Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig

Jonathan S. Bakin and Norman M. Weinberger

Department of Psychobiology and Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717 (U.S.A.)

(Accepted 10 July 1990)

Key words: Auditory cortex; Conditioning; Learning; Plasticity; Receptive fields

To determine if classical conditioning produces general or specific modification of responses to acoustic conditioned stimuli (CS), frequency receptive fields (RF) of neurons in guinea pig auditory cortex were determined before and up to 24 h after fear conditioning. Highly specific RF plasticity characterized by maximal increased responses to the CS frequency and decreased responses to the pretraining best frequency (BF) and other frequencies was observed in 70% of conditioning cases. These opposing changes were often sufficient to produce a shift in tuning such that the frequency of the CS became the new BF. CS frequency specific plasticity was maintained as long as 24 h. Sensitization training produced general increased responses across the RF without CS specificity. The findings indicate that associative processes produce systematic modification of the auditory system's processing of frequency information and exemplify the advantages of combining receptive field analysis with behavioral training in the study of the neural bases of learning and memory.

INTRODUCTION

The concept of a neuron's receptive field (RF) has proven to be very useful in attacking a fundamental problem in neurobiology: how does a brain provide an internal representation of the external world? Operationally, the receptive field of a cell reflects the parameters of sensory stimuli that alter its physiological activity, usually indicated as a change in the probability of discharge. Receptive field analysis has proven seminal for understanding the functional organization of sensory systems. Furthermore, it has played an important role in the discovery of neuronal plasticity in sensory cortex following sensory deprivation or peripheral denervation during development and adulthood, in the visual, somatosensory, and auditory cortices. The actual underlying mechanisms and behavioral consequences of such cortical receptive field plasticity are currently under intensive study.

In contrast, receptive field analysis has been neglected in the study of learning and memory, despite overwhelming documentation that sensory cortex is involved in integrative processes such as learning. This oversight is particularly troublesome because much contemporary inquiry in cognitive and behavioral sciences, neurosciences, and neural modeling concerns how brains acquire and represent information. While there is no dearth of research in the neurophysiology of learning and memory, the results usually pertain to changes in the excitability of neurons, rather than to information processing per se.

To clarify this distinction, we may consider the large body of literature which shows that associative processes during classical conditioning induce changes in response to an acoustic conditioning stimulus (CS) in the auditory cortex. Interpretation of increased response to the CS (e.g. a tone) can be made in terms of increased excitability, but cannot be made in terms of information processing. The increased response could be due either to a general increase in responsivity to all (or most) acoustic frequencies, or it could reflect a highly specific modification of the way in which frequency information is processed. Training and testing with one tonal frequency (the CS) does not provide the data critical to distinguish between these alternatives. However, receptive field analysis does provide a way to resolve the issue. The 'general excitability' hypothesis predicts that responses to many frequencies will be facilitated following conditioning with one frequency. That is, the receptive field will exhibit stronger responses across its domain. Alternatively, the 'information processing' hypothesis predicts that following conditioning, the receptive field will be systematically modified to emphasize the frequency used as the conditioned stimulus.

These alternatives were tested by Diamond and Weinberger by determining the frequency RF of neurons in non-primary auditory (secondary [AII] and...
ventral ectosylvian [VE]) cortical fields during classical conditioning of the pupillary dilation response in the cat. They found that the processing of frequency information was systematically modified during learning. Conditioning, but not sensitization training, produced changes in response that were largest at the frequency of the CS. Furthermore, this CS frequency specificity (CS-FS) remained during the retention times available (e.g., 30 min). Finally, extinction training (CS alone) produced a reversal of the CS-FS modification of the receptive fields.

The discovery that the frequency tuning properties of neurons in the cerebral cortex depend not only on stimulus parameters but also on the acquired significance of a stimulus, raises a multitude of questions concerning its characteristics and mechanisms. Elsewhere, we have set forth a preliminary model of the mechanisms of receptive field plasticity during learning. The present experiment concerns further characterization of this phenomenon. Specifically, we asked whether such receptive field plasticity develops in primary auditory cortex, whether it is long lasting and whether it can be observed in another order of mammals. Some of these findings have been reported in abstracts.

**MATERIALS AND METHODS**

**Subjects and preparation**

The subjects were 8 adult male Hartley guinea pigs (350–600 g, Hilltop Farms). Prior to surgery, they were given i.p. injections of atropine sulfate (0.02 mg/kg) and diazepam (8 mg/kg) followed 20 min later by a single injection of sodium pentobarbital (Nembutal, 20 mg/kg). If necessary, additional i.p. injections of sodium pentobarbital (10 mg/kg) were administered to maintain satisfactory anesthesia levels. Animals were placed on a heating pad and prepared for sterile surgery. The skull was held firmly in place by blunt ear bars using a stereotaxic apparatus. An antibiotic ophthalmic ointment (Terramycin) was liberally applied to both eyes. The pina were clamped and resected, and the wounds were allowed to heal with a topical antibiotic (Panalog). The calvarium was cleaned and an acrylic pedestal anchored by stainless steel screws was affixed to the skull. The pedestal contained two 0.5-in. spacers that allowed fixation of the animals head to the stereotaxic apparatus during recording sessions. One of the skull screws served as the reference electrode. Following drying of the pedestal, the animal was released from the earbars and was held only by the spacers. A craniotomy was performed over the left auditory cortex and the dura mater was removed under microscopic observation.

Auditory cortex was located according to the characteristic pattern of cerebral vasculature at and immediately caudal to the sylvian fissure, plus evoked potential magnitude and latency changes. An electrode array was lowered using a non-slip guide and nickel evoked-potentials were monitored. Upon attainment of sharp large (>500 µV) evoked potentials on a majority of electrodes, the electrode advancement was halted (mean depth = 1136 µm, range = 1000–1400 µm) and the exposure was filled with a layer of fine powdered gel foam. The array was cemented in place and the skin was sutured around the base of the pedestal. Animals were allowed to recover in an incubator.

