Final Progress Report for the New Jersey Commission on Spinal Cord Research
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Preamble:

The following is a summary of the investigations that were supported by funds supplied by the New Jersey Commission on Spinal Cord Research.

Tenascin-C Domains in Experimental Spinal Cord Injury
Awarded: June, 2001-2004

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N.B. I took over the *nominal position as PI* of this project on the retirement of Dr. Herbert Geller, the original PI of the project. His postdoctoral fellow Dr. Huaying Li. did most of the work, whose salary was supported by the grant. He has left the university and much of the original data is either lost or is incomprehensible. What follows is my best effort to reconstruct the finding found within the project.

1. Goals:

The peptide fragment VFDFNVLK derived from the fnD fibronectin repeat of tenascin-C (see Figure 1) has activity to promote neurite growth and guidance in cell culture. We therefore tested this peptide in rat model of spinal cord injury.

2. The Findings:

ABSTRACT and Aims of the Project

Astrocyte-derived factors that are thought to play an important role in promoting neurite outgrowth and guidance during development (e.g., Smith et al 1986; Letourneau et al 1994). One important extracellular matrix protein is tenascin-C, which is transiently expressed along the borders of migratory pathways in the developing cortex (Steindler et al 1989, 1990). In addition, tenascin-C is expressed in glial scars following injury to the central nervous system (Lochter et al., 1991; McKeon et al., 1991; Laywell et al., 1996). The peptide fragment *VFDNFVLK* derived from the fnD fibronectin repeat of tenascin-C

(see Figure 1) has activity to promote neurite growth and guidance in cell culture (Meiners et al 1999, 2001; Mercado et al 2004). We therefore tested this peptide in rat model of spinal cord injury. Adult Sprague female rats (2 -3 months) were subject do a dorsal hemisection on the right side at T8 (Brown-Sequard Syndrome). Some animals were implanted with an Alzet osmotic minipump to deliver the peptide or PBS for a period of either 1 week or 2 weeks after the lesion. There were 7 groups of animals: a) Intact no pump; b) Intact with pump (PBS); c) Lesion No pump; d) Lesion with pump and PBS; and e) Lesion with pump and peptide. All animals were allowed to survive approximately 1 month. One week prior to euthanasia, the animals are labelled with retrograde and/or anterograde tracers. For anterograde tracing, 10% BDA in TBS pH8 was ionophoretically injected into the sensorimotor cortex. For retrograde tracing 0.3 - 0.5 ml Fast Blue 2% in PBS was injected into the lesion side, one segment caudal to the lesion. Coronal sections for retrograde tracing

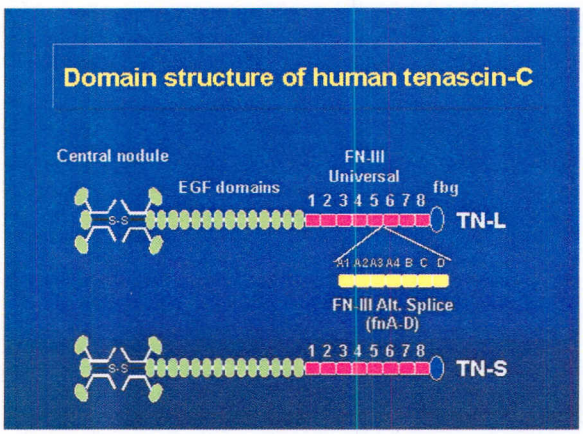

Figure 1

MATERIALS & METHODS

Adult Sprague females rats (2 -3 months) were subjected to a dorsal hemisection on the right side at T8.severing the corticospinal tract (CST). Each experimental group had 8 animals (see Table 1) (adult, female, SD rat, weighting 155-165 grams. The animals were anesthetized with a mixture of acepromazine (1 mg/100 grams) and Ketamine-HCL (10 mg/100 grams). A laminectomy was performed at T7, exposing the spinal cord. The dura was cut and was followed by a hemisection of the dorsal columns and underlying corticospinal pathways.

