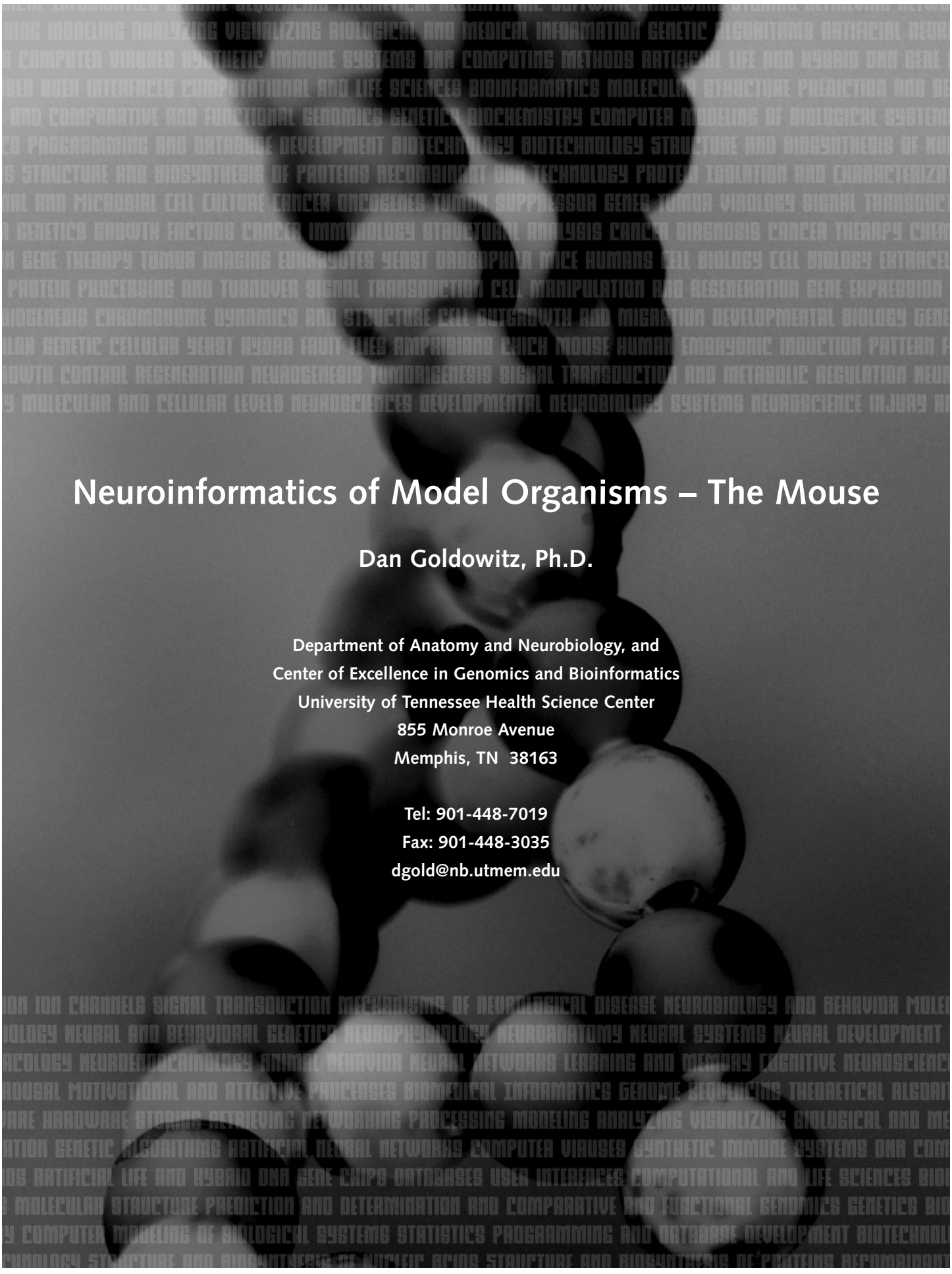

Neuroinformatics of Model Organisms – The Mouse

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I. INTRODUCTION

In the past, the choice of experimental subject was driven by specific physiological traits or historical precedence. Thus, one looked at the pig heart due to its similarity to the human, one used rats or pigeons for the analysis of behavior because of the large literature that supported this effort, and likewise for the analysis of higher visual function using the cat. The current era now relies on commonality (the genome) and the conservation of function (e.g., homeodomain proteins or signaling molecules do remarkably similar tasks in a worm cell as a monkey cell). With the unification of species as a shared and common source for neurobiological inquiry, informatics plays a critical role in collating and relating the data obtained from various organisms to obtain insights that would have been difficult from the study of a single organism. This component of the short course will give a quick overview of the various species that have assumed "model organism" status in the modern era of functional genomics and bioinformatics. Then, the mouse is chosen for a more in depth example of the use of model organisms. As with each component of this Short Course, this syllabus and presentation provides a survey of the tools available to the researcher to develop research programs and solve problems using the model organism approach. In this vein, web pages and screen shots are provided to help the student appreciate the incredible resources that are available on the World Wide Web and foster the easy access of data that will facilitate the appreciation and use of bioinformatic tools for the use and analysis of model organisms in neuroscientific inquiries.

