Time Course of Conjugated Neuronal Firing in the Motor Cortex of Conditioned Cats

A. V. Bogdanov and A. G. Galashina

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The activity of neurons able to generate impulses synergistically at intervals of not more than 0.5 msec (conjugated neuronal firing) was analyzed in the motor cortex of cats with two forms of learned behavior (a conditioned response to time and a conditioned defensive response to sound). It is shown that conjugated neuronal firing is a process based on spontaneous impulse traffic and that its nonuniform distribution in time is determined by changes occurring in cellular structures during adaptive behavior.

Key Words: *conditioned response; multineuronal activity; cross-correlation analysis; conjugated neuronal firing*

The cross-correlation analysis of cell-to-cell interactions, introduced in 1967 [10], can demonstrate only the presence or absence of a functional connection between neurons, whereas analysis of the times at which impulses making up the peak on a histogram arise provides information on the structure of interneuronal connections. Accordingly, some scientists [5-8] began singling out such impulses for a secondary analysis by the method of autocorrelation, which showed that the distribution of conjugated impulses in time was nonuniform. Although this method failed to reveal at which particular moments the neurons worked in a synergistic manner, the information it yielded proved very useful because it allowed neuronal activity in animals to be considered in relation to their behavioral responses. Our early studies of this kind addressing the structure of conjugated discharges [4,9] indicated that, in real time, the intervals between conjugated impulses can vary widely from a few milliseconds to several seconds. More recently we proved that the nonuniform distribution of conjugated impulses is not a random phenomenon [3]. Specifically, our analysis of conjugated neuronal discharges in animals that had learned a conditioned response to time showed a concentration of these discharges at the times when the conditioned response was being performed. In the experiment reported here, we tried to find out whether the nonuniform time distribution of conjugated impulses is peculiar to the form of learning in which the conditioned stimulus is time or whether it is a more general property developed by cellular structures of the brain during learning.

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow

MATERIALS AND METHODS

The experiment was carried out on four cats under conditions of free behavior. Multineuronal activity was recorded in all cats from the cortical representation of a foreleg with 50-micron Nichrome electrodes [1] implanted under Nembutal anesthesia. After series of impulses emitted by individual neurons were singled out from the total multineuronal activity by the shapes of their component spikes [2], cross-correlation histograms were constructed with a step of 0.5 msec and an epoch of 25 msec [1]. Thereafter, a secondary analysis of the peaks on the histograms was performed, evaluating in real time the instants at which conjugated impulses occurred and using the technique of polynomial smoothing to reveal the main trends in impulse distribution.

In two of the cats, a conditioned defensive response to sound (CR_S) was elaborated by delivering clicks (conditioned stimulus) at a frequency of 10 Hz

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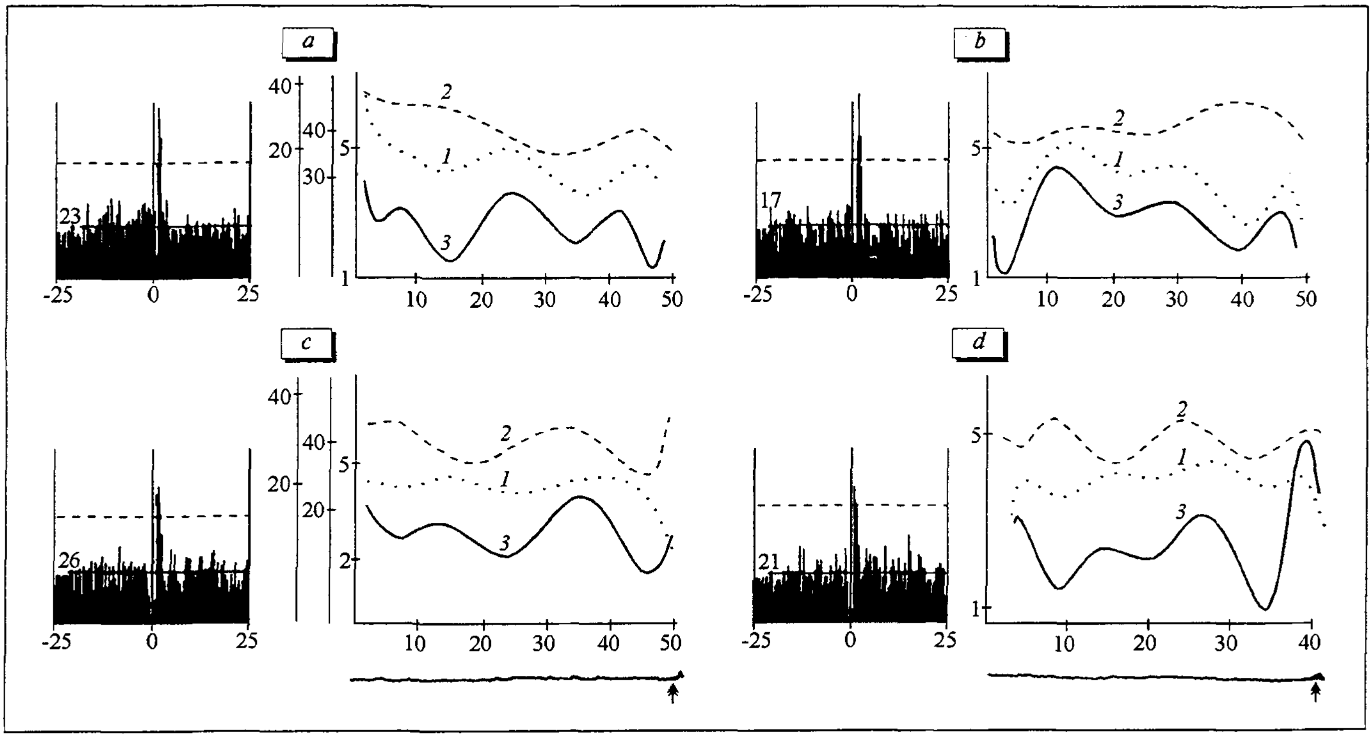

