ELECTRIC DIPOLE THEORY OF CHEMICAL SYNAPTIC TRANSMISSION

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ABSTRACT In this paper we propose that chemicals such as acetylcholine are electric dipoles which when oriented and arranged in a large array could produce an electric field strong enough to drive positive ions over the junction barrier of the postsynaptic membrane and thus initiate excitation or produce depolarization. This theory is able to explain a great number of facts such as cleft size, synaptic delay, nonregeneration, subthreshold integration, facilitation with repetition, and the calcium and magnesium effects. It also shows why and how acetylcholine could act as excitatory or inhibitory transmitters under different circumstances. Our conclusion is that the nature of synaptic transmission is essentially electrical, be it mediated by electrical or chemical transmitters.

I. INTRODUCTION

The term "synapse" which means "to clasp" in Greek was introduced by Sherrington in 1897 for the junctional region between two nerve cells. The connotation has been loosely extended since to a connection, a contact, a near contact or even a crossing between neurons or between a nerve and a muscle fiber. In many cases, there is a gap or a cleft across a synapse but in some other cases such as is often found in fish, a synapse is a real physical joint. It is because of the existence of these two structural types of synapses, the gap and the gapless, that the problem of electrical transmission across a synapse developed.

The nature of synaptic transmission has been in speculation for almost a hundred years. Dubois-Reymond (1877) first suggested that synaptic transmission could be either chemical or electrical, more probably the former. Since then, great controversy has run across the two rival schools, the chemical school led by Dale and Loewi and the electrical school by Erlanger, Gasser, and Lorente de Nó. The situation just before 1959 was that the chemical hypothesis had won overwhelming support and the electrical theory was finally to be eliminated. The year of 1959 was a turning point. In that year and thereafter, studies of synapse and synaptic transmission in fishes notably by Furshpan (1959, 1964) and Furukawa (1963) have established firm and unequivocal evidence for electrotonic transmission. The progress made in this

direction is so fast and so drastic that Bennett was led to make the following provocative statements (1966). "Considering only physiological data, known instances of excitatory chemical transmission are fewer in number than known instances of electrotonic transmission. From a superficial point of view, electrotonic transmission is simpler than chemical and the widespread occurrence of regions of membrane fusion and electrical coupling between some nerve cells might be an argument for the *greater primitiveness* of electrotonic junctions. It might be wondered why chemical transmission developed at all."

It appears now, that there is little question about the evidence and the principle of electrotonic synaptic transmission. Though there is an enormous amount of data on chemical transmission, its working principle is yet to be established. Furthermore, there are a great number of facts which remain unexplained. This paper is an attempt to perform this double task: first, to find a physical principle, and then to explain as many of the known facts under this principle as is possible.

It is well-known that if a nerve is to be excited, positive ions, Na in the normal case, have to go into the interior. To make this possible, the net force on each positive ion (Na) must be directed inward, or at least be zero. Neglecting ion-ion interaction, there are two main forces on each ion. The first is the electrical force (f_1) exerted by the dipole layer at the surface of the membrane. This force is effective if the ion in question is near the edge of or in the junction barrier and is proportional to the barrier potential. The second force is the diffusional force (f_2) arising from the concentration gradients of the ions concerned. In the resting state, f_1 on Na is outward, f_2 is inward, and $f_1 > f_2$, and hence Na ions are kept out. In section II, we shall calculate f_1 and f_2 for Na ions to prove the above statement.

There are several ways to excite a nerve in principle. The first is to reduce f_1. Since f_1 is proportional to the barrier potential, one can reduce f_1 by lowering the junction barrier. This is usually done by depolarization. The second is to increase f_2. This may be achieved by raising the Na concentration in the external solution. The third way is to add a third force f_3 such that

$$f_1 + f_2 + f_3 \geq 0 \tag{1}$$

where "greater than" means "directed inward." This condition is our principle for chemical synaptic transmission and will be referred to as the "force condition" in this paper. The f_3 is to be provided by chemical molecules such as acetylcholine (ACh) which are considered as electric dipoles. In section III, detailed calculations will be given on f_3 and other related quantities. From these calculations, we shall show why a large cleft space (greater than 150 A) is required and why there is a large synaptic delay in chemical transmission.

The inhibitory action of ACh is considered in section IV. Many other facts observed in chemical transmission will be discussed in sections V–VII. This is the first time a physical theory has been applied to chemical synaptic transmission. Such a

new and unusual approach may shed some light onto the mystery of one of the most important neural phenomena.

II. FORCES ON Na IN NERVES

In normal cases, nerve excitation invariably involves flow of Na ions into the interior of nerve membrane. In this section we shall calculate the two forces f_1 and f_2 on Na ions which are in the immediate vicinity of the junction barrier at the membrane surface. Prior to this calculation, a physical picture of a nerve membrane needs be described briefly. This is illustrated in Fig. 1. According to the Danielli-Davson model (1935), a cell membrane has two dipole layers, one on each side, with the positive end facing the membrane. In the aqueous phase in the immediate vicinity of the dipole, there is an excess of positive ions whose concentration is greater than the bulk concentration in the solution. In a nerve axon, the excess positive ions in the external solution are normally the Na ions which will go inward during excitation.

