ELECTROPHYSIOLOGICAL INVESTIGATION OF DISCRETE SKILLED MOVEMENTS IN RATS

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Konorski's concept of instrumental conditioning (Konorski and Miller 1933, Konorski 1967) extended the applicability of Pavlov's theory to voluntary movement and bridged thus the gap between respondent and operant behavior which appeared to be unsurmountable to some critics of Pavlov. Konorski was able to explain how apparently random reactions which are a complex product of orienting behavior, instincts, and acquired experience can be changed by association with reinforcers (food, pain) into conditioned reactions, often emitted without any obvious antecedent conditioned stimulus (CS).

The absence of the CS considerably limited electrophysiological analysis of the self-paced voluntary movements. Whereas in the classical conditioning the impulse traffic can be traced from the CS input to the conditioned reflex (CR) output, in many types of instrumental conditioning CR is the only outstanding event. Computer methods recently made it possible to analyze the activity immediately preceding the discrete voluntary movement and to attempt an "up-stream" exploration of the preparatory neural events. The contingent negative variation (Walter et al. 1964), "Bereitschaftspotential" (Kornhuber and Deecke 1965) and motor potentials (Vaughan et al. 1968) described in man are analogous to similar phenomena accompanying performance of instrumental reactions in animals (Vaughan et al. 1970, Rosenfeld and Fox 1972, Megirian et al. 1974c). Electrical potentials are usually accompanied by excitability changes which can help to localize the plastic process and to determine its time course. They can be investigated by electrical stimulation of
brain (for review see Doty 1969) which was up to now, however, almost exclusively used for analysis of respondent behavior.

Both electrical potentials and excitability changes reflect not only the elaboration of output commands but also the continuous control of their performance and activation of nonspecific auxiliary mechanisms (arousal, attention, motivation). The purpose of the present study is to assess the significance of the above factors in the so called “handedness” in rats (Peterson 1934). Hungry rats offered food pellets from a narrow transparent tube eventually learn to reach into the tube and to grasp the food with one forelimb. Usually the left or right paw is consistently preferred. The movement is impaired by damage to a small region of the motor cortex contralateral to the preferred paw (Peterson and Devine 1963), which corresponds to the overlapping sensory and motor representation of the forelimb (Hall and Lindholm 1974).

METHODS

Fifty six male hooded rats (Druckrey strain) aged 2 to 3 months were used. The animals were reduced to 80% of their body weight and maintained on 24 hr food deprivation schedule with water freely available. The handedness experiments were performed in a plastic chamber (30 × 18 × 15 cm) with a circular opening in the center of the front wall, 5 cm above the floor (Fig. 1). A perspex feeder, a tube 45 mm long with 11 mm internal diameter, was attached to the wall opening from the outside. Small pellets (30–50 mg) prepared from Larsen’s wet chow were introduced into the feeder and their distance from the feeder entrance was controlled by an adjustable piston. A plastic ring fitting the outside diameter of the feeder was equipped with a miniature light bulb facing a phototransistor. The photoelectric system could be fixed in
a suitable position between the feeder entrance and the food, so that the reaching forelimb interrupted the beam and reduced the current across the phototransistor.

Food deprived rats were introduced into the apparatus and offered food placed close to the feeder entrance. The pellets were gradually moved into the feeding tube until the animal could only reach them with one forepaw. Most rats preferred to perform this movement with the left or right forepaw, the preference stabilizing after 2–3 days of training. Fifty pellets were given per day and training continued until the animals were able to get pellets placed 2–3 cm deep into the feeder.

Well trained animals were anesthetized with Nembutal (40 mg/kg) and implanted with various types of recording and stimulating electrodes. Ball tipped silver wire electrodes were used for recording from cortical surface, twisted stainless steel wire electrodes (200 μm diameter, 1 mm tip separation) for stimulation of deep structures. Silver tubes (2.0 mm outside and 1.4 mm inside diameter, 10 mm long) contacting the cortical surface served both for drug application and EEG recording. Between experiments the cannulae were closed with tightly fitting mandrels. Two silver screws (2 mm diameter) placed 7 mm rostral to bregma were used as reference and ground electrodes, respectively. All electrodes were connected to a subminiature 5-pin transistor socket and the whole implant was fixed to the skull with anchoring screws and acrylic resin.

The animal was connected by a light weight suspended cable to the EEG input and placed inside the training chamber. Referential EEG recordings were made with a conventional EEG apparatus with filters set to pass 1.5–70 Hz without attenuation. Low resistance of recording electrodes (approximately 10 kΩ), a tightly woven cable and good contact in the head socket were usually sufficient to ensure artifact-free recording during reaching, when the rat assumed a standing position in front of the feeder and performed only small head movements. To further limit the movement artifacts, a miniature operational amplifier placed in the cable connector was used in some experiments as signal follower, with the output impedance of 1 kΩ.

