Induction of Long-Term Receptive Field Plasticity in the Auditory Cortex of the Waking Guinea Pig by Stimulation of the Nucleus Basalis

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Learning induces neuronal receptive field (RF) plasticity in primary auditory cortex. This plasticity constitutes physiological memory as it is associative, highly specific, discriminative, develops rapidly, and is retained indefinitely. This study examined whether pairing a tone with activation of the nucleus basalis could induce RF plasticity in the waking guinea pig and, if so, whether it could be retained for 24 hr. Subjects received 40 trials of a single frequency paired with electrical stimulation of the nucleus basalis (NB) at tone onset. The physiological effectiveness of NB stimulation was assessed later while subjects were anesthetized with urethane by noting whether stimulation produced cortical desynchronization. Subjects in which NB stimulation was effective did develop RF plasticity and this was retained for 24 hr. Thus, activation of the NB during normal learning may be sufficient to induce enduring physiological memory in auditory cortex.

The search for the mechanisms of learning and memory continues to be a central problem in neuroscience. The approach of recording neurophysiological correlates of behavioral learning is advantageous in potentially locating a site of (probably distributed) storage and in providing an approximation of the actual acquired information. A combination of neurophysiological correlates of behavioral learning with a controlled stimulation of designated circuits has the additional benefit of providing for tests of candidate mechanisms of a behaviorally induced neurophysiological index of information storage by determining whether direct activation of a designated brain circuit produces the same neurophysiological correlates as those that are induced during behavioral learning.

However, there are actually very few neurophysiological correlates of behavioral learning that meet the criteria for indexing the storage of memories, particularly in the cerebral neocortex, which is generally agreed to be a major site of acquired information. In the case of associative declarative memory (as opposed to procedural or sensorimotor skill memory), these criteria include the following: The neurophysiological phenomenon must be (a) induced during normal learning in behaving subjects, (b) associative, (c) highly specific to the information acquired, (d) induced very rapidly, and (e) retained for behaviorally relevant durations (e.g., weeks or months).

The only neurophysiological phenomenon in the cerebral neocortex that currently meets all of these criteria is learning-induced receptive field (RF) plasticity. This is induced during Pavlovian (classical) conditioning in which a pure tone conditioned stimulus (CS) is paired with an aversive unconditional stimulus (US). This stimulus–stimulus association, which develops more rapidly than does stimulus–response association (Konorski, 1967; Lennartz & Weinberger, 1992, 1994; Schlosberg, 1937), produces systematic modification of neuronal frequency RFs in the primary auditory cortex (ACx). In general, frequency tuning is shifted toward or even to the frequency of the CS because responses to the CS frequency increase, whereas responses to the pretrained best frequency (BF; i.e., the frequency at the peak of the tuning curve) and other frequencies decrease or change less.

Associative RF plasticity is induced by normal learning experiences in waking animals (Bakin, Lepan, & Weinberger, 1992; Bakin & Weinberger, 1990), is highly specific to the CS frequency (Bakin et al., 1992; Edeline & Weinberger, 1993), develops in only a few trials (Edeline, Pham, & Weinberger, 1993), and is retained for weeks and months (Weinberger, Javid, & Lepan, 1993). Because learning-induced RF plasticity has all of these characteristics of behavioral memory, it may be said to constitute physiological memory.

The cholinergic projections to the ACx from the nuclei basalis (NB; Hartgraves, Mensah, & Kelly, 1982; Johnston, McKinney, & Coyle, 1979, 1981; Lehman, Nagy, Atmadja, & Fibiger, 1980; Mesulam, Muñson, Levey, & Wainer, 1983) have been hypothesized to be critically involved in learning-induced RF plasticity in the ACx (Weinberger, Ashe, Diamond, et al., 1990; Weinberger, Ashe, Metherate, et al., 1990). It has long been known that blockade of the central cholinergic system impairs learning, particularly that involving the forebrain, both in animals (Deutsch, 1971; reviewed...
whether indeed pairing a tone with NB stimulation in the cortex, then it should be demonstrable in the waking state. The purpose of the current experiment was to determine effective sites in the NB.

