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PRESYNAPTIC HYPERPOLARIZATION: A ROLE FOR FINE AFFERENT FIBRES

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As nerve impulses arrive at the spinal cord and enter the terminal arborization of afferent fibres, they are subject to a pre-synaptic control mechanism. This mechanism has its effect by modulating the membrane potential of the terminal parts of the fibre. Previous studies have shown that presynaptic control can decrease the postsynaptic effect of arriving impulses (Howland, Lettvin, McCulloch, Pitts & Wall, 1955; Frank & Fuortes, 1957; Eccles, 1961). This paper provides evidence that the presynaptic control mechanism is in continuous operation and that arriving nerve impulses may either increase or decrease the membrane potential of neighbouring fibres and, therefore, may either facilitate or inhibit pre-synaptically.

The evidence that depolarization of the terminal arborization reduces the excitatory effectiveness of impulses has been extensively reviewed by Eccles (1961). The evidence that hyperpolarization of the terminal arborization will increase the effectiveness of impulses comes mainly from studies of post-tetanic potentiation where the positive after-potentials of impulses in a high frequency volley add to each other and leave a long-lasting hyperpolarization in the terminals of those fibres which have carried the volley (Lloyd, 1949; Wall & Johnson, 1958). There are two theories to explain the coupling of the effectiveness of the impulse to the membrane potential. One suggests that the amount of hypothetical transmitter substance released is controlled by the presynaptic membrane potential (see Takeuchi & Takeuchi, 1962). The other suggests that the membrane potential may control the transmission of impulses into terminal arborizations by blocking them (reviewed by Wall, 1964). These two theories are not exclusive of each other.

The membrane potential of the terminals of afferent fibres in mammals cannot be measured directly because of their small diameter. On the other hand, changes of the membrane potential of terminals with respect to the parent axons can be measured very easily as the dorsal root potential (DRP) (Barron & Matthews, 1938; Lloyd & McIntyre, 1949). A more

direct and localized measure of membrane potential is to test excitability of the arborization with micro-electrode stimulation (Wall, 1958).

The first evidence that the arrival of a volley of nerve impulses at the cord was followed by prolonged depolarization both of the active fibres which had carried the volley and of their passive neighbours was provided by Barron & Matthews (1938). They measured the DRP between the cut end of a dorsal root and its root entry zone. It was shown later (Wall, 1958) that this potential measured primarily the depolarization of the terminals of the large afferent cutaneous fibres. The mechanism which brings about this depolarization is still under debate. Wall (1962) attributes it to the activity of the small cells of substantia gelatinosa, while Eccles, Kostyuk & Schmidt (1962) believe it is generated by the activity of deeper cells. This issue is discussed elsewhere (Wall, 1964). Whatever the origin of the depolarization may be, there is no doubt that it produces presynaptic inhibition either by block (Howland *et al.* 1955; Wall, McCulloch, Lettvin & Pitts, 1955), or by the decreased synaptic excitatory effect of impulses of decreased height (Frank, 1959; Eccles, Eccles & Magni, 1961).

There have been three types of experiment reporting hyperpolarization of terminal arborizations. The first is post-tetanic potentiation, which has already been mentioned. This phenomenon is restricted to the active fibres and, unlike the other phenomena discussed here, seems to be produced entirely by the properties of active presynaptic membrane without involving the activity of other cells. The next is the report by Lloyd (1952) of a long small postive DRP, DR VI, which followed the large negative DRP. It was observed only in unanaesthetized spinal cord preparations. One other type of positive DRP has been briefly reported by Lundberg & Vyklicky (1963). They observed that a large negative DRP produced by impulses descending from the mid-brain could be decreased by the arrival of a peripheral afferent volley. This phenomenon is probably the same as the second type of hyperpolarization reported in this paper. That is, the positivity is with respect to the base line observed just before stimulation. In Lundberg & Vyklicky's experiment this base line would be the peak of the large negative DRP.

The experiments to be described proceeded in three stages. First, we considered the possibility that some level of presynaptic inhibition might be continually present and that it was being generated by the 'resting' afferent barrage which arrives at the spinal cord. Next, having found signs of a tonically active mechanism, we investigated the role played by small diameter peripheral nerve fibres in setting the level of the membrane potential of the terminal arborization of cutaneous nerve fibres. Lastly, we manipulated the intensity of the afferent barrage in various fibre groups and investigated the effect of arriving volleys when the presynaptic control

mechanism has been set at various levels. As will be seen, we have found evidence that presynaptic facilitation occurs as well as inhibition, and that activity in fine fibres presynaptically facilitates the effects of large fibres by hyperpolarizing the terminal arborization of these larger fibres.

METHODS

All experiments were carried out on spinal cats. Under ether anaesthesia, the carotid arteries were ligated, the cord sectioned at C1 and the basilar artery cut and occluded by pressure. After this, no further ether was administered. The animals were paralysed by intravenous gallamine triethiodide. The lumbar enlargement and popliteal fossa were opened and exposed tissue was covered with paraffin oil or, on occasions, with silicone oil (Dow Chemical Corp. No. 666). Temperature of the body and oil baths was maintained at 38° C. The results to be reported were seen only in animals with an execllent blood circulation in the spinal cord. Small arteries on the lateral surface of the cord could be seen pulsating vigorously and there was no sign of sludging of blood in any exposed vessels observed through a dissecting microscope. We used only healthy cats weighing more than 2 kg and found it important to give the initial anaesthesia with fresh anaesthetic ether and to clean surfaces over which the ether passed. Roots were dissected out under the microscope. It was found important to avoid even slight traction on dorsal roots since this resulted in repetitive depolarization of their terminals and the generation of large dorsal root reflexes. Animals with an adequate cord circulation and no local damage showed the long positive DRP, DR VI, of Lloyd (1952).

All recordings were made through cathode followers. Direct current amplification was by means of a Tektronix 2A63 leading to a Tektronix 502. Great care had to be taken to isolate the stimulus from ground in order to prevent current spread. This was particularly important here because we were forced to use relatively high voltages, up to 20 V, in order to stimulate peripheral C fibres. For relatively small nerves such as the sural, isolation through General Radio type 578-A transformers was adequate but for larger nerves it was necessary to use a battery-operated stimulator which was actuated by a pulse to a switching diode.

