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PROPERTIES OF SYNAPTIC LINKAGE FROM LONG RANGING AFFERENTS ONTO DORSAL HORN NEURONES IN NORMAL AND DEAFFERENTED CATS

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SUMMARY

1. In intact cats, dorsal horn cells sometimes respond to afferents entering the cord three or four segments more rostral. If dorsal roots near a segment have been cut for at least 4 weeks, many more cells respond to these long ranging afferents. Using extra- and intracellular recording, we examined the change in the nature of the connectivity of the long ranging afferents from dorsal roots L3 and L4 onto cells in L7.

2. In intact animals 33% of all cells in L7 recorded extracellularly and 48% of those recorded intracellularly exhibited responses to electrical stimulation of skin whose afferents entered the spinal cord over dorsal roots L3 and L4. Most of these responses had a long latency and followed high frequency stimulation poorly.

3. Thirty-eight to fifty-one days after cutting dorsal roots L5–S2, 76% of cells recorded intracellularly in L7 responded to long ranging afferents, in contrast to 48% in the intact cord. In these preparations 48% of all cells responded in less than 5 msec versus 12% in intact cats. Furthermore, 21% of the cells had natural receptive fields in the distant dermatome versus 1% in intact animals.

4. Chronic deafferentation of a segment increased the number of cells responding to afferents arriving over distant dorsal roots and increased the speed and power of the responses.

INTRODUCTION

We are concerned here with the nature and plasticity of a widespread subliminal fringe which exists beyond the natural cutaneous receptive field of dorsal horn cells. In L7 of the adult cat, the natural receptive field of almost all cells more than 100 μm medial to the root entry zone lies on the foot or toes (Brown & Fuchs, 1975). Lateral to this line, cells have their receptive field on the leg. Amongst these lateral cells, a small number (21%) have extensive receptive fields which reach as far as the L4 dermatome (Devor & Wall, 1976). In the present experiments, we find 1% of all L7 dorsal cells have such fields. Thus almost all cells subserve the dermatome of their own segment and are supplied with afferents over nearby dorsal roots.

Electrical stimulation of skin or dorsal roots shows that substantial numbers of

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cells respond to afferents originating from skin distant from their natural receptive field (Merrill & Wall, 1972; Devor, Merrill & Wall, 1977). Afferents have been shown to distribute more widely in the cord than would be predicted from the natural receptive field of cord cells (Wall & Werman, 1976). However, the latency and frequency following of cells responding to these long ranging afferents suggest that many are driven by polysynaptic inputs (Devor *et al.* 1977). Whatever the linkage, it is evident that the synchronisation of afferent volleys produced by electrical stimulation reveals the existence of an extensive subliminal fringe on many cells.

The interest in this subliminal fringe is increased by the observation that deafferentation of cells either by cutting nearby roots (Basbaum & Wall, 1976; Dostrovsky, Millar & Wall, 1976) or by cutting peripheral nerve (Devor & Wall, 1978) is followed by the appearance of cells which can be driven by natural stimulation of long ranging afferents. This shift of receptive field naturally raised the question of whether existing subliminal connexions have been strengthened or whether intact afferent fibres have formed new connexions on deafferented cells. Thus far, all observations have depended on extracellular recording. Here we use intracellular recording which should provide a more accurate assessment of the extent of the subliminal fringe and of changes following deafferentation.

METHODS

Adult cats were prepared for examination of the lumbar enlargement either following decerebration or Dial-urethane anaesthesia (0·75 mg/kg) as previously described (Devor *et al.* 1977). The spinal cord was transected at T13. The animals were paralysed with gallamine and artificially ventilated. For extracellular recording, glass KCl-filled pipettes of 2–4MΩ resistance were used. For intracellular recording, 10–25 MΩ electrodes, filled with 2 M-sodium citrate were used. Each cord was searched from just lateral to the root entry zone to the medial edge of the dorsal horn in L7 to a depth of 2 mm. Electrode tracks were located by cutting the electrode followed by dehydraation and clearing (Wall & Werman, (1976).

For extracellular recording, each spontaneously active unit which could be satisfactorily isolated as a single unit had its natural receptive field identified by first roughly rubbing over the surface of the flank, leg, foot and toes. It was then defined in more detail with brush, touch and pressure probes. Then the latency of electrical responses from the middle of its natural receptive field was determined by placing two 25 gauge needles in the skin and stimulating with a square pulse (5 mA, 0·1 msec, 1 Hz). Each cell was also tested to see if it would respond to electrical stimulation of other limb regions and also skin of the flank using the same type of electrodes and pulses. The flank electrodes were placed subcutaneously just rostral to mid thigh on the lateral abdominal wall. At the end of the experiment, dorsal roots were dissected free and cut, and it was shown by recording from the distal end of cut rootlets that the afferent volley from the flank stimulus arrived only over rootlets L3 and L4. During intracellular recording, a pair of ball electrodes was placed on dorsal columns in L5 and a regular stimulus was applied while the fine micro-electrode was lowered into the dorsal horn. Units responding transynaptically with a repetitive discharge to the dorsal column stimulus and displaying signs of intracellular penetration (stable membrane potential 25–70 mV, evidence of e.p.s.p.s and i.p.s.p.s, positive going action potentials) were chosen for study. The peripheral stimuli were the same as those used for extracellular recording. Deep cells sensitive only to joint movement and/or muscle palpitation were not studied.

