Muscarinic dependence of nucleus basalis induced conditioned receptive field plasticity

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Receptive field (RF) plasticity in primary auditory cortex of adult animals, specifically selective increased response to a tonal conditioned stimulus (CS) relative to other frequencies, can be induced both by behavioral conditioning and by pairing a tone with stimulation of the nucleus basalis (NB). This study determined whether cortical muscarinic receptors are necessary for NB-induced RF plasticity. Single units in layers II–IV were studied in Urethane anesthetized adult rats. The cortex was perfused with saline or saline + atropine sulfate. Conditioning, 30 trials of pairing a tone with NB stimulation, produced a significant CS-specific response increase ($n = 8$). Local atropine blocked NB-induced RF plasticity, actually resulting in CS-specific response decrease ($n = 6$). Therefore, NB-induced RF plasticity requires engagement of muscarinic receptors in auditory cortex. *NeuroReport* 12:1537–1542 © 2001 Lippincott Williams & Wilkins.

Key words: Atropine; Cholinergic system; Learning; Memory

INTRODUCTION

Learning systematically alters frequency receptive fields in the primary auditory cortex. For example, following classical conditioning, responses to the frequency of the tonal conditioned stimulus are increased relative to responses to other frequencies [1]. This receptive field plasticity has all of the characteristics of major forms of memory: it is associative, highly specific, discriminative, rapidly developing, retained for long periods, and exhibits consolidation [2]. Receptive field plasticity at a local cortical level was predicted to yield an increased representation of the conditioned stimulus frequency across auditory cortex [3], which was subsequently found [4].

In 1990, a cholinergic model hypothesized that cortical memory storage, indexed by RF plasticity, is normally induced by appropriately timed pairing of a tone with an unconditioned stimulus (e.g. shock), activating the nucleus basalis which in turn releases acetylcholine (ACh) that acts through cortical muscarinic receptors [3]. Consistent with this model, pairing a tone with direct stimulation of the nucleus basalis induces receptive field plasticity similar to learning-induced plasticity [5–8].

However, mechanisms of plasticity induced by stimulation of the nucleus basalis are not yet known. Atropine applied to auditory cortex blocks the EEG desynchronization caused by NB stimulation that induces RF plasticity [5]. Congruously, NB stimulation produces RF plasticity only if it also produces EEG desynchronization, suggesting that ACh release is necessary for the induction of the plasticity [7]. Cholino-toxic NB lesions block NB-induced functional reorganization of the auditory cortex [8]. These findings suggest that associative RF plasticity induced by pairing a tone with NB stimulation requires ACh release and engagement of muscarinic receptors in the auditory cortex. This experiment tested the hypothesis.

MATERIALS AND METHODS

Surgery: Male Sprague–Dawley rats ($n = 12$; weight 413 ± 78 g, mean ± s.d.; Charles River) were anesthetized with urethane (1.6 g/kg, i.p.; Sigma, St Louis, MO). Major surgical, electrophysiological, and histological techniques were similar to those described elsewhere [5]. The head was affixed to a support by attachment to a skull pedestal of dental cement and ear bars were removed. All procedures were performed under sterile conditions in accordance with standards for the care and use of laboratory animals established by the NIH.

A calibrated speaker was fixed at the entrance to the left ear canal. Dura was removed around the contralateral auditory cortical surface, which was perfused with warmed (37°C) saline or artificial cerebrospinal fluid (ACSF) at the rate of 0.15–0.3 ml/min. A concentric bipolar stainless steel stimulating electrode was lowered (AP −2.3, L 4.2) toward the nucleus basalis at a 45° angle in the frontal plane, while bipolar electrical stimuli (0.2 ms pulses, 200–500 μA, 100 Hz, 100–600 ms) [9] were delivered via stimulus isolation units. The electrode was advanced until EEG desynchronization could be consistently observed from an epipial electrode in auditory cortex. A microelectrode (2–3 MΩ) was lowered until a cluster of neurons with a signal-to-noise ratio of at least 3:1 was isolated (Fig. 1a). Recordings were made in layers II–IV (300–10 000 Hz,

