J Physiology (Paris) (1996) 90, 353–360

Possible involvement of neuromodulatory systems in cortical Hebbian-like plasticity

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Summary — Plasticity of neuronal covariances (functional plasticity) is controlled by behavior (Ahissar *et al* (1992) *Science* 257, 1412–1415). Whether this behavioral control involves neuromodulatory systems was tested by examining the effect of acetylcholine (ACh) and noradrenaline (NE) on functional plasticity in anesthetized animals and by comparing the effects of these neuromodulators in an anesthetized preparation to that of behavior in awake animals. Local iontophoretic applications of these drugs during manipulations of activity covariance in guinea pig auditory cortex did not mimic the behavioral control of functional plasticity that was previously observed in awake monkeys. Thus, the hypotheses according to which these neuromodulators control functional plasticity independent of their concentration and time of release were not supported by our data. The significant plasticity induced nevertheless, by some of the conditionings in the presence of ACh and NE, suggests that factors, other than those that were experimentally controlled, could regulate this plasticity. These factors could be among others the timing of drug(s) applications relative to the conditioning time, the local concentrations of the drug(s) and/or the site of application with respect to the relevant synapses.

acetylcholine / noradrenaline / attention / auditory cortex / cross-correlation / neuromodulator / Hebbian synapse / synaptic plasticity

Introduction

Functional plasticity in the behaving monkey behaves according to a rule that can be considered a derivative of the generalized Hebbian rule (Brown *et al*, 1990) translated to covariance measurements (Frégnac *et al*, 1988; Ahissar *et al*, 1992a; Ahissar and Ahissar, 1994; Frégnac and Shulz, 1994). Ongoing covariances remain higher after an induced increment and remain lower after an induced decrement. However, in contrast to the prediction of the basic Hebbian and covariance rules (Sejnowski, 1977), an ongoing covariance is not sufficient for inducing neuronal plasticity, but rather a change in this covariance is required (see Frégnac and Shulz, 1989). This requirement for a change in covariance avoids the runaway problem, which is inherent in the Hebbian-covariance rules (Ahissar and Ahissar, 1994). Thus, the learning rule that the neuronal pairs in the auditory cortex of the monkey obey is designated as the 'steady-state covariance rule'.

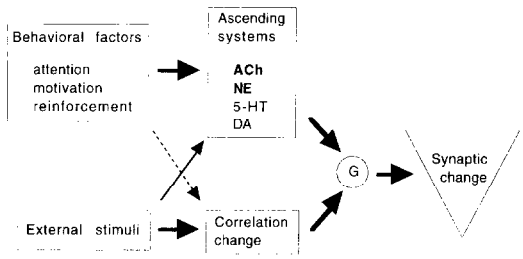
Functional plasticity depends upon the behavioral state of the monkey (Ahissar *et al*, 1992a). When a change in covariance is imposed during the performance of a behavioral task, this change almost always outlasts the conditioning period. The change in covariance that remains following the conditioning is roughly equal, on the average, to the square root of the change in covariance that is evident during the conditioning. In contrast, following imposed changes in correlated activity during non-behaving conditionings, covariances change only occasionally, the changes are smaller, and their dependency on the induced covariance changes is weaker. Based on these results, a working model for a neuromodulatory control of functional plasticity was developed. This model was tested by examining whether local applications of ACh and/or NE at the vicinity of the recorded cells in anesthetized guinea pigs can mimic the behavioral control of such neuronal plasticity that was observed in the monkey.

Materials and methods

The working model

In this model, behavioral factors affect single synapses in the brain through diffuse ascending systems (see fig 1A and Crow, 1968; Kety, 1970). In the monkey, this control appears to be a gain control, *ie* the behaviorally-triggered agents magnify, rather than enable, the synaptic changes, since weak changes are observed also in the non-behaving state (Ahissar *et al*, 1992a). However, this constraint is not strong, since: i) the monkey could be partially attentive to the stimuli even when no reinforcement is given; and ii) slow covariance fluctuations (independent of the conditioning timing) could cause a weak artificial correlation between the induced and lasting covariance changes. Thus, the behavioral control is assumed here to be of either gain or gating type (G). The model assumes, as a first approximation, that the major interaction between the behavioral factors and the sensory events takes place in the changing synapses. However, other interactions (broken and solid thin arrows) probably play important roles as well.