For the chronic deafferentation, cats were anaesthetized with Nembutal (35 mg/kg). Under sterile conditions, a hemilaminectomy of vertebrae L5–L7 was performed and the epidural fat removed by suction. This revealed the roots and ganglia of L5–S1. The dural cuff of each root medial to the ganglion was slit and, using a fine glass hook, jeweller's forceps and scissors, the dorsal root was dissected free of the ventral root and sectioned filament by filament. This procedure can be carried out without c.s.f. leakage and with preservation of the blood supply. The

entire S2 root was crushed with a fine haemostat. The exposed roots were covered with gelfoam and the incision closed in layers. With antibiotics, all cats recovered uneventfully. Thirty-eight to fifty-one days after deafferentation, the cords were searched in an acute experiment and finally the cord was fixed for histological examination. This revealed no meningitis, root and dorsal column demyelination and, unlike longer survivors (Basbaum & Wall, 1976), no significant gross atrophy or distortion of the dorsal horn in the L7 segment.

RESULTS

Extracellular recording of units in intact cord

Recordings were made from 272 units in the intact L7 dorsal horn in twenty-six cats. In 269 cells the natural receptive field was restricted to the leg or foot or toes and only in three did it extend rostrally to include the region in which the flank electrical stimulus was applied. However, eighty-nine of the cells (33%) responded to the flank electrical stimulus. This over-all figure hides an important difference between cells depending on their location in the dorsal horn and the location of their receptive field. For 206 cells located in the medial two thirds of the dorsal horn the receptive field was on the foot or toes. Of these cells twenty-eight (14%) responded to the distant stimulation. For seventy-nine cells located more laterally, the receptive field tended to be on the upper or lower leg. Of these lateral cells, sixty-one (79%) fired in response to the distant stimulus. The cells had cutaneous response characteristics previously described for laminae IV and V (Wall, 1967). The distribution of latencies from the flank stimulus for the eighty-nine responding cells is given in Fig. 1*A*. It will be noticed that only four cells fired within 5 msec of the stimulus. These followed stimuli at more than 10 Hz with a fixed latency. For the rest, as latency increased, variability of the discharge was enhanced and the ability to follow high stimulus repetition rates was diminished. The threshold for the flank-evoked response was very close to threshold for the afferent volley and similar to that for receptive field stimulation, indicating that large peripheral A fibres were involved in mediating these effects.

Intracellular recording

A total of ninety-four cells were penetrated and held for sufficient time to carry out the necessary stimuli and to measure latencies. Immediately on penetration, a membrane potential of 60–70 mV was recorded but this often decayed to 30–50 mV. In some units, the spike mechanism became inactivated (Hongo, Jankowska & Lundberg, 1968) but e.p.s.p.s and i.p.s.p.s remained evident. For each cell, we verified that each observed response was a true transmembrane potential by comparing the intracellularly recorded response to the field potential recorded extracellularly. The response of each cell was examined in single sweeps and with an average of 8–32 sweeps following stimulation so that small responses could be detected.

Intact preparations

Thirty-three cells were examined in nine cats. Receptive fields were located for thirty-one. Twenty-one of the natural receptive fields were on foot or toes and nine were on the upper or lower leg. None extended rostrally into the region of the distant stimulus. However, fifteen of these thirty-one cells responded to electrical stimuli at the distant site (eight of twenty-two medial cells with foot or toe receptive field and

seven of nine lateral cells with leg receptive field). The distribution of latencies is shown in Fig. 1*B*. It will be noted that four of thirty-three cells responded (with e.p.s.p.s) in less than 5 msec, compared with four of 272 cells recorded extracellularly which responded with spikes in less than 5 msec.

