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Transient changes of electrical activity in the rat barrel cortex during conditioning

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Abstract

To reveal the dynamics of neurophysiological changes in the rat barrel cortex induced by conditioned stimulation we recorded the local niicro-electroencephalographic (EEG) activity and evoked potentials (EPs) in barrel cortex to stimulation of a single vibrissa before and after pairing it with a mild electric shock applied to the rat's tail. Following the introduction of the reinforcing stimulus, the amplitude of the first negative component of evoked potentials in the cortex on the conditioned side grew in relation to the same component of control potentials, evoked by stimulation of the opposite symmetrical vibrissa. This change was accompanied by a latent decrease in spectral power of the EEG within the alpha and beta frequency bands in both hemispheres. The observed changes in both of these electrical manifestations of enhanced neuronal activity reverted after two (EP) or three (EEG) days of conditioning. These results are discussed in relation to the putative activity of neuromodulatory systems. © 1998 Elsevier Science B.V.

Keywords: Plasticity; Evoked potentials; EEG; FFT; Bairel field; Rat

1. Introduction

Adult rat barrel cortex exhibits a variety of plastic changes during altered sensory experience [3,31]. Concurrent stimulation of a pair of vibrissae causes the neurons in corresponding barrels/columns to increase their responses to either whisker [15,16]. Similar results are obtained when all but two adjacent vibrissae are trimmed close to the skin [17,18]. Classical conditioning at maturity, in which whisker stroking is paired with positive or aversive reinforcement can also produce functional plasticity. As shown in mice, conditioning can increase metabolic labelling with 2-deoxyglucose (2-DG) within the cortical representation of involved vibrissae [32]. To specify the dynamics of sensory cortical plasticity requires a less remote methodology, with temporal resolution better than that delivered by 2-DG autoradiography. For this reason, we adapted an established electrophysiological procedure for evoking extracellular field potentials (EPs). Although these responses are highly stereotyped [27] and the underlying neuronal activity overlaps spatially and temporally [4], detailed analysis of EPs have been shown to be useful for studying the functional dynamics of cortical activation during physiological stimulation of vibrissae [1,19,20,27].

Previous studies on rats have shown that EPs in visual cortex differ during sleep and wakefulness [11,14,47]. There is substantial evidence that evoked potentials in the sensory cortical areas depend also on the level of alertness or attention which is manifested by changes of the spectral power in underlying 'spontaneous' electroencephalo-graphic (EEG) activity [21,24,47,52]. It is widely believed that the source of cortical activation originates within the brain stem [21,24] and basal forebrain [12,21,37] neuro-modulatory projections. Such modulation could underlie the increase in amplitude of EPs in rat auditory cortex when meaningless clicks became classical conditioning stimuli [35]. This hypothesis is supported by the recent experiments of Storozhuk et al. [49] who showed neuromodulatory dependent changes of activity of the cells in the cat sensorimotor cortex during conditioning. Similar enhancement of evoked potential and cell responses to

Abbreviations: EEG, intracortically recorded micro-electroencephalo-graphic activity; EP, evoked potential; FFT, Fast Fourier Transform; CS, conditioned stimulus; US, unconditioned stimulus; HVS, high voltage spindle; PBS, phosphate buffered saline

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conditioned stimuli occurred in the cat visual cortex during instrumental conditioning paradigms [30,43].

In the present study, we establish the dynamics of alterations in transmission in barrel cortex induced by sensory conditioning using the evoked potential technique in conjunction with spectral analysis of local EEGs.

2. Materials and methods

2.1. Preparatory procedures

Eight hooded male rats (R22-R29), weighing from 300 to 400 g, were used for these experiments. During the habituation sessions rats were kept in a specially designed chamber which restrained body movements but allowed us free access to the whiskers. Animals were accustomed to this holder until they showed no signs of fear. This took some 2-3 weeks of training for 40 min each day, rewarded by food (cheese, bananas, sweet porridge—whatever they preferred) at the end of each session. After this initial training surgery was performed. Rats were premedicated with atropine (0.4 mg/kg body weight, s.c.) and anesthetized with chloral hydrate (1 ml of 3.5% solution per 100 g body weight, i.p.). Additional doses of l/5th of the primary dose were added when necessary. On both sides of the skull two holes (2-3 mm posterior and 5-6 mm lateral to bregma) were drilled to expose parts of the barrel field. Two electrodes (see below) were placed symmetrically on each barrel cortex at the level of layer IV (450-780 /xm) and fixed with dental cement (Duracryl) for later chronic EEG recording. Two screws cemented in the center of the skull were used to fix the rat's head to the holder during the subsequent behavioral experimental sessions. The wound was rinsed with local antiseptic and the animals were left for a few days recovery.

