In: M. Kossut (Ed.) Plasticity of Adult Barrel Cortex. (2001) Graham Publ. Corp. Johnson City, TN, 229-239.

MODIFICATION OF EVOKED POTENTIALS IN THE RAT'S BARREL CORTEX INDUCED BY CONDITIONING STIMULI

Andrzej Wróbel and Ewa Kublik
Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland

Abstract

Recording of potentials evoked (EPs) by whisker deflection in the barrel cortex of awake rat allow for accurate, long-lasting, on line observation of modification in cortical processing. The classical conditioning procedure with aversive reinforcement coursed specific, one-two days long increase of cortical EP amplitudes. The enhanced EPs were preceded by about 40 ms activation of local field potential. The data from anesthetized animals suggest that this activation may be partly due to cholinergic input from nucleus basalis.

1. INTRODUCTION

The constant flow of background sensory information activates in the primary cortex a spatiotemporal map of the external world which is habituated to low value in order to detect the new transients. Any lasting change in the background stimulation should accordingly reorganize this process. The broken vibrissa, for example, could distort the sensory map if a special build-in mechanism would not exist to allow for immediate detecting and successive sealing of discontinuity in incoming activation. Similarly, one would expect that other modality input of transient behavioral importance could also modify the sensory cortical processing.

In this report we review the search for a mechanism which allows the cortical network to rapidly recognize, and consequently adjust to steady, contextual stimulation. It was obvious that such investigation should be perform on behaving animal with precise time resolution of recorded cortical activity. The investigated system should also provide punctilious stimulation and focal recording. The vibrissa-barrel system of the rat seems to be ideally suited to fulfill all these requirements. Aversive classical conditioning was chosen as a model for enviromental change since it allows for simple delivery of the two associated stimuli. Additionally, this paradigm was previously shown to induce short-lasting changes in metabolic labelling within the cortical representation of stimulated vibrissae of the alert mice

(19).

2. VIBRISSA EVOKED POTENTIALS IN THE BARREL CORTEX

To reveal the dynamics of conditioned activation of the barrel cortex of the conscious rat we recorded evoked potentials (EPs) to stimulation of single vibrissa during a classical paradigm (16). This old electrophysiological technique offers several advantages. In opposite to unitary cell responses the stable evoked potentials can be recorded from one electrode for a long time (up to half of a year in our experiments) and therefore allow for tracing of activity changes accompanying the long-lasting conditioning procedure. The time resolution in such recordings is limited only by computer software. Although single EP reflects a summation of membrane currents from a large population of neighboring neurones the spatial attenuation of the field largely constrains the measured signal. Indeed, we found that field recorded by a single electrode was activated mainly within the principal column and only a small fraction of the surrounding barrel cortex (see Fig. 3B - N1 response area, comp. also Ref. 4). Such spatial distribution closely resamble the adequate measure of the receptive fields of single barrel units (Fig. 3 in Ref. 1). The spatial resolution of EP-method was shown to be sufficient to enable paired conditioning between neighboring vibrissae (13).

The typical EP contains fields originating from many subthreshold neuronal currents which can not be traced with electrode recording extracellular activity from single units. Analyzing such small currents enables for estimation of the subthreshold, modulatory inputs to the barrel column. Finally, we have previously shown that principal component analysis allows for extracting independent responses of supra- and infragranular pyramidal cell groups from EPs recorded with single electrod (17). Such analysis provides therefore the possibility for observation of the activation dynamics of main output pathways from the barrel column (for reviews see: 10, 1). Due to all above advantages the single EP contains information which otherwise require time consuming effort of recording from many individual neurons of different layers.

On the beginning of the experiment animals were accustomed to rest in a restraining apparatus for a few days, after which implantation surgery was performed. Monopolar electrodes were dipped symmetrically in both barrel cortices at the approximate level of layer IV and special device mounted to the scull in order to fix the animal's head to a holder during the subsequent experimental sessions. The fixation allowed to glue two principal vibrissae on both sides of the snout to the stimulators. The

principal vibrissa was selected from others by greatest amplitude of response from the appropriate electrode. Few following days of habituation was allowed for different rats to become accustomed to a restrained whisker. This could be recognized not only by the behavioral calm but most easy by stabilization of recorded EPs (16, 24).

