Contents

Foreword to the Second Edition	xiii
Foreword to the First Edition	xv
Preface to the Second Edition	xvii
Preface to the First Edition	xix
Introduction	xxiii

1. Whole-Brain Imaging

Structural Brain Imaging Techniques	2
Cerebral Angiography	3
Computerized Tomography	4
Magnetic Resonance Imaging	6
Diffusion MRI	12
Functional Brain Imaging Techniques	14
Functional Magnetic Resonance Imaging	14
Positron Emission Tomography	17
Single-Proton Emission Computerized Tomography	19
Electroencephalography	20
Magnetoencephalography	20
Optical Imaging	22
Functional Imaging Experimental Design and Analysis	24
Planning the Experiment	25
Conducting the Experiment	29
Manipulating Neural Activity during an Experiment	31
Postexperimental Data Analysis	32
Conclusion	35
Suggested Reading and References	37

2. Animal Behavior

Considerations for Choosing and Performing a Behavioral Assay	40
Choosing an Appropriate Model Organism	40
Choosing an Appropriate Behavioral Paradigm	41
Variability in Individuals	42
Using Animal Behavior as a Model for Human Behavior	43

vi Contents

Rodent Behavioral Paradigms	44
Locomotor Activity	44
Motor Coordination and Balance	45
Sensory Function	48
Nociception	50
Spatial Learning and Memory	52
Nonspatial Learning and Memory	54
1 0	56
Attention and Impulsivity Reward-Related Behaviors	56
	57
Social Behaviors	58
Anxiety	60
Depression	61
Drosophila Behavioral Paradigms	_
Locomotor Behavior	62
Flight	62
Sensory Function	63
Learning and Memory	65
Social Behaviors	65
Caenorhabditis elegans Behavioral Paradigms	65
Locomotor Behavior	66
Sensory Behavior	66
Nonhuman Primate Behavioral Paradigms	67
Conclusion	69
Suggested Reading and References	69

3. Stereotaxic Surgeries and In Vivo Techniques

Stereotaxic Surgeries	74
Stereotaxic Surgeries in Rodents	76
Stereotaxic Surgeries in Nonhuman Primates	79
Implants for Long-Term Access to the Brain	80
Sealable Chambers	80
Cannulae	80
Cranial Windows	81
Measuring Neural Activity In Vivo	81
Electrophysiology	81
Fluorescent Activity Indicators	82
Measuring Neural Chemistry In Vivo	82
Microdialysis	82
Voltammetry and Amperometry	84
Manipulating Neural Activity In Vivo	86
Conclusion	86
Suggested Reading and References	86

4. Electrophysiology

A Brief Review of the Electrical Properties of Neurons	90
The Electrophysiology Rig	92
Categories of Electrophysiology Recordings	93

Extracellular Recording	99
Intracellular Recording	105
Patch Clamp Techniques	105
Electrophysiology Tissue Preparations	108
In Vitro Recordings	108
In Vivo Recordings	112
Manipulating Neural Activity during	
Electrophysiology Experiments	113
Conclusion	114
Suggested Reading and References	114

5. Microscopy

Essential Principles of Microscopy	118
Fundamental Parameters in Microscopy	118
The Design of a Compound Microscope	121
The Design of a Stereomicroscope	125
Light Microscopy	126
Fluorescence Microscopy	128
Epifluorescent Microscopy	129
Confocal Microscopy	129
Two-Photon Microscopy	132
Total Internal Reflection Fluorescence Microscopy	132
Electron Microscopy	133
Transmission Electron Microscopy	134
Scanning Electron Microscopy	134
Electron Tomography	135
Preparing and Interpreting Microscopy Data	136
Image Processing	136
Interpreting Images	137
Conclusion	139
Suggested Reading and References	143

6. Visualizing Neural Structure

Tissue Preparation	146
Fixation	146
Embedding	147
Sectioning	147
Visualizing Morphology	150
Cell Body Stains	150
Fiber Stains	152
Golgi Stain	152
Intracellular and Juxtacellular Labeling	153
Visualizing Gene and Protein Expression	156
In Situ Hybridization	156
Immunohistochemistry	158
Enzymatic Histochemistry	160
Reporter Genes	160

Visualizing Circuitry	162
Anterograde and Retrograde Tracers	162
Transsynaptic Labeling	164
Conclusion	165
Suggested Reading and References	165

7. Visualizing Neural Function

Static Markers of Activity	168
Assaying Neural Activity in Fixed Tissue	168
Assaying Cellular Function in Fixed Tissue	169
Visualizing Neural Activity	171
Imaging Voltage	171
Imaging Calcium Dynamics	173
Imaging Synaptic Transmission	176
Visualizing Protein Function	177
Time-Lapse Imaging with Reporter Genes	177
Fluorescence Resonance Energy Transfer	177
Bimolecular Fluorescence Complementation	180
Fluorescence Recovery after Photobleaching	180
Photoactivation and Photoconversion	180
Conclusion	181
Suggested Reading and References	181

