Acetylcholine-Dependent Induction and Expression of Functional Plasticity in the Barrel Cortex of the Adult Rat

VALÉRIE EGO-STENGEL,[1] DANIEL E. SHULZ,[1] SEBASTIAN HAIDARLIU,[2] RONEN SOSNIK,[2] AND EHUD AHISSAR[2]

[1]*Unité de Neurosciences Intégratives et Computationnelles, Institut de Neurobiologie Alfred Fessard, Centre National de la Recherche Scientifique, 91198 Gif sur Yvette, France; and* [2]*Department of Neurobiology, The Weizmann Institute of Science, 76100 Rehovot, Israel*

Received 26 September 2000; accepted in final form 21 February 2001

Ego-Stengel, Valérie, Daniel E. Shulz, Sebastian Haidarliu, Ronen Sosnik, and Ehud Ahissar. Acetylcholine-dependent induction and expression of functional plasticity in the barrel cortex of the adult rat. *J Neurophysiol* 86: 422–437, 2001. The involvement of acetylcholine (ACh) in the induction of neuronal sensory plasticity is well documented. Recently we demonstrated in the somatosensory cortex of the anesthetized rat that ACh is also involved in the expression of neuronal plasticity. Pairing stimulation of the principal whisker at a fixed temporal frequency with ACh iontophoresis induced potentiations of response that required re-application of ACh to be expressed. Here we fully characterize this phenomenon and extend it to stimulation of adjacent whiskers. We show that these ACh-dependent potentiations are cumulative and reversible. When several sensori-cholinergic pairings were applied consecutively with stimulation of the principal whisker, the response at the paired frequency was further increased, demonstrating a cumulative process that could reach saturation levels. The potentiations were specific to the stimulus frequency: if the successive pairings were done at different frequencies, then the potentiation caused by the first pairing was depotentiated, whereas the response to the newly paired frequency was potentiated. During testing, the potentiation of response did not develop immediately on the presentation of the paired frequency during application of ACh: the analysis of the kinetics of the effect indicates that this process requires the sequential presentation of several trains of stimulation at the paired frequency to be expressed. We present evidence that a plasticity with similar characteristics can be induced for responses to stimulation of an adjacent whisker, suggesting that this potentiation could participate in receptive field spatial reorganizations. The spatial and temporal properties of the ACh-dependent plasticity presented here impose specific constraints on the underlying cellular and molecular mechanisms.

INTRODUCTION

The study of the required conditions for the induction of neuronal plasticity in the adult primary sensory cortices has led to the implication of neuromodulators in this process. Acetylcholine (ACh) released in the cortex from fibers originating in the nucleus basalis magnocellularis (NBM) is a major candidate (Dykes 1997; Singer 1990). Indeed, ACh micro-iontophoresis (Greuel et al. 1988; Metherate and Weinberger 1989; Metherate et al. 1987, 1988a,b) or stimulation of the NBM (Bakin and Weinberger 1996; Edeline et al. 1994; Kilgard and Merzenich 1998b; Tremblay et al. 1990a,b) during the repetitive presentation of a stimulus is sufficient to induce long-lasting modifications of neuronal responses. Furthermore, cortical map reorganization and neuronal receptive field changes in sensory cortices were shown to be blocked by lesions of the cholinergic system (Baskerville et al. 1997; Bear and Singer 1986; Sachdev et al. 1998) or by cholinergic antagonists (Maalouf et al. 1998). Thus increased levels of ACh in the cortex provide the adequate neurochemical environment for the induction of plasticity (Dykes 1997; Singer 1990).

By contrast, the requirements for ACh during the expression phase of plasticity have not been extensively studied. In the olfactory cortex, ACh exerts a differential effect on thalamocortical versus intracortical pathways (Hasselmo and Bower 1993). Based on these observations, these authors proposed that increased levels of ACh promote learning of new information by enhancing afferent inputs and enabling plasticity, whereas decreased cholinergic levels facilitate retrieval (Hasselmo and Bower 1993). However, behavioral studies have shown instances in which retrieval of a newly acquired memory depends on the similarity between the endogenous neurochemical state that develops after training and the one that develops during testing [endogenous state-dependent learning (discussed in Izquierdo 1984)]. This suggests that at the cellular level, retrieval of an ACh-induced plasticity could be improved by the presence of ACh during testing (Zornetzer 1978). We have recently reported that in the barrel cortex of anesthetized rats, ACh plays a dual role in neuronal plasticity: it is essential both during the induction and the expression phases (Shulz et al. 2000). Herein, we analyzed the effects of applying consecutive sensori-cholinergic pairing protocols, investigated the retrieval kinetics, and tested to see if the ACh-dependent plasticity occurred when stimulating nonprincipal whiskers as well. The latter analysis was motivated by the fact that previous studies on plasticity in the barrel cortex using whisker-pairing protocols have shown that enhancement in response was prominent for the intact adjacent whisker as well as for the principal whisker (Armstrong-James et al. 1994; Diamond et al. 1993).

Address for reprint requests: D. E. Shulz, U.N.I.C., UPR CNRS 2191, Institut de Neurobiologie Alfred Fessard, CNRS, 91198 Gif sur Yvette, France (E-mail: shulz@iaf.cnrs-gif.fr).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

METHODS

Animal preparation

Twenty-four adult male Wistar albino rats weighing 300 ± 25 g obtained from the Animal Breeding Unit of The Weizmann Institute of Science were used for these experiments. Maintenance, manipulations, and surgery were according to institutional animal welfare guidelines that meet the National Institutes of Health standards. The animals received an injection of atropine methyl nitrate (0.3 mg/kg im), a derivative of atropine that does not cross the blood-brain barrier (Weiner 1980), and were anesthetized with urethan (1.5 g/kg ip). Supplementary doses of urethan (0.15 g/kg ip) were administered when necessary throughout the experiment to maintain an adequate level of anesthesia, indicated by the absence of eyeblink reflex or response to hindpaw pinch. Body temperature was maintained at 37°C using a temperature-regulated heating pad.

The animal was mounted in a stereotaxic frame with a modified head holder without ear bars, which allowed free access to the somatosensory cortex and to vibrissae (Haidarliu 1996). A local anesthetic (lidocaïne, 2%) was injected subcutaneously in all skin incisions. The right scalp and temporal muscle were resected. A 3 × 3 mm craniotomy was made to expose the right posteromedial barrel subfield (PMBSF; P_1–P_4, L_4–L_7 from Bregma) (Chapin and Lin 1984). The dura was opened. A dental cement cup was made surrounding the skull opening and was filled with saline to prevent drying of the cortex. Vibrissae were clipped on the left side of the snout to a length of 1 cm.

Electrophysiological recording and iontophoresis

Neural activity was recorded extracellularly with a multi-electrode array composed of one or two tungsten-in-glass electrodes (TE, 0.2–0.8 MΩ at 1 kHz) and one or two combined electrodes (CE) mounted within a metallic guide tube (Haidarliu et al. 1995). The CEs were composed of a tungsten-in-glass electrode surrounded by six glass micropipettes for simultaneous iontophoresis and recording. The six barrels were filled with acetylcholine chloride (1 M, pH 4.5) and sodium chloride (3 M) for current balance. In three experiments, one iontophoresis barrel was filled with atropine sulfate (0.1 M, pH 4.5). Results reported in this paper do not involve atropine iontophoresis. Retaining currents of −10 nA were used to prevent drugs from leaking. During periods of ejection, balanced 20- to 80-nA currents were applied. The CEs and TEs were lowered independently using a multi-electrode microdrive system. Signals were amplified and filtered for spike activity (0.5–8 kHz). For each recording electrode, up to three single units were isolated using a template-matching spike sorter (MSD-2; Alpha-Omega, Nazareth, Israel). The shape of action potentials was continuously inspected to ensure that the same neurons were recorded throughout the protocols. When action potential waveforms could not be discriminated, multi-unit data were collected either by defining a template encompassing several waveforms or by amplitude sorting. Spike times were acquired on a computer at 1 kHz.

Whisker stimulation and pairing protocol

Once units were isolated, vibrissae were at first manually deflected while monitoring the extracellular signal. For each unit, the principal whisker was defined as the whisker eliciting the maximal neuronal response. This whisker was chosen for computer-controlled stimulation. Since the electrodes in the array could be located in different barrels, in some cases simultaneously recorded units did not have the same principal whisker. We selected the principal whisker of units recorded by a CE for subsequent stimulation. Hence for some of the other units, the stimulated whisker was an adjacent whisker rather than their principal whisker. We inserted the selected whisker in a short Teflon tubing attached to a linear electromagnetic vibrator (Schneider 1988). Stimulation was automatically controlled by the data-acquisition computer and consisted of pulses of 5-ms rise time followed by 5-ms fall time, producing a 160 μm rostrocaudal deflection at ~5 mm from the follicle of the deflected whisker.

We determined the response to deflections of the vibrissae at temporal frequencies from 2 to 11 Hz (Fig. 1*A*). For each frequency, stimuli were always applied in blocks of 12 consecutive trains of 4 s + 1-s inter-train interval; each block of stimulation thus lasted 60 s. The temporal-frequency tuning curve (TFTC) of each unit was determined by deflecting the principal vibrissa at different frequencies

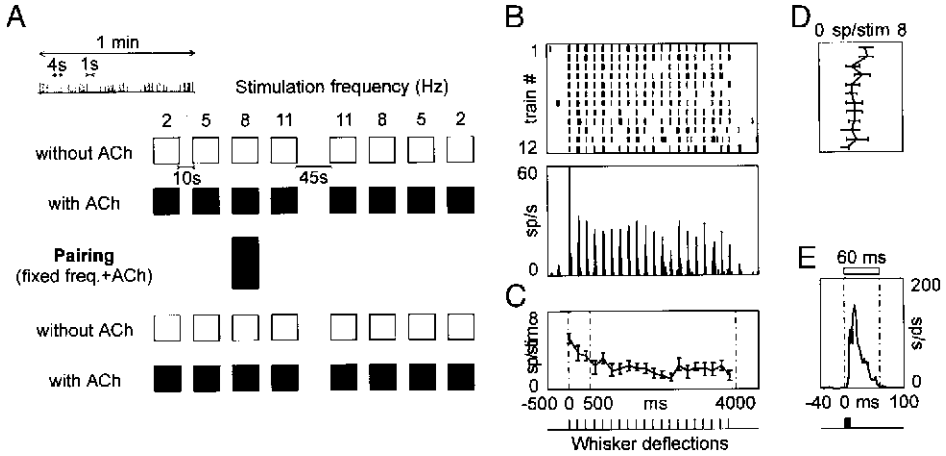

FIG. 1. *A*: stimulation protocol. Whisker deflections were applied in blocks (represented by squares) of 12 consecutive trains of 4 s + 1-s inter-train interval. Frequencies were presented in the following order: 2, 5, 8, 11, (45- to 110-s interval), 11, 8, 5, 2 Hz with an inter-block interval of 10 s. The response of the unit was first determined without acetylcholine (ACh; white squares) then during ACh iontophoresis (black squares). During the pairing protocol, a "double" block of 24 trains of stimulation at a single frequency (on this example, 8 Hz; black rectangle) was accompanied by ACh iontophoresis. The response of the unit was tested twice after pairing, once without and once with ACh. *B*: raster plot of the action potentials of a unit during 1 block (12 trains) of mechanical stimulation at 5 Hz and corresponding peristimulus time histogram (PSTH); bin, 10 ms. *C*: kinetics of the response within trains of stimulation. The spike count in response to each deflection in the trains of stimulation was averaged across the 12 trains. Times of whisker deflections are shown *below* the graph. Dashed lines indicate the beginning of trains of stimulation and the temporal window defined for analysis of the steady state (500–4,000 ms). *D*: steady-state average spike count for each of the 12 trains of stimulation. *E*: PSTH of the response to all deflections at 5 Hz; bin, 1 ms (black bar, 10-ms long stimulus). The dashed lines indicate the temporal window used to quantify the spike count in response to each deflection (0–60 ms).

in the following order: 2, 5, 8, 11, (in a few cases 14), (45-s interval), (14), 11, 8, 5, 2 Hz, with inter-block intervals of 10 s. The 45-s interval was designed to effectively separate the two blocks of stimulation at the highest frequency. Consequently, responses at each frequency were obtained from two blocks of 12 trains of stimuli each. The total number of deflections ranged from 192 at 2 Hz to 1,056 at 11 Hz. In a few cases ($n = 19/208$), 14 Hz was also tested.