The electrical responses in the barrel cortex were evoked by brief deflection (2-3 ms, 100 µm) of the principal whisker at different interstimuli intervals (20-40 s) delivered at random order. Each evoked potential (Fig. 1A) consisted of three main components: P1, with a peak latency of about 5-6 ms, N1 reaching maximum at about 10 ms and P2, at about 20-25 ms. The P1 component could be attributed to the incoming activation of the thalamo-cortical fibers since it was the only component surviving the most severe, reversible cooling of the cortex (12). The N1 component reflects mostly the postsynaptic activation of the pyramidal cells in layers II, III, V and VI since their apical dendrites extending perpendicularly to the cortical surface form natural dipoles easily detected by microelectrodes (5). We estimated that contribution of the pyramidal neurones activity to the N1 component was 75% in the nonanaesthetized rats (17).

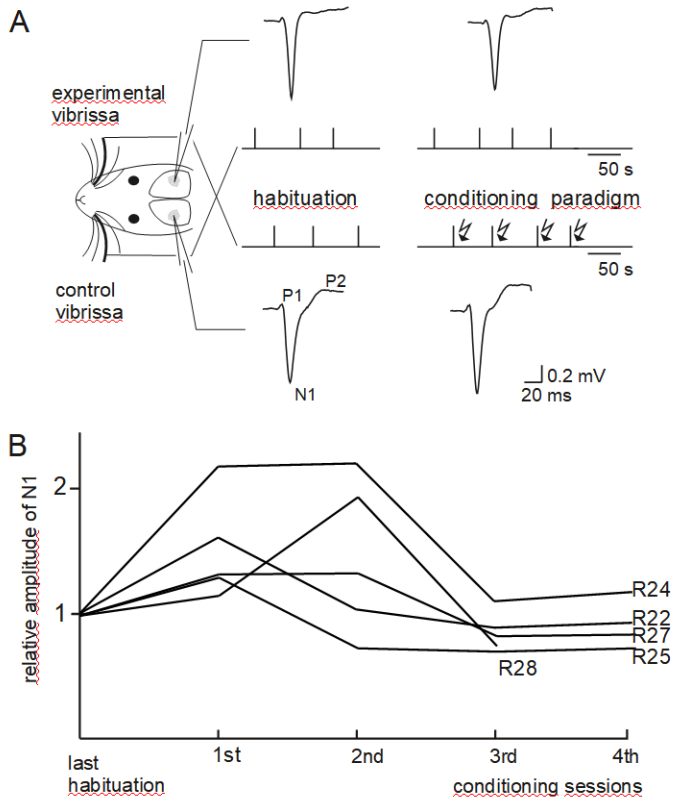

Fig. 1. A, Evoked potentials obtained by stimulation of symmetrical vibrissae (marked by thicker lines) on both sides of the snout in Rat 25. About 80 potentials were averaged in each case. P1, N1, P2- main components of typical EP in the barrel cortex. Nete constant amplitude of P1 and increase of N1 and P2 after introduction of

reinforcing stimulus on the conditioned (left) side of the cortex. Broken arrows indicate mild electric shock (US) applied to the tail200 ms after vibrissa stimulation (CS, marked by short vertical lines on time axes). Monopolar recordings from layer IV. Positivity is upwards as it is on all other figures showing Eps and LFPs. **B,** Relative N1 amplitude (average amplitude on the conditioned side devided by average amplitude on the control side) in five consecutive experimental sessions, normalized to the ratio calculated for the last habituation session. Note greater N1 amplitudes in first and second conditioned day as measured in experimental side of the cortex, when compared to the control.

