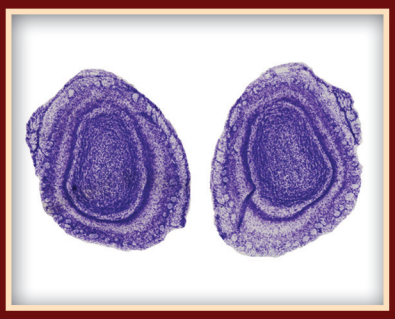
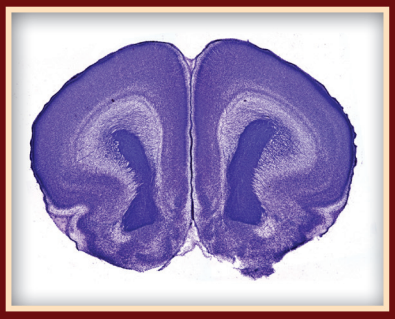
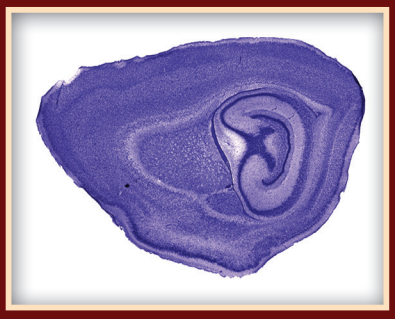
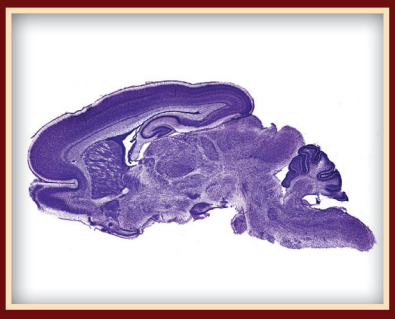
Atlas of the

