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# Targeted Spinal Cord Therapeutics Delivery: Stabilized Platform and Microelectrode Recording Guidance Validation

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**Key Words**
Microinjection platform · Molecular therapeutics · Microelectrode recording · Therapeutics delivery · Intraspinal targeting

**Abstract**
***Background/Aims:*** No validated delivery technique exists for accurate, reproducible delivery of biological therapies to discrete spinal cord targets. To address this unmet need, we have constructed a stabilized platform capable of supporting physiologic mapping, through microelectrode recording, and cellular or viral payload delivery to the ventral horn. ***Methods:*** A porcine animal model (n = 7) has been chosen based upon the inherent morphologic similarities between the human and porcine spine. Animals underwent physiologic mapping and subsequent microinjection of a green-fluorescent-protein-labeled cell suspension. Sacrifice (t = 3 h) was performed immediately following behavioral assessment. ***Results:*** Histologic analysis has supported our ability to achieve localization to the ipsilateral ventral horn in the spinal cord. Complications included death due to malignant hyperthermia (n = 1), hindlimb dysfunction attributable to epidural hematoma (n = 1), and hindlimb dysfunction attributable to cord penetration (n = 2). ***Conclusions:*** These results indicate an ability to achieve accurate targeting, but the elevated incidence of neurologic morbidity will require further studies with longer follow-ups that incorporate procedural and equipment modifications that will allow for a reduced number of cord penetrations and will account for observed cardiorespiratory-associated cord movement. These initial results reinforce the challenges of translating biological restorative therapies from small to large animal models and ultimately to humans.

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## Introduction

The delivery of cellular and molecular therapies to the spinal cord holds significant promise for the treatment of traumatic, autoimmune, degenerative and functional spinal cord diseases, all of which are thought to result from compromised neural connectivity, due to a combination of axonal disruption and cell body death. In the attempt to address the pathogenic mechanisms underlying these conditions, therapeutic strategies have been proposed that promote neuroprotection, axonal regeneration or cell-based neurorestoration through replacement of lost neural tissue or supporting stroma. A variety of in vitro and small animal in vivo experiments demonstrate motor neuron protection and axonal regeneration

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through the delivery of genes for trophic factors (e.g. IGF-1, GDNF) antiapoptotic proteins (e.g. XIAP, Bcl-xL) or siRNA capable of inactivating toxic gene products (SOD1 siRNA) [1–6]. Vectors designed for the delivery of these genes can be injected directly into the spinal cord (in vivo gene transfer) or delivered to cells which are subsequently transplanted into the spinal cord (ex vivo gene transfer) [7, 8]. As an example of cellular neurorestoration, recent experiments demonstrate that motor neurons derived from embryonic stem cells can provide functional recovery in rats that have undergone Sindbis-virus-induced motor neuron loss and suffered from attendant paralysis. Repair required both intraparenchymal cell transplant in combination with a suppression of myelin inhibitory signaling and the appropriate trophic support for the advancing axon [9]. Despite the success of these disparate therapeutic approaches in small animal models, clinical translation of these approaches will require a safe and accurate means for controlled intraspinal delivery to the site of interest.

Our laboratory and others have previously described the use of intramuscular and peripheral nerve injection as a means to deliver viral vectors to motor neurons via retrograde axonal transport [10]. However, attempts to scale this technique up to large animals have failed when using the current generation of vectors. Moreover, retrograde transport is only capable of motor neuron delivery, whereas gene therapy for the treatment of multiple sclerosis, spinal cord injury and even motor neuron disease may depend on vector delivery to white matter tracts, other neuronal classes and glia (e.g. astrocytes and oligodendrocytes) within the spinal cord. Similarly, much has been written about the capacity of stem cells to home in on areas of pathology [11, 12]. However, this feature only applies to undifferentiated cells, not biomanufactured cells of a particular lineage. Moreover, dosing accuracy may prove elusive given low or variable migration into the spinal cord. Thus, rational methods for safe and accurate spinal cord injection are necessary for the near term translation of cell- and gene-based spinal cord therapies.

In contrast to small animal models, where spinal cord injections are routinely performed without guidance or stabilization, translation of parenchymal injection-dependent therapies to human trials may require one or both. Some form of guidance is recommended to improve localization of the therapy to the tract or neurons of interest and to reduce the incidence of off-target sequelae. Stabilization has the dual functions of increasing the targeting precision while also reducing sequelae as the spinal cord can require cannulation for a period of several minutes during the targeting and infusion process. Recent successes in cell-based therapy experimentation and the proprietary development of viral vectors designed for direct parenchymal injection both beg the need for a technology capable of targeted, localized administration of a biologic payload in translation from small to large animal model studies.

