*J. Physiol.* (1968), **199**, *pp.* 511–532
*With 7 text-figures*
*Printed in Great Britain*



# CORD CELLS RESPONDING TO FINE MYELINATED AFFERENTS FROM VISCERA, MUSCLE AND SKIN

BY B. POMERANZ, P. D. WALL AND W. V. WEBER

*From the M.R.C. Cerebral Functions Research Group, Department of Anatomy, University College London, W.C.1, England and Department of Biology and Research Laboratory of Electronics, M.I.T., Cambridge, Mass. U.S.A.*

(*Received* 21 *February* 1968)

SUMMARY

1. Micro-electrode recordings were made in the thoracic cord of acute spinal cats. Cells, which were located in the histologically defined lamina 5, responded both to the fine myelinated afferents from the splanchnic nerve and to afferents from the skin. Splanchnic afferents inhibit the effect of converging cutaneous inputs for periods up to 150 msec. Skin stimuli may also inhibit the effect of afferent nerve impulses from viscera. Some cells respond monosynaptically to the splanchnic afferents, others indirectly.

2. Fine myelinated afferents from gastrocnemius (group 3) stimulate lamina 5 cells which also have cutaneous receptive fields. Cutaneous and group 3 muscle afferents interact by mutual inhibition in their effect on the cells.

3. Fine myelinated afferents from skin excite lamina 5 cells. The cutaneous responses of lamina 5 cells contrast with those of lamina 4 cells in the following respects: (*a*) the receptive fields are larger, (*b*) they respond with an increased latency to A$\beta$ afferents, (*c*) there is a low pressure threshold at the edge, (*d*) they respond to a wide range of pressure stimuli from light brush to heavy pinch applied to the centre of the receptive fields and (*e*) they respond to A$\Delta$ afferents.

4. Lamina 5 cells receive fine myelinated afferents either from viscera or from muscle or from skin. Lamina 4 receives large myelinated afferents from skin and lamina 6 receives large myelinated afferents from muscle. The results suggest the hypothesis that some fine myelinated afferents form a class of afferents which signal the state of tissue, and end on lamina 5 cells.

## INTRODUCTION

Rexed (1952) subdivided the gray matter of the spinal cord of the cat on the basis of the size, shape and distribution of the cell bodies. He concluded that the large cell region of the dorsal horn was subdivided into three laminae which he numbered laminae 4, 5 and 6. Wall (1967) investigated these laminae by recording from single cells with micro-electrodes and found that cells in Rexed's laminae 4 and 5 responded to cutaneous stimulation while cells in lamina 6 responded to limb movement. In the present paper, we have continued this physiological analysis of the subdivisions of the dorsal horn cells by searching for those cells which respond to the small myelinated afferent fibres from viscera, muscle and skin. The splanchnic, gastrocnemius and sural nerves were chosen as convenient sources of these afferent fibres since the splanchnic nerve originates in viscera, the gastrocnemius nerve in muscle, and the sural nerve in skin.

Small myelinated afferent fibres with diameters of 1–5 $\mu$ and conduction velocities of 4–35 m/s are found in the splanchnic, gastrocnemius and sural nerves (Patton, 1965). In the splanchnic nerve, some of these fibres are stimulated by movements of the small intestine (Bessou & Perl, 1966) while others respond only to intense stimuli (Gernandt & Zotterman, 1946). In muscle nerves, the small myelinated afferents are the group 3 afferents and most are stimulated by pressure or noxious stimulation (Paintal, 1960; Bessou & Laporte, 1961). In skin nerves, the small myelinated fibres form the delta group which contains both fibres which respond to light pressure (Hunt & McIntyre, 1960) and others which only transmit impulses when the skin is damaged (Burgess & Perl, 1967).

The region of termination of fine visceral afferents in the spinal cord has been investigated by Weber (1966) and by Selzer & Spencer (1967) using both the techniques of single unit recording and of electrical field plotting. They conclude that cells receiving visceral afferents lie in the ventral part of the dorsal horn. Fine muscle afferent fibres and the interneurones on which they end have been included in the flexor reflex afferent pathways by Lundberg (1964). Wickelgren (1967) reports that some lamina 5 cells respond to group 3 muscle afferents. Lundberg (1964) has also placed the cutaneous delta fibres and the interneurones which receive them in the flexor reflex afferent pathway. Fetz (1968) noted that the dorsal horn cells which responded to a wide range of cutaneous pressure stimuli lay ventral to a layer of cells which responded only to light and medium pressure. It is therefore apparent that evidence has been accumulating which points to the ventral part of the dorsal horn as containing cells which respond to the fine myelinated afferents from viscera, muscle and skin.

The central effects of visceral afferents may interact with those of soma-

tic origin. Downman (1955) showed that stimulation of the smaller myelinated fibres in the splanchnic nerve or stimulation of an intercostal nerve evokes a discharge in other intercostal nerves. He further showed that these two types of afferent volley interact with each other so that in the decerebrate cat one volley facilitates the reflex effects of the other, while in the spinal animal there was an inhibitory interaction. Franz, Evans & Perl (1966) recorded from sympathetic preganglionic rami in spinal cats and showed that certain fibres responded both to somatic and to splanchnic nerve stimulation. Fine myelinated afferents in both the somatic and the visceral nerves had to be stimulated in order to evoke the reflex effects. The reflex discharge evoked by one nerve was markedly depressed for several hundred milliseconds by a conditioning volley to another nerve. Weber (1966) discovered certain single cells in the spinal cord which responded to both splanchnic and to somatic nerve stimulation. Similarly, Selzer & Spencer (1967) recorded from single cells in the upper lumbar cord which responded to pelvic visceral afferents and to somatic stimulation.

In the experiments described in this paper, we set out to locate those cells which respond to the fine myelinated afferents and to describe the interactions on those cells of inputs of different origins.

## METHODS

All experiments were carried out on cats prepared under ether anaesthesia with occlusion of the basilar and carotid arteries and section of the spinal cord at C1. The animals were then given artificial respiration, paralysed with gallamine triethiodide and the administration of ether was stopped. Extracellular single unit recordings were made with fluid filled, 2–5 MΩ, glass micro-electrodes by the methods previously described (Wall, 1967). The stimulus sites on peripheral nerves were prepared by dissecting the nerve free from surrounding tissue over a distance of 15–20 mm, without section of the nerve. A sheet of paraffin wax (Parafilm 'M', American Can Co.) was then slipped under the freed section of nerve. Fine insulated wire with a diameter of 200 $\mu$ was brought from the stimulus isolation unit to the stimulus site. On the end of each wire, 3–4 mm of 50 $\mu$ platinum wire was attached. The platinum wire was slipped under the nerve and gently wrapped around it so that no tension was exerted on the nerve. Two such wires separated by 5 mm were placed around the nerve and then embedding wax, at a temp. of 40–45° C, was dropped on until all exposed nerve and wire was covered. The wax did not affect impulse conduction and prevented drying or flooding of the exposed region. The lightness and flexibility of the electrodes and their fixed apposition to the nerve allowed free movement of the region without change of the position of the electrodes with respect to the nerve. In many experiments, additional pairs of electrodes were placed on more proximal parts of the nerve so that they could be used either to record the volley generated by the first pair or to generate afferent volleys with a shorter peripheral conduction distance. Four types of natural stimulation of skin were used; brush, touch, mild pressure produced by picking up a fold of skin, heavy pressure by pinching a skin fold. For electrical stimulation of skin, fine hypodermic needles or Michel wound clips were used as stimulating electrodes.