**Recording electrodes and cluster characteristics**

Electrode arrays consisted of 6–12 formvar-coated tungsten (0.002 in., California Fine Wire Co.) wires arranged in two rows.

![Fig. 1. Quality and stability of cluster recordings. Pre-training (A) and immediate post-training (B) recordings taken from the same electrode prior to and after behavioral conditioning. Pre-training (C) and immediate post-training (D) recordings taken from the same electrode presented in (A) and (B). Note faster time sweep. This cluster consisted of two waveforms.](image)

Electrodes were spaced approximately 300 µm apart and held in place by an Amphenol connector strip. The array was coated with several layers of epoxy and baked until dry. Following drying, the impedances of the electrodes were individually lowered by passing current until they measured approximately 3–10 MΩ (reference 1 kHz signal). Unit activity was usually present beginning on the second day and could remain for a period of several weeks. Multiple unit clusters were recorded most often but it was possible to record from single units (n = 3). Data reported here were from recordings that showed waveform stability throughout the experiment (Fig. 1). Recordings averaged less than 4 cells per cluster.

**Recording sessions and receptive field determination**

The unanesthetized animal was supported and restrained by a vinyl hammock held within a metal frame located inside an acoustic chamber (AIC Industries). Its pedestal was bolted to a rigid post. A calibrated speaker (AIWA) was placed firmly against the contralateral external ear surface by means of a flexible support. Care was taken to ensure that the head was held at a level appropriate to the animal's normal posture during quiet waking in its home cage. Guinea pig heart rate shows adaptation and stability in this situation.

Electrodes were monitored every few days for signs of tone-evoked discharges. If an electrode revealed satisfactory discharge characteristics (identifiable waveforms, 3:1 signal-to-noise ratio or better, Fig. 1) then a complete receptive field characterization was performed. Using a computer-controlled digital frequency synthesizer (Wavetek 5100), an intensively filtered tone bursts (50 or 200 ms duration, inter-tone intervals of 550 or 400 ms, respectively, rise-fall 5 ms) was presented to the ear contralateral to the recording site. Sound levels were calibrated at the speaker (0–90 dB SPL, Bruel and Kjaer condenser microphone type 4134 and sound level preamp type 2204, Hewlett-Packard 3581A wave analyzer).
TABLE I

Behavioral and neuronal measures

<table>
<thead>
<tr>
<th>Group</th>
<th>Behavioral data</th>
<th>Neuronal data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Trials to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>criterion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Conditioned</td>
<td>9</td>
<td>4.1</td>
</tr>
<tr>
<td>Frequency Specific</td>
<td>(6)</td>
<td>4.5</td>
</tr>
<tr>
<td>General Increase</td>
<td>(3)</td>
<td>3.3</td>
</tr>
<tr>
<td>Sensitization</td>
<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>Comparison</td>
<td>U</td>
<td>P</td>
</tr>
<tr>
<td>Conditioned vs. Sensitization</td>
<td>0</td>
<td>****</td>
</tr>
<tr>
<td>Frequency Specific vs Sensitization</td>
<td>0</td>
<td>**</td>
</tr>
<tr>
<td>Frequency Specific vs General</td>
<td>6.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>General Inc. vs. Sensitization</td>
<td>0</td>
<td>*</td>
</tr>
</tbody>
</table>

* P < 0.01; ** P < 0.025; *** P < 0.001; **** P < 0.0001, n.s., non-significant. x, Animals that did not reach criterion within 30 trials were assigned a value of 30.

Because calibrations at the tympanic membrane cannot be accomplished in waking, behaving animals*. The tone series consisted of 11 ascending frequencies that covered the range of responsiveness for the particular cluster of cells. The frequencies used varied across animals, but were constant for a given animal across all of its recording sessions. This series was repeated 20 times at a near threshold intensity. Following presentation of the set at a particular intensity, the series was repeated at 3–6 higher intensities until saturation was reached**. Thus, for every animal, responses to a range of frequencies over a range of intensities were recorded. Receptive fields from the same electrode were determined immediately prior to training and during 3 retention time sessions: immediately post, 1 h post, and also 24 h post-training, if possible.

Neuronal activity (300 Hz to 3000 Hz, Dagan 2400 preamplifier), was amplified, discriminated (SA-131, voltage discriminator), and monitored on a storage oscilloscope (Tektronix 5111) as well as recorded on tape (Hewlett Packard 3964A). A laboratory computer (PDP 11/73) stored the times of occurrence of action potentials (voltage detector pulses) and stimuli, and was used for quantitative data analysis and to construct peristimulus time histograms.

Behavioral training

Animals underwent behavioral training in an acoustic isolation chamber distinct from the recording chamber. Animals were placed in a plexiglass box (12 in. high by 10 in. wide by 5 in. deep) located 3 in. away from an 8 in. full-range speaker. The sides of the chamber had numerous small holes. A small amount of electrode cream (Bendix EKGsol) was applied to the paws. Conditioning trials consisted of a 10 s 80 dB tone (conditioned stimulus, CS; intensity measured inside the plexiglass box) followed at tone offset by a 2 s shock (unconditioned stimulus, US) delivered through the chamber's grid floor (Grass S-48 stimulator and constant current isolation unit, 1–4 mA). Sensitization trials were either the tone or the shock and were presented explicitly unpaired. Conditioned animals received from 10 to 30 trials of paired stimulation while sensitization animals received 30 trials each of the CS and the US. Intervall intervals averaged 90 seconds apart (range = 60–120 s).

Animals were observed through a window in the acoustic chamber for the entire duration of training. All animals displayed orienting responses (head turning toward the speaker) at tone onset that habituated during training. Behavioral responses that developed during training included chewing, freezing, head movement, and rearing during tone presentation, and were recorded as evidence of conditioned responses (CR). The acquisition criterion was CRs on 3 consecutive trials. If this criterion was not met, the animal was assigned the number of training trials presented.

