Experimental Microsurgical Repair of Spinal Roots

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THREE DIFFERENT METHODS of nerve repair were evaluated in an experimental model of spinal root injury. In adult rats, dorsal L4 roots were cleanly severed and repaired by microsurgical techniques. Anastomosis was performed by direct end-to-end suture, the arterial sleeve technique, or the interposition of a nerve graft. Results were evaluated 7, 10, and 14 weeks after surgery. Regeneration was studied by light and electron microscopy, showing a fair regenerative pattern in each group. The endoneurial connective response, including neovascularization, was more prominent after grafting. The artery sleeve technique is a very tedious procedure, and fibrosis around the artery and arachnoiditis were intense. A lack of continuity was found in 3 of 12 direct sutures. In conclusion, the best method for the reparation of nerve roots seems to be the interposition of a nerve graft. (Neurosurgery 33:1083–1088)

Key words: Nerve regeneration, Nerve repair, Nerve root

The development of microsurgical techniques has permitted an accurate and satisfactory reparation of peripheral nerve lesions. The microsurgical repair of peripheral nerve injuries is possible because of the supporting connective tissue of the peripheral nerve. The perineurium packs groups of nerve fibers in fascicles, and the epineurium isolates these fascicles from the neighboring tissues (22). Microsurgical techniques permit the neurolysis and suture of nerve fascicles, manipulating only these connective elements of the nerve. However, the intracranial part of the cranial nerves and the spinal roots have several important differences when compared with their peripheral part, namely, the absence of the perineurium and the epineurium and, therefore, the absence of any fascicular pattern (21, 22). These structural differences lead to a special nerve root response to injury (23) and provide an additional difficulty for surgical repair.

Different experimental studies have demonstrated the presence of an active nerve regeneration after crushing or severing dorsal or ventral roots in rats or cats (3, 10–13). There are also some clinical reports and clinical series communicating successful repairs of spinal or cranial nerve roots (1, 2, 5, 9, 14–20). Several surgical techniques have been used for the reparation of these nerve structures, including direct end-to-end suture, sutureless techniques with fibrin glue, and nerve grafts. However, there is a lack of experimental studies dealing with the selection of the best method for the surgical reparation of nerve roots.

In this experimental work on rats, we have used three surgical techniques for the reparation of dorsal roots of the cauda equina, namely, direct end-to-end suture, nerve graft interposition, and arterial sleeve technique. The results were evaluated by a study of the degree and quality of nerve regeneration on the spinal cord side of the suture line and the connective response into and around the repaired root.

MATERIAL AND METHODS

Male Wistar rats weighing 225 g were used for the experiment. The animals were obtained from the Breeding Facilities of the Faculty of Medicine of Cádiz. The animals were anesthetized with a mixture of ketamine (60 mg/kg) and diazepam (6 mg/kg) administered intraperitoneally and were maintained in a spontaneous ventilation chamber. The lumbar spine was exposed by the use of a midline posterior approach, and the laminae of L3 and L4 were removed. The dura mater was then opened with the tip of a needle, exposing the cauda equina and the lower spinal cord. The dorsal L4 root was identified at the preganglionar level and cleanly severed in the intradural space. The roots were repaired by different surgical techniques. In 12 rats, a direct microsurgical end-to-end anastomosis was carried out with Ethilon 10/0. In each case, no more than two stitches were necessary to put the proximal and distal root stumps in close contact. In 15 rats, severed roots were repaired by the use of an arterial sleeve. For this purpose, common carotid arteries were removed from donor rats. Arteries were placed in a recipient filled with a heparinized Ringer lactate solution bubbled with 95% O_2 and 5% CO_2, and adventitial layers were removed. Then, the artery was longitudinally opened. An 8- to 10-mm segment of the artery was then transferred to the cauda equina and the proximal and distal stumps of the root were placed in close contact over the inner aspect of the arterial wall. The artery was then wrapped around the root and closed up by interrupted sutures with Ethilon 10/0. Finally, in a third group of animals (12 rats), the root section was repaired by the place-

