was measured by presentation of a tone series (50 ms duration; 5 ms cos2 ramp; 300 Hz to 32 kHz in 0.075 log10 steps, or about 0.25 octave) in 10 dB steps from 2 to 72 dB SPL. Each frequency-intensity combination was presented five times con-
secutively at a rate of 1 Hz. BBN stimulation was achieved by presentation of Gaussian noise bursts (50 ms duration; 5 ms cos^2 ramp) presented in 10 dB steps from –8 to 72 dB SPL RMS. Assuming a bandwidth of 37.7 kHz (range of the stimulus delivery system), 72 dB RMS would constitute a spectrum level of 26.0 dB SPL. Each noise burst stimulus was presented 15 times consecutively at a rate of 1 Hz.

Data acquisition

Multunit (referred to hereafter as "units") recordings were made with 1-MΩ tungsten microelectrodes insulated with Parylene C or polyimide tubing (MicroProbe, Potomac, MD). Electrodes were advanced at an angle perpendicular to the pial surface using a hydraulic or piezoelectric microdrive unit (David Kopf Instruments, Tujunga, CA). For sites on the gyral surface (e.g., CL, AI, R), the electrode angle ranged from about 30–45°. Penetrations targeting the lower bank of the lateral sulcus (LS; e.g., CM, AI, RM, R) were made by a vertical approach through the overlying parietal cortex. The angle of penetration for these sites was slightly oblique, depending on location within the LS. The locations of recording sites in auditory cortex were identified in reconstructed serial sections of each brain using established cytoarchitectonic criteria and tonotopic gradients. In each case, recording sites were spaced by 0.5 mm and tended to be concentrated in one auditory cortical area. Thus in no single case was a complete map of more than one area obtained. By compiling the results across cases, the number of recording sites was determined for each auditory cortical area: A1 (37), CM (48), R (5), RM (2), CL (7). Neurons in layer III and IV were targeted by advancing the electrode in 50-μm steps from the pial surface at depths ranging from 500 to 1,200 μm. A composite of the recording sites over all cases included in this report is shown in Fig. 3.

Stimulus presentation and data acquisition were controlled by Brainware software (TDT) and TDT hardware. In most electrode penetrations, single channel recordings were made, but in some penetrations, recordings were obtained simultaneously from two to four electrodes at approximately the same depth, separated by 0.5 or 1 mm. Signals were amplified (10,000 times) and filtered (300-3,000 Hz). Waveforms and timestamps of spikes (500 or 1,000 ms; re: stimulus onset) were saved on a computer hard drive for off-line sorting and analysis. At the end of each recording procedure, electrolytic marker lesions (10 μA for 10 s) were made in key locations to facilitate reconstruction of recording sites.

Data analysis

In this report, our analyses concern only recordings from A1 and CM. Neurophysiological data were analyzed using custom software in Matlab (Mathworks, Lowell, MA) to obtain response profiles for each stimulus set. Responses were plotted in raster format or as peristimulus time histograms (PSTHs). If the parameters to be analyzed were not distributed normally, model-free statistical tests were used as indicated.

Response latency and firing rate

For all stimuli used in this study, firing rate (spikes/s) was calculated from the number of spikes summed over all trials from response onset to 50 ms after stimulus onset. This value was used to obtain the FRA for tones and rate-level functions for BBN. Response latencies were defined as the minimum time at which the firing rate first became greater than six times the SD of the baseline multunit spontaneous firing rate (Fig. 2). Baseline was calculated from stimulus onset to 10 ms after stimulus onset. A bin size ranging from 0.1 to 1.0 ms was used to optimize temporal resolution depending on relative response strength and level of spontaneous activity.

FRA

The FRA of each multunit cluster (Fig. 3) was calculated from responses to tones at each frequency-intensity combination in the stimulus set. For the analyses included in this report, Fc (center frequency) was also estimated at each stimulus intensity from the firing rate-weighted average of octave-transformed frequencies at that intensity, as in Eq. 1

\[ F_c = \exp \left( \sum_{i} r \log(f_i) / \sum_{i} r_i \right) \] (1)

where \( r \) equals the firing rate, and \( f \) is the tone frequency, both indexed by \( i \). The characteristic frequency (CF) was taken as the Fc at the lowest intensity for which a significant response was obtained [i.e., threshold \( (TF_{CF}) \)]. Thus CF was equal to Fc at threshold. Threshold could not be determined for some units, because neurons were responsive at levels below the intensity range minimum (i.e., 2 dB SPL). In these cases, CF was estimated at 2 dB SPL from Eq. 1.

Tuning bandwidth (BW) was estimated at every intensity. For a given intensity, each frequency was expressed as a partial octave (0.25 octave/bin), reflecting the resolution of the FRA stimulus set. The octave intervals at which a response was measured were summed as an index of the BW at that intensity. In addition, mean bandwidth (MBW) was calculated from the BWs at all sound intensities to which the unit responded.

