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FACTORS FORMING THE EDGE OF A RECEPTIVE FIELD: THE PRESENCE OF RELATIVELY INEFFECTIVE AFFERENT TERMINALS

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SUMMARY

A specialized type of spinal cord cell has its cell body in lamina IV and has a small low threshold cutaneous receptive field which is remarkable for its abrupt edge. No signs could be found of a subliminal fringe to this field since its size remains fixed during wide excursions of the cell's excitability. Reversible blocking of peripheral nerves and dorsal roots showed that the afferents responsible for exciting these cells following natural stimuli, run in a restricted area of peripheral nerve and dorsal root. When the fibres necessary to sustain the natural stimulus receptive field were blocked, it was shown that other large myelinated fibres in neighbouring roots were still capable of firing the cell monosynaptically following electrical stimulation of the root or periphery although no natural stimuli were able to change the cell's excitability. It is necessary to divide the afferent synapses on such cells into a class which is highly effective in firing the cell on natural stimulation and a second class which has no effect yet detected following natural stimuli but which can fire the cell monosynaptically if synchronously activated by electrical stimulation. Suggestions are made for possible presynaptic and post-synaptic mechanisms which might divide the effect of arriving impulses into two such classes.

INTRODUCTION

In a recent study, Wall & Egger (1971) found that certain cells in the thalamic nucleus ventralis posterolateralis of the rat changed their receptive fields from leg to arm some days after the cells had been deafferented by destruction of nucleus gracilis. There are two likely classes of explanation for this phenomenon. One is that the intact afferents from the cuneate nucleus had sprouted and occupied sites left vacant by the degeneration

of the afferents from nucleus gracilis. The other is that cuneate afferents were already present and became effective following withdrawal of the axons from n. gracilis. Physiological experiments to investigate these alternatives are difficult in the thalamus because of the many convergent inputs. It was decided to investigate the problem on a particular type of cell in spinal cord where manipulation of inputs can be achieved with greater control.

The cells chosen for investigation were a variety of cell in lamina IV of cat lumbar cord whose properties have been extensively described (Wall, 1960; Taub, 1964; Wall, 1967; Fetz, 1968; Pomeranz, Wall & Weber, 1968; Brown, 1971). Their characteristics are that they have small cutaneous receptive fields; they are excited monosynaptically by myelinated afferents: many of them send axons into dorsolateral ipsilateral white matter. Within the receptive field, hair movement and light pressure excite the cell. The edge of the receptive field for these natural stimuli appears to be abrupt (Taub, 1964; Brown, 1971). The edge of the excitatory receptive field is not formed by an inhibitory surround although there may be inhibitory zones, usually separated from the excitatory region (Taub, 1964; Brown, 1971). Furthermore, the size of the receptive field appears remarkably stable when the cell's excitability is varied. The excitability of the cell was judged by the threshold for peripheral stimuli, by the frequency of ongoing activity in the absence of intentional stimuli and by the number of impulses in the repetitive discharge following a single stimulus. Excitability was increased by post-tetanic potentiation, by strychnine, by heating the skin of the receptive field (Wall, 1960) and by removal of descending inhibition by reversible cold block of thoracic cord in a decerebrate animal (Wall, 1967; Brown, 1971). Excitability of the cells was decreased by barbiturate anaesthesia, prolonged asphyxia (Wall, 1960) pyramidal tract stimulation (Fetz, 1968) and brain stem stimulation (Taub, 1964). None of these procedures varied the size of the cells' receptive field. Even the ongoing activity of the cell could not be influenced by natural stimuli outside the receptive field. This series of negative results contrasts strongly with the marked changes of receptive field size produced in lamina V cells by similar manipulations (Wall, 1967; Hillman & Wall, 1969).

It is evident therefore that the boundary of the receptive field for this type of cell can neither be the product of inhibition nor of the transition from a liminal zone to a subliminal fringe. One might expect that afferent fibres might originate from skin outside the excitatory receptive field and would terminate on the cell, facilitate it but be unable to excite the cell. These fibres could be called the 'subliminal fringe' of the effective excitatory afferents. Evidently there is no subliminal fringe of afferent inner-

vation because if it existed we would expect the receptive field size to expand and contract with factors which change the cells excitability. This suggests an anatomical substrate of the receptive field in which a group of afferents converge from a limited area of skin on to a cell and make contact with the cell with a high safety factor of transmission. It further suggests that afferents from skin close to but not within the receptive field either fail to make any contact with the cell or, if they do, these contacts are ineffective in firing the cell even in extreme hyperexcitability. This last suggestion not only summarizes an apparent conclusion from the observed physiology of the cells in adult animals but it also defines the limits of the accuracy with which connexions must be formed during development.

Two equivocal observations had been made which favoured the possibility that a type of connexion existed from skin to these cells which was incapable of firing the cells when natural stimuli were used. Wall (1960) had made progressive cuts through dorsal roots while recording from the axons of lamina IV cells in order to determine the distribution of afferents within the dorsal roots as they coursed from the periphery to converge on to the cell under observation. He found that many roots could be cut without any apparent interference with the cell's response to peripheral stimuli. Finally, a rootlet was cut and the cell failed to respond to natural stimuli. The obvious conclusion was that all the necessary afferents had been running in this critical rootlet. However, it was noted that on sectioning rootlets at some distance from the critical rootlet, the cell responded with a burst of impulses. Furthermore, when these distant rootlets were electrically stimulated, the cell responded showing that the rootlet contained fibres capable of exciting the cell in spite of the fact that no change had been seen in the peripheral receptive field when the rootlet was sectioned. The other observation was by Taub (1964) who noted that electrical stimulation of nerves subserving skin distant to the receptive field would excite the cells weakly. The example given was of a cell whose receptive field was limited to lateral thigh and yet stimulation of sural nerve which subserves skin of ankle and foot also excited the cell. These paradoxical results of a difference of receptive field depending on the use of electrical or natural stimuli were not followed up partly because it was not certain if they simply demonstrated highly indirect effects such as release from an inhibition.