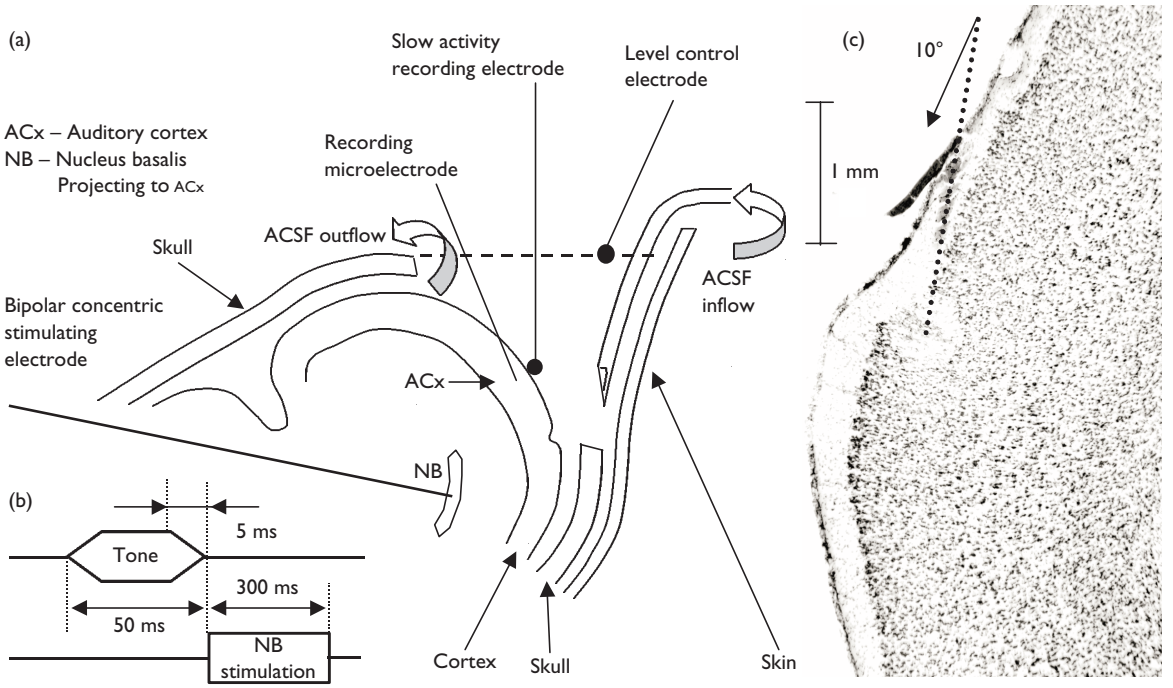

Fig. 1. (**a**) A schematic of the experimental setup. (**b**) Graphic representation of a single trial of the conditioning protocol. (**c**) A photomicrograph showing a marking lesion made of a recording site in layers II/III following an experiment. The dotted line indicates the electrode's trajectory at a 10° angle, ending at the point of the electrolytic lesion.

×10 000) because the perfusion solution was more likely to reach cells in these layers than cells in the infragranular layer (Fig. 1c).

Experimental protocol: There were two groups, conditioning (COND) and conditioning plus atropine (COND + ATS). For both groups, RFs were determined before and after conditioning by presentation of isointensity ascending sequences of 15 pure tone bursts (frequency steps 1/32 to 1/8 octaves, duration 100 ms, rise/fall time 5 ms, inter-tone interval 0.55 s, intensity 50–90 dB) delivered 10–20 times at 2 s intervals. Frequencies were selected to minimally span each cell's RF, and thus varied across recording sites. All stimulus parameters for a cell were identical before and after conditioning. The intensity chosen was based on its ability to elicit significant ($p < 0.05$, *t*-test) responses relative to the level of preceding background activity to several of the test frequencies. RFs were re-determined every 10 min (2–10 times).

A frequency that was not the best frequency (BF) was selected to serve as the conditional stimulus [5]. Subjects received 30 trials of the CS (50 ms, rise/fall time 5 ms, same intensity as RF determination, inter-tone interval 60 ± 5 s) paired with NB stimulation, delivered at tone offset (Fig. 1b). Five to ten minutes post-conditioning, RFs were collected 4–17 times (40–170 min post-conditioning). The detailed duration of post-training effects was not analyzed in this acute study because chronic studies have shown at least 24 h retention [7]. Several postconditioning RFs provided ample opportunity either to observe RF plasticity in the COND group or ascertain that RF plasticity did not develop in the COND + ATS group. More RFs were obtained in the latter group (1 3.7 ± 2.6) than in the COND group (9.0 ± 4.5), increasing chances of detecting plasticity under atropine, thus avoiding a false negative conclusion.