Rate level calculations for BBN stimuli

The BBN response threshold was identified as the lowest intensity at which a significant response was elicited by a BBN stimulus. Rate-level functions were calculated from the average spike rate over 15 trials at each intensity in the stimulus set. For each rate-level function, a nonmonotonicity index (NMI) was calculated from the ratio of the firing rate at 72 dB SPL to the maximal firing rate, subtracted from 1. Related measurements were SPL_{max}\_FRC, the intensity at which a unit responded maximally, and SPL_{min}, the intensity at which response latency was minimum.

A principal component analysis (PCA) of the rate-level functions was also performed to account for the variability in their shapes, rather than characterizing functions by their values at a particular intensity. For PCA, rate-level functions were treated as nine-dimensional vectors, corresponding to the number of BBN intensities examined. The mean vector was subtracted from each vectors, after normalization of its length. Then, principal components (PCs) were obtained as eigenvectors of a covariance matrix of vectors, which constituted orthogonal axes in nine-dimensional space. The PC score of individual vectors was estimated from the projection of that vector onto each PC. For each PC, the proportion of the total variance accounted for was obtained from the corresponding eigen-values of the covariance matrix.

Perfusion and histology

At the end of the recording experiment, a lethal dose of pentobarbital was administered. Just after cardiac arrest, the animal was perfused through the heart with cold (4°C) saline, followed by cold 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. Immediately following the perfusion, the brains were removed and photographed. The cerebral hemispheres were separated from the thalamus and brain stem, blocked, and placed in 30% sucrose for 1–3 days. The cerebral hemispheres were cut perpendicular to the LS in the caudal to rostral direction at 40 μm. In each series of sections, every sixth section was processed for: 1) fluorescent microscopy; 2) biotinylated dextranamine (BDA) or cholera toxin subunit β (CTB; Molecular Probes, Eugene, OR); 3) myelin (Gallyas 1979); 4) acetylcholinester-
ase (Geneser-Jensen and Blackstad 1971); 5) stained for Nissl substance with thionin; and 6) parvalbumin immunohistochemistry.

Reconstruction of serial sections

Areas of auditory cortex were identified by architectonic criteria established in New and Old World monkeys (see Hackett et al. 1998a, 2001), including marmoset monkeys (de la Mothe et al. 2002). Electrode tracks and electrolytic marker lesions were identified in each section and used to reconstruct surface maps of electrode penetrations. Figure 1 contains photomicrographs showing the cytoarchitecture (Nissl stain) unique to A1, CM, and adjacent areas. Briefly, the core areas A1 and R (Fig. 1, A and B) exhibit koniocellular features typical of A1 in other species, except that layer IV is narrower in R and pyramidal cells in layer III are slightly larger. Caudal to A1, CM (Fig. 1C) is characterized by a broad layer III populated by large pyramidal cells that exhibit poor radial alignment. Layer IV is distinct, but narrower than in A1, and layer V is more densely populated. Medial to A1, layer III in CM (Fig. 1D) is less broad, but the large pyramidal cells remain a prominent feature. Rostral to CM and medial to R is RM (Fig. 1E). Compared with CM, pyramidal cells in lower layer III are smaller in RM and radial alignment in all layers is more distinct. The lateral belt areas ML and CL (Fig. 1, F and G) have a distinct layer V containing a distinct population of large pyramidal cells. Radial organization is particularly distinct in ML from the white matter through layer III, whereas the pyramidal cells in layer III of CL are less orderly, as in CM. The retrolateral area (Ri) (Fig. 1H) occupies the fundus of the lateral fissure medial to CM on the lower bank and S2 on the upper bank. Laminar compression is pronounced and columnar spacing is broad, creating a distinct border with CM.

Results

Responses to tones

CATEGORIZATION OF RESPONSE PROFILES. The response profiles of multiunit response profiles were sorted into onset, sustained, onset-sustained, or pause types, adopting previously established criteria (Recanzone 2000). Profiles with an onset transient (onset or onset-sustained), like those in Fig. 2, were clearly the most prominent in both areas. Proportions of neurons with those types were similar in A1 (28/37) and CM (41/48) (Fisher’s exact test, $P > 0.05$). The relatively high proportion of neurons with onset-sustained profiles obtained under ketamine anesthesia compares favorably with recordings in awake monkeys (Lu et al. 2001; Recanzone 2000) but contrasts with pentobarbital or isoflurane anesthesia, in which onset response profiles for brief stimuli were typical (Cheung et al. 2001a). We did not observe clear offset transients in our data sample. Instead, there usually was a rebound increase in firing rate in both A1 and CM. In these cases, the rebound most often followed a period of suppression that began immediately after the onset transient.

