Acta Neurobiol. Exp. 1970, 30: 81–94

A NEW HYPOTHESIS CONCERNING THE MECHANISM OF FORMATION OF THE CONDITIONED REFLEX

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A number of facts and observations seem to indicate that conditioned connections (temporary connections) are true neural pathways between certain parts of the brain and that conditioned reflexes after elaboration do not differ essentially from unconditioned reflexes. Furthermore it was assumed that conditioned connections preexist and become effective as a result of the procedure of conditioning (Konorski 1948). According to the most widespread opinion, conditioned connections are established as a result of changes in the region of synapses; that is, changes would occur in already existing synaptic structures — "potential" synapses (Barondes 1965). In favour of this opinion are the data of electron microscopy showing that the number of synaptic structures in the cerebral cortex reaches its maximum relatively early in development and does not subsequently increase appreciably (Aghajanian and Bloom 1967), although the individually acquired activity of the central nervous system develops mainly at later stages. Oscillographic studies of "conditioned evoked potentials" (Buser and Roger 1957) have also led to the conclusion that the connections revealed after the elaboration of a conditioned reflex pre-exist and are only facilitated as a result of this procedure (Fessard 1960). However, this assumption cannot be regarded as absolute, i.e. the possibility of formation of new synapses cannot be ruled out (Aghajanian and Bloom 1967, Beritashvili 1968).

According to Eccles (1964), the transformation of "potential" into "actual" synapses is caused by repeated synaptic activity and may be due either to the formation of increased amounts of transmitter, or to the enhanced readiness of its secretion, or else to a growth of synaptic structures. But, of course, conditioning cannot be accounted for entirely

by changes in the synapses during their increased activity because it is based essentially on the coincidence in time of the conditioned and unconditioned stimuli. According to Anokhin (1968), the impulses generated by indifferent and unconditioned stimuli meet on the membrane of the same cell; the subsynaptic membranes enter into closest chemical interaction with the neuroplasm, with a subsequent change in the RNA code and formation of protein molecules which are the storers of the formed association; in the last instance the question is thus transferred to the field of biochemistry. According to Jasper (1967), the mechanisms governing the growth and consolidation of inborn, unconditioned connections may be similar to those established in the course of conditioning. Already Ariens-Kappers asserted that the phylogenesis of neural connections is similar to the development of associative connections in the life of the individual (quoted from Beritov 1932). Examining the possible mechanism of formation of temporary connections Beritov (1932) attached some importance to the electrotonic spread of biocurrents along the neural circuits of the cortex and to the development of myelin sheaths of the nerve fibres; as we shall see below, these latter ideas are of special interest in connection with the proposed new hypothesis of the mechanism of formation of conditioned connections.

The factual data and theoretical considerations underlying the proposed hypothesis will be set forth below.

1. The use of direct current amplifiers has made it possible to establish that when a sufficiently strong volley of impulses reaches a given point of the cortex a slow negative potential lasting hundreds of milliseconds develops on its surface after an initial, comparatively brief evoked potential. Rhythmic stimulation gives rise to a negative d-c shift, but when the stimulation is discontinued the potential returns to its initial level usually in a few seconds. These electric reactions were recorded in the somatosensory region of the cortex upon stimulation of spinal nerves and the skin (Arduini et al. 1957, Roitbak 1965), in the optic cortex upon light stimulation (Pearlman 1963), and in auditory cortex during the action of sounds (Gumnit 1960). In experiments with chronically implanted electrodes the potential shifts of the potential of the cortical surface turned out to be very variable in localization, amplitude and sign (Rowland and Goldstone 1963); they are in an intricate manner connected with the arousal reaction (Caspers 1961); apparently both specific and nonspecific afferent systems participate in these processes (Lickey and Fox 1966).

