Daily variation and appetitive conditioning-induced plasticity of auditory cortex receptive fields

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Abstract
Long-term modification of cortical receptive field maps follows learning of sensory discriminations and conditioned associations. In the process of determining whether appetitive – as opposed to aversive – conditioning is effective in causing such plastic changes, it was discovered that multineuron receptive fields, when measured in rats under ketamine-sedation, vary substantially over the course of a week, even in the absence of classical conditioning and electrode movement. Specifically, a simple correlation analysis showed that iso-intensity frequency response curves of multunit clusters and local field potentials recorded from auditory cortex are nonstationary over 7 days. Nevertheless, significant plastic changes in receptive fields, due to conditioned pairing of a pure tone and electrical stimulation of brain reward centres, are detectable above and beyond these spontaneous daily variations. This finding is based on a novel statistical plasticity criterion which compares receptive fields recorded for three days before and three days after conditioning. Based on a more traditional criterion (i.e. one day before and after conditioning), the prevalence of learning-induced changes caused by appetitive conditioning appears to be comparable to that described in previous studies involving aversive conditioning.

Introduction
In the past decade it has become increasingly clear that sensory receptive fields, and corresponding sensory-topic maps, can be modified throughout adulthood in mammals. This plasticity is believed to be adaptive such that neural resources devoted to processing sensory information can be re-allocated to those particular features of the environment, determined by past experience, to be most behaviourally relevant. Such learning-induced changes have been observed in auditory, somatosensory and visual systems (for recent reviews see Scheich et al., 1997; Buonomano & Merzenich, 1998; Edeline, 1999).

Frequency receptive fields of neurons at both cortical and subcortical levels of the mammalian auditory system can be rapidly modified by many learning paradigms, including conditioned pairing of tone and footshock (Bakin & Weinberger, 1990; Edeline & Weinberger, 1991; Gao & Suga, 1998), aversive differential conditioning (Edeline & Weinberger, 1993; Ohl & Scheich, 1996), instrumental avoidance conditioning (Bakin et al., 1996) and habituation (Condon & Weinberger, 1991). Modifications can also be induced by direct experimental manipulations, including application of pharmacological agents (Ashe et al., 1989; McKenna et al., 1989; Metherate & Weinberger, 1989; Zhang et al., 1997), conditioned pairing of tone and electrical stimulation of subcortical nuclei (e.g. Gonzalez-Lima & Scheich, 1984, 1986; Bakin & Weinberger, 1996), direct electrical stimulation of auditory cortex (Cruikshank & Weinberger, 1996; Maldonado & Gerstein, 1996; Yan & Suga, 1998) and presentation of intense acoustic stimuli (Willott & Lu, 1982; Calford et al., 1993).

Several chronic manipulations (applied for weeks) have been shown to induce long lasting plastic changes in auditory receptive fields. These include sudden (Robertson & Irvine, 1989) and progressive (Willott et al., 1993) cochlear lesions, stimulation of cochlea with implanted electrodes (Dinse et al., 1997), discrimination training (Recanzone et al., 1993) and pairing of tones with direct stimulation of nucleus basalis (Kilgard & Merzenich, 1998).

Only one short-term (< 1 h) manipulation has been demonstrated to produce long-term (up to 8 weeks) plastic changes of auditory cortex receptive fields; conditioned pairing of tone and footshock (Weinberger et al., 1993). For the present investigation we ask whether appetitive conditioning (pairing of tone and reward) has a similarly powerful and enduring effect on the receptive fields of auditory cortical neurons as this aversive conditioning paradigm. ‘Reward’ consists of direct electrical stimulation of the medial forebrain bundle (MFB) in the region of lateral hypothalamus and ventral tegmental area (Olds, 1962). As we set out to answer the above question we found that the frequency response curves (FRCs) of multunit clusters and local field potentials in rat auditory cortex vary from day to day, even in the absence of conditioned pairing. In order to interpret properly any observed modification of FRCs after training, it is important to understand the nature and magnitude of these ‘spontaneous’ daily variations. However, this phenomenon has not been closely investigated. Therefore, in the first part of this study we employ a simple correlation analysis to show that these variations do not represent random, or stochastic, variability about an otherwise stationary FRC, but instead are a progressive change of the neuronal receptive fields. In the second part we compare the relative frequency of observable receptive field modification induced by appetitive conditioning to the relative frequency of observable changes due to
these spontaneous daily variations. We argue that observed changes can be more confidently attributed to learning-induced plasticity when such a comparison to the spontaneous ‘plasticity’ is undertaken. This work has appeared previously in abstract form (Kisley & Gerstein, 1999a).

Materials and methods

Surgical preparation and implantation of chronic electrodes

All procedures were in accordance with the Institutional Animal Care and Use Committee at the University of Pennsylvania and the Guidelines of the United States National Institutes of Health for use of animals in biomedical research. Female albino rats weighing 250–350 g (~10–15-week-old) were anaesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (8 mg/kg). Periodic supplemental doses of anaesthetic were administered to maintain surgical anaesthesia during implantation of electrodes. Glycopyrrolate (0.06 mg/kg), a muscarinic antagonist, was injected subcutaneously at the beginning of surgery to reduce respiratory tract secretions. Body temperature was monitored with a rectal probe, and maintained at 37 °C with a heating pad.