II. THE FLY, WORM AND FISH

Various communities of researchers have established websites that offer easy portals to appreciation of countless numbers of organisms. Three such examples are provided below that have come to support community research in the fruit fly, the nematode, and the zebra fish. Each of these sites provides excellent user support, access to discussion groups, informative introductions into the organism, atlases, its gross anatomy, development, and genetics. In addition, each of these sites notes upcoming events and meetings, recent publications, links to various researchers and research projects, and (importantly) resources. Finally, there are special features of each site that make them interesting to visit such as: an "online atlas and database of the Drosophila nervous system" that serves as a great tutorial to find out about the neuroanatomy of the fly, the historical account of how Sydney Brenner came to the nematode to study development and neurobiology, and "Zebrafish K–12" that provides a tribute to Georege Stresinger, the "founding father" of zebrafish research and guides for laboratory experiments and much more.

The genetic resources at these sites are formidable. Both the fly and the worm have full genome sequence and the tools are available to search and query these genomes. Zebrafish is the least mature of all three model systems, but there is a zebrafish genome project that is in full swing. At all three sites are lists of mutant stocks and their availability.

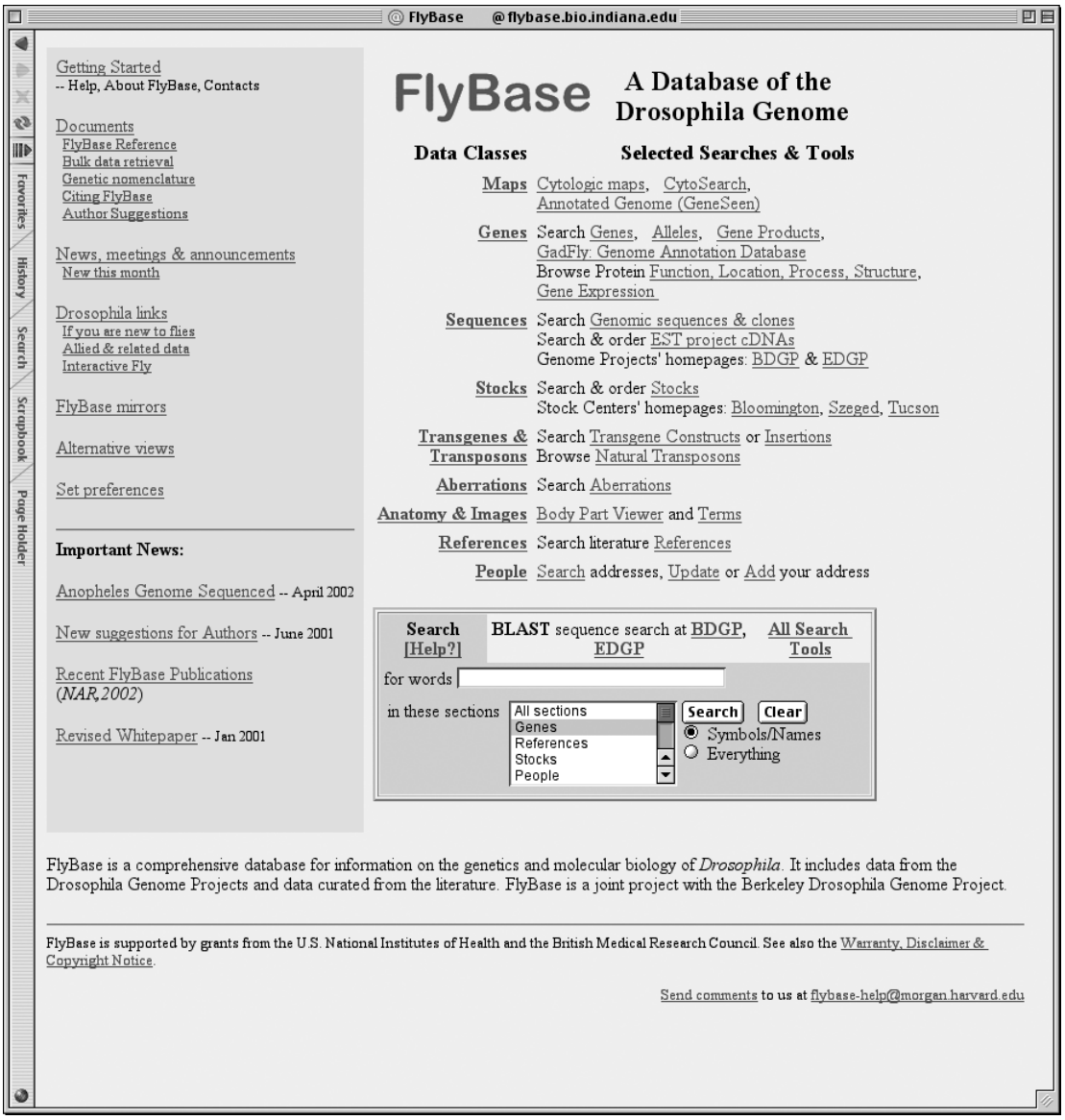
A. Drosophila melanogaster
http://www.flybase.org/

B. Caenorhabditis elegans
http://elegans.swmed.edu/

Worm Breeder's Gazette
http://elegans.swmed.edu/wli/tocs/wbg07.1.html

C. Danio rerio
http://zfin.org/

A screen shot from the Drosophila Flybase website is shown below. The material in the center of the screen gives a good idea of the various tools one has available to explore the use of the fruit fly in research. The "Getting Started" clickable in the upper-left is a good place to get to get an idea of navigating the site.