ment between the proximal and distal stumps of a nerve graft taken from a donor rat. In these experiments, a 5-mm-long nerve graft was obtained from the peroneal branch of the sciatic nerve and was secured to the severed root with one or two 10/0 Ethilon stitches. For the operative procedures, a gentle microsurgical technique was used. After anastomosis, repaired roots were slipped among cauda equina roots, the dura mater was closed with interrupted 10/0 sutures, and the muscle and skin were sutured in layers. No spine immobilization was used. No drugs were administered during the follow-up. Animals were identified and logged in standard colony conditions.

Sets of animals from each experimental group were anesthetized as described above 7, 10, and 14 weeks after surgery; the lumbar spine was again approached for the microanatomical exploration of the cauda equina and the removal of the repaired roots. The cut roots were then oriented and fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1 mol/L) for 4 hours at 4°C. The specimens were washed in buffer, postfixed in 1% osmium tetroxide in cacodylate buffer (0.1 mol/L) for 2 hours, dehydrated, and placed in Epon overnight at 70°C. Transverse semithin sections, 1 μm thick, were systematically obtained from the ganglionic and spinal cord sides of the repair site and were examined by light microscopy after being stained with toluidine blue. Selected areas were cut for electron microscopy. Ultrathin sections were cut and stained with uranile acetate and lead citrate and were then examined under a JEOL-1200 EX/Temscan electron microscope.

RESULTS

Suture group

The examination under surgical microscope during necropsy showed that the suture area was thinner than the root and had a brownish-yellow color. There were very few adherences to neighboring roots. In three cases, the root was found to be interrupted, making it difficult to find the ganglionic side of the root, which is always far away from the site of reparation.

Light microscopy study showed a very irregular pattern of regeneration, with many Schwann cells. On the side of the spinal cord, the number of nerve fibers was scarce. The fibers were small, both in size and in myelin thickness (*Fig. 1, left*).

An ultrastructural study showed a regenerative pattern with a number of Schwann cells enveloped in their basement membranes and harboring one or more regenerative axons. These cells had a huge nucleus and a large cytoplasm with a very developed endoplasmic reticulum and numerous mitochondria and organelles. It is evident that Schwann cells had multiplied during the earlier stages of regeneration. Regenerative axons contained many neurotubules, neurofilaments, mitochondria, and vesicular bodies. Unmyelinated axons were also seen. There was a very limited connective reaction, without fibroblastic cells and with a scarcely extracellular collagen, seen as thin fibrils oriented parallel to the longitudinal axis of the nerve fibers (*Fig. 1, right*).

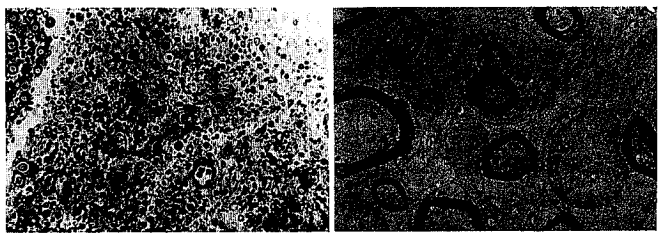

FIGURE 1. End-to-end direct suture group. Microphotographs of transverse sections of repaired roots. *Left*, regenerative pattern 7 weeks after surgery (toluidine blue, ×200). *Right*, electron microscope study showing many hyperactive Schwann cells with myelinated fibers. The endoneurium has some collagen filaments (magnification, ×5000).

Artery sleeve reparation

Observation under an operative microscope showed a segmentary thickness of the repaired root. There was a dense fibrosis around the artery graft with adherences among the roots. In all cases, continuity was found at the repair level.