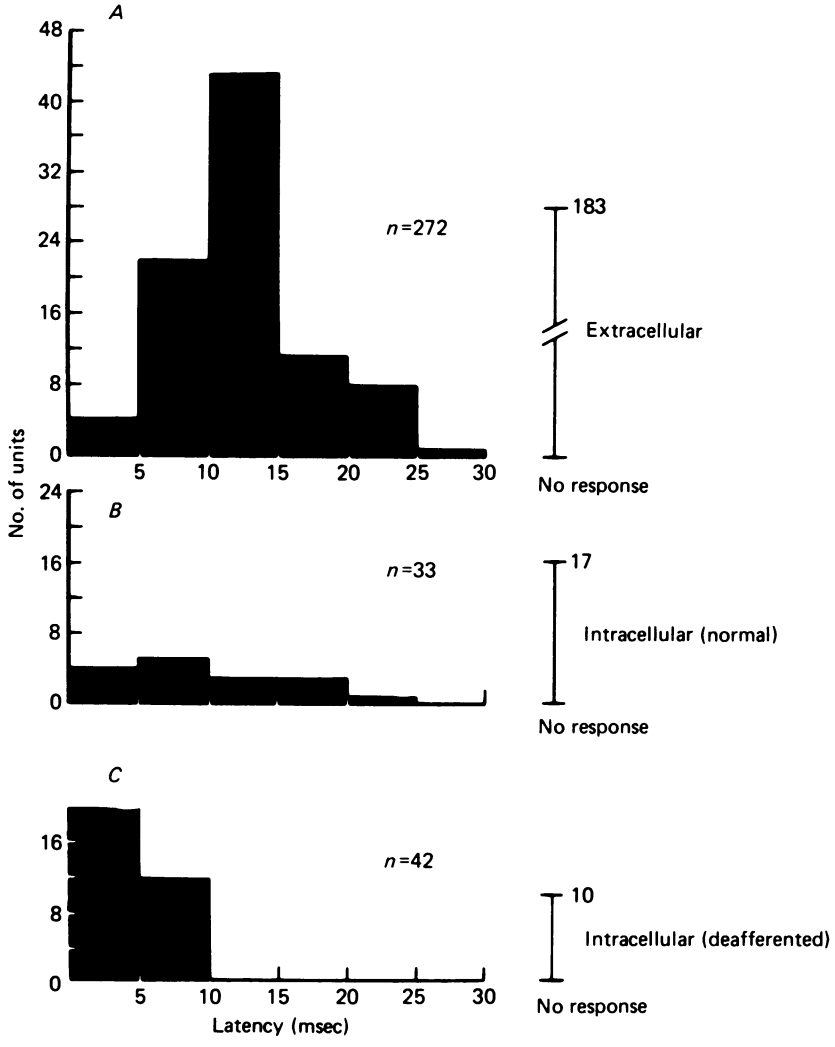

Fig. 1. Distribution of latencies to first sign of excitation for *A*, extracellular spike responses in intact preparations; *B*, intracellular e.p.s.p. responses in intact preparations; and *C*, intracellular responses in chronically deafferented cats.

If the electrical stimulus was applied directly to the natural receptive field, the initial response was excitatory, often including repetitive discharge (Fig. 2*A*). This was repeatable from stimulus to stimulus at 1 Hz (Figs. 2 and 5). At higher stimulus intensities a mixture of depolarizing and hyperpolarizing responses was often observed (Hongo *et al.* 1968). In Fig. 3 the responses to a series of four stimuli of increasing intensity is shown. The lowest strength evoked only an e.p.s.p. Raising the stimulus strength gave rise to a repetitive burst of seven impulses which was abolished

by the appearance of inhibition as the stimulus was increased further. All the studies described here were carried out using stimulus strengths equivalent to the maximum value shown in this Figure (i.e. substantially above threshold for the initial e.p.s.p.).

Stimulation at points outside the receptive field evoked more complex responses, often beginning with an i.p.s.p. (Figs. 2*C* and *D*). The e.p.s.p.s were not constant (Figs. 2*B*, *C* and *D*) even at 1 Hz. In particular, flank stimuli usually evoked depolarising responses with variable latencies which often preceded or were mixed with i.p.s.p.s (Fig. 4). More rarely, flank stimuli produced regular and repeatable

