INVESTIGATING THE HUMAN SPINAL SENSORIMOTOR PATHWAYS THROUGH FUNCTIONAL MAGNETIC RESONANCE IMAGING

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- 44 articles using task- or resting-state spinal fMRI were identified and analyzed
- We mapped the spinal fMRI sensory and motor activation across these studies
- The well-known functional organization of the spinal cord can be observed with fMRI
- fMRI can provide spinal data on larger scales, including on brain-spine interactions
- Spinal fMRI has specific advantages when used in clinical research and practice

INVESTIGATING THE HUMAN SPINAL SENSORIMOTOR PATHWAYS THROUGH FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Abstract:

Most of our knowledge about the human spinal ascending (sensory) and descending (motor) pathways comes from non-invasive electrophysiological investigations. However, recent methodological advances in acquisition and analyses of functional magnetic resonance imaging (fMRI) data from the spinal cord, either alone or in combination with the brain, have allowed us to gain further insights into the organization of this structure. In the current review, we conducted a systematic search to produced somatotopic maps of the spinal fMRI activity observed through different somatosensory, motor and resting-state paradigms. By cross-referencing these human neuroimaging findings with knowledge acquired through neurophysiological recordings, our review demonstrates that spinal fMRI is a powerful tool for exploring, *in vivo,* the human spinal cord pathways. We report strong cross-validation between task-related and resting-state fMRI in accordance with well-known hemicord, postero-anterior and rostro-caudal organization of these pathways. We also highlight the specific advantages of using spinal fMRI in clinical settings to characterize better spinal-related impairments, predict disease progression, and guide the implementation of therapeutic interventions.

Keywords: spinal-cord fMRI; sensorimotor pathways; motor; proprioception; touch; resting-state

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1 Introduction to the spinal cord fMRI technique and its challenges

The spinal cord is responsible for transmitting neural signals from the brain to the muscles (i.e., motor information), and from the body periphery back to the brain (i.e., somatosensory information). Ample evidence also indicates that the integration of these processes takes place at each level of the central nervous system (CNS), that is at both spinal cord and brain levels, such that the continuous flow of afferent inputs from multiple sensory sources modulates and updates the motor output. Most of our

knowledge about sensorimotor processes at the spinal level comes from research in animal models due to the complexity and ethical issues related to its direct accessibility in humans. In fact, traditional investigations of human spinal cord functions have been relied upon indirect electrophysiological measurements through the coupling of electromyogram recordings with electrical or magnetic stimulations of peripheral nerves, the cerebellum or cortical regions of the brain; (Pierrot-Deseilligny and Burke, 2005, 2012), and more recently using trans-spinal direct current stimulation as well (tsDCS; for references see Nardone et al., 2015). While these approaches have helped to understand better the time course of spinal cord processes, they give limited insight into the precision with regards to their spatial localization within this structure. In the current review, we show that spinal cord functional magnetic resonance imaging (fMRI) has the potential to, not only complement the results obtained through these traditional electrophysiological approaches, but also to provide a larger scale overview of the spinal network functional organization at multiple spinal segments and its interaction with supraspinal centres.

fMRI is a non-invasive technique allowing the indirect detection and localization, *in vivo,* of task-related neural activity as well as of functional networks based upon the analysis of spontaneous activity recorded at rest (i.e., resting-state [rs-fMRI]). Despite the fact that spinal fMRI was acquired for the first time using a motor task paradigm as early as 1996 (Yoshizawa et al., 1996), the number of studies employing this imaging method at the spinal level has remained very low compared to that of cerebral fMRI studies, as the former encompasses the following technical challenges (for review see, Tinnermann et al., 2020). First, the size of the spinal cord is small relative to the achievable imaging resolution, with the cross-section diameter at its largest segment (i.e., cervical level) being approximately 9 mm and 13 mm on the antero-posterior and medio-lateral axes, respectively. Second, the spinal cord is located deep inside the spine canal, which leads to lower coil sensitivity compared to other regions. Third, the spinal cord follows a long-extended rostro-caudal curvature and is also surrounded by different types of tissue, including bones, cartilage (intervertebral disks) and cerebrospinal fluid that produce inhomogeneities in the magnetic field and that can vary along the spinal cord (Stroman et al., 2014; Verma and Cohen-Adad, 2014). Finally, the imaging quality can be severely affected by the participants' physical movements such as swallowing or snoring, as well as by physiological noise arising from respiratory and cardiovascular sources that create non-rigid motion artefacts (Brooks et al., 2008; Eippert et al., 2017a; Kong et al., 2012; Piché et al., 2009). Yet, in the last 10 years, the optimisation of image acquisition protocols at high-field strength, as well as the development of novel radiofrequency coils, advanced shimming procedures (Barry et al. 2018) and pulse sequences designed for selective fields-of-view (Finsterbusch, 2013) have improved significantly the image quality in spinal fMRI.

In fact, such technological and methodological advances can explain the recent significant increase in the number of studies that have investigated spinal cord functions, non-invasively, in humans using this imaging approach, and more specifically the underlying neurophysiological basis for somatosensory and motor responses.

2 *Objectives*

In the current review, we propose to achieve four main objectives. First, we describe the structural and functional organization of the human spinal cord, as revealed by anatomical and electrophysiological studies (section 3). After presenting evidence demonstrating that spinal fMRI is a reliable method to detect spinal neural activity indirectly (section 4), we then summarize the results of all relevant studies which used different paradigms designed to assess spinal activity related to somatosensory stimulation, motor tasks, and resting state (section 5). Next, we synthesize the findings derived from

these investigations and cross-referenced them with our previous neurophysiological knowledge on spinal pathways (section 6). On this basis, we discuss their validity in characterizing the functional organization of the human spinal sensory and motor pathways *in vivo*. Finally, we discuss the potential of employing spinal fMRI in clinical practice, both for the development of new neurological biomarkers and assessment of the disease progression, as well as for its importance in guiding clinicians in their choice of therapeutic approaches (section 7).

3 *Human organization of the sensory and motor spinal pathways*

The spinal cord is located in the vertebral column and extends rostro-caudally from the brainstem to the lower back (lumbar region). This long, thin and ovoid-shaped tubular structure measures approximately 43-45 cm in length and has a variable diameter cross-sectionally (6-13 mm) with a larger diameter at the cervical level (Purves, 2018). The topographical organization of the spinal cord along the rostro-caudal orientation follows two different nomenclatures: one uses the vertebrae (vertebral level), while the other utilizes the nerve roots (spinal level) as landmarks. Importantly, both classifications do not exactly align and can vary across subjects (Cadotte et al., 2015). Yet, it is more standard (in neurophysiology studies, for example) to describe the position of spinal neural activity according to the 31 nerve roots (i.e., 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal, Figure 1) along the spinal cord. Each spinal segment gives rise to fanning rootlets over a rostro-caudal extent, which converge into pairs of dorsal and ventral roots (Figure 1). The dorsal roots are composed of afferent fibers that transmit sensory information from the skin, muscles, and visceral organs, and contain bodies of the corresponding sensory neurons in the dorsal root ganglia (DRG). By contrast, the ventral roots contain efferent fibers that transmit the motor commands from the brain to the muscles, and consist of the axons of motoneurons whose cell bodies are located in the ventral part of the spinal cord. The dorsal and ventral roots converge into a pair of mixed spinal nerve (a left and a right branch, respectively). The group of muscles innervated by a single ventral root is called a myotome, while a dermatome usually represents the skin area that provides sensory information through the same dorsal root (Keegan and Garrett, 1948). Thus, spinal nerves are distributed to specific body parts, with the upper cervical segments (C1-C4) being dedicated to the face and neck, the lower cervical and first thoracic segments (C4-T1) to the upper limbs, the thoracic segments (T1-T12) to the trunk and the lower spinal segments (L1-S2) to the lower limbs (Purves, 2018).

Figure1 around here, color, 2 columns

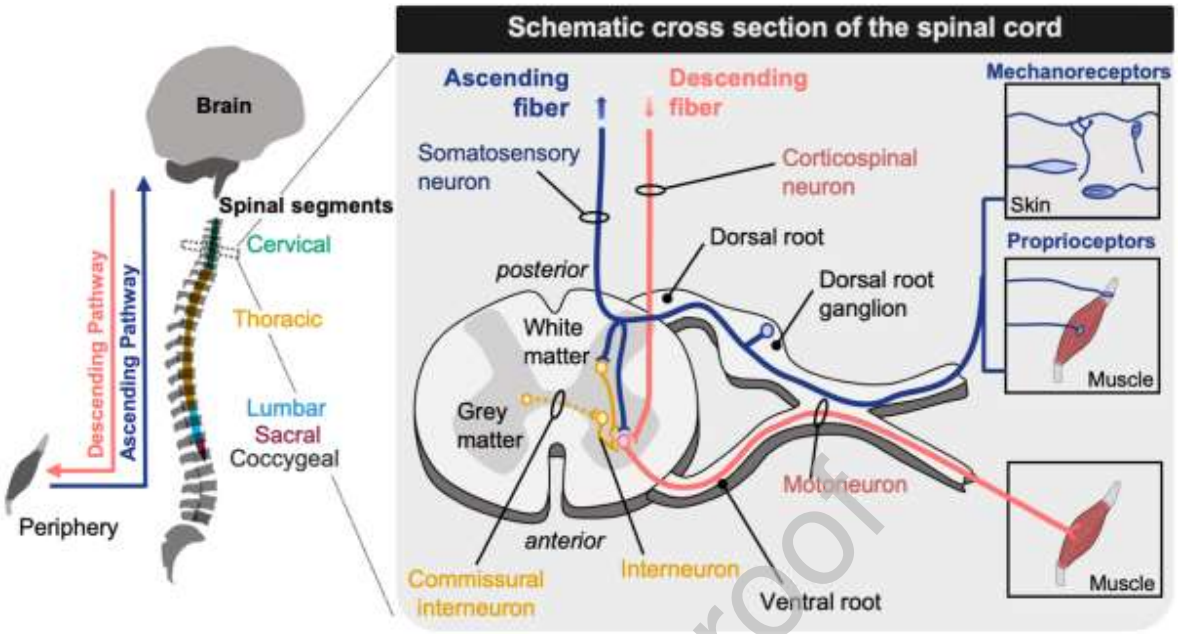

Figure 1 (color) around here

Figure 1 | Schematic representation of the overall anatomical organization of the spinal cord as well as its sensory and motor pathways. Left column: The descending motor tract (corticospinal pathway) transmit the neural signal from the brain to the muscle (pink), while the ascending sensory tract (dorsal column pathway) sends information from the body periphery to the brain (blue). Right column: A schematic cross section of the spinal cord is represented on the right side. The circuitry of motoneurons (pink), somatosensory neurons (blue) and interneurons (yellow) are schematized.

Similar to the brain, the spinal cord contains grey and white matter (GM and WM, respectively). However, unlike the topography of the GM and WM at the cerebral level, the spinal GM is organized in a butterfly-shaped column (when seen in transversal plane, Figure 1) that is surrounded by WM. The GM includes cell bodies and axons of both interneurons and motoneurons, as well as axons of peripheral sensory neurons. The WM consists mainly of myelinated ascending and descending fibers forming fiber tracts (bundle of myelinated axons). The spinal WM contains different types of tracts between the spinal cord and supraspinal structures, such as the brainstem, cerebellum and cerebrum (Lévy et al., 2015). The ascending pathways are responsible for the transit of sensory information from peripheral receptors, including mechanoreceptors, proprioceptors, thermoreceptors and nociceptors to the brain and include the dorsal column, spinothalamic and spinocerebellar tracts. By contrast, the descending pathways are involved in transmitting motor information from the brain to the muscles through corticospinal, reticulospinal, tectospinal and vestibulospinal tracts. In the transversal plane, the spinal GM can be divided into two anterior and two posterior horns. Motoneurons are located in the ventral horns, while the dorsal horns mostly contain sensory neurons that receive input from the DRG neurons. Interneurons, which are actually present in both horns and intermediate zones, have different function: they can relay the sensorimotor inputs, modulate the motoneurons activity, transmit information to the opposite horn, transmit information to adjacent upper and lower segments or more distant segments (propriospinal neurons), as well as send information to supraspinal centers (Maxwell and Soteropoulos, 2020; Zavvarian et al., 2020). Neural processing within the spinal cord occurs mainly through activity from populations of neurons and their synapses located in the GM, according to a

segmented functional organization along the rostro-caudal plane (i.e., corresponding to myotomes and dermatomes serving different parts of the body).

Altogether, the GM and WM support the transmission of sensory and motor signals through and within the spinal cord. Indeed, the descending motor command driving an intentional action arises from the somato-topically organized population of cortical neurons, which are predominantly located in the frontal and parietal cortices (Brodmann areas 1-7, Penfield and Boldrey, 1937). The corticospinal fibers descend through subcortical WM (i.e., corona radiata) and then through the medulla oblongata, where ~90% cross the midline and follow the dorso-lateral columns of the spinal WM (lateral corticospinal tract); the remaining 10% running into the ventral column and crossing the midline at spinal level (ventral corticospinal tract). The corticospinal axons terminate mostly in the hemicord contralateral to the cortical hemisphere of origin (motor decussation), but ipsilateral to the target limb. Those originating from the motor cortex synapse with motoneurons in the ventral horn (cortico-motoneuronal connexions) and with interneurons. As for the sensory command, fibers follow two main tracts, which constitute the ascending system. The first tract includes the DRG neurons as first-order somatosensory neurons (from proprioceptors and mechanoreceptors). They project their axons on spinal GM and send a collateral branch to the dorsal columns, reaching the second order neurons located in the medulla oblongata. These then send projection across the midline before connecting the third interneurons in the thalamus, and project to the somatosensory cortex contralateral to the spinal dorsal root of origin (sensory decussation). The other ascending tract is composed of axons of spinal interneurons (activated by nociceptors and thermoreceptors), which cross the midline and reach the spino-thalamic tract (lateral parts of the spinal WM), and then projects onto the somatosensory cortex, after thalamic relay (Purves, 2018).

The anatomical organization of the spinal cord described above gives rise to a precise underlying functional organization that follow three patterns: 1) each spinal segment transmits information from/to the corresponding body part (e.g., upper or lower limbs), 2) right and left sides of the GM relay information from/to the corresponding body sides (e.g., left or right limb), while 3) the antero-posterior division mostly emphasizes the distinction between motor (anterior) and sensory (dorsal) neuronal endings.

4 *Spinal cord fMRI: a reliable method to detect indirect neural activity*

Similar to its cerebral counterpart, fMRI of the human spinal cord aims to investigate the neural correlates, notably, of task-related activity using motor or sensory stimulation paradigms, as well as functional connectivity linked to intrinsic neural activity at rest. To do so, two different types of physiological signals have been used to date: the BOLD (blood oxygen level dependent, for review see Heeger and Ress, 2002) and, to a lesser extent, the SEEP (signal enhancement by extra-vascular water proton) contrasts (Stroman et al., 2003). The fMRI signal obtained through these two contrasts is thought to arise from local vascular changes attributed to the activity of a neuronal population. However, the surrounding vascular organization of the spinal cord can also lead to BOLD activation outside the spinal cord. Even if these unexpected BOLD responses are not necessarily false positives, it's now possible to reduce the influence of surface draining veins by excluding surrounding tissue (segmentation) and by using random-effects group analysis approaches.

Evidence supporting the reliability of the hemodynamic changes in the spinal grey matter also comes from several animal studies (Lawrence et al., 2004; Piché et al., 2017; Wu et al., 2019; Yang et al., 2015). For example, Lawrence et al. (2004) were the first to compare measurements of neural activity using

fMRI and immunohistochemistry in rats' spinal cord. They found a good correspondence between MRI functional signals and immunohistochemical markers (c-fos) of neural activity during nociceptive stimulation. Interestingly, two recent studies in non-human primates (Wu et al., 2019 and rats (Piché et al., 2017) have also revealed a tight coupling between these spinal local field potentials and spinal cord blood flow measures, thus supporting the general notion that fMRI reflects both excitatory and inhibitory synaptic activities. Together, these findings thus confirm that spinal fMRI is a reliable tool for studying neural activity, hence explaining the increase in the use of this methodological approach to explore spinal functions and dysfunctions in both healthy and diseased human participants.

