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INVESTIGATION OF THE INFLUENCE OF MAGNETIC FIELDS WITH DIFFERENT PARAMETERS ON ELECTRICAL ACTIVITY OF MOLLUSC NEURONS

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A growing body of evidence now suggests that magnetic field (MF)may influence on pineal gland and retina of vertebrates. Some electrophysiological studies have shown that single stepwise change of vertical or horizontal constant component of the earth´s magnetic field (MF) by 0.05 mT is enough to cause a considerable change in spontaneous activity of neurons of pigeons[19], guinea pigs[18], rats[16] and electrical activity of human retina[10]. Similar alteration of MF affects the nocturnal level of cAMF[17], that of melatonine and key ferments of its synthesis in pineal gland and retina of birds and mammalia[4,15;23]. These data suggests that along with light the natural magnetic field may have a "Zeiggeber" function at organizing the circadian rhythmicity[4]. The role of weak MF as a synchronizer of circadian behaviour rhythms has been demonstrated in direct experiments[2,3].

Recently a fruitful approach to clearing up the mechanism of effect of low frequency MF has been outlined. In papers by Liboff et al. on the study of the effect of combination of a weak constant and low frequency alternating MF on Ca^{2+}- and K^{+}-dependent cell reaction[12-14,22] a simple empirical expression was obtained connecting the amplitude of constant field with the frequency of the variable one under which the effects have been observed. The derived formula, at least formally,coincides with the expression for the frequency of cyclotron resonance of ions which activation effects were observed in experiments.

It is unlikely, however, that by means of such a mechanism the MF effects on pineal gland and retina could be explained. First, the maximum efficiency under the action of MF combination was observed for approximate equality of amplitudes of constant and variable fields[13,22]. In experiments on pineal gland and retina the variable component comparable by amplitude with the constant was apparently missing. Secondly, in pineal gland neutron reactions on MF there was a number of special features difficult to explain due to ion "activation". One of them that could be called "memory" consists in the following. Alteration of the character of electrical activity continued a long time, even after field returned into the initial state[16,18,19]. In a similar way MF affected the secretory activity. So, the lowered level of melatanine was observed during two hours after a single change of the field[23]. Another peculiarity consisted in a "trigger" character of the reaction - a part of cells lost their sensitivity to the field repeat changes after they reacted to the first action[16,18,19].

In order to clarify the mechanism of MF effect on pineal gland and retina it is necessary to clear out whether their reaction was caused by the MF changing in time or it was determined by the effect of the constant field. The solving of this question determines the choice of a class of physical phenomena that could be a basis for the mechanism of MF interaction with cells. If the effect of MF was determined by the rate of its changing in time one could assume that the definite part in interaction is played by electrical component since at MF alteration the electrical field is induced.

The effect of static MF (magnetic component) could manifest itself either due to the Lorentz force affecting the trajectory of moving charged particles (as, for example, in the above mechanism based on ion cyclotron resonance[12]) or at the field interaction with the particles having the magnetic momentum (e.g., the electron spin in mechanisms of magnetic field effect based on optical "pumping" in rhodopsin molecules[11] and in photo-inducible electron transfer process[27]) or magnetite crystals[9].

Another question, arising when reading the literature on the MF effect on pineal gland, is as follows: whether pinealocytes are able to percieve directly the MF or their reaction is determined by neuronal and/or hormonal effects from other formation.So,for the blinded rats in contrast with the normal ones MF did not affect the synthesis melatonine[15],whereas for the pigeons the section of optic nerves or administration of the beta-adrenergetic blocker did not prevent the caused MF alternations of neuron electrical activity,that is,in author's opinion, provided an evidence in favor of autonomity of pineal gland reaction[20].

Special features of the procedure of the above experiments, as well as the complex organization of cerebrum of vertebrates do not permit to solve definitely the problems posed above. It seems reasonable to study the regularities of MF effect on any more primitively organized system.

In our experiments we studied the effect of MF of different parameters on the electrical activity (EA) of neurons of Lymnaea stagnalis mollusc[5-8]. The simplicity of organization of nervous system as compared to the brain of vertebrates allows one to interpret more unambiguosly the experimental results.

MATERIALS AND TECHNIQUES

Isolated brain of mollusc L. stagnalis was placed into the 2.5 ml chamber with the Ringer solution duct. Shells of ganglion were cut slightly, so that surface became accessible to microelectrode insertion. Intracellular electrical activity (EA) of neurons was registered by glass microelectrodes filled with 2 M KCl. Horizontal MF was created using 32 cm Helmholtz coils. The Helmholtz coil system was possible to rotate around its vertical axis by means of a mechanical drive. Except of the mentioned cases, the direction of the field being generated coincided with the earth's direction. The experiments on the MF effect were started in 30-40 min after the microelectrode insertion into neuron, when a stationary level of neuron EA was achieved.

