J. Physiol. (1959) 145, 326–335

SLOW POST-SYNAPTIC POTENTIALS RECORDED FROM THE GIANT MOTOR FIBRE OF THE CRAYFISH

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(*Received* 30 *July* 1958)

Two types of post-synaptic response have been recorded from the giant motor fibres in the nerve cord of the crayfish (Furshpan & Potter, 1959). These were the rapid excitatory post-synaptic potentials which arose at the giant motor synapses and slower potentials of unknown origin. Further study has indicated that the second type of response is associated with an inhibitory effect and it resembles the *I* potentials of crayfish stretch receptor neurones in several respects (Kuffler & Eyzaguirre, 1955). An added point of interest is that transmission across these synapses appears to be brought about by a 'chemical' mechanism, unlike the 'electrical' mechanism described in the preceding paper.

METHODS

The methods for preparing, stimulating and recording from the ventral nerve cord of *Astacus fluviatilis* were identical to those used before (Furshpan & Potter, 1959). In most experiments the giant motor fibre ('post-fibre') was impaled with two micro-electrodes at various places between the mid line of the nerve cord and the point at which the fibre leaves the third ganglionic root (see Fig. 1 of the preceding paper, p. 290). Solutions could be run through the chamber without dislodging the electrodes, at a rate of flow such that the effects of drugs were often seen within 15 sec.

RESULTS

The two types of post-synaptic potentials (p.s.p.'s) produced in the giant motor fibre by stimulation of the nerve cord are shown in Fig. 1*a*. Following the initial excitatory potentials, which arose at the giant motor synapses (Furshpan & Potter, 1959), there were smaller, slower p.s.p.'s. The two responses could be readily distinguished by several criteria, among which was their difference in time course. It can be seen from Table 1 that there was no overlap between the values for rise time and for time from onset to half-decline.

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The slow potentials were also seen in the absence of cord stimulation (Fig. 1*b*) in all but a few cases. The discharge was very variable in frequency and sometimes appeared to be rhythmic. The time course of the spontaneously arising slow potentials is given separately in Table 1.