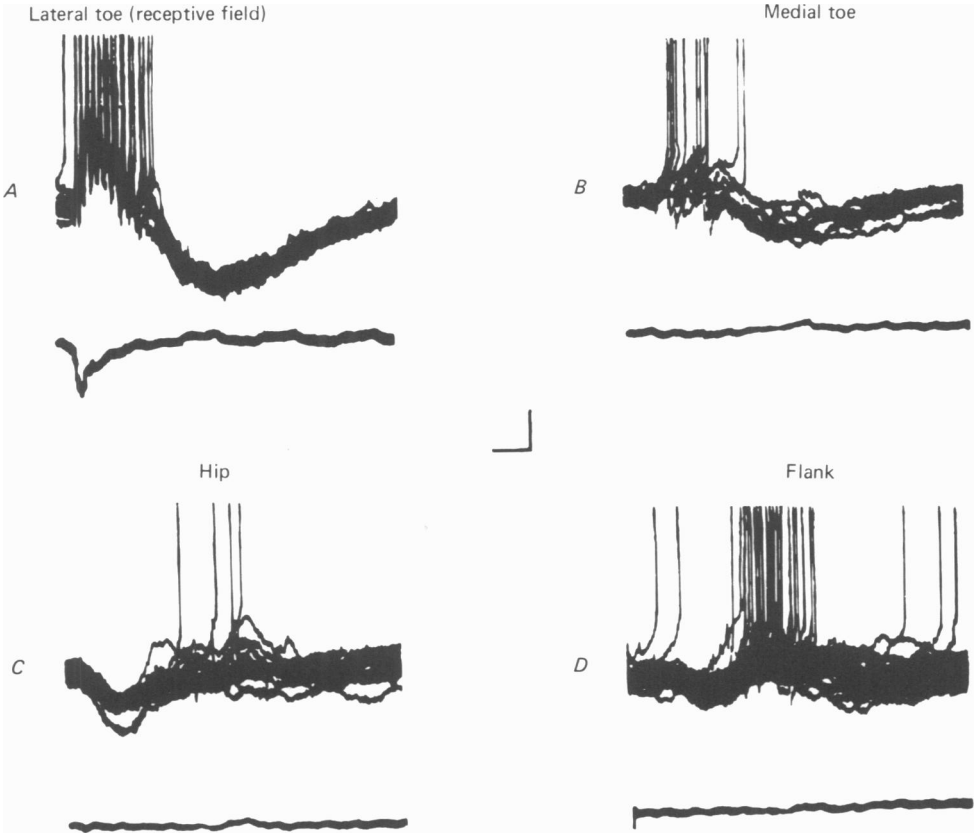

Fig. 2. Responses of a single cell to stimulation of different positions on the body surface. Extracellular records are below each response. Receptive field was brush-touch on lateral toe with pressure on lateral foot and ankle. Depth of the cell was 1·4 mm. Note the reproducibility of the response from receptive field stimulation compared to the others. In this and subsequent Figures, spikes (retouched) were attenuated by input stage of tape recorder which was adjusted to display the synaptic potentials. 10–15 superimposed sweeps. Membrane potential was −25 mV. Calibrations 10 msec, 5 mV.

depolarising responses (Fig. 4*B*, 2). It will be seen in Figs. 4*A*1, 4*A*2 and 4*B*1 that e.p.s.p.s often occurred irregularly, a fact which would have been hidden by averaging but which appears in superimposed sweeps. Responses at short latencies (4–12 msec) to stimulation of long ranging afferents were more or less constant at

1 Hz but generally failed at 10 Hz (Fig. 5). The ability to follow high stimulus rates improved as the latency diminished; one very small e.p.s.p. at 4 msec latency barely followed at 20 Hz. Such cells tended to have proximal receptive fields. The meaning of these latencies can be judged from the observation that the minimum time from the flank stimulus point to the L4 dorsal root was measured as 0·9 msec and from the L4 dorsal root to L4 afferent fibres in the L7 segment as 1·0 msec. These are minimum times and slower afferent impulses arrive over a period of at least 2–5 msec (Wall & Werman, 1976).

