

Long-Term Consolidation and Retention of Learning-Induced Tuning Plasticity in the Auditory Cortex of the Guinea Pig

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The major goal of this study was to determine whether classical conditioning produces long-term neural consolidation of frequency tuning plasticity in the auditory cortex. Local field potentials (LFPs) were obtained from chronically implanted adult male Hartley guinea pigs that were divided into conditioning ($n = 4$) and sensitization control ($n = 3$) groups. Tuning functions were determined in awake subjects for average LFPs (~ 0.4 to 36.0 kHz, -20 to 80 dB) immediately before training as well as 1 h and 1, 3, 7, and 10 days after training; sensitization subjects did not have a 10-day retention test. Conditioning consisted of a single session of 30 to 45 trials of a 6-s tone (CS, 70 dB) that was not the best frequency (BF, peak of a tuning curve), followed by a brief leg shock (US) at CS offset. Sensitization control animals received the same density of CS and US presentations unpaired. Heart rate recordings showed that the conditioning group developed conditioned bradycardia, whereas the sensitization control group did not. Local field potentials in the conditioning group, but not in the sensitization group, developed tuning plasticity. The ratio of responses to the CS frequency versus the BF were increased 1 h after training, and this increase was retained for the 10-day period of the study. Both tuning plasticity and retention were observed across stimulus levels (10–80 dB). Most noteworthy, tuning plasticity exhibited consolidation (i.e., developed greater CS-specific effects across retention periods), attaining asymptote at 3 days. The findings indicate that LFPs in the auditory cortex have three cardinal features of behavioral memory: associative tuning plasticity, long-term retention, and long-term consolidation. Potential cellular and subcellular mechanisms of LFP tuning plasticity and long-term consolidation are discussed. © 2002 Elsevier Science

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INTRODUCTION

The discovery that sensory cortex directly participates in learning and memory originated in studies of local field potentials (LFPs) in the auditory cortex during classical conditioning. Learning was found to produce increased responses in the auditory cortex to acoustic conditioned stimuli (CS) (Galambos, Sheatz, & Vernier, 1955). However, sensory cortical plasticity in learning did not become incorporated into general conceptions of brain function, in the field of either learning/memory or sensory physiology, despite subsequent replication and extension of these findings with single-unit and multiple-unit recordings and verification of the fact that the effect was associative, that is, due to learning itself rather than to arousal or other nonassociative variables (reviewed in Weinberger & Diamond, 1987).

The use of a hybrid experimental approach incorporating basic design elements of both fields has altered the understanding of the role of sensory cortex in learning. Instead of recording responses to the CS only during training trials, frequency receptive fields (RFs) were obtained before and at various intervals after training. Receptive field analysis revealed that learning produces highly specific and systematic changes in the frequency tuning of cells in the primary auditory cortex (ACx) (Bakin & Weinberger, 1990). For example, classical conditioning and instrumental avoidance learning increase the magnitude of response to the frequency of the CS relative to that of the pretraining best frequency (BF, peak of a tuning curve) and other frequencies, whose responses often decrease. These simultaneous changes of opposite sign are often sufficient to actually shift RF tuning to the frequency of the CS, resulting in an expanded representation of behaviorally significant stimuli in cortical frequency maps (Recanzone, Schreiner, & Merzenich, 1993). This RF plasticity is highly specific to the frequency of the CS (e.g., ± 0.1 octaves), is associative (not found in sensitization control groups), is discriminative (increased response to a CS+ tone and decreased response to a CS- tone), develops rapidly (within five trials), lasts indefinitely (tracked to 8 weeks), and consolidates over the brief periods studied (minutes to an hour) (reviewed in Weinberger, 1998, 2001). Thus, RF plasticity has the cardinal features of memory. It is hypothesized to constitute a neural "memory code" for the representation of acquired stimulus importance, specifically, the greater the behavioral significance, the larger the number of cells tuned to that stimulus (Weinberger, 2001, *in press*).

It is well known that memories are not fixed at the time of learning but are susceptible to postlearning events, such as head trauma, that produce retrograde amnesia. Postlearning treatments, such as the release of stress hormones, can strengthen memories beyond normal levels. In all cases, susceptibility to postlearning effects decreases over time; the exact time course (e.g., hours, days, months) depends on various circumstances, such as the task and the amount of training. This time-dependent reduction in susceptibility of memory may reflect a gradient of increase in the strength of memory after initial learning in the absence of further training (reviewed in McGaugh, 2000). However, there have been few attempts to directly observe temporally graded increases in neural correlates of memory.

Prior studies of neural consolidation in mammals have discovered shifts in the loci of involvement after training for neural discharges (Freeman & Gabriel, 1999), glucose uptake (Bontempi, Jaffard, & Destrade, 1996; Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Sif et al., 1991) and PET recording (Shadmehr & Holcomb, 1997). While prior research has emphasized shifting loci of neural events after training, this does not imply that all neural substrates of memories become redistributed. A distinction has been made between regions that store specific sensory aspects of an experience from those that serve other necessary functions. For example, it has been proposed that cortical memory storage is strengthened by repeated activation of neocortical sensory representations by the hippocampus, which itself has a reduced role over time (Zola-Morgan & Squire, 1990). Thus, in addition to changes in the distribution of involved brain systems during consolidation, one might expect an actual strengthening of neural substrates of stored sensory events over days or longer. To the best of our knowledge, such long-term neural consolidation has not been reported. As noted above, the ACx develops CS-specific tuning plasticity that has the major features of memory, including short-term (minutes to an hour) consolidation. The goal of this study was to determine whether the ACx develops long-term (days) neural consolidation.

In the current study, we analyzed the tuning of LFPs from 1 h to 10 days after a single session of classical conditioning. Local field potentials exhibit greater long-term stability than do unitary discharges because they represent the massed responses of neural elements over a larger cortical area, reducing susceptibility to loss of data over time (Bullock, 1997). Moreover, LFPs exhibit good frequency tuning (Galli, Lifshitz & Adrian, 1971; Tunturi, 1944; Walloch, 1975; Woolsey & Walzl, 1942). Finally, the frequency tuning of LFPs does not drift over weeks (Galván, Chen, & Weinberger, 2001). The study of long-term consolidation requires, first, that LFPs first develop tuning plasticity and, second, that they exhibit long-term retention. Neither of these features had been studied previously, but their investigation was a natural part of the current experimental design. Some of these findings have been reported in abstract (Galván, Chen, & Weinberger, 1998).

MATERIALS AND METHODS

Subjects and Surgical Preparation

The subjects were 7 male adult Hartley guinea pigs (Hilltop Farms), weighing 370 to 690 g at the time of surgery. They were housed in groups of 2 or 3 in standard guinea pig cages, with ad libitum food and water, on a 12-h light–dark cycle (lights on at 7 a.m.). On the day of surgery, they were premedicated with atropine sulfate (0.22 mg/kg i.p.) and diazepam (9.0 mg/kg i.p.), followed by sodium pentobarbital (25 mg/kg i.p.) 15 min later. Supplements of sodium pentobarbital (8.3 mg/kg i.p.) were administered as needed to maintain a state of areflexia. Body temperature was maintained at 37°C with the use of a homeothermic heating pad (Harvard Apparatus, Cambridge, MA), and ophthalmic ointment was applied to keep the eyes moist. Subjects were mounted in a stereotaxic instrument (Kopf, Tujunga, CA); the scalp was resected after subcutaneous administration of lidocaine, and the calvaria was cleared. Stainless steel screws were threaded into several small burr holes, a silver ball electrode was placed in a burr hole near bregma to serve as the reference electrode, and a craniotomy was performed over the left auditory cortex.

A pedestal of dental acrylic was constructed into which threaded spacers were embedded, and these were bolted to a rigid support, allowing removal of ear bars.

The auditory cortex was identified physiologically by recording local field potentials elicited by clicks using a roving microelectrode. A general frequency map was then obtained using tones of various frequencies. The guinea pig's tonotopic auditory cortex consists of two major mirror image areas: an anterior field with low- to high-frequency organization along the anterior-posterior axis and a posterior field with the reverse organization (Redies, Sieben, & Creutzfeldt, 1989; Robertson & Irvine, 1989). The dura was removed and an electrode array was lowered slowly into the cortex using a Narishige (Tokyo) stepping microdrive (Model SM21) and fixed after the surface positive LFP reversed (~ 900 – $1100 \mu\text{m}$ depth). As this reversal takes place in the region of the border between layers III and IV, consistent with a current sink in layer IV, the recording sites were in layer IV or below (Borbély, 1970; Creutzfeldt, Watanabe, & Lux, 1966; Mitzdorf, 1985). The electrode array consisted of a linear arrangement of four to eight Teflon-coated tungsten wire electrodes (0.004 inches, Calif. Fine Wire, Carlsborg, WA) in a Wire-Pro (Salem, NJ) connector strip. The distance between adjacent electrodes was $\sim 550 \mu\text{m}$, and impedance at 1.0 kHz was $\sim 0.5 \text{ M}\Omega$. The brain was covered with a layer of Gelfoam (Upjohn/Pharmacia, Kalamazoo, MI), and the electrode array was affixed to the pedestal with dental acrylic. An antibiotic ointment (Panalog, Solvay, Mendota Heights, MN) was applied before suturing the scalp. Subjects were given a subcutaneous injection (2–6 ml) of physiological saline at body temperature, given an additional injection of atropine sulfate (0.22 mg/kg i.p.), and allowed to recover in an incubator before being returned to the vivarium. All procedures were performed in accordance with the University of California, Irvine, animal research committee and the National Institute of Health animal welfare guidelines.

Acoustic Stimulation and Recording of LFPs

Pure tone stimuli were generated by a Wavetek digital synthesizer and a digital attenuator bank (Model 5100, WWG, San Diego), controlled by a minicomputer (Digital 11/73, Digital Equipment Corp., Cambridge, MA), and delivered to a calibrated 1.5-inch speaker. Rise and fall times of tone bursts were 5 ms (S84-04 acoustic gate, Coulbourn Instruments, Lehigh Valley, PA). The speaker housing was placed at the entrance to the ear canal contralateral to the recording sites and calibrated at this position ($.0002 \text{ dyne/cm}^2$, Model 4134 condenser microphone, B & K, Copenhagen, Denmark) because calibration at the tympanic membrane requires invasive procedures that are stressful to waking animals (Suga & Manabe, 1982). Tone-evoked LFPs were recorded by a multiple-channel amplifier (EX-1000, gain = 1000, 1–300 Hz, Dagan, Minneapolis, MN) and digitized on a 386 computer (AST Technologies, Irvine, CA) using commercial software (Datawave Technologies, Longmont, CO). During conditioning and sensitization training, shock was applied to the hind limbs via foil cuffs by a physiological stimulator (Model S-88, Grass Instruments, Quincy, MA).

Tuning was determined sequentially at 11 stimulus intensities (descending order, 80 to -20 dB) by presenting 20 repetitions of an ascending frequency sequence of either 11 or 14 tones (number was constant within a subject) across the frequency range of ~ 0.4

to 36.0 kHz (half-octave steps, 100-ms tone duration bursts, 10-ms rise/fall times, 800-ms intertone intervals, 1.5-s intersequence interval).