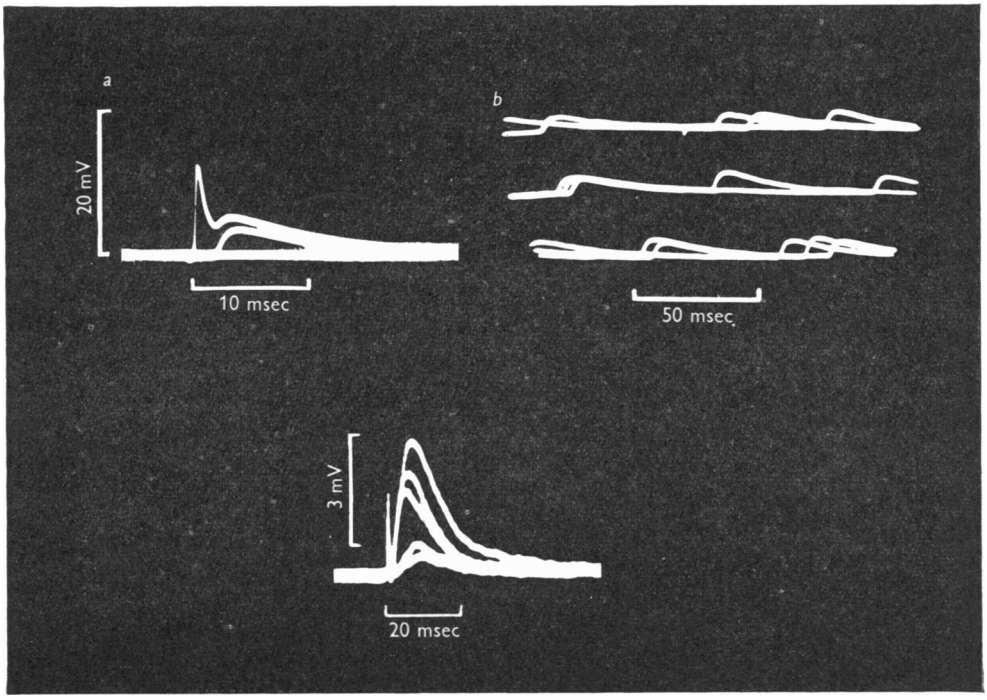

Fig. 1*a*: Excitatory and slow p.s.p.'s on two successive sweeps. On the first sweep only a slow p.s.p. was recorded. On the second cord stimulation strength was increased, exciting the lateral giant fibre; the slow potential summed with the falling phase of the larger excitatory p.s.p. *b*: Spontaneous slow p.s.p.'s. Several sweeps superimposed in each line. The base line was displaced 10 mV between lines. *c*: All-or-nothing increments in the size of slow potentials. Cord stimulation strength was increased after each of the five superimposed sweeps.

TABLE 1. Time courses of the excitatory, the slow and the spontaneously arising slow p.s.p.'s. The range is given in brackets beneath the mean and S.E. The onset was determined by extrapolating the approximately linear part of the rising phase to the base line

Type of p.s.p.	Number of post-fibres	Rise time (msec)	Time from onset to half-decline (msec)
Excitatory (lateral pre-fibre)	28	0·45±0·02 (0·31–0·91)	1·4±0·1 (0·83–3·2)
Slow	22	3·1±0·19 (1·8–4·0)	12·0±0·84 (7·7–24·0)
Spontaneous slow	20	3·8±0·16 (1·7–5·2)	14·7±1·11 (5·7–29·5)

The slow p.s.p.'s never exceeded 10 mV in amplitude and at the resting level of post-fibre membrane potential appeared as depolarizations in all cases but one. (In this preparation slow p.s.p.'s were seen only when the membrane potential was displaced from its resting value, except at very high stimulation intensity.) Increasing the strength of the stimulus usually produced all-or-nothing increments in the size of the slow response (Fig. 1*c*), indicating that several presynaptic nerve fibres were involved. The largest number of such all-or-nothing steps was seven.

In Fig. 2 the spontaneous slow potentials are compared with those which arose in response to cord stimulation. The similarity in the sizes of the two potentials strongly suggests that the spontaneous p.s.p.'s are not analogous to the miniature end-plate potentials of vertebrate muscle (e.g. Fatt & Katz, 1952), but are due to the all-or-nothing firing of the presynaptic axons. In a few preparations the spontaneous p.s.p.'s repeatedly occurred in several different sizes, suggesting that more than one of the 'slow' 'pre-fibres' was discharging.

Dependence of the size and sign of the p.s.p.'s on post-fibre membrane potential

Fig. 3 shows the effect of superimposing the slow potentials on electrotonic pulses. The p.s.p.'s were reduced in size during a small depolarizing pulse and reversed in sign when the depolarization was larger. In this case the membrane potential at which the p.s.p.'s changed sign (the 'reversal potential') was 8–10 mV below the resting level. When the membrane was hyperpolarized the amplitude of the p.s.p.'s was increased over that at resting potential. The dependence of the p.s.p. amplitude on post-fibre membrane potential is shown graphically, for another experiment, in Fig. 4. The relationship is approximately linear over a range of about 30 mV of membrane potential with a slope of 0·54. An alternative way of describing the effect is that the slow p.s.p. tended to diminish, by a constant fraction, any electrotonic displacement of the membrane potential from the reversal level; and in this case the slow p.s.p. amounted to 54% of the deviation from reversal potential. In the seven experiments in which this calculation could be made, and in which apparently maximal cord stimulation was employed, the mean amplitude of the slow response was 49% (27–75%) of the departure from reversal potential.