The major technical problem in these experiments was the generation of an afferent volley limited to impulses in fine fibres with no impulses being produced in the large fibres. We tried one after another of the methods previously used for the generation of fine fibre volleys and reviewed by Douglas & Ritchie (1962). Three methods seemed suitable for our purposes, but we found on attempting to use them that they had to be rejected. Pressure block could not be used because it was irreversible, uncontrollable, and did not produce complete separation of C fibres from A fibres. It was true that pressure applied to a nerve produced a greater reduction of transmission in large fibres than in small ones, but the selectivity was too poor for our purposes. We then attempted to use the technique developed by Kuffler & Vaughan-Williams (1953) for the isolation of A delta from larger A fibres in the frog. We wished to separate C from A impulses in a mammal but were unable to do so. The technique consists of stimulating a cut peripheral nerve with the cathode on the cut end and the anode at some distance proximally. A prolonged square wave is applied to the nerve and impulses are initiated at the cathode. Impulses travelling with a high velocity in the larger fibres arrive at the proximal anode and are blocked. The square wave is then removed and the slower-travelling impulses which have not yet reached the anode may proceed unhindered. The problem with this clever method, which makes it unusable in mammals if the size of the initial stimulus is large enough to evoke impulses in delta and C fibres, is that the removal of the stimulus generates impulses in the large A fibres as an 'off' stimulus. We spent considerable time forming the shape of the stimulus to produce various linear and sigmoidal declining phases in an attempt to remove the anodal block so gently that we did not generate

an off response. We never succeeded in achieving the triple requirement of generating a C volley, blocking the A volley, and not generating an 'off' response as we removed the block. We failed with the technique on the sural nerve both when we used Kuffler and Vaughan-Williams' single pair of electrodes and when we attempted to evoke the volley at the end of the nerve with one pair of electrodes and then to block the A volley with an independent battery-operated pair of blocking electrodes.

We turned next to the technique originally developed by Bishop & Heinbecker (1935) and used extensively by Laporte & Bessou (1958). In this method, a tetanus is applied to the nerve for a period of some seconds and then it is observed that single shocks applied to the nerve for some short time after the end of the tetanus fail to produce impulses in the large fibres. There are two obvious disadvantages to the technique. First, the spinal cord has been pre-conditioned by the tetanus which has been used to establish the block. Secondly, the block is unstable and therefore it is difficult to do any but the simplest experiments. We found on examining the method a third objection which made it quite useless for our purposes. After the tetanus and during the apparent block period, we found clear signs that asynchronous volleys of impulses in large A fibres were being generated from the region of the electrodes which had delivered the tetanus. Therefore, it was clear that, while the method did in fact establish preferential block of large fibres, it also generated a low level but continuous asynchronous barrage in the very fibres one wished to block.

From a consideration of the way in which the tetanus block must work, we developed a much simpler method which had been mentioned briefly by Kuffler & Gerard (1948). Bishop & Heinbecker (1935) produced the block by high frequency stimulation, but factors other than the impulses must be involved because the stimulus needed to elicit the block is grossly supramaximal for the blocked fibres. Therefore, it seemed reasonable to us that the fibre membrane under the stimulus electrodes was being charged to some level which an arriving impulse could no longer discharge. The block following the supramaximal tetanus stimulation presumably lasts until this charge leaks off. We therefore decided to try the effect of simple polarization on a nerve to see if we could establish a steady preferential block of large fibres. We placed the nerve on two trough silver–silver chloride electrodes. Each trough was 6 mm long and separated by 3 mm. The contact of nerve to metal was through cotton soaked in mammalian Ringer's solution. A battery is connected across the two polarizing electrodes with the anode proximal. As the current is raised, a stage is reached at which impulses are continuously generated from the cathode, many of which pass beyond the anode. A further increase of current introduces a block of large A fibres about the anode and the current can be increased until only C fibre impulses will pass the anode. Provided that low resistance contact between the nerve and electrodes has been established, the block can be applied reversibly for periods of minutes. Details of currents and electrode placement are provided below. We presume that this preferential block works because the amount of current flowing into and out of a nerve fibre is determined in part by the fibre's internal resistance which will vary with the square of the fibre diameter. Therefore, we can assume that a continuous anodal block will be established in the same order as impulses are generated at the cathode. That is to say, anodal block will appear first in the largest fibres as the blocking current is increased.

RESULTS

Steady state depolarization of terminal arborizations

Barron & Matthews (1938) reported in their original paper on DRPs that a tetanus delivered to a peripheral nerve would produce a steady negative DRP for the duration of the tetanus. In 1957, following a suggestion by Dr W. McCulloch that the spinal cord might be generating a

dorsoventral steady resting potential, a number of us in this laboratory (R. Gesteland, J. Y. Lettvin, and P. D. Wall, unpublished results) observed a steady dorsal cord potential which was influenced by the position of the leg. In the present experiments, we dissected a rootlet from the L6 dorsal root, cutting the peripheral end of the rootlet. The rootlet was placed on well chlorided silver hooks with one on the peripheral end and one close to, but not touching, the cord. Gentle, steady pressure was then applied to a single toe pad. Throughout the period of pressing, the central electrode became 150 μV negative to the peripheral electrode, a negative steady DRP, Fig. 1*a*. With the leg hanging down, a board was raised so that the foot was resting lightly on the board and again during the period of contact, the DRP moved about 150 μV negative. Movement of joints without variation of skin pressure produced very little effect, so that it was concluded that the steady DRP was produced mainly by cutaneous afferents rather than by joint or muscle afferents. The position of the pressure point on the skin of the leg had a marked effect on the shape of the DRP recorded on rootlets from either L5 or L6. The effect of pressure on the foot is shown in Fig. 1*a* where it will be noted that removal of the pressure is followed by a swing of the DRP to a positive value. As the stimulus point was moved to dermatomes more caudal than the one represented in the recording root, the size of the negative shift with the onset of pressure diminished while the size of the positive swing when the stimulus was removed increased. Pressure in the perianal region produced very little negative response in an L5 rootlet, but there was a large positive swing when the pressure was removed. The interesting apparent spatial relationship between stimulus point and response will not be discussed further here.

