Reorganization in Awake Rat Auditory Cortex by Local Microstimulation and Its Effect on Frequency-Discrimination Behavior

SANJIV K. TALWAR AND GEORGE L. GERSTEIN
Department of Neuroscience, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received 6 June 2000; accepted in final form 5 June 2001

INTRODUCTION

A characteristic feature of sensory organization within the neocortex is the ordered representation of external stimulus parameters in the form of maps. The primary auditory (AI) cortex has sound frequency, reflected in the frequency response characteristics of individual neurons, systematically represented across its surface in a tonotopic arrangement. This functional organization has been demonstrated in a large number of species and by a varied number of methodologies (for reviews, see de Ribaupierre 1997; Pickles 1982). The mapping of AI by electrophysiological methods relies on identifying the frequency within a neuron’s receptive field (tuning curve) to which it is maximally sensitive—its characteristic frequency (CF).

A rich literature exists showing that cortical maps, like AI in the auditory cortex, are dynamically organized or plastic and demonstrate a capability for reorganization to an extent that they “. . . reflect their input histories and change in representational detail continuously, throughout life” (Merzenich 1987). A large number of studies in sensory cortices that mainly involve manipulating cortical input or conditioning learning procedures can be adduced as support of this view (for reviews, see Irvine and Rajan 1996; Jenkins et al. 1990; Weinberger 1993; Weinberger and Diamond 1987; Weinberger et al. 1990). In the auditory cortex, a variety of conditioning procedures have been shown to affect the frequency selectivity of individual auditory neurons such that there is an increase in cortical representation of behaviorally relevant frequencies (e.g., Bakin and Weinberger 1990). Similarly, sensory deafferentation by partial lesioning of the cochlea results in an expanded representation of frequencies adjacent to the lesioned range (Robertson and Irvine 1989).

It is of particular interest to know if such experimental changes in cortical stimulus representation have any intrinsic significance for perception by an animal. Some observations have indicated the possibility that an increased area of cortical representation may result in better processing of relevant stim...
ulii. For example, mice strains with a higher proportion of cortical neurons responding to certain frequencies appear to have better discrimination performance around these frequencies (Kulig and Willott 1984). As a different example reflecting this principle, studies in monkeys and humans who have undergone amputations have shown an increase in cortical representation of skin regions surrounding the amputations (Merzenich et al. 1983, 1984; Yang et al. 1994). In parallel, post-amputation perceptual changes are known to occur; these include a progressive increase in the sensitivity and discrimination ability of the limb stump (Teuber et al. 1949).

Improvements in sensory perception, however, occur more naturally as a consequence of long-term practice in specific behavioral tasks (Anderson 1980), a phenomenon often referred to as perceptual learning. Auditory frequency discrimination, for example, is a behavior that undergoes perceptual learning; i.e., it shows gradual improvements in discrimination ability with long-term training (Campbell and Small 1963; Demany 1985). These behavioral gains, additionally, are limited to the actual discriminanda frequencies used in training (Prosen et al. 1990; Recanzone et al. 1993; Talwar and Gerstein 1998). In this context, the studies by Recanzone et al. (1993) are especially germane: using primate subjects, they showed that improvement in frequency-discrimination performance is correlated with specific increases in the cortical representation of frequencies at which the animals are trained. Identical observations have also been made in the somatosensory system following improvements in somatosensory tasks (Recanzone et al. 1992b).

These studies support the important notion that cortical reorganization, particularly an increase in cortical representation, is the process that underlies perceptual learning (Jenkins et al. 1990; Merzenich et al. 1988). Nevertheless, the actual significance of cortical reorganizations observed in any given experimental situation remains difficult to evaluate. For example, it seems reasonable to suppose that changes in cortical representations seen in the general plasticity experiment must bear consequences for specific behaviors. The near ubiquitous expansion of central representation following either the alteration of normal cortical input or conditioning raises the issue of its perceptual consequences on one hand and of its relevance to perceptual learning on the other.

This study addresses two questions: does the increase in cortical representation of a specific stimulus parameter have any direct behavioral correlates and, specifically, is an increase in cortical representation sufficient for perceptual improvement?

We attempted to tackle these questions by reversing the traditional approach of plasticity experiments: instead of studying cortical reorganizations consequent to an experimental situation like conditioned learning, we first induced similar cortical changes and then examined if they could affect an animal’s perception. As a model, we artificially induced cortical plasticity in the AI map of the rat by focally stimulating with a weak electric current (intra-cortical microstimulation or ICMS); thereafter we investigated if this cortical reorganization could affect the rat’s ability to discriminate frequencies. Note that ICMS has been shown to effectively induce representational changes very similar to those seen following the traditional learning experiment (Bedenbaugh 1993; Dinse et al. 1993; Maldonado and Gerstein 1996a,b; Recanzone et al. 1992a; Sirois 1993). Specifically, in the anesthetized rat AI cortex, the main effect of ICMS is that, depending on the site of stimulation, it increases the cortical representation of particular frequencies (as well as the levels of correlated activity within this enlarged area) (Maldonado and Gerstein 1996a,b). These changes appear to be similar to those that accompany improvements in an animal’s ability to discriminate frequencies with practice (Recanzone et al. 1993), with the exception that they are temporary and disappear within a few hours.

The results of our study are structured at three different levels: the frequency response characteristics of AI cells and their overall organization are examined in both anesthetized and awake animal, the effect of ICMS on AI organization is studied in the awake animal, and the effect of ICMS-induced reorganization on the rat’s frequency-discrimination ability is evaluated.

**Methods**

**Experimental protocol**

Figure 1 depicts the overall sequence of events in the study. Rats were first trained to make fine frequency discriminations around a test frequency of 5 kHz. After the task was learned, an array of fine wire electrodes were implanted into the AI. A week after recovery, the CF of each implanted AI site was determined and one of these sites was chosen to be the site of future ICMS. The rats were then briefly retrained to make discriminations around a new test frequency. This new test frequency corresponded to the CF of the chosen ICMS site. Thereafter, the main ICMS/behavioral experimental phase began: subjects were required to make fine discriminations around the new test frequencies before and after ICMS enlarged its cortical representation. Differences in pre- and post-ICMS frequency-discrimination performance were statistically evaluated. Over the time course of the experiment, at three separate times, several recordings of AI frequency response were made.

**Subjects**

Thirty-six female albino rats (Charles River), 275–300 g in weight, started this study. Of these, 10 subjects (R1–R10) were selected to complete the ICMS/behavioral experiment on the basis of several electrophysiological criteria (see Results). Subjects were housed in pairs and maintained in a 12-h light cycle. They were trained daily and had free access to food; water, however, was restricted to an hour after training and was otherwise available as primary reinforcement in the operant chamber (subjects were allowed full water access for 6 days before and after electrode implantation). Under this regimen, subjects maintained 80–85% of their ad libitum weight. Additional subjects, C1–C4, served in control experiments that first evaluated the effects of ICMS in the cortex of awake animal. The care and use of animals conformed to National Institutes of Health guidelines: “Preparation and Maintenance of Higher Animals During Neuroscience Experiments” (NIH Publication No. 94-3207, 1994).

**Sound field**

The acoustic setting and its calibration for behavioral testing have been detailed in previous studies (Talwar and Gerstein 1998, 1999).
Briefly, a sound-transparent cage suspended from the ceiling of an anechoic sound-attenuated room served as the operant chamber. Sensory manipulanda consisted of a nosing hole with an in-built photo-electric switch in the front wall and an observing lever on the left wall. Sine waves, shaped into tone-bursts with a rise and fall time of 5 ms, were delivered through a speaker situated above the nosing hole; below the nosing hole a spout delivered water rewards. No standing waves were detected in the test cage, and the area of the cage within which the rat moved approximated a free field. All behavioral tests were carried out at 50 dB SPL. Being a sound-calibrated environment, the same cage also served as the platform on which neuronal responses were recorded.

Behavioral testing procedures

Two separate behavioral paradigms, which differed mainly in their cognitive difficulty, were used to test the rat’s frequency-discrimination ability. The two tasks are fully detailed elsewhere (Talwar and Gerstein 1998, 1999); their important features are described in the following text. Both tasks involved identifying a small frequency difference ($\Delta f$) from a test frequency ($f$). A subject’s ability in detecting this small frequency difference was evaluated before and after ICMS increased the AI frequency representation of $f$. The $\Delta f$ frequency difference was around known threshold levels for the rat, while $f$ depended on the CF of the chosen ICMS site (the frequency representation of which was increased by ICMS).