Additionally, the neuromodulatory actions of ACh (Woody, Swartz, & Gruen, 1978) have been implicated in adult sensory cortical plasticity. For example, ACh produces long-lasting facilitation of responses to cutaneous stimulation when applied either cortically (Metherate, Tremblay, & Dykes, 1988a, 1988b) or released by stimulation of the basal forebrain (Rasmussen & Dykes, 1988; Tremblay, Warren, & Dykes, 1990; Webster et al., 1991). Of particular relevance, the involvement of muscarinic receptors in the cortex appears to be necessary for RF plasticity of a simple form of learning: sensory preconditioning (Delacour, Houcine, & Costa, 1990). Of greatest relevance, ACh has been implicated in RF plasticity in the ACx. Iontophoretic application of muscarinic agonists (McKenna, Ashe, & Weinberger, 1989) or anticholinesterases (Ashe, McKenna, & Weinberger, 1989) produces lasting modification of frequency tuning. Stimulation of the NB produces atropine-sensitive lasting modification of evoked responses in the ACx, including facilitation of field potentials, cellular discharges and excitatory postsynaptic potentials elicited by medial geniculate stimulation (Metherate & Ashe, 1991, 1993), and facilitation of neuronal discharges to tones (Edeline, Hars, Maho, & Hennevin, 1994; Edeline, Maho, Hars, & Hennevin, 1994; Hars, Maho, Edeline, & Hennevin, 1993). Furthermore, pairing a tone with the iontophoretic application of muscarinic agonists produces pairing-specific, atropine-sensitive modification of RFs that include shifts of tuning to or toward the frequency of the paired tone (Metherate & Weinberger, 1990). Finally, neurons in the NB develop discharge plasticity to stimuli that signal reinforcement (Maho, Hats, Edeline, & Hennevin, 1995; Richardson & DeLong, 1986).

It has been found recently that pairing a tone with stimulation of the NB can induce RF plasticity in the ACx, in urethane-anesthetized guinea pigs (Bakin & Weinberger, 1996). The effects remained 30 min after pairing, the longest time tested. Also, they were found to be associative because sensitization control subjects failed to develop RF plasticity. The involvement of muscarinic receptors in the ACx was implicated because application of atropine to the ACx also blocked EEG desynchronization produced by stimulation of effective sites in the NB.

If behavioral learning in the waking state normally engages the NB to promote information storage in the cortex, then it should be demonstrable in the waking state. The purpose of the current experiment was to determine whether indeed pairing a tone with NB stimulation in the waking guinea pig would be sufficient to induce RF plasticity. A second goal was to determine, in the case of such plasticity, whether its duration would approximate behaviorally relevant retention periods (e.g., 24 hr).

Method

Experimental Overview

An NB-stimulating electrode and cortical electrodes were implanted in adult guinea pigs during preparatory surgery. After a recovery period (7–25 days), they were prepared for a chronic experiment, in which a tone presentation was paired with NB stimulation. ACx RFs were determined for all responsive electrodes (i.e., those with frequency-tuned units) before and after pairing a non-BF tone with NB stimulation. Posttraining RFs were obtained at several intervals on the day of training (Day 1) and 24 hr later (Day 2).

Subjects and Preparatory Surgery

Adult male Hartley guinea pigs (342–504 g) were premedicated with atropine sulfate (0.22–0.33 mg/kg ip) and diazepam (9.0 mg/kg ip) to produce neuralgia 10 min before anesthetization with sodium pentobarbital (initial anesthetic dose = 25 mg/kg ip). Tail-pinch and eyeblink reflexes were assessed, and additional supplements of sodium pentobarbital (8.33 mg/kg) were administered to ensure a satisfactory reflexive state. The guinea pigs’ temperature (37 °C) was maintained using an adjustable heating pad. The guinea pigs were mounted in a stereotaxic instrument using blunt ear bars, and an ophthalmic ointment was applied (oxytetracycline) to prevent infection and drying of the cornea. A parasagittal incision was made and the skull exposed. An acrylic pedestal, containing fixation points for later attachment to a stereotaxic frame, was affixed to the surface of the skull and was anchored by several stainless steel screws. A silver ball (0.25 cm in diameter) was placed on the surface of the cortex to serve as a reference electrode during electrophysiological recording.

