to attach and spread on a substrate. Because most nontransformed animal cells must attach to a substrate before they can grow (*9*, *15*), the failure to culture hydra cells may have resulted in part from the use of unsuitable substrates. The use of mesoglea as a substrate for isolated hydra cells might represent, therefore, a major step toward their successful culture.

Mesoglea is probably not a prime factor in causing graft rejection (Table 2). For example, cells from *H. attenuata* stick and spread on mesoglea produced by *H. viridis* epithelial cells but will not form cell junctions with the cells themselves; grafts between portions of individuals of the two species separate in situ (*16*). Hence the cause of graft rejection between different hydra species may lie not with cell-mesogleal interactions but rather with cell-cell interactions.

In contrast to hydra cells, mammalian and *Drosophila* cells did not find hydra mesoglea suitable for attachment and instead tended to stay rounded when contacting the mesoglea. When the dish was agitated, the cells rolled onto the surrounding plastic and spread there (Fig. 1, d and e), as though avoiding the mesoglea. Just as the mammalian cells did not attach well to the mesoglea, hydra cells did not attach and spread on substrates suitable for most mammalian cells (Table 1). The amphibian cells, on the other hand, stuck and spread well on both mesoglea and plastic.

Should we expect mammalian cells to attach to an extracellular matrix produced by an animal as primitive as the hydra? Animals from the phylum Cnidaria were the first to evolve epithelia, and the mesoglea may represent an early progenitor of vertebrate basement membranes. Hydra mesoglea, for example, is homologous to vertebrate basement membrane in a number of ways (*3*, *5*): it contains a collagen-like component, as evidenced by its amino acid composition and the presence of a glucosylgalactose disaccharide unit; there is an increase in the amounts of hydroxylysine and neutral sugars relative to vertebrate collagen; and it is continually secreted by the overlying epithelial layers. If the vertebrate basement membrane did evolve from the mesoglea, we may be able to use the latter as a model to determine which components or arrangement of components are recognized by epithelial cells.

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9 September 1980

Giant Synaptic Potential Hypothesis for Epileptiform Activity

Abstract. *According to one hypothesis, the paroxysmal depolarizing shift observed in the penicillin model of epilepsy results from a giant excitatory postsynaptic potential. This hypothesis has recently been questioned, primarily because it has never been subjected to rigorous experimental examination. Four quantitative predictions were derived from this hypothesis and tested in CA3 pyramidal neurons of the hippocampus. The four critical predictions concern the behavior of the paroxysmal depolarizing shift under current- and voltage-clamp conditions as a function of membrane potential. The experiments confirmed all four predictions.*

One of the most extensively studied animal models of epilepsy is provided by the topical application of penicillin to the mammalian cortex (*1–4*). Periodic interictal epileptiform discharges are recorded in the electrocorticogram after this treatment. The intracellular correlate of these interictal events consists of a sudden 20- to 50-mV depolarization, which lasts for 50 to 100 msec. This sudden depolarization was originally termed a paroxysmal depolarization shift (PDS) by Matsumoto and Ajmone-Marsan (*1*). The mechanism underlying the PDS has been extensively studied because it is believed to bear directly on our understanding of the cellular basis of epilepsy. The most influential hypothesis regarding the mechanism of PDS generation is that the PDS is a giant compound excitatory postsynaptic potential (EPSP) (*2*). According to this hypothesis, penicillin increases the efficacy or recruitment of recurrent excitatory circuits, which in turn are responsible for the giant EPSP. The giant EPSP hypothesis does not specify the mechanism whereby penicillin has this effect on recurrent excitation, but it might be imagined to occur by a reduction in feed-forward or recurrent inhibition, since we have recently demonstrated that penicillin blocks all detectable inhibitory miniature synaptic potentials in hippocampal neurons (*5*).