The COND and COND + ATS groups were studied under identical conditions except that the latter group had the addition of the muscarinic blocker atropine sulfate (ATS, 140–420 mg/kg, 0.21–0.62 mM) in either saline or ACSF. Because state differences can affect expression of ACh-dependent sensory cortical plasticity, ATS was applied during conditioning and postconditioning periods to preserve the same cortical state during the acquisition and retention phases [10]. The continued presence of ATS following pairing does not affect the expression of plasticity once it has been formed [11]. In both groups, a quiet period of 5–10 min preceded the start of conditioning, to allow time for ATS diffusion. A relatively high ATS concentration was used to promote effective concentration of the drug in the cortex.

Data analysis: Spike waveforms were separated with cluster analysis. The mean discharge rate in response to each frequency was used to construct frequency receptive fields. To determine the effects of a treatment, the data were normalized to control for overall differences in response to different tones within a cell before conditioning and to allow comparison of average changes between groups despite differences in the absolute levels of response. Normalization consisted of dividing the post-conditioning responses by the preconditioning responses,

on a tone-by-tone basis, providing an index of the relative change independent of absolute change.

The average discharge rates during presentation of the CS frequency and the frequencies immediately lower and higher were pooled for all tuning functions separately for preconditioning and post-conditioning. The ratios for each frequency were then calculated (postconditioning/preconditioning). For the CS frequency, this ratio is referred to as H2. The ratios for the adjacent lower and higher frequencies were averaged, yielding a value referred to as H1. The response to the CS relative to the average of the two adjacent frequencies was determined as a new ratio, H2/H1, the index of specific relative change. A value of 1.0 indicates no difference; larger and smaller values indicate increases and decreases in response to the CS relative to its adjacent frequencies, respectively. The magnitude of this specificity index was also analyzed with respect to the relative frequency distance between the conditioned stimulus and the best frequency (RD = the CS–BF distance as a fraction of the total receptive field size where each receptive field size is normalized to 1.0). Electrode sites were determined by locating electrolytic lesions (Fig. 1c) made after each experiment, preceding sacrifice and cardiac perfusion.

RESULTS

General characteristics: Included subjects had to have effective stimulation of the NB, i.e. 3–9 s (4.36 ± 1.50 s) of EEG desynchronization. The average stimulation level for these subjects was 378 ± 125 μA (range 200–500 μA). Single cells all exhibited statistically significant discharge rate increase in response to at least 3 (up to 11, 7.8 ± 2.6) of 15 frequencies in the RF. Fourteen single cells meeting this criteria were obtained from 12 subjects (COND = 8; COND + ATS = 6). Histological examination showed no difference in electrode depth ($p > 0.80$, two-tailed *t*-test) or in the distribution of cells in layers II, III or IV. There were no differences between groups in the sites of the NB stimulating electrodes ($p > 0.25$).

Cells in this study exhibited marked onset responses followed by continued tonic discharges at a lower level (Fig. 2). The COND and COND + ATS groups did not differ functionally before the conditioning treatment, based on measures of response to the BF: onset latency (11.9 ± 6.5 ms and 19.2 ± 15.3 ms, respectively; $p > 0.30$, two-tailed *t*-test), response duration (88.1 ± 41.0 ms and 75.0 ± 25.2 ms, respectively; $p > 0.45$) or response discharge rate (93.2 ± 51.3 imp/s and 125.2 ± 56.0 imp/s, respectively; $p > 0.25$). There was no difference in the relative distance between the BF and CS frequencies (0.221 ± 0.124 and 0.266 ± 0.096, respectively; $p > 0.45$).

Effects of conditioning and atropine: Conditioning produced CS-specific relative increased responses. For the example given in Fig. 2, after pairing 15.0 kHz (70 dB) with NB stimulation (300 ms, 400 μA), responses at many frequencies increased. However, responses to the CS frequency of 15.0 kHz increased relatively more than did responses to 14.5 and 15.5 kHz, its two adjacent frequencies. The results of quantitative analyses of the average discharge rates during the entire tone duration for this cell are presented in Fig. 3a,b. Figure 3a shows that conditioning did not change the pre-training BF (16.0 kHz), but significantly increased responses to the CS (15.0 kHz; $p < 0.001$; *t*-test) and frequencies around it (14.0, 14.5, 15.5 and 16.0 kHz, $p < 0.001$; 17.0 kHz, $p < 0.002$). The specificity of response enhancement is highlighted in Fig. 3b. Following conditioning, the greatest relative increase within the RF was at the CS frequency, to 189% of preconditioning; the BF increased only to 153%. The H2/H1 ratio (see Materials and Methods) for this cell was 1.12, i.e. enhancement of the response to 15.0 kHz (CS) was 12% larger than enhancement of the average responses for 14.5 and 15.5 kHz.