The pedestal was bolted to a stereotaxic support, and the ear bars were removed before ACx electrode implantation. A unilateral craniotomy over the left ACx was performed and dura mater resected under microscopic observation. The ACx was located by the species-characteristic cerebral vasculature. Acoustic responsiveness was confirmed by monitoring click evoked potentials at the cortical surface and during electrode implantation. To maximize the number of recordings from a given waking animal, we used chronically implanted arrays of electrodes. An array consisted of seven electrodes (varying in length from 250 to 1,750 μm and increasing in length by 250-μm increments, sterilized Teflon-coated tungsten wire, 50 μ in diameter) in a helical pattern, with each electrode being spaced approximately 200 μm from other electrodes, and the longest electrode was positioned in the middle of the helix. A stimulating electrode (bipolar twisted 100 μm, Formvar insulated stainless steel) was stereotaxically positioned into the left NB (target = 4.5 mm lateral, 0.6 to –1.6 anterior–posterior from bregma, and 6.0–8.25 ventral from pial surface) and affixed using dental acrylic. The incision was sutured, a topical antibiotic (Panalog, Solvay Animal Health, Mendota Heights, MN) was applied, and the guinea pigs were allowed to recover in an incubator. After recovery they were returned to their home cage, where they had continual access to food and water (12-hr light–dark cycle). All procedures were performed according to the guidelines of the University of California Irvine Animal Research Committee and National Institutes of Health.
Recording Sessions and RF Determinations

Unit clusters were recorded from responsive electrodes that had acceptable waveforms and clear-frequency tuning. Neuronal activity (2-5 waveforms) was amplified, filtered (300-3000 Hz), voltage discriminated, and stored in a Brainwave workstation (Brainwave Systems, Broomfield, CO). The spike-level discriminator was kept constant within an electrode for all RFs for a single experiment once the voltage was set for the pretraining RF determinations.

Acoustic Stimulation

The workstation generated a calibrated isointensity ascending frequency series of tone bursts (rise and fall = 10 ms, duration = 80 ms, and intertone interval = 400 ms). The discharges to 20 repetitions of these isointensity frequency series were determined at either 70 or 80 dB. The neural data were digitized (16 kHz per electrode average sampling rate, 32 points per waveform) and stored on a 386 computer with a data acquisition software package (DataWave Technologies, Longmont, CO).

Pairing Tone and NB Stimulation

Guinea pigs received 40 trials of a 1.0-s tone (CS) paired with NB stimulation, 500 ms at CS offset. The intertrial interval was 30-90 s (M = 60 s). The CS intensity matched the intensity used to assess RF stability during the pretraining sessions. For each guinea pig, the CS frequency was selected so that it was not the pretraining BF, to permit detection of possible shifts in tuning. The choice of CS frequency was based on selecting a frequency that exhibited responses 50% or more of the response of the BF, to permit easy detection of either an increased or decreased response. This resulted in the selection of CS frequencies that generally were within 0.5 octave of the BF.

Heart rate was monitored during the training sessions, and EEG was monitored during the terminal experiment. Heart rate and EEG were both filtered (1-1000 Hz), amplified (DAM 50, 10,000 gain; WPL, Sarasota, FL), and written out on a polygraph (Grass Model 7, Grass Instruments, Quincy, MA).

Quantification of Neuronal Responses

Frequency RFs were calculated for each assessment of auditory responsiveness before training (pretraining; "Pre 1-3") and seven posttraining retention intervals by subtracting the average discharge during a pretone period (200 ms) from the average discharge during a temporal window for each tone (10-30 ms window, which was the period of tuned onset responses). The evoked discharge to the 20 presentations of each tone was plotted against frequency. An evoked response across the frequency at the single intensity constituted an RF. The three pretraining RFs were averaged. RF difference functions for each posttraining retention period (Day 1, immediate, 20, 40, and 60 min; Day 2, 24 hr, 20 min, and 40 min later) were calculated by subtracting the postraining values from the pretraining average. These difference functions revealed the training effects.

For statistical analyses, we determined changes in the ratio of responses (spike rate) to the CS frequency and to the pretraining BF. The change in the CS:BF ratio was calculated as follows for each posttraining time period: \( \Delta \text{CS:BF} = (\text{post-CS/post-BF}) - (\text{pre-CS/pre-BF}) \).

Increases in \( \Delta \text{CS:BF} \) indicated a relative increase in the magnitude of response to the CS frequency after treatment, whereas decreases indicated the converse.

Training Protocol

The awake guinea pigs were placed in a hammock suspended from a frame for 1-3 hr for 1-4 days to adapt them to the experimental environment, making it possible to record neural responses without excessive movement artifacts. The guinea pigs’ head was affixed to the stereotaxic frame via the pedestal and positioned to approximate their normal waking posture, and their paws were allowed to rest on a supporting surface. A small speaker (Aiwa; calibrated through 0-90 dB SPL with a Bruel and Kjaer Type 4134 condenser microphone, 2204 sound level preamplifier, and Hewlett-Packard 3581A wave analyzer) contained in a housing was placed firmly against the ear canal contralateral to the ACx containing the electrode array. Multiple tone sequences were presented during adaptation. The entire apparatus was contained in an acoustic chamber (IAC Industries, Bronx, NY).

