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Acetylcholine produces stimulus-specific receptive field alterations in cat auditory cortex

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Frequency receptive fields (RFs) were determined before and after pairing iontophoretic administration of acetylcholine (ACh) with a repeated single-frequency stimulus in the auditory cortex of barbiturate-anesthetized cats. In 58% of the cells, the paired ACh + tone treatment produced subsequent alterations of frequency RFs. In half of these cases, the RF modifications were highly specific to the frequency that had been paired with ACh. Atropine antagonized the frequency-specific effects of ACh, suggesting that they were mediated via muscarinic cholinergic receptors.

Plasticity of receptive fields (RFs) in sensory cortex can be produced during various forms of experience. Visual cortical RFs can be altered during manipulations of visual input[18] as well as by selective attention[11]. Somatosensory cortical RFs are also alterable by attention[4,12] and a variety of peripheral manipulations[7,13]. In the auditory cortex, RFs may be dependent on behavioral state[10], and previous studies from this laboratory have shown that alterations of auditory cortical RFs that develop during classical conditioning[17] are highly restricted to the frequency of the conditioned stimulus (CS)[2]. While phenomena of sensory cortical plasticity are well documented, the neuromodulatory mechanisms underlying these processes remain unclear. We are investigating the role of acetylcholine (ACh) in auditory cortical plasticity in light of its known influence on learning processes[16] and on other forms of neuronal plasticity in sensory cortex[1,9].

During iontophoretic administration of ACh in sensory cortex, responses of single neurons to visual[14,15], somatosensory[3,8] and auditory[6] stimuli are generally facilitated. These effects are largely mediated via muscarinic cholinergic receptors[3,6,8,14]. Modulation of sensory responses can outlast the application of ACh by tens of minutes[3,9,15] provided that it is administered simultaneously with sensory stimulation rather than sequentially[9]. Still unknown is whether ACh produces a prolonged general change in excitability or whether its long-lasting effects specifically alter processing of the sensory stimulus with which it was simultaneously 'paired' To answer this question, we restricted sensory input in the presence of ACh to a single tonal frequency and determined frequency RFs before and after this treatment.

Adult cats were prepared for multiple recording sessions during an initial surgery by the placement of skull screws and an acrylic pedestal on the skull. This permitted fixation of the head and access to the bone overlying auditory cortex during each session. Both the initial surgery and subsequent recording sessions were conducted using pentobarbital sodium anesthesia (Nembutal; 35 mg/kg initially, supplemented as needed to maintain areflexia). During each session, a burr hole was drilled in the bone overlying auditory

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cortex, the dura was cut, and a 7-barrel micropipette inserted. The central recording barrel contained 1 M NaCl and the outer drug barrels contained ACh chloride (1 M, pH 4.5), sodium glutamate (0.5 M, pH 8), atropine sulphate (25 mM in 0.155 M NaCl, pH 5) and 1 M NaCl for current balance (all drugs from Sigma). Drugs were ejected using a 6-channel iontophoresis unit (Medical Systems) and were retained by 10 nA currents of the appropriate polarity. A computer-controlled acoustic delivery system produced pure tones via a frequency generator (Wavetek 114; sine wave distortion <1.0% from 1 Hz to 100 kHz) and calibrated earphone (Aiwa) positioned within the auditory meatus contralateral to the recording site. This system produced isointensity tones when measured at the speaker. The acoustic calibration and standard electrophysiological recording techniques used have been described previously[6].