Experimental design

Following completion of the pre-training receptive field characterization, the animal was removed from the recording chamber and placed in the conditioning chamber. The animal underwent either classical conditioning or sensitization training. Immediately following the conclusion of the training session, the animal was returned to the recording chamber. The immediate post-training receptive field was determined. The animal remained in the hammock for 1 h at which time the 1-h post-training receptive field was determined. After completion of the receptive field characterization, the animal was returned to its home cage in the vivarium. Twenty-four hours after the completion of the behavioral training session, the animal was returned to the recording chamber and restrained for another characterization of the receptive field. Some recordings at the 1 and 24 h time period were rejected due to a change in or loss of

* This method was based upon previously established techniques when a closed system cannot be used in awake animals. Although sound pressure levels at the tympanic membrane could not be measured, this is not critical for interpretation of the findings because stimuli were essentially the same before and after training and the consistent findings (see Results) could not be explained by putative changes in sound pressure level.

** In one subject, acceptable recordings were obtained from two electrodes. The receptive fields of the two recordings were determined sequentially with the order of electrode testing constant across RF determinations.
waveforms present at the pre- and immediate post-conditioning periods. In 4 cases for which subjects had excellent neuronal activity following 24 h, animals were retrained 1–7 days later, using a different CS frequency and a different electrode. Neuronal data were obtained from ten cases of conditioning and three cases of sensitization.

The choice of the frequency to be used as the conditioned stimulus (CS) was based on the pre-training receptive fields. The CS frequency had to elicit an identifiable response at some intensity, but not elicit a maximal response at most intensities, i.e., not be the best frequency (BF).
Fig. 2. Classical conditioning causes a CS frequency-specific increase. In all figures receptive fields are quantified by subtracting spontaneous activity from the tone-evoked activity in the PSTHs. RF difference functions show evoked activity (filled squares) and spontaneous activity (open circles). The frequency of the CS is indicated by a filled arrowhead and the pre-training best frequency by an open arrowhead. Inspection of pre-conditioning (A), immediate post-conditioning (C), 1 h post-conditioning (F), and 24 h post-conditioning (I) peristimulus histograms and raster reveals an increase at the CS frequency (6.0 kHz, filled arrowhead) and decreases at other frequencies, including the pre-training best frequency (10.0 kHz, open arrowhead). The increase at the CS was present immediately following conditioning and developed until reaching a maximum value at the longest retention interval tested (24 h). The decreases at other frequencies were also present immediately and developed over time, reaching a maximum at 24 h post-conditioning. The corresponding receptive field functions B-D-G-K are given in the middle column. Subtracting the pre-conditioning RF (B) from the post-conditioning RFs (D-G-K) reveals the effect of conditioning and is shown as RF difference functions (E-H-L, right column). Note the maximal increase at the CS frequency and the decreases at higher frequencies including the pretraining best frequency. Also shown is the difference in spontaneous activity (pre-tone intervals) obtained during the same recording sessions (open circles). Note that the spontaneous activity was unrelated to the RF changes. At all time points, there was a decrease in the spontaneous activity while the response to the CS frequency increased. Latency to onset response for this cluster was always less than 30 ms. Inset: example of measurement of the difference in change between the CS and the BF (A), and the 50% bandwidth of the peak at the CS frequency (B).

Analysis of neuronal data

Frequency receptive fields (RF) for the set of 20 repetitions of the tone sequences were calculated by subtracting the average discharge during the pre-tone period (500 ms) from the average discharge during each tone, thus obtaining a measure of tone-evoked activity. The evoked response to the 20 presentations of each tone was averaged. These values were calculated for each intensity presented at each time point, and were plotted vs. the frequency of the stimulus.

The effect of training was assessed by calculating RF difference functions. The pre-training RF was subtracted from the post-training RF of the same intensity. The following RF difference functions were calculated for all intensities when possible: immediate post-training, 1 h post-training, and 24 h post-training.

Comparisons across animals and group means were made on normalized difference scores. Scores were normalized with reference both to magnitude of change and frequencies tested. In order to equally weight the contribution of each animal’s difference score, all group means were based on percentage change from pre-conditioning baseline receptive fields. A percentage difference score response function was calculated by dividing the difference score by the absolute value of the largest difference score obtained for a tone within that same intensity/time point set, and the result was multiplied by 100 for a percentage value:

\[
\text{Difference score} = \frac{\text{Maximum difference score}}{\text{Maximum difference score}} \times 100\%
\]

Because different frequency ranges were used for the different animals, a common frequency reference was established using an octave scale. Frequencies were grouped and averaged according to quarter octave intervals across the range of ± 1.5 octaves centered at the CS frequency.

Frequency specific criteria

In order for an animal to be classified as exhibiting a frequency specific change, two separate criteria classes had to be satisfied: (a) a CS-frequency specificity requirement, and (b) a reliability requirement. The frequency-specific criteria were (1) the largest change in the RF difference function had to be at the CS frequency, (2) the bandwidth at 50% of the CS peak had to be less than 1/2 octave, and (3) the difference score at the CS frequency had to be at least 50% greater that the difference score for the pre-training best frequency. The largest increase at the CS is a very restrictive criterion, as increases at frequencies as close as 0.04 octave were not considered as CS frequency-specific. The bandwidth criterion eliminated general increases in which the CS frequency happened to be the largest change. The CS-BF criterion emphasized the CS selective effects by requiring greater change at the CS frequency than at the most potent pre-training frequency, the best frequency. The reliability criterion was that the same CS frequency-specific effect had to occur either at (1) the same intensity during two or more retention time session, or (2) at two or more intensities during the same retention session. The probability that a recording site would satisfy all of the criteria by chance is approximately 0.004**.