By recording the EPs to stimulation of all vibrissae in the barrel field we could map the activation fields for both N1 and P2 components (Fig. 3A). N1 had a smaller field diameter with steeper weighing function than P2, which was evoked in most of the barrel field (comp. also Ref. 4, 11). The comparison of excitation, latency and polarity of these two main components allowed us to attribute their main origins either to the barrel corresponding to the principal whisker (N1) or to the surrounding barrel cortex (P2).

3. TRANSIENT ACTIVATION OF BARREL CORTEX INDUCED BY CONDITIONING

After habituation of the background responses during first two sessions a mild electric shock (US) was applied to the rat's tail 200 ms delayed to each stimulation of the right vibrissa (CS). Since the position of the electrodes implanted in both barrel field varied in relation to the surface of the cortex and the principal barrel centers, the shapes of EPs also varied. We therefore measured amplitudes of only largest, N1 components: control on the right barrel field and conditioned on the left (Fig. 1A). Together with the first US application, the amplitude of the N1 component of EP on the conditioned side of the cortex grew significantly in relation to that evoked by stimulation of symmetrical, contralateral vibrissa (Fig. 1, comp. also Ref. 16). This enhancement ceased eventually after two - three days of conditioning despite continuous reinforcement (Fig. 1B). The equal level of the N1 amplitudes measured in both cortical fields remind subsequently constant during three consecutive days (sessions 4-6, not shown).

The hemispheric specificity of the transient increase of N1 component 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. Although we do not have direct evidence for the cortical origin of observed plastic modifications (for discussion see Ref. 16), such interpretation is favored by the fact that P1 component, which we have attributed to the thalamic volley (11), reminded constant in most of our experiments (e.g.

Fig. 1A). If prominent plastic changes would originate at thalamic level, P1 component should have adequately alter. The possibillity that small part of plastic modification observed in the adult barrel cortex may be of thalamic origin has been reported recently (2).

The unspecific effect of the noxious stimulation during conditioning procedure could be observed by its arousing influence on activity in both cortical hemispheres. It was manifested by uniform bilateral decrease of the power of the local field potentials as measured within 7-20 Hz frequency band during intertrial periods following the unconditioned stimulation. This general effect was most prominent at the second and third days of conditioning and then ceased. It seems adequate to speculate that the recruitment of plastic modifications in the barrel cortex is based on classical hebbian mechanism and requires both specific and unspecific activation (comp. also Chapter 5 and Ref. 16).

4. RAPID DYNAMICS OF THE CONTEXTUAL CORTICAL ACTIVATION

After proving that the observed changes of EPs were hemisphere-specific we examined detailed modifications of the components of evoked potentials. Few methodological improvements were introduced in this part of experiment. The electrodes were placed on the same side of the head as reinforcing electrode mounted on the rat's ear. Such close arrangement of electrodes delivering CS and US stimuli further improved the US reinforcing effect. To allow direct comparison between the control and conditioned EPs we have started the US application in the middle of the first conditioning session.

The revised experimental paradigm allowed observation that the increase of the N1 ad P2 amplitudes started already with the few first applications of the reinforcing stimulus (Figs. 2, see also Ref. 23, 24). In fact, the largest increase of the amplitude of these components was typically seen after second up to tenth CS-US pairing and then the responses stabilized at lower but still elevated level throughout the rest of the session (Fig. 3A). Such a dynamics of EP modifications was repeatedly observed within the group of six rats (Fig. 2B).