In addition to evidence demonstrating that the spinal BOLD is a valid indirect measure of neuronal populations, technological advances in spinal cord imaging over the last 10-15 years have ameliorated drastically the reliability of the results, and have expanded the possible applications for functional spinal cord investigations. First, the spatial localization of spinal fMRI signal has been improved through a variety of structural spinal MRI techniques both during image acquisition and preprocessing, hence allowing the precise delineation of the different tissue types within the spinal cord. For instance, fMRI studies of the spinal cord at 3T and 7T incorporate anatomical T_1-weighted, T_2-weighted or T_2*-weighted scans, which make it possible to distinguish between different spinal cord tissues (butterfly-shaped GM, WM and CSF). In terms of anatomical image pre-processing, quasi-automatic segmentation methods for the WM, GM and CSF, including surface-based, intensity-based or image-based methods, have also been recently developed, thus permitting a precise characterization of the anatomical structure of the spinal cord in both healthy individuals and patients with spinal pathologies (De Leener et al., 2016). Second, the development of new fMRI acquisition protocols at high and ultra-high field strengths, shimming procedures, optimized pulse sequences and selective field-of-view techniques, combined with more sophisticated functional preprocessing methods such slice-wise correction (De Leener et al., 2017) and physiological noise modeling (Brooks et al., 2008; Eippert et al., 2017a) have dramatically increased the reliability of spinal fMRI findings. Finally, the development of a standard template of the spinal cord (e.g., PAM50; De Leener et al., 2018) provides now the possibility to average results across subjects while reducing selection and registration biases, and then perform group-based analyses as well as compare groups of participants in the same reference space, similar to standard procedures used for brain fMRI data.

5 *Selection of studies using spinal fMRI in conjunction with somatosensory, motor and resting-state paradigms*

5.1 Study selection (Figure 2)

For this review, we conducted a systematic search of spinal fMRI studies that used different somatosensory, motor and resting-state paradigms to assess spinal activity. The search was performed on November 19, 2020, using the ISI Web of Science, and included the following databases: Web of Science core collections, MEDLINE, Scielo, Biological abstracts and Current contents connect. We selected studies that included the two following terms in their title, abstract or keywords: 1) "functional neuroimaging" OR "functional imaging" OR "functional magnetic resonance imaging" OR "fMRI" AND 2) "spinal cord" OR "spinal" OR "spine". We selected only studies that: 1) were published in English, 2) contained original data (i.e., no reviews, opinion papers etc.), 3) employed experimental paradigms that included somatosensory stimulation (proprioceptive or tactile stimulation), motor tasks or spinal rs-fMRI, while excluding those that used painful, thermal, emotional stimuli or sexual arousal (as the latter render difficult the separation between sensory stimulation and motor actions, and may even recruit the autonomic nervous system), and 4) provided data from healthy participants. Following such criteria, a total of 44 articles were then selected for the present literature review. Supplementary information

and tables for the risk of bias assessment (Tables S1, S2, S3), as well as tables related to the preprocessing steps and statistical analyses (Tables S4, S5, S6) recently proposed by the Committee on Best Practices in Data Analysis and Sharing (COBIDAS) are available in supplementary materials and tables.

Figure 2 around here, Black and white, 2 columns

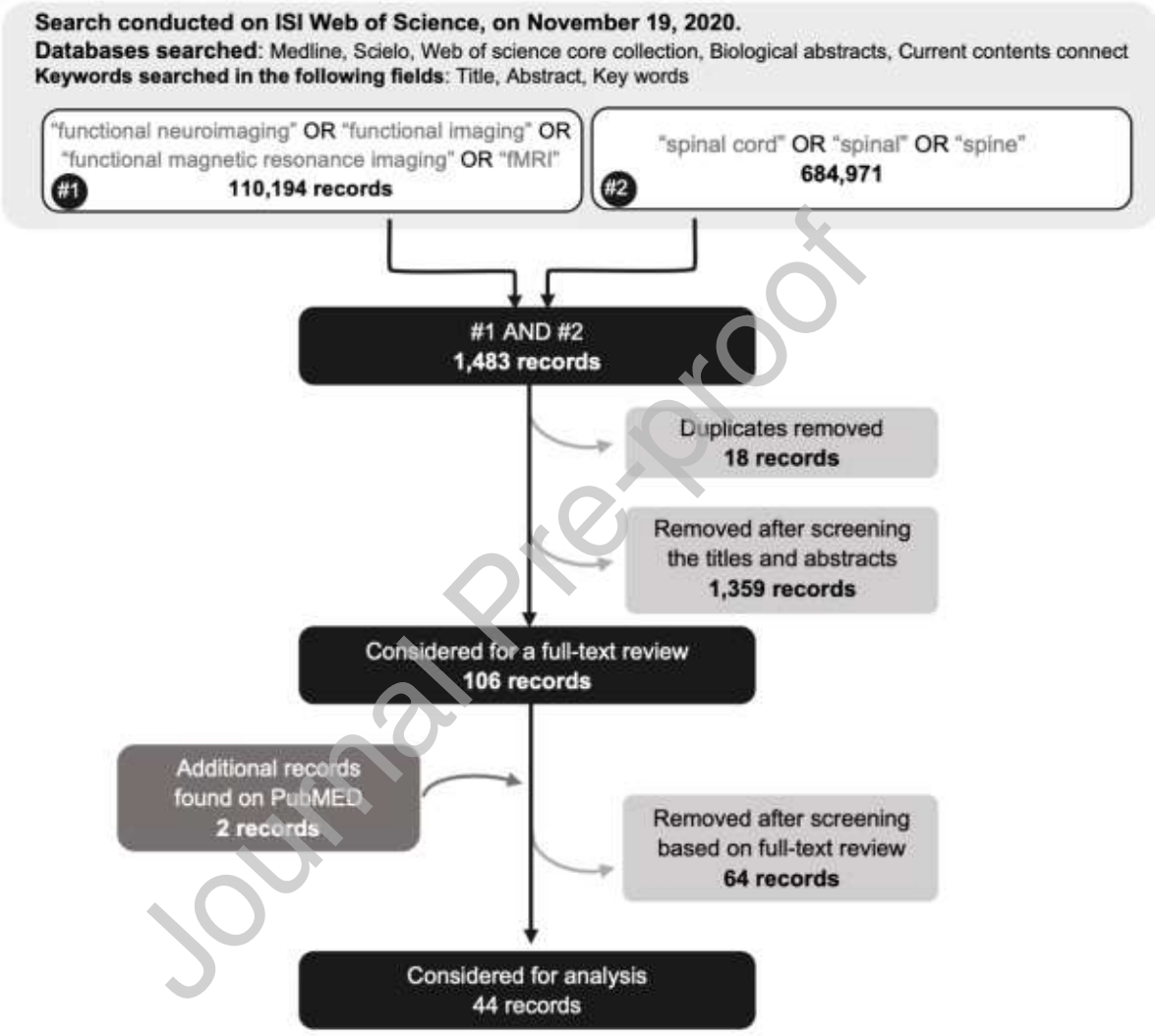

Figure 2 | Flow diagram summarizing the steps involved in the present systematic review. The initial search yielded a total of 1483 articles. Screening and eligibility assessments were then performed by two independent authors (CL and OL). All titles and abstracts were read (1483 articles), and duplicates removed (18 articles). Then, based on content of the title and abstract, we identified 106 potentially eligible studies that were subsequently reviewed based on the full text in order to retain those that met the inclusion criteria. Given that PubMED may include records that are not present in MEDLINE, we conducted a separate search on PubMED using the same search syntax. The results identified two additional records, hence a total of 44 articles were selected for this systematic literature review.

5.2 Heterogeneity in the methods used between studies

It is important to mention that the selected papers varied greatly in terms of the level of methodological details reported in each study, although they all included basic information, such as the number of participants, MRI strength-field, type of pulse sequences (e.g., echo gradient or spin echo), as well as the spatial and temporal resolutions at which scans were acquired (Table1, 2 and 3). Consequently, as Figure 3 illustrates, one can see that from 1996 to now, there has been an increase in the number of participants *per* study (from 4 to 56). The studies have been carried out at increasing field strengths (from 0.2T to 7T) and scans have been collected at higher spatial resolutions, as reflected in the decrease in slice thickness (from 10 to 2 mm). Furthermore, because of the small number of participants and the poor spatial resolution at which scans were acquired, earlier studies mainly reported qualitative results at the subject level, whereas the more recent ones have systematically been reporting the results of group analyses. We believe that these changes reflect the evolution in technological advances observed over the years in spinal cord imaging, which have improved the reliability of the results drastically and have expanded the possible applications of functional spinal cord investigations.

Figure 3 around here, color, 2 columns

Figure 3 | Evolution in methods associated with spinal cord imaging as a function of the publication year. A- Number of participants across the years. The different colors represent groups of studies published in four separate time periods (<2006 (n=7): yellow; 2006-2010 (n=14): green; 2011-2015 (n=7): blue; 2016-2020 (n=15): red). **B-** Changes in MRI field-strengths (0.2T, 1.5T, 3T, 7T from the light pink to the dark pink color, respectively) with which scans were acquired during the same four time periods (<2006, 2006-2010, 2011-2015,2016-2020). **C-** Changes in spatial resolution used during scanning, again across the four time periods.

5.3 Description of the experimental paradigms used in spinal fMRI

In this section, we summarize the results of the 44 studies that used spinal fMRI to investigate the neural substrates associated with a sensory or motor task at this level of the CNS, or that have measured the spinal cord functional activity at rest (Table1, 2 and 3). Note that the term "somatosensory" used in the current review refers to touch and proprioception, as painful and thermal paradigms were not included.

a. Studies using somatosensory stimulation paradigms and spinal fMRI

The number of studies conducted to date that have used somatosensory stimulation in healthy adults to examine the neural bases of proprioceptive and tactile information at the spinal level, is relatively small (n=14). Moreover, with the exception of three studies (Kornelsen et al., 2013; Kornelsen and Stroman, 2004; Lawrence et al., 2008), most of them have focused on the effects of sensory stimulation in the upper limbs (Table 1). Of these, only four research groups have investigated muscle proprioception using passive limb movements (Agosta et al., 2008b; Kornelsen and Stroman, 2004; Valsasina et al., 2008) and transcutaneous electrical stimulation of the median nerve (Backes et al., 2001). Investigations of the neural correlates within the spinal cord that are related to touch have been carried out using a variety of stimuli applied to the skin, such as air puffs (Stroman and Ryner, 2001), static pressure stimuli (Agosta et al., 2009b; Brooks et al., 2012; Ghazni et al., 2010; Rocca et al., 2012; Stracke et al., 2005; Valsasina et al., 2008), dynamic brushing (Ghazni et al., 2010; Summers et al., 2010; Weber et al., 2020), superficial mechanical vibration (Kornelsen et al., 2013; Lawrence et al., 2008;

Valsasina et al., 2008) and transcutaneous electrical stimulation of the median nerve (Backes et al., 2001).

Table 1| Studies using somatosensory paradigms and spinal fMRI. The nature of the stimulation, the body part involved as well as the number of participants (n), the age range and the MRI field-strength are reported below. The rostro-caudal extent of the data acquisition (coverage) and the peak of activity related to the task are then indicated in the last two columns.

Studies	**Stimulation type** Body part	N [Age] (years)	Field-Strength Contrast/Sequence	x y z (mm) Orientation	TE/TR (ms)	Coverage	Peak(s) of Activity
Backes et al., 2001	**Electrical stim.** R median nerve	7 [20-33]	1.5 T BOLD / EPI	-/- / 8 and 5 Sag. + Axial	50/68	C4-T1*	C4-T1*
Stroman et Ryner 2001	**Air puffs** R or L palm	15 n.d.	1.5 T BOLD / GE-EPI	-/-/7.5 Axial	36-96/ -	C6-T1*	C6-T1*
Kornelsen et Stroman 2004	**Passive movement** R and L ankle	6 (5 men) n.d.	1.5 T SEEP / FSE	-/- / 7.5 Axial	42.3/ -	L1-S3	L1-S3
Stracke et al., 2005	**Tactile pressure** R thumb R 3th finger R 5th finger	9 (7 men) [24-33]	1.5 T BOLD / GE-EPI	1.8/1.8/4 Axial	50/2000	C1-T1*	C3, C4, C6 C3, C4, C7 C3, C4, C8
Agosta et al., 2008b	**Passive movement** R wrist	10 HC (4 men) [21-55]	1.5T FSE	1.2/1.2/4 Sag.	71/2700	C5-C8	C5-C6
Lawrence et al., 2008	**Skin vibration** R biceps R wrist R or L palm R patella R achille tendon	7 (4 men) [28.5 ± 6]	1.5 T SEEP / FSE	0.9/0.9/7 Axial	40/6000	C4-C7* C6-T2* C6-T2* T10-L1* T10-L1*	C5* C7* C6-C7* T12* T11-T12*
Valsasina et al., 2008	**Passive movement** – R wrist **Pressure** –R palm	12 (5 men) [37.7 ± 11]	1.5 T SEEP / FSE	0.3/0.3/7 Axial	11/2850	C5-C8 C5-C8	C6-C7 C6-C7
Agosta et al., 2009b	**Tactile pressure** R palm	12 (4 men) [21-56]	1.5 T SEEP / FSE	0.3/0.3/7 Axial	42.5/1065	C5-C8	C6-C7
Ghazni et al., 2010	**Pressure** - R thumb **Brushing** – R thumb	8 (1 men) [18-26]	3 T SEEP / FSE	1/1/2 Sag. + Axial	-	Thal-T1*[1]	C7-C8 C7-C8
Summer et al., 2010	**Brushing** – L hand	11 (5 men) [20-34]	3T BOLD / GE-EPI	1/1/4 Axial	23/1000	C4-C7*	C5-C8
Brooks et al., 2012	**Tactile pressure** R base of thumb L base of thumb	18 (11 men) [22-40]	3T BOLD / GE-EPI	-/-/4 Axial	39/1000	C4-T1*	C5-C4* C7-T1*
Rocca et al., 2012	**Pressure** – R palm	20 HC (7 men) [37.3]	1.5 T FSE	0.4/0.4/7 Axial	11/2850	C5-C8	C5-C8
Kornelsen et al., 2013	**Skin vibration** R torso	15 (7 men) [18-25]	3T BOLD / FSE	1.5/1.1/2 Axial	38/1000	T1-T12*	T1-T12*
Weber et al., 2020	**Tactile brushing** R or L shoulder R or L 3th finger	29 (7 male) [36 ± 11]	3 T BOLD / GE-EPI	1/1/3 Axial	30/2000	C3-C7*	C5-C7 C5-C7

R: Right, L: Left, HC: healthy control, SCI: spinal cord injury. BOLD: Blood Oxygen Level Dependent, SEEP: Signal Enhancement by Extra-vascular water Proton, EPI: Echo Planar Image, GE: Gradient Echo, FSE: Fast Spine Echo. C: Cervical, L: Lumbar, S: Sacral, T: Thoracic, Thal: Thalamus. Coverage column: * indicates that the location of the spinal activity corresponds to a vertebral level, while the location with no symbol refers to a spinal segment.

[1] The analysis was carried out on 8 predetermined regions (thalamus, midbrain, pons, medulla, C5, C6, C7, and C8

b. Studies using motor paradigms and spinal fMRI

A plethora of neuroimaging studies have also investigated the cerebral correlates of motor control and motor learning in humans by employing a vast array of paradigms requiring upper-limb movements with different levels of complexity. The results of those studies have revealed the critical role that the basal ganglia, cerebellum and motor areas forming the cortico-striatal and cortico-cerebellar systems (Dayan and Cohen, 2011; Doyon and Benali, 2005; Hardwick et al., 2018) in movement production and procedural memory (i.e., motor skill learning). Comparatively, less than two dozen studies have employed similar paradigms to investigate the spinal correlates of motor control and skill learning, despite the fact that the first spinal fMRI investigation was conducted more than 25 years ago by Yoshizawa and collaborators (1996) who used a simple voluntary fist clenching task in healthy individuals. The latter authors found an increase in BOLD signal during the motor task as compared to rest, with grey matter activation being localized primarily in the ventral horn of the cervical spinal cord, ipsilateral to the executed movements. Interestingly, versions of this motor task were then employed in many subsequent spinal fMRI studies (Backes et al., 2001; Giulietti et al., 2008; Islam et al., 2019; Ng et al., 2006; Smith and Kornelsen, 2011; Stroman et al., 1999; Stroman and Ryner, 2001), as fist clenching is best suited to generate increased activity at different segments of the spinal cord due to the involvement of several muscles from the forearm and fingers in the task. More recently, however, finger tapping paradigms (Bouwman et al., 2008; Govers et al., 2007; Maieron et al., 2007; Ng et al., 2008; Smith and Kornelsen, 2011; Vahdat et al., 2015; Xie et al., 2009), as well as those that employ simple movements of a given body segment (Barry et al., 2020; Kinany et al., 2019; Madi et al., 2001; Weber et al., 2016) have also been carried out to identify the spinal neural correlates associated with movements of different parts of the upper limbs. Our systematic review revealed only one study that measured the spinal activity elicited by a motor task involving movements of the lower limbs in healthy adults (Kornelsen and Stroman, 2004). Thus, to date, spinal neural correlates of lower limb movements are too sparse to provide clear conclusions; due mainly to the fact that spinal fMRI of the thoracic and lumbar segments is more challenging than at the cervical level because of the smaller cord diameter and the greater physiological noise, such as breathing.

