Cortical microstimulation in auditory cortex of rat elicits best-frequency dependent behaviors

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Abstract
Electrical activation of the auditory cortex has been shown to elicit an auditory sensation;
however, the perceptual effects of auditory cortical microstimulation delivered through
penetrating microelectrodes have not been clearly elucidated. This study examines the
relationship between electrical microstimulus location within the adult rat auditory cortex and
the subsequent behavior induced. Four rats were trained on an auditory frequency
discrimination task and their lever-pressing behavior in response to stimuli of intermediate
auditory frequencies was quantified. Each trained rat was then implanted with a microwire
array in the auditory cortex of the left hemisphere. Best frequencies (BFs) of each electrode in
the array were determined by both local field potential and multi-unit spike-rate activity evoked
by pure tone stimuli. A cross-dimensional psychophysical generalization paradigm was used
to evaluate cortical microstimulation-induced behavior. Using the BFs of each electrode, the
microstimulation-induced behavior was evaluated relative to the auditory-induced behavior.
Microstimulation resulted in behavior that was dependent on the BFs of the electrodes used for
stimulation. These results are consistent with recent reports indicating that
electrophysiological recordings of neural responses to sensory stimuli may provide insight into
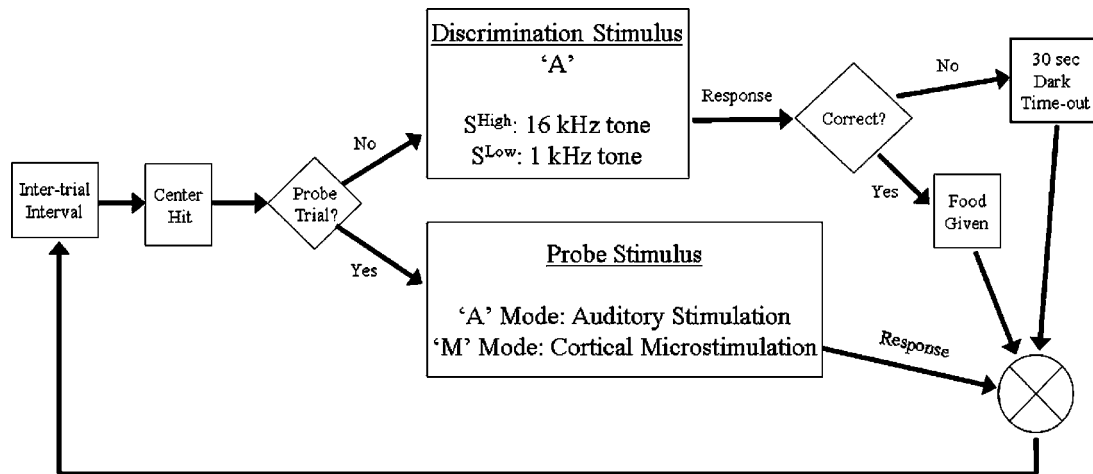the sensation generated by electrical stimulation of the same sensory neural tissue.

Introduction

A set of now-classical studies introduced the use of electrical
stimulation of human sensory cortex to elicit sensory
perceptions (Brindley and Lewin 1968a, Dobelle *et al* 1973,
Penfield and Rasmussen 1950). While groundbreaking, these
studies were limiting in terms of the relatively high stimulation
currents and unreliable percepts that resulted, in part, from
the use of surface electrodes for epicortical stimulation. The
use of penetrating intracortical microelectrodes addresses the
high stimulation level issue by bringing the sites of activation
closer to the target neuronal population to result in lower
stimulation thresholds and more confined stimulus volumes

(Bartlett and Doty 1980, Ronner *et al* 1981, Stoney *et al* 1968).
More detailed investigation of the efficacy of intracortical
microstimulation to transmit sensory information directly to
sensory cortex is driven by developments in microelectrode
technologies, which now provide a means to establish chronic
intracortical neural interfaces having precise two- or three-
dimensional arrangement of stimulating sites.

Cortical microstimulation can be utilized to provide a
'virtual' signal that subjects use in the processing of local
information. Cortical stimulation of sensorimotor areas can
be utilized as a conditional stimulus in a classical conditioning
paradigm (Woody and Engel 1972). Newsome and colleagues
have shown that a primate's perceptual decisions can be
biased by microstimulation of the middle temporal visual
cortical area (Groh *et al* 1997, Salzman *et al* 1990, 1992,
Seidemann *et al* 1998). Recent studies by Romo and

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Figure 1. Trial behavioral flowchart. Each daily testing session consisted of approximately 275 trials. The context of the trial presentations randomly varied; 75% were discrimination trials (auditory task), and the remaining 25% were probe trials. On a discrimination trial, correct auditory tone identification of either 16 kHz or 1 kHz was rewarded by a 4.5 mg food pellet; incorrect identification was reinforced by a 30 s dark time-out. During probe trials, the subject was presented an atypical stimulus of one of two modes. The 'A' mode recorded the subject's responses to tones of intermediate auditory frequencies. The 'M' mode recorded the subject's responses to electrical microstimulation in the auditory cortex. After subject responses to the probe stimuli were recorded, the next trial was initiated; the probe trials were never reinforced. Only one probe mode was used in a given testing session, resulting in two possible sets of behavioral data, A–A (auditory task, auditory probe) and A–M (auditory task, microstimulation probe).

colleagues have shown that microstimulation of the primate somatosensory cortex using putative temporal stimulation parameters provides salient sensory information to behaving primates (Romo *et al* 2000, 1998). In order to further understand cortical neural coding for the development of cortical neuroprostheses, elucidation of both the properties of the electrical stimulus as well as encoding strategies to increase the information capacity of cortical microstimulation are required.

