J. Physiol. (1987), **382**, 331–354
With 14 text-figures
Printed in Great Britain

AN INTRACELLULAR STUDY OF SPINOCERVICAL TRACT CELL RESPONSES TO NATURAL STIMULI AND SINGLE HAIR AFFERENT FIBRES IN CATS

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(*Received 26 February 1986*)

SUMMARY

1. Intracellular recordings were made from spinocervical tract (s.c.t.) neurones in cats anaesthetized with chloralose and paralysed with gallamine triethiodide.

2. In one series of experiments the cells' receptive fields were examined with the use of natural stimuli. Hair movement within the impulse firing zone of the cell evoked excitatory post-synaptic potentials (e.p.s.p.s) from which impulses were generated; in addition, in the majority of s.c.t. cells tested, areas were found within the impulse firing zone where hair movement elicited both e.p.s.p.s and inhibitory post-synaptic potentials (i.p.s.p.s). Outside the firing zones, both regions evoking e.p.s.p.s and regions evoking i.p.s.p.s were observed in all neurones examined in detail (ten cells). The responses of these neurones to a variety of natural stimuli showed the receptive fields of s.c.t. cells to be more complex than previously thought.

3. In a second series of experiments, intracellular recordings from s.c.t. cells were combined with intracellular recording and stimulation of single dorsal root ganglion cells belonging to group II hair follicle afferent fibres. When the afferent fibres innervated skin within the impulse firing zone of the s.c.t. cell, single afferent impulses evoked e.p.s.p. complexes consisting of both mono- and polysynaptic components; no i.p.s.p.s were observed in response to single hair follicle afferent impulses or to trains. Although the monosynaptic e.p.s.p. component was often large and had a fast rise time, s.c.t. cell impulses usually arose from the later components. Afferent fibres innervating the central region of the s.c.t. cell firing zones tended to evoke relatively large e.p.s.p.s with fast rise times. The rise times and amplitudes of the e.p.s.p.s evoked by afferent fibres from the periphery, however, varied between afferent fibres but included the slowest and smallest in the total sample of synaptically coupled pairs. Afferent fibres from outside the s.c.t. cell's firing zone were usually ineffective in setting up post-synaptic potentials, but one group III hair follicle afferent fibre, from an inhibitory receptive field component, gave rise to i.p.s.p.s.

4. The effects of pairs and trains of afferent impulses at intervals of 10, 25, 50, 100 and 200 ms were examined. At 25 ms the response to the second afferent impulse was

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profoundly less than that evoked by the first and was still substantially reduced at 200 ms interval. In all synaptically coupled pairs studied, the e.p.s.p. complex evoked by the second afferent impulse was smaller in amplitude than that evoked by the first. This reduction appeared to be limited to the later components with the monosynaptic component unaffected.

5. There was no evidence for recurrent inhibitory or facilitatory effects in the s.c.t. system.

6. The results are discussed in relation to previous work and tentative wiring diagrams of the neuronal circuits involved are presented.

INTRODUCTION

Careful and systematic examination of the receptive fields (impulse firing zone) of spinocervical tract (s.c.t.) neurones reveals a gradient of sensitivity across the fields from periphery to centre; the most sensitive part of the field to movement of hairs is at or near the centre (Brown, Noble & Rowe, 1986). When small groups of hairs in two separate parts of the s.c.t. cell's receptive field are moved simultaneously, the response of the cell is not a linear summation of the responses to each separate stimulus. The response is usually less than a linear summation and may be no greater than the response to one stimulus alone. In the preceding papers (Brown, Koerber & Noble, 1987*a*, *b*) it has been shown that (1) a single impulse in a single group II hair follicle afferent fibre from within the firing zone of a s.c.t. neurone always increases the probability of that neurone firing; indeed, in about 50 % of hair follicle afferent–s.c.t. neurone pairs of this type, a single hair afferent impulse evokes at least one impulse in the s.c.t. neurone in 50 % of the trials; (2) in the vast majority of such neuronal pairs the latencies of the responses to single hair afferent impulses indicate a polysynaptic linkage; (3) the increased probability of firing produced by a single hair afferent impulse is followed by a prolonged period of reduced responsiveness (to a subsequent afferent impulse) that lasts for about 1500 ms.

These results led us to pose a number of questions about the organization of the hair follicle afferent–s.c.t. system. For example: was the gradient of sensitivity across s.c.t. neurone fields due to varying strengths of synaptic input or to inhibitory processes? How much of the failure of two simultaneous inputs to summate was due to an active inhibitory process and, if so, was any of the inhibition acting directly on the s.c.t. cell as post-synaptic inhibition? Even though a single hair afferent impulse increased the firing probability of a s.c.t. cell with a latency generally indicative of a polysynaptic mechanism, how effective was the monosynaptic component; previous work (Brown & Noble, 1982) had shown that all group II hair follicle afferent fibres from within the receptive field of a s.c.t. neurone had monosynaptic connexions with the neurone? Was the depressed response of the hair afferent fibre–s.c.t. cell circuitry that occurs after a single hair afferent impulse due to post-activation depression, post-synaptic inhibition acting on the s.c.t. cell or to some inhibitory process acting presynaptically to the s.c.t. cell?

In order to provide some insight into these problems we have extended the analysis to the intracellular level. Intracellular recordings have been made from s.c.t.

neurones while (1) stimulating their receptive fields with natural stimuli, and (2) producing known inputs to them by intracellular activation of single hair follicle afferent neurones.

METHODS

All experiments were performed on cats anaesthetized with α-chloralose (70 mg kg^{-1}), after induction of anaesthesia with halothane in a $N_2O:O_2$ mixture, and paralysed with gallamine triethiodide. Full details of the surgery performed, methods for assessing anaesthetic level, for stimulating receptive fields with controlled air jets, intracellular stimulation of dorsal root ganglion cells belonging to hair afferent fibres, and for identifying s.c.t. neurones have been described previously (see Brown *et al.* 1987*a*, *b*).

Two series of experiments were performed. In the first (four cats, 2·3–2·5 kg weight) intracellular recordings were made from identified s.c.t. neurones and their receptive fields were examined in detail using air jets, toothed clips applied to the skin and hand-held probes. In the second (seven cats, 2·3–3·4 kg weight) intracellular recordings were made from s.c.t. neurones and their responses to known inputs from single hair follicle afferent fibres were examined. The known inputs were produced by intracellular stimulation of dorsal root ganglion cells. In this second series of experiments the receptive fields of the s.c.t. neurones were also examined in detail and the data added to that of the first series. In all experiments the intracellular recordings from the s.c.t. neurones were made with glass micro-electrodes filled with a solution of potassium citrate (2 mol/l^{-1}) having impedances of 5–10 MΩ.

RESULTS

Responses of s.c.t. neurones to natural stimulation of the skin

A total of twenty-five s.c.t. neurones were examined for their responses to natural stimulation of the skin. First, their excitatory receptive fields were located approximately by delineating their impulse firing zone during extracellular recording. Upon penetration of the neurone (resting membrane potentials between −60 and −75 mV measured by d.c. shift recorded on penetration and exit of electrode), the impulse firing zone was confirmed (and was not obviously different from that delineated earlier) and then the firing zone and adjacent areas of skin were examined for presence of (1) subliminal fringes, where excitatory post-synaptic potentials (e.p.s.p.s) but no impulses could be elicited, and (2) areas from within and from outside the excitatory receptive field, where inhibitory post-synaptic potentials (i.p.s.p.s) could be elicited. Receptive fields (the term includes the impulse firing zone, any subliminal fringe and any inhibitory areas) were often complex and for ease of description will be described in terms of the responses elicited from within the impulse firing zone, from subliminal fringes and from inhibitory areas outside the impulse firing zone. Of the twenty-five neurones examined, all had their impulse firing zones stimulated by air jets to search for areas giving i.p.s.p.s. A full, detailed examination of the areas surrounding the impulse firing zone was, however, only carried out on ten neurones because it was very time consuming.

Responses from within the impulse firing zone

Responses to air jets. Air jets applied to positions within the impulse firing zone produced e.p.s.p. complexes at all points and these were usually of sufficient

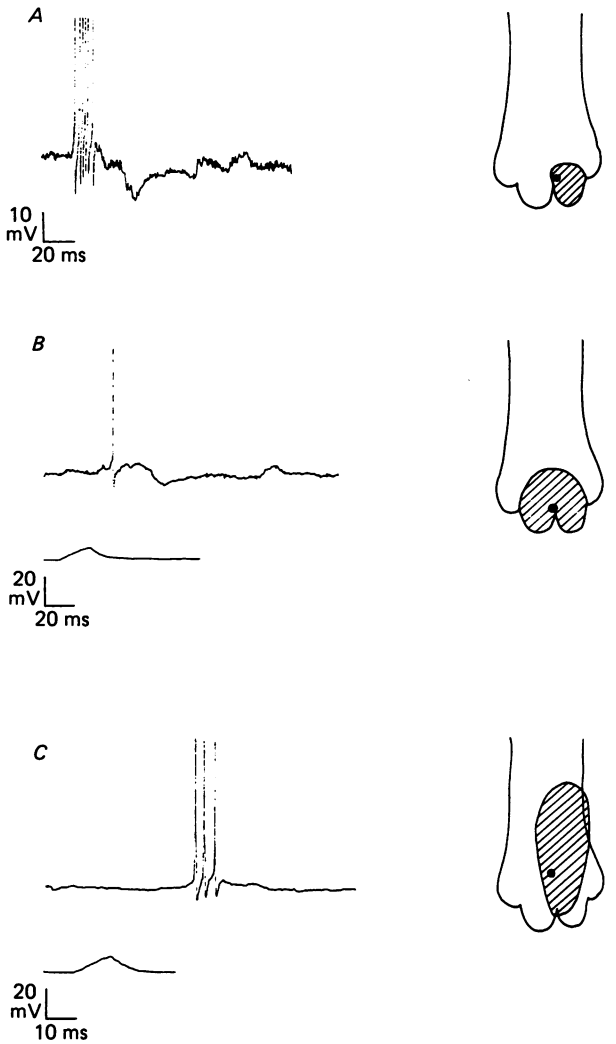

Fig. 1. Post-synaptic potentials recorded with intracellular electrodes from three s.c.t. neurones (*A*, *B* and *C*) in response to discrete air jets applied within the impulse firing zone (hatched on outline of cat hind paw with the location of the air jet in each case indicated by a filled circle). The lower traces in *B* and *C* are an analogue of the air jet. Voltage calibrations refer to the intracellular records. In *A* and *B* e.p.s.p.–i.p.s.p. complexes were typically recorded; in *C* no i.p.s.p. could be recorded in response to repeated stimuli. For details see text.

