Final Narrative Report
New Jersey Commission on Spinal Cord Research

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Body of Report

1. Original Aims of the Project:

1. To provide subsidized microarray services to researchers studying spinal cord injury.
2. To host microarray and animal database services for all spinal cord researchers.
3. To build and operate data mining algorithms to discover associations within our databases.

The purpose of this project was to provide low-cost comprehensive microarray and data analysis services for spinal cord researchers in New Jersey and worldwide. Working with our collaborators, we provided rat oligo microarrays, performed hybridization services, and offered data hosting on our custom web database. Our group included technical staff for performing assays, staff dedicated to creating and maintaining databases and online analytical algorithms and assistance with data reduction and interpretation. Applying these advanced technologies in a cost-effective manner to a broad collection of research projects aimed at curing spinal cord injury certainly accelerated progress and supported a state-wide effort to serve spinal cord patients in New Jersey.

2. Project Successes

We have been extraordinarily successful with this project. Immediately upon initiation of the project, we identified an alternative, cost-effective microarray database (BASE; Saal et al., 2002) which we implemented very quickly. We added functionality to this public-domain scientific software package, and distributed worldwide. We have performed a large number of collaborative microarray studies for a range of researchers. These studies are working their way into publications and grants. We initiated the design and construction of a spinal cord injury-specific animal database, and we have an initial working model. And finally, we have mapped the requirements for data mining algorithms that will be built in the final year of the project.

The BASE Microarray Database. Our objective was to implement a solution with web access, a well-structured database, support for multiple platforms and non-sophisticated users built with a scalable, open architecture. We initially proposed to use a "thin client" technology supporting a server-side, Oracle-driven package running on a parallel computer. However, when BASE was first released early summer 2002, we realized that we could better manage the porting of this package to a powerful, dual-processor Linux-based server in our own lab. Furthermore, the open architecture of BASE allowed us to customize key aspects of the user interface, and to link this database with our eventual animal database. No commercial database package allowed us to customize and add the animal database.

We were one of the first sites to successfully implement BASE. BASE was created at the University of Lund, Sweden, under the GNU General Public License (the same license used by Linux). This provides that all modifications to the original code must be made public, so that all users may benefit from dispersed support of the architecture. We installed it on a Dell Precision dual-CPU server running RedHat Linux 7.3. BASE uses MySQL as its underlying database container, PHP as the programming interface to the database, with results output via Apache web-service software. Some of the computational algorithms are implemented in C and are

called on the server. BASE provides MIAME-compliant storage of all experimental data (Brazma et al., 2001), which is required for publication of most microarray experiments.

We quickly realized that BASE, while a powerful and reliable method for storing and presenting data, lacked in data analysis. We designed and released a small package for interfacing BASE with GeneSpring via ODBC. This has been widely used by other labs throughout the world to combine the storage capacity and organization of BASE with the power of analysis in GeneSpring.

We also programmed a C implementation of principal components analysis (PCA) that runs as a "plug-in" within BASE. This was also released under the GNU General Public License, and has been widely distributed.

Collaborative Microarray Studies. Our many collaborative studies are summarized in Table 1. In general, the ability to offer microarray services at a reduced cost (subsidized by this award) has enabled many researchers to extend their work significantly. A good example is our study with Dr. Michal Schwartz of the Weizmann Institute. Dr. Schwartz's lab had been co-culturing activated T-lympocytes with microglia, with the hypothesis that diffusible substances regulated genes in the target microglia, affecting their response to injury. We screened co-cultured vs. non-co-cultured microglia, and identified several responses indicative of interferon γ (IFNγ) stimulation. Further experiments with cultured microglia showed that T-cells could be replaced with IFNγ addition, and that both treatments specifically upregulated GLT-1 mRNA, which encodes a glutamate transporter. Because of these experiments, Dr. Schwartz was able to hypothesize that T-cells regulate the ability of microglia to buffer toxic glutamate from the spinal cord injury site, and that this cell-cell regulation appears to be mediated by IFNγ. Subsequent experiments proved this hypothesis by showing specific uptake of glutamate by microglia. Other collaborations are summarized in Table 1.

