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RESEARCH ARTICLE

Anatomic conditions for bypass surgery between rostral (T7–T9) and caudal (L2, L4, S1) ventral roots to treat paralysis after spinal cord injury

Habib Bendella[a,1], Svenja Rink[b,1], Aliona Wöhler[c], Janna Feiser[d], Andre Wilden[c], Maria Grosheva[e], Hans-Jürgen Stoffels[f], Carolin Meyer[g], Marilena Manthou[h], Makoto Nakamura[a], Doychin N. Angelov[c,*]

[a] Department of Neurosurgery, University of Witten/Herdecke, Cologne Merheim Medical Center (CMMC), Cologne, Germany
[b] Department of Prosthetic Dentistry, School of Dental and Oral Medicine, University of Cologne, Cologne, Germany
[c] Department of Anatomy I, University of Cologne, Germany
[d] Praxis "Med 360 Grad", Burger Straße 213, 42859 Remscheid, Germany
[e] Department of Otorhinolaryngology, University of Cologne, Germany
[f] Medizin Foto Köln, University of Cologne, Germany
[g] Department of Orthopedics and Trauma Surgery, University of Cologne, Germany
[h] Department of Histology and Embryology, Aristotle University Thessaloniki, Greece

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ABSTRACT

Severe spinal cord injuries cause permanent neurological deficits and are still considered as inaccessible to efficient therapy. Injured spinal cord axons are unable to spontaneously regenerate. Re-establishing functional activity especially in the lower limbs by reinnervation of the caudal infra-lesional territories might represent an effective therapeutic strategy.

Numerous surgical neurotizations have been developed to bridge the spinal cord lesion site and connect the intact supra-lesional portions of the spinal cord to peripheral nerves (spinal nerves, intercostal nerves) and muscles. The major disadvantage of these techniques is the increased hypersensitivity, spasticity and pathologic pain in the spinal cord injured patients, which occur due to the vigorous sprouting of injured afferent sensory fibers after reconstructive surgery.

Using micro-surgical instruments and an operation microscope we performed detailed anatomical preparation of the vertebral canal and its content in five human cadavers. Our observations allow us to put forward the possibility to develop a more precise surgical approach, the so called "ventral root bypass" that avoids lesion of the dorsal roots and eliminates sensitivity complications.

The proposed kind of neurotization has been neither used, nor put forward. The general opinion is that *radix ventralis* and *radix dorsalis* unite to form the spinal nerve inside the dural sac. This assumption is not accurate, because both radices leave the dural sac separately. This neglected anatomical feature allows a reliable intravertebral exposure of the dura-mater ensheathed ventral roots and their damage-preventing end-to-side neurorrhaphy by interpositional nerve grafts.

1. Introduction

Traumatic spinal cord injury (SCI) triggers neural degeneration and rampant axotomy that evolves over time with different grades of motor and sensory deficits (Wyndaele and Wyndaele, 2006). There are currently no definitive therapies to significantly improve outcome following SCI (Fouad and Pearson, 2004). At the acute stage, the only available treatment with proven – though limited – efficacy, is surgery. Performed within the first hours after injury, the surgical treatment allows to decompress the spinal cord (by remov-

* Corresponding author at: Institut 1 für Anatomie der Universität zu Köln, Joseph-Stelzmann-Strasse 20, D-50931 Köln, Germany.
E-mail addresses: habib@bendella.de (H. Bendella), svenjarink@web.de (S. Rink), awoehler@web.de (A. Wöhler), janna.feiser@med360grad.de (J. Feiser), beluga2000@gmx.de (A. Wilden), maria.grosheva@uk-koeln.de (M. Grosheva), hans-jürgen.stoffels@live.de (H.-J. Stoffels), carolin.meyer@uk-koeln.de (C. Meyer), marilenamanthou@yahoo.gr (M. Manthou), NakamuraM@kliniken-koeln.de (M. Nakamura), angelov.anatomie@uni-koeln.de (D.N. Angelov).
[1] Contributed equally and share first authorship.