**TASK 1.** Subjects R1–R5 were trained to identify a change in the frequency ($\Delta f$) of a target tone from an ongoing train of tones presented at test frequency $f$ (Talwar and Gerstein 1998). The $f + \Delta f$ target signal was presented randomly at 5–35 s after the start of a series of $f$ tones in which tone sound duration and inter-tone interval were 250 and 350 ms, respectively. A nosing response within 1.75 s of target tone onset was scored as a hit and rewarded; lack of response in this period was a miss. Hits or misses successfully terminated a series; the next series began after a 2- to 4-s time-out. Nosing responses outside the hit window counted as false alarms, aborted the series, and resulted in a 8- to 12-s time-out. A test session consisted of 40 tone series ($\Delta f$ was constant within a session). Our previous study showed that rats are capable of detecting frequency differences as small as 3% ($f$) when tested in this paradigm (threshold performance).

**TASK 2.** In contrast to subjects R1–R5, subjects R6–R10 were tested in a more difficult discrete trial task (Talwar and Gerstein 1999). Subjects pressed the observing lever and were presented with a sequential pair of pure tones either of matching frequency ($f, f + \Delta f$) or nonmatching frequency ($f, f + \Delta f$). Tone duration/interval was 500/100 ms. A test session consisted of 200 trials with the probability of any trial containing $f + \Delta f$ being equal to 0.4. A nosing response to $f + \Delta f$ within 2 s from its onset counted as a hit. Responses to $f$ were false alarms. Not responding to $f + \Delta f$ or to $f$ was a “miss” and “correct rejection,” respectively. In this type of procedure, rats typically report threshold values of around $\Delta f = 6–7\%$. As with task 1, $\Delta f$’s remained constant within a test session.

For all behavioral sessions, hit and false alarm probabilities were calculated and performance measured using the theory of signal detection (Green and Swets 1966). Specifically, the rat’s ability to discriminate a small frequency difference ($\Delta f$) from $f$ was measured using the signal detection index $A’$ (Grier 1971; Pollack and Norman 1964). The index $R1$ measured response bias (Frey and Collier 1973). The computational formulae used to calculate these indices, where $h$ = hit probability and $fa$ = false alarm probability, were

$$A’ = \frac{1}{2}\sqrt{1 - \left(\frac{h - fa}{1 - (h - fa)}\right)^2},$$

$$R1 = \left[\frac{h + fa - 1}{1 - (h - fa)^2}\right].$$

The performance index $A’$ varies between 0 and 1; a measure of 0.5 indicates chance performance (i.e., $h = fa$) while $A’ = 1$ represents perfect performance. A value around $A’ = 0.84$ represents threshold performance (Talwar and Gerstein 1998). In contrast to $A’$, $R1$ varies between $-1$ and 1. Values of $R1$ near 0 indicate neutral bias—i.e., an equal tendency on the part of a subject to make a go (nosing) or no-go response under ambiguous signal conditions. Values of $R1$ near 1 indicate a strong bias to make go responses when subjects are unsure of the status of a signal.

All subjects were initially trained at 5 kHz with $\Delta f$’s set around threshold (~5% in task 1 and 7% in task 2). After training was completed electrodes were implanted into the AI cortex. A week after implantation, each subject was briefly acclimatized (3–4 sessions) to making discriminations around a new test frequency $f$. This new test frequency corresponded to the CF of an AI electrode site that was chosen to be the site of future stimulation. Testing for the effects of ICMS on frequency-discrimination behavior then began. First, the rat’s pre-ICMS frequency-discrimination performance was tested at a given $\Delta f$. Subjects then underwent a 4-h period of ICMS to enlarge the cortical $f$ representation. After this period, the rat’s post-ICMS frequency-discrimination performance at the same $\Delta f$ was re-evaluated. The actual magnitude of $\Delta f$ varied among subjects, and was chosen so that normal discrimination performance measured around $A’ = 0.75–0.80$ (the slope of the psychometric function in this region tends to be steepest). Every day, for 16 days, subjects underwent paired pre and post ICMS sessions; of these, 6 days were controls during which rat’s under went sham-ICMS sessions in which the physical arrangements were the same as actual ICMS sessions except that stimulation current was switched off.

Electrode assembly and implantation

Electrodes were an array of eight parylene C insulated 25 $\mu$m Nichrome wires (Redi Ohm-800) with their tips cut to a 45° bevel and soldered onto a 10-pin connector (MicroTech). Electrode tips were spaced at 300 $\mu$m. Impedance of each electrode was around 0.3 M$\Omega$ measured at 1 kHz. A 100-$\mu$m stainless steel wire served as the reference electrode. Anesthesia was induced by a mixture of ketamine (70 mg/kg) and xylazine (7.2 mg/kg) injected intra-peritoneally. 0.1 mg of glycopyrrolate (a synthetic atropine analogue) was injected intramuscularly to prevent excess bronchial secretions. 1–2 supplement doses ($\frac{1}{4}$ of induction dose) were sufficient to maintain surgical levels of anesthesia through the procedure. Body temperature was maintained at 37°C. During implantation, subjects were positioned in a stereotaxic apparatus in which the contralateral ear bar had been drilled ($\frac{3}{16}$-in diameter) so that a small loud speaker (Motorola) could be fitted on the end. The speaker had a near flat frequency response between 4 and 50 kHz ($\pm 4$ dB). Skin and fascia were incised sagitally in the dorsal midline of the head and reflected until clean bone was exposed. Four bone anchor stainless steel screws (size 0–80-1⁄16-in) were placed in the skull top. The dorsotemporal skull was exposed and a craniotomy (~3 × 2 mm) made directly overlying the Te1 auditory field (stereotaxic coordinates: Paxinos and Watson 1986; vascular patterns on the brain surface also provide an easy reference to the primary auditory cortex: Sally and Kelly 1988). The dura was nicked, and the electrode array, positioned in the rostral-caudal axis, was moved into the cortex by an electronically driven microdrive (Burleigh) to a depth of 0.8 mm (approximately corresponding to layer IV of the auditory cortex). The reference wire was wound around bone anchor screws. Final positioning of electrodes was determined on the basis of a strong response to sound clicks and tones delivered through the contra lateral ear bar. Multunit frequency response was evaluated on-line and repeated every 10 min. When these were judged stable the area surrounding the electrodes was packed with sterile gel foam. The electrode wires were first affixed in place (with acrylic) to the surrounding bone after which the entire assembly was bent upright and cemented normal to the skull top. While under anesthesia, subjects were transferred to the operant
Cage so that the frequency response of all implanted sites could be determined within a sound calibrated environment. Seven subjects were implanted in the left cortex, including all subjects trained on the discrete trial task in which the positioning of the observing lever made the left cortex contra lateral to the sound source. This precaution was taken because there is some evidence that frequency perception may specifically be related to sound originating in the contra lateral acoustic space (Jenkins and Merzenich 1984).

**Recording apparatus**

During recordings, a head-stage connector, housing operational amplifiers configured as followers, plugged into the connectors cemented on the rat’s skull. Signals passed through a preamplifier (gain = 50) and were filtered (f3, ab band-pass = 0.5–5 kHz) and amplified. In the awake animal, the head stage was built into the ends of flexible 24-gauge wires, which passed to the preamplifier through a swivel connector above the operant cage. Signals were fed through A/D converters, treated by digital signal processors (Plexon Scientific) and recorded on a PC. Software (Plexon Scientific) controlled amplification, filtering, and spike display. A separate circuit using a controlling PC-generated sound stimuli. Each spike and acoustic event was tagged with their times of occurrence and stored for later analysis. During recording the analog signal was fed through an amplitude detector, which output a digital signal when waveforms exceeded an (adjustable) amplitude. Local software provided real-time analysis.

**Auditory stimuli and analysis of response**

Pure tones at 15 logarithmically spaced frequencies from 4 to 40 kHz were used to determine frequency response characteristics of cells. All tones had a linear rise and fall time of 5 ms and duration of 100 ms. Tone intervals were 500 ms in the anesthetized animal; in awake animals, intervals varied from 0.5 to 2 s (in the awake animal, acoustic stimuli at unpredictable intervals more consistently resulted in an acoustic response). For most recordings, tone intensity was fixed at 50 dB SPL. Occasionally, however, tone intensities were also varied, from 20 to 70 dB SPL in 10-dB steps. For all recordings, each stimulus (i.e., each frequency/intensity combination) was presented 20 times at random. Anesthetized subjects were placed in the center of the cage floor with the sound source exactly contra lateral to the implanted cortex. For awake recordings, satiated subjects were free to move around the calibrated environment of the operant cage.

Recordings generally only included spike waveforms for which the signal-to-noise ratio was more than 3:1. Where possible, waveforms were first sorted using principal component (PC) analysis (Abeles and Goldstein 1970). Well-separated clusters of dots in the PC plot—the projection of a waveform onto the first two principal components—represented spike waveforms. These waveforms were called well-isolated spikes and unsorted multiunit activity (specified in a library of waveforms—represented spike waveforms). Where possible, waveforms were added and delivered through a constant current stimulus isolator (WPI A395). During ICMS subjects were placed in a 12 × 10 × 10-in Plexiglas cage. The stimulus current was fed from the cage ceiling through a flexible cable that plugged into the electrode assembly on the rat’s skull. A switch dictated the AI electrode site to be stimulated. No acoustic stimuli were presented during this period.