In Fig. 4 the depolarization at which reversal occurred was about 3 mV. In sixteen experiments in which the intracellular electrodes were filled with 3 M-KCl (Coombs, Eccles & Fatt, 1955), the mean reversal potential was 8·6 mV below the resting level (range, 3–20 mV). In seven other experiments, in which the electrodes contained 0·6 M-K_2SO_4 or 2·5 M sodium citrate, the mean reversal depolarization was 6·0 mV (1–12·5 mV). In most experiments the

resting potential of the post-fibre was not known and the absolute reversal membrane potential could not be determined.

The above observations are qualitatively similar to those which have been made at several different junctions (e.g. Fatt & Katz, 1951, 1953; Coombs *et al.* 1955; Burke & Ginsborg, 1956). In each case the results were explained in terms of a chemical transmitter which increased the conductance of the post-junctional membrane to one or more ions. The transmitter would thus

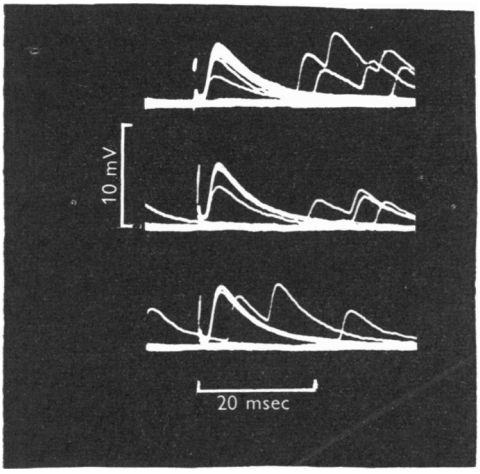

Fig. 2

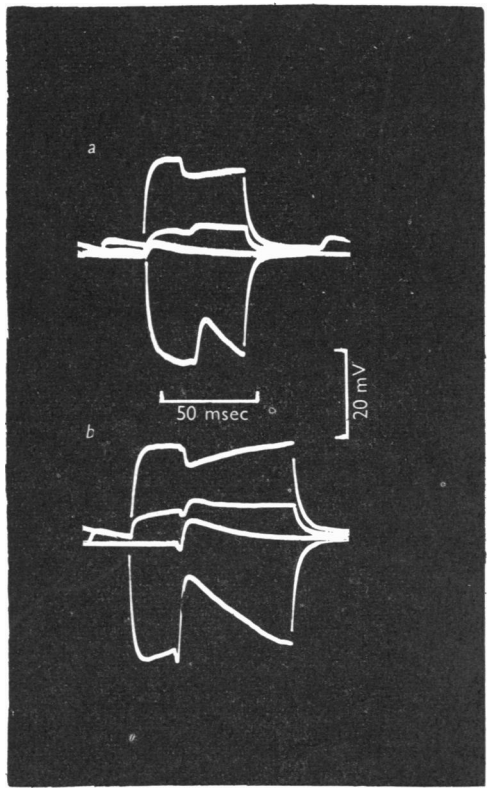

Fig. 3

Fig. 2. Comparison of the time courses of slow p.s.p.'s which arose spontaneously and in response to stimulation of the cord. In each of the three frames, which are all from the same experiment, graded slow p.s.p.'s which follow immediately after the shock artifact were produced by gradually increasing the stimulus strength on a number of successive sweeps. The frequency of the spontaneous potentials was increased by the stimulation.

Fig. 3. Effect of displacing the membrane potential of the post-fibre on slow p.s.p.'s (spontaneously arising in *a*; in response to cord stimulation in *b*). Catelectrotonus (upward deflexions) and anelectrotonus (downward deflexions) were produced by passing pulses of current with a micro-electrode inserted less than 0·2 mm peripheral to the recording electrode.

tend to shift the membrane potential towards the electrochemical equilibrium for those ions; and the size and sign of the resulting p.s.p. would depend on the value of the membrane potential in relation to this equilibrium level (Fatt & Katz, 1953). It seems very likely that the same type of 'chemical' mechanism accounts for the slow p.s.p.'s. In any event, their reversal cannot be explained by the 'electrical' transmission found at the giant motor synapses (Furshpan & Potter, 1958), and thus two quite different types of junctional mechanism are present on the same post-fibre.

