Final Report

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1. Original aims of the project.

1. Continue development of our SCI-base animal database to extend reporting functions, add new data tables for new functional assays, add data input validation, and improve dependability and functionality. Client technical support will be provided on an ongoing basis.
2. Work with Dr. Frank P.T. Hamers to integrate his algorithm for the BBB locomotor function scoring system into SCI-Base, and to add the new BMS (Basso Mouse Scale).
3. Work with Dr. Hamers to develop, validate, and integrate new software and hardware for the MASCIS device interface.

2. Project successes.

While we held true to our original goals, many of the steps taken to implement our goals evolved over the time of the project. These kinds of adjustments in strategy are often advantageous to build on new knowledge or new methods.

In Aim 1 we proposed to continue development of our SCI-base database for use in spinal cord research laboratories. We did continue to expand the database system and to support its use, primarily within the Keck Center. The software was downloaded by at least four groups: University of Texas Medical Branch, University of Louisville, Acorda, Inc., and Otsuka Maryland Medicinal Labs. After assisting several sites with downloading, installing, and customizing the database, we found that many of them did not have the complex needs that we do in the Keck Center, so most external sites have discontinued use of SCI-base. This is not to be considered a failure, however, since every one of the sites testing the software found valuable strategic benefits of specific aspects of SCI-base and they subsequently built databases using many of our concepts. If we only led the way to promote organization of spinal cord injury data from animal studies, our contribution was successful.

Aim 2 sought to integrate an algorithm from Dr. Frank Hamers into the SCI-base database. We tested this algorithm extensively and determined that it did not produce accurate scoring, according the strict interpretation of the BBB and BMS scales. Therefore, we rejected the use of this algorithm.

We subsequently learned of a novel instrument for quantifying locomotor behavior in spinal cord injured rats—the DigiGait device. This system images the dynamic footprints of a rodent walking on a transparent treadmill and quantifies a large set of parameters based on the

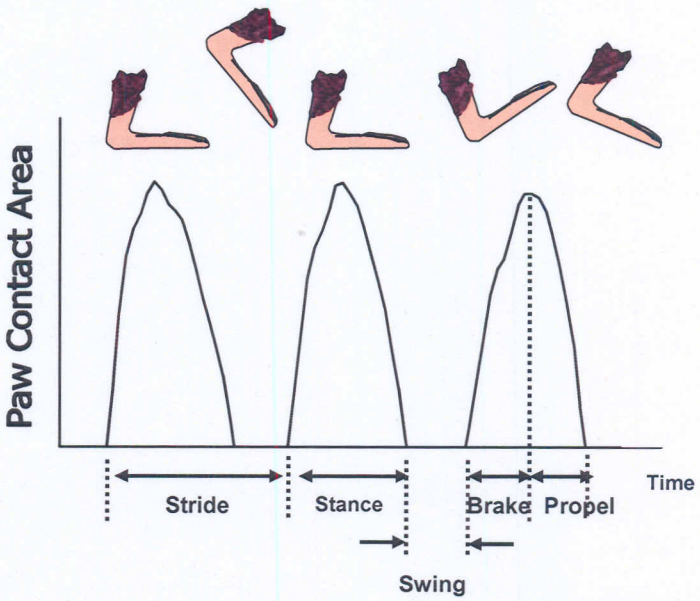

Figure 1. Schematic of gait dynamics, with definition of the different components of stride.

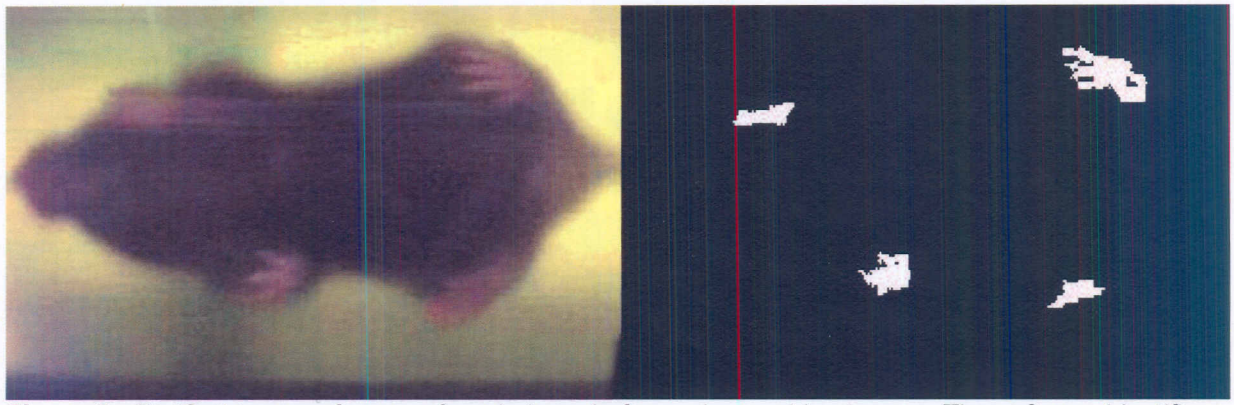

Figure 2. DigiGait image of mouse from below. Left panel, raw video image. The software identifies the color of the paws and subtracts all colors that do not correspond to the paw colors. Front paw size and dynamics are different than hind paw size and dynamics. (Right panel, gray-scale of paws only.) The area of the paw print can be mapped over time, as a paw appears and disappears during the braking, propulsion, and swing phases of gait, providing a dynamic signal of sequential strides.

standards of gait dynamics (Fig. 1). We requested and received permission to use a portion of this award to contribute to the purchase of a DigiGait system.

