ACTA NEUROBIOL. EXP. 1979, 39: 517-536

Lecture delivered at the Warsaw Colloquium on Instrumental Conditioning and Brain Research May 1979

NEURONAL MECHANISMS OF CONDITIONED PLACING REACTIONS IN CATS

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Abstract. Neuronal correlates of conditioned placing reaction of cat's forepaw were studied. The conditioned reaction evoking by tactile stimulation of the paw's ventral side had the same motor pattern, consisting mainly in successive flexion and extension of elbow joint, as the placing reaction evoked by paw's dorsal side stimulation in naive animals. The activity of single neurons from the m. biceps representation area in pericruciate motor cortex and VL thalamic nucleus was recorded. As a result of learning the excitatory response of cortical neurons to paw's ventral side stimulation in 20–50 ms post-stimulus interval was 2–2.5 times more than the response to the same stimulation in naive cats. This short-latency increase of response was not accompanied by modifications of sensory inflow to the motor cortex or movement related afferentation changes arriving from VL nucleus. There were no marked differences in excitability of cortical neurons of naive and trained animals as well. The results suggest a functional plasticity of the neuronal net in the motor cortex, consisting of a change in the efficiency of connections between neurons receiving sensory determined afferent excitation and the functional groups of neurons controlling the contraction of different muscles.

INTRODUCTION

Plastic changes and their initial localization related to the neuronal mechanisms of conditioned reflexes are not yet known. The present research is devoted to the neurophysiological investigation of a model of

learning based on the neuronal reorganization in the placing reaction of cats. The placing reaction in naive animals is evoked by tactile stimulation of the dorsal side of the forepaw. The conditioned reflex procedure results in the elaboration of the placing reaction to tactile stimulation of the ventral surface of the paw, which in naive animals initially blocks the dorsal placing reaction.

The extirpation of the motor cortex results in the irreversible disappearence of the placing reaction (4). The transcortical sensorimotor connections participating in placing reaction performance have been recently investigated (3), and are a part of the complicated system of transcortical reflex arcs participating in the initiation and control of movements (12). Therefore, the investigation of changes in the activity of motor cortical neurons occurring in parallel with plastic modification in the placing reaction is important for estimating the role of the motor cortex in the formation and alteration of motor habits.

METHODS

The experiment was conducted out on adult unanesthetized cats, weighing 3.0 to 4.5 kg, that had their heads fixed in a frame with implanted screws. The forepaws remained free, but could not be seen by the cat. The conditioned placing reaction to ventral stimulation was reinforced with milk given automatically by a tube placed in the cat's mouth (8). Tactile stimulation of the paw was made by a special device ("toucher") with a photocell and light source used for the recording of tactile stimulation. The light beam crossed by the paw was recorded, and errors of measurement were less than 10 ms.

The silver electrodes implanted in m. biceps and m. triceps for chronical registration of EMG were connected by a female connector located on the animal's head with nichrome wire introduced subcutaneously. Extracellular recording of cortical neurons was done by tungsten microelectrodes introduced into the brain by means of a mechanical driver. The dura mater was not removed. The recording of nerve cells activity was made in that area of the pericruciate motor cortex where superficial electrical stimulation evoked elbow flexion.

The signals from the nerve cell were directed to a preamplifier placed on the animal's head and recorded on film and magnetic tape. The analysis of the experimental data was performed by a 512 channel analyzer (LP — 4050, NOKIA), and poststimulus time histograms (PST) were recorded on a plotter and printed. Spikes of several neurons having equal amplitudes and reactions to the stimuli presented were registered together. Microstimulation of the cortex was done through the recording

electrode with trains of rectangular negative pulses. The duration of the pulse trains was 25 ms, the interpulse interval was 2.5 ms, and the duration of a single pulse was from 0.15 to 0.3 ms. The amplitude of the stimulating current ranged from 5 to 50 μA (16). Antidromic identification of pyramidal tract neurons was done by bipolar electrodes introduced into the pyramidal tract at the P11 or P3 level.

RESULTS

Plasticity of the cat's placing reaction

The placing reaction in naive, awake cats is elicited by touching the dorsal side of the forepaw, and consists of a sequence of movements resulting in the ventral surface of the paw contacting an object (support). The shoulder, elbow and wrist joints muscles of the forepaw all participate in this movement (Fig. 1*A*). The movement of the elbow joint, however, is the core of placing reaction. The placing reaction evoked by touching the dorsal side of the paw (Sd) consists of a succession of m. biceps and m. triceps contractions. Tactile stimulation of the ventral side of paw (Sv) of the naive animal evokes extention (action of m.

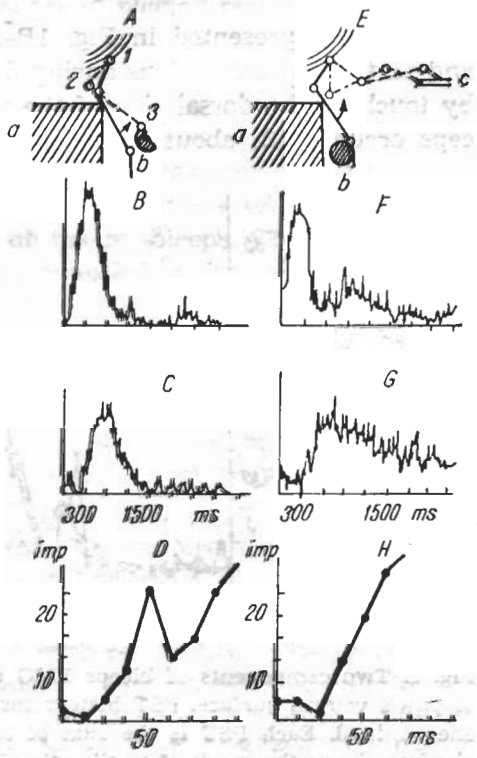


Fig. 1. The cat's placing reaction evoked by tactile stimulation of the dorsal (*A*, unconditioned reaction) in naive animals and ventral (*E*, conditioned reaction) side of the forepaw. 1–3, shoulder, elbow and wrist joints respectively; *a*, stand under the paw; *b*, tactile stimulus; *c*, platform (support) for placing the paw. Averaged PST of EMG m. biceps (*B*, *F*, *D*, *H*) and m. triceps (*C*, *G*) of unconditioned (B–D) and conditioned (F–H) reactions. Abscissa, time (in ms) starting from touching the paw. Ordinate, number of crossings of the pre-set level by EMG potentials. Number of trials: *B*, *C*, *F*, *G* = 20; *D* = 129; *H* = 152.

triceps) and blocks the placing reaction to the dorsal stimulation. Thus, the initial sensorimotor relations for the elbow joint may be briefly described as follows: Sd — BIC, Sv — TRI, SvN (Sd — BIC) (N — sign of negation).