Recording sites in auditory cortex

The auditory cortex of the guinea pig consists of 3 tonotopic fields plus several surrounding non-tonotopic fields. These are aligned in a rostral-caudal direction and have been termed fields S, A and DC, respectively7.8. Field S is extremely small and was not the site of recordings in this study. Fields A and DC (called fields I and II by Hellweg et al.20, and rostral, caudal by Robertson and Irvine26) respectively receive projections from the tonotopic auditory thalamic nucleus, the ventral medial geniculate37. Field A is the largest field and has a frequency representation of low to high in the ventrostral to dorsocaudal dimension. Field DC has a mirror image frequency organization to field A. Based upon these mirror images and the directions of frequency organization, it might be thought that field A is homologous or analogous to the anterior auditory field3 and that field DC has this same relationship to field

---

* Average tone discharge was calculated for a ‘window’ set to minimally frame the evoked response. Most windows were onset windows, but two were whole-tone windows (onset mean duration = 30 ms, n = 10; whole-tone duration = 200 ms, n = 2).

** The probability of obtaining a CS frequency-specific effect by chance due to the use of multiple measures was based on the conjoint probabilities of the following: (a) largest change at the CS frequency = 1/11 (11 tones in a receptive field); (b) bandwidth criterion = 1/2 (change centered on the CS frequency could have been smaller or larger than the criterion); (c) increase 50% greater than BF = 1/2 (this is highly conservative as 1/2 is the probability that the CS would have a larger change than the BF; the requirement of a 50% difference reduces this probability but the amount cannot be determined directly. The conjoint probabilities are 1/11 × 1/2 × 1/2 = 1/44. Since all three criteria had to be met twice, the probability of this is 1/44 × 1/44 = 1/1936. However, since there were at least two time points and an average of four intensities, there were at least 8 opportunities for the criteria to be met. This increases the probability to 1936/8 = 242 or P = 0.004.
A: Pre-Conditioning

B: Pre-Conditioning RF

C: 1 Hour Post-Conditioning

D: 1 Hour Post-Conditioning RF

E: Post-RF minus Pre-RF

Fig. 3. Classical conditioning can result in the CS frequency becoming the best frequency. Pre-conditioning (A) and 1 h post-conditioning (C) PSTHs and rasters show that conditioning can result in the CS frequency (9.0 kHz, filled arrowhead) becoming the best frequency. Receptive fields are quantified in (B) and (D). Receptive field difference function (E) reveals a maximal increase at the CS frequency and maximal decrease at the pre-training best frequency (9.5 kHz, open arrowhead). Also graphed is the spontaneous activity difference function for the same sessions. Note that there was no CS-specific change in spontaneous activity. Latency to onset of response was less than 20 ms.

At28 as delineated in the cat. However, additional detailed anatomical and physiological studies are required before this conclusion is warranted. Our own pilot studies verified the basic frequency organization. In the present experiment, examination of best frequencies along the rostral-caudal dimension within an electrode array revealed that recordings were obtained from the largest field (A); for example, adjacent electrodes separated by 300 μm rostral-caudal had best frequencies of 12.0 and 16.0 kHz, respectively. Some recordings from posterior electrodes were likely to have been obtained from field DC, but in the absence of a detailed frequency map, which could not be done during implantation of the chronic electrode array, no firm conclusion can be drawn. Responses of neuron clusters themselves were consistent with the characteristics of previous reports from primary auditory cortex in the waking cat10 and monkey29. As the goal of this study was to record from tonotopic auditory fields, the absence of clear attribution of each recording site either to field A or DC is not a severe limitation. For purposes of exposition, hereafter we refer to the recording sites as from ‘primary’ or ‘tonotopic’ auditory cortex.

RESULTS

Behavior

Conditioning led to the development of conditioned responses, including chewing, lateral and vertical head movement, and rearing, with an increasing probability of
Fig. 4. CS frequency-specific increase with decrease at other frequencies produces different post-conditioning receptive fields. The consistent increase at the CS frequency and the decreases at other frequencies produces different shapes of frequency receptive fields depending on the shape of the pre-training receptive field. Pre-conditioning (A,D), immediate post-conditioning (B,E), and RF difference functions (C,F) from two different animals. In A-B-C, conditioning created a secondary peak centered on the CS frequency in a previously smooth receptive field. In D-E-F, conditioning led to a smoothing of a previously complex pre-training receptive field.

occurrence later in tone presentation (latency = 4–10 s) in all animals. Consistent CRs were not observed in the sensitization group. Conditioned animals reached the behavioral criterion significantly faster than sensitization animals, none of which attained criterion ($U = 0, P < 0.01$, Table 1).

Neuronal data

Effects of conditioning on receptive fields

CS frequency-specific increases. As noted above, conditioning resulted in CS frequency-specific increases of
receptive fields in 70% (7/10) of the immediate post-training recording cases*. The CS frequency-specific criteria could have been met by an increased response to the CS frequency without decreased responses to other frequencies, but this was not observed. CS frequency-specific increases consisted of an increase in response to the CS frequency and a decrease in response to other frequencies, particularly to the pre-training best frequency. Fig. 2 illustrates such a case. Note the evolution of the increase at the CS frequency and consistent

* One subject yielded recordings from two electrodes. The CS was 12.0 kHz, the BFs at the electrodes were 10.0 and 16.0 kHz. Both recordings exhibited CS frequency-specific plasticity.
Fig. 6. Conditioning can result in a general increase in receptive fields. Prior to conditioning, the best frequency was 9.0 kHz (A,B). In this example, conditioning led to a general increase in the receptive field. Immediately following conditioning (C,D,E) the maximal increase was not at the CS frequency (12.0 kHz). The increase in the receptive field grew over time. This case failed to meet the CS-BF or the bandwidth criteria at 24 h, and failed to meet all of the criteria at earlier time points.

decrease at other frequencies, including the pre-training best frequency. This effect was long lasting, as evidenced by retention of the increased response to the CS at 1 h and 24 h post-training. Sixty-seven percent (4/6) of the CS frequency-specific cases yielding recordings for 24 h demonstrated the same effect 1 h and 24 h after training.