2.2. *Conditioning procedure*

On the day of surgery, all whiskers were cut to the length of 15 mm. Low tone audio-speakers with a 30 mm long stick of balsa wood glued to the membrane were used for whisker stimulation. The end of the stick was attached to the whisker approximately 10 mm from the snout. With this arrangement single square wave pulses of 2 ms duration delivered to the speaker produced a 1 mm vertical movement of the whisker. An average of 8 evoked potentials (EPs) from each whisker was initially registered to choose the best responding whisker for further experiments.

The experiment consisted of five to six consecutive daily sessions. During one session, lasting for about an hour, the symmetrical whiskers on both sides of the snout received 20 trains of 5 stimuli each (with 1 Hz frequency within the train), delivered alternately to the left and right side with a time interval changing between 20, 30 and 40 s. During the first two sessions (marked 1H and 2H) the animal was allowed to habituate to the vibrissae stimulation. With the third session, the stimulation of the right vibrissa (CS) was paired with mild electrical stimulation (0.05-0.25 mA, 50 Hz, 1 s) of the tail's skin (US), with a 200-250 ms delay from the last stimulus in the train. The electrical stimulus was measured for each animal separately and kept always just suprathreshold in order to minimize the possible jitter in personal traits [46]. The whisker on the left side was stimulated without reinforcement for recording control potentials at symmetrical positions within the opposite barrel field. An experiment on each animal consisted of 3-4 conditioning sessions (marked 1C, 2C. 3C and 4C).

2.3. Recording and histology

Tungsten electrodes of 50 /xm diameter, sharpened and insulated with lacquer, were used for monopolar micro-EEG recordings (abbreviated as EEG) with the screw in the nasal bone used as a reference. Simultaneous EEG signals from 4 electrodes were amplified (1000X , input resistance 5 MH), filtered (from 1 Hz to 1 kHz) and stored on magnetic tape using a RACAL V-store recorder. Two other channels were used to register stimulus markers (7 of 8 rats) and comments on the animal's behavior (all animals). All taped data were examined for integrity and epochs with artifacts were excluded from further analysis. The EEG signal was digitized off-line with a 400 Hz sampling frequency and stored in a PC compatible computer. The interstimulus intervals (lasting about 10-40 s) were used for FFT calculations. For evoked potentials analysis, epochs of 100 ms duration commencing 10 ms prior to stimulus were digitized at a frequency of 6666 Hz and averaged for the whole daily session (about 100 single EPs). During the episodes of high-voltage spindles (HVS) vibrissa stimulation did not evoke any noticeable responses (see Section 3). Such events encompassed less then 15% of all stimulations and appropriate trials were excluded from averaging. The experiment lasted about two weeks after the surgery. Only one electrode failed to record during this period.

At the end of the experiment animals were overdosed with Nembutal and perfused with PBS and 4% paraformaldehyde in PBS. Brains were removed, and both cortices flattened [50]. Cortical slices, 50 *fim* thick, were cut tangentially to the surface, air dried and stained with cresyi violet to reveal the barrel map. Electrode positions were verified from easily visible traces of gliosis. Histological analysis in all rats showed that the localization of the electrodes on the two opposing barrel fields differed no more than an additional barrel diameter. Detailed analyses were carried out on recordings from the pairs of electrodes located most symmetrically within the opposing barrel fields (the data connected with continuous lines in Fig. 3A, C and D). In practice, the results from the remaining pairs of electrodes showed no differences to those from the

Table 1
The significance of the overall EEG spectral power decrease following application of the US stimulus

FF1 frequency bands	Consecutive experimental sessions				
	2H	1C	2C	3C	4C
1—4Hz	–	–	–	–	–
4—6Hz	–	–	–	–	–
7—12 Hz	–	–	0.035	0.035	–
13—20 Hz	–	–	0.035	0.035	–
20-40Hz	–	–	–	–	–
40—50Hz	–	–	–	–	–

–: Nonsignificant; 0.035 confidence level.

optimal ones (see broken lines in Fig. 3A, C and D). This finding assured us that the summed potentials, registered in our experiment, were rather insensitive to small displacements of electrode positions within the barrel field. Analogously, Di and Barth [19], found that a similarly pronounced negative component of the EP (N1, see below) can be recorded from the same electrode after peripheral stimulation of several neighboring vibrissae. From the above analysis we consider that N1 amplitudes recorded from all implanted pairs of electrodes could be used and directly compared for evaluation of experimental/control ratios. Five animals passed all criteria satisfactorily for EP analysis and comparison.

2.4. Statistical evaluation

Multivariate analysis of variance was used to reveal differences between EP amplitudes during subsequent sessions (control and conditioned). The Mest was then applied for establishing the significance of differences. The nonparametric sign test was used for establishing the global effects within the whole experimental group for both EPs amplitudes and EEG spectral power changes, within chosen frequency bands (see Table 1).