Fig. 1. Conjugated firing by one neuronal pair of a cat before (*a* and *b*) and during (*c* and *d*) the elaboration of a conditioned response to time (CR_T), *c* and *d* corresponding to the 12th and 15th presentation of the reinforcer (meat), respectively. To the left of the curves are cross—correlation histograms where the figures 23, 17, 26, and 21 are the average number of impulses per bin and the dashed lines indicate the level of significance (0.997). The curves show the distribution of initial (*1* and *2*) and conjugated (*3*) neuronal firing. Abscissa: real time in seconds; ordinate: number of conjugated impulses per step of analysis. The two lines on the bottom are chest mechanograms (recording time 1.5 min) where the arrows mark the times of meat presentation.

for 5 sec followed immediately by five electric shocks of threshold strength at the same frequency via electrodes applied to the foreleg. Enhancement of the myogram from the stimulated foreleg during the delivery of the conditioned stimulus was taken as evidence that the response had taken place. The myogram and artifacts from the clicks and electrostimulation were taperecorded. The impulse activity of 45 neuronal pairs recorded during the 30-60 sec elapsing before the delivery of the unconditioned stimulus was analyzed for each cat.

In the other two cats, a conditioned reflex to time (CR_T) was developed by regularly presenting meat pellets (reinforcing stimulus) at 1.5-minute intervals, so that, to take them, the cat had to push the curtain concealing the feeding trough. The pushing of the curtain and the time mark were recorded automatically. The impulse activity of 38 neuronal pairs recorded only in the second half of the conditioned response interval (in the phase of active anticipation) was analyzed for each cat.

RESULTS

Before the CR_T was established, several functional connections that remained unchanged right up to the end of the experiment were identified between neurons of the motor cortex in the cats. Consider one of those connections over the period of four 50-second intervals (Fig. 1). It can be seen that the control histograms (*a* and *b*) have virtually the same pattern as the test histograms (*c* and *d*) (20 min later). The strength of the connection was 9.9 in the first control fragment (*a*), 7.8 in the second control fragment (*b*), 7.6 before the 12th food presentation, and 7.4 before the 15th, i.e., the strength of the connection was similar in three of the four fragments. This and the patterns of the histograms indicate that the functional connection between the neurons under study hardly changed at this stage of CR_T elaboration (the direction, latency, and strength of the connection remained virtually unaltered). However, as the distribution of conjugated impulses in real time shows, the neurons began to change their interrelated activities after the 9th to 14th presentations of food and did so just as it was presented. A secondary analysis of the control data on this functional connection revealed only slight variations in the real time distribution of conjugated impulses, coinciding with variations in the firing frequencies of both neurons. Conjugated impulses were clustered just at or, at any rate, near the end of the interval before the

12th food presentation and invariably at its very end before the 15th presentation. These findings show that temporal variations in conjugated impulses may be a more sensitive indicator of the changes taking place in the cortex in the course of learning than the distribution of initial (prelearning) impulse sequences.