In this paper we return to the problem of what factors form the boundary of these cells' receptive field and ask if the synchronous activation of afferents from outside the receptive field can demonstrate the existence of afferents whose effect following natural stimuli is so weak that they cannot form a subliminal fringe.

METHODS

The experiments reported here were carried out on forty-five adults cats with body weights in excess of 2 kg. They were either decerebrate or spinal with the section at C1 or anaesthetized with a solution of allobarbitone, monoethyl urea and urethane in water, purified over charcoal and made up to substitute for the Dial–urethane CIBA solution, 0·6 ml./kg, which is no longer available. All animals were paralysed with gallamine triethiodide and maintained on a drip of 4% dextrose in saline. The lumbar enlargement was exposed and stabilized by an open pneumothorax and by propping the segment from which recordings were to be taken. The general preparation was similar to that previously described (Hillman & Wall, 1969).

Recordings were made with glass micro-electrodes filled with near saturated potassium chloride with resistances between 10 and 50 MΩ. The recordings were taken from selected axons in the dorsolateral white matter of spinal cord in segments L2–4. There were three reasons for recording from axons rather than from dorsal horn cells by the extracellular method. First, electrical stimuli of peripheral nerves or of dorsal roots were to be used. These stimuli result in very large massed potentials within the cord which submerge extracellularly recorded unit spikes particularly in the early phase of the massed cord potential when it is rising steeply. Exact measurement of latency of unit spikes was crucial to this experiment. The presence of a unit spike on a steeply rising base line would have prohibited accurate identification of the spike's initiation point. The second reason for axon recording at a distance from the cell body was that, in many of the experiments, it was necessary to manipulate the roots during the course of recording. The inevitable movement of nearby cord would have displaced the recording electrode. Even where mechanical manipulation was not carried out during recording, the stimulation and blocking electrode were located close to the region of the cell bodies from which recording would have been taken. These electrodes and their menisci restricted visibility of cord surface and restricted free movement of the microelectrode. Therefore it was necessary to use the less convenient axon recording method in a region of cord some segments rostral to the cell body. The dorsolateral white matter was exposed by dissection of arachnoid membrane and by section of dorsal rootlets which were reflected medially to expose the dorsolateral funiculus.

In many preparations dorsal roots were cut either during or before recording. A common preparation was one in which all dorsal roots from L2 to coccygeal segments were cut leaving only the two largest roots either L6 and L7 or L7 and S1. All root sections were carried out under a dissecting microscope in such a way as to preserve the radicular artery. First, arachnoid was stripped off the root. Then the rostral or caudal end of the root was gently everted so that its ventral surface could be inspected for the artery which always lies relatively free on the ventral surface of dorsal root. Once identified, the artery was separated from the root for a few millimetres by blunt dissection and by a very small glass probe. With the artery freed, it was then safe to proceed with a complete section of the root. The preservation of these arteries is not merely necessary for the convenience of an absence of bleeding but because cord excitability drops if extensive root sections include arterial destruction.

A number of methods of reversible block were tried. Cold block of peripheral nerve was achieved by the method previously used (Wall, 1967) by packing the nerve with 1 cm cubes of Ringer ice. Abolition of response to stimulation of the medial popliteal was readily achieved but as we shall see we were not able to achieve complete insulation of the lateral popliteal which suffered some cooling although not enough to block. For blocking a dorsal root, local anaesthesia with 1 or 2% lignocaine (Xylo-

caine, Astra) was used. All neighbouring roots and spinal cord were covered with low melting point paraffin wax. The root to be blocked was left with about 15 mm exposed and contained in an open well whose walls and bottom were made of paraffin wax. Stimulating electrodes were placed around the distal region of the root and completely sealed in place with paraffin wax. This allowed central recordings to be made, to follow the course and completion of the block. Lignocaine was then placed in the well containing the exposed root and left until a complete block was

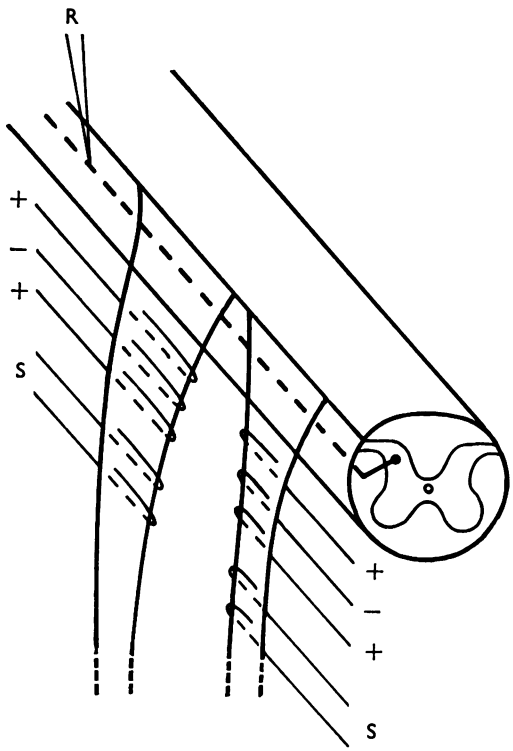

Fig. 1. The arrangement of stimulating, blocking and recording electrodes. The pairs marked S were stimulating electrodes, one pair on each root. The three more proximal electrodes on each root carried the blocking currents (see text). Recordings were made with micropipettes from the axons in the dorsolateral funiculus or dorsal column (in the case of primary afferents).