For each animal, the spectral power changes were revealed by means of a specially developed Curve Discrimination test (CD-test). The test was applied to Fast Fourier Transform (FFT) courses averaged separately from two classes of FFTs. Those FFT classes corresponded to different experimental periods in one session—those after stimuli applied to the control vibrissa and these after the CS + US complexes. The successive steps of the CD-test were performed along the frequency axis starting with the lowest frequency. Within a single step, we calculated the cumulative sums of the averaged FFTs and the difference between such integrals. This difference was interpreted as a measure of a difference between the two averaged FFTs. When applied to both classes of FFTs the probability distributions for such an integral were also determined. This procedure allowed us to find a confidence level for the computed difference between both integrals.

The probability distribution of the cumulative sum was determined from actual dispersion of FFT values. By calculating the standard error at a given frequency, it was possible to evaluate the width of the above Gaussian distribution and consequently the probability distribution for the cumulative sum. This distribution is a convolution of the FFT values probability distribution at a given frequency and the cumulative distribution evaluated in the former step of the testing procedure. When starting from the lowest frequency the subsequent cumulative distributions are determined directly from those previously computed. The confidence level for the difference between the FFT curves was calculated with the use of the probability distribution of difference between the integrals which was a convolution of cumulative distributions of the two FFT classes.

3. Results

3.1. Evoked potential data

Typical averaged evoked potentials, recorded from two electrodes placed symmetrically in the barrel cortex of the conditioned and control hemispheres in the unanesthetized rat are shown in Fig. 1. Characteristic components of the response evoked by vibrissa stimulation at monopolar electrodes [20] may be easily traced in all EPs presented in this figure. The response starts with a small positive deflection (PI) followed by a large negative wave with a peak latency of about 12-15 ms (N1), terminating with a long latency positive wave (P2). Since the position of the electrodes in relation to the surface of the cortex and the barrel center varied, the shapes of EPs also varied, with the two positive components not always well measurable (e.g., smaller PI and P2 components on the control EPs in Fig. 1; compare also Siegel and Sisson [46]). Therefore, we decided to measure only the amplitude of N1 which could

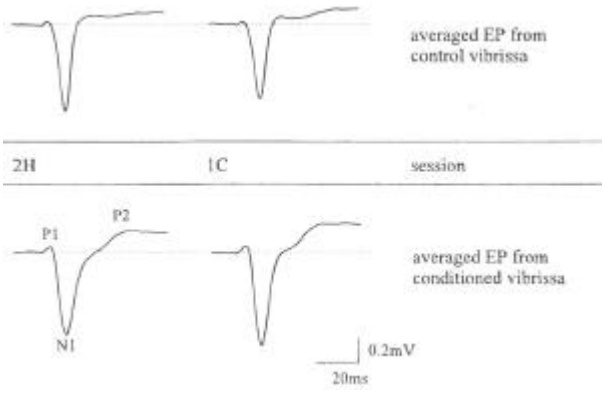


Fig. 1. Averaged evoked potentials obtained by stimulation of symmetrical vibrissae on both sides of the snout in Rat 25. About 80 potentials were averaged in each case. Symbols: 2H = second habituation day; 1C = first conditioning session; N1 = negative component measured in this experiment. Positivity **is** upwards.

consistently be recorded and calculated by computer software. Even with this limitation we did observe, in each recording site, a substantial variation of the N 1 component evoked by consecutive stimulations throughout all sessions (Fig. 2). This variation was not much higher than that observed in the anaesthetized preparation however [2,19,20,42] and can be explained by changes in the concurrent underlying EEG activity [2,7,9]. Despite the large variance of measured EP amplitudes statistical analysis clearly confirmed their alteration with conditioning (see below).

During first two experimental sessions the animals were allowed to habituate to the fixation and stimulation of the whiskers. The related behavioral signs of discomfort were barely seen only during beginning of the first habituation session (1H) and the evoked potentials amplitude stabilized also rather quickly. We allowed the second daily session for further habituation of the control responses but a small decline of N1 amplitude could be observed during remaining days of experiment (Fig. 2).

Figure 2 presents the values of all N1 amplitudes (5 measures from each CS train) throughout the last habituation (2H) and three consecutive conditioning sessions (1C, **2C,** 3C), in Rat 28. The average amplitudes of N1 waves as measured in EPs obtained for stimulation of control and conditioned vibrissae on both sides of the snout, did not differ significantly neither in session 2H nor in session **3C.** Both amplitude values in sessions 3C were, however smaller as compared to those measured in sessions 2H. This small decreasing trend of the EPs could result from progression of underlying habituation process in both hemispheres. When compared to control, it is easy to notice that EPs on the conditioned side were greater during **the** first two conditioning sessions (1C, 2C). The increase **in** EP amplitudes on the conditioned side was observed immediately after the first US stimulus presented, as we have reported previously [56].

