ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1997, 57: 247-253

Interneuronal cortical connections and intertrial responses in appetitive instrumental learning

Galina Kh. Merzhanova

Department of Conditioned Reflexes and Physiology of Emotions, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, 5A Butlerov St., Moscow 117865, Russia

Short communication

Abstract. The organization of interneuronal cortical connections in intertrial periods was studied in 4 cats trained to perform the delayed appetitive instrumental response to a visual conditioned stimulus (CS). Crosscorrelational analysis revealed changes in intra- and intercortical neuronal networks of the visual and motor cortical projection areas. Depending on the form of behavior in the intertrial period, i.e., the presence or absence of the acquired instrumental response, the functional connections of either informational (time delays of less than 30 ms) or motivational (time delays in the range of 60-100 ms) character dominated between the neurons of the motor and visual cortical areas.

Key words: correlated neuronal activity, instrumental appetitive response, intertrial responses, interneuronal connections, cat

The intertrial period is not unimportant in conditioning experiments. Under the same environmental conditions animals show various tonic and phasic instrumental responses. The intertrial responses (ITRs) resemble those to the CS and appear at both early and late stages of conditioning (Skipin et al. 1955, Plonskaja 1959, Pakovich 1971, Dolbakyan 1991, Zielinski 1993). Behavioral data indicate that at early stage of conditioning the ITRs appear due to formation of temporary connections between contextual stimuli and a motor center (Pakovich 1971), whereas at late phase of conditioning the activation of backward temporary connections from reinforcing structures (Asratyan 1981) is the chief cause of ITRs. For instance, isolated presentation of the reinforcing stimulus (food), after elaboration of conditioned responses (CRs) to a passive lifting of a dog's forelimb, starts to elicit the lifting of forelimb. Electrical stimulation of the lateral hypothalamus, applied on a background of a previously well-established instrumental appetitive CR produced the same instrumental response (Grastyan et al. 1956, Andersen and Wyrwicka 1957, Miller 1961, Delgado 1964, Merzhanova 1988).

In our first study (Merzhanova and Dorokhov 1980) we tried to find the neurophysiological manifestations of backward conditioned connections by recording neuronal activity in alert cats trained to perform milk-reinforced conditioned reflexes, elaborated in response to electostimulation of visual structures (optical radiation or lateral geniculate nucleus). In these experiments 2 ml of milk were administered directly into the oral cavity through an indwelling cannula which excluded many situational appetitive signs. In well-established conditioned reflexes, the patterns of neuronal activity in the visual cortex (projection of CS) were similar in cases of an isolated CS presentation and isolated milk application. The latter served as a test for the activation of backward conditioned connections from a "feeding" center to the primary signal area in the visual cortex. However, the pattern of neuronal activity in the visual cortex was different in the case of milk presentation to naive cats. The next step in the investigation of backward conditioned connections was application of crosscorrelation analysis to multi-unit activity recorded from the visual and sensorimotor cortical regions (Merzhanova 1988). In these experiments the interrelations were analyzed between visual and motor neurons. Different time delays were revealed between the cooperative neuronal activity of these regions. The interneuronal visual-motor connections with the delay (the time of appearance of the impulse of one neuron after the discharge of another) of up to 30 ms were more evident in trained animals (the so called "informational connections"), while interconnections within the range of 90-120 ms were more typical for the alimentary motivational state of an animal (the so called "motivational connections") (Merzhanova 1986). The statistical interneuronal connections with different time delays both from visual to motor neurons (forward direction) and *vice versa* (backward direction) suggested a complex character of neurophysiological manifestations of two-way connections.

The aim of the present experiment was to investigate the cooperative neuronal activity of the visual and motor cortical areas during intertrial intervals (ITI) with and without the occurrence of intersignal instrumental responses (ITRs) and to confirm the role of backward conditioned connections, which occur during intertrial periods at the stage of stable appetitive motivated instrumental responding.

The experimental sequence included acquisition of delayed appetitive instrumental responses to a CS (continuous light), recording of spontaneous multi-unit activity in cortical structures and statistical crosscorrelational analysis of cooperative activity of neurons under study.

