Nonlinear Pharmacological Modulation of Conditioning in the Neocortex

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Abstract. The conditioned defence reflex was elaborated by pair combination of direct stimulation of the motor cortex and electrocutaneous stimulation in awake nonimmobilized rabbit. This conditioning was organized mainly in the cortical part close to the stimulating electrode in the cortex. Influence of modulation of functional state on the observed phenomenon by means of application of GABA solution on the hypothetical place of the formation of the conditioning was examined. S-shaped dependence of effects on concentration of GABA solution was found. The hypothesis of the simultaneous modulation of processes of excitement and inhibition was used to analyse empirical dependences. Saturation effects (100% appearance of same alterations) were taken into account. The model was suitable for description of the discovered nonlinear pharmacological modulation of physiological processes.

Key words: Brain, Cortex, Neuronal population, Conditioning, GABA, Mathematical model.

1. Introduction

One of the most fundamental problems of modern biophysics is probably to determine the relationship between the level of the processes underlying learning and the parameters of the factors modulating them. This trend of investigations is necessary to consider biophysical mechanisms of adaptation, training and memory and to forecast and to control the functional state of the brain.

An application of pharmacological influences on the brain functions may be the optimum method for solution of the abovementioned problem. There is substantial evidence of the pharmacological modulation of conditioning. Most of the available data are concerned with the systemic administration of drugs. However, there is a great number of brain structures that contribute to organization of the conditioning. The specific set of such structures is determined by the kind of the conditioning and is obscure in most experimental situation. At the same time, the total effect of drugs depends, as a rule, on the complex structural integration in the brain, which can result in different directions of the effects on systemic and local applications of drugs. Therefore it is desirable to develop such an experimental procedure which will permit the experimenter to localize the conditioning and to affect it pharmacologically.

The purpose of the present study was preliminary to elaborate the method permitting localization of both the pharmacological effect and the brain function (learning) and then on this basis to investigate the dependence of behavioural events upon the intensity of the pharmacological influence and to try to describe it mathematically.

In order to change the functional state of cortical neuronal populations where the conditioning formed under used experimental approaches the solutions of γ -aminobutyric acid (GABA) were applied to the cortex. GABA was chosen as, firstly, it is known to be the 'universal' inhibitory transmitter [2, 5, 12] and, secondly, drugs synthesized on the basis of GABA and its derivatives are used in clinics to treat disturbances of memory [8].

2. Pharmacological Modulation of Cortical Conditioning by GABA

The method suggested complies with the described requirements, as distinct from conventional approaches. The point is that the conditioning elaborated by combination of direct stimulation of the motor cortex and electrocutaneous stimulation in all probability occurred not far from the cortical stimulating electrodes used in our experiments [3, 6]. It is found that in cats the blink conditioning reflex is elaborated on stimulation of the motor cortex. Moreover the latency of a conditioned reaction is about 12 msec, which is only 4 msec more than the time necessary in order that neuronal impulses can reach appropriate muscles from the cortex [13]. Hence the organization of the conditioning reflex can take place mainly in stimulated neuronal populations of the cortex [3, 6]. Moreover such a experimental approach does not require any solution of questions of fixation and reproduction of information.

The experiments were carried out in 40 awake nonimmobilized rabbits. In the whole the experimental procedure was similar to one in electrophysiological experiments on investigation of a conditioning on neuronal level [2, 4, 5]. Details of the experimental procedure were described in another paper [3]. Conditioned reflexes were elaborated by combination of the direct stimulation of the motor cortex and the electrocutaneous stimulation of the contralateral hind. The electrical stimulation of the cortex was done by means of coaxial electrodes using rectangular current pulses 0.1 msec duration and intensity (8 V) sufficient for the cortical evoked responses but insufficient for the locomotor reactions. The intervals between the conditioning stimulus (the stimulation of the cortex) and the reinforcing stimulus (the electrocutaneous stimulation) were 5 sec and between these pairs of stimuli were 20 to 40 sec. 5 single conditioning stimuli (the stimulation of the cortex) at a time were applied after every 20 pairs of combinations of stimuli. Within one experiment each animal received 200 pairs of the combined stimuli and 50 single conditioning stimuli. The main criterion of the conditioning was the locomotor activity on omission of the reinforcing stimulus (the reproduction of the reaction on the electrocutaneous stimulation).

The drugs were applied to the motor cortex through a trepanized hole (which was also used to insert the stimulating electrodes) 20 to 30 minutes before the onset of experimentation. In each rabbit the following solutions of GABA were used: 0.1, 0.5, 1, 2, 5, 10, 20% ($9.7 \cdot 10^{-2}$ M = 1%). All solutions were applied to the cortex surface in 20 μ l portions. The intervals between experiments were more than 5 days. On the whole, 360 experiments were carried out in 40 rabbits (including application of the physiological solution and a pseudoconditioning). Statistical significance of the difference between events under GABA application of each concentration and those under application of the physiological solution was estimated using chi-square criterion for alternative distributions.