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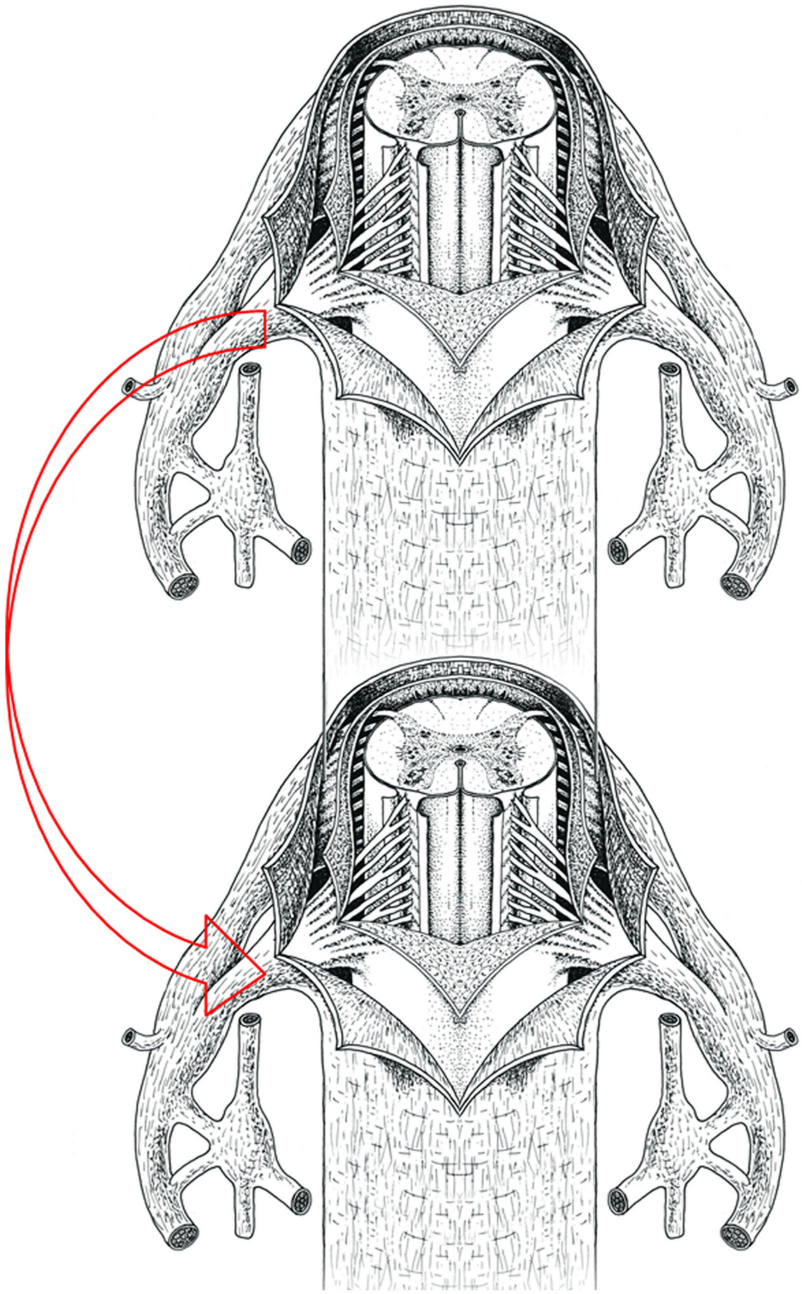

Fig. 1. Schematical drawing illustrationg the proposed bypass between the rostral and caudal ventral roots. Adapted from Krstic (1978) Tafel 174.

ing bony fragments and blood cloths that could injure the spinal cord further), to correct the spinal deformation and to stabilize the spine.

During the last decades, many transplant strategies have been developed which attempt to re-connect the intact supra- and infra-lesional portions of the injured spinal cord. Grafts of different nature have been interposed between the rostral and caudal stumps of the injured/sectioned spinal cord (bridge "cord to cord"). Particularly peripheral nerves, Schwann cells, olfactory ensheathing glial cells, ependymal or stem cells, have proven to be able to promote axonal regeneration in the spinal cord of adult rats leading to encouraging results but with very limited functional recovery. Nevertheless, the "cord to cord" grafting techniques carry the main and challenging issue to overcome the obstacle represented by the rostral and caudal "glial barriers" (Dam-Hieu et al., 2004, 2006).

