

Fig. 3. Quantal release of transmitter in a giant synapse of the stellate ganglion. Nerve impulse activity had been abolished by tetrodotoxin ( $3 \times 10^{-7}$  g/ml.). The presynaptic fibre is depolarized with electric pulses applied through an intracellular electrode. The records are samples of the postsynaptic potentials (lower traces) from a series obtained during stimulation with constant intensity. In some trials the pulse evokes a miniature synaptic potential after variable delay. Below: diagram of experiment. The presynaptic fibre is shown with most of its branches. Only the giant synapses on the axons which run on the fourth and last stellar nerves are shown. The recording electrode is inside the fourth giant axon. The diameter of this axon, measured 3 mm away from the ganglion, was  $65\mu$ .

observation that there is mutual electric coupling between different postsynaptic giant axons, though with great attenuation; this is possibly due to axon branches of the nerve cells supplying more than one of the giant fibres.

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### Input-Output Relation of a Single Synapse

THE rate of secretion of a transmitter substance from a nerve terminal is known to be controlled by the membrane potential of the terminal. This has been shown, for example, at the neuromuscular junction by registering the frequency of miniature end-plate potentials when electric currents are sent through the motor nerve endings<sup>1</sup>. More recently, the use of tetrodotoxin<sup>2</sup> has made it possible to eliminate initiation of impulses on either side of the junction, and so to study the graded relation between electrical "input" and "output" of a synapse, undisturbed by any regenerative potential change. With this method it has been found that brief pulses of depolarization, locally imposed on a motor nerve ending, elicit graded end-plate potentials the size of which can reach or even surpass that normally produced by a nerve impulse<sup>3</sup>. To obtain additional information, it was necessary to use a preparation in which direct measurements of the membrane potential can be made on both sides of the junction. The so-called giant synapse of the squid<sup>4</sup> is suitable for this purpose: it is possible to insert microelectrodes in the pre- as well as post-synaptic nerve fibres of this preparation, close to their region of synaptic contact, and there is now good evidence that chemical transmission operates at this synapse<sup>5</sup>. The experiments reported here

were made on the stellate ganglion of the squid *Loligo vulgaris* at the Naples Zoological Station. Similar work carried out at Woods Hole has been reported by Bloedel, Gage, Llinás and Quastel<sup>6</sup>.

The main results, illustrated in Figs. 1 and 2, were obtained by placing two microelectrodes into the presynaptic fibre (one for passing current, and the other for recording membrane potential), and one recording electrode into the postsynaptic axon. The region of synaptic contact extended over a length of 0.5–1 mm, and the siting of the presynaptic recording electrode in relation to this synaptic region was of great importance. Several arrangements were tried, sometimes in the same preparation: reversing stimulation and recording within the presynaptic fibre, reinserting one or both electrodes at different distances and repeating observations. It became clear that the local depolarization produced by a brief pulse is

attenuated appreciably in the terminal branch, especially when the depolarization is intense. If both "pre"-electrodes are placed upstream, as in the experiment of Fig. 1, with the recording probe intermediate between the stimulating electrode and the synapse, then it is at least certain that the recorded presynaptic depolarization exceeds that occurring at the points of synaptic activity farther downstream. The "true" curve must, therefore, lie somewhat to the left of those shown in Fig. 1, B and C.

Fig. 2 shows another curve. This was obtained when the "pre"-recording electrode had been placed farther downstream, into the region of synaptic contact itself. In addition the preparation had become more fully equilibrated in a solution rich in calcium, which may account for some of the differences between Figs. 1 and 2.

To determine the initial "foot" of these curves, a higher amplification was used for the postsynaptic response. In