*Method of recording.* A method of recording and display (Wall, 1960) allows the response

to 150 or more pairs of stimuli to be presented in one figure so that one can see any variability in the response and the effects of interaction as the time interval between the stimuli is changed. The system can be explained by reference to Fig. 4*A*. This is the record of an experiment in which there was one recording micro-electrode near a dorsal horn cell, and two sites of electrical stimulation, the splanchnic nerve and the skin. The figure is read from above downwards. The stimulus artifact from cutaneous stimulation gives a spot whose size and position on the screen is adjusted to give a continuous vertical line as the stimulus is repeated once every second. This line is at the zero point on the abscissa which represents the separation in time of the two stimuli; it is scaled $\pm 100$ msec on either side of the zero. The artifact from splanchnic nerve stimulation similarly produces a continuous line which approaches the vertical line as the interval between the pair of stimuli is decreased on repetition. The recording of the cell firing produces a dot on the screen. At the start of the experiment (top of Fig. 4*A*), the splanchnic stimulus leads the cutaneous stimulus by 100 msec and the cell fires about 15 msec after the splanchnic stimulus. There is no firing of the cell in response to the cutaneous stimulus. The same result is found as the time interval between the stimuli is reduced by equal increments until they are coincident (first crossing point of the continuous lines). In the following section the splanchnic stimulus progressively lags behind the cutaneous stimulus, and the unit now responds to both stimuli. In the lower half of the figure, the temporal relationship between the stimuli is progressively reversed until the initial condition is reached after 150 pairs of stimuli.

## RESULTS

### *Thoracic cord*

#### 1. *Effects of splanchnic nerve stimulation*

*A. Afferent volley.* Two pairs of electrodes, separated byabout 3 cm, were placed on the splanchnic nerve above the diaphragm. When stimulating pulses were applied to one pair of electrodes, a compound action potential was recorded on the other pair (Fig. 1). When the stimulus strength was twenty times threshold, the compound action potential had three components produced by impulses travelling at 50–77 m/s, 8·5–12 m/s and 1·1–1·4 m/s.

*B. Cord cells responding to splanchnic stimulation.* The dorsal horn of thoracic segments 7, 8 and 9 was searched with micro-electrodes. If the stimulus intensity to the splanchnic was low so that only the fastest component of the compound action potential was observed on the nerve, a small brief field potential was recorded in the ipsilateral dorsal quadrant of the cord at the expected time of the arrival of the high velocity afferent volley. This showed that the impulses had arrived in the spinal cord but no single cells were ever discovered which responded to these impulses transmitted by the larger myelinated fibres in the splanchnic nerve. If the stimulus intensity on the splanchnic nerve was raised so that an afferent volley was generated which travelled at 8·5–12 m/s many cells were detected in deeper parts of the dorsal horn which responded with a burst of repetitive firing after the arrival in the cord of this slower afferent volley. The number of impulses in the repetitive discharge varied from 2 to 12

impulses and the duration of the discharge varied from 2 to 16 msec. Latency and synaptic delay was calculated from data of the type shown in Fig. 2, for seventy-eight cells. The latency of the first response was measured, first, when the distal pair of electrodes generated the afferent volley and, secondly, when the proximal pair were used to stimulate. The difference of the two latencies and the distance between the pairs of

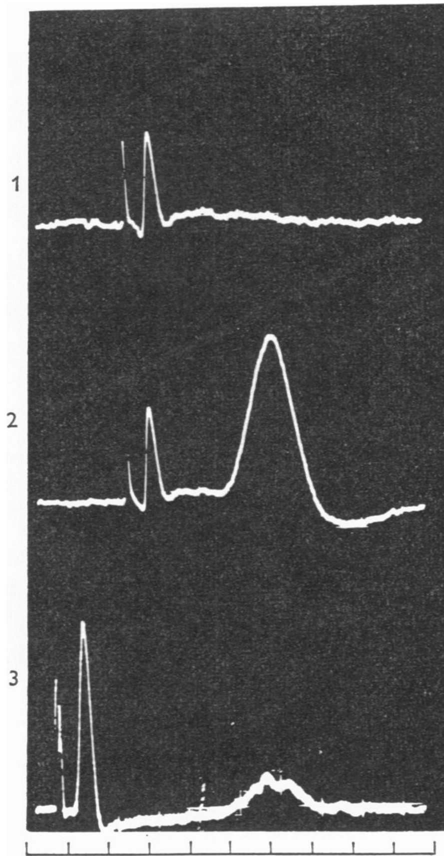


Fig. 1. Compound action potential recorded on splanchnic nerve. Pairs of stimulating and recording electrodes were placed on the intact nerve *in situ* at a distance of 3 cm from each other on the course of the nerve above the diaphragm in a spinal cat.

1. Stimulus 2·5 times threshold produces only the fastest component of the compound action potential containing impulses travelling at 50–77 m/s. Time: 1 msec/division.

2. Stimulus five times threshold produces a second component of the compound action potential with impulses travelling at 8·5–12 m/s. Time: 1 msec/division.

Stimulus twenty times threshold produces a third component made up of impulses travelling at 1·1–1·4 m/s. Time: 5 msec/division.

stimulating electrodes allowed the calculation of the conduction velocity of those fibres responsible for firing the cell. The result showed that cells were fired by afferents travelling at a conduction velocity of 10–30 m/s. The sympathetic chain and rami had been cut so that splanchnic afferent impulses could only enter the cord over the dorsal root of segments T8 and T9. This limitation of entry point allowed the conduction distance between the stimulus point and the observed cell to be measured fairly

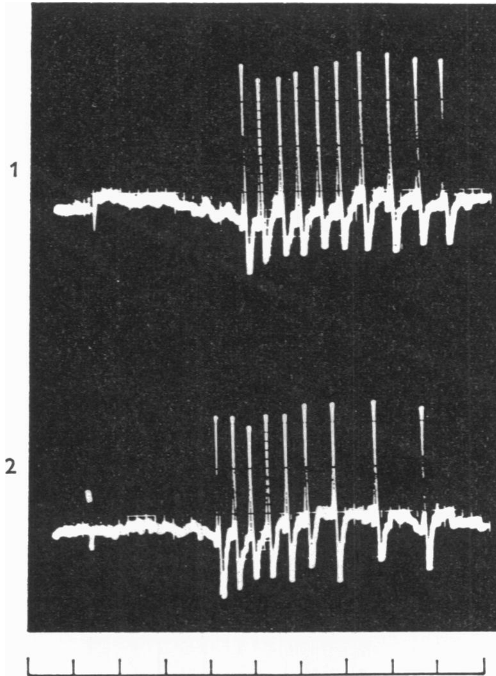


Fig. 2. Repetitive responses of cell in lamina 5 of T8 segment of spinal cord following a single stimulation of splanchnic nerve. The stimulus intensity was sufficient to generate the AΔ component of the compound action potential.