The current study utilizes a large animal model to explore targeted cell delivery into the ventral horn of the spinal parenchyma, testing the hypothesis that accurate cell delivery can be achieved from a stabilized platform under the guidance of physiologic mapping by microelectrode recording (MER) data and with confirmation by peripherally measured motor evoked potentials. The results presented herein indicate a potential role for MER as a means to achieve guidance during intraparenchymal injection through mapping the gray and white matter boundaries of the spinal cord. Evoked motor potentials can be elicited with microstimulation within the cord. Histologic analysis supports ventral horn targeting and localization of the infused payload. Neurologic examination, assessed through pre- and postoperative sensory and motor assessments, revealed a significant rate of compromise in spinal cord function in the immediate postoperative period. This observation suggests a necessity for experimental and procedural modifications in the evolution of these techniques and the devices that support them.

## Materials and Methods

*Cells*

All infused pigs were injected with green-fluorescent-protein (GFP)-labeled neural progenitor cells, prepared in a similar manner, from E14 rat cortex. Cells were injected at a concentration of 200,000 cells/μl at a rate of 1 μl/min with a total injected volume of 10 μl.

*Surgical Technique*

Two-level laminectomies were performed on 7 wild-type female pigs weighing between 30 and 40 kg, at the position of L3/4 or L4/5 based upon spinous process size. The position was gauged by counting rostrally from the sacrum and caudally from T12. The pig was placed in the prone position, and the operative field was prepared with alcohol and betadyne prior to draping. A 16-cm incision was created over the spine and a multilevel laminectomy performed over the lumbar spinal cord. Following laminectomy, a 2-cm incision was made through the dura with the aid of a dural guide, exposing the spinal cord. The dura was reflected away from the pial layer using 4-0 Nurolon suture. Pial incision at the injection site was performed with a No. 11 blade. At this point, the stabilized microinjection platform was attached to the

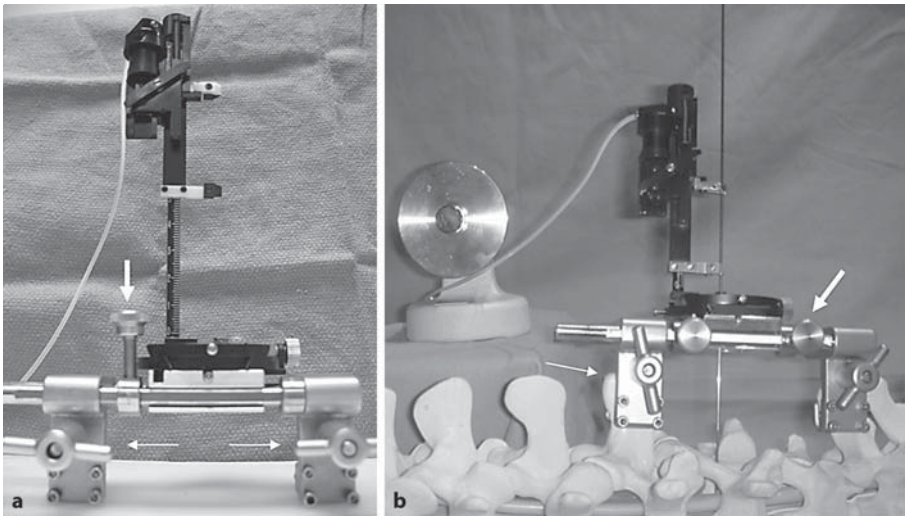


**Fig. 1.** Prototype platform and drive. **a** Platform base with spinous process C clamps visible (thin arrows). Drive platform locking screws (thick arrows). Electrode guidance platform and drive assembly are in black. **b** Assembled platform and drive attached to a laminectomy model. Fine adjustment depth controller seen in the background.