The first obvious question to be asked of this potential is: what is its relation to the DRP evoked by a single shock to a dorsal root? If the two potentials have the same origin, they might be expected to occlude each other since the phasic DRP has an easily attained maximum. We therefore dissected out a second rootlet from L6 and placed its peripheral end on stimulating electrodes. A 1 V, 0·1 msec square wave stimulus was delivered 5 times a second. Each stimulus evoked a negative DRP in the neighbouring recording rootlet lasting about 100 msec. These responses are recorded as a thick band made up of the closely spaced DRPs in Figs. 1*b* and 1*c*. When the pressure stimulus to the foot is applied (Fig. 1*b*) the base line rises, but the peak of the evoked DRP remains at the same height. In Fig 1*c* the same experiment is carried out except that a 1 cm diameter disc vibrating at 100 c/sec is placed lightly on the foot and again it will be seen that the potential evoked by the vibration occludes the potential evoked by the shocks to the dorsal rootlet. These results show that if there is a steady afferent barrage in cutaneous fibres, there will be a continuous

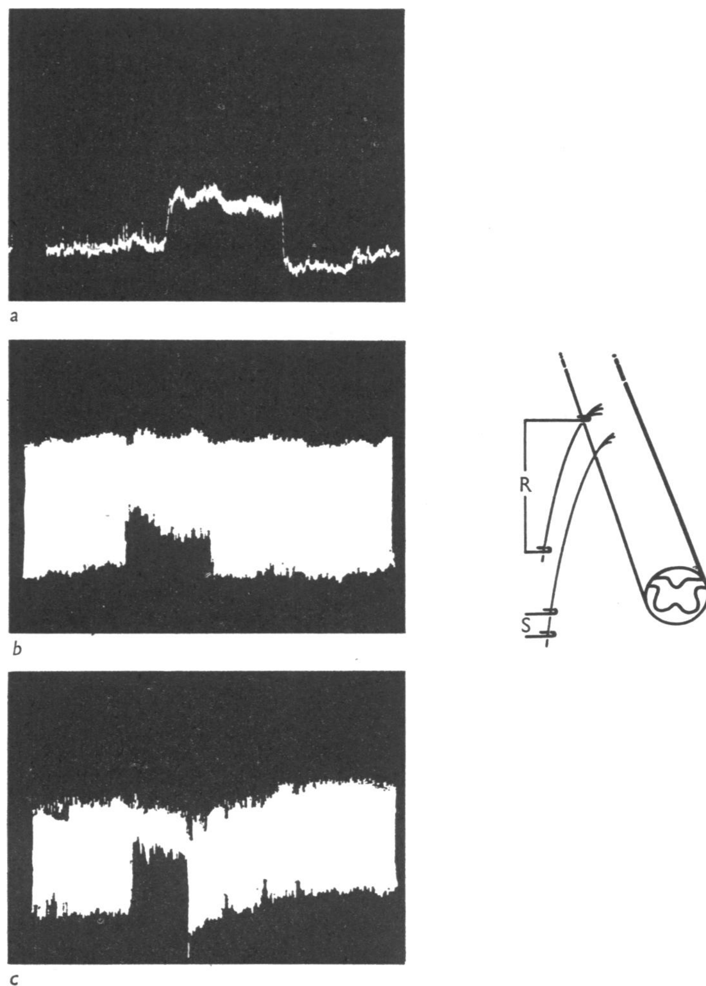

Fig. 1. Continuous generation of negative DRP. Recording made on L6 dorsal rootlet. Frequency range of recording 0–600 c/s. Duration of recording 50 sec. In frame *a*, light pressure was applied to one toe pad and produced a 150 μV steady negative DRP throughout the stimulus. In frame *b*, a phasic DRP was evoked 5 times/sec by applying a 1 V 0·1 msec stimulus to the end of a second rootlet from L6. The thick band is made up of these DRPs placed side by side so that the top of the band represents the height of the maximal DRP which can be produced under these conditions and the bottom of the line represents the starting potential from which the phasic DRP takes off. The foot pad was gently pressed for 12 sec and produced a steady negative DRP which occluded the phasic DRP. In frame *c*, a 1 cm diameter metal plate vibrating at 100 c/sec was placed gently against the foot and evoked a 200 μV steady negative DRP which occluded the phasic DRP which was being produced in the same way as in frame *b*.

generation of a negative DRP. This DRP occludes the ordinary phasic DRP evoked by single shocks to a dorsal rootlet.

Hyperpolarization of terminal arborizations by fine fibres

The phenomenon as we first saw it is shown in Fig. 2. The lateral popliteal nerve was cut, dissected free, and placed on stimulating electrodes. A single shock was given of sufficient intensity to produce impulses in all fibres with conduction velocities down to about 2 m/sec. This volley produced the DRP shown in Fig. 2*a*. This potential consists mainly of the

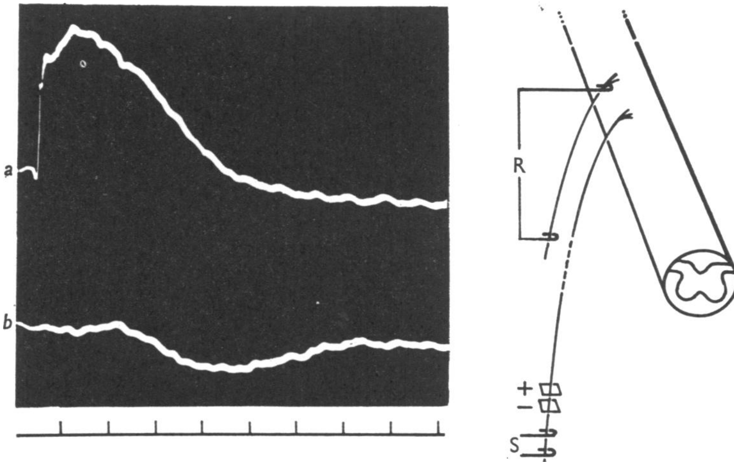

Fig. 2. Purely positive DRP. Stimulus 15 V, 0·5 msec applied to cut end of lateral popliteal nerve. Recording on dorsal rootlet of L6. Time marks 20 msec. Frame *a* shows the normal passive DRP consisting of a large negative phase 250 μV in height, followed by a prolonged smaller positive phase. In frame *b*, the preferential block was introduced by running a current of 5 mA between the polarizing electrodes with the anode proximal. The DRP produced by the same stimulus as in frame *a* is now purely positive.

large negative component, DR V, of Lloyd & McIntyre (1949). It is followed by a small prolonged positive DRP, DR VI (Lloyd, 1952). The nerve was resting on polarizing electrodes, and by interposing an anodal block between the stimulus point and the spinal cord we prevented impulses in the larger nerve fibres from penetrating through the block and reaching the cord (see Methods). As we increased the polarizing current, we observed a decrease in the height of the early negative DRP and an increase of the later positive DRP. Finally, when the polarizing current running through this large diameter nerve was about 5 mA, we observed a purely positive DRP, Fig. 2*b*.

