Effects of GABA and Picrotoxin on Temporary Bonds in Neuronal Populations of the Motor Cortex

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INTRODUCTION

The temporary bonds within neuronal populations revealed by changes in the neuronal activity can be formed directly in the cerebral cortex. As a result of paired stimulations applied to either two subcortical brain structures or two different sites of the cortex itself, cortical neurons acquire the ability to generate, in the absence of a supporting stimulus, impulse activity characterized by an altered pattern [1]. The set of such changes is linked to the moment of the application of the supporting stimulus. It consists of the elements reproducing a response to the real stimulus and activity patterns that differ in their pattern from the evoked responses observed later. The above types of activity rearrangements may develop when impulsion arrives at the cortex through different pathways: the results from the specific (stimulation of the medial lemniscus, ML) or nonspecific (stimulation of the reticular nucleus in the midbrain tectum, RF) stimulations, or from the recurrent influences (stimulation of the pyramidal tract, PT). This indicates that similar intracortical mechanisms are involved in the formation of a temporary bond under these conditions. However, the probability of each rearrangement is determined by the specific pathway by which the impulses arrive at the cortex.

Further study of the above phenomena with help of pharmacological analysis should be an object of intense interest; the direct application of pharmacologically active substances to the cortex may be most useful for that. Such an approach excludes the occurrence of reflected effects caused by the influences of substances used on other brain structures. In addition, it allows one to influence the synaptic transmission in the entire neuronal population involved in the temporary bond formation, rather than only the neuron examined, which cannot be considered the only site responsible for the above phenomena. We have already analyzed the role of cholinergic transmission during temporary bond formation in the populations of cortical neurons [2, 3]. The aim of the present work was to study the effect of the changes in the GABAergic transmission upon the temporary bond described above. It seemed worthwhile because GABA is commonly considered to be a dominant inhibitory transmitter in the brain cortex [4-7]. In addition, despite the contradictions in the experimental data, the GABA-based drugs are used in clinical practice to cure memory disturbances [8]. Some of the preliminary results obtained with the use of GABA application were discussed earlier [1, 3].
Methods

The experiments were performed on 53 awake non-immobilized New Zealand rabbits (males) weighing 2.5 to 3.0 kg. The electrodes for stimulation of the ML, RF, and PT were inserted in advance under nembutal anesthesia [2, 3].

The brain structures were stimulated with 0.1-msec rectangular pulses. As in our previous experiments, paired stimuli were applied to two distinct brain structures (the ML, RF, or PT) at an interpulse interval of 1.2 sec. The interval between the pairs of stimuli varied from 10 to 60 sec. The number of combinations used to test a single situation exceeded 100. After every 20 combinations, the first stimulus only was applied five times in succession. The recording techniques, the activity registration, localization of the cells, and their identification as pyramidal neurons were described earlier [2]. If the supporting stimulus was missing, the evoked neuronal activity and the rearrangements in the spike trains were examined with the use of the computer analysis of poststimulus histograms [2]. Three types of histograms were used, with the 50-msec-, 500-msec-, and 5-sec-long epochs analyzed, and with 1-, 10-, and 20-msec-long bins, respectively. The histograms of the first and second types were plotted separately for each of the combined stimuli and for the cases when the supporting stimulus was missing. The third type of histograms allowed us to cover an interval including the results obtained when either both stimuli were combined or only the first stimulus was used, while the second one was missing. The reasons why the three types of analyses were used in parallel had been explained earlier [2].

The solutions at a volume of 20 μl were applied to the motor cortex through the trepanation-hole site where impulse activity was recorded 20-30 min before the experiment, and once more before testing. The 1% GABA solution (9.7·10^{-2} M; Sigma, USA) and 0.5% picrotoxin solution (8.3·10^{-3} M, Sigma, USA) were used. These concentrations were close to those used conventionally in the neurophysiological experiments when the substances are applied to the cortex, and corresponded to the doses usually injected into the inner brain structures [9-11]. In control experiments, physiological saline was applied.

After the experiments, both the localization of stimulating electrodes and the absence of pathological changes in the cortex were controlled histologically.

The statistical analysis of the resulting data was similar to that previously described [2]. The presence of the neuronal responses and the activity changes observed in the absence of the supporting stimulus, as well as the similarity or diversity of the responses, were estimated using the χ²-test. The numbers of neurons with different types of reactivity, as well as the effects of different substances applied, were compared as the alternative distributions using the χ²-test. Standard errors for the variance shares in the case of an alternative distribution were calculated (Figs. 2-4).

Results

The impulse activity was recorded from 366 neurons. Combined stimulations of the brain structures resulted in formation of a temporary bond. The spike train rearrangements occurring at a time when the supporting stimulus was missing were recognized as main indications of the temporary bond.

Figure 1 shows the neuronal impulsation and the corresponding poststimulus variation curves obtained when the two stimuli were combined or when the second one was missing. The rearrangements of the impulsation were observed both in the neurons sending and not sending their axons to the PT. However, they were observed significantly more often in the cells located in the upper layers of the cortex than in the cells located in its deep layers (P < 0.05-0.01); a relative border was drawn at the level of 1100 μm [12]. The manifestations of the temporary bonds in the case of different sequences of brain stimulations were analyzed earlier [1].

The results obtained with the use of different stimulation sequences are analyzed together. We used this approach because, first, the number of times each pair of stimuli was applied (its share of the total number of stimuli) was approximately similar for each solution. Second, the effects of substances observed when different brain structures were stimulated were of similar direction. Third, it seemed reasonable to look for common features in the pharmacological modulation of temporary bonds in the cortical neuron populations at this stage of our study.

During the analysis, our attention was drawn to the configuration of the changes in neuronal activity in the absence of a supporting stimulus. These changes were classified as generation of activity with a pattern either similar to, or different from, that of the evoked responses. The time of the onset of such changes was fixed. The changes in the spike trains observed in the absence of the supporting stimulus could be related either to one of the above types or to both types.

A 50-msec-Long Analysis Epoch. Figure 2 shows the data on the total number of cases in which the spike train was rearranged in the absence of the supporting stimulus, the cases when response patterns were partly reproduced, and the cases when the spike trains differed from the response patterns.

Application of GABA decreased the probability of impulsion rearrangement at the moment when the missing stimulus should appear (Fig. 2A, 2). The decrease was significant both when the total number of