Two control TFTCs were determined before pairing: one in the absence of iontophoresis and a second one during ACh iontophoresis. We then applied a pairing protocol consisting of one block of 24 trains of stimulation (each of 4 s + 1-s inter-train interval) of the vibrissa at one fixed temporal frequency (5, 8, or 11 Hz) accompanied with ACh iontophoresis. Following pairing, two test TFTCs were determined again, one without ACh and one with ACh.

The temporal stability of response and the eventual effect of the application of ACh during the second control TFTC were tested on 40 units for which the protocol was identical except that the pairing period between the control and test TFTCs was omitted ("unpaired" group). The sequence of stimulation was in those cases: TFTC without ACh, TFTC with ACh, TFTC without ACh, and TFTC with ACh.

Data analysis

Recordings were monitored on-line by inspecting a rate meter for each unit (firing rate as a function of time) and data analysis was performed off-line (Matlab). Units that had a discharge rate less than 2 spikes/s (including the spontaneous activity) in response to deflections of the principal whisker at 2 Hz were considered as unresponsive units and analyzed separately.

Unit responses to stimulation were plotted as raster diagrams (Fig. 1*B*). The response of a unit to a deflection of the vibrissa was defined as the spike count in a fixed temporal window chosen to contain the entire response (0–60 ms; restricted to 50 ms for 23 units for which an inhibition phase started at 50 ms; see Fig. 1*E*). Response during stimulation trains was composed of an initial adapting phase during the first 500 ms followed by a steady-state response. Only deflections between 500 and 4,000 ms of each train were included for quantification of the steady-state regime. Conversely, the first deflection of each train was analyzed separately. Peristimulus time histograms (PSTHs) were constructed for each stimulation frequency by averaging the instantaneous firing rate of the unit relative to the onset of deflection of the vibrissa (Fig. 1*E*). One-millisecond bins were used, and smoothing was achieved by convolution with a right triangle of area 1 and base 4 ms. Note that due to the periodic nature of the stimulation, especially for the higher frequencies, the activity that precedes the stimulus in each PSTH corresponds to the tonic activation of the unit during the stimulus train and cannot be considered as a spontaneous activity. To estimate the decrease of the response within a train and the kinetics of response from train to train, the spike count was averaged respectively for individual deflections across the 24 trains (Fig. 1*C*) and for deflections in the steady state of each train (Fig. 1*D*). TFTCs were obtained by plotting the average spike count as a function of the frequency of stimulation.

We looked for specific changes in the response of the unit at each frequency compared with other frequencies independently of global modifications of excitability. To this purpose, the relative strength of the response to a given frequency was quantified by the weighted ratio $WR = (Rf - AvgR)/(Rf + AvgR)$, where Rf is the response to stimulation at a given frequency and AvgR is the averaged response to stimulation at all other frequencies. This ratio, which takes values from −1 to +1, was calculated independently for each of the 24 trains of stimuli and for each frequency. To assess the effect of pairing, the 24 values obtained before and after pairing were statistically compared [2-tailed Kolmogorov-Smirnov (KS), significance level $P < 0.01$]. This comparison was performed independently for each frequency and for the two test conditions, without and with ACh. When several pairings were performed on the same units and to keep the initial state comparable among units, only the first paired frequency was considered for quantifying the percentage of modified units. The effect was assessed systematically on the test period immediately after the last pairing at that frequency. Average values are displayed as means ± SE unless indicated otherwise.

Histology

At the end of seven experiments, small electrolytic lesions were made at known depths using 3- to 5-μA current applied twice for 2 s through one of the tungsten-in-glass electrodes. The animal was given a lethal dose of thiopentone (0.5 ml ip per animal) and perfused transcardially with saline followed by a fixative solution (2.5% glutaraldehyde, 0.5% paraformaldehyde, and 5% sucrose in 0.1 M phosphate buffer, pH 7.4). Tangential or coronal sections (50 or 60 μm) were cut through the right PMBSF and stained for cytochrome oxidase to visualize barrels. The laminar positions of the lesions in coronal sections were used to establish a correspondence between the depth of the electrode penetration and the layer recorded from. This relation enabled us to estimate the laminar location of each cell from its recording depth.

RESULTS

Two hundred and eight units were recorded in the somatosensory cortex of adult rats during at least one complete stimulation protocol. Twenty-two units were unresponsive to whisker stimulation (see METHODS) and were analyzed separately. Of the remaining 186 units, 134 (62 single units and 72 multi-units) were recorded by a combined electrode (CE) and 52 (24 single units and 28 multi-units) by a tungsten-in-glass electrode (TE).

Spontaneous and evoked activity in control conditions

The spontaneous activity of units was quantified over periods of 45 to 110 s prior to any pharmacological stimulation. Single and multi-units recorded by the TEs had an average spontaneous firing rate similar to single and multi-units recorded by the CEs (TEs: 12.1 ± 2.1 spikes/s; CEs: 12.9 ± 1.4 spikes/s; 2-tailed Student's *t*-test, $P > 0.7$), suggesting that the geometry of the combined electrodes did not introduce a sampling bias.

The stimulated whisker was mechanically deflected at frequencies ranging from 2 to 11 Hz. Units responded with phasic increased activity after each deflection. The raster plots displayed in Fig. 2 show the response of a cortical unit to two blocks of stimulation (12 4-s trains each) at 2, 5, 8, and 11 Hz. The first deflection of each train, which in all cases was preceded by a 1-s stimulation-free period, elicited a comparable discharge rate whatever the frequency of stimulation. The following deflection occurred after a variable time interval depending on the stimulus frequency, from 500 ms at 2 Hz to 91 ms at 11 Hz, and produced a smaller response. This decrease in evoked activity from one deflection to the following was prominent for shorter intervals, i.e., higher frequencies of stimulation. After this transient kinetics, the response reached a steady-state level that decreased with increasing stimulation frequencies. Almost all units exhibited these low-pass filter characteristics: in 180/186 cases the response to 5-Hz stimulation was lower than the response to 2-Hz stimulation (this difference reached significance in 98 cases; 1-tailed Mann-Whitney *U* test, $P < 0.01$). In a few cases, however, the

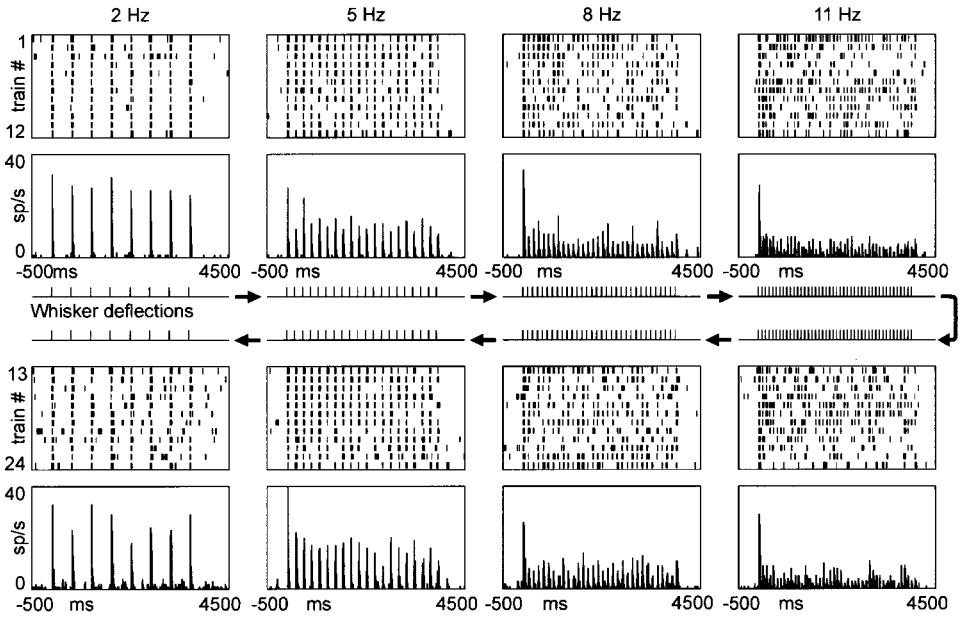

FIG. 2. Low-pass filter characteristic and temporal stability of the response to stimulation at different frequencies. Raster plots and PSTHs for consecutive blocks of stimulation at 2, 5, 8, and 11 Hz (*top half*, from left to right) and 11, 8, 5, and 2 Hz (*bottom half*, from right to left, experimental time is indicated by the →) are displayed for a cortical multi-unit recording at depth 694 μm; bin, 10 ms.

steady-state response to 5-Hz stimulation was significantly greater than to 2-Hz stimulation (n = 6/186). This specific tuning property was not correlated to other cell parameters (depth, spontaneous and evoked levels of activity). Figure 2 also demonstrates temporal stability of responses during the recording because the responses to stimulation at the same frequency during different blocks (which for 2 Hz for example were done at 10 min interval) were unchanged.

The response to each deflection was quantified by the number of action potentials in the temporal window 0–60 ms after the onset of deflection. By averaging this spike count across trains, the kinetics of the discharge rate during the train was compared across frequencies. Figure 3*A* displays the average kinetics for all single units recorded in the barrel corresponding to the stimulated whisker (*left*, n = 63) and in adjacent barrels (*right*, n = 13). For both populations, the response to the first deflection of the train was constant across frequencies, whereas the response to following deflections rapidly decreased and stabilized at different plateau values depending on the stimulation frequency. This adaptation phenomenon usually did not occur at 2 Hz (red lines in Fig. 3*A*), indicating the lack of lasting effect 500 ms after the onset of whisker deflection, and was strongest at 11 Hz. The low-pass filter characteristic was observed both when the principal whisker or an adjacent whisker was stimulated. In the latter case, however, steady-state responses at higher frequencies (8 and 11 Hz) were on average indistinguishable from spontaneous activity.

We investigated whether the response evolved from one

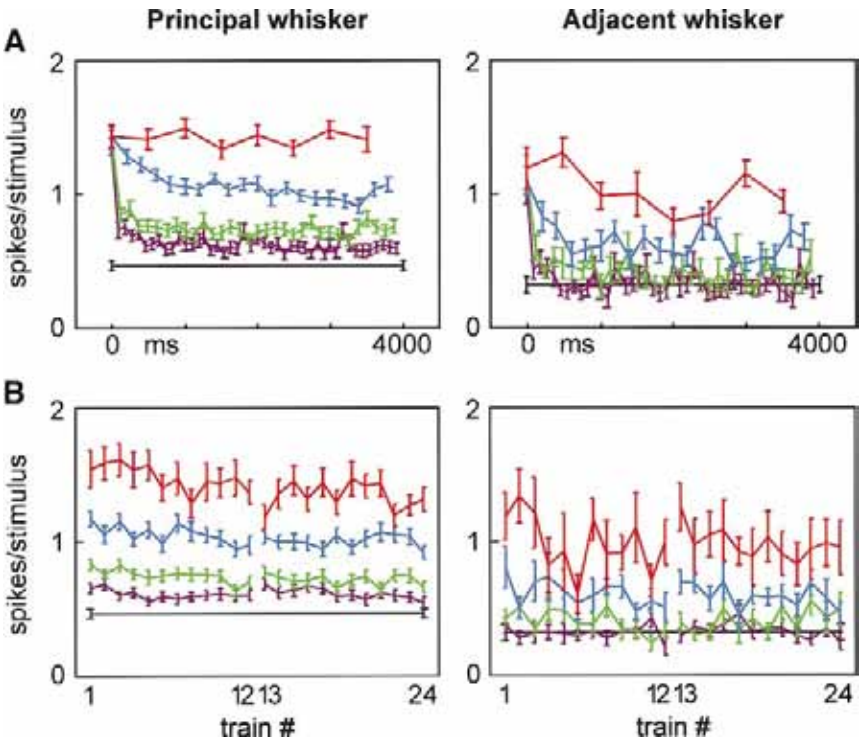

FIG. 3. Amplitude and kinetics of the response to stimulation of the principal and adjacent whiskers at different temporal frequencies. *A*: average spike count in response to each of the deflections within a train for stimulation at 2 (red), 5 (blue), 8 (green), and 11 Hz (purple) for all single units for which the principal whisker (*left*, n = 63) or an adjacent whisker (*right*, n = 13) was stimulated. The black line indicates the level of spontaneous activity (adjusted to a 60-ms window to be comparable with responses). *B*: steady-state average spike count for the 2 blocks of stimulation (color code as in *A*). Because of the intrinsic disparity in the levels of evoked activity for different units, responses were normalized, before averaging, by the mean level of evoked activity of each unit. To express the normalized response in a spikes/stimulus scale, the result was then multiplied by the average evoked activity across the population.

train to the next as stimulation at one fixed frequency was presented. This was done by plotting the steady-state value of the response (calculated as the average of individual deflection responses in the 500- to 4,000-ms window of each train) as a function of the train number. Figure 3*B* displays the result of this analysis for the same populations as in Fig. 3*A*. No systematic trend was observed from one train to another or from the first block of stimulation (*trains 1–12*) to the second block (*trains 13–24*). This confirms that the adaptation rate and the evoked activity of barrel cortex neurons was stable over the course of a TFTC protocol.