On Day 1 of the experiment, wound clips were placed on the guinea pigs’ thorax to monitor heartbeat. After placing them in the hammock in the position used during adaptation, we assessed the effectiveness of the NB-stimulating electrode. They were given NB stimulation trains at ascending current levels (from 100 \( \mu \)A increased in 50-100-\( \mu \)A increments) to determine the current level that would not produce a behavioral response as determined by observing the animals and their heart rate. The average current was 325 \( \mu \)A (100-400 \( \mu \)A, monophasic, 0.2-ms square wave, 200 Hz, total train duration = 500 ms, delivered by a Grass S88 stimulator and stimulus isolation unit).

An initial RF of a unit cluster was determined by presenting a tone sequence 20 times at a single intensity (70 or 80 dB; intersequence interval = 1.2 s). Several frequency ranges were used to determine the frequency representation of the cluster. After RFs had been determined, one frequency range was selected for the experiment and repeated several times at 20-min intervals to assess the stability of the RF.

After RF determination, the guinea pigs received conditioning (paired tone–NB stimulation) trials. Immediately after training, the RF was determined again using the identical tone series previously presented. RF characterization was repeated at intervals of 20, 40, and 60 min on Day 1. The animals were then returned to their home cage. On Day 2, the guinea pigs were placed in the hammock approximately 24 hr after Day 1 pairing. After a 15-min adaptation period, RFs were determined in exactly the same manner as on Day 1 (the “24-hr” period). Additional RFs were determined 20 (“24[20]”) and 40 (“24[40]”) min later. The animals were then returned to their home cage.

Terminal Experiment

In the main experiment we determined whether NB stimulation could induce RF plasticity. Independent assessment of the physiological effectiveness of NB stimulation also was obtained by determining whether it was sufficient to produce a predicted change in the EEG of the ACx, a shift from high-voltage slow waves to low-voltage fast waves (i.e., EEG desynchronization). This could not be accomplished in the waking state because the EEG is already desynchronized. Previous researchers have found that so-called “cortical arousal” cannot be assessed with the EEG when it is highly desynchronized (e.g., Rowland, 1967). We confirmed this in pilot studies in which spectral analysis of the EEG obtained in waking guinea pigs before, during, and immediately after both
A. EEG Desynchrony to BasF Stimulation

1. 

BF stim

2. 

BF stim

3. 

500 uV

BF stim

B. No EEG Desynchrony to BasF Stimulation

1. 

BF stim

2. 

DOOR OPENED

500 uV

5 sec

BF stim

Figure 1. A1–A3: Three consecutive examples of electroencephalographic (EEG) desynchrony to nucleus basalis (NB) stimulation (stim) in a urethane-anesthetized guinea pig (interstimulus interval = 30–60 s, 500 μA). Before NB stimulation, the animal exhibited large-amplitude, slow-wave activity in the auditory cortex. The NB stimulation produced a bout of EEG desynchrony characterized by a decrease in EEG amplitude and an increase in fast-wave activity. The duration of desynchrony was approximately 10–15 s after each stimulation, and the EEG gradually returned to prestimulation levels. B1: Example of no discernible EEG desynchrony to stimulation (1,500 μA). B2: However, this same animal did exhibit normal EEG desynchronization to sensory stimulation, such as opening the door of the acoustic chamber, indicating that failure of brain stimulation was not attributable to inability to desynchronize under its level of anesthesia. BF = best frequency. BasF = basal forebrain.

novel sensory stimulation and electrical stimulation of the NB showed identical power spectra (Weinberger, 1997, unpublished results). Therefore, we conducted the assessment of NB effects on the EEG in each animal during a terminal experiment on a day after training, in which the animals were anesthetized with urethane (1.5 g/kg ip). The stimulation train parameters were the same as those used during the main experiment. However, if desynchronization was not obtained with repeated attempts, then stimulation intensity was increased in steps of approximately 100 μA to a maximum of 1,500 μA. Click evoked potentials also were obtained to verify that recording sites in the ACx were located in deep layers, indicated by an initial negative response to clicks.

Histology

After completion of the terminal experiment, a lesion current was passed through the NB-stimulating electrode (5 μA for 5 s).
The guinea pigs were overdosed with sodium pentobarbital (100 mg/kg), perfused with formalin–saline, and the brain collected and processed for Nissl staining (40-μm frozen sections) to determine the site of the stimulating electrode tip.

