

Preface

Neuroscience is one of the most dynamic disciplines in biology. The inherent interest of the nervous system is not alone sufficient to explain the explosion of work in this field. The last decade has seen considerable conceptual progress, but technical advances leading to the introduction of new methods in laboratories across the world have also played a major part in driving the field forward. The rapid transfer of technical information and experience is the partner of profound conceptual change.

The techniques for investigating the nervous system that have been developed require familiarity with electrophysiology, mouse genetics, optics, brain anatomy, and many other methods. More than most areas of biology, neuroscience is a multidisciplinary activity. Although it is hard to be comprehensive in this diverse field, our goal in creating this manual is to provide a source for core techniques in the systems and behavioral aspects of neuroscience.

The methods in *Short Protocols in Neuroscience: Systems and Behavioral Methods* are shortened versions of methods published in *Current Protocols in Neuroscience* (CPNS). This compendium includes step-by-step descriptions for a broad range of systems and behavioral neuroscience methods. The methods are complete and easy to follow, and the book is easier to use at the bench than the parent volumes. *Short Protocols* is ideal for graduate students and postdoctoral fellows who are familiar with the background and theory found in CPNS, and sufficient detail is provided to allow experienced investigators to use it as a stand-alone bench guide.

Although mastery of the techniques presented in this manual, and its companion, *Short Protocols in Neuroscience: Cellular and Molecular Methods*, will allow the reader to design and complete experiments in neuroscience, this manual is not intended to be a substitute for an advanced training program in neuroscience, nor for any number of comprehensive textbooks in the field. In addition to becoming thoroughly knowledgeable of the terms and concepts of neuroscience, readers should gain first-hand experience in basic techniques and safety procedures by working in a supervised neuroscience laboratory.

HOW TO USE THIS MANUAL

Format and Organization

Subjects in this manual are organized by chapters, and protocols are contained in units. Protocol units, which constitute the bulk of the book, generally describe a method and include one or more protocols with listings of materials and steps. Recipes for unique reagents and solutions are included in *APPENDIX 1*, and literature references are included in the *References* section at the end of the book. The book also includes several overview units which contain theoretical discussions that lay the foundation for subsequent protocol units.

Protocols

Many units in the manual contain groups of protocols, each presented with a series of steps. One or more *basic* protocols are presented first in each unit and generally cover the recommended or most universally applicable approaches. *Alternate* protocols are provided where different equipment or reagents can be employed to achieve similar ends, where the starting material requires a variation in approach, or where requirements for the end product differ from those in the basic protocol. *Support* protocols describe additional steps that are required to perform the basic or alternate protocols; these steps are separated from the core protocol because they might be applicable to other uses in the manual, or because they are performed in a time frame separate from the basic protocol steps.

Reagents and Solutions

Reagents required for a protocol are itemized in the materials list before the procedure begins. Many are common stock solutions, others are commonly used buffers or media, while others are solutions unique to a particular protocol. All recipes are presented in *APPENDIX 1*. It is important to note that the names of some of these special solutions might be similar from unit to unit (e.g., SDS sample buffer) while the recipes differ. To minimize confusion, the appropriate unit for a given recipe, except for those for commonly used solutions, is given parenthetically in the appendix.

NOTE: Deionized, distilled water should be used in the preparation of all reagents and solutions and for all protocol steps. When sterile solutions are required, that will be indicated in the protocol.

Equipment

When special equipment is required for an experiment, that equipment is listed in the Materials list for the protocol. The Materials list does not include every item used; rather it includes items that might not be readily available in the laboratory, items that have particular specifications, and items that require special preparation or temperatures.

Standard pieces of equipment used in the modern neuroscience laboratory are listed in the accompanying box. These items are used frequently in the protocols in this manual and are not necessarily listed in the Material list.

Commercial Suppliers

Throughout the manual we have recommended commercial suppliers of chemicals, biological materials, and equipment. In some cases, the noted brand has been found to be of superior quality, or it is the only suitable product available in the marketplace. In other cases, the experience of the author

Standard Equipment

Applicators, cotton-tipped and wooden

Autoclave

Balances, analytical and preparative

Beakers

Bench protectors, plastic-backed (including "blue pads")

Biohazard disposal containers and bags

Bottles, glass and plastic

Bunsen burners

Cell harvester, for determining radioactivity uptake in 96-well microtiter plates

Centrifuges, low-speed (6,000 rpm) and high-speed (20,000 rpm) refrigerated centrifuges and an ultracentrifuge (20,000 to 80,000 rpm) are required for many procedures. At least one microcentrifuge that holds standard 0.5- and 1.5-ml microcentrifuge tubes is essential. It is also useful to have a tabletop swinging-bucket centrifuge with adapters for spinning 96-well microtiter plates.

Clamps

Cold room or cold box, 4°C

Computer (IBM-compatible or Macintosh) and printer

Containers, assortment of plastic and glass dishes for gel and membrane washes

Coplin jars or staining dishes, glass, for 75 × 25-mm slides

Cryovials, sterile—e.g., Nunc

Cuvettes, plastic disposable, glass, and quartz

Darkroom and developing tank, or X-Omat automatic X-ray film developer (Kodak)

Desiccators (including vacuum desiccators) and desiccant

Dry ice

Filtration apparatus, for collecting acid precipitates on nitrocellulose filters or membranes

Flasks, glass (e.g., Erlenmeyer, beveled shaker)

Forceps

Fraction collector

Freezers, -20° and -80°C

Geiger counter

Gel dryer

Gel electrophoresis equipment, at least one full-size horizontal apparatus and one horizontal minigel apparatus, one vertical full-size and minigel apparatus for polyacrylamide protein gels, and specialized equipment for two-dimensional protein gels