After elaboration of a conditioned reflex (for example, by association of sound with electrocutaneous stimulation) the formerly "indifferent" stimulation begins to evoke in the focus of unconditioned stimulation,

a reaction similar to the one evoked by the unconditioned stimulation. This concerns both the evoked potentials (Buser and Roger 1957) and the activity of individual neurons; studies of unit discharges in the somatosensory cortex have established that after the elaboration of a conditioned reflex there appears a similarity in the reactions to conditioned and unconditioned stimuli, that is under the action of conditioned stimulation the given cortical neuron imitates the changes in spike discharges evoked by unconditioned stimulation (Shulgina 1967, Vasilevsky 1968). Thus after the elaboration of the conditioned reflex the conditioned stimulation begins to excite approximately the same complexes of neurons as does unconditioned stimulation. As to the d-c potentials, their records do not show such constant and simple results of duplicating the effect of the unconditioned stimulation (Rowland 1961).

The slow negative potential of the cortical response to direct electric stimulation has been studied in great detail (Goldring and O'Leary 1960, Roitbak 1965). This is a local electrical reaction recorded on a territory with a radius of 3 mm around the stimulating electrodes (Fig. 1AB); the slow negative potential evoked by the second stimulus is reduced with intervals of less than 1 sec (Fig. 1C); on rhythmic stimulation the potential shifts to the negative side and upon discontinuation of stimulation it returns to the initial level in a few sec (Fig. 1E); during the slow negative potential the discharges of neurons in the given point of the cortex are depressed (Fig. 1D). It is interesting to note that under the

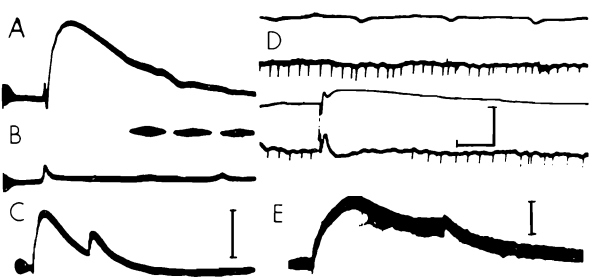

Fig. 1. Slow negative potentials on the surface of the cortex. A, slow negative potential at a distance of 0.5 mm from the stimulating electrodes; B, its absence at a distance of 3 mm; time markings, 200 msec; C, weakening of the slow negative potential to the second stimulus applied 0.9 sec after the first, voltage calibration, 1 mv; D, macroelectrode lead from the surface of the cortex (upper curves) and extracellular microelectrode lead from a neuron in the cortex (lower curves), time calibration, 70 msec, voltage calibration, 1 mv and 0.25 mv for upper and lower curves respectively; E, negative d-c shift recorded at a distance of 1.5 mm from the stimulated point, frequency of stimulation 100 per sec, stimulation lasted 3 sec, voltage calibration, 1 mv. All experiments performed on cats under deep Nembutal anesthesia

action of morphine and other analgesics these potentials are eliminated (Roitbak 1969).

2. When studying the mechanism of conditioned reflex formation, we must take into consideration the fact that the brain consists of nerve cells and neuroglial cells (there are ten times as many of the latter) and the greater part of the surface of neurons is in contact with the glial elements. The opposition of the membranes of the neurons and glial cells separated by narrow clefts about 150 A wide is of paramount physiological importance (Galambos 1961, 1965, Kuffler and Nicholls 1966, Roitbak 1968).

The assumption that the slow negative potential on the surface of the cortex is of neuroglial origin (Roitbak 1963) is now supported by a number of facts. Firstly, it was established that it is eliminated by certain doses of X-rays without any change in the dendritic potentials and primary responses (Roitbak 1969); these doses of X-rays cause noticeable morphological changes only in the neuroglial elements (Maxwell and Kruger 1965). Secondly, it was shown that the slow negative potential on the surface of the cortex to direct stimulation occurs simultaneously with depolarization of the membrane of the "idle" cell at the given point of the cortex (Karahashi and Goldring 1966); the "idle" cells of the cortex were histologically identified as neuroglial (Kelly et al. 1967, Grossman and Hampton 1968) and they are apparently oligodendrocytes (Kelly et al. 1967). It appears that the glial cells of the cortex are also depolarized upon stimulation of the thalamus (Karahashi and Goldring 1966, Grossman and Hampton 1968) and "spontaneously" (Grossman and Hampton 1968).

