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## *Designer Mice*

The disease is inherited. Family pedigrees indicate an autosomal dominant gene. Linkage analyses reveal one strongly associated chromosomal locus. Mapping identifies the gene. The cDNA for the gene is sequenced. The anatomical distribution of the gene is primarily in the brain. The symptoms of the disease are neurological and psychiatric. There is no effective treatment. The disease is ultimately lethal.

Your mission, should you choose to accept it, is to develop a treatment for the disease. Replacement gene therapy is the best hope. But you don't know the gene product, you don't know its function, and you don't know if gene delivery would be therapeutic. Where do you start?

These days, you may choose to start with a targeted gene mutation, to generate a mutant mouse model of the hereditary disease. A DNA construct containing a mutated form of the responsible gene is developed. The construct is inserted into the mouse genome. A line of mice with the mutated gene is generated. Characteristics of the mutant mice are identified in comparison to normal controls. Salient characteristics relevant to the human disease are quantitated. These disease-like traits are then used as surrogate markers for evaluating the effectiveness of treatments. Putative therapies are administered to the mutant mice. A treatment that prevents or reverses the disease traits in the mutant mice is taken forward for further testing as a potential therapeutic treatment for the human genetic disease. Gene therapy, based on targeted gene replacement of the missing or incorrect gene in the human hereditary disease, is described in Chapter 14. In the future, medicine may shift emphasis from treating the symptoms to administering replacement genes that efficiently and permanently cure the disease.

Targeted gene mutation in mice has revolutionized biomedical research. *Transgenic* mice have extra copies of a gene, or copies of a new gene, inserted into the mouse genome. Mice with additional copies of a normal gene enable the investigation of the functional outcomes of the overexpression of the gene product. Additions of a

new gene that is not normally present in the mouse genome, such as the aberrant form of a human gene linked to a disease, enable the investigation of the functional outcomes of the expression of the disease gene. For example, the mutated form of the human *huntingtin* gene is added to the mouse genome to generate a mouse model of Huntington's disease. *Knockout* mice have a disrupted gene that is nonfunctional. The *null mutant homozygous* knockout mouse is deficient in both alleles of a gene; the *heterozygote* is deficient in one of its two alleles for the gene. The *genotype* designation  $-/-$  represents the null mutant,  $+/-$  represents the heterozygote, and  $+/+$  represents the *wildtype normal control*. The *phenotype* is the set of observed traits of the mutant line of mice. Phenotypes include pathological, biochemical, anatomical, physiological, and behavioral characteristics.

Targeted mutations of genes expressed in the brain are revealing the mechanisms underlying normal behavior and behavioral abnormalities. Mouse models of human neuropsychiatric diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, anorexia, anxiety, ataxias, autism, bipolar disorder, depression, drug and alcohol addiction, Huntington's disease, obesity, Parkinson's disease, and schizophrenia, are characterized in part by their behavioral phenotypes.

This book will introduce the novice to the rich literature of behavioral tests in mice. Careful readers will learn how to optimize the application of these tests for behavioral phenotyping of their line of mutant mice. Based on our experiences, our laboratory is working toward a unified approach for the optimal conduct of behavioral phenotyping experiments in mutant mice. Recommendations are offered in Chapter 13 for a three-tiered sequence of behavioral tests, applicable to the range of behavioral domains regulated by genes expressed in the mammalian brain.

## SCOPE

This book is designed as an overview of the field of behavioral neuroscience, as it can be applied to the behavioral phenotyping of transgenic and knockout mice. Molecular geneticists may browse through the chapters relevant to their gene, to get ideas for possible tests to try. Behavioral neuroscientists who have no experience with mutant mice may wish to read about the latest genetic technologies, the behavioral tests that have been used to study mice with targeted gene mutations, and some of the successful experiments published in the genetics literature. Behavioral geneticists who are expert in one area of mouse behavior may find the chapters describing other areas of mouse behaviors to be useful for expanding their research repertoire.

Chapters are organized around behavioral domains, including general health, neurological reflexes, developmental milestones, motor functions, sensory abilities, learning and memory, feeding, sexual and parental behaviors, social behaviors, and rodent paradigms relevant to fear, anxiety, depression, schizophrenia, reward, drug addiction, neurodevelopmental disorders such as autism, and aging diseases such as Alzheimer's. Each chapter begins with a brief history of the early work in the field and the present hypotheses about mechanisms underlying the expression of the behavior. A list of general review articles and books is offered for each topic, encouraging the interested reader to gain more in-depth knowledge of the relevant literature. Chapter lengths vary, reflecting the number of established tests available for mice in each behavioral domain, and the current number of publications of mutant mice displaying interesting behavioral phenotypes within each behavioral domain.