In 43% of the CS frequency-specific cases (3/7) this increase in response to the frequency of the CS, coupled with the decrease at the pre-training BF and other frequencies, was strong enough to result in the CS becoming the new best frequency. Fig. 3 illustrates such a shift in receptive field tuning. The post-training receptive field shows a peak at the CS frequency where a 'notch' had been observed prior to training. This example also demonstrates the high degree of specificity which can be obtained. The pre-training best frequency was 9.5 kHz, and the CS was chosen to be 9.0 kHz, approximately one-tenth of an octave away. The RF difference function shows that conditioning led to maximum increase at the CS and a maximum decrease at the original BF. In addition, the magnitude of the effect was large; the response to the CS increased by 34.2 spikes/s, or 83.0% greater than its original value, and the response to the BF decreased by 35.8 spikes/s, or 35.3% less than its original value.

This pattern of increased response to the frequency of the CS and decreased response to other frequencies was consistent regardless of the particular shape of the
Fig. 7. Sensitization can cause a general increase in the receptive field. Prior to conditioning, the best frequency was 26.0 kHz (A,B). Following sensitization training, there was a general increase in the receptive field that was present immediately (C,D,E), grew over time (F,G,H), and was retained for 24 h (I,K,L). The CS frequency never showed maximal increases nor did the BF exhibit a decrease (E,H,L). Note the large increases in 50% bandwidth, evident in the PSTHs as well as in the RF difference functions.

receptive field prior to conditioning (e.g. Fig. 4C,F).

Thus, the post-conditioning receptive field shapes could be quite different, depending on the pre-training shape of the RF. Fig. 4 presents two examples.

Fig. 4A shows a RF with a smooth increase in response to the pre-training BF of 16.0 kHz. Following conditioning with a CS of 12.0 kHz, the RF was no longer smooth, with a new peak present at the CS frequency (Fig. 4B).

A complementary case is shown in Fig. 4D, which has a multi-peaked RF prior to training, with the BF = 30.0
kHz and smaller peaks centered at 16.0 kHz and 26.0 kHz. Following training with a CS of 22.0 kHz, a smooth receptive field with a single peak at 26.0 kHz was obtained.

Although all recordings classified as CS frequency-specific did meet the criteria (see Materials and Methods), in some of these cases there were interesting RF changes at other time points or intensities or both which failed to meet all of the criteria. The most interesting of these were cases in which there was little or no increase at the frequency of the CS but decreases at most other frequencies, including the BF. Fig. 5 presents such a case, in which the greatest decrease is at the pre-training BF. These decreases also resulted in a shift of receptive field tuning in which the frequency of the CS became the new BF.

Group data obtained from the 7 immediate post-training retention sessions exhibiting CS frequency-specific increases are summarized in Fig. 8A and Table I. The mean increase at the CS frequency was 76.4%. Note also the clear reduction of response at frequencies higher and lower than the CS frequency, i.e., side-band suppression. The high degree of frequency specificity of this plasticity is demonstrated by the fact that values were at or below pre-training levels within ± one quarter of an octave.

General increases

Thirty percent (3/10) of the cases revealed general increases in the receptive field when tested immediately following training. These were long-lasting; all of the general increases were still present at the 1 h post-training retention session, and the case tested 24 h post-training still exhibited a general increase across its receptive field. In no cases did conditioning cause a general increase in the response function that resulted in a tuning curve shift where the CS became the new best frequency.

An example of a general increase is illustrated in Fig. 6. Note that immediately following conditioning, the receptive field difference function showed a peak maximal at a non-CS frequency, and that this peak grew over time. At 24 h post-training, the maximal increase was at the CS frequency. This case failed the CS-frequency specific criteria; at two time points the maximal change was not at the CS, at all 3 time points the CS–BF difference was less than 50%, and at two time points the 50% bandwidth was greater than 0.5 octaves.

Group data obtained for general increases immediately post-training are presented in Fig. 8B. The mean increase at the CS was 53.0%. Tuning around the CS frequency was broad and there were no decreases in the receptive field (Table I).

**A. CS-FREQUENCY SPECIFIC**

**B. GENERAL INCREASE**

**C. SENSITIZATION**

Fig. 8. Normalized group data for receptive field difference functions. CS frequency-specific (A), general increase (B), and sensitization (C) group RF difference functions obtained from normalized individual case difference functions. The frequency axis is distance from the CS frequency (arrow) in octaves. Note the maximal change at the CS and the sideband inhibition present only in the CS frequency-specific group. Both General and Sensitization groups had increased responses that included the CS frequency, but neither had sideband inhibition. Bandwidth of the CS frequency-specific increase was significantly narrower than either the general increase or the sensitization groups. (frequency specific n = 7, general increase n = 3, sensitization n = 3).

**Effects of sensitization on receptive fields**

All animals that underwent sensitization training (n = 3) developed a general increase in receptive field tuning that was present at several intensities. These increases could last up to 24 h and never resulted in the CS
becoming the best frequency. An example is presented in Fig. 7. Like both CS frequency-specific and general increases, this change could evolve over time. Note the large 50% bandwidth present at 1 h and 24 h post-training (0.97 and 1.18 octaves, respectively). Group data for sensitization are given in Fig. 8C. The mean increase at the CS was 39.2%, the bandwidth was broad, and there were no decreases in the receptive field (Table 1).

Comparisons across groups

Conditioning produced a larger increase at the CS frequency and a narrower 50% peak bandwidth than did sensitization training (both $U = 3$, $P < 0.025$). Frequency-specific cases were significantly different from sensitization cases and from general increase cases on all 3 criterion measures (Table I, Mann–Whitney, all values $P < 0.02$). No differences were found between the general increase cases and the sensitization cases (Mann–Whitney, all values $P > 0.1$).

Differences across groups cannot be explained by variation in stimuli presented as no statistical differences in intensity ranges or number of intensities presented were found (Mann–Whitney, all values $P > 0.05$). In addition, no differences were found across any group in the original best frequency, the CS chosen, or the distance between the BF and the CS in octaves (Mann–Whitney, all $P > 0.05$).