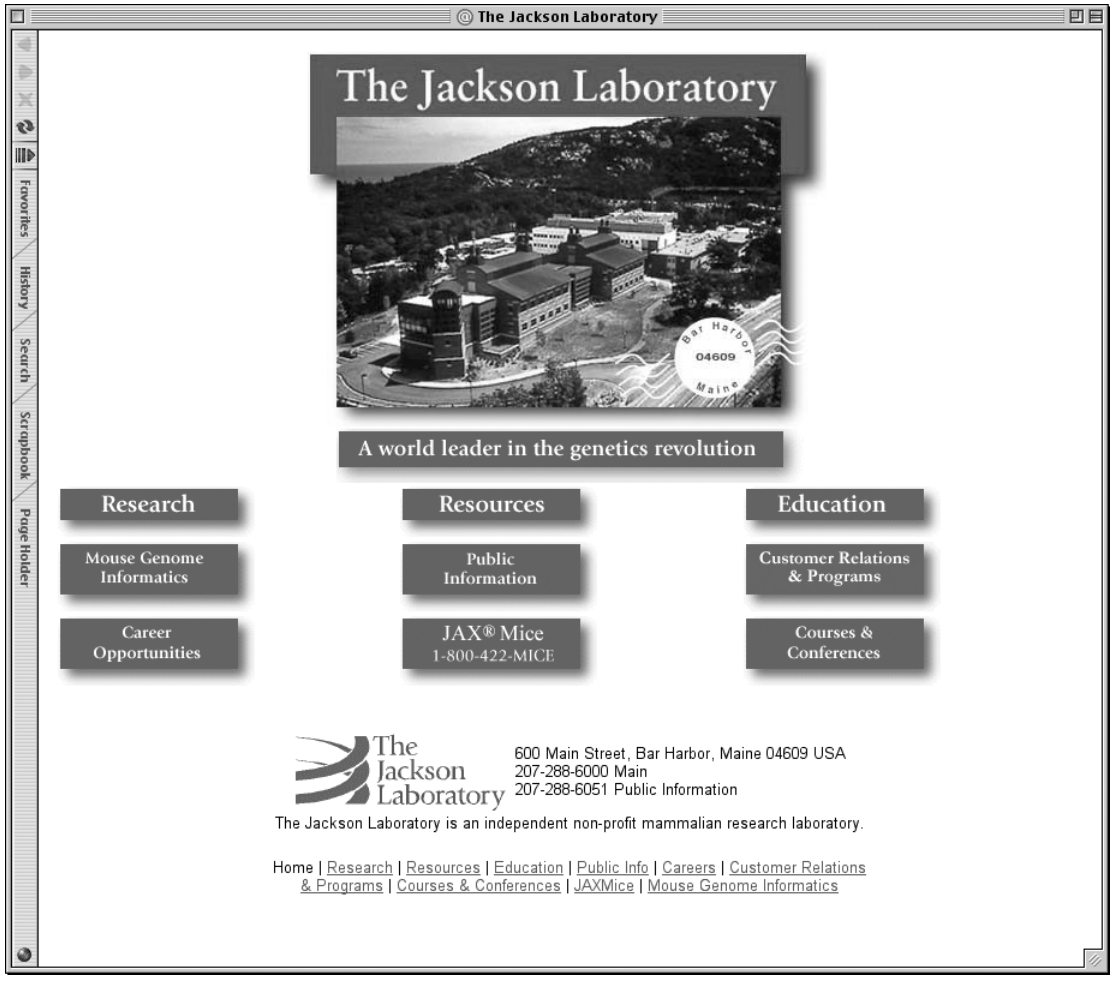


III. THE MOUSE

The laboratory mouse, Mus musculus, has come to occupy a central place in the biologist's armentarium of tools to explore the genetic underpinnings of brain and behavior. The humble beginnings of mouse research as an off-shoot of "mouse fanciers" and Miss Abbie Lathrop and her "fancy" mice that were brought into the laboratory by Dr. E. Castle in the early 1900s are quite an amazing start to the current state of numerous, large corporate entities that are involved in the creation of mouse mutants and functional genomics. Just as impressively, several federal governments have recognized the value of the mouse in biomedical research and have funded large scale research efforts to sequence the mouse genome, analyze various phenotypes, and mutagenize the genome to identify gene function.

The starting point for any journey into mouse-dom must begin at the Jackson Laboratories. One of Castle's students, Clarence Little, helped found the Labs and establish its primary mission in 1929. Located in what must be one of the more idyllic research settings in the world, Bar Harbor, Maine,

WWW.JAX.ORG



this laboratory has provided the wildtype and mutant strains of mice and the single-minded (save for small diversions with dogs and rats) intellectual and research commitment for the past 75 years that has been the dominant force in mouse biology and genetics. Visit the laboratory website to find out about the corporate end of Jackson Resource Services and then visit the nervecenter of information about the mouse at informatics.jax.org (A.1.). If you are an occasional user of mice or are a "lifer" the listserver maintained by the informatics group at JAX is a great repository of all things essential and arcane (A.2.). One can learn different means to keep track of pups with various labeling systems or why the term "murine" does not specifically apply to the mouse.