Standard tests are then presented in detail. Highlighted are those tasks that have been extensively validated in mice. Demonstrations of genetic components of task performance are described, including experiments comparing inbred strains of mice (strain distributions), naturally occurring mutants (spontaneous mutations), chemical mutagenesis (ENU), quantitative trait loci linkage analysis (QTL), and gene expression patterns (DNA microarrays). Experimental design and specific behavioral tasks are presented as simply as possible. Extensive references are included for each behavioral test, to encourage extensive reading of the primary experimental literature from experts on each topic.

Illustrations are provided for the most frequently used behavioral tasks. Photographs of the equipment or diagrams of the task accompany the text. Samples of representative data are shown. The data presentation is designed to indicate the qualitative and quantitative results that can be expected when the task is properly conducted.

Each chapter includes the results of elegant experiments in which these tasks are successfully applied to characterize transgenic and knockout mice. Examples are limited to the behavioral phenotypes. A more global discussion including physiological, anatomical, and neurochemical phenotypes is beyond the scope of this book.

Classics in the neuroscience literature are listed at the end of this chapter. Background literature for individual behavioral domains is referenced at the end of each chapter. These chapter lists offer good books, special issues of journals, review articles, and primary literature focused on the hypotheses and current body of knowledge for each behavioral domain. A wealth of original publications, describing specific methods and results in detail, is referenced within the text and listed at the end of the book. The referenced literature is designed to provide helpful examples but does not attempt to compile a comprehensive survey. The field is moving too fast. As this second edition goes to press for a 2006 publication date, only articles appearing by late 2005 are included.

The concept behind each chapter is to present simple descriptions of the abundance of behavioral paradigms, developed over the long, illustrious history of behavioral neuroscience. The secondary goal is to provide access to the primary literature and selected reviews. The tertiary goal is to highlight some of the excellent behavioral neuroscience laboratories conducting these types of experiments. Many of these behavioral neuroscientists may be willing to provide intensive training, and/or to engage in collaborative research, to phenotype a new transgenic or knockout mouse.

## MESSAGE TO MOLECULAR GENETICISTS

Welcome to the world of behavioral neuroscience! Animal behavior has fascinated human observers throughout history. Systematic studies of animal behavior began over 100 years ago with Darwin's observations on finches of the Galapagos Islands, in which feeding behavior correlated with beak shape and ecological niche (Darwin, 1839). The 1973 Nobel Prize in Medicine was awarded to Konrad Lorenz, Nikolaas Tinbergen, and Karl von Frisch for their elegant ethological studies of naturalistic animal behavior (von Frisch, 1967; Lorenz, 1974; Tinbergen, 1974). Behavioral genetics grew out of observations of species and strain differences. Present-day behavioral neuroscience and behavioral genetics focus on mechanisms underlying observed behaviors, and on the interaction of genetic, anatomical, physiological, biochemical, and environmental factors. Behavioral neuroscientists are found in universities, research

institutes, biotechnology and pharmaceutical companies, usually within departments of neuroscience, psychology, biology, zoology, pharmacology, and psychiatry. At the annual meetings of the Society for Neuroscience in the United States, which are attended by more than 30,000 neuroscientists, approximately 15% of the lectures and poster presentations represent behavioral neuroscience research.

### **Caveat to Molecular Geneticists**

*This is not a “how-to” manual.* Animal behavior is too complex and requires too much training to be attempted for the first time directly from this book alone. As in any field of science, behavioral research has evolved proper experimental designs and controls that must be correctly applied for the data to be interpretable. Little things, such as how to handle the mouse to reduce stress, can greatly affect the results of a behavioral experiment. Like microinjecting an oocyte or operating a DNA sequencer, the tricks of the trade are best learned from the experts. You don't want to waste your time reinventing the wheel. Setting up the tasks described without a behavioral neuroscientist collaborator is not recommended.

Instead, this book is a brief overview of what is available. The descriptions of behavioral tests and experimental design are provided as an initial framework for your thinking and planning. After the orientation provided in these chapters, you will be knowledgeable about the wide range of behavioral tests available for investigating your mice.

The first step is to define your hypotheses about the gene of interest. The second step is to choose the relevant tests. The third step is to develop a collaboration with a reputable behavioral neuroscience laboratory. With a few phone calls, you are likely to find a good behavioral neuroscientist in your geographic area who is willing to work with you on behavioral testing of your knockouts. The names of some of the best laboratories appear within the referenced primary publications in each chapter. Many universities have mouse behavioral phenotyping core facilities available to their investigators. Or you may prefer to contract with a company that conducts rodent behavioral tests on a fee-for-service basis. Contact information for companies is listed in Chapter 2. In addition a set of good review articles and books is provided for each behavioral domain in each chapter. These can help you to identify optimal potential collaborators to contact.