The placing reaction is characterized by a number of plastic properties. Repeated stimulation of the dorsal side results in the extinction of the placing reaction. The reinforcement of the placing reaction with milk resulted in an increase in its probability of occurrence and shortening of its latency. In animals trained for 1–3 wk to raise the paw on the platform in response to touching of the ventral side the functional connection, Sv — TRI becomes ineffective, being replaced by a newly formed connection Sv — BIC (Fig. 1*E*). In addition the functional connection Sd — BIC of 3 animals was extinguished, which was achieved with great difficulty and required some thousands of administrations of dorsal stimulation of the paw without reinforcement. New functional relations for these animals may be briefly presented as follows: Sd — O, Sv — BIC.

EMG of the biceps and triceps during the performance of placing reactions by naive and trained animals

The data presented in Fig. 1*B–D* show the succession of m. biceps and m. triceps contractions during the performance of the reaction evoked by touching the dorsal side of the paw. The earliest activation in m. biceps occurs with about 30 ms latency. Figure 1*F–H* presents examples

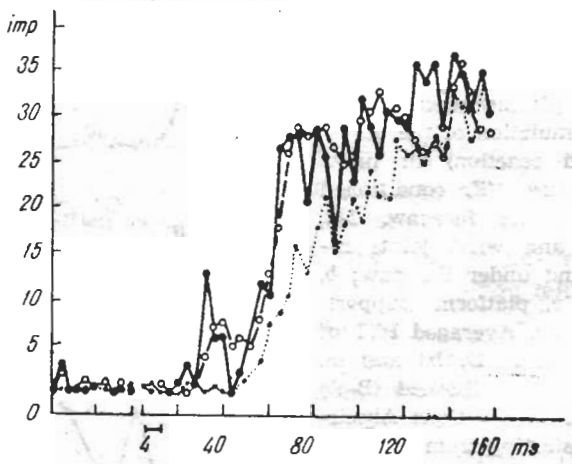


Fig. 2. Two components of biceps EMG during the placing reaction to stimulation of paw's ventral surface. PST histograms of biceps EMG of 3 different sessions in one animal. Each PST is the sum of 50 single trials. The horizontal line below abscissa shows the mark of tactile stimulation. Abscissa, time (ms); ordinate, number of impulses per 4 ms.

of the EMG of biceps and triceps in trained animals, that performed the placing reaction in response to stimulation of the ventral side of the paw (Fig. 1*E*). Comparison of the biceps and triceps EMG during performance of dorsal placing (naive animals) and conditioned placing reaction to ventral tactile stimulation (trained animals) show similar dynamics of m. biceps and m. triceps activity in both cases (Fig. 1*B, F, C* and *G*). The extended time scale shows the average latency of m. biceps activation, was about 30 ms (cf. Fig. 1*D* and *H*). The latency of the paw movement was measured as the time between the moment of touching and the moment of the paw's lifting from the toucher surface. It lasted not less than 40–50 ms in both cases.

On some PSTs there are two components in the EMG changes of the biceps: a first small component of about the 30 ms latency and a main component having 40–60 ms of latency (Fig. 2). These components join and become indistinguishable, especially on the PST if the level of motor readiness is high and the performance of the placing reaction is fast.

General functional properties of the neurons in the motor cortex participating in the performance of placing reactions

The activity of 430 neurons of the pericruciate motor cortex in 7 cats related to the representation of m. biceps and m. triceps was recorded during the placing reaction to tactile stimulation of the dorsal surface of the paw and during the conditioned placing reaction to ventral stimulation. Figure 3*A* shows the area of pericruciate cortex where superficial stimulation evoked contraction of the m. biceps. The same figure presents examples of neuronal activity recorded in this area during the performance of the placing reaction (Fig. 3*C*) and the EMG of the m. biceps reaction (Fig. 3*B*) during microstimulation of the cortex through the recording electrode.

The activity of neurons in the motor cortex was considered as related to m. biceps contraction if: (i) during the placing reaction the increase of neuronal spike frequency coincided with the EMG (Fig. 4); and (ii) stimulation of the cortex through the recording electrode elicited the EMG response (Fig. 3*B*). The majority of neurons were recorded in the deep layers of the cortex, in the region most effective for EMG activation by cortical microstimulation. A number of them were identified as neurons of the pyramidal tract with conduction velocity ranging from 10 to 60 m/s. The threshold for the cortical microstimulation in this region did not exceed 5 μA. The modifications of cortical neuronal activity occurring simultaneously and in parallel with the contraction

of the respective muscles and the animal's movement will be henceforth referred to as changes of the motor type.

Figure 10*A* shows the summarized PST of neuronal activity in the motor cortex representation of the forepaw flexion of two animals (solid line) during performance of the placing reaction in response to stimulation of the paw's ventral surface. The curve shown is divided into two components. Besides the main motor type component associating with EMG changes, the first component also can be distinguished. The latter increased for 50 ms after tactile stimulation, that is before the initiation of the paw's removal from the surface of toucher. Such a component

