What's Wrong With My Mouse?

Behavioral Phenotyping of Transgenic and Knockout Mice

Second Edition

Jacqueline N. Crawley, Ph.D.

WILEY-INTERSCIENCE
A John Wiley & Sons, Inc., Publication

What's Wrong With My Mouse?

What's Wrong With My Mouse?

Behavioral Phenotyping of Transgenic and Knockout Mice

Second Edition

Jacqueline N. Crawley, Ph.D.

WILEY-INTERSCIENCE
A John Wiley & Sons, Inc., Publication

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data:

Crawley, Jacqueline N.
What's wrong with my mouse? : behavioral phenotyping of transgenic and knockout mice / Jacqueline N. Crawley. —2nd ed.
p. ; cm.
Includes bibliographical references.
ISBN: 978-0-471-47192-9 (cloth)
1. Neurogenetics—Animal models. 2. Transgenic mice—Behavior. I. Title.
[DNLM: 1. Mice, Transgenic. 2. Behavior, Animal. 3. Disease Models, Animal. 4. Genetics, Behavioral—methods. 5. Mice, Knockout. 6. Phenotype. QY 60.R6 C911w 2006]
QP356.22.C73 2006
573.8′480724—dc22 2006033565

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

To Andy and Barry,
For the privilege of sharing your genes

Contents

Preface

Five years is not a long time in science. Imagine my amazement at the stunning number of new publications describing elegant behavioral phenotyping of transgenic and knockout mice. When the first edition of *What's Wrong With My Mouse?* was completed in 1999, approximately 500 papers had been published on behavioral phenotypes of mice with targeted gene mutations. Since then the literature has quintupled, with at least 2000 new papers between 2000 and 2005. *Science* magazine's *Breakthroughs of the Year* 2005* highlights genes linked to brain disorders, emphasizing that "connecting the dots between genetics and abnormal behavior has been anything but child's play." Functional consequences of dysfunctional genes, allelic variations, susceptibility genes, protective genetic backgrounds, and environmental modulation are increasingly revealed by careful analyses of the behaviors of mutant mice.

Writing the first edition of *What's Wrong With My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice* was a challenge in summarizing the large number of well-validated behavioral tests for mice. The challenge for the second edition was to read the exponentially expanding new literature on targeted gene mutations and then select just a few good examples to insert into existing chapters for each behavioral domain. Two new chapters are added. Chapter 12, "Neurodevelopment and Neurodegeneration," illustrates the application of behavioral phenotyping methods to some of the best mouse models of human brain diseases. Important genetic models of neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis, employ robust behavioral phenotypes in mutant mice to test hypotheses about the causes of the neurodegenerative process, and to evaluate the efficacy of potential treatments. Mutant mouse models of neurodevelopmental diseases, including mental retardation and autism, provide intriguing new leads about genes regulating the exquisite biological processes controlling brain development. Chapter 13, "Putting It All Together," outlines strategies for comprehensive behavioral phenotyping. Recommendations are offered for designing the sequence of behavioral tests that

Science* **310: 1880–1885, December 23, 2005.

best addresses your hypothesis about the targeted gene, while incorporating the critical controls to rule out artifactual interpretations. Remarkably, some forefront techniques mentioned in the first edition's Chapter 12, "The Next Generation" are now established methodologies, including DNA microarrays and viral vector delivery of genes into the brain. Revised Chapter 14, "The Next Generation," contains discussion of new bioinformatics tools, including interactive websites that compile comprehensive phenotypes of each mutant mouse, database mining to discover single nucleotide polymorphisms across inbred strains of mice, and the role of micro RNAs in gene silencing.