TONOTOPIC MAPS AND RELATED FEATURES. An orderly representation of CF was found in both A1 and CM. Shown in Fig. 3 are schematic diagrams of cases 2–6 showing the distribution of CF by location relative to the borders of A1 with surrounding areas. The CF distribution in A1 was tonotopically organized. On the lateral surface, the CF progression in A1 decreased from caudal to rostral and reversed at a border determined to be R. In cases 2 and 6, for example, the lowest CFs were located in the rostrolateral portion of A1. Caudally, the CF gradient increased, reversing at the border with area CL (cases 2, 4, and 5). Within the LS, areas CM and medial A1 were explored by vertical penetrations (cases 3–5). The caudomedial portion of A1 was populated by high CF units. The gradient reversed rapidly beyond the border with CM. Broadly tuned or high CF (>10 kHz) units were concentrated within about 1 mm of the border, whereas units with lower CFs (<5 kHz) populated the caudal extent of CM. Thus the tonotopic gradient along the rostro-caudal axis in CM seemed to change steeply from high-to-low CF. While this could have been due to coarse mapping of this area, penetrations were evenly spaced with a grid interval no >500 μm in most instances. This suggests that there may have been relatively few units with an intermediate CF (5 kHz < CF < 10 kHz) in CM, and that these were located within a narrow band of cortex. Inspection of the distributions of CF in A1 and CM (Fig. 4, bottom) confirms these observations. Excluding the subpopulation of broadly tuned units, the CFs of units sampled in CM were biased toward low or high frequencies. In comparison, the CF distribution in A1 peaked around 4 kHz and was fairly flat

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**FIG. 1.** Sections of the marmoset monkey superior temporal cortex (case 02–60) stained for Nissl substance to reveal laminar cytoarchitecture of individual areas. Roman numerals denote cortical layer delimited by black lines at the left of each panel. A: area auditory 1 (A1; core). B: area rostral (R; core). C: caudomedial area (CM; belt, caudal to A1). D: area CM (belt, medial to A1). E: area rostromedial (RM; medial belt). F: area middle lateral (ML; belt lateral to A1). G: area caudolateral (CL; belt, lateral to CM). H: area retrolateral area (Ri; fundus of lateral fissure medial to CM).

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FIG. 2. Response latency for tone stimulation of 3 recording sites in A1 and CM. Each set of panels (left, middle, right) corresponds to 1 recording site. Each panel contains raster plots of spikes recorded at 1 stimulus intensity (2–72 dB SPL) plotted as a function of stimulus frequency (300 Hz–32 kHz, 5 rows/trials per frequency). Response latency at each frequency in the raster plot is indicated by the vertical black bar, which spans 5 rows. Left: responses of a broadly tuned cluster in CM (case 02–76). Middle: low characteristic frequency (CF; 1.9 kHz) cluster in CM (case 02–60). Right: high CF (15.9 kHz) cluster in A1 (case 02–60).

FIG. 3. Frequency tuning and CF topography. Top: schematic drawing of the marmoset superior temporal region showing recording sites (filled circles) compiled across all cases included in this study. The lateral sulcus (LS) was graphically unfolded to show the auditory and somatic sensory areas located on the upper and lower bank of the fissure. Red, CF>10 kHz; yellow, 5 kHz<CF<10 kHz; blue, CF<5 kHz; black, broadly tuned; white, somatic sensory response. STG, superior temporal gyrus; CiS, circular sulcus. Inset: right hemisphere of the marmoset brain highlighting the location of auditory cortical areas (box). Bottom right: schematic topographic maps of recording sites for each case. Within each panel (case), the CF of each recording site is indicated by grid color (color scale bar). Black squares indicate recording sites of broadly tuned units. Recording sites are spatially segregated by about 500 μm. The location of the LS is shown by the dashed black line. Borders between areas are indicated by thinner dashed gray line. Bottom left: examples of frequency-response areas (FRA) recorded from 1 site in A1 and 13 sites in CM of case 4. Black asterisk denotes same location shown at right. In each panel, the FRA of 1 multiunit cluster is shown. Color scale indicates percentage of maximum response (spike rate) for that cluster, where red is 100% and blue is 0%. Red line within each FRA tracks the center frequency (Fc) calculated at each intensity.
through 16 kHz. The distributions of CF in A1 and CM were significantly different [2-tailed Kolmogorov-Smirnov (K-S) test, \( P < 0.01 \)], suggesting that a proportional bias in the representation of CF may differentially characterize the two areas.

Further analyses of the FRAs obtained in A1 and CM revealed that stimulus intensity had variable effects on responses to tones in both areas. First, THCF values were lower in CM (Fig. 4, top left). All thresholds were 52 dB SPL or lower, most often at 22 dB SPL for A1 and 2 dB SPL for CM. However, the lowest tone intensity in our FRA stimulus set was 2 dB SPL, so actual thresholds could have been lower for some units. Overall, the THCF distribution in CM was shifted significantly to lower intensities compared with A1, regardless of whether the broadly tuned units were included in (1-tailed KS-test; \( P < 0.01 \)) or excluded from (1-tailed KS-test; \( P < 0.05 \)) the sample.