A solution to this problem could be to interpose nerve grafts between the intact rostral spinal cord and the peripheral nervous system ("cord to periphery" or "neurotization of the infra-lesional cord"). Support to this strategy (Bertelli et al., 1994; Horvat et al., 1988; Tadie et al., 2002; Konya et al., 2008) was provided by results from experimental studies, showing that:

- avulsed ventral nerve roots can be reimplanted into the spinal cord (Carlstedt et al., 1986, 1993, 1995; Carlstedt, 1991; Cullheim et al., 1989);
- spinal motoneurons can successfully reinnervate denervated muscles even after redirection of the ventral root axons (Thulin and Carlsson, 1969; Richardson et al., 1980; Shonnard and Wakefield, 1983; Liu et al., 1999, 2001; Zhou et al., 2014).

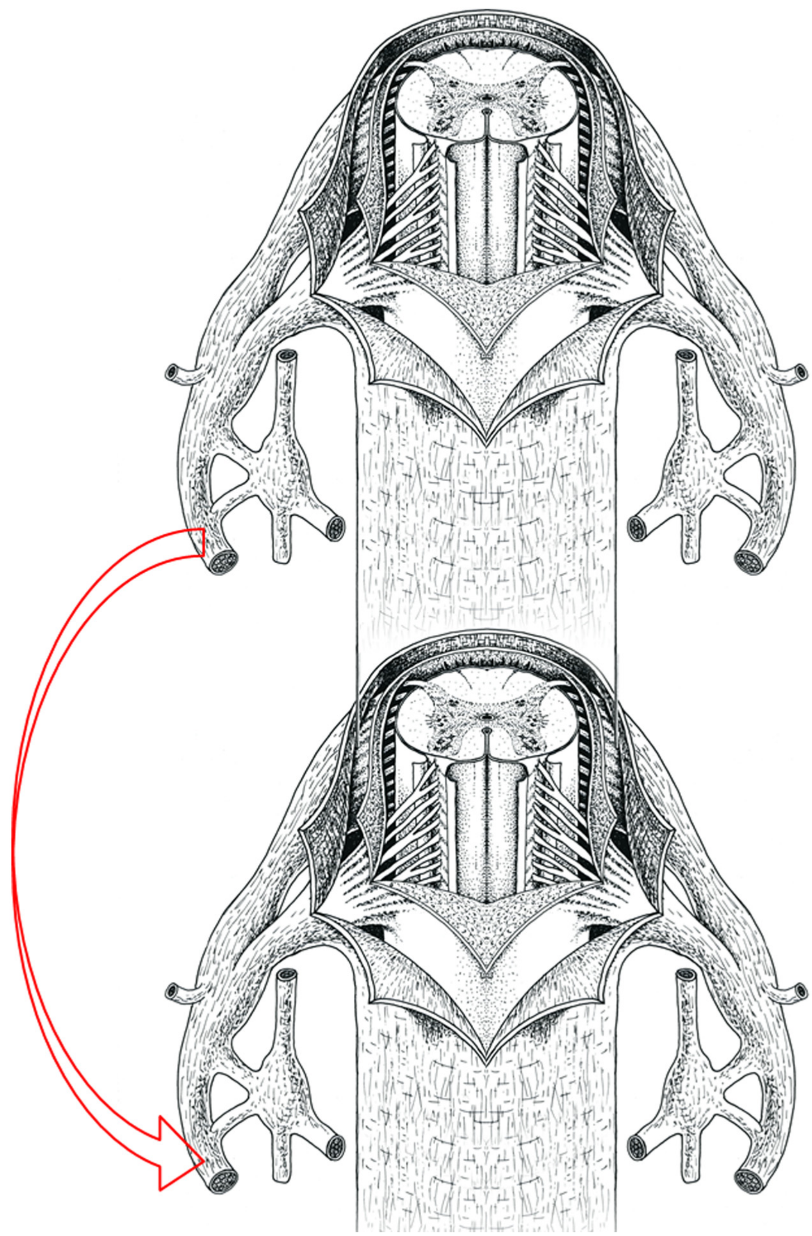

Fig. 2. Schematical drawing illustrationg the commonly used bypass between the rostral and caudal spinal nerves or their *rami ventrales* (intercostal nerves). Adapted from Krstic (1978) Tafel 174.

