Neuroanatomy of the Spinal Cord: Current Research and Future Prospects

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Introduction

In this article my brief is to provide a succinct state of the art commentary on the neuroanatomy of the spinal cord. This is a field in which major developments in methodology are providing important information on the neuronal circuitry of the spinal cord and this work complements our existing knowledge of its basic structure and pathways. One of the most interesting areas of development has been in the application of neuroanatomy in combination with physiology and neurochemistry in order to provide a correlation between structure and function. In this article I will focus on three different but related areas to illustrate some of the current trends and future prospects for basic research on spinal cord neuroanatomy.

Spinal cord circuitry and connections

Much recent anatomical work has focused on the use of anterograde and retrograde axonal transport of specific tracers in order to examine neuronal pathways within the spinal cord. This approach gives detailed information on the exact location of the cells of origin of specific pathways and on the distribution of their terminals. The information obtained generally agrees with the results of earlier studies but provides more detail and may detect new pathways. For example, anterograde tracer studies have identified terminations of descending bulbospinal pathways in the superficial dorsal horn and in ventral horn motoneuronal cell groups (Kuypers and Huisman, 1982). Retrograde transport studies have shown the uneven distribution of spinothalamic projection neurons at different spinal cord levels (Kemplay and Webster, 1986). Some of the results of spinal cord tract tracing and intracellular labelling (see below) studies are shown in Figure 1. So far, tract tracing studies have mainly been at the light microscopic level but it is technically possible to extend them to the ultrastructural level. This allows one to identify inputs and targets of identified cells and thus build up a picture of synaptic circuitry. Such studies cannot be carried

Figure 1 Diagram showing some of the basic cell types and connections of the spinal cord. This scheme is based on the results of classic neuroanatomical studies supplemented by data from the tract tracing and intracellular HRP techniques described in the text. For more detail see Brown, 1981, 1982; Kemplay and Webster, 1986; Kuypers and Huisman 1982; Perl, 1984. All cells and connections occur bilaterally in the spinal cord but for simplicity I^a afferents, interneurons, motoneurons and ascending tracts (stippled) are represented on the left half of the diagram while descending tracts (hatched) and cell bodies of spinothalamic projection neurones are represented on the right half. Visceral and autonomic afferents and efferents are not shown, nor has any attempt been made to take account of segmental differences. Roman numerals indicate the laminae of Rexed and as far as possible the location of cells and the site of termination of projections in the different laminae have been shown accurately. For clarity certain details have been omitted from the diagram but are described in the legend below.

I^a afferent neurones comprise two broad classes. Small neurones convey information mainly about cutaneous pain and temperature sensibility (A δ , C conduction velocity) and terminate mainly in laminae I (A δ), II (C), V (A δ) and X (A δ , not shown). Large neurones convey information from cutaneous touch and hair receptors (A α , A β), muscle spindles (IA, II) and tendon organs (IB) and generally have axons which ascend in the dorsal columns (DC) to terminate in the dorsal column nuclei. However, these neurones also have collaterals which enter the spinal grey matter to terminate in laminae III (A α , A β hair follicle afferents), III–VI (A α , A β low threshold mechanoreceptors), VI, VII, IX (IA spindle), IV–VII, IX (II spindle) and V–VII (IB tendon).

Spinothalamic (ST) projection neurones are located in laminae I, IV–VI, VII (not shown) and VIII (not shown) and send axons across the midline to ascend in the ventrolateral (VL) funiculus. A few cells (mainly in lamina VIII) have ipsilateral projections (not shown). Also contained within the VL funiculus although not shown are the axons of ventral spinocerebellar neurones and various spinobulbar pathways including spinotectal, spinoreticular, spino-olivary, spinovestibular and spinopontine projections. The cells of origin of many of these pathways are located in similar laminae to those of the spinothalamic pathway and some cells have projections both to the thalamus and to more caudal bulbar locations. In the dorsal funiculus run axons of the dorsal spinocerebellar tract (DSC) derived from cells of Clarke's column which is located in laminae VI, VII between the third lumbar (L3) and eighth cervical (C8) segments. The spinocervical tract in the dorsolateral funiculus and the post-synaptic dorsal column system are not shown on the diagram. These pathways have been best characterised in the cat but are thought also to occur in humans.