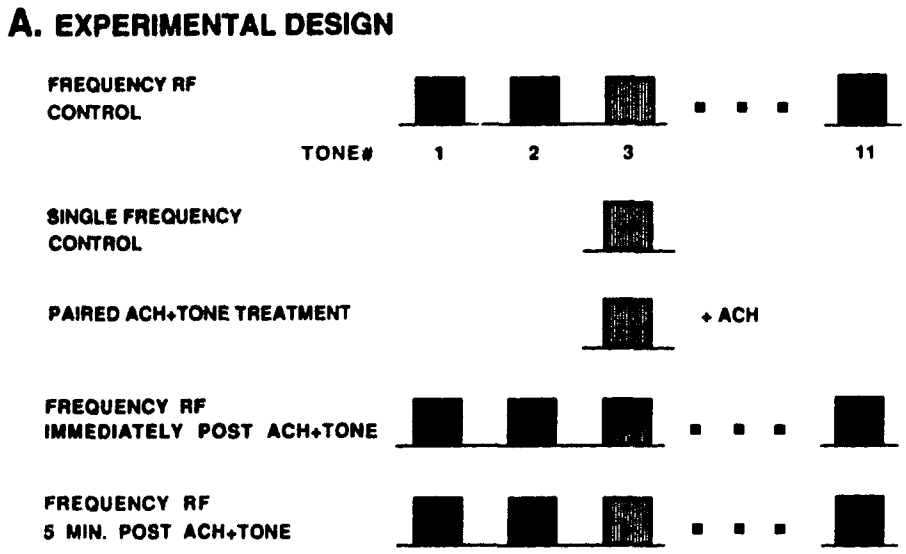
A laboratory computer (PDP 11/73) stored the times of occurrence of individual action potentials and later was used to construct peristimulus time histograms and to count the number of discharges during each tone and during the 300 ms period immediately preceding each tone. A cell's frequency RF was determined by the response (spikes/s) evoked by each of eleven 200 ms tones (see Fig. 1A) with the spontaneous activity (spikes/s) in the 300 ms preceding each tone subtracted from each response. Changes produced in the cell's RF subsequent to pairing ACh with the single-frequency tone were quantified by subtracting the control RF tone responses from the respective tone responses in the post-ACh RF. The resulting difference score (i.e. post-ACh tone response minus control tone response) for each of the 11 tones comprised a difference function showing the change from control across frequency. To permit comparisons among cells, each cell's difference function was normalized by dividing each tone's difference score by the absolute value of the difference score for the tone that exhibited the greatest change from control, and multiplying the result by 100%. Thus, the normalized difference function comprised values determined by applying the following formula to each tone response in the RF:

$$\frac{\text{difference score for tone response}}{\text{absolute value (maximum difference score in RF)}} \times 100\%$$

When the discharge of a single neuron was isolated in auditory cortex by the advancing microelectrode, its frequency RF was determined using a sequence of 11 isointensity tones as illustrated schematically in Fig. 1A. Next, the cell's response to a single frequency was determined. ACh was then administered iontophoretically and auditory stimulation using the same frequency was repeated. No systematic differences were observed between using continuous ACh current and 200 ms ACh current pulses, either during or immediately preceding each tone burst. In accordance with previous findings from auditory cortex[6], ACh generally produced excitatory effects during pairing with a single frequency; these data will be presented elsewhere. The RF was re-determined following application of ACh. Fig. 1B displays the magnitudes of altered responses for all cells, separately for paired and non-paired frequencies, within 5 min of pairing ACh and a tone. These distributions are significantly different ($\chi^2 = 53.72$, df 10, $P < 0.001$), indicating that during the post-ACh determination of the RF, responses to tones previously paired with ACh were altered differently than responses to non-paired tones. The difference is attributable to the fact that a disproportionate number of cells ($n = 22$, 24%) developed the greatest decrease in response (−100%) at the frequency paired with ACh (Fig. 1B,*). In other words, the effect of ACh on some cells was to produce the greatest decrease in response to the paired frequency with lesser changes to non-paired frequencies (i.e. a frequency-specific decrease). Less frequently observed changes in RFs were frequency-specific increases ($n = 5$) and general changes in excitability indexed by increased ($n = 16$) or decreased ($n = 10$) responses across a broad range of frequencies; these will be presented elsewhere.

