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Spinal Processing: Anatomy and Physiology of Spinal Nociceptive Mechanisms

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Spinal processing: anatomy and physiology of spinal nociceptive mechanisms

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The processing of nociceptive input that occurs at the spinal level represents the first stage of effective control over its access to higher regions of the central nervous system. Recent developments in both the anatomy and physiology of nociceptive processing pathways at this level are beginning to yield an integrated understanding of structure and function. Most small afferent axons terminate in the more superficial laminae of dorsal horn, but technical difficulties have, until recently, prevented analysis of the functional properties of identified small fibres.

A direct input of nociceptive afferents on to particular dorsal horn neurons is difficult to establish in view of the slow impulse conduction in these fibres and the small size of target neurons in the substantia gelatinosa.

The small cells themselves are being analysed for relations between structure and function, using physiological, intracellular staining and immunocytochemical techniques to characterize their properties. They appear to be a highly heterogeneous population with many sub-classes, whether typed according to the transmitter they contain, e.g. enkephalin, to their physiological responses: whether excitatory or inhibitory to nociceptive and other inputs, or to both.

The multireceptive neurons that project out of the dorsal horn toward supraspinal regions are, in general, located in deeper laminae and are likely to receive nociceptive information through polysynaptic pathways. The nocireceptive neurons in lamina I, which receive exclusively nociceptive inputs from myelinated and non-myelinated afferents project, at least in part, to thalamic and brain stem regions. Polysynaptic nociceptive pathways in dorsal horn may be subject to different controls from neurons in laminae I and II. Tonic descending inhibition is operative on the former and it is becoming clearly established that descending systems such as those containing noradrenaline, can regulate the access of nociceptive information to higher levels. The mechanisms of such descending controls and the importance of their interaction with segmental control systems, such as those involving the dynorphin opioids, are just beginning to be understood.

Many somatosensory neurons in dorsal horn, both the large cells, some of which project supraspinally, and the small cells of superficial laminae, receive convergent nociceptive and non-nociceptive inputs. Although solely nociresponsive neurons are clearly likely to fill a role in the processing and signalling of pain in the conscious central nervous system, the way in which such useful specificity could be conveyed by multireceptive neurons is difficult to appreciate. The question of specificity of nociceptive pathways at higher levels and that of the role, if any, of multireceptive cells in pain signalling will be important future targets for research.

1. Introduction

Peripheral sensory receptors encode various parameters of stimuli delivered to the tissues and 'specific' sensory receptors are present in all mammals. The nociceptors are distinguished by their relatively high thresholds to stimuli; this indeed is the basis of their classification. Interest in the mechanisms of nociception at the spinal level can therefore properly be directed at the question of the way in which activity entering in afferent fibres from nociceptors is processed. A major advance has been made in the last decade in unravelling the distribution of dorsal root afferent fibres once they enter the spinal cord, following the introduction of intracellular labelling of individual identified afferent units with horseradish peroxidase (HRP) by Brown and colleagues in Edinburgh and by Jankowska and colleagues in Gothenberg. The precision achieved by these methods allows very positive statements to be made about the local organization of the incoming afferent fibres in relation to the functional properties of the receptor (see Brown 1981). Most of the published evidence deals with mechanoreceptors that send information along the larger myelinated afferent fibres. The axons from different kinds of mechanoreceptor form distinctive arborizations of their collaterals in the dorsal horn, as well as sending a major branch rostrally in the dorsal columns. It has been established unequivocally that most synaptic contacts between the mechanoreceptors and second order neurons are not in the superficial dorsal horn: they are in Rexed's lamina III and deeper. In contrast the smaller dorsal root afferent units, among which are to be found the nociceptors, are less well studied because of technical difficulties. Several firm statements can, however, be made on the basis of older knowledge reinforced by more recent studies (figure 1). First, the axons enter and terminate in the spinal cord close to the segment of entry: they do not send long collaterals up the dorsal columns (Szentagothai 1964). Secondly, the zones of termination are principally in the superficial dorsal horn (laminae I and II) (Réthelyi 1977; Light & Perl 1979*a*, *b*; Gobel *et al.* 1981; Réthelyi *et al.* 1982).

Since the small myelinated (i.e. Aδ) and non-myelinated (i.e. C) dorsal root afferent fibres comprise a mixed population with respect to peripheral specificity (Cervero & Iggo 1980; Brown 1981) it is not sufficient, in the context of nociception, simply to trace the terminations of the small fibres. The physiological characteristics of the afferent units must also be established. Very few such attempts have been made, notably by Light & Perl (1979), Réthelyi *et al.* (1982) and Perl (1984). The collaterals of mechanical nociceptors with myelinated (Aδ) axons had two zones of termination, in lamina I and around the I/II_o border and more deeply in lamina V. In contrast, the type D hair follicle afferents, which are sensitive mechanoreceptors (Brown & Iggo 1967) terminated more deeply in the dorsal horn in laminae III and IV, with no terminals in laminae I or II_o (Réthelyi *et al.* 1982). The non-myelinated afferent units have been more resistant to single unit analysis. Perl (1984) reports that in a small sample of such units in primate and guinea-pig there were endings in the substantia gelatinosa (lamina II) and also in deeper laminae. In view of the well-established existence of a large number of nociceptors with C-afferent fibres it is clearly necessary that this problem of the C fibres and their termination be pursued vigorously.

A further development of the labelled identified afferent unit studies has enabled the synaptic organization to be examined, and here again the versatility of HRP is evident. Electron microscopy using HRP-filled afferent units is providing new insights. The e.m. pictures provide direct evidence of synaptic contacts made by the nociceptor afferents with dendrites in lamina I and in addition show what are judged to be axo-axonal synaptic junctions

