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DIFFERENT PROPERTIES OF SYNAPSES BETWEEN A SINGLE SENSORY NEURONE AND TWO DIFFERENT MOTOR CELLS IN THE LEECH C.N.S.

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SUMMARY

In leech ganglia, an individual sensory cell that responds specifically to noxious mechanical stimulation of the skin (N cell) excites two different motoneurones. One raises the annuli of the skin into ridges (the AE cell), while the other innervates longitudinal muscles and thereby shortens the body segment (L cell). A comparison has been made of the way in which these two synapses behave when their common presynaptic cell is stimulated in various conditions.

1. Using previously described criteria, N sensory cells have been shown to make monosynaptic chemical connexions with both the AE and L motoneurones (Nicholls & Purves, 1972). Following a single stimulus, the excitatory synaptic potential recorded in the AE motoneurone was only about one tenth the size of that in the L cell (approximately 0·5 mV compared to 5 mV). Trains of impulses in the same N sensory cell gave rise to synaptic potentials in the AE and the L motoneurones that underwent phases of facilitation and depression; the facilitation, however, was characteristically greater and longer lasting at synapses upon the AE motoneurone.

2. The differences between the two synapses were accentuated in Ringer fluid containing increased concentrations of Ca and also in the cold. Under both of these conditions repetitive firing by the N sensory cell could give rise to synaptic potentials in the AE motoneurone which progressively increased in amplitude, while those in the L motoneurone became smaller.

3. The results suggest that the differences in synaptic transmission can be accounted for by variations in the amount of transmitter released at the presynaptic N cell terminals, rather than by differences in the

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post-synaptic cells. The animal's behaviour corresponds to expectations from the physiology of the synapses.

INTRODUCTION

At neuromuscular synapses of lobsters and crayfish, it has been shown that the various terminals of a single motor nerve fibre do not all behave in the same way. Some synapses facilitate greatly during repetitive stimulation – that is, the synaptic potentials recorded in the muscle fibre become progressively larger. In contrast, at other junctions made by the same motoneurone on different muscle fibres, there is much less facilitation (see Atwood & Bittner, 1971; Frank, 1973). The presynaptic nerve terminals rather than the muscle fibres have been shown to be the site of these differences, some terminals releasing more transmitter than others. These observations have raised the possibility that the properties of nerve cell terminals may depend upon the type of post-synaptic cell with which they make contact (Frank, 1973).

For a number of reasons, it would be of interest to determine whether differences in the effectiveness of transmission also occur at different synapses made by an individual nerve cell in the C.N.S. For example, trains of impulses in an individual neurone could activate two post-synaptic cells at different times and different rates. if facilitation were to grow more rapidly at one synapse than the other. With this type of information it might be possible to explain certain behavioural responses in terms of the synaptic mechanisms involved. A further aspect of the problem is whether a post-synaptic neurone in the C.N.S. can determine the release characteristics of the presynaptic terminals that impinge upon it. Here we are concerned primarily with an investigation of the first point, namely, how transmission varies at the different synapses made by a neurone.

A favourable preparation for examining this problem is provided by the C.N.S. of the leech. In each segmental ganglion one can readily identify individual sensory and motor nerve cells and trace their connexions by recording with intracellular micro-electrodes. From earlier work it is known that the mechanosensory cells that respond to noxious mechanical stimuli (N cells) make monosynaptic excitatory synapses upon a identified motoneurone (the L cell), which innervates longitudinal muscle fibres and shortens the segment (Nicholls & Purves, 1970, 1972). In the present investigation we have established that these same sensory N cells also make excitatory synapses upon a different motoneurone, the annulus erector (AE) cell, which causes the skin to be raised into ridges (Stuart, 1970). The effect of this cell's action is very obvious and makes

the skin surface look ridged, like a concertina. Squeezing or pressing the skin therefore leads to a dual reaction, shortening of the segment and erection of the skin annuli. It was clear from observing the animal that the two reflexes followed different time courses; the shortening occurred abruptly and was poorly maintained, while the annuli became erect more slowly but stayed up for longer. This prompted us to measure the facilitation and depression occurring at the synapses upon the L and AE motoneurones to see whether differences in synaptic mechanisms could account for the behaviour we observed.