A. Working model



B. Hypotheses

order	ACh	NE	timing	concen.
1	+			
1		+		
2	+	+		
2	+		+	
2	+			+
2		+	+	
2		+		+
3	+	+	+	
3	+	+		+
3	+		+	+
3		+	+	+
4	+	+	+	+

+ , neuronal plasticity depends upon

Fig 1. Working model and hypotheses of neuronal mechanisms underlying behavioral control of synaptic plasticity.

Two neuromodulators, ACh and NE, were utilized. We assumed that neuromodulatory control by ACh and NE could depend on: i) synergistic operation of the two drugs; ii) the exact concentration of the drug(s) at the vicinity of the synapses under control (hereafter, concentration or intensity of release); and iii) the exact time relationships between drug release and the induction of a covariance change (hereafter, timing of release). These assumed factors could either affect the nature of the neuromodulatory control, *eg* change the polarity of the synaptic modification, or slightly modulate the parameters of the control, *eg* the gain of the synaptic modification. Herein, dependency on a factor of neuromodulatory control refers only to the former. Control of neuronal plasticity by ACh and/or NE could be of first- or higher-order (fig 1B). According to the first-order hypotheses, ACh or NE control neuronal plasticity independently of each other and of the intensity and timing of their release. Second-order hypotheses are: i) ACh and NE synergistically control neuronal plasticity; ii) ACh or NE control of neuronal plasticity depends on the neuromodulator concentration; and iii) ACh or NE control of neuronal plasticity depend on the timing of neuromodulator release. Third-order hypotheses include different combinations of three of these four possible variables, and the fourth-order hypothesis suggests that the neuromodulatory control of neuronal plasticity requires synergistic operation of ACh and NE and depends on both timing and concentration.

Recordings, iontophoresis, and cellular conditioning

Pairs of neurons in the auditory cortices of anesthetized guinea pigs were recorded using special combined electrodes (CEs) through which cholinergic and noradrenergic agonists could be iontophoretically applied (Haidarliu *et al*, 1995). Briefly, the electrode set-up consisted of two types of electrodes included within a metallic guide tube: two regular tungsten-in-glass electrodes (TEs; 0.2–0.8 MΩ at 1 kHz; median, 0.3 MΩ) and two CEs, which consisted of a tungsten electrode (0.2–0.8 MΩ) within the central barrel of a 7-barreled glass pipette. The horizontal distance between the electrode tips was 300 to 600 μm. The guide tube was brought close to the exposed dura, and each of the four electrodes was introduced independently into the auditory cortex with a multi-electrode microdrive system. Four spike sorters (MSD-2, Alpha-Omega, Nazareth, Israel), one for each electrode, were used to isolate single- and multi-unit spikes.

For the iontophoretic application of neuromodulatory drugs, the CE was filled with the desired solutions before insertion into the guide tube. The six barrels were filled either with an aqueous solution of ACh chloride (1 M, pH 4.5), carbamylcholine chloride (carbachol; CCh; 1 M, pH 6.0), (–)arterenol bitartrate (NE; 0.5 M in a 100 μM ascorbic acid solution, pH 4.5), or NaCl (3 M). The impedance of the glass barrels varied from 10 to 100 MΩ (median, 30 MΩ), depending on the width of the barrel orifice and the solution with which filled. Retaining currents of –5 to –10 nA were used to prevent the drugs from leaking. The ejection currents used were 5 to 200 nA (positive current). Since the effects of the neuromodulating drugs (which were applied iontophoretically) persisted for at least several minutes, conditioning, occurring just after the end of the drug ejection, was considered as being in temporal overlap and contiguity with the drug application.