The experiment was carried out on four adult freely moving cats with chronically implanted nichrome electrode bundles (4-6 wires, each electrode 50 μm in diameter) in the visual and motor cortical areas. Training of the delayed instrumental appetitive response to the CS continued until 80% of correct responses. After preliminary training to press a pedal for food reinforcement, the light CS was introduced. The lamp was placed under the transparent cover of the pedal and lit for 10 s (the delay period). After pressing the pedal by a cat at the end of the delay period in 10 s, meat reinforcement (5 g) was given automatically. During the intertrial intervals, pressing the pedal was not effective for food. Multi-unit activity was recorded in the visual (area 18) and motor (area 4) cortical regions in deep (5-6) layers.

A statistical method for crosscorrelational analysis of interneuronal relations was used (Gerstein 1970, Kruger 1983). The selection of spikes was made according to their shape (Buch-Wiener et al. 1990). Cross- and autocorrelograms were constructed with 2 and 10 ms bins. Crosscorrelograms of the asymmetrical type were used, with extremes above the confidence level (3 σ relative to mean values) and short duration of dependence index (2-4 ms) (Fig. 1). The cooperative activity of visual and motor cortical neurons was analyzed in intertrial inter-

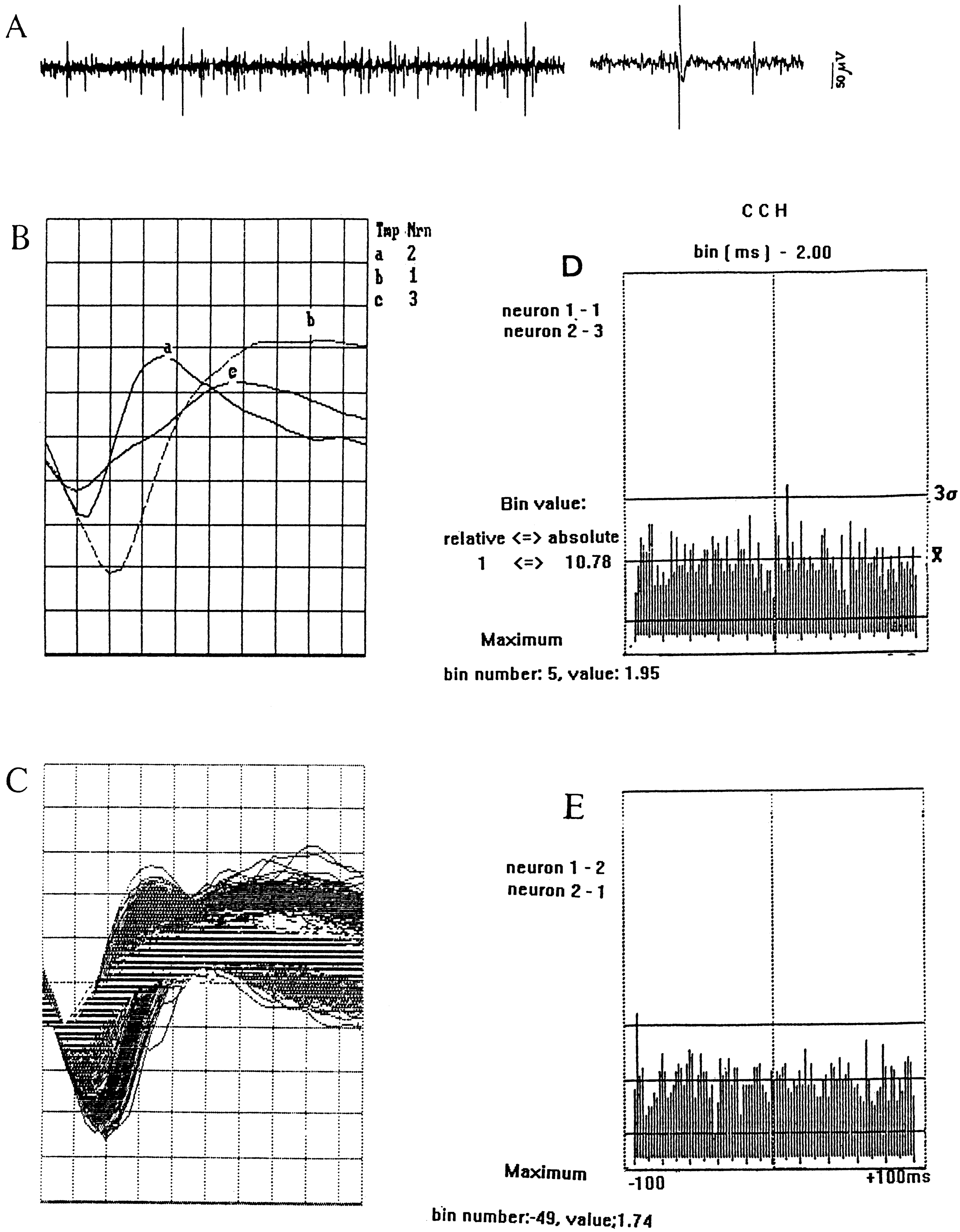

Fig. 1. Analysis of multi-unit activity. A, original recording of multi-unit activity in the motor cortex of an awake cat. B, specimens of 3 neuronal action potentials sorted by spike forms. C, superpositions of action potentials selected by specimens in multi-unit activity. D, E, examples of cross-correlational histograms (CCH); ordinate: numbers of interspike intervals, abscissa: 100 ms, $\overline{X}$ - the mean frequency of interspike intervals of histogram; 3 σ - the level of significance of peaks.

vals with and without intertrial movements. Among them, 116 fragments (30 s each) of multi-unit activity were analyzed, which corresponded to the ITIs without the intersignal instrumental movements (ITRs), while 95 fragments (30 s each) corresponded to the periods with their appearance in the trained cats. The crosscorrelational method allowed us to find: the temporal delay within cooperating neuronal pairs, and b) the direction of relations (from the visual to motor neurons or *vice versa*), judging from the appearance of picks in the left or right branches of crosscorrelogram.

Stimulation of motor points under chronic conditions in the 4 cats caused flexion of the right (instrumental) forelimb. Anatomical verification showed the electrode location in the motor (area 4) and visual (area 18) cortical fields.