The anatomical consequences of spinal cord injury and protein application were assessed by using of anterograde tract tracing with biotinylated dextran (**BDA**) (Molecular Probes, Inc.) and/or retrograde labeling with the fluorescent tracer Fast Blue (**FB**) (Sigma). The animals were allowed to survive 4 weeks following injury.

The experimental groups were:

a) ***Intact control*** animals had no spinal cord lesion: half had an Alzet mini-osmotic pump implanted to deliver PBS at T8 and the other half did not, BDA was injected into the sensorimotor cortex and/or FB injected at T9.
b) ***Injury control*** The CST was cut at T8 (right side) and no pump was implanted, 1 week prior to sacrifice, BDA was injected into sensorimotor cortex, or the retrograde tracer FB was injected at T9.

c) ***Lesion plus pump*** Again, the lesion was at T8 (right side). An Alzet osmotic mini pump was implanted to deliver the peptide or PBS to the lesion site for a period of either 1 week or 2 week. Three weeks following injury and pump implantation this animals the animals were injected with BDA or FB. These rats were allowed to survive for 1 additional week for a total of 4 weeks.

At the end of the experiment all rats were transcardially perfused with 4% paraformaldehyde and the tissue sectioned on a cryostat for histological processing.
FB-labeled neurons were counted in the sensorimotor cortex, red nuclei (RN), and lateral vestibular nuclei (LVE).

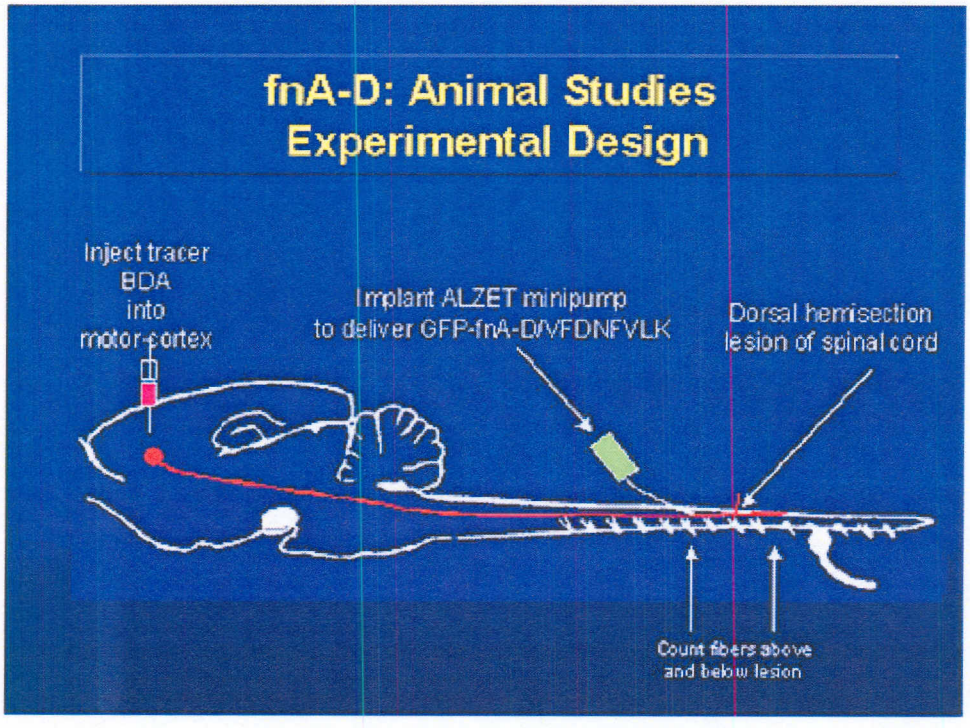

Figure 2: Graphical summary of the experimental methods.