Consider now the distribution of conjugated neuronal discharges recorded when a cat was displaying a well-learned CR_S (as indicated by a response rate of 70% during the experiment). Fig. 2, *a* reflects a situation in which the cat demonstrated a well-defined reaction (as evidenced by the surge on the myogram) to the conditioned stimulus. By the time of the response, the firing frequency of both neurons had increased, as had the clustering of conjugated impulses. At first sight, everything seems to be clear here: neuronal activation in the cortical projection zone where the "working" foreleg is represented was manifested in a sharply increased frequency of neuronal firing with a resultant rise in the number of conjugated impulses. However, as can be seen in Fig. 2, *b*, variations in the distribution of these impulses mirror variations in the firing frequency of only one neuron, while the increases in the firing frequency of both neurons and in the clustering of conjugated impulses during the action of the conditioned stimulus occur in the absence of a conditioned response. This suggests that both the temporal variation in the frequency of initial impulse series and the distribution pattern of conjugated impulses were associated with learning, for otherwise no change in neuronal activity in the absence of an effector reaction would have been observed at the time of conditioned stimulus delivery.

Figure 2, *c* depicts a situation differing from the two preceding ones. The cat made two movements several seconds before the conditioned stimulus was delivered and exhibited one weak conditioned response. In this situation, the two neurons develop reciprocal changes in firing frequency, and the time course of the distribution curve for conjugated impulses is now distinct from those of the two curves describing alterations in the frequency of initial impulse series. The clustering of conjugated impulses is maximal at the time when the animal is displaying the conditioned response. The increased clustering of conjugated impulses in this instance was unlikely to have resulted from an altered pattern of firing frequency of one of the two neurons. Indeed, the firing frequency of one of them at the time of conditioned stimulus delivery did not exceed its average values recorded for the fragment shown in Fig. 2, while the firing frequency of the other neuron increased later. It should be noted that only the third (conditioned-response) movement made by the cat was accompanied by a marked increase in the clustering of conjugated impulses.

The last stage in our study of interneuronal correlations was analysis of the histograms, which demonstrated mutually dependent relations between neurons in several simultaneously registered neuronal pairs (Fig. 3, *a-c*). We chose for illustration that fragment of the experiment during which a cat was performing movements repeatedly (Fig. 3, *d*) and so its reaction to the conditioned stimulus cannot be regarded as a conditioned response, although the cat had learned the CR_S well. The conjugated impulses

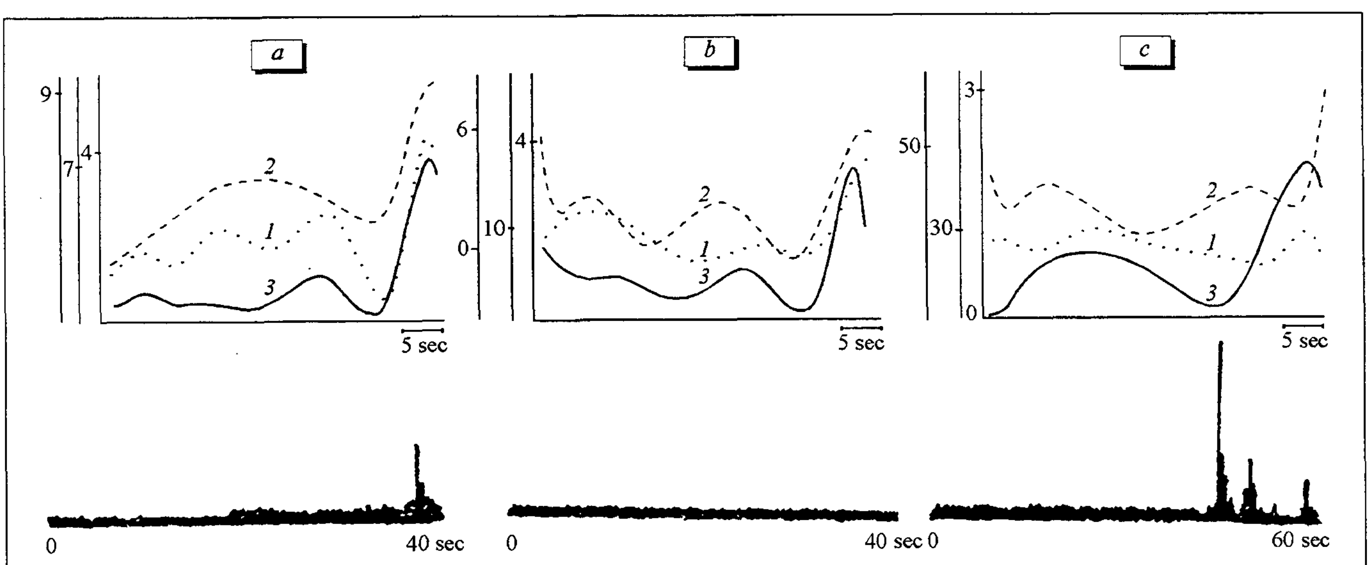

Fig. 2. Conjugated neuronal firing in a cat that has learned the conditioned response to sound (CR_S). The curves show the distribution of the initial (*1* and *2*) and conjugated (*3*) neuronal firing. Abscissa: real time; ordinate: number of impulses per step of analysis. Below are myograms of foreleg muscle. *a*) response; *b*) no response; *c*) weak response after a strong intersignal reaction.