1. The stimulus was applied at a distance of 9·5 cm from the cord segment which contained the responding cell.

2. The stimulus was applied at a distance of 6·5 cm from the cell's segment. It will be noticed that the latency of the cell's response is shorter for the shorter conduction distance. Time: 2 msec/division in both cases.

accurately. Therefore, since the conduction velocity and distance were known, the synaptic delay could be calculated. In the segment containing the roots carrying arriving impulses, many cells were found in which the delay between arrival of the afferent volley and response of the cell was 0·5–1·5 msec. These cells may be assumed to have responded monosynapti-

cally to the afferent fibres. Many other cells were detected in the same region which responded with a latency of 2–15 msec after the arrival of the entering volley and these cells must be presumed to be in indirect contact with splanchnic afferents. No lateral horn cells were detected which responded antidromically to the peripheral stimulus.

*C. Location of cells responding to splanchnic afferents.* Mapping experiments were carried out by the method previously used (Wall, 1967). The splanchnic nerve was stimulated once a second at an intensity well above the threshold for fine myelinated afferents. First of all, a micro-electrode was made to penetrate the cord along a track close to the mid line and recordings were made at 50 $\mu$ intervals during the penetration. When the electrode had penetrated to a depth of 2 mm, it was cut off and left in position for later location by the clearing technique (Wall, 1967). Next, a second micro-electrode was placed in the cord on a track parallel to the first and slightly lateral to it. This process was continued until the entire dorsal horn had been searched for cells responding to the splanchnic afferent volley. Maps were completed in six cats and three of the maps are shown in Fig. 3. The outlines of the dorsal and ventral horns were traced from the actual specimen of the cleared spinal cord containing the micro-electrodes. A point was marked on each electrode track at which the electrode had first recorded cells in the dorsal part of the dorsal horn which responded to cutaneous stimulation but not to splanchnic nerve stimulation. These cells will be discussed in the next section. A line was drawn across the dorsal horn connecting these points on each electrode track. This line is the more dorsal of the two lines drawn in the dorsal horns shown in Fig. 3. As each electrode penetrated more deeply into the dorsal horn, a point was reached at which cells were encountered which responded to the splanchnic volley. The depth at which these cells were first encountered was marked on each electrode track and a second more ventral line was drawn across the dorsal horn. The zone between the two lines which had been located by micro-electrode recording corresponds roughly to Rexed's histological lamina 4. The remainder of dorsal horn ventral to the second line which contained cells responding to the splanchnic afferents is roughly the same as Rexed's histological lamina 5. Thoracic dorsal horn does not contain a histologically recognizable lamina 6.

### 2. *Cells responding to cutaneous stimuli*

While searching for cells responding to splanchnic stimulation, all cells were examined for cutaneous responses. The dorsal horn was mapped for cells responding to cutaneous stimulation by the same method and at the same time as it was mapped for splanchnic responses. The zone between the two lines drawn in the dorsal horn in Fig. 3 was found to contain cells

with the characteristics of lamina 4 cells previously described in the lumbar enlargement (Wall, 1967). The receptive fields were relatively small, 1–2 cm, and the cells within the lamina were arranged in a clear topographic map with medial cells having ventrally placed receptive fields

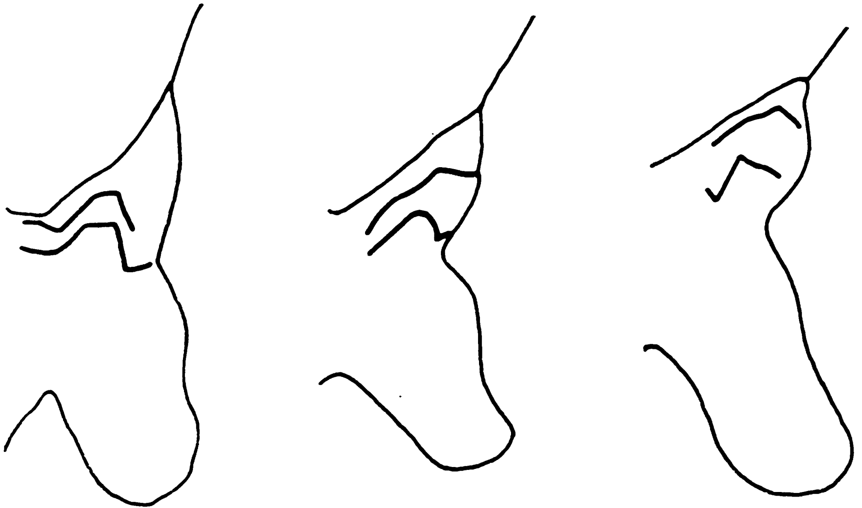

Fig. 3. Cord maps showing the location of regions in which cells were encountered which responded to cutaneous and splanchnic stimulation. The outline maps are traced from three separate experiments on thoracic segments 7, 8 and 9. Each dorsal horn had been explored by at least eight parallel electrode tracks which had been located. The more dorsal line in each dorsal horn is drawn between points on the electrode tracks at which cells were first encountered which responded to light brushing of the skin. In the region between the two lines in the dorsal horn, recordings were made from cells which generated action potentials of more than 50 $\mu$V, responded to brushing of the skin and never responded to splanchnic nerve stimulation.

When the electrodes penetrated to the more ventral of the two lines, cells were encountered which responded to splanchnic nerve stimulation. These cells also had cutaneous receptive fields considerably larger than those of the more dorsal cells.

and lateral cells receiving from dorsal areas within the dermatome of the segment. Electrical stimulation of either the receptive field or the intercostal nerve produced firing of these cells within 0·5–1·5 msec of the entering volley so that they can be considered to be monosynaptically connected to A$\beta$ afferent fibres. The cells responded to brush, touch and light pressure within their receptive field. Some cells adapted rapidly if the stimulus was maintained. If the pressure on the skin fold was increased to a heavy pinch, no cells in this lamina increased their firing rate.

When the electrodes penetrated to the region whose upper boundary is

marked by the more ventral line in Fig. 3, there were five changes in the property of the cells which differentiated these deeper cells from the more superficial cells. The size of the receptive field expanded to involve about one third of the dermatome. This was the same sudden expansion which had been observed at the lumbar lamina 4–5 junction (Wall, 1967). The receptive fields changed from having a relatively uniform threshold over the entire field as seen in lamina 4 to having a marked gradient of pressure threshold. Most responded to brush in the centre but heavy pinching was required at the edge of the receptive field. Some cells did not respond to brushing and required a light pressure stimulus even in the middle of their receptive fields. In the middle of the receptive field, the cells exhibited a 'wide dynamic range' (Mendell, 1966). That is to say that, unlike the lamina 4 cells, if the pressure was increased from light touch to heavy pinch, the firing frequency of the cell increased with each increment of pressure stimulus. As in the lumbar region, the cells in lamina 5 responded to electrical stimulation of A$\beta$ afferents but with a longer latency than the cells in lamina 4. Twenty-six pairs of cells were examined; one cell of each pair was in lamina 4 and the other cell was immediately ventral to the first in lamina 5. These pairs of cells were examined for their latency of response to an afferent A$\beta$ volley generated by electrical stimulation applied directly to the skin of the cells' receptive fields. Two pairs showed no latency shift but the remainder showed a shift of 0·5–2·0 msec, average 0·9 msec. This latency shift had been previously interpreted as suggesting that the lamina 5 cells were stimulated by the lamina 4 cells which received the A$\beta$ input. In some preparations, the lamina 5 cells showed a marked habituation to repeated light brushing within their receptive field, a phenomenon previously described in lumbar lamina 5 cells in the decerebrate cat (Wall 1967) and the spinal rat (Wall, Freeman & Major, 1967). The final difference between lamina 4 and lamina 5 cells was that many of the cells with lamina 5 cutaneous characteristics responded to splanchnic nerve stimulation.