Adaptation, Training, and Tuning Retention Tests

After 4 to 5 days of recovery, subjects were adapted to hammock restraint for several sessions over 2 to 3 weeks in two different acoustic rooms (IAC, Bronx, NY), later used for obtaining tuning functions and behavioral training, respectively. The subject rested in a vinyl hammock, and the head was affixed to a rigid support via attachments to the skull pedestal. Frequency tuning was determined during adaptation sessions to determine which recording sites yielded clearly tuned LFPs and to ensure that tuning did not drift over days. Stability data have been reported elsewhere (Galván et al., 2001). Thirteen electrodes yielded stable tuned LFPs during adaptation.

Before training, subjects were divided into two groups: classical conditioning ($n = 4$, tuning from nine probes) and sensitization control ($n = 3$, tuning from four probes). There was no significant difference between groups in the location of electrodes within the mirror image primary auditory cortical fields based on the frequency map obtained during surgery (conditioning: anterior = 7, posterior = 2; sensitization control: anterior = 3, posterior = 1; chi-square, $p > .05$). There was no significant difference in pretraining best frequencies (chi-square, $p > .05$). Tuning functions were determined immediately preceding a single training session as well as at 1 h and 1, 3, 7, and 10 days after training. (The sensitization group was not tested at a 10 days because of the lack of RF plasticity for all preceding periods.) The tonal frequency selected as the conditioned stimulus was chosen to be different from the pretraining BF, based on the stable tuning functions determined during adaptation. The purpose of selecting a non-BF tone was to determine whether conditioning produces increased response magnitude of the CS frequency relative to the pretraining best frequency, as previously found for unit discharges in the auditory cortex (Bakin & Weinberger, 1990; Edeline & Weinberger, 1993; Weinberger, Javid, & Lapan, 1993). The CS frequencies were selected to span ~ 0.5 to 2.5 octaves above and below the pretraining BF because this was the general range of pretraining responses. We used a range of frequency distances to avoid conclusions based on a specific or highly limited distance.

On the day of training, pretraining tuning functions were obtained. Then each subject was taken to a differently sized and furnished acoustic room in another part of the laboratory and was placed in a hammock with the pedestal fixed to a rigid support to prevent head movements and to maintain a constant relation of the speaker to the ear. Testing and training took place in different environments to minimize possible generalization of fear from the training to the testing chamber. After training, subjects exhibited no distress when placed in the testing chamber, to which they had been well adapted. The acoustic stimulation system used for training was identical to that used for RF determination. The conditioning group first received several trials of CS alone (6 s, 70 dB) (habituation), and then the CS was paired with a shock (US) to the hind limbs (500 ms train, 200 pps, 2 ms duration, 1.5–2.5 mV) at CS offset for 30 to 45 trials (ITI mean = 90 s, range = 30–150 s). US intensity was set to levels that were minimal to elicit consistent unconditioned limb withdrawal. The sensitization control group received the same habituation and training protocol except that the CS and US were explicitly unpaired, in a pseudo-random manner (no more than three CS or US in a row), with the same overall stimulus

density. The purpose of the habituation trials was to make certain that subjects could hear and respond to the tone and exhibit behavioral plasticity in the form of a reduction of response with tone repetition.

After training, subjects were returned to their home cages for approximately 1 h and then brought back to the original recording chamber, at which time the 1-h posttraining RFs were obtained. Subjects were returned to the vivarium, and RFs were obtained for up to 10 days, as indicated above.

Heart Rate Recording and Analysis

The electrocardiogram was recorded during training sessions via two stainless steel wires inserted subcutaneously on either side of the thorax. It was amplified (gain = 1000, bandpass = 10–100 Hz, DAM 50, WPI Instruments, Sarasota, FL), displayed on an oscilloscope, and processed by a voltage discriminator that was set to provide an output pulse that coincided with each heartbeat. Output pulses were sent either to a rate meter, whose output (beats per minute) was continually written out on a chart recorder (bandpass 0–75 Hz, Model 7, Grass Instruments) or digitized and stored by a CED data acquisition system and PC computer (Cambridge Electronic Instruments, Cambridge, UK). Responses to tones were invariably slowing of heart rate. The cardiac deceleration response to the CS was quantified for each trial by subtracting the minimum heart rate during the CS from the heart rate immediately preceding CS presentation. The mean cardiac deceleration response was calculated for habituation and for each five-trial block of training. Because animals received different numbers of habituation trials, the data for each subject were normalized; the mean response for each block of conditioning or sensitization was divided by the mean response during habituation and was expressed as percentage change from habituation as $[(\text{training/habituation}) \times 100] - 100$.

A value of zero indicates no change from mean tone-evoked bradycardia during habituation. Positive values indicate more bradycardia than the mean, while negative values indicate bradycardia less than the mean response during habituation (a value of negative 100 would be equal to no bradycardia to the CS).

Analysis of Tuning Functions

Tone-evoked LFPs consisted of a very small and inconsistent positivity (P1, ~8–12 ms), followed by a large and consistent negativity (N1, ~15–20 ms) and a smaller and longer latency positivity of variable amplitude (P2, ~30–40 ms) (Borbély, 1970; Borbély & Hall, 1970). Off-line analysis was accomplished using the Experimenter's Workbench software package (DataWave, Longmont, CO). The mean baseline (~6 ms following stimulus onset, always preceding any cortical response) to peak or valley values was calculated for the P1, N1, and P2 components over the 20 tone repetitions of every combination of frequency and intensity for all days of recording. The P1 components were too small and inconsistent to analyze. The P2 data were analyzed and found not to exhibit systematic tuning and are not reported here. The N1 component was systematically tuned and is the subject of this article. The characteristic frequency (CF) was the frequency eliciting a response at threshold. The threshold was determined by two independent blind assessments, performed on different occasions by two experimenters, to be the lowest

intensity with a sharp and consistent N1 potential, provided that the CF also elicited an N1 potential above threshold.

The amplitudes of the N1 were determined and analyzed. However, in a previous study of long-term recording in the absence of training, we found that while frequency tuning does not drift, absolute amplitudes can vary randomly over periods of 10 days or more (Galván et al., 2001). Therefore, the N1 amplitude for each probe was normalized within each daily tuning function for each intensity by dividing the response to each tone by the value of the largest response for that intensity. Normalization allowed comparison of responses from different days within an animal without affecting the shape of the tuning functions.

The magnitude of the normalized averaged N1 response to each frequency was used to generate the tuning function for each intensity at each recording period. To assess changes after training, difference functions were generated by subtracting the pretraining tuning curve from each of the posttraining tuning curves for each intensity. The magnitude of response at the frequency that had been used in conditioning or sensitization (hereafter called CS frequency) was compared to the response at the pretraining BF for the pretraining period and each of the posttraining periods for all intensities. The ratio (hereafter called CS/BF ratio) is $[CS/(CS + BF)] \times 2$. The ratio would equal 1.0 if the magnitude of response to the CS frequency were equal to the magnitude of response to the BF. (This formula was used to avoid obtaining infinite values from a direct division of the CS magnitude by the BF magnitude, as would occur if responses to the pretraining BF became zero after training.) This CS/BF ratio was computed for every tuning function.

Data were not included in the ratio computation (i.e., further analyzed) in two situations. First, data were rejected if the CS were the same frequency as the BF. While the CS frequency was selected to be different from the BF, this decision was based on the vast majority of BFs across tone intensity. But sometimes the BF was not identical at all intensities, and it happened to be the same as the CS frequency in 8 of 112 pretraining tuning functions. Second, data were rejected if there was no response to the CS frequency both before and after training. In these cases (12 of 461 pre- and posttraining tuning functions), the CS frequency was considered to be outside the receptive field of the recording site in question.

Statistical analyses for both cardiac and LFP data were performed using nonparametric tests because the data were not normally distributed. Wilcoxon tests were used for within-group paired comparisons, the Mann–Whitney test was used for between-group comparisons, and the Page Trend Test for Ordered Alternatives was used to evaluate consolidation (Siegel & Castellan, 1988). The latter, as a one-tailed test, was appropriate because neural consolidation in this study was predicted to be increasing strength of effect over time.

Histology

After completion of the protocol, subjects were euthanized with an overdose of sodium pentobarbital. The brains were perfused with saline and formalin and removed for examination. Frozen sections (40 μm) were obtained and stained with cresyl violet. Electrode tracks could be detected in many cases, and the depths were consistent with recording sites in layer IV or below. However, the inversion of the LFP with depth was used as the defining criterion of recording below layer III (Borbély, 1970).

RESULTS

Behavioral Conditioning

Presentation of the CS alone at the beginning of the protocol produced the typical cardiac deceleration (bradycardia) “orienting” response, which became smaller on subsequent habituation trials. The conditioning group reversed this habituation trend and developed conditioned bradycardia during subsequent CS–US pairing. By contrast, the sensitization control group did not develop conditioned bradycardia but rather exhibited continual lessening of CS-evoked bradycardia during training, approaching no response. Data from both groups are presented in Fig. 1, in which 0% change is the mean level of CS-evoked bradycardia during the preceding tone-alone (habituation) trials. Note that mean responses during the first five trial blocks of training were not different for the conditioning and sensitization groups and that their negative values near zero change indicate a small decrease of CS-evoked bradycardia from the mean bradycardia during the preceding tone-alone trials. This is consistent with continued habituation of tone-evoked bradycardia during the first block of training.

However, beginning with the second block of trials, CS-evoked bradycardia increased for the conditioning group but decreased (continued to habituate) for the sensitization group. These opposite trends continued to develop throughout the balance of the 30 trials of training. Across the six blocks of training, the conditioning group showed a trend of increasing bradycardia (Page Trend Test, $Z_L = 3.69$, $p < .001$), while the sensitization control group showed a trend of decreasing bradycardia (Page Trend Test, $Z_L = 2.91$, $p < .001$). By the sixth block of training, the conditioning group exhibited CS-evoked bradycardia that was 65% greater than the mean of the preceding tone-alone trials. By contrast, the sensitization control group exhibited a 75% loss of CS-evoked bradycardia

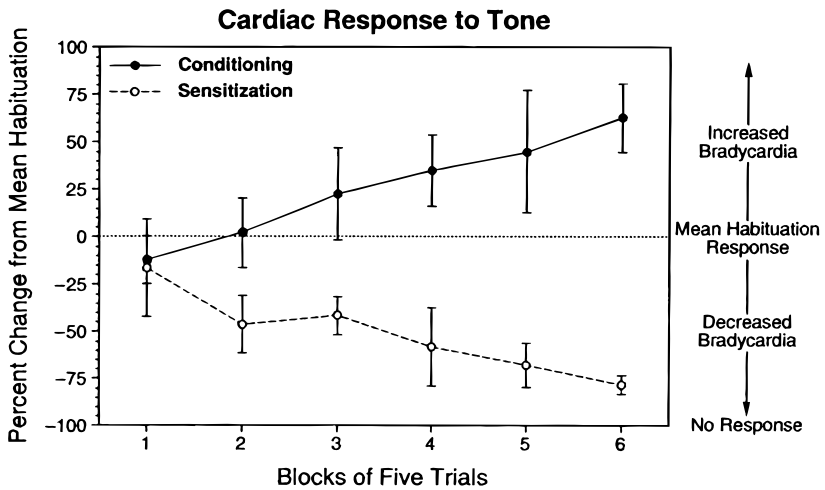


FIG. 1. Mean (\pm SE) cardiac deceleration (bradycardia) for the conditioning and sensitization control groups during 30 training trials, relative to mean bradycardia responses during preceding habituation trials (not shown). Zero on the ordinate indicates no difference from the mean of habituation. Positive values indicate increased bradycardia, and negative values indicate decreased bradycardia, compared to the mean of habituation. Note that the conditioning group exhibits growth of conditioned bradycardia, while the sensitization group approaches the level of no response. Groups differed significantly during blocks 4 to 6.

from the mean of the tone-alone trials. The differences between the two groups were statistically significant for blocks starting with the fourth block (Mann–Whitney tests, all p 's < .05). The findings indicate that the CS-evoked bradycardia in the conditioning group constituted an associative cardiac conditioned response, whereas the loss of bradycardia in the sensitization group constituted continued habituation of the cardiac “orienting” response (reviewed in Sokolov, 1963).