Results

The results are presented in an unusual order because the effects of NB stimulation during the terminal experiments proved to be important. Therefore, we first present the findings of the terminal experiment, including a histological summary. This is followed by analysis of the relationship between RF changes and the results of the terminal experiment.

Effectiveness of NB Stimulation

During the terminal experiment under urethane anesthesia, all guinea pigs exhibited large-amplitude, slow-wave activity in the ACx. Stimulation of the NB consistently produced a shift to low-voltage, fast-wave activity in the animals (i.e., EEG desynchronization; see Figure 1A). However, NB stimulation was ineffective in half (4 of 8) of the animals. This failure occurred despite the use of higher levels of current, to a maximum of 1,500 μA. Figure 1B1 shows an example of a failure of NB stimulation to produce EEG desynchronization. It might be thought that such failures simply reflected deeper levels of anesthesia. However, in such cases, EEG desynchronization was easily produced by sensory stimulation, such as entry into the acoustic chamber by the experimenter (see Figure 1B2).

A summary of the histology is provided in Figure 2. All stimulation sites were located in the ventral to lateral-medial globus pallidus and the adjacent ventral caudate putamen, in the region corresponding to the origin of projections from the NB to the ACx. The noneffective sites tended to be dorsal (n = 3) or ventral (n = 1) but were within the medial–lateral range of the effective group.

Another possibility is that failure to obtain desynchronization in the terminal experiment might have reflected some differential treatment of the two groups during the pairing treatments (e.g., higher current levels in the noneffective group that might have produced local neuropathology). However, current levels were low, and there was no difference between groups in the current levels used (effective = 293 ± 128 μA; noneffective = 342 ± 65 μA). Mann–Whitney U(28) = 89.5, p > .4. For the remaining results, we refer to the effective group as the “desynch” group and the ineffective group as the nondesynch group.

Relationship of RF Plasticity to Effectiveness of NB Stimulation

Desynchrony to NB Stimulation

The pairing of a tone with stimulation of the NB produced RF plasticity for the desynch group. Specifically, responses to the CS frequency increased relative to responses to the pretraining BF. Figure 3 shows an example of this effect.

Peristimulus time histograms and accompanying rasters show that before training, the BF was 6.0 kHz (see Figure 3A1). The CS was selected to be 5.0 kHz, and the guinea pig was then given the standard training protocol. Figure 3A2 shows the data for the 20-min posttraining period. After pairing, responses to the CS frequency were increased, whereas those to the pretraining BF were decreased. These opposite changes were sufficiently large to produce a shift in tuning, so that the CS frequency became the new BF. Corresponding quantified tuning curves are given in Figures 3B1 and 3B2. A difference tuning function (20 min postraining minus pretraining) in Figure 3B3 shows that the largest increase in response at any frequency was at the frequency of the CS, whereas there was no increase at the pretraining BF.

This RF plasticity also was observed to be stable on Day 2, 24 hr after pairing. Figure 4 shows an example, with tuning curves and difference functions for postraining and the three retention periods after 24 hr. Tuning shifted from 12 kHz to the CS frequency of 14 kHz (see Figure 4A). Corresponding tuning difference functions (see Figure 4B) show that the responses to the CS frequency increased; in this case, the largest increase was actually at the frequency immediately higher than the CS frequency (16 kHz). These functions also show that the response to the BF and several other frequencies decreased.
Figure 3. A: Example of peristimulus time histograms and rasters for a guinea pig in which nucleus basalis (NB) stimulation produced a change in frequency tuning. A1: Pretraining peristimulus time histogram (PSTH) recorded from a cluster that responded from 3.5 to 10.0 kHz. The best frequency (BF) was 6.0 kHz. The conditioned stimulus (CS) was selected to be 5.0 kHz (arrow). A2: Posttraining records 20 min after 40 trials of paired tone-NB stimulation. The PSTH showed an increased response to the CS frequency and a decreased response to the pretraining BF of 6.0 kHz. These opposite changes were sufficient to produce a tuning shift of the posttraining BF to the CS frequency (arrow). B: Quantified receptive fields (RFs) for this animal. B1: The three pretraining PSTHs (one shown in A1) were quantified and averaged to ensure stability and establish a pretraining RF. B2: The pretraining and 20-min posttraining RFs showing the tuning shift to the CS frequency. B3: RF difference function obtained by subtracting the pretraining RF from the 20-min posttraining RF. In this example, the CS frequency had the largest response increase and the BF a slight decrease.
Receptive Field Plasticity One Day Post Training

Receptive Fields

1. 