### 3. *Interaction of splanchnic and cutaneous volleys*

Since the cells which responded to splanchnic afferents also responded to cutaneous stimulation, it was important to study the interactions of these convergent inputs. This was done by stimulating both the splanchnic nerve and the cutaneous receptive field of the single cells under examination and by varying the time interval between the two stimuli. The results from two cells are shown in Fig. 4 where cell *A* showed the most commonly observed interaction and cell *B* a less frequent type. For cell *A*, the stimulus strengths were adjusted so that the cell responded with one or two

impulses when either the splanchnic or the cutaneous stimuli were applied by themselves. If the splanchnic stimulus preceded the cutaneous stimulus, the cutaneous response was inhibited. For this cell, the duration of the inhibition was found to be 150 msec. In the illustration, the responses of the cell are shown when the splanchnic stimulus was gradually shifted

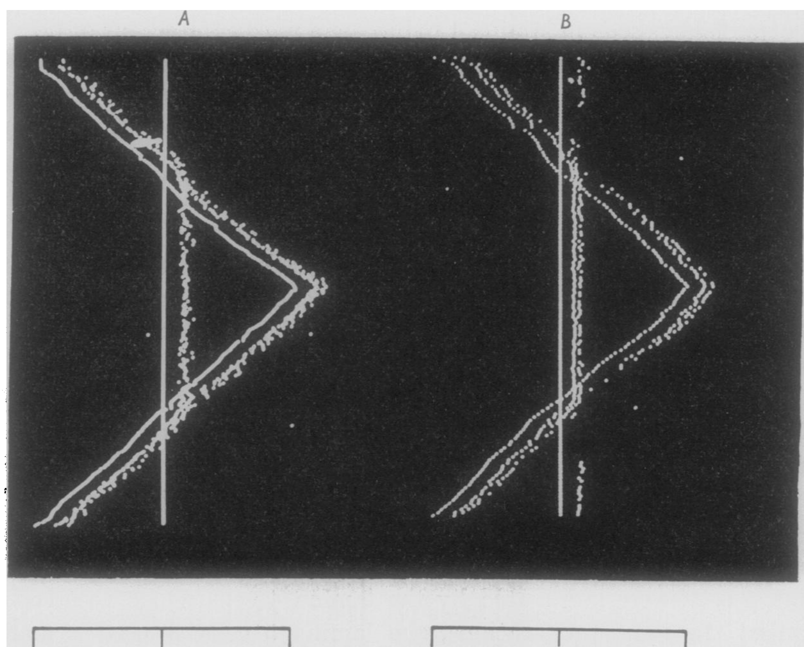


Fig. 4. Interaction of splanchnic and cutaneous afferent volleys on two lamina 5 cells, *A* and *B*, in segment T8. Paired stimuli were delivered once a second to AΔ splanchnic fibres and to the cutaneous receptive field of the cell. One hundred and fifty pairs of stimuli and responses are shown. The time interval between conditioning and test stimuli was progressively varied. The response to cutaneous stimulation is shown at a fixed position while the splanchnic stimulus was moved. At the top, splanchnic stimulation was given 100 msec before cutaneous stimulation and then moved step by step until, in the middle of the picture, cutaneous stimulation is given 100 msec before splanchnic stimulation (see Methods). The splanchnic volley inhibited the cutaneous response while the cutaneous volley had no effect on the splanchnic response in cell *A* and produced a weak inhibition in cell *B*. Time marks: 100 msec.

from 100 msec before the cutaneous stimulus to 100 msec after it. Where the splanchnic stimulation occurred earlier than the cutaneous stimulation, the cutaneous response was completely inhibited. When the two stimuli were synchronous, there was a partial summation of the two responses. Where the cutaneous stimulus preceded the splanchnic stimulus, there was no observable effect of the cutaneous stimulus on the responses of the

cell to the splanchnic volley. In cell *B*, the two inputs given by themselves each produced two impulses from the cell. If the splanchnic stimulus preceded the cutaneous by 15–100 msec, the illustration shows that the response of the cell to the cutaneous volley was partially inhibited so that the cell fired only once instead of twice. If the splanchnic stimulus preceded the cutaneous by 0–15 msec, the cutaneous response was completely inhibited. When the cutaneous stimulus preceded the splanchnic, it will be seen that in this cell, unlike cell *A*, the cutaneous stimulus inhibited the splanchnic response for a period of about 25 msec. In these experiments the cutaneous stimulus was firing off a far smaller number of peripheral nerve fibres than the splanchnic nerve stimulus.

We wondered if the reason why the splanchnic volley inhibited the cutaneous response while the cutaneous afferents usually failed to inhibit the splanchnic response was because the size of the cutaneous volley was so much smaller than the splanchnic volley. To test this suggestion, we stimulated the entire intercostal nerve at the costovertebral junction instead of stimulating the skin through hypodermic needles placed in the receptive field of the cell under observation. Under these conditions, it was found that the volley from the intercostal nerve always inhibited responses produced by splanchnic stimulation. The duration of the inhibition produced by intercostal nerve stimulation was about equal to that which followed splanchnic stimuli. The duration varied from cell to cell between 100 and 200 msec.

## *Lumbar cord*

1. *Effects of gastrocnemius nerve stimulation*

A. *Afferent volley.* The nerve to the gastrocnemius was dissected free in the popliteal fossa and placed on pairs of stimulating and recording electrodes. When a stimulus at twice the threshold intensity was applied to the nerve, a compound action potential was recorded from the nerve. This wave was produced by impulses in the large motor fibres and the group 1 and 2 sensory fibres. When the stimulus intensity was raised to ten times threshold, a later component of the compound action potential was recorded. This component was produced by impulses in the group 3 afferents.

*B. Cord cells responding to the afferent volley.* Gastrocnemius afferents enter the cord in the rostral S1 and caudal L7 dorsal root filaments. The exact location of the entry points was determined in each cat by local stimulation on the surface of dorsal rootlets and by recording the size of the antidromic volley on the nerve to gastrocnemius. A search was then carried out in segments L7 and S1 for cells which responded to afferent impulses from gastrocnemius. The method of mapping was the same as that used to search for cells in the thoracic cord which responded to

splanchnic afferents. Cells were located in the dorsal horn which responded to group 3 afferent nerve impulses but did not respond to group 1 or 2 afferents. These cells would only respond to gastrocnemius nerve stimulation if the intensity of the stimulus was 2·5–10·0 times the threshold for the largest axons. The cells fired repetitively for as long as 10 msec following the arrival of a maximal group 3 volley. In the lateral part of dorsal horn, close to the root entry zone of the gastrocnemius afferent fibres, many cells responded within less than 1 msec of the entry of the group 3 afferent impulse volley. These cells can therefore be assumed to be monosynaptically connected to the group 3 afferents. In other parts of segments L 7 and S 1, cells could be found which also responded when, and only when, a group 3 volley was generated, but these cells responded with a longer latency and were presumed to be fired indirectly by a polysynaptic pathway.