Second, as the FRAs in Fig. 3 suggest, Fc varied with intensity. Even though the correlation between THCF and CF was not significant for either A1 or CM at threshold (\( P > 0.05 \); Fig. 4, top right), the Fc tended to shift to lower frequencies at higher intensities. In Fig. 5, the difference between Fc\(_{72\text{dB}}\) and CF (Fc\(_{72\text{dB}}\) − CF) was plotted against CF for all units in A1 and CM. There was a significant negative correlation between the shift in tuning (Fc\(_{72\text{dB}}\) − CF) and CF in the two areas (\( r^2 = 0.391 \) for A1, \( r^2 = 0.609 \) for CM, \( P < 0.01 \) for both), but correlation coefficients were not significantly different (\( P > 0.1 \)). Thus in both areas the Fc of low CF units stayed relatively constant, whereas the Fc of high CF units shifted to lower values as intensity increased.

**TUNING BANDWIDTH.** Tuning bandwidth was assessed in several ways. First, a global estimate of responsiveness to tones was obtained from MBW (see METHODS). Comparison of the MBW distributions in A1 and CM (Fig. 6) revealed that the A1 distribution was shifted toward significantly narrower tuning compared with CM (1-tailed K-S test, \( P < 0.05 \)). A distinct subpopulation of CM units was identified that was responsive across most of the FRA stimulus range (Fig. 2, left). These units, defined by a MBW\(_{>6\text{octaves}}\) were categorized as broadly tuned. In our sample, only one A1 unit was found to be broadly tuned, with a THCF of 22 dB SPL, whereas there were nine broadly tuned CM units, all with a THCF at 2 dB SPL. The proportion of broadly tuned units in CM (9/48) was significantly greater than A1 (1/37) (Fisher’s exact test, \( P < 0.05 \)). Broadly tuned units were also treated separately in the calculation of CF (Fig. 4, “Broad”).

**FIG. 4.** Distributions of CF and threshold CF (THCF) of units in A1 and CM. **Bottom:** distributions of CF of units in A1 (black) and CM (gray). Broadly tuned units are shown separately for both areas (right). **Top left:** distributions of THCF of units in A1 and CM. **Top right:** scatter plot of A1 and CM units, plotting the THCF of each unit against its corresponding CF. The large symbol (bottom right in scatter plot) represents 9 broadly tuned units in CM. Otherwise, each symbol represents 1 unit/cluster. The x- and y-axes of the scatter plot are common to CF distribution histogram (bottom) and THCF distribution histogram (top left), respectively. Note, in subsequent figures, A1 and CM units are designated by black and gray shading, respectively.

**FIG. 5.** Relationship between Fc and stimulus intensity in CM and A1. The difference between Fc at 72 dB and the CF at threshold (Fc\(_{72\text{dB}}\) − CF) is plotted against CF for units in A1 (black dots) and CM (gray dots). Regression lines for each are also shown.

**FIG. 6.** MBW distributions of neurons in A1 and CM. MBW was estimated for each unit by averaging BWs (expressed in octaves) at all 8 levels in the FRA stimulus set (2–72 dB SPL, 10-dB step).
indicated in black and populated the rostral (high CF) end of CM. This suggested the possibility that there exists a subarea of broadly tuned units in CM.

Next, BW was calculated in 10 dB steps from THCF to 40 dB above. (i.e., BW_{TH} to BW_{40}). Note that many units were responsive over several octaves at THCF. For some units, the absolute intensity for the BW_{30} and BW_{40} calculations were above the ceiling intensity (72 dB SPL), so BW at 72 dB was used. As Fig. 7 shows, the BWs widened with intensity for units in A1 and CM. Comparison of the distributions at BW_{TH} to BW_{20} revealed no significant differences between areas (2-tail K-S test, \( P > 0.05 \)). At BW_{30} and BW_{40}, however, the BWs of CM units were wider compared with A1 (\( P < 0.05 \)). The proportion of units in both areas with BWs < 1.5 octaves was not significantly different from BW_{TH} to BW_{20} (Fisher’s exact test, \( P > 0.05 \)), but was significant for BW_{30} (\( P < 0.01 \)), where no CM units had BW_{30} < 1.5 octaves.

While the foregoing indicates that tuning in A1 and CM was similar only at low intensities, these calculations were based on intensity relative to THCF. Since THCF differed among neurons (Fig. 4), we reanalyzed BWs based on absolute intensity, irrespective of THCF (Fig. 8). At the lowest intensity (2 dB SPL), which was below THCF for most units, BWs were distributed over the full six-octave range, with a slight bias toward narrower BWs. As intensity increased, the number of responsive units increased, and the BW distributions shifted to higher values in both areas. Comparisons of median BWs and their distributions indicated that median BWs at 2 and 12 dB SPL were not significantly different among units in A1 and CM (Mann-Whitney \( U \) test; \( P > 0.05 \)), whereas, at higher absolute intensities, median BW tuning in CM was significantly greater than in A1 (\( U \) test, \( P < 0.05 \)). Therefore these results confirm those based on intensity relative to THCF.

