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TITLE: Development of Practical and Rapid Field-Based Therapies for SCI

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14. ABSTRACT We have designed a set of experiments to test candidate therapies for spinal cord injury (SCI) that could be applied in the battlefield and other acute traumatic settings. Specifically, we aim to optimize a combinatorial treatment to augment behavioral recovery following severe spinal cord contusion. These strategies include: 1) augmentation of the intrinsic growth state of an injured neuron by elevating cyclic AMP levels, and 2) modifying the extrinsic toxic/inhibitory spinal cord environment by transplantation of cells secreting growth factors.					
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Introduction:

We have designed a set of experiments to test candidate therapies for spinal cord injury (SCI) that could be applied in the battlefield and other acute traumatic settings. Specifically, we aim to optimize a combinatorial treatment to augment behavioral recovery following severe spinal cord contusion. These strategies include: 1) augmentation of the *intrinsic* growth state of an injured neuron by elevating cyclic AMP levels, and 2) modifying the *extrinsic* toxic/inhibitory spinal cord environment by transplantation of cells secreting growth factors. We have identified allogeneic bone marrow stromal cells (BMSC) as a potential source for cell transplantation for the following reasons:

- 1) BMSCs obtained from unrelated bone marrow donors can be obtained, expanded, genetically modified and banked to serve as a readily and rapidly available cell source to treat patients with spinal cord injury.
- 2) Cells can be rapidly thawed and transplanted into an SCI patient during spinal decompression surgery.
- 3) Cells are allogeneic and thus will be eliminated by the host after approximately two weeks. Delayed cell removal is advantageous because these cells will provide *transient* growth factor expression to minimize secondary injury that occurs days and weeks following the initial injury. In addition, these cells will establish an early scaffold that will support Schwann cell migration into the lesion site, thus providing a late scaffold to potentially support axonal growth.

Specific Aim 1: Establish the Feasibility, Time Frame, and In Vivo Survival of Allogeneic Bone Marrow Stromal Cells.

Specific Aim 2: Examine Anatomical and Behavioral Outcomes of Battlefield-Relevant Treatments After Acute Contusive SCI.

Body:

In the study period we made substantial progress in the approved Statement of Work. We transplanted allogeneic marrow stromal cells into sites of acute spinal cord contusion. Upon consultation with neurosurgeons, both civilian and military, we identified the 2-day post-lesion time point as an appropriate time point for cell transplantation. This is based on: 1) Clinical relevance: the majority of spinal decompression surgeries occur within 2-day post-injury and transplantation of cells at this time would be highly desirable; 2) MSCs do not survive as well when grafted into a 1-day spinal cord contusion. These BMSCs have been modified to secrete the neurotrophin brain-derived neurotrophic factor (BDNF).

We performed several experiments to optimize grafting of cells into a severe thoracic contusion using the Infinite Horizon device. We used a model of severe spinal cord injury because it more accurately reflects the most common type of human injury, severe spinal cord injury. Rats were subjected to either a 250-kDyn or 400-kDyn force to the T9 thoracic spinal cord. Open field locomotor analysis using the Basso-Beattie-

Bresnahan (BBB) scoring scale indicated that both groups recovered some locomotor function. With a 250-kDyn injury, rats recovered above a score of 9 indicating they could support their weight on the hindlimbs (Figure 1). In contrast, the 400-kDyn-injured rats recovered to BBB scores of 5-6. However, the 400-kDyn lesion, while more clinically-relevant, expanded substantially and contained multiple septations. This type of lesion makes cell transplantation much more difficult, resulting in sub-optimal cell filling of the lesion site (Figure 2). Despite sub-optimal filling, we continued with the experiment to examine the effect of BMSC transplantation into acute spinal cord contusion.

We did not detect an effect of transplantation of BMSCs on locomotion up to 6 weeks post-transplantation (Figure 3). Because the BMSCs did not completely fill this type of lesion site, we then developed a spinal cord crush model, which results in a smaller lesion cavity that lacks septations, and BBB scores more similar to those deficits occurring after human injuries (Figure 4). With this type of crush lesion, we are able to completely fill the lesion site with our BMSCs (Figure 5). However, we did not detect an effect on locomotion up to 10 weeks post-transplantation (Figure 6). We did detect an effect of BMSC treatment on conditioned place preference (CPP) after injury, suggesting an exacerbation of pain. That is, transplantation with BMSCs resulted in rats spending significantly less time in an area where they received normally non-noxious tactile stimulation of the hindpaws (Figure 7). This type of analysis can be interpreted as tactile allodynia, wherein rats that receive a stimulus that is typically non-noxious now interpret the stimulus as noxious.