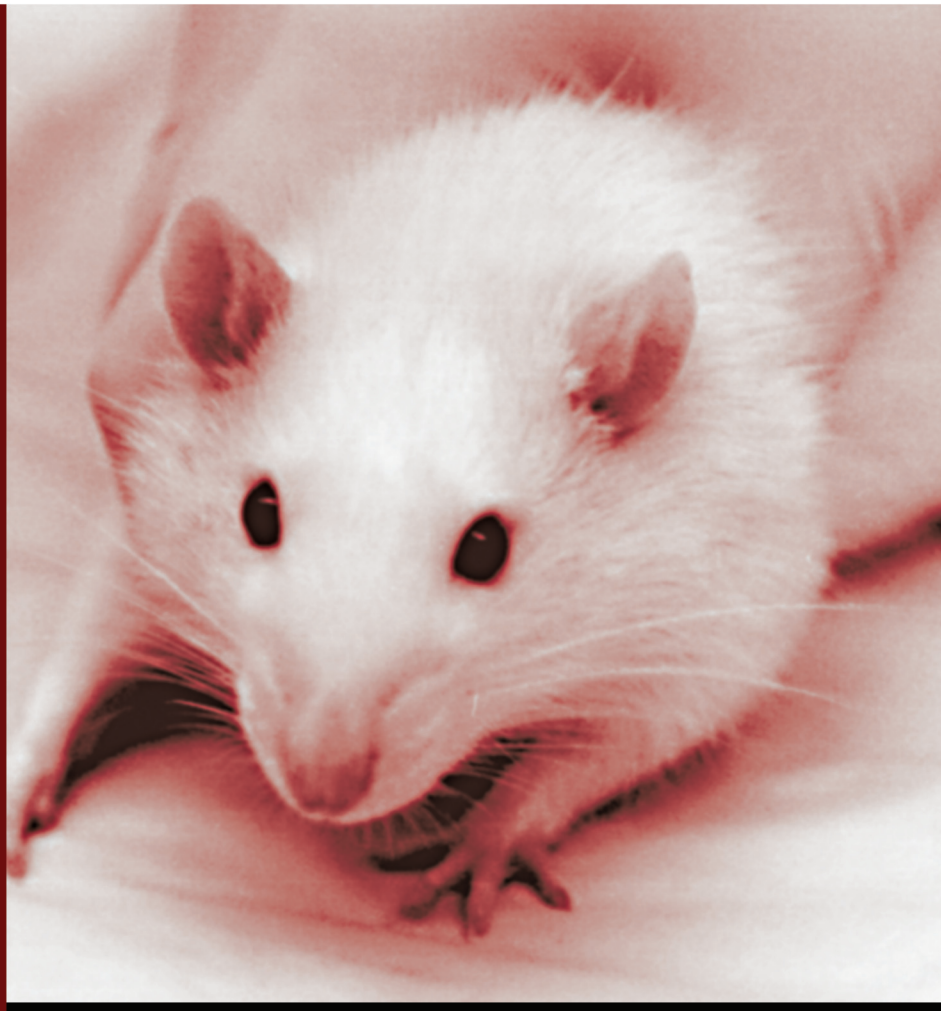
NEONATAL RAT BRAIN











Renuka Ramachandra
Thyagarajan Subramanian

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Dedication

We dedicate this scientific work to our parents who inspired us to follow our interests and provided unwavering support to us during all our struggles and successes.

Contents

Preface

The rat brain develops enormously after birth and the changes are very rapid. We have attempted to prepare a photographic atlas representing the neonatal rat brain at postnatal (P) days P-1, P-7, and P-14. This atlas illustrates the main anatomical features at these three ages.

It will serve the needs of researchers and students who are interested in postnatal development, slice cultures, developmental disorders, neuroanatomy, neuropharmacology, neurobiochemistry, and neuropathology of rats. We hope that this atlas will provide a template for comparative studies with other species and numerous animal models of brain pathology in rats.

Acknowledgments

We are grateful to the following individuals for making this atlas work possible through their moral, emotional, technical, and scientific support: David Good, Keith Elmslie, Kala Venkiteswaran, Sriram S. Shanmugavelandy, Christopher Lieu, Anand Rao, Mathew Berk, Timothy Gilmour, and Barbara Norwitz. We also acknowledge financial support for this work from research grants to Thyagarajan Subramanian from the National Institutes of Health (NS42402), Commonwealth of Pennsylvania Tobacco Settlement Biomedical Research Fund, and the Penn State University Brain Repair Research Fund. We also thank the U.S. Department of Health for the Physician Scientist Research Award.

About the Authors

Renuka Ramachandra is a postdoctoral researcher at the AT Still University in Kirksville, Missouri. She received her PhD degree in neurophysiology from India and her initial postdoctoral training at the National Brain Research Centre in India, a premier neuroscience institute. Dr. Ramachandra worked on the effect of barrel cortex lesions in rats and on the effects of drugs on the developing rat brain. She also worked as a research scientist in the embryonic stem cell group at Reliance Life Sciences, Mumbai, India, where she differentiated human embryonic stem cells into oligodendrocytes for spinal cord injury treatment. She went on to use her skills and knowledge of basic electrophysiology, brain anatomy, and histological techniques to develop an in vitro basal ganglia slice culture model in the Department of Neurology at Penn State College of Medicine. This model is used to study in vitro cell transplantation. During the development of this model, she recognized the need for a neonatal rat brain atlas. Dr. Ramachandra created this atlas for her project and decided to publish it so that it would be available to all neuroscientists and students who need one.