METHODS

The procedure has been described in detail in earlier papers (see Nicholls & Baylor, 1968; Nicholls & Purves, 1972). A sensory and a motor cell were impaled simultaneously with micro-electrodes filled with 4 M potassium acetate and having resistances of 50–100 MΩ. The positions of the N sensory cell and the AE and L motor cells are shown in Fig. 1. All three cells can be unambiguously recognized by their morphological and physiological characteristics. The N cell that was used for these studies is the one situated more medially in the ganglion, since it gave rise to larger synaptic potentials on the AE cell. In normal Ringer fluid, impalement of the AE cell often causes it to fire; when this occurred the membrane was hyperpolarized by current passed through the micro-electrode.

The L cell is situated dorsally on the opposite surface of the ganglion. To impale both it and the medial N cell simultaneously was technically difficult. Hence in the earlier experiments of Nicholls & Purves (1970, 1972) the laterally situated N cell was primarily used. Nevertheless, with the ganglion pinned out appropriately, it is possible to impale the medial N cell and the L cell simultaneously. Confirming earlier work, we have found that the two N cells act in a similar manner upon the L cell.

Ringer fluid, which flowed continually past the preparation, contained (mM); NaCl, 115; KCl, 4; $CaCl_2$, 1·8; glucose, 12·3; Tris-maleate, neutralized in pH 7·4 with NaOH, 10 mM. When necessary, the concentration of $CaCl_2$ was increased by isotonic substitution for NaCl. In general, trains of 20 impulses at different frequencies were initiated in the sensory N cell by stimuli applied through the micro-electrode. Before the train, stimuli were applied once every 30 sec to avoid depression (see Nicholls & Purves, 1972). Recovery was assessed by stimulating first at 1/10 sec, then with a single shock once every 2 min with at least 10 min between trains.

The usual procedure was to study the effects in the AE or the L cell of not more than 2 or 3 trains initiated in a single N cell. In a few experiments the same N cell was tested on both motoneurones.

To measure facilitation, the peak value of the second synaptic potential was measured from the extrapolated tail of the preceding one and compared to the first synaptic potential as described by Mallart & Martin (1967). In leech ganglia as in other invertebrates, the synaptic connexions are all situated within the complex neuropile. There are none on the cell bodies.

RESULTS

The connexions of N sensory cells to the annulus erector motoneurone

An impulse in the medially situated N sensory cell gave rise to an excitatory synaptic potential in the AE motoneurone. In normal Ringer fluid the synaptic potential following a single impulse was small, usually 0·3–1·0 mV. Because of the large number of 'spontaneous' excitatory and inhibitory potentials that are recorded in the AE cell in Ringer fluid, we cannot say whether the fluctuations represent variations in quantum content. Nor is it clear whether the small size is due to a low quantum content or to the distance from the cell body to the synapse within the neuropile.

A point of importance for the subsequent analysis of facilitation was to determine whether the pathway from the N cell to the AE cell was direct or involved an interneurone. Several features of the synaptic potential in the AE cell showed that it arose monosynaptically like that recorded in the L cell (Nicholls & Purves, 1970). Strongly suggestive of a monosynaptic connexion was that unitary synaptic potentials in the AE cell followed impulses in the N cell in a one-to-one manner and arose at a constant latency of less than 2 msec. Cooling to 4° C increased the delay to about 10–20 msec, which is comparable to that seen at other synapses in the leech at that temperature (Nicholls & Purves, 1972). A critical test, however, was provided by the finding that hyperpolarization of the presynaptic cell with a large, brief current resulted in the appearance of a 50–100% increase in the synaptic potential recorded in the AE motoneurone. Current could spread from the cell body of the presynaptic neurone to its terminals when transmitter was liberated, thus excluding the presence of an interneurone. An example is shown in Fig. 1 (see also Nicholls & Purves, 1972). As one would expect for a chemically mediated synapse the synaptic potential in the AE cell was abolished in high Mg (20 mM) and restored when 15 mM-Ca was added to the Mg fluid.