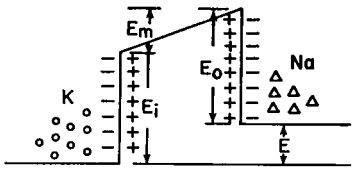

FIGURE 1 Potential profile across a nerve membrane in the resting state.

Fig. 1 shows that the observed "membrane potential" is the sum of three potentials, the two junction potentials E_i and E_o, and the "true" membrane potential. According to Johnson, Eyring, and Polissar (1954), the junction potentials are of the same order as the observed membrane potential. Since the latter is usually about 70 mv, it is not unreasonable to take $E_o \sim 20$ mv. The junction width is not more than 10 A. Therefore the electrical force f_1 exerted by the dipole layer on a Na ion in the immediate vicinity of the dipole would be in the order of

$$f_1 \simeq -qE_o/\Delta s = -20 \times 10^{-3}/10^{-7}$$
$$= -2 \times 10^5 \text{ ev/cm.} \quad (2)$$

The minus sign indicates an outward direction.

Without electrical barriers, the Na ions in the external solution would diffuse inward by the force

$$f_2 = kT\nabla \log [\text{Na}] = kT[\log [\text{Na}]_o/[\text{Na}]_i]/W \quad (3)$$

where [Na] means Na concentration and W, the thickness of the membrane. Taking $kT = 0.025$ ev (at room temperature), $[\text{Na}]_o/[\text{Na}]_i \sim 10$, $W \sim 100$ A, we

obtain

$$f_2 = 5.7 \times 10^4 \text{ ev/cm.}$$

Therefore the total force (neglecting ion-ion interaction) on a Na ion at the edge of the dipole barrier would be

$$F(\text{Na}) = f_1 + f_2 = -2 \times 10^5 + 5.7 \times 10^4$$
$$= -1.43 \times 10^5 \text{ ev/cm.} \quad (4)$$

This means that in the resting state, Na ions in the external solution are forced to stay out mainly because of the electrical barrier at the membrane surface. The problem of exciting the nerve is essentially how to reduce or counteract this negative (outward) force at the edge of the junction barrier. This is our starting point for treating chemical synaptic transmission.

III. THE THIRD FORCE IN SYNAPSE

In chemical synaptic transmission, chemical molecules such as acetylcholine (ACh) are released from vesicles at the nerve terminals upon the arrival of nerve impulses. The molecules are injected into a cleft space of the order of 150–200 A and excite the postsynaptic membrane with a latency (or synaptic delay) of 0.3–10 msec after the peak of the presynaptic potential. In extreme cases, the latency could be as long as 300 msec. Chemical transmission is less obvious or absent in synaptic cleft much less than 150 A.

In this section we shall explain how the excitatory action of ACh can be brought forth. Next we shall show from calculations why a cleft space of no less than 150 A is needed and why the synaptic delay is so long for chemical transmission. We shall start from a basic property of molecules. A molecule is either nonpolar or polar electrically, depending on whether the centers of gravity of its positive and negative charges coincide or not. Unless a molecule has a symmetrical structure, it is more often than not an electric dipole. An ACh molecule has a nitrogen pole at one end and a carboxyl group at the other and it is very likely an electric dipole. Its longitudinal chain has six bonds. Assuming 1.5 A for each bond, the chain would be about 10 A long. Its dipole moment would be in the order of

$$p = qL = 1.6 \times 10^{-19} \times 10^{-9} = 1.6 \times 10^{-28} \text{ coul-m}$$

by taking the polar charge equal to 1 electron unit.

When ACh molecules are released into the cleft, their dipole orientations will be initially in random directions. However, as they approach in close proximity to the postsynaptic membrane, the ACh in the foremost front will become oriented one after another under the influence of the near field of the dipole layer at the mem-

brane surface. The first layer of ACh dipoles when fully oriented will extend the field backwards and hence the later-coming ACh will follow suit. The process will go on and on and finally a multilayer ACh dipole array is formed in the cleft space. This is shown in Fig. 2. As described before, there are excess Na ions right close to the membrane junction barrier. Each Na ion in this region is acted upon by three forces, f_1, f_2, and f_3, the last being produced by the ACh dipole array. The Na ions will be driven into the postsynaptic membrane if

$$f_1 + f_2 + f_3 \geq 0 \tag{5}$$

or

$$f_3 \geq -(f_1 + f_2) = 1.43 \times 10^5 \text{ ev/cm.}$$

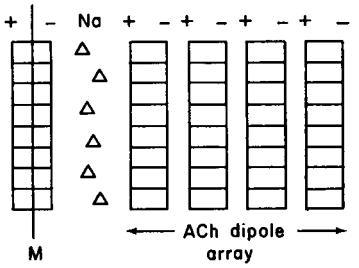

FIGURE 2 The mediation of an ACh dipole array for synaptic transmission.

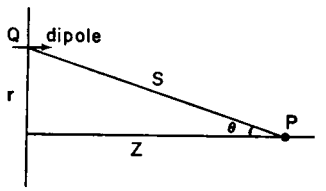

FIGURE 3 The field produced at P by a dipole at Q.

In order to produce f_3 of this magnitude, the ACh dipole array must reach a critical size. We shall now calculate this critical size.