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

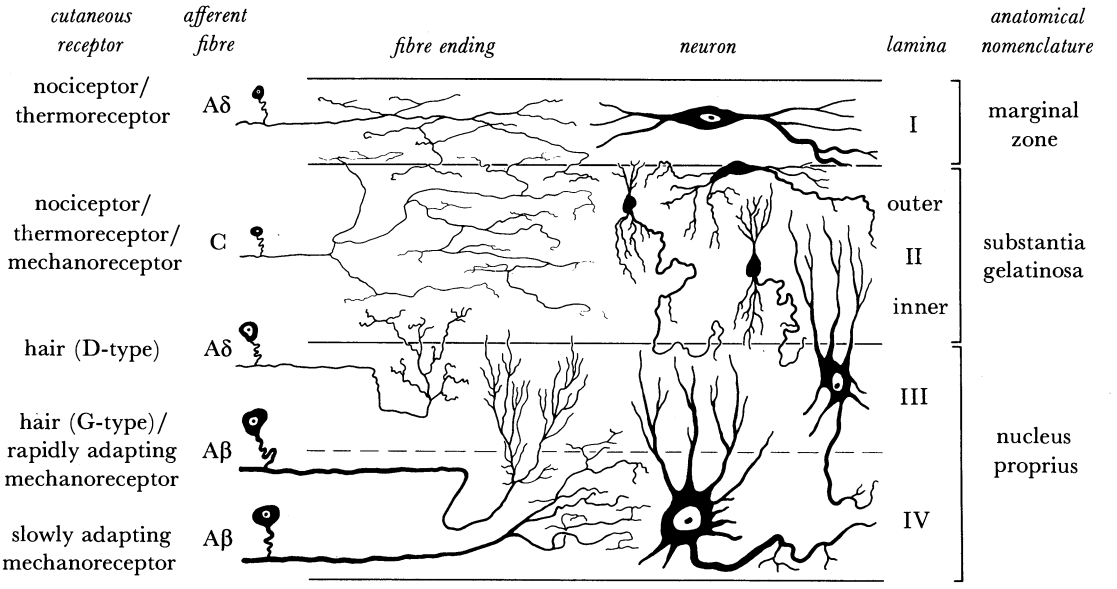

FIGURE 1. Schematic diagram of the neuronal organization of, and afferent input to, the superficial dorsal horn. The diagram represents a transverse section of the dorsal horn and illustrates the afferent fibre endings and neuronal elements present in the first four laminae of the dorsal horn. To the left of the diagram the types of afferent fibre and relevant receptor groups associated to them are listed. Fibre endings in the dorsal horn are schematized diagrams taken from published morphological studies. Neurons in the diagram represent standard types of neuron in the superficial dorsal horn. The following types have been illustrated: (from top to bottom) a marginal cell, an s.g. limiting cell, two s.g. central cells and two neurons of the nucleus proprius, the more superficial of which has dendrites penetrating lamina II. Indicated at the right of the diagram are the laminar division of the superficial dorsal horn and corresponding anatomical nomenclature. (From Cervero & Iggo (1980).)

on the nociceptor afferent terminals themselves (Gobel *et al.* 1980). Since, as will be discussed shortly, the majority of dendrites in lamina I are those of neurons with cell bodies in lamina I, the conclusion can be reached that the dendrites are indeed likely to include those of nociceptor-driven neurons in lamina I. The further advance of labelling both presynaptic and postsynaptic neurons, currently being developed by Brown and his colleagues (Brown 1981) for larger tract neurons (d.c.p.s., s.c.t.) in laminae III and IV, has not yet been achieved for the superficial laminae. It is, therefore, necessary to fall back on less precise techniques using implanted HRP, taken up and transported by an undifferentiated population of neurons. These methods show clearly that the afferent fibres form complex arborizations in lamina I. Two kinds have been distinguished (Gobel *et al.* 1980) related to Aδ and to C afferent fibres. The latter are considered to give rise to the very fine (0.3 μm diameter) axonal arborizations in lamina I, and therefore their connections become relevant to the mechanisms of nociception, even though their actual function is not yet established. Both the smaller myelinated (Aδ) and the non-myelinated (C) terminals form glomeruli (Kerr 1966) in which the large afferent terminal is almost completely surrounded by and in synaptic contact with other neural processes including dendritic spines. Gobel *et al.* (1981) interpret their e.m. results as indicating that several synaptic interactions can involve the afferent terminal of the glomerulus. It can synapse on dendritic spines or shafts, and can receive axo-axonic and dendro-axonic synapses. Until the various components are identified it becomes difficult to do more than speculate on the functional implications, but results such as these establish the possibility of considerable modification of the excitatory competence of the afferent fibre.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2. Control of receptor sensitivity

Several mammalian sensory systems, like those in non-vertebrates, can have the excitability of the receptor modified by mechanisms that operate in the periphery. The eye, ear and muscle spindle all possess these attributes. In contrast, the cutaneous sensory system lacks any effective peripheral control, although receptors can be affected by peripherally acting chemicals. Instead, the first stage of effective control operates in the dorsal horn (and dorsal column nuclei for sensitive mechanoreceptors). The new structural information (briefly reviewed above) shows that such modification could occur at the primary synapses likely to be those of nociceptor units, and physiological evidence for such an action on C fibre terminals has come from Hentall & Fields (1979) who measured excitability changes, indicative of primary afferent depolarization.

3. Neuronal end-stations of nociceptors

The next logical step is to consider the neurons of the dorsal horn that are affected by an input from the nociceptors. Here also the use of intracellular labelling, particularly with HRP, has allowed individual cells to be evaluated functionally and subsequently analysed morphologically. Concurrent labelling of both the afferent fibre and its target neuron will yield unequivocal evidence of monosynaptic connection but this has so far been successful only for larger axons and neurons, such as group II cutaneous afferent fibres and d.c.p.s. cells (Bannatyne *et al.* 1984) or Ia muscle afferent units and motoneurons (Brown & Fyffe 1980). The difficulties of verification of a monosynaptic linkage, when the afferent input is in slowly-conducting fibres, makes the prospect of such visualization enticing.

Several laboratories have published accounts of the functional characteristics of neurons in the dorsal horn, from lamina I to V or deeper. It is interesting to recall briefly the stages by which the gradual elucidation of neuronal morpho-functional characteristics has been achieved. Initially, only extracellular evidence from electrophysiological recording was available on interneurons (see, for example, Kolmodin & Skoglund (1960)) or intracellular records from larger neurons activated by cutaneous myelinated afferent fibres (see, for example, Eccles & Lundberg (1959)). These early studies were of neurons in lamina III or deeper. The report by Christensen & Perl (1970) on extracellular records of neurons in lamina I sparked off a great deal of interest, and showed that these smaller neurons were accessible to unit recording and that some of them could be excited only by nociceptors. The complexity of the neuronal organization in the dorsal horn made mandatory more accurate knowledge of the location of the neurons recorded. This was first provided by the use of extracellular recording with micropipettes containing a dye, such as pontamine sky blue (Hellon 1971), which replaced the less reliable depth gauge measurements and gave some assurance that the recording electrodes were where they were thought to be. Even so, uncertainty continued as to the actual neural element that generated the electrical activity that was recorded, i.e. soma, dendrite or axon.