Once a surgical plane of anaesthesia was reached (assessed by pedal-withdrawal reflex), the rat’s head was shaved and fixed in a stereotaxic frame. Skin, muscle, and connective tissue were removed from the top and left temporal portion of the skull. Small stainless-steel screws, to serve as recording reference and anchors for the dental acrylic cap, were inserted into drilled holes at several locations on the top of the skull. A small cranioectomy, ~1 mm in diameter, was centred 5.0 mm posterior and 1.0 mm lateral (to the animal’s right) of bregma. A twisted pair of 100 μm stainless steel microwires (California Fine Wire, Grover Beach, CA, USA) were lowered through the craniotomy and to 8 mm directly ventral from dura. This location corresponds to the MPF at the level of the lateral hypothalamus and ventral tegmental area (Nieuwenhuys et al., 1982; Paxinos & Watson, 1997). Self-stimulation through electrodes at these coordinates has been shown to cause extremely powerful ‘approach’ behaviour, with virtually no associated ‘avoidance’ behaviour (Olds & Olds, 1963). These electrodes were fixed in place with dental acrylic.

A 2 × 1 mm cranioectomy for the recording electrodes was centred at 5 mm posterior and 4 mm ventral of bregma, corresponding to Te1, left primary auditory cortex (Paxinos & Watson, 1997). After cutting the dura, a linear array of four or eight 50 μm stainless steel microwires (impedance <500 kΩ at 1 kHz; interelectrode spacing ~200 μm) was rapidly inserted into the brain, then slowly (~10 μm/min) lowered to the infragranular layers (0.8–1.0 mm below dura). Before insertion, these electrodes were orientated perpendicular to the surface of the brain. Dental acrylic was used to fix the array in place, and then to make a protective cap over the entire exposed skull and around the connectors for the stimulation and recording channels. The skin at the edges of the acrylic cap was sutured, and the animal was placed in an isolated recovery cage. Recording sessions began two weeks after surgery.

One week after surgery, and again after all recording was completed, physiological/behavioural verification of stimulating electrode placement in the intended brain reward centre was made. Specifically, rats were allowed to activate a nose-poke and consequently receive an electrical stimulus identical to that used for conditioned pairing (see below). In all cases, after no more than four trials, the animals would begin poking rapidly and repeatedly. Although they attempted to continue poking, we removed them from the cage after a maximum of 15 trials. Localization of stimulating electrodes to the intended brain areas was also visualized with gross histological examination in half of the conditioned animals. Briefly, animals were anaesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (8 mg/kg). DC current of 50 μA was passed through the stimulating electrodes for 20 s. Animals were perfused intracardially with saline, followed by 10% formalin. Brains were removed and placed in 10% formalin for 24 h, then 30% sucrose–formalin for 1 week. Frozen sections (40 μm) were examined with light microscopy (not shown).

Daily acquisition of frequency response curves

Each day the animals were sedated with just enough ketamine (~65 mg/kg i.p.) to allow head-restraint without any struggling or signs of distress. This also provided a consistent behavioural state from day to day. The precise dose differed between rats, but once an effective dose was established it was used every day for each individual animal. Care was taken to avoid using doses large enough to cause synchronized bursting of cortical neurons, which has been shown to strongly modulate evoked responses in this system (Kisley & Gerstein, 1999b). Typically, animals were just starting to move their hindlimbs at the end of the 35 min recording session. Other than head-restraint, no painful or distressing procedures took place during these recording sessions.

At 10, 20, and 30 min after intraperitoneal injection of the ketamine, iso-intensity FRCs were obtained from multiunit clusters and local field potentials recorded from the microwires chronically implanted in auditory cortex. Auditory stimulation consisted of ten pure tones, logarithmically spaced from 5–31.06 kHz. Tones were produced by computer-controlled operation of a Voltage Variable Systems model 404 sine wave generator and model 271 attenuator (Philadelphia, PA, USA). Free-field stimulation was achieved with a speaker (#40-1377, Radio Shack, Fort Worth, TX, USA) located directly in front of the rat, 10 cm from the ears (head held by connection between a 10-pin plug and connector on head). After amplification (D-75 power amplifier, Crown, Elkhart, IN, USA) and postattenuation (#31-1957, Optimus, Fort Worth, TX, USA), tone intensity at the ear was 50 dB sound pressure level (SPL), which is well above the albino rat’s behavioural threshold across this frequency range (<20 dB SPL; Fay, 1988). For this speaker, intensity variation for 50 dB tones across a 15-cm by 15-cm square region was previously determined to be ±5 dB for all frequencies (calibrated with a 6.25-mm microphone, Bruel & Kjaer, Denmark). Since the rats’ heads were fixed to a location in this sound field to within 0.25 cm each day, daily variation of sound intensity was very small. Tones were 100 ms in duration, with 5 ms rise and fall time. For each FRC, ~50 tones of each frequency were presented in random order. A total of 500 ms elapsed between the end of one tone and the beginning of the next. All experiments were carried out in a double-walled, sound-attenuated room.