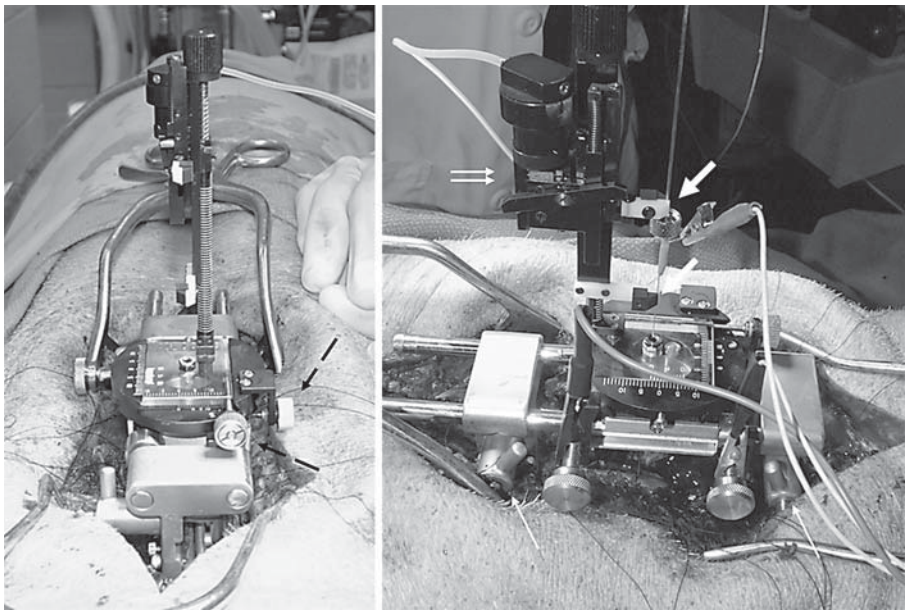

**Fig. 2.** Platform and drive in vivo. C clamps are attached to spinous processes (thin arrows). Depth is controlled by hydraulic drive (double arrows). Electrode is stabilized by guiding arms (thick arrows). Lateral positioning is controlled by analog dials (dashed arrows).

appropriate spinous processes, allowing the device to span the laminectomy. Next, the microdrive (Narishige MO-97) was attached to the stabilization platform, as depicted in figures 1 and 2. The hydraulic microdrive was used for controlled advancement of the electrode and cannula. The microelectrode fit snugly within the outer cannula with the tip retracted 0.75 mm into the cannula until continuous contact was made with the dorsal surface of the spinal cord. The electrode was advanced into the spinal cord, with documentation of depths when recordings were taken from individual neurons. The spinal cord was penetrated on an orthogonal trajectory to the cord surface at a point 2 mm medial to the dorsal root entry zone. As necessary, either 1 or 2 passes were made with a microelectrode to confirm accurate targeting. The cell suspension was infused at a rate of 1μl/min 1 mm above the gray-white junction at the base of the ventral horn, as confirmed by MER. The cannula was left in place for 5 min following infusion to minimize cell suspension reflux along the cannula track.

Following removal of the injection cannula, the entire apparatus was removed and the incision was closed in 4 layers. The dura was closed with a running stitch, in a watertight fashion, using 4-0 Nurolon suture. A strip of Gelfoam was placed over the closure. A 0 Vicryl suture was used for the deep muscular layer obliterating the dead space and reducing the potential for development of an epidural hematoma. The second layer, the fascia, was also closed with 0 Vicryl suture in a watertight fashion. The dermal layer was closed with 2-0 Vicryl sutures followed by skin stapling.

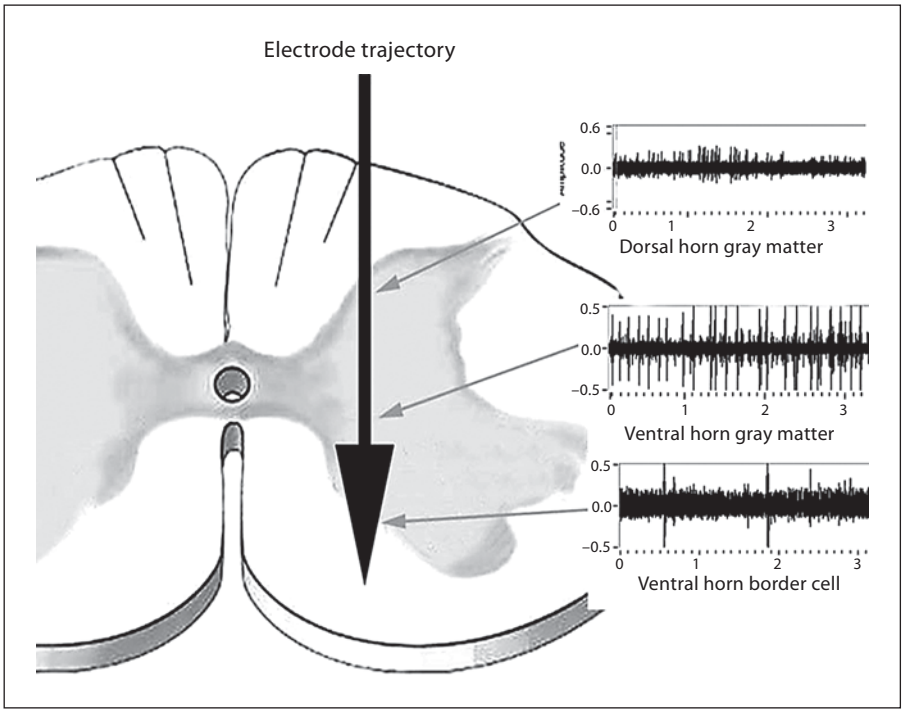


**Fig. 3.** Spinal cord MER technique. Depth-dependent nature of recordings taken by microelectrode within the gray matter. MER can be used to ensure lateral localization (gray vs. white matter) and to approximate the appropriate depth for infusion into the ventral horn. Positional confirmation is attained by measuring evoked motor unit activation secondary to stimulation in the ventral horn.