The collaboration is often arranged such that the behavioral neuroscience laboratory or core facility conducts all of the behavioral phenotyping experiments independently. Intellectual input from the molecular genetics group generates the hypotheses to address, suggests the behavioral tests to conduct, and contributes to the interpretation of the results. In this model, a scientific collaboration is set up along the lines of a program project or a center grant, with equitable agreements to share funding and authorships. Alternatively, your molecular genetics group may wish to learn the behavioral techniques from a good behavioral neuroscience laboratory, and then set up the experiments in your own laboratory. In this model the senior molecular geneticist visits the behavioral neuroscience laboratory and observes the techniques directly. Post-doctoral fellows and graduate students in the molecular genetics laboratory then spend several weeks or months in the laboratory of the behavioral neuroscience collaborators, learning the intricacies of conducting the experiments. Courses in mouse behavioral research that may provide additional useful training are listed at the end of this chapter.

This approach ensures that the behavioral concepts and techniques employed in the molecular genetics laboratory are consistent with the highest standards of behavioral neuroscience research, and that the methods used will be acceptable for publication in the best journals. This book will provide you with sufficient background for informed discussion of behavioral tests in the course of your first conversations with potential behavioral neuroscience collaborators.

## MESSAGE TO BEHAVIORAL NEUROSCIENTISTS

Behavioral neuroscience has a new tool. Transgenic and knockout mice with mutations in genes linked to human diseases provide excellent models of the behavioral traits characterizing many human genetic disorders. Behavioral abnormalities in these mouse models offer quantitative surrogate markers for the symptoms of the human disease. Reversal or prevention of the behavioral abnormalities in the mouse model can be used as a powerful preclinical endpoint, to assess the efficacy of new treatments for a genetic disease.

Targeted gene mutation offers an alternative approach to investigating endogenous mechanisms underlying behavior. Gene mutations may provide information that complements findings based on electrolytic or neurochemical lesions. For example, null mutation of a gene critical for the functioning of one hippocampal layer may be a superior alternative to an electrolytic hippocampal lesion. Deletion of the gene for a neurotransmitter receptor is analogous to administering a receptor antagonist. This technique is especially useful in understanding the behavioral relevance of a receptor subtype for which no pharmacological antagonists are available. Disruption of a developmental gene can test hypotheses about the role of a neurotrophic factor in neuronal migration or synapse formation. Addition of extra copies of a gene for a hypothalamic neuropeptide can address the outcome of excessive quantities of that neuropeptide on feeding behaviors and the etiology of obesity. Single gene mutations can be used to explore the interactions of genetic and environmental factors, such as social and parental behaviors in mutants missing receptors for olfactory signaling cues. As the technology advances, gene mutations are targeting specific brain structures at specific periods of development or during discrete experimental time points in the adult mouse.

This book is designed to provide you with an introduction to the transgenic and knockout technology, and approaches to optimize its application to behavioral neuroscience research. Chapter 2 presents descriptions of the molecular genetics of the gene targeting vector, generation of the chimera, and breeding strategies. Chapter 14 discusses quantitative trait loci analysis, DNA microarray analysis, chemical- and radiation-induced mutagenesis, viral vector gene delivery, RNA silencing, conditional mutations expressed only in one tissue type, and inducible mutations expressed at controlled time periods. Illustrations and diagrams were chosen to clarify these complex molecular technologies. References to primary publications, review articles, and books are offered at the end of the chapter for each topic, chosen for the interested reader wishing to learn more about molecular genetics. A list of Web sites for further information on the mouse and human genome projects, databases of mouse phenotypes, sources of mutant mice, and overviews of mouse behavioral genetics are provided at the end of Chapter 2.

This book is further designed to give concrete examples of experimental design, methods, and optimization of specific tasks for behavioral phenotyping of mutant

mice. Tasks originally developed for mice, and rat tasks that have been successfully adapted for mice, are highlighted. Strategies for converting rat tasks to mouse tasks are included. Cases in which rat tasks apparently cannot be performed by mice are stated. Examples are given of proper and improper behavioral methods that have been used to characterize transgenic and knockout mice.

For behavioral neuroscientists working on one type of behavior, who wish to learn more about tasks for another type of behavior, extensive references are provided within each chapter for each behavioral domain. These publications can be used to obtain specific details of experimental methods. Your excellent behavioral neuroscience laboratories may be highlighted, as suggested contacts for geneticists to consult for more information or to obtain additional training in a specific behavioral task.