Cortical signals were buffered near the head with follower circuits, then passed to preamplifier, amplifier and digital signal processing hardware (Plexon, Dallas, TX, USA). Before the preamplifier, the signal lines were split into extracellular action potential (‘unit’) and local field potential channels. Unit data were filtered between 300 and 3000 Hz, and candidate spike-waveforms crossing a fixed threshold were sampled at 40 kHz. Action potential waveforms were stored for off-line analysis. Field potentials were filtered between 0.5 and 300 Hz, and sampled continuously at 1 kHz. All signals were referenced to a ground wire attached to the skull screws.

For multiunit FRCs, response amplitude at each frequency was computed as the average number of spikes arising between 5 and
40 ms after tone onset. The multiunit cluster recorded from each electrode was considered to consist of all threshold crossings, regardless of waveform size or shape. Threshold levels were arbitrary (typically around three standard deviations beyond noise), but held fixed across days for each individual electrode. In order to ensure that we were recording from the same population of neurons from day to day, electrode stability was assessed by examining spike waveforms from the first and last days of a 7-day recording block. This was done by projecting the action potential waveforms onto the first and second principal components that had been computed from a library of action potential recordings from rat auditory cortex. This computation is expressed graphically as a scatter plot where each point represents a single spike waveform. It has been shown that electrode movement alters these scatter plots (Abeles & Goldstein, 1977; Snider & Bonds, 1998). About 5% of 7-day recording blocks were determined to be contaminated by electrode instability, and, thus, excluded from further analysis. An example of multiunit activity and two examples of stability assessment are shown in Fig. 1. Average firing rates of multiunit clusters across days were also examined to ensure that no progressive changes (which might indicate tissue damage) were occurring.

FRCs were also constructed from tone-evoked local field potentials recorded from one electrode in six of the eight rats. Response amplitude was calculated as the difference between the peak of the initial positive deflection (6–10 ms after tone onset) and the trough of the primary negative wave (18–28 ms after tone onset) of the average evoked field potential for each frequency. Subsequent analysis was identical to that carried out for multiunit clusters.

**Classical Conditioning**

At least 3 weeks after surgery, six animals were trained with a conditioned pairing paradigm. No recording took place on the day of conditioning. While freely moving about a small shielded cage the animal was presented with 30 trials – random intertrial intervals between 50 and 120 s – consisting of a 2.5 s tone (conditioned stimulus) immediately followed by 0.5 s of electrical stimulation (unconditioned stimulus) through the electrodes in the lateral hypothalamus/ventral tegmental area, contralateral to the recording electrodes in the auditory cortex. The tone was fixed at one of the ten frequencies used for generating the FRCs. The intensity of the tone was 50 dB SPL (± 5 dB, see above for calibration) throughout the wire-mesh cage. The electrical stimulation was a 200-Hz sine-wave, with a peak amplitude of ± 100 μA. The electrical stimulation parameters (duration, frequency and amplitude) are within the range of parameters previously found to be effective in eliciting self-stimulation behaviour in rats (reviewed by Olds, 1962).

All animals, within five to ten conditioned pairing trials, exhibited a clear and consistent conditioned emotional response during the conditioned stimulus (i.e. tone). This reaction, alternatively labelled ‘freezing’ or conditioned suppression, was comparable to the description of that used to verify the establishment of conditioned associations in studies of aversive conditioning-induced neural plasticity (e.g. Bakin & Weinberger, 1990; LeDoux & Muller, 1997).

In the same cage, and before conditioned pairing took place, the animal was presented with a random tone series consisting of all ten frequencies, in a manner identical to that used for acquiring the FRCs. This preconditioning acoustic stimulation was intended to allow time for habituation to the physical and auditory environments. Three of six animals experienced the conditioned pairing paradigm twice. In these instances, the conditioning sessions were separated by at least 2 weeks, and different tone frequencies were used.

In order to assess whether any observed learning-induced plasticity required both the tone and the electrical stimulation, we repeated the exact same conditioning paradigm with only tones or only MFB stimulation. These control experiments were carried out on the same animals. Half of the animals experienced conditioned pairing first, then tone-alone, then MFB stimulus-alone sessions. The other half of the animals experienced a tone-alone session first, then MFB stimulus-alone, then conditioned pairing of tone and stimulus.
Long-term analysis of frequency response curves

All FRCs presented in this report were normalized. Specifically, the amplitude of response at each frequency was divided by the mean response amplitude for all ten frequencies of a given FRC. This allowed analysis of daily fluctuation of relative, rather than absolute, response magnitude at each sound frequency (frequency selectivity, rather than overall firing rate, is the variable of interest). For daily analysis, an average of the three normalized FRCs – taken 10, 20, and 30 min after ketamine injection – was computed. For two of eight rats, recordings were made at 15 and 45 min after ketamine injection, in which case the mean curves were computed from these two time-points. To exclude frequency receptive fields that varied excessively, even during the course of a 30 min recording session, we excluded those multiunit clusters that had mean intraday correlation coefficients (i.e. between FRCs taken at 10, 20, and 30 min of the same day) below 0.8. Five of 43 multiunit clusters were removed because of this restriction.