According to Kuffler (1968), the neuroglial cells in the cortex are depolarized by the same mechanism that was shown in the experiments on glial cells of amphibians, that is under the action of K^+ ions liberated from the excited neural elements (Kuffler and Nicholls 1966). But another mechanism may be also assumed, since it was shown by electron microscope that the glial cells are in contact with the endings of the axon terminals containing synaptic vesicles (Mugniani and Walberg 1964), and it was discovered that the membrane of the glial cells of the cortex is depolarized by ACh and GABA (Krnjević and Schwartz 1967); consequently it cannot be excluded that the neuroglial cells in the cortex are depolarized by the action of chemical mediators which either excite or inhibit the neurons.

Thus, when a volley of impulses reaches the given neuron-glial complex of the cortex, it produces postsynaptic potentials and the electrical discharges in the nerve cells on the one hand, and a prolonged depolarization of neuroglial cells, on the other. The depolarization of

neuroglial cells may serve as a signal for their trophic activity (Kuffler and Nicholls 1966).

3. The known function of oligodendrocytes is the formation of myelin sheaths of nerve fibres in the developing central nervous system by encasing the naked axon cylinder in the processes of the oligodendrocytes (Bunge et al. 1962); when the glial process spirals round the axon, the cytoplasm it contains becomes obliterated and the plasma membranes draw closer together, forming a myelin sheath; the mechanism is similar to that of myelination in the peripheral nervous system (Bunge et al. 1961). The connection of the membrane of oligodendrocytes with the myelin sheaths is shown also in the cerebral cortex (Mugniani and Walberg 1964).

Oligodendrocytes form myelin not only during the development of the central nervous system, but also in adult cerebral tissue: oligodendrocytes surrounding and encasing axons and then forming myelin were seen in a culture of adult cerebral tissue on the 14th day of incubation (Ross et al. 1962). The capacity of oligodendrocytes for myelination in the mature central nervous system clearly manifests itself under conditions of recovery from experimentally produced demyelination in vivo and in situ (Bunge et al. 1961, Aparicio et al. 1968), in remyelination the newly forming myelin sheaths often consist of only 2–3 layers. The tendency of the oligodendrocyte processes to "spiral" round the neural elements is manifested in an excessive, grotesque form during their pathologically intense growth in the oligodendrogliomas (Robertson and Vogel 1962). Thus, in the mature central nervous system oligodendrocytes retain their ability to form myelin; this is a complex and still incompletely elucidated process that has a number of aspects (Schjeide et al. 1968).

ACh increases the motor activity of glial cells pulsating movements and movements round the neuron and along the axon (Geiger 1963), the movement of a glial cell may be evoked by electric stimulation: at the parameters of the stimulus at which depolarization of its membrane is evoked (as a result of a "dielectric breakdown" — Wardell 1966) it may contract (Hild and Tasaki 1962). It may be assumed that depolarization of the oligodendrocyte membrane serves as a signal for myelin formation.

4. The appearance of a clearly defined ability to form conditioned reflexes in the ascending series of verbrates coincides with the differentiation of neuroglia and, especially, with the appearance of oligodendroglia. This conclusion may be drawn from comparing the few electron microscopic data on the phylogeny of neuroglia with the data on the phylogeny of higher nervous activity.

No true conditioned reflexes are elaborated in lampreys, in which

only the so-called "summational" reflexes can be formed (Sergeyev 1967). A capacity to form conditioned reflexes is clearly manifest in bony fish (Beritashvili 1968, Salzinger et al. 1968), while the formation of higher forms of associations appears in reptiles and fully develops in mammals (Sergeyev 1967).

In lampreys neuroglia do not differentiate into astroglia and oligodendroglia and there are no myelinated fibres in the central nervous system (Bertolini 1964). In addition to ependymal glia sharks have an astroglia which performs two functions — those of a hematoencephalic barrier and of myelination; they are the first vertebrates to have myelinated fibres (Bakay and Lee 1966). In bony fish there is a differentiation into astroglia and oligodendroglia, but there are few oligodendrocytes and few of these are associated with myelin sheaths. The brain of reptiles contains a large number of oligodendrocytes and it is often possible to trace their connection with the myelin sheaths of axons (Kruger and Maxwell 1967).