Table 2 | Studies using motor task paradigms and spinal fMRI. The nature of the motor task, the body part involved as well as the number of participants (n), the age range and the MRI field-strength used in each study are reported. The rostro-caudal extent of the acquired data (coverage) and the peak of activity related to the task are then indicated in the last two columns.

Studies	Task Body part	N [age] years	Field-Strength Contrast/Sequence	x y z (mm) Orientation	TE/TR (ms)	Coverage	Activity peak
Yoshizawa et al., 1996	**Clenching** R hand	4 men [38 ± 11]	1.5 T BOLD/spoiled-GE	-/-/5 Sagittal	10/500	C7-C8*	C7-C8*
Stroman et al., 1999	**Clenching** R hand	25 (14 men) [19-66]	3 T BOLD/spoiled-GE	-/-/5 Sag. + Axial	30/80	C6-T1*	C8
Backes et al., 2001	**Clenching** L or R hands	11 (9 men) [20-33]	1.5 T BOLD / EPI	-/-/8 or 5 Sag. + Axial	50/68	C1-T1*	C4-C7*
Madi et al., 2001	**Flexion** - R elbow **Extension** - R wrist **Abduction** - R 5th finger **Weight holding** – R hand	3 men 6 men 6 men 4 men [20-50]	1.5 T BOLD / GE-EPI	-/-/4 Sag -/-/5 Sag -/-/5 Sag -/-/6 Sag	50/3000	C4-T2*	C5-C6* C6-C7* C7-T1* C5-C6/C8-T1*
Stroman et Ryner 2001	**Clenching** L or R hand with a ball	15 n.d	1.5 T BOLD / GE-EPI	0.9/0.9/7.5 Axial	36-96/-	C6-C8*	C7*
Kornelsen et Stroman 2004	**Pedaling** L and R Ankle	6 (5 men) n.d	1.5 T SEEP / FSE	-/-/7.5 Axial	42.5/-	L1-S3	L1-S3
Ng et al., 2006	**Clenching** L and R hands	14/28 included [20-36]	0.2 T SEEP / FSE	-/-/10 -	24/1000	C5-T1	C5-C6
Govers et al., 2007	**Finger tapping** R fingers	12 (5 men) [18-25]	1.5 T BOLD / GE-EPI	-/-/3 Axial	50/3000	C5-T2*	C7*
Maieron et al., 2007	**Finger tapping** L or R fingers	13 (7 men) [21-44]	3 T BOLD / GE-EPI	1.2/1.2/4.5 Oblique	32/1000	C5-T1*	C5-T1*
Bouwman et al., 2008	**Finger tapping** L or R fingers	10 (7 men) [25-40]	3 T BOLD / FSE + GE-EPI	1/1/2.8 Axial	35-20/5890-486	C1-T2*	C5-T1*
Giulietti et al., 2008	**Clenching** R hand with a ball	7 [34.4 ± 3.7]	1.5 T BOLD / GE-EPI	0.9/0.9/9 Axial	32-100/6000	C5-C7*	C5-C6
Ng et al., 2008	**Finger tapping** R or L fingers	10 men [18-25]	3 T BOLD / GE-EPI	1/1.3/5 Axial	15/2500	C1-C7*	C5-C7*
Xie et al., 2009	**Finger tapping** Fingers	8 (6 men) [21-38]	1.5 T SEEP / FSE	-/-/2.8 or 7 Sag. + Axial	42/1065	C4-T2*	C5-C7*
Smith et Kornelsen 2011	**Button press** R fingers	14 (6 men) [22]	3T FSE	1/1/2 -	38/1000	C1/T1*	C6-C7
Vahdat et al., 2015	**Finger tapping (MSL)** R fingers	25 (11men) [24] (median)	3 T BOLD / GE-EPI	2.5/2.5/4 Axial	20/2500	Whole brain and C1-C7*	C7
Weber et al., 2016	**Isometric contraction** L or R wrist	11 (5 men) [37.7 ± 1.9]	3 T BOLD / GE-EPI	1/1/3 Axial	34/2500	C4-T1*	C7
Islam et al., 2019	**Clenching** L and R hands	9 n.d	3T BOLD / SE-EPI	-/-/4	30/2400	Whole brain and T2*	C8
Kinany et al., 2019	**Extension** – R & L wrist **Adduction** – R & L wrist **Abduction**– R & L fingers	19 (8men) [26 ± 3.4]	3 T BOLD / GE-EPI	1/1/3 Axial	30/2500	C3-T1*	C5-C6 C7-C8 C6-C8
Barry et al., 2020	**Extension** – R or L finger	7 (4 men) [25.7 ± 4.5]	3 T Bold / GE	1/1/5 Axial	10/36.5	C5-C7	C7

R: Right, L: Left, MSL: Motor sequence learning. BOLD: Blood Oxygen Level Dependent, SEEP: Signal Enhancement by Extra-vascular water Proton, *EPI: Echo Planar Image, GE: Gradient Echo, FSE: Fast Spine Echo*. C: Cervical, L: Lumbar, S: Sacral, T: Thoracic. In the coverage column, the * indicates that the location of the spinal activity corresponds to a vertebral level, while the location with no symbol refers to a spinal segment.

c. Studies using spinal rs-fMRI

The temporal and spatial organizations of spontaneous neural activity during rest have been widely investigated at the brain level, as they are thought to reveal the intrinsic connectivity between distinct functional networks (Biswal et al., 1995; Damoiseaux et al., 2006; Raichle, 2015). Indeed, resting-state studies have consistently revealed distinct and reproductible cerebral networks, such as the default mode, the dorsal and ventral attentional, the sensorimotor and the visual networks, to name only a few (for review see Raichle 2015). Interestingly, such organization in functional connectivity at rest has also been observed at the subcortical level, for example within the brainstem, cerebellum as well as between basal ganglia and cortex (Beissner et al., 2014; Di Martino et al., 2008; Guell et al., 2018). These observations led subsequently to the investigation of resting-state fluctuations at the spinal cord level in order to assess the extent to which this functional signature could be generalized to the entire CNS. Our systematic search yielded a total of 14 studies that used spinal rs-fMRI, most of them being published in the last 5 years. In this section, we thus describe the different approaches used in these studies that sought to characterize the functional connectivity during resting-state in the spinal cord (Table 3).

Wei et al. (2010) were the first to investigate spontaneous BOLD signal activity in the humans cervical cord using an independent component analysis (ICA) approach. They found acceptable levels of reproducibility of the independent component maps across sessions at the individual level, but did not report any group data. These authors mentioned that signal changes from the primary independent components occurred mainly in the frequency band of the physiological respiratory cycle (0.27-0.33 Hz), thus making difficult the interpretation of the neural origin of these observations. In response to this limitations, however, physiological noise modelling methods have subsequently been developed in order to increase the quality of the spinal cord data by removing the non-neuronal signal contribution observed in BOLD activity, hence allowing for the detection of more reliable spinal functional connectivity patterns in the spinal cord (Brooks et al., 2008).

In addition to ICA approaches (Kong et al., 2014; San Emeterio Nateras et al., 2016; Vahdat et al., 2020; Wei et al., 2010) that capture temporally synchronised spinal networks at rest, temporal correlations between specific spinal regions of interest (seed-based correlation) have often been employed to measure functional connectivity within the spinal cord, or between the spinal cord and brain (Barry et al., 2016, 2014; Conrad et al., 2018; Eippert et al., 2017b; Harita and Stroman, 2017; Liu et al., 2016; San Emeterio Nateras et al., 2016; Vahdat et al., 2020; Weber et al., 2018).

The latter approaches are not the only options available today, however. To complement the resting-state measures of functional connectivity characterizing the spinal networks that can be detected through ICA and seed-based approaches, Kinany et al. (2020) have very recently proposed a dynamic functional connectivity approach called innovation-based co-activation patterns (iCAPs). This method provides spatiotemporal connectivity maps, which correspond to neural networks that display similar changes in patterns of sustained activity over time.

Yet, the studies described above mainly investigated resting-state connectivity in spinal regions with a limited rostro-caudal extent, and thus did not allow the full exploration of networks across different levels of the CNS. To fill this gap, two recent studies extended the resting-state networks investigation from the cervical spinal cord to the brainstem (Harita et al., 2019) and from the spinal cord to the whole brain (Vahdat et al., 2020). The latter employed both ICA and seed-based connectivity approaches in combination with an innovative rs-fMRI data acquisition technique allowing for simultaneous scanning of both cervical spinal cord and the brain.

In summary, in the past 5 years, there has been a significant increase of interest in exploring the spinal cord spontaneous activity using rs-fMRI. However, studies have focused mainly on the cervical spinal cord and data on the lumbar level are lacking. Functional activity of the spinal cord networks at rest can now be reliably investigated using both classical, static (ICA and seed-based) and dynamic (iCAP) approaches, with simultaneous acquisition of the cervical spinal cord and the brain offering a new perspective to evaluate the functional connectivity throughout the entire sensorimotor network.

Table 3 | Resting-state studies using spinal fMRI. The type of analyses as well as the number of participants (n), age range and the MRI field-strength are reported. The field of view (coverage) of the acquisition is also mentioned. The last column corresponds to the functional connectivity networks observed in the study.

Authors	N [Age] years	Field-Strength Contrast/Sequence	x y z (mm) Orientation	TE/TR (ms)	Type of analyses	Coverage	Networks
Wei et al., 2010	10 men [19-35]	1.5 T BOLD/GE-EPI	-/-/5 Axial	40/3000	ICA	C5-T1*	-
Barry et al., 2014	22 (11 men)$ [28.4 ± 8.8]	7 T BOLD/GE-EPI	0.9/0.9/4 Axial	8/17	seed-based	C2-C5*	Dorso-Dorsal Ventro-Ventral Dorso-Ventral
Kong et al., 2014	20 men [26.45 ± 3.9]	3 T BOLD/GE-EPI	1/1/3 Axial	44/1890	ICA	C4-T1*	Dorso-Dorsal Ventro-Ventral
San Emeterio Nateras et al., 2016	9 men [29 ± 6.4]	3 T BOLD/EPI	1/1/3 Axial	26/2000	ICA seed-based	C1-C4*	Dorso-Dorsal Ventro-Ventral Dorso-Ventral
Barry et al., 2016	23 (11men) [25.7 ± 4.5]	7 T BOLD/GE-EPI	0.9/0.9/4 Axial	7.8/18	seed-based	C2-C5*	Dorso-Dorsal Ventro-Ventral
Liu et al., 2016	24 (18 men) [30 ± 5]	3T BOLD/GE-EPI	1.2/1.2/4 Axial	30/2000	seed-based		Dorso-Dorsal Ventro-Ventral
Eippert et al., 2017b	20 men$ [26.5 ± 3.9]	3T BOLD/GE-EPI	1/1/5 Axial	44/1890	seed-based	C4-T1*	Dorso-Dorsal Ventro-Ventral
Harita and Stroman, 2017	16 (2 men)$$ [21 ± 2]	3 T BOLD/FSE	1/1/4.5 Axial	76/2250	seed-based	Medulla-C6*	Dorso-Dorsal Ventro-Ventral
Conrad et al., 2018	56 (27 men)$ [29.2 ± 9.8]	7 T BOLD/GE-EPI	0.9/0.9/4 -	8/17	seed-based	C2-C5*	Dorso-Dorsal Ventro-Ventral Dorso-Ventral
Weber et al., 2018	23 (16 men)# [28.3 ± 2.4]	3T BOLD/GE-EPI	1/1/3 Axial	30/2500	seed-based	C3-C7*	Rostro-Caudal Dorso-Dorsal Ventro-Ventral Dorso-Ventral
Harita et al., 2019	16 (2 men)$$ [21 ± 2]	3T BOLD/FSE	1.5/1.5/2 Axial	76/6750	cluster-based	Brainstem-T2*	Dorso-Dorsal Ventro-Ventral Dorso-Ventral
Vahdat et al., 2020	24 (11 men) [25.1]	3T BOLD/GE-EPI	1.2/1.2/5 Axial	30&33/3050	ICA seed-based	Whole brain C4-T1*	Dorso-Dorsal Ventro-Ventral Spino-Cerebral
Kinany et al., 2020	22 (11 men) [28.5 ± 3.5]	3T BOLD/GE-EPI	1/1/3 Axial	30/2000	iCAPs	C1-T1*	Ventro-Ventral WM tracts

R: Right, L: Left, MSL: Motor sequence learning. BOLD: Blood Oxygen Level Dependent, EPI: Echo Planar Image, GE: Gradient Echo. ICA: Independent Component Analysis, iCAPs: Innovation-driven CoActivation Patterns. C: Cervical, L: Lumbar, S: Sacral, T: Thoracic

$ same participants were used in Eippert al. 2017b and Conrad et al. 2018

$$ same participants were used in Harita and Stroman, 2017 and Harita et al. 2019

#: same participants were used in Weber et al. 2018 and Weber et al. 2016

Coverage column: We put * for spinal location that correspond to vertebral level and no symbol when it's related to spinal segment.

6 *Spinal fMRI corroborates the existence of the two main spinal pathways?*

In this section, we describe and discuss the results of studies reviewed in the previous section with the goal of assessing, for the first time, the extent to which the typical functional organization in the ascending and descending pathways of the spinal cord described in electrophysiological studies, can also be revealed through *in vivo* fMRI investigations of this structure in humans. To this end, we assess whether the results strongly corroborate the expected spinal cord functional organization in hemicords (right or left sides), motor and sensory (anterior and posterior horns) and segmental (upper or lower limb) circuits (Figure 4). Finally, we then try to shed light on the neural processes that may explain the fMRI data observed during a motor or sensory task, as well as at rest.

