

## EXCITATORY ACTIONS OF SINGLE IMPULSES IN SINGLE HAIR FOLLICLE AFFERENT FIBRES ON SPINOCERVICAL TRACT NEURONES IN THE CAT

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### SUMMARY

1. In cats under chloralose anaesthesia single dorsal root ganglion cells with axons innervating hair follicles were stimulated intracellularly to produce single impulses. At the same time single spinocervical tract (s.c.t.) neurones were recorded extracellularly, from their axons in the upper lumbar cord.

2. When the receptive field of the afferent fibre was contained within the impulse firing zone of the s.c.t. cell, a single afferent impulse increased the probability of firing of the neurone. In thirty-nine pairs of units, where the afferent fibre had a group II conduction velocity, coupling was very efficient and for seventeen pairs the single afferent impulse produced one or more impulses in the s.c.t. cell in at least 90% of trials. The mean number of impulses evoked in s.c.t. cells by a single group II afferent impulse was 1.47. The latencies of the impulses ranged from 1.5 to 14.0 ms, with times to peak and total durations of 2.5–17.5 ms and 4.5–28.0 ms respectively. For two pairs of units where the afferent fibre had a group III conduction velocity the effectiveness of single afferent impulses was much less and the latencies, but not the durations, of the impulses were longer (12 and 17 ms).

3. When the receptive field of the hair follicle afferent fibre was outside, but close to, the firing zone of the s.c.t. neurone there was no indication that single afferent impulses affected the probability of neuronal discharge for thirteen of fifteen pairs of units. Weak excitation was observed in two pairs and this was clear only when two or more afferent impulses were employed.

4. There was a tendency for hair follicle afferent fibres with their receptive fields at or near the centre of the s.c.t. cell's firing zone to be most effective, producing shorter latency responses with more impulses at higher frequencies. When the afferent's field was peripherally located in the s.c.t. neurone's firing zone there was a wide range of responses but these included those with the longest latencies and very few impulses.

5. The results are discussed with reference to previous work on the spinocervical tract and to the known actions of single impulses on other neuronal types.

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Suggestions are made for the possible excitatory neuronal circuits linking hair follicle afferent fibres to the s.c.t. neurones.

#### INTRODUCTION

More than 90 % of spinocervical tract (s.c.t.) neurones are excited by moving hairs of the coat (see Brown, 1981). When single hairs, or a small cluster of hairs, are moved with an electronically controlled mechanical stimulator the stimulus-response curves generated show that s.c.t. neurones faithfully transmit the input arriving over the primary afferent fibres; in fact some amplification takes place (Brown & Franz, 1969). Each hair follicle is, however, innervated by several afferent fibres and it is not clear how much of this effect is due to the convergence of these fibres onto single s.c.t. neurones or how much might be attributed to factors intrinsic to the transmission between single hair follicle afferent fibres and s.c.t. neurones.

The security of transmission between single cutaneous afferent fibres and s.c.t. neurones was demonstrated by Hongo & Koike (1975). They applied discrete electrical stimulation to the skin and showed that around threshold levels of stimulation excitatory post-synaptic potentials (e.p.s.p.s) with both mono- and polysynaptic components were evoked in s.c.t. cells. Because of their all-or-nothing nature these compound potentials were attributed to the effect of single impulses generated in single hair follicle afferent fibres. The monosynaptic component was large, ranging from 1 to 18 mV in amplitude and the polysynaptic component was usually even larger, ranging up to 27 mV. Impulses in the s.c.t. cell generally arose from the polysynaptic component. For the monosynaptic component, the rise times were faster and the amplitudes were larger when the e.p.s.p.s were evoked from the centre as compared with the periphery of the s.c.t. neurone's receptive field. Hongo & Koike (1975) suggested that hair follicle afferent fibres from the central regions of the s.c.t. cell's receptive field would make a greater number of synaptic contacts with the cell and these would be more proximally located in comparison with afferent fibres from the peripheral regions of the s.c.t. neurone's field. This prediction was directly confirmed, at the level of the light microscope, by Brown & Noble (1982).

In the experiments to be reported in the present paper we have (1) further examined the contribution a single impulse in a single hair follicle afferent fibre can make to the discharge of a s.c.t. neurone; and (2) examined the relative efficacy of this contribution with respect to the location within or proximity to the neurone's receptive field. To do this we have excited single dorsal root ganglion cells (hair follicle afferent), by intracellular current pulses, and simultaneously recorded extracellularly responses from single s.c.t. neurones. In one accompanying paper (Brown, Koerber & Noble, 1987*a*) the effects of pairs of impulses in the afferent fibre have been studied and in another (Brown, Koerber & Noble, 1987*b*) the analysis of the response of the s.c.t. cell has been taken to the intracellular level. An account of some preliminary results has been published (Brown, Koerber, Noble, Rose & Snow, 1984).

## METHODS

Experiments were performed on eighteen young adult cats (2.2–2.5 kg body weight), one animal being used for a control experiment (see below). All animals were anaesthetized with  $\alpha$ -chloralose ( $70 \text{ mg kg}^{-1}$ ) after induction of anaesthesia with 4% halothane in a  $\text{N}_2\text{O}:\text{O}_2$  mixture and paralysed with gallamine triethiodide. For details of the methods used for assessing and maintaining the

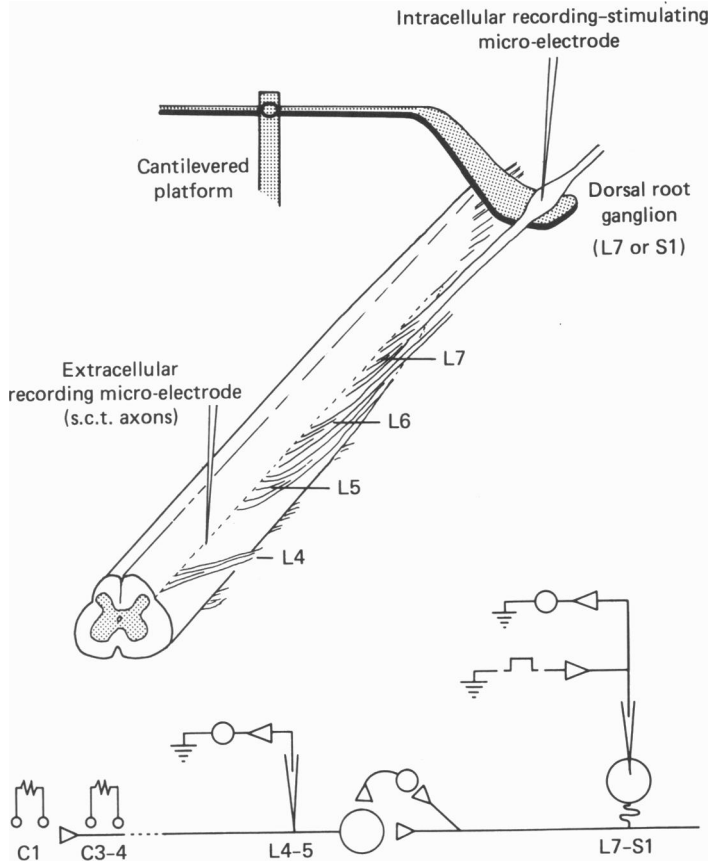


Fig. 1. The experimental arrangement. The upper drawing indicates the cantilevered platform supporting the dorsal root ganglion and the recording site in the upper lumbar cord for the spinocervical tract axons. The lower diagram represents the arrangement in a more stylized manner and shows, from the left: the placement of stimulating electrodes above and below the termination of s.c.t. axons at C1–C2, the recording micro-electrode at L4–L5, the mono- and polysynaptic excitatory connexions from a hair follicle afferent fibre whose cell body is impaled at the L7–S1 ganglion by a micro-electrode used for both intracellular recording and current passing. For further information see the text.

physiological condition of the preparation see Brown, Noble & Rowe (1986). Laminectomies were performed to expose the C1–C4 cord segments (except in the control experiment) and the lumbosacral cord and dorsal root ganglia L7 and S1 were also exposed. The ipsilateral sciatic nerve was exposed in the thigh and placed, in continuity, on a pair of Ag–AgCl or Pt stimulating electrodes.