Previous studies have shown that that Mongolian gerbils (Scheich and Breindl 2002), cats (Rousche and Normann 1999) and rats (Rousche *et al* 2003) are able to detect microstimulation of auditory cortex. Further, Rousche *et al* showed that cortical microstimulation of increasing intensity was more readily detected. This result paralleled auditory tone intensity detection behavior. Interestingly, the detection results were similar regardless of the stimulus location within the auditory cortex, even though the rat primary auditory cortex is tonotopically organized (Sally and Kelly 1988); however, since a detection paradigm was used, the spectral-based sensation effects of stimulus location could not be determined. Human studies have shown that surface stimulation of human primary auditory cortex evokes perceptions that contain a pitch (Dobelle *et al* 1973, Penfield and Perot 1963). The pitch was reported to depend on electrode location within the primary auditory field, which was theorized to be attributed to the arrangement of iso-frequency bands into a tonotopic map.

The objective of this study was to determine whether microstimulation within iso-frequency bands in the primary auditory cortex would elicit behavior that was consistent with that elicited by the corresponding auditory tone. A cross-dimensional psychophysical task was used to determine the relative sensations of auditory and cortical stimulation in the adult 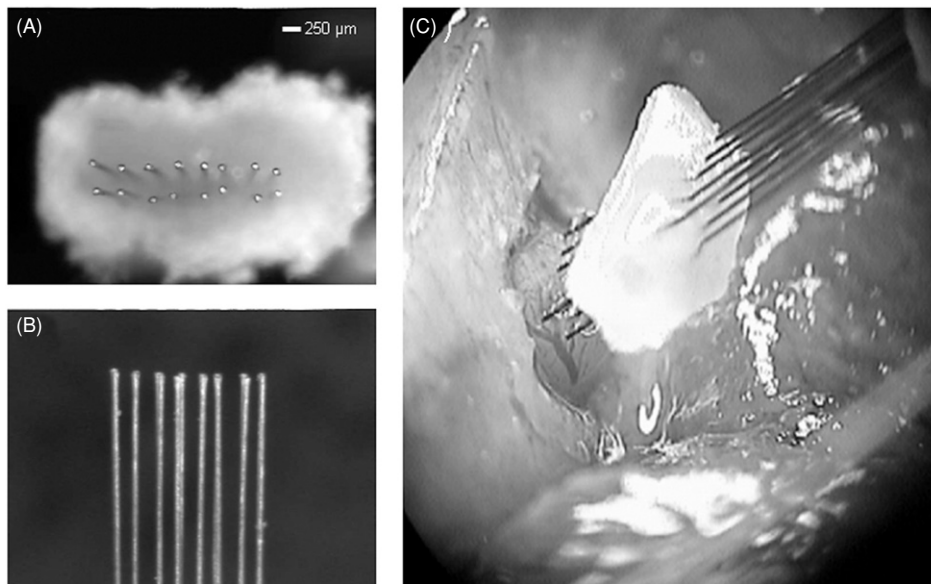white rat. Cortical microstimulation elicited behaviors relative to the best frequency (BF) of the electrode location that were correlated with behavior evoked by acoustic stimulation of the same frequency. These results demonstrate that spatially distributed microstimulation in auditory cortex provides 'virtual' auditory cues with distinct spectral content.

Methods

Behavioral training

Four male, naïve Sprague-Dawley rats (250–300 g) were trained in an auditory discrimination task. Initially, the rats were food deprived to 80% of their free-feeding weight. Subjects were generally trained 5–6 days a week, and given food on off days to maintain their food deprivation weight. Subjects responded in standard operant conditioning behavioral boxes (Med Associates, St Albans, VT) located within an anechoic room. The response wall of the test box included three side-by-side retractable response levers approximately 4 inches above the cage floor. A house light at the rear of the box was utilized for both illumination and negative reinforcement. The behavioral apparatus was controlled and monitored by software developed in-house, running on a PC interfaced with digital input–output hardware (System II, Tucker-Davis Technologies, Gainesville, FL). This equipment was also used to generate all auditory stimuli used in the experiment. The auditory stimuli were delivered via a speaker (Yamaha NS-10M Studio, Yamaha Corporation, Buena Park, CA) located 1 m directly above the test box. The system was calibrated to a position directly above the center lever and delivered a near-flat frequency response between 500 Hz and 32 kHz.

A discrimination task in a forced-choice psychophysical paradigm was used to assess stimulus generalization (figure 1).

Figure 2. Chronic microwire rat auditory cortex preparation. Arrays of 50 μm polyimide-coated tungsten microwires were assembled into an array consisting of two rows of eight electrodes. Electrode centers were spaced approximately 250 μm apart. (A) Top view of a sample electrode array viewed from the tip. (B) Side view of the same electrode array. (C) Electrode array just prior to insertion into left hemisphere auditory cortex. The electrode array insertion site was first located stereotaxically, and subsequently confirmed via vascular landmarks. The array was positioned to avoid major vascular injury and implanted between the large anterior and posterior dorsoventral vessels that characterize the primary auditory cortex in the rat.