Histological Examination of the Lesion Site

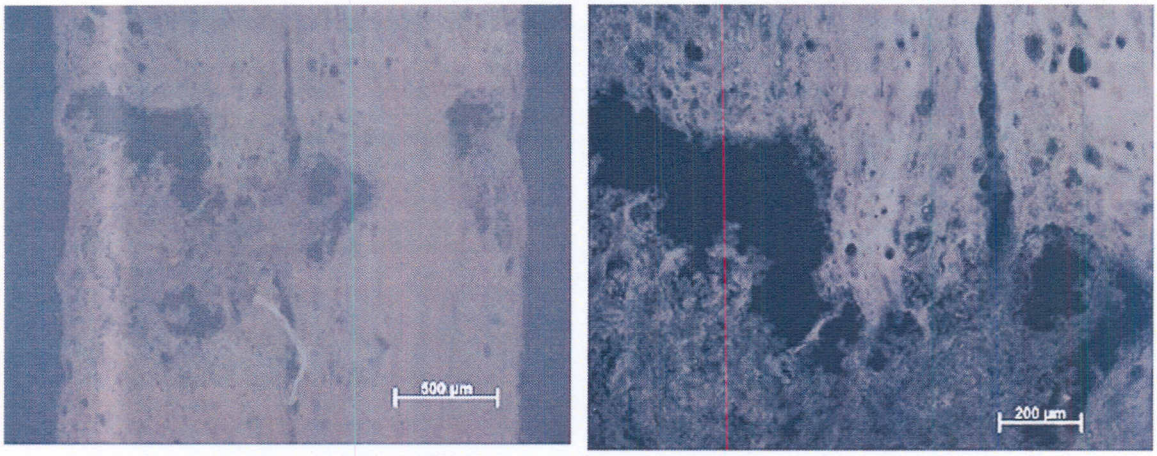

Figure 3: **Lesion Site.** Dark-field photomicrographs illustrating the extent of the lesion site. A Lesion was made on the right side at T8. Left: Low Power. Right: Higher magnification vies.

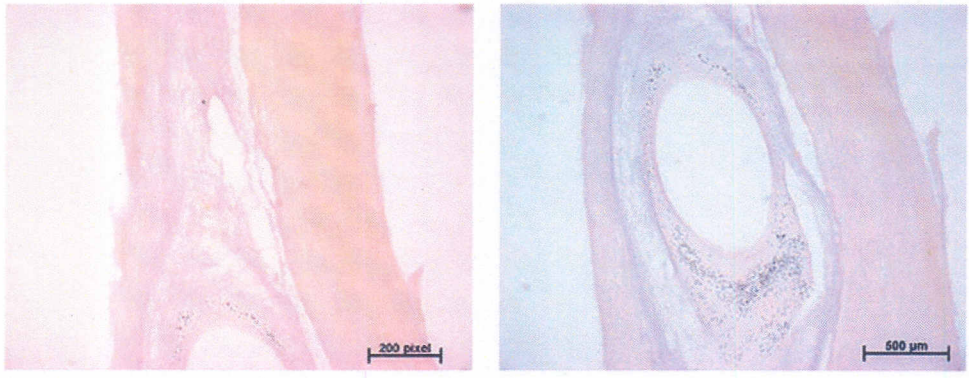

Figure 4: Bright-field photomicrographs illustrating spinal cord damage resulting implantation of an Alzet mini-osmotic pump that was used to deliver protein fragments or vehicle to the injury site.

Anterograde tracing with BDA

Anterograde tracing: 10% BDA was injected into the sensorimotor cortex to anterogradely label corticospinal tract axons.