The behavior of animals in intertrial intervals changed after stabilization of the instrumental appetitive response to light as a CS. In most cases, the animals were located near the pedal and feeder, looked at it, and "waited" for the CS presentation without any movement. Occasionally, during an intertrial period the cats would press the pedal with the "instrumental" paw, but did not get reinforced. The duration and character of such pressings were similar to those observed in response to the sporadic CS. For example, if an animal held the paw on the pedal during the entire delay period of a trial, an intertrial pedal pressing was also prolonged and lasted for the time equal to the duration of the delayed period. If the instrumental response to the light CS was short and appeared at the end of the delay, movements were also short and imitated the original CR.

Numbers of neuronal connections within both motor and visual cortical microareas during intertrial periods differed, depending on whether the intersignal motor responses occurred or not. In the absence of instrumental movements during the intertrial period, the number of neurons working cooperatively in the motor cortex was significantly greater ($P<0.05$) than that observed in relation with appearance of the instrumental movement (Fig. 2A). A similar tendency was observed for neuronal interconnections in the visual cortex, though here the results of the comparison were not significant ($P>0.05$) (Fig. 2B). In the visual cortex, the number of interconnections with interspike intervals below 30 ms was significantly greater ($P<0.05$) than that with intervals longer than 30 ms. This finding was found in situations both with the presence and absence of the intertrial reactions, as well as for neurons of the motor cortex (Fig. 2A and B, right). Summarizing the data concerning interneuronal interactions between closely spaced neighbouring cells in the visual and motor cortex areas, it may be concluded that the time intervals of waiting for the CS were characterized by a greater number of conjugated neuronal pairs than the intervals with intertrial movements. At the same time, the analysis showed a predominant appearance of cellular interactions with delays in the range of 0-30 ms in both cases.

Conjugated neurons in the two investigated structures were also found. The number and characteristics of the neuronal pairs were compared in trained animals depending on the behavior during intertrial periods. In this case, particular attention was given to the forward (from visual to motor neurons) and backward (from motor to visual) connections. During the intertrial periods without the instrumental movements, the number of forward interneuronal connections characterized by different temporal delays were almost equal (Fig. 3A, a), while the backward interactions of the motivational type in the range of 60-100 ms were observed significantly more frequently ($P<0.05$) than those of the informational (0-30 ms) type (Fig. 3A, b). During intertrial periods with instrumental movements, the backward interactions within the range 0-30 ms occurred significantly more frequently than the functional connections with longer temporal delays (Fig. 3B, b). The temporal distribution of forward connections was similar (Fig. 3B, a).