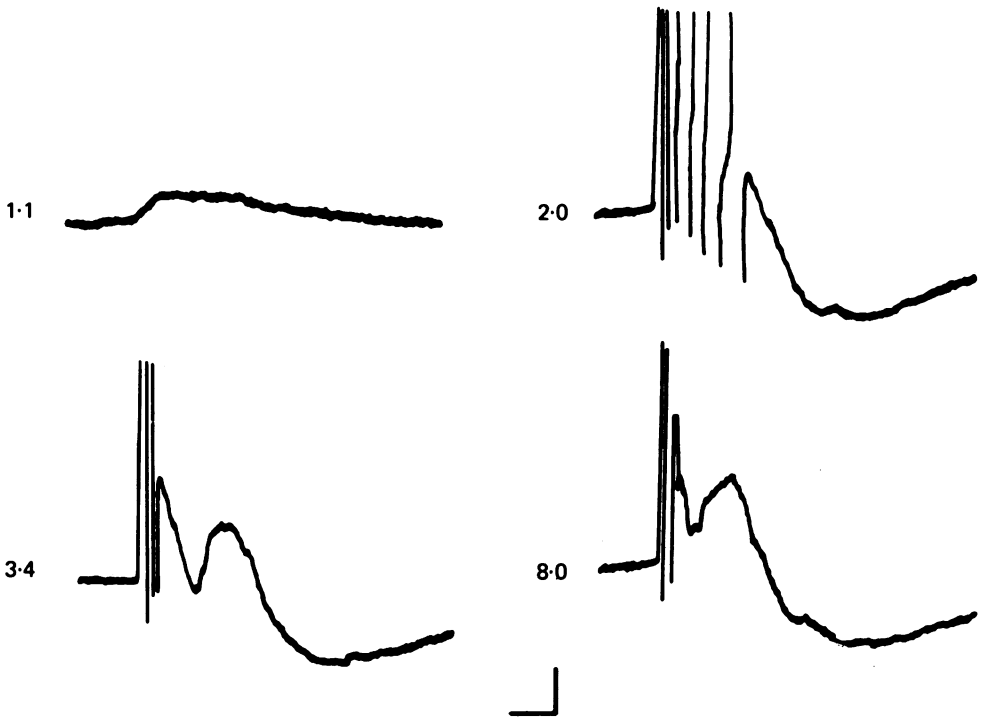

Fig. 3. Demonstration of mixed excitation and inhibition from electrical stimulation of the skin in the receptive field. Single sweeps. Cell was located 1·3 mm from the surface. Membrane potential was 60 mV. Brush-touch receptive field was between lateral and second toe. Records display the response of the cell to electrical stimulation of the receptive field where threshold is defined in terms of the appearance of the post-synaptic response. Further discussion in text. Calibration 5 msec, 1·5 mV.

Acutely deafferented preparations

In two cats, the lumbar enlargement was exposed and dorsal roots L5–S2 were acutely sectioned within the dura. The L7 segment was searched for cells. Nineteen cells were penetrated and held for sufficient time to examine their responses to peripheral stimuli. As expected, they failed to respond to leg stimuli and their responses to flank stimulation did not differ significantly from those seen in the intact animal.

Chronically deafferented preparations

Forty-two cells in L7 were penetrated and held for sufficient time to examine their response characteristics in eight cats which had survived 38–51 days after roots L5–S2 had been sectioned. A preliminary extracellular search in each animal showed that many cells located throughout the dorsal horn had acquired natural receptive

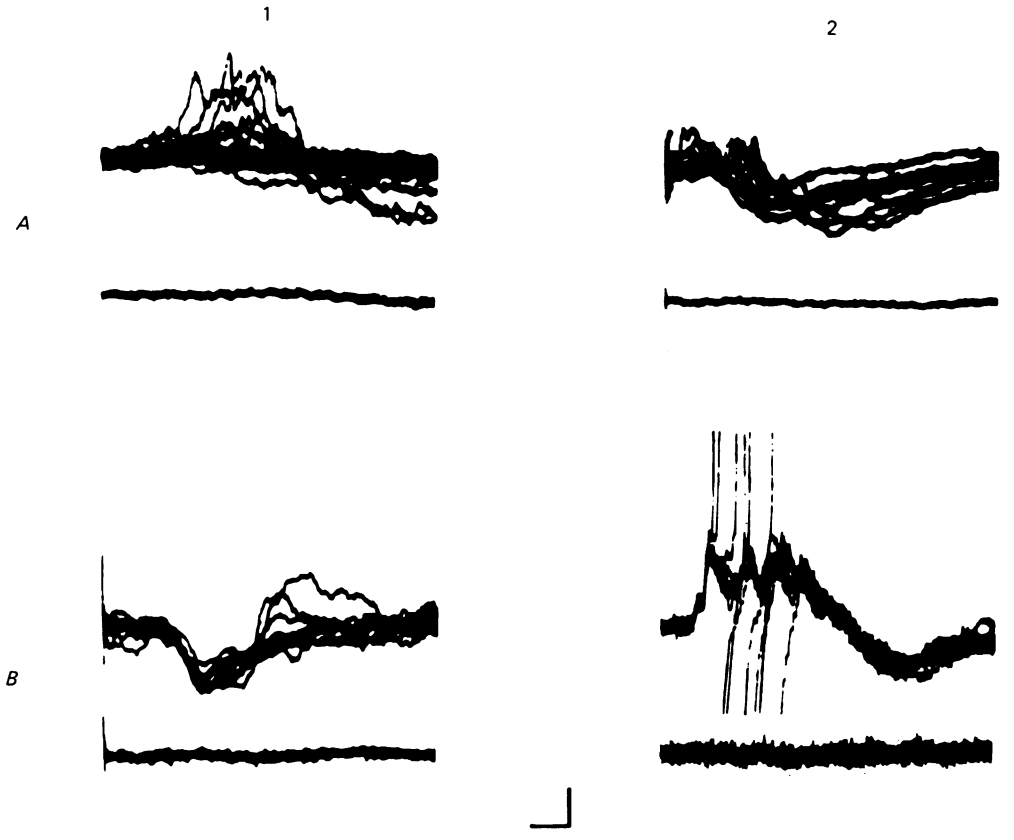

Fig. 4. Examples of responses of four cells recorded in L7 to stimulation of the flank (long ranging afferents). 10–15 sweeps. Extracellular responses below each. The two cells in row *A* are from one cat and those from row *B* are from another. *A*1, depth 1·5 mm, receptive field was brush-touch on second most lateral toe and pinch on distal foot, membrane potential was −50 mV. *A*2, depth 1·2 mm, brush-touch receptive field was on distal foot and pinch on proximal foot which caused hyperpolarisation of the cell, membrane potential was −50 mV. *B*1, depth 1·4 mm, receptive field was brush-touch on proximal lateral foot and ankle, membrane potential was −60 mV. *B*2, depth 1·7 mm, receptive field was brush-touch on the proximal lateral leg, membrane potential −50 mV. Calibrations *A*1: 10 msec, 2 mV; *A*2: 10 msec, 5 mV; *B*1: 5 msec, 1·2 mV; *B*2: 5 msec, 2 mV.