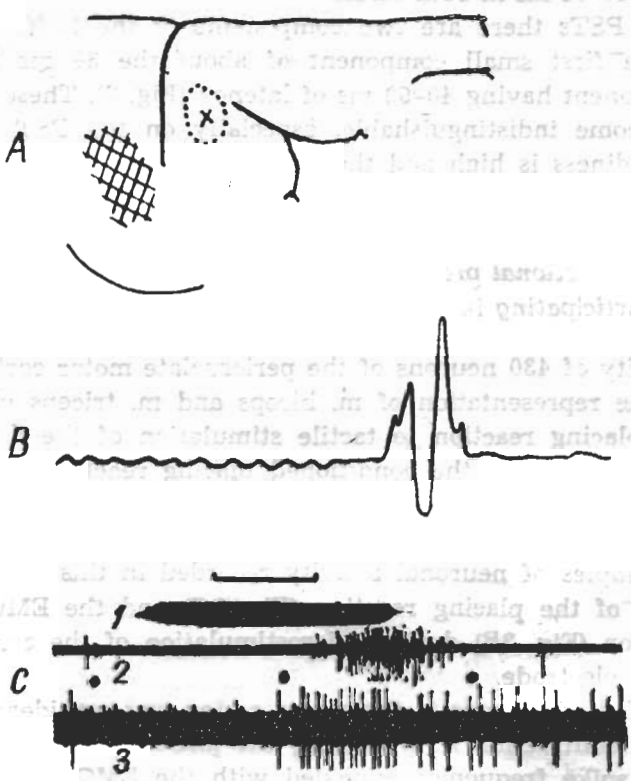
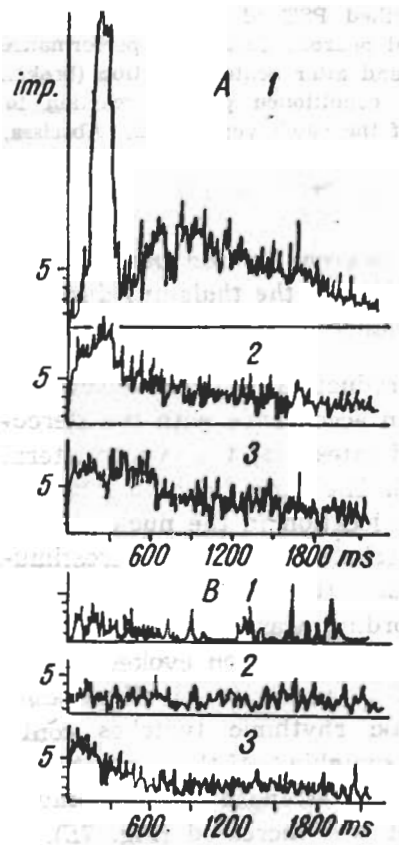


Fig. 3. *A*, The recording area of neuronal activity (dashed) in the cat's cortex. *B*, m. biceps EMG reaction to microstimulation of the motor cortex (12 μA) through the recording electrode. Time mark, 14 ms. *C*, the activity changes of motor cortex neurons and m. biceps EMG during the placing reaction. Recording of neuronal activity was done at the same cortical point stimulation of which resulted in the effect presented on Fig. 3*B*: 1, tactile stimulation; left extreme point of the dark stripe corresponds to beginning of tactile stimulation; the decrease in the dark stripe corresponds to the moment of the paw withdrawal from the tactile stimulus; 2, m. biceps EMG; 3, neuron's activity; time calibration, 0.25 s.



is also distinguished in the reactions of single neurons (Fig. 5). Temporal parameters of the first component in neuronal activity correspond to parameters of the first component observed in the EMG of the biceps (Fig. 2). However this component is more noticeable in the activity of cortical neurons (cf. Fig. 10*A* and Fig. 2).

The first component in the activity of some neurons remained almost without change in the absence of the paw's motor reaction (for example after acute extinction of the placing reaction, Figs. 4–3, and 5*D*). However, in the activity of other neurons of the motor cortex, the same changes in time were observed only in conjunction

Fig. 4. PST histograms of neuronal reactions (2, 3) and m. biceps EMG (1) during the placing reaction (*A*) and after acute extinction of the placing reaction (*B*). Abscissa, time (ms) after beginning of tactile stimulation; ordinate, number of impulses per 10 ms. Each PST is a sum of 10 realizations.

with forepaw movement (Figs. 4–2 and 5*A–C*). The latencies of reaction of cortical neurons in both cases did not exceed 20 ms (Fig. 6), although it is difficult to determine the exact latencies since the method of measurement was not precise enough. The above-mentioned properties of the first component in neuronal activity during the performance of placing reactions allows one to consider it to be the sensory determined response evoked by incoming tactile afferentation to the motor cortex.

Since the latency of this sensory type of reaction in motor cortical neurons is about 20 ms and the time necessary for the transmission of the cortical signal to muscles, in accordance with the data of microstimulation, is about 10 ms, the minimal time of signal transmission from skin receptors to respective muscles through the motor cortex must be about 30 ms. This period coincides with the minimal latency of the biceps EMG reaction to a tactile stimulus observed in the experiment.

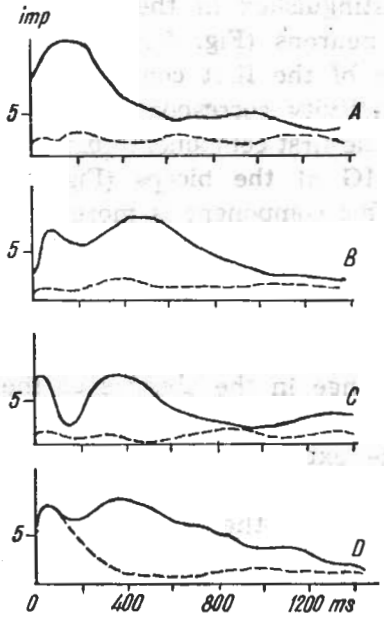


Fig. 5. Smoothed PST of activity changes of motor cortical neurons during the performance (solid lines) and after acute extinction (broken lines) of the conditioned placing reaction to stimulation of the paw's ventral side. Abscissa, time (ms) after beginning of tactile stimulation; ordinate, the number of impulses per 10 ms.

Activity of neurons in the ventrolateral nucleus (VL) of the thalamus during performance of a placing reaction

The introduction of microelectrodes was made in accordance with the stereotaxic coordinates of the ventrolateral nucleus (15). The main functional criteria of electrode location in the nucleus were the characteristic effects of microstimulation through the recording microelectrode. Recording was performed in the area in which stimulation evoked a local flexion of the forepaw in the elbow joint. Characteristic rhythmic twitches continuing after switching off the current were observed if the strength of the stimulating current was increased (Fig. 7*E*). In this area neurons were recorded with increasing activity during the active flexion of the forelimb at the elbow joint. The activity of 51 neurons in VL was

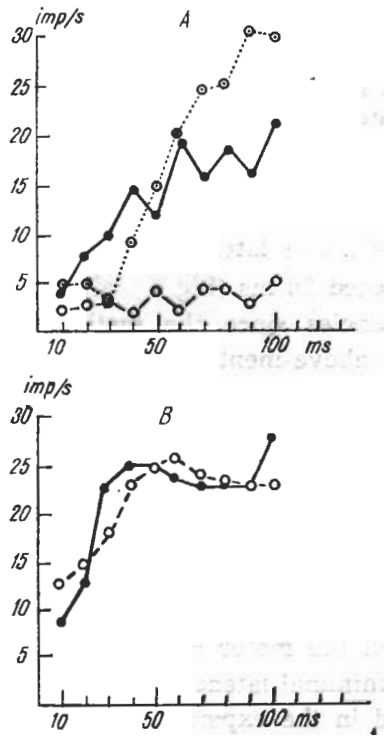


Fig. 6. PST histograms of reactions of the cortical neurons. *A*, averaged PST histogram of the activity of 6 neurons; *B*, averaged PST histogram of the activity of 5 neurons. During the investigation of each neuron 10 trials were summarized; the number of EMG trials corresponds to number of trials used for analysis of neuronal activity. Solid lines, neuronal activity during the placing reaction; broken lines, in the absence of movement after acute extinction of the placing reaction; dotted lines, changes of EMG m. bicep activity during the movement. Abscissa, time (ms) following the beginning of tactile stimulation; ordinate, averaged number of impulses per 1 s.