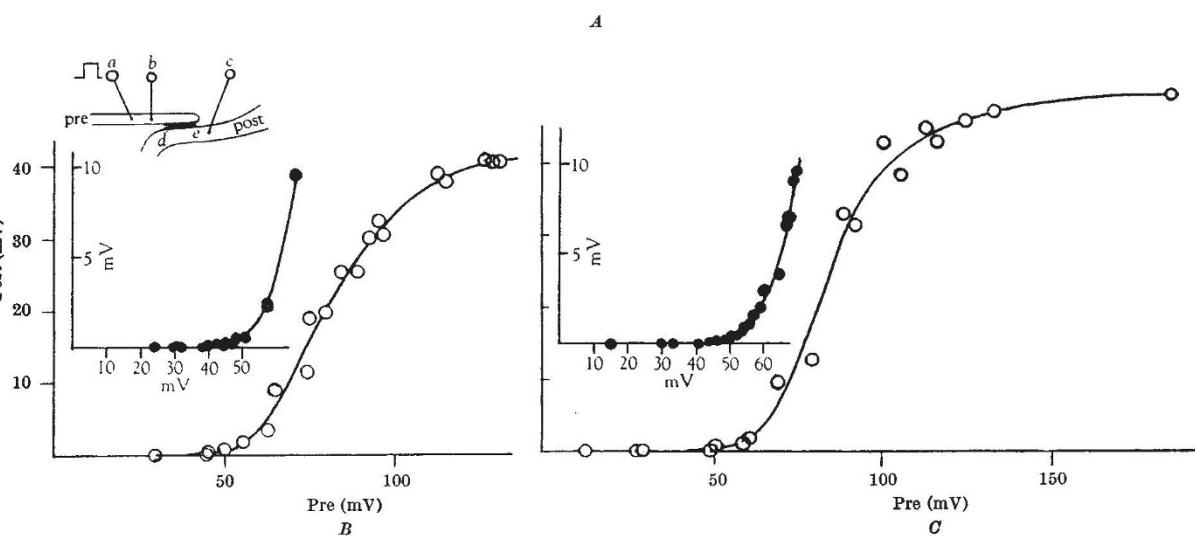
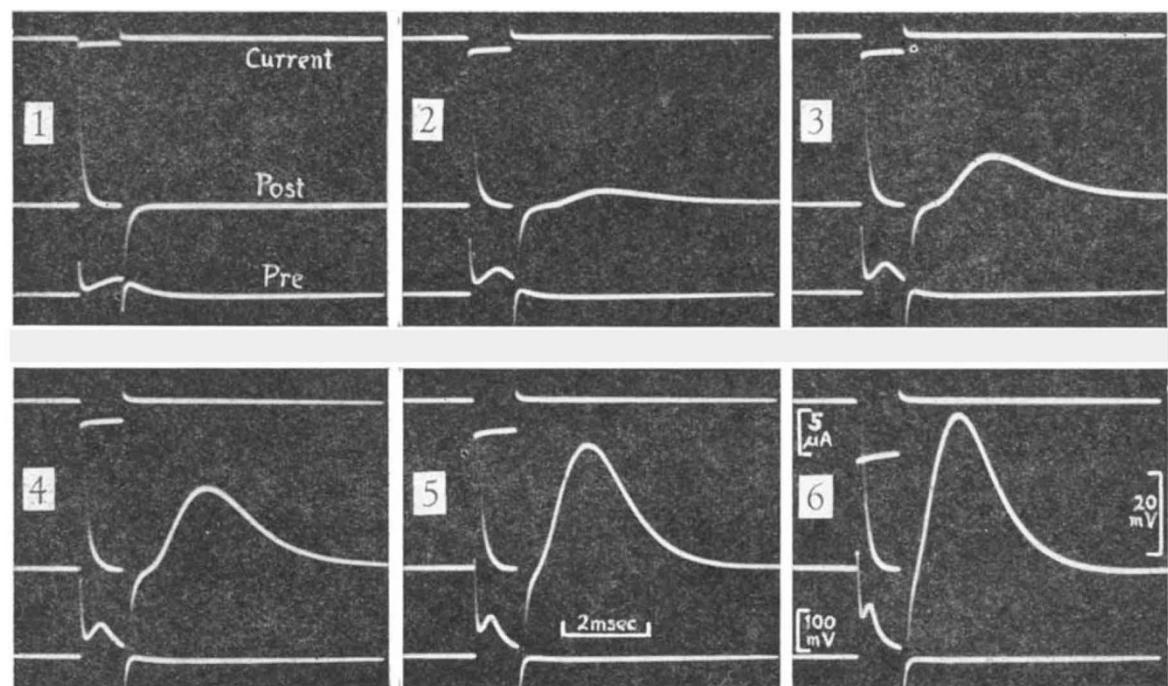


Fig. 1. Arrangement of electrodes is shown in upper diagram of Fig. 1B. Pre: presynaptic terminal. Post: postsynaptic giant axon. Length of synaptic contact  $d-e$ : 0.8 mm;  $a$ : Current-passing electrode;  $b$ : "pre"-recording electrode;  $c$ : "post"-recording electrode. Distances:  $a-d$ , 0.7 mm;  $b-d$ , 0.35 mm;  $e-d$ , 0.3 mm. Fig. 1B, Sample recordings. Fig. 1C, Input-output relation obtained with 1 msec current pulses. Abscissa, presynaptic depolarization; ordinate, postsynaptic response. Fig. 1D, Inset, initial part of curve in greater detail. Fig. 1E, From same synapse after external calcium concentration had been raised from 11 to 58 mM. Temperature in these experiments was about 10°C; and concentration of tetrodotoxin  $2 \times 10^{-7}$  g/ml.

several cases, considerable amplitude fluctuations were seen in the postsynaptic potential when operating in this "threshold" region of the synaptic transfer curve<sup>7</sup>. These fluctuations are indicative of a quantal release mechanism similar to that occurring at the neuromuscular junction<sup>8</sup>.