DEPENDENCE OF NEURON REACTION ON THE RATE OF MF ALTERATION

In order to clarify the dependence of neuron reaction on MF alteration in time,the experiments were carried out on the influence of a single MF impulse of triangular shape, which rate of edges changing varied within a wide range from $5x10^{-3}$ to $2x10^2$ mT/s[5]. The impulse of 1 mT amplitude with the rates of both edges being less or equal to 10^{-2} mT/s did not cause a noticeable neuron reaction. However, under action on these neurons by the impulse one edge of which had the rate increased up to 0.1 mT/s and more, in 30%, and in some series even in 70% cases, changes in neuron EA were observed (Fig.1). Similarly, to the experiments on pineal gland[16,19]for spontaneously active cells the increase or diminishing of activity frequency was observed, whereas for cells without spontaneous activity, changing of membrane potential was by 5-10 mV. Reaction latent period varied from experiment to experiment within 7 to 60 s (usually 10-20 s). Time of reaction complete development was as high as ten secons up to a few minutes. Similarly to pineal cells, reaction of a part of cells has an irreversible character (observations during 30-40 min), however in some experiments initial character of EA recovered in 10-15 min having been affected by MF impulse. Another peculiari-

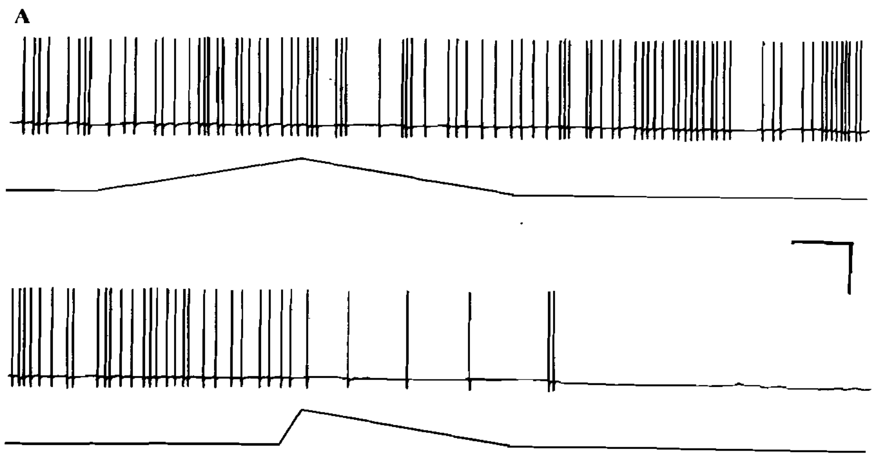

Fig.1. The influence on EA of neurons of MF impulse of 1 mT amplitude with drop rate of 10^{-2} mT/s (upper) growth rate and 0.1 mT/s (bottom). Calibration here and in other figures: horizontal bar - 30 s, vertical bar - 40 mV.

ty of mollusc neuron reaction that brings them together with pinealocytes is that for the major part of cells after reaction development to the first impulse the repeated action does not cause additional reaction.

THE EFFECT OF A SERIES OF MF IMPULSES ON NEURONS

Especially vividly the "trigger" character of the reaction and the phenomenon of "memory" display when investigating a possibility of summation of effects of a few MF impulses with amplitude less than the threshold ones.

In contrast to pinealocytes and retina mollusc neuron reaction to MF has a less sensitivity to MF amplitude. Thus, if for the reaction of pineal gland neurons 0.05 mT was a significant amplitude, and for some cells even 10^{-4} mT[19] was enough, then for mollusc neurons when affected with 0.1 mT amplitude impulse there was no reaction observed[5]. It turned out however, that under the action of a few impulses of such amplitudes it was possible to initiate changing of neuron EA[6] (Fig.2). The efficiency of action depended on a period of impulse sequence. At 15 s period reaction was observed for 14 among 15 neurons under study, and at 60 s change of EF was