Two examples of cells that developed frequency-specific decreases in their RFs are shown in Fig. 2. In Fig. 2A, pairing ACh with a 5 kHz tone essentially abolished the response to that frequency. This decrease was restricted to the paired frequency; in contrast, adjacent frequencies developed enhanced responses. This RF alteration persisted for at least 20 min after pairing ACh with the tone. The duration of frequency-specific RF modifications ranged from 5 to over 20 min.

Although ACh did not generally change the size of



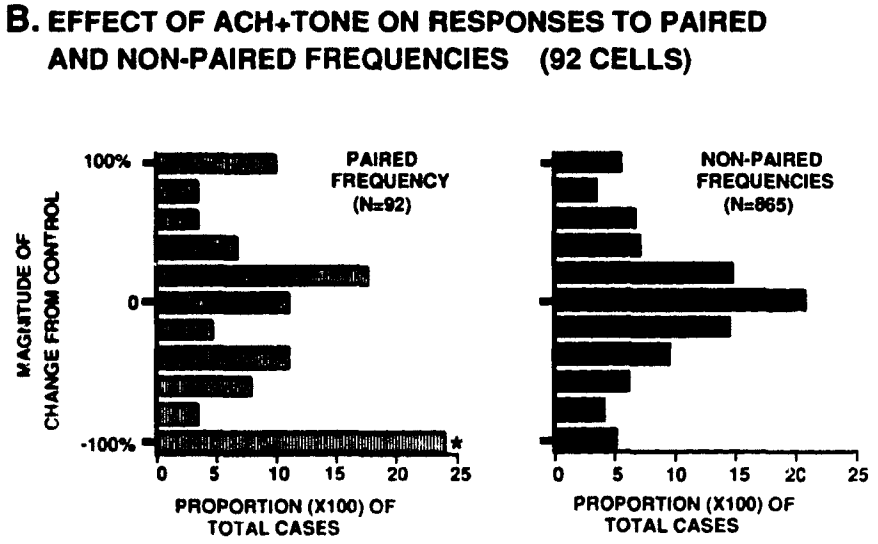


Fig. 1. A: the frequency RF of a single neuron was determined by its response to an ascending sequence of 11 isointensity pure tones (200 ms duration each, 600 ms between tones), here illustrated schematically. These sequences were selected so that tones spanned the cell's response range in 0.5, 1 or 2 kHz steps. The sequence was repeated 20 times (total 3 min duration). Next, a single, generally non-best, frequency to which the cell responded (tone 3 in the sequence illustrated above) was presented alone (25 repetitions, tone duration 200 ms, 1.3 s between tones, total 45 s duration) and then presented during iontophoretic administration of ACh (20–100 nA). The cell's frequency RF was re-determined when the ACh current was turned off, and at 3–5 min intervals subsequently. The data obtained during these procedures consist of spontaneous and tone-evoked discharges. For each tone, the preceding spontaneous activity was subtracted from the evoked response. B: distribution of normalized difference scores obtained for the tones paired with ACh (range 0.5–32 kHz) as well as the combined distributions (not significantly different from each other, $P > 0.1$) of responses to all non-paired frequencies (range 0.5–30 kHz). For each cell, the tone response displaying the maximum change from control had a normalized difference score of ± 100% (for the maximum change being an increase or decrease, respectively); lesser changes to other tones were scaled to this value, and responses not different from control had a score of 0%. The two distributions are significantly different ($\chi^2 = 53.72$, df 10, $P < 0.001$) due to the high number of cells in which the maximum change from the control RF was a decreased response at the paired frequency (*). Without the −100% bins the distributions are not significantly different: $\chi^2 = 15.99$, df 9, $P > 0.05$.