Finally, since the FC of high CF units shifted to lower values as intensity increased (Fig. 5), we considered that BW may correlate with CF. The subpopulation of broadly tuned units was excluded, there was no clear correlation between CF and BW_{TH}, BW_{10}, or BW_{20} (\( P > 0.05 \), both areas). In CM only, BW_{30} and BW_{40} were significantly correlated with CF, as BW became wider at lower intensities than in A1 (\( P < 0.05 \)). In both areas, however, there was a positive correlation between CF and BW at 62 and 72 dB SPL (\( P < 0.05 \)). Thus units with higher CFs tended to have wider bandwidths at higher intensities in both A1 and CM.

MINIMAL RESPONSE LATENCIES FOR TONES. In Fig. 9A, the distributions of minimal response latency for tones are plotted for A1 and CM. Median response latency in CM (13.4 ms) was significantly shorter than in A1 (17.8 ms) (\( U \) test, \( P < 0.001 \)). The latency difference between areas is comparable to the difference between AAF and A1 in other mammals (see DISCUSSION). The correlations between minimal latency and CF were not significant in either A1 or CM (\( P > 0.7 \); Fig. 9B). In addition, while minimal latencies were generally obtained from responses to the highest tone intensity (72 dB SPL), the correlation between FC_{72dB} and minimal latency was not significant in either area (\( P > 0.3 \)). Last, since the frequency at which minimal latency was obtained (F_{minL}) did not necessarily correspond to FC_{72dB}, we also quantified the correlation between F_{minL} and minimum latency. This was also not significant in either area (\( P > 0.5 \)), consistent with the fact that F_{minL} was significantly correlated with both CF and FC_{72dB} in A1 and CM (\( P < 0.005 \)). Thus there was no clear relationship

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**FIG. 7.** BW distributions of A1 and CM units at/above THCF. Distributions of BW at 10, 20, 30, and 40 dB above THCF of individual units of A1 and CM are shown from bottom to top. BW bin size is 0.5 octave. Results include broadly tuned units.

**FIG. 8.** BW distributions of A1 (black bars) and CM (gray bars) units as a function of tone intensity (2–72 dB SPL, 10-dB steps). BW bin size is 0.5 octave. Results include broadly tuned units.
RELATIONSHIP BETWEEN FIRING RATE AND LATENCY. Most units in A1 and CM exhibited an inverse-like relationship between latency (and its variance) and firing rate across the range of the FRA stimulus set (Fig. 10A). To determine whether there were any differences in this relationship between A1 and CM, the following exponential decay function was applied,

\[ Y = Y_0 + A \times \exp(-BX) \]

(X = firing rate, Y = latency, \(Y_0\) = asymptote of Y, \(A\) = the difference between estimated latency at zero firing rate and \(Y_0\), and \(B\) = rate of change in latency depending on firing rate). Application of this function approximated a linear fit after log-transformation of latencies and preserved the trend toward lower variance for stronger responses (DeWeese et al. 2003; Heil 1997; Mendelson et al. 1997). The asymptotes (\(Y_0\)) of most units in A1 (18.0 ms) and CM (15.1 ms) were significantly correlated (Fig. 10B) with minimal latencies (\(r^2 = 0.313\) for A1, \(r^2 = 0.692\) for CM; \(P < 0.001\)) after excluding a few units with erratic fitted curves. Accordingly, \(Y_0\) was significantly lower for units in CM compared with A1 (2-tailed K-S test, \(P < 0.01\)), since minimal latencies were also shorter in CM.

FC ESTIMATION BY RESPONSE LATENCY. The relationship between response latency and firing rate in both A1 and CM suggested to us that response parameters calculated from firing rate could also be estimated by response latency. To test this hypothesis, \(F_{c_{\text{Latency}}}\) was estimated at each intensity in the FRA stimulus set by substituting the inverse response latency for \(r_i\) (firing rate) in Eq. 1. At all intensities, \(F_{c_{\text{Latency}}}\) was correlated with Fc based on firing rates in both areas (\(P < 0.001\)), indicating that Fc can be reliably predicted from \(F_{c_{\text{Latency}}}\). Significant biases (sign test, \(P < 0.05\)) were identified for some units in A1 and CM, but these were not systematic in either direction in A1 or CM.