Figure 4 around here, color, two columns

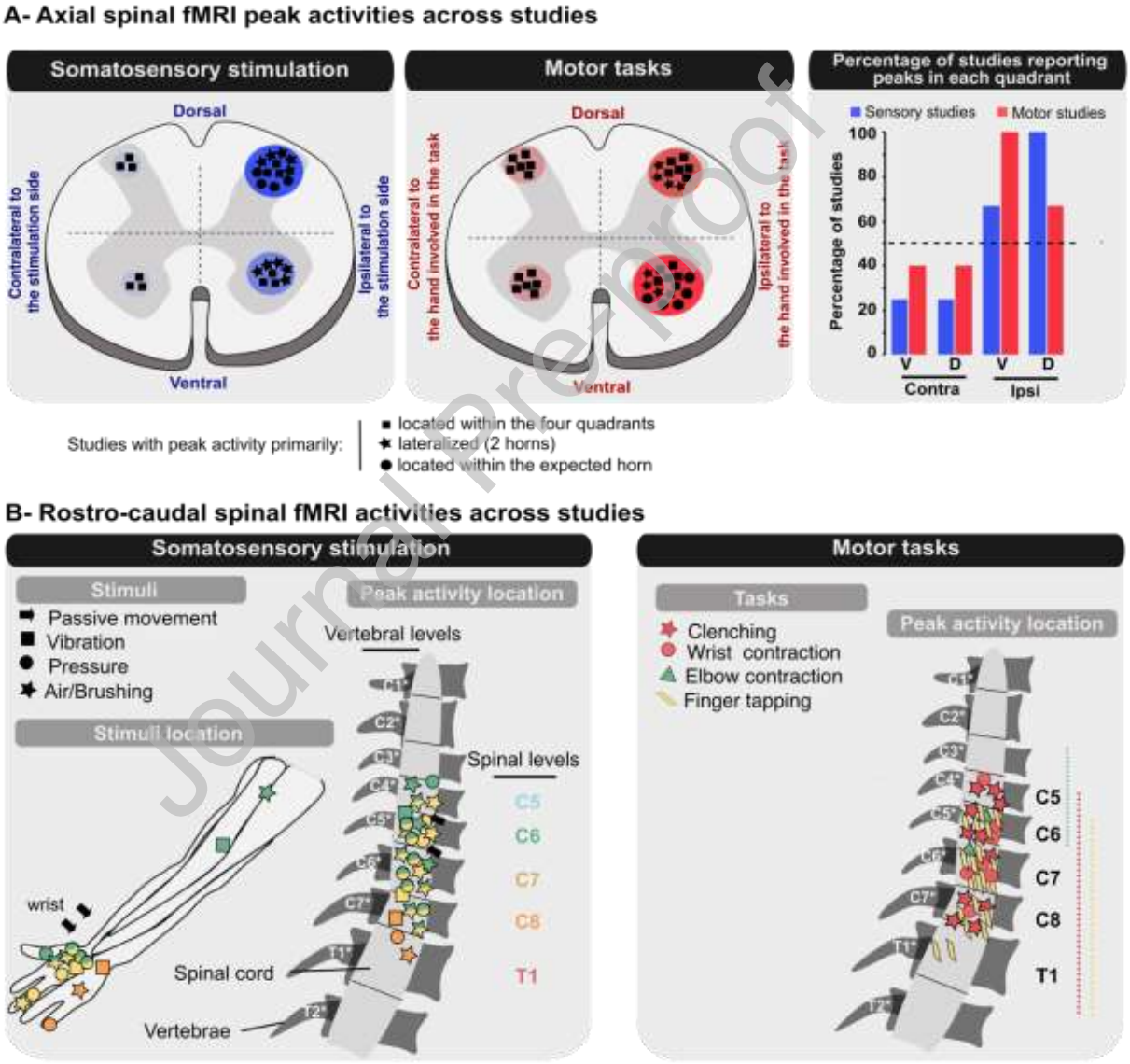

Figure 4 | Schematic representation of peaks of activity found in spinal fMRI studies using ipsilateral somatosensory stimulation (blue, n=12) or motor task performed using an upper limb (red, n=15) A- Schematic representations in the axial plane at the cervical level of the spinal cord. Activation peaks are presented in the four spinal horns: ventral-contralateral, dorsal-contralateral, ventral-ipsilateral and dorsal-ipsilateral. The terms "contralateral" and "ipsilateral" relate to

the side of the stimulation or the upper limb used to perform the motor tasks. The symbols represent the main peak(s) of activity in each study, which were observed either in the expected horn (one circle), in the ventral and dorsal ipsilateral horns (one star in the two horns) or in the four quadrants (one square in all four horns). The colored circle illustrates the proportion of studies found in each quadrant, with a larger size and a more intense color when the proportion is greater. The graph in the right column represents the values of this peak proportion for each quadrant and each type of experimental, somatosensory (blue) or motor (red) paradigms. Note that most studies have shown activity on the ipsilateral side of the spinal cord that extends to the ventral and dorsal horn. Furthermore, when studies reported preferential peaks of activity in the ventro-dorsal direction, the latter was mostly located in the ventral horn for motor studies and in the dorsal horn for somatosensory studies. **B- Schematic representations in rostro-caudal plane (C2-T1 spinal segments)**. The left display represents the location of somatic stimulation applied to the upper limb throughout the studies. The dark lines on the arm refer to different dermatomes according to Keegan and Garrett (1948) and the color correspond to the expected spinal level for the stimulated dermatome. The right illustration represents the locations of peak activities for different motor paradigms which include elbow (green), fingers (yellow) or wrist and finger (red). The color lines at the right of the spine represent the expected segment for the myotome involved in the task according to by Schirmer et al. (2011).

Note that we illustrate peak activations at the group level when reported. When studies reported only results of individual analysis, we illustrate the peak location corresponding to for the largest number of participants. V= Ventral, D= Dorsal, C= cervical, T= Thoracic, *: vertebrae level.

6.1 Lateralization of spinal fMRI activity corroborates hemicord pathways

a. Synopsis of findings reported in the literature

Most of the research groups that used ipsilateral sensory stimulation paradigms (n=9/12) have reported functional spinal cord activity changes that were predominantly detected on the ipsilateral side of the stimulation (Stroman and Ryner, 2001; Lawrence et al., 2008; Valsasina et al., 2008; Agosta et al., 2009b; Ghazni et al., 2010; Brooks et al., 2012; Rocca et al., 2012; Kornelsen et al., 2013; Weber et al., 2020, see Figure 4A). For example, such a pattern of findings has been reported by Brooks and colleagues (2012) who investigated the laterality of the BOLD signal related to pressures applied to the right or left eminence thenar (i.e., base of thumb). More specifically, statistical comparisons between the two hemicords revealed activity that was significantly greater within the expected cervical segment ipsilateral to the stimulation as compared to that of the contralateral side. These observations have also been corroborated in a very recent study, which found a significant lateralization of the activity at both subject and group levels in the ipsilateral hemicord during tactile brushing of the shoulder or third digit skin (Weber et al., 2020). A study in non-human primates has also confirmed the reliability of those previous results by combining electrophysiological recordings and fMRI during tactile stimuli of the digit (Wu et al., 2019). The authors found that tactile stimulation evoked both electrophysiological and BOLD signals co-localized primarily in the ipsilateral dorsal horn, as well as in the contralateral dorsal horn, albeit to a smaller extent.

Contrary to the results using sensory stimulation, lateralization of the motor-related spinal cord activity has been less consistent in studies involving unimanual motor tasks (Figure 4A). Indeed, six studies failed to demonstrate activity lateralization in the spinal cord as their pattern revealed bilateral activations (Backes et al., 2001; Bouwman et al., 2008; Govers et al., 2007; Madi et al., 2001; Ng et al., 2008; Stroman and Ryner, 2001), whereas nine others reported activity in both hemicords with a predominance for the ipsilateral side (Barry et al., 2020; Giulietti et al., 2008; Maieron et al., 2007; Smith and Kornelsen, 2011; Stroman et al., 1999; Vahdat et al., 2015; Weber et al., 2016; Xie et al., 2009; Yoshizawa et al., 1996). Of these studies, four used statistical approaches to assess the level of laterality (Bouwman et al., 2008; Maieron et al., 2007; Ng et al., 2008; Weber et al., 2016), but only two of them detected a significant degree of laterality at the spinal level (Maieron et al., 2007; Weber et al., 2016). For example, the most recent of these studies sought to evaluate the degree of lateralization by

statistically comparing the levels of activity in both left and right hemicord during either left or right wrist movement (Weber et al., 2016). The results revealed a significant lateralization of the activity towards the hemicord, ipsilateral to the limb executing the motor task, which was observed reliably across different sessions. Moreover, the authors supplemented these results with a multi-voxel pattern analysis that successfully differentiated between the experimental conditions e.g., left and right motor executions, at the C6 and C7 vertebral level. Altogether, the present findings suggest that although fMRI activity lateralization dominates across studies involving unilateral motor tasks, it is not a consistent feature and future research will be needed to identify the reasons that could explain this heterogeneous pattern of results.

In addition to task-related findings, studies investigating functional connectivity at rest have consistently reported the presence of bilateral spinal cord networks. Indeed, using the ICA approach, Kong et al. (2014) were able to identify separate resting-state networks involving the ventral and dorsal horns, on the left and right sides of the spinal cord, respectively, with good reliability and reproducibility. Similar bilateral networks were also reported by Barry et al. (2014) at 7T who used predefined regions of interests to assess the levels of correlation between distinct spinal regions. Interestingly, the authors demonstrated that correlations between the left and right dorsal horns, as well as between left and right ventral horns were reliable across participants. In fact, the existence of such a bilateral spinal functional connectivity has been reported and replicated in all of the most recent studies (Barry et al., 2016; Conrad et al., 2018; Eippert et al., 2017b; Harita et al., 2019; Harita and Stroman, 2017; Kinany et al., 2020; Liu et al., 2016; San Emeterio Nateras et al., 2016; Vahdat et al., 2020; Weber et al., 2018), hence demonstrating the robustness and reproducible nature of the resting-state functional connectivity at different segments of the human spinal cord.

b. Potential mechanisms underlying unilateral and bilateral fMRI activity

Corroborating the organization of the well-known spinal circuitries established by previous electrophysiology work, fMRI activations during somatosensory stimulation or motor tasks have been reported primarily in the ipsilateral horns. In fact, while all studies using motor paradigms have revealed activations in the ventral horns that were ipsilateral to the effectors performing the task, 40% of them have also reported activity in the contralateral dorsal and ventral horns (i.e., bilateral activations; Figure 4A). Although still unclear, this apparent inconsistency in the lateralization of the spinal cord activity during unimanual motor tasks could be explained in different ways. First, it is well known that axons from the reticulospinal tracts in humans, mainly involved in controlling axial and proximal muscles, innervate lower motor neuron bilaterally in the more medial parts of the ventral horn (Purves, 2018). Second, at the brain level, if unilateral movements are preferentially associated with the activation of contralateral motor areas, the recruitment of ipsilateral motor areas is also involved (Orban et al., 2010).This activation in both cerebral hemispheres could thus be the direct consequence of the activity observed in the two horns of the spinal cord. Third, muscle relaxation is not a passive phenomenon, but rather it involves a tonic level of motoneuron activity in order to maintain the muscle tone (i.e., the resting level of tension in a muscle) necessary to respond optimally to a voluntary or reflex command (Pierrot-Deseilligny and Burke, 2012). Finally, there is ample electrophysiological evidence supporting the presence of spinal interneurons with axonal projections that cross the spinal cord to the opposite side (for review see Maxwell and Soteropoulos, 2020). These interneurons, called commissural interneurons, are known to contain axons that project within the same segment, or between spinal segments by crossing at another segmental level, different from its origin (Matsuyama et al., 2006). To date, these have been mainly described in cats and rodents at the lumbar level where they seem to play a role in locomotor coordination and posture, although electrophysiological studies of human lumbar

spinal cord activity are also revealing a similar organization (Hanna-Boutros et al., 2014; Mrachacz-Kersting et al., 2018). Although there is limited evidence for the existence of commissural connections at the cervical level in non-human primates, it suggests that commissural interneurons are also involvement in the coordination of upper limb movements required for object manipulation (Soteropoulos et al., 2013).

At the brain level, the processing of sensory and motor information requires, in part, correlations between homotopic regions of the two hemispheres (Stark et al., 2008); and as such, brain rs-fMRI studies have reported the existence of bilateral networks reflecting the synchronization between the bilateral sensory and motor areas at rest. Furthermore, a strong lateralization of cerebral activity of the motor and sensory regions of the brain has also been observed during unilateral motor or sensory tasks, respectively. Interestingly, the results reported here from both resting-state and task-related spinal fMRI studies provide support for a similar organization of functional activity at the spinal cord level. In view of this mirror organization between these two structures, one can reasonably speculate that top-down signals from the brain may also play a role in the bilateral organization of spinal networks at rest. However, to date the fMRI research showing the bilateral axis of symmetry of the CNS has mainly focused on the brain organization. The current review, however, shows that the spinal fMRI techniques provide the opportunity to study the same functional organization *in vivo*, in humans, at the spinal level. More importantly, the latest innovative approaches allowing for the simultaneous scanning of the brain and cervical spinal cord represent valuable tools in assessing the topological organization of functional activity at rest as well as during tasks or stimulations, at multiple levels of the CNS. To this end, a recent fMRI study conducted by Doyon and his colleagues (Vahdat et al., 2020) was the first to provide evidence of activity lateralization at rest in the whole sensorimotor network, at both the brain and spinal levels. The authors showed that spinal cord spontaneous activity is primarily correlated with contralateral brain areas, findings that are consistent with decussation of afferent and efferent pathways described in section 3.

In sum, fMRI studies using task-related and resting-state paradigms have successfully revealed the organization of the spinal cord as two hemicords. Although during unilateral tasks, the spinal horn ipsilateral to the involved limb seems to be recruited preferentially, at rest, the activity of the two hemicords seems to be strongly coupled. Although still conjectural, evidence suggests that this coupling could reflect different physiological origins, such as brain-driven information, tonic activity of spinal neurons or commissural interneuron activity (Figure 2B). In addition, it is now possible, thanks to simultaneous fMRI of the brain and spinal cord, to observe the hemicord organization of sensorimotor pathways throughout the CNS and to assess the cross-organization of sensory and motor projections between the brain and spinal cord.

6.2 Dorso-ventral organization of spinal fMRI activity corroborates somatosensory and motor pathways

a. Synopsis of the findings reported in the literature

Only a few sensory stimulation studies have demonstrated prominent activation in the dorsal (sensory) horn, at the level of the stimulated dermatome (Lawrence et al., 2008; Ghazni et al., 2010; Rocca et al., 2012; Kornelsen et al., 2013, see Figure 4A). Instead, authors have often reported that the activation is also spreading toward the ventral horn (Agosta et al., 2008a, 2009b; Backes et al., 2001; Brooks et al., 2012; Stroman and Ryner, 2001; Summers et al., 2010; Valsasina et al., 2008; Weber et al., 2020). Such a pattern of results is consistent with those observed using motor paradigms, which elicit activations in both ventral and dorsal parts of the spinal cord as well. For example, Kinany et al. (2019) investigated

BOLD signal changes in the spinal cord during bimanual wrist extension, wrist adduction and finger abduction. The results showed activations that were not only localized in the ventral horns of the spinal cord (56.44%), but extended also dorsally as subjects were performing any of the three types of movements. Using a multi-voxel pattern analysis approach, the authors were then able to decode the pattern of activity specific to each of the three types of movements in the anterior hemicord, as well as in the posterior part, hence suggesting that the activity in these two parts of the spinal cord is task specific.

In addition to task-related paradigms, data based on rs-fMRI acquisitions have provided solid evidence of distinct dorsal-dorsal and ventral-ventral spinal networks, which have been replicated in most studies (Barry et al., 2016, 2014; Conrad et al., 2018; Eippert et al., 2017b; Harita et al., 2019; Harita and Stroman, 2017; Kong et al., 2014; Liu et al., 2016; San Emeterio Nateras et al., 2016; Vahdat et al., 2020; Weber et al., 2018). In fact, the level of reproducibility of findings related to the dorsal-dorsal and ventral-ventral spinal networks has been high, not only in humans, but across species (Chen et al., 2015; Wu et al., 2019), despite a variety of acquisition, processing and analysis techniques employed. By contrast, dorso-ventral resting-state networks have been less consistent (Barry et al., 2014; Conrad et al., 2018; Harita et al., 2019; San Emeterio Nateras et al., 2016; Weber et al., 2018). Moreover, most of these studies have revealed higher correlations between the left and right ventral horns than between the left and right dorsal ones (Barry et al., 2014, 2014; Conrad et al., 2018; Harita and Stroman, 2017; Kinany et al., 2020; Kong et al., 2014). Altogether, the results suggest that different patterns of activity can be observed depending on the experimental paradigm: studies involving motor or sensory tasks revealing co-activation of the dorsal and ventral horns, while rs-fMRI studies showing a consistent dorso-ventral division.

b. Potential mechanisms underlying ventro-dorsal organization of spinal fMRI activity

All task-related spinal fMRI studies reviewed here (Section 5) reported consistent activations in the ventral (for motor paradigms) and dorsal (for sensory paradigms) ipsilateral to the task or stimulation, the latter being consistent with the fact that ventral and dorsal grey matter are composed primarily of motoneurons and sensory neurons, respectively. Yet, two thirds of these studies have also found ipsilateral activations in the opposite horn (Figure 4A) indicating a spread of activation towards the dorsal horn during motor tasks, and towards the ventral horn during sensory simulation. Such a pattern of findings could be partly due to methodological limitations, such as the spatial resolution in axial planes and the spatial smoothing of the activity. Yet, it is also possible that those results are related to neurophysiological mechanisms underpinned by a top-down regulation of the signal (Schomburg, 1990; Willis and Coggeshall, 2004) and early local sensorimotor integration during motor contraction. Indeed, a complex spinal circuitry resides at the spinal level between sensory neurons, motoneurons and interneurons, thus leading to various connexions such as proprioceptive and cutaneous reflex loops, facilitation and recurrent inhibition of excited motoneurons as well as reciprocal inhibition between motoneurons of antagonist muscles (Pierrot-Deseilligny and Burke, 2012).