Pairing-induced plasticity of the response to principal-whisker stimulation

A full pairing protocol was applied on 119 units recorded by the CEs. Units for which the principal whisker ($n = 105$) and an adjacent whisker ($n = 14$) were stimulated were analyzed separately for assessing the percentage of modifications.

Frequency-specific modifications of response were observed following pairing of ACh iontophoresis with stimulation of the principal whisker at a fixed temporal frequency. Figure 4 shows two examples of significant potentiations of the response to stimulation at the paired frequency. For the cortical unit in Fig. 4*A1*, submitted to a pairing at 8 Hz, the response to 8-Hz stimulation was enhanced after pairing when tested with ACh iontophoresis (KS, $P < 1.10^{-8}$). The potentiation was revealed only for the paired frequency and exclusively when the unit was tested with ACh (KS, $P = 0.3$ for the test without ACh). As seen in this example, the modifications of the response following each deflection could be accompanied by an increase in the tonic level of activity within the train. In control conditions, this tonic level was constant with stimulation frequency or increased concentrations of ACh. Its modification after pairing was thus unexpected. We quantified this component of the response as the integrated spike count in the 20 ms preceding each deflection. Statistical analysis was conducted for this additional set of values. Both the phasic (due to each whisker deflection) and tonic (due to the entire train of deflections) components of the response were increased in the example of Fig. 4*A1* (KS, $P < 1.10^{-4}$ for each component). In Fig. 4*B1*, pairing ACh iontophoresis with whisker stimulation at 11 Hz on a different cortical unit also resulted in a significant enhancement of response at the paired frequency (KS, $P < 1.10^{-7}$; $P < 1.10^{-4}$ for each component of the response). The TFTCs summarize the frequency specificity of the potentiation; TFTCs computed before and after pairing overlap for all frequencies except the paired ones (Fig. 4, *A2* and *B2*).

Frequency-specific changes in response were quantified for each unit by calculating the difference (after minus before pairing) in relative strength of the response to stimulation at each frequency (WR). The ratio WR is not affected by global multiplicative changes in responsiveness; changes in WR in-

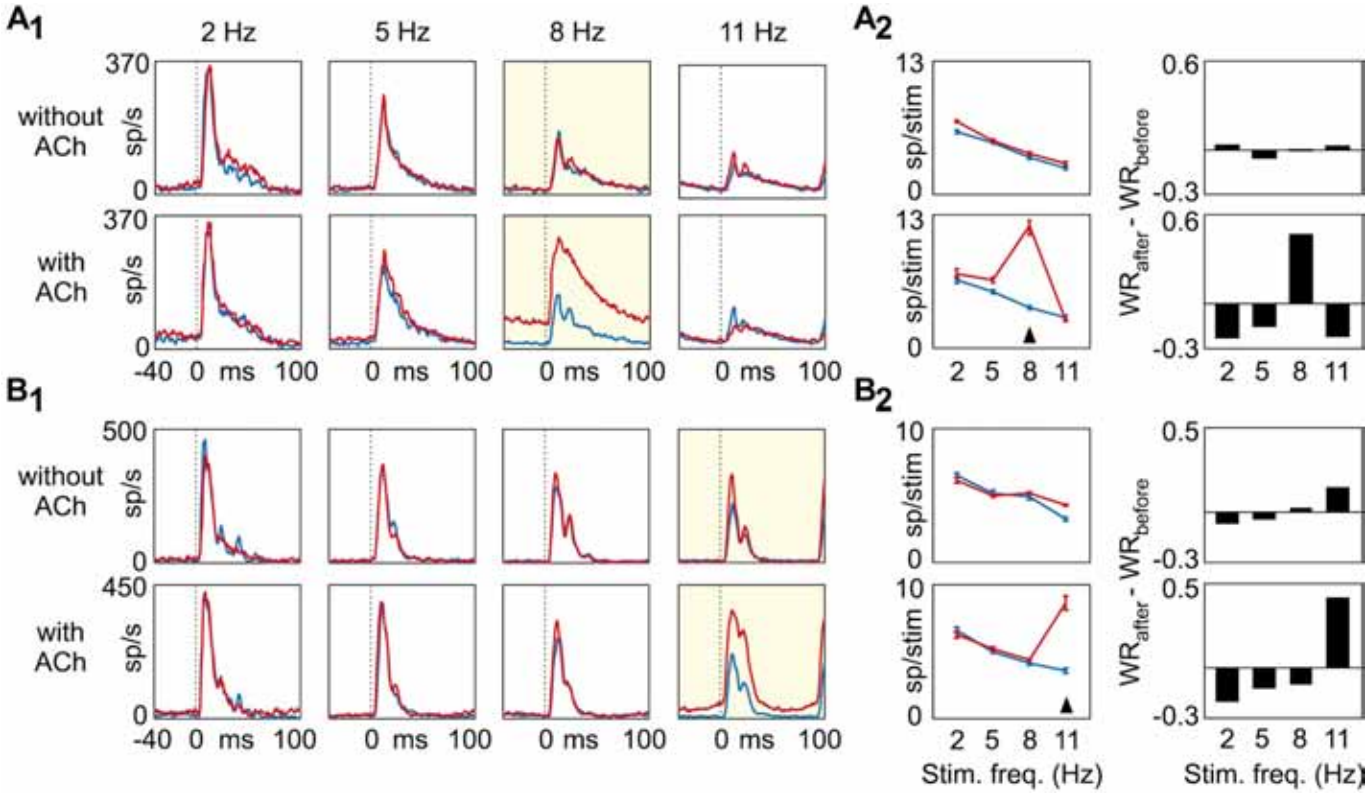

FIG. 4. Plasticity of cortical responses expressed only during ACh application in 2 different units for principal-whisker stimulation. PSTHs of response before (blue) and after (red) pairing superimposed, for the 2 test conditions, without and with ACh, for multi-unit recordings at depth 1,347 μm (*A1*) and depth 986 μm (*B1*). In these and subsequent PSTHs, - - - at *time 0* indicates the onset of deflections and yellow shading indicates the paired frequency. *A2* and *B2*: temporal-frequency tuning curves (TFTCs, average spike count ± SE) and response differences ($WR_{after} - WR_{before}$, see METHODS for definition of WR) for the same units. Pairing at 8 (*A*) and 11 Hz (*B*) resulted in an enhanced response to the paired frequency when tested with ACh (KS, $P < 1.10^{-7}$). When tested without ACh, no change (*A*, KS, $P = 0.3$) or a smaller increase in response was observed (*B*, KS, $P < 0.01$).

dicate changes in response at one frequency relative to the responses at other frequencies. The cortical units of Fig. 4 showed an increased absolute response for the paired frequency after pairing and no change for unpaired frequencies. Consequently, the relative strength in response (WR) to the paired frequency was increased and those to the unpaired frequencies were decreased (Fig. 4, *A2* and *B2*, *right*).

Each unit was tested for statistically significant modifications of its relative response (expressed by WR) at the paired frequency. Overall, 29% of the units had a significantly modified response after pairing when tested with ACh and the majority of these changes were potentiations (18 of 30). Twenty-one percent of the units had a modified response when tested without ACh, and these changes were mainly decreases in response (13 of 22). Similar results were obtained when the analysis was restricted to single units: 8 cells of 53 showed a modified response when tested without ACh, whereas 16 showed a modified response when tested with ACh of which 75% were potentiations.

We studied whether these changes in response resulted from the fixed-frequency pairing by comparing response modifications obtained after ACh pairing and response modifications observed when units were only repeatedly tested without and with ACh (see METHODS). Figure 5 displays the result of such repetitive testing for two cortical units. In Fig. 5*A*, PSTHs of response before and after pairing show no frequency-specific modification of response when tested either without ACh or with ACh (KS, $P > 0.05$). Similarly for the cell depicted in Fig. 5*B*, although a general decrease in response occurred, we did not observe a significant potentiation of the response at one frequency compared with others (blue vs. green PSTHs in Fig. 5*B;* KS, $P > 0.05$). However, a frequency-specific ACh-dependent potentiation to stimulation at 5 Hz was revealed after pairing ACh iontophoresis with stimulation at that frequency (red vs. blue PSTHs; KS, $P < 1.10^{-5}$), indicating that the effect was caused by the pairing.

We compared the modifications of response observed for units submitted to repetitive testing ("unpaired" group) and units submitted to the pairings ("paired" group). The cumulative distribution of changes expressed with ACh were significantly different for these two populations (1-tailed Mann-Whitney *U* test, $P < 0.001$) (Shulz et al. 2000) and revealed that the potentiations of response could be attributed to the effects of the pairings whereas the depressions could be explained, at least on a statistical background, by the ACh-induced variability. Moreover, the TFTC reorganization appeared to be different in the two groups: whereas changes in the paired group were highly specific to the paired frequency, and thus exhibited a sharp peak at that frequency, in the unpaired group, changes were usually distributed across frequencies. To demonstrate this difference in the profile of changes, we averaged response ratio (WR) changes for all units showing a statistically significant potentiation at any frequency, separately for units in the paired and unpaired groups. As expected, in the paired group, there was a significant enhancement in response at the paired frequency compared with changes at other frequencies (ANOVA, $P < 0.01$). By contrast, in the unpaired group, changes in response at all frequencies were similar (ANOVA, $P > 0.3$), which indicates that there was no "natural" tendency for spontaneous potentiations at one particular frequency within the range used here. Second, we quantified the peak observed in the profile of changes by calculating for the two groups of cells the difference between the changes at the paired frequency (for the unpaired group, the maximally enhanced frequency) and the average change at the two neighboring frequencies (i.e., ± 3 Hz). This value, which measures the contrast between the response change at the peak and at neighboring frequencies, was significantly higher in the paired group than in the unpaired group (2-tailed Student's *t*-test, $P < 0.0004$). This result confirms that when changes occurred due to the pairings, they were specific to the paired frequency, whereas changes observed after repetitive testing corresponded to global changes in excitability that generalized to neighboring frequencies (Fig. 5).

Pairing-induced plasticity of the response to adjacent-whisker stimulation

We conducted a separate analysis for the 14 units recorded by CEs, submitted to at least one pairing protocol, and for which we stimulated one adjacent whisker instead of the principal whisker. As for principal-whisker stimulation, frequency-specific and ACh-dependent modifications of response were observed following pairing. The cortical cell of Fig. 6*A* exhibited a weak response to whisker stimulation at 8 Hz in control conditions. After pairing ACh iontophoresis with stimulation at that frequency, the response to 8 Hz was enhanced when testing with ACh iontophoresis (KS, $P < 1.10^{-5}$), whereas it was unchanged when testing without ACh (KS, $P = 0.9$).

Population analysis confirmed that the effects of the pairing protocols were not restricted to units located in the barrel corresponding to the stimulated whisker. Six units of 14, which were stimulated via a nonprincipal whisker, showed a significant potentiation of response to stimulation at the paired frequency when tested with ACh after pairing, which was significantly more (χ^2, $P < 0.05$) than the percentage for principal-whisker stimulation (42 vs. 17%). For both populations of cells, the potentiations observed during testing with ACh were maximal for the paired frequency compared with unpaired frequencies (1-tailed Student's *t*-test, $P < 0.05$), whereas no significant difference was observed when tested without ACh (1-tailed Student's *t*-test, $P > 0.15$; Fig. 6*B*). Additionally, both populations of units showed cumulative and reversible effects and similar kinetics of the expression of modifications; therefore they were grouped in one large dataset for the description of these characteristics.