Collaborator	Location	Funding Source	Description	Status
Martin Grumet & Hedong Li	Keck Center, Rutgers University	NJCSCR	Comparison of radial glial-like cells to identify radial glial-specific genes	Li et al. (2003); several new grants resulted from this collaboration.
Ronald P. Hart	Keck Center Rutgers University	NJCSCR	Identification of inflammatory responses to SCI	Nesic et al. (2002); Pan et al. (2002, 2004); Carmel (2004), Cizcova (2004)
Wise Young	Keck Center Rutgers University	Spinal Cord Injury Project	Estrus-cycle effects on SCI; minocyline targets; Tylenol targets; erythropoeitin	Manuscripts in prep.
Marie Filbin	Hunter College, NYC	NIH (Filbin); Bryon Riesch Foundation (Hart)	Genes correlating with neurite extension on inhibitory substrates	Zixuan et al., Submitted.
Martin Marsala	UCSD	NIH	Genes mediating preconditioning ischemia in spinal cord	Carmel et al. (2004); Cizkova (2004). NIH grant submission 6/1/05.
Michal Schwartz	Weizmann Institute, Israel	Proneuron, Ltd.	Genes regulated by T-cell activation in spinal microglia.	Shaked et al. (2005)
Philip Popovich	Ohio State University	NIH	T-cell specific gene responses in spinal cord injury; microglial responses; Toll-like receptors	NIH R01 funded 2004 (Hart as subcontractor); Jones et al. (2002, 2005), Kigerl, Submitted.
Wilma Friedman	Rutgers University, Newark	NJCSCR	Neurotrophin responses in spinal astrocytes and neurons.	Preliminary studies complete; full experiment in progress
Mary Bunge	Miami Project	CRPF	MDP (endotoxin) response in injured spinal cord	Preliminary study completed, full study completed elsewhere.
Patrick Sullivan	University of Kentucky	NIH	Mitochondrial responses to spinal cord injury vs. head trauma	Sullivan et al. (2004)

Table 1. Collaborating spinal cord research projects.

SCIBase Animal Database. The largest undertaking of this project was the animal experimental database. Our goal was to record every medical event during animal SCI studies, so that functional observations may be linked to microarray results; linking physiological responses to gene expression. The concept also provides a convenient tracking system for traditional SCI experiments. Many other laboratories have expressed interest in this software.

For the first seven months of the project, we employed a bioinformatics specialist who is also a licensed veterinarian (Dr. Gregory Voronin). This proved to be immensely useful during the design and trial phase of the project. Dr. Voronin, through his experience working for Rutgers

Laboratory Animal Services, was intimately familiar with all stages of animal care, husbandry, locomotion and complications. This knowledge was incorporated into the design of our database. Unfortunately, Dr. Voronin recently chose to return to private practice for personal reasons.

With our database foundation intact, we hired Mr. Jeffrey Weeks to continue the project. Mr. Weeks, a C4 quadriplegic, is an experienced professional software developer and database expert, who was recruited to our project through his work on the CareCure community (http://carecure.org), where his is a moderator of the spinal cord discussion group. Mr. Weeks had previously given us occasional help with BASE implementation and moderation. We were very lucky to obtain such a senior-level software designer.

Our original implementation of the database used Java-based layering technologies (Jakarta Stuts, JDO, Tomcat). While this proved to be quite flexible for the developer, and was valuable during our early trials and changes, we have decided to port the design to PHP and MySQL, the same architecture used for BASE. We have found PHP to be an easy-to-use and powerful language. We believe it will be easier not only for us to maintain, but it will also be easier for other scientific groups to implement our database. Furthermore, this architecture will run on Windows, UNIX, Linux or Macintosh computers with no changes to the underlying code.

The database stores census data about each animal, and links individual "clinical care events." These include weights, urine output, urinary complications, postoperative complications, and all drugs delivered. For each event, choices are limited to our approved animal protocols, so the database may be used as a care guide by staff. Summary care reports are sent directly to a networked laser printer for archival notebooks, and these sheets contain a bar-code for immediate updating of the records for that animal. This system allows a care worker (with a laptop computer, an 802.11b [wireless network] connection, and a bar code reader) to visit the animal quarters, call up records specific for each animal, update records, provide care, document care, store, and print all data.