Although in some respects the synapses of the N cell on the AE and the L motoneurones appear similar, there were consistent quantitative differences. First, as mentioned already, the synaptic potentials were smaller in the AE cell, 0·3–1·0 mV compared to about 2–10 mV in the L cell. Second, besides forming a chemical synapse, the N cell was in addition electrically coupled to the AE cell but not the L cell. However, the coupling, which did not rectify, was weak so that it could be detected only when large currents were passed into the N cell, considerably beyond the range of those produced by an impulse. Thus, at room temperature one cannot see the electrical component of the synaptic potential when

chemical transmission is blocked by Mg. A further difference between N cell synapses on the AE and the L cells was that repetitive firing often evoked a polysynaptic inhibitory potential on the AE cell. This sometimes made it difficult to analyse events occurring during a train.

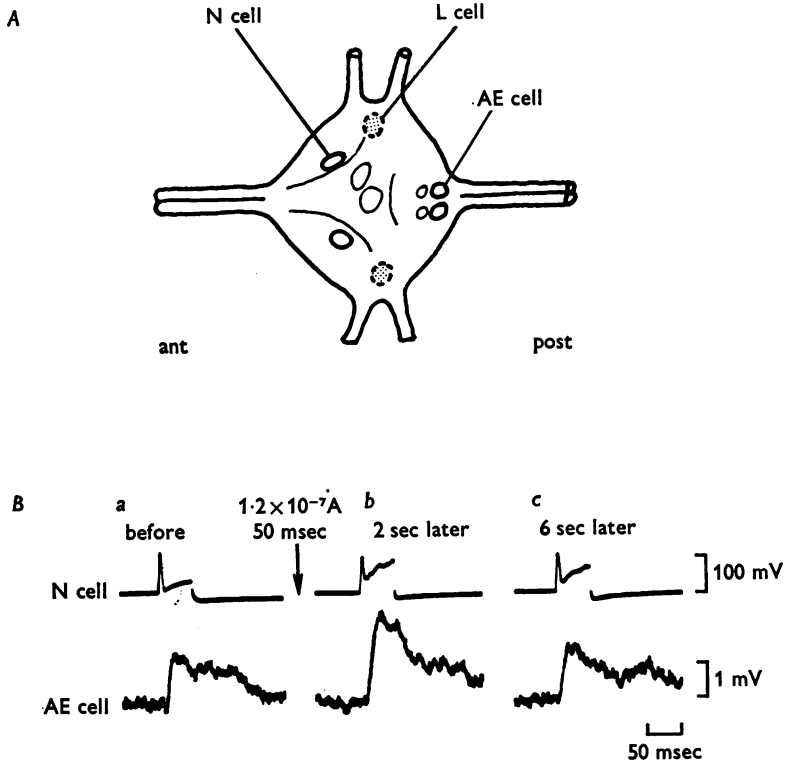

Fig. 1. Intracellular recordings from a nociceptive (N) sensory cell and the annulus erector motoneurone (AE cell) to demonstrate monosynaptic connexions. *A* shows the positions of these cells and also that of the L motoneurone which lies on the deep, dorsal surface of the ganglion. *B* shows that the synaptic potential in the AE cell was increased after a brief hyperpolarizing current pulse was delivered to the N cell. An increase of this type would not occur if an interneurone were interposed in the pathway. The bathing fluid contained 15 mM-Ca throughout to simplify the measurement of synaptic potentials (see text).

Facilitation following a single impulse

Fig. 2 shows the difference in facilitation at the synapses on AE and L motoneurones following a single impulse in an N cell. At both synapses a second impulse in the N cell gave rise to a larger synaptic potential, but the effect was far more marked in the AE cell. The graph shown for the L cell agrees well with results obtained by Nicholls & Purves

