

Regular Article

Combined delivery of neurotrophin-3 and NMDA receptors 2D subunit strengthens synaptic transmission in contused and staggered double hemisectioned spinal cord of neonatal rat

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Received 29 June 2005; revised 21 September 2005; accepted 11 October 2005

Available online 11 November 2005

Abstract

We investigated whether administration of neurotrophin-3 (NT-3) and NMDA-2D-expressing units, found previously to enhance transmission in neonatal rat spinal cord, strengthens synaptic connections in the injured neonatal cord. We employed electrophysiological methods to evaluate the strength of synaptic transmission to individual motoneurons in the contusion and staggered double hemisection spinal cord injury (SCI) models. SCI at caudal thoracic levels (T11–T12) was carried out at postnatal day 2 (P2). Plugs containing NT-3-secreting fibroblasts and NR2D-expressing HSV-1 amplicons (HSVnr2d) were implanted above the lesion. Control animals were treated with an amplicon-expressing β -galactosidase (HSVlac). After 8–10 days of treatment, the rats were sacrificed and spinal cords were removed for intracellular recording. Untreated contused cords preserved a fraction of white matter and weak monosynaptic responses were observed through the injury region. However, no synaptic connections were observed in control cords receiving double hemisection injury. Combined treatment with NT-3 and HSVnr2d strengthened monosynaptic connections in contused cords and induced the appearance of weak but functional multisynaptic connections in double hemisectioned cords. In contrast, treatment with either NT-3 or HSVnr2d alone failed to induce appearance of synaptic responses through the hemisectioned region. These results suggest that chronic treatment with NT-3 secreting fibroblasts combined with facilitated function of NMDA receptors by HSVnr2d treatment strengthens connections that survive incomplete SCI and therefore that such combined treatment might facilitate recovery of function following SCI.

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Keywords: Spinal cord injury; EPSP; Motoneuron; NR2D; Viral vector; NT-3; HSV amplicon; Engineered fibroblasts; Plasticity

Introduction

Some neuronal connectivity often remains following acute spinal cord injury (SCI), and limited functional recovery has often been observed in rats and people with spinal cord contusion. However, the degree of recovery is variable, and synaptic plasticity in surviving pathways is an important component of this recovery process (Raineteau and Schwab,

2001). The aim of this research was to devise strategies to strengthen synaptic effects of the surviving descending fibers after spinal cord injury by investigating their functional synaptic projections to individual motoneurons.

Our previous studies revealed that neurotrophin NT-3 applied acutely (Arvanov et al., 2000) or chronically via engineered fibroblasts (Arvanian et al., 2003) enhances the intracellularly recorded synaptic response of lumbar motoneurons to stimulation of segmental and descending inputs. The acute action of NT-3 requires functional NMDA receptors (Arvanian and Mendell, 2001). It was markedly potentiated in older neonates by the virally delivered NR-2D regulatory

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subunit of the NMDA receptor that enhances NMDA receptor function in motoneurons by decreasing NMDAR Mg^{2+} blockade (Arvanian et al., 2004). Therefore, in the present study, we examined whether combined delivery of NT-3 secreted from engineered fibroblasts (Horner and Gage, 2002) and NMDAR-2D expression via HSV-1 amplicon vector transduction (Bowers et al., 2001) strengthens the existing weak connections to intracellularly recorded motoneurons through the partially injured neonatal rat spinal cord.

Two spinal cord injury models were employed in this study: contusion and staggered contralateral hemisections (double hemisection). The latter leaves a tissue bridge that allows non-severed cortico-spinal tract neurons to reconnect through undamaged tissue (Bernstein and Stelzner, 1983). We found that combined treatment of the injured animals with NT-3 secreting fibroblasts and HSV-1 amplicon expressing the NR2D subunit strengthened surviving monosynaptic connections in the contused cord and induced the appearance of functional but weak multisynaptic connections through the double hemisected injury region.

Materials and methods

Spinal cord injury, implantation of neurotrophin-producing fibroblasts, HSV-1 amplicon transductions, as well as intracellular recordings, were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at SUNY-Stony Brook.

Preparation of collagen plugs containing NT-3 or β -galactosidase (β -Gal) secreting fibroblasts

Genetically engineered NT-3 or β -Gal producing cultured rat fibroblast cells were suspended in 0.6% glucose-PBS to a final concentration of 0.4×10^6 cells/ μ l, and a volume of approximately 2 μ l was inserted in collagen plugs, as previously described (Kawaja and Gage, 1992; McTigue et al., 1998).