To assess whether observed variation in FRCs was due to random fluctuation about a fixed curve or due to progressive receptive field changes we performed a simple correlation analysis on all available 7-day ‘blocks’ of tuning curves taken from the multiunit clusters and field potentials of individual electrodes. The correlation coefficient was computed between all pairs of averaged tuning curves (day 1 vs. day 2, day 1 vs. day 3, … day 2 vs. day 3, … etc.). These coefficients were plotted as a function of ‘lag’ between curves (1 day, 2 days, … 6 days), and a straight line was fit to the data. The slope of this line, hereafter referred to as the correlation slope, indicates whether a FRC was fixed or whether it was nonstationary across days; if the curve was changing then the correlation coefficient should go down as the number of days between curves increases, thus, leading to a negative correlation slope. However, if the receptive field was stationary, and exhibiting only random (i.e. stochastic) fluctuations, the correlation between any two FRCs in the 7-day block should be the same on average, leading to a correlation slope of zero. To ensure that the analysis itself was not biased towards negative correlation slopes, it was repeated using the same 7-day blocks after random scrambling (without replacement) of the daily FRCs. If not biased, the analysis should provide an estimate of zero correlation slope for these randomly shuffled data.

Learning-induced plasticity of FRCs was approached in a different manner, still using 7-day blocks. For each day of conditioning, and for each electrode, the magnitude of response at the conditioned frequency was compared between the 3 days before and the 3 days after conditioned pairing. In particular, ‘plasticity’ required that the response magnitude to the conditioned frequency increased significantly according to a two-distribution, lower-tailed t-test (P < 0.01) between the 3 pre- and postconditioning days. This analysis requires the assumption that the two sampled populations consist of FRCs recorded from the multiunit cluster before and after conditioning. In the event that three consecutive days of recorded FRCs were not available, the data from the nearest day was used (this occurred in four of 33 cases involving conditioned pairing of tone and electrical stimulation of brain reward centres).

To determine if the likelihood of observing plasticity after classical conditioning was greater than that observed during normal daily experience, the same plasticity criterion was applied to every available 7-day block recorded in the absence of conditioning. Since these data were not associated with any particular tone frequency, the number of plasticity tests performed was increased by using all ten frequencies in each FRC. To further increase confidence in the estimate of spontaneously occurring plasticity, all overlapping 7-day blocks were analysed. Assuming the resulting proportion is a reliable estimate of the likelihood of plastic changes in the absence of conditioned pairing, normal-curve approximation of the binomial distribution can be used to assess the significance of observed prevalence of plastic changes after conditioning.

Results

Of 50 functional microwires implanted in the auditory cortex of eight rats, 38 were utilized for further analysis. These 38 exhibited obvious multiunit activity (e.g. Fig. 1) and FRCs that satisfied a within-session consistency criterion (see Materials and methods). The latter requirement prevented the inclusion of frequency receptive fields that exhibited extreme variability even over the course of 30 min. Such highly variable curves were generally associated with multiunit clusters that exhibited peak onset firing rates only two or three times background rates. Peak onset firing rates of more stable tuning curves were between five and 20 times average background firing rates. Best frequencies (i.e. frequencies of maximal response for each FRC) for multiunit clusters varied across the entire range of frequencies presented (5–31.06 kHz), but were most commonly found between 6.13 and 13.79 kHz. This frequency range corresponds to the albino rat’s highest behavioural sensitivity (reviewed by Fay, 1988). Because only ten logarithmically spaced frequencies were used, best frequencies could not be precisely resolved. We occasionally observed FRCs with two clear peaks. Since these tuning curves were obtained from multiunit clusters, it could not be determined whether such double-peaks were due to contributions from two differently tuned populations of neurons, or from individual neurons with ‘multipeaked’ tuning curves (e.g. Sutter & Schreiner, 1991).

Daily variation of frequency response curves

Receptive fields of multiunit clusters were analysed in 7-day blocks. As discussed in the Materials and methods section and as illustrated in Fig. 1, if an electrode moved during the 7-day period, the data obtained from that electrode were not analysed. A total of 54 multiunit cluster (there were more 7-day blocks than electrodes because several animals were recorded from for two 7-day blocks) and nine local field potential 7-day blocks were available for analysis.

A rather extreme example of daily variation of a multiunit receptive field across 7 days is demonstrated in Fig. 2. Note that there were three different best frequencies for this multipeaked tuning curve over the course of 1 week. The plot in the upper right of Fig. 2A suggests that the three tuning curves taken within a single day were generally more consistent than tuning curves taken across days. Quantitatively only a trend was observed; the average correlation coefficient was 0.944 between curves taken during a single recording session at 10 and 20 min after ketamine injection, vs. 0.919 between curves taken at 10 min one day and 20 min the subsequent day (t-test, P < 0.1). Note that, across days, the response magnitude to some frequencies (e.g. 9.19 kHz) changed progressively and others changed more suddenly (e.g. 11.26 kHz).