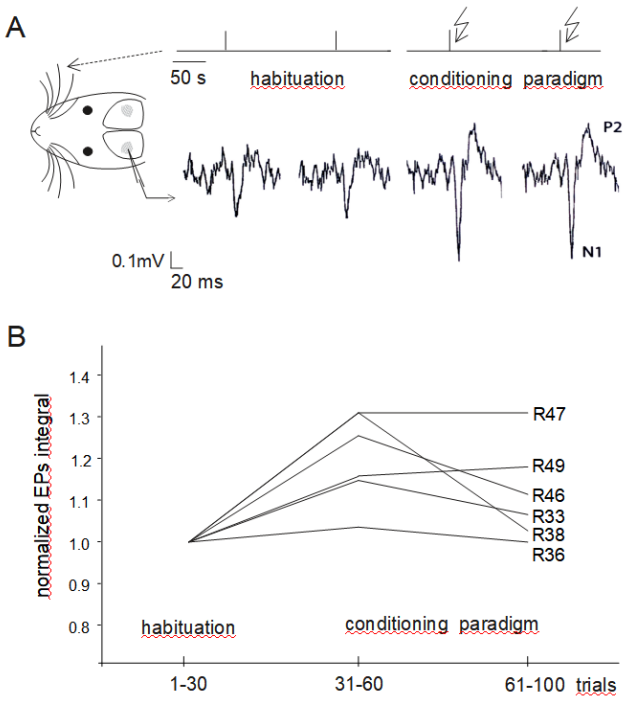

Fig. 2. A, Paradigm of second experiment and avoked potentials obtained by stimulation of principal vibrissa in Rat 38. The individual variability of Eps was lowered by averaging the EPs from three consecutive trials (the current, preceding and following). Two last averaged EPs from habituated period are shown to the left, second and third of the conditioned part of the session, to the right. N1,P2- negative and positive EP components. Note increase of both of these components with introduction of mild electric shock (US, broken arrows) applied to the ear at the same side of the snout (left) as stimulated vibrissa (CS,short vertical markers on time axis mark moment of vibrissal deflection).Bipolar recordings. **B,** EP integrals, averaged within groups of 30 consecutive records and normalized to the to the value of the first group (1-30). EP integral was measured as modulus of area between N1 and P2 curves and steady voltage of starting value.

It is worth mentioning that large amplitude EPs were also observed together with application of other contextual, aversive stimulation (24). For example, gluing of the principal vibrissa to the stimulator during first session produced an unpleasant, arousing situation because it prevented the possibility of voluntary whisker movements. Such vibrissal restraint in the first habituation session was accompanied by EPs of large amplitude which gradually decreased in parallel with the putative habituation process (within 2-4 days in most rats). Cliping up the electrode that delivered the US to the rat's ear was another stimulus which enhanced the amplitude of EPs and habituated similarly, within one to three days. Finally, we have also found that dynamics of modifications of the EP amplitude dependent on the behavioral value of US. For example, the weaker US current was used for conditioning the smaller amplitude changes, and with longer delay, were observed.