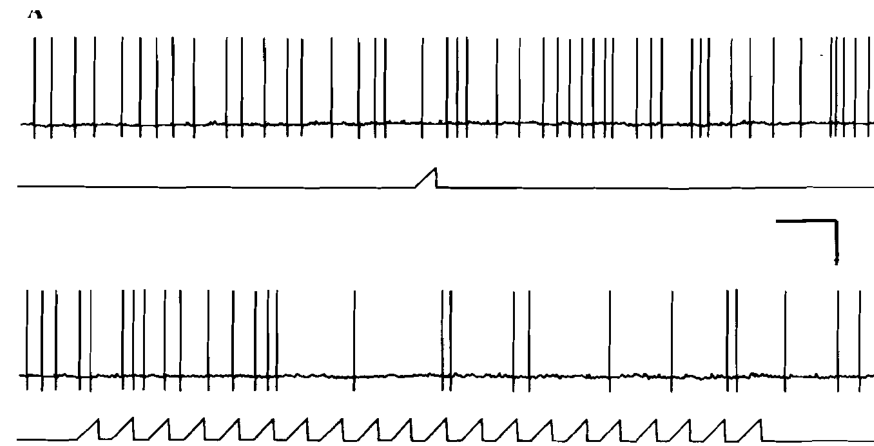

Fig.2. The effect on neutron EA of MF single impulse of 0.1 mT amplitude (rate of growth is 10^{-2} mT/s, the drop rate - 0.1 mT/s) and a series of impulses with the same parameters with 15 s sequence interval.

observed only for 9 from 19 neurons investigated. The quantity of MF impulses sufficient for the neutron reaction starting varied in different experiments from 3 to 10 (in the majority of experiments it was 5-8). Thus, the summary energy in this case was compared with the energy of a single impulse of MF of 1 mT amplitude. This provides an evidence in favor of a threshold character of neutron reaction.

The possibility of summation of effect of MF impulses separated in time assumes a low rate of dissipation of energy obtained as a result of individual event and could help to explain the effect of small fluctuations of MF on biosystems, for example, during the disturbance of the earth´s magnetic field.

REACTION OF NEUTRONS ON CHANGING MF DIRECTION AND ON MF CHANGE IN TIME

In refs.[19,23] the influence of the change of MF vector direction on secretor and elecrtical activity of pinealocytes was observed. The change of MF direction, however, was achived due to current variation in Helmholtz coil and thus simultaneously the MF change in time took place. The question about possibility of effect of changing the MF direction on biosystems also arises in connection with a popular hypothesis about a magnetite nature of magnetoreception of animals[9]. In case of its validity the reaction of biosystems could be expected independently of changing MF direction or MF changing in time.

We have carried out the experiments (not published data) in which on one and the same neurons there was registered their reaction to changing the direction of MF horizontal vector when turning the preliminarily energized coil to 90^0 around vertical axis and also to changing of MF in time due to current change in coil. Rule of MF changing in both cases was similar: in the first case -5 s turn, 15 s break and 5 s turn to the initial state, in the second case -5 s growth, 15 s "Plateau" and 5 s drop. Amplitude of the field in both cases was 1 mT.

The experiments demonstrated that neuron reaction is observed as a rule only under action of MF impulse of trapezoidal shape, that is, as MF changing in time. Among 52 neurons investigated 17 neurons have reacted to MF impulse and only 2-to change in the field direction. The latter two cases most probably are explained by mechanical vibration or a high sensitivity of these cells to changing MF, since because of inevitable small inhomogeneity of the field between Helmholtz coil the field change in time will go at coil turning, too.

NEURON REACTION TO HARMONIC AND PULSED MF

The dependence of neuron reaction on the rate of MF alteration permits one to assume that the MF effect goes via induction of rotational electrical field (REF). However, interaction with electrical field apparently differs from a simple mechanism based on the Faraday induction. It is indicated in the experiments where a small efficiency of harmonic fields inducing REF was shown comparable by amplitude with that induced by a "rapid" edge of the asymmetric impulse. In ref.[8] the same neurons were affected with the harmonic MF with 0.1 and 1 Hz frequencies and a single impulse of asymmetric shape with the 1 mT/s rate of rapid edge. The field amplitude in both cases was 1 mT/s. The following picture was observed in the experiments. Neurons did not, as a rule, react to influence of harmonic MF. So, for the effect of 0.1 Hz frequency field there was no changing EA observed in any experiment (n = 12), and for effect of the 1 Hz frequency field the reaction was observed in six neurons from the 16 investigated. Under action on the same neurons by a single impulse the majority of neurons (24 from 28 investigated) had an expressed change in character of activity (Fig.3) including six ones where the sinusoidal field had caused insignificant alterations.