achieved as judged by a cessation of central response to the peripheral stimulus. An attempt was then made to remove the local anaesthetic by irrigation of the root with saline. The method was abandoned after several trials because complete removal of the lignocaine could not be achieved with irrigation of up to 1 hr. Although the central response returned, the latency of the central response remained somewhat slower than it had been before the blockade. We therefore turned back to the use of anodal block. The reason why this had not been tried in the first place was that we know from previous experience (Mendell & Wall, 1964) that this method as usually used is unsuitable for application to an intact nerve but is suitable where the block

is applied to a sectioned nerve dissected free at its distal end. In the usual method, two blocking electrodes are placed on a nerve with the anode proximal. If the nerve is intact and therefore in contact with the animal at both ends of the free length, there are two current paths between the blocking electrodes. One path passes directly along the nerve from anode to cathode and achieves the required hyperpolarization under the anode. The second current path, with only a slightly higher resistance, passes from the anode proximally along the nerve to enter the main mass of tissue then flows in the body, to the distal end of the nerve and from here flows to the cathode on the nerve. This second pathway has the effect of placing a virtual cathode proximal to the blocking anode and therefore of generating a steady barrage of impulses which enter the cord throughout the period of block. The excitation was quite unacceptable in our experiments. The continuous firing could be eliminated by mounting two flanking anodes around a central cathode on the root (Fig. 1). This arrangement has the effect of making two regions of the root exactly equipotential so that no current flows in the secondary circuit out of the root and through the body. Recordings were made both from fibres in dorsal root central to the block and from dorsal horn cells supplied by fibres from the blocked root and no signs of excitation were detected when the blocking current was applied through the three electrode arrangement whereas they were usually observed if only two blocking electrodes were used. The blocking electrodes were loops of chlorided silver wire and on occasions contact was improved by the application of agar–saline. The electrodes were applied with an approximate 10 mm separation between each electrode. Distal to the blocked zone, a stimulating pair of electrodes was always placed on the roots to test for cord cell responses to root stimulation, and to test for the completion of the block. Block was usually achieved with currents of 50–100 μA. Since detailed testing of receptive fields had to be carried out, it was necessary to maintain these blocking currents for many minutes. On removal of the block, it was required for our tests that the reactivity of the central cell under examination should return to its control value as measured by response to root and peripheral stimuli. This return could be verified during the early parts of an experiment but after a total of about 15 min of block had been maintained, there were the beginnings of irreversible damage to the root so that latencies of response failed to return to control values. If further sessions of block were attempted, the root eventually became permanently blocked. However, the time available for genuinely reversible blocking was sufficient for several cells to be examined in each animal. The arrangements for stimulation, recording and amplification were standard and have been described before (Wall, 1967).

RESULTS

The results described in this paper were obtained from a specific type of cell whose cell bodies lie in lamina IV and whose axons project in the dorsolateral white matter (Wall, 1960; Fetz, 1968; Pomeranz *et al.* 1968). This type is defined by the following characteristics: (1) it responds to light brushing of the skin or hair of its receptive field and fails to increase the frequency of response if the strength of a pressure stimulus is raised from a mild to heavy pressure; (2) it responds to electrical stimulation of myelinated afferents but fails to increase its response if unmyelinated afferents are included in the volley; (3) it has a small receptive field (for example, less than the size of one toe if it is located on toes or not larger

than 20 mm if more proximally placed on the foot); (4) the edge of the receptive field for brushing or for von Frey hair stimulation seems abrupt and, beyond this edge, heavy pressure or pinching fails to produce a response; (5) the cell's axon at L 3 carries an impulse less than 2 msec after stimulation of the peripheral part of dorsal roots L7 or S1. Axons which fulfil these five criteria are quite easily located in dorsolateral white matter but they are mixed with axons from other types of cells with larger receptive fields, longer latencies and a larger dynamic range. The results described here apply specifically to the defined type and do not apply to the other types which were also examined and whose properties will be discussed in a later paper.

Comparison of receptive field (RF) with natural and local electrical stimuli

After an initial rough inspection, the receptive fields of lamina four cells were mapped out with a graded series of von Frey hairs. The threshold for firing appeared uniform over the entire receptive field without patches or spots of high or low threshold. Movement of single hairs was adequate to excite the cell. Beyond the edge of the RF, no firing could be produced with these threshold stimuli. Pinching the skin with forceps similarly failed to modify the cells firing if applied with steady pressure outside the RF. Since the excitatory region is extremely sensitive, rapidly applied stabbing or tapping stimuli are misleading if applied outside the low threshold RF because of mechanical spread of the distortion from the point of application of the stimulus. Therefore it is not possible to say if sudden heavy pressure stimuli applied outside the low threshold region are or are not effective. We have stressed in the introduction that the low threshold RF has an abrupt edge which does not move with changes of excitability of the cell. This phenomenon was again confirmed in this series of experiments by examining the receptive field in the decerebrate state and again following cold block of lower thoracic cord (Wall, 1967). If as in Fig. 2, the receptive field is on the most lateral toe, it is possible by clamping the foot and a medial toe to test this medial toe with vigorous rapid stimuli and to show that here at least the cell fails to respond to such stimuli. Electrical stimuli were then applied first by placing two 30-gauge hypodermic needles into the middle of the most sensitive zone. A single square-wave pulse, duration 0·05 msec, was then applied and the voltage raised until the cell responded to each stimulus. This voltage was taken as threshold and was recorded with the latency of the cell's response. In the example shown in Fig. 2, the threshold voltage was 1 and the latency 6·9 msec. This procedure was repeated using this voltage at the stimulus points shown in the diagram. Stimulus points were separated by 1 cm along four distal-proximal lines; lateral, mid-plantar, medial and mid-dorsal. It is shown that the cell

responded at nine such stimulus points which lay outside the low threshold receptive field. Of course if the stimulus intensity was raised, the cell would respond from even further distant points but the significance of these responses is highly doubtful because of the probability of current spread either to the sensitive zone or to deep lying peripheral nerves. Even the responses at the threshold voltage could be attributed to current spread but this seems less likely and therefore it does appear that the receptive field for local electrical stimulation is larger than that for natural stimuli. The