Cellular conditioning paradigms were applied to manipulate neuronal covariances by pairing auditory stimuli (unconditioned stimuli) with neuronal activities (Ahissar *et al*, 1992a). The unconditioned stimulus was an acoustic stimulus that effectively activated the target neuron, and during conditioning was applied immediately (< 4 ms) after every spike of the trigger neuron that appeared with an interval of at least 50 ms after the preceding spike. The auditory stimulus duration was 30 ms. Cross-correlograms between the trigger and the target neurons were calculated as previously described (Perkel *et al*, 1967; Ahissar *et al*, 1992b). The asynchronous gain (ASG) measurement (Abeles, 1982; Ahissar *et al*, 1992a) was used to estimate the functional coupling between neurons. The ASG equals the area under a peak of a correlogram that is above the expected correlation if the neurons were firing independently, and within a specific time lag range, divided by the number of triggering spikes used to construct the correlogram.

Results

Eighty-five neuronal pairs that showed signs of correlated activity before conditioning were selected for analysis. The covariance changes obtained in the guinea pig were compared to those obtained in a previous study in the monkey (Ahissar *et al*, 1992a), where similar auditory stimuli, conditioning paradigms, and recording techniques were utilized, but with conventional TE electrodes and no drug applications. In the absence of drug applications ($n = 63$), conditionings of the guinea pig neuronal pairs yielded results that resembled those from the non-behaving monkeys. Lasting covariance changes occurred only occasionally, were usually small, and the dependency of the lasting changes on induced changes was weak (fig 2).

The first-order hypothesis, in which ACh controls neuronal plasticity independently of timing or concentration, was tested by applying the conditioning paradigms together with local applications of ACh or the muscarinic agonist carbachol (CCh: 40–200 nA), to 21 neuronal pairs. Conditionings that were accompanied by ACh or CCh applications did not show significant dependency of lasting changes on the induced covariance changes (fig 2). Thus, ACh applications did not mimic behavioral control in the monkey and the first-order hypothesis for ACh was rejected. Data obtained from tests with NE alone were not conclusive, since there was usually a strong inhibitory effect of NE applications that impaired covariance measurements (data not shown).

The first of the second-order hypotheses, in which ACh and NE synergistically control neuronal plasticity, was tested by combined administration of ACh (or CCh; 40–200 nA) and NE (5–60 nA) during the conditionings of 22 neuronal pairs. Conditionings that were accompanied by simultaneous applications of ACh (or CCh) and NE did not show significant dependency of lasting changes on the induced covariance changes (fig 2), thus, the first of the second-order hypotheses was also rejected. In several cases, this second-order conditioning paradigm yielded significant lasting modifications of functional couplings, however, these modifications were not necessarily constrained by the steady-state covariance rule.

An example of a significant neuromodulatory effect on plasticity of a neuronal pair (circled in fig 2) that does obey the steady-state covariance rule is depicted in figures 3 and 4. In this example: i) both neurons were recorded from the CE electrode which was used to eject the drugs; ii) the NE current was high relative to the current usually used; and iii) the duration of drug application was short relative to durations usually used. The target neuron (unit 1) exhibited an oscillatory autocorrelation (fig 3A). This oscillatory pattern, which was 'mapped' into most of the computed cross-correlograms (fig 3B; see Perkel *et al*, 1967), was not included in the measurements of functional couplings; only the near-zero peak (0–7 ms) was considered. The pre-conditioning cross-correlograms of this neuronal pair showed a weak correlation that became significantly strengthened only when the conditioning period had been accompanied by the simultaneous applications for 1 min of CCh (40 nA) and NE (40 nA) from the glass pipettes of the recording electrode (fig 3B, left column). This strengthening was partially extinguished during the following 6 min. In contrast, conditioning alone (fig 3B, middle column) or application of drugs alone (fig 3B, right column) did not produce significant lasting strengthening of the coupling. Functional coupling of this neuronal pair was quantified using the ASG measurement (Abeles, 1982; Ahissar *et al*, 1992a). The ASG that was computed for the near-zero peak of the cross-correlations (0–7 ms; fig 4B) deviated from baseline near the beginning of the depicted period (time ≈ 0; reason unknown), following the first drug application, during the conditioning without drugs, and following the conditioning in the presence of drugs. The latter deviation was the largest and longest lasting. Conditionings induced large increments of the covariance at time lags that corresponded to the latency of the response of unit 1 (see fig 3B), and these covariances were depicted by the ASG values computed for the 0–50 ms time-delay range of the cross-correlation (fig 4C). Some of the modifications of the functional coupling were accompanied by modifications of the average firing rates of the two units (fig 4D). The increased activity level of unit 1 following the conditioning in the presence of CCh and NE (fig 4D) might reflect increased excitability (Shulz *et al*, in press) or increased synaptic input, the latter of which was supported in the behaving monkey (Ahissar *et al*, 1992a).