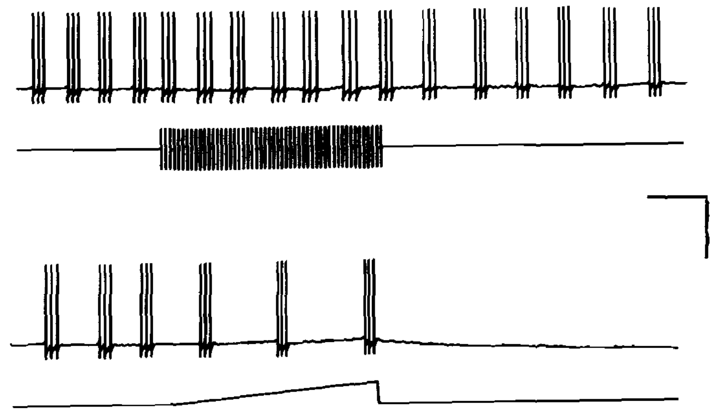

Fig.3. The effect on neuron EA of sinusoidal MF with 1 Hz impulse frequency and growth rate of 10^{-2} mT/s and 1 mT/s drop rate. In both cases the amplitude is 1 mT.

NEURON REACTION ON IMPULSES OF MF OF SYMMETRICAL AND ASYMMETRICAL SHAPE

In order to clarify the reasons for a small efficiency of harmonic MF the effect of its single oscillation range was imitated by impulse of MF with linearly changing fronts[18]. Symmetrical impulse of MF of triangular form with 1 mT/s edge rates caused the change of EH of only 2 from 19 neurons investigated. However, for the majority of these nonreacting neurons (namely 16 from 17) the reaction was observed when they underwent the action of an impulse which rate for one of the edge (leading edge or trailing edge) was diminished up to 0.01 mT/s (i.e. up to the rate being outside the range of effective values), and the rate of the other edge was unaffected (Fig.4).

The presence of neuron reaction both to the leading and trailing edges of asymmetrical impulse permits one to assume that in the case of symmetrical impulse each front separately could cause neuron reaction. In such a case its absence in response to symmetrical impulse apparently could be explained due to "obliteration" of the effect of leading edge of the impulse because of the influence of trailing edge, that is the process caused by changing MF reversible. The phenomenon of reversibility is easy to understand if to assume that the effect of MF goes due to REF. Really, the direction of electrical fields induced by the leading and traling edges is opposite and, re-

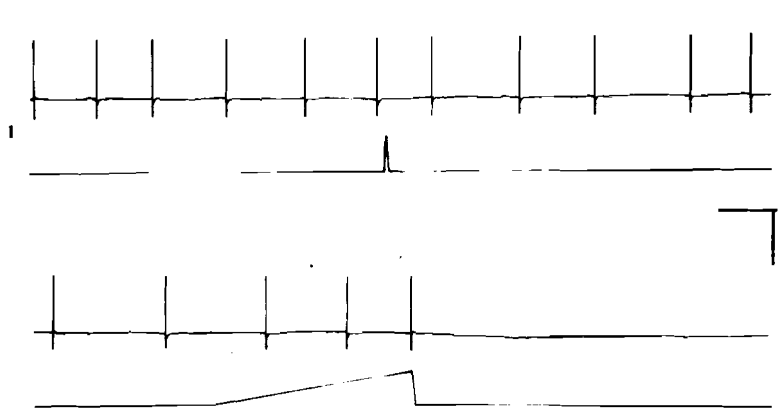

Fig.4. The effect on neutron EA of symmetrical (growth rate and drop rate - I mT/s) and asymmetrical (growth rate - 10^{-2} mT/s, drop rate - 1 mT/s) of I mT MF amplitude impulses.

spectively, the interaction of charged particles with them differs by sign. In the case of symmetrical impulse of MF the amplitudes of electrical fields induced by the leading and trailing edges compensate one another. That does not occur at the action of asymmetrical impulse. This is just the reason for great efficiency of asymmetrical MF as compared with the symmetrical ones (including the harmonic ones, too).

NEURON REACTION TO THE IMPULSES OF TRAPEZOIDAL SHAPE

The previous experiments have shown that the impulses with linearly changing edges could be convenient tool to investigate "primary" process of electromagnetic field interaction with neural cells. To understand the mechanism of MF interaction with neurons and to search for the optimum regimes of action the investigation of kinetic characteristics of functioning a hypothetical "receptor" are of interest, in particular, clearing up of a period duration during which the action of impulse leading edge could be "obliterated" by the effect of the trailing edge.