In all experiments the L7 or S1 dorsal root ganglion was supported on a cantilevered platform

(Fig. 1). Small slits were made in the connective tissue sheath of the ganglion and glass micropipette electrodes filled with 3 M-KCl (8–12 M $\Omega$  at 1 kHz) inserted into the ganglion with a stepping motor drive while the short arm of the cantilevered platform was tapped gently by hand; this manoeuvre greatly facilitating intracellular penetration of ganglion cells. During attempts to penetrate ganglion cells the ipsilateral sciatic nerve was stimulated once every 500 ms so that extracellular recordings of ganglion cell impulse activity could be observed and the receptive field of the afferent unit determined. Once a stable penetration had been obtained the dorsal root ganglion cell was excited to fire single impulses by passing depolarizing pulses of current (less than 1–14 nA, 0.1–0.3 ms square waves) through the intracellular micro-electrode while continuously monitoring the response of the cell. The repetition rate for the ganglion cell stimulation was once every 1500 ms. This rate was chosen because faster rates of stimulation led to reduced s.c.t. cell responses. In fact a repetition rate of once every 1500 ms did lead to a reduction of response in some s.c.t. units when compared with once every 3000 ms but the faster rate was chosen as a compromise between stability of the response and the time needed to capture enough responses for each unit. The effects of stimulus repetition rate are considered in more detail in an accompanying paper (Brown *et al.* 1987a).

In the main series of experiments simultaneous extracellular recordings were made with 4 M-NaCl-filled micro-electrodes from axons of s.c.t. neurones as they ran in the dorsolateral funiculus at upper lumbar levels. S.c.t. axons were identified electrophysiologically by their antidromic excitation from the ipsilateral dorsolateral funiculus at C3 and their lack of such excitation from C1. The experimental arrangement is shown in Fig. 1.

Receptive fields were examined with the aid of hand-held brushes and probes and plotted on large photographic prints of the hind limb. Toothed clips were used to excite mechanical nociceptors. S.c.t. neurones were divided into those excited to fire by hair movement alone and those excited to fire by both hair movement and heavy pressure and pinch of the skin. There were no differences in the responses of these two types to single hair follicle afferent fibre impulses. In the present experiments, s.c.t. cells responding only to pressure and pinch were, except for one neurone, not utilized for study. The data was taped for subsequent analysis with Neurolog modules.

*Control experiment.* One animal was prepared as those in the main series except that no cervical laminectomy was performed. Small fascicles of the L7 dorsal root were prepared and placed on pairs of recording electrodes (three pairs). Once a L7 dorsal root ganglion cell had been impaled the fascicles were examined for unitary activity locked to impulses evoked by intracellular excitation of the ganglion cell. Because muscle afferent fibre activity submerged most of the single impulses locked to the ganglion cell impulses we had to crush the sciatic nerve. Unfortunately we were therefore unable to examine the receptive fields of the ganglion cells. We did, however, record their peripheral conduction velocities and rejected axons conducting at greater than 80 m s<sup>-1</sup> (presumed group I muscle afferent fibres for the most part, see Results). The central latencies, from the dorsal root ganglion to the dorsal root recording electrodes were measured.

## RESULTS

In the present experiments and in those reported in accompanying papers (Brown *et al.* 1987a, b) it was important to determine whether the intracellular current pulse to the dorsal root ganglion cell evoked only those impulses seen on the simultaneous intracellular recording and that (a) no other ganglion cells were excited, (b) no additional impulses were evoked in the ganglion cell under study (e.g. elicited at the initial axon segment and not seen in the record from the cell's soma), and (c) the impulse initiated in the soma actually travelled along the axon in the dorsal root to the spinal cord. For these reasons we performed a control experiment.

### *Control experiment*

For eighteen dorsal root ganglion cells excited by intracellular current pulse injection, single action potentials time-locked to the intracellularly evoked impulse

were recorded from a dorsal root fascicle. Axons conducting at greater than  $80 \text{ m s}^{-1}$  were rejected in order to avoid biasing the sample with an over-representation of group I muscle afferent fibres and to restrict the sample to axons conducting in the velocity range similar to that of most of the hair follicle afferent fibres recorded in the main series of experiments which, with the exception of four  $A\delta$  fibres, was  $53\text{--}88 \text{ m s}^{-1}$ . Thus, the peripheral (sciatic to dorsal root ganglion) conduction velocities in the sample used ranged from  $51$  to  $79 \text{ m s}^{-1}$ .

All eighteen pairs of recordings showed a one-to-one relationship between the impulse initiated by intracellular current injection in the dorsal root ganglion cell and the action potential recorded from a dorsal root fascicle (Fig. 2). Each pair of records was examined over many minutes whilst the ganglion cell was excited at rates of  $0\cdot67\text{--}500 \text{ Hz}$ . No signs of extra impulses in the dorsal rootlet recordings were ever observed.

Eleven of the units were tested with pairs or short trains of three to four stimuli. Nine units were tested at  $500 \text{ Hz}$  and followed the stimuli in a one-to-one fashion (Fig. 2C); the remaining two units were tested at  $250 \text{ Hz}$  and they also followed the stimuli faithfully.

After testing the cell in this way, the micro-electrode was withdrawn to a position just outside the dorsal root ganglion cell and attempts were made to fire the cell by extracellular current pulses. The micro-electrode was withdrawn in small steps until the d.c. level returned to zero and evoked action potentials could still be recorded at amplitudes of the order of  $15\text{--}20 \text{ mV}$ . None of the cells tested could be fired by pulses of  $23 \text{ nA}$  amplitude and  $1 \text{ ms}$  duration.

The control experiment shows that no impulses additional to those recorded through the intracellular stimulating electrode were evoked by current pulses with the amplitudes and durations used in the main series of experiments (less than  $1\text{--}14 \text{ nA}$ ,  $0\cdot1\text{--}0\cdot3 \text{ ms}$ ) and that impulses were not evoked in other dorsal root ganglion cells by such stimuli spreading to excite them extracellularly. Obviously, we cannot know whether impulses recorded from the soma of a ganglion cell always reach the cord. At best we can state that where we were able to find the dorsal rootlet carrying the axon of the impaled cell we could always demonstrate one-to-one following.

#### *Main series of experiments*

##### *Effects of single impulses in hair follicle afferent fibres on the discharge of s.c.t. neurones*

*The sample of hair follicle afferent fibre-s.c.t. neurone pairs.* The present sample consists of two sets of afferent fibre-neurone pairs. In one the receptive field of the hair follicle afferent fibre was contained within the firing zone (extracellularly recorded excitatory receptive field) of the s.c.t. neurone, and in the other the receptive field of the hair follicle afferent fibre was outside the firing zone of the s.c.t. neurone. The first set we shall call pairs with contained fields and the second set pairs with separated fields. There were forty-one pairs with contained fields made up of thirty-three hair follicle afferent units and forty s.c.t. neurones, some of the individual hair follicle afferent fibres or s.c.t. cells being common to more than one pair. Of the thirty-three hair follicle afferent fibres, thirty-one had peripheral conduction velocities of  $53\text{--}88 \text{ m s}^{-1}$  and two had conduction velocities of  $22$  and  $24 \text{ m s}^{-1}$ . A large number of pairs of units with separated fields were recorded but

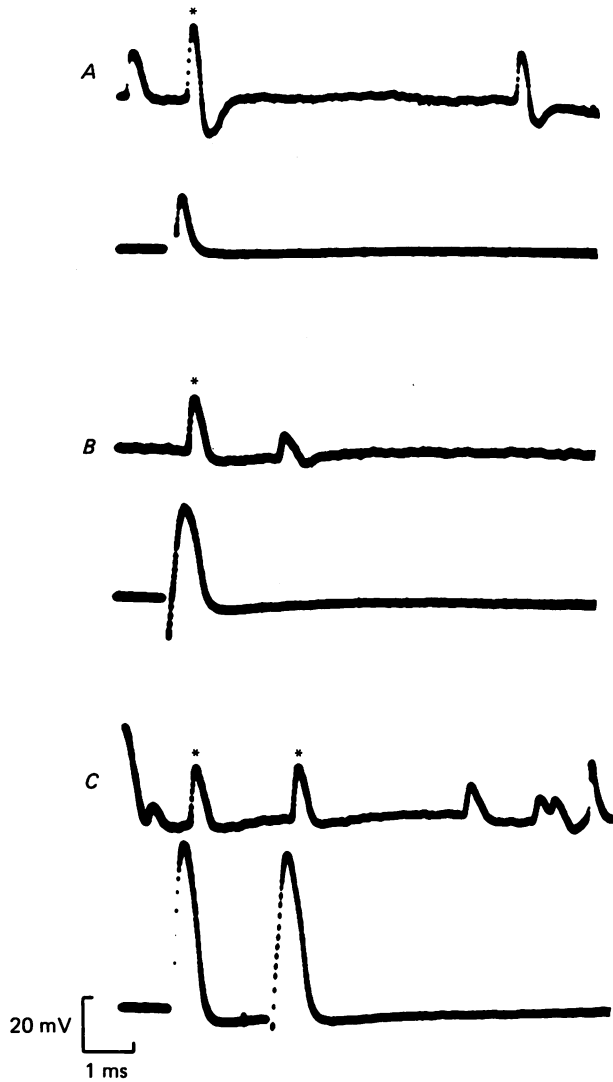


Fig. 2. The control experiment. In each pair of records, the upper trace is a recording from a dorsal root filament and the lower trace the intracellularly evoked and recorded action potential in a dorsal root ganglion cell. *A* and *B* show two separate units, the action potential in the upper member of each pair that is marked with an asterisk being the impulse identified as belonging to the afferent fibre of the ganglion cell. Although action potentials from other fibres are present in the upper traces, these are not synchronized with the lower trace and only a single action potential is evoked in the fibre belonging to the intracellularly excited afferent neurone. *C* shows, for another pair of recordings, the response in the fibre (upper trace) to a pair of impulses evoked at an interval of 2 ms. There were two impulses recorded in the dorsal root filament that were synchronized with the two evoked in the ganglion cell.