For statistical evaluation of this increase, we normalized the averages of amplitudes in session 2H to 100%. The multivariate analysis of variance applied to data grouped in successive experimental sessions, for control and conditioned vibrissa, showed strong interaction between sessions and vibrissae for each animal (F = 6.5 to 41, $p < 0.0001$). This indicated that changes of N 1 amplitude in subsequent sessions significantly differed for control vs. conditioned vibrissa. Evoked potentials amplitudes were significantly larger on the conditioned side compared to the control side, during session 1C in each data set ($p< 0.001$, for four rats and $p < 0.09$ for one animal; Mest).

The increase of EP amplitudes with conditioning has been confirmed by the sign test for the whole group of animals as follows. The amplitudes of N1 in daily sessions for all rats were averaged for each recording site. The normalized averages obtained from the recordings on the side contralateral to the conditioning whisker were then divided by the averages obtained from corresponding potentials evoked by stimulating the control whisker, assuming that they record activity from a symmetrical set of barrels (see Section 2). The normalized ratios (plotted **in** Fig. 3A), calculated for all data from symmetric pairs of electrodes increased with introducing the US reinforcement in the first conditioning session (1C). The relative increase in N1 in the first conditioning session in comparison to the last habituation was found to be statistically significant in the whole group of animals (p <0.01; data from only one pair of electrodes for each rat was taken for analysis—see Section 2). On the following day—during the second conditioning session (2C)—we observed a further relative increase in N1 for 5 recording sites (in 3 animals) and a decrease in these values for the remaining 4

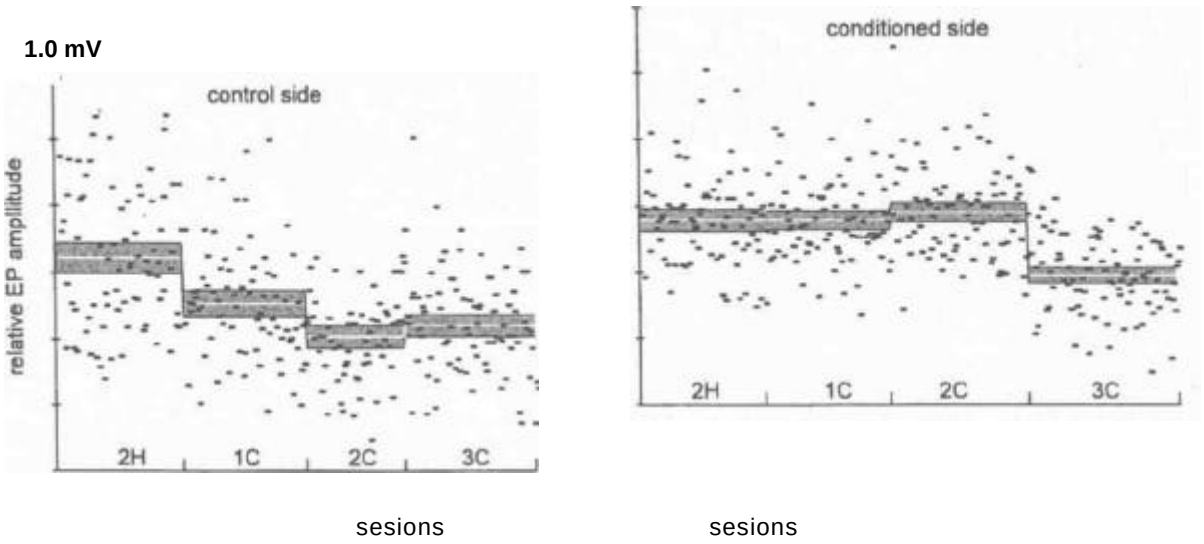


Fig. 2. The amplitudes of the negative component N1 measured in single conical EPs during the last habituation and three conditioned sessions in Rat 28. The results from following sessions arc separated by dashes on the abscissa. The average values for each session are plotted as straight lines with 2 SEM corridors. Note greater N1 amplitudes in day 1C and 2C on the conditioned side when compared to the control. A small trend for decreasing amplitudes accompanying the habituation process is evident for both experimental and control sides. Some stimuli applied during periods of strong HVS episodes fail to evoke significant responses and were excluded from the analysis (sec Section 2). This led to truncation in the corresponding data series as in session 2C for control vibrissae stimulation.