recorded altogether. The changes of neurons in VL during performance of the placing reaction are similar to the changes of neurons in the motor cortex (Fig. 7 *A–D*). However, the first component is not observed. None of the recorded neurons in VL reacted to tactile stimulation in the absence of movement. Figure 8 shows the histograms of reaction latency distribution in the motor cortical neurons, VL neurons and biceps EMG. The latency was considered as the time to attain half of the reaction in a given PST. Figure 8 *A* shows the reaction latency distribution in motor cortical neurons to tactile stimulation of the paw in the absence of mo-

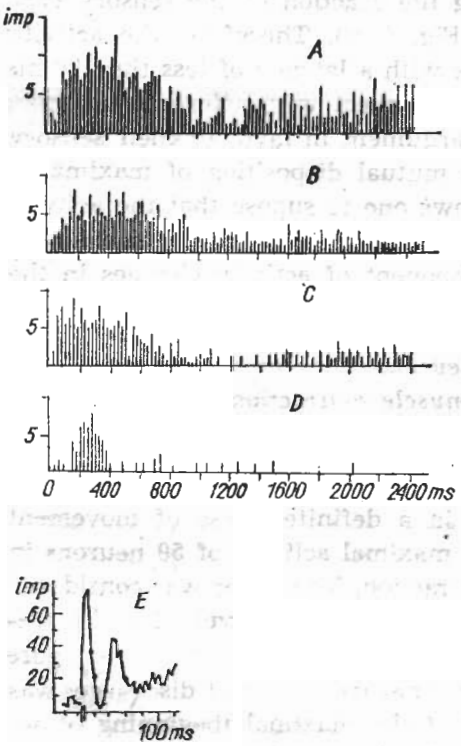


Fig. 7. Activity changes of neurons in the ventrolateral thalamic nucleus during the placing reaction (A–D). Abscissa, time (ms) after beginning of tactile stimulation; ordinate, number of impulses per 25 ms. Each PST histogram, the sum of 10 trials. **E**, PST histogram of biceps EMG in response to electrical stimulation of the ventrolateral nucleus (showed by arrows). Abscissa, time (ms); ordinate, the number of impulses per 4 ms.

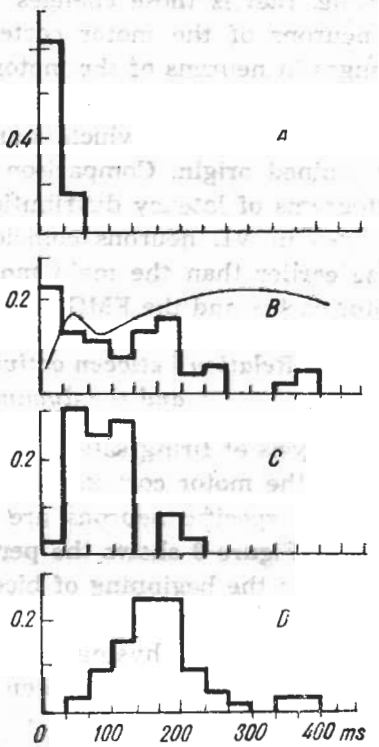


Fig. 8. Histograms of the distribution of latencies of neuronal reactions in motor cortex (*A*, *B*), the ventrolateral thalamic nucleus (*C*) and biceps EMG (*D*) to tactile stimulation of the paw which evoked the placing reaction (*B–D*) and did not evoke a motor reaction after acute extinction of the placing reaction (*A*). Abscissa, latency (ms); ordinate, relative frequency of occurrence at a given latency. For explanation see text.

vement (after acute extinction of the conditioned motor reaction); Figure 8 *B* shows the reaction latency distribution in the case of the same tactile stimulation evoking the conditioned motor reaction of the paw. Also presented here is the summarized curve of the activity changes in neurons in the area of cortical representation of forelimb flexions during movement (see Fig. 10 *A*). Figure 8 panels *C* and *D* show the distributions of reaction latencies for neurons of VL and motoneurons (EMG) under the same conditions. It is necessary to point out that the changes of neuronal activity in VL with a latency less than 30 ms are practically missing, that is those changes forming the reaction of the sensory type in neurons of the motor cortex (cf. Fig. 8 *A*). Therefore, the activity changes in neurons of the motor cortex with a latency of less than 30 ms and respective changes in EMG do not connect with afferentation from VL to motor cortex, which is another argument in favor of their sensory determined origin. Comparison of the mutual disposition of maxima on histograms of latency distributions allows one to supose that the activity changes in VL neurons coincided with the development of movement arise earlier than the main motor component of activity changes in the motor cortex and the EMG of biceps.

Relation between activity of neurons in the motor cortex and the dynamics of muscle contraction

Analysis of firing rate changes in the neurons of the forelimb flexion area in the motor cortex, developing in association with movement, showed that specific neurons are active in a definite phase of movement (11, 13). Figure 9 shows the periods of maximal activity of 50 neurons in relation to the beginning of biceps contraction. The latter was considered as the moment of reaching a half of the maximal level of EMG frequency in a given hystogram. The length of each segment on the figure corresponds to the period when the neuronal frequency of discharges was increasing from a level equal to half of the maximal (beginning of segment) to the maximal (end of segment). As may be seen, the periods of maximal increase in the neuronal discharge frequencies are distributed equally enough in relation to the start of flexion. This distribution does not depend on the dispersion of EMG maxima positions (vertical marks on Fig. 9). For neurons of the motor cortex, the time of switching on correlated positively with the moment of achieving the maximal reaction ($r = 0.78$; $P < 0.005$) and with the time of the switching off of neuronal activity ($r = 0.29$; $P < 0.025$). The difference in periods of maximal activity, was characteristic for different neurons, since the temporal parameters of the reactions of every single neuron in successive recordings remained approximately permanent.

