LETTERS TO THE EDITOR

Comment on Wei's Theory of Synaptic Conduction

Dear Sir:

Although the problem of axonal impulse conduction has received theoretical consideration (with some significant success beginning with the Hodgkin-Huxley [1952] work), synaptic impulse transport eludes the firm quantitative, but nevertheless, phenomenological description enjoyed by axonal phenomena. Serious theoretical investigation in this area is warranted. Wei (1968) attempted to begin to fill this void and his work constitutes a provocative first step. However, it seems to me his theory is based on a questionable model of the conduction process.

Specifically, Wei begins with the undisputed observation that a nerve impulse arriving at the presynaptic membrane induces the release of acetylcholine (ACh). He then suggests that the ACh molecules released into the synaptic cleft form a particular ordered dipolar array, apparently an electret. Wei associates with this ordered array an electric field of sufficient strength to induce the depolarization of the postsynaptic membrane system. The result, he claims, is the stimulation of a propagating action potential, a nerve impulse, in the next segment of the nerve system. The formation of the dipolar array appears to me to be a most questionable feature of his theory.

The validity of Wei's model mechanism is suspect for two related reasons: one thermodynamic and the other electrostatic. Thermodynamically the synaptic system itself appears to offer little in the way of ancillary structure or chemical and physical processes likely to support the mechanism devised by Wei. It seems improbable that the randomly released ACh could in any way spontaneously form an ordered state. The free energy change for the process would be positive and of large magnitude; i.e., nonspontaneous. This follows as the associated entropy change must be negative and of large magnitude. It further seems unlikely that by any mechanism the system enthalpy could be sufficiently negative to counter the large negative entropy change for the formation of such an ordered state. Such a situation apparently would involve the stimulation, by the arriving nerve impulse, of some large scale process of an appropriate character to result in an enthalpy change more negative than the $-T\Delta S$ contribution.

Assume, for the sake of argument, however, that an ordered dipolar array forms in the synaptic cleft. Wei suggests that the array has the configuration illustrated schematically in Fig. 1. Electrostatically, this configuration is extremely hard to prepare. In fact, systems in which such configurations exist have been prepared at considerable energetic expense. For example, the well-known formation of electrets requires one to cool dipolar substances, classically organic dipolar waxes, from melts to form solids in the presence of strong, externally applied electric fields (Gutmann, 1948).

Wei suggests that for the synaptic system the leading ACh molecules in the collection of molecules diffusing across the cleft arrive in the postsynaptic region and orient themselves in the "field" of the postsynaptic membrane. Succeeding molecules orient themselves on top of those already aligned to form the array illustrated in Fig. 1. However, such an orientation effect probably cannot propagate even though there are strong orientation forces at specific

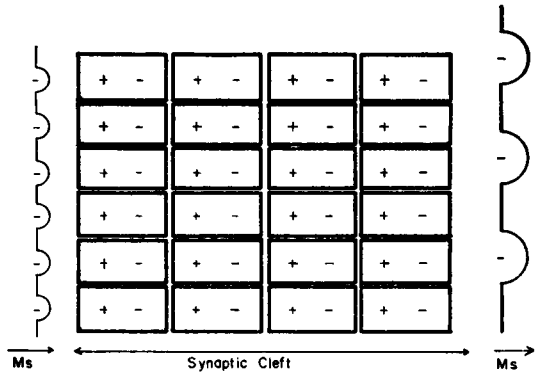

FIGURE 1

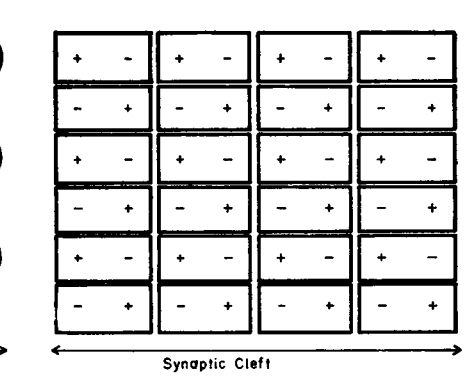

FIGURE 2

FIGURE 1 A schematic representation of Wei's (1968) mediation of an ACh dipolar array for synaptic conduction. In Wei's model the region here labeled Ms (i.e., membrane surface) includes a layer of sodium cations (Na^+), although there is no peremptory reason to believe a uniform layer should exist even for double layer formation.

FIGURE 2 A schematic representation of a more likely (but still not probable) configuration for a dipolar array. Here we reasonably assume the spacing of the anionic sites in the membrane surface is sufficient to accomodate the configuration of ACh molecules shown. Indeed, between the anionic membrane sites it is likely there is adsorbed mobile cation (such as Na^+, K^+, and Ca^{++}) which enhances the reasonableness of this representation over Wei's.

sites on the postsynaptic membrane. There is in the synaptic cleft considerable water, as solvent, as well as numerous other ionic and neutral molecular species. Somehow all this additional material must be displaced in the process of the formation of the array. Moreover, the vesicles on the postsynaptic membrane, at which excitatory action takes place, are not uniformly distributed on a regular, smooth surface. The epitaxial-like growth of the ordered array seems even less likely in view of this.