DISCUSSION

The current findings reveal that classical conditioning produces substantial, systematic and highly specific modifications of frequency receptive fields in primary auditory cortex of the guinea pig. Specifically, responses to the frequency of the conditioned stimulus are increased while responses to other frequencies, particularly the pre-training best frequency, are reduced. These coordinated changes can be sufficient to shift the tuning curve so that the frequency of the CS becomes the new best frequency. Furthermore, this receptive field plasticity is long-lasting, being present at retention intervals of one and even 24 h. Additionally, the changes in tuning are highly selective: the bandwidth centered on the CS frequency averaged only 0.21 octaves (Table I, Fig. 8). This high degree of selectivity is noteworthy given the absence of frequency discrimination training, which would be expected to produce even sharper tuning effects.

Controls for non-associative and state effects

That this RF plasticity is associative is indicated by the absence of CS-FS effects in the animals trained on the sensitization paradigm; they developed a general increase in response across their receptive fields. This notwithstanding, it might be thought that the RF plasticity was due to a change in arousal level during the determination of receptive fields, such that arousal was highest during presentation of the CS frequency and lowest during presentation of non-CS frequencies. However, using sensitive indices, such as pupillary dilation and heart rate, our previous studies of receptive field plasticity found no evidence of tonic or phasic arousal during RF determinations11,14. Furthermore, the present study provides several reasons for ruling out the possibility that putative state changes produced CS-FS receptive field plasticity.

First, such an explanation is extremely implausible as it would require an extraordinary pattern of increased and decreased arousal to 50–200 ms tones presented at a rate of 1.6/s. Furthermore, this pattern would have to be repeated as many as twenty times during RF determination to obtain the consistent results evident in the rasters. Finally, such an arousal pattern would have to be extremely discriminative as responses to stimuli only 0.06 (Fig. 3) or 0.08 (Fig. 4) octaves from the CS frequency exhibited decreased responses after conditioning.

Second, if CS-related arousal, rather than alteration in the processing of frequency information, is responsible, then CS frequency-specific plasticity should have been observed in animals which developed CS-related arousal, i.e., animals that developed behavioral conditioned responses. However, some of these animals did not develop CS specific plasticity, but rather exhibited general increases in response (Fig. 6 and 8B).

Third, putative phasic arousal evoked by the CS frequency during RF determination could not have altered responses to that tone because the latencies of tuned neuronal responses were 10–40 ms, whereas the shortest latency sign of cortical arousal is greater than 100 ms50. Nonetheless, it might be that arousal effects evoked by the first CS presentation during RF determination would be present preceding subsequent presentations of the CS frequency. However, this predicts that responses to the first CS presentation post-conditioning should be smaller than to succeeding presentations. Inspection of rasters fails to reveal such a pattern (e.g., Figs. 2 and 3).

Fourth, if RF difference functions reflect changes in arousal, then the background (inter-tone) discharge rate should reflect this patterned change in excitability. However, comparisons of background vs evoked difference functions show that these two measures are unrelated; background changes are more or less the same across frequencies whereas evoked discharges are decidedly and significantly frequency-specific (Figs. 2–5).

Relation to previous findings

This experiment is the first study of the effects of
learning upon receptive field plasticity in primary auditory cortex. It considerably extends the previous reports of Diamond and Weinberger\textsuperscript{10,11}. They found CS-specific receptive field plasticity in non-tonotopic auditory fields (AII and VE) of the muscle-blocked cat, using the pupillary dilation CR as the index of association; they also reported retention data for about 30 min postconditioning. The current experiment differs from their work in several respects: type of cortical field (primary vs secondary); order of mammal (rodentia vs carnivora); type of preparation (undrugged vs muscle-blocked); conditioned response (general somatic vs pupillary dilation); retention interval (1 and 24 h vs 30 min). Therefore, CS-FS receptive field plasticity is not a highly restricted phenomenon but rather develops across the several major experimental variables studied to date.

Another difference between the previous work and this experiment was that we recorded from clusters of 2–3 waveforms using a low impedance chronic electrode, rather than from single neurons using a high impedance acute electrode, in order to obtain data at longer retention intervals. This recording technique did not prevent the detection of RF plasticity in the present study. Diamond and Weinberger\textsuperscript{10,11} reported that, in addition to increased responses, some single neurons developed a decreased response to the frequency of the CS. Because of the very small number of waveforms in the clusters recorded here, it is unlikely that decreased responses developed but were masked, as the cluster discharge would likely be sensitive to this type of change\textsuperscript{*}. Non-tonotopic fields such as AII and VE may be involved in more complex aspects of information processing during learning than are tonotopic fields, as they exhibit decreases as well as increases to the CS-frequency.

Receptive field plasticity has also been reported in studies of selective attention in previously trained animals. For example, spatial attention in highly trained monkeys is accompanied by decreased responses to unattended stimuli in non-primary visual fields; however, there was no facilitation to attended stimuli\textsuperscript{31}. Increased non-spatial attention is accompanied by increased responses to attended stimuli\textsuperscript{42}. We found increased responses to the CS frequency as well as decreased responses to non-CS frequencies. Therefore, the two types of selective attention may each employ one of the two processes which are evident at early stages of associative learning, i.e., either decreased response to less important stimuli or increased response to important stimuli.

Receptive field plasticity has been observed in sensory cortex following selective deprivation of sensory input, both during development and in adulthood. As subjects presumably have the opportunity to learn to adjust their behavior to compensate for the sensory loss during the postmanipulation period, this plasticity may involve learning\textsuperscript{**}. The highly controlled paradigm of classical conditioning may be an optimal condition under which to detect receptive field plasticity caused by learning. The development of plasticity may be slower in the days following partial sensory deprivation because of the less consistent relationship between a stimulus parameter and reinforcement, with consequent intermixing of both reinforced and non-reinforced (i.e. extinguished) relationships.