Figure 10 *A* shows the summarized and averaged reactions of cortical neurons connected functionally with the biceps and the EMG of this muscles during performance of a motor task. Reactions were averaged with steps of 10 ms (0–100 ms) and 100 ms (100–900 ms). As seen on Fig. 10 *A*, *B* the summarized neuronal activity of the motor cortex and EMG changes of the respective muscles are proportional to each other during the whole period of the reaction performance with the exception

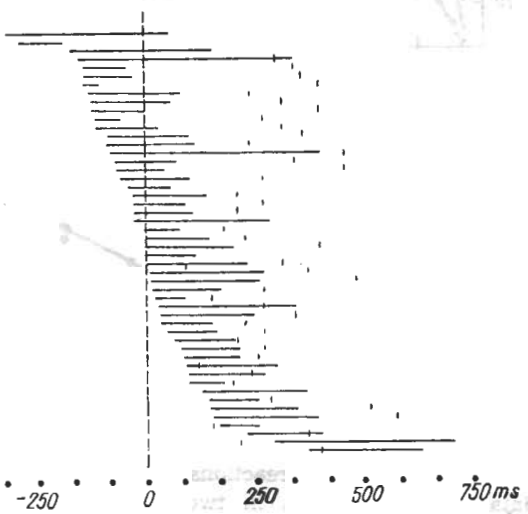


Fig. 9. The distribution of periods of highest activity of 50 neurons during the placing reaction in relation to the beginning of m. bicep contraction. Vertical broken line, the moment of reaching half of the maximal activity of biceps EMG in given PST histogram. The beginning of each horizontal line, the moment of reaching half of the maximal level of the neuron's reaction; the end, maximum of reaction. Vertical marks, maxima of EMG. Each horizontal line is based on an averaged PST histogram (10 trials). Time is shown in ms.

of the short interval of 50–100 ms from the start of stimulation. During the reaction, there is the obvious change of the proportion for neuronal and EMG activity; it increased during the interval of 100 to 700 ms in comparison with the interval of 0 to 50 ms (Fig. 10 *B*). As has already been mentioned, the changes of neuronal and EMG activity in the 0 to 50 ms interval were predominantly sensory determined. These changes in the activity of motor cortical neurons were expressed much better than in the EMG of the corresponding muscle.

Simultaneous with the increase of neuronal activity in the cortical area of biceps, the initial phase of the conditioned placing reaction was characterized by reciprocal supression of neuronal activity in the cortical area of triceps (Fig. 11).

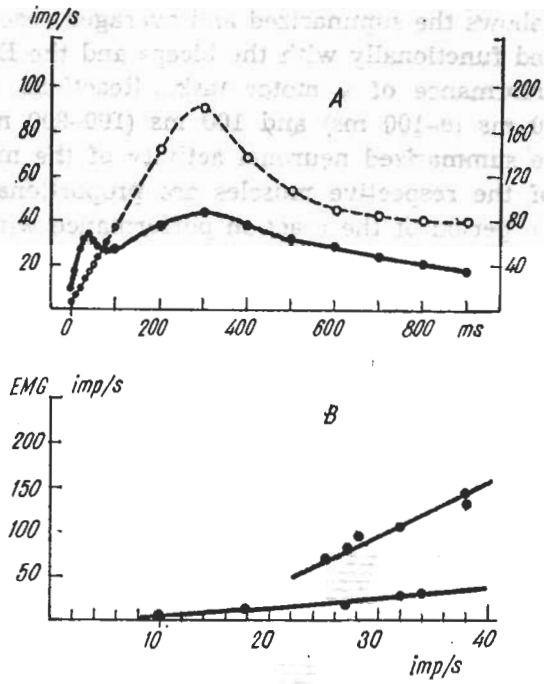


Fig. 10. *A*, PST histograms of neuronal reactions in the m. bicep cortical area (dark circles, averaged data from 34 neurons in two animals) and of m. biceps EMG (open circles) during conditioned placing reaction to ventral stimulation of the paw. Other denotations as in Fig. 6. The right scale, EMG; left neuronal activity. *B*, Relation of averaged neuronal activity in the m. bicep cortical area and biceps EMG according to data presented in Fig. 10 *A*. Abscissa, the average frequency of neuronal activity; ordinate, EMG activity in corresponding moments after tactile stimulation. The lines are drawn according to the regression equations. 1, regression line for 0–50 ms interval: $y_1 = -3.5 + x$, $r = 0.97$; 2, regression line for 100–700 ms interval: $y_2 = 6x - 85.5$, $r = 0.99$

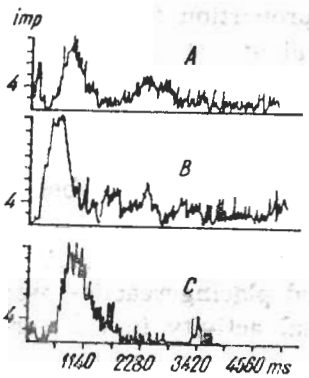


Fig. 11. PST histograms for neuronal activity (*A*) and EMG of m. biceps (*B*) and EMG of m. triceps (C) during the placing reaction. Other denotations as in Fig. 4.

The localization of the skin receptive fields of cortical neurons having projections to m. biceps and m. triceps was studied by tactile stimulation of the dorsal and ventral sides of distal parts of the paw. We selected neurons with a stable sensory component of responses to tactile stimulation, remaining even in the absence of the motor reaction of the paw. The reactivity of cortical neurons to the tactile stimulation coming from the paw skin surface was investigated in 4 naive and 5 trained animals (Table I). The neurons of the biceps cortical area are excited mainly from the dorsal side of the paw representing the receptive field for the initial phase of the placing reaction. 14 of 65 neurons (21%) in the m. biceps cortical area of the animals, performing placing reaction to stimulation of the paw's dorsal side, responded to that kind of stimulation in the absence of paw movement with stable reactions of the sensory type (Fig. 12). Only about 8% (11 of 136) of the neurons of the biceps

TABLE I

The distribution of neurons activated by tactile stimulation in the absence of forepaw movement among the neurons in m. biceps and m. triceps cortical areas. Sd, dorsal surface; Sv, ventral surface

Groups of neurons	Type of tactile stimulation of paw	Naive animals	Trained animals	
		$n = 4$ Sd–BIC Sv–TRI	$n = 2$ Sd–BIC Sv–BIC	$n = 3$ Sd–O Sv–BIC
Neurons of m. biceps cortical area	Sd	14 of 65 (21%)		5 of 70 (7%)
	Sv	11 of 136 (8%)		
		4 of 41 (10%)	7 of 95 (7.4%)	
Neurons of m. triceps cortical area	Sd	0 of 14		
	Sv	14 of 14		

cortical area reacted to stimulation of the paw's ventral side in naive (S_d — BIC) and trained (S_v — BIC) animals.