Gloves, plastic and latex, disposable and asbestos

Graduated cylinders

Heating blocks, variable temperature up to 100°C; these thermostat-controlled metal heating blocks that hold test tubes and/or microcentrifuge tubes are very convenient for carrying out enzymatic reactions

Heat-sealable plastic bags and sealing apparatus

Hemocytometer

Hoods, chemical (fume), microbiological safety, and laminar flow (tissue culture)

Hot plates, with or without magnetic stirrer

Ice buckets

Ice maker

Incubators, 37°C for bacteria and humidified 37°C, 5% CO₂ for tissue culture

Kimwipes, or equivalent lint-free tissues

Lab coats

Light box, for viewing gels and autoradiograms

Liquid nitrogen and Dewar flask

Lyophilizer

Magnetic stirrers, (with heater is useful)

Markers, including indelible markers and china-marking pencils

Microcentrifuge, Eppendorf-type, maximum speed 12,000 to 14,000 rpm

Microcentrifuge tubes, 1.5-ml and 0.5-ml

continued

of that protocol is limited to that brand. In the latter situation, recommendations are offered as an aid to the novice neuroscientist in obtaining the tools of the trade. Experienced investigators are therefore encouraged to experiment with substituting their own favorite brands.

Addresses, phone numbers, facsimile numbers, and URLs of all suppliers mentioned in this manual are provided in *APPENDIX 3*.

References

Short Protocols gives only a limited number of the most fundamental references as background.

These are listed at the end of each unit. Full bibliographic information for these and cited references are given in the *References* at the end of this book. Readers who would like a more extensive entry into the literature for background and application of methods are referred to the appropriate unit in *Current Protocols in Neuroscience*.

Safety Considerations

Anyone carrying out these protocols may encounter the following hazardous or potentially hazardous materials: (1) radioactive substances, (2) toxic chemicals and carcinogenic or teratogenic

Standard Equipment, *continued*

Microscope, standard optical model (optionally with epifluorescence or phase-contrast illumination) and inverted microscope for tissue culture

Microscope slides and coverslips

Mortar and pestle

Ovens, drying, hybridization, vacuum, and microwave

Paper cutter, large size, for 46 × 57-cm Whatman paper sheets

Paper towels

Parafilm

pH meter

pH paper

Pipet bulbs, or battery-operated pipetting devices—e.g., Pipet-Aid (Drummond Scientific)

Pipets, Pasteur and graduated, glass and plastic, serological (1-ml to 25-ml)

Pipettors, adjustable delivery, volume ranges 0.5 to 10 μ l, 10 to 200 μ l, and 200 to 1000 μ l. It is best to have one set of these three sizes for each full-time researcher and sets dedicated for radioactive and PCR experiments.

Plastic wrap, UV-transparent (e.g., Saran Wrap)

Pliers, needle nose

Polaroid camera

Power supplies, 300-V power supplies are sufficient for polyacrylamide gels; 2000- to 3000-V is needed for some applications

Racks, for test tubes and microcentrifuge tubes

Radiation shield, Lucite or Plexiglas

Radioactive waste containers, for liquid and solid waste

Razor blades

Refrigerator, 4°C

Ring stands and rings

Rotator, end-over-end

Rubber bands

Rubber policeman

Rubber stoppers

Safety glasses

Scalpels and blades

Scintillation counter

Scissors

Sectioning equipment, cryostat microtome, sliding microtome (with stage and knife), Vibratome

Shakers, orbital and platform, room temperature or 37°C. An enclosed shaker (e.g., New Brunswick Controlled Environment Incubator Shaker) that can spin 4-liter flasks is essential for growing 1-liter *E. coli* cultures; a rotary shaking water bath (New Brunswick R76) is useful for growing smaller cultures in flasks

Spectrophotometer, UV and visible

Speedvac evaporator (Savant)

Stir-bars, assorted sizes

Surgical equipment, scale for weighing animals, electric razor, syringes, hypodermic needles, dissection instruments (scissors, scalpels, forceps, hemostats, retractor, bone drill, sutures), operating microscope (optional), heating pad, sterile gauze

Tape, masking and electrician's

Thermometers

Timer

UV cross-linker (e.g., Stratalinker from Stratagene)

UV light sources, long- and short-wave, stationary or hand-held

UV transilluminator

Vacuum aspirator

Vacuum line

Vortex mixers

Wash bottles, plastic and glass

Water baths, variable temperature up to 80°C

Water purification equipment, e.g., Milli-Q system (Millipore) or equivalent

X-ray film cassettes and intensifying screens

reagents, (3) pathogenic and infectious biological agents, and (4) recombinant DNA constructs. Check the guidelines of your particular institution with regard to use and disposal of these hazardous materials. Only limited cautionary statements are included in this manual. Users are responsible for understanding the dangers of working with hazardous materials and for strictly following the safety guidelines established by manufacturers as well as local and national regulatory agencies. Radioactive substances must be used only under the supervision of licensed users, following the guidelines of the National Regulatory Commission (NRC).

Animal Handling

Many protocols call for use of live animals (usually rats or mice) for experiments. Prior to conducting any laboratory procedures with live subjects, the experimental approach must be submitted in writing to the appropriate Institutional Animal Care and Use Committee (IACUC). Written approval from this committee is absolutely required prior to undertaking any live-animal studies. Some specific animal care and handling guidelines are provided in the protocols where live subjects are

used, but check with your IACUC guidelines to obtain more extensive guidelines.

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RECOMMENDED BACKGROUND READING

Becker, J.B., Breedlove, M.S., and Crews, D. (eds.) 1993. Behavioral Endocrinology. MIT Press, Cambridge, Mass.

Jacqueline N. Crawley, Charles R. Gerfen, Michael A. Rogawski, David R. Sibley, Phil Skolnick, and Susan Wray