Thyagarajan Subramanian is a professor of neurology and neural and behavioral sciences at Penn State University Hershey Medical Center in Hershey, Pennsylvania. Dr. Subramanian received his initial medical training from Calicut Medical College in Calicut, India and his graduate training in developmental neurobiology and neural cell transplantation under the guidance of Dr. R. D. Lund at the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. He completed his neurology residency at the University of Pittsburgh followed by fellowship training in neurological research and movement disorders at Emory University in Atlanta, Georgia under the guidance of Drs. Mahlon R. DeLong, Ray L. Watts, and Roy A. E. Bakay. Dr. Subramanian's research interests are in developmental neurobiology, basal ganglia physiology, cell transplantation, gene therapy, and experimental neurotherapeutics. Using a variety of animal models, cell and tissue culture techniques, in vivo electrophysiology, and detailed histology, Dr. Subramanian and co-workers have described several novel findings in central nervous system (CNS) response to transplantation. Among his major scientific contributions are his work on creating an MRI based brain atlas of the Rhesus monkey, description of the effects of immunomodulation on host responses to CNS xenografting, and the description of the use of retinal pigment epithelial cell grafts for CNS repair. Dr. Subramanian has directed the medical neuroscience course for medical and graduate students and trained numerous medical students, as well as neurology, neurosurgery, physiatry, psychiatry, and geriatric residents. He has served as the education committee chair of the American Society for Neural Therapy and Repair (ASNTR) and has trained numerous scientists and physicians who now work as independent investigators in many institutions all over the world.

Introduction

Rodents have frequently been used to model a number of neurological disorders. The neonatal rat in particular has been used in many developmental studies and extensively for preparing slice culture models that permit the study of interactions among different regions of the brain during development. Neonatal rats have also been used as models for neurotoxicological screening and mechanistic studies (Becker and Liu, 2006; Noraberg, 2004). During our attempts to develop a neonatal rat brain slice culture model of basal ganglia development, we realized that there was no published neonatal rat brain atlas. This took us by surprise as we knew of quite a few adult rat brain atlases. To remedy this situation, we developed this atlas showing representative development of the rat brain between postnatal day 1 (P-1) and postnatal day 14 (P-14). A number of animals from each litter were euthanized on the designated postnatal dates and the brains were sectioned, stained, photographed, and annotated to prepare this atlas.

This atlas is an effort to provide a guide to the neonatal rat brain. It contains a comprehensive set of histological images of the newborn rat brain from P-1, P-7, and P-14. Although we prepared brain sections for all the ages from P-1 to P-14, we have chosen to present these three ages as representative to provide a template of developmental maturation of the neonatal rat brain at various stages. Further, the inclusion of every age between P-1 and P-14 would needlessly add pages to this atlas without adding value. Additional images will be made available as electronic resources for individuals who seek images not represented in this volume. P-0 was covered earlier in *The Atlas of the Developing Rat Nervous System* (Paxinos et al., 1994), so we started at P-1, proceeded to P-7 (the midpoint in neonatal development), and concluded at P-14. This atlas contains both coronal and sagittal sections for all the three age groups. The P-1 section contains 30 coronal plates and 14 sagittal plates; P-7 includes 27 coronal plates and 24 sagittal plates. The final P-14 section shows 41 coronal plates and 21 sagittal plates. Each set consists of contiguous sections from individual animals with no substitutions or omissions. The sections were prepared carefully to ensure that their orientations were maintained and were consistently of the highest quality. The selections were based on the structural variability represented. Care was taken to minimize tears and distortions. Fixation and staining cause minimal amounts of shrinkage and damage to tissues. However, we feel that the structural details are well preserved.

This atlas has certain unique features. The sections are Nissl stained with cresyl violet—the most common staining technique used in the neurosciences. Future editions will include specific immunostains and special stains in the same format as these Nissl stains. The photomicrographs achieve high resolution and clarity. The structures are directly labeled on the images, making it easier for readers to correlate data. The electronic version will allow labels to be removed so the atlas can be used as a teaching tool.

Animal Preparation

Sprague-Dawley rat pups were used for this work. Pregnant dams from Charles River Laboratories (Wilmington, MA) were received on day 13.5 of gestation and housed in standard laboratory conditions, with 12-hour dark and light cycles and administration of food and water ad libitum. All procedures complied with guidelines issued by the National Institutes of Health and were approved by Penn State University's Institutional Animal Care and Welfare Committee. After birth, rat pups ranging in age from P-1 to P-14 were sacrificed at the same time every day. The sexes of the newborns were not taken into consideration. The neonates were decapitated and the brains were removed carefully and postfixed in 4% paraformaldehyde for 3 to 4 days. This allowed preservation of the brain structures and made it easier to isolate the brains from the delicate skulls. The brains were then cryoprotected in 15% sucrose in phosphate buffered saline (PBS) and then in 30% sucrose in PBS.