*C. Location of cells responding to group* 3 *muscle afferents.* The L 7–S 1 junction was mapped with regularly spaced micro-electrode tracks. The method used and the results obtained with cutaneous stimulation were similar to those previously reported (Wall, 1967). In the dorsal horn there was a dorsal lamina containing cells with small cutaneous receptive fields, a middle lamina with cells with greatly expanded cutaneous receptive fields and a ventral lamina containing cells which responded to passive movement of the leg. These three laminae which were delineated by recording from single cells corresponded roughly to Rexed's histological laminae 4, 5 and 6. The cells in lamina 4 never responded under any circumstances to stimulation of the gastrocnemius nerve. As soon as lamina 5 was entered and cells were encountered with enlarged cutaneous receptive fields, it was found that these cells were the ones which responded to group 3 afferent volleys but showed no response whatsoever to stimulation of group 1 or 2 afferent volleys. On deeper penetration, provided that the electrode was in the lateral half of the dorsal horn and close to the root entry zone of gastrocnemius afferents, cells were encountered with lamina 6 characteristics, that is to say, they responded to passive movement of the legs, particularly ankle flexion. Lamina 6 cells in the lateral half of the lamina responded to group 1 afferent volleys with a central latency of less than 1 msec after the arrival in the cord of the group 1 afferent volley. It is apparent therefore that in segments L 7 and S 1, lamina 5 contains the cells which respond monosynaptically to group 3 gastrocnemius afferents. Lamina 5 also contains cells which respond polysynaptically to these afferents.

2. *Cells responding both to cutaneous stimuli and to group* 3 *muscle afferents*

All those cells in lamina 5 which responded monosynaptically to gastrocnemius group 3 afferents had cutaneous receptive fields on the calf, Achilles tendon or heel. In other words they had a cutaneous receptive field which overlay some part of the muscle or its insertion. In contrast to the cells in direct contact with the group 3 afferents, the cells which responded with a latency of more than 1 msec after the arrival of the afferent volley had receptive fields on the sole of the foot and on the toes. Details of these receptive fields will be given below.

3. *Interaction of group* 3 *muscle afferent volleys and cutaneous volleys*

The cutaneous receptive fields of the lamina 5 cells receiving gastrocnemius group 3 afferents mainly lie in skin supplied by sural nerve. We therefore chose to study the interaction of volleys from the sural and gastrocnemius nerves rather than to stimulate the skin directly. The advantage of sural stimulation was, first, that we could generate a larger and more synchronous volley than could have been produced from skin stimulation and, secondly, that we could ensure by insulation of the stimulating electrodes that no stimulus spread to underlying muscle occurred. The results are shown for four different cells in Figs. 5 and 6. In Fig. 5, cell 1*A*, the gastrocnemius nerve was fired at thirty times the stimulus intensity for group 1 afferents and the cell responded with a multiple burst. The sural nerve was stimulated at 1·5 times the threshold for the largest A$\beta$ fibres and the cell responded twice. When the two volleys interacted, there was a slight inhibition of the sural response by the group 3 afferents and a weak inhibition of the group 3 response by conditioning from the sural. If the stimulus intensity on the sural was raised to fifteen times threshold (cell 1*B*) the inhibition of the gastrocnemius response by the preceding sural volley became very much stronger and the gastrocnemius inhibition of the sural effect disappeared. The other three cells, viz. Fig. 5, cell 2 and Fig. 6, cells 1 and 2, show variations of this balance between the mutual inhibitory effects of one volley on the other. Figure 6 cell 1*A* shows an inhibition of the sural response by group 3 afferents while there is no inhibition of the response to the group 3 afferent volley produced by threshold sural stimulation. This result is similar to that shown in Fig. 4*A* where splanchnic stimulation inhibited the response to cutaneous stimulation but cutaneous stimulation failed to inhibit the splanchnic response. However, if the stimulus strength to the sural nerve was raised to 1·5 times threshold, as in Fig. 6, 1*B*, the sural stimulus was then followed by an inhibition of the response to the group 3 afferents from gastrocnemius. Many of the cells, which responded to both

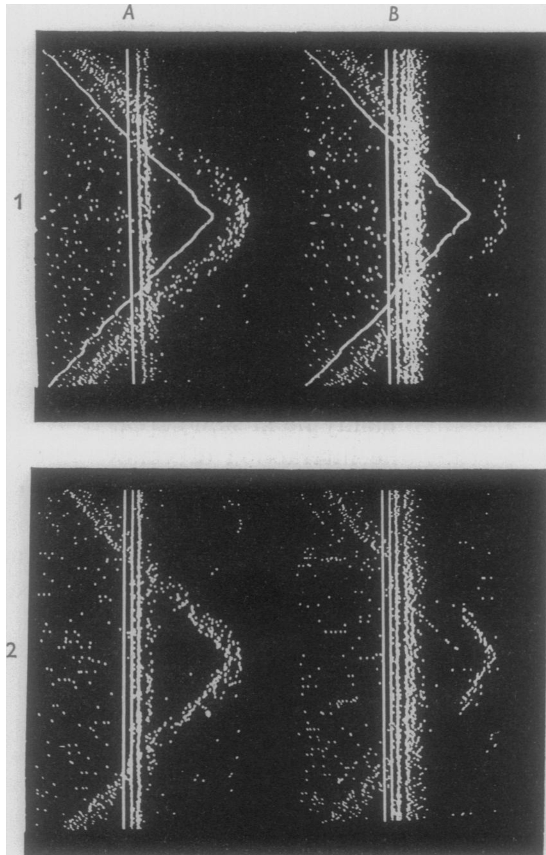


Fig. 5. Interactions on lamina 5 cells of gastrocnemius and sural nerve volleys. The method of recording is the same as that shown in Fig. 4. The vertical lines are the records of the stimulus artifacts and responses of a single cell to sural nerve stimulation. The timing of the gastrocnemius nerve stimulation was varied with respect to the sural nerve stimulus. Stimuli were given at 3/sec in pairs. At the top of each picture the stimulus to the gastrocnemius preceded that to the sural by 50 msec. The time interval between the gastrocnemius and sural stimuli was shifted regularly until half way down each picture the sural stimulus preceded that to the gastrocnemius by 50 msec. The lower half of each picture shows the effect of reversing the direction of shift of time interval between the two stimuli. Each picture shows the response of the cell to 300 pairs of stimuli.

Cell 1*A*. Sural stimulus $1\cdot5\times$ threshold for $A\beta$ fibres. Gastrocnemius stimulus $30\times$ threshold for group 1 fibres. Cell 1*B*. Sural stimulus $15\times$ threshold for $A\beta$ fibres. Gastrocnemius stimulus $30\times$ threshold for group 1 fibres.