Laminar location of cells expressing pairing-induced potentiations

Histological localization of the recording sites was performed after seven experiments in which small electrolytic lesions were made at the end of the recordings. Using the known depths of those recordings, we established a layer-depth correspondence and used this relation to estimate the laminar location of other recording sites for which we had the direct reading of the electrode microdrive. Based on this estimation, cells expressing a potentiation of the response to the paired frequency when tested with ACh were exclusively found in layers IV and V of the barrel cortex (layer IV, $n = 14/75$; layer V, $n = 10/31$). Layers II, III, and VI, which were less explored

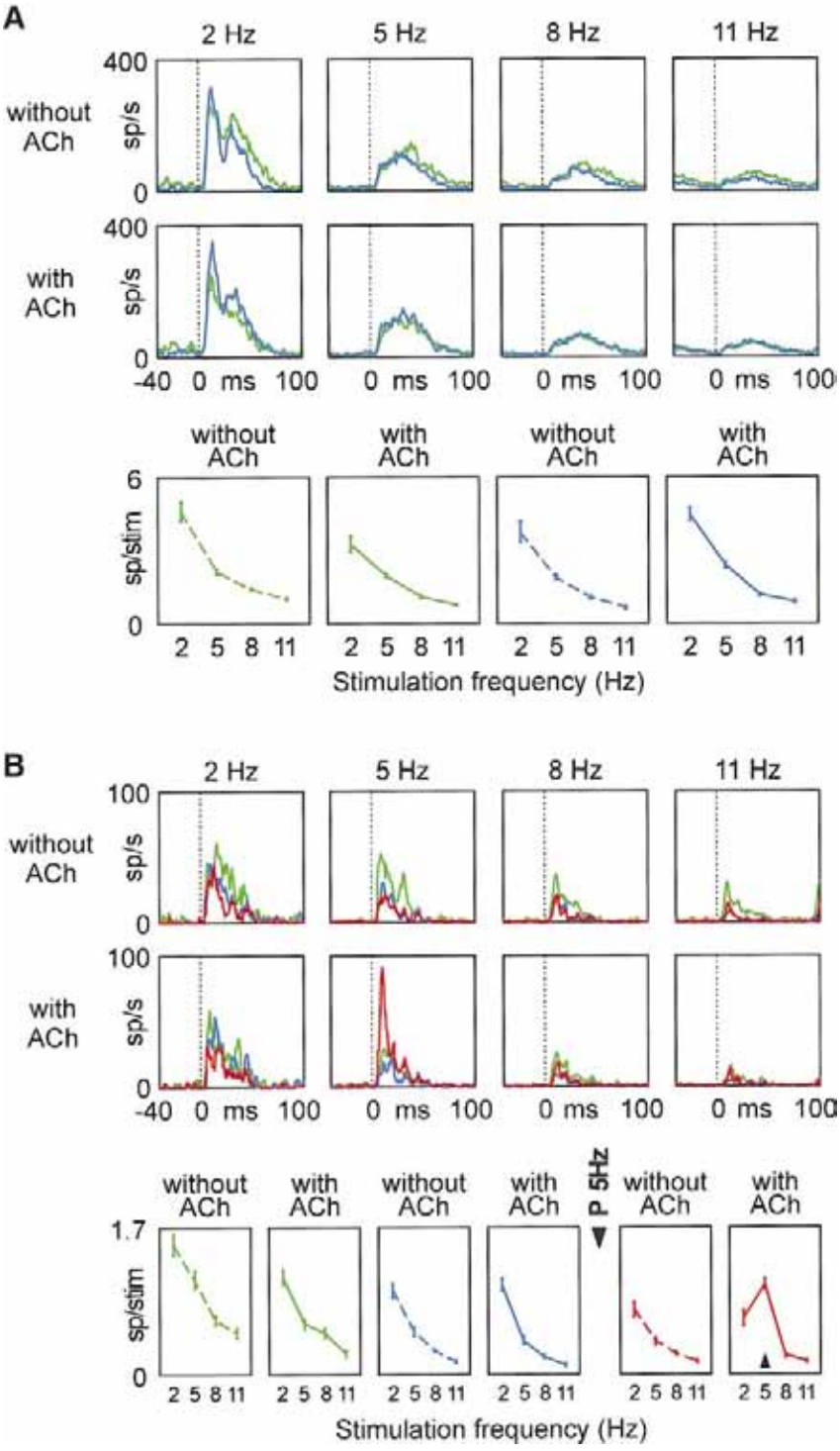

FIG. 5. Repetitive testing induced no frequency-specific changes in response in two different units. *A*, *top*: PSTHs of response during the 1st (green) and 2nd (blue) tests superimposed for the 2 test conditions, without and with iontophoresis of ACh, for a multi-unit recording at depth 1,344 μm. *Bottom*: TFTCs for the same unit for tests without and with ACh in chronological order. - - -, correspond to tests without ACh. No frequency-specific significant change was observed for any frequency tested and in any of the 2 conditions (KS, $P > 0.05$). *B*: PSTHs and TFTCs of response during the 1st (green) and 2nd (blue) tests and after a series of 3 pairings at 5 Hz (red) for a single unit recorded at depth 726 μm. A significant decrease was found for 8 Hz when tested for the second time with ACh (blue vs. green curve; KS, $P < 0.01$) even though the general profile of the TFTC is unchanged; no significant change was observed for other frequencies in the 2 conditions (KS, $P > 0.01$). This cell expressed frequency-specific plasticity when tested with ACh after pairing at 5 Hz (KS, $P < 1.10^{-5}$; the arrow head indicates the paired frequency) but not when tested without ACh (KS, $P = 0.4$).

in our study, did not show such plasticity (layers II and III, $n = 0/6$; layer VI, $n = 0/7$). However, the difference in the proportion of potentiated cells across layers was not significant (χ^2, $P = 0.10$).

Pairing-induced modifications are not transferred through the intracortical network

Within the multi-electrode array, units recorded by the CEs were presumably directly reached by iontophoresed ACh, whereas units recorded by the TEs were beyond the range of diffusion of ACh (Haidarliu, Shulz, and Ahissar, unpublished results). Indirect effects of ACh could be mediated, however, through modulation of network activity. We investigated whether the response of units recorded by the TEs were modified after the pairing protocol. Of 23 single units, only one showed an increased response to stimulation at the paired frequency when tested with ACh. This was significantly less than for units recorded by the CEs (χ^2, $P < 0.05$); this was consistent with the limited diffusion volume of ACh in the

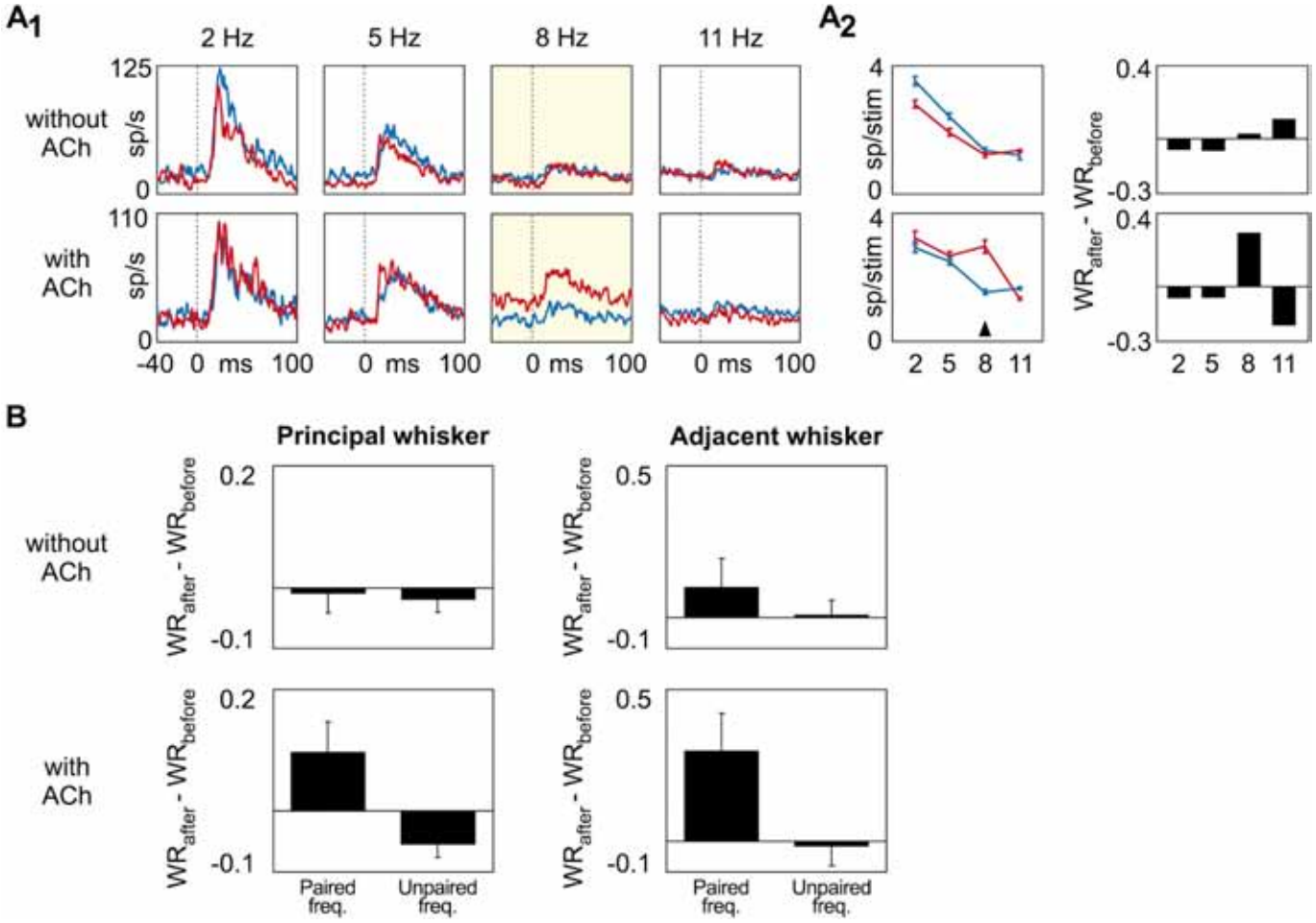

FIG. 6. Plasticity of cortical responses expressed only during ACh application for adjacent-whisker stimulation. *A1*: PSTHs of response before (blue) and after (red) pairing superimposed for the 2 test conditions, without and with ACh, for a single-unit recording at depth 1,136 μm at the border of barrels D1 and E1 during stimulation of whisker D2. *A2*: TFTCs and response differences for the same cell. Pairing at 8 Hz resulted in an enhanced response to the paired frequency when tested with ACh (KS, $P < 1.10^{-5}$), whereas no change (KS, $P = 0.9$) was observed when tested without ACh. *B*: average response differences for paired and unpaired frequencies; these averages were calculated for all units expressing a significant potentiation of response at any of the tested frequencies to avoid any bias toward the paired frequency (principal whisker, $n = 40$; adjacent whisker, $n = 7$).

cortex and suggested that the change in the activity of units reached by iontophoresed ACh did not induce significant changes in the activity of distant units through the cortical network.

Effects of pairing on unresponsive units

Twenty-two units were initially unresponsive to whisker stimulation; 8 of these units were recorded by a CE and submitted to at least one complete pairing protocol (19 protocols were applied in total for the 8 units). We did not observe the appearance of a response to stimulation at any of the tested frequencies in any of these cases when tested with ACh (KS, $P > 0.05$), suggesting that the response potentiations revealed for initially active units resulted from increases in the discharge rates of the recorded units and not from the addition of de novo responses of previously silent units.