The usual evidence for and against the giant EPSP hypothesis is indirect and unconvincing (*2–4*). We therefore derived and tested certain quantitative predictions of the central tenet of this hypothesis; namely, that the PDS is a very large EPSP. The first prediction is that the frequency or probability of occurrence of PDS's should be unaltered by changes in the membrane potential. By contrast, if the PDS were purely an intrinsic regenerative membrane event, its frequency or probability of occurrence should be affected by changing the membrane potential. Second, if the PDS is a large EPSP, its amplitude should be a monotonically decreasing function of the membrane potential in accordance with the decrease in synaptic driving force. Third, if the PDS is synaptic in origin, it should be possible to reverse its polarity by depolarizing the cell beyond the synaptic equilibrium potential. Fourth, if the PDS is a *giant* EPSP, then the synaptic currents underlying the PDS should be large relative to those accompanying normal spontaneously occurring EPSP's. A set of similar quantitative predictions can be derived for the assumption that the PDS is a large EPSP caused by a decrease in conductance (*6*).

Until recently, it was not possible to evaluate these predictions experimentally in mammalian cortical neurons, since an adequate test requires changing the membrane potential to extreme voltages under both current- and voltage-clamp conditions. We have developed methods

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that now make it possible to explore these predictions under current clamp at membrane potentials ranging from −150 to +65 mV and under voltage clamp from −100 to 0 mV. We used the hippocampal slice preparation because it offers a number of experimental advantages and because it has been used in much of the recent research on penicillin-induced epileptiform activity. We have restricted our analysis to pyramidal neurons of the CA3 region of the hippocampus because (i) most of the in vivo work on experimental epilepsy in the hippocampus has been done on CA3 neurons (*2*); (ii) CA3 neurons appear to be the pacemakers for the spontaneous interictal events (*7*); and (iii) much is known about the synaptic microphysiology and active and passive membrane properties of these cells (*5, 8-11*).

Hippocampal slices, prepared from guinea pigs (300 to 500 g), were maintained in vitro (*8-10*) and continuously perfused with an oxygenated saline of the usual ionic composition, to which sodium penicillin G (2000 I.U., 3.4 m*M*) was added in some cases. In order to inject the required amount of current into these cells (up to 10 nA) while accurately recording the membrane potential, we used a single-electrode clamp circuit (*9, 10*) in conjunction with relatively low impedance (30 to 50 megohms) 2*M* Cs_2SO_4-filled glass microelectrodes especially designed and tested for this purpose. Injection of Cs^+ from these pipettes typically doubled or tripled the resting input resistance, possibly by blocking resting and active potassium conductances (*10*). This enabled us to alter the membrane potential by passing less current and to minimize changes in input resistance resulting from changes in voltage. The recording electrodes were visually guided to the stratum pyramidale layer of the CA3 region, which consists predominantly of the cell bodies of pyramidal neurons.

Normally, CA3 neurons display spontaneous burst firing and respond to a depolarizing current with a brief burst of action potentials (*10, 11*). The frequency of these bursts is highly voltage-dependent. Small hyperpolarizing currents cause a decrease in their frequency, and a 10- to 20-mV hyperpolarization of the membrane potential can prevent them completely. In our experiments, spontaneous extracellular field potentials were never observed before penicillin was added to the bathing solution, and thus the normal burst firing of CA3 neurons is probably asynchronous. However, periodic field potentials were recorded extracellularly from all healthy slices (Fig. 1A, trace 3) within minutes after penicillin perfusion was begun.

Intracellular recordings from CA3 neurons in penicillin-treated slices displayed sudden spontaneous periodic depolarizations of the membrane potential (Fig. 1A, trace 1), which we call PDS's because of their similarity to those first described in vivo (*1, 2*). The intracellular PDS was always in synchrony with the extracellular field. Each PDS was followed by a long-lasting increase in input conductance (as measured by trains of brief constant-current pulses) of approximately 500 msec duration. This increase in input conductance usually resulted in a hyperpolarization (Fig. 1A, trace 1), which increased in amplitude when the cell was depolarized. In this report, we distinguish between the early events responsible for the PDS and later events associated with the post-PDS increase in