To assess quantitatively whether these variations represented stochastic variability about an otherwise stationary tuning curve, or progressive changes from day to day, we employed a simple idea; if the receptive fields were nonstationary over 7 days, then the average correlation between response curves should decrease as the number of intervening days increases (i.e. they should gradually become less similar to each other). If the receptive field was fixed and the observed variation is simply due to random variability, then the average correlation between curves should be independent of the
number of intervening days. The analytical implementation of this idea is exemplified in Fig. 3 (see Methods for details). It can be seen that, for this more typical multiunit cluster during this 7-day block, there were progressive changes across days in response magnitude at some tone frequencies. As expected, the correlation between tuning curves decreased as the lag (in days) between curves increased (Fig. 3C). This is summarized by considering the slope of a line fit to the scatter-plot of correlation coefficients ('correlation slope'). A negative slope indicates a nonstationary receptive field, whereas, a slope of zero indicates random variability about a fixed curve. In this instance the slope was $-8.40 \times 10^{-3}$. A population of such slopes must be considered in order to make a statistical assessment.

The correlation slopes for all 54 multiunit clusters are shown in Fig. 4A. This histogram clearly shows that, for the population of 7-day blocks, the correlation slope was negative (one-distribution, upper-tailed $t$-test, $P < 10^{-15}$). Therefore, the average correlation between FRCs decreased as the number of intervening days increased, supporting the idea that the observed variations of receptive fields were progressive and not simply random. The mean correlation slope for all 7-day blocks was $-4.98 \times 10^{-3}$ (SD = 4.92 $\times 10^{-3}$).

Although not shown, the population of 7-day blocks taken from evoked local field potentials showed basically the same behaviour as the data from multiunit clusters. The mean correlation slope for these FRCs was $-5.30 \times 10^{-3}$ ($n = 9$, SD = 4.23 $\times 10^{-3}$). Like the population of multiunit clusters, the mean correlation slope was
An example of learning-induced plasticity is illustrated in Fig. 5. It can be seen that, for the multiunit cluster recorded from this electrode, conditioned pairing of a 6.13-kHz tone and electrical stimulation of brain reward centres produced a clear enhancement of response to the conditioned frequency. The significance of this enhancement was assessed with a lower-tailed, two-distribution $t$-test ($P < 0.01$) between the response magnitude to the conditioned frequency for the 3 days before and the 3 days after conditioning (see Materials and methods for assumptions regarding populations and sampling). In this case, the response to the conditioned frequency did increase significantly, and is, thus, considered to be ‘plasticity’. The response magnitude to the frequency immediately above the conditioned frequency was significantly decreased as a result of conditioned pairing (Fig. 5A and B). The local field potential evoked response recorded from the same electrode was also significantly enhanced at the conditioned frequency (Fig. 5C and D).

Another example of significant learning-induced plasticity of a multiunit FRC is shown in Fig. 6. In this case the conditioned frequency became the best frequency after conditioning. An adjacent frequency band was also enhanced, though not significantly. Note that the sudden and sustained change in the FRC caused by the conditioned pairing is obvious even in the presence of day-to-day variability. Although there appears to be further increase in response magnitude at the conditioned frequency from day one to day three postconditioning (i.e. putative ‘consolidation’), it is difficult to attribute this change to the training event; as shown above, FRCs often show this degree of modification simply as a result of everyday experience.

To determine whether the observed learning-induced plasticity occurred more often after conditioned pairing of tone and reward than it did during normal everyday experience, the plasticity criterion was applied to all available 7-day blocks acquired in the absence of conditioned pairing. For this analysis, day four was considered pseudo-conditioning day. Of the 2200 available $t$-tests (see Materials and methods), 2.6% exhibited significant enhancement that qualified as plasticity. Because this proportion was based on so many trials, we assumed that it reliably estimated the actual likelihood of observing plastic changes with our criterion in the absence of conditioning. Of the 33 available $t$-tests (across six rats) involving conditioned pairing of tone and electrical brain stimulation, 18.2% qualified as plasticity. The difference between these two proportions is significant (normal-curve approximation of binomial distribution, $P < 0.01$). Thus, appetitive conditioning does induce significant changes in the FRCs above and beyond that which is observed in the absence of conditioning. Incidentally, utilizing an upper-tailed $t$-test criterion – effectively considering significant decreases at the conditioned frequency as ‘plasticity’ – did not uncover a significant effect of training (3.0% after training compared to 2.6% spontaneously, $z = 0.05$).

To determine whether the induction of receptive field plasticity required both the tone and electrical brain stimulation, we repeated the conditioning paradigm but with either tones only or MFB stimulation only (half of the animals experienced these nonassociative tasks before the conditioned pairing task). For tone-alone, 6.7% of the 30 $t$-tests passed the plasticity criterion. For stimulus-alone, 2.1% of the 240 available $t$-tests were significant (normalized response magnitude at all ten frequencies was used for analysing the stimulus-alone paradigm). Neither of these proportions were significantly different from the likelihood of observing plastic changes in the absence of conditioned pairing ($z = 0.05$).