**Possible functional roles**

Frequency-specific receptive field plasticity was evident immediately following conditioning, which generally consisted of 30 trials. Although this is rapid for neuronal plasticity, it may be a substantial underestimate of how quickly RF are modified during learning. Note that animals exhibited behavioral conditioned responses very quickly, averaging less than 5 trials to criterion (Table I). Therefore, RF plasticity may have developed equally rapidly. Of note, frequency-specific RF plasticity developed only in animals that developed behavioral CRs. However, associative learning (indexed behaviorally) is not sufficient for such plasticity, as some conditioned animals developed behavioral CRs but general increases in neural response. Associative learning may be a necessary condition, as no sensitization animals developed CS-FS plasticity.

This behavioral/neural dissociation supports the view that the cortical plasticity is not in a ‘series circuit’ between the CS and a particular CR that is evoked at the time of CS presentation\textsuperscript{45,48,49}. Among the possible functions served by RF plasticity are stimulus

\textsuperscript{*} It might be argued that the post-training population of cells was not identical to the pre-training population, and that cells lost or gained during training were responsible for the differences in tuning curves observed. This is extremely unlikely for the following reasons: (1) Post-training waveforms were compared to photographs of pre-training waveforms and any subject whose post-training recordings suggested the waveform population had changed was eliminated from further consideration. (2) This argument requires that additional cells recorded during post-conditioning sessions were tuned to the CS frequency and not to adjacent frequencies, in order to result in post-conditioning CS frequency-specific increases and non-CS frequency decreases observed. (3) In addition, this putative recruitment of cells would have had to occur only in animals trained in conditioning because CS-specific effects were not found in animals that underwent sensitization training.

\textsuperscript{**} Learning effects cannot explain immediate ‘unmasking’ of weak inputs that has been observed in the somatosensory system\textsuperscript{5,7}.
constancy\textsuperscript{58}, selective attention\textsuperscript{48}, integration of an acoustic stimulus over time\textsuperscript{26,51}, pattern discrimination\textsuperscript{32}, short-term memory\textsuperscript{41}, long-term memory\textsuperscript{13}, and the determination of frequency relationships involved in the abstraction of pitch\textsuperscript{33,32}.

Toward mechanisms of associative receptive field plasticity

A noteworthy aspect of the present findings is that conditioning produces opposite effects at the frequencies of the CS and the BF (plus other non-CS frequencies), i.e., increased responses to the former and decreased responses to the latter. Recently, we have hypothesized that learning-induced frequency-specific receptive field plasticity is not simply projected onto the auditory cortex from subcortical or other cortical regions, but rather reflects the cortical integration of 3 distinct subcortical influences\textsuperscript{2,46,47}: (a) a frequency-specific, non-plastic, tonotopic projection from the ventral division of the medial geniculate nucleus (MGv); (b) a non-frequency-specific, highly plastic, diffuse projection from the magnocellular division of the medial geniculate (MGm)\textsuperscript{13,16,17,35,40}, and (c) a global neuromodulatory input (e.g., the cholinergic muscarinic input from the basal forebrain). Specifically, it is thought that synapses of pyramidal cells in the auditory cortex activated by the CS-frequency during training trials are strengthened whereas synapses not engaged by the CS-frequency tend to be weakened, both changes being dependent upon increased excitability of the post-synaptic cell\textsuperscript{7,46,47}.

Analogues of classical conditioning in sensory cortex are relevant to the issue of possible mechanisms. For example, sequential stimulation of two vibrissae reduces inhibition and/or increases responses of rat barrel field neurons to stimulation of the first vibrissa (‘conditioned stimulus’) alone\textsuperscript{5}. More detailed receptive field data have been reported in the visual cortex. Pairing depolarizing or hyperpolarizing current with effective and non-effective visual stimuli produces shifts in receptive fields to the non-preferred stimuli which appear to depend on stimulus pairing\textsuperscript{15}. The use of neuromodulators as ‘unconditioned stimuli’ can also produce specific changes in receptive fields in the somatosensory\textsuperscript{23}, visual\textsuperscript{19}, and auditory cortices. Regarding the latter, the application of acetylcholine (ACh), or an anticholinesterase, can produce systematic shifts in frequency receptive fields which seem to require the involvement of muscarinic receptors\textsuperscript{14,25,30}. More directly related to the present findings, the pairing of ACh alone with a non-best frequency tone is effective in producing receptive field modifications that are highly specific to the paired stimulus\textsuperscript{30}.

Thus, highly specific modifications of cortical receptive fields, including increased responses to a non-best stimulus and decreased responses to an initially preferred stimulus, are observed with direct manipulations of sensory cortex. Similarly, we have found an increased response to the CS frequency and decreased responses to non-CS frequencies. Therefore, the present findings provide a behavioral context within which the analogue data may be evaluated. The similarity between the basic findings of this investigation and those of the analogues supports the view that behavioral conditioning produces CS-specific receptive field plasticity in the sensory neocortex of the conditioned stimulus by mechanisms which involve neuromodulators operating at the level of the cerebral cortex\textsuperscript{7,46,47}.

Enlarged cortical representations of controlled behavioral stimuli have been found in trained animals\textsuperscript{22,42}. It seems quite possible that the representation of frequency across the auditory cortex is systematically altered by associative learning. Frequencies which we selected as conditioned stimuli were specifically not the BF of the cells we recorded from but must have been the BF of other neurons. For cells in which the CS and the BF are the same, we would expect an increased response at the CS (BF) frequency and decreased responses to other frequencies. Across the frequency representation, both these cells and cells which shift their BF to or toward the CS frequency would consequently yield an enlarged representation of the CS frequency and decreased representation of other frequencies\textsuperscript{46,47}.