One of the limitations of the present method is the lack of a fully adequate presynaptic voltage control, in spite of the use of tetrodotoxin. Strong maintained current pulses produce only a transient peak of depolarization in the pre-fibre, because the gradual rise of potassium conductance ("delayed rectification") is not abolished by the toxin<sup>2,9</sup>. Thus, associated with the changes in amplitude (which are presented in the graphs) there were also changes in time course and in spatial attenuation of the presynaptic potentials, and more work will be needed to control these factors independently.

An attempt was made to overcome these limitations by loading the terminal with tetraethylammonium (TEA) ions. This substance, if applied intracellularly, is known to interfere with the rise of potassium conductance<sup>10</sup>, and after prolonged ionophoretic infusion of TEA into the presynaptic axoplasm, it was indeed possible to produce well maintained intense depolarizations of the terminal. Under these conditions, a very interesting feature appeared. As the internal potential was displaced progressively, from the resting level of -65 mV, past zero, to increasingly positive potentials, a point was reached beyond which the postsynaptic response became reduced and eventually suppressed during the period of the presynaptic potential change, and only made its appearance after the termination of the latter (Fig. 3). This is precisely what was predicted on the basis of recent experiments on the neuromuscular junction<sup>11</sup>. The

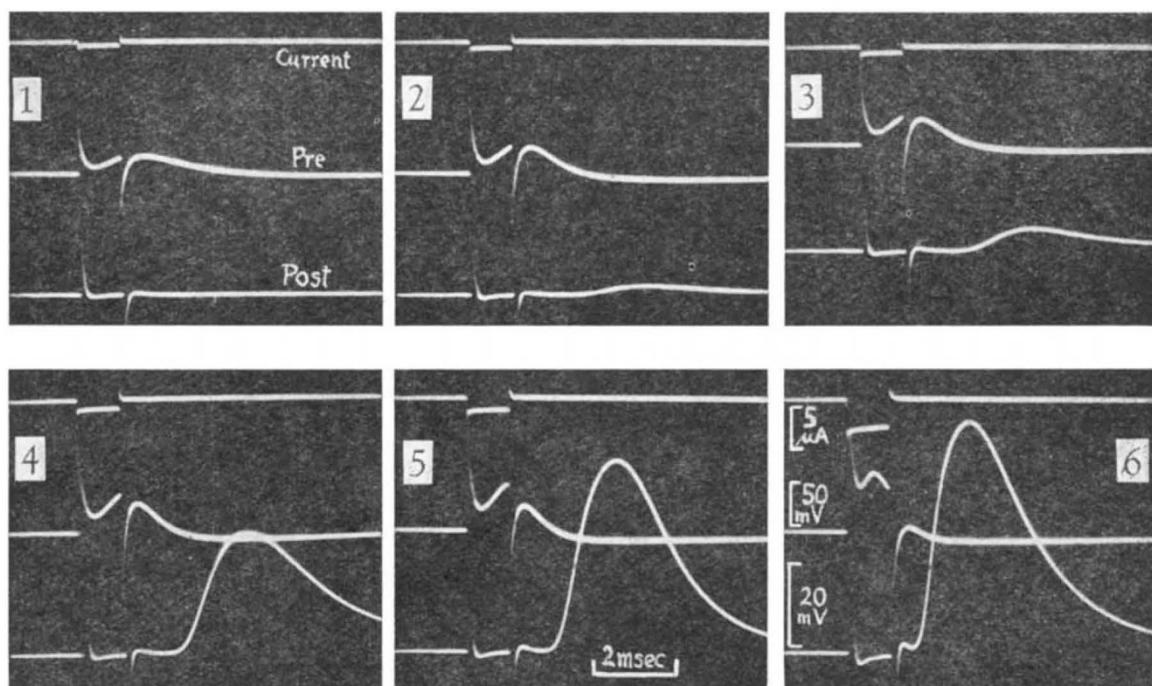


Fig. 2. From same preparation after re-positioning presynaptic electrodes. Distances (upper diagram in Fig. 2B): *a-d*, 0.6 mm; *d-b*, 0.35 mm. Pre-recording electrode is now within the region of synaptic contact. Sample recordings in Fig. 2A, and "input-output" curves in Fig. 2B.