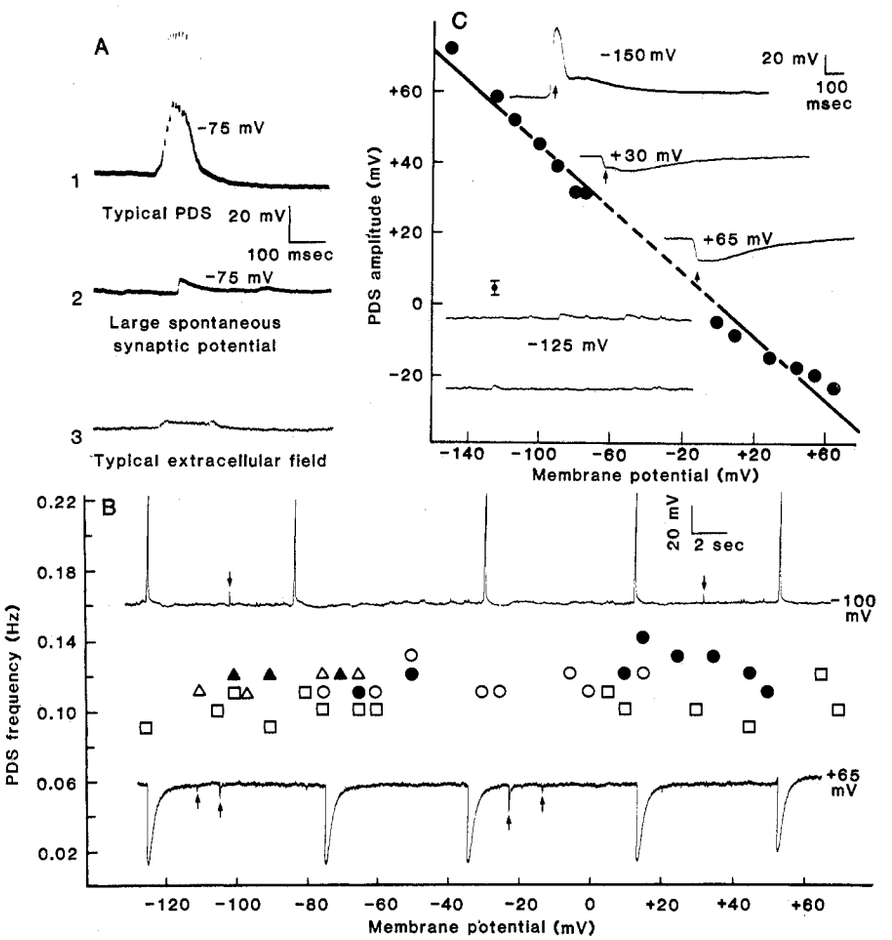

Fig. 1. Current-clamp experiments on the influence of membrane potential on the frequency, amplitude, and polarity of PDS's. (A) Trace 1 illustrates a typical PDS at −75 mV. The action potentials superimposed on the top of the slow depolarization have been clipped. This PDS was immediately preceded by a large, spontaneous EPSP, shown in trace 2. A typical spontaneous positive-going field recorded when the intracellular electrode was removed from the cell soma is shown in trace 3. The brief field potentials associated with multiple population spikes from the CA3 region are superimposed on this recording but are difficult to see at this gain setting and sweep speed. This record, however, demonstrates that the extracellular field potential associated with the PDS is positive-going and cannot account for the reversal of the PDS shown in (C) [see (*3*)]. (B) Influence of membrane potential on the frequency of interictal events in several cells, indicated by different symbols. Open and closed symbols represent slices from different animals. Chart records of the interictal event are illustrated at membrane potentials of −100 and +65 mV. The amplitude of these events is attenuated by the frequency response (100 Hz) of the penwriter. At +65 mV, the PDS is reversed in polarity, as are the spontaneous EPSP's, which are indicated by the arrows. The apparent increase in PDS duration at +65 mV is due to the outward currents (Fig. 2B) associated with the post-PDS conductance increase. (C) Influence of membrane potential on the amplitude and polarity of the PDS, taken from the same cell illustrated in (A). The influence of membrane potential on the PDS amplitude and polarity is illustrated in the upper part of the figure at three different membrane potentials. The amplitude of the PDS was measured at the time indicated by the arrow. Note that the post-PDS increase in conductance is associated with a depolarization at −150 mV and a hyperpolarization at more depolarized potentials. Examples of spontaneous EPSP's recorded from another cell are illustrated in the lower part of this figure. The cell was bathed in normal saline and hyperpolarized to −125 mV.The mean amplitude (± standard deviation) of a representative series of 30 of these EPSP's is plotted above the traces.