*Anesthetic Technique*

The following regimen was used for induction: ketamine 20 mg/kg, xylazine 1 mg/kg and atropine 0.02 mg/kg. For sedation, acepromazine 0.1 mg/kg (maximum dose 3 mg i.m.) was given. For pain, morphine 0.5 mg/kg i.v. and ketoprofen 1–1.5 mg/kg i.v. was given. Additionally, morphine was given every 3 h at 0.5 mg/kg i.v. If the pig began to recover from sedation at less than 1 h since the previous morphine dose, additional medication was withheld. If it had been greater than 1 h, additional morphine was provided. If the pig recovered quickly from sedation, 1–3 mg of thiopental was given as supplemental chemical restraint. To facilitate the recording of evoked motor potential, rapidly reversible non-depolarizing paralytics were employed only during exposure of the bony anatomy of the lumbar spine, prior to performance of the laminectomy.

*Platform, Hydraulic Drive and Infusion Pump*

The prototype injection platform was fabricated in the Department of Biomedical Engineering Prototype Lab in the Lerner Research Institute at the Cleveland Clinic. The device attaches to the spinous processes rostral and caudal to the gap created by the laminectomy. Attachment is secured by pressure clamps on the rostral and caudal spinous process. As depicted in figure 1, the distance between spinous process clamps can be adjusted to accommodate a variety of laminectomy lengths. Once rigidly attached to the platform stage, the microdrive is capable of additional rostrocaudal and mediolateral electrode or cannula position adjustment. Electrode and cannula depth were controlled with a Narishige model MO-97 hydraulic drive. A programmable Harvard p99 pump (Harvard, Inc.) connected to a Hamilton syringe by silastic tubing was used for purposes of cell suspension infusion.

*MER Technique and Stimulation*

MER was initially attempted with the Recording MicroSyringe system (Crist Instrument Co., Inc.) which combines an infusion port and recording electrode. The poor quality of the recordings obtained with the Recording MicroSyringe in pig 1 led to the adoption of an Alpha Omega microrecording electrode (Alpha Omega Ltd.) for all remaining procedures. The platinum/iridium electrode had an impedance of 300–500 kΩ measured at 1 kHz. The microelectrode was advanced slowly along the trajectory to facilitate isolation of neuronal activity, with visual and auditory representation of cell electrical activity providing the basis for depth assessment, as demonstrated in figure 3. The loss of cellular activity and a decrease in background activity marked the transition from the ventral horn to the white matter.

Once the appropriate location had been reached as defined by the gray-white junction at the floor of the ventral horn (MER), motor evoked potentials were generated. Low-frequency, pulsatile square wave stimulation was delivered to the ventral horn through the microelectrode. As the amplitude of the stimulation was titrated, visual inspection of the animal was performed in order to identify the activated muscle group. Surface EMG electrodes were placed over the activated area for the recording purposes. Based upon the observations of recorded action potentials and background resting activity, the position within the ventral horn was confirmed. In many cases, an additional pass was required to delineate the limits of the gray matter.

*Histology*

Following sacrifice, the spinal cords were fixed in 4% PFA for 10 days. The following day, they were transferred to 30% sucrose in PBS for 24–48 h until dehydrated, as determined by loss of buoyancy. The excised cord segments were frozen with isopen-