Plasticity of Frequency Tuning

An averaged tone-evoked response recorded from an awake guinea pig is shown in Fig. 2A. The negative wave (N1) exhibits systematic frequency tuning (Galván et al., 2001). Figure 2B presents examples of average LFPs across a wide range of frequencies (0.97–30.00 kHz) and intensities (–20 to 80 dB). In this case, the characteristic frequency (threshold frequency) is 7.78 kHz, and the threshold is 0 dB. Note that the bandwidth of response decreases systematically as stimulus intensity decreases. Figure 2C provides the tuning curve for the N1 component at 50 dB for the data in Fig. 2B. The frequency specificity, low threshold, and narrowing of bandwidth are the same characteristics previously reported for single-unit discharges (reviewed in Aitkin, 1990).

During the pretraining period, there were no significant differences between the conditioning and sensitization control groups in absolute LFP amplitude (tuned N1 component, pooled across intensity) (CS frequency, Mann–Whitney, $z = 0.52$, $p > .05$; BF, $z = 0.13$, $p > .05$). Thereafter, amplitudes changed over days. In the conditioning group, the CS values increased from pretraining to day 10, from $161.5 (\pm 18.9 \text{ SE})$ to $207.0 (\pm 20.8) \mu\text{V}$, (Wilcoxon, $z = 2.25$, $p < .05$), while responses to the BF decreased (301.0 ± 28.8 vs $253.9 \pm 22.2 \mu\text{V}$, $z = 2.91$, $p < .01$). The sensitization controls exhibited no significant changes to the CS frequency, but responses to the BF decreased from pretraining ($244.8 \pm 22.3 \mu\text{V}$) to day 7 ($196.8 \pm 24.6 \mu\text{V}$) ($z = 3.28$, $p < .001$).

The opposite changes in absolute amplitude for the conditioning group indicate a differential associative effect for the CS frequency versus the BF. However, as indicated above, absolute amplitudes change over days in the absence of any training (Galván et al., 2001). The confounding effects of time per se with training effects compromises the interpretation of findings based solely on absolute amplitudes. Therefore, statistical analyses of training effects were limited to measures of relative change of response.

Associativity. Inspection of tuning curves before conditioning and at the 1 h postconditioning retention period showed that frequency tuning had changed. The most characteristic change was an increase in response to the frequency that had been used as the CS, relative to changes at other frequencies in the tuning function. In many cases, responses to other frequencies, usually including the preconditioning BF, decreased.

Figure 3 presents examples from 3 subjects illustrating a variety of effects, all of which exhibited differential effects on the CS frequency and the preconditioning BF. In the first example (Fig. 3A, left), responses to the CS frequency increased, while responses to the BF were unchanged. The difference curve (Fig. 3A, right) shows that the CS frequency exhibited the maximal increase in the tuning function, while the response to the BF did not change and responses to several other frequencies decreased. Figure 3B shows a case in which responses to the CS frequency increased, while responses to most other frequencies, including the preconditioning BF, decreased. A third example of associativity at the 1-h

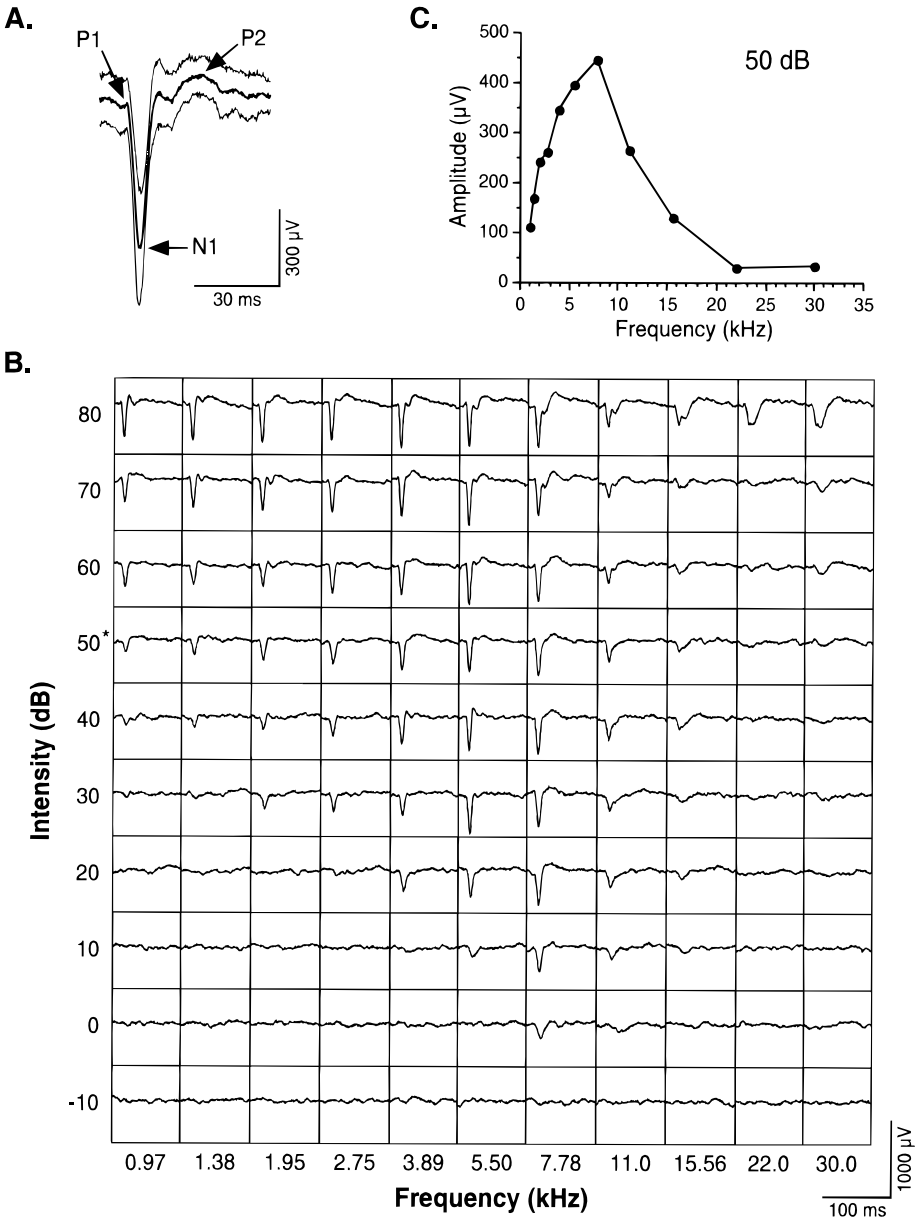


FIG. 2. Example of tuned LFPs. (A) An average potential, indicating the N1 component that is tuned to frequency, the small inconsistent P1, and the longer latency P2 component that is not tuned. (B) Average potentials across frequency and intensity. Note the decreasing bandwidth as stimulus level is reduced from 80 dB. The threshold was 0 dB, and the characteristic frequency at this level was 7.78 kHz. (C) A tuning function for the responses shown in panel B at 50 dB.

retention period is presented in Fig. 3C. The pretraining tuning curve was double-peaked, with BFs at 5.6 and 22.0 kHz (Fig. 3C, left). The CS was chosen to be a frequency between the two peaks, 11.0 kHz. Both pretraining BFs were decreased 1 h after training. In this case, the increase at the CS frequency was not the largest increase; rather, the CS and adjacent frequencies “filled in” the tuning curve between peaks (Fig. 3C, right).

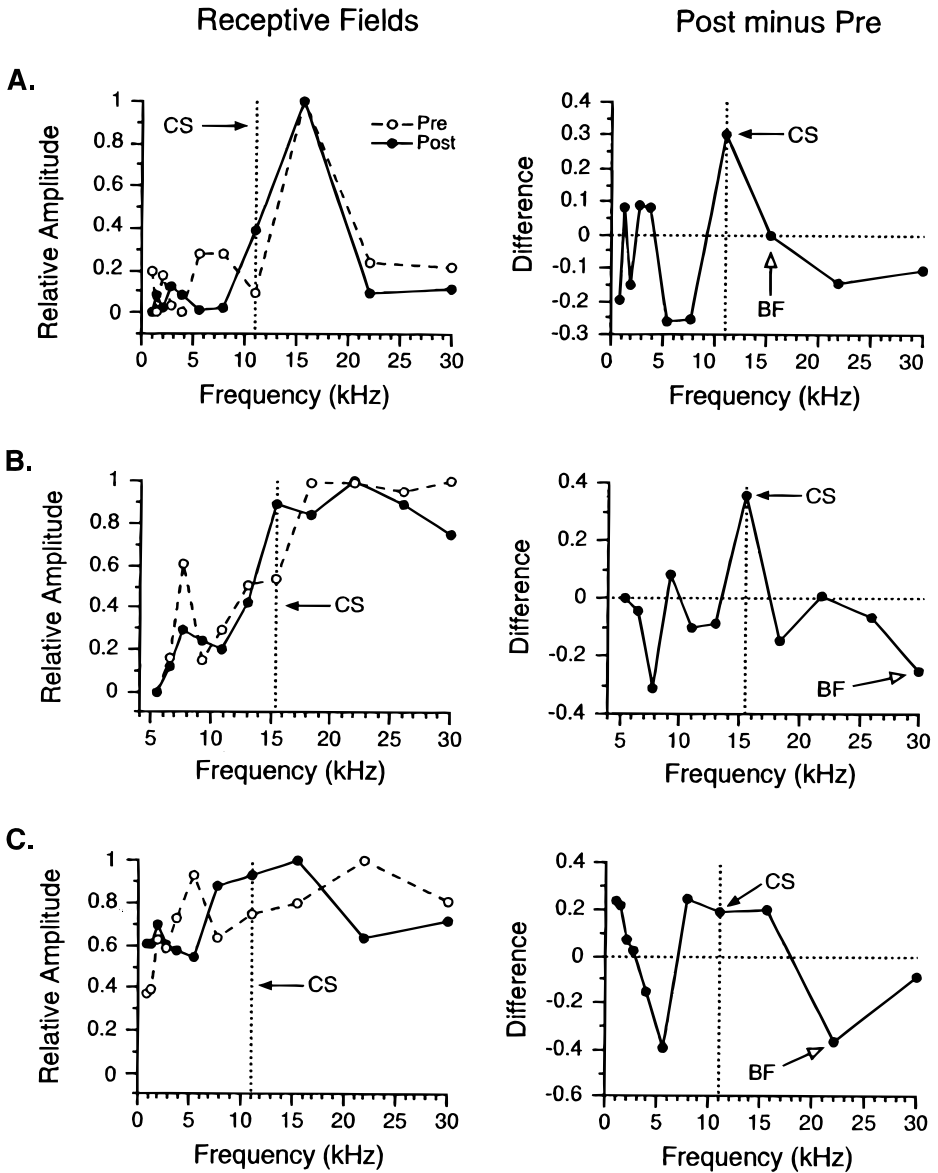


FIG. 3. Three examples of tuning curves from different probes before and 1 h after conditioning (left column) and their difference curves (postconditioning minus preconditioning tuning curve) (right column). (A) The CS was 11.0 kHz. Response to the CS frequency increased 1 h after training, and this was the greatest increase in the tuning difference function (postconditioning minus preconditioning). The magnitude of response to the CS frequency versus the BF was 0.167 at preconditioning and 0.564 at 1 h. Data were obtained at 10 dB. (B) The CS frequency was 15.56 kHz. Response to the CS frequency increased 1 h after training, and this was the largest increase; responses to the BF and several other frequencies decreased. The pretraining CS/BF ratio was 0.695, which increased to 1.086 1 h after training. Data were obtained at 30 dB. (C) An example of tuning functions at 80 dB, showing characteristic broader tuning (see also Fig. 2). The preconditioning tuning curve was double-peaked. The CS frequency of 11.0 kHz was selected to be between the peaks. The responses to the CS and adjacent frequencies increased, while responses to the BF and some other frequencies decreased, 1 h after training. The CS/BF ratio increased from 0.856 to 1.191. Arrows in tuning difference functions indicate CS and BF in this figure as well as in Figs. 5, 6, 9, and 10.