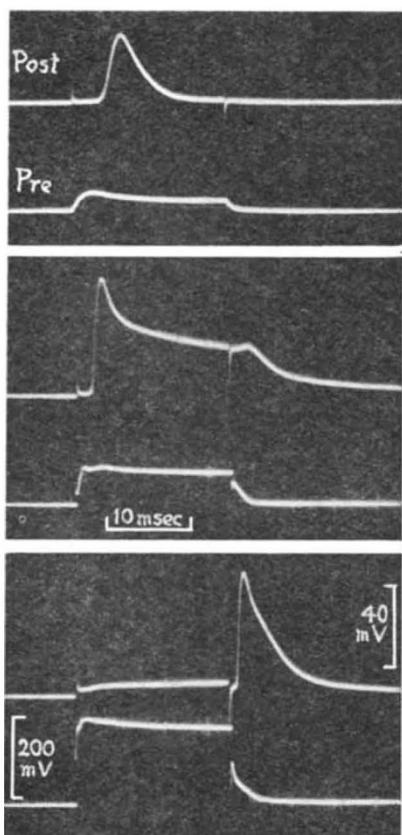


Fig. 3. The current-passing electrode was inserted into the pre-fibre at the beginning of the synaptic contact region, the pre-recording electrode 0.28 mm farther down along the synapse. The current electrode contained a concentrated solution of tetraethylammonium bromide and had been used to load the terminal ionophoretically with TEA. Three pairs of pre- and post-potentials are shown, the applied current pulse being increased from above downwards.

conclusions of that work were that one of the first steps, from depolarization to transmitter release, is the movement towards the inside of the axon membrane of a positively charged substance (probably calcium ions or a calcium compound  $\text{CaR}^+$ ). Reversal of the membrane potential would have a dual effect: it would open a gate for calcium ions (or for a positively charged calcium compound), but at the same time oppose the inward movement of cations. The former action rises and falls with a phase lag, while the latter is an immediate ionophoretic effect, synchronous with the imposed potential change. On this hypothesis one would expect that a sufficiently large positive displacement of the internal potential "would completely prevent inward movement of the postulated Ca-compound, and that transmitter release could in principle be delayed until the end of the pulse regardless of its duration"<sup>11</sup>, a prediction which is well borne out by the present experiment. At the squid giant synapse as well as at the neuromuscular junction, external calcium ions are indispensable for synaptic activity<sup>12</sup>. We regard our present results as further support for the view that inward movement of calcium, or of a calcium compound carrying net positive charge, is an early link in the "electro-secretory coupling" process.

Some other points of interest emerge from these observations. (i) The "threshold" for eliciting a detectable postsynaptic response (that is, for evoking transmitter release) is a brief depolarization of about 25–40 mV. This is higher than the normal threshold for spike initiation (about 15 mV); and thus no synaptic potential is likely to be obtained by local application of current normally, until a full-size action potential is elicited in the presynaptic axon. (ii) As at the neuromuscular junction, it is

possible, after tetrodotoxin, to evoke large postsynaptic potentials by local depolarization of the terminal without involving the inward sodium currents necessary for normal electric excitation. The largest postsynaptic potential observed in these experiments was 63 mV, in an axon of 66 mV resting potential. This, presumably, comes close to the "equilibrium potential" of the activated postsynaptic membrane (see ref. 13), and it is quite probable that the plateau reached by the input-output curves in Figs. 1 and 2 can be attributed to such a postsynaptic saturation effect. There is no evidence that the rate of transmitter release had reached maximum intensity at that point of the curve.

The fact that such large synaptic effects can be produced by a "passive" depolarization of the terminal raises once more the question of whether active propagation of an impulse right up to the end of the presynaptic element is a requirement, or merely a safeguard, for synaptic transmission. This question, however, remains somewhat academic, for there is good evidence that the impulse does travel into the synaptic region of the axon, at this as well as at the skeletal neuromuscular junction<sup>14</sup>.

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### Increased Tolerance to Intravenous Hypertonic Saline in Patients with Essential Hypertension

THE application of hypertonic solutions speeds the appearance of clinical signs of cellular dehydration in proportion to the impermeability of the cellular membrane. Intravenous infusion of hypertonic saline, which remains effectively in the extracellular fluid space, stimulates cellular dehydration more intensely than an equivalent amount of urea, which readily penetrates cell membranes<sup>1,2</sup>. Rapid intravenous application of a hypertonic solution of sodium chloride brings about a hypertonic expansion of the physiologically active part of the extracellular fluid space<sup>3,4</sup>. There is evidence that intravenous loading with strongly hypertonic salt solutions is partially dependent on the mean arterial blood pressure if the renal function is not or is only slightly affected.

Experiments were carried out with four healthy persons (one man and three women, aged 21–42 yr), with normal