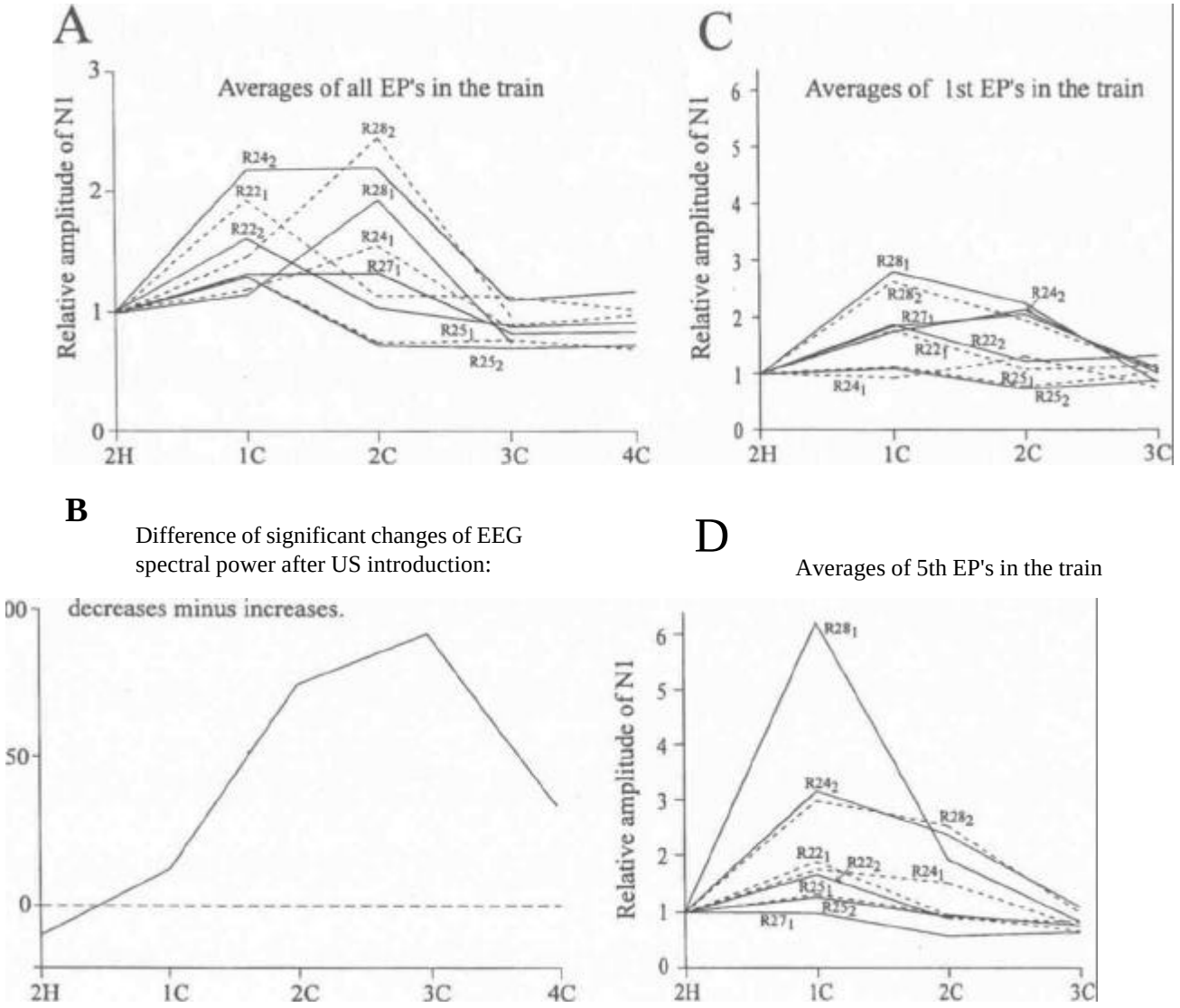


Fig. 3. (A) Relative N1 amplitudes (amplitude on the conditioned side/amplitude on the control side) in three consecutive sessions normalized to the same ratio calculated for the second habituation session. Subscripts 1 and 2 marking data from different animals (R22, R24, R25, R27, R28) refer to recordings obtained from anterior and posterior pairs of electrodes in the same animal. The continuous lines connect data points obtained from electrode pairs most symmetrically located in two barrel fields (see Section 2). The broken line correspond to data from other electrode pairs in all animals. (B) Number of significant decreases minus number of significant increases of EEG power following all CS-US applications as measured in all frequency bands for all animals during consecutive experimental days. (C, D) Relative N 1 amplitudes as presented in A. but calculated separately for first (C) and fifth (D) stimuli in the conditioning train. Data from 5 rats are presented in A, C, D and for 8 animals in B. See Section 2 for details.

paired sites (in 2 animals). These differences could be due to different dynamics for the acquisition of the train related conditioned response. As can be seen in Fig. 3C and D the relative increase in amplitude starts and ceases more rapidly for the 5th stimuli than for the 1st ones. In fact, the relative amplitudes of the 5th EPs in the train (which proceeded immediately the US) reached maximal values during the first session for all recording sites (Fig. 3D). On the third conditioning day (3C) normalized ratios for paired EPs in all animals reverted to their original values. This relative level of the N 1 wave subsequently remained constant over three consecutive days (4C-6C) as found for the four rats which were investigated over this longer period of time (not shown).