The neurons of the m. triceps cortical area associated with the extension of the forelimb at elbow joint (Fig. 13) on the contrary are excited during tactile stimulation in the absence of movement exclusively from the ventral side of the paw (Fig. 13 *C*), that is, from the receptive field of the extension reflex. The stimulation of the dorsal surface of the paw was ineffective (Fig. 13 *B*) or decreased the activity of these neurons.

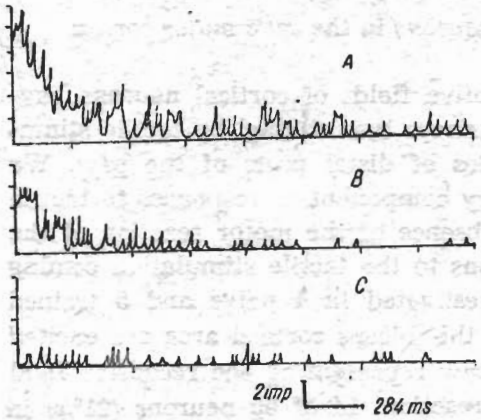


Fig. 12. PST histograms of neuronal activity in the motor cortex of a naive animal. *A*, during the placing reaction; *B*, in response to dorsal stimulation after the acute extinction of the placing reaction; *C*, in response to ventral stimulation. Other denotations as in Fig. 4.

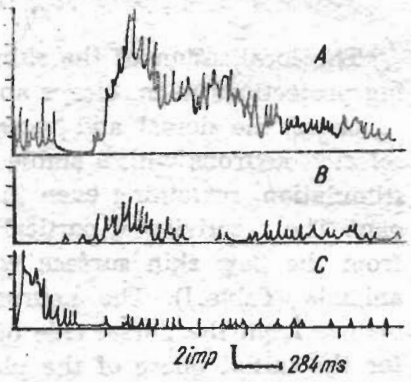


Fig. 13. PST histograms of neuronal activity in the motor cortex and EMG of the m. triceps of a naive animal. PST of EMG of the m. triceps (*A*) and neuronal activity (*B*) during the placing reaction evoked by dorsal stimulation. *C*, reaction to ventral stimulation in the absence of movement. Other denotations as in Fig. 4.

Functional connections of neurons in the motor cortex after elaboration of the conditioned placing reaction

As was seen from the above data, the process of learning consisted in a change in the functional significance of tactile stimulation of the paw's ventral surface. In naive animals touching the area of the pads on the paw's ventral surface evokes the extension reflex and blocks the placing reaction. After training touching the paw's ventral side evoked the flexion and placing reaction on the support as shown on the Fig. 1. The conditioned reaction to ventral stimulation as far as the motor pattern is concerned, was similar to the initial placing reaction evoked by touching the paw's dorsal side in naive animals.

The changes that occurred in the properties of activity in neurons of the motor cortex as a result of learning may be described as following. Tactile stimulation of the paw's ventral side resulted in conditioned placing reaction evoked the same average response of neurons in the biceps cortical area in the 20–50 ms post-stimulus interval as stimulation of the paw's dorsal side resulted in the placing reaction in naive animals (Fig. 14 *A*). Figure 14 *A* shows the averaged reaction of neurons in the biceps cortical area to tactile stimulation of the paw's dorsal side resulted

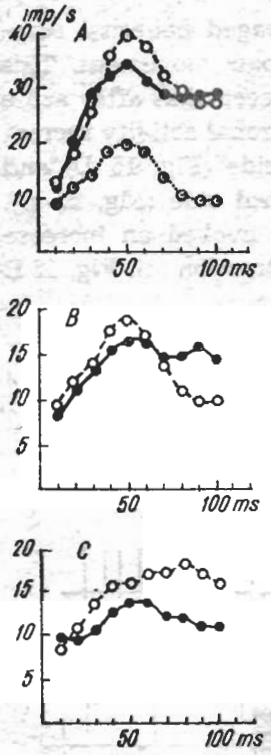


Fig. 14. Averaged PST histograms of neuronal reactions in the m. biceps cortical representation area. *A*, during placing reaction to ventral (dark circles, averaged data from 42 neurons) and dorsal (open circles, 36 neurons) stimulation; open circles with dot inside shows response to ventral stimulation which did not evoke placing reactions in naive animals (36 neurons). *B*, reactions to ventral stimulation in the absence of forepaw movement; dark circles, trained animals after acute extinction of the conditioned placing reaction (24 neurons); open circles, naive control animals (36 neurons); *C*, reactions to dorsal stimulation in the absence of forepaw movement; open circles, naive control animals after acute extinction of the dorsal placing reaction; dark circles, trained animals (24 neurons). Other denotations in Fig. 6.

in the placing movement in naive animals (Sd — BIC, open circles) and the averaged reaction to tactile stimulation of the paw's ventral side resulted in the placing movement in trained animals (Sv — TRI, close circles). The third curve on this figure (open circles with point inside) shows the averaged reaction of neurons in the biceps cortical area of naive animals to stimulation of the ventral surface, which was ineffective in producing a placing reaction. The effective stimulation resulted in placing movement (Sd in naive animals, Sv — in trained ones) evoked an approximately equal increase of discharge frequency in the cortical pool of neurons related to m. biceps contraction. The figure shows the identity of the reaction latency in both cases as well.

Elaboration of the conditioned placing reaction does not change the relative number of neurons in the m. biceps cortical area being steadily excited during tactile stimulation of the paw's ventral side, idependent of presence or absence of the movement reaction.

From 41 neurons in naive animals (Sv — TRI; Sd — BIC) activated during the m. biceps contraction only 4 neurons (10%) were excited by stimulation of the paw's ventral side in the absence of movement. Among the trained animals (Sv — BIC) 7 neurons of 95 (about 7%) were excited by the same stimulus. Figure 14 *B* shows the averaged neuronal reactions in the m. biceps cortical area to stimulation of the paw's ventral side in the absence of movement in naive animals (Sv — TRI, open circles) and to ventral stimulation in trained animals (Sv — BIC) after acute extinction of the conditioned placing reaction (close circles). The comparison of

these curves does not reveal any difference in averaged neuronal reactions to ventral stimulation in the absence of forepaw movement. This conclusion is illustrated also by figure 15. It can be seen that after acute extinction of the conditioned placing reaction the neuronal activity increased during the tactile stimulation of paw's dorsal side (Fig. 15 *D*) and was inhibited during stimulation of the paw's ventral side (Fig. 15 *C*). However, the stimulation of the paw's ventral side evoked an increase of neuronal activity in association with m. biceps contraction (cf. Fig. 15 *B* and Fig. 15 *C*).