The amplified outputs of two selected channels were fed into the analog-to-digital converter of a LINC 8 computer, programmed for online averaging of the motor potentials. At 1 msec intervals the computer sampled the EEG and the output of the photoelectric circuit. The last 256 msec of the EEG record were continuously stored in one part of the core memory, the most recent sample replacing the oldest entry in the loop. The photoelectrically indicated movements of the forepaw interrupted the updating of the preextension loop and commanded the com-
puter to store the subsequent 256 samples in another part of the core memory. Pre- and postextension activity was averaged in groups of 32 responses. The averages were plotted on an incremental plotter (CALCOMP 565) and stored on digital magnetic tape for further processing.

Electrical pulses (0.1 msec, 0–50 v) applied through a stimulus isolation unit were used for stimulation, which could be applied at regular intervals or triggered by reaching after a 10–522 msec delay. Light flashes were synchronized in the same way. The stimulus delivery initiated EEG sampling by the computer. With the next reach the extension-stimulus delay was increased by a predetermined interval (usually 32 or 64 msec) and after three increments the original delay was used again. The averaged evoked potentials were computed separately for 32 responses accumulated with the four different delays.

Seizure activity evoked by application of 1%/ picrotoxin (5 μl) into the silver cannula was recorded together with the photoelectric signals of reaching on a four-channel FM tape recorder and processed off-line on the LINC 8 computer programmed for plotting pre- and postreach histograms of epileptic spike occurrence during 512 msec before and 512 msec after forelimb extension.

RESULTS

Motor potentials

Using backward and forward averaging of EEG activity preceding and following the reaching movement, Megirian et al. (1974a) found in the forelimb region of the sensorimotor cortex a prominent surface negative wave (50 μv) culminating 28 ± 3 msec after the extension signal, preceded by a lower positivity (maximum −36 ± 0.9 msec before extension). A less clear late positivity corresponded probably to the ensuing flexion. The averaged motor potentials were best expressed in the hemisphere contralateral to the reaching forepaw and decreased in rostrocaudal direction, their amplitude dropping to zero in the visual cortex (Fig. 2). Increased difficulty of the task (reduced size of pellets, partially obliterated approach to the feeder, partial paralysis of the forelimb by Novocain infiltration) increased the amplitude of the averaged motor responses both in contralateral and ipsilateral hemispheres.

Similar averaged motor potentials were found in cats (Rosenfeld and Fox 1972) trained to reach into an opening in the wall. A surface positive wave appeared in the postcruciate sulcus contralateral to the reaching forelimb approximately 50 msec before movement and a sur-
Fig. 2. Topographic distribution of the average motor potentials in the cerebral cortex of a left-handed rat. Each curve represents average of 32 responses. Extension of the left paw into the feeder is indicated by the vertical dotted line. The rectangular pulse below the last recording corresponds to the average duration of extension. Calibration: 100 msec, 100 μv. Positivity upwards.

face negative wave attained maximum about 20 msec after the onset of movement. In both cases it is difficult to decide which part of the response is due to pure preparatory activity and which is already affected by the somatosensory feedback from the moving limb. Amplitude of the averaged motor potentials is inversely related to the temporal separation of their different components from the triggering movement. This finding is not only due to poor synchronization of distant events but may also reflect the spatiotemporal convergence of activity to the motor centers and indicate shortlasting preparation of simple movements of this type.

_Nonspecific auxiliary mechanisms_

Performance of the reaching movement is impossible without preliminary information about the position of the target with respect to the body and without feedback control of the ensuing movement. It can be argued that reaching implies continuous checking of limb position in the feeder by somesthesis and kinesthesis and to a lesser extent also by vision. The significance of the feedback is maximal just before completion of the movement and abruptly decreases after the food is seized.
or (in case of unsuccessful reaches) after the onset of flexion. The corresponding changes in selective attention and general arousal are too diffuse and asynchronous to be reflected in the gross EEG activity, but they can be detected by testing stimuli applied in different phases of the movement to sensory pathways or integrating centers.

Megirian et al. (1974b) explored visual excitability during reaching. Flashes applied within 256 msec before forelimb extension evoked averaged visual responses the early components of which were 40–45% lower than in the responses evoked outside this interval. When flashes were triggered by extension, the late components of the averaged visual responses were increased at extension-flash delays of 40–50 msec, that is at the onset of flexion.