There are several excellent books that can serve as a great starting point to find out about the biology of the mouse, its use in genetic analysis of the brain, and the background and essentials of mouse genetics and maintaining a mouse colony. Three such books are listed in A.3.a-c. Very conveniently, the book by Silver is available online and free-of-charge (A.3.c.). These books are important resources for understanding what is involved in setting up a colony of mice, basic mouse biology, and understanding the numerous issues involved in mouse husbandry and behavior. Recently, CRC press has initiated "Laboratory Animal Sciences Titles" that include a "bestseller" by Suckow et al. entitled The Laboratory Mouse and several titles concerning the phenotypic analysis of mutant mice, vision, and hearing.

Multiple chapters, reviews and books are appearing annually that deal with the behavioral analysis of mice, with a focus on transgenic and knockout lines (A.4.a-d, g, h). Probably the most complete and extensively referenced book of this genre is that by Jackie Crawley, entitled What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice. This book represents the accumulated wisdom of one laboratory's extensive efforts to behaviorally characterize many neurological mutant mice. A very comprehensive review of the behavioral testing and outcome of almost 200 mutated genes (largely of the knockout variety) was published by Bolivar et al. (Mammalian Genome, 11[2000]260-274). This article, also available online (A.4.g) details all the behavioral tests done on each of the published mutants, and whether there was a mutant phenotype and the direction of the deviance. The Williams' lab at nervenet.org maintains an extensive database of known/reported phenotypes of recombinant inbred strains of mice and their investigations into the quantitative neuroanatomy of the mouse brain (A.4.f). Community collaborative efforts, such as nervenet.org, are invaluable tools for the researcher to discover data on inbred and mutant strains of mice (A.4.e,h)

A good road map to the mouse brain is an essential tool for any neuroscientist interested in using the mouse as a model organism. The neuroanatomical atlas that the first generation of mouse neurogeneticists was raised on was one by Sidman et al. (Atlas of the Mouse Brain and Spinal Cord, Richard Sidman, M.D., Jay B. Angevine, Jr., Ph.D., Elizabeth Taber Pierce, Ph.D., Harvard University Press, Cambridge Mass, 1971). This atlas has been out of print for some time but there is a web-incarnation of that Atlas created by Dr. Sidman (A.5.a). It is in color and has a nice 3-D section that allows one to visualize pathways and structures in the mouse brain. Commercial publications are coming out on an annual basis. There is a new Paxinos and Franklin atlas that is quite comprehensive with coronal and sagittal sections that include acetylcholinesterase staining patterns (A.5.b). Another recent and in-depth atlas that compares the brains of two of the most commonly used inbred strains for neuroscientific research (C57BL/6 and 129/Sv) is by Hof et al. (A.5.b).

Although these atlases allow one to navigate through the adult mouse CNS, the developing nervous system has its special challenges as many obvious cell groups in the adult are part of a homogenous neuroepithelium and only exist as

"primordial" which need a specialist's guide for identifying these indistinguishable structures. Several atlases exist which look at various times during development, either part of the whole embryo or specially focused on the CNS (A.5.c). More generally, researchers centered at the University of Edinburgh have put together an excellent developmental atlas of the mouse (http://genex.hgu.mrc.ac.uk/) that not only includes annotations of structures throughout embryogenesis but will include a comprehensive map of gene expression. Their 3-D images are stunning and quite useful tso visualize the developing brain and embryo in three dimensions.

Individual investigators have also developed a wealth of resources for the researcher to find out about mouse neurogenetics in general and the neuroanatomy of the mouse in particular.. There is a site at the University of Toronto maintained by Jeff Henderson and this site has a set of neat tools to explore the mature mouse nervous system. Another home-grown site that has made it into the Science NetWatch is that of Rob Williams (www.mbl.org) that provides a unique opportunity to view the brains of a large collection of recombinant inbred strains, whose brains have been celloidin-embedded and sectioned in the horizontal and coronal plane. In addition, an ever-evolving feature at this site is the ability to examination histological material on a Zeiss microscope over the internet. The iScope is a public resource and is being supported by the Human Brain Project (NIMH) and NSF. Finally, the Goldowitz imaging laboratory has teamed-up with MicroBrightfield to establish a brain atlas and interactive posting of phenodeviant brains using the VirtualSlide technology. (A.5.d) (Supported by NIA and the 7 NIH Institutes that support the Mouse Mutagenesis Program).