amplitude to fire cells at most trials, even when the air pressure and jet duration were restricted in attempts to demonstrate only the post-synaptic potential complexes. For most (twenty) of the twenty-five s.c.t. cells tested, however, positions were found within the firing zone where application of the air jet elicited i.p.s.p.s in addition to the excitatory responses and these were seen both in cells responding to hair movement only and to hair movement and pressure on the skin. Figs. 1, 2 and 3 show examples of responses, that include i.p.s.p. complexes, to air jets applied within the

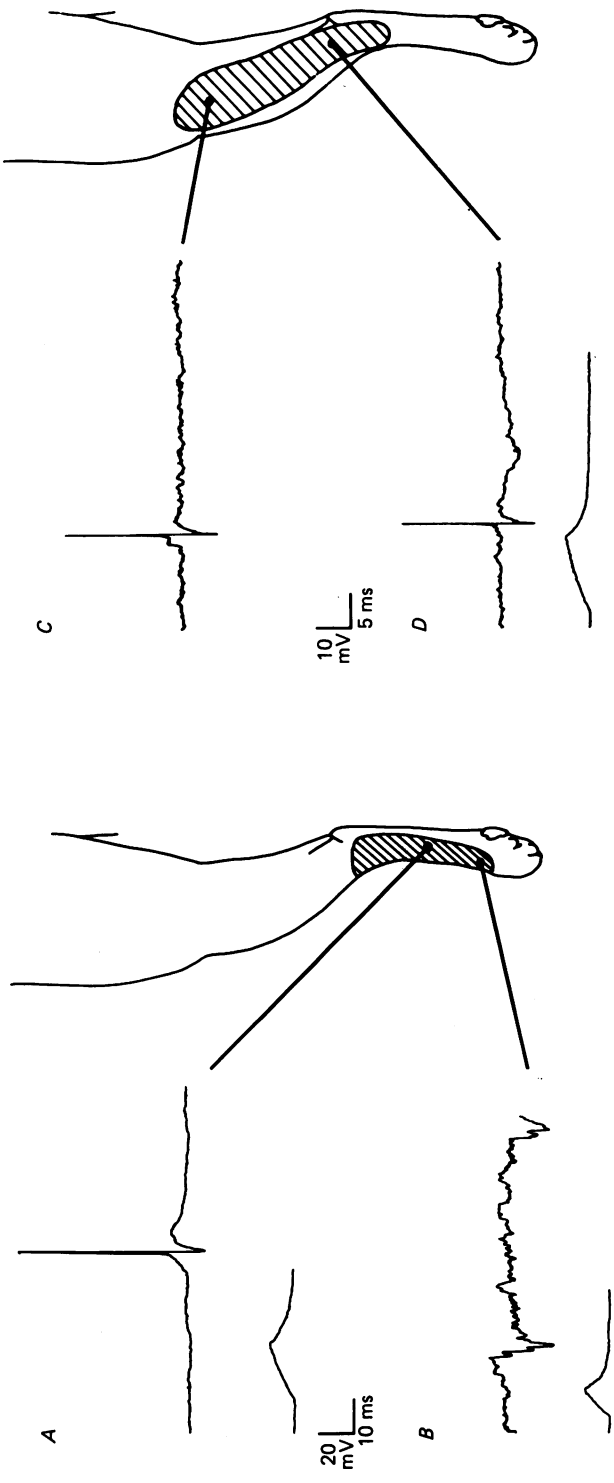

Fig. 2. Post-synaptic potentials recorded from two s.c.t. cells in response to air jets applied at two separate locations within their impulse firing zones (hatched on outline of cat hind limb). For each cell air jets applied at one location (*A* and *C*) evoked only an e.p.s.p. from which single action potentials were generated. At the more distal locations (*B* and *D*) e.p.s.p.–i.p.s.p. complexes were typically evoked. Note differences in time scale between *A* and *B* and between *C* and *D*. Voltage calibrations refer to intracellular record in each case.

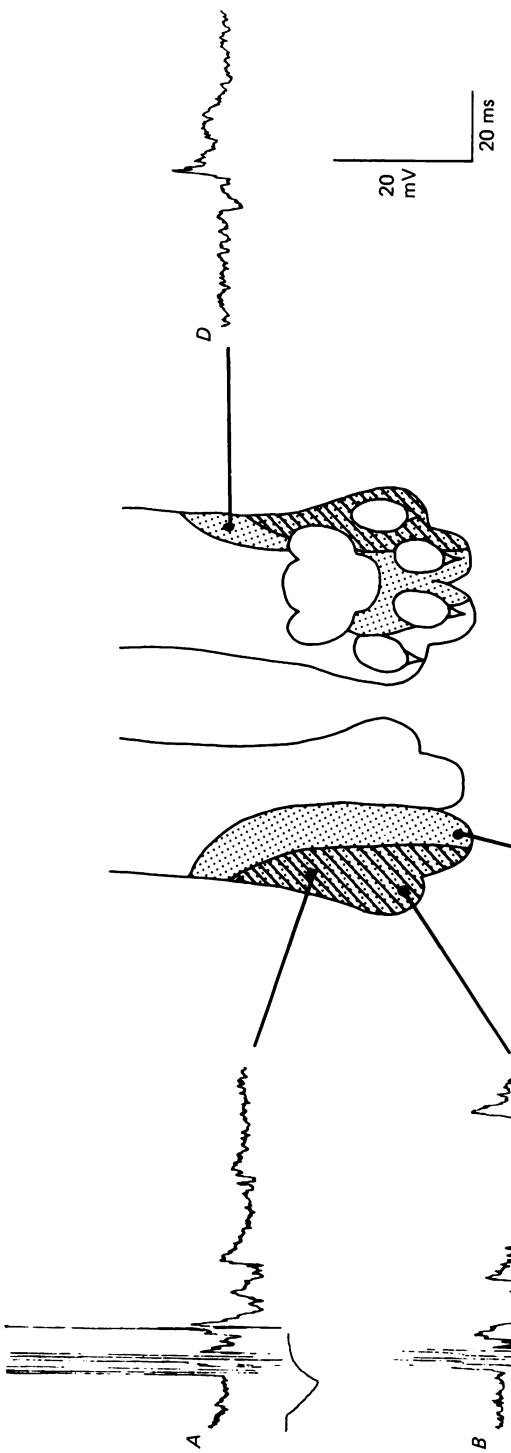

Fig. 3. Post-synaptic potentials recorded in response to air jets applied at four locations (*A*, *B*, *C* and *D*) indicated within the impulse firing zone (hatched) and inhibitory zone (stippled) of the receptive field of a s.c.t. meurone. The entire receptive field was mapped by applying similar air jets at locations both within and outside the field. In this case e.p.s.p.–i.p.s.p. complexes were recorded at all locations within the firing zone of the cell. The action potentials in *A* and *B* have been clipped in the respective traces. The voltage calibration refers to the intracellular records. The lower trace in *A* represents the analogue of the air jet.

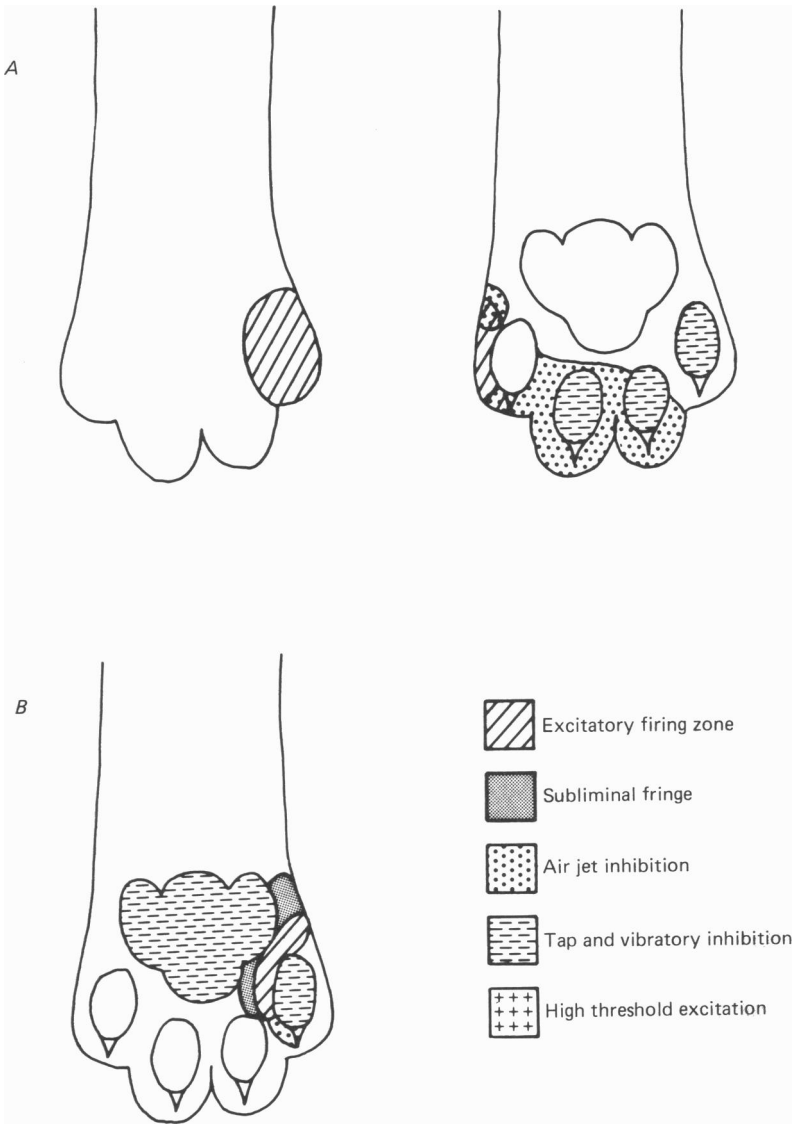

Fig. 4. Receptive fields of two s.c.t. cells (*A* and *B*) recorded with intracellular microelectrodes and mapped by moving hairs with an air jet, applying pressure with a toothed clip, tapping the field with a small probe and by applying vibratory stimuli with a tuning fork (frequency 500 Hz). Representations of the various parts of the receptive field (both inhibitory and excitatory) are as indicated in the key and drawn on the outline of the cat hind paw. The subliminal fringe refers to an area from which e.p.s.p.s but not action potentials could be evoked when hairs were moved by application of an air jet; the excitatory firing zone is that area from which such stimuli caused the cell to fire action potentials.