Briefly, the system consists of an all-aluminum superstructure that includes a DC motor-driven transparent belt. Mice walk upon the top-surface of the belt. Motor speed can be set via a motor controller with digital display. The system is configured with a high-speed digital video camera to image the mice on the belt from underneath (Fig. 2).

Custom software converts the images of limb placement into maps of individual limb dynamics, describing the complete gait cycle through sequential stance-swing phases. The software was automated to identify the binary code for the color of a mouse's paw and subtract all colors that do not correspond within a threshold of that color. All images are then converted to a gray-scale so that what remains are white paw-prints against a black background (Figure 2, right).

As an example of the usefulness of the DigiGait measurements, we compared two groups of rats that were treated with a proprietary compound under contract with a commercial partner. In this experiment 8 animals received standard spinal cord injury and a control treatment, and 8 animals received injury plus the proprietary compound. Two weeks after injury, there was no significant difference in BBB score (Control 9.57±0.58 vs. Treatment 10.57±0.48, mean±SEM, p=0.21). However,

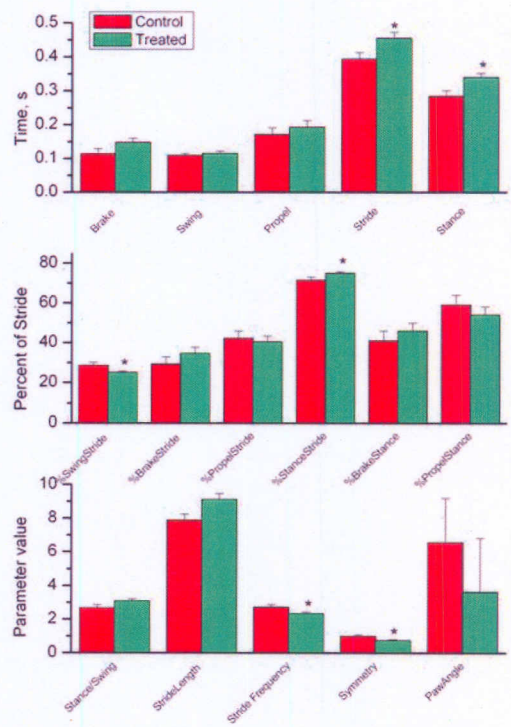

Figure 3. DigiGait parameters measured for two groups of SCI rats (n=8) treated with a compound (Treated) or vehicle (Control). Asterisks denote significance at $p<0.05$.

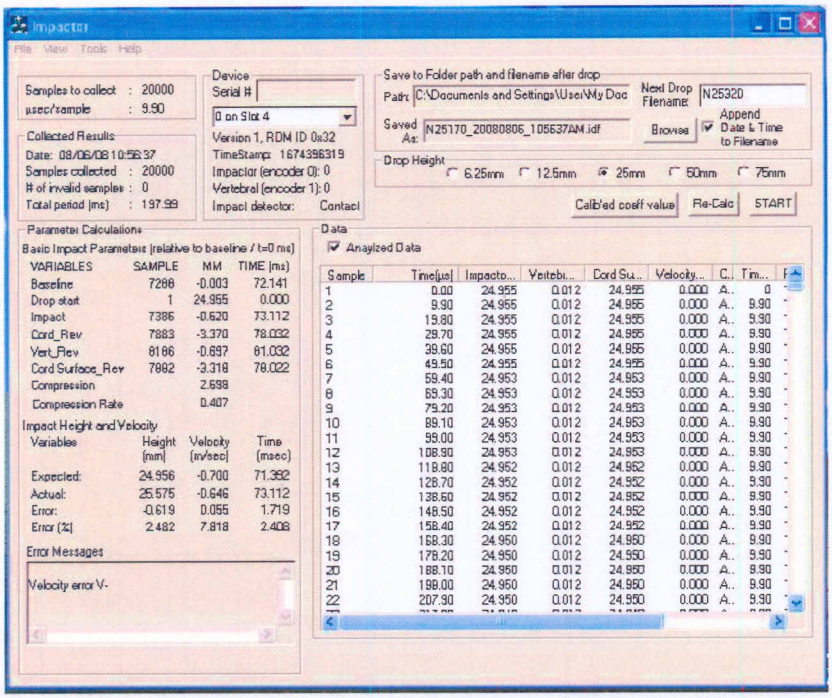

Fig. 4. Screenshot of the new V.7 Impactor software for Windows XP designed and executed under this project.

slight but significant differences were observed in stride time, stance time, %swing, %stance, stride frequency and stride symmetry ($p<0.05$, Fig. 3). This proves that the DigiGait measurements can reveal group differences that are not significant by BBB scoring.