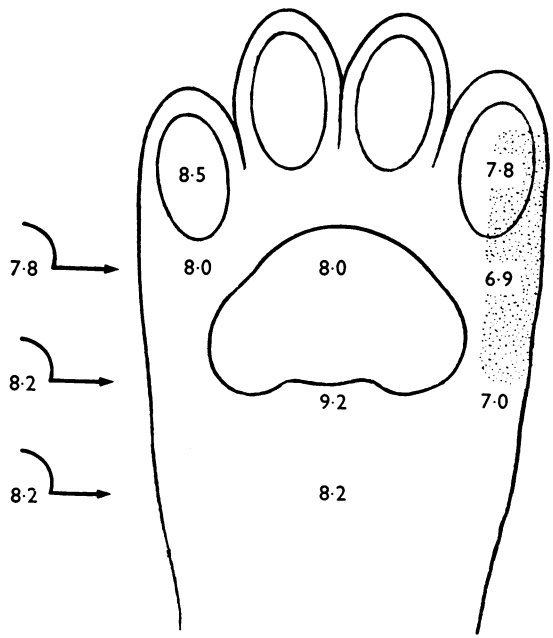

Fig. 2. The receptive field of a dorsal horn cell. The cell responded when the region on the lateral toe indicated with stippling was lightly brushed or tapped with von Frey hairs. The numbers indicate the positions of electrical stimuli and latency of response. The electric stimulus was the same size for all points shown. The three numbers at the left refer to stimulus positions on the dorsum of the foot.

point on the medial toe is particularly relevant since heavy abrupt pressure stimuli to this toe did not evoke a response but threshold electrical stimuli did. It will be noted that the latency of response varied from 6·9 msec in the middle of the RF to 9·2 msec proximal to the foot pad. It is not possible to differentiate between the possibility that these different latencies are produced either by indirect firing of the cell within the cord by polysynaptic connexions or by temporal dispersion of the afferent volley in fine peripheral terminals of the cutaneous afferents. It is clear that there is a

considerable slowing in peripheral terminals because the conduction distance from recording electrode to stimulus point in the middle of the receptive field was 390 mm and, assuming a single synaptic delay of 1 msec, this implies an average peripheral conduction velocity of 66 m/sec. However, for the same cell, stimulation of the nerve at the ankle gave an average peripheral conduction velocity of 78 m/sec. We can conclude that while peripheral studies give some indication that the electrical RF is larger than the pressure RF, we cannot state this with certainty because of problems of electrical and mechanical stimulus spread nor can we be certain that the mechanisms of synaptic response of the cell are the same for the two types of stimulation. It was therefore necessary to move proximally for test stimulation, in order to allow differential blocking and to decrease problems of temporal dispersion of the arriving volleys.

Role of medial and lateral popliteal nerves in carrying afferents to single lamina IV cells

In decerebrate cats, the lumbar enlargement was prepared for recording and axons of cells showing the required RF properties were selected. The popliteal fossa was opened and some 5 cm of both the lateral and medial popliteal nerves were dissected free, but left intact. Stimulating electrodes were installed on each nerve and the entire exposed area was covered with a low melting point paraffin wax to cover all of the lateral popliteal and both stimulating electrode pairs but leaving approximately 4 cm of the medial popliteal exposed. This region was covered with saline. A large number of cells were encountered which responded to stimulation of either branch of the sciatic nerve. The largest number of cells had their receptive fields on the toes or foot as would be expected. The foot is subserved by the medial and lateral popliteal nerves. The commonest finding was that the latency of the cells' response was the same ($\pm$ 0·2 msec) irrespective of which nerve was stimulated. The speed of response was consistent with the cells being fired monosynaptically by afferents in the Aβ range of conduction velocity. Less commonly there were larger differences. For example a cell with its RF between toes 2 and 3 responded 2·8 msec after lateral popliteal stimulation but did not respond until 20·0 msec after medial popliteal stimulation. Even less commonly, cells were encountered which responded only to one of the two nerves. The phenomenon reported by Taub (1964) was seen where cells with their RFs outside the area subserved by the sciatic nerve still responded to medial and lateral popliteal stimulation. However, in all such cases, the latency of response was longer than that observed for units whose RFs lay on feet or toes. For example, a unit with its RF on the tail responded at 3·2 and 3·1 msec to medial and lateral stimuli. Another tail unit responded at 6·5 and 6·3 msec to these

stimuli. A unit whose RF was on proximal lateral thigh responded to medial popliteal in 5·6 msec and to lateral popliteal in 5·8 msec. The conduction distance in this case from stimulating electrodes at the knee to the recording electrode at L4 was 160 mm and the afferent volley began to arrive in the lumbar enlargement at 2 msec. Since all of the cells examined responded monosynaptically to an Aβ volley from the nerve subserving their receptive field, it is apparent that cells responding with these long latencies from distant nerves are probably firing by way of some polysynaptic mechanism. This does not apply to the cells with RFs on the foot which appeared to respond monosynaptically to stimulation of either major branch of the sciatic. We therefore decided to see what happened to the receptive field if one of the nerves was blocked. To give an example, a cell with a small receptive field on the tip of toe 3 responded at 2·75 msec to stimulation of the medial popliteal and 2·6 msec to the lateral. Cubes of Ringer saline ice were then packed around the exposed length of the medial popliteal. The latency of response of the cell to medial popliteal stimulation became more and more prolonged and finally no response could be obtained by maximal stimulation of the nerve. The cell still responded perfectly to lateral popliteal stimulation but the latency had risen from its control value of 2·6–2·8 msec. Under this condition, the receptive field of the cell appeared completely unchanged either in size or threshold although the ongoing activity of the cell had decreased. It was evident that the receptive field did not depend on the fibres which stimulation showed must run in the medial popliteal nerve. In another example in another preparation a cell with its RF on the proximal part of toe 3 responded at 3·8 and 4·2 msec, to medial and lateral stimulation. Cold block of the medial nerve was completed at which time the latency to lateral nerve stimulation rose to 4·6 msec. In this case the receptive field was completely abolished and here one must conclude that the medial popliteal was necessary for the normal connexion of the periphery to the cell but the lateral branch still contained fibres capable of firing the cell with electrical stimuli but incapable of firing the cell with natural stimuli.