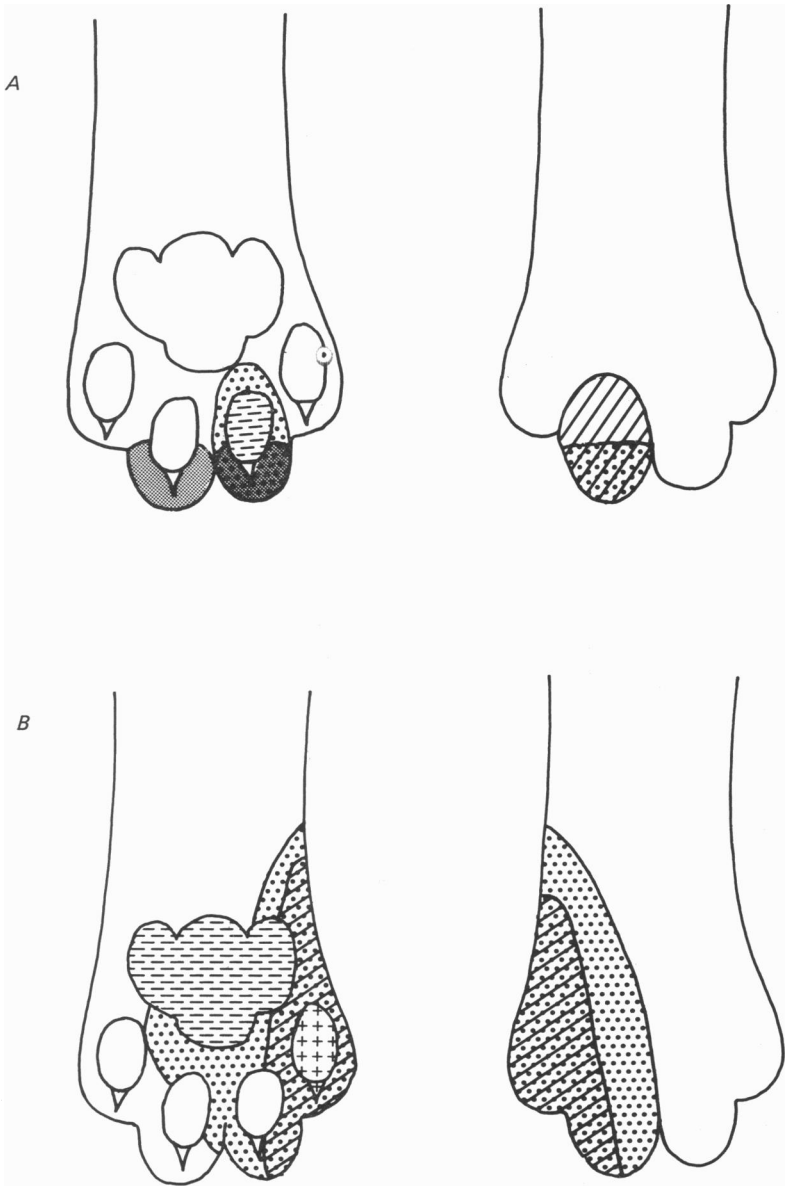

Fig. 5. Receptive fields of two s.c.t. cells (*A* and *B*) recorded with intracellular microelectrodes and mapped as in Fig. 4. Representations of the various parts of the receptive fields are as indicated in the key to Fig. 4.

firing zones of five s.c.t. neurones. A typical feature of the i.p.s.p. complexes was that they occurred later than the e.p.s.p.s, generally at 25–35 ms after the onset of the excitatory response, although occasionally an obvious i.p.s.p. at earlier times (15 ms) was observed and in one unit i.p.s.p.s followed the e.p.s.p.s by only 2–3 ms. It is, of course, possible that inhibitory processes occur much sooner but only become apparent after the e.p.s.p. complex has diminished. The i.p.s.p. complexes generally

lasted for 20–50 ms and no impulses occurred when they were present. In one cell the i.p.s.p. complex lasted for over 200 ms. Inhibitory post-synaptic potentials were not, however, recorded in response to stimuli applied at all areas tested within the s.c.t. cell firing zones, even when the response from other areas included i.p.s.p.s (see Fig. 2).

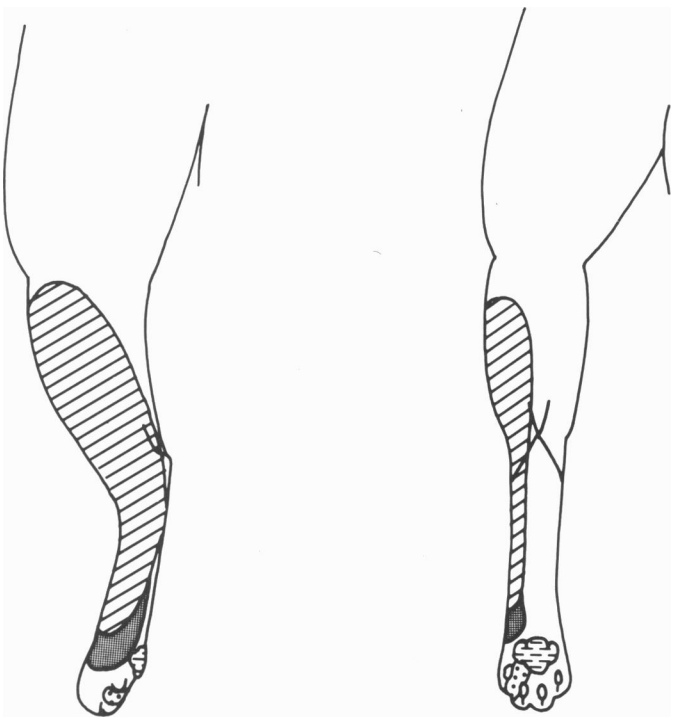

Fig. 6. Receptive field of a s.c.t. cell recorded as in Figs. 4 and 5 using the same key. In this case the inhibitory receptive field was separated from the excitatory field by an area of skin from which no response could be evoked.

Response to pinch. In neurones that responded with excitation to both hair movement and pressure within the firing zone, the application of a toothed clip (painful to the experimenters) led to excitatory responses. In two of three units tested that were excited by hair movement only, the application of a clip to the skin of the firing zone elicited i.p.s.p.s.

Responses from outside the impulse firing zone

Excitatory response. Small areas adjacent to the impulse firing zone from which e.p.s.p.s could be elicited were observed in four of the ten neurones whose fields were subjected to the most detailed examination (Figs. 4*B* and 5*A*). These areas were always very small (smaller than the firing zone) and adjacent to the firing zone. In one cell (Fig. 5*B*) the 'subliminal fringe' was on the glabrous skin of a toe pad and responded to pinch: in all other situations the e.p.s.p.s were evoked in response to air jets and to brushing the hairs.

Inhibitory receptive fields. Inhibitory areas were found for all ten s.c.t. neurones

examined in detail, and these included cells responding to both hair movement and skin pressure (pinch) as well as to hair movement only. These inhibitory areas produced i.p.s.p.s when stimulated and adequate stimuli included (in different cells) air jets to both hairy and glabrous skin, vibration (tuning fork at 500 Hz) applied