The greatest reward for writing a scientific book is to see it used in advancing biomedical research. Thank you for the many communications I received about your successful uses of *What's Wrong With My Mouse?* in your ongoing experiments. It is beautiful to see the scientific community effectively applying information from the first edition—batteries of accepted behavioral tests to characterize a new mutation, backcrossing into a single genetic background, proper controls for procedural abilities, multiple corroborative tasks within a behavioral domain—thereby enhancing the rigor of experimental findings. Fascinating questions received from readers inspired many of the new inclusions in the second edition. Truly it was those emails out of the blue from unknown students and renowned molecular geneticists, plus the positive reinforcement of seeing *What's Wrong With My Mouse?* in labs and offices that I visited while giving lectures over the past five years, that convinced this behavioral neuroscientist to engage in the drudgery of a book update. Further I am grateful to Luna Han, the Life and Medical Sciences book editor at John Wiley & Sons who talked me into the second edition, Thomas Moore, Wiley's Senior Editor who shepherded the project to completion, Editorial Assistant Ian Collins, Senior Production Editor II Danielle Lacourciere, and Art Director Dean Gonzalez, for their essential guidance and much-needed practical and moral support. I hope this book continues to offer ideas that will aid neuroscientists and geneticists in your exciting future discoveries, to elaborate our understanding of how genes shape behavior, and to employ the behavioral phenotypes of transgenic and knockout mice in developing cures for human genetic diseases.

JACQUELINE N. CRAWLEY, PH.D.
CHEVY CHASE, MARYLAND, USA
March 2006

Preface to the First Edition

Targeted mutation of genes expressed in the nervous system is an exciting new research field that is forging a remarkable amalgam of molecular genetics and behavioral neuroscience. My laboratory in Bethesda has been the fortunate recipient of visits from many molecular geneticists over the past five years, who come to ask, "What's wrong with my mouse? Can you tell us what behaviors are abnormal in our null mutants? And how *do* you measure behavior, anyway?"

We have had some remarkable opportunities to collaborate with outstanding molecular geneticists in the National Institutes of Health Intramural Research Program and throughout the world on investigations of the behavioral effects of mutations in genes expressed in the mouse brain. Each of these collaborations has been a learning experience, increasing our understanding of the optimal experimental design for analyzing behavioral phenotypes of mutant mice. What are the best tests to address each specific hypothesis? Which methods work best for mice? Which rat tasks can be adapted for mice? What are the correct controls? What are the hidden pitfalls, lurking artifacts, false positives, and false negatives? Which statistical tests are most sensitive for detection of the genotype effect? What is the minimum number of animals necessary for each genotype, gender, and age? Our laboratory and many others are gradually working out the best methods for behavioral phenotyping of transgenic and knockout mice.

In the same conversations, molecular geneticists frequently asked me to recommend a book they could consult to learn more about behavioral tests for mice. Apparently the scientific book publishers are receiving similar queries. Ann Boyle and Robert Harington at John Wiley & Sons, convinced of a real need for such a book, sweet-talked me into filling the void. *What's Wrong With My Mouse?* is written for these pioneering molecular geneticists, and for the talented students who will be the next driving force in moving the field forward.

On a personal level, I would like to express deep appreciation to all of my behavioral neuroscientist colleagues around the world for their outstanding work, past, present, and

future. Your contributions to the excellence and abundance of mouse behavioral tests provide the foundation for the rapidly expanding scientific discoveries forthcoming from behavioral phenotyping studies of transgenic and knockout mice. This book is a testament to your accomplishments.

JACQUELINE N. CRAWLEY, PH.D.
CHEVY CHASE, MARYLAND
July, 1999

Acknowledgments

The author expresses sincere gratitude to the many colleagues who generously contributed photographs, drawings, and graphics from their research programs:

Carol Barnes, University of Arizona (*Figure 6.20A*)

Caroline and Robert Blanchard, University of Hawaii (*Figure 9.6*)

Igor Branchi and Enrico Alleva, Istituto Superiore di Sanita, Rome (*Figures 8.8A, 12.1, 12.2*)

Richard Brown, Dalhousie University (*Table 3.3*)

Katrina Cuasay, Joanna Hill, and Jacqueline Crawley, National Institute of Mental Health, Bethesda, MD (*Figure 5.1A*).