Let us first calculate the field produced by a single layer of ACh dipoles along the axis. Assume the layer is in a circular form of radius R. Consider a dipole at a distance r from the center. The potential produced at point P (Fig. 3), by this dipole is (Kip, 1962),

$$V = \frac{p}{4\pi\epsilon}\frac{\cos\theta}{S^2} = \frac{p}{4\pi\epsilon}\frac{z}{S^3}$$

$$= \frac{p}{4\pi\epsilon} \times z[r^2 + z^2]^{-3/2}$$

where p is the dipole moment. If n is the density of dipoles, then the resultant poten-

tial produced by all the dipoles of this circular layer is

$$V_1 = \frac{npz}{4\pi\epsilon} \int_0^R \frac{2\pi r\, dr}{[r^2 + z^2]^{3/2}}$$

$$= \frac{np}{2\epsilon}\left[1 - \frac{z}{[R^2 + z^2]^{1/2}}\right].$$

The electric field at P along the z-axis is then

$$F_z = \frac{-\partial V_1}{\partial z} = \frac{np}{2\epsilon}\left[\frac{1}{[R^2 + z^2]^{1/2}} - \frac{z}{[R^2 + z^2]^{3/2}}\right].$$

If $z \ll 0.1\ R$, the above formula reduces to

$$F_z = \frac{np}{2\epsilon R} \tag{6}$$

which is independent of z. According to Eccles (1957), a typical synaptic knob is about 2 μ in diameter separated by a cleft space of about 200 A from the post-synapse. In that case $R = 1\ \mu$ and z is no greater than 200 A, and so the above formula (6) should be almost exact. Since F_z is independent of z, the field produced by n layers will be simply NF_z.

To calculate F_z we need to know n, the density of dipoles per unit area (square meter). Taking 3 A as the spacing between two adjacent parallel dipoles, we have in MKS units,

$$n = 1/(3 \times 10^{-10})^2 = 1.1 \times 10^{19} \text{ per m}^2.$$

The dipole moment of ACh has been obtained before as

$$p = 1.6 \times 10^{-28} \text{ coul-m.}$$

We also know

$$\epsilon = 80 \times (36\pi \times 10^9)^{-1}$$

$$= 7.2 \times 10^{-10} \text{ coul/v-m (for water)}$$

$$R = 1\ \mu = 10^{-6} \text{ m}$$

where the radius (R) of the ACh disc is taken equal to that of a typical synaptic knob. This is somewhat justified because of the possible existence of a diffusional barrier around and extended from the knob in the cleft space (Eccles 1957, p. 216, Fig. 78).

With the above values substituted in equation 6, we find

$$F_z = 1.1 \times 10^6 \text{ v/m} = 1.1 \times 10^4 \text{ v/cm.}$$

The electrical force on a monovalent cation will be

$$f_z = qF_z = 1.1 \times 10^4 \text{ ev/cm}.$$

In order to produce a total force $f_3 = 1.43 \times 10^5$ ev/cm, it would require

$$N = f_3/f_z = 1.43 \times 10^5/1.1 \times 10^4 = 13 \text{ layers}.$$

This means that once a 13 layer ACh dipole array is formed, the condition

$$f_1 + f_2 + f_3 \geq 0$$

will be fulfilled and the Na ions close to the junction barrier of the postsynaptic membrane will be driven inwards, thus depolarizing that membrane. Since each layer is 10 A thick (the length of an ACh dipole), a close-packed array of 13 layers will be at least 130 A. This is the critical size of the array over which the above "force condition" will be surely fulfilled. This critical size is independent of the layer thickness but is fixed by the magnitude of f_3 because the critical size

$$S_c = N \cdot L = \frac{f_3}{f_z} \cdot L = \frac{f_3}{K \cdot L} \cdot L = f_3/K \tag{7}$$

since f_z is proportional to the dipole moment $p = q \cdot L$.

The above calculation shows very clearly that a cleft space must be larger than 130 A in order to accommodate a big ACh dipole array which can produce a sufficiently strong field for synaptic transmission. This also explains how the excitatory action of ACh comes about and on what condition. The calculated *minimum* size for cleft space required for chemical transmission is in close agreement with the empirical condition, that is, 150–200 A.

Next we shall calculate the number of ACh dipoles in a 13 layer array. The number of dipoles in each layer is

$$N(1) = NA = 1.1 \times 10^{19} \times \pi(10^{-6})^2 = 3.5 \times 10^7.$$

In 13 layers,

$$N(13) = 4.6 \times 10^8.$$

This number is meaningful in two counts. First, we need to understand that it will take some time for each dipole to orient itself in the desired direction. This transient time in average would be in the same order of the dipole relaxation time. In aqueous solutions, the dipole relaxation time is usually in the range of 10^{-10}–10^{-12} sec (Fröhlich, 1958). The total time required for $N(13)$ dipoles to orient themselves in the same general direction would be in the range of 0.46–46 msec which is well within the range of the observed synaptic delay (0.3–300 msec). Second, according to

Birks and MacIntosch (1961), the *maximum* rate of release of ACh into cleft is 2.8×10^{-9} g/min or 2×10^{9} molecules/msec. However, ACh will be hydrolyzed through the catalysis of choline esterase at the rate of 10^{9} molecules/msec (Oser, 1965). Thus the net rate of production of ACh during *maximum* activity is 10^{9} molecules/msec. In 5 msec, the maximum total number of ACh available in the cleft would be about 5×10^{9} molecules. The actual population could be considerably less than this figure. From the calculation made before, the number of ACh dipoles in a 13 layer array is 4.6×10^{8} which should be the *minimum* number of ACh required for mediating synaptic transmission. That the calculated number is one-tenth of the maximum number available and perhaps comparable with the actual population of ACh in order of magnitude lends another quantitative support to the theory.