Discussion

Neuronal plasticity in the auditory cortices of anesthetized and of behaving animals was studied using the cross-correlation technique, which allows examination of neuronal interactions based on simultaneous extracellular recordings. Herein, correlated activities are assumed to be the outcome of given synaptic substrates, and changes in correlated activities (functional plasticity) are assumed to reflect changes in the underlying synaptic weights. The nature of the synaptic changes, however, cannot be directly estimated from the functional changes. Thus, synaptic 'learning' rules can only

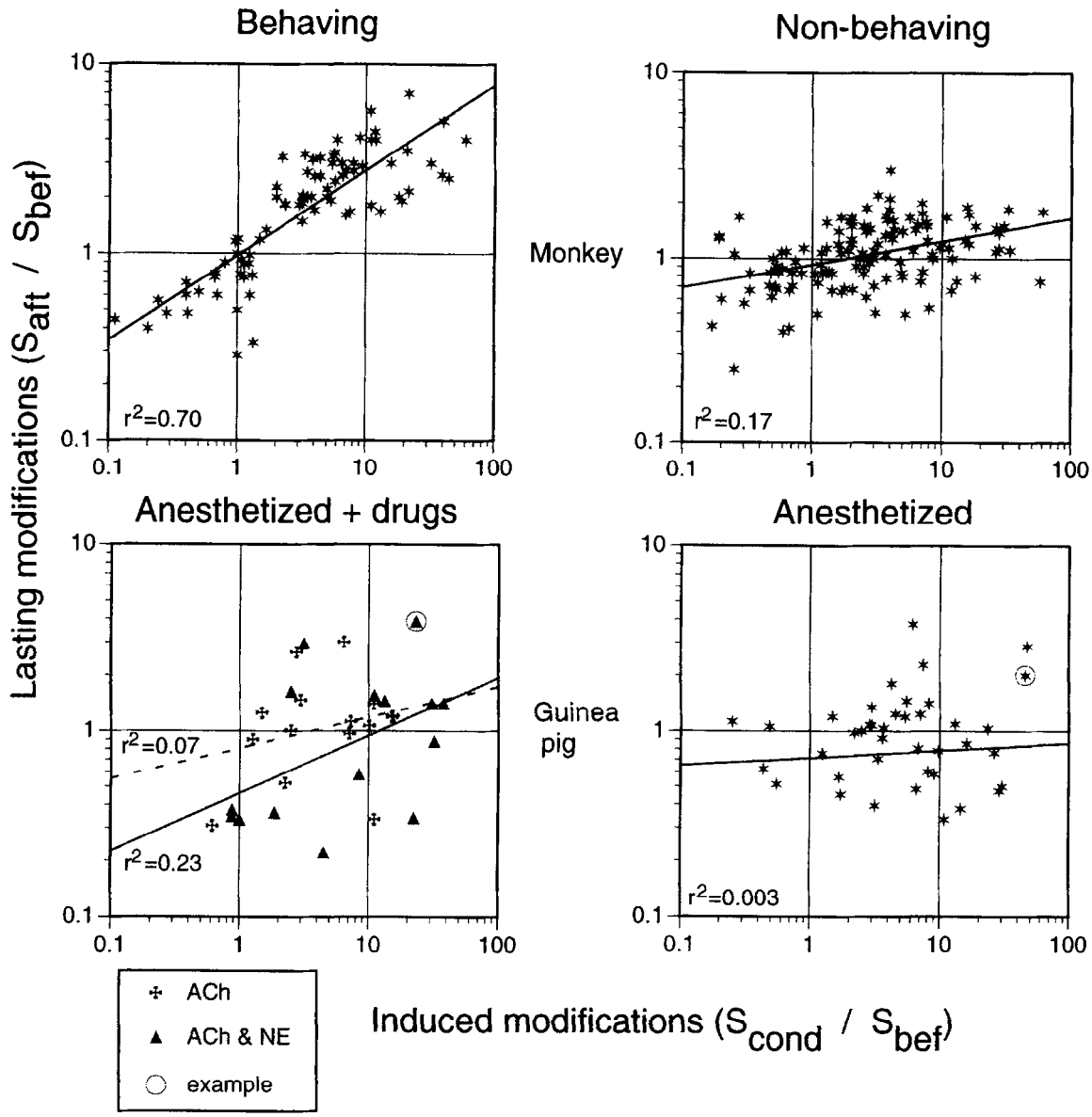


Fig 2. Dependence of lasting covariance modifications on induced covariance modifications in the guinea pig and the monkey. S_{bef}, strength before conditioning; S_{cond}, strength during conditioning; S_{aft}, strength after conditioning. The strength of the connection was quantified by computing the asynchronous gain (ASG) from the crosscorrelograms (see *Materials and methods*). In these log-log presentations, only neuronal pairs were included that exhibited positive ratios, *ie* did not change the sign of the ASG during or after conditioning. Each symbol represents the average values for several conditioning blocks of one neuronal pair. *, no drug. Upper panels were adapted from Ahissar *et al* (1992).

be qualitatively constrained by the findings described here. For quantitative validation, the synaptic rules must be translated into 'functional learning rules', which relate to changes in neuronal covariances. We believe that covariance measurements (also referred to as measures of functional couplings, functional or effective connections, and neuronal contingencies) are appropriate for probing the circuit level, and thus, for studying the causal relationships between behavior and neuronal plasticity. Functional 'learning rules' determine the causal relationships between abrupt changes in neuronal covariances and the resulting changes in the ongoing co-