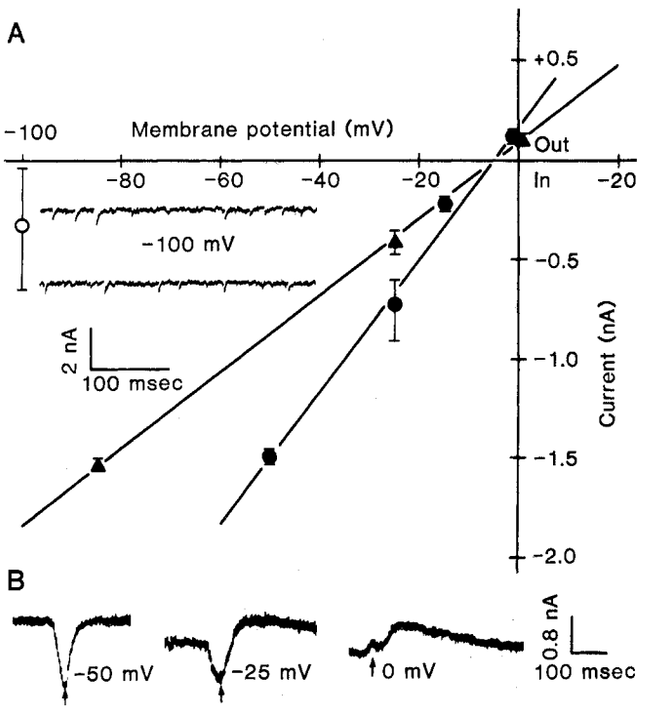

Fig. 2. Voltage-clamp analysis of the currents underlying the PDS as a function of membrane potential. Data points represent the mean of two or more successive clamp records. (A) Closed symbols illustrate the voltage dependence of the PDS currents in two cells. In both cells, the apparent reversal potential is about −5 mV. Examples of spontaneous synaptic currents recorded at −100 mV are illustrated in the lower left, and their mean amplitude and standard deviation are plotted to the left of the traces (open circle). (B) The waveform of the currents associated with the PDS is shown at three different holding potentials. Measurements of the early currents underlying the PDS were taken at the points indicated by the arrows. Note that at −25 mV the early currents are inward, and the late currents, which correspond in time to the post-PDS increases in conductance, are outward. At 0 mV, both the early and late currents are outward.

conductance, since our experiments deal specifically with the giant EPSP hypothesis for the early events underlying the PDS.

The first prediction from the giant EPSP hypothesis is that the probability of occurrence or frequency of the PDS should be unaffected by the membrane potential. In Fig. 1B, the frequency of spontaneously occurring PDS's is plotted against membrane potential for four cells. As the membrane potential was changed from −150 to +65 mV, a remarkably constant frequency of PDS occurrence was observed, even from cells in different slices. The average frequency was 0.11 Hz. Chart records of the PDS from one cell at −100 mV and at +65 mV (Fig. 1B, insets) show that at +65 mV, the PDS is always negative-going, but occurs at nearly the same frequency as at −100 mV. The arrows point to spontaneous EPSP's (which are reversed at +65 mV).

