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CHARACTERISTICS OF SPINAL NEURONES RESPONDING TO CUTANEOUS MYELINATED AND UNMYELINATED FIBRES*

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SUMMARY

1. Spike discharges were recorded from neurones in the lumbar spinal cord in cats anaesthetized by barbiturate.

2. The neurones were examined systematically for various physiological parameters and for their location. Especially the neurones situated in the dorsal horn were classified for the following parameters: mono- or polysynaptic linkage to myelinated afferents; type of natural stimuli which excited the neurones; depth from the cord surface; number of impulses discharged upon a cutaneous A fibre stimulus; steady-state discharge in the absence of intentional stimulation.

3. All neurones were also tested as to whether or not they responded to volleys in cutaneous C fibres. Of 111 units which were activated by the A fibres in nerves from the hairy skin, 57 (= 51 %) responded to C volleys in those nerves too.

4. By blocking conduction in the A fibres using polarizing currents it was shown that the responses to C fibre volleys were partially or totally suppressed by a preceding discharge of the neurone in response to an A volley. Using search stimuli which were suprathreshold for C fibres one cell out of 36 could be found which responded only to afferent volleys in C fibres.

5. About half of all neurones were shown to be connected monosynaptically to cutaneous A fibres, as was judged from the synaptic delay. The other half were polysynaptically linked to the A fibres. Both mono- and polysynaptic neurones were found in all layers of the dorsal horn. About 15 % of the cells had additional input from muscle Group II and/or III fibres via polysynaptic pathways.

6. Subdividing the A and A + C responsive neurones according to their mono- (M) or polysynaptic (P) connexions yielded the following subsamples: MC, 39 %; PC, 15 %; MA, 13 %; PA, 33 %. Most MC neurones

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had, and most PA units had not, a spontaneous discharge. About half of the PA cells could not be driven by natural skin stimulation. The majority of MC units responded specifically to movement of hairs.

7. A model was proposed hypothesizing two pathways in the dorsal horn, one showing convergence of A and C fibres and the other not. Some relations concerning other observations on C fibre effects were discussed.

INTRODUCTION

A number of investigations has been performed on responses induced in the central nervous system by volleys in unmyelinated or C fibres, which constitute the bulk of fibres particularly in the cutaneous nerves. Such work has been met with considerable interest both by the neurophysiologists and the neurologists, because C fibres have long been accepted as participating in the transmission of painful stimuli. The original view that the C fibres represent a specific nociceptive system was a simplification, since it is known now that the receptive endings of a large proportion of this fibre group responds to innocuous mechanical and thermal stimulation (Douglas & Ritchie, 1957; Douglas, Ritchie & Straub, 1960; Iggo, 1959, 1960; Hensel, Iggo & Witt, 1960; Iriuchijima & Zotterman, 1960; Bessou & Perl, 1969). The physiological signification of these latter fibres is unknown as yet.

During the last few years various reflexes have been recorded following afferent C volleys, using electrophysiological methods. In ventral roots motor reflexes occur, which sum with those elicited by cutaneous myelinated fibres and therefore might be considered as flexor reflexes (Koll, Haase, Schütz & Mühlberg, 1961; Franz & Iggo, 1968). In dorsal roots a C-DRP (dorsal root potential evoked by C afferents) was measured (Zimmermann, 1968*a*; Franz & Iggo, 1968) which could be interpreted as a presynaptic depolarization (C-PAD) generated in myelinated fibre terminations (Jänig & Zimmermann, 1971). These latter findings were in contrast to a report of a DRP of opposite sign, i.e. of presynaptic hyperpolarization following a C volley (Mendell & Wall, 1964). Furthermore, in neurones of the spinal cord the axons of which ascend in the dorsolateral tract (Mendell & Wall, 1965; Mendell, 1966) especially in the spino-cervical tract (Gregor & Zimmermann, 1969; Gregor, 1971) and in the anterolateral tract (Manfredi & Castellucci, 1969; Manfredi, 1970) excitatory actions were described consequent upon volleys in cutaneous C fibres. Exceptionally prominent reflexes following C volleys occur in the sympathetic trunk (Schmidt & Weller, 1970), which probably account for the observation of profound cardiovascular reactions evoked by such stimuli (Ranson, 1921; Laporte & Montastruc, 1957).

The interconnecting pathway from the afferent C fibres to all these nervous system activities has to originate in the dorsal horn, in which structure end all afferent fibres of cutaneous nerves (Szentágothai, 1964; Réthelyi & Szentágothai, 1969; Scheibel & Scheibel, 1968, 1969). We have therefore investigated, in a systematic approach, the afferent connexions of C fibres to the dorsal horn neurones of the cat. A similar investigation has been reported on the rhesus monkey (Wagman & Price, 1969). The aims of our study were (1) to examine the patterns of responses elicited in the neurones by electrical stimulation of cutaneous C fibres, (2) to evaluate the proportion of cells which do have C input and (3) to test whether neurones receiving C input form a special population of cells as revealed by their physiological parameters. Part of our results have appeared in a preliminary communication (Gregor & Zimmermann, 1969).

METHODS

The experiments were performed on twenty adult cats (weight from 2·2 to 3·0 kg) anaesthetized by pentobarbitone sodium (Nembutal, initial dose 40 mg/kg injected I.P.; repeated doses of 4 mg/kg were given I.V. at intervals of 2–5 hr, as required) and immobilized with gallamine triethiodide (Flaxedil, repeated doses of 10 mg/kg, given I.V.). The animals were set on artificial respiration. The mean blood pressure of the cats was continuously recorded by a catheter in the right common carotid artery and was kept above 90 mm Hg if necessary by infusion of dextran solution (Macrodex). The rectal temperature of the animals was kept between 37 and 38° C by electrical heating of the ventral body surface.

Preparations. The lumbar spinal cord was exposed by a laminectomy from the L 1 to the S 1 vertebrae. A cord transection was performed at L 1. The animals were rigidly held in a frame by a pair of clamps affixed to the T 13 vertebrae and to the iliac crests. The spinal cord was covered by warmed paraffin oil (kept at 37° C by electrical heating). The following nerves of the left leg were exposed in a paraffin oil pool: The sciatic nerve (SC), the superficial peroneal (SP), the medial branch of the sural nerve (SU), the nerves to the knee flexors: posterior biceps + semitendinosus (PBST) and to the hip extensors: anterior biceps + semimembranosus (SMAB). The temperature in this pool usually was at 30–33° C. The PBST and SMAB were cut peripherally and put on electrodes for electrical stimulation. The SC, SP and SU nerves were left in continuity and mounted on electrodes for stimulation (S_1, S_2, S_4 in Fig. 1). The mass action potentials were recorded from the SP and SU nerves by separate electrodes (R_1, R_4 in Fig. 1). The SU nerve had an additional electrode pair (B in Fig. 1) placed between the S_1 and R_1 electrodes and used to polarize the nerve in order to perform a selective block of the myelinated fibres. Between the electrode pairs S_1, B and R_1 the SU nerve was left in its normal connective tissue environment for a length of about 10–15 mm. Care was taken to preserve a good blood supply of the nerve sections which were freed from the connectives for the mounting of the electrodes. The stimulation electrode of the SP (S_4 in Fig. 1) was placed distally to the branching of the nerves to peroneus brevis and tertius muscles, thus activating cutaneous afferent fibres only.