There were three obvious deficiencies in this experiment. We were

stimulating and blocking a large mixed nerve which entered the cord over many segments. The thickness of the nerve made it unlikely that we could produce a uniform current distribution throughout the whole cross-section of the nerve. To overcome these two objections, we turned to the sural nerve, which is a purely cutaneous nerve with its major roots of entry in segments L 7 and S 1. The major deficiency, however, was that we did not know the exact input to the cord. We attempted therefore to place bipolar recording electrodes on the sural nerve proximal to the stimulating electrodes and to the selective blocking electrodes. The resulting diphasic recording was inadequate for our purposes because we could see no signs of the lower velocity components of the compound action potential. Since it was evidently necessary for us to obtain monophasic recordings in order to get an adequate picture of the slower components of the input volley, we next carried out the experiments shown in Fig. 3. The sural nerve was dissected free, cut peripherally and placed on stimulating electrodes with selective blocking electrodes proximal. The rootlets which contained the largest group of afferent fibres from the sural nerve were located in each cat by searching over the surface of the entering rootlets with a pair of stimulating bipolar electrodes. During this procedure, the two electrodes on the end of the sural nerve were used to record the antidromic volley evoked by the searching electrodes. A rootlet in the L 7 segment which contained a minor fraction of the input was selected and cut 25 mm from its entry into the spinal cord. The central end of this rootlet was now dissected free and placed on recording electrodes in order to record the passive DRP evoked by the sural volley. The peripheral end of the rootlet was dissected free and placed on recording electrodes in order to measure the fraction of the afferent volley which ran in that rootlet. The results are shown in Fig. 3. The reader is cautioned to remember that the two traces, *A* for the input volley and *B* for the DRP, are independent of each other. The input trace *A* is moving at 10 times the speed of the DRP trace *B*. In Fig. 3*a*, the stimulus was adequte to evoke impulses in all *A* fibres, and the expected DRP is shown in *B*. In Fig. 3*b*, the polarizing current was turned on to sufficient intensity to block all impulses travelling at speeds above 50 m/sec, giving rise to a DRP with only a small negative phase. In Fig. 3*c*, the blocking current was raised so that no impulses arrived at the cord in the faster fibres. The DRP evoked by this volley is purely positive and delayed 60 msec. However, no clear sign of the input volley can be seen on the upper trace. The method has two obvious disadvantages. We were forced to sample the input volley from the rostral edge of the fan of entering fibres from the sural nerve, and one cannot be sure that the fraction monitored is representative of the whole. The second problem is that with the long conduction distance, more than 160 mm,

the slower components of the compound action potential are so dispersed that they cannot be recorded.

We therefore developed the method shown in Fig. 4. The sural nerve was dissected free from the mid-thigh to the lower leg and then folded back

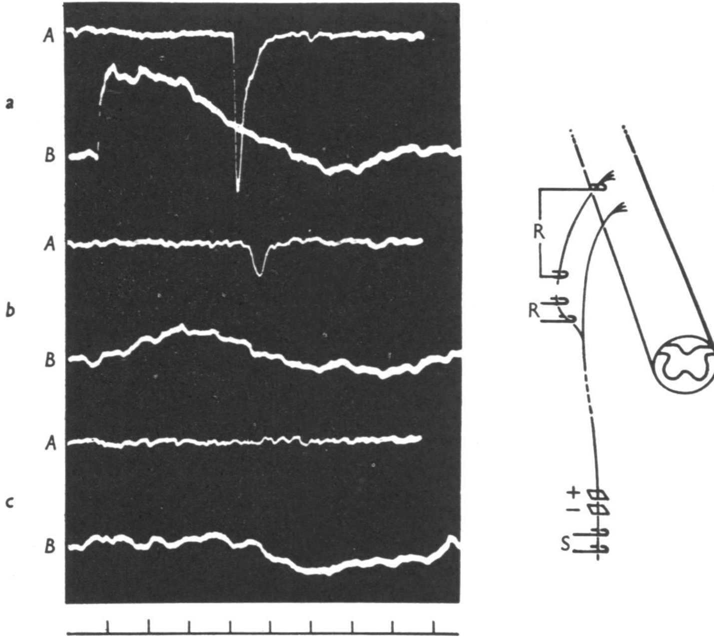

Fig. 3. Effect of preferential blocking on DRPs and on input recorded on a root. The stimulus was 10 V for 0·2 msec applied to the cut end of the sural nerve. Recording *A* was made on the peripheral part of a cut dorsal rootlet in L 7 to record a sample of the incoming volley. The time base for this trace is 2 msec per division. Recording *B* was made from the central part of a cut rootlet of L 7 to record the DRP. The time base for this trace is 20 msec per division and it therefore should not be aligned with trace *A*. Frame *a* shows the input (*A*) and the DRP (*B*) recorded when the full volley arrives at the cord from the sural nerve. Frame *b* shows the DRP and input when the fastest group of A fibres have been blocked by a polarizing current of 50 μA. Frame *c* shows the positive DRP and the apparent absence of input when all faster conducting A fibres have been blocked by a polarizing current of 100 μA.

on itself. The double portion of the nerve was then placed on the stimulating and selective blocking electrodes. Recording electrodes were placed on the end of the sural. The antidromic volley recorded on these electrodes was identical with the orthodromic volley which proceeded up the other arm of the sural nerve, entered the cord and evoked a DRP, recorded on a sectioned rootlet dissected from the rostral part of L 7 dorsal root. Figure 4*a*, trace *B* shows the compound action potential recorded on the end of