IV. INHIBITORY ACTION OF ACh

Besides excitatory mediation, ACh can also produce inhibitory action under certain circumstances, for example, in the vagus nerve of the heart. One wonders how the same substance plays opposite roles at different places. To seek the answer, we must consider the chemical character of ACh.

ACh consists of two parts, choline and acetic acid. In liquids, it can be hydrolyzed through the catalysis of choline esterase. Ample evidence indicates that choline esterase resides on the surface of the cell membrane and one of its main functions is to shorten the lifetime of ACh. It was thought that as an ACh molecule touched upon the membrane surface, its positive end (choline) was snatched by and hence bound to the anionic site of choline esterase, thus ending its life (Wilson et al., 1950).

Here we ought to look again at the membrane structure. A cell membrane, according to the Danielli-Davson model consists of a bimolecular lipid leaflet covered with proteins on both sides. The lipid is usually in the form of lecithin which has a choline for its polar end. The choline is most likely on the positive side of the dipole layer at the membrane surface. Now after the hydrolysis of ACh, another choline is bound at least temporarily to the surface. It is conceivable that this new choline may swing around and come to choline base (the positive side) of the surface dipoles, thus adding to their strength. If a large number of new dipoles were created this way, the junction barrier would be heightened. This would upset the force condition and thus cause inhibitory action. However, by metabolism, the newly added dipoles may not stay for too long before they are enzymatically hydrolyzed and start another cycle of ACh synthesis.

From the discussion given in this and the previous sections, ACh would produce either excitatory or inhibitory action depending on the size of the cleft space, the release rate of ACh from the nerve ending, and the concentration of choline esterase on the membrane surface. If the first two factors are large and the last one comparably small, ACh would be an excitatory transmitter, otherwise, inhibitory.

There is ample evidence to support this statement; the most recent evidence is the following. Larramendi et al. (1967) found that the synaptic vesicles within basket, Golgi, and Purkinje terminals (inhibitory) were significantly smaller than in mossy and parallel fibers (excitatory). Since smaller vesicles would contain smaller amounts of ACh, this finding implies that the rate of release of ACh from inhibitory terminals would be smaller than that from excitatory terminals. A low release rate means a small dipole density n, in equation 6. In that case, the theory predicts inhibitory rather than excitatory action of ACh on the postsynaptic membrane, which is in general agreement with the finding on the nature of the synapses.

V. NONREGENERATION AND INTEGRATION

It has been often stated that nerve propagation along an axon is "regenerative" (Hodgkin, 1951; Katz, 1966) but synaptic transmission is "non-regenerative" (Katz, 1966). The mechanisms behind these terms have not been well understood. In fact, events such as the change in Na permeability, the entry of Na, and the change in membrane potential occur in both cases. Then why is the sequence of events regenerative in one case but not in the other?

The answer to this question could be inferred from the different ways by which the force condition is fulfilled. According to our theory, in the case of nerve conduction, f_1 is reduced, while in synaptic transmission, f_3 is added. Though both result in the entry of Na into the membrane, the other consequences of f_1-reduction and of f_3-addition are not the same. Here, some elaboration on the molecular mechanism of f_1-reduction is necessary.

As described before (see Fig. 1) there are two layers of dipoles, one on each side of the membrane and their positive ends reside in the membrane. When a membrane is hyperpolarized, that is, a positive potential to the exterior (Fig. 4 *A*), the field (E_h) is in a direction to firmly lock the dipoles in their present orientation. Those dipoles such as number 3 in Fig. 4 *A*, which are a little off the original orientation, will be forced back by the field. Thus the hyperpolarizing potential tends to align *all* dipoles parallel to one another. Hence the dipoles are really "hyperpolarized" and the junction barrier becomes "heightened." However, if a membrane is depolarized, that is, a negative potential to the exterior (Fig. 4 *B*), the field direction and the dipole orientations are parallel. Though in principle the field tends to turn dipoles to the antiparallel direction, the amount of work required is so large that

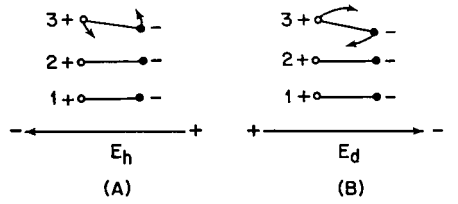

FIGURE 4 Dipole orientations and rotations by (*A*) a hyperpolarizing field E_h, and (*B*) a depolarizing field E_d.