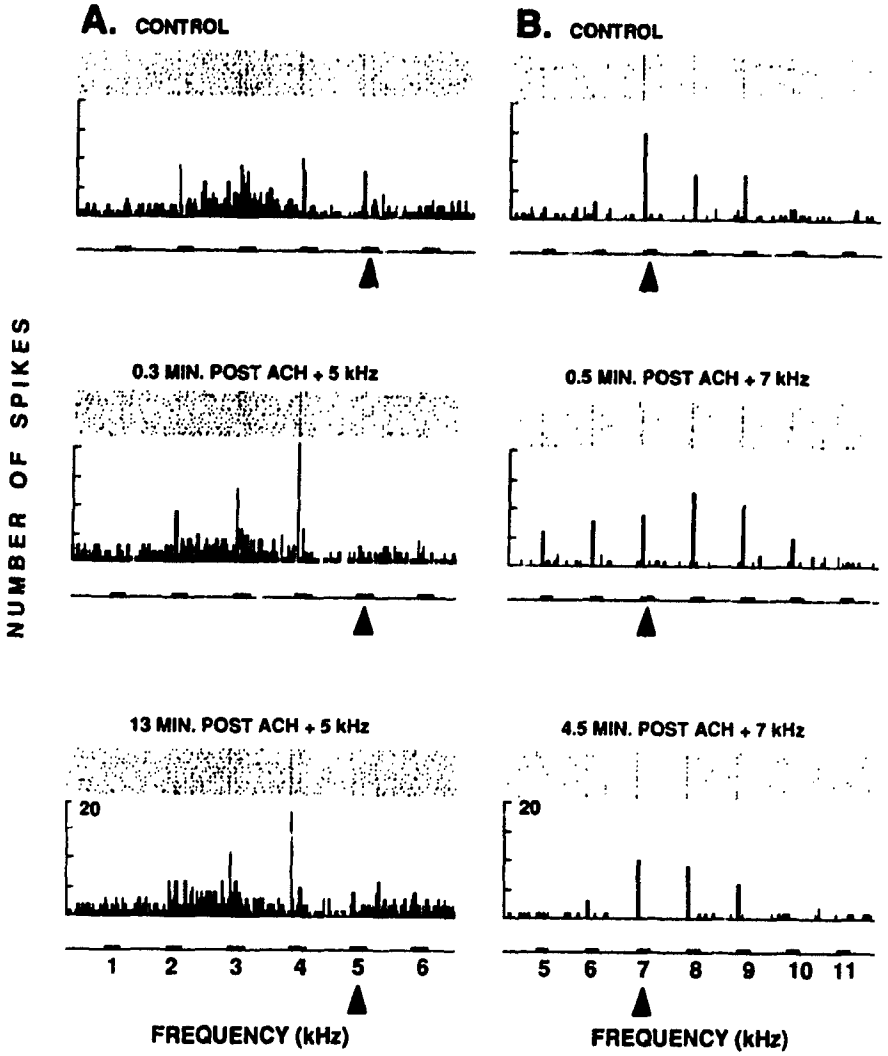


Fig. 2. Record for two cells showing frequency-specific receptive field changes produced by pairing ACh administration with a repeated, single-frequency tone. A: control receptive field delineated by a sequence of 11 isointensity tones (90 dB intensity at the earphone) ranging from 1 to 11 kHz in 1 kHz steps (only 1–6 kHz shown here, no response from 6–11 kHz). Raster plot above histogram depicts responses during each of 20 trials. The cell responded above spontaneous levels to tones from 2–5 kHz. When ACh administration (35 nA) was paired with a 5 kHz tone, the evoked response was enhanced with no change in the level of spontaneous activity (not shown). Immediately following the ACh application, a second RF determination revealed a greatly decreased response to the paired tone (5 kHz, arrowhead) and enhanced responsiveness at the immediately adjacent 4 kHz tone. The frequency-specific effect was observed at 5 and 10 min intervals after ACh (not shown). At 13 min (A, bottom) the effect of the paired treatment on the RF was still present, although beginning to dissipate. Complete recovery was not observed even 20 min after ACh. B: another cell responded to tones from 7 to 9 kHz in the control situation (stimulation at 80 dB). After pairing a 7 kHz tone with a 30 nA dose of ACh, the post-ACh RF displayed a decrease in response to the paired frequency and an increase in adjacent frequency responses. These effects were accompanied by a RF expansion so that the range of responsive frequencies approximately doubled. Note also that the best frequency shifted from 7 to 8 kHz during this process. Near complete recovery was seen 4.5 min after the ACh treatment with 7 kHz again being the best frequency and the RF returning to its control size. Bin width is 10 ms in A and B.