If we continue to hold to the hypothesis that an ordered array forms spontaneously, then in view of the electrostatic considerations which apply in the absence of any strong external electric field, the system assumes the electrostatically neutral, inert configuration illustrated in Fig. 2. This is the case even in the presence of some specific attachment of ACh at the postsynaptic membrane.

Further, on the basis of statistical mechanical arguments we conclude that neither ordered array is possible in the synaptic cleft. This follows from the fact that the release of ACh into the synaptic cleft is followed by the normal diffusion of this substance within the cleft region. Even though this region is small, the transport processes involved are necessarily brownian, i.e., stochastic. Moreover, the energies necessary to dispel all competing substances plus the energy necessary to form the energetically less likely ordered array in Fig. 1 as opposed to the more likely array of Fig. 2 together cast doubt as to the likelihood a dipolar array can exist in any form. Due to statistical-mechanical, thermal fluctuations the motions of the individual ACh molecules are and remain random. This enforced randomness is universal except possibly in the region just neighboring the postsynaptic membrane where double layer effects are important. The existence of thermodynamic fluctuations alone seems to militate against the spontaneous formation of any conceivable type of ordered array in the synaptic cleft in the absence of any significant, strong, and long-ranged external fields or additional metabolic processes.

Should the formation of a solid array be a type of "crystallization" phenomenon, which is the only possible way such an ordered array could form, then the surroundings must be capable of removing a considerable quantity of energy, the quantity alluded to in the preceding paragraph. On the basis of all the arguments listed above it appears that the basic model Wei proposed will require some compelling, direct experimental substantiation before it can be accepted.

I must express an additional objection to Wei's model system. An ancillary hypothesis to Wei's original model states that the dipolar character of the ACh molecule is primarily important in the synaptic activation mechanism. This hypothesis is misleading. ACh is a quaternary amine. Moreover, it contains an ester group. It is more likely, however, that it is the quaternary amine part of the molecule which is important in the activation process. As is known from equilibrium as well as kinetic studies of ionic association on exchange polymers, quaternary amine groups strongly associate with anionic sites (Helfferich, 1962). Certainly, this strongly ionized character of the ACh quaternary amine group will overrule any much weaker dipolar character the molecule possesses. As a separate example illustrating this behavior of the quaternary amine group, consider the relative behavior of morphine and quaternized morphine (Triggle, 1965). Morphine, as is known, acts to block nerve impulse conduction at the synaptic level, although the blockage is not complete. Upon quaternization, however, the quaternized morphine acts in a manner very similar to tubocurarine salts (Triggle, 1965). Tubocurare and quaternized amines are generally believed to block sites on the postsynaptic membrane normally available to ACh.

If Wei's model appears not to stand the test of physical reasonableness, what then is to replace it?

Without attempting the formal development of a theory of synaptic conduction here, I am led nevertheless to suggest that the action of ACh in effecting the synaptic transmission of nerve impulses takes place entirely at sites on the postsynaptic membrane surface. A reasonable mechanism requires the formation of local, nonequilibrium double layer distributions brought about by the rapid accumulation of ACh at postsynaptic sites, i.e., the vesicles. Double layer potentials are certainly of magnitude sufficiently great to effect changes, and these changes can be characterized by membrane depolarization.

It is known that ACh-esterase deactivates the action of ACh itself. There is a possible, reasonable explanation of this. The de-esterification process produces acetate anion. This anion can compete with membrane anionic sites for association with ACh as well as choline. The salts of weak acids and bases are not completely ionized in aqueous ionic solutions, i.e., a significant quantity of the salt is in an associated form. Choline as well as ACh can form such weak acid-base salts. The result of the action of acetate anion at the membrane surface would be the "regeneration" of anionic attachment sites. This concept is strengthened by the observation that (*a*) large quantities of ACh can depolarize bare segments of unmyelinated axons (Feldberg et al., 1940), yet insufficient quantities of the esterase are present to enter into the process. Moreover, the structure of the bare axon bears no direct resemblance to the synaptic region. Further, (*b*) quaternary amine salts also depolarize the axon membrane system both along bare axon segments and in the synapse (Triggle, 1965). In the case of the depolarization of excitable membranes by quaternary amines, the initial depolarization is followed by inactivation of the membrane due to the continued presence of these quaternary amine cationic species.

In conclusion, then, I would urge Wei's model of the synaptic transmission process be subjected to careful questioning as to its validity. On the basis of considerable experimental evidence there now seem to be compelling reasons to suspect his model is not tenable. I would also urge the de-emphasis of the investigation of the dipolar properties of ACh as related in

any directly significant way to the synaptic conduction process. This is not to say that dipolar properties of ACh may not be important. Certainly, they may have some effect on the formation of nonequilibrium double layer structures. In place of Wei's model I suggest the reasonableness of physical models based on the consideration of the formation of nonequilibrium double layer structures around the anionic attachment regions (at the vesicles) in the postsynaptic portions of the cleft region. I would also suggest that it is the ionic character of ACh salts, namely the chloride and hydroxide, which figures most importantly in these synaptic conduction processes as opposed to Wei's suggestion of the importance of the dipolar effects.

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P. P. SCHMIDT
Department of Chemistry
Oakland University
Rochester, Michigan 48063
and
William Ramsay and Ralph Forster Laboratories
University College
London, W.C. 1, England