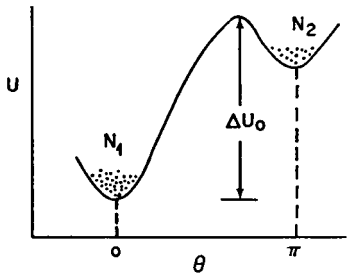

FIGURE 5 Energy states of dipoles vs. rotation.

most of the dipoles will remain in their original orientation. However, if the field strength exceeds a certain threshold, many dipoles will be turned around in accordance with the antiparallel rule. The situation is shown in Fig. 5. There are now dipoles populated in two states whose orientations differ by 180°. Since their dipole moments are opposite, the resultant barrier potential will be proportional to $(N_1 - N_2)$. The effect of the depolarizing field is to decrease N_1 and increase N_2. Hence the dipole layer is really "depolarized" and the barrier potential lowered. Since f_1 is proportional to the barrier potential, it is reduced by depolarization.

According to Wei's theory of nerve conduction (1966, 1967), a nerve axon is like a transistor from the standpoint of charge configuration, potential profile, and charged particle physics. However, the transistor gain can be realized only if the "emitter" (outer) junction barrier is lowered. The reason for this transistor action can be found in any electronics book. When a membrane is depolarized, the "emitter" junction is indeed lowered, and therefore one should expect gain, amplification, or regeneration in nerve conduction. In synaptic transmission, according to the present theory, the junction barrier is not lowered but instead an f_3 is introduced by the ACh dipole array. In fact, this f_3 would tend to hyperpolarize the junction dipoles at the surface of the postsynaptic membrane. In this case, the Na ions are driven by f_3 over the junction barrier and the membrane takes no transistor action on the entering Na. One should then expect nonregeneration in chemical synaptic transmission.

One of the most important functions performed by the central neurons is the integration of messages converging onto it from many other nerve cells. It is established that the integration takes place at synapses and at the subthreshold level of the membrane potential (Eccles, 1953, 1964; Katz, 1966). The question is how and exactly where the integration is done. According to our theory, the third force f_3 is

$$f_3 = Nf_z = \frac{Nnp}{2\epsilon R} \text{ (ev/m)} \tag{8}$$

where N is the number of layers of the ACh dipole array and n, the dipole density per unit area. The product Nn, has contributions from all the nerve terminals converging onto a central neuron.

Let

$$M = nN.$$

Then

$$M = \sum_k M_k , \tag{9}$$

where M_k is the contribution of ACh from the kth nerve cell. M_k can be positive or negative; the latter means annihilation of ACh by choline esterase. Now it should be clear how integration is performed. It must take place in the cleft rather than on the postsynaptic membrane. Even if each M_k is in the subthreshold range and unable to excite the postsynaptic membrane, the total M could be large enough to bring f_3 to the critical value such that the force condition is fulfilled. Then impulses will be initiated in response to this integrated action. The integration is *electrical* in nature in the sense that only the integration of f_3 is important rather than of M dipoles in random directions.

I wish to emphasize that equations 1, 8, and 9 possibly provide the physical basis and understanding of neuronal integration. This formalism might be helpful for qualitative analysis of integrative actions in the nerve system.

VI. FACILITATION AND SUPPRESSION

Experimental evidence has indicated that the release of ACh from nerve endings and/or their action on the postsynaptic membrane can be facilitated or suppressed by electrical interference or certain substances. Some of these factors and their effects are summarized as below.

(*a*) A hyperpolarizing pulse applied to a nerve terminal during the falling phase of its action potential can suppress transmitter release; a depolarizing pulse can potentiate the release (Katz and Miledi, 1967 *a*).

(*b*) If one lowers the normal Ca concentration and adds Mg to the muscle bath, the amount of ACh delivered by an impulse can be reduced to a very low level (Del Castillo and Engback, 1954; Katz and Miledi, 1965; Katz, 1966).

(*c*) The amount of transmitter released by each impulse increases with repetition (Katz, 1966).

(*d*) Within a certain range, lengthening the depolarizing pulse increases the rate of the transmitter release (Katz and Miledi, 1967 *c*).

In order to interpret these facts, we shall start from the interaction of a dipole with an electric field. The interaction energy is given by

$$U = p \cdot E \tag{10}$$

where p is the dipole moment, and E, the electric field. Since everybody likes to stay in the lowest possible energy state, equation 10 tells us that a dipole will move from a region of high field to that of low field. We shall employ this principle to consider the effects on the release of ACh.

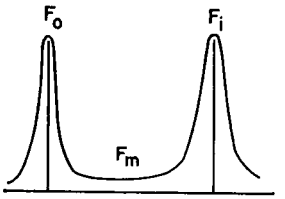

FIGURE 6 Field profile across a nerve membrane in the resting state.

In the quiescent state, ACh molecules are contained in vesicles whose front doors are closed by the presynaptic membrane (Eccles, 1964). Since ACh is considered an electric dipole, the door should mean an electric barrier of high field. The field profile across a membrane is shown in Fig. 6. This profile is simply a plot by differentiation of the potential profile shown in Fig. 1. Here F_o and F_i represent the fields at the outer and inner junctions while F_m, that in the membrane. It can be seen that F_o and F_i act as double doors, and F_m, something like a trap. Under these conditions, ACh cannot come out of the vesicles.