In contrast, all spinal rs-fMRI studies reviewed here (Section 5 and Table 3) identified segregated dorsal and ventral bilateral networks, with only 5 studies reporting dorso-ventral connectivity. The segregated ventral and dorsal networks observed at rest could be driven by the continuous integration of somatosensory information (dorsal horn), tonic level of motoneuron activity to maintain muscle tone and intrinsic motor coordination for autonomic functions such as breathing (ventral horn).

In fact, this idea of local intrinsic activities at rest coincides with the spatially segregated organization of the resting-state activity patterns found across different spinal segments. Alternatively, the dorso-ventral division at rest could also reflect a continuous somatotopic communication between the brain and spinal cord through the descending pathway (motor), and between the spinal cord and brain

through ascending pathways (sensory). The latter hypothesis is strongly supported by a recent study from our group that measured resting-state activity at different levels of the CNS using functional images of the brain and the spinal cord acquired simultaneously (Vahdat et al. 2020). The results revealed that the ventral and dorsal horns were functionally correlated with brain areas associated with motor and somatosensory functions, respectively (Figure 5), hence supporting the possibility of functional sub-divisions in the spinal cord. Finally, we also noticed a lower consistency of the dorso-dorsal networks compared to the ventro-ventral networks across rs-fMRI studies. The latter finding could be related to structural differences between the dorsal and ventral horns or to differences in baseline activity levels due to the tonic activity of motoneurons.

In sum, ascending (sensory) and descending (motor) pathways appear to operate distinctly at rest resulting in both: continuous communication between the brain and spinal cord and strong bilateral ventral and dorsal horn communication for local integration. In contrast, ascending and descending pathways strongly communicate with each other during tasks, probably to quickly adapt motor behaviors and motor command through sensory feedback and reflex loops.

Figure 5 around here, color, 2 columns

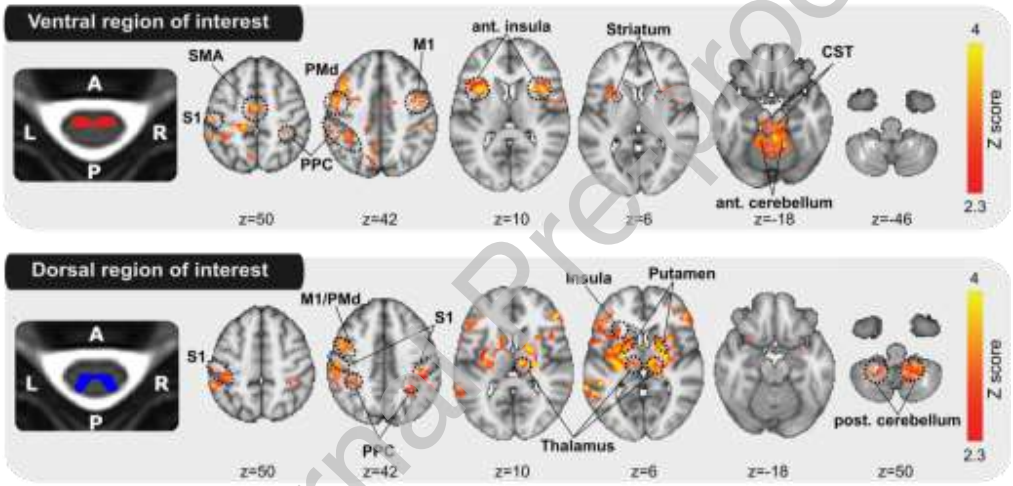

Figure 5 | Functional connectivity between spinal cord and brain during resting-state. The ventral horns (red, top) and the dorsal horns (blue, bottom) region of interests used for functional connectivity analysis and their associated brain functional connectivity maps are represented. As shown, functional connectivity with ventral and dorsal spinal cord were mostly significant with the brain sensorimotor network, in particular ventral spinal region was strongly connected with bilateral frontal motor areas and anterior cerebellum and dorsal spinal cord with bilateral primary somatosensory cortex, bilateral posterior parietal cortex, insula and posterior cerebellum. CST: corticospinal tract, M1: primary motor area, PMd: premotor dorsal area, PPC: posterior parietal cortex, S1: primary somatosensory cortex, A: anterior, P: posterior, L: Left, Right: right. Illustration was modified from Vahdat et al. 2020.

6.3 Rostro-caudal extent of spinal fMRI activity corroborates spinal segmental organization

a. Synopsis of the findings reported in the literature

As expected, somatosensory stimulation of the lower limbs has revealed activations in the caudal spinal cord segments (Kornelsen and Stroman, 2004; Lawrence et al., 2008), whereas stimulation of the upper limbs has been associated with more rostral foci of BOLD activity (Agosta et al., 2008a, 2009b; Backes et al., 2001; Brooks et al., 2012; Ghazni et al., 2010; Lawrence et al., 2008; Rocca et al., 2012; Stracke et al., 2005; Stroman and Ryner, 2001; Summers et al., 2010; Valsasina et al., 2008; Zhong et al., 2017). For

example, to investigate rostro-caudal organization of the spinal cord, Kornelsen et al. (2013) have evaluated the BOLD response along the entire thoracic spinal cord during skin vibrations delivered at different locations on the trunk, and showed activation in the corresponding thoracic spinal segments (T8 to T11 vertebrae). Similarly, activations located at the C5, C7, C6/7, T11, T11/T12 vertebrae have also been found during vibratory stimulation of the biceps, wrist, palm, patella and Achilles' tendon, respectively (Lawrence et al., 2008). Finally, increased activation in the C6/C7 spinal segment has been observed consistently during palm, wrist and thumb stimulation (Table 1), corresponding to the expected spinal segment localization of the stimulated dermatome (Figure 4B).

Such rostro-caudal organization of the spinal cord activity has also been observed during the execution of motor tasks. Madi et al. (2001) asked six participants to perform contractions with their right finger, wrist or elbow, which are known to recruit different myotomes. As expected, they found activation foci mostly located in the anticipated segmental level, at the site of the muscle innervation (elbow: C5-C6 vertebrae, wrist: C6-C7 vertebrae, finger: C7-T1 vertebrae). Importantly, the latter findings have been replicated in another study, which involved other myotomes recruited during bilateral wrist extension, wrist adduction and finger abduction, and included a larger group of subjects (n=19, Kinany et al., 2019). Again, these authors reported an increase in BOLD signal that followed a rostro-caudal organization associated with the three upper limb movements, and that corroborated with expected maps of spinal cord functional organization. Thus, across studies, the predicted rostro-caudal functional organisation has been mainly observed between C6-C7 during elbow contractions, C6-C8 when subjects are engaging in wrist movements (wrist contraction or clenching) and between the C6-T1 segments when they are required to produce finger tapping movements (Figure 4B).

Spatially-segregated patterns of resting-state activity have also been consistently reported in the rostro-caudal orientation using seed-based or ICA functional connectivity approaches (Barry et al., 2014; Eippert et al., 2017b; Kong et al., 2014; Liu et al., 2016; Weber et al., 2018). For example, such a functional organization in networks with limited rostro-caudal extent has been confirmed by Kong and colleagues (2014), who showed that intrinsic spinal cord networks never extended beyond the length of a given vertebrae. Recently, dynamic resting-state functional connectivity approaches provided additional support for this principle of organization (Kinany et al., 2020). In this study, the authors found not only strong functional coupling within the same spinal segment, but also anti-coupling patterns between different spinal segmental levels.

However, it is important to mention that the spread of activity toward non-expected spinal level has also been observed in different sensory stimulation or motor task studies. For example, Weber et al. (2020) showed at group level that tactile stimuli of the shoulder and third finger did not only give rise to activity in the expected C5-C7 segment, but that it extended along the rostro-caudal axis. Individual results indicating high inter-subject variability in the spatial localization of the activity as activation maps overlapped for only 14 of 24 participants. In addition, Strake et al, (2005) showed that somatosensory stimulation give rise not only to activations within the corresponding stimulated dermatomes (i.e. C6: thumb ,C7: 3th finger, C8: 5th finger) but in to an unexpected recruitment of the C3 and C4 spinal segments as well. Furthermore, such extension of activation to upper spinal segments have been reported systematically across participants during a finger taping task (Govers et al., 2007) and at group level during isometric contraction of the left wrist (Weber et al., 2016).

b. Potential physiological processes underlying rostro-caudal organization

As demonstrated above, spinal cord fMRI has been used successfully to demonstrate the overall functional organization of the spinal cord in the rostro-caudal direction (Figure 4B). Indeed, the localization of activations observed in these fMRI studies, although sometimes widespread, are

consistent with the heuristic map of myotomal (Schirmer et al., 2011) and dermatomal innervations (Keegan and Garrett, 1948) previously described in humans. These neural maps illustrate the fact that afferents from the same skin area and muscle group enter the spinal cord at the same segmental level, and that the cortical motor commands controlling the same muscle group also project to the same segment. It is also interesting to mention that fMRI results of the somatosensory studies were consistent regardless of the type of stimuli used (passive limb movements, air puffs, static pressure stimuli, dynamic brushing, superficial mechanical vibration and transcutaneous electrical stimulation of the median nerve). This observation might be partly due to the fact that a single tactile stimulus can activate different classes of mechanoreceptors (e.g. Meissner's corpuscles, Merkel's discs, Pacian's corpuscles, Ruffini's endings), all of which have the property of sending sensory information into the spinal cord ipsilaterally at the corresponding segmental level (for a review, see Ackerley and Kavounoudias, 2015).

Interestingly, the spatially segregated patterns of activity observed at rest also mirror the somatotopic distribution of sensory and motor signals from/to different body parts throughout the entire ascending and descending pathways (Barry et al., 2014; Eippert et al., 2017b; Kinany et al., 2020; Kong et al., 2014; Liu et al., 2016; Weber et al., 2018). Yet in addition to those segregated networks, two studies that investigated anti-correlation and anti-coupling in the spinal cord and have reported opposite patterns between segments (Kinany et al., 2020; Kong et al., 2014), the latter being related to intersegmental neurons that work in phase-lags for locomotion (Namba and Mulloney, 1999) or support the intersegmental inhibition for respiratory processes (McBain et al., 2016).

Although the activation peak at the group level seems to reflect the anatomical organization, most task-related fMRI studies have also reported a spread of BOLD responses in adjacent segments. Several physiological and methodological factors could be at the origin of these results. The first hypothesis is related to the well-known variability between individuals regarding the anatomical organization of the spinal cord. Indeed, an early study involving dissections of the spinal cord in human cadavers has revealed a strong inter-individual variability in the correspondence between spinal segments defined using the vertebrae *versus* those using the nerve rootlets; i.e., the more typical marker for the segmental division of the spinal cord (Lang and Bartram, 1982). Using structural MRI of the cervical spinal cord, Cadotte et al. (2015) have supported such findings, as they reported both consistent intra-individual rostro-caudal distribution of nerve rootlet in 20 healthy subjects, but a large inter-individual variability regarding the correspondence between spinal and vertebral level. Thus, to improve the correspondence of the neuroanatomy of the spinal cord between individuals, future studies will need to improve their normalisation strategies by, for example, establishing a segmental division at the individual level or using probabilistic segmental maps from a large sample of participants. Second, spread of the activation could be due to the experimental paradigms used. For example, fist clenching involves not only complex coordinated muscle contractions of the hand, but of the forearm as well. Furthermore, isometric contractions of the wrist can also lead to additional finger contractions. Thus, to identify the effect of muscle activity on BOLD spinal data more systematically, we believe that electromyographic signals should be recorded in conjunction with spinal cord fMRI acquisitions. Similarly, studies using sensory stimuli such as nerve stimulation, brushing, passive movement or vibration are rarely limited to the stimulation of a single dermatome and therefore may induce responses in adjacent segmental levels. Third, fMRI acquisition parameters such as axial orientation and thickness of the slices could also explain the lack of precision in the rostro-caudal plane. Altogether, the origin of the spread of activity to adjacent segments is still unclear and should be carefully considered in future studies.

In addition to the spread of activities toward an adjacent segment, activations engaging distant rostral spinal segment have also been observed during simple motor tasks or sensory stimuli. Such pattern of activation could be underpinned by the activity of different neurons located in the spinal cord, whose axonal endings project onto different segments of the spine. In fact, animal studies have demonstrated the existence of propriospinal neurons, with long-distance projections, which are involved in long motor reflexes and allow rapid correction of movement errors (for review see Flynn et al., 2011). In humans, such neurons have also been reported in the cervical cord through electrophysiological examination (for review see Pierrot-Deseilligny and Marchand-Pauvert, 2002). The cervical propriospinal neurons are located rostrally (C_3-C_4 segments) and are involved in the integration of somatosensory inputs to adjust upper limb motor command. This neural substrate could thus explain the rostral fMRI activities observed in four reviewed studies during tactile stimuli of the fingers (Stracke et al., 2005) or hand movements (Govers et al., 2007; Ng et al., 2008; Weber et al., 2016). However, it is difficult to assess the consistency and significance of this observation in past spinal fMRI studies given that a majority of them have limited their functional acquisitions to a field of view between the C_4-T_1 vertebrae (see Table 1 & Table 2). Thus, it would be interesting in the subsequent studies to acquire spinal fMRI data covering the entire cervical spinal cord, including the first cervical segments, in a more systematic way to obtain an overview of the spinal sensorimotor processing from the upper limbs.

Altogether, the rostro-caudal segregation of resting networks corresponds to the organization in spinal segments observed according to the dermatome or myotome recruited during tasks (e.g., sensory or motor). In other words, for each segment of the spinal cord, the neural substrates that support those behaviors appear to show a synchronous functional connectivity at rest, hence resulting in intrinsic spinal networks. However, future studies should more carefully consider individual anatomical variability in order to elucidate the methodological or physiological component of the spread of activities to adjacent segments.

6.4 Spinal fMRI activation is modulated during tasks and reveals local plasticity

In addition to the spatial functional organization of the spinal cord described above, some studies have reported significant correlations between fMRI signal changes and those related to the intensity (Madi et al., 2001) or complexity (Maieron et al., 2007; Ng et al., 2008) of movements generated during motor learning tasks. At the brain level, it is commonly accepted that the neural response, as measured with fMRI, is proportional to the intensity of proprioceptive or tactile stimulation (Landelle et al., 2020) or is correlated with improvement in performance on a motor skill learning task (Orban et al., 2010). However, to our knowledge, no study using a somatosensory paradigm has examined the link between somatosensory perception and fMRI responses in the spinal cord.

Doyon's group has recently investigated the neural correlates of motor learning using a novel *f*MRI protocol that allowed the simultaneous acquisition of both brain and cervical spinal cord images (Vahdat et al., 2015). In this study, participants were required to perform two motor sequence learning tasks with their non-dominant hand: a simple and a complex sequence of finger movements. The authors showed greater activation and a larger spatial extent in the complex compared to the simple learning motor task within the C6-C8 spinal segments (Figure 6). Importantly, the authors also found significant changes in cervical activation that was modulated in association with behavioral improvements, and that was linearly independent from the task-related signal in the brain structures known to be involved in this type of task. These results provide support for the existence of spinal cord intrinsic plasticity that contributes to the acquisition of new motor skills in humans, distinctly from that seen at the brain level. Such findings are consistent with a previous electrophysiological study from the same group who measured the Hoffman reflex (a measure of spinal cord sensorimotor excitability) in

the forearm and revealed persistent decrease of the reflex amplitude over the course of motor sequence learning (Lungu et al., 2010). Interpreting their simultaneous brain-spinal cord fMRI results in conjunction with this electrophysiological finding, Vahdat et al. (2015) proposed that the increase in spinal cord BOLD response during learning may be due to the establishment of neural plasticity related to the presynaptic inhibition of motoneurons.

Interestingly, in the same study (Vahdat et al. 2015), functional connectivity analyses revealed that the correlation between the spinal cord activity and that of the primary sensory motor cortex gradually decreased over the course of complex motor learning, while that between the spinal cord and the medial cerebellum increased. This observation is consistent with the idea that motor skill learning induces specific patterns of functional plasticity that encompass both the brain (Dayan and Cohen, 2011; Doyon and Benali, 2005; Hardwick et al., 2018), as well as the spinal cord circuits.