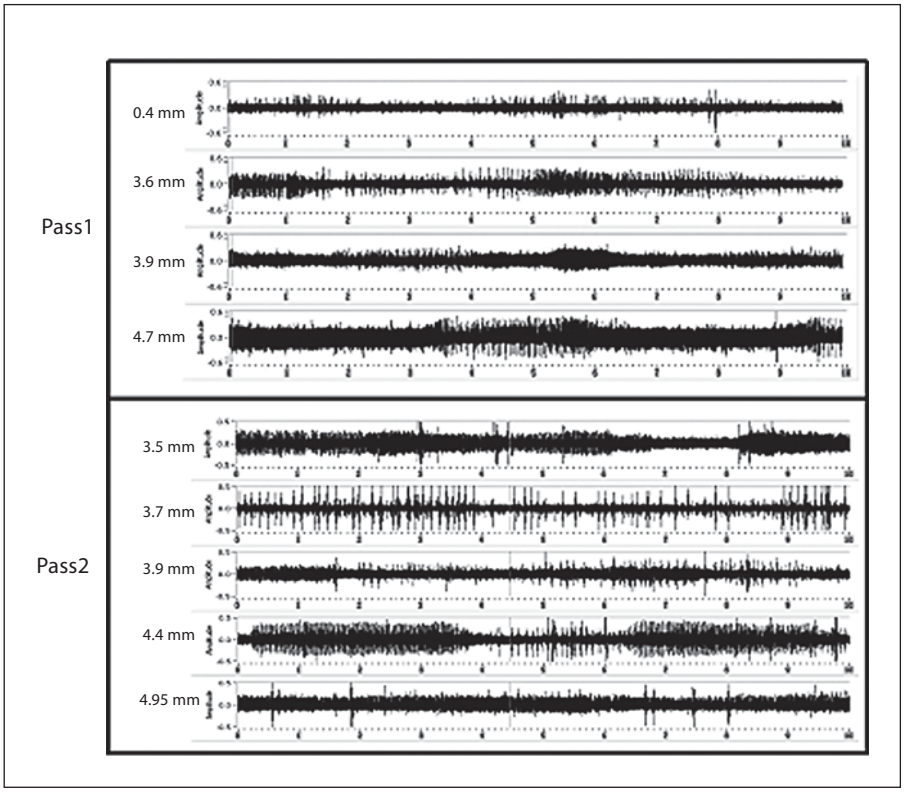


**Fig. 4.** Representative examples of the MER traces obtained from pig 3. Depth recordings for both passes are shown. In pass 1, the recordings, which resembled white matter at successively deep readings, prompted extraction of the electrode and a second attempt at a unique trajectory. Pass 2 was noted by tracings resembling gray matter. Following confirmation by motor unit assessment of evoked potentials, appropriate positioning is confirmed.

tane and cut into 25-μm sections with a cryostat (Jung Frigocut 2800N, Cambridge Instruments). At 100-μm intervals, 3 sections were obtained. Two of the sections were stained with HE, and a third was left unstained for GFP visualization.

*Behavioral Assessments*

Both pre- and postoperative (t = 3 h following closure) behavioral assessments were gathered to assess neurologic morbidity associated with the cell infusion procedure. Sensory evaluation utilized a von Frey anesthesiometer with a 400-gram rigid tip applicator. The pigs were assessed on the carpal pad at all 4 limbs, with the forelimbs providing baseline data. Scoring was based upon active limb withdrawal or vocalization. Motor evaluation was measured using both a modified Basso, Beattie, Bresnahan (BBB) scale and a Tarlov scale. The BBB scale was initially developed as a test for a small animal model but has recently been validated for use in a large animal model [13, 14].

## Results

*Ventral Horn Targeting and Localization*

Operative assessment of ventral horn targeting was accomplished by a combination of MER and spinal cord motor evoked potentials with assessment of motor unit activity and conduction, respectively. Figure 4 provides representative tracings at various depths of electrode penetration. Two passes were made with the recording electrode if conclusive recordings were not attained on the first pass. The pigs that required a second pass are indicated in table 1. The second pass of the electrode into the spinal cord appeared to elicit predominately cellular recordings, whereas the first pass MER often indicated that the electrode track coursed through the white matter. When a second pass was required, the electrode was displaced either in the mediolateral plane or in the rostrocaudal plane prior to penetration. MER-based assessment of localization was dependent upon both auditory and visual interpretation of the data. Spinal cord motor evoked potentials provided confirmation that the ventral horn had been reached. Figure 5 shows a sample motor evoked potential as a result of stimulation of the ventral cord. Together, cellular recordings and microstimulation with motor unit assessment provided positional confirmation. Dual confirmation was achieved before proceeding with injection.

*Histologic Analysis*

Figure 6 presents an example of the histological data attained following postoperative sacrifice. Figure 6a

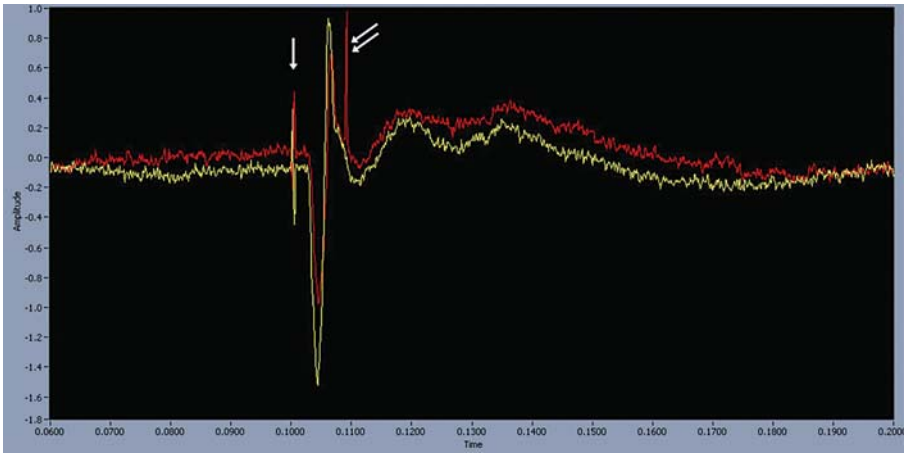


**Fig. 5.** Motor evoked potential elicited by single-pulse microstimulation of the ventral horn. Two independent superimposed samples demonstrate reproducibility of the evoked response. Each waveform represents a 140-ms sample of data, including a 40-ms of pre-stimulus baseline terminated by the stimulation artifact (single, white arrow) and followed by a 100-ms post-stimulus recording window. The stimulation artifact is followed shortly (<4 ms) by a large biphasic physiological response. The double arrows identify a small electrical artifact occurring within the recorded signal.