HSV amplicon vector construction and packaging

Packaging of helper virus-free amplicon vector stocks and subsequent virus purification and determination of amplicon virus titers via both expression- and transduction-based methodologies were carried out as previously described (Bowers et al., 2000, 2001). Vectors carried genes for either NR2D (HSVnr2d) or β -galactosidase (HSVlac), as well as the reporter gene, green fluorescent protein.

Surgical procedure for the staggered double lesion, injection of vectors and implantation of fibroblasts

Two-day-old (P2) Sprague–Dawley rats were anesthetized by hypothermia by placing them on a latex glove in contact with a bed of ice for 10 min. Under a dissecting microscope, the skin and muscles overlying the midthoracic cord at T11–T12 were separated and retracted, and the underlying spinal cord segment was exposed. The cord was carefully lifted from

the bone using a thin spatula and the sharp blade of an iridectomy scissors was placed in the vertical position on the surface of the cord at the midline at T11. Keeping the blade in the vertical position, it was pushed down until it penetrated the entire thickness of the cord and emerged from the other side. Using both blades of the scissors, the left lateral and ventral funiculus were then lesioned unilaterally. While keeping the cord on the spatula, the mirror procedure was repeated on the right side of the cord at T12.

HSV-1 vectors were then injected directly into the lesion on both sides. Using a Hamilton microliter syringe with a 33-gauge needle, virus ($\sim 10^4$ viral particles) was injected directly into the lesioned area at T11 and T12 (2 injections of 1 μ l each). Subsequently, a plug containing fibroblasts was implanted on top of the lesioned area. The collagen plug (shaped as a half cylinder 1 mm in diameter and 1.5 mm long) was placed flat side down directly on the spinal cord and held loosely in place with durafilm (Codman and Schurtleff, Inc.). Finally, the muscle and skin were sutured in layers with 5-0 silk sutures and the wound was covered with sesame oil to prevent rejection of the pup by the mother (see Arvanian et al., 2003). Pups were kept warm and were returned to the mother when they became active. Maternal grooming was sufficient to maintain post-injury bladder expression.

Contusion injury

P2 rats were anesthetized by hypothermia for 10 min, the skin and muscles overlying the midthoracic cord at T11–T12 were separated and retracted, and the underlying spinal cord segment was exposed. Rats were placed in special tray with a wax mold to stabilize them and aligned under the center of the Infinite Horizon (IH) Impactor mouse force probe. In order to account for the protective effect of cold anesthesia, the injury was initiated after pups took their first spontaneous inhalation. Rats received 30-kilodyne contusion injury and spinal cord was examined for even bilateral bruising. HSV-1 and NT-3 were administered, muscle and skin were sutured in layers, the wound was covered with sesame oil and pups were kept warm as described above. Upon recovery from anesthesia and before returning them to the mother, the pups were observed to exhibit hindlimb paralysis.

Electrophysiology

After 8–10 days of treatment, the rats were prepared for electrophysiological recording using methods previously described in detail (Arvanov et al., 2000; Arvanian et al., 2005). Experimenters were blinded as to treatment of the injured rats. Rats were anesthetized in halothane (10 ml in 5 l volume) and decapitated; the spinal cord (spanning segments from approximately T1 to S3) was removed from the animal and a region from S3 to L1 was hemisected leaving the injured thoracic region undisturbed. The left hemicord was pinned to a Sylgard-coated surface in the recording chamber. The chamber was superfused (10 ml/min) with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5,

MgSO₄ 2.0, NaHCO₃ 25, NaH₂PO₄ 1.2, dextrose 11, aerated with 95% O₂/5% CO₂ (pH 7.4, 30°C). The VLF was dissected free of the spinal cord at T2 (Pinco and Lev-Tov, 1994). Suction stimulating electrodes were attached to peeled VLF axon bundles for activation of descending inputs to motoneurons, to the cut L5 dorsal root for activation of segmental inputs to motoneurons, and to the L5 ventral root for identification of recorded cells as motoneurons by antidromic activation. Intracellular recordings in lumbar spinal motoneurons in the L5 segment were obtained using sharp microelectrodes (resistance 70–110 MΩ, filled with 3 M potassium acetate). Ten stimuli of 50 μs duration and 0.05 Hz stimulation rate were delivered separately to DR and VLF at an intensity sufficient to evoke the maximum monosynaptic potential for DR (about 100 μA) and to evoke the maximum response for VLF in non-injured cords (about 500 μA; Arvanov et al., 2000). Short latency (4 to 10 ms) VLF responses were judged to be monosynaptic if they exhibited little amplitude and latency fluctuation compared to later polysynaptic components when the responses to successive single trials were superimposed (Arvanian et al., 2003, 2004). All cells displayed a resting membrane potential of –63 mV to –67 mV.