we only examined fifteen pairs in sufficient detail to provide quantitative data. These fifteen pairs were made up of ten hair follicle afferent fibres, including two  $A\delta$  fibres conducting at 22.5 and 24 m s<sup>-1</sup>, and fourteen s.c.t. neurones. The additional pairs with separated fields that we observed were examined only cursorily as the two receptive fields were some distance apart and there were no signs that the hair follicle afferent fibre had any excitatory influence on the s.c.t. neurone. Weak inhibitory effects would not have been noticed against the generally low background activity in the s.c.t. neurones.

*Pairs of units with contained receptive fields.* In the present context the term 'contained receptive fields' means that the receptive field of the hair follicle afferent fibre was contained within that area of skin from which the s.c.t. neurone could be caused to discharge impulses when the hairs were moved with hand-held brushes or probes. Obviously, when determined by extracellular recording from a s.c.t. neurone the receptive field is in fact the impulse firing zone under the conditions of the experiment. Only intracellular recording or a manoeuvre that raised the excitability of the neurone would reveal any surrounding subliminal fringe in the field. The impulse firing zone of a s.c.t. neurone is usually stable during the period of recording, but its edge can only be determined with limited accuracy (to 1–2 mm) using hand-held probes.

The most striking observation made in this series of experiments on pairs of neurones with contained fields was that a single impulse in a single hair follicle afferent fibre was capable of increasing the probability of firing of the s.c.t. neurone. The group II hair follicle afferent fibres were much more efficacious on the whole than the two group III ( $A\delta$ ) fibres and results for the two sets of fibres will be presented separately.

*Group II hair follicle afferent fibres.* The sample of thirty-nine pairs of units consisted of thirty-one hair follicle afferent fibres and thirty-eight s.c.t. neurones. When a single action potential was generated in a single dorsal root ganglion neurone at a rate of once every 1500 ms (faster rates led to a reduction in response, see Brown *et al.* 1987a) an impulse discharge of at least one action potential could be observed in the s.c.t. neurone in twenty-one of the pairs (54 %) in at least 50 % of trials (see Fig. 3).

For thirty-seven pairs of units sufficient trials were recorded to allow post-stimulus time histograms to be produced (more than 100 trials for thirty-two pairs, twenty to eighty trials for the remaining pairs). Single action potentials generated in the afferent fibres evoked impulses in the s.c.t. neurones with success rates of 2–100 %, with a mean of 65 % and a standard deviation of 34.33 % (Fig. 3). The success rate is defined as the percentage of trials in which at least a single action potential was evoked in the s.c.t. neurone during the period of increased firing probability indicated by the post-stimulus time histogram. There were only three pairs with a success rate of less than 10 % and seven pairs had success rates of 90 % or greater. The pairs with success rates of less than 10 % might be considered not to show a significant response but each of them had a much greater response when two impulses 5 ms apart were evoked in the hair follicle afferent neurone, and these responses occurred over a similar time course to the small responses to the single impulse (Fig. 4A). Furthermore, the two pairs with the smallest response (2 and 3 %) had s.c.t. neurones with a very

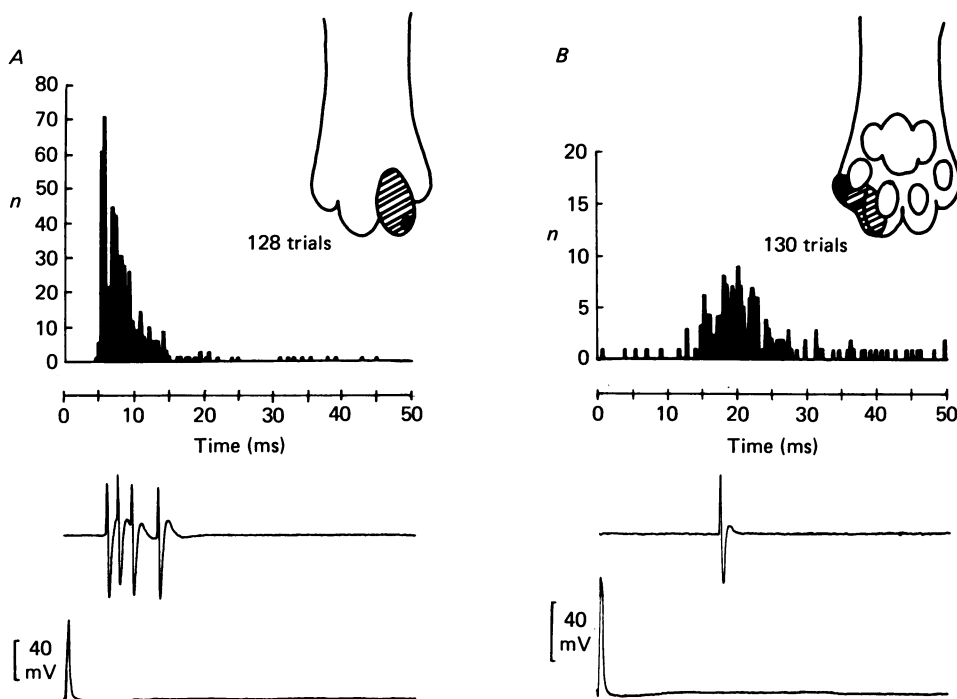


Fig. 3. Responses of single s.c.t. neurones to single hair follicle afferent impulses. In both *A* and *B* the upper part of the Figure shows a post-stimulus time histogram (bin width: 0.4 ms) of the s.c.t. neurone responses to single hair follicle inputs, with the locations of their receptive fields indicated on the Figurines, the s.c.t. firing zone being shown hatched and the hair follicle afferent's receptive field as a filled area. The lower part of each Figure shows a typical single response recorded from the s.c.t. neurone and, at the bottom, the intracellularly recorded and elicited action potential in the hair follicle afferent neurone.

low rate of resting activity (less than one impulse a second) and the evoked response was clearly discernible. In chloralose-anaesthetized cats s.c.t. neurones carry a 'background' or 'resting' discharge. For the present sample of neurones this discharge ranged from 0.1 to 25.3 impulses  $s^{-1}$ , with the majority (78%) discharging at between 1.0 and 8.0 times a second. In all of the post-stimulus time histograms shown this 'background' activity has not been taken into account. Figs. 3 and 4 show examples of the range of post-stimulus time histograms evoked in a variety of s.c.t. neurones by single impulses in hair follicle afferent fibres.