Although interesting results were being obtained with this type of experiment, they were not pursued for the following reasons: (1) since the latency of response to stimulation of the unblocked nerve rose, it was not possible to decide if some significant cooling of this nerve had occurred or if the excitability of the cell has dropped because the ongoing afferent barrage had been decreased by the intended block; (2) complete recovery from the block took surprisingly long times, often over 30 min, which strained the limits of holding units; (3) the distance between stimulus and recording points was too large to state with confidence that the cells were

responding monosynaptically; (4) the worst problem was that the technique did not allow alternate checking of either nerve which was clearly necessary to define the relative role of the two nerves. For these reasons, the site of manipulation and technique of block were changed.

Section and stimulation of dorsal rootlets to determinine the course of afferents converging on to single cell

In eight animals, a single cell was selected with a typical restricted receptive field. The outline of the receptive field was marked on the skin. The cord had been fully stabilized at the recording point. Then, under the dissecting microscope, roots were carefully cut step by step preserving the radicular arteries. This progressive rhizotomy either started at the rostral end of the lumbar enlargement at L4 and moved caudally or it started with coccygeal segments and worked rostrally. After each rootlet had been cut, the receptive field of the unit was tested. It was noted in each case that a region was reached where the section of each rootlet was accompanied by a shower of action potentials from the unit but no change could be detected in the receptive field, or in the character of the electrically evoked response. In six of the cells a rootlet was then sectioned which completely abolished the peripheral receptive field. In two cells, section of one rootlet abolished about half of the receptive field and section of the neighbouring rootlet completed the deafferentation of the cell. It was certain that recordings were still being made from the now deafferented cells because ongoing activity of the unit could still be recorded with the characteristic spike height and shape of the unit spike and because electrical stimulation of the sectioned rootlets still evoked a repetitive burst in the cells. These results which repeat those previously reported (Wall, 1960) could mean either that all the significant afferents capable of firing the cell in response to natural stimuli run in one or two rootlets or it could mean that many afferents run in over many rootlets, and that they are so redundant in their action on the cell that section of many rootlets results in no significant change of the receptive field until the final cuts abolish the minimal number of redundant fibres.

Once a cell had been identified and the roots supplying afferents to the cell had been cut, it was then possible to stimulate the cut roots to detect the latency of response of the cell to stimulation of fractions of the root. Each root was divided into 3–5 fractions and mounted on stimulating pairs of electrodes. The results for four cells are shown in Fig. 3. It will be seen that for each cell, the rootlets which contained excitatory afferents spread over two segments. In these four cells, each responded to a fraction of either S1 and L7 or L7 and L6. For each cell, there was a fraction of a root which on stimulation made the cell respond with a mimimal latency.

Rostral and caudal to these minimum latency rootlets, there were rootlets which fired the cell with slightly longer latencies. These flanking rootlets were stimulated with the same threshold voltage used on the fastest rootlet. Beyond these rootlets, the cell failed to respond to rootlet stimulation at 5 times the threshold voltage used on the most sensitive rootlet. In cell *A*, Fig. 3, one rootlet in the middle of L7 failed to fire the cell although rootlets flanking it did make the cell fire at a short latency. It is always possible that the dissection had damaged the relevant fibres in this rootlet. We can conclude that electrical stimulation of rootlets shows that

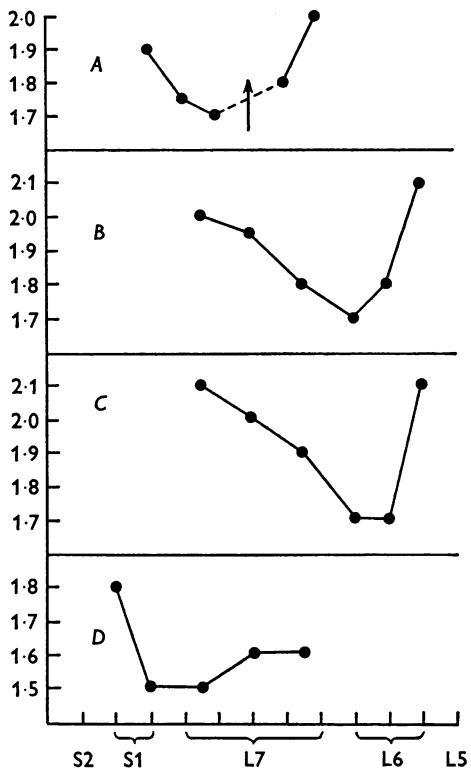

Fig. 3. Latencies for four dorsal horn cells to stimulation of fractions of lumbar and sacral dorsal roots. The stimuli were applied to the roots as shown along the horizontal scale. The vertical scale in each case shows the corresponding latencies in msec. The arrow in *A* indicates that stimulation of middle rootlet in L7 failed to drive the cell (see text).

afferents capable of firing the cell at short latency are widely distributed and exist in rootlets whose section apparently failed to change the extent and sensitivity of the receptive field of the cell. We are left then with the question of whether these widely distributed afferents are effective but

redundant or are they ineffective in making the cell respond to natural stimuli? This can only be decided by alternate block of fractions of the arriving afferents.