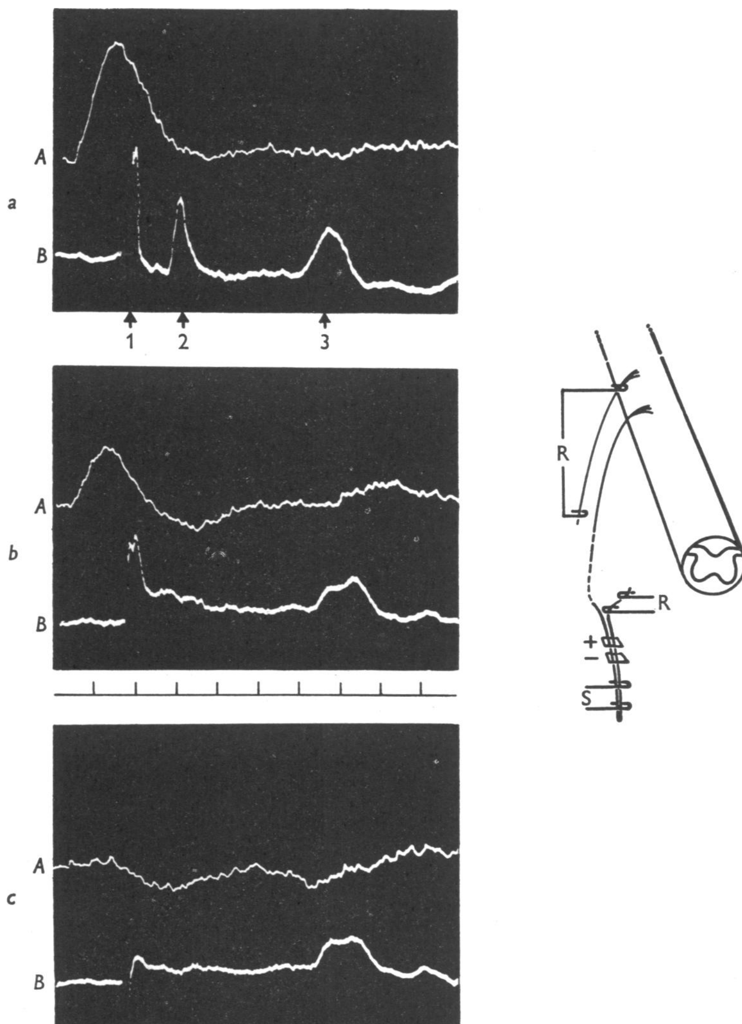

Fig. 4. Recording of DRP and the complete input volley which evoked it. Stimulus applied at point where sural nerve is doubled back on itself, 20 V for 0·5 msec. Recording *A* shows DRP evoked by sural volley. Time base 50 msec per division. Recorded on a rostral rootlet of L7. Recording *B* shows the sural volley recorded antidromically on the end of the sural nerve. Time base for this trace is 5 msec per division. The compound action potential shows a number of components which are discussed in the text. Frame *a* shows the DRP (*A*) recorded when the full volley arrives at the cord from the sural nerve. Frame *b* shows the DRP produced when the fastest A component has been blocked by a polarizing current of 50 μA. Frame *c* shows a two-phase positive DRP recorded when the input consists only of impulses in a group of slow A fibres and in the C fibres. The block was produced here by a current of 100 μA.

the sural nerve when a stimulus of 20 V and 0·5 msec duration was delivered. There are three large components marked. One has an amplitude off the screen and contains the stimulus artifact and the fastest A components. The second A component (A delta) can be seen after phase 1 with a conduction velocity about 3 m/sec. Component 2 is produced by repetitive firing of the fastest A fibres as a consequence of the very high stimulus strength. Component 3 shows the C fibre volley with a conduction velocity beginning at about 1 m/sec. All conduction velocities were checked by varying the conduction distance. This complex volley produces the DRP shown on a slower time base in Fig. 4*a*, trace *A*.

Next, in Fig. 4*b*, the selective block was applied with a current of 50 μA and it is apparent the fastest fibres have been blocked; their repetitive firing has disappeared and the DRP has lost much of its negative component and the positive has increased. It will be noted that the A delta and C fibre impulses have been slightly slowed down as a result of their passage through the region of block. Next, in Fig. 4*c*, the blocking current is increased to 100 μA. The disurbance seen in the region of the previous wave 1, is now entirely stimulus artifact. Some A delta impulses can be seen travelling at 2 m/sec and these produce a positive DRP. Finally, the C fibre component appears and produces a second positive DRP. If the blocking current is increased further, the delta input disappears and so does the first positive DRP. These results appeared consistently in cat after cat.

Effect of barbiturate anaesthesia on positive dorsal root potentials

If a light anaesthetic dose of intravenous barbiturate, 50 mgm pentobarbitone/kg, was given, the positive dorsal root potential produced by the fine fibre afferents was immediately and completely abolished. As previously reported by Lloyd (1952), such a dose of barbiturate markedly affects the DRP produced by a single A volley. It produces a marked prolongation of the negative component from about 90 msec to more than 300 msec and at the same time it abolishes the positive component.

Effect of varying the steady state dorsal root potential on the shape of phasically evoked dorsal root potentials

From the above experiments, a picture appears of two opposing effects resulting from the arrival of a mixed volley. The first is a negative DRP; the other, a positive one. We therefore wondered what would happen if we produced a steady depolarization of the endings, and therefore, a steady negative DRP, and then fired in a mixed volley. The steady depolarization as shown in section 1 can be produced by tetanus of a peripheral nerve, by steady pressure on the skin or by vibration. The results shown here, Fig. 5, were produced by tetanus of a dorsal root, and similar results were obtained

by skin pressure or vibration. The DRP was recorded from a rootlet of L 6. First, dorsal root L 5 was stimulated and produced the typical passive DRP shown on the left in Fig. 5*a*. 150 msec later, dorsal root S 2 was stimulated and produced a second smaller DRP shown on the right of Fig. 5*a*. The strength of stimulus to both roots was adequate to produce impulses in most A fibres. Next, a continuous tetanus was applied to a rootlet of L 7 at 500/sec, 0·05 msec duration with a strength adequate to produce impulses in A fibres. A consequence was to produce a continuous

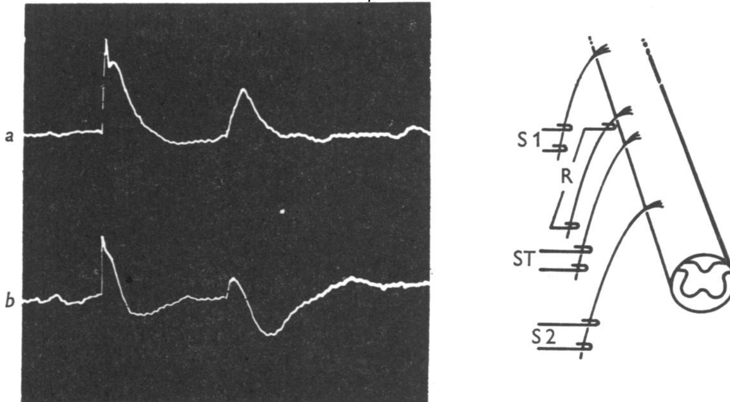

Fig. 5. Effect of tonic DRP on shape of phasic DRP. Recordings of DRP made on rootlet of L 6. The first stimulus (S 1) was applied to L 5 dorsal root and evokes the DRPs seen on the left. 150 msec later, a stimulus was applied to the S 2 dorsal root. The stimuli to these two roots were 2 V 0·1 msec. Trace *a* shows the two DRPs, the one evoked by S 1 on the left, and that produced by S 2 on the right. Trace *b*, a continuous tetanus was now applied to a rootlet of L 7 at 500 pulses/sec, 0·05 msec duration, 0·5 V intensity (ST). This tetanus produced a steady negative DRP. Under these conditions, the two stimuli, S 1 and S 2, now produce DRPs with a reduced negative phase and a greatly enhanced positive phase.

negative dorsal root potential. The effect on the phasic DRPs can be seen in Fig. 5*b*. The negative phase is decreased and the positive is enhanced. The effect is much greater on the DRP evoked by the caudal root than on the one from the rostral root. The effect was found on stimulating, tetanizing, and recording from many combinations of roots whenever the tetanized root was interposed between the stimulated root and the recording root. The rate of decline of the negative slow potential is greatly increased by the presence of the tetanization.