In another series of experiments responses in sensorimotor cortex were evoked by electrical stimulation of the ventrolateral thalamic nucleus, medial thalamic nuclei or of the caudate nucleus in the hemisphere.

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Fig. 3. Changes of the average evoked potentials to stimulation of ventrolateral thalamic nucleus applied at regularly increasing delays (indicated in msec) after extension. The trace starts simultaneously with the thalamic stimulus. R, random stimulation uncorrelated with reaching. Responses in the sensorimotor cortex ipsilateral to the stimulating electrodes. Calibration 200 msec, 500 μv, positivity upwards.
contralateral to the preferred forelimb. The computer controlled application of the stimulus cycled four different extension-stimulus delays ranging from 10 to 234 msec. A typical experiment is illustrated by Fig. 3. Stimulation of ventrolateral thalamus by single pulses evokes a complex response in the ipsilateral cortex, characterized by an early component partly obscured by the stimulus artefact and by a late surface negative wave culminating 100 msec after stimulus application. Thalamic stimulus applied already 10 msec after extension elicits a response with the late component augmented. With increasing extension-stimulus delay, the late wave grows, reaches maximum at 74 msec and gradually declines afterwards. At the height of the effect repetitive activity with the frequency of 10 Hz was enhanced.

The above results reveal increased tendency to synchronization after termination of the movement, but small changes of the initial components of the response preclude conclusions about earlier effects. A method introduced by Johnson and Hanna (1970, 1972) can help to characterize the changes occurring during movement in more detail. When the ventrolateral thalamic nucleus in cats is stimulated by a pair of pulses the response to the second pulse increases linearly as a function of the interpulse interval over the range from 70 to 130 msec. Peripheral stimuli applied within 25 msec about the first conditioning pulse reduce the slope of the recovery cycle to a minimum.

In the double stimulus experiments the first pulse was triggered by the extension signal with a 10 msec delay and the second pulse followed at variable delays ranging from 32 to 224 msec. A typical experiment with stimulation of the head of the caudate nucleus is illus-

Fig. 4. Changes of the average evoked responses to the double pulse stimulation of the ipsilateral caudate nucleus. Only the responses to the second stimulus are shown. The numbers indicate the duration of the interpulse interval in milliseconds. IR, random stimulation applied independently on reaching. IT, the first stimulus was triggered with a 10 msec delay by reaching. Other description as in Fig. 3.
trated by Fig. 4, showing responses of the ipsilateral sensorimotor cortex to the second stimulus only. When the stimuli were applied independently of reaching, amplitude of the second response characteristically increased and reached maximum at the interpulse interval of 96 msec. Stimuli synchronized with reaching did not show the excitability increase — the second response remained low at all interpulse intervals.

In another cases the cycle was not so clearly expressed when the stimuli were applied outside reaching, but the response at 32 and 64 msec interpulse intervals were usually lower when stimulation was synchronized with reaching. Example of such experiment is given in Fig. 5,

![Fig. 5. Average evoked responses to double pulse stimulation of the ipsilateral ventrolateral thalamus. Other description as in Fig. 4.](image)

showing response of sensorimotor cortex to double pulse stimulation of the ventrolateral thalamus. Although the initial component was not much affected, there are complex modifications of the late waves.

Results of experiments of the above type are summarized in Fig. 6 and 7, showing the amplitude of the early and late components of the averaged cortical responses to single pulse (Fig. 6) and double pulse (Fig. 7) stimuli either triggered by reaching or independent on reaching. In spite of considerable interindividual variability due to electrode placement and different stimulus intensity, the summarized results confirm the statistically significant flattening of the recovery cycle for the reaching triggered stimuli. This effect is better expressed in the ipsilateral than in the contralateral hemisphere and attenuates at 100 to 150 msec interpulse intervals. The early components of the cortical response to
Fig. 6. Changes of average evoked response to single pulse stimulation of the thalamic nuclei contralateral to the preferred forepaw. I and C, responses in the cortex ipsilateral and contralateral to the thalamic stimulus; E and L, early and late components of the average evoked response. Ordinate, mean amplitude of the early (E) or late (L) components of the average evoked response; abscissa, time from extension to the thalamic stimulus. R, responses obtained with random stimuli.

Fig. 7. Changes of average evoked responses to double pulse stimulation of the thalamic nuclei contralateral to the preferred forepaw. R and T, random stimulation and extension-triggered stimulation. Ordinate, mean amplitude of the early (E) or late (L) components of the average evoked response to the second stimulus; Abscissa, interval between the paired stimuli. Other description as in Fig. 6.
ipsilateral single pulses are decreased by the preceding movement whereas late components are enhanced. No such changes are seen in the hemisphere contralateral to stimulation.