Ongoing analyses:

- 1) Quantification of spared tissue at lesion site following BMSC transplantation into acute spinal cord crush
- 2) Quantification of spared myelin at lesion site following BMSC transplantation into acute spinal cord crush
- 3) Quantification of BMSC survival 3 days and 10 weeks post-transplantation
- 4) Quantification of Schwann cell infiltration 3 days post-BMSC transplantation
- 5) Quantification of spared/sprouted axons within, around and below crush lesion site

Key Research Accomplishments:

- A model of severe spinal cord injury crush has been successfully developed that more accurately reflects the nature of human injury.
- Acute transplantation of allogeneic BMSCs, with or without BDNF over-expression, does not promote locomotor recovery following severe spinal cord crush or contusion.
- BMSC transplantation potentially leads to tactile allodynia.

Reportable outcomes: In progress.

Conclusion:

Currently, there are no effective treatments for spinal cord injury (SCI). We successfully developed a model of severe SCI in rats that more accurately models the most common form of human SCI. We then determined whether grafting of a practical, readily available cell source of bone marrow stromal cells genetically engineered to secrete BDNF would improve anatomical and functional outcomes in this severe SCI model. Treatment with BDNF-secreting BMSCs did not improve functional outcomes after severe SCI. Anatomical analyses are still in progress. Despite negative outcomes in this experiment, we believe that this work achieved the important goal of developing a better model of SCI that can allow more rapid and cost effective screening of potential therapies for SCI, without requiring the expense and time of human trials. That is, a therapy that fails to show efficacy in this severe rodent model of SCI may not merit translation to human trials. In the future, we will use this advanced model to screen other potential therapies, including neural stem cell studies that are currently in progress as a result of work performed under this grant.

References: None

Appendices: None

Supporting Data:

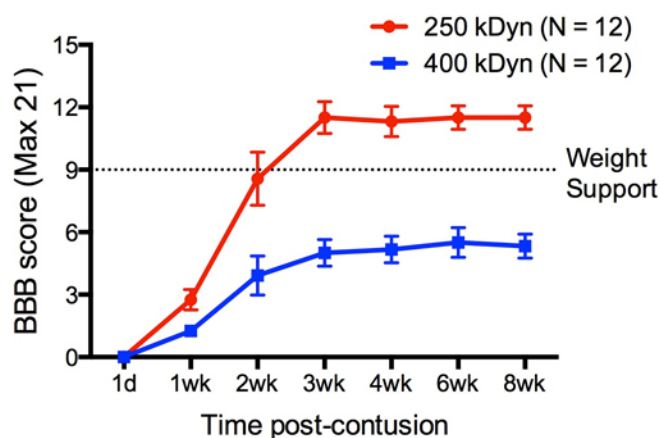
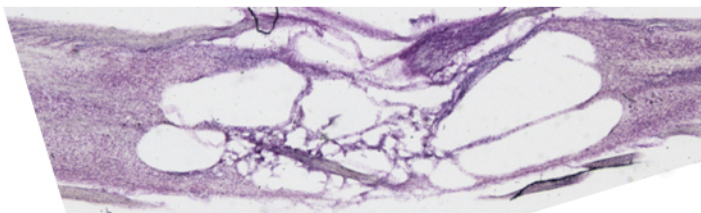


Figure 1: (A) Locomotor analysis following contusion of the thoracic T9 spinal cord using either a 250-kDyn or 400-kDyn force. (B) Horizontal section of the 400-kDyn contusion lesion site stained with cresyl violet



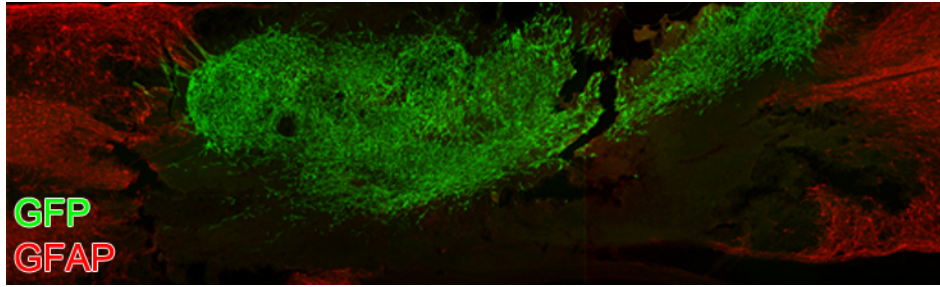


Figure 2: GFP+ BMSCs transplanted into a 2-day T9 contusion do not completely fill lesion site 3 days post-transplantation as identified with GFAP immunolabeling.