The average number of action potentials evoked in the s.c.t. cells by each hair follicle afferent impulse ranged from 0.02 to 4.71 ( $1.47 \pm 1.27$ , mean  $\pm$  s.d.,  $n = 38$ ). The latencies of the responses, measured from the onset of the impulse evoked in the dorsal root ganglion cell to the first obviously increased bin in the post-stimulus time histogram ranged from 1.5 to 14 ms ( $5.03 \pm 2.46$ , mean  $\pm$  s.d.,  $n = 37$ ). Times to peak and total response durations were 2.5–17.5 ms and 4.5–28 ms respectively. There was a strong positive correlation between the number of action potentials evoked by the single hair follicle afferent impulse and the frequency at which they occurred (number of action potentials/response duration; the rate of background activity being sub-



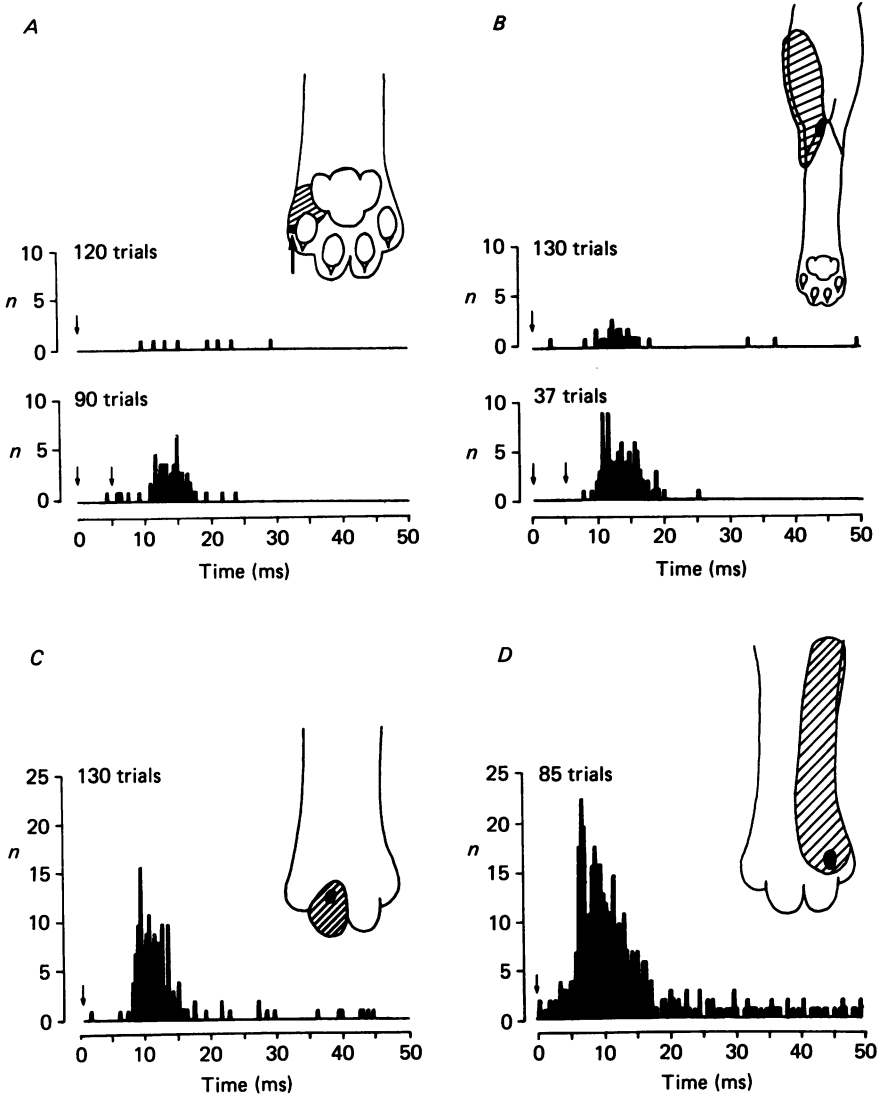


Fig. 4. Range of responsiveness of s.c.t. neurones to single hair follicle afferent impulses. *A* and *B* show two of the pairs of units with weak responses (3 and 16% success rates) to single impulses (upper histograms) and that they both produced more vigorous responses to pairs of impulses at an interval of 5 ms (arrows). *C* and *D* show two pairs with stronger coupling; 60 and 96% success rates respectively. The bin width in all histograms is 0.4 ms. The inset in each part of the Figure shows the relative positions of the s.c.t. cell's firing zone (hatched) and the hair follicle afferent's receptive field (filled).

tracted from the calculated frequency) and also significant negative correlations between the latency of the response and both the response frequency and the number of action potentials evoked (Fig. 5). In other words responses with short latencies tended to consist of more impulses at higher frequencies than those with long latencies but the higher frequency responses, whilst containing a larger number of action potentials, were not spread out in time, and there was no correlation between

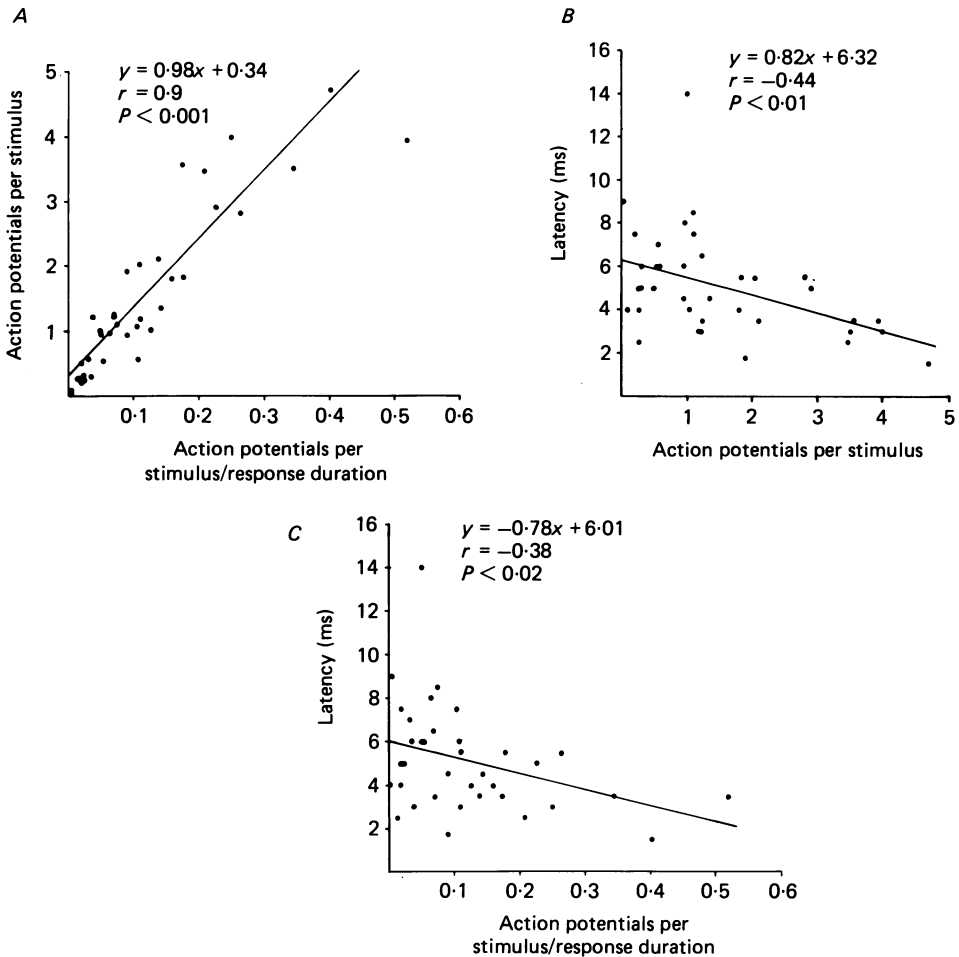


Fig. 5. Relationships between various parameters of the s.c.t. cell responses to single hair follicle impulses. *A* shows the relationship between the number of impulses evoked and the frequency at which they occurred. *B* and *C* show the relationship between the latencies of the responses and the number of action potentials evoked and the response frequency respectively. In each graph the line has been fitted by the method of least squares, and both the correlation coefficient ( $r$ ) and the significance level ( $P$ , Student's  $t$  test) are given.

the duration of a response and the number of impulses it contained. Furthermore, there was no correlation between the conduction velocity of the hair follicle afferent and the latency of the response evoked in the s.c.t. neurone ( $P > 0.1$ , Student's  $t$  test) so the early responses were not due to the earlier arrival of impulses or the larger diameter of the faster conducting afferent fibres. In fact the control experiment had shown that for primary afferent fibres with peripheral conduction velocities of  $51\text{--}79\text{ m s}^{-1}$  the latency between the onset of the impulse in the dorsal root ganglion and its recording in a dorsal root filament near the spinal cord ranged from  $0.2$  to  $0.5$  ms so only a difference of some  $0.3$  ms can be accounted for by differences in conduction velocity in the present sample of hair follicle afferent fibres ( $53\text{--}88\text{ m s}^{-1}$ ).