If you have decided to take the plunge and maintain mice, whether as a large colony or just a few knockouts or transgenics, it would be of great help to rely on other sources than occasional words of advice from your vets or local mouse guru. The resources noted above (the MGI listserve, Silver textbook, etc) are available and will provide you with a strong grounding in the essentials of mouse husbandry. Silver's text is quite excellent in providing the background and details of mouse maintenance. Many of the essentials of the reproduction and physiology of mice can be found in the "Biology of the Laboratory Mouse". As problems or questions arise that are pertinent to your research effort, there is a large community of researchers who are generous with their thoughts and opinions. There are formal courses that are offered in the specialized areas of genetics (A.6.a), behavior (A.6.b), and various technical and scientific issues (A.6.c). Finally, there is an organized group, the International Mammalian Genome Society (see C), which is largely dedicated to the use of the mouse in genetic research. There are several public sources for obtaining programs to help manage larger animal colonies and two of these are available at http://mickey.utmem.edu/main/databases.html (A.4.f).

A. Getting information

1. The Jackson Laboratory — http://www.jax.org/ , http://www.informatics.jax.org/
2. Forum for topics in mouse genetics digest mgi-list@lists.informatics.jax.org
   a. Check out the WEB version of mgi-list at http://www.informatics.jax.org/mgihome/lists/lists.shtml
3. Books devoted to the use of mice in biomedical research.
   a. "Biology of the Laboratory Mouse" — JAX lab staff
   b. "Mouse Genetics: Concepts and Applications" by Lee Silver http://www.informatics.jax.org/silver/
   c. A recent series of books from CRC Press, in their Laboratory Animal Science titles deals with many aspects of the mouse — www.crcpress.com
4. Behavioral analysis of the mouse
   a. "Techniques for the Genetic Analysis of Brain and Behavior: Focus on the Mouse" – Goldowitz et al. (eds), Elsevier, 1992.
   b. "Neurobehavioral Genetics: Methods and Applications" – B.C. Jones and P. Mormede (eds), CRC Press, 1999.
   c. "What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice", J. N. Crawley, Wiley, 2000.
   d. ILAR issue vol. 41, #3, 2000 devoted to "Mouse Behavioral Models in Biomedical Research. Issue available online at: http://www4.nas.edu/cls/ijhome.nsf/44bf87db309563a0852566f2006d63bb/df6569ad9156a7e6852568eb00587bfa?OpenDocument
   e. Mouse Phenome Project — http://aretha.jax.org/pub-cgi/phenome/mpdcgi
   f. Mouse Phenotype Databases — http://mickey.utmem.edu/main/databases.html
   g. Excellent review of behavioral phenotypes in mutant mice http://www.wadsworth.org/BMS/genomics/transgenics.htm
   h. Also look at www.mymouse.org, a site maintained by Jeff Noebels and co-workers aimed at an online collaborative for the phenotypic analysis of mutant mice.
5. Neuroanatomical analysis of the mouse.
   a. The Sidman Atlas and its web version: http://www.hms.harvard.edu/research/brain/atlas.html
   b. Other atlases of the adult mouse CNS. George Paxinos and Keith Franklin (The Mouse Brain in Stereotaxic Coordinates 2nd edition, Academic Press, San Diego, 2001). A more detailed and comprehensive atlas has been produced by Hof et al. (Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv, Patrick R. Hof, Warren G. Young, Floyd E. Bloom, Pavel V. Belichenko, Marco R. Celio, Elsevier Science, 2001).
   c. Atlases of the developing mouse nervous system – These would include Jacobowitz and Abbott's Chemoarchitectonic Atlas of the Developing Mouse Brain, CRC Press, 1998; Schambra et al., Atlas of the Prenatal Mouse Brain, Acad. Press, 1991; and μMRI Atlas of Mouse Development at http://mouseatlas.caltech.edu/ .
   d. Sites too numerous to mention can be found as links at these locations www.mbl.org, www.neurophenotyping.utmem.edu,

http://www.phm.utoronto.ca/~jeffh/neuromouse.htm

6. Courses for basic learning and continuing education
   a. JAX/Johns Hopkins – Mammalian Genetics Course, http://www.jax.org/courses/documents/shortcourse02_html.htm
   b. IBANGS – Summer school in Behavioral Genetics, http://www.ibngs.org/bni/eighth.html
   c. The Jackson Labs, Woods Hole and Cold Springs Harbor offer courses related to the mouse and/or bioinformatics, and their websites should be checked for their yearly offerings. An international effort for the phenotypic analysis of the mouse has had one meeting (http://www.medizin.uni-koeln.de/dekanat/fd/Transgenic_mice/ "Behavioural Phenotyping of Mouse Mutants" February 17-19, 2000 in Cologne, Germany), and plans for another, so this may be an additional resource for further learning and education.