**Preliminary ICMS experiments**

In the rat AI cortex, ICMS-induced changes have been documented in the anesthetized subject (Maldonado and Gerstein 1996a,b). Similar effects in the awake animal, however, could not be assumed and needed to be first demonstrated. Our study, therefore examined this issue in four separate control subjects (C1–C4). These subjects did not take part in the later behavioral experiments.

Under anesthesia an electrode array was implanted in AI and a week of recovery followed. Thereafter, awake subjects were placed in the operant cage while auditory stimuli were presented and recordings obtained. Initial FRHs were obtained and labeled as pre-ICMS recordings. A specific AI site was then chosen to be stimulated. Animals were transferred to the ICMS chamber in which a 4-h period of ICMS was given. Thereafter, FRHs were again determined (post-ICMS recordings). After another period of 4 h, spent in the animals’ home cage, recordings were repeated (4 h post-ICMS).

**RESULTS**

Recordings from AI were obtained at three stages of the experiment (see Fig. 1). The first stage contained recordings obtained just after electrode implantation while animals were still anesthetized. The second stage was recordings in the chronic animal 1 wk after implantation in both awake and anesthetized condition. Here awake recordings were first obtained after which an induction anesthetic dose was given and recordings continued. The third stage had recordings obtained at the end of the experiment, again in both the awake and anesthetized condition. These recordings were obtained 2 wk after second-stage recordings.

At each recording stage, several sequential FRHs were constructed for all electrode sites (≥6 sequential FRHs; 20 stimulus repetitions; 15 logarithmically spaced frequencies from 4 to 40 kHz; 2- to 5-min intervals; 50 dB SPL). For second-stage recordings, in addition to the usual FRHs, a complete “tuning
curve was constructed by also varying intensities from 20 to 70 dB SPL.

Note that the main ICMS/behavioral phase of the experiment took place between the second and third stages of recordings. Subjects were selected for the main ICMS/behavior experiments only if second-stage recordings satisfied several criteria. First, at least three consecutive electrode sites within the array were required to demonstrate a strong short latency response to BF tones, with good frequency selectivity, and clear tonotopy. Second, signal quality was also required to be satisfactory (signal-noise ratio $\geq 3:1$). Electrode sites that fulfilled these criteria were considered to be within the AI field. The results reported in this study are only from such data.

**Single-unit isolation**

Our study used fixed fine wires to record from AI over a long duration of time. In the acute preparation well-separated point clusters indicating different single units were usually obtained in the principal component plane; sometimes, however, excess brain noise led to blurring of cluster boundaries, a problem that was, in part, traceable to superposition of waveforms from two or more nearby cells. To quantify the degree of this problem, we sifted through $10^5$ recorded spikes, looking for waveforms exhibiting more than one peak with the aid of an identification algorithm based on local maxima. A number of spontaneous spikes (3.78%) had multiple peaks. By contrast, in the set of waveforms occurring in response to a tone (the acoustic time window of interest), 10.13% exhibited more than one peak, indicating that spike superimposition is a relatively large source of waveform sorting error in rat AI. To minimize this error, we report only from cells that formed clearly delineated clusters during principal component analysis.

Over the long term, a period of around 2–3 wk, we found that fixed fine wires are capable of recording from the same population of cells (see following text). Although multiunit activity in the 10 subjects selected for the main behavioral experiment remained relatively stable for the duration of the experiment, there occurred some degradation in signal quality with time such that by the third recording stage very few single units were clearly identifiable. In general, individual waveforms tended to shrink, coalescing somewhat into a relatively amorphous population that became difficult to sort. In the acute preparation (the first recording stage), we obtained a single cell yield of 2.4 cells per electrode. By contrast, the cell yield in second-stage recordings, obtained 1 wk later, was limited to 0.26.

The observations that follow relate to 112 AI cells from first-stage acute recordings. Where possible, comparisons are also made between neural responses in the anesthetized and awake condition, drawing from the cell population isolated in second-stage recordings. This population comprised of 36 cells. Although this is a relatively small sample, the fact that the same cells were recorded across both anesthetized and awake conditions adds interest to the observations. Inferences for single units are also made on the basis of multiunit recordings since these constituted the bulk of data from second-stage recordings.

**Characteristics of strongly responsive cells**

Figure 2A shows the spontaneous firing-rate distribution in the AI cell population under surgical depths of anesthesia. Mean and median firing rates were 2.9 and 2.44 Hz, respectively. Given the spontaneous rates of firing in multiunit recordings, which was usually between 20 and 30 Hz (at the amplitude thresholds we set), these average rates estimate that multiunit recordings generally contained $\sim 10–12$ cells. Compared to cells in the anesthetized animal, cells in the awake animal demonstrated a much higher rate of firing. Figure 2B shows the firing rate distribution in 36 cells recorded in the awake subject while Fig. 2C shows the scatter of individual firing rates of these 36 cells recorded while subjects were in both anesthetized and awake conditions. The mean increase in firing rate in these cells, from the anesthetized to awake condition, was 4.8 Hz. Under anesthesia, neurons exhibited a strong tendency to fire periodically, in distinctive rhythmic bursts often synchronous across electrodes, and with a frequency of $\sim 1–3$ Hz. All 36 cells recorded in the second stage demonstrated this feature under anesthesia; by contrast none of the same cells showed a strong periodic rhythm in their awake activity [straightforward autocorrelograms (not shown) demonstrated the difference].

The best-recognized AI neuronal characteristic was a short-latency ($<20$ ms) response to the onset of pure tones presented at or near a neuron’s BF. In general, the neurons we recorded from showed fairly large differences in the strengths of their evoked responses, in the range of frequencies to which they were sensitive and in the stability of their frequency response across recordings. In the interests of imposing some order on the data, we divided the cell population on the basis of their response strength to BF tones at 50 dB SPL (see METHODS).

Of the 112 cells that were isolated from acute first-stage recordings, roughly $\frac{2}{3}$ ($n = 40$) were classified as acoustically strong and another $\frac{1}{3}$ ($n = 34$) as weak. The remaining $\frac{1}{3}$ ($n = 38$) did not respond to tones. In second-stage recordings, of the 36 cells under anesthesia, 14 cells responded strongly, 10 responded weakly, while 12 did not respond. We found that cells that were classified as acoustically strong or weak under anesthesia maintained their respective strengths in the awake condition. However, 10 of the 12 cells that did not respond under anesthesia emerged with a clear acoustic response when awake (8 weak, 2 strong).

**Characteristics of strongly responsive cells**

Figure 3, A–D, shows the different temporal patterns of response, in the form of peristimulus time histograms (PSTHs), identified within the strongly responsive AI cell population (from acute, first-stage recordings). The standard neuronal response to the onset of a BF tone was a phasic short latency on response, followed by a period of suppression of varying
This pattern was seen in ~50% of neurons. In another 30% of neurons this ON response was followed by one (usually) or more long latency response components (Fig. 3B). Two other kinds of responses were also encountered: ~8% of neurons were ON-OFF cells and demonstrated a strong phasic response to both the onset and offset of tones (Fig. 3C); ~12% of cells demonstrated some form of tonic or “through” response to tones that continued for the duration of the stimulus (Fig. 3D). The preceding percentages were obtained by statistical comparison of the firing rate of each neuron in the time window of interest, relative to the level of its spontaneous activity. This was done in a manner identical to that adopted for identifying AI neurons responsive to tones (see METHODS). For example, a significant OFF component was identified by comparing responses in the relevant time window (5–50 ms after tone offset) with spontaneous firing rates in the 100 ms before tone-presentation, averaged over 100 presentations (t-test at α = 0.05). In general, multunit PSTHs also demonstrated temporal response patterns similar to that of single cells. From all multunit recordings, we found that 62% of AI sites showed a simple ON response, 25% an additional long latency component, 10% a through response, and 3% an ON-OFF response. Other response patterns could not be identified.

In second-stage recordings, strongly acoustic neurons were either simple ON cells or had an additional long-latency component. In these recordings, anesthetized cells maintained their temporal characteristics in the awake condition. Except for the higher spontaneous firing rates, the PSTHs of awake cells were similar to their anesthetized PSTHs.

Neurons that demonstrated “strong” evoked responses also showed strong tuning characteristics—that is, they demonstrated clear frequency selectivity and were very stable in their frequency response characteristics and BFs across sequential recordings. For each neuron, frequency selectivity and BF were determined from FRHs recorded at 50 dB SPL. In general we found that strong neurons differed in their tuning bandwidth to a considerable degree and, in most cases, were tuned to only one range of frequencies. Occasionally, however, cells were tuned to two separate frequency regions (see Fig. 6 for example single-unit FRHs). We defined such multipeaked tuning curves by the presence of a secondary peak with amplitude ≥0.4 of the primary peak. By this definition, 25% of cells exhibited multipeaked tuning curves (BFS were taken to correspond with the higher peak).