A semithin section study showed that the lumen of the artery was filled with regenerative fibers and Schwann cells. There were areas containing abundant small myelinated axons, regular in caliber and myelin thickness. However, in other areas, axons were few in number and of smaller caliber. The elastic layers of the artery wall were identified in all cases, and there was no reactive or inflammatory reaction against the graft. However, the graft was surrounded by some layers of connective tissue (*Fig. 2, left*). The breaks in the artery wall between sutures permit the entrance of this connective tissue. A very small number of nerve fibers were seen escaping from the artery lumen through the breaks in the artery wall (*Fig. 2, right*). An ultrastructural study showed findings similar to those of the study of sutured roots.

Nerve graft group

Nerve grafts were identified on the roots as being portions that were thicker and harder than the root itself. There were no adherences between the graft and the roots. In all cases, both stumps of the repaired root remained in continuity.

A light microscopy study showed a fair pattern of regeneration on the spinal cord side of the root. The graft was surrounded by a well-defined layer of connective tissue arranged in a circular pattern (epineurium) that acts as a barrier, limiting the entry of reactive tissue and the exit of nerve fibers (*Fig. 3,*

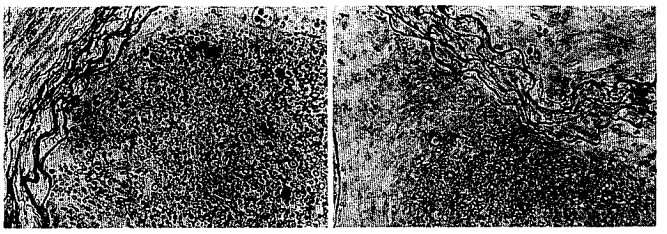

FIGURE 2. Arterial sleeve group. Microphotographs of transverse sections of repaired roots (toluidine blue, ×200). *Left*, regenerative pattern 14 weeks after surgery. *Right*, detail of a break in the wall of the artery, showing the exit of some regenerative fibers and the entrance of bands of connective tissue.

center left). Inside the graft, the histological appearance varies according to the time elapsing between section and repair. Seven weeks after suture, it is possible to see many cords of reactive Schwann cells without signs of further innervation. Among these cords, groups of degenerative fibers can be seen. Later, there are visible fibers of different diameters in different stages of myelination and numerous Schwann cells.

An electron microscopy study shows that the cords are formed by reactive Schwann cells grouped by sheaths made by very thick basement membranes. The Schwann cells had a large cytoplasm with numerous organelles, namely a well-developed rough endoplasmic reticulum. The cell membranes are folded (*Fig. 3, upper left*). Some bands had unmyelinated regenerative nerve fibers. Ten and 14 weeks after surgery, the bands are more numerous but are thinner, with one or two Schwann cells and some nerve fibers. The cytoplasm of the Schwann cells is smaller and possesses few organelles. Most of the axons included inside the bands are myelinated and, although they are small, have a regular diameter and myelin thickness. There are large amounts of collagen, arranged in bundles, oriented in the same direction as the nerve fibers (*Fig. 3, upper right*). Some fibroblastic-type cells were found in the endoneourium. Among the collagen fibrils, there are clusters of unmyelinated axons packed by Schwann cell prolongations and surrounded by a basement membrane (*Fig. 3, lower left*). A well-defined limit can be seen between the nerve fibers and the perineurium layers (*Fig. 3, center right*). There is also a vigorous capillary sprouting arranged in a longitudinal pattern, with tight junctions between the endothelial cells (*Fig. 3, lower right*).

DISCUSSION

Regeneration of spinal roots

Nathaliel and Pease (12, 13) studied the regeneration of dorsal roots after crush injury and found myelinated axons as early as 12 days after the lesion (12, 13). These regenerating sprouts are associated with Schwann cells, forming the Buengner bands. Initially, the Schwann cells possessed simultaneously multiple sprouts in different stages of myelination, although eventually only one of them became fully myelinated (12, 13). Normal dorsal roots have some Schwann cells, and a few days after injury, they show a marked hypertrophy and multiplication. All of these features of root regeneration are similar to those seen in peripheral nerves. However, the paramount point in root regeneration is the absence of connective tissue participation (12, 13).