If the presynaptic membrane is depolarized, the outer and then the inner junction barriers will be sufficiently lowered and hence the double doors are open, releasing ACh. The release rate of ACh will depend in the number of vesicles being opened which in turn will be proportional to the area of the membrane under depolarization. The said area should be proportional to the duration of the depolarizing pulse if that duration does not exceed a certain limit. Beyond that limit, the membrane will be automatically repolarized (dipoles relaxing to the lower energy states, Fig. 5), and the double doors are closed.

When the presynaptic membrane is hyperpolarized, the junction barriers are heightened, and hence the double doors are more firmly closed or will be closing from the open position. Hence the release of ACh will be suppressed or slowed.

The effects of Ca^{++} and Mg^{++} on synaptic transmission can be understood from their physical properties. Firstly, both Ca^{++} and Mg^{++} carry a double electronic charge. Because of the stronger Coulombic interactions, these ions will be in closer contact with the dipole layer than the monovalent ions (Na^+, K^+). If Ca^{++} and Na^+ have equal bulk concentrations, their concentrations at the surface of the membrane could be as high as 100 to 1 (Brown and Danielli, 1964). Secondly, as *bare* ions, Ca^{++} is larger in size than Mg^{++} because the former has eight more outer electrons. Thus the positive nuclear charge of Mg^{++} is less screened by the outer electrons than that of Ca^{++}. In water, the less-screened nuclear charge of Mg^{++} will attract more polar water molecules. As a result, a hydrated Mg^{++} ion will be larger than a hydrated Ca^{++} ion, their radii being 4.65 A (Mg^{++}) and 3.21 A (Ca^{++}) (Stern and Amis, 1959) in contrast with the bare ion radii, 0.65 A (Mg^{++}) and 0.99 A (Ca^{++}) (Pauling, 1960). The much larger size of (Mg^{++}) ions (the parentheses indicate "hydrated") would make them difficult to enter the membrane. The (Ca^{++}) ions on the other hand could easily penetrate into the membrane not only because of their smaller size, but also because of their easy association with chloride ions to

form $CaCl_2$. Their antagonistic effects on synaptic transmission can now be explained on this basis.

The (Mg^{++}) ions in synapse would produce three effects. Firstly, their standing firm close to the presynaptic membrane would erect an additional barrier and thus block the pathway of ACh into the cleft. Secondly, if there are a sufficient number of (Mg^{++}) in the cleft, their huge size (4·65 A) would reduce the effective space of the cleft and prohibit the ACh dipole array from reaching the critical size to fulfill the force condition. Thirdly, the intervening (Mg^{++}) ions of double charge and large size could disarrange the ACh dipoles so that their collective concerted action becomes impossible. For the above reasons, the (Mg^{++}) ions can be regarded as, and are indeed, inhibitory agents in synaptic transmission.

The entry of (Ca^{++}) into the membrane could produce two effects. If they stay in the junction barrier, they would neutralize some of the negative charges and hence reduce the barrier height (Kavanau, 1965). This would lower F_o and F_i (Fig. 6), the double doors to the vesicle. Furthermore, the presence of calcium in the membrane, particularly in the form of $CaCl_2$, would lead to the "running out" of almost all of the water from between the lamellae, leaving a single-mixed lipid (Kavanau, 1965). This is known as the Palmer-Schmitt effect (1941). Since water is polar but lipid is nonpolar (its polar group is counted and included in the dipole layer), the running-out of water would lead to the lowering of the dielectric constant of the membrane. With the surface charge density unchanged, this would increase the electric field in the membrane. Therefore, the field profile across the presynaptic membrane would change from that shown in Fig. 6, to something like the contour of a shallow dish. In other words, the height of the double doors to the vesicles would be drastically reduced by the introduction of (Ca^{++}). Such an interpretation can account for a number of observations on the (Ca^{++}) effects on the release of ACh (Katz and Miledi, 1965, 1967 *a*, 1967 *b*, 1967 *c*). It should be understood that the entry of (Ca^{++}) into the membrane can be facilitated mostly by depolarizing the membrane (reducing the barrier). Hence either lengthening the depolarizing pulse or increasing its frequency would bring in more (Ca^{++}) and thus speed the rate of release of ACh as observed.

VII. PERMEABILITY CHANGES BY ACh

It has been observed that the permeability changes of the postsynaptic membrane to Na, K, and Cl caused by ACh vary from cell to cell. The current interpretations are that a membrane has channels or pores of at least two sizes whose walls may be positively charged, negatively charged, or neutral (Eccles, 1964, 1966). By assuming the opening of a suitable type (size and charge) of channels by the "transmitters," one would never fail to explain the permeability changes and the excitatory or inhibitory actions just as observed. The fundamental difficulties of this charged-channel model are:

(*a*) The physical existence of channels in a lipid membrane,

(*b*) What makes charge accumulate on the inner wall of the channel? and

(*c*) What mechanism opens the gate of a particular type channel?

Without indicating the answer to these questions, the model is too artificial to be of value.

Even the widely used concept of membrane permeability is questionable both academically and practically. Some definition could lead to a paradox (Johnson, Eyring, and Polissar, 1954). In the membrane theory and also in experiment, the permeability of a nerve membrane depends on almost everything: time, temperature, membrane potential, ion concentrations, membrane structure, etc. Thus to compare the results from two similar experiments is often difficult and sometimes confusing. And the question as to whose claim is more trustworthy may not be settled for years. For these reasons, many of the results on the permeability changes by the action of chemical transmitters must be received with care and caution.