3.2. Fourier analysis of EEC activity

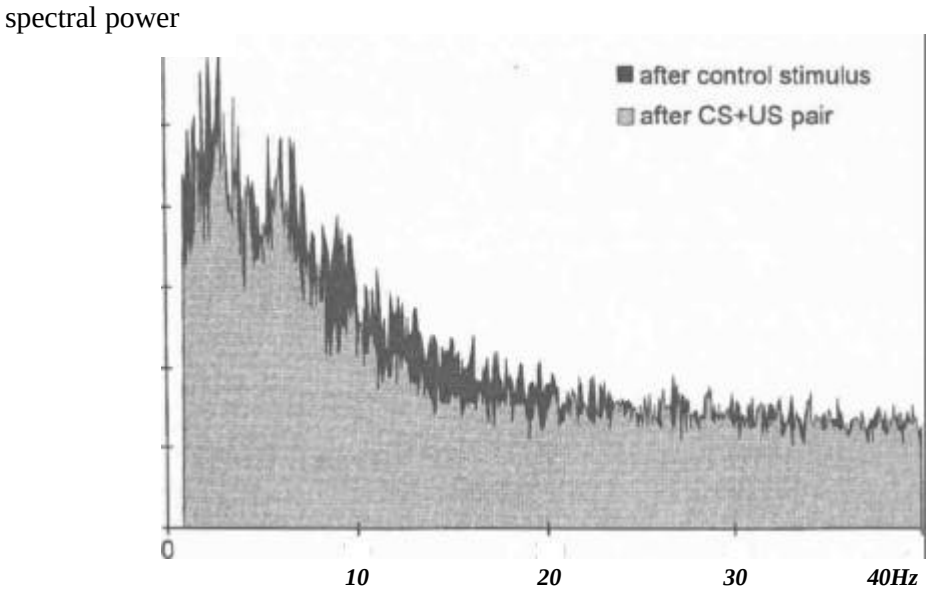
It has been reported previously that the 'degree of desynchronization' of the EEG (i.e., shift of the spectral power from lower to higher frequencies) is positively correlated with the amplitude of evoked responses recorded in sensory cortex [21,46]. In attempting to trace any reorganization of cortical activity during conditioning we calculated the Fast Fourier Transform (FFT) of the EEG. We analyzed recordings which were approximately 10-20 s

long, taken from interstimulus periods during the experiment. Fourier spectra derived and averaged from all such periods and all electrodes during the second conditioning session (2C) from one rat are shown in Fig. 4. Close inspection of the histograms shows that the introduction of the conditioning stimulus was associated with a lowered power of the spectrum in 8-20 Hz band.

In some of the FFT histograms we observed a peak around 10 Hz which was associated with presence of the high-voltage spindles (HVS) activity characteristic for the motionless, awake rat [12]. The HVS events measured in the intertrial intervals lasted from 0.5 to 10 s, which agree with previously reported values [12]. We observed such activity in all but two experimental animals. The HVS episodes appeared simultaneously at all recording sites but their relative amplitudes differed irregularly. There was a tendency for HVS events to shorten during the interstimuli intervals which followed the application of US. This observation is in accordance with the observed statistically significant decrease in spectral power within the 7-12 Hz band in relevant experimental periods (Table 1).

For statistical analysis of the power spectrum changes we divided the spectrum into relevant EEG frequency bands: delta (1-4 Hz), theta (4-6 Hz), alpha (7-12 Hz), beta (13-20 Hz) and high beta plus gamma (21-50 Hz). The specially developed test for statistical evaluation of curve differences (CD-test, see Section 2) was applied to the spectral data from each session. Significant differences in spectral power at each recording site were detected **in** all bands for periods following conditioned vs. control stimuli. Since no significant differences between hemispheres related to the conditioning procedure were found, for further statistical evaluation we considered data gathered from all recording sites. We qualified the unidirectional differences (increases/decreases) of FFT power observed parallely at all four, or at least three electrodes (with the remaining one unbiased) as the US related changes of FFT within a given frequency band and session. The significance of these changes over the whole experimental group of animals was verified by the sign test. Independently from the hemisphere, a significant decrease in the EEG spectral power in alpha and beta bands was detected after US application during conditioning sessions (2C and 3C). The spectral power after CS-US pairing was found to be lower than that measured in the intertrial period after differential, control stimulus (Table 1).