The study of regeneration in our groups of direct end-to-end suture and reparation with arterial sleeves confirms these findings. Buengner bands in roots repaired with nerve grafts are more prominent, and the connective response is evident, with many collagen fibrils, some fibroblastic cells, and a rich neovascularization. The repair of spinal dorsal roots is followed by a satisfactory degree of nerve regeneration, without significant differences in relation to the surgical technique.

In our experimental study, we have used dorsal roots of the cauda equina as representatives of any nerve in its intrathecal trajectory, although there are differences in nerve regeneration between sensory and motor fibers. The most important task of the regenerative sprouts after dorsal root injury is the entry and growth into the spinal cord, thereby making functional connections. This was beyond the purposes of our study and has been reviewed by others (8). However, the regeneration of dorsal root fibers is almost absent in the central nervous system, particularly because of the development of a glial basement membrane.

Clinical and experimental studies

Nerve regeneration has been studied after experimental lesions of cranial nerves and spinal roots by the use of the classic direct end-to-end suture or nerve grafting. However, only a few studies were designed for the selection of the most useful surgical technique. Before the development of microsurgical techniques, Moyer et al. (10) repaired spinal nerve roots using the arterial sleeve method described earlier by Weiss (24). In our study, we have used a posterior modification of this technique, using veins for sciatic repair in rats (4, 6).

From a clinical point of view, there are few studies or case reports communicating the microsurgical reconstruction of nerve roots. This procedure has been rejected in brachial plexus avulsion because the roots are detached from the spinal cord surface, retract, become atrophic, and remain unidentifiable during surgical exploration (22). However, the construction of cauda equina roots has been strongly recommended by the use of direct surgical end-to-end anastomosis, microsurgical neurolysis, or anastomosis between intercostal nerves and cauda equina roots (14). The facial trunk in the cerebellopontine angle is the cranial nerve most frequently damaged during intracranial procedures (1, 2, 19). Samii (15–17) describes several procedures for the reparation of the facial nerve after acoustic neuroma surgery, including direct end-to-end microsurgical suture or nerve grafting in cases of large defects. Reports of the direct repair of other intracranial nerves, especially those that travel through the cavernous sinus, have been also described (5, 9, 18, 20).

Selection of the surgical technique

In our study, we have compared three surgical techniques. The epineurium and the perineurium are almost absent in spinal roots and in the intracranial part of the cranial nerves. From a technical point of view, this fact makes end-to-end suture very difficult because the lack of connective tissue favors damage to the root during manipulation or suturing. The final result is injury of both stumps of the root, perturbing the satisfactory continuity of the root and the strength of the suture line. The arterial sleeve avoids direct manipulation and the placement of stitches in both root stumps. Arteries, veins, and pseudosynovial tubes have all been used as biological autografts for the reparation of peripheral nerve lesions (4, 6, 7, 24). However, it is a tedious technique, and the contact between both root stumps is not assured after surgery. Moreover, a connective reaction around the artery was very strong in our experience and involved neighboring roots that could potentially be damaged. Conversely, nerve grafts eliminate the prob-