Subjects were positively reinforced via single food pellets (P J Noyes, 45 mg rodent diet I, Lancaster, NH) for correct responses to trains of auditory stimuli. Initially, all three levers were retracted, and the house light was illuminated. The subjects were signaled to start a single trial by the extension of the center lever. In order to minimize spurious trial initiations, trials were subject-initiated by two recorded presses of this center response lever. Subsequently, the center lever was retracted, and a train (250 ms on, 250 ms off) of five pure-tone bursts was delivered. Stimulus trains were utilized instead of long stimulus durations in order to limit the total stimulus duration for the later microstimulation experiments. Auditory training stimuli trains at either 1 kHz, or 16 kHz were delivered at 70 dB SPL. Upon completion of the auditory stimulus presentation, the two outer levers were extended. A fixed-ratio response paradigm was utilized, and in order to stabilize impulsive behavior, subjects were reinforced only after four responses on a given lever within 7 s of outer lever presentation. Responses were designated correct and positively reinforced for a left-hand lever response to the 1 kHz stimulus or a right-hand lever response to the 16 kHz stimulus. Left-hand responses to the 16 kHz stimulus or right-hand responses to the 1 kHz stimulus were designated incorrect and negative reinforcement was given in the form of a 30 s dark timeout. A response was considered null, and negatively reinforced, if the subject did not respond within the 7 s response window. Null response trials in all of the training or testing were rare (zero for >95% of the sessions) and were not used in the behavioral data analysis. The general training paradigm generally required several months of training.

Auditory generalization behavioral testing

Upon criterion performance of the auditory training paradigm (above 90% for three consecutive days), psychophysical curves were created to assess auditory generalization behavioral performance. The standard auditory 1 kHz or 16 kHz reinforced stimuli were delivered, indicating left- or right-hand behavioral cues respectively; however, in the generalization-testing paradigm, approximately 25% of the trials were randomly chosen as 'probe' trials in which stimuli of intermediate auditory frequencies were presented, and the subsequent behavior recorded. The probe trials were never reinforced. The rats' generalization behavior in response to four auditory probe frequencies was tested. The auditory probe frequencies were chosen as tones spaced evenly on a logarithmic scale (1740 Hz, 3030 Hz, 5280 Hz and 9190 Hz). Hereto, this test is referred to as 'A–A' (the standard auditory discrimination task, 'A', coupled with auditory probe testing, 'A'). Daily testing sessions continued until each subject received 200 positive rewards. Due to the random trial nature of the probe presentation in the experiment, the exact number of daily trials varied, but was on average approximately 275. Daily testing sessions lasted approximately 80 min.

Surgical implantation

After three consecutive successful auditory generalization-testing sessions, each rat was chronically implanted with an array of microelectrodes. Electrode array construction and surgical implantation have been described in a previous publication (Williams *et al* 1999). Briefly, the electrode arrays consisted of two rows of eight, 50 μm diameter, polyimide coated tungsten microwires (figures 2(A) and (B)). Electrode

spacing in a given row and between rows was 250 μm, resulting in an electrode footprint of 1.75 mm by 0.25 mm. Literature reports have identified the anterior–posterior extent of rat primary auditory cortex as 1.9 mm (Kilgard and Merzenich 1999) and 2.5 mm (Sally and Kelly 1988). Thus, our electrode array was designed to span approximately 70–90% of primary auditory cortex. Animals were anesthetized with a combination of Ketamine 75.0 mg kg^{-1}, Xylazine 7.5 mg kg^{-1} and Acepromazine 1.5 mg kg^{-1}. The scalp was removed over the left hemisphere, and a 4 mm × 4 mm crainiotomy was performed at 4 mm lateral and 5 mm posterior to bregma. The dura mater was removed and the tissue moistened with sterile saline. The auditory cortex was located stereotaxically and from vascular landmarks as identified in previous studies (Sally and Kelly 1988). Viewed through a microscope, the electrode array was positioned at the surface of the brain between the large anterior and posterior dorsoventral vessels that have been shown in literature to delineate the auditory cortex in the rat (figure 2(C)). The array was rapidly inserted into cortex until visual pia mater penetration was confirmed. The electrode array was then retracted to the previously defined cortical surface and subsequently lowered to 600 μm. Gelfoam© was positioned on the brain around the electrodes to serve as a protective barrier, and the array was affixed to stainless steel bone screws in the cranium with polymethyl-methacralate. All procedures complied with the United States Department of Agriculture guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee.

Cortical microstimulation behavioral testing

After surgical recovery (approx. 1.5 weeks), the rats were tested to ensure that their behavior to the auditory task was unchanged. In all cases, subject behavior returned to pre-surgical level in less than two sessions.

In order to assess cortical microstimulation, the method of intermixing probe trials with the standard reinforced auditory discrimination trials was again employed. Daily testing sessions were conducted in an identical manner as the A–A testing sessions. The trials were subject initiated by center lever presses, and the trial stimulation was presented after center lever retraction. Approximately 75% of the trials were the normal auditory task 1 kHz or 16 kHz cues ('A'), and were reinforced appropriately. The resultant 25% of the trials were microstimulation probe trials, where the cue was cortical microstimulation delivered through a single electrode ('M'). These data are hereto referred to as 'A–M'. Due to electrode array geometry, daily A–M testing sessions were conducted on only a single row.