Responses to noise bursts

RATE-LEVEL FUNCTIONS. Analyses of responses to BBN stimuli revealed no significant differences between A1 and CM. Response thresholds were uniformly low (Fig. 11A; 2-tailed K-S test, \(P > 0.05\)), and mean rate-level functions were monotonic and saturating for most neurons in both areas, with similar SD over the intensity range (Fig. 11B). A two-way ANOVA (A1 or CM and BBN intensity) showed a significant effect of BBN intensity, but not of cortical area or the interaction. Calculation of the non-monotonicity index (NMI) (see METHODS) revealed that a small number of neurons in A1 (5/26) and CM (9/43) could be classified as nonmonotonic (index values \(>0.5\), i.e., 50% reduction in firing rate at 72 dB re: maximum firing rate). Comparisons of the NMI and related parameters (i.e., SPLmax FR, SPLmin L) were not significant between areas (2-tailed K-S, \(P > 0.05\)).
Whereas the NMI has revealed differences between A1 and PAF in the cat (Stecker et al. 2003), the analysis revealed no difference between A1 and CM in the marmoset. However, we noted that the SD of rate-level functions was large, even at the lowest intensity (Fig. 11B). Therefore PCA was used to determine whether neurons in A1 and CM were actually different by accounting for the shapes of the functions across the intensity range. The values of all PCs showed a unimodal distribution around the mean rate-level function in both areas and no significant difference between areas (2-tailed K-S test, \( P > 0.05 \)).

**MINIMAL RESPONSE LATENCIES FOR BBN STIMULI** Figure 12 shows the distributions of minimal response latencies to BBN (left) and tones (bottom) for units in A1 and CM. As was the case for tones, median BBN latencies in CM (median, 14.30 ms) were significantly shorter than in A1 (median, 20.90 ms; \( U \) test, \( P < 0.001 \)). Within each area, the minimal response latencies for tones and BBN were well correlated (A1: \( r^2 = 0.186 \); CM: \( r^2 = 0.575 \), \( P < 0.05 \) for both areas; Fig. 12, top right) and not significantly different (Wilcoxon signed-rank test, \( P > 0.5 \) for both areas). A two-way ANOVA of log-transformed minimal latencies with A1 or CM as the area factor and BBN or tones as the stimulation factor revealed an effect of areas (\( P < 0.001 \)) but not stimulus type or their interaction (both \( P > 0.05 \)).

**DISCUSSION**

The goal of this study was to explore differences in the response properties of neurons in areas A1 and CM of the marmoset monkey auditory cortex. Multunit recordings indicated that both areas were tonotopically organized and shared a high CF border, but the tonotopic gradient was more compressed in CM. Units in both areas had similar robust onset responses with short response latencies, intensity-dependent tuning bandwidth, and largely monotonic BBN rate-level functions. In contrast, CM units had shorter minimal response latencies, lower thresholds for tones, greater expansion of bandwidth at higher intensities, and a population of broadly tuned units. In comparison with A1 and CM, neurons in the lateral belt areas were poorly responsive to tones and noise bursts under ketamine-xylazine anesthesia. The data support the hypothesis that A1 and CM play distinct functional roles in the processing of sound, but also suggest that neuron responses in CM overlap more with A1 than with the lateral belt areas.
Tonotopic organization

The location and tonotopic organization of A1 was comparable with that of previous studies of the marmoset monkey (Aitkin et al. 1986, 1988; de la Mothe et al. 2002; Luetthke et al. 1989; Nagarajan et al. 1998) and other New World species as well (owl monkey: Fitzpatrick and Imig 1980; Imig et al. 1977; Morel and Kaas 1992; Recanzone et al. 1993; 1999; squirrel monkey: Cheung et al. 2001a; Forbes and Moskowitz 1974). In A1, the lowest CF units (<5 kHz) were concentrated rostrally and laterally on the lateral surface of the STG, whereas the highest CF units were found caudally and medially, within the lateral fissure. CFs ranged from 1 to 23 kHz, with an overrepresentation between 2 and 16 kHz. At higher intensities, however, responses were found to tones ≤32 kHz.

CM was opposed to A1 caudally and medially. From the caudal border of A1, CM extended about 2 mm toward the caudal terminus of the LS and medially for about 1 mm from A1 toward the fundus and the junction with the retrolinsorial area. Units with high CF (>10 kHz) were located near the A1 border, and low CF units were located caudally. Although CM has not been completely mapped before, these results are in agreement with studies in other primates, in which either a reversal or disruption in the tonotopic gradient was encountered at the A1/CM border (Cheung et al. 2001a; Imig et al. 1977; Kosaki et al. 1997; Merzenich and Brugge 1973; Rauschecker et al. 1997; Recanzone et al. 1999, 2000a; Selezneva et al. 2003). Thus CM is the only field to share a high-frequency border with A1 in monkeys, comparable to the anterior auditory field (AAF) in cats, ferrets, and other mammals.

Broadly tuned CM units were found near the A1 border, intermixed with high CF units. Caudally, there was an abrupt transition from high to low CFs, but intermediate CFs (5 kHz < CF < 10 kHz) were rarely encountered, suggesting that this CF range is underrepresented in CM. This compression could be accounted for by the small size of CM relative to A1. In A1 isofrequency contours are spaced at about 1 mm/octave over some 5 mm (Aitkin et al. 1986), whereas CM extends no more than 2.5 mm beyond the border of A1. Assuming that CF contours are spaced at similar intervals in CM, the smaller size of CM could account for the absence of the intermediate CF representation. At moderate to high intensities, where the Fc of high CF units shifted to lower frequencies, high CF units were responsive to the intermediate frequencies. Accordingly, the frequency response in CM is more uniform at higher intensities. A discontinuous CF gradient was also apparent in maps of the AAF in studies of both the cat (Andersen et al. 1980; Lee et al. 2004; Phillips and Irvine 1982; Reale and Imig 1980; Tian and Rauschecker 1994) and ferret (Kowalski et al. 1995).