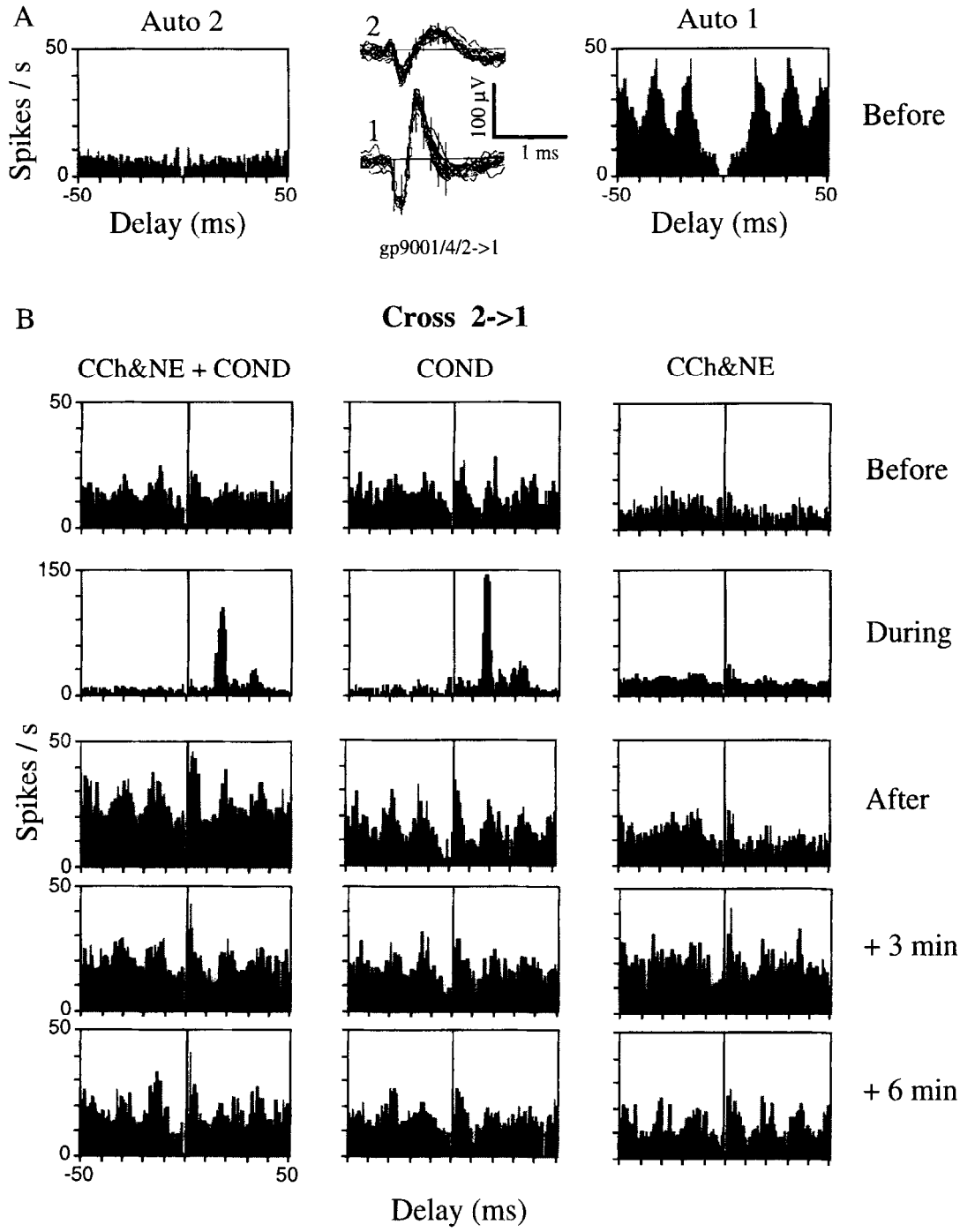


Fig 3. An example in which simultaneous application of CCh and NE during conditioning induced functional plasticity in the guinea pig. **A**. Auto-correlations (auto) and spike shapes (middle) of the two neurons. The sorting templates are superimposed on top of the spike shapes (eight rectangles with vertical lines). **B**. Cross-correlograms obtained before (–3 min), during, and after (immediately, + 3 min, and + 6 min) conditioning. Computing times: 2 min, except for during the conditioning period (2nd row), when they were (left to right) 4, 3, and 4 min. Binwidth of correlograms 1 ms. The data corresponding to this figure are shown in a circle in figure 2.