The electromyogram and the mechanogram were registered from the excited hind, as well as the frequency of respiratory motions. Their stationarity was examined and the number of disturbances was registered within 5 sec before application of the conditioning stimulus, within the interval between the conditioning and the reinforcing stimuli, and a 5 second interval after application of the reinforcing stimulus. On omitting of the reinforcing stimulus the analysis procedure was the same as in case of application of combined stimuli. The histograms of 'disturbances' were plotted in 0.5 sec steps.

For comparing the reaction intensities between the experiments, the reaction/background ratio was determined. For each series with the five-fold omission, the following coefficients was calculated:

$$K = \frac{P \cdot Br}{Bp \cdot R}$$

where P is the amplitude of the reproduction peak on the averaged histogram for the given series with the omissions; Bp is the mean background for the same histogram; R is the amplitude of the reaction of 20 preceding combinations to the reinforcing stimulus; Br is the mean background for the latter histogram. Then 10 values obtained in one experiment with application of some GABA solution were compared in pairs with analogous values from another experiment with application of the physiological solution (in one and the same animal) and the number of cases with the enhancement and the weakening of the reaction after drug application was considered. Thus, 8 rows of 10 values were obtained in each of 40 rabbits (on the whole, 3200 values).

On the basis of these investigations it was found that application of GABA solutions to the site of the conditioning stimulus produces modulation of the conditioning. A non-monotonic relationship between the effects and solution concentrations was revealed, which was shown up both in the level and in the direction of the influences. The total evidence thus obtained is presented in Figure 1 where the percentage ratios of the number of the enhanced or weakened reproductions of the reactions during the omissions of the reinforcing stimulus to the total number of events under application of various solutions are given. At low GABA concentrations the enhanced reproduction of responses prevailed. The reproduction of

Figure 1. Effect of GABA on the conditioning reproduction of the locomotor reaction during omission of the reinforcing stimulus. The ordinate is the percentage of cases, the abscissa is the concentration of GABA solution. The scale of GABA concentrations is close to a logarithmic one. The solid line denotes the enhancement of the reproduction, the dotted line denotes the weakening of one.

reactions showed a tendency to the weakening with increasing concentration. At the concentration of 20% the reproduction of the reactions during the omissions of the reinforcing stimulus was strongly weakened or absent at all.

Figure 2 shows the difference between the number of events with the enhancement of the reproduction of reactions and the number of those with the weakening of them, which emphasizes the observed effects. S-shaped dependence of investigated effects upon intensity of pharmacological influences is demonstrated.

3. Mathematical Model of Modulation of Conditioning by GABA

The next attempt to analyze the dependence of the parameters studied on the intensity of pharmacological actions was a mathematical description of this relationship.

A relatively simple and popular method of mathematical description of processes under study is the use of polynomials [1]. In our case the theoretical curves close to experimental ones were obtained using the polynomial of the 6th degree. Based of this approach, one can define the form of dependence of effects studied on intensity of pharmacological action and predict particular values of the functions for a greater set of arguments than it was in real experiments. However, it should be noted that there was a very high sensitivity of the theoretical regressions to the value of coefficients which were rather numerous. Besides, these equations though being of practical importance had no clear physiological sense. Hence, to analyse further experimental dependences, the model was required which would be relatively simple ('crude') and not without physiological sense.

Figure 2. The difference between the number of cases with the enhancement of the conditioning reproduction and the number of with the weakening of one under GABA application. The ordinate is the percentage of cases with dominant effects of GABA in the direction of the enhancement of the conditioning reproduction (up) and of the weakening of one (down). The abscissa is the concentration of GABA solution. The scale is close to a logarithmic one.

The following requirements to the model were propounded:

1. 'Saturability' of the effect: the event cannot occur more frequently than in 100% of cases;
2. 'Probability approach': a potential event can or cannot occur;
3. The model should be simple enough, i.e. be of a general character [10];
4. The models to describe regressions for increasing and decreasing physiological parameters should be similar to each other, since, first, both effects occur in the same neuronal substrate with the same output based on the initially similar pharmacokinetics, second, for both of them the above listed principles must be observed.

There is a viewpoint that the biological activity of drugs is linearly connected with the dose logarithm [11]. Similar approaches are frequently used in description of radiobiological effects [9]. When analyzing biotransformations of drugs in the organism, linear mathematical models are used as a sum of exponentials whose number is determined by process complexity [7]. However, all these and similar approaches cannot comply with the abovementioned principles.

The most frequently used mathematical model of nonlinear processes in vivo and in vitro is the equation of Michaelis-Menten used alone or within complex multi-component models [10]. In terms of pharmacokinetics it looks as follows:

$$V = \frac{V_m \cdot C}{K_m + C},$$

where C is the concentration of drugs or a substrate, V_m is the maximum possible rate of a process occurring on complete saturation of the enzyme with the substrate, K_m is Michaelis' constant equal to the substance concentration at which the process rate is $V_m/2$ [7].