Electrodes within an array were labeled such that electrode 1 was the most anterior electrode on the dorsal row. Subsequent electrodes on the dorsal row were labeled through electrode 8. Electrode 9 was labeled as the most anterior electrode on the ventral row and the rest of the electrodes on the ventral row were labeled through electrode 16. Four cortical microstimulation probe stimuli were tested on a given row in order to maintain consistency between the A–M and A–A testing sessions. On A–M1 sessions the four different probe stimuli were microstimulation delivered through electrodes 1, 3, 5 or 7. A–M2 probe stimuli were microstimulation delivered through electrodes 9, 11, 13 or 15.

Microstimulation pulse trains consisted of cathodic first, charge-balanced, biphasic square-wave pulses (250 μs pulse width) delivered at 200 Hz and 68 μA. This stimulus intensity was chosen for two reasons. First, a calculated estimation of current spread based on parameters reported in the literature led to minimal effective stimulation radii (100 μm) between neighboring electrodes at 68 μA (Nunez 1981, Stoney *et al* 1968). Second, in several literature references, 68 μA was a sufficient microstimulation level to ensure that the stimulus was behaviorally robust (Rousche *et al* 2001, Tehovnik 1996). A waveform generator (WaveTek, Everett, WA) was used to generate the pulse train, which was delivered through an optical stimulus isolator (A–M Systems, Carlsborg, WA) in constant-current stimulation mode. The cortical microstimulation stimulus intensity was confirmed using a 1 kΩ resistor circuit prior to testing. The cranial stainless-steel screws served as the stimulation return pathway.
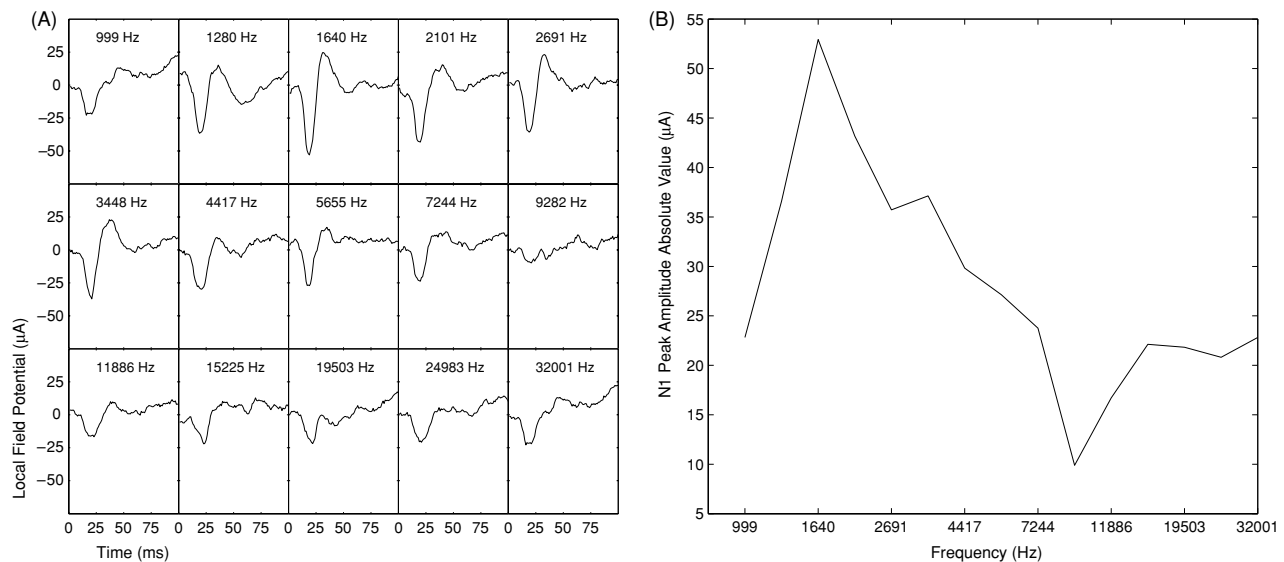
The temporal parameters of the microstimulation were chosen to mimic the temporal envelope of the auditory stimuli. Microstimulation pulse trains were delivered in five bursts (250 ms on, 250 ms off). The behavioral apparatus software recorded responses to both the task stimuli and the probe stimuli. The microstimulation-evoked behavior was evaluated based on BFs of the electrodes as discussed below.

Cortical microstimulation control

A control experiment was conducted to ensure that no environmental behavioral cues were affecting behavioral discrimination. In the control experiment the subject was reinforced for correct discrimination of the standard auditory task. Session probe stimuli were constructed with the same parameters and probability as the previous A–M sessions; however, in this experiment the stimulus isolator was turned off, ensuring that the animal would not receive cortical microstimulation. Behavioral responses to both the normal auditory discrimination trials and the control probe trials were tabulated and analyzed.

Electrophysiological recording and analysis

Electrophysiological recordings in response to auditory stimulation were conducted under anesthesia using the aforementioned ketamine/xylazine/acepromazine mixture. Recording sessions occurred subsequent to the microstimulation behavioral sessions. Anesthetized subjects were positioned on the cage floor in the center of the calibrated environment. Frequency response characteristics of local field potentials (LFPs) and simultaneously recorded multi-unit clusters were determined from 15 logarithmically spaced pure tones ranging in frequency from 1 kHz to 32 kHz delivered at 70 dB SPL. Tone intervals were 100 ms on, 900 ms off. Each frequency was randomly repeated 48 times. All tones had a 5 ms cosine gated rise and fall time.