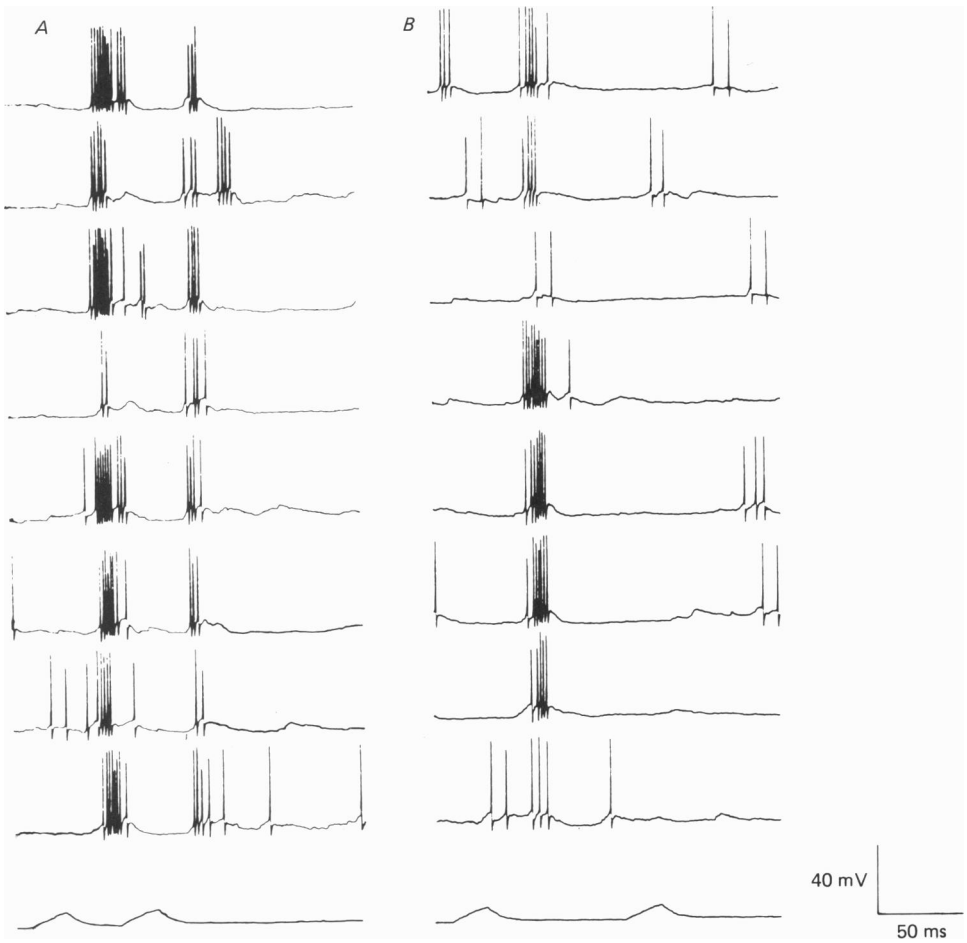

Fig. 7. Intracellular recordings of the response of a s.c.t. cell to two air jets (conditioning and test stimuli) applied at the same location within the cell's excitatory receptive field. Sequential recordings of the response with conditioning–test intervals of 50 and 100 ms are presented in *A* and *B* respectively. The response to the test stimulus is considerably less than that evoked by the conditioning stimulus with an interval of 50 ms and is all but absent with the interval set at 100 ms. Voltage calibration refers to the intracellular records. Analogues of the air jets are shown in the bottom traces in *A* and *B*.

to glabrous skin and toothed clips applied to both hairy and glabrous skin. Examples of the inhibitory receptive fields and i.p.s.p.s evoked from outside the cells' firing zones are shown in Figs. 3, 4, 5 and 6. The inhibitory fields were either adjacent to the firing zone (and appeared to be extensions of the inhibitory components contained within that zone), or were separated from the firing zone by skin from which no apparent responses were evoked (see Fig. 6).

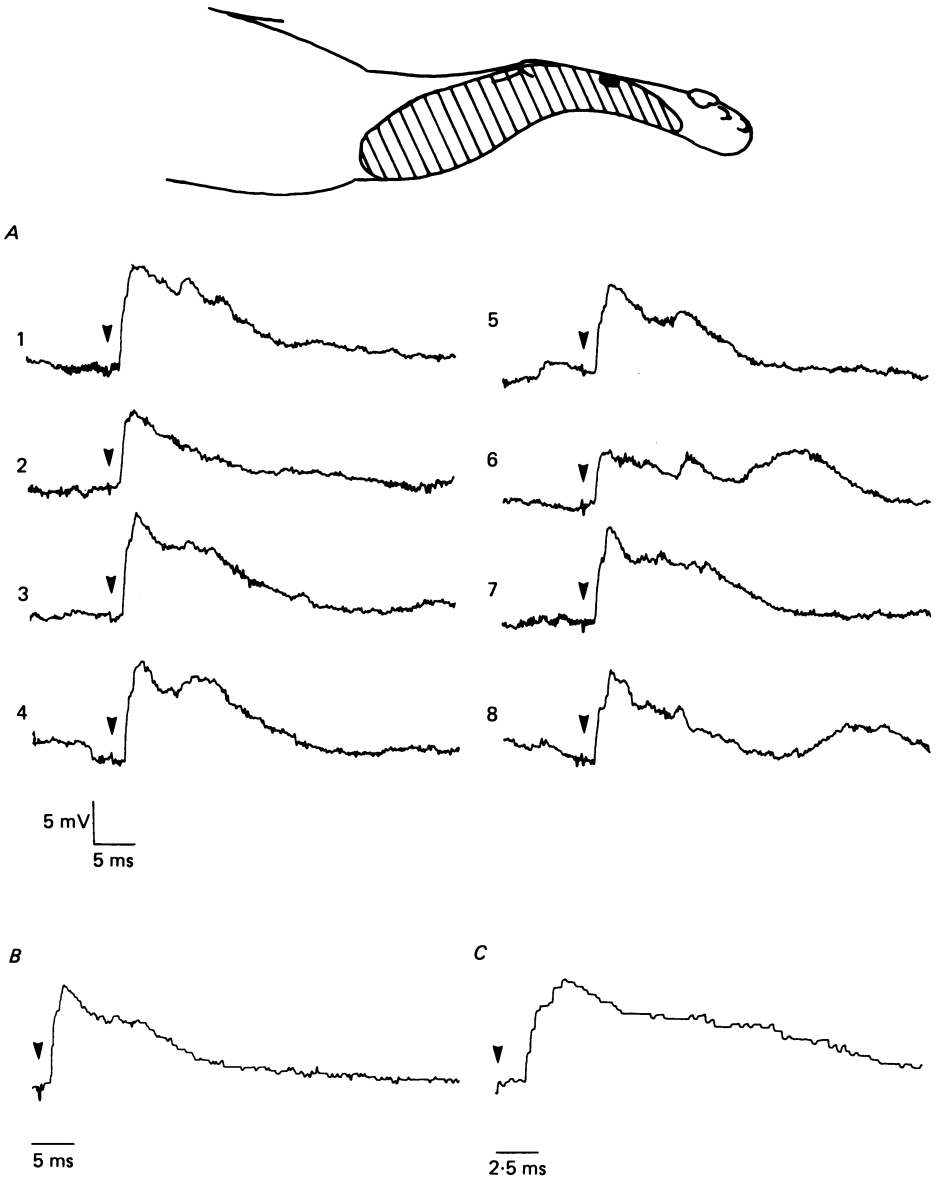

Fig. 8. *A*, post-synaptic potentials recorded in a s.c.t. cell and evoked by single action potentials generated by passing current pulses into a dorsal root ganglion cell of a group II hair follicle afferent fibre. The receptive field of the afferent fibre (indicated by the filled circle) was contained within the receptive field of the s.c.t. neurone (hatched on outline of the cat hind limb). Trials 1–8 are sequential recordings but have been selected to reveal only the post-synaptic potentials; trials in which action potentials were generated have been omitted. Arrowheads indicate time at which a single action potential was generated in the afferent fibre. *B* and *C*, average of potential records shown in *A* at slow (*B*) and fast (*C*) time sweeps.

Responses to paired air jets. Intracellular recordings were made of the responses of seven s.c.t. cells when two air jets (conditioning and test stimuli with inter-stimulus intervals of 25, 50 and 100 ms) were applied at one location within the firing zone of each cell. In each case, the response to the test stimulus was considerably less than that evoked by the conditioning air jet (Fig. 7). These reduced responses were not accompanied by long-lasting changes in membrane potential and occurred at each of the inter-stimulus intervals employed.

Conclusions

The detailed examination of s.c.t. neurones' receptive fields carried out during intracellular recording has revealed that these fields are complex. This result confirms and extends the much earlier observations of Hongo, Jankowska & Lundberg (1968). The use of intracellular recording has revealed the inhibitory components contained within the impulse firing zone of most s.c.t. cells and also the small subliminal areas and the inhibitory receptive fields from outside the firing zone.

Responses of s.c.t. neurones to impulses in single hair follicle afferent fibres

In this series of experiments simultaneous intracellular recordings were made from a dorsal root ganglion cell innervating hair follicles and from a s.c.t. neurone. The ganglion cell was caused to fire impulses by means of intracellular current pulses passed through the recording electrode. The effects of these impulses on the s.c.t. neurone were examined and these effects could also be conditioned by a variety of manoeuvres. In twelve afferent fibre–s.c.t. neurone pairs the hair follicle receptive field was contained within the firing zone of the s.c.t. cell (contained pairs), whereas in five pairs the hair afferent fibre receptive field was outside, but close to, the firing zone of the s.c.t. cell.

Contained pairs

Effects of single hair follicle afferent impulses. A total of twelve hair follicle afferent fibre–s.c.t. cell pairs with contained fields was studied. The sample consisted of nine afferent fibres and twelve s.c.t. neurones; that is, some afferent fibres were common to more than one pair (one being studied with three s.c.t. neurones and one with two). All the afferent fibres had conduction velocities within the group II range (48·5–68·1 m s^{-1}) and the s.c.t. neurones included those excited by hair movement alone as well as those excited by both hair movement and pressure.

A single impulse in the afferent fibre produced an e.p.s.p. complex in all the s.c.t. cells. Examples of these responses are shown in Figs. 8, 9 and 10. In all but one of the pairs with contained fields the latency of the initial component of the e.p.s.p.

Fig. 9. Post-synaptic potentials recorded in three s.c.t. cells (*A*, *B* and *C*) and evoked by single action potentials generated by passing current pulses into a dorsal root ganglion cell of a single group II hair follicle afferent fibre. In this case the receptive field of the afferent fibre was contained within the excitatory fields of all three s.c.t. neurones and was synaptically coupled with all three. The three records in each case are sequential but have been selected as in Fig. 8 to reveal only the post-synaptic potentials. Arrowheads indicate time at which a single action potential was generated in the afferent fibre.