In order to compare the prevalence of plastic changes induced by conditioned pairing of tone and reward to that of tone and

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**Learning-induced plasticity of frequency response curves**

How do learning-induced modifications of frequency receptive fields compare to the changes described above which occurred in the absence of conditioning? To answer this question, FRCs of multiunit clusters were again analysed in 7-day blocks, with conditioned pairing occurring on day four. As in the previous section, only data for which the electrode did not appear to move were analysed.

**Fig. 4.** Multiunit FRCs are nonstationary over the course of 1 week. (A) Histogram of correlation slopes taken from all available multiunit cluster 7-day blocks. Dotted line indicates slope of zero, which would be expected if daily variations were simply due to random variability about an otherwise stationary FRC. Mean of distribution $= -4.98 \times 10^{-3}$, SD $= 4.92 \times 10^{-3}$. (B) Histogram of correlation slopes from the same 7-day blocks shown in A after random shuffling of the FRCs within each block. As expected, this data is centred around a slope of zero, indicating that the analysis is not biased towards generating negative correlation slopes.

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found to be significantly less than zero (one-distribution, upper-tailed $t$-test, $P < 0.001$).

In order to ensure that our correlation analysis was not biased towards negative correlation slopes, we repeated it after randomly shuffling the multiunit FRCs within each 7-day block. Randomly scrambling each 7-day block results, as expected, in a population of correlation slopes centred around zero (Fig. 4B). Since there are thousands of random permutations for the FRCs within each 7-day block, we can repeat the shuffling many times to generate a more reliable estimate of the actual mean correlation slope. Scrambling each 7-day block 200 times leads to a total of 10800 correlation slopes, the mean of which is $0.00 \times 10^{-3}$ (SD $= 5.80 \times 10^{-3}$).
punishment, the present data were re-analysed using a slightly modified version of the criterion employed by Weinberger et al. (1993). This criterion states that learning-induced plasticity has taken place if the difference between the normalized response to the conditioned frequency on the day after conditioning, and that taken just before conditioning, is greater than 0.1 for a majority of sound intensities. Whereas, our criterion involves comparison of response magnitudes for multiple days before and after learning (and, thus, allows a statistical assessment), that of Weinberger et al. (1993) utilizes responses for only 1 day pre- and postconditioning. The other important difference is that, whereas, Weinberger et al. (1993) normalized the response to the conditioned frequency by the response to the preconditioning best frequency, we normalized by the mean response strength for all frequencies in the FRC. Unlike the study of Weinberger et al. (1993) we did not record evoked neural activity for multiple tone intensities. Therefore, we cannot employ the exact same criterion. Nevertheless, applying a 1-day pre- and postconditioning criterion with normalization by the best frequency to our data, we find that conditioned pairing of tone and reward produced detectable plastic changes 36.4% of the time (n = 33). Conditioned pairing of tone and punishment (footshock) produced plasticity 45% of the time (Weinberger et al., 1993; 24 h after training, n = 20). These proportions are not significantly different at any reasonable significance level (two-proportion, binomial distribution).

It is useful to know whether the likelihood of observing learning-induced plasticity depends upon the relationship between a conditioned frequency and the particular shape of a FRC. To address this, we assessed correlation between changes in response amplitude at the conditioned frequency and (i) the preconditioning response amplitude at the conditioned frequency as compared to (and normalized by) the mean response amplitude for all frequencies; and (ii) the octave distance (and direction) between the conditioned frequency and the preconditioning best frequency. Regarding the first comparison, it was found that conditioned frequencies with relatively low response magnitudes before conditioned pairing were more likely to exhibit large learning-induced increases in response. Figure 7 shows how an increase in response to the conditioned frequency depends on the normalized response at that frequency before conditioning (averaged over 3 days). The correlation between these values was −0.59 (P < 0.001). It can be seen in this figure that the 1-day pre- or postconditioning criterion classified as plastic increases two points which actually had decreased mean responses (as computed from 3-day averages) at the conditioned frequency. Since this criterion is only based on response amplitudes one day before and one day after conditioning, daily variability can cause such misclassifications.

We did not find a similar linear correlation between the octave distance from the preconditioning best frequency to the conditioned frequency and the magnitude of observed learning-induced plasticity (correlation coefficient = 0.0091). Although a nonlinear dependence between octave distance and the size of aversive conditioning-induced changes has been demonstrated (Gao & Suga, 1998), we do not have sufficient data to determine whether a similar relationship applies to the appetitive paradigm. Finally, we note that the average activity across all frequencies of the FRCs did not change significantly as a result of appetitive conditioning (paired t-test between 3 days pre- and postconditioning, P < 0.1).
Discussion

Of necessity, the present study consisted of two parts. In the first part it was demonstrated that multiunit cluster and local field potential receptive fields in rat primary auditory cortex are nonstationary when measured daily (under Ketamine sedation) for a period of 1 week. Classical conditioning, however, was shown in part two to induce changes in receptive fields that are detectable above and beyond these spontaneous variations. The latter point was shown with two statistical tests. First, paired $t$-tests were used to compare the mean response strength at the conditioned frequency for the three days before and three days after classical conditioning. Then, a test of binomial probabilities was implemented to demonstrate that significant plastic changes, as assessed by $t$-test, occur significantly more often after classical conditioning than as a result of the spontaneous daily variations described in part one. For the first time it has been shown that conditioned pairing of an acoustic stimulus and reward produces enduring (at least 3 days) modification of auditory receptive fields, and that it does so about as often as conditioned pairing involving punishment.