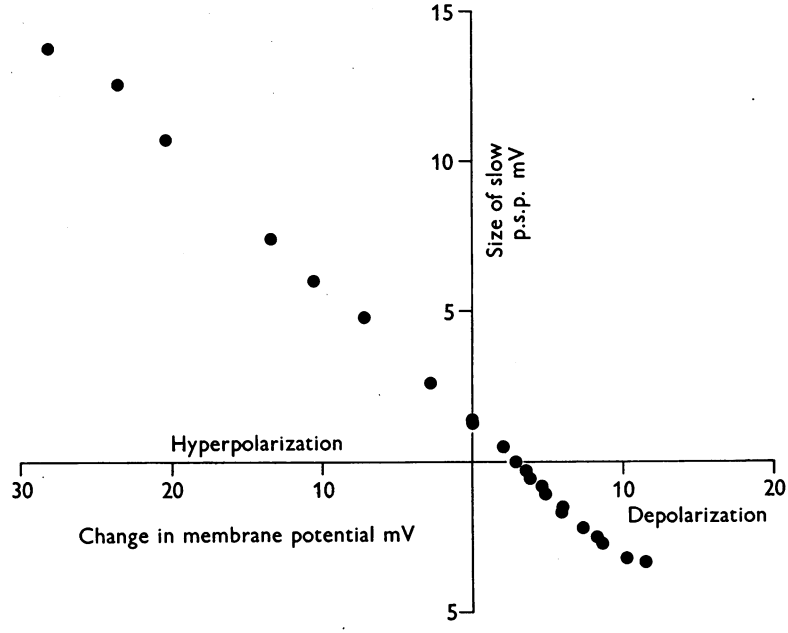

Fig. 4. Changes in the size and sign of the slow p.s.p.'s with changes in post-fibre membrane potential. Depolarizing slow p.s.p.'s are plotted above the horizontal axis. At resting potential the p.s.p.'s were 1·4 mV in size; they reversed sign about 3 mV below resting potential.

Interaction of the slow potentials with the p.s.p.'s of the giant motor synapses

In all the above experiments the reversal depolarization was considerably less than the threshold depolarization. This suggested that the slow p.s.p.'s would be associated with inhibitory effects, and the interaction between the two types of responses was examined. It was found that the excitatory p.s.p.'s were always diminished when superimposed on the rising phase of the slow potential; but when they coincided with the declining phase, they were reduced in some preparations and augmented in others (Fig. 5). The mean maximum reduction, in fourteen experiments, was 20% (6–53%). The augmentation of the excitatory p.s.p. was due to its summation with the slow

potential. The effect was usually slight and the largest increase observed (Fig. 5*b*) was 10%.

There is reason for believing that, in the intact animal, the percentage reduction might be greater and the augmentation effect absent. It is possible that the disparity which was usually observed between the reversal and resting levels of membrane potential was due to experimental damage (see Discussion). If the two levels were coincident, the 'inhibitory action' would not be accompanied by any potential change (Fatt & Katz, 1953) and summation between excitatory and slow potentials could not occur. The 'inhibitory' effect would be enhanced because it tends to restore, by a constant fraction, any displacement of membrane potential from the reversal level and the further (i.e. at higher membrane potential) this level is from the peak of the excitatory p.s.p., the larger the reduction which results. If the reversal potential were at or near the resting level, there could be little doubt that the slow p.s.p.'s would have an inhibitory effect.