Continued improvements in electrode and related techniques next made the smallest neurons, those in the substantia gelatinosa, a reasonable target, but even four years ago there was still considerable uncertainty, or scepticism, about claims that substantia gelatinosa neurons could actually be recorded from (see Cervero & Iggo 1980). The resolution of these

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

uncertainties has come from the use of intracellular labelling with HRP or lucifer yellow (Light *et al.* 1979; Bennett *et al.* 1980; Molony *et al.* 1981; Woolf & Fitzgerald 1983). This is indeed a technical 'tour de force', when it is realized that the diameters of the soma are generally less than 10 μm.

The scale of the problem of neuronal identification can be gauged from estimates of cell density in the superficial dorsal horn. Cervero & Iggo (1980) concluded that in the substantia gelatinosa there were 75000–90000 neurons per millimetre of spinal cord in the cat, on one side. An electrophysiological experiment is often considered a success if half-a-dozen such neurons are satisfactorily recorded from, and one or two marked intracellularly. It is clear, therefore, that the available sample of substantia gelatinosa neurons is a trivial fraction of the total population. Even more striking, is the fact that the 'large' ascending tracts that originate from the spinal dorsal horn, such as the s.c.t. and d.c.p.s., are *in toto* a numerically insignificant proportion of the total population of dorsal horn neurons. Very firm estimates by Brown (Brown *et al.* 1980) indicate that the s.c.t. from the lumbo-sacral cord of the cat comprises 500–800 neurons on each side and Bennett *et al.* (1983) make a similar claim for the d.c.p.s. system. Clearly, the majority of dorsal horn neurons are segmental or propriospinal, a fact long familiar to morphologists (Kerr 1966).

4. Functional characteristics of nociceptor responsive dorsal horn neurons

Electrophysiological methods provide a direct way of establishing the functional characteristics of neurons excited by noxious stimuli. One reason why such an approach is needed is that other approaches, such as investigations of motor reflexes, behaviour or sensation, are all dealing with the consequences of action and interaction in polysynaptic pathways. Necessary though this latter kind of evidence is, it does not pinpoint the exact steps in the processing of information.

The outcome of these kinds of neurophysiological experiments has been to yield, in rats, cats, rabbits and monkeys, a generally agreed picture. The dorsal horn neurons can be classed using several parameters, in a manner agreed by the IUPS Somatosensory and Pain Commission (Brown & Réthelyi 1981), on the basis of (*a*) their responses to naturally applied stimuli, (*b*) their location in the dorsal horn and (*c*) the destination of their axons. There are two main kinds of nociceptor-responsive neurons, those that are excited only by noxious stimuli and those excited by both noxious and innocuous stimuli. Each of these categories is an omnibus, containing a variety of subtypes. An example of a subtype of the former is the population of nociceptive neurons in lamina I that project to the nucleus submedius of the thalamus (Craig & Burton 1981), and of the latter are the multireceptive neurons of the s.c.t. that are excited by hair follicle afferents and by thermal nociceptors (Cervero *et al.* 1977) or the substantia gelatinosa neurons, that are propriospinal and are excited by myelinated mechanoreceptors and inhibited by nociceptors (Cervero *et al.* 1979*a*). Thus the broad primary classification has quickly to be expanded to generate a more detailed inventory. Nevertheless, just as the apparent confusion of peripheral sensory receptors has yielded to systematic order, so too, it can be predicted, will the dorsal horn neurons.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

5. Multireceptive dorsal horn neurons

In addition to their termination in the superficial dorsal horn described above, axons from cutaneous nociceptors have been found to have a second principal region of termination deeper in the dorsal horn, in lamina V (Réthelyi *et al.* 1982). Electrophysiological recording in these deeper laminae has found a very small number of nociceptor-specific neurons; by far the greater proportion of neurons in the deeper laminae of the dorsal horn with a cutaneous noxious input are excited by cutaneous mechanoreceptors as well, i.e. they are multireceptive. Such cells have in fact been found throughout the dorsal horn, and indeed appear to form the majority of the 'electrophysiological' population. Many of the largest dorsal horn neurons situated in laminae III–V have these characteristics, including those that send their axons into ascending tracts: spinothalamic, dorsal column postsynaptic, spinocervical, spinoreticular (see Willis, this symposium). Diversity is also present in their morphological characteristics, including particularly the distribution of their dendrites. In addition to these, many small neurons in lamina II and some lamina I neurons are multireceptive.

Although many of the deeper neurons can be monosynaptically excited by the myelinated cutaneous fibres (their dendrites and axonal arborizations are co-terminous in lamina III and below), their direct excitation by nociceptive afferents is more uncertain. This is especially true for the C-nociceptors, most of which apparently terminate in the superficial laminae (see Brown (1982) for review and Perl (1984)). It is probable, therefore, that many of the multireceptive neurons are in polysynaptic relation to nociceptors, a factor of importance in consideration of the inhibitory control of nociception. Some of the large neurons, such as the d.c.p.s., do however send dendrites into the superficial dorsal horn and could therefore receive a monosynaptic nociceptive input.

A typical response of a multireceptive lamina IV neuron is illustrated in figure 2, with several characteristic features, such as (1) a brief burst at high frequency when activated by large myelinated axons, the brevity possibly being accounted for by polysynaptic inhibition (Hongo *et al.* 1968); (2) long latency, sustained discharge on C fibre activation, with a pronounced after discharge, especially in response to noxious thermal stimulation.

Within the broad category of multireceptive neurons that enter ascending tracts, sub-categories can be made. Examples are the s.c.t. neurons that are excited by hair follicle afferents and nociceptors (Brown & Franz 1969) which can be further subdivided according to their responses to noxious thermal stimuli (Cervero *et al.* 1977) and the d.c.p.s. neurons, which differ from the s.c.t. in so far as they can be excited by other cutaneous mechanoreceptors in addition to hair follicles, as well as by thermal nociceptors (Angaut-Petit 1975).