To gain an idea of relative broadness of tuning within the anesthetized cell population the bandwidth at 0.5 FRH response amplitude was used as an index. These data were first logarithmically transformed (log10 [fr2] – log10 [fr1], where fr1 and fr2 represent the frequency range to which a cell was sensitive to at 50% of its maximal response). Figure 4A plots individual bandwidths against BFS in the entire strongly acoustic neuronal population. The scatter of the data does not indicate any systematic relationship of tuning broadness with frequency if a log transform is employed. Note that even for cells tuned to the same BF there existed a fair amount of variation in tuning bandwidth at 50 dB SPL although the majority could be said to be narrowly tuned.

There was little change in the tuning characteristics of individual neurons in the awake condition. Figure 4, B and C, makes a direct comparison of bandwidth and BFs for 14 cells in both awake and anesthetized conditions. The FRHs were constructed over 100 stimulus repetitions at 50 dB SPL. The two scatter plots show that BFs tended to remain unaltered; tuning bandwidth, however, appeared to be slightly larger in...
awake cells. A Wilcoxon test on these data indicated that awake bandwidths were significantly larger ($P < 0.05$).

Stability of tuning in strongly responsive cells was measured by examining changes in FRFs over six sequential recorded FRHs (5-min intervals each with 20 stimulus repetitions, $n = 40$ anesthetized neurons). Given the stimulus set we used to identify FRFs, the probability that a given neuron would change its BF across two sequential recordings was calculated as 0.08. This BF tuning stability of strong cells was seen in both the anesthetized and the awake condition. We observed, however, that the overall FRHs of neurons in the awake rat tended to be more noisy than when they were anesthetized (this was not quantified).

**Response latency**

Response latency was measured as time to first spike after BF tone onset. Minimum response latency of individual neurons was estimated from the cumulative response histogram, which is derived from the PSTH (e.g., see Nicolelis et al. 1999). In the anesthetized cell population, mean response latency was 17 ms, with a range of 8–26 ms (tones of 5-ms rise time, 50 dB SPL steady-state amplitude, 20 stimulus repetitions). Figure 5A makes a comparison of response latency in the awake and anesthetized state ($n = 14$ neurons recorded in the second stage; data from 100 stimulus presentations in each condition). The scatter of the data shows that minimum latencies in the awake cell were smaller than when it was anesthetized. On the average, neurons responded 3.96 ms earlier to stimuli in the awake state than in the anesthetized state. This difference was statistically significant ($P < 0.01$, $t$-test).

The relationship between minimum latency and steady-state tone intensity was also examined using data from second-stage recordings. In the anesthetized animal, a clear inverse relationship existed between minimum latencies and tone intensity even though the slopes of these functions as well as latency at each intensity varied among recorded cells. This inverse relationship, however, appeared to vanish in the awake animal. From first stage acute recordings 34 cells were labeled as strongly responsive neurons in comparison to strongly acoustic neurons. In addition to smaller evoked response, these cells also significantly differed from strongly responsive cells along other dimensions—viz., response latency and stability of frequency response. Mean response latency was 26 ms with a range of 20–35. Stability of frequency response was measured by noting their FRFs across six individual sequentially recorded FRHs. The probability that their BF would change across two sequential recordings was calculated as 0.61; i.e., it was likely that a given recording would show a BF dissimilar to the BF of the previous recordings. Bandwidths (at 0.5 FRH response amplitude), similarly, tended to be very variable, ranging uniformly from widths spanning less than 2 frequency-stimulus

*FIG. 5.* Minimum response latencies (RL) of AI neurons. A: response latencies of strongly responsive neurons in both awake and anesthetized conditions (steady-state 50 dB SPL tones at BF, with a rise time of 5 ms). B and C: the latency-intensity function for 5 neurons in the anesthetized and awake conditions, respectively.

Weakly responsive cells

Units that showed weak acoustic responses comprised roughly another third of the total anesthetized state cell population and 50% of the awake state population (anesthetized cells showing no acoustic response often emerged with clear, although weak, responses in the awake condition). From the standpoint of frequency mapping within AI, the response properties of this class of cells were difficult to fit into a consistent framework and were the main source of variability in the data. Weakly acoustic units only appeared to demonstrate an ON response, often at longer response latencies. The tuning of these cells was generally ambivalent; BFs and FRH functions tended to be labile across sequential recordings. Occasionally, their PSTHs (20 stimulus repetitions) showed no acoustic response to any frequency in the stimulus set. Longer PSTH averaging was often needed to observe a clear acoustic response.

From first stage acute recordings 34 cells were labeled as weak. As noted in METHODS, five sequentially recorded FRHs (20 stimulus presentations each at 50 dB SPL) were summed to identify their ON acoustic response in comparison to strongly acoustic neurons. In addition to smaller evoked response, these cells also significantly differed from strongly responsive cells along other dimensions—viz., response latency and stability of frequency response. Mean response latency was 26 ms with a range of 20–35. Stability of frequency response was measured by noting their FRFs across six individual sequentially recorded FRHs. The probability that their BF would change across two sequential recordings was calculated as 0.61; i.e., it was likely that a given recording would show a BF dissimilar to the BF of the previous recordings. Bandwidths (at 0.5 FRH response amplitude), similarly, tended to be very variable, ranging uniformly from widths spanning less than 2 frequency-stimulus
spacings to more than 11 frequency spacings. Additionally, 0.68 of weakly responsive neurons showed more than one significant peak in their FRHs.

**Multiunit response and tonotopic organization in AI**

Around 95% of AI sites showed a strong acoustic response in multiunit recordings. All AI sites (i.e., multiunit recordings) showed a monotonic increase of evoked discharge with intensity without evidence of saturation (20–70 dB SPL). BFs at 50 dB SPL almost always corresponded with the true CF of the AI site, making these measures interchangeable. At high intensities, FRHs tended to flatten out with an increase in the range of frequencies to which the site was responsive. In general, there did not appear to be any significant difference in the FRHs recorded in the anesthetized or awake condition with the exception that awake FRHs tended to be broader and noisier.

Figure 6 illustrates the general relationship we found between the tuning of cell populations and those of its individual member neurons. In the figure, the multiunit FRHs of four example AI sites (A–D) are shown along with the FRHs of four strongly responsive individual cells isolated within each recording (A–C are from anesthetized recordings, while D is from an awake subject). In general, multiunit FRHs were generally smoother and had a larger bandwidth in comparison with the FRHs of individual cells in the population. The details of individual FRHs belonging to the same cell population sometimes varied considerably in that they were often highly selective to different, although nearby, frequency regions. Nevertheless, as Fig. 6 indicates, the actual BFs of individual cells, if not coincident with the population BF, did not differ by much. For these data, no individual neuron registered a BF more than two frequency spacings away from its population BF.

Of interest was the amount that individual BFs differed from the BFs their populations expressed. For each strongly acoustic cell isolated in first stage recordings, we calculated the difference between its BF and that of its population (averaged over 100 stimulus presentations); this difference was expressed in octaves. Thus a neuron tuned to 16 kHz in a population tuned to 8 kHz would have a BF 1 octave away from that of its population. The histogram in Fig. 7 gives the distribution of these differences (n = 42 strongly responsive neurons). About 50% of neurons recorded at a given AI site shared the BFs of their population; an additional 30% had BFs within 0.24 octaves of their population. (Note that fine wire electrodes are generally capable of recording multiunit activity at distances of ~100 μm.)

In the anesthetized animal, multiunit FRHs showed a clear change in their BFs along the rostral-caudal axis of AI with...
high frequencies being represented rostrally and low frequen-
cies caudally. In the awake animal, since multiunit FRHs were
noisier, a clear tonotopic organization was sometimes obscured
when averaged over insufficient stimuli. With 100 stimulus
presentations, however, a clear tuning gradient was usually
seen. Figure 8, as an example, compares the expression of
tonotopy in the anesthetized and awake condition by plotting
BFs across electrode sites in one animal. In general, the dif-
ference in BFs as well as the dissimilarity in tuning curves
between any two AI sites depended on the cortical distance
between them and appeared to be independent of the state of
the animal, whether anesthetized or awake.

Stability of tuning

Of specific interest to this study was the degree of tuning
stability exhibited by AI sites over successively obtained FRHs
within a recording stage and over the long term, i.e., over the
time course of the experiment.