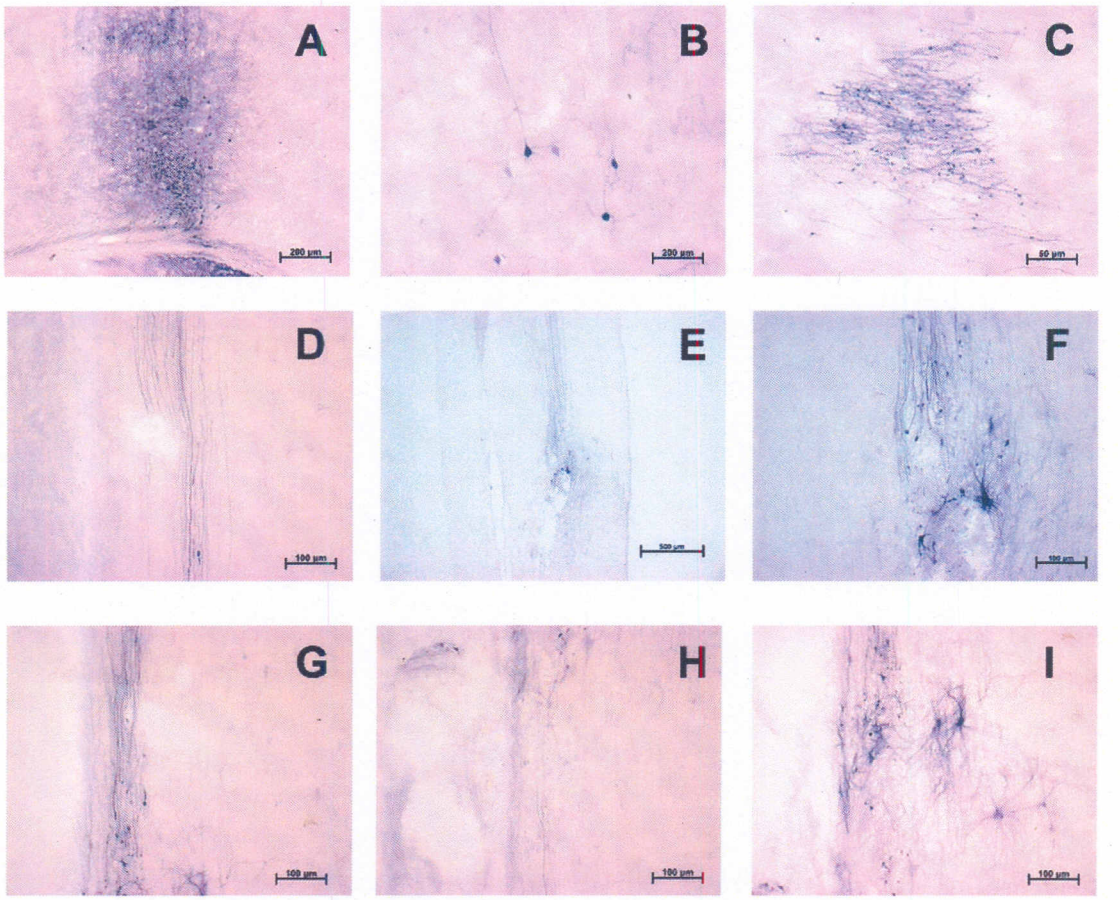

Figure 5: A. BDA injection site, labeled sensorimotor cortex. B. BDA-labeled cortical neurons. C. BDA-labeled corticospinal tract axons in the midbrain. D. BDA-labeled corticospinal tract at C7, rostral to the injury. E – F. After lesion, degenerating corticospinal tract axons were revealed with BDA.
Left: Low Power, Right: Higher Power of the same field. G – I. Anterogradely marked CST axons show sprouting in an animals with VFDFNVLK peptide application