The second prediction is that the amplitude of the PDS should be a monotonically decreasing function of the membrane potential. A plot of the PDS amplitude versus membrane potential in the range −150 to +65 mV (Fig. 1C) confirms the prediction. Since no correction was made for nonlinear summation (*12*) or changes in input resistance as a function of voltage, it is not surprising that the data show some deviation from strict linearity. No current-clamp measurements were made in the region between −75 and 0 mV because Johnston *et al.* (*10*) showed that a voltage-dependent, slow, inward current, probably carried by calcium ions, is activated at membrane potentials above (more positive than) −50 mV. We wanted to minimize the concomitant activation by the PDS of this inward current. At membrane potentials above −75 mV the PDS would depolarize the cell above −50 mV (Fig. 1C). We did, however, explore this range of membrane potentials, between −75 and 0 mV, under voltage-clamp conditions (see Fig. 2A), as described below.

The third prediction from the giant EPSP hypothesis is that it should be possible to reverse the polarity of the PDS when the subsynaptic membrane is depolarized beyond the synaptic equilibrium potential (*6*). This prediction is confirmed in Fig. 1, B and C. The PDS is entirely hyperpolarizing at membrane potentials more positive than 0 mV. The reversal potential appears to lie in the range −10 to +10 mV. However, the actual value cannot be determined accurately from the data presented in Fig. 1C, since we have not taken into account such factors as nonlinear summation and changes in the input resistance, and, as mentioned above, we were unable to collect uncontaminated data in current clamp at potentials between −75 and 0 mV.

The fourth prediction is that the synaptic currents associated with the PDS should be large in comparison to those responsible for spontaneously occurring EPSP's. For this prediction, voltage clamping is required. Although we have shown that hippocampal neurons are electrically compact (*13*), we cannot claim to have a perfect space clamp of the subsynaptic region. Nevertheless, the use of the soma voltage clamp should substantially reduce the effect of voltage-dependent changes in membrane conductance. In Fig. 2A, a current-voltage (*I-V*) plot is shown for the early currents underlying the PDS in two cells. Three voltage-clamp records are shown at −50, −25, and 0 mV in Fig. 2B. The *I-V* curve suggests an apparent reversal potential (*14*) of −5 mV for the currents underlying the PDS in both cells. The conductance increase in these two cells is 19 and 32 nS. For comparison, the mean and standard deviation of the amplitude of spontaneously occurring synaptic currents from a cell in normal saline is also shown in Fig. 2A. This cell was illustrated because it displayed relatively large spontaneous synaptic currents. In order to increase the amplitude of these currents so that we could resolve them more clearly above the background noise levels, the cell was hyperpolarized to −100 mV. Even at this hyperpolarized potential, the currents averaged only 0.3 nA, and this may be an overestimate of the mean since the smallest currents are probably buried in the background noise. Although it was not possible to clamp the PDS's at −100 mV in the two cells illustrated in Fig. 2A, if we extrapolate the *I-V* relationship to this potential, we can estimate that the presumed synaptic currents underlying these PDS's would be at least six times larger than those associated with spontaneously occurring EPSP's in normal saline. The amplitude of the PDS is compared to the amplitude of spontaneously occurring EPSP's in Fig. 1C.

Recently, the giant EPSP hypothesis was challenged, partly because of a lack of supporting evidence (*3*, *4*). The alternative hypothesis suggested in these reviews (*3*, *4*) is that the PDS is simply due to an endogenous regenerative membrane current which, in the absence of synaptic inhibition, is triggered by EPSP's of normal amplitude. Although the giant EPSP hypothesis can more economically account for our data (*15*), we note that one of its provisions, which we have not tested, is that the giant EPSP is due to recurrent excitation.

Our experiments have greatly strengthened the giant EPSP hypothesis by confirming four key quantitative pre-

dictions of its central tenet. We are not suggesting that the membranes of these cells lack the intrinsic capability of bursting; it is well known that they can burst, even in the absence of synaptic input. The intrinsic, voltage-dependent, slow, inward current described by Johnston *et al.* (*10*) may add to the depolarization produced by the postulated summated EPSP, a possibility which can now be explored further by voltage-clamp experiments.