### **Caveat to Behavioral Neuroscientists**

*This is a guidebook, not a comprehensive or scholarly review of behavioral neuroscience methods.* Please understand that this is not a textbook, nor a review, nor an in-depth analysis of theory. Rather, the goal of this book is a brief introduction to behavioral neuroscience for the novice. Chapters are designed as overviews of the behavioral domains that are most likely to be useful in behavioral phenotyping of mutant mice. The behavioral tests chosen for presentation are primarily those that work well with mice, and have been used with some success in some cases to characterize mutant mice. Many interesting and important behavioral paradigms are not discussed because they have not yet been fully validated for mice, or replicated across several laboratories, or have limited applicability to experiments with mutant lines of mice. Chapters are of unequal lengths, reflecting the abundance of good tests for mice in some behavioral domains and the dearth of well-characterized tests for mice in other behavioral domains.

Of course, this book cannot replace a thorough reading of the many excellent books and review articles representing the breadth and depth of behavioral research. Instead, recommended textbooks and review articles are listed in each chapter. Small samplings of the multitude of original data papers are referenced in the text.

Finally, the descriptions of the methods are intentionally superficial. Behavior is not a cookbook discipline. In the opinion of this author, molecular geneticists are best advised to seek training or collaboration with a good behavioral neuroscience laboratory, such as yours, rather than set up the behavioral tasks independently.

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### Special Issues of Journals

- Behavior Genetics* **26**(3), May 1996 Special Issue: *Molecular Genetic Approaches to Mammalian Brain and Behavior*.
- Behavior Genetics* **27**(4), July 1997 Special Issue: *The Genetics of Obesity*.
- Behavioural Brain Research* **95**(1), September 1998 Special Issue: *Behavioural Neurogenetics*.
- Brain Research* **835**, 1999 Special Issue: *Knockouts and Mutants: Genetically Dissecting Brain and Behavior*.
- Hormones and Behavior* **31**(3), June 1997 Special Issue: *Single Gene Mutations, Gene Knockouts and Behavioral Neuroendocrinology*.
- Learning and Memory* **5**(4–5), September/October 1998 Special Issue: *Transgenic/Knockout Approaches in Neurobiology*.
- Nature Neuroscience* **7**(5), May 2004: *Scaling up Neuroscience*.
- Neuropeptides* **36**(2–3), April/June 2002 Special Issue: *Transgenics and Knockouts with Mutations in Genes for Neuropeptides and Their Receptors*.
- Psychopharmacology* **132**(2), July 11, 1997, Special Issue: *Behavioral and Molecular Genetics*.
- Psychopharmacology* **174**(4) August 2004 Special Issue: *Molecular Genetics and Psychopharmacology*.

### Neuroscience Methods Series

- Current Protocols in Neuroscience*. Wiley.
- Journal of Neuroscience Methods*. Elsevier.
- Methods in Neuroscience*. Academic Press.
- Neuroscience Protocols*. Elsevier.
- Techniques in the Behavioral and Neural Sciences*. Elsevier.

### Courses in Mouse Behavior

- Cold Spring Harbor Laboratory Courses in Mouse Behavioral Analysis*. Summer 2003 course was organized by Stephen Anagnostaras and Mark Mayford, held at Cold Spring Harbor Laboratory, New York, USA. <http://meetings.cshl.org/2003/2003c-maze.htm>
- EMBO/FENS Practical Course in Mouse Transgenics and Behavior*. July 2003 course was organized by David P. Wolfer, Hans-Peter Lipp, and Richard G. M. Morris, held at the University of Zurich, Switzerland. <http://www.dpwolfer.ch/mouse-course>
- International Summer School on Behavioral Neurogenetics. August 2004 course was organized by Robert W. Williams, Douglas Matthews, Byron Jones, and Dan Goldowitz, held at the University of Tennessee, Memphis, Tennessee, USA. <http://tmmouse.org/documents/BehNeurog-SummerSchool04.pdf>; and Second Annual Experimental Neurogenetics Mouse Workshop,



organized by Dan Goldowitz and members of the Tennessee Mouse Genome Consortium, May 2005, Memphis, Tennessee, USA.

*JAX Neurogenetics Conference*, organized by Wayne Frankel. June 2004 course held at The Jackson Laboratory, Bar Harbor, Maine, USA. <http://www.jax.org/courses/events/coursedetails.do?id=28>

*The Laboratory Mouse in Vision Research*, Organized by John Macauley, Maureen McCall, and Patsy Nishina, October 2004 course held at The Jackson Laboratory, Bar Harbor, Maine, USA <http://www.jax.org/courses/events/coursedetails.do?id=42>

*Walk This Way: Gait Dynamics in Rodent Models of Human Diseases*. August 2005 workshop organized by Mouse Specifics, Inc., Cambridge Life Sciences Center, Cambridge, MA.