The results are presented as mean ± SEM. We recorded VLF-EPSPs from 3 to 7 motoneurons in each spinal cord. The response from all recorded cells in each cord was averaged and used for statistical analyses, i.e., degrees of freedom were derived from the number of animals, not the total number of cells. Data were compared first by carrying out one-way ANOVA (Sigmastat 2.0). If significant differences were observed between the groups, the Student–Newman–Keuls test was used for pairwise multiple comparisons between them. All illustrated responses are the largest obtained from each treatment group. Data in uninjured rats obtained from previously published experiments (Arvanov et al., 2000; Arvanian et al., 2004) were used for some comparisons. In other cases, where animal size was an important variable (e.g.,

for measurements of latency where conduction distance is a crucial variable), comparisons were made to data obtained from untreated littermates of the treated animals at the same age.

Histology

After completion of electrophysiological recording, the spinal cord was prepared for morphological evaluation of the injury level (e.g., vertebral level of the damage). The cord was placed in 4% PFA for 1 h and then transferred to 30% sucrose in PBS for cryoprotection for 48 h. A 1-cm segment of cord at the contusion or in the zone of double hemisection was embedded in paraffin and horizontal sections were cut on a cryostat at 10 μm. The sections were thaw-mounted onto double-substrated glass slides (chrom-alum-gelatin and poly-L-lysine) and slides were stored at –20°C until staining. The sections were stained with cresyl violet as previously described (Van Hartesveldt et al., 1986). Sections from contused cords were stained with Luxol Fast Blue and cresyl violet to simultaneously visualize white matter myelin integrity and cellular morphology, viewed using a Zeiss Axioskop upright microscope and images were captured using a Spot RT camera. Captured images were examined using ImagePro Plus software (Media Cybernetics, Inc.).

Results

In order to evaluate the strength of descending connections to individual lumbar motoneurons through the injury region we recorded the responses evoked by stimulation of VLF fibers at T2 (above the injury) in L5 motoneurons (below the injury). Recordings were performed in cords from P10 to P12 rats that received either contusion or staggered double hemisection injury at T11–T12 level at P2.

In rats receiving the contusion injury (Fig. 1), the contusion was visible in histological sections as a loss of neurons in the