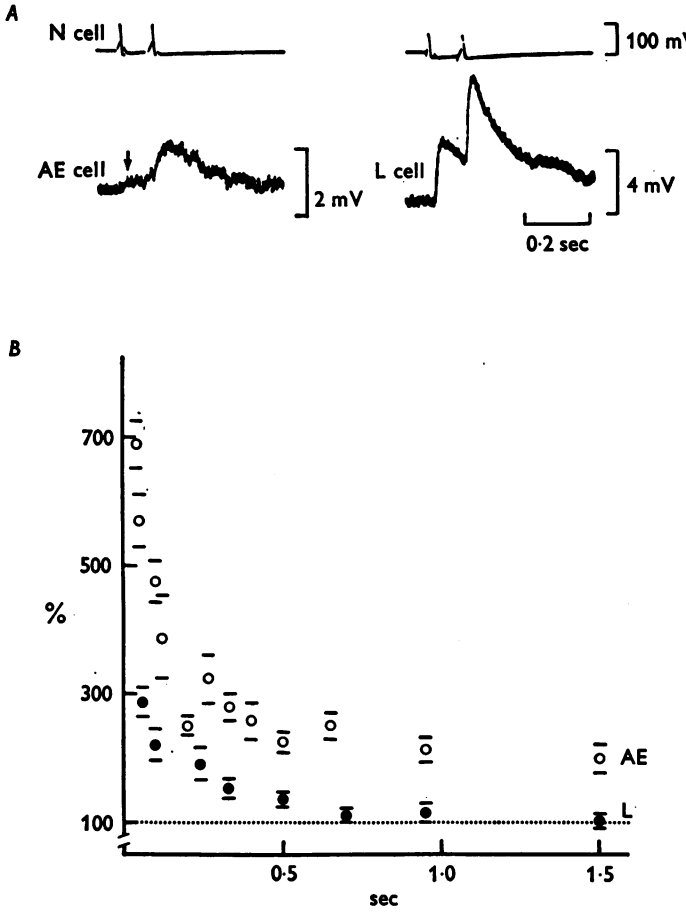

Fig. 2. Comparison of the facilitation in AE and L motor cells following single impulses in an N cell. Paired stimuli were delivered once every minute. *A* shows recordings from the two motoneurones. The first synaptic potential in the AE cell, indicated by the arrow, is considerably smaller than the second. The synapse to the L cell is less facilitated with a similar interval between impulses in the N cell. *B* is a graph of the size of the second synaptic potential expressed as a percentage of the first (ordinate) plotted against the interval between impulses in the N cells (abscissa, sec). For AE (○) and L cells (●) the bars represent the S.E. of mean. Each point is the average of twenty to forty measurements in most instances and at least six measurements in the others. The control value of the synaptic potentials (100%) in the AE cells was $0{\cdot}4 \pm 0{\cdot}06$ mV and in the L cells was $3{\cdot}5 \pm 0{\cdot}02$ mV. These experiments were made in Ringer fluid containing 1·8 mM-Ca.

(1972), for which the laterally situated N cell was used. Features of the facilitation at the AE cell synapse were its greater amplitude and longer duration. The potentials grew to seven times their original size and continued to be twice as large for more than 1 sec after a single impulse. From this one would expect facilitation at the AE cell synapse to be apparent at low frequencies. We shall see that the results obtained with paired shocks could not be used to predict events occurring during a train: the effects observed with more than two impulses were too complex, owing to non-linear summation of facilitation and the build up of depression.