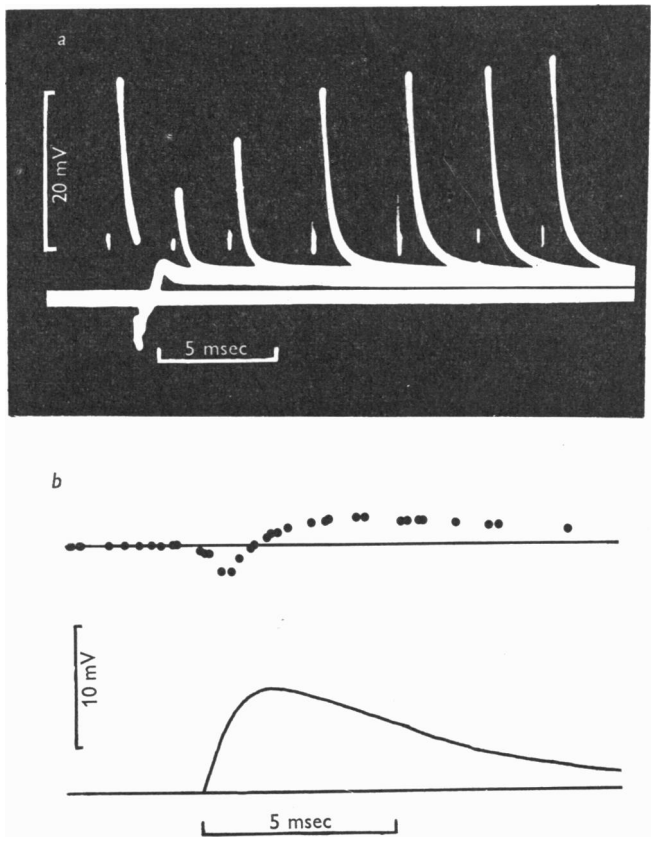

Fig. 5. Depression of excitatory p.s.p.'s by slow potentials. *a*: Seven excitatory p.s.p.'s produced by internal stimulation of the lateral pre-fibre were successively superimposed on a slow potential. The rising phase of the slow potential is obscured by a small excitatory p.s.p. which arose at another giant motor synapse. *b*: Tracing of a similar experiment. The peaks of the 'giant' p.s.p.'s are indicated by dots; in the absence of a slow potential these p.s.p.'s were 20 mV in amplitude (line drawn parallel to the base line).

The action of gamma-aminobutyric acid on the post-fibre

Gamma-aminobutyric acid (GABA) has recently been found to inhibit the contraction of crayfish muscle (McLennan, 1957) and to mimic some of the effects of the inhibitory synapses on crayfish stretch-receptor neurones (Edwards & Kuffler, 1957). It was of interest, therefore, to see if GABA would also imitate some of the effects of the slow p.s.p.'s.

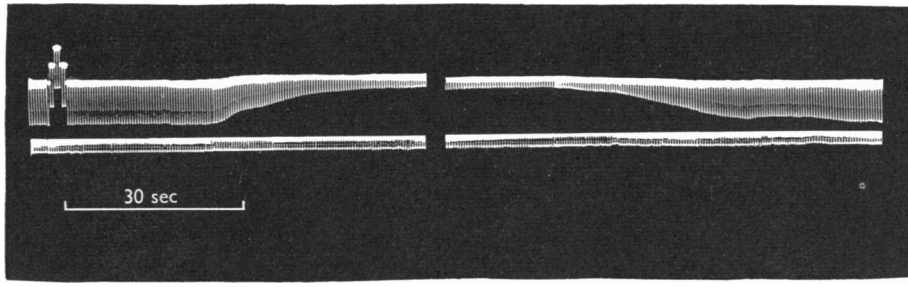

Fig. 6. The effect of 3×10^{-5} g/ml. GABA on post-fibre membrane potential and resistance. Lower trace: 20 msec pulses of hyperpolarizing current passed with an internal electrode. Upper trace: potential changes in the post-fibre recorded with another micro-electrode, hyperpolarizations downward. At the signal on the left (two 10 mV steps) van Harreveld's solution containing GABA began to flow into the chamber. After about 25 sec the post-fibre membrane potential fell slightly and the anelectrotonic potentials began to decrease in size. The break in the record represents a period of about 100 sec during which GABA continued to flow into the chamber. At the moment when the record recommences, fresh van Harreveld's solution was introduced to wash out the GABA.