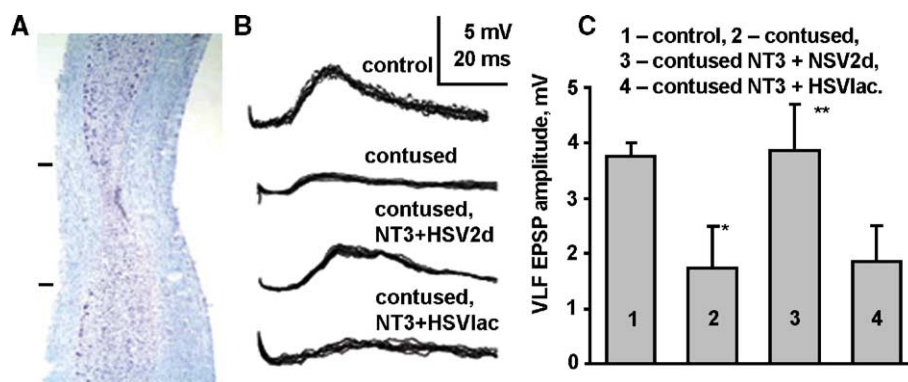


Fig. 1. Evaluation of contused cords: weak monosynaptic connections to L5 motoneurons preserved in neonatal spinal cord after contusion injury and these connections are strengthened following 8–10 days of combined treatment with NT-3 and HSVnr2d. (A) Cresyl violet stained horizontal section through the ventral horn at contusion zone from P12 cord. Rostral is on top. Note the absence of motoneurons in the contusion zone (horizontal lines). (B) Typical superimposed responses of L5 motoneuron (below injury) induced by stimulation of VLF at T2 (above injury), recorded from P10 to P12 rats treated as follows: non-injured (control), contused non-treated (contused), and contused treated with NT-3 and HSVnr2d (contused, NT-3 + HSVnr2d). All responses displayed stimulus artifact at left and are the largest for each treatment group. (C) Data summarized for experiments under conditions described in B. * and ** denote $P < 0.05$ for comparison of peak amplitude of the EPSP at 4–7 ms latency for monosynaptic response between contused versus control groups (*) and contused treated versus contused non-treated groups (**), respectively. All rats received contusion injury at P2 at T11–T12 level and responses of L5 motoneurons evoked by stimulation of the descending VLF fibers were recorded intracellularly at P10–P12.

gray matter (most apparently the large caliber motor neurons) at the injury site, and concomitant narrowing of the gray matter parenchyma (Fig. 1A). From the appearance of the Luxol Fast Blue staining, myelination appeared grossly normal in these animals that received neonatal contusions. Functional monosynaptic connections from VLF were preserved through the injury region in all rats (Fig. 1B). The mean latency in untreated contused animals was 5.6 ± 1.1 ms ($n = 5$) which is comparable to the latency of monosynaptic responses of non-injured littermates (5.8 ± 1.3 ms, $n = 4$). This is consistent with the appearance of normal myelination in the contused animals. Contused littermates that received NT-3 plus HSVnr2d treatment exhibited similar values of latency (5.5 ± 1 ms, $n = 4$). However, the monosynaptic VLF-evoked responses in untreated contused cords were significantly smaller in amplitude than monosynaptic responses in non-injured cords (Figs. 1B and C), declining from a mean of 3.8 ± 0.2 mV ($n = 57$) to a mean of 1.7 ± 0.7 mV ($n = 5$). Treatment of contused cords with NT-3 alone had no significant effect 1.9 ± 0.6 mV ($n = 3$), but treatment with NT-3 and HSVnr2d resulted in recovery to 3.9 ± 0.8 mV ($n = 4$), similar to values in intact cords (Fig. 1C).

Staggered double hemisection injury was more severe (Fig. 2), leaving only a small bridge of connecting tissue (Fig. 2A). No responses could be recorded in L5 motoneurons in response to VLF stimulation at T1/2 from rats that received double hemisection injury with no subsequent treatment (Fig. 2B, double hemisected non-treated, 18 motoneurons from 5 cords). No responses could be recorded in rats that received double hemisection and treatment with HSVnr2d alone (Fig. 2B, HSVnr2d alone, 16 motoneurons from 5 cords). In double hemisected cords treated with NT-3 alone, small polysynaptic responses were recorded in only 1 of 15 motoneurons (Fig. 2B) recorded from a total of 5 cords. However, EPSPs evoked by VLF stimulation above the injury were recorded in all 11 motoneurons recorded in 4 of the 5 double hemisected cords

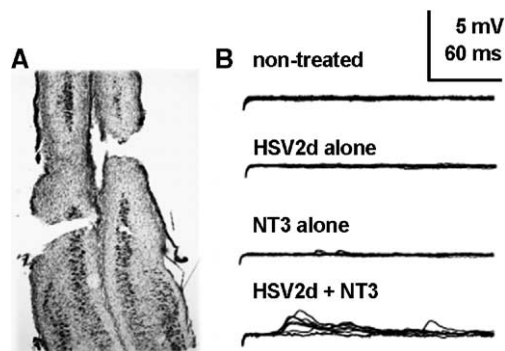


Fig. 2. Evaluation of double hemisected cords. (A) Cresyl violet-stained horizontal section at staggered hemisection zone from P12 cord. (B) Superimposed traces from motoneurons at L5 (below injury) exhibiting largest response to VLF stimulation at T2 (above injury). These responses were recorded at P10–12 after double hemisection at P2 in rats in the following treatment groups (top to bottom traces): non-treated; treated with HSV2d and fibroblasts secreting β -Gal (HSVnr2d alone); treated with HSVlac and fibroblasts secreting NT-3 (NT3 alone); and treated with HSVnr2d and fibroblasts secreting NT-3 (HSVnr2d + NT-3). Note appearance of multi-synaptic connections in a double hemisected cord from a rat that received combined HSVnr2d and NT-3 treatment.