We shall use the force condition to show that the permeability measured *in vitro* could be quite different from that *in vivo*. The minimum f_3 required in the force condition is

$$f_3 = -(f_1 + f_2) = -qE + kT\nabla \log (C_k) \tag{11}$$

where (C_k) is the concentration of the Kth ion species. This equation shows that the minimum f_3 for Na will be different from that for Cl not only because the (C_k)'s are different but also because the q's are of opposite sign. Suppose that in the natural environment (the external medium is rich in both Na and Cl), the minimum f_3 to push Na in is smaller than that to pull Cl out, i.e.,

$$f_3 \text{ (Na in)} < f_3 \text{ (Cl out)}.$$

If the actual f_3 produced by ACh is between the above two values, then one would observe influx of Na but not efflux of Cl. He would conclude that the membrane is depolarized by the influx of Na and the action of ACh is to open the Na channel. Another person may do the experiment by using an external solution which is completely free of Cl. In this case, f_2 for Cl will be outward and its magnitude would be very great. Then

$$f_3 \text{ [Cl out]} \ll f_3 \text{ [Na in]}.$$

That is, even an extremely weak field produced by ACh or perhaps some small stray field could draw a large efflux of Cl. The membrane is surely depolarized by the efflux of Cl and one could say that the ACh had opened the Cl channel. The interpretation in each case may be regarded as correct for its own experiment but it is certainly wrong for the *other* experiment.

In most experiments measuring the permeabilities of a biological membrane,

people used to prepare and change solutions of different compositions and they interpreted their results based on the *in vitro* conditions to mean the mechanisms *in vivo*. The force condition indicates that the interpretation could be entirely incorrect if one has not examined the two conditions (in vitro and in vivo) carefully. Therefore it is better not to propose any charged channel model without great deliberation on the real situation.

VIII. DISCUSSION

The theory as proposed in the above is a new attempt to understand the mechanism of chemical synaptic transmission from the standpoint of physical principles. Since this is a different approach from current thinking, questions could be raised concerning some vague and unmentioned points in the theory. In this regard, we should like to make some general remarks before touching upon specific questions.

The proposed theory is not a hypothesis in the usual sense of the word. A hypothesis is a new idea which finds no first principle available for its support at the time when it is proposed. Examples are "the earth is flat" hypothesis, the photon hypothesis of light, the spin hypothesis of electrons, etc. The theory which we proposed here is not of this kind. Instead, it is solidly based on the following well-known principles: (*a*) Newton's first and second laws of dynamics: these are restated and formulated into our force condition. Thus the force condition is a first principle which should be valid under any circumstance. (*b*) Molecular structure and electric dipole moment: According to Fröhlich (1958), "A non-polar molecule must have a point of symmetry defined in such a way that the distribution of charges along (or near) any straight line passing through it must be symmetrical with respect to this point." The molecular structure of ACh is such that no point of symmetry can be found, and therefore it must be a dipolar molecule. (*c*) Dipole orientation under the field: As Newton's second law dictates, any dipole (electric or magnetic) will reorient itself to the direction of the field unless it is barred by the other forces. Thus from a physicist's standpoint, the proposed theory is not a hypothesis but a derivative from the laws of dynamics and the law of symmetry. Based on these few principles calculations were made and interpretations were given which have all shown good agreement with the known facts in chemical synaptic transmission.

The first question one may ask is if there is any experimental evidence for the electric dipole moment of ACh. At present, we are not aware of any direct experiment which might have been done for this study. Theory and evidence usually do not come at the same time. This means that more work need be done along this line. The second question is how the specificity of ACh and the role of AChE are related to the theory. The theory predicts that if AChE is inactivated, then the ACh dipole array would stay forever in the cleft space. In that case, the force condition would be satisfied all the time, resulting in overstimulation of the nerve until it is exhausted. Nerve gases such as DFP (di-isopropyl-fluoro-phosphate) just bear this out (Liener, 1966).

The theory differs from the prevailing chemical hypothesis in that the exciting action on nerve by ACh should take place before not after ACh touches AChE on the postsynaptic membrane. The action of nerve gases indicates that the idea embodied in the present theory is more tenable for if the postsynaptic excitation were the result of chemical reaction between ACh and AChE in contact, then when the latter is inactivated by nerve gases, the postsynaptic membrane should be in the resting state, contrary to the observation. In section IV, we have explained the inhibitory action of ACh on the basis of its hydrolysis through the catalytic action of AChE. Thus the present theory has the specificity of ACh and the role of AChE well built-in and has made it clear how they perform their functions in excitatory and in inhibitory actions.