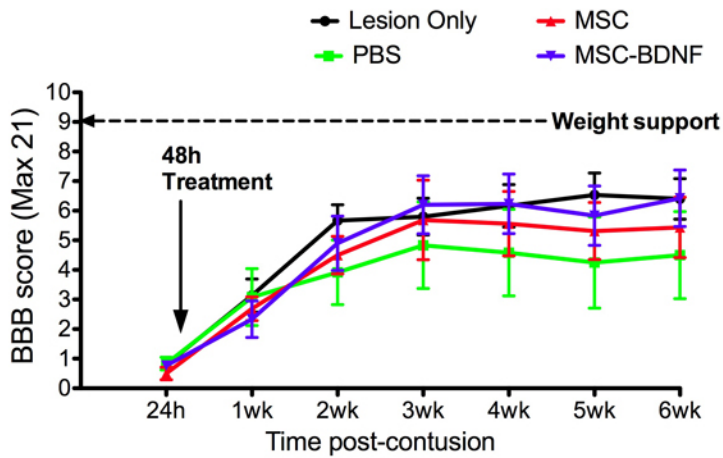


Figure 3: Locomotor analysis following a T9 contusion. 2 days post-contusion the lesion sites were re-exposed (Lesion only), injected with PBS (PBS), BMSCs (MSC), or BMSCs expressing BDNF (MSC-BDNF).

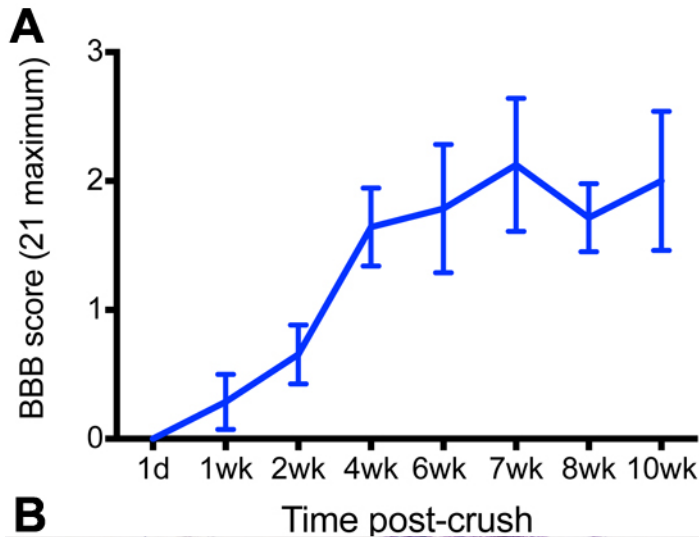
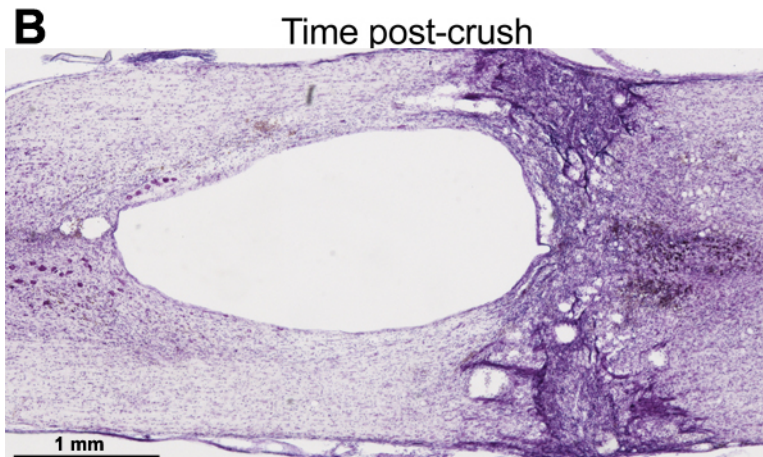


Figure 4: (A) Locomotor analysis following a T9 crush. (B) Horizontal section of the crush lesion site stained with cresyl violet.