fields on the flank, confirming previous work (Basbaum & Wall, 1976). Of the forty-two cells examined intracellularly, thirty-two (76%) responded to electrical stimulation of the distant afferents, compared to 48% in the intact animals. A more striking difference was that twenty (48%) responded with latencies of less than

5 msec, compared to 12% in the intact preparation. These short latency e.p.s.p.s were constant in amplitude and latency and always followed at high stimulation frequencies, occasionally up to and beyond 100 Hz (Fig. 6). At 100 Hz, a clear shortening of the e.p.s.p. was observed (Fig. 6), presumably reflecting a depression of later polysynaptic components at high stimulation rates. In these deafferented animals nine out of forty-two cells (21%) had natural receptive fields on the flank,

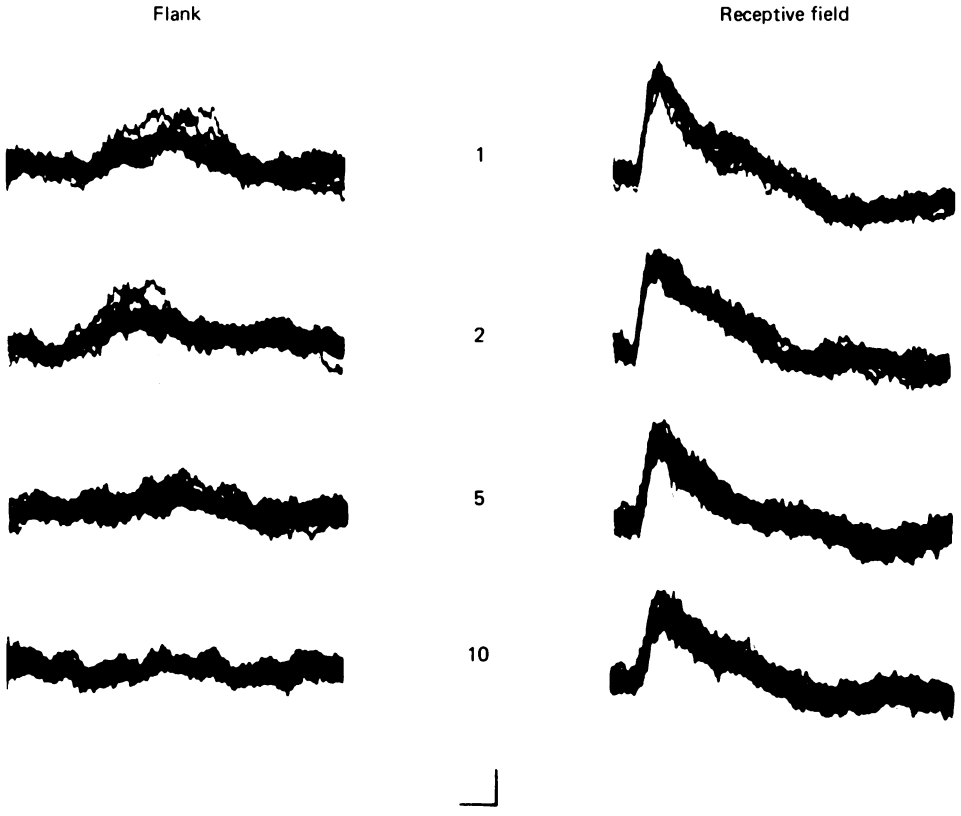

Fig. 5. Ability of e.p.s.p. to follow higher rates of stimulation in the hip receptive field than the flank. Stimulation rates of 1, 2, 5 and 10 Hz. Unit had brush-touch receptive field on hip. Membrane potential = −50 mV. Depth from surface 1·2 mm. 10–15 superimposed sweeps. Calibration 5 msec and 1 mV.

compared to fewer than 1% in intact preparations. Whereas the cells with flank receptive fields in intact preparations were all located in the extreme lateral part of dorsal horn, requiring penetrations lateral to the root entry zone, the locally deafferented cells with such receptive fields were scattered throughout the grey matter. In three of the eight animals, the initial extracellular search revealed a few cells with distal receptive fields suggesting that some dorsal roots had not been divided completely. This unintentional partial deafferentation of the cord offered an advantage since medial cells could be detected which presumably retained at least part of their original receptive field. The responses of three such cells to flank stimuli are shown in Fig. 7. Each of these cells had natural receptive fields on foot or toes and yet