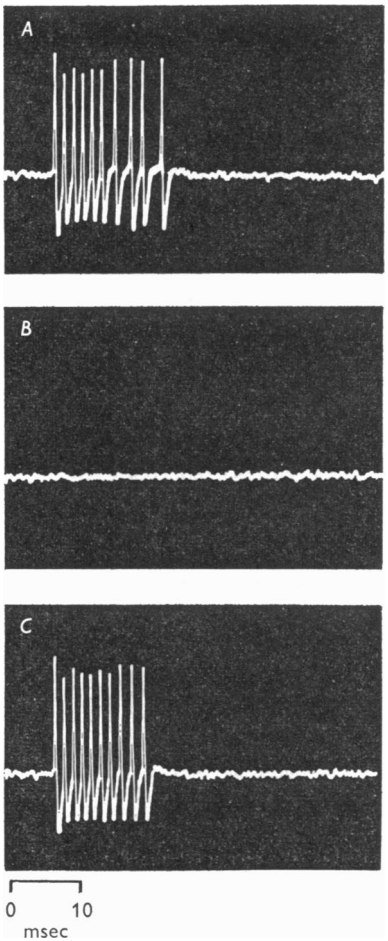

Fig. 4. Response of a dorsal horn cell to electrical stimulation in its receptive field. *A* shows the response to a single stimulus before anodal block, *B* during the block and *C* immediately after the block.

The effect on receptive field of block of either of two dorsal roots

For reasons discussed in the Methods section, we settled on a method of anodal blocking of intact dorsal roots using three blocking electrodes on each root. All dorsal roots were cut from coccygeal segments to L4, leaving intact the two largest roots either S1 and L7 or L7 and L6. Stimulating electrodes were mounted on peripheral parts of the two intact roots.

Fibres were selected from the dorsolateral fasciculus at L3–4 which responded in less than 2 msec to stimulation of both roots independently. This procedure selected cells of the required type whose afferents, like those in Fig. 3, were evidently included in the two intact dorsal roots. Blocking electrodes were mounted on each intact dorsal root in the arrangement show in Fig. 1. A number of checks were necessary to show that the block was satisfactory for this type of experiment. Reversibility was checked by making sure for each cell and for each block that the cell returned to its control state after the termination of the block. Cells were checked before and after for their RFs, their latency to root and peripheral stimulation and the length of repetitive firing to root and peripheral stimulation (Fig. 4). As discussed in the Methods section, experiments were terminated when reversibility failed. The next point checked was the possibility that the blocking electrodes simply raised the threshold at the root stimulation point so that the electrical stimulus fell below threshold but the root was not in fact blocked. This was checked by three methods. The stimulus intensity on the root was raised to 2–4 times threshold and the blocking current raised until the central cell failed to respond to the root stimulus which was being applied peripheral to the blocking electrodes. At this stage, recordings were made on a peripheral nerve, such as the sural nerve, to detect the antidromic volley which ran backwards down the sensory fibres from the stimulus point on the dorsal root. If the blocking current had prevented impulses from being generated at the stimulus point, no antidromic volley would have been recorded. In fact the blocking and stimulus voltages were always arranged so that such a volley could be recorded and therefore one was certain that an afferent volley was being generated. Abolition of the response from stimulation applied directly to the skin of the receptive field was another way of being sure that the effects of an afferent volley were being blocked since it is not likely that current applied to a dorsal root would spread to nerve fibres in the foot. It was necessary to check that the afferent volley was truly blocked and not simply desynchronized. This check was acehived by recording from single afferent fibres either at the root entry zone or in the dorsal column (Fig. 5). These recordings showed that the single impulse resulting from the root stimulus was absent during the block and not delayed. This type of recording also allowed one to be sure that the triplet arrangement of blocking electrodes, anode–cathode–anode, was not itself producing firing within the afferent fibres. Such firing was observed during the application of very low level currents which were insufficient to block and it was observed for a few seconds after abrupt removal of the block. Other reasons for believing that the blockade was not itself generating an afferent barrage were first that the central cells usually decreased their

ongoing activity during block presumably due to the abolition of the normal ongoing afferent barrage of peripheral origin and second that no increase of activity was observed on peripheral nerves during blockade. Finally, it was important to be sure that the block was limited to the root in question and had not affected the response of the other root. This was checked by measuring the threshold for response to stimulation of one root while blocking its neighbour (Fig. 6). It was found that there there was no spread of the block from one root to the next.

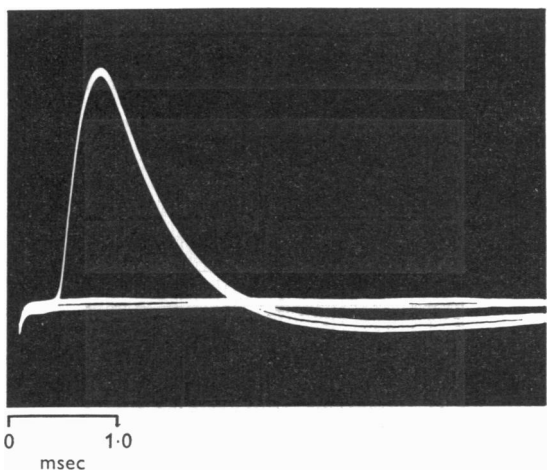

Fig. 5. The response of a single primary afferent fibre recorded in the dorsal column to four shocks delivered to its dorsal root distal to the blocking electrodes. Two shocks were given before the block and two during the block.

All the necessary criteria of cell type, afferent distribution and successful block for a sufficient time to examine the receptive field were achieved for sixteen cells with anodal block of the roots and for a further four cells using xylocaine block of one root. In eighteen of these twenty cells, the results were unequivocal. When one root was blocked, the low threshold receptive field completely disappeared while, when the other root was blocked, no discernible difference could be detected in the low threshold receptive field. In all cells but one, the root which was essential for the RF was the root whose stimulation produced the lowest latency of response. In all cases when the essential root was blocked and no RF was detectable, electrical stimulation of the unblocked root still produced a response of the cell in less than 2 msec. For example, a unit with its receptive field on the dorsum of the foot between toes 1 and 2 responded to stimulation of the S1 root with 0·5 volts for 0·01 msec after a latency of 1·75 msec. It

responded to stimulation of the L7 root with the same stimulus at 1·65 msec. When the S1 root was blocked no change could be detected in the RF while when the L7 root was blocked, the RF disappeared. If electrical stimuli were delivered to the skin in the middle of the RF through hypodermic needles, the unit responded to a shock of 0·8 V for 0·1 msec with a latency of 6·8 msec. Blocking S1 did not change this response but if L7 was blocked,