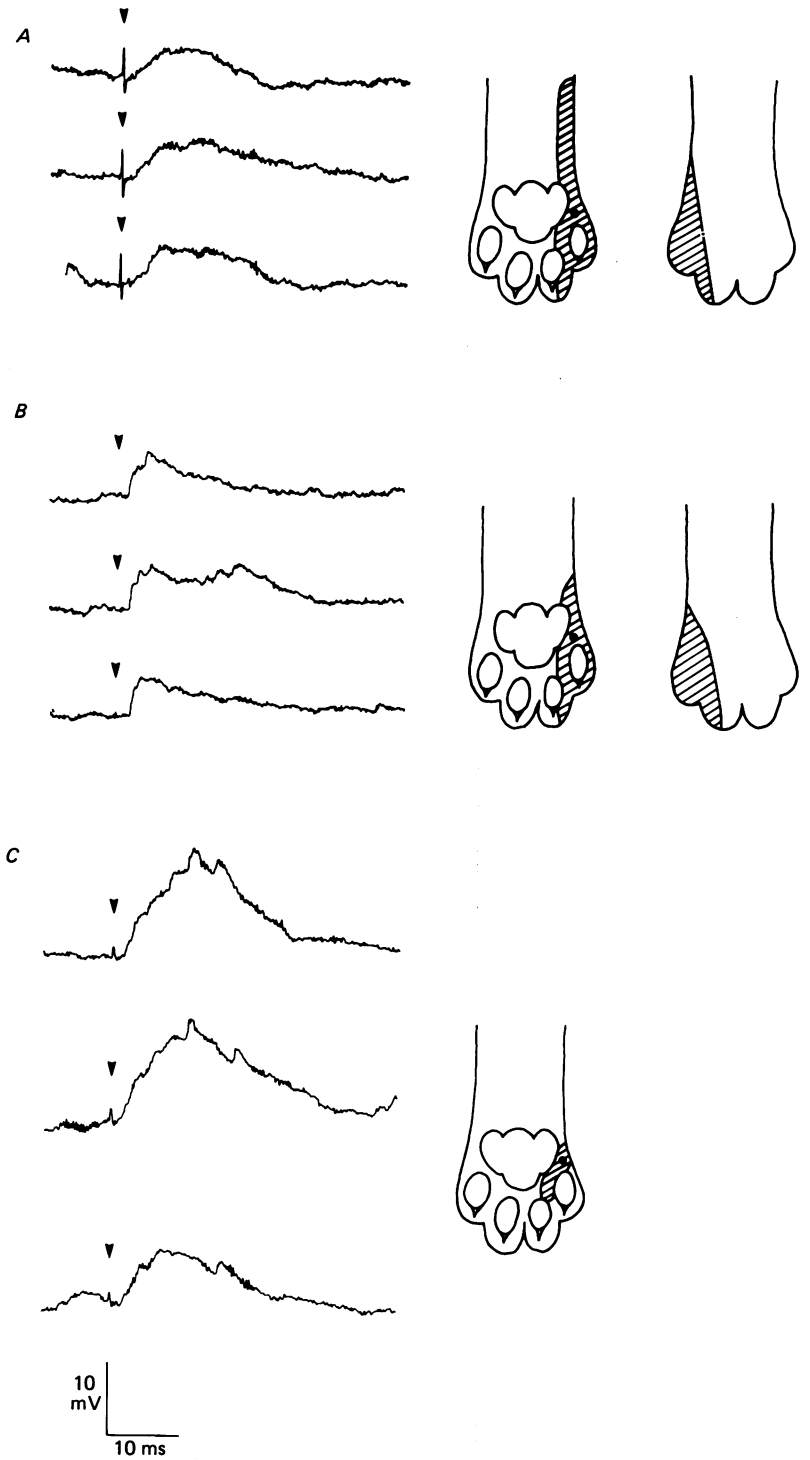

Fig. 9. For legend see opposite.

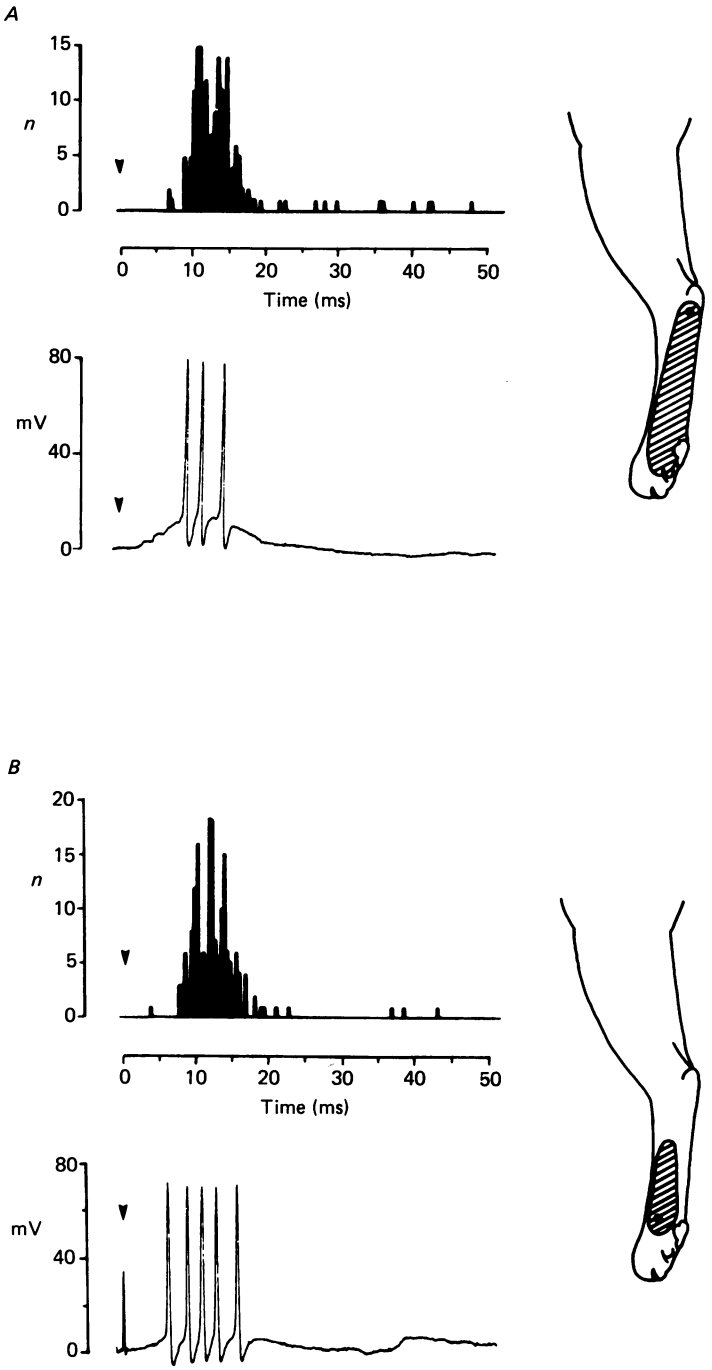

Fig. 10. *A*, intracellular recording (lower trace) of the response of a s.c.t. cell evoked by generating a single action potential (onset time indicated by arrow) in a group II hair follicle afferent fibre as described in Fig. 8. The upper trace presents a post-stimulus time histogram accumulated from responses in seventy-five trials recorded extracellularly. *B*, as in *A* but for a different synaptically coupled hair afferent–s.c.t. cell pair. The post-stimulus time histogram is accumulated from fifty-five trials. Bin widths: 0·4 ms. In each case the receptive field of the afferent fibre (filled circle) was contained and was situated peripherally within that of the s.c.t. cell (hatched on inset Figurine).

complex was 2·0 ms or less, indicating that this early part of the response was produced by monosynaptic connexions between the hair follicle afferent fibre and the s.c.t. neurones. In all pairs, however, this early component was followed by further e.p.s.p. components; the total duration of the complex being 30–40 ms. There was considerable variation in the rise times and amplitudes of these excitatory potentials, even though the latencies and durations were all very similar. As may be seen in Figs. 8–10 some responses had early (monosynaptic) components with very fast rise times (less than 0·5 ms) and large amplitudes (between 8 and 12 mV). Other responses had smaller monosynaptic components (1 mV or less) and the e.p.s.p. complex grew slowly from this over the succeeding 5–10 ms to reach a peak which could be as great as 20 mV. In contrast to the results of Hongo & Koike (1975), where the later components tended to be larger than the monosynaptic ones and to have a distinct peak, in the present sample of single hair fibre–s.c.t. responses in nearly all cases the later components grew out of the monosynaptic component to reach a maximum within 5 ms of onset and then decayed from this peak. The decay, although usually irregular, was such that later components did not rise above the amplitude of the early peak. Nevertheless, even where the monosynaptic component was large and had a fast rise time, impulses arose a few (usually two to three) milliseconds after the onset of the e.p.s.p. Where the e.p.s.p. grew more gradually from a small monosynaptic component the impulses arose after 5–12 ms (Fig. 10*A*).

In general (see Fig. 9*B*), and in confirmation of Hongo & Koike (1975) and our earlier observations (Brown *et al.* 1987*a*), hair follicle afferent fibres from the central parts of the impulse firing zone of the s.c.t. cell had more powerful monosynaptic e.p.s.p. components than afferent fibres from the peripheral parts. But hair follicle afferent fibres from the periphery produced a wide variety of responses and, even though the smallest and slowest monosynaptic e.p.s.p.s were evoked by these afferents, in some pairs of cells they were capable of eliciting large and fast e.p.s.p.s (see Fig. 8).

In striking contrast to the post-synaptic potentials evoked by applying an air jet to the s.c.t. cell's firing zone, when i.p.s.p.s were commonly elicited (see above), single hair afferent fibre impulses never evoked observable i.p.s.p.s in the present sample of contained pairs. In none of the many hundreds of individual trials examined was it possible to find any evidence of an i.p.s.p. and neither was it possible to reveal the presence of an i.p.s.p. time locked to the afferent input by means of averaging responses uncomplicated by s.c.t. impulse discharge (see Fig. 8).

Effects of pairs and trains of afferent impulses. The effects of pairs of single hair follicle afferent impulses at intervals of 10, 25, 50, 100 and 200 ms were examined. At 10 ms (Fig. 11*B*) there was summation of the responses (or facilitation of the second response over the first in other pairs of cells); but the total duration of the impulse activity in the s.c.t. cell was not longer than that of the post-stimulus time histogram built up from the response to a single afferent impulse alone. At 25 ms the response to the second afferent impulse was profoundly less than that evoked by the first impulse; this was usually such that the second response would now only rarely lead to impulse initiation in the s.c.t. cell even when the coupling between the fibre and the cell was strong enough to elicit at least one impulse in the majority of trials (Fig. 11*C*).