Cell 2*A*. Sural stimulus $1\cdot5\times$ threshold for $A\beta$ fibres. Gastrocnemius stimulus $50\times$ threshold for group 1 fibres. Cell 2*B*. Sural stimulus $15\times$ threshold for $A\beta$ fibres. Gastrocnemius stimulus $50\times$ threshold for group 1 fibres.

afferent volleys, produced impulses with a low average frequency in the absence of intentional stimulation. This 'spontaneous' activity was also inhibited by the arrival of afferent volleys from either nerve. The 'spontaneous' activity produced the random scattering of dots to the left of the stimulus artifacts in Figs. 5 and 6. The absence of these random dots after the arrival of the afferent volleys illustrates the silencing of the

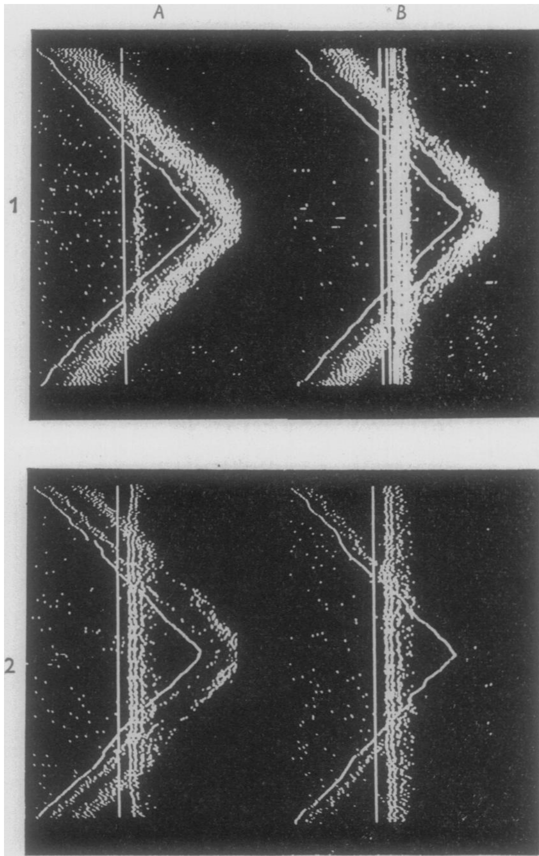


Fig. 6. Interactions on lamina 5 cells of gastrocnemius and sural nerve volleys. As in Fig. 5, each picture shows the response of a single cell in lamina 5 to pairs of stimuli to gastrocnemius and sural nerves. Three hundred pairs of stimuli and responses are shown. The diagonal lines are made by the gastrocnemius volley which was varied from 50 msec before to 50 msec after the sural volley.

Cell 1*A*. Sural stimulus 1 × threshold for A$\beta$ fibres. Gastrocnemius stimulus 30 × threshold for group 1 fibres. Cell 1*B*. Sural stimulus 1·5 × threshold for A$\beta$ fibres. Gastrocnemius stimulus 30 × threshold for group 1 fibres.

Cell 2*A*. Sural stimulus 15 × threshold for A$\beta$ fibres. Gastrocnemius stimulus 30 × threshold for group 1 fibres. Cell 2*B*. Sural stimulus 15 × threshold for A$\beta$ fibres. Gastrocnemius stimulus 3 × threshold for group 1 fibres.

'spontaneous' activity of the cells. In summary, the results show that a high intensity stimulus to either nerve inhibited the responses produced by a low intensity stimulus to the other nerve for periods of 45–200 msec.

*Lumbar cord B*

*Effects of sural nerve stimulation*

*A. Afferent volley.* The sural nerve was dissected free either in the popliteal fossa or on the surface of gastrocnemius, and stimulating and recording electrodes were installed on the nerve. The compound action potential produced by gradually increasing strengths of stimuli was recorded. Here, as with the splanchnic and gastrocnemius nerves, a slowly conducting group of nerve impulses, the A delta group, could be recorded.

*B. Cord cells responding to the afferent volley.* The dorsal horn was searched with micro-electrodes in the L 7–S 1 segments and stimuli of various amplitudes were given to the sural nerve. The search and mapping procedure was the same as that used for the splanchnic and gastrocnemius nerves. Two clearly separated types of cells were found. One responded with a brief repetitive discharge when an $A\beta$ volley was generated in the sural nerve but, when the stimulus was increased above this level, there was no further prolongation of the burst length. The second type, illustrated in Fig. 7, also responded to an $A\beta$ volley. However, as the stimulus intensity was increased, so that the afferent volley contained impulses in smaller and smaller fibres, the second type of cell, unlike the first, responded with a more and more prolonged burst of repetitive firing. The extent of this prolongation varied from cell to cell. In some cells the repetitive discharge did not extend beyond 50 msec and in others it lasted longer than 1 sec. As the stimulus strength increased, the repetitive discharge did not increase in a smooth fashion. The prolongation took place in a series of stages marked by the appearance of relatively high frequency bursts. In the cell shown in Fig. 7*A* there were seven clear stages in the prolongation of the repetitive charge. The last of these stages was triggered by the inclusion in the afferent volley of impulses in non-myelinated C fibres when the stimulus intensity exceeded 10 V for 0·1 msec. In the second cell, Fig. 7*B*, only two bursts were set off by the myelinated fibres and a third was evoked by non-myelinated afferents. Most of the cells of the second type produced 3–7 bursts of discharge during their repetitive discharge following the arrival of a volley set off by a supramaximal stimulus to the sural nerve. Mendell (1966) has reported this 'banding' of the repetitive discharge. The extension of the duration of repetitive firing was caused by recruitment of more and more fibres in the sural volley and was not due to current spread to other nerves. To test for the possibility of stimulus spread, the nearby gastrocnemius muscle was inspected

under a dissecting microscope for signs of twitches associated with the stimulus and, since none were seen it is reasonable to assume that the stimulating current was confined to the sural nerve.

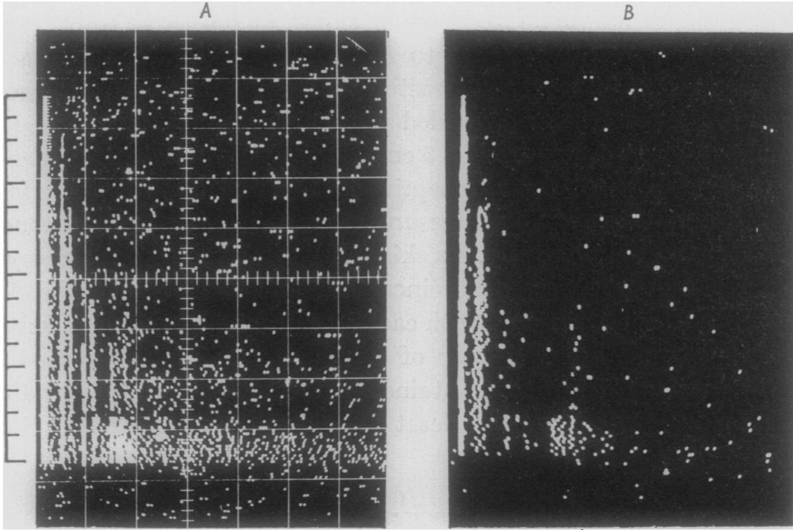


Fig. 7. Effect of increasing stimulus strength to sural nerve on responses of lamina 5 cells. Recordings from two cells, *A* and *B*, in lamina 5. Each nerve impulse produced a single dot and, if the nerve impulses occurred at high frequency, the dots fused to produce horizontal lines. Each picture shows the result of 112 stimuli given to the sural nerve at 1/sec. The responses to each stimulus are shown as a horizontal line of dots. The length of each line from left to right represents 1 sec. The stimulus strength was raised by sixteen steps. The beginning of each step is marked on the scale on the left of picture *A*. The results of the lowest stimulus are shown at the top of each picture. The stimulus strength to the sural nerve was increased in sixteen steps from 1 V for 0·01 msec to 20 V for 0·1 msec. Seven responses to each of the sixteen steps are shown.