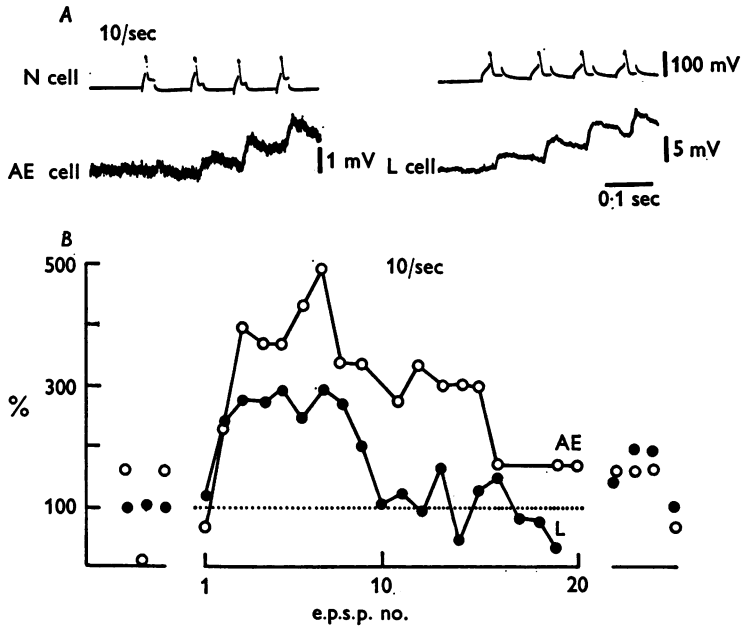

Fig. 3. Facilitation of synaptic potentials in AE and L motor cells during trains of impulses in N cells at 10/sec in normal Ringer fluid. *A* shows the first few potentials and *B* is a graph of the size of the synaptic potential plotted against its ordinal number in the train for the AE cell (○) and the L cell (●). The dotted line is the average value before the train. Points before the train were obtained once every 30 sec and after the train at 10 sec intervals. The synaptic potentials in the AE cell facilitate more and do not become depressed to below the original size.

Facilitation during trains of impulses

In normal Ringer fluid, the synaptic potentials recorded in the L motoneurone showed both facilitation and depression. During trains at 10/sec the potentials first grew larger and then became smaller than the control values (Fig. 3). The connexions on to the AE cell showed similar

features, but with greater facilitation and less depression. Thus, in the examples shown in Fig. 3, during a train of impulses at 10/sec the synaptic potentials recorded in the AE cell increased to about five times their original size and thereafter remained larger than before. In contrast those in the L cell increased by a factor of only about 2·5 and then became depressed compared to the initial value. Differences between the potentials recorded in the L cell and in the AE cell were also apparent with trains at frequencies of 1/sec, 2/sec and 15/sec.

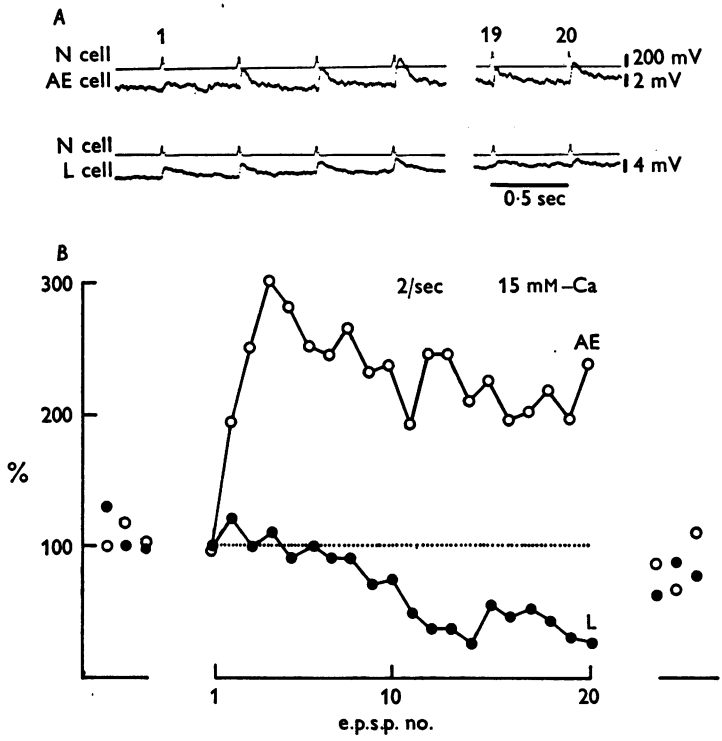

Fig. 4. Trains of impulses in N cells in fluid containing 15 mM-Ca instead of 1·8. Records from AE and L cells at the beginning and end of trains at 2/sec are shown in *A*. *B* shows that while the synaptic potentials in the AE cell (○) were facilitated, those in the L cell (●) were depressed. Intervals between tests were 30 sec before the train, and 10 sec after the train. The dotted line is the average value of synaptic potentials before the train.

The contrast in responses was, however, most clearly seen in experiments in which the Ca concentration of the bathing fluid was increased to 15 mM. Under these conditions the synaptic potentials are larger and, in the case of the AE cell, easier to measure. Fig. 4 illustrates the effect

of trains at 2/sec. In confirmation of earlier findings, the synaptic potentials in the L cell showed mainly depression at this frequency in high Ca (see Text-fig. 4 in Nicholls & Purves, 1972). In the same concentration of Ca, potentials recorded in the AE cell were greatly facilitated throughout the train.

