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The spinal cord of the common marmoset (*Callithrix jacchus*)

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ABSTRACT

The marmoset spinal cord possesses all the characteristic features of a typical mammalian spinal cord, but with some interesting variation in the levels of origin of the limb nerves. In our study Nissl and ChAT sections of the each segment of the spinal cord in two marmosets (Ma5 and Ma8), we found that the spinal cord can be functionally and anatomically divided into six regions: the prebrachial region (C1 to C3); the brachial region (C4 to C8) – segments supplying the upper limb; the post-brachial region (T1 to L1) – containing the sympathetic outflow, and supplying the hypaxial muscles of the body wall; the crural region (L2 to L5) – segments supplying the lower limb; the postcrural region (L6) – containing the parasympathetic outflow; and the caudal region (L7 to Co4) – supplying the tail. In the rat, mouse, and rhesus monkey, the prebrachial region consists of segments C1 to C4 (with the phrenic nucleus located at the C4 segment), and the brachial region extends from C5 to T1 inclusive. The prefixing of the upper limb outflow in these two marmosets mirrors the finding in the literature that a large C4 contribution to the brachial plexus is common in humans.

1. Introduction

Mapping the spinal cord in a single species like the marmoset may seem at first to be a trivial exercise, since it is widely believed that spinal cord anatomy is both simple and consistent among mammals. However, there is a paucity of accurate anatomical detail on the mammalian spinal cord, and this paper reveals that there is much to learn about interspecific variation in mammalian spinal cord anatomy.

There were only a small number of attempts to map the mammalian spinal cord in the twentieth century. The first of these was the classic monograph of Bruce (1901) on the human spinal cord. Bruce presented high quality images of fiber-stained and Nissl-stained sections of all segments of a well-fixed human spinal cord. The images are accompanied by detailed description of the arrangement of the gray matter and the motor neuron groups. While the original printed monograph is difficult to locate, an electronic copy of the manuscript held by Cornell University is now available (http://www.archive.org/details/cu31924024791406).

The next important development in mapping the spinal cord was the construction of a scheme for mapping the laminae of spinal cord gray matter by Rexed (1952). Using this scheme, Rexed (1954) published an atlas of the cat spinal cord. The laminar scheme of Rexed has proved a valuable addition to the understanding of spinal cord anatomy, and has been incorporated into the great majority of published experimental studies on mammalian and non-mammalian vertebrates. A notable attempt to make a new spinal cord atlas using Rexed's laminae is that of Noback and Harting (1971), who published a diagrammatic summary of spinal cord anatomy of the rhesus monkey, but this work did not include images of all spinal segments. Next, Gunnar Grant's group published an atlas of the spinal cord of the rat (Molander et al., 1984, 1989), but this lacked an analysis of motor neuron groups.

Since 2000, comprehensive atlases of the spinal cord of the rat and mouse were published as part of a book on mammalian spinal cord anatomy (Watson et al., 2009a,b). A collaboration between Watson and Tokuno resulted in the preparation of complete sets of spinal cord sections of marmoset, Japanese monkey, and rhesus monkey, and these are publicly available on the website http://marmoset-brain.org. The availability of these sections

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prompted the publication of a second book that includes atlases of the spinal cords of rat, mouse, marmoset, rhesus monkey, and human (Sengul et al., 2013).

1.1. Spinal cord segmentation

Before moving to a consideration of the marmoset spinal cord anatomy, we must deal with the issue of apparent and real 'segmentation' in the spinal cord. The spinal cord is customarily divided into segments according to the vertebral level of emergence of each spinal nerve. For example, the human spinal cord is said to have 31 segments – 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and one coccygeal. This scheme is handy for descriptive purposes but it has significant limitations. First of all, there is not an exact correlation between vertebral regions and the functional regions of the spinal cord (see below). Secondly, there is no external or microscopic anatomical distinction between adjacent spinal cord 'segments,' in that they do not display anatomical boundaries or lineage restriction during development (Lim et al., 1991; Stern et al., 1991). By comparison, the segments of the hindbrain are clearly formed by lineage restriction (Fraser et al., 1990). It may be concluded that the spinal cord segments are not intrinsic; instead they are simply territories defined by the segmental (somite defined) opportunities for exit of the spinal nerves. It may be said that the apparent segmental arrangement is imposed on the spinal cord by the adjacent vertebrae.

On the other hand, the spinal cord can be divided into a sequence of six regions that are defined by anatomy and function (Watson and Sidhu, 2009), and which correlate with territories defined by gene expression during development (Philippidou and Dasen, 2013). The value of subdivision of the spinal cord into functional regions was hinted at by Langley (1921), who showed in a diagram that the autonomic preganglionic regions were positioned on either side of the lumbar enlargement. Watson and Sidhu (2009) extended this idea by pointing out that the limb motor neuron regions were also discrete regions with minimal overlap at the rostral and caudal ends. The resultant scheme is based on the ease with which the enlargements devoted to the supply of the upper and lower limb can be recognized. The importance of the schema is the way that it correlates with *Hox* gene expression patterns in the spinal cord (see below).