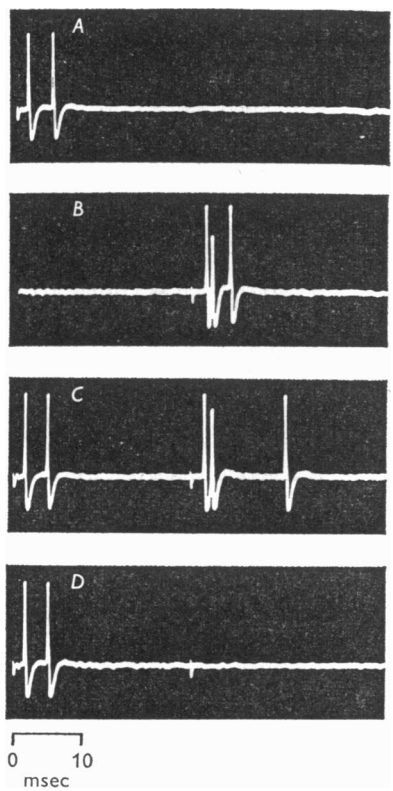

Fig. 6. The responses of a dorsal horn cell with the electrodes arranged as in Fig. 1, on the L6 and L7 dorsal roots. In *A* L6 was stimulated alone, in *B* L7 was stimulated alone, in *C*, both were stimulated and in *D*, both were stimulated but L7 was anodally blocked.

the stimulus had to be raised to 15 V 0·5 msec and the cell responded with a latency of 9·0 msec. With L7 blocked, this unit failed to respond even to strong pressure or sudden pinches to the foot even though movement of a few hairs produced vigorous firing without block of L7. For many of the cells whose low threshold receptive field disappeared with the block of one root, vigorous stabbing or pinching stimuli in the RF or nearby would produce a very brief response, often only a single impulse, and even this

response would often habituate if the stimulus was repeated. In those cells where an electrical response could still be obtained by direct stimulation of the receptive field after the critical root had been blocked, the threshold and latency of response were always markedly increased. There were two exceptions to this apparent complete dependence of the low threshold receptive field on one root. One such cell had a 20 mm receptive field on the lateral ankle. Block of L7 led to a disappearance of the distal 4 mm of the field while block of S1 removed about 15 mm of the RF leaving a zone from which von Frey hair stimulation produced a very much reduced number of spikes. Evidently for this cell the critical afferents for the low threshold response were shared between L7 and S1. The other exceptional cell had its receptive field on leg and perineum with a large area and therefore was doubtful in its classification as a lamina 4 cell. For this cell, block of neither root completely abolished the receptive field. We conclude that, with these two exceptions, the afferents responsible for the low threshold pressure receptive field ran in a single root. Afferents existed in the neighbouring root which could not produce firing following natural stimuli. These neighbouring roots contained afferents with low electrical thresholds and high conduction velocities which were capable of firing the cell with short latencies following electrical stimulation of the root.

DISCUSSION

There are three phenomena whose relations we now need to discuss: (1) a type of cell exists with a small cutaneous receptive field with fixed boundaries without signs of a subliminal fringe; (2) the fibres necessary for the cell's response to this receptive field run in a restricted group of dorsal rootlets; (3) flanking this restricted zone of rootlets, there are dorsal rootlets containing fibres which excite the cell if they are stimulated electrically, but are incapable of exciting the cell following natural stimuli. There are three locations at which the mechanism for this ineffectiveness might exist. First, the ineffectiveness of flanking afferents could be produced by an interneurone interposed between the afferents and the recorded cell. Secondly, both the effective and ineffective afferents could terminate monosynaptically on the cell but the latter fail to depolarize the cell sufficiently unless their action is synchronized by electrical stimulation. And, the third possibility is that the terminals of the ineffective fibres could be normally blocked and only carry impulses in the presence of synchronous activity in neighbouring structures.

There is good reason to believe that the cells in question are in monosynaptic contact with at least some afferents from dorsal roots. The cell bodies whose firing pattern fits the described characteristics lie in lamina

IV (Wall, 1967). Cells in this region receive large myelinated dorsal root afferents which arborize and make extensive contacts with both cell body and dendrites (Cajal, 1909; Szentagothai, 1964; Scheibel & Scheibel, 1968). Electrical stimulation of the dorsal root nearest to the cell body produces a soma spike which has a synaptic delay of 0·8–1·2 msec (with a latency variation for a particular cell of less than 0·1 msec) and follows repetitive stimulation at more than 200/sec. If it is accepted that these responses fulfil the criteria for monosynaptic excitation, we need then to ask the same question of the responses produced by the flanking rootlets. As seen in Fig. 3, these rootlets produced responses with longer latencies. In the near neighbours to the critical root, the increase was 0·1 msec, not sufficient to justify the postulation of a second synapse. At the extreme edges the additional latency was as high as 0·4 msec. These responses from the most distant rootlet stimulation retained the characteristic small variation of latency of less than 0·1 msec for any one cell and an ability to follow at stimulus rates higher than 200/sec. Here too the additional delay could be attributed to conduction delay in fine afferent terminal branches or to the time taken for the excitatory post-synaptic potential to rise above the cell's threshold. Therefore, even for the extreme edges of the dorsal root zone capable of firing the cell, it is not necessary to postulate the interposition of an additional neurone between afferents and the examined cell, but it remains possible that such a cell exists.