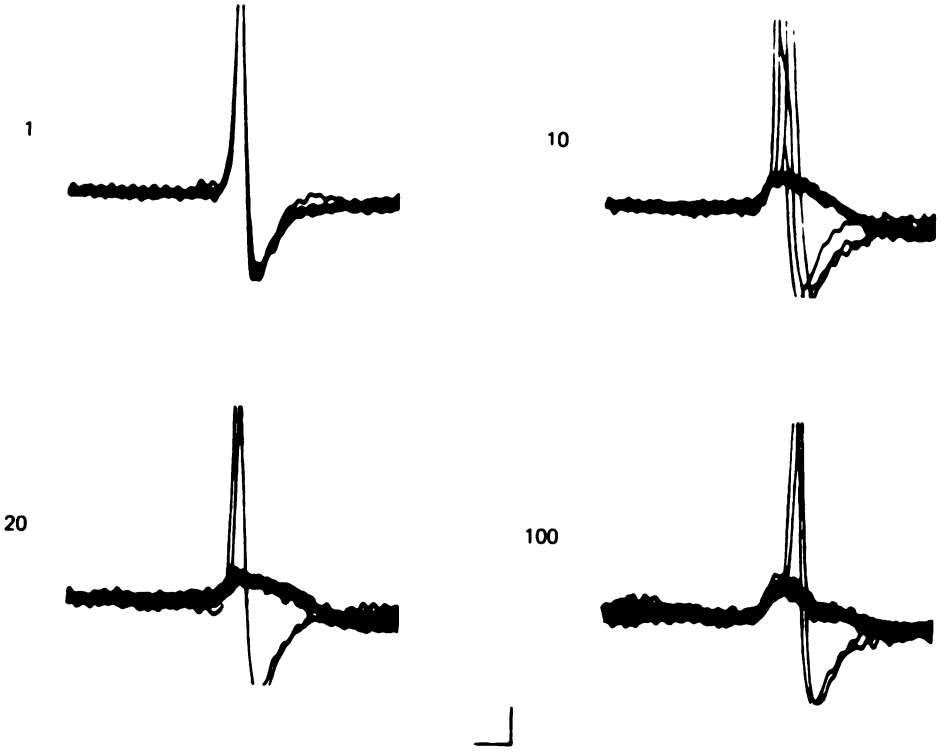

Fig. 6. Ability of cell in chronically deafferented preparation to follow high frequency stimulation delivered to the flank (long ranging afferents). 10–12 superimposed sweeps for each. This cell had a large brush-touch receptive field on the flank of the animal including the (electrical) stimulus position. There was a small brush-touch sensitive region on the upper lateral leg not connected with the main receptive field. Note the ability of the cell to generate constant amplitude e.p.s.p.s even at 100 Hz. The low frequency cut-off was set at 100 Hz to avoid base line jitter. Action potential of this cell was 40 mV (but attenuated by tape recorder input stage). Calibration 1 msec, 5 mV.

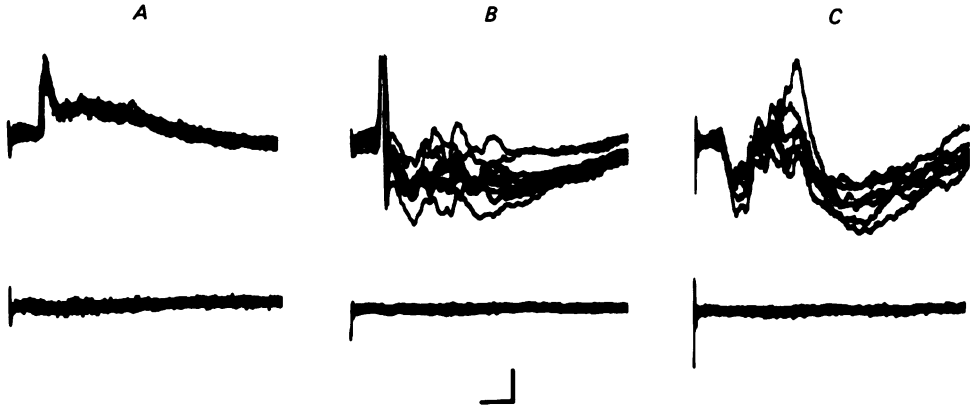

Fig. 7. Response of three cells in the same chronically deafferented preparation to identical flank stimulation. 10–12 superimposed sweeps. Extracellular responses below. Note the regular early e.p.s.p. in *A*, the early e.p.s.p. and spike followed by variable late e.p.s.p. in *B* and the early i.p.s.p. followed by a variable late e.p.s.p. in *C*. Further discussion in text. The membrane potentials were -25, -60 and -65 mV for these three cells. Calibration 5 msec and 2 mV.

two of them (Figs. 7*A* and 7*B*) had a short-latency reliable excitatory input from the flank, a combination never observed in the non-deafferented preparation. A third cell (Fig. 7*C*) exhibited a more typical response for a cell with a distal receptive field consisting of an early i.p.s.p. followed by a later and variable e.p.s.p.