In the human spinal cord, the upper limb is commonly supplied by spinal nerves C5 to T1 and the lower limb is supplied by L2 to S1. At the caudal end of the upper limb enlargement there is an abrupt change to a long region that gives rise to the sympathetic preganglionic nerves. Next, the sympathetic outflow region ends immediately before the lower limb enlargement. In a similar manner to the sympathetic outflow, the parasympathetic preganglionic nerve outflow region commences immediately after the caudal end of the lower limb enlargement. The parasympathetic outflow region is short (S2–S4 in humans), and is succeeded by the spinal cord region that supplies the tail. To complete this six-segment functional subdivision, we can add that the segments between the hindbrain and the upper limb enlargement supply the muscles of the neck, including the muscles of the diaphragm, which migrate from the neck muscle group during development. In summary, the spinal cord can be represented as containing six functionally defined, non-overlapping regions on the basis of motor neuron distribution alone; these are the neck muscle region, the upper limb muscle region, the sympathetic outflow region, the lower limb muscle region, the parasympathetic outflow region, and the tail muscle region (Fig. 1).

A feature of this subdivision into six regions is the ease with which they can be defined histologically. The boundaries of the regions can be confidently identified by the presence or absence of only two features – the lateral motor column (LMC) and the preganglionic (intermediolateral) column (PGC) (Fig. 1). The LMC is a lateral bulge of the ventral horn formed by the presence of cholinergic limb motor neurons in the limb enlargements. The PGC is also a collection of cholinergic motor neurons, but these neurons are sparser and smaller than those of the LMC, and are more dorsally placed in the spinal cord gray matter. The PGC contains the preganglionic autonomic neurons. These two motor neuron patterns are mutually exclusive, in that areas with an LMC do not have a PGC, and vice versa. The neck and tail regions are defined by the fact that they possess neither an LMC nor a PGC.

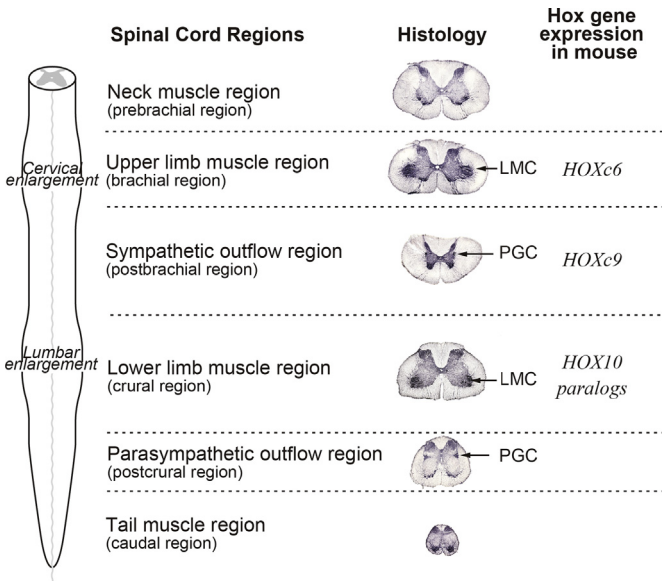

Fig. 1. A diagram to show the subdivision of the mammalian spinal cord into six regions. The drawing on the far left and the column 'Spinal Cord Regions' show the position of the cervical and lumbar enlargements of the spinal cord in relation to the autonomic regions. The column headed 'Histology' shows images of ChAT sections of each of the six regions. The limb enlargements are characterized by the presence of a lateral motor column (LMC) and the autonomic regions containing a preganglionic column (PGC). The neck (prebrachial) and tail (caudal) regions have neither an LMC nor a PGC. The column on the right indicates that the limb enlargements and the sympathetic outflow region are marked by particular patterns of hox gene expression in the mouse.

Because the LMC and the PGC are both made up of cholinergic neurons, the simplest way to divide the spinal cord into its functional regions is to stain spinal cord sections with acetylcholinesterase (AChE) histochemistry or choline acetyltransferase (ChAT) immunohistochemistry.

Strong support for the subdivision of the spinal cord into a small number of functional regions comes from studies of *Hox* gene expression in the chick and the mouse (Dasen et al., 2003; Guthrie, 2004): the upper limb (brachial) region is defined by the expression of *Hoxc6* (Dasen et al., 2003); the sympathetic outflow (postbrachial) region is defined by the expression of *Hoxc9* (Dasen et al., 2003; Philippidou and Dasen, 2013), and the lower limb (crural) region is defined by the expression of *Hox10* paralogs (Carpenter, 2002; Lance-Jones et al., 2001; Lin and Carpenter, 2003; Wu et al., 2008) (Fig. 1).

1.2. The scope of this report

This report is based primarily on a series of images of transverse sections of marmoset spinal cord which first became available on the website http://marmoset-brain.org, and which were subsequently published (accompanied by line diagrams) as a book (Sengul et al., 2013). The present report is the first critical analysis of these published images.

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