Figure 6 around here, color, one column

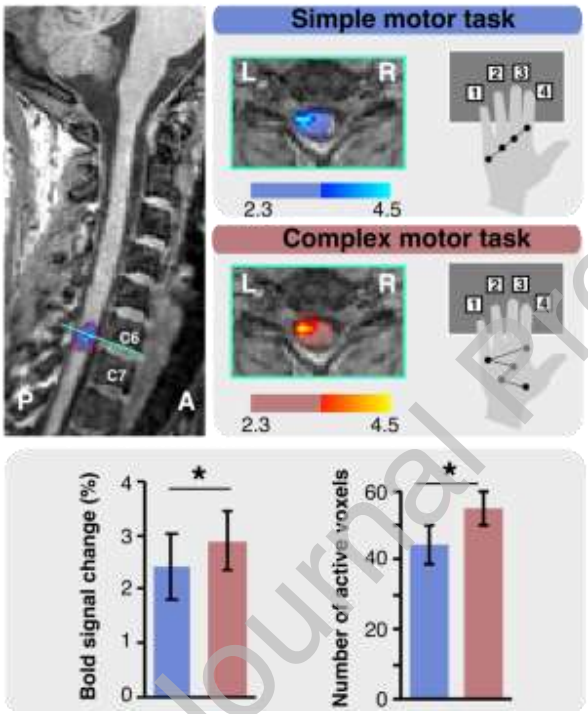

Figure 6 | Spinal cord activity correlated with behavioural improvements in motor performance. The activation maps represent the results of the main effect analysis related to the practice of two different motor sequences with the left fingers: one simple (in blue) and one complex (in red) sequence. In both conditions, the activation peaks were located in C7 spinal segment, and on the ipsilateral side of the finger movements. The graph on the left shows that the mean amplitude of the BOLD signal change was significantly higher in the complex, as compared to the simple sequence condition, while the graph on the right shows a significant difference in the number of active voxels (spatial extension) between the complex and simple sequence condition. Data from Vahdat et al. 2015.

Altogether, this body of research demonstrates that spinal fMRI in humans proves to be a powerful tool for studying, *in vivo,* the sensorimotor neural pathways. This review highlights a strong coherence between the results reported by task-related and rs-fMRI studies. Indeed, results from several investigations have consistently revealed: 1) the predominance for lateralization of the activation during task as well as a strong coupling between left and right hemicords at rest, 2) evidence not only

for the spreading of activation in both anterior and posterior horns during both sensory and motor task, but an antero-posterior segregation at rest, and 3) a limited rostro-caudal extent of the activation in all experimental paradigms. These results are in line with well-known hemicord (lateralization), sensory-motor (posterior-anterior) and rostro-caudal (spinal segment) organization of spinal pathways.

7 Applications to clinical research

In regard to the use of spinal fMRI in clinical settings and for clinical research, this method has the crucial advantage of providing *in vivo* spinal cord functional data, while being non-invasive and relatively accessible, both in terms of the range of patients that can be assessed, as well as the MR scanner manufacturers. One of its main uses in clinical setting is in comparing groups of participants (i.e., healthy vs. controls), as well as in the evaluation of functional changes over time in the same individual. As compared to most electrophysiological techniques, spinal fMRI can be employed to study and assess multiple spinal segments at the same time, with a good in-plane resolution within each of them, thus offering a major advantage in clinical practice, as discussed in detail below.

Yet, one of the main disadvantages is that the majority of spinal cord fMRI study done to date has been limited to the cervical cord. Further works are therefore needed to reduce our knowledge gap regarding the lower part of the spinal cord, as the lower segments have important functional roles in postural and gait control and may be associated with clinical dysfunction.

7.1 Using spinal fMRI to characterize and predict diseases progression affecting the spinal cord

Spinal cord disfunction is present in a number of neurological disorders including spinal cord injury (SCI), motoneurons diseases (MNDs), multiple sclerosis (MS) as well as during normal and pathological aging.

To date, outcome measures used in routine clinical practice to better characterize the neuronal changes underlying spinal cord disorders are based upon physical assessments, neurophysiological measurements (i.e., electromyography, compound muscle action potential, sensory or motor evoked potentials), anatomical imaging (MRI) and biological samplings (neurofilaments quantification). However, as these measurements provide incomplete information, a combination of them is needed to determine the level of residual functioning. Yet again, together they do not allow to describe fully the extent of the lesion(s) in the rostro-caudal and axial planes.

Thus, while the use of anatomical MRI data helps to locate structural abnormalities, the latter must be combined with behavioral performance to better understand the underlying functional alterations. In this review, we provide evidence that spinal fMRI is a promising tool for exploring the underlying neural substrate subserving various spinal cord functions. Furthermore, although it is currently used primarily for research purposes, we conjecture that the latter has great potential as a tool useful for clinical evaluations. The advantage of fMRI as a biomarker is to study spontaneous changes in the activity of spinal cord networks, not only during resting state, but also during a specific task that patients may have difficulty in performing. In addition, one can expect that in some diseases, functional connectivity changes will appear before measurable morphological changes. For example, compensatory mechanisms or activity changes related to changes in neurotransmission could be observed in fMRI and would allow early diagnosis of the disease.

The number of investigations that used spinal fMRI to examine plastic changes related to spinal abnormalities has grown significantly in the past ten years. Most of these studies have employed task-related spinal fMRI paradigms in patients with SCI. They have shown that BOLD activity can be detected below the level of injury, even though the patients could not feel the stimuli (Stroman, 2002; Stroman et al., 2004), as well as a greater increase in the activation magnitude in patients compared to healthy participants following sensory stimulation (Cadotte et al., 2012; Zhong et al., 2017). Interestingly, spinal fMRI studies in MS patients have also reported increased activation in cervical cord as compared to control subjects, with a more bilateral extent of the activation in response to tactile stimulations (Agosta et al., 2009a, 2008a; Valsasina et al., 2012, 2010) and passive movements (Agosta et al., 2008b). Altogether, these studies thus provide evidence of bilateral network overactivations related to somatosensory stimulation in both SCI and MS patients. The latter results are consistent with the reduction in lateralization of brain activity during somatosensory stimulation and motor tasks observed in MNDs, MS and SCI patients (for reviews see Shen et al., 2015; Peterson and Fling, 2018; Wang et al., 2019), as well as during normal (Landelle et al., 2020; Ward et al., 2008) and pathological aging (Wu et al., 2015). Given that this phenomenon is observed both in the brain and spinal cord, future studies should address this issue directly by carrying out simultaneous brain and spinal cord acquisitions (Tinnermann et al., 2017; Vahdat et al., 2020, 2015) in order to better understand the interactions in neural changes observed in sensorimotor network between these two levels of the CNS.

While task-related spinal fMRI offers new insights into disease-related changes in the brain and spinal cord, the spinal rs-fMRI also provides the opportunity to characterize these changes in a way that is more accessible in clinical practice. Indeed, the same resting-state protocols can be used regardless of the degree of physical or cognitive limitation of the patients (given that there is no task at hand). Moreover, the rs-fMRI will not only allow clinicians to compare patients to their healthy counterparts, but also to evaluate changes of the functional connectivity and architecture within the same patient over time. Thus, functional connectivity at rest in spinal cord could be a good candidate to be a clinical biomarker of disease progression and recovery, treatment success, as well as a predictor of disease evolution, provided that future studies will present evidence of pattern reproducibility in longitudinal spinal rs-fMRI studies.

7.2 Using spinal fMRI to monitor treatment approaches

Pathology of the spinal cord often leads to severe sensorimotor impairment involving unilateral or bilateral alteration of the sensory and motor pathways below the segment presenting the spinal abnormality. The use of drug treatments or rehabilitation methods to improve the sensorimotor capacities of patients have been tested in combination with brain fMRI in order to highlight the potential for functional restoration after such interventions. For example, brain investigation of MS patients have shown that brain neuroplasticity can be modulated by pharmacological treatment (Mainero et al., 2004) and by rehabilitation regimens, such as sensorimotor training (Tomassini et al., 2012). To our knowledge, no studies have investigated the effect of this type of treatment on the spinal cord function, although changes in resting-state connectivity related to spinal MS lesions have been recently observed (Conrad et al., 2018). Thus, spinal fMRI appears promising for evaluating the reorganization of the sensorimotor networks in the spinal cord and for demonstrating the benefits of such interventions.

Spinal fMRI for preoperative mapping has also shown to be very promising in SCI patients given the great heterogeneity of lesions between individuals. For example, epidural electrical stimulation of the spinal cord is an innovative technique being used to improve recovery after a spinal cord injury. Using matrix of electrodes applied dorsally to the lumbosacral spinal cord, this technique aims to activate

spinal circuits involved in motor function by recruiting proprioceptive afferents (for review, see Courtine and Sofroniew, 2019). Combined with an intense rehabilitation protocol, such electrical stimulations delivered with precise spatiotemporal patterns has been found to improve postural and locomotor abilities in patients with spinal cord injury (Wagner et al., 2018). Spinal cord fMRI recordings in those patients would therefore provide valuable information on the functional state of the sensorimotor circuits before and after treatment. Furthermore, establishing a diagnosis of the remaining functional activity in the spinal cord could be relevant to predict success of treatment and could be used as part of the inclusion process.

In summary, spinal fMRI has been used to date in a relatively narrow area of clinical research, but our review suggests that it may hold a very promising future. To be successful, such clinical applications should be undertaken by combining state-of-the-art fMRI acquisition and processing techniques developed over the last few years, with a good understanding of the physiological mechanisms underlying the observed functional activity.

8 Limitation and recommendation:

In section 1, we described the technical challenges and limitations (e.g., small dimension of the spinal cord, deep structure surrounded by different types of tissue, long-extended rostro-caudal curvature, physiological noise, and physical movements) that researchers have been dealing with when acquiring spinal fMRI data. Thanks to technological advances in this field, however, it's now possible to optimize the image acquisition protocols and data processing to significantly improve the image quality in spinal fMRI (section 4). Indeed, we strongly recommend following the guidelines for improving fMRI acquisition methods that have been introduced over the last decade to maximize signal-to-noise ratios and resolution, and that have been well described in a recent review article (Cohen-Adad, 2017; Stroman et al., 2014; Tinnermann et al., 2020). In addition to technological advances, it is recommended that the experimenter positions the participant carefully (cervical cord almost straight and to minimize the curvature of the neck). A fat saturation and stabilization device for MRI (Sadpad™) should also be positioned around the neck and chest to reduce body movements and increase the magnetic field homogeneity across the cervical spine, particularly near the laryngopharyngeal region. Finally, it is important to inform the participant to try not to swallow during the scans or at least to do so very gently.

In addition to the challenge of acquiring images covering the spinal cord or the brain and spinal cord simultaneously, data processing must follow recent guidelines. While standard neuroimaging software (e.g. SPM, FSL, AFNI, BrainVoyager, nilearn toolbox …) have been developed to perform pre-processing and statistical analysis of the brain, standard processing pipelines for the spinal cord are just beginning to emerge. The most recent studies (Islam et al., 2019; Kinany et al., 2020, 2019; Vahdat et al., 2020; Weber et al., 2020, 2018, 2016) and review papers article (Cohen-Adad, 2017; Stroman et al., 2014; Tinnermann et al., 2020) are now referring to the Spinal Cord Toolbox (SCT), an open-source software developed for processing spinal cord MRI data. SCT offers a standardization of the processing pipelines although some steps still have to be done or corrected manually (e.g. segmentation, vertebral labeling). In addition to preprocessing, physiological noise modelling should be applied carefully to increase the reliability of spinal cord fMRI results. We recommend to combine physiological noise modeling that account for cardiac and respiratory cycle based on the RETROICOR technique (Glover et al., 2000) with the CompCor method based on principal components derived from noise in regions-of-interest (e.g CSF) (Benhzadi et al. 2007). Despite such recommendations, signal losses may occur and measurements of the tSNR and inter-scan motion will allow the experimenter to exclude some participants or scans. Finally, the development of more standardized acquisition and pre-processing

pipelines is gradually being accompanied by more reproducible group analyses. To provide an overview of the preprocessing and analysis steps used in the 44 studies included in this review, we have reported this information in supplementary tables (Tables S4, S5, S6). Future brain and spinal-cord fMRI studies will need to carefully consider the recent recommendation proposed by the Committee on Best Practices in Data Analysis and Sharing (COBIDAS) to improve the reproducibility of analysis methods and the confidence in the published results.

9 *Concluding remarks*

Human spinal cord fMRI investigating somatosensory and motor systems generated much excitement in recent years through a wide variety of experimental paradigms. Although the development of reliable spinal fMRI methods has been a long and slow process due to the many technical challenges inherent to this technique, the present review demonstrates that this particular application of functional neuroimaging has resulted in major advances in the field. Indeed, the body of research reviewed here not only confirms and support the basic notions acquired over the years on the physiological organization of the spinal cord, but also show that these studies increase our understanding of the complex role of the spinal cord in the processing of sensorimotor information required for movement perception, execution and learning; processes that are typically studied mainly in 'higher' brain areas by the neuroimaging community. By cross-referencing the results obtained at the fMRI macroscopic level in humans with our previous knowledge of the spinal ascending (sensory) and descending (motor) pathways from mainly electrophysiological observations, we demonstrate here that spinal fMRI is a powerful non-invasive tool for exploring human spinal cord pathways on a large scale, as well as at multiple levels of the CNS. Indeed, we highlight strong cross-validation between task-related and resting state fMRI results to investigate ascending and descending pathways in accordance with well-known hemi-body (lateralization), sensory-motor (posterior-anterior) and rostro-caudal (spinal segment) organization of spinal pathways. The field has also accumulated consistent evidence of different activity patterns depending on whether participants are involved in a sensory stimulation paradigm, motor task or at rest.

Moreover, spinal fMRI presents distinct advantages over the electrophysiological techniques in terms of *in vivo* imaging of multiple segments and 3D localization of activity. Although future studies will need to carefully consider its inherent technical challenges, we believe that the current advances provide a good bridgehead to expand the use of spinal fMRI in clinical settings and clinical research to better characterize diseases affecting this structure, predict their progression, and guide therapeutic interventions. Finally, the simultaneous acquisition of both brain and cervical spinal cord fMRI data is a very effective tool in the study of healthy and pathological neurophysiological sensorimotor mechanisms in vivo, encompassing both motor and sensory systems as a whole.

Data and Code Availability

The data used for the figure 6 were acquired by Vahdat et al. 2015 and are available here:
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ackerley, R., Kavounoudias, A., 2015. The role of tactile afference in shaping motor behaviour and implications for prosthetic innovation. Neuropsychologia 79, 192–205. https://doi.org/10.1016/j.neuropsychologia.2015.06.024

Agosta, F., Valsasina, P., Absinta, M., Sala, S., Caputo, D., Filippi, M., 2009a. Primary Progressive Multiple Sclerosis: Tactile-associated Functional MR Activity in the Cervical Spinal Cord. Radiology 253, 209–215. https://doi.org/10.1148/radiol.2532090187

Agosta, F., Valsasina, P., Caputo, D., Rocca, M.A., Filippi, M., 2009b. Tactile-associated fMRI recruitment of the cervical cord in healthy subjects. Hum. Brain Mapp. 30, 340–345. https://doi.org/10.1002/hbm.20499

Agosta, F., Valsasina, P., Caputo, D., Stroman, P.W., Filippi, M., 2008a. Tactile-associated recruitment of the cervical cord is altered in patients with multiple sclerosis. NeuroImage 39, 1542–1548. https://doi.org/10.1016/j.neuroimage.2007.10.048

Agosta, F., Valsasina, P., Rocca, M.A., Caputo, D., Sala, S., Judica, E., Stroman, P.W., Filippi, M., 2008b. Evidence for enhanced functional activity of cervical cord in relapsing multiple sclerosis. Magn. Reson. Med. 59, 1035–1042. https://doi.org/10.1002/mrm.21595

Backes, W.H., Mess, W.H., Wilmink, J.T., 2001. Functional MR imaging of the cervical spinal cord by use of median nerve stimulation and fist clenching. AJNR Am J Neuroradiol 22, 1854–1859.