And also that CNS axons can regrow through a peripheral nerve graft that was long enough to bridge over the avulsed nerve roots (Bertelli et al., 1994; Liu et al., 1998). All that implies, that "there is some evidence that peripheral nerve bridging is worth pursuing" (Tator, 2006). Nevertheless, the "glial barrier" problem in after any CNS-surgery is still present.

In our present proposal for possible surgical treatment of the SCI sequelae we avoid any contact with CNS. In analogy with the neurotization paradigm, we propose the insertion and end-to-side suture of an interpositional nerve graft (IPNG) between rostral (above the lesion) and caudal (below the injury) ventral roots (*radices ventrales*) of the spinal nerves (Fig. 1).

The advantage of such a bypass, connecting only the ventral roots, but not the proximal spinal nerves or the intercostal nerves (with motor and sensory axons; Hauge, 1991; Tok et al., 1991; Zhao et al., 1997; Lim and Baskaran, 2001; Liu et al., 2003; Vialle et al., 2006; Oppenheim et al., 2009; Haque et al., 2012) (Fig. 2), is that the vigorous sprouting of injured afferent sensory fibers after reconstructive surgery (Hulsebosch and Coggeshall, 1981; James et al., 2017) is avoided. This is very important, because it might increase the already existing hypersensitivity and pathologic pain in SCI patients.

This kind of neurotization has been neither used, nor even put forward. Most studies are focused on "cord to periphery" neurotizations using intercostal nerves or IPNG to connect the spinal cord to the nerve roots (i.e. the proximal spinal nerves). The possible reasons for this omission are, in our opinion, two. First, the ventral roots are relatively "difficult" to expose, one needs to search for them deeply to the dorsal roots and the spinal ganglion. Second, neurosurgeons may have not come to this idea, because a lot of them share the opinion that *radix ventralis* and *radix dorsalis* unite to form the spinal nerve inside the dural sac (Joukal et al., 2016; Rohen et al., 2016; Sakka et al., 2016; Fig. 3).

This is not true, both radices leave the dural sac separately (Warwick and Williams, 1973, 1980; Waldeyer and Mayet, 1980; Berry et al., 1995; Tillmann, 1997; Newell, 2005). This anatomical