Seven of the eight cells developed a CS-specific increase in their H2/H1 ratios, i.e. a value > 1.0. To determine if the specificity of NB-induced plasticity depends on the relation between the frequencies of the conditioned stimulus and the best frequency, we calculated the correlation between the relative CS-BF distance and the magnitude of the conditioned H2/H1 response ratios in the COND group. This relationship was statistically significant and is best characterized by a parabolic function ($r_{xy} = 0.77$, $p < 0.05$, two-tailed test; Fig 3e). The top of the curve corresponds to the relative CS–BF distance of 0.27, yielding at that point an H2/H1 ratio of 1.12 (i.e. 12% increase). The numerical solution of the parabolic equation yielded the relative distance (RD) range of 0.17–0.35 within which one should expect the occurrence of the top 5% of the H2/H1 ratios. Six cells in the COND group happened to lie within that range. They developed a significant CS-specific rise in response to 110.9 ± 7.2% (i.e. 10.9% increase, $p < 0.02$, two-tailed *t*-test).

The COND + ATS group did not develop CS-specific increased responses. Figure 3c shows an example of the effect of ATS on pairing a tone (8.0 kHz, 70 dB) and NB stimulation (300 ms, 500 μA). There was no frequency-specific increase but rather a decrease in response to the CS ($p < 0.002$) and to nearby frequencies: 3 kHz ($p < 0.01$), 4 kHz ($p < 0.0002$), 5 and 6 kHz ($p < 0.002$), 7 kHz ($p < 0.05$) and 12 kHz ($p < 0.001$), relative to the preconditioned responses. Responses to frequencies outside the RF for this neuron (< 3 kHz) were unaffected. The decline is seen more clearly in Fig. 3d. Following conditioning, there was a 52% decline in response to the CS frequency. The H2/H1 ratio was 0.73, indicating a greater decrease for the CS frequency than for the adjacent frequencies (i.e. a frequency-specific decrease). Five of the six cells exhibited an H2/H1 ratio < 1.0 (Fig. 3e).

In contrast to the COND group, there was no correlation between the relative CS–BF distance and the magnitude of changes of H2/H1 response ratios in the COND + ATS group. (Fits of linear, exponential and parabolic functions all were insignificant, $p > 0.05$.) Four cells in this group fell within the COND group's optimal RD range (Fig. 3e). They showed a significant decline in response, to 79.1 ± 15.5% in H2/H1 ratios ($p < 0.05$, one-tailed *t*-test). Therefore, while the two groups had cells within the same range for maximum CS-specific effects, only the COND group developed specific facilitation of response while ATS actually produced a CS-specific decrease.

The COND and COND + ATS groups exhibited significant opposite CS-specific changes following conditioning. Statistical tests were conducted both for the total number