Cumulative effects of consecutive pairing protocols

On 57 units, we performed one to three additional pairing protocols at the same frequency after the first pairing. We observed cumulative effects until a maximal enhancement of response to stimulation at the paired frequency was reached. Figure 7 displays the results on two cortical cells submitted to three consecutive pairings at 5 (Fig. 7*A*) and 8 Hz (Fig. 7*B*). In the first example, two pairing protocols were necessary to reveal ACh-dependent plasticity at the paired frequency (1st pairing, KS, $P > 0.9$; 2nd pairing, KS, $P < 0.005$ compared with initial control), and a third pairing further enhanced the potentiation (KS, $P < 1.10^{-6}$). The time course of the potentiation through the three pairings is depicted in Fig. 7*A2*. Note that the TFTC as well as the WR value for the paired frequency remained unchanged when tested without ACh (KS, $P > 0.4$ for all pairings) even though tests without and with ACh alternated during the experiment. In the second example, potentiation of the response to stimulation at the paired frequency during ACh iontophoresis was already present after the first pairing (KS, $P < 1.10^{-4}$) and reached its maximum after the second pairing (KS, $P < 1.10^{-4}$). As confirmed by the consecutive values of WR (Fig. 7*B2*), the potentiation was saturated after the second pairing since a third pairing did not further increase the relative strength of the response of the cell

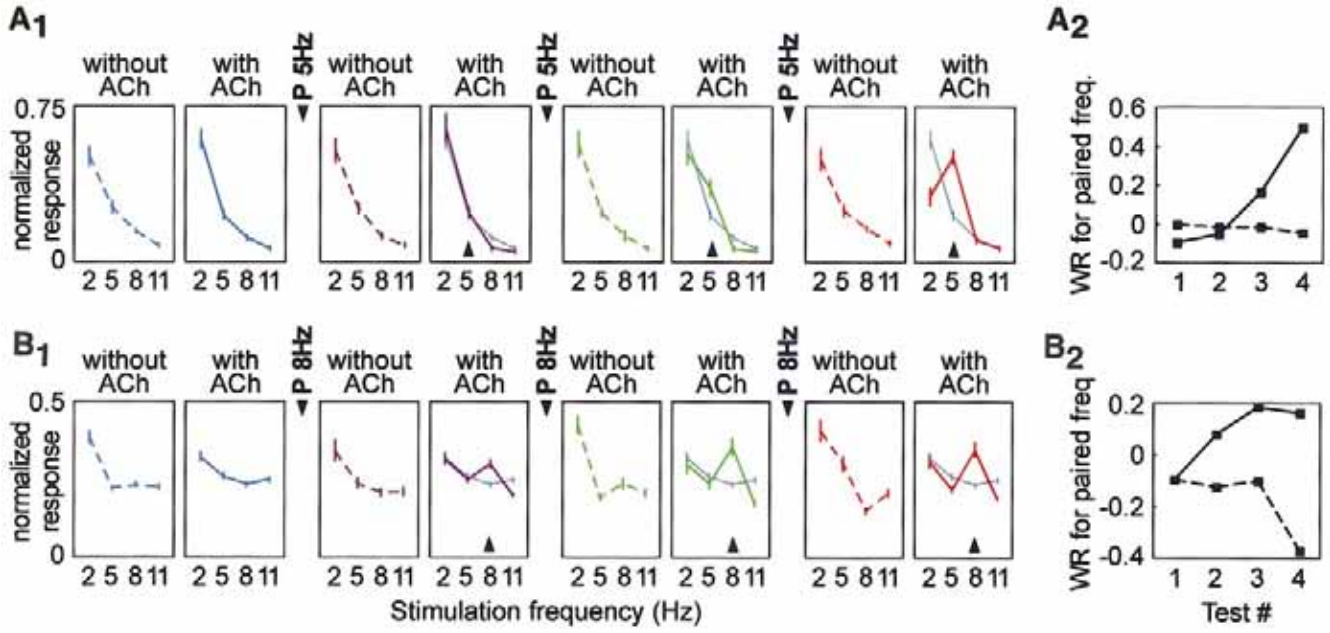

FIG. 7. Two examples of cumulative effects of successive pairings at the same stimulation frequency (*A*, 5 Hz; *B*, 8 Hz). *A1* and *B1*: TFTCs before (blue), after 1st (purple), 2nd (green), and 3rd (red) pairings are depicted in chronological order for a single unit recorded at depth 726 μm (*A*) and a single unit recorded at depth 1,050 μm (*B*). Dashed curves, tests without ACh. ▲, the paired frequency. Average responses (±SE) to the different frequencies of stimulation are expressed as a fraction of the summed response to the 4 tested frequencies (normalized response). To facilitate visual comparisons, TFTCs obtained with ACh before pairing were superimposed (thin lines) on TFTCs obtained after pairing. *A2* and *B2*: response ratio (WR, see METHODS for definition) for the paired frequency before and after each of the pairing protocols when tested without ACh (dashed line) and with ACh (solid line). In *A*, whereas the response to the paired frequency (5 Hz) was not modified when tested with ACh after the 1st pairing (KS, $P > 0.9$), it was significantly enhanced after the 2nd pairing (KS, $P < 0.01$ compared with initial control) and further more after the 3rd pairing (KS, $P < 1.10^{-6}$). In none of these cases was the response without ACh significantly modified (KS, $P > 0.4$). In *B*, the response to the paired frequency (8 Hz) was significantly enhanced after each of the three pairings compared with initial control when tested with ACh (KS, $P < 1.10^{-4}$). When tested without ACh, no change (1st and 2nd pairing, KS, $P > 0.4$) or a decrease in response was observed (3rd pairing, KS, $P < 1.10^{-5}$).

for the paired frequency. With this unit, the noncholinergic tests revealed constant WRs (KS, $P > 0.2$), except for the last pairing, after which it was significantly reduced (KS, $P < 1.10^{-5}$). Whether this reduction was related to the saturation of the ACh-dependent expression is not known.

Of the 57 units tested, 11 units exhibited a significantly increased response to stimulation at the paired frequency during ACh iontophoresis after a series of several pairings, whereas only 5 of these units showed significant modifications after the first pairing. On average for these 11 units, the relative response to stimulation at the paired frequency when tested with ACh was significantly increased after the first pairing (2-tailed Student's *t*-test, $P < 0.002$; Fig. 8, *right*) and was further potentiated by the second and third pairings at the same paired frequency (2-tailed Student's *t*-test compared with initial control, 2nd pairing, $P < 1.10^{-4}$, $n = 11$; 3rd pairing, $P < 0.05$, $n = 4$). In contrast, tests of response without ACh (- - -) or to stimulation at unpaired frequencies (Fig. 8, *left*) did not reveal any change (2-tailed Student's *t*-test, $P > 0.3$ in each case).

Frequency-specificity and reversibility of the modifications

To confirm the specificity of changes for the paired frequency, we performed pairings at 5, 8, or 11 Hz. These three frequencies were equally effective in the induction of ACh-dependent potentiations of response (respectively, 3/10, 14/73, and 7/36 significant potentiations; χ^2, $P > 0.7$). Furthermore the enhancement of response after pairing at one frequency could be reversed by a second pairing at a different frequency, resulting in a relative decrease in response to stimulation at the initially potentiated frequency and an increase at the newly paired frequency. Figure 9 displays this switch in response enhancement for a cortical unit submitted to two consecutive

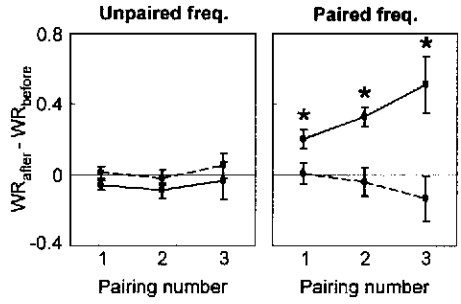

FIG. 8. Average cumulative effects of successive pairings at the same paired frequency. Response differences ($WR_{after} - WR_{before}$, mean ± SE) were averaged for all units expressing a significant enhancement of response after the last pairing at the same paired frequency ($n = 11$). - - -, tests without ACh; —, tests with ACh. *Left*: response differences for unpaired frequencies. None of the data points is significantly different from control (2-tailed Student's *t*-test, $P > 0.3$). *Right*: response differences for the paired frequency. The response was significantly enhanced after each successive pairing compared with initial control for tests with ACh (*, 2-tailed Student's *t*-test, $P < 0.05$). Changes were not significant for tests without ACh (2-tailed Student's *t*-test, $P > 0.3$).

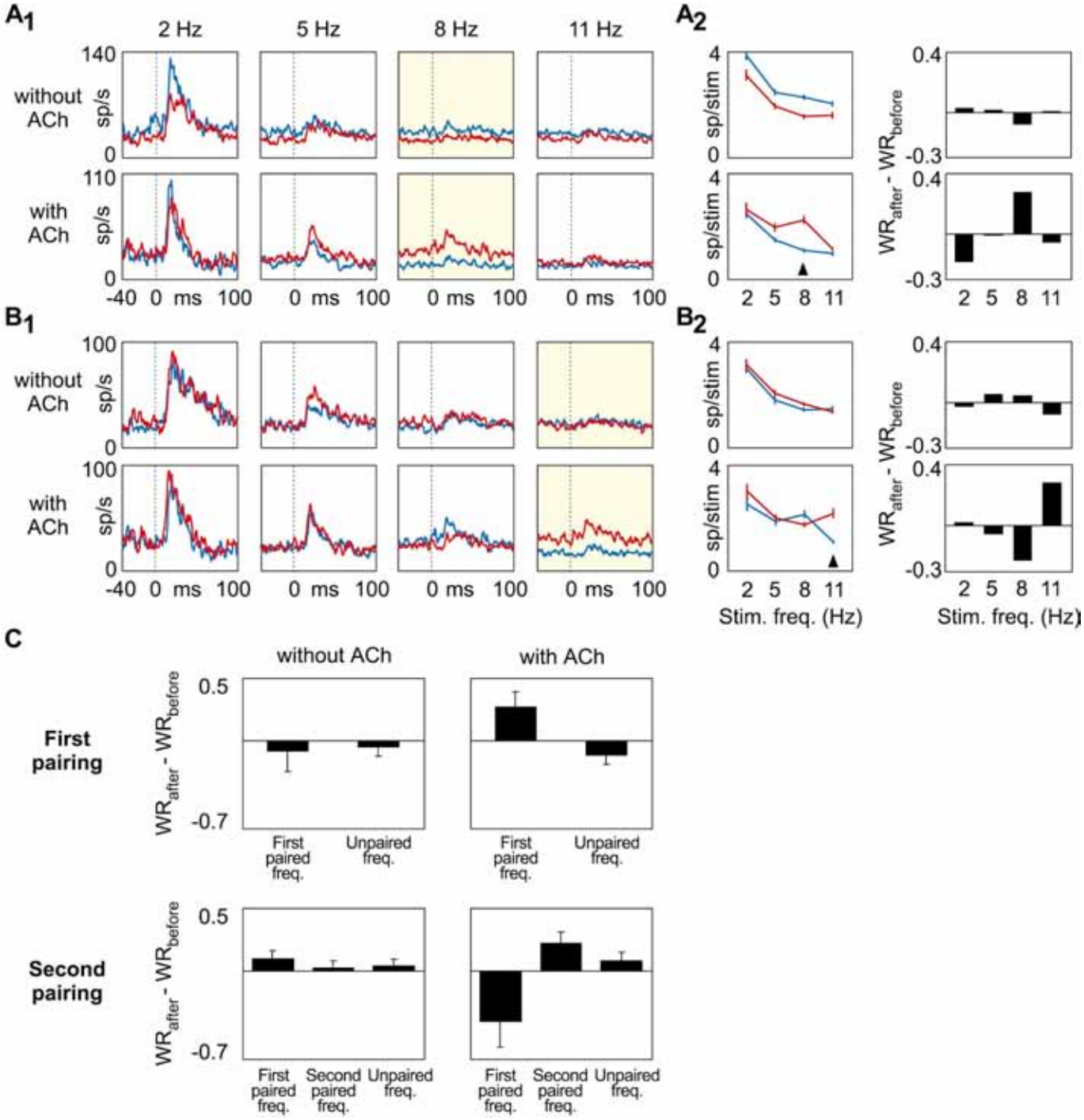

FIG. 9. Reversal of the response potentiation by a 2nd pairing at a different frequency. PSTHs of response before (blue) and after (red) a 1st pairing at 8 Hz (*A1*) and a 2nd pairing at 11 Hz (*B1*), for a single unit recorded at depth 1,310 μm. *A2* and *B2*: TFTCs and response changes ($WR_{after} - WR_{before}$). After the 1st pairing, the response to the paired frequency (8 Hz) was significantly modified when tested with ACh (KS, $P < 0.0002$). The 2nd pairing reversed this effect and induced a frequency-specific change for the newly paired frequency (11 Hz, KS, $P < 1.10^{-6}$). Notice that the response to the initially paired frequency (8 Hz) was significantly depressed by the 2nd pairing (KS, $P < 0.0002$). *C*: response differences (mean ± SE) for paired and unpaired frequencies averaged across a group of units that expressed a significant potentiation for the 1st paired frequency and on which we applied a subsequent pairing with a 2nd paired frequency ($n = 5$).

pairings, first at 8 Hz (Fig. 9*A;* KS, $P < 0.0002$) and then at 11 Hz (Fig. 9*B;* KS, $P < 1.10^{-6}$).

Overall, 29 units were tested with two different pairing frequencies. In five cases, the response to stimulation at the first paired frequency was potentiated after pairing when tested with ACh. Figure 9*C* shows the average response differences for paired and unpaired frequencies when tested without and with ACh for these units. After the first pairing, response at the paired frequency under ACh iontophoresis was enhanced compared with unpaired frequencies (1-tailed Student's *t*-test, $P <$

0.01). The second pairing induced an ACh-dependent enhancement of response for the newly paired frequency (1-tailed Student's *t*-test, $P < 0.05$ compared with all other frequencies) and a decrease in response to stimulation at the initially paired frequency (1-tailed Student's *t*-test, $P < 0.001$). These results suggest that the frequency selectivity of units is affected both by potentiations of response to stimulation at paired frequencies and depotentiations of response for other previously enhanced frequencies.