Relation of positive dorsal root potential to excitability of terminal arborization

It has been shown several times (Wall, 1958; Eccles, 1961; Lundberg & Vyklicky, 1963) that negative DRPs represent a depolarization of the terminal arborization of some of the fibres in the dorsal root. Since a

positive DRP might represent a process of a different kind, it was necessary to show that the positive DRPs we have described were in fact associated with a decrease of excitability of the terminal arborization and therefore

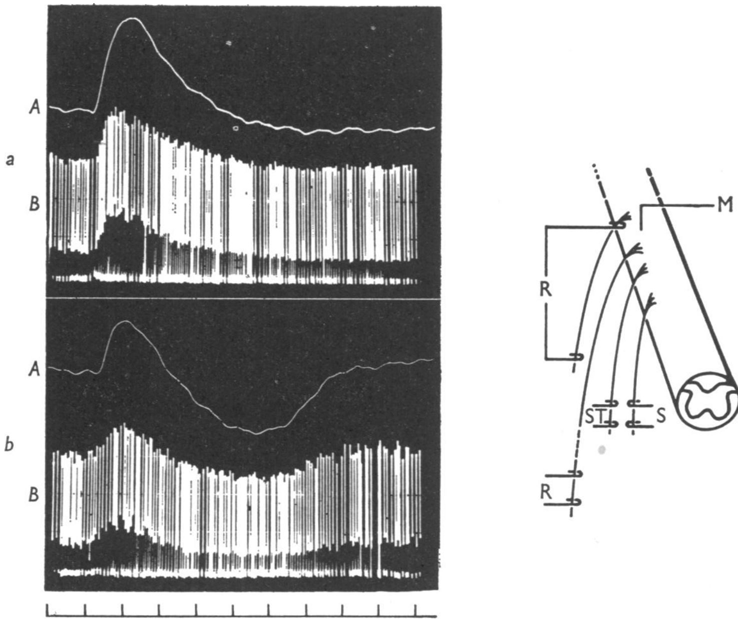

Fig. 6. Association of negative and positive DRPs with excitability changes in the terminal arborization. Trace *A* is the dorsal root potential evoked in an L 7 dorsal rootlet by stimulation of dorsal root S 2 with 2 V, 0·1 msec. Frame *a* is the normal situation, and frame *b* shows the modified DRP which is produced if a steady negative DRP is evoked by an ongoing tetanus of an L 7 rootlet at 500 pulses/sec, 0·05 msec duration, 0·5 V intensity. Trace *B* shows the change in height of the antidromic volley recorded on the end of the sural nerve when a stimulus is applied through a micro-electrode in the terminal arborization of the sural nerve, at the indicated phases of the DRP. (For details, see text.) Each frame was produced by superimposing 120 records of paired conditioning and test stimuli with the C–T interval changed to sweep over the full duration of the DRP. Time base 20 msec per division. Frame *a* shows that, during the normal negative DRP, there is an increased excitability of the terminals. Frame *b* shows that when there is a positive DRP following a small negative DRP, the excitability increases during the negative phase and decreases during the positive phase.

with an increase of membrane potential. The method of testing and precautions which must be taken has been previously described (Wall, 1958). In Figs. 6*a*, trace *A* and 6*b*, trace *A*, DRPs are shown which are similar to the two right-handed DRPs shown in Fig. 5. They are the DRPs produced in L 7 by a single stimulus to S 2 dorsal root; in Fig. 6*b*, trace *A* a continuous tetanus is applied to a rootlet between the stimulated and recording root and, as before, it is apparent that the tetanus produced a decreased negative

DRP and an exaggerated positive DRP. Figure 6*a*, trace *B* and Fig. 6*b*, trace *B* show the testing of excitability of the terminal arborization during the two types of DRP. The terminal's excitability was tested by placing a stimulating micro-electrode in the dorsal horn of the cord in rostral S1 in the region of the terminals of the sural nerve in layer IV of Rexed. The micro-electrode was glass-covered cylindrical 12 μ platinum wire with the end ground to a needle point. When a 0·1 msec pulse was applied to the micro-electrode negative with respect to a distant diffuse anode, an antidromic volley was generated in the afferent fibres of the sural nerve and appeared out on the cut end of the nerve where they were recorded. The area of the A alpha component of the antidromic action potential was recorded as a single vertical line. When the length of the line decreases, it means that a fixed strength of stimulus within the cord stimulated fewer afferent fibres antidromically, and therefore the excitability of the terminals is decreased. The time between the conditioning stimulus to the dorsal root and the test stimulus to the terminals is varied. A pair of test and conditioning stimuli was given every 2 sec. The *B* records in Fig. 6 are made by superimposing 120 test records of the antidromic volley taken during the indicated stages of the DRP. Figure 6*a* repeats the previously published result and shows that the terminals of the sural nerve increase their excitability during a negative DRP. Figure 6*b* shows that the terminals decrease their excitability during the positive DRP.

Effect of C fibre impulses on reflex effects of A fibre impulses.

We have shown above that an afferent volley, consisting only of C fibres, produces a positive DRP. A tetanus of C fibre impulses produces a tonic positive DRP. A positive DRP is associated with a hyperpolarization of the A afferents. Taking these three facts together, it is now of considerable interest to observe the reflex effect of the A afferents and the C afferents, singly and in combination. In our experiments, we saw no reflex effects of C fibres if they were fired in isolation. We have tried single volleys, multiple bursts, and continuous tentanus, and have not yet seen a ventral root output as a result of any form of isolated C fibre input from the sural nerve. C fibre stimulation was arranged as previously shown in Fig. 4. A long length of sural was dissected free, doubled on itself, and placed on stimulating and blocking electrodes. We recorded the afferent C volleys, but do not show them here. We cut the ventral root S1 and placed it on recording electrodes. No combination of C volleys produced a reflex on the ventral root. We used small fractions of the ventral root on which it is easy to observe an asynchronous tonic output, and saw no such output with tetanization of the C fibres, although an output was easily recorded if any A fibre impulses were present in the afferent volley. Figure 7*C*

shows the absence of response on a part of the ventral root S1 during a maximal C fibre tetanus at 200/sec produced by 20 V 0·5 msec stimuli with a 100 μA blocking current. The small vertical lines in the record are stimulus artifacts. Next, the polysynaptic reflex evoked by fast A fibres was produced by placing stimulating electrodes on the sural nerve proximal to the two pairs of electrodes used to produce the C fibre volley. A shock