Figure 3. Acoustic frequency tuning from local field potentials in auditory cortex. (A) Fifteen separate 100 ms tonepips with frequencies logarithmically spaced from 1 kHz to 32 kHz were delivered at 70 dB. The waveforms represent the average local field potential on a single electrode evoked by 48 repetitions of each tonepip stimulus. (B) The BF for this electrode from an N1 peak analysis was 1640 Hz.

Electrophysiological data were recorded on a multichannel acquisition processor (MAP) simultaneously for the 16 channels at 40 kHz (Plexon Inc, Denison, TX). The data were filtered and amplified either for LFP recording (1–300 Hz, gain = 10 000) or for multi-unit recording (250 Hz–8 kHz, programmable gain). The LFP responses were analyzed based on a previous study showing that the N1 peak of the LFP responses of the primary auditory cortex of guinea pig is tuned in the same way as single unit responses and exhibit this tuning over long-time periods (Galvan *et al* 2001). The LFP data from the first 50 ms after each stimulus onset were examined for auditory tuning. The LFP data were averaged for each of the fifteen tones and the frequency with the largest negative deflection from the 50 ms mean was determined to be the BF. When present on a recording electrode multi-unit responses were averaged as a function of the stimulus frequency, and the stimulus eliciting the largest response over the 100 ms tone duration was determined to be the multi-unit BF. When both LFP and multi-unit BF measurements were available on an electrode the overall BF of the electrode was determined as the average of the two measurements.

Statistical evaluation of microstimulation-induced behavior

In order to evaluate the behavior induced by cortical microstimulation predictions of the BF of each stimulated electrode were made based on the microstimulation-induced behavior. First the auditory generalization data were modeled for each subject by a logarithmic model. Then the predicted BF of the sensation could be calculated by evaluating this model at each behavioral data point. The predicted BFs for each stimulated electrode were plotted relative to the actual BF from the electrophysiological data and a correlation analysis was performed.

Results

In order to quantify microstimulation-induced behavior, both auditory-induced electrophysiological responses and auditory-induced behavior characterization were characterized. The BFs across the electrode array were determined via analysis of auditory evoked LFP and multi-unit spike-rate fluctuations. Microstimulation-induced behavior was then evaluated relative to the natural auditory-evoked behavior to obtain a prediction of the sensation of the single electrode microstimulation. These data were evaluated relative to the electrophysiological BF recorded at each electrode.
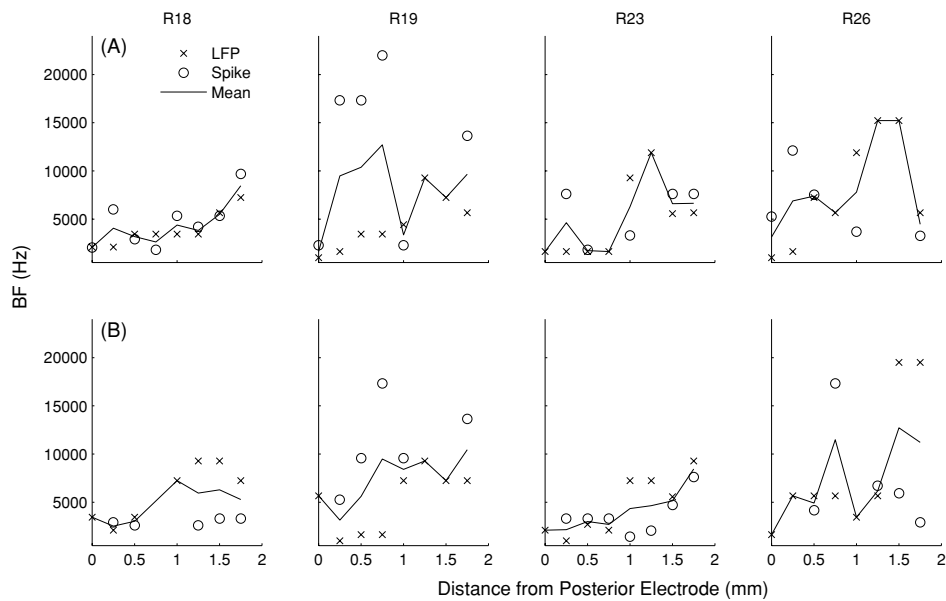
Auditory response electrophysiology