Analysis of group data focused on changes in the magnitude of response to the frequency of the CS versus the magnitude of responses to the preconditioning BF. The criterion for an associative effect was a significant increase in CS/BF ratios at the first (1 h) retention period. The mean CS/BF ratios pooled across intensity are shown for the conditioning group, in the period preceding and 1 h following training, in Fig. 4A. The ratio for the 1-h retention period was significantly larger (Wilcoxon, $z = 2.19$, $p < .05$).

The CS/BF ratios for the sensitization control group are presented in Fig. 4B. In contrast to the conditioning group, the control group did not develop a significant increase from the pretraining period (Wilcoxon, $p > .05$). This lack of effect was not due to putative pretraining differences between groups because there were none. The mean pretraining CS/BF ratios were not significantly different (Mann–Whitney, $p > .05$). The range of pretraining BFs did not differ (Mann–Whitney, $p > .05$). Neither did the octave frequency distance between the CS frequency and the BF (Mann–Whitney, $p > .05$). Thus, increased relative response to the CS frequency can be attributed to associative processes.

Long-term retention. Long-term retention was assessed by comparing CS/BF ratios before training to those obtained 10 days after training. Tuning plasticity was retained for the 10-day period of this study. Figure 5A illustrates additional data for the recording presented in Fig. 3A, which showed associative effects at 1 h. Figure 5A presents tuning functions for 1 and 10 days. This figure reveals that the CS-specific effects present at 1 h were maintained at the 1- and 10-day retention periods; the differences in relative CS amplitude were ~ 0.50 and ~ 0.55 at 1 and 10 days, respectively. In this case, there was no change in response at the BF. The example in Fig. 5B shows that associative effects can become stronger by the post-10-day retention period. Thus, in the difference curve for day 1 (Fig. 5B, right), the response to the CS frequency showed a difference in relative amplitude of ~ 0.25 as part of a somewhat broad increase, while responses to the BF were unchanged. By contrast, 10 days after training, the CS increase was ~ 0.45 , the difference curve had sharpened, and responses to the preconditioning BF had decreased.

The mean CS/BF ratio, pooled across intensity, for the conditioning group at 10 days was 0.858, which was significantly greater than the pretraining ratio of 0.623 (Wilcoxon, $z = 4.62$, $p < .0001$). The longest retention test for the sensitization control group was 7 days. This group exhibited no difference between the pretraining and 7-day retention period (Wilcoxon, $z = 0.30$, $p > .05$), as might have been expected from its lack of effect

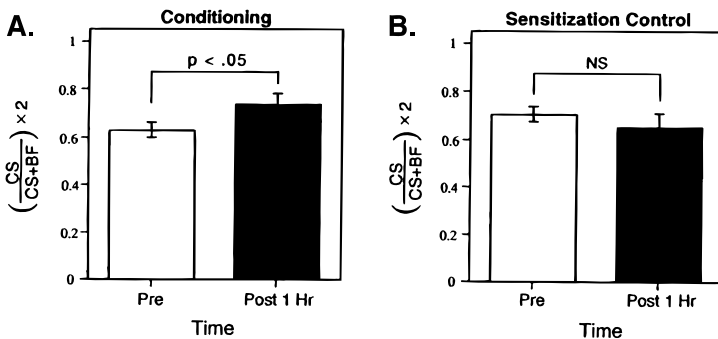


FIG. 4. Group mean CS/BF ratios for test of associativity. (A) In the conditioning group, 1 h after training, the CS/BF ratio was significantly greater than the pretraining ratio. (B) In the sensitization control group, 1 h after training, there was no significant change in the CS/BF ratio.

in the analysis of associativity. For comparison purposes, the conditioning group showed a significant increase in CS/BF ratio from pretraining at 7 days (Wilcoxon, $z = 5.38$, $p < .0001$).

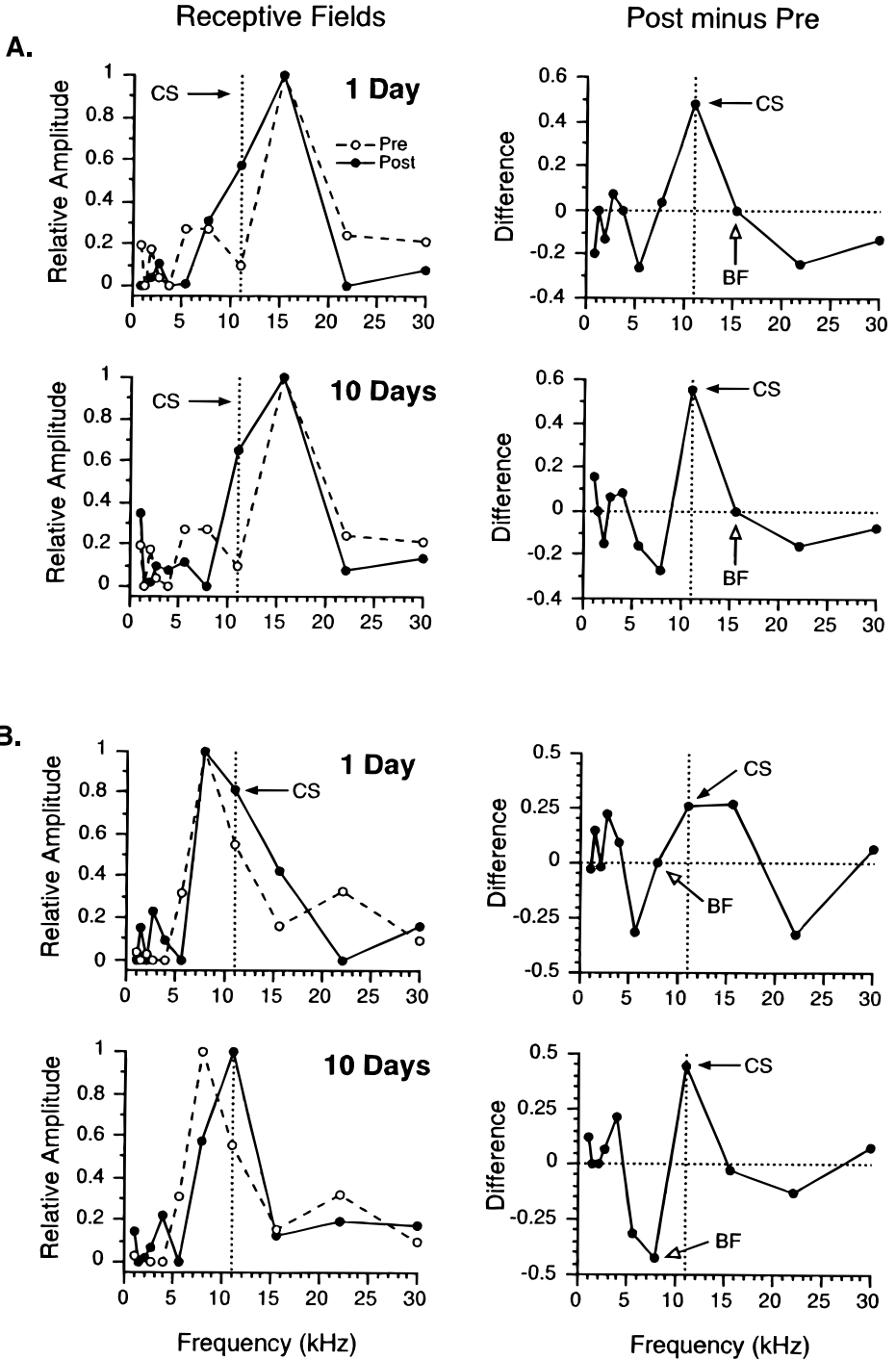
Subjects were trained with a CS intensity of 70 dB, and previous analyses focused on data pooled across intensity. This raises the question of whether the effects of conditioning were confined to the range of intensities around the training intensity or developed across stimulus levels. Figure 6 presents an example of tuning plasticity across intensity for the 10-day retention period. At each intensity, responses to the frequency of the CS became larger relative to most other frequencies. The difference functions show that the CS frequency exhibited either the maximum increase in response (30, 40, or 80 dB) or nearly the maximum increase in response (20, 50, or 70 dB). Responses to the preconditioning BF were decreased at every intensity.

Analysis of group CS/BF ratios at each intensity (10–80 dB) pooled across postconditioning periods revealed larger ratios compared to the preconditioning period for most intensities. The differences attained statistical significance for 10, 30, 40, 60, 70, and 80 dB (Wilcoxon, p 's $< .05$ to $.0001$) (Fig. 7). The largest increase was at 10 dB, the group threshold, at which responses to the CS frequency actually became greater than responses to the preconditioning BF.

Consolidation. Consolidation was operationally defined as a significant increasing trend in CS/BF ratios from 1 h to 10 days postconditioning. An indication of consolidation is present in the example previously shown in Fig. 5B, for which CS/BF ratios increased from 1 to 10 days. Mean conditioning group CS/BF ratios are shown in Fig. 8A. Note that all ratios, from 1 h to 10 days, were greater than the ratio for the preconditioning period (dashed line). These increases are statistically significant for each of the postconditioning retention tests (Wilcoxon: 1 h, $z = 2.19$, $p < .05$; 1 day, $z = 2.87$, $p < .01$; 3 days, $z = 3.91$, $p < .0001$; 7 days, $z = 5.38$, $p < .0001$; 10 days, $z = 4.62$, $p < .0001$). Of greatest importance regarding consolidation, there was an increasing trend over days. This trend was statistically significant (Page Trend Test, $Z_L = 1.93$, $p < .05$).