Stimulation and blocking. Electrical stimuli were delivered by a Grass S 8 stimulator via isolation units. The amplitudes of the square pulses used to activate the myelinated or A fibres only were in the range of up to 1·5 V, the duration was 0·1 msec.

The stimulus strengths were routinely expressed in multiples of the threshold (T) of the most excitable fibres in each nerve as revealed from the volley recorded from that nerve or from the dorsal roots (L6/L7 segmental level). Stimuli of 1·5 V were usually at about $8T$, which is above threshold of all fibres in the Groups I, II and of most fibres in Group III. The stimuli to excite the unmyelinated or C fibres of the SU and SP nerves were 0·5 msec in duration and had amplitudes of up to 15 V, which yielded maximum compound volleys of these fibres. Transient current polarization of the SU nerve via the electrode pair B (Fig. 1) was performed in order to generate a cathodal conduction block of the A fibres. The method had been described previously (Zimmermann, 1968*b*); in the following only the most important features and the methodical amendments are described. The polarizing currents were applied

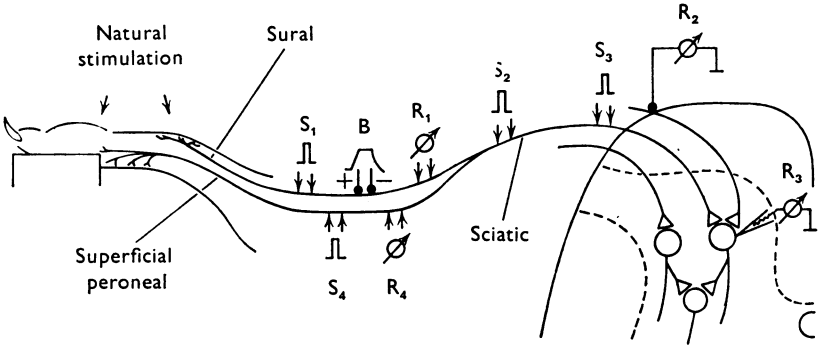

Fig. 1. Schematic drawing of the experimental set up. Electrical stimuli were applied by electrodes S_1, S_2, S_4 and S_3 to the respective nerves as shown, or to the dorsal roots. The cutaneous nerves SU (sural) and superficial peroneal (SP) were left in continuity, thus allowing activation of afferent fibres by natural (mechanical) stimulation. R_1, R_4 recorded the nerve volleys, R_2 the dorsal root volleys and the spinal cord surface potentials. The micro-electrode R_3 recorded from single cells within the spinal cord. The electrode pair B was connected to a current generator (isolated from ground), which delivered polarizing currents to the SU nerve.

through ball shaped (1 mm in diameter) electrodes B, spaced by about 8 mm. The distal pole (anode) of this electrode pair was usually located just at the distal end of the sections (about 15–20 mm in length) which was dissected free. In order to prevent local high current densities the contact to the nerve was established by a droplet of lymph fluid or blood. The current source was isolated from ground in order to provide a well defined current path. The current generator had been described in detail previously (Zimmermann, 1968*b*). The time course of the current was arranged to yield a rise in two ramp intervals. The maximum current strength reached about 2 sec after the onset was adjusted in order to effect a total conduction block in the A fibres, but leaving conduction intact in the C fibres. This state could be reached in most experiments. The first step of the polarizing current acted as a stimulus to some thick myelinated fibres. This step was adjusted so that a further increase (second ramp) did not stimulate any fibres in the nerve. The stimulation of myelinated fibres by the onset of the blocking current was anteceded for an appropriate interval in order to prevent interference of its effects in the spinal cord with those evoked by the selective C input under study. Usually an interval of 2·5 sec was sufficient for this

purpose. The expenditure of this transient polarization was put up with the aim to keep current load of the nerve as small as possible, this providing a stable and highly selective blocking condition for a long time.

Recordings. Afferent volleys were recorded by electrode pairs from the SU and SP nerves (R_1 and R_4 in Fig. 1) or by a single ended electrode from the cord dorsum (R_2). This latter electrode was also used to measure the N-wave (Bernhard, 1953; Bernhard & Widen, 1953), which is constituted by the extracellular currents during post-synaptic activity of dorsal horn neurones. After amplification by a low level preamplifier (Tektronix type 122) the recordings were displayed on an oscilloscope (Tektronix type 565) and photographed by a camera (Grass C4). Glass micro-electrodes filled with 3 M-KCl and having d.c. resistances from 15 to 30 MΩ were used to record intra- and extracellularly from neurones (R_3 in Fig. 1). The indifferent electrode was the large area ground electrode located in the muscles of the dorsum. Impedance matching was performed by a cathode follower having a field effect transistor as the head stage; its input resistance was above 10^{13} Ω. The neurone's discharges were displayed on an storage oscilloscope (Tektronix type 564) and photographed with a conventional camera (Leica M1).

Procedure. The size of the N-wave measured systematically along the cord by R_2 (Fig. 1) was taken to determine the regions of maximum afferent inflow from the SP and SU nerves. The N-wave size is plotted in Fig. 2*A* against the length coordinate of the cord, the extensions of three segments (L6, L7, S1) are indicated below. Examples of recordings are given in B and C after 4 *T* stimulation of the SU and SP nerves respectively. The records each were taken at the positions labelled by *x*, *y*, *z* in *A*. Usually sites of maximum N-waves were different for both nerves. At an intermediate level revealing activity from both nerves (corresponding to site *y* in Fig. 2) the pia arachnoidea was opened with fine watchmaker forceps. The micro-electrode was inserted with a micromanipulator at a distance from 0·3 to 1·7 mm lateral to the posterior median sulcus at an angle of 15° from the vertical sagittal plane (see Fig. 5*C*). The three space co-ordinates of the micromanipulator were read when the micro-electrode made contact with the cord surface, and tracks were then made into the cord not less than 0·1 mm apart in both head-to-tail and side-to-side axes up to a depth of at least 2·3 mm from the cord dorsum. During the slow penetration of the micro-electrode (about 10 μ/sec) search stimuli at a strength of 1·5 V (about 8–10 *T*) were delivered to the nerves SC, PBST and SMAB, except in three experiments in which search stimuli were applied to the SU and SP nerves at a strength supramaximal for C fibres. Each neurone found was investigated for as many as possible of the following parameters: an axon projection into the dorso-lateral funiculus; the stimulus strength of the various nerves for a spike to appear, i.e. the threshold T_n of the neuron; the latency of the first spike at $2T_n$; the steady-state discharge; the number of spikes after stimuli in the various nerves; the responses upon different mechanical skin stimuli; the location of the neurone. Details will be described in the appropriate section of the results. Moreover, all the units were tested whether they could be activated via C fibres in the SP and SU nerves.