This diversity is further compounded by the fact that the tract axons arise from only a small part of the total population of multireceptive neurons in the dorsal horn, particularly if the substantia gelatinosa is included. The fact that so many neurons received convergent excitatory input should also be considered in the light of the inhibitory convergence within the same pool of neurons, from the same receptors. In addition, the output of a single neuron must be determined by the geometry of both monosynaptic and polysynaptic connections, which must be very complex in these neurons with convergent inputs. Furthermore, the functional roles of these neurons may be influenced by the observer, a kind of Heisenberg effect in which the interest of the investigator introduces a bias as a result of the way in which the system

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

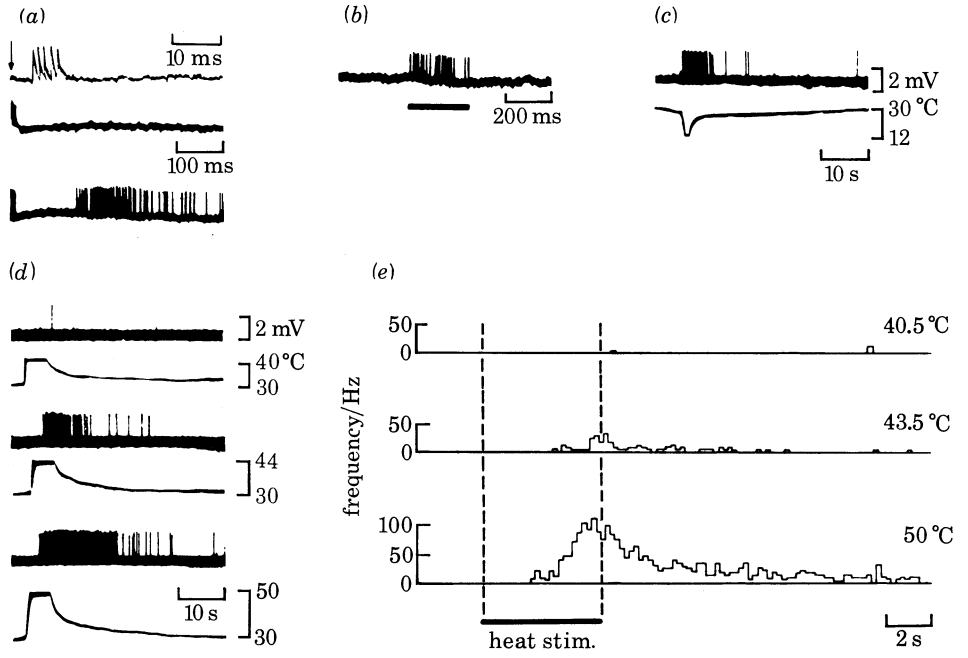

FIGURE 2. Response of a class 2 neuron to various kinds of stimuli. (*a*) Upper two traces, electrical stimulation at twice threshold of both plantar nerves; lowermost trace, electrical stimulation at 250 times threshold of the plantar nerves, which was above the threshold of C-fibres. (*b*) Hair movement (indicated by the bar below) on the plantar surface of the foot. (*c*) Skin cooling by ethylchloride evaporation; the skin surface temperature recorded by a thermocouple is shown below. (*d*) Heating the skin of the foot-pad by radiation to 40, 44 and 50 °C, respectively; the skin surface temperatures measured with a thermocouple are shown below each record. (*e*) Time course of discharge frequencies plotted as histograms, during and after heating the foot-pad to various temperatures. Duration of heat stimulus indicated by the bar below. Spinalized animal. Unit was 1750 μm from cord dorsum and had polysynaptic connection to group II afferents. (From Handwerker *et al.* (1975).)

is being investigated. In the present circumstances it should always be considered that our interest in nociception and pain tends to under-emphasize other possible functions such as a general alerting mechanism, or reflex regulation of muscle activity.

6. NOCICEPTIVE NEURONS

The earlier uncertainty about the existence of 'nociceptor-specific' neurons and a 'nocispecific' pathway has been resolved by the vigorous experimental analysis of the responses to noxious stimuli that followed the report of Christensen & Perl (1970).

Nociceptor-specific neurons in lamina I of the dorsal horn have been reported by several laboratories. Some of them are the large Waldeyer neurons; morpho-functional studies of these neurons (Light *et al.* 1979; Bennett *et al.* 1980; Molony *et al.* 1981; Lima & Coimbra 1983; Réthelyi *et al.* 1983; Woolf & Fitzgerald 1983; Perl 1984) established that they are relatively large, with dendrites distributed rostro-caudally principally as a flattened sheet over the surface of the dorsal horn. Some of them are excited exclusively by mechanical nociceptors with Aδ axons, corresponding to class 3a of Cervero *et al.* (1976). A characteristic feature of these neurons is that they are normally silent in the absence of an applied stimulus and are brought into persistent activity by an appropriate and maintained noxious stimulus. Other

Waldeyer marginal cells are excited by both mechanical nociceptors and thermal nociceptors (class 3b of Cervero *et al.* 1976), and by Aδ and C afferent fibres. These neurons are more likely to carry a 'background' discharge that can be enhanced by repetitive electrical stimulation of C fibres. Characteristic Waldeyer neurons with these patterns of input have been filled with HRP (Light *et al.* 1979; Molony *et al.* 1981). However, not all Waldeyer cells are nociceptor-specific: Réthelyi *et al.* (1983) described HRP-stained Waldeyer neurons, some of which were selectively nociceptive, while others were excited by both light tactile and by noxious stimuli. These latter may include at least some of the neurons reported by several groups to be excited by converging inputs from nociceptors and mechanoreceptors (Handwerker *et al.* 1975; Cervero *et al.* 1976; Woolf & Fitzgerald 1983).

The dendrites of Waldeyer neurons bear synapses formed by axons and since many are excited only by noxious stimuli it can be concluded that there are probably monosynaptic connections from the nociceptors. Some of these neurons are known to project beyond the lumbosacral enlargement of the spinal cord (Handwerker *et al.* 1975; Cervero *et al.* 1976) and may reach the thalamus (see Willis, this symposium; Carstens & Trevino 1978; Craig & Burton 1981) and thus provide a direct path for the nociceptive input to reach the brain. The axons are relatively slow, conducting at less than 15 m s^{-1}, but myelinated.

However, not only are all Waldeyer cells not nociceptive, but also they constitute only a small proportion, probably less than 5%, of the cell population of lamina I, the remainder being small cells of uncertain physiological characteristics which merge with the outer layer (II_o) of the substantia gelatinosa (Lima & Coimbra 1983). It would therefore appear probable that some of the extracellular recordings from nociceptive neurons located in lamina I originated in these small neurons, and different laboratories have described small HRP-stained nociceptive neurons situated close to the lamina I/II border (Light *et al.* 1979; Bennett *et al.* 1980; Molony *et al.* 1981; Réthelyi *et al.* 1983). A variety of morphological characteristics have been reported. For example, limiting cells of Cajal (Ramon y Cajal 1909) with fan-shaped ventrally oriented dendrites (the stalked cells of Gobel 1975; 1978) have been illustrated in papers by Perl (1984) for an Aδ nociceptor-specific neuron at the lamina I/II border, by Molony *et al.* (1981) for a neuron with the same location and orientation that carried background activity at 1–6/s and was inhibited by a variety of natural stimuli and excited by electrical stimulation of the tibial nerve; by Woolf & Fitzgerald (1983) for a neuron about 75 μm below the dorsal white matter, with 3/s background discharge, that was excited by noxious stimuli, and by Gobel *et al.* (1980) for a cell at the I/II_o border responding to noxious inputs in Aδ and C fibres. It thus becomes possible to put together a group of neurons from different laboratories and obtained under different, but no doubt equally arduous, conditions that share common morphological and functional properties, but about which differences persist. This heterogeneity, both of morphology and function appears to be even more marked for the 'islet' cells of Gobel ('central' cells of Cajal), a view also expressed by Woolf & Fitzgerald (1983) and by Perl (1984). Thus , some 'islet' neurons in inner lamina II are excited only by light tactile stimuli, and on extracellular recording are discharged only by an input in the C-mechanoreceptors, whereas others with similar morphology are multireceptive.