The answer to the interesting question of why, despite the considerable similarity in the structure of the brain of lampreys and fish (Polyakov 1964) and the similarity in the electric reactions of different parts of the brain to peripheral stimulation in these animals (Karamyan 1963), there are substantial differences in the character of the formation of conditioned connections (Sergeyev 1967) can apparently be given if we consider the sharp difference between these animals in the sense of the neuroglial composition of the brain and, hence, the absence in one case, and the presence in the other case, of myelinated fibres in the central nervous system.

5. It is now believed that the electric properties of the presynaptic terminals may differ from those of the parent fibre, the reasons for it probably being the chemoceptors covering part of their surface, special properties of the membrane, etc. (Eccles 1964, Shapovalov 1966). In the neuromuscular apparatus of the crayfish excitation does not actively spread in the presynaptic terminals (Dudel 1963). In the long terminals of the motor axon in the neuromuscular junctions of the frog excitation actively spreads at the rate of about 0.3 m/sec (Braun and Schmidt 1966, Katz and Miledi 1968), it is not clear, however, whether this applies to the region of the last 5–10 μm. An active spread of excitation to the presynaptic terminals is not obligatory for all types of synaptic junctions (Katz and Miledi 1968), different types of chemically operating synapses are apparently subject to different variations in the sense of the effectiveness with which the presynaptic impulse penetrates the terminal. In the cases of blocked excitation, at any distance from the synaptic junction, the transmitter must be released by means of an electrotonically spreading action potential. In experiments with blocking the excitation

with tetrodotoxin it was shown that electric pulses applied to the nerve fibre evoke transmitter release if the terminal is electrotonically depolarized and there are Ca^{++} ions in the external medium; the amount of mediator released depends on the strength and duration of the pulse electrotonically reaching the terminal, i.e. depends on the number of coulombs in the pulse (Katz and Miledi 1967). Thus the amount of transmitter released into the synaptic cleft is greater, other things being equal, the more electricity passes through the membrane of the terminal.

Most of the synapses in the cerebral cortex are formed by nonmyelinated presynaptic terminals (Eccles 1964). With the aid of an electron microscope they can be traced to a distance of 4–5 μm before they synapse; in the cortex there are also quite a few presynaptic fibres in which the myelin ends near the synaptic bouton — at a distance of 0.5–2–0 μm (Gray 1959, Khattab 1968).

The length constant (λ) for the electrotonic spread of the action potential along a terminal 1.5 μm in diameter is 250 μm (Katz and Miledi 1968), for presynaptic terminals 0.1 μm and less in diameter — their diameter in the cortex (Khattab 1968) — λ must be about several μm, i.e. in the presynaptic terminals of the cortex the action potential must spread electrotonically with a sharp decrement.

In Tasaki's experiments (1953) the treatment of the myelin sheath with saponin resulted in an increased leakage of the action potential through the myelin sheath and a sharp reduction in the strength of the current directed outward through the nearest node of Ranvier. In the myelin sheath the layers corresponding to the single membranes of Schwann cells are located at a distance of 85 A from each other. The resistance of each layer is 500 ohm/cm^2. There must be an optimum thickness of the myelin sheath which offers the best conditions for the spread of local current circuits (Hodgkin 1964).

Since the amount of transmitter released by a synaptic terminal is a function of the amount of electricity passing through its membrane, the presence or absence of a myelin sheath round the presynaptic region of the terminal must be of enormous importance to the efficiency of synaptic transmission in the cerebral cortex.