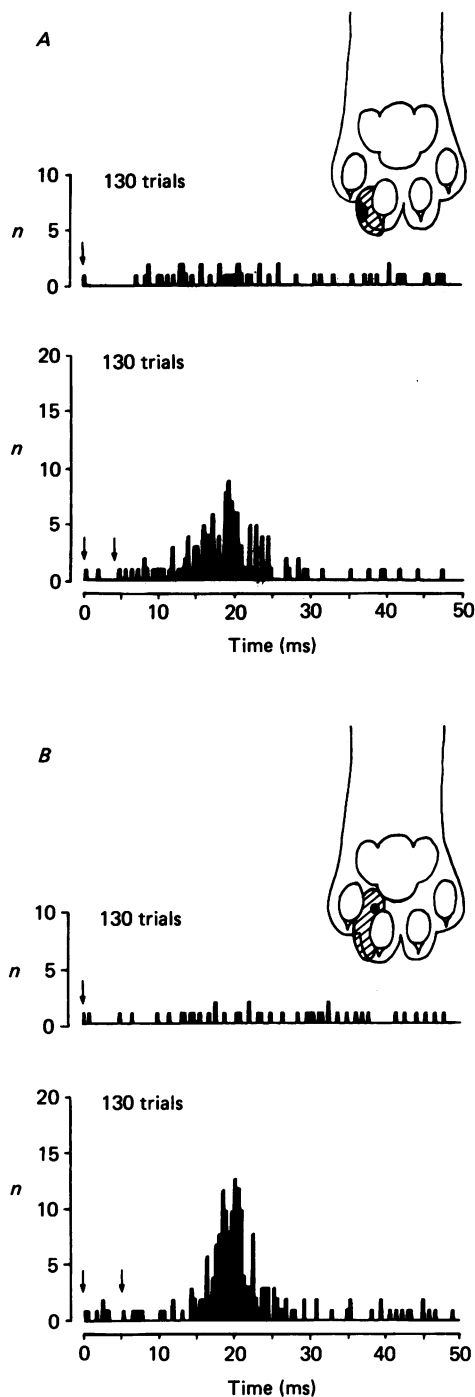


Fig. 6. Responses of s.c.t. neurones to impulses in group III hair follicle afferent fibres. Two pairs of units are shown (*A* and *B*). In each, a single afferent impulse is relatively ineffective in causing the s.c.t. neurone to fire but a pair of impulses at 5 ms separation produces a much stronger response (bin width: 0.4 ms). Note the long latencies of these responses in comparison with those evoked by group II afferent fibres, and the fact that the response durations are similar to those evoked by group II fibres.

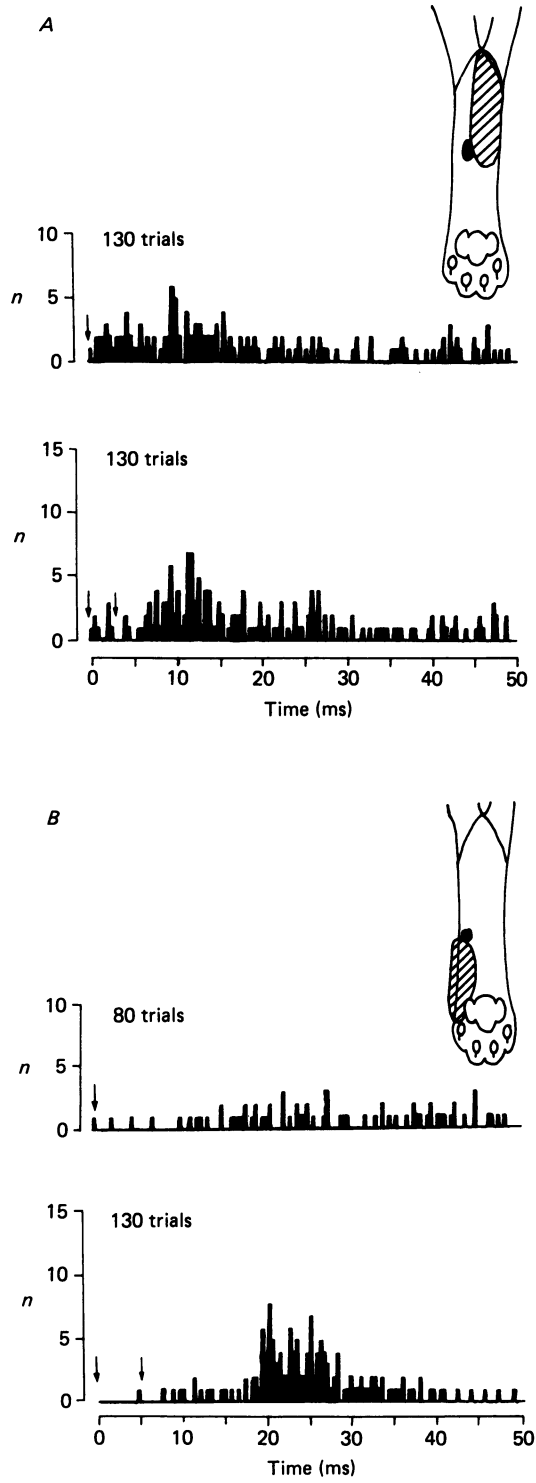


Fig. 7. For legend see opposite.

*Group III hair follicle afferent fibres.* Group III (A $\delta$ ) axons excited by hair movement innervate down hair follicle receptors, in contrast to group II axons which innervate the follicles of the larger guard (and tylotrich) hairs (Brown & Iggo, 1967). Differences in the effects of impulses in the two sets of axons on s.c.t. neurones would indicate the relative effectiveness of the inputs and the neuronal circuitry involved. Only two pairs of units having group III hair follicle afferent fibres with receptive fields contained within the firing zone of a s.c.t. cell were recorded. But the properties of the two pairs were similar. Single impulses in the group III axons were relatively ineffective in increasing the firing probability of the s.c.t. neurones (8% success rates in both pairs; 0.25 and 0.21 impulses stimulus<sup>-1</sup>) and the responses had long latencies of 17.0 and 12.0 ms. These latencies are at and beyond the higher end of the range for group II-excited neurones (1.5–14.0 ms) but the durations of the responses, at 13.0 and 14.5 ms, were well within the group II-excited range (4.5 to 28.0 ms). For both pairs of units excitation with two impulses at intervals of 5–25 ms led to a much greater response (Fig. 6).

*Pairs of units with separated receptive fields.* Fifteen pairs of units with separated fields were studied in detail. Generally, the receptive field of the hair follicle units was within 1–3 mm of the edge of the firing zone of the s.c.t. neurone, but for two pairs the fields were about 8–10 mm apart. Pairs with the hair follicle receptive field close to the firing zone of the s.c.t. cell were examined for the presence of subliminal fringes around the s.c.t. firing zone (but see above for the caveat about the accuracy of delineating the edge of the firing zone).

The fifteen pairs consisted of eight group II hair follicle axons paired with eleven s.c.t. neurones (twelve pairs) and two group III axons paired with three s.c.t. cells (three pairs), although one of the s.c.t. cells of the latter group only responded to pressure and pinch and had apparently no hair follicle excitatory input when tested with a hand-held brush.

For only two of the fifteen pairs (one group II and one group III axon) was there any evidence for the hair follicle afferent fibre causing an increased probability of neuronal firing and then the evidence was clear only when a pair of impulses was evoked in the hair follicle axon at 3 or 5 ms intervals (Fig. 7). One pair showed a possible weak effect of two impulses at 5 ms separation. The remaining twelve pairs showed no discernible action of the hair follicle unit (see Fig. 10 for examples). In these 'negative' pairs, peri- as well as post-stimulus time histograms were examined to allow a better appreciation of the pre-stimulus background activity of the s.c.t. neurone.

*Influence of receptive field location on the effectiveness of the hair follicle afferent input*

In the present sample of thirty-nine pairs of units with group II axons and contained fields very few of the hair follicle units had receptive fields at or near the centre of the s.c.t. cell's firing zone. But those that were at the centre (two) or up

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Fig. 7. Responses evoked by hair follicle afferent fibres with receptive fields outside the firing zone of s.c.t. cells. *A* and *B* show responses from the only two pairs of units with clear effects; in both, a pair of afferent impulses at 5 ms intervals was necessary to raise the response much above background (bin width: 0.4 ms). The afferent fibre in *B* had a group III conduction velocity.