Retrograde tracing with Fast Blue

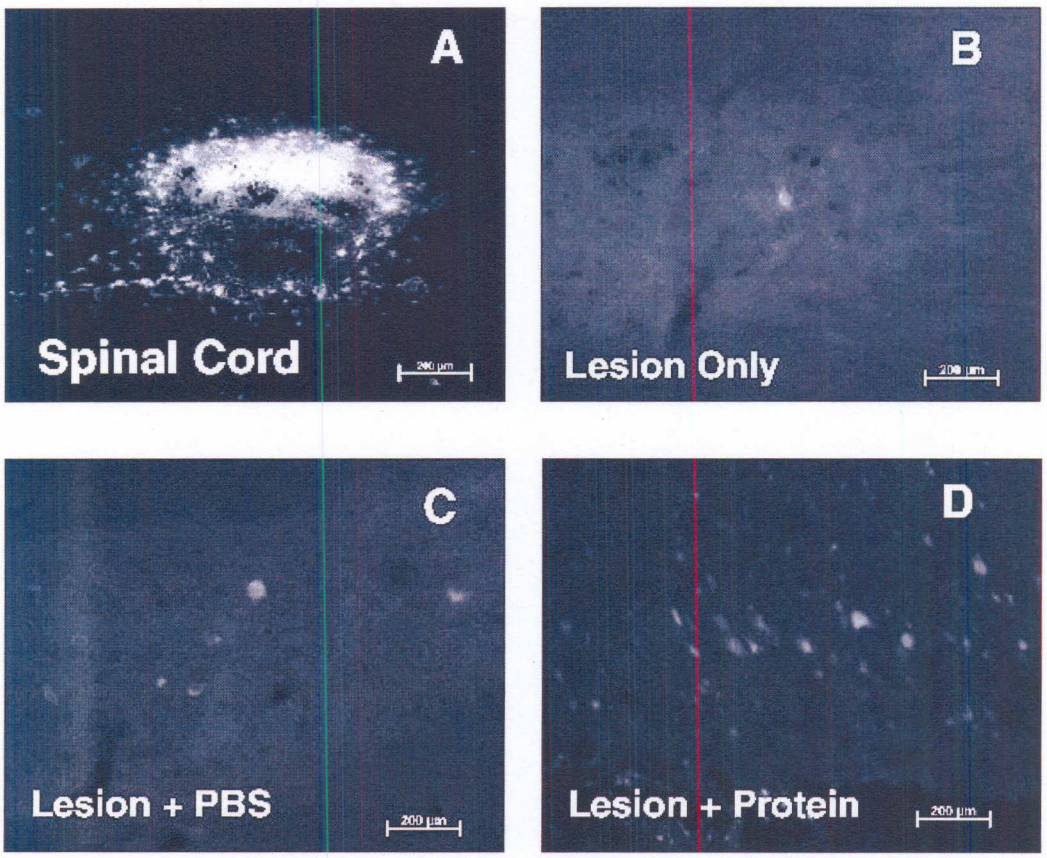

Figure 6 **Retrograde tracing,**: In order to determine if descending fibers were "encouraged" to grow past the lesion site 2% Fast Blue was injected into the spinal cord at T9, one segment caudal to the lesion site. The animals were allowed to survive on additional week