To characterize the dynamics of the EEG changes throughout the whole experiment, we calculated the number of statistically significant decreases and increases of the EEG spectral power in all specified bands, which followed all US presentations in all animals. The differences between the global number of decreases and increases as obtained for each session are presented in Fig. 3B. The course of the function on Fig. 3B clearly shows the transient surplus of decreases evoked by the CS-US pair in the global power of cortical EEG. This function is, however, delayed for about a day when compared with the EP changes shown in Fig. 3A. It reaches a maximum at the third conditioning session during which the EP amplitude is dropping back to its control level. Such a close correspondence between both functions presumably represent some related underlying neuronal processes.



Rg. 4. An example of smoothed FFT spectra calculated from EEG data registered from barrel fields of Rat 28. Dark histogram represents FFT spectrum averaged from all inter-trial intervals following stimulation of the conditioned vibrissa, whereas lighter histogram represent averaged FFT from corresponding intervals after pairing CS with unconditioned stimulus. Both averages were calculated in session 2C from all four electrodes implanted in two hemispheres. Note decreased power from about 7 to 20 Hz. observed in both hemispheres for intertrial intervals following US. Spectra were cut above 40 Hz for clarity.

4. Discussion

In this study, we have shown that the amplitude of the N1 component of potential evoked (EP) in rat barrel cortex by conditioned vibrissa stimulation increases transiently in relation to the same component of the control potential. This hemispheric specificity proves that observed EP changes cannot be referred to any unspecific sensitization mechanism caused by the presence of noxious stimuli throughout the experiment but to the true conditioned response. The unspecific alertness instead, was manifested by uniform bilateral decrease in EEG power within the alpha and beta frequency bands which followed conditioning trials (see below).

The specific increase in the EP amplitude observed during conditioning suggests that information processing of the external stimulus drives the plastic changes at the sensory cortex. We cannot be sure, however, whether these changes take place solely in the cortex or whether they might partly originate in the thalamus as have been proposed by some investigators [4,25,33]. In support of the possible cortical origin of the conditioning evoked changes is the finding by Teich et al. [51], that intact auditory cortex is crucial for active inhibition of conditioned responses during an extinction procedure. We hypothesize below that the observed transient increase of the N1 component is also attributed to changes in cortical activity.

Single cell response enhancements lasting up to an hour were observed in the barrel cortex after acute concomitant stimulation of two whiskers in the rat [16]. Plastic changes were also observed with a different procedure in which 24 h 'pairing' of untrimmed whiskers during normal behavior led to transient enhancement of cell responses in supra- and infragranular layers in corresponding cortical columns [18]. The same procedure, prolonged for three days, resulted in a more persistent plasticity exhibited by barrel cells [17]. These results are especially interesting, assuming that the N1 peak we have measured in the barrel cortex originates mostly from pyramidal cells located in the infra- and supragranular layers [20]. Our results support therefore a notion about transient change in connectivity of pyramidal cells. These changes could then be transferred to layer IV granular cells [4] whose changes would not be realized in our study since they lack apical dendrites, and contribute much less to evoked potentials [20].

The modification of the N1 wave we observed was immediate and started with first US reinforcement but in most cases ceased in the next day session. Similarly, a rapid development of single cell receptive field plasticity was observed during classical conditioning in guinea pig auditory cortex by Edeline et al. [22]. These authors argued that together with specificity and endurance, such a rapid course of acquisition satisfies all criteria for processes involved in the formation of associative memory.

An enhancement of EP amplitude to clicks in aversive classical conditioning was found in the rat auditory cortex by Mark and Hall [35]. Although this phenomenon persisted for up to six days of extinction, it was shown to be non-specific and could be observed at the same recording site when the conditioned reaction was evoked by shifting to photic instead of auditory stimulation. From this. Hall and Mark [26] attributed the increase in amplitude of evoked potentials to a fear reaction rather than to a conditioning process. By contrast, the changes in the amplitude of the EP found in our study were stimulus-specific. Similarly, changes in potentials evoked by conditioning stimuli were observed during instrumental paradigms in the visual system of the cat [30] and the dog [43]. In both experiments, the induced responses persisted throughout the experiment. Whether the long effects in these changes, compared to our transient changes, were due to the conditioning procedure or to changes in thalamo-cortical synap-tic efficacy [4] remains to be investigated.