B. Data sharing
   1. Mouse neurogenetics and QTL analyses and tools: www.nervenet.org (Rob Williams magnanimous gift to the mouse community) and http://www.complextrait.org/ (website of the Complex Trait Consortium
   2. Mouse Phenome Project – http://aretha.jax.org/pub-cgi/phenome/mpdcgi

C. The International Mouse Genome Society and the journal, Mammalian Genome — http://imgs.org/

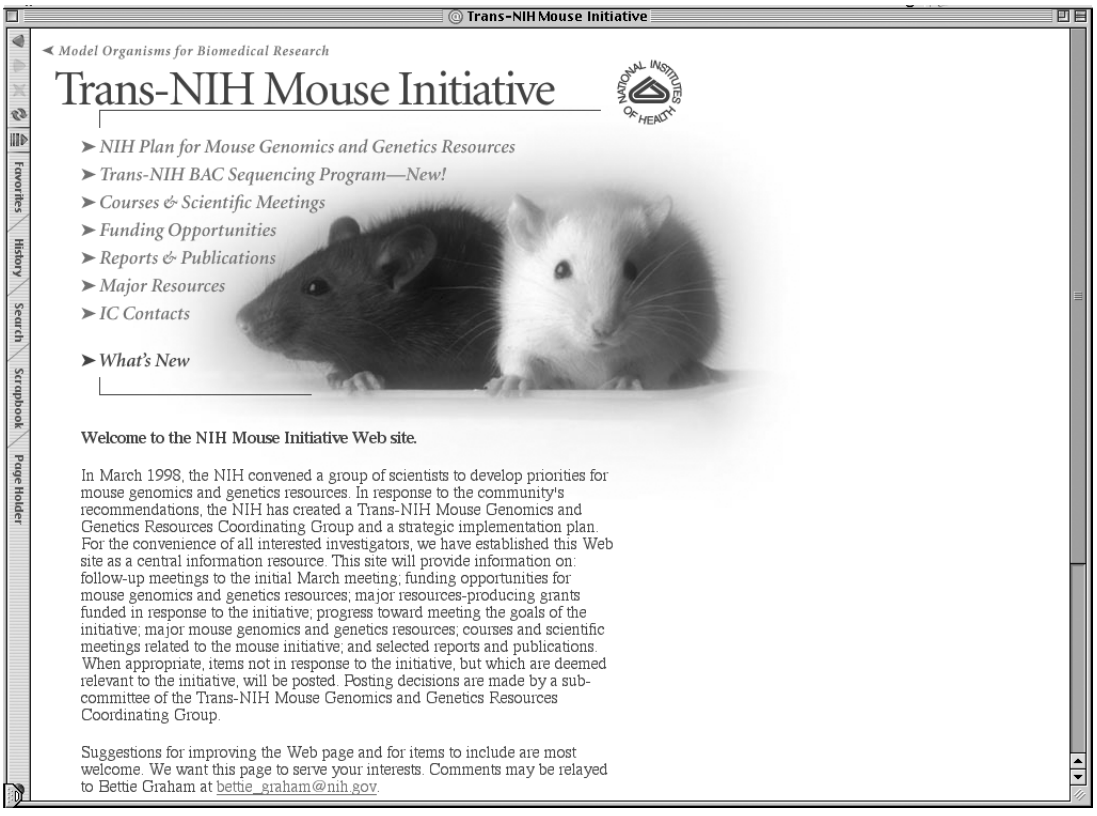
IV. MOUSE MODELS

TRANS–NIH MOUSE INITIATIVE

What has been termed "the post genomic era" or "functional genomics" is upon us, which is to say there is now no excuse to take the 3x109 or so base pairs of DNA in mouse or man and find out what they are doing in terms of the biology of the organism. It became clear to the research community and NIH that this new era would best be served by focusing on the mouse – both in terms of establishing its genomic sequence and understanding its functional genomics using mutational methodologies. This becomes a major issue of informatics – the acquisition of large amounts of data (genetic and phenotypic) and the relational nature of that data. The earliest efforts in the neurosciences began with a fruitful collaboration between Richard Sidman and the Jackson Laboratory that has been summarized in a listing of all the mice with spontaneous mutations that resulted in a neurological phenotype (at that time; THE GREY BOOK ref)(A). As the technology became available, this pioneering work was followed by creation of mutants by overexpression or insertional mutagenesis with transgenic mice (B) and then the willful creation of specific mutations using homologous recombination (C). A hybrid approach using gene-trap techniques can attack larger classes of molecules, some of which have not been previously defined (C).

It was recognized that a more wide-ranging approach that could attack the whole genome and create various mutant phenotypes for a singe gene was also needed. The pioneering work in the fruit fly and the zebra fish pointed the way. British and German efforts began large scale mutagenesis of the mouse using N-ethyl-N-nitrosurea (ENU). The

WWW.NIH.GOV/SCIENCE/MODELS/MOUSE



NIH had convened think-groups to arrive at some decisions about the use of the mouse as a model organism and considered ENU mutagenesis as an important component in this effort (http://www.nih.gov/about/director/reports/mgenome.htm, and http://www.nih.gov/science/models/mouse/reports/actionplan.html). The Japanese, Australian and Canadian governments have initiated similar efforts (see D). These efforts are being consolidated as an international mouse mutagenesis consortium that should enhance the sharing of reagents and data (see http://www.nih.gov/science/models/mouse/reports/summary_imm_consortium.html).

One of the major US-funded efforts focused on obtaining ENU-induced mutations in mice that had neurological phenotypes (http://www.nih.gov/science/models/mouse/funding/rfa_mmpnsb.html). Three groups were funded under this effort, and their progress can be monintored online at the Jackson Lab, Northwestern University, and Tennessee Mouse Genome Consortium websites (D).