Inspection of individual temporal functions revealed that some recording sites did not exhibit an increasing trend over days. Three recording sites showed an immediate increase at 1 h that was maintained without further growth over 10 days. We refer to this pattern as “immediate asymptote” (Fig. 8A1). The retention ratios across these recordings were significantly greater than their pretraining ratios (Wilcoxon, p 's $< .05$ to $.001$), but their retention periods did not differ from each other (paired tests of all retention period combinations, Wilcoxon, all p 's $> .05$). An example from the immediate asymptote category is shown in Fig. 9. The increase in CS/BF ratio from 0.619 at pretraining to

FIG. 5. Long-term retention of tuning curve plasticity for two cases. Data illustrated are for pretraining and 1- and 10-day retention periods. (A) The CS was 11.0 kHz. Responses to the CS had increased, while responses to other frequencies were essentially unchanged or decreased, 1 day after conditioning. This same pattern was maintained at 10 days. The CS/BF ratios increased from 0.167 (pretraining) to 0.730 (1 day) and 0.786 (10 days). (These data were from the same probe and stimulus intensity [10 dB] as the data shown in Fig. 3A.) (B) The CS was 11.0 kHz; data were obtained at 10 dB. This case showed increased effects from day 1 to day 10. The pretraining ratio was 0.711, posttraining 1 day was 0.897, and posttraining 10 days was 1.270. The frequency of the CS became the new BF at 10 days because responses to the CS frequency increased while responses to the BF decreased greatly, at 10 days.



1.006 at 1 h later (Fig. 9A) was generally maintained without growth to 10 days (Figs. 9B–E). The magnitude of CS/BF increase was smaller at 7 days, indicating that long-term retention need not be maintained at a constant level across 10 days. Responses to the preconditioning BF decreased at all retention periods.

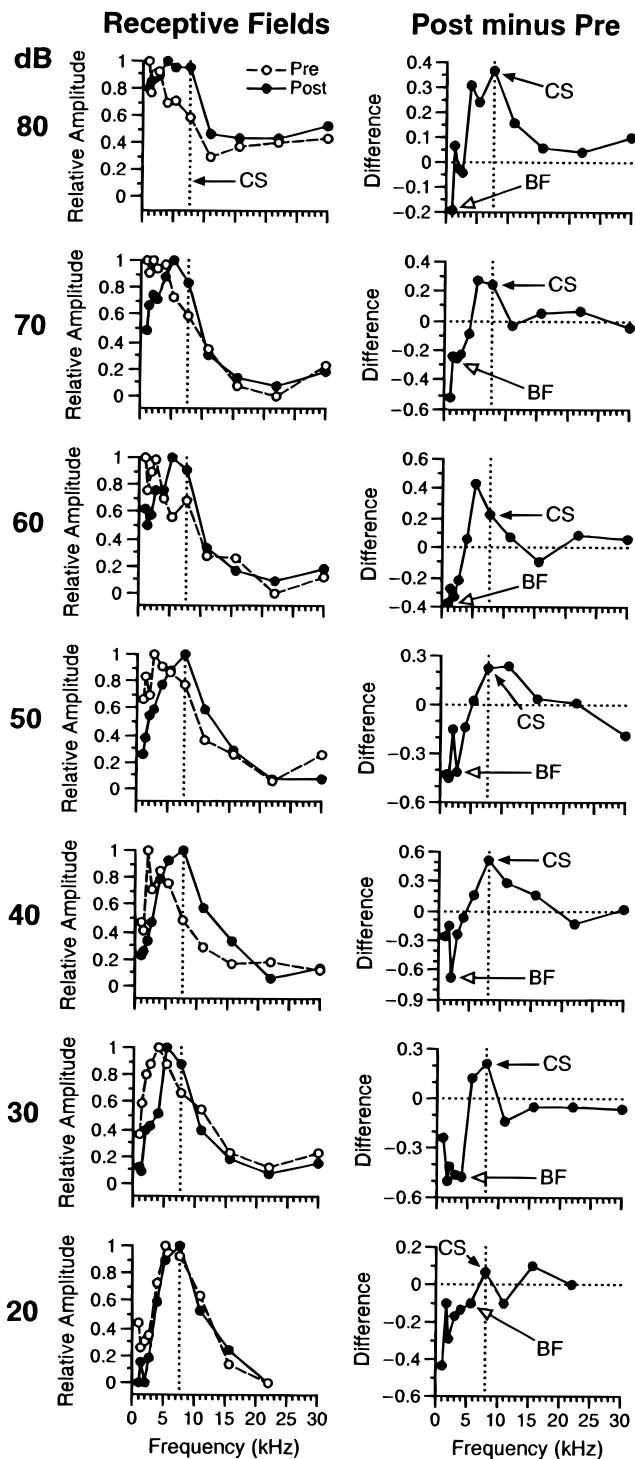
The actual consolidation pattern was more common, obtained at six recording sites (Fig. 8A2). This function exhibited a significant trend (Page Trend Test, $Z_L = 2.26$, $p > .01$). Consolidation was characterized by increased CS/BF ratios over the first 3 days of retention and maintenance of the 3-day level 7 and 10 days after conditioning. The CS/BF ratios were significantly larger than the pretraining value at the 1-, 3-, 7-, and 10-day retention periods (Wilcoxon, p 's $< .05$ to $.0001$). Note that there was no significant difference at the 1-h period. Thus, consolidation may begin within 1 h but not attain statistical levels of growth until 1 day after training.

An example of the consolidation pattern is shown in Fig. 10. There were increased responses to many frequencies 1 h after conditioning. Increased responses became more specific to the frequency of the CS at the 1-day retention period, at which time this frequency exhibited the maximum increased response (Fig. 10B). Maximal increased responses at the CS frequency also occurred at 3, 7, and 10 days (Figs. 10C–E). Decreases in response to the preconditioning BF were evident in all postconditioning periods, and the CS frequency became the new BF on day 10 (Fig. 10E).

Additional analyses of the immediate asymptote and consolidation groups revealed two pretraining differences that might help to account for their distinctive posttraining patterns. Figures 8A1 and 8A2 suggest that the pretraining CS/BF ratio was higher for the immediate asymptote group. This difference was statistically significant (0.752 vs 0.560, respectively, Mann–Whitney, $z = 2.27$, $p < .05$). This difference may arise from a differential distance between the CS frequency and the pretraining BF. In an inverted “U” tuning function, CS frequencies that happened to be closer to the BF are more likely to have a greater magnitude of response (i.e., approach the magnitude of response to the BF) than are frequencies at a greater distance (e.g., Figs. 2C, 3A, 5, and 6). Indeed, the octave distance between the CS frequency and the pretraining BF was also significantly smaller for the immediate asymptote group than for the consolidation group (0.684 vs 1.236 octaves, respectively, Mann–Whitney, $z = 3.33$, $p < .001$). Two subjects yielded data from recording sites that were in both subgroups (immediate asymptote and consolidation). Thus, different temporal dynamics can develop after training within different parts of the auditory cortex within an animal.

The sensitization control group (Fig. 8B) exhibited no significant increasing trend over days (Page Trend Test, $p > .05$). The small increase from 1 h to 3 days was not significant either (Wilcoxon, $p > .05$).

FIG. 6. Examples of tuning and tuning difference functions across stimulus intensity. Data are for 10 days postconditioning. The CS frequency was 7.78 kHz. After training, responses to all of the preconditioning BFs were reduced, whereas responses to the CS frequency were increased. At each stimulus intensity, tuning shifted toward (30, 60, 70, or 80 dB) or to (20, 40, or 50 dB) the frequency of the CS, and tuning difference functions generally showed increased responses to the frequency of the CS that were either maximal (30, 40, or 80 dB) or near maximal (20, 50, or 70 dB). CS/BF ratios: 80 dB (pre = 0.738, post = 1.085); 70 dB (pre = 0.740, post = 1.265); 60 dB (pre = 0.810, post = 1.184); 50 dB (pre = 0.872, post = 1.262); 40 dB (pre = 0.655, post = 1.500); 30 dB (pre = 0.800, post = 1.254); 20 dB (pre = 0.965, post = 1.054).



DISCUSSION

Overview of Findings

The current results show that LFP tuning plasticity develops, is retained for 10 days (the longest period tested), and (most important) exhibits long-term consolidation. Although subjects were trained with a CS level of 70 dB, tuning plasticity developed across stimulus intensities (10–80 dB), indicating that its expression generalizes beyond the specific stimulus level used during learning. Local field potential tuning plasticity is not limited to a certain frequency range because the pretraining BFs of the nine probes in the conditioning group ranged from 1.50 to 25.70 kHz. The absolute amplitude of responses to the CS frequency increased, while responses to the pretraining BF decreased in the conditioning group. However, as noted above, findings based on changes in absolute amplitude should be interpreted with caution because amplitude may change spontaneously over days (Galván et al., 2001). The opposite signs of change for the CS frequency and the BF do suggest that changes in absolute amplitude reflect associative processes to some extent rather than purely random factors. Consistent with this possibility, LFP amplitude changes show the same directionality as do changes of unit discharge rates in studies of receptive field plasticity (Bakin, South, & Weinberger, 1996; Bakin & Weinberger, 1990; Edeline, Pham, & Weinberger, 1993; Edeline & Weinberger, 1993; Weinberger et al., 1993).

While the conditioning group exhibited consolidation (i.e., temporally graded increase in the CS/BF ratio), further analysis showed that six of nine recording sites yielded a significant gradient of increasing CS/BF ratio over days and three sites did not. The latter attained asymptote at 1 h and retained this increase for 10 days. Analysis of pretraining factors showed that the immediate asymptote group happened to have CS frequencies that had significantly smaller octave distances to the pretraining BF and, as might be expected because of such proximity, also had a significantly greater CS/BF ratio than the consolidation subgroup. In short, the immediate asymptote recordings had relatively stronger responses than did those that required 3 days to reach asymptote. These findings suggest that the rate of development of neural plasticity (immediate vs time dependent) depends on the initial relative strength of response to the CS frequency. Stronger responses are augmented quickly, probably attaining the maximum change possible, whereas weaker responses, which may increase over time at a given rate, require more days to achieve their maxima. It is possible that the immediate asymptote sites are also subject to a continual influence to increase their strength but that the current means of detecting such a process is limited by a ceiling effect. Perhaps other cellular or molecular methods could reveal such dynamics.