Synaptic delay. In order to decide whether a unit under study was mono- or polysynaptically linked to the periphery an estimation of the central latency was performed by two methods, M1 and M2. M1 was a direct measurement of the central latency upon a dorsal root stimulus. Two stimulation electrodes (only one indicated by S_3 in Fig. 1) were arranged on neighbouring roots supplying the region of micro-electrode recording. The minimum latency of the first spike was selected and was corrected by subtraction of 0·2 msec for intraspinal presynaptic conduction time. This value was an average found when recording intracellularly from primary afferent fibres within the cord under identical conditions of dorsal root stimulation

(M. Gregor & M. Zimmermann, unpublished observations). M2 was a more indirect extrapolative method, which was based upon the well known relationships between the diameter of a nerve fibre, its threshold, and its conduction velocity. From the stimulus strength at threshold T_n of a given unit the presumptive peripheral conduction time was calculated. The calculated conduction time was then subtracted from the total latency between the stimulus and the first spike of the unit's discharge at $2T_n$ to yield the synaptic delay. A similar approach has been described in detail (Rosenberg, 1970). The direct comparison between both methods exhibited the values found by M2 often to deviate considerably from those measured directly

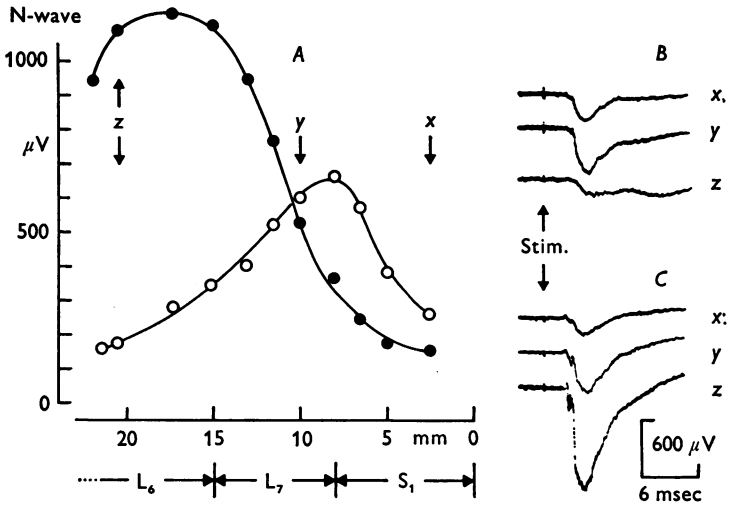

Fig. 2. Spatial variation of the N-wave along the lumbosacral cord. The N-wave was recorded from the cord surface following $4T$ stimulation of the SU and SP nerves; specimens from different loci x, y, z are given in B and C, respectively. In A the sizes of the N-waves (ordinate) are plotted in relation to the length co-ordinate of the cord (abscissa); the extensions of the cord segments involved are indicated below the abscissa. Measurements were taken from the SP (●) and from the SU (○) nerves respectively; x, y, z denote the loci at which the records shown in B and C were taken. Negative deflexions are downwards in B and C.

by M1 (up to ± 0·5 msec). However, in the experiments using M2 only this method could be used for many units to decide whether they were mono- or polysynaptically connected to the afferent A fibres (see context to Fig. 6*B*). The central delays (M2) were always longer upon stimulation of the muscle nerves than they were upon stimulation of a mixed (SC) or a skin nerve (SU, SP). Therefrom it can be concluded that in the dorsal horn neurones the measurement of the central delay by dorsal root stimulation (method M1) yields values which are representative for cutaneous afferent pathway.

Anatomical procedures. To locate the neurones the micro-electrode in the last track of an experiment was cut off and left in its position which was identified histologically in frozen sections. By the aid of the three space co-ordinates of the electrode tip and the track's direction the locations of all the neurones of that experiment were recon-

structed. The nerve length between the stimulating electrodes and the recording electrodes on the nerves and on the spinal cord were measured at the end of the experiment for the purpose of calculating peripheral conduction velocities.

RESULTS

1. *Response of spinal neurones upon stimulation of A and C fibres*

When recording from spinal neurones sometimes a late discharge appeared, when the intensity of the stimulus was raised above threshold for the C fibres. As shown below (Figs. 3 and 4) this late discharge is positively correlated with the C volley in the nerve, the latency being accounted for by the slow conduction in the afferent C fibres (about 1 m/sec).

The lower trace of Fig. 3*A* is the response of a dorsal horn neurone upon an afferent volley in the A and C fibres of the SU nerve. The upper trace shows the record from that nerve, displayed appropriately to monitor the action potential of the C fibres starting at about 50 msec. The compound action potential of the A fibres at about 1 msec after the beginning of the sweep is off the screen at that gain, its amplitude being about 10 times larger than that of the C potential. The first part of the neurone's discharge in Fig. 3*A* beginning a few msec after the stimulus and lasting for about 100 msec was present also when the stimulus strength was below threshold of the C fibres. This early discharge obviously was due to the volley in the A fibres (Group II and III) and will be called the A discharge, or A response, of the neurone. The late discharge, starting at 200 msec, was present, however, only with stimuli above threshold of the C fibres, in the experiment of Fig. 3 the C volley had to have an appreciable size before the late discharge (the C response of the neurone) appeared (Fig. 3*B*). When blocking the sural A fibres by transient polarization of the nerve proximal to the stimulus (see Fig. 1) the A discharge of the unit disappeared, but the C discharge grew markedly larger (Fig. 3*C*). Fig. 3*E* plots quantitatively the decrease of the A response of the same unit (●) and the increase of the C response (○) with increasing strength of the blocking current, thus reducing the number of A fibres contributing to the A volley. All A fibres had ceased to conduct in this experiment when the blocking current exceeded 80 μA. In Fig. 3*F* the relationship is shown between the size of the large diphasic component of the C volley (abscissa) and the C discharge (ordinate) when the A fibres were either blocked completely (○) or where not blocked (●). From this plot it can be seen that at the threshold of the C response of the neurone the C volley had already attained a considerable size when the myelinated fibres were not blocked (●, see also Fig. 3*B*), whereas under the conditions of a pure afferent C

volley the threshold of the C response was slightly above that of the nerve (○, see also Fig. 3*D* and the vertical dashed lines in Fig. 4).