*C. Location of cord cells responding to sural AΔ afferents.* It was found by mapping that the cells, which responded only to the A$\beta$ volley, all lay within lamina 4. Cells which showed a prolongation of their repetitive discharge if AΔ were included in the afferent volley, were found to lie deeper in the dorsal horn than cells with the brief discharge. In all these experiments, the sural nerve had been left intact so that it was possible both to stimulate the nerve electrically and to test the skin with natural stimuli. The cells in lamina 4, which responded only to A$\beta$ volleys, had small receptive fields, about 1–2 cm across, on the lower leg or foot. All responded to light brushing and to light touch and some increased their discharge if the skin fold was picked up. None showed a further increase of discharge if the skin fold was pinched. All adapted to a steady

stimulus, some within a few impulses while others maintained their discharge for seconds. The threshold within the whole area of the receptive field seemed uniform when tested by manual stimulation. No attempt was made to subdivide these cells into subclasses based on location, receptive field size, adaptation, pressure range or length of repetitive discharge.

If the deeper cells responding to AΔ fibres were tested with natural stimulation of the skin they contrasted with the lamina 4 cells in several ways. The receptive fields expanded proximo-distally within the S1–L7 dermatomes up to a length of 4–5 cm. There was a marked gradient of pressure sensitivity within the receptive field so that light brushing in the centre stimulated the cell but pressure was required at the edge. Finally, and perhaps most significantly, if a skin fold was picked up in the receptive field and the pressure gradually increased to a hard pinch, the firing frequency of the cell increased with each increment of pressure in contrast to lamina 4 cells. The frequency of discharge gradually decreased if a heavy pressure stimulus was maintained but some cells would maintain an increased firing frequency for at least 1 hr.

## DISCUSSION

The results show that there are cells in the region of lamina 5 which are excited by fine myelinated afferents from either viscera, muscle or skin. All of these cells also had a cutaneous receptive field and there were inhibitory interactions between the fine myelinated input and the afferents from the cutaneous receptive field. We failed to detect cells which responded only to visceral afferents or to group 3 muscle afferents. Such specific cells may exist in the population of cells from which recordings cannot be made with presently available micro-electrodes.

The acute spinal cat was chosen as a preparation on which to begin the investigation of these cells. There are reasons to believe that their responses may be different in the decerebrate or freely moving animal. Downman (1955) showed that viscero-somatic reflexes and the interaction of inputs were strikingly different in the spinal and decerebrate cat. Lundberg (1964) has reviewed the mechanisms responsible for the classical observation that section of spinal cord in the decerebrate animal facilitates flexor reflexes and inhibits stretch reflexes. Wall (1967) showed that spinal cord block in the decerebrate animal greatly facilitated the cutaneous response of lamina 5 cells and found that impulses descending from the head inhibited the cutaneous input and facilitated the proprioceptive input to lamina 6 cells. For these reasons, we now plan to extend the observations on these cells so that the effect of convergent inputs may be observed in decerebrate and other preparations. In describing the responses of these cells, it is

important to consider not only the activity of other parts of the nervous system but also the state of the cardiovascular system. We have not reported here results obtained from animals in shock with poor circulation observed in cord surface vessels because we noted that cells in lamina 4 and lamina 5 failed to respond to natural cutaneous stimulation in these animals although brief repetitive discharges could still be recorded from lamina 5 cells following splanchnic stimulation.

We failed to detect cells responding to the large diameter group of myelinated afferents in the splanchnic nerve. This fits with other types of evidence suggesting that these afferents may not play a role in generating local segmental reflexes. Widen (1955) showed that they did not produce a slow wave in the cord. Downman (1955) stimulated the largest group of splanchnic afferents without producing muscular reflexes and Franz *et al.* (1966) were unable to record a sympathetic discharge produced by their stimulation.

We have shown here that cells in the region of Rexed's lamina 5 contrast in their response properties with the cells in lamina 4 in the following ways: (1) larger cutaneous receptive fields, (2) response to smaller diameter myelinated afferents, (3) longer latency of response to cutaneous Aβ afferents, (4) wider dynamic range of response to cutaneous pressure stimuli, (5) slower adaptation of responses to continuous pressure stimuli. In a previous paper (Wall, 1967), the distinction between the cells in the two laminae had depended on the interpretation of quantitative differences of response and it remained a possibility that there was a continuous dorso-ventral gradient of gradually shifting properties as suggested by A. Taub (1966, personal communication). The results presented here now provide a clear qualitative difference between the two populations of cells since the more dorsal group do not respond to the fine myelinated afferents of visceral and muscle origin while the more ventral group does respond. Similarly, a clear ventral border to the region of lamina 5 cells can be drawn because lamina 5 cells respond only to group 3 muscle afferents while lamina 6 cells respond to groups 1 and 2. We did not search in lateral and ventral horns for cells responding to visceral afferents.

In previous papers by Wall (1960, 1967) there was no report of the important difference between lamina 4 and lamina 5 cells with respect to the range of pressure stimuli to which they responded. There were two reasons for this. Much of the earlier work depended on recording from axons in the dorso-lateral column which at that time were thought to have a uniform origin so that variations of the range of pressure sensitivity were thought of as variations within a single group of cells. In the more recent mapping experiments (Wall, 1967) intense pressure stimuli to the skin were avoided so that the skin would not be damaged for subsequent

stimuli and so that the stimulus would not spread mechanically to distant structures. Mendell (1966) defined a type of fibre in the dorsolateral tract (DLT) which had a wide dynamic range of response to pressure stimuli and also responded to unmyelinated fibres. Lundberg & Oscarsson (1961) reported that only those units in the DLT with wide dynamic range could be fired by group 3 muscle afferents while the units with a narrow range could only be fired by skin A$\beta$ fibres (i.e. lamina 4). Fetz (1968) found that some 20 % of DLT fibres originated in lamina 5 and the rest in lamina 4. He suggests that the deeper cells tended to have a wider dynamic range although he did not use heavy pinch stimuli. He also showed that pyramidal tract stimulation had a greater effect on cells with wide dynamic range. Wickelgren (1967) showed that a group of dorsal interneurones which habituated to repeated cutaneous stimuli tended to have the common properties of wide dynamic range, long latency of response, prolonged repetitive discharge, high spontaneous activity and perhaps larger receptive field size. The results of these workers tend to support the contention of this paper that there are in the dorsal part of the dorsal horn two types of cell responding to cutaneous stimulation.