We released the database and published its specifications (Weeks and Hart, 2004). A subsequent NJCSCR project was funded in part to continue development of the SciBase database (04-3031-SCR-E-0).

3. Project Challenges

The third specific aim, to develop data mining algorithms, was largely precluded by the commercial availability of excellent data mining tools. Therefore, we adapted our attention to obtaining, implementing, training and utilizing these commercial tools. We were an early adopter of the BASE database and we contributed software for some useful algorithms (such as principal components analysis). We then licensed GeneSpring and adapted its database access functions to draw data from BASE. We also adapted the R statistical language to provide more customized data analysis (for example: Pan et al., 2004). One of our publications was essentially a "tour de force" comparing commonly-used data filtering and clustering techniques to demonstrate that these algorithms tend to select the same results from the dataset. More recently, we move to a more sophisticated database/analysis package, GeneTraffic. During each phase of this process, we worked to implement our original goals.

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

This project has served as a bellwether of the use of functional genomics in spinal cord injury. Several leading labs, including Allan Faden's group at Georgetown and Paul Reier's lab at Florida, built on our early findings and made use of our analysis methods (Di Giovanni et al., 2003; Velardo et al., 2004; De Biase et al., 2005; Di Giovanni et al., 2005a; Di Giovanni et al., 2005b). We contributed directly to the work of several leaders in the field, such as Marie Filbin (manuscript submitted), Michal Schwartz (Shaked et al., 2005), Phil Popovich (Jones et al., 2002; Jones et al., 2005), Pat Sullivan (Sullivan et al., 2004), and Marty Grumet (Li et al., 2003). Finally, we helped to bring Martin Marsala into the realm of spinal cord injury by capitalizing on their elegant ischemia model (Carmel et al., 2004; Cizkova et al., 2004). I believe that we have contributed to the field's understanding of the inflammatory response to SCI, the potential for protection by preconditioning, and the identification of regeneration-associated genes.

5. Plans to continue this research

The research begun under this project seeded new work in many labs. Several grants have been funded (Popovich, NIH; Hart, Grumet; NJCSCR) and more are in progress (Grumet and Hart, NIH, 5.7%ile awaiting funding decision; Marsala NIH submitted 6/05).

6. Publications included in report

Software Development:

BASE plug-in for principal components analysis, Voronin and Hart

Database connection from BASE to GeneSpring, DeLong, Weeks, Faulk and Hart

Traditional Publications:

Carmel, J.B., A. Galante, P. Soteropolos, P. Tolias, M. Recce, W. Young and R. P. Hart (2001) Profiling gene expression following spinal cord injury reveals spreading inflammation and neuronal loss. Physiological Genomics 7:201-213.

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Jones, T.B., D.M. Basso, A. Sodhi, J.Z. Pan, R.P. Hart, R.C. MacCallum, S. Lee, C.C. Whitacre and P.G. Popovich (2002) Pathological central nervous system autoimmune disease triggered by traumatic spinal cord injury: Implications for autoimmune vaccine therapies. Journal of Neuroscience. 22: 2690-2700.

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Li, H., Y. Berlin, R.P. Hart and M. Grumet. (2003) Microtubules are critical for radial glial morphology. Glia 44: 37-46.

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Carmel JB, Kakinohana O, Mestril R, Young W, Marsala M, Hart RP (2004) Mediators of ischemic preconditioning identified by microarray analysis of rat spinal cord. ExpNeurol 185:81.

Cizkova D, Carmel JB, Yamamoto K, Kakinohana O, Sun D, Hart RP, Marsala M (2004) Characterization of spinal HSP72 induction and development of ischemic tolerance after spinal ischemia in rats. ExpNeurol 185:97.

De Biase A, Knoblach SM, Di Giovanni S, Fan C, Molon A, Hoffman EP, Faden AI (2005) Gene expression profiling of experimental traumatic spinal cord injury as a function of distance from impact site and injury severity. Physiol Genomics 22:368-381.

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