Recording data consisted of LFP and multi-unit activity evoked by auditory stimuli across four, 16-channel microelectrode arrays implanted in the auditory cortex of the four behaviorally trained rats. An example of stimulus-triggered and averaged LFP data from a single electrode is shown in figure 3. In this example, 1640 Hz produced the largest N1 peak deflection from the mean, and was determined to be the BF. The negative wave exhibits systematic tuning at frequencies neighboring 1640 Hz.
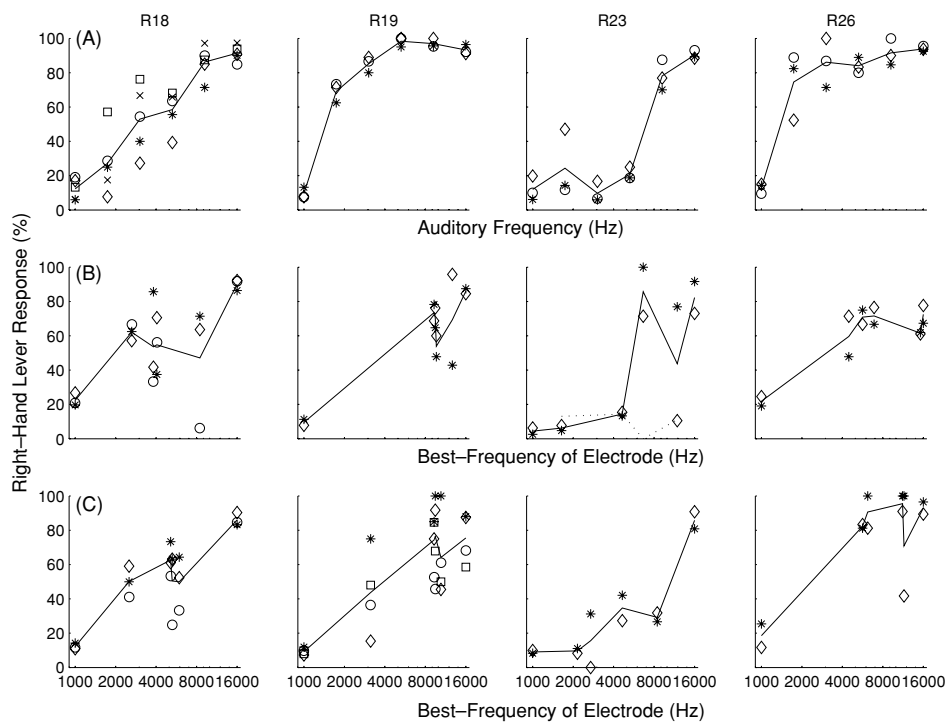
BF data were evaluated for each subject for both the dorsal and ventral row of the array as shown in figure 4. From 64 channels across the four rats studied, LFP BFs were found from 999 Hz to 19 503 Hz, spanning nearly the entire spectrum used for auditory stimulation in this study. The multi-unit BFs spanned a similar range, from 1432 Hz to 21 965 Hz.

A–A behavior results

The subjects reliably performed the discrimination of the 1 kHz and 16 kHz frequencies. Example data from 14 sessions over 4 rats are shown in figure 5(A). Each column represents data

Figure 4. Best frequency maps in each of four rats. Local field potential and spike-rate best frequencies on the dorsal row (A) and ventral row (B) of the electrode array in each rat. The line represents the running mean of local field potential and spike-rate BF estimations. In general, BF increases as the anterior distance along the array increases.

Figure 5. Auditory and microstimulation generalization curves. (A) Each marker type represents data from a single auditory generalization testing session plotted as a function of auditory stimulus frequency. Columns represent data from the four subjects. The ordinate represents the per cent of the responses on the right-hand lever. (B), (C) Microstimulation generalization curves from stimulation sessions on the dorsal row (B) or ventral row (C) of the electrode array, respectively. Subjects were rewarded for responding correctly to acoustic 1 kHz and 16 kHz tones. Microstimulation trials are plotted relative to the BF of the electrode used to provide the electrical stimulation. A control experiment using zero microstimulation current on probe trials is shown as the dotted line for the dorsal row (B) of subject R23.

from an individual subject. Daily sessions are represented by individual data markers, and the sliding mean of the sessions is indicated by the solid line. The overall performance of the discrimination task for these 14 sessions was 91.4 ± 0.9%

(mean ± standard error). The rats consistently responded to auditory frequencies intermediate of the 1 kHz and 16 kHz reinforced frequencies. Across animals the response curves increased in a general monotonic fashion, such that higher

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frequency auditory cues resulted in more right-hand lever responses. There was a marked behavioral difference between subjects; however, the behavior within a subject was generally consistent across testing sessions.
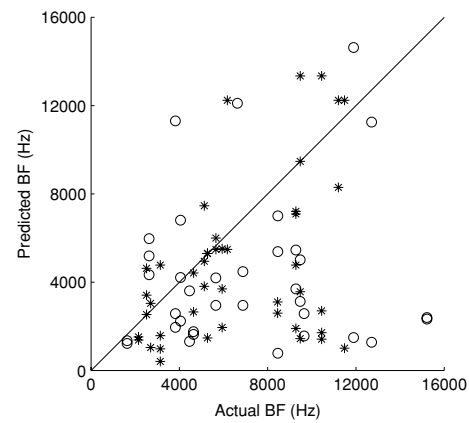
A–M behavior results

Figures 5(B) and (C) show the A–M results plotted versus the BF of the electrode used for stimulation for the dorsal and ventral rows, respectively. Again, daily sessions are indicated by individual data markers, and the solid line indicates the sliding mean of the sessions. Overall, 20 A–M sessions were performed across the four subjects. Microstimulation of the different electrodes resulted in similar lever-pressing behavior to the auditory trials. Microstimulation probe trials resulting in null responses were extremely rare (zero for 19 of the 20 sessions). Further, the response latency for microstimulation probe trials (1663 ± 633 ms; mean $\pm$ standard deviation) was significantly shorter (*t*-test, $p < 0.01$) than the auditory probe trials (2109 ± 848 ms; mean $\pm$ standard deviation). These data suggest that microstimulation can provide a robust sensory cue for behavior.

A control experiment was conducted to ensure that the electrical stimulation apparatus provided no confounding behavioral cues (shown by the dotted line in figure 5(B) for subject R23). In this experiment, the session was conducted as a normal A–M session, except that the electrical stimulus isolator was turned off, ensuring that no microstimulation cues were delivered to the subject. Given the experimental design utilizing lever retraction and extension the subject readily responded on all trials; however, the subject responses to any of the control stimuli were not significantly different from each other (*t*-test, $p < 0.05$). This result suggests that these trials provided no differential information to the subject. The low percentage of right-lever responses on these control trials is indicative of a left-lever bias for this subject.