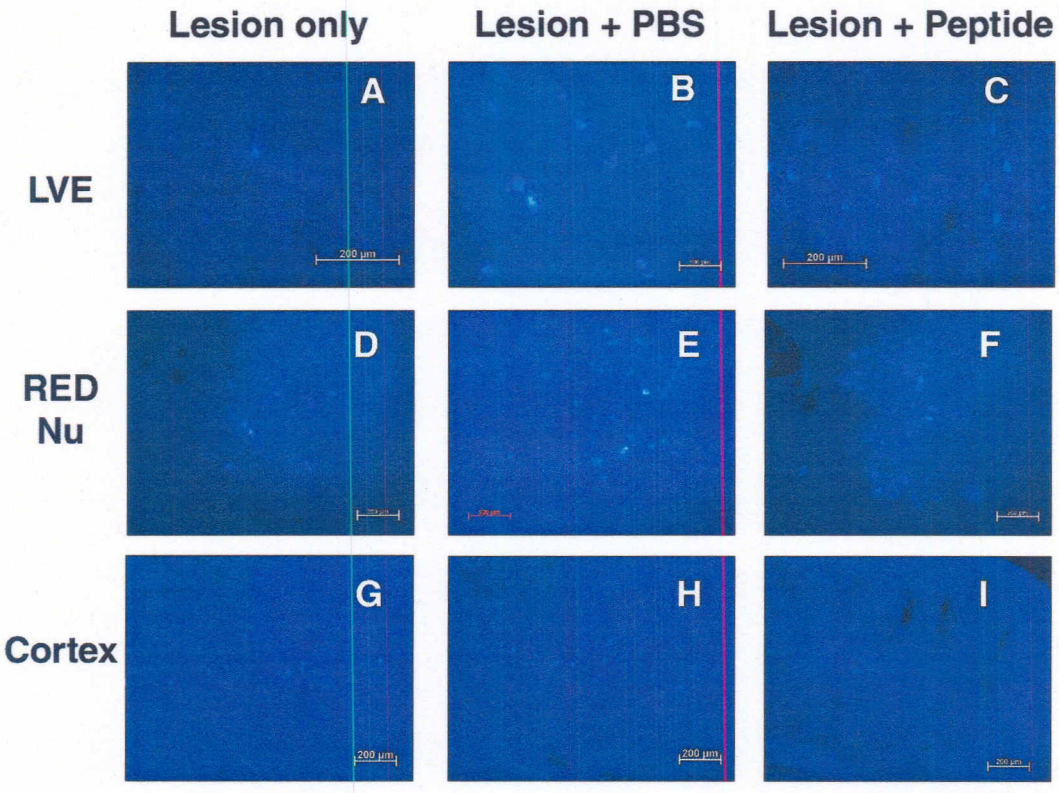

Figure 7 Retrograde labelling of brainstem and cortical projections to the spinal cord. This figure presents typical examples of fast-blue (FB) labelled cells in lateral vestibular nucleus (LVE), the red nucleus (Red Nu) and the conteralateral cortex in animals that were subjected either lesion only, lesion and a pump filled with PBS (Lesion + PBS) or lesion and a pump filled with peptide. More FB+ cells were detected following administration of the peptide than in either of the other conditions, see Table 1 below.

Table 1

Summary of Retrogradely Marked Cells					
Group	Experiment	Spinal Cord	LVE	RN	Cortex
1	no lesion	2155	388	357	102
2	lesion only	240	114	74	20
3	Lesion + PBS pump	314	81	53	36
4	Lesion + Peptide pump	1512	265	227	98
Fast Blue (0.1 ~ 0.2 l of 2% FB) was injected into T9 (1 segment caudal to the lesion) 3 weeks after lesioning. Animals were sacrificed 1 week later and labeled cells were counted in each of the regions above. Labeled cells in spinal cord were counted at segments C1 – T7.					

SUMMARY

Delivery of the tenascin-C-derived peptide VFDFNVLK appeared to reduce axonal degeneration and increase axonal sprouting following a dorsal hemisection at spinal segment T8 of adult rats spinal cord.

Without treatment with VFDFNVLK, the majority of damaged CST axons die back varying distances from lesion site—The distance of this "die-back" increased with survival time: At 7 days following injury, labeled CST fibers were about 0~50 mm rostral to the injury; at 14 days this distance increased to 100 mm; and, at 28 days it was 100~250 mm. Nonetheless, we have seen some damaged CST axons to spontaneously exhibit some regeneration over short distances, and some "sprouting" of undamaged CST axons.
The tenascin-C-derived peptide VFDFNVLK may alter the microenvironment of the lesion site, allowing damaged CNS axons to re-grow past the injury. This is supported by our observation of increased numbers of FB-labeled neurons in the sensorimotor cortex, RN and LVE following treatment with the VFDFNVLK peptide.

Reference List

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4. Implications for future study and clinical treatment

Although the data obtained in this project is suggestive that tenascin-C-derived peptide VFDFNVLK may be a useful compound in the treatment of spinal cord injury, considerable work need to be done to confirm it potential utility.

5. Plans for Continuation of this work.

As of now I have no plans to continue these investigations

6. Publications:

No publications have been generated from this study. Since Drs Li and Geller have left the university, no further analysis can be generated.