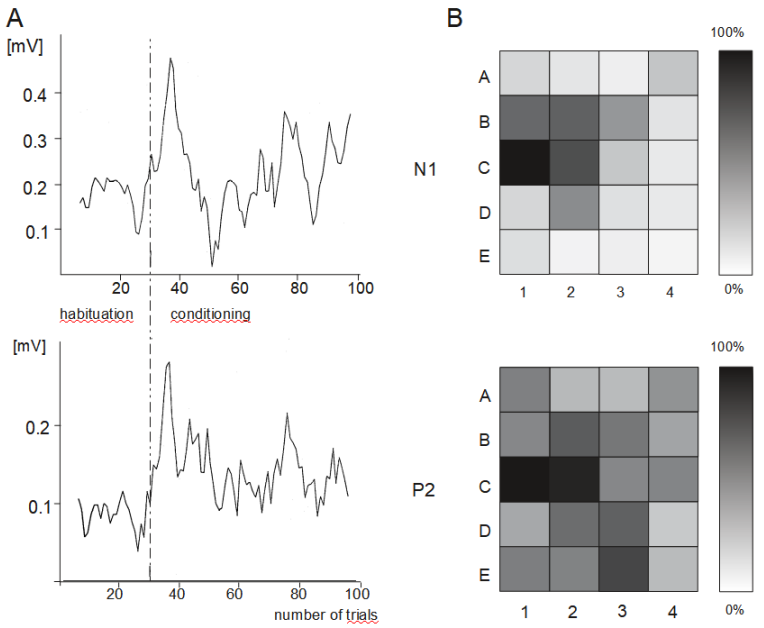

Fig. 3. A, N1 and P2 amplitudes measured from single, consecutive evoked potentials durind session in wich US was applied for the first time after 30th CS, and then continously paired with it. Note large, transient increase of both amplitudes during first 16 paired stimulations followed by less eminent enhancement phase starting from 59th stimulation and lasting until the end of the session. **B,** Normalized N1 and P2 response maps within the barrel field. The map was taken before the conditioning session shown in **A.** The bipolar electrode was placed in C1 barrel column.
Amplitude of the averaged EP (20m recordings) to stimulation of principal vibrissa was taken for 100% and marked as black box within the scheme of barrel field matrix.
The averaged amplitudes of N1 and P2 values recorded with the same electrode after stimulation of other vibrissae were shadowed with corresponding gray density scale. Note steeper weighing function of N1 than P2 activation fields.

We do not know the cellular mechanisms underlying the observed enhancement of EP amplitude on the conditioned side of the cortex (for detailed discussion see Ref. 16). It is reasonable to suggest that this enhancement originated from more cells being excited in the barrel column during facilitatory action of neuromodulatory inputs activated by aversive stimulation (comp. 6, 14, 18, 20, 22). The cholinergic input, for example, have been recently shown to have most prominent impact on the muscarinic receptors at apical dendrites of pyramidal cells (21) which could lead to the increase of N1 component amplitude. The relatively short time course of sensitization and internalization of cholinergic receptors (21) parallels the dynamics of the transient increase and return of EP amplitudes, exactly as observed in our experiments (Figs. 1B, 2B and 3). Transient action is exerted on the cortical network also by noradrenergic input (18). The other possible hypothesis for the declining phase of the observed effects would involve active habituation of unavoidable aversive stimuli (16). Whatever the mechanism, the transient modifications of EPs during conditioning procedure is obviously accompanied by parallel modulation of activation of the

large part of the barrel cortex.

5. MODULATORY ACTIVATION OF THE BARREL CORTEX PRECEDES TEMPORARY INCREASE OF VIBRISSA EVOKED POTENTIALS

We tried to reach a more global insight into the activation mechanisms accompanying conditioning by studying the low frequency components of the local field potential (LFP) measured with the same microelectrode as used for evoked potentials recording. In order to do so we analyzed data with the lower filter cut-off set at 0.1 Hz in believe that possible modulatory inputs could be traced above this frequency (20). All EPs from the experimental session were classified into two classes according to the relative contribution of their subcomponents by means of specially designed algorithm (24). The resulting class 1 EPs were all of small N1 and P2 components and were most frequently found during habituated period of experimental session, just before US introduction (compare average class 1 EP in Fig. 4A and EPs amplitudes numbered 1-20, 22-31 and 47-58 in the experiment showed in Fig. 3). In opposite, the class 2 EPs had large N1 and P2 components and appeared most frequently after first thirty paired CS-US stimulations (class 2 averaged EP in Fig. 4A and corresponding EP amplitude values numbered 21, 32-46 and 59-100 in Fig. 3). It is obvious that appearance of class 2 EP parallells period of enhanced cortical activity.

Asking for possible neuromodulatory action shaping the cortical responses one should study the related background activation of the responding cells. We have therefore separately averaged the EPs of both classes together with their preceding, one second long, LFPs (25). The results indicated that the enhancement of the N1 component was always led by a positive shift of LFP level (Fig. 4A). This positivity was systematically observed in the group of five rats, within 40 ms period preceded the occurrence of class 2 EPs (Fig. 4B). Since the vibrissal stimulation was independent from LFP fluctuations such a correlation indicates that both events are tightly related. The recording electrodes used in this experiment were located at the level of layer V and it is reasonable to hypothesize that the current source observed before "activated" EP class corresponds to sink in superficial layers. Such a sink could be most probably coursed by neuromodulatory fibers ending in layer I in close relation to apical dendrites of pyramidal cells from layers II, III and V (7, 20, 21).