Robert Drugan, University of New Hampshire (*Opening to Chapter 10*)

Steven Dunnett, Rebecca Carter, and Jenny Morton and Steve Dunnett, Cardiff University and Cambridge University (*Figures 4.4, 4.8, 4.12, 5.3A*)

Gerry Dawson, Merck Neuroscience Research Centre (*Figure 6.32*)

William Falls, University of Vermont (*Figure 10.3*)

Deborah Finn and John Crabbe, Oregon Health Sciences University, Portland, OR (*Figure 11.2*)

Nick Grahame and Chris Cunningham, Oregon Health Sciences University (*Figure 11.3*).

Howard Eichenbaum and Aras Petrulis (*Figure 6.22*)

Andrew Holmes, National Institute on Alcoholism and Alcohol Abuse, Rockville, MD (*Figures 6.31, 10.9A,B,C, 12.7*)

Dina Lipkind, Yoav Benjamini, and Ilan Golani, Tel Aviv University (*Figure 4.2*)

Timothy Moran, Johns Hopkins University (*Figure 7.9*)

Sheryl Moy, University of North Carolina (*Figures 12.4C,D*)

Randy Nelson, Johns Hopkins University (*Figure 9.4*)

Patrick Nolan, Mammalian Genetics Unit, Medical Research Council, Harwell, UK (*Figure 14.7*)

Richard Palmiter, University of Washington (*Openings to Chapters 2 and 7*)

Richard Paylor, Baylor College of Medicine (*Figures 2.7, 6.6, 10.15, Table 3.4*)

Petko Petkov, The Jackson Laboratory, Bar Habor, ME (*Figure 2.6*).

Marina Picciotto and Venetia Zachariou, Yale University and University of Crete (*Figure 11.11*).

Emilie Rissman, University of Virginia (*Figure 8.2A*)

John Robinson, State University of New York at Stony Brook (*Figure 6.29A*)

Selen Tolu, National Institute of Mental Health (*Figures 12.4A,B*).

Harry Shair, New York State Psychiatric Institute (*Figures 8.8B, 12.3*)

Uri Shalev and Ina Weiner, Tel Aviv University (*Figure 10.13*)

George Uhl, National Institute on Drug Abuse (*Figure 11.5*)

Douglas Wahlsten, University of Windsor (*Figure 13.1*)

Jeanne Wehner, Institute of Behavioral Genetics, University of Colorado (*Figures 2.4, 14.5, Table 2.1*)

Scott Wersinger, University of Buffalo (*Figures 9.7*)

Craige Wrenn, Drake University (*Figures 5.1B, 6.24B, 6.24C*)

Jeana Yates, Northwestern University (*Table 14.2*)

Scott Young, National Institute of Mental Health (*Figure 8.6B*)

The author's admiration and appreciation is extended to Jonathan Klein, the outstanding artist who contributed his creative illustrations:

Openings to Chapters 3, 5, 8, 9, and 14

Gratitude goes to John Ward, the impressive photographer whose patience captured the images of mice performing behavioral tasks:

Figures 4.1A, 4.1B, 4.5, 4.6A, 5.2, 6.3A, 6.3B, 6.11B, 6.14, 6.18, 6.26A,B, 7.2, and 10.7A,B, Opening to Chapter 4

Special thanks to Andy Wolfe for his creative design:

Opening to Chapter 11

The author is grateful to the many excellent companies that manufacture behavioral test equipment or products used in generating and testing transgenic and knockout mice, for their generosity in contributing the following photographs and schematic diagrams:

Accuscan (*Figure 7.8*)

Affymetrix, Inc. (*Figure 14.15*)

American Association of Laboratory Animal Science (*Figure 13.2*)

Clontech (*Figure 14.2*)