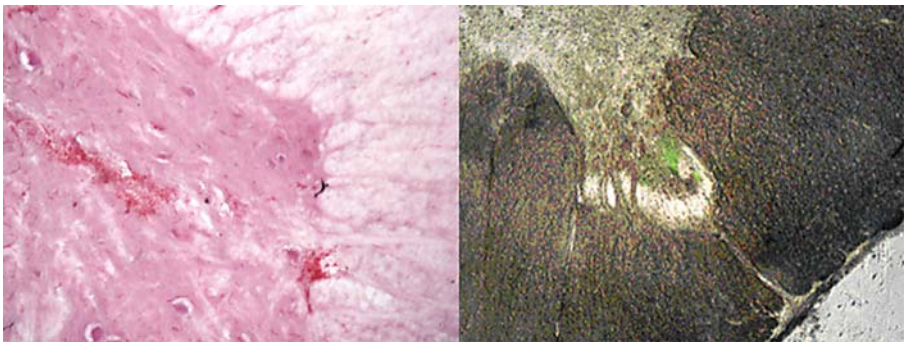

**Fig. 6.** Histologic targeting confirmation. Both HE (×20) (left) and fluorescence microscopy (×5) (right) have been used to confirm ventral horn targeting of the E14-harvested rat neural progenitors, ex vivo modified to encode GFP.

demonstrates an HE-stained section showing injected cells in the ventral horn. Figure 6b shows a similar fluorescence image with GFP-positive cells localized to the ventral horn.

*Behavioral Assessment*

Pre- and postoperative behavioral assessment was completed on 5 of the 7 pigs (pigs 3–7). Five of the 7 pigs received pre- and postoperative motor and sensory assessment with the von Frey anesthesiometer, while one of the pigs was assessed with gross pain stimuli postoperatively (pigs 3–7) and pig 2, respectively. One of the pigs (pig 1) succumbed to malignant hyperthermia, a condition that has an elevated incidence in the porcine animal model with use of inhalation anesthetics. Pig 2 developed a postoperative epidural hematoma which resulted in bilateral, symmetric paraparesis. This pig had received a preoperative course of NSAIDS; the regimen was subsequently adjusted to the use of opioid-based pain medications.

Motor assessment data, presented in table 1, provide evidence as to the neurologic morbidity associated with

**Table 1.** Behavioral assessment – motor

| Pig | Preoperative | | Postoperative | | Passes |
|---|---|---|---|---|---|
| | BBB | Tarlov | BBB | Tarlov | |
| 1 | 21 | 4 | – | – | 1 |
| 2 | 21 | 4 | 6 | 2 | 2 (RC) |
| 3 | 21 | 4 | 21 | 4 | 2 (ML) |
| 4 | 21 | 4 | 9 | 3 | 2 (RC) |
| 5 | 21 | 4 | 21 | 4 | 2 (RC) |
| 6 | 21 | 4 | 21 | 4 | 2 (ML) |
| 7 | 21 | 4 | 10 | 3 | 1 |

Both pre- and postoperative values for the BBB and Tarlov scale are given. Additionally, the number of passes required for infusion is provided. Pig 1 succumbed to malignant hyperthermia and could not be assessed. ML = Mediolateral; RC = rostrocaudal (indicates position of second pass entry point relative to first pass). Pig 2: postoperative epidural hematoma with symmetric weakness and sensory deficits. Only weakness was noted in the remaining pigs with abnormal scores and was unilateral in the other cases.

electrode penetration, cannulation or mass effect from fluid introduction into the ventral horn of the spinal cord. In no scenario were sensory deficits noted. However, procedure-associated neurologic morbidity occurred in pigs 4 and 7. Therefore, neurologic morbidity directly attributable to intraspinal recording and infusion was noted. Further, in the 6 pigs that were assessed with MER, multiple electrode passes were required in 5 cases. In context, the data presented in table 1 and figure 7 indicate that sensory function was not disrupted and that procedure-associated neurologic morbidity was localized to the ventral horn.