Specific cooperative cellular activity in cortical points of representation of paired (CS-US) stimuli in the process of conditioning has been investigated in several studies (Gassanov et al. 1985, Merzhanova 1988, Gassanov 1991, Vaadia et al. 1991, Ahissar et al. 1992). The obtained data suggest that networks of neurons formed in the process of the learning reflect a special state of cells which E.A. Asratyan called as the "local conditioned reflex" (Asratyan 1978, 1981). He assumed that connections between the foci were formed both in the cortical projections of CS and of the reinforcing stimulus as a result of the combined effects of two stimuli. In present work such conditioned neuronal reconstructions were recorded during intertrial periods both in the visual and sensorimotor cortical areas. The results of the analysis of interneuronal relationships performed by us favor two-way conditioned connections.

Functional differentiation of the neuronal interstructural interaction is of particular interest. In the intertrial period not accompanied by instrumental movements, interactions of the motivational character dominated with

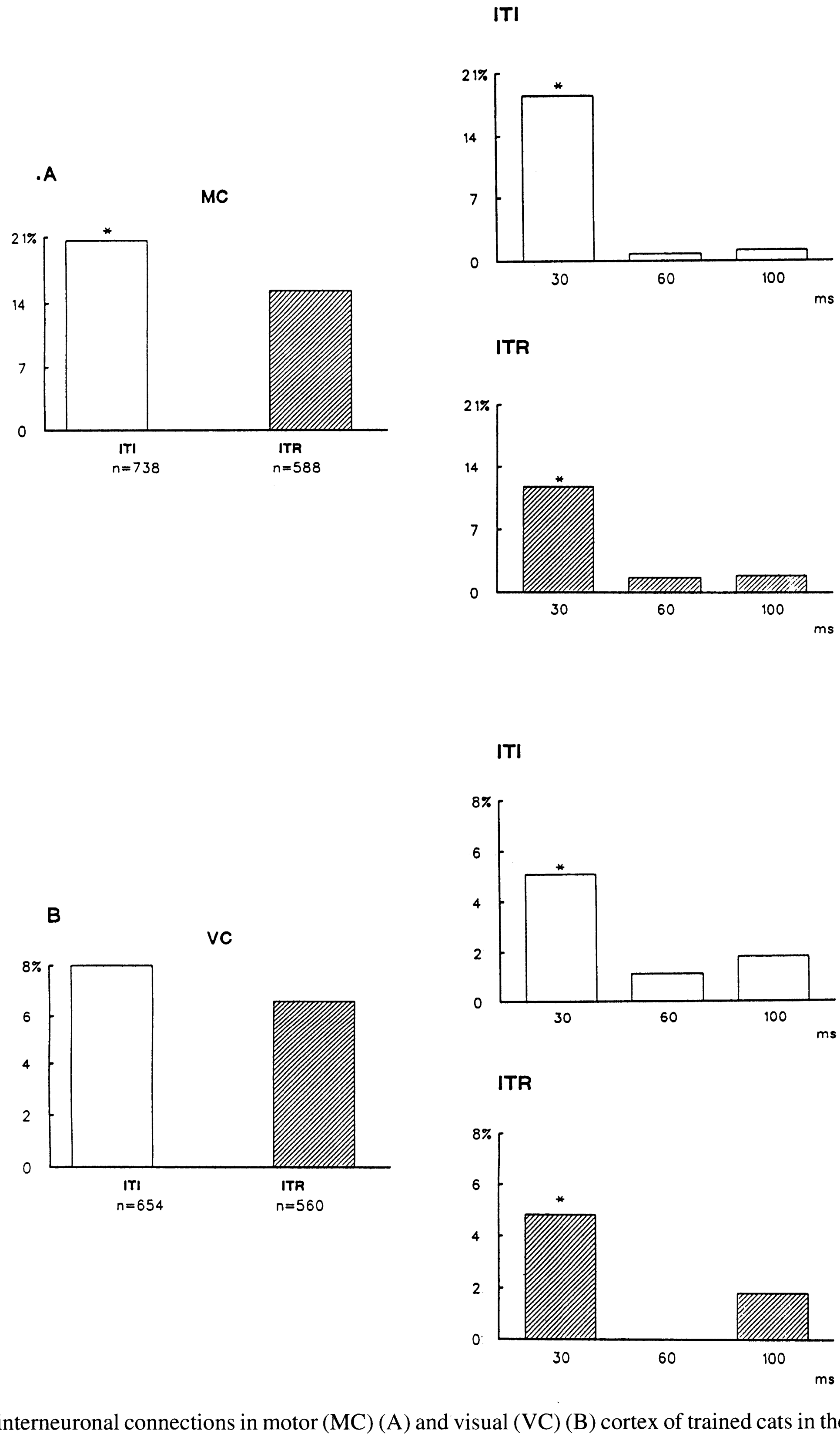

Fig. 2. Identified interneuronal connections in motor (MC) (A) and visual (VC) (B) cortex of trained cats in the intertrial period with the absence (ITI) and presence (ITR) of instrumental movement. Ordinate: the number of identified connections %; *n*, number of investigated histograms; to the right: distribution of identified connections throughout time delays; abscissa: time, ms; $*P<0.05$; the levels of significance are given according to the statistical method.

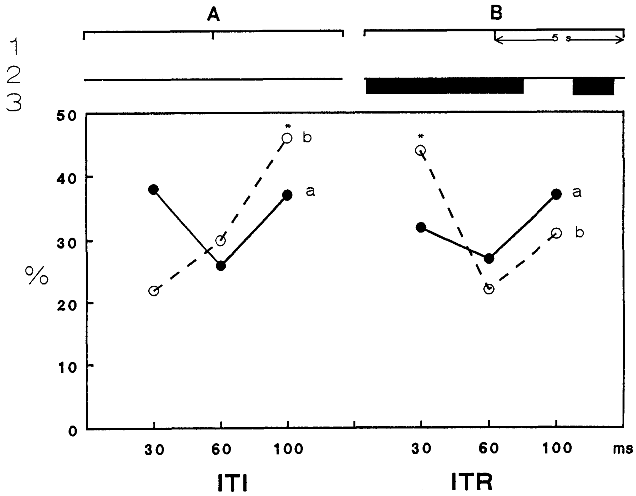

Fig. 3. Types of functional interneuronal connections according to the time delays of cooperatively working neuronal pairs with the absence (ITI) (A) and presence (ITR) (B) of instrumental movements in the intertrial period. 1, 5 s; 2, heavy line marks pedal pressing; 3, number of identified functional connections, % (ordinate); a, forward (from visual to motor neurones) directions; b, backward (from motor to visual neurons) directions; abscissa: time delays, ms; $^*P<0.05$.