6. After a series of impulses the probability of transmitter release at the neuromuscular junction remains higher for a period of several minutes. This manifests itself in an increased frequency of miniature e.p.p.'s. (Brooks 1956) and in the phenomenon of post-tetanic potentiation, i.e. in an increased frequency of "spontaneous" release of quanta of the transmitter and an increased amount of transmitter released by the nervous impulses. The phenomenon of post-tetanic potentiation clearly

manifests itself in the cerebral cortex in experiments with electric stimulation of its surface (Roitbak 1955).

The striking dependence of the postsynaptic potentials of the neuromuscular junction on the concentration of Ca^{++} ions in the external medium led to the assumption that post-tetanic potentiation is connected with Ca^{++} (Katz and Miledi 1965); this assumption has a number of facts in its favour (Gage and Hubbard 1966). In the cerebral cortex Ca^{++} acts similarly: a temporary application of Ringer's solution containing an increased amount of Ca^{++} to the surface of the cortex resulted in a considerable increase in dendritic potentials; these postsynaptic potentials returned to their initial amplitude in 200 min or so (Roitbak and Oniani 1967).

Depolarization of nerve fibres is the cause of the movement of Ca^{++} ions from the membrane into the external and internal medium (Gage and Hubbard 1966). In examining the subsequent fate of Ca^{++} it is necessary to take into consideration not only the processes operating on the membrane of the terminals after their depolarization — recombination of Ca^{++} with sites of the membrane (Gage and Hubbard 1966), but also the following circumstance: the fate of Ca^{++} in extracellular space must depend on the presence in it of macromolecules not yet identified and possessing an ability to bind or fix the ions (Adey 1967, John 1967).

Thus, after depolarization of the presynaptic terminals the physicochemical state of their membranes and the composition of the intercellular medium surrounding them remain altered; this alteration "chemical trace") disappears gradually, in the course of a few minutes.

The proposed hypothesis consists in the following. Under the action of "indifferent" stimulation the excitation reaches, along the pre-existing neural pathways, the "potential" synapses on the neurons in the focus of unconditioned stimulation. Owing to the anatomic relations established during the ontogenesis namely the multiplicity of appropriate afferent fibres extending to the given neuron neuroglial module of the cortex, the effectiveness of the connections with it and, possibly, the character of impulsation (prolonged impulses in the thin afferent fibres that are excited, for example, by pain stimulation) the unconditioned stimulation must, as a rule, evoke, in addition to a spike discharge of the neurons, an activation of the neuroglial cells and a prolonged depolarization of their membranes, which is a signal to the oligodendrocytes for myelin formation that thus becomes possible. The possibility is translated into reality if the processes of the oligodendrocyte contact a naked terminal which has been depolarized, i.e. during the change in the physico-chemical state of its membrane and the composition of the medium in the cleft between them. Enclosed in a glial process and then encased in

myelin the axon terminal is more effective: a stronger electric current electrotonically reaches the synaptic terminal and evokes release of a larger number of mediator quanta; the terminal becomes active in the sense of evoking excitation (or inhibition) of the postsynaptic element — the connection has been formed and can to a certain extent be improved by the increased number of layers in the myelin sheath (Fig. 2).

On the basis of this hypothesis it is possible to answer the question of why the elaboration of a conditioned reflex requires certain temporal relations between the stimuli. Usually a conditioned reflex is elaborated when an "indifferent" stimulation precedes the unconditioned stimulation, the interval between them may reach 20 min and even more. After depolarization of the presynaptic terminals the "chemical trace" persists,