Explicit in this effort is that what we find in mouse will be directly translatable to what is found in the human, and that genetic mutant mice can serve as models for human dysfunction. The most comprehensive treatment of genetic diseases in man, of which mice may be able to model, is the online version of "On Mendelian Inheritance in Man" (E). A "dysmorphology database" has been established that with an easy click allows one to get thumbnail sketches of human and mouse syndromes. Finally, one might want to get mutant mice as a tool in their research program. There are a variety of sources other than the Jackson Laboratories (F). The NIH-NCRR has recently bestablished four Mouse Mutant Resource Centers where transgenic and knockout lines of mice are maintained and distributed. The European mutagenesis and knockout groups have also consolidated their mutant mouse resources and will distribute mutant mice. Finally, the NIH Neuromutagenesis centers will soon have a distribution arm where one can get ENU-induced mutants of varying neurological phenotypes.

A. Spontaneous mutants (see JAX)
http://www.jax.org/resources/documents/
B. Transgenesis [see T-base (free); Biomednet (costly), Pathbase (coming online)]
http://www.jax.org/resources/documents/imr/ – The Induced Mutant Resource at JAX
http://tbase.jax.org/ – The transgenic/targeted mutant database maintained at JAX
http://research.bmn.com/mkmd – Presented by Elsevier Press
C. Targeted mutagenesis
German effort, http://tikus.gsf.de/ and http://genetrap.de
Skarnes, http://socrates.berkeley.edu/~skarnes/resource.html
Lexicon, http://www.lexicon-genetics.com/omnibank/omnibank.htm
Deltagen, http://www.deltagen.com/demo/index.html
D. ENU mutagenesis programs – PUT IMMC website here
The Tennessee Mouse Genome Consortium
http://www.tnmouse.org/neuromutagenesis/index.html
Northwestern, http://genome.northwestern.edu/
Jackson labs, http://www.jax.org/nmf/documents/about.html
Baylor, http://www.mouse-genome.bcm.tmc.edu/ENU/ENUexperiment.asp
McLaughlin Inst., http://www.montana.edu/wwwmri/enump.html
Harwell, http://www.mgu.har.mrc.ac.uk/mutabase/
German, http://www.gsf.de/ieg/groups/enu-mouse.html
Japanese, http://www.gsc.riken.go.jp/e/group/mousegrE.html
Australian, http://jcsmr.anu.edu.au/group_pages/mgc/mutagenesis/mutagenesis.html
Canadian, http://cmhd.mshri.on.ca/
Novartis program, website not circulated
E. Human and mouse-human mutant information
OMIM – http://www.ncbi.nlm.nih.gov/Omim/
Human-Mouse Dysmorphology Database
http://www.hgmp.mrc.ac.uk/DHMHD/dysmorph.html
F. Distribution and getting mice
NIH resources: www.mmrrc.org and coming soon a Distribution Center for the three NIH-funded Neuromutagenesis Centers.
European resources – www.emmanet.org
The Jackson Induced Mutant Resource – http://www.jax.org/resources/documents/imr/

V. Gene mapping and homology

In David Deitcher's presentation, you will receive detailed information about the mining of genomic data. In this section, various sites are provided where one can obtain this data for the mouse (A). Although the public effort to sequence the mouse genome is basically completed, it is still in the construction stage which precedes annotation and identification of transcriptional units. What is useful in getting a more complete view of the mouse sequence data and gene identification is to take advantage of the high degree of homology between mouse and man and access the more complete human DNA sequence (B). Furthermore, the state of the public sequence is also amenable to searches for homologous genes in other species that might be elaborated in the mouse and suggest gene families.

A. NCBI—Mouse genome information
http://www.ncbi.nlm.nih.gov/genome/guide/mouse/
http://www.ncbi.nlm.nih.gov/genome/seq/MmHome.html

UK site for mouse genome – Ensembl
http://www.ensembl.org/Mus_musculus
(see below)