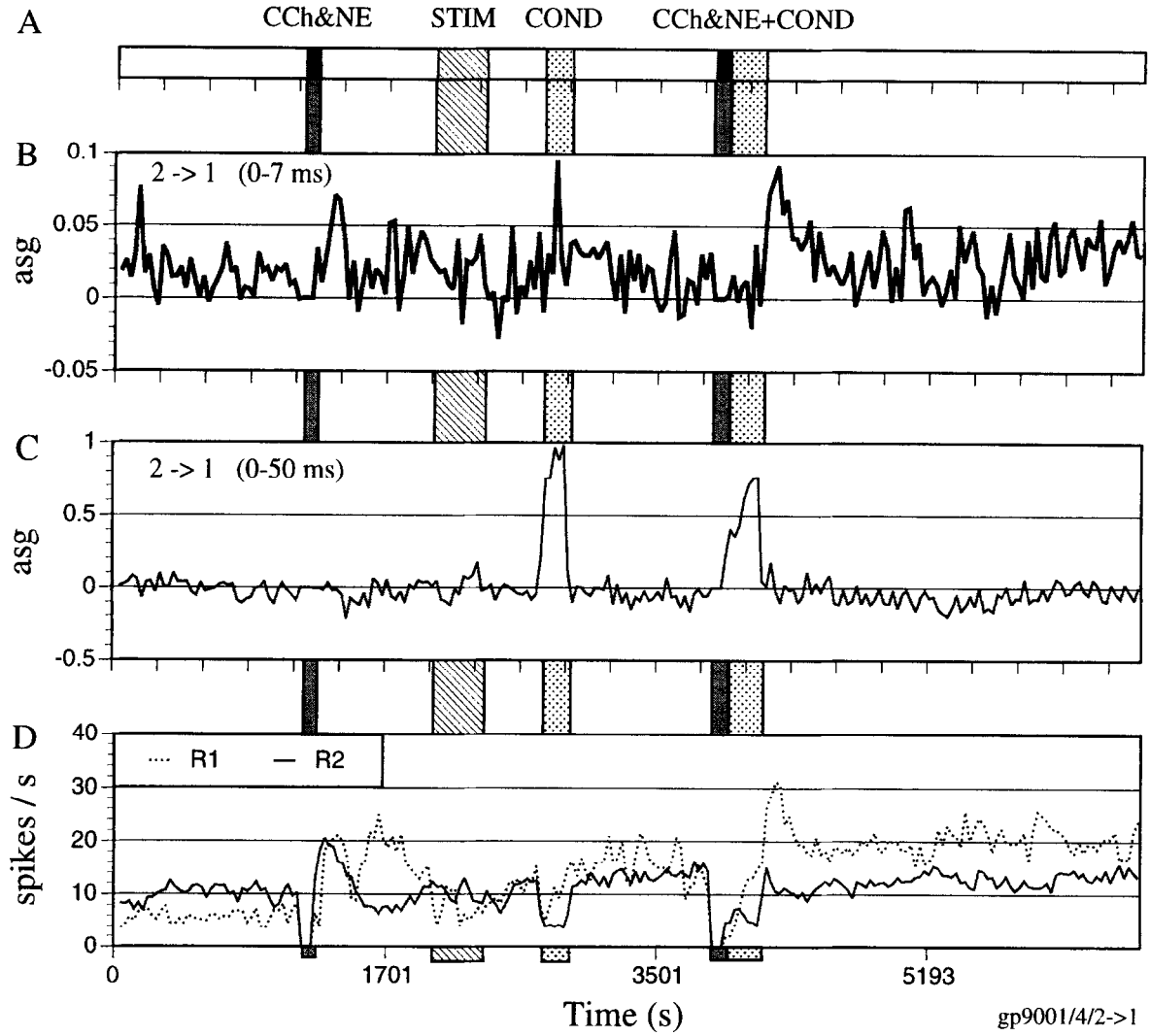


Fig 4. Time course of the conditionings of figure 3. **A.** Protocol. **B.** ASG for the near-zero correlation peak (0–7 ms, see fig 3B) as a function of time. **C.** ASG for the near-zero + the delayed (stimulus-induced) correlation peaks (0–50 ms, see fig 3B), as a function of time. **D.** Average firing rates of unit 1 (R1) and unit 2 (R2), as a function of time. STIM, period during which acoustic stimuli were randomly applied to determine the receptive field of unit 1. The data corresponding to this figure are shown in a circle in figure 2.

variances, *ie* the formation of memory traces of neuronal covariances (see Sejnowski, 1977).