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2 June 1980; revised 28 August 1980

Minor Planet Satellites

Reitsema (*1*) challenged the reliability of the observational data from which Binzel and I (*2*) concluded that satellites of minor planets are numerous and commonplace. I offer the following replies to the questions he raised.

The confirmed satellite of 532 Herculina was detected by an experienced occultation observer, J. McMahon, along with the occultation by Herculina itself and five other occultation events. Reitsema refers to the five unconfirmed visual observations as "apparently spurious." Yet McMahon reported all six secondary disappearances and reappearances as having the same sharp, distinctively occultation appearance as the Herculina event itself. With nearly four magnitudes of light loss involved, events caused by atmospheric turbulence were out of the question. His timings of the Herculina event, the ninth and tenth timings in his series of 14, agreed well with those of other observers. And the only secondary event long enough to have had a counterpart in the Lowell lightcurve did appear there at exactly the location McMahon's timings indicated. I do not know of any valid basis for sugesting that McMahon's other events are spurious.

Reitsema gives low credence to visual occultation observations, but in my experience such observations are highly reliable. It is one of my major duties at the Naval Observatory to reduce and analyze both visual and photoelectric lunar occultation timings, which are currently made at the rate of 10,000 per year. I have analyzed 140,000 such timings. Less than 10 percent of these observations need to be rejected, and most of those because of positional errors in the star catalog used for reductions, errors in the station coordinates, or problems beyond the control of the observer. Grazing occultations are particularly relevant to the asteroid situation, since they are observed mainly by amateurs, at remote field locations, with portable telescopes, under adverse climatic and environmental conditions, and involve the possibility of a miss or an unknown number of disappearances and reappearances over about a 5-minute interval. The important difference from asteroids is that each observer's report can be checked for consistency with those of the observers on either side of him, typically 500 to 1000 feet (0.15 to 0.3 km) away, and in this way again it has been established that typical observations, including a sample of inexperienced observers and averaged over all manner of telescopes and field conditions, are nearly 90 percent reliable. Secondary events, which seem to be the rule with asteroids, simply do not occur for lunar occultations, except in poor seeing with a star close to the threshold of visibility. In the latter situation the observer is aware that the flickering is probably atmospheric from its extremely rapid and random nature. There is no reason I know of why the 90 percent reliability of visual lunar occultation timings should not also be valid for minor planet occultations. However, the minor planet occultations have been more carefully investigated than most, and I expect the reliability of the reported secondary events to be higher than average. The observations listed as "probable" in (*2*) would be particularly difficult to explain in any other way.

Photoelectric recordings of occultations can be spurious for different reasons, but there are not many ways to get spurious photoelectric events without realizing it at the time. The photoelectric method provides numerous checks, the strongest of which is on the occulted light level, which in general is neither the background level nor the zero level. For 18 Melpomene it was easy for photoelectric observers to distinguish the star's light, the light from Melpomene, their combined light, the empty field light, and the zero light levels. Clouds have never in my experience produced an intensity change so rapid as to be confused with a true occultation, and in any event the light level is not usually the same for clouds and occultations.

Further, there is no real possibility of confusing drifts out of aperture with occultations. Even without sidereal drive, the seeing disks of the stars would produce slower light changes than a true occultation (assuming one has enough time resolution to see the difference). Under ordinary circumstances, it takes 10 seconds or more for seeing disks of stars to enter or leave a diaphragm. The two spurious events mentioned by Reitsema for the 13 Egeria occultation would have to be shown in a figure that displays some of the lightcurve before their misinterpretation as occultations could be judged as näive or realistic.

The last point concerns our conclusion in (*2*) that minor satellites are numerous and commonplace. After the 6 Hebe event, I argued that the secondary observation could not have been a satellite because the odds were great against one observer seeing Hebe's only satellite by chance, and even greater against his having looked at the only minor planet with a satellite. I did not accept the con-

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