The curves in Fig. 3*F* exhibit steep increases at volley sizes of 400 μV (○) and 600 μV (●) respectively. The abscissa value comprises, however, only the large early component of the C volley, the later components recruited at high stimulus strength where neglected. In Fig. 4*A* we have therefore plotted the C discharge of the neurone alternatively in relation to the stimulus strength applied to the SU nerve, either without (●) or

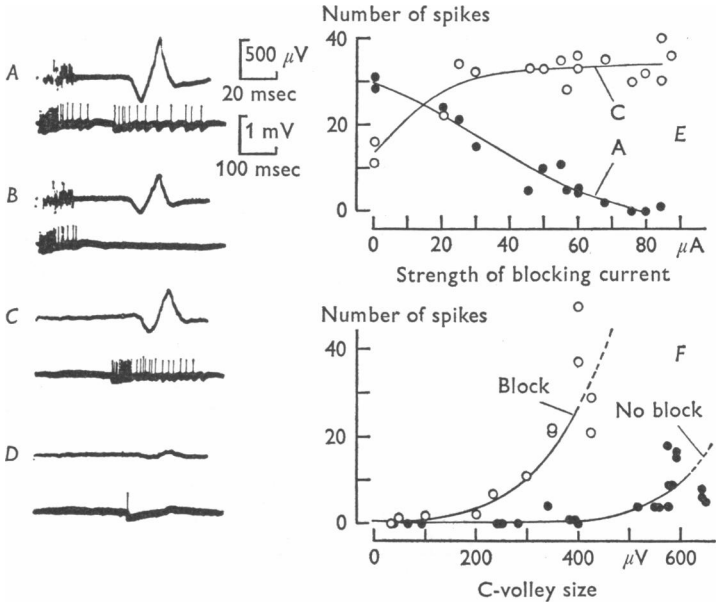

Fig. 3. Effect of differential blocking of the A fibres upon the activity of a dorsal horn neurone.

A–D: each pair of records shows the spike discharge of the cell (lower trace) and the simultaneously recorded compound action potential from the SU nerve (upper trace) following stimulation of this nerve. Note different time scales in the upper and lower traces. The stimulus was a square pulse of 15 V, 0·5 msec in duration in *A* and *C*, 4 V in *B* and 3·5 V in *D*. In *C* and *D* all A fibres were blocked by a polarizing current of 80 μA applied to the nerve between stimulating and recording sites (see Fig. 1). The A volley was off the screen in *A* and *B*.

E: the ordinate plots the spike discharges due to the *A* and *C* volleys (●, ○ respectively) at different strength (abscissa) of the blocking current. Stimulus as in *A*.

F: the C discharge (ordinate) beginning at 200 msec after the stimulus is plotted in relation to the size of the C volley, either with (○) or without (●) simultaneous block of the A fibres.

All results from the same neurone, located in a depth of 1600 μ from the cord dorsum.

with (○) concomitant blocking of the A fibres. It is clearly seen that the C discharge of the neurone increased even in a stimulus range in which the large diphasic component of the volley remained constant (Fig. 4*B*). It was supposed therefore that recruitment of a considerable proportion of C fibres occurred even at a stimulus strength beyond 7 V. Such recruitment could be observed directly when recording from fine nerve strands containing only a few single C fibres, as is demonstrated by the fine dashed curve (▲, right-hand ordinate) in Fig. 4*B*. This line indicates the cumulative distribution of the electrical thresholds of a sample of 127 single C fibres

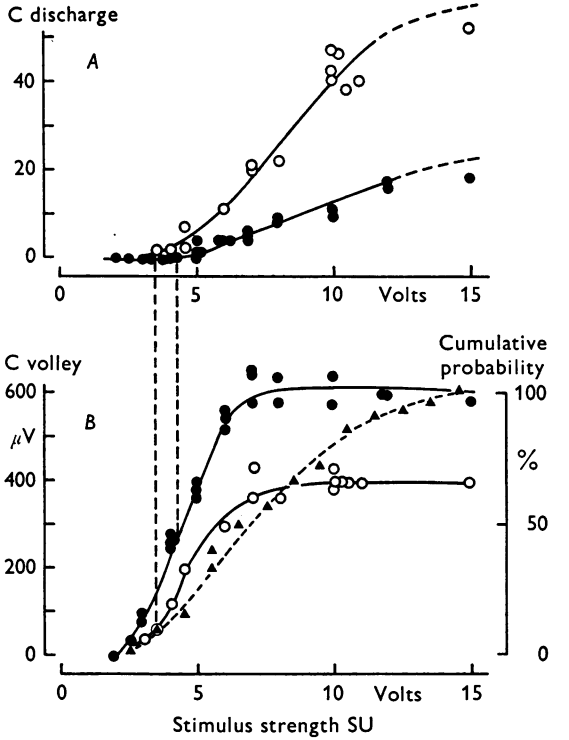

Fig. 4. Effect of varying the stimulus strength on the nerve volley and on the discharge of a neurone.

A: the ordinate plots the number of spikes in the C discharge in relation to the strength of the stimulus (abscissa) applied to the SU nerve, either with (○) or without (●) conduction block in the A fibres performed by a polarizing current of 90 μA.

B: Amplitude of the C volley (ordinate) in the SU nerve in relation to the stimulus strength (abscissa), when a polarizing current was either applied to the nerve (○) or not (●). The thin dashed line (▲) and the right-hand ordinate scale indicate the recruitment of C fibres as taken from the threshold distribution of 127 single cutaneous C fibres (M. Zimmermann, unpublished). The vertical dashed lines connecting the graphs in *A* and *B* indicate the volley sizes at the discharge thresholds. Same unit as in Fig. 3.

from the sural and plantar nerves, i.e. the recruitment of fibres at increasing stimulus strength. The recruitment curve approximately parallels the increase in the C discharge of the neurone (Fig. 4*A*, ○). Thus it appears that the amplitude of the C volley is not an adequate measure of the C fibres recruited in a nerve.

Most neurones of this paper in which a response upon stimulation of C fibres occurred yielded similar characteristics: if no block of the A fibres was applied a C response appeared when the volley (i.e. the large diphasic component) had a size ranging from 20 to 50% of the maximum value, whereas under blocking conditions the C discharge appeared at a volley size of about 10% of maximum.