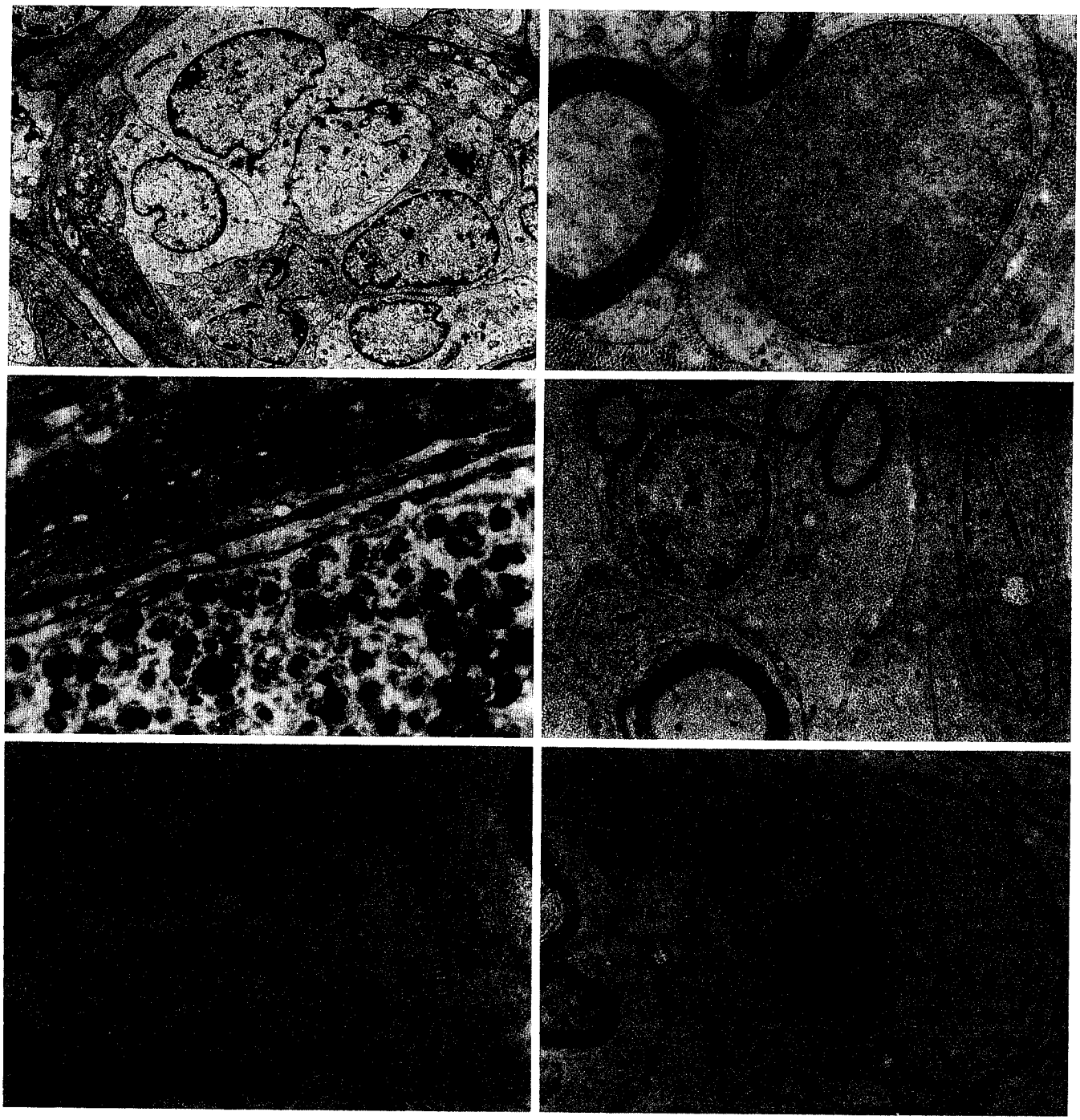

FIGURE 3. Nerve graft group. Microphotographs of transverse sections of the nerve graft. ***Upper left,*** Buengner's band 7 weeks after surgery (magnification, ×3000). ***Upper right,*** high-power magnification showing regenerated myelinated fibers. The endoneurium is rich in collagen filaments (magnification, ×12,000). ***Center left,*** toluidine-stained semithin section showing the regenerative pattern adjacent to the perineurium (magnification, ×400). ***Center right,*** electron microscopy study of this area, showing some myelinated and unmyelinated sprouts surrounded by an evident endoneurium and in close contact with perineurial fibroblastic cells (magnification, ×6000). ***Lower left,*** cluster of unmyelinated fibers (magnification, ×15,000). ***Lower right,*** endoneurial capillary surrounded by a basal membrane and showing tight unions between adjacent endothelial cells (magnification, ×6000).

lem of tension on the suture line and facilitate the suture of the root stumps; also, the peripheral arachnoiditis is very small. In addition, a peripheral nerve graft should have many fibroblast-like and Schwann cells related to the local production of nerve growth factors; this favors and guides nerve regeneration.