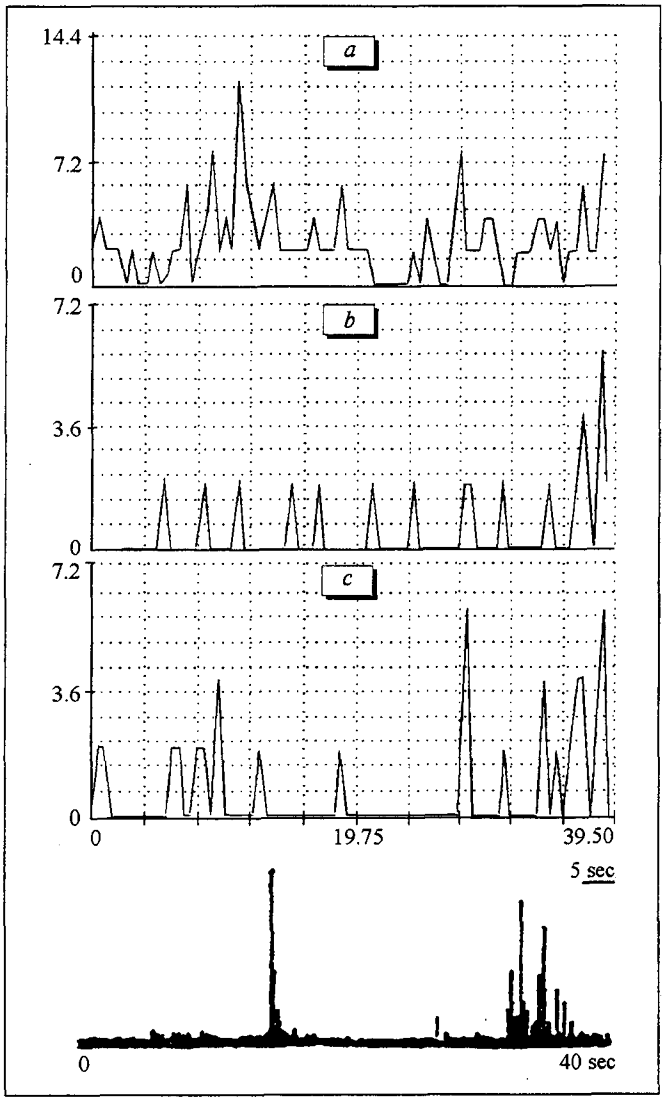

Fig. 3. Conjugated firing by three neuronal pairs (*a*, *b*, and *c*) simultaneously recorded during a conditioned response to sound (CR_S). Abscissa: real time in seconds; ordinate: number of impulses per step of analysis. *d*) myogram.

emitted by one of the neuronal pairs (Fig. 3, *a*) are concentrated at a time when the animal has not yet made the intersignal movement. The conjugated impulses of another pair (Fig. 3, *b*) are concentrated when the conditioned stimulus is delivered, while those of a third pair (Fig. 3, *c*) show two concentration peaks. One of these coincides in time with the intersignal movement and the other, with the conditioned stimulus delivery. In this situation (Fig. 3, *c*) both neurons appear to be implicated in the intersignal reaction as well as in the reaction timed to occur when the conditioned stimulus was delivered.

The three situations in Fig. 3 illustrate selective activation of neurons belonging to one pool or, at any rate, located very close to each other. The fragment we chose indicates that the CR_S by an animal that has learned it well can still be manifested at the level of interaction between individual pairs of neurons even if the behavioral manifestations of that response are not apparent. Since in our previous study the same result was obtained for the CR_T [3], we can conclude that the manifestation of conditioned responses at the level of interneuronal interaction reflects a general property shared by cellular structures of a learning brain rather than being specific to one form of learning; that conjugated neuronal firing is a process in its own right, based on spontaneous impulse traffic; and that the concentration of conjugated impulses may be determined by changes occurring in cellular structures during learning.

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