Kinetics of the potentiated responses

Of the 24 cases of significant potentiations, the tonic component of the response was significantly increased in 9 cases (see Fig. 4*A* for an example of enhanced tonic component and Fig. 5*B* for potentiation of the phasic response following each deflection only). In four of these nine cases, the increase in the absolute stimulus-locked spike count could be fully explained by the change in the tonic component. These results indicated that the modification was not temporally restricted to the epochs of afferent input activation; rather they suggest a prolonged change in activity during stimulation trains.

Retrieval of the potentiated response required both stimulation at the paired frequency and the application of ACh. Since stimulation at the paired frequency was presented in blocks of 12 trains, the potentiation could in principle either appear de novo during each stimulation train, in which case the response to the first deflection of the trains should not be potentiated, or could develop during the entire block, affecting the response to all deflections in the trains. A comparison of the potentiations of the responses to the first deflections with those of the responses to the subsequent deflections revealed similar potentiations. Figure 10, *A* and *B*, shows the results for the three different groups of significantly modified units submitted to pairings at 5-, 8-, and 11-Hz stimulation. Both the response to the first deflection of the train and the response during the steady state were significantly increased after pairing when testing with ACh (2-tailed paired Student's *t*-test, 1st deflection, $P < 1.10^{-4}$, steady state, $P < 1.10^{-10}$). Thus the potentiation has a slow kinetics compatible with the time scale of a block of stimulation (tens of seconds) rather than that of a single train (seconds).

Sequential averaging of triplets of trains for units potentiated at 8 Hz (Fig. 10*C*) depicts this slow retrieval kinetics. During the first three trains, the average potentiation was not significant (2-tailed paired Student's *t*-test, $P > 0.5$). However, during the remaining nine trains, potentiation was significant for the entire train, including the first deflection (2-tailed paired Student's *t*-test, $P < 0.02$ for each 3-train average).

This slow kinetics developed de novo for each block of stimulation at the paired frequency. Figure 11*A* shows four examples of the time course of steady-state response potentiation when units were tested with ACh at the paired frequency.

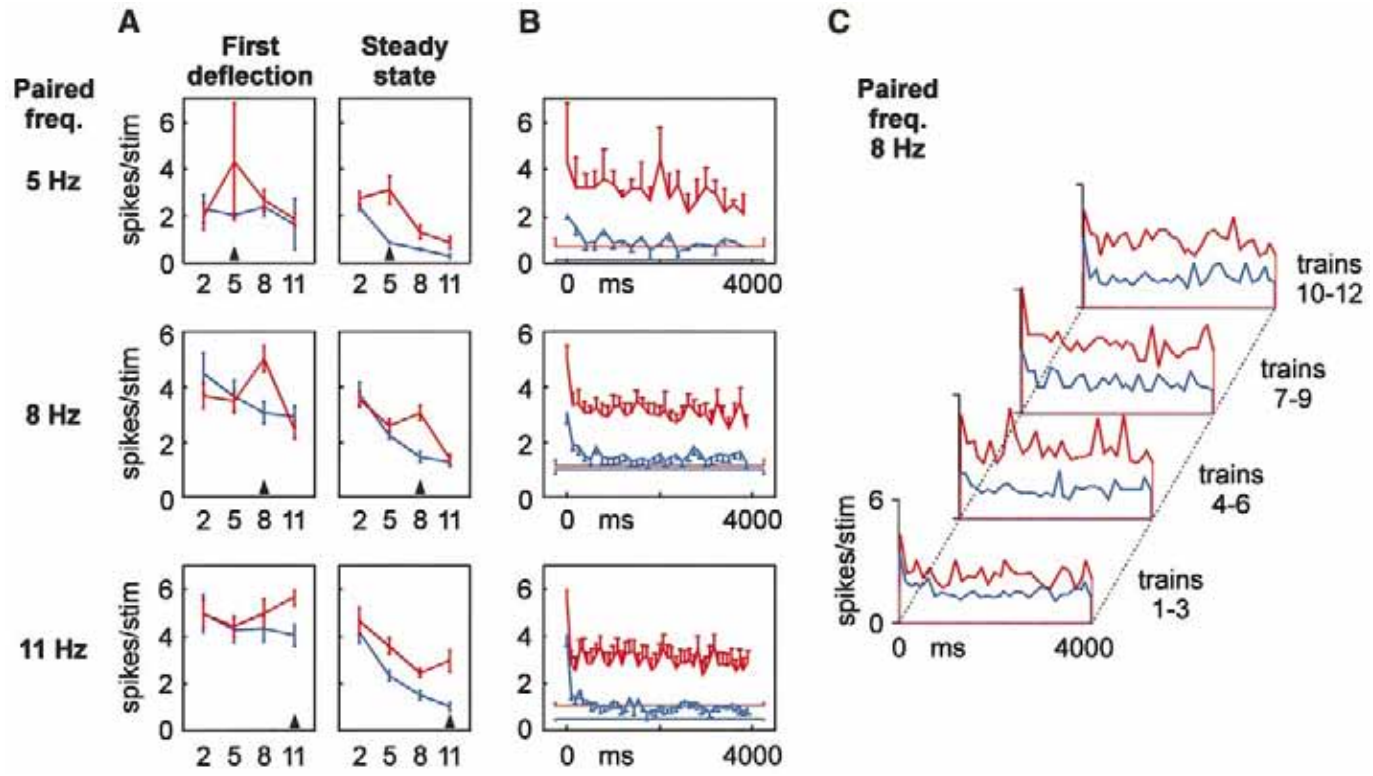

FIG. 10. Potentiation of the response to the 1st and subsequent whisker deflections within trains of stimulation. *A*: TFTCs before (blue) and after (red) pairing obtained during ACh iontophoresis for the response to the 1st deflection of the trains (*left*) and the response in the steady state (*right*), averaged across all units that expressed a significant potentiation for the paired frequency after pairing at 5 (*top*; $n = 3$), 8 (*middle*; $n = 14$) and 11 Hz (*bottom*; $n = 7$). *B*: average spike count in response to each of the deflections for trains at the paired frequency before (blue) and after (red) pairing for the same 3 groups of units (same normalization as in Fig. 3). *C*: average spike count in response to each of the deflections for *trains 1–3*, *4–6*, *7–9*, and *10–12* of the 2 blocks of stimulation at the paired frequency before (blue) and after (red) pairing, for the group of units paired at 8 Hz and expressing a significant potentiation (same normalization as in Fig. 3).

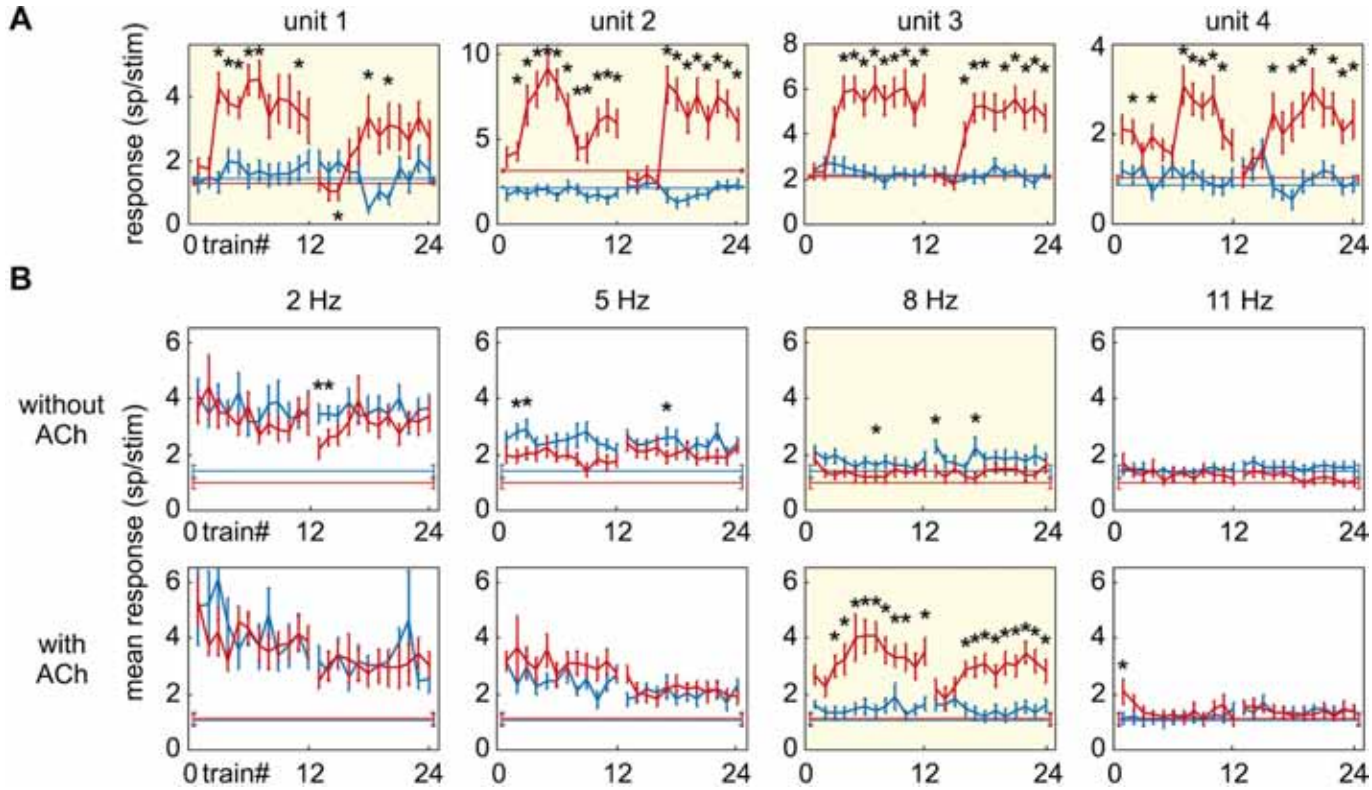

FIG. 11. Slow kinetics of the response potentiation in the steady state. *A*: average spike count before (blue) and after (red) pairing in response to the 2 blocks of 12 trains of stimulation at the paired frequency during ACh iontophoresis, for four different units submitted to a pairing at 8 Hz. Straight lines indicate spontaneous activity levels. Asterisks indicate significant changes in response after pairing compared with before pairing (two-tailed paired Student's *t*-test, $P < 0.05$). *B*: average spike count before (blue) and after (red) pairing in response to the 2 blocks of 12 trains of stimulation at 2, 5, 8 and 11 Hz and in the two test conditions without and with ACh, for the group of units paired at 8 Hz and expressing a significant potentiation (same normalization as in Fig. 3). Asterisks indicate significant changes in response after pairing compared with before pairing (two-tailed paired Student's *t*-test, $P < 0.05$).

The potentiation was expressed after a delay of two to five trains at the beginning of each block of stimulation. Averaging across all units expressing a significant potentiation at 8 Hz (Fig. 11*B*) revealed that the potentiation was statistically significant after a delay of two trains for the first block and three trains for the second block of stimulation (2-tailed paired Student's *t*-test, $P < 0.05$). By contrast, no specific time course was observed on the response to other frequencies during ACh iontophoresis. Note however that the response to the first train of stimulation at 11 Hz, which follows the first block of stimulation at the paired frequency, was potentiated after pairing, indicating a prolonged effect of the potentiation between blocks. When units were tested without ACh, no change in response was observed or a depression without a specific time course which occasionally reached significance.

DISCUSSION

Pairing ACh iontophoresis with mechanical deflections of the principal vibrissa at a given frequency produced specific potentiations of the response of barrel cortex neurons selective of the paired frequency, the expression of which depended on the presence of ACh. Herein, we demonstrated that these modifications were frequency-specific, cumulative, could be saturated and reversed. This ACh-dependent potentiation also occurred and at an even higher proportion when an adjacent vibrissa rather than the principal vibrissa of the recorded neuron was stimulated. The potentiation was not immediately expressed on the presentation of the paired frequency but rather required several trains of stimulation at that frequency to be expressed.