Solutions of GABA more concentrated than about 2×10^{-5} g/ml. were found to produce a marked decrease in resistance, and a slight (2–10 mV) fall in the membrane potential, of the post-fibre. The effect of 3×10^{-5} g/ml. GABA is illustrated in Fig. 6. In this case the maximum fall in membrane potential was 4 mV and even before washing with normal solution (beginning after the gap in the record) the potential level had partially returned to its resting value. The decrease in membrane resistance is shown by the reduction in the amplitude (by a factor of five) of the anelectrotonic potentials. It can be seen that the effect was reversible; and on each of three repetitions of the procedure, similar results were obtained. The figure does not give a measure of the rapidity of the GABA action. The recorded time course was probably determined, for the most part, by the rate at which the drug solution flowed into the chamber and mixed with the normal solution. A decrease in the membrane resistance of the stretch-receptor neurones during GABA application has recently been reported by Kuffler & Edwards (1958). When the membrane potential was near the resting level, no potential change occurred during the GABA action; while in stretch-depolarized cells GABA gave rise to a hyperpolarization. With their

method of rapid drug application this hyperpolarization appeared within a fraction of 1 sec.

In five experiments, 5×10^{-5} g/ml. GABA reduced the amplitude of electrotonic potentials by a mean factor of about eight (range, about 4–12), and caused a mean fall in membrane potential of 6 mV (4–10 mV). As would be expected from its effect on post-fibre membrane resistance, GABA also brought about a pronounced depression of the excitatory p.s.p.'s. In six experiments the excitatory potentials were decreased in amplitude by a mean factor of about six (3–10) in 5×10^{-5} g/ml. GABA.

Inasmuch as GABA reproduces some of the effects of the presumed transmitter at the slow synapses, it might be asked if the two substances have the same action on the post-fibre. If in both cases the conductance increase involves the same ion or ions, then the equilibrium potential for both effects would be identical. There is insufficient information about the spatial distribution of either the 'slow' synapses or the sites sensitive to GABA to make an exact determination of the equilibium level in either case. Nevertheless, it is apparent that the two levels are not very different. The reversal potential gives a rough measure of the equilibrium level for the slow p.s.p.'s (Burke & Ginsborg, 1956) and, provided the conductance change is very large, the depolarization caused by GABA approaches the equilibrium potential for that effect. In the six experiments in which the comparison could be made, the mean difference between the slow-potential reversal level and the depolarization caused by 5×10^{-5} g/ml. GABA, was only 1·2 mV (0–3 mV); and in the two cases in which the disparity was largest there was evidence that the GABA effect was not maximal. The mean depression of excitatory potentials by 5×10^{-5} g/ml. GABA was about five times larger than that produced by the slow p.s.p.'s. This is probably not incompatible with the suggestion that GABA and the slow p.s.p.'s affected the conductance to the same ion or ions. The action of the transmitter was relatively brief, whereas that of GABA was prolonged and, possibly, on a larger number of sites in the post-fibre membrane.

The effect of GABA on the lateral giant pre-fibre was also tested. In three of the four experiments, pre-fibre membrane resistance and potential were quite unaffected by 5×10^{-5} g/ml. GABA. In the fourth case the slight reduction in membrane potential and resistance was accounted for by the fact that the lateral giant motor synapse did not show the usual rectification and thus the reduced membrane resistance of the post-fibre short-circuited the pre-fibre.