The mechanism underlying this reduced responsivenes to the second of a pair of

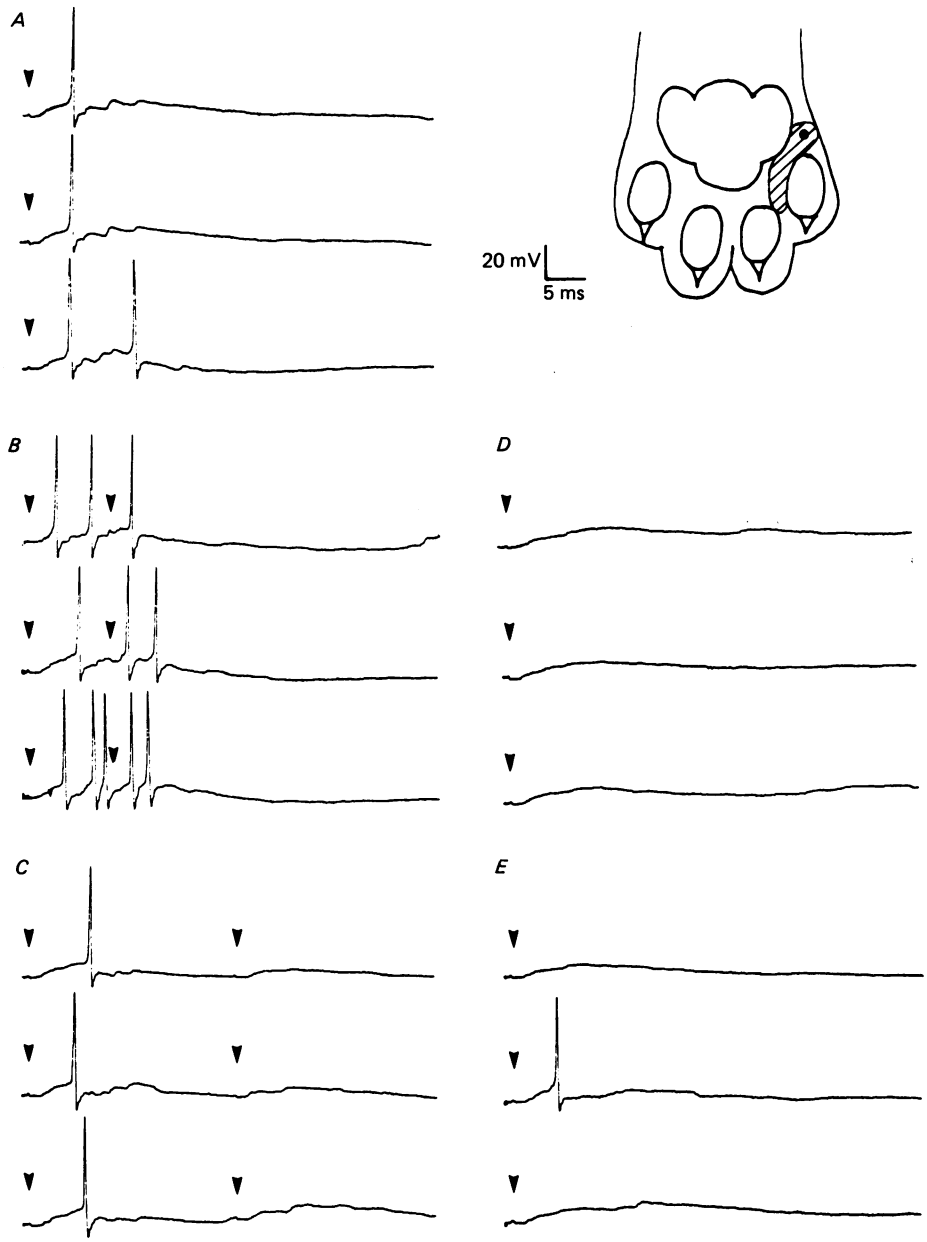

Fig. 11. Responses (recorded intracellularly) of a s.c.t. cell to single and pairs of action potentials generated (indicated by arrows) in a single group II hair follicle afferent fibre. *A*, response to a single afferent impulse; *B*, *C*, *D* and *E* responses to pairs of afferent impulses at 10, 25, 100 and 200 ms respectively. In *D* and *E* only the responses to the second of the pair of impulses are given. Respective locations of the afferent fibre (filled circle) and s.c.t. (hatched) receptive fields were generated in the cell body of the afferent fibre as described in Fig. 8.

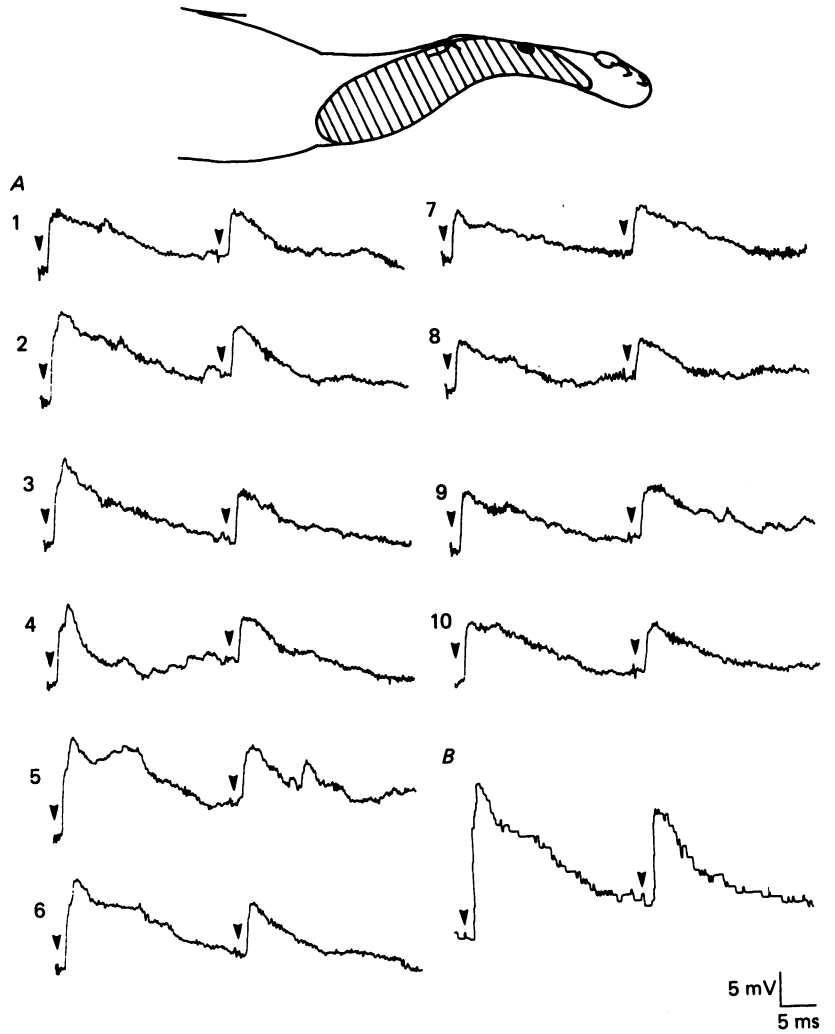

Fig. 12. Post-synaptic potentials recorded from a s.c.t. cell and evoked by generating pairs of action potentials (25 ms interval) in a group II hair follicle afferent fibre. Arrows indicate time of onset of afferent impulses generated in the dorsal root ganglion. Records 1–10 are sequential but have been selected as in Fig. 8 to reveal only the post-synaptic potentials (records with action potentials have been excluded). *B*, average of records presented in *A*. Note the reduction of the later components of the second e.p.s.p.

afferent impulses was indicated by examination of averaged post-synaptic potentials (Fig. 12). In all pairs studied, the e.p.s.p. evoked by the second afferent impulse was smaller in amplitude than that evoked by the first impulse, and this reduction was limited essentially to the later components. The monosynaptic component of the e.p.s.p. complex appeared unaffected. This action on the later e.p.s.p. components was especially easy to observe when trains of afferent impulses were elicited in the dorsal root ganglion cell: at 200 Hz, six impulses, the later components were almost completely absent whereas the monosynaptic component remained.

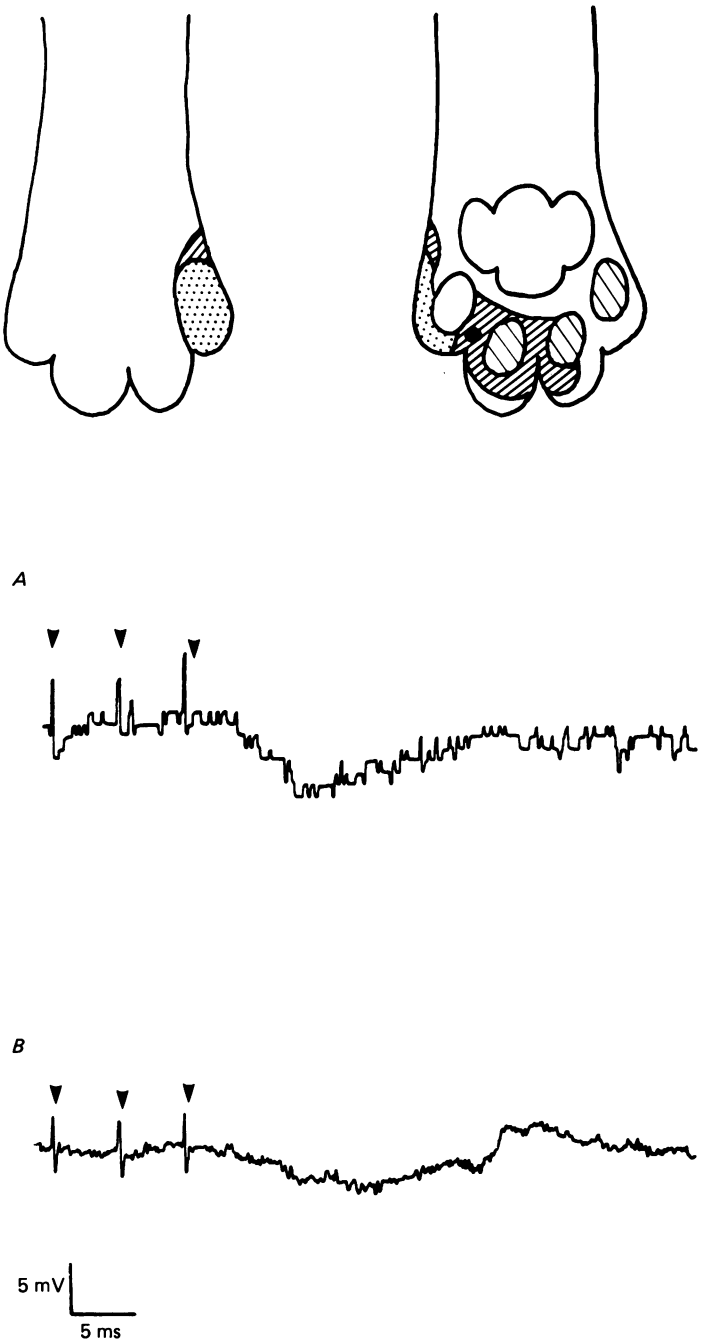

Fig. 13. For legend see opposite.