In section II, we have chosen the value for E_o, the barrier potential at the outer junction of the membrane to be 20 mv on which the calculations in section III are based. One may wonder if this value for E_o was chosen with reason. To arrive at this value, we have resorted to the following guide lines. First, in the resting state not many Na ions enter into the membrane. According to our force condition, this requires $f_1 \gg f_2$, the value for the latter has been given in section II. This means that E_o should be much larger than 5.7 mv. Second, since ions could have a thermal energy kT (about 25×10^{-3} ev at room temperature 25°C), the E_o must not be much lower than 25 mv, for otherwise, thermal excitation of the nerve even without stimulation would be excessive at 25°C, a fact not usually observed. However, at higher temperatures, man (and other animals as well) often tires easily and is sluggish to stimulation, an indication of frequent thermal agitation in the nerve. This, according to our thinking, implies that the E_o would be in the neighborhood of 25 mv but not much higher. Third, the ion distribution in energy is usually taken to be Boltzmann. This means that not all ions are in the ground state. Those ions in the higher energy states will see a barrier somewhat less than the full junction barrier. Guided by the above considerations, we thought that an effective value of 20 mv for E_o would not be unreasonable.

In this paper, we have not taken ion-ion interactions into consideration. There are several reasons for this neglect based on our experience in similar problems (electron-electron interaction or in general, many-body problems) encountered in solid-state physics. The first and the simplest reason is that the mathematical work involved if the problem is to be treated properly and honestly is insurmountable. To make the mathematics manageable, one has either to take drastic approximations or to propose some specific or even unrealistic models. The end result is uncertain and may hardly be worth the effort in view of the fact that the present liquid-state theory is not as fully developed as the solid-state theory. Second, the work and the achievement of Felix Bloch (1928), the founder of modern solid-state physics has produced a far-reaching influence on our thinking in the treatment of complex interactions. In his first and famous formulation of Bloch theorem and Bloch functions,

he *completely neglected* the seemingly very important Coulomb interactions between electrons. Though having been under severe attack for many decades, Bloch's simple theory, to the amazement of everyone has stood the test of time and has become firmly established in solid-state physics. But the puzzle remains: How can electron-electron interactions be neglected or why do their effects not show up? These questions were finally answered by Bohm and Pines in a series of papers published in the *Physical Review* from 1951 to 1953. Their main result is this. The Coulomb interaction between electrons could in principle lead to two kinds of interactions, the long-range and the short-range ones. The long-range interaction is a collective mode plasma oscillation which in metal cannot be excited under ordinary circumstances because of the high energy (10 ev) requirement. The short-range interaction is a screened interaction which in metal cannot go farther than the interatomic distance. Thus at long last, Bloch's neglect of Coulomb interaction between electrons is fully justified. What the implications of Bohm and Pines' results in ion-ion interactions in solutions may be is not too clear at this stage and will not be clear until a thorough study of the situation is made. This is beyond the scope of the present paper.

The force condition $f_1 + f_2 + f_3 > 0$, as we have stated before, is a first principle for nerve excitation. Here we wish to amplify its significance and also clarify this condition. First, since it is a first principle, it is valid under any circumstance. For example, in the normal case, this condition should be applicable to Na ions, while in the perfused case in which some other ion species is used as substitute for Na ions, the force condition would apply to that ion species. Second, the f_3 in the condition can be any external force other than the junction barrier force f_1 and the diffusional force f_2. If the f_3 can be known a priori or can be determined by some independent means, then the barrier potential could be determined by the minimum f_3 to fulfill the force condition, i.e. by the observation of the smallest f_3 to cause nerve excitation. Third, since the ion energy distribution is Boltzmann, they would not see one and the same barrier height, E_o. Rather, each ion will see an effective barrier height $E_o' = E_o - E_k$, where E_k is the internal energy of the ion in question. Strictly speaking, the f_1 should be determined by E_o' rather than by E_o. For excitation of the "all-or-none" type, ions in the ground state would be required to enter into the membrane because of their largest population. However, if the barrier height E_o is great, and the available f_3 is not sufficiently large, the ground-state ions may never get excited to the top of the barrier. In that case, only the ions in the higher states which see much reduced effective barrier could overcome the barrier with a small assistance of f_3. Since the populations in the higher states are much smaller than that in the ground state, the potential produced as the result of the entrance of these few energetic ions may not be of the "all-or-none" type but of the "subthreshold" or the "miniature" type. The occurrence of the miniature motor end-plate potential (epp) could at least partly be explained on this basis. Fourth, in the case of chemical synaptic transmission, the f_3 will depend on the available ACh concentration which

in turn will depend on the release rate of ACh from vesicles, the annihilating rate by AChE, the sizes of the cleft space, and the synaptic knob. If a drug could affect any of the above factors, it would produce an effect on synaptic transmission. What effect a particular drug will produce is beyond the scope of the present work.

Undoubtedly, there are many other questions which may not find answers from this theory simply because the needed principles and/or the required experimental data are not available. It is to be understood that every theory has its domain of validity and limitations. The theory which we propose is no exception to this rule.

IX. CONCLUSION

In this paper, we have employed one of the very fundamental concepts in physics ("force") and set up a simple principle (the "force condition") as the basis for nerve excitation. That ACh molecules are taken as electric dipoles is more a fact than a hypothesis. The ion-dipole and field-dipole interactions have been well discussed together with calculations in some detail to explain for the first time many important facts observed in chemical synaptic transmission. Our conclusion is that the nature of synaptic transmission is *essentially electrical*, be it mediated by electrical or chemical transmitters. Each type of transmitter is suitable and effective for the given conditions of a synapse. This would not only resolve the long-held controversy but also show a new approach, the physical approach, which may lead to a better understanding of one of the most important phenomena in the nerve system.

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