DISCUSSION

The reversal in the sign of the slow p.s.p.'s cannot be accounted for by the junctional rectifier mechanism found at the giant motor synapses. On the other hand, the behaviour of the slow p.s.p.'s resembled that of the inhibitory potentials at certain peripheral junctions in crustaceans (Fatt & Katz, 1953;

Kuffler & Eyzaguirre, 1955), and the 'chemical' mechanism of transmission that was proposed in those cases can also be applied to the present one. Thus, both 'chemical' and 'electrical' synapses are apparently present on the same post-fibre.

The recorded reversal level would have differed from the equilibrium potential if there was spatial decrement of potentials between the electrodes and the synapses, or if the latter were distributed over the length of the post-fibre (Burke & Ginsborg, 1956). The apparent equilibrium potential would also have varied with changes in the resting potential. In addition, the equilibrium level would differ from that in the intact animal if the normal distribution of ions across the post-fibre membrane was altered by the experimental procedure. The small difference between the mean reversal levels recorded with micro-electrodes containing different salts suggests that leakage from the electrodes was not an important factor. Diffusion of salts would be expected at the cut end of the post-fibre and at other regions damaged by dissection and micro-electrode insertion, but the importance of these effects in altering the equilibrium potential is not known. In the absence of more information about the 'normal' level of the equilibrium potential and the reflex connexions of the pre-fibres, the function of the slow p.s.p.'s cannot be decided conclusively. We have not demonstrated inhibition as such, but only a depression of subthreshold excitatory p.s.p.'s. The mean depression in fourteen experiments was only 20%, and in most of the cases there was also a slight augmentation of the excitatory potential, due to its summation with the declining phase of the slow p.s.p. On the other hand, it should be pointed out that the percentage depression does not provide a good measure of the effectiveness with which impulse transmission is prevented. For example, a slow potential would not depress at all an excitatory p.s.p. which was just equal to the reversal depolarization, even though it might reduce and bring below threshold a larger excitatory potential. A better measure of the intensity of the 'inhibitory action' is given by the percentage depression of that fraction of the excitatory p.s.p. which exceeded the reversal depolarization. In the five experiments in which this could be determined a mean value of 28% (9–69%) was found, whereas in the same experiments the mean depression of the total amplitude of the excitatory potential was only 13·5% (6–40%).

There is an additional factor which might have contributed to the relatively slight depression obtained in some of the experiments. A number of pre-fibres give rise to the slow response and there was no way of knowing if all of them had been stimulated, or whether those axons which did fire made synaptic contact with the post-fibre close to the relevant giant motor synapse.

SUMMARY

1. A description is given of small slow post-synaptic potentials which arose in giant motor fibres, either spontaneously or as the result of stimulation of the dorsal surface of the nerve cord. In size and time course these p.s.p.'s differed markedly from the excitatory 'giant' p.s.p.'s.

2. The sign of the slow p.s.p.'s was dependent on the level of the membrane potential of the giant motor fibre. At membrane potentials above a particular level (the 'reversal potential') the p.s.p.'s appeared as depolarizations; below this level they appeared as hyperpolarizations. The mean reversal potential was about 7 mV below the resting potential of the giant motor fibres. The observation of such a reversal point is regarded as evidence that a 'chemical' transmitting mechanism must be responsible for the production of these slow p.s.p.'s.

3. The slow potentials depressed the excitatory p.s.p.'s arising at the giant motor synapses. However, it cannot be stated whether they constitute a mechanism which is normally involved in regulating transmission across these synapses.

4. Gamma-aminobutyric acid in concentrations of $3–5 \times 10^{-5}$ g/ml. mimicked some of the effects of the slow p.s.p.'s.

It is a pleasure to thank Professor B. Katz for continual advice and encouragement during the course of the work, and Dr P. Fatt for his helpful suggestions and comments. Mr J. L. Parkinson provided invaluable help, and frequent technical assistance was received from Audrey M. Paintin and K. S. Copeland. Financial support has been received from the National Science Foundation and the U.S. Public Health Service.

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