All the above effects express the activity of thalamic mechanisms mediating the interaction of different sensory modalities and of the non-specific arousal system with the motor centers. Modification of averaged evoked responses is not limited to the motor cortex but can be observed in the entire projection of the stimulated point. This indicates that the results are due to excitability changes in the stimulated region rather than in the area of recording.

Focal seizures and reaching

The excitability changes described in the preceding sections cannot be traced back to the premovement period by extension–triggered stimulation. In an attempt to assess the excitability of motor cortex before and after movement (Islam and Bureš 1975) focus of interictal seizure activity was established by local application of 10% picrotoxin on the cortical areas contralateral or ipsilateral to the preferred forepaw. After clear-cut epileptic spikes appeared in the EEG record from the picrotoxin treated area, food was placed into the feeder and reaching was recorded together with the EEG. Resulting interictal activity did not usually interfere with reaching even when the focus was established in the sensorimotor cortex controlling the forepaw and single spikes were accompanied by jerking movements of the limb. Reaching was halted by paroxysmal discharge in the contralateral but not in the ipsilateral cortex. Frequency of spikes generated in the ipsilateral focus during the short series of repetitive reaches (lasting from 0.3 sec to several sec) was increased with respect to the background activity. In the contralateral foci slow (less than 0.5Hz) and fast (more than 0.5Hz) interictal activity was increased and decreased during reaching, respectively.

Finer peri-reach changes of spike incidence were revealed by computer analysis performed in eight rats. Spikes occurring during 512 msec before and 512 msec after the extension signal were accumulated in 16-msec classes of a histogram distribution. Typical results are illustrated in Fig. 8. Histogram A is based on accumulation of 1536 reaches. Spike incidence in the focus contralateral to the preferred forelimb was decreased −350 to −80 msec before reaching (minimum at −160 msec). The spike incidence was briefly increased during extension and remained stable during postreach interval. Gross analysis showed that the spontaneous discharge rate of 1.395 Hz dropped during reaching to 1.150 Hz.
Fig. 8. Examples of typical peri-reach histograms of epileptic spikes. Abscissa, time in msec before (negative values) and after (positive values) the onset of extension; Ordinate, number of spikes in 16 msec bins. The histogram A is based on the analysis of 1536 reaches, histograms B, C and D on 512 reaches. Histograms A, C and D are from contralateral, histogram B, from ipsilateral epileptic foci.

Histogram B represents a prolonged pre-reach increase of discharge rate in the ipsilateral focus. A similar tonic increase of interictal activity in a contralateral focus is shown in histogram C where it is combined with an additional brief increment (64 msec) immediately after extension. Histogram D illustrates a contralateral focus with a clearly developed preextension and postextension inhibition.

Although the histograms varied from animal to animal, increase of activity coincident with the extension was a common finding both in ipsilateral and contralateral foci (in 11 out of 13 experiments). The central peak was often preceded by inhibition which was more prominent in the contralateral foci. The postextension inhibition was less expressed and irregular. The results indicate that excitability changes associated with movement may precede extension by several hundreds milliseconds.

The interpretation of preextension excitability changes is not simple, however, since the measuring procedure is not irrelevant for the subsequent reaching. This is true both for electrical stimulation and for the chemically generated spikes. The decrease of the spike incidence in the
pre-reach interval can be due either to increased inhibition accompanying elaboration of reaching or it may express decreased probability of reaching in the interval immediately following the spike. Both interpretations are not mutually exclusive. The situation can be best described by interference of two mutually independent processes. The early phases of movement elaboration can be reset by spikes generated in the critical motor region and repetitive discharge prevents the reaching altogether. On the other hand, the movement once started increases the probability of spike discharge which in this phase does not prevent successful performance of the reaching. If the reaching induced synchronization of the focal discharge is prominent, postmovement reduction of spike incidence can simply reflect refractoriness of the spike generating mechanism.

**DISCUSSION**

The experimental evidence reviewed in this article shows that performance of a discrete reaching movement involves both specific and nonspecific mechanisms contributing to the concomitant changes of electrical activity and brain excitability. Correlates of the specific mechanisms can be recognised by their spatially limited distribution: the averaged motor potentials attain maximal amplitude in the forelimb projection area and rapidly attenuate with distance. Cortical epileptic focus interferes with reaching only when localized in the forelimb area contralateral to the preferred forepaw. Nonspecific mechanisms cause diffuse effects appearing simultaneously in both hemispheres. Changes of visual evoked potentials as well as some changes of thalamic and caudate excitability belong to this category. The purpose of the nonspecific mechanisms is to ensure selective attention to relevant stimuli and suppression of interfering signals. The reduction of responses to testing stimuli can be due to occlusion (most neurons in the pool are already activated), lateral inhibition (processing of the relevant signals inhibits the activity of adjacent neurons) or simply to phasic modulation of the level of arousal. Tight interconnection of the specific and nonspecific mechanisms makes their accurate separation almost impossible.