To further evaluate the microstimulation-BF relationship, the data for all four subjects were evaluated cumulatively, as shown in figure 6. Based on logarithmic fits to the auditory generalization data shown in figure 5(A), the microstimulation behavioral data were used to estimate the frequency of the induced sensation (predicted BF). These data were then plotted relative to the actual BF of each electrode, as determined by the electrophysiological measurements. Data from the dorsal and ventral row are depicted by circles and stars data markers, respectively. Correlation analysis of the cumulative predicted and actual BFs showed a significant positive correlation ($R = 0.26$, $p < 0.05$). The cumulative ventral row data showed a stronger correlation ($R = 0.40$, $p < 0.01$) than the dorsal row data ($R = 0.12$, $p = 0.54$). The highest individual subject correlation was found for subject R23 ($R = 0.56$, $p < 0.05$).

Discussion

The objective of this study was to investigate the behavioral effects associated with microstimulation of the auditory cortex in the adult rat model. Subjects were required to behaviorally respond to cortical microstimulation probe trials randomly

Figure 6. The relationship of the electrophysiologically determined BF to the behavioral BF. Based on a logarithmic model of the auditory generalization behavior, a predicted BF for the microstimulation trials was calculated. These data are positively correlated with the actual BF of each electrode as determined by local field potential and spike-rate measurements ($R = 0.26$, $p < 0.05$).

inserted between trials of an auditory frequency discrimination task. The microstimulation-induced responses were analyzed relative to each microelectrode's auditory electrophysiology characteristics determined via LFP recording.

The sensation basis of auditory cortex stimulation

Animal stimuli-detection behavior in response to penetrating visual cortical stimulation was first evaluated by Bartlett and Doty (1980) in the primate and more recently by Troyk *et al* (2003) and Bradley *et al* (2005). Subsequently, Rousche and Normann (1999) assessed stimuli-detection behavior in response to auditory cortical stimulation first in the cat model and later in the rat model (Rousche *et al* 2003). These studies show that subjects readily respond to single electrode stimulation in a sensory detection paradigm. Recently, Scheich and Briendl (2002) evaluated penetrating auditory cortex stimulation in a discrimination paradigm in the Mongolian gerbil. The gerbils were able to discriminate spatial, temporal and spatio-temporal patterns of two-electrode stimulation. In this study we added to this literature, evaluating the spectral dependence of the auditory sensations evoked by single electrode microstimulation in different spatial locations within auditory cortex.

The results in figure 6 show that the basic spectral component of the elicited sensations was positively correlated with increasing BFs in the auditory cortex. This is consistent with visual cortex studies of systematic variation of sensation with microstimulation location. Both Brindley and Lewin (1968b) and Dobelle *et al* reported that surface visual cortex stimulation produced phosphenes that roughly corresponded to classical map expectations (Dobelle and Mladejovsky 1974).

Temporal stimulation parameters

The electrical stimulus pulse train used in this study was designed to match the temporal envelope of the auditory stimulus. Using surface stimulation of visual cortex, Dobelle

reported 10–15 s of continuous stimulation before the elicited phosphenes faded (Dobelle and Mladejovsky 1974). Using penetrating stimulation, Schmidt *et al* (1996) reported a phosphene duration of 930 ms before the sensation faded. Dobelle employed 1 s pulse trains for surface auditory cortex stimulation, and did not publish any subject reports of the auditory sensation fading (Dobelle *et al* 1973). The electrical stimulation used in this study consisted of five 250 ms pulse trains (with 250 ms inter-train intervals). The durations of these pulse trains were well below the stimulus durations that led to fading sensation in the human studies. Therefore, it is likely that the sensation in this study persisted for the full 250 ms. Schmidt *et al* (1996) noted an accommodation to repeated stimulation that decreased the brightness of the phosphenes produced. Over 5 repetitions, the intensity dropped 20–30%. Our stimulus trains may have resulted in accommodation, which likely would have decreased the loudness of the perceived sensation (Dobelle *et al* 1973).

The effect of temporal patterning of cortical microstimulation on sensation is still unknown. The 200 Hz bipolar stimulus used in this study would theoretically correspond to a 200 Hz amplitude-modulated sound; however, cortical neurons typically do not follow such fast modulation frequencies. The temporal patterning in this study was chosen to follow the paradigm used in other cortical microstimulation studies. However, it is noted that this abnormal temporal pattern in cortex may have contributed to some of the variability in the results. It is well documented that auditory cortex neurons exhibit 'on' responses to stimuli; however, whether this feature can be captured via microstimulation using a modulated pulse rate is not yet known. In primate somatosensory cortex, Romo *et al* (1998) reported identical behavioral responses to periodic and aperiodic cortical microstimulus trains of the same mean frequency. This suggests, at least for the train durations and stimulation modulation rates reported in that study, that aperiodic temporal information may not convey additional stimulus information.

Spatial and temporal pattern of microstimulation

Sensory stimulus encoding occurs in distributed spatial and temporal patterns (Chapin and Nicolelis 1999, Furukawa *et al* 2000, Villa and Abeles 1990). Given this characteristic, more sophisticated temporal and spatial microstimulation patterning is required for stimulus equivalency between natural auditory and electrical stimulation. The possibility of increasingly sophisticated temporal and spatial patterning of microstimulation is intimately tied to elegant device improvements. Micromachined device technologies offer intricate control over device parameters. Hetke *et al* (2003) demonstrated three-dimensional site spacing geometry in a cortical recording and stimulating device. The study presented here focused on the application basis of cortical microstimulation and thus utilized microwire arrays. However, several investigators in our laboratory have recently described success in utilizing complex micromachined devices for long-term cortical recording (Kipke *et al* 2003, Vetter

et al 2004). These recent device developments indicate that complex spatial and temporal stimulation patterns in long-term preparations may be a possibility and provide a device framework for further cortical microstimulation neuroprosthetic research.