We have already referred to e.m. studies of HRP labelled small superficial dorsal horn neurons (Gobel *et al.* 1980) which showed that they are in postsynaptic relation to the large terminal swellings of nociceptive afferent fibres in the glomeruli described earlier. The

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

conclusion to be drawn is that there is monosynaptic excitatory drive from nociceptors to these neurons. Electrophysiological testing for monosynaptic connections from primary afferent fibres can be done with some confidence for the myelinated axons, where the conduction times are short and not likely to exceed the synaptic delay, so that reliance can be placed on latency measurements; monosynaptic connections to lamina III cells from mechanoreceptive afferents are convincingly demonstrated by these means. However, the electrophysiological search for monosynaptic connections from C fibres, although such must indubitably exist, is more difficult. In our own laboratories, intracellular recording from small nociceptive neurons around the lamina I-II border showed several interesting features. First, the rate of background firing (generally under 10/s) of the neurons was loosely correlated with the recorded membrane potential, and tended to be lower at high membrane potentials. Secondly, there was usually continuous ongoing synaptic activity, both e.p.s.ps and i.p.s.ps, even when the cells were not discharging impulses (figure 3). Thirdly, when the neurons

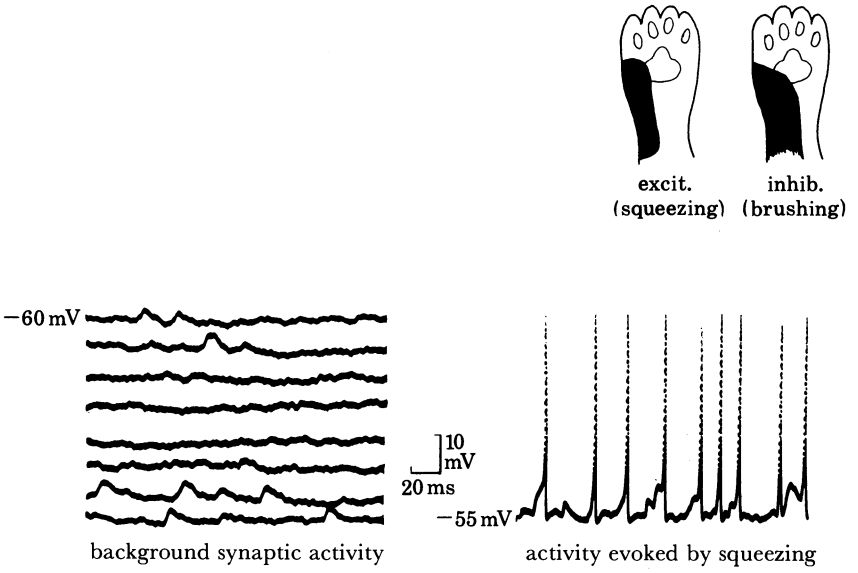

FIGURE 3. Intracellular d.c. recordings from a nociceptive s.g. neuron in a chloralose-anaesthetized cat. The recorded resting membrane potential was −60 mV, and the neuron exhibited considerable background postsynaptic potential activity without any output of action potentials. Firm squeezing of the excitatory receptive field resulted in depolarization of the membrane to −55 mV and firing of action potentials, each arising from an e.p.s.p. (Brushing of the inhibitory receptive field resulted in hyperpolarization of the membrane). (From Steedman *et al* (1983).)

discharged an impulse, the action potential spike usually took off from a clear e.p.s.p. (Steedman *et al.* 1983). Fourthly, intracellular recording from substantia gelatinosa neurons with axons in Lissauer's tract (Cervero *et al.* 1979*b*) yielded monosynaptic e.p.s.ps from small myelinated axons, presumably afferent. Fifthly, electrical stimulation of peripheral nerves and/or dorsal roots above C-threshold caused some substantia gelatinosa neurons, recorded intracellularly, to discharge impulses at a latency of about 250 ms (figure 4). Two features of this discharge are of interest: (1) the latencies were remarkably constant, given their duration, and varied by not more than ±2 ms, i.e., less than 1% and (2) a maximum of only four impulses was discharged in response to supra-maximal stimulation of C fibres in the peripheral nerve (Iggo *et al.* 1984). The very constant latency, taken together with the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