Columbus Instruments (*Figures 3.3, 4.7, 4.10, 7.8*)

Hamilton-Kinder (*Figure 3.4*)

Lafayette Instrument Company, Inc. (*Figure 4.9*)

Med Associates, Inc. (*Figure 6.26 concept, 10.11*)

Mini-Mitter Company (*Figure 4.11A*)

New Behavior (*Figure 3.5*)

San Diego Instruments (*Figure 5.3A, 6.3E, 6.11A*)

Stoelting Company (*Figures 4.3A, 5.5, 5.6, 5.7*)

Taconic Farms, Inc (*Figure 2.1, 2.3*)

Permission for the use of previously published figures was kindly granted by the following journals:

Academic Press (*Figures 3.2, 8.2B, Table 9.1, Table 9.2*)

American Physiological Society (*Figure 7.5*)

American Psychological Association (*Figures 6.16, 6.17, 6.21, 6.23, 6.29B, 6.32B*)

American Society for Clinical Investigation (*Figure 14.14*)

American Society for Pharmacology and Experimental Therapeutics (*Figure 11.8*)

Annals of the New York Academy of Sciences (*Figure 10.1*)

Blackwell (*Figures 2.8; 12.1*)

Cell Press (*Figures 3.3A,B, 4.11B, 6.1, 6,2, 7.4, 8.3A, 8.4, 9.2, 9.5B, 10.17, Tables 5.2, 10.4*)

Cold Spring Harbor Laboratory Press (*Figure 2.6, Table 13.1*).

Elsevier Science (*Figures 2.10, 5.6B, 6.3D, 6.6, 6.10, 6.22, 6.25, 6.28, 7.9, 8.1B, 8.5, 9.7, 10.10, 10.12, 10.14, 11.6, Tables 5.1, 7.2, 10.1, 10.2, 10.3, 10.4, 11.1, 12.5A,B, 12.6, 12.9, Frontpiece to Chapter 13*)

John Wiley & Sons, Inc. (*Figures 2.2, 2.4, 4.4, 4.8, 6.3C, 6.19, 6.24, 6.27, 6.32A,B, 7.11, 8.7, 9.3A,B, 9.6, 10.3A,B, 10.4, 10.8A, 10.9B, 12.2, 12.3, Tables 2.1, 2.4, 12.1, 12.10*)

Lippincott Williams & Wilkins (*Figures 2.10 by Montkowski Brain Research, 5.4, Table 10.4*)

Nature (*Openings to Chapters 2 and 7, Figures 4.1B, 11.7, 12.8. Table 14.2*)

Nature Genetics (*Figure 2.5, 2.9, 4.6B, 4.4, 6.12A, 11.4, 14.4, 14.5, 14.9, Table 14.1*)

Nature Medicine (*Table 7.2*)

Oxford University Press (*Figures 7.1, 7.6, 7.7, 7.10, 14.8*)

Plenum Publishing Company (*Figures 8.3B, 9.5A, 10.7*).

Proceedings of the National Academy of Sciences USA (*Figures 6.4, 6.5, 6.7, 6.12B, 12.7, 14.11*)

Science (*Figures 6.20B, Opening to Chapter 7,7.3, 10.2, 11.10, 14.6, 14.12, 14.13, Table 7.1*)

Scientific American (*Figure 14.10*).

Society for Neuroscience (*Figures 4.12, 6.9, 10.16, 10.18*)

Springer-Verlag (*Figures 2.7, 3.1, 5.3B, 6.15, 10.5, 10.6, 10.8B, 10.15, 11.9, Table 2.2, 2.3, 3.1, 6.1*)

Warner Brothers, Inc., representative Paula Allen informally agreed to the use of a Pinky and The Brain drawing and quote (*Opening to Chapter 6*)

Karl Herrup, Case Western Reserve University, used the phrase "What's Wrong With My Mouse?" as the title of a 1996 Short Course sponsored by the Society for Neuroscience. Dr. Herrup and the Society for Neuroscience kindly approved the use of "What's Wrong With My Mouse" as the title for the present book.