Some implications of receptive field plasticity for neural mechanisms of learning and memory

Sensory neurophysiology and the neurobiology of learning and memory are both concerned with how nervous systems process information but have generally done so in parallel rather than intersecting lines of inquiry\textsuperscript{48}. Sensory neurophysiology has concentrated on the use of anesthetized subjects, for which learning is not an issue. Neurophysiology of learning has focused on immediate behavioral responses, thus ignoring sensory structures which may not be essential for an immediate conditioned response, e.g., sensory neocortex.

The current line of inquiry demonstrates that a combination of the approaches of both fields can help resolve issues fundamental to the neurobiology of learning and memory. In the present case, associative learning produces a highly specific change in the processing of frequency information rather than a general increase in response, which is seen to be the result of sensitization. Receptive field analysis was critical to resolve this issue. Both conditioning and sensitization produced increased responses to the CS frequency. If only the CS frequency had been tested following training, them conditioning and sensitization could be thought to produce the same qualitative effect. Receptive field analysis revealed that
the procedures produce qualitatively different effects, as only conditioning produced a shift in tuning, decreases at the pre-training best frequency, and sideband inhibition.

Finally, the approach used here shows that, among the initial events in associative learning, there is a re-tuning of frequency receptive fields in favor of the CS which accompanies the acquisition of information concerning CS-US relationships. We think it likely that having acquired and stored such information, an organism can use it to both generate a variety of specific behavioral responses and to combine it with other information to produce new cognitive structures in the service of immediate and future adaptive functions.

Acknowledgements. This research was supported by ONR N00014-87-K-0433 and an unrestricted grant from the Monsanto Company to N.M.W., and from a Chancellor’s Irvine Fellowship and NIMH Pre-Doctoral Training Grant MH14599 to J.S.B. We wish to express our thanks to Cheryl Condon for invaluable assistance in early stages of the study, J. Coni for assistance in the cortical mapping studies, J. M. Cassady for computer programs, and Jacque Weinberger for extensive secretarial assistance.

REFERENCES

16 Gabriel, M., Miller, J.D. and Saltwick, S.E., Multiple unit activity of the rabbit medial geniculate nucleus in conditioning, extinction and reversal, Physiol. Psychol., 4 (1976) 124-134.
17 Gabriel, M., Saltwick, S.E. and Miller, J.D., Conditioning and reversal of short-latency multiple-unit responses in the rabbit medial geniculate nucleus, Science, 189 (1975) 1108-1109.
33. Pantelis, C., Hoke, M., Lutkenhoner, B. and Lehnhertz, K., 
Tonotopic organization of the auditory cortex: pitch versus 
34. Pelleg-Toiba, R. and Wollberg, Z., Tuning properties of auditory 
cortex cells in the awake squirrel monkey, Exp. Brain Res., 74 
35. Polonskaya, L.U., Conditioned reaction of neurons in the 
magnocellular part of the medial geniculate body, J. Higher 
cortex in cats reared after unilateral cochlear ablation in the 
37. Redies, H., Brandner, S. and Creutzfeldt, O.D., Anatomy of the 
auditory thalamocortical system of the guinea pig, J. Comp. 
subdivisions in the auditory cortex of the guinea pig, J. Comp. 
39. Robertson, D. and Irvine, D.R., Plasticity of frequency organi-
zation in auditory cortex of guinea pigs with partial unilateral 
40. Ryugo, D.K. and Weinberger, N.M., Differential plasticity of 
morphologically distinct neuron populations in the medial geniculate 
body of the cat during classical conditioning, Behav. 
41. Sakurai, Y., Thalamocortical, hippocampal, and auditory neu-
ronal activity related to auditory working memory process in the 
42. Spinelli, D.N. and Jensen, F.E., Plasticity: the mirror of 
experience, Science, 208 (1977) 75–78.
43. Spitzer, H., Desmones, R. and Moran, J., Increased attention 
enhances both behavioral and neuronal performance, Science, 
44. Suga, N. and Manabe, T., Neural basis of amplitude spectrum 
representation in auditory cortex of the mustached bat, J. 
45. Weinberger, N.M., The neurophysiology of learning: a view 
from the sensory side. In L. Squire and N. Butters (Eds.), The 
Neurophysiology of Memory, Guilford Press, New York, 1984, 
pp. 489–503.
46. Weinberger, N.M., Ashe, J.H., Metherate, R., McKenna, T.M., 
Diamond, D.M. and Bakin, J.S., Retuning auditory cortex by 
learning: a preliminary model of receptive field plasticity, 
47. Weinberger, N.M., Ashe, J.H., Metherate, R., McKenna, T.M., 
Diamond, D.M., Bakin, J.S., Lennartz, R.C. and Cassady, 
J.R., Neural adaptive information processing: a preliminary 
model of receptive field plasticity in auditory cortex during 
Pavlovian conditioning. In L. Moore and M. Gabriel (Eds.), 
Neurocomputation and Learning: Foundations of Adaptive Net-
48. Weinberger, N.M. and Diamond, D.M., Physiological plasticity 
in auditory cortex: rapid induction by learning, Prog. Neuro-
49. Weinberger, N.M., Diamond, D.M. and McKenna, T.M., Initial 
events in conditioning: plasticity in the pupillomotor and 
auditory systems. In G. Lynch, J.L. McEachron and N.M. 
Weinberger (Eds.), Neurobiology of Learning and Memory, 
50. Weinberger, N.M. and Lindsley, D.B., Behavioral and electro-
encephalographic arousal to contrasting novel stimulation, Science, 
144 (1964) 1355–1367.
51. Weinberger, N.M. and McKenna, T.M., Sensitivity of single 
neurons in auditory cortex to contour: toward a neurophysiology 
52. Whitefield, I.C., The role of auditory cortex in behavior. In A. 
Peters and E.G. Jones (Eds.), Cerebral Cortex Association and 
53. Weis, T.N. and Hubel, D.H., Single cell responses in striate 
cortex of kittens deprived of vision in one eye, J. Neurophysiol., 
26 (1963) 1003–1017.
54. Weis, T.N. and Hubel, D.H., Comparison of the effects of 
unilateral and bilateral eye closure on cortical unit responses in 