Similar results were obtained in high Ca at other frequencies. In a few experiments the same N cell was stimulated with a train while recordings were made from first the AE cell, then the L cell, and finally the AE cell again. The results were similar to those shown in Fig. 4.

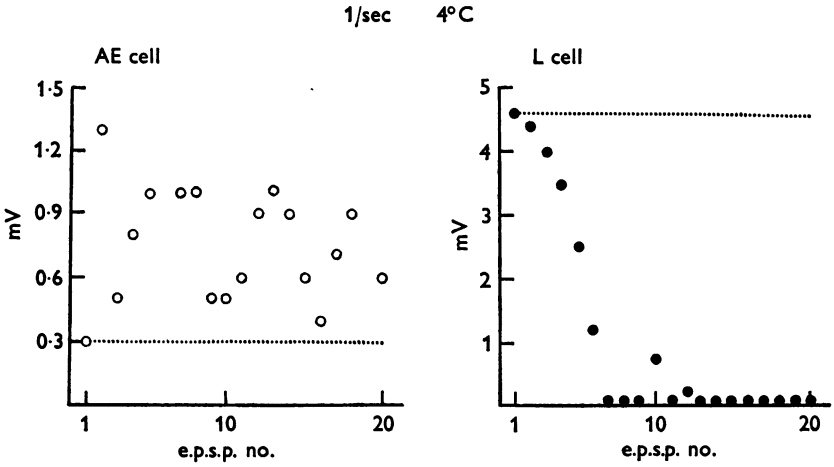

Fig. 5. Different effects of reduced temperature on synaptic transmission from N cells to AE cell (○) and L cell (●). At 4° C the synaptic potentials in the AE cell are facilitated during a train at 1/sec. Those in the L cell decline to zero after about ten impulses (see also Nicholls & Purves, 1972, Text-fig. 9).

Effects of lowered temperatures on synaptic transmission

In the cold, leeches exhibit normal but slowed reflexes. The frequency of sensory impulses initiated by standard mechanical stimuli to the skin is reduced and the duration of action potentials increased. In the cold, as at room temperature, trains of impulses in the N cell give rise to characteristically different potentials in the AE and the L motoneurones.

At 4° C transmission between the N cell and the L motoneurones becomes rapidly depressed, so that the synaptic potentials cannot be seen after approximately 10 impulses (see Fig. 5, and also Text-fig. 9 in Nicholls & Purves, 1972). At the same time the synaptic potentials in the AE cell behaved differently by remaining larger than the control size throughout a train of 20 impulses at 1/sec (Fig. 5). At both synapses, the synaptic delay at 4° C was increased to about 10–20 msec; in the

cold the electrical component of transmission from the N cell to the AE cell could sometimes be seen as a small depolarization. All the effects of cooling on synaptic transmission were reversed within a few minutes by rewarming to room temperature.

Connexions of sensory cells of different modality to the AE motoneurone

At crustacean neuromuscular junctions Frank (1973) has shown that all the presynaptic endings on a given muscle fibre have similar characteristics of facilitation. This suggests that the post-synaptic cell may somehow influence the properties of the incoming fibres. Accordingly it was of interest to determine the properties of a variety of sensory inputs to the AE and the L motoneurones.

The experiments described so far were all made with the N sensory cell that is situated medially in the ganglion. Tests were also made with the more lateral N sensory cell and with the two pressure-sensitive cells (P cells) which lie anterior to the AE cell (see Nicholls & Baylor, 1968). In general the results were similar to those reported above. The principal difference with the lateral N cell was that the synaptic potentials recorded in the AE motoneurone were even smaller than those evoked by the medial N cell. Furthermore, they were harder to measure during trains since they were occasionally followed by inhibitory potentials.

With trains of impulses P cells, like N cells, gave rise to greater facilitation at synapses upon the AE cell than the L cell. The potentials in the AE cell from the P cell also showed less depression in the cold than those in L cells. In addition, inhibitory potentials frequently occurred in the AE cell during brief trains of impulses in P cells. Electrical coupling from P cells to the AE cell was strong enough that in the presence of 20 mM-Mg a small potential could still be seen in the AE cell with each impulse.