The first of these issues was examined in sequential second
stage recordings at 60 AI sites in the anesthetized subject
(recordings at 5-min intervals, with 20 stimulus repetitions at
50 dB SPL). For each AI site its “true” BF was defined as the
mean BF across eight successive FRHs (mean BF was obtained
by averaging over the logarithms of individual BFs and taking
the antilog). As we did earlier, a measure of the variability in
individually recorded BFs was phrased as the following ques-
tion: how much would the BF from a single recording be
expected to differ from the true BF of the underlying AI site?
200 FRHs were randomly selected from these data; for each
FRH, the algebraic difference between its BF and the true BF
of the site it was recorded from was expressed in terms of
octaves. Figure 9 plots the distribution of these octave differ-
ces; it shows a rough Gaussian distribution, giving an indi-
cation of measurement variability in AI. From the distribution,
we estimate that 95% of recorded BFs (each from 20 stimulus
presentations) in rat AI would be expected to lie within 0.25
octaves of the true BF of any given AI site. Nevertheless, the
exact degree of stability varied with individual sites with some
sites being much more stable than others. Curiously, we ob-
served that the stability of tuning at any given AI site was
strongly correlated with the “smoothness” of its tuning func-
tion judged visually. Figure 10 gives an idea of the ranges of
smoothness in FRH functions that we encountered in rat AI.
The data are from a subject in which all eight electrodes were
within AI. Sites 6 and 7 demonstrate what we considered as
smooth FRH functions. By contrast, sites 3 and 4 demonstrate
“roughness” or “jaggedness.”

Of greater importance was the possibility of changes in the
tuning characteristics of AI sites over the ICMS/behavioral
phase of the experiment, i.e., between the second and third
recording stage. Note that the AI location to be stimulated
(ICMS site) was selected from second stage recordings. The
discriminanda frequency for behavioral testing was chosen to
correspond with the BF of this site because ICMS was intended
to increase its central representation. Naturally, if the location of electrode tips changed over the duration of behavioral testing, or if AI sites were otherwise demonstrably unstable in their long-term tuning properties, the choice of the behavioral test frequency may have become meaningless. Figure 11 plots the true BF of the ICMS site as well as the true BFs of the AI sites immediately adjacent (rostral and caudal) to it, both before and after the ICMS/behavioral phase of the experiment (data from all 10 subjects). The figure shows that BFs recorded in AI tended to remain stable over this 2-wk time period (the little drift that is present appears to be random). The coefficient of linear regression for each data group was: rostral $R^2 = 0.95$; caudal $R^2 = 0.96$; ICMS site $R^2 = 0.97$ (all associated with $P < 0.01$). Thus in each subject, the behavioral test frequency corresponded to the frequency that enlarged in central representation due to ICMS. We note, however, that 3 rats in addition to the 10 subjects reported here, did, in fact, show a significant difference in the BFs of their ICMS site over the duration of the experiment; these subjects were rejected for the final experiment. Even so, the preceding findings indicate that flexible wires are generally capable of tracking the same cell population over a relatively long period of time.

In the context of Fig. 11 and long-term tuning shifts, another observation should be made: learning experiments have generally shown that exposure to a behaviorally significant frequency may increase its cortical representation (e.g., Recanzone et al. 1993). Thus in addition to the short-term ICMS-induced changes, there was a possibility for AI sites to exhibit longer duration plastic receptive field changes over the experiment because of learning exposure to the behavioral test frequency itself. These learning-induced changes, if any, would be expected to occur at sites adjacent to the ICMS site (since these sites, by definition, were the nearest to the AI location coding for the test frequency; receptive field shifts at these sites would be expected to be toward ICMS site tuning). Figure 11, however, indicates that brief learning exposure to the test frequency did not affect the BFs of nearby AI sites; i.e., no strong learning-induced changes were visible in our subjects, at least over a 2-wk testing period.

Effects of ICMS in AI of awake subjects

Previous studies in our lab have shown that ICMS has a strong but relatively short-duration effect in reorganizing the AI map of anesthetized rats (Maldonado and Gerstein 1996a,b). Here we report that similar results are also seen in the
awake animal. As noted earlier, the subjects of these subexperiments (C1–C4), did not partake in the main behavioral experiment. The protocol of these subexperiments was nearly identical to that followed by Maldonado and Gerstein (1996a). A week after electrode-array implantation, tonotopic organization in AI was first confirmed in all subjects. (A total of 28 electrodes across the 4 subjects were judged to have been implanted in AI.) A specific AI site, which showed BFs clearly different from that of adjacent sites, was chosen as the stimulation ICMS site. Six sequential FRHs (each 20 stimuli, 4–40 kHz, at 50 dB SPL) were constructed at each of three time periods: pre ICMS, post ICMS, and at 4 h post ICMS.

Figure 12 shows the effect of ICMS in the awake AI cortex of rats using data from an example subject in whom the effect of cortical stimulation was striking. The panels in the center column show the average FRHs of the ICMS site at the three different times of the experiment: pre, post, and 4 h post-ICMS. The panels to the left and right of the center column show the corresponding FRHs of the two electrodes adjacent to the ICMS site (caudal and rostral). To quantify ICMS effects, we looked for changes in the evoked response to the frequency that corresponded to the BF of the stimulated site. Specifically, at the adjacent sites we measured changes in evoked response to the frequency that corresponded to the BF of the stimulated site. This test, therefore focused on the most likely locus of receptive field change. In the example data of Fig. 12, the BF of the ICMS site before stimulation was 12.7 kHz. The BFs of the AI sites caudal and rostral to the ICMS site were 5.56 and 17.6 kHz respectively (not highlighted in the figures). The response amplitude at the the adjacent sites to the BF of the ICMS site was caudal = 0.25 and rostral = 0.52. As the panels in the center row show (marked by the vertical arrow), the evoked response at the two adjacent sites to tones of 12.7 kHz—the BF of the ICMS site—increased greatly in post ICMS recordings. After a 4 h period of ICMS the response amplitude at the two adjacent sites had increased to caudal = 0.50 and rostral = 0.84. The panels in the third row show that by the time an additional period of 4 h had elapsed the response amplitude in the two adjacent sites had reverted to approximate their pre ICMS levels. Note also that over this experimental period the FRHs of the stimulated site itself did not appear to change significantly.

The preceding analysis was extended to all four subjects and to all electrode sites that were adjacent (300 μm) as well as to those that were one removed (600 μm) from the ICMS site. Thus 16 electrode sites from four subjects were available whose FRHs could be paired as pre and post ICMS. In these data, our test sample was, again, the evoked response (normalized) of the electrode site at the BF of the stimulated site. Table 1 summarized the data. It lists mean pre- and post-ICMS response amplitudes to the BF of the stimulated site at all 16 other sites. A paired t-test on these data showed that, as a group, electrode sites 300 μm distant from the ICMS site demonstrated a significant increase in auditory response to the BF of the stimulated site (P < 0.01). In comparison, electrodes that were 600 μm distant did not do so. Next, we looked at each electrode site individually. As previously noted, six FRHs were constructed at each stage of recording: pre, post, and 4 h post ICMS.
TABLE 1. Effect of ICMS on frequency representation in AI cortex of the awake rat

<table>
<thead>
<tr>
<th>Subject</th>
<th>Electrode Location Relative to ICMS Site</th>
<th>Distance From ICMS Site, μm</th>
<th>Pre ICMS Evoked Response</th>
<th>Post ICMS Evoked Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>C</td>
<td>300</td>
<td>0.305</td>
<td>0.431</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>300</td>
<td>0.190</td>
<td>0.134</td>
</tr>
<tr>
<td>R</td>
<td>C</td>
<td>600</td>
<td>0.171</td>
<td>0.213</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>600</td>
<td>0.009</td>
<td>0.001</td>
</tr>
<tr>
<td>R</td>
<td>C</td>
<td>300</td>
<td>0.251</td>
<td>0.501</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>300</td>
<td>0.524</td>
<td>0.840</td>
</tr>
<tr>
<td>R</td>
<td>C</td>
<td>600</td>
<td>0.212</td>
<td>0.247</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>600</td>
<td>0.017</td>
<td>0.006</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>300</td>
<td>0.293</td>
<td>0.335</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>300</td>
<td>0.682</td>
<td>0.801</td>
</tr>
<tr>
<td>C</td>
<td>R</td>
<td>600</td>
<td>0.139</td>
<td>0.214</td>
</tr>
<tr>
<td>R</td>
<td>C</td>
<td>600</td>
<td>0.467</td>
<td>0.618</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>300</td>
<td>0.303</td>
<td>0.620</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>300</td>
<td>0.542</td>
<td>0.725</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>600</td>
<td>0.422</td>
<td>0.551</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>600</td>
<td>0.291</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Evoked responses to the best frequency of the stimulated site were measured before and after intracortical microstimulation (ICMS) at electrode sites 300 and 600 μm distant from the stimulated site. These are entered in the fourth and fifth column of the table. Significant increases in evoked response after ICMS are denoted with an underline. Location of electrode is relative to that of the ICMS site and is denoted with either C = caudal or R = rostral. Note that evoked response at a given electrode site (to the best frequency of the ICMS site) is normalized by the maximum evoked response exhibited by the site.