that received combined treatment with HSVnr2d and NT-3 (Fig. 2B, HSVnr2d + NT3). All 4 motoneurons recorded from a fifth cord in this group failed to generate VLF-responses. The VLF-responses evoked in NT-3 and HSVnr2d treated cords appeared to be polysynaptic. These responses displayed long latency (23.6 ± 5.3 ms, $n = 4$ in double hemisected HSVnr2d + NT3 group compared to 5.8 ± 1.3 ms for monosynaptic responses in the non-injured group), and considerable fluctuation in both latency and amplitude compared with the monosynaptic component in non-injured group. The motoneurons recorded in these experimental groups all displayed a resting membrane potential ranging from -63 mV to -67 mV and generated antidromic action potentials and monosynaptic responses in response to stimulation of the L5 dorsal root. Thus, the motoneurons were viable, and capable of generating synaptic responses independently of whether they received VLF input (not shown).

Discussion

The major finding is that combined delivery of NT-3 secreted from engineered fibroblasts and NMDAR-2D subunits via HSV-1 amplicon-mediated transduction strengthened the connections made to motoneurons by VLF fibers projecting through the injured spinal cord in both contusion and double hemisection injury models in neonatal rats. These treatments were tested because of their ability individually to enhance projections to motoneurons from intact fibers in uninjured neonates (Arvanian et al., 2003, 2004). The present experiments were performed in neonates because in vitro recording is limited to rats younger than P14 (Fulton and Walton, 1986). Also, neonatal rats exhibit a greater amount of anatomical reorganization and behavioral recovery in response to spinal cord injury than the adult rat (Stelzner and Weber, 1979; Kunkel-Bagden and Bregman, 1990; Bregman et al., 1993; Firkins et al., 1993; Diener and Bregman, 1998). The greater neuronal plasticity in neonates facilitates comparison of treatments that strengthen synaptic connections. Such findings may provide guidance for therapeutic intervention in injured adults.

The contusions carried out in these experiments preserved a fraction of VLF axons and their monosynaptic connections through the injury region; the monosynaptic responses in non-treated contused cords were reduced to about one half the magnitude observed in control rats (see Fig. 1). Spinal cords isolated from neonatal rats that received double hemisection with no subsequent treatment exhibited no synaptic connectivity via VLF. Consistent with these observations, contusion produced only transient functional impairments in the neonates, while neonatal rats that received double staggered lesion displayed permanent impairment of motor function measured as an inability to use their hindlimbs to swim and to stand to explore their environment (rearing) (Arvanian et al., in press).

We did not determine whether strengthening of VLF projections to motoneurons resulted in behavioral improvement. However, in recent experiments, we used LSD, which also strengthens NMDAR-mediated projections (Arvanov et

al., 1999), in conjunction with NT-3 in the double hemisection-injured neonatal rat and found that hindlimb rearing and swimming improved significantly compared to untreated rats (Arvanian et al., in press). In these experiments, we also detected strengthening of VLF projections similar to that observed in the present experiments, i.e., emergence of weak polysynaptic projections. It was not possible to ascribe the behavioral recovery specifically to strengthening of VLF connections. However, it seems reasonable to conclude that the functional effects of the NT-3 + LSD treatment contributed to behavioral recovery. Thus, it seems possible that the treatment with NT-3 and NR2D as carried out here would elicit behavioral improvement similar to that observed in our previous experiments.

The inability of NT-3 alone delivered chronically from P2 to P10 to promote recovery of apparently intact VLF projections in the contused cord was surprising in view of its ability to enhance VLF projections under these conditions in the intact cord (Arvanian et al., 2003). One possibility is a reduction in expression of *trkC*, the receptor for NT-3 as a result of the contusion. The expression of another neurotrophin receptor, *trkB*, has been shown to be reduced in the contused spinal cord (Liebl et al., 2001), and the effects of exogenous BDNF on the AMPA/kainate receptor-mediated responses of cells in lamina II are substantially reduced in contused neonates (Garraway et al., 2005). However, the fact that NT-3 became effective when delivered in conjunction with NR2D suggests that the VLF-EPSPs elicited after contusion were not from a subset of VLF fibers that had survived the contusion and whose synapses onto motoneurons were normal in all respects. Rather, it appears that the contusion injury had damaged the VLF fibers or at least their NMDAR-mediated synapses such that recovery from Mg^{2+} block was required for NT-3 to exert its effect. It is not known what specifically is disrupted in contused fibers, for example, whether axonal transport is completely normal. We did observe normal myelination in contrast to the demyelination often observed after adult contusion (Blight, 1983; Cao et al., 2005). However, we cannot determine whether this represents a difference between neonates and adults, or is due to the relatively low impact of the contusion injury (Cao et al., 2005).