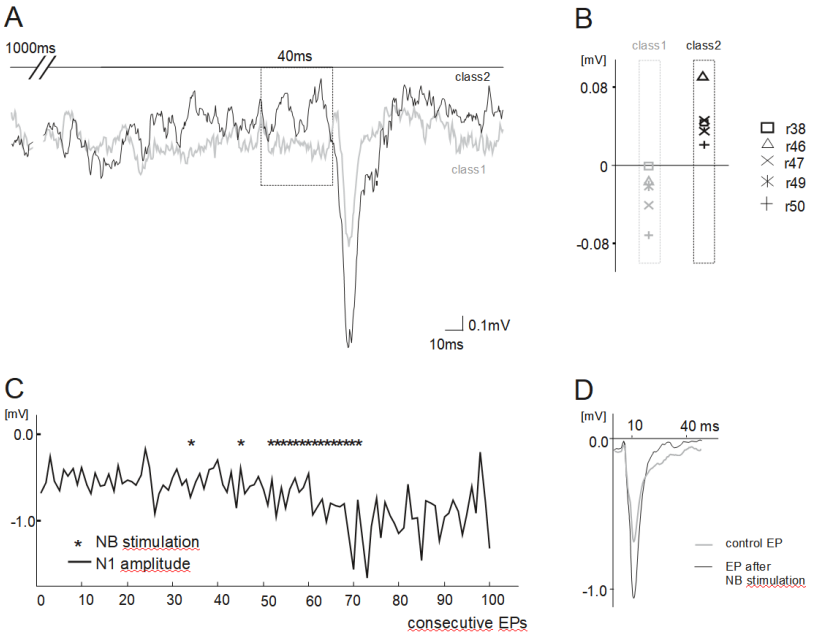

Fig. 4. A, Evoked potentials averaged within classes grouped according to the relative magnitude of their subcomponents (Rat 46; see text for details). EPs of class 1 were most frequently recorded in rats habituated to CS and those of class 2 from animals aroused by US introduction. **B,** Averaged values of local field potential measured during 40 ms preceded the occurrence of potential of the corresponding classes shown in A. Data from 5 rats. Note positive shift of LFP accompanying appearance of class 2 EPs. Lower filter set at 0.1 Hz. **C,** Experiment on anaesthetized rat. N1 amplitudes were measured after vibrissa deflection every 20 s. Stars indicate those trials which were measured by NBM stimulation (200 uA, 0,5 ms long rectangular stimuli, with 20 Hz for 1 S). **D,** Averaged Eps (20 recordings) obtained before and after NBM stimulation.

One of the neuromodulators which has been shown to selectively enhance efficacy of cortical synapses is acetylcholine (15, 21). In awake rats the cholinergic neurons of nucleus basalis magnocellularis (NBM) participate in enhancement of cortical activity (3). The projection from NBM and cholinergic receptors in the cortex seem to play an important role also in cortical plasticity induced by conditioning (9, 13). We have therefore run a control experiment on anaesthetized rats in which each vibrissa stimulation was preceded by electrical stimulation of NBM. Although direct electrical stimulation hardly reminds physiological activation we could observe very similar dynamics of vibrissa evoked potentials in the barrel cortex as observed earlier in behaving animals. The similar enhancement of N1 amplitude was accompanying the first twenty trials reinforced by US applied on the ear in nonanesthetized rat (Fig. 4A) and paired with NBM stimulations in sleeping animal (Fig. 4C,D). The longer trains of NBM stimuli led to less consistent results (comp. Ref. 8). The two described experiments support the notion that acetylcholine may play regulatory role in information processing and plasticity within barrel cortex.

Acknowledgements

We thank dr. P. Musiał who took part in all described experiments. E.K. was granted with doctoral fund from the State Committee for Scientific Research No. 4P05A.054.11.

ABBREVIATIONS USED

EP evoked potential
LFP local field potentials, intracortically recorded micro-electroencephalographic activity
CS conditioned stimulus
US unconditioned stimulus