Separated pairs

Five pairs of units with clearly separated fields were studied and, in addition, one pair where there was doubt as to whether the receptive field of the hair follicle afferent fibre was inside or outside the s.c.t. cell's firing zone (see above). Of the five pairs with separated fields, four had hair afferent fibres with group II conduction velocities and for none of these were any post-synaptic potentials observed in the s.c.t. cell when single or pairs of impulses were evoked. One of the separated pairs consisted of a s.c.t. neurone and a group III hair follicle afferent fibre (conduction velocity 31 m s^{-1}) in which the receptive field of the afferent fibre was situated within an area adjacent to the firing zone of the s.c.t. cell but from which i.p.s.p.s were evoked by an air jet (the receptive field of this s.c.t. neurone is shown in Fig. 13). A train of three impulses (5 ms interval) in the hair follicle afferent fibre elicited an i.p.s.p. in the s.c.t. cell with a latency of 15 ms (from the first afferent impulse) and a total duration of 20 ms.

Absence of recurrent effects in the s.c.t.

In order to examine for the presence of recurrent actions in the s.c.t. system we applied the following tests: direct excitation of s.c.t. cells by intracellular current pulses, conditioning with such direct responses either the effect of a single hair follicle afferent impulse in a dorsal root ganglion cell (a member of a contained pair) or a response elicited by an air jet on the s.c.t. cell's firing zone. Intracellularly evoked action potentials were set up in two neurones. Single directly evoked action potentials and, more importantly, trains of three to six impulses failed to elicit any signs of either e.p.s.p.s or i.p.s.p.s in the neurones. Conditioning with directly evoked impulses failed to affect both the response to single impulses or trains of impulses in a hair follicle afferent fibre and the response to excitation produced by an air jet to the firing zone of the cell.

DISCUSSION

The experiments described in the present paper clarify some of the issues raised by the results of the preceding ones (Brown *et al.* 1986, 1987*a*, *b*) but they also raise many questions. Thus, it is now established that applying an air jet (with the intention of moving hairs) within the firing zone of a s.c.t. neurone leads to e.p.s.p.s, from which impulses arise and, at many locations, i.p.s.p.s in addition. This

Fig. 13. Inhibitory post-synaptic potential recorded in a s.c.t. cell and evoked by generating a train of three impulses in a group III hair follicle afferent fibre. *A*, average of ten sweeps (1500 ms interval). *B*, trace of a single response. Arrows indicate artifacts recorded on generating impulses in the cell body of the afferent fibre in the dorsal root ganglion. The receptive fields of the afferent fibre and s.c.t. cell are shown on the outline of the cat hind paw. In this instance the excitatory field of the s.c.t. cell has been represented by stippling. The inhibitory field of the s.c.t. cell determined by application of air jets to the fur is represented by the hatching on the hairy skin; hatching on the foot pads represents inhibition produced by applying a tuning fork (500 Hz) and tapping the glabrous skin. The receptive field of the afferent fibre (filled circle) was outside the excitatory field of the s.c.t. cell but was included in the inhibitory receptive field.

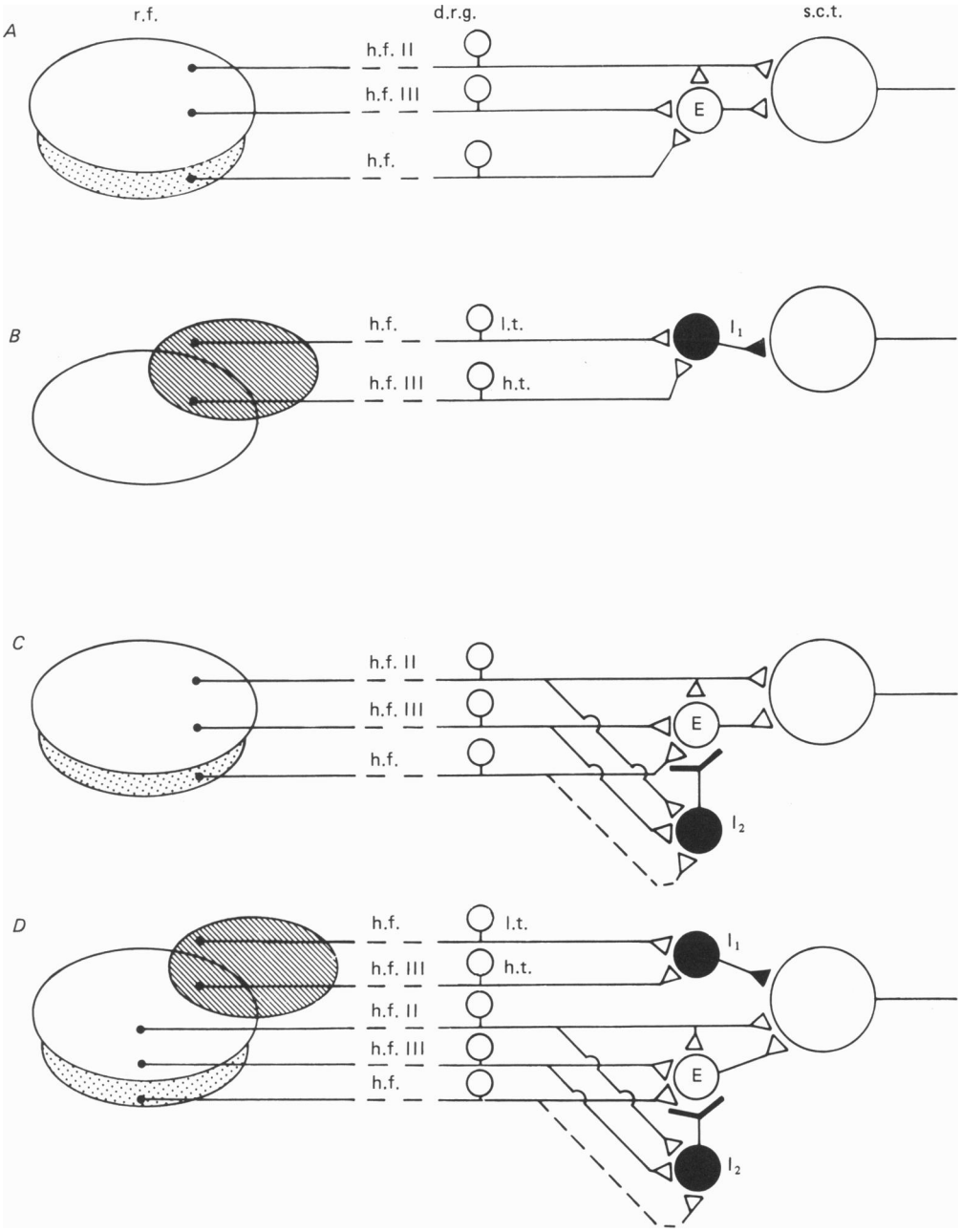

Fig. 14. Schematic representation of the probable neuronal circuitry mediating hair follicle afferent input (h.f.) to s.c.t. neurones. R.f. = s.c.t. receptive field; d.r.g. = dorsal root ganglion; s.c.t. = s.c.t. cell; E = excitatory interneurone; I = inhibitory interneurones; l.t., low threshold; h.t., high threshold. The hatched area represents the inhibitory receptive field; stippled area represents subliminal fringe; open elipse represents excitatory receptive field. For details see text.

observation was made on nearly all cells studied and indicates that s.c.t. cells have overlapping excitatory and inhibitory fields and also provides some evidence that at least part of the failure of two simultaneous inputs to summate linearly (Brown *et al.* 1986) is due to concurrent inhibitory processes that include post-synaptic inhibition. But the story is more complicated than this: for although a single action potential, or a train of action potentials, in a single hair follicle afferent fibre from within the firing zone evokes e.p.s.p.s in the s.c.t. cells it affects, we could find no sign of any i.p.s.p.s in the present experiments. It seems, therefore, that either a single hair follicle afferent fibre by itself is incapable of producing i.p.s.p.s that are large enough to be observed, even on averaged responses, or the air jet stimulus is providing an input additional to group II hair follicle input. A clue to this puzzle may be the observation that a single group III hair follicle afferent fibre, albeit from within an obvious inhibitory receptive field, was capable of eliciting i.p.s.p.s in a s.c.t. neurone with a latency, from the ganglion cell, of about 15 ms. Unfortunately, all of our sample of coupled pairs had a group II hair afferent as its fibre component and we have not yet observed the effects of group III afferent fibres from within the firing zone. But the air jet will undoubtedly activate group III hair follicle afferent fibres and the latency of the i.p.s.p.s produced, around 25 ms following the onset of the e.p.s.p.s, is about the right order of magnitude to be due to group III fibre activation. Even so, group III fibres are not simply inhibitory in their action. Movement of down hairs, which are innervated by group III axons, excites s.c.t. neurones (Brown & Franz, 1969) and activity of single group III axons from within the s.c.t. cell's firing zone can also excite them and this excitation lasts for at least 25 ms (Brown *et al.* 1987*a*).