In order to analyze the dependence of neurophysiological effects on the intensity of pharmacological action, let us use the description of pharmacokinetics processes. The functional shifts toward predominance of excitation or inhibition must be associated with the rate of biotransformation of their regulating substances. Both the increase and the decrease of the parameters registered are finally determined by chemical events and, hence, by their rate which depends on the dose of the substance. Since the neurophysiological effects can indirectly reflect biotransformation processes, an additional coefficient is required which will relate them to each other.

As applied to neurophysiological effects, the above equation can be rewritten in the following form:

$$Y = \frac{C \cdot X}{p + X},$$

where Y is the effect itself, C is the maximum effect, X is the concentration of drug, p is a coefficient analogous to that of Michaelis, K is the coefficient defining the relationship between the effect and biotransformation characteristics.

The additional coefficient K has to meet the following requirements:

1. The value of the coefficient K must possess the limitations: $0 < K < 1$;
2. This coefficient must possess different parameters for the effects of the enhancement and the weakening of physiological indices;
3. The coefficient K cannot be a constant value since its significance determines by a drug dose, i.e. intensity of pharmacological influence;
4. The sum of additional coefficients for cases with the enhancement and the weakening of physiological indices under the same drug concentration cannot exceed one: $K_1 + K_2 \leq 1$, as the sum of physiological affects cannot exceed 100%.

After some thorough examination of possible versions the following formulae were chosen:

$$K_1 = \frac{r}{r + X^2} \quad \text{for effects of the enhancement of physiological indices and}$$

$$K_2 = \frac{X^2}{r + X^2} \quad \text{for effects of the weakening of physiological indices,}$$

where X is the concentration of a drug.

The physiological essence of these coefficients is the facilitation of the weakening of the conditioning as the concentration of GABA solution increases and the facilitation of the enhancement of the conditioning as the concentration of one decreases.

Consequently, the proposed equation had the following final kind:

$$Y1 = \frac{r1}{r1 + X^2} \cdot \frac{C \cdot X}{p1 + X} \quad \text{for effects of the enhancement of physiological indices and}$$

$$Y2 = \frac{X^2}{r2 + X^2} \cdot \frac{C \cdot X}{p2 + X} \quad \text{for effects of the weakening of physiological indices,}$$

where C is the maximum effect, i.e. 100% of cases, X is the concentration of a drug, p and r are the appropriate coefficients.

On the basis of the latter equations curves were drawn using the series of 12 values (0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 30, 40, 50) for all 4 coefficients ($p1$, $p2$, $r1$, $r2$). Then these curves were compared with empirical ones by means of the chi-square criterion and Man-White's test. The obtained theoretical curves are partly presented in Figures 3 and 4.

It was found that the effects of the enhancement of physiological indices by GABA application were described by the abovementioned method if $p1 = 0.05$, 0.1, 0.5 and $r1 = 10$. For the analogous description of the effects of the weakening physiological indices the following values of the coefficients were needful: $p2 = 0.1$, 0.5, 1 and $r2 = 5$, 10, 20. In these cases the null hypotheses were not refused ($P > 0.05$).

The proposed equations were sufficient for the description of the sum and the difference of the effects of the enhancement and the weakening of physiological indices under the application of GABA solutions of different concentration.

The consideration of the coefficients suitable for the description of the dependence of the conditioning on intensity of pharmacological influence shows that the effects of the enhancement differ from the effects of the weakening rather the coefficients p , than by the coefficients r which are very close or equal. In case of the enhancement of physiological indices the coefficients p were about 10 times smaller than those in case of the weakening.

Thus, the suggested mathematical model is suitable to describe the modulation of processes of excitement and inhibition by GABA under conditioning and general effects of the relation of these processes.

Figure 3. Some series of curves obtained on the basis of the proposed equation for effect of the enhancement of physiological indices. The values of r are 50 (up), 40, 30, 20, 10, 5, 2, 1, 0.5, 0.1 (down) in each part of the figure. The values of p and X are showed in the figure. The solid lines denote the theoretical curves. In the last part of the figure the dotted line denotes the empirical curve.

4. Conclusion

In awake nonimmobilized rabbit it was established that the conditioned defence reflex was elaborated by pair combination of the direct stimulation of the motor cortex and the electrocutaneous stimulation during one experiment. This conditioning was organized mainly in neuronal populations near the stimulating electrode in the cortex. GABA application to the site of the cortex stimulation produced the modulation of the conditioning. Nonlinear dependence of the effects on the drug concentration was found. The modulation of the conditioning by GABA apparently included facilitation of processes of excitement and inhibition.

The mathematical model for the control of the conditioning by GABA was proposed on the basis of the modification of the equation of Michaelis-Menten. The model is virtually able to describe as a 'crude' version the nonlinear pharmacological modulation of neurophysiological parameters and to systematize the data on reverse effects of one and the same substance. In reality, there can be a more

Figure 4. Some series of curves obtained on the basis of the proposed equation for effect of the weakening of physiological indices. The values of r are 0.1 (up), 0.5, 1, 2, 5, 10, 20, 30, 40, 50 (down) in each part of the figure. The other designations as in Figure 3.

complex dependence of the effects on the intensity of pharmacological action than it is considered in the model since there is a very great number of constituents and interactions between them.

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