The dominance of a particular CF range may have behavioral significance, as in the bat auditory cortex (Suga et al. 1983). In marmosets, intermediate and high-frequency components (>5 kHz) dominate the vocal spectrum (Wang et al. 1995, 2000), suggesting that vocal calls are processed differently in the high and low CF domains of CM. Wang et al. (1995) reported that units with CF < 4 kHz were poorly responsive to twitters, consistent with tuning properties. They also noted that the first phrase of the twitter call primarily evoked responses from units with CF > 9 kHz, whereas the second phrase in the call evoked responses from units with CF > 5 kHz. These results suggest that the greatest activity in CM evoked by a twitter call would involve the high CF and broadly tuned units. Indeed, we have observed that high CF and broadly tuned units in CM respond robustly to twitter calls (Kajikawa et al. 2003). While the behavioral relevance of these observations is not known, it seems clear that complex sounds are processed differently in A1 and CM, and also within each area.

Frequency tuning

Overall, frequency tuning was significantly broader in CM than A1, consistent with previous observations in primates (Aitkin et al. 1986; Fu et al. 2003; Imig et al. 1977; Kosaki et al. 1997; Luetthke et al. 1989; Merzenich and Brugge 1973; Rauschecker et al. 1997; Recanzone et al. 1999, 2000a; Selezneva et al. 2003). This is also one of the few clear differences between A1 and AAF in cats and other mammals (Eggermont 1998; Knight et al. 1977; Kowalski et al. 1995; Schreiner and Urbas 1988; Rutkowski et al. 2003; Tian and Rauschecker 1994). The difference remained significant even after the units classified as broadly tuned were removed from the analysis, suggesting that broader tuning is a general feature of neurons in CM. The differences between A1 and CM were, however, intensity-dependent. At low intensities (within 20 dB of threshold) tuning bandwidth in the two areas was comparable. Only at higher intensities did the difference become significant. These findings underscore the observation that tuning among auditory cortical neurons is dependent on stimulus intensity (Phillips et al. 1994). These results also reveal that the receptive fields in A1 and CM are actually quite similar compared with the lateral belt and parabelt regions, where neurons are poorly responsive to tones in anesthetized animals (Rauschecker et al. 1995).

Response latency

In studies of auditory cortex, spike timing has become an important topic. In addition to the encoding of detailed temporal structure (Liang et al. 2002; Lu and Wang 2004; Lu et al. 2001), the information contained within the spike train is useful in distinguishing neuronal populations (e.g., Mendelson et al. 1997; Schreiner and Urbas 1988) and for the encoding of stimulus parameters such as sound location (Brugge et al. 1996; Eggermont and Moseop 1998; Malone et al. 2002; Middlebrooks et al. 1994, 1998). In this study, response latency was inversely related to intensity and firing rate for most units in A1 and CM (e.g., Phillips et al. 1985). The nature of this relationship was such that the variance decreased exponentially at higher firing rates, also consistent with studies of A1 in other mammals (DeWeese et al. 2003; Heil 1997; Mendelson et al. 1997). Furthermore, the consistency of this relationship was great enough to allow prediction of Fc on the basis of response latencies, suggesting that spectral information is redundantly represented in both firing rate and latency in A1 and CM.

With respect to latency differences between A1 and CM, we found that minimum response latencies for both tones and noise bursts were significantly shorter in CM. Within each area, latencies were almost identical for tones and noise. For A1, minimum response latencies were comparable to those recorded by others from the layer III/IV band in anesthetized New and Old World primates (Brosch et al. 1999; Cheung et al. 2001a,b; Recanzone et al. 1999), which are some 10–15 ms
shorter than for awake-behaving animals (Durif et al. 2003; Liang et al. 2002; Recanzone et al. 2000a; Scott et al. 2000). Between-study comparisons of response latencies for CM were less conclusive since few data are available. Scott et al. (2000) reported that some of the shortest latencies encountered in their survey of A1, ML/CL, and CM were found in CM. In contrast, Recanzone et al. (2000a) found no significant difference in average minimum response latencies between A1 and CM, but noted that neurons in A1 had the shortest peak latencies. However, both of these macaque studies reported significantly longer latencies in the lateral belt areas adjacent to A1 and CM. Similarly, response latencies in the AAF of other mammals have been found to be either shorter (Eggermont 1998; Kowalski et al. 1995; Norena and Eggermont 2002; Rutkowski et al. 2003) or the same as (Knight 1977; Phillips and Irvine 1982) latencies in A1 compared with significantly longer latencies in AII or PAF.