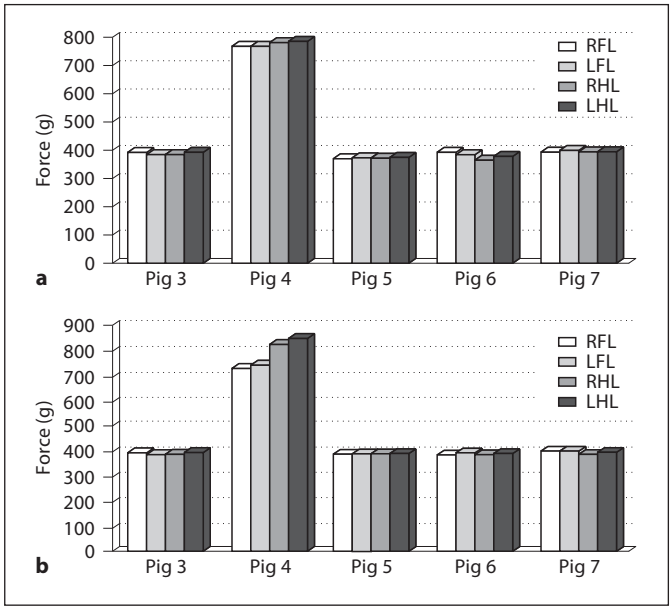


**Fig. 7.** Behavioral assessment – sensory. Grams of force required to achieve limb withdrawal or vocalization. Sensory information is not presented for either pig 1 or 2. The figure legend indicates the tested carpal pad (RFL = right forelimb; LFL = left forelimb; RHL = right hindlimb; LHL = left hindlimb. Values are not presented for pig 1, as it did not survive the operation, nor for pig 2, as it developed an epidural hematoma and subsequently a symmetric paraparesis. A diminished response to gross pain stimuli was present. **a** Preoperative. **b** Postoperative.

## Discussion

The results of this study provide preliminary data for a new approach aimed at achieving directed localization of pharmacologic or biologic agents tailored towards parenchymal delivery within the spinal cord and specifically the ventral horn in this study. Accurate targeting of the ventral horn was achieved in all scenarios by MER and peripherally measured evoked potentials elicited by microstimulation. In contrast to MER in the deep brain nuclei, general anesthesia fails to suppress neuronal activity in the spinal cord. Thus, MER is feasible within the spinal cord of the sedated mammal. Moreover, it is possible to evoke a localized response by microstimulation of the ventral horn. This provides a means for confirming the cannula tip location in the ventral horn. In addition, it provides the added advantage of identifying the muscles innervated by the transplanted spinal cord segment. In this study, consistent activation of the quadratus lumborum muscle was noted. Because the pig spinal cord extends the full length of the thecal sac, this may indicate targeting to more rostral gray matter than would be found in the human at a comparable spinal level. In the context of phase I trials focused on the safety of injections, this methodology would also allow for the identification of motor units in the region of transplantation. The function of these motor units can be compared to those of segments that were not transplanted in order to establish preliminary efficacy data for transplant-related motor neuron protection.

The clinical precedence of the dorsal root entry zone procedure [15, 16] and cordotomy demonstrate that the spinal cord can tolerate shallow penetration with thin gauge needle/electrodes. However, in the current study, 40% of pigs assessed pre- and postoperatively (pigs 3–7) demonstrated neurological compromise 3 h after surgery. This morbidity did not correlate with the use of single or double electrode passes arguing against number of passes being the critical variable in injection safety. Importantly, because the Alpha Omega electrode does not accommodate injection, all animals except the first required at least 2 passes, first with the electrode, then with the cannula. Thus, double penetration through a single pial opening may be the cause of the high rate of neurological compromise. Nevertheless, attendant morbidity was still associated with cell infusion, leading to the conclusion that infusion-related increases in local hydrostatic pressure could account for the observed postoperative hindlimb motor dysfunction. In addition, the lumbar cord was noted to move with animal ventilation (respiratory) and to pulsate in synchrony with the heart rate (cardioballistic). This movement against a rigid needle in place for the duration of a slow injection may also have contributed to morbidity. Finally, because this initial exploratory study was only continued for 3 h after recovery to limit concerns from the Cleveland Clinic Institutional Animal Care and Use Committee, it is possible that some degree of the currently observed morbidity is a transient phenomenon.

Direct injection to the spinal cord has occurred only rarely in human trials and as infrequently in large animal models [17]. Though this procedure is carried out frequently in small animal models to test proof of principle hypotheses, direct injection of unguided, unstabilized therapies has raised the fear that the treatment would pose more harm than good, as a result of direct trauma to the spinal cord parenchyma. Attempts to translate either biologic or pharmacologic therapies to clinical trials, then, have focused on systemic or intrathecal delivery, with peripheral administration of viral vectors remaining largely an experimental procedure. Both forms of administration fail to achieve localization and so increase the risk of off-target effects, while minimizing overall therapeutic efficacy due to poor penetration of the blood-brain barrier.