Forelimb extension arbitrarily used as the synchronizing signal of the reaching movement is close to the onset of the extension-grasp-flexion sequence which can be repeated up to 3 to 5 times per second. The extension-flexion interval of 80–120 msec (Megirian et al. 1974a) corresponds well to the duration of the averaged motor potential. Similarly the increased synchronization observed in the excitability measurements
coincides with the onset of flexion which abruptly resets the arousal level, gradually increasing over the approach phase of the movement.

Whereas the postextension changes of EEG activity and brain excitability can be determined rather accurately the exact duration of the preextension phase is uncertain. Taking into account that the inter-reach interval can be as short as 200 msec, it is improbable that the preparatory activity lasts longer than 100 msec. The early positivity of the averaged motor potential may precede extension by 50 msec. The movement associated changes of interictal activity in the epileptic focus appear even earlier but, as already pointed out, they reflect also the influence of the focal discharge on reaching. Perhaps the gradual increase of spike incidence in the 100 msec preceding extension (Fig. 8A) may express the gradual increase of excitability which is further augmented during movement.

The changes preceding the actual reaching may correspond, in accordance with Konorski’s (1967) concept of skillful movements, to the activation of kinesthetic gnostic units, which receive messages from other gnostic fields (visual, somesthetic) and are facilitated by the motivational system. To each movement corresponds a specific set of kinesthetic gnostic units the activation of which is transmitted through the corresponding projective units and descending pathways to the muscles. The gnostic fields of the kinesthetic analyser are formed as a result of motor learning and represent a sort of “motor engrams” for skilled movements. The gnostic fields allow performance of the movement even in absence of the peripheral kinesthetic feedback, the role of which is reduced to a correcting mechanism. It is conceivable that changes occurring during movement are due not only to sensory feedback, but that they represent the read-out of subsequent segments of the motor program. Such possibility is supported by the observation that the averaged motor potential of reaching is not suppressed but enhanced by novocainization of the preferred forelimb in spite of a considerable decrease of peripheral sensory influx (Megirian et al. 1974a).

As pointed out by Konorski (1967) behavioral motor acts are programmed not only at the cortical level. Importance of basal ganglia for operant behavior was demonstrated by Hansing et al. (1968) and by Levine et al. (1971), who reported impairment of bar pressing with the contralateral forepaw after unilateral lesion to the caudate nucleus or globus pallidus. Changes of caudate excitability during movement may express specific rather than nonspecific mechanisms. Konorski (1967) ascribes an important role in the organization of voluntary acts to the cerebellum, which receives through the cortico-ponto-cerebellar pathways a copy of the commands sent to the executive organs, and through spino-
cerebellar pathways slightly delayed information about the execution of these commands. Comparison of these two streams of information is essential for appropriate correction of the movement pattern which is mediated by cerebello-cortical pathways passing through the ventral lateral nucleus of thalamus (Yoshida et al. 1966). Some changes of thalamic excitability may be due to such feedback control of motor cortex, others reflect nonspecific changes in the general memory system (Thompson 1974) which comprises the posterior thalamic nuclei.

SUMMARY

The neurophysiological mechanisms of voluntary movement were studied in rats trained to retrieve food pellets from a narrow feeding tube. Reaching movements were photoelectrically detected and used to trigger a computer program averaging the EEG activity preceding and/or following the movement and to trigger delayed stimulation of thalamic centers or caudate nucleus. The average motor potential accompanying reaching attained maximum amplitude (50 μV) in the motor cortex contralateral to the preferred forepaw. Thalamic and caudate stimuli applied during 100 msec after reaching evoked responses with diminished early and enhanced late components and increased tendency to synchronization. When the first pulse of a pair of pulses was applied 10 msec after reaching, the recovery cycle monitored by the response to the second pulse was flattened at interpulse intervals shorter than 100–150 msec. An epileptic focus established in the motor cortex was activated by reaching with the ipsilateral forepaw. Reaching also increased activity of slow, but decreased the discharge rate of fast contralateral foci. Peri-reach histograms of interictal spikes showed brief activation of discharge during extension, preceded and followed by more prolonged inhibition. The results are discussed with reference to Konorski's concept of skillful movements.

REFERENCES


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