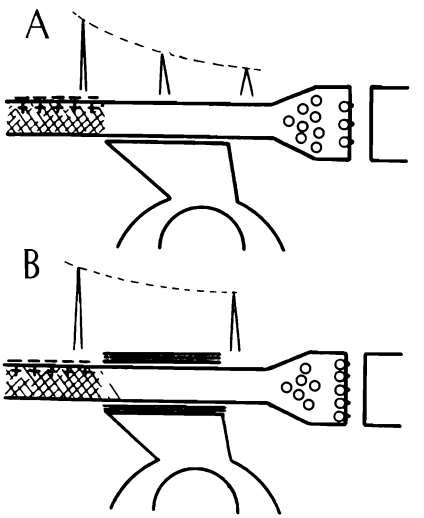

Fig. 2. Schematic representation of the main principle of the proposed hypothesis of formation of conditioned (temporary) connections. Represented: presynaptic terminal, synaptic terminal with vesicles, synaptic cleft and postsynaptic element; oligodendrocyte with process, region occupied by process of excitation spreading along terminal; electrotonically spreading action potentials below block of excitation in terminal. A, "potential" synapse: presynaptic region of terminal not encased in myelin, and decrement of action potential in its electronic spread is large, an amount of transmitter insufficient to excite or inhibit the neuron is released into the cleft. B, formation of conditioned (temporary) connection: as a result of combining in time the excitation of the given presynaptic terminal during the action of "indifferent" stimulation with the activation of the oligodendrocyte during the action of unconditioned stimulation the process of the oligodendrocyte encases the presynaptic region of the terminal in a myelin sheath; the decrement of the action potential during its electronic spread diminishes, a large amount of transmitter is released into the cleft, and the postsynaptic neuron is effectively excited or inhibited

decreasing gradually for many minutes; the unconditioned stimulation, which serves as a signal for myelin formation, can therefore be effective a long time after the moment of application of the "indifferent" stimulation. A conditioned (avoidance) reflex may also be elaborated at a reverse sequence of combinations, for which purpose "indifferent" stimulation must be applied immediately after the end of the unconditioned stimulation or within 1–3 sec (Beritov 1932). The depolarization of the oligodendrocyte membrane ends comparatively soon after the discontinuance of unconditioned stimulation, and in a few seconds it becomes probably subthreshold as a signal to myelin formation; usually therefore upon application of "indifferent" stimulation after more than 1–3 sec no conditions are created for myelination, which, as was mentioned above, consist in a coincidence in time of sufficiently intensive depolarization of the oligodendrocyte with the physico-chemical change in the cleft between its processes and the presynaptic terminals of the "potential" synapses.

Since any sufficiently strong peripheral stimulation evokes in the corresponding region of the cortex a considerable reaction of both neurons and neuroglia another stimulation may in accordance with the aforesaid principle become connected with any peripheral stimulation and lead to formation of associative conditioned connections.

In connection with the proposed hypothesis the following facts assume special interest.

1. In rats raised under conditions of "pressure" of the external environment — trained and solving problems — the cerebral cortex was thicker and heavier than in those raised in isolation. Measurements of the amount of true and false cholinesterase showed that the cortex had grown thicker and heavier through an increase in the mass of neuroglia (Diamond et al. 1964). These data satisfy one of the main requirements of the hypothesis, namely, that the formation of conditioned connections must be accompanied by an increase in the mass of neuroglia because of the growth of the spiralling oligodendrocyte processes.

2. The action of X-rays in a dose of 700 r on the brain of newborn rats destroys only a few nerve cells, but sharply affects myelination; moreover, certain changes in the biochemical composition of myelin are observed, and the oligodendrocytes are smaller than normal (Schjeide et al. 1968). It is well known that after exposure of rats at an early age to X-rays, elaboration of conditioned reflexes is considerably delayed or becomes impossible (Voyevodina 1967). These data meet one of the basic requirements of the hypothesis, namely, that a disturbance in myelination must be accompanied by a disturbance in elaboration of conditioned reflexes.

CONCLUSION

A new hypothesis of the mechanism of formation of conditioned connections is proposed. It is postulated that depolarization of the oligodendrocyte membrane is a signal for myelination and this process requires that at the moment of depolarization of the oligodendrocyte there should be in the intercellular clefts between its processes and the presynaptic terminals a physico-chemical "trace" of the preceding excitation of these terminals. The enclosure in the glial process and the myelin encasement of the presynaptic region of the terminal create favourable conditions for the electrotonic spread of the action potential, and the nervous impulse thus releases more mediator: the synapse in the cerebral cortex changes form a "potential" to an "actual" synapse.

I am indebted to prof. I. Beritashvili and to prof. J. Konorski for much helpful discussion and criticism.

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Received 5 May 1969