In the present paper, evidence has been provided to support the claim that a stabilized platform can be used to achieve accurate targeting of infused cells to the ventral horn with the use of MER and motor evoked potentials as tools for guidance. In this preliminary series, attendant morbidity was noted to be high, potentially a function of the short time elapsed between surgical closure and behavioral assessment. The data accrued, as well as clinical knowledge, support the fact that electrode penetration of the spinal cord is tolerated, suggesting that the observed motor function morbidity was at least partially due to local infusion-related motor neuron and white matter compression or edema. This observation has led to the initiation of future studies designed to examine procedure-associated morbidity at extended time points. The proposed survival study should provide data regarding: potential reversibility of the observed postoperative neurologic morbidity, potential for minimization of this morbidity by optimization of cell infusion volume and rate, and localization of the infused cells. The hypothesis that respiratory movement in the spinal cord contributes to morbidity may be tested by temporarily holding ventilation after maximizing oxygen saturation during recording and infusion. Alternatively, injection at an alternate site (e.g. the cervical cord) may minimize respiratory-associated cord movement. Further, injection will be attempted with the use of predetermined coordinates (coordinate-based microinjection) in the attempt to reduce the required number of cord penetrations. This targeting strategy will be assessed for accuracy and analyzed to determine whether the additional cord penetrations required for physiologic mapping have contributed to the observed neurologic morbidity.

## References


1 Garrity-Moses ME, Teng Q, Krudy C, Yang J, Federici T, Boulis NM: X-Linked inhibitor of apoptosis protein gene-based neuroprotection for the peripheral nervous system. Neurosurgery 2006;59:172–182; discussion 172–182.

2 Garrity-Moses ME, Teng Q, Liu J, Tanase D, Boulis NM: Neuroprotective adeno-associated virus Bcl-xL gene transfer in models of motor neuron disease. Muscle Nerve 2005; 32:734–744.

3 Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH: Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. Science 2003;301:839–842.

4 Miller TM, Kaspar BK, Kops GJ, Yamanaka K, Christian LJ, Gage FH, Cleveland DW: Virus-delivered small RNA silencing sustains strength in amyotrophic lateral sclerosis. Ann Neurol 2005;57:773–776.

5 Vincent AM, Feldman EL, Song DK, Jung V, Schild A, Zhang W, Imperiale MJ, Boulis NM: Adeno-associated viral-mediated insulin-like growth factor delivery protects motor neurons in vitro. Neuromolecular Med 2004;6:79–85.

6 Wang LJ, Lu YY, Muramatsu S, Ikeguchi K, Fujimoto K, Okada T, Mizukami H, Matsushita T, Hanazono Y, Kume A, Nagatsu T, Ozawa K, Nakano I: Neuroprotective effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. J Neurosci 2002;22: 6920–6928.

7 Davies JE, Huang C, Proschel C, Noble M, Mayer-Proschel M, Davies SJ: Astrocytes derived from glial-restricted precursors promote spinal cord repair. J Biol 2006;5:7.

8 Hsieh J, Aimone JB, Kaspar BK, Kuwabara T, Nakashima K, Gage FH: IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. J Cell Biol 2004; 164:111–122.

9 Deshpande DM, Kim YS, Martinez T, Carmen J, Dike S, Shats I, Rubin LL, Drummond J, Krishnan C, Hoke A, Maragakis N, Shefner J, Rothstein JD, Kerr DA: Recovery from paralysis in adult rats using embryonic stem cells. Ann Neurol 2006;60:32–44.

10 Federici T, Boulis NM: Gene-based treatment of motor neuron diseases. Muscle Nerve 2006;33:302–323.

11 Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, Frenkel D, Li J, Sidman RL, Walsh CA, Snyder EY, Khoury SJ: Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci USA 2004;101: 18117–18122.

12 Penn MS, Bakken EE: Heart-brain medicine: where we go from here and why. Cleve Clin J Med 2007;74(suppl 1):S4–6.

13 Basso DM, Beattie MS, Bresnahan JC: A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995;12:1–21.

14 Fukuda S, Nakamura T, Kishigami Y, Endo K, Azuma T, Fujikawa T, Tsutsumi S, Shimizu Y: New canine spinal cord injury model free from laminectomy. Brain Res Brain Res Protoc 2005;14:171–180.

15 Friedman AH, Nashold BS Jr: DREZ lesions for relief of pain related to spinal cord injury. J Neurosurg 1986;65:465–469.

16 Gorecki JP, Nashold BS: The Duke experience with the nucleus caudalis DREZ operation. Acta Neurochir Suppl 1995;64:128–131.

17 Mazzini L, Mareschi K, Ferrero I, Vassallo E, Oliveri G, Boccaletti R, Testa L, Livigni S, Fagioli F: Autologous mesenchymal stem cells: clinical applications in amyotrophic lateral sclerosis. Neurol Res 2006;28:523–526.