David Kupfer, University of Pittsburgh, used the phrase "give your mouse a physical" (Chapter 3), at a workshop on mouse behaviors sponsored by Dr. Norman Anderson, Director of the Office of Behavioral and Social Sciences Research, National Institutes of Health, Bethesda, MD, July 13, 1998.

Personal thanks to Ann Boyle, Robert Harington, Luna Han, and Thomas Moore, at John Wiley & Sons, Inc.

Very special thanks to Barry Wolfe, for his infinite kindness and understanding.

1

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

What's Wrong With My Mouse? By Jacqueline N. Crawley

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. Postdoctoral 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.

BACKGROUND LITERATURE

Alcock J (1989). *Animal Behavior*. Sinauer Associates, Sunderland, MA.

Buccafusco JJ (2000). *Methods of Behavioral Analysis in Neuroscience* CRC Press, Boca Raton, FL.

Baker C (2004). *Behavioral Genetics*. American Association for the Advancement of Science and The Hastings Center, Washington, DC.

Becker JB, Breedlove SM, Crews D (1993). *Behavioral Endocrinology*. MIT Press, Cambridge.

Bloom FE, Young WG (1995). *Brain Browser*. Academic, San Diego.

Bolivar V, Cook M, Flaherty L (2000b). List of transgenic and knockout mice: Behavioral profiles. *Mammalian Genome* **11**: 260–274.

Campbell IL, Gold LH (1996). Transgenic modeling of neuropsychiatry disorders. *Molecular Psychiatry* **1:** 105–120.

Crawley JN, Ed. (1997 and ongoing supplements). Chapter 8 Behavioral Neuroscience. In *Current Protocols in Neuroscience*, J Crawley, C Gerfen, M Rogawski, D Sibley, P Skolnick, W Wray, R McKay, Eds. Wiley, New York (print version and CD-ROM).

Crusio WE, Gerlai RT, Eds. (1999). *Handbook of Molecular-Genetic Techniques for Brain and Behavior Research*. Elsevier, New York.

Darwin C (1839–1843). *Zoology of the Voyage of the H.M.S. Beagle, Part 3 Birds*, Smith Elder and Company, London.

Dowling JE (1998). *Creating Mind: How the Brain Works*. Norton, New York.

Foster G (1997). *Chemical Neuroanatomy of the Prenatal Rat Brain*. Oxford University Press, New York.

Franklin BJ, Paxinos G (1996). *The Mouse Brain in Stereotaxic Coordinates*. Academic, San Diego.

Goldowitz D, Wahlsten D, Wimer RE, Eds. (1992). *Techniques for the Genetic Analysis of Brain and Behavior: Focus on the Mouse*. Elsevier, Amsterdam.

Grandin T (1998). *Genetics and the Behavior of Domestic Animals*. Academic, San Diego.

Griffin DR (1985). Animal consciousness. *Neuroscience and Biobehavioral Reviews* **9:** 615–622.

Jacobowitz DM, Abbott LC (1998). *Chemoarchitechtonic Atlas of the Developing Mouse Brain*. CRC Press, Boca Raton, FL.

JAX Notes (2003). The importance of understanding substrains in the genomic age. The Jackson Laboratory 491: 1–3.

Jones BC, Mormede P (1999). *Neurobehavioral Genetics: Methods and Applications*. CRC Press, Boca Raton, FL.

Kandel ER, Schwartz JH, Jessel TM (2000). *Principles of Neural Science*, 4th ed. McGraw-Hill, New York.

Kaufman MH (1992). *Atlas of Mouse Development*. Academic, San Diego.

Lorenz K (1971). *Studies in Animal and Human Behavior*. Harvard University Press, Cambridge, MA.