The connexions of the sensory cells that respond to touch (T cells) and the AE motoneurone were not examined systematically because the chemical component was weak and appeared to be polysynaptic. The electrical coupling of T cells to the AE cell was more powerful than that from N or P cells.

DISCUSSION

The synapses made by N sensory cells on the annulus erector and the large longitudinal motoneurones have several properties in common. Both synapses are chemical and monosynaptic. Both differ from electrical synapses by varying in effectiveness during trains of impulses at frequencies within the range produced by mechanical stimulation of the skin (Nicholls & Purves, 1970, 1972). But in several respects the two synapses differ

from each other, especially in the degree of facilitation and depression. One clear example is provided by the effects of single impulses to the presynaptic N cell. At similar intervals the potential recorded in the AE cell is increased far more than that in the L cell and the increase persists for considerably longer. Moreover, during brief trains, the synaptic potentials can be increased in the AE cell while they are actually decreased in the L cell. These differences are particularly marked in the cold and in raised Ca concentrations. A descriptive summary of these results would be that synaptic transmission to the AE cell facilitates more and depresses less than that to the L cell.

In theory, the differences between the synapses might be attributed to (1) different properties of the presynaptic terminals of the N cell or (2) differences in the post-synaptic receptors. For example, with trains of impulses one set of N cell terminals might increase transmitter liberation more than others. Alternatively, for a post-synaptic mechanism, the receptors on the L neurone might become saturated or desensitized. If this occurred, increased amounts of transmitter would not be able to produce larger potentials. Since the identity of the chemical transmitter is not known, no direct test of the post-synaptic alternative can be done. However, our experiments provide indirect evidence in favour of a variation in the release of transmitter. For example, in 10 mM-Mg the synaptic potential recorded in an L cell becomes reduced in size and therefore less desensitization would be expected. Even in this solution the facilitation in L cells was significantly smaller than in AE cells (see also Nicholls & Purves, 1972).

Furthermore, there is no obvious post-synaptic effect that could account for the pronounced depression on the L cell synapse at 4° C when, at 1/sec, successive synaptic potentials decline rapidly to zero with no changes in the membrane potential of the L cell.

An alternative explanation of these results, variations in the amount of transmitter released, seems to be more likely. At neuromuscular synapses of frogs and crustacea it has been shown that greater facilitation occurs at terminals that release fewer quanta and give rise to small synaptic potentials (Rahamimoff, 1968; Atwood & Bittner, 1971). If N cell terminals were to release more transmitter on to the L cell than on to the AE cell, then a difference in depletion of available transmitter could explain the relatively decreased facilitation and increased depression. High Ca concentrations which increase transmitter release (del Castillo & Katz, 1954) and cooling, which prolongs the duration of the action potential, both increase the amplitude of synaptic potentials (Nicholls & Purves, 1972) and give rise to a concomitant increase in depression.

Our findings are consistent with the idea that the release properties

of a presynaptic terminal depend upon the type of post-synaptic cell with which it makes contact. Although we did not make extensive studies with P sensory cells, it was clear that they behaved like N cells, producing larger synaptic potentials with less facilitation in the L cell. It will be of interest to see whether other cells can be found which synapse on both the AE and the L cells and to examine their characteristics of facilitation and depression.

So far there is little information about analogous situations in the C.N.S. of vertebrates. One example is provided by the branches of Ia afferent fibres which have characteristically different effects at two different synapses. One branch causes a large, powerful, excitatory potential in the neurones of Clarke's column while another evokes only a small subthreshold potential in spinal motoneurones (Kuno & Miyahara, 1968). It is not yet known how these synapses behave with trains of impulses.

A satisfactory aspect of these studies is the general agreement between the properties of synaptic transmission and the behavioural reactions one observes in response to mechanical stimuli. By facilitating to a different extent at two terminals, the synaptic potentials produced by a sensory cell can trigger two events in sequence, shortening followed by annulus erection. It would be of interest to know how widespread such differential effects are in setting up timed sequential reactions to stimuli.

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