In contrast to contused cords, where combined treatment with NT-3 and NR2D induced strengthening of preserved monosynaptic connections, the VLF-evoked responses in the NT-3 + HSVnr2d treated double hemisectioned cord lacked a monosynaptic component and displayed properties of multisynaptic responses, i.e., a high degree of fluctuation in latency and amplitude (see Fig. 2B). Since in the case of double hemisection the white matter of the hemicord at the recording site was completely removed during surgery (see Fig. 2A), we surmise that these multisynaptic responses are due to strengthening of synaptic connections through the spared gray matter at the site of injury, probably between the hemisections. As in contused cords, we assume that both NT-3 and NR2D were necessary either because local damage in the gray matter between the hemisections prevented chronically delivered NT-3 from potentiating responses as would be possible in the

undamaged spinal cord, or because NMDA receptor-mediated synaptic transmission to interneurons in that region of the gray matter required removal of Mg^{2+} block. It is interesting that a small polysynaptic response was recorded in one motoneuron from a rat treated with NT-3 alone. This suggests that NT-3 alone did exert an effect but that it was very weak due to Mg^{2+} block of NMDA receptor mediated EPSPs in the gray matter between the hemisections.

These studies illuminate possible strategies for improving conduction through the damaged spinal cord in adults. Although it is known that neurotrophins, specifically NT-3 or BDNF, can promote sprouting or growth of intact or damaged axons, respectively, their ability to elicit synaptic responses from neurons caudal to injury may require additional support, particularly for NMDA receptor-mediated transmission. It will be necessary to carry out such studies in adults to determine the effectiveness of this strategy in improving recovery of the damaged spinal cord.

Acknowledgments

We thank Dr. J. Petruska, Ms. H. Manuzon, Mr. W. Narrow, Ms. C. Engessar-Cesar and the Statistical Consulting Unit at SUNY-Stony Brook for assistance. This study was supported by grants from the Christopher Reeve Paralysis Foundation and NIH 2 RO1 NS 16996 (LMM), the Nathan Shock Center at the University of Rochester (WJB and HJF) and NIH NS36420 (HJF), and the NY State Spinal Cord Injury Foundation (VLA).

References

- Arvanian, V.L., Mendell, L.M., 2001. Removal of NMDA receptor Mg^{2+} block extends the action of NT-3 on synaptic transmission in neonatal rat motoneurons. *J. Neurophysiol.* 86, 123–129.
- Arvanian, V.L., Homer, P.J., Gage, F.H., Mendell, L.M., 2003. Chronic Neurotrophin-3 strengthens synaptic connections to motoneurons in the neonatal rat. *J. Neurosci.* 23, 8706–8712.
- Arvanian, V.L., Bowers, W.J., Petruska, J.C., Motin, V., Manuzon, H., Narrow, W.C., Federoff, H.J., Mendell, L.M., 2004. Viral delivery of NR2D subunits reduces Mg^{2+} block of NMDA receptor and restores NT-3-induced potentiation of AMPA-kainate responses in maturing rat motoneurons. *J. Neurophysiol.* 92, 2394–2404.
- Arvanian, V.L., Motin, V., Mendell, L.M., 2005. Comparison of metabotropic glutamate receptor responses at segmental and descending inputs to motoneurons in neonatal rat spinal cord. *J. Pharmacol. Exp. Ther.* 312, 669–677.
- Arvanian, V.L., Manuzon, H., Davenport, M., Bushell, G., Mendell, L.M. and Robinson, J.K., in press. Combined treatment with neurotrophin-3 and lsd facilitates behavioral recovery from double-hemisection spinal injury in neonatal rats *J. Neurotrauma*.
- Arvanov, V.L., Liang, X., Russo, A., Wang, R.Y., 1999. LSD and DOB: interaction with 5-HT_{2A} receptors to inhibit NMDA receptor-mediated transmission in the rat prefrontal cortex. *Eur. J. Neurosci.* 11, 3064–3072.
- Arvanov, V.L., Seebach, B.S., Mendell, L.M., 2000. NT-3 evokes an LTP-like facilitation of AMPA/kainate receptor-mediated synaptic transmission in the neonatal rat spinal cord. *J. Neurophysiol.* 84, 752–758.
- Bernstein, D.R., Stelzner, D.J., 1983. Plasticity of the corticospinal tract following midthoracic spinal injury in the postnatal rat. *J. Comp. Neurol.* 221, 382–400.
- Blight, A.R., 1983. Cellular morphology of chronic spinal cord injury in the cat: analysis of myelinated axons by line-sampling. *Neuroscience* 10, 521–543.