Difference Functions

2. 

3. 

Figure 4. Receptive field (RF) plasticity 24 hr after training. A1–A3: Quantified RFs, normalized to the maximum response, observed 1 day after training are plotted with the pretraining RF (Subject 10) for three retention tests: 24 hr, and 20 min (24[20]), and 40 min (24[40]) later. Before training on Day 1, the best frequency (BF) was 12.0 kHz and the conditioned stimulus (CS) (arrow) was selected to be 14.0 kHz. The BF shift to the CS frequency was maintained at all three retentions tests. B1–B3: The corresponding difference functions show increased responses to the CS (arrow) frequency, with the largest increase at the frequency immediately higher than the CS frequency. The difference functions also show that the response to the BF and several other frequencies decreased.
to changes in RFs during the pairing protocol, we analyzed both the magnitude of changes, indexed by the CS:BF ratio, and the specificity of RF changes using measures described in a later section.

**CS:BF changes.** The group results for the CS:BF ratio for Day 1 are summarized in Figure 6A. For the desynch group, for the four retention periods (immediately, 20 min, 40 min, and 60 min), training produced increases in the CS:BF ratio. That is, there was a relative increase in the rate of discharge to the frequency of the CS compared with the pretraining BF at all time points on Day 1. By contrast, the nondesynch group exhibited no comparable increases, and small decreases were observed. Figure 6B shows the findings for Day 2. The desynch group still showed increases in CS:BF ratios 24 hr after training at all three retention periods. The nondesynch group also showed increased ratios, although they were smaller than for the desynch group.

Wilcoxon signed-ranks tests were calculated to determine any effects at each individual time period. The CS:BF ratio measures at 20 and 60 min were significantly greater than for the pretraining period for the desynch group: 20 min, \( Z(15) = 2.84, p = .005 \); 60 min, \( Z(15) = 2.329, p = .02 \). However, the nondesynch group showed no significant change from the CS:BF ratio of the pretraining period. No other time periods differed significantly. To determine any differential effects between the desynch and nondesynch groups, we performed Mann-Whitney tests for all time periods. There was a differential effect at 20 min, \( U(28) = 46, p = .01 \), with the desynch group having a larger increase relative to the nondesynch group, which exhibited no change in the neural responses. Finally, seven of seven of the time periods showed a larger increase for the desynch group (sign test, \( p < .01 \)).

**Specificity of RF plasticity.** To determine the degree of specificity of the RF plasticity, we computed the average difference functions centered on the CS for neural responses (difference function = postraining spike count minus pretraining spike count) \( \pm 1.5 \) octaves from the CS frequency. Figure 7 shows changes to RFs during the pairing protocol, we analyzed both the magnitude of changes, indexed by the CS:BF ratio, and the specificity of RF changes using measures described in a later section.

**No Desynchrony to NB Stimulation**

The recordings from the nondesynch group failed to exhibit such plasticity. Figure 5 shows an example. Before pairing, the BF was 16 kHz and the CS was selected to be 12 kHz (see Figure 5-1). After pairing, the BF was still 16 kHz, and, in fact, there was little or no change at any frequency (see Figures 5-2 and 5-3).

**Group Comparisons (Desynch Vs. Nondesynch)**

To determine whether the differential effectiveness of NB stimulation, assessed in the terminal experiment, was related
CS/BF Changes

Figure 6. Changes in the conditioned stimulus (CS)-to-best frequency (BF) ratios (CS:BF posttraining minus CS:BF pretraining) for both groups (desynchronized and nondesynchronized) for each retention period after training. A: Changes on Day 1. For the desynch group, training produced facilitated responses at the CS frequency relative to the BF across all four time periods. The 20-min and 60-min periods were significantly greater than the pretraining measure for the desynch group (Wilcoxin signed-ranks test). The nondesynch group did not show any significant changes and exhibited small changes, mainly decreases. The desynch group had a significantly larger increase in neural responses than the nondesynch group at 20 min (line with arrows; Mann-Whitney test). B: CS:BF changes on Day 2, 24 hr after pairing. Both groups showed increases, but larger increases occurred for the desynch group. However, there were neither statistically significant changes from pretraining nor differences between groups. BasF = basal forebrain. EEG = electroencephalographic.

frequency showed a positive slope (CS at local maximum) in 11 of 14 cases (binomial test, p < .025).