A critical factor in the formation of memory traces of neuronal covariances is behavior (Ahissar *et al*, 1992a). The hypotheses we outlined here for the possible neuronal implementations of this behavioral control (fig 1A) were inspired by the intuitive descriptions of Crow and Kety (Crow, 1968; Kety, 1970), in which the main mediation role was assigned to the diffuse ascending systems. In the majority of experimental studies of the effects of ACh and NE on neuronal plasticity (during development, in adults, and in *in vivo* and *in vitro* preparations), ACh and NE were shown to be capable of modulating neuronal plasticity (Metherate *et al*, 1987, 1988; Greuel *et al*, 1988; Dahl and Sarvey, 1989; Johnston *et al*, 1989; Brocher *et al*, 1992; Hasselmo and Barkai, 1995; reviewed in Rauschecker, 1991; Kasamatsu, 1987; Weinberger, 1993; Hasselmo, 1995). Moreover, a combined action of ACh and NE has been reported to be more potent than an independent action of each (Bear and Singer, 1986, Brocher *et al*, 1992; reviewed in Rauschecker, 1991). Thus, ACh

and NE were chosen as the primary neuromodulators for the control of neuronal plasticity in this study. High variability in the effects produced by the repetition of similar paradigms using these drugs is characteristic of these studies (Sillito, 1986; Rauschecker, 1991; Ahissar and Ahissar, 1994). However, the variability in the functional coupling found between different neuronal pairs (fig 2), and sometimes even the variability of the plasticity induced by different conditionings for the same neuronal pair (data not shown), suggest that the source of this variability might be endogenous.

A particular hypothesis was supported or rejected by comparing the results obtained with paradigms obeying a hypothesis with those obtained in behaving monkeys (fig 2). The 'baseline' of functional plasticity obtained in the anesthetized guinea pig was compared with that obtained in non-behaving monkeys (fig 2, right column). For both cases, the dependency of lasting modifications on induced modification was weak (see r^2 values), significant lasting modifications occurred only occasionally, and residual influence of the intermixed other paradigms could occur. Examples of the latter are that the monkey could sometimes be attentive in the 'non-behaving' state and that the neurons in the guinea pig could still be under the influence of drugs applied during other intermingled conditionings.

The validity of the two hypotheses of neuromodulatory control of functional plasticity that do not require accurate control of timing and concentration, and thus, can be tested using local iontophoretic drug applications, was examined. The results do not support neither the first order hypothesis, in which ACh controls, and the first of the second order hypotheses (the only one which was experimentally tested), in which ACh and NE synergistically control neuronal plasticity independently of timing or concentration. However, the significant plasticity induced by some of the conditionings that were accompanied by drugs, especially when ACh and NE were applied simultaneously (figs 2–4), suggests that factors, other than those that were experimentally monitored, control this plasticity.

At the present stage of this study, still in progress, we cannot exclude that these factors could be the timing of drug(s) applications relative to the conditioning time and/or the local concentrations of the drug at the vicinity of the relevant synapses. The mechanism regulating the timing and/or intensity of the endogenous release of ACh and/or NE could be tuned for learning during: i) evolution; and ii) ontogenetic development. In the first case, tuning should be common to all individuals, and pooling data originating from different subjects may be justified. In contrast, in the second case, the putative activation of cholinergic and noradrenerdic systems in a given learning situation would depend on the past experience of the animal, and the expression of the tuning process could vary between subjects. Therefore, a high inter-subject variability can be expected, possibly calling for careful interpretations of averaged data, especially at early stages of the parametric search for the relevant causative variables.

Acknowledgments

This study was supported by the Israel Science Foundation, administered by the Israel Academy of Sciences and Humanities, and by the Minerva Foundation, Munich, Germany. EA was supported by the Alon Fellowship, Israel. SH was supported by the Center for Absorption in Science, Ministry of Absorption, Israel. DES was supported by the MDRI-CNRS, HFSP (RG 69/93) and the French Ministry of Foreign Affairs during his visits to The Weizmann Institute.

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