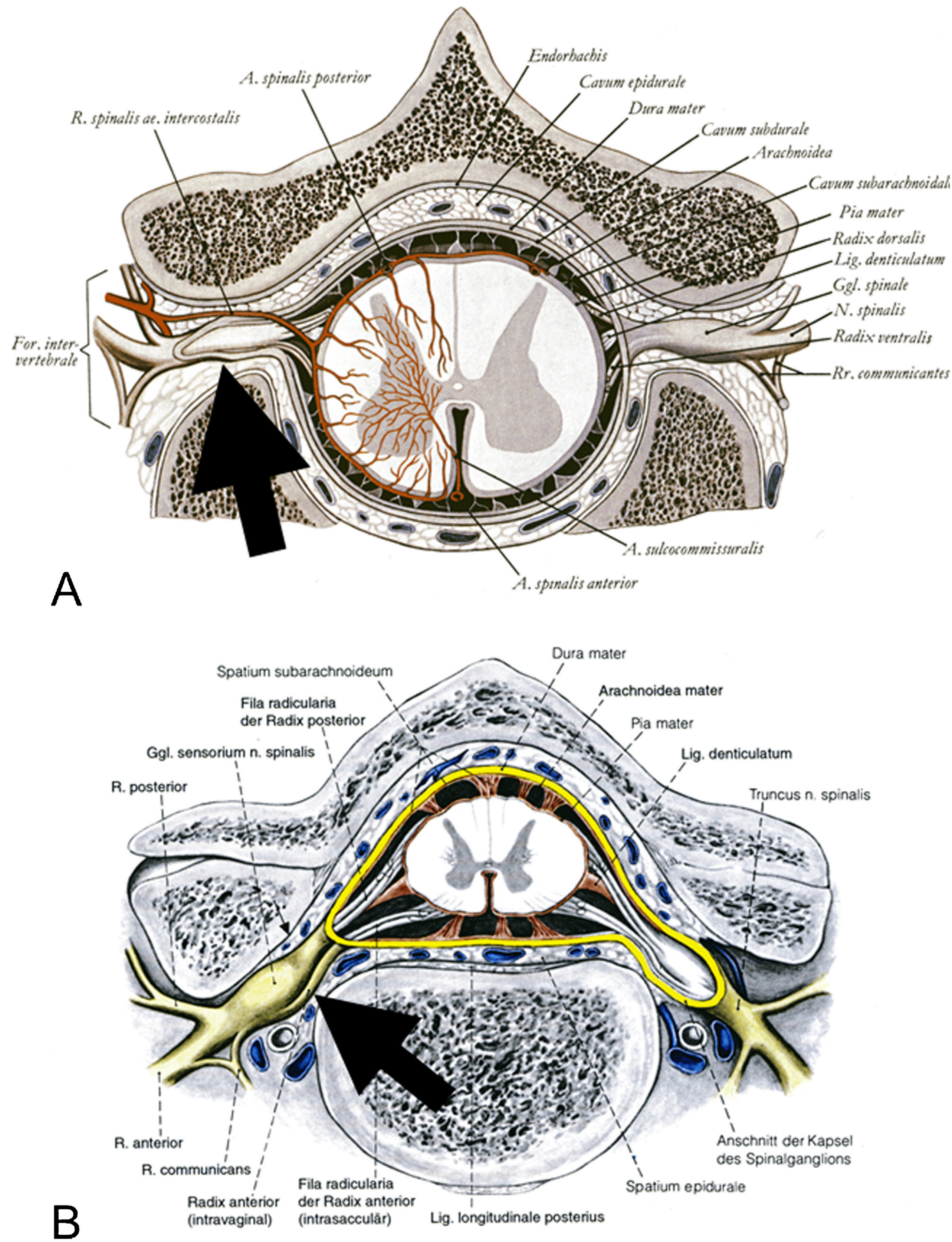

Fig. 3. (A) Schematical drawing of a cross section through the human spinal cord illustrating the intra-arachnoidal course of the ventral and dorsal roots and their intradural unification at the level of the intervertebral foramen (*arrow*). Note that the spinal ganglion has been cut tangentially so that its surface is deprived from dura mater spinalis. Adapted from Waldeyer and Mayet (1980). (B) Similar drawing showing that the ventral and dorsal roots perforate dura mater spinalis separately before their unification at the level of the intervertebral foramen (*arrow*). Note that the spinal ganglion is intact and its surface is covered by dura mater. Adapted from Tillmann (1997).

feature allows a reliable intravertebral exposure of the dura-mater ensheathed ventral roots.

2. Materials and methods

This anatomical study was carried out in 5 human cadavers that had been initially dissected by students during practical anatomical courses in the winter semesters 2016/2017 and 2017/2018 at the Anatomical Institute, University of Cologne, Germany. The extensive additional dissectional work to distinguish and identify the intravertebral ventral roots of the thoracic spinal nerves was performed by S. Rink, H. Bendella, A. Wöhler, J. Feiser, A. Wilden and D.N. Angelov.

For the anatomical teaching courses all cadavers had been fixed in 3% aqueous formalin solution. Cadavers were not specified according to gender or age of the donor, but according to their registration number. The entire study was performed with permission of the Ethic Committee of the Faculty of Medicine, University of Cologne Nr. 16-576 from January 11, 2017.

The vertebral canal was approached from the dorsal aspect of the body. All autochthonous muscles of the medial tract (mm. interspinales, mm. intertransversarii, m. semispinalis, mm. rotatores longi and brevi) were carefully mobilized and removed. Thereafter several thoracic spinous processes together with their laminae and pedicles were resected by carving initially just medial to the adjacent articular processes (left and right). The vertebral canal was thus opened and *dura mater spinalis* exposed. After careful further removal of the articular processes by means of a bone forceps, the *foramina intervertebralia* were opened.

Micro-dissections followed under a surgical microscope (OpMi-1, Zeiss) at $\times$6.0 magnification with the aid of micro-surgical instruments (forceps FD284, FM24, FM3, scissors FM11, FM13 purchased from Aesculap AG). Findings were documented in photographs taken by a Nikon D610 digital camera equipped with an AF-S MICRO NIKKOR 105 mm macro objective and by an Olympus DP21 digital camera.