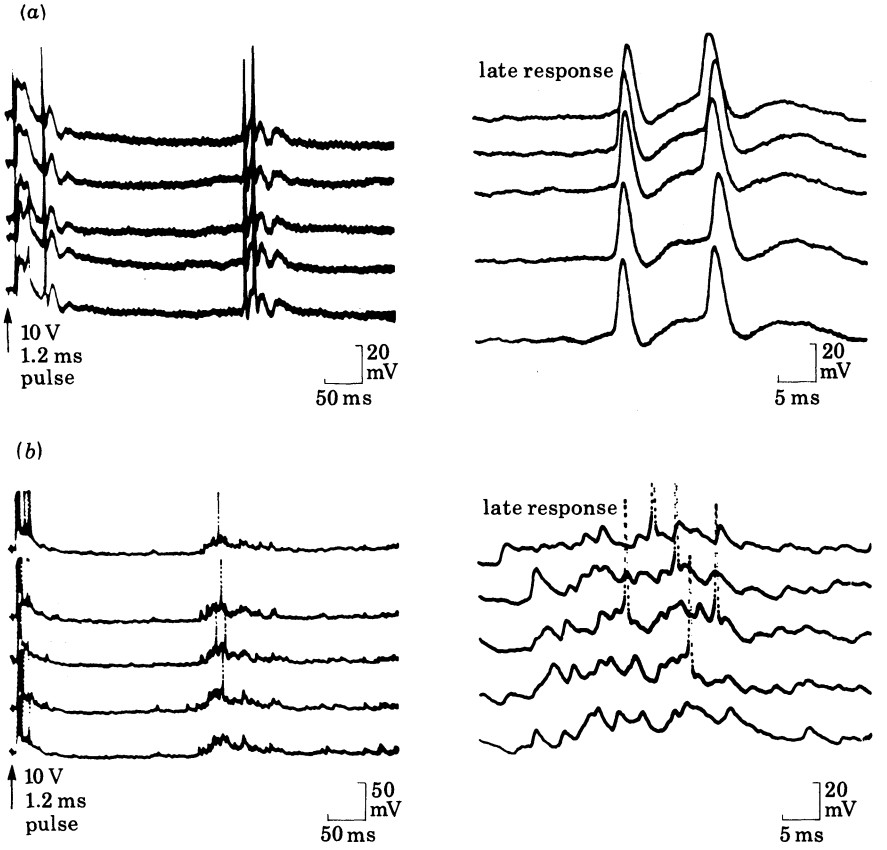

FIGURE 4. Intracellular recording of the activity evoked in a nociceptive s.g. neuron (*a*) by repeated single-shock electrical stimulation of the peroneal nerve, demonstrating the constant latency and consistency of the responses to C-fibre stimulation. This is contrasted with the variable latency and inconsistency of the C-fibre evoked responses of a multireceptive neuron in lamina IV (*b*). The right panels show the late C-fibre evoked responses expanded in time.

antecedent depolarizations from which the action potentials arose, is good, though perhaps hardly sufficient, evidence for monosynaptic connection. A similar conclusion was reached, on the basis of extracellular recording, by Fitzgerald & Wall (1980).

There is thus evidence that at least some small superficial dorsal horn neurons receive direct and possibly exclusive input from cutaneous nociceptors, an example of which is shown in figure 5; the question of the destination of their axons will be dealt with by a later speaker, but there is strong evidence that a large proportion are interneurons projecting only short distances, only a few being the site of origin of long ascending axons. HRP staining has shown axons terminating within the superficial dorsal horn, descending into deeper laminae (Bennett *et al.* 1980; Molony *et al.* 1981) and occasionally projecting into Lissauer's tract. There would thus appear to be a multiplicity of morphological types of nociceptive neurons in the superficial dorsal horn, both with respect to dendritic tree geometry and axonal projection.

The brevity of the nociceptive responses of substantia gelatinosa neurons to a single volley in afferent C fibres (figure 4*a*) is, however, a distinctive feature given the very vigorous and sustained discharge so easily aroused in larger, multireceptive neurons in laminae III and IV (figure 2*a*). Several explanations can be offered including: (1) the synaptic potency of substantia gelatinosa cell excitation by nociceptive afferents may be low and/or the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

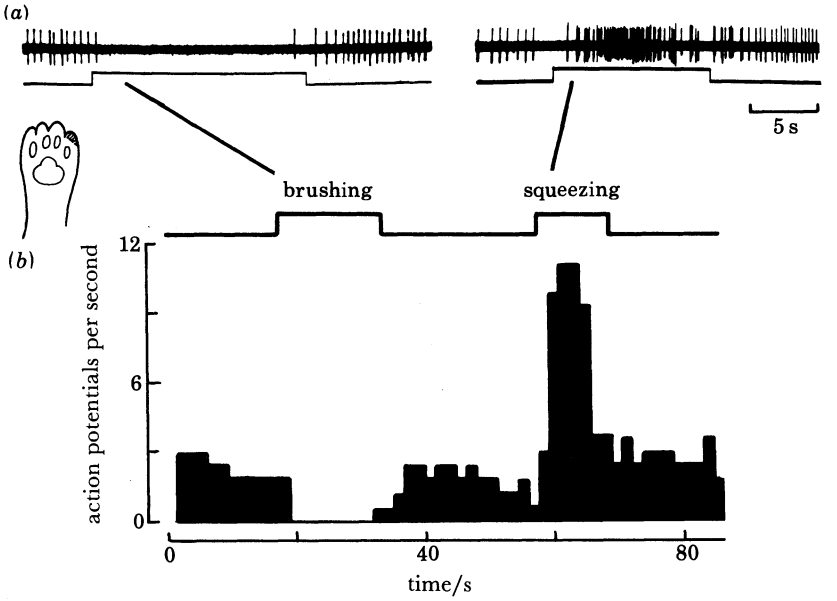

FIGURE 5. Responses of an s.g. neuron to natural stimulation of its cutaneous receptive field. (*a*) Extracellular recording; (*b*) density histogram of action potentials collected in 1.6 s bins. (From Steedman & Molony (1983).)

persistence of transmitter action at the afferent terminals may be brief; (ii) the substantia gelatinosa cells may not be capable of sustained discharge at high frequency. However, some substantia gelatinosa neurons show sustained responses to other, presumably monosynaptic inputs (Cervero *et al.* 1979*a*; Woolf & Fitzgerald 1983). Clearly these differences need to be considered in the context of activation of multiple pathways within the dorsal horn. Further possible explanations, therefore, for the observed differences between superficial and deeper neurons are that the sustained, higher frequency discharges require repetitive input in the afferent fibres (as would normally occur during natural stimulation of the nociceptors), or self-sustaining recurrent excitatory loops operating via multisynaptic pathways or converging polysynaptic excitatory inputs rather than direct monosynaptic excitation. The glomeruli in which at least some of the dorsal root afferents end certainly provide ample opportunity for synaptic interactions that might affect the potency of individual inputs.

7. INHIBITORY ACTIONS

All neuronal activity expresses a balance of excitation and inhibition and the dorsal horn neurons are no exception. Several laboratories, including my own, have given full weight to this interaction, and have been as concerned to explore the inhibitory responses as the excitatory. Inhibition at the level of the dorsal horn has several origins. It can come from supraspinal sources, as will be more fully documented by Fields and Duggan (this symposium), or be elicited at the segmental level by afferent input (figure 5). Segmental inhibition evoked by myelinated axons of cutaneous origin and particularly effective against the excitatory actions of small myelinated and unmyelinated fibres has been described for other dorsal horn neurons (see Brown (1981) for review). The supraspinal sources may be many, and express themselves in the dorsal horn by several processes. 5-HT is present in raphé-spinal neurons

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

and probably acts at several levels of c.n.s., including the dorsal horn, where it has been visualized by immunocytochemical and radioautographic techniques by Dubner and colleagues (Nishikawa *et al.* 1983). Noradrenaline is another transmitter of supraspinal origin likely to be involved in the processing of nociceptive input in the dorsal horn. Recent work by S. M. Fleetwood-Walker and her colleagues has shown convincingly that noradrenaline has a powerful selective antagonistic action on noxious excitatory inputs to large multireceptive transmission neurons (such as the s.c.t.) that project to higher levels. Furthermore, this effect is mediated at α_2 adrenergic receptors remote from the cell bodies of the s.c.t. neurons in laminae III–V. A possible site of action is in the substantia gelatinosa where, as has already been discussed, the nociceptive afferent fibres are known to terminate. Exclusively nociceptive neurons can also be inhibited by an indirect action of noradrenaline. Clearly, therefore, interneurons are important in mediating descending influences as well as those of the segmental afferent inputs. The descending monoaminergic systems may not normally be very active (see for example, Soja & Sinclair 1983), but appear to be highly activated by noxious levels of cutaneous stimulation (Tyce & Yaksh 1981).