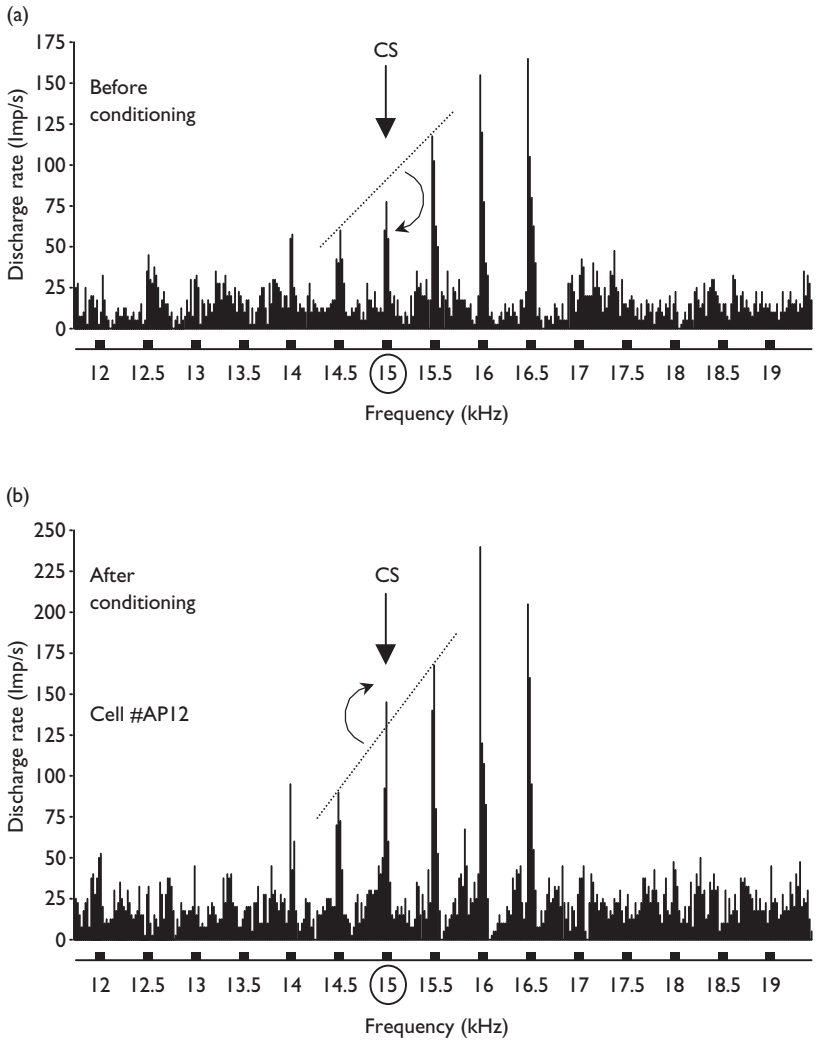

Fig. 2. Examples of peristimulus time histograms (PSTH; 20 ms bins) for a cell from the COND group. (**a**) before and (**b**) after pairing. The CS frequency was 15.0 kHz. The dotted lines in (a) and (b) connect the peaks of responses to the adjacent lower (14.5 kHz) and higher (15.5 kHz) frequencies. Indication that pairing the CS with NB stimulation produced a CS-specific facilitation of response is provided by noting the relationship of the peak response to the CS frequency to the dotted line: it is below the line before pairing but above the line after pairing (curved arrows). However, quantitative analyses were based on the number of discharges throughout the duration of tones, not merely the peak responses (see text). Number of tone repetitions for each PSTH was 20.

of cells in each group and for those cells lying within the optimal RD (Fig. 3f). For the total number of cells, the H2/H1 ratios between groups differed significantly ($p < 0.03$, two-tailed t-test). The H2/H1 ratio for the COND group showed an increase to 1.074 ± 0.093 ($p < 0.05$, one-tailed t-test, $n = 8$) while it decreased for the COND + ATS group to 0.857 ± 0.170 ($p < 0.05$, one-tailed t-test, $n = 6$). For cells falling within the optimal RD, increases for the COND group were naturally larger, but decreases for the COND + ATS group were also larger. The difference between groups was significant ($p < 0.02$, two-tailed t-test). The overall differences between the groups were 21.7% for all cells and 31.8% for cells in the optimal RD (Fig. 3f).

DISCUSSION

This experiment follows a long line of inquiry on the role of the cholinergic nucleus basalis in sensory cortical plasticity, in which acute studies of sensory cortex were hypothesized to have implications for memory [12]. For example, both application of ACh to the somatosensory cortex and NB stimulation can produce long term changes of cutaneous responsiveness; pairing cutaneous stimulation with NB stimulation can produce persistent enhancement of responses and intracortically applied atropine blocks this effect [13]. Similar effects occur in auditory cortex (e.g. [14]). Parallel studies of somatosensory and auditory cortices, using protocols analogous to associative conditioning or direct studies of learning, showed that receptive fields are changed to emphasize the processing and representation of signal stimuli [1,4,15]. Other studies have supported the hypothesis that ACh released from the NB into sensory neocortex mediates learning-induced CS-specific receptive field plasticity [16,17]. ACh can increase thalamocortical transmission and sensory responsiveness in a manner