ICMS. For each electrode site and subject, therefore, six FRHs and after ICMS were available for comparison. A t-test on these data (α < 0.01) showed that six of eight AI sites 300 μm away from the stimulated site significantly increased their response to the BF of the stimulated site (in Table 1, these are underlined). In addition two electrode sites ~600 μm distant from the stimulated site also showed significant increases in their evoked response to the BF of the stimulated site. It was therefore concluded that ICMS significantly increases the AI frequency representation of the stimulated site in the awake animal. Thus changes in frequency representation were common at AI regions 300 μm away from the stimulated region; occasionally, changes in frequency representations could even be observed as far as 600 μm away.

TABLE 2. Testing parameters and ICMS effect on behavior

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test Frequency, fkHz</th>
<th>Frequency Difference, % of f</th>
<th>Pre ICMS</th>
<th>Post ICMS</th>
<th>Pre Control</th>
<th>Post Control</th>
<th>True Increase</th>
<th>True RI Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>20.72</td>
<td>5.2</td>
<td>0.78</td>
<td>0.80</td>
<td>0.79</td>
<td>0.78</td>
<td>0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>R2</td>
<td>20.72</td>
<td>4.8</td>
<td>0.74</td>
<td>0.75</td>
<td>0.78</td>
<td>0.72</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>R3</td>
<td>7.72</td>
<td>4.1</td>
<td>0.79</td>
<td>0.80</td>
<td>0.76</td>
<td>0.81</td>
<td>-0.04</td>
<td>-0.13</td>
</tr>
<tr>
<td>R4</td>
<td>12.65</td>
<td>5.6</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
<td>0.01</td>
<td>-0.07</td>
</tr>
<tr>
<td>R5</td>
<td>28.79</td>
<td>4.2</td>
<td>0.77</td>
<td>0.81</td>
<td>0.76</td>
<td>0.83</td>
<td>-0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>R6</td>
<td>20.72</td>
<td>7.1</td>
<td>0.78</td>
<td>0.78</td>
<td>0.76</td>
<td>0.82</td>
<td>-0.06</td>
<td>-0.16</td>
</tr>
<tr>
<td>R7</td>
<td>33.93</td>
<td>7.9</td>
<td>0.78</td>
<td>0.80</td>
<td>0.80</td>
<td>0.77</td>
<td>0.05</td>
<td>-0.05</td>
</tr>
<tr>
<td>R8</td>
<td>24.42</td>
<td>8.1</td>
<td>0.76</td>
<td>0.81</td>
<td>0.79</td>
<td>0.72</td>
<td>0.12</td>
<td>-0.08</td>
</tr>
<tr>
<td>R9</td>
<td>12.65</td>
<td>6.3</td>
<td>0.75</td>
<td>0.81</td>
<td>0.77</td>
<td>0.80</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>R10</td>
<td>14.91</td>
<td>6.9</td>
<td>0.80</td>
<td>0.79</td>
<td>0.81</td>
<td>0.83</td>
<td>-0.03</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Effects of ICMS on frequency-discrimination behavior

The ability of 10 rats to discriminate frequencies around a test frequency was tested before and after ICMS enlarged its cortical representation. As noted in METHODS, pre and post ICMS sessions were run daily over 16 days. Of these, 6 days were designated as controls, during which no ICMS was actually given—i.e., the rats underwent sham ICMS sessions (control days were chosen randomly). Thus the behavioral data consisted of a rat's performance in pre and post ICMS sessions (10 paired data points) as well as pre and post sham ICMS sessions (6 paired data points).

Table 2 summarizes the results from the final experiment. The first two columns list the frequency and the mean frequency difference at which each subject was tested. Test frequencies varied in individual subjects, corresponding to BFs of AI sites chosen for stimulation. The table then lists mean frequency-discrimination performance—measured as A’—in all four sample groups: pre and post ICMS sessions and pre and post sham ICMS sessions. (Standard deviations for discrimination performance ranged from 0.031 to 0.076.) We sometimes observed that normal rats tended to perform better during the second session of the day. Therefore we made a comparison between changes in performance after ICMS and changes in performance after sham-ICMS session. Column 8 of Table 2 evaluates the quantity mean (post – pre) ICMS performance – mean (post – pre) sham ICMS performance. To test for significance for each subject, a t-test specifically compared two data groups: 10 (post – pre) ICMS data points and 6 (post – pre) sham ICMS data points. This test (α = 0.01) showed that only one rat, R8, tested in the discrete trial task, demonstrated a significant increase in frequency-discrimination performance which could be ascribed wholly to the effects of ICMS. Thus if ICMS had any effect on performance it was a weak one, sufficient perhaps to improve performance in a small fraction of subjects.

In addition to discrimination ability, the possible affects of ICMS on an animals’ decision behavior was examined by measuring response bias (RI) levels before and after ICMS. Mean response bias tended to be higher in post-ICMS sessions. A comparison with controls, however, showed that the increase in performance after ICMS was not sufficient to be observed as far as 600 μm away.
in post-ICMS response bias was a nonspecific change that could not be ascribed to the effects of ICMS. As column 8 did for discrimination performance, Column 9 lists the quantified mean (post – pre) ICMS response bias – mean (post – pre) sham ICMS response bias. None of these data represented significant increases in response bias after ICMS relative to that after sham-ICMS. It was likely that differences in response bias across the two behavioral sessions, which were spaced by 4 h spent in the ICMS chamber, were due to changes in motivational states. Post ICMS sessions took place very late in the evenings when rats, being nocturnal, were more active in making “go” responses to ambiguous signals. Previous psychophysical studies have indicated that in some rats, higher response bias is associated with better frequency-discrimination performance (see Talwar and Gerstein 1999), which may explain why some subjects also appeared to perform better during the 2nd session of the day. We concluded that ICMS does not have a significant affect on the frequency-discrimination behavior of the rat, either on its sensory perception or on its decision strategies.

During both pre and post ICMS test sessions, while the rats were engaged in their tasks, recordings from AI were continuously obtained. These data were examined for evidence that during post ICMS sessions the representation of the test frequency was in fact increased in the AI cortices of our subjects. Because of the limited number of single cells that could be sorted, we looked only at multiunit activity. As before, we focused on spike activity at the two electrode sites that were adjacent to the site of AI stimulation. At these sites, the evoked response to each test frequency tone was first determined; the average evoked response before ICMS was then compared with the average evoked response after ICMS.

Each subject’s recordings from two adjacent AI sites were available. During recordings, amplitude thresholds were set so that rates of spontaneous multi-neural activity were generally between 20 and 30 spikes/s. For all test tones in a session (all sessions contained 100) the average rate of spike activity (spikes/s) in the 8- to 50-ms time window after tone onset was calculated. The rate of spontaneous activity, calculated from the 100-ms interval before tone presentation was subtracted from the response rate. This differential rate was averaged over all tones to give average evoked response at the adjacent site to the BF of the ICMS site (i.e., to the behavioral test frequency). Every ICMS—or sham ICMS—session was thus associated with paired data points, the (mean) evoked response before and after ICMS/sham-ICMS (each subject underwent 10 ICMS and 6 sham-ICMS sessions). For each adjacent electrode, a paired t-test evaluated the significance of changes in the evoked response both after ICMS and after sham ICMS sessions (\( \alpha = 0.05 \)).

Figure 13 summarizes the results. For all subjects, and each adjacent electrode, we show mean increases in evoked response after ICMS sessions as well as control (sham ICMS) sessions. Figure 13 organizes the data by electrode group (caudal or rostral) and by subject. Significant increases in evoked response (\( P < 0.01 \)) are marked (+; a t-test compared evoked responses before and after ICMS). The AI cortices of 8 of 10 subjects showed a significant increase in evoked response to the test frequency in at least one adjacent AI site. This analysis, therefore, confirmed that in post ICMS sessions, cortical stimulation had in fact increased the AI representation of the behavioral test frequency in the majority of subjects. As the figure also shows, changes in evoked response after sham-ICMS sessions, by comparison, were variable and were that which could be expected by chance. An additional interesting observation can also be made: subject R8, the only rat whose frequency-discrimination performance did improve as a result of ICMS, also showed accompanying large increases in evoked response in both adjacent electrodes. The scale of these changes appeared to be larger than those seen in most other subjects.
subjects. Nevertheless across all animals, the data did not show a significant correlation between changes in evoked response after ICMS and changes in behavior. Therefore notwithstanding the data from subject R8, we concluded that a significant increase in cortical representation of specific frequencies is not sufficient to improve frequency-discrimination performance.

**DISCUSSION**

The central aim of this study was to investigate the possible behavioral consequences of altering cortical AI frequency mapping in the rat using weak electric stimulation. In the process, some observations on rat AI frequency response characteristics and its functional organization were made. Fixed fine wires were used to record from and deliver stimulation to AI sites over a prolonged time period. We found that this technique made it generally possible to track the same group of cells over a period of 2–3 wk. This experimental requirement can sometimes be important and was essential for this study. However, the cost of using fixed fine wires, at least in the rat AI region, is a diminishing single-unit yield with time. The first, and major, problem is a slow degradation of signal-noise ratio; the contraction of individual waveforms with time has the effect of increasing their similarity. The second problem is the relatively large amount of spike superimposition that the AI region appears to exhibit during stimulus presentation. The AI region appears to be physiologically dense, with cells tending to fire synchronously especially in response to stimuli. In our view, the first of these problems in particular, seems to be relatively intractable at present; it is insufficiently mentioned in the literature and remains a limiting problem in studying the neuronal underpinnings of behavior.