Barry, R.L., Conrad, B.N., Maki, S., Watchmaker, J.M., McKeithan, L.J., Box, B.A., Weinberg, Q.R., Smith, S.A., Gore, J.C., 2020. Multi-shot acquisitions for stimulus-evoked spinal cord BOLD fMRI. Magn Reson Med mrm.28570. https://doi.org/10.1002/mrm.28570

Barry, R.L., Rogers, B.P., Conrad, B.N., Smith, S.A., Gore, J.C., 2016. Reproducibility of resting state spinal cord networks in healthy volunteers at 7 Tesla. NeuroImage 133, 31–40. https://doi.org/10.1016/j.neuroimage.2016.02.058

Barry, R.L., Smith, S.A., Dula, A.N., Gore, J.C., 2014. Resting state functional connectivity in the human spinal cord. eLife 3, e02812. https://doi.org/10.7554/eLife.02812

Barry, R.L., Vannesjo, S.J., By, S., Gore, J.C., Smith, S.A., 2018. Spinal cord MRI at 7T. NeuroImage 168, 437–451. https://doi.org/10.1016/j.neuroimage.2017.07.003

Beissner, F., Schumann, A., Brunn, F., Eisenträger, D., Bär, K.-J., 2014. Advances in functional magnetic resonance imaging of the human brainstem. NeuroImage 86, 91–98. https://doi.org/10.1016/j.neuroimage.2013.07.081

Biswal, B., Zerrin Yetkin, F., Haughton, V.M., Hyde, J.S., 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar mri. Magnetic Resonance in Medicine 34, 537–541. https://doi.org/10.1002/mrm.1910340409

Bouwman, C.J.C., Wilmink, J.T., Mess, W.H., Backes, W.H., 2008. Spinal cord functional MRI at 3 T: Gradient echo echo-planar imaging versus turbo spin echo. NeuroImage 43, 288–296. https://doi.org/10.1016/j.neuroimage.2008.07.024

Brooks, J.C.W., Beckmann, C.F., Miller, K.L., Wise, R.G., Porro, C.A., Tracey, I., Jenkinson, M., 2008. Physiological noise modelling for spinal functional magnetic resonance imaging studies. NeuroImage 39, 680–692. https://doi.org/10.1016/j.neuroimage.2007.09.018

Brooks, J.C.W., Kong, Y., Lee, M.C., Warnaby, C.E., Wanigasekera, V., Jenkinson, M., Tracey, I., 2012. Stimulus Site and Modality Dependence of Functional Activity within the Human Spinal Cord. Journal of Neuroscience 32, 6231–6239. https://doi.org/10.1523/JNEUROSCI.2543-11.2012

Cadotte, D.W., Bosma, R., Mikulis, D., Nugaeva, N., Smith, K., Pokrupa, R., Islam, O., Stroman, P.W., Fehlings, M.G., 2012. Plasticity of the Injured Human Spinal Cord: Insights Revealed by Spinal Cord Functional MRI. PLoS ONE 7, e45560. https://doi.org/10.1371/journal.pone.0045560

Cadotte, D.W., Cadotte, A., Cohen-Adad, J., Fleet, D., Livne, M., Wilson, J.R., Mikulis, D., Nugaeva, N., Fehlings, M.G., 2015. Characterizing the Location of Spinal and Vertebral Levels in the Human Cervical Spinal Cord. AJNR Am J Neuroradiol 36, 803–810. https://doi.org/10.3174/ajnr.A4192

Chen, L.M., Mishra, A., Yang, P.-F., Wang, F., Gore, J.C., 2015. Injury alters intrinsic functional connectivity within the primate spinal cord. Proc Natl Acad Sci USA 112, 5991–5996. https://doi.org/10.1073/pnas.1424106112

Cohen-Adad, J., 2017. Functional Magnetic Resonance Imaging of the Spinal Cord: Current Status and Future Developments. Seminars in Ultrasound, CT and MRI 38, 176–186. https://doi.org/10.1053/j.sult.2016.07.007

Conrad, B.N., Barry, R.L., Rogers, B.P., Maki, S., Mishra, A., Thukral, S., Sriram, S., Bhatia, A., Pawate, S., Gore, J.C., Smith, S.A., 2018. Multiple sclerosis lesions affect intrinsic functional connectivity of the spinal cord. Brain 141, 1650–1664. https://doi.org/10.1093/brain/awy083

Courtine, G., Sofroniew, M.V., 2019. Spinal cord repair: advances in biology and technology. Nat Med 25, 898–908. https://doi.org/10.1038/s41591-019-0475-6

Damoiseaux, J.S., Rombouts, S.A.R.B., Barkhof, F., Scheltens, P., Stam, C.J., Smith, S.M., Beckmann, C.F., 2006. Consistent resting-state networks across healthy subjects. Proceedings of the National Academy of Sciences 103, 13848–13853. https://doi.org/10.1073/pnas.0601417103

Dayan, E., Cohen, L.G., 2011. Neuroplasticity Subserving Motor Skill Learning. Neuron 72, 443–454. https://doi.org/10.1016/j.neuron.2011.10.008

De Leener, B., Fonov, V.S., Collins, D.L., Callot, V., Stikov, N., Cohen-Adad, J., 2018. PAM50: Unbiased multimodal template of the brainstem and spinal cord aligned with the ICBM152 space. NeuroImage 165, 170–179. https://doi.org/10.1016/j.neuroimage.2017.10.041

De Leener, B., Lévy, S., Dupont, S.M., Fonov, V.S., Stikov, N., Louis Collins, D., Callot, V., Cohen-Adad, J., 2017. SCT: Spinal Cord Toolbox, an open-source software for processing spinal cord MRI data. NeuroImage 145, 24–43. https://doi.org/10.1016/j.neuroimage.2016.10.009

De Leener, B., Taso, M., Cohen-Adad, J., Callot, V., 2016. Segmentation of the human spinal cord. Magn Reson Mater Phy 29, 125–153. https://doi.org/10.1007/s10334-015-0507-2

Di Martino, A., Scheres, A., Margulies, D.S., Kelly, A.M.C., Uddin, L.Q., Shehzad, Z., Biswal, B., Walters, J.R., Castellanos, F.X., Milham, M.P., 2008. Functional Connectivity of Human Striatum: A Resting State fMRI Study. Cerebral Cortex 18, 2735–2747. https://doi.org/10.1093/cercor/bhn041

Doyon, J., Benali, H., 2005. Reorganization and plasticity in the adult brain during learning of motor skills. Current Opinion in Neurobiology 15, 161–167. https://doi.org/10.1016/j.conb.2005.03.004

Eippert, F., Kong, Y., Jenkinson, M., Tracey, I., Brooks, J.C.W., 2017a. Denoising spinal cord fMRI data: Approaches to acquisition and analysis. NeuroImage 154, 255–266. https://doi.org/10.1016/j.neuroimage.2016.09.065

Eippert, F., Kong, Y., Winkler, A.M., Andersson, J.L., Finsterbusch, J., Büchel, C., Brooks, J.C.W., Tracey, I., 2017b. Investigating resting-state functional connectivity in the cervical spinal cord at 3 T. NeuroImage 147, 589–601. https://doi.org/10.1016/j.neuroimage.2016.12.072

Finsterbusch, J., 2013. Functional neuroimaging of inner fields-of-view with 2D-selective RF excitations. Magnetic Resonance Imaging 31, 1228–1235. https://doi.org/10.1016/j.mri.2013.03.005

Flynn, J.R., Graham, B.A., Galea, M.P., Callister, R.J., 2011. The role of propriospinal interneurons in recovery from spinal cord injury. Neuropharmacology 60, 809–822. https://doi.org/10.1016/j.neuropharm.2011.01.016

Ghazni, N.F., Cahill, C.M., Stroman, P.W., 2010. Tactile Sensory and Pain Networks in the Human Spinal Cord and Brain Stem Mapped by Means of Functional MR Imaging. AJNR Am J Neuroradiol 31, 661–667. https://doi.org/10.3174/ajnr.A1909

Giulietti, G., Giove, F., Garreffa, G., Colonnese, C., Mangia, S., Maraviglia, B., 2008. Characterization of the functional response in the human spinal cord: Impulse-response function and linearity. NeuroImage 42, 626–634. https://doi.org/10.1016/j.neuroimage.2008.05.006

Glover, G.H., Li, T.Q., Ress, D., 2000. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. Magn Reson Med 44, 162–167. https://doi.org/10.1002/1522-2594(200007)44:1<162::aid-mrm23>3.0.co;2-e

Govers, N., Béghin, J., Van Goethem, J.W.M., Michiels, J., van den Hauwe, L., Vandervliet, E., Parizel, P.M., 2007. Functional MRI of the cervical spinal cord on 1.5 T with fingertapping: to what extent is it feasible? Neuroradiology 49, 73–81. https://doi.org/10.1007/s00234-006-0162-4

Guell, X., Schmahmann, J.D., Gabrieli, J.D., Ghosh, S.S., 2018. Functional gradients of the cerebellum. eLife 7, e36652. https://doi.org/10.7554/eLife.36652

Hanna-Boutros, B., Sangari, S., Karasu, A., Giboin, L.-S., Marchand-Pauvert, V., 2014. Task-related modulation of crossed spinal inhibition between human lower limbs. Journal of Neurophysiology 111, 1865–1876. https://doi.org/10.1152/jn.00838.2013

Hardwick, R.M., Caspers, S., Eickhoff, S.B., Swinnen, S.P., 2018. Neural correlates of action: Comparing meta-analyses of imagery, observation, and execution. Neuroscience & Biobehavioral Reviews 94, 31–44. https://doi.org/10.1016/j.neubiorev.2018.08.003

Harita, S., Ioachim, G., Powers, J., Stroman, P.W., 2019. Investigation of Resting-State BOLD Networks in the Human Brainstem and Spinal Cord. Neuroscience 404, 71–81. https://doi.org/10.1016/j.neuroscience.2019.02.009

Harita, S., Stroman, P.W., 2017. Confirmation of resting-state BOLD fluctuations in the human brainstem and spinal cord after identification and removal of physiological noise: Resting-State BOLD fMRI in the Human Brainstem and Spinal Cord. Magn. Reson. Med. 78, 2149–2156. https://doi.org/10.1002/mrm.26606

Heeger, D.J., Ress, D., 2002. What does fMRI tell us about neuronal activity? Nat Rev Neurosci 3, 142–151. https://doi.org/10.1038/nrn730

Islam, H., Law, C.S.W., Weber, K.A., Mackey, S.C., Glover, G.H., 2019. Dynamic per slice shimming for simultaneous brain and spinal cord fMRI. Magn Reson Med 81, 825–838.

https://doi.org/10.1002/mrm.27388

Keegan, J.J., Garrett, F.D., 1948. The segmental distribution of the cutaneous nerves in the limbs of man. Anat. Rec. 102, 409–437. https://doi.org/10.1002/ar.1091020403

Kinany, N., Pirondini, E., Martuzzi, R., Mattera, L., Micera, S., Van de Ville, D., 2019. Functional imaging of rostrocaudal spinal activity during upper limb motor tasks. NeuroImage 200, 590–600. https://doi.org/10.1016/j.neuroimage.2019.05.036

Kinany, N., Pirondini, E., Micera, S., Van De Ville, D., 2020. Dynamic Functional Connectivity of Resting-State Spinal Cord fMRI Reveals Fine-Grained Intrinsic Architecture. Neuron S0896627320305687. https://doi.org/10.1016/j.neuron.2020.07.024

Kong, Y., Eippert, F., Beckmann, C.F., Andersson, J., Finsterbusch, J., Büchel, C., Tracey, I., Brooks, J.C.W., 2014. Intrinsically organized resting state networks in the human spinal cord. Proc Natl Acad Sci USA 111, 18067–18072. https://doi.org/10.1073/pnas.1414293111

Kong, Y., Jenkinson, M., Andersson, J., Tracey, I., Brooks, J.C.W., 2012. Assessment of physiological noise modelling methods for functional imaging of the spinal cord. NeuroImage 60, 1538–1549. https://doi.org/10.1016/j.neuroimage.2011.11.077

Kornelsen, J., Smith, S.D., McIver, T.A., Sboto-Frankenstein, U., Latta, P., Tomanek, B., 2013. Functional MRI of the thoracic spinal cord during vibration sensation. J Magn Reson Imaging 37, 981–985. https://doi.org/10.1002/jmri.23819

Kornelsen, J., Stroman, P.W., 2004. fMRI of the lumbar spinal cord during a lower limb motor task. Magn. Reson. Med. 52, 411–414. https://doi.org/10.1002/mrm.20157

Landelle, C., Anton, J.-L., Nazarian, B., Sein, J., Gharbi, A., Felician, O., Kavounoudias, A., 2020. Functional brain changes in the elderly for the perception of hand movements: A greater impairment occurs in proprioception than touch. NeuroImage 220, 117056. https://doi.org/10.1016/j.neuroimage.2020.117056

Lang, J., Bartram, C.T., 1982. [Fila radicularia of the ventral and dorsal radices of the human spinal cord]. Gegenbaurs Morphol Jahrb 128, 417–462.

Lawrence, J., Stroman, P.W., Bascaramurty, S., Jordan, L.M., Malisza, K.L., 2004. Correlation of functional activation in the rat spinal cord with neuronal activation detected by immunohistochemistry. NeuroImage 22, 1802–1807. https://doi.org/10.1016/j.neuroimage.2004.04.001

Lawrence, J.M., Stroman, P.W., Kollias, S.S., 2008. Functional magnetic resonance imaging of the human spinal cord during vibration stimulation of different dermatomes. Neuroradiology 50, 273–280. https://doi.org/10.1007/s00234-007-0338-6

Lévy, S., Benhamou, M., Naaman, C., Rainville, P., Callot, V., Cohen-Adad, J., 2015. White matter atlas of the human spinal cord with estimation of partial volume effect. NeuroImage 119, 262–271. https://doi.org/10.1016/j.neuroimage.2015.06.040

Liu, X., Zhou, F., Li, X., Qian, W., Cui, J., Zhou, I.Y., Luk, K.D.K., Wu, Ed.X., Hu, Y., 2016. Organization of the intrinsic functional network in the cervical spinal cord: A resting state functional MRI study. Neuroscience 336, 30–38. https://doi.org/10.1016/j.neuroscience.2016.08.042

Lungu, O., Frigon, A., Piché, M., Rainville, P., Rossignol, S., Doyon, J., 2010. Changes in Spinal Reflex Excitability Associated With Motor Sequence Learning. Journal of Neurophysiology 103, 2675–2683. https://doi.org/10.1152/jn.00006.2010

Madi, S., Flanders, A.E., Vinitski, S., Herbison, G.J., Nissanov, J., 2001. Functional MR imaging of the human cervical spinal cord. AJNR Am J Neuroradiol 22, 1768–1774.