3. Results

The intervertebral foramina were further widened and the dorsal root ganglia were exposed (arrows in Fig. 4A). Using a blunt probe, one could slightly displace the dorsal root ganglion upwards or downwards and reach the ventral root (arrow in Fig. 4B) below it.

The careful deep preparation under the microscope allowed the removal of the entire dural sac (without the spinal ganglia) with its content (Fig. 5A). This, in turn, enabled us to inspect and photograph the dorsal and ventral roots at higher magnifications (Fig. 5B).

These high-power photographs, allow a very good detection of the ventral roots which, in turn, minimizes the risk to damage them during bypass surgery.

Our results show that a small incision in the dural sheath of the rostral ventral root is possible. Thus, a predegenerated IPNG can be inserted into the local recess of the subarachnoidal space and fixed to the dura by an epineural end-to-side suture. The intact ventral root axons are not going to be transected. A slight damage to the motor axons (e.g. by a crush) proximally to the IPNG-insertion will trigger the collateral branching of numerous axons (Hulsebosch and Coggeshall, 1981), which will grow along the pre-degenerated IPNG and reach the caudal ventral root.

4. Discussion

Why were the unaffiliated ventral roots overseen and neglected by the surgeons for so many years? One possible answer may be

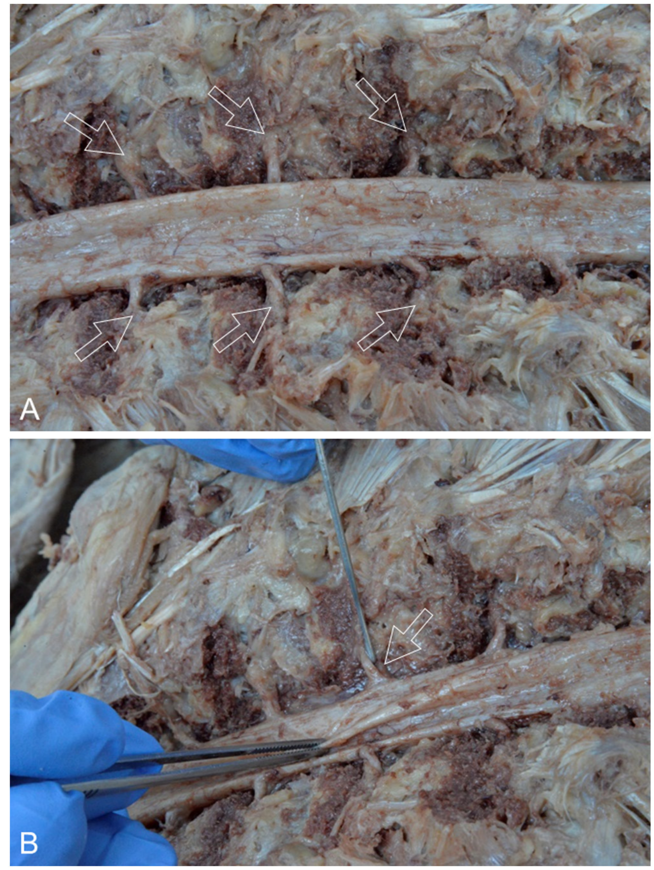

Fig. 4. (A) Widely opened vertebral canal reveals the dorsal aspect of dura mater spinalis, the dorsal roots and the spinal ganglia (*arrows*). (B) A slight displacement of the dorsal root by a blunt probe reveals the ventral root (*arrow*).

found in numerous “classical” drawings of transversely sectioned spinal cord with its roots and meninges. When the section plane passes tangentially through the spinal gangion, it looks as if both radices unite in the (sub)arachnoidal space, i.e. within the dural sac (Fig. 3B, right side; Tillmann, 1997; Netter, 2008). When the section plane, however, passes above the dura-mater ensheathed spinal ganglion, it is evident, that both radices perforate dura mater separately and unite at the level of the spinal ganglion (Fig. 3B, left side; Tillmann, 1997; Netter, 2008).