DISCUSSION

We have shown here that many dorsal horn cells in intact preparations respond to afferents entering the cord over distant roots although most of these responses appear to be produced by weak or polysynaptic pathways. After deafferentation by cutting nearby dorsal roots, many more cells respond to both natural and electrical stimulation of the long ranging afferents and the speed and reliability of many of these responses approaches that expected for monosynaptic connexions. The general observations confirm those previously seen (Basbaum & Wall, 1976). The reason for the present experiments with intracellular recording was to ask whether the responses observed in the deafferented cords were novel or only an exaggeration of responses present in intact preparations.

We must first ask if our method of sampling could have inadvertently selected cells of a particular type. In each animal, search tracks were made throughout the dorsal horn with care being taken not to prefer one region over another. Unlike a previous paper (Devor *et al.* 1977), where cells were selected which responded to distant root stimulation, we here examined each isolated cell and asked if it responded to afferents from L3 and L4. Since we know that a rare type of cell exists in intact cord L7 segment which has a large natural receptive field extending rostrally onto the abdomen (Devor & Wall, 1976), it is possible that we were selecting only such cells in the deafferented animals. Such cells might appear to have only a flank receptive field, having lost the leg part of their receptive field as a result of the cutting of the dorsal roots supplying the leg. There are three reasons why we can reject this possibility. The first is that the deafferented cells responding to flank stimuli were scattered through the entire medial to lateral extent of the dorsal horn whereas the cells with natural flank receptive fields are all located on the lateral margin of dorsal horn. The deafferented cords showed no gross atrophy, so that medial displacement of lateral cells is improbable. Secondly, such cells were rare in intact cord (21 % of *lateral* cells and 1 % of all cells) whereas they were common throughout deafferented cord. Thirdly, the unintentional incomplete deafferentation in three animals allowed the observation of medial cells still with a foot or toe receptive field and yet which also responded briskly and reliably to flank stimulation quite unlike cells in intact animals. The necessity to use a search stimulus while attempting intracellular penetration might have biassed the sample in favour of cells connected to long ranging afferents. Since dorsal roots were to be cut, it was necessary to use dorsal column search stimuli in both groups of animals and it is therefore apparent that the cells located by this method are necessarily driven by those fibres which remain in the dorsal column.

From the results on intact animals, we can first ask if intracellular recording revealed a more extensive subliminal fringe than was apparent with extracellular recording. The over-all number of penetrated cells may be too small to provide

significant results for this general question. However it is true that 48 % (sixteen of thirty-three) of the penetrated cells produced depolarising responses to long ranging afferents, whereas 33 % (eighty-nine of 272) of intact cells exhibited spikes on extracellular recording. What is significantly different is that under 5 % of the extracellular responses occurred within 5 msec of the stimulus whereas 25 % of the intracellular responses occurred in this time. If we included those cells which failed to respond at all, 2 % of the cells responded in less that 5 msec by extracellular recording versus 12 % recorded intracellularly. The fastest recorded conduction time of afferent impulses from the peripheral stimulus to the region of the recorded cells was 2 msec. Therefore it is unlikely that more than a very small number of these responses were monosynaptic particularly in view of the variability in latency and inability to follow high stimulus repetition rates (Berry & Pentreath, 1976). There is no doubt, however, that many cells, even with intracellular recording and with averaging of many sweeps, failed to show any excitatory response to distant stimuli although it remains possible that very small e.p.s.p.s with variable latency went undetected even after averaging. These non-responding cells occurred particularly in medial dorsal horn and had distal natural receptive fields.

In the chronically deafferented cords, it is clear that all measures of response to long ranging afferents showed an increase in comparison to cells examined in intact animals. The percentage of cells responding to electrical stimulation rose from 48 % to 76 %. Of these intracellular responses, the percentage occurring within 5 msec of the stimulus rose from 25 % to 63 %. The regularity of latency and frequency following markedly increased. Cells with natural receptive fields on the flank rose from 1 % to 21 %. These cells were no longer confined to the lateral border of the dorsal horn but were spread throughout. Clearly there had been a considerable increase in the number of responding cells. The analysis of e.p.s.p. properties suggests further that there were many more cells which were candidates for having a powerful and monosynaptic input from long ranging afferents. To this extent we can conclude that the increased responsiveness resulted from new connexions rather than the strengthening of previously subliminal ones. These may have been produced by sprouting of intact afferents onto deafferented cells from which we recorded (Liu & Chambers, 1958; Murray & Goldberger, 1974). However, it remains a possibility that the connexions so commonly observed after deafferentation were the result of unmasking of synapses which were present but non-functional (i.e. produced no e.p.s.p.s) in the intact animal (Wall, 1977). A further possibility is that some of these new connexions were polysynaptic with increases in synaptic efficacy occurring further back in pathway between afferents and the interneurones studied here.

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