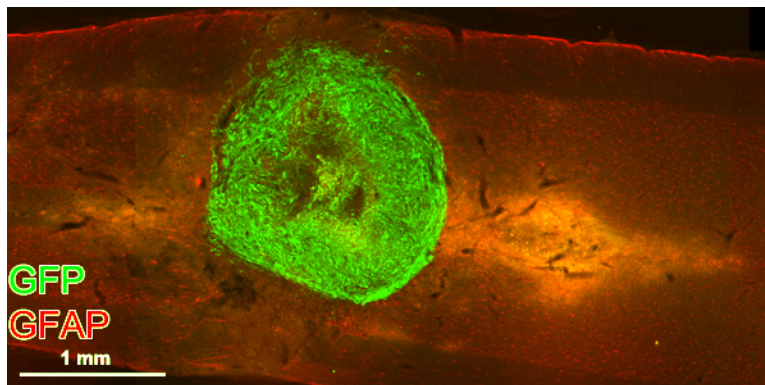


Figure 5: GFP+ BMSCs transplanted into a 2-day T9 crush completely fill lesion site 3 days post-transplantation as identified with GFAP immunolabeling

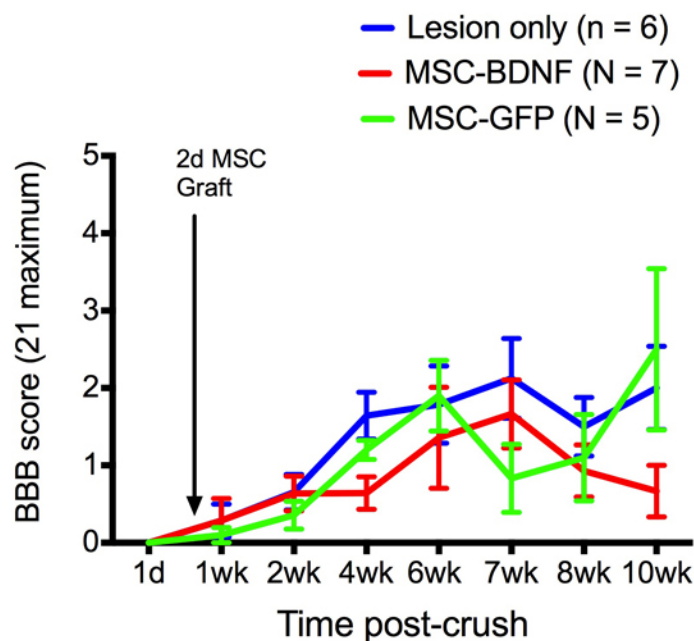


Figure 6: Locomotor analysis following T9 crush and transplantation with BMSCs expressing BDNF (MSC-BDNF) or the control gene, GFP (MSC-GFP).

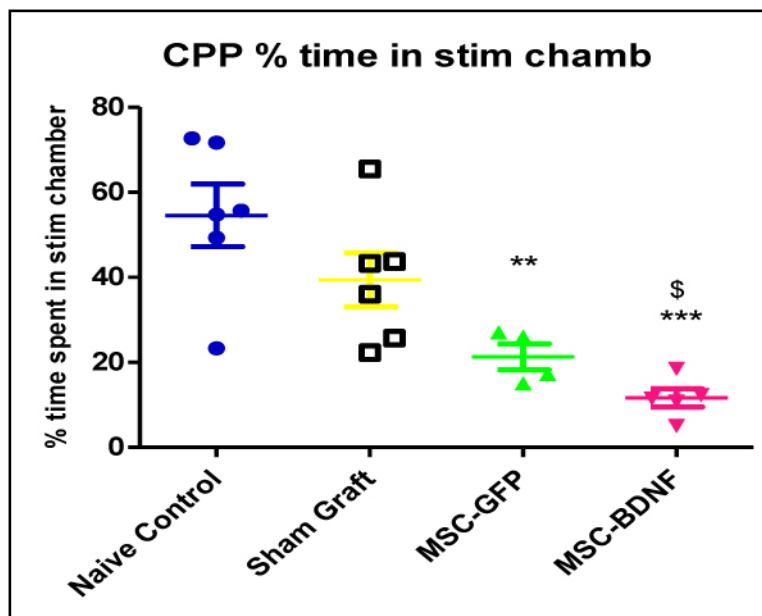


Figure 7: Conditioned Place Preference (CPP) following T9 crush. Rats receiving BMSC transplants spent less time in the non-noxious tactile stimulation chamber.
 ** p<0.01 compared to Naïve control
 *** p<0.001 compared to Naïve control
 \$ p<0.05 compared to Sham graft