The nondesynch group continued to have small or no response changes at the CS frequency, whereas there were increases at nearby frequencies across the time periods on Day 1. This effect also was present but smaller on the 2nd day. A slope analysis indicated that the change in response at the CS frequency was at a local minimum in 11 of 14 cases (binomial test, p < .001).

The differential effect between the two groups (desynch minus nondesynch) showed a specific increase at the CS frequency at all periods after the immediate posttraining period (see Figure 7C). The effect broadened somewhat over time and was greatly increased through the 2nd day, with the largest increase occurring at the CS frequency, or for 24(20), an adjacent frequency.

To quantify the changes in response at the frequency of the CS relative to the other frequencies, we computed a response index (RI) using the difference functions from Figures 7A and 7B: RI = [(CS/mean response of frequencies ranging from ± 1.5 octaves) - 1]. (If RI = 0, then CS = mean response; if RI > 0, then CS > mean; if RI < 0, then CS < mean.) The RI revealed that the CS frequency for the desynch group had significantly larger responses than the mean of all responses for seven of seven retention periods (binomial test, p < .01), whereas the nondesynch group showed the opposite effect: seven of seven smaller responses than the mean response (binomial test, p < .01; see Figure 8).

Discussion

In this study we investigated whether pairing a tone with activation of the NB could produce CS-specific RF plasticity in the ACx of the waking guinea pig. Two groups were established from the results of the terminal experiment: a group that exhibited electrocortical desynchronization of the ACx to NB stimulation (desynch) and a group that did not desynchronize to the stimulation (nondesynch). The desynch group developed CS-specific RF plasticity, characterized by an increased response to the CS frequency with decreased or no response changes at other frequencies. The plasticity was retained for 24 hr, the longest retention interval tested. The RF plasticity was essentially the same as that produced using a similar protocol in the anesthetized guinea pig (Bakin & Weinberger, 1996). Thus, these results extend the effect to the waking state, and to behaviorally relevant long-term retention (i.e., 24 hr). The findings also are similar to the RF plasticity that develops during behavioral conditioning in waking animals (e.g., Bakin & Weinberger, 1990; Edeline et al., 1993; Edeline & Weinberger, 1993; Weinberger et al., 1993).

By contrast, the nondesynch group exhibited no change or
Figure 7. Normalized group receptive field (RF) difference functions (posttraining RFS minus pretrained RFs, centered on the CS frequency [arrow]) for all seven retention periods. A: RF difference functions for the desynch group. The dotted lines in A and B represent the 75% bandwidth (BW) markers. Note the absence of a strong effect immediately after training, the development of a conditioned stimulus (CS)-specific increase at 20 min, which is less pronounced at 40 min and particularly at 60 min but larger and centered at or adjacent to the CS frequency at all three retention periods 24 hr later. B: RF difference functions for the nondesynch group. In contrast to the desynch group, there was an absence of a CS-specific increase at any of the seven retention intervals. Rather, the change in the CS frequency is consistently at a local minimum. C: The difference between the desynch and nondesynch groups; the average difference function for the nondesynch group (see Figure 7B) was subtracted from the corresponding average difference function of the desynch group (see Figure 7A). This reveals the overall differential effect and shows that the maximal increase in response was largely centered on the frequency of the CS.
decreased response at the CS frequency, coupled with increased responses to adjacent frequencies. This produced a local minimum in the RF at the CS frequency (e.g., Figure 7B). The effects for both groups were specific and long-lasting, being evident at all three retention periods 24 hr after training. Longer retention intervals will be needed to determine the ultimate duration of this NB-induced RF plasticity.

Our protocol was not designed to test for the associativity of pairing effects because previous findings in the anesthetized preparation showed that CS-specific RF plasticity that is induced by pairing a tone with NB stimulation is associative (Bakin & Weinberger, 1996). However, one cannot assume that identical processes occur in the waking state, in which the arousal level of an organism is much greater than in the anesthetized state. Therefore, no claims of associativity are made for our findings, and this issue is currently being addressed using a discrimination protocol.