Daily variation of frequency receptive fields

Before discussing the significance of the finding that receptive fields change progressively and ‘spontaneously’, it is necessary to consider potential caveats associated with our methodology. First, and foremost, this conclusion depends upon having obtained recordings from the same multiunit clusters from day to day. We ensured that only stable 7-day blocks of FRCs were analysed by assessing the relative consistency of principal component scatter plots for the action potential waveforms recorded from each electrode. It has previously been shown that electrode movement of only 10 $\mu$m can have an effect on these scatter plots (Fig. 9 of Abeles & Goldstein, 1977; see also Snider & Bonds, 1998). It is also relevant that FRCs taken from evoked field potentials varied nonrandomly over the course of a week. Since these signals represent summation over a relatively large volume (up to 1 mm from the tip of the electrode, Bullock, 1997), it is unlikely that small electrode movements could produce such significant alterations in the field potential receptive fields.

All analysis in the present report was applied to normalized FRCs. That is, for each neuron population recording, the evoked response to each tone frequency was divided by the average response to all frequencies. This manipulation was employed because the relative, rather than absolute, firing rate of a neuron (or neurons) determines pitch-selectivity, or ‘tuning’. While reducing the day-to-day random variability of the FRCs, normalization by itself does not introduce nonrandom variations. Since all frequencies in an FRC are normalized by the same value, tuning curve shape is not affected. Further, although normalization can be problematic when firing rates are very low, this should not have been an issue in the present study since...
Variability were excluded from further analysis (see Results).

It is important to remember that all signals used in the present study were taken from populations of neurons. This restriction was of necessity, not of choice. In our experience, extracellular action potential waveforms recorded acutely with microwires from rat primary auditory cortex all exhibit relatively similar shapes, making sorting by principal components analysis somewhat difficult, though quite tractable. Difficulty becomes near impossibility in chronic recordings where the signal to noise ratio is reduced (e.g. Figure. 1C and D), presumably because of gliosis around the electrode (e.g. Rousche & Normann, 1998; Turner et al., 1999). Even when sophisticated single-wire sorting techniques are applied to acutely recorded waveforms from other cortical areas of larger species, classification errors can occur (Gray et al., 1995). Although we have had significant success isolating single units from acute recordings with tetrodes, similar attempts with fixed-electrode chronic recordings have failed, perhaps because of long-term tissue damage caused by the large, twisted bundle of wires.

It might be argued that the gradual changes in receptive fields observed in the present study were due, in fact, to damage caused by the electrodes, and subsequent reorganization of the tonotopic map. This is unlikely because we observed such systematic changes even in rats that had been chronically implanted for 7 months (not shown). Further, we would expect to see changes in the principal component scatter plots of action potential waveforms if the physical health and/or organization of the cortex surrounding the electrode tip was changing during a 7-day block.

Unfortunately we cannot be certain that giving daily doses of ketamine is not in fact what caused the auditory receptive fields to be nonstationary. However, in order to be certain that learning-induced changes observed in the receptive fields were due to plastic changes, rather than simply selective attention to a particular tone frequency, it was necessary to record from a basically unconscious animal. This condition also prevented the animal’s general behavioural state from increasing the variability between days. Because anaesthesia depth can modulate sensory receptive fields in general (Duncan et al., 1982; Armstrong-James & George, 1988; Wörgötter et al., 1998; Friedberg et al., 1999) and auditory cortex evoked activity in particular (Kisley & Gerstein, 1999b), we were careful to give the same dose of ketamine every day, to record at fixed postinjection intervals and then average together FRCs taken at these time points.

Although not quantified, we observed many different types of receptive field changes. For example, some FRCs exhibited drifting best frequencies (both upwards and downwards), while others showed relatively stable best frequencies and systematically changing response magnitudes in the side-bands. Because of this, and because cortical FRCs display an incredible diversity of shapes, we chose to compare receptive fields across days with correlation coefficients rather than using more traditional auditory neurophysiology measures (e.g. characteristic frequency and bandwidth). Any type of nonrandom variation in a FRC’s shape will result in a negative correlation slope. Further studies will be necessary to understand in detail precisely what types of changes occur and their relevance for the frequency selectivity of cortical neurons and neuron populations.

The finding of nonstationary receptive fields depends upon the total duration of measurement used for the correlation analysis. It is possible that, over longer time-scales, the FRCs are basically stable. This would be the case if, for example, the receptive fields oscillate or follow a random walk on a time-scale of months. We certainly do not expect that the pitch-selectivity of a cortical neuron continues to change monotonically throughout the life of an animal. Regardless, it can be concluded from our study that detectable changes occur over the course of 24 h (or less), and they do not cycle during seven or fewer days. Although not shown, we analysed several 14-day blocks of data, and came to basically the same conclusion. Here we present data from 7-day blocks only because increasing the length of analysis block increases the likelihood of electrode instability (Liu et al., 1999).