**Frequency response characteristics in AI**

The rat AI cortex was first mapped by Sally and Kelly (1988) using an anesthetized preparation (Equithesin). More recently, Gaese (1997) has also made observations on its frequency-response characteristics. In this study we added to this literature, noting some of the frequency response properties of rat AI under ketamine anesthesia and making a direct comparison with its characteristics in the awake animal (recordings approximated cortical layer IV). In the context of this study, “awake” refers to the disinterested animal moving freely within the acoustic field with no behavioral significance attached to stimuli.

We classified single units into strongly responsive, weakly responsive, and nonresponsive neurons on the basis of their evoked response strength to BF tones. Weakly responsive cells differ from strongly responsive cells in that they either show a smaller relative change in firing rate in response to stimuli and/or respond only on some stimulus presentations. It should be stressed that the strong/weak cell classification we employed served only to organize the data because a large amount of variability in frequency response characteristics appears to exist within rat AI. We attempt, however, to create a clearer picture of the AI region by this seemingly arbitrary division. For example, the classical literature describes the AI cells as exhibiting a short-latency (<20 ms) phasic response to CF tones and set within a cortical field arranged tonotopically (at least under anesthesia). Our findings, however, indicate that this view can only be supported by considering neurons that are, in fact, strongly responsive to acoustic stimuli. These strongly acoustic cells appear only to form a third of the cell population in the ketamine-anesthetized animal. Their temporal response patterns can be classified into at least four different types and may be analogous to those found in the AI region of other species (de Ribaupierre 1997; Pickles 1982). The FRHs of strongly responsive neurons demonstrated strong tuning to a relatively narrow range of frequencies, with around a fourth of them being tuned to two nonoverlapping frequency bands. Their frequency response functions were very stable. Under a logarithmic transform, their tuning bandwidths appeared to be independent of BF. In the awake animal, the preceding frequency-response characteristics of strongly acoustic cells were maintained; the only measurable difference was that cells tended to exhibit larger bandwidths when awake, which may indicate a greater sensitivity to acoustic stimuli.

By contrast, the frequency-response characteristics of weakly acoustic cells were difficult to classify systematically. Despite being labeled as weak, these cells all showed a clear on phasic response to BF tones when averaged over a large number of stimuli. However, their FRHs in individual recordings tended to be very labile, and sometimes no tuning to a specific frequency range could be inferred. The data indicate a strong correlation between the strength of evoked response and stability of tuning in individual cells. From a frequency-tuning standpoint the presence of weakly acoustic cells, naturally, degrades the traditional tonotopic-map view of the AI field. It is possible that many anesthetized studies report a neat single-unit AI tonotopic organization because they unconsciously adopt methodologies that lead to sampling and reporting only from strongly acoustic cells. When mapping the AI field, the CFs of neurons are usually determined audiovisually from only a few stimulus passes. This approach, while accurately mapping strongly acoustic neurons, tends to overlook weakly acoustic units.

A possible and simple explanation of the origins of weakly acoustic cells is that tonal stimuli we used did not adequately fall into their response areas in terms of best intensity and sound location (reducing signal/noise ratio and making it a measurement problem). These variables are known to influence firing rates, although in ways that cannot be predicted from an AI location since the details of their AI mapping does not appear to be systematic (de Ribaupierre 1997; Pickles 1982). Intensity and sound location were held constant in our studies with anesthetized animals. In the awake animal, sound location varied with the position of the head; furthermore, differential sound shadowing by the pinnae (Wiener et al. 1966) and masking noises by a subject’s movements (Imig and Weinberger 1970) can also affect firing rates. Nevertheless, we averaged over a very large number of stimuli, and FRHs were expected to be independent of local firing-rate variations. Additionally, note that weakly acoustic cells maintained their response characteristics in both anesthetized and awake recording. Another possible explanation for our findings, then, is that the properties of the weakly responsive population may be weighted toward acoustic input that may differ from that of the strongly responsive population (although, strictly speaking, both populations lie at the 2 ends of a response strength continuum). Recent work in the rat auditory system (Winer et al. 1999) has shown two separate projections from the medial
geniculate body (MGB) to the AI. The first of these, arising from the ventral nucleus, is the classical lemniscal line input with all the hallmarks of the primary acoustic pathway and is likely to be responsible for the response properties of the strongly acoustic AI cell population. The second projection, from the medial nucleus, has hitherto not been well defined in the rat. It is a more diffuse system, with a coarse topographic mapping. It is possible that this projection underlies the ill-defined response properties of the weak cell population. A plausible explanation of the AI role of such cells is to separately mediate attention and behavior in the awake animal while strongly acoustic cells maintain an ongoing frequency analysis. An assembly of strongly acoustic cells would be expected to discharge on every stimulus presentation (thus coding for frequency), while the firing of weakly acoustic neurons could be regulated by context. Our weakly acoustic cell may correspond with literature reports of AI cells that in the awake animal appear to be influenced by the context within which stimuli are presented (e.g., Ahissar et al. 1992; Hubel et al. 1959; Miller et al. 1972). Note also that the medial division of the MGB, in contrast to the ventral division, shows a robust plasticity (Gerfen and Weinberger 1983; Weinberger and Diamond 1987). Lesions of this nucleus lead to a cognitive deficit (Glassman et al. 1975; Kelly and Judge 1985), indicating that this projection channel is concerned with behavioral states. (Weakly responsive cells may thus be more responsive to stimuli that are more complex than the ones we used.) A clearer idea of the origins and response properties of weakly acoustic cells on the AI may, therefore, require a differential analysis of their properties while training animals in tasks that require them to attend to stimuli. Additional support for some of the preceding speculations comes also from electrophysiological findings in the AI cortex of other animal species. For example, in the guinea pig, South and Weinberger (1995) have specifically compared AI multiunit frequency response to the response of its individual units along three separate dimensions—CF, threshold, and bandwidth. Working from a different analytic standpoint, based on PSTH patterns, their study has also suggested the presence of two different populations of frequency responsive cells that may be similar to the two populations indicated here.

We evaluated the issue of tonotopy from multiunit or population activity. Each electrode recording generally comprised a population of ~10 active cells, likely to exist within an approximate volume of 100 μm diam. Under anesthesia, FRHs at any given AI site almost invariably showed a strong acoustic response. Their BF exhibited a clear tonotopic organization across the rostral-caudal axis of AI, consistent with the findings of Sally and Kelly (1988). Their tuning properties tended to be stable, both in sequential recordings within a recording session, as well as in the long term, a period of 2 wk. Across sequential recordings (20 stimuli each) our results indicate that there is a 95% probability that a sample BF would lie within 0.25 octaves of the true BF of an AI site (which had been measured by very large averages). Our AI sites showed a fairly large range in their degree of stability with some sites consistently showing more instability than others. Given that strongly acoustic cells typically showed very little variability in their frequency response, this suggests the presence of different local densities of weakly acoustic neurons. Our findings appear to suggest that the ratio of strongly to weakly acoustic neurons may determine the stability of response at a given AI location.

At any given AI site, individual BFs of strongly acoustic cells tended to cluster around the population BF. Over the entire strongly acoustic cell data 90% of individual units exhibited BFs <0.24 octaves away from the population average. In terms of BF, this is consistent with the widely held idea that nearby AI regions code for similar frequencies, although it appears that, as a computational map, the rat AI is not very tightly organized, perhaps reflecting the rats’ relatively poor frequency-discrimination ability (Talwar and Gerstein 1998). The details of frequency response functions of neighboring cells sometimes differed to a considerable extent, being tuned to different, although nearby, range of frequencies. [The previous cited study by South and Weinberger (1995) has made similar observations in the AI cortex of the guinea pig: it found that 1 class of frequency responsive cells at a given AI site tends to be tightly matched by CF. This class appears to correspond to the strongly acoustic neurons of our study.] It was not surprising, then, that multiunit frequency response functions were much broader than individual cell tuning. Our observations suggest that multiunit tuning curves can be considered as an average tuning measure summed over the underlying population and weighted toward the properties of strongly acoustic cells. In this regard, an interesting observation is that smoothness of the tuning function at any AI site was highly correlated with the stability of its frequency response. Smoothness of tuning would be expected if the underlying cell population is mainly comprised of strongly acoustic cells tuned to similar CFs.