Nevertheless, the present results, based on anatomical observations in human cadaver material, indicate that the performance of a ventral root bypass via end-to-side neurorhaphy is possible. Furthermore, we are convinced, that the serious technical difficulties can be overcome by the neurosurgeons. This is why, in the following discussion, we shall concentrate mostly on general neuro-biological, but not on the technical aspects of the proposed surgical treatment.

4.1. No support from animal experiments

Despite cumbersome measurements on ratś intercostal nerves, the results of which put forward the suggestion that these nerves could be used as experimental nerve grafts (Üstüner et al., 1996), we are not aware of any small-animal studies with ventral root bypass after SCI. A possible explanation is that ventral roots in rats are thin and vulnerable and very easy to tear (Fig. 6).

In their study in cats (Thulin and Carlsson, 1969) connected ventral roots after overleaping (bridging) one segment but without IPNG. Positive evidence of functional efferent neuromuscular reconnections was obtained and a method suitable for bridging a

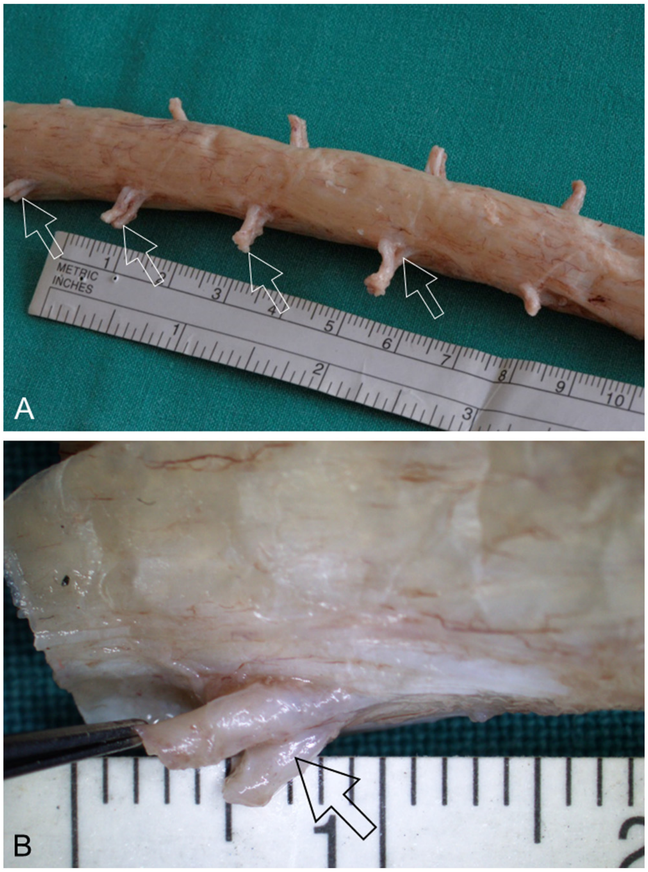

Fig. 5. (A) Following careful deep preparation under the microscope the entire dural sac (without the spinal ganglia) was removed from the vertebral canal. (B) Inspection and photography of the specimen under the Olympus DP21 digital camera revealed the ventral root more clearly.