2. *The proportion of units having C input*

In our experiments we recorded from a total of 245 neurones. Twelve of these had an axon in the dorsolateral funiculus, they were omitted from this investigation since the chances are that these axons run in the spinocervical tract. The C input to this special population of dorsal horn cells will be examined in a separate investigation (M. Gregor & M. Zimmermann, in preparation). Of the remaining 233 cells, 180 were located in the dorsal horn, including the first to fifth laminae of the cytoarchitectonic subdivision (Fig. 5*A*; Rexed, 1952). Most statements of the following results concern these dorsal horn cells.

The proportion of neurones in the dorsal horn which received inputs via afferent C fibres was calculated from those experiments in which systematic tracking and testing for C input was performed (see Methods). These include 150 cells which were found with search stimuli in the SC + PBST + SMAB nerves. No unit was found in the dorsal horn which responded to PBST or SMAB, but not to SC stimuli; thus, all 150 neurones were contacted by myelinated afferents from the SC nerve. Fifty-seven units of the sample responded also to the C volleys in the SP and/or SU nerves, i.e. 38% of dorsal horn neurones receive both A and C input. This percentage is, however, not representative, since A and C afferents are compared covering peripheral areas of quite different sizes, i.e. those of the SC and the SP + SU nerves respectively. When considering the A and C input of the same nerves, the following numbers arise: from 111 neurones activated by A volleys in the SP and/or SU 57 responded also to a C volley, i.e. 51%. This value of 51% is considered to be a representative value of the proportion of dorsal horn cells, having afferent connexion to both myelinated and unmyelinated fibres. In all these units an A response could be elicited at least from the same nerve which produced the C response. There was no instance of a neurone responding to the C fibres in one nerve but only to the A fibres in another nerve (cf., however, the context of Fig. 9).

The fifty-seven C-responsive cells of our dorsal horn samples were reached by the C input either via the SU nerve alone (fourteen units), via the SP alone (twenty-three) or via both nerves (twenty). Thus thirty-four units responded to a C volley in the SU nerve. Two of them were recognized to be C responsive only under the condition of a selective C volley, i.e. during blocking of the A fibres. The blocking method was not applied to the SP nerve, therefore it might be that C input via this nerve escaped detection in some cases. However, from the corresponding number in the SU nerve mentioned above (two of thirty-four) we do not think this to be likely.

A subtotal of thirteen units out of the sample responding to the A fibres in the SP and/or SU nerves (111 units) could be activated also via myelinated afferents in the muscle nerves PBST and/or SMAB. The threshold of these stimuli were in the range between 1·3 and 13*T* (mean value 6*T*), thus excluding Ia and Ib activation and rather indicating afferent pathways termed as 'flexor reflex afferents' (Eccles & Lundberg, 1959; Holmqvist, Lundberg & Oscarsson, 1960). In this subsample the proportion of units having cutaneous C input was 54 %, which is practically identical to the value determined to be representative for the total sample.

From the neurones located in the ventral horn (not included in the following analysis) seven were identified by antidromic spike invasion to be motoneurones. In two of these a C volley in the SU nerve produced a discharge at a latency of about 200 msec. This observation is in accordance with the occurrence of ventral root reflexes upon C volleys in cutaneous nerves (Koll *et al.* 1961; Franz & Iggo, 1968; W. Jänig & M. Zimmermann, unpublished observations). One of the two C responsive motoneurones was a PBST motoneurone, thus the C fibres evoked a flexor reflex. The other motoneurones were not identified as belonging to a flexor or to an extensor muscle.

3. *The location of the neurones within the spinal cord*

In most experiments micro-electrode tracks were performed systematically as described in methods. In Fig. 5*C* an example of such a histological reconstruction of one experiment is given. Each point or circle represents the exact location of a neurone, the points indicating the neurones which could be excited by C fibres of either the SU or SP nerves. The density of neurones in Fig. 5*C* is not uniform, because the micro-electrode had to be exchanged several times during this series; the ease of location and retention of neurones differs with different electrodes, even when these have apparently identical physical characteristics (e.g. electrical resistance). This non-uniformity disappeared when superimposing the results of several experiments (Fig. 5*B*).

The results of eleven experiments in which a topographic reconstruction

of the units was performed are superimposed on to an average cross-section, which is shown in Fig. 5*B*. In all experiments tracking was performed at least up to a depth of 2·3 mm from the dorsal surface as is indicated by the horizontal line in Fig. 5*B*. The region above this line includes the laminae I to V in the cytoarchitectonic subdivision of the gray matter (Rexed, 1952, 1954), as is seen by comparing Fig. 5*B* with Fig. 5*A*. No exceptional positions of the units which were excited by C fibres can be seen from Fig. 5*B*.

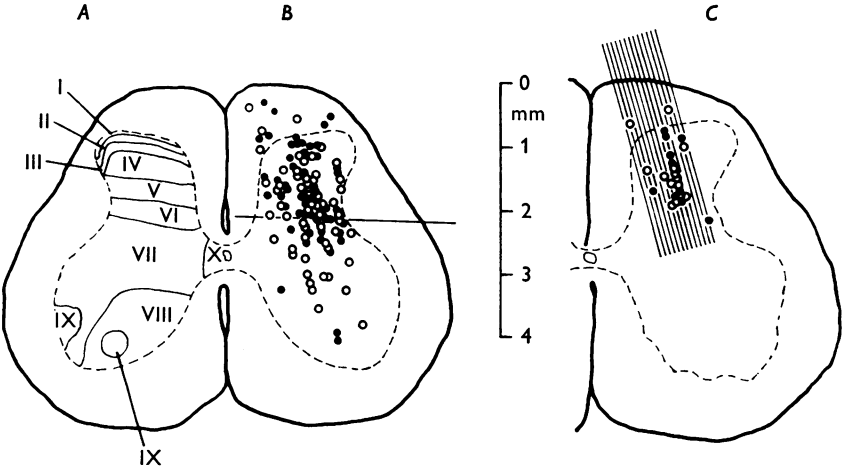

Fig. 5. The location of spinal neurones responding either to C and/or A volleys in cutaneous nerves.

A: location of the various cytoarchitectonic layers in the spinal cord (L 7 segmental level) according to Rexed (1952, 1954).

B: location of the neurones found in eleven experiments superimposed on to a cord section of average dimensions. The reconstruction of the sites was accomplished using the spatial co-ordinates of the micro-electrode tip. The units responded either to A volleys (○) or to A + C volleys (●) in the SU and/or SP nerves. The horizontal line indicates the depth up to which systematic tracking was performed with the micro-electrodes.