The brains were positioned and frozen on sucrose blocks made on the base of a sliding microtome that was then used to cut the brains at 50-µm thickness for both coronal and sagittal views. For sagittal sections, each brain was cut along the midline carefully and placed with the midline facing the block. Care was taken to retain and mount every section cut. We noted all missing sections so that we could calculate the correct distance from the midline for the sagittal sections.

Section Processing

Every section was mounted on polylysine-coated slides, air dried, and stained for Nissl bodies using cresyl violet. In short, the slides with the sections were passed through the solutions for 3 minutes each in the following order except where noted: 100% ethanol, 100% ethanol, 95% ethanol, 75% ethanol, water (single dip), cresyl violet (3 to 4 minutes), water, 75% ethanol, 95% ethanol, 100% ethanol, 100% ethanol, and xylene. The slides were cover slipped using DPX mounting media and left to dry for 2 days before image capture.

To make 500 ml of 0.5% cresyl violet (pH about 3.9), we mixed 2.5 g cresyl echt violet, 300 mL water, 30 mL 0.1 M sodium acetate (13.6 g granular sodium acetate in 92 mL water), and 170 mL 1.0 M acetic acid (29 mL glacial acetic acid added to 471 mL water). This solution was mixed at least 7 days on a magnetic stirrer and then filtered.

Imaging

Using Neurolucida software (Version 8, MBF Biosciences), photomicrographs of all the serial sections were captured. We used the virtual slice feature of Neurolucida that allowed us to capture the images at 4× magnification in smaller blocks and finally merge them to yield a holistic image of an entire section. In digital format, the images can be zoomed in without losing much of the detail. The images were post processed in Adobe Photoshop to clear up the background. No changes were made to the actual photomicrographic images captured.

Labeling

The images were labeled based on the nomenclature used by Paxinos, with some modifications to suit the need of the atlas. For the P-1 rat brain, we labeled most structures using *The Atlas of the Developing Rat Nervous System* (Paxinos et al., 1994). Because the P-7 and P-14 brain structures are similar to those of adult rat brains, we followed the nomenclature of *The Rat Brain in Stereotaxic Coordinates* (Paxinos et al., 1998).

The labeling of the respective structures was done on the actual photographic images rather than on the classic line diagrams that most atlases utilize. This allows the user to correlate the structures and their names easily. We decided not to demarcate the areas on the brain as the brain is a very plastic structure and the areas could not be distinguished from each other easily. The major parts of the brain were labeled via Adobe Photoshop without the inclusion of minor details.

We used cresyl violet stain to identify the structures. This posed limitations on identifying some of the smaller nuclei that required special staining. Most of the structures were identified based on the proximity to the surrounding structures and outstanding landmarks; for example, striatum was identified based on its patchy matrix and close proximity to the corpus callosum.

The most difficult structures to identify were the thalamic nuclei in the P-1 brains. We restricted our labeling to major nuclei only to avoid confusion. Since the brain develops very quickly in the first few days after birth, it is very difficult to demarcate the developing nuclei without in-depth knowledge of that area of research. The other structure that posed certain limitations in labeling was the cerebellum of the P-1 brain. The lobules are very different from those of the adult brain.

We tried to keep most of the sagittal brain sections intact. However, we encountered a few sections in which the cerebellum could not be kept intact with the rest of the brain. The distances between adjacent plates of the sagittal sections were estimated from the midline using the section thickness; the midline was considered the absolute zero.

This photographic atlas can assist neuroscientists and students to identify and understand the developing rat brain structure. However, it does not cover stereotaxic coordinates.