In the awake animal, multiunit tuning functions tended to be broader and more unpredictable than in the anesthetized animal. This observation can be explained by the presence of a larger proportion of labile, weakly acoustic cells in awake recordings as well as the increased sensitivity of awake cells. Note that a majority of cells not responding under anesthesia became weakly responsive when awake; thus awake recordings may be expected to consist of ~50–60% of weakly acoustic units. Nonetheless, long averages also showed clear changes of CFs across AI, similar to the anesthetized subject. The demonstration that tonotopy is also well expressed in the awake animal seems to be at odds with the literature. To date, few studies have investigated this property in the awake animal; their findings are generally unsupportive of a clear tonotopy. Current literature highlights the unpredictable and complicated nature of acoustic response in the awake animal (de Ribaupierre 1997; Pickles 1982). It is possible that this view has been based on the activity of individual units, which in the awake animal—as our findings indicate—is composed of a large fraction of weakly acoustic cells that, in fact, do exhibit labile tuning properties. Multiunit tuning measures, and signal averaging over a large number of stimulus presentations, may therefore be necessary to clearly demonstrate a tonotopic organization in the awake animal. Another factor also may be methodological: in our experience presenting stimuli at unpredictable intervals more consistently guaranteed an acoustic response. Such presentation may guard, to some extent, against what can be described as “habituation” in the awake disinterested cortex—the tendency for evoked discharge to reduce with highly periodic stimuli.
Central reorganizations and behavior

ICMS of AI caused a rapid, and reversible, reorganization of the AI frequency organization in the awake animal with a time course of hours. This finding has previously been documented in the AI region of ketamine-anesthetized rats. The new finding is that this effect also extends to the AI cortex of the awake rat. This suggests that, as in anesthetized subjects, an unsupervised Hebbian learning rule—where a positive contingency between the increased activities of pre and post synaptic neurons strengthen their connections whereas a negative contingency weakens it—is a sufficient condition for local plasticity to develop in the cortex of awake animal (see Dinse et al. 1993).

Stimulation of a specific AI location changed the receptive fields of cells in nearby regions so that they became more responsive to the BF of the stimulated cells. In effect, therefore, ICMS increases the central representation of a specific frequency also in the awake animal. In our study, changes were almost always observable at distances of 300 μm from the stimulated AI region, indicating large-scale tuning shifts within the AI map similar to that seen in learning situations. Occasionally, tuning shifts could be recorded at distances as far as 600 μm from the stimulated site.

One aim of our study was to examine if the preceding ICMS affects in AI could be manifest in changes in acoustic perception. In the event, our results showed that ICMS-induced plasticity in the AI cortex of the rat had weak or no affect on its frequency-discrimination behavior. The statistics showed that 1 subject of 10 significantly improved performance as a direct consequence of ICMS-induced plasticity, while there was no effect on the rat’s cognitive decision behavior. The conclusion we therefore draw is that an increased area of central frequency representation is insufficient to alter frequency-discrimination behavior in the rat. We have of course not eliminated the possibility that some other acoustic or perceptual behavior was affected.

Our results may be relevant for studies that record an increase in the cortical representation of an external stimulus parameter following on experimental manipulations either in the auditory or other sensory systems. Examples are auditory conditioning (see Ahissar and Ahissar 1994; Weinberger 1993) and peripheral deafferentation (Merzenich et al. 1984; Robertson and Irvine 1989). Our results may also be relevant for the general phenomenon of perceptual learning. Recanzone et al. (1993) showed that improvement in frequency-discrimination ability at a particular frequency is correlated with an increase in that cortical frequency representation. Their studies suggest that improvements in the ability to discriminate frequencies—an example of perceptual learning—may depend on the ability of the nervous system to increase central stimulus representation. For frequency-discrimination learning at least, our results indicate that an increase in central representation, by itself, does not confer behavioral gains in frequency discrimination. Thus while an increase in central representation is not sufficient to account for perceptual learning, it may be still be necessary.

We should examine several possibilities that might explain why our ICMS-induced increases in AI frequency representation did not lead to improvement of frequency-discrimination performance. One simple idea might be that ICMS did not change frequency representation in AI sufficiently to cause changes in performance—suggesting that real world behaviors are robust and likely to be immune to focal changes in the parametric nature of central representation. However, we note that the extent of the ICMS-induced reorganization seen here in the waking rat AI cortex does not seem to be significantly different from that which accompanies perceptual learning of frequency discrimination in the monkey (Recanzone et al. 1993) or of those accompanying auditory conditioning and peripheral deafferentation (Robertson and Irvine 1989; Weinberger 1993). Another idea might be that the effects of ICMS in AI were too transient to have influenced performance. However, the initial ICMS control experiments we performed indicated that changes in tuning curves after ICMS were significant at least over the 20- to 30-min recording duration, a period of time that was longer than the duration of the behavioral tasks. The behavioral task we used here was actually designed to take the transient nature of ICMS into account. Finally, yet another possible explanation for the lack of behavioral effects of ICMS reorganization in AI is that an animal’s ability to discriminate frequencies may not, in fact, be dependent on the AI region of its cortex. Thus the test of frequency discrimination may have been inappropriate to examine for possible changes in behavior and testing along auditory dimensions other than frequency may have been necessary to uncover other possible changes in perception. Indeed, a large number of ablation studies over the past four decades have shown that the auditory cortex appears not to be necessary for frequency discrimination (for a comprehensive review, see Pickles 1982). This body of evidence, naturally, makes it difficult to evaluate the significance of the findings reported by Recanzone and colleagues (1993), as well as of our findings. Accordingly we recently re-examined the role of AI in hearing (Talwar et al. 2000, 2001) by applying an acute and reversible bilateral inactivation of the AI cortex of rats (with the GABA agonist muscimol) while the animals performed a simple auditory task. These experiments showed that the AI cortex is, in fact, intimately and acutely involved in both sound detection and in discriminating sound frequencies—findings that lend vitality to the present results as well as those of Recanzone et al. (1993).

It is likely, therefore, that our results serve to highlight the differences between central reorganizations due to natural learning and those due to artificial reorganizations through techniques like ICMS; these differences in reorganization directly relate to their possible perceptual correlates. For example, an important variable in plasticity experiments is the behavioral context of the putative remapping stimulus. Although ICMS-induced remapping simulates the plastic changes seen following auditory learning, there may be major differences in the actual reorganizations that make learning-induced changes more meaningful for the animal. Cortical electric stimulation has no behavioral significance attached to it; it adds cells to neuronal assemblies without any meaningful context. In this respect, it is similar to some other remapping experiments that may also, ultimately, be behaviorally irrelevant—for example, the large-scale reorganization seen by pairing an auditory stimulus with stimulation of the nucleus basalis (Bakin and Weinberger 1996; Kilgarrd and Merzenich 1998). In contrast, all demonstrated plasticity that occurs as a consequence of learning is strongly linked with the biological significance of the stimulus and involves adding neurons to the processing assemblies within the context of explicit task...
knowledge. The relevance of context in perceptual improvement can be inferred from the literature even at the cellular level. In the monkey, for example, the strength of functional connections between neurons has been shown to strongly depend on behavioral significance of the stimulus (Ahissar et al. 1992). It is possible, therefore that cell assemblies in the behaving animal follow Hebbian rules that differ from those in the unattending awake subject: they may be strongly supervised in addition to being behaviorally gated (Ahissar and Ahissar 1994).

The relevance of context can also be inferred from the extent of changes seen in natural learning. ICMS demonstrates that cortical plasticity can be mediated primarily through intracortical networks and can occur independently of changes in subcortical structures (Sirois 1993). By contrast, learning paradigms involve plastic changes throughout higher-order auditory nuclei as well as the hippocampus and amygdala (Edeline and Weinberger 1991; Edeline et al. 1990; Muramoto et al. 1993; Weinberger 1993; Weinberger et al. 1990) and may well also include centrifugal control of perception (Zhang et al. 1997). It is possible that if electrical stimulation of AI regions can be tied to biological significance—i.e., if stimulation can be made relevant for the animal within a stimulus-reward situation—this would lead to larger, and more meaningful, changes within the auditory map. Experimental designs that produce a rapid method of remapping the cortex with implicit context may be needed to demonstrate changes in behavior. Many such simple experimental remappings, not using ICMS, have been reported. These often use negative shock reinforcement tied to particular tone frequencies (e.g., Bakin and Weinberger 1990). Currently, we are exploring similar methods to rapidly inducshifts in mapping and perception but within a positive reinforcement scheme (Kisley and Gerstein 2001).

In the end, there remain questions about the actual behavioral significance of learning induced plasticity itself. Although Recanzone et al. (1993) showed that the area of frequency representation was correlated with improvement in performance, at least one study, that of Edeline and Weinberger (1993), demonstrates that cortical plasticity can develop following difficult frequency-discrimination training even though subjects actually fail to discriminate. It is likely, then, that factors specific to the learning task play a role in dictating the mode of plasticity expression, suggesting that behavioral improvement requires task-specific control. Thus it still remains to determine under what conditions frequencies that gain extra representation, gain behavioral benefits (see Ahissar and Ahissar 1994). Extension of the methods reported in this study may be a useful approach to directly answer this intriguing question.

REFERENCES