In the process of evaluating the behavioral meaning of the compound CSs used in the experiment, we found that potentials evoked by the last stimulus in the train changed earlier than the first ones (Fig. 3C and D). One possible interpretation for this observation is a gradual shift of the CS conditioned value from the last stimulus in the train, to the first one. During conditioning the aversive meaning of the last stimulus seemed to be gradually shifted toward the beginning of the train.

The transient increase in amplitude of EPs was accompanied in our experiment by latent decreases in the EEG spectral power in alpha (7-12 Hz) and beta (13-20 Hz) bands. The relationship between the EEG *desynchronization' (decrease in low frequency power) and the amplitude of the EP has been reported previously [11,16,21,47]. Our report is, to our knowledge, the first which quantifies this effect by statistical evaluation of powers in the Fourier spectra. The main effect observed was a shortening of high voltage spindle (HVS) episodes which activity Buzsaki et al. [12] found to be characteristic for behavioral immobility in the conscious rat. These authors suggested that HVS might reflect the mechanism of closing the thalamic gate for incoming sensory information. They argued that cholinergic activation on the neocortex is exerted by means of two mechanisms: direct excitatory action and suppression of reticular inhibitory input on relay thalamic nuclei. The last, should be manifested by suppression of the thalamic rhythm-generator mechanism resulting in a shift of cortical activity from HVS to 'desynchronization'—exactly as found in our experiment. Whether such a mechanism should be accompanied by an increase of power within the high frequency bands can be debated. Our statistical analysis does not allow for such an inference (Table 1).

The observation that an aversive US was followed by EEG changes could be explained by its arousal property. These effects were, however, lagging in relation to EP changes. In fact, the maximally significant decrease in EEG power was observed on the third conditioning day.

This was after the EP amplitude had returned to its starting value. Such delayed changes might, therefore, represent an active habituation process. Teich et al. [51] have shown that auditory cortex plays a crucial role in inhibiting a previously reinforced response during an extinction procedure in a classical conditioning experiment. It is, however, possible that retention of the learned connection might still be present at a single cell level [54].

The bilateral changes in EEG power agree well with their postulated neuromodulatory origin. Although basal forebrain cholinergic projections are unilateral the aversive stimulus applied on the tail would result in global cortical arousal [21,36,37,41,48]. There are strong evidences that also conditioning processes in the cortical level are supported by modulatory cholinergic mechanisms [36]. When studying neuronal mechanism underlying development of conditioned eyeblink reflex. Woody and Gruen [55] have shown that acetylcholine increases input resistance and the excitability of layer V neurons of cat motor cortex by decreasing an outward membrane current. Such a mechanism may act in local neuronal processes and therefore be input-specific. Since cellular conditioning was facilitated during behavioral experiments by glutamate application these authors have postulated that it may indeed accelerate acetylcholine-induced conditioning [55].

Barrel cortex neuron plasticity in conscious animals following a 'pairing* paradigm is also influenced by cholinergic mechanisms [15]. Similarly, Hars et al. [28] showed recently that the cholinergic input to cortex facilitates tone-evoked responses in auditory cortex cells in the awake rat. This notion was supported using the evoked potential method in sleeping animals [38,39,44]. Recently, Jacobs and Juliano [29] provided evidence that cholinergic depletion resulting in a decrease in neuronal response to evoked stimuli, transiently impairs an animal's ability to perceive and appropriately respond to sensory information.

Edeline et al. [21] and Metherate et al. [40] by studying auditory cortex in urethane anaesthetized rats concluded that the specific activation of cortical neurons combined with cholinergic action could provide adequate conditions for selective changes in response occurring during conditioning procedure. They found also that only a strong cholinergic input is able to produce the long-lasting facilitation of the evoked neuronal responses whereas a weak cholinergic activation brings only transient changes. On the other hand, the effects of strong stimulation of the basal forebrain nuclei might result in decreased sensory responses in the cortex [21,53]. We do not think that the transitory effect obtained in our experiment might be in any sense due to the strength of stimulation. We would rather argue (see above) that its mechanism is based on an active neuronal process accompanying mild but persisting and unavoidable aversive US.

We focused our discussion on cholinergic neuromodulatory action. It is worth mentioning the possible involvement of the noradrenergic system in the observed phenomena which seems to target onto the supra- and infragranular layers [5,36] producing desynchronization of cortical EEG [8]. Noradrenergic neurones of the locus coeruleus were found to participate in inducing the temporary alert state proceeding the subsequent specific reaction of the animal [6,24,45]. This activation usually disappears when the behavioral response is well established [10,34,45]. Finally, the glutamatergic pathways from non-specific, diffuse thalamic nuclei could also contribute to the observed effects [36], possibly by accelerating the underlying neuronal processes [55]. This action might not be, however, related to the well known augmenting mechanism [13,23] since our stimuli were delivered with 1 Hz, well below the frequency required for evoking augmenting responses[13].

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