Descending pathways shown on the diagram are the dorsolateral (D) and ventral (V) bulbospinal (BS) and corticospinal (CS) tracts. Bulbospinal pathways include rubrospinal (terminating in laminae V–VII), vestibulospinal (laminae VII, VIII), reticulospinal (laminae

VII, VIII), raphespinal (I, II, V, VII–X), hypothalamospinal (I–III, VII, X) and tectospinal (VI, VII) tracts.

Interneurons are located in all areas of the spinal cord but are shown only in laminae II, III and VIII. Some of those in lamina VIII (Renshaw cells) are innervated by recurrent motoneurone collaterals. Not shown are the numerous short axon neurones which innervate adjoining laminae or which project for only a few spinal cord segments (propriospinal cells).

Motoneurons are shown as either α (innervating extrafusal muscle fibres) or γ (innervating intrafusal fibres).

Figure 2 Enkephalin immunoreactivity in human post-mortem spinal cord. Unpublished work. P. Anand and J.V. Priestley. Monoclonal leu/met enkephalin antibody provided by AC Cuello (Montreal).

(A) Immunoreactive fibres in the dorsal horn of the spinal cord. Staining is most intense in the superficial layers (laminae I, II) and in a diffuse meshwork of fibers in the area of the dorso-lateral fasciculus.

(B) Varicose immunoreactive fibres in the dorsolateral fasciculus.

Scale bars = 500 μ m (A) and 50 μ m (B)

Figure 3 Diagram showing possible synaptic circuitry in the substantia gelatinosa deduced from ultrastructural immunocytochemical studies on the neuropeptides substance P and enkephalin. From Priestley, Somogyi and Cuello, 1982. Substance P (SP) containing primary afferent fibres participate in four different types of synapse: 1, synapses onto lamina I marginal zone projection neurones (Mg); 2, synapses onto a class of substantia gelatinosa interneurones (SG); 3, synapses onto a second class of substantia gelatinosa interneurones called islet cells (Is); 4, axoaxonic synapses received from certain interneurones. Enkephalin is found in some islet cells and so may interact with substance P in at least four different ways: a, both peptides innervate marginal zone neurones; b, both peptides innervate substantia gelatinosa interneurones; c, substance P innervates enkephalin containing islet cells; d, enkephalin terminals synapse presynaptically on substance P terminals.

I, II indicate laminae I and II. T indicates transmission neurones located in deeper laminae (IV–VI).

out in humans but comparative studies in different species may reveal principles of spinal cord organisation which will be useful in clinical analyses.

Intracellular electrophysiological studies

It is possible to record intracellularly from individual cells and axons, characterise their properties and projections electrophysiologically, and then inject them with horseradish peroxidase (HRP) enzyme. The injected HRP can then be revealed histochemically and the morphology and ultrastructure of the injected cells examined in the light and electron microscope. This powerful technique has been used to map the pattern of termination within the spinal cord of different classes of primary afferent fibres and to examine the dendritic arborisation and axonal projections of certain spinal cord interneurones and projection neurones (Lagerbäck, Ronnevi, Cullheim and Kellerth, 1981; Brown, 1981; Perl, 1984; see also Fig. 1). At the ultrastructural level, neurones pre- and post-synaptic to the labelled cell can be visualised and possible neuronal circuits can be identified (Gobel, Falls, Bennett *et al.*, 1980). This type of analysis involves the painstaking reconstruction of the structure and synaptic organisa-

tion of very small numbers of cells. It is tempting to make broad generalisations about spinal cord organisation from these few results but this must only be done with great care. However, the approach is one of the few techniques which gives a direct correlation between neuronal structure and function and will certainly make important contributions in the coming years.