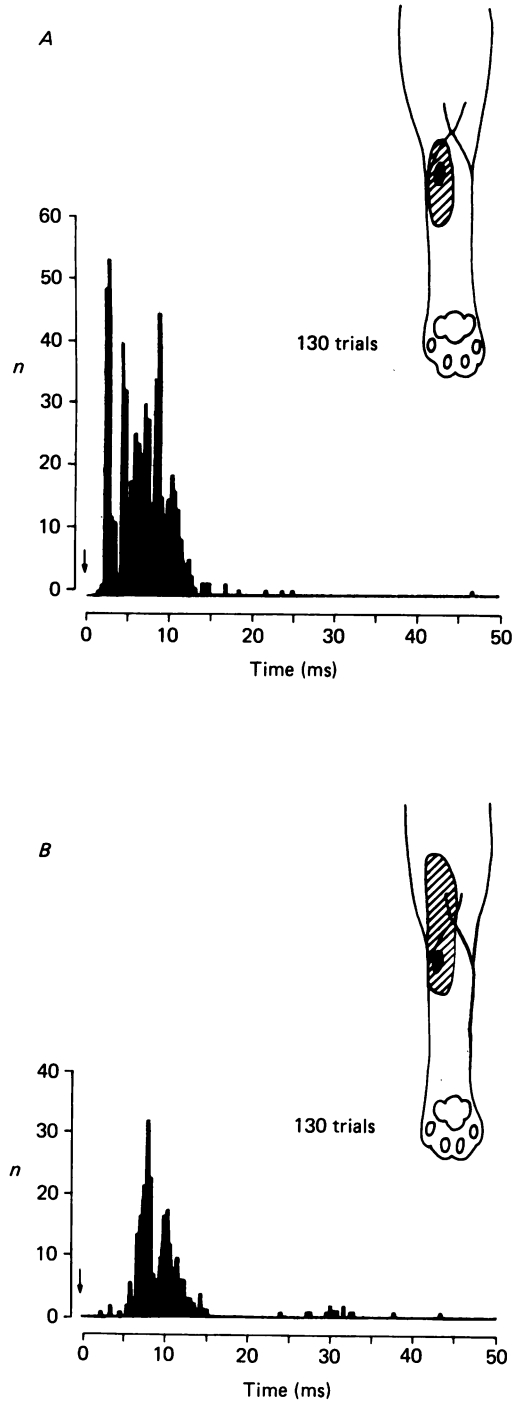


Fig. 8. Influence of receptive field position on the response. *A* and *B* show responses from two pairs of units in which the hair follicle afferent fibre was common to each pair, but was near the centre of the s.c.t. cell's firing zone in *A* and at the periphery in *B*. Both pairs show quite effective coupling but the latency of the response in *A* is much shorter (bin width: 0.4 ms).

to about midway between the centre and the periphery (five) included the two pairs with the shortest latency responses to a single hair follicle afferent impulse (1.5 and 1.75 ms), three of the six pairs that responded with an average of more than three impulses in the s.c.t. neurone to a single action potential in the hair follicle afferent fibre, and three of the five pairs in which a single impulse in the hair follicle afferent fibre always fired the s.c.t. cell. When the hair follicle receptive field was located at or near the periphery of the s.c.t. cell's firing zone there was a wide range of effectiveness: from short latencies (down to 2.5 ms) to long ones (up to 14 ms), from little increase in firing probability ( $0.08$  impulses stimulus<sup>-1</sup>) to considerable increases (up to  $3.99$  impulses stimulus<sup>-1</sup>) and from rarely causing the s.c.t. cells to fire (3% success) to always causing at least one impulse in the s.c.t. cell (100% success).

In general when the hair follicle fibre's receptive field was at or near the middle of the s.c.t. neurone's firing zone then a single impulse in the afferent fibre led to earlier and greater (more impulses at a higher frequency) discharge in the s.c.t. cell. For peripherally located hair follicle receptive fields there was a wide range of actions but on the average the responses were later and of less degree (see Figs. 3, 4, 8 and 11).

Where a single hair follicle unit was paired with more than one s.c.t. cell the influence of field position could be examined. Fig. 8 shows two pairs in which an afferent fibre was coupled with two s.c.t. neurones. In Fig. 8A the hair follicle axon's field was centrally placed and the latency of the response was shorter than in Fig. 8B where the hair follicle field was more peripherally located, even though in both sets of results the hair follicle fibre was quite effective in firing the neurone.

#### *Independence from post-synaptic effects*

One s.c.t. neurone was common to two pairs of units. In these pairs the properties of the response were not determined solely by the s.c.t. neurone (Fig. 9). The two hair follicle axons had similar conduction velocities and their receptive fields were quite close together, and yet the responses had different latencies (4.0 and 6.0 ms), times to peak (5 and 13.5 ms) and durations (8 and 18 ms), and even though similar numbers of action potentials were evoked in each case (1.02 and 0.98 impulses stimulus<sup>-1</sup>) the effectiveness for eliciting at least one action potential per trial differed between the two pairs (100 and 70%).

#### *Actions of a single hair follicle afferent fibre on eight different s.c.t. cells*

In one experiment we were able to maintain intracellular recording and stimulation from a single dorsal root ganglion cell long enough to acquire useful data on eight s.c.t. neurones, and in many respects the results from these eight pairs summarize the results for the whole sample. These results will be described in detail. Figs. 10 and 11 show the receptive fields of the afferent fibre and the eight s.c.t. neurones and post-stimulus time histograms in response to exciting the hair follicle afferent unit. Four of the s.c.t. neurones had firing zones just outside the hair follicle unit's receptive field and none showed any evidence of synaptic coupling even when two or three impulses were evoked in the ganglion cell (Fig. 10), or when the excitability of the s.c.t. cell was raised by placing a clip on the s.c.t. cell's firing zone or by brushing the hairs in the firing zone (not illustrated). In contrast, for the four pairs

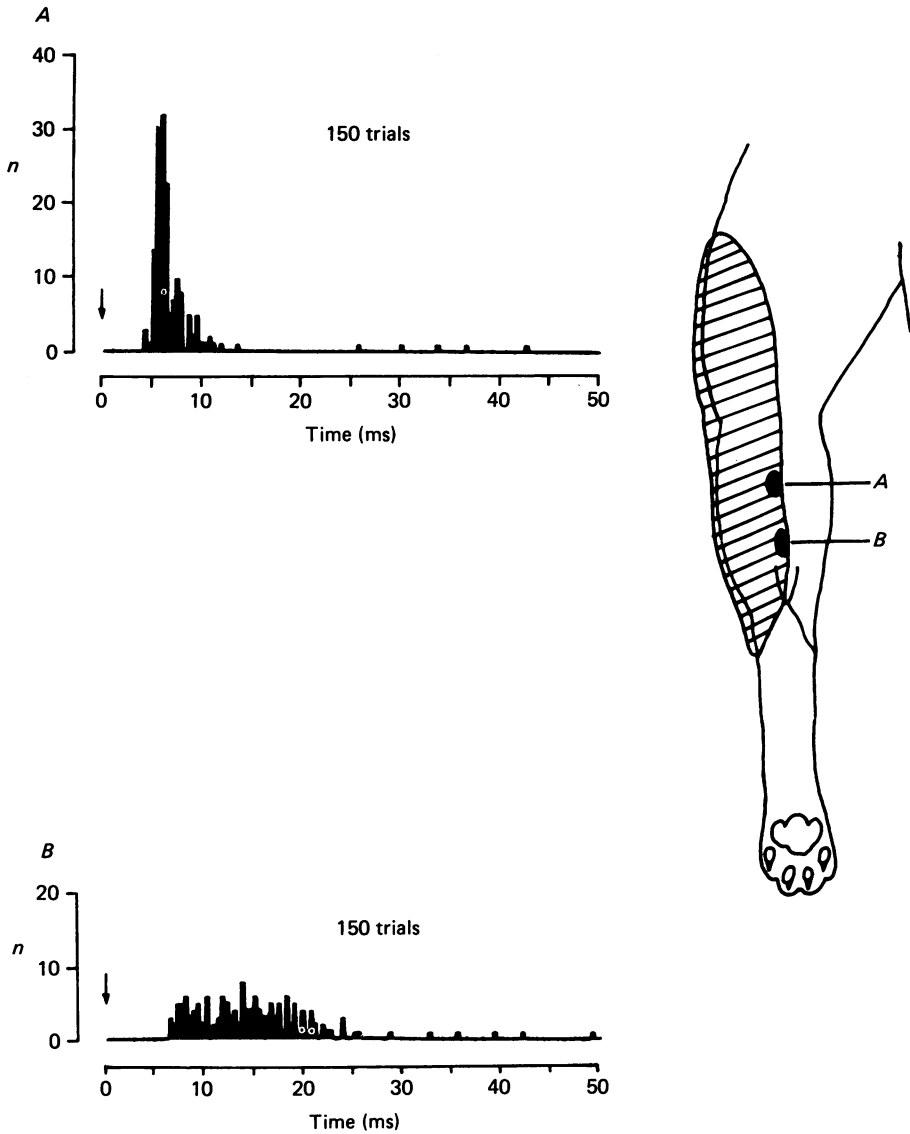


Fig. 9. Independence of responses from the post-synaptic cell. *A* and *B* show responses from two pairs of units in which the s.c.t. neurone was common to both (bin width: 0.4 ms). *A* shows more effective coupling than *B* with shorter latency, time to peak and duration.

where the hair follicle field was inside the s.c.t. cells' firing zones, single impulses in the ganglion cell always led to an increased probability of firing in the s.c.t. neurone (Fig. 11).