The CS-specific local minimum that developed in the nondesynch group is similar to the effect of habituation training on frequency RFs. Condon and Weinberger (1991) used a simple paradigm in the waking guinea pig, in which a given frequency was repeated at the rate of 1.25/s for several minutes. This produced a long-lasting (tested to ~1 hr) specific decreased response at the repeated frequency with no change or even increases at adjacent ("sideband") frequencies. From an operational viewpoint, the nondesynch group probably underwent the same type of habituation paradigm because, although the tone was presented on each trial, apparently there was no effective unconditioned stimulus, rendering the protocol for this group a "tone-alone" protocol. The major difference between the design of the previous explicit habituation study and the current study was that the current experiment involved a much slower rate of tone presentation (average of 1/min) and only 40 tone presentations instead of hundreds of stimuli. This might account for the fact that the absolute magnitude of response decrement at the "CS" frequency was less in the current study. In fact, actual decreased responses (below zero) were observed only at 40 and 60 min (see Figure 7B). Thus, the major absolute effect was an increased response to adjacent lower and higher frequencies. Another interpretation is that the nondesynch group developed a general increase in response in the range of the CS frequency but that a habituatory process prevented this increase at the CS frequency. Further studies are needed to clarify the results.

It might be thought that the RF plasticity in the desynch group resulted not from the cholinergic activation of the NB but that it was aversive. This seems extremely unlikely on several grounds: First, we were careful to keep stimulus levels below those that would evoke any detectable behavioral response, including changes in heart rate. We found no evidence that NB stimulation was aversive. Second, there was no significant difference in stimulation levels between...
groups. If the NB stimulation effects in the desynch group were due to its aversive nature, rather than to its engagement of the cholinergic system that produces EEG desynchronization, then changes in RFs should have been similar for both groups. However, the effects were not similar and were related to the effectiveness of the NB stimulation to produce EEG desynchronization.

The physiological differences that occurred during the terminal experiment did not seem to have a precise relationship to anatomical location, so neurohistological findings alone appear to be insufficient to gauge the effectiveness of stimulation. This indicates the need to have independent measures of the effectiveness of brain stimulation experiments, at least for the area investigated in this experiment.

The weak immediate effect in the desynch group is of interest. Although it was in the predicted direction of a CS-specific increase, it increased markedly at later retention periods. This weak effect was probably not due to masking by habituation processes that might accompany conditioning processes because the habituation effect was not transient and was clearly present at retention intervals at which the CS specific increases were stronger. It is possible that the effects reflect a genuine "incubation" process because similar effects have been found in earlier studies of learning-induced RF plasticity in behaving animals (Edeline & Weinberger, 1993). By contrast, they were not present in the previous experiment of NB-induced RF plasticity in the anesthetized animal (Bakin & Weinberger, 1996). Parametric experiments are needed to better understand the relation between NB-induced and RF plasticity in different preparations.

The results are consistent with the "cholinergic hypothesis" of learning-induced RF plasticity in the ACx of awake animals (e.g., Weinberger, Ashe, Diamond, et al., 1990; Weinberger, Ashe, Metherate, 1990). However, several questions need to be addressed further. We have already noted the need to determine whether the effects in the waking animal are associative. Regarding the putative involvement of the cholinergic system, it must be recognized that there are GABAergic cells in the NB that project to the neocortex (Fisher, Buchwald, Hull, & Levine, 1988). Although it seems paradoxical that excitation of GABAergic neurons terminates on GABAergic cells within the neocortex, it is known that at least some of these neurons terminate on GABAergic cells within the neocortex (Freund & Gulyas, 1991; Freund & Meskenaite, 1992). Thus, their excitation by electrical stimulation might very well produce inhibition of cortical GABAergic cells, resulting in a disinhibition of their target cells. If sufficient target cells change state, they might produce cortical desynchronization (Metherate et al., 1992).

Another possibility is that NB stimulation produced RF plasticity independent of its ability to desynchronize the EEG through a process not yet known. Certainly, attribution of our effects to the cholinergic system, possibly acting at muscarinic receptors in the ACx, must remain tentative, awaiting appropriate pharmacological and other studies that directly manipulate the NB cholinergic influences on the ACx.

Finally, our findings may very well apply to adult RF plasticity in other primary sensory cortices (reviewed by Weinberger, 1995). For example, reorganization of the somatosensory cortex after peripheral deafferentation seems to depend on an intact NB cholinergic system (Juliano, Ma, & Eslin, 1991). In addition, the "conditioned response" of neurons in the barrel cortex that develops to stimulation of a "CS whisker" after pairing it with stimulation of an effective "unconditioned stimulus whisker" is abolished by the local application of atropine (Delacour et al., 1990). Thus, pairing somatosensory stimulation with NB stimulation might induce specific RF and map plasticity in the somatosensory cortex.

References


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