We have seen that a single impulse in group II hair follicle afferent fibre not only evokes a profound excitatory response but also produces a marked depression of transmission through the s.c.t. that is maximal at about 25 ms and lasts for up to 1500 ms, which is much longer than the duration of i.p.s.p.s produced by an air jet. We have been unable to produce any evidence either for a recurrent inhibitory effect or for an inhibition acting directly on the s.c.t. cell itself. Furthermore, transmission was depressed even when the conditioning afferent impulse evoked only e.p.s.p.s and not action potentials in the s.c.t. cell; the depression was not, therefore, due to a post-activation depression produced by the discharge of the s.c.t. cell concerned.

The present results have confirmed and extended those of Hongo *et al.* (1968) on the receptive field organization of s.c.t. cells. Extracellular recording indicates that most s.c.t. cells have relatively simple receptive fields, when tested with mechanical stimulation, consisting of (a) a firing zone that responds to either hair movement or hair movement plus high threshold mechanical stimulation, and (b) in some units, inhibitory fields that tend to respond mainly to high threshold inputs and be remote from the firing zone (Brown & Franz, 1969), but intracellular recording establishes that s.c.t. receptive fields are complex. In addition to the inhibitory components that occur within the firing zone, there are also areas of skin, adjacent to or separated from the firing zone, from which i.p.s.p.s may be elicited and there are also small adjacent areas of skin from which e.p.s.p.s may be elicited. A common location for inhibitory effects, in neurones with firing zones on the toes, was the glabrous skin of the central pad or the toe pads and this inhibition was elicited by gentle mechanical stimulation

including vibration with a tuning fork, indicative of input from sensitive mechanoreceptors in the glabrous skin. In addition, other inhibitory areas were responsive to moving hairs or to high threshold mechanical stimulation. The low threshold inhibition evoked from glabrous skin would be expected to influence excitatory transmission through the s.c.t. during locomotion. In addition to the above some s.c.t. neurones may be excited and/or inhibited by noxious heat stimuli (Brown & Franz, 1969; Cervero, Iggo & Molony, 1977).

Intracellular recording has also revealed, in confirmation of Hongo & Koike (1975) and as expected from our earlier experiments (Brown *et al.* 1987*a*), that a single hair follicle afferent impulse in an axon from the firing zone of the s.c.t. neurone evokes an e.p.s.p. complex. This e.p.s.p. complex nearly always has a monosynaptic component, in agreement with Brown & Noble (1982) who showed, for a sample of intracellularly stained pairs of hair follicle fibres and s.c.t. cells, that there were monosynaptic connexions between them if the afferent fibre innervated skin within the firing zone of the neurone. Furthermore, the monosynaptic component of the response may be very large and have a fast rise time, but this is not necessarily the case. There was a little evidence that afferent fibres from the central parts of a s.c.t. neurone's field had especially prominent monosynaptic e.p.s.p.s. Peripherally located ones had monosynaptic e.p.s.p. components that ranged widely in amplitude and rise time, again in agreement with Hongo & Koike (1975) and with expectations from the extracellular experiments (Brown *et al.* 1987*a*).

In the first intracellular study of s.c.t. neurones, Hongo *et al.* (1968) described disynaptic i.p.s.p.s in response to cutaneous nerve stimulation as a characteristic feature of s.c.t. neurone responses. We have not seen such disynaptic i.p.s.p.s in response to identified hair follicle afferent input and such responses are noticeable by their absence in the records of Hongo & Koike (1975). We conclude that the disynaptic i.p.s.p.s recorded by Hongo *et al.* (1968) were produced by electrical stimulation of whole peripheral nerves. Obviously, the results of Hongo *et al.* (1968) indicate that i.p.s.p.s with a short (disynaptic) latency do affect s.c.t. cells. We suggest that they may have arisen from the sensitive mechanoreceptive inhibitory zones to the receptive fields, such as those in glabrous skin or those near the cell's firing zone.

It is now established that inhibitory mechanisms acting on transmission of information through the s.c.t. are especially effective on the polysynaptic inputs to the system. This has been thought likely for a considerable time (e.g. Brown, 1971; Brown, Kirk & Martin, 1973; Hongo & Koike, 1975). There is, however, a twist to the story; single hair follicle afferent fibres activate s.c.t. cells through both mono- and polysynaptic pathways and it is the polysynaptic component of this input that is effectively inhibited. With the exception of group III hair follicle afferent fibres (Brown *et al.* 1987*a*), which appear to have polysynaptic excitatory connexions, it is not known how other inputs from within the firing zone, i.e. from the high threshold receptors, activate s.c.t. cells. Since they are very effectively inhibited by a variety of descending and segmental pathways (see Brown, 1981) it seems likely that their input may be via polysynaptic connexions.

Hongo *et al.* (1968) noted that antidromic activation of s.c.t. cells, and stimulation of the dorsolateral funiculus at strengths below antidromic firing threshold for the

single cell under study, failed to produce post-synaptic potentials in the cell. They conclude there was no recurrent action in the s.c.t. system. We have confirmed this using a more direct approach.

Conclusions

The results of the experiments presented in this and the preceding papers (Brown *et al.* 1987*a*, *b*) allow a series of circuit diagrams to be drawn which suggest the probable neuronal circuitry involved (Fig. 14). Fig. 14*A* shows the excitatory circuits suggested by the extracellular experiments of Brown *et al.* (1987*a*). The intracellular experiments have confirmed the monosynaptic nature of the input from within the impulse firing zone of the s.c.t. neurone. The polysynaptic nature of the excitatory input from any subliminal fringe area is not confirmed, but remains extremely likely. Fig. 14*B* shows the post-synaptic inhibitory input from both inside and outside the firing zone. The origin of the inhibition from inside the zone is not yet determined, but certainly arises from mechanoreceptors sensitive to a very gentle air jet. Since single group II hair follicle afferent fibres appear not to evoke i.p.s.p.s, we tentatively identify the inhibition evoked from inside the impulse firing zone as due to activity in group III hair follicle afferent fibres, but it could conceivably be due to slowly adapting type I units. The i.p.s.p.s evoked from outside the firing zone may be due, in any one s.c.t. cell, to a variety of sources: hair follicle receptors innervated by group II or III axons, slowly adapting type I receptors in hairy skin, low threshold mechanoreceptors in glabrous skin (Pacinian corpuscles, Krause endings or slowly adapting type I receptors) or high threshold mechanoreceptors in hair or glabrous skin (heat nocireceptors have not been tested). Fig. 14*C* shows the organization of excitatory connexions to s.c.t. cells from hair follicle afferent fibres (as shown in Fig. 14*A*) but with the addition of an interneuronal network (depicted as a single neurone) brought into play by activity in single group II hair follicle afferent fibres. Since such activity fails to elicit an i.p.s.p. in s.c.t. cells it is shown acting on transmission in the interneuronal chain. The mechanisms are left indeterminate; we have no direct evidence as to whether it is pre- or post-synaptic, although suggest it is both. Furthermore, since there are no axo-axonic synapses on hair follicle afferent boutons that contact s.c.t. cells (Maxwell, Fyffe & Brown, 1984) the monosynaptic connexion between the afferent fibre and the s.c.t. cell is left free of influence from the interneuronal system. Finally, in Fig. 14*D*, we show a tentative scheme for the minimal circuitry involved in the results presented in this paper and the preceding ones (Brown *et al.* 1987*a*, *b*).

We wish to thank Miss S. Brass, Mrs A. Corbett, Mr C. M. Warwick and Mrs J. Wood for excellent technical assistance. The work was supported by a Programme Grant from the Medical Research Council. Animals were held in the Wellcome Animal Research Laboratory, Faculty of Veterinary Medicine, University of Edinburgh.

REFERENCES

BROWN, A. G. (1971). Effects of descending impulses on transmission through the spinocervical tract. *Journal of Physiology* **219**, 103–125.

BROWN, A. G. (1981). The spinocervical tract. *Progress in Neurobiology* **17**, 59–96.

BROWN, A. G. & FRANZ, D. N. (1969). Responses of spinocervical tract neurones to natural stimulation of identified cutaneous receptors. *Experimental Brain Research* **7**, 213–249.

BROWN, A. G., KIRK, E. J. & MARTIN, H. F. (1973). Descending and segmental inhibition of transmission through the spinocervical tract. *Journal of Physiology* **230**, 689–705.

BROWN, A. G., KOERBER, H. R. & NOBLE, R. (1987*a*). Excitatory actions of single impulses in single hair follicle afferent fibres on spinocervical tract neurones in the cat. *Journal of Physiology* **382**, 291–312.

BROWN, A. G., KOERBER, H. R. & NOBLE, R. (1987*b*). Actions of trains and pairs of impulses from single primary afferent fibres on single spinocervical tract cells in the cat. *Journal of Physiology* **382**, 313–329.

BROWN, A. G. & NOBLE, R. (1982). Connexions between hair follicle afferent fibres and spinocervical tract neurones in the cat: the synthesis of receptive fields. *Journal of Physiology* **323**, 77–91.

BROWN, A. G., NOBLE, R. & ROWE, M. J. (1986). Receptive field profiles and integrative properties of spinocervical tract cells in the cat. *Journal of Physiology* **374**, 335–347.

CERVERO, F., IGGO, A. & MOLONY, V. (1977). Responses of spinocervical tract neurones to noxious stimulation of the skin. *Journal of Physiology*, **267**, 537–558.

HONGO, T. & KOIKE, H. (1975). Some aspects of synaptic organization in the spino-cervical tract cell in the cat. In *The Somatosensory System*, ed. KORNHUBER, H. H., pp. 218–226. Stuttgart: Georg Thieme.

HONGO, T., JANKOWSKA, E. & LUNDBERG, A. (1968). Post-synaptic excitation and inhibition from primary afferents in neurones of the spinocervical tract. *Journal of Physiology* **199**, 569–592.

MAXWELL, D. J., FYFFE, R. E. W. & BROWN, A. G. (1984). Fine structure of normal and degenerating primary afferent boutons associated with characterized spinocervical tract neurons in the cat. *Neuroscience* **12**, 151–163.