Other kinds of descending influences are tonically active since large multireceptive dorsal horn neurons are known to be under potent descending inhibition, in so far as their vigorous responses to noxious stimulation in reversibly spinal animals may be completely suppressed when supraspinal influences are free to operate, whereas the mechanoreceptive inputs may be equally effective in the two states (Handwerker *et al.* 1975). Once again an explanation for this selective inhibition must be sought at indirect, possibly more superficial loci, in particular in the substantia gelatinosa since there is now good morphological evidence that functionally identified substantia gelatinosa neurons send their axons into lamina III and below (Bennett *et al.* 1980; Molony *et al.* 1981). For these reasons the responses of nociceptive and of multireceptive neurons in lamina I and the substantia gelatinosa during reversible spinal block are of direct relevance. An effective way of examining descending influences is to use reversible block of the lower thoracic cord while recording from neurons in the lumbar dorsal horn. Nociceptive neurons in lamina I, both projecting and non-projecting, were found to be relatively or completely unaffected by reversible block of the spinal cord (Cervero *et al.* 1976) and thus in conditions known to produce complete selective suppression of the powerful noxious activation of large multireceptive cells that is so conspicuous in the spinal state. This evidence would appear to rule out the segmental involvement of such nociceptive neurons in the tonic descending inhibition. Alternative candidates are neurons in the substantia gelatinosa, for which however there is less information. In a brief report Cervero *et al.* (1979*c*) presented results for six substantia gelatinosa neurons, in four of which no changes in background activity or cutaneous receptive fields were found, and in the remaining two only small or transient changes were evident when the spinal block was applied or removed. This is, of course, a very small sample on which to base a conclusion that tonic supraspinal inhibition does not operate through the substantia gelatinosa, but at least it does not provide any positive evidence. Substantia gelatinosa neurons can, however, be potently inhibited by electrical stimulation with electrodes on Lissauer's tract several segments rostral to the site of recording in the lumbar cord (Cervero *et al.* 1979*d*). The available evidence is insufficient to identify the actual fibres involved. Dubuisson & Wall (1980) in contrast to Cervero *et al.* (1979*d*) found no sign of inhibition of neurons in the superficial dorsal horn when they stimulated the d.l.f., and that units with an ongoing discharge were facilitated, especially those

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

responding to intense peripheral stimulation. If these latter neurons are in monosynaptic connection with the d.l.f. and involved in inhibiting tract neurons they must themselves be inhibitory or act through a polysynaptic chain.

In general, knowledge of the detailed neuronal organization of the normal dorsal horn is far from complete although under intensive investigation. Attention has already been drawn to the difficulty of establishing in electrophysiological experiments whether slowly conducting afferent fibres make monosynaptic connections with a neuron. Indeed, in most of the published accounts the only information available concerns the conditions leading to firing of the neuron, defining only whether the effective input was carried in myelinated or non-myelinated axons and what kind of natural stimulus was used with no indication as to the pathways involved. A particular difficulty arises from the generally powerful inhibition of nociceptor-driven (as well as other) neurons by the fast conducting group II afferent fibres so that, when electrical stimulation of a peripheral nerve or dorsal root provides the afferent input, the more slowly conducting fibres have to express themselves on a background of inhibition. The nociceptive lamina I neurons certainly do not require such a background, excitatory or otherwise, in order to be activated since if all the myelinated fibres in a peripheral nerve are blocked, noxious thermal stimulation is still effective, through the C-nociceptors, in exciting such neurons (Iggo 1974).

These difficulties are, to a degree, reduced by new information coming from immunocytochemical methods combined with HRP labelling, using both light and electron microscopy. Enkephalin-containing neurons are abundant in the superficial dorsal horn, and include Waldeyer neurons of lamina I and 'stalked' and 'islet' cells of lamina II_o (Bennett *et al.* 1982; Glazer & Basbaum 1983). E.m. examination of serial sections has thrown light on the synaptic arrangements, although it is not able to specify the functional identity of the neural elements. In laminae I and II, the enkephalin (ENK) is contained in dendrites which in lamina I appear to be, in part, postsynaptic to afferent terminals that could be from nociceptors. There are also contacts between ENK neurons and ENK profiles, presumably of other ENK dorsal horn neurons.

ENK-containing axons were small in lamina II but both large and small in lamina I and the majority of such axons were presynaptic to unlabelled dendrites (Hunt *et al.* 1980; Aronin *et al.* 1981; Glazer & Basbaum 1984). At least some of these form synapses on projection neurons although such a connection has so far only been established for large multipolar neurons in lamina V (Ruda 1982). The Waldeyer neurons in lamina I include some that are nociceptor specific, but since Glazer & Basbaum (1983) report that the majority of ENK axons made contact with unlabelled dendrites, ENK-containing Waldeyer neurons which project supraspinally may not be in direct synaptic contact with other ENK-containing Waldeyer neurons. Small local neurons containing 'dynorphin' opioids are also present in the superficial dorsal horn (Botticelli *et al.* 1981). Other physiologically active peptides could also be involved.