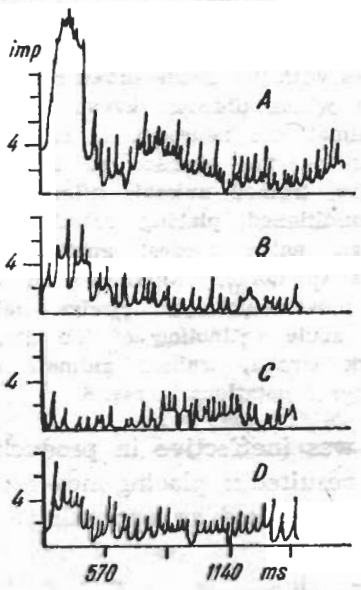


Fig. 15. PST histograms of EMG of the m. biceps (*A*) and neuronal activity (*B–D*) in the m. biceps cortical area of a trained animal. *B*, neuronal reaction during the placing reaction evoked by ventral stimulation. *C*, *D*, reaction to ventral and dorsal stimulation respectively with no movement. Other denotations in Fig. 4.

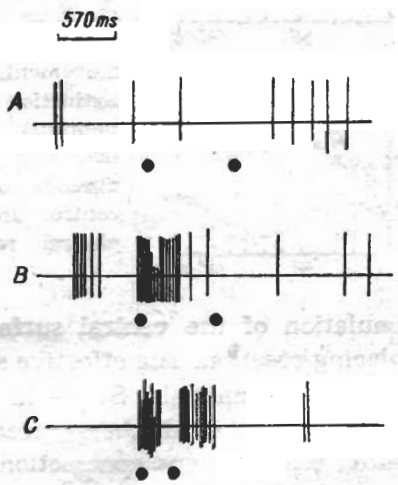


Fig. 16. Reactions of a neuron in the m. triceps cortical area of a trained animal to the touching of the dorsal (*A*) and ventral (*B*) side of the paw without movement and during performance of the placing reaction to ventral side stimulation (*C*). Recordings of neuronal activity on paper tape. The first point corresponds to beginning of tactile stimulation; the second, to the end of tactile stimulation (*A*, *B*) and paw withdrawal from the toucher surface during the placing reaction (*C*).

The retention of stable sensory connections to the motor cortical neurons after learning was supported by the investigation of neuronal reactions in m. triceps cortical area (Fig. 16). The neurons of this area of

trained animals were excited by tactile stimulation of the paw's ventral side, although the ventral stimulation became ineffective as referred to m. triceps contraction.

Other results were obtained concerning the inputs to m. biceps cortical area after chronic extinction of the initial placing reaction to dorsal side stimulation in animals which performed the conditioned placing reaction to ventral side stimulation. A decrease in the number of neurons in the m. biceps cortical area responding to dorsal stimulation up to 7% (5 of 70 neurons) was observed in this case. All of these neurons were recorded in the motor cortex of the animal with incomplete extinction of the reaction to stimulation of paw's dorsal side.

Figure 14, *C* shows the reaction of the neurons in naive (S_d — BIC, open circles) and trained (S_v — BIC, S_d — 0, close circles) animals to stimulation of the paw's dorsal side in the absence of movement. Chronic extinction of the dorsal placing reaction under condition of retention of responses to stimulation of ventral side decreased the efficiency of dorsal tactile stimulation with respect to neurons of the m. biceps cortical area.

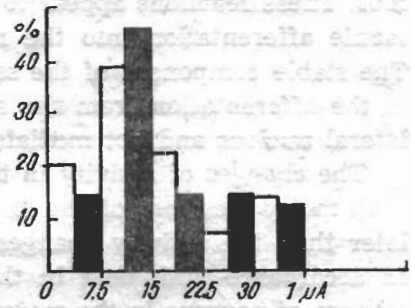


Fig. 17. Histograms of threshold currents evoking the m. biceps contraction. Microstimulation of the motor cortex. Dashed columns, data from trained animals (50 measurements); open, data from naive animals (36 measurements). Abscissa, current (μA); ordinate, percent of stimulations at a given threshold.

A threshold current evoking the electrical response in the m. biceps of naive and trained animals (ranging from 5 to 50 μA) was passed through the microelectrode at the recording points of the neurons excited in parallel with the m. biceps contraction. The comparison of histograms (Fig. 17) of the threshold current distribution for m. bicep EMG responses in trained (dashed columns) and naive animals (open columns) shows that there is no essential difference in excitability between the populations of cortical neurons controlling the forepaw flexions.

DISCUSSION

In the activity of recorded neurons we distinguished the excitatory reactions of two types: 1) changes related to the active contraction of different muscles and to movement and 2) a sensory-determined compo-

nent of reaction to tactile stimulation of the paw. This division, initially based on the observations of temporal dynamics in the activity of neurons in the motor cortex during performance of the placing reaction, are confirmed by other findings:

1. There are different relations of neuronal activity and EMG biceps during temporal intervals corresponding to the first sensory-determined component and the main motor component;

2. The changes of neuronal activity recorded in the ventrolateral nucleus of the thalamus occur with a latency corresponding to the main component of activity in neurons of the motor cortex;

3. The short-latency responses of some neurons of the motor cortex to tactile stimulation were retained in the absence of paw movement.

These data suggest a different origin of the first and the main components in neuronal activity of the motor cortex (2). Stability and short latency of neuronal reactions of the sensory type (about 20 ms) are comparable with respective characteristics of the reactions in neurons of the sensory cortical area and ventralis posterolateralis nucleus of the thalamus. These reactions appear to be determined by the entrance of specific tactile afferentation into the motor cortex through the lemniscal path. The stable component of the sensory response is likely a direct response to the afferentation from the sensorimotor cortex and ventralis posterolateral nucleus and not mediated through interneurons.

The changes of activity in the neurons of the motor cortex associated with motor reactions of the forepaw develop in parallel and partly arise later than the activity changes in neurons of the ventrolateral nucleus. In accordance with data in the literature is seems that the motor discharge of neurons in the motor cortex is partly determined by incoming excitation from the cerebellar nuclei through ventrolateral nucleus (2, 17).

The placing reaction is characterized by some plastic properties worth studying in order to investigate the neuronal mechanisms of formation and extinction of motor habits. First, the placing reaction disappears when there is no constant reinforcement. Second, a number of instrumental motor reflexes can be elaborated using the placing reaction as an unconditioned reflex. The motor pattern of these instrumental reflexes is similar to the motor pattern of the placing reaction. We elaborated the conditioned placing reaction in response to the stimulation of the paw's ventral side. This stimulation in naive cats initially blocks and inhibits the placing reaction to stimulation of the paw's dorsal side.