Caveats aside, we believe the observation of nonrandom changes in FRCs was due to actual modification of the functional organization of auditory cortex receptive fields. It cannot yet be determined whether these changes happened spontaneously or as a result of the rats’ daily experiences. Nor can the behavioural relevance (if any) of these changes be appreciated from the present data. Nevertheless, these findings have implications for past and future studies of long-term receptive field plasticity. For example, analysing several days before conditioning allows a more reliable estimate of each multunit cluster’s receptive field, and its natural rate of variation. As discussed above, a criterion using only one day’s receptive fields pre- and postconditioning doubled the estimated number of multunit clusters classified as exhibiting significant plastic changes by our criterion (which is statistical and compares curves from multiple days). This suggests that the day-to-day variability of FRCs could lead to errors when estimating the prevalence of significant and detectable learning-induced changes.

Learning-induced plasticity of frequency receptive fields

Given the very strong behavioural significance associated with electrical stimulation of the MFB (Olds, 1962), we were not surprised to find that conditioned pairing involving such a reward would produce detectable changes in the receptive field of cortical neurons. Previously it has been shown that conditioned pairing of sound and stimulation of lateral hypothalamus leads to modified evoked responses in the auditory system (Woody et al., 1994). However, it was not clear until the present study whether: (i) receptive fields, in particular, can be modified by such appetitive conditioning and (ii) the modifications endure for days, as opposed to hours, beyond the training event. Our results are consistent with the idea that the modification of evoked response is due to actual plastic changes rather than selective attention (during the postconditioning measurement phase of the experiment), because the FRCs were altered even when assessed while the animals were under sedation. However, attentional modulation cannot be completely ruled out since the animals were generally beginning to move near the end of the recording period. Previous studies have shown definitive plastic changes after aversive conditioning (Lennartz & Weinberger, 1992; Weinberger et al., 1993) and discrimination training (Recanzone et al., 1993) where receptive fields were subsequently measured in fully anaesthetized animals. One very important issue that remains to be addressed – for which the present data are too scant – is the detailed relationship between the shape of an FRC before appetitive conditioning, the frequency of the conditioning tone and the resulting receptive field changes.

In order to determine whether the learning-induced changes observed in the present study required both a tone and stimulation of brain reward centres, we repeated the training but with stimulus-alone and tone-alone paradigms. Although self-stimulation behaviour has been shown to produce structural changes in several brain regions including hippocampus and cortex (Shankaranarayana Rao et al., 1999), we found that the prevalence of significant modifications in auditory cortex frequency receptive fields after stimulus-alone training was essentially the same as that after no training. Training with a pure tone in the absence of brain stimulation, however, did
produce a slight—though not significant—increase in the percentage of receptive fields that exhibited plastic changes. While still unexplained, this finding is consistent with previous studies demonstrating elevated probability of plastic changes after tone-alone training (Gao & Suga, 1998; Yan & Suga, 1998). However, as with these aversive conditioning studies, the prevalence of plasticity was clearly highest after associative training. The effects of strict ‘nonassociative’ training (i.e., presentation of both tones and brain stimulation during a single session but without temporal pairing) were not assessed in this study.

From the present results we cannot determine the exact mechanism of appetitive conditioning-induced plasticity of auditory receptive fields. Further experiments are required to decide whether this type of learning-induced plasticity involves some or all of the same neural circuits, still under debate, believed to be responsible for aversive conditioning (LeDoux & Muller, 1997; Gao & Suga, 1998; Weinberger, 1998). In addition, it is too early to tell whether conditioned pairing of tone and reward in general, rather than pairing of tone and direct electrical stimulation of the brain in particular, is the necessary element for inducing plasticity. The MFB contains fibers from many different modulatory brain centres (Nieuwenhuys et al., 1982), some of which might be directly or indirectly involved in learning-induced plasticity of cortical receptive fields (e.g. cholinergic nuclei of the basal forebrain: Bakin & Weinberger, 1996; Kilgard & Merzenich, 1998).

Regardless of the precise mechanism of learning-induced plasticity, we would ultimately like to characterize the behavioural relevance of the additional neural resources apparently devoted to processing stimuli at the frequency of the conditioning tone after the pairing described in this and previous reports. A recent study from our laboratory (S. K. Talwar & G. L. Gerstein, unpublished observation) showed that expansion of a given sound frequency’s representation by intracortical microstimulation does not improve an animal’s frequency-discrimination abilities (but other abilities associated with cortical function including spatial-discrimination were not assessed). Is the same true of plasticity evoked with this more behaviourally relevant appetitive conditioning paradigm? The answer to this question has implications for the physiological basis of perceptual learning, and perhaps, for the future of noninvasive compensatory treatment of individuals with selective brain damage.

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Abbreviations
FRC, frequency response curve; MFB, medial forebrain bundle.

References