Spontaneous and evoked activity in control conditions

Spontaneous activity and responses to mechanical stimulation in control conditions were similar to those obtained previously (Ahissar et al. 1997, 2000). Each train of deflections produced a maximal response to the first deflection in the train followed by a rapid decrease until the response stabilized at its steady-state value. Typically, temporal-frequency tuning curves showed decreasing steady-state responses to increasing frequencies of stimulation. The decrease in cortical response to deflections within a train of stimulation agrees with previous studies demonstrating suppressive effects for this range of inter-stimulus intervals (Shimegi et al. 1999; Simons 1985). This phenomenon may be partially explained by the decreased afferent activity from the thalamus in anesthetized animals (Ahissar et al. 2000; Diamond et al. 1992). Thalamocortical and intracortical mechanisms are also likely to participate. A decremental response of the thalamocortical connection arising from lemniscal thalamic nuclei was described for frequencies above 5 Hz (Castro-Alamancos and Connors 1996c). Also,

paired-pulse depression has been demonstrated in vitro on individual intracortical excitatory postsynaptic potentials in neocortex (Markram and Tsodyks 1996; Thomson et al. 1993) and could theoretically result in low-amplitude response to high frequencies of afferent inputs (Abbott et al. 1997). Additionally, intracellular recordings have revealed inhibitory postsynaptic potentials evoked by vibrissal stimulation that weaken subsequent responses occurring at inter-stimulus intervals in the time range studied here (Carvell and Simons 1988; Moore and Nelson 1998). The potentiations of response observed after pairing might result in part from a change in these thalamocortical and intracortical response properties (see following text).

Characteristics and effectiveness of the sensori-cholinergic pairings

Previous studies of ACh-dependent functional plasticity in the adult rat barrel cortex have mainly employed global manipulations of the cholinergic innervation, such as lesion of the NBM by the immunotoxin 192 IgG-saporin (Baskerville et al. 1997; Sachdev et al. 1998) or cholinergic blockade using systemically injected atropine sulfate (Maalouf et al. 1998). The disruption of the cortical cholinergic activity produced by these protocols consistently resulted in a reduced plasticity in the barrel cortex, demonstrating the critical role of ACh in the induction of plasticity. However, these studies did not investigate the requirement for ACh during the induction and the expression phases of plasticity. To determine the implication of ACh in these two phases, we designed a protocol in which the local concentration of ACh could be increased by iontophoresis. This technique enabled us to pair a specific sensory stimulation with ACh and test the response to a range of different stimuli both without and with ACh for the same units.

During the pairing protocol, mechanical stimulation at a fixed frequency associated with ACh iontophoresis lasted 2 min, a duration only twice longer than the duration of a single block at the same temporal-frequency during the control and test periods. Nonetheless, this short pairing protocol was sufficient to rapidly induce plasticity. The effect was not saturated after one pairing; the modifications could be further enhanced by applying additional pairing protocols. The short duration of pairing and the cumulative effect of a series of pairings suggest that we used a relatively "weak" protocol [for comparison, consider the extensive pairings performed in Kilgard and Merzenich (1998a)]. This is also supported by the fact that the modifications could be rapidly reversed by applying a second pairing at a different frequency. These considerations raise the possibility that modifications analogous to those revealed after pairing might have also been induced during the control periods in particular during the control period with ACh before pairing, i.e., the second TFTC determination. During that period, stimulation was applied in blocks of 1 min each, first from the lowest to the highest frequency and then in reverse order. It is possible that plastic changes were induced for each frequency and immediately reversed by stimulation at the next frequency. Furthermore some of these changes may have lasted beyond the duration of the TFTC. To estimate the incidence of lasting changes following TFTCs with ACh, we performed experiments during which pairing was not applied but the control and test periods were maintained. Indeed, unpaired units also exhibited significant modifications. However, there was a qualitative difference between the modifications obtained with and without pairing. Whereas the modifications obtained without pairing were generalized across a range of frequencies, those obtained after pairing were specific to the paired frequency. We conclude that even though the presence of ACh during the TFTC induces modifications, the presentation of multiple frequencies does not allow any single frequency to be strongly associated with the ACh.

Special care was taken to ensure that ejection currents did not affect neuronal activity. First, currents used for drug applications were in the range of 20–80 nA. These intensities usually do not by themselves affect neuronal activities (Purves 1981; Shulz et al. 1997). Second, balanced ejections were applied to minimize extracellular potential changes near the tip of the electrode. Indeed, our results revealed that the time course of the effects was generally too slow to be caused by DC effects. Moreover, the blockade of the effects by atropine (Shulz et al. 2000) confirmed the specific action of ACh on cholinergic receptors. Another strong indication that current effects were not involved comes from the fact that even though ACh was iontophoresed continuously during the test after pairing, the modifications were frequency specific. The potentiation revealed after pairing, including the increased background activity, was only observed within trains of stimulation at the paired frequency. If these modifications were due to current effects, they should presumably affect the evoked activity during the entire ACh iontophoresis period, a phenomenon that we did not observe.

Characteristics of the modifications of response induced by sensori-cholinergic pairings

Modifications observed after pairing were mainly increases of response to stimulation at the paired frequency, revealed only when ACh was re-supplied. The response at temporal frequencies other than the paired frequency was unchanged or changed to a lesser extent. Previous investigations in the auditory (Bakin and Weinberger 1996; Bjordahl et al. 1998; Edeline et al. 1994; Kilgard and Merzenich 1998b; Metherate and Weinberger 1989, 1990) and the somatosensory (Metherate et al. 1987, 1988b; Rasmusson and Dykes 1988; Tremblay et al. 1990b) cortex have shown that pairing sensory stimulation with either ACh iontophoresis or NBM stimulation produces lasting changes in response for the paired stimulus. Cortical mapping of the primary auditory area has confirmed these stimulus-specific alterations and has shown that the cortical area devoted to the paired tone frequency was increased after extensive pairing of NBM stimulation and tone presentation (Kilgard and Merzenich 1998a). In our study, the cortical area activated by the paired whisker is not expected to be significantly increased, first because only responses to a single stimulation frequency were potentiated, and second because we used a relatively weak pairing. Even though only one third of the neurons submitted to a pairing showed significant modifications, this subset of cells exhibited robust modifications and a consistent pattern of plasticity, including selectivity for the paired frequency, summation, saturation and reversibility. This percentage of changes may be due to the experimental methods used, which might not reveal the whole population potentially expressing plasticity, or alternatively may result

from the fact that ACh influence is restricted to a subset of cortical cells.

In all the aforementioned studies, the plastic changes were measured during testing without ACh iontophoresis or stimulation of the NBM. By contrast in our protocol, the expression of frequency-specific potentiations depended on the presence of ACh during testing. Part of this discrepancy can be explained by the fact that protocols used previously in the literature generally employed more massive pairings that could eventually render the expression of plasticity less dependent on the presence of ACh. It would be also interesting to determine whether, in those cases, retesting with ACh unmasks more robust and consistent changes. The implication of ACh in the expression of plasticity in the barrel cortex is supported by a study (Delacour et al. 1990) made in the awake rat using a whisker-pairing protocol. The pairing resulted in an enhanced response, the expression of which was blocked by the exogenous application of atropine, indicating that the effect depended on the effectiveness of endogenous ACh. As this study was performed on awake animals, ACh might have been already present at a sufficient concentration at the time of sensory pairing to induce plasticity, and during tests of response to express changes (Sarter and Bruno 1997). The "control" and "test" periods would then be equivalent to our "with ACh" condition, whereas the "atropine" periods would correspond to our "without ACh" condition.

Possible mechanisms of the pairing-induced potentiation of response

Possible mechanisms underlying the induction and expression of the plasticity described here are highly constrained by characteristics such as the frequency specificity of the expressed potentiation, the dependence on ACh, and the kinetics observed during retrieval.

In control conditions, barrel cortex neurons exhibited decreased spike counts in response to increasing frequencies of stimulation. This is due to the decrease in evoked activity from one deflection to the following within a train. The cortical mechanisms involved in this low-pass filtering might have been affected by the pairings. In particular, the removal of the rapid decrease in response within trains at the paired frequency should produce an increase in the steady-state response and could contribute to the transformation to a band-pass filtering described here after pairing. However, we report in addition that after pairing, the response to the first deflection of each train was also significantly enhanced, indicating a prolonged effect from one train to another within blocks of stimulation. This increase in response cannot be explained by modifications of the kinetics within trains and must be attributed to an independent mechanism. Moreover, the analysis of the time course of the potentiation of response revealed a delay in its establishment lasting tens of seconds. These results imply that the expression of plasticity under ACh involves a mechanism triggered by the presentation of the paired temporal frequency, developing with a slow kinetics, and, once established, retained during at least 1 s (the inter-train interval).

The enhanced responses could result from plasticity at the level of the thalamocortical connections, the intracortical circuitry, or changes in the intrinsic properties of the cortical cells. ACh diffusion diameter in cortical tissue during prolonged iontophoresis was estimated in the 300-μm range (Haidarliu and Ahissar, unpublished observations), supporting the idea of a localized effect of ACh in our protocol. We also report a lack of expressed modifications in units distant from the iontophoresis electrode (recorded by a TE). Taken together, these findings argue in favor of a local modification induced and expressed by the neuronal microcircuits in the immediate vicinity of the electrode.

Repetitive stimulation at 7–14 Hz of some thalamocortical pathways induces a rapid enhancement of cortical responses known as the augmenting response (Castro-Alamancos and Connors 1996a; Morison and Dempsey 1942). One interesting hypothesis is that our pairing would result in the involvement of these augmenting responses. However, the augmenting response develops during the first two to three stimulations of thalamocortical pathways, whereas we did not observe rapid increases in response within trains but rather rapid decreases. Also, the augmenting response is modulated by the behavioral state of the animal (Castro-Alamancos and Connors 1996b; Steriade and Morin 1981) and is abolished during states of arousal, when the cortical release of ACh is high. In our case, on the contrary, the increased effects were observed during ACh application.

In anesthetized rats, thalamic neurons respond in a low-pass manner similar to cortical neurons (Ahissar et al. 2000; Diamond et al. 1992). Consequently at the cortical level, all thalamocortical projections that are active at high frequencies are also presumably active at low frequencies. This implies that the sets of afferents active for different temporal frequencies of stimulation strongly overlap. Because of this lack of input separability, it is hardly conceivable that the frequency specificity of the modifications could arise from synaptic changes in a subset of thalamocortical connections. Indeed, such synaptic changes would also be expressed at frequencies lower than the paired frequency. We did not find such an asymmetry in the profile of changes after pairing. Our observations are thus more compatible with a participation of intracortical mechanisms (see also Fox et al. 2000; Wallace and Fox 1999).

The expression of these intracortical changes was shown to be specific to the paired frequency. Several hypotheses concerning the underlying cellular mechanisms are considered. First, similar to the mechanisms generating the augmenting response (Castro-Alamancos 1997; Castro-Alamancos and Connors 1996a), specific membrane conductances can be activated or de-inactivated within a precise time window after each spike (or short burst of spikes), resulting in an increased response to afferent activity at a specific frequency. However, this mechanism should reset a few hundred of milliseconds after the end of each train of stimulation. Thus the response to the first deflection of the following train in our protocol should not be potentiated since the inter-train interval exceeds that time window. Our observation that the response to the first deflection of each train of stimulation was also potentiated led us to reject this hypothesis.

Second, recent studies have shown that a single synapse may exhibit different time constants of rapid depression and facilitation so that, depending on the value of these parameters, it transmits information with different filtering characteristics (Markram and Tsodyks 1996; Tsodyks and Markram 1997). In this framework, stimulation of the whisker at one frequency would be preferentially propagated through the cortical net-

work by synapses transmitting efficiently that frequency. Synaptic plasticity was interpreted by these authors in terms of modifications of the frequency dependence of transmission (Markram et al. 1998a). Thus the band-pass TFTCs observed after pairing could be explained by band-pass dependence of synaptic transmissions on the frequency of stimulation. However, the frequency dependence of the net synaptic effect is usually of a low-pass or high-pass nature (Gupta et al. 2000; Markram et al. 1998b). Modifications in synaptic time constants could increase or decrease the cutoff frequencies of these transfer functions but could not create a band-pass transfer function for an individual synapse. Thus our results could be explained in this framework only if changes in high- and low-pass synapses occurred in a coordinated manner in the cortical network to produce a net band-pass transmission.

Another possibility is that each frequency selectively activates a subset of neuronal circuits. As we usually did not observe frequency-specific cells for frequencies higher than 2 Hz before pairing, we have to suppose that these circuits are bound together by other means than an absolute increase in activity, for example, through synchronization of their different members. Other potential circuits are those that contain oscillatory units (Ahissar 1998). Frequency-specific changes can be induced in these circuits by changing their working parameters such as the input-output transfer functions of the oscillatory units (Ahissar et al. 1997) or of the other members of the circuit. In agreement with this explanation is the observation that oscillatory neurons of the somatosensory cortex often exhibit slow locking in kinetics (Ahissar, unpublished observations), with a time scale that is similar to that of the slow retrieval kinetics observed herein.