a cell's RF, such changes did not preclude frequency-specific effects. Despite an increase in RF size following the pairing of ACh with a 7 kHz tone (Fig. 2B), the frequency-specific decrease is evident in the response to 7 kHz combined with an increase in responses to adjacent frequencies.

To determine the profile of change in RFs for frequency-specific decreases, changes in response were averaged as a function of distance (in kHz) from the paired frequency. Fig. 3A reveals both the magni-

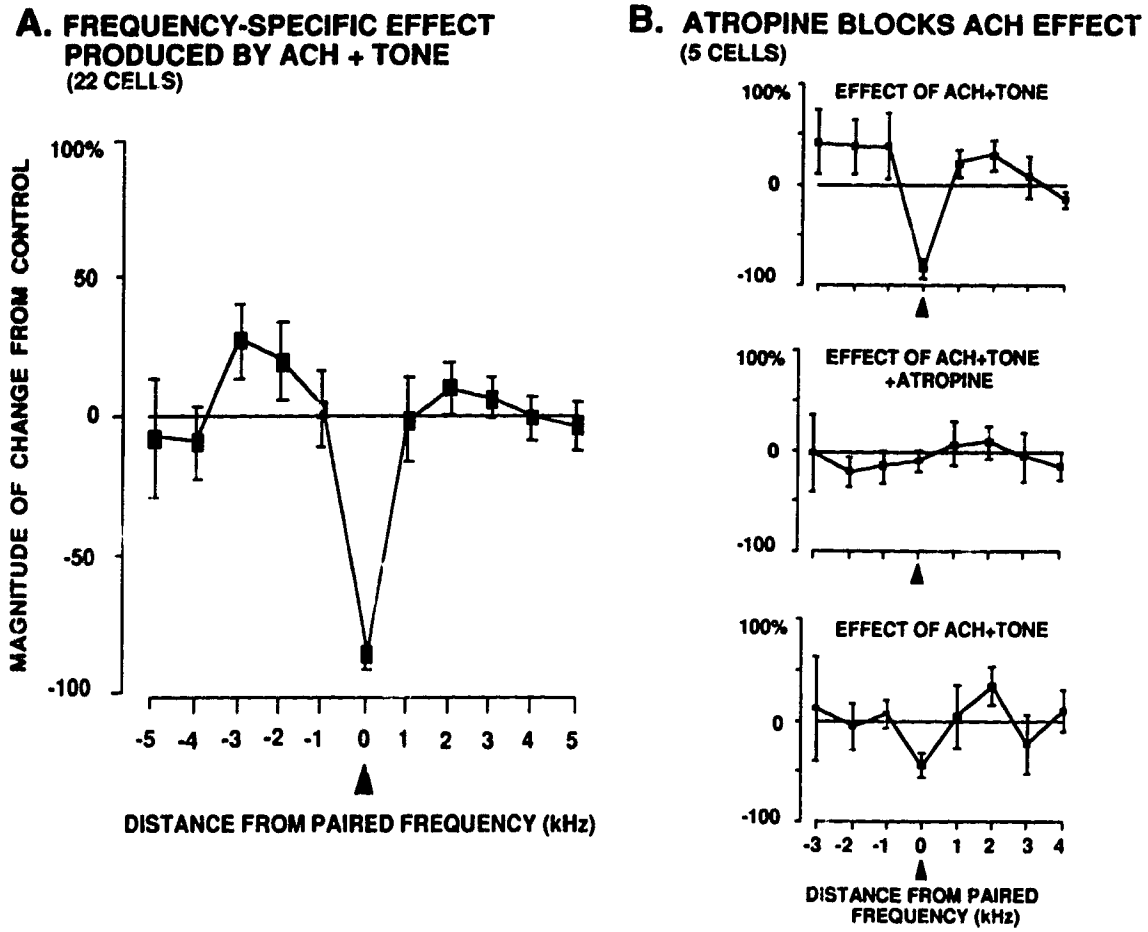


Fig. 3. A: mean normalized difference scores (± S.E.) of 22 cells displaying frequency-specific decreases in responsiveness at the paired frequency (arrowhead) within 5 min following the pairing of ACh with a tone. RF changes for each cell were aligned so that the paired frequency difference score was plotted at '0' and difference scores for the other, non-paired frequencies were plotted in 1 kHz steps from the paired tone. These scores were then averaged for the 22 cells. In addition to the decrease that is highly specific to the paired tone, enhancement of responses to nearby frequencies is also seen, although the magnitude of this effect and the range of frequencies affected varied among cells. Each data point it the mean of 22 values or fewer, since some cells were not responsive up to ± 5 kHz from the paired frequency, and some cell's RFs were sampled in 2 kHz steps. (Actual values from left to right, n = 6, 10, 10, 16, 18, 22 (paired), 17, 22, 16, 20, 14). B: frequency-specific effects of pairing ACh with a tone are blocked by atropine administered iontophoretically. The average, normalized difference curves for 5 cells under these conditions show that the frequency-specific decrease and enhancement of responses to adjacent frequencies due to ACh are blocked by the muscarinic antagonist atropine (mean dose 59 nA). Partial recovery can be seen afterwards (mean 15.9 min post-atropine). Each data point is the mean (± S.E.) of 3–5 values.

tude and degree of specificity of the effect. The average decrease at the paired frequency is large (–87%) whereas responses 1 kHz away from the paired frequency are at control levels. Although responses to frequencies close to the paired tone were often increased (Fig. 2), the extent and magnitude of this sideband enhancement varied among cells and is thus only moderate in the averaged data. Frequency-specific effects were attributable to the pairing of ACh and tones, since they were evident neither following the mere passage of time nor following acoustic stimulation alone.