Subsequent work with the DigiGait system has been invaluable in assessing locomotor behavior recovery after spinal contusion and it has helped us to evaluate several potential therapies. While much of this work is done under commercial contract and is protected by the terms of our contract, we expect to be allowed to begin publishing selected results soon.

Aim 3 sought to bring the MASCIS device software up to date and integrate it with the database. This has proven to be a larger and more challenging goal than we envisioned. We previously found a commercial hardware interface card with an included set of software tools to allow us to develop the required interface. However, this supplier soon terminated production of the card, ending our efforts.

We subsequently contracted out development of a Windows-based MASCIS interface to a private company in China. The current version (v7.5, see Fig. 4) of Impactor software was original designed in a DOS environment using the Pascal language. Over the years, minor changes have been made to address software glitches. As computer technology evolves, there is a growing need to adapt the Impactor software to new operating systems. We selected Window XP as the operating system on which the new Impactor software will be based one because it is a mature and stable system and wildly available to end-users. A tremendous effort has been put into the software adaptation including designing a new acquisition hardware and rewriting the software codes.

We have accomplished the following points and are still revising the software. The current software is the 7th revision and has incorporated more than 170 comments and suggestions. We anticipate that the Impactor software will be finished by the end of this calendar year.

1. Selected and modified US Digital PCI-3E-S-S3797card as the acquisition hardware
2. Selection of 12.5, 25, 50 mm heights
3. Universal file system: the graph and data are in commonly-used file system formats so they can be opened without the Impactor software.

4. The software can be used on all Window XP computers regardless the clock speed (this was not true for the DOS version).
5. Simplified the calibration procedure so the end-user can insert a calibration file into the system
6. Raw data (20,000 data points) can be displayed, checked individually, printed and sent by email.
7. Impact graphs can display different lines, and the region of interest can be selected and displayed.
8. Graphs can be printed as dots-and-line in either color or monochrome.
9. All impactor data can be search by file name, project name, date.
10. Time stamp is automatically generated in every acquisition.

Release of this new software and hardware will be welcomed by the many labs using the MASCIS injury model throughout the world.

3. Project challenges.

As outlined above, changing situations caused us to continuously re-evaluate our strategies for completing our goals. After several delays, we believe we have successfully completed the initial goals.

4. Implications for future research and/or clinical treatment.

The Windows MASCIS interface will have the largest impact on the field, since more than 300 labs throughout the world depend on this instrument.

The DigiGait has proven to be useful in evaluating proprietary compounds and the results of these studies will be published shortly. It is quite likely that one or more of the compounds tested will be taken to human clinical trials soon.

The SCI-base database continues to track all data produced in the Keck Center and is a key element in our preclinical animal trials performed for a wide array of corporate collaborators.

5. Plans to continue this research, including applications submitted to other sources for ongoing support.

All technologies developed under this award continue to be used day-to-day in the Keck Center as we search for effective therapies. Every contract and grant application relies upon these important tools. At this time we do not see a need to continue development of these technologies but they will be a part of every project going forward.

6. List and include a copy of all publications emerging from this research, including those in preparation.

SCI-base database:

- Iseda, T., T. Okuda, N. Kane-Goldsmith, M. Mathew, S. Ahmed, Y.W. Chang, W. Young and M. Grumet (2008) Single, high-dose intraspinal injection of chondroitinase reduces glycosaminoglycans in injured spinal cord and promotes corticospinal axonal regrowth after hemisection but not contusion., J Neurotrauma 25: 334-49.

- Hasegawa, K., Y.W. Chang, H. Li, Y. Berlin, O. Ikeda, N. Kane-Goldsmith and M. Grumet (2005) Embryonic radial glia bridge spinal cord lesions and promote functional recovery following spinal cord injury, Exp Neurol 193: 394-410.
- Matsumoto, M., T. Ichikawa, W. Young and N. Kodama (2008) Glutamine synthetase protects the spinal cord against hypoxia-induced and GABA(A) receptor-activated axonal depressions., Surg Neurol 70: 122-8.
- Hashimoto, M., D. Sun, S.R. Rittling, D.T. Denhardt and W. Young (2007) Osteopontin-deficient mice exhibit less inflammation, greater tissue damage, and impaired locomotor recovery from spinal cord injury compared with wild-type controls., J Neurosci 27: 3603-11. Abstract | PubMed

DigiGait:

- Adamson, C. and M. Grumet, SiRNA inhibition of rho kinase mRNA in injured spinal cord. In preparation.
- Adamson, C. Sankar, Seiji, Joanne, Levi, Y., Grumet. Transplantation of neurotrophin-expressing bone marrow stem cells following spinal cord injury.

MASCIS software interface:

- (A single technical report will likely be prepared to publicize this new interface).