Immunocytochemistry

A third approach which is changing our understanding of the structure and function of the spinal cord is immunocytochemistry. This technique allows specific antigens to be localised in tissue sections at the light and electron microscopic level and has many applications in the field of neuroscience (Cuello, Priestley and Sofroniew, 1983). In the spinal cord one of the most fruitful applications has been for mapping the distribution of putative neurotransmitters (Hunt, 1983) and, unlike some of the techniques described earlier, this approach can be applied to human post mortem tissue (Pearson, Brandels and Cuello, 1982; see also Fig. 2). At the ultrastructural level it is possible to identify transmitters contained in particular synaptic circuits (Priestley, Somogyi and Cuello, 1982; see Fig. 3). As in other areas of the brain, most work in recent years has

Table Transmitter candidates for the various spinal cord neuronal systems shown in Figure 1.

In some systems there are numerous candidates. It is not proposed that all these are authentic transmitters but only that all have been localised to a particular neuronal system and therefore must be considered as candidates. The excitatory amino acids glutamate (glu) and aspartate (asp) are candidate transmitters in many systems. However, present techniques cannot distinguish between cells which use amino acids as transmitters and cells which use them simply for general cellular metabolism.

Neuronal system	Transmitter candidate
<i>Primary afferent neurones</i>	
A δ , C	substance P, substance K, somatostatin, CCK, CGRP, bombesin/GRP, dynorphin, VIP, PHI, CRF, angiotensin II, enkephalin, vasopressin, oxytocin, galanin, monoamines, ATP
IA, IB, II, A α , A β	glu ?, asp ?
<i>Ascending pathways</i>	
Spinothalamic	Not known. glu ?, asp ?
Spinocerebellar	Not known. glu ?, asp ?
Spinobulbar	Not known. glu ?, asp ?
<i>Descending pathways</i>	
Corticospinal	Not known. glu ?, asp ?
Bulbosplinal	Mainly not known. NA, enkephalin, CCK, substance P, AcCh ?, glu?, asp ?, histamine?
Raphespinal	5-HT, substance P, TRH, CCK
Hypothalamospinal	DA, vasopressin, oxytocin, somatostatin, β -endorphin, ACTH
<i>Interneurones</i>	
Laminae I, II, X, IML	Enkephalin, neurotensin, somatostatin, CCK, substance P, NPY, dynorphin, FMRF-NH ₂ , CRF, VIP, GABA, 5-HT
Other laminae	A few peptide cells, GABA, AcCh, gly?
<i>Motoneurones</i>	AcCh, CGRP

Abbreviations: IML, autonomic intermediolateral cell column; CCK, cholecystokinin; CGRP, calcitonin gene related peptide; VIP, vasoactive intestinal polypeptide; CRF, corticotropin releasing factor; GRP, gastrin releasing peptide; NA, noradrenaline; AcCh, acetylcholine; TRH, thyrotropin releasing hormone; 5-HT, 5-hydroxytryptamine; DA, dopamine; ACTH, adrenocorticotrophic hormone; NPY, neuropeptide Y; CRF, corticotropin releasing factor; GABA, γ -aminobutyric acid.

focused on neuropeptides and there are now about 20 peptides which are known to occur in the spinal cord and which may be transmitters. However, the peptides are not homogeneously distributed but appear to be concentrated in particular areas such as the substantia gelatinosa of the dorsal horn and the intermediolateral sympathetic cell column. It is now possible to use immunocytochemistry to localise 'classical' transmitters such as amino acids (glutamate, γ -amino butyric acid) or acetylcholine and the identification of spinal cord systems which use these transmitters is likely to be a major focus for research in the next few years. At present, there are many candidate transmitters for small diameter primary afferent fibres and for dorsal horn interneurons but very little is known about transmitters in other spinal cord systems (Fig. 1 and Table). Another new and important research area involves the localisation of antigens which are specific for particular spinal cord cell types (Dodd and Jessell, 1985). These antigens are not transmitters but probably play diverse roles in cell recognition and neuronal development. Their identification is likely to provide important information on the processes which control spinal cord development.

Conclusion

In this brief review I have attempted to highlight some of the key areas of current basic research on the neuroanatomy of the spinal cord. One theme has come up repeatedly, namely the circuitry and synaptic connections of spinal cord neurones. We already have a very good knowledge of the basic neuronal constituents of the spinal cord and these are summarised in Figure 1. Hopefully over the next 25 years we will acquire a fuller understanding of the connections these different constituents make with one another and we will be able to relate spinal cord circuitry to physiology, neurochemistry and pharmacology.

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