The fact that two patterns of posttraining tuning plasticity emerged is of both practical and theoretical importance. From a practical standpoint, the wide range of CS–BF distances employed (see Methods) enabled the detection of both immediate asymptote and gradual consolidation. If all CS–BF distances had been relatively small (~ 0.5 octaves), then the gradual consolidation pattern probably would not have been observed and vice versa. From a theoretical point of view, memory consolidation has been assumed to represent the strengthening of an initially fragile unitary memory over time. However, the immediate asymptote pattern suggests that information storage can consist of two entities: an immediate strong and lasting memory and a gradually strengthened memory based on the “recruitment” of neurons with weaker initial responses to the CS frequency. But the immediate

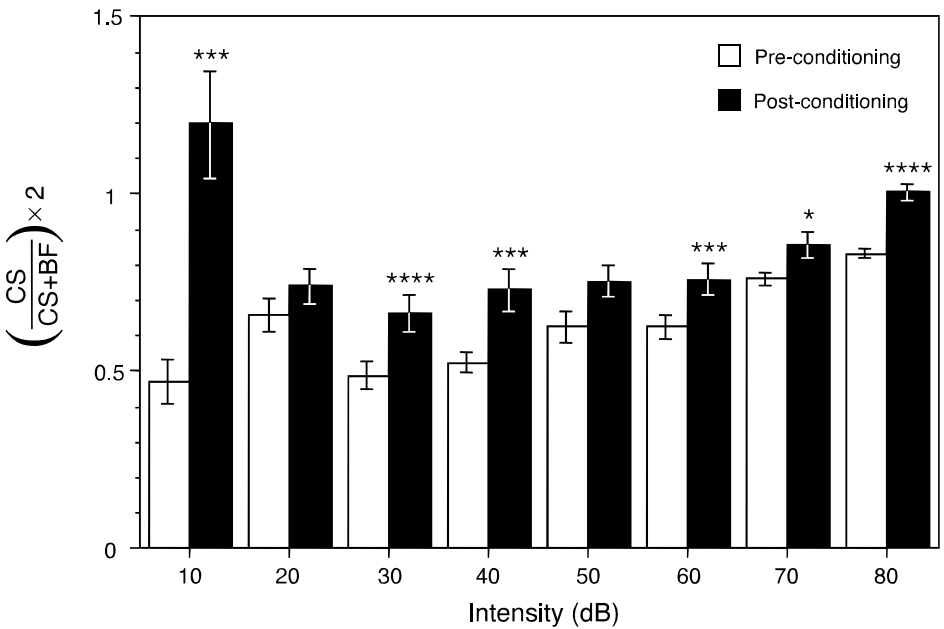


FIG. 7. Conditioning group mean CS/BF ratios at each stimulus intensity for the preconditioning versus postconditioning periods, pooled across retention periods. The postconditioning ratios were significantly greater than the preconditioning ratios at six of eight stimulus levels. * $p < .05$; *** $p < .001$; **** $p < .0001$. Note that 10 dB exhibited the greatest increase; its postconditioning ratio increased from 0.47 to 1.20. Ratios greater than 1.00 indicate that responses to the CS became larger than responses to the BF at 10 dB.

strong memory is comprised of a relatively small number of cells (i.e., those whose initial responses are stronger because they are tuned near the pretraining BF). Thus, the strong immediate memory trace might be insufficient to produce immediately strong memory at the behavioral level. It would be interesting to determine whether the immediate asymptotic RF plasticity is necessary for the gradual consolidation of other neurons by selectively inactivating cells whose tuning is near that of the pretraining BF.

Local field potential tuning plasticity is associative because it developed in the conditioning group but not in the sensitization control group. The conditioning group also developed cardiac conditioned responses in contrast to the sensitization control group, which exhibited continued habituation of response to tone. The related behavioral and LFP findings in the conditioning group do not imply a causal relationship in either direction. This is a separate issue not addressed by this study. We have hypothesized that the storage of learned information in the auditory cortex (as well as in other cortical regions) subserves a wide variety of cognitive processes and adaptive behaviors rather than being tied to a single behavioral sign of association, such as a particular conditioned response (Lennartz & Weinberger, 1992a; Weinberger, 1995). Thus, the analysis of cardiac conditioned responses is intended to show only that LFP plasticity develops when a tone acquires behavioral importance to animals, as in the conditioned group, but not when it does not, as in the sensitization group.

Despite strong evidence that tuning plasticity is associative, two nonassociative explanations have been offered: spontaneous drift of tuning and increased arousal. Previously, both have been ruled out for unit discharge RF plasticity (reviewed in Weinberger, 1998).

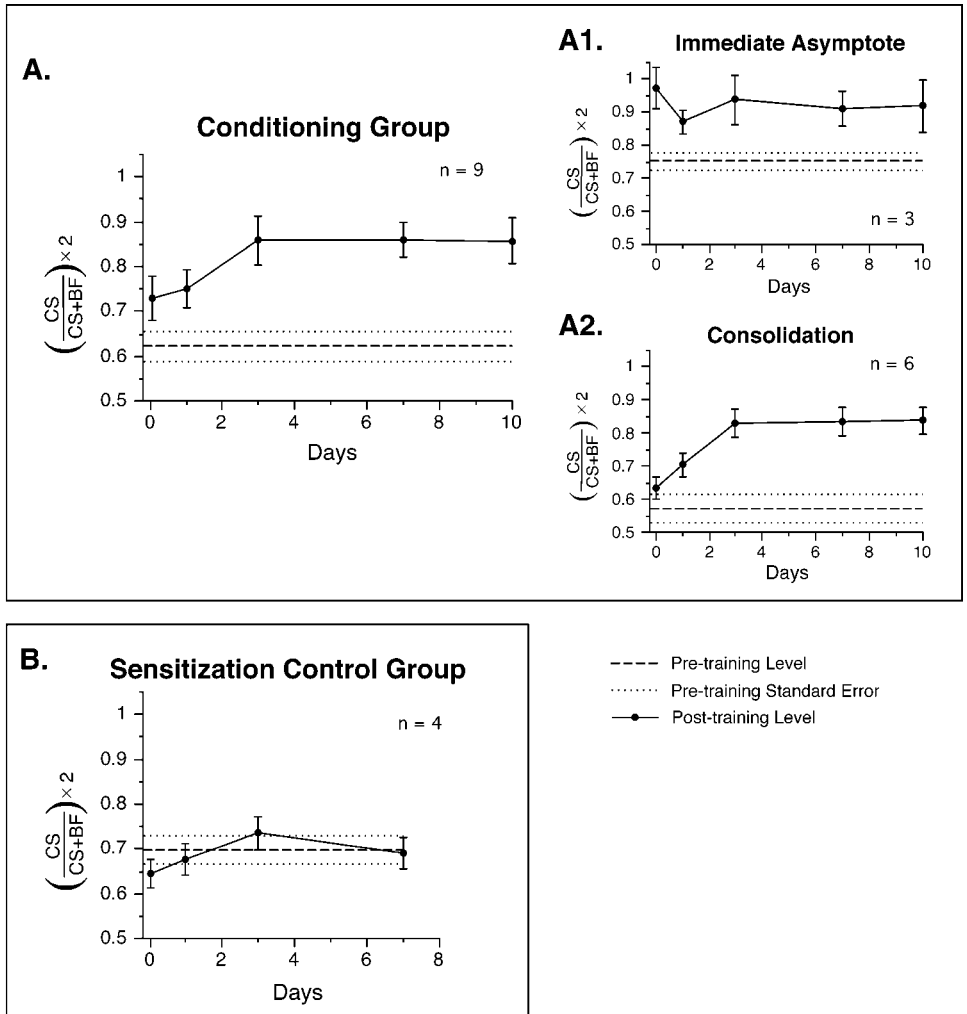
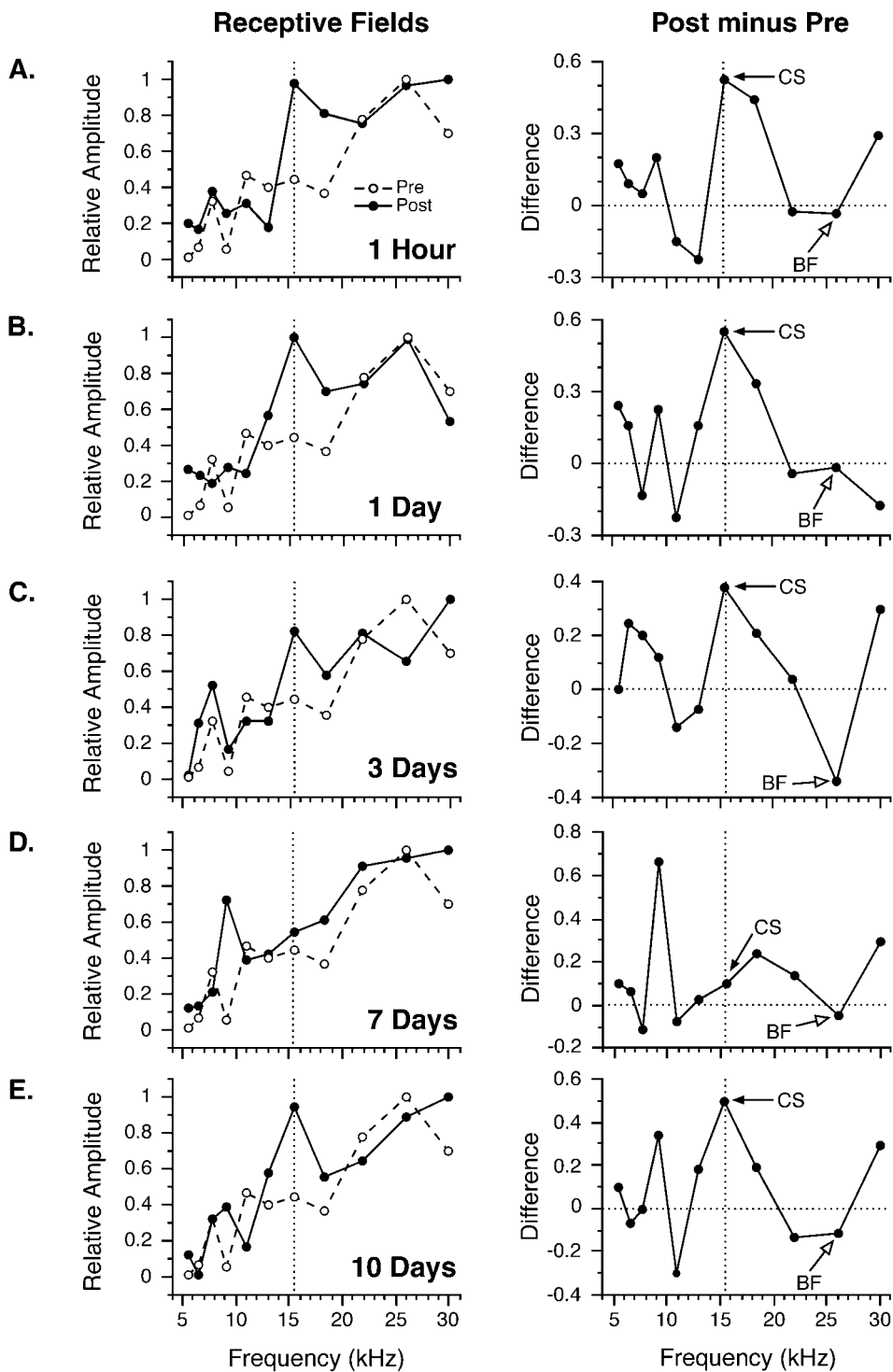


FIG. 8. Group mean (\pm SE) CS/BF ratios for each recording period, pooled across intensity levels. The pretraining CS/BF ratio is indicated by the dashed line; dotted lines denote ± 1 SE. (A) Conditioning group data. The retention period ratios were each significantly greater than the preconditioning ratio, and the trend from posttraining 1 h to posttraining 10 days was also significant. Asymptote was attained at 3 days. (A1) Mean data for a subset of the probes ($n = 3$) that displayed an “immediate asymptote” pattern. Ratios increased 1 h after training and were maintained above pretraining levels for 10 days, with no significant trend over time. CS/BF ratios for 1 h to 10 days were not significantly different from each other. (A2) Mean data for the majority of probes ($n = 6$) that demonstrated a significant trend of increasing CS/BF ratios across days. The asymptote of this consolidation function was attained at 3 days. CS/BF ratios from 3 to 10 days were not significantly different from each other. (B) Group data for sensitization controls. No posttraining periods were significantly greater than the pretraining ratio, and there was no significant trend.