Frequency discrimination and generalization

Here we used a frequency discrimination and generalization behavioral task to determine the individual subjects' behavior relative to frequency. Although the speaker and testing box were calibrated at the position of the animal, an important possible confound exists in the relationship between frequency and intensity at the tympanic membrane. Thus, the animals may have utilized a combination of frequency and intensity to accomplish the discrimination. However, this strategy presumably would not have had an effect on the microstimulation trials, since the microstimulation was always provided at the same intensity.

The frequency generalization data for three of the four subjects exhibited a pronounced categorization shape rather than the characteristic sigmoidal shape. This may be due to an undersampling of the frequency domain near the subjects' point of subjective equality. Interestingly, the frequency at which the responses shifted from the left-hand lever to the right-hand lever was not identical for those three subjects. However, importantly, the behavior of the subjects over the course of multiple sessions was consistent, allowing a fairly robust determination of sensation based on behavior. This consistency was necessary to predict the spectral basis of the sensation evoked by microstimulation from the behavior.

Electrophysiological recording and BF distribution

This study determined auditory best frequencies under anesthesia. Recordings were not conducted in the awake state due to the small amplitudes of LFP recordings, and the recording artifact induced by movement; however, several studies have reported that BFs of single units do not change from awake to anesthetized states in rat auditory cortex (Gaese and Ostwald 2001, Talwar and Gerstein 2001). Several studies have described the complexity of auditory response properties in awake preparations (deCharms *et al* 1998, Gaese and Ostwald 2003), as well as the long-term dynamic nature of auditory cortex response properties (Witte *et al* 1999). These studies indicate that the BF-based analysis conducted here was valid, but also indicate that further studies are merited utilizing better devices and more thorough auditory cortical response measures in awake animal preparations.

Previous literature reports have shown that LFP recordings provide a robust measure of tuning across the electrode array, which is not significantly different from multiunit responses (Norena and Eggermont 2002). Our results generally show consistent tuning between LFP and multiunit responses; however, some of the electrode sites exhibited large differences between the LFP and multi-unit tuning. These differences are a possible confound in the microstimulation induced behavior results and may contribute to some of the unexplained behavioral variance.

Recently, Galvan *et al* (2001) reported long-term frequency of tuning of LFPs in auditory cortex of guinea pig. Our recordings in auditory cortex of the rat produced tuning curves from 999 Hz to 24 983 Hz. This is slightly lower than the upper ranges of ~40 kHz found by Sally and Kelly (1988); however, most of the responses >30 kHz found by Sally and Kelly were proximal, or even anterior to the large anterior dorsoventral vessel in primary auditory cortex. Our electrode arrays were intentionally implanted significantly caudally of this vessel in order to avoid vascular injury. This may have biased our electrode placement in the lower frequency ranges. Additionally, the lower frequency representations of our rats may have been expanded due to training and microstimulation-induced plasticity, as discussed below.

Cortical reorganization

Due to the increasing potential failure of recording capabilities with lifetime of the microelectrode array, implantation did not occur until after the rats were successfully trained in the 1 kHz/16 kHz discrimination task. It has been shown that behavioral training alters the representation of sensory stimuli in cortex (Bakin *et al* 1996, Recanzone *et al* 1992, 1993, Suga *et al* 2002). Furthermore, cortical microstimulation has been shown to change the functional organization of cortex (Chowdhury and Suga 2000, Dinse *et al* 1993, Maldonado and Gerstein 1996, Nudo *et al* 1990, Talwar and Gerstein 2001). In the current study, training was conducted for several months at 1 kHz and 16 kHz. Additionally, several sessions of microstimulation were conducted before BF measurements were made. Our electrophysiological results show an over-representation of the lower frequencies, with 100% of the stimulated electrodes having BFs of 16 kHz or lower.

The expanded cortical representations of the lower frequencies may have influenced the results of the microstimulation-induced behavior. The results from figure 6 show a lower correlation of microstimulation-induced behavior with BF, relative to the correlation of the auditory-induced behavior with frequency. If, in fact, the frequency representation within auditory cortex was distorted, this may have tended to diminish the strength of the BF-dependent microstimulation behavior and therefore may have contributed to some of the uncorrelated variance in the data.

In conclusion, the data suggest that cortical microstimulation in different locations in auditory cortex provides a behaviorally relevant auditory sensation. Furthermore, the behavior evoked by this sensation is dependent on the best frequency of the tissue local to the implanted electrode. In order to quantify the information-carrying capacity of chronically implanted electrodes in the auditory cortex for a sensory neuroprosthesis, more parameters require investigation. Putatively, more knowledge of the sensations elicited by penetrating electrical stimulation of sensory cortex would allow for better engineering of the stimulus and electrodes to increase the information transfer. In our studies, the subject was not required to respond to other components of the stimulus (for example, bandwidth, background stimuli and temporal parameters); thus the

sensation cannot be fully described. However, this study validates further exploration of the electrical stimulus perceptual parameters of penetrating auditory cortical stimulation.

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