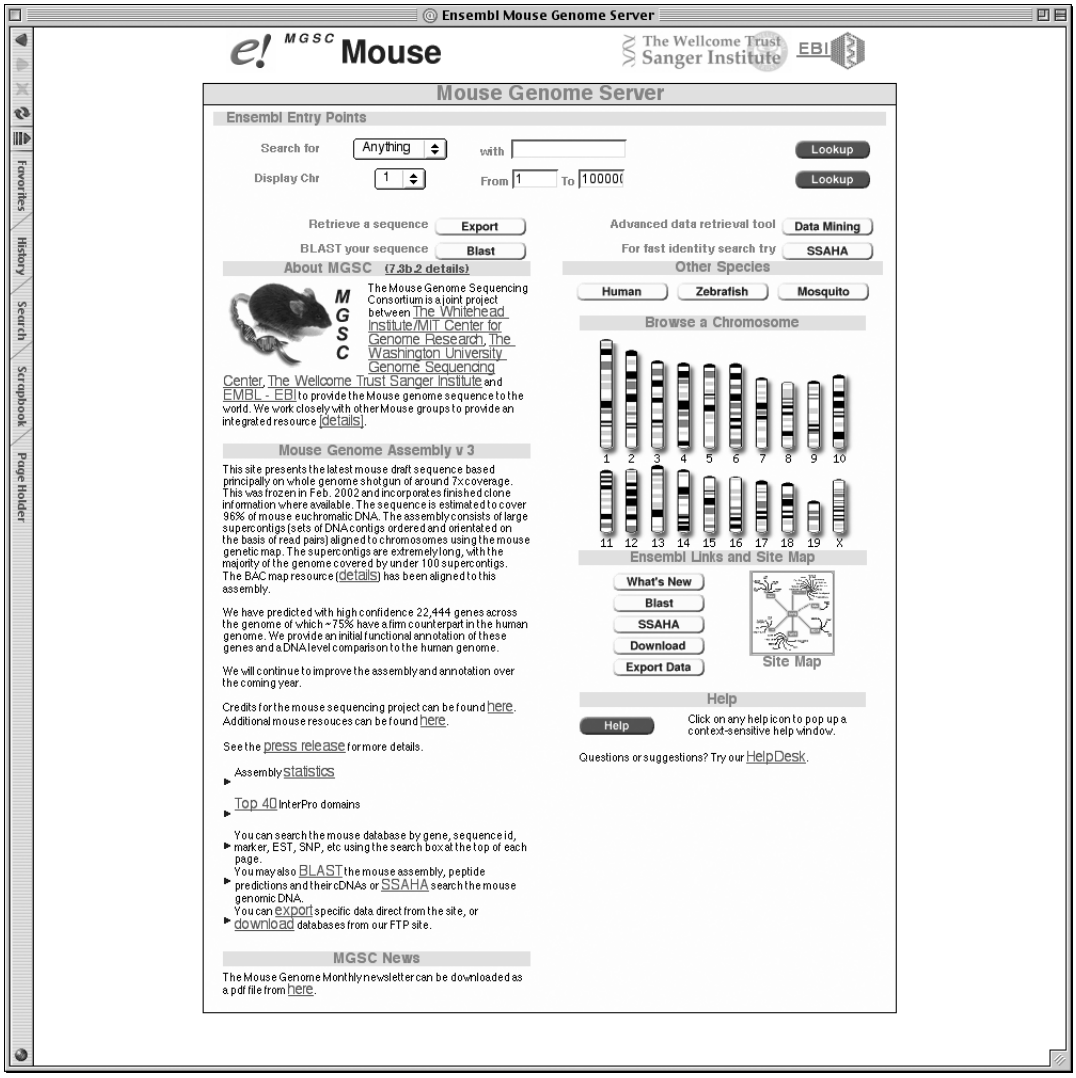
B. MGI 2.6 mouse <–> human comparative maps
http://www.informatics.jax.org/reports/homologymap/mouse_human.shtml

Human
http://genome.ucsc.edu/index.html,
http://www.ncbi.nlm.nih.gov/genome/guide/human/

C. Zebrafish
http://zfin.org/
http://www.sanger.ac.uk/Projects/D_rerio

Drosophila
http://www.fruitfly.org/annot/

Nematode
http://elegans.swmed.edu/genome.shtml



VI. PRACTICAL PROBLEM IN MOUSE NEUROGENETICS

Your late colleague, Dr. X, has bequeathed you a transgenic line of mice that has an unusual phenotype. The transgenic mice have abnormal prepulse inhibition and just hang, with limited struggling, when suspended by their tail. Although this signals a mouse of some predictive validity for various models of psychiatric disorders, NIMH is overflowing with mice of varying phenotypes – it wants to have genes with function. So, being a former researcher on the comparative anatomy of the avian cortex, what do you do?

Setting up a colony
- Internal approvals, working with external sources
- In-house help to set up a colony; outside help in setting up a colony
- Breeding: record keeping is critical
- Determining mode of inheritance

Mapping the mutant locus
- Identifying the strains for a mapping cross
- Making your cross; backcross or intercross
- Analyzing data to map your mutation to a chromosomal region

Identifying the mutant gene (sub cM attack)
- High resolution cross (1,000 or so offspring)

Candidate gene approach (multi cM attack)
- Human homology

Microarray/gene expression analysis
- Verification of candidate
  - Expression (mRNA or protein)
  - DNA sequence
  - Transgenic (knockin/knockout; transgenic)

VII. PERSPECTIVES

This presentation is meant to serve as a roadmap to the neuroscientist who would like to use the mouse as a model research organism. The "Practical Problem" (VI) is meant to get the neurons firing about how one would use the resources outlined above. The field is growing rapidly and a consideration of coming developments is important. The other presentations of this Neuroinformatics course provide critical complements to approaching the genomics of nervous system function.

A. Completion of the mouse genomic sequence and its annotation (see above).

B. Gensat program – NIH's support of two efforts to map the expression of all genes in the brain at different developmental stages. One is a high throughput in situ hybridization approach and the other proposes to use BAC transgenic mice to map gene expression in vivo.

C. The fine mapping of complex traits – the Complex Trait Consortium (http://www.complextrait.org/)

D. The co-ordination of genomic, phenotypic, proteomic, anatomical and other databases for synthesis of information and discovery. See http://www.npaci.edu/DICE/Neuro/, the website of Ellisman and Martone and colleagues that proposes a Neurosciences Federated Database. Sounds like Star Trek for the inveterate neuroscientist.