In conclusion, the reparation of severed spinal roots or cranial nerves is technically feasible in selected cases of accidental lesions after the resection of spinal or intracranial nerve tumors. In these cases, our experimental study shows that the best surgical procedure is reparation with peripheral nerve grafts.

ACKNOWLEDGMENTS

The author thanks Olga Aliseda and Emilio de la Orden for their laboratory work. This work was supported by a grant from the 'Junta de Andalucía' to Group 3080.

Received, October 28, 1992.
Accepted, June 2, 1993.
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COMMENTS

Dr. González-Darder reported the microsurgical repair of sectioned dorsal L4 spinal roots in the rat. The author compared three types of surgical repair using histological and electron microscopic studies. Many articles have reported the surgical repair of peripheral nerves or cranial nerves with end-to-end anastomosis or with interposition nerve grafting techniques, but very few have reported the subsequent construction of the spinal roots.

This is a well-written article, with meticulous microsurgical technique and excellent electron microscopic examinations. In my clinical experience, direct end-to-end anastamosis provided the best recovery of nerve function in cranial nerves. The results of this article demonstrate that the best result was with interposition grafting, which is surprising. In many spinal root injuries, the injury includes avulsion at the root entry zone. If the author could demonstrate succession in the repair of the entry root zone avulsion, it would be interesting and would have additional application to clinical cases. Also, if the author could perform the spinal root construction in the anterior roots, the investigator could probably observe the recovery of motor function clinically in addition to histologically. This article not only contributes to the understanding of the regeneration and subsequent innervation of spinal roots but provides important clinical application for the construction of the injured spinal root.

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Dr. González-Darder reports three different repair techniques of sectioned intraspinal dorsal roots in a rat model. The experimental groups are direct end-to-end suture, arterial sleeve (allograft carotid artery) anastomosis, and nerve graft interposition (allograft peroneal nerve). The results showed that 33% of the directly repaired roots had separated at the time of follow-up. The arterial sleeve anastomoses were characterized by a localized inflammatory reaction and apparent subsequent generation of the injured axons. The nerve graft group demonstrated no inflammatory response and microscopic evidence of nerve regeneration. The functional outcome of these grafts was not assessed.

This study addresses an important point in the surgical repair of nerve injuries. The author confirms that it is imperative to avoid tension on the repair site of an intraspinal root. This may in part be due to the lack of supporting connective tissue in the spinal roots, as the author describes. However, even in peripheral nerves with sufficient supporting structures, repairs under tension have a poorer outcome than a slack repair (2).

The other area of comment is the choice of a nerve allograft for the nerve repair and the lack of an inflammatory response. From this study, it is unclear why an allograft carotid artery would incite a large inflammatory response, but an allograft peripheral nerve would not. In a rat model of peripheral nerve allograft repairs, Bain (1) had demonstrated an immune response with graft rejection that could be modified by the use of cyclosporin A. This reduction in nerve allograft rejection was seen histologically and clinically. Additionally, as pointed out in the text, the use of a peripheral nerve allograft in the central nervous system with its supporting cells and neurotrophic factors may confer an advantage over other graft material such as the arterial sleeve.

The author presents experimental support in a rat model for the use of nerve grafts for the anatomic repair of intraspinal dorsal root nerve gaps. This investigation is of value by virtue of its novel approach and location. The repair of ventral roots would be an interesting follow-up to the study. This is as exciting area of research with potential applications in several areas of neurosurgery.

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