C: same as in *B*, the results are however from a single experiment. The oblique lines indicate the micro-electrode tracks performed in this experiment. Micro-electrodes were exchanged several times during the series.

In the following paragraphs both groups of cells with and without input via afferent C fibres are examined for possible discriminanda contained in their response parameters.

4. *Synaptic linkage to the myelinated afferents*

Measurements of central latencies were used to yield a classification of the neurones whether being mono- or polysynaptically connected with the periphery. In Fig. 6*A* the abscissa of each point or circle represents the

synaptic delay of an individual cell (method M1), the ordinate its depth from the dorsal surface of the cord. As in all other figures the points and circles designate the neurones which respond to an afferent C volley and which do not respond, respectively. Delays of up to 1·2 msec signal a monosynaptic connexion to the afferent fibres, whereas delays above 1·2 msec point to a polysynaptic afferent pathway. The value of 1·2 msec provides a reliable separation of mono- and polysynaptic units (Hunt & Kuno, 1959; Wall, 1960; Armett, Gray & Palmer, 1961).

The units labelled by an arrow in Fig. 6*A* were activated in addition by stimulation of the PBST and/or SMAB nerves. The nerve thresholds of these muscle responses were in the range of 1·5–13*T*, the afferents involved therefore belonging to Groups II and III fibres. The synaptic delay of the muscle input (estimated by method M2) ranged from 2 to 6 msec; we concluded therefore these pathways to be polysynaptic. Afferent input of this type (muscle Group II and III and cutaneous Group II) has been reported to evoke a flexor reflex, and the afferents involved have been generally designated therefore as 'flexor reflex afferents' (Eccles & Lundberg, 1959; Holmqvist *et al.* 1960; Eccles, Kostyuk & Schmidt, 1962*b*). No neurones were found within the dorsal horn which had monosynaptic input from these muscle afferents, therefore we conclude that these fibres relay on to cells in deeper regions.

No correlation of the synaptic delay is visible in Fig. 6*A* with respect to the location of the unit; at each lamina in the gray matter there are neurones exhibiting either mono- or polysynaptic activation following dorsal root stimulation. This is in contrast to findings reported previously (Wall, 1967). It can be seen, however, that in the monosynaptic group the majority of the units received a C input, whereas in the polysynaptic group the inverse was true. The proportions of neurones either having C input or not are given quantitatively in Fig. 6*B* by the height of the columns with stippled or with white areas respectively. The dotted continuations of the columns indicate the results when units are added to either group in which the central delay following activation via the SC nerve was estimated by the more indirect procedure M2. Herewith only neurones were included having delays of either below 0·7 msec or above 1·7 msec, thus accounting for the margin of uncertainty inherent in the M2 method/($\pm$ 0·5 msec; see Methods). The total sample in Fig. 6*B* comprises 72 neurones, of which thirty-seven were mono- and thirty-five polysynaptically linked to cutaneous A fibres. Thus we have found both types of cells at equal probabilities. However, A and A + C neurones were found to be distributed in different proportions within the mono- and polysynaptic samples. This finding will be considered in more detail in the following paragraph.

5. *Spontaneous activity and discharges upon stimulation of the nerves and the skin*

On the basis of the criteria, synaptic delay and C responsiveness, we subdivided the dorsal horn neurones into four classes (Fig. 7*A*): monosynaptic cells with C response (MC), polysynaptic cells with C response (PC) and, correspondingly, mono- and polysynaptic cells without C input (MA, PA). The percentages of either class of the total sample (which is identical to that of Fig. 6*B*) are as follows: MA 13%, PA 33%, MC 39%, PC 15%. It is especially obvious from Fig. 7*A* that the PA units are more numerous than the MA units to approximately the same extent as the PCs are outnumbered by the MCs.

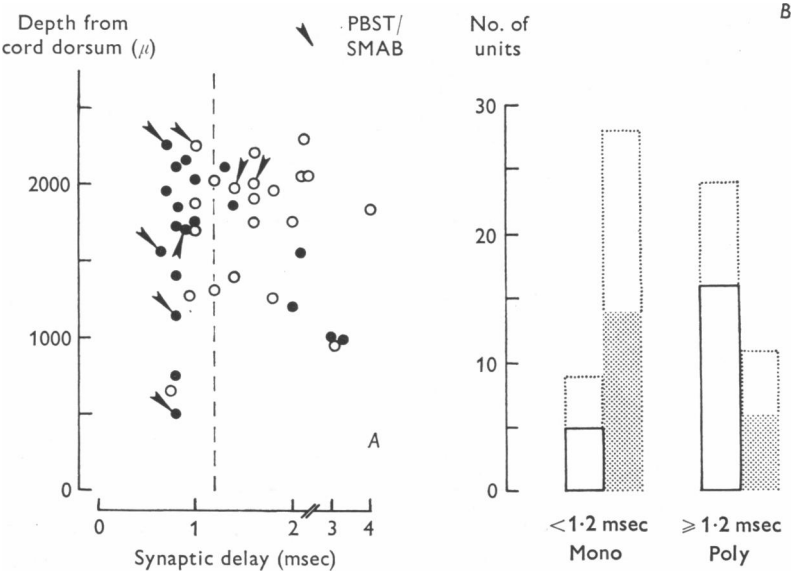

Fig. 6. The synaptic delay of dorsal horn neurones.

A: each point plots the synaptic delay (abscissa) and the depth from the cord surface (ordinate) of a single neurone, either responding to A+C volleys (●) or to A volleys (○) in the SU and/or SP nerves. The synaptic delay was determined by dorsal root stimulation (M1, see Methods). The arrows indicate units having additional input from muscle nerves (PBST and/or SMAB, Groups II, III). The dashed line separates neurones having monosynaptic (left) and polysynaptic (right) linkage to dorsal root fibres. Results from five experiments.

B: the height of the columns (vertical scale) indicates the number of mono- and polysynaptic neurones, the white (continuous line) and stippled columns belonging to units responding to A volleys and to A+C volleys respectively; same units as in *A*. The dotted extensions include the appropriate units, the synaptic delay of which was determined indirectly by subtraction of the peripheral conduction time from the total latency upon a stimulus given to the sciatic (SC) nerve (method M2).