Maieron, M., Iannetti, G.D., Bodurka, J., Tracey, I., Bandettini, P.A., Porro, C.A., 2007. Functional Responses in the Human Spinal Cord during Willed Motor Actions: Evidence for Side- and Rate-Dependent Activity. Journal of Neuroscience 27, 4182–4190. https://doi.org/10.1523/JNEUROSCI.3910-06.2007

Mainero, C., Inghilleri, M., Pantano, P., Conte, A., Lenzi, D., Frasca, V., Bozzao, L., Pozzilli, C., 2004. Enhanced brain motor activity in patients with MS after a single dose of 3,4-diaminopyridine. Neurology 62, 2044–2050. https://doi.org/10.1212/01.WNL.0000129263.14219.A8

Matsuyama, K., Kobayashi, S., Aoki, M., 2006. Projection patterns of lamina VIII commissural neurons in the lumbar spinal cord of the adult cat: An anterograde neural tracing study. Neuroscience 140, 203–218. https://doi.org/10.1016/j.neuroscience.2006.02.005

Maxwell, D.J., Soteropoulos, D.S., 2020. The mammalian spinal commissural system: properties and functions. Journal of Neurophysiology 123, 4–21. https://doi.org/10.1152/jn.00347.2019

McBain, R.A., Taylor, J.L., Gorman, R.B., Gandevia, S.C., Butler, J.E., 2016. Human intersegmental reflexes from intercostal afferents to scalene muscles: Human intersegmental reflexes in respiratory muscles. Exp Physiol 101, 1301–1308. https://doi.org/10.1113/EP085907

Mrachacz-Kersting, N., Gervasio, S., Marchand-Pauvert, V., 2018. Evidence for a Supraspinal Contribution to the Human Crossed Reflex Response During Human Walking. Front. Hum. Neurosci. 12, 260. https://doi.org/10.3389/fnhum.2018.00260

Namba, H., Mulloney, B., 1999. Coordination of Limb Movements: Three Types of Intersegmental Interneurons in the Swimmeret System and Their Responses to Changes in Excitation. Journal of Neurophysiology 81, 2437–2450. https://doi.org/10.1152/jn.1999.81.5.2437

Nardone, R., Höller, Y., Taylor, A., Thomschewski, A., Orioli, A., Frey, V., Trinka, E., Brigo, F., 2015. Noninvasive Spinal Cord Stimulation: Technical Aspects and Therapeutic Applications: Spinal Cord Stimulation. Neuromodulation: Technology at the Neural Interface 18, 580–591. https://doi.org/10.1111/ner.12332

Ng, M.C., Wong, K.K., Li, G., Lai, S., Yang, E.S., Hu, Y., Luk, K.D., 2006. Proton-density-weighted spinal fMRI with sensorimotor stimulation at 0.2 T. NeuroImage 29, 995–999. https://doi.org/10.1016/j.neuroimage.2005.08.011

Ng, M.-C., Wu, E.X., Lau, H.-F., Hu, Y., Lam, E.Y., Luk, K.D., 2008. Cervical spinal cord BOLD fMRI study: Modulation of functional activation by dexterity of dominant and non-dominant hands. NeuroImage 39, 825–831. https://doi.org/10.1016/j.neuroimage.2007.09.026

Orban, P., Peigneux, P., Lungu, O., Albouy, G., Breton, E., Laberenne, F., Benali, H., Maquet, P., Doyon, J., 2010. The multifaceted nature of the relationship between performance and brain activity in motor sequence learning. NeuroImage 49, 694–702. https://doi.org/10.1016/j.neuroimage.2009.08.055

Penfield, W., Boldrey, E., 1937. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 60, 389–443. https://doi.org/10.1093/brain/60.4.389

Peterson, D.S., Fling, B.W., 2018. How changes in brain activity and connectivity are associated with motor performance in people with MS. NeuroImage: Clinical 17, 153–162. https://doi.org/10.1016/j.nicl.2017.09.019

Piché, M., Cohen-Adad, J., Nejad, M.K., Perlbarg, V., Xie, G., Beaudoin, G., Benali, H., Rainville, P.,

2009. Characterization of cardiac-related noise in fMRI of the cervical spinal cord. Magnetic Resonance Imaging 27, 300–310. https://doi.org/10.1016/j.mri.2008.07.019

Piché, M., Paquette, T., Leblond, H., 2017. Tight neurovascular coupling in the spinal cord during nociceptive stimulation in intact and spinal rats. Neuroscience 355, 1–8. https://doi.org/10.1016/j.neuroscience.2017.04.038

Pierrot-Deseilligny, E., Burke, D., 2005. The Circuitry of the Human Spinal Cord: Its Role in Motor Control and Movement Disorders, 1st ed. Cambridge University Press. https://doi.org/10.1017/CBO9780511545047

Pierrot-Deseilligny, E., Burke, D.J., 2012. The circuitry of the human spinal cord: spinal and corticospinal mechanisms of movement. Cambridge University Press, Cambridge, England ; New York.

Pierrot-Deseilligny, E., Marchand-Pauvert, V., 2002. A cervical propriospinal system in man. Adv Exp Med Biol 508, 273–279. https://doi.org/10.1007/978-1-4615-0713-0_33

Purves, D. (Ed.), 2018. Neuroscience, Sixth edition. ed. Oxford University Press, New York.

Raichle, M.E., 2015. The Brain's Default Mode Network. Annual Review of Neuroscience 38, 433–447. https://doi.org/10.1146/annurev-neuro-071013-014030

Rocca, M., Absinta, M., Valsasina, P., Copetti, M., Caputo, D., Comi, G., Filippi, M., 2012. Abnormal cervical cord function contributes to fatigue in multiple sclerosis. Mult Scler 18, 1552–1559. https://doi.org/10.1177/1352458512440516

San Emeterio Nateras, O., Yu, F., Muir, E.R., Bazan, C., Franklin, C.G., Li, W., Li, J., Lancaster, J.L., Duong, T.Q., 2016. Intrinsic Resting-State Functional Connectivity in the Human Spinal Cord at 3.0 T. Radiology 279, 262–268. https://doi.org/10.1148/radiol.2015150768

Schirmer, C.M., Shils, J.L., Arle, J.E., Cosgrove, G.R., Dempsey, P.K., Tarlov, E., Kim, S., Martin, C.J., Feltz, C., Moul, M., Magge, S., 2011. Heuristic map of myotomal innervation in humans using direct intraoperative nerve root stimulation: Clinical article. SPI 15, 64–70. https://doi.org/10.3171/2011.2.SPINE1068

Schomburg, E.D., 1990. Spinal sensorimotor systems and their supraspinal control. Neuroscience Research 7, 265–340. https://doi.org/10.1016/0168-0102(90)90008-3

Shen, D., Cui, L., Cui, B., Fang, J., Li, D., Ma, J., 2015. A Systematic Review and Meta-Analysis of the Functional MRI Investigation of Motor Neuron Disease. Front. Neurol. 6. https://doi.org/10.3389/fneur.2015.00246

Smith, S.D., Kornelsen, J., 2011. Emotion-dependent responses in spinal cord neurons: A spinal fMRI study. NeuroImage 58, 269–274. https://doi.org/10.1016/j.neuroimage.2011.06.004

Soteropoulos, D.S., Edgley, S.A., Baker, S.N., 2013. Spinal Commissural Connections to Motoneurons Controlling the Primate Hand and Wrist. Journal of Neuroscience 33, 9614–9625. https://doi.org/10.1523/JNEUROSCI.0269-13.2013

Stark, D.E., Margulies, D.S., Shehzad, Z.E., Reiss, P., Kelly, A.M.C., Uddin, L.Q., Gee, D.G., Roy, A.K., Banich, M.T., Castellanos, F.X., Milham, M.P., 2008. Regional Variation in Interhemispheric Coordination of Intrinsic Hemodynamic Fluctuations. Journal of Neuroscience 28, 13754–13764. https://doi.org/10.1523/JNEUROSCI.4544-08.2008

Stracke, C.P., Pettersson, L.G., Schoth, F., Möller-Hartmann, W., Krings, T., 2005. Interneuronal systems of the cervical spinal cord assessed with BOLD imaging at 1.5 T. Neuroradiology 47, 127–133.

https://doi.org/10.1007/s00234-004-1318-8

Stroman, P., 2002. Mapping of Neuronal Function in the Healthy and Injured Human Spinal Cord with Spinal fMRI. NeuroImage 17, 1854–1860. https://doi.org/10.1006/nimg.2002.1305

Stroman, P.W., Kornelsen, J., Bergman, A., Krause, V., Ethans, K., Malisza, K.L., Tomanek, B., 2004. Noninvasive assessment of the injured human spinal cord by means of functional magnetic resonance imaging. Spinal Cord 42, 59–66. https://doi.org/10.1038/sj.sc.3101559

Stroman, P.W., Nance, P.W., Ryner, L.N., 1999. BOLD MRI of the human cervical spinal cord at 3 tesla. Magn Reson Med 42, 571–576. https://doi.org/10.1002/(sici)1522-2594(199909)42:3<571::aid-mrm20>3.0.co;2-n

Stroman, P.W., Ryner, L.N., 2001. Functional MRI of motor and sensory activation in the human spinal cord. Magnetic Resonance Imaging 19, 27–32. https://doi.org/10.1016/S0730-725X(01)00226-0

Stroman, P.W., Tomanek, B., Krause, V., Frankenstein, U.N., Malisza, K.L., 2003. Functional magnetic resonance imaging of the human brain based on signal enhancement by extravascular protons (SEEP fMRI). Magn. Reson. Med. 49, 433–439. https://doi.org/10.1002/mrm.10831

Stroman, P.W., Wheeler-Kingshott, C., Bacon, M., Schwab, J.M., Bosma, R., Brooks, J., Cadotte, D., Carlstedt, T., Ciccarelli, O., Cohen-Adad, J., Curt, A., Evangelou, N., Fehlings, M.G., Filippi, M., Kelley, B.J., Kollias, S., Mackay, A., Porro, C.A., Smith, S., Strittmatter, S.M., Summers, P., Tracey, I., 2014. The current state-of-the-art of spinal cord imaging: Methods. NeuroImage 84, 1070–1081. https://doi.org/10.1016/j.neuroimage.2013.04.124

Summers, P.E., Ferraro, D., Duzzi, D., Lui, F., Iannetti, G.D., Porro, C.A., 2010. A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord. NeuroImage 50, 1408–1415. https://doi.org/10.1016/j.neuroimage.2010.01.043

Tinnermann, A., Büchel, C., Cohen-Adad, J., 2020. Cortico-spinal imaging to study pain. NeuroImage 117439. https://doi.org/10.1016/j.neuroimage.2020.117439

Tinnermann, A., Geuter, S., Sprenger, C., Finsterbusch, J., Büchel, C., 2017. Interactions between brain and spinal cord mediate value effects in nocebo hyperalgesia. Science 358, 105–108. https://doi.org/10.1126/science.aan1221

Tomassini, V., Johansen-Berg, H., Jbabdi, S., Wise, R.G., Pozzilli, C., Palace, J., Matthews, P.M., 2012. Relating Brain Damage to Brain Plasticity in Patients With Multiple Sclerosis. Neurorehabil Neural Repair 26, 581–593. https://doi.org/10.1177/1545968311433208

Vahdat, S., Khatibi, A., Lungu, O., Finsterbusch, J., Büchel, C., Cohen-Adad, J., Marchand-Pauvert, V., Doyon, J., 2020. Resting-state brain and spinal cord networks in humans are functionally integrated. PLoS Biol 18, e3000789. https://doi.org/10.1371/journal.pbio.3000789

Vahdat, S., Lungu, O., Cohen-Adad, J., Marchand-Pauvert, V., Benali, H., Doyon, J., 2015. Simultaneous Brain–Cervical Cord fMRI Reveals Intrinsic Spinal Cord Plasticity during Motor Sequence Learning. PLOS Biology 13, e1002186. https://doi.org/10.1371/journal.pbio.1002186

Valsasina, P., Agosta, F., Absinta, M., Sala, S., Caputo, D., Filippi, M., 2010. Cervical cord functional MRI changes in relapse-onset MS patients. Journal of Neurology, Neurosurgery & Psychiatry 81, 405–408. https://doi.org/10.1136/jnnp.2009.187526

Valsasina, P., Agosta, F., Caputo, D., Stroman, P.W., Filippi, M., 2008. Spinal fMRI during proprioceptive and tactile tasks in healthy subjects: activity detected using cross-correlation, general linear model and independent component analysis. Neuroradiology 50, 895–902. https://doi.org/10.1007/s00234-008-

0420-8

Valsasina, P., Rocca, M.A., Absinta, M., Agosta, F., Caputo, D., Comi, G., Filippi, M., 2012. Cervical cord FMRI abnormalities differ between the progressive forms of multiple sclerosis. Hum. Brain Mapp. 33, 2072–2080. https://doi.org/10.1002/hbm.21346

Verma, T., Cohen-Adad, J., 2014. Effect of respiration on the B $_0$ field in the human spinal cord at 3T: Effect of Respiration on B $_0$ Field in Human Spinal Cord. Magn. Reson. Med. 72, 1629–1636. https://doi.org/10.1002/mrm.25075

Wagner, F.B., Mignardot, J.-B., Le Goff-Mignardot, C.G., Demesmaeker, R., Komi, S., Capogrosso, M., Rowald, A., Seáñez, I., Caban, M., Pirondini, E., Vat, M., McCracken, L.A., Heimgartner, R., Fodor, I., Watrin, A., Seguin, P., Paoles, E., Van Den Keybus, K., Eberle, G., Schurch, B., Pralong, E., Becce, F., Prior, J., Buse, N., Buschman, R., Neufeld, E., Kuster, N., Carda, S., von Zitzewitz, J., Delattre, V., Denison, T., Lambert, H., Minassian, K., Bloch, J., Courtine, G., 2018. Targeted neurotechnology restores walking in humans with spinal cord injury. Nature 563, 65–71. https://doi.org/10.1038/s41586-018-0649-2

Wang, W., Xie, W., Zhang, Q., Liu, L., Liu, J., Zhou, S., Shi, J., Chen, J., Ning, B., 2019. Reorganization of the brain in spinal cord injury: a meta-analysis of functional MRI studies. Neuroradiology 61, 1309–1318. https://doi.org/10.1007/s00234-019-02272-3

Ward, N.S., Swayne, O.B., Newton, J.M., 2008. Age-dependent changes in the neural correlates of force modulation: an fMRI study. Neurobiol Aging 29, 1434–46.

Weber, K.A., Chen, Y., Paliwal, M., Law, C.S., Hopkins, B.S., Mackey, S., Dhaher, Y., Parrish, T.B., Smith, Z.A., 2020. Assessing the spatial distribution of cervical spinal cord activity during tactile stimulation of the upper extremity in humans with functional magnetic resonance imaging. NeuroImage 217, 116905. https://doi.org/10.1016/j.neuroimage.2020.116905

Weber, K.A., Chen, Y., Wang, X., Kahnt, T., Parrish, T.B., 2016. Lateralization of cervical spinal cord activity during an isometric upper extremity motor task with functional magnetic resonance imaging. NeuroImage 125, 233–243. https://doi.org/10.1016/j.neuroimage.2015.10.014

Weber, K.A., Sentis, A.I., Bernadel-Huey, O.N., Chen, Y., Wang, X., Parrish, T.B., Mackey, S., 2018. Thermal Stimulation Alters Cervical Spinal Cord Functional Connectivity in Humans. Neuroscience 369, 40–50. https://doi.org/10.1016/j.neuroscience.2017.10.035

Wei, P., Li, J., Gao, F., Ye, D., Zhong, Q., Liu, S., 2010. Resting state networks in human cervical spinal cord observed with fMRI. Eur J Appl Physiol 108, 265–271. https://doi.org/10.1007/s00421-009-1205-4

Willis, W.D., Coggeshall, R.E., 2004. Sensory Mechanisms of the Spinal Cord: Volume 1: Primary Afferent Neurons and the Spinal Dorsal Horn Volume 2: Ascending Sensory Tracts and their Descending Control.

Wu, T., Hou, Y., Hallett, M., Zhang, J., Chan, P., 2015. Lateralization of brain activity pattern during unilateral movement in Parkinson's disease: Motor Lateralization in Parkinson's Disease. Hum. Brain Mapp. 36, 1878–1891. https://doi.org/10.1002/hbm.22743

Wu, T.-L., Yang, P.-F., Wang, F., Shi, Z., Mishra, A., Wu, R., Chen, L.M., Gore, J.C., 2019. Intrinsic functional architecture of the non-human primate spinal cord derived from fMRI and electrophysiology. Nat Commun 10, 1416. https://doi.org/10.1038/s41467-019-09485-3

Xie, C., Kong, K., Guan, J., Chen, Y., He, J., Qi, W., Wang, X., Shen, Z., Wu, R., 2009. SSFSE sequence functional MRI of the human cervical spinal cord with complex finger tapping. European Journal of

Radiology 70, 1–6. https://doi.org/10.1016/j.ejrad.2008.01.003

Yang, P.-F., Wang, F., Chen, L.M., 2015. Differential fMRI Activation Patterns to Noxious Heat and Tactile Stimuli in the Primate Spinal Cord. Journal of Neuroscience 35, 10493–10502. https://doi.org/10.1523/JNEUROSCI.0583-15.2015

Yoshizawa, T., Nose, T., Moore, G.J., Sillerud, L.O., 1996. Functional Magnetic Resonance Imaging of Motor Activation in the Human Cervical Spinal Cord. NeuroImage 4, 174–182. https://doi.org/10.1006/nimg.1996.0068

Zavvarian, M.-M., Hong, J., Fehlings, M.G., 2020. The Functional Role of Spinal Interneurons Following Traumatic Spinal Cord Injury. Front. Cell. Neurosci. 14, 127. https://doi.org/10.3389/fncel.2020.00127

Zhong, X.-P., Chen, Y.-X., Li, Z.-Y., Shen, Z.-W., Kong, K.-M., Wu, R.-H., 2017. Cervical spinal functional magnetic resonance imaging of the spinal cord injured patient during electrical stimulation. Eur Spine J 26, 71–77. https://doi.org/10.1007/s00586-016-4646-6