8. Manipulating Neural Activity

	Experimental Logic of Manipulating Neural Activity	186
	Physical Manipulation	187
	Ablation	187
	Thermal Cooling	188
	Electrical Manipulation	189
	Pharmacological Manipulation	189
	Genetic Manipulation	191
	Pharmacogenetic Manipulation	192
	Optogenetic Manipulation	193
	Optogenetic Transgenes	193
	Methods of Delivering Light to the Nervous System	195
	Strategies for Studying Neural Circuits Using Optogenetics	196
	Neuromodulation Techniques Used in Humans	197
	Conclusion	197
	Suggested Reading and References	200
9.	Identifying Genes and Proteins of Interest	

A Brief Review of Genes and Proteins	204
The Central Dogma of Molecular Biology	204
Deoxyribonucleic Acid	204

Transcription	206
Translation	208
Genetic Screens	209
Forward Genetic Screen	210
Reverse Genetic Screen	213
In Silico Screens	214
BLAST	214
Ensembl	214
Molecular Screens	214
cDNA Microarray Screen	215
RNAi Screen	216
Conclusion	216
Suggested Reading and References	217

10. Molecular Cloning and Recombinant DNA Technology

Isolating DNA Fragments	220
Restriction Enzymes	220
Polymerase Chain Reaction	221
Isolation and Characterization of DNA Fragments Using Gel	
Electrophoresis	226
Cloning DNA	227
Vectors	227
Ligation	229
Transformation	231
Purifying DNA from Host Cells	231
Identifying DNA	231
DNA Sequencing	233
Nucleic Acid Hybridization Techniques	234
Conclusion	236
Suggested Reading and References	236

11. Gene Delivery Strategies

Physical Gene Delivery	239
Microinjection	240
Electroporation	241
Biolistics	243
Chemical Gene Delivery	244
Calcium Phosphate Transfection	244
Lipid Transfection	246
Viral Gene Delivery	247
Adenovirus	248
Canine Adenovirus	248
Herpes Simplex Virus	249
Adeno-Associated Virus	250
Lentivirus	250

x Contents

	Conclusion	250
	Suggested Reading and References	251
12.	Making and Using Transgenic Organisms	
	Transgenes	254
	Reporter Genes	254
	Genes Used to Measure Neural Activity	254
	Genes Used to Manipulate Neural Activity	256
	Genes Used to Ablate Neurons	257
	Genes Used to Disrupt Endogenous Gene Function	257
	Overexpression of Endogenous Genes	258
	The Transgenic Construct	258
	Binary Transgenic Systems	261
	The Gal4/UAS System	262
	The Cre/Lox System	263
	The Flp/Frt System	264
	The Tet-off/Tet-on System	264
	Making Transgenic Organisms	265
	Making Transgenic Mice	265
	Making Transgenic Flies	267
	Making Transgenic Worms	267
	Making Other Transgenic Organisms Conclusion	269 269
		269 270
	Suggested Reading and References	2/0
13.	Manipulating Endogenous Genes	
	Classical Gene Targeting Methods	274
	Knockout Mouse	275
	Knockin Mouse	282
	Conditional Knockout Mouse	282
	Nuclease-Based Gene Targeting Methods	284
	Disrupting Gene Products	287
	RNA Interference (RNAi)	287
	Morpholinos	289
	Dominant Negatives	290
	Conclusion	291
	Suggested Reading and References	291
14.	Cell Culture Techniques	
	Cell Culture Equipment and Reagents	296
	Equipment	296
	Culture Media	298
	Immortalized Cell Lines	298
	Primary Cell and Tissue Culture	301
		- • •

Slice Cultures	302
Explant Cultures	303
Dissociated Cell Cultures	303
Stem Cell Cultures	304
Embryonic Stem Cells	304
Neural Stem Cells	305
Induced Pluripotent Stem Cells	306
Manipulating Cells in Culture	307
Transfection and Infection	307
Co-Culture Systems	307
Pharmacology	308
Antibody Interference	308
Conclusion	308
Suggested Reading and References	309

15. Biochemical Assays and Intracellular Signaling

Introduction to Signal Transduction and Intracellular Signaling	313
Fundamental Tools Used to Study Proteins	313
Making and Using Antibodies	314
Purifying Proteins	318
Investigating Protein Expression	320
Western Blot	320
Enzyme-Linked Immunosorbent Assay	323
Radioimmunoassay	324
Immunohistochemistry	325
Immunoelectron Microscopy	326
Reporter Proteins	327
Investigating Protein–Protein Interactions	327
Co-Immunoprecipitation	327
Protein Affinity Chromatography	328
Yeast Two-Hybrid Assay	330
Investigating Posttranslational Modifications	331
PTM-Specific Assays	336
PTM-Specific Antibodies	337
Investigating Protein–DNA Interactions	337
Electrophoretic Mobility Shift Assay	338
Chromatin Immunoprecipitation	339
Luciferase Assay	341
Conclusion	342
Suggested Reading and References	342

Glossary	345
Index	377