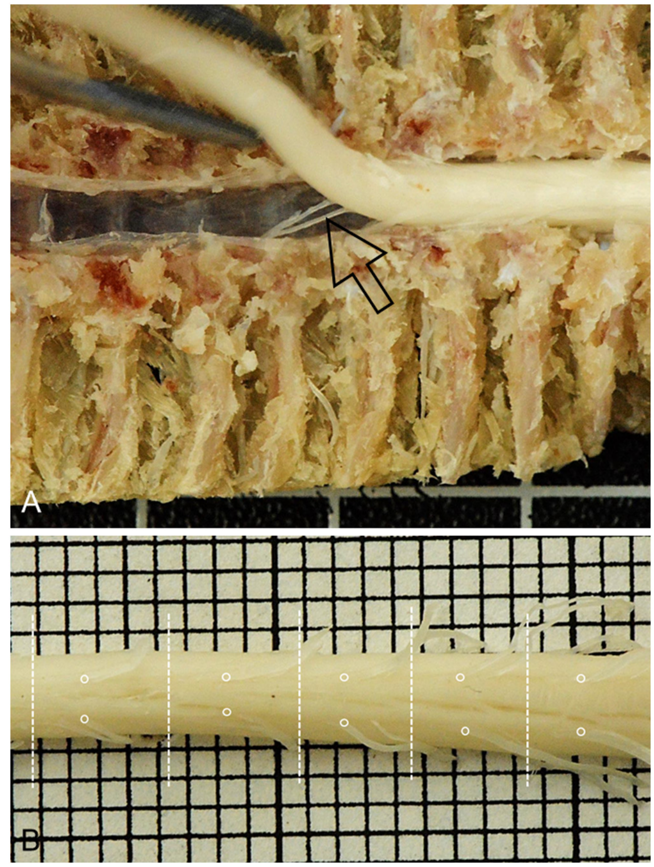

Fig. 6. (A) Vertebral canal and spinal cord of a rat after transcardial perfusion fixation with 4% solution of paraformaldehyde in phosphate-buffered saline. The *arrow* points at two very thin ventral roots. (B) The subsequent removal of this rat spinal cord from the vertebral canal shows one more time how thin and vulnerable are the ventral roots. Small circles indicate the places where the ventral roots leave the spinal cord parenchyma. The dashed lines delineate the spinal cord segments with a length of appr. 4 mm in the rat.

cord transection and supplying motor activity to distal levels by approximating ventral roots from above and below the transection was suggested (Thulin and Carlsson, 1969).

4.2. Justification to use end-to side neurorrhaphy

End-to-side neurorhaphy, which was originally described in the late 19th century, has regained attention because of the finding that axon elongation occurs even if nerve stumps are not directly attached to each other (Viterbo et al., 1994). In particular, by suturing the end of the donor nerve to the side surface of the recipient nerve, an “inflow type” of nerve regeneration (regenerated axons extend through an epineural window into the windowed nerve) is established (Okouchi et al., 2008; Okouchi et al., 2009). In inflow type end-to-side neurorhaphy, 52.8% of axon fibers regenerated, compared to 89% in outflow type end-to-side neurorhaphy (regenerated axons extend in the opposite direction) (Tateshita et al., 2018).

4.3. Anticipated effect(s) of ventral radicular bypass

The nerve supply to muscles is determined during the embryonic development and is specific for every muscle. This is why, a successfully transplantated nerve supply – expressed by functional recovery – should rely on neural plasticity (Ramer et al., 2005).

The initial question is, whether we can expect that motor input from the spinal cord above the injury – if brought below the lesion site to intact ventral roots – is able to improve recovery of motor functions which were originally executed by motoneurons in the lower portion of the spinal cord. If “yes”, this would mean that the motor neurons above the lesion are able to re-innervate paralyzed muscles (along an appropriate scaffold) and improve their functional recovery.

For example, human anatomy teaches that the muscles involved in the flexion of the femur in the hip joint are innervated by ventral roots L_2–L_4 and those involved in the extension – by ventral roots L_4–S_1. Thus, if we circumvent a putative lesion site e.g. at the level of roots Th_8–Th_{10} (most contusive injuries are 2–3 segments long (Dietz and Fouad, 2014)) and suture the bypassing scaffold to the ventral root L_4, flexion and extension in the hip joint should be theoretically possible. In addition, one should try to connect more than 2 ventral roots in order to improve the functional recovery. In support of this assumption we may point out the sufficient number of axons in the ventral roots: 8467 ± 1019 at level C7, 6538 ± 892 at level T12, 9169 ± 1160 at level L3, and 8253 ± 1419 at level S1 (Liu et al., 2015).

This in turn would mean that ventral horn motoneurons projecting to spinal nerves above and below the lesion, i.e. approximately 3 segments apart, have identical potential from which similar features may develop. Is this true? We do not know, and are not aware of any studies on specific features of the human ventral horn motoneurons along the rostro-caudal axis.

Despite these accounts of functional recovery, SCI bypass models that connect ventral roots are subject to one important limitation: redirected nerve fibers must grow into IPNG, caudal ventral roots and through the length of peripheral nerves to reach their targets and recover function. The distance between the rostral roots and distal targets (lower leg muscles, for example) is rather long, which may require years before function occurs. Since this length of time may prevent meaningful muscle recovery, as substantial muscle atrophy and irreversible fibrosis can occur in that time frame (Scelsi et al., 1982), regular treatment with physical therapy is recommended.

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