#### DISCUSSION

The present results establish that there is an extremely strong excitatory synaptic linkage between single hair follicle afferent fibres, conducting in the group II range, and s.c.t. neurones when the cutaneous receptive field (firing zone) of the s.c.t.



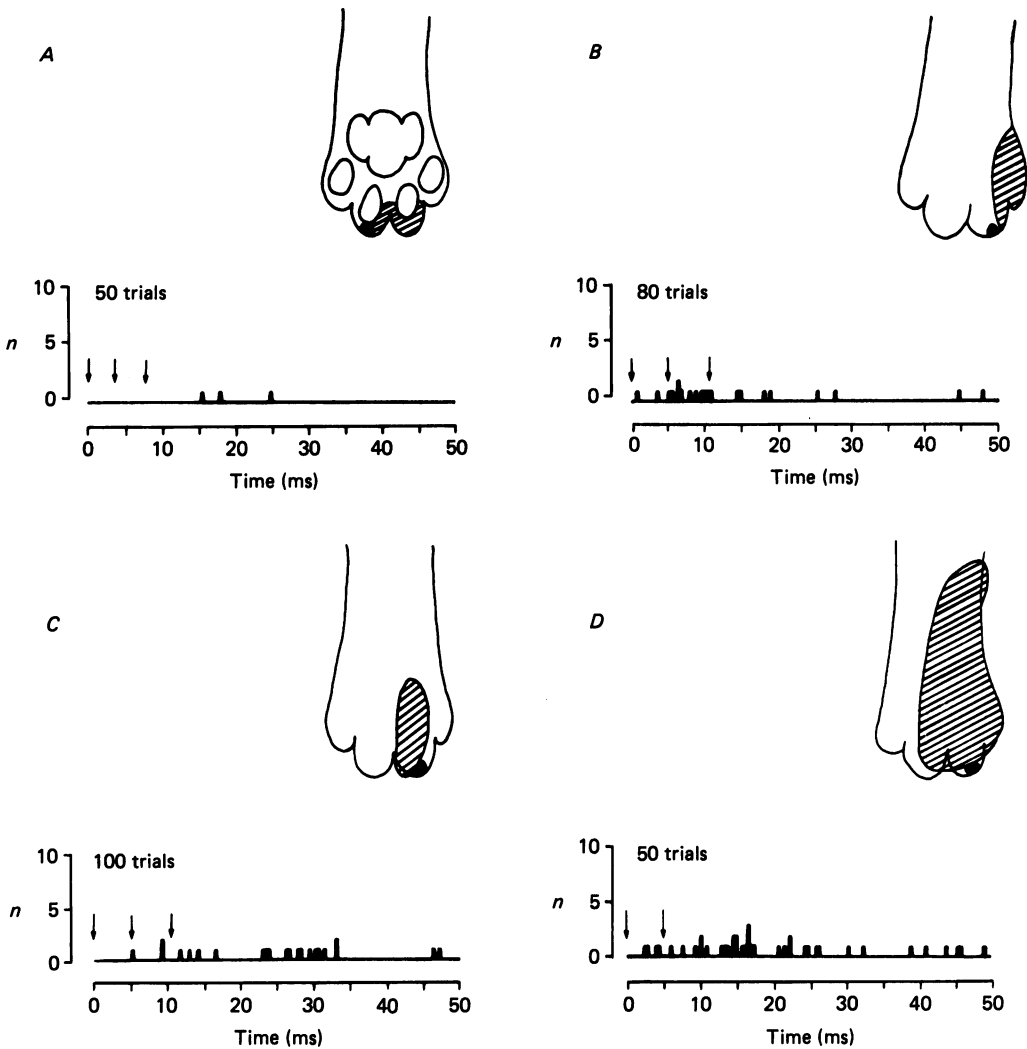


Fig. 10. Actions of a single hair follicle afferent fibre on four different s.c.t. neurones. Note that the hair follicle afferent fibre's receptive field was on the tip of toe IV and extended onto both the dorsal and ventral surface of the toe. For further details see the text. The bin width in all histograms is 0.4 ms.

neurone contains the receptive field of the hair follicle axon. The strength of synaptic coupling is such that a single impulse in the hair follicle fibre will lead to an impulse discharge in the majority of s.c.t. neurones whose receptive fields contain that of the afferent fibre. In the present sample 54% of hair follicle afferent-s.c.t. neurone pairs showed a response of at least one impulse in the s.c.t. cell to a single impulse in the hair follicle afferent fibre in more than 50% of trials. Group III hair follicle afferent fibres, which innervate follicles of the down hairs (Brown & Iggo, 1967), are also quite strongly coupled to s.c.t. neurones whose firing zones contain their fields, but less strongly than the group II axons.

These results are dependent on the adequacy of the technique used to elicit a single

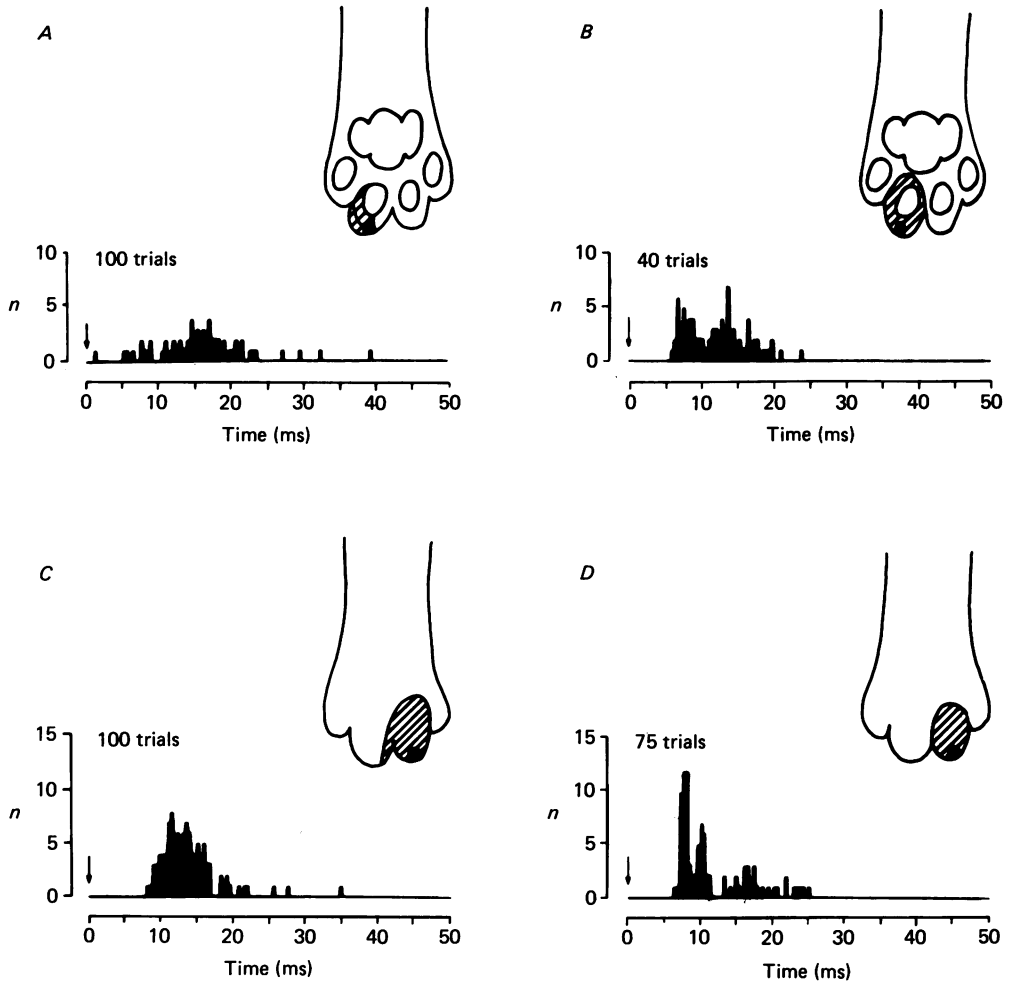


Fig. 11. Actions of the same single hair follicle afferent fibre as in Fig. 10 on another four different s.c.t. neurones. For further details see legend to Fig. 10 and the text.

impulse in a single afferent fibre. The control experiment has shown that the technique of intracellular stimulation of dorsal root ganglion cells used in the experiments only evokes the action potentials seen on the simultaneous recording: other dorsal root ganglion cells are not caused to fire by the strengths and durations of current used. What cannot be established with the methods used is that the evoked impulse actually reaches the spinal cord. In the present context, however, this problem is not important: for all hair follicle afferent fibre-s.c.t. cell pairs studied, when the receptive field of the afferent fibre was contained within the firing zone of the neurone, single impulses elicited in the afferent neurone's soma always led to an increased probability of firing in the s.c.t. cell. Therefore, if a significant proportion of the impulses elicited in a ganglion cell failed to reach the axon's terminals in the spinal cord (for whatever reason) then the effect would be that we have underestimated the effects of single hair follicle afferent impulses on s.c.t. neurones and that the synaptic linkage is even stronger than described herein.