- Bowers, W.J., Howard, D.F., Federoff, H.J., 2000. Discordance between expression and genome transfer titering of HSV amplicon vectors: recommendation for standardized enumeration. *Mol. Ther.* 1, 294–299.
- Bowers, W.J., Howard, D.F., Brooks, A.I., Halterman, M.W., Federoff, H.J., 2001. Expression of vhs and VP16 during HSV-1 helper virus-free amplicon packaging enhances titers. *Gene Ther.* 8, 111–120.
- Bregman, B.S., Kunkel-Bagden, E., Reier, P.J., Dai, H.N., McAtee, M., Gao, D., 1993. Recovery of function after spinal cord injury: mechanisms underlying transplant-mediated recovery of function differ after spinal cord injury in newborn and adult rats. *Exp. Neurol.* 123, 3–16.
- Cao, Q., Zhang, Y.P., Iannotti, C., DeVries, W.H., Xu, X.M., Shields, C.B., Whittemore, S.R., 2005. Functional and electrophysiological changes after graded traumatic spinal cord injury in adult rat. *Exp. Neurol.* 191 (Suppl. 1), S3–S16.
- Diener, P.S., Bregman, B.S., 1998. Fetal spinal cord transplants support growth of supraspinal and segmental projections after cervical spinal cord hemisection in the neonatal rat. *J. Neurosci.* 18, 779–793.
- Firkins, S.S., Bates, C.A., Stelzner, D.J., 1993. Corticospinal tract plasticity and astroglial reactivity after cervical spinal injury in the postnatal rat. *Exp. Neurol.* 120, 1–15.
- Fulton, B.P., Walton, K., 1986. Electrophysiological properties of neonatal rat motoneurons studied in vitro. *J. Physiol.* 370, 651–678.
- Garraway, S.M., Anderson, A.J., Mendell, L.M., 2005. BDNF-induced facilitation of afferent-evoked responses in lamina II neurons is reduced after neonatal spinal cord contusion injury. *J. Neurophysiol.* 94, 1798–1804.
- Horner, P.J., Gage, F.H., 2002. Regeneration in the adult and aging brain. *Arch. Neurol.* 59, 1717–1720.
- Kawaja, M.D., Gage, F.H., 1992. Morphological and neurochemical features of cultured primary skin fibroblasts of Fischer 344 rats following striatal implantation. *J. Comp. Neurol.* 317, 102–116.
- Kunkel-Bagden, E., Bregman, B.S., 1990. Spinal cord transplants enhance the recovery of locomotor function after spinal cord injury at birth. *Exp. Brain Res.* 81, 25–34.
- Liebl, D.J., Huang, W., Young, W., Parada, L.F., 2001. Regulation of Trk receptors following contusion of the rat spinal cord. *Exp. Neurol.* 167, 15–26.
- McTigue, D.M., Horner, P.J., Stokes, B.T., Gage, F.H., 1998. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J. Neurosci.* 18, 5354–5365.
- Pinco, M., Lev-Tov, A., 1994. Synaptic transmission between ventrolateral funiculus axons and lumbar motoneurons in the isolated spinal cord of the neonatal rat. *J. Neurophysiol.* 72, 2406–2419.
- Raineteau, O., Schwab, M.E., 2001. Plasticity of motor systems after incomplete spinal cord injury. *Nat. Rev., Neurosci.* 2, 263–273.
- Stelzner, D.J., Weber, E.D., Prendergast, J., 1979. A comparison of the effect of mid-thoracic spinal hemisection in the neonatal or weanling rat on the distribution and density of dorsal root axons in the lumbosacral spinal cord of the adult. *Brain Res.* 172, 407–426.
- Van Hartesveldt, C., Moore, B., Hartman, B.K., 1986. Transient midline raphe glial structure in the developing rat. *J. Comp. Neurol.* 253, 174–184.