Evidence that the frequency-specific decrease is mediated via muscarinic receptors is presented in Fig. 3B. Atropine blocked the frequency-specific decrease and also the sideband enhancement produced by ACh. As we observed unchanged waveforms and unchanged discharges to acoustic stimulation following atropine + ACh (Fig. 3B, middle), the blockade was not due to an 'anesthetic' effect of atropine.

Also, atropine blockade shows that the frequency-specific depression could not be due merely to repetition of the single-frequency tone.

These data show that following single-frequency stimulation in the presence of ACh, cortical RFs can be altered in a manner highly specific to the paired frequency. A dramatic decrease in response occurs at the paired tone, often accompanied by enhanced responses at other, nearby frequencies. The mechanisms involved in this phenomenon remain to be determined, but since active neuronal elements are more likely to be modified during ACh administration than inactive ones[9,19] cholinoceptive inhibitory interneurons[5] activated by both the single tone and ACh during the paired treatment may be involved in the lasting decreased responsiveness to the paired frequency. The enhancement at other, non-paired frequencies could be a more general postsynaptic effect, and the observation that frequency-specific decreases also occur without sideband enhancement (unpublished findings) suggests that separate mechanisms underly the two effects.

Thus, stimulus-specific RF alterations can be produced subsequent to acoustic stimulation in the presence of ACh. Frequency RF alterations that are specific to a behaviorally meaningful tone have been observed in the auditory cortex of cats during learning[2,17], and other RF changes in sensory cortices have been attributed to changes in attention[4,10-12]. Further studies are needed to examine the role of ACh in these and other forms of sensory cortical RF plasticity.

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