Lorenz KZ (1974). Analogy as a source of knowledge. *Science* **185:** 229–234.

Lorenz KZ (1981). *The Foundations of Ethology*. Springer, New York.

Mak RW (1998). *The Gene Knockout FactsBook*. Academic, San Diego.

Marler P, Hamilton WJ, Eds. (1968). *Mechanisms of Animal Behavior*. Wiley, New York.

Nelson RJ (2005) *An Introduction to Behavioral Endocrinology*. Sinauer, Sunderland, MA.

Nelson RJ, Young KA (1998). Behavior in mice with targeted disruption of single genes. *Neuroscience and Biobehavioral Reviews* **22:** 453–462.

Paxinos G (1985). *The Rat Nervous System*. Academic, New York.

Paxinos G, Franklin KBJ (2000, 2003). *The Mouse Brain in Stereotaxic Coordinates.* Academic, San Diego.

Plomin R, DeFries JC, McClearn GE, Rutter M (1998). *Behavioral Genetics*. Freeman, New York.

Schambra UB, Lauder JM, Silver J (1991). *Atlas of the Prenatal Mouse Brain*. Academic, New York.

Silver LM (1995). *Mouse Genetics*. Oxford University Press, New York.

Squire LR, Bloom FE, McConnell SK, Roberts JL, Spitzer NC, Zigmond MJ (2002). *Fundamental Neuroscience*, 2d ed. Academic Press, New York.

Swanson, L (1998). *Brain Maps: Structure of the Rat Brain*. Elsevier Science, New York (print version and CD-ROM).

Tinbergen N (1974). Ethology and stress diseases. *Science* **185:** 20–27.

Von Frisch K (1967). *The Dance Language and Orientation of Bees*. Belknap, Cambridge, MA.

Wahlsten D, Crabbe JC (2006). Behavioral testing. In *The Mouse in Biomedical Research*, volume III, in press.

Wahlsten D, Metten P, Phillips TJ, Boehm SL, Burkhart-Kasch S, Dorow J, Doerksen S, Downing C, Fogarty J, Rodd-Henricks K, Hen R, McKinnon CS, Merrill CM, Nolte C, Schalomon M, Schlumbohn JP, Sibert JR, Wenger CD, Dudek BC, Crabbe JC (2003). Different data from different labs: Lessons from studies of gene-environment interaction. *Journal of Neurobiology* **54**: 283–311.

Whishaw IQ, Kolb B (2004). *The Behavior of the Laboratory Rat: A Handbook with Tests.* Oxford University Press, Oxford.

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://tnmouse.org/documents/BehNeurogSummerSchool04.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.

Mice expressing the rat metallothionen-growth hormone fusion gene grew significantly larger than their littermates. *(From the cover of Nature, Vol. 300, December 16, 1982; Palmiter et al., 1982.)*

2

Of Unicorns and Chimeras

Targeted gene mutation technologies began in the 1980s (Jaenisch, 1976, 1988; Costantini and Lacy, 1981; Gordon and Ruddle, 1981; Harbers et al., 1981; Wagner et al., 1981a, 1981b; Jaenisch, 1988; Pascoe et al., 1992; Doetschman, 1991; Smithies, 1993; Bronson and Smithies, 1994; Smithies and Kim, 1994; Capecchi, 1989, 1994). Building on manipulations of yeast and fruitfly genomes, mouse mutations multiplied. The first big success in detecting a phenotype relevant to behavior in a transgenic mouse appeared in 1982. The cover illustration of the December 16 issue of *Nature*, shown on the opposite page, excited the popular imagination with the dramatic results of the elegant experiments by Richard Palmiter and co-workers at the University of Washington (Palmiter et al., 1982). A growth hormone overexpressing transgenic mouse was much larger than normal littermate control mice of the same age and gender, as a result of more rapid weight gain. Technical advances in targeted gene mutations in mammals raised hopes that the new technology could be applied to discovering the role of individual genes in normal and abnormal behavioral processes. This dream moved into the realm of reality over the past decade. Many excellent books and review articles describe the techniques for generating transgenics, knockouts, knock-ins, conditional mutations, inducible mutations, and further elegant genome manipulations (Bradley et al., 1992; Hogan et al., 1994; Accili, 2000; Joyner, 2000; Jackson, 2000; Gossmann et al., 2000; Rülicke and Hübscher, 2000; Nestler et al., 2001; Hofker et al., 2002; Nagy et al., 2002; Wolfer et al., 2002; Tecott and Wehner, 2001; Tecott, 2003; Tenenbaum et al., 2004).