The intrathecal injection of drugs has proved to be a powerful method for assessing the involvement of opioids such as the enkephalins, their antagonists and other neurotransmitters in nociception (Yaksh 1984). The results establish very clearly that the opioids of μ, δ (and perhaps now also κ (Han & Xie 1984)) types are involved in nociception at the spinal level and thus provide affirmative evidence that the opioid-containing neurons are also implicated. A more direct assessment comes from the use of ionophoretic techniques, employing selective

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

μ, δ and κ agonists. These more detailed studies have become necessary, following the intrathecal experiments that demonstrated the differing effects of the various agonists. Duggan *et al.* (1977) brought forward evidence that morphine was more effective in causing selective reduction of nociceptor-induced discharge of lamina IV neurons if it was ejected in the substantia gelatinosa, rather that at the level of the soma in lamina IV. In contrast, the opioid peptide, [Met]enkephalinamide, was selective from the substantia gelatinosa to within 100 μm of the soma (Duggan *et al.* 1981). The opioids used in these experiments, however, suffer the disadvantage that they are not particularly selective for opioid receptor subtypes. Using selective agonists and multireceptive neurons with long ascending projections, Fleetwood-Walker *et al.* (1984) have now shown a powerful and selective antinociceptive action of the endogenous κ-selective-agonist, dynorphin(1-13), rather than the μ- or δ agonists ([D-Ala^2, MePhe^4, Glyol^5]enkephalin and [D-Ala^2, D-leu^5]enkephalin). This effect of dynorphin is unlikely to be a direct action on the s.c.t. neurons examined, and furthermore, it is reversed by a specific α_2 adrenergic antagonist (figure 6), revealing a hitherto unknown interaction of

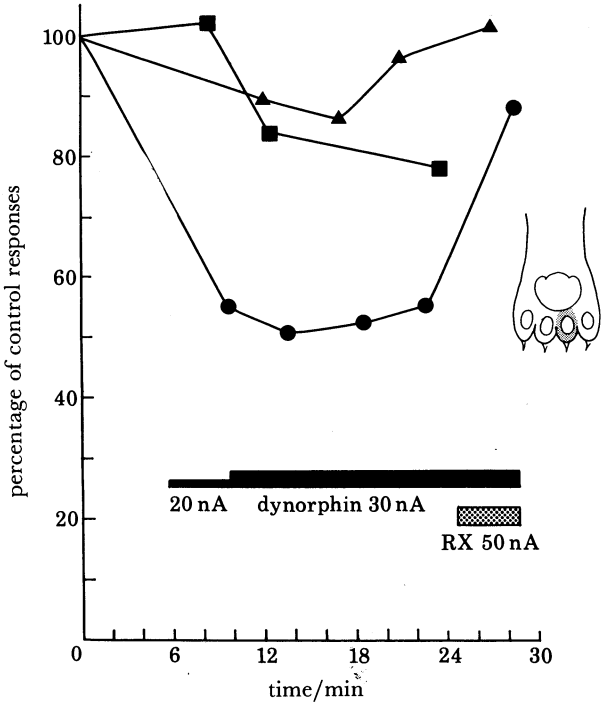

FIGURE 6. Antagonism of the selective effect of dynorphin (1–13) by an α_2 antagonist (RX 781094) in the spinal cord of the cat. This shows an example of an identified (s.c.t.) neuron, where dynorphin (1–13) applied iontophoretically close to the cell body, has produced a selective reduction in the response to noxious heat (circles), but not the responses to innocuous brush (triangles), or iontophoretically applied (±)-homocysteic acid (squares). (The receptive field of this neuron is shown in the inset.) The effect is rapidly reversed by iontophoretic application of the α_2 antagonist, RX781094, suggesting a novel mechanism for the mediation of segmental antinociceptive effects of κ opioids through the activation of descending catecholamine tracts.

local segmental opioid systems with descending noradrenergic systems. The neurons at which these interactions occur may prove to be of particular importance in mediating both a descending influence on nociceptive processing and a segmental influence that may be exerted through activation of the descending tract's terminals. The site of integration of these influences is within the superficial dorsal horn and would seem likely to be the substantia gelatinosa.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

8. Functional implications

This survey of the morphology and physiology of the dorsal horn in relation to nociception, leads to the conclusion that nociceptors can activate (through both direct and indirect routes) and inhibit a variety of dorsal horn neurons. Two features stand out. First, there is evidence that a substantial number of neurons in the most superficial layers of the dorsal horn are capable of transmitting to supraspinal levels a quantitatively valid account of noxious stimuli delivered to the peripheral tissues. It is reasonable to attribute to these neurons the capacity to encode the intensity and locality of a noxious stimulus. The system is affected by both local and descending controls, although not apparently under powerful tonic descending control. The central pathway is, to some degree at least, in the spinothalamic system (see Willis, this symposium). Secondly, there is another group of neurons, some at least with large somata and with rapidly conducting axons in the ascending pathways, that are powerfully excited by both mechanoreceptors and nociceptors. What part do they play in nociception and pain? Their excitation by both innocuous mechanoreceptors and noxious inputs would be thought to lead to sensory confusion. Yet there is no doubt that activation of a single mechanoreceptor can evoke an 'elementary' sensation, the quality of which depends on the kind of afferent unit that is active (Ochoa & Torebjörk 1983). A common characteristic of many of the multireceptive neurons is the selective supraspinal inhibition, both tonic and phasic, that can suppress their responses to noxious stimulation. An explanation for these effects may lie in part in the more complex excitatory route from the nociceptors. This would offer internuncial targets on the polysynaptic nociceptor path at which both descending and segmental inhibition could act. Yet other mechanisms can be conceived that would allow the output of these multireceptive neurons to have a sensory role in nociception. For example, the direct activation of the ascending, purely nociceptive neurons, e.g. of the spino-thalamo-cortical pathway, might 'set' the system so that an input along multireceptive routes could be interpreted as an indication of nociceptive input. A general alerting function may perhaps be important too.

It is nevertheless difficult, if the multireceptive neurons are involved, to account for the precise quantifiable sensations aroused by repetitive electrical stimulation of a mechanoreceptive cutaneous axon, that totally lacks any penumbra of 'pain'. Such an afferent input would activate, in parallel, the dorsal column system and one or more of the less direct dorsal horn systems, such as the s.c.t., d.c.p.s. or s.t.t., including their multireceptive elements. Yet, in such tests in conscious human subjects, there is no report of discomfort or pain (Ochoa & Torebjörk 1983).

The present evidence is, therefore, that nociception in the context of pain can only clearly be identified as a property of the nocispecific neurons, such as those in lamina I of the spinal cord. The very numerous multireceptive neurons, at this stage of our knowledge, have to be accorded a 'non-specific' role. One such is a contribution to the sensory-discriminative aspects of pain. On the other hand, and quite distinct from a sensory role, they must be accorded an important place in reflexes at both segmental and more complex levels, and may also have a general alerting function at these levels. Nevertheless, the spinothalamic tract apparently contains a majority of neurons with multireceptive characteristics, and these activate neurons that in their turn project to the primary and/or second somatosensory areas of the cortex. Do they have a sensory role, and what is it?

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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