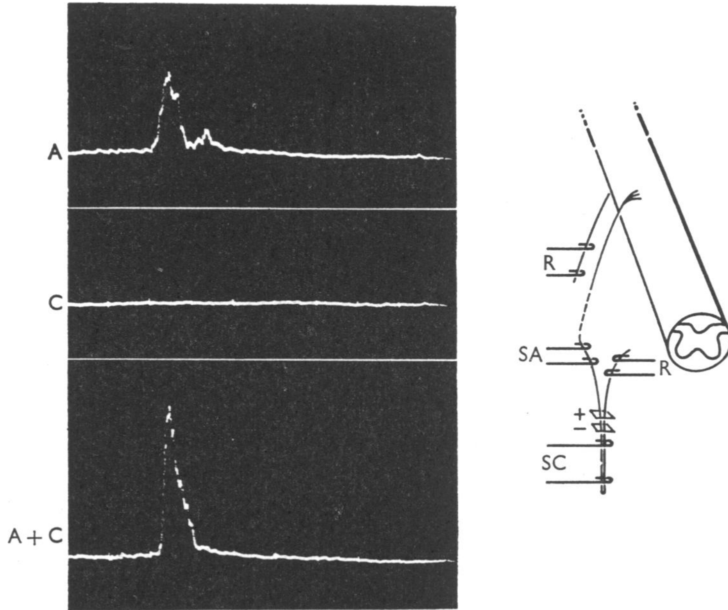

Fig. 7. C fibre tetanus facilitates reflex evoked by A fibre volley. The top record shows reflex which appeared on ventral rootlet from S1 when a 0·5 V 0·05 msec stimulus was applied at SA on the sural nerve. This stimulus was adequate only for the fastest component of the A fibres. This volley was monitored on the end of the sural nerve, but is not shown here. The middle record shows the absence of activity on ventral root S1 when a continuous C fibre tetanus was evoked in the sural nerve at 200/sec by stimuli of 20 V 0·5 msec. The method of producing a pure C fibre volley is that shown in Fig. 4. The preferential block was established with a current of 100 μA. Stimulus artifacts on this trace at 5 msec. The lowest record shows that the C fibre tetanus, which produces no reflex itself, greatly facilitates the reflex produced by A alone. The C tetanus was continuous, the A stimulus was exactly that shown on the A trace.

of 0·5 V 0·05 msec duration was applied, and the resulting reflex is shown in Fig. 7*A*. When a fast A fibre volley was superimposed on top of the C fibre tetanus, there was a marked potentiation of the reflex, Fig. 7, *A* & *C*.

DISCUSSION

We would like to discuss these observations in terms of a series of postulates.

Postulate 1. The spinal cord is normally continuously bombarded by some cutaneous afferent impulses

There have been many reports of 'spontaneously' active cutaneous fibres (Hensel, Iggo & Witt, 1960; Wall, 1960; Hunt & MacIntyre, 1960, etc). Furthermore, apart from these fibres which carry impulses in the absence of any obvious stimulation, there are many non-adapting or, more usually, partially adapting pressure receptors. Since an animal normally has its feet on the ground, in fact as well as metaphorically, these pressure points will generate a continuous barrage. In the artificial situation of the physiologist's animal, there are, in addition, the various incisions and damage points which will contribute to the general cutaneous afferent barrage.

Postulate 2. One effect of this barrage is to hold the terminals of large cutaneous afferents partially depolarized

We have shown in the first series of experiments reported above that very light pressure to the skin produces a continuous negative DRP. We have also shown that this DRP occludes the DRP evoked by a single volley and therefore we can assume that they are two aspects of the same mechanism. As described in the introduction, a negative dorsal root potential implies that the terminals are depolarized with respect to the parent axons. The cord is also being continuously bombarded by proprioceptive afferents. Eccles and his colleagues (reviewed by Eccles, 1964) have shown that short bursts of impulses in these fibres will also result in depolarization of the terminals of cutaneous afferents. We can assume, therefore, that there is normally continuous activity of the mechanism which generates negative DRPs and, therefore, continuous depolarization. We can be quite certain from our results that if one toe pad is in light, steady contact with the ground, there will be steady depolarization of some terminals. It should be emphasized that the mechanism for generating terminal depolarization affects not only the fibres which carry the afferent barrage, but also their passive neighbours. A by-product of the presence of a tonic DRP which occludes the phasic DRP will be that reports on various manipulations which increased and decreased the height of the DRP will now have to be re-investigated to see if they were the consequence of a steady shift of the base line.

Postulate 3. Afferent impulses in fine fibres hyperpolarize the terminals of large afferent cutaneous fibres

We have shown above that, if a pure C fibre volley is fired into the cord, an entirely positive DRP is generated. So far, this is the only central effect

of such a volley which we have detected. This contrasts with the work of Laporte & Bessou (1958), who reported that multiple volleys of C fibre afferents produced a ventral root reflex. As discussed above, we did not find their method of blocking A fibres satisfactory. They recorded their input volley diphasically, which is so insensitive that low intensity delta volleys would not have been detected. There is a more intriguing explanation of their results. As reported above, the tetanic method of block used by Laporte and Bessou not only blocks the larger fibres, but also generates a continuous asynchronous volley in the A fibres beyond the block. It seems possible now that the C fibre volley they were generating produced a hyperpolarization of the terminals of the A fibres, and by this mechanism potentiated the effect of the arriving A volleys. Similar arguments now make it very difficult to interpret other reports in the literature of central effects of C fibres (such as Collins & Randt, 1960) where C volleys have simply been added to A volleys by gradually increasing the strength of the peripheral stimulus. The appearance of some late phenomenon after a huge universal volley has been delivered from a mixed peripheral nerve may be the result of repetitive firing, or it may be due to a potentiation of a large fibre system from hyperpolarization of its endings by the effect of fine fibres as described above. It will be necessary to investigate apparent delayed central effects of a large volley for these phenomena before attributing them directly to the arrival of impulses in C fibres.