If we turn to the second explanation where all fibres terminate monosynaptically but some are incapable of firing the cell with natural stimulation, there are certain testable predictions. Intracellular recording in such a situation should record small excitatory post-synaptic potentials (e.p.s.p.s) when the ineffective afferents are fired by natural stimulation. Such recordings are possible for at least brief periods (Hongo *et al.* 1968) but we have not yet attempted them in this situation. In the absence of intracellular recording, one might expect that the presence of provoked e.p.s.p.s would interact with the mechanism which produces the ongoing activity of the cell. One would predict that natural stimuli even if incapable of initiating a spike by themselves would increase the probability of the cell producing a 'spontaneous' action potential. Visual inspection of ongoing activity with and without intermittent peripheral stimulation has not so far revealed this tendency but experiments are now in progress with quantitative measurement to see if such an interaction can be detected.

It seems then that it is necessary to postulate two classes of synaptic mechanism one highly effective and the other highly ineffective. Hints of a similar problem appear in the work of others. For example, in the trigeminal nucleus, there are cells with a similar anatomy and physiology to

those in lamina IV (Gobel & Dubner, 1969; Kerr, 1970). These cells show a mismatch between the anatomy and physiology suggesting the presence of ineffective terminals. The first fact is that trigeminal afferent terminals arborize in a restricted zone of the nucleus (Aström, 1953). The second fact is that the natural stimulus receptive field of the cells on which these afferents terminate is small and restricted to less than one division of the trigeminal nerve (Wall & Taub, 1962). However, the dendrites of these cells extend well into zones of the nucleus supplied by afferents from the other divisions of the fifth nerve and yet there is no sign of physiological response when these distant zones of skin are stimulated (Darian-Smith *et al.* 1963). Could there be a post-synaptic explanation for the apparent subdivision of terminals into two separate classes? The effectiveness of synapses in firing a cell will decrease with distance from the triggering point because of the electrotonic properties of a dendritic tree (Rall, 1962). This effect will presumably be exaggerated if the synaptic contact areas become smaller with distance from the cell body as is the case in trigeminal nucleus cells (Gobel & Dubner, 1969). A step could be introduced in this gradual diminution of the effectiveness of synapses with distance if there were sudden constrictions of the dendrites interposed between the synapse and the cell body. Constrictions or 'beading' does appear in the secondary dendrites of both the cord and trigeminal cells (Scheibel & Scheibel, 1968; Gobel & Dubner, 1969). These constrictions would be points of high intracellular resistance which would decrease the electronic coupling between distant dendrites and the cell body. A further point of sudden constriction occurs at the base of dendritic spines. In fish motoneurones, Diamond, Gray & Yasargil (1970) presented evidence that synaptic end knobs on the long dendritic spines were in poor electrotonic contact with the rest of the cell. Spines up to 3 μm long exist on the trigeminal cells (Gobel & Dubner, 1969) but are shorter on the lamina IV cell dendrites (Scheibel & Scheibel, 1968). Therefore the possibility exists that distant synapses in these cells are electrotonically insulated from the cell body either by constrictions at the base of spines or by dendritic constrictions. These constrictions could introduce the required separation of synaptic effectiveness by dividing a class of proximal synapses from a distant class. If many of the distant synapses were simultaneously activated by electrical stimulation, then a local spike might be generated within the distant dendrite or spine and this would have sufficient amplitude to propagate a detectable disturbance into the cell proximal to the constriction. In support of this suggestion, evidence exists for dendritic spikes within the lamina IV cells (Wall, 1965). If the distant synaptic contacts had to generate a dendritic spike before they could affect the excitability of the cell, this, in addition to the constriction, would separate them into a separate class from the class of more

proximal synapses which are in effective electronic contact with the trigger zone.

Finally, we must consider the possibility that there is a presynaptic mechanism which renders certain types of afferent ineffective. Katz & Miledi (1963) for the frog motoneurone and Kuno (1964) for the cat motoneurone have shown that the probability of release of a quantum of transmitter for each impulse is less than one in some terminals. This provides a possible presynaptic mechanism for ineffective terminals which would on average emit so little transmitter that their effect would be undetectable unless many were synchronized by electrical stimulation. On synchronous firing, the probabilities of the individual terminals would all be added together thus making firing likely. This should be testable either by intracellular recording or by a statistical analysis of the effects of repeated natural stimulation in the ineffective zone where such stimuli should tend occasionally to increase the probability of 'spontaneous' firing. Visual inspection has so far failed to show this effect but detailed analysis is in progress. It has been known for some time that intermittent conduction block occurs even in the large diameter collaterals of peripheral afferents which run in dorsal column (Barron & Matthews, 1935) and these observations have recently been extended to other dorsal root branch points (Chung, Raymond & Lettvin, 1970). Failure of conduction in some branches of motor axons has been shown (Krnjević & Miledi, 1959). Induced blocking has been shown in dorsal root terminal arborizations (Howland, Lettvin, Pitts, McCulloch & Wall, 1965). Unblocking of conduction produced by activity in neighbouring afferents has been shown by Chung *et al.* (1970). If intermittent blocking and stimulated blocking and unblocking occur in afferent fibres, then there is the possibility that certain terminals suffer a continuous blockade due either to internal factors such as fibre diameter and membrane potential or to external factors. Normally impulses arriving from the parent axon would fail to influence the cell because of the failure to penetrate the terminal. If, however, a synchronous volley arrived in many neighbouring fibres, the block might be temporarily relieved by the mutual field interaction of impulses in neighbouring fibres (Katz & Schmitt, 1942). Evidence has been presented for the possible existence of inactive terminals of motor axons to eye musculature in fish (Mark, Marotte & Johnstone, 1970).

One inevitably asks what could be the possible function of the ineffective contacts. They could have a past or present or future function. In the past, they might have been effective at some stage of embryonic development. At some stage the cell might have a much wider receptive field from which the small adult receptive field crystallizes as a result of mutal interaction between those terminals which are normally synchronously active. The

ineffective terminals would remain as ghosts of the cell's childhood. The contacts might have a present function under some conditions of raised excitability which we have yet to discover. Finally, it is possible that the endings might become functional as a result of some plastic change triggered by the degeneration of neighbours or as a result of novel activity patterns.

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