The observation of the region of termination of splanchnic afferents confirms the slow wave analysis of Weber (1966) that the main activity was set off in the neck region of the dorsal horn. Similar conclusions were also reached for a visceral input entering the cord over the L4 dorsal root by Selzer & Spencer (1967) and by Selzer (1967). These authors also noted a powerful inhibition of the visceral input by the somatic and a mutual depolarization of the terminals of the two types of afferent fibres suggesting that at least part of the inhibition was presynaptic. We would now attribute the preponderant inhibition of the visceral input by the somatic, reported by Selzer & Spencer (1967), as being due to the larger size of the somatic input volley. The results reported here tend to support the suggestion made by Wall (1967) that lamina 4 units project on to lamina 5 cells and that this pathway explains why lamina 5 cells respond to A$\beta$ cutaneous afferents with a delay. More experiments are required to make clear the synaptic relations between A$\beta$ and delta cutaneous afferents and the cells of lamina 4 and lamina 5. We have presented evidence that some cells respond directly to small myelinated afferents while others respond with such a long latency that they cannot be directly in contact with afferents. Lamina 5 is histologically characterized by its reticulated appearance caused by the many longitudinally running bundles of axons (Rexed, 1952). These axons may interconnect the cells of the region and may be responsible for the spread of firing within the lamina at considerable distances from the cells which have received the input volley from fine myelinated afferents.

If the cells described in lamina 5 are the only ones to receive visceral afferents, then it must be that their activity triggers visceral pain reactions. It is therefore of considerable interest to note that these cells also have a cutaneous receptive field. Of the many theories of referred pain, this finding lends support to the theory of Ruch (1965) that interneurones would be found in the cord on which visceral and cutaneous impulses converge. If these are the cells which trigger pain reactions, then it is clear that the signal for pain must be some level of prolonged high frequency firing since very light pressure stimuli also evokes some discharge. It is also clear that the interactions between arriving afferent volleys and the effects of descending impulses which control the cells' excitability (Wall, 1967; Fetz, 1968) must be studied in detail.

Lamina 5 runs as a continuous structure through all segments of the spinal cord. We have shown that its cells in different regions receive small myelinated fibres from a visceral, a muscle and a skin nerve. One might therefore speculate that lamina 5 receives fine myelinated afferents from all types of tissue. Muscle is a specialized tissue with specialized end organs such as spindles and Golgi tendon organs but it may also contain general tissue receptors which may be attached to group 3 afferents. Similarly, skin has specialized endings such as those around hair follicles and touch corpuscles but in addition it may have general tissue receptors attached to some of the AΔ fibres. A final step in this train of speculation would be to suggest that lamina 4 receives the specialized cutaneous afferents, lamina 5 receives general tissue afferents and lamina 6 receives the specialized muscle afferents.

The authors would like to thank Miss Diane Major for help with the experiments and Drs Downman, Fetz, Mendell, Taub and Wickelgren for helpful discussions. The work was supported by the Medical Research Council of Great Britain and the Foundation Fund for Research in Psychiatry. One of us (W.V.W.) began work on this topic as part of his Ph.D. project directed by Professor C. B. B. Downman at the Royal Free Hospital Medical School.

## REFERENCES

Bessou, P. & Laporte, Y. (1961). Etude des recepteurs musculaires innerves par les fibres afferents du Groupe III (fibres myelinisées). *Archs ital. Biol.* **99**, 293–321.

Bessou, P. & Perl, E. R. (1966). A movement receptor of the small intestine. *J. Physiol.* **182**, 404–426.

Burgess, P. R. & Perl, E. R. (1967). Cutaneous nociceptors. *Fedn Proc.* **26**, 492.

Downman, C. B. B. (1955). Skeletal muscle reflexes of splanchnic and intercostal nerve origin. *J. Neurophysiol.* **18**, 218–235.

Fetz, E. (1968). Pyramidal tract effects on interneurons in the cat lumbar dorsal horn. *J. Neurophysiol.* **31**, 69–80.

Franz, D. N., Evans, M. H. & Perl, E. R. (1966). Characteristics of viscerosympathetic reflexes in the spinal cat. *Am. J. Physiol.* **211**, 1292–1298.

Gernandt, B. & Zotterman, Y. (1946). Intestinal pain: an electrophysiological investigation on mesenteric nerves. *Acta physiol. scand.* **12**, 56–72.

HUNT, C. C. & MCINTYRE, A. K. (1960). An analysis of fibre diameter and receptor characteristics of myelinated cutaneous afferents in cat. *J. Physiol.* **153**, 99–112.

LUNDBERG, A. (1964). Physiology of spinal neurons. *Prog. Brain Res.* **12**, 197–221.

LUNDBERG, A. & OSCARSSON, O. (1961). Three ascending spinal pathways. *Acta physiol. scand.* **51**, 1–16.

MENDELL, L. M. (1966). Physiological properties of unmyelinated fibre projection to the spinal cord. *Expl Neurol.* **16**, 316–332.

PAINTAL, A. S. (1960). Functional analysis of Group 3 afferent fibres of mammalian muscles. *J. Physiol.* **152**, 250–270.

PATTON, H. D. (1965). Special properties of nerve trunks and tracts. *Physiology and Biophysics*, ed. RUCH, T. C. & PATTON, H. D. 19th ed.. pp. 73–91. Philadelphia: Saunders.

REXED, B. (1952). The cytoarchitectonic organization of spinal cord in cat. *J. comp. Neurol.* **96**, 415–456.

RUCH, T. C. (1965). Pathophysiology of pain. *Physiology and Biophysics*, ed. RUCH, T. C. & PATTON, H. D. 19th edn., pp. 345–353. Philadelphia: Saunders.

SELZER, M. E. (1967). Interactions between visceral and cutaneous afferent nerve fibres in the spinal cord of cat. Ph.D. thesis, New York University Medical School.

SELZER, M. E. & SPENCER, W. A. (1967). Convergence and reciprocal inhibition of visceral and cutaneous afferents in the spinal cord. *Fedn Proc.* **26**, 433.

WALL, P. D. (1960). Cord cells responding to touch, damage and temperature of the skin. *J. Neurophysiol.* **23**, 197–210.

WALL, P. D. (1967). The laminar organisation of dorsal horn and effects of descending impulses. *J. Physiol.* **188**, 403–423.

WALL, P. D., FREEMAN, J. H. & MAJOR, D. (1967). Dorsal horn cells in spinal and freely moving rats. *Expl Neurol.* **19**, 519–529.

WEBER, W. V. (1966). Comparison of activity in thoracic spinal cord of visceral and somatic origins. Ph.D. Thesis. University of London.

WICKELGREN, B. G. (1967). Habituation of spinal interneurons. *J. Neurophysiol.* **30**, 1404–1423 and 1424–1438.

WIDEN, L. (1955). Cerebellar representation of high threshold afferents from splanchnic nerve. *Acta physiol. scand.* suppl.. **117**, 1–69.