Postulate 4. Afferent impulses in large fibres depolarize the terminals of neighbouring large afferent cutaneous fibres

The general correctness of this has been apparent since the work of Barron and Matthews in 1935. However, we need to discuss the definition of large and fine fibres. Pure positive DRPs are produced by C fibres and by A fibres with a conduction velocity up to about 5 m/sec. These slow A delta fibres not only produce a positive DRP, but also a ventral root reflex. When fibres of a larger diameter are fired, the larger the fibre diameter the larger the negative component of the DRP. Thus, the group of large fibres appear to have a mixed effect. This mixed effect is shown clearly in the experiments where we evoked a steady depolarization of the endings by a tetanus and then fired in a volley of impulses in A fibres. The mechanism for producing a negative DRP was already almost fully in action, and therefore the A volley could only demonstrate its tendency to produce a positive DRP preceded by a small negative DRP (Fig. 5). We cannot say if the fibres producing the positive DRP are the same as those evoking the negative one. The very largest fibres seemed to produce a purely negative DRP. The relative positive DRP shown by Lundberg & Vyklicky (1963) was presumably of this type, where the mixed effect of a large afferent volley

is unmasked when it arrives at a time of a large negative dorsal root potential.

Postulate 5. Hyperpolarization of terminals is produced by inhibition of the tonic depolarizing mechanism

There is no evidence for this postulate, but it represents the simplest hypothesis to tie together the observed phenomena. We have shown evidence that the depolarization of the terminals is produced by activity in the small cells of substantia gelatinosa (Wall, 1962). It is suggested that the large fibres excite these cells and the fine fibres inhibit them. There is an anatomical basis for this opposing action of the two types of fibre. Szentogothai (1964) has shown that fine afferent fibres penetrate into the substantia gelatinosa from the dorsal surface while larger fibres penetrate from the ventral surface. The cells of substantia gelatinosa, he believes, do not send axons outside the region of substantia gelatinosa. The cells are arranged in a general dorso-ventral orientation. We suggest that the large fibres will tend to end on the ventral dendrites of the cell and are excitatory, while the fine fibres will end on the dorsal dendrites of the cell and will inhibit.

Postulate 6. Barbiturate blocks the hyperpolarizing effect

We have shown that the positive DRP evoked by a fine fibre volley is abolished by barbiturate. Lloyd (1952) had already shown that the small positive component of a DRP is abolished by barbiturate. This suggests that an action of barbiturate might be to prevent the ability of an afferent volley to turn off the negative DRP-generating mechanism. The effect of barbiturate is of some importance in the discussion on the mechanism which generates the negative DRP. Eccles *et al.* (1962) believed that the negative DRP was produced by a transmitter substance put out by a group of internuncials, D cells, which fire for about 20 msec after the arrival of various afferent volleys. They postulated that the long time course of the negative DRP was taken up by the removal of the depolarizing transmitter substance. However, they knew that barbiturate greatly prolonged the negative DRP, but reduced the response of their D cells. They therefore had to introduce the highly unlikely hypothesis that barbiturate reduced the amount of their hypothetical transmitter which was released, but at the same time greatly potentiated its action. We believe that this passive theory to explain the decline of the negative DRP is now quite untenable. We have shown above that the time constant of the decline can be altered over a wide range. This makes it far more likely that the negative DRP is under continuous active control by cells whose activity may be increased or decreased fairly rapidly. It is therefore suggested that the ordinary phasic DRP is produced by the continuous activity of

substantia gelatinosa cells whose activity is increased during a rising phase of a negative DRP, and decreased partly under the influence of inhibitory processes, during the falling phase. This inhibition appears particularly sensitive to barbiturate.

Postulate 7. The post-synaptic excitatory effectiveness of an impulse is determined in part by the presynaptic membrane potential

The general reasons for this statement were given in the introduction. It is obvious that if presynaptic hyperpolarization is to potentiate the effect of an impulse, the cell on which the impulse arrives must have a 'subliminal fringe' or, in other words, it must be able to increase its output to an increased input. There is all the usual evidence that the cells of lamina IV on which the large cutaneous afferents end do, in fact, have a substantial subliminal fringe. The most recent evidence (Taub, 1963) shows that the cutaneous receptive fields of these cells can be varied.

Postulate 8. The effectiveness of a large cutaneous fibre input is partly determined by the preceding balance of large and small afferent fibre activity acting through their opposing presynaptic effects

This postulate is reached by combining the preceding postulates. The effectiveness of an impulse in the large cutaneous fibres depends on the presynaptic membrane potential, which in turn is under the control of large fibre afferents which tend to depolarize it, and fine afferents which tend to hyperpolarize it. We have shown that it is possible either to hyperpolarize or depolarize passive terminals by afferent volleys. It has been known since the work of Hagbarth & Kerr (1954) that descending volleys from the head may also affect the DRP. We therefore expect that numerous central mechanisms will also be discovered which control the membrane potential of afferent terminals.

We have shown in Fig. 7 a simple example of the action of fine fibres on the post-synaptic effect of large fibre volley. All indications suggest that this is presynaptic facilitation. The situation is the complementary opposite of presynaptic inhibition which has been generally accepted. The C fibre volley produces a terminal hyperpolarization in the A fibres and the cells on which the A fibres end have a subliminal fringe. The C fibre volley produces no reflex output by itself, but greatly facilitates the reflex produced by an A volley. The final link in the proof of presynaptic facilitation would be to show that the C fibres produce little or no subliminal facilitation of the post-synaptic cells. This we have not yet been able to do, and so for the present we can only say that three of the four requirements for presynaptic facilitation have been shown to exist, and that the fourth has not yet been tested.

SUMMARY

1. Experiments are described in spinal cats which show that the steady arrival of cutaneous impulses at the cord results in a steady depolarization of passive afferent terminals.

2. By the use of a method for preferential blocking of large fibres by anodal polarization, it is shown that impulses in fine fibres produce a hyperpolarization of the terminals of large fibres. This effect is blocked by barbiturate.

3. If terminals are held steadily depolarized, the arrival of impulses in A fibres hyperpolarizes the terminals of passive neighbours. Positive dorsal root potentials are associated with hyperpolarization of the terminals of large diameter cutaneous fibres. C fibre volleys hyperpolarize the terminals of cutaneous A fibres and facilitate the reflex evoked by the A fibres.

4. The results are used to support the following postulates:

(*a*) The spinal cord is normally continuously bombarded by some cutaneous afferent impulses.

(*b*) One effect of this barrage is to hold the terminals of large cutaneous afferents partially depolarized.

(*c*) Afferent impulses in fine fibres hyperpolarize the terminals of large afferent cutaneous fibres.

(*d*) Afferent impulses in large fibres depolarize the terminals of neighbouring large afferent cutaneous fibres.

(*e*) Hyperpolarization of the terminals is produced by inhibition of the tonic depolarizing mechanism.

(*f*) Barbiturate blocks the hyperpolarizing effect.

(*g*) The post-synaptic excitatory effectiveness of an impulse is determined partly by the presynaptic membrane potential.

(*h*) The effectiveness of a large diameter cutaneous fibre input is partly determined by the preceding balance of large and small afferent fibre activity acting through their opposing presynaptic effects.

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