With this aim the effect on neurons of impulses of MF of trapezoidal shape with different duration of a "plateau", that is with a different interval impulse edges was investigated[7]. The duration of the "plateau" increased correspondingly to a serial number of the impulse and was 0, 1, 5, 10 and 15 s (Fig.5). In these experiments the effect of impulse of MF with

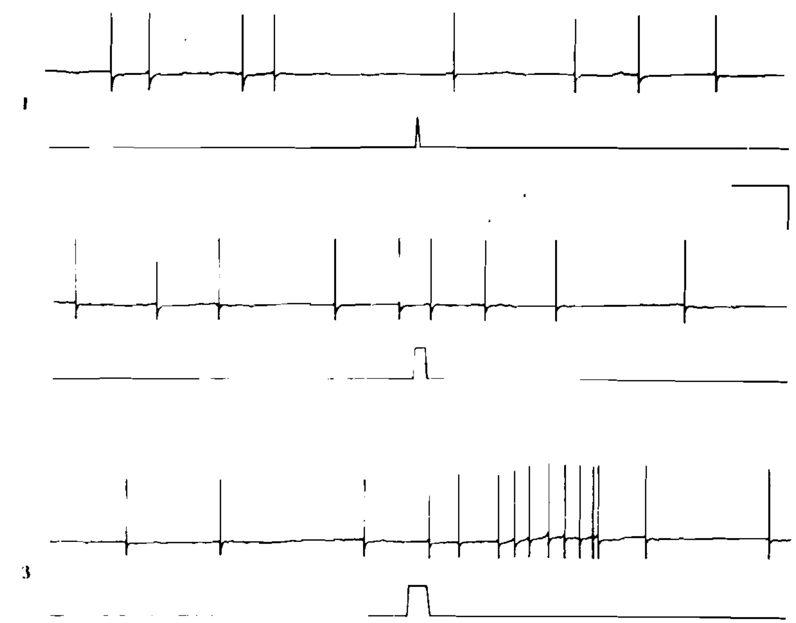

Fig.5. The effect on neuron EA of impulses of MF of trapezoidal shape with time interval between edges 0.5 and 10 s. The rate of growth and drop for all the impulses is 1 mT/s, amplitude -I mT.

the plateau zero duration caused changing of EA of one neuron from 23 investigated. To action of impulse of MF with 1 s plateau duration the EA change also occurred at one neuron, with 5 s "plateau" duration - at 11 neurons. In two neurons of this series we have not achieved EA changes by means of impulses of MF of all the investigated parameters. The control experiments have shown that the increase in neuron sensitivity indeed is linked with increasing the time interval between edges and not with the increasing quantity of acting impulses.

Thus, the process caused in neurons by changing MF is reversible during less than 10 s. Taking into account that the latent period preceeded to neuron EA change in the majority of our experiments exceeded 10 s probably it is reasonable to select a separate reversible stage directly connected with REF "reception" that "starts" the process responsible for the neuron EA changes being observed.

The performed experiments have shown that the character of reactions of mollusc brain neurons and pineal gland to MF have much in common. First, this concerns the so-called "memory" and "trigger" character of the reaction with respect to pineal gland. The frequency of spontaneous electrical activity for both objects could change both towards the increase and towards the diminishing. The quantity of cells sensitive to MF in our experiments as well as in experiments on pineal gland[16,18,19] was approximately 30% of the number of investigated (more than 1500 during 1982 to 1988), however, in some periods of time (for example, in summer 1982) their number was considerably greater. Presently, it is difficult to say is the reason for their sensitivity variation.

Changes of neuron electrical activity observed in our experiments are probably linked with magnetosensitivity of just these cells from which recording was performed. Only in some cases after the MF action the appearance of postsynaptic activity was observed. A possibility of hormonal effects due to increasing the activity of secretor neurons is also of low probability, since the ganglion shells were incised and so neurons were washed continuously with Ringer solution.

In review[1] the data are presented pointing to a probable role of cell membranes in cell interactions with electromagnetic fields. Since neuron EA is directly connected with cell membrane penetrability, our data could serve as an additional confirmation of this hypothesis.

The dependence of neuron reaction on the rate of MF change in time proves that the effect of MF on cells goes due to the electrical and not to magnetic fields as proposed in some theoretical studies[11,21]. Evidently, also in this case the energy absorbed by cell is small and the interaction of the field with the "elementary receptor" could be based on its independence on thermal noises and/or collective effects. A possibility of summation of some separate MF impulses and long lasting reversible state discovered in our experiments points to the stability of the "receptor" to termal fluctuation of environment and thus confirms the value of the above hypothesis.

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