Probably the most challenging constraint imposed by our data is that, whatever the underlying mechanism, its expression depends on ACh. The differential effect of ACh on the multiple types of interneurons in the cortex (Kawaguchi 1997; Xiang et al. 1998) enables switching between different neuronal circuits. Thus ACh may switch between circuits that are tuned to different frequencies (Ahissar et al. 1997) by changing the balance between recurrent inhibitory pathways (Reyes et al. 1998). Further studies are needed to distinguish between these possible explanations.

We thank Y. Frégnac for helpful comments on the manuscript.

This work was supported by Programme International de Cooperation Scientifique Centre National de la Recherche Scientifique, Ministère des Affaires Etrangères Français, Association Franco-Israélienne pour la Recherche Scientifique et Technique, Human Frontier Science Program, the Abramson Family Foundation (USA), and the MINERVA Foundation (Germany).

REFERENCES

ABBOTT LF, VARELA JA, SEN K, AND NELSON SB. Synaptic depression and cortical gain control. *Science* 275: 220–224, 1997.

AHISSAR E. Temporal-code to rate-code conversion by neuronal phase-locked loops. *Neural Comput* 10: 597–650, 1998.

AHISSAR E, HAIDARLIU S, AND ZACKSENHOUSE M. Decoding temporally encoded sensory input by cortical oscillations and thalamic phase comparators. *Proc Natl Acad Sci USA* 94: 11633–11638, 1997.

AHISSAR E, SOSNIK R, AND HAIDARLIU S. Transformation from temporal to rate coding in a somatosensory thalamocortical pathway. *Nature* 406: 302–306, 2000.

ARMSTRONG-JAMES M, DIAMOND ME, AND EBNER FF. An innocuous bias in whisker use in adult rats modifies receptive fields of barrel cortex neurons. *J Neurosci* 14: 6978–6991, 1994.

BAKIN JS AND WEINBERGER NM. Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. *Proc Natl Acad Sci USA* 93: 11219–11224, 1996.

BASKERVILLE KA, SCHWEITZER JB, AND HERRON P. Effects of cholinergic depletion on experience-dependent plasticity in the cortex of the rat. *Neuroscience* 80: 1159–1169, 1997.

BEAR MF AND SINGER W. Modulation of visual cortical plasticity by acetylcholine and noradrenaline. *Nature* 320: 172–176, 1986.

BJORDAHL TS, DIMYAN MA, AND WEINBERGER NM. Induction of long-term receptive field plasticity in the auditory cortex of the waking guinea pig by stimulation of the nucleus basalis. *Behav Neurosci* 112: 467–479, 1998.

CARVELL GE AND SIMONS DJ. Membrane potential changes in rat SmI cortical neurons evoked by controlled stimulation of mystacial vibrissae. *Brain Res* 448: 186–191, 1988.

CASTRO-ALAMANCOS MA. Short-term plasticity in thalamocortical pathways: cellular mechanisms and functional roles. *Rev Neurosci* 8: 95–116, 1997.

CASTRO-ALAMANCOS MA AND CONNORS BW. Cellular mechanisms of the augmenting response: short-term plasticity in a thalamocortical pathway. *J Neurosci* 16: 7742–7756, 1996a.

CASTRO-ALAMANCOS MA AND CONNORS BW. Short-term plasticity of a thalamocortical pathway dynamically modulated by behavioral state. *Science* 272: 274–277, 1996b.

CASTRO-ALAMANCOS MA AND CONNORS BW. Spatiotemporal properties of short-term plasticity sensorimotor thalamocortical pathways of the rat. *J Neurosci* 16: 2767–2779, 1996c.

CHAPIN JK AND LIN CS. Mapping the body representation in the SI cortex of anesthetized and awake rats. *J Comp Neurol* 229: 199–213, 1984.

DELACOUR J, HOUCINE O, AND COSTA JC. Evidence for a cholinergic mechanism of "learned" changes in the responses of barrel field neurons of the awake and undrugged rat. *Neuroscience* 34: 1–8, 1990.

DIAMOND ME, ARMSTRONG-JAMES M, AND EBNER FF. Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus. *J Comp Neurol* 318: 462–476, 1992.

DIAMOND ME, ARMSTRONG-JAMES M, AND EBNER FF. Experience-dependent plasticity in adult rat barrel cortex. *Proc Natl Acad Sci USA* 90: 2082–2086, 1993.

DYKES RW. Mechanisms controlling neuronal plasticity in somatosensory cortex. *Can J Physiol Pharmacol* 75: 535–545, 1997.

EDELINE JM, HARS B, MAHO C, AND HENNEVIN E. Transient and prolonged facilitation of tone-evoked responses induced by basal forebrain stimulations in the rat auditory cortex. *Exp Brain Res* 97: 373–386, 1994.

FOX K, GLAZEWSKI S, AND SCHULZE S. Plasticity and stability of somatosensory maps in thalamus and cortex. *Curr Opin Neurobiol* 10: 494–497, 2000.

GREUEL JM, LUHMANN HJ, AND SINGER W. Pharmacological induction of use-dependent receptive field modifications in the visual cortex. *Science* 242: 74–77, 1988.

GUPTA A, WANG Y, AND MARKRAM H. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science* 287: 273–278, 2000.

HAIDARLIU S. An anatomically adapted, injury-free headholder for guinea pigs. *Physiol Behav* 60: 111–114, 1996.

HAIDARLIU S, SHULZ D, AND AHISSAR E. A multi-electrode array for combined microiontophoresis and multiple single-unit recordings. *J Neurosci Methods* 56: 125–131, 1995.

HASSELMO ME AND BOWER JM. Acetylcholine and memory. *Trends Neurosci* 16: 218–222, 1993.

IZQUIERDO I. Endogenous state dependency: memory depends on the relation between the neurohumoral and hormonal states present after training and at the time of testing. In: *Neurobiology of Learning and Memory*, edited by Lynch G, McGaugh JL, and Weinberger NM. New York: Guilford, 1984, p. 333–350.

KAWAGUCHI Y. Selective cholinergic modulation of cortical GABAergic cell subtypes. *J Neurophysiol* 78: 1743–1747, 1997.

KILGARD MP AND MERZENICH MM. Cortical map reorganization enabled by nucleus basalis activity. *Science* 279: 1714–1718, 1998a.

KILGARD MP AND MERZENICH MM. Plasticity of temporal information processing in the primary auditory cortex. *Nat Neurosci* 1: 727–731, 1998b.

MAALOUF M, MIASNIKOV AA, AND DYKES RW. Blockade of cholinergic receptors in rat barrel cortex prevents long-term changes in the evoked potential during sensory preconditioning. *J Neurophysiol* 80: 529–545, 1998.

MARKRAM H, GUPTA A, UZIEL A, WANG Y, AND TSODYKS M. Information processing with frequency-dependent synaptic connections. *Neurobiol Learn Mem* 70: 101–112, 1998a.

MARKRAM H AND TSODYKS M. Redistribution of synaptic efficacy between neocortical pyramidal neurons. *Nature* 382: 807–810, 1996.

MARKRAM H, WANG Y, AND TSODYKS M. Differential signaling via the same axon of neocortical pyramidal neurons. *Proc Natl Acad Sci USA* 95: 5323–5328, 1998b.

METHERATE R, TREMBLAY N, AND DYKES RW. Acetylcholine permits long-term enhancement of neuronal responsiveness in cat primary somatosensory cortex. *Neuroscience* 22: 75–81, 1987.

METHERATE R, TREMBLAY N, AND DYKES RW. The effects of acetylcholine on response properties of cat somatosensory cortical neurons. *J Neurophysiol* 59: 1231–1252, 1988a.

METHERATE R, TREMBLAY N, AND DYKES RW. Transient and prolonged effects of acetylcholine on responsiveness of cat somatosensory cortical neurons. *J Neurophysiol* 59: 1253–1276, 1988b.

METHERATE R AND WEINBERGER NM. Acetylcholine produces stimulus-specific receptive field alterations in cat auditory cortex. *Brain Res* 480: 372–377, 1989.

METHERATE R AND WEINBERGER NM. Cholinergic modulation of responses to single tones produces tone-specific receptive field alterations in cat auditory cortex. *Synapse* 6: 133–145, 1990.

MOORE CI AND NELSON SB. Spatio-temporal subthreshold receptive fields in the vibrissa representation of rat primary somatosensory cortex. *J Neurophysiol* 80: 2882–2892, 1998.

MORISON RS AND DEMPSEY EW. A study of thalamo-cortical relations. *Am J Physiol* 135: 281–292, 1942.

PURVES RD. *Microelectrode Methods for Intracellular Recordings and Ionophoresis.* New York: Academic, 1981.

RASMUSSON DD AND DYKES RW. Long-term enhancement of evoked potentials in cat somatosensory cortex produced by co-activation of the basal forebrain and cutaneous receptors. *Exp Brain Res* 70: 276–286, 1988.

REYES A, LUJAN R, ROZOV A, BURNASHEV N, SOMOGYI P, AND SAKMANN B. Target-cell-specific facilitation and depression in neocortical circuits. *Nat Neurosci* 1: 279–285, 1998.

SACHDEV RN, LU SM, WILEY RG, AND EBNER FF. Role of the basal forebrain cholinergic projection in somatosensory cortical plasticity. *J Neurophysiol* 79: 3216–3228, 1998.

SARTER M AND BRUNO JP. Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. *Brain Res Rev* 23: 28–46, 1997.

SCHNEIDER W. The tactile array stimulator. *John Hopkins APL Tech Digest* 9: 39–43, 1988.

SHIMEGI S, ICHIKAWA T, AKASAKI T, AND SATO H. Temporal characteristics of response integration evoked by multiple whisker stimulations in the barrel cortex of rats. *J Neurosci* 19: 10164–10175, 1999.

SHULZ DE, COHEN S, HAIDARLIU S, AND AHISSAR E. Differential effects of acetylcholine on neuronal activity and interactions in the auditory cortex of the guinea-pig. *Eur J Neurosci* 9: 396–409, 1997.

SHULZ DE, SOSNIK R, EGO V, HAIDARLIU S, AND AHISSAR E. A neuronal analogue of state-dependent learning. *Nature* 403: 549–553, 2000.

SIMONS DJ. Temporal and spatial integration in the rat SI vibrissa cortex. *J Neurophysiol* 54: 615–635, 1985.

SINGER W. Ontogenetic self-organization and learning. In: *Brain Organization and Memory: Cells, Systems and Circuits*, edited by McGaugh JL, Weinberger NM, and Lynch G. New York: Oxford Univ. Press, 1990, p. 211–233.

STERIADE M AND MORIN D. Reticular influences on primary and augmenting responses in the somatosensory cortex. *Brain Res* 205: 67–80, 1981.

THOMSON AM, DEUCHARS J, AND WEST DC. Large, deep layer pyramid-pyramid single axon EPSPs in slices of rat motor cortex display paired pulse and frequency-dependent depression, mediated presynaptically and self-facilitation, mediated postsynaptically. *J Neurophysiol* 70: 2354–2369, 1993.

TREMBLAY N, WARREN RA, AND DYKES RW. Electrophysiological studies of acetylcholine and the role of the basal forebrain in the somatosensory cortex of the cat. I. Cortical neurons excited by glutamate. *J Neurophysiol* 64: 1199–1211, 1990a.

TREMBLAY N, WARREN RA, AND DYKES RW. Electrophysiological studies of acetylcholine and the role of the basal forebrain in the somatosensory cortex of the cat. II. Cortical neurons excited by somatic stimuli. *J Neurophysiol* 64: 1212–1222, 1990b.

TSODYKS MV AND MARKRAM H. The neural code between neocortical pyramidal neurons depends on neurotransmitter release probability. *Proc Natl Acad Sci USA* 94: 719–723, 1997.

WALLACE H AND FOX K. Local cortical interactions determine the form of cortical plasticity. *J Neurobiol* 41: 58–63, 1999.

WEINER N. Atropine, scopolamine, and related antimuscarinic drugs. In: *The Pharmacological Basis of Therapeutics*, edited by Goodman LS and Gilman A. New York: Macmillan, 1980, p. 121.

XIANG Z, HUGUENARD JR, AND PRINCE DA. Cholinergic switching within neocortical inhibitory networks. *Science* 281: 985–988, 1998.

ZORNETZER SF. Neurotransmitter modulation and memory: a new pharmacological phrenology? In: *Psychopharmacology: A Generation of Progress*, edited by Lipton MA, Di Mascio A, and Killam KF. New York: Raven, 1978, p. 646.