An outstanding observation was that C responsiveness was highly correlated with spontaneous activity of the cells: about 75 % of C neurones (MC + PC) had a spontaneous discharge in the absence of intentional stimulation, whereas in the MA + PA group the corresponding proportion was 30 %. This is demonstrated in Fig. 7*A*: the white parts of the columns represent the units which were spontaneously active, the black areas those which were not. It is seen from Fig. 7*A* that such silent units constitute the bulk of cells in the PA subsample. When considering the discharge elicited in the neurones by a 4*T* stimulus delivered to the SU or SP

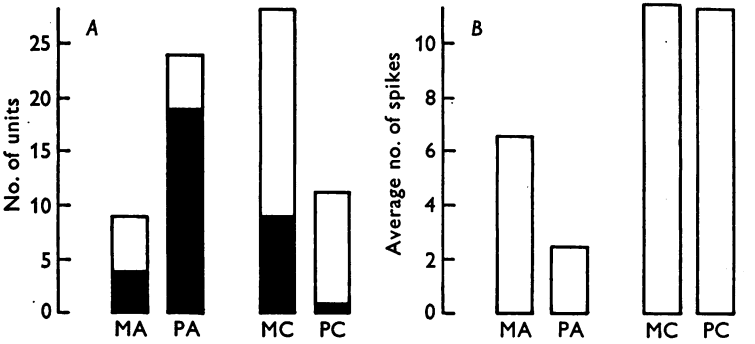

Fig. 7. Spontaneous activity and discharges upon A volleys of the neurones.
A: the lengths of columns indicate the numbers of neurones in either subsample: mono- (M) and polysynaptic (P) units, activated either by A input only (A) or by A and C input (C). The white or black sections of the columns comprise the units which had or had not a spontaneous discharge, respectively.
B: the column height shows the average number of spikes discharged upon a 4*T* stimulus to the SU or SP nerves in either subsample, as in *A*.

nerves additional discriminanda appear. In Fig. 7*B* the average numbers of spikes in this A discharge are figured for four subsamples, i.e. MA, PA, MC, PC. No differences can be seen in the average A discharges of the MC and PC neurones. By contrast, the number of impulses in the PA units is less than half of that of the MA cells. Thus, the lack of a spontaneous discharge and a rather minor response to an A volley are combined characteristics of most PA units.

Part of the experiments were aimed to evaluate the possibility that C input to the neurones is correlated with their response characteristics following natural skin stimulation. In a total of sixty-four cells five types could be discerned on the basis of mechanical stimulation, as is depicted in Fig. 8. Twenty-six of the units responded to movement of hairs only; in most cases an oral air puff was sufficient as a stimulus (first column in Fig. 8). When applying steady pressure of moderate intensity to the receptive field these units discharged at a high rate at the onset of the

stimulus; they became, however, silent when movement of the stimulator had ceased. Nine other units responded steadily to such pressure stimuli only (third column), whereas in seven units a steady response upon skin pressure could be recorded and, in addition, they were excited by moving a few hairs (second column). Twelve units could be excited only by stimuli of high intensity (fourth column). In the fifty-four neurones responding to either mechanical stimulus the proportion of units fired by C input was about 70 %; no significant deviations from this average value were present in the four subgroups. However, in the ten units which did not respond to any mechanical skin stimulation (right-hand column in Fig. 8) no C response occurred; they were all PA units lacking a spontaneous discharge.

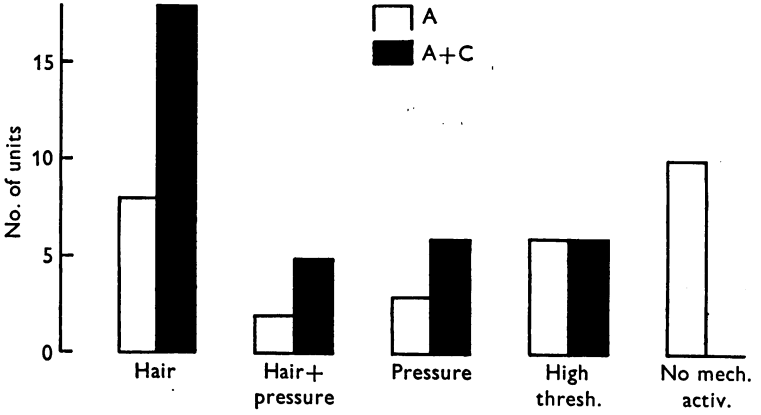

Fig. 8. Excitation upon adequate skin stimulation. The neurones are grouped according to their excitability by various natural skin stimuli, as is indicated above. In each group units are subdivided whether responding or not to C volleys (black or white columns respectively).

Summing up, no correlations of the C input of a neurone with the mode of response upon natural skin stimulation could be detected. However, it was a prominent finding that the majority of PA units (1) did not discharge spontaneously, (2) had a low excitability by electrical stimulation of cutaneous nerves, and (3) were not activated by mechanical skin stimulation. These properties will be considered in the Discussion.

6. *Neurones responding only to a volley in C fibres*

In three experiments the stimulus given to the SU and SP nerves during the micro-electrode tracking was supramaximal for the C fibres. This was done in order to find some cells responding to afferent input via C fibres only, thus escaping detection with the normally used search stimulus (8*T*). During these experiments thirty-five units were found responding to

both the A and C afferents of the SU and/or SP nerves. These cells therefore could have been detected also by the 8*T* search stimulus usually given to the SC nerve. However one unit was found in these experiments which responded exclusively to C input, as is illustrated in Fig. 9*A*. When the stimulus was above threshold of the C fibres in the SU nerve, a discharge of five spikes was evoked in the unit at a latency of more than 200 msec (second and third records in Fig. 9*A*; the uppermost record shows the volley in the SU nerve). Between the two recordings in A the stimulus strength was lowered below the threshold of the C fibres, as is seen in the nerve record in *B*. The discharge of the cell was absent in this case (Fig. 9*B*, lower record). The unit was lost before further measurements could be done. Its location in the cross-section of the L7 segment is shown in Fig. 9*C*. Probably units of this type are rather small cells or/and occur only rarely in the cord.

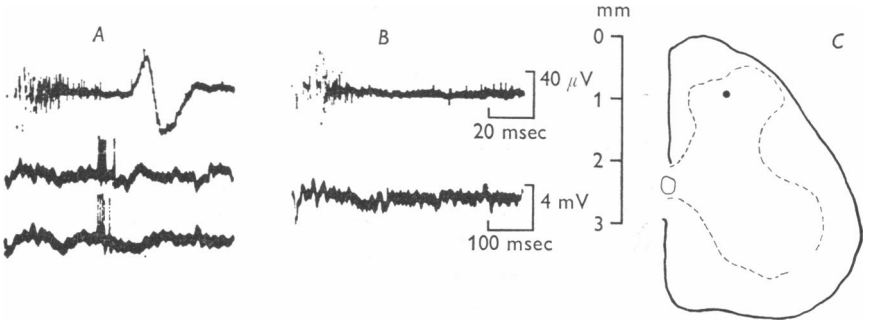

Fig. 9. Discharge characteristics and location of a neurone responding only to C volleys.