temporal delays of 60-100 ms, directed from motor to visual neurons. Execution of pedal pressing movements raised the number of informational interactions (within 0-30 ms range) of the backward direction. It may be suggested that the functional connections directed from motor to visual neurons during the intertrial period are a manifestation of the backward conditioned connections by Asratyan. In accordance with his ideas, such functional connections develop in a direction from the structures of the reinforcing reflex, i.e., motivational, to motor and signal structures (Asratyan 1981). The balance between such interactions during the intertrial period is functionally dependent on the presence or absence of instrumental movements. The influence of a reinforcing appetitive stimulus is addressed both directly to visual (Merzhanova and Berg 1989) and motor cortical cells (Merzhanova 1988).

Some authors relate the appearance of intertrial reactions to the imperfection of conditioned reflex to a signal (Pakovich 1981). However, our observations indicate that intertrial reactions of animals appear not only at the stage of unstable performance, but after consolidation of specialized conditioned reflexes as well. Therefore, we suspect that intertrial reactions are performed due to activation of the backward conditioned connections and are governed by the influences from the structures of reinforcing appetitive reflexes correlated with individual - typological characteristics of higher nervous activity of animals. The main purpose of reactions which proceed by mechanisms of backward connections is the creation of conditions for the perfect perception of phasic CSs enhancing the excitability of the signal structures (Asratyan 1980, Simonov 1987, Merzhanova 1988). In conclusion it may be assumed that the visual-motor coordination in well trained animals is of a dynamic character during the intertrial period, when there are fluctuations in control of one of the reinforcing structures over the other. In such a case the regulating function is connected either with information transmission from motor to visual neurons (in the cases of occurrence of motor reflexes) or with motivational influences (in the cases of conditioned "waiting"). From our point of view this phenomenon can be explained in context of formation of the backward temporary connections.

I would like to thank Mr. P. Buch-Winer and Mr. I. Volkoff for computer programs of multi-unit analysis, Mrs. Yu. Nikitina for technical assistance, and Prof. K. Zieliński and Dr. G. Grygoryan for helpful comments on the manuscript. This work was supported in part by the International Science Foundation (Grant No. 5O300) and by the Russian Foundation for Fundamental Investigations (project No. 96-04-48197).