FIG. 9. An example of a recording exhibiting the “immediate asymptote” pattern. The CS frequency was 15.56 kHz. (A) Responses to the CS frequency increased 1 h after training. (B–E) The enhanced response was maintained at 1, 3, 7, and 10 days after training. However, the CS/BF ratio was lower at 7 days, showing that retention was not necessarily at a constant level on all days. The preconditioning CS/BF ratio was 0.619, posttraining 1 h was 1.006, posttraining 1 day was 1.005, posttraining 3 days was 1.114, posttraining 7 days was 0.732, and posttraining 10 days was 1.270. Data were obtained at 70 dB.



Nonetheless, Kisley and Gerstein (1999) suggested that spontaneous changes of tuning could be mistaken for long-term tuning plasticity (but see Kisley & Gerstein, 2001). However, such putative tuning drift should be the same for conditioning and sensitization control groups, and tuning plasticity developed only in the former. Moreover, LFP tuning does not spontaneously drift over days and weeks (Galván et al., 2001).

Putative increased arousal has been suggested for increased LFP responses to the CS during training trials (Molnár, Karmos, Csépe, & Winkler, 1988). While training trials may involve a performance confound of increased arousal, determination of tuning before and after training apparently does not have this problem. In the current experiment, subjects were trained in one room while tuning functions were obtained in a different room. Acoustic context also differed. During training trials, the CS tone was 6 s at 70 dB and had a mean intertrial interval of 90 s. During tuning determination, the frequency of the CS (and all other tones) was 0.1 s in duration, embedded in a sequence with many other tones and intensities. In a previous study using a highly similar design, subjects produced conditioned responses only during training trials, never to the CS or any other frequency during tuning determination (Diamond & Weinberger, 1989). Thus, subjects do not regard a brief tone that is presented in a rapid tonal sequence in another room to be the CS that predicts shock during training trials. Also, RF plasticity induced in the waking state is expressed during tuning determination with subjects under deep general anesthesia in which arousal is absent (Lennartz & Weinberger, 1992b; Weinberger et al., 1993). Current findings also are inconsistent with an arousal confound for LFPs. The latency of the N1 component of the LFP is 15 to 20 ms, a latency that precedes any known behavioral or neural sign of increased arousal, whose latency is ~ 100 ms or more (e.g., Weinberger & Lindsley, 1964). Also, an arousal explanation would predict a decreased effect due to habituation or extinction over days of repeated tones in the absence of pairing with shock. But tuning plasticity exhibited consolidation.

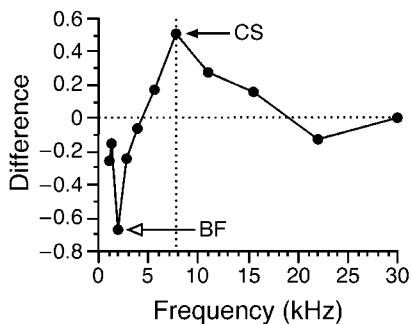
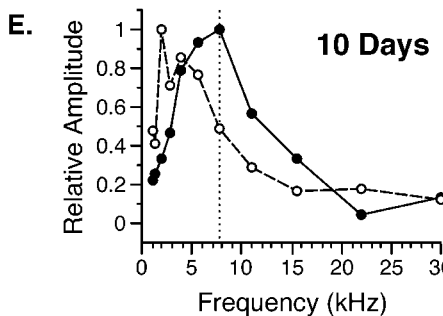
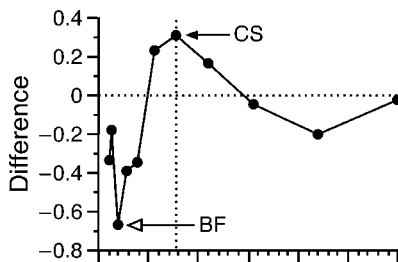
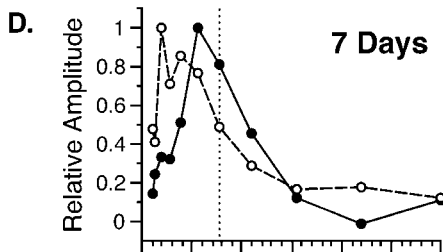
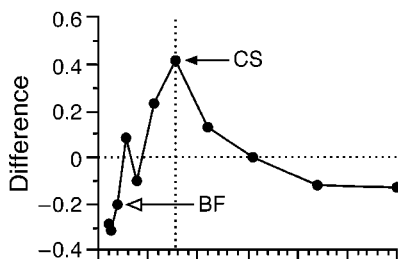
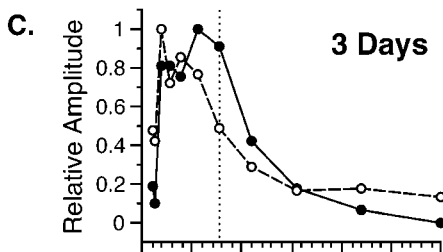
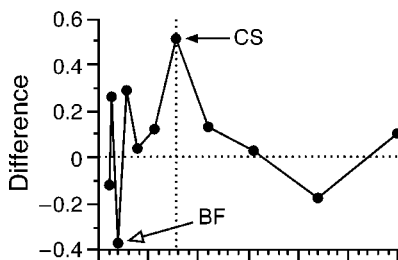
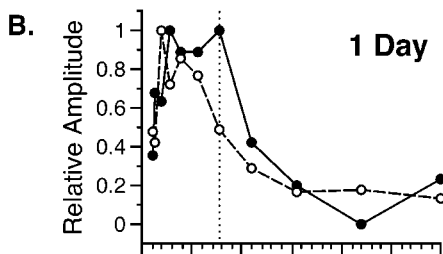
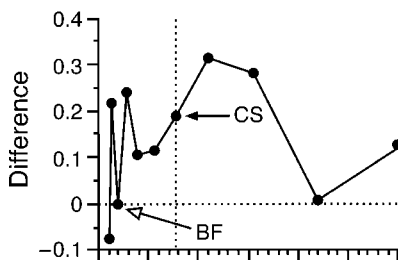
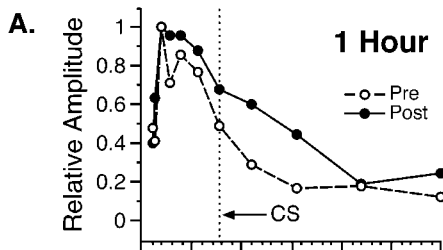
Relation to Previous Receptive Field Findings

This experiment appears to be the first on associative tuning plasticity of LFPs. Previously, RF plasticity has been studied in the auditory cortex for cellular discharges (reviewed in Weinberger, 1998). The current findings suggest that an increased representation for CS frequencies should be found in cortical maps, as reported for unit activity (Recanzone et al., 1993). This remains to be studied. Unit RF plasticity also exhibits short-term consolidation (i.e., increased strength of effect in the absence of further training) for tested periods of up to 1 h after training (Edeline et al., 1993; Edeline & Weinberger, 1993). There is also some evidence for long-term consolidation. Retention tests given for

FIG. 10. An example of a recording that showed the consolidation pattern (i.e., an increase in CS/BF ratio across days). The CS was 7.78 kHz. The preconditioning BF was 1.95 kHz. (A) At 1 h postconditioning, there was only a small increase in the CS/BF ratio, from 0.655 to 0.807. (B) At the 1-day retention period, the RF changes became more specific to the frequency of the CS, which exhibited the maximum increased response across the tuning curve, and the CS/BF ratio increased to 1.228. (C) At 3 days, in this case, the CS/BF ratio was smaller (1.064) than at 1 day, indicating that consolidation functions are not necessarily monotonic. (D) The CS/BF ratio increased to 1.414 at 7 days. (E) The CS/BF ratio reached its maximum level of 1.500 at the 10-day retention period. Stimulus intensity was 40 dB.

Receptive Fields

Post minus Pre



up to 8 weeks after a single training session have shown an increasing effect (Weinberger et al., 1993). However, these data were obtained from a diminishing population of recordings due to the loss of acceptable recordings over weeks. Those data might have reflected “survivor bias” (i.e., maintained recordings might have had larger effects than those that were lost). Thus, those findings cannot be interpreted unambiguously as having shown long-term consolidation.

Analysis of LFP consolidation indicated that the conditioning group exhibited significant plasticity starting 1 h after training but that a breakdown of this group into immediate asymptote versus consolidation subgroups revealed something else (Fig. 8). Although the consolidation subgroup showed an increase in CS/BF ratio as early as 1 h posttraining, this change did not attain statistical significance until 1 day after training. This finding contrasts with unit discharge studies that exhibited RF plasticity immediately after training (reviewed in Weinberger, 1995, 1998). However, the difference may be more apparent than real. None of the unit RF studies examined long-term consolidation, as was done here. If that had been accomplished and the data had been analyzed in a similar manner, then they might have revealed that the significant group effects immediately or soon after training also reflected subpopulations of recordings, one attaining asymptote immediately and the other over periods of days.

The extent to which LFP tuning plasticity develops in other conditioning situations, such as instrumental conditioning and discrimination training, and the rate of its development remain to be determined. Local field potential and cellular discharge tuning plasticity need not exhibit identical characteristics, particularly as they represent related but different underlying neuronal processes (see later section on “Possible Mechanisms of LFP Tuning Plasticity and Consolidation”). However, the current study does extend the study of tuning plasticity beyond that previously accomplished with recordings of cellular discharges. It was possible to obtain reliable LFP data over the long-term period of the study. In so doing, it was possible not only to determine that the plasticity was retained over 10 days but also that it grew in strength over a period of 3 days following the training session.

The long-term retention observed here contrasts with the shorter term retention of 3 to 5 days for learning-induced plasticity in the somatosensory cortex of the mouse, induced by pairing stimulation of a row of whiskers with a tail shock (Siucinska & Kossut, 1996). This transient effect can readily be understood as an instance of weak learning due to the use of an unusual training protocol in which the intertrial interval was actually shorter than the CS-US interval. Such a design would be expected to produce weaker effects (Mackintosh, 1974; Rescorla, 1988).

Neural Consolidation

Previous studies of neural consolidation have employed several protocols, none of which was identical to that used in the current case. In perhaps the only other neurophysiological study reporting consolidation, Freeman and Gabriel (1999) reported a shift in the loci of associative neural responses in rabbits trained to avoid foot shock. After a single session of training, subjects underwent a second training session either immediately or 48 h later. In the group receiving a second session immediately, CS-elicited cellular discharges increased in the antero-dorsal (AD) and medial dorsal (MD) thalamic nuclei and in the anterior cingulate cortex. However, in the second group, the passage of 48 h

between sessions produced increased responses in other structures, specifically in the antero-ventral (AV) nucleus and in the posterior cingulate cortex.