As a result of learning, the ventral stimulus evoking the conditioned placing reaction evokes a significantly more intense increase of short-latency excitatory activity changes in the neurons of m. biceps cortical area. This increase might be supposed to be determined by the increase

of sensory afferentation coming to the neurons in the biceps cortical area from the sensory cortex (SmI) and the nucleus ventralis posterolateral (1, 5, 18), i.e., by the redistribution in "addresses" of specific afferent excitation on the motor cortical neurons. This is not the case. It was found that the relative number of neurons in m. bicep cortical area responding to stimulation of the paw's ventral side in the absence of the placing reaction does not changes. A distinct specificity of reactions to ventral stimulation in m. triceps cortical neurons is preserved despite the depression of the reaction, Sv — TRI, and the elaboration of a new opposite reaction, Sv — BIC. The reactions of neurons in SM1 sensory cortex to tactile and other kinds of specific stimulation are characterized by stability, being independent of previous learning, motor readiness and functional state of the animal (6, 19). So it seems that elaboration of the conditioned placing reaction does not result in efficiency changes in direct pathways connecting tactile receptors with the motor cortex or ending points of SM1 and VPL afferents on motor cortical neurons.

The lack of excitability changes in cortical output elements points to motor cortical interneurons as a probable place of plastic changes providing an increase of responses of biceps cortical area neurons to stimulation of the paw's ventral side, which evokes the conditioned placing reaction. The comparison of the data obtained with other data (6, 7, 9-11, 14, 20, 21) allows us to conclude that there is a high plasticity of the neuronal net of the motor cortex.

The investigation of the strict organization and physiological mechanism of plasticity of interneuronal connections in the motor cortex is the task of a further study. It probably will bring us closer to an understanding of some of the mechanisms of instrumental conditioned reflexes.

We thank Prof. E. N. Sokolov for valuable comments on the manuscript.

REFERENCES

1. ALBE-FESSARD, D. 1975. The motor cortex as the reflectory center. *In* A. S. Batuev (ed.), Sensornaya organizatsiya dvizhenii. Izdat. Nauka.
2. AMASSIAN, V. E., WEINER, H. A. and ROSENBLUM, M. 1972. Neural system subserving the tactile placing reaction: a model for the study of higher level control of movement. Brain Res. 40: 171–178.
3. ASANUMA, H., STONEY, S. D. A. Jr., ABZUG, C. 1968. Relationship between afferent input and motor outflow in cat motor sensory cortex. J. Neurophysiol., 31: 670–681.
4. BARD, P. 1933 Studies on the cerebral cortex. I. Localized control of placing and hopping reactions in the cat and their normal management by small cortical remnants. Arch. Neurol. Psychiatr. 30: 40–74.

5. EVARTS, E. V. 1974. Precentral and postcentral cortical activity in association with visually triggered movement. J. Neurophysiol. 37: 373–381.
6. EVARTS, E. V. and TANJI, J. 1974. Gating of motor cortex reflexes by prior instruction. Brain Res. 71: 479–494.
7. FETZ, E. E. and FINOCCHIO, D. V. 1975. Correlation between activity of motor cortex cells and arm muscles during operantly conditioned response patterns. Exp. Brain Res. 23: 217–240.
8. FOX, S. S. and RUDELL, A. P. 1970. Operant controlled neural events: functional independance in behavioral coding by early and late components of visual cortical evoked response in cats. J. Neurophysiol. 33: 548–561.
9. KOTLYAR, B. I., MAIOROV, V. I. and SAVCHENKO, E. I. 1975. Models of learning based on plastic properties of placing reaction in the cats (in Russian). Zh. Vyssh. Nervn. Deyat. Im. I. P. Pavlova, 25: 967–973.
10. MAIOROV, V. I., KOTLYAR, B. I. and SAVCHENKO, E. I. 1977. The participation of cat's motor cortex neurons in afferent reorganization of placing reaction (in Russian). Zh. Vyssh. Nervn. Deyat. Im. I. P. Pavlova 27: 931–940.
11. MAIOROV, V. I., SAVCHENKO, E. I. and KOTLYAR, B. I. 1977. The transformation of the afferent tactile signal to the motor command in the motor cortex of the cat. Neurophysiologia 9: 115–123.
12. MURPHY, J. T., WONG, V. C. and KWAN, H. C. 1975. Afferent-efferent linkages in motor cortex for single forelimb muscles. J. Neurophysiol. 38: 990–1016.
13. PORTER, R. and LEWIS, M. 1975. Relationship of neuronal discharges in the precentral gyrus of monkeys to the performance of arm movements. Brain Res. 103: 201–213.
14. SAVCHENKO, E. I., MAIOROV, V. I. and KOTLYAR, B. I. 1976. The activity of cat's motor cortex neurons in the course of realization of the conditional placing reaction (in Russian). Zh. Vyssh. Nervn. Deyat. Im. I. P. Pavlova 26: 65–72.
15. SNIDER, R. S. and NIEMER, W. T. 1961. A stereotaxic atlas of the cat brain. Univ. Chicago Press, Chicago.
16. STONEY, S. D., THOMPSON, W. D. and ASANUMA, H. 1968. Excitation of pyramidal tract cells by intracortical microstimulation: effective extend of stimulating current. J. Neurophysiol. 31: 656–669.
17. THACH, W. T. 1978. Correlation of neural discharge with pattern and force of muscular activity, joint position and direction of intended next movement in motor cortex and cerebellum. J. Neurophysiol. 41: 654–676.
18. THOMPSON, W. D., STONEY, S. D. and ASANUMA, H. 1970. Characteristics of projections from primary sensory cortex to motosensory cortex in cats. Brain Res. 22: 15–27.
19. TOWE, A. L., TYNER, C. F. and NYQUIST, J. K. 1976. Facilitatory and inhibitory modulation of wide field neuron activity in postcruciate cerebral cortex of the domestic cat. Exp. Neurol. 50: 734–756.
20. VORONIN, L. L. 1976. Cellular mechanisms of conditioned activity. Zh. Vyssh. Nervn. Deyat. Im. I. P. Pavlova 26: 705–719.
21. WOODY, C. D., VASSILEVSKY, N. N. and ENGEL, J. 1970. Conditioned eye blink: unit activity at coronal-precruciate cortex of the cat. J. Neurophysiol. 33: 851–864.

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