A: in the middle and lower traces the responses of a neurone are displayed upon a stimulus which was supramaximal for the A and C fibres in the SU nerve; the nerve record is shown in the upper trace. Note different voltage and time calibrations, as indicated at the corresponding records in *B*.

B: record from the SU nerve (upper trace) and from the same neurone as in *A* (lower trace) at a stimulus strength which was subthreshold for the C fibres. This record was taken between the neurone responses shown in *A*.

C: the dot shows the location of the neurone, reconstructed from the spatial co-ordinates of the micro-electrode tip upon a frozen section of the cord (L7 segment).

DISCUSSION

The classes of neurones found in our study can be arranged to several pathways, as are depicted in Fig. 10*A* and *B*, respectively. What is schematically drawn in this diagram as a 'neurone' means rather a population of cells than a single cell; the cascaded link between mono- and polysynaptic units includes some convergence and divergence within

either pathway. In Fig. 10*A* the pathway is drawn which exhibits convergence of A and C fibres. Those units which receive the A afferents monosynaptically (MC) represent the largest proportion of the total sample (39 %). Convergence occurred always of the A and C fibres from the same nerve or nerves. Two possibilities exist for the mode of access of the

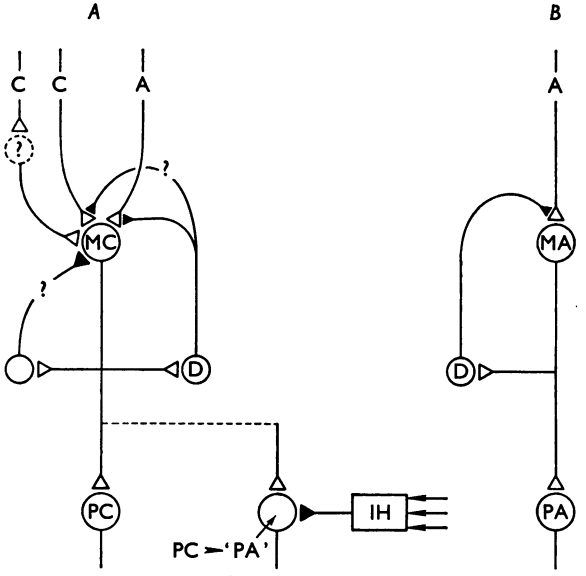

Fig. 10. Schematic diagram of the dorsal horn pathways in relation to the convergence of A and C fibres.

A: summarizes the findings of MC and PC cells which receive both A and C input. Two possible inhibitory feed-back pathways are drawn (indicated by black synaptic terminals). The input from the C fibres occurs either by monosynaptic contact or by specialized interneurones. Polysynaptic units (PC) are drawn in cascade.

In order to account for the large proportion of polysynaptic neurones which do not respond to C input a large part of the higher order neurones in the A+C pathway are assumed to be under the influence of a powerful steady-state inhibitory system (IH).

B: summarizes the assumed pathway of neurones having afferent connexion only with A fibres. For details see text.

C fibres to the MC neurones, which are both incorporated into Fig. 10*A*: (1) the C fibres too have monosynaptic endings; (2) specialized neurones are intercalated between the C afferents and the MC neurones. One interneurone fitting the demand of a separate C input was found in our study (Fig. 9); one cell having similar firing properties had been observed previously in the cat (Hunt & Kuno, 1959) and two in the monkey dorsal horn (Wagman & Price, 1969). Statements on a possible role of these cells

in the convergent pathway of A and C fibres will be speculative until a systematic investigation provides data on their frequency of occurrence and on their location.

Because the C discharge of the MC cells is suppressed by a preceding A discharge (see Figs. 3 and 4), an inhibitory feed-back must be postulated (inhibitory neurones indicated by black synaptic terminals in Fig. 10). This inhibition might act either post-synaptically upon the MC cells or presynaptically upon the C fibre terminals. Thus far a decision between both alternatives is not possible. In extreme cases the inhibition might be so strong as to suppress the C response totally as revealed by the neurones in which C input could be found only after blocking the A fibres (see Results). A detailed knowledge of the neuronal circuitry might be important for the functional interpretation of this inhibitory influence of A volleys on to the C input.

There is evidence that presynaptic depolarization occurs in a large fraction of myelinated dorsal root fibres following stimulation of cutaneous C fibres (Franz & Iggo, 1968; Zimmermann, 1968*a*; Jänig & Zimmermann, 1971). It was shown that this so-called C-PAD (primary afferent depolarization generated by C fibres) exhibits convergence with that PAD evoked by A fibre stimuli (A-PAD). Our results indicate the convergence to occur already on to second-order neurones, the MC cells.

It had been concluded previously that the interneurones which generate the PAD, the so-called D-cells, must have the following characteristics: repetitive discharge upon a single afferent volley and at least disynaptic afferent connexions (Eccles, Kostyuk & Schmidt, 1962*a*). Neurones which fit these requirements in the pathway of the C-PAD, i.e. D-cells having both A and C input, are contained in the PC sample.

The neurones which do respond to an A volley but not to a C volley are summarized in Fig. 10*B*. The post-synaptic inhibitory feed-back has been omitted here, though its presence should be suggested. The presynaptic inhibition, however, is well established in all cutaneous Group II fibre pathways (Jänig, Schmidt & Zimmermann, 1968).

One difficulty arises when the numbers of mono- and polysynaptic neurones are considered quantitatively. Although in the total sample mono- and polysynaptic units appear at equal proportions, they are unsymmetrically partitioned in both the pathways (see Figs. 6*B* and 7*A*). There are no reasons to expect different relationships of divergence and convergence in the A and A + C pathway. Thus an alternative explanation has to be sought to account for the preponderance of the PA units and the scarcity of the PC units. In Fig. 10*A* we propose a possible interpretation for this phenomenon: a part of the polysynaptic units in the A + C pathway are affected by a strong steady-state inhibition, indicated by 'IH'

in the Figure. The assumption of such an influence should concern about two thirds of these polysynaptic cells, which accordingly are turned into PA units and thus result in the disproportional number of PA cells in our experiments. The proposed tonic inhibitory action upon a large proportion of polysynaptic neurones is fully compatible with the prominent characteristics of most PA units described in the results. Such a tonic inhibitory influence could provide a mechanism for the control of sensory inflow, which should be especially powerful when operated by a descending system. Further experiments are needed to decide whether such descending influences exist on to those PA cells which presumably are inhibited PC cells. A possible candidate for a descending modulatory function is the pyramidal tract, stimulation of which exerts inhibitory effects on to superficially located dorsal horn neurones and excitatory (disinhibitory?) effects on to neurones located in the deeper layers of the dorsal horn (Fetz, 1968).

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