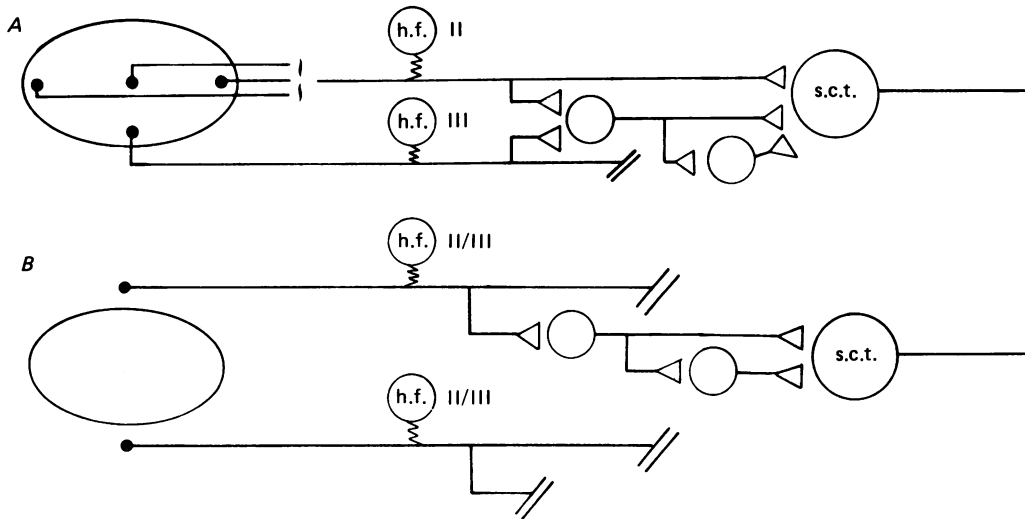


Fig. 12. Diagrammatic summary of the tentative neuronal circuitry suggested by the present experiments. Although each hair follicle afferent fibre and each s.c.t. neurone represents a single cell, the excitatory interneurons (unlabelled) represent sets of neurones. H.f., hair follicle afferent neurone; II, group II afferent fibre; III, group III afferent fibre; II/III, group II or group III afferent fibre; s.c.t., s.c.t. neurone. For further details see the text.

This ability of single cutaneous axons to cause s.c.t. neurones to fire in response to an input that is the minimum possible is in strong contrast to the actions of single muscle spindle primary afferent fibres (Ia) on motoneurons where summation of activity from several afferent fibres is required to cause neuronal firing (see, for example, Burke & Rudomin, 1977), and of single group I muscle afferent fibres on dorsal spinocerebellar tract neurones where summation is also required (Tracey & Walmsley, 1984). It is, however, similar to the actions of single slowly adapting type I cutaneous afferent fibres on certain dorsal horn neurones (Brown, Moraff & Tapper, 1974; Tapper & Wiesenfeld, 1980) and also brings to mind the strong excitatory action a single climbing fibre has on a single cerebellar Purkinje cell (Eccles, Llinás & Sasaki, 1966). How general this ability of a single afferent impulse to fire a cell might be, remains to be clarified. But the results do emphasize that it is a mistake to think that all neuronal excitatory connexions (that operate with impulses) behave like the Ia afferent fibre- $\alpha$ -motoneurone system.

In spite of the strong excitatory linkage between single hair follicle afferent fibres and s.c.t. neurones, only four pairs of units had response latencies indicative of possible monosynaptic coupling between the members of the pair. That is, only four of the pairs showed clear increases in firing at latencies of 2.5 ms or less following the initiation of the impulse in the dorsal root ganglion cell. It seems reasonable to take 2.5 ms as the cut-off point for monosynaptic excitation. If 0.4 ms is allowed for conduction time from the dorsal root ganglion cell to the cord, a further 0.2 ms for intraspinal conduction, 0.5 ms for minimal synaptic delay and 0.5 ms for conduction along the s.c.t. axon to the recording site, then a minimal latency of the order of 1.6 ms for monosynaptic activation results. Stimulation of the sciatic nerve in the

high produced latencies of s.c.t. cell responses of 2.5–4.0 ms and the peripheral component of this time, for the fastest hair follicle afferent fibres, would be about 1 ms and the monosynaptic latency from dorsal root ganglion to the recording site in the upper lumbar cord would be 1.5–3.0 ms. It is conceivable that a 2.5 ms cut-off might exclude some monosynaptic actions, especially if the monosynaptic e.p.s.p. has a slow rise time. In the present sample three pairs of units had central latencies of 3.0 ms and five pairs had latencies of 3.5 ms. But the majority of pairs had latencies in excess of 3.5 ms and presumably this indicates di- or polysynaptic coupling. The results might, therefore, appear puzzling because Brown & Noble (1982) showed, for a set of hair follicle afferent fibre–s.c.t. neurone pairs injected with horseradish peroxidase, that when the hair follicle axon had a receptive field contained within the excitatory cutaneous receptive field of the s.c.t. neurone, monosynaptic connexions between the pair could always be observed at the light microscopical level. The answer to this presumably lies in the observations of Hongo & Koike (1975) who showed that when the skin within the receptive field of a s.c.t. neurone was stimulated around threshold then either no response was recorded from within the s.c.t. neurone or a complex e.p.s.p. was evoked consisting of a monosynaptic component followed by polysynaptic components. Furthermore, the polysynaptic component was usually of greater amplitude than the monosynaptic component and impulses usually arose from this later part of the response. The present results fit precisely with this observation of Hongo & Koike (1975).

The present results provide a little evidence for the presence of a subliminal fringe around the firing zone of the excitatory receptive field of s.c.t. neurones. Only two of fifteen pairs of units showed clear evidence that input from a single hair follicle afferent fibre with its receptive field just outside the s.c.t. neurone's firing zone could raise the probability of cell firing. However, this sort of experimental test is probably too insensitive to reveal very weak actions.

When the present results, together with those of Hongo & Koike (1975) and Brown & Noble (1982), are taken into account tentative diagrams of the excitatory microcircuitry for the hair follicle afferent input to s.c.t. neurones may be drawn. Such diagrams are shown in Fig. 12. All group II hair follicle axons with their receptive fields inside the firing zone of a s.c.t. neurone's excitatory receptive field have monosynaptic connexions with the neurone and, in addition, each afferent fibre also excites the neurone via interneurons (Fig. 12*A*). Some hair follicle afferent fibres with receptive fields close to the firing zone of a s.c.t. neurone may excite that neurone through an interneuronal chain (Fig. 12*B*). Group III hair follicle afferent fibres seem to be arranged such that those with fields within the firing zone of a s.c.t. neurone probably do not excite the neurone monosynaptically but do so through a chain of interneurons (Fig. 12*A*) although we have no direct evidence to exclude a monosynaptic connexion; the arrangement for group III afferent fibres from just outside the s.c.t. cell's firing zone is probably similar to that for group III axons (Fig. 12*B*). What the degree of convergence onto the various interneurons in the excitatory chain might be is subject to speculation, but presumably is considerable otherwise the numbers of such interneurons would be extremely large and it is known (Maxwell, Fyffe & Brown, 1984) that about half of the presumed excitatory synapses on s.c.t. neurones arise from primary (hair follicle) afferent fibres.

A single group II hair follicle afferent fibre has been estimated to provide monosynaptic connexions to between 100 and 150 s.c.t. neurones (Brown, Rose & Snow, 1980). This set of s.c.t. neurones will consist of those cells whose firing zone contains the receptive field of the afferent fibre. From the present results it can be estimated that one impulse arriving over the afferent fibre may, if the conditions are favourable, lead to at least one impulse being evoked in fifty to seventy-five of these s.c.t. neurones. Fifty to seventy-five neurones represents about 6–10% of the population of s.c.t. neurones subserving the hind limb (Brown, Fyffe, Noble, Rose & Snow, 1980). It is concluded that a single action potential in a single hair follicle afferent fibre may be a significant event as far as the central nervous system is concerned.

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