Ahissar E., Vaadia E., Ahissar M., Bergman H., Arieli A., Abeles M. (1992) Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. Science 257: 1412-1415.

Andersson B., Wyrwicka W. (1957) The elicitation of a drinking motor conditioned reaction by electrical stimulation of the hypothalamic drinking area in the goat. Acta Physiol. Scand. 41: 194.

Asratyan E.A. (1978) Des liasons nerveuses bidirectionells. La Resherche. 9: 237-245.

Asratyan E.A. (1981) Two-way connections as a general neurophysiological principle (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 31: 3-11.

Buch-Wiener P.V., Volkoff I.V., Merzhanova G.Kh. (1990) Collector of spikes (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 40: 1194-1199.

Delgado J.M.R. (1964) Free behavior and brain stimulation. Intern. Rev. Neurobiol. 6: 349-449.

Dolbakyan E.E. (1991) Conditioned avoidance reflex and intertrial responses cardiac-motor relationships (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 41: 267-280.

Gassanov U.G. (1991) Systems neuronal activity in learning. In: Neuronal cooperativity (Ed. J. Kruger). Springer Verlag, Berlin, p. 280-298.

Gassanov U.G., Merzhanova G.Kh., Galashina A.G. (1985) Interneuronal relations within and between cortical areas during conditioning in cats. Behav. Brain Res. 15: 137-146.

Gerstein G.L. (1970) Functional association of neurons: detection and interpretation. The neurosciences: second study program (Ed. F.O. Schmidt). Academic Press, New York, p. 648-661.

Grastyan E., Lissak K., Kekesi K. (1956) Facilitation and inhibition of conditioned alimentary and defensive reflexes by stimulation of the hypothalamic and reticular formation. Acta Physiol. Hung. 9: 133.

Kruger J. (1983) Simultaneous individual recordings from many cerebral neurons: techniques and results. Rev. Physiol. Biochem. Pharmacol. 98: 178-233.

Merzhanova G.Kh. (1986) Temporal interneuronal relationships in the cortex of the cat brain as a function of the level of alimentary motivation (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 36: 520- 527.

Merzhanova G.Kh. (1988) Neuronal manifestation of two-way connections in conditioning. Pavlovian J. Biol. Sci. 23: 135-142.

Merzhanova G.Kh., Berg A.I. (1989) Cortico-hypothalamic interneuronal correlation during learning in cats. Neurosci. Behav. Physiol. 19: 466-474.

Merzhanova G.Kh., Dorokhov V.B. (1980) Neuronal activity during formation of backward conditioned connections (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 30: 279-287.

Miller N.E. (1961) Learning and performance motivated by direct stimulation of the brain. In: Electrical stimulation of the brain (Ed. D.E. Sheer). Univ. Texas Press, Austin, p. 387.

Pakovich B.I. (1971) Nature of intersignal appearing in early period of conditioning (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 21: 904-913.

Pavlov I.P. (1936) Foreword to the paper by J.Konorski and S.Miller (in Russian). Trudi Fisiol. Lab. Akad. I.P. Pavlova 6: 115-118.

Plonskaja E.I. (1959) On the problem of the physiological mechanism of intersignal movements (in Russian). Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova 9: 593-601.

Simonov P.V. (1987) Two-way conditioning as neurophysiologycal number of spikes basis of behavior motivation. Neuroscience (suppl. to vol. 22). The II World Congress of Neuroscience (IBRO). Budapest, August 16-21. Pergamon Press, Oxford, P. 488 (1492R).

Skipin G.V., Antonova A.A., Aslanova I.F., Vinnik R.L. (1955) Functional nature of named voluntary alimentary movements in dogs (in Russian). Trudi Inst. Vyssh. Nervn. Deyat. Seria Physiologia. 1: 27-36.

Vaadia E., Ahissar E., Bergman H., Lavner Y. (1991) Correlated activity of neurons: a neural code for higher brain functions? In: Neuronal cooperativity (Ed. Kruger). Springer Verlag, Berlin, p. 249-276.

Zielinski K. (1993) Intertrial responses in defensive instrumental learning. Acta Neurobiol. Exp. 53: 215-229.

Received 28 February 1996, accepted 19 May 1997