The present chapter provides a brief overview of the types of targeted gene mutations in current use. The primary focus of this chapter is on the steps following the generation of the first founder mouse. Breeding strategies tailored to the needs of behavioral phenotyping are presented. Background strains that have been effectively used to breed mutant lines are recommended for various behavioral domains. Housing, transportation,

What's Wrong With My Mouse? By Jacqueline N. Crawley

group size, group composition, and animal welfare requirements are described, to meet the special demands of behavioral phenotyping. Original literature cited in the text, and review articles cited at the end of this chapter, provide more in-depth information for the interested reader. The listings at the end of this chapter include background readings on DNA constructs, embryonic stem cell lines, breeding strategies, mouse handbooks, companies that provide breeding and genotyping services, companies that design and manufacture behavioral test equipment, and academic organizations and companies that generate and phenotype mutant mice. Web sites relevant to these topics are included.

GENERATING A TARGETED GENE MUTATION

The process of developing a transgenic or knockout mouse begins with an identified gene. If the gene has not yet been sequenced, a useful targeting vector cannot be designed.

Transgenic is defined by the insertion of a gene. Transgenic mice may have a new gene added, for example, the human gene for a hereditary disease such as Huntington's (Carter et al., 1999), or extra copies of an existing gene, for example, the corticotropin releasing factor gene in order to investigate excessive expression of this stress-related hormone and neurotransmitter (Stenzel-Poore et al., 1994). Transgenic techniques involve microinjection of the DNA construct containing the transgene into the pronucleus of a fertilized mouse oocyte. The DNA construct also contains a reporter gene, such as β-galactosidase (lacZ), with a nuclear localization signal (nls). The reporter gene is simultaneously driven by the promoter for the transgene. LacZ positive cells indicate the presence of the transgene in the cell. Concentrations of the reporter gene, the transgene, and the gene product are assayed to determine the overexpression level of the gene in the tissue of interest. Anatomical mapping of the localization of the reporter gene, the transgene, and the gene product is used to describe the distribution of the transgene in the brain, or to more precisely delineate the specific neurons expressing a known gene product during stages of development (Jacobowitz and Abbott, 1997; Itoh et al., 1998). Transgenic methods are diagrammed in Figure 2.1. Each mouse that develops from a microinjected egg is a potential *founder* of a mutant line. The success rate of the technique is proportional to the number of eggs injected, because insertions through homologous recombinations are random, infrequent events. The more lottery tickets you buy, the better your chances of winning.

Knockout mice represent a loss of function or a null mutation, meaning a mutated gene that does not synthesize its protein. Knockout mice are generated by a different set of techniques (Wynshaw-Boris et al., 1999; Ledermann, 2000). Instead of a gene insertion, knockouts have a mutation introduced into a carefully chosen exon of the cDNA of the gene. The mutation is usually a selective deletion of a portion of DNA that is critical for the expression of the gene product. The gene for resistance to an antibiotic drug, such as the neomycin resistance gene (Neo^r), is inserted within the DNA construct as a marker. The deletion and insertion usually shift the reading frame for the DNA, rendering incorrect reading of the triplet base pair codes for the amino acids comprising the gene product. A typical targeting vector is shown in Figure 2.2.