Given current models of auditory cortical processing in primates, in which the belt areas are thought to mediate a second level of processing (Kaas and Hackett 2000; Rausscher 2000; Rausscher et al. 1997; Recanzone 2000), it is difficult to understand how minimum response latencies in CM could be shorter than A1 (this study; Scott et al. 2000) or the same as A1 (Recanzone et al. 2000a), especially when latencies in the lateral belt are clearly longer. Moreover, we found that lateral belt neurons were poorly responsive under ketamine anesthesia, whereas neurons in A1 and CM responded vigorously. Taken together, these findings suggest that the functional organization of CM is not typical of the lateral belt areas. This issue is discussed further below.

Accounting for similarities and differences in A1 and CM

To the extent that neuronal activity in a given cortical area reflects the convergence of cortical and subcortical inputs, an accounting of those connections is essential to understanding the functional organization of that area. For areas A1 and CM, the anatomical features generally support the key findings of the present and previous studies, but some questions remain.

By definition, the dominant inputs to the core region arise from the MGv, whereas the principal inputs to the belt and parabelt areas include the broadly tuned neurons of the nonlemniscal thalamic nuclei (e.g., MGd, MGe, suprageniculate, limitans, pulvinar) and feedforward projections from the core (Burton and Jones 1976; de la Mothe et al. 2002; Hackett et al. 1998b; Jones and Burton 1976; Molinari et al. 1995; Morel and Kaas 1992; Morel et al. 1993; Pandya and Rose 1993; Pandya et al. 1994; Rausscher et al. 1997). Accordingly, neurons in the belt areas would be expected to exhibit broader tuning and longer response latencies than neurons in the core areas, which receive lemniscal inputs from the MGv (Molinari et al. 1995). Although the data are limited, at present, the connections of CM are further distinguished by a dominant projection to layer IV from the anterodorsal division (MGad) of the MGC (de la Mothe et al. 2002; Hashikawa et al. 1995; Molinari et al. 1995). The significance of this projection concerns the similarities between the MGad and the MGv. Both nuclei contain small densely packed cells and have the highest density of parvalbumin-immunoreactive (PV-IR) cells in the MGC (Molinari et al. 1995). In addition, both nuclei appear to receive inputs from the central nucleus of the inferior colliculus through a PV-IR pathway ascending in the brachium of the inferior colliculus (Molinari et al. 1995). Therefore the MGv and MGad may be the recipients of parallel projections from the primary (lemniscal) auditory pathway. In that respect, the MGad may be comparable to the lateral division of the posterior group (Pol) in cats, as suggested by Jones (1997). This is an intriguing possibility, because the Pol is tonotopically organized and comprised of neurons with narrow tuning and short latencies comparable to those of the MGv (Imig and Morel 1984, 1985a,b). Accordingly, the primary-like responses in A1 and CM could be driven by independent inputs from the MGv and MGad, respectively. Given the neurophysiological similarities between A1 and CM, this hypothesis deserves further evaluation.

With respect to the influence of corticocortical connections, the relationship between A1 and CM is also uncertain. Injections of tracers into both areas of New and Old World primates have produced widely disparate results (Aitkin et al. 1988; Cipolloni and Pandya 1989; Fitzpatrick and Imig 1980; Galaburda and Pandya 1983; Morel and Kaas 1992; Morel et al. 1993). While most of these studies revealed that A1 projects to CM, the laminar specificity and topographic arrangement of those projections were inconsistently identified across studies. Therefore it is unclear whether neurons in A1 can drive responses in CM (i.e., feedforward projection) or whether those inputs are largely modulatory. This information relates directly to issue concerning serial processing between A1 and CM. In addition, the anatomical data do not reveal whether neurons in CM are integrating across frequency bands in A1 or if the connections are topographic and favor matched frequency bands. This information would help to determine the source of broader tuning in CM. Detailed anatomical studies are needed to clarify answer these questions.

Technical considerations

Direct comparison of these results with those of previous studies is limited by a number of variables, including species differences, state of arousal, method of sound delivery, laminar recording depth, data acquisition, and analysis. Given these factors and the small number of studies available for comparison, it is clear that further investigation is needed before significant modifications of the primate model are warranted. A significant limitation of this and previous studies in which two cortical areas were compared has been the reliance on sequential single-channel recordings distributed across more than one animal. The use of multichannel recordings to simultaneously record from neurons in two or more areas of interest would reduce variability and enhance the value of future comparative studies.

In conclusion, multiunit recordings in the marmoset monkey auditory cortex revealed that CM can be distinguished from A1 on the basis of responses to tones and noise burst stimuli. The results also indicated that responses in CM were more similar to those in A1 than to areas of the lateral belt cortex in ketamine-anesthetized marmosets. While the similarities and differences between areas can be partially accounted for by known anatomical features, detailed insights into the relationship between A1 and CM will require additional studies and refinement of the primate model of auditory cortex.
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