Studies of metabolic activity, using between-group designs, also have revealed shifts of locus over time. Sif et al. (1991) saw regional differences in [^{14}C]2-deoxyglucose (2-DG) labeling in mice sacrificed either 15 or 220 min after 2 days of training in an appetitive conditioning task. Subcortical (and, to a lesser extent, cortical) areas were activated soon after training, whereas approximately 3.5 h after training, cortical areas were more activated while subcortical areas tended to lose significant 2-DG labeling. Mice trained in an eight-arm radial maze also exhibited a shift of activation from subcortical to cortical areas 3 h posttraining (Bontempi et al., 1996). Across a longer time span, spatial training is accompanied by shifts of 2-DG uptake from the hippocampus to the cortex between 5 and 25 days after training (Bontempi et al., 1999). The involvement of different, and perhaps more, areas is compatible with increased functional strength of the neural substrates of memory.

Bertaina-Anglade, Tramu, and Destrade (2000) measured c-Fos expression in mice trained for 5 days on an operant appetitive task and sacrificed at different time points either after the 1st, 2nd, or 5th day of training. c-Fos expression increased at a 60-min time period. On day 1, the CA3, anterior cingulate, and occipital and parietal lobes demonstrated increased levels, whereas on day 2, hippocampal formation, subiculum, entorhinal cortex, and post cingulate cortex levels increased.

Shifts in locus of activation have also been reported in humans. Shadmehr and Holcomb (1997) used PET to study learning of a motor skill in which subjects had to move a handle to a target against a stable force and then during recall 5.5 h later. A comparison of immediate versus delayed recall revealed that the behavior was not significantly different between the two conditions, but activity had decreased in prefrontal cortex and concomitantly increased in parietal, premotor, and cerebellar cortex over that span of time.

Comparison of the current findings with those of these previous studies is not straightforward because of several differences in protocol. The current experiment used a single session of classical conditioning and a within-subjects design. Previous animal studies have employed multiple training sessions of instrumental learning, usually with a between-subjects design. Of these differences, the use of single versus multiple training sessions provides the greatest obstacle to integrating findings because it is difficult to separate consolidation effects from training effects (see also Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999). Nonetheless, there are points of similarity. Thus, while shifts of maximum metabolic activity have been observed from subcortical structures to the cortex, increased cortical activity, albeit of a lower level, has been observed minutes after training (e.g., Bontempi et al., 1996). The current experiment focused on a single locus, the ACx, so shifts of activity across days could well have occurred in other regions of the brain. Therefore, the current findings should be seen as complementary to previous reports of shifting loci during consolidation. In short, increasing strength of plasticity in a sensory processing system seems to be one facet of neural consolidation, while shifts in areas of involvement is another.

A particularly novel aspect of the current study concerns the specificity of effects. The current findings reveal that the neural consolidation is specific to the behaviorally significant stimulus, in this case the tonal frequency of the CS. Such specificity can be revealed best by the use of many stimuli along a sensory dimension (e.g., frequency) and the

determination of neural tuning before and at several intervals following training. In the current experiment, 0.5-octave frequency steps were used; hence, the frequency specificity of tuning plasticity could not be determined with greater precision. This half-octave level may be considered a provisional upper limit for the specificity of LFP tuning plasticity and its consolidation. Future studies of tuning should employ smaller frequency steps to determine the limits of consolidation specificity.

It is often incorrectly assumed that memory consolidation consists of a monotonic increasing function of memory strength. Under this assumption, some of the results reported here are problematic because they exhibit nonmonotonic features. For example, Fig. 10 shows a case in which, despite an overall growth of CS/BF ratios, there was a single time point where a “dip” in the consolidation function was observed. However, behavior is not necessarily characterized by monotonic increasing posttraining functions. A well-studied example is the “Kamin effect,” in which subjects exhibit a period of reduced performance preceding a renewed increase in learned behavior (Kamin, 1957). Such temporary impairments may reflect periods of incomplete overlap between successive phases of memory (e.g., short term, intermediate, long term). The nonmonotonic neural effects reported here have a precedent. Bontempi et al. (1996) reported immediate and 3-h increases in 2-DG uptake in many areas of the brain but found no significant effects 1 h after training. The complexities of neural substrates of memory consolidation are only beginning to be delineated, but it is unlikely that they will prove to consist only of monotonic functions (see also Mauelshagen, Parker, & Carew, 1996).

Possible Mechanisms of LFP Tuning Plasticity and Consolidation

Local field potentials in the cerebral cortex are thought to largely represent extracellularly recorded synchronous excitatory postsynaptic potentials (EPSPs) (Creutzfeldt et al., 1966; Mitzdorf, 1985), with little (if any) contribution from cellular spikes (Humphrey, 1968). Consistent with this interpretation, unit discharges in the auditory cortex tend to occur on the rising phase of LFPs (Konig, Pujol, & Marty, 1972; Wolpaw, 1979). Local field potentials and unit discharges, recorded simultaneously from the same microelectrode in the auditory cortex, can exhibit highly similar spectral and temporal properties. For example, their frequency tuning is highly similar, as is their ability to follow rates of click repetition (Eggermont, 1996; Eggermont & Smith, 1995). However, LFPs and unit discharges also can exhibit certain differences. For example, LFP thresholds are ~ 10 dB below unit thresholds; because of their similarity of tuning, threshold best frequencies of LFPs “predict” best frequencies of cellular discharges at their own higher stimulus thresholds (Galván, Chen, & Weinberger, 1997). These relationships are also consistent with the view that LFPs represent population EPSPs.

Threshold LFPs might provide information that is not accessible from stimulus-evoked unit discharges, which may be absent at very low stimulus levels. In this regard, LFP tuning plasticity was observed at a stimulus level of 10 dB (Fig. 7), the lowest level at which LFPs could be obtained reliably. It is interesting that LFP tuning plasticity, indexed by increased CS/BF ratios after training, was greatest at 10 dB; this was the only intensity at which responses to the CS frequency became larger than responses to the pretraining BF. While this observation suggests that LFP plasticity at 10 dB indexed tuning changes below threshold for cellular discharges, resolution of this issue will require future studies

involving simultaneous recording of cellular discharges and LFPs and, if possible, intracellular recordings.

The N1 LFP recorded in this study from infragranular layers appears to represent one or more current sinks in the cortical depths. This interpretation is based on several factors. First, surface recordings yield a positive LFP component, P1, that has approximately the same latency as the subdural N1 (~15–20 ms) and that inverts below layer II/III (Barth & Di, 1990; Borbély, 1970; Kaga, Hink, Shinoda, & Suzuki, 1980; Knight & Brailowsky, 1990; McGee, Kraus, Comperatore, & Nicol, 1991; Steinschneider et al., 1992). From CSD analysis, it has been argued that in the monkey, this depth N1 reflects a single current sink in layer IV and a deeper current source (Steinschneider et al., 1992). However, using both CSD and principal components analysis, Barth and Di (1990) have shown in the rat that nearly all of the variance of this depth N1 can be accounted for by two populations of vertically oriented pyramidal cells in supragranular and infragranular layers that are activated in an overlapping, asynchronous manner. An important implication is the inadequacy of a model of strictly sequential vertical information processing, necessarily replaced by one that also includes parallel processing to account for this component of the LFP.

This analysis indicates the high degree of complexity facing some lines of future study. Since the N1 appears to be a composite of extracellular recorded EPSPs from spatially separate but temporally overlapping populations, it will be difficult to determine the contributions of each population. The tuning plasticity may result from any of a number of combinations of change within one or both populations, and these need not be the same on every occasion. Thus, while LFP recordings have proven to be beneficial in the delineation of consolidation in the auditory cortex, they may be severely limited in future studies of local circuit processes underlying learning-induced tuning plasticity. Nonetheless, a first step should be to perform CSD analysis to directly characterize the nature of changes in current flows involved in LFP tuning plasticity.

Cholinergic mechanisms are known to be sufficient to induce RF plasticity of unit discharges. Thus, nonaversive stimulation of the nucleus basalis can serve as a proxy for foot shock in conditioning analogs (Bakin & Weinberger, 1996; Kilgard & Merzenich, 1998), and this is dependent on the activation of muscarinic receptors in the auditory cortex (Miasnikov, McLin, & Weinberger, 2001). Therefore, similar cholinergic mechanisms may be involved in LFP plasticity.

Associative learning was accompanied by an increase in the absolute amplitude of LFPs evoked by the frequency of the conditioned stimulus and a decrease in the absolute amplitudes of LFPs evoked by the pretraining BF (but see caveats above regarding absolute amplitudes over days). These results are consistent with opposite changes in synaptic strength that might be produced by LTP-like and LTD-like mechanisms, respectively. Local field potential facilitation via an LTP-like mechanism has been reported to reveal increased synaptic strength in the rat motor cortex after motor skill learning (Rioult-Pedotti, Friedman, & Donoghue, 2000). Changes in synaptic strength are best detected by direct intracellular recordings, which were not feasible in this multi-day study. Intracellular recordings might be obtainable during short-term behavioral conditioning because tuning plasticity develops in the auditory cortex in only five trials (Edeline et al., 1993). *In vitro* studies of auditory cortex using temporal samplings of posttraining retention/consolidation intervals are also possible.

However, one cannot assume that LFP tuning plasticity reflects changes in synaptic

strength. Because LFPs reflect the contributions of many cells, the same effects might have been caused by changes in the number of contributing cells, none of which had developed changes in synaptic strength. This could result from changes in the thalamo-cortical response to tones, perhaps reflecting a subcortical locus of tuning plasticity. The major source of thalamic input to the auditory cortex, the ventral medial geniculate nucleus, does develop CS-specific receptive field plasticity during behavioral cardiac conditioning. However, this plasticity is short term, dissipating by 1 h posttraining (Edeline & Weinberger, 1991). Therefore, the long-term retention and consolidation observed in this study cannot be attributed completely to the ventral medial geniculate. Nonetheless, direct mechanistic studies are needed to distinguish between putative changes in synaptic strength and changes in number of contributing elements.

Cellular/Molecular mechanisms of time-dependent changes in memory, or the analogs of memory (e.g., changes in synaptic strength), are beginning to be delineated in several systems, for example, *Aplysia* (Bartsch et al., 1995; Ghirardi, Montarolo, & Kandel, 1995; Mauelshagen et al., 1996; Sutton & Carew, 2000), *Drosophila* (Tulley, Preat, Boynton, & Del Vecchio, 1994; Yin et al., 1994), *Hermisenda* (Crow, Xue-Bian, & Siddiqi, 1999), and the mammalian hippocampus (Abel et al., 1997; Frey, Krug, Reymann, & Matthies, 1988; Kang & Schuman, 1996; Nguyen, Abel, & Kandel, 1994). In each case, multiple temporal phases of plasticity have been identified during retention periods following the induction of plasticity by learning or by direct activation of neural pathways. Three temporal phases have been identified (short, intermediate, and long term), based on differential involvement of various molecular processes. For example, within the dentate gyrus, CA1, and CA3 regions of the hippocampus, a late LTP (L-LTP), which emerges after 2 to 3 h, requires protein synthesis (Frey et al., 1988) or RNA synthesis (Nguyen et al., 1994), while an early LTP (E-LTP), which is retained for 1 to 3 h, does not. Although the current time-dependent increase in tuning plasticity cannot yet be segmented into temporally distinct phases on the basis of mechanism, its particularly long time course suggests that the consolidation observed here likely reflects more than one process. Long time courses suggest the involvement of both transcriptional and translational processes in the induction of the lasting change in plasticity (Bartsch et al., 1995; Frey et al., 1988; Kang & Schuman, 1996; Yin et al., 1994). The neural consolidation reported here now provides an opportunity to address these types of issues in the cerebral cortex.

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