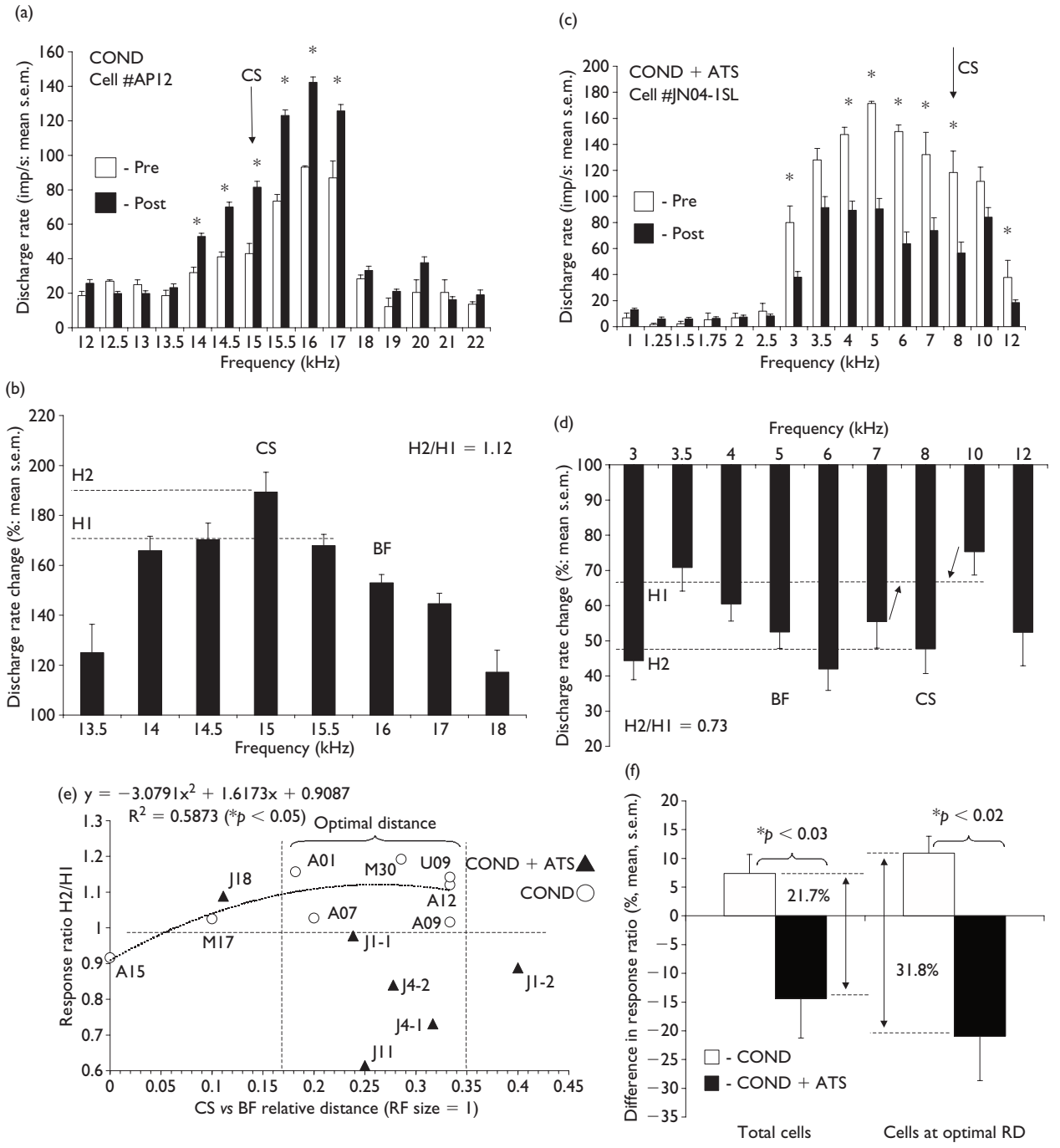

Fig. 3. The effects of pairing a tone with NB stimulation alone or with atropine on receptive fields. (**a**) Average responses to the frequency of the CS and other frequencies (70 dB) obtained before (left bars) and following (13 sets; right bars) conditioning for cell #AP12 from the COND group. (**b**) Percentage change in response to several frequencies following conditioning relative to response to the same frequency before conditioning. Level of 100% on ordinate indicates relative magnitude of response before pairing. The maximal increase was at 15.0 kHz, the CS frequency. (**c**,**d**) Effects of pairing for cell JN04-1SL from the COND + ATS group. The cell developed a general decline in responsiveness that was not quite specific to the CS frequency. (**e**) Specificity of change (H2/H1 ratios) for all cells as a function of the relative distance (RD) between the CS and the BF; the figure bracket indicates the extent of the relative distance range corresponding to the optimal distance, i.e., highest 5% of the H2/H1 ratios based on the parabolic equation calculated for the COND group. Data for the COND + ATS group could not be fit to a parabolic function; within the same range of RD corresponding to the top 5% of the COND group, the cells of the COND + ATS group show an average decrease of 20.9%. (F) Change in H2/H1 ratios for the total population of cells (left) and for cells within the optimal RD range for CS-specific increases (right).

sensitive to cholinergic antagonists [16,18,19]. Moreover, NB stimulation can be substituted for a peripheral unconditioned stimulus to induce CS-specific RF plasticity in the auditory cortex, like that observed following learning [5–8].

The present findings show that receptive field plasticity induced by NB stimulation requires the engagement of muscarinic receptors in the auditory cortex. This report documents the findings from 14 single cells from 12 subjects. Although this number may not be considered large, it was sufficient to meet statistical criteria. It should be noted that the H2/H1 specificity index showed virtually no overlap between the COND *vs* COND + ATS groups (Fig. 3e). The number of cells reported here reflects the long duration and arduousness of the experiments. Several other subjects yielded partial data that failed to meet the inclusion criteria specified above.

The specific increases in the COND group and the specific decreases in the COND + ATS group could occur on a background of general increased or decreased responsitivity, respectfully. The use of relative measures of change precluded the masking of specific effects by any general effects. CS-specific RF plasticity is known to develop during learning in behaving animals despite general changes in overall responsiveness (e.g. see Figure 2 in [1]). The general reduced responses produced by atropine may have reflected a reduction of excitation mediated by muscarinic receptors, without affecting inhibition produced via nicotinic receptors [20]. Atropine not only blocked the induction of RF plasticity, but also produced a CS-specific decrement. This is similar to frequency-specific RF decrement in auditory cortex during habituation [21]. The cells of the COND + ATS group actually may have been exposed to a functional habituation paradigm to the CS because atropine appears to nullify the physiological muscarinic effects of NB stimulation, resulting in a virtual tone alone paradigm.

The role of the nucleus basalis in cortical RF plasticity has been extended in several ways. First, the degree of specificity of RF plasticity was found to depend upon the relative distance of the frequency of the CS from the best frequency. The optimal distance was about one-quarter of the receptive field size (0.27 RD for the current data). This has a practical value in providing guidelines for future studies, particularly to avoid false negative conclusions by using a disadvantageous frequency distance. A more general issue concerns the mechanisms underlying the existence of an optimal range, neither too close nor too far from the best frequency of a cell. While the current study can shed no light on this factor, the findings need to be accounted for in cell-circuit models of RF plasticity.

Second, the present results extend prior findings of plasticity in layers V–VI [5–7] to layers II, III and IV. A further extension concerns the types of cellular responses to tone. Previous studies have been primarily concerned with cells whose major response feature is a marked brief onset response, often with subsequent suppression and a reduced level of discharge. In contrast, the cells studied here were characterized by onset responses with strong tonic discharges, usually lasting for most of the tone duration (Fig. 2a,b). Thus, NB-induced RF plasticity can be induced in all layers of auditory cortex and in cells that exhibit both phasic and tonic responses to tone.

RF plasticity may also involve non-cholinergic mechanisms activated by NB stimulation. For example, the NB contains GABAergic cells projecting to auditory cortex [22] and NB stimulation can produce disinhibition of inhibitory cortical cells without releasing ACh [23]. These might act synergistically with muscarinic receptor activation to promote frequency-specific facilitation. Additionally, frequency-specific response decreases to tones paired with ACh applied to auditory cortex iontophoretically have previously been attributed to muscarinic activation of inhibitory cortical cells [14].

CONCLUSION

Presentation of tone followed by stimulation of the nucleus basalis produced specific facilitation of frequency receptive fields in the primary auditory cortex. This plasticity was blocked by cortical perfusion with atropine sulfate, which resulted in frequency-specific response reduction. As NB-induced RF plasticity has the same characteristics